AMIDOALKYLATION OF PYRIMIDINE BASES OF NUCLEIC ACIDS

B. M. Khutova, S. V. Klyuchko, and L. P. Prikazchikova

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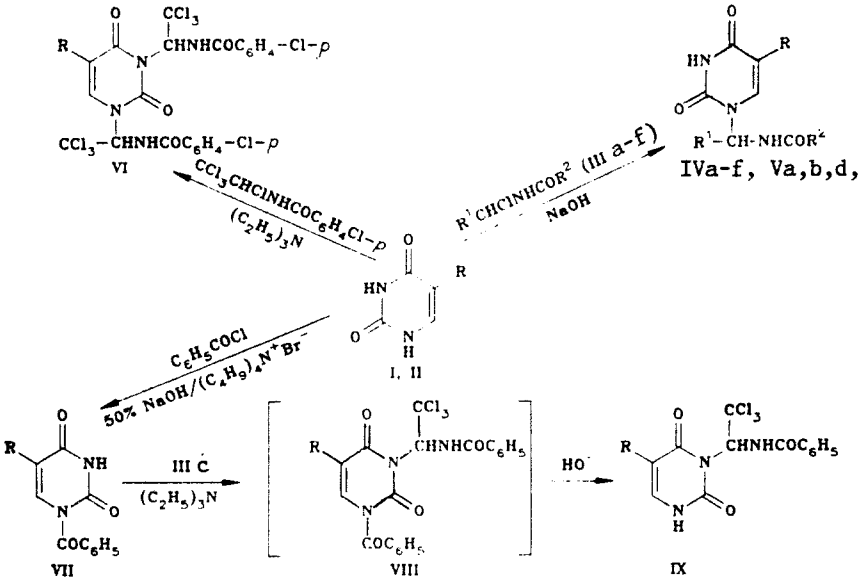
The reaction of uracil, thymine, and cytosine with acid N-α-chloroalkylamides gave N-substituted derivatives of these compounds. Conditions for the selective amidoalkylation of pyrimidine bases in the 1 position were found.

The chemical modification of pyrimidine bases of nucleic acids under mild conditions is important both for the synthesis of biologically active compounds and for the study of the transformations of nucleotides and nucleic acids.

In the present research we studied the amidoalkylation of uracil, thymine, and cytosine. The sulfonamidomethylation of a trimethylsilyl derivative of uracil was described in [1]. We have found that uracil and thymine react readily with various acid chloroalkylamides in aqueous acetone at 0°C in the presence of an alkali to give $N_{(1)}$-substituted derivatives IV and V.

In the case of the reaction of uracil (I) with a p-chlorobenzoic acid 1,2,2,2-tetrachloroalkylamide it was shown that in the presence of triethylamine amidoalkylation takes place at the two ring nitrogen atoms to give 1,3-disubstituted derivative VI.



I, IV, VI—IX R=H; II V R=CH_3; III, IV, V a R^1=CCl_3, R^2=$C(CH_3)_3$; b R^1=CCl_3, R^2=OCH_3; c R^1=CCl_3, R^2=C_6H_5; d R^1=CCl_3, R^2=C_4H_3O; e R^1=H, R^2=C_6H_5; f R^1=COC_6H_5, R^2=C_6H_5

A low-field shift of the signal of the 6-H proton ($\Delta\delta$ 0.67 ppm) as compared with unsubstituted uracil is observed in the PMR spectra of IV and V; this indicates substitution at the ring $N_{(1)}$ atom. Furthermore, N_3-substituted uracils were obtained. From [2] we know of the selective $N_{(1)}$-benzoylation of uracil under interphase-catalysis conditions. The benzoyl protective group was removed after benzoylation and amidoalkylation, and $N_{(3)}$-substituted uracil IX was obtained. A bathochromic shift of the long-wave absorption maximum is observed in the UV spectra of solutions in alkali for $N_{(3)}$-substituted uracils [3]. This shift of the long-wave absorption maximum was manifested for IX, whereas it is absent in the case of IV. This also confirms the presence of a side chain attached to the $N_{(1)}$ atom of IV and V.

Cytosine is acetylated beforehand to protect the amino group in order to obtain N-substituted derivatives. Acetylcytosine XI reacts under mild conditions at 0°C with amidoalkylating agents III to give N-substituted derivatives XII (see scheme below).

