[Chem. Pharm. Bull. 34(2) 893—896 (1986)]

5-Fluorouracil Derivatives. XI.[1] Synthesis of 1-Hexylcarbamoyl-5-fluorouracil Metabolites

SHOICHIRO OZAKI,*,[a] YUTAKA WATANABE,[a] TOSHIO NAGASE,[a] YOSHIMASA IKE,[b] and HARUKI MORI[b]

Faculty of Engineering, Ehime University,[a] Bunkyo-cho, Matsuyama 790, Japan, and Research Center, Mitsui Toatsu Chemicals,[b] Kasama-cho, Totsuka-ku, Yokohama 247, Japan

(Received June 14, 1985)

Main metabolites of 1-hexylcarbamoyl-5-fluorouracil, *i.e.*, 1-(5-carboxypentylcarbamoyl)-, 1-(3-carboxypropylcarbamoyl)-, 1-(5-hydroxyhexylcarbamoyl)- and 1-(5-oxohexylcarbamoyl)-5-fluorouracils, were synthesized on a large scale.

Keywords——5-fluorouracil; 1-hexylcarbamoyl-5-fluorouracil; antitumor agent; 1-(5-carboxypentylcarbamoyl)-5-fluorouracil; 1-(3-carboxypropylcarbamoyl)-5-fluorouracil; 1-(5-hydroxyhexylcarbamoyl)-5-fluorouracil; 1-(5-oxohexylcarbamoyl)-5-fluorouracil; metabolite; 1-hexylcarbamoyl-5-fluorouracil

1-Hexylcarbamoyl-5-fluorouracil (HCFU, **1**) was synthesized by us[2] as a masked form of 5-fluorouracil and its antitumor activity was studied by Hoshi *et al.*[3] This antitumor agent is now commercially available from Mitsui Pharmaceutical Inc. and is in clinical use.

The metabolic studies of HCFU in rats,[4] rabbits,[5] dogs,[5] mice[6] and humans[7-9] have revealed that the main metabolites are 1-(5-carboxypentylcarbamoyl)-5-fluorouracil (CPEFU, **2**), 1-(3-carboxypropylcarbamoyl)-5-fluorouracil (CPRFU, **3**), 1-(5-hydroxyhexylcarbamoyl)-5-fluorouracil (HHCFU, **4**) and 1-(5-oxohexylcarbamoyl)-5-fluorouracil (OHCFU, **5**), as well as 5-fluorouracil (5-FU) and its metabolites such as α-fluoro-β-alanine and 6-aminohexanoic acid.

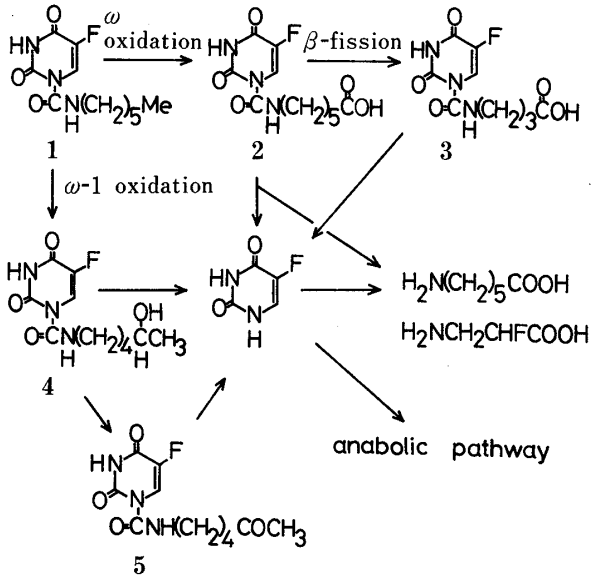

Chart 1. Possible Metabolic Pathways of HCFU

In order to study the toxicity and the antitumor activity of these metabolites and also to obtain authentic samples for comparison, we have prepared **2**, **3**, **4**, and **5** in large quantities. Studies on their toxicity[10,11] and antitumor activities[12] and comparison of the synthetic samples with samples obtained from human serum[7,9] have already been reported. Even though synthetic methods for these metabolites were briefly described by us in the patent,[13] methods for large-scale synthesis have not been reported in detail. We now wish to describe in detail the syntheses of these HCFU metabolites.

