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Sparsomycin Analogs. II.[1] Synthesis and Biological Activities of 5-Carboxy-6-methyluracil Derivatives[2]

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In order to study the structure–activity relationship of sparsomycin, an antitumor antibiotic, 26 sparsomycin-related compounds (**3**—**5**) were synthesized and their antibacterial activities and lytic actions on Ehrlich ascites carcinoma cells were tested.

Keywords——sparsomycin; sparsomycin analog; 5-carboxy-6-methyluracil derivative; antibacterial activity; sheet method

Several studies have already been reported on compounds related to sparsomycin[3] (Fig. 1), an antitumor antibiotic, but none with biological activity superior to that of sparsomycin has ever been found.[4-7] It was shown that all of the compounds with any biological activity (antitumor activity[4,5] or inhibiting activity for protein synthesis on bacterial ribosomes[6,7]) have D-configuration, which is the same as that of sparsomycin.

The unique structural and biological properties of sparsomycin prompted us to investigate the structure–activity relationship of sparsomycin in further detail. In the present report, we describe the syntheses of 26 kinds of 5-carboxy-6-methyluracil derivatives lacking the ethylene moiety of the acryloyl portion of sparsomycin and the evaluation of their antibacterial potencies and lytic actions on Ehrlich ascites carcinoma cells. Three types of derivatives were synthesized, as shown in Fig. 2. The first type consisted of the compounds **3a**—**i** whose A moiety (Fig. 2) was one of nine amino acid esters, *i.e.*, glycine methyl ester, L- and D-alanine methyl esters, L- and D-serine methyl esters, L- and D-*S*-methylcysteine methyl esters, L- and D-methionine methyl esters. The second type consisted of **4a**—**i** whose A moiety was one of the corresponding amino acids. Similarly, the A moiety of the last type was one of the corresponding amino alcohols.

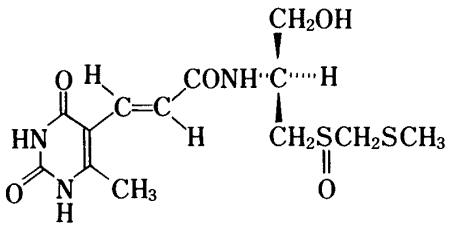
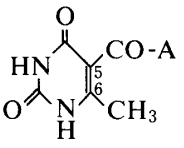


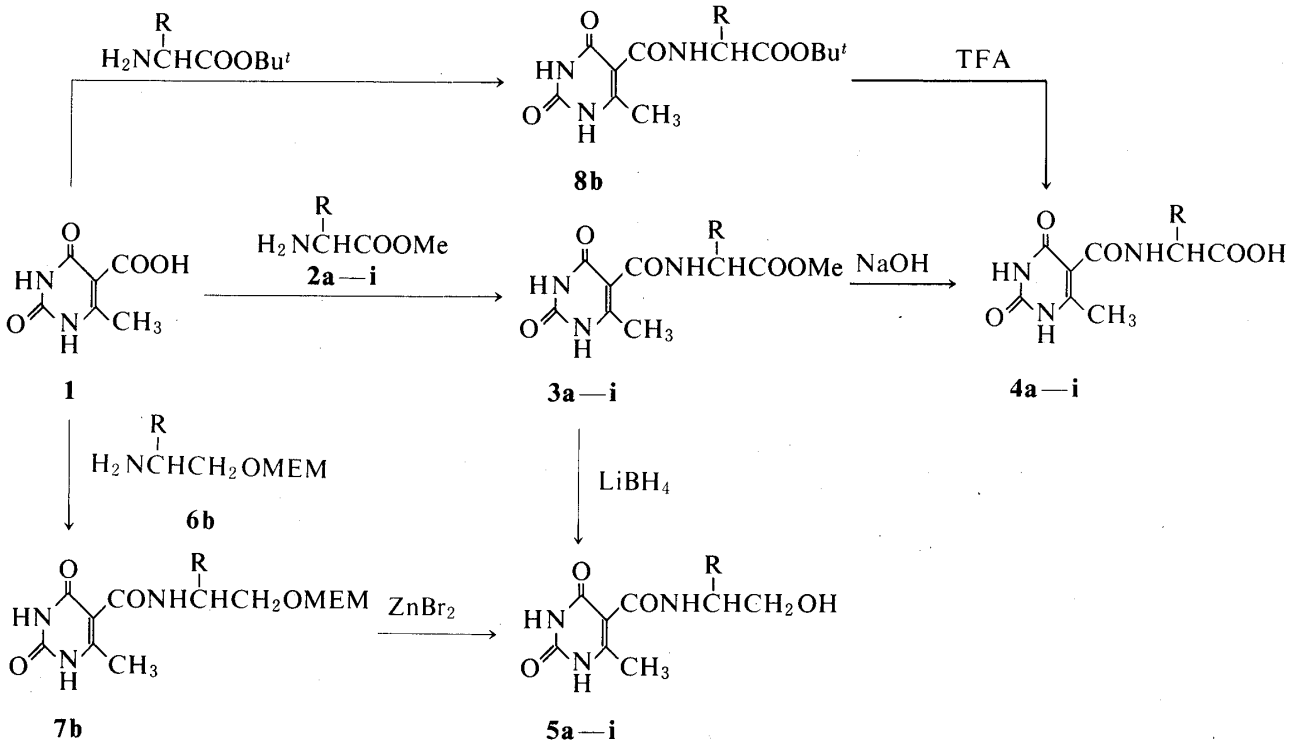
Fig. 1. Structure of Sparsomycin



A = amino acid esters (**3a**—**i**), amino acids (**4a**—**i**), and amino alcohols (**5a**—**i**)

