# 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

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**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.

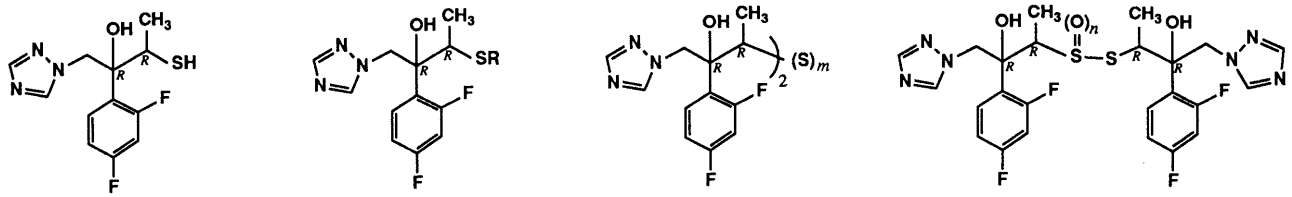
We have continued chemical modification studies on the thiol **1** in order to increase the antifungal activity as well as to improve the physicochemical properties, stability and water-solubility.

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*.

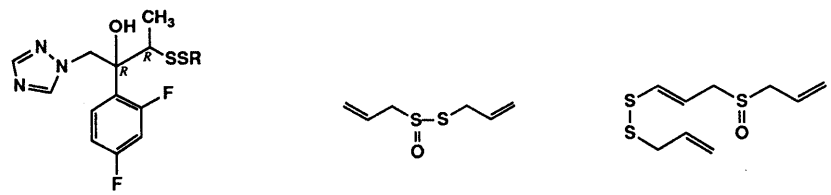
In this paper we describe the synthesis of various polysulfides **3**, **4** and **5** (Charts 2 and 3, Table II) and their antifungal activity against *C. albicans* TA *in vitro* and *in vivo* (Table I).

