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

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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 us²⁾ 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⁾ mice⁶⁾ and humans⁷⁻⁹⁾ 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.

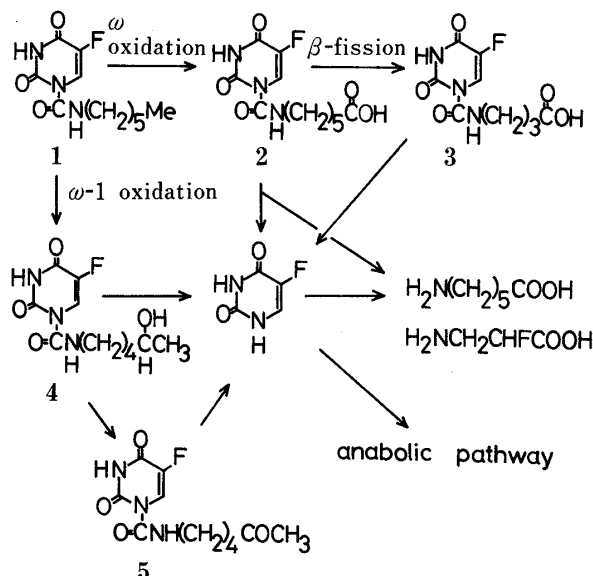


Chart 1. Possible Metabolic Pathways of HCFU.

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 activities¹²⁾ 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.

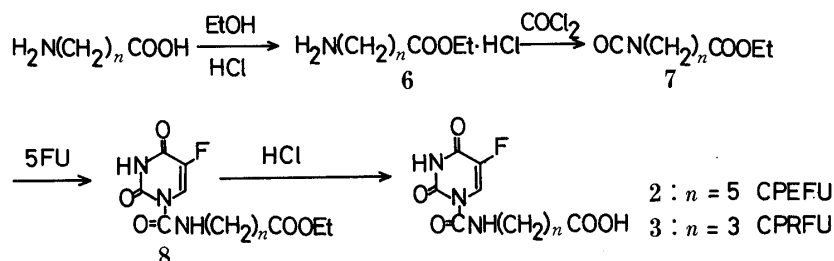


Chart 2

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-ethoxycarbonylpentylcarbamoyl)-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 environment 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-oxohexanenitrile (**10**) in good yield. The reaction of **10** with ethylene glycol gave 5,5-ethylenedioxyhexanenitrile (**11**) in very good yield. Reduction of **11** with lithium aluminium hydride gave 5,5-ethylenedioxyhexylamine (**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**).

