Optically Active Antifungal Azoles. XIII.[1] Synthesis of Stereoisomers and Metabolites of 1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (TAK-456)

Takashi Ichikawa,*[,a] Masami Yamada,[b] Masashi Yamaguchi,[c] Tomoyuki Kitazaki,[b] Yoshihiro Matsushita,[a] Keiko Higashikawa,[a] and Katsumi Itoh[a]

Medicinal Chemistry Research Laboratories I,[a] Medicinal Chemistry Research Laboratories II[b] and Drug Analysis & Pharmacokinetic Research Laboratories,[c] Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 17–85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532–8686, Japan. Received March 16, 2001; accepted June 5, 2001

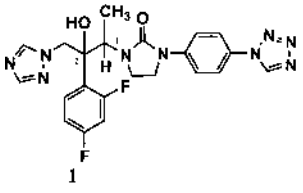
1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone [(1*R*,2*R*)-1: TAK-456] is a new antifungal agent selected as a candidate for clinical trials. The three stereoisomers [(1*S*,2*R*)-, (1*S*,2*S*)- and (1*R*,2*S*)-1] of this compound were prepared as authentic samples to determine the enantiomeric and diastereomeric purity of TAK-456 as well as to compare their *in vitro* antifungal activity. Pharmacokinetic studies of TAK-456 using rats identified the existence of metabolites in the liver homogenate. The structures of the major metabolites were assigned as 4-hydroxy-2-imidazolidinone (3) and/or 5-hydroxy-2-imidazolidinone (4), based on HPLC and LC/MS/MS analyses. These hydroxylated compounds, 3 and 4, were prepared by reduction of the corresponding imidazolidinediones, 11 and 12, and confirmed to be identical to the metabolites by HPLC. *In vitro* antifungal activities of the three stereoisomers and the synthesized metabolites were considerably weaker than TAK-456.

Key words TAK-456; optically active antifungal triazole; stereoisomer; metabolite; stereocontrolled synthesis; antifungal activity

We have recently reported the synthesis and potent antifungal activity of 1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone [(1*R*,2*R*)-**1**: TAK-456][2] and the water-soluble triazolium salt **2** (TAK-457: prodrug of TAK-456)[1] (Chart 1). Since these compounds exhibited potent therapeutic activities against various models of candidiasis and aspergillosis using mice, TAK-456 and TAK-457 were designated as the candidates for development of two types of formulation for oral and intravenous administrations, respectively, in clinical use.[3]

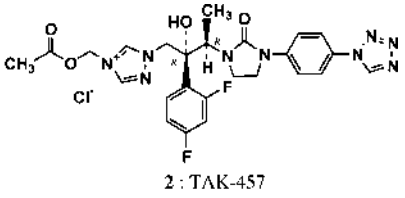
In the course of development of these antifungal agents, the three stereoisomers [(1*S*,2*R*)-, (1*S*,2*S*)- and (1*R*,2*S*)-**1**] were needed as authentic samples to determine the enantiomeric and diastereomeric purity of the TAK-456 bulk synthesis product, as well as to compare the *in vitro* antifungal activities of the stereoisomers.

In addition, pharmacokinetic studies on TAK-456 using rats revealed the existence of some metabolites in the liver homogenate. The structures of the major metabolites were assigned as 1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-hydroxy-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (**3**: Chart 1) and/or 1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-

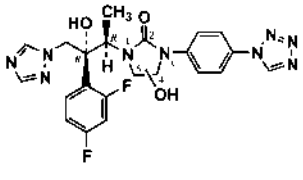


1

(1*R*, 2*R*) : TAK-456
(1*S*, 2*R*)
(1*S*, 2*S*)
(1*R*, 2*S*)



2 : TAK-457



3 (4-OH)
4 (5-OH)

Chart 1

* To whom correspondence should be addressed. e-mail: Ichikawa_Takashi@takeda.co.jp

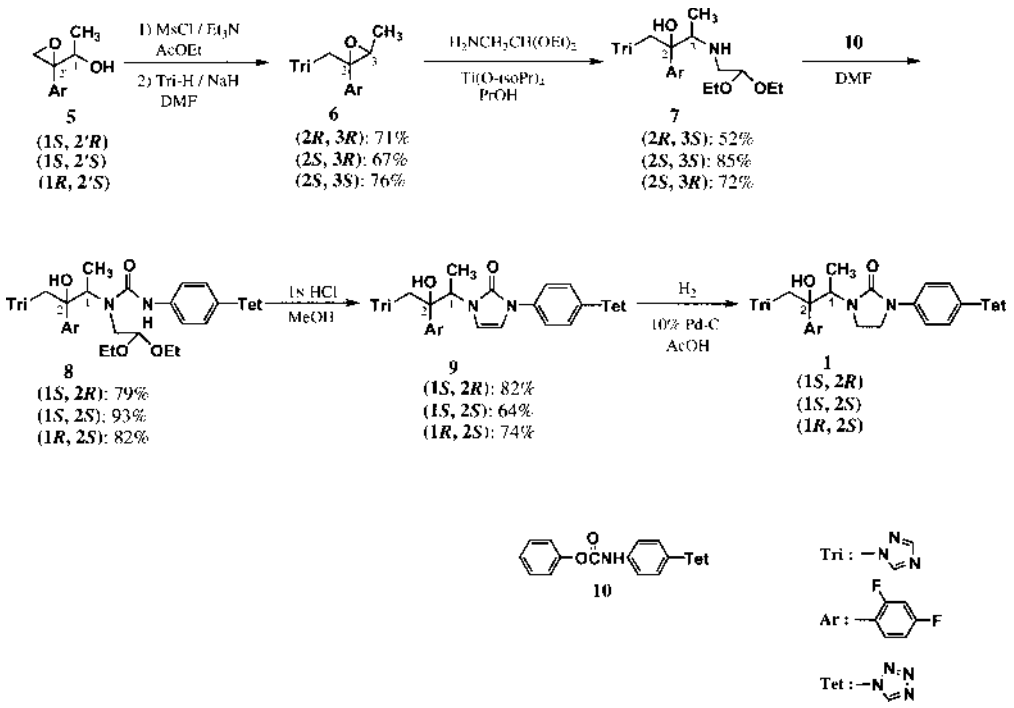


Chart 2

1,2,4-triazol-1-yl)propyl]-5-hydroxy-3-[4-(1*H*-1-tetrazolyl)-phenyl]-2-imidazolidinone (**4**: Chart 1) based on the results of HPLC and LC/MS/MS analyses. Definitive structural determination and synthesis of the metabolites were necessary in order to use these compounds as the authentic samples for future metabolic studies in various animals.

In this paper, we describe the stereocontrolled synthesis of the three stereoisomers of TAK-456 and the structural determination of the metabolites by chemical synthesis, as well as the *in vitro* antifungal activities of these compounds in comparison with TAK-456.

Synthesis We have previously reported an improved route for the synthesis of TAK-456 starting from (2*R*,3*S*)-2-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)methyl-3-methyloxirane [(2*R*,3*S*)-**6**],[2b] which was derived from methyl (*R*)-lactate by stereocontrolled synthesis.[4a] Preparation of the three stereoisomers was carried out using optically active oxiranes **6**[4a,c,e—g] with the appropriate stereochemistry as the key intermediates *via* the same synthetic route used for TAK-456 as shown in Chart 2.

Thus, the optically active oxiranylethanol (1*S*,2′*R*)-**5**[4b,d,e] was converted to the oxirane (2*R*,3*R*)-**6**[4a] in 71% yield by reaction with methanesulfonyl chloride (MsCl) in the presence of triethylamine (Et_3N) followed by treatment with the sodium salt of 1*H*-1,2,4-triazole (Tri-H).[5] Compound (2*R*,3*R*)-**6** was reacted with 2,2-diethoxyethylamine [$H_2NCH_2CH(OEt)_2$] in the presence of titanium tetraisopropoxide [$Ti(O\text{-}isoPr)_4$] in propanol (PrOH) to give the ring-opened product (2*R*,3*S*)-**7** as an oil in 52% yield, which was then treated with phenyl 4-(1*H*-1-tetrazolyl)phenylcarbamate (**10**)[2] at 80 °C in *N*,*N*-dimethylformamide (DMF) to give the urea (1*S*,2*R*)-**8** (79% isolated yield). Subsequent intramolecular cyclization of (1*S*,2*R*)-**8** under acidic conditions using 1 N hydrochloric acid in methanol (1 N HCl–MeOH) gave the imidazolone (1*S*,2*R*)-**9** in 82% yield which was followed by catalytic hydrogenation of (1*S*,2*R*)-**9** over palladium on carbon (Pd–C) in acetic acid (AcOH) to afford (1*S*,2*R*)-**1**. For synthesis of the other two stereoisomers, the oxiranes **6** with (2*S*,3*R*)- and (2*S*,3*S*)-configurations were prepared from the oxiranylethanols, (1*S*,2′*S*)-**5**[4b,e] and (1*R*,2′*S*)-**5**,[4b] respectively. These oxiranes were converted to the corresponding imidazolidinones, (1*S*,2*S*)-**1** and (1*R*,2*S*)-**1**, *via* the same sequence of reactions used for (1*S*,2*R*)-**1**. The physicochemical properties for the three stereoisomers prepared are shown in Table 1 together with those for TAK-456.[2a] The diastereomeric (% de) and enantiomeric (% ee) purities were determined by HPLC on an octadecyl silica (ODS) column and a chiral stationary column, respectively. The four stereoisomers are each of sufficient purity to enable comparison of their *in vitro* antifungal activities.

During preliminary pharmacokinetic studies on TAK-456, HPLC analysis of rat liver homogenate was carried out after oral administration of TAK-456. Some peaks in the chromatograph were observed at shorter retention times compared with that of TAK-456 as shown in Fig. 1 and were considered to be metabolites with increased hydrophilicity. The major metabolite (peak A) was analyzed by LC/MS/MS and the molecular weight was determined to be 497; *i.e.*, 16 mass units larger than that of TAK-456, suggesting that oxidation had occurred on the skeleton of TAK-456. Since the ethylene moiety in the imidazolidin-2-one nucleus appears to be the most susceptible to metabolic oxidation in TAK-456, the structures of the major metabolites were thought to be the 4- and/or 5-hydroxy-2-imidazolidinones, **3** and/or **4**. We expected that the hydroxy-2-imidazolidinone could be smoothly dehydrated to form the 2-imidazolone under acidic conditions and thus the rat liver homogenate containing the metabolites was treated with 6 N HCl and then analyzed by HPLC. As expected, peak A disappeared and another strong peak appeared at the same retention time as that of the 2-imi-

Table 1. Physicochemical Properties of Stereoisomers (**1**)

