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Synthetic studies of thiazoline and thiazolidine-containing natural products – 2. Total synthesis of the antimycoplasma antibiotic micacocidin

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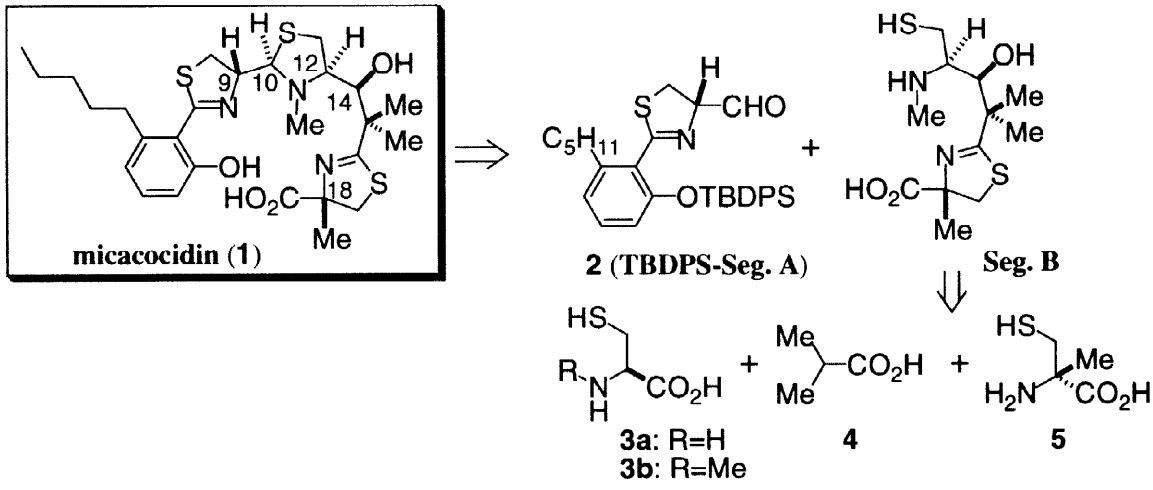
Abstract

Synthesis of the right half of micacocodin (segment B) and subsequent completion of total synthesis of the antimycoplasma antibiotic micacocidin is described. The desired *S*-configuration at C-14 secondary carbinol was obtained by stereoselective reduction of the preceding ketone in accordance with the Cram rule. Condensation of two labile segments, A and B, was achieved in the presence of potassium acetate. The chiral center at C-10 was finally isomerized to the natural configuration through formation of the Zn complex.

Keywords: antibiotics; thiazolines; thiazolidines; chelation.

In our preceding paper [1], we reported synthesis of protected segment A (**2**) of the antimycoplasma antibiotic micacocidin (**1**) [2], in which phosphorus pentachloride-mediated thiazoline constructing reaction was applied. In this paper, we describe the synthesis of the right half of **1** (segment B), and condensation of these two segments leading to **1** [3].



Scheme 1. Retrosynthetic analysis

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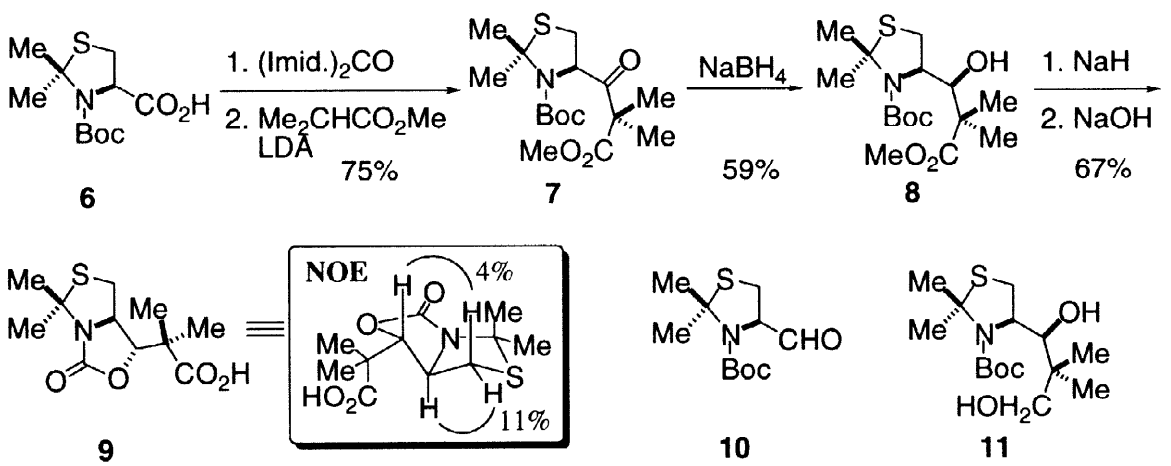
To introduce three chiral centers into segment B, we planned to use cysteine for C-12 and C-18, while stereoselective reaction was used for C-14 configuration. Construction of the central thiazolidine ring through condensation of segments A and B, and stereochemical control on the configuration at the C-10 carbon were performed with reference to model experiments described in the previous report [1]. Thus, we retrosynthesized segment B to L-cysteine (**3a**), isobutyric acid (**4**) and 2-methyl-*S*-cysteine (**5**) (**Scheme 1**). An initial attempt starting with *N*-methyl-L-cysteine (**3b**) was unsuccessful because the *N*-methyl-oxazolidinone ring corresponding to **14** resisted cleavage [4], so herein we began the synthetic study with **3a**.

