Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2475–2486

BIOORGANIC & MEDICINAL CHEMISTRY

Design and Synthesis of Potential Inhibitors of the Ergosterol Biosynthesis as Antifungal Agents

Sung-Kee Chung,[a,*] Ki-Wha Lee,[a] Heui Il Kang,[b] Chika Yamashita,[c] Makiko Kudo[c] and Yuzo Yoshida[c]

[a]*Department of Chemistry, Division of Molecular & Life Sciences, Pohang University of Science & Technology, Pohang 790-784, South Korea*

[b]*Yuhan Research Center, Gunpo, Kyunggi-do 435-030, South Korea*

[c]*School of Pharmaceutical Sciences, Mucogawa Women's University, Nishinomiya 663-8179, Japan*

Received 14 March 2000; accepted 26 June 2000

Abstract—A series of azolylmethyloxolane derivatives with modified sterol side-chain structures, designed as potential dual functional inhibitors of cytochrome P450 14α-demethylase (14DM) and Δ^{24}-sterol methyltransferase (24-SMT) based on the common characteristic features of 24-aminosterols and azole antifungal agents, were synthesized and evaluated for their antifungal activities and inhibitory activities of 14DM and 24-SMT. Among these compounds, imidazolylmethyloxolane derivatives **28a** and **28b** showed potent in vitro antifungal activities comparable to those of itraconazole. However, the in vitro bioactivities have not been linearly translated into in vivo protection data for some unknown reasons.

Introduction

During the past two decades, the frequencies of invasive and systemic fungal infections have increased dramatically in the population with altered immunity. Unfortunately, the available drugs have shown only limited utility in many cases by reason of drug toxicity and resistance. Thus, there is a need for novel therapies for serious fungal diseases as well as for the management of the legions of topical fungal infections.[1]

Ergosterol is the dominant sterol in most fungi with the notable exception of the Oomycete genera *Pythium* and *Phytophthora*, which apparently do not synthesize any sterol. Both ergosterol and cholesterol, the major mammalian sterol, are synthesized from acetyl-CoA via a series of enzymatic reactions. Lanosterol represents the key branching point in the biosynthesis of ergosterol and cholesterol, since the biosynthetic steps leading to lanosterol are common to both fungi and animals. Conversion of lanosterol to ergosterol involves multistep processes that are catalyzed by membrane-bound enzymes. The precise sequence in which these reactions occur appears to be dependent upon fungal species, but in most fungi, with a possible exception of *Saccharomyces*, the first step is the C-24 methylation and it is followed by the sequential demethylations at C-14 and C-4. Once the methylation and demethylations have taken place, various double bond transformations occur and the exact order of the double bond transformation may also vary depending on organisms.[2] It is known that *S*-adenosyl-L-methionine: Δ^{24}-sterol methyltransferase (24-SMT) and cytochrome P450-linked monooxygenase component of lanosterol 14α-demethylase (14DM) are the key enzymes involved in the fungal ergosterol biosynthesis.[3]

Several groups have shown that sterols with a heteroatom substituent at C-24 or C-25 are effective inhibitors of the 24-SMT in fungi.[4] In the previous papers we reported the synthesis and antifungal activities of some steroid derivatives, which were designed as potential inhibitors of 24-SMT. Remarkably potent in vitro antifungal activities were observed with 24-aminocholesterol (**1**) and 24-aminolanosterol.[5] Many of the azole antifungal agents, such as fluconazole and itraconazole that are widely used today, are believed to inhibit the 14DM in the ergosterol biosynthesis.[1b] Since the target of 14DM is the 14α-methyl group of lanosterol, a logical inhibitor could be a lanosterol derivative with a heme binding component at the 14α-methyl position. Indeed, 14α-imidazolylmethyllanosterol (**2**) was reported to have in vitro antifungal activities.[6] Furthermore, it was envisioned that aryloxolanes might serve as isosteres for

*Corresponding author. Tel.: +82-562-279-2103; fax: +82-562-279-2020; e-mail: skchung@chem.postech.ac.kr

0968-0896/00/$ - see front matter
PII: S0968-0896(00)00177-2

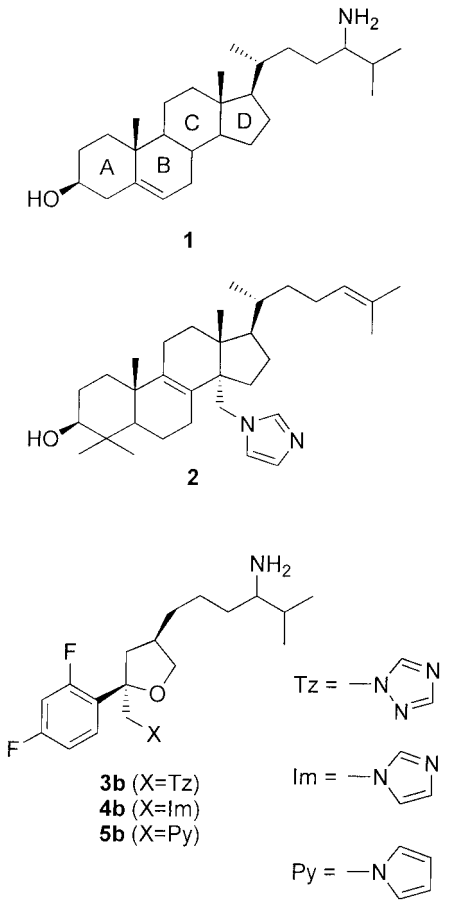


Figure 1.

the steroidal skeleton for the development of antifungal agents.[7]

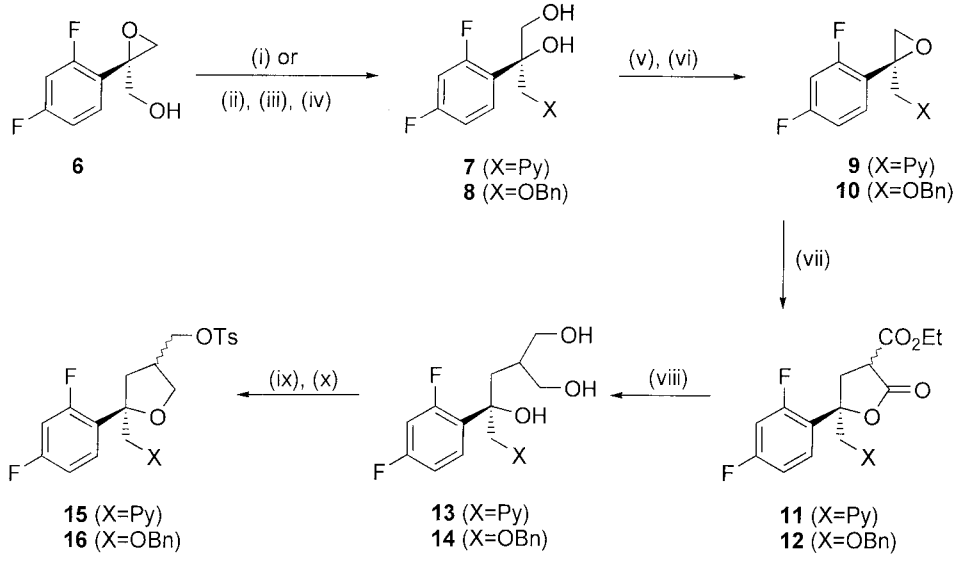
Thus, we designed azolylmethyloxolane derivative, **3b** and **4b** as potential dual functional inhibitors of cytochrome P450 14DM and 24-SMT by combining the structural features of 24-aminosterols and azole antifungal agents.

The pyrrolylmethyloxolane derivative **5b** was also designed for the purpose of comparison. We anticipated that the oxolane with (*2R*)-*trans*-stereochemistry would be a better structural mimic of the sterol skeleton. The 2β-aromatic ring and the oxolane ring of **3b** and **4b** might be regarded as the B and D rings of the steroids **1** and **2**, and the 4-(4-amino-5-methylhexyl) group and the 2α-azolylmethyl group of **3b** and **4b** as the 17-(24-aminoalkyl) side chain of **1** and the 14α-imidazolylmethyl group of **2**, respectively.

Results and Discussion

Adopting the literature procedures of Saksena,[8] which utilized the Sharpless–Katsuki asymmetric epoxidation, we readily prepared the pyrrolyl (**15**) and the benzyl intermediates (**16**) (Scheme 1). Reaction of the known epoxy alcohol[8] (**6**) with pyrrole in the presence of NaH in DMF provided the pyrrolyl diol (**7**) in 46% yield. And, protection of the epoxy alcohol (**6**) by THP, followed by reaction with neat benzyl alcohol in the presence of NaH and acid methanolysis, gave the benzyl diol (**8**) in 84% overall yield. The primary alcohol functionality of the diols (**7** and **8**) was converted to mesylate, and then cyclized in the presence of NaH in DMF to the oxiranes (**9** and **10**) in ca. 80% yield. Reaction of the oxiranes (**9** and **10**) with malonate anion in DMSO provided the lactones (**11** and **12**) as a mixture of diastereomeric isomers in good yield. Reduction of the lactones (**11** and **12**) with $LiBH_4$ generated in situ in ethanol cleanly provided the triols (**13** and **14**) in ca. 90% yield. Conversion of the two primary alcohol functionalities of the triols (**13** and **14**) into tosylate, followed by treatment with NaH in boiling toluene, gave the tosylates (**15** and **16**) in ca. 80% overall yield.

Diastereomeric **16a** and **16b** could be readily separated by repeated column chromatography and fractional crystallization in a 4:6 ratio,[9] but diastereomers of

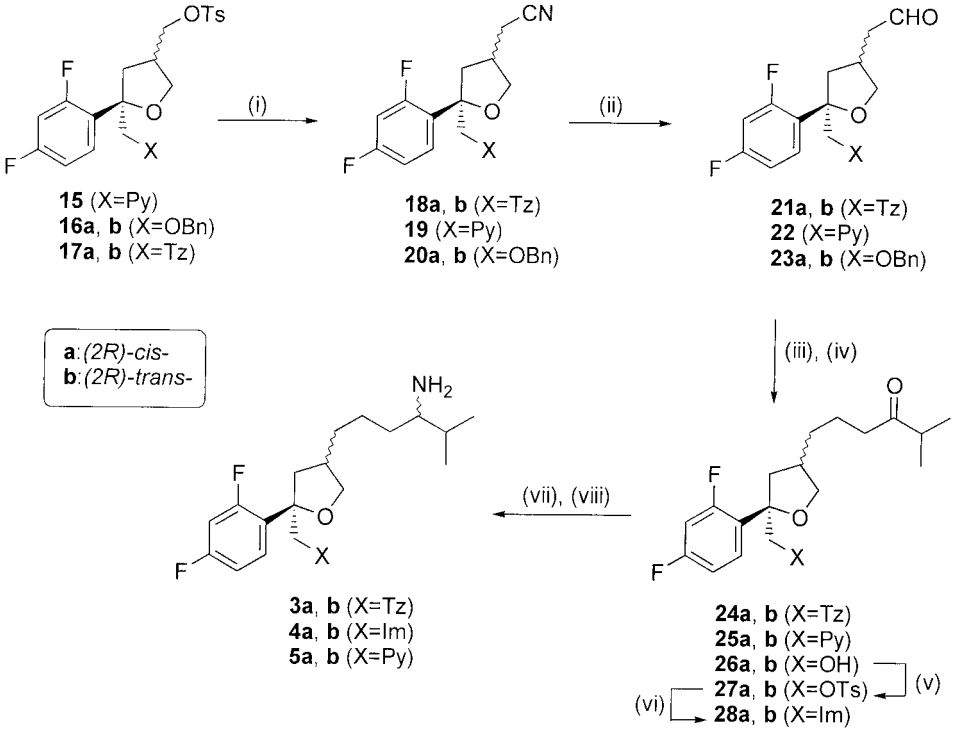


Scheme 1. (i) Pyrrole, NaH, DMF, 50–60 °C; (ii) DHP, PPTS, CH_2Cl_2, rt; (iii) BnOH, NaH, 50–60 °C; (iv) TsOH, MeOH, rt; (v) MsCl, TEA, CH_2Cl_2, rt; (vi) NaH, DMF, rt; (vii) diethyl malonate, NaH, DMSO, 75–85 °C; (viii) LiCl, $NaBH_4$, EtOH, rt; (ix) TsCl, TEA, DMAP, CH_2Cl_2, rt; (x) NaH, toluene, reflux.

