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Synthesis of 3-Amino-3-(5-fluorouracil-1-yl)propionic Acid and 4-Amino-4-(5-fluorouracil-1-yl)butyric Acid Derivatives

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3-Acylamino-3-(5-fluorouracil-1-yl)propionates (**3a, b**) and 4-acylamino-4-(5-fluorouracil-1-yl)butyrates (**3c—e**) were synthesized from 3-acylamino-3-carboxypropionates (**1a, b**) and 4-acylamino-4-carboxybutyrates (**1c—e**), respectively, *via* electrochemical oxidation. Catalytic hydrogenolysis of the benzyl esters (**3a, c, d**) gave the corresponding carboxylic acids (**4a′, c′, d′**) without cleavage of the geminal diamine moieties.

Keywords——5-fluorouracil; substitution reaction; geminal diamines; 3-acylamino-3-(5-fluorouracil-1-yl)propionates; 4-acylamino-4-(5-fluorouracil-1-yl)butyrates; aspartic acid derivatives; glutamic acid derivatives; anodic oxidation; 3-acetamidoacrylates

5-Fluorouracil is well-known to exhibit antitumor activity.[2] It has, however, a serious drawback for clinical use in that it shows strong toxicity. For this reason, recent efforts have increasingly been focused on the search for "masked" compounds of 5-fluorouracil with reduced toxicity.[3] Various substituents have been introduced at the N^1-position of 5-fluorouracil[4] on the premise that the derivatives would be susceptible to degradation in biological processes to release 5-fluorouracil. It seems likely that the structural feature of the substituents, especially the nature of the chemical bonds between 5-fluorouracil and the substituents are a major determinant of toxicity. Most recently, derivatives having a geminal diamine moiety (Fig. 1) have been suggested to decompose with the elimination of 5-fluorouracil[5,6] in biological processes.

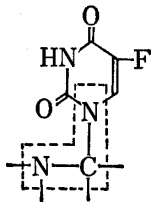


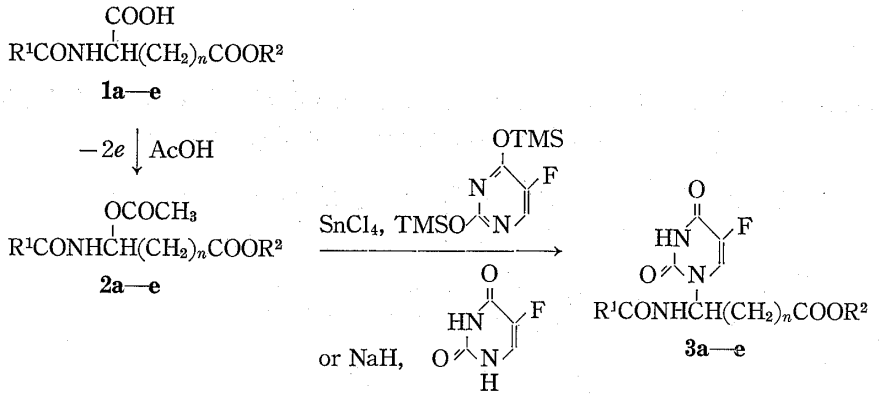
Fig. 1. Geminal Diamine Moiety of 5-Fluorouracil Derivatives

We have been interested in the synthesis and antitumor activities of 5-fluorouracil containing amino acids in which geminal diamine skeletons are involved. In the previous paper,[5] we reported a general method for the synthesis of 2-(pyrimidin-1-yl)-2-amino acids. In this article, a synthesis of 3-amino-3-(5-fluorouracil-1-yl)propionic acid and 4-amino-4-(5-fluorouracil-1-yl) butyric acid derivatives is described.

3-Amino-3-(5-fluorouracil-1-yl)propionates

A synthesis of 3-acylamino-3-(5-fluorouracil-1-yl)propionates (**3a, b**) involves the replacement of the 3-carboxylic acid groups of 3-acylamino-3-carboxypropionates (aspartic acid