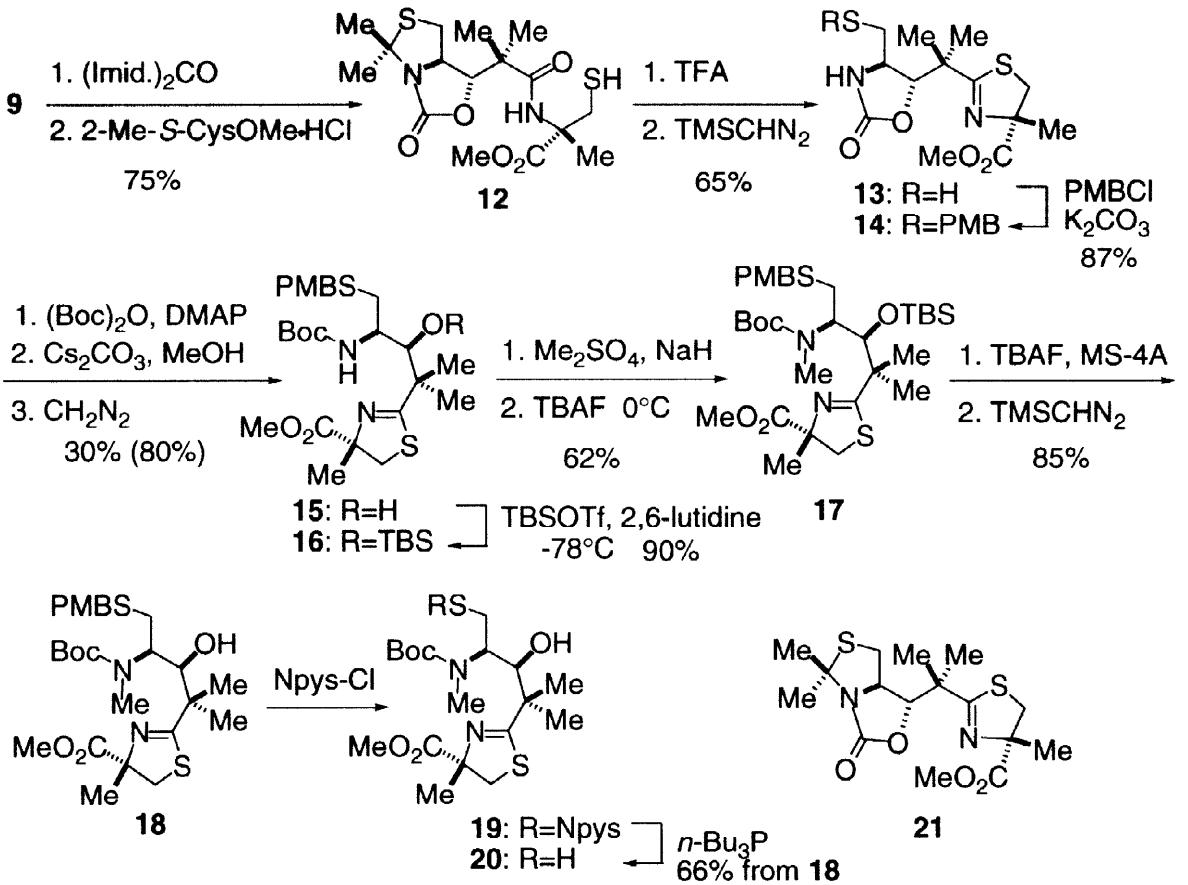
Synthesis of segment B

Elongation of the carboxyl moiety in thiazolidine **6** [5], which was prepared from L-cysteine (**3a**) via a two-step reaction, was achieved by carbonyldiimidazole treatment and subsequent condensation with methyl isobutyrate to yield keto-ester **7**. Reduction of **7** with sodium borohydride proceeded with sufficient stereoselectivity in accordance with the Cram rule as expected to yield the desired *S*-alcohol **8**, along with a trace amount of *R* isomer and an over-reduced diol **11**, both of which were readily separated by chromatography. Compound **8** was then converted to **9** through construction of an oxazolidinone ring with sodium hydride and subsequent hydrolysis. The configuration of **9** was confirmed by NOE examination as shown in **Scheme 2**. When the elongation reaction was performed with aldehyde **10** [6] and methyl isobutyrate, the unwanted *R*-alcohol was predominant (*R*:*S*=ca.3:2 by NMR).



Scheme 2.

Next, condensation of **9** with 2-methyl-*S*-cysteine methyl ester hydrochloride [7] was performed using carbonyldiimidazole to yield peptide **12**. Treatment of **12** with trifluoroacetic acid in refluxing toluene accomplished cyclization of the *N*-acylcysteine moiety with concomitant removal of the acetonide protecting moiety. Since the methyl ester moiety was also hydrolyzed during the reaction, the product was treated with (trimethylsilyl)diazomethane to yield thiol **13**. When the cyclization reaction of **12** was carried out under dehydration conditions, for example with a Dean-Stark trap, the major product was **21**, and attempts to remove the acetonide moiety resulted in simultaneous cleavage of the thiazoline ring. Then, the thiol residue in **13** was protected with a *p*-methoxybenzyl (PMB) group.



