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Sparsomycin Analogs. IV.[1] Synthesis and Antitumor Activity of Pyrimidine-5-carboxamides and (*E*)-β-(Pyrimidin-5-yl)-acrylamides

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Various pyrimidine-5-carboxamides (**14**, **16**, **18**, **20**, and **21**) and (*E*)-β-(pyrimidin-5-yl)acrylamides (**15**, **17**, **19**, and **22**) were synthesized as sparsomycin analogs, and their antitumor activity was examined by cell growth inhibition assay against mouse leukemia L5178Y cells *in vitro*. Synthesis was carried out by condensation of appropriate acids (**4**, **6**, **10**, and **12**) and amino acid methyl esters (**13**) by the mixed anhydride method using isobutyl chlorocarbonate. The condensation product was converted to the corresponding acid and alcohol derivatives by hydrolysis and $LiBH_4$ reduction. The compounds having an ethylene linkage at the C-5 position and an ester moiety at the terminal amino acid functionality (**15b**, and **17b—g**) exhibited remarkable antitumor activity.

Keywords——sparsomycin; sparsomycin analog; pyrimidine-5-carboxamide; (*E*)-β-(pyrimidin-5-yl)acrylamide; antitumor activity

In 1962, sparsomycin was first isolated from the culture filtrate of *Streptomyces sparsogenes*,[2] although the structure was finally established only a few years ago due to its unique features (Fig. 1).[3] This antibiotic has attracted the interest of a number of chemists because of its broad spectrum of antitumor activity.[4] There have been several studies dealing with the synthesis of sparsomycin analogs.[5-11] These studies can be categorized into two classes. The first involves modification of the amino alcohol part related to the chirality of both carbon and sulfoxide sulfur atoms. For example, Liskamp *et al.* have recently reported that octylsparsomycin exhibits stronger antitumor activity *in vitro* than that of sparsomycin.[11a] The other class of studies deals with modification of the uracil acrylic acid part, with attention focused on exchange of the ring system from uracil to another type, such as benzene or furan.[5] However, there has been no study dealing with direct modification of the uracil ring.

In this paper, we describe the synthesis of pyrimidine ring-modified analogs and examination of their antitumor activities by observing the inhibition of cell growth of mouse leukemia cells *in vitro*.

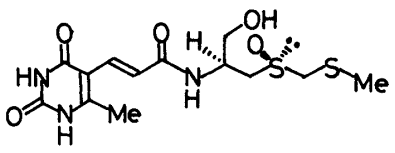


Fig. 1. Structure of Sparsomycin (S_c–R_s) (**1**)

Results and Discussion

The synthetic routes employed for the preparation of intermediate acids (**4**, **6**, **10**, and **12**) are outlined in Chart 1. 5-Formylpyrimidine (**3**), the key intermediate for the *N*,*N*-dimethyl series, was prepared from 6-methyluracil (**2**) in accordance with the previously reported method.[12] Oxidation of **3** with potassium persulfate in the presence of a catalytic amount of silver nitrate gave pyrimidine-5-carboxylic acid (**4**) in excellent yield. On the other hand, treatment of **3** with triphenylcarboethoxymethylenephosphorane in benzene at 80 °C gave the acrylate (**5**). The isolation of **5** was unsuccessful, and thus KOH–MeOH hydrolysis of the resulting ester **5** was carried out without isolation to afford (*E*)-*β*-(pyrimidin-5-yl)acrylic acid (**6**) exclusively in the *trans* configuration, as determined from photo-isomerization and nuclear magnetic resonance (NMR) spectra.[13] Another series of dimethoxy acids (**10** and **12**) was obtained from 5-halo-2,4-dimethoxypyrimidines (**8** and **9**), prepared by halogenation of **7**.[14] After the halogen lithium exchange of **8** with *n*-BuLi, followed by treatment with carbon dioxide, the 5-carboxylic acid (**10**) was obtained. (*E*)-*β*-(2,4-Dimethoxypyrimidin-5-yl)acrylic acid (**12**) was prepared by basic hydrolysis of the corresponding methyl ester (**11**), which was easily prepared from the coupling of 5-iodopyrimidine (**9**) with methyl acrylate in excellent yield.[15]

The condensation of carboxylic acids (**4**, **6**, **10**, and **12**) with amino acid methyl esters (**13**) was performed by the mixed anhydride (MA) method using isobutyl chlorocarbonate (BCC) in moderate to excellent yields.[16] The basic hydrolysis and reduction with lithium borohydride of the products afforded the corresponding acids (**18**—**20**) and alcohol derivatives (**21** and **22**). However, in the reduction of the *N*,*N*-dimethyl condensation products (**14** and

