Total Synthesis of Antibiotic Althiomycin[1)]

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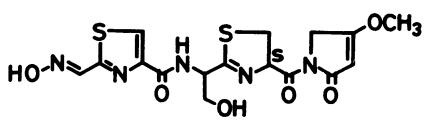
Total Synthesis of antibiotic althiomycin has been achieved starting from D-cysteine. The imide bond between thiazoline and the pyrrolinone part was constructed by coupling reaction of sodium salt of pyrrolinone with cysteine active ester or by photoreaction with diketene. The hydroxymethyl group attached on the carbon adjacent to C-2 of the thiazoline ring, was introduced by aldol condensation posterior to the thiazoline ring formation. The thiazole part was introduced in a final step in whole process of the total synthesis of the antibiotic. The synthetic althiomycin was identical with the natural antibiotic in all respects.

Althiomycin (**1**) was first isolated from *Streptomyces althioticus* by Umezawa and his collaborators in 1957[2)] and then from other microorganisms by Sensi *et al.*[3)] as well as Kunze *et al.*[4)] This antibiotic inhibits the protein synthesis in both Gram-positive and negative bacteria.[5)] The structural determination of althiomycin was carried out by Umezawa *et al.*,[6)] Kirst *et al.*,[7)] and us.[8)] Althiomycin has two asymmetric centers, one of which, C-4 of the thiazoline ring, was deduced to be of *S* configuration, corresponding to D-cysteine residue, while the *exo* carbon atom adjacent to C-2 of the thiazoline ring, is still ambiguous, because this antibiotic is always isolated as the mixture of epimers concerning this asymmetric carbon. The geometric configuration of the aldoxime has been determined as *E* form from the result of X-ray analysis of the methanolysis product of althiomycin[7)] as well as our synthetic study.[8)] In order to investigate the structure-activity relationship of althiomycin, we first attempted the total synthesis of this antibiotic.

The synthetic pathway is based on the "stepwise elongation method" from C-terminal to N-terminal. Concerning the preparation of pyrrolinone part, 2,4-pyrrolidinedione (**2**) synthesized according to the method of Lowe *et al.*[9)] was methylated with ethereal diazomethane to give 4-methoxy-3-pyrrolin-2-one (**3**) (yield 52.9%) as the major product and 2-methoxy-4-one isomer (**4**) (7.1%) as the minor one. The mixture was purified and separated by silica-gel column chromatography using the developing solvent of ethyl acetate–methanol 9:1. The predominant product (**3**) is completely identical with naturally derived pyrrolinone in all respects. As a preliminary experiment of acylation of the pyrrolinone, acid chloride method is solely successful among various kinds of acylation methods applied.



Althiomycin (1)

Fig. 1.

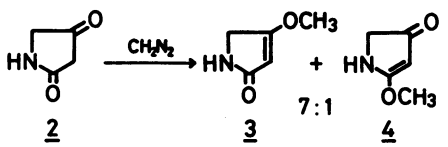


Fig. 2.

Secondly, the synthesis of the thiazoline part was performed by coupling method of the imino ether of *N*-protected glycine and D-cysteine ester. *N,S*-Ditrityl-D-cysteine diphenylmethyl ester (**6**) was prepared from D-cysteine *p*-toluenesulfonate *via* successive procedures of tritylation of mercapto and amino groups and esterification with diphenyldiazomethane. Both trityl (Trt) groups were removed with silver nitrate to give cysteine derivative with free amino and mercapto groups (**7**). The compound was coupled with ethyl benzyloxycarbonylaminoacetimidate[10)] to obtain the thiazoline ester (**8**) (yield 73.9%). Removal of diphenylmethyl (DPM) group afforded the thiazolinecarboxylic acid (**9**).

With reference of results of the preliminary acylation of the pyrrolinone, the coupling of the thiazolinecarboxylic acid (**9**) with methoxypyrrolinone (**3**) was attempted using several carboxylic activation methods such as acid chloride, active ester, mixed anhydride, dicyclohexylcarbodiimide–4-dimethylaminopyridine, *N*-ethyl-5-phenylisoxazolium-3-sulfonate (Woodward's reagent "K"), azide, or triphenylphosphine-di-2-pyridyl disulfide (Mukaiyama's method). Amino activation methods like triethyloxonium tetrafluoroborate (Meerwein's reagent), 2-chloro-1-methylpyridinium iodide,[11)] phosphazo, phosphite ester, and trimethylsilylation–acid chloride method[12)] were also tried. However, the desired coupling product (**10**) could never be obtained by these methods because of a low reactivity of the imino group of the pyrrolinone and an instability of the thiazoline ring. Therefore, we next tried to apply a photoreaction of the thiazolinecarbonyl azide (**12**) with diketene according to the method exploited in the case of substituted benzoyl azides by Kato *et al.*[13)] The corresponding azide (**12**) was synthesized from carboxylic acid **9** *via* successive procedures of conversion to the protected hydrazide with *t*-butyl carbazate, water-soluble carbodiimide (*N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide), and

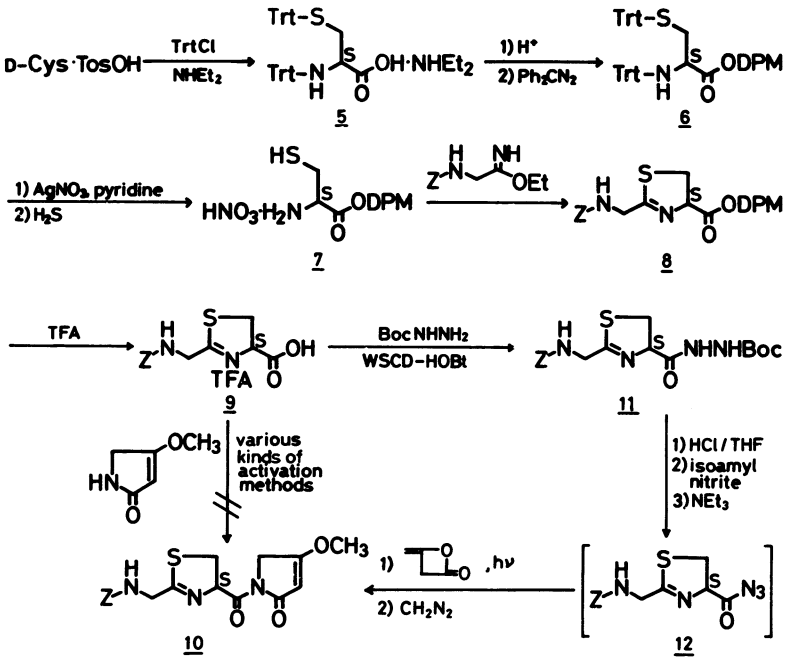


Fig. 3.