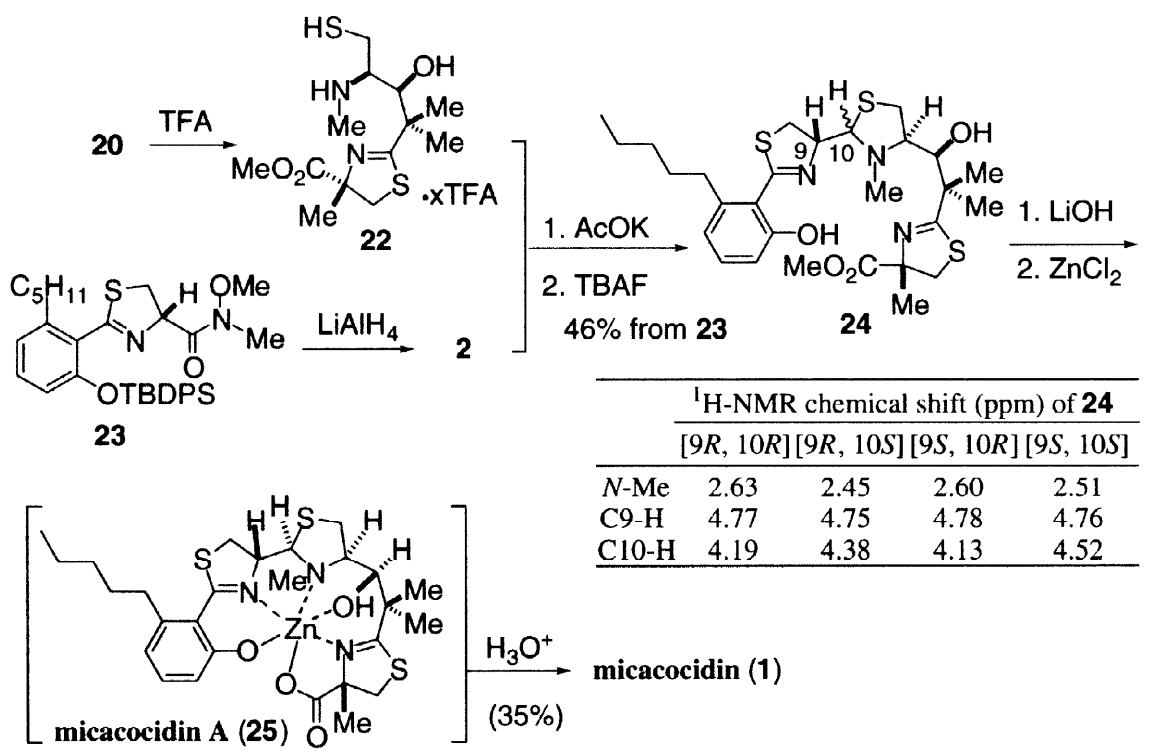
Scheme 3.

Cleavage of the oxazolidinone ring in **14** was performed as follows. Introduction of a *t*-butoxycarbonyl (Boc) group and subsequent treatment with methanolic cesium carbonate [8], followed by remethylation of the hydrolyzed methoxycarbonyl group, afforded **15** with concomitant recovery of **14**. After protection of newly formed secondary alcohol with a *t*-butyldimethylsilyl (TBS) group, *N*-methylation of **16** was effected by treatment with

dimethyl sulfate and sodium hydride. Since recovered **16** was not readily separable from **17** by chromatography, the product contaminated with **16** was then treated with a small amount of tetrabutylammonium fluoride (TBAF) at low temperature to selectively desilylate **16**. Pure **17** thus obtained was afresh treated with TBAF in the presence of 4A molecular sieves (4A-MS), followed by remethylation of the hydrolyzed ester to afford **18**. Regeneration of the thiol residue in **18** was achieved by substituting a 3-nitro-2-pyridinesulfenyl (Npys) group for PMB and subsequent treatment with tributylphosphine [9] to provide Boc-protected segment B **20**. **(Scheme 3)**

Total synthesis of micacocidin (1)

The Boc group in **20** was removed by treatment with trifluoroacetic acid to give amino-thiol **22** which was ready for condensation reaction. On the other hand, as mentioned in our preceding report [1], the TBDPS-protected segment A (**2**) was fairly labile, so that segment A was preserved as Weinreb amide **23**. Condensation of alkaline sensitive **22** and acid sensitive **2**, generated from **23** by $LiAlH_4$ treatment immediately before use, was achieved in the presence of potassium acetate, and subsequent desilylation with TBAF afforded micacocidin methyl ester **24** as a mixture of diastereomers involving C-10 (main) and C-9 configurations.



	^{1}H-NMR chemical shift (ppm) of **24**			
	[9*R*, 10*R*]	[9*R*, 10*S*]	[9*S*, 10*R*]	[9*S*, 10*S*]
N-Me	2.63	2.45	2.60	2.51
C9-H	4.77	4.75	4.78	4.76
C10-H	4.19	4.38	4.13	4.52

Scheme 4.

After hydrolysis of the terminal ester group with LiOH, treatment of the resulting acid with zinc chloride resulted in isomerization of the C-10 configuration intensifying natural chirality via micacocidin A (**25**) formation (9*R*:9*S* = ca.3:1, by ^{1}H-NMR). Finally, purification of the product by HPLC furnished micacocidin (**1**). (**Scheme 4**)

Synthetic micacocidin thus obtained was identified with the natural sample by comparison of their behavior on HPLC and spectroscopic properties (IR, NMR, MS, $[\alpha]_D$).

Experimental section

General. ^{1}H-NMR spectra were recorded on a JEOL GSX-270 or JMN A-400 spectrometer, and other instruments used to obtain physical data and experimental conditions for chromatography were the same as described in the preceding paper [1].

Ketone 7. To an ice-cold solution of acid **6** [5] (16.53 g) in THF (62.0 ml) was added carbonyldiimidazole (10.0 g, 1.00 eq.). The mixture was stirred at the same temperature for 30 min and then at room temperature for 2 h. The reaction mixture was diluted with AcOEt, washed with water and brine. The AcOEt layer was dried over Na_2SO_4 and concentrated under reduced pressure to give crude acylimidazole (18.15 g).