compound **15** were not separable at this stage. We also obtained the known triazolyl intermediate (**17a**,**b**) according to the procedures of Saksena.[8] Treatment of the tosylates (**15**, **16a**,**b** and **17a,b**) with potassium cyanide gave the cyano compounds (**18a**,**b**, **19** and **20a**,**b**) in almost quantitative yield.[10] Reduction of the nitriles with DIBAL in toluene gave the aldehydes (**21a**,**b**, **22** and **23a**,**b**) in ca. 65% yield.[11] The ketones (**24a**,**b**, **25** and **26a**,**b**) were readily prepared by the Horner–Wadsworth–Emmons reaction[12] between the corresponding aldehydes (**21a**,**b**, **22** and **23a**,**b**) and dimethyl 3-methyl-2-oxobutylphosphonate, followed by hydrogenation (H_2/Pd–C/EtOH) in ca. 70% overall yield. At this stage the diastereomers (*2R*)-*cis*-isomer **25a** and (*2R*)-*trans*-isomer **25b** were separated in a 4:6 ratio, and assigned on the basis of 2D NOESY experiment in which a cross peak was observed between the resonances at δ 1.47 (m, 1H) and δ 2.55 (m, 1 H) in **25b**.[13] Tosylation of the hydroxyketones (**26a**,**b**) under the standard conditions proceeded cleanly. Nucleophilic displacements of the 2α-methyltosylate in a neopentyl-like system such as **27a**,**b** are expected to be difficult, although there are some known exceptions.[14] Among the several conditions examined the best results were obtained in neat *N*,*N*′-dimethylpropyleneurea (DMPU) at 100 °C. Thus, displacement of the tosyl group in **27a**,**b** with sodium imidazole in DMPU gave **28a**,**b** in ca. 30% yield. At this stage it was possible to assign the stereochemistry of **28a**,**b** by comparison of the NMR data of **24b** obtained from **27b** with sodium triazole with those of **24a,b** that were obtained from the known **17a,b**. The ketones (**24a**,**b**, **25a**,**b** and **28a**,**b**) were converted to the corresponding oximes by treatment with $NH_2OH{\cdot}HCl$ in pyridine and ethanol. Attempts to reduce oximes to amines with H_2/PtO_2,[15a] $NaBH_4/TiCl_4$,[15b] or LAH[15c] were not successful. However, it was found that reduction of the oximes with $NaBH_4/NiCl_2{\cdot}6H_2O$ in methanol provided the target amines (**3a**,**b**, **4a**,**b** and **5a**,**b**) in good yield.[16] Under this reduction condition, dehalogenated products that are inseparable by column chromatography were also obtained in about 7% yield on the basis of NMR analysis[17] (Scheme 2).

The in vitro antifungal activities of the imidazoles, triazoles and pyrroles were assessed against a number of fungal strains. The MIC values of these compounds are listed in Table 1 with those of fluconazole and itraconazole as the reference standards. The inhibitory activities of the imidazole, triazole and pyrrole compounds were also examined against rat liver microsomal lanosterol 14DM[18] and recombinant *Saccharomyces cerevisiae* 24-SMT.[19] The results of the 14DM inhibitory experiments are summarized in Table 2.

Several conclusions may be drawn from the data of in vitro antifungal activities and enzymic bioassays. First, our goal of enhancing the bioactivity via the dual inhibitions of 14DM and 24-SMT is not fully realized, since the designed molecules (**3**–**5**) do not show more potent activities than 24-aminocholesterol or the reference standards. The meager antifungal activities displayed by **3**–**5** may be due to the interference of either 14DM or 24-SMT. The fact that the (*2R*)-*cis*-isomers (**3a** and **4a**) have slightly higher activities than the (*2R*)-*trans*-isomers (**3b** and **4b**) may suggest that these activities are more likely due to the inhibition of 14DM rather than



Scheme 2. (i) KCN, DMSO, 50–55 °C; (ii) DIBAL, toluene; (iii) dimethyl 3-methyl-2-oxobutylphosphonate, DBU, LiCl, MeCN, rt; (iv) H_2, Pd–C, EtOH, rt; (v) TsCl, TEA, DMAP, CH_2Cl_2, rt; (vi) Na-imidazole, DMPU, 100 °C; (vii) NH_2OHHCl, pyridine, EtOH, rt; (viii) NaBH4, $NiCl_2{\cdot}6H_2O$, MeOH.

Table 1. In vitro antifungal activities (MIC, μg/mL)

Fungal strain	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**	**24a**	**24b**	**25a**	**25b**	**28a**	**28b**	FCZ[a]	ICZ[b]
C. albicans B. 02630	>100	>100	>100	>100	100	100	>100	>100	>100	>100	50	25	>100	>100
C. albicans A. 10231	>100	>100	100	>100	100	100	>100	>100	>100	>100	25	25	>100	>100
C. albicans CI-2	100	>100	100	>100	100	100	100	>100	>100	>100	50	25	>100	>100
C. albicans IFO. 1385	6.25	12.5	1.56	12.5	25	12.5	<0.1	<0.1	>100	>100	<0.1	<0.1	12.5	0.78
C. tropicalis A. 13803	>100	>100	100	100	100	100	>100	>100	>100	>100	50	50	>100	>100
C. pseudotropicalis K. 11658	>100	>100	100	>100	100	100	6.25	50	>100	>100	1.56	12.5	12.5	0.39
C. krusei K. 11655	>100	>100	>100	>100	>100	100	12.5	100	>100	>100	3.13	25	25	0.39
C. parapsilosis A. 7330	nt	nt	>100	>100	100	50	nt	nt	>100	>100	1.56	12.5	6.25	0.78
T. glabrata B. 16205	>100	>100	50	>100	100	50	50	50	>100	>100	1.56	3.13	100	50
Cry. neoformans B.42419	nt[c]	nt	25	100	25	25	nt	nt	>100	>100	0.78	1.56	25	0.39
Cry. neoformans IFM. 40092	nt	nt	3.13	25	25	12.5	nt	nt	>100	>100	<0.1	<0.1	3.13	<0.1
Cry. neoformans A. 34144	50	100	50	100	25	25	6.25	12.5	>100	>100	0.78	1.56	12.5	0.39
A. fumigatus B. 19119	>100	>100	>100	>100	100	50	50	100	>100	>100	3.13	3.13	>100	0.39
A. niger A. 16404	>100	>100	>100	>100	100	50	>100	>100	>100	>100	25	12.5	>100	1.56
T. mentagrophytes A. 9129	>100	>100	100	100	50	25	25	50	>100	>100	3.13	6.25	100	<0.1
T. mentagrophytes B. 32663	100	>100	25	25	25	12.5	100	6.25	>100	>100	1.56	1.56	12.5	<0.1

[a]Fluconazole.
[b]Itraconazole.
[c]Not tested.

Table 2. Inhibition of rat liver microsomal lanosterol 14DM

Compound	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**	**24a**	**24b**	**25a**	**25b**	**28a**	**28b**
% Activity to control	57.9	83.4	15.9	67.7	87.4	86.9	6.0	8.9	97.6	99.9	0	0

24-SMT, since a similar stereochemistry dependence is observed in their inhibitory effects on 14DM activity. On the other hand, the activities of **5a** and **5b** are more likely due to the inhibition of 24-SMT, because pyrrole derivatives having no nitrogen atom that can bind to the heme iron of 14DM show little inhibitory effect on 14DM activity (Table 2). This suggests the important role of the amino-prenyl side chain on the inhibition of 24-SMT, as suggested previously.[5] Second, some unexpectedly potent activities are observed with the imidazole-containing keto compounds **28a** and **28b** as opposed to **25a** and **25b**, and these activities may be attributed to the inhibition of 14DM. These compounds were found to completely inhibit the 14DM activity at 10 μM (Table 2). Despite the similar strong inhibitory effect on 14DM activity **24a** and **24b** did not show strong antifungal activities. The azole compounds **3a** and **4a** with the amino-prenyl side chain also show some inhibitory effects on 14DM activity, but very low antifungal activities. These results indicate that the enzyme 14DM shows considerably higher affinity for the compounds having the oxo-prenyl side chain than those with the amino-prenyl derivatives. The comparison of the inhibitory effects of the amino compounds (**3** and **4**) and the keto compounds (**24** and **28**) indicates that the affinity of 14DM is higher for imidazole than for triazole. The 14DM inhibitory effects are generally higher with the (*2R*)-*cis*-isomers than the (*2R*)-*trans*-isomers. This stereochemistry dependence is in line with the observed in vitro antifungal activities of these compounds. The activities of the (*2R*)-*cis*-isomer (**28a**) are found to be more potent than those of the (*2R*)-*trans*-isomer (**28b**) against all strains of fungi tested, and they are better than those of fluconazole and almost comparable to those of itraconazole. This observation indicates that 14DM more favorably interacts with (*2R*)-*cis*-isomer than (*2R*)-*trans*-isomer, as with other reported cases.[8]

Since it is known that the correlations between in vitro and in vivo activities are generally unreliable in case of azole antifungals,[20] in vivo tests were also performed with murine systemic candidiasis models. The therapeutic effects on lethal models causing total death of untreated groups within 3–4 days of infection are summarized in Table 3. All the mice treated with oral dose of fluconazole (2 and 10 mg/kg) once daily for 3 days

Table 3. Antifungal in vivo efficacy against murine candidiasis

Compound	Dose	Lethal number of mouse after infection ($n=8$) at day							
	(mg/kg)	1	2	3	4	5	6	7	Death rate
28a	2	1	1	4	2	—	—	—	100
	10	1	1	3	2	1	—	—	100
28b	2	2	4	2	—	—	—	—	100
	10	0	4	4	—	—	—	—	100
Fluconazole	2	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0
Control		3	1	3	1	—	—	—	100

survived until day 7 after infection. However, all the infected mice died within 5 days when treated with **28a** or **28b** at the same dose range, although **28a** showed slightly higher therapeutic efficacy than **28b**. It is evident that unfortunately the potent in vitro activities of **28a** and **28b** were not translated into in vivo activities.

Conclusion

In an attempt to develop potential dual functional inhibitors that might have antifungal activities by inhibiting both the C-14 demethylation and the C-24 methylation step of the ergosterol biosynthesis, we have synthesized a series of azolylmethyloxolane derivatives with modified sterol side chain structures. It is apparent that the imidazole and triazole groups play a major role in the antifungal activities observed with compounds **3**, **4**, **24** and **28**, likely due to the inhibition of 14DM. The pyrrole compounds (**5** and **25**) are not as effective, as they are devoid of the nitrogen ligand necessary for binding with heme iron. In particular the imidazole-containing keto compounds **28a** and **28b** were found to possess potent in vitro antifungal activities. However, the in vitro bioactivities have not been linearly translated into in vivo protection data for some unknown reasons, although the rapid metabolic transformation may be suspected.