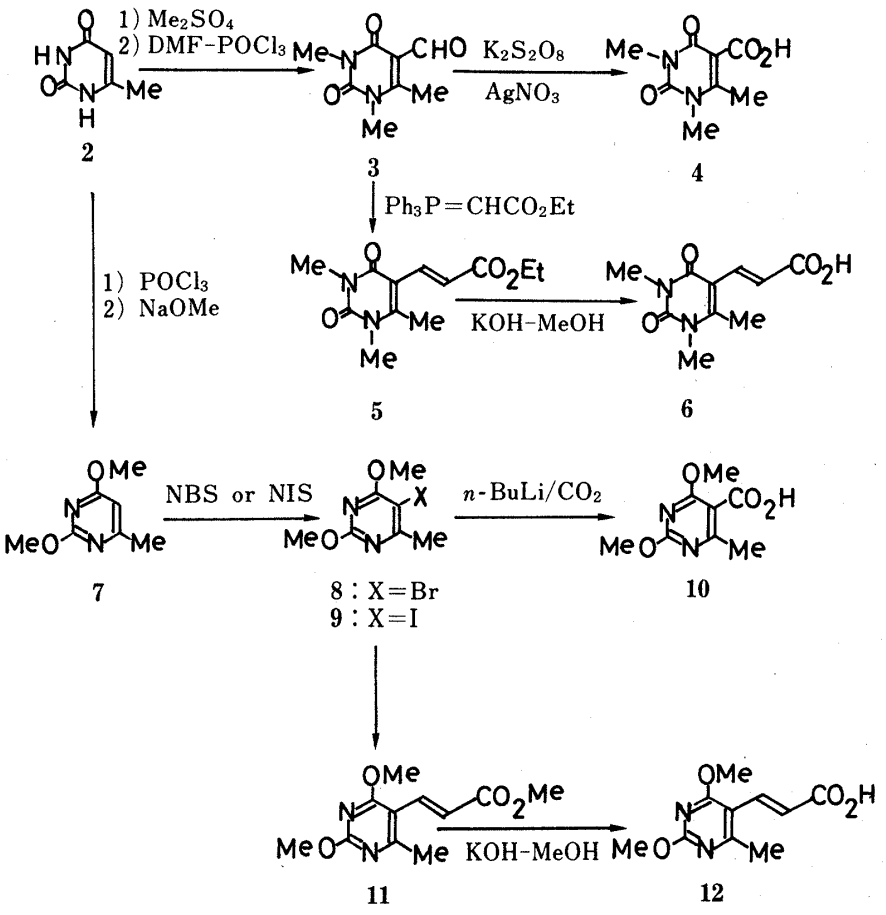
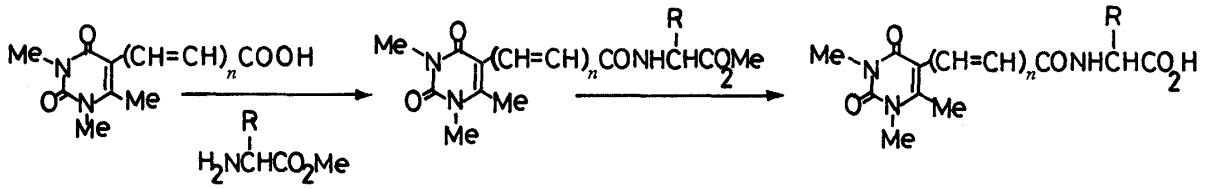