Fig. 2. 5-Carboxy-6-methyluracil Derivatives

The routes of synthesis of these compounds are shown in Chart 1. First, 5-carboxy-6-methyluracil (**1**) prepared in the manner reported previously[1] was condensed with amino acid methyl esters (**2a**—**i**) by the mixed anhydride (MA) method[9] using isobutylchlorocarbonate (BCC) to give *N*-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)amino acid methyl esters (**3a**—**i**) in 54—81% yields. Each compound in the **3a**—**i** group was identified by mass (MS) spectroscopy, elemental analysis (Table I) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy (Table II).



a: R=H
b: R=CH_3 (L)
c: R=CH_3 (D)
d: R=CH_2OH (L)
e: R=CH_2OH (D)
f: R=$CH_2CH_2SCH_3$ (L)
g: R=$CH_2CH_2SCH_3$ (D)
h: R=CH_2SCH_3 (L)
i: R=CH_2SCH_3 (D)

MEM=$CH_2OCH_2CH_2OCH_3$

Chart 1

TABLE I. *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-amino Acid Methyl Esters

Compd.	Yield (%)	Recryst. solvent	$[\alpha]_D^{28}$ [a] (°)	MS (*m/z*)	Formula	Analysis (%) Calcd (Found) C	H	N
3a	73	MeOH		241 (M^+)	$C_9H_{11}N_3O_5$	43.20 (43.10)	4.83 4.72	16.79 16.81)
3b	76	MeOH	−1.6	255 (M^+)	$C_{10}H_{13}N_3O_5$	47.05 (46.95)	5.13 5.17	16.46 16.51)
3c	74	MeOH	+2.4	255 (M^+)	$C_{10}H_{13}N_3O_5$	47.05 (47.00)	5.13 5.18	16.46 16.38)
3d	63	MeOH	+5.2	240 (M−31)	$C_{10}H_{13}N_3O_6$	44.28 (43.97)	4.83 4.79	15.55 15.25)
3e	68	MeOH	−4.3	241 (M-30)	$C_{10}H_{13}N_3O_6$	44.28 (43.90)	4.83 4.83	15.55 15.30)
3f	65	MeOH	−6.6	315 (M^+)	$C_{12}H_{17}N_3O_5S$	45.71 (45.49)	5.43 5.37	13.33 13.44)
3g	71	MeOH	+7.0	315 (M^+)	$C_{12}H_{17}N_3O_5S$	45.71 (45.77)	5.43 5.33	13.33 13.30)
3h	54	MeOH	−25.4	301 (M^+)	$C_{11}H_{15}N_3O_5S$	43.85 (43.58)	5.02 5.07	13.95 14.15)
3i	81	H_2O	+25.4	301 (M^+)	$C_{11}H_{15}N_3O_5S$	43.85 (43.89)	5.02 5.04	13.95 14.05)

a) c=1.0, DMSO.

TABLE II. ^{1}H-NMR Chemical Shifts [δ (ppm)from Tetramethylsilane in DMSO-d_6] of Compounds **3a**—**i**

	CH_3	$CHC\underline{H}_2CH_2$	SCH_3	6-CH_3	CH_2S	$COOCH_3$	$C\underline{H}_2OH$	CH	OH	CONH	N^1H	N^3H
3a				2.48 (s)		3.70 (s)		4.05 (d,2H)		9.46 (t)	11.55 (br, 2H)	
3b	1.36 (d)			2.47 (s)		3.71 (s)		4.45 (m)		9.52 (d)	11.51	11.60
3c	1.36 (d)			2.47 (s)		3.70 (s)		4.45 (m)		9.51 (d)	11.51	11.60
3d				2.51 (s)		3.69 (s)	3.69	4.31 (qua)	5.18 (br)	9.70 (d)	11.44	11.53
3e				2.53 (s)		3.71 (s)	3.71	4.52 (qua)	5.18 (br)	9.80 (d)	11.56	11.64
3f		1.60—2.16 (m)	2.06 (s)	2.43 (s,3H; t,2H)		3.69 (s)		4.56 (qua)		9.40 (d)	11.40	11.48
3g		1.60—2.16 (m)	2.03 (s)	2.40 (s,3H; t,2H)		3.64 (s)		4.30 (qua)		9.30 (d)	11.27	11.35
3h			2.09 (s)	2.50 (s)	2.95 (d)	3.70 (s)		4.72 (qua)		9.78 (d)	11.47	11.54
3i			2.11 (s)	2.52 (s)	2.96 (d)	3.72 (s)		4.74 (qua)		9.84 (d)	11.57	11.64