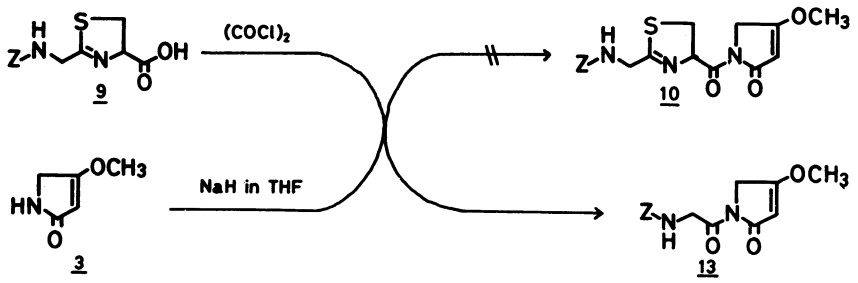


Fig. 4.

1-hydroxybenzotriazole, and then azidation with isopentyl nitrite followed by removal of *t*-butoxycarbonyl (Boc) group. A mixture of the azide (**12**) thus obtained without isolation and diketene distilled just before use was irradiated with a high-pressure mercury lamp through a quartz filter (HALOS PIH-100) in chloroform for 6 h at 0 °C under nitrogen atmosphere. The product was methylated with diazomethane to give the desired thiazoline-pyrrolinone compound (**10**). This route was, however, found to be disadvantageous concerning yield and reproducibility despite of searches of the good reaction condition.

Thus, we reinvestigated the coupling of the thiazoline part with the pyrrolinone to improve this synthesis. The defects in the above reaction seemed to be due to a low nucleophilicity of the imino group of the pyrrolinone ring. Therefore, we next tried a coupling of thiazolinecarbonyl chloride with sodium salt of the pyrrolinone prepared from **3** and sodium hydride. Actually the reaction afforded only glycylpyrrolinone (**13**), but not the desired thiazoline-pyrrolinone derivative (**10**), presumably because of the situation that C-2 of the thiazoline ring is more reactive than the carbonyl carbon of the acid chloride under these conditions. From results mentioned above, we then attempted the formation of the thiazoline ring after coupling of the cysteine active ester with sodium salt of the pyrrolinone. Each active ester (**14**—**16**) prepared from *N*-hydroxysuccinimide (HONSu), *N*-hydroxy-5-norbornene-*endo*-2,3-dicarboximide (HONb), or *p*-nitrophenol (HONp), was coupled with sodium salt of the pyrrolinone respectively. The products from both ONb and ONp esters were the cysteinylpyrrolinone (**18**), whereas that from ONSu ester was found to be *N*-[3-(cysteinyloxyaminocarbonyl)propionyl]-3-pyrrolin-2-one derivative (**17**) formed by ring opening of the succinimide. By comparison of two reactions using ONb and ONp esters, the former is superior to the latter both in respects of easiness in the treatment of the active esters and $[\alpha]_D$ of the product. The protected cysteinylpyrrolinone (**18**) thus obtained was deprotected with silver nitrate and hydrogen sulfide,

and then coupled with the imidic ether of benzyloxycarbonylglycine to afford the thiazoline-pyrrolinone derivative (**10**) in 39% yield. Formations of the thiazoline ring and the imide bond between the thiazoline and the pyrrolinone part were confirmed in long-range coupling (1Hz) between the *exo* methylene of C-2 and the proton of C-4 in the thiazoline ring, and downfield shift (0.3 ppm) of methylene protons of the pyrrolinone ring in NMR respectively.

The aldol condensation for the thiazoline moiety with aqueous 35% formaldehyde solution in dimethyl sulfoxide at room temperature for 3 d afforded the hydroxymethyl compound (**19**) and its anhydro derivative in ratio of about 1:1 on TLC. However, when the aldol condensation was carried out by use of paraformaldehyde instead of formalin in degassed and anhydrous dimethyl sulfoxide, only the desired hydroxymethyl derivative was obtained as a sole product. The result of this reaction seems to be much dependent to the solvent used since this type of reaction for model compounds gave the β-hydroxy derivative in aprotic polar solvents such as dimethyl sulfoxide or *N*,*N*-dimethylformamide, while, in a protic solvent like methanol its anhydro derivative was obtained.[14]

The thiazole moiety, namely (*E*)-2-(hydroxyiminomethyl)-4-thiazolecarbonyl azide (**29**), was synthesized as shown in the scheme. For ethyl diethoxyacetate (**20**)[15] amidation, dehydration, and addition of hydrogen sulfide were successively carried out to result in the production of diethoxythioacetamide (**23**), which was then coupled with ethyl 3-bromopyruvate[16] to yield thiazole acetal (**24**). After removal of the acetal, the aldehyde was converted to oximes (**26**, **27**) with hydroxylamine hydrochloride. The (*E*)-aldoxime (**26**) was separated from (*Z*)-isomer (**27**) by silica-gel column chromatography and fractional recrystallization. The compound (**26**) thus obtained was then converted into thiazolecarbonyl azide (**29**) in the usual way.

Deprotection of the thiazoline-pyrrolinone compound **19** with anhydrous hydrogen fluoride followed by coupling with the thiazole part by the azide method was carried out under following conditions; the thiazoline-pyrrolinone derivative (**19**) was dissolved in anisole, and then anhydrous hydrogen fluoride was introduced to the solution at −78 °C. After deprotection procedure for 20 min at 0 °C, hydrogen fluoride

