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Synthesis and Morphological Reversion Activity on *src*^{ts}NRK Cells of Pyrimidinylpropanamide Antibiotics, Sparsomycin, Sparoxomycin A_1, A_2, and Their Analogues

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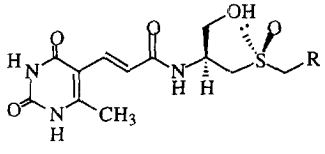
Abstracts: Three pyrimidinylpropanamide antibiotics sparsomycin (**1**), sparoxomycins A1, A2 (**2**, **3**), and also six analogues (**4**-**9**) have been synthesized by employing asymmetric sulfide oxidation conditions as a key step. Sparsomycin (**1**) and its alkyl analogues (**5**-**7**) showed higher morphological reversion activities on *src*^{ts}NRK cells than **2** and **3**.

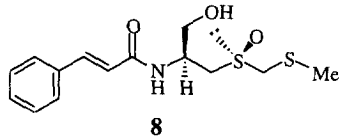
Sparsomycin (**1**), an inhibitor of protein biosynthesis, is a metabolite of *Streptomyces sparsogenes* or *Streptomyces cuspidosporus*.[1-3] The structure of **1** is closely related to that of sparoxomycins A1 (**2**) and A2 (**3**), which were isolated from a culture broth of *Streptomyces sparsogenes* SN-2325 as new members of the pyrimidinylpropanamide antibiotic in 1996,[4a] differing only in their oxidation level at sulfur atom. The sparoxomycins (**2** and **3**) converted transformed morphology of temperature-sensitive mutant Rous sarcoma virus-infected NRK cells (*src*^{ts}NRK cells) to normal morphology at a wide range of concentrations without cytotoxicity.[4b] The sparsomycin (**1**) has antitumor activity; however, there are no reports regarding normalization of the phenotype of oncogene-transformed cells by **1** and its analogues.[5] Therefore, we became interested in designing and synthesizing analogues of these pyrimidinylpropanamide antibiotics, to evaluate their biological properties. We wish to report here a novel and stereoselective synthesis of natural products (**1**-**3**), and their analogues (**4**-**9**) as well as their morphological reversion activity on *src*^{ts}NRK cells.[6]

Figure 1. Structures of sparsomycin (**1**), sparoxomycins A_1, A_2 (**2**, **3**), and their analogues **4**-**9**.



sparsomycin (**1**): R = SCH_3
sparoxomysin A_1 (**2**): R = $S^R(=O)CH_3$
sparoxomysin A_2 (**3**): R = $S^S(=O)CH_3$

4: R = H
5: R = SCH_2CH_3
6: R = $SCH_2CH_2CH_2CH_3$
7: R = $SCH(CH_3)CH_2CH_3$



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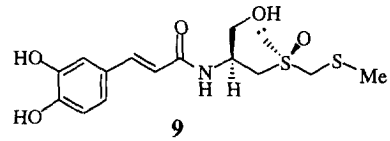

