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Polyfunctional Derivatives of Isocytosine. Effect of Hydration on Prototropic Tautomerism of 2-(2-Hydroxyethyl)amino-6-methylpyrimidin-4(3*H*)-one

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Abstract — Hydration of 2-(2-hydroxyethyl)amino-6-methylpyrimidin-4(3*H*)-one forms an equilibrium mixture of 4-oxo-3,4-dihydro and 4-hydroxy tautomers. The intermediate in mutual transitions of these tautomers has a zwitter ionic structure. The equilibrium shifts to the 4-oxo-3,4-dihydro form as the polarity of the medium decreases.

The prototropic tautomerism of 2-amino-4-oxopyrimidines and its dependence on specific solvation has repeatedly been studied on isocytosine as an example. In a neutral aqueous solution, isocytosine exists as a mixture of 4-oxo-1,4-dihydro and 4-oxo-3,4-dihydro tautomers [1, 2], the latter prevailing in less polar media [1, 3, 4]. Existence of the 4-hydroxy tautomer of isocytosine and its zwitter ionic analog is not ruled out completely, but is considered unlikely by formal reasons [2]. At the same time, the 4-oxo-3,4-dihydro and 4-hydroxy tautomers form a pair involved in intermolecular proton transfer on isocytosine hydration [5].

The aim of the present work was to study the effect of hydration on the prototropic tautomerism of a polyfunctional isocytosine derivative, 2-(2-hydroxyethyl)-amino-6-methylpyrimidin-4(3*H*)-one (**I**), by UV spectroscopy.

The prototropic tautomerism of substituted isocytosine **I** in ethanol have been by Agai *et al.* who provided unambiguous evidence to show that this compound exists exclusively as the 4-oxo-3,4-dihydro tautomer.

Compound **I** was synthesized by amination of 6-methyl-2-(methylsulfanyl)pyrimidin-4(3*H*)-one (**II**) with excess 2-aminoethanol in rigid conditions, and thio ether **II**, by S-methylation of 6-methyl-2-thiouracil (**III**) with equimolar amount of dimethyl sulfate in aqueous alkali. Unlike the other known syntheses of compound **I** via cyclocondensation of 2-(hydroxyethyl)guanidine with ethyl acetoacetate [7] and amination of thio ether **II** with a mixture of 2-aminoethanol and its hydrochloride [8], our procedure is more facile to realize.

Similar reactions of 3,6-dimethyl-2-(methylsulfanyl)pyrimidin-4(3*H*)-one (**IV**) and 1,6-dimethyl-2-(methylsulfanyl)pyrimidin-4(1*H*)-one (**V**) with 2-aminoethanol gave the model compounds 2-(2-hydroxyethyl)amino-3,6-dimethylpyrimidin-4(3*H*)-one (**VI**) and 2-(2-hydroxyethyl)amino-1,6-dimethylpyrimidin-4(1*H*)-one (**VII**), respectively, and required methyl sulfides **IV** and **V**, by exhaustive S,N-dimethylation of thioxoketone **III** with excess dimethyl sulfate by our modified procedure [9].

The other model compound 2-(4-methoxy-6-methylpyrimidin-2-yl)aminoethyl acetate (**X**) was synthesized by consecutive acetylation of substituted isocytosine **I** with excess acetic anhydride in absolute pyridine, exchange chlorination of 2-(6-methyl-4-oxo-3,4-dihydropyrimidin-2-yl)aminoethyl acetate (**VIII**) with phosphoryl chloride, and methoxylation of 2-(4-chloro-6-methylpyrimidin-2-yl)aminoethyl acetate (**IX**) with sodium methylate in absolute methanol. Contrary to expectations, the latter reaction is not accompanied by elimination of the acetyl protective group even with a 3-fold excess of sodium methylate, and deacetylation of ester **X** in aqueous alkali gives rise to 2-(4-methoxy-6-methylpyrimidin-2-yl)aminoethyl acetate (**X**) that is impossible to crystallize from various solvents because of its extreme hygroscopicity. By the same reason, pyrimidine **XI** hydrochloride could not be isolated crystalline by treatment with saturated HCl solutions in absolute diethyl ether or ethanol.

