SYNTHESIS AND BIOLOGICAL ACTIVITY OF 7,8-POLYMETHYLENEPURINES

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This report is a continuation of our studies on the synthesis of 7,8-polymethylene derivatives of purine and the systematic search for biologically active substances among these compounds. The synthesis and biological activity of the isomeric 8,9-polymethylenepurines have been studied in detail [2]. However, purine derivatives containing polyethylene chain in position 7 and 8 have received very little study [5].

These studies were started using cyclic N-cyanamides (Ia-c), which are easy to synthesize by the reaction of O-methylbutyro-, valero- and caprolactimes with cyanamide [3]. The literature contains a number of examples of the use of these compounds for preparing N-substituted N'-cyanamidines, which can be cyclized using the Thorpe-Ziegler reaction to produce amino derivatives of imidazole [9-11].

The first stage of these studies required a method for N-alkylating amidines Ia-c at the cyclic NH group to be developed. Given that cyanamide is very acidic, while N-cyanamidines are extremely weakly basic [4], it seemed logical to prepare these compounds through metal derivatives of amidines Ia-c. Bromoacetic ester was selected as the alkylating agent, because the presence of a ethoxycarbonylmethyl group at position 1 of the amidines makes the methylene unit sufficiently basic for the Thorpe-Ziegler reaction. Attempts to use the potash-acetone system were unsuccessful: alkylation did not occur even at the solvent's boiling temperature. A successful alkylation reaction was obtained by using the sodium salts of amidines I, prepared using a suspension of metallic sodium in toluene. However, this method gave low yields of reaction products, and was quite complex for the preparative point of view. The best approach was to use a suspension of potash in dimethylformamide (DMF) as the base, which allowed the reaction of Ia-c with chloroacetic ester to take place easily at 80°C, and the desired N-ethoxycarbonylmethyl-N'-cyanamidines (IIa-c) were produced with satisfactory yields. While the iminopyrrolidine derivatives of series IIa were obtained in the solid form and was characterized by elemental analysis, the six- and seven-membered analogs were isolated as oils by column chromatography, and their structures were determined by mass spectroscopy.

The N-ethoxycarbonylmethyl-N'-cyanamidines IIa-c thus prepared were able to undergo intramolecular cyclization by the Thorpe-Ziegler reaction. This was achieved by treatment with sodium ethanoate in ethanol with gentle heating, which produced amino derivatives of polymethyleneimidazoles (IIIa-c).

The pyrimidine ring could be closed to make the purine systems of interest by using a variety of one-carbon components, such as urea, thiourea, guanidine, etc. [8]. We selected dimethylformamide diethylacetal, which was added to condensation reactions with aminoimidazoles IIIa-c. This reaction produced amidines (IVa-c) at high yield. The pentamethylene derivative IVc could not be extracted in the solid phase, and was used in the next step without purification.

The pyrimidine ring was closed to prepared polymethylenehypoxanthines (Va-c) by incubating compounds IVa-c at 120°C for 8 h in an autoclave, in ethanolic ammonia at ten-fold excess.

The first step in this heterocyclization reaction is probably transamination of the amidine group; stabilization of the intermediate amidines (VIa-c) occurs because of intramolecular condensation, which eliminates an ethanol molecule to form the tricyclic purines V. The structure of the resulting hypoxanthine derivatives Va-c was confirmed by elemental analysis and by mass and NMR spectroscopy. Thus, for example, the structure of compound Va was easily deducted from its ^{1}H NMR spectrum, which contained methylene proton signals as triplets at