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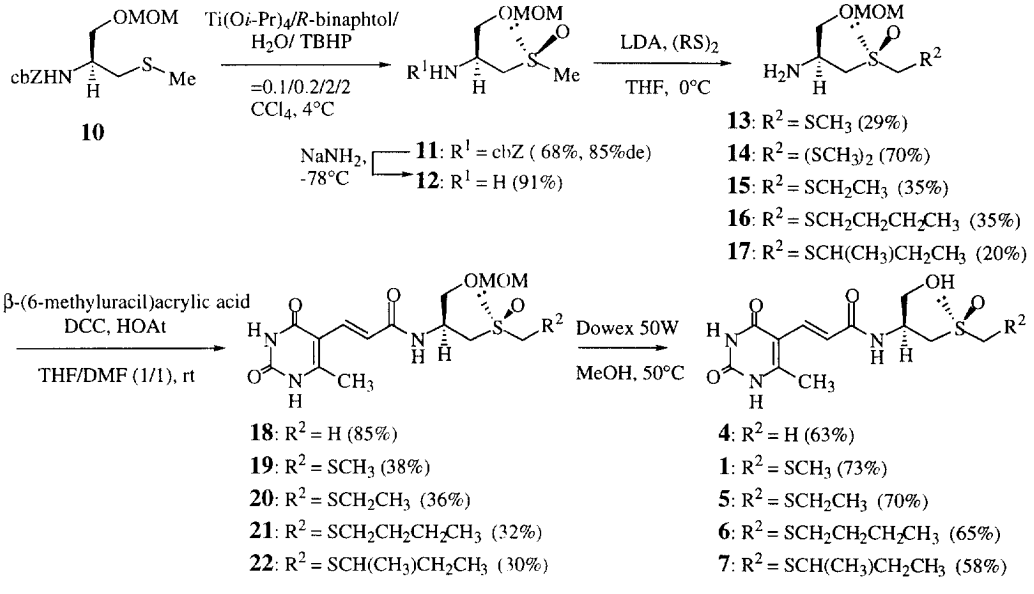
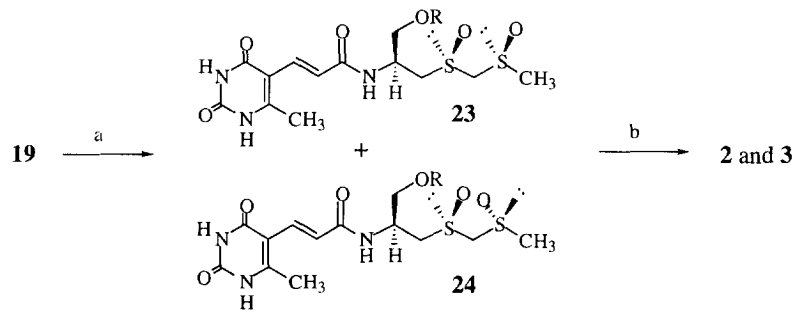
The synthetic pathway to **1-9** is based on the following procedure which improved a shortcoming in Helquest's protocol[2] developed in the synthesis of sparsomycin (**1**). In order to achieve the stereoselective synthesis, the oxidation of sulfide (**10**) to the chiral sulfoxide (**11**) under asymmetric oxidation conditions[7-11] was studied. After several attempts,[12] diastereoselective oxidation was found to proceed catalytically in the presence of a titanium complex produced from Ti(O*i*-Pr)$_4$ and chiral binaphtol.[9] When 0.2 mol equiv. of *R*-(+)-binaphtol was used as a ligand at 4℃ in CCl_4, *S* configuration of sulfoxide (**11**) was isolated in 68 % yield with 85 % diastereomeric excess (de).[13] Single recrystallization of the diastereomixture from hexane-CH_2Cl_2 afforded optically pure (*S*)-**11**, mp. 113-114 ℃, $[\alpha]_D^{19}$ = +87.1 (c 0.19, CH_2Cl_2).[14] *S*-Sulfoxide (**11**) was subjected to sulfenylation after removal of the benzyloxycarbonyl (cbZ) group under dissolved metal conditions (91%). The sulfenylation of **12** to dithioacetal mono-oxide (**13**) was difficult to reproduce even under the reported conditions.[2] Only the double sulfenylation product (**14**) was obtained in 70 % by the addition of dimethyl disulfide after treatment of amine (**12**) with 2 equiv. of lithium diisopropylamide (LDA). When 1 equiv. of dimethyl disulfide was first added to a THF solution of **12** and then LDA (1.5 equiv.) was added in two portions (reverse addition), sulfenylation proceeded to give the desired dithioacetal mono-S-oxide (**13**) in 29 % (53 % based on consumed material) along with 11% of **14**. The coupling of β-(6-methyluracil)acrylic acid and **13** was achieved by 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzo-7-azatriazole (HOAt) to give **19** in 38 %. Hydrolytic removal of the MOM group with Dowex 50W in MeOH afforded **1**, mp 204-206℃, in 73 % yield. The synthetic material was spectroscopically (IR, ^{1}H-NMR, ^{13}C-NMR, MS) identical with natural sparsomycin, and also had specific rotation, $[\alpha]_D^{25}$ = +65.1° (c 0.28, H_2O), in good agreement with the literature value [lit.[2b] $[\alpha]_D^{25}$ = +65.1 (c 0.37, H_2O)]. The alkyl (Et, nBu and isoBu) analogues (**5-7**) and methyl sulfoxide analogue (**4**) were also prepared by employing the sequences similar to that developed for the synthesis of **1** from **12**. These results are summarized in **Scheme 1**.

Scheme 1. Synthesis of sparsomycin (**1**) and its analogues (**4-7**).

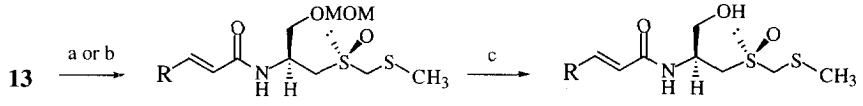


Scheme 2. Synthesis of sparoxomycin A1 (**2**) and A2 (**3**).



reagents and conditions: a) $NaIO_4$, $MeCN/H_2O$=1/1, **23/24** = 1/1, 77%
b) Dowex 50W, MeOH, 50°C, 80% and separatioin by HPLC, 14% for **2**, 12% for **3**.

Scheme 3. Synthesis of cinnamide derivatives (**8** and **9**).