Experimental

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-360 or Rudolph Autopol polarimeter. Infra-red (IR) spectra were recorded on a Bomem M100-C15, Bio-Rad FTS-40, or Nicolet 20SXC FT-IR spectrometer with the compound in thin film on an NaCl plate or KBr pellet. Proton nuclear magnetic resonance (^{1}H NMR; 300 MHz) spectra were taken on a Bruker DPX 300 or Varian Gemini 2000 FT-NMR spectrometer. Chemical shifts are reported in δ ppm relative to tetramethylsilane as an internal standard. Gas chromatography (GC) was performed on an HP 5890 Series Plus with Quadrex 400-5HT capillary column. Mass spectra were determined on a Kratos MS 25 RFA (EI and FAB) system. High resolution MS were measured by Korea Basic Science Center at Taejon, Korea or Inter-University Center for Natural Science Research Facilities in Seoul National University at Seoul, Korea.

Analytical TLC was performed on Silica gel 60 F_{254} TLC plates (E. Merck, 0.25 mm layer thickness) and spots were made visible by ultraviolet (UV) irradiation, and/or by staining with iodine or spraying with *p*-anisaldehyde solution followed by charring with a heat gun. Column chromatography was carried out on Silica gel 60 (E. Merck, 230–400 mesh). All reactions were carried out under N_2 or Ar atmosphere in oven-dried glassware, all commercial chemicals were used as obtained, and all solvents were carefully dried and distilled by standard methods prior to use.

(2*S*)-[2-[2-(2,4-Difluorophenyl)]oxiranyl]methanol (6). The epoxy alcohol **6** was prepared according to the method developed by Saksena et al.:[8] $[\alpha]_D^{25}-37.66°$ ($c=1.15$, MeOH) [lit.[8] $[\alpha]_D^{25}-36.7°$ ($c=1$, MeOH)]; IR (film) ν 3397, 2930, 1613 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.70 (br, 1H), 2.83 (d, $J=5.1$ Hz, 1H), 3.28 (d, $J=5.1$ Hz, 1H), 4.02 (d, $J=12.7$ Hz, 2H), 6.83 (m, 2H), 7.36 (m, 1H); MS (EI) m/z 187 ($M^+ + 1$), 186 (M^+).

(2*R*)-2-(2,4-Difluorophenyl)-3-(1*H*-pyrrol-1-yl)-1,2-propanediol (7). To a suspension of sodium hydride (60% mineral oil dispersion, 1.41 g, 35.0 mmol, washed with *n*-pentane) in DMF (45 mL) with stirring at 0 °C was added pyrrole (5.6 mL, 80.71 mmol). The mixture was allowed to warm up to room temperature. When the mixture became clear, a solution of **6** (4.2 g, 22.56 mmol) in DMF (15 mL) was added. The mixture was then stirred at 50–60 °C for 2.5 h, after which the solvent was distilled off under reduced pressure and the residue was partitioned between EtOAc (100 mL) and water (50 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give **7** (2.64 g, 46.2%) as an oil: $[\alpha]_D^{27}-60.80°$ ($c=0.22$, $CHCl_3$); IR (film) ν 3457, 1617, 1598, 1499, 1421, 1272 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.31 (br, 1H), 3.41 (s, 1H), 3.79 (app t, 2H), 4.27 (app t, 2H), 6.06 (app t, $J=2.1$ Hz, 2H), 6.50 (t, $J=2.1$ Hz, 2H), 6.78–6.87 (m, 2H), 7.41–7.50 (m, 1H); MS (EI) m/z 253 (M^+); HRMS (EI) calcd for $C_{13}H_{13}O_2NF_2$ 253.0914 (M^+), found 253.0916.

(2*S*)-3-Benzyloxy-2-(2,4-difluorophenyl)-1,2-propanediol (8). A solution of **6** (4.78 g, 25.66 mmol), 3,4-dihydro-2*H*-pyran (3.51 mL, 38.47 mmol), and pyridinium *p*-toluenesulfonate (0.15 g, 0.59 mmol) in CH_2Cl_2 (72 mL) was stirred overnight at room temperature. The mixture was washed with aqueous solution of 5% $NaHCO_3$ (30 mL), water (30 mL) and brine (30 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give (*2S*)-2-(2,4-difluorophenyl)-2-[(tetrahydropyran-2-yloxy)methyl]oxirane (6.22 g) as an oil. The product obtained above in THF (10 mL) was added to a solution of sodium hydride (60% mineral oil dispersion, 1.41 g, 35.0 mmol, washed with *n*-pentane) in benzyl alcohol (17 mL) at room temperature. The mixture was stirred at 50–60 °C for 24 h. Then, it was poured into ice–water (50 mL), followed by extraction with EtOAc (150 mL). The organic layer was washed with brine (30 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was treated with *p*-toluenesulfonic acid monohydrate (4.53 g, 23.81 mmol) in MeOH (70 mL) at room temperature for 0.5 h. Then the solvent was distilled off under reduced pressure and the residue was diluted with EtOAc (150 mL). The organic layer was washed with aqueous solution of 5% $NaHCO_3$ (30 mL×2) and brine (30 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give **8** (6.36 g, 84.1% yield from **6**) as an oil: $[\alpha]_D^{20}$ 4.77° ($c=0.86$, MeOH); IR (film) ν 3431,

2871, 1616, 1500, 1422, 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (t, $J=6.6$ Hz, 1H), 3.66–3.72 (m, 2H), 3.76 (dd, $J=9.6$, 2.1 Hz, 1H), 3.96 (dd, $J=9.6$, 1.2 Hz, 1H), 4.05 (ddd, $J=11.4$, 6.0, 2.4 Hz, 1H), 6.74 (m, 1H), 6.88 (m, 1H), 7.21–7.24 (m, 2H), 7.28–7.35 (m, 3H), 7.68 (m, 1H); MS (FAB) m/z 295 ($M^+ + 1$); HRMS (FAB) calcd for $C_{16}H_{17}O_3F_2$ 295.1146 ($M^+ + 1$), found 295.1143.

(2*R*)-2-(2,4-Difluorophenyl)-2-[(1*H*-pyrrol-1-yl)methyl] oxirane (9). To a solution of **7** (3.5 g, 13.82 mmol) and triethylamine (3.1 mL, 22.24 mmol) in CH_2Cl_2 (54 mL) at 0 °C was added methanesulfonyl chloride (1.25 mL, 16.15 mmol), and then the mixture was stirred at room temperature for 2 h. The mixture was washed with aqueous solution of 5% NaH_2PO_4 (20 mL×2) and brine (20 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo to give (2*R*)-2-(2,4-difluorophenyl)-1-methanesulfonyloxy-3-(1*H*-pyrrol-1-yl)-2-propanol as an oil in quantitative yield. To a solution of the product obtained above in DMF (65 mL) was added sodium hydride (60% mineral oil dispersion, 0.61 g, 15.2 mmol), and then the mixture was stirred at room temperature for 4 h. The solvent was distilled off under reduced pressure and the residue was partitioned between EtOAc (100 mL) and water (50 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give **9** (2.61 g, 80.3% yield from **7**) as an oil: $[\alpha]_D^{25}$ 7.94° ($c=0.20$, $CHCl_3$); IR (film) ν 1619, 1599, 1507, 1497, 1424, 1276, 1108, 964 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.80 (app t, $J=5.5$ Hz, 2H), 4.12 (d, $J=15.1$ Hz, 1H), 4.47 (d, $J=15.1$ Hz, 1H), 6.08 (app t, $J=2.2$ Hz, 2H), 6.59 (app t, $J=2.2$ Hz, 2H), 6.75–6.85 (m, 2H), 7.15–7.19 (m, 1H); MS (EI) m/z 235 (M^+); HRMS (EI) calcd for $C_{13}H_{11}ONF_2$ 235.0809 (M^+), found 235.0814.

(2*R*)-2-Benzyloxymethyl-2-(2,4-difluorophenyl)oxirane (10). By the same method as described for **9**, **8** (6.58 g, 22.35 mmol) was converted to **10** (5.15 g, 83.3% yield from **8**) as an oil: $[\alpha]_D^{18}$ 20.11° ($c=0.78$, $CHCl_3$); IR (film) ν 3065, 3032, 2912, 2862, 1619, 1601, 1507, 1425, 1270, 1098, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.80 (d, $J=5.7$ Hz, 1H), 3.13 (d, $J=5.1$ Hz, 1H), 3.65 (dd, $J=11.7$, 1.5 Hz, 1H), 4.02 (dd, $J=11.7$, 1.5 Hz, 1H), 4.56 (s, 2H), 6.79 (m, 1H), 6.86 (m, 1H), 7.21–7.25 (m, 2H), 7.26–7.33 (m, 3H), 7.44 (m, 1H); MS (FAB) m/z 277 ($M^+ + 1$); HRMS (FAB) calcd for $C_{16}H_{15}O_2F_2$ 277.1040 ($M^+ + 1$), found 277.1035.

Ethyl (5*R*-*cis*)- and (5*R*-*trans*)-5-(2,4-difluorophenyl)-2-oxo-5-[(1*H*-pyrrol-1-yl)methyl]-3-tetrahydrofurancarboxylate (11). To a suspension of sodium hydride (60% mineral oil dispersion, 0.89 g, 22.2 mmol, washed with *n*-pentane) in DMSO (27 mL) was added diethyl malonate (3.72 mL, 24.5 mmol). After stirring at room temperature for 1 h, a solution of **9** (2.57 g, 10.93 mmol) in DMSO (5 mL) was added to the mixture. The mixture was then stirred at 75–80 °C for 30 h. It was poured into a well-stirred mixture of aqueous solution of 5% NaH_2PO_4 (50 mL), brine (50 mL) and EtOAc (150 mL), and partitioned. The aqueous layer was extracted with EtOAc (50 mL). The organic layers were combined, washed with brine (30 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give **11** (2.68 g, 70.2%) as an oil: IR (film) ν 1791, 1738, 1600, 1502, 1448, 1291, 1158, 967 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.21 (t, $J=7.2$ Hz, 2.1H), 1.29 (t, $J=7.2$ Hz, 0.9H), 2.31 (t, $J=10.2$ Hz, 0.7H), 2.76–2.85 (m, 1H), 2.91–3.01 (m, 1H), 3.49 (dd, $J=11.0$, 9.3 Hz, 3H), 4.07–4.17 (m, 1.3H), 4.20–4.31 (m, 2H), 4.36–4.49 (m, 0.7H), 6.02 (t, $J=2.4$ Hz, 0.6H), 6.24 (t, $J=2.1$ Hz, 1.4H), 6.54 (t, $J=2.4$ Hz, 0.6H), 6.71 (t, $J=2.4$ Hz, 1.4H), 6.76–6.86 (m, 0.6H), 6.88–6.99 (m, 1.4H), 7.18–7.25 (m, 0.3H), 7.56–7.64 (m, 0.7H); MS (EI) m/z 349 (M^+); HRMS (EI) calcd for $C_{18}H_{17}O_4NF_2$ 349.1126 (M^+), found 349.1120.

Ethyl (5*R*-*cis*)- and (5*R*-*trans*)-5-benzyloxymethyl-5-(2,4-difluorophenyl)-2-oxo-3-tetrahydrofurancarboxylate (12). By the same method as described for **11**, **10** (4.41 g, 15.96 mmol) was converted to **12** (5.35 g, 85.9%) as an oil: IR (film) ν 3065, 2983, 2869, 1788, 1738, 1617, 1601, 1503, 1453 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (t, $J=6.9$ Hz, 3H), 2.66–2.83 (m, 1H), 3.10–3.27 (m, 1H), 3.51–3.56 (m, 1H), 3.70–3.85 (m, 1H), 4.05 (t, $J=10.0$ Hz, 0.75H), 4.16 (t, $J=6.9$ Hz, 0.25H), 4.21 (q, $J=6.9$ Hz, 2H), 4.57 (dd, $J=19.2$, 12.0 Hz, 2H), 6.78–6.93 (m, 2H), 7.23–7.39 (m, 5H), 7.49–7.61 (m, 1H); MS (FAB) m/z 391 ($M^+ + 1$); HRMS (FAB) calcd for $C_{21}H_{21}O_5F_2$ 391.1357 ($M^+ + 1$), found 391.1342.

