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Polymethylene Derivatives of Nucleic Bases with ω-Functional Groups. III.[1] *N*-[7-(2-Oxocyclohexyl)-7-oxoheptyl]-Substituted Pyrimidines

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Abstract—New polymethylene derivatives of nucleic bases containing a β-dioxo function at the ω-position were synthesized by alkylation of uracil, thymine, and cytosine with 1-(7-chloroheptanoyl)cyclohexan-2-one, and their physicochemical properties were studied.

Key words: *alkylation, nucleosides, polymethylene analogues*

INTRODUCTION

We have previously shown that polymethylene derivatives of nucleic bases with various functional groups at the ω-position of their carbon chains (more than four carbon atoms in length) are convenient and promising tools for studying the enzymes of nucleic exchange [1, 2].[3] For example, we found that such thymine and uracil derivatives can effectively interact with the reverse transcriptase site responsible for the recognition of the $tRNA^{Lys}$ anticodon and activate this enzyme. The study of interaction with DNA topoisomerase I demonstrated that these derivatives only slightly inhibit the enzyme, the activity of the uracil derivatives being higher than that of the thymine derivatives.

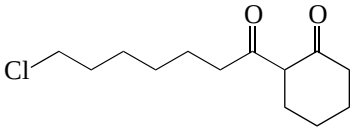
It has recently been demonstrated that 4-aryl-2,4-dioxobutanoic acids are the effective inhibitors of HIV-1 integrase. The mechanism of action and the peculiarities of binding to the HIV-1 integrase are still not clear, but the presence of β-dioxo fragment was found to be one of the factors determining the activity of these compounds [6]. The keto–enol tautomerism inherent in β-diketones might contribute to the interaction with the corresponding enzymes or receptors, since the pH values around the enzyme active sites could substantially differ from the physiological values.

No compounds containing β-diketone fragments in the ω-position of carbon chain have yet been described among a few polymethylene derivatives of nucleic bases derivatives with functional groups. Moreover, simple synthetic schemes for their preparation are not obvious. In this work, we describe the synthesis of the first pyrimidine-derived compounds of this type.

RESULTS AND DISCUSSION

We chose the alkylation of a nucleic base or its protected derivative with 2-(7-chloroheptanoyl)cyclohexanone (**I**) for the preparation of new polymethylene derivatives of nucleic bases bearing a β-diketone function in the ω-position.

The alkylating reagent (**I**) was prepared, using the procedure described by Nesmeyanov *et al.* [7], by the acylation of cyclohexanone morpholine enamine [8] with 7-chloroheptanoyl chloride under mild conditions. The subsequent acidic hydrolysis of the resulting acylated enamine led to the target 1,3-diketone (**I**). Note that a considerable amount of side product with a substantially lower boiling point arose in this reaction; its structure was not determined.



(**I**)

As we shown in [2], the alkylation of 2,4-dihydroxypyrimidines with alkyl halides in DMF in the presence of DBU results in moderate yields of N^1-substituted derivatives (method A). The corresponding and N^1-[7-(2-oxocyclohexyl)-7-oxoheptyl] derivatives of uracil (**II**) and thymine (**IV**) were obtained by this method in 30–40% yields. Silica gel column chromatography allowed the separation of target derivatives (**II**) and (**IV**) from N^1,N^3-bisalkylated products (**III**) and (**V**), respectively; the admixtures of them were ~5%.

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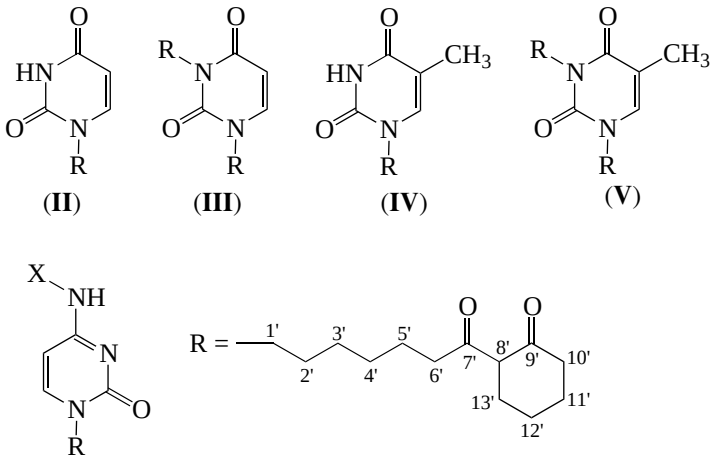
[3] Abbreviations: DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene.