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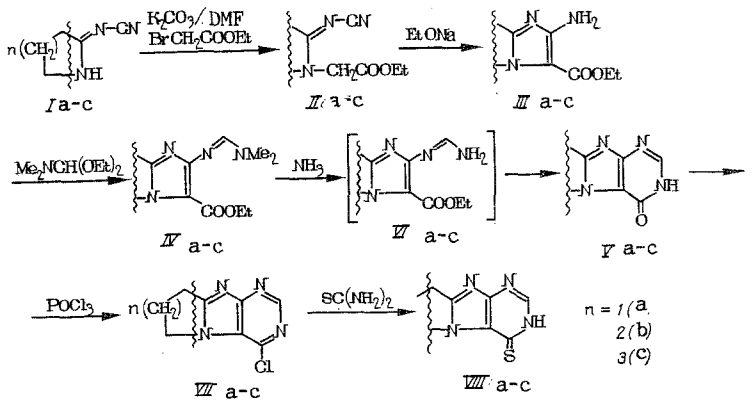
2.92 and 4.20 ppm (α-CH_2, γ-CH_2) and as a quintet at 2.58 ppm (β-CH_2); the pyrimidine CH- and NH- proton signals were singlets at 7.89 and 12.22 ppm respectively.

Hypoxanthines could be thionylated by the classic method of substituting the 6-oxo group, by treating them with phosphorus pentasulfide in pyridine [6]. However, since the 6-chloro derivatives are of interest in their own right, for preparing other 6-substituted purines, we selected another widely used method for preparing these mercapto derivatives. The interaction of hypoxanthines Va-c with $POCl_3$ in the presence of $Et_3N \cdot HCl$, followed by degradation of the intermediate chloroxy complexes with aqueous alkali was used to synthesize the tricyclic 6-chloropurines (VIIa-c). These were easily converted into the 6-mercapto derivatives (VIIIa-c) by treatment with thiourea in ethanol followed by degradation of the isothiouronyl salt with aqueous alkali.



Thus, combination of the N-ethoxycarbonylmethylation of the N'-cyanamidines with the Thorpe-Ziegler cyclization, followed by purine synthesis was found to be a suitable method for synthesizing the 7,8-polymethylene derivatives of the 6-mercaptopurines.

EXPERIMENTAL (CHEMICAL)

The mass spectra of the compounds synthesized here were taken on a MAT-112 spectrometer, with an ionizing voltage of 50 eV, an ionization chamber temperature of 140°C; ^1H NMR spectra were taken on an X-linked-200 spectrometer, using trimethylsilane as the internal standard. Melting temperatures were measured on a Boetius heating block.

1-Ethoxycarbonylmethyl-2-cyanoiminopyrrolidine (IIa)

Method A. N-cyanamidine Ia (27 g, 248 mmole) was added to a suspension of 6 g (260 mmole) of sodium in 400 ml of dry toluene; the mixture was boiled for 30 min, after which 5 ml of ethanol was added and the reaction was boiled for another 30 min. Solvent was then evaporated from the reaction to a vapor temperature of 110°C, the reaction was cooled to 5-10°C, and 34.5 g (280 mmole) of chloroacetic ester was added dropwise at a rate that prevented the temperature from increasing by more than 5°C. The reaction was then stirred for 1 h, and filtered; the filtrate was evaporated and the residue was shaken with hexane, and compound IIa was collected by filtration, with $M^+ = 195$.

Method B. N-cyanamidine Ia (27 g, 220 mmole) was added to a suspension of 35 g (250 mmole) of potash in 75 ml of DMF, along with 27 g (220 mmole) of chloroacetic ester, and the mixture was heated to 80°C for 1 h. The reaction was then filtered and evaporated, and the residue was shaken with hexane.

1-Ethoxycarbonylmethyl-2-cyanoiminopiperidine (IIb)

This was prepared from compound Ib by a method analogous to that used for compound IIa.

1-Ethoxycarbonylmethyl-2-cyanoiminohexahydroazepine (IIc)

This was prepared from compound Ic by a method analogous to that used for compound IIa.

1,2-Trimethylene-4-amino-5-ethoxycarbonylimidazole (IIIa)

Compound IIa (26 g) was added to a solution of EtONa, prepared by mixing 150 ml absolute ethanol with 1 g Na, and the reaction was boiled for 15 min and evaporated. The residue was

