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Synthesis and Activity of Pyrimidinylpropenamide Antibiotics: The Alkyl Analogues of Sparsomycin*

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Facile syntheses of sparsomycin (3) and its four analogues (4–7) based on diastereoselective oxidation of sulfide, sulfenylation, and coupling of 6-methyluracylacryllic acid with monooxodithioacetal amine, are described. Studies on the biological activity of morphological reversion on *src*^{ts}-NRK cells were also carried out.

Key words: total synthesis; *src*^{ts}-NRK cell; sparoxomycin A1; pyridinylpropenamide antibiotic; structure-activation relationship

Initial studies.We have previously reported that the pyrimidinylpropenamide antibiotics, sparoxomycins A1 and A2 (**1** and **2**), showed remarkably flat reversion activity on the transformed morphology of temperature-sensitive mutant Rous sarcoma virus-infected NRK cells (*src*^{ts}-NRK cells) to normal morphology in a wide range of concentration without cytotoxicity.[1,2] The structures of **1** and **2** are closely related to the inhibitor of protein biosynthesis, sparsomycin (**3**), which is a secondary metabolite of *Streptomyces sparsogenes*[3] or *Streptomyces cuspidosporus*,[4] only differing in the oxidation level at the sulfur atom. Wiley and Nackellar elucidated the structure of **3** in 1970,[5] while Ottenheijm and coworkers deduced the absolute configuration in 1981.[6] Sparsomycin (**3**) displayed broad-spectrum *in vitro* activity against a variety of gram-negative and gram-positive bacteria, and showed potent antitumor activity. The biological activity resulted from its ability to inhibit the peptide bond-forming step of protein biosynthesis by interacting with the large ribosomal subunit.[7–10] These potentially important biological activities have made **3** an attractive target for a potential antineoplastic compound.[11] Since the first synthetic study of sparsomycin in 1976,[12] synthetic studies of sparsomycin,[13–15] total synthesis,[16–18] biosynthesis,[19,20] and structure-activity relationship studies[21–27] have been widely reported. There have been many reports on the antitumor, antibacterial, antifungal, and antiviral properties; however, normalization of the phenotype of oncogene-transformed cells by **3** and its analogues has not previously been reported. We have already reported the syntheses of **1**–**3** and six analogues. Sparsomycin (**3**) and its alkyl analogues, ethyl and butyl, showed higher morphological reversion activities than those of **1** and **2**.[28] This paper reports in full our observations regarding a novel and stereoselective synthesis of the alkyl analogues of sparsomycin, ethylsparsomycin (**4**), butylsparsomycin (**5**), allylsparsomycin (**6**), and of benzylsparsomycin (**7**), as well as the biological activity of morphological reversion on *src*^{ts}-NRK cells.[29] The work on sparoxomycins A1 and A2 and their analogues was not reinvestigated.

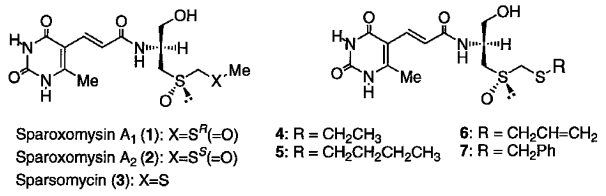

Fig. 1. Structures of the Target Compounds.

Retrosynthetic analysis. A retrosynthetic analysis was carried out based on Helquist's protocol[18] as summarized in Scheme 1. The structure of sparsomycin might enable it to be produced from 6-methyluracylacryllic acid (**8**) and the hydroxy-protected

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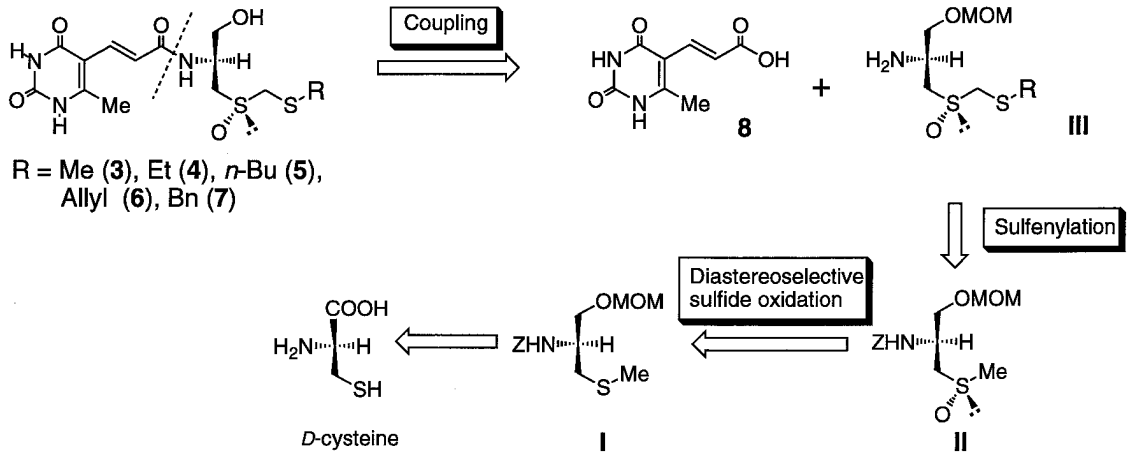
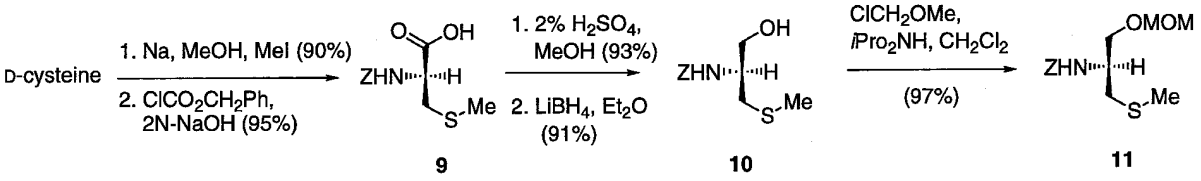
Abbreviations: Cbz, benzyloxycarbonyl; MOM, methoxymethyl; TBHP, *tert*-butyl hydroperoxide; DET, diethyl tartrate; LDA, lithium diisopropylamide; THF, tetrahydrofuran; DME, ethylene glycol dimethyl ether; DMF, *N*,*N*-dimethylformamide; HMPA, hexamethylphosphoramide; TMEDA, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; DCC, dicyclohexyl carbodiimide; HOBt, 1-hydroxybenzotriazole; HOAt, 1-hydroxy-7-azabenzotriazole



Scheme 1. Retrosynthesis of Sparsomycin and Its Alkyl Analogues.



Scheme 2. Sulfide Preparation from D-Cysteine.

amino dithioacetal mono-*S*-oxide part (**III**) and to couple these two building blocks by amide formation. The alkyl sulfenyl group of **III** was prepared by sulfenylation of the methyl sulfoxide (**II**) with alkyl disulfide. In order to avoid any separation of the diastereomeric mixture of sulfoxides, diastereoselective oxidation of sulfide **I** to **II** was studied. The starting sulfide was derived from D-cysteine.

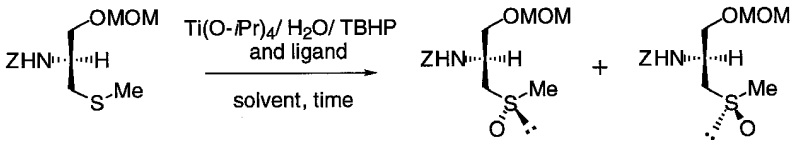
Results and Discussion

Diastereoselective oxidation of the sulfide

Esterification of D-cysteine and subsequent Cbz protection afforded *S*-methyl D-cysteine *N*-Cbz derivative **9** in an 85% yield. The carboxyl group was converted to alcohol **10** by a two-step sequence in an 84% yield, involving methyl ester formation with 2% H_2SO_4/MeOH and subsequent lithium borohydride reduction. The hydroxy group was protected by MOM ether **11** in a 97% yield. All reactions were relatively simple and easy to perform (5 steps, 77% overall yield) compared with the previously reported synthesis (7 steps, 52% overall yield).[18]

Diastereoselective oxidation of the sulfide to sulfoxide was studied. Many useful methods for asymmetric sulfide oxidation have been reported in the literature.[30] After several attempts to obtain the highest yield and selectivity, the titanium complex in the presence of water produced from Ti(O*i*-Pr)$_4$ and tartaric acid derivatives reported by Kagan[31-33] and 1,1′-bi-2-naphthol reported by Uemura[34,35] were found to achieve the required diastereoselective oxidation. The results are summarized in Table 1. When MOM-protected sulfide **11** was oxidized by TBHP (a decane solution) in CCl_4 with 1.0 eq. of Ti(O*i*-Pr)$_4$ and H_2O without a ligand, slow reactivity and poor diastereo-selectivity were observed (entry 1). The reaction in the presence of 2.0 eq. of (2*R*,3*R*)-(+)-DET as a ligand at −20°C enabled diastereomers *Sc*Ss-**12** and *Sc*Rs-**12** of sulfoxide[36] to be isolated in a 44% yield with 24% d.e. selectivity by an HPLC analysis (entry 2). Desired *Sc*Ss-**12** was provided in an 86% chemical yield with 65% d.e. selectivity by using (2*S*,3*S*)-(−)-DET as the ligand (entry 3). Catalytic oxidation was achieved by using 1,1′-bi-2-naphthol. The addition of 0.05 eq of (*R*)-(+)-1,1′-bi-2-naphthol catalyzed selective sulfide oxidation[33,34] to give *Sc*Ss-**12** in a 74% yield with 72% d.e. selectivity at 20°C in 1 h. Interestingly, (*S*)-(−)-1,1′-bi-2-naphthol catalyzed oxidation in a 74% yield to afford *Sc*Ss-**12**, but with no selectivity (entry 4 *vs.* 5). The reaction in a toluene solvent gave a comparable chemical yield with slightly lower selectivity of 65% d.e. (entry 6). The CH_2Cl_2 solvent gave high chemical yield of 90% but low selectivity of 33% d.e. (entry 7). The reaction was optimized by using 0.2 equiv. of (*R*)-(+)-1,1′-bi-2-naphthol. When the reaction was performed at 0°C for 2.5 h, the selectivity was increased to 87% d.e. with a 59% yield (entry 8). The reaction at −10°C required a prolonged reaction time to provide a slightly increased 75% chemical yield with 85% d.e. (entry 9). A lower reaction temperature provided a better chemical yield of 78%, however, the d.e. selectivity dropped to 77% (entry

