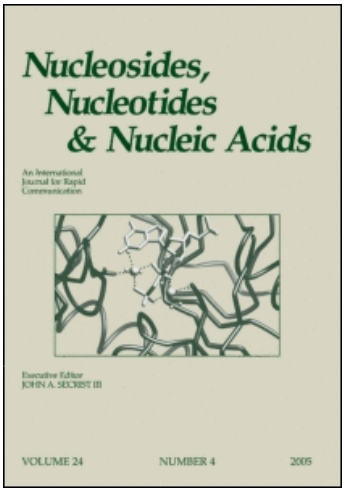

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SYNTHESIS OF 1-(2-HYDROXY-3-METHOXYPROPYL)URACILS AND THEIR ACTIVITY AGAINST L1210 AND MACROPHAGE RAW 264.7 CELLS

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ABSTRACT

The title compounds were obtained from appropriate 5-substituted uracil derivatives and 1,2-oxy-3-methoxypropane in the presence of sodium hydride. Under similar conditions 5-iodouracil gave 2-methoxymethyl-2,3-dihydro-oxazolo[3,2-c]pyrimidine-5,7-dione as a result of intramolecular *cine* type nucleophilic substitution. Cytotoxicity of 1-(2-hydroxy-3-methoxypropyl)-5-substituted uracil derivatives against L1210 and macrophage RAW 264.7 cells in vitro was examined.

INTRODUCTION

N-Alkylated heterocycles containing one or more hydroxy groups in alkyl backbone play an important role in antiviral and antitumor therapy.[1–3] Acyclovir and *S*-DHPA are well-known compounds of this class.[2] In our

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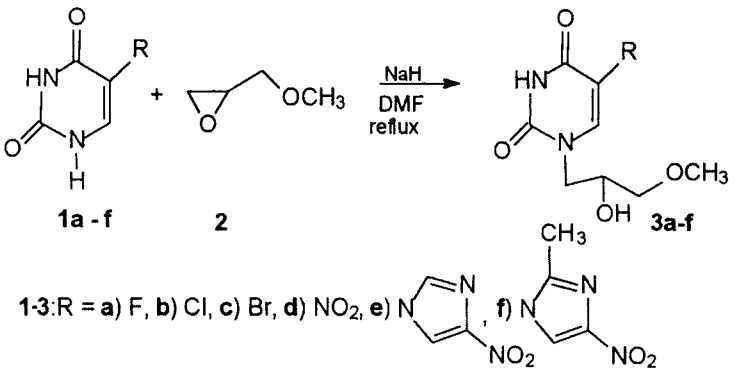
research we concentrated on uracil derivatives, which are less common. 5-Fluorouracil has showed high anticancer activity and is applied in acute leukemia diseases.[1] 1-(2-Hydroxyethoxymethyl)-6-thiophenyl)thymine (HEPT) and its 6-thio-(3,5-dimethylphenyl) analogue inhibit HIV-1 replication in MT-4 cells.[1] Recently we have reported method of *N*-alkylation of uracils using 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) and methoxymethyloxirane as the alkylating agent.[4] Moderate yields of the products and formation of dialkyl derivatives as by-products, prompted us to search for more effective methods of uracil *N*-alkylation. Uracil and its 5-halogen-substituted derivatives as *NH*-acids are characterized by rather low acidity (pK_a (in H_2O) = 9.4 for uracil and 7.9 for 5-fluorouracil).[5]

CHEMISTRY

When triethylamine, potassium carbonate or sodium hydride were used as deprotonating agents in acetonitrile solution, the reaction between uracils and methoxymethyloxirane proceed very slowly and yields of the products were low. Similar results were obtained even in the presence of 50% excess (in respect to uracil derivative) of potassium carbonate in dimethylformamide solution. Much better results were obtained when reaction was performed in the presence of catalytic amounts of sodium hydride in boiling anhydrous dimethylformamide (DMF). To avoid formation of undesired dialkylated products, methoxymethyloxirane 0.93 mmole was used on 1 mmole of uracil derivative (Sch. 1).