The UV spectra of compound **I** in neutral aqueous solutions [c $(0.5–1.5)\times10^{-4}$ M] contain a broad asymmetric band with a maximum at 272 nm ($\log\varepsilon$ 3.71) and a shoulder at 285 nm (Fig. 1a). At the same time, the optical density of solutions of compound **I**

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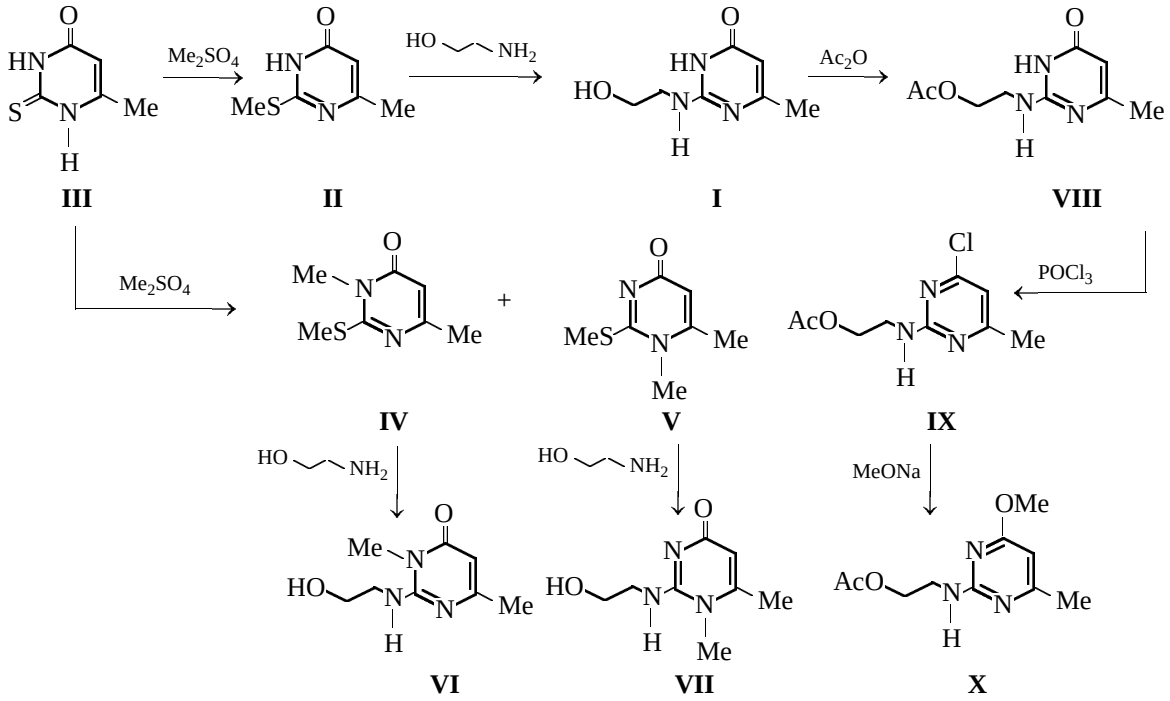


linearly varies with concentration only in the range $(0.5–1.25)\times10^{-4}$ M, whereas at higher concentrations, negative deviations from the Bouguer law are observed. The possible reason for this phenomenon is hydration of isocytosine **I** followed by prototropic rearrangement.

To obtain evidence for hydration, we compared the spectra of compound **I** in water, ethanol, and DMF and found out that the absorption band gets narrower and suffers a bathochroic shift to 289 (ethanol) and 290 nm (DMF), accompanied by a hyperchromic effect [log ε 3.98 (ethanol) and 4.00 (DMF)], as the polarity of the solvent decreases (the normalized Dimroth–Reichrdt polarity parameters E_T^N for water, ethanol, and DMF are 1.000, 0.654, and 0.404, respectively [10]) (Fig. 1b). Such a negative solvatochromism of isocytosine **I** is a qualitative criterion of its hydration.