Table 1. Diastereoselective Oxidation of the *S*-Methyl-D-cysteinol Derivatives to Methyl Sulfoxide



11 *ScSs*-**12** *ScRs*-**12**

Entry	Ligand	Eq.	Temp (°C)	Time (h)	Solvent	Yield (%[a])	de (%[b])	Configuration of sulfur atom
1	—	0	0	16	CCl_4	47	6	*S*s
2	(2*R*,3*R*)-(+)-DET	2.0	−20	4	CCl_4	44	24	*R*s
3	(2*S*,3*S*)-(−)-DET	2.0	−20	4	CCl_4	86	65	*S*s
4	(*R*)-(+)-1,1′-bi-2-naphthol	0.05	20	1	CCl_4	74	72	*S*s
5	(*S*)-(−)-1,1′-bi-2-naphthol	0.05	20	1	CCl_4	74	0	—
6	(*R*)-(+)-1,1′-bi-2-naphthol	0.05	20	1	toluene	74	65	*S*s
7	(*R*)-(+)-1,1′-bi-2-naphthol	0.05	20	1	CH_2Cl_2	90	33	*S*s
8	(*R*)-(+)-1,1′-bi-2-naphthol	0.2	0	2.5	CCl_4	59	87	*S*s
9	(*R*)-(+)-1,1′-bi-2-naphthol	0.2	−10	9.5	CCl_4	75	85	*S*s
10	(*R*)-(+)-1,1′-bi-2-naphthol	0.2	−20	15	CCl_4	78	77	*S*s

[a]Isolation yield by SiO_2 column chromatography. [b]The diastereomeric excess (de) was determined by an HPLC analysis with an RP-18 column (Mightysil®, 250×4.6 mm, 5 μm, MeOH/H_2O, 40/60, 1.0 ml/min).

10). The desired optically pure compound, *ScSs*-**12**, was easily obtainable by a single recrystallization from CH_2Cl_2/hexane (1/5), mp 113–114°C, $[\alpha]_D^{19}$ +87.2° (*c* 0.2, CH_2Cl_2). The *S* configuration at the sulfur atom of *ScSs*-**12** was confirmed by a spectroscopic data comparison with the literature value.[18)]

Construction of the dithioacetal mono-S-oxide

The amino-protecting Cbz Group of *ScSs*-**12** was removed under dissolved metal conditions to afford methyl sulfoxide **13** in a 91% yield. The dithioacetal mono-*S*-oxide structure was subjected to sulfenylation of the α-sulfinyl carbanion, which has already been reported by Helquist.[15,18)] However, under the reported conditions, sulfenylation was quite difficult to reproduce, and the trithioacetal mono-*S*-oxide (**14b**) was only isolated in a 47% yield. After several trials, in which LDA (1.0 equiv.) was slowly added dropwise to a THF solution of a mixture of **13** and dimethyl disulfide (1.0 equiv.) (reverse addition), the desired sulfenylation proceeded to obtain dithioacetal mono-*S*-oxide (**14a**) in a 29% yield (52% based on the consumed material) contaminated with 11% of **14b**.[37)] Use of a solvent such as ether, DME or DMF, and HMPA or TMEDA as the additive did not improve the yield of **14a**. Although improvement of the sulfenylation conditions without the formation of trithioacetal mono-*S*-oxide (**15b–18b**) is still necessary, under the reverse addition conditions, sulfenylation provided ethyl, *n*-butyl, allyl and benzyl dithioacetal mono-*S*-oxides (**15a–18a**) in 35%, 36%, 7.5% and 7% yields, respectively (Scheme 3).

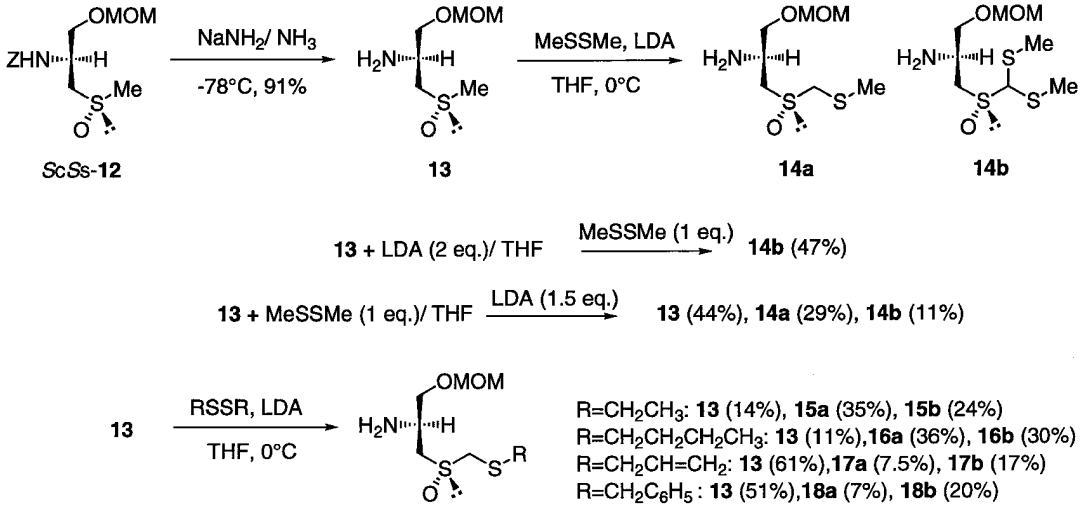
Synthesis of sparsomycin and its alkyl analogues

The coupling between the acid part (**8**) and amine part (**III**) was next examined. The coupling of **8**[38)] and **13** with dicyclohexyl DCC and HOBt in DMF gave amide **19** in a 56% yield. This coupling yield of **19** was improved to 85% by using HOAt.[39)] Treatment of **19** with 1 M HCl/MeOH resulted in an inseparable epimeric 1/1 mixture of sulfoxides which was contaminated. Ion exchange resin was very useful for deprotection toward the polar sparsomycin analogues to avoid a tedious isolation work-up and sulfoxide epimerization. The MOM group was removed with Dowex® 50W in MeOH to give alcohol **20** in a 65% yield.

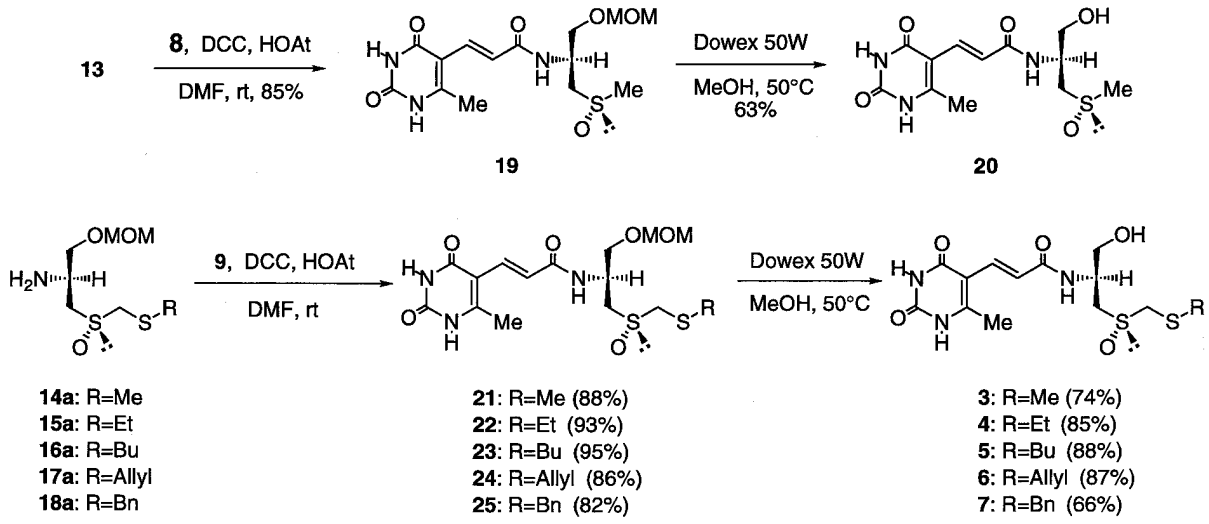
Sparsomycin (**3**) and its alkyl analogues, ethylsparsomycin (**4**), n-butylsparsomycin (**5**), allylsparsomycin (**6**) and benzylsparsomycin (**7**), were synthesized from (**14a–18a**) by following the optimized conditions *via* MOM-ethers (**21–25**) in good yields (Scheme 4). The optical rotation and melting point of synthetic sparsomycin (**3**), $[\alpha]_D^{25}$ +65.1° (*c* 0.28, H_2O) and mp 204–206°C (decomposing), are in good agreement with the literature values, $[\alpha]_D^{25}$ +65.1° (*c* 0.37, H_2O)] and mp 207–210°C (decomposing).[3)] All these new compounds gave satisfactory NMR and IR data together with HRMS. We stored sparoxomycin and its analogues (**3–7**) in the dark after their recrystallization from MeOH, because the trans double bond tended to easily isomerize to the *cis*-isomer in a solution.