The alkylation of cytosine with chloride (**I**) by method A led to a multicomponent mixture, and we succeeded in the isolation of the target (**VIa**) only in 6% yield. Therefore, other synthetic ways for the preparation of this product were tried. In particular, cytosine protected at the exo amino group with a benzoyl residue was alkylated with chloride (**I**) by method A to give (**VIb**). The removal of the N^4-benzoyl group using 5 M ammonia in methanol (24 h at 20°C) [9] followed by the purification of product on a Dowex 50 × 8 (H^+ form) column led to the target diketone (**VIa**) in a total yield of 27%.

Another way involved the alkylation of cytosine sodium salt, prepared with sodium hydride in DMF (method B). This allowed us both to avoid the protection of exocyclic amino group (as in method A) and to increase the yield of the target cytosine derivative (**VIa**) to 43%.



(**VIa**), (**VIb**) **a**: X = H; **b**: X = Bz.

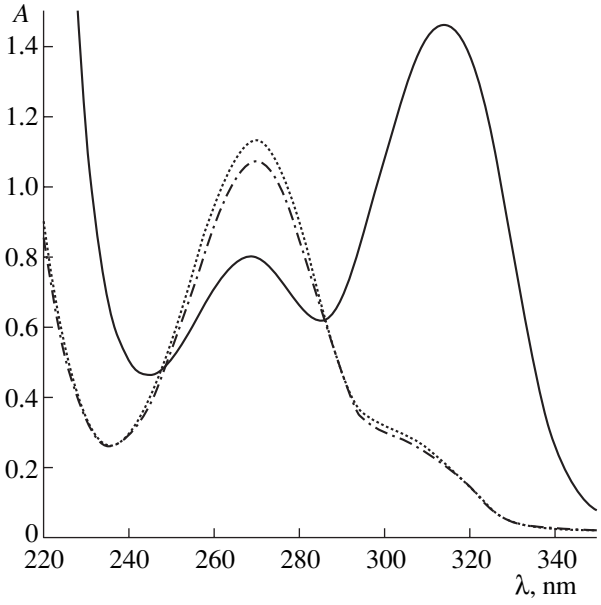
The structures of the alkylated pyrimidines (**II**)–(**VI**) were confirmed by NMR and UV spectroscopy and mass spectrometry (see Tables 1, 2 and the Experimental section).

Note that, according to UV spectra, the β-diketone fragments in (**II**)–(**VI**) are only slightly enolized in acid and neutral aqueous solutions. A pH increase causes an increase in the portion of enol form, which manifests itself in the appearance of absorption maximum at 315 nm. The UV spectra of thymine derivative (**IV**) exemplify this dependence (figure). On the other hand, according to NMR spectra, the enolization of the β-diketone fragments is nearly complete (more than 90%) in anhydrous media (DMSO or $CDCl_3$).

EXPERIMENTAL

Uracil, thymine, and cytosine were from Sigma (United States); DBU was from Aldrich (United States); sodium hydride (80% suspension in mineral oil) was from Fluka (Switzerland); ion-exchange resin Dowex 50 × 8 was from Serva (Germany).

Solvents were purified and dried using standard protocols [10]. UV spectra were recorded on a Cary 50 spectrophotometer (Varian, Australia) at pH 1, 7, and 14. The values of λ_{max} are given in nm (ε, M^{-1} cm^{-1}). Mass spectra were registered on an MS-30 mass spectrometer (Kratos, Japan); electron impact was used as the ionization method. NMR spectra were registered on a Bruker AMXIII-400 spectrometer (Germany) with the working frequency of 400 MHz for H^1 and 100 MHz



UV spectrum of N^1-[7-(2-oxocyclohexyl)-7-oxoheptyl]uracil (**II**); pH 1 (-·-); pH 7 (···); pH 14 (—).