Compd.	Yield (%)	mp (°C) (Solvent)[b]	Analysis (%) Calcd (Found) C	H	N	^{1}H-NMR δ	IR (KBr) cm^{-1}	$[\alpha]_D$ {°C} (*c*) [Solvent]	de[c] (%)	ee[d] (%)
(1*R*,2*R*)-**1**:	TAK-456[a]	211—213 (EtOH)	$C_{22}H_{21}F_2N_9O_2$ 54.88 (54.79)	4.40 (4.42)	26.18 (26.00)	1.08 (3H, d, *J*=7 Hz), 3.69—4.14 (4H, m), 4.52 (1H, d, *J*=14 Hz), 4.65—4.80 (1H, m), 5.12 (1H, d, *J*=14 Hz), 5.35 (1H, br), 6.74—6.84 (2H, m), 7.36—7.49 (1H, m), 7.68 (2H, d, *J*=9 Hz), 7.77 (1H, s), 7.82 (2H, d, *J*=9 Hz), 7.87 (1H, s), 8.98 (1H, s) (in $CDCl_3$)	3400, 3120, 1680, 1610, 1520, 1498, 1480	−60.6° {20} (1.0) [MeOH]	>99	>99
(1*S*,2*R*)-**1**	30	206—207 (EtOH)	$C_{22}H_{21}F_2N_9O_2$ 54.88 (54.92)	4.40 (4.30)	26.18 (26.12)	1.37 (3H, d, *J*=7 Hz), 3,42—3.74 (4H, m), 4.56 (1H, d, *J*=14 Hz), 4.63 (1H, q, *J*=7 Hz), 4.88 (1H, d, *J*=14 Hz), 6.16 (1H, s), 6.77—6.87 (1H, m), 7.01—7.13 (1H, m), 7.32—7.44 (1H, m), 7.62 (2H, d, *J*=9 Hz), 7.70 (1H, s), 7.79 (2H, d, *J*=9 Hz), 8.29 (1H, s), 10.00 (1H, s) (in DMSO-d_6)	3241, 3135, 1705, 1680, 1524, 1429, 1271	−91.7° {20} (1.0) [DMSO]	>99	95.3
(1*S*,2*S*)-**1**	19	210—211 (EtOH)	$C_{24}H_{21}F_2N_7O_3$ 54.88 (54.64)	4.40 (4.39)	26.18 (26.16)	1.09 (3H, d, *J*=7 Hz), 3,68—4.13 (4H, m), 4.52 (1H, d, *J*=14 Hz), 4.72 (1H, q, *J*=7 Hz), 5.12 (1H, d, *J*=14 Hz), 5.35 (1H, br), 6.73—6.85 (2H, m), 7.37—7.49 (1H, m), 7.68 (2H, d, *J*=9 Hz), 7.77 (1H, s), 7.82 (2H, d, *J*=9 Hz), 7.87 (1H, s), 8.98 (1H, s) (in $CDCl_3$)	3400, 3121, 1682, 1617, 1524, 1424, 1267	+60.7° {20} (1.0) [MeOH]	>99	99.9
(1*R*,2*S*)-**1**	19	207—208 (EtOH)	$C_{24}H_{21}F_2N_7O_3$ 54.88 (55.01)	4.40 (4.38)	26.18 (26.00)	1.37 (3H, d, *J*=7 Hz), 3,42—3.74 (4H, m), 4.56 (1H, d, *J*=14 Hz), 4.63 (1H, q, *J*=7 Hz), 4.88 (1H, d, *J*=14 Hz), 6.16 (1H, s), 6.78—6.86 (1H, m), 7.01—7.12 (1H, m), 7.32—7.45 (1H, m), 7.62 (2H, d, *J*=9 Hz), 7.69 (1H, s), 7.78 (2H, d, *J*=9 Hz), 8.29 (1H, s), 10.00 (1H, s) (in DMSO-d_6)	3243, 3135, 1705, 1680, 1524, 1429, 1271	+88.1° {20} (1.0) [DMSO]	>99	94.3

a) Reported in ref. 2*a*. *b*) Recrystallization solvent. *c*) Determined by HPLC using an ODS column. *d*) Determined by HPLC using Chiralpak AS.

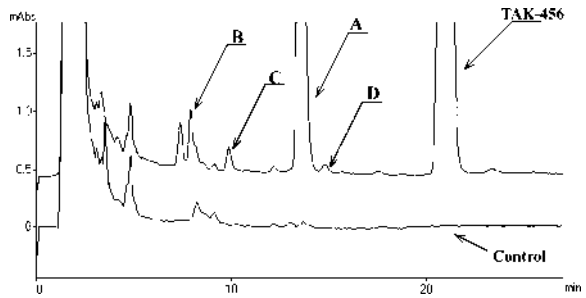


Fig. 1. Chromatograms of Rat Liver Homogenate

dazolone (1*R*,2*R*)-**9**.[2a] Furthermore, LC/MS/MS analysis indicated that its molecular weight was identical to that of (1*R*,2*R*)-**9**. On the basis of the above analytical results, we planned to synthesize **3** and **4** to confirm the structure of the major metabolites, as well as to determine the stereochemistry of the new chiral center attached to the hydroxyl group.

Our initial retrosynthetic analysis for compounds **3** and **4** is shown in Chart 3. The final step is reduction of the imidazolidinediones, **11** and **12**, to the corresponding hydroxyimidazolidinones, **3** and **4**. Compounds **11** and **12** may be derived from the esters **13** and **14** which could be used as the precursors for ring-closure and could be prepared *via* appropriate functionallization of (2*R*,3*R*)-3-amino-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (**15**).[6]

The synthetic routes that were initially investigated are shown in Chart 4. Compound **15** was allowed to react with ethyl bromoacetate ($BrCH_2COOEt$) in the presence of Et_3N to give the *N*-substituted glycinate **16** in 80% yield. Then, compound **16** was reacted with the carbamate **10** to give **13** under similar conditions to those used for the synthesis of **8**. In this case, condensation and the subsequent ring-closure proceeded smoothly, and the desired imidazolidine-2,4-dione **11** was obtained in 78% isolated yield.

The reduction of **11** with sodium borohydride ($NaBH_4$) was carried out in ethanol (EtOH) at 0 °C. The HPLC and TLC analyses of the reaction mixture indicated that two products, a less polar product and a more polar product, were formed in a ratio of approximately 2 : 1.[7] Treatment of the products with a catalytic amount of hydrochloric acid gave the imidazolone (1*R*,2*R*)-**9**, exclusively.[8] Therefore, these were considered to be the epimers of the desired 4-hydroxy-2-imidazolidinones (**3a**, **b**) resulting from the newly formed chiral center attached to the hydroxyl group in the imidazolidinone nucleus. During separation of the two epimers by chromatography, it was suspected that epimerization occurred to some extent on the silica gel, because clear separation could not be attained. The less polar epimer (**3a**: major product) was obtained as colorless prisms by recrystallization of the residue from the eluate containing **3a** as the major component. Purification of the more polar epimer (**3b**: minor product) was carried out by ODS column chromatography under neutral conditions affording **3b** as an amorphous powder. In order to examine the liability of **3a**, **b** to epimerization, the **3a**-enriched mixture (**3a** : **3b**=97 : 3) was heated at 50—60 °C in EtOH. HPLC analysis of the resulting solution indicated that the ratio of **3b** increased with time and that equilibrium was reached after 6 h with a ratio of **3a** : **3b**=44 : 56.[8]

The physicochemical properties of **3a** and **3b** are shown in Table 2 and support the structural assignment. However, the stereochemistry of the newly formed chiral center at the 4-position in the imidazolidinone nucleus could not be deter-

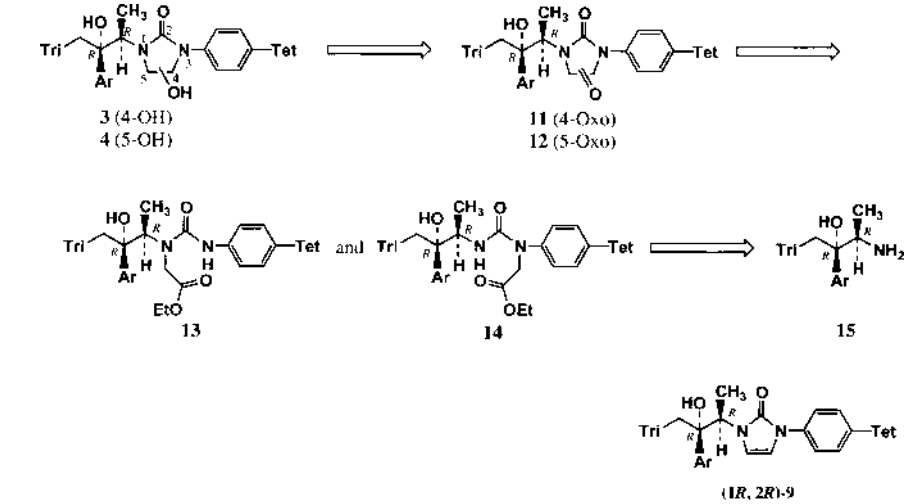


Chart 3

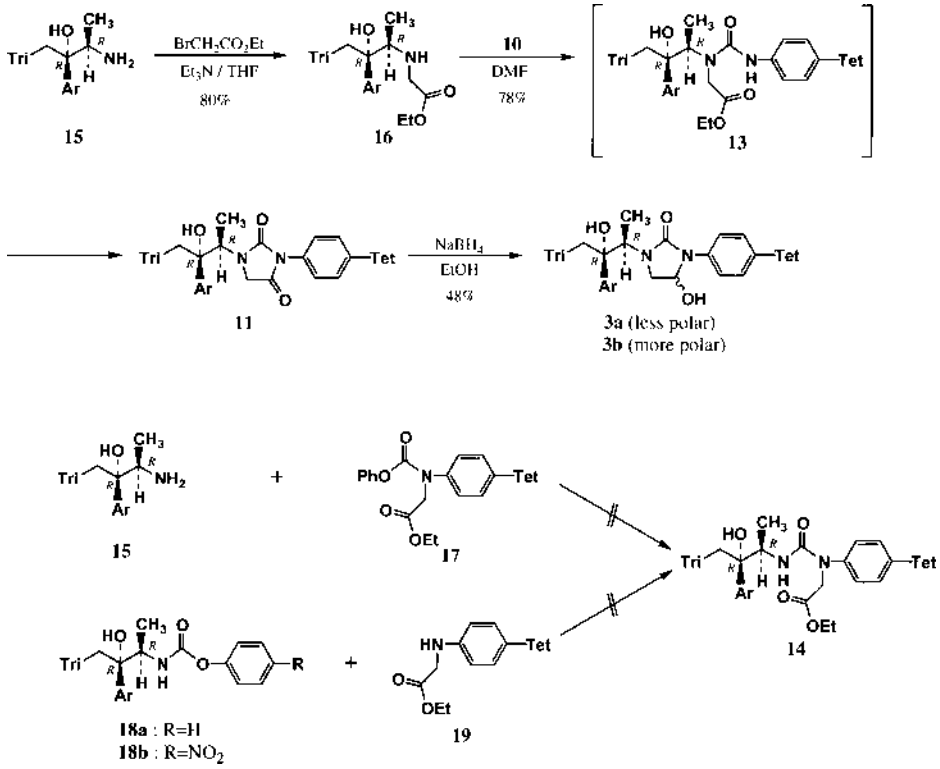


Chart 4

mined by spectroscopic methods such as NMR. Thus crystalline **3a** was submitted to X-ray crystallographic analysis and the stereochemistry of the 4-position in **3a** was determined to be (*S*)-configuration relative to the known (*R*)-configurations of the two chiral centers in the trisubstituted butanol skeleton as shown in Fig. 2. Therefore, the configuration of the 4-position in the other epimer **3b** was assigned to be (*R*). Compounds **3a** and **3b** prepared above were analyzed by HPLC using the same conditions as used for analysis of rat liver homogenate and confirmed to be identical to the peaks B and C (Fig. 1), respectively.