Under a nitrogen atmosphere, methyl isobutyrate (14.0 ml, 2.10 eq.) was added dropwise over 10 min to a solution prepared by adding LDA (43.7 ml of 2.0 M, 1.50 eq.) at -78°C into Et_2O (140 ml). This mixture was stirred at the same temperature for 25 min. Then, the crude acylimidazole (18.15 g) solution in THF (100 ml) was added over 30 min, and this mixture was stirred at the same temperature for a further 30 min. The reaction was quenched with 10 % aq.citric acid, and the mixture was extracted with AcOEt. The organic layer was taken and washed with water and brine, dried over Na_2SO_4, then concentrated under reduced pressure. The residue was purified by chromatography (silica 500 g, AcOEt/hexane = 1/6) to give **7** (16.45 g, 75%). Mp 45-48 °C; $[\alpha]^{24}_D$ -59.2 (c 1.01, $CHCl_3$); IR ν_{max} 2976, 2933, 1717, 1683, 1363, 1171 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.44 (3H, s), 1.45 (9H, s), 1.54 (3H, s), 1.75 (3H, br-s), 1.83 (3H, br-s), 2.92 (1H, br-s), 3.23 (1H, br-s), 3.74 (3H, s), 5.29 (1H, br-s); Anal. calcd for $C_{16}H_{27}NO_5S$: C, 55.63; H, 7.88; N, 4.05. found: C, 55.48; H, 7.77; N,4.18.

Secondary alcohol 8. To an ice-cold solution of ketone **7** (16.45 g, 47.6 mmol) in EtOH (71.0 ml) was slowly added $NaBH_4$ (1.80 g, 1.00 eq.), and this mixture was stirred at room temperature overnight. The reaction mixture was poured into ice-cold sat.aq.NH_4Cl slowly, and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, then concentrated under reduced pressure. The residue was chromatographed (silica 500 g, AcOEt/hexane = 1/6 to 1/2) to afford **8** (9.80 g, 59%) and over-reduced diol **11** (3.69 g, 24%). **8**; Mp 60-63 °C; $[\alpha]^{24}_D$ -40.0 (c 1.00, $CHCl_3$); IR ν_{max} 3483, 2977, 2933, 1727, 1694,

1657, 1366, 1170 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.25 (3H, s), 1.31 (3H, s), 1.48 (9H, s), 1.78 (6H, s), 2.38 (1H, d, J= 12.2 Hz), 3.17 (1H, dd, J= 12.2, 5.5 Hz), 3.38 (1H, br-s), 3.70 (3H, s), 3.90 (1H, t, J= 9.2 Hz), 4.56 (1H, dd, J= 9.2, 5.5 Hz); Anal. calcd for $C_{16}H_{29}NO_5S$: C, 55.31; H, 8.41; N, 4.03. found: C, 55.11; H, 8.03; N,4.21. **11**; Mp 128-129 °C; $[\alpha]^{24}_D$ -16.8 (c 1.00, $CHCl_3$); IR ν_{max} 3367, 3254, 2971, 2868, 1662, 1394, 1168 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.98 (3H, s), 1.06 (3H, s), 1.51 (9H, s), 1.80 (3H, s), 1.81 (3H, s), 2.48 (1H, d, J= 12.0 Hz), 3.12 (1H, br-s), 3.29 (1H, dd, J= 12.0, 5.3 Hz), 3.47 (1H, t, J= 8.1 Hz), 3.56 (1H, dd, J= 11.7, 5.8 Hz), 3.64 (1H, dd, J= 11.7, 7.4 Hz), 3.86 (1H, br-s), 4.74 (1H, dd, J= 8.1, 5.3 Hz); EIMS *m/z* 639 $[2M+H]^+$, 320 $[M+H]^+$, 264, 220; HR-EIMS *m/z* 320.1909 $[M+H]^+$ (calcd 320.1896 for $C_{15}H_{30}NO_4S$); Anal. calcd for $C_{15}H_{29}NO_4S$: C, 56.40; H, 9.15; N, 4.38. found: C, 56.19; H, 9.00; N,4.34.

Carboxylic acid 9. To an ice-cold solution of alcohol **8** (9.80 g, 28.2 mmol) in THF (70.0 ml) was slowly added NaH (60%, 1.35 g, 1.20 eq.), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into ice-cold sat.aq.NH_4Cl slowly, and the whole was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, then concentrated under reduced pressure. The residue was purified by chromatography (silica 320 g, AcOEt/hexane = 1/4) to yield a white solid (methyl ester of **9**, 5.54 g).

A mixture of the solid (5.54 g, 20.3 mmol) and NaOH (0.97 g, 1.20 eq.) in MeOH (30.0 ml) and water (10.0 ml) was stirred under reflux overnight. After cooling to room temperature, MeOH was removed in vacuo, and the residue was taken up in water and washed with AcOEt. The aqueous phase was acidified (pH 3-4) with aq.HCl, and then extracted twice with AcOEt. The combined organic phase was washed with brine, dried over Na_2SO_4, then concentrated under reduced pressure to afford pure **9** (4.87 g, 67 % from **8**) as a white solid. Recrystallization of the solid from AcOEt-hexane gave colorless crystals (3.59 g). Mp 182-185 °C; $[\alpha]^{24}_D$ -5.0 (c 1.01, MeOH); IR ν_{max} 3273, 2978, 2938, 1735, 1370, 1264 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.30 (3H, s), 1.38 (3H, s), 1.69 (3H, s), 1.98 (3H, s), 3.04 (1H, dd, J= 10.4, 9.8 Hz), 3.12 (1H, dd, J= 10.4, 5.5 Hz), 4.31 (1H, ddd, J= 9.8, 6.1, 5.5 Hz), 4.45 (1H, d, J= 6.1 Hz); Anal. calcd for $C_{11}H_{17}NO_4S$: C, 50.95; H, 6.61; N, 5.40. found: C, 50.84; H, 6.53; N,5.45.

