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Polymethylene Derivatives of Pyrimidine Nucleic Bases Bearing ω-Functional Groups[1]

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Abstract—N^1-Acyclic derivatives of pyrimidine bases (uracil, thymine, and cytosine) with hydrophobic polymethylene chains containing various functional groups in an ω-position of the alkyl substituent were synthesized. Their physicochemical properties and inhibitory effect on the HIV reverse transcriptase and human DNA topoisomerase I were studied.

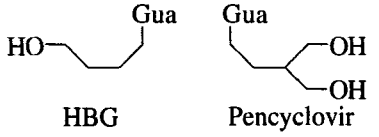
Key words: alkylation, nucleosides, polymethylene analogues

INTRODUCTION

Non-glycoside nucleoside analogues are useful as potential pharmacological agents and models for studying mechanisms of action of the nucleic metabolism enzymes [1–3].

Although syntheses and studies of biological properties of non-glycoside nucleoside and nucleotide analogues have been carried out since the 1980s, only recently appeared publications on the polymethylene analogues with four or more carbon atoms.

9-(4-Hydroxybutyl)guanine (HBG) a structural analogue of acyclovir, is widely used in herpes treatment [4]. Like acyclovir, HBG is a substrate for the HSV-1 and HSV-2 thymidine kinases, but not for the cell kinase. Pencyclovir, a selective antiherpes agent, can also be assigned to the same class of compounds [5].



The inhibitors of purine nucleoside phosphorylase are used for treatment of some diseases and pathologies, such as leukemia, rejection of transplanted organs, autoimmune dysfunctions, rheumatoid arthritis, and psoriasis. The crystallographic study revealed three discrete regions in the enzyme active site: guanine-binding site, phosphate-binding site, and hydrophobic pocket. It was shown that 9-polymethyleneguanine ω-phosphonates comprising a large hydrophobic fragment are excellent inhibitors of purine nucleoside phosphorylase [6].

In this context, promising is the development of methods of synthesis of modified nucleic bases bearing a polymethylene chain with various functional groups in an ω-position. Here, we describe the synthesis and properties of the uracil, thymine, and cytosine derivatives.

RESULTS AND DISCUSSION

The synthesis is based on alkylation of nucleic bases with odd-numbered ω-chlorocarboxylates and ω-polymethylenechlorohydrins derived therefrom or their acetates. These derivatives are easily available using reactions of the products of ethylene–carbon tetrachloride radical telomerization as described in classical works by A. N. Nesmeyanov *et al.* [7]. We used δ-chlorovaleric, ω-chloroenanthic, and ω-chloropelargonic acids, containing 5, 7, and 9 carbon atoms, respectively.

The standard dehydrohalogenation reagents for alkylation of pyrimidine bases in aprotic solvents are potassium or cesium carbonate. Few examples are known of the use of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for this purpose [8, 9], for example, in the alkylation of thymine with 1-chloro-4-acetoxycyclohexene-2 [9]. Currently, nitrogen-containing organic bases are widely used in organic chemistry [10], and we chose to study their effect on the yield of alkylation products in the reaction of thymine with ethyl δ-chlorovalerate. Potassium carbonate, DBN (1,5-diazabicyclo[4.3.0]non-5-ene, pK_a 13.5), DBU (pK_a 11.6), and TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene, $pK_a > 14$) were compared [10].

As Table 1 shows, optimal conditions of the thymine alkylation are DMF as a solvent and DBU as a dehydrohalogenation reagent (method A). The reaction mixture (uracil or thymine, ethyl ω-chlorocarboxylate, DBU, DMF) was kept for 10–30 h at 80–100°C (TLC con-

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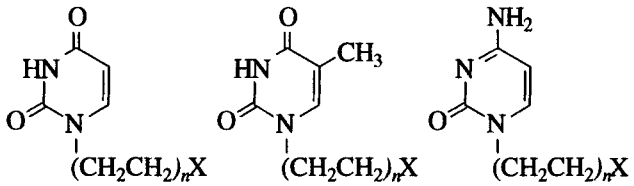
Table 1. Thymine alkylation with ethyl δ chlorovaleric acid (15 h, 80°C)

Reagent ratio*	Solvent	Base	Yield, %	
			monoproduct**	bisproduct***
1 : 1 : 2	DMSO	K_2CO_3	31.5	27.2
1 : 1.5 : 1.5	DMF	DBU	49.2	39.5
1 : 1.5 : 1.5	DMF	DBN	37.2	19.6
1 : 1.5 : 1.5	DMF	TBD	42.2	35.6

*Thymine–ethyl δ chlorovalerate–base ratio.
**N^1-(γ-Ethoxycarbonyl-*n*-butyl)thymine.
***N^1,N^3-bis-(γ-Ethoxycarbonyl-*n*-butyl)thymine.

trol), and, after standard treatment, the corresponding uracil (**Ia**, **Id**, **Ig**) or thymine (**IIa**, **IId**, **IIg**) derivatives were isolated by column chromatography on silica gel (Table 2).