Biological Activity

With sparsomycin and its analogues in hand, the morphological reversion activity on *src*^{ts}-NRK cells was determined in a concentration-dependent manner. The relationship between the structure and antitumor activity of sparsomycin and its analogues



Scheme 3. Construction of the Dithioacetal Mono-*S*-oxides.



Scheme 4. Syntheses of Sparsomycin (**3**) and Its Analogues (**4**–**7**).[1)]

has previously been reported for leukemia L1210 cells. The ID_{50} values of the sparsomycin analogues rose as their lipophilicity increased, this tendency being similar to the study on the inhibition of HeLa S3 colony formation.[40)] All of the assay samples were used after being recrystallized from MeOH as white prisms. The results are presented in Table 3. Although all the analogues exhibited morphological reversion activity on *src*^{ts}-NRK cells, sparsomycin (**3**) showed the highest activity (7.8 μM). It is interesting that the morphological reversion activity on *src*^{ts}-NRK cells and the lipophilicity of sparsomycin were not parallel. The minimal effective concentration (MEC) of the ethyl and allyl analogues (**4** and **6**) was twice at 15.6 μM, of the butyl analogue (**5**) was four times at 31.3 μM, of the benzyl analogue (**7**) and MOM-ethers (**19**, **21** and **22**) was 8 times at 62.5 μM that of sparsomycin (**3**). MOM-ether **23** was 16-fold less potent than **3** to show 125 μM. Interestingly, methyl sulfoxide (**20**) showed no activity at 530 μM. The synthesized cinnamide, catechol and pyridine derivatives instead of the pyrimidinylpropenamide group[28)] also did not show any activity at 530 μM. These findings suggest that the pyrimidinylpropenamide group was essential and that the monooxodithioacetal group also played an important role in exhibiting the morphological reversion activity.

Conclusion

Sparsomycin (**3**) and its four analogues (**4**–**7**) were synthesized by diastereoselective oxidation of sulfide, sulfenylation, and coupling of the 6-methyluracylacryllic acid and monooxodithioacetal amine parts. Their morphological reversion activity on *src*^{ts}-NRK cells was also studied to find that the pyrimidinylpropenamide group was essential and the monoox-

Table 2. Flat Reversion Activity on the Transformed Morphology of *src*^{ts}-NRK Cells

Compound	*src*^{ts}-NRK, MEC (μM)
MOM-**20** (**19**)	62.5
20	>530.0[a]
MOM-**3** (**21**)	62.5
MOM-**4** (**22**)	62.5
MOM-**6** (**23**)	125.0
sparsomycin (**3**)	7.8
4	15.6
5	31.3
6	15.6
7	62.5

[a] No activity at 530 μM.

odithioacetal group also played an important role in exhibiting the morphological reversion activity.

Experimental

General. All melting point (mp) data were measured with Yamato MP-21 melting point apparatus and are uncorrected. Optical rotation values were measured in $CHCl_3$, H_2O or MeOH with a Horiba SEPA-300 high-sensitivity polarimeter. Synthetic sulfoxides *ScSs*-**12** and *ScRs*-**12** were analyzed in a Mightysil® RP-18 GP column (Kanto Chemical Co., Japan, 250×4.6 mm, 5 μm) using 40% MeOH in H_2O as the eluent. Retention times (^{t}R) and integrated ratios were obtained from a Hewlett-Packard 3390A recorder. IR spectra were recorded as neat for oils, and as KBr discs for solids, with a Shimadzu OR-8000 spectrometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ or CD_3OD by JEOL JNM EX-400 and JEOL JNM LA-400 instruments, while low- and high-resolution mass spectra (MS) were measured with a JEOL JMS AX-500 spectrometer at 70 or 15 eV. Fuji Silica Chemical Ltd. BW-300 silica gel was used for column chromatography.

(S)-N-(Benzyloxycarbonyl)-O-(methoxymethyl)-S-methyl-D-cysteinol S-oxide (12). An oven-dried, 300-ml three-necked flask equipped with a septum and a nitrogen inlet was charged with Ti(O*i*-Pr)$_4$ (0.63 g, 2.2 mmol), (*R*)-(+)-1,1′-bi-2-naphthol (1.25 g, 4.4 mmol) and carbontetrachloride (50 ml). To the vigorous stirred reaction mixture at 0°C was slowly added H_2O (0.79 ml, 44 mmol) *via* syringe, stirring being continued for 30 min. A CCl_4 (20 ml) solution of *N*-(benzyloxycarbonyl)-*O*-(methoxymethyl)-*S*-methyl-D-cysteinol (**11**, 6.6 g, 22 mmol) and 5–6 M decane solution of TBHP (8 ml, 40–48 mmol) were added, and the resulting mixture was stirred at −10°C for 9.5 h. The reaction was quenched with H_2O (20 ml) at that temperature. The reaction mixture was warmed to rt, the undissolved material was separated by Celite filtration, and the mixture was extracted with CH_2Cl_2 (3×100 ml). The combined organic layers were washed with brine (300 ml) and then dried over Na_2SO_4. Concentration and subsequent purification by silica gel column chromatography (CH_2Cl_2/MeOH, 30/1) afforded a mixture of *ScSs*-**12** and *ScRs*-**12** (5.2 g, 75%) as a colorless solid. The diastereomeric excess (de) was determined by an HPLC analysis to be 85% (Mightysil® RP-18, 250×4.6 mm, 5 μm, 40% MeOH/H_2O, 1.0 ml/min) ^{t}R(*ScSs*)-**12**, 14.2 min (92.5%), ^{t}R(*ScRs*)-**12**, 15.3 min (7.5%).

The mixture of sulfoxides (**12**, 5.2 g) was recrystallized from CH_2Cl_2/hexane (1/5) to give a 4.10 g (79%) of *ScSs*-**12** as colorless needles, mp 113–114°C (CH_2Cl_2/hexane), *Rf* 0.15 (CH_2Cl_2/MeOH, 20/1), $[\alpha]_D^{25}$ +87.2° (*c* 0.20, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.36–7.29 (5H, m, Ph), 5.77 (1H, br d, NH, *J*=7.5 Hz), 5.10 (2H, s), 4.63 (2H, s), 4.36–4.28 (1H, m), 3.78 (1H, dd, *J*=3.4, 10.0 Hz), 3.74 (1H, dd, *J*=5.9, 10.0 Hz), 3.35 (3H, s), 3.04 (1H, dd, *J*=6.6, 13.0 Hz), 2.97 (1H, dd, *J*=4.9, 13.0 Hz), 2.62 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz): 155.7, 136.3, 128.4, 128.2, 128.0, 96.6, 68.4, 66.6, 56.1, 55.3, 47.5, 39.1; IR ν_{max} (neat) cm^{-1}: 3326 (m), 2928 (m), 1726 (m), 1687 (s), 1541 (s), 1466 (w), 1309 (m), 1273 (m), 1142 (m), 1039 (m), 1026 (m), 727 (w), FAB-MS *m/z* (rel. int.): 318 (10), 317 (22), 316 (MH^+, 100), 284 (8), 176 (9), 164 (6), 91 (100); FAB HRMS *m/z*: calcd. for $C_{14}H_{22}O_5NS$ (MH^+), 316.1218; found, 316.1202.

(S)-O-(methoxymethyl)-S-methyl-D-cysteinol S-oxide (13). *N*-(benzyloxycarbonyl)-*O*-(methoxymethyl)-*S*-methyl-D-cysteinol *S*-oxide (**12**, 100 mg, 0.32 mmol) was placed in the three-necked flask equipped with a dry ice condenser. A mixture of dry ice and acetone was placed in the condenser, and the bottom flask was cooled in a dry ice-acetone bath. Ammonia gas was introduced and condensed (ca. 10 ml) in the flask, and sodium (18 mg, 0.8 mmol) was added to the flask. After several minutes, the dark blue solution was gradually decolorized, and reaction was then quenched by adding ammonium chloride (0.28 g). The liquid ammonia was allowed to evaporate, and the residue was extracted with CH_2Cl_2 (3×10 ml). The combined organic layers were washed with brine (20 ml) and then dried over Na_2SO_4. Concentration afforded the amine as a red oil. This material was purified by silica gel short-column chromatography (CH_2Cl_2/MeOH, 10/1) and subsequent Kugelrohr distillation to afford 52.2 mg (91%) of **13** as a colorless oil, bp 170–175°C (0.45 mmHg), *Rf* 0.25 (CH_2Cl_2/MeOH, 10/1), $[\alpha]_D^{22}$ +99.8° (*c* 1.97, $CHCl_3$), ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.64 (2H, s), 3.62–3.55 (2H, m), 3.50 (1H, dd, *J*=7.1, 10.7 Hz), 3.37 (3H, s), 2.83 (1H, dd, *J*=2.7, 13.0 Hz), 2.68 (1H, dd, *J*=9.7, 13.0 Hz), 2.64 (3H, s), ^{13}C-NMR ($CDCl_3$, 100 MHz): 96.6, 71.9, 59.3, 54.9, 45.9, 38.6, IR ν_{max} (neat) cm^{-1}: 3400 (m), 3285

(m), 2926 (m), 1674 (m), 1296 (m), 1213 (m), 1149 (m), 1111 (m), 1037 (s), 956 (m), 916 (m), 692 (w), FAB-MS *m/z* (rel. int.): 187 (7), 183 (10), 182 (MH^+, 100), 150 (20), 118 (6), 86 (23), 45 (30); FAB HRMS *m/z*: calcd. for $C_6H_{16}O_3NS$ (MH^+), 182.0851; found, 182.0877.

