Electrophilic addition to the multiple bond of 1-carboxymethyl-5-fluorouracil

I. B. Chernikova, M. S. Yunusov, and A. G. Mustaphin*

Ufa Institute of Chemistry, Ufa Federal Research Centre, Russian Academy of Sciences, 71 prosp. Oktyabrya, 450054 Ufa, Russian Federation. E-mail: inna.b.chernikova@yandex.ru

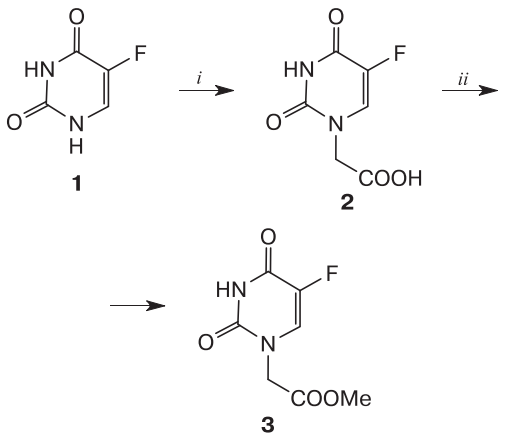
1-Carboxymethyl-5-fluoro-5-halogeno-6-hydroxy-5,6-dihydrouracils and 1-carboxymethyl-5-fluoro-6-hydroxy-5-nitro-5,6-dihydrouracil were synthesized in high yields by oxidative halogenation or nitration of 1-carboxymethyl-5-fluorouracil. Oxidative halogenation or nitration of 5-fluoro-1-(methoxycarbonylmethyl)uracil in acidic media results in the addition of the corresponding groups at the C(5) atom of uracil and simultaneous hydrolysis of the ester group. Bimolecular ions of the obtained compounds were detected in the negative ion mass spectra.

Key words: 5-fluorouracil, electrophilic addition, halogenation.

A number of uracil derivatives have been shown to exhibit antitumor[1—6] and antiviral activity.[7—10] At the same time, the reported derivatives possessing an antiviral activity differ considerably in their structures from 5-chloro-5-fluoro-6-hydroxy-5,6-dihydrouracil, for which we formerly revealed a pronounced antiviral activity against different strains of influenza virus.[11] In the search for new antiviral drugs among analogs of 5-chloro-5-fluoro-6-hydroxy-5,6-dihydrouracil, compounds containing an acetic acid residue at position 1 were synthesized.

1-Carboxymethyl-5-fluorouracil (**2**) was prepared from 5-fluorouracil (**1**) and chloroacetic acid according to a described method.[12] The corresponding methyl ester **3** was synthesized by boiling acid **2** in methanol in the presence of sulfuric acid (Scheme 1).[13]

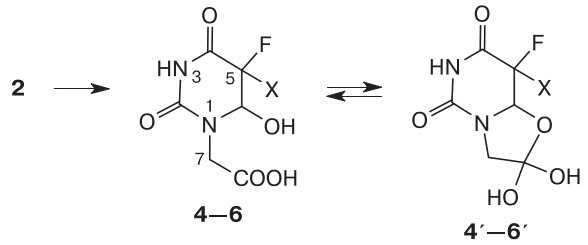
Scheme 1



Reagents and conditions: *i*. $ClCH_2COOH$ (1.35 eq.), KOH (4 eq.), H_2O, ~20 °C, 5 h; *ii*. MeOH, H_2SO_4 (cat.), 60 °C, 3 h.

Oxidative halogenation and nitration of compound **2** were carried out using previously developed methods.[14] The mechanism of the electrophilic addition to uracil derivatives was already discussed.[14—15] Bromination of compound **2** with a $KBr—H_2O_2$ mixture in 20% H_2SO_4 for 5 h gave bromohydrin **4** in 67% yield (Scheme 2). A twofold prolongation of the reaction increased the yield to 83%.

