ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2013, Vol. 39, No. 3, pp. 299–305. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © A.V. Semakov, A.A. Blinkov, G.P. Gaenko, A.G. Vostrova, J.G. Molotkovsky, 2013, published in Bioorganicheskaya Khimiya, 2013, Vol. 39, No. 3, pp. 338–345.

Synthesis and Properties of Lipophilic Derivatives of 5-Fluorouracil

A. V. Semakov, A. A. Blinkov, G. P. Gaenko, A. G. Vostrova, and J. G. Molotkovsky[1]

Shemyakin—Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, 117997 Russia

Received December 12, 2012; in final form, December 24, 2012

Abstract—A series of N^1-acyl derivatives of 5-fluorouracil (5-FU) bearing the residues of palmitic, *p*-myristoylaminobenzoic, *p*-oleoylaminobenzoic, and adamantane-1-carboxylic acids have been synthesized. The relative hydrolysis rates for the derivatives under physiological conditions (pH 7.2 and 37°C) have been determined, and it has been shown that the resistance of these compounds to hydrolysis increases as the steric accessibility of the amide group at residue N^1 of 5-FU decreases. The derivatives easily incorporate into the lipid bilayer; their liposomal preparations show a marked cytostatic activity on human breast lymphoma cells (LD50 ~1 μM) and are of interest as potential antitumor preparations. In addition, a fluorescent analogue of the above derivatives, 1-[8-(3-perylenyl)octanoyl]-5-fluorouracil, has been synthesized, which is intended for studying the behavior of 5-FU derivatives in cells and tissues by instrumental methods.

Keywords: 5-fluorouracil, N-acyl derivatives, cytostatic activity, lipophilic predrugs, liposomes, fluorescent analogue

DOI: 10.1134/S1068162013030138

INTRODUCTION

5-Fluorouracil is an antimetabolite antitumor agent, which was first obtained in 1957 [1] and is also currently used to advantage in clinical practice owing to a wide spectrum of activity against various, primarily solid, tumors and the capacity to permeate through the hematoencephalic barrier [2]. An additional important point is the relatively low cost of 5-FU. However, the substantial systemic toxicity of 5-FU and the relatively rapid development of resistance of tumors to it [3, 4], problems common to some degree to all ATAs, necessitate a search for more perfect ways of its application, primarily those that enhance the selectivity of the incorporation of the drug into malignant cells and tissues compared with normal, thereby increasing its therapeutic index.

One of these ways is the design of derivatives that are 5-FU precursors, including those that increase the lipophilicity of the 5-FU molecule. Lipophilicity facilitates the incorporation of drugs into tumor cells (where 5-FU is released), which differ from normal by a higher fluidity and disorder of membranes [5]. Among the first derivatives was 1-furyl derivative, which showed the efficacy of this approach [6]. At present, hundreds of 5-FU derivatives of different kinds are known (see reviews [7–9]). Clinical trials of some of these derivatives showed that their therapeutic index is much higher compared with the original 5-FU; nevertheless, this effect still remains limited.

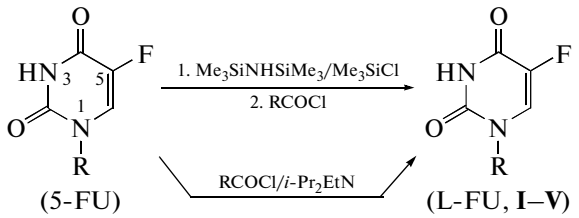
The possibility of incorporating 5-FU into the aqueous interior of liposomes was studied; however, the small water-soluble molecule was found to pass through liposomal bilayers with a great velocity [10], which cast some doubt upon the possibility of using these preparations as ATAs. It was proposed to retain 5-FU in the aqueous space of liposomes as a part of the copper complex; this preparation showed a higher effectiveness in (in vivo) experiments compared with native 5-FU [11]. A common disadvantage of these approaches is the complicated methodology of incorporating the water-soluble medicinal substances into the aqueous interior of liposomes and the incompleteness of incorporation.

L-FUs incorporated into nanosized carriers, primarily liposomes, to be more precise, liposomal bilayers, have great advantages in this respect (see reviews [12, 13]). These are the ease and completeness of incorporation of L-FU into liposomes, low losses of the drug in the blood flow, and the possibility of its direct transmembrane transfer to tumor cells; the latter can change the intracellular traffic of the preparation.

The efficacy of a drug in the liposomal form compared with its native form increases also as a result of the so-called passive transport due to a higher permeability of defective capillary walls in tumors for liposomes [14]. A further increase in the selectivity of drug delivery to tumors is afforded by the incorporation of

Abbreviations: ATA, antitumor agent; 5-FU and L-FU, 5-fluorouracil and its lipophilic derivative; DIPEA, diisopropylethylamine; ePC, egg phosphatidylcholine; HBL, human breast lymphoma; PAB, *p*-aminobenzoyl.

[1] Corresponding author: phone/fax: 8 (495) 330-6601; e-mail: igmol@ibch.ru.



