

Optically Active Antifungal Azoles. II.¹⁾Synthesis and Antifungal Activity of Polysulfide Derivatives of (2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-mercapto-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol

Akihiro TASAKA,* Norikazu TAMURA, Yoshihiro MATSUSHITA, Ryogo HAYASHI, Kenji OKONOGI and Katsumi ITOH

Pharmaceutical Research Laboratories III, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan. Received August 21, 1992

In an effort to find potent antifungal agents, a variety of optically active triazole derivatives with a polysulfide structure, **3**, **4** and **5**, were prepared and evaluated for antifungal activity against *Candida albicans* *in vitro* and *in vivo*. The symmetrical polysulfides **3** ($m=2-4$) were obtained by an oxidative coupling reaction of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-mercapto-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (**1**) or by the treatment of its thiocarbonate derivative **8** with potassium *tert*-butoxide. The unsymmetrical disulfides **5** were synthesized by the reaction of the thiol **1** with Bunte salts **11** or the thiosulfinate **12** or by the reaction of the thiocarbonate **8** with various thiols **13**. All of these polysulfides showed potent antifungal activity against candidosis in mice.

Keywords polysulfide; optically active antifungal azole; triazolylmercaptobutanol; antifungal activity; cytochrome P450_{14DM} inhibitor; structure-activity relationship

In the previous paper,¹⁾ we described the chiral synthesis of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-mercapto-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (**1**), which is a key intermediate in the synthesis of optically active antifungal triazoles **2**. In the course of our research, the thiol **1** was found to have potent antifungal activity *in vitro* as well as *in vivo*, and the (2*R*,3*R*)-configuration of **1** was found to be essential for its potent *in vivo* activity. However, the thiol **1** is somewhat susceptible to air-oxidation, and its water-solubility is not high enough for formulation as an injectable product.

We have continued chemical modification studies on the thiol **1** in order to increase the antifungal activity as well as to improve the physicochemical properties, stability and water-solubility.

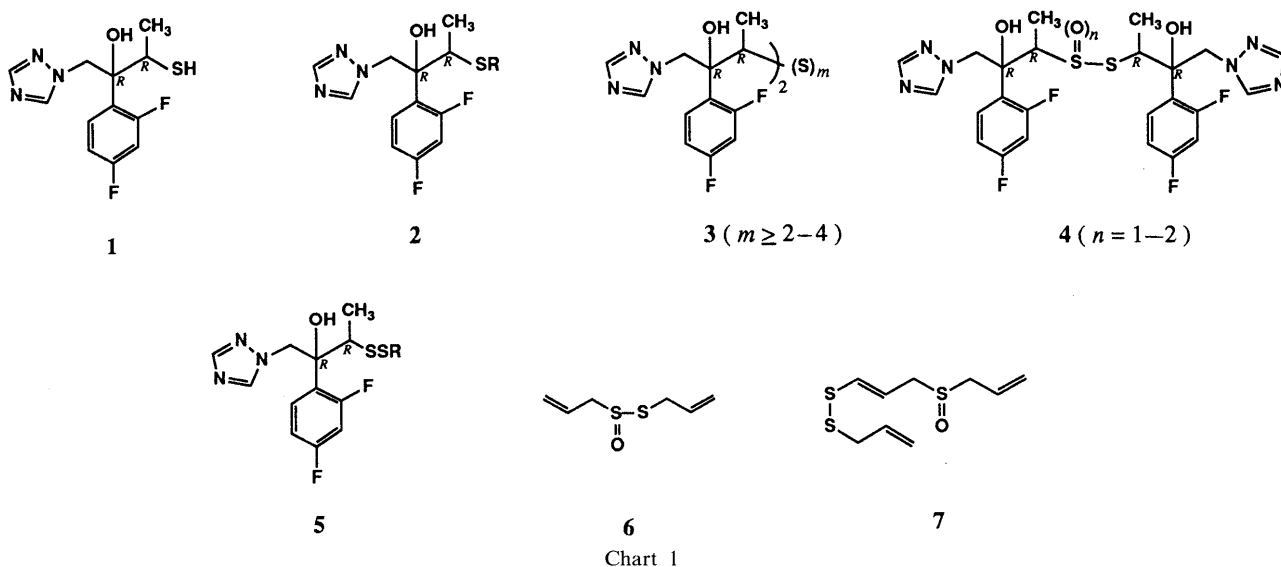
The extract of *Allium sativum* has been reported to show potent antifungal activity, and the active components were identified as polysulfides such as allicin (**6**), ajoene (**7**), diallyl trisulfide, *etc.*²⁾ This prompted us to incorporate a polysulfide structure into the triazole antifungals, and

we designed the symmetrical polysulfide **3** ($m \geq 2$), its oxygenated derivatives **4** and the unsymmetrical disulfides **5** as possible target compounds. It was thought that disulfides, **3** ($m=2$) and **5**, would not be susceptible to air-oxidation and that introduction of hydrophilic functional groups into the substituent R in **5** would increase the water-solubility. Since disulfides have been reported to regenerate the parent thiols by the action of a redox system *in vivo*,³⁾ the polysulfides **3-5** described above were considered to be precursors which could generate the thiol **1** *in vivo*.

In this paper we describe the synthesis of various polysulfides **3**, **4** and **5** (Charts 2 and 3, Table II) and their antifungal activity against *C. albicans* TA *in vitro* and *in vivo* (Table I).

Synthesis

First, synthesis of the symmetrical polysulfides **3a-c** and the oxygenated derivatives **4a-b** was investigated (Chart 2). The thiol **1** was treated with 1 eq of iodine in