(4*R*)-4-(2,4-Difluorophenyl)-2-hydroxymethyl-5-(1*H*-pyrrol-1-yl)-1,4-pentanediol (13). To a cooled solution of **11** (2.68 g, 7.67 mmol) and lithium chloride (1.14 g, 26.89 mmol) in absolute EtOH (60 mL) was added in portions sodium borohydride (1.02 g, 26.96 mmol), and then the mixture was stirred at room temperature for 28 h. MeOH (20 mL) and water (3 mL) were added to the mixture. After stirring for additional 1.5 h, the solvents were distilled off under reduced pressure. The residue was extracted with warm EtOH (60 mL). The extract was filtered, and then the filtrate was concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give **13** (2.12 g, 88.8%) as a solid: $[\alpha]_D^{27}$ −47.92° ($c=0.22$, $CHCl_3$); IR (film) ν 3440, 2936, 2884, 1615, 1597, 1497, 1420, 1286, 1270, 1091, 966 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (br, 1H), 1.71–1.78 (m, 1H), 1.85 (br, 1H), 2.29 (d, $J=14.7$ Hz, 1H), 2.75 (br, 1H), 3.41–3.50 (m, 3H), 3.63–3.68 (m, 1H), 4.12 (d, $J=13.8$ Hz, 1H), 4.22 (d, $J=14.4$ Hz, 1H), 4.65 (br, 1H), 6.05 (app t, $J=2.4$ Hz, 2H), 6.53 (app t, $J=2.4$ Hz, 2H), 6.78–6.88 (m, 2H), 7.47–7.56 (m, 1H); MS (EI) m/z 312 ($M^+ + 1$), 311 (M^+); HRMS (EI) calcd for $C_{16}H_{19}O_3NF_2$ 311.1333 (M^+), found 311.1332.

(4*R*)-5-Benzyloxy-4-(2,4-difluorophenyl)-2-hydroxymethyl-1,4-pentanediol (14). By the same method as described for **13**, **12** (5.56 g, 14.24 mmol) was converted to **14** (4.76 g, 94.8%) as an oil: $[\alpha]_D^{18}$ −3.68° ($c=0.54$, $CHCl_3$); IR (film) ν 3350, 2928, 2870, 1615, 1598, 1498, 1419, 1270, 968 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.53 (m, 1H), 1.92 (dd, $J=14.4$, 8.7 Hz, 1H), 2.05–2.12 (m, 1H), 2.26 (br, 1H), 3.09 (br, 1H), 3.40–3.50 (m, 2H), 3.52–3.70 (m, 3H), 3.74 (dd, $J=9.3$, 0.9 Hz, 1H), 4.10 (br, 1H), 4.48

(dd, $J=15.9$, 12.3 Hz, 2H), 6.71–6.78 (m, 1H), 6.86–6.93 (m, 1H), 7.15–7.18 (m, 2H), 7.26–7.34 (m, 3H), 7.61–7.69 (m, 1H); MS (EI) m/z 353 ($M^+ + 1$), (FAB) m/z 353 ($M^+ + 1$); HRMS (FAB) calcd for $C_{19}H_{23}O_4F_2$ 353.1564 ($M^+ + 1$), found 353.1566.

(*2R-cis*)- and (*2R-trans*)-2-(2,4-Difluorophenyl)-4-[[(4-methylphenyl)sulfonyloxy]methyl]-2-[(1*H*-pyrrol-1-yl)methyl] tetrahydrofuran (15). To a solution of **13** (2.0 g, 6.42 mmol), 4-(dimethylamino)pyridine (80 mg, 0.65 mmol) and triethylamine (2.17 mL, 15.57 mmol) in CH_2Cl_2 (26 mL) at 0 °C was added in portions *p*-toluenesulfonyl chloride (2.72 g, 14.27 mmol). After stirring at room temperature for 15.5 h, the solvent was distilled off under reduced pressure and the residue was diluted with EtOAc (70 mL). The organic layer was washed with aqueous solution of 5% NaH_2PO_4 (15 mL), 5% $NaHCO_3$ (15 mL) and brine (15 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo to give (*4R*)-4-(2,4-difluorophenyl)-2-hydroxy-2-[[(4-methyl-phenyl)sulfonyloxy]methyl]-5-(1*H*-pyrrol-1-yl)pentyl 4-methylbenzenesulfonate as an oil. The crude product was treated with sodium hydride (60% mineral oil dispersion, 1.5 g, 37.5 mmol) in refluxing toluene (90 mL) for 4 h. After cooling, insoluble excess sodium hydride was filtered off and filter cake was washed with EtOAc (50 mL). The combined organic layers were washed with aqueous solution of 5% NaH_2PO_4 (15 mL), 5% $NaHCO_3$ (15 mL) and brine (15 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give **15** (2.34 g, 84.2% yield from **13**) as an oily mixture of (*2R*)-*cis*- and (*2R*)-*trans*-isomers in a 4:6 ratio: IR (film) ν 2977, 2930, 2871, 1616, 1598, 1496, 1363, 1289, 1269, 1177 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.69 (dd, $J=12.9$, 6.9 Hz, 0.4H), 1.80–1.87 (m, 0.6H), 2.06 (sept, $J=7.2$ Hz, 0.6H), 2.92–2.46 (m, 4.4H), 3.47–3.55 (m, 1H), 3.60–3.78 (m, 2H), 3.87 (app t, $J=9.0$ Hz, 0.4H), 3.95–4.09 (m, 2.6H), 5.98 (app t, $J=2.1$ Hz, 0.8H), 6.04 (app t, $J=2.1$ Hz, 1.2H), 6.54–6.58 (m, 2H), 6.75–6.84 (m, 2H), 7.26–7.44 (m, 3H), 7.65 (d, $J=8.1$ Hz, 1.2H), 7.73 (d, $J=8.4$ Hz, 0.8H); MS (EI) m/z 448 ($M^+ + 1$), 447 (M^+); HRMS (EI) calcd for $C_{23}H_{23}O_4NF_2S$ 447.1316 (M^+), found 447.1317.

(*2R-cis*)-2-Benzyloxymethyl-2-(2,4-difluorophenyl)-4-[[(4-methylphenyl)sulfonyloxy]methyl]tetrahydrofuran (16a) and (*2R-trans*)-2-benzyloxymethyl-2-(2,4-difluorophenyl)-4-[[(4-methylphenyl)sulfonyloxy]methyl]tetrahydrofuran (16b). By the same method as described for **15**, **14** (4.68 g, 13.27 mmol) was converted to **16** (5.18 g, 79.92%). Repeated column chromatography of **16** eluting with EtOAc:hexane (1:4, v/v) and careful fractional crystallization from EtOAc–hexane afforded the less polar (*2R*)-*cis*-isomer **16a** (1.50 g, 23.1%) as a solid, which was recrystallized from EtOAc–hexane: mp 75–75.5 °C; $[\alpha]_D^{19}$ −12.04° ($c=0.93$, $CHCl_3$); IR (film) ν 2865, 1615, 1598, 1497, 1363, 1270, 1177, 1098, 966 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.11 (dd, $J=13.2$, 6.6 Hz, 1H), 2.28 (ddd, $J=13.2$, 8.1, 2.4 Hz, 1H), 2.42 (s, 3H), 2.51 (m, 1H), 3.53 (dd, $J=10.5$, 1.8 Hz, 1H), 3.79 (dd, $J=9$, 5.4 Hz, 1H), 3.89 (dd, $J=9.2$, 6.9 Hz, 1H), 4.0–4.12 (m, 2H), 4.49 (s, 2H), 6.7–6.77 (m, 1H), 6.81–6.88 (m, 1H), 7.17–7.21 (m, 2H), 7.27–7.34 (m, 5H), 7.48–7.56 (m, 1H), 7.71–7.75 (m, 2H); MS (FAB) m/z 489 ($M^+ + 1$); HRMS (FAB) calcd for $C_{26}H_{27}O_5F_2S$ 489.1547 ($M^+ + 1$), found 489.1552, and (*2R*)-*trans*-isomer **16b** (2.19 g, 33.8%) as an oil: $[\alpha]_D^{20}$ 16.49° ($c=1.0$, $CHCl_3$); IR (film) ν 2952, 2864, 1615, 1599, 1498, 1363, 1269, 1177, 1099, 967 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.77 (ddd, $J=13.2$, 6.6, 2.1 Hz, 1H), 2.44(s, 3H), 2.57 (ddd, $J=13.2$, 8.4, 2.7 Hz, 1H), 2.81 (m, 1H), 3.47 (dd, $J=10.2$, 1.8 Hz, 1H), 3.58–3.63 (m, 2H), 3.73–3.86 (m, 2H), 4.15 (dd, $J=9$, 6.9 Hz, 1H), 4.50 (s, 2H), 6.67–6.74 (m, 1H), 6.77–6.83 (m, 1H), 7.18–7.21 (m, 2H), 7.24–7.33 (m, 5H), 7.47–7.55 (m, 1H), 7.67–7.72 (m, 2H); MS (FAB) m/z 489 ($M^+ + 1$); HRMS (FAB) calcd for $C_{26}H_{27}O_5F_2S$ 489.1547 ($M^+ + 1$), found 489.1552.

(*2R-cis*)-2-(2,4-Difluorophenyl)-4-[[(4-methylphenyl)sulfonyloxy]methyl]-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (17a) and (*2R-trans*)-2-(2,4-difluorophenyl)-4-[[(4-methylphenyl)sulfonyloxy]methyl]-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (17b). The tosylates **17a** and **17b** were prepared from **6** according to the method developed by Saksena et al.[8] **17a**: mp 97–99 °C [lit.[8] mp 96–98 °C]; $[\alpha]_D^{25}$ −41.57° ($c=0.9$, $CHCl_3$) [lit.[8] $[\alpha]_D^{25}$ −39.6° ($c=1$, $CHCl_3$)]; IR (film) ν 1616, 1501, 1361, 1272, 1176 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.90 (m, 1H), 2.46 (m, 5H), 3.57 (dd, $J=9.1$, 6.2 Hz, 1H), 3.69 (dd, $J=9.8$, 7.5 Hz, 1H), 3.84 (dd, $J=9.8$, 5.6 Hz, 1H), 3.98 (dd, $J=8.9$, 7.2 Hz, 1H), 4.46 (d, $J=14.4$ Hz, 1H), 4.54 (d, $J=14.3$ Hz, 1H), 6.82 (m, 2H), 7.33 (m, 3H), 7.75 (m, 3H), 8.03 (s, 1H); MS (FAB) m/z 450 ($M^+ + 1$); and **17b**: $[\alpha]_D^{25}$ −19.77° ($c=1.0$, $CHCl_3$); IR (film) ν 1610, 1502, 1360, 1272, 1176 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.91 (ddd, $J=13.6$, 7.5, 2.0 Hz, 1H), 2.14 (m, 1H), 2.63 (ddd, $J=13.5$, 8.4, 2.3 Hz, 1H), 3.56 (dd, $J=8.8$, 7.7 Hz, 1H), 3.74 (m, 2H), 3.98 (dd, $J=8.9$, 6.8 Hz, 1H), 4.31 (d, $J=14.3$ Hz, 1H), 4.50 (d, $J=14.2$ Hz, 1H), 6.80 (m, 2H), 7.34 (m, 3H), 7.65 (s, 1H), 7.67 (s, 1H), 7.81 (s, 1H), 8.04 (s, 1H); MS (FAB) m/z 450 ($M^+ + 1$).