Scheme 2



Compound	X	Yield (%)
4, 4′	Br	83
5, 5′	Cl	78
6, 6′	NO_2	75

Reagents and conditions: *i*. KBr (2 eq.), 20% H_2SO_4, 33% H_2O_2 (3 eq.), ~20 °C, 10 h, or *ii*. HCl (3 eq.), 33% H_2O_2 (4 eq.), CH_2Cl_2, ~20 °C, 5 h, or *iii*. H_2SO_4, 67% HNO_3, 0→20 °C, 5 h.

Chlorination of **2** using KCl under analogous conditions did not result in the desired product substituted at position 5; only starting compound **2** was isolated. However, chlorination of **2** in a two-phase medium[16] gave chlorohydrin **5** in 78% yield. Nitration of uracil **2** with an $H_2SO_4—HNO_3$ mixture at 0—10 °C for 5 h produced the corresponding nitro compound in 75% yield (see Scheme 2).

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It is worth noting that methylene protons of the CH_2COOH moieties in obtained compounds **4—6** are not equivalent and appear in the ^1H NMR spectrum as two doublets, whereas they are equivalent in starting 1-carboxymethyl-5-fluorouracil (**2**). Nonequivalence of these protons in compounds **4—6** is probably due to the presence of the OH group in position 6 and the formation of a hydrogen bond between this OH group and the carboxyl group, as well as due to the existence of the ring-chain tautomerism leading to the formation of equilibrium structures **4′—6′** (see Scheme 2). This is indicated by the preservation of the proton nonequivalence in the ^1H NMR spectra at 70 °C. Note that the nonequivalence of protons at the C(7) atom is also observed for methyl ester **7**. In the ^{13}C NMR and ^1H NMR spectra of compounds **4—6**, the spin-spin interactions of the F atom with the C(4), C(5) and C(6) atoms, as well as with the proton at the C(6) atom are observed. In the mass spectra of negative ions, the $[M - H]^-$ ion peaks with masses of 283 and 239 and isotopic distributions characteristic of monobromo or monochloro derivatives, respectively, were observed for compounds **4** and **5**. Bimolecular ions with masses of 567 and 479, which contain two Br or Cl atoms, respectively, were detected for these compounds in addition. A similar observation was made for nitro derivative **6** as well.

The presence of bimolecular negative ions in the mass spectra of compounds **4—6** can be explained by the initial formation of radical anions following by the formation of anions $[M - H]^-$ (Scheme 3). Probably, the formation of radical anions **III** is more preferable because of their capability to be stabilized by formation of equilibrium structures **IV** and **V**. The latter can react with initial molecules **I**, forming bimolecular anion $[2 M - H]^-$, *e.g.*, of type **VI**.

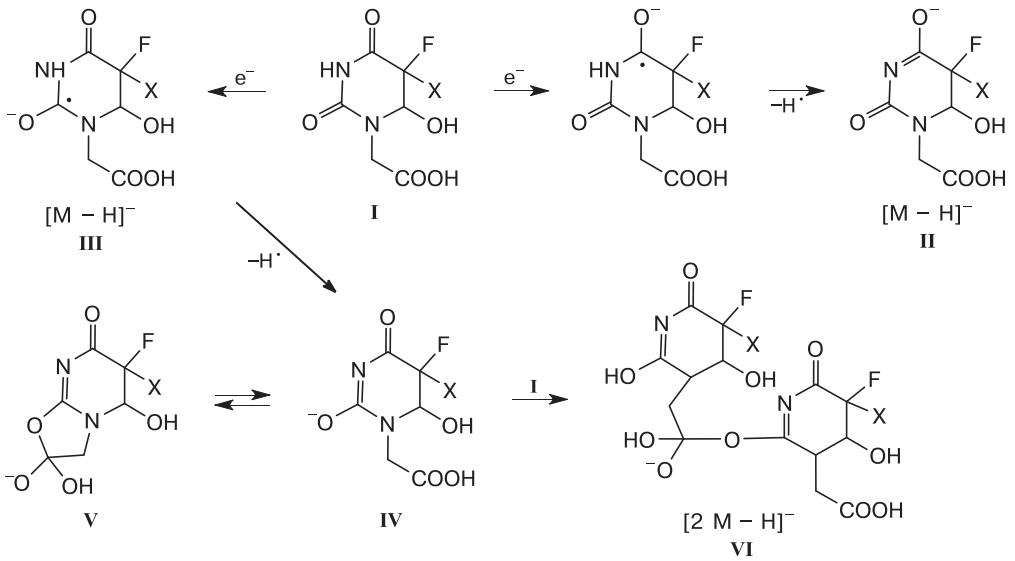
The spatial structures of compounds **4—6** likely correspond to those of analogous 5-fluorouracil derivatives substituted at position 5, in which the OH group at the C(6) atom acquires an axial position due to anomeric effect, whereas the F atom acquires an equatorial position.[14] Formerly we showed that according to the calculations at the B3LYP/6-311+G(d,p)//B3LYP/6-311G(d,p) + IEFPCM level of theory, as well as the results of the GIAO modelling of the ^{13}C NMR spectra, the equatorial orientation of the fluorine atom and the axial orientation of the hydroxyl group at the position 6 of the dihydrouracil ring are characteristic of 5,6-substituted 5-fluorouracils.[14]