(I a–i) **(II a–i)** **(III a–i)**

a, **b**, **c**: $n = 2$; **d**, **e**, **f**: $n = 3$; **g**, **h**, **i**: $n = 4$

a, **d**, **g**: X = COOEt; **b**, **e**, **h**: X = COOH;
c, **f**, **i**: X = CH_2OH

The aforementioned reaction conditions were also suitable for the preparation of uracil and thymine hydroxy derivatives using the alkylation with α,ω-polymethylenechlorohydrin acetates (Table 2, method E). The acetyl group was removed by alkaline hydrolysis.

Cytosine alkylation under these conditions resulted in a complex, hard-to-separate mixture. Therefore, alkylation of the cytosine sodium salt was used. A cytosine suspension in DMF was treated with sodium hydride, ethyl ω-chlorocarboxylate (1.1 eq) was added, and the mixture was kept at 80–100°C for 20–30 h.

This method afforded good yields of cytosine derivatives on alkylation by both chlorocarboxylates and acetates of polymethylenechlorohydrins (Table 3, methods B and F, respectively).

The conversion of cytosine to uracil derivatives was carried out using either the bisulfite approach [11] or lithium hydroxide [12]. We found that the reflux of cytosine polymethylene derivatives with 4 M water–methanol LiOH solution gives the corresponding uracil analogues in good yields. Physicochemical characteristics of the uracil derivatives obtained by this approach and by the direct alkylation of uracil coincide.

The acid hydrolysis of the ethoxycarbonyl group was accomplished by refluxing compounds (**I–III a**, **d**, **g**) in 3 M hydrochloric acid. Yields and some characteristics of carboxylic acids (**I–III b**, **e**, **h**) are given in Tables 2 and 3.

The ester group was reduced to the hydroxymethyl one by lithium aluminum hydride in tetrahydrofurane. The hydroxyl-bearing derivatives obtained by various methods had the same characteristics. As is seen in Tables 2 and 3, direct alkylation of pyrimidines with α,ω-polymethylenechlorohydrin acetates followed by deprotection afforded better yields than reduction of ethoxycarbonyl derivatives of nucleic bases.

The structure of the compounds synthesized was confirmed by the UV, NMR, and mass spectra data (Tables 2 and 3 and the Experimental section).

The compounds synthesized were studied as potential inhibitors of HIV reverse transcriptase and human DNA topoisomerase I, which are the major targets of most of the known antiviral [13] and antitumor [14–16] agents. The inhibitory properties toward these enzymes are given in Table 4. As is seen, these compounds, except **Id**, **Ig**, **IIi**, and **IIIf**, did not inhibit the polymerization reaction even at rather high concentrations (1 mM). At the same time, some derivatives not only inhibited the enzyme at this concentration, but also accelerated polymerization. A considerable activation of reverse transcriptase (21–50%) was observed for compounds **Ia**, **IIe**, **IIf**, and **IIc**. We showed earlier that the $tRNA^{Lys}$ (the natural enzyme primer) interaction with the regulatory subunit p51 of the reverse transcriptase functional dimer p51/p66 caused its conformational changes and, hence, the enzyme activation in the polymerization reaction on poly(A) · oligo(dT) or in other template–primer complexes [17, 18]. Later, it was shown that 7–10-mer $tRNA^{Lys}$ fragments containing nucleotide units of the anticodon loop were able to activate the enzyme. The second essential tRNA region affecting its activating ability was found to be the 3'-terminal CCA sequence [19, 20]. The removal of this sequence from the tRNA resulted in a noticeable decrease in its activating properties. Various nucleosides, dNMP, and short oligonucleotides could not activate the enzyme. Thus, we were first to find the thymine and uracil derivatives that can effectively interact with the region of reverse transcriptase involved in the rec-

Table 2. Method of synthesis, yields, melting points, and UV spectral data of N^1-uracil and -thymine derivatives (**I**) and (**II**)

Compound	Method*	Yield, %	T_{mp}, °C	UV, λ_{max}, nm (ε)		
				pH 1	pH 7	pH 14
(Ia)	A	48.1	68–70	267(9700)	267(9600)	265(7100)
(Ib)	C	95.3	135–136	266(8600)	266(8500)	265(6200)
(Ic)	G	90	124–125	266(9900)	266(9500)	265(7100)
(Id)	A	42.1	59–60	267(9900)	266(9700)	265(7800)
(Ie)	C	98	125–126	266(8700)	266(8600)	265(6600)
(If)	E	57.5	85–87	266(8900)	266(8950)	265(6400)
(Ig)	A	40	67–69	267(9900)	267(9800)	265(7100)
(Ih)	C	97	130–132	266(8900)	266(9100)	265(6900)
(Ii)	E	45	100–102	266(8900)	266(9050)	265(6800)
	G	80	101–103	266(8900)	266(9100)	265(6900)
(IIa)	A	43.3	82–83	272(9300)	272(9600)	270(7300)
(IIb)	C	94	131–133	272(8500)	272(8900)	270(6300)
(IIc)	E	47	174–176	272(9000)	271(9200)	270(6900)
(IId)	A	59.9	80–81	272(8500)	272(8900)	270(6500)
(IIe)	C	91	124.5–125.5	272(8700)	272(8500)	270(6400)
(IIf)	E	48.3	131–132	272(8800)	271(9000)	270(6800)
(IIg)	A	60.1	65–67	272(8600)	272(9000)	270(6800)
(IIh)	C	95	108–110	272(8900)	272(9200)	270(6800)
(IIi)	E	37.3	126–128	272(8800)	272(9100)	270(6600)

* See the Experimental section.