Chart 1



4: $n=0$
6: $n=1$

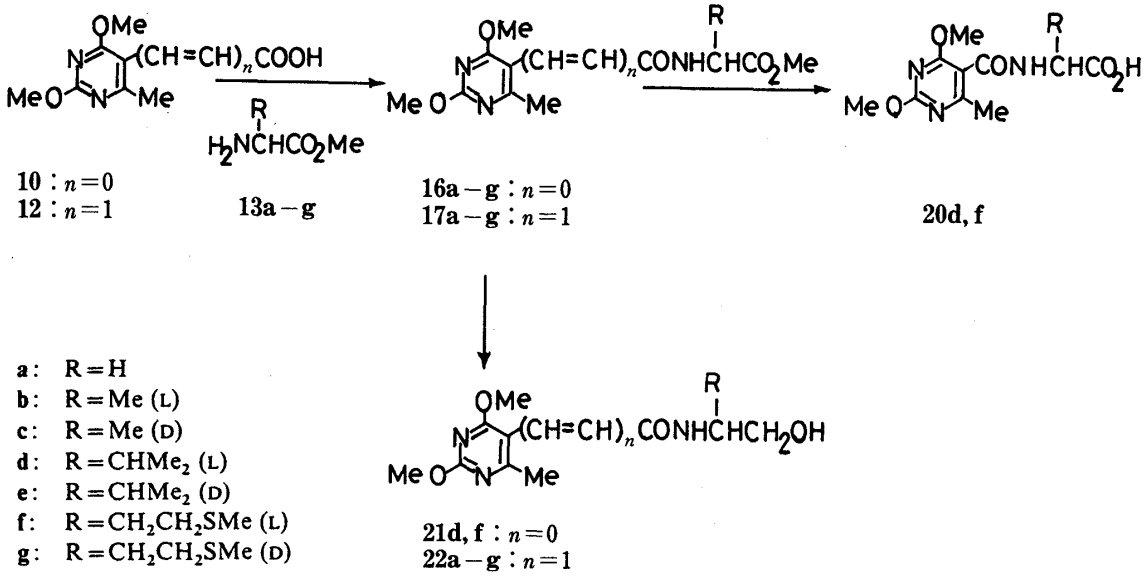
13a—g

14a—g: $n=0$
15a—g: $n=1$

18a: $n=0$
19a, b, d, f: $n=1$

a: R=H
b: R=Me (L)
c: R=Me (D)
d: R=$CHMe_2$ (L)
e: R=$CHMe_2$ (D)
f: R=CH_2CH_2SMe (L)
g: R=CH_2CH_2SMe (D)

Chart 2



10: $n=0$
12: $n=1$

13a—g

16a—g: $n=0$
17a—g: $n=1$

20d, f

21d, f: $n=0$
22a—g: $n=1$

a: R=H
b: R=Me (L)
c: R=Me (D)
d: R=$CHMe_2$ (L)
e: R=$CHMe_2$ (D)
f: R=CH_2CH_2SMe (L)
g: R=CH_2CH_2SMe (D)

Chart 3

15), none of the desired product was obtained due to the further reduction of the pyrimidine ring internal double bond between the C-5 and C-6 positions (Charts 2 and 3). These results are summarized in Table I.

The antitumor activities of the newly synthesized sparsomycin analogs were evaluated from the percent inhibition of deoxyribonucleic acid (DNA) synthesis calculated from the incorporation of [methyl-^{3}H]thymidine into L5178Y murine lymphoma cells *in vitro*. As shown in Table II, the reference compound, sparsomycin, inhibited the growth of L5178Y cells (99.4%) at a concentration of 200 μg/ml. The antitumor activities of synthesized analogs are also shown in Table II. From a comparison of the antitumor activity in relation to the terminal functionality, the order of antitumor activity *in vitro* is as follows: ester > alcohol > carboxylic acid except for the **21** series compounds. In particular, acids (compounds **18—20** series) showed only slight activity. Among the *N,N*-dimethyl type analogs, **15** series compounds having an ethylenic double bond were more active than the **14** series compounds, and similar results were obtained between the dimethoxy type analogs **16** and **17**. These results indicate that the ethylenic double bond at the C-5 position of the pyrimidine ring has an important role in the activity. The stereochemistry of the amino acids was also related to antitumor activity. In both the **14** and **16** series compounds (without an