TABLE 1. Physicochemical Properties of the Compounds Synthesized

Compound	Yield, %	Melting temperature, °C (solvent)	Molecular formula
IIa	57**	78—80, water	$C_9H_{13}N_3O_2$
IIb*	—	—	$C_{10}H_{15}N_3O_2$
IIc*	—	—	$C_{11}H_{17}N_3O_2$
IIIa	82	142—3, ethyl acetate	$C_9H_{13}N_3O_2$
IIIb	73	110—1, hexane	$C_{10}H_{15}N_3O_2$
IIIc	68	111—3, ethanol + H_2O, 1:10	$C_{11}H_{17}N_3O_2$
VIa	81	120—2, toluene	$C_{12}H_{18}N_4O_2$
VIb	65	97—8, toluene	$C_{13}H_{20}N_4O_2$
VIc	—	—	$C_{14}H_{22}N_4O_2$
Va	73	>300, H_2O	$C_2H_2N_4O$
Vb	68	274—6, H_2O	$C_9H_{10}N_4O$
Vc	57	279—81, H_2O	$C_{10}H_{12}N_4O$
VIIa	92	179—82, isopropanol	$C_8H_7N_4Cl$
VIIb	84	220—2, isopropanol	$C_9H_9N_4Cl$
VIIc	86	162—3, isopropanol	$C_{10}H_{11}N_4Cl$
VIIIa	65	>300 dimethylformamide, 1:1	$C_8H_2N_4S$
VIIIb	71	>300 dimethylformamide, 1:1	$C_9H_{10}N_4S$
VIIIc	75	>300 dimethylformamide 1:1	$C_{10}H_{12}N_4S$

*The structure was determined by IR and mass spectroscopy.
†Method A; method B gave 77%.

then resuspended in water, filtered, and washed with water. The mother fluid was extracted with $CHCl_3$, dried over $CaCl_2$ evaporated, and the residue was resuspended in water to yield additional IIIa.

1,2-Pentamethylene-4-amino-5-ethoxycarbonylimidazole (IIIc)

This was prepared from compound IIc by a similar method used for compound IIIa.

1,2-Trimethylene-4-(N,N-dimethylaminomethylene)-amino-5-ethoxycarbonylimidazole (IVa)

A mixture of 1.95 g (10 mmole) of imidazole IIIa and 1.72 ml (10 mmole) of dimethylformamide acetal in 15 ml of dry toluene was boiled for 2 h, after which another 0.86 ml (5 mmole) of acetal was added, and the reaction was boiled for another 2 h. The reaction was then evaporated, filtered, and the product was washed with hexane. M^+ was 250.

1,2-Tetramethylene-4-(N,N-dimethylaminomethylene)-amino-5-ethoxycarbonylimidazole (IVb)

This was prepared from imidazole IIIb by a similar method used for compound IVa. M^+ was 264.

1,2-Pentamethylene-4-(N,N-dimethylaminomethylene)-amino-5-ethoxycarbonylimidazole (IVc)

This was prepared from imidazole IIIc by a similar method used for compound IVa. M^+ was 278.

1,6-Dihydro-1H-7,8-trimethylenepurine-6-one (Va)

A mixture of 1 g of imidazole IV and 15 ml of 20% ethanolic ammonia was heated in an autoclave at 120°C for 8 h, after which the reaction mixture was evaporated and filtered, and the residue was washed with ethanol. The ^1H NMR spectrum (DMSO-d_6) was: 2.58 (2H, quintet, β-CH_2); 2.92 (2H, triplet, γ-CH_2); 4.20 (2H, triplet, α-CH_2); 7.89 (1H, singlet, 4-CH); 12.22, wide singlet, NH).

1,6-Dihydro-1H-7,8-tetramethylenepurine-6-one (Vb)

This was prepared from imidazole IVb using a reaction similar to that used for the preparation of compound Va.

TABLE 2. Antitumor Activity of Compounds

Compound	Tumor strain	Amount given dose, mg/kg	I_T	K_p
Va	Jenson sarcoma	100	23	—5
Vc		110	18	—9
VII a		60	20	+4
VII c		30	20	+1
VIIIa		60	20	—1
VIIIc		110	14	—5
Va	M-1 sarcoma	110	40	—6
Vc		116	29	—16
VII a		120	34	+5
VII c		70	43	—7
VIIIa		110	19	—4
VIII c		110	10	—7
Va	B-16 melanoma	50	10	—1
Vc		50	30	—5
VII a		50	21	—5
VII c		20	10	—6
VIII a		50	20	+3
V a	L 1210	100	0	—4
V c		112	20	—
Vc		110	0	—
VIIa		112	0	—
VIIc		63	0	—
VIII a		110	0	—
VIII c		112	0	—

1,6-Dihydro-1H-7,8-pentamethylenepurine-6-one (Vc)

This was prepared from imidazole IVc using a reaction similar to that used for the preparation of compound Va.