The presence in molecule **I** of two potential hydration centers, amide and guanidine, necessitates assessment of the degree of involvement of each of them in hydrogen bonding. Comparing the UV spectra

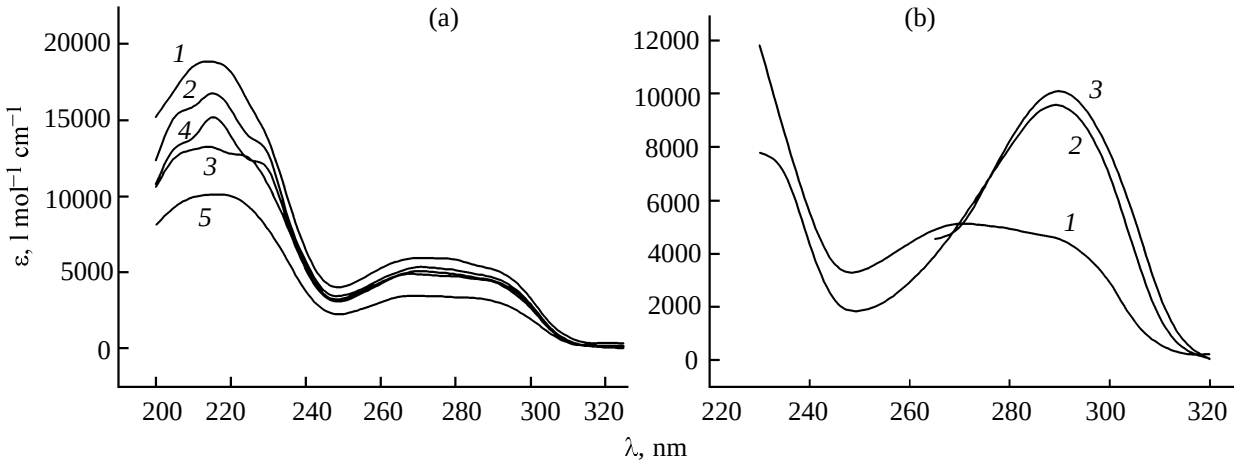


Fig. 1. UV spectra of 2-(2-hydroxyethyl)amino-6-methylpyrimidin-4(3*H*)-one (**I**) in (a) water [concentration (*1*) 0.5×10^{-4}, (*2*) 0.75×10^{-4}, (*3*) 10^{-4}, (*4*) 1.25×10^{-4}, and (*5*) 1.5×10^{-4} M], (b) (*1*) water, (*2*) ethanol, and (*3*) DMF.

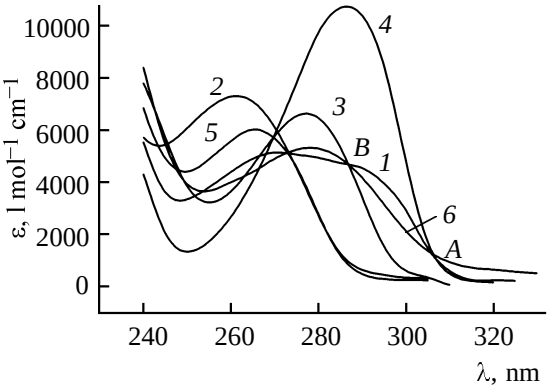


Fig. 2. UV spectra of the (*1*) neutral, (*2*) cationic, and (*3*) anionic forms of 2-(2-hydroxyethyl)amino-6-methylpyrimidin-4(3*H*)-one (**I**), (*4*) methylisocytosine **VI**, (*5*) methylisocytosine **VII**, and (*6*) ester **X**.