Peptide 12. An ice-cold solution of acid **9** (3.89 g, 15.0 mmol) in THF (30.0 ml) was treated with carbonyldiimidazole (2.55 g, 1.05 eq.), and stirred at room temperature for 1.5 h. This mixture containing crude acylimidazole was added to an ice-cold solution of 2-Me-*S*-CysOMe•HCl [7] (4.18 g, 1.5 eq.) in DMF (30.0 ml) over 5 min. After stirring at room temperature for 3 h, the reaction was quenched with sat.aq.NH_4Cl, and extracted with AcOEt. The organic phase was taken and washed with brine, dried over Na_2SO_4, then evaporated under reduced pressure. The residue was purified by chromatography (silica 270 g, AcOEt/hexane = 2/3) to afford **12** (4.40 g, 75%). $[\alpha]^{28}_D$ -40.6 (c 1.00, $CHCl_3$); IR ν_{max} 3363,

2980, 2934, 2567, 1743, 1657, 1520, 1371, 1260 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.29 (3H, s), 1.32 (3H, s), 1.60 (3H, s), 1.61 (1H, dd, *J*= 9.2, 5.5 Hz), 1.68 (3H, s), 1.95 (3H, s), 2.99 (1H, dd, *J*= 11.0, 9.2 Hz), 3.07 (1H, dd, *J*= 11.0, 5.5 Hz), 3.15 (1H, dd, *J*= 14.0, 8.6 Hz), 3.35 (1H, dd, *J*= 14.0, 9.2 Hz), 3.80 (3H, s), 4.36 (2H, m), 6.79 (1H, br-s); LSIMS *m/z* 781 $[2M+H]^+$, 391 $[M+H]^+$, 331, 219; HR-LSIMS *m/z* 391.1360 $[M+H]^+$ (calcd 391.1361 for $C_{16}H_{27}N_2O_5S_2$).

Thiol 13. A mixture of **12** (4.00 g, 10.2 mmol), TFA (10.0 ml) and toluene (90.0 ml) was heated with stirring under reflux for 3 days. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in Et_2O (45.0 ml) and MeOH (5.00 ml), and treated with (trimethylsilyl)diazomethane (2.00 M in hexane) dropwise until generation of N_2 gas ceased. After quenching the methylation with AcOH, the reaction mixture was diluted with AcOEt, washed with sat.aq.NH_4Cl and brine, dried over Na_2SO_4, and then evaporated under reduced pressure. The residue was purified by chromatography (silica 150 g, AcOEt /hexane = 2/1) to afford **13** as an oil (2.22 g, 65%). $[\alpha]^{24}_D$ -2.0 (c 0.50, $CHCl_3$); IR ν_{max} 3270, 2978, 2934, 2563, 1741, 1606, 1236 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.28 (3H, s), 1.36 (3H, s), 1.49 (1H, dd, *J*= 10.1, 7.8 Hz), 1.51 (3H, s), 2.58 (1H, ddd, *J*= 13.9, 10.1, 7.8 Hz), 2.90 (1H, ddd, *J*= 13.9, 7.8, 3.3 Hz), 3.12 (1H, d, *J*= 11.4 Hz), 3.66 (1H, d, *J*= 11.4 Hz), 3.79 (3H, s), 3.80 (1H, m), 4.47 (1H, d, *J*= 3.8 Hz), 5.77 (1H, br-s); EIMS *m/z* 333 $[M+H]^+$, 201; HR-EIMS *m/z* 333.0930 $[M+H]^+$ (calcd 333.0943 for $C_{13}H_{21}N_2O_4S_2$).

Thiazolidine 21. A mixture of **12** (391 mg, 1.00 mmol), TFA (1.00 ml) and toluene (9.00 ml) was heated with stirring under reflux for 3 days. During the reaction, H_2O generated *in situ* was removed with an equipped Dean-Stark trap. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in AcOEt, washed with sat.aq.$NaHCO_3$ and brine, dried over Na_2SO_4, and then evaporated under reduced pressure. The residue was purified by chromatography (silica 15 g, AcOEt /hexane = 1/2 to 1/1) to afford **21** (207 mg, 56%) and **13** (100 mg, 30 %). **21**; $[\alpha]^{24}_D$ -7.2 (c 0.50, $CHCl_3$); IR ν_{max} 2979, 2936, 1759, 1610, 1370, 1236 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.30 (3H, s), 1.37 (3H, s), 1.53 (3H, s), 1.67 (3H, s), 1.97 (3H, s), 3.00 (1H, dd, *J*=10.4, 9.8 Hz), 3.10 (1H, dd, *J*= 10.4, 5.5 Hz), 3.12 (1H, d, *J*= 11.6 Hz), 3.68 (1H, d, *J*= 11.6 Hz), 3.78 (3H, s), 4.35 (1H, dt, *J*= 9.8, 5.5 Hz), 4.46 (1H, d, *J*= 5.5 Hz); EIMS *m/z* 373 $[M+H]^+$, 201; HR-EIMS *m/z* 373.1259 $[M+H]^+$ (calcd 373.1256 for $C_{16}H_{25}N_2O_4S_2$).

Thiazoline 14. To an ice-cold solution of **13** (2.22 g, 6.68 mmol) in DMF (13.4 ml) was added *p*-methoxybenzyl chloride (1.09 ml, 1.20 eq.) and K_2CO_3 (1.38 g, 1.50 eq.), and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and then evaporated under reduced pressure. The residue was chromatographed (silica 140 g, AcOEt /hexane = 3/2) to give **14**

(2.64 g, 87%). $[\alpha]^{28}_{D}$ -19.8 (c 1.00, $CHCl_3$); IR ν_{max} 3283, 2971, 2941, 1751, 1609, 1511, 1248 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.23 (3H, s), 1.29 (3H, s), 1.46 (3H, s), 2.49 (1H, dd, J= 13.4, 9.2 Hz), 2.83 (1H, dd, J= 13.4, 3.7 Hz), 3.09 (1H, d, J= 11.6 Hz), 3.64 (1H, d, J= 11.6 Hz), 3.70 (2H, s), 3.74 (1H, m), 3.77 (3H, s), 3.80 (3H, s), 4.36 (1H, d, J= 3.7 Hz), 5.14 (1H, br-s), 6.86 (2H, d, J= 8.6 Hz), 7.22 (2H, d, J= 8.6 Hz); LSIMS *m/z* 905 $[2M+H]^+$, 453 $[M+H]^+$, 331, 121; HR-LSIMS *m/z* 453.1523 $[M+H]^+$ (calcd 453.1518 for $C_{21}H_{29}N_2O_5S_2$).