TABLE I. Physical and Chemical Data for Sparsomycin Analogs

Compd. No.	Yield (%)	mp (°C) (Solvent)	$[\alpha]_D^{21}$ [a] (°)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
14a	82	106—107 (Pet. ether–MeOH)		$C_{11}H_{15}N_3O_5$	49.07 (49.03	5.64 5.83	15.61 15.73)
14b	49	112—113 (*n*-Hexane–MeOH)	−5.3	$C_{12}H_{17}N_3O_5$	50.88 (50.98	6.05 6.06	14.83 14.90)
14c	61	108—109 (*n*-Hexane–MeOH)	+5.1	$C_{12}H_{17}N_3O_5$	50.88 (50.83	6.05 6.07	14.83 15.09)
14d	71	70—71 (*n*-Hexane–MeOH)	+9.8	$C_{14}H_{21}N_3O_5$	54.01 (53.98	6.80 6.72	13.50 13.22)
14e	69	—[b]	−8.4	$C_{14}H_{21}N_3O_5$	High mass 341.148 (341.148)		
14f	65	90—91 (Pet. ether–MeOH)	−8.6	$C_{14}H_{21}N_3O_5$	48.97 (48.98	6.17 6.16	12.24 12.30)
14g	54	92—93 (Pet. ether–MeOH)	+7.9	$C_{14}H_{21}N_3O_5S$	48.97 (48.95	6.17 6.22	12.24 12.28)
15a	64	184—184.5 (MeOH)		$C_{13}H_{17}N_3O_5$	52.87 (52.96	5.80 5.84	14.23 14.30)
15b	60	193—193.5 (Pet. ether–MeOH)	−29.8	$C_{14}H_{19}N_3O_5$	54.36 (54.46	6.19 6.27	13.59 13.63)
15c	38	192—193 (Pet. ether–MeOH)	+30.2	$C_{14}H_{19}N_3O_5$	54.36 (54.19	6.19 6.16	13.59 13.63)
15d	66	151—152 (*n*-Hexane–MeOH)	−22.6	$C_{16}H_{23}N_3O_5$	56.96 (56.82	6.87 6.88	12.46 12.82)
15e	70	149—149.5 (*n*-Hexane–MeOH)	+21.5	$C_{16}H_{23}N_3O_5$	56.96 (56.97	6.87 6.87	12.46 12.46)
15f	51	130—130.5 (*n*-Hexane–MeOH)	−43.8	$C_{16}H_{23}N_3O_5$	52.02 (52.13	6.28 6.20	11.38 11.21)
15g	72	124—125 (*n*-Hexane–MeOH)	+40.3	$C_{16}H_{23}N_3O_5S$	52.02 (52.36	6.28 6.38	11.38 11.45)
16a	70	88—90 (*n*-Hexane–$CHCl_3$)		$C_{11}H_{15}N_3O_5$	49.07 (49.26	5.64 5.58	15.61 15.50)
16b	75	97—98 (Ether)	−10.4	$C_{12}H_{17}N_3O_5$	50.88 (51.22	6.05 6.08	14.83 14.69)
16c	68	95—97 (Ether)	+12.2	$C_{12}H_{17}N_3O_5$	50.88 (51.09	6.05 5.92	14.83 15.06)
16d	44	45—46 (Pet. ether–$CHCl_3$)	+4.9	$C_{14}H_{21}N_3O_5$	54.01 (54.50	6.80 6.92	13.50 13.11)
16e	73	42—43 (Pet. ether–$CHCl_3$)	−5.5	$C_{14}H_{21}N_3O_5$	54.01 (54.49	6.80 6.96	13.50 12.92)
16f	69	81—82 (Pet. ether–MeOH)	−24.3	$C_{14}H_{21}N_3O_5$	48.97 (48.87	6.17 6.44	12.24 11.96)
16g	40	81—82 (Pet. ether–MeOH)	+23.9	$C_{14}H_{21}N_3O_5S$	48.97 (49.05	6.17 5.92	12.24 11.98)
17a	78	150—151 (*n*-Hexane–MeOH)		$C_{13}H_{17}N_3O_5$	52.87 (52.50	5.80 5.76	14.23 14.12)
17b	83	135—137 (Pet. ether–$CHCl_3$)	−9.5	$C_{14}H_{19}N_3O_5$	54.36 (54.24	6.19 6.25	13.59 13.43)
17c	74	139—141 (Pet. ether–$CHCl_3$)	+9.4	$C_{14}H_{19}N_3O_5$	54.36 (53.92	6.19 5.91	13.59 13.57)
17d	69	158—160 (*n*-Hexane–MeOH)	+3.2	$C_{16}H_{23}N_3O_5$	56.96 (57.13	6.87 6.88	12.46 12.46)
17e	68	154—156 (*n*-Hexane–MeOH)	−2.2	$C_{16}H_{23}N_3O_5$	56.96 (56.74	6.87 6.89	12.46 12.55)

TABLE I. (continued)