(*2R-cis*)-4-Cyanomethyl-2-(2,4-difluorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (18a). A solution of **17a** (0.93 g, 2.07 mmol) and potassium cyanide (0.2 g, 3.10 mmol) in DMSO (10 mL) was stirred at 55 °C for 15 h. After cooling, the mixture was diluted with EtOAc (50 mL). The organic layer was washed with water (15 mL×3), dried over anhydrous magnesium sulfate, and concentrated in vacuo to give a solid, which was recrystallized from EtOAc–hexane to give **18a** (0.53 g, 84.5%): mp 113.5–114.4 °C; $[\alpha]_D^{22}$ −57.33° ($c=0.5$, $CHCl_3$); IR (film) ν 2246, 1615 cm^{-1}; ^1H NMR (CDCl3) δ 2.08 (dd, $J=13.0$, 8.6 Hz, 1H), 2.20 (d, $J=6.9$ Hz, 2H), 2.47 (m 1H), 2.63 (m, 1H), 3.56 (dd, $J=8.9$, 7.2 Hz, 1H), 4.11 (dd, $J=8.8$, 7.1 Hz, 1H), 4.49 (d, $J=14.4$ Hz, 1H), 4.64 (d, $J=14.4$ Hz, 1H), 6.85 (m, 2H), 7.36 (m, 1H), 7.83 (s, 1H), 8.10 (s, 1H); MS (EI) m/z 305 ($M^+ + 1$).

(*2R-trans*)-4-Cyanomethyl-2-(2,4-difluorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (18b). By the same method as described for **18a**, **17b** (1.69 g, 3.76 mmol) was converted to **18b** (1.12 g, 97.9%) as an oil after purification by column chromatography (EtOAc–hexane gradient): $[\alpha]_D^{22}$ −50.04° ($c=0.5$, $CHCl_3$); IR

(film) ν 2247, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.01 (m, 2H), 2.26 (d, $J=6.4$ Hz, 2H), 2.90 (m, 1H), 3.57 (t, $J=8.6$ Hz, 1H), 4.08 (m, 1H), 4.33 (d, $J=14.3$ Hz, 1H), 4.56 (d, $J=14.2$ Hz, 1H), 6.87 (m, 2H), 7.48 (m, 1H), 7.86 (s, 1H), 8.08 (s, 1H); MS (EI) m/z 305 ($M^+ + 1$); HRMS (EI) calcd for $C_{15}H_{15}ON_4F_2$ 305.1214 ($M^+ + 1$), found 305.1226.

(2*R*-*cis*)- and (2*R*-*trans*)-4-Cyanomethyl-2-(2,4-difluorophenyl)-2-[(1*H*-pyrrol-1-yl)methyl]tetrahydrofuran (19). By the same method as described for **18a**, **15** (2.66 g, 5.96 mmol) was converted to **19** (1.74 g, 97.2%) as a solid, which was recrystallized from EtOAc–hexane: IR (film) ν 3103, 2943, 2867, 2248, 1617, 1598, 1497, 1422, 1289, 1269, 1137 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.78–1.98 (m, 2.6H), 2.20 (d, $J=6.6$ Hz, 1.2H), 2.39–2.51 (m, 0.6H), 2.66 (ddd, $J=12.8$, 7.2, 3.3 Hz, 0.6H), 3.50 (t, $J=8.4$ Hz, 0.6H), 3.65 (dd, $J=9.8$, 4.8 Hz, 0.4H), 3.91–4.20 (m, 3H), 6.09–6.12 (m, 2H), 6.65–6.69 (m, 2H), 6.81–6.90 (m, 2H), 7.44–7.59 (m, 1H); MS (EI) m/z 302 (M^+); HRMS (EI) calcd for $C_{17}H_{16}ON_2F_2$ 302.1231 (M^+), found 302.1239.

(2*R*-*cis*)-2-Benzyloxymethyl-4-cyanomethyl-2-(2,4-difluorophenyl)tetrahydrofuran (20a). By the same method as described for **18a**, **16a** (1.44 g, 3.04 mmol) was converted to **20a** (0.9 g, 88.8%) as an oil after purification by column chromatography (EtOAc–hexane gradient): $[\alpha]_D^{18}$ −2.80° ($c=1.21$, $CHCl_3$); IR (film) ν 3065, 2939, 2865, 2247, 1615, 1599, 1497, 1422, 1270, 1104, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.30–2.45 (m, 2H), 2.50–2.60 (m, 3H), 3.55 (dd, $J=10.5$, 1.8 Hz, 1H), 3.68 (dd, $J=10.5$, 1.8 Hz, 1H), 3.82 (dd, $J=8.7$, 4.2 Hz, 1H), 3.93–3.98 (m, 1H), 4.57 (s, 2H), 6.73–6.81 (m, 1H), 6.83–6.90 (m, 1H), 7.24–7.37 (m, 5H), 7.52–7.60 (m, 1H); MS (FAB) m/z 344 ($M^+ + 1$); HRMS (FAB) calcd for $C_{20}H_{20}O_2NF_2$ 344.1462 ($M^+ + 1$), found 344.1458.

(2*R*-*trans*)-2-Benzyloxymethyl-4-cyanomethyl-2-(2,4-difluorophenyl)tetrahydrofuran (20b). By the same method as described for **18a**, **16b** (2.14 g, 4.51 mmol) was converted to **20b** (1.43 g, 95.1%) as an oil after purification by column chromatography (EtOAc–hexane gradient): $[\alpha]_D^{20}$ 19.03° ($c=1.19$, $CHCl_3$); IR (film) ν 3064, 2944, 2863, 2247, 1615, 1600, 1499, 1423, 1269, 1106 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.86–1.95 (m, 1H), 2.30 (d, $J=6.9$ Hz, 2H), 2.76–2.87 (m, 2H), 3.49 (dd, $J=10.5$, 1.5 Hz, 1H), 3.62 (dd, $J=8.4$, 6.9 Hz, 1H), 3.65 (dd, $J=10.2$, 1.5 Hz, 1H), 4.24–4.29 (m, 1H), 4.54 (s, 2H), 6.73–6.80 (m, 1H), 6.84–6.90 (m, 1H), 7.22–7.35 (m, 5H), 7.59–7.67 (m, 1H); MS (FAB) m/z 344 ($M^+ + 1$); HRMS (FAB) calcd for $C_{20}H_{20}O_2NF_2$ 344.1462 ($M^+ + 1$), found 344.1461.

(2*R*-*cis*)-2-(2,4-Difluorophenyl)-4-formylmethyl-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (21a). To a solution of **18a** (0.49 g, 1.6 mmol) in toluene (13 mL) at −78 °C was added dropwise diisobutylaluminum hydride (1 M/toluene, 2 mL) over a period of 30 min. After stirring at the same temperature for 30 min, the mixture was allowed to warm up to room temperature, and then was stirred for 4 h. The mixture was cooled to 10 °C, and a saturated aqueous solution of NH_4Cl was added to the mixture, which was extracted with EtOAc (50 mL). The extract was washed with brine (15 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane, 2:1, v/v) to give **21a** (0.26 g, 52.1%) as a solid: mp 62.4–63.6 °C; $[\alpha]_D^{22}$ −56.4° ($c=0.5$, $CHCl_3$); IR (film) ν 2729, 1722 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (m, 4H), 4.21 (m, 1H), 2.47 (m 1H), 4.47 (d, $J=14.3$ Hz, 1H), 4.63 (d, $J=14.3$ Hz, 1H), 6.84 (m, 2H), 7.36 (m, 1H), 7.84 (s, 1H), 8.12 (s, 1H), 9.67 (s, 1H); MS (EI) m/z 308 ($M^+ + 1$).

(2*R*-*trans*)-2-(2,4-Difluorophenyl)-4-formylmethyl-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (21b). By the same method as described for **21a**, **18b** (1.0 g, 3.29 mmol) was converted to **21b** (0.41 g, 40.4%) as an oil: $[\alpha]_D^{22}$ −30.4° ($c=0.5$, $CHCl_3$); IR (film) ν 2729, 1722 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.83 (m, 1H), 2.16 (m, 1H), 2.40 (m, 2H), 3.39 (t, $J=8.8$ Hz, 1H), 4.20 (dd, $J=8.4$, 6.9 Hz, 1H), 4.36 (d, $J=14.2$ Hz, 1H), 4.54 (d, $J=14.2$ Hz, 1H), 6.84 (m, 2H), 7.43 (m, 1H), 7.84 (s, 1H), 8.09 (s, 1H), 9.64 (br, 1H); MS (EI) m/z 308 ($M^+ + 1$).

(2*R*-*cis*)- and (2*R*-*trans*)-2-(2,4-Difluorophenyl)-4-formylmethyl-2-[(1*H*-pyrrol-1-yl)methyl]tetrahydrofuran (22). By the same method as described for **21a**, **19** (1.5 g, 4.96 mmol) was converted to **22** (1.04 g, 68.7%) as an oil: IR (film) ν 2942, 2724, 1724, 1616, 1598, 1497, 1422, 1269, 1136, 963 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.66–1.78 (m, 1.2H), 1.98–2.10 (m, 0.6H), 2.26–2.36 (m, 1.6H), 2.38–2.50 (m, 1H), 2.67 (ddd, $J=13.2$, 8.0, 3.6 Hz, 0.6H), 3.34 (t, $J=8.7$ Hz, 0.6H), 3.42 (dd, $J=8.3$, 7.2 Hz, 0.4H), 4.0–4.20 (m, 3H), 6.07–6.09 (m, 2H), 6.62–6.66 (m, 3H), 6.79–6.86 (m, 2H), 7.43–7.54 (m, 1H), 9.61 (app t, $J=1.2$ Hz, 1H); MS (EI) m/z 305 (M^+); HRMS (EI) calcd for $C_{17}H_{17}O_2NF_2$ 305.1227 (M^+), found 305.1225.

(2*R*-*cis*)-2-Benzyloxymethyl-2-(2,4-difluorophenyl)-4-(formylmethyl)tetrahydrofuran (23a). By the same method as described for **21a**, **20a** (0.86g, 2.51 mmol) was converted to **23a** (0.56 g, 64.1%) as an oil: $[\alpha]_D^{18}$ 2.54° ($c=1.36$, $CHCl_3$); IR (film) ν 2941, 2862, 2723, 1723, 1614, 1600, 1497, 1420, 1269, 1106, 1031, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (dd, $J=12.8$, 8.4 Hz, 1H), 2.42 (ddd, $J=12.6$, 6.9, 2.4 Hz, 1H), 2.50–2.74 (m, 3H), 3.60–3.72 (m, 3H), 4.16 (dd, $J=8.4$, 6.6 Hz, 1H), 4.55 (s, 2H), 6.71–6.79 (m, 1H), 6.84–6.90 (m, 1H), 7.21–7.34 (m, 5H), 7.55–7.64 (m, 1H), 9.71 (s, 1H); MS (FAB) m/z 347 ($M^+ + 1$); HRMS (FAB) calcd for $C_{20}H_{21}O_3F_2$ 347.1459 ($M^+ + 1$), found 347.1457.