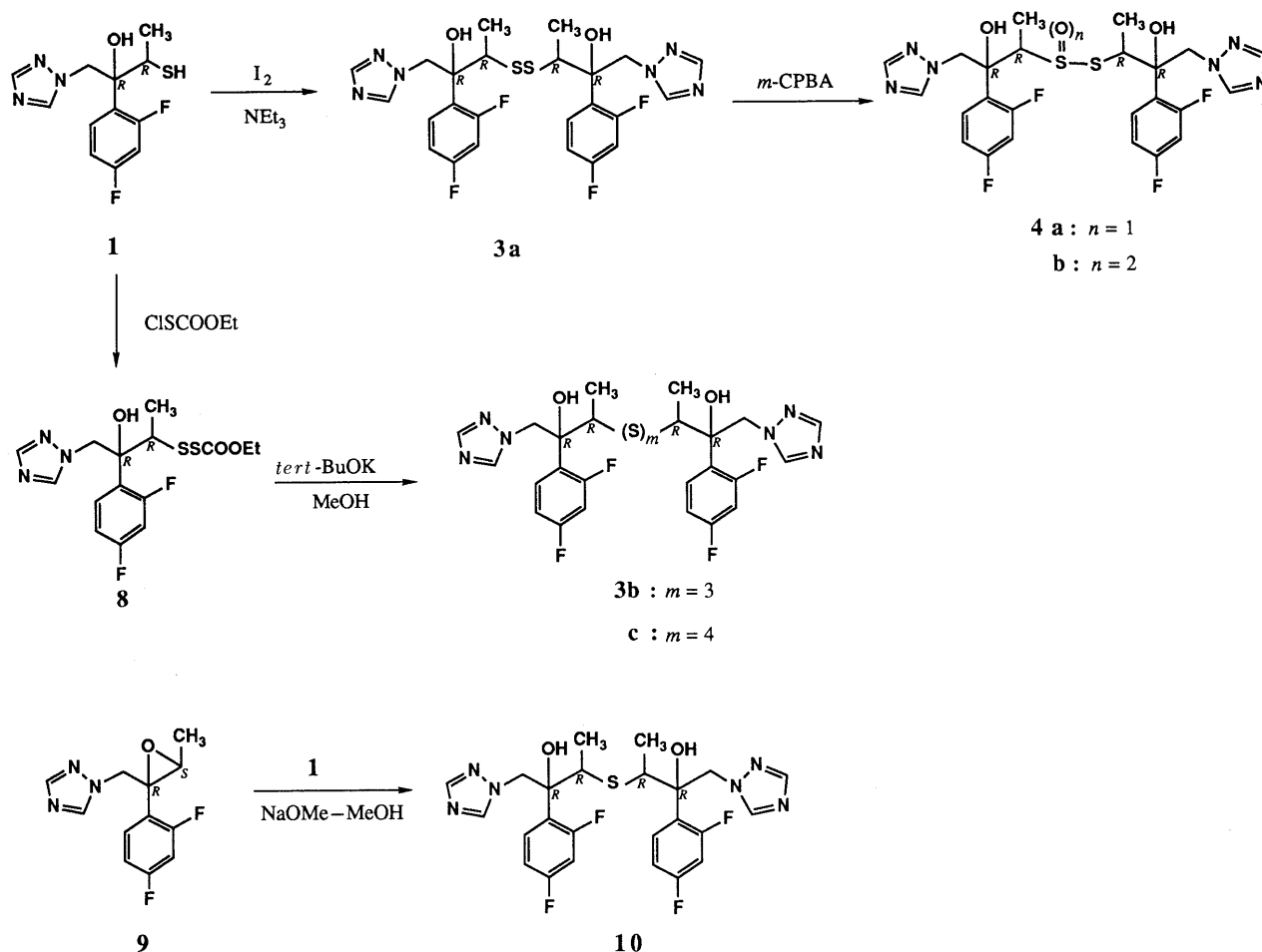


Chart 2

dichloromethane (CH_2Cl_2) to give **3a** in a 57% yield. The yield was increased to 88% by the addition of triethylamine to neutralize the hydrogen iodide formed in the course of the reaction. The oxidation of **3a** with 1 eq of *m*-chloroperbenzoic acid (*m*-CPBA) gave the mono-oxygenated product **4a** in a 37% yield. The dioxygenated compound **4b** was prepared in a 24% yield by the treatment of **3a** with 2 eq of *m*-CPBA. Both **4a** and **4b** were purified by flash chromatography on silica gel. The sulfonylthio structure of **4b** was confirmed by the chemical shifts of the α -protons [$-\text{CH}(\text{CH}_3)-$] in the nuclear magnetic resonance spectrum ($^1\text{H-NMR}$). The α -protons of **4b** were observed at δ 4.09 (1H) and 4.30 (1H), which were assigned to $-\text{CH}(\text{CH}_3)-\text{S}-$ and $-\text{CH}(\text{CH}_3)-\text{SO}_2-$, respectively.

Harpp and Granata reported an excellent method for the preparation of polysulfides, *i.e.* the self-coupling reaction of sulfenylthiocarbonate derivatives in the presence of a base.⁴⁾ Thus, the thiol **1** was converted to ethyl (1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyldithiocarbonate (**8**) in a quantitative yield upon reaction with ethoxycarbonylsulfenyl chloride at 0°C in methanol (MeOH). Compound **8** was treated with 1 eq of potassium *tert*-butoxide to form a mixture of polysulfides **3**. Purification and isolation were carried out by reverse phase column chromatography using CPO-223L[®](ODS) to give the trisulfide **3b** and the tetrasulfide **3c** in 52% and 4.5% yields, respectively.

Analysis of the reaction mixture by high performance liquid chromatography (HPLC) showed the coexistence of higher polysulfides **3** ($n \geq 5$), which had long retention times compared with those of **3b** and **3c**. Attempted isolation of these higher polysulfides was unsuccessful because of their instability.

The evaluation of *in vivo* antifungal activity revealed that the symmetrical polysulfides **3a-c** were highly active against candidosis. These compounds seemed to be converted to the thiol **1** to a certain extent *in vivo*. Therefore, the activity of **3** might be due to the formation of **1** *in vivo*. Thus, we were interested in the activity of the sulfide **10**, which has a structure similar to that of **3** but would not be converted to **1** *in vivo*. The ring-opening reaction of the oxirane **9**¹⁾ with the thiol **1** in the presence of sodium methoxide (NaOMe) under reflux in MeOH gave the sulfide **10** in a 37% yield (Chart 2).

Next, the synthesis of the unsymmetrical disulfides was undertaken according to the methods shown in Chart 3. In the case of the synthesis of the disulfides **5a-c**, the thiol **1** was used as the starting material and allowed to react with sodium thiosulfate derivatives (**11**), which are called Bunte salts [method A]. The thiosulfinate derivative **12** was also a good reagent to react with **1** and produced **5d** in a 43% yield [method B]. Purification of these disulfides was carried out by silica gel chromatography.