## 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



**1** **2** **3** ($m\geq 2$–4) **4** ($n=1$–2)



**5** **6** **7**

Chart 1

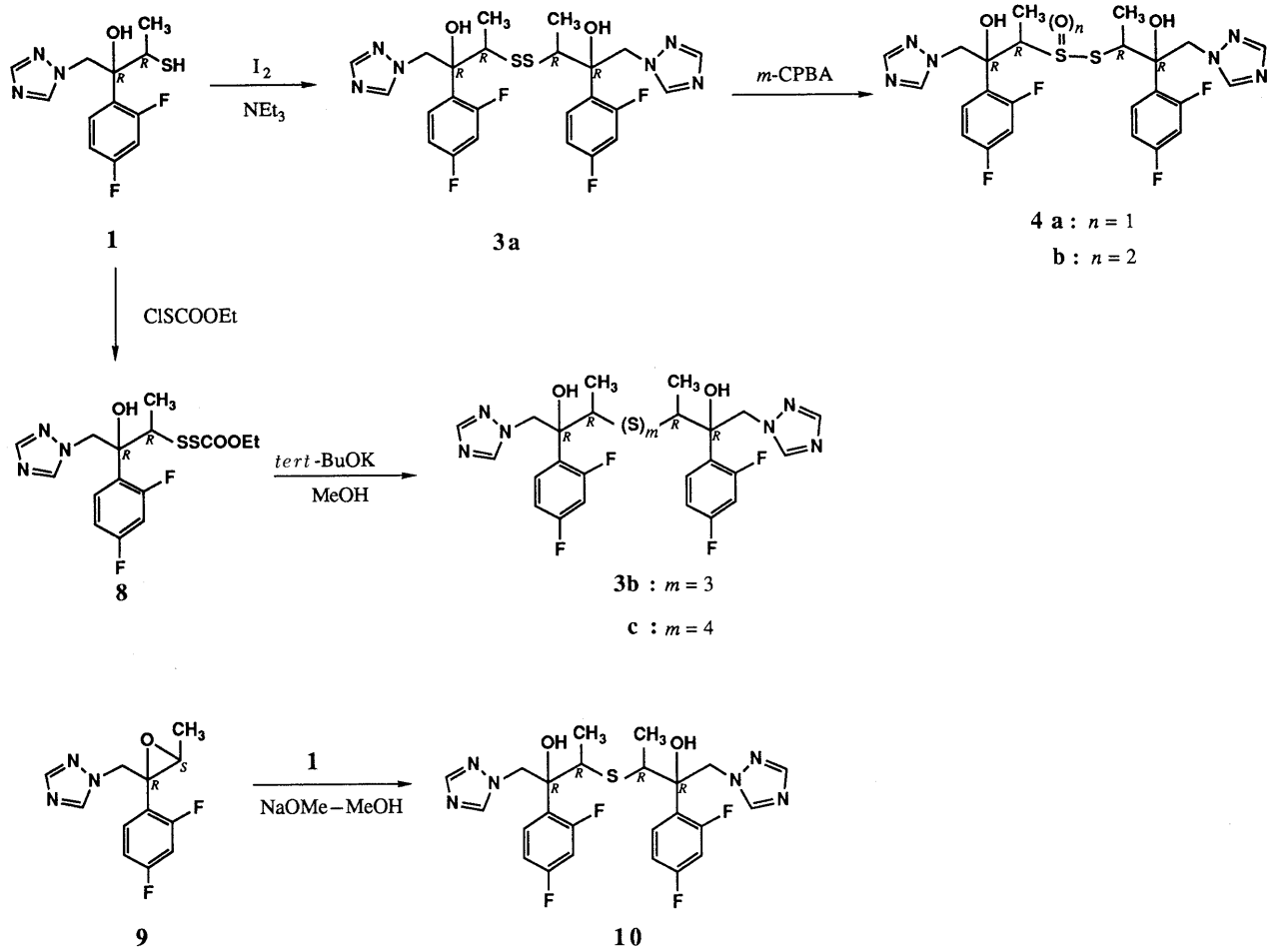



Chart 2

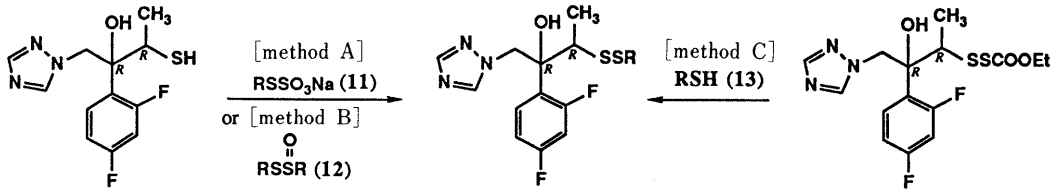
dichloromethane ($CH_2Cl_2$) to give **3a** in a 57% yield. The yield was increased to 88% by the addition of triethylamine to neutralize the hydrogen iodide formed in the course of the reaction. The oxidation of **3a** with 1 eq of *m*-chloroperbenzoic acid (*m*-CPBA) gave the monooxygenated product **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 [–CH(CH$_3$)–] in the nuclear magnetic resonance spectrum ($^1$H-NMR). The α-protons of **4b** were observed at $\delta$ 4.09 (1H) and 4.30 (1H), which were assigned to –CH(CH$_3$)–S– and –CH(CH$_3$)–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-



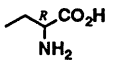
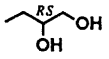
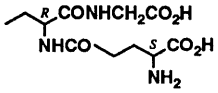
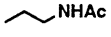
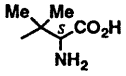
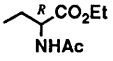
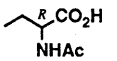

| | R | method | | R | method |
|---|---|---|---|---|---|
| 5a |  | A | 5g |  | C |
| 5b |  | A | 5h |  | C |
| 5c |  | A | 5i |  | C |
| 5d |  | B | 5j |  | C |
| 5e |  | C | 5k |  | C |
| 5f |  | C | 5l |  | C |

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 method[1] and an agar-dilution method[7] 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, μg/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** described above did not provide a remarkable improvement of *in vitro* antifungal activity.