These esters **3a**—**i** were then hydrolyzed with 1 N NaOH to give the carboxylic acids (**4a**—**i**) in 44—76% yields. Compound **4b** was also synthesized by another route. Compound **1** was condensed with *tert*-butyl L-alaninate[10] by the MA method to give *tert*-butyl *N*-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-alaninate (**8b**), followed by treatment with trifluoroacetic acid (TFA) to give **4b**. Since the specific rotation of **4b** obtained by treatment with TFA from **8b** was identical with that of **4b** obtained by hydrolysis from **3b**, it is clear that racemization did not occur in the saponification reaction. Tables III and IV show spectral data and other physicochemical data for **4a**—**i**. In the ^{1}H-NMR spectra of **4a**—**i**, the signals of methyl protons of the esters (δ 3.64—3.72 ppm) had disappeared. In the MS, **4d** and

TABLE III. *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)amino Acids

Compd.	Yield (%)	$[\alpha]_D^{28}$ [a] (°)	MS (*m/z*)	Formula	Analysis (%) Calcd (Found) C	H	N
4a	63		227 (M^+)	$C_8H_9N_3O_5$	42.30 (42.09	3.99 3.96	18.50 18.75)
4b	50	+12.0	241 (M^+)	$C_9H_{11}N_3O_5 \cdot 1/2H_2O$	43.20 (43.20	4.83 4.68	16.79 16.90)
4c	44	−12.0	241 (M^+)	$C_9H_{11}N_3O_5 \cdot 1/2H_2O$	43.20 (43.02	4.83 4.70	16.79 16.79)
4d	59	+18.6	239 (M−18)	$C_9H_{11}N_3O_6$	42.03 (41.85	4.31 4.43	16.34 16.18)
4e	45	−16.8	239 (M−18)	$C_9H_{11}N_3O_6$	42.03 (41.93	4.31 4.26	16.34 16.36)
4f	76	+ 2.6	301 (M^+)	$C_{11}H_{15}N_3O_5S$	43.85 (43.18	5.02 4.92	13.95 13.74)
4g	62	− 1.6	301 (M^+)	$C_{11}H_{15}N_3O_5S$	43.85 (43.40	5.02 4.94	13.95 13.87)
4h	49	−16.6	287 (M^+)	$C_{10}H_{13}N_3O_5S$	41.81 (41.03	4.56 4.51	14.63 14.38)
4i	59	+16.9	242 (M-45)	$C_{10}H_{13}N_3O_5S$	41.81 (41.35	4.56 4.63	14.63 14.68)

a) c=1.0, DMSO.

4e gave m/z 239 $(M-H_2O)^+$, and **4i** gave m/z 242 $(M-COOH)^+$, but all other compounds provided a molecular ion (M^+) peak.

On the other hand, the esters **3a**—**i** were reduced with lithium borohydride to give the corresponding alcohols **5a**—**i** in 33—58% yields (Table V). In the ^{1}H-NMR spectra of **5a**—**i** (Table VI), the signals of methyl protons of the esters had disappeared, and new signals due to $C\underline{H}_2OH$ appeared at δ 4.73—4.80 ppm. In the MS of **5a**—**i** (Table V), all of them except **5b** showed mainly the dehydroxymethyl ion (M–31). The reduction products, **5d** and **5e**, from **3d** and **3e** are identical. Compound **5b** was also obtained by another method. That is, **1** was

TABLE IV. ^{1}H-NMR Chemical Shifts [δ (ppm) from Tetramethylsilane in DMSO-d_6] of Compounds **4a**—**i**[a]

	CH_3	$CHC\underline{H}_2CH_2$	SCH_3	6-CH_3	CH_2S	$C\underline{H}_2OH$	CH	CONH	N^1H	N^3H
4a				2.52 (s)			4.01 (d, 2H)	9.50 (t)	11.20—12.00 (br)	
4b	1.38 (d)			2.50 (s)			4.42 (qui)	9.58 (d)	11.00—12.00 (br)	
4c	1.36 (d)			2.50 (s)			4.41 (qui)	9.58 (d)	11.00—12.00 (br)	
4d[b]				2.54 (s)		3.80 (double gua)	4.50 (m)	9.80 (d)	11.20—12.00 (br)	
4e[b]				2.54 (s)		3.77 (double gua)	4.45 (m)	9.72 (d)	11.00—11.80 (br)	
4f		1.68—2.28 (m)	2.09 (s)	2.47 (s)	2.55 (m)		4.55 (m)	9.51 (d)	11.62	11.72
4g		1.68—2.28 (m)	2.09 (s)	2.48 (s)	2.56 (m)		4.56 (m)	9.51 (d)	11.58	11.67
4h			2.13 (s)	2.55 (s)	2.98 (d)		4.72 (m)	9.87 (d)	11.20—12.00 (br)	
4i			2.13 (s)	2.55 (s)	2.99 (d)		4.72 (m)	9.87 (d)	11.20—12.00 (br)	

a) The carboxyl protons in all compounds were unobservable.
b) The hydroxyl proton was unobservable.