For the synthesis of the disulfides **5e-l** which contain various functional groups in the substituent R, an alter-

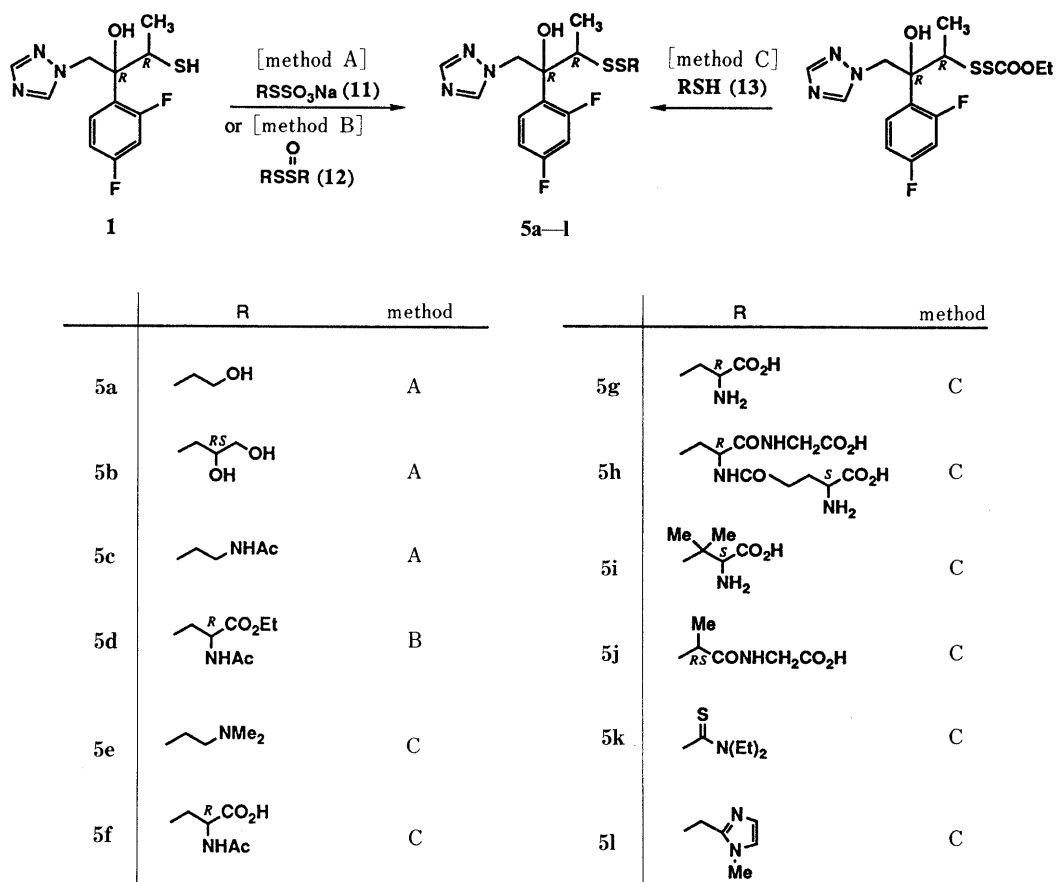


Chart 3

native method was investigated. Brois *et al.* reported that the reaction of sulfenylthiocarbonates with thiols gave unsymmetrical disulfides in good yields.⁵⁾ We applied this method for the synthesis of **5e–l** [method C]. The reaction of **8** with various thiols **13** in MeOH, aqueous MeOH or aqueous ethanol (EtOH) in the presence of a trace amount of triethylamine gave the desired unsymmetrical disulfides. Reverse phase column chromatography using ODS, highly porous polymer resin (CHP-20) or silica gel chromatography gave compounds **5e–l** in 39–67% yields. Among these compounds, the glutathione-conjugate **5h** was found to have a remarkably high water-solubility (>10%) as well as a high stability.⁶⁾

Antifungal Activity The polysulfides (**3–5** and **8**) and a related compound (**10**) were evaluated for antifungal activities against *C. albicans* TA *in vitro* and *in vivo*, and the results are shown in Table I. The *in vitro* assay was carried out by a paper disc method¹⁾ and an agar-dilution method⁷⁾ on yeast nitrogen base (YNB) and polypeptone-yeast extract-glucose (PYG) media at pH 7.0. The activities are expressed as the diameter of the growth inhibition zone (mm) around the paper disc soaked in a solution of 1 mg/ml of test compound and as minimum inhibitory concentration (MIC, $\mu\text{g/ml}$). All of the compounds prepared inhibited the growth of *C. albicans* TA in the paper-disc assay; however, their MIC values were higher than that of the thiol **1**, except in the cases of **3c**, **5k** and **8**. It is noteworthy that the compounds (**5a–j**) which contain a hydrophilic moiety showed weak activity in MIC tests. The chemical modifications of the thiol **1**

TABLE I. Antifungal Activity of Polysulfides (**3–5** and **8**) and a Related Compound (**10**) against *C. albicans* TA

Compound	<i>In vivo</i> (in mice) ED ₅₀ (mg/kg) <i>p.o.</i>	<i>In vitro</i>		
		Disc (1 mg/ml) Diameter (mm) YNB	MIC ($\mu\text{g/ml}$) YNB	PYG
1	0.19	37	12.5	50
3a	0.18	20	50	25
3b	0.35	25	25	>100
3c	0.50	20	12.5	25
4a	0.50	28	50	50
4b	0.50	23	50	50
5a	0.35	40	100	50
5b	0.50	25	>100	100
5c	0.35	30	100	50
5d	0.50	20	100	100
5e	0.50	25	100	100
5f	0.22	20	100	100
5g	0.18	20	100	100
5h	0.50	20	100	100
5i	0.50	17	100	100
5j	0.09	20	>100	>100
5k	0.50	20	12.5	25
5l	<0.25	37	50	100
8	0.50	20	3.1	25
10	2.0	20	>100	50
Fluconazole	0.29–0.35	18	>100	100

described above did not provide a remarkable improvement of *in vitro* antifungal activity.

C. albicans TA-infected mice were used for the *in vivo*

assay,¹⁾ and the activity is expressed in terms of ED₅₀ (mg/kg, the dose of the compound which allowed 50% of infected mice to survive after oral administration). All of the polysulfides prepared showed potent *in vivo* activity (ED₅₀, 0.1–0.5 mg/kg). Among them, the symmetrical disulfide **3a** and the conjugates with *N*-acetyl cysteine (**5f**), cysteine (**5g**), 2-mercaptopropionylglycine (**5j**) and 2-mercapto-1-methylimidazole (**5l**) were found to be as potent as the thiol **1**. Furthermore, the glutathione conjugate (**5h**), which has high water-solubility, had potent activity comparable to that of fluconazole. On the other hand, the symmetrical sulfide **10** had only moderate activity (ED₅₀, 2 mg/kg) and was about ten times less potent than the symmetrical disulfide **3a**.