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

| Compound | *In vivo* (in mice) $ED_{50}$ (mg/kg) *p.o.* | *In vitro* Disc (1 mg/ml) Diameter (mm) YNB | *In vitro* MIC (μg/ml) YNB | *In vitro* MIC (μg/ml) 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 |

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

assay,[1] and the activity is expressed in terms of $ED_{50}$ (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 ($ED_{50}$, 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 ($ED_{50}$, 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 physicochemical 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. $^1$H-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 $F_{254}$ 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 $CH_2Cl_2$ (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 $CH_2Cl_2$ (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 $Na_2SO_3$, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50 g, $CH_2Cl_2$–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)propyldithiocarbonate (8, Table II)** The thiol **1** (7.0 g, 24.5 mmol) was added portionwise to a solution of ethoxycarbonylsulfenyl chloride[8] (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 $NaHCO_3$, dried over $MgSO_4$ 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 ($Et_2O$) 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**—**3c** 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–$H_2O$, 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/cm$^2$). Elution with MeOH–$H_2O$ (8 : 2, v/v) followed by precipitation from EtOH–$H_2O$ 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)propylthiosulfinyl]-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 $CH_2Cl_2$ (1 ml) was added to a solution of **3a** (240 mg, 0.42 mmol) in $CH_2Cl_2$ (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 $CH_2Cl_2$–AcOEt–MeOH (5 : 5 : 1, v/v) followed by precipitation from $Et_2O$ 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–$H_2O$–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)propylthiosulfonyl]-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 $CH_2Cl_2$ (4 ml) at 0 °C. After being stirred for 2 h at room temperature, the mixture was washed with 5% aqueous $NaHCO_3$, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (15 g). Elution with $CH_2Cl_2$–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 $MgSO_4$ 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–$Et_2O$ gave **10** (1.98 g, 37%) as a colorless crystalline powder.

