SYNTHESIS AND ANTIVIRAL ACTIVITY OF NEW UNSATURATED PYRIMIDINE ACYCLONUCLEOSIDES

A. A. Ozerov, M. S. Novikov,
A. K. Brel', O. T. Andreeva,
G. V. Vladykov, E. I. Boreko,
L. V. Korobchenko, and S. G. Vervetchenko

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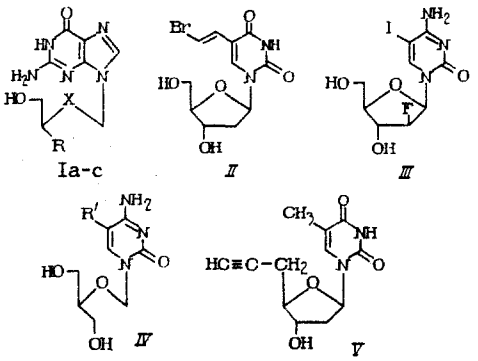
The discovery of the unique antiherpetic properties of acyclovir [ACV, (Ia)] [7, 20] stimulated much interest in the search for new acyclic analogs of the nucleosides and the study of their antiviral action. This led to the synthesis of acyclonucleosides similar in structure to ACV: gancyclovir [GCV, (Ib)] [12, 15] and 9-(4-hydroxy-3-hydroxymethylbutyl)guanine (Ic) [10, 23].



R=H (Ia), CH_2OH (Ib,c); X=O (Ia, b), CH_2 (Ic);
R'=H, F, Cl, Br, I, CH_3 (IV).

The investigation of the mechanism of antiviral action showed a general trend for (Ia-c): the compounds are activated in cells infected with herpes virus by the phosphorylation with viral thymidine kinase, and inhibit viral DNA-polymerase in the presence of triphosphates [8, 19, 22]. An analogous mechanism of antiviral action is found for the pyrimidine nucleosides 5-E-(2-bromovinyl)-2'-desoxyuridine [BVDU, (II)] [5, 6] and 1-(2'-fluoro-2'-desoxy-β-D-arabinofuranosyl)-5-iodocytosine (III) [11, 22, 24], which exhibit vigorous antiherpetic activity. Acyclic analogs of pyrimidine nucleosides, similar in structure to (Ia) and (Ib), either showed weak activity against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) or were inactive [4, 9, 13, 16-18]. The 1-(1,3-dihydroxypropoxymethyl)cytosines (IV) were synthesized only recently; they showed effective inhibition of the replication of the Epstein-Barr virus and the cytomegalovirus in vitro [3].

A different mechanism of antiviral action is possessed by 5'-ethynylthymidine (V). Having a triple carbon–carbon bond at the position 5 of the ribosyl residue, (V) inhibited the viral thymidine kinase in the cells infected with HSV-1, and did not inhibit the cellular thymidine kinase. Moreover, (V) showed effective inhibition of the thymidine kinase produced by viral strains resistant to ACV and BVDU [14]. However, acyclic analogs of 5'-ethynylthymidine or other acyclonucleosides having a terminal carbon–carbon multiple bond and showing marked antiviral activity have been unknown until the present time.

The present work describes the synthesis of some unsaturated pyrimidine acyclonucleosides and presents results of the investigation into their antiviral aactivity.

The starting point of the synthesis was the isolation of the allyl 1-chloroalkyl esters (VIa-c) by the Anri reaction from aldehydes and allyl alcohol according to the known method

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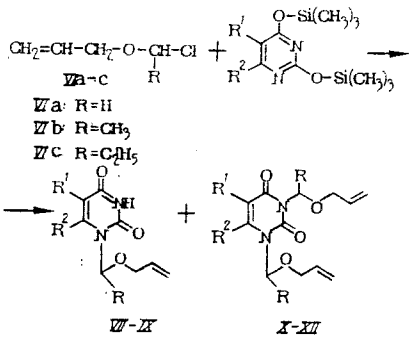
TABLE 1. Structure and Physicochemical Properties of Allyloxyalkyl Derivatives of Uracil (VIIa-h), (VIIIa-d), (IXa-d), (Xa-d), (XIa-c), and (XII)-(XVI)