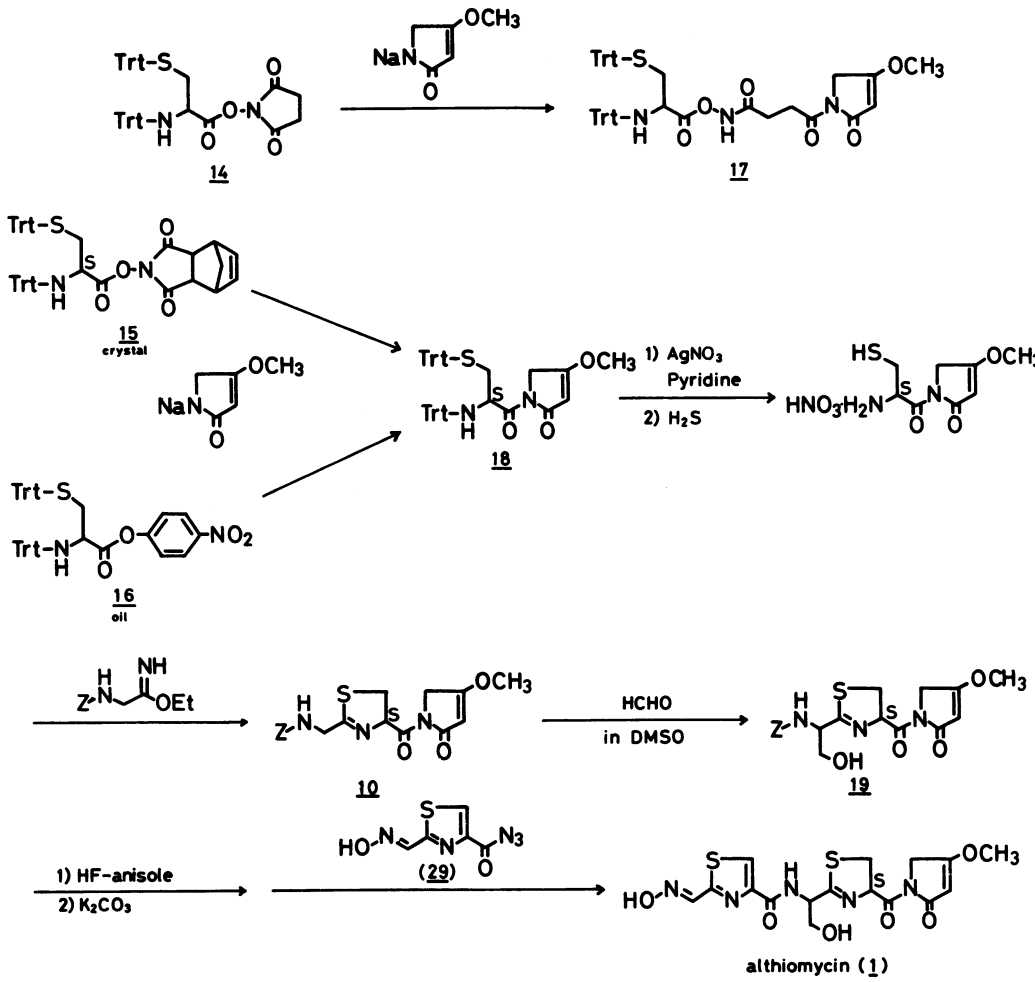


Fig. 5.

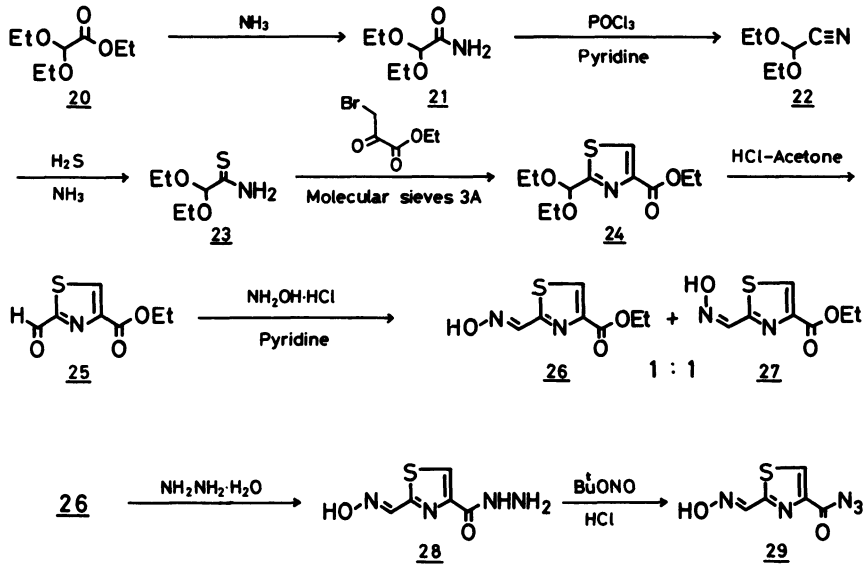

NH3
POCl3
Pyridine
20
21
22
H2S
NH3
Molecular sieves 3A
23
24
HCl-Acetone
NH2OH·HCl
Pyridine
25
26
1 : 1
27
NH2NH2·H2O
28
BuONO
HCl
29

Fig. 6.

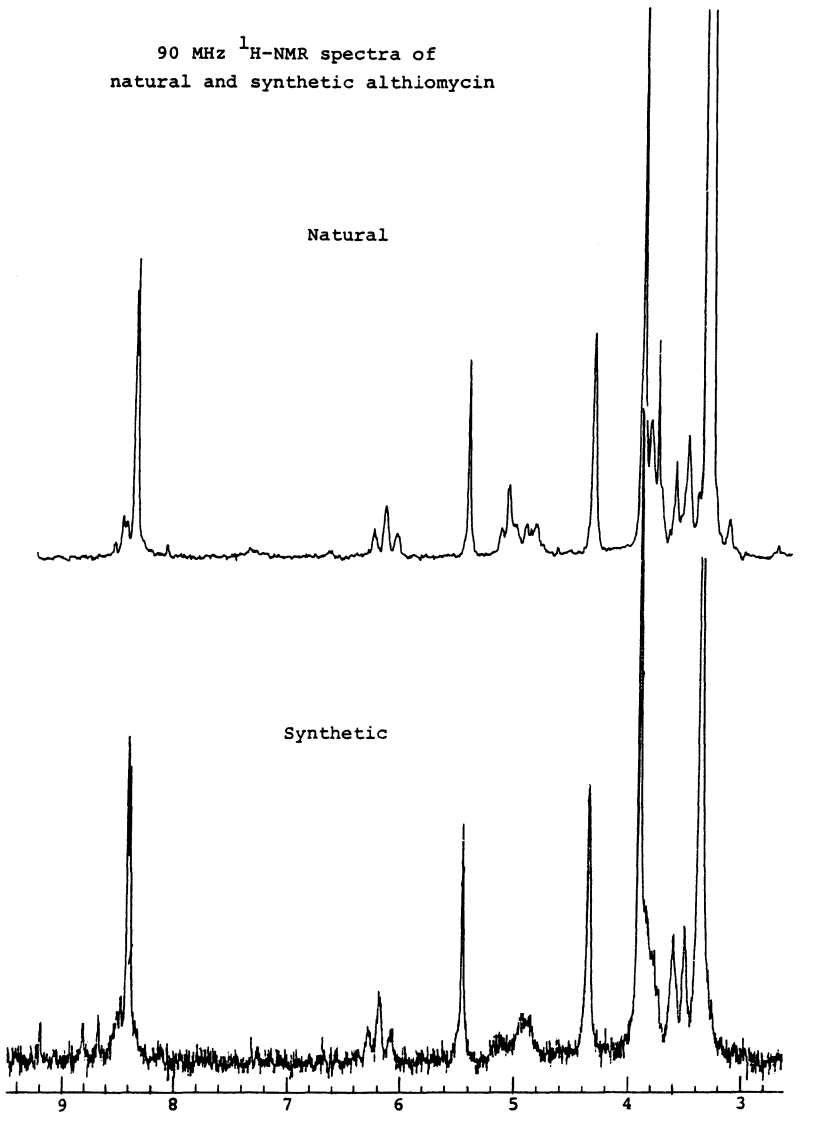

90 MHz 1H-NMR spectra of
natural and synthetic althiomycin
Natural
Synthetic
9
8
7
6
5
4
3

Fig. 7.