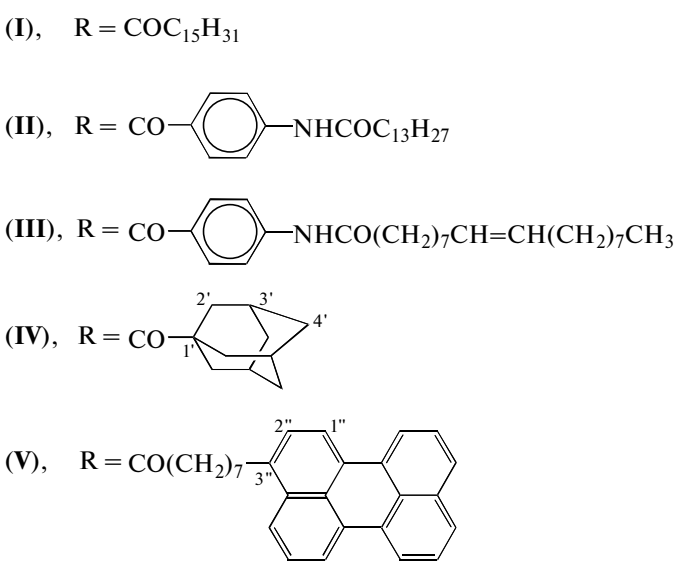


Fig. 1. Scheme of synthesis of lipophilic 5-FU derivatives.

ATAs into liposomes loaded with ligands affine for the surface components of tumor cell membranes, e. g., with antibodies to specific proteins (see reviews [15, 16]).

In the choice of L-FU intended for the incorporation into liposomes, its *N*-acyl derivatives, primarily fatty acid derivatives, merit attention. In physicochemical parameters, their chains best fit nonpolar residues of liposomal bilayers, promoting in such a way the incorporation of L-FU into them. The methods of *N*-acylation of 5-FU [17, 18] and related heterocycles [19] have been well elaborated; in this case, the acylation of 5-FU occurs as a rule at the N^1-position of the heterocycle. However, the study of the properties of some acylated heterocycles showed that aliphatic N^1-acyl derivatives of 5-FU have a low stability; in aqueous systems and under weakly acid conditions, they are easily hydrolyzed [20, 21]. Thus, the lifetime ($t_{1/2}$) of N^1-dodecanoyl-5-FU is ~5 min at pH 7.4 and 37°C [20]. As far as one can judge, 1-stearoyl-5-FU has approximately the same stability; at pH > 8, the stability of this compound is substantially higher [21]. On the other hand, as stated by Sun et al. [22], the 5-FU *o*-toluyl derivative incorporated into liposomes is quite stable.

Because we had a wide experience in synthesizing and applying lipophilic derivatives of melphalan, methotrexate, and other ATAs and their liposomal preparations, which showed a high efficacy [23], we set ourselves the task of obtaining similar derivatives of 5-FU, which, on the one hand, should be capable of transferring 5-FU in the composition of liposomes into tumors and releasing it there (not earlier) and, on the other hand, be quite stable in operation and in storage. In this case, we had to bear in mind that chemically firm *N*-derivatives of 5-FU possess no antitumor activity (see, e. g., [24]); therefore, L-FU to be designed had to be sufficiently (but not extremely) labile to release the active principle.

N-Acyl L-FUs were chosen for this purpose because of the ease of their synthesis and presumed facility of incorporation into liposomes; the major problem was to choose acids whose derivatives would satisfy the above criteria and would not form additional toxic compounds upon intracellular release of 5-FU. In the present paper, the synthesis of a series of novel lipophilic *N*-acyl L-FUs (**I**)–(**IV**), their stability in aqueous medium, and their cytotoxic activity in vitro are described. In addition, we describe here the synthesis of the fluorescently labeled [8-(3-perylenyl)octanoyl analogue (**V**); we synthesized this derivative considering the necessity of further study of the intracellular traffic and metabolism of lipophilic L-FU derivatives by instrumental methods.

1-Palmitoyl-5-FU (**I**) was chosen as a reference standard. This substance is similar to 1-stearoyl-5-FU whose properties are described in detail in [21]. When choosing other derivatives for the study, we were guided by few available data on their stability in aqueous medium. Although the authors of [22] present no direct evidence on the stability of the *o*-toluyl 5-FU derivative (to which the 3-acyl structure is assigned) to hydrolysis, the indirect evidence suggests that this compound is more stable than the stearoyl analogue [21]. We also proposed that this greater stability is due to a lesser steric accessibility of the amide bond in the toluyl derivative compared with the stearoyl derivative. It was logical to try *N*-aroyl L-FUs; however, aroyl residues had to be modified by increasing their lipophilicity, since the data reported in [22] indicate that the *o*-toluyl 5-FU derivative is poorly retained in the liposomal bilayer. Therefore, two other L-FUs, (**II**) and (**III**), were obtained by incorporating into 5-FU the PAB residue additionally lipophilized by the *N*-myristoyl or the *N*-oleoyl group, respectively. Finally, N^1-(adamantane-1-carbonyl)-5-FU (**IV**) as a 5-FU derivative with the most restricted access to the amide group was synthesized (Fig. 1).

