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Polymethylene Derivatives of Nucleic Bases with ω-Functional Groups: VI.[1] [8-(2-Oxocyclohexyl)-9-oxooctyl]pyrimidines as Potential Inhibitors of Pyrimidine Phosphorylases

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Abstract—New polymethylene derivatives of nucleic bases with β-diketo function in ω-position were prepared by alkylation of uracil, thymine, and cytosine. Their physicochemical properties and effect on the *E. coli* uridine and thimidine phosphorylases were studied.

Key words: alkylation, β-diketones, nucleosides, polymethylene analogues, pyrimidine phosphorylases

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INTRODUCTION

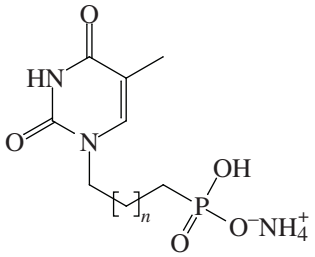
Polymethylene derivatives of nucleic bases bearing various functional groups at the end of the hydrocarbon chain are convenient tools for studying enzymes of nucleic exchange.[3] α,ω-Fragments of these structures can interact with the enzyme active sites, whereas the hydrophobic polymethylene chains regulate the distance between substituents in α,ω-positions of the potential inhibitor.

We had earlier synthesized pyrimidine and pyrine polymethylene derivatives with terminal alkoxycarbonyl, carboxyl, and hydroxyl groups [2]. Similar thymine and uracil derivatives were found to effectively interact with the HIV reverse transcriptase region responsible for the $tRNA^{Lys}$ anticodon recognition and activate the enzyme. It was also shown that these derivatives weakly inhibit DNA topoisomerase I. Their biological activities depend on the length of polymethylene linker.

When continuing the methodical studies of polymethylene derivatives of nucleic bases, we thought it reasonable to synthesize and study the compounds containing β-dicarbonyl groups in the ω-positions with the aim to evaluate the effects of the β-diketone fragment and/or keto–enol tautomerism on biological activity of these derivatives. β-Diketones are known to be good chelating agents of bivalent metals. This property determines high inhibitory activity of some members of this class toward the hepatitis C virus RNA dependent RNA polymerase [3–5] and HIV integrase [6–8]. β-Dicarbonyl compounds isolated from various natural sources display a wide spectrum of biological activities [9]. Among them, there are potent antioxidants, anticancer, antiviral, antimicrobial, and antifungal agents. Many synthetic β-diketones were also found to be biologically active [9].

Earlier we reported preparation and physicochemical properties of [7-(2-oxocyclohexyl)-7-oxoheptyl]pyrimidines [10] and purines [11]. β-Dicarbonyl groups in these compounds are separated from the nucleic base with a hexamethylene chain.

Preparation of compounds of formula (**I**) effectively inhibiting *E. coli* thymidine phosphorylase was described in [12]. The inhibitor's pyrimidine residue is localized in the thymine binding site, whereas the phosphonate group placed 8–10 Å away (8–9 methylene units) occupies the inorganic phosphate binding site.



(**I**) $n = 6, 7$

Thymidine phosphorylase is one of the key enzymes of the alternate pathway of biosynthesis and catabolism of pyrimidine 2'-deoxynucleosides. It is involved in reversible transformations of pyrimidine 2'-deoxynu-

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[3] Abbreviations: TP, thymidine phosphorylase from *E.coli* (EC 2.4.2.4); UP, uridine phosphorylase from *E.coli* (EC 2.4.2.3); and DBU, 1,8-diazabicyclo[5.4.0]undece-7-ene.

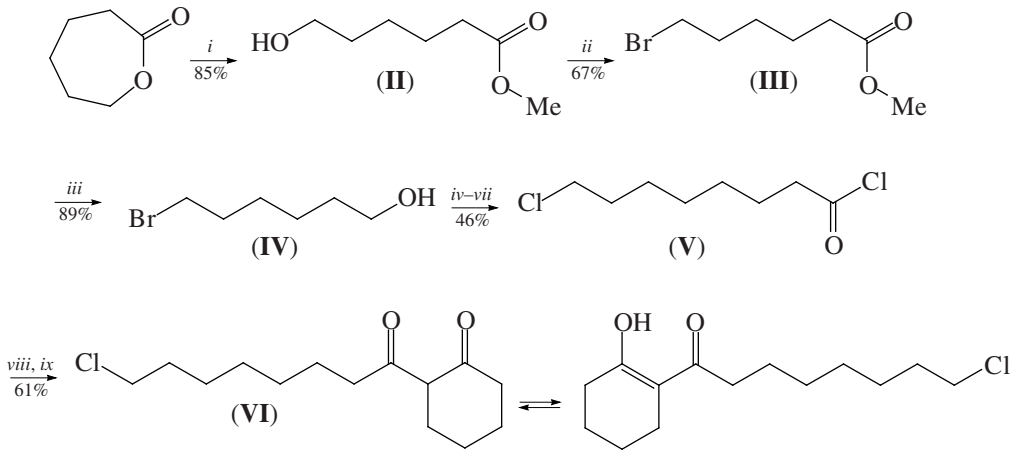
cleosides to 2'-deoxy-α-*D*-ribose-1-phosphate and pyrimidine. Bacterial enzymes of this type are used in practice for commercial production of such essential therapeutic products of nucleoside nature as cladribine, fludarabine, and ribavirin [13]. In addition, thymidine phosphorylase may serve as a rather adequate model for screening of inhibitors of human thymidine phosphorylase, thus promoting further interest to this enzyme. An increased level of thymidine phosphorylase is observed in tumor cells of different types [14]. Moreover, there is some evidence about its involvement in angiogenesis and apoptosis [15].