1) Location: *3-16-89 Kashima, Yodogawa-ku, Osaka 532, Japan.*
2) C. Heidelberger, N.K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R.J. Schnitzer, E. Pleven, and J. Scheiner, *Nature*(London), **179**, 663 (1957).
3) M. Yasumoto, I. Yamawaki, T. Marunaka, and S. Hashimoto, *J. Med. Chem.*, **21**, 738 (1978) and references cited therein.
4) S. Ozaki, Y. Ike, H. Mizuno, K. Ishikawa, and H. Mori, *Bull. Chem. Soc. Jpn.*, **50**, 2406 (1977) and references cited therein.
5) T. Nishitani, T. Iwasaki, Y. Mushika, and M. Miyoshi, *J. Org. Chem.*, **44**, 2019 (1979).
6) The stability of geminal diamines in aqueous solutions has been discussed by several authors. See, for example, G. Moad and S.J. Benkovic, *J. Am. Chem. Soc.*, **100**, 5495 (1978).



a: $n=1$, $R^1=Me$, $R^2=CH_2Ph$
b: $n=1$, $R^1=Me$, $R^2=Et$
c: $n=2$, $R^1=Me$, $R^2=CH_2Ph$
d: $n=2$, $R^1=Ph$, $R^2=CH_2Ph$
e: $n=2$, $R^1=PhCH_2O$, $R^2=CH_2Ph$

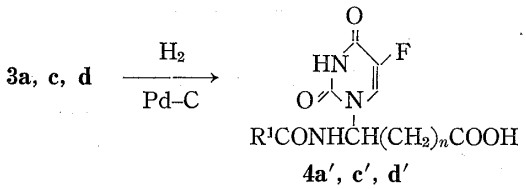
TMS=Si(Me)$_3$

Chart 1

derivatives) (**1a, b**) with N^1-position of 5-fluorouracil *via* electrochemical oxidation, as depicted in Chart 1. Benzyl 4-acetamido-3-acetoxypropionate (**2a**), which was obtained by anodic oxidation of compound (**1a**),[7] was allowed to react with 2,4-bis(trimethylsilyl)-5-fluorouracil in the presence of stannic chloride at −30° to afford benzyl 3-acetamido-3-(5-fluorouracil-1-yl)propionate (**3a**) in 27% yield. In this reaction, the elimination products, *trans*- and *cis*-3-acetamidoacrylic acid benzyl esters, were formed in 62% yield; the ratio of the *trans* and *cis* isomers was 3: 1. Compound (**3b**) was also prepared under the same conditions.

Although the reaction of the acetoxy compound (**2a, b**) with 5-fluorouacil was carried out in the presence of sodium hydride under the conditions employed in the previous paper,[5] no formation of the desired substiution products was detected; the elimination products were formed exclusively.

A facile elimination reaction to N-acyl-2,3-dehydroamino acids[8] was observed on treating 2-(pyrimidin-1-yl)-2-amino acid with a base.[5] It should be noted that the 3-acylamino-3-(5-fluorouracil-1-yl)propionates (**3a, b**) obtained here are more labile than 2-(pyrimidin-1-yl)-2-amino acids under basic conditions, due to the presence of the rather acidic C-2 protons of the amino acid moieties in compounds (**3a, b**). For example, when compound (**3a**) was treated with one molar equivalent of sodium acetate, it was quantitatively converted to a mixture of 5-fluorouracil and *trans*- and *cis*-3-acetamidoacrylic acid benzyl esters. Accordingly, in the above coupling reactions, the reaction should be quenched carefully by using one molar equivalent of sodium hydrogen carbonate with respect to stannic chloride.



a′: $n=1$, $R^1=Me$
c′: $n=2$, $R^1=Me$
d′: $n=2$, $R^1=Ph$

Chart 2

In order to obtain a derivative bearing an unprotected carboxylic acid moiety, compound (**3a**) was hydrogenolyzed in dioxane over palladium on charcoal. The desired carboxylic acid (**4a′**) was obtained in 98% yield without appreciable cleavage of the geminal diamine moiety.

7) T. Iwasaki, H. Horikawa, K. Matsumoto, and M. Miyoshi, *J. Org. Chem.*, **42**, 2419 (1977).
8) Elimination reactions of 2-functionalized-2-amino acids into the corresponding 2,3-dehydroamino acids have been reported. See T. Iwasaki, H. Horikawa, K. Matsumoto, and M. Miyoshi, *Bull. Chem. Soc. Jpn.*, **52**, 826 (1979) and references cited therein.

The coupling position of 5-fluorouracil in these derivatives (**3a, b, 4a'**) was confirmed by the UV spectral data. The derivatives gave UV spectra characteristic of N^1-alkylated 5-fluorouracils,[5,9] for example, compound (**3a**) shows absorption maxima at almost the same wavelength in methanol (278 nm) and 0.01 N NaOH (279 nm).

The yields and some properties of the derivatives are shown in Table 1.