To obtain a fluorescent probe simulating behavior of other L-FUs, 5-FU was acylated by 8-(3-perylenyl)octanoic acid. This acid was chosen for the following reasons. The 3-perylenyl fluorophore is chemically rather stable and highly sensitive; its spectral charac-

teristics (excitation at 400–450 nm, emission at 450–500 nm) are convenient for confocal microscopy and flow-cytofluorimetry studies. The fluorophore-carrying acid was chosen so that the total length of the fluorescently labeled acyl was close to the length of palmitoyl, one of the most widely occurring fatty acid residues of cell membranes.

8-(3-Perylenyl)octanoic acid was synthesized according to the scheme described for the homologous 8-(3-perylenyl)butanoic acid [25]: perylene was acylated with an excess of suberic acid dichloride according to Friedel–Crafts to give 7-(3-perylenoyl)heptanoic acid, which was then reduced according to Wolff–Kishner/Huang–Minlon to give 8-(3-perylenyl)octanoic acid. This acid was obtained in a similar way by Hermetter and Oskolkova; the synthesis is briefly described in a patent [26].

The palmitoyl derivative (**I**) was synthesized by two alternative methods: the acylation of 5-FU with acid chloride in the presence of a base [17, 18] or through a trimethylsilyl 5-FU derivative [19, 21]. The two methods give close results; the fluorescently labeled analogue (**V**) was obtained using the second method, and the other derivatives (**II**)–(**IV**) were synthesized by the first method, which is more convenient in experiment. The structures of (**I**)–(**V**) were confirmed by the data of ^{1}H NMR, mass, UV, and fluorescence spectra (see the Experimental section).

All L-FUs (**I**)–(**V**) possess no marked solubility in water but incorporate easily into ePC liposomes in the molar ratio 1 : 9 (see the Experimental section); the gel filtration of liposomal preparations through Sephadex LH-20 showed an almost 100% incorporation (data not shown). It should be noted that none of the fractions was found to contain substantial amounts of 5-FU upon gel filtration (as determined by TLC), although liposomes were prepared by dispersion in aqueous buffer with pH 7.2; presumably, the incorporation into the liposome bilayer strongly shields the amide bond of the acyl residue, thereby preventing hydrolysis.

As was mentioned above, the stability of the preparations in aqueous media is one of their most important characteristics, although it remains still unclear how great must be their optimal stability to achieve the maximum antitumor activity and therapeutic index and in what media. The significance of the minimum stability of L-FUs for convenient manipulation during isolation, purification, preparation of compounds, etc. is evident from the fact that the most unstable of our L-FUs, palmitoyl-5-FU (**I**), is completely decomposed on purification by chromatography on silica gel (the stability is higher in reverse-phase chromatography in aprotic solvents). The most stable derivative adamantoyl-5-FU (**IV**) withstands flash chromatography on silica gel; L-FUs of intermediate stability (**II**) and (**III**) endure rapid filtration through silica gel and reverse-phase chromatography (see the Experimental section). TLC of L-FUs (**I**)–(**III**) on silica gel was performed only in nonaqueous systems.

First-order constants of the rate of hydrolysis of lipophilic 5-FU derivatives in acetonitrile–20 mM Tris-HCl, pH 7.2, at 37°C

Derivative	k, s^{-1}
(**I**)	0.31
(**II**)	0.12
(**III**)	0.13
(**IV**)	0.055

At this step of the investigation, we restricted ourselves to a comparison of the hydrolysis rates of (**I**)–(**IV**) in a system containing Tris-HCl buffer, pH 7.2, at 37°C, i. e., under conditions close to those under which ATAs are in clinical use. Owing to a moderate solubility of L-FUs in aqueous solutions, the hydrolysis was carried out in a mixture acetonitrile–buffer 4 : 1; the course of hydrolysis was controlled densitometrically after the separation of the starting L-FU and 5-FU by TLC (see the Experimental section).

The hydrolysis of all L-FUs tested is described by the first-order kinetics, which is indicated by the exponential time dependence of substrate decrease (data not shown); the rate constants are given in the table. The mechanism of hydrolytic decomposition of *N*-acyl (aroyl) 5-FU derivatives has not been studied; the information on this subject is scanty, and it is only known that the stability of 1-stearoyl-5-FU is minimal at pH ~ 4 [21]. However, it is clear that the resistance to hydrolytic cleavage of the above-described L-FUs (**I**)–(**IV**) increases in the following order: palmitoyl-5-FU < *N*-myristoyl-PAB-5-FU = *N*-oleoyl-PAB-5-FU < adamantoyl-5-FU, which fully agrees with the increase in the steric restriction of access to the amide bond of the acyl group. At the same time, the close cytotoxic activity of L-FUs (**I**)–(**IV**) (Fig. 2) strongly suggests that, in tumor cells, they all undergo hydrolysis with the release of the active starting compound, 5-FU.