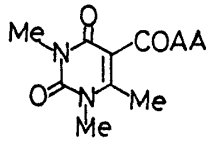
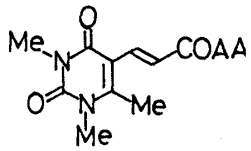
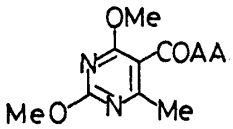
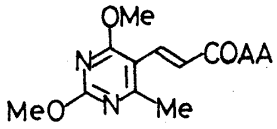
Compd. No.	Yield (%)	mp (°C) (Solvent)	$[\alpha]_D^{21}$ [a] (°)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
17f	59	152—153 (*n*-Hexane–MeOH)	−31.9	$C_{16}H_{23}N_3O_5$	52.02 (52.03	6.28 6.28	11.38 11.18)
17g	59	148—149 (*n*-Hexane–MeOH)	+32.1	$C_{16}H_{23}N_3O_5S$	52.02 (52.55	6.28 6.31	11.38 11.40)
18a	88	191—192 (Pet. ether–MeOH)		$C_{10}H_{13}N_3O_5$	47.06 (47.26	5.13 5.14	16.47 16.56)
19a	99	249—250 (AcOEt–MeOH)		$C_{12}H_{15}N_3O_5$	48.16 (47.99	5.73 5.74	14.04 13.87)
19b	69	251—252 (AcOEt–MeOH)	+5.2	$C_{13}H_{17}N_3O_5$	52.87 (52.95	5.80 5.84	14.23 14.22)
19d	83	226—227 (AcOEt–MeOH)	+7.2[c]	$C_{15}H_{21}N_3O_5$	55.72 (56.30	6.55 6.57	13.00 12.90)
19f	61	99—100 (*n*-Hexane–MeOH)	−25.2	$C_{15}H_{21}N_3O_5S$	50.70 (50.11	5.96 5.74	11.83 11.74)
20d	83	148—149 (*n*-Hexane–MeCN)	+14.4	$C_{13}H_{19}N_3O_5$	52.51 (52.70	6.44 6.58	14.13 14.12)
20f	77	142—145 (*n*-Hexane–MeCN)	−23.1	$C_{13}H_{19}N_3O_5S$	47.41 (46.99	5.82 5.85	12.76 12.46)
21d	41	148—149 (*n*-Hexane–MeOH)	+9.7	$C_{13}H_{21}N_3O_4$	55.11 (55.22	7.47 7.59	14.83 14.49)
21f	81	103—105 (AcOEt–MeOH)	−25.4	$C_{13}H_{21}N_3O_4S$	49.51 (49.09	6.71 6.60	13.33 12.79)
22a	79	110—112 (*n*-Hexane–AcOEt)		$C_{12}H_{17}N_3O_4$	53.92 (53.67	6.41 6.52	15.72 15.55)
22b	72	162—163 (Ether–$CHCl_3$)	+7.2	$C_{13}H_{19}N_3O_4$	55.50 (55.74	6.81 6.78	14.94 15.01)
22c	77	165—167 (Ether–$CHCl_3$)	−6.5	$C_{13}H_{19}N_3O_4$	55.50 (55.66	6.81 6.65	14.94 14.85)
22d	56	158—159 (Ether–$CHCl_3$)	−14.9	$C_{15}H_{23}N_3O_4$	58.23 (57.98	7.49 7.59	13.58 13.23)
22e	62	160—162 (Ether–$CHCl_3$)	+16.8	$C_{15}H_{23}N_3O_4$	58.23 (58.32	7.49 7.25	13.58 13.65)
22f	44	142—143 (*n*-Hexane–AcOEt)	−30.9	$C_{15}H_{23}N_3O_4S$	52.77 (52.44	6.79 6.40	12.31 12.38)
22g	59	148—149 (*n*-Hexane–AcOEt)	+34.0	$C_{15}H_{23}N_3O_4S$	52.77 (52.40	6.79 6.75	12.31 12.35)

a) $c = 1.0$, MeOH. *b*) Was not solidified. *c*) $c = 1.0$, DMSO.

ethylenic double bond), the derivatives of D-amino acids were more effective than those of L-amino acids, whereas **15** series compounds afforded the opposite results. It is noteworthy that both **17** and **22** series compounds, which exhibit remarkable activities, were little affected by the stereochemistry except for **22f**. Although the **15** and **17** series compounds, containing the same ethylenic double bond at the C-5 position, differed only in the replacement of two methoxy groups on the pyrimidine ring, their antitumor activities were markedly different. This suggests that replacement of the oxo group on the pyrimidine ring by a methoxy group enhances antitumor activity. The effects of various alkoxy groups on the pyrimidine ring will be presented in a subsequent publication.

From our observations on the relationship between the structure of sparsomycin analogs and antitumor activity, it is clear that the existence of two alkoxy groups in the pyrimidine

TABLE II. Antitumor Activity of Sparsomycin Analogs toward L5178Y Cells *in Vitro*[a]

Analogs	Terminal	Amino acid (AA) part (L) Gly (a)	Ala (b)	Val (d)	Met (f)	(D) Ala (c)	Val (e)	Met (g)
	COOMe (**14**)	2.2	1.5	0	0	17.9	43.9	10.5
	COOH (**18**)	8.4	—[b]	—	—	—	—	—
	COOMe (**15**)	2.0	99.6	61.7	70.0	33.4	55.5	69.9
	COOH (**19**)	0	0	0	0	—	—	—
	COOMe (**16**)	21.3	0	7.6	13.6	49.5	59.8	62.8
	COOH (**20**)	—	—	21.9	31.4	—	—	—
	CH_2OH (**21**)	—	—	0	0	—	—	—
	COOMe (**17**)	46.0	99.9	85.1	96.9	80.6	89.6	84.6
	CH_2OH (**22**)	62.0	54.4	77.0	0	62.0	78.3	72.9

a) Values are percent inhibition by the analogs at a concentration of 200 μg per ml *vs.* control. Under the same conditions, sparsomycin gave 99.4% inhibition. *b*) Not tested.

ring and also the ethylenic double bond at the C-5 position is favorable for the expression of antitumor activity in this type of sparsomycin analog. These considerations should facilitate the design of compounds that are more effective against human neoplasms. Studies on the antitumor activity and toxicity of these analogs in mice are in progress.

Experimental

Chemicals——All melting points are uncorrected. Optical rotations were obtained with a JASCO DIP-4 digital polarimeter. Infrared (IR) absorption spectra were recorded on a JASCO IRA-2 spectrometer, and NMR spectra on a JEOL JNM-MH-100 spectrometer (with tetramethylsilane as an internal standard). All new compounds were identified by IR and NMR spectroscopy. Satisfactory elemental analyses are indicated in Table I.