Next, we attempted to prepare the 2,5-dione **12** according to the synthetic plan shown in Chart 3. However, reaction of **15** with the phenylcabamate **17** to obtain the precursor **14** did not give the desired product and resulted in decomposition or recovery of the starting materials. Another approach to prepare **14** by reaction of compound **18a** or more reactive **18b** with the glycinates **19** also failed to proceed successfully.

We then turned our efforts to investigating a new route which involves ring closure of the carbamate **22** to the 2,5-dione **12** as the key step (Chart 5). We carried out the synthesis of **22** *via* two routes.

Firstly, the ethyl glycinate **19** was hydrolyzed to the corresponding acid **20** in 74% yield, which was then condensed with the amine **15** in the presence of diethyl cyanophosphonate (DEPC) and Et_3N in DMF to obtain the amide **21** (88% isolated yield). Compound **21** was allowed to react with ethyl chlorocarbonate (ClCOOEt) to give the desired product **22** in

Table 2. Physicochemical Properties of 1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4- and 5-Hydroxy-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinones

Compd.	mp (°C) (Solvent)[a)]	Analysis (%) Calcd (Found) C H N	^{1}H-NMR (in DMSO-d_6) δ	IR (KBr) cm^{-1}	$[\alpha]_D$ {°C} (*c*) MeOH
3a	211.5—212.5 (Acetone–AcOEt)	$C_{22}H_{21}F_2N_9O_3$ 53.12 4.25 25.34 (53.06 4.38 25.38) [SI-MS: 498 (MH^+)]	0.98 (3H, d, *J*=7.0 Hz), 3.37 (1H, d, *J*=11 Hz), 4.17 (1H, dd, *J*=11, 7.4 Hz), 4.53 (1H, d, *J*=14 Hz), 4.78 (1H, q, *J*=7.0 Hz), 4.87 (1H, d, *J*=14 Hz), 5.79 (1H, d, *J*=16 Hz), 5.83 (1H, d, *J*=16 Hz), 6.69 (1H, d, *J*=8.8 Hz), 6.86—6.96 (1H, m), 7.09—7.34 (2H, m), 7.63 (1H, s), 7.88 (2H, *J*=9.0 Hz), 8.00 (2H, d, *J*=9.0 Hz), 8.27 (1H, s), 10.05 (1H, s).	3438, 3134, 1688, 1615, 1522, 1426, 1260, 1206, 1134, 1105	−37.9° {20} (1.0)
3b	Amorphous	$C_{22}H_{21}F_2N_9O_3 \cdot 1/2AcOEt$ 53.23 4.65 23.28 (52.96 4.59 23.03) [SI-MS: 498 (MH^+)]	0.92 (3H, d, *J*=7.0 Hz), 3.72 (1H, d, *J*=10 Hz), 3.80—3.89 (1H, m), 4.70 (1H, d, *J*=14 Hz), 4.73—4.87 (1H, m), 4.85 (1H, d, *J*=14 Hz), 5.81 (1H, d, *J*=15 Hz), 5.84 (1H, d, *J*=15 Hz), 6.71 (1H, d, *J*=8.6 Hz), 6.91—7.00 (1H, m), 7.20—7.36 (2H, m), 7.69 (1H, s), 7.80 (2H, d, *J*=9.0 Hz), 8.00 (2H, d, *J*=9.0 Hz), 8.27 (1H, s), 10.06 (1H, s)	3123, 2984, 1701, 1617, 1524, 1431, 1267, 1207, 1140, 1094	—[b)]
4a	Amorphous	$C_{22}H_{21}F_2N_9O_3$ [SI-MS: 498 (MH^+)]	1.10 (3H, d, *J*=7.0 Hz), 3.73 (1H, d, *J*=10 Hz), 4.22—4.31 (1H, m), 4.52 (1H, d, *J*=14 Hz), 4.60—4.78 (1H, m), 4.83 (1H, d, *J*=14 Hz), 5.59 (1H, br), 6.59 (1H, br), 6.86—6.95 (1H, m), 7.13—7.24 (1H, m), 7.29—7.40 (1H, m), 7.60 (1H, s), 7.90 (4H, s), 8.27 (1H, s), 10.06 (1H, s).	3108, 1676, 1618, 1524, 1267, 1138	—
4b	153—154 (AcOEt)	$C_{22}H_{21}F_2N_9O_3 \cdot 1/2H_2O$ 52.17 4.38 24.89 (52.26 4.54 24.77) [SI-MS: 498 (MH^+)]	1.03 (3H, d, *J*=7.0 Hz), 3.84 (1H, d, *J*=10.6 Hz), 4.26 (1H, dd, *J*=10.6, 7.2 Hz), 4.61 (1H, d, *J*=15 Hz), 4.65—4.96 (2H, m), 4.93 (1H, d, *J*=15 Hz), 5.43 (1H, d, *J*=6.2 Hz), 6.00—6.60 (1H, br), 6.86—6.94 (1H, m), 7.16—7.36 (2H, m), 7.59 (1H, s), 7.91 (4H, s), 8.20 (1H, s), 10.07 (1H, s).	3258, 2718, 1709, 1620, 1609, 1524, 1420, 1258, 1136, 1044	−74.8° {20} (0.5)

a) Recrystallization solvent. *b*) Not measured.

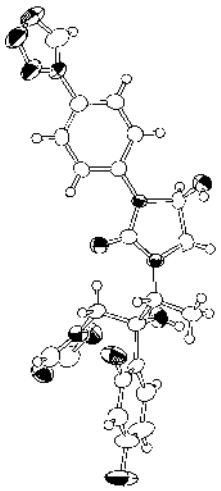


Fig. 2. Molecular View of **3a**

75% yield. The second route starting from the *tert*-butyl glycinate **23** also proceeded successfully. Compound **23** was reacted with ClCOOEt to obtain the carbamate **24** (85% yield). The *tert*-butyl moiety of **24** was removed by treatment with hydrogen chloride and the resulting acid **25** (85% yield) was condensed with **15** in the presence of DEPC to obtain **22** in 87% isolated yield. In contrast to **13**, compound **22** was stable and ring closure to **12** did not occur under neutral conditions even at elevated temperature (80→100 °C). Albericio and Barany reported that tetrabutylammonium fluoride (TBAF) is an effective catalyst for ring-closure reactions to obtain hydantoins.[9)] Thus we investigated the conversion of **22**→**12** using this catalyst. However, although use of TBAF did give the desired compound **12**, the conversion of **22** to **12** was low and we attempted to improve the yield of **12** by modifying the reaction conditions. Nevertheless, even under the optimized conditions, the isolated yield of the desired **12** was only 27% with the 23% recovery of **22**.

The 2,5-dione **12** was reduced to the 5-hydroxy compound **4** under conditions similar to those used for the synthesis of **3a**, **b**. Formation of two products, **4a** (less polar, minor) and **4b** (more polar, major), was observed on TLC[10)] which were considered to be epimers analogous to **3a**, **b**.[11)] However, in contrast with the case of **3a**, **b**, these two epimers are stable on silica gel and the less polar epimer **4a** could be purified by column chromatography and was obtained as an amorphous powder in 13% isolated yield. The more polar epimer **4b** was obtained in 67% isolated yield as colorless prisms by recrystallization after silica gel chromatography. The physicochemical properties of **4a** and **4b** are shown in Table 2. The stereochemical assignment of 5-position was also carried out by X-ray crystallographic analysis using a crystal of **4b**, and the stereochemistry of the 5-carbon atom in **4b** was determined to be (*S*)-configuration as shown in Fig. 3. Therefore, the configuration of the 5-position of the other epimer **4a** was assigned to be (*R*).

Compounds **4a** and **4b** prepared above were examined by HPLC under the same conditions as used for analysis of the rat liver homogenate, and confirmed to be identical to the peak A (Fig. 1). At present, it is unclear whether peak A includes a single or both epimers **4a**, **b**, because the epimers were not separable on ODS under the conditions used in this analysis.

In addition to the identification of the metabolites **3** and **4** by HPLC, the small peak D (Fig. 1) was identified as the imidazolone (1*R*,2*R*)-**9**, which was presumed to be generated from the metabolites **3** and **4** *via* dehydration.

Antifungal Activity The stereoisomers [(1*S*,2*R*)-, (1*S*,2*S*)-, (1*R*,2*S*)-**1**] and the metabolites (**3a**, **b** and **4a**, **b**) prepared in this report were evaluated for *in vitro* antifungal activity for comparison with TAK-456, and the results are shown in Table 3. The activity is expressed as the minimum inhibitory concentration (MIC, μg/ml). The MIC values for

yeast type fungi such as *Candida* and *Cryptococcus* species were determined by an agar dilution method using RPMI 1640 medium under 20% CO_2,[12] and MIC values for *Aspergillus* species were measured using the same medium under ordinary air. As can be seen in Table 3, the antifungal activities of the stereoisomers and the metabolites against various fungi are considerably weaker compared to those of TAK-456. The order of potency among the stereoisomers is (1*R*,2*R*)≫(1*S*,2*R*)≥(1*S*,2*S*)>(1*R*,2*S*), which is similar to our previous results obtained in a series of optically active 1,2,3-trisubstituted butanols.[4a—c] Among the metabolites, **4a** and **4b** showed some activity, however the potency is about ten times less than that of TAK-456.