TABLE V. *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)amino Alcohols

Compd.	Yield (%)	$[\alpha]_D^{28}$[a] (°)	MS (m/z)	Formula	Analysis (%) Calcd (Found) C	H	N
5a	45		195 (M−18) 182 (M−31)	$C_8H_{11}N_3O_4$	45.07 (44.64	5.20 5.03	19.71 19.27)
5b	33	+6.5	227 (M^+)	$C_9H_{13}N_3O_4$	47.57 (47.43	5.77 5.78	18.49 18.51)
5c	41	−7.3	209 (M−18) 196 (M−31)	$C_9H_{13}N_3O_4$	47.57 (47.15	5.77 5.86	18.49 18.50)
5d[b]	44		212 (M−31)	$C_9H_{13}N_3O_5$	44.45 (44.36	5.39 5.45	17.28 17.41)
5e[b]	35		212 (M−31)	$C_9H_{13}N_3O_5$	44.45 (44.28	5.39 5.40	17.28 17.19)
5f	50	−15.4	256 (M−31)	$C_{11}H_{17}N_3O_4S$	45.98 (45.82	5.96 5.95	14.62 14.32)
5g	47	+14.5	256 (M−31)	$C_{11}H_{17}N_3O_4S$	45.98 (45.76	5.96 5.95	14.62 14.23)
5h	41	−47.6	242 (M−31)	$C_{10}H_{15}N_3O_4S$	43.95 (43.54	5.53 5.61	15.37 15.23)
5i	58	+50.2	242 (M−31)	$C_{10}H_{15}N_3O_4S$	43.95 (43.40	5.53 5.60	15.37 15.04)

a) c=1.0, DMSO.
b) **5d** and **5e** are equivalent each other.

TABLE VI. ^{1}H-NMR Chemical Shifts [δ (ppm)from Tetramethylsilane in DMSO-d_6] of Compounds **3a—i**

	$CH_3CHCH_2CH_2$	SCH_3	6-CH_3	CH_2S	CH_2OH	CH	OH	CONH	N^1H	N^3H
5a		2.45 (s)			3.20—3.70 (m, 4H)		4.76	9.08 (t)	11.39	11.51
5b	1.10 (d)	2.44 (s)			3.47 (d)	3.95 (m)	4.76 (br)	8.95 (d)	11.38	11.50
5c	1.11 (d)	2.45 (s)			3.48 (d)	3.95 (m)	4.80 (br)	8.94 (d)	11.37	11.49
5d		2.49 (s)			3.53 (d, 4H)	3.87 (m)	4.73 (br, 2H)	9.13 (d)	11.42	11.53
5e		2.49 (s)			3.53 (d, 4H)	3.87 (m)	4.75 (br, 2H)	9.11 (d)	11.40	11.50
5f	1.78 (m)	2.07 (s)	2.42 (s)	2.54 (m)	3.44 (double qua)	3.96 (m)	4.80 (br)	8.85 (d)	11.44 (br, 2H)	
5g	1.77 (m)	2.06 (s)	2.42 (s)	2.54 (m)	3.44 (double qua)	3.96 (m)	4.77 (br)	8.86 (d)	11.36	11.48
5h		2.09 (s)	2.49 (s)	2.66 (qua)	3.55 (double qua)	4.06 (m)	4.80 (br)	9.23 (d)	11.44	11.54
5i		2.13 (s)	2.52 (s)	2.68 (qua)	3.57 (double qua)	4.05 (m)	4.80 (br)	9.30 (d)	11.53	11.63

condensed with β-methoxyethoxymethyl (MEM) ether of alaninol (**6b**) by the MA method to give MEM ether of **5b** (**7b**). Subsequently, **7b** was deprotected with zinc bromide to give **5b**.

Thus, 26 kinds of sparsomycin-related compounds, **3a—i**, **4a—i**, and **5a—i**, were synthesized.

The antibacterial activities of the newly synthesized sparsomycin-related compounds were tested by the standard method recommended by the Japanese Society of Chemotherapy.[11] In this test, sparsomycin was employed as the control. None of the synthetic compounds showed antibacterial activity even at a concentration of 200 μg/ml against any of the 8 kinds of the test microorganisms, whereas sparsomycin showed potent activity. Lytic actions on Ehrlich ascites carcinoma cells were also tested by the sheet method.[8] None of the 26 compounds showed lytic action.

These results suggest that the ethylene moiety of the acryloyl portion and/or the side chain of the amino alcohol portion of sparsomycin are essential for biological activity. Further studies on this series of analogs, in which the amino alcohol portion of sparsomycin is replaced by other amino alcohols, amino acids, and amino acid esters, are in progress.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were taken on a JASCO IRA-2 spectrometer, MS on a JEOL JMS-D100, ultraviolet (UV) spectra on a Hitachi 323 recording spectrophotometer, optical rotations on a JASCO DIP-4 digital polarimeter, and ^{1}H-NMR spectra on a JEOL JNM-MH-100 using tetramethylsilane as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; qua, quartet; qui, quintet; m, multiplet; br, broad. Thin layer chromatography (TLC) was performed on silica gel GF_{254} (Merck). *Rf* values refer to the following solvent systems: Rf_1, MeCOEt: Me_2CO: H_2O (7:2:1); Rf_2, EtOH: MeOH: H_2O (50:45:5); Rf_3, $CHCl_3$: MeOH: AcOH (95:5:3).