These results suggest that the *in vivo* activity of the polysulfides, **3**–**5**, might be mainly due to the thiol **1** which is regenerated *in vivo*. The slight differences in the *in vivo* activity might reflect their bioavailability, pharmacokinetics and/or rate of regeneration of the thiol **1**.

In conclusion, the conversion of the thiol **1** to the polysulfide provided compounds with desirable physico-chemical properties, *i.e.*, stability and water-solubility, without a decrease in *in vivo* antifungal activity. Further biological evaluation of these compounds is in progress.

Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a Hitachi 215 spectrometer. ¹H-NMR spectra were taken on a Varian Gemini-200 (200 MHz) spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. The optical rotations were recorded with a JASCO DIP-181 or DIP-370 digital polarimeter. The secondary ion mass spectra (SIMS) were obtained on a Hitachi M-80A mass spectrometer.

Reactions were followed by thin-layer chromatography (TLC) on TLC plates, Silica gel 60 F₂₅₄ precoated (E. Merck), or by HPLC using ODS column (A303; Yamamura Chemical Laboratories Co.). Chromatographic separations were carried out on Silica gel 60 (0.063–0.200 mm, E. Merck), ODS (CPO-223L[®], pre-packed column, 22 mm × 300 mm, Kusano Kagakukikai Co.) or highly porous polymer resin (CHP-20, 0.15–0.5 mm, Mitsubishi Chemical Industries).

Bis[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl] Disulfide (3a**, Table II)** A solution of iodine (0.93 g, 3.68 mmol) in CH₂Cl₂ (120 ml) was added dropwise to a mixture of **1**¹⁾ (2.0 g, 7.0 mmol) and triethylamine (1.95 ml, 14 mmol) in CH₂Cl₂ (100 ml) over a period of 30 min with stirring at 0 °C. After being stirred for 30 min at 0 °C, the mixture was washed with aqueous Na₂SO₃, dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50 g, CH₂Cl₂–AcOEt–MeOH, 10:10:1, v/v). The combined eluents were concentrated *in vacuo*, and the residue was dissolved in EtOH (5 ml). The resulting solution was diluted with water (100 ml) at 0 °C. The precipitates were collected by filtration to give **3a** (1.74 g, 88%) as a colorless amorphous powder.

Ethyl (1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propylidithiocarbonate (8**, Table II)** The thiol **1** (7.0 g, 24.5 mmol) was added portionwise to a solution of ethoxycarbonylsulfenyl chloride⁸⁾ (4.14 g, 29.4 mmol) in EtOH (70 ml) over a period of 5 min with stirring at 0 °C. After being stirred for 20 min at room temperature, the mixture was concentrated *in vacuo*. The oily residue was dissolved in AcOEt (100 ml), and the resulting solution was washed with aqueous NaHCO₃, dried over MgSO₄ and evaporated *in vacuo* to give **8** (9.44 g, 99%) as a colorless oil. A part of this compound was converted to the hydrochloride by treatment with HCl (4 M solution in AcOEt), and this was crystallized from diethyl ether (Et₂O) to yield a colorless crystalline powder.

Bis[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl] Trisulfide (3b**, Table II) and Bis[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl] Tetrasulfide (**3c**, Table II)** A solution of potassium *tert*-butoxide (0.56 g, 5.0 mmol)

in MeOH (2 ml) was added to a solution of **8** (2.2 g, 5.6 mmol) in MeOH (20 ml) at –78 °C, and the mixture was then stirred at 0 °C for 5 min. At the end of the reaction, five products (**3a**–**3e** and two unidentified polysulfides) were detected by HPLC under the following conditions: A-303 column (ODS, 4.6 mm i.d. × 250 mm, Yamamura Chemical Laboratories Co.); mobile phase, MeOH–H₂O, 8:2, v/v; flow rate 0.8 ml/min; detection, UV at 254 nm. The retention times were 7.9, 11.3, 15.6, 23.6 and 35.7 min, respectively. Acetic acid (0.3 ml) was added to the mixture, and the solvent was evaporated off *in vacuo*. The residue was purified by reverse-phase chromatography using a pre-packed column (CPO-223L[®], 10–20 kg/cm²). Elution with MeOH–H₂O (8:2, v/v) followed by precipitation from EtOH–H₂O gave **3b** (0.88 g, 52%) as a white amorphous powder. The eluent containing a less polar compound was concentrated under reduced pressure to give **3c** (79 mg, 4.5%) as a white amorphous powder.

(2*R*,3*R*)-3-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propylthiosulfenyl]-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (4a**, Table II)** A solution of *m*-CPBA (85 mg, 0.49 mmol) in CH₂Cl₂ (1 ml) was added to a solution of **3a** (240 mg, 0.42 mmol) in CH₂Cl₂ (6 ml) at 0 °C. The resulting mixture was stirred for 20 min at 0 °C and concentrated to *ca.* 2 ml *in vacuo*. The residue was purified by flash chromatography on silica gel (20 g). Elution with CH₂Cl₂–AcOEt–MeOH (5:5:1, v/v) followed by precipitation from Et₂O gave **4a** (89 mg, 37%) as a white amorphous powder.

Compound **4a** consists of two diastereomers, and the ratio of isomers was determined to be 2:1 by HPLC analysis under the following conditions: A-303 column (ODS, 4.6 mm i.d. × 250 mm, Yamamura Chemical Laboratories Co.), mobile phase, MeOH–H₂O–AcOH, 7:3:0.02, v/v; flow rate 0.8 ml/min; detection, UV at 254 nm. Their retention times were 8.5 and 11.2 min.

(2*R*,3*R*)-3-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propylthiosulfenyl]-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (4b**, Table II)** *m*-CPBA (142 mg, 0.82 mmol) was added to a solution of **3a** (200 mg, 0.35 mmol) in CH₂Cl₂ (4 ml) at 0 °C. After being stirred for 2 h at room temperature, the mixture was washed with 5% aqueous NaHCO₃, dried over MgSO₄ and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (15 g). Elution with CH₂Cl₂–AcOEt–MeOH (5:5:1, v/v) gave **4b** (48 mg, 24%) as a white amorphous powder.