Table 1. Comparisons of synthetic and natural althiomycin

		Synthetic	Natural
$[\alpha]_D^{20}$ (Methyl Cellosolve)		+22.3°	+20.8°
UV λ_{max}^{MeOH} (ε_{max})		223 nm (34100)	223 nm (38700)
		240 nm (26300)	240 nm (29900)
		286 nm (8200)	286 nm (8310)
TLC[a]	$CHCl_3$-MeOH 9:1	0.33	0.33
	Benzene-AcOEt 1:3	0.45	0.45
	Benzene-acetone 1:1	0.51	0.51
HPLC	31% CH_3CN[b]	7.4 min	7.4 min
	CH_3CN-MeOH-H_2O[c] (1:2:7)	25.4 min	25.4 min

a) Merck Kieselgel F_{254}. b) Nagel Nucleosil $7C_{18}$ (6 mm×250 mm), 1.5 ml/min. c) Waters Radialpak μ-Bondapak C_{18} (8 mm×100 mm), 1.0 ml/min

Table 2. Minimum inhibitory concentration of natural and synthetic althiomycin

Test organism	Synthetic	Natural
Staph. aureus ATCC 6538P	25	25
Staph. aureus MS353	25	25
Staph. aureus MS353 C36	25	25
Staph. aureus MS353 A0	25	25
Staph. aureus 0116	25	25
Staph. aureus 0119	25	25
Staph. aureus 0126	25	25
Staph. aureus 0127	25	25
Staph. epidermidis sp-al-1	25	25
Strept. pyogenes N.Y.5	3.1	3.1
Strept. pyogenes 1022	3.1	3.1
Strept. faecalis 1501	100	100
Strept. agalactiae 1020	6.3	6.3
Sarcina lutea ATCC 9341	1.6	1.6
Micrococcus flavus ATCC 10240	3.1	3.1
Corynebact. diphtheriae P.W.8	0.8	0.8
Bac. subtilis ATCC 6633	25	25
E. coli NIHJ-JC2	>100	>100
E. coli B	50	50
Klebs. pneumoniae ATCC 10031	6.3	6.3
Salm. typhosa H 901	>100	>100
Salm. enteritidis Gaertner	>100	>100
Shigella flexineri type 3a	50	25
Shigella sonnei E33	>100	>100
Proteus vulgaris OX19	50	50
Serratia carcescens	>100	>100
Ps. aeruginosa LAM1095	>100	>100

was evaporated off *in vacuo* as quickly as possible. The residual oil was neutralized with potassium carbonate, and coupled with thiazolecarbonyl azide in *N,N*-dimethylformamide for 3 h at room temperature. The product after purification by preparative silica-gel TLC (chloroform–methanol 9:1) was completely identical with natural althiomycin as shown in Table 1. Antibacterial activities of the synthetic althiomycin were also quite identical with those of the natural antibiotic (Table 2).

Experimental

All melting points are uncorrected. The IR spectra were determined on a JASCO A-100 grating infrared spectrophotometer; and the ^1H- and ^{13}C-NMR spectra, on a Varian XL-100-15 NMR spectrometer, a Jeol FX-90Q NMR spectrometer, a Jeol JNM-PMX 60 NMR spectrometer, and a Varian T-60 NMR spectrometer. The chemical shifts are given in δ, with TMS as the internal standard. The UV spectra were obtained with a Hitachi 124 spectrophotometer. The electron-impact and field-desorption mass spectra were obtained with a Jeol JMS-OlSG-2 mass spectrometer and Hitachi RM-50 spectrometer. The optical rotations were obtained with a Perkin-Elmer 141 polarimeter. The high performance liquid chromatography was performed on a Yanako liquid chromatograph model L-2000 and Shimadzu LC-5A liquid chromatograph. TLC was carried out by the ascending method on a Merck Kieselgel F_{254} and Woelm Kieselgel F_{254}.

4-Methoxy-3-pyrrolin-2-one (3). To a solution of 2,4-pyrrolidinedione (**2**)[9] (1.19 g, 12.0 mmol) in 100 ml of acetonitrile, was added excess diazomethane in ether and allowed to react for 10 h. After evaporation of excess diazomethane at 50 °C, the solution was concentrated *in vacuo* to give brown needles. Purification and separation by silica-gel column chromatography gave pale yellow solid (R_f 0.80 on TLC using a developing solvent chloroform–methanol 7:1). This solid was recrystallized from ethyl acetate and hexane; yield 0.72 g (53%). Mp 128—128.5 °C. Found: C, 53.05; H, 6.21; N, 12.36%. Calcd for $C_5H_7NO_2$: C, 53.09; H, 6.24; N, 12.38%. EI-MS: *m/z* (rel intensity) 113 (100), 98 (14), 86 (50), 83 (31), 70 (100), and 28 (52). 60 MHz ^1H-NMR ($CDCl_3$) δ=7.0 (1H, br.), 5.18 (1H, s), 3.98 (2H, s), 3.85 (3H, s).

1-Acetyl-4-methoxy-3-pyrrolin-2-one. To a solution of **3** (100 mg, 0.885 mmol) in 0.9 ml of chloroform, was added triethylamine (134 mg, 1.33 mmol) and acetyl chloride (208 mg, 2.65 mmol). A mixture was stirred for 2 h under ice-cooling and then diluted with chloroform. After washing with saturated aqueous sodium hydrogencarbonate and brine, the organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification by preparative silica-gel TLC (chloroform–methanol 9:1) gave

colorless needles; yield 121 mg (100%). Mp 92—92.5 °C. Found: C, 54.05; H, 5.86; N, 8.94%. Calcd for $C_7H_9NO_3$: C, 54.19; H, 5.85; N, 9.03%. EI-MS *m/z* (rel intensity), 155 (64), 113 (86), 99 (83), 70 (47), and 43 (100). 60 MHz ^{1}H-NMR ($CDCl_3$) δ=5.20 (1H, s), 4.30 (2H, s), 3.90 (3H, s), 2.50 (3H, s).

N,S-Dityryl-D-cysteine Diethylamine Salt (5). To a solution of D-cysteine *p*-toluenesulfonate (13.1 g, 44.7 mmol) in 85 ml of degassed water, a solution of diethylamine (24.6 ml, 0.237 mol) in 85 ml of water was added dropwise with stirring for 5 min at 0 °C. To the reaction mixture, a solution of trityl chloride (37.4 g, 0.134 mol) in 290 ml of ether was added dropwise with a vigorous stirring for 10 min at −20 °C. After additional stirring for 1.5 h, the reaction mixture was extracted with chloroform and washed with water. The organic layer was concentrated *in vacuo* and residual oil was dissolved in 300 ml of 99% ethanol. To the solution, diethylamine (8 ml) was added and colorless prisms precipitated was collected; yield 27.2 g (89.8%). Mp 193—194 °C (dec). $[\alpha]_D^{20}$ −62.1° (*c* 1.50, chloroform). Found: C, 79.38; H, 6.72; N, 3.91; S, 4.50%. Calcd for $C_{45}H_{46}N_2O_2S$: C, 79.61; H, 6.83; N, 4.13; S, 4.72%.