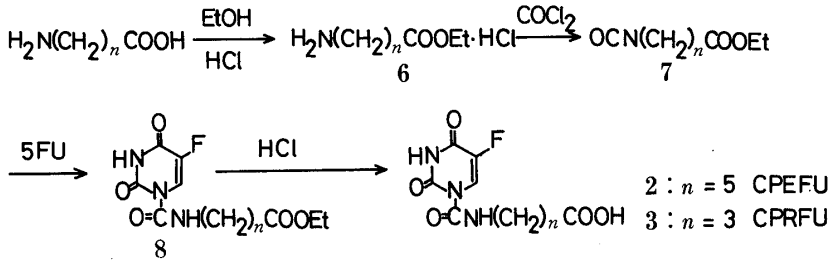

Chart 2

The reaction of 6-aminohexanoic acid and 4-aminobutyric acid with ethanol in the presence of dry hydrogen chloride gave ester hydrochlorides **6a** and **6b**. They were reacted with phosgene to afford ethyl 6-isocyanatohexanoate (**7a**) and ethyl 4-isocyanatobutyrate (**7b**). The reaction of 5-FU with **7a** and **7b** gave 1-(5-ethoxycarbonylpentylcarbamoyl)-5-fluorouracil (**8a**) and 1-(3-ethoxycarbonylpenthylcarbamoyl)-5-fluorouracil (**8b**). Hydrolysis of these products with concentrated hydrochloric acid afforded **2** and **3**, respectively. It is remarkable that the carbon–nitrogen bond between the nitrogen atom of 5-FU and the carbonyl group is not degraded by boiling in strongly acidic hydrochloric acid, even though this bond may be gradually hydrolyzed at 36.5 °C (body temperature) in neutral aqueous conditions. This is the reason why HCFU is a good antitumor agent which decomposes gradually in tumor cells. Since HCFU is administered orally, it must be stable in the stomach, where the enviroment is strongly acidic.

Compounds **4** and **5** were prepared by the method shown in Chart 3. The reaction of ethyl acetoacetate with acrylonitrile in the presence of sodium ethoxide gave ethyl 1-cyano-4-oxo-3-pentanecarboxylate (**9**). Alkaline hydrolysis of **9** at 100 °C gave 5-oxohexanitrile (**10**) in good yield. The reaction of **10** with ethylene glycol gave 5,5-ethylenedioxyhexanitrile (**11**) in very good yield. Reduction of **11** with lithium aluminium hydride gave 5,5-ethylenedioxy-hexylamine (**12**). The amine **12** was reacted with 1-chlorocarbonyl-5-fluorouracil (obtained from 5-FU and phosgene) to afford 1-(5,5-ethylenedioxyhexylcarbamoyl)-5-fluorouracil (**13**).

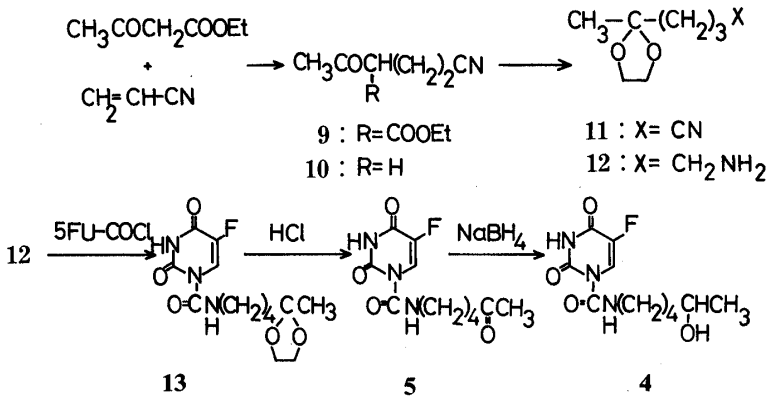

Chart 3

Hydrolysis of **13** afforded **5**. Reduction of **5** with sodium borohydride gave **4**.

The materials obtained from the serum of humans,[7-9] rats,[4] mice,[6] and dogs[5] were found to be identical with **2**, **3**, **4**, and **5**. The materials were also used for studies on toxicity[10,11] and antitumor activity.[12]

Experimental

Ethyl 6-Isocyanatohexanoate (7a)——Phosgene gas (330 g, 3.34 mol) was bubbled at room temperature through a mixture of ethyl 6-aminohexanoate hydrochloride (**6a**, 596.74 g, 3.049 mol) and toluene (4 l). The reaction mixture was heated at 60—110 °C for 2 h, additional phosgene (165 g, 1.67 mol) was added, and then the mixture was distilled to afford **7a** (412.05 g, 72.94%). bp, 120 °C/9 mmHg.