Table 3. Method of synthesis, yields, melting points, and UV spectral data of N^1-cytosine derivatives (**III**)

Compound	Method*	Yield, %	T_{mp}, °C	UV, λ_{max}, nm (ε)		
				pH 1	pH 7	pH 14
(IIIa)	B	51	194–195	284(10100)	276(9600)	274(9600)
(IIIb)	C	97	179–180	284(10200)	277(8800)	275(8600)
(IIIc)	F	52	198–201	282(10100)	276(9900)	274(9200)
(IIId)	B	77.7	175–177	283(10200)	276(9900)	275(9600)
(IIIe)	C	98	180–182	282(9800)	276(9800)	274(8900)
(IIIf)	D	59.2	151–153	283(9400)	275(9300)	274(8900)
	F	49.0	153–155	283(10100)	275(9900)	274(8900)
(IIIg)	B	55	182–184	283(10100)	276(9900)	274(9400)
(IIIh)	C	90	218–219	283(10300)	278(9800)	274(9300)
(IIIi)	D	44	158–160	282(10600)	276(10700)	274(9900)
	F	46	159–161	282(10500)	276(10600)	274(9600)

* See the Experimental section.

ognition of the $tRNA^{Lys}$ anticodon (UUC) and the enzyme activation. The activation level for compound **Ia** (50%) was thereby only six or two times lower than that of $tRNA^{Lys}$ and its oligonucleotide analogues.

Some heterocyclic compounds, for example, camptothecin, are known to be specific inhibitors of DNA topoisomerase I and are widely used as antitumor agents [14–16]. In this context, we evaluated the inhib-

itory properties of the new series of compounds using protocols described in [21, 22]. Most of the compounds inhibited the enzyme, although the effect was incomplete even at high concentrations (1 mM). Interestingly, the highest inhibitory properties were displayed by uracil derivatives **Ia–If**.

EXPERIMENTAL

Solvents were purified and dried using standard methods. Uracil, thymine, cytosine, Tris, sodium dodecyl sulfate, and glycerol were from Sigma (United States); EDTA was from Serva (Germany); agarose was from Lachema (Czech Republic). UV spectra were recorded on a Specord UV VIS spectrophotometer (Germany). Mass spectra were registered on a MS 902 AEI Manchester device (United States). NMR spectra were registered on a Bruker AMX 400 spectrometer (Germany) at 300 K. Chemical shifts are in ppm, and spin-coupling constants are in Hz. TLC was performed on Kieselgel 60 F_{254} plates (Merck, Germany) using the chloroform–ethanol systems (1) 19 : 1, (2) 18 : 2, (3) 17 : 3, (4) 14 : 6, and (5) 10 : 10. Column chromatography was carried out on silica gel L 40/100 (Chemapol, Czech Republic). Radioactivity was determined on paper filters FN-16 (Filtrac, Germany) on a counter MINI-Beta (LKB-Vallac, Sweden). Electrophoresis was run in 0.8% agarose gel in Tris-acetate buffer (pH 7.5), and gels were stained with ethidium bromide (0.5 μg/ml).

The HIV reverse transcriptase assay. The polymerization reaction catalyzed by reverse transcriptase was performed at 30°C for 30 min [23]. The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 5 mM dithiothreitol, 0.5 mM EDTA, 80 mM KCl, 50 μM [^{3}H]dTTP, and 1.8 OU_{260}/ml poly(A) template at the saturating concentration. Primer $d(pT)_{16}$ at the optimal concentration (5×10^{-6} M) and the synthesized compounds (10^{-3} M) were added to this solution. The polymerization reaction was initiated by the addition of the enzyme (0.02–2 U), and the mixture was incubated for 1 h. During the incubation, aliquots (5–10 μl) were taken after each 5–20 min and loaded onto dry FN-16 filters (15 × 15 mm) presoaked in 5% chloroacetic acid. The filters were washed seven times with 5% chloroacetic acid at 0°C for 10 min, then with acetone cooled to 5°C to remove the acid, and dried, and their radioactivity was determined on a MINI-Beta toluene scintillation counter.

The human DNA topoisomerase I assay. The activity of DNA topoisomerase was evaluated by the degree of transition of supercoiled plasmid DNA Bluescript to the relaxed form at 30°C for 15 min. The reaction mixture (20 μl) contained 50 mM Tris-HCl buffer (pH 8.0), 0.05 M NaCl, bovine serum albumine (30 μg/ml), 0.5 mM dithiothreitol, 0.2 mM EDTA, 10% glycerol, supercoiled DNA (10 μg/ml) and the compounds under study at the concentration of 10^{-3} M.