(2*R*-*trans*)-2-Benzyloxymethyl-2-(2,4-difluorophenyl)-4-(formylmethyl)tetrahydrofuran (23b). By the same method as described for **21a**, **20b** (1.23 g, 3.57 mmol) was converted to **23b** (0.92 g, 74.2%) as an oil: $[\alpha]_D^{19}$ 13.81° ($c=0.91$, $CHCl_3$); IR (film) ν 2944, 2860, 2724, 1723, 1615, 1600, 1497, 1421, 1269, 1104, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.74 (ddd, $J=12.9$, 8.4, 2.4 Hz, 1H), 2.36–2.52 (m, 2H), 2.70–2.80 (m, 1H), 2.84–2.97 (m, 1H), 3.44 (t, $J=8.4$ Hz, 1H), 3.53 (dd, $J=10.5$, 1.5 Hz, 1H), 3.65 (dd, $J=10.2$, 1.5 Hz, 1H), 4.31 (dd, $J=8.1$, 6.9 Hz, 1H), 4.54 (s, 2H), 6.71–6.79 (m, 1H), 6.83–6.90 (m, 1H), 7.20–7.33 (m, 5H), 7.60–7.68 (m, 1H), 9.71 (t, $J=1.5$ Hz, 1H); MS (FAB) m/z 347 ($M^+ + 1$); HRMS (FAB) calcd for $C_{20}H_{21}O_3F_2$ 347.1459 ($M^+ + 1$), found 347.1458.

Dimethyl 3-methyl-2-oxobutylphosphonate. To a solution of dimethyl methylphosphonate (11.2 g, 90 mmol) in THF (70 mL) at −78 °C was added dropwise *n*-butyllithium (1.6 M/hexane, 56 mL) over a period of 30 min, and then the mixture stirred at the same temperature for 1 h. Isobutyryl chloride (3.14 mL, 30 mmol) was added dropwise to the mixture. After stirring at the same temperature for 30 min, a saturated aqueous solution of NH_4Cl was added to the mixture, which was diluted with EtOAc (150 mL) and acidified with 1 N HCl solution. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by vacuum distillation to give the title compound (4.35 g, 74.8%): bp 98–100 °C (2.1 torr); IR (neat) ν 2955, 1711, 1464, 1252, 1035 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.13 (d, $J=6.9$ Hz, 6H), 2.83 (sept, $J=6.9$ Hz, 1H), 3.14 (d, $J=22.5$ Hz, 2H), 3.77 (s, 3H), 3.81 (s, 3H); MS (EI) m/z 194 (M^+).

(*2R-cis*)-2-(2,4-Difluorophenyl)-4-(5-methyl-4-oxohexyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (24a). To a stirred mixture of dimethyl 3-methyl-2-oxobutylphosphonate (153 mg, 0.79 mmol), 1,8-diazabicyclo-[5,4,0]undecen-7-ene (120 mg, 0.79 mmol) and lithium chloride (33 mg, 0.79 mmol) in acetonitrile (4 mL) at room temperature was added a solution of **21a** (220 mg, 0.72 mmol) in acetonitrile (3 mL). After stirring at the same temperature for 3 h, the mixture was diluted with EtOAc (50 mL). The organic layer was washed with aqueous solution of 5% NaH_2PO_4 (20 mL) and brine (20 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give (*2R-cis*)-2-(2,4-difluorophenyl)-4-(5-methyl-4-oxo-2-hexenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (244 mg, 90.9%) as an oil. A solution of the product (210 mg, 0.56 mmol) obtained above in absolute EtOH (10 mL) was stirred overnight with 5% palladium–charcoal (30 mg) under an H_2 atmosphere at room temperature. The catalyst was filtered off using Celite and the filtrate was concentrated in vacuo to give **24a** (171 mg, 81.0%) as an oil, which was used without further purification for the next reaction: $[\alpha]_D^{22}$ −32.38° ($c=0.5$, $CHCl_3$); IR (film) ν 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (s, 3H), 1.07 (s, 3H), 1.21 (m, 2H), 1.45 (m, 2H), 1.70 (dd, $J=12.5$, 10.6 Hz, 1H), 2.05 (m, 1H), 2.38 (t, 2H), 2.47 (m, 1H), 2.54 (m, 1H), 3.37 (t, 1H), 4.10 (t, 1H), 4.51 (d, $J=14.3$ Hz, 1H), 4.60 (d, $J=14.3$ Hz, 1H), 6.82 (m, 2H), 7.36 (m, 1H), 7.80 (s, 1H), 8.11 (s, 1H); MS (EI) m/z 378 ($M^+ + 1$); HRMS (EI) calcd for $C_{20}H_{26}O_2N_3F_2$ 378.1993 ($M^+ + 1$), found 378.1978.

(*2R-trans*)-2-(2,4-Difluorophenyl)-4-(5-methyl-4-oxohexyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (24b). Method A. By the same method as described for **24a**, **21b** (352 mg, 1.15 mmol) was converted to **24b** (266 mg, 67.8% yield from **21b**) as an oil: $[\alpha]_D^{22}$ −26.64° ($c=0.5$, $CHCl_3$); IR (film) ν 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04 (s, 3H), 1.07 (s, 3H), 1.16 (m, 2H), 1.46 (m, 3H), 1.74 (m, 1H), 2.35 (t, $J=7.1$ Hz, 2H), 2.54 (m, 1H), 2.78 (m, 1H), 3.33 (m, 1H), 4.06 (t, 1H), 4.30 (d, $J=14.1$ Hz, 1H), 4.54 (d, $J=14.1$ Hz, 1H), 6.84 (m, 2H), 7.46 (m, 1H), 7.86 (s, 1H), 8.11 (s, 1H); MS (EI) m/z 378 ($M^+ + 1$); HRMS (EI) calcd for $C_{20}H_{26}O_2N_3F_2$ 378.1993 ($M^+ + 1$), found 378.1986.

Method B. **24b** was obtained, by the same method as described for **28a** from **27b** (59 mg, 0.12 mmol), as an oil (27 mg, 46.3%) after purification by column chromatography (acetone–hexane gradient). NMR spectra of this product were identical with those of **24b**, obtained from the known **17b**.

(*2R-cis*)-2-(2,4-Difluorophenyl)-4-(5-methyl-4-oxohexyl)-2-[(1*H*-pyrrol-1-yl)methyl]tetrahydrofuran (25a) and (*2R-trans*)-2-(2,4-difluorophenyl)-4-(5-methyl-4-oxohexyl)-2-[(1*H*-pyrrol-1-yl)methyl]tetrahydrofuran (25b). By the same method as described for **24a**, **22** (890 mg, 2.92 mmol) was converted to **25** (762 mg, 79.4% yield from **22**) as an oil. Repeated column chromatography of **25** eluting with EtOAc:hexane (1:20, v:v) afforded the more polar (*2R*)-*cis*-isomer **25a** (167 mg, 17.4%) as an oil: $[\alpha]_D^{24}$ −22.03° ($c=0.53$, $CHCl_3$); IR (film) ν 2933, 2870, 1711, 1615, 1599, 1496, 1421, 1289, 1269, 1135 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (d, $J=6.9$ Hz, 6H), 1.12–1.26 (m, 2H), 1.38–1.54 (m, 3H), 2.54 (sept, $J=6.9$ Hz, 1H), 3.40 (t, $J=8.7$ Hz, 1H), 4.05 (t, $J=7.8$ Hz, 1H), 4.17 (s, 2H), 6.03 (app t, $J=2.4$ Hz, 2H), 6.60 (app t, $J=2.4$ Hz, 2H), 6.76–6.83 (m, 2H), 7.37–7.45 (m, 1H); MS (EI) m/z 376 ($M^+ + 1$), 375 (M^+); HRMS (EI) calcd for $C_{22}H_{27}O_2NF_2$ 375.2010 (M^+), found 375.2001; and (*2R*)-*trans*-isomer **25b** (307 mg, 32.0%) as an oil: $[\alpha]_D^{24}$ −10.07° ($c=0.57$, $CHCl_3$); IR (film) ν 2939, 2854, 1711, 1616, 1597, 1496, 1422, 1289, 1269, 1091, 964 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06 (d, $J=6.9$ Hz, 6H), 1.09–1.17 (m, 2H), 1.36–1.54 (m, 3H), 1.59–1.69 (m, 1H), 2.32 (t, $J=7.2$ Hz, 2H), 2.46–2.62 (m, 2H), 3.28 (dd, $J=9.6$, 8.1 Hz, 1H), 3.95–4.11 (m, 3H), 6.07 (app t, $J=2.1$ Hz, 2H), 6.63 (app t, $J=2.1$ Hz, 2H), 6.77–6.85 (m, 2H), 7.47–7.56 (m, 1H); MS (EI) m/z 376 ($M^+ + 1$), 375 (M^+); HRMS (EI) calcd for $C_{22}H_{27}O_2NF_2$ 375.2010 (M^+), found 375.2014.

(*2R-cis*)-2-(2,4-Difluorophenyl)-2-hydroxymethyl-4-(5-methyl-4-oxohexyl)tetrahydrofuran (26a). By the same method as described for **24a**, **23a** (527 mg, 1.52 mmol) was converted to **26a** (362 mg, 75.9% yield from **23a**) as an oil: $[\alpha]_D^{19}$ −10.76° ($c=1.49$, $CHCl_3$); IR (film) ν 3466, 2969, 2933, 2871, 1709, 1614, 1601, 1497, 1269, 1136, 967 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.07 (d, $J=6.9$ Hz, 6H), 1.34–1.44 (m, 2H), 1.46–1.59 (m, 2H), 1.94 (dd, $J=12.2$, 10.5 Hz, 1H), 2.04–2.15 (m, 2H), 2.33 (ddd, $J=11.9$, 6.9, 2.4 Hz, 1H), 2.43 (t, $J=6.9$ Hz, 2H), 2.56 (sept, $J=6.9$ Hz, 1H), 3.56 (t, $J=8.4$ Hz, 1H), 3.70 (ddd, $J=11.6$, 5.4, 1.2 Hz, 1H), 3.80 (ddd, $J=11.6$, 8.3, 2.1 Hz, 1H), 4.14 (t, $J=8.1$ Hz, 1H), 6.74–6.82 (m, 1H), 6.83–6.89 (m, 1H), 7.46–7.54 (m, 1H); MS (FAB) m/z 327 ($M^+ + 1$); HRMS (FAB) calcd for $C_{18}H_{25}O_3F_2$ 327.1772 ($M^+ + 1$), found 327.1786.

(*2R-trans*)-2-(2,4-Difluorophenyl)-2-hydroxymethyl-4-(5-methyl-4-oxohexyl)tetrahydrofuran (26b). By the same method as described for **24a**, **23b** (847 mg, 2.45 mmol) was converted to **26b** (568 mg, 71.6% yield from **23b**) as an oil: $[\alpha]_D^{21}$ −7.83° ($c=1.19$, $CHCl_3$); IR (film) ν 3464, 2969, 2937, 2871, 1710, 1615, 1600, 1497, 1421, 1269, 1135, 1056, 967 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.07 (d,

$J=7.2$ Hz, 6H), 1.26 (dd, $J=15.3$, 8.1 Hz, 2H), 1.49–1.60 (m, 2H), 1.71 (ddd, $J=12.9$, 9.3, 2.1 Hz, 1H), 2.21 (dd, $J=7.2$, 6.6 Hz, 1H), 2.31–2.44 (m, 3H), 2.55 (dd, $J=13.8$, 6.9 Hz, 1H), 2.60–2.69 (m, 1H), 3.40 (dd, $J=9.3$, 8.4 Hz, 1H), 3.60 (ddd, $J=11.5$, 6, 1.2 Hz, 1H), 3.70 (ddd, $J=11.3$, 7.8, 1.5 Hz, 1H), 4.16 (t, $J=8.1$ Hz, 1H), 6.78–6.85 (m, 2H), 7.51–7.59 (m, 1H); MS (FAB) m/z 327 ($M^+ + 1$); HRMS (FAB) calcd for $C_{18}H_{25}O_3F_2$ 327.1772 ($M^+ + 1$), found 327.1758.