5-Carboxy-1,2,3,4-tetrahydro-1,3,6-trimethyl-2,6-dioxopyrimidine (4)——A catalytic amount of silver nitrate (40 mg, 0.3 mmol) was added to a stirred solution of 5-formylpyrimidine (**3**, 5 g, 27 mmol)[12] and potassium persulfate (7.4 g, 27 mmol) in water (50 ml) at 40 °C. The reaction mixture was stirred for a further 2 h, and within 20 min the first crystals of the acid (**4**) appeared. After cooling, the precipitate was collected and recrystallized from acetonitrile to yield **4** (5.1 g, 87%). mp 218—220 °C. IR ν_{max}^{Nujol} cm^{-1}: 1710, 1660, 1620. NMR (DMSO-d_6) δ: 3.60 (s, 3H, NMe), 3.43 (s, 3H, NMe), 2.92 (s, 3H, Me), COOH was absent. *Anal.* Calcd for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.54; H, 5.00; N, 14.29.

(*E*)-β-(1,2,3,4-Tetrahydro-1,3,6-trimethyl-2,4-dioxopyrimidin-5-yl)acrylic Acid (6)——A solution of 5-formylpyrimidine (**3**, 1 g, 5.5 mmol) and triphenylcarboethoxymethylenephosphorane (2.1 g, 6 mmol) in benzene (20 ml) was heated under reflux for 4 h. After cooling, the solvent was removed *in vacuo*, and 10% KOH (20 ml) and MeOH (20 ml) were added to the residue. The resulting mixture was stirred for 6 h at room temperature, and then MeOH was removed *in vacuo*. The residue was acidified with 10% HCl and the precipitate was collected and recrystallized from acetonitrile and MeOH to yield **6** (0.6 g, 48%). mp 263—266 °C. IR ν_{max}^{Nujol} cm^{-1}: 1710, 1660, 1615. NMR (DMSO-d_6) δ: 7.50 (d, 1H, J=14 Hz, =CH), 6.90 (d, 1H, J=14 Hz, =CH), 3.43 (s, 3H, NMe), 3.20 (s, 3H, NMe), 2.43 (s, 3H,

Me), COOH was absent. *Anal.* Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.50. Found: C, 53.36; H, 5.30; N, 12.71.

5-Carboxy-2,4-dimethoxy-6-methylpyrimidine (10)——A 1.5 M solution of *n*-BuLi (6.3 ml, 9.5 mmol) was added dropwise to a stirred solution of 5-bromopyrimidine (**8**, 2 g, 8.6 mmol)[13] in anhydrous ether (200 ml) under nitrogen at −70 °C. The resulting mixture was stirred for a further 15 min, and then poured into dry-ice-saturated ether (100 ml). The reaction mixture was allowed to warm up to room temperature, and then added to water (150 ml). The aqueous layer was separated, acidified with 10% HCl, and then extracted three times with 100 ml of AcOEt. The extract was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from acetonitrile to yield **10** (1.53 g, 90%). mp 142—144 °C. IR ν_{max}^{Nujol} cm^{-1}: 1680, 1580. NMR (DMSO-d_6) δ: 10.51 (s, 1H, COOH), 4.08 (s, 3H, OMe), 4.04 (s, 3H, OMe), 2.63 (s, 3H, Me). *Anal.* Calcd for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.55; H, 5.14; N, 14.20.

(*E*)-β-(2,4-Dimethoxy-6-methylpyrimidin-5-yl)acrylic Acid (12)——A solution of methyl (*E*)-β-(2,4-dimethoxy-6-methylpyrimidin-5-yl)acrylate (**11**, 1.61 g, 65.4 mmol)[14] in 10% KOH (100 ml) and MeOH (100 ml) was stirred for 2 h at room temperature. After removal of the MeOH *in vacuo*, the residue was acidified with 10% HCl and then extracted three times with 100 ml of AcOEt. The extract was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from acetonitrile to yield **12** (1.36 g, 92%). mp 208—209 °C. IR ν_{max}^{Nujol} cm^{-1}: 1690, 1620, 1580. NMR (DMSO-d_6) δ: 11.82 (s, 1H, COOH), 7.88 (d, 1H, J=14 Hz, =CH), 6.68 (d, 1H, J=14 Hz, =CH), 4.10 (s, 3H, OMe), 4.03 (s, 3H, OMe), 2.60 (s, 3H, Me). *Anal.* Calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.50. Found: C, 53.54; H, 5.39; N, 12.48.