Table 4. The relative activity of HIV reverse transcriptase (RT) and human DNA topoisomerase I (TOPO) (the enzyme activity in the absence of the inhibitor is taken as 100%; the inhibitor concentration is 10^{-3} M)

Compound	Relative activity, %		Compound	Relative activity, %	
	RT	TOPO		RT	TOPO
(Ia)	150	41	**(IIh)**	89	73
(Id)	74	38	**(IIIb)**	49	92
(Ig)	70	44	**(IIIe)**	95	100
(IIa)	88	53	**(IIIh)**	95	71
(IId)	103	91	**(If)**	100	42
(IIg)	106	78	**(IIc)**	138	97
(IIId)	97	76	**(IIf)**	124	95
(IIIg)	100	97	**(IIi)**	86	81
(Ie)	60	44	**(IIIc)**	97	84
(IIb)	60	96	**(IIIf)**	74	95
(IIe)	121	89	**(IIIi)**	103	92

After 15-min incubation, a solution containing glycerol (50%), sodium dodecyl sulfate (6%), and Bromphenol Blue (0.1%) was added. The reaction was stopped by cooling to –20°C. The ratio of the supercoiled and relaxed DNA forms was determined by electrophoresis followed by taking photographs, film scanning, and data processing using the GelPro program (Microsoft, United States).

Uracil and thymine alkylation with ethyl ω-chlorocarboxylates (Method A). Ethyl ω-chlorocarboxylate (30 mmol) and DBU (30 mmol) were added to a suspension of uracil or thymine (20 mmol) in anhydrous DMF (30 ml). The reaction mixture was kept for 15–20 h at 80–100°C (TLC monitoring). After evaporation, the residue was dissolved in chloroform (20–25 ml); the solution was washed with water, dried with anhydrous sodium sulfate, evaporated, and chromatographed on a silica gel column (200 g) in a gradient of ethanol in chloroform (0–10%).

Cytosine alkylation with ethyl ω-chlorocarboxylates (Method B). Sodium hydride (22 mmol) as a 60% suspension in mineral oil was added to a cytosine suspension (20 mmol) in anhydrous DMF (30 ml), after 30-min stirring at room temperature ethyl ω-chlorocarboxylate (30 mmol) was added, and the reaction mixture was kept at 80–100°C for 20–30 h (TLC) and treated as in Method A.

The preparation of N^1-(ω-carboxyalkyl)pyrimidines by acid hydrolysis of ethoxycarbonyl derivatives (Method C). A solution of an ethoxycarbonyl derivative (3 mmol) in 3 M hydrochloric acid (20 ml) was refluxed for 3 h with TLC monitoring. The reaction mixture was evaporated and coevaporated with water.

The products were recrystallized from water–ethanol mixtures containing 10 to 50% ethanol.

The preparation of N^1-(ω-carboxyalkyl)pyrimidines by reduction of ethoxycarbonyl derivatives with lithium aluminum hydride in tetrahydrofuran (Method D). A solution of ethoxycarbonyl derivative (5 mmol) in tetrahydrofuran (15 ml) was added to a suspension of lithium aluminum hydride (500 mg, 13 mmol) in tetrahydrofuran (50 ml) at stirring for 20 min, and the reaction mixture was refluxed. After the reaction was completed (TLC), the mixture was cooled, and ethyl acetate (60 ml) and water (15 ml) were added. The mixture was refluxed for 30 min, cooled to room temperature, and evaporated. The residue was dissolved in 3 M HCl, adjusted to pH 7–8 with concentrated aqueous ammonia, and evaporated. The product was extracted with hot ethyl acetate; the extracts were pooled, evaporated, and chromatographed on a silica gel column in a gradient of ethanol (0–10%) in chloroform.

Alkylation of uracil and thymine with ω-chlorohydrin acetates (Method E). To a suspension of uracil or thymine (20 mmol) in anhydrous DMF (30 ml) were added ω-chlorohydrin acetate (30 mmol) and DBU (30 mmol). The reaction mixture was kept for 15–20 h at 80–100°C (TLC), and the product was isolated as described in method A. The resulting acetates were treated with 5 M methanolic ammonia to remove the acetyl protection.

Alkylation of cytosine with ω-chlorohydrin acetates (Method F). To a suspension of cytosine (20 mmol) in anhydrous DMF (30 ml) was added sodium hydride (22 mmol, 60% suspension in mineral oil), the mixture was stirred for 30 min at room temperature, and ω-chlorohydrin acetate (30 mmol) was added. The reaction mixture was kept at 80–100°C for 15–20 h (TCL), and the products were isolated and purified as described in method A. The acetyl groups were removed by treatment with 5 M methanolic ammonia.

