Chemical Modification of Fumagillin. III.[1] Modification of the Spiro-epoxide

Shogo MARUI,* Toshihiro YAMAMOTO, Katsuichi SUDO, Hiroshi AKIMOTO, and Shoji KISHIMOTO

Pharmaceutical Research Laboratories III, Takeda Chemical Industries, Ltd., 17–85 Juso-honmachi 2-chome, Yodogawa-ku, Osaka 532, Japan. Received August 15, 1994; accepted November 9, 1994

The spiro-epoxy group of fumagillol (2) was selectively modified and several analogues of AGM-1470 (3) with a (dialkyl)-β-hydroxyethylsulfonium moiety were prepared. These analogues were found to inhibit angiogenesis induced by basic fibroblast growth factor in the rat micropocket assay. They also inhibited the growth of M5076 cells *in vivo*, but did not affect the body weight change of the tested mice during the assay.

Key words fumagillin; AGM-1470; sulfonium analogue; anti-angiogenic activity; antitumor activity

Growth of solid tumors is thought to be dependent on angiogenesis, and so inhibition of angiogenesis should provide a new approach to cancer therapy.[2] Recently Ingber *et al.*[3] reported that fumagillin (**1**) showed potent anti-angiogenic activity, but it appeared to be toxic. Therefore, we started chemical modification of **1** in an effort to obtain analogues retaining the potent anti-angiogenic activity of **1** but having less toxicity. In previous papers,[1] we reported chemical modifications of the 6-hydroxyl group of fumagillol (**2**), a degradation product of **1**, and found that the compounds obtained by these chemical modifications possess anti-angiogenic activity. For example, AGM-1470 (**3**) showed potent anti-angiogenic activity and also found exhibited potent antitumor activity.[3,4] However, **3** is a rather hydrophobic compound, and introduction of a hydrophilic group should improve the water-solubility.

It is known that the spiro-epoxide of fumagillol (**2**) is more reactive to the nucleophilic attack of hydrides than the other epoxide.[5] This suggests that selective modification of the spiro-epoxide would be possible. Therefore, we examined conversion of the spiro-epoxide of **2** to the (dialkyl)-β-hydroxyethylsulfonium moiety, and some sulfonium analogues of **3** were prepared. We expected that the (dialkyl)-β-hydroxyethylsulfonium moiety would endow the molecule with hydrophilicity and that such a synthetic equivalent to an epoxide would show similar biological activities. Here we report the synthesis and biological activities of sulfonium analogues of **3**.

Chemistry

The spiro epoxide of fumagillol (**2**) could be selectively opened by treatment with sodium thiomethoxide to give **4** (yield, 69%), and **4** afforded **5** in 83% yield upon reaction with chloroacetyl isocyanate. The dimethylsulfonium compound **6** was obtained quantitatively by the methylation of the methylthio group of **5**.

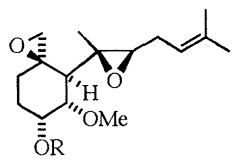
Although the benzylmethylsulfonium compound **8a** could be prepared in a similar manner, isolation of **8a** from some by-products was difficult. However, it was found that the following method gives a better result. Compound **4** was treated with benzyl bromide, and then the secondary hydroxyl group was carbamoylated. It was found that the benzylation reaction of **4** can be accelerated by the addition of AgBr. Compounds with a substituent on the phenyl ring (**8b—e**) were prepared by the same method (Chart 2).

Compounds **8a—e** were 1 : 1 mixtures of diastereomers because of a newly formed asymmetric center at the sulfur atom. Therefore compound **12** was synthesized as a related compound with no asymmetric center at the sulfur atom (Chart 3).

Fumagillol (**2**) was treated with 2-mercaptomethylbenzyl alcohol[6] in the presence of sodium methoxide to give **9** in 92% yield. The hydroxyl group at the benzyl position of **9** could be selectively mesylated and **10** was obtained. It was found that compound **10** is a relatively unstable compound and spontaneous cyclization reaction at the sulfur atom occurred gradually at room temperature to give **11**. Therefore crude **10** was submitted to the next reaction without isolation, or heated in CH_2Cl_2 at 30 °C for 24 h (yield of **11** from **9**, 96%). A chloroacetylcabamoyl group was introduced by the treatment of **11** with chloroacetyl isocyanate and exchange of the counter anion occurred with Cl^- derived from NaCl, which was used in the course of extraction, to give **12** in 29% yield.

As reported in the previous paper,[1b] the amino analogues of fumagillol (**2**) also have anti-angiogenic activity. Therefore the ureido analogue of **12** was synthesized (Chart 4).

6-Oxo-6-deoxyfumagillol (**13**)[1b] gave **14** in 85% yield by the reaction with 2-mercaptomethylbenzyl alcohol in the presence of sodium methoxide. Reductive amination of **14** using $AcONH_4$ and $NaBH_3CN$ gave **15** (yield, 58%), and **16** was obtained in 64% yield by treatment with



fumagillin (**1**) : R = CO(CH=CH)$_4$CO$_2$H

fumagillol (**2**) : R = H

AGM-1470 (**3**) : R = CONHCOCH$_2$Cl

Chart 1

* To whom correspondence should be addressed.