The proposed stereochemistry agrees with the influence of the anomeric effect on α-substituted saturated *N*(*O, S*)-heterocycles, in which, as shown in many examples, the OH group located in the α-position to the heteratom in the heterocycle is usually axially oriented.[17]

Compounds **4—6** are amorphous, which precludes their X-ray analysis. Taking into account the conditions, under which the oxidative halogenation and nitration were carried out, one may expect that the electrophilic additions should proceed according to the monomolecular mechanism via an intermediate C(6)-carbocation being planar in the case of uracil derivatives, which determinates the predominant role of the anomeric effect.[17] Under the used conditions, the orientation of the substituents at the C(5) and C(6) atoms may vary and differ from the initial one. In this case, the influence of the anomeric effect should also predominate.

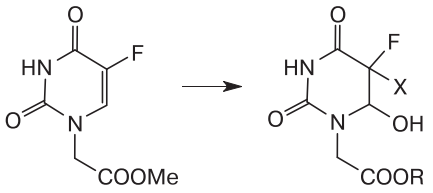
Bromination of 5-fluoro-1-(methoxycarbonylmethyl)-uracil (**3**) using the $KBr—H_2O_2$ system in 20% H_2SO_4 gave 5-bromo-1-carboxymethyl-5-fluoro-6-hydroxy-5,6-dihydrouracil (**4**) in 86% yield (Scheme 4). Simultaneously with bromination at position C(5) of the uracil ring,

Scheme 3



hydrolysis of the ester group occurred in the acidic medium. Similarly to chlorination of **2**, chlorination of compound **3** with the use of KCl for 5 h under the same conditions leaved the starting compound intact. Chlorination in the two-phase system resulted in the formation of two products, one of which is acid **5**, while the other is its methyl ester **7**. 1-Carboxymethyl-5-fluoro-6-hydroxy-5-nitro-5,6-dihydrouracil (**6**) was obtained in 84% yield upon nitration of compound **3** with the H_2SO_4—HNO_3 mixture at 0—10 °C for 5 h (see Scheme 4).

Scheme 4



3 **4—7**

Compound	X	R	Yield (%)
4	Br	H	86
5	Cl	H	—
6	NO_2	H	84
7	Cl	Me	—

Reagents and conditions: *i*. KBr (2 eq.), 20% H_2SO_4, 33% H_2O_2 (3 eq.), ~20 °C, 5 h, or *ii*. HCl (3 eq.), 33% H_2O_2 (4 eq.), CH_2Cl_2, ~20 °C, 5 h, or *iii*. H_2SO_4, 67% HNO_3, 0→20 °C, 5 h.

In conclusion, 5-halogeno-6-hydroxy and 6-hydroxy-5-nitro derivatives of uracil were synthesized for the first time by oxidative halogenation and nitration of 1-carboxymethyl-5-fluorouracil and its methyl ester with the purpose of their further involvement in a study of the dependence between the structure and antiviral activity in the series of uracil derivatives.