Deamination of cytosine to the corresponding uracil derivatives with lithium hydroxide (Method G). A cytosine derivative (1 mmol) and ethanol (2 ml) were added to a 4 M solution of lithium hydroxide (8 ml), and the mixture was refluxed for 12 h. After the reaction was over (TLC), the mixture was cooled, neutralized with 3 M hydrochloric acid, and evaporated. The product was extracted with hot chloroform and, after evaporation, recrystallized from water.

N^1-(δ-Ethoxycarbonyl-*n*-butyl)uracil (Ia). R_f 0.32 (1). ^{1}H NMR ($CDCl_3$): 1.22 (3H, t, $J_{1'',2''}$ 7, H2''), 1.68 (4H, m, H2', H3'), 2.32 (2H, t, $J_{3',4'}$ 6.7, H4'), 3.70 (2H, t, $J_{1',2'}$ 7, H1'), 4.15 (2H, q, H1''), 5.75 (1H, d, $J_{5,6}$ 7.9, H5), 7.12 (1H, d, H6), 9.8 (1H, s, NH). MS: *m/z* 241 [M^+]. Calc. for $C_{11}H_{16}N_2O_4$: 240.

N^1-(ω-Ethoxycarbonyl-*n*-hexyl)uracil (Id). R_f 0.34 (1). ^{1}H NMR ($CDCl_3$): 1.26 (3H, $J_{1'',2''}$ 7.1, H2''), 1.37 (4H, m, H3' and H4'), 1.63 (3H, m, H5'), 1.70 (2H, m, H2'), 2.20 (2H, t, $J_{5',6'}$ 7.3, H6'), 3.72 (2H, t, $J_{1',2'}$ 7.3, H1'), 4.12 (2H, q, H1''), 5.75 (1H, d, $J_{5,6}$ 7.8, H5), 7.2 (1H, d, H6), 9.15 (1H, s, NH). MS: *m/z* 269 [M^+], Calc. for $C_{13}H_{20}N_2O_4$: 268.

N^1-(ω-Ethoxycarbonyl-*n*-octyl)uracil (Ig). R_f 0.36 (1). ^{1}H NMR ($CDCl_3$): 1.25 (3H, t, $J_{1'',2''}$ 7.1, H2''), 1.35 (8H, m, H3'–H6'), 1.65 (2H, m, H7'), 1.70 (2H, m, H2'), 2.22 (2H, t, $J_{7',8'}$ 7.3, H8'), 3.75 (2H, t, $J_{1',2'}$ 7.3, H1'), 4.12 (2H, q, H1''), 5.75 (1H, d, $J_{5,6}$ 7.8, H5), 7,15 (1H, d, H6), 9.18 (1H, s, NH). MS: *m/z* 297 [M^+]. Calc. for $C_{15}H_{24}N_2O_4$: 296.

N^1-(δ-Ethoxycarbonyl-*n*-butyl)thymine (IIa). R_f 0.72 (2). ^{1}H NMR ($CDCl_3$): 1.22 (3H, t, $J_{1'',2''}$ 7.3, H2''), 1.66 (4H, m, H2'and H3'), 1.87 (3H, s, 5-Me), 2.32 (2H, t, $J_{3',4'}$ 7, H4'), 3.60 (2H, t, $J_{1',2'}$, 6.7, H1'), 4.15 (2H, q, H1''), 7.15 (1H, s, H6), 9.12 (1H, s, NH). MS: *m/z* 255 [M^+]. Calc. for $C_{12}H_{18}N_2O_4$: 254.

N^1-(ω-Ethoxycarbonyl-*n*-hexyl)thymine (IId). R_f 0.33 (1). ^{1}H NMR ($CDCl_3$): 1.22 (3H, t, $J_{1'',2''}$ 7.3, H2''), 1.33 (4H, m, H3' and H4'), 1.62 (4H, m, H2' and H5'), 1.88 (3H, s, 5-Me), 2.26 (2H, t, $J_{5',6'}$ 7.3, H6'), 3.65 (2H, t, $J_{1',2'}$ 7.3, H1'), 4.15 (2H, q, H1''), 6.95 (1H, s, H6), 8.98 (1H, s, NH). MS: *m/z* 283 [M^+]. Calc. for $C_{14}H_{22}N_2O_4$: 282.

N^1-(ω-Ethoxycarbonyl-*n*-octyl)thymine (IIg). R_f 0.36 (1). ^{1}H NMR ($CDCl_3$): 1.22 (3H, t, $J_{1'',2''}$ 7.3, H2''), 1.30 (8H, m, H3'and H6'), 1.60 (4H, m, H2' and H7'), 1.88 (3H, s, 5-Me), 2.30 (2H, t, $J_{7',8'}$ 7.3, H8'), 3.65 (2H, t, $J_{1',2'}$ 7.3, H1'), 4.11 (2H, q, H1''), 6.95 (1H, s, H6), 9.0 (1H, s, NH). MS: *m/z* 311 [M^+]. Calc. for $C_{16}H_{26}N_2O_4$: 310.