Based on the above discussed facts, we believed the synthesis of N^1-[8-(2-oxocyclohexyl)-8-oxooctyl]pyrimidines, the study of their properties, and evaluation of inhibition of *E. coli* thymidine and uridine phosphorylases would be well motivated. It was also rational to compare biological properties of the compounds synthesized in this work with those of similar derivatives described earlier [1].

RESULTS AND DISCUSSION

Apparently, a reasonable strategy for the synthesis of target β-diketones is alkylation of uracil, thymine, and cytosine with the corresponding β-diketone containing a halogen atom in the ω-position relative to the keto function.

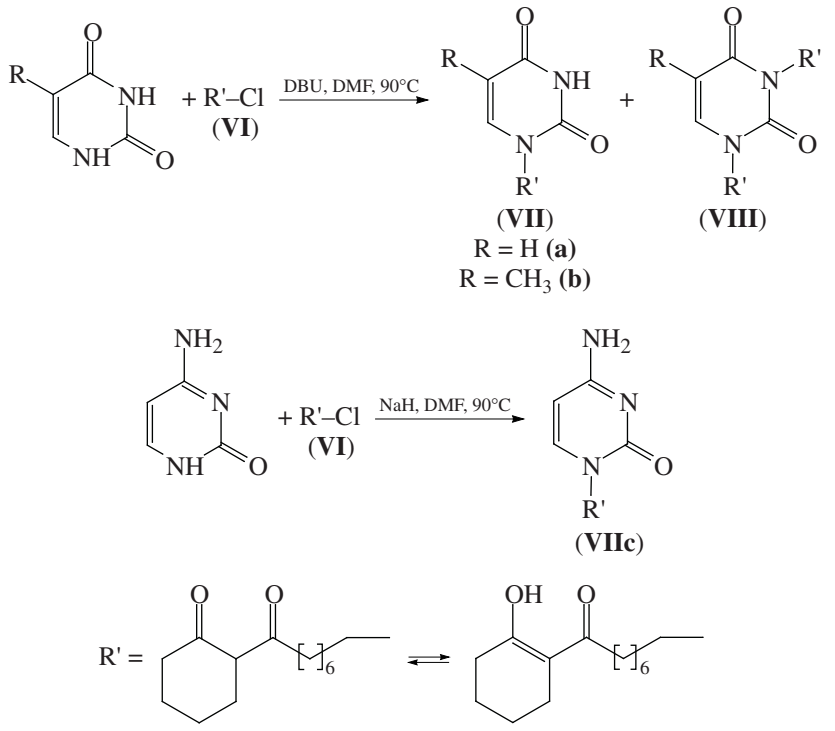
An alkylating agent 2-(8-chloroctanoyl)cyclohexanone (**VI**) was obtained at the first stage (Scheme 1). The key compound for realization of this scheme is 6-bromohexan-1-ol (**IV**). It is commercially available, but its price is unreasonably high. Therefore, ε-caprolactone was used as an alternative starting compound for its synthesis.



Scheme 1. *i*) MeOH/TosOH; *ii*) PBr_3/Et_2O; *iii*) $LiAlH_4/Et_2O$, 0°C; *iv*) diethyl malonate/NaOEt/dioxane, Δ; *v*) HCl/H_2O (1 : 1), Δ; *vi*) 180–190°C; *vii*) $SOCl_2/CHCl_3$; *viii*) 1-morpholinecyclohex-1-ene/$Et_3N/CHCl_3$, Δ; *ix*) H_2SO_4/H_2O.

Two-step preparation of ethyl 6-hydroxyhexanoate (**II**) by hydrolysis of ε-caprolactone under alkaline conditions followed by treatment with ethyl iodide has been published [16], but this procedure requires considerable amounts of organic solvents. The method of preparation of ethyl 6-hydroxyhexanoate (**II**) by alcoholysis of ε-caprolactone catalyzed by *p*-toluenesulfonic acid similarly to [17], where sulfuric acid was used as a catalyst, proved to be more effective. Treatment of compound (**II**) with phosphorus tribromide in ether gave bromide (**III**), which was smoothly reduced with lithium aluminum hydride to target 6-bromohexan-1-ol (**IV**). The reaction of compound (**IV**) with diethyl malonate followed by hydrolysis and decarboxylation of the substituted malonate under standard conditions yielded 8-hydroxyoctanoic acid, which, without purification, was transformed with thionyl chloride to the corresponding 8-hydroxyoctanoyl chloride (**V**). The target alkylating agent (**VI**) was obtained in a high yield by interaction of chloride (**V**) with 4-(1-cyclohexen-1-yl) morpholine under the Stork reaction conditions [18, 19].