Thiazoline alcohol 15. A mixture of oxazolidinone **14** (2.50 g, 5.52 mmol), $(Boc)_2O$ (1.33 g, 1.10 eq.) and DMAP (34.0 mg, 0.05 eq.) in CH_2Cl_2 (16.6 ml) was stirred at room temperature for 30 min, and then the mixture was concentrated under reduced pressure. A solution of the residue in MeOH (27.6 ml) was treated with Cs_2CO_3 (3.60 g, 2.00 eq) and stirred at room temperature for 22 h. The reaction was quenched with 10% aq.citric acid and extracted twice with AcOEt. The combined organic phase was washed with brine, dried over Na_2SO_4, and then evaporated under reduced pressure. A solution of the residue in Et_2O (45.0 ml) and MeOH (5.00 ml) was treated with ethereal diazomethane, which was prepared from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and KOH, dropwise at 0°C, until generation of N_2 gas ceased. The reaction was quenched with AcOH, and concentrated under reduced pressure to yield a residue which was chromatographed (silica 100 g, AcOEt /hexane = 1/4 to 2/1) to afford **15** (878 mg, 30%) and recovered **14** (1.64 g, 66%). **15**; $[\alpha]^{28}_{D}$ -29.4 (c 1.00, $CHCl_3$); IR ν_{max} 3418, 3295, 2973, 2932, 1739, 1705, 1609, 1512, 1249, 1173 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.24 (3H, s), 1.36 (3H, s), 1.43 (9H, s), 1.57 (3H, s), 2.52 (1H, dd, J= 14.0, 5.5 Hz), 2.67 (1H, dd, J= 14.0, 8.5 Hz), 3.08 (1H, d, J= 11.6 Hz), 3.61 (1H, d, J= 11.6 Hz), 3.73 (2H, s), 3.78 (3H, s), 3.79 (3H, s), 3.86 (1H, br-s), 3.95 (1H, br-q, J= 9.7 Hz), 5.17 (1H, br-d, J= 10.4 Hz), 5.48 (1H, br-s), 6.83 (2H, d, J= 8.6 Hz), 7.29 (2H, d, J= 8.6 Hz); LSIMS *m/z* 527 $[M+H]^+$, 230, 202, 121; HR-LSIMS *m/z* 527.2244 $[M+H]^+$ (calcd 527.2250 for $C_{25}H_{39}N_2O_6S_2$).

***N*-Me-TBS-ether 17.** To a solution of alcohol **15** (436 mg, 0.83 mmol) and 2,6-lutidine (0.38 ml, 4.00 eq.) in CH_2Cl_2 (4.10 ml) was added TBSOTf (0.38 ml, 2.00 eq.) dropwise over 5 min at -78°C, and the mixture was stirred at the same temperature for 30 min. The reaction was quenched with sat.aq.$NaHCO_3$ and extracted with AcOEt. The AcOEt extract was washed with water and brine, dried over Na_2SO_4, and then evaporated under reduced pressure. The residue was purified by chromatography (silica 25 g, AcOEt /hexane = 1/5) to afford **16** (479 mg, 90%). IR ν_{max} 3453, 2954, 2932, 1735, 1711, 1610, 1512, 1250, 1175 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.10 (3H, s), 0.11 (3H, s), 0.92 (9H, s), 1.23 (3H, s), 1.27 (3H, s), 1.44 (9H, s), 1.56 (3H, s), 2.49 (1H, dd, J= 13.4, 7.9 Hz), 2.60 (1H, dd, J= 13.4, 6.1 Hz), 3.10 (1H, d, J= 11.6 Hz), 3.65 (1H, d, J= 11.6 Hz), 3.71 (2H, d, J= 2.4 Hz), 3.77 (3H, s), 3.78 (3H, s), 3.92 (1H, br-q, J= 7.9 Hz), 4.17 (1H, br-s), 5.09 (1H, br-d, J= 8.5 Hz), 6.82 (2H, d, J= 8.5 Hz), 7.27 (2H, d, J= 8.5 Hz).

To a solution of **16** (479 mg, 0.75 mmol) and Me_2SO_4 (942 mg, 10.0 eq.) in DMF (7.50 ml) was slowly added NaH (60%, 239 mg, 8.00 eq.). The mixture was stirred at room temperature for 30 min and then at 75 °C overnight. After cooling to room temperature, the reaction mixture was diluted with AcOEt and Et_2O, and then the organic phase was washed with sat.aq.NH_4Cl, water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in THF (6.00 ml) and treated with TBAF (1.00 M in THF, 0.19 ml, 0.25 eq.) at 0°C, and stirred at the same temperature for 20 min. The reaction mixture was poured into 5% aq.$KHSO_4$ and extracted with AcOEt. The AcOEt extract was washed with brine, dried over Na_2SO_4, then evaporated under reduced pressure. The residue was purified by chromatography (silica 25 g, AcOEt /hexane = 1/5 to 1/3) to give **17** (304 mg, 62%) and recovered **15** (55.0 mg, 14%). **17**; $[\alpha]^{24}_D$ -26.4 (c 0.50, $CHCl_3$); IR ν_{max} 2955, 2856, 1739, 1689, 1610, 1512, 1252, 1173 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, 120°C) δ 0.07 (6H, s), 0.90 (9H, s), 1.16 (3H, s), 1.23 (3H, s), 1.44 (9H, s), 1.45 (3H, s), 2.64 (2H, m), 2.73 (3H, s), 3.11 (1H, d, J= 11.5 Hz), 3.61 (1H, d, J= 11.5 Hz), 3.69 (3H, s), 3.70 (2H, s), 3.75 (3H, s), 4.31 (1H, d, J= 3.4 Hz), 4.46 (1H, br-s), 6.85 (2H, d, J= 8.3 Hz), 7.22 (2H, d, J= 8.3 Hz); LSIMS *m/z* 655 $[M+H]^+$, 555, 121; HR-LSIMS *m/z* 655.3276 $[M+H]^+$ (calcd 655.3271 for $C_{32}H_{55}N_2O_6S_2Si$).