General Procedure for Condensation of Acids (4 and 6) with Amino Acid Methyl Esters (13a—g)——BCC (1.5 g, 11 mmol) and *N*-methylmorpholine (1.1 g, 11 mmol) were added to a stirred solution of acid (10 mmol) in dimethylformamide (DMF) (20 ml) at 0 °C. The resulting mixture was stirred for 15 min at 0 °C, then a precooled solution of amino acid methyl ester hydrochloride (11 mmol) and *N*-methylmorpholine (1.1 g, 11 mmol) in DMF (20 ml) was added. The whole mixture was further stirred for an appropriate period (5—12 h). After removal of the solvent, water (50 ml) was added to the residue, which was then extracted with $CHCl_3$ (70 ml × 3). The extract was washed successively with 10% HCl, 10% Na_2CO_3 and brine, and then dried over Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel ($CHCl_3$: AcOEt = 9 : 1 as an eluent solvent) to yield the condensation product. The results of these procedures are summarized in Table I. An example is described below.

Methyl *N*-(1,2,3,4-Tetrahydro-1,3,6-trimethyl-2,4-dioxo-5-pyrimidinylcarbonyl)glycinate (14a)——This was prepared from **4** (2 g, 10.1 mmol) and methyl glycinate hydrochloride (1.39 g, 11.1 mmol). Recrystallization from petroleum ether and methanol gave pure **14a** (2.23 g, 82%). mp 106—107 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200, 1740, 1700, 1660. NMR ($CDCl_3$) δ: 9.30 (1H, br s, NH), 4.16 (2H, d, J=7 Hz, CH_2), 3.75 (3H, s, Me), 3.52 (3H, s, Me), 3.37 (3H, s, Me), 2.78 (3H, s, Me). MS *m/z*: 269 (M^+).

General Procedure for Hydrolysis of Condensation Products (14, 16, and 17)——A condensation product (3 mmol) was dissolved in NaOH solution [1 N NaOH (20 ml) and MeOH (20 ml] and the solution was stirred for several hours at room temperature. MeOH was removed *in vacuo*, and the residue was extracted with $CHCl_3$ (20 ml × 3). The aqueous layer was acidified with 10% HCl solution and then extracted three times with 40 ml of AcOEt. (When a precipitate appeared, it was collected and then purified by recrystallization.) The extract was dried over Na_2SO_4, and then concentrated *in vacuo*. The residue was recrystallized from hexane–MeOH or petroleum ether–MeOH to yield the amino acid. The results of these procedures are summarized in Table I. An example is described below.

***N*-(1,2,3,4-Tetrahydro-1,3,6-trimethyl-2,4-dioxo-5-pyrimidinylcarbonyl)glycine (18a)**——This was prepared from **14a** (300 mg, 1.1 mmol). Recrystallization from petroleum ether and methanol gave pure **18a** (251 mg, 88%). mp 191—192 °C. IR ν_{max}^{Nujol} cm^{-1}: 3350, 1710, 1640, 1605. NMR (DMSO-d_6) δ: 8.85 (1H, s, NH), 3.96 (2H, d, J=5 Hz, CH_2), 3.45 (3H, s, Me), 3.29 (3H, s, Me), 2.63 (3H, s, Me), COOH is absent. MS *m/z*: 255 (M^+).

General Procedure for Reduction of Condensation Products (16 and 17)——$LiBH_4$ (2 mmol) was added stepwise to a stirred solution of a condensation product (1 mmol) in tetrahydrofuran (THF) (20 ml), and the resulting mixture was stirred for 5 h. After cooling in an ice-bath, the reaction mixture was quenched with MeOH : H_2O = 9 : 1 and then the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel (AcOEt : MeOH = 9 : 1 as an eluent solvent) to yield the amino alcohol. The results of these procedures are summarized in Table I. An example is described below.

***N*-(2,4-Dimethoxy-6-methyl-5-pyrimidinylcarbonyl)-L-valinol (21d)**——This is prepared from **16d** (200 mg, 0.64 mmol). Recrystallization from hexane and methanol gave pure **21d** (75 mg, 41%). mp 148—149 °C. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1650, 1580. NMR ($CDCl_3$) δ: 6.75 (1H, d, J=8 Hz, NH), 4.1—3.6 (4H, m, CH, CH_2, OH), 4.00 (6H, s, OMe × 2), 2.47 (3H, s, Me), 2.1—1.8 (1H, m, CH), 0.98 (3H, d, J=8 Hz, Me), 0.91 (3H, d, J=8 Hz, Me). MS *m/z*: 283 (M^+).