7,8-Trimethylene-6-chloropurine (VIIa)

A mixture of 3.88 g (20 mmole) of compound Va, 15 ml of $POCl_3$ and 1 g of $Et_3N \cdot HCl$ was boiled for 2 h. Excess $POCl_3$ was then distilled off, and the residue was dissolved in $CHCl_3$, poured over ice, and neutralized with NaOH solution to pH 7. The organic layer was separated off, dried, evaporated, and shaken with petroleum ether.

7,8-Tetramethylene-6-chloropurine (VIIb)

This was prepared from compound Vb using a reaction similar to that used for the preparation of compound VIIa.

7,8-Pentamethylene-6-chloropurine (VIIc)

This was prepared from compound Vc using a reaction similar to that used for the preparation of compound VIIa.

1,6-Dihydro-1H-7,8-trimethylenepurine-6-thione (VIIIa)

A mixture of 1 g (5 mmole) of chloropurine VIIa and 0.39 g (5.5 mmole) of thiourea was boiled in 25 ml absolute ethanol for 1.5 h, after which the solvent was evaporated. The residue was boiled for 5 min with 40 ml of 15% NaOH, cooled, and neutralized with acetic acid to pH 7; the precipitate was collected by filtration and washed with water and acetone. $M^+ = 192$.

1,6-Dihydro-1H-7,8-tetramethylenepurine-6-thione (VIIIb)

This was prepared from chloropurine VIIb using a reaction similar to that used for the preparation of compound VIIIa.

1,6-Dihydro-1H-7,8-pentamethylenepurine-6-thione (VIIIc)

This was prepared from chloropurine VIIc using a reaction similar to that used for the preparation of compound VIIIa.

Elemental analysis agreed with calculated values. Table 1 shows the yields and physicochemical properties of the compounds synthesized.

EXPERIMENTAL (BIOLOGICAL)

Antiviral activity was studied using herpes simplex virus type I, antigenic type L_2 and influenza virus A/Bethesda (H_2N_2), grown in primary trypsinized chick embryo fibroblasts (CEF) and in white mongrel mice (14-16 g).

Virus-inhibiting activity was studied in cell cultures, after preliminary determination of maximum tolerable concentrations (MTC) for CEF cells. Virus was adsorbed for 1 h, and compounds were added to the cell monolayers at the MTC and lower concentrations. Results were assessed in terms of the ability of compounds to prevent the viral cytopathic effects (CPE) and to reduce the infectious titers from the control values.

The therapeutic activity of the compounds was studied in mice with generalized herpes and with influenza pneumonia, following intranasal inoculation. Compounds were given p.o. at doses of 25-100 mg/kg once daily for 5 days. Activity was expressed in terms of the reduction in lethality in treated animals as compared to untreated controls.

Two of the six compounds (Va and VIIIc) inhibited herpes simplex virus reproduction at a concentration of 10 μg/ml, preventing the CPE and reducing the infectious titer by 1.25 log (TCD_{50} 50% tissue culture infectious dose). These compounds were inactive in mice with generalized herpes. Of the three compounds studied with respect to influenza virus, Va, Vc, and VIIIc, only Va had therapeutic effects in mice with pneumonia, a dose of 60 mg/kg/day reducing lethality by 30%.

These results show that the antiviral activity of 7,8-polymethylene-6-mercaptopurines against viruses with DNA and RNA genomes was not high, but suggest that a search for potential antiviral agents among these heterocyclic compounds might be valuable.