Table 1. ^{1}H NMR spectral data of the synthesized compounds (δ, ppm; *J*, Hz)

Protons	(II)	(III)	(IV)	(V)	(VIa)	(VI·)
H3	8.99 (1 H, s)	–	7.99 (1 H, c)	–	–	–
H5	5.68 (1 H, d, $J_{5,6}$ 7.8)	5.64 (1 H, d, $J_{5,6}$ 7.8)	–	–	5.6 (1 H, d, $J_{5,6}$ 7.7)	5.63 (1 H, d, $J_{5,6}$ 7.8)
H6	7.14 (1 H, d, $J_{5,6}$ 7.8)	7.1 (1 H, d, $J_{5,6}$ 7.8)	6.95 (1 H, s)	6.92 (1 H, s)	7.9 (1 H, d, $J_{5,6}$ 7.7)	6.88 (1 H, d, $J_{5,6}$ 7.8)
5-Me	–	–	1.9 (3 H, c)	1.87 (3 H, c)	–	–
4-NH_2	–	–	–	–	6.75 (2 H, s)	–
4-NHBz	–	–	–	–	–	7.24 (1 H, s)
PhCO-	–	–	–	–	–	*o*–7.89 (2 H, d, $J_{o,m}$ 7.5) *m*–7.5 (2 H, m) *p*–7.59 (1 H, t, $J_{p,m}$ 7.8)
H1'	3.72 (2 H, t, $J_{1',2'}$ 7.2)	3.86 (2 H, t, $J_{1',2'}$ 7.5)	3.67 (2 H, t, $J_{1',2'}$ 7.5)	3.88 (2 H, t, $J_{1',2'}$ 7.8)	3.65 (2 H, t, $J_{1',2'}$ 7.2)	3.89 (2 H, t, $J_{1',2'}$ 7.2)
H1"	–	3.66 (2 H, t, $J_{1",2"}$ 7.2)	–	3.65 (2 H, t, $J_{1",2"}$ 7.2)	–	–
H2'-H5'	1.36 (8 H, m)	1.36 (16 H, m)	1.36 (8 H, m)	1.36 (16 H, m)	1.36 (8 H, Ï)	1.36 (8 H, m)
H2"-H5"	–		–		–	–
H6'	2.41 (2 H, t, $J_{6',5'}$ 7.5)	2.34 (4 H, m)	2.4 (2 H, t, $J_{6',5'}$ 7.5)	2.35 (4 H, m)	2.41 (2 H, t, $J_{6',5'}$ 7.5)	2.41 (2 H, t, $J_{6',5'}$ 7.5)
H6"	–		–		–	–
9'-OH	15.9 (1 H, s)	15.9 (1 H, s)	15.9 (1 H, s)	15.92 (1 H, s)	15.9 (1 H, s)	15.9 (1 H, s)
9"-OH	–	15.95 (1 H, s)	–	15.96 (1 H, s)	–	–
H10', H13'	2.3 (4 H, m)	2.3 (8 H, m)	2.3 (4 H, m)	2.3 (8 H, m)	2.3 (4 H, m)	2.3 (4 H, m)
H10", H13"	–		–		–	–
H11', H12'	1.38 (4 H, m)	1.38 (8 H, m)	1.38 (4 H, m)	1.38 (8 H, m)	1.38 (4 H, m)	1.38 (4 H, m)
H11", H12"	–		–		–	–

for C^{13} at 300K in DMSO-d_6 (if not stated otherwise); tetramethylsilane was used as an internal reference; chemical shifts are given in ppm and coupling constants in Hz. TLC was performed on Kieselgel 60 F_{254} plates (Merck, Germany); elution in chloroform (system A) or 19 : 1 chloroform–ethanol (system B). Column chromatography was carried out on silica gel L40/100 (Chemapol, Czech Republic).