We showed earlier that DBU is useful as a base at the last stage of preparation of ω-substituted polymethylene purine and pyrimidine derivatives (Scheme 2) [10, 11]. In this work we obtained 25% N^1-[8-(2-oxocyclohexyl)-8-oxooctyl] uracil (**VIIa**) and 16% -thymine (**VIIb**) derivatives by heating of uracil or thymine, respectively, with chloride (**VI**) (method A). The products of N^1,N^3-alkylation (**VIII**) are comparatively easily separated by silica column chromatography.



Scheme 2.

In the case of cytosine, higher alkylation yields were achieved by reaction with the alkylating agent of cytosine sodium salt obtained by interaction of cytosine with sodium hydride in DMF rather than by using DBU as a base (method B) [10]. The structures of the synthesized pyrimidines (**VIIa**)–(**VIIc**) were confirmed by NMR and mass-spectrometry data (see Tables 1 and 2 and Experimental section).

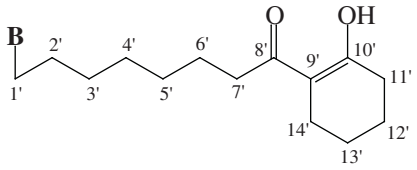
Under the conditions of registration of NMR spectra (DMSO-d_6 and $CDCl_3$ as solvents, 25°C) β-dicarbonyl groups of compounds (**VI**), (**VIIa**), (**VIIc**), and (**VIII**) predominantly exist in the enol form, which was supported by the resonance at 15.9 ppm in ^{1}H NMR spectra (Table 1) inherent for the protons involved in the formation of tight hydrogen bonds as well as by the group of peaks at 106, 181, and 201 ppm in ^{13}C NMR spectra (Table 2) corresponding to enolized β-dicarbonyl groups. In our case, of two carbonyl groups, endocyclic or exocyclic group could be enolized. The *endo*-structure was ascribed to compounds (**VI**), (**VIIa**)–(**VIIc**), and (**VIII**) by analogy with 2-acetylcyclohexanones, for which this form is predominant in nonpolar solvents, whereas in 2-acetylcyclopentanones the exocyclic carbonyl group is enolized due to rigidity of the cyclopentane ring [20].

Compounds (**VIIa**) and (**VIIb**) as well as earlier described diketo pyrimidine derivatives (**IXa**) and (**IXb**), whose nuclear base residue is separated from the β-dicarbonyl group with six methylene units [10], were studied as inhibitors of *E. coli* thymidine and uridine phosphorylase. Inhibitory properties of cytosine derivative (**VIIc**) were not studied because cytidine is neither a substrate nor an inhibitor of these enzymes [21].

The enzymes were isolated from *E. coli* superproducer strains using the known procedure [22]. Unnatural substrate 4-thiothymidine was used for studying the effect of compounds (**VIIa**), (**VIIb**), (**IXa**), and (**IXb**) on the phosphorolysis reaction catalyzed by *E. coli* thymidine and uridine phosphorylases. Due to the absorbance maximum at 335 nm it can be used for spectrophotometric determination of inhibitory activities of the compounds whose absorption lies within the absorbance range of a natural substrate (for thymidine, λ_{max} 267 nm) [22].

For derivatives (**VIIa**) and (**VIIb**) bearing one more methylene unit if compared with compounds (**IXa**), and (**IXb**), TP and UP inhibition constants differed by one order of magnitude (Table 3), which is correlated with the data for compounds of type (**I**) [12]. Thus derivatives (**VIIa**) and (**VIIb**) (K_i 65 μM) are bound to TP 5–7 times more effectively than thymidine (K_m 330 μM). As the formation of the active site of *E. coli* thymidine phosphorylase is associated with major amino acids (His85, Lys190, Lys191, and Arg171) it seemed rational to study the inhibitory activity of previously described thymine and uracil derivatives (**Xa**) and (**Xb**) [2] containing an ω-carboxylic group joined to the heterocyclic base with a hexamethylene linker. However, these derivatives were inactive towards both phosphorylases (Table 3).

Table 1. ^{1}H NMR spectra (δ, ppm) of compounds (**VIIa**)–(**VIIc**)



Proton	Compound		
	(**VIIa**)*	(**VIIb**)**	(**VIIc**)***
H3 (1 H)	10.29, s	11.13, s	–
H5 (1 H)	5.52, d, *J* 7.8	–	5.62, d, *J* 7.3
H6 (1 H)	7.12, d, *J* 7.8	7.5, s	7.54, d, *J* 7.3
H1' (2 H)	3.56, t, *J* 7.16	3.60, t, *J* 7.16	3.58, t, *J* 7.16
H2'–H6', H12', H13' (14 H)	1.16–1.5, m	1.27–1.6, m	1.25–1.6, m
H7' (2 H)	2.21, t, *J* 7.16	2.42, t, *J* 7.16	2.41, t, *J* 7.16
10'-O*H* (1 H)	15.81, s	15.98, s	15.98, s
H11', H14' (4 H)	2.13, m	2.27, m	2.26, m

Notes: * NMR spectra of compound (**VIIa**) were registered in $CDCl_3$; in other cases, in DMSO-d_6.