Diphenylmethyl N,S-Dityryl-D-cysteinate (6). To a suspension of *N,S*-dityryl-D-cysteine diethylamine salt (19.9 g, 29.4 mmol) in 300 ml of ether, 10 ml of 3M aqueous (1 M= 1 mol dm^{-3}) citric acid was added with vigorous stirring for 40 min at 0 °C. The ethereal solution was washed with water and dried over anhydrous magnesium sulfate. The solution was concentrated *in vacuo* to 100 ml. To the solution, diphenyldiazomethane (5.70 g, 29.4 mmol) was added. After stirring for 1 d, the insoluble material was filtered off and the solution was concentrated *in vacuo*. The residue was recrystallized from ether and methanol; yield 19.3 g (84.8%). Mp 155—156 °C. $[\alpha]_D^{20}$ −44.4° (*c* 0.468, acetone). Found: C, 83.75; H, 5.87; N, 1.90; S, 4.09%. Calcd for $C_{54}H_{45}NO_2S$: C, 84.01; H, 5.88; N, 1.81; S, 4.15%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=7.30 (40H, m), 7.10 (1H, s), 6.30 (1H, s), 3.50 (1H, br.), 2.80 (1H, d), 2.40 (2H, d).

Diphenylmethyl D-Cysteinate Nitrate (7). To a solution of diphenylmethyl *N,S*-dityryl-D-cysteinate (**6**) (19.2 g, 24.9 mmol) in 25 ml of benzene, a solution of silver nitrate (4.65 g, 27.4 mmol) and pyridine (2.20 ml, 27.4 mmol) in 40 ml of methanol was added and stirred for 1 d at room temperature. Through the reaction mixture, hydrogen sulfide was bubbled with stirring for 30 min at room temperature. Silver sulfide was filtered off and the filtrate was concentrated *in vacuo*. The residue was recrystallized from methanol and ether; yield 6.30 g (72.2%). Mp 123—125 °C. $[\alpha]_D^{20}$ +15.7° (*c* 2.84, *N,N*-dimethylformamide). Found: C, 54.95; H, 5.35; N, 7.78; S, 8.65%. Calcd for $C_{16}H_{18}N_2O_5S$: C, 54.84; H, 5.18; N, 8.00; S, 9.15%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=7.30 (10H, s), 6.93 (1H, s), 4.50 (1H, t, *J*=4Hz), 3.30 (2H, d, *J*=4Hz).

Diphenylmethyl (S)-2-(Benzyloxycarbonylaminomethyl)-2-thiazoline-4-carboxylate (8). To a solution of ethyl (benzyloxycarbonylamino)acetimidate[10] (4.25 g, 18.0 mmol) in 120 ml of absolute ethanol, diphenylmethyl D-cysteinate nitrate (6.30 g, 18.0 mmol) was added. The reaction mixture was stirred for 16 h at room temperature and concentrated *in vacuo*. The residual oil was dissolved in ethyl acetate and washed with water. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Recrystallization from acetone-hexane gave colorless crystals; yield 6.12 g (73.9%). Mp 94—96 °C. $[\alpha]_D^{14}$ −51.9° (*c* 1.15, benzene). Found: C, 67.76; H, 5.30; N, 6.06; S, 7.09%. Calcd for $C_{26}H_{24}N_2O_4S$: C, 67.81; H, 5.26; N, 6.08; S, 6.96%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=7.45 (15H, s), 6.88 (1H, s), 5.40 (1H, br.), 5.13 (1H, dt, *J*=2Hz, 10Hz), 5.08 (2H, s), 4.18 (2H, dd, *J*=2Hz, 7Hz), 3.52 (2H, d, *J*=10 Hz).

(S)-2-(Benzyloxycarbonylaminomethyl)-4-carboxy-2-thiazolinium Trifluoroacetate (9). A mixture of diphenylmethyl (*S*)-2-(benzyloxycarbonylaminomethyl)-2-thiazoline-4-carboxylate (6.12 g, 13.3 mmol) and trifluoroacetic acid (19.8 ml, 0.266 mol) was stirred for 30 min at 0 °C. To the reaction mixture, 200 ml of ether was added. The precipitate was collected and washed with ether, yield 4.43 g (81.6%). Mp>200 °C. 60 MHz ^{1}H-NMR (CF_3COOD) δ=7.34 (5H, s), 5.24 (2H, s), 4.70 (1H, br. t), 4.27 (2H, s), 3.74 (2H, br. d).

(S)-N'-(t-Butoxycarbonyl)-2-(benzyloxycarbonylaminomethyl)-2-thiazoline-4-carbohydrazide (11). To a solution of **9** (4.43 g, 10.9 mmol) in 20 ml of tetrahydrofuran, *t*-butyl carbazate (3.01 g, 22.8 mmol), 1-hydroxybenzotriazole (3.08 g, 22.8 mmol), and *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (4.37 g, 22.8 mmol) were added successively and the mixture was stirred for 2 h at 0 °C. After additional stirring for 16 h at room temperature, the reaction mixture was concentrated *in vacuo* and dissolved in ethyl acetate. The solution was washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate. The solution was concentrated *in vacuo*. Purification by silica-gel column chromatography (120 g, benzene–ethyl acetate 3:1) gave colorless oil; yield 2.60 g (58.7%). Found: *m/z* 408.1458. Calcd for $C_{18}H_{24}N_4O_5S$: *m/z* 408.1466. Found: C, 52.76; H, 6.31; N, 12.54; S, 6.61%. Calcd for $C_{18}H_{24}N_4O_5S \cdot 0.5C_4H_8O_2$: C, 53.08; H, 6.24; N, 12.38; C, 7.09%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=8.63 (1H, d, *J*=3Hz), 7.30 (5H, s), 6.85 (1H, d, *J*=3Hz), 6.00 (1H, br.t, *J*=6 Hz), 5.06 (2H, s), 5.03 (1H, tt, *J*=2Hz, 9Hz), 4.10 (2H, dd, *J*=2Hz, 6Hz), 3.53 (2H, d, *J*=9Hz), 1.45 (9H, s).