25: R = C_6H_5 (65%)
26: R = $(HO)_2C_6H_3$ (38%)

8: R = C_6H_5 (65%)
9: R = $(HO)_2C_6H_3$ (62%)

reagents and conditions: a) *trans*-cinnamoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt b) *trans*-3,4-dihydroxycinnamic acid DCC, HOAt, DMF, rt c) Dowex 50W, MeOH, 50°C

The 1:1 mixture of dioxodithioacetal **2** and **3** was prepared by $NaIO_4$ oxidation[15,16] of **19** followed by removal of the MOM group in 62 % (2 steps). Sparoxomycins A1 (**2**) and A2 (**3**) were isolated by careful ODS column chromatography and HPLC (Mightysil RP-18) in 14 % for **2** and 12 % for **3**, respectively (**Scheme 2**).[17] The cinnamide and catechol derivatives (**8** and **9**) were synthesized from **13**. The 65 % yield of **25** was obtained from coupling of (*E*)-cinnamoyl chloride with **13** under Et_3N, DMAP conditions. The analogue (**26**) was also obtained from coupling of 3,4-dihydroxycinnamic acid with **13** under DCC, HOAt conditions in 38 % yield. Hydrolytic removal of the MOM group of **25** and **26** with Dowex 50W in MeOH afforded **8** and **9** in 65% and 62 %, respectively (**Scheme 3**).

The morphological reversion activity on *src*^{ts}NRK cells (a gift from Dr. Y. Uehara, National Institute of Infectious Diseases) was assessed with synthetic compounds (**1**-**9** and **19**).[18] The results shown in **Table 1** disclose that the morphological reversion activity of **1** was found to be 6.5 μM and this activity was 80 times more potent than that of **2** and **3**. The alkyl analogues **5**, **6** and **19**, and **7** were 3-, 10-, and 30-fold less potent than **1**. The methyl sulfoxide (**4**), cinnamide and catechol derivatives (**8** and **9**) did not show any activities at 530 μM. These findings suggest that the pyrimidinylpropanamide group is essential and the monooxodithioacetal group plays an important role in exhibiting the morphological reversion activity.

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Table 1. Morphological reversion activity of *src*^{ts}NRK cells

Compound	MEC (μM)[a]
sparsomycin (**1**)	6.5
sparoxomycin A1 (**2**)	530
sparoxomycin A2 (**3**)	530
MOM-**1** (**19**)	60
4	no activity[b]
5	20
6	60
7	180
8	no activity[b]
9	no activity[b]

[a] minimal effective concentration. [b] no activity at 530μM

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12. We searched for the oxidation conditions under which the highest yield and selectivity could be obtained; thus, the combination of a protecting group on the hydroxy group, a ligand for catalyst (tartaric acid derivatives, binaphtols, salen and camphor-sulfonyloxaziridine derivatives), the effects of the solvent (CCl_4, CH_2Cl_2, toluene), and the reaction temperature were each studied.
13. The diastereomer excess was determined by HPLC analysis to be 85 % (Mightysil RP-18, MeOH/H_2O, 4/6, 1.0 mL/min) ^{t}R(*S*)-**11**, 14.2 (92.5 %); ^{t}R(*R*)-**11**, 15.3 (7.5 %).
14. The spectroscopic data (IR, ^{1}H-NMR) had in good agreement with the literature value.[2b] Data for optically pure (*S*)-**11**; Rf: 0.15 (CH_2Cl_2/MeOH, 20/1); ^{1}H NMR ($CDCl_3$, 400MHz) 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 ($CDCl_3$, 100MHz) 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 (neat) 3326 (m), 2928 (m), 1726 (m), 1687(s), 1541(s), 1466 (w), 1309 (m), 1273 (m),1142(m), 1039 (m), 1026(m), 727 (w); FABMS (*m/z*) 318 (10), 317 (22), 316 (MH^+, 100), 284 (8), 176 (9), 164 (6), 91 (100); Exact MS (*m/z*) calcd. for $C_{14}H_{22}O_5NS$, 316.1218; found, 316.1202.
15. Under the stereoselective oxidation conditions for **10** to **11**, the reaction did not proceed.
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17. Synthetic materials were spectroscopically (IR, ^{1}H NMR, ^{13}C NMR, MS) identical with natural sparoxomycin A_1 and A_2.[4a]
18. The morphological reversion activity on *src*^{ts}NRK cells was assessed with HPLC pure compounds as follows; The cells were cultured in EAGLE's minimal essential medium (MEM) supplement with 10 % calf serum (CS, Hyclone Laboratories, Logan, Utah) at permissive temperature (32℃) or at nonpermissive temperature (39℃). The cells ($1x10^5$ cells/ml) maintained at 32℃ were seeded into a 96-well microtiter plate and cultured for two hours at 32℃ in 5 % CO_2 atmosphere. Solution of various concentration of the compounds (5 μl each) was added and morphological reversion of *src*^{ts}NRK cells were observed under a microscope after 18 to 20 hours incubation at 32℃.