N^1-(δ-Ethoxycarbonyl-*n*-butyl)cytosine (IIIa). R_f 0.22 (2). ^{1}H NMR (DMSO-d_6): 1.22 (3H, t, $J_{1'',2''}$ 7.3, H2''), 1.60 (4H, m, H2' and H3'), 2.30 (2H, t, $J_{3',4'}$ 7.3, H4'), 3.65 (2H, t, $J_{1',2'}$ 7.3, H1'), 4.10 (2H, q, H1''), 5.62 (1H, d, $J_{5,6}$ 7, H5), 6.75 (2H, s, NH_2), 7.42 (1H, d, H6). MS: *m/z* 240 [M^+]. Calc. for $C_{11}H_{17}N_3O_3$: 239.

N^1-(ω-Ethoxycarbonyl-*n*-hexyl)cytosine (IIId). R_f 0.24 (2). ^{1}H NMR (DMSO-d_6): 1.22 (3H, t, $J_{1'',2''}$ 7.3, H2''), 1.30 (4H, m, H3' and H4'), 1.52 (2H, m, H5'), 1.60 (2H, m, H2'), 2.20 (2H, t, $J_{5',6'}$ 7.3, H6'), 3.65 (2H, t, $J_{1',2'}$ 7.3, H1'), 4.10 (2H, q, H1''), 5.60 (1H, d, $J_{5,6}$ 7.1, H5), 6.75 (2H, s, NH_2), 7.40 (1H, d, H6). MS: *m/z* 268 [M^+]. Calc. for $C_{13}H_{21}N_3O_3$: 267.

N^1-(ω-Ethoxycarbonyl-*n*-octyl)cytosine (IIIg). R_f 0.32 (3). ^{1}H NMR (DMSO-d_6): 1.20 (3H, t, $J_{1'',2''}$ 7.3, H2''), 1.35 (8H, m, H3'–H6'), 1.50 (2H, m, H7'), 1.60 (2H, m, H2'), 2.20 (2H, t, $J_{7',8'}$ 7.3, H8'), 3.62 (2H, t, $J_{1',2'}$ 7.3, H1'), 4.15 (2H, q, H1''), 5.60 (1H, d, $J_{5,6}$ 7.1, H5), 6.75 (2H, s, NH_2), 7.40 (1H, d, H6). MS: *m/z* 296 [M^+]. Calc. for $C_{15}H_{25}N_3O_3$: 295.

N^1-(δ-Carboxy-*n*-butyl)uracil (Ib). R_f 0.39 (4). ^{1}H NMR (DMSO-d_6): 1.46 (2H, m, H3'), 1.56 (2H, m, H2'), 2.22 (2H, t, $J_{3',4'}$ 7.2, H4'), 3.60 (2H, t, $J_{1',2'}$ 7.0, H1'), 5.55 (1H, d, $J_{5,6}$ 7.9, H5), 7.60 (1H, d, H6), 11.15 (1H, s, NH), 11.90 (1H, s, COOH). MS: *m/z* 213 [M^+]. Calc. for $C_9H_{12}N_2O_4$: 212.

N^1-(ω-Carboxy-*n*-hexyl)uracil (Ie). R_f 0.12 (1). ^{1}H NMR (DMSO-d_6): 1.26 (4H, m, H3' and H4'), 1.48 (2H, m, H5'), 1.55 (2H, m, H2'), 2.17 (2H, d, $J_{5',6'}$ 7.3, H6'), 3.62 (2H, t, $J_{1',2'}$ 7.2, H1'), 5.50 (1H, d, $J_{5,6}$ 7.9, H5), 7.50 (1H, d, H6), 11.10 (1H, s, NH), 11.90 (1H, s, COOH). MS: *m/z* 241 [M^+]. Calc. for $C_{11}H_{16}N_2O_4$: 240.

N^1-(ω-Carboxy-*n*-octyl)uracil (Ih). R_f 0.13 (1). ^{1}H NMR (DMSO-d_6): 1.30 (8H, m, H3' and H6'), 1.50 (2H, m, H7'), 1.55 (2H, m, H2'), 2.15 (2H, t, $J_{7',8'}$ 7.3, H8'), 3.65 (2H, t, $J_{1',2'}$ 7.3, H1'), 5.50 (1H, d, $J_{5,6}$ 7.9, H5), 7.50 (1H, d, H6), 11.10 (1H, s, NH). MS: *m/z* 269 [M^+]. Calc. for $C_{13}H_{20}N_2O_4$: 268.

N^1-(δ-Carboxy-*n*-butyl)thymine (IIb). R_f 0.42 (3). ^{1}H NMR (DMSO-d_6): 1.55 (2H, m, H3'), 1.65 (2H, m, H2'), 1.88 (3H, s, 5-Me), 2.25 (2H, t, $J_{3',4'}$ 7.3, H4'), 3.62 (2H, t, $J_{1',2'}$ 7.3, H1'), 7.35 (1H, s, H6), 10.90 (1H, s, NH), 11.80 (1H, s, COOH). MS: *m/z* 227 [M^+]. Calc. for $C_{10}H_{14}N_2O_4$: 226.