(S)-2-(Benzyloxycarbonylaminomethyl)-2-thiazoline-4-carbohydrazide Dihydrochloride. The protected hydrazide **11** (2.60 g, 6.37 mmol) was dissolved in 4.67 M hydrogen chloride in tetrahydrofuran (27.3 ml, 0.127 mol) and allowed to react for 2 h at 0 °C. To the reaction mixture, 300 ml of ether was added and the precipitate was collected; yield 2.05 g (84.4%). Mp 132—134 °C (dec). Found: C, 38.59; H, 5.18; N, 14.62; S, 8.03; Cl, 17.67%. Calcd for $C_{13}H_{18}N_4O_3SCl_2 \cdot H_2O$: C, 39.10; H, 5.05; N, 14.03; S, 8.41; Cl, 17.76%.

N,S-Dityryl-D-cysteine 5-Norbornene-endo-2,3-dicarboximidyl Ester (15). To a suspension of *N,S*-dityryl-D-cysteine diethylamine salt (**5**) (2.98 g, 3.86 mmol) in 200 ml of ether, 2 ml of 3 M aqueous citric acid was added. The reaction mixture was vigorously stirred for 30 min at 0 °C. An organic layer was washed with water and dried over anhydrous magnesium sulfate. The solution was concentrated *in vacuo* and the residual oil was dissolved in 15 ml of tetrahydrofuran. To the solution, *N*-hydroxy-5-norbornene-*endo*-2,3-dicarboximide (1.04 g, 5.79 mmol) and dicyclohexylcarbodiimide (0.950 g, 4.63 mmol) were added and stirred for 2 h at 0 °C. After additional stirring for 10 h at room temperature, the reaction mixture was concentrated *in vacuo*. The residual oil was dissolved in ethyl acetate, and the insoluble material was filtered off. The filtrate was washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue

was recrystallized from ethyl acetate and hexane; yield 2.88 g (97.3%). Mp 194—195 °C (dec). $[\alpha]_D^{18}$ −63.4° (*c* 1.45, chloroform). Found: C, 78.10; H, 5.61; H, 3.67; S, 4.21%. Calcd for $C_{50}H_{42}N_2O_4S$: C, 78.31; H, 5.52; N, 3.65; S, 4.18%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=7.30 (30H, m), 7.00 (1H, br. s), 6.07 (2H, br. s), 3.78 (1H, m), 3.50 (2H, br. s), 3.25 (2H, br. s), 2.50 (2H, br. d), and 1.45—1.85 (2H, br. m).

1-(N,S-Dirityl-D-cysteinyl)-4-methoxy-3-pyrrolin-2-one (18). To a solution of 4-methoxy-3-pyrrolin-2-one (**3**) (5.60 g, 49.6 mmol) in 155 ml of anhydrous tetrahydrofuran, 60% sodium hydride (1.59 g, 39.7 mmol) was added. The mixture was heated under reflux for 10 min. To the solution, *N,S*-ditrityl-D-cysteine 5-norbornene-*endo*-2,3-dicarboximidyl ester (**15**) (24.0 g, 33.1 mmol) was added with vigorous stirring for 20 min at room temperature. Ethyl acetate was added to the reaction mixture, and the solution was washed with saturated aqueous sodium hydrogencarbonate and brine. An organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was recrystallized from ethyl acetate and hexane; yield 20.3 g (87.9%). Mp 214—215 °C (dec). $[\alpha]_D^{21}$ −23.6° (*c* 2.02, chloroform). Found: C, 78.40; H, 5.94; N, 3.89; S, 4.50%. Calcd for $C_{46}H_{40}N_2O_3S$: C, 78.83; H, 5.75; N, 4.00; S, 4.57%. 100 MHz ^{1}H-NMR ($CDCl_3$) δ=7.0—7.6 (30H, m), 4.86 (1H, s), 4.84 (1H, br. s), 3.78 (3H, s), 3.77 (1H, d, *J*=18 Hz), 3.43 (1H, br. t), 3.34 (1H, d, *J*=18 Hz), 2.80 (1H, dd, *J*=6 Hz, 12 Hz), 2.58 (1H, dd, *J*=4 Hz, 12 Hz). 22.5 MHz ^{13}C-NMR ($CDCl_3$) δ=175.9, 172.2, 168.8, 146.1, 145.0, 129.8, 129.0, 127.8, 127.5, 126.5, 126.2, 94.3, 71.2, 66.5, 58.5, 54.4, 48.2, 36.8.

1-[(S)-2-(Benzyloxycarbonylaminomethyl)-2-thiazoline-4-carbonyl]-4-methoxy-3-pyrrolin-2-one (10). *i)* Via *Photoreaction*: To a suspension of (*S*)-2-(benzyloxycarbonylaminomethyl)-2-thiazoline-4-carbohydrazide dihydrochloride (1.33 g, 3.48 mmol) in 32 ml of degassed ethanol-free chloroform, isopentyl nitrite (0.673 ml, 3.44 mmol) and 4.20 M hydrogen chloride in tetrahydrofuran (0.815 ml, 3.44 mmol) were added at −18 °C and the stirring was continued for 25 min at −18 °C. The reaction mixture was neutralized with triethylamine (1.54 ml, 11.1 mmol). To the solution, diketene (2.65 ml, 3.48 mol) was added and irradiated with a 100 watt high-pressure mercury lamp (HALŌS PIH-100) with a quartz filter under nitrogen atmosphere at 0 °C for 6 h. After concentration *in vacuo*, the residual oil was dissolved in 10 ml of chloroform and methylated with excess ethereal diazomethane with stirring for 2.5 h. After evaporation of diazomethane under reduced pressure, the solution was washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. Purification by silica-gel preparative TLC (benzene–ethyl acetate 1:3) gave colorless oil; yield 67.7 mg (5.0%).

ii) Via *Sodium Salt of Pyrrolinone*: To a suspension of 1-(*N,S*-dirityl-D-cysteinyl)-4-methoxy-3-pyrrolin-2-one (**18**) (1.14 g, 1.63 mmol) in the mixture of 5.70 ml of methanol and 4.00 ml of benzene, pyridine (144 μl, 1.79 mmol) and silver nitrate (304 mg, 1.79 mmol) were added and stirring was continued for 4 h at room temperature. Hydrogen sulfide was bubbled through the reaction mixture for 20 min at room temperature. Silver sulfide was filtered off and the filtrate was concentrated *in vacuo*. To a solution of the residual oil in 22 ml of 99% ethanol, ethyl (benzyloxycarbonylamino)acetimidate[10] (385 mg, 1.63 mmol) was added and stirred for 20 h at room temperature. After concentration, the residual oil was dissolved in ethyl acetate and washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was recrystallized from ethyl acetate and hexane; yield 250 mg (39.4%). Mp 185—186 °C (dec). $[\alpha]_D^{20}$ +21.0° (*c* 0.879, chloroform). Found: C, 55.54; H, 4.94; N, 10.73; S, 8.02%. Calcd for $C_{18}H_{19}N_3O_5S$: C, 55.52; H, 4.92; N, 10.79; S, 8.23%. 100 MHz ^{1}H-NMR ($CDCl_3$) δ=7.36 (5H, s), 6.24 (1H, tt, *J*=1 Hz, 9 Hz), 5.54 (1H, br. s), 5.16 (1H, s), 5.15 (2H, s), 4.30 (2H, s), 4.26 (2H, dd, *J*=1 Hz, 5 Hz), 3.90 (3H, s), 3.61 (1H, dd, *J*=6 Hz, 9 Hz), and 3.50 (1H, dd, *J*=6 Hz, 9 Hz). 22.5 MHz ^{13}C-NMR ($CDCl_3$) δ=176.6, 171.9, 169.5, 169.0, 155.9, 136.4, 128.5, 128.0, 94.5, 78.1, 67.1, 58.8, 48.5, 43.7, and 35.7.