7-Chloroheptanoylcyclohexanone-2 (I). A solution of 7-chloroheptanoyl chloride (36.6 g, 0.2 mol) in dry chloroform (100 ml) was added dropwise under stirring to a solution of *N*-(cyclohexen-1-yl)morpholine [8] (37 g, 0.22 mol) and triethylamine (35.5 ml, 0.25 mol) in chloroform (250 ml), and the mixture was kept at 20°C for 14 h. A solution of concentrate H_2SO_4 (60 ml) in water (80 ml) was added dropwise under vigorous stirring, and the mixture was refluxed under stirring for 4 h and cooled. The organic layer was separated, the aqueous fraction was extracted with chloroform (2 × 100 ml), the organic extracts were combined, dried with Na_2SO_4, and evaporated. The residue was distilled in a vacuum to give 29.7 g (60.7%) of (**I**); bp 147–149°C/1 mmHg; ^{1}H NMR ($CDCl_3$): 1.02 (2 H, m, H4'), 1.08 (2 H, m, H5'), 1.24 (2 H, m, H6'), 1.30 (4 H, m, H4 and H5), 1.40 (2 H, m, H3'), 1.94 (4 H, m, H6, H3), 2.05 (2 H, t, $J_{2',3'}$ 7.0, H2'), 3.16 (2 H, t, $J_{7',6'}$ 6.5, H7'), and 15.6 (1 H, s, 1-OH); ^{13}C NMR: 22.75 (C4), 22.99 (C5), 23.99 (C3'), 25.82 (C3), 27.65 (C5'), 29.96 (C4'), 31.58 (C6'), 35.81 (C2'), 44.0(C6'), 44.98 (C7'), 105.59 (C2), 179.8 (C1), and 200.58 (C1'); MS, *m*/*z*: 244.8 $[M]^+$; calc. for $C_{13}H_{21}ClO_2$: 244.8.

Alkylation of pyrimidine bases with 7-chloroheptanoylcyclohexanone-2 using DBU as a base (method A). Alkylating reagent (**I**) (3.7 g, 15 mmol) and DBU (2.3 g, 15 mmol) were added to a suspension of pyrimidine base or its protected derivative (10 mmol) in anhydrous DMF (25 ml). The reaction mixture was kept at 80–100°C for 20 h (TLC monitoring). The mixture was cooled and evaporated to dryness. The residue was suspended in chloroform and chromatographed on a silica gel column (5 × 28 cm) eluted with a gradient of ethanol (0 ⟶ 10%) in chloroform. The target fractions

Table 2. ^{13}C NMR spectral data of the synthesized compounds

Carbon atoms	Compound, δ, ppm					
	(II)	**(III)**	**(IV)**	**(V)**	**(VIa)**	**(VI·)**
C2	150.9	151.3	150.8	151.3	156.4	166.2
C4	163.75	162.95	164.1	163.65	165.5	170.0
C5	102.25	101.5	107.8	109.6	93.8	93.5
C6	144.5	142.0	140.5	138.2	141.4	148.9
5-Me	–	–	12.4	12.9	–	–
PhCO-	–	–	–	–	–	213.0 (CO), 127.7 (*m*), 129.2 (*o*), 133.2 (*p*)
C1'	48.9	49.6	48.6	49.3	50.1	51.1
C1"	–	41.0	–	41.3	–	–
C2'	31.25	32.7	31.25	31.09	31.25	31.25
C2"	–	32.3	–	31.03	–	–
C3'	28.9	28.76	28.9	28.97	28.9	28.9
C3"	–	28.73	–	28.87	–	–
C4'	26.4	27.73	26.4	26.7	26.5	26.5
C4"	–	26.67	–	26.3	–	–
C5'	29	31.08	29.2	28.8	29.0	29.0
C5"	–	31.02	–	27.4	–	–
C6'	36.8	36.7	36.8	36.7	36.8	36.8
C6"	–	36.6	–	36.6	–	–
C7'	201.4	201.3	201.4	201.3	201.4	201.4
C7"	–	201.1	–	201.2	–	–
C8'	106.9	106.6*	106.9	106.7*	106.9	106.9
C8"	–		–		–	–
C9'	181.8	181.62	181.8	181.64	181.8	181.8
C9"	–	181.56	–	181.57	–	–
C10'	28.9	28.7	28.8	28.9	28.9	28.7
C10"	–	28.8	–	28.7	–	–
C11'	21.8	21.61	21.8	23.61	21.8	21.8
C11"	–	21.57	–	21.58	–	–
C12'	23.0	24.07	23.0	22.81	23.0	23.0
C12"	–	23.83	–	22.78	–	–
C13'	24.0	23.71**	24.0	23.7**	24.3	24.2
C13"	–		–		–	–

* C8' and C8" resonances are overlapped.
** C13' and C13" resonances are overlapped.

were evaporated, and the residue was recrystallized from water–ethanol mixtures or ethanol.