Bis[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl] Sulfide (10**, Table I)** A mixture of **1** (2.85 g, 10 mmol) and **9** (2.51 g, 10 mmol) and NaOMe (1 M solution in MeOH, 20 ml) was heated at 70 °C with stirring for 15 h. After being cooled, the mixture was partitioned between AcOEt (150 ml) and aqueous HCl (1 M solution, 20 ml). The organic layer was washed with water, dried over MgSO₄ and concentrated *in vacuo*. The residue was chromatographed on silica gel (50 g). Elution with hexane–AcOEt (1:1, v/v)→AcOEt–MeOH (10:1, v/v) followed by recrystallization from AcOEt–Et₂O gave **10** (1.98 g, 37%) as a colorless crystalline powder.

(2*R*,3*R*)-2-(2,4-difluorophenyl)-3-(2-hydroxyethylthio)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (5a**, Table II)** Method A: Aqueous NaOH solution (1 M, 1 ml) was added to a mixture of **1** (0.3 g, 1.05 mmol) and sodium *S*-(2-hydroxyethyl)thiosulfate⁹⁾ (**11a**, 0.75 g, 4.1 mmol) in MeOH (30 ml) with stirring at 0 °C. Stirring was continued for 30 min at 0 °C, and the mixture was diluted with water (20 ml), acidified (pH 4) with diluted aqueous HCl and extracted with CH₂Cl₂ (50 ml). The extract was washed with water, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by chromatography on silica gel. Elution with CH₂Cl₂–acetone (1:1, v/v) gave **5a** (0.24 g, 63%) as a colorless oil. This oil was treated with HCl (4 M solution in AcOEt) and the product was crystallized from AcOEt to give **5a**·HCl as a colorless crystalline powder.

The reaction of **1** with sodium *S*-(2,3-dihydroxypropyl)thiosulfate (**11b**) or *S*-(2-acetylminoethyl)thiosulfate (**11c**) was carried out in the same manner as described above to yield compounds **5b** and **5c**, which were treated with HCl (4 M solution in AcOEt) to give the corresponding hydrochlorides (Table II).

Bunte Salts (11a**–**c**)** Bunte salts were prepared by the reaction of sodium thiosulfate with the corresponding halides. A typical example is as follows. A solution of *N*-(2-chloroethyl)acetamide (10 g, 82.2 mmol) in EtOH (60 ml) was added to a solution of sodium thiosulfate (20.4 g, 82.2 mmol) in water (60 ml). The mixture was refluxed for 3 h and concentrated *in vacuo*. Et₂O (200 ml) was added to the residue to precipitate crude **11a** (14 g) as a colorless powder, which was used for the next step without purification.

***N*-Acetyl-*S*[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-**

TABLE II. Polysulfide Derivatives of (2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-mercapto-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol

Compd.	Yield (%)	mp (°C) (Solvent) ^{a)}	Formula	Analysis (%)			¹ H-NMR δ (in CDCl ₃)	IR $\nu_{\text{max}}^{\text{KBr}}$ (cm ⁻¹)	[α] _D (c, %) {°C} in MeOH
				Calcd	Found	N			
3a	88	AP ^{b)}	C ₂₄ H ₂₄ F ₄ N ₆ O ₂ S ₂	50.70 (50.55)	4.25 4.22	14.78 14.62)	1.24 (6H, d, <i>J</i> = 7 Hz), 3.60 (2H, q, <i>J</i> = 7 Hz), 5.02 (2H, d, <i>J</i> = 1.4 Hz), 5.05 (4H, s), 6.68–6.84 (4H, m), 7.30–7.42 (2H, m), 7.79 (2H, s), 7.83 (2H, s)	3400, 1610, 1500, 1420, 1270, 1138	–78.2 (1.0) {23}
3b	52	AP	C ₂₄ H ₂₄ F ₄ N ₆ O ₂ S ₃ · 1/2H ₂ O	47.28 (47.53)	4.13 4.18	13.78 13.65)	1.30 (6H, d, <i>J</i> = 7 Hz), 3.74 (2H, q, <i>J</i> = 7 Hz), 4.99 (6H, s), 6.71–6.85 (4H, m), 7.27–7.43 (2H, m), 7.78 (2H, s), 7.80 (2H, s)	3400, 1620, 1505, 1424, 1270, 1138	–303.5 (0.5) {25}
3c	4.5	AP	C ₂₄ H ₂₄ F ₄ N ₆ O ₂ S ₄ · H ₂ O	44.30 (44.56)	4.03 3.67	12.91 12.76)	1.31 (6H, d, <i>J</i> = 7 Hz), 3.82 (2H, q, <i>J</i> = 7 Hz), 5.02 (2H, s), 5.04 (2H, s), 5.14 (2H, d, <i>J</i> = 1.6 Hz), 6.65–6.84 (4H, m), 7.25–7.45 (2H, m), 7.81 (2H, s), 7.82 (2H, s)	3420, 1620, 1505, 1420, 1275, 1140	–278.8 (0.43) {23}
4a	37	AP	C ₂₄ H ₂₄ F ₄ N ₆ O ₃ S ₂	49.31 (49.07)	4.14 4.29	14.38 13.96)	1.17–1.45 (6H, m), 3.85–4.20 (2H, m), 4.80–5.65 (6H, m), 6.70–6.88 (4H, m), 7.30–7.52 (2H, m), 7.74 (1H, s), 7.80 (1H, s), 7.89 (1H, s), 7.93 (1H, s)	3420, 1615, 1500, 1420, 1270, 1135	–67.2 (0.34) {23}
4b	24	AP	C ₂₄ H ₂₄ F ₄ N ₆ O ₄ S ₂	47.99 (47.65)	4.03 3.89	13.99 13.97)	1.37 (3H, d, <i>J</i> = 7 Hz), 1.38 (3H, d, <i>J</i> = 7 Hz), 4.09 (1H, q, <i>J</i> = 7 Hz), 4.30 (1H, dq, <i>J</i> = 1.4, 7 Hz), 4.92–5.15 (3H, m), 5.35–5.50 (3H, m), 6.68–6.88 (4H, m), 7.23–7.45 (2H, m), 7.76 (1H, s), 7.80 (1H, s), 7.82 (1H, s), 7.83 (1H, s)	3400, 1615, 1500, 1420, 1310, 1270, 1130	–78.8 (0.42) {23}
5a · HCl	63	118–120 (EA)	C ₁₄ H ₁₇ F ₂ N ₃ O ₂ S ₂ · HCl	42.26 (42.08)	4.56 4.54	10.56 10.63)	1.14 (3H, d, <i>J</i> = 7 Hz), 2.80–2.92 (2H, m), 3.61–3.74 (3H, m), 4.82 (1H, d, <i>J</i> = 14 Hz), 5.00 (1H, d, <i>J</i> = 14 Hz), 6.84–6.93 (1H, m), 7.07–7.29 (2H, m), 7.80 (1H, s), 8.56 (1H, s)	3200, 1610, 1500, 1420, 1270, 1150	–62.4 (1.0) {23}
5b · HCl	23	95–97 (Et ₂ O)	C ₁₅ H ₁₉ F ₂ N ₃ O ₃ S ₂ · HCl · 1/2H ₂ O	41.23 (41.02)	4.84 4.61	9.62 9.81)	1.06–1.18 (3H, m), 2.71–2.87 (1H, m), 2.98–3.09 (1H, m), 3.33–3.48 (2H, m), 3.58–3.77 (2H, m), 4.77–5.02 (2H, m), 6.82–6.98 (1H, m), 7.08–7.29 (2H, m), 7.87 (1H, s), 8.64 (1H, s)	3200, 1610, 1500, 1270, 1140	–57.4 (1.0) {23}
5c · HCl	44	91–93 (Et ₂ O)	C ₁₆ H ₂₀ F ₂ N ₄ O ₂ S ₂ · HCl · 3/2H ₂ O	44.74 (44.34)	5.40 5.04	13.04 13.07)	1.12 (3H, d, <i>J</i> = 7 Hz), 1.83 (3H, s), 2.81–2.92 (2H, m), 3.33–3.45 (2H, m), 3.59 (1H, q, <i>J</i> = 7 Hz), 4.85 (1H, d, <i>J</i> = 14 Hz), 4.99 (1H, d, <i>J</i> = 14 Hz), 6.87–6.96 (1H, m), 7.11–7.29 (2H, m), 7.87 (1H, s), 7.92–8.14 (1H, m), 8.63 (1H, s)	3250, 1650, 1610, 1500, 1410, 1270	–55.9 (0.62) {23}
5d · HCl	43	90–92 (PE)	C ₁₉ H ₂₄ F ₂ N ₄ O ₄ S ₂ · HCl	44.66 (44.64)	4.93 4.95	10.96 10.73)	1.07–1.19 (6H, m), 1.90 (3H, s), 2.92–3.22 (2H, m), 3.58 (1H, q, <i>J</i> = 7 Hz), 4.12 (2H, q, <i>J</i> = 7 Hz), 4.59–4.70 (1H, m), 4.81 (1H, d, <i>J</i> = 14 Hz), 5.00 (1H, d, <i>J</i> = 14 Hz), 6.87–6.96 (1H, m), 7.09–7.29 (2H, m), 7.80 (1H, s), 8.51 (1H, s)	3250, 1740, 1680, 1610, 1500, 1420, 1370	–104.2 (1.0) {23}
5e · HCl	51	AP	C ₁₆ H ₂₂ F ₂ N ₄ OS ₂ · 2HCl · 2H ₂ O	38.63 (38.67)	5.67 5.56	11.26 11.48)	1.17 (3H, d, <i>J</i> = 7 Hz), 2.80 (6H, d, <i>J</i> = 4.8 Hz), 3.13–3.48 (4H, m), 3.62 (1H, q, <i>J</i> = 7 Hz), 4.91 (2H, s), 6.87–7.01 (1H, m), 7.10–7.32 (2H, m), 7.79 (1H, s), 8.49 (1H, s)	3400, 1620, 1505, 1420, 1275, 1135	–43.6 (0.55) {23}
5f	65	132–140 (Et ₂ O)	C ₁₇ H ₂₀ F ₂ N ₄ O ₄ S ₂ · 3/2H ₂ O	43.11 (43.33)	4.89 4.91	11.83 11.30)	1.08 (3H, d, <i>J</i> = 7 Hz), 1.89 (3H, s), 2.60–3.80 (3H, m), 4.26–4.40 (1H, m), 4.77 (1H, d, <i>J</i> = 14 Hz), 4.97 (1H, d, <i>J</i> = 14 Hz), 6.73–6.87 (1H, m), 7.05–7.35 (2H, m), 7.61 (1H, s), 8.44 (1H, s)	3400, 1650, 1615, 1500, 1420, 1270, 1138	–105.2 (0.53) {23}
5g	67	163–165 (dec.) (H ₂ O)	C ₁₅ H ₁₈ F ₂ N ₄ O ₃ S ₂ · 1/2H ₂ O	43.57 (43.58)	4.63 4.55	13.55 13.40)	1.12 (3H, d, <i>J</i> = 7 Hz), 3.20–3.25 (2H, m), 3.60 (1H, q, <i>J</i> = 7 Hz), 3.71 (1H, m), 4.79 (1H, d, <i>J</i> = 14 Hz), 4.96 (1H, d, <i>J</i> = 14 Hz), 6.82–6.96 (1H, m), 7.08–7.32 (2H, m), 7.63 (1H, s), 8.42 (1H, s)	3420, 1615, 1500, 1382, 1270, 1135	–239.4 (0.5) {25}
5h	57	AP	C ₂₂ H ₂₈ F ₂ N ₆ O ₇ S ₂ · H ₂ O	43.41 (43.57)	4.97 5.25	13.81 13.51)	1.13 (3H, d, <i>J</i> = 7 Hz), 1.90 (2H, m), 2.37 (2H, m), 2.85–3.40 (4H, m), 3.51 (1H, q, <i>J</i> = 7 Hz), 3.69 (1H, d, <i>J</i> = 5 Hz), 4.61 (1H, m), 4.78 (1H, d, <i>J</i> = 14 Hz), 4.95 (1H, d, <i>J</i> = 14 Hz), 6.89–6.96 (1H, m), 7.06–7.30 (2H, m), 7.65 (1H, s), 8.32 (1H, s)	3400, 1738, 1650, 1500, 1410, 1270, 1130	–113.1 (0.5) {25}
5i	39	167–169 (H ₂ O)	C ₁₇ H ₂₂ F ₂ N ₄ O ₃ S ₂ · H ₂ O	45.32 (45.40)	5.37 5.51	12.44 12.48)	1.08 (3H, d, <i>J</i> = 7 Hz), 1.35 (3H, s), 1.51 (3H, s), 3.35 (1H, s), 3.61 (1H, q, <i>J</i> = 7 Hz), 4.87 (1H, d, <i>J</i> = 14 Hz), 4.98 (1H, d, <i>J</i> = 14 Hz), 6.82–6.96 (1H, m), 7.10–7.28 (2H, m), 7.63 (1H, s), 8.34 (1H, s)	3380, 1610, 1485, 1380, 1270, 1130	+103.8 (0.5) {25}
5j	65	AP	C ₁₇ H ₂₀ F ₂ N ₄ O ₄ S ₂	45.70 (45.89)	4.51 4.79	12.59 12.21)	1.08 (3H, d, <i>J</i> = 6.6 Hz), 1.42 (3H, d, <i>J</i> = 6.8 Hz), 3.55–4.10 (5H, m), 4.80 (1H, d, <i>J</i> = 14 Hz), 4.96 (1H, d, <i>J</i> = 14 Hz), 6.80–6.95 (1H, m), 7.00–7.30 (2H, m), 7.65 (1H, s), 8.29 (1H, s)	3400, 1720, 1650, 1615, 1500, 1275, 1138	–105.2 (0.53) {23}
5k · HCl	58	108–109 (Et ₂ O)	C ₁₇ H ₂₂ F ₂ N ₄ OS ₃ · HCl	43.53 (43.39)	4.94 4.95	11.94 11.71)	1.12 (3H, d, <i>J</i> = 7 Hz), 1.17–1.33 (6H, m), 3.62–4.08 (5H, m), 4.95 (1H, d, <i>J</i> = 14 Hz), 5.35 (1H, d, <i>J</i> = 14 Hz), 6.83–6.97 (1H, m), 7.09–7.27 (2H, m), 7.94 (1H, s), 8.79 (1H, s)	3150, 1620, 1500, 1420, 1270	–284 (0.96) {25}
5l	42	112–113 (IPE)	C ₁₇ H ₁₉ F ₂ N ₅ OS ₂	49.62 (49.51)	4.65 4.74	17.02 17.08)	1.13 (3H, d, <i>J</i> = 6.8 Hz), 3.53 (1H, q, <i>J</i> = 6.8 Hz), 3.73 (3H, s), 3.84 (1H, d, <i>J</i> = 14 Hz), 4.18 (1H, d, <i>J</i> = 14 Hz), 4.77 (1H, d, <i>J</i> = 14.4 Hz), 4.99 (1H, d, <i>J</i> = 14.4 Hz), 6.68–6.82 (2H, m), 6.96 (1H, s), 7.03 (1H, s), 7.40–7.53 (2H, m), 7.70 (1H, s), 8.15 (1H, s)	3050, 1600, 1490, 1420, 1260	–15.7 (1.5) {25}
8 · HCl	99	106–112 (Et ₂ O)	C ₁₅ H ₁₇ F ₂ N ₃ O ₃ S ₂ · HCl	42.30 (42.21)	4.26 4.30	9.86 9.79)	1.14 (3H, d, <i>J</i> = 7 Hz), 1.28 (3H, t, <i>J</i> = 7 Hz), 3.65 (1H, dq, <i>J</i> = 1.4, 7 Hz), 4.36 (2H, q, <i>J</i> = 7 Hz), 4.82 (1H, d, <i>J</i> = 14.4 Hz), 5.11 (1H, d, <i>J</i> = 14.4 Hz), 6.86–7.02 (1H, m), 7.10–7.30 (2H, m), 7.85 (1H, s), 8.59 (1H, s)	3200, 1735, 1610, 1500, 1420, 1270, 1130	–171.9 (1.0) {23}
10	37	206–209 (EA–Et ₂ O)	C ₂₄ H ₂₉ F ₄ N ₆ O ₂ S	53.73 (53.68)	4.51 4.50	15.66 15.45)	1.25 (6H, d, <i>J</i> = 6.8 Hz), 3.49 (2H, q, <i>J</i> = 6.8 Hz), 4.93 (2H, d, <i>J</i> = 14 Hz), 5.15 (2H, s), 5.17 (2H, d, <i>J</i> = 14 Hz), 6.70–6.85 (4H, m), 7.27–7.50 (2H, m), 7.80 (2H, s), 7.85 (2H, s)	3380, 1610, 1590, 1495, 1415, 1375	–95.2 (1.0) {25}