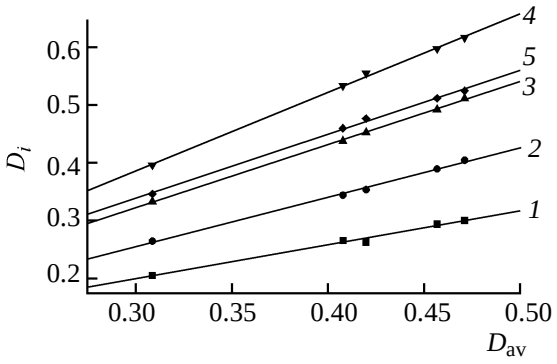


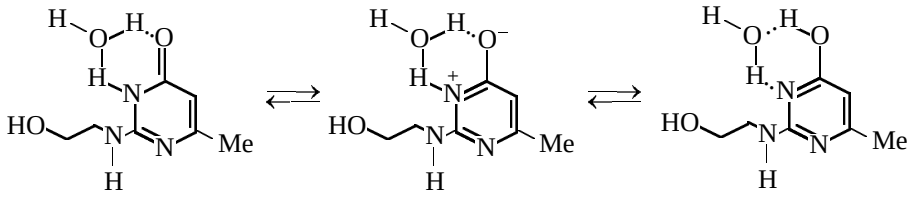
Fig. 3. Optical density of solution *i* (D_i) vs. average optical density (D_{av}) of other solutions at various wavelengths (the numbers in the figure correspond to the numbers of solutions in the table).

of compound **I** at various pHs with those of methylisocytosine **VII** and ester **X** one can see that the spectra of the neutral (pH 7) and anionic (pH 12) forms of the former compound are similar to the spectrum of ester **X**, implying preferential hydration of the amide fragment (Fig. 2). Had the guanidine fragment been hydrated, the spectra of the neutral and cationic (pH 2) forms of compound **I** would have been similar to that of methylisocytosine **VII**, which is not the case.

Even though the spectra of isocytosine **I** and ester **X** are similar to each other, the band at 272 nm in the spectrum of the former compound is asymmetric and thus difficult to assign to individual absorption of the 4-hydroxy tautomer. On the other hand, the fact that the spectra of compound **I** at varied concentration lack the isobestic point allows no suggestions as to the number of tautomers in the solution. In this connection we made use of the *n*-component test [11] and found out the solutions of isocytosine **I** are not one-component: The optical density of any of them D_i linearly varies with the average optical density D_{av} of all the solutions at any wavelength, however, the necessary condition for this dependence to pass through the origin is not fulfilled. By contrast, the ratio of the optical densities of two solutions at any wavelength is constant within error (see table), implying that the solutions of compound **I** contain two tautomers. Of the possible tautomers, the most probable are 4-oxo-3,4-dihydro (**Ia**) and 4-hydroxy (**Ib**) tautomers. Evidence for this suggestion is provided by the presence of an isosbestic point at 306 nm in the spectra of substituted isocytosine **I**, methylisocytosine **VI**, and ester **X** (Fig. 2a). The use of ester **X** rather than its H deacetylated analog **XI** as a model compound is validated by the similarity of the spectra of compound **I** and its acetate **VIII**. In addition to the aforesaid we should note that, as judged from the presence in the spectra of the neutral and anionic forms of isocytosine **I** and ester **X** of an isosbestic point at 287 nm (Fig. 2b), interconversions

Optical densities (D_h) and their functions for solutions of substituted isocytosine **I**

Solution (1)–(5) or optical density function	D_λ at λ				
	250 nm	260 nm	270 nm	280 nm	290 nm
1	0.204	0.265	0.300	0.294	0.262
2	0.264	0.344	0.405	0.389	0.353
3	0.333	0.438	0.511	0.492	0.452
4	0.395	0.533	0.616	0.597	0.554
5	0.345	0.460	0.524	0.511	0.476
D_{av}	0.308	0.408	0.471	0.456	0.419
$D_1 - D_{av}$	–0.104	–0.143	–0.171	–0.162	–0.157
$D_2 - D_{av}$	–0.044	–0.064	–0.066	–0.067	–0.066
$D_3 - D_{av}$	0.025	0.030	0.040	0.036	0.033
$D_4 - D_{av}$	0.087	0.125	0.145	0.141	0.135
$D_5 - D_{av}$	0.037	0.052	0.053	0.055	0.057
$(D_1 - D_{av})/(D_2 - D_{av})$	2.36	2.23	2.59	2.42	2.37
$(D_2 - D_{av})/(D_2 - D_{av})$	1	1	1	1	1
$(D_3 - D_{av})/(D_2 - D_{av})$	–0.56	–0.46	–0.60	–0.54	–0.50
$(D_4 - D_{av})/(D_2 - D_{av})$	–1.98	–1.95	–2.19	–2.10	–2.05
$(D_5 - D_{av})/(D_2 - D_{av})$	–0.84	–0.81	–0.80	–0.82	–0.86



Ia **A** **Ib**

of tautomers **Ia** and **Ib** occur via intermediate formation of zwitter ion **A**.