** For compound (**VIIb**), the resonance of 5-CH_3 was at 1.75 ppm, s, 3 H.

*** For compound (**VIIc**), the resonance of 4-NH_2 was at 7.00, s, 2 H.

To conclude, we synthesized new polymethylene β-diketo derivatives of pyrimidine nucleic bases. The compounds inhibiting *E. coli* thymidine and uridine phosphorylases were found among them. Their inhibitory activities were higher than those of similar compounds with shorter linkers.

EXPERIMENTAL

Uracil, thymine, cytosine were from Sigma (United States); 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), sodium hydride (80% suspension in mineral oil), phosphorus tribromide from Fluka (Switzerland); *p*-toluenesulfonic acid monohydrate from Pierce (United States); ε-caprolactone, lithium aluminum hydride, diethyl malonate, sodium methylate, $CDCl_3$, and DMSO-d_6 were from Acros Organics (Belgium).

Solvents were purified and dehydrated as described earlier [23]. TLC was performed on Kieselgel 60 F_{254} plates (Merck, Germany) with the following eluting systems: chloroform (1); chloroform–ethanol 19.5 : 0.5 (2); 19 : 1 (3); and 17 : 3 (4). The compounds were visualized by UV absorption at 254 nm. β-diketones were detected with $FeCl_3$. Column chromatography was performed on silica gel L 40/100 (Chemapol, Czech Republic).

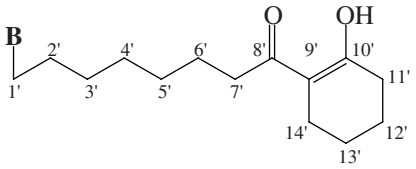
Mass spectra were registered on a MS 30 spectrometer (Kratos, Japan), with electron impact as an ionization method. NMR spectra were registered (δ, ppm, *J*, Hz) on a Bruker AMXIII-400 spectrometer (Germany) with a working frequency 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei at 300 in $CDCl_3$ and DMSO-d_6.

Methyl 6-hydroxyhexanoate (II). ε-Caprolactone (45 ml, 0.4 mol) was added under stirring to a solution of *p*-toluenesulfonic acid (3 g) in absolute methanol (150 ml), and the mixture was kept at room temperature for 18 h. Methanol was evaporated, saturated sodium hydrocarbonate solution (40 ml) and chloroform (70 ml) were added to the oil-like residue, the organic layer was separated, and the aqueous layer was extracted with chloroform (3 × 50 ml). The extracts were combined, dried with sodium sulfate, the solvent was evaporated, and the residue was distilled in a vacuum to give 49.7 g (85%); bp 130–133°C/18 mm Hg. ^{1}H NMR ($CDCl_3$): 1.37 (2 H, m, C*H*$_2$CH$_2$CH$_2$OH); 1.54 (2 H, m, C*H*$_2$CH$_2$COOCH$_3$); 1.67 (2 H, m, C*H*$_2$CH$_2$OH); 2.21 (2 H, t, *J* 7.2, C*H*$_2$COOCH$_3$); 3.41 (2 H, t, *J* 6.5, C*H*$_2$OH); 3.54 (3 H, s, COOC*H*$_3$). ^{13}C NMR ($CDCl_3$): 23.9 (*C*H$_2$CH$_2$CH$_2$OH); 26.1 (*C*H$_2$CH$_2$COOCH$_3$); 32.0 (*C*H$_2$CH$_2$OH); 33.5 (*C*H$_2$COOCH$_3$); 44.4 (*C*H$_2$OH); 51.1 (COO*C*H$_3$); 173.4 (*C*OOCH$_3$).

Methyl 6-bromohexanoate (III). A solution of methyl ester (**II**) (45.2 g, 0.31 mol) in absolute diethyl ether (150 ml) was added dropwise under stirring and cooling (0°C) to phosphorous tribromide (19 ml, 0.2 mol). After the addition was over, cooling was stopped and the reaction mixture was kept at room temperature overnight. The ether was removed at 20°C, the residue was evaporated for 40 min on a rotary evaporator in a vacuum of a water-jet pump at a water bath temperature of 90°C. Ice (100 g) and chloroform (50 ml) were added to the residue. The organic layer was separated, and the aqueous layer was extracted with chloroform (3 × 50 ml). The extracts were combined, washed with saturated sodium hydrocarbonate solution (150 ml), dried with sodium sulfate, the solvent was evaporated, and the residue was distilled in a vacuum to give 43.2 g of (**III**) (67%); bp 122–125°C/22 mm Hg. ^{1}H NMR ($CDCl_3$): 49 (2 H, m, C*H*$_2$CH$_2$CH$_2$Br); 1.64 (2 H, m, C*H*$_2$CH$_2$COOCH$_3$); 1.88 (2 H, m, C*H*$_2$CH$_2$Br); 2.32 (2 H, t, *J* 7.3, C*H*$_2$COOCH$_3$); 3.42 (2 H, t, *J* 6.5, C*H*$_2$Br); 3.54 (3 H, s, COOC*H*$_3$). ^{13}C NMR ($CDCl_3$): 24.1 (*C*H$_2$CH$_2$COOCH$_3$); 27.6 (*C*H$_2$CH$_2$CH$_2$Br); 32.4 (*C*H$_2$COOCH$_3$); 33.5 (*C*H$_2$CH$_2$Br); 34.1 (*C*H$_2$Br); 51.1 (COO*C*H$_3$); 173.4 (*C*OOCH$_3$).