***N*-Me-alcohol 18.** A mixture of **17** (304 mg, 0.46 mmol), THF (4.60 ml), MS-4A (600 mg, x 2 wt) and TBAF (1.00 M in THF, 1.16 ml, 2.50 eq.) was stirred at room temperature for 1.5 h. The MS-4A was removed by filtration and the AcOEt filtrate was washed with 5% aq.$KHSO_4$ and brine, dried over Na_2SO_4, then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (5.00 ml) and MeOH (1.00 ml), and treated with (trimethylsilyl)diazomethane (2.00 M in hexane) dropwise, until generation of N_2 gas ceased. The reaction was quenched with AcOH, and concentrated under reduced pressure. The residue was purified by chromatography (silica 15 g, AcOEt /hexane = 1/5) to give **18** (213 mg, 85%). $[\alpha]^{24}_D$ -73.5 (c 0.40, $CHCl_3$); IR ν_{max} 3322, 2975, 2835, 1735, 1684, 1610, 1512, 1248, 1171 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, 130°C) δ 1.21 (6H, s), 1.44 (9H, s), 1.45 (3H, s), 2.67 (1H, dd, J= 13.2, 5.4 Hz), 2.73 (1H, m), 2.74 (3H, s), 3.13 (1H, d, J= 11.2 Hz), 3.60 (1H, d, J= 11.2 Hz), 3.69 (2H, s), 3.70 (3H, s), 3.75 (3H, s), 3.84 (1H, d, J= 3.4 Hz), 4.30 (1H, br-s), 6.85 (2H, d, J= 8.5 Hz), 7.22 (2H, d, J= 8.5 Hz); LSIMS *m/z* 541 $[M+H]^+$, 441, 121; HR-LSIMS *m/z* 541.2403 $[M+H]^+$ (calcd 541.2406 for $C_{26}H_{41}N_2O_6S_2$).

Thiol 20. To an ice-cold solution of **18** (161 mg, 0.30 mmol) in CH_2Cl_2 (6.00 ml) was added freshly prepared Npys-Cl (85.0 mg, 1.50 eq.), and stirred at same temperature for 30 min. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed (silica 10 g, AcOEt /hexane = 2/3 to 3/1) to give an oily product (**19**, 150 mg).

To a solution of **19** (150 mg, ca.0.26 mmol) in acetone (4.00 ml) and water (1.00 ml) was added *n*-Bu_3P (65.0 μl, 1.00 eq.), and stirred at room temperature for 20 min. The acetone

was removed in vacuo and the residue was taken up in AcOEt. The AcOEt extract was washed with 10% aq.citric acid and brine, dried over Na_2SO_4, then concentrated under reduced pressure. The residue was purified by chromatography (silica 8.0 g, AcOEt /hexane = 1/3) to afford **20** (83.0 mg, 66% from **18**). $[\alpha]^{25}_D$ -73.2 (c 0.50, $CHCl_3$); IR ν_{max} 3333, 2974, 2560, 1735, 1690, 1601, 1445, 1366, 1168 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6, 120°C) δ 1.21 (3H, s), 1.22 (3H, s), 1.43 (9H, s), 1.45 (3H, s), 1.80 (1H, t, J= 7.6 Hz), 2.73 (1H, br-d, J= 13.4 Hz), 2.77 (3H, s), 2.81 (1H, m), 3.15 (1H, d, J= 11.5 Hz), 3.60 (1H, d, J= 11.5 Hz), 3.71 (3H, s), 3.86 (1H, d, J= 3.7 Hz), 4.13 (1H, br-s); LSIMS *m/z* 421 $[M+H]^+$, 347, 321, 230; HR-LSIMS *m/z* 421.1838 $[M+H]^+$ (calcd 421.1831 for $C_{18}H_{33}N_2O_5S_2$).

Micacocidin methyl ester 24. To an ice-cold solution of **20** (40.0 mg, 0.10 mmol) in CH_2Cl_2 (2.00 ml) was added TFA (0.40 ml), and stirred at the same temperature for 10 min, then at room temperature for 1 h. The resulting mixture was then concentrated under reduced pressure to afford TFA salt **22** (62.0 mg, quant.) as a pale yellow oil. The product **22** thus obtained was subjected to the next reaction without purification. **22**; IR ν_{max} 3308, 2976, 2444, 1739, 1683, 1436, 1203, 1136 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.29 (1H, m), 1.33 (3H, s), 1.48 (3H, s), 1.58 (3H, s), 2.81 (3H, br-s), 2.98 (2H, m), 3.12 (1H, m), 3.24 (1H, d, J= 11.6 Hz), 3.60 (1H, d, J= 11.6 Hz), 3.81 (3H, s), 3.97 (1H, br-s), 7.95 (3H, br-s).