a) Crystallization solvents: E, ethanol; Et₂O, diethyl ether; EA, ethyl acetate; M, methanol; PE, petroleum ether; IPE, diisopropyl ether. b) Amorphous powder.

1,2,4-triazol-1-yl)propylthio]cysteine Ethyl Ester (5d, Table II) Method B: A mixture of *N,N'*-diacetyl-L-cystine diethyl ester¹⁰⁾ (929 mg, 2.4 mmol) and hydrogen peroxide (30% solution in water, 0.41 ml, 3.6 mmol) in 50% aqueous acetic acid (12 ml) was allowed to stand for 22 h at room temperature. A solution of **1** (700 mg, 2.4 mmol) in EtOH (25 ml) was added to the mixture, and the pH was adjusted to 8 by addition of 20% aqueous NaOH at 0°C. The resulting mixture was stirred at room temperature for 90 min and concentrated *in vacuo*. The residue was extracted with CH₂Cl₂ (50 ml), and the extract was washed with water, dried over MgSO₄ and concentrated *in vacuo*. The residue was chromatographed on silica gel (500 g). Elution with AcOEt–MeOH (50:1, v/v) gave **5d** (0.45 g, 43%) as a colorless oil, which was treated with HCl (2 M solution in AcOEt) followed by crystallization from petroleum ether to give **5d**·HCl (0.37 g) as a colorless crystalline powder.

(2R,3R)-2-(2,4-Difluorophenyl)-3-(2-dimethylaminoethylthio)-1-(1H-1,2,4-triazol-1-yl)-2-butanol (5e, Table II) Method C: *N,N*-Dimethyl-2-mercaptoethylamine hydrochloride (0.13 g, 0.92 ml) was added to a solution of **8** (0.30 g, 0.77 mmol) in MeOH (4 ml) with stirring at room temperature. The mixture was stirred for 30 min at room temperature and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (30 g, AcOEt–MeOH, 2:1, v/v) followed by treatment with HCl (4 M solution in AcOEt) to give **5e**·HCl (0.19 g, 51%) as a white amorphous powder.