The antitumorigenic activity of the synthesized compounds was studied; the N-substituted derivatives of uracil, thymine, and cytosine were inactive with respect to lympholeucosis P-388.

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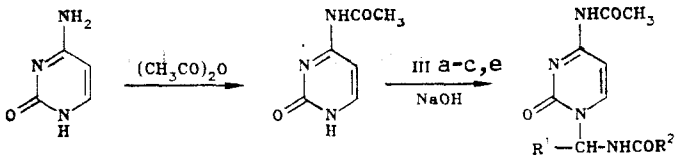
TABLE 1. Characteristics of IV and XII

Compound	Empirical formula	mp, °C	PMR spectra, δ, ppm (J, Hz)						Yield, %
			5-H, d	6-H, d	—CH—, d	—NH—, d	uracil NH	other signals	
IV a	$C_{11}H_{14}Cl_3N_3O_3 \cdot CH_3OH$	242...243	5,79 (J=8,2)	8,23 (J=8,2)	7,4 (J=9,9)	8,47 (J=9,9)	11,6	3,36 (s, 9H, $C(CH_3)_3$)	68
IV b	$C_8H_8Cl_3N_3O_4 \cdot CH_3OH$	224...226	5,76 (J=8,1)	7,8 (J=8,1)	6,98 (J=10,7)	9,17 (J=10,7)	11,6	3,63 (s, 3H, OCH_3); 3,42 (s, 3H, methanol-CH_3)	47
IV c	$C_{13}H_{10}Cl_3N_3O_3$	239...240	5,76 (J=8,2)	8,20 (J=8,2)	In the phenyl-proton region	9,6 (J=10,2)	11,6	7,52...7,81 (m, 6H, C_6H_5, CH)	67
IV d	$C_{11}H_8Cl_3N_3O_4$	256...258							39
IV e	$C_{12}H_{11}N_3O_3$	178...179	5,52 (J=9,9)	7,84 (J=9,9)	—	9,41 t	11,24	5,09 (d, 2H, CH_2, J=8,2); 7,42...7,74 (m, 5H, C_6H_5)	53
IV f	$C_{19}H_{15}N_3O_4$	229...231	4,80 (J=8,2)	6,94 (J=8,2)	In the phenyl-proton region	8,99 (J=10,2)	10,59	6,58...6,74 (m, 11H, $2C_6H_5$, CH)	52
XII a	$C_{13}H_{17}Cl_3N_4O_3$	221...223	7,24 (J=8,0)	8,62 (J=8,0)	7,68 (J=10,0)	8,49 (J=10,0)	—	1,16 (s, 9H, $C(CH_3)_3$); 3,40 (s, 3H, CH_3); 11,03 (s, 1H, NH—$COCH_3$)	47
XII b	$C_{10}H_{11}Cl_3N_4O_4$	198...200	7,37 (J=7,7)	7,88 (J=7,7)	7,45 (J=9,9)	9,02 (J=9,9)	—	3,41 (s, 3H, CH_3); 3,61 (s, 3H, OCH_3); 10,81 (s, 1H, NH—$COCH_3$)	40
XII c	$C_{15}H_{13}Cl_3N_4O_3$	197...199							99
XII e	$C_{14}H_{13}N_4O_3$	246...248	7,15 (J=8,0)	8,20 (J=8,0)	—	9,53 t	—	3,45 (s, 3H, CH_3); 5,68 (d, 2H, CH_2, J=7,0); 7,46...7,89 (m, 5H, C_6H_5); 10,79 (s, 1H, NH—$COCH_3$)	67

TABLE 2. Characteristics of V

Compound	Empirical formula	mp, °C	PMR spectra, δ, ppm (J, Hz)						Yield, %
			CH_3, s	6-H, s	—CH—, d	—NH—, d	uracil NH	other signals	
Va	$C_{12}H_{16}Cl_3N_3O_3$	268...270	1,79	8,14	7,42 (10,2)	8,4 (10,2)	11,6	3,48 (s, 9H, $C(CH_3)_3$)	39
Vb	$C_9H_7Cl_3N_3O_4 \times \times CH_3OH$	242...245	1,77	7,8	7,0 (10,0)	9,06 (10,0)	11,58	3,5 (s, 3H, methanol-CH_3); 3,66 (s, 3H, OCH_3)	25
Vd	$C_{12}H_{10}Cl_3N_3O_4$	277...278	1,80	8,14	7,4 (10,0)	9,4 (10,0)	11,63	—	37
Ve	$C_{13}H_{13}N_3O_3$	228...230	1,75	In the phenyl-proton region	—	9,41 (t)	11,26	5,10 (d, 2H, CH_2, J=5,5); 7,23...7,89 (m, 6H, C_6H_5, 6-H)	23