In conclusion, we have carried out the synthesis of the three stereoisomers of TAK-456 [(1*S*,2*R*)-, (1*S*,2*S*)-, (1*R*,2*S*)-**1**] and established an efficient route for synthesis of the four metabolites, **3a**, **b** and **4a**, **b**. The stereoisomers and the metabolites exhibited considerably weaker antifungal activity against various fungi compared with TAK-456. These metabolites **3** and **4** were confirmed to exist in the rat liver

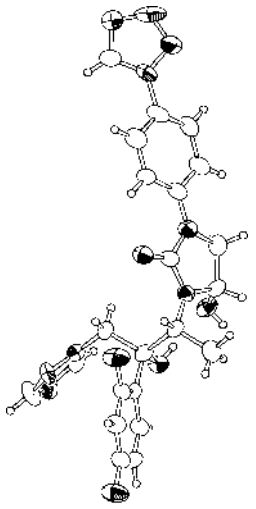


Fig. 3. Molecular View of **4b**

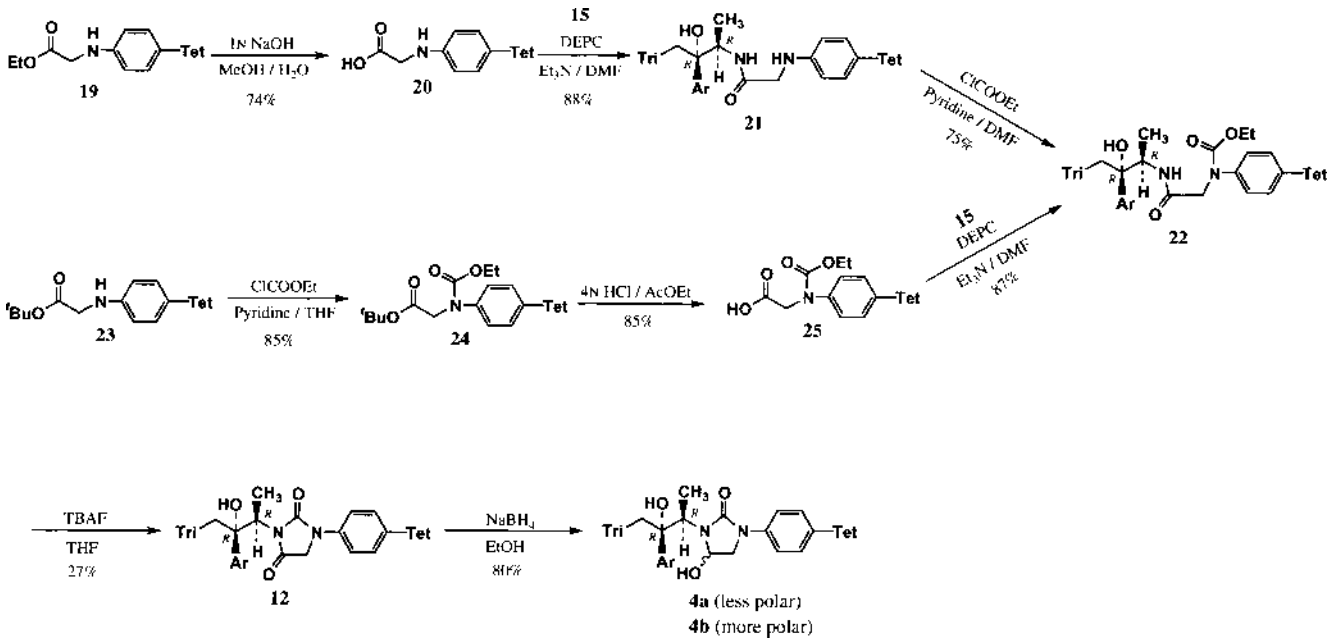


Chart 5

Table 3. *In Vitro* Antifungal Activities

Fungus	Strain	MIC (μg/ml)							
		(1*R*,2*R*)-**1** TAK-456	(1*S*,2*R*)-**1**	(1*S*,2*S*)-**1**	(1*R*,2*S*)-**1**	**3a**	**3b**	**4a**	**4b**
Candida albicans	IFO 0583	0.016	1	2	>16	4	4	0.25	0.25
	TA	0.016	1	2	>16	4	4	0.25	0.5
	TIMM1756	0.03	4	4	>16	16	8	1	0.5
	CA383	4	>16	>16	>16	>16	>16	>16	>16
Candida tropicalis	IFO 10241	0.25	16	>16	>16	>16	16	4	2
Candida krusei	IFO 1162	2	>16	>16	>16	>16	>16	>16	>16
Candida utilis	IFO 0619	0.25	8	8	16	>16	>16	4	4
Cryptococcus neoformans	TIMM1740	0.25	>16	>16	>16	>16	>16	16	8
Aspergillus fumigatus	IFO 6344	0.5	>64	>64	>64	>64	>64	64	32
	437	0.5	>64	>64	>64	>64	>64	64	32
	TIMM1728	0.5	>64	>64	>64	>64	>64	64	32
Aspergillus niger	IFO 4414	1	>64	>64	>64	>64	>64	64	32

homogenate after oral administration of TAK-456. Detailed pharmacokinetic profiles of the candidates, TAK-456 and TAK-457, will be reported in due course.

Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with JASCO IR-810 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad. The secondary ion mass spectra (SI-MS) were measured with a Hitachi M-80A mass spectrometer. The optical rotations were recorded with a Jasco DIP-181 or DIP-370 digital polarimeter.

Reactions were carried out at room temperature unless otherwise noted and followed by TLC on Silica-gel 60 F_{254} precoated TLC plates (E. Merck) or by HPLC using an ODS column (A-303, 4.6 mm i.d.×250 mm, YMC Co., Ltd.). Standard work-up procedures were as follows. The reaction mixture was partitioned between the indicated organic solvent such as ethyl acetate (AcOEt) and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions: water, 5% aqueous sodium carbonate solution (aqueous $NaHCO_3$), saturated sodium chloride (NaCl) solution (brine), 1 N aqueous sodium hydroxide solution (1 N NaOH) and 1 N HCl. Extracts were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered and evaporated *in vacuo.* Chromatographic separations were carried out on Silica-gel 60 (0.063—0.200 mm, E. Merck) or an ODS column (CPO-273L, pre-packed column, 22 mm×300 mm, Kusano Kagaku Kikai Co.) using the indicated eluents.

(1*S*)-1-[(2*R*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethanol [(1*S*,2′*R*)-5] This compound was prepared according to the method described in our previous report.[4d)]

(1*S*)-1-[(2*S*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethanol [(1*S*,2′*S*)-5] A mixture of 2-(2,4-difluorophenyl)-2-[(1*S*)-1-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)ethyl]oxirane[13)] (49.6 g), pyridinium *p*-toluenesulfonate (4.3 g) and EtOH (600 ml) was stirred for 1.5 h at 55 °C, then concentrated *in vacuo* and worked up (AcOEt; water, brine). The residue was purified by flash chromatography on silica gel [hexane–AcOEt, 20 : 1→10 : 1→5 : 1, v/v] to give (1*S*)-1-[2-(2,4-difluorophenyl)-2-oxiranyl]ethanol (23.5 g, 70%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.22 (3H, m), 1.78, 2.03 (1H), 2.80, 2.92 (1H), 3.28—3.31 (1H, m), 4.02—4.18 (1H, m), 6.76—6.95 (2H, m), 7.32—7.48 (1H, m).

This oil (21.1 g) and 3,5-dinitrobenzoyl chloride (31.7 g) were dissolved in tetrahydrofuran (THF, 360 ml). Et_3N (19.1 ml) was added dropwise to this solution at 0 °C. After having been stirred for 2 h, the mixture was washed (water, aqueous $NaHCO_3$) and concentrated *in vacuo.* The residue was chromatographed on silica gel (hexane–AcOEt, 20 : 1→10 : 1→5 : 1, v/v) and crystallized from AcOEt–MeOH (1 : 1, v/v, 100 ml) to give [(1*S*)-1-[(2*S*)-2-(2,4-difluorophenyl)-2-oxiranyl]ethyl] 3,5-dinitrobenzoate (15.5 g, 38%) as colorless needles. mp 108.5—109.5 °C. *Anal.* Calcd for $C_{17}H_{12}F_2N_2O_7$: C, 51.79; H, 3.10; N, 7.10. Found: C, 51.81; H, 3.07; N, 7.15. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, dd, J=6.6, 1.6 Hz), 3.01 (1H, d, J=4.6 Hz), 3.23 (1H, d, J=4.6 Hz), 5.34 (1H, q, J=6.6 Hz), 6.85—7.06 (2H, m), 7.48—7.59 (1H, m), 9.13 (2H, d, J=2.2 Hz), 9.25 (1H, t, J=2.2 Hz). IR (KBr): 1734, 1549, 1346, 1279, 1169 cm^{-1}. $[\alpha]_D^{20}$: +23.2° (c=1.0, $CHCl_3$).

A 1 N NaOH solution (36 ml) was added dropwise to a solution of the dinitrobenzoate prepared above (7.04 g) in MeOH (350 ml). The mixture was stirred for 1 h, then 1 N HCl (18 ml) was added. The whole was concentrated *in vacuo* and worked up (AcOEt; brine) to afford a residue, which was purified by chromatography on silica gel (AcOEt–hexane, 1 : 4→1 : 3, v/v) to give (1*S*,2′*S*)-**5** (3.5 g, 98%) as a pale yellow oil. This alcohol was identical to the authentic sample[4b)] upon direct comparison.

(1*R*)-1-[(2*S*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethanol [(1*R*,2′*S*)-5] Triphenylphosphine (13 g), benzoic acid (6.05 g) and diethyl azodicarboxylate (40% in toluene, 21.6 g) were added to an ice-cooled solution of (1*S*,2′*S*)-**5** (4.96 g) in THF (100 ml). The mixture was stirred for 1 d, then worked up (AcOEt; aqueous $NaHCO_3$, water, brine), and the residue was chromatographed on silica gel (AcOEt–hexane, 1 : 30, v/v) to give [(1*R*)-1-[(2*S*)-2-(2,4-difluorophenyl)-2-oxiranyl]ethyl] benzoate (5.15 g, 68%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, d, J=6.6 Hz), 2.89 (1H, d, J=5.2 Hz), 3.28 (1H, d, J=5.2 Hz), 5.36 (1H, q, J=6.6 Hz), 6.75—6.93 (2H, m), 7.38—7.60 (4H, m), 7.95—8.00 (2H, m). IR (neat): 1719, 1617, 1601, 1508, 1453, 1427, 1267, 1096 cm^{-1}.

A 28% sodium methoxide–MeOH solution (4.12 ml) was added dropwise to an ice-cooled solution of the benzoate prepared above (5.0 g) in MeOH (190 ml). The mixture was stirred overnight, then 1 N HCl (23 ml) was added. The whole was concentrated *in vacuo* to afford a residue, which was submitted to chromatography on silica gel (AcOEt–hexane, 1 : 6, v/v) to give (1*R*,2′*S*)-**5** (2.87 g, 87%) as a colorless oil. This alcohol was identical to the authentic sample[4b)] upon direct comparison.

(2*R*,3*R*)-2-(2,4-Difluorophenyl)-3-methyl-2-(1*H*-1,2,4-triazol-1-yl)-methyloxirane [(2*R*,3*R*)-6] Et_3N (5.43 ml) was added dropwise to an ice-cooled solution of (1*S*,2′*R*)-**5** (5.2 g) and MsCl (4.46 g) in AcOEt (132 ml). The mixture was stirred for 30 min, then worked up (AcOEt; water, aqueous $NaHCO_3$, brine) to give [(1*S*)-1-[(2*R*)-2-(2,4-difluorophenyl)-2-oxiranyl]-ethyl] methanesulfonate as a colorless oil, which was used for the next step without purification.

Tri-H (6.28 g) was added to a stirred mixture of sodium hydride (NaH, 60% in oil, 3.12 g) and DMF (140 ml) at 0 °C. The whole was stirred for 10 min, then the mesylate prepared above was added. The resulting mixture was stirred for 5.5 h at 50 °C, and worked up (AcOEt; water, brine). The residue was purified by chromatography on silica gel (AcOEt–hexane, 55 : 45→3 : 2, v/v) to give (2*R*,3*R*)-**6** [4.6 g, 71% from (1*S*,2′*R*)-**5**] as a colorless oil. The oxiranes, (2*S*,3*R*)-**6** and (2*S*,3*S*)-**6**, were prepared according to the same way as above from the corresponding oxiranylethanols, (1*S*,2′*S*)-**5** and (1*R*,2′*S*)-**5**, in yields of 67% and 76%, respectively. These oxiranes prepared were identical to the authentic samples,[4a)] respectively, upon direct comparison.