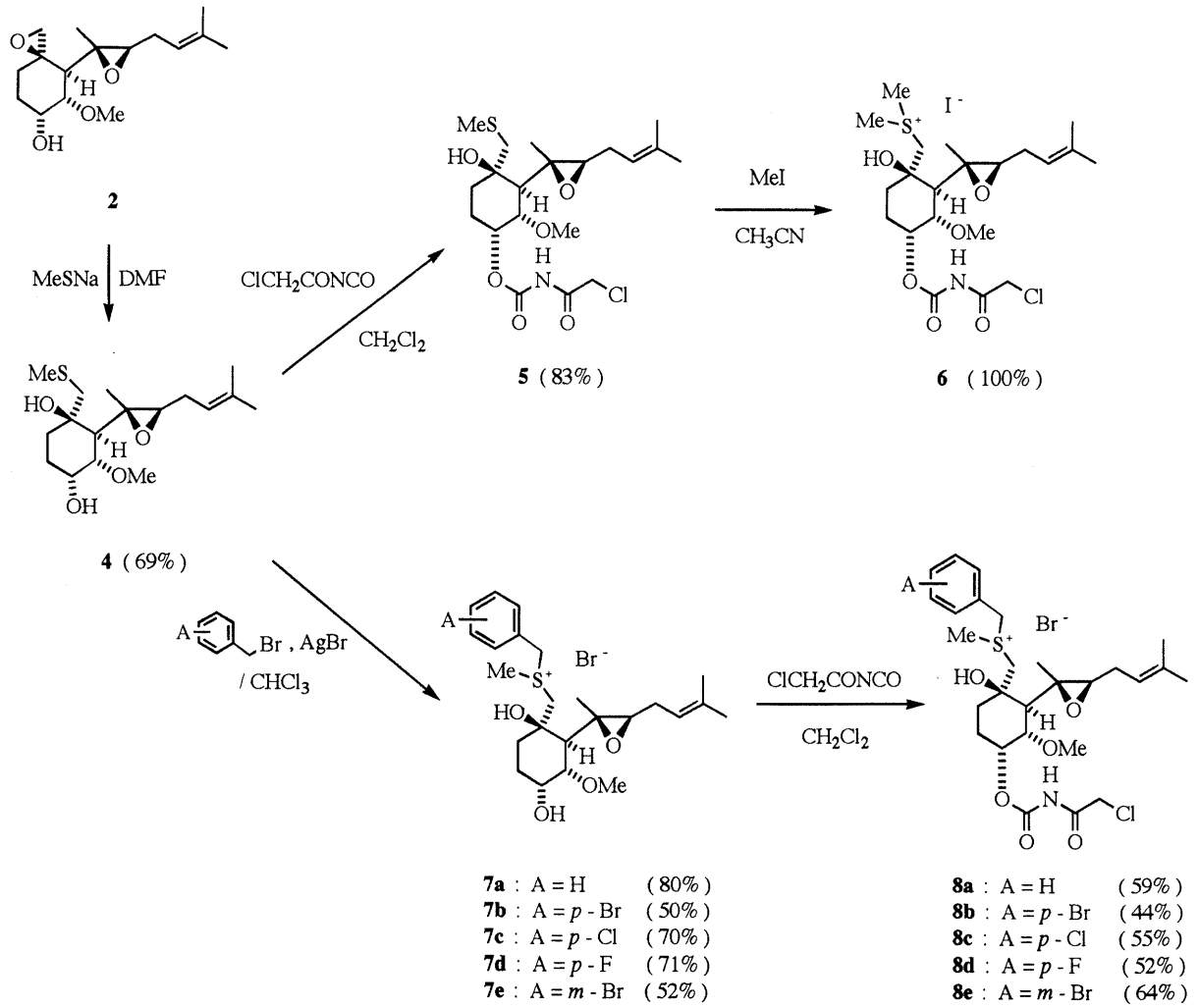


Chart 2

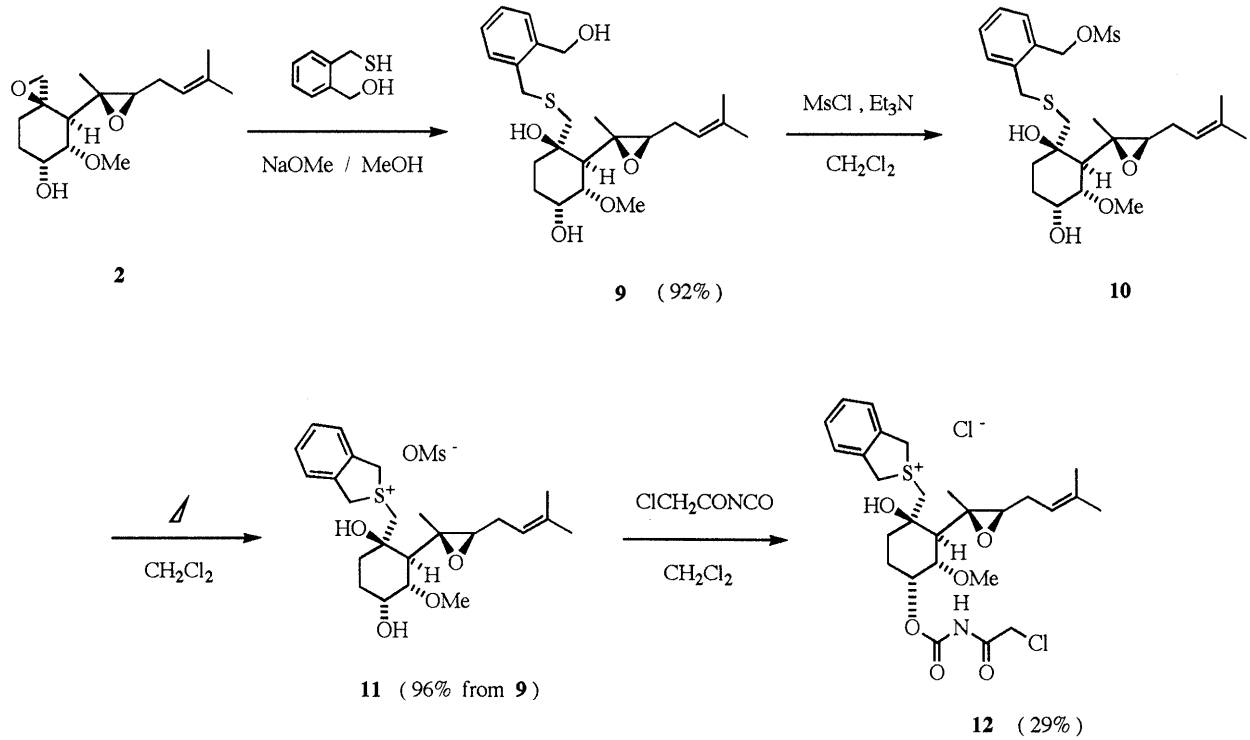
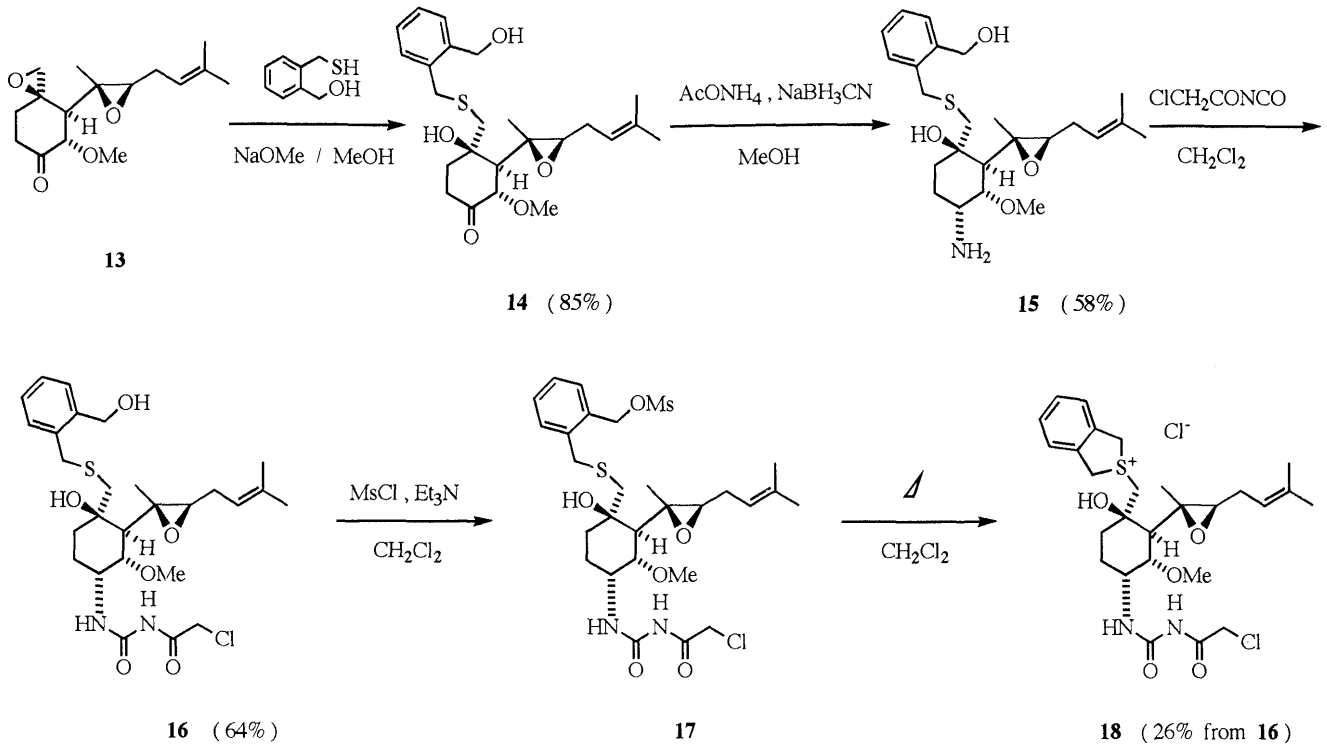


Chart 3



13

14 (85%)

15 (58%)

16 (64%)

17

18 (26% from 16)

Chart 4

chloroacetyl isocyanate. The hydroxyl group at the benzyl position of **16** was mesylated and the resulting **17** was heated in CH_2Cl_2 at 30 °C for 24 h to give **18** (yield, 26% from **16**).

Biological Activities and Discussion

The sulfonium compounds obtained above were found to show anti-angiogenic activity in the rat corneal micropocket assay. For example, administration of **8a** inhibited the angiogenesis induced by basic fibroblast growth factor (bFGF) in 75% of corneas. Next, the antitumor activity of the sulfonium analogues against M5076 cells was examined *in vivo* (Table 1).

All the sulfonium compounds showed antitumor activity. Some relation between the electronic effect of the substituents on the phenyl ring of **8a**—**e** and the anti-tumor activity was observed, but the effect of these substituents was not clear-cut.

Compound **12**, which has a cyclic sulfonium moiety and therefore no asymmetric center at the sulfur atom, showed the most potent antitumor activity. The activity seems to be almost equal to that of AGM-1470 (**3**).[4] Compound **18**, the ureido analogue of **12**, also showed antitumor activity, although its activity is not so potent as that of **12**.

Yamaoka *et al.* reported that the body weight change of mice treated with **3**, was mildly affected.[4] However, in the case of sulfonium analogues, the body weight of tested mice increased similarly to that of the control mice. This may suggest low toxicity of the sulfonium compounds.

Water-solubility of **12**, which showed the most potent antitumor activity among these sulfonium compounds, was then compared with that of **3**. Compound **12** (25 mg) was added to 1 ml of distilled water and the mixture was stirred vigorously for 30 min. Most of **12** dissolved. On the other hand, **3** remained insoluble when similarly treated. The supernatants were subjected to HPLC analysis, and the concentration estimated for **3** was 1 mg/ml, while that of **12** was 22 mg/ml.

Table 1. Antitumor Activity of Sulfonium Analogues

Compound No.	Dose (mg/kg/d)	T/C (%)	Body weight change[a] (g)
3[b]	15	13	—[c]
6	30	36	+0.1
8a	30	20	0.0
8b	20	21	+0.2
8c	20	27	+0.3
8d	20	29	0.0
8e	20	14	+0.1
12	20	9	+0.2
18	20	22	+0.3

a) Mean difference of body weight from control. *b*) Cited from ref. 4. *c*) See text and ref. 4. T/C, tested/control.