When sodium hydride was added to a suspension of 5-fluorouracil **1a** in anhydrous DMF, the reaction mixture became yellowish. After equilibration for 1 hour at 25°C, methoxymethyloxirane **2** was added dropwise and the resulting mixture was refluxed for several hours. When TLC analysis indicated complete disappearance of **2**, the reaction solution was cooled down to



Scheme 1.

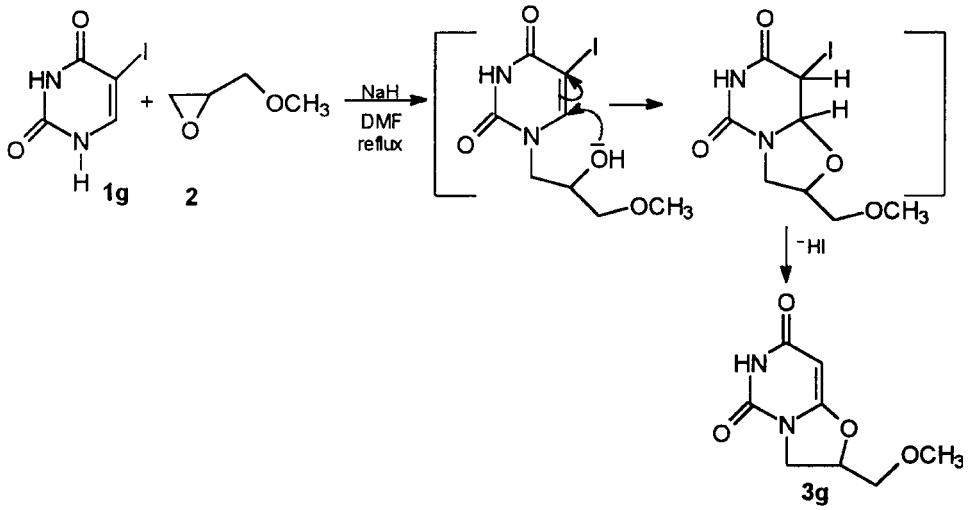
Table 1. *N*-Alkylated 5-Substituted Uracils

Compd.	Yield % (A-C)*	M.p.°C	Formula	Elemental Analysis					
				Calculated			Found		
				C	H	N	C	H	N
3a	72 (A)	106–107	$C_8H_{11}FN_2O_4$ (218.18)	44.04	5.08	12.84	43.86	4.99	12.60
3b	64 (A)	118–119	$C_8H_{11}ClN_2O_4$ (234.64)	40.95	4.73	11.94	40.65	4.71	11.88
3c	52 (B)	154–155	$C_8H_{11}BrN_2O_4$ (279.08)	34.43	3.97	10.04	34.63	4.20	10.26
3d	89 (A)	162–163	$C_8H_{11}N_3O_6$ (245.19)	39.19	4.52	17.14	38.99	4.79	16.75
3e	37 (A)	232–233	$C_{11}H_{13}N_5O_6$ (311.25)	42.45	4.21	22.50	42.21	4.05	22.26
3f	70 (C)	131–132	$C_{12}H_{15}N_5O_6$ (325.28)	44.31	4.65	21.53	44.09	4.43	21.37

*(A-C) Solvent systems for column chromatography.

room temperature, carefully neutralized by addition of acetic acid and evaporated to dryness under reduced pressure. *N*-Alkylation product **3a** was isolated in 72% yield after column chromatography. Similar conditions were applied for others uracil derivatives (Table 1). The product structures were established based on ^{1}H and ^{13}C NMR spectra as well as results of elemental analyses. Ambident uracil anions can be alkylated on both nitrogen and oxygen atoms. Formation of *O*-alkylated products was reported early.[6,7] Regiochemistry of compounds **3a**–**3f** was ascertained from their NMR spectra. Thus, in the ^{13}C NMR spectra, signals for C-4 and C-2 appear in the region characteristic for *N*-1 alkylated pyrimidines (160 and 150 ppm respectively); therefore their presence excludes formation of *O*-alkylated products under the conditions employed by us.