The unexpected similarity of the UV spectra of compound **I** in the protic ethanol and aprotic DMF makes us to suggest that its tautomerization is associated not only with specific hydrogen bonding, but also with the high polarity of the medium. Actually, addition of the less polar ethanol to aqueous solutions of isocytosine **I** produces a sharp bathochromic shift of the absorption maximum of this compound, that overtakes the absorption maximum of methylisocytosine **VI** at ethanol concentrations above 20 vol%. In other words, increase of the polarity of the ethanol–water mixture shifts the absorption maximum of compound **I** hypsochromically (Fig. 4). These data provide unambiguous evidence to show that the equilibrium mixture of tautomers **Ia** and **Ib** can exist only in aqueous solution whose polarity parameter E_T^N is no lower than 0.95. In solutions with E_T^N 0.95–30.92, the 4-hydroxy tautomer **Ib** fast converts into the 4-oxo-3,4-dihydro tautomer **Ia**, and it is the latter form in which isocytosine **I** exists exclusively in solutions with $E_T^N < 0.92$.

EXPERIMENTAL

The UV spectra were obtained on an SF-26 spectrophotometer in quartz cells 1 cm thick at solution concentrations of 10–4 M, unless otherwise specified.

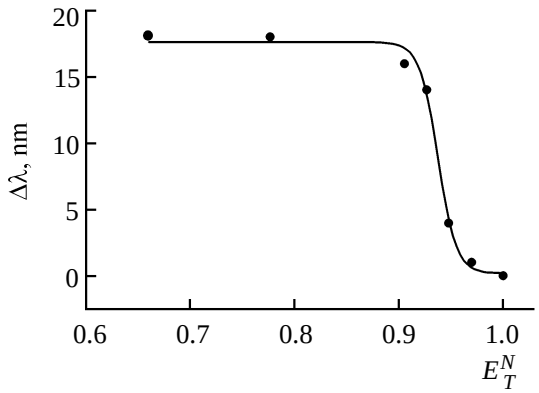


Fig. 4. Change of the absorption maximum (Δλ) of 2-(2-hydroxyethyl)amino-6-methylpyrimidin-4(3*H*)-one vs. polarity of the medium (E_T^N).

The ^{1}H NMR spectra were measured on a Bruker AC-200 instrument (200.13 MHz) in DMSO-$\mathbf{d_6}$, internal reference residual DMSO proton signals. Elemental analysis was performed on a Hewlett–Packard B-185 analyzer.

The purity of the synthesized compounds was controlled by TLC on Silufol UV-254 plates in the systems 1-butanol–acetic acid–water, 1:1:1 (A), chloroform–methanol, 9:1 (B), acetone–hexane, 2:1 (C), and acetone–hexane, 1:1 + 2 drops of pyridine (D). Development was performed with UV light.

The normalized Dimroth–Reichardt parameters E_T^N for 5:95, 10:90, 15:85, 20:80, and 50:50 (vol%) mixtures were obtained by interpolation [12].

6-Methyl-2-(methylsulfanyl)methylpyrimidin-4(3*H*)-one (**II**) and 6-methyl-2-thiouracil (**III**) were synthesized as described in [13].

2-(2-Hydroxyethyl)amino-6-methylpyrimidin-4(3*H*)-one (I). A mixture of 6.24 g of thio ether **II** and 4.88 g of 2-aminoethanol was heated at 140°C until methanethiol no longer evolved. After cooling to 40°C, the reaction mixture was diluted with 15 ml of water, the suspension that formed was neutralized with acetic acid, and the precipitate that formed was filtered off and recrystallized from water. After drying at 80°C for 10 h, 5.2 g (77 %) of compound **I** was obtained, mp 209°C (published data [6, 7]: mp 204–205°C), R_f 0.52 (A). ^{1}H NMR spectrum, δ, ppm: 2.00 s (3H, Me), 3.31 m (4H, CH2), 4.84 br.s (1H, OH), 5.39 s (1H, CH), 6.43 br.s (1H, NH_e), 10.47 br.s (1H, NH).