The sulfonium compounds obtained above, especially **12**, seem to possess favourable profiles of toxicity and solubility compared to **3**.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DPI-181. Infrared (IR) spectra were taken on a JASCO IR-810 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini-200, and chemical shifts are given in ppm with tetramethylsilane as the internal standard. Mass spectra (MS) were obtained on a Hitachi RMU-6D. HPLC analysis was carried out using a Waters M-6000A apparatus.

(1*R*,2*R*,3*S*,4*R*)-4-Hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]-1-[(methylthio)methyl]cyclohexanol (4)

Thiomethoxide (2.23 g) was added to a solution of fumagillol (**2**, 3.00 g) in *N*,*N*-dimethylformamide (DMF) (6 ml) and the reaction mixture was stirred for 1 h at room temperature. Water (20 ml) was then added and the products were extracted with diisopropyl ether. The extract was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt : hexane = 1 : 2) and recrystallized from hexane to give **4** (2.43 g, 69%) as colorless crystals, mp 52—53 °C, $[\alpha]_D^{24}$ −57.5° (*c* = 0.20, $CHCl_3$). IR (KBr): 3380, 2930, 1375, 1335, 1120, 1080, 1040 cm^{-1}. NMR ($CDCl_3$) δ: 1.45 (3H, s), 1.55 (1H, m), 1.66 (3H, s), 1.74 (3H, s), 1.65—1.90 (4H, m), 2.10—3.05 (3H, m), 3.30 (1H, m), 3.35 (3H, s), 4.22 (1H, m), 5.19 (1H, m). *Anal.* Calcd for $C_{17}H_{30}O_4S$: C, 61.78; H, 9.15. Found: C, 61.66; H, 9.30.

(1*R*,2*R*,3*S*,4*R*)-4-(Chloroacetylcarbamoyl)oxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]-1-[(methylthio)methyl]-cyclohexanol (5) Chloroacetyl isocyanate (0.46 ml) was added to a solution of **4** (1.50 g) in dichloromethane (15 ml) with ice-cooling. The reaction mixture was stirred for 20 min, and then diluted with AcOEt (60 ml). The AcOEt solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt : hexane = 1 : 2) and recrystallized from ether–hexane to give **5** (1.70 g, 83%) as colorless crystals, mp 101–102 °C, $[\alpha]_D^{26}$ −81.5° (*c* = 0.20, $CHCl_3$). IR (KBr): 3440, 3260, 2930, 1760, 1720, 1545, 1525, 1255, 1190, 1025 cm^{-1}. NMR ($CDCl_3$) δ: 1.46 (3H, s), 1.66 (3H, s), 1.74 (3H, s), 1.60—1.90 (4H, m), 2.05—2.55 (3H, m), 2.21 (3H, s), 2.84 (1H, d, *J* = 13 Hz), 2.93 (1H, t, *J* = 7 Hz), 2.99 (1H, d, *J* = 13 Hz), 3.32 (3H, s), 3.34 (1H, m), 4.51 (2H, s), 5.19 (1H, m), 5.45 (1H, m). *Anal.* Calcd for $C_{20}H_{32}ClNO_6S$: C, 53.38; H, 7.17; N, 3.11. Found: C, 53.34; H, 7.14; N, 3.03.

[[(1*R*,2*R*,3*S*,4*R*)-4-(Chloroacetylcarbamoyl)oxy-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]-methyl]dimethylsulfonium Iodide (6) Methyl iodide (0.82 ml) was added to a solution of **5** (500 mg) in CH_3CN (2 ml). The reaction mixture was stirred for 8 h, and then concentrated *in vacuo*. The residue was triturated with ether to give **6** (701 mg, quantitative) as a white powder, $[\alpha]_D^{26}$ −49.0° (*c* = 0.20, $CHCl_3$). IR (KBr): 3410, 2960, 2920, 1775, 1690, 1510, 1210, 1030 cm^{-1}. NMR (CD_3OD) δ: 1.47 (3H, s), 1.69 (3H, s), 1.75 (3H, s), 1.65—1.95 (4H, m), 2.10—2.60 (3H, m), 3.01 (3H, s), 3.07 (3H, s), 3.12 (1H, t, *J* = 6 Hz), 3.46 (1H, m), 3.70 (1H, d, *J* = 13 Hz), 4.03 (2H, s), 4.05 (1H, d, *J* = 13 Hz), 5.24 (1H, m), 5.48 (1H, m). *Anal.* Calcd for $C_{21}H_{35}ClINO_6S \cdot 4H_2O$: C, 37.99; H, 6.53; N, 2.11. Found: C, 38.07; H, 6.36; N, 2.16.

Preparation of Compounds 7a—e. Typical Procedure: Benzyl[[1*R*,2*R*,3*S*,4*R*)-1,4-dihydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]methyl]methylsulfonium Bromide (7a) Silver bromide (228 mg) was added to a solution of **4** (1.00 g) and benzyl bromide (1.8 ml) in chloroform (1 ml) and the mixture was stirred for 4 h. Precipitates were removed and the filtrate was concentraged *in vacuo*. The residue was triturated with ether to give **7a** (1.22 g, 80%) as a white powder, $[\alpha]_D^{25}$ −26.9° (*c* = 0.20, $CHCl_3$). IR (KBr): 3400, 2920, 1455, 1380, 1120, 1080, 1045 cm^{-1}. NMR (CD_3OD) δ: 1.43 (3H, s), 1.68 (3H, s), 1.75 (3H, s), 1.55—1.90 (4H, m), 2.10—2.55 (3H, m), 2.83 (1.5H, s), 2.99 (1.5H, s), 3.10 (1H, t, *J* = 6 Hz), 3.27 (1H, m), 3.33 (1.5H, s), 3.35 (1.5H, s), 3.54 (1H, m), 3.86—4.12 (1H, m), 4.24 (1H, m), 4.50—4.92 (2H, m), 5.24 (1H, m), 7.48 (2H, m), 7.69 (2H, m).