The reaction of **8** with *N*-acetylcysteine was carried out in a similar manner, and precipitation from Et₂O gave **5f** (Table II) as a colorless crystalline powder.

S-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propylthio]-L-cysteine (5g, Table II) A solution of L-cysteine hydrochloride monohydrate (1.58 g, 9.0 mmol) in water (5 ml) and triethylamine (1.25 ml, 9.0 mmol) were added successively to a solution of **8**·HCl (1.93 g, 4.5 mmol) in MeOH (15 ml) with stirring at 0°C. The mixture was stirred at room temperature for 1 h, diluted with water (20 ml) and then purified by chromatography using CHP-20 resin (120 ml). Elution with MeOH–H₂O (1:1→2:1) followed by crystallization of the product from water gave **5g** (1.22 g, 67%) as colorless needles.

S-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propylthio]glutathione (5h, Table II) A solution of glutathione (reduced form, 6.57 g, 16.45 mmol) in water (49 ml) and triethylamine (0.23 ml, 1.65 mmol) were added successively to a solution of **8** (6.4 g, 16.45 mmol) in MeOH (96 ml) with stirring at 0°C. The mixture was stirred at room temperature for 4 h, then aqueous HCl (1 M solution, 1.6 ml) was added. The mixture was concentrated to ca. 30 ml *in vacuo* and purified by chromatography using CHP-20 resin (400 g, MeOH–H₂O, 1:2→1:1, v/v). Concentration of fractions containing **5h** *in vacuo* followed by lyophilization gave **5h** (5.5 g, 57%) as a white amorphous powder.

S-[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propylthio]-D-penicillamine (5i, Table II) D-Penicillamine (0.14 g, 0.92 mmol) and triethylamine (9 mg, 0.08 mmol) were added successively to a solution of **8** (0.30 g, 0.77 mmol) in MeOH (6 ml) with stirring at 0°C. The mixture was stirred at room temperature for 1 h and concentrated *in vacuo*. The residue was purified by chromatography using a pre-packed column (CPO-223L®). Elution with MeOH–H₂O (2:1, v/v) followed by crystallization of the product from water gave **5i** (0.13 g, 39%) as colorless needles.

The reaction of **8** with sodium 2-mercaptopropionylglycine and the purification of the product were carried out in a similar manner to that described above. Precipitation from Et₂O gave compound **5j** (Table II) as a white amorphous powder.

[(1R,2R)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1H-1,2,4-triazol-1-yl)propyl] N,N-Diethylaminothiocarbonyl Disulfide (5k, Table II) Sodium diethyldithiocarbonate (1.35 g, 7.9 mmol) was added to a solution of **8**·HCl (0.64 g, 1.6 mmol) in aqueous EtOH (EtOH, 20 ml, H₂O, 15 ml). The resulting mixture was stirred at room temperature for 1 h, diluted with water (15 ml) and extracted with CH₂Cl₂ (50 ml, 20 ml). The extracts were combined, washed with water, dried over MgSO₄ and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (100 g, hexane–AcOEt, 3:1, v/v) followed by treatment with HCl (4 M solution in AcOEt) gave **5k**·HCl (0.41 g, 58%) as a colorless crystalline powder.

The reaction of **8**·HCl with 2-mercaptomethyl-1-methylimidazole¹¹⁾ (**13i**) was carried out in a similar manner to that described above, and purification of the product by silica gel chromatography followed by crystallization from diisopropyl ether gave **5l** (Table II) as colorless needles.

Antifungal Activity The methods of *in vitro* paper disc assay and *in vivo* assay were described in the previous paper.¹⁾ The MIC values were determined by the conventional method⁷⁾ on YNB and PYG media buffered to pH 7.0. The cultures were incubated at 28°C for 2 d.

Acknowledgments We thank Dr. S. Kishimoto and Dr. M. Suzuki for their encouragement throughout this work.

References and Notes

- 1) A. Tasaka, N. Tamura, Y. Matsushita, K. Teranishi, K. Okonogi and K. Itoh, *Chem. Pharm. Bull.*, **41**, 1035 (1993).
- 2) a) Y. Yamada and K. Azuma, *Antimicrobio. Agents Chemother.*, **11**, 743 (1977); N. Caporaso, S. M. Smith and R. H. K. Eng, *ibid.*, **23**, 700 (1983); L. E. Davis, J.-K. Shen and Y. Cai, *ibid.*, **34**, 651 (1990); b) E. Block, *J. Am. Chem. Soc.*, **106**, 8295 (1984); Y. Yoshida, S. Kasuga, N. Hayashi, T. Ushiroguchi, H. Matsuura and S. Nakagawa, *Appl. Environ. Microbiol.*, **53**, 615 (1987).
- 3) A. Holmgren, *J. Biol. Chem.*, **254**, 9113 (1979).
- 4) D. N. Harpp and A. Granata, *Tetrahedron Lett.*, **35**, 3001 (1976).
- 5) S. T. Brois, J. F. Pilot and W. W. Barnum, *J. Am. Chem. Soc.*, **92**, 7629 (1970).
- 6) The thiol **1** was stable when it was stored in a crystalline state in a refrigerator, but was gradually oxidized to form the disulfide **3a** in solution. In contrast, the disulfide **3a** was remarkably stable even in solution. No decomposition was observed when an aqueous solution of **3a** was allowed to stand for 1 week at 40°C. The unsymmetrical disulfides, **5g** and **5h**, were also stable in a neutral medium.
- 7) C. Brass, J. Z. Shainhouse and D. A. Stevens, *Antimicrobio. Agents Chemother.*, **15**, 763 (1979).
- 8) L. Field and R. Ravichandran, *J. Org. Chem.*, **44**, 2624 (1979).
- 9) S. Yurugi, *Yakugaku Zasshi*, **74**, 1157 (1954).
- 10) B. Aleksiev and S. Stoev, *Pharmazie*, **24**, 305 (1969).
- 11) J. P. Street, K. I. Skoreg, R. S. Brown and R. G. Ball, *J. Am. Chem. Soc.*, **107**, 7669 (1985).