(2*R*,3*S*)-3-(2,2-Diethoxyethyl)amino-2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol [(2*R*,3*S*)-7] A mixture of (2*R*,3*R*)-**6** (4.17 g), $Ti(O\text{-}isoPr)_4$ (7.35 ml), $H_2NCH_2CH(OEt)_2$ (48.3 ml) and PrOH (50 ml) was stirred under reflux for 5.5 h under a nitrogen atmosphere. The solvent and $H_2NCH_2CH(OEt)_2$ were distilled off under reduced pressure. The residue was diluted with AcOEt (50 ml). 2 N NaOH (50 ml) saturated with NaCl was added and the resulting mixture was stirred for 1 h. A milky precipitate was filtered off and washed with AcOEt (50 ml). The filtrate and the washing were combined and extracted with 1 N HCl (50 ml×3). The aqueous extracts were combined and neutralized with 2 N NaOH saturated with NaCl (75 ml) at 0 °C. The whole was extracted with AcOEt (150 ml). The extract was washed with water (100 ml) and brine (100 ml), successively, dried over $MgSO_4$ and evaporated *in vacuo.* The residue was purified by chromatography on silica gel (AcOEt–hexane, 4 : 1, v/v) and crystallized from AcOEt–hexane to give (2*R*,3*S*)-**7** (3.3 g, 52%) as a white crystalline powder. mp 81—82 °C. *Anal.* Calcd for $C_{18}H_{26}F_2N_4O_3$: C, 56.24; H, 6.82; N, 14.57. Found: C, 56.06; H, 6.52; N, 14.29. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, d, J=7 Hz), 1.06—1.13 (1H, br), 1.16 (3H, t, J=7 Hz), 1.25 (3H, d, J=6.6 Hz), 2.23 (1H, dd, J=12, 5.2 Hz), 2.57 (1H, dd, J=12, 5.8 Hz), 3.20 (1H, q, J=6.6 Hz), 3.33—3.68 (4H, m), 4.34 (1H, dd, J=5.8, 5.2 Hz), 4.56 (1H, d, J=14 Hz), 4.81 (1H, d, J=14 z), 5.03 (1H, s), 6.69—6.81 (2H, m), 7.37—7.50 (1H, m), 7.69 (1H, s), 8.08 (1H, s). IR (KBr): 3320, 2976, 1617, 1599, 1499, 1418, 1273, 1123, 1061, 966 cm^{-1}. $[\alpha]_D^{20}$: −29.4° (c=0.99, MeOH).

(2*S*,3*S*)-**7**: The oxirane (2*S*,3*R*)-**6** was allowed to react with $H_2NCH_2CH(OEt)_2$ to obtain (2*S*,3*S*)-**7** in the same manner as described above. Yield 85% (a pale yellow oil). *Anal.* Calcd for $C_{18}H_{26}F_2N_4O_3$: C, 56.24; H, 6.82; N, 14.57. Found: C, 56.11; H, 6.70; N, 14.57. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, d, J=6.6 Hz), 1.00—1.20 (1H, br), 1.23 (6H, t, J=7 Hz), 2.67 (1H, dd, J=12, 4.6 Hz), 2.95 (1H, dd, J=12, 6.4 Hz), 3.11 (1H, q, J=6.6 Hz), 3.48—3.80 (4H, m), 4.55 (1H, dd, J=6.4, 4.6 Hz), 4.75 (1H, d, J=14 Hz), 4.86 (1H, s), 4.89 (1H, d, J=14 Hz), 6.70—6.81 (2H, m), 7.33—7.45 (1H, m), 7.76 (1H, s), 7.93 (1H, s). IR (neat): 2976, 1617, 1501, 1273, 1136, 1100, 1063, 964 cm^{-1}. $[\alpha]_D^{20}$: +70.2° (c=1.0, MeOH).

(2*S*,3*R*)-**7**: The oxirane (2*S*,3*S*)-**6** was allowed to react with $H_2NCH_2CH(OEt)_2$ to obtain (2*S*,3*R*)-**7** in the same manner as described above. Yield 72% (a white crystalline powder). mp 82—83 °C (AcOEt–hexane). *Anal.* Calcd for $C_{18}H_{26}F_2N_4O_3$: C, 56.24; H, 6.82; N, 14.57. Found: C, 56.49; H, 6.87; N, 14.78. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, d, J=7 Hz), 1.13—1.20 (1H, br), 1.16 (3H, t, J=7 Hz), 1.26 (3H, d, J=6.6 Hz), 2.23 (1H, dd, J=12, 5.2 Hz), 2.57 (1H, dd, J=12, 5.8 Hz), 3.20 (1H, q, J=6.6 Hz), 3.33—3.70 (4H, m), 4.34 (1H, dd, J=5.8, 5.2 Hz), 4.56 (1H, d, J=14 Hz), 4.81 (1H, d, J=14 Hz), 5.03 (1H, s), 6.69—6.81 (2H, m), 7.37—7.49 (1H, m), 7.68 (1H, s), 8.07 (1H, s). IR (KBr): 3314, 2976, 1615, 1599, 1499, 1418, 1273, 1121, 1063, 966 cm^{-1}. $[\alpha]_D^{20}$: +31.2° (c=1.0, MeOH).

1-(2,2-Diethoxyethyl)-1-[(1*S*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-urea [(1*S*,2*R*)-8] A mixture of (2*R*,3*S*)-**7** (2.85 g), **10** (1.88 g) and DMF (29 ml) was stirred for 6 h at 80 °C under a nitrogen atmosphere. The mixture was cooled and worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (AcOEt–hexane, 8.5 : 1, v/v→AcOEt–MeOH, 8 : 1, v/v) to give (1*S*,2*R*)-**8** (3.33 g, 79%) as a white powder.

SI-MS (*m/z*): 572 (MH^+). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 1.52 (3H, d, J=7 Hz), 3.21 (2H, br), 3.44—3.99 (5H, m), 4.55 (1H, d, J=14 Hz), 4.52—4.61 (1H, m), 4.76 (1H, d, J=14 Hz), 6.72—6.86 (2H, m), 7.33 (2H, d, J=9 Hz), 7.42—7.55 (1H, m), 7.57 (2H, d, J=9 Hz), 7.66 (1H, s), 7.83 (1H, s), 8.20 (1H, s), 8.52 (1H, s), 8.93 (1H, s). IR (KBr): 3308, 3114, 2978, 1651, 1522, 1499, 1472, 1273, 1132 cm^{-1}. $[\alpha]_D^{20}$: +169.4° (c=1.0, MeOH).

(1*S*,2*S*)-**8**: Compound (2*S*,3*S*)-**7** was converted to (1*S*,2*S*)-**8** by the reaction with **10** under the same condition as described above. Yield 93% (a white crystalline powder). mp 179—181 °C [AcOEt–diisopropyl ether ($isoPr_2O$)]. *Anal.* Calcd for $C_{26}H_{31}F_2N_9O_4$: C, 54.63; H, 5.74; N, 22.05. Found: C, 54.38; H, 5.37; N, 22.07. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (1.5H, d, J=7 Hz), 1.24 (1.5H, d, J=7 Hz), 1.31—1.40 (6H, m), 3.52—4.09 (7H, m), 4.39 (0.5H, d, J=14 Hz), 4.56 (0.5H, d, J=14 Hz), 4.76 (0.5H, m), 4.93—5.08 (1H, m), 5.26—5.47 (1.5H, m), 6.73—6.83 (2H, m), 7.32—7.68 (5.5H, m), 7.79 (0.5H, s), 7.82 (0.5H, s), 8.24 (0.5H, s), 8.85 (0.5H, br), 8.94 (0.5H, s), 8.96 (0.5H, s), 9.15 (0.5H, s). IR (KBr): 3312, 3112, 2978, 1651, 1524, 1499, 1470, 1248, 1134 cm^{-1}. $[\alpha]_D^{20}$: +110.4° (c=1.0, MeOH).

(1*R*,2*S*)-**8**: Compound (2*S*,3*R*)-**7** was converted to (1*R*,2*S*)-**8** by the reaction with **10** under the same condition as described above. Yield 82% (a white powder). SI-MS (*m/z*): 572 (MH^+). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7 Hz), 1.32 (3H, t, J=7 Hz), 1.51 (3H, d, J=7 Hz), 3.21 (1H, br), 3.44—3.98 (6H, m), 4.55 (1H, d, J=14 Hz), 4.52—4.63 (1H, m), 4.75 (1H, d, J=14 Hz), 6.71—6.86 (2H, m), 7.33 (2H, d, J=9 Hz), 7.42—7.55 (1H, m), 7.57 (2H, d, J=9 Hz), 7.66 (1H, s), 7.83 (1H, s), 8.20 (1H, s), 8.52 (1H, s), 8.92 (1H, s). IR (KBr): 3312, 3131, 2978, 1651, 1524, 1499, 1472, 1273, 1132 cm^{-1}. $[\alpha]_D^{20}$: −172.4° (c=1.0, MeOH).

1-[(1*S*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-(1*H*,3*H*)-imidazolone [(1*S*,2*R*)-9] 1 N HCl (46.5 ml) was added to a solution of (1*S*,2*R*)-**8** (2.66 g) in MeOH (47 ml). The mixture was stirred for 5 h at 55 °C and cooled. After having been neutralized with 2 N NaOH (23.5 ml), the whole was evaporated *in vacuo* to remove MeOH and extracted with AcOEt–THF (1 : 1, v/v; 120 ml). The extract was washed with water (50 ml) and brine (50 ml), successively, dried over $MgSO_4$ and evaporated *in vacuo.* The residue was purified by silica gel column chromatography (AcOEt → AcOEt/MeOH, 7 : 1, v/v) and crystallized from AcOEt–THF to give (1*S*,2*R*)-**9** (1.83 g, 82%) as a white crystalline powder. mp 203—204 °C. *Anal.* Calcd for $C_{22}H_{19}F_2N_9O_2$: C, 55.11; H, 3.99; N, 26.29. Found: C, 55.08; H, 4.19; N, 26.10. ^{1}H-NMR ($CDCl_3$) δ: 1.61 (3H, d, J=7 Hz), 4.57 (1H, d, J=14 Hz), 4.82 (1H, q, J=7 Hz), 4.88 (1H, d, J=14 Hz), 6.31—6.46 (3H, m), 6.63—6.78 (2H, m), 7.35—7.47 (1H, m), 7.64 (2H, d, J=9 Hz), 7.73 (2H, d, J=9 Hz), 7.75 (1H, s), 8.06 (1H, s), 8.99 (1H, s). IR (KBr): 3125, 1684, 1617, 1526, 1429, 1271, 1256, 733 cm^{-1}. $[\alpha]_D^{20}$: −97.1° [c=1.0, dimethylsulfoxide (DMSO)].