**(2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-(2-hydroxyethyldithio)-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)thiosulfate[9] (**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 $CH_2Cl_2$ (50 ml). The extract was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by chromatography on silica gel. Elution with $CH_2Cl_2$–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-acetylaminoethyl)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*. $Et_2O$ (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 (%) Calcd (Found) C | H | N | $^1$H-NMR $\delta$ (in $CDCl_3$) | IR $\nu_{max}^{KBr}$ ($cm^{-1}$) | $[\alpha]_D$ ($c$, %) {°C} in MeOH |
|---|---|---|---|---|---|---|---|---|---|
| **3a** | 88 | AP[b] | $C_{24}H_{24}F_4N_6O_2S_2$ | 50.70 (50.55) | 4.25 (4.22) | 14.78 (14.62) | 1.24 (6H, d, $J$=7 Hz), 3.60 (2H, q, $J$=7 Hz), 5.02 (2H, d, $J$=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 | $C_{24}H_{24}F_4N_6O_2S_3 \cdot 1/2H_2O$ | 47.28 (47.53) | 4.13 (4.18) | 13.78 (13.65) | 1.30 (6H, d, $J$=7 Hz), 3.74 (2H, q, $J$=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) SIMS ($m/z$): 601 ($MH^+$) | 3400, 1620, 1505, 1424, 1270, 1138 | −303.5 (0.5) {25} |
| **3c** | 4.5 | AP | $C_{24}H_{24}F_4N_6O_2S_4 \cdot H_2O$ | 44.30 (44.56) | 4.03 (3.67) | 12.91 (12.76) | 1.31 (6H, d, $J$=7 Hz), 3.82 (2H, q, $J$=7 Hz), 5.02 (2H, s), 5.04 (2H, s), 5.14 (2H, d, $J$=1.6 Hz), 6.65—6.84 (4H, m), 7.25—7.45 (2H, m), 7.81 (2H, s), 7.82 (2H, s) SIMS ($m/z$): 633 ($MH^+$) | 3420, 1620, 1505, 1420, 1275, 1140 | −278.8 (0.43) {23} |
| **4a** | 37 | AP | $C_{24}H_{24}F_4N_6O_3S_2$ | 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) SIMS ($m/z$): 585 ($MH^+$) | 3420, 1615, 1500, 1420, 1270, 1135 | −67.2 (0.34) {23} |
| **4b** | 24 | AP | $C_{24}H_{24}F_4N_6O_4S_2$ | 47.99 (47.65) | 4.03 (3.89) | 13.99 (13.97) | 1.37 (3H, d, $J$=7 Hz), 1.38 (3H, d, $J$=7 Hz), 4.09 (1H, q, $J$=7 Hz), 4.30 (1H, dq, $J$=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) SIMS ($m/z$): 601 ($MH^+$) | 3400, 1615, 1500, 1420, 1310, 1270, 1130 | −78.8 (0.42) {23} |
| **5a**·HCl | 63 | 118—120 (EA) | $C_{14}H_{17}F_2N_3O_2S_2 \cdot HCl$ | 42.26 (42.08) | 4.56 (4.54) | 10.56 (10.63) | 1.14 (3H, d, $J$=7 Hz), 2.80—2.92 (2H, m), 3.61—3.74 (3H, m), 4.82 (1H, d, $J$=14 Hz), 5.00 (1H, d, $J$=14 Hz), 6.84—6.93 (1H, m), 7.07—7.29 (2H, m), 7.80 (1H, s), 8.56 (1H, s) (in DMSO-$d_6$) | 3200, 1610, 1500, 1420, 1270, 1150 | −62.4 (1.0) {23} |
| **5b**·HCl | 23 | 95—97 ($Et_2O$) | $C_{15}H_{19}F_2N_3O_3S_2 \cdot HCl \cdot 1/2H_2O$ | 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) (in DMSO-$d_6$) | 3200, 1610, 1500, 1270, 1140 | −57.4 (1.0) {23} |
| **5c**·HCl | 44 | 91—93 ($Et_2O$) | $C_{16}H_{20}F_2N_4O_2S_2 \cdot HCl \cdot 3/2H_2O$ | 44.74 (44.34) | 5.40 (5.04) | 13.04 (13.07) | 1.12 (3H, d, $J$=7 Hz), 1.83 (3H, s), 2.81—2.92 (2H, m), 3.33—3.45 (2H, m), 3.59 (1H, q, $J$=7 Hz), 4.85 (1H, d, $J$=14 Hz), 4.99 (1H, d, $J$=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) (in DMSO-$d_6$) SIMS ($m/z$): 403 ($MH^+$) | 3250, 1650, 1610, 1500, 1410, 1270 | −55.9 (0.62) {23} |