Compound	R	R1	R2	Yield, %	mp, °C (n_D^{20})	Empirical formula
VIIa	H	H	H	84,1	114—116	$C_8H_{10}N_2O_3$
VIIb	H	F	H	65,0	50—51	$C_8H_9N_2O_3F$
VIIc	H	CP	H	78,3	92—94	$C_8H_9N_2O_3Cl$
VIId	H	Br	H	92,9	99—101	$C_8H_9N_2O_3Br$
VIIe	H	CH_3	H	77,4	118—119	$C_9H_{12}N_2O_3$
VIIf	H	H	CH_3	54,6	113—115	$C_9H_{12}N_2O_3$
VIIg	H	Br	CH_3	57,3	130—132	$C_9H_{11}N_2O_3Br$
VIIh	H	H	COOH	49,7	103—105	$C_9H_{10}N_2O_5$
VIIIa	CH_3	H	H	88,0	41—42	$C_9H_{12}N_2O_3$
VIIIb	CH_3	F	H	76,4	99—100	$C_9H_{11}N_2O_3F$
VIIIc	CH_3	H	CH_3	23,2	(1,5286)	$C_{10}H_{14}N_2O_3$
VIIId	CH_3	Br	CH_3	34,4	157—159	$C_{10}H_{13}N_2O_3Br$
IXa	C_2H_5	H	H	91,2	59—60	$C_{10}H_{14}N_2O_3$
IXb	C_2H_5	F	H	73,6	59—61	$C_{10}H_{13}N_2O_3F$
IXc	C_2H_5	Br	H	61,9	111—112	$C_{10}H_{13}N_2O_3Br$
IXd	C_2H_5	H	CH_3	10,1	(1,5284)	$C_{11}H_{16}N_2O_3$
Xa	H	H	H	90,0	(1,5226)	$C_{12}H_{16}N_2O_4$
Xb	H	F	H	89,0	(1,5102)	$C_{12}H_{15}N_2O_4F$
Xc	H	Cl	H	86,1	(1,5316)	$C_{12}H_{15}N_2O_4Cl$
Xd	H	CH_3	H	78,8	(1,5214)	$C_{13}H_{18}N_2O_4$
XIa	CH_3	F	H	73,1	(1,5020)	$C_{14}H_{19}N_2O_4F$
XIb	CH_3	H	CH_3	52,2 a	(1,5104)	$C_{15}H_{22}N_2O_4$
XIc	CH_3	Br	CH_3	37,4 b	(1,5336)	$C_{15}H_{21}N_2O_4Br$
XII	C_2H_5	H	CH_3	50,3 c	(1,4999)	$C_{17}H_{25}N_2O_4$
XIII	H	H	H	83,0	136—137	$C_{15}H_{15}N_3O_3$
XIV	H	H	H	90,0	218—219	$C_8H_{11}N_3O_2$
XV	H	H	H	72,8	47—49	$C_{24}H_{40}N_2O_4$
XVI	H	H	H	61,5	116—118	$C_8H_{12}N_2O_3$

[a]The compounds (VIIIc) and (XIa) are formed simultaneously.
[b]The compounds (VIIId) and (XIc) are formed simultaneously.
[c]The compounds (IXd) and (XII) are formed simultaneously.

proposed for allyl chloromethyl ether [2]. The absence of the absorption bands of the HO and C=O groups from the IR spectra testified to the high purity of the chloroethers (VIa-c) obtained.



The reaction of equimolar amounts of the allyl 1-chloroalkyl ethers (VIa-c) with 2,4-bis(trimethylsilyloxy)pyrimidine and its 5-substituted derivatives at room temperature in a dry solvent (methylene chloride, chloroform, tetrachloromethane, 1,2-dichloroethane, or acetonitrile) for 24 h led exclusively to the N(1)-monoallyloxyalkyl derivatives (VIIa-e), (VIIIa, b), and (IXa-c) with the yield of 62-93% (Table 1). The presence of the methyl group at the position 6 of the pyrimidine ring lowered the rate of the N(1)-alkylation significantly; this is probably caused by steric factors. For this reason, both 1-(allyloxyalkyl)-6-methyluracils (VIIIc, d), (IXd) as well as the 1,3-di(allyloxyalkyl)-6-methyluracils (XIb,c), (XII) are formed in the course of the reaction. The increase in the size of the radical R in the allyl 1-chloroalkyl ethers from H to CH_3 and C_2H_5 resulted in a regular decrease in the selectivity of the N(1)-monoalkylation. Thus, if 1-(allyloxymethyl)-6-methyluracil (VIIf) was obtained with the yield of 54.6%, then 1-(allyloxy-1-ethyl)-6-methyluracil (VIIIc) and 1-(allyloxy-1-propyl)-6-methyluracil (IXd) were produced in the corresponding yields of 23.2 and 10.1% under the strictly identical conditions. The analogous trend is also observed for 5-bromo-6-methyluracil.