The cytostatic activity of L-FU-loaded liposomes in HBL cells was estimated by the standard trypan blue test. The IC_{50} value was determined by extrapolating the data on cell survival depending on the concentration of liposome preparations added to the medium (Fig. 2). The IC_{50} values for all preparations tested were close to ~1 μM and exceeded the IC_{50} value for L-FU seven to ten times. The lower cytostatic activity of the liposomal forms of the ATA is a natural phenomenon and was described many times (see, e. g., [27]). It should be noted that, in in vivo experiments, the reverse situation can occur (and is often observed), namely, a higher antitumor activity of liposome preparations carrying lipophilic prodrugs of ATAs as compared with these agents per se (see, e. g., [28]).

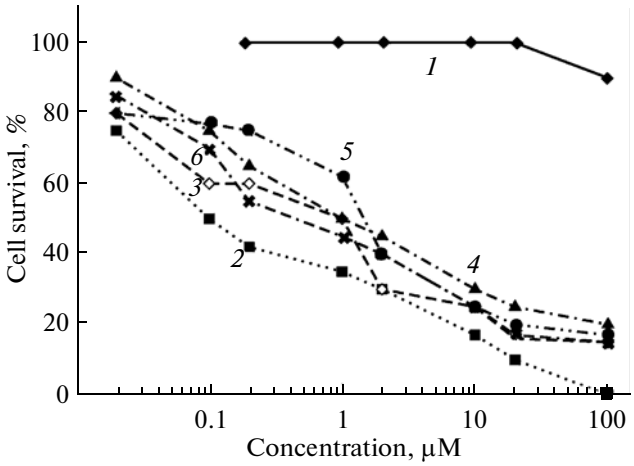


Fig. 2. Effect on the growth of HBL cells in vitro of (*1*) control (empty) ePC liposomes, (*2*) free 5-FU, (*3*) 1-palmitoyl-5-fluorouracil (I), (*4*) 1-(*p*-myristoyl-PAB-5-fluorouracil (II), (*5*) 1-(*p*-oleoyl-PAB-5-fluorouracil (**III**), and (*6*) 1-adamantoyl-5-fluorouracil (IV), incorporated into liposomes containing L-FU–ePC 1 : 9 (mol/mol); incubation for 48 h. On the abscissa, the concentration of 5-FU (for curves *2*–*6*); curve *1* was plotted for the ePC liposome concentrations corresponding to ePC concentrations for curves *2*–*6*.

In conclusion, the results of this study suggest that some *N*-acyl derivatives of 5-FU having a sufficient stability in aqueous media may be of substantial interest as promising ATAs, considering the wide spectrum of activity of the initial agent.

EXPERIMENTAL

The chemicals used: *p*-aminobenzoic acid, DIPEA, hexamethyldisilazane, trimethylchlorosilane, 5-fluorouracil (5-FU) (Sigma, United States); oleic and adamantane-1-carboxylic acids, perylene, pyridine (Fluka, Germany); silica gel 60 and DC-Alufolien Kieselgel-60 plates for TLC (Merck, Germany); and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) (Avanti, United States). Other reagents were from Reakhim (Russia). DIPEA was distilled over ninhydrin, then over powdery KOH; methanol was distilled over magnesium methylate, and chloroform and methylene chloride were distilled over phosphorus pentoxide. Other solvents were used after conventional purification. PBS, pH 7.2, was prepared from a concentrate (Sigma, United States).

Thionyl chloride was distilled over linseed oil, and palmitoyl chloride was obtained by the boiling of palmitic acid with an excess of thionyl chloride in benzene followed by distillation, bp 110°C/1 mmHg. In a similar way, myristoyl chloride was obtained from myristic acid, bp 136–141°C/2 mmHg, and adamantane-1-carbonyl chloride, from adamantane-1-carboxylic acid, bp ~130°C. Oleoyl chloride was obtained by the reaction of thionyl chloride with oleic acid in the presence of calcined Na_2CO_3 (3 h at 25°C); excessive $SOCl_2$ was removed by evaporation with dry toluene. 8-(3-Perylenyl)octanoic acid, mp 220–223°C (decomp.), was obtained as described in [25]; its ^{1}H NMR spectrum and behavior in TLC correspond to those described in [26].

Column chromatography was carried out using silica gel Kieselgel 60 and LiChrosorb RP-18 (for reverse-phase chromatography) (Merck). For TLC, plates with the fluorescent indicator Kieselgel 60 F_{254} and without indicator Kieselgel 60 (Merck), as well as plates with reverse phase RP18 F_{254s} were used. Detection in TLC was with phosphomolybdic acid (A), by UV irradiation (B) (5-FU and its derivatives quench the fluorescence of the indicator), and with an acidified $KMnO_4$ solution (C) (5-FU and L-FU appear as yellow spots against the rose-colored background). Evaporation in vacuo was carried out at 8–15 mmHg and a temperature of <40°C.

Mass spectra were recorded on a MALDI TOF mass spectrometer UltraFlex (Bruker Daltonics, Germany): N_2 laser, 337 nm, matrix 2.5-dihydroxybenzoic acid, and on an ESI-TOF mass spectrometer MX-5311 (IAP RAS, St. Petersburg) with nanospray ionization and registration of positive ions; ^{1}H NMR spectra (δ, ppm, *J*, Hz) were recorded on a Bruker WM-700 device (United States). Electronic spectra of substances were measured on an SF-256UVI spectrometer (LOMO Fotonika, St. Petersburg) in ethanol; fluorescence spectra (in ethanol) were recorded on a Hitachi F-4000 spectrofluorimeter (Japan); the slit width at excitation and emission was 3 nm.