1-(5-Ethoxycarbonylpentylcarbamoyl)-5-fluorouracil (8a)——A mixture of 5-FU (241.14 g, 1.85 mol), **7a** (412.05 g, 2.22 mol) and pyridine (800 ml) was heated at 90 °C for 3 h, then allowed to cool. Pyridine was evaporated off and the residue was dissolved in CH_2Cl_2 (3 l). The organic layer was washed with hydrochloric acid (1 l) and dried over Na_2SO_4. Evaporation of CH_2Cl_2 and washing with ethyl ether gave **8a** (531.2 g, 90.9%). Recrystallization of **8a** from ethanol gave pure **8a**. mp, 108 °C. *Anal.* Calcd for $C_{13}H_{18}FN_3O_5$: C, 49.52; H, 5.75; N, 13.32. Found: C, 49.61; H, 5.61; N, 13.19.

1-(5-Carboxypentylcarbamoyl)-5-fluorouracil (2)——A mixture of **8a** (475.3 g, 1.507 mol) and conc. HCl (4055 g) was heated at 80 °C. Within 10 min, **8a** was dissolved and then a new white solid was soon precipitated. After cooling at 0 °C and sludging with ice water, the solid was filtered off, washed with cold ethanol and dried to afford **2** (342.9 g, 79.21%). mp, 146 °C. NMR (DMSO-d_6) δ: 1.2—1.7 (6H, m, CH_2), 2.2 (2H, t, CH_2CO), 3.3 (2H, m, CH_2NH), 8.4 (1H, d, C_6-H), 9.2 (1H, br, $NHCH_2$). *Anal.* Calcd for $C_{11}H_{14}FN_3O_5$: C, 46.00; H, 4.91; N, 14.62. Found: C, 45.89; H, 4.81; N, 14.55.

Ethyl 4-Isocyanatobutyrate (7b)——Ethyl 4-aminobutyrate hydrochloride (**6b**, 116.0 g, 0.692 mol) and phosgene (138 g, 1.38 mol) were reacted as described for the preparation of **7a**. Yield: 61.72 g (56.8%) of **7b**. bp, 81 °C/6 mmHg.

1-(3-Ethoxycarbonylpropylcarbamoyl)-5-fluorouracil (8b)——**7b** (61.70 g, 0.393 mol) and 5-FU (51.0 g, 0.39 mol) were reacted as described for the preparation of **8a**. Yield: 97.14 g (86.4%) of **8b**. mp, 153 °C. *Anal.* Calcd for $C_{11}H_{14}FN_3O_5$: C, 46.00; H, 4.91; N, 14.62. Found: C, 46.11; H, 4.96; N, 14.91.

1-(3-Carboxypropylcarbamoyl)-5-fluorouracil (3)——**8b** (94.0 g, 0.327 mol) and conc. HCl (680 ml) were heated at 80 °C for 25 min. Cooling at 10 °C gave crystals, which were filtered off, washed with cold water, and dried to afford **3** (76.08 g, 89.7%). mp, 154 °C. NMR (DMSO-d_6) δ: 1.75 (2H, m, CH_2), 2.30 (2H, t, CH_2CO), 3.31 (2H, m, CH_2NH), 8.35 (1H, d, C_6-H), 9.15 (1H, br, $NHCH_2$). *Anal.* Calcd for $C_9H_{10}FN_3O_5$: C, 41.71; H, 3.89; N, 16.20. Found: C, 41.52; H, 3.81; N, 16.01.

1-Cyano-4-oxo-3-pentanecarboxyrate (9)——Ethyl acetoacetate (522.2 g, 3.73 mol) was added to a sodium ethoxide solution (prepared from 3 g of sodium in 400 ml of ethanol), then acetonitrile (198 g, 3.73 mol) was added dropwise at 40—45 °C in 3 h. The solution was kept at this temperature for 1 h and ethanol was distilled off. The residue was washed with 200 ml of water containing 10 ml of acetic acid, and then distilled to afford **9** (325 g, 47.6%). bp, 140 °C/4 mmHg. *Anal.* Calcd for $C_9H_{13}NO_3$: C, 59.00; H, 7.15; N, 7.64. Found: C, 58.92; H, 7.27; N, 7.30.

5-Oxohexanitrile (10)——A mixture of **9** (310 g, 1.69 mol), H_2O (3000 ml) and Na_2CO_3 (300 g) was heated under reflux for 4 h. After cooling, K_2CO_3 (100 g) was added and then the solution was extracted with ether three times (500 ml + 300 ml + 300 ml) and with dichloromethane four times (500 ml + 3 × 300 ml). After drying with Na_2SO_4, the solvent was evaporated off and the residue was distilled to give **10** (182.3 g, 96.9%), boiling at 134 °C/34 mmHg. NMR (neat) δ: 1.82 (2H, t, J = 7 Hz, CH_2), 2.08 (3H, s, CH_3), 2.40 (2H, t, J = 7 Hz, CH_2CN), 2.59 (2H, t, J = 7 Hz, $COCH_2$). IR (neat): 2950, 2240 (CN), 1712 (s, C=O), 1428, 1370, 1165 cm^{-1}.