The synthesis of 1-(allyloxymethyl)cytosine (XIV) was accomplished by means of the alkylation of the trimethylsilyl derivative of N(4)-benzoylcytosine with allyl chloromethyl ether and the subsequent removal of the benzoyl protecting group in the intermediate 1-(allyloxymethyl)-N(4)-benzoylcytosine (XIII) by a methanolic solution of ammonia saturated at 0°C. The utilization of the trimethylsilylderivative of the unsubstituted cytosine as the substrate did not guarantee the acceptable yield of the desired product.

The saturated analog of 1-(allyloxymethyl)uracil [AMU, (VIIa)], namely 1-(propoxymethyl)uracil (XVI), was obtained analogously by the condensation of 2,4-bis(trimethylsilyloxy)pyrimidine with propyl chloromethyl ether.

For the purpose of studying the structure–activity dependence, a lipophilic analog of AMU – 1-(allyloxymethyl)-3-palmitoyluracil (XV) – was also synthesized. The initial AMU (VIIa) was converted to the 4-O-trimethylsilyl derivative, which was acylated by palmitoyl chloride according to a known method [21] giving the final product with the yield of 72.8%.

The structure of the compounds synthesized was confirmed by the data of PMR spectroscopy.

EXPERIMENTAL (CHEMICAL)

The purity of the compounds obtained was monitored by the method of thin layer chromatography on plates of Silufol UV-254 using the 1:1 mixture of chloroform–ethyl acetate (A), and the 9:1 mixture of chloroform–methanol (B) as eluents; development was performed with iodine vapor. The IR spectra were registered on the "Hitachi-225" spectrometer using a thin layer. The PMR spectra were registered on a "Tesla BS-567A" spectrometer (100 MHz) using the FT regime and solutions of deuterochloroform or dimethylsulfoxide-D_6; the internal standard was HMDS. The data of the elemental analysis satisfy the calculated values.

General Method for the Isolation of 1-(Allyloxyalkyl)uracils (VII)-(IX) and 1,3-Di-(allyloxyalkyl)uracils (XIb,c) and (XII). The corresponding pyrimidine base (0.020 mole) is boiled in 50 ml of hexamethyldisilazane in the presence of 1 ml of trimethylchlorosilane until the formation of a clear solution is achieved. The excess of the hexamethyldisilazane is removed in vacuo. The resulting trimethylsilyl derivative (the yield of 95-99%) is dissolved in 50 ml of a dry aprotic solvent prior to the addition of the equimolar amount of the corresponding allyl 1-chloroalkyl ether at room temperature with stirring and the protection from the atmospheric moisture. The mixture is stirred for 1 day at room temperature. Water (50 ml) is added, and the mixture is stirred for 1 h; the organic layer is separated, and the aqueous layer is extracted fivefold with 25 ml portions of chloroform. The combined extracts and the organic layer are dried with magnesium sulfate prior to the filtration and the concentration in vacuo. The residue is chromatographed on a column with silica gel (50 by 1.5 cm) with the initial elution of the dialkylation product with chloroform, and the elution of the 1-(allyloxyalkyl)uracils (VII)-(IX) with the system B. The fractions containing the object products, according to the TLC data, are combined and concentrated in vacuo.

1,3-Di(allyloxyalkyl)uracils (X)-(XII). These compounds are obtained analogously with the utilization of 2 equivalents of the corresponding allyl 1-chloroalkyl ether for 1 equivalent of the trimethylsilyl derivative of the pyrimidine base.