General procedure for sulfenylation. A 0.9 M LDA solution was prepared from 0.75 ml (5.2 mmol) of diisopropylamine, 3.3 ml (5.2 mmol) of a 1.57 M hexane solution of *n*-BuLi and 1.75 ml of THF. 1.5 Equivalents of the LDA solution was slowly added (within 10 min) to a stirred THF solution of (*S*)-*O*-(methoxymethyl)-*S*-methyl-D-cysteinol *S*-oxide (**13**) and alkyl disulfide at 0°C, and stirring was continued for 30 min at that temperature. The reaction was quenched by adding MeOH, and the mixture was allowed to warm to rt. After the mixture had been concentrated *in vacuo*, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 20/1 to 10/1) to afford a di-sulfenylation product (**b**) from the first fraction and mono-sulfenylation product (**a**) from the second fraction as a colorless oil. The starting material (**13**) was recovered as a colorless oil from the third fraction. All compounds were obtained as colorless oils.

(R)-O-(Methoxymethyl)-S-((methylthio)methyl)-D-cysteinol S-oxide (14a). Sulfenylation according to the general procedure, using **13** (282 mg, 1.56 mmol), methyl disulfide (181 μl, 1.56 mmol) and an LDA solution (2.6 ml, 2.34 mmol) in THF (5 ml) afforded a colorless oil which was purified by silica gel column chromatography to give **14b** (46 mg, 11%) from the first fraction and **14a** (102 mg, 29%) from the second fraction. The starting material (**13**, 124 mg, 44%) was recovered from the third fraction. Data for **14a**: *Rf* 0.28 (CH_2Cl_2/MeOH, 20/1), $[\alpha]_D^{31}$ +130.6° (*c* 1.29, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.64 (2H, s), 3.76 (1H, d, *J*=13.7 Hz), 3.68 (1H, d, *J*=13.7 Hz), 3.58 (1H, dd, *J*=5.3, 9.5 Hz), 3.55 (1H, dd, *J*=3.5, 9.5 Hz), 3.52–3.45 (1H, m), 3.33 (3H, s), 2.87 (1H, dd, *J*=10.2, 12.9 Hz), 2.81 (1H, dd, *J*=3.5, 12.9 Hz), 2.33 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz): 96.6, 72.4, 56.5, 55.4, 55.1, 46.3, 17.2; IR ν_{max} (neat) cm^{-1}: 3400 (m), 3285 (m), 2926 (m), 2826 (w), 1674 (m), 1410 (w), 1296 (m), 1213 (w), 1150 (m), 1111 (s), 1038 (s), 957 (m), 916 (m); FAB-MS *m/z* (rel. int.): 230 (13), 229 (17), 228 (MH^+, 83), 183 (67), 107 (100); FAB HRMS *m/z*: calcd. for $C_7H_{18}O_3NS_2$ (MH^+), 228.0728; found, 228.0737. Data for **14b**: ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.63–4.60 (3H, s, $OC\underline{H}_2O$ and $C\underline{H}(SMe)_2$), 3.65 (1H, dd, *J*=4.7, 11.5 Hz), 3.65–3.57 (1H, m), 3.52 (1H, dd, *J*=5.4, 11.5 Hz), 3.38 (3H, s), 3.02–3.00 (2H, m), 2.38 (3H, s), 2.37 (3H, s), ^{13}C-NMR ($CDCl_3$, 100 MHz): 96.6, 72.5, 71.9, 55.4, 55.0, 46.6, 15.7, 15.4; FAB-MS *m/z* (rel. int.): 274 (MH^+).

(R)-O-(Methoxymethyl)-S-((ethylthio)methyl)-D-cysteinol S-oxide (15a). Sulfenylation according to the general procedure, an using **13** (270 mg, 1.49 mmol), ethyl disulfide (184 μl, 1.49 mmol) and LDA solution (2.5 ml, 2.25 mmol) in THF (5 ml), afforded a colorless oil which was purified by silica gel column chromatography to give **15b** (108 mg, 24%) from the first fraction and **15a** (126 mg, 35%) from the second fraction. The starting material (**13**, 37 mg, 14%) was recovered from the third fraction. Data for **15a**: *Rf* 0.32 (EtOAc/MeOH, 7/1), $[\alpha]_D^{20}$ +34.5° (*c* 2.4, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.61 (2H, s), 3.78 (1H, d, *J*=13.7 Hz), 3.74 (1H, d, *J*=13.7 Hz), 3.60–3.55 (2H, m), 3.52–3.45 (1H, m), 3.33 (3H, s), 2.91 (1H, dd, *J*=12.2, 13.7 Hz), 2.82 (1H, dd, *J*=2.7, 13.7 Hz), 2.70 (2H, q, *J*=7.3 Hz), 1.27 (3H, t, *J*=7.3 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz): 96.6, 72.4, 56.4, 55.4, 52.8, 46.3, 27.6, 14.5; IR ν_{max} (neat) cm^{-1}: 3600–3080 (br s), 2930 (m), 1559 (w), 1507 (w), 1456 (m), 1269 (w), 1149 (m), 110 (m), 1111 (m), 1038 (s), 916 (w); FAB-MS *m/z* (rel. int.): 264 ($[M+Na]^+$, 28), 243 (13), 242 (MH^+, 100), 180 (16), 95 (99), FAB HRMS *m/z*: calcd. for $C_8H_{20}O_3NS_2$ (MH^+), 242.0885; found, 242.0898.

(R)-O-(Methoxymethyl)-S-((n-butylthio)methyl)-D-cysteinol S-oxide (16a). Sulfenylation according to the general procedure using **13** (270 mg, 1.49 mmol), butyl disulfide (283 μl, 1.49 mmol) and LDA solution (2.5 ml, 2.25 mmol) in THF (5 ml) afforded a colorless oil which was purified by silica gel column chromatography to give **16b** (161 mg, 30%) from the first fraction and **16a** (143 mg, 36%) from the second fraction. The starting material (**13**, 30 mg, 11%) was recovered from the third fraction. Data for **16a**: *Rf* 0.35 (EtOAc/MeOH, 10/1), $[\alpha]_D^{20}$ +43.3° (*c* 0.73, $CHCl_3$), ^{1}H-NMR (400 MHz, $CDCl_3$): 4.66 (2H, s), 3.80 (1H, d, *J*=13.9 Hz), 3.76 (1H, d, *J*=13.9 Hz), 3.61 (1H, dd, *J*=4.1, 10.7 Hz), 3.64–3.59 (1H, m), 3.51 (1H, dd, *J*=7.1, 10.7 Hz), 3.37 (3H, s), 2.97 (1H, dd, *J*=9.9, 12.9 Hz), 2.81 (1H, dd, *J*=2.7, 12.9 Hz), 2.74 (2H, t, *J*=7.3 Hz), 1.60 (2H, quintet, *J*=7.3 Hz), 1.43 (2H, sextet, *J*=7.3 Hz), 0.92 (3H, t, *J*=7.3 Hz), ^{13}C-NMR (100 MHz, $CDCl_3$): 96.6, 72.3, 56.3, 55.4, 53.2, 46.4, 33.5, 31.4, 21.7, 13.5, IR ν_{max} (neat) cm^{-1}: 3360 (w), 2957 (m), 2930 (m), 2874 (s), 1670 (w), 1460 (w), 1380 (w), 1150 (m), 1111 (m), 1038 (s), 918 (m); FAB-MS *m/z* (rel. int.): 272 (4), 271 (5), 270 (MH^+, 35), 192 (6), 167 (17), 149 (100), 103 (27), 61 (48), FAB HRMS *m/z*: calcd. for $C_{10}H_{24}O_3NS_2$ (MH^+), 270.1198; found, 270.1298.

(R)-O-(Methoxymethyl)-S-((allylthio)methyl)-D-cysteinol S-oxide (17a). Sulfenylation according to the general procedure, using **13** (181 mg, 1.00 mmol), allyl disulfide (80%, 181 μl, 1.0 mmol) and a 0.5 M LDA solution (2.0 ml, 1.00 mmol) in THF (10 ml), afforded a colorless oil which was purified by silica

gel column chromatography to give **17b** (56.1 mg, 17%) from the first fraction and **17a** (18.9 mg, 7.5%) from the second fraction. The starting material (**13**, 111 mg, 61%) was recovered from the third fraction. Data for **17a**: $[\alpha]_D^{31}$ +103.3° (*c* 0.68, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.76 (1H, ddt, *J*=17.5, 10.1, 7.3 Hz), 5.22 (1H, dd, *J*=1.0, 10.0 Hz), 5.21 (1H, dd, *J*=1.0, 17.5 Hz), 4.64 (2H, s), 3.74 (1H, d, *J*=13.7 Hz), 3.71 (1H, d, *J*=13.7 Hz), 3.62–3.48 (3H, m), 3.36 (3H, s), 3.31 (2H, d, *J*=7.3 Hz), 2.91 (1H, dd, *J*=9.8, 12.7 Hz), 2.81 (1H, dd, *J*=3.0, 12.7 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz): 132.4, 119.4, 96.6, 72.4, 56.5, 55.4, 50.7, 46.4, 35.6; IR ν_{max} (neat) cm^{-1}: 3368 (m), 2928 (m), 1636 (w), 1404 (w), 1150 (m), 1112 (m), 1040 (s), 920 (m); FAB-MS *m/z* (rel. int.): 254 (MH^+, 1.2), 253 (4.4), 179 (74), 148 (24), 130 (27), 87 (100), 72 (39); FAB HRMS *m/z*: calcd. for $C_9H_{19}O_3NS_2$ (MH^+), 253.0806; found, 253.0829.