General Procedure for the Synthesis of Compounds 3a—i——A solution of 5-carboxy-6-methyluracil (**1**) (3.40 g, 20 mmol) in dimethylformamide (DMF) (50 ml) was cooled to $-10°$C, then BCC (2.73 g, 20 mmol) and triethylamine (TEA) (2.14 g, 21 mmol) were added. The mixture was stirred for 15 min at $-10°$C, then a precooled solution of an amino acid methyl ester hydrochloride (**2a—i**) (22 mmol) and TEA (2.24 g, 22 mmol) in DMF was added, and the whole was stirred for 1 h at $-5°$C then overnight at room temperature. The liquid phase was then separated, the solvent was removed *in vacuo*, and the residue was triturated with cold H_2O. The crude product was filtered off with suction and recrystallized from MeOH to give the condensation product (**3a—i**). Table I shows the yields, optical rotations, MS data and elemental analyses of the products. The ^{1}H-NMR data are listed in Table II. Other physicochemical properties of the products are as follows.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)glycinate (**3a**): Colorless needles, mp 276°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3525, 3290, 1730, 1650, 1625, 1570, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 270 (9380). Rf_1, 0.69; Rf_2, 0.52.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-alaninate (**3b**): Colorless needles, mp 228—230°C. IR ν_{max}^{Nujol} cm^{-1}: 3330, 3290, 1745, 1735, 1715, 1665, 1625, 1585, 1515. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 268.5 (10640). Rf_1, 0.73; Rf_2, 0.62.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-alaninate (**3c**): Colorless needles, mp 228—229.5°C. IR ν_{max}^{Nujol} cm^{-1}: 3330, 3290, 1745, 1735, 1715, 1665, 1625, 1585, 1515. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 268.5 (9480). Rf_1, 0.73; Rf_2, 0.61.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-serinate (**3d**): Colorless needles, mp 240°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3550, 3400, 3325, 3280, 1745, 1710, 1665, 1620, 1575, 1525. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 270.5 (10800). Rf_1, 0.61; Rf_2, 0.60.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-serinate (**3e**): Colorless needles, mp 242°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3550, 3410, 3325, 3280, 1745, 1710, 1660, 1620, 1575, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 271 (10971). Rf_1, 0.61; Rf_2, 0.59.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-methioninate (**3f**): Colorless needles, mp 176°C. IR ν_{max}^{Nujol} cm^{-1}: 3320, 3270, 1745, 1720, 1665, 1625, 1580, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (11500). Rf_1, 0.82; Rf_2, 0.62.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-methioninate (**3g**): Colorless needles, mp 174—176.5°C. IR ν_{max}^{Nujol} cm^{-1}: 3320, 3270, 1745, 1720, 1660, 1625, 1580, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (9945). Rf_1, 0.82; Rf_2, 0.62.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-*S*-methyl-L-cysteinate (**3h**): Yellow crystals, mp 179—180°C. IR ν_{max}^{Nujol} cm^{-1}: 3290, 1745, 1720, 1660, 1620, 1580, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 271 (10590). Rf_1, 0.82; Rf_2, 0.62.

Methyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-*S*-methyl-D-cysteinate (**3i**): Colorless needles (from H_2O), mp 179—182°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3280, 1745, 1725, 1660, 1620, 1580, 1525, 1495. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 271 (9770). Rf_1, 0.82; Rf_2, 0.61.

General Procedure for the Synthesis of Compounds 4a—i——One of **3a—i** (2 mmol) was dissolved in NaOH solution [1 N NaOH (5 ml) and H_2O (4 ml)] and the solution was stirred for 30 min at room temperature. The reaction mixture was acidified with 1 N HCl under ice-water cooling. The precipitate was filtered off with suction, washed with cold H_2O and recrystallized from H_2O to give the product **4a—i**. Table III shows the yields, optical rotations, MS data and elemental analyses of the products. The ^{1}H-NMR data are listed in Table IV. Other physicochemical properties of the products are as follows.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)glycine (**4a**): White crystals, mp 283—284°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3280, 1740, 1650, 1580, 1520. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269.5 (9630). Rf_1, 0.04; Rf_2, 0.20.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-alanine (**4b**): Colorless needles, mp 254—255°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3170, 1760, 1740, 1720, 1670, 1640, 1610, 1585, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (10720). Rf_1, 0.11; Rf_2, 0.37.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-alanine (**4c**): Colorless needles, mp 251—254°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3175, 1760, 1745, 1720, 1665, 1640, 1605, 1585, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (10110). Rf_1, 0.11; Rf_2, 0.36.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-serine (**4d**): Colorless needles, mp 235°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3500, 3180, 1715, 1680, 1630, 1550, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 271 (9640). Rf_1, 0.03; Rf_2, 0.23.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-serine (**4e**): Colorless needles, mp 235°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3500, 3430, 3250, 3180, 1720, 1685, 1665, 1605, 1575, 1540. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 271 (9300). Rf_1, 0.04; Rf_2, 0.22.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-methionine (**4f**): White crystals, mp 110—120°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3580, 3430, 3170, 1740, 1725, 1665, 1630, 1565, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 270 (11260). Rf_1, 0.14; Rf_2, 0.41.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-methionine (**4g**): White crystals, mp 110—120°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3425, 3160, 1740, 1720, 1670, 1630, 1570, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 270 (9990). Rf_1, 0.15; Rf_2, 0.40.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-*S*-methyl-L-cysteine (**4h**): Yellow crystals, mp 106°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 1710, 1660, 1620, 1575, 1525. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 270.5 (11830). Rf_1, 0.15; Rf_2, 0.43.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-*S*-methyl-D-cysteine (**4i**): White crystals, mp 165—167°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3330, 1705, 1665, 1620, 1525. UV $\lambda_{max}^{H_2O}$ nm (ϵ):271.5 (10400). Rf_1, 0.15; Rf_2, 0.42.