1-[(4S)-2-[1-(Benzyloxycarbonylamino)-2-hydroxyethyl]-2-thiazoline-4-carbonyl]-4-methoxy-3-pyrrolin-2-one (19). To a solution of 1-[(*S*)-2-(benzyloxycarbonylaminomethyl)-2-thiazoline-4-carbonyl]-4-methoxy-3-pyrrolin-2-one (**10**) (50.0 mg, 0.129 mmol) in 500 μl of degassed dimethyl sulfoxide, pulverized paraformaldehyde (77.0 mg, 2.57 mmol) was added. The mixture was stirred for 7 d at room temperature. The insoluble material was filtered off and the filtrate was dissolved in ethyl acetate. The solution was washed with water and dried over anhydrous magnesium sulfate. The solution was concentrated *in vacuo*. The residue was lyophilized; yield 52.7 mg (97.6%). Mp 71—73 °C. 100 MHz ^{1}H-NMR ($CDCl_3$) δ=7.34 (5H, s), 6.29 (1H, m), 5.88 (1H, m), 5.16 (1H, s), 5.13 (2H, s), 4.75 (1H, m), 4.28 (2H, s), 3.95—4.08 (3H, m), 3.90 (3H, s), 3.48 (1H, d, *J*=6 Hz), and 3.35 (1H, d, *J*=6 Hz). 22.5 MHz ^{13}C-NMR ($CDCl_3$) δ=177.0, 176.9, 169.7, 169.5, 156.6, 136.3, 128.5, 128.0, 94.4, 78.2, 67.1, 66.5, 58.9, 52.7, 48.4, and 34.1.

Diethoxyacetamide (21). A solution of ethyl diethoxyacetate[15] (42.7 g, 0.243 mol) in 2 l of 99% ethanol, was saturated with ammonia in a pressure bottle and kept at room temperature for 2 d. Concentration *in vacuo* gave colorless plates; yield 36.4 g (100%). Mp 80—81 °C. IR (Nujol) 1670 cm^{-1}. Found: C, 48.70; H, 8.91; N, 9.49%. Calcd for $C_6H_{13}NO_3$: C, 48.96; H, 8.90; N, 9.52%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=6.8 (2H, br.), 4.88 (1H, s), 3.73 (2H, q, *J*=7 Hz), 1.27 (6H, t, *J*=7 Hz).

Diethoxyacetonitrile (22). To a solution of diethoxyacetamide (**21**) (1.00 g, 6.80 mmol) in 16 ml of pyridine, phosphoryl chloride (1.15 g, 7.49 mmol) was added dropwise with stirring at −18 °C for 30 min. After additional stirring for 30 min, the reaction mixture was poured into about 100 g of cracked ice carefully. The mixture was extracted with ether and washed with 1M hydrochloric acid, brine, saturated aqueous sodium hydrogencarbonate, and brine successively. The organic layer was dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residual oil was distilled under reduced pressure and collected a fraction boiling at 63—63.5 °C/ 22 mmHg: yield 0.78 g (89%). Found: C, 55.82; H, 8.69; N, 10.92%. Calcd for $C_6H_{11}NO_2$: C, 55.79; H, 8.59; N, 10.85%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=5.48 (1H, s), 3.73 (4H, q, *J*=7 Hz), and 1.25 (6H, t, *J*=7 Hz).

Diethoxythioacetamide (23). *i) From 21*: To a solution of diethoxyacetamide (**21**) (4.00 g, 27.2 mmol) in 50 ml of dry benzene, phosphorus pentasulfide (2.00 g, 9.00 mmol) was added portionwise with stirring during a period of 10 min at room temperature. After additional stirring for 10 min, an insoluble material was removed by decantation, and the

supernatant was washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was recrystallized from benzene and hexane; yield 2.86 g (64.3%).

ii) From **22**: Through a solution of diethoxyacetonitrile (**22**) (580 mg, 4.50 mmol) in 300 ml of dry methanol containing 18 g of ammonia, hydrogen sulfide was bubbled with stirring for 16 h at room temperature. After hydrogen sulfide was removed by aeration, the solution was concentrated *in vacuo*. Recrystallization from benzene–hexane gave colorless needles; yield 0.73 g (100%). Mp 81—82 °C. IR (Nujol) 1600 cm^{-1} ($\nu_{C=S}$). Found: C, 44.18; H, 8.06; N, 8.54; S, 19.50%. Calcd for $C_6H_{13}NO_2S$: C, 44.15; H, 8.03; N, 8.58; S, 19.69%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=8.2 (2H, br.), 5.10 (1H, s), 3.75 (4H, q, J=7 Hz), and 1.20 (6H, t, J=7 Hz).

Ethyl 2-(diethoxymethyl)-4-thiazolecarboxylate (24). Ethyl 3-bromopyruvate[16] (0.37 g, 1.9 mmol) was added to a solution of diethoxythioacetamide (**23**) (0.37 g, 2.3 mmol) in 4 ml of dry ethanol, and the mixture was heated under reflux in the presence of Molecular Sieves 3A (about 1 g) for 30 min. The solution was concentrated *in vacuo*, extracted with ethyl acetate and washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification by preparative TLC (chloroform–methanol 50:1) gave colorless oil; yield 0.49 g (100%). Found: C, 51.19; H, 6.68; N, 5.25; S, 11.79%. Calcd for $C_{11}H_{17}NO_4S$: C, 50.95; H, 6.61; N, 5.40; S, 12.36%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=8.30 (1H, s), 5.78 (1H, s), 4.48 (2H, q, J=7 Hz), 3.77 (4H, q, J=7 Hz), 1.40 (3H, t, J=7 Hz), and 1.25 (6H, t, J=7 Hz).