N^1-(ω-Carboxy-*n*-hexyl)thymine (IIe). R_f 0.46 (3). ^{1}H NMR (DMSO-d_6): 1.30 (4H, m, H3' and H4'), 1.60 (2H, m, H2' and H5'), 1.90 (3H, s, 5-Me), 2,20 (2H, t, $J_{5',6'}$ 7.3, H6'), 3.60 (2H, t, $J_{1',2'}$ 7.3, H1'), 7.40 (1H, s, H6), 10.90 (1H, s, NH), 11.90 (1H, s, COOH). MS: *m/z* 254 [M^+]. Calc. for $C_{12}H_{18}N_2O_4$: 254.

N^1-(ω-Carboxy-*n*-octyl)thymine (IIh). R_f 0.50 (3). ^{1}H NMR (DMSO-d_6): 1.30 (8H, m, H3' and H6'), 1.55 (2H, m, H2' and H7'), 1.85 (3H, s, 5-Me), 2.22 (2H, $J_{7',8'}$ 7.3, H8'), 3.65 (2H, t, $J_{1',2'}$ 7.3, H1'), 7.30 (1H, s, H6), 10.95 (1H, s, NH). MS: *m/z* 283 [M^+]. Calc. for $C_{14}H_{22}N_2O_4$: 282.

N^1-(δ-Carboxy-*n*-butyl)cytosine (IIIb). R_f 0.23 (5). ^{1}H NMR (DMSO-d_6): 1.50 (2H, m, H3'), 1,60 (2H, m, H2'), 2.20 (2H, t, $J_{3',4'}$ 7.3, H4'), 3.75 (2H, t, $J_{1',2'}$ 7.3, H1'), 6,15 (1H, d, $J_{5,6}$ 7.6, H5), 8,05 (1H, d, H6), 8,65 (1H, s, NH_2), 9.70 (1H, s, NH_2), 12.00 (1H, s, COOH). MS: *m/z* 212 [M^+]. Calc. for $C_9H_{13}N_3O_3$: 211.

N^1-(ω-Carboxy-*n*-hexyl)cytosine (IIIe). R_f 0.36 (5). ^{1}H NMR (DMSO-d_6): 1.27 (4H, m, H3' and H4'), 1.49 (2H, m, H5'), 1.60 (2H, m, H2'), 2.22 (2H, t, $J_{5',6'}$ 7.3, H6'), 3.73 (2H, t, $J_{1',2'}$ 7.3, H1'), 6.10 (1H, d, $J_{5,6}$ 7.6, H5), 8.00 (1H, d, H6), 8.65 (1H, s, NH_2), 9.65 (1H, s, NH_2), 12.00 (1H, s, COOH). MS: *m/z* 240 [M^+]. Calc. for $C_{11}H_{17}N_3O_3$: 239.

N^1-(ω-Carboxy-*n*-octyl)cytosine (IIIh). R_f 0.35 (4). ^{1}H NMR (DMF-d_7): 1.30 (8H, m, H3' and H6'), 1.55 (2H, m, H7'), 1.63 (2H, m, H2'), 2.22 (2H, t, $J_{7',8'}$ 7.3, H8'), 3.72 (2H, t, $J_{1',2'}$ 7.3, H1'), 5.80 (1H, d, $J_{5,6}$ 7.6, H5), 7.1 (2H, s, NH_2), 7.70 (1H, d, H6). MS: *m/z* 268 [M^+]. Calc. for $C_{13}H_{21}N_3O_3$: 267.

N^1-(ω-Hydroxy-*n*-amyl)uracil (Ic). R_f 0.20 (3). ^{1}H NMR (DMSO-d_6): 1.25 (2H, m, H3'), 1.40 (2H, m, H4'), 1.55 (2H, m, H2'), 3.35 (2H, t, $J_{5',4'}$ 6.6, H5'), 3.60 (2H, t, $J_{1',2'}$ 7.1, H1'), 3.95 (1H, s, OH), 5.50 (1H, d, $J_{5,6}$ 7.63, H5), 7,60 (1H, d, H6), 11.00 (1H, s, NH). MS: *m/z* 199 [M^+]. Calc. for $C_9H_{14}N_2O_3$: 198.

N^1-(ω-Hydroxy-*n*-heptyl)uracil (If). R_f 0.47 (3). ^{1}H NMR (DMSO-d_6, 50°C): 1.26 (6H, m, H3' and H5'), 1.36 (2H, m, H6'), 1.56 (2H, m, H2'), 3.35 (2H, t, $J_{6',7'}$ 6.6, H7'), 3.60 (2H, t, $J_{1',2'}$ 7.1, H1'), 3.95 (1H, s, OH), 5.50 (1H, d, $J_{5,6}$ 7.6, H5), 7,60 (1H, d, H6), 11,10 (1H, s, NH). MS: *m/z* 227 [M^+]. Calc. for $C_{11}H_{18}N_2O_3$: 226.