1-Palmitoyl-5-fluorouracil (I). *A*. Palmitoyl chloride (215 μL, 0.58 mmol) was added dropwise to a suspension of 5-FU (50 mg, 0.385 mmol) in a mixture of anhydrous dioxane (2 mL) and DIPEA (137 μL, 0.58 mmol) in an atmosphere of argon, and the mixture was stirred for 1 h at 40–50°C. The course of the reaction was controlled by TLC in benzene—ethyl acetate 7 : 1, on silica gel F_{254}; detection: A, B, and C. The mixture was evaporated in vacuo, and the residue was washed with heptane at 50°C, crystallized from benzene, and dried in vacuo. The yield of derivative (**I**), which was completely identical to the preparation obtained by the method *B*, was 120 mg (85.3%).

B. A mixture of 5-FU (200 mg, 1.54 mmol), hexamethyldisilazane (5.4 mL, 26 mmol), and trimethylchlorosilane (1.54 mL, 12 mmol) was stirred for 4 h at 110–120°C (bath), the excess of silylating reagents was removed in vacuo, acetonitrile (10 mL) and palmitoyl chloride (467 μL; 423 mg, 1.54 mmol) were added to the residue (colorless liquid), and the mixture was stirred for 4 h at 80°C. The course of the reaction was controlled by TLC in benzene–ethyl acetate 7 : 1, on a plate with the fluorescent indicator; detection: A, B, and C. The precipitate formed after cooling to room temperature was aspirated after 6 h, washed with cold benzene and crystallized again from benzene.

Derivative (**I**) was obtained as a colorless powder; yield 470 mg (83%); mp 99–101°C; R_f 0.6 in the above indicated system; UV, λ_{max}, nm (ε, M^{-1} cm^{-1}): 272 (9000); ESI-MS, *m/z*: 391.229 $[M + Na]^+$ $C_{20}H_{33}FN_2O_3Na$, *M* 391.238), 130.56 $[M - acyl + H]^+$; ^1H NMR: 0.89 (3 H, t, CH_3), 1.26 (24 H, br m $(CH_2)_{12}$), 1.72 (2 H, m, $C\underline{H}_2CH_2CO$), 3.12 (2 H, m, CH_2CO), 8.30 (1 H, d, J_{F-H} 5, CF=CH), 8.34 (1 H, br s, NH).

1-(*p*-Myristoylaminobenzoyl)-5-fluorouracil (II). *p*–Myristoylaminobenzoic acid was obtained by the acylation of *p*-aminobenzoic acid by an equivalent amount of myristoyl chloride in pyridine (3 h at 10°C and 1 h at 25°C). After conventional treatment and crystallization from the chloroform–acetone mixture, the yield was 80%. TLC: system benzene–ethyl acetate–AcOH 75 : 15 : 2; R_f 0.39 (detection: A); mp 224–226°C; UV, λ_{max}, nm (ε, M^{-1} cm^{-1}): 273 (3.4×10^4). Literature data [29]: mp 220–222°C.

DMF (5 μL) was added to a suspension of *p*-myristoylaminobenzoic acid (3 g) in a mixture of $SOCl_2$ (10 mL) and benzene (30 mL), and the mixture was stirred for 2 h and allowed to stand for 3 days. Then the solution was filtered and dried at 20–30 Pa; the resulting *p*-myristoyl-PAB chloride was used without additional purification. The completeness of the formation of acid chloride was controlled by converting a sample of the latter to methyl ether (TLC: R_f 0.56 in the above indicated system) after the treatment with the CH_3OH–pyridine mixture.

A solution of *p*-myristoyl-PAB chloride (155 mg, 0.42 mmol) in dry dioxane (2 mL) was added to a suspension of 5-FU (50 mg, 0.385 mmol) in dry dioxane (1 mL) and DIPEA (134 μL, 0.77 mmol) under stirring in an atmosphere of argon, and the mixture was stirred at 40°C until the complete dissolution of 5-FU (~1 h). The course of the reaction was controlled by TLC on RP-18 F_{254s} plates in the system MeOH–water 9 : 1; R_f of 5-FU was ~0.9, and of product (**II**), 0.34. The mixture was evaporated in vacuo, and the residue (amorphous mass) was crystallized at –15–20°C from CH_2Cl_2 and then from toluene at –15–20°C after which it was dried at 20–30 Pa. Yield of product (**II**) 51 mg (30%); mp 145–147°C; UV, λ_{max}, nm (ε, M^{-1} cm^{-1}): 278 (3.1×10^4), 311 (3.1×10^4); MALDI-MS, *m/z*: 482.253 $[M + Na]^+$; ^1H NMR (CD_3OD-$CDCl_3$): 0.85 (3 H, t, *J* 7.1, CH_3), 1.22 (18 H, m, $(CH_2)_9$), 1.34 (2 H, m, $C\mathbf{H}_2CH_3$), 1.57 (2 H, m, $C\mathbf{H}_2CH_2CO$), 2.36 (2 H, t, *J* 7.5, CH_2CO), 7.57–7.61 (4 H, m, Ar), 7.73 (1 H, d, J_{F-H} 7, CF=CH).