Table I. Yields and Some Properties of 5-Fluorouracil Derivatives

Compd. No.	Yield (%)	mp (°C) (dec.)	UV λ_{max}^{MeOH} nm (log ε)	Analysis (%) Calcd (Found) C	H	N	F	NMR (DMSO-d_6), δ
3a	27	196	268 (3.93)	55.01 (54.91	4.62 4.63	12.03 12.09	5.44 5.69)	1.86 (3H, s), 3.03 (2H, d), 5.11 (2H, s), 5.9—6.4 (1H, m), 7.32 (5H, s), 7.87 (1H, d, J=7 Hz), 8.78 (1H, d), 11.73 (1H, br s)
3b	22	196	268 (3.94)	45.99 (46.18	4.91 4.88	14.63 14.55	6.62 6.38)	1.18 (3H, t), 1.88 (3H, s), 2.94 (2H, d), 4.06 (2H, q), 5.9—6.4 (1H, m), 7.87 (1H, d, J=7 Hz), 8.74 (1H, d), 11.73 (1H, br s)
3c	60	190	269 (3.50)	56.19 (56.18	4.99 4.99	11.57 11.50	5.23 5.11)	1.89 (3H, s), 2.14 (2H, t), 2.3—2.7 (2H, m), 5.09 (2H, s), 5.7—6.2 (1H, m), 7.36 (5H, s), 7.83 (1H, d, J=7 Hz), 8.66 (1H, d) 11.73 (1H, br s)
3d	57	213	269 (3.94)	62.11 (61.95	4.74 4.68	9.88 9.84	4.47 4.54)	2.0—2.8 (4H, m), 5.12 (2H, s), 6.1—6.6 (1H, m), 7.36 (5H, s), 7.4—8.0 (5H, m), 8.06 (1H, d, J=7 Hz), 9.08 (1H, d), 11.81 (1H, br s)
3e	59	173	266 (3.81)	60.65 (60.55	4.87 4.94	9.23 9.21	4.17 4.08)	2.14 (2H, t), 2.2—2.7 (2H, m), 5.09 (4H, s), 5.7—6.2 (1H, m), 7.35 (10H, s), 7.83 (1H, d, J=7 Hz), 8.24 (1H, d), 11.76 (1H, br d)
4a'	98	193	269 (3.89)	41.70 (41.53	3.89 4.02	16.21 16.04	7.33 7.18)	1.90 (3H, s), 2.89 (2H, d), 5.8—6.3 (1H, m), 7.89 (1H, d, J=7 Hz), 8.76 (1H, d), 11.5—12.0 (1H, br)
4c'	98	185	271 (3.92)	43.96 (43.60	4.43 4.61	15.38 14.95	6.95 6.69)	1.89 (3H, s), 2.0—2.4 (4H, m), 5.7—6.2 (1H, m), 7.82 (1H, d, J=7 Hz), 8.62 (1H, d), 11.4—12.1 (1H, br)
4d'	96	203	270 (4.00)	53.73 (53.78	4.21 4.29	12.53 12.56	5.67 5.81)	2.0—2.5 (4H, m), 6.1—6.5 (1H, br), 7.4—8.0 (5H, m), 8.00 (1H, d, J=7 Hz), 9.06 (1H, d), 11.5—12.2 (1H, br)

4-Acylamino-4-(5-fluorouracil-1-yl)butyrates

4-Acylamino-4-(5-fluorouracil-1-yl)butyrates (**3c—e**) were obtained from the corresponding 4-acylamino-4-carboxybutyrates (glutamic acid derivatives) according to Chart 1.

Benzyl 4-acetamido-4-carboxybutyrate (**1c**) was electrolyzed in a mixture of acetic acid and tetrahydrofuran (3:1) using a graphite anode-graphite cathode in a nondivided cell to afford the acetoxy compound (**2c**) in a good yield. Making use of the procedure reported by us,[5,10] the coupling reaction of the acetoxy compound (**2c**) with 5-fluorouracil in the presence of sodium hydride proceeded smoothly to afford compound (**3c**) in 63% yield. The other compounds (**3d, e**) were also obtained from the corresponding butyric acid derivatives (**1d, e**) in good yields.

Hydrogenolysis of compounds (**3c, d**) under the conditions described above led to the formation of the corresponding unprotected carboxylic acids (**4c', d'**) in almost quantitative yields.