6-Bromohexan-1-ol (IV). Lithium aluminum hydride (4.7 g, 0.124 mol) was gradually added under cooling (0°C) and stirring to a solution of methyl ester (**III**) (43.2 g, 0.21 mol) in anhydrous ether (200 ml) in a three-necked flask supplied with a backflow condenser and a mechanical stirrer. Cooling was removed, and the mixture was stirred for another 2 h. The reaction mixture was cooled again to 0°C and water

Table 2. ^{13}C NMR spectra (δ, ppm) of compounds (**VIIa**)–(**VIIc**)



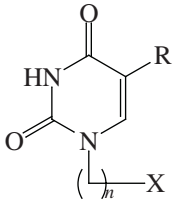
Carbon atom	Compound			Carbon atom	Compound		
	(**VIIa**)*	(**VIIb**)**	(**VIIc**)		(**VIIa**)	(**VIIb**)	(**VIIc**)
C2	150.9	150.8	155.8	C6'	23.3	22.9	22.9
C4	164.1	164.2	165.8	C7'	36.2	36.2	36.2
C5	101.4	108.3	93.0	C8'	200.9	201.7	201.5
C6	144.4	141.3	146.0	C9'	106.2	106.7	106.6
C1'	48.2	47.0	48.6	C10'	181.2	180.7	181.0
C2'	28.6	28.5	28.6	C11'	30.6	30.5	30.5
C3'	28.4	28.3	28.3	C12'	23.6	23.5	23.5
C4'	25.7	25.6	25.8	C13'	21.1	21.1	21.1
C5'	28.4	28.4	28.5	C14'	22.3	22.2	22.3

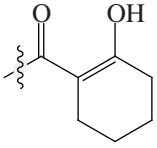
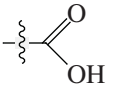
Notes: * NMR spectra of compound (**VIIa**) were registered in $CDCl_3$; in other cases, in DMSO-d_6.
** For compound (**VIIb**), the resonance of 5-CH_3 was at 11.8 ppm.

(7.1 ml), 20% sodium hydroxide (6.2 ml), water (16.8 ml), and 40% sodium hydroxide (19.3 ml) were successively added. The ether layer was decanted, and the residue was extracted with dichloromethane (4 × 70 ml). The combined extracts were washed with water (50 ml), dried with sodium sulfate, evaporated, and the residue was distilled in a vacuum to give 33.4 g (89%); bp 128–130°C/18 mmHg (published data: bp 105–106°C/5 mmHg [24].

8-Chlorooctanoyl chloride (V). A mixture of diethyl malonate (53 ml, 0.35 mol) and a solution of sodium ethylate (8.6 g, 0.12 mol) in dioxane (70 ml) were stirred at room temperature up to complete dissolution of the initially formed disodium diethylmalonate. Then a solution of 6-bromohexan-1-ol (**IV**) (22 g, 0.12 mol) in dioxane (10 ml) was added dropwise under stirring, and the reaction mixture was refluxed for 2 h. Dioxane was evaporated, water and chloroform (50 ml of each) were added, the organic layer was separated, and the aqueous layer was extracted with chloroform (2 × 50 ml). The extracts were combined, dried with sodium sulfate, and the solvent and excess of diethylmalonate were evaporated in a vacuum. Diluted hydrochloric acid (1 : 1, 20 ml) was added to the residue, the mixture was refluxed for 4 h, evaporated, and the residue was heated on a bath at 180–190°C for 40 min, cooled, and 20% NaOH (30 ml) was carefully added. The resulting solution was heated on a bath at 100°C for 20 min, cooled and carefully acidified with concentrated HCl up to pH 1. Chloroform (50 ml) was added, the organic layer was separated, and the aqueous phase was extracted with chloroform (4 × 50 ml). The extracts were dried with sodium sulfate, evaporated, and the residual oil was dissolved in chloroform (25 ml). Thionyl chloride (29 ml,

Table 3. Inhibition constants for pyrimidine phosphorylases with polymethylene derivatives of nucleic acids with ω-functional groups



Compound				K_i, μM	
N	R	*n*	X	TP*	UP
(**VIIa**)	H	7		65	10
(**VIIb**)	CH_3	7		65	90
(**IXa**)	H	6		500	220
(**IXb**)	CH_3	6		600	600
(**Xa**)	H	6		>1000	>1000
(**Xb**)	CH_3	6		>1000	>1000

Note: * For TP, thymidine is a substrate (K_m 330 μM) and uridine is an inhibitor (K_i 60 μM).