[(4-Bromophenyl)methyl][[(1*R*,2*R*,3*S*,4*R*)-1,4-dihydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]methyl]methylsulfonium Bromide (7b) White amorphous powder, $[\alpha]_D^{25}$ −26.2° (*c* = 0.20, $CHCl_3$). IR (KBr): 3400, 2920, 1490, 1380, 1120, 1070, 1045 cm^{-1}. NMR (CD_3OD) δ: 1.43 (3H, s), 1.68 (3H, s), 1.75 (3H, s), 1.55—1.90 (4H, m), 2.10—2.55 (3H, m), 2.82 (1.5H, s), 2.99 (1.5H, s), 3.10 (1H, t, *J* = 6 Hz), 3.27 (1H, m), 3.33 (1.5H, 3.35 (1.5H, s), 3.54 (1H, m), 3.85—4.10 (1H, m), 4.24 (1H, m), 4.50—4.92 (2H, m), 5.24 (1H, m), 7.48 (2H, m), 7.69 (2H, m).

[(4-Chlorophenyl)methyl][[(1*R*,2*R*,3*S*,4*R*)-1,4-dihydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]-methyl]methylsulfonium Bromide (7c) White amorphous powder, $[\alpha]_D^{25}$ −30.4° (*c* = 0.20, $CHCl_3$). IR (KBr): 3390, 2920, 1490, 1380, 1095, 1045 cm^{-1}. NMR (CD_3OD) δ: 1.43 (3H, s), 1.68 (3H, s), 1.75 (3H, s), 1.55—1.90 (4H, m), 2.10—2.55 (3H, m), 2.82 (1.5H, s), 2.99 (1.5H, s), 3.09 (1H, t, *J* = 6 Hz), 3.27 (1H, m), 3.33 (1.5H, s), 3.35 (1.5H, s), 3.54 (1H, m), 3.90—4.10 (1H, m), 4.24 (1H, m), 4.50 (0.5H, d, *J* = 18 Hz), 4.71 (1H, s), 4.91 (0.5H, d, *J* = 18 Hz), 5.23 (1H, m), 7.53 (4H, m).

[(4-Fluorophenyl)methyl][[(1*R*,2*R*,3*S*,4*R*)-1,4-dihydroxy-3-methoxy-2-[(2R,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]-methylsulfonium Bromide (7d) White amorphous powder, $[\alpha]_D^{25}$ −35.1° (*c* = 0.20, $CHCl_3$). IR (KBr): 3390, 2920, 1510, 1225, 1120, 1045 cm^{-1}. NMR (CD_3OD) δ: 1.43 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 1.55—1.90 (4H, m), 2.10—2.55 (3H, m), 2.80 (1.5H, s), 2.98 (1.5H, s), 3.10 (1H, t, *J* = 6 Hz). 3.25 (1H, m), 3.33 (1.5H, s), 3.35 (1.5H, s), 3.53 (1H, m), 3.85—4.10 (1H, m), 4.24 (1H, m), 4.60 (0.5H, d, *J* = 17 Hz), 4.71 (1H, s), 4.92 (0.5H, d, *J* = 18 Hz), 5.23 (1H, m), 7.25 (2H, m), 7.59 (2H, m).

[(3-Bromophenyl)methyl][[(1*R*,2*R*,3*S*,4*R*)-1,4-dihydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]-methyl]methylsulfonium Bromide (7e) White amorphous powder, $[\alpha]_D^{25}$ −2.9° (*c* = 0.20, $CHCl_3$). IR (KBr): 3410, 2920, 1435, 1380, 1225, 1070, 1040 cm^{-1}. NMR (CD_3OD) δ: 1.44 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 1.55—1.90 (4H, m), 2.10—2.55 (3H, m), 2.83 (1.5H, s), 2.98 (1.5H, s), 3.10 (1H, t, *J* = 6 Hz), 3.25 (1H, m), 3.33 (1.5H, s), 3.35 (1.5H, s), 3.53 (1H, m), 3.85—4.10 (1H, m), 4.25 (1H, m), 4.60 (0.5H, d, *J* = 17 Hz), 4.69 (1H, s), 4.83 (0.5H, d, *J* = 17 Hz), 5.23 (1H, m), 7.35—7.85 (4H, m).

Preparation of Compound 8a—e. Typical Procedure: Benzyl[[(1*R*,2*R*,3*S*,4*R*)-4-(chloroacetylcarbamoyl)oxy-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]methyl]-methylsulfonium Bromide (8a) Chloroacetyl isocyanate (0.20 ml) was added to a solution of **7a** (1.00 g) in CH_2Cl_2 (3 ml) with ice-cooling and the reaction mixture stirred for 30 min. Water was added and the product was extracted with AcOEt. The extract was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 20 : 1) to give **8a** (730 mg, 59%) as a white amorphous powder, $[\alpha]_D^{26}$ −54.5° (*c* = 0.22, $CHCl_3$). IR (KBr): 3410, 2830, 1780, 1755, 1710, 1515, 1220, 1190, 1025 cm^{-1}. NMR (CD_3OD) δ: 1.42 (3H, m), 1.68 (3H, s), 1.74 (3H, s), 1.65—2.00 (4H, m), 2.10—2.60 (3H, m), 2.82 (1.5H, s), 3.00 (1.5H, s), 3.12 (1H, m), 3.30—3.75 (8H, m), 4.03 (1H, m), 4.45—5.05 (4H, m), 5.25 (1H, m), 5.45 (1H, m), 7.53 (4H, m). *Anal.* Calcd for $C_{27}H_{39}BrClNO_6S \cdot 2.5H_2O$: C, 48.69; H, 6.66; N, 2.10. Found: C, 48.66; H, 6.35; N, 2.29.