*(R)-O-(Methoxymethyl)-S-((benzylthio)methyl)-D-cysteinol S-oxide (**18a**).* Sulfenylation according to the general procedure, using **13** (181 mg, 1.00 mmol), benzyl disulfide (98% purity, 251 mg, 1.00 mmol) and a 0.5 M LDA solution (2.0 ml, 1.00 mmol) in THF (10 ml), afforded a colorless oil which was purified by silica gel column chromatography to give **18b** (84.9 mg, 20%) from the first fraction and **18a** (20.2 mg, 7%) from the second fraction. The starting material (**13**, 92.0 mg, 51%) was recovered from the third fraction. Data for **18a**: *Rf* 0.32 (EtOAc/MeOH, 7/1), $[\alpha]_D^{30}$ +110.1° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.34–7.33 (5H, m), 4.63 (2H, s), 3.92 (1H, dd, *J*=4.2, 8.8 Hz), 3.66 (1H, d, *J*=13.7 Hz), 3.59 (1H, d, *J*=13.7 Hz), 3.58 (1H, dd, *J*=4.2, 8.8 Hz), 3.59–3.52 (1H, m), 3.48 (1H, dd, *J*=5.1, 8.8 Hz), 3.36 (3H, s), 2.87 (1H, dd, *J*=9.2, 12.7 Hz), 2.81 (1H, dd, *J*=3.5, 12.7 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz): 136.4, 129.2, 127.6, 96.6, 72.3, 56.5, 55.4, 51.2, 46.4, 36.9; IR ν_{max} (neat) cm^{-1}: 3370 (s), 2928 (m), 1601 (w), 1459 (w), 1150 (m), 1111 (m), 1038 (s), 916 (w), 704 (m); FAB-MS *m/z* (rel. int.): 326 ($[M+Na]^+$, 18), 305 (16), 304 (MH^+, 84), 180 (20), 137 (47), 91 (100); FAB HRMS *m/z*: calcd. for $C_{13}H_{22}O_3NS_2$ (MH^+), 304.1041; found, 304.1060.

*General procedure for coupling between of (E)-3-(6-methyl-5-uracilyl)-2-propeonic acid (**8**) and the amine part (**III**).* A solution of amine part **III** in DMF was added to a DMF solution of (*E*)-3-(6-methyl-5-uracilyl)-2-propeonic acid (**8**), DCC, and HOAt. The reaction mixture was stirred for 12 h at room temperature. The reaction was concentrated, and the residue was directly purified by silica gel column chromatography (CH_2Cl_2/MeOH, 9/1) and subsequent concentration of the appropriate fraction *in vacuo* to give the indicated yields of products as white solids.

*(E)-(Ss)-N-[2-(Methylsulfinyl)-1-[(methoxymethyloxy)methyl]ethyl]-3-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinyl)-2-propenamide (**19**).* Coupling according to the general procedure, using **8** (157.6 mg, 0.93 mmol), **13** (140.0 mg, 0.77 mmol), DCC (191 mg, 0.93 mmol), and HOAt (126.0 mg, 0.93 mmol) in DMF (5 ml), afforded a solid which was purified by silica gel column chromatography to give 235.3 mg (85%) of **19** as a white solid, mp 124–127°C, *Rf* 0.25 (EtOAc/MeOH, 3/1), $[\alpha]_D^{27}$ +121.5° (*c* 0.56, MeOH); ^{1}H-NMR (D_2O, 400 MHz): 7.19 (1H, d, *J*=15.5 Hz), 6.87 (1H, d, *J*=15.5 Hz), 4.56 (2H, s), 4.49–4.41 (1H, m), 3.63 (1H, dd, *J*=4.8, 10.7 Hz), 3.58 (1H, dd, *J*=5.8, 10.7 Hz), 3.22 (3H, s), 2.99 (1H, t, *J*=11.5 Hz), 2.98 (1H, dd, *J*=8.8, 11.5 Hz), 2.59 (3H, s), 2.19 (3H, s); ^{13}C-NMR (D_2O, 100 MHz): 169.9, 165.3, 157.5, 152.3, 133.2, 121.1, 106.4, 97.0, 69.7, 56.2, 55.9, 45.6, 38.1, 17.5; IR ν_{max} (KBr) cm^{-1}: 3300 (m), 3063 (w), 3002 (w), 2950 (m), 2911 (m), 2820 (m), 1659 (s), 1633 (s), 1547 (s), 1339 (m), 1254 (m), 1172(w), 1109 (s), 10518 (s), 1024 (s), 972 (m), 767 (m); FAB-MS *m/z* (rel. int.): 362 (22), 361 (48), 360 (MH^+, 100), 182 (83), 179 (58); FAB HRMS *m/z*: calcd. for $C_{14}H_{22}O_6N_3S$ (MH^+), 360.1229; found, 360.1217.

*O-(Methoxymethyl)sparsomycin (**21**).* Coupling according to the general procedure, using **8** (23.5 mg, 0.12 mmol), **14a** (23.7 mg, 0.104 mmol), DCC (24.8 mg, 0.12 mmol), and HOAt (16.3 mg, 0.12 mmol) in DMF (1 ml), afforded a solid which was purified by silica gel column chromatography to give 35.6 mg (88%) of **21** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, mp 115–117°C (decomposing), *Rf* 0.35 (EtOAc/MeOH, 8/1), $[\alpha]_D^{23}$ +44.0° (*c* 2.2, H_2O); ^{1}H-NMR (D_2O, 400 MHz): 7.20 (1H, d, *J*=15.3 Hz), 6.87 (1H, d, *J*=15.3 Hz), 4.56 (2H, s), 4.52–4.46 (1H, m), 3.94 (1H, d, *J*=13.9 Hz), 3.77 (1H, d, *J*=13.9 Hz), 3.65 (1H, dd, *J*=4.7, 10.7 Hz), 3.60 (1H, dd, *J*=5.6, 10.7 Hz), 3.22 (3H, s), 3.08 (1H, dd, *J*=10.2, 13.5 Hz), 3.02 (1H, dd, *J*=4.1, 13.5 Hz), 2.18 (3H, s), 2.11 (3H, s); ^{13}C-NMR (D_2O, 100 MHz): 169.8, 165.3, 157.3, 152.1, 133.2, 121.2, 106.5, 97.0, 69.7, 55.9, 55.3, 53.6, 45.6, 17.4, 16.8; IR ν_{max} (KBr) cm^{-1}: 3440 (s), 3280 (m), 2820 (w), 1720 (s), 1655 (s), 1620 (m), 1610 (m), 1530 (m), 1460 (w), 1440 (m), 1350 (w), 1300 (w), 1210 (w), 1150 (w), 1125 (m), 1045 (m), 1000 (w), 918 (w); FAB-MS *m/z* (rel. int.): 408 (19), 407 (23), 406 (MH^+, 66), 360 (16), 344 (16), 228 (25), 179 (100); FAB HRMS *m/z*: calcd. for (MH^+) $C_{15}H_{24}O_6N_3S_2$, 406.1107; found, 406.1110.

*O-(Methoxymethyl)ethylsparsomycin (**22**).* Coupling according to the general procedure, using **8** (58.5 mg, 0.3 mmol), **15a** (66.4 mg, 0.28 mmol), DCC (62.0 mg, 0.3 mmol), and HOAt (41 mg, 0.3

mmol) in DMF (1 ml), afforded a solid which was purified by silica gel column chromatography to give 107 mg (93%) of **22** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism: mp 169–172°C (decomposing), *Rf* 0.4 (EtOAc/MeOH, 6/1), $[\alpha]_D^{23}$ +46.8° (*c* 2.4, MeOH); ^{1}H-NMR (400 MHz, D_2O): 7.29 (1H, d, *J*=15.6 Hz), 6.92 (1H, d, *J*=15.6 Hz), 4.58 (2H, s), 4.56 (1H, m), 4.00 (1H, d, *J*=14.1 Hz), 3.85 (1H, d, *J*=14.1 Hz), 3.67 (1H, dd, *J*=4.7, 10.5 Hz), 3.63 (1H, dd, *J*=5.3, 10.5 Hz), 3.25 (3H, s), 3.14 (1H, dd, *J*=8.7, 13.7 Hz), 3.02 (1H, dd, *J*=3.7, 13.7 Hz), 2.61 (2H, q, *J*=7.5 Hz), 1.12 (3H, t, *J*=7.5 Hz); ^{13}C-NMR (100 MHz, D_2O): 170.0, 165.7, 157.7, 152.5, 133.4, 121.2, 106.6, 97.0, 69.8, 55.9, 53.8, 53.1, 45.7, 28.0, 17.5, 14.9; IR ν_{max} (KBr) cm^{-1}: 3447 (m), 2955 (m), 2936 (m), 2826 (m), 1717 (s), 1684 (s), 1608 (s), 1456 (m), 1340 (m), 1302 (m), 1213 (w), 1151 (s), 1034 (m), 862 (w); FAB-MS *m/z* (rel. int.): 422 (3), 421 (5), 420 (MH^+, 15), 404 (6), 388 (11), 356 (8), 328 (8), 284 (6), 242 (10), 179 (66), 76 (100); FAB HRMS *m/z*: calcd. for $C_{16}H_{26}O_6N_3S_2$ (MH^+), 420.1263; found, 420.1262.