(1*S*,2*S*)-**9**: Compound (1*S*,2*S*)-**8** was converted to (1*S*,2*S*)-**9** under the same condition as described above. Yield 64% (a white crystalline powder). mp 196—197 °C (AcOEt–$isoPr_2O$). *Anal.* Calcd for $C_{22}H_{19}F_2N_9O_2$: C, 55.11; H, 3.99; N, 26.29. Found: C, 55.07; H, 4.05; N, 26.27. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, d, J=7 Hz), 4.22 (1H, d, J=14 Hz), 5.03 (1H, q, J=7 Hz), 5.15 (1H, d, J=14 Hz), 5.46 (1H, br), 6.74—6.88 (4H, m), 7.42—7.54 (1H, m), 7.76 (1H, s), 7.83 (2H, d, J=9 Hz), 7.87 (1H, s), 7.97 (2H, d, J=9 Hz), 9.08 (1H, s). IR (KBr): 3400, 3127, 1682, 1615, 1524, 1429, 1254, 1140 cm^{-1}. $[\alpha]_D^{20}$: +15.4° (c=1.0, MeOH).

(1*R*,2*S*)-**9**: Compound (1*R*,2*S*)-**8** was converted to (1*R*,2*S*)-**9** under the same condition as described above. Yield 74% (a white crystalline powder). mp 204—205 °C (AcOEt–THF). *Anal.* Calcd for $C_{22}H_{19}F_2N_9O_2$: C, 55.11; H, 3.99; N, 26.29. Found: C, 54.84; H, 3.94; N, 25.99. ^{1}H-NMR ($CDCl_3$) δ: 1.61 (3H, d, J=7 Hz), 4.57 (1H, d, J=14 Hz), 4.81 (1H, q, J=7 Hz), 4.88 (1H, d, J=14 Hz), 6.34—6.46 (3H, m), 6.63—6.78 (2H, m), 7.35—7.47 (1H, m), 7.63 (2H, d, J=9 Hz), 7.73 (2H, d, J=9 Hz), 7.75 (1H, s), 8.06 (1H, s), 8.98 (1H, s). IR (KBr): 3127, 1682, 1617, 1526, 1429, 1271, 1256 cm^{-1}. $[\alpha]_D^{20}$: +99.0° (c=1.0, DMSO).

1-[(1*S*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone [(1*S*,2*R*)-1: Table 1] A solution of (1*S*,2*R*)-**9** (1.03 g) in AcOH (75 ml) was hydrogenated over 10% Pd–C (50% wet, 1.03 g) for 24 h under an atmospheric pressure. The catalyst was filtered off and washed with AcOH (10 ml). The filtrate and the washing were combined and concentrated *in vacuo.* The concentrate was worked up (AcOEt; water, aqueous. $NaHCO_3$, brine). The residue was purified by silica gel column chromatography (AcOEt–MeOH, 7 : 1, v/v) and crystallized from EtOH to give (1*S*,2*R*)-**1** (0.31 g, 30%) as white powdery crystals.

(1*S*,2*S*)-**1** and (1*R*,2*S*)-**1** (Table 1): These stereoisomers were prepared in the same manner as above from the corresponding optically active imidazolones [(1*S*,2*S*)-**9** and (1*R*,2*S*)-**9**].

The % de of the stereoisomers was determined by HPLC using an ODS column under the following conditions: mobile phase, MeOH–water–AcOH, 7 : 3 : 0.02, v/v; flow rate, 0.8 ml/min; detection, UV at 262 nm. The % ee of the stereoisomers was determined by HPLC using a chiral stationary phase column (Chiralpak AS 4.6 mm i.d.×250 mm, Daicel Chemical Industries, Tokyo, Japan) under the following conditions: mobile phase, hexane–EtOH, 3 : 7, v/v; flow rate, 0.8 ml/min; detection, UV at 262 nm.

Ethyl *N*-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]glycinate (16) A mixture of **15** (1.04 g),[6] THF (15 ml), $BrCH_2COOEt$ (0.65 ml) and Et_3N (1.08 ml) was stirred for 22 h under a nitrogen atmosphere. An additional amount of $BrCH_2COOEt$ (0.22 ml) and Et_3N (0.27 ml) was added to the reaction mixture. The whole was stirred for further 63 h under a nitrogen atmosphere and concentrated *in vacuo.* The concentrate was dissolved in AcOEt (70 ml) and washed with 5% aqueous. $NaHCO_3$ (35 ml) and brine (35 ml). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo.* The residue was purified by silica gel column chromatography (AcOEt–hexane, 1 : 3 → 1 : 4, v/v) to give **16** (1.1 g, 80%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, dd, J=6.6, 1.2 Hz), 1.30 (3H, t, J=7 Hz), 1.46 (1H, br), 3.11 (1H, dq, J=6.6, 1.8 Hz), 4.22 (2H, q, J=7 Hz), 4.82 (1H, d, J=14 Hz), 4.95 (1H, d, J=14 Hz), 4.96 (1H, s), 6.69—6.81 (2H, m), 7.36—7.49 (1H, m), 7.76 (1H, s), 7.94 (1H, s). IR (KBr): 3331, 2980, 1736, 1618, 1597, 1501, 1420, 1273, 1138 cm^{-1}. SI-MS (*m/z*): 355 (MH^+).

1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2,4-imidazolidinedione (11) A mixture of **16** (0.92 g), **10** (0.88 g) and DMF (10 ml) was stirred at 80 °C for 20 h under a nitrogen atmosphere. After having been cooled, the mixture was diluted with AcOEt (100 ml) and washed with water (50 ml). The aqueous layer was extracted with AcOEt (30 ml). The AcOEt layers were combined and washed with 0.1 N HCl (50 ml), water (50 ml) and brine (50 ml), successively. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo.* The residue was purified by silica gel column chromatography (AcOEt–hexane, 6 : 1 → AcOEt–acetone, 6 : 1, v/v) and crystallized from AcOEt–$isoPr_2O$ to give **11** (1.0 g, 78%) as white powdery crystals. mp 136—137 °C. *Anal.* Calcd for $C_{22}H_{19}F_2N_9O_3$: C, 53.33; H, 3.87; N, 25.44. Found: C, 53.10; H, 3.89; N, 25.15. ^{1}H-NMR ($CDCl_3$) δ: 1.13 (3H, d, J=7 Hz), 4.27 (1H, d, J=19 Hz), 4.47 (1H, d, J=14 Hz), 4.69 (1H, d, J=19 Hz), 5.00 (1H, dq, J=7, 1.8 Hz), 5.17 (1H, d, J=14 Hz), 5.39 (1H, d, J=1.8 Hz), 6.75—6.87 (2H, m), 7.34—7.46 (1H, m), 7.76—7.88 (6H, m), 9.06 (1H, s). IR (KBr): 3385, 3127, 1771, 1715, 1617, 1524, 1424, 1207 cm^{-1}. $[\alpha]_D^{20}$: −65.6° (c=1.0, MeOH).

(4*S*)-1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-hydroxy-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (3a: Table 2) and (4*R*)-1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-hydroxy-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (3b: Table 2) Compound **11** (0.70 g) was added to a stirred solution of $NaBH_4$ (0.11 g) in EtOH (60 ml) at 0 °C under a nitrogen atmosphere. The mixture was stirred at 0 °C under a nitrogen atmosphere. During stirring, $NaBH_4$ (0.11 g×3) was added to the mixture at 30 min intervals. After the final addition of $NaBH_4$, the whole was stirred for further 1.5 h at 0 °C under a nitrogen atmosphere and diluted with AcOEt (100 ml). The solution was washed with brine (50 ml×3), dried over $MgSO_4$ and evaporated *in vacuo.* The residue was purified by silica gel column chromatography (AcOEt → AcOEt–acetone, 8 : 1, v/v). The first eluate containing **3a** as the major component was evaporated *in vacuo.* The residue was crystallized from AcOEt to give a white crystalline powder, which was recrystallized from acetone-AcOEt to give **3a** (0.22 g, 31%) as colorless prisms. The second eluate containing **3a** and **3b** in a ratio of 36 : 64 was evaporated *in vacuo* to give a white residue (0.12 g, 17%), which was separated using ODS column chromatography [acetonitrile (MeCN)–water, 3 : 7, v/v; flow rate 0.3 ml/min]. The fractions containing **3b** was combined and evaporated *in vacuo.* The residue was dissolved in AcOEt (100 ml) and washed with water (50 ml) and brine (40 ml), successively. The AcOEt layer was dried over $MgSO_4$ and evaporated *in vacuo* to give **3b** (0.042 g, 6%) as a white amorphous powder.

Ethyl *N*-[4-(1*H*-1-Tetrazolyl)phenyl]glycinate (19) A mixture of 1-(4-aminophenyl)-1*H*-tetrazole (6.0 g),[2a,b] THF (100 ml), $BrCH_2COOEt$ (4.56 ml) and Et_3N (5.7 ml) was stirred for 4 h at 50 °C. An additional amount of $BrCH_2COOEt$ (4.56 ml) and Et_3N (5.7 ml) was added to the reaction mixture. The whole was stirred for further 16 h at 50 °C. After having been cooled, the mixture was worked up (AcOEt; water, brine). Diethyl ether (Et_2O, 100 ml) was added to the residue and the resulting precipitates were collected by filtration to give **19** (8.77 g, 95%) as a white powder. *Anal.*

Calcd for $C_{11}H_{13}N_5O_2$: C, 53.43; H, 5.30; N, 28.32. Found: C, 53.43; H, 5.25; N, 28.06. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (3H, t, J=7 Hz), 3.99 (2H, d, J=6 Hz), 4.14 (2H, q, J=7 Hz), 6.58 (1H, br), 6.75 (2H, d, J=8.8 Hz), 7.56 (2H, d, J=8.8 Hz), 9.85 (1H, s). IR (KBr): 3384, 3140, 1725, 1611, 1534, 1231, 828 cm^{-1}.

Ethyl *N*-Phenoxycarbonyl-*N*-[4-(1*H*-1-tetrazolyl)phenyl]glycinate (17) Pyridine (0.65 ml) and phenyl chlorocarbonate (PhOCOCl, 1.21 ml) were added dropwise to a solution of **19** (1.0 g) in a mixture of DMF (10 ml) and THF (10 ml). After having been stirred for 5 h, the whole was worked up (AcOEt; water). The residue was purified by silica gel column chromatography (AcOEt–hexane, 2 : 1, v/v) to give **17** (0.90 g, 61%) as a colorless oil. *Anal.* Calcd for $C_{18}H_{17}N_5O_4$: C, 58.85; H, 4.66; N, 19.06. Found: C, 58.72; H, 4.65; N, 18.89. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7 Hz), 4.28 (2H, q, J=7 Hz), 4.51 (2H, s), 7.11—7.40 (5H, m), 7.68 (1H, d, J=8.8 Hz), 7.76 (1H, d, J=8.8 Hz), 9.03 (1H, s).

Phenyl *N*-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]carbamate (18a) PhOCOCl (0.41 ml) was added dropwise to a solution of **15** (1.07 g) in a mixture of pyridine (0.24 ml) and DMF (20 ml). After having been stirred for 1 h, the whole was worked up (AcOEt; water). The residue was purified by silica gel column chromatography (AcOEt–hexane, 1 : 1→5 : 2, v/v) and crystallized from Et_2O–isoPr$_2O$ to give **18a** (1.0 g, 85%) as white powdery crystals. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, d, J=7 Hz), 4.48–4.56 (1H, m), 4.63 (1H, d, J=14 Hz), 5.04 (1H, d, J=14 Hz), 5.25 (1H, d, J=1.4 Hz), 5.65 (1H, d, J=9 Hz), 6.72—6.82 (2H, m), 7.15—7.43 (6H, m), 7.79 (1H, s), 7.83 (1H, s).