5-Nitrouracil is the strongest acid ($pK_a = 5.50$) in the series of investigated uracils. Its addition to the oxirane can occur without deprotonating agent. Strong effect of 5-nitro group activates ^{1}*N*-position only, what can be easily deduced from resonance structures of this derivative. In light of that, the high yield of **3d** observed in a reaction without a catalyst is not surprising. Alkylation of 5-iodouracil (**1g**) using **2** in the presence of sodium hydride gave different result. After standard work-up the product was isolated in 40% yield, but it exhibited spectroscopic properties different from other synthesized uracil derivatives. Signal for H-6, usually appearing in ^{1}H NMR spectra at 8–9 ppm, in this case was found at 4.94 ppm thus shifted to higher field by about 3 ppm Also a signal of C-5 in ^{13}C NMR spectra was shifted to 80.71 ppm and that of C-1′ to 43.48 ppm. In contrast, a signal of C-2′ was shifted to lower field (74.77 ppm). Based on these spectral data and elemental analysis, we suggest the structure **3g** for the product from 5-iodouracil. Formation of **3g** can be explained in terms of *cine* type intramolecular nucleophilic



Scheme 2.

substitution of iodine atom following preliminary common *N*-alkylation as it is presented in Sch. 2.

BIOLOGY

Compounds **3a**–3**f** were subjected to cells-growth/cells-kill test based on mitochondrial dehydrogenase activity assay (MTT method)[10–13] in L1210 and macrophage RAW 264.7 cells. None of the compounds has showed significant activity at cell density of 20,000 cells/mL. The growth of L 1210 leukemia cells (density 50,000 cells/mL) was inhibited to 80% by **3f** at 10 μM. For comparison, 5-fluorouracil reduced the growth by 51% at the same concentration. Other compounds were inactive to leukemia cells on concentration up to 100 μM. Macrophage RAW 264.7 is more sensitive to the synthesized uracil derivatives. At cells density 20,000/mL **3b** and **3d** (concentration 10 μM) exhibited inhibition to 80% and 88% respectively. 5-Fluorouracil showed 27% inhibition at the same concentration. At the highest cell density (50,000 cells/mL) compounds **3b** and **3d** reduced cell growth to 87% at 10 μM concentration. **3b** and **3d** reduced growth to 86% and 5-fluorouracil to 9% at conc. 50 μM.

EXPERIMENTAL

Chemistry: NMR spectra were recorded at 300 MHz for ^{1}H NMR and 75.5 MHz for ^{13}C NMR on a Varian spectrometer in DMSO-d_6 solution with

TMS as an internal standard. Chemical shifts are in ppm. Column chromatography was performed on silica gel (230–400 mesh, Merck). TLC plates (Merck, silica gel $60F_{254}$) were visualized in UV light and in iodine chamber. Solution of MeOH in $CHCl_3$ was used as eluent; system A: 10:90; B: 1:99; C: 3:97. Uracils **1a–d** and **1g** were purchased from Aldrich. Compounds **1e–f**[8] and **2**[9] was obtained and had properties according to reported data.

Alkylation of Uracils 3a-g (A General Procedure)

To a suspension of **1** (3 mmole) in anhydrous DMF (12 mL sodium hydride (0.02 g 0.7 mmole) was added at 25°C while stirring. The reaction mixture was equilibrated for 1 h in anhydrous conditions, and then heated to boiling, followed by dropwise addition of **2** (0.25 g 2.8 mmole). The resulting mixture was heated under reflux and monitored by TLC. After complete disappearance of spot of **2** on TLC (iodine chamber), the resulting solution was cooled down to room temperature, carefully neutralized with glacial acetic acid and evaporated to dryness, followed by co-evaporation with water (2×10 mL). The residual oil was purified by column chromatography using system A, B or C. The products were recrystallized from methanol.