Alkylation of sodium salt of cytosine with 7-chloroheptanoylcyclohexanone-2 (method B). Sodium hydride (0.33 g, 11 mmol) was added to a suspension of cytosine (1.2 g, 10 mmol) in anhydrous DMF (25 ml). After 30-min stirring at 20°C, the alkylating reagent (**I**) (2.9 g, 12 mmol) was added, and the reaction mixture was kept at 80–100°C for 20 h (TLC monitoring). The solvent was evaporated, and the residue was shaken in a mixture of water (20 ml) and chloroform (50 ml). The organic layer was separated, the aqueous phase was

extracted with chloroform (5 × 70 ml), and the combined extracts were dried with anhydrous sodium sulfate. The evaporated residue was chromatographed on a silica gel column as described above. The target fractions were evaporated, and the residue was recrystallized from ethanol.

***N*^{1}-[7-(2-Oxocyclohexyl)-7-oxoheptyl]uracil (II)** was obtained by method A in a yield of 35%; R_f 0.59 (A); mp 79–81°C (1 : 3 water–ethanol); UV: pH 1, 270 (17700); pH 7, 270 (18 800); pH 14, 269 (13 200) and 315 (24100); MS, *m*/*z* 320 [M^+],calc. for $C_{17}H_{24}N_2O_4$: 320.

***N*^{1},*N*^{3}-Bis[7-(2-Oxocyclohexyl)-7-oxoheptyl]uracil (III)** was obtained by method A in a yield of 3%; R_f 0.23 (B). UV: pH 1, 286 (11400); pH 7, 286 (12300); pH 14, 280 (9600) and 313 (22 400); MS, *m*/*z*: 529 $[M]^+$; calc. for $C_{30}H_{44}N_2O_6$: 529.

***N*^{1}-[7-(2-Oxocyclohexyl)-7-oxoheptyl]thymine (IV)** was obtained by method A in a yield of 39%; R_f 0.645 (A); mp 119–120°C (1 : 2 water–ethanol); UV: pH 1, 275 (14500); pH 7, 276 (16200); pH 14, 278 (12000) and 315 (19 800); MS, *m*/*z*: 334 $[M]^+$; calc. for $C_{18}H_{26}N_2O_4$: 334.

***N*^{1},*N*^{3}-Bis[7-(2-Oxocyclohexyl)-7-oxoheptyl]thymine (V)** was obtained by method A in a yield of 4%; R_f 0.31 (A). UV: pH 1, 290 (17900); pH 7, 290 (19000); pH 14, 285 (17700), 313 (25200); MS *m*/*z*: 543 $[M]^+$; calc. for $C_{31}H_{46}N_2O_6$: 543.

***N*^{1}-[7-(2-Oxocyclohexyl)-7-oxoheptyl]cytosine (VIa)** was obtained by method A in a yield of 6% and by method B in a yield of 43%; R_f 0.07 (A); mp 157–159°C (ethanol). UV: pH 1, 286 (15800); pH 7, 277 (11400); pH 14, 281 (11400) and 315 (16400); MS, *m*/*z*: 319 $[M]^+$; calc. for $C_{17}H_{25}N_3O_3$: 319.

***N*^{1}-[7-(2-Oxocyclohexyl)-7-oxoheptyl]-*N*^{4}-benzoylcytosine (VIb)** was obtained by method A in a yield of 30.5%; R_f 0.67 (A); mp 132–134°C (4 : 5 water–ethanol); MS, *m*/*z*: 423.5 $[M]^+$; calc. for $C_{24}H_{29}N_3O_4$: 423.5.

Removal of benzoyl protective group. Cytosine derivative (**VIb**) (0.25 g, 0.6 mmol) was kept in 5 M ammonia solution in methanol at room temperature. After 24 h (TLC monitoring), the reaction mixture was evaporated, the residue was dissolved in ethanol (15 ml), and applied onto a Dowex 50 × 8 (H^+ form) column. The column was washed with water and alcohol. The product was eluted with a 4 : 1 ethanol–conc. aqueous ammonia mixture (150 ml). The eluate was evaporated to give 0.17 g (89%) of (**VIa**), whose physicochemical characteristics were identical to those of the derivative obtained by the methods A and B.

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