[(4-Bromophenyl)methyl][[(1*R*,2*R*,3*S*,4*R*)-4-(chloroacetylcarbamoyl)-oxy-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)-oxiran-2-yl]cyclohexyl]methyl]methylsulfonium Bromide (8b) White amorphous powder, $[\alpha]_D^{25}$ −43.5° (*c* = 0.20, $CHCl_3$). IR (KBr): 3400, 2930, 1780, 1755, 1720, 1485, 1200, 1070 cm^{-1}. NMR (CD_3OD) δ: 1.43 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 1.50—2.00 (4H, m), 2.10—2.55 (3H, m), 2.86 (1.5H, s), 3.01 (1.5H, s), 3.10 (1H, t, *J* = 6 Hz), 3.34 (1.5H, s), 3.37 (1.5H, s), 3.45 (1H, m), 3.57 (1H, m), 3.90—4.15 (1H, m), 4.43 (2H, s), 4.60 (0.5H, d, *J* = 13 Hz), 4.73 (1H, s), 4.91 (0.5H, d, *J* = 13 Hz), 5.23 (1H, m), 5.45 (1H, m), 7.49 (2H, m), 7.69 (2H, m). *Anal.* Calcd for $C_{27}H_{38}Br_2ClNO_6S$: C, 46.33; H, 5.47; N, 2.00. Found: C, 46.12; H, 5.78; N, 2.29.

[(4-Chlorophenyl)methyl][[1*R*,2*R*,3*S*,4*R*)-4-(chloroacetylcarbamoyl)-oxy-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)-oxiran-2-yl]cyclohexyl]methyl]methylsulfonium Bromide (8c) White amorphous powder, $[\alpha]_D^{24}$ −45.2° (*c* = 0.22, $CHCl_3$). IR (KBr): 3410, 2930, 1785, 1755, 1720, 1525, 1495, 1200, 1080 cm^{-1}. NMR (CD_3OD) δ: 1.43 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 1.60—1.95 (4H, m), 2.10—2.55 (3H, m), 2.85 (1.5H, s), 3.01 (1.5H, s), 3.09 (1H, t, *J* = 6 Hz), 3.33 (1.5H, s), 3.34 (1.5H, s), 3.45 (1H, m), 3.58 (1H, m), 3.90—4.20 (1H, m), 4.43 (1H, s), 4.44 (1H, s), 4.66—4.98 (2H, m), 5.23 (1H, m), 5.46 (1H, m), 7.55 (4H, m). *Anal.* Calcd for $C_{27}H_{38}BrCl_2NO_6S$: C, 49.47; H, 5.84; N, 2.14. Found: C, 49.65; H, 5.46; N, 2.34.

[[(1*R*,2*R*,3*S*,4*R*)-4-(Chloroacetylcarbamoyl)oxy-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]-methyl][(4-fluorophenyl)methyl]methylsulfonium Bromide (8d) White amorphous powder, $[\alpha]_D^{24}$ −53.5° (*c* = 0.22, $CHCl_3$). IR (KBr): 3410, 2930, 1785, 1720, 1510, 1230, 1200 cm^{-1}. NMR (CD_3OD) δ: 1.43 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 1.50—1.95 (4H, m), 2.10—2.55 (3H, m), 2.84 (1.5H, s), 3.00 (1.5H, s), 3.10 (1H, t, *J* = 6 Hz), 3.33 (1.5H, s), 3.35 (1.5H, s), 3.45 (1H, m), 3.58 (1H, m), 3.90—4.20 (1H, m), 4.43 (1H, s), 4.44 (1H, s), 4.70 (0.5H, d, *J* = 13 Hz), 4.87 (1H, s), 4.99 (0.5H, d, *J* = 13 Hz), 5.23 (1H, m), 5.46 (1H, m), 7.26 (2H, m), 7.61 (2H, m). *Anal.* Calcd for $C_{27}H_{38}BrClFNO_6S$: C, 50.75; H, 5.99; N, 2.19. Found: C, 50.93; H, 6.28; N, 2.38.

[(3-Bromophenyl)methyl][[(1*R*,2*R*,3*S*,4*R*)-4-(chloroacetylcarbamoyl)-oxy-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)-oxiran-2-yl]cyclohexyl]methyl]methylsulfonium Bromide (8e) White amorphous powder, $[\alpha]_D^{22}$ −38.3° (*c* = 0.20, $CHCl_3$). IR (KBr): 3420, 2930, 1785, 1755, 1715, 1520, 1385, 1200 cm^{-1}. NMR (CD_3OD) δ: 1.43

(3H, s), 1.68 (3H, s), 1.73 (3H, s), 1.50—1.95 (4H, m), 2.10—2.60 (3H, m), 2.87 (1.5H, s), 3.01 (1.5H, s), 3.11 (1H, t, $J=6$ Hz), 3.32 (1.5H, s), 3.34 (1.5H, s), 3.45 (1H, m), 3.57 (1H, m), 3.90—4.15 (1H, m), 4.43 (1H, m), 4.64 (0.5H, d, $J=10$ Hz), 4.73 (1H, s), 4.92 (0.5H, d, $J=10$ Hz), 5.22 (1H, m), 5.46 (1H, m), 7.35—7.90 (4H, m). *Anal.* Calcd for $C_{27}H_{38}Br_2ClNO_6S$: C, 46.33; H, 5.47; N, 2.00. Found: C, 46.10; H, 5.74; N, 2.25.