O-(Methoxymethyl)n-butylsparsomycin (23). Coupling according to the general procedure, using **8** (53.2 mg, 0.27 mmol), **16a** (61.0 mg, 0.23 mmol), DCC (56.0 mg, 0.27 mmol), and HOAt (37.0 mg, 0.27 mmol) in DMF (1 ml), afforded a solid which was purified by silica gel column chromatography to give 95.6 mg (95%) of **23** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, mp 203–204.5°C (decomposing), *Rf* 0.28 (EtOAc/MeOH, 8/1), $[\alpha]_D^{23}$ +31.6 (*c* 1.1, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): 7.44 (1H, d, *J*=15.3 Hz), 7.21 (1H, d, *J*=15.3 Hz), 4.64 (2H, s), 4.66–4.61 (1H, m), 4.01 (1H, d, *J*=13.9 Hz), 3.87 (1H, d, *J*=13.9 Hz), 3.69 (2H, d, *J*=5.1 Hz), 3.35 (3H, s), 3.23 (1H, dd, *J*=10.3, 13.2 Hz), 3.07 (1H, dd, *J*=3.4, 13.3 Hz), 2.76 (2H, t, *J*=7.1 Hz), 1.60 (2H, quintet, *J*=7.3 Hz), 1.40 (2H, sextet, *J*=7.3 Hz), 0.91 (3H, t, *J*=7.3 Hz); ^{13}C-NMR (100 MHz, CD_3OD): 169.5, 164.8, 156.3, 151.9, 133.0, 122.1, 106.7, 97.7, 70.1, 55.7, 55.0, 54.4, 46.3, 34.3, 32.7, 22.7, 16.9, 13.9; IR ν_{max} (KBr) cm^{-1}: 2957 (m), 2934 (m), 2826 (w), 1723 (s), 1717 (s), 1674 (s), 1609 (m), 1539 (m), 1456 (m), 1304 (m), 1302(m), 1211 (m), 1151 (m), 1113 (m), 1039 (m), 916 (w); FAB-MS *m/z* (rel. int.): 450 (13), 449 (25), 448 (MH^+, 100), 373 (14), 324 (16), 264 (5), 242 (44), 180 (6), 148 (8); FAB HRMS *m/z*: calcd. for $C_{18}H_{30}O_6N_3S_2$ (MH^+), 448.1577; found, 448.1577.

O-(Methoxymethyl)allylsparsomycin (24). Coupling according to the general procedure, using **8** (14.5 mg, 0.074 mmol), **17a** (15.6 mg, 0.062 mmol), DCC (515.2 mg, 0.074 mmol), and HOAt (10.4 mg, 0.074 mmol) in DMF (1 ml), afforded a solid which was purified by silica gel column chromatography to give 22.9 mg (86%) of **24** as a white solid, mp 192–193°C (decomposing), *Rf* 0.21 (CH_2Cl_2/MeOH, 10/1), $[\alpha]_D^{30}$ +98.9° (*c* 0.21, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): 7.44 (1H, d, *J*=15.2 Hz), 7.22 (1H, d, *J*=15.2 Hz), 5.81 (1H, ddt, *J*=10.0, 17.1, 7.3 Hz), 5.20 (1H, dd, *J*=1.2, 17.1 Hz), 5.19 (1H, dd, *J*=1.0, 10.0 Hz), 4.66 (2H, s), 4.66–4.58 (1H, m), 3.97 (1H, d, *J*=13.9 Hz), 3.80 (1H, d, *J*=13.9 Hz), 3.68 (2H, d, *J*=5.1 Hz), 3.36 (2H, d, *J*=7.3 Hz), 3.35 (3H, s), 3.21 (1H, dd, *J*=10.4, 13.4 Hz), 3.09 (1H, dd, *J*=3.6, 13.4 Hz), 2.35 (3H, s); ^{13}C-NMR (100 MHz, CD_3OD): 169.5, 164.8, 156.2, 151.6, 134.4, 133.0, 122.1, 119.4, 106.7, 97.7, 70.0, 55.7, 54.9, 52.2, 46.2, 36.6, 16.9; IR ν_{max} (KBr) cm^{-1}: 3260 (m), 2920 (m), 2820 (m), 1719 (s), 1655 (s), 1650 (s), 1541 (s), 1522 (s), 1509 (s), 1459 (m), 1341 (m), 1113 (m), 1040 (m), 999 (m); FAB-MS *m/z* (rel. int.): 454 ($[M+Na]^+$, 8), 433 (5), 432 (MH^+, 20), 398 (27), 373 (26), 254 (20), 179 (78), 87 (100), FAB HRMS *m/z*: calcd. for $C_{17}H_{26}O_6N_3S_2$ (MH^+), 432.1263; found, 432.1271.

O-(Methoxymethyl)benzylsparsomycin (25). Coupling according to the general procedure, using **8** (15.5 mg, 0.08 mmol), **18a** (20.0 mg, 0.66 mmol), and HOAt (10.8 mg, 0.08 mmol) in DMF (1 ml), afforded a solid which was purified by silica gel column chromatography to give 26.0 mg (82%) of **25** as a white solid, mp 217.5–219°C (decomposing), $[\alpha]_D^{23}$ +109.5 (*c* 0.31, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): 7.43 (1H, d, *J*=15.4 Hz), 7.35–7.29 (2H, m), 7.26–7.21 (1H, m), 7.22 (1H, d, *J*=15.4 Hz), 4.63 (2H, s), 4.64–4.55 (1H, m), 3.94 (2H, s), 3.88 (1H, d, *J*=13.9 Hz), 3.70 (1H, d, *J*=13.9 Hz), 3.67 (2H, d, *J*=5.2, Hz), 3.34 (3H, s), 3.18 (1H, dd, *J*=10.2, 13.4 Hz), 3.05 (1H, dd, *J*=3.9, 13.4 Hz), 2.34 (3H, s); ^{13}C-NMR (100 MHz, CD_3OD): 169.3, 164.7, 156.0, 151.8, 138.1, 132.9, 130.3, 129.7, 128.5, 122.1, 106.7, 97.6, 69.9, 55.8, 54.9, 52.8, 46.1, 37.9, 16.9, IR (KBr) 3285 (m), 2960 (m), 2822 (m), 1719 (s), 1701 (s), 1694 (s), 1541 (s), 1439 (m), 1350 (m), 1300 (m), 1221 (m), 1148 (m), 1109 (m), 1034 (s), 770 (w), 698 (w); FAB-MS *m/z* (rel. int.): 504 ($[M+Na]^+$, 21), 483 (7), 482 (MH^+, 20), 179 (34), 91 (100); FAB HRMS *m/z*: calcd. for $C_{21}H_{28}O_6N_3S_2$ (MH^+), 482.1420; found, 482.1419.

General procedure for MOM deprotection. Dowex® 50W (H^+) was added to a solution of the MOM ether in MeOH. The suspension was stirred for 2 h at 50°C, and the Dowex resin was filtered off. Concentration and subsequent purification by silica gel column chromatography (CH_2Cl_2/MeOH, 10/1) gave the deprotected products as white solids.

(E)-(Ss)-N-[1-(Hydroxymethyl)ethyl-2-(methylsulfinyl)]-3-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinyl)-2-propenamide (20). Deprotection according to the general procedure, using **19** (53.6 mg, 0.15 mmol) and Dowex® 50W (H^+) (100 mg) in MeOH (10 ml), afforded a solid which was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 9/1) to give 29.6 mg (63%) of **20** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, ^{1}H-NMR (400 MHz, CD_3OD): 7.27 (1H, d, J=15.6 Hz), 6.92 (1H, d, J=15.6 Hz), 4.40–4.34 (1H, m), 3.66 (1H, dd, J=4.6, 11.7 Hz), 3.58 (1H, dd, J=5.8, 11.7 Hz), 3.05–2.96 (2H, m), 2.65 (3H, s), 2.28 (3H, s); ^{13}C-NMR (100 MHz, CD_3OD): 169.7, 165.0, 156.9, 152.0, 132.9, 122.0, 106.7, 64.1, 56.9, 47.9, 38.6, 17.3; IR ν_{max} (KBr) cm^{-1}: 3300 (s), 2950 (s), 2826 (s), 1710 (s), 1671 (s), 1603 (s), 1541 (s), 1429 (s), 1356 (m), 1306 (m), 1047 (s); FAB-MS m/z (rel. int.): 318 (17), 317 (33), 316 (MH^+, 100), 224 (58), 179 (33), 138 (33); FAB HRMS m/z: calcd. for $C_{12}H_{18}O_5N_3S$ (MH^+), 316.0967; found, 316.0934.

Sparsomycin (3). Deprotection according to the general procedure using **21** (62 mg, 0.153 mmol) and Dowex® 50W (H^+) (100 mg) in MeOH (10 ml) afforded a solid, which was purified by silica gel column chromatography to give 41 mg (74%) of **3** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, *Rf* 0.1 (EtOAc/MeOH, 6/1), $[\alpha]_D^{25}$ +65.1° (*c* 0.28, H_2O), mp 204–206°C (decomposing); ^{1}H-NMR (400 MHz, D_2O): 7.22 (1H, d, J=15.3 Hz), 6.88 (1H, d, J=15.5 Hz), 4.43–4.36 (1H, m), 3.94 (1H, d, J=13.9 Hz), 3.77 (1H, d, J=13.9 Hz), 3.62 (1H, dd, J=4.9, 11.7 Hz), 3.55 (1H, dd, J=5.8, 11.7 Hz), 3.01 (2H, m), 2.20 (3H, s), 2.10 (3H, s); ^{13}C-NMR (100 MHz, D_2O): 170.0, 165.4, 157.4, 152.2, 133.1, 121.3, 106.6, 63.7, 55.3, 53.5, 47.4, 17.4, 16.8; IR ν_{max} (KBr) cm^{-1}: 3400 (w), 2820 (m), 1720 (s), 1660 (s), 1542 (s), 1433 (s), 1360 (m), 1308 (m), 1080 (w), 1010 (s), 978 (m), 860 (w), 780 (w), 536 (s); FAB-MS m/z (rel. int.): 384 ($[M+Na]^+$, 10), 363 (10), 362 (MH^+, 39), 346 (5), 207 (44), 179 (39), 115 (100); FAB HRMS m/z: calcd. for $C_{13}H_{20}O_5N_3S_2$ (MH^+), 362.0844; found, 362.0854.