3,6-Dimethyl-2-(methylsulfanyl)pyrimidin-4(3*H*)-one (IV) and 1,6-dimethyl-2-(methylsulfanyl)pyrimidin-4(1*H*)-one (V). Thioxoketone **III**, 28.4 g, was added to a solution of 18 g of NaOH in 72 ml of water. When compound **III** had dissolved completely, 50.4 g of dimethyl sulfate was added dropwise with vigorous stirring. The reaction mixture was heated at 70°C for 2 h and then cooled to 20°C over the course of 12 h. The precipitate that formed was filtered off and recrystallized from water. After drying in a vacuum over P_2O_5, 14 g (41%) of methyl sulfide **IV** was obtained, mp 92°C (published data [9]:

mp 94°C), R_f 0.79 (B). Found, %: C 48.58; H 4.60; N 16.28. $C_7H_{10}N_2OS$. Calculated, %: C 49.41; H 5.88; N 16.47.

Hydrolysis of the reaction product with conc. HCl (100°C, 1 h) gave 3,6-dimethyluracil, mp 274°C (decomp.) (published data [14]: mp 274°C), R_f 0.55 (C). UV spectrum, λmax, nm (log ε): 260 (3.93) (pH 7), 280 (4.04) (pH 12); the bathochromic shift of the absorption maximum in the UV spectrum of 3,6-dimethyluracil in going from neutral to alkaline solution suggests [15] the 4-oxo-3,4-dihydro configuration of sulfide **IV**.

The mother liquor after separation of compound **IV** was treated with chloroform (4 × 50 ml), the first extract was separated and three following were combined {by contrast to the original procedure [9], this procedure makes it possible to obtain methyl sulfide **V** containing no admixture of isomer **IV**}. The chloroform was removed from the combined extract, and the residue was recrystallized from water. After drying in a vacuum over phosphorus pentoxide, 1.5 g (4.4%) of methyl sulfide **V** was obtained, mp 229°C (published data [9]: mp 207°C), R_f 0.46 (B). Found, %: C 48.46; H 4.98; N 16.14. $C_7H_{10}N_2OS$. Calculated, %: C 49.41; H 5.88; N 16.47.

Hydrolysis of the reaction product with conc. HCl (100°C, 1 h) gave 1,6-dimethyluracil, mp 230°C (published data [16]: mp 224–225°C), R_f 0.34 (C). UV spectrum, λ_{max}, nm (log ε): 266 (4.00) (pH 7), 265 (3.88) (pH 12); the absence of bathocromic shift of the absorption maximum in the UV spectrum of 1,6-dimethyluracil in going from the neutral to alkaline solution suggests [15] the 4-oxo-1,4-dihydro configuration of methyl sulfide **V**.

2-(2-Hydroxyethyl)amino-3,6-dimethylpyrimidin-4(3*H*)-one (VI). A mixture of 1.02 g of methyl sulfide **IV** and 0.73 g of 2-aminoethanol was heated at 150°C until methanethiol no longer evolved. After cooling to room temperature, the reaction mixture was diluted with 3 ml of water, and the precipitate was filtered off and recrystallized from water. After drying in a vacuum over phosphorus pentoxide, 0.44 g (40%) of methylisocytosine **VI** was obtained, mp 194°C (published data [8]: mp 186–187°C), R_f 0.36 (A). ^{1}H NMR spectrum, δ, ppm: 2.03 s (3H, Me), 3.24 s (3H, NMe), 3.39, 3.42, 3.44 t (2H, CH_2), 3.53, 3.56 d (2H, CH_2), 4.60 br.s (1H, OH), 5.45 s (1H, CH), 6.83 br.s (1H, NH_e). Found, %: C 52.89; H 7.73; N 22.94. $C_8H_{13}N_3O_2$. Calculated, %: C 52.46; H 7.10; N 22.95.