(1*R*,2*R*,3*S*,4*R*)-1-[[(2-Hydroxymethyl)phenyl]methylthio]methyl-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexan-1,4-diol (9) 2-Mercaptomethylbenzyl alcohol (655 mg) was added to a solution of sodium methoxide (28% solution in MeOH) (3 ml) in MeOH (3 ml). The reaction mixture was stirred for 10 min, and then fumagillol (**2**, 1.00 g) was added with ice-cooling and stirred for 1 h at room temperature. Water was added and the product was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt : hexane = 20 : 1) to give **9** (1.43 g, 92%) as a colorless oil, $[\alpha]_D^{25}$ −15.4° ($c=0.20$, $CHCl_3$). IR (neat): 3400, 2920, 1450, 1370, 1080, 1040 cm^{-1}. NMR ($CDCl_3$) δ: 1.39 (3H, s), 1.66 (3H, s), 1.75 (3H, s), 1.55—1.85 (4H, m), 2.00—2.55 (3H, m), 2.84 (1H, d, $J=13$ Hz), 2.93 (1H, d, $J=13$ Hz), 2.94 (1H, t, $J=6$ Hz), 3.28 (1H, m), 3.32 (3H, s), 3.86 (1H, d, $J=13$ Hz), 3.96 (1H, d, $J=13$ Hz), 4.20 (1H, m), 4.77 (2H, br d, $J=6$ Hz), 5.19 (1H, m), 7.20—7.50 (4H, m).

(1*R*,2*R*,3*S*,4*R*)-1-[[(2-Methanesulfonyloxymethyl)phenyl]methylthio]methyl-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexan-1,4-diol (10) Methanesulfonyl chloride (160 μl) was added to a solution of **9** (900 mg) and Et_3N (0.58 ml) in CH_2Cl_2 (3 ml) with ice-cooling and the reaction mixture was stirred for 15 min. Water was added and the product was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give crude **10** (1.13 g). The crude compound was used in the next reaction without purification. NMR ($CDCl_3$) δ: 1.40 (3H, s), 1.67 (3H, s), 1.74 (3H, s), 1.45—1.90 (4H, m), 2.00—2.55 (3H, m), 2.89 (2H, s), 2.93 (1H, t, $J=6$ Hz), 2.95 (3H, s), 3.27 (1H, m), 3.33 (3H, s), 3.85 (1H, d, $J=13$ Hz), 3.96 (1H, d, $J=13$ Hz), 4.21 (1H, m), 5.20 (1H, m), 5.42 (1H, d, $J=12$ Hz), 5.49 (1H, d, $J=12$ Hz), 7.25—7.50 (4H, m).

2-[[(1*R*,2*R*,3*S*,4*R*)-1,4-Dihydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]methyl]-1,3-dihydrobenzo-[*c*]thiophenium Methanesulfonate (11) A solution of crude **10** (1.13 g) in CH_2Cl_2 (1 ml) was stirred at 30 °C for 24 h. The reaction mixture was concentrated *in vacuo*. The residue was triturated with ether to give **11** (1.02 g, 96% from **9**) as a white powder, $[\alpha]_D^{25}$ −2.7° ($c=1.29$, $CHCl_3$). IR (KBr): 3400, 2925, 1195, 1055, 1050 cm^{-1}. NMR (CD_3OD) δ: 1.28 (3H, s), 1.63 (3H, s), 1.72 (3H, s), 1.50—2.45 (7H, m), 2.70 (3H, s), 3.02 (1H, m), 3.34 (3H, s), 3.40 (1H, m), 3.45 (1H, d, $J=13$ Hz), 3.89 (1H, d, $J=13$ Hz), 4.78 (1H, d, $J=14$ Hz), 4.95—5.25 (4H, m), 5.49 (1H, m), 7.40—7.60 (4H, m).

2-[[(1*R*,2*R*,3*S*,4*R*)-4-(Chloroacetylcarbamoyl)oxy-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl-[methyl]-1,3-dihydrobenzo[*c*]thiophenium Chloride (12) Chloroacetyl isocyanate (0.46 ml) was added to a solution of **11** (920 mg) in CH_2Cl_2 (5 ml) with ice-cooling and the reaction mixture was stirred for 15 min. Water was added, followed by NaCl. The products were extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 20 : 1) to give **12** (300 mg, 29%) as a white amorphous powder, $[\alpha]_D^{22}$ −36.8° ($c=0.22$, $CHCl_3$). IR (KBr): 3400, 2930, 1785, 1755, 1720, 1525, 1200 cm^{-1}. NMR (CD_3OD) δ: 1.28 (3H, s), 1.63 (3H, s), 1.71 (3H, s), 1.50—2.45 (7H, m), 3.02 (1H, m), 3.33 (3H, s), 3.51 (1H, m), 3.52 (1H, d, $J=13$ Hz), 3.93 (1H, d, $J=13$ Hz), 4.23 (2H, s), 4.86 (1H, d, $J=16$ Hz), 5.00—5.25 (4H, m), 5.49 (1H, m), 7.40—7.60 (4H, m). *Anal.* Calcd for $C_{27}H_{37}Cl_2NO_6S \cdot H_2O$: C, 54.73; H, 6.63; N, 2.36. Found: C, 54.65; H, 6.64; N, 2.40.

(1*R*,2*R*,3*S*)-1-[[(2-Hydroxymethyl)phenyl]methylthio]methyl-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]-4-oxocyclohexanol (14) 2-Mercaptomethylbenzyl alcohol (655 mg) was added to a solution of sodium methoxide (28% solution in MeOH) (0.51 ml) in MeOH (1.5 ml). The reaction mixture was stirred for 10 min, and then 6-oxo-6-deoxy-fumagillol (**13**, 300 mg) was added with ice-cooling and the reaction mixture was stirred for 1 h at room temperature. Water was added and the product was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt : hexane = 20 : 1) to give **14** (397 mg, 85%) as a colorless oil, $[\alpha]_D^{25}$ −76.5° ($c=0.22$, $CHCl_3$). IR (neat): 3440, 2920, 1720, 1450, 1105, 1040 cm^{-1}. NMR ($CDCl_3$) δ: 1.39 (3H, s), 1.66 (3H, s), 1.73 (1H, m), 1.74 (3H, s), 2.00—2.55 (4H, m), 2.40 (1H, t, $J=6$ Hz), 2.65—3.05 (3H, m), 2.95 (1H, d, $J=6$ Hz), 3.39 (3H, s), 3.84 (1H, d, $J=12$ Hz), 3.92 (2H, d, $J=6$ Hz), 4.01 (1H, m), 4.77 (2H, d, $J=6$ Hz), 5.18 (1H, m), 7.20—7.45 (4H, m).