Ethylsparsomycin (4). Deprotection according to the general procedure, using **22** (15.4 mg, 37.0 μmol) and Dowex® 50W (H^+) (500 mg) in MeOH (10 ml), afforded a solid which was purified by silica gel column chromatography to give 11.7 mg (85%) of **4** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, mp 221.5–222.5°C (decomposing), *Rf* 0.2 (EtOAc/MeOH, 6/1), $[\alpha]_D^{23}$ +64.6° (*c* 0.81, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): 7.42 (1H, d, J=15.3 Hz), 7.24 (1H, d, J=15.3 Hz), 4.51–4.44 (1H, m), 4.02 (1H, d, J=13.9 Hz), 3.88 (1H, d, J=13.9 Hz), 3.68 (1H, dd, J=5.4, 10.9 Hz), 3.66 (1H, dd, J=5.4, 10.9 Hz), 3.19 (1H, dd, J=10.2, 13.1 Hz), 3.06 (1H, dd, J=3.7, 13.1 Hz), 2.76 (2H, q, J=7.6 Hz), 2.35 (3H, s), 1.27 (3H, t, J=7.6 Hz); ^{13}C-NMR (100 MHz, CD_3OD): 169.6, 164.8, 156.2, 151.9, 132.8, 122.3, 106.8, 64.6, 54.7, 54.1, 48.2, 28.5, 16.9, 15.1; IR ν_{max} (KBr) cm^{-1}: 3250 (m), 2970 (m), 2934 (m), 2818 (m), 1713 (s), 1668 (s), 1540 (m), 1447 (m), 1311 (w), 1219 (w), 1084 (w), 1011 (m), 860 (m); FAB-MS m/z (rel. int.): 398 ($[M+Na]^+$, 45), 377 (9), 376 (MH^+, 33), 176 (100); FAB HRMS m/z: calcd. for $C_{14}H_{22}O_5N_3S_2$ (MH^+), 376.1001; found, 376.1003.

n-Butylsparsomycin (5). Deprotection according to the general procedure, using **23** (48.0 mg, 0.11 mmol) and Dowex® 50W (H^+) (500 mg) in MeOH (10 ml), afforded a solid which was purified by silica gel column chromatography to give 38.3 mg (88%) of **5** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, mp 225–226°C (decomposing), *Rf* 0.2 (EtOAc/MeOH, 6/1), $[\alpha]_D^{23}$ +89.2° (*c* 0.82, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): 7.42 (1H, d, J=15.4 Hz), 7.23 (1H, d, J=15.4 Hz), 4.51–4.44 (1H, m), 4.02 (1H, d, J=14.0 Hz), 3.88 (1H, d, J=14.0 Hz), 3.69 (2H, d, J=5.4 Hz), 3.20 (1H, dd, J=10.5, 13.4 Hz), 3.06 (1H, dd, J=3.7, 13.4 Hz), 2.74 (2H, t, J=7.3 Hz), 2.34 (3H, s), 1.58 (2H, quint, J=7.3 Hz), 1.40 (2H, extet, J=7.4 Hz), 0.90 (3H, t, J=7.4 Hz); ^{13}C-NMR (100 MHz, CD_3OD): 169.6, 164.8, 156.2, 151.9, 132.8, 122.3, 106.8, 64.6, 54.8, 54.4, 48.2, 34.3, 32.7, 22.7, 17.0, 14.0; IR ν_{max} (KBr) cm^{-1}: 3260 (m), 2960 (m), 2932 (m), 2870 (m), 1706 (s), 1655 (s), 1541 (m), 1508 (m), 1458 (m), 1320 (w), 1225 (w), 1036 (w), 1019 (m); FAB-MS m/z (rel. int.): 405 (9), 404 (MH^+, 32), 388 (28), 250 (18), 239 (58), 179 (100); FAB HRMS m/z: calcd. for $C_{16}H_{26}O_5N_3S_2$ (MH^+), 404.1314; found, 404.1312.

Allylsparsomycin (6). Deprotection according to the general procedure, using **24** (12.0 mg, 28.0 μmol) and Dowex® 50W (H^+) (100 mg) in MeOH (5 ml), afforded a solid which was purified by silica gel column chromatography to give 9.4 mg (87%) of **6** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, mp 225–226°C (decomposing), $[\alpha]_D^{30}$ +66.9° (*c* 0.28, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): 7.43 (1H, d, J=15.3 Hz), 7.22 (1H, d, J=15.3 Hz), 5.81 (1H, ddt, J=10.0, 17.1, 7.4 Hz), 5.20 (1H, qd, J=1.4, 17.4 Hz), 5.18 (1H, qd, J=1.0, 10.0 Hz), 4.46 (1H, dddd, J=3.9, 5.0, 5.6, 10.3 Hz), 3.96 (1H, d, J=13.9 Hz), 3.79 (1H, d, J=13.9 Hz), 3.69 (1H, dd, J=5.0, 11.0 Hz), 3.66 (1H, dd, J=5.6, 11.0 Hz), 3.36 (1H, dt, J=7.3, 1.0 Hz), 3.18 (1H, dd, J=10.3, 13.4 Hz), 3.08 (1H, dd, J=3.9, 13.4 Hz), 2.35 (3H, s); ^{13}C-NMR (100 MHz, CD_3OD): 169.6, 164.8, 156.2, 151.9,

134.4, 132.9, 122.3, 119.4, 106.8, 64.6, 54.8, 52.3, 48.2, 36.6, 16.9; IR ν_{max} (KBr) cm^{-1}: 3600–3800 (m), 1717 (s), 1675 (s), 1617 (s), 1540 (m), 1458 (s), 1430 (s), 1354 (s), 1341 (s), 1300 (m), 1217 (m), 1088 (m), 986 (s), 864 (m); FAB-MS *m/z* (rel. int.): 389 (24), 388 (MH^+, 69), 179 (100); FAB HRMS *m/z*: calcd. for $C_{15}H_{21}O_5N_3S_2$ (MH^+), 388.1001; found, 388.0990.

Benzylsparsomycin (7). Deprotection according to the general procedure, using **25** (14.1 mg, 0.029 mmol) and Dowex® 50W (H^+) (100 mg) in MeOH (5 ml), afforded a solid which was purified by silica gel column chromatography to give 8.4 mg (66%) of **7** as a white solid. An assay sample was obtained by crystallization from MeOH as a white prism, mp 226.5–228°C (decomposing), $[\alpha]_D^{23}$ +51.1° (*c* 0.40, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): 7.44 (1H, d, *J*=15.3 Hz), 7.36–7.30 (4H, m), 7.26–7.20 (1H, m), 7.22 (1H, d, *J*=15.3 Hz), 4.48–4.42 (1H, m), 3.95 (2H, s), 3.90 (1H, d, *J*=13.9 Hz), 3.73 (1H, d, *J*=13.9 Hz), 3.67 (1H, dd, *J*=4.9, 12.2 Hz), 3.65 (1H, dd, *J*=5.4, 12.2 Hz), 3.15 (1H, dd, *J*=10.2, 12.2 Hz), 3.05 (1H, dd, *J*=3.7, 13.2 Hz), 2.34 (3H, s); ^{13}C-NMR (100 MHz, CD_3OD): 169.6, 164.8, 156.2, 151.9, 138.4, 132.9, 130.5, 129.8, 128.6, 122.3, 106.8, 64.6, 54.9, 52.9, 48.1, 37.9, 16.9; IR ν_{max} (KBr) cm^{-1}: 3260 (m), 3060 (m), 2920 (m), 2815 (m), 1719 (s), 1665 (s), 1655 (s), 1603 (s), 1541 (s), 1509 (s), 1440 (m), 1347 (m), 1302 (m), 1020 (m), 866 (w), 702 (w); FAB-MS *m/z* (rel. int.): 460 ($[M+Na]^+$, 4), 439 (3), 438 (MH^+, 8), 179 (43), 91 (100); FAB HRMS *m/z*: calcd. for $C_{19}H_{24}O_5N_3S_2$ (MH^+), 438.1157; found, 438.1148.

Biological Activity

Morphological reversion activity. The morphological reversion activity on *src*^{ts}-NRK cells, which were kindly presented by Dr. Y. Uehara of National Institute of Infectious Diseases, was assessed. The cells were cultured in EAGLE's minimal essential medium (MEM) supplemented with 10% calf serum (CS, Hyclone Laboratories, Logan, Utah, USA) at the permissive temperature (32°C) or at nonpermissive temperature (39°C). The cells (1×10^5 cells/ml) maintained at 32°C were seeded into a 96-well microtiter plate and cultured for two hours at 32°C in a 5% CO_2 atmosphere. Various concentrations of the compound solution (5 μl each) were added, and morphological reversion of *src*^{ts}-NRK cells was observed under a microscope after 18 to 20 hours of incubation at 32°C.