4-Nitrophenyl *N*-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)]-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]carbamate (18b) 4-Nitrophenyl chlorocarbonate (0.89 ml) was added dropwise to a solution of **15** (1.07 g) in a mixture of pyridine (0.32 ml) and THF (20 ml). After having been stirred for 1 h, the whole was worked up (AcOEt; water). The residue was purified by silica gel column chromatography (AcOEt–hexane, 1 : 1→3 : 1, v/v) to give **18b** (0.34 g, 20%) as a white powder. ^{1}H-NMR ($CDCl_3$) δ: 1.03 (3H, d, J=7 Hz), 4.48—4.57 (1H, m), 4.63 (1H, d, J=14 Hz), 5.02 (1H, d, J=14 Hz), 5.30 (1H, d, J=1.4 Hz), 5.76 (1H, d, J=14 Hz), 6.74—6.87 (2H, m), 7.31—7.44 (3H, m), 7.81 (1H, s), 7.85 (1H, s), 8.24—8.32 (2H, m).

***N*-[4-(1*H*-1-tetrazolyl)phenyl]glycine (20)** 1 N NaOH (25.4 ml) and water (100 ml) were added to a solution of **19** (5.23 g) in a mixture of THF (150 ml) and MeOH (100 ml). After having been stirred for 30 min, the mixture was neutralized with AcOH (4 ml) and concentrated *in vacuo.* The concentrate was dissolved in a mixture of AcOEt (100 ml) and THF (50 ml). The solution was washed with water (100 ml), dried over $MgSO_4$ and concentrated *in vacuo.* The precipitated crystals were collected by filtration and dried over phosphorous pentoxide (P_2O_5) at 40 °C *in vacuo* to obtain **20** (3.84 g, 74%) as pale brown powdery crystals. mp 202—203 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.89 (2H, s), 6.48 (1H, br), 6.74 (2H, d, J=8.8 Hz), 7.55 (2H, d, J=8.8 Hz), 9.84 (1H, s). IR (KBr): 3393, 2892, 1725, 1613, 1534, 1217, 828 cm^{-1}.

***N*-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-2-[4-(1*H*-1-tetrazolyl)phenyl]amimoacetamide (21)** DEPC (0.72 ml) and Et_3N (0.6 ml) were added successively to a mixture of **15** (1.07 g), **20** (0.87 g) and DMF (30 ml) at 0 °C. After having been stirred for 1 h, the mixture was worked up (AcOEt; water). The residue was purified by chromatography on silica gel (AcOEt–THF, 2 : 5, v/v) and the eluate was concentrated *in vacuo.* The precipitated crystals were collected by filtration and dried over P_2O_5 *in vacuo.* Compound **21** (1.65 g, 88%) was obtained as white powdery crystals. mp 89.5—90.5 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, d, J=7 Hz), 3.97 (2H, s), 4.37 (1H, d, J=14 Hz), 4.81 (2H, q, J=7 Hz), 4.95 (1H, d, J=14 Hz), 6.68—6.82 (3H, m), 7.02 (1H, br), 7.21—7.38 (1H, m), 7.51 (2H, d, J=8.8 Hz), 7.79 (2H, d, J=8.8 z), 8.89 (1H, s). IR (KBr): 3333, 1665, 1613, 1526, 1501, 1273, 1140 cm^{-1}. $[\alpha]_D^{20}$: −53.1° (c=1.0, MeOH).

***tert*-Butyl *N*-[4-(1*H*-1-Tetrazolyl)phenyl]glycinate (23)** A mixture of 1-(4-aminophenyl)-1*H*-tetrazole (1.02 g),[2a,b] DMF (30 ml), *tert*-butyl chloroacetate (1.36 ml), Et_3N (1.32 ml) and potassium iodide (KI, 10.5 g) was stirred for 15 h at 50 °C under a nitrogen atmosphere. An additional amount of *tert*-butyl chloroacetate (20.4 ml), Et_3N (15.8 ml) and KI (31.5 g) was added to the mixture. The whole was stirred for further 5 d at 50 °C, cooled and worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (AcOEt–hexane, 1 : 1, v/v) and crystallized from AcOEt to give **23** (0.49 mg, 28%) as white powdery crystals. mp 104—105 °C. *Anal.* Calcd for $C_{13}H_{17}N_5O_2$: C, 56.71; H, 6.22; N, 25.44. Found: C, 56.75; H, 6.19; N, 25.55. ^{1}H-NMR ($CDCl_3$) δ: 1.51 (9H, s), 3.86 (2H, d, J=5.2 Hz), 4.68 (1H, br), 6.67—6.73 (2H, m), 7.44—7.50 (2H, m), 8.85 (1H, s). IR (KBr): 3368, 1723, 1611, 1534, 1235, 1161 cm^{-1}.

***tert*-Butyl *N*-Ethoxycarbonyl-*N*-[4-(1*H*-1-tetrazolyl)phenyl]glycinate (24)** Pyridine (0.30 ml) and ClCOOEt (0.36 ml) were added dropwise to a solution of **23** (0.43 g) in a mixture of DMF (2 ml) and THF (8 ml). After the mixture was stirred for 2 h, an additional amount of ClCOOEt (0.36 ml) was added. After having been stirred for 15 h, the whole was diluted with AcOEt (50 ml), washed with 5% aqueous phosphoric acid solution (30 ml), water (30 ml) and brine (20 ml), successively. The AcOEt layer was dried over $MgSO_4$ and concentrated *in vacuo.* The concentrate was purified by silica gel column chromatography (AcOEt–hexane, 1 : 1, v/v) and crystallized from AcOEt–isoPr$_2O$ to give **24** (0.46 mg, 85%) as white powdery crystals. mp 113—114 °C. *Anal.* Calcd for $C_{16}H_{21}N_5O_4$: C, 55.32; H, 6.09; N, 20.16. Found: C, 55.55; H, 5.91; N, 20.41. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7 Hz), 1.49 (9H, s), 4.23 (2H, q, J=7 Hz), 4.28 (2H, s), 7.55 (2H, d, J=9 Hz), 7.70 (2H, d, J=9 Hz), 8.98 (1H, s). IR (KBr): 3123, 2982, 1742, 1709, 1522, 1231, 1155 cm^{-1}.

***N*-Ethoxycarbonyl-*N*-[4-(1*H*-1-tetrazolyl)phenyl]glycine (25)** 4 N Hydrogen chloride–AcOEt solution (20 ml) was added to a solution of **24** (0.49 g) in AcOEt (10 ml). After having been stirred for 17 h, the mixture was diluted with AcOEt (70 ml). The AcOEt solution was washed with brine (40 ml) and water (40 ml), successively, dried over $MgSO_4$ and evaporated *in vacuo* to give **25** (0.38 g, 85%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7 Hz), 4.2 (2H, q, J=7 Hz), 4.4 (2H, s), 7.5 (2H, d, J=8.8 Hz), 7.73 (2H, d, J=8.8 Hz), 8.00 (1H, br), 9.07 (1H, s). IR (KBr): 3125, 2986, 1705, 1607, 1522, 1381, 1213 cm^{-1}.

Ethyl *N*-[2-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]amino-2-oxoethyl]-*N*-[4-(1*H*-1-tetrazolyl)-phenyl]carbamate (22) 1) Pyridine (0.49 ml) and ClCOOEt (0.59 ml) were added successively to a solution of **21** (1.2 g) in DMF (20 ml) at 0 °C. After having been stirred for 17 h, the mixture was worked up (AcOEt; water). The residue was purified by silica gel column chromatography (AcOEt → AcOEt–THF, 5 : 1, v/v) and crystallized from isoPr$_2O$ to give **22** (1.02 g, 75%) as white powdery crystals. mp 183.5—184.5 °C. *Anal.* Calcd for $C_{24}H_{25}F_2N_9O_4$: C, 53.23; H, 4.65; N, 23.28. Found: C, 53.12; H, 4.79; N, 23.06. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, d, J=6.6 Hz), 1.26 (3H, t, J=7.2 Hz), 4.11—4.92 (5H, m), 4.90 (1H, q, J=6.6 Hz), 4.93 (1H, d, J=14 Hz), 5.26 (1H, s), 6.53—6.82 (3H, m), 7.35—7.50 (1H, m), 7.67—7.79 (4H, m), 8.99 (1H, s). IR (KBr): 3316, 1699, 1617, 1522, 1273, 1140 cm^{-1}. $[\alpha]_D^{20}$: −17.4° (c=1.0, MeOH).

2) DEPC (0.21 ml) was added dropwise over a period of 5 min to a mixture of **25** (0.38 g), **15** (0.35 g), Et_3N (0.2 ml) and DMF (11.5 ml) at 0 °C under a nitrogen atmosphere. After having been stirred for 1.5 h, the mixture was worked up (AcOEt; water, brine). The residue was purified by silica gel column chromatography (AcOEt–THF, 5 : 1→1 : 1, v/v) and crystallized from isoPr$_2O$–AcOEt to give **22** (0.61 g, 87%) as white powdery crystals.

1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2,5-imidazolidinedione (12) TBAF (9.23 ml) was added dropwise over a period of 5 min to a solution of **22** (5.0 g) in THF (200 ml). After having been stirred for 1 h, the mixture was stirred for further 8 h at 30 °C. AcOH (0.52 ml) was added to the mixture, and the resulting mixture was worked up (AcOEt; brine). The residue was purified by silica gel column chromatography (AcOEt–Et_2O, 4 : 1, v/v) to give **12** (1.23 g, 27%) as a white powder with recovery of **22** (1.15 g, 23%). *Anal.* Calcd for $C_{22}H_{19}F_2N_9O_3 \cdot 1/2$AcOEt: C, 53.43; H, 4.30; N, 23.37. Found: C, 53.40; H, 4.36; N, 23.31. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, d, J=7 Hz), 4.58—5.02 (5H, m), 5.83 (4/7H, s), 5.91 (3/7H, s), 6.89—6.97 (1H, m), 7.16—7.26 (1H, m), 7.32—7.48 (1H, m), 7.61 (1H, s), 7.92—8.03 (4H, m), 8.27 (1H, d, J=4 Hz), 10.10 (1H, s). IR (KBr): 3345, 3129, 1771, 1705, 1618, 1524, 1208, 1138 cm^{-1}. $[\alpha]_D^{20}$: −54.9° (c=1.0, MeOH).

(5*R*)-1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-5-hydroxy-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (4a: Table 2) and (5*S*)-1-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-5-hydroxy-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (4b: Table 2) $NaBH_4$ (0.55 g) was added to a solution of **12** (2.56 g) in EtOH (170 ml) at 0 °C under a nitrogen atmosphere. After having been stirred for 2.5 h at 0 °C, an additional amount of $NaBH_4$ (0.1 g) was added to the mixture. The whole was stirred for further 2.5 h at 0 °C and diluted with AcOEt (350 ml), water (170 ml) and brine (170 ml). AcOEt layer was separated and the aqueous layer was extracted with AcOEt (100 ml). The AcOEt layers were combined, washed with water (10 ml×2), dried over $MgSO_4$ and evaporated *in vacuo.* The residue was submitted silica gel column chromatography (AcOEt–acetone, 12 : 1, v/v). The eluate containing **4a** and **4b** was evaporated *in vacuo* and the residue was crystallized from acetone–MeOH–AcOEt to give the more polar product **4b** (1.71 g, 67%) as colorless prisms. The mother liquor of this crystallization was evaporated *in vacuo* and the residue was submitted to sil-

ica gel column chromatography (AcOEt–acetone–hexane, 1 : 1 : 1, v/v) to obtain the less polar product **4a** (0.34 g, 13%) as a white amorphous powder.