EXPERIMENTAL (BIOLOGICAL)

The antiviral properties of the compounds were determined in experiments on tissue cultures in respect to HSV-1, the virus of variola vaccine (VVV), classical avian plague virus (CAPV), vesicular stomatitis virus (VSV), Venezuelan equine encephalomyelitis (VEE), and ECHO 6 by th emethods of the "screening test" and the reduction of the plaques under an agar covering. Investigations with the ECHO virus were performed on passaged cultures of human embryonic cutaneous-muscle cells. With the respiratory-syncytial (RS) type, investigations were performed on a culture of transplanted rabbit lung cells. With the remaining viruses, investigations were performed on initially trypsinized chick embryo fibroblasts. Criteria of antiviral action were the presence of zones of the suppression of plaque formation with the investigation by the "screening test," as well as the decrease in the viral titer by the action of the substances studied by comparison with the untreated control with the investigation by the plaque reduction method. The method of investigation and the evaluation of the results obtained were previously described in detail [1].

TABLE 2. Characterististics of the Antiviral Action of the Unsaturated Pyrimidine Acyclonucleosides

Compound	Virus	Method of plaque reduction		
		concentration, μg/ml	titer of the virus, log PFU/ml	decrease of the viral titer by comparison with the control, log PFU/ml
VIIa	HSV	800	⩽3,50	⩾1,54
		400	⩽3,50	⩾1,54
		200	⩽3,50	⩾1,54
		100	⩽3,50	⩾1,54
		0	5,04	—
VIIf	VVV	400	⩽2,00	⩾1,69
		200	3,65	0,04
		100	3,67	0,02
		0	3,69	—
VIIIb	VSV	800	⩽3,00	⩾1,74
		400	3,95	0,79
		200	4,31	0,43
		0	4,74	—
VIIIc	RS	800	⩽4,50	⩾1,50
		400	⩽4,50	⩾1,50
		200	5,61	0,39
		0	6,00	—
VIIId	RS	400	⩽4,50	⩾1,50
		200	⩽4,50	⩽1,50
		100	5,65	0,35
		0	6,00	—
IXc	VVV	400	⩽1,80	⩾1,54
		200	⩽1,80	⩾1,54
		100	3,03	0,31
		0	3,34	—
Xa	HSV	800	⩽3,70	⩾1,88
		400	⩽3,70	⩾1,88
		200	5,29	0,29
		0	5,58	—
Xc	RS	400	⩽4,00	⩾1,43
		200	⩽4,00	⩾1,43
		100	4,85	0,58
		0	5,43	—
XIb	VVV	100	⩽1,80	⩾1,54
		50	3,12	0,22
		25	3,23	0,11
		0	3,34	—
XIII	VVV	200	⩽3,00	⩾1,64
		100	⩽3,00	⩾1,64
		50	3,30	1,34
		25	4,23	0,41
		0	4,64	—
XIV	VVV	200	⩽3,00	⩾1,64
		100	⩽3,00	⩾1,64
		50	4,20	0,44
		0	4,64	—
XVI	VVV	800	⩽4,00	⩾1,60
		400	5,45	0,15
		200	5,52	0,08
		0	5,43	—

TABLE 3. Effectiveness of the Antiviral Action of 1-(Allyloxymethyl)uracil (VIIa) using the Model of Experimental Herpetic Meningoencephalitis of White Mice

Dose, mg/kg	Lethality, %	Index of protection, %	P
200	25,0±11,1	65,51	0,01
100	15,0±8,2	79,59	0,001
10	25,0±11,1	65,51	0,01
1	37,5±12,5	47,36	0,05
Placebo	73,9±9,3	—	—

The effectiveness of 1-(allyloxymethyl)uracil in animal experiments was tested using the model of experimental herpetic meningoencephalitis. White mice were infected with 10-100 LD_{50} of the HSV-1 (Koptev) ip. The infected animals were separated into groups each of which received a particular dose of the substance from 200 to 1 mg/kg in the form of a suspension in 0.5% starch gel of the volume 0.1 ml intragastrically for 7 days, twice a day, 3 h after infection. The effectiveness of the antiviral action of the compound was evaluated from the decrease in the lethality in the groups by comparison with the control.