Experimental

The ^1H and ^{13}C NMR spectra were recorded at a constant temperature of 298 K using a Bruker Avance-III 500 pulsed spectrometer operating at frequencies of 500.13 MHz (^1H) and 125.76 MHz (^{13}C) and equipped with a 5 mm broad-band multinuclear (PABBO) probe and the Z-axis gradient unit. Solvent signals were used as references. The ^{13}C NMR spectra with proton decoupling were recorded using the following parameters: the 29.8 kHz spectral window, the 3.2 μs exciting pulse (30°) duration, the 2 s relaxation delay, the number of points was 64 K, the number of scans was 512—2048. The DEPT-90 and DEPT-135 experiments were performed to assist the interpretation of the ^{13}C NMR spectra. Two-dimensional spectra were obtained using the standard multipulse sequences available in the spectrometer software. Mass spectra were recorded using a Shimadzu LCMS-2010 EV spectrometer with quadrupole mass analyzer (manual syringe injection, mixed acetonitrile-chloroform solutions of samples, the 95 : 5 acetonitrile-water mixture as an eluent, flow rate of 0.1 mL min^{-1}) operating in the positive and negative ion modes at the capillary potentials of 4.5 and −3.5 kV, respectively; the atmospheric-pressure chemical ionization (APCI) was used as an ionization method, the APCI interface, heater and vaporizer temperatures were 250, 200 and 230 °C, respectively, the interface capillary voltage was 25÷−25 V. The flow rate of the nebulizing gas (nitrogen) was 2.5 L min^{-1}. Elemental analysis was carried out using a EURO-3000 elemental analyzer.

5-Bromo-1-carboxymethyl-5-fluoro-6-hydroxy-5,6-dihydrouracil (4). To a mixture of 1-carboxymethyl-5-fluorouracil (**2**) (0.10 g, 0.53 mmol) or 5-fluoro-1-(methoxycarbonyl)methyluracil (**3**) (0.11 g, 0.53 mmol) and KBr (0.13 g, 1.06 mmol) in 20% H_2SO_4 (2.30 mL), 33% H_2O_2 (0.16 mL, 1.59 mmol) was added dropwise, and the resulting mixture was stirred for 5 h at room temperature. Then, the reaction mixture was diluted with water and extracted with diethyl ether. The combined extracts were washed with water, dried over Na_2SO_4, the solvent was evaporated, the product was re-precipitated from an acetone-hexane (1 : 7) mixture. A white powder was obtained. In the case of substrate **2**, the yield of **4** was 67% (0.10 g), upon the twofold prolongation of the reaction, the yield increased to 83% (0.12 g). In the case of substrate **3**, the yield of **4** was 86% (0.13 g). ^1H NMR (DMSO-d_6) δ: 3.97 (d, 1 H, H_a(7), J = 17.40 Hz); 4.15 (d, 1 H, H_b(7), J = 17.40 Hz); 5.45 (d, 1 H, H(6), J = 4.50 Hz); 7.35 (br.s, 1 H, OH); 11.25 (br.s, 1 H, H(3)). ^{13}C NMR (DMSO-d_6) δ: 48.19 (s, C(7)); 83.77 (d, C(6), J = 26.40 Hz); 91.12 (d, C(5), J = 199.95 Hz); 150.88 (s, C(2)); 163.84 (d, C(4), J = 18.90 Hz); 169.89 (s, C(8)). MS, m/z (I_{rel} (%)): 283 [M − H]$^-$ (90), 285 (80); 567 [2 M − H]$^-$ (32), 569 (100), 571 (42). Found (%): C, 24.54; H, 2.07; Br, 29.03; N, 9.85. $C_6H_6BrFN_2O_5$. Calculated (%): C, 25.28; H, 2.12; Br, 28.03; N, 9.83.