| **5d**·HCl | 43 | 90—92 (PE) | $C_{19}H_{24}F_2N_4O_4S_2 \cdot HCl$ | 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, $J$=7 Hz), 4.12 (2H, q, $J$=7 Hz), 4.59—4.70 (1H, m), 4.81 (1H, d, $J$=14 Hz), 5.00 (1H, d, $J$=14 Hz), 6.87—6.96 (1H, m), 7.09—7.29 (2H, m), 7.80 (1H, s), 8.51 (1H, s) (in DMSO-$d_6$) SIMS ($m/z$): 475 ($MH^+$) | 3250, 1740, 1680, 1610, 1500, 1420, 1370 | −104.2 (1.0) {23} |
| **5e**·HCl | 51 | AP | $C_{16}H_{22}F_2N_4OS_2 \cdot 2HCl \cdot 2H_2O$ | 38.63 (38.67) | 5.67 (5.56) | 11.26 (11.48) | 1.17 (3H, d, $J$=7 Hz), 2.80 (6H, d, $J$=4.8 Hz), 3.13—3.48 (4H, m), 3.62 (1H, q, $J$=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) (in DMSO-$d_6$) SIMS ($m/z$): 389 ($MH^+$) | 3400, 1620, 1505, 1420, 1275, 1135 | −43.6 (0.55) {23} |
| **5f** | 65 | 132—140 ($Et_2O$) | $C_{17}H_{20}F_2N_4O_4S_2 \cdot 3/2H_2O$ | 43.11 (43.33) | 4.89 (4.91) | 11.83 (11.30) | 1.08 (3H, d, $J$=7 Hz), 1.89 (3H, s), 2.60—3.80 (3H, m), 4.26—4.40 (1H, m), 4.77 (1H, d, $J$=14 Hz), 4.97 (1H, d, $J$=14 Hz), 6.73—6.87 (1H, m), 7.05—7.35 (2H, m), 7.61 (1H, s), 8.44 (1H, s) SIMS ($m/z$): 447 ($MH^+$) | 3400, 1650, 1615, 1500, 1420, 1270, 1138 | −105.2 (0.53) {23} |
| **5g** | 67 | 163—165 (dec.) ($H_2O$) | $C_{15}H_{18}F_2N_4O_3S_2 \cdot 1/2H_2O$ | 43.57 (43.58) | 4.63 (4.55) | 13.55 (13.40) | 1.12 (3H, d, $J$=7 Hz), 3.20—3.25 (2H, m), 3.60 (1H, q, $J$=7 Hz), 3.71 (1H, m), 4.79 (1H, d, $J$=14 Hz), 4.96 (1H, d, $J$=14 Hz), 6.82—6.96 (1H, m), 7.08—7.32 (2H, m), 7.63 (1H, s), 8.42 (1H, s) (in DMSO-$d_6$+$D_2O$) SIMS ($m/z$): 405 ($MH^+$) | 3420, 1615, 1500, 1382, 1270, 1135 | −239.4 (0.5) {25} |
| **5h** | 57 | AP | $C_{22}H_{28}F_2N_6O_7S_2 \cdot H_2O$ | 43.41 (43.57) | 4.97 (5.25) | 13.81 (13.51) | 1.13 (3H, d, $J$=7 Hz), 1.90 (2H, m), 2.37 (2H, m), 2.85—3.40 (4H, m), 3.51 (1H, q, $J$=7 Hz), 3.69 (1H, d, $J$=5 Hz), 4.61 (1H, m), 4.78 (1H, d, $J$=14 Hz), 4.95 (1H, d, $J$=14 Hz), 6.89—6.96 (1H, m), 7.06—7.30 (2H, m), 7.65 (1H, s), 8.32 (1H, s) (in DMSO-$d_6$+$D_2O$) SIMS ($m/z$): 591 ($MH^+$) | 3400, 1738, 1650, 1500, 1410, 1270, 1130 | −113.1 (0.5) {25} |
| **5i** | 39 | 167—169 ($H_2O$) | $C_{17}H_{22}F_2N_4O_3S_2 \cdot H_2O$ | 45.32 (45.40) | 5.37 (5.51) | 12.44 (12.48) | 1.08 (3H, d, $J$=7 Hz), 1.35 (3H, s), 1.51 (3H, s), 3.35 (1H, s), 3.61 (1H, q, $J$=7 Hz), 4.87 (1H, d, $J$=14 Hz), 4.98 (1H, d, $J$=14 Hz), 6.82—6.96 (1H, m), 7.10—7.28 (2H, m), 7.63 (1H, s), 8.34 (1H, s) (in DMSO-$d_6$) SIMS ($m/z$): 433 ($MH^+$) | 3380, 1610, 1485, 1380, 1270, 1130 | +103.8 (0.5) {25} |
| **5j** | 65 | AP | $C_{17}H_{20}F_2N_4O_4S_2$ | 45.70 (45.89) | 4.51 (4.79) | 12.59 (12.21) | 1.08 (3H, d, $J$=6.6 Hz), 1.42 (3H, d, $J$=6.8 Hz), 3.55—4.10 (5H, m), 4.80 (1H, d, $J$=14 Hz), 4.96 (1H, d, $J$=14 Hz), 6.80—6.95 (1H, m), 7.00—7.30 (2H, m), 7.65 (1H, s), 8.29 (1H, s) (in DMSO-$d_6$) | 3400, 1720, 1650, 1615, 1500, 1275, 1138 | −105.2 (0.53) {23} |