9) A. Albert, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 2, ed. by W.W. Zorbach and R.S. Tipson, Wiley Interscience, New York, 1973, pp. 47—123.
10) Y. Ozaki, T. Iwasaki, H. Horikawa, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, **44**, 391 (1979).

The 4-acylamino-4-(5-fluorouracil-1-yl)butyric acid derivatives obtained above are far more stable than 2-(5-fluorouracil-1-yl)-2-amino acids derivatives[5] and 3-acylamino-3-(5-fluorouracil-1-yl)propionic acid derivatives under both basic and acidic conditions.

These compounds were charaterized by determination of their spectral data and by elemental analyses. The results are summarized in Table 1.

Evaluation of the antitumor activities of the 5-fluorouracil derivatives is in progress and the results will be reported elsewhere.

Experimental

Melting points were measured using a Yamato melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-27G infrared spectrophotometer. NMR spectra were obtained using a Hitachi Perkin-Elmer R-20 high-resolution NMR spectrometer with tetramethylsilane as an internal standard. UV spectra were measured on a Hitachi EPS-3T spectrometer. Mass spectra were taken with a Hitachi M-60 mass spectrometer. The electrolyses were carried out with a Hokuto Potentio-Galvanostat PGS-108 (2A-120V) attached to a Hokuto HA-108A coulomb meter.

Preparation of Compounds (1)——Compounds (**1a, b**) and compounds (**1c—e**) were prepared from aspartic acid and glutamic acid, respectively, by the usual procedure.[11] The physical constants of these compounds are given elsewhere.[12]

Preparation of Compounds (2a—e)——Compounds (**2a, b**) were prepared according to the method developed in our laboratory.[7] Compounds (**2c—e**) were synthesized as follows. The carboxylic acid (**1d**) (13.6 g, 0.04 mol) and sodium acetate (0.8 g, 0.01 mol) were dissolved in a mixture of 60 ml of acetic acid and 20 ml of tetrahydrofuran. The solution was put in an ordinary beaker (4 cm in diameter and 10 cm in height), and electrolyzed at a constant current of 0.4 A at 15—20° using a graphite anode (3×4 cm^2)-graphite cathode in a nondivided cell. After the theoretical amount of current had been passed, the electrolyzed solution was evaporated to dryness *in vacuo* below 30°. The residue was extracted with ethyl acetate. The extract was washed once with water, dried over magnesium sulfate, and then evaporated to dryness *in vacuo*. On leaving the resulting residue to stand at −30°, crystals appeared. The crystals were recrystallized from ethyl acetate–hexane to give 12.2 g (86%) of compound (**2d**) as colorless needles: mp 87—88°; IR (Nujol) 3320, 1737, 1731, 1650 cm^{-1}; NMR ($CDCl_3$) δ: 2.03 (3H, s), 1.9—2.7 (4H, m), 5.10 (2H, s), 6.3—6.8 (1H, m), 7.30 (5H, s), 7.1—8.0 (6H, m, Ph+NH); MS (*m/e*): 296 (M–AcO), 295, 204, 189, 173, 161, 160, 146, 141, 106, 105, 91, 77, 65, 56. Compounds (**2c, e**) were also prepared under the same conditions. The physical constants of compounds (**2c, e**) were as follows. Compound (**2c**): colorless syrup; IR (film): 2390 (br), 1736 (br), 1667 (br) cm^{-1}; NMR ($CDCl_3$) δ: 1.95 (3H, s), 2.03 (3H, s), 2.0—2.7 (4H, m), 5.17 (2H, s), 6.2—6.8 (1H, m), 7.1—7.5 (1H, br), 7.35 (5H, s); MS (*m/e*): 234 (M–AcO), 233, 192, 174, 164, 146, 142, 127, 108, 107, 98, 92, 91, 86, 79, 77, 65, 56, 43. Compound (**2e**): faintly yellow syrup; IR (film): 3320 (br), 1726, 1715 cm^{-1}; NMR ($CDCl_3$) δ: 1.7—2.2 (4H, m), 2.02 (3H, s), 5.07 (4H, s), 6.2—6.7 (1H, m), 7.29 (10H, s), 7.8—8.3 (1H, br); MS (*m/e*): 326 (M–AcO), 325, 282, 234, 221, 218, 192, 180, 151, 146, 141, 125, 108, 107, 92, 91, 79, 77, 71, 65, 43.

These acetoxy compounds are unstable to heat, and accordingly should be kept at least below −30°.