1-Carboxymethyl-5-chloro-5-fluoro-6-hydroxy-5,6-dihydrouracil (5). To 1-carboxymethyl-5-fluorouracil (**2**) (0.10 g, 0.53 mmol) or 5-fluoro-1-(methoxycarbonyl)methyluracil (**3**) (0.11 g, 0.53 mmol) in CH_2Cl_2 (1.00 mL), 34% HCl (0.15 mL, 1.60 mmol) was added under stirring at room temperature, then, 33% H_2O_2 (0.21 mL, 2.12 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 5 h. Then, the reaction mixture was diluted with water and extracted with diethyl ether. The combined extracts were washed with water, dried over Na_2SO_4, the solvent was evaporated, the product was re-precipitated from a mixture of acetone with hexane (1 : 7). A white powder was obtained. In the case of substrate **2**, the yield of **5** was 78% (0.10 g). In the case of substrate **3**, the total yield of **5** and **7** (in the 7 : 3 ratio) was 0.13 g. ^1H NMR (DMSO-d_6) δ: 3.95 (d, 1 H, H_a(7), J = 17.50 Hz); 4.10 (d, 1 H, H_b(7), J = 17.50 Hz); 5.35 (d, 1 H, H(6), J = 1.70 Hz); 7.60 (br.s, 1 H, OH); 11.90 (br.s, 1 H, H(3)). ^{13}C NMR (DMSO-d_6) δ: 48.44 (s, C(7)); 82.93 (d, C(6), J = 27.67 Hz); 97.30 (d, C(5), J = 255.29 Hz); 150.84 (s, C(2)); 163.04 (d, C(4), J = 27.66 Hz); 168.73 (s, C(8)). MS, m/z (I_{rel} (%)): 239 [M − H]$^-$ (100), 241 (30); 479 [2 M − H]$^-$ (84), 481 (58), 483 (5%). Found (%): C, 29.11; H, 2.39; Cl, 30.01; N, 14.81. $C_6H_6ClFN_2O_5$. Calculated (%): C, 29.96; H, 2.51; Cl, 14.74; N, 11.64.

5-Chloro-5-fluoro-6-hydroxy-1-(methoxycarbonyl)methyl-5,6-dihydrouracil (7). ^1H NMR (DMSO-d_6) δ: 3.66 (s, 3 H, H (9)); 3.99 (d, 1 H, H_a(7), J = 17.50 Hz); 4.20 (d, 1 H, H_b(7), J = 17.50 Hz); 5.36 (d, 1 H, H(6), J = 1.70 Hz); 7.65 (br.s, 1 H, OH); 11.38 (d, 1 H, H(3), J = 3.46 Hz). ^{13}C NMR (DMSO-d_6)

δ: 48.02 (s, C(7)); 52.32 (s, C(9)); 83.05 (d, C(6), J = 26.56 Hz); 97.33 (d, C(5), J = 254.81 Hz); 150.86 (s, C(2)); 163.03 (d, C(4), J = 27.22 Hz); 169.93 (s, C(8)).

1-Carboxymethyl-5-fluoro-6-hydroxy-5-nitro-5,6-dihydrouracil (6). 1-Carboxymethyl-5-fluorouracil (**2**) (0.10 g, 0.53 mmol) or 5-fluoro-1-(methoxycarbonylmethyl)uracil (**3**) (0.11 g, 0.53 mmol) was gradually dissolved under stirring in concentrated H_2SO_4 (0.30 mL, ρ = 1.8356 g mL^{-1}). After complete dissolution of the starting compound, the mixture was cooled to 0 °C, 67% HNO_3 (0.20 mL, ρ = 1.399 g mL^{-1}) was added dropwise to the mixture, and the resulting mixture was kept at 0—10 °C for 5 h. The mixture was diluted with water, neutralized with $NaHCO_3$ to pH = 3 and extracted with diethyl ether. The combined extracts were washed with water, dried over Na_2SO_4, the solvent was evaporated, the product was re-precipitated from a mixture of acetone with hexane (1 : 7). A white powder was obtained. In the case of substrate **2**, the yield of **6** was 75% (0.10 g); in the case of substrate **3**, the total yield of **6** was 84% (0.11 g). ^{1}H NMR (DMSO-d_6) δ: 3.90 (d, 1 H, H_a(7), J = 17.75 Hz); 4.25 (d, 1 H, H_b(7), J = 17.75 Hz); 5.55 (d, 1 H, H(6), J = 3.80 Hz); 7.90 (br.s, 1 H, OH); 11.65 (br.s, 1 H, H(3)). ^{13}C NMR (DMSO-d_6) δ: 46.55 (s, C(7)); 78.80 (d, C(6), J = 25.20 Hz); 107.34 (d, C(5), J = 245.20 Hz); 150.47 (s, C(2)); 158.68 (d, C(4), J = 26.40 Hz); 170.02 (s, C(8)). MS, m/z (I_{rel} (%): 250 $[M - H]^-$ (100); 501 $[2 M - H]^-$ (9). Found (%): C, 28.63; H, 2.33; N, 16.89. $C_6H_6FN_3O_7$. Calculated (%): C, 28.70; H, 2.41; N, 16.73.