0.4 mol) was added to the solution under cooling (0°C) and stirring. Cooling was removed, and the reaction mixture was kept for 12 h at room temperature, refluxed for 1.5 h, evaporated, and the residue was distilled twice in a vacuum to give 10.9 g (46%) of compound (**V**); bp 137–140°C/15 mm Hg. ^{1}H NMR ($CDCl_3$): 1.28–1.40 (6 H, m, CH_2CH_2CH_2CH_2CH_2Cl); 1.65–1.75 (4 H, m, CH_2CH_2COCl, CH_2CH_2Cl); 2.84 (2 H, t, *J* 7.2, CH_2COCl); 3.47 (2 H, t, *J* 6.5, CH_2Cl). ^{13}C NMR ($CDCl_3$): 24.8 (*C*H_2CH$_2$COCl); 26.4 (*C*H_2CH$_2$CH$_2$-COCl); 28.0 (*C*H_2CH$_2$CH$_2$Cl); 28.1 (*C*H_2CH$_2$CH$_2$-CH$_2$Cl); 32.3 (*C*H_2CH$_2$Cl); 44.8 (*C*H_2Cl); 46.8 (*C*H_2COOCl); 173.4 (*C*OOCl).

2-(8-Chlorooctanoyl)cyclohexanone (VI). A solution of 8-chlorooctanoyl chloride (**V**) (8.8 g, 44 mmol) in chloroform (15 ml) was added dropwise under stirring to a solution of 1-morpholinylcyclohex-1-ene (11 g, 66 mmol) and triethylamine (11 ml, 75 mmol) in dry chloroform (70 ml), and the reaction mixture was kept for 14 h at 20°C. A solution of concentrated H_2SO_4 (19 ml) in water (24 ml) was added for 4 h under vigorous stirring at 20°C. The mixture was refluxed for 4 h under vigorous stirring, cooled, the organic layer was separated, the aqueous layer was extracted with chloroform (2 × 100 ml), the extracts were combined, dried with sodium sulfate, evaporated, and the residue was distilled in a vacuum to give 6.9 g (60.6%); bp 175–177°C/1 mmHg. Mass: *m/z* 258.8 [*M*]$^+$, calc. 258.8 ($C_{14}H_{23}ClO_2$). ^{1}H NMR ($CDCl_3$): 1.20–1.75 (14 H, m, H2–H6, H12, H13); 2.25 (4 H, m, H11, H14); 2.33 (2 H, t, *J* 7.5, H7); 3.46 (2 H, t, *J* 6.5, H1); 15.95 (1 H, s, 10-O*H*). ^{13}C NMR ($CDCl_3$): 21.53 (C13); 22.73 (C14); 23.68 (C6); 23.99 (C12); 26.55 (C4); 28.54 (C3); 29.03 (C5); 31.00 (C2); 32.41 (C11); 36.61 (C7); 44.85 (C1); 106.54 (C9); 181.6 (C10); 201.16 (C8).

Alkylation of nucleic bases using DBU as a base (method A). The alkylating agent (**VI**) (1.3 g, 5 mmol) and DBU (0.9 ml, 6 mmol) were added to a suspension of a nucleic base (5 mmol) in anhydrous DMF (10 ml) and the mixture was heated for 20 h at 80–100°C. The reaction course was monitored by TLC. The reaction mixture was cooled and evaporated in a vacuum, the residue was suspended in a minimal volume of chloroform and purified on a silica gel (200 g) column (5 × 28 cm) eluted with a gradient of ethanol in chloroform (0 ⟶ 20%). The fractions containing the target product were evaporated and the residue was recrystallized.

Alkylation of sodium salt of cytosine (method B). Sodium hydride (0.17 g, 5.6 mmol) was added to a suspension of cytosine (5.5 g, 5 mmol) an anhydrous DMF (10 ml). The mixture was stirred for 30 min at 20°C and the alkylating agent (**VI**) (1.45 g, 5.6 mmol) was added. The mixture was heated at 80–100°C for 20 h. The reaction course was monitored by TLC. DMF was evaporated in vacuum, and the residue was shaken with a mixture of water (20 ml) and chloroform (50 ml). The organic phase was separated, the aqueous phase was extracted with chloroform 5 × 30 ml), the extracts were combined, dried with sodium sulfate, the solvent was evaporated, and the residue was chromatographed on a column (for conditions, see method A). The fractions containing the target product were evaporated and the residue was recrystallized.

***N*^{1}-[8-(2-Oxocyclohexyl)-8-oxooctyl]uracil (VIIa)** was obtained by method A in a yield of 25%. R_f 0.48 (3), mp 105–106°C (EtOAc–heptane, 1 : 2). Mass: *m/z* 334.4 [*M*]$^+$, *m/z* 335.4 [*M* + *H*]$^+$, calc. [*M*]$^+$ 334.4 ($C_{18}H_{26}N_2O_4$). For NMR spectra, see Tables 1 and 2.

***N*^{1}-[8-(2-Oxocyclohexyl)-8-oxooctyl]thymine (VIIb)** was obtained by method A in a yield of 16%. R_f 0.645(2), mp 92–94°C (EtOAc–heptane, 1 : 2). Mass: *m/z* 348.4 [*M*]$^+$, *m/z* 349.4 [*M* + *H*]$^+$, calc. [*M*]$^+$ 348.4 ($C_{19}H_{28}N_2O_4$). For NMR spectra, see Tables 1 and 2.