1-(2-Hydroxy-3-methoxypropyl)-5-fluorouracil (3a). ^{1}H NMR: $\delta = 11.76$ (s, 1H, NH), 7.92 (d, 1H, $J = 7.2$ Hz, H-6), 5.22 (d, 1H, $J = 5.4$ Hz, OH), 3.83 (dd, 2H, $J = 5.1$ Hz, 11.1 Hz, H-1′), 3.45–3.49 (m, 1H, H-2′), 3.40–3.31 (m, 2H, H-3′), 3.27 (s, 3H, CH_3O).

1-(2-Hydroxy-3-methoxypropyl)-5-chlorouracil (3b). ^{1}H NMR: $\delta = 11.79$ (s, 1H, NH), 8.03 (s, 1H, H-6), 5.23 (d, 1H, $J = 5.7$ Hz, OH), 3.86 (m, 2H, H-1′), 3.48 (m, 1H, H-2′), 3.31 (m, 2H, H-3′), 3.26 (s, 3H, CH_3O). ^{13}C NMR: $\delta = 159.65$ (C-4), 150.31 (C-2), 146.48 (C-6), 93.62 (C-5), 74.33 (C-3′), 66.45 (C-2′), 58.35 (CH_3O), 51.39 (C-3′).

1-(2-Hydroxy-3-methoxypropyl)-5-bromouracil (3c). ^{1}H NMR: $\delta = 11.79$ (s, 1H, NH), 8.03 (s, 1H, H-6), 5.23 (d, 1H, J = 5.1 Hz, OH), 3.90 (m, 2H, H-1′), 3.45 (m, 1H, H-2′), 3.31 (m, 2H, H-3′), 3.26 (s, 3H, CH_3O). ^{13}C NMR: $\delta = 159.40$ (C-4), 150.31 (C-2), 144.11 (C-6), 105.18 (C-5), 74.33 (C-3′), 66.45 (C-2′), 58.34 (CH_3O), 51.36 (C-1′).

1-(2-Hydroxy-3-methoxypropyl)-5-nitrouracil (3d). ^{1}H NMR: $\delta = 9.06$ (s, 1H, H-6), 5.33 (d, 1H, $J = 5.6$ Hz, OH), 4.06 (dd, 1H, $J = 3.2$ Hz, 13.6 Hz, H-1′$_a$), 3.85 (m, 1H, H-2′), 3.68 (dd, 1H, $J = 8.7$ Hz, 13.6 Hz, H-1′$_b$), 3.33 (m, 2H, H-3′), 3.27 (s, 3H, CH_3O). ^{13}C NMR: $\delta = 154.96$ (C-4), 151.45 (C-2),

149.28 (C-6), 124.29 (C-5), 74.06 (C-3′), 66.12 (C-2′), 58.38 (CH_3O), 52.22 (C-1′).

1-(2-Hydroxy-3-methoxypropyl)-5-(4-nitromidazol-1-yl)uracil (3e). ^{1}H NMR: δ = 12.01 (s, 1H, NH), 8.47 (d, 1H, $J = 1.5$ Hz, H-5im), 8.31 (s, 1H, H-6), 7.94 (d, 1H, $J = 1.5$ Hz, H-2im), 5.30 (m, 1H, OH), 3.90 (m, 2H, H-1′), 3.58 (m, 1H, H-2′), 3.35 (m, 2H, H-3′), 3.26 (s, 3H, CH_3O). ^{13}C NMR: δ = 159.44 (C-4), 149.91 (C-2), 146.92 (C-4im), 143.12 (C-6), 135.76 (C-2im), 121.98 (C-5im), 118.91 (C-5), 74.30 (C-3′), 66.58 (C-2′), 58.58 (CH_3O), 58.41 (C-1′).