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References

1. R. Duschinsky, E. Pleven, C. Heidelberger, *J. Am. Chem. Soc*., 1957, **79**, 4559.
2. C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven, J. Scheiner, *Nature*, 1957, **179**, 663.
3. F. J. Ansfield, A. R. Curreri, *J. Nat. Cancer Inst.*, 1959, **22**, 497.
4. R. D. Safaev, V. I. Babskyi, V. A. Suriko, V. S. Khilko, 2007, http://www.oncology.ru/specialist/journal_oncology/archive/0408/015.pdf.
5. D. V. Mal´tseva, G. S. Zakharova, S. A. Rodin, A. G. Tonevitsky, *Russ. Chem. Bull.*, 2018, **57**, 2148.
6. Yu. I. Murinov, S. A. Grabovskii, N. N. Kabal´nova, *Russ. Chem. Bull.*, 2019, **68**, 946.
7. A. R. Gimadieva, Yu. N. Tchernyshenko, A. G. Mustaphin, I. B. Abdrachmanov, *Bash. Khim. Zhurn*. [*Bashkir J. of Chem.*], 2007, **14**, No. 3, 5 (in Russian).
8. A. G. Mustaphin, A. R. Gimadieva, A. K. Fattakhov, I. B. Abdrachmanov, *Vestn. Bash. Un-ta* [*Bull. Bashkir Univ.*], 2010, **15**, No. 3, 575 (in Russian).
9. A. G. Mustaphin, A. R. Gimadieva, I. B. Abdrachmanov, G. A. Tolstikov, *Khimiya v Interesah Ustoich. Razvitiya* [*Chemistry for Sustainable Development*], 2008, **16**, No. 6, 735 (in Russian).
10. A. R. Gimadieva, *Syntez, modificatsii i biologitcheskaya activnost uracilov* [*Synthesis, Modifications and Biological Activity of Uracils*], Izd-vo Gilem, Ufa, 2013, 176 pp. (in Russian).
11. I. B. Chernikova, S. L. Khursan, M. Yu. Eropkin, M. S. Yunusov, *Pharm. Chem. J. (Engl. Transl.)*, 2019, **53**, 108.
12. D.-Z. Li, Q.-Z. Zhang, C.-Y. Wang, Y.-L. Zhang, X.-Y. Li, J.-T. Huang, H.-Y. Liu, Zh.-D. Fu, H.-X. Song, J.-P. Lin, T.-F. Ji, X.-D. Pan, *Eur. J. Med. Chem*., 2017, **125**, 1235.
13. X.-H. Xu, G.-M. Yao, Y.-M. Li, J.-H. Lu, Ch.-J. Lin, X. Wang, Ch.-H. Kong, *J. Nat. Prod.*, 2003, **66**, 285.
14. I. B. Chernikova, S. L. Khursan, L. V. Spirikhin, M. S. Yunusov, *Chem. Heterocycl. Compd*., 2015, **51**, 568.
15. V. G. Kasradze, I. B. Ignat´eva, R. A. Khusnutdinov, K. Yu. Suponitsky, M. Yu. Antipin, M. S. Yunusov, *Chem. Heterocycl. Compd.*, 2012, **48**, 1018.
16. I. B. Chernikova, I. Z. Sagadatova, M. S. Yunusov, R. F. Talipov, *Russ. J. Org. Chem.*, 2019, **55**, 325.
17. A. J. Kirby, *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*, Springer, Berlin, 1983, 149 pp.

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