***N*^{1},*N*^{3}-Bis-[8-(2-oxocyclohexyl)-8-oxooctyl]thymine (VIIIb)** was obtained by method A in 2% yield as oil. R_f 0.17 (1). Mass: *m/z* 570.8 [*M*]$^+$, 571.8 [*M* + *H*$^+$], calc. *M* 570.8 ($C_{33}H_{50}N_2O_6$). ^{1}H NMR ($CDCl_3$): 1.20–1.70 (28 H, m, H2'–H6', H2''–H6'', H12', H12'', H13', H13''); 1.88 (3 H, s, 5-CH_3); 2.27 (8 H, m, H11', H11'', H14'', H14''); 2.35 (4 H, m, H7', H7''); 3.65 (2 H, t, *J* 7.2, H1''); 3.88 (2 H, t, *J* 7.5, H1'); 6.92 (1 H, s, H6); 15.95 (1 H, s, 10''-O*H*); 15.98 (1 H, s, 10'-O*H*). ^{13}C NMR ($CDCl_3$): 12.91 (5-*C*H_3); 21.59 (2 C, C13', C13''); 22.79 (2 C, C14', C14''); 23.73 (2 C, C12', C12''); 24.13 (C6''); 24.83 (C6'); 26.24 (C4''); 26.73 (C4'); 27.44 (C2'); 28.92 (2 C, C2'', C5'); 28.98 (C5''); 29.05 (C3''); 29.18 (C3'); 31.08 (2 C, C11', C11''); 36.73 (2 C, C7', C7''); 41.32 (C1''); 49.34 (C1'); 106.61 (2 C, C9', C9''); 109.55 (C5); 138.20 (C6); 151.27 (C2); 163.66 (C4); 181.67 (2 C, C10', C10''); 201.15 (2 C, C8'', C8').

1-[8-(2-Oxocyclohexyl)-8-oxooctyl]cytosine (VIIc) was obtained by method B in yield 38%. R_f 0.43 (4), mp 142–143°C (ethanol). Mass: *m/z* 333.4 [*M*]$^+$, 334.5 [*M* + *H*]$^+$, calc. [*M*]$^+$ 333.4 ($C_{18}H_{27}N_3O_3$). For NMR spectra, see Tables 1 and 2.

Enzyme isolation and purification. The *E. coli* strains BL21(DE3)pERTPHO1 (TP superproducer) and BL21(DE3)pERUPHO1 (UP superproducer) were cultivated as described in [25]. The grown cells were pelleted by centrifugation for 20 min at 6000 *g*, resuspended in 20 mM Tris buffer (20 mM Tris–HCl and 2 mM EDTA, pH 7.5) and degraded by ultrasonication at 4°C. The mixture was centrifuged for 30 min at 10000 *g* for separation from insoluble cell components. Further purification was performed by desalting of the resulting supernatant with ammonium sulfate (20% saturation). Insoluble proteins were centrifuged for 20 min at 12000 *g*. For precipitation of target proteins, the supernatant was saturated with ammonium sulfate up to 80% and centrifuged for 30 min at 17000 *g*. The precipitate containing the enzyme was dissolved in 20 mM Tris buffer (20 mM Tris–HCl, 2 mM EDTA, and 50 mM NaCl, pH 7.5) and chromatographed on a Sephadex G-100 column. The fractions containing the enzyme were saturated with ammonium sulfate up to 80%, and the residue was separated by centrifugation

for 30 min at 17000 *g*, dissolved in phosphate buffer (10 mM KH_2PO_4, pH 7.3), and dialyzed against the same buffer.

The enzyme yield from 10 g of crude cells was 300–400 mg with specific activity of 45 U/mg (UP) and 120 U/mg (TP). Based on electrophoresis data, the purity of the isolated proteins exceeded 95%. The obtained values of specific activities agreed with the published data [26].