Ethyl 2-Formyl-4-thiazolecarboxylate (25). To a solution of ethyl 2-(diethoxymethyl)-4-thiazolecarboxylate (**24**) (1.59 g, 6.13 mmol) in 125 ml of acetone, 1 M hydrochloric acid (12.5 ml) was added and heated under reflux for 45 min. The reaction mixture was concentrated *in vacuo*, and the residual oil was extracted with ethyl acetate and washed with saturated aqueous sodium hydrogencarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The residue was recrystallized from ether and hexane; yield 1.13 g (100%). Mp 67—68 °C. IR (Nujol) 1720 and 1695 cm^{-1}. Found: C, 45.54; H, 3.75; N, 7.55; S, 17.25%. Calcd for $C_7H_7NO_3S$: C, 45.39; H, 3.81; N, 7.56; S, 17.31%. 60 MHz ^{1}H-NMR ($CDCl_3$) δ=10.13 (1H, d, J=1 Hz), 8.63 (1H, d, J=1 Hz), 4.53 (2H, q, J=7 Hz), and 1.43 (3H, t, J=7 Hz).

Ethyl (E)- and (Z)-2-(Hydroxyiminomethyl)-4-thiazolecarboxylate (26, 27). To a solution of ethyl 2-formyl-4-thiazolecarboxylate (**25**) (8.14 g, 44.0 mmol) in 70 ml of dry ethanol, pyridine (70.0 ml, 0.871 mol) and hydroxylamine hydrochloride (13.0 g, 0.187 mol) were added and heated under reflux for 1 h. The reaction mixture was concentrated *in vacuo*, and water (100 ml) was added to the residual oil. The precipitate was recrystallized from ethyl acetate and hexane; yield 8.04 g (91.4%). This solid is a mixture of (*E*)- and (*Z*)-aldoxime in ratio of about 1:1. Fractional recrystallization of the mixture from either ethyl acetate–hexane or methanol–ether gave colorless needles (**26**) and colorless powder (**27**). (*E*)-isomer **26**: Mp 183—184 °C. Found: C, 42.08; H, 4.01; N, 13.85; S, 15.91%. Calcd for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; H, 14.00; S, 16.01%. 100 MHz ^{1}H-NMR (DMSO-d_6): δ=12.24 (1H, s), 8.50 (1H, d, J=1 Hz), 8.40 (1H, d, J=1 Hz), 4.38 (2H, q, J=7 Hz), 1.36 (3H, t, J=7 Hz); (*Z*)-isomer **27**: mp 163—164 °C. Found: C, 42.09; H, 4.01; N, 13.87; S, 16.04%. Calcd for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 14.00; S, 16.01%. 100 MHz ^{1}H-NMR (DMSO-d_6) δ=13.12 (1H, br. s), 8.67 (1H, d, J=1 Hz), 8.10 (1H, d, J=1 Hz), 4.39 (2H, q, J=7 Hz), 1.36 (3H, t, J=7 Hz).

(E)-2-(Hydroxyiminomethyl)-4-thiazolecarbohydrazide (28). To a solution of ethyl (*E*)-2-(hydroxyiminomethyl)-4-thiazolecarboxylate (**26**) (4.50 g, 22.5 mmol) in 40 ml of *N,N*-dimethylformamide, 100% hydrazine hydrate (24.0 ml, 0.494 mol) was added at 0 °C and allowed to react for 18 h at 5 °C. Colorless needles precipitated was collected and washed with a small amount of methanol; yield 3.50 g (89.7%). Mp>200 °C. Found: C, 32.30; H, 3.30; N, 29.70; S, 17.35%. Calcd for $C_5H_6N_4O_2S$: C, 32.25; H, 3.25; N, 30.09; S, 17.22%. 60 MHz ^{1}H-NMR (DMSO-d_6) δ=8.40 (1H, s), 8.32 (1H, s).

(E)-2-(Hydroxyiminomethyl)thiazole-4-carbonyl Azide (29). To a suspension of (*E*)-2-(hydroxyiminomethyl)-4-thiazolecarbohydrazide (**28**) (500 mg, 2.69 mmol) in 3 ml of *N,N*-dimethylformamide, *t*-butyl nitrite (350 μl, 2.96 mmol) and 4.67 M hydrogen chloride in tetrahydrofuran (1.27 ml, 5.92 mmol) were added at −50 °C. The reaction mixture was stirred for 3 h at −30 °C, extracted with ethyl acetate and washed with water. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was recrystallized from acetone and hexane; yield 330 mg (62.3%). Mp 165—166 °C (dec). IR (Nujol) 2160 cm^{-1}. Found: C, 30.69; H, 1.64; N, 35.04; S, 16.58%. Calcd for $C_5H_3N_5O_2S$: C, 30.46; H, 1.53; N, 35.52; S, 16.26%. 60 MHz ^{1}H-NMR ($CDCl_3$–CD_3OD 1:1) δ=8.33 (1H, s), 8.32 (1H, s).

Althiomycin (1). To a solution of **19** (79.0 mg, 0.189 mmol) in 204 μl of anisole, anhydrous hydrogen fluoride (about 1 ml) was introduced at −78 °C within 10 min and stirred for a period of 20 min at 0 °C. Hydrogen fluoride was evaporated off *in vacuo* at 0 °C for 20 min. To a solution of the product in 1 ml of *N,N*-dimethylformamide, anhydrous potassium carbonate (781 mg, 5.66 mmol) was added at −78 °C. The reaction mixture was vigorously stirred for 5 min at room temperature under reduced pressure. Under cooling with Dry Ice–methanol bath, (*E*)-2-(hydroxyiminomethyl)-4-thiazolecarbonyl azide (**29**) (37.1 mg, 0.189 mmol) was added to the reaction mixture, which was stirred for 30 min at 0 °C and then for 2.5 h at room temperature. Ethyl acetate was added to this solution and washed with 0.1 M potassium phosphate buffer (pH 7.15) and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Purification by silica-gel preparative TLC using a developing solvent chloroform–methanol (9:1) gave colorless powder; yield 14.8 mg (17.9%). Mp 175—176 °C (dec). $[\alpha]_D^{27}$ +22.3° (*c* 0.065, Methyl Cellosolve). UV_{max} (CH_3OH) 223 nm (ε 34000), 240 nm (ε 25800), 286 nm (ε 8460). Found: C, 43.78; H, 4.38; N, 13.97; S, 12.95%. Calcd for $C_{16}H_{17}N_5O_6S_2 \cdot 0.5\ H_2O \cdot 0.5\ C_4H_8O_2$: C, 43.89; H, 4.50; N, 14.22; S, 13.02%. 90 MHz ^{1}H-NMR (DMSO-d_6) δ=8.40 (1H, br.s), 8.37 (1H, s), 8.35 (1H, s), 6.16 (1H, dt, J=3 Hz, 9 Hz), 5.43 (1H, s), 5.10 (1H, m), 4.90 (1H, m), 4.33 (2H, s), 3.89 (3H, s), 3.83 (2H, m), and 3.50 (2H, t).

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