Antifungal Activity A double concentration of RPMI-1640 medium (Gibco BRL, Grand Island, N.Y.) was prepared with 0.3 M morpholinepropanesulfonic acid (MOPS; Dojindo, Tokyo, Japan) buffer (pH 7.0), sterilized by filtration through a membrane filter (pore size, 0.45 μm) and mixed with an equal volume of 3.0% agar (Wako, Osaka, Japan) which had been autoclaved at 121 °C for 15 min and kept at 55 °C. The agar medium (9.9 ml) was then poured into petri dishes containing 0.1 ml of serial dilutions of antifungal agents dissolved in DMSO and allowed to solidify. About 10^3 CFU of fungal cells suspended in saline was inoculated with a multiple inoculator (Sakuma, Tokyo, Japan) onto the agar plates prepared above. For the measurement of *in vitro* antifungal activities against *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida utilis* and *Cryptococcus neoformans*, the plates were incubated in a CO_2 incubator at 35 °C for 20 h. After MICs for *Candida* species were determined, the plates were incubated in a CO_2 incubator for an additional 48 h to determine MICs for *Cryptococcus neoformans.* The MIC was defined as the lowest concentration of an antifungal agent giving no visible growth or causing almost complete inhibition of growth. In the other hand, the plates inoculated with *Aspergillus fumigatus* and *Aspergillus niger* were incubated in an ordinary incubator at 35 °C for 20 h. The MIC was defined as the lowest concentration of an antifungal agent giving no visible growth.

Analysis of Rat Liver Homogenate The animals used in this study were male CD (SD) IGS rats (8-weeks old; Charles River Japan Inc., Kanagawa, Japan). They were fed laboratory chow (CE-2, CLEA Japan Inc., Tokyo, Japan), had free access to water and were housed in temperature- and humidity-controlled rooms (20 to 26 °C, 40 to 75% R.H.) with 12 h light-dark cycles for more than a week before use.

TAK-456 was suspended in aqueous 0.5% methylcellulose solution at a dose of 30 mg/5 ml/kg and administered orally to fed animals. The liver of rats was excised under ether anesthesia at 4 h after dosing. The liver was immediately homogenized with a 4-fold amount (w/v) of ice-cold saline. The homogenate was kept frozen at −80 °C until analysis.

The rat liver homogenate (2 ml) was deproteinized with MeCN (3 ml). After centrifugation at 19000 ***g*** for 10 min, to the supernatant (4 ml) was added 0.01 N aqueous ammonium acetate solution (0.01 N aqueous NH_4OAc, 4 ml) adjusted to pH 6.0 with AcOH. The solution (100 μl) was injected to an HPLC and analyzed. To the supernatant (1 ml) was added 6 N HCl (0.2 ml) and the resulting mixture was incubated at 37 °C for 20 min. After addition of 1 N aqueous NaOH (0.12 ml), the mixture (100 μl) was injected to an HPLC and analyzed. The eluates corresponding to the peak A and the new strong peak appeared after HCl-treatment were individually collected, and the aliquot (50 μl) was injected to an LC/MS/MS to determine the molecular weight.

HPLC conditions: The HPLC system consisted of an LC-10AD pump, an SIL-10A autosampler, a CTO-10AC column oven, an SPD10AV UV-detector, and a CLASS-LC10 workstation (all from Shimadzu Co., Kyoto, Japan). The analytical column was an L-Column ODS (5 mm particle size, 4.6 mm i.d.×150 mm, Chemicals Evaluation and Research Institute, Tokyo, Japan). The mobile phase was a mixture of 0.01 N aqueous NH_4OAc (pH 6.0, adjusted with AcOH) and MeCN (68 : 32, v/v). The column temperature and the flow-rate were 40 °C and 1.0 ml/min, respectively. UV detection was carried out at 274 nm.

LC/MS/MS conditions: A 717 puls autosampler (Nihon Waters K. K., Tokyo, Japan), an LC-10AD pump (Shimadzu Co., Kyoto, Japan) and a Perkin-Elmer Sciex API 3000 triple quadrupole mass spectrometer (Perkin-Elmer Sciex, Canada) with a turbo ionspray source for atomospheric pressure ionization were used for the analysis. Data processing was achieved with the PE software (Sample Control version 1.4 and Multivew version 1.4). The analytical column was an L-column ODS (2.1 mm i.d.×150 mm). The mobile phase was a mixture of 0.01 N aqueous ammonium formate solution (pH 3.0, adjusted with formic acid) and MeCN (1 : 1, v/v). The column temperature and the flow-rate were 40 °C and 0.2 ml/min, respectively. The ionspray and orifice voltage, and temperature were set at 5400 V, 50 V, and 425 °C, respectively. Data acquisition was in the positive ionization Q1 scan mode using 1 ms dwell time per transition.

X-Ray Crystallographic Analysis A single crystal (0.40×0.20×0.06 mm) of **3a** was obtained by recrystallization from acetone. A single crystal (0.80×0.12×0.05 mm) of **4b** was obtained by recrystallization from mixture of aceton and AcOEt. The reflection data collected on a Rigaku AFC5R diffractometer with graphite monochromated Cu$K\alpha$ radiation. The structure were solved by direct methods using the program SIR92[14] for **3a**, and using the program SYSTEM90[15] for **4b**. The structures were then refined by the full-matrix least-squares refinement (SHELXL-97[16]) with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms. Crystal data for **3a**; $C_{22}H_{21}F_2N_9O_3$; M=497.46; orthorhombic, space group $P2_12_12_1$ (#19), a=15.750 (2), b=23.339 (4), c=6.214 (4) Å, V=2284 (1) Å^3, Z=4, D_c=1.446 g/cm^3, $R1$=0.046, $wR2$=0.128 for 1194 observed reflections with $I>2\sigma(I)$. Crystal data for **4b**; $C_{22}H_{21}F_2N_9O_3 \cdot 1/2CH_3COOC_2H_5$; M=541.52; orthorhombic, space group $P2_12_12_1$ (#19), a=18.166 (4), b=32.932 (5), c=8.519(5) Å, V=5096 (2) Å^3, D_c=1.411 g/cm^3, Z=8, $R1$=0.073, $wR2$=0.252 for 1779 observed reflections with $I>2\sigma(I)$.

Acknowledgements We thank Drs. S. Kishimoto and A. Miyake for their encouragement throughout this work. We also thank Mr. T. Yoshida for the biological assay.

References and Notes

1) Part XII: Ichikawa T., Kitazaki T., Matsushita Y., Yamada M., Hayashi R., Yamaguchi M., Kiyota Y., Okonogi K., Itoh K., *Chem. Pharm. Bull*., **49**, 1102—1109 (2001).
2) *a*) Kitazaki T., Ichikawa T., Tasaka A., Hosono H., Matsushita Y., Hayashi R., Okonogi K., Itoh K., *Chem. Pharm. Bull*., **48**, 1935—1946 (2000); *b*) Ichikawa T., Kitazaki T., Matsushita Y., Hosono H., Yamada M., Mizuno M., Itoh K., *ibid*., **48**, 1947—1953 (2000).
3) *a*) Tsuchimori N., Hayashi R., Kitamoto N., Asai K., Kitazaki T., Iizawa Y., Itoh K., Okonogi K., *Antimicrob. Agents Chemother*., submitted; *b*) Hayashi R., Kitamoto N., Iizawa Y., Ichikawa T., Itoh K., Kitazaki T., Okonogi K., *ibid*., submitted.
4) *a*) Tasaka A., Tamura N., Matsushita Y., Teranishi K., Hayashi R., Okonogi K., Itoh K., *Chem. Pharm. Bull*., **41**, 1035—1042 (1993); *b*) Tasaka A., Kitazaki T., Tsuchimori N., Matsushita Y., Hayashi R., Okonogi K., Itoh K., *ibid*., **45**, 321—326 (1997); *c*) Tasaka A., Tsuchimori N., Kitazaki T., Hiroe K., Hayashi R., Okonogi K., Itoh K., *ibid*., **43**, 441—449 (1995); *d*) Kitazaki T., Tamura N., Tasaka A., Matsushita Y., Hayashi R., Okonogi K., Itoh K., *ibid*., **44**, 314—327 (1996); *e*) Konosu T., Miyaoka T., Tajima Y., Oida S., *ibid*., **39**, 2241—2246 (1991); *f*) Konosu T., Tajima Y., Miyaoka T., Oida S., *Tetrahedron Lett*., **32**, 7545—7548 (1991); *g*) Konosu T., Miyaoka T., Tajima Y., Oida S., *Chem. Pharm. Bull*., **40**, 562—564 (1992).
5) This method has been already reported in ref. 4*e*.
6) *a*) Konosu T., Tajima Y., Takeda N., Miyaoka T., Kashihara M., Yasuda H., Oida S., *Chem. Pharm. Bull*., **39**, 2581—2589 (1991); *b*) Bartroli J., Turmo E., Belloc J., Form J., *J. Org. Chem*., **60**, 3000—3012 (1995).
7) The ratio was determined by HPLC on an ODS column under the following conditions: mobile phase, MeOH–H_2O–AcOH, 7 : 3 : 0.02, v/v; flow rate, 0.8 ml/min; detection UV at 262 nm.
8) This conversion was monitored by HPLC using an ODS column and the conditions shown in ref. 7.
9) Albericio F., Barany G., *Int. J. Protein Res*., **30**, 177—205 (1987).
10) These epimers were not separable on an ODS column under the conditions shown in ref. 7.
11) It was confirmed that the treatment of **4a**, **b** with hydrochloric acid gave (1*R*,2*R*)-**9** similarly to the case of **3a**, **b**.
12) The agar dilution method for *in vitro* susceptibility testing of antifungal agents under 20% CO_2 was developed in our laboratories: Yoshida T., Jono K., Okonogi K., *Antimicrob. Agents Chemother*., **41**, 1349—1351 (1997).
13) Synthesis of this compound was carried out starting from ethyl (*S*)-lactate according to the method described in ref. 4*a*. This oxirane is a mixture of (2*S*)-2-(2,4-difluorophenyl)-2-[(1*S*)-1-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)ethyl]oxirane and (2*R*)-2-(2,4-difluorophenyl)-2-[(1*S*)-1-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)ethyl]oxirane in a ratio of *ca* 4 : 1 and was used without separation.
14) Altomare A., Burla M. C., Camalli M., Cascarano M., Giacovazzo C., Guagliardi A., Polidori G., *J. Appl. Cryst*., **27**, 435 (1994).
15) Yonggeng H., Min G., Lipu L., Peixin H., *Acta Cryst*., **A50**, 748—753 (1994).
16) SHELXL-97: Sheldrick G. M. (1997). Program for the refinement of crystal structures. University of Göttingen, Germany.