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REFERENCES

1. Kritzyn, A.M. and Komissarov, V.V., Bioorg. Khim., 2005, vol. 31, pp. 609–615; *Rus. J. Bioorg. Chem.*, 2005, vol. 31, pp. 549–555.
2. Makinsky, A.A., Kritzyn, A.M., Ul'yanova, E.A., Zakharova, O.D., and Nevinsky, G.A., Bioorg. Khim., 2000, vol. 26, pp. 735–742; *Rus. J. Bioorg. Chem.* 2000, vol. 26, pp. 662–668.
3. Summa, V., Petrocchi, A., Matassa, V.G., Taliani, M., Laufer, R., De Francesco, R., Altamura, S., and Pace, P., *J. Med. Chem.,* 2004, vol. 47, pp. 5336–5339.
4. Biswal, B.K., Cherney, M.M., Wang, M., Chan, L., Yannopoulos, C.G., Bilimoria, D., Nicolas, O., Bedard, J., and James, M.N.G., *J. Biol. Chem.*, 2005, vol. 280, pp. 18202–18210.
5. Summa, V., Petrocchi, A., Pace, P., Matassa, V.G., De Francesco, R., Altamura, S., Tomei, L., Koch, U., and Neuner, P., *J. Med. Chem.,* 2004, vol. 47, pp. 14–17.
6. Wai, J.S., Egbertson, M.S., Payne, L.S., Fisher, T.E., Embrey, M.W., Tran, L.O., Melamed, J.Y., et al., *J. Med. Chem.*, 2000, vol. 43, pp. 4923–4926.
7. Grobler, J.A., Stillmock, K., Hu, B., Witmer, M., Felock, P., Espeseth, A.S., Wolfe, A., Egbertson, M., Bourgeois, M., Melamed, J., Wai, J.S., Young, S., Vacca, J., and Hazuda, D.J., *Proc. Natl. Acad. Sci. USA,* 2002, vol. 99, pp. 6661–6666.
8. Long, Y.-Q., Jiang, X.-H., Dayam, R., Sanchez, T., Shoemaker, R., Sei, S., and Neamati, N., *J. Med. Chem.*, 2004, vol. 47, pp. 2561–2573.
9. Kel'vin, A.V., *Current Organic Chemistry*, 2003, vol. 7, pp. 1691–1711.
10. Kritzyn, A.M. and Komissarov, V.V., Bioorg. Khim., 2004, vol. 30, pp. 487–492; *Rus. J. Bioorg. Chem.,* 2004, vol. 30, pp. 436–440.
11. Kritzyn, A.M., Vesalainen, J., and Komissarov, V.V., Bioorg. Khim., 2005, vol. 31, pp. 288–294; *Rus. J. Bioorg. Chem.* 2005, vol. 31, pp. 256–262.
12. Esteban-Gamboa, A., Balzarini, J., Esnouf, R., De Clercq, E., Camarasa, M.-J., and Perez-Perez, M.-J., *J. Med. Chem.,* 2000, vol. 43, pp. 971–983.
13. Konstantinova, I.D., Leont'eva, N.A., Galegov, G.A., Ryzhova, O.I., Chuvikovsky, D.V., Antonov, K.V., Esipov, R.S., Taran, S.A., Verevkina, K.N., Feofanov, S.A., and Miroshnikov, A.I., *Bioorg. Khim.,* 2004, vol. 30, pp. 613–620; *Rus. J. Bioorg. Chem.,* 2004, vol. 30, pp. 553–560.
14. Moghaddam, A., Zhang, H.T., Fan, T.P.D., Hu, D.E., Lees, V., Turley, H., Fox, S.B., Gatter, K.C., Harris, A.L., and Bicknell, R., *Proc. Natl. Acad. Sci. USA*, 1995, vol. 92, pp. 998–1002.
15. Matsushita, S., Nitanda, T., Furukawa, T., Sumizawa, T., Tani, A., Nishimoto, K., Akiba, S., Miyadera, K., Fukushima, M., Yamada, Y., Yoshiba, H., Kanzaki, T., and Akiyama, S., *Cancer Res.,* 1999, vol. 59, pp. 1911–1916.
16. Bookser, B.C., Kasibhatla, S.R., Appleman, J.R., and Erion, M.D., *J. Med. Chem.*, 2000, vol. 43, pp. 1495–1507.
17. Friess, S.L., *J. Am. Chem. Soc.*, 1949, vol. 71, pp. 2571–2575.
18. Hünig, S., Lücke, E., and Benzing, E., *Chem. Ber.*, 1958, vol. 91, pp. 129–133.
19. Stork, G., Brizzolara, A., Landesman, H., Szmuszkovicz, J., and Terrell, R., *J. Amer. Chem. Soc*, 1963, vol. 85, pp. 207–222.
20. Jones, R.A. and Stokes, M.J., *Tetrahedron*, 1984, vol. 40, pp. 1051–1060.
21. Panova, N.G., Alekseev, K.S., Kuz'michev, A.S., Shcheveleva, E.V., Gavryushov, S.A., Polyakov, K.M., Kritsyn, A.M., Mikhailov, S.N., Esipov, R.S., and Miroshnikov, A.I., *Biokhimiya* (Moscow), 2007, vol. 72, pp. 27–35.
22. Panova, N.G., Shcheveleva, E.V., Alekseev, K.S., Mukhorotov, V.G., Zuev, A.N., Mikhailov, S.N., Esipov, R.S., Chuvikovskii, D.V., and Miroshnikov, A.I., *Mol. Biol.* (Moscow), 2005, vol. 38, pp. 907–913.
23. Tietze, L. and Eicher, T., *Reaktionen und Synthesen im organische-chemischen Praktikum und Forschungslaboratorium*, New York: Georg Thieme Verlag Stuttgart, 1991. Translated under the title *Preparativnaya organicheskaya khimiya*, Moscow: Mir, 1999.
24. Degering, E.F. and Boatright, L.G., *J. Am. Chem. Soc.*, 1950, vol. 72, pp. 5137–5139.
25. Esipov, R.S., Gurevich, A.I., Chuvikovsky, D.V., Chupova, L.A., Muravyova, T.I., and Miroshnikov, A.I., *Protein Expr. Purif.*, 2002, vol. 24, pp. 56–60.
26. Krenitsky, T.A., Koszalka, G.W., and Tuttle, J.V., *Biochemistry*, 1981, vol. 20, pp. 3615–3621.