1-(*p*-Oleoylaminobenzoyl)-5-fluorouracil (III) was obtained by the above-described method from *p*-aminobenzoic acid (1.08 g, 7.86 mmol) and oleoyl chloride (2.37 mL, 8.14 mmol). The product was chromatographed on silica gel (45 g) by the elution with $CHCl_3$–ethyl acetate–AcOH 95 : 5 : 1. The yield of *p*-oleoylaminobenzoic acid was 2.43 g (76.9%); R_f 0.37 in the same system (detection: A); mp 201–204°C; UV, λ_{max}, nm (ε, M^{-1} cm^{-1}): 274 (3.3×10^4), ESI-MS, *m/z*: 401.305 $[M + H]^+$.

p-Oleoylaminobenzoic acid was converted to acid chloride (dense oil, quantitative yield) as described above, which was then used without additional purification.

A solution of *p*-myristoyl-PAB chloride (0.31 g, 0.75 mmol) in dry dioxane (2 mL) was added under stirring in an atmosphere of argon to a suspension of 5-FU (106 mg, 0.81 mmol) in dry dioxane (2 mL) and DIPEA (0.26 mL, 1.4 mmol), and the mixture was stirred at 60°C until the complete dissolution of 5-FU (~2 h). The course of the reaction was controlled as described for analogue (**II**). The mixture was allowed to stand for 18 h at 25°C and then evaporated twice with benzene. The solution of the residue in benzene (50 mL) was washed with water (2 × 30 mL, separation of phases by centrifugation), dried over Na_2SO_4, and evaporated. The filtration of the residue through silica gel (1 g) in benzene–acetate 2 : 1 and then through LiChrosorb RP-18 (1 g) in a mixture of acetonitrile with 5% chloroform yielded 345 mg (87%) of 1-*p*-oleoyl-PAB-5-FU (**III**) as a chromatographically individual colorless powder; R_f ~ 0.5 in benzene–ethyl acetate 2 : 1; mp 112–115°C; UV, λ_{max}, nm (ε, M^{-1} cm^{-1}): 284 (3.6×10^4), 311 (3.5×10^4); MALDI-MS, *m/z*: 536.301 $[M + Na]^+$; ^1H NMR (CD_3OD–$CDCl_3$): 0.88 (3 H, t, *J* 7.0, CH_3), 1.22 (18 H, m, $(CH_2)_9$), 1.33 (2 H, m, $C\mathbf{H}_2CH_3$), 1.58 (2 H, m, $C\mathbf{H}_2CH_2CO$), 2.38 (2 H, t, *J* 7.4, CH_2CO), 4.37 (1 H, q, *J* 5.5, =CH), 5.69 (q, *J* 5.5, =CH), 7.65–7.77 (4 H, m, Ar), 7.86 (1 H, d, J_{F-H} 7, CF=CH), 8.45 (1 H, s, NH).

1-Adamantoyl-5-fluorouracil (IV). 5-FU (100 mg, 0.77 mmol) was acylated with 1-adamantoyl chloride (139 mg, 0.7 mmol) in dioxane (1 mL) and DIPEA (0.3 mL) as described for analogue (**III**). The resulting mixture was purified by flash chromatography on silica gel (3 g), the column was washed with chloroform, and the product was eluted with chloroform–ethyl acetate–*t*-BuOH 70 : 10 : 1 to give a substance with R_f 0.26 (control by TLC in the same system). The yield of product (**IV**) 61 mg (30%); mp >166°C (decomposition); UV, λ_{max}, nm (ε, M^{-1} cm^{-1}): 274 (4400); MALDI-MS, *m/z*: 315.135 $[M + Na]^+$, 292.113 $[M]^+$, 273.052 $[M - F]^+$; ^1H NMR (CD_3OD–$CDCl_3$): 1.76 (6 H, m, 4'-H), 1.94 (3 H, m, 3'-H), 2.07 (6 H, m, 2'-H), 7.38 (1H, br s, CF=CH).

1-[8-(3-Perylenyl)octanoyl-5-fluorouracil (V). Freshly calcined sodium carbonate (16 mg, 0.15 mmol) was added to a solution of 8-(3-perylenyl)octanoic acid (30 mg, 76 μmol) in dry methylene chloride (1 mL), then thionyl chloride (45 μL, 0.61 mmol) was added under stirring in an atmosphere of argon, and the mixture was agitated for 4 h. The mixture was extracted with dry ether, and the extract was evaporated, concentrated by evaporation in dry benzene in vacuo, and dried for 1 h at 30 Pa. The acid chloride of

8-(3-perylenyl)octanoic acid was used in the subsequent reaction without additional purification.