Synthesis of 4b *via* 8b——*tert*-Butyl *N*-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-alaninate (**8b**): BCC (0.4 ml, 3.2 mmol) and TEA (0.46 ml, 3.5 mmol) were added to a solution of **1** (0.50 g,

2.9 mmol) in DMF (6 ml) at −10°C with stirring. Stirring was continued for 15 min, then a solution of *tert*-butyl L-alaninate[10] (0.42g, 2.9 mmol) in DMF (6 ml) was added and the reaction mixture was stirred for 1 h at −5°C and then overnight at room temperature. The solvent was removed *in vacuo* and the oily residue was triturated with a small amount of H_2O. The precipitate formed was collected, washed with H_2O, and recrystallized from MeOH to give white crystals (**8b**) : 0.57 g (67%). mp 185—185.5°C (dec.). $[\alpha]_D^{15}$: +13.5° (c=1, DMSO). Rf_3, 0.47. ^{1}H-NMR (DMSO-d_6) δ: 0.95 (3H, d, CH_3), 1.40 (9H, s, OBu^t), 2.42 (3H, s, 6-CH_3), 4.23 (1H, qua, CH), 9.26 (1H, d, CONH), 11.00—11.32 (2H, br, ring NH). *Anal.* Calcd for $C_{13}H_{19}N_3O_5$: C, 52.51; H, 6.44; N, 14.13. Found: C, 52.36; H, 6.42; N, 14.05. MS m/z: 297 (M^+).

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-alanine (**4b**): A mixture of **8b** (0.70 g, 2.4 mmol) and TFA (15 ml) was stirred for 3 h at room temperature. The reaction mixture was concentrated *in vacuo* and the oily residue was triturated with H_2O (2 ml). The precipitate formed was collected, washed with H_2O and recrystallized from H_2O to give white crystals (**4b**): 0.49 g (86%) mp 254—255°C (dec.). $[\alpha]_D^{28}$: +12° (c=1, DMSO). Rf_1, 0.11; Rf_2, 0.37; Rf_3, 0.12. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (3H, d, CH_3), 2.50 (3H, s, 6-CH_3), 4.42 (1H, qua, CH), 9.58 (1H, d, CONH), 11.20—12.40 (2H, br, ring NH). *Anal.* Calcd for $C_9H_{11}N_3O_5$: C, 44.82; H, 4.60; N, 17.42. Found: C, 44.80; H, 4.78; N, 17.50. MS m/z: 241 (M^+). IR ν_{max}^{Nujol} cm^{-1}: 3350, 3180, 1760, 1740, 1720, 1670, 1640, 1610, 1580, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (10000).

General Procedure for the Synthesis of Compounds 5a—i——A suspension of one of **3a—i** (2 mmol) in tetrahydrofuran (THF) was added with stirring to a solution of $LiBH_4$ (0.11 g, 5 mmol) in THF and the mixture was stirred for 5 h at room temperature. The reaction mixture was cooled, diluted with 50% EtOH (20 ml) and stirred with Dowex-50 to give a solution of about pH 5. The resin was filtered off, the filtrate concentrated to dryness *in vacuo* and the boric acid removed by coevaporation several times with MeOH–benzene (2:1). The residue was recrystallized from H_2O to give the reduction product (**5a—i**). Table V shows the yields, optical rotations, MS data and elemental analyses of the products. The ^{1}H-NMR data are listed in Table VI. Other physicochemical properties of the products are as follows.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)glycinol (**5a**): Colorless plates, mp 240—244°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3420, 3250, 1720, 1660, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 268.3 (11290). Rf_1, 0.42; Rf_2, 0.56.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-alaninol (**5b**): Colorless plates, mp 240—242°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3450, 3150, 1745, 1725, 1665, 1640, 1565, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 267.5 (10650). Rf_1, 0.51; Rf_2, 0.58.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-alaninol (**5c**): Colorless plates, mp 238—244°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3460, 3150, 1745, 1725, 1665, 1635, 1565, 1510. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 267.5 (10130). Rf_1, 0.52; Rf_2, 0.59.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)serinol (**5d, 5e**): i) **5d** (from **3d**). Slightly yellow crystals, mp 251—253°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3360, 3175, 1710, 1695, 1680, 1625, 1585, 1530. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (11710). Rf_1, 0.38; Rf_2, 0.55.