| **5k**·HCl | 58 | 108—109 ($Et_2O$) | $C_{17}H_{22}F_2N_4OS_3 \cdot HCl$ | 43.53 (43.39) | 4.94 (4.95) | 11.94 (11.71) | 1.12 (3H, d, $J$=7 Hz), 1.17—1.33 (6H, m), 3.62—4.08 (5H, m), 4.95 (1H, d, $J$=14 Hz), 5.35 (1H, d, $J$=14 Hz), 6.83—6.97 (1H, m), 7.09—7.27 (2H, m), 7.94 (1H, s), 8.79 (1H, s) (in DMSO-$d_6$) | 3150, 1620, 1500, 1420, 1270 | −284 (0.96) {25} |
| **5l** | 42 | 112—113 (IPE) | $C_{17}H_{19}F_2N_5OS_2$ | 49.62 (49.51) | 4.65 (4.74) | 17.02 (17.08) | 1.13 (3H, d, $J$=6.8 Hz), 3.53 (1H, q, $J$=6.8 Hz), 3.73 (3H, s), 3.84 (1H, d, $J$=14 Hz), 4.18 (1H, d, $J$=14 Hz), 4.77 (1H, d, $J$=14.4 Hz), 4.99 (1H, d, $J$=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 ($Et_2O$) | $C_{15}H_{17}F_2N_3O_3S_2 \cdot HCl$ | 42.30 (42.21) | 4.26 (4.30) | 9.86 (9.79) | 1.14 (3H, d, $J$=7 Hz), 1.28 (3H, t, $J$=7 Hz), 3.65 (1H, dq, $J$=1.4, 7 Hz), 4.36 (2H, q, $J$=7 Hz), 4.82 (1H, d, $J$=14.4 Hz), 5.11 (1H, d, $J$=14.4 Hz), 6.86—7.02 (1H, m), 7.10—7.30 (2H, m), 7.85 (1H, s), 8.59 (1H, s) (in DMSO-$d_6$) | 3200, 1735, 1610, 1500, 1420, 1270, 1130 | −171.9 (1.0) {23} |
| **10** | 37 | 206—209 (EA–$Et_2O$) | $C_{24}H_{29}F_4N_6O_2S$ | 53.73 (53.68) | 4.51 (4.50) | 15.66 (15.45) | 1.25 (6H, d, $J$=6.8 Hz), 3.49 (2H, q, $J$=6.8 Hz), 4.93 (2H, d, $J$=14 Hz), 5.15 (2H, s), 5.17 (2H, d, $J$=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; $Et_2O$, 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 ester[10] (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 $CH_2Cl_2$ (50 ml), and the extract was washed with water, dried over $MgSO_4$ 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.

**(2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-(2-dimethylaminoethyldithio)-1-(1*H*-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 $Et_2O$ gave **5f** (Table II) as a colorless crystalline powder.

***S*-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-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–$H_2O$ (1 : 1→2 : 1) followed by crystallization of the product from water gave **5g** (1.22 g, 67%) as colorless needles.

***S*-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-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–$H_2O$, 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*-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-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–$H_2O$ (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 $Et_2O$ gave compound **5j** (Table II) as a white amorphous powder.

**[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-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, $H_2O$, 15 ml). The resulting mixture was stirred at room temperature for 1 h, diluted with water (15 ml) and extracted with $CH_2Cl_2$ (50 ml, 20 ml). The extracts were combined, washed with water, dried over $MgSO_4$ 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-methylimidazole[11] (**13l**) 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 method[7] on YNB and PYG media buffered to pH 7.0. The cultures were incubated at 28 °C for 2 d.

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**References and Notes**

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