Antitumor Assay——Roswell Park Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal calf serum and 50 μg/ml of kanamycin (RPMI-FCS) was used as the cell culture medium. Mouse L5178Y lymphoma cells (10^5) in 1 ml of RPMI-FCS were prepared. All samples were dissolved in DMSO at a concentration

of 20.2 mg/ml. Cell suspension (200 μl) and sample solution (2 μl) were mixed in a Costar 3096 micro-tissue culture plate (Costar, Cambridge, Mass.). In this case, the final sample concentration was 200 μg/ml. As a control group, the same amount of cell suspension and 2 μl of DMSO were mixed. The plate was incubated in a CO_2 incubator at 37 °C for 44 h. [Methyl-^{3}H]thymidine (0.4 μCi in 10 μl of saline: specific activity 20 Ci/mmol) purchased from New England Nuclear (Boston, Mass.) was added as a precursor to each well and incubated for 4 h. L5178Y cells were exposed to the sample during the assay period (48 h). Cells were harvested on a glass-fiber disk (Whatman Ltd., Madison, England). The disk was successively washed with 10% ice-cold trichloroacetic acid (TCA) and water, and then dried. Radioactivity was determined with a Beckman LS9000 liquid scintillation counter (Beckman Instruments Inc., Irvine, Calif.) using toluene–PPO–POPOP counting solution [PPO, 2,5-diphenyloxazole; POPOP, 2,2-*p*-phenylenebis(5-phenyloxazole)]. Each experiment was performed in triplicate. Inhibition of DNA synthesis was calculated from the incorporation of ^{3}H into the TCA-insoluble fraction of cells on the disk using the following formula;

$$\text{percentage inhibition } (\%) = (A - B)/A \times 100,$$

where A is the average ^{3}H count of the control group and B is that of the treated sample.

References and Notes

1) A part of this work was presented at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1986. Part III: S. Kanatomo, S. Nagai, K. Ohki, T. Hase, C. Nomura, and E. Okezaki, *Chem. Pharm. Bull.*, **32**, 4625 (1984).
2) A. D. Argoudelis and R. R. Herr, *Antimicrob. Agents Chemotherapy*, **1962**, 780.
3) H. C. J. Ottenheijm, R. M. J. Liskamp, and M. W. Tijhuis, *Tetrahedron Lett.*, **1979**, 387; H. C. J. Ottenheijm, R. M. J. Liskamp, S. P. J. M. van Nispen, H. A. Boots, and M. W. Tijhuis, *J. Org. Chem.*, **46**, 3273 (1981); H. C. J. Ottenheijm, R. M. J. Liskamp, P. Helquist, S. W. Lauker, and M. S. Shekhani, *J. Am. Chem. Soc.*, **103**, 1720 (1981).
4) S. P. Owen, A. Dietz, and G. W. Camiener, *Antimicrob. Agents Chemotherapy*, **1962**, 772.
5) R. J. Dubois, C.-C. L. Lin, and B. L. Michel, *J. Pharm. Sci.*, **64**, 825 (1975); C.-C. L. Lin and R. J. Dubois, *J. Med. Chem.*, **20**, 337 (1977).
6) R. Vince, J. Brownell, and C. K. Lee, *Biochem. Biophys. Res. Commun.*, **75**, 563 (1977); C. K. Lee and R. Vince, *J. Med. Chem.*, **21**, 176 (1978).
7) S. Kanatomo, S. Nagai, T. Hase, K. Ohki, C. Nomura, and E. Okezaki, *Chem. Pharm. Bull.*, **31**, 135 (1983).
8) S. S. Duke and M. R. Boots, *J. Med. Chem.*, **26**, 1556 (1984).
9) J. Zemlicka and A. Bhuta, *J. Med. Chem.*, **25**, 1123 (1982).
10) G. A. Flynn and D. W. Beight, *Tetrahedron Lett.*, **25**, 2655 (1984); G. A. Flynn and R. J. Ash, *Biochem. Biophys. Res. Commun.*, **114**, 1 (1983).
11) *a*) R. M. J. Liskamp, J. H. Colstee, H. C. J. Ottenheijm, P. Lelieveld, and W. Akkerman, *J. Med. Chem.*, **27**, 301 (1984); *b*) L. A. G. M. van den Broek, R. M. J. Liskamp, J. H. Colstee, P. Leliveld, M. Remacha, D. Vazquez, J. P. G. Ballesta, and H. C. J. Ottenheijm, *ibid.*, **30**, 325 (1987).
12) S. Senda, K. Hirota, G.-N. Yang, and M. Shirahashi, *Yakugaku Zasshi*, **91**, 1372 (1971).
13) Irradiation of **6** in acetonitrile afforded the *Z*-isomer and its NMR spectra exhibits doublets at δ 6.78 and 6.05 (J = 11 Hz) due to the olefinic protons.
14) T. Nishiwaki, *Tetrahedron*, **22**, 2401 (1966).
15) A. Wada, J. Yamamoto, T. Hase, S. Nagai, and S. Kanatomo, *Synthesis*, **1986**, 555.
16) It is well known that the racemization does not occur under these reaction conditions. G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **88**, 1338 (1966).