5-FU (5 mg, 36 μmol) was boiled and stirred for 4 h in an atmosphere of argon with hexamethyldisilazane (128 μL, 0.61 mmol) and trimethylchlorosilane (45 μL, 0.34 mmol) as described for the synthesis of (**I**). After cooling, the excess of silylating agents was removed in vacuo. The residue was diluted with dry acetonitrile (250 μL), a half of the above-described acid chloride of 8-(3-perylenyl)octanoic acid in the form of a solution in dry acetonitrile was added, and the mixture was stirred for 4 h at 80°C. The course of the reaction was controlled by TLC (Kieselgel 60 F_{254}) in benzene–ethyl acetate 7 : 1; detection: A and B. The reaction mixture was evaporated twice with benzene, and product (**V**) was separated by reprecipitation with toluene from the chloroform solution at −10°C and flash chromatography on reverse phase RP-18 (Merck) in acetonitrile–methylene chloride. Yield: 8 mg (40%); the product was obtained as a dark red amorphous substance having no clearly defined melting point (decomposition >150°C); R_f 0.7 in the above-indicated system, detection: A and B; UV, λ_{max}, nm (ε, M^{-1} cm^{-1}): 260 (6.1×10^4), 423 (4.2×10^4), 447 (5.9×10^4); MALDI-MS, *m/z*: 507.212 $[M + H]^+$; ^{1}H NMR: 1.27 (6 H, br m, $(C\underline{H}_2)_3CH_2CH_2COO$), 1.63 (2 H, m, $C\underline{H}_2CH_2COO$), 1.70 (2 H, m, $ArCH_2C\underline{H}_2$), 2.56 (2 H, t, $J_{2\text{-}3}$ 7.5 Hz, CH_2COO), 3.07 (2 H, t, $J_{7\text{-}8}$ 7.5 Hz, $ArCH_2$), 7.17 (1 H, d, *J* 8.0 Hz, Ar), 7.40–7.55 (3 H, m, Ar), 7.66 (2 H, m, Ar), 7.73 (2 H, m, Ar), 7.80 (1 H, d, *J* 8.1 Hz, Ar), 8.07–8.14 (3 H, m, Ar; =CH). Fluorescence (in ethanol ~0.5 μM): λ_{ex} 423, 447 nm, λ_{em} 461, 484 nm.

Kinetics of hydrolysis of 5-FU derivatives. A solution of a derivative (5 mg) in a mixture of acetonitrile (4 mL) and 20 mM Tris-HCl buffer, pH 7.2, (1 mL) was incubated at 37°C and stirred for 3 h. Every 30 min, 5-μL samples were taken by a microsyringe. Plates for TLC were preliminarily developed in acetonitrile–chloroform 4 : 1 to remove contaminants and activated. Samples for the analysis of (**I**)–(**III**) were applied to reverse-phase RP18 F_{254s} plates and developed in acetonitrile; for the analysis of (**IV**), Kieselgel 60 F_{254} plates (development in benzene–ethyl acetate 1 : 1) were used. During UV irradiation, spots as well as 5-FU and L-FU were denoted on plates; the fluorescence *D* in the zone of spots was estimated on a Shimadzu CS-920 densitometer (Japan) at 270 nm. The decomposition constant for 5-FU (*k*) was calculated by the first-order kinetics equation: $kt = \ln(D_0/D)$.

Liposomes. Aliquots of solutions of lipids and L-FU (10 mol % of the total amounts of lipids) in chloroform–methanol 2 : 1 were mixed, evaporated, and dried in vacuo (30 Pa, 2 h) in a round-bottomed flask. PBS, pH 7.2, was added to the resulting lipid film. Then the sample was frozen six times in liquid nitrogen and thawed at 40°C. The resulting suspension was extruded 20 times through a filter with a pore size of 100 nm (Nucleopore, United States) using a miniextruder (Avanti Polar Lipids, United States). The content of 5-FU in liposomal preparations was 10 mM.

Cytotoxic activity of L-FU. HBL cells in the logarithmic growth phase were incubated for 48 h in a 24-well plate in RPMI 1640 culture medium supplemented with 7% fetal serum containing 5×10^3 cells/well with a liposomal preparation containing 10 mol % L-FU with respect to lipid, with 5-FU (addition in the form of a 10 mM aqueous solution; the final 5-FU concentration from 0.01 to 10 μM), or an aliquot of empty liposomes of the same lipid concentration (control). The number of viable cells was determined by the standard trypan blue test; the survival (%) was estimated by the equation (number of live cells in the experiment/number of live cells in the control) × 100.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 09-04-00313). The authors would like to thank I.A. Boldyrev (IBCH RAS) for help in data processing.