ii) **5e** (from **3e**). White crystals, mp 252—253°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3370, 3190, 1740, 1700, 1685, 1630, 1590, 1535. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (11970). Rf_1, 0.38; Rf_2, 0.55.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-methioninol (**5f**): White crystals, mp 183—188°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3170, 1740, 1725, 1660, 1630, 1550, 1500. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 268.5 (8590). Rf_1, 0.68; Rf_2, 0.58.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-D-methioninol (**5g**): White crystals, mp 187—192°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3290, 3180, 1740, 1660, 1630, 1550, 1500. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 268.5 (10360). Rf_1, 0.67; Rf_2, 0.59.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-*S*-methyl-L-cysteinol[1] (**5h**): White crystals, mp 203—207°C (dec.). IR ν_{max}^{Nujol} cm^{-1}: 3300, 3180, 1740, 1720, 1640, 1560, 1500. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (9590). Rf_1, 0.66; Rf_2, 0.58.

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-*S*-methyl-D-cysteinol (**5i**): White crystals, mp 201—206°C (dec.) IR ν_{max}^{Nujol} cm^{-1}: 3300, 3180, 1740, 1725, 1660, 1560, 1500. UV $\lambda_{max}^{H_2O}$ nm (ϵ): 269 (9460). Rf_1, 0.67; Rf_2, 0.59.

Synthesis of 5b *via* 7b——*O*-Methoxyethoxymethyl-L-alaninol (**6b**): i) *N*-Carbobenzoxy-L-alaninol: Ethereal diazomethane was added to a solution of carbobenzoxy-L-alanine[12] (2.23 g, 10 mmol) in ether (20 ml) until the yellow color remained. The ether was evaporated off under reduced pressure to give oily methyl carbobenzoxy-L-alaninate (2.33 g). This was dissolved in THF (50 ml) and $LiBH_4$ (0.26 g, 12 mmol) was added to the solution. The mixture was stirred for 1 h at room temperature, then chilled H_2O (3 ml) was added and the formed precipitate was separated. The filtrate was evaporated to dryness under reduced pressure and the oily residue was dissolved in ether. The insoluble material was separated by filtration and the filtrate was evaporated to dryness. The oily residue was triturated with petroleum ether and the precipitate was collected and recrystallized from ether–petroleum ether to give *N*-carbobenzoxy-L-alaninol (1.40 g, 67%) as colorless needles. mp 65—68°C. $[\alpha]_D^{25}$: −14.3° (c=2.0, EtOH). Rf_3, 0.50. IR ν_{max}^{KBr} cm^{-1}: 3350, 3325, 1650, 1560. *Anal.* Calcd for $C_{11}H_{15}NO_3$: C, 63.12; H, 7.22; N, 6.72. Found: C, 63.15; H, 7.38; N, 6.69. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, d, CH_3), 3.00 (1H, s, OH, exchangeable), 3.40 (3H, m, $CHCH_2$), 5.15 (2H, s, CH_2Ph), 7.40 (5H, s,

Ph-H).

ii) *N*-Carbobenzoxy-*O*-methoxyethoxymethyl-L-alaninol: A solution of MEM chloride (1.86 g, 15 mmol) in CH_2Cl_2 (5 ml) was added to a solution of *N*-carbobenzoxy-L-alaninol (2.00 g, 10 mmol) and diisopropylethylamine (2.45 g, 19 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred for 3 h at 25°C, then the solvent was evaporated off. The oily residue was extracted with AcOEt (50 ml) and the insoluble material was separated by filtration. The filtrate was evaporated to dryness under reduced pressure to give an oily material (2.79 g, 94%). This was used in the following reaction without further purification. Rf_3, 0.65. IR ν_{max}^{KBr} cm^{-1}: 3350, 2925, 1720, 1650. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, d, CH_3), 3.42 (3H, s, OCH_3), 3.64 (2H, d, $OC\underline{H}_2CH$), 3.25—3.70 (7H, m, OCH_2O, CH, OCH_2CH_2O), 5.08 (2H, s, CH_2Ph), 5.30 (1H, d, NH), 7.32 (5H, s, Ph-H). MS *m/z*: 297 (M^+).

iii) *O*-Methoxyethoxymethyl-L-alaninol (**6b**): A solution of *N*-carbobenzoxy-*O*-methoxyethoxymethyl-L-alaninol (1.30 g, 4.4 mmol) in MeOH (30 ml) was catalytically reduced in the presence of 10% Pd-C (0.01 g) in a usual manner. Pd-C was separated by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in ether and the solution was dried over anhydrous $MgSO_4$. The solvent was evaporated off and an oily material (0.65 g, 90%) was obtained. This was used in the following reaction without further purification. Rf_3, 0.20. IR ν_{max}^{KBr} cm^{-1}: 3350, 2925, 1200—1000 (C-O-C). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, d, CH_3), 3.15 (2H, d, NH_2), 3.42 (3H, s, OCH_3), 3.64 (2H, d, CH_2O), 3.25—3.70 (7H, m, CH, OCH_2O, OCH_2CH_2O).