1-(2-Hydroxy-3-methoxypropyl)-5-(2-methyl-4-nitromidazol-1-yl)uracil (3f). ^{1}H NMR: δ = 11.98 (s, 1H, NH), 8.28 (s, 1H, H-6), 8.24 (s, 1H, H-5im), 5.25 (m, 1H, OH), 3.89 (m, 2H, H-1′), 3.48 (m, 1H, H-2′), 3.34 (m, 2H, H-3′), 3.28 (s, 3H, CH_3O), 2.27 (s, 3H, CH_3). ^{13}C NMR: δ = 159.76 (C-4), 150.16 (C-2), 146.44 (C-4im), 145.97 (C-6), 145.65 (C-2im), 123.62 (C-5im), 109.69 (C-5), 74.20 (C-3′), 66.33 (C-2′), 58.39 (CH_3O), 51.72 (C-1′), 12.47 (CH_3).

2-Methoxymethyl-2,3-dihydro-oxazolo[3,2-c]pyrimidine-5,7-dione (3g). Yield 0.25 g (40%) System (A). M.p. 175 – 176°C. ^{1}H NMR: δ = 10.89 (s, 1H, NH), 5.16 (m, 1H, H-2′), 4.94 (s, 1H, H-5), 4.05 (t, 1H, $J = 9.4$ Hz, H-3′$_a$), 3.74 (dd, 1H, $J = 6.6$ Hz, 9.4 Hz, H-3′$_b$), 3.66 (dd, 1H, $J = 2.6$ Hz, 11.5 Hz, H-1′$_a$), 3.58 (dd, 1H, $J = 4.6$ Hz, 11.5 Hz, H-1′$_b$), 3.36 (s, 3H, CH_3O). ^{13}C NMR: δ = 164.74 (C-4), 162.44 (C-2), 147.84 (C-6), 80.71 (C-5), 74.77 (C-2′), 71.62 (C-3′), 58.57 (CH_3O), 43.48 (C-1′). Anal. Calcd. for $C_8H_{10}N_2O_4 \times 0.5\ H_2O$ (207.19): %C 46.39; %H 5.35; %N 13.52. Found: %C 46.11; %H 5.35; %N 13.24.

Biology: The cells growth/cells kill measuring based on mitochondrial dehydrogenase activity assay (MTT).[10–13]

Material and reagents: Cells lines mouse lymphocytic leukemia L1210 and mouse monocyte-macrophage RAW 264.7 were used. RPMI 1640 medium with glutamine, fetal bovine serum, penicillin, streptomycin was purchased from Gibco BRL Life Technology, USA. 3-(4,4-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dodecylsulfate sodium salt (SDS) and dimethylformamide were purchased from Sigma Aldrich Company, Germany. Colony was incubated in ASSAB incubator KEBO Lab, Sweden.

The L1210 and RAW 264.7 cells were cultured in medium containing RPMI 1640 with glutamine, 10% fetal bovine serum, penicillin (1000 U/mL) and streptomycin (100 μg/mL) in atmosphere of air with 5% CO_2 in ASSAB incubator at 37°C. After 3 days the cell density was estimated in Burker chamber. To assays cell densities 20,000 cells/mL and 50,000 cells/mL were used. The assay was performed in 96-well micro plate. Uracil derivatives at indicated concentration were added to cells suspension. The assay mixture

was incubated for 6 days and 20 μL MTT (1.1 mmole) was added. After 3 h 100 μL of SDS was added and incubation was continued for 24 h. Absorbance of formazane (reduced form of MTT) was measured at 550 nm and percentage of survival cells was calculated from equilibrium:

$$P = \frac{A_a - A_0}{A_c - A_0} \times 100\%$$

P = percentage of survived cells
A_a = absorbance of formazane in supernatant from assay suspension
A_0 = absorbance of background
A_c = absorbance of blank supernatant.

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