(*2R-cis*)-2-(2,4-Difluorophenyl)-4-(5-methyl-4-oxohexyl)-2-[[(4-methylphenyl)sulfonyloxy]methyl]tetrahydrofuran (27a). To a solution of **26a** (158 mg, 0.48 mmol), 4-(dimethylamino)pyridine (6 mg, 0.05 mmol) and triethylamine (0.14 mL, 0.97 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added in portions *p*-toluenesulfonyl chloride (138 mg, 0.72 mmol). After stirring at room temperature for 7 h, the solvent was distilled off under reduced pressure, and the residue was diluted with EtOAc (40 mL). The organic layer was washed with aqueous solution of water, 5% citric acid (10 mL) and brine (15 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give **27a** (229 mg, 98.5%): $[\alpha]_D^{20}-8.38°$ ($c=1.03$, $CHCl_3$); IR (film) ν 2970, 2934, 2872, 1711, 1615, 1599, 1498, 1363, 1177, 968 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.07 (d, $J=6.9$ Hz, 6H), 1.29–1.40 (m, 2H), 1.40–1.55 (m, 2H), 1.84 (dd, $J=12.5$, 10.2 Hz, 1H), 1.96–2.10 (m, 1H), 2.31 (ddd, $J=12.4$, 7.2, 2.7 Hz, 1H), 2.39–2.44 (m, 5H), 2.56 (sept, $J=6.9$ Hz, 1H), 3.51 (t, $J=8.4$ Hz, 1H), 4.05 (t, $J=7.8$ Hz, 1H), 4.16 (dd, $J=10.5$, 0.9 Hz, 1H), 4.24 (d, $J=10.5$ Hz, 1H), 6.62–6.69 (m, 1H), 6.79–6.85 (m, 1H), 7.27–7.29 (m, 2H), 7.44–7.52 (m, 1H), 7.64–7.67 (m, 2H); MS (FAB) m/z 481 ($M^+ + 1$); HRMS (FAB) calcd for $C_{25}H_{31}O_5F_2S$ 481.1860 ($M^+ + 1$), found 481.1865.

(*2R-trans*)-2-(2,4-Difluorophenyl)-4-(5-methyl-4-oxohexyl)-2-[[(4-methylphenyl)sulfonyloxy]methyl]tetrahydrofuran (27b). By the same method as described for **27a**, **26b** (142 mg, 0.43 mmol) was converted to **27b** (206 mg, 98.5%) as an oil: $[\alpha]_D^{22}-5.74°$ ($c=1.03$, $CHCl_3$); IR (film) ν 2969, 2939, 2871, 1710, 1615, 1598, 1498, 1363, 1177, 966 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06 (d, $J=6.9$ Hz, 6H), 1.18–1.28 (m, 2H), 1.45–1.57 (m, 1H), 1.62–1.70 (m, 1H), 2.30–2.44 (m, 6H), 2.50–2.65 (m, 2H), 3.34 (dd, $J=9.2$, 8.4 Hz, 1H), 4.0 (dd, $J=10.1$, 0.9 Hz, 1H), 4.08 (t, $J=7.5$ Hz, 1H), 4.16 (dd, $J=10.2$, 0.6 Hz, 1H), 6.64–6.71 (m, 1H), 6.78–6.85 (m, 1H), 7.28–7.31 (m, 2H), 7.48–7.57 (m, 1H), 7.65–7.69 (m, 2H); MS (FAB) m/z 481 ($M^+ + 1$); HRMS (FAB) calcd for $C_{25}H_{31}O_5F_2S$ 481.1860 ($M^+ + 1$), found 481.1866.

(*2R-cis*)-2-(2,4-Difluorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-4-(5-methyl-4-oxohexyl)tetrahydrofuran (28a). A solution of **27a** (183 mg, 0.38 mmol) and imidazole sodium salt (90 mg, 0.90 mmol) in *N*,*N*′-dimethylpropyleneurea (3 mL) was stirred at 100 °C for 44 h. The solvent was distilled off under reduced pressure, and the residue was partitioned between EtOAc (30 mL) and water (15 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (acetone–hexane gradient) to give **28a** (40 mg, 27.9%): $[\alpha]_D^{21}-31.71°$ ($c=0.1$, $CHCl_3$); IR (film) ν 2970, 2935, 2871, 1710, 1615, 1600, 1498, 1269, 1136, 1076, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.06 (d, $J=6.9$ Hz, 6H), 1.16–1.27 (m, 2H), 1.35–1.52 (m, 2H), 1.55 (dd, $J=12.5$, 10.8 Hz, 1H), 1.98–2.10 (m, 1H), 2.36–2.46 (m, 3H), 2.54 (sept, $J=6.9$ Hz, 1H), 3.44 (t, $J=9$ Hz, 1H), 4.09 (t, $J=8.1$ Hz, 1H), 4.24 (dd, $J=17.1$, 14.4 Hz, 2H), 6.76–6.85 (m, 2H), 6.86 (s, 1H), 6.92 (s, 1H), 7.32–7.40 (m, 2H); MS (FAB) m/z 377 ($M^+ + 1$): HRMS (FAB) calcd for $C_{21}H_{27}O_2N_2F_2$ 377.2041 ($M^+ + 1$), found 377.2039.

(*2R-trans*)-2-(2,4-Difluorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]-4-(5-methyl-4-oxohexyl)tetrahydrofuran (28b). By the same method as described for **28a**, **27b** (200 mg, 0.42 mmol) was converted to **28b** (51 mg, 32.6%) as an oil: $[\alpha]_D^{21}-20.0°$ ($c=0.1$, $CHCl_3$); IR (film) ν 2969, 2938, 2870, 1709, 1616, 1598, 1499, 1269, 1107, 964 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (d, $J=6.9$ Hz, 6H), 1.11–1.19 (m, 2H), 1.36–1.54 (m, 2H), 1.61–1.79 (m, 2H), 2.34 (t, $J=7.2$ Hz, 2H), 2.46–2.58 (m, 2H), 3.34 (t, $J=9.3$ Hz, 1H), 4.04–4.19 (m, 3H), 6.77–6.85 (m, 2H), 6.87 (s, 1H), 6.95 (s, 1H), 7.40 (s, 1H), 7.42–7.49 (m, 1H); MS (FAB) m/z 377 ($M^+ + 1$); HRMS (FAB) calcd for $C_{21}H_{27}O_2N_2F_2$ 377.2041 ($M^+ + 1$), found 377.2048.

(*2R-cis*)-4-(4-Amino-5-methylhexyl)-2-(2,4-difluorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (3a). A solution of **24a** (111 mg, 0.29 mmol), hydroxylamine hydrochloride (33 mg, 0.48 mmol), and pyridine (77 μL, 0.95 mmol) in absolute EtOH (4 mL) was stirred overnight at room temperature. The solvent was distilled off under reduced pressure, and the residue was partitioned between EtOAc (30 mL) and water (15 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc–hexane gradient) to give the corresponding oxime (95 mg, 82.3%). To a solution of the oxime (63 mg, 0.16 mmol) and $NiCl_2{\cdot}6H_2O$ (77 mg, 0.32 mmol) in MeOH (2 mL) at −30 °C was added sodium borohydride (61 mg, 1.61 mmol), and then the mixture was stirred at the same temperature for 30 min. The mixture was allowed to warm up to room temperature. After stirring for 1.5 h, the solvent was distilled off under reduced pressure. The residue was dissolved in aqueous solution of 10% HCl (1 mL). The mixture was basified with diluted ammonium hydroxide and partitioned between EtOAc (20 mL) and water (5 mL). The organic layer was washed with water (5 mL×2), dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH, 15:1.1:0.15, v:v:v) to give **3a** (54 mg, 89.1%): IR (film) ν 3376, 3308, 2918, 1611 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (d, $J=6.7$ Hz, 3H), 0.87 (d, $J=6.8$ Hz, 3H), 1.1–1.4 (m, 6H), 1.56 (m, 1H), 1.69 (m, 3H), 2.05 (m, 1H), 2.47 (m, 2H), 3.37 (t, 1H), 4.10 (t, $J=7.8$ Hz, 1H), 4.50 (d, $J=14.2$ Hz, 1H), 4.61 (d, $J=14.3$ Hz, 1H), 6.82 (m, 2H), 7.37 (m, 1H), 7.81 (s, 1H), 8.11 (s, 1H); MS (FAB) m/z 379 ($M^+ + 1$); HRMS (FAB) calcd for $C_{20}H_{29}ON_4F_2$ 379.2309 ($M^+ + 1$), found 379.2307.

(2*R*-*trans*)-4-(4-Amino-5-methylhexyl)-2-(2,4-difluorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]tetrahydrofuran (3b). By the same method as described for **3a**, **24b** (173 mg, 0.46 mmol) was converted to **3b** (95 mg, 81.6% yield from **24b**) as an oil: IR (film) ν 3375, 3306, 2915, 1609 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (dd, $J=6.8$, 0.9 Hz, 3H), 0.87 (d, $J=6.8$ Hz, 3H), 1.09–1.36 (m, 6H), 1.50–1.60 (m, 4H), 1.73 (m, 1H), 2.43 (m, 1H), 2.78 (m, 1H), 3.33 (t, $J=9.1$ Hz, 1H), 4.06 (t, $J=7.6$ Hz, 1H), 4.30 (d, $J=14.1$ Hz, 1H), 4.54 (d, $J=14.1$ Hz, 1H), 6.84 (m, 2H), 7.47 (m, 1H), 7.86 (s, 1H), 8.11 (s, 1H); MS (FAB) *m*/*z* 379 ($M^+ + 1$); HRMS (FAB) calcd for $C_{20}H_{29}ON_4F_2$ 379.2309 ($M^+ + 1$), found 379.2325.

(2*R*-*cis*)-4-(4-Amino-5-methylhexyl)-2-(2,4-difluorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]tetrahydrofuran (4a). By the same method as described for **3a**, **28a** (33.9 mg, 0.09 mmol) was converted to **4a** (20.5 mg, 60.3%) as an oil: IR (film) ν 3373, 3301, 2931, 2867, 1615, 1599, 1498, 1269, 1107, 1076, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (dd, $J=6.9$, 0.6 Hz, 3H), 0.87 (d, $J=6.9$ Hz, 3H), 1.10–1.22 (m, 2H), 1.26–1.36 (m, 4H), 1.49–1.60 (m, 4H), 2.02–2.09 (m, 1H), 2.37–2.45 (m, 2H), 3.43 (t, $J=9$ Hz, 1H), 4.09 (t, $J=7.8$ Hz, 1H), 4.24 (dd, $J=16.2$, 15 Hz, 2H), 6.76–6.84 (m, 2H), 6.86 (t, $J=1.2$ Hz, 1H), 6.92 (t, $J=1.2$ Hz, 1H), 7.32–7.41 (m, 2H); MS (FAB) *m*/*z* 378 ($M^+ + 1$); HRMS (FAB) calcd for $C_{21}H_{30}ON_3F_2$ 378.2357 ($M^+ + 1$), found 378.2358.

(2*R*-*trans*)-4-(4-Amino-5-methylhexyl)-2-(2,4-difluorophenyl)-2-[(1*H*-imidazol-1-yl)methyl]tetrahydrofuran (4b). By the same method as described for **3a**, **28b** (44.7 mg, 0.12 mmol) was converted to **4b** (17.6 mg, 39.3%) as an oil: IR (film) ν 3375, 3301, 2933, 2868, 1616, 1598, 1498, 1269, 1106, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (d, $J=6.9$ Hz, 3H), 0.87 (dd, $J=6.9$, 0.9 Hz, 3H), 1.11–1.24 (m, 4H), 1.24–1.36 (m, 2H), 1.40–1.58 (m, 3H), 1.68–1.79 (m, 2H), 2.39–2.45 (m, 1H), 2.49–2.58 (m, 1H), 3.34 (t, $J=9$ Hz, 1H), 4.04–4.19 (m, 3H), 6.79–6.87 (m, 3H), 6.95 (s, 1H), 7.40–7.50 (m, 2H); MS (FAB) *m*/*z* 378 ($M^+ + 1$); HRMS (FAB) calcd for $C_{21}H_{30}ON_3F_2$ 378.2357 ($M^+ + 1$), found 378.2367.