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-*O*-methoxyethoxymethyl-L-alaninol (**7b**): BCC (0.62 ml, 4.8 mmol) and TEA (0.67 ml, 4.8 mmol) were added dropwise to a solution of **1** (0.75 g, 4.4 mmol) in DMF (10 ml) at −10°C and the mixture was stirred for 15 min. A solution of **6b** (0.65 g, 4.4 mmol) in DMF (10 ml) was then added and the whole was stirred for 1 h at −5°C. Stirring was continued at room temperature overnight, then the solvent was evaporated off under reduced pressure. The oily residue was triturated with H_2O to afford a crude product (1.25 g, 91%). After recrystallization from H_2O, a white crystalline powder (0.68 g, 50%), mp 201—201.5°C, was obtained. $[\alpha]_D^{25}$: +32.0° (*c*=1, DMSO). Rf_3, 0.20. IR ν_{max}^{KBr} cm^{-1}: 3050, 2950, 1720. *Anal.* Calcd for $C_{13}H_{21}N_3O_6$: C, 49.52; H, 6.71; N, 13.33. Found: C, 49.42; H, 6.90; N, 13.30. UV λ_{max}^{DMSO} nm (ϵ): 269.5 (9000). ^{1}H-NMR (DMSO-d_6) δ: 1.38 (3H, d, CH_3), 2.46 (3H, s, 6-CH_3), 3.25—4.20 (7H, m, CH, OCH_2CH_2O, OCH_2O), 4.25 (3H, s, OCH_3), 4.40 (2H, d, $CHC\underline{H}_2O$), 9.44 (1H, d, CONH), 11.44 (1H, s, N^1H), 11.56 (1H, s, N^3H). MS *m/z*: 315 (M^+), 256 (M−59).

N-(1,2,3,4-Tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinylcarbonyl)-L-alaninol (**5b**): $ZnBr_2$ (1.10 g, 5 mmol) was added to a solution of **7b** (0.32 g, 1 mmol) in dry THF (8 ml), and the solution was stirred for 20 h at room temperature. After addition of H_2O (2 ml), the mixture was evaporated to dryness under reduced pressure, and the crystalline residue was triturated with chilled H_2O (2 ml×4). The crude crystals (0.17 g, 78%) were recrystallized from H_2O to give colorless plates (0.15 g, 64%), mp 240—242°C (dec.). $[\alpha]_D^{27}$: +7.8°(*c*=1, DMSO). IR ν_{max}^{Nujol} cm^{-1}: 3450, 3150, 1745, 1725, 1665, 1640, 1565, 1510. *Anal.* Calcd for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.77; N, 18.49. Found: C, 47.41; H, 5.79; N, 18.47. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, d, CH_3), 2.40 (3H, s, 6-CH_3), 3.95 (1H, m, CH), 3.25—4.40 (3H, br, CH_2OH), 9.00 (1H, d, CONH), 11.44 (1H, s, N^1H), 11.56 (1H, s, N^3H). MS *m/z*: 227 (M^+), 209 (M−18), 196 (M−31). UV λ_{max}^{DMSO} nm (ϵ): 268 (10000).

Test for Antibacterial Potency——The test for antibacterial potency (MIC measurement) was carried out according to the standard method recommended by the Japanese Society of Chemotherapy, *i.e.*, the agar plate dilution method.[11]

i) Samples: Sparsomycin (Upjohn) and sparsomycin-related compounds synthesized by us were dissolved in 2% DMSO (dimethylsulfoxide, Wako Junyaku) solution at a concentration of 200 μg/ml, and subjected to serial two-fold dilution with sterile H_2O.

ii) Strains: Eight strains of bacteria preserved at Hokuriku Seiyaku Company were used. *Staphylococcus aureus* FDA 209P JC-1; *Streptococcus pyogenes* COOK; *Bacillus subtilis* ATCC 6633; *Escherichia coli* NIHJ JC-2; *Krebsiella pneumoniae* PCI 602; *Salmonella typhi* 901; *Pseudomonas aeruginosa* IFO 3445; *Proteus vulgaris* OX-19.

iii) Culture medium: For bacterial growth, Trypticase Soy Broth (BBL) was used. For the measurement of sensitivity, Heart Infusion Agar (Nissui Seiyaku) was used.

Test for Lytic Action on Carcinoma Cells——The sheet method[8] was employed to test the lytic action on Ehrlich ascites carcinoma cells of 26 synthetic sparsomycin-related compounds. To prepare the test sample, 2 mg of the material was dissolved in 4 ml of 0.025 M phosphate buffer (pH 7.0).

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