REFERENCES

1. Heidelberger, C., Chaudhuri, N.K., Danneberg, P., Mooren, D., Griesbach, L., Duschinsky, R., Schnitzer, R.J., Pleven, E., and Scheiner, J., *Nature,* 1957, vol. 179, pp. 663–666.
2. Noordhuis, P., Holwerda, U., Van der Wilt, C.L., Van Groeningen, C.J., Smid, K., Meijer, S., Pinedo, H.M., and Peters, G.J., *Ann. Oncol.,* 2004, vol. 15, pp. 1025–1032.
3. Chen, Z.X. and Breitman, T.R., *Cancer Res.,* 1994, vol. 54, pp. 3494–3499.
4. Peters, G.J., Backus, H.H., Freemantle, S., van Triest, B., Codacci-Pisanelli, G., Van der Wilt, C.L., Smid, K., Lunec, J., Calvert, A.H., Marsh, H., McLeod, H.L., Bloemena, E., Meijers, G., Jansen, G., Van Groeningen, C.J., and Pinedo, H.M., *Biochim. Biophys. Acta*, 2002, vol. 1587, pp. 194–205.
5. Funaki, N.O., Tanaka, J., Kohmoto, M., Sugiyama, T., Ohshio, G., Nonaka, A., Yotsumoto, F., Takeda, Y., and Imamura, M., *Oncol. Rep.*, 2001, vol. 8, pp. 527–532.
6. Giller, S.A., Zhuk, R.A., and Lidak, M.Yu., *Dokl. Akad. Nauk SSSR*, 1967, vol. 176, pp. 332–225.
7. Malet-Martino, M.P., Jolimaitre, P., and Martino, R., Curr. Med. Chem, *Anti-Cancer Agents*, 2002, vol. 2, pp. 267–310.
8. Pan, X., Wang, C., Wang, F., Li, P., Hu, Z., Shan, Y., and Zhang, J., *Curr. Med. Chem.*, 2011, vol. 18, pp. 4538–4556.
9. Alvarez, P., Marchal, J.A., Boulaiz, H., Carrillo, E., Velez, C., Rodriguez-Serrano, F., Melguizo, C., Prados, J., Madeddu, R., and Aranega, A., *Expert Opin. Ther. Patents,* 2012, vol. 22, pp. 107–123.

10. Elorza, B., Elorza, M.A., Frutos, G., and Chantres, J.R., *Biochim. Biophys. Acta*, 1993, vol. 1153, pp. 135–142.
11. Thomas, A.M., Kapanen, A.I., Hare, J.I., Ramsay, E., Edwards, K., Karlsson, G., and Bally, M.B., *J. Control. Release*, 2011, vol. 150, pp. 212–219.
12. Murakami, T., *Biotechnol. J.*, 2012, vol. 7, pp. 762–767.
13. Kumar, P., Gulbake, A., and Jain, S.K., *Crit. Rev. Ther. Drug Carrier Syst.*, 2012, vol. 29, pp. 355–419.
14. Straubinger, R.M., Arnold, R.D., Zhou, R., Mazurchuk, R., and Slack, J.E., *Anticancer Res.*, 2004, vol. 24, no. 2.
15. Yu, B., Tai, H.C., Xue, W., Lee, L.J., and Lee, R.J., *Mol. Membr. Biol.*, 2010, vol. 27, pp. 286–298.
16. Basile, L., Pignatello, R., and Passirani, C., *Curr. Drug Deliv.*, 2012, vol. 9, pp. 255–268.
17. Tada, M., US Patent No. 3971784, 1976.
18. Ishida, T., Nishimura, D., Sugawara, T., and Ooka, T., US Patent No. 4088646, 1978.
19. Robins, M.J. and Hatfield, P.W., *Can. J. Chem.*, 1982, vol. 60, pp. 547–553.
20. Jolimaitre, P., Malet-Martino, M., and Martino, R., *Int. J. Pharm.,* 2003, vol. 259, pp. 181–192.
21. Yu, B.T., Sun, X., and Zhang, Z.R., *Arch. Pharm. Res.*, 2003, vol. 26, pp. 1096–1101.
22. Sun, W., Zhang, N., Li, A., Zou, W., and Xu, W., *Int. J. Pharm.,* 2008, vol. 353, pp. 243–250.
23. Vodovozova, E.L., Moiseeva, E.V., Gaenko, G.P., Bovin, N.V., and Molotkovsky, J.G., *Russ.. Bioterapevt. Zh.*, 2008, vol. 7, no. 2, pp. 24–33.
24. Nishimoto, S., Hatta, H., Ueshima, H., and Kagiya, T., *J. Med. Chem.,* 1992, vol. 35, pp. 2711–2712.
25. Grechishnikova, I.V., Mikhalev, I.I., and Molotkovsky, J.G., *Bioorg. Khim.*, 1995, vol. 21, pp. 70–76.
26. Hermetter, A. and Oskolkova, O., US Patent No. 7772402, 2010.
27. Menezes, D.E.L., Kirchmeier, M.J., Gagne, J.F., Pilarski, L.M., and Allen, T.M., *J. Liposome Res.*, 1999, vol. 9, pp. 199–228.
28. Vodovozova, E.L., Moiseeva, E.V., Grechko, G.K., Gayenko, G.P., Nifant'ev, N.E., Bovin, N.V., and Molotkovsky, J.G., *Eur. J. Cancer*, 2000, vol. 36, pp. 942–949.
29. DeVries, V.G., Largis, E.E., Miner, T.G., Shepherd, R.G., and Upelacis, J., *J. Med. Chem.*, 1983, vol. 26, pp. 1411–1423.

Translated by S. Sidorova