(2*R*-*cis*)-4-(4-Amino-5-methylhexyl)-2-(2,4-difluorophenyl)-2-[(1*H*-pyrrol-1-yl)methyl]tetrahydrofuran (5a). By the same method as described for **3a**, **25a** (107.4 mg, 0.29 mmol) was converted to **5a** (44.4 mg, 74.9% yield from **25a**) as an oil: IR (film) ν 2928, 2868, 1615, 1599, 1496, 1421, 1289, 1269 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83–0.89 (m, 6 H), 1.11–1.40 (m, 6 H), 1.50–1.66 (m, 2H), 1.92–2.09 (m, 1H), 2.35 (ddd, $J=12.2$, 6.9, 2.7 Hz, 1H), 2.46–2.60 (br, 3H), 3.40 (app t, $J=8.1$ Hz, 1H), 4.05 (t, $J=8.1$ Hz, 1H), 4.17 (s, 2H), 6.03 (app t, $J=2.1$ Hz, 2H), 6.61 (t, $J=2.1$ Hz, 2H), 6.75–6.83 (m, 2H), 7.37–7.45 (m, 1H); MS (EI) *m*/*z* 376 (M^+); HRMS (EI) calcd for $C_{22}H_{30}ON_2F_2$ 376.2326 (M^+), found 376.2332.

(2*R*-*trans*)-4-(4-Amino-5-methylhexyl)-2-(2,4-difluorophenyl)-2-[(1*H*-pyrrol-1-yl)methyl]tetrahydrofuran (5b). By the same method as described for **3a**, **25b** (193 mg, 0.51 mmol) was converted to **5b** (92 mg, 82.5% yield from **25b**) as an oil: IR (film) ν 2932, 2855, 1616, 1598, 1496, 1422, 1289, 1269, 1114 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (d, $J=6.6$ Hz, 3H), 0.88 (d, $J=6.6$ Hz, 3H), 1.10–1.20 (br, 4H), 1.26–1.35 (m, 2H), 1.52–1.70 (m, 3H), 1.89 (br, 2H), 2.46 (br, 1H), 2.54–2.62 (m, 1H), 3.29 (app t, $J=8.1$ Hz, 1H), 3.93–4.11 (m, 3H), 6.06 (t, $J=2.1$ Hz, 2H), 6.62 (t, $J=2.1$ Hz, 2H), 6.77–6.85 (m, 2H), 7.47–7.53 (m, 1H); MS (EI) *m*/*z* 376 ($M^+ + 1$), 376 (M^+); HRMS (EI) calcd for $C_{22}H_{30}ON_2F_2$ 376.2326 (M^+), found 376.2332.

Antifungal bioassays

The in vitro antifungal activities were assayed by microdilution method[21] with Kimmig's broth. Cultures grown on yeast malt extract agar were used to prepare the inoculums which were adjusted to 1×10^4 CFU/mL. Test compounds were serially diluted to provide a range of 0.05–100 μg/ml. Microtiter plates were incubated for 48 h at 30 °C, and minimal inhibitory concentration (MIC) was determined as the concentration showing 90% inhibition of growth by visual inspection relative to drug-free control.

The in vivo efficacy against systemic murine candidiasis was measured as follows. Male ICR mice weighing 23–25 g were infected with *Candida albicans* B02630 by injecting $0.7–1.0 \times 10^7$ CFU into tail vein. Test compounds suspended in 70% PEG 200 were orally administered at the dose range of 2–10 mg/kg by gavage once daily for 3 days starting at 1 h post-infection. The survival rates were recorded for a period of 7 days.

Inhibition assay of rat liver microsomal lanosterol 14-demethylase

The enzyme was prepared from 3–4 mg of rat liver microsomes and 10 mg protein of post-microsomal supernatant. And 46.9 nmol of lanosterol dispersed with Tween 80 was used as the substrate. Lanosterol 14-demethylase activity under these conditions was 0.6–1.0 nmol/min/mg protein of microsomes. Concentrations of inhibitors were 10 μM. Reactions were run at 37 °C for 10 min under constant shaking in air, and sterols were extracted from the reaction mixture after saponfication. The analyses of the lanosterol fraction separated with TLC and the calculations of the 14-demethylase activities were done according to the previous method.[22]

Acknowledgements

We thank POSTECH/RIST/POSCO for the financial support of this work. We are grateful to Professor W. David Nes and Dr. Zhonghua Jia for the inhibition assays of our compounds against Δ^{24}-sterol methyltransferase.

References and Notes

1. (a) Georgiev, V. St. *Ann. N. Y. Acad. Sci.* **1988**, *544*, 1. (b) Clark, A. M. In *New Approach for Antifungal Drugs*; Fernanades, P. B., Ed.; Birkhauser: Boston, 1992; pp 1–19. (c) Tuite, M. F. *TIBTECH*. **1996**, *14*, 219. (d) Balkovec, J. M. In *Ann.*

Rep. Med. Chem.; Bristol, J. A., Ed.; Academic Press: San Diego, 1998; Vol. 33, pp 173–116. (e) Andriole, V. T. *J. Antimicrob. Chemother.* **1999**, *44*, 151.
2. (a) Nes, W. R., McKean, M. L. *Biochemistry of Steroids and Other Isoprenoids*; University Park Press: Baltimore, 1977. (b) Schroepfer, G. J., Jr. *Ann. Rev. Biochem.* **1982**, *51*, 555. (c) Mercer, E. I. *Pesticide Sci.* **1984**, *15*, 133.
3. Adams, J. L., Metcalf, B. W. In *Comprehensive Medicinal Chemistry*; Hansch, C.; Sammes, P. G.; Taylor, J. B., Eds.; Pergamon: Oxford, 1990; Vol. 2, pp 333–364.
4. (a) Nes, W. D., Guo, D., Zhou, W. *Arch. Biochem. Biophys.* **1997**, *342*, 68. (b) Ator, M. A., Schmidt, S. J., Adams, J. L., Dolle, J. M., R. E.; Kruse, L. I., Frey, C. L., Barone *J. Med. Chem.* **1992**, *35*, 100. (c) Acuna-Johnson, A. P., Oehlschlager, C., Pierce, A. M., Pierce, H. D., Jr., Czyzewska, E. K. *Bioorg. Med. Chem.* **1997**, *5*, 821. (d) Beuchet, P., Dherbomez, M., Elkiel, L., Charles, G., Letourneux, Y. *Bioorg. Med. Chem.* **1999**, *9*, 1599.
5. (a) Chung, S.-K., Ryoo, C. H., Yang, H. W., Shim, J.-Y., Kang, M. G., Lee, K. W., Kang, H. I. *Tetrahedron* **1998**, *54*, 15899. (b) Chung, S.-K., Ryoo, C. H., Yang, H. W., Shim, J.-Y., Kang, H. I. *Kor. J. Med. Chem.* **1998**, *8*, 10. (c) Chung, S.-K., Shim, J.-Y., Kang, M. G., Lee, K. W., Kang, H. I. *ibid.* **1998**, *8*, 14. (d) A recent enzymic assay showed that indeed these compounds are potent inhibitors of 24-SMT; pers. comm. with Professor W. D. Nes.
6. Cooper, A. B.; Wright, J. J.; Ganguly, A. K.; Desai, J.; Loebenberg, D.; Parmegiani, R.; Feingold, D. S.; Sud, I. J. *J. Chem. Soc., Chem. Commun.* **1989**, *898*.
7. Aryloxazolidines,[23a] arylmorpholines,[23b] and aryloxathianes[23c] were designed and synthesized to simulate lanosterol skeleton.
8. Saksena, A. K.; Girijavallabhan, V. M.; Lovery, R. G.; Pike, R. E.; Desai, J. A.; Ganguly, A. K.; Hare, R. S.; Loebenberg, D.; Cacciapuoti, A.; Parmegiani, M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2023.
9. (a) Diastereomeric purities were confirmed by GC after conversion of the tosylate to the corresponding cyanide. The optical purities of both compounds were >96% de. (b) GC conditions: HP 5890 Series Plus gas chromatography; Quadrex 400-5HT Cap. Column 0.25 mm×15 m; inj. temp. 400 °C; det. temp. 400 °C; 70–400 °C (15 °C/min); split 1:20.
10. Proudfoot, J. R.; Li, X.; Djerassi, C. *J. Org. Chem.* **1985**, *50*, 2026.
11. Cohen, N.; Eichel, W. F.; Lopresti, R. J.; Neukom, C.; Saucy, G. *J. Org. Chem.* **1976**, *41*, 3512.
12. Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183.
13. Both compounds were of high diastereomeric purity (>94% de) on the basis of NMR.
14. (a) March, J. *Advanced Organic Chemistry*; 3rd ed.; John Wiley & Sons: New York, 1985; p 229 and references therein. (b) Saksena, A. K., Girijavallabhan, V. M., Lovery, R. G., Pike, R. E., Wang, H., Ganguly, A. K., Morgan, B., Zaks, A.; Puar, M. S. *Tetrahedron Lett.* **1995**, *36*, 1787.
15. (a) Secrist, J. A. III, Logue, M. W. *J. Org. Chem.* **1972**, *37*, 335. (b) Kano, S., Tanaka, Y., Sugino, E., Hibino, S. *Synthesis* **1980**, 695. (c) Wang, S. S., Sukenik, C. N. *J. Org. Chem.* **1985**, *50*, 5448.
16. (a) Ipaktschi, J. *Chem. Ber.* **1984**, *117*, 856. (b) Nose, A., Kudo, T. *Chem. Pharm. Bull.* **1981**, *29*, 1159.
17. It is known that removal of halogen from aromatic rings can be effected by $NaBH_4$ with aid of metal salts. See (a) Egli, R. A. *Helv. Chim. Acta* **1968**, *51*, 2090. (b) Lin, S-.T., Roth, J. A. *J. Org. Chem.* **1979**, *44*, 309.
18. It is known that the inhibitory effects of azole compounds on mammalian sterol 14DM are qualitatively the same as those on the yeast enzyme.[22]
19. Nes, W. D. Unpublished results. Enzyme assay with zymosterol and AdoMet as substrates and 24-SMT showed that all compounds would mildly inhibit 24-SMT. The concentrations of zymosterol and inhibitors were 50 μM.
20. (a) Boyle, F. T., Ryley, J. F., Wilson, R. G. In *Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents*; Fromtling, R. A., Ed.; J. R. Prouse Science: Barcelona, 1987; pp 31–41. (b) Anaissie, E. J., Karyotakis, N. C., Hachem, R., Dignani, M. C., Rex, J. H., Paetznick, V. *J. Infect. Dis.* **1994**, *170*, 384.
21. National Committee for Clinical Laboratory Standards, 1992. Reference method for broth dilution for antifungal susceptibility testing of yeast; proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, PA, USA.
22. Aoyama, Y.; Yoshida, Y. *Biochem. Biophys. Res. Commun.* **1991**, *178*, 1064.
23. (a) Konosu, T., Tajima, Y., Takeda, N., Miyaoka, T., Kasahara, M., Yasuda, H., Oida, S. *Chem. Pharm. Bull.* **1990**, *38*, 2476. (b) Bartroli, J., Turmo, E., Algueró, M., Boncompte, E., Vericat, M. L., García-Rafanell, J., Forn, J. *J. Med. Chem.* **1995**, *38*, 3918. (c) Miyauchi, H., Tanio, T., Ohashi, N. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2377.