Preparation of Compounds (3a, b) (Typical Procedure)——2,4-Bis(trimethylsilyl)-5-fluorouracil (2.74 g, 0.01 mol) and the acetoxy compound (**2a**) (2.79 g, 0.01 mol) were dissolved in 20 ml of acetonitrile. Stannic chloride (0.58 ml, 0.005 mol) was added dropwise at −30° under vigorous stirring. The stirring was continued for 30 min at the same temperature. The reaction was quenched by the addition of 5 ml of water containing 2.02 g of sodium hydrogen carbonate. The organic layer was separated, and the aqueous layer was further extracted with ethyl acetate. The combined organic layer was dried over magnesium sulfate and then evaporated to dryness *in vacuo*. The residue was treated with 100 ml of chloroform and the insoluble materials were filtered off. The filtrate was evaporated to dryness *in vacuo* and the resulting crystals were triturated with ether. The crystals were collected by filtration to give 0.94 g (27%) of compound (**3a**), which yielded colorless needles on recrystallization from ethyl acetate–hexane. The physical constants of compound (**3a**) are shown in Table I.

The filtrate was evaporated to dryness *in vacuo* and the residue was subjected to silica gel chromatography using chloroform–ethanol (20: 1) as an eluent to afford 0.36 g of *cis*- and 1.00 g of *trans*-3-acetamidoacrylic acid benzyl esters. The *cis* and *trans* isomers showed *Rf* values of 0.83 and 0.42, respectively, on TLC with chloroform–ethanol (20: 1) as a developing solvent. The pure *cis*-isomer was obtained as colorless needles by recrystallization from hexane: mp 74—75°; IR (Nujol): 3350, 1715, 1690, 1618 cm^{-1}; NMR ($CDCl_3$) δ: 2.10 (3H, s), 5.15 (1H, d, J=9.0 Hz), 5.16 (2H, s), 7.32 (5H, s), 7.44 (1H, d, d, J=9, 12 Hz), 10.0—10.8 (1H,

11) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, 1961, pp. 883—943.

12) G.R. Pettit, "Synthetic Peptides," Vol. 1, Van Nostrand Reinhold Company, New York, 1970, pp. 9—78.

br); *Anal.* Calcd for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39%. Found: C, 65.52; H, 5.99; N, 6.45%. The pure *trans*-isomer was obtained as colorless needles by recrystallization from ethyl acetate–hexane: mp 104—106°; IR (Nujol): 3320, 1718, 1693, 1626 cm^{-1}; NMR ($CDCl_3$) δ: 2.07 (3H, s), 5.16 (2H, s), 5.52 (1H, d, $J=14$ Hz), 7.32 (5H, s), 8.03 (1H, d, d, $J=14$, 12 Hz), 9.11 (1H, d, $J=12$ Hz); *Anal.* Calcd for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39%. Found: C, 65.91; H, 6.01; N, 6.31%.

Preparation of Compounds (3c—e) (Typical Procedure)——5-Fluorouracil (1.95 g, 0.015 mol) was dissolved in 30 ml of dimethylformamide, then 65% sodium hydride (0.55 g, 0.015 mol) was added in one portion and the mixture was stirred for 30 min at 50°. The mixture was cooled to 5—10° under ice cooling. The acetoxy compound (**2c**) (4.40 g, 0.015 mol) dissolved in 10 ml of dimethylformamide was added dropwise under vigorous stirring, and the reaction mixture was stirred for a further 50 min at the same temperature. Water (150 ml) was added to the mixture, and the pH of the solution was adjusted to 6—7 by addition of acetic acid. The crystals that appeared were collected by filtration to give 2.6 g of compound (**3c**). A second crop (0.68 g) was obtained from the filtrate. Recrystallization from ethyl acetate afforded the pure compound (**3c**) as colorless needles. The physical constants are shown in Table I.

Compound (**3d, e**) were also prepared under the same conditions.

Preparation of Compounds (4a′, c′, d′) (Typical Procedure)——The benzyl ester (**3a**, 0.5 g, 0.0014 mol) was dissolved in 50 ml of dioxane and the solution was subjected to hydrogenolysis over 10% Pd–C (0.1 g) at atmospheric pressure. After the theoretical amount of hydrogen had been absorbed, the catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo*. The resulting crystals were triturated with ethyl acetate. The crystals (0.36 g, 98% yield) were crystallized from ethanol to give colorless needles of compound (**4a′**). The physical constants of this compound are shown in Table I.

Compounds (**4c′, d′**) were also prepared under the same conditions.

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