2-(2-Hydroxyethylamino)amino-1,6-dimethylpyrimidin-4(1*H*)-one (VII) was prepared in a similar way from 0.76 g of methyl sulfide **V** and 0.55 g of 2-aminoethanol. Yield 0.14 g (17%), mp 226°C (decomp.) (published data [17]: mp 255°C), R_f 0.29 (A). ^{1}H NMR spectrum, δ, ppm: 2.17 s (3H, Me), 3.28, 3.35 m (5H, CH_2 + NMe), 3.49, 3.52 d (2H, CH_2), 4.76 s (1H, OH), 5.40 s (1H, CH), 6.84 s (1H, NH_e). Found, %: C 51.51; 6.55; N 22.02. $C_8H_{13}N_3O_2$. Calculated, %: C 52.46; H 7.10; N 22.95.

2-(6-Methyl-4-oxo-3,4-dihydropyrimidin-2-yl)-aminoethyl acetate (VIII). Acetic anhydride, 1.53 g, was added with vigorous stirring to a hot (80°C) suspension of 1.69 g of compound **III** in 3 ml of absolute pyridine. The mixture was then heated at 100°C for 1 h and cooled to room temperature. The precipitate that formed was filetered off and recrystallized from water. After drying at 80°C for 10 h, 1.16 g (55%) of acetate **VIII** was obtained, mp 173°C, R_f 0.27 (B). ^{1}H NMR spectrum, δ, ppm: 2.02 s (6H, Ac + Me), 3.49, 3.52 d (2H, CH_2), 4.07, 4.10, 4.13 t (2H, CH_2), 5.37 s (1H, CH), 6.40 br.s (1H, NH_e), 10.49 br.s (1H, NH). UV spectrum, λ_{max}, nm (log ε): 270 (3.70), 285 sh. Found, %: C 51.22; H 6.46; N 19.85. $C_9H_{13}N_3O_3$. Calculated, %: C 51.18; H 6.16; N 19.91.

2-(4-Chloro-6-methylpyrimidin-2-yl)aminoethyl acetate (IX). A mixture of 2.41 g of acetate **VIII** and 16.7 g of phosphoryl chloride was refluxed for 3 h and excess phosphoryl chloride was removed in a vacuum. Finely crushed ice was added to the residue, the resulting suspension was neutralized with 9% aqueous ammonia, and the precipitate was filtered off and dried in a vacuum. After recrystallization from diethyl ether and drying in a vacuum, 0.94 g (36%) of chloropyrimidine **IX** was obtained, mp 124°C, R_f 0.66 (D). ^{1}H NMR spectrum, δ, ppm: 1.99 s (3H, Ac), 2.26 s (3H, Me), 3.50, 3.53, 3.55 t (2H, CH_2), 4.07, 4.10, 4.13 t (2H, CH_2), 6.46 s (1H, CH), 7.45 s (1H, NH_e). Found, %: C 46.87; H 5.33; N 17.96. $C_9H_{12} \cdot ClN_3O_2$. Calculated, %: C 47.05; H 5.22; N 18.30. The mother liquor was reduced by 3/4, the precipitate that formed was recrystallized from absolute ethanol gave an additional 0.04 g (1.5%) of chloropyrimidine **IX**. Its mixed sample with the main portion of the reaction product showed no melting point depression.

2-(4-Methoxy-6-methylpyrimidin-2-yl)aminoethyl acetate (X). Sodium, 0.14 g, dissolved in 5 ml of absolute methanol was added dropwise with vigorous stirring to a hot (50°C) solution of 0.94 g of chloropyrimidine **IX** in 20 ml of absolute methanol. The mixture was refluxed for 3 h, the precipitate was filtered off, and the filtrate was evaporated in a vacuum to dryness. The residue was refluxed with 10 ml of absolute diethyl ether, the undissolved residue was filtered off, and the filtrate was cooled to –25°C. A precipitate formed and was filtered off,

washed with hexane, and dried in a vacuum to obtain 0.29 g (32%) of ester **X**, mp 58°C, R_f 0.35 (D). ^{1}H NMR spectrum, δ, ppm: 1.88 s (3H, Ac), 2.16 s (3H, Me), 3.33, 3.35, 3.38 t (2H, CH_2), 3.48, 3.51, 3.54 t (2H, CH_2), 3.80 s (3H, OMe), 5.77 s (1H, CH), 6.49 s (1H, NH_e). We failed to perform elemental analysis of the product because of its decomposition.

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