5,5-Ethylenedioxyhexanitrile (11)——A mixture of **10** (66.69 g, 0.6 mol), ehtylene glycol (217 g, 3.5 mol), *p*-toluenesulfonic acid (3.2 g) and toluene (7 l) was heated. Two liters of toluene was distilled off in 7 h. The reaction mixture was cooled and washed with water (1 l) saturated with $NaHCO_3$ and then with water (1 l). The toluene solution was dried with Na_2SO_4 and toluene was distilled off to give **11** (91.54 g, 98.3%). NMR (neat) δ: 1.22 (3H, s, CH_3), 1.64 (4H, m, $(CH_2)_2$), 2.35 (2H, m, CH_2CN), 3.92 (4H, m, OCH_2CH_2O).

5,5-Ethylenedioxyhexylamine (12)——A solution of **11** (91.5 g, 0.59 mol) in ethyl ether (100 ml) was added dropwise to a mixture of $LiAlH_4$ (56.9 g, 1.50 mol) and ethyl ether (2 l). The reaction mixture was heated for 3 h under reflux, then cooled. H_2O (57 ml), 15% NaOH aqueous solution (57 ml) and H_2O (171 ml) were added, and the mixture was filtered. The white precipitate was washed well with ethyl ether. The combined ethyl ether solution was dried with Na_2SO_4 and concentrated to give **12** (78.6 g, 83.7%), boiling at 63.5 °C/3 mmHg. NMR (neat) δ: 1.22 (3H, s, CH_3), 1.41 (6H, m, $(CH_2)_3$), 2.60 (4H, s, $CH_2 + NH_2$), 3.81 (4H, s, OCH_2CH_2O), IR (neat): 3370, 2940 (S), 2880, 1600, 1460, 1380, 1220, 1060 (S), 947, 850 cm^{-1}.

1-(5,5-Ethylenedioxyhexylcarbamoyl)-5-fluorouracil (13)——Phosgene gas (4.75 g, 0.048 mol evolved from trichloromethyl chloroformate 4.75 g) was passed at 5 °C through a cooled solution of 5-fluorouracil (5.2 g, 0.04 mol) in pyridine (110 ml). A mixture of **8** (6.0 g, 0.0377 mol) and triethylamine (8.88 g, 0.0879 mol) was added at 0—7 °C and

the resulting mixture was stirred for 30 min at 5 °C then for 30 min at 20 °C. The pyridine hydrochloride was filtered off and pyridine was evaporated off. CH_2Cl_2 (300 ml) was added to the residue, and 5-fluorouracil (1.2 g) was filtered off. The filtrate was evaporated and the residue was recrystallized from ethanol to give **13** (5.8 g, 48.8%). mp, 124 °C. NMR ($CDCl_3$) δ: 1.30 (3H, s, CH_3), 1.62 (4H, m, $(CH_2)_3$), 3.41 (2H, q, $J=7$ Hz, $NHCH_2$), 3.97 (4H, s, OCH_2CH_2O), 8.45 (1H, d, $J=7$ Hz, C_6-H), 9.08 (1H, t, $J=7$ Hz, NH), 9.87 (1H, br, N_3-H). IR (KBr): 3290, 3050, 1740 (C=O), 1715, 1525, 1342, 1270, 1220, 1095, 1040, 850, 760 cm^{-1}. *Anal.* Calcd for $C_{13}H_{18}FN_3O_5$: C, 49.52; H, 5.75; F, 6.03; N, 13.32. Found: C, 49.80; H, 6.00; F, 6.11; N, 13.20.