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References

1) Ubukata, M., Morita, T., Kakeya, H., Kobinata, K., Kudo, T., and Osada, H., Sparoxomycins A1 and A2, new inducers of the flat reversion of NRK cells transformed by temperature sensitive rous sarcoma virus, I. Taxonomy of the producing organism, fermentation and biological activity. *J. Antibiotics*, **49**, 1096–1100 (1996).
2) Ubukata, M., Morita, T., Uramoto, M., and Osada, H., Sparoxomycins A1 and A2, new inducers of the flat reversion of NRK cells transformed by temperature sensitive rous sarcoma virus, II. Isolation, physico-chemical properties and structure elucidation. *J. Antibiotics*, **49**, 65–70 (1996).
3) Argoudelis, A. D., and Herr, R. R., Sparsomycin, a new antibiotic II. Isolation and characterization. *Antimicrob. Ag. Chemother.*, 780–786 (1962).
4) Higashide, E., Hasegawa, T., Shibata, M., Mizuno, K., and Akaike, H., Studies on the Streptomycetes. *Streptomyces cuspidosporus nov. sp* and the antibiotic sparsomycin and tubercidin produced thereby. *Ann. Rept. Takeda Res. Lab.*, **25**, 1–14 (1966).
5) Wiley, P. F., and Mackellar, F. A., The structure of sparsomycin. *J. Am. Chem. Soc.*, **92**, 417–418 (1970).
6) Ottenheijm, H. C. J., Liskamp, R. M. J., Helquist, P., Lauher, J. W., and Shekhani, M. S., Absolute configuration of sparsomycin. A chiroptical study of sulfoxides. *J. Am. Chem. Soc.*, **103**, 1720–1723 (1981).
7) Lazaro, E., Felix, A. S., van den Broek, L. A. G. M., Ottenheijm, H. C. J., and Ballesta, J. P. G., Interaction of the antibiotic sparsomycin with the ribosome. *Antimicrob. Ag. Chemother.*, 10–13 (1991).
8) Lazaro, E., van den Broek, L. A. G. M., Felix, A. S., Ottenheijm, H. C. J., and Ballesta, J. P. G., Biochemical and kinetic characteristics of the interaction of the antibiotic sparsomycin with prokaryotic and eukaryotic ribosomes. *Biochemistry*, **30**, 9642–9648 (1991).
9) Theocharis, D. A., and Coutsogeorgopoulos, C., Mechanism of action of sparsomycin in protein synthesis. *Biochemistry*, **31**, 5861–5868 (1992).
10) Porse, B. T., Kirillov, S. V., Awayz, M. J., Ottenheijm, H. C. J., and Garrett, R. A., Direct crosslinking of the antitumor antibiotic sparsomycin, and its derivatives, to A2602 in the peptidyl transferase center of 23S-like rRNA within ribosome-tRNA complexes. *Proc. Natl. Acad. Sci. USA*, **96**, 9003–9008 (1999).
11) For a review of sparsomycin, see: Ottenheijm, H. C. J., van den Broek, L. A. G. M., Ballesta, J. P. G., and Zylicz, Z., Chemical and biological aspects of sparsomycin, an antibiotic from streptomyces. *Progress in Medicinal Chemistry*, **23**, 220–268 (1986).
12) Ottenheijm, H. C. J., van Nispen, S. P. J. M., and Sinnige, M. J., Synthesis of *S*-deoxo-(*R*)-sparsomycin. *Tetrahedron Lett.*, 1899–1902 (1976).
13) Ottenheijm, H. C. J., and Liskamp, R. M. J., Approaches to the antibiotics sparsomycin. An

efficient synthesis of the cysteinol mono-oxodithioacetal moiety. *Tetrahedron Lett.*, 2437–2438 (1978).
14) Ottenheijm, H. C. J., Liskamp, R. M. J., and Tijhuis, M. W., Total synthesis of enantiomeric sparsomycin. *Tetrahedron Lett.*, 387–390 (1979).
15) Helquist, P., and Shekhani, M. S., Total synthesis of (R_c)-sparsomycin. *J. Am. Chem. Soc.*, **101**, 1057–1059 (1979).
16) Ottenheijm, H. C. J., Liskamp, R. M. J., van Nispen, S. P. J., Boots, H. A., and Tijhuis, M. W., Total synthesis of the antibiotic sparsomycin, a modified uracil amino acid monoxodithioacetal. *J. Org. Chem.*, **46**, 3273–3283 (1981).
17) Liskamp, R. M. J., Zeegers, H. J. M., and Ottenheijm, H. C. J., Synthesis and ring-opening reactions of functionalized sultines. A new approach to sparsomycin. *J. Org. Chem.*, **46**, 5408–5413 (1981).
18) Wang, D.-R. H., Helquist, P., and Shekhani, M. S., Total synthesis of sparsomycin. Approaches using cysteine and serine inversion. *J. Org. Chem.*, **50**, 1264–1271 (1985).
19) Parry, R. J., Li, Y., and Gomez, E. E., Biosynthesis of the antitumor antibiotic sparsomycin. *J. Am. Chem. Soc.*, **114**, 5946–5959 (1992).
20) Parry, R. J., Hoyt, J. C., and Li, Y., The biosynthesis of sparsomycin. Further investigations of the biosynthesis of the uracil acrylic acid moiety. *Tetrahedron Lett.*, 7497–7500 (1994).
21) Lin, C.-C. L., and Dubois, R. J., Pyrimidinylpropenamides as antitumor agents. Analogues of the antibiotic sparsomycin. *J. Med. Chem.*, **20**, 337–341 (1977).
22) Lee, C. K., and Vince, R., Effect of sparsomycin analogues on the puromycin-peptidyl transferase reaction on ribosomes. *J. Med. Chem.*, **21**, 176–179 (1978).
23) Zemlicka, J., and Bhuta, A., Sparsophenicol: A new synthetic hybrid antibiotic inhibiting ribosomal peptide synthesis. *J. Med. Chem.*, **25**, 1123–1125 (1982).
24) Duke, A. A., and Boots, M. R., Synthesis and biological evaluation of sparsomycin analogues. *J. Med. Chem.*, **26**, 1556–1561 (1983).
25) Liskamp, R. M. J., Colstee, J. H., Ottenheijm, H. C. J., Lelieveld, P., and Akkermann, W., Structure-activity relationships of sparsomycin and its analogues. Octylsparsomycin: The first analogue more active than sparsomycin. *J. Med. Chem.*, **27**, 301–306 (1984).
26) Van den Broek, L. A. G. M., Liskamp, R. M. J., Colstee, J. H., Leilieveld, P., Remacha, M., Vazquez, D., Ballesta, J. P. G., and Ottenheijm, H. C. J., Structure-activity relationships of sparsomycin and its analogues. Inhibition of peptide bond formation in cell-free systems and L1210 and bacterial cell. *J. Med. Chem.*, **30**, 325–333 (1987).
27) Van den Broek, L. A. G. M., Lazaro, E., Zylicz, Z., Fennis, P. J., Missler, F. A. N., Leilieveld, P., Garzotto, M., Wanger, D. J. F., Ballesta, J. P. G., and Ottenheijm, H. C. J., Lipophilic analogues of sparsomycin as strong inhibitors of protein synthesis and tumor growth: Structure-activity relationship study. *J. Med. Chem.*, **32**, 2002–2015 (1989).
28) Preliminary communication, see: Nakajima, N., Enomoto, T., Matsuura, N., and Ubukata, M., Synthesis and morphological reversion activity on *src*^{ts}-NRK cells of pyrimidinylpropenamide antibiotics, sparsomycin, sparoxomycins A1, A2, and their analogues. *Bioorganic & Medicinal Chemistry Letters*, **8**, 3331–3334 (1998).
29) Uehara, Y., Hori, M., Takeuchi, T., and Umezawa, H., Screening of agents which convert transformed morphology of rous sarcoma virus-infected rat kidney cells to normal morphology: identification of an active agent as herbimycin and its inhibition of intracellular *src*kinase. *Jap. J. Cancer Res. (Gann)*, **76**, 672 (1985).
30) Kagan, H. B., ''Catalytic Asymmetric Synthesis'', ed. Ojima, I., VCH, New York, pp. 203–226 (1993).
31) Pitchen, P., and Kagan, H. B., An efficient asymmetric oxidation of sulfides to sulfoxides. *Tetrahedron Lett.*, **25**, 1049–1052 (1984).
32) Pitchen, P., Dunach, E., Deshmukh, M. N., and Kagan, H. B., An efficient asymmetric oxidation of sulfides to sulfoxides. *J. Am. Chem. Soc.*, **106**, 8188 (1984).
33) Zhao, S. H., Samuel, O., and Kagan, H. B., Asymmetric oxidation of sulfides mediated by chiral titanium complexes: mechanistic and synthetic aspects. *Tetrahedron*, **43**, 5135–5144 (1987).
34) Komatsu, N., Nishibayashi, Y., Sugita, T., and Uemura, A., Catalytic asymmetric oxidation of sulfides to sulfoxides using *R*-(+)-binaphthol. *Tetrahedron Lett.*, **33**, 5391–5394 (1992).
35) Komatsu, N., Hashizume, M., Sugita, T., and Uemura, A., Catalytic asymmetric oxidation of sulfides to sulfoxides with *tert*-butyl hydroperoxide using binaphthol as a chiral auxiliary. *J. Org. Chem.*, **58**, 4529–4533 (1993).
36) Sc*S*s: *S* configuration at the carbon and sulfur atoms. Sc*R*s: *S* configuration at the carbon and *R* configuration at the sulfur atoms.
37) Reaction for 30 min at 0°C. The yields obtained after 60 min at 0°C are as follows: **17a** (20%), **17b** (25%), **16** (41%).
38) This compound was prepared from commercially available 5-hydroxymethyl-6-methyluracil (Sigma) by Dubois procedure.[22]
39) Carpino, L. A., 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive. *J. Am. Chem. Soc.*, **115**, 4397–4398 (1993).
40) ID_{50} values of HeLa S3 colony formation inhibition (μM) were as follows: **19** (13.4), **20** (3.1), **21** (1.6), **3** (0.8), **4** (0.7), **5** (0.6), **6** (0.8), **7** (0.6).