N^1-(ω-Hydroxy-*n*-nonyl)uracil (Ii). R_f 0.52 (3). ^{1}H NMR (DMSO-d_6): 1.25 (10H, m, H3' and H7'), 1.39 (2H, m, H8'), 1,55 (2H, m, H2'), 3.35 (2H, t, $J_{8',9'}$ 6.6, H9'), 3.62 (2H, t, $J_{1',2'}$ 7.1, H1'), 3.95 (1H, s, OH), 5.50 (1H, d, $J_{5,6}$ 7.63, H5), 7.62 (1H, d, H6), 11.05 (1H, s, NH). MS: *m/z* 254 [M^+]. Calc. for $C_{13}H_{22}N_2O_3$: 254.

N^1-(ω-Hydroxy-*n*-amyl)thymine (IIc). R_f 0.25 (3). ^{1}H NMR (DMSO-d_6): 1.25 (2H, m, H3'), 1.35 (2H, m, H4'), 1.65 (2H, m, H3'), 1.8 (3H, s, 5-Me), 3.35 (2H, t, $J_{4',5'}$ 6.6, H5'), 3,62 (2H, t, $J_{1',2'}$ 7.1, H1'), 3.95 (1H, s, OH), 7.40 (1H, s, H6), 11.05 (1H, s, NH). MS: *m/z* 213 [M^+]. Calc. for $C_{10}H_{16}N_2O_3$: 212.

N^1-(ω-Hydroxy-*n*-heptyl)thymine (IIf). R_f 0.53 (3). ^{1}H NMR (DMSO-d_6): 1.30 (6H, m, H3' and H5'), 1.37 (2H, m, H6'), 1.62 (2H, m, H2'), 1.8 (3H, s, 5-Me), 3.35 (2H, t, $J_{6',7'}$ 6.6, H7'), 3.60 (2H, t, $J_{1',2'}$ 7.1, H1'), 3.95 (1H, s, OH), 7.40 (1H, s, H6), 11.0 (1H, s, NH). MS: *m/z* 241 [M^+]. Calc. for $C_{12}H_{20}N_2O_3$: 240.

N^1-(ω-Hydroxy-*n*-nonyl)thymine (IIi). R_f 0.63 (3). ^{1}H NMR (DMSO-d_6): 1.30 (10H, m, H3' and H7'), 1.40 (2H, m, H8'), 1.60 (2H, m, H2'), 1.8 (3H, s, 5-Me), 3.35 (2H, t, $J_{9',8'}$ 6.6, H9'), 3.62 (2H, t, $J_{1',2'}$ 7.1, H1'), 3.95 (1H, s, OH), 7.35 (1H, s, H6), 10.95 (1H, s, NH). MS: *m/z* 269 [M^+]. Calc. for $C_{14}H_{24}N_2O_3$: 268.

N^1-(ω-Hydroxy-*n*-amyl)cytosine (IIIc). R_f 0.15 (5). ^{1}H NMR (DMSO-d_6): 1.22 (2H, m, H3'), 1.35 (2H, m, H4'), 1.60 (2H, m, H2'), 3.42 (2H, t, $J_{4',5'}$ 6.6, H5'), 3.65 (2H, t, $J_{1',2'}$ 7.1, H1'), 4.0 (1H, s, OH), 5.60 (1H, d, $J_{5,6}$ 7.1, H5), 6.75 (2H, s, NH_2), 7.40 (1H, d, H6). MS: *m/z* 198 [M^+]. Calc. for $C_9H_{15}N_3O_2$: 197.

N^1-(ω-Hydroxy-*n*-heptyl)cytosine (IIIf). R_f 0.32 (4). ^{1}H NMR (DMSO-d_6): 1.30 (6H, m, H3' and H5'), 1.40 (2H, m, H6'), 1.60 (2H, m, H2'), 3.40 (2H, t, $J_{6',7'}$ 6.6, H7'), 3.65 (2H, t, $J_{1',2'}$ 7.1, H1'), 4.0 (1H, s, OH), 5.62 (1H, d, $J_{5,6}$ 7.1, H5), 6.75 (2H, s, NH_2), 7.40 (1H, d, H6). MS: *m/z* 226 [M^+]. Calc. for $C_{11}H_{19}N_3O_2$: 225.

N^1-(ω-Hydroxy-*n*-nonyl)cytosine (IIIi). R_f 0.46 (4). ^{1}H NMR (DMSO-d_6): 1.25 (10H, m, H3' and H7'), 1.40 (2H, m, H8'),1.53 (2H, m, H2'), 3.36 (2H, t, $J_{9',8'}$ 6.6, H9'), 3.60 (2H, t, $J_{1',2'}$ 7.1, H-1'), 4.2 (1H, s, OH), 5.60 (1H, d, $J_{5,6}$ 7.1, H5), 6.85 (2H, s, NH_2), 7.50 (1H, d, H6). MS: *m/z* 254 [M^+]. Calc. for $C_{13}H_{23}N_3O_2$: 253.

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