1-(5-Oxohexylcarbamoyl)-5-fluorouracil (5)——A mixture of **13** (2.9 g, 9.2 mmol), conc.HCl (10 ml) and methanol (100 ml) was kept at room temperature (30 °C) for 14 h. Methanol was evaporated off, the residue was dissolved in CH_2Cl_2 (100 ml), and the solution was washed with H_2O (100 ml). The aqueous layer was washed with CH_2Cl_2 (100 ml) and then ethyl acetate (50 ml). The organic layers were combined, dried with Na_2SO_4 and evaporated to dryness. The residue was recrystallized from ethanol to give **5** (2.51 g, 51.1%). mp, 115 °C. NMR ($CDCl_3$) δ: 1.60 (4H, m, CH_2CH_2), 2.13 (3H, s, CH_3), 2.50 (2H, m, CH_2CO), 3.42 (2H, m, $NHCH_2$), 8.50 (1H, d, $J=7$ Hz, C_6-H), 9.12 (1H, t, $NHCH_2$), 10.0 (1H, b, N_3-H). IR (KBr): 3300, 3090, 3050, 2850, 1742 (C=O), 1690, 1530, 1342, 1272, 1225, 1095, 1040, 870 cm^{-1}. *Anal.* Calcd for $C_{11}H_{14}FN_3O_4$: C, 48.71; H, 5.20; F, 7.01; N, 15.49. Found: C, 48.41; H, 5.02; F, 6.99; N, 15.18.

1-(5-Hydroxyhexylcarbamoyl)-5-fluorouracil (4)——Sodium borohydride (6.27 g, 0.166 mol) was added to a mixture of **5** (40.90 g, 0.151 mol) and methanol (2 l) at 5—10 °C in 1 h. The reaction mixture was kept at this temperature for 30 min, then the methanol was evaporated off under reduced pressure. The residue was dissolved in CH_2Cl_2 (5 l) and washed with conc. HCl (200 ml). The organic layer was dried with Na_2SO_4 and the solvent was evaporated off. The residue was recrystallized from ethanol to give **4** (18.0 g, 43.7%). mp, 127 °C. NMR (DMSO-d_6) δ: 1.04 (3H, d, CH_3), 1.44 (6H, m, CH_2), 3.28 (2H, m, $NHCH_2$), 3.56 (1H, m, CH), 4.26 (1H, br, OH), 8.34 (1H, d, C_6-H), 9.10 (1H, t, $NHCH_2$), 12.20 (1H, s, N_3-H). IR (KBr): 3360, 3080, 2820, 1740 (C=O), 1695, 1545, 1342, 1280, 1095 cm^{-1}. *Anal.* Calcd for $C_{11}H_{16}FN_3O_4$: C, 48.35; H, 5.90; F, 6.95; N, 15.37. Found: C, 48.36; H, 5.86; F, 6.59; N, 15.37.

References

1) Preceding paper in this series, S. Ozaki, Y. Watanabe, T. Hoshiko, T. Nagase, T. Ogasawara, H. Furukawa, A. Uemura, K. Ishikawa, H. Mori, A. Hoshi, M. Iigo, and R. Tokuzen, *Chem. Pharm. Bull.*, **34**, 150 (1986).
2) S. Ozaki, Y. Ike, H. Mizuno, K. Ishikawa, and H. Mori, *Bull. Chem. Soc. Jpn.*, **50**, 2406 (1977).
3) A. Hoshi, M. Iigo, A. Nakamura, M. Yoshida, and K. Kuretani, *Gann*, **67**, 725 (1976).
4) T. Kobari, K. Tan, M. Kumakura, S. Watanabe, I. Shirakawa, H. Kobayashi, A. Ujiie, Y. Miyama, H. Namekawa, and H. Yamamoto, *Xenobiotica*, **8**, 547 (1978).
5) T. Kobari, Y. Iguro, A. Ujiie, H. Namekawa, Proceedings of the 11th ICC and the 19th ICAAC American Society of Microbiology, 1980, p. 1584.
6) M. Iigo, A. Hoshi, and K. Kuretani, *Cancer Chemother. Pharmacol.*, **4**, 189 (1980).
7) A. Kono, M. Tanaka, S. Eguchi, and Y. Hara, *J. Chromatogr.*, **163**, 109 (1979).
8) A. Kono, Y. Hara, S. Eguchi, and M. Tanaka, *J. Chromatogr.*, **182**, 125 (1980).
9) Y. Koyama, *Gan To Kagakuryoho*, **7**, 1181 (1980).
10) K. Horigome, K. Ozeki, T. Matsushima, Z. Hemmi, K. Maruyama, H. Majima, and Y. Sakai, *Rinsho Yakuri*, **11**, 27 (1980).
11) K. Horigome, K. Muramoto, K. Arai, Z. Hemmi, and K. Sakai, *Rinsho Yakuri*, **11**, 17 (1980).
12) A. Hoshi, M. Yoshida, M. Inomata, M. Iigo, N. Ando, and K. Kuretani, *J. Pharmacobio-Dyn.*, **3**, 478 (1980).
13) Mitsui Toatsu Chemicals Inc., Japan. Patent Open 57-85374 (1982) [*Chem. Abstr.* **98**, 16713*u* (1983)].