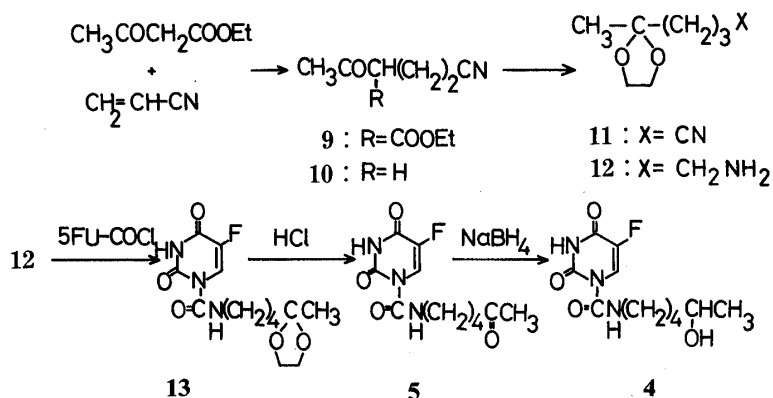


Chart 3

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 dogs⁵⁾ 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 CH₂Cl₂ (3 l). The organic layer was washed with hydrochloric acid (1 l) and dried over Na₂SO₄. Evaporation of CH₂Cl₂ 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 C₁₃H₁₈FN₃O₅: 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, CH₂), 2.2 (2H, t, CH₂CO), 3.3 (2H, m, CH₂NH), 8.4 (1H, d, C₆-H), 9.2 (1H, br, NHCH₂). *Anal.* Calcd for C₁₁H₁₄FN₃O₅: 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 C₁₁H₁₄FN₃O₅: 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, CH₂), 2.30 (2H, t, CH₂CO), 3.31 (2H, m, CH₂NH), 8.35 (1H, d, C₆-H), 9.15 (1H, br, NHCH₂). *Anal.* Calcd for C₉H₁₀FN₃O₅: 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 C₉H₁₃NO₃: 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), H₂O (3000 ml) and Na₂CO₃ (300 g) was heated under reflux for 4 h. After cooling, K₂CO₃ (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 Na₂SO₄, 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, CH₂), 2.08 (3H, s, CH₃), 2.40 (2H, t, *J* = 7 Hz, CH₂CN), 2.59 (2H, t, *J* = 7 Hz, COCH₂). 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), ethylene 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 NaHCO₃ and then with water (1 l). The toluene solution was dried with Na₂SO₄ and toluene was distilled off to give **11** (91.54 g, 98.3%). NMR (neat) δ: 1.22 (3H, s, CH₃), 1.64 (4H, m, (CH₂)₂), 2.35 (2H, m, CH₂CN), 3.92 (4H, m, OCH₂CH₂O).

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 LiAlH₄ (56.9 g, 1.50 mol) and ethyl ether (2 l). The reaction mixture was heated for 3 h under reflux, then cooled. H₂O (57 ml), 15% NaOH aqueous solution (57 ml) and H₂O (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 Na₂SO₄ and concentrated to give **12** (78.6 g, 83.7%), boiling at 63.5 °C/3 mmHg. NMR (neat) δ: 1.22 (3H, s, CH₃), 1.41 (6H, m, (CH₂)₃), 2.60 (4H, s, CH₂ + NH₂), 3.81 (4H, s, OCH₂CH₂O), 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. CH₂Cl₂ (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 (CDCl₃) δ: 1.30 (3H, s, CH₃), 1.62 (4H, m, (CH₂)₃), 3.41 (2H, q, *J* = 7 Hz, NHCH₂), 3.97 (4H, s, OCH₂CH₂O), 8.45 (1H, d, *J* = 7 Hz, C₆-H), 9.08 (1H, t, *J* = 7 Hz, NH), 9.87 (1H, br, N₃-H). IR (KBr): 3290, 3050, 1740 (C=O), 1715, 1525, 1342, 1270, 1220, 1095, 1040, 850, 760 cm⁻¹. Anal. Calcd for C₁₃H₁₈FN₃O₅: 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 CH₂Cl₂ (100 ml), and the solution was washed with H₂O (100 ml). The aqueous layer was washed with CH₂Cl₂ (100 ml) and then ethyl acetate (50 ml). The organic layers were combined, dried with Na₂SO₄ and evaporated to dryness. The residue was recrystallized from ethanol to give **5** (2.51 g, 51.1%). mp, 115 °C. NMR (CDCl₃) δ: 1.60 (4H, m, CH₂CH₂), 2.13 (3H, s, CH₃), 2.50 (2H, m, CH₂CO), 3.42 (2H, m, NHCH₂), 8.50 (1H, d, *J* = 7 Hz, C₆-H), 9.12 (1H, t, NHCH₂), 10.0 (1H, b, N₃-H). IR (KBr): 3300, 3090, 3050, 2850, 1742 (C=O), 1690, 1530, 1342, 1272, 1225, 1095, 1040, 870 cm⁻¹. Anal. Calcd for C₁₁H₁₄FN₃O₄: 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 CH₂Cl₂ (5 l) and washed with conc. HCl (200 ml). The organic layer was dried with Na₂SO₄ 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, CH₃), 1.44 (6H, m, CH₂), 3.28 (2H, m, NHCH₂), 3.56 (1H, m, CH), 4.26 (1H, br, OH), 8.34 (1H, d, C₆-H), 9.10 (1H, t, NHCH₂), 12.20 (1H, s, N₃-H). IR (KBr): 3360, 3080, 2820, 1740 (C=O), 1695, 1545, 1342, 1280, 1095 cm⁻¹. Anal. Calcd for C₁₁H₁₆FN₃O₄: 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.

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