To an ice-cold solution of **23** [1] (31.0 mg, 5.42×10^{-5} mol) in THF (1.50 ml) was added $LiAlH_4$ (2.50 mg, 1.20 eq.), and the mixture was stirred at the same temperature for 30 min. The reaction was quenched with AcOEt, and then the mixture was poured into sat.aq.NH_4Cl and extracted with AcOEt. The AcOEt layer was washed with water and brine, dried over Na_2SO_4, then concentrated under reduced pressure.

Under a nitrogen atmosphere, to a suspension of the residue and AcOK (80.0 mg, 15.0 eq.) in CH_2Cl_2 (1.50 ml) was added a solution of TFA salt **22** (42.0 mg, 1.50 eq.) in CH_2Cl_2 (1.00 ml) dropwise over 30 min, and the mixture was stirred at room temperature for 19 h. The reaction mixture was diluted with AcOEt, washed with 5% aq.$KHSO_4$ and brine, and dried over Na_2SO_4. Evaporation of the AcOEt phase under reduced pressure gave a pale yellow oil (55.0 mg).

To an ice-cold solution of the oil (55.0 mg) in THF (2.00 ml) was added TBAF (1.00 m in THF, 65.0 µl, 1.20 eq.), and stirred at room temperature for 15 min. The reaction mixture was diluted with AcOEt and washed with 5% aq.$KHSO_4$ and brine, dried over Na_2SO_4, then concentrated under reduced pressure. The residue was purified by chromatography (silica 5.0 g, AcOEt /hexane = 2/5) to give micacocidin methyl ester (**24**) (14.4 mg, 46 % from **23**), as a mixture of 4 diastereomers. IR ν_{max} 3333, 2957, 2870, 1735, 1582, 1449, 1289, 1207 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) [major isomer; 9*R*,10*R*] δ 0.90 (3H, t, J= 6.9 Hz), 1.31 (3H, s), 1.34 (3H, s), 1.36 (4H, m), 1.52 (3H, s), 1.61 (2H, m), 2.63 (3H, s), 2.92 (1H, dd, J= 11.7, 4.5 Hz), 2.95 (2H, m), 3.11 (1H, d, J= 11.4 Hz), 3.14 (1H, dd, J= 11.4, 7.6 Hz), 3.24 (1H, dd, J= 11.7, 6.9 Hz), 3.40 (1H, td, J= 6.8, 4.5 Hz), 3.46 (1H, dd, J= 11.4, 8.7 Hz), 3.51 (1H, d,

J = 6.6 Hz), 3.62 (1H, d, J = 11.4 Hz), 3.78 (3H, s), 4.19 (1H, d, J = 9.2 Hz), 4.77 (1H, ddd, J = 9.2, 8.7, 7.6 Hz), 6.70 (1H, dd, J = 7.6, 1.3 Hz), 6.85 (1H, dd, J = 8.2, 1.3 Hz), 7.21 (1H, dd, J = 8.2, 7.6 Hz).

Micacocidin (1). To an ice-cold solution of micacocidin methyl ester **24** (a mixture of 4 diastereomers, 12.0 mg, 2.06×10^{-5} mol) in THF (1.00 ml) and water (0.25 ml) was added LiOH·H_2O (1.80 mg, 2.10 eq.), and stirred at room temperature for 30 min. The reaction mixture was diluted with AcOEt and washed with 5% aq.$KHSO_4$ and brine, dried over Na_2SO_4, then concentrated under reduced pressure to give crude micacocidin as a mixture of 4 diastereomers.

To a solution of the residue in MeOH (1.00 ml) and water (0.25 ml) was added $ZnCl_2$ (42.0 mg, 15.0 eq.), and stirred at room temperature overnight. The mixture was diluted with AcOEt and washed with 5% aq.$KHSO_4$ to release the zinc ion. The organic layer was washed with brine, dried over Na_2SO_4, then concentrated under reduced pressure to give crude micacocidin (8.50 mg, ca.3:1 mixture of 2 diastereomers of C-9 configuration).

The crude micacocidin (6.0 mg) was purified by HPLC [ODS HG-5 (50 x 250 mm), 75 % MeOH + 1 mM phosphate buffer (pH 7), 7.5 ml/min, det. UV 254 nm] to yield pure micacocidin (3.0 mg, Rt; 20.3 min) and C-9 isomer (1.2 mg, Rt; 18.5 min). **1**; $[\alpha]^{22}_D$ -65.3 (c 0.93, MeOH); IR ν_{max} 3064, 2924, 2857, 1729, 1581, 1464, 1290, 1211 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.90 (3H, t, J = 6.9 Hz), 1.31 (3H, s), 1.32 (3H, s), 1.36 (4H, m), 1.58 (3H, s), 1.61 (2H, m), 2.63 (3H, s), 2.94 (3H, m), 3.16 (1H, dd, J = 11.4, 7.6 Hz), 3.17 (1H, d, J = 11.5 Hz), 3.26 (1H, dd, J = 11.2, 7.2 Hz), 3.34 (1H, td, J = 6.9, 4.0 Hz), 3.47 (1H, dd, J = 11.4, 8.7 Hz), 3.61 (1H, d, J = 6.8 Hz), 3.65 (1H, d, J = 11.5 Hz), 4.22 (1H, d, J = 9.1 Hz), 4.76 (1H, td, J = 8.9, 7.6 Hz), 6.64 (1H, br-s), 6.71 (1H, dd, J = 7.6, 1.2 Hz), 6.86 (1H, dd, J = 8.2, 1.2 Hz), 7.21 (1H, t, J = 7.8 Hz); HR-LSIMS m/z 566.2183 $[M+H]^+$ (calcd 566.2179 for $C_{27}H_{39}N_3O_4S_3$).

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