X XI XII a-c,e

III, XII a $R^1=CCl_3$, $R^2=C(CH_3)_3$; b $R^1=CCl_3$; $R^2=OCH_3$; c $R^1=CCl_3$, $R^2=C_6H_5$; e $R^1=H$, $R^2=C_6H_5$

EXPERIMENTAL

The UV spectra were recorded with a Specord spectrophotometer. The PMR spectra of solutions in deuterochloroform were obtained with a Bruker WP-200 spectrometer with hexamethyldisiloxane (HMDS) as the external standard.

The results of elementary analysis for C, H, N, and Cl were in agreement with the calculated values.

The characteristics of the synthesized IV, V, and XII are presented in Tables 1 and 2.

$N_{(1)}$-Amidoalkyl Derivatives of Uracil (IVa-f), Thymine (Va,b,d,e), and Acetylcytosine (XIIa-c,e). A 10-ml sample of uracil was added to a solution of 10 mmole of sodium hydroxide in 20 ml of water and, after it had dissolved completely, a solution of the corresponding N-(1-chloroalkyl)amide in 20 ml of acetone was added with stirring and cooling to 0°C. The solution was stirred for 30 min, after which the solvent was removed in vacuo, and the residue was neutralized with 1 N HCl solution. The resulting precipitate was removed by filtration and crystallized from methanol.

N,N-1,3-Bis[2,2,2-trichloro-1-(p-chlorobenzoylamino)-1-ethyl]uracil (VI, $C_{22}H_{14}Cl_3N_4O_4$). A solution of 1.6 g (5 mmole) of the p-chlorobenzoic acid 1,2,2,2-tetrachloroalkylamide in 20 ml of absolute acetonitrile was added dropwise to a suspension of 0.6 g (5 mmole) of uracil and 0.5 g (5 mmole) of triethylamine in 20 ml of absolute acetonitrile, and the suspension was refluxed for 11 h. The solution was then evaporated in vacuo, and the residual oil was triturated with 20 ml of ether. The precipitate was removed by filtration to give a product with mp 191-192°C (from CCl_4). PMR spectrum: 5.75 (1H, d, 5-H, J = 8.2 Hz), 7.55-7.84 (10H, m, $2C_6H_4$, 2CH), 8.20 (1H, d, 6-H, J = 8.2 Hz), 9.72 ppm (2H, d, 2NH, J = 9.9 Hz). The yield of VI was 0.46 g (15%).

$N_{(1)}$-Benzoyluracil (VII). This compound was obtained by the method in [1].

$N_{(3)}$-[2,2,2-Trichloro-1-benzoylamino-1-ethyl]uracil (IX, $C_{13}H_{10}Cl_3N_3O$). A 1.35-g (45 mmole) sample of the benzoic acid 1,2,2,2-tetrachloroalkylamide was added dropwise to a solution of 0.6 g (30 mmole) of 1-benzoyluracil and 0.3 g (30 mmole) of triethylamine in 20 ml of absolute acetonitrile, and the suspension was refluxed for 2 h. The solution was evaporated in vacuo, and the residue was refluxed with 50 ml of absolute benzene. The mixture was filtered to give 0.3 g (73%) of triethylamine hydrochloride, and the filtrate was evaporated in vacuo. The residual oil was refluxed in 10 ml of butyl alcohol for 4 h, and the resulting colorless precipitate was removed by filtration to give a product with mp 240-241°C (from methanol). PMR spectrum: 5.62 (1H, d, 5-H, J = 7.5 Hz), 7.50-7.74 (7H, m, C_6H_5, CH, 6-H), 9.11 (1H, d, NH—CH, J = 10 Hz), 11.52 ppm (1H, s, uracil NH). The yield of IX was 0.12 g (13%).

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