Antitumor activity was studied in ten experiments using 120 mongrel male rats (110-120 g) with Jensen sarcoma, M-1 sarcoma, and in 180 BDF_1 mice (20-22 g) with B-16 melanoma and L-1210 leukemia. Compounds were given i.p. in 10% polyvinylpyrrolidone daily for 7 days, starting 5 days after tumor transplantation. Animals were killed with ether 24 h after the last dose, and tumor weights and changes in body weight were measured. The index of tumor growth inhibition (I_t, %) and the coefficient of body growth (K_p) were determined. Positive values of K_p indicate a greater, and negative values a lesser, increase in body weight during the experiment as compared with controls. When mice carried L-1210 leukemia, compound dosage was started 24 h after tumor transplantation, and were given daily for 5 days. The duration of life was compared with that in controls [7].

Table 2 shows that the compounds had low toxicity after single i.p. doses; lethal doses were greater than 230 mg/kg. However, compound VIIc had cumulative toxicity: animals died after two doses of 100 mg/kg. Compounds VIIIa and VIIIc had no antitumor activity in these experiments. None of the compounds was effective in rats with L-1210 leukemia. Compound Vb had slight antitumor activity in rats with B-16 melanoma (I_t = 30%). Compounds Va, VIIa, and VIIc had weak antitumor activity in rats with M-1 sarcoma (I_t = 40%, 34%, and 43% respectively). The combination of antiviral and antitumor activities indicates that further searching for biological activity among the 7,8-polymethylenepurines is worthwhile.

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SYNTHESIS AND ANTIVIRAL ACTIVITY OF CROWN-CONTAINING PURINES

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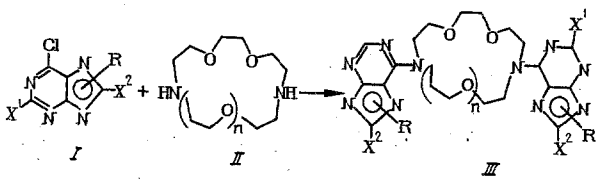
Purines containing macrocyclic fragment substituents have not previously been described. Considering the biological importance of purine derivatives and their ability to form complexes [1], we have studied the isolation of purines with fragments containing azamacroheterocyclic nuclei and have investigated their biological activities.

Methods based on the use of halogen-substituted purines (I) and diazacrown ethers (II) could be among the best routes for synthesizing this type of structure. Boiling the initial reagents in anhydrous ethanol gives an extremely slow reaction, probably because of the relatively low mobility of the halogen atoms in the chloropurines I, and also because of the low basicity of the nitrogen atoms in the azacrown ethers II.



I II III

X^1 = Cl (Ia, c, IIIa, b, e, f), H (Ib, IIIc, d)
X^2 = Cl (Ia, IIIa, b), H (Ib, c, IIIc-f)
R = H (Ia, b, IIIa-d), CH_3 (Ic, IIIe, f)
n = 1 (Ia, IIIa, c, e), 2 (Ib, IIIb, d, f)

The reaction occurs much more rapidly in the autoclave in anhydrous ethanol at 160°C.

Kinetic studies showed that the reactivity of N-substituted purines for amines changes in the sequence 6 > 2 > 8. Nucleophilic substitution in 2,6,8-trichloropurine and 7-methyl-2,6-dichloropurine occurs in position 6, as in classical examples of amination [2-4]. When diazacrown ethers are used as the amines, the reaction occurs at two nitrogen atoms, i.e. at a purine:diazacrown ether ratio of 2:1. Only in the case of the reaction of 6-chloropurine with diaza-18-crown-6 are the products obtained as a mixture of the mono- and disubstituted crown ethers. In all probability, substitution would always involve two nitrogen atoms if the reaction were carried out at high pressure and temperature, and only a large excess of the crown ether or a very short reaction time would result in the predominance of the monosubstituted crown ether.

The initial chloropurines I were prepared by standard methods [5-7]. The structures of compounds III were confirmed after synthesis by spectral analysis (Table 1).

The antiviral activity and acute toxicity of compounds IIIa-f were studied, and the results are presented in Table 2.

EXPERIMENTAL (CHEMICAL)

IR spectra were taken on a Perkin-Elmer 580B in KBr tablets. Mass spectra were taken on a Varian MATCH-5 with an ionizing voltage of 70 eV and a temperature a little above the

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