The data obtained showed that 12 of the 28 unsaturated pyrimidine acyclonucleosides studied had antiviral activity (Table 2).

The compounds (VIIf), (IXc), (XIb), (XIII), and (XIV) exhibited weak activity toward the VVV. A weak degree of activity was shown by the compounds (VIIIc), (Xa), (XIa), and (XVI) toward the HSV. At the same time, the compound (VIIa) exhibited a marked capacity for the inhibition of the replication of the herpes virus. A decrease in the viral titer to a value equal to 1.54 log PFU/ml was noted for all the concentrations studied.

The compounds (XIIIc,d) and (Xc) inhibited the replication of the RS-virus to an insignificant extent.

It should be noted that all the compounds studied were inactive toward the CAPV, VSV, and the virus of VEE.

Proceeding from the data obtained, it can be concluded that the introduction of the methyl group (VIIe), fluorine (VIIb), or chlorine (VIIc) at the position 5 of the basic structure of 1-(allyloxymethyl)uracil leads to the complete loss of antiherpetic activity, whereas the introduction of bromine (VIId) increases the toxicity of the compound. The loss of antiviral activity is also observed with the introduction of the carboxyl group (VIIh) at the position 6. The presence of a methyl group at the position 6 decreases the antiherpetic activity, but the activity toward the RS-virus thereby appears (VIIIc,d) and (Xc). Activity is also lost with the introduction of additional substituents into the side chain. For example, 1-(allyloxymethyl)uracil (VIIa) is highly active toward the HSV, but its homologs (VIIIa) and (IXa) are inactive. The analogous dependence is observed in the transition form the monoallyloxyalkyl derivatives to the diallyloxyalkyl derivatives (X)-(XII).

Attention is drawn to the circumstance that the unsubstituted N(3) position of the pyrimidine ring is required for the presence of high activity since the introduction of an acyl substituent, e.g. palmitoyl (XV), leads to the complete loss of the antiviral activity of the basic compound (VIIa). The carbon to carbon double bond at the end of the side chain is also important. The saturated analog 1-(allyloxymethyl)uracil (XVI) exhibited very weak activity toward the herpes viruses.

The study of the antiviral activity of 1-(allyloxymethyl)uracil (VIIa) using the model of experimental herpetic meningoencephalitis of mice showed that this compound gives a reliable decrease in the lethality of the animals, and exhibits marked therapeutic action at doses of 1-200 mg/kg (Table 3). The acute 24 h toxicity LD_{50} (mice, intragastric) thereby comprises > 1000 mg/kg.

The data obtained permit consideration of the further expedient search for inhibitors of viral activity in the series of unsaturated pyrimidine acyclonucleosides.

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HYPOLIPIDEMIC ACTIVITY OF RACEMIC 18-METHYL-D-HOMO-B-NOR-9-ISOESTRONE

V. E. Ryzhenkov, A. A. Prokop'ev, I. Yu. Kameneva, I. V. Ishchenko, and A. G. Shavva

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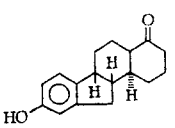
The use of estrogens is known to reduce experimental hypercholesterolemia and the degree of development of atherosclerosis [3, 13]. There are also data indicating that the permeability of the aorta to atherogenic lipoproteins (LP) is reduced under the influence of estrogens [16]. However, the use of estrogens for the prophylaxis and therapy of atherosclerosis is limited as a result of their side effects, in particular, an increase in the level of triglycerides (TG) in the blood plasma, which substantially increases the hazard of thrombus formation [17]. In view of this, a search is being made for modified estrogens with reduced hormonal effects or none at all, but at the same time, in view of the structural similarity, exhibiting hypolipidemic and antiatherosclerotic properties. Only a few such compounds, possessing reduced estrogenic activity and rather pronounced hypolipidemic activity, are currently known [2, 6, 12]. In this work we investigated 18-methyl-D-homo-B-nor-9-isoestrone (MHI), the synthesis of which was described earlier [7]. The presence of a five-membered B ring in this compound instead of the six-membered or expanded D ring leads to a substantial change in the geometry of the molecule, which was also responsible for the substantial decrease in specific hormonal action of such compound [11]. Estrodiol was used for comparison.



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