(1*R*,2*R*,3*S*,4*R*)-4-Amino-1-[[(2-hydroxymethyl)phenyl]methylthio]-methyl-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexanol (15) $NaBH_3CN$ (304 mg) was added to a solution of **14** (1.07 g) and $AcONH_4$ (1.86 g) in MeOH 825 ml). The reaction mixture was stirred for 1 h, then concentrated *in vacuo*, and the residue was dissolved in AcOEt. The AcOEt solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$: MeOH : conc. NH_4OH = 20 : 1 : 0.1) to give **15** (629 mg, 58%) as a colorless oil, $[\alpha]_D^{25}$ −28.4° ($c=0.22$, $CHCl_3$). IR (neat): 3440, 2930, 1450, 1380, 1090, 1040 cm^{-1}: NMR ($CDCl_3$) δ: 1.35—1.90 (4H, m), 1.39 (3H, s), 1.66 (3H, s), 1.74 (3H, s), 2.05—2.55 (3H, m), 2.90 (2H, s), 2.96 (1H, t, $J=6$ Hz), 3.25 (1H, m), 3.28 (3H, s), 3.52 (1H, m), 3.87 (1H, d, $J=13$ Hz), 3.99 (2H, d, $J=13$ Hz), 4.74 (1H, d, $J=12$ Hz), 4.81 (1H, d, $J=12$ Hz), 5.19 (1H, m), 7.20—7.45 (4H, m).

(1*R*,2*R*,3*S*,4*R*)-4-(*N'*-Chloroacetylureido)-1-[[(2-hydroxymethyl)-phenyl]methylthio]methyl-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexanol (16) Chloroacetyl isocyanate (0.22 ml) was added to a solution of **15** (629 mg) in CH_2Cl_2 (20 ml) with ice-cooling, and the reaction mixture was stirred for 10 min. Water was then added and the product was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and then concentrated *in vacuo*. The residue was chromatographed on silica gel (AcOEt : hexane = 3 : 2) to give **16** (514 mg, 64%) as a white amorphous powder, $[\alpha]_D^{25}$ −37.1° ($c=0.22$, $CHCl_3$). IR (KBr): 3420, 2925, 1705, 1545, 1490, 1235, 1200 cm^{-1}. NMR ($CDCl_3$) δ: 1.40 (3H, s), 1.45—2.55 (6H, m), 1.65 (3H, s), 1.73 (3H, m), 2.65 (1H, t, $J=6$ Hz), 2.83 (1H, d, $J=13$ Hz), 2.96 (1H, t, $J=6$ Hz), 2.97 (1H, d, $J=13$ Hz), 3.30 (3H, s), 3.35 (1H, dd, $J=4$, 11 Hz), 3.86 (1H, d, $J=13$ Hz), 3.94 (1H, d, $J=13$ Hz), 4.15 (2H, s), 4.47 (1H, m), 4.75 (1H, dd, $J=6$, 13 Hz), 4.81 (1H, dd, $J=6$, 13 Hz), 5.17 (1H, m), 7.20—7.45 (4H, m), 8.25 (2H, m).

(1*R*,2*R*,3*S*,4*R*)-4-(*N'*-Chloroacetylureido)-1-[[(2-methanesulfonyl-oxymethyl)phenyl]methylthio]methyl-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]-3-methoxycyclohexanol (17) Methanesulfonyl chloride (73 μl) was added to a solution of **16** (500 mg) and Et_3N (0.25 ml) in CH_2Cl_2 (5 ml) with ice-cooling and the reaction mixture was stirred for 10 min. Water was added and the product was extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give crude **17** (530 mg). The crude compound was used in the next reaction without purification. NMR ($CDCl_3$) δ: 1.40 (3H, s), 1.65 (3H, s), 1.73 (3H, s), 1.45—1.95 (4H, m), 2.00—2.55 (3H, m), 2.81 (1H, d, $J=14$ Hz), 2.94 (3H, s), 2.97 (1H, d, $J=14$ Hz), 2.98 (1H, t, $J=6$ Hz), 3.30 (3H, s), 3.35 (1H, m), 3.86 (1H, d, $J=13$ Hz), 3.96 (1H, d, $J=6$ Hz), 4.16 (3H, br s), 4.48 (1H, m), 5.26 (1H, m), 5.46 (2H, s), 7.15—7.55 (4H, m), 8.42 (1H, m).

2-[[(1*R*,2*R*,3*S*,4*R*)-4-(*N'*-Chloroacetylureido)-1-hydroxy-3-methoxy-2-[(2*R*,3*R*)-2-methyl-3-(3-methyl-2-butenyl)oxiran-2-yl]cyclohexyl]-methyl]-1,3-dihydro-benzo[*c*]thiophenium Chloride (18) A solution of crude **17** (530 mg) in CH_2Cl_2 (2 ml) was stirred at 30° for 24 h. Water was added to it, followed by NaCl. The products were extracted with AcOEt. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 20 : 1) to give **18** (137 mg, 26% from **16**) as a white amorphous powder, $[\alpha]_D^{22}$ −31.8° ($c=0.21$, $CHCl_3$). IR (KBr): 3400, 3290, 2930, 1750, 1540, 1490, 1205 cm^{-1}. NMR (CD_3OD) δ: 1.31 (3H, s), 1.64 (3H, s), 1.72 (3H, s), 1.55—2.45 (7H, m), 3.07 (1H, t, $J=7$ Hz), 3.31 (3H, s), 3.52 (1H, d, $J=13$ Hz), 3.56 (1H, dd, $J=4$, 10 Hz), 3.91 (1H, d, $J=13$ Hz), 4.19 (2H, s), 4.42 (1H, m), 4.81 (1H, d, $J=16$ Hz), 4.95—5.25 (4H, m), 7.40—7.60 (4H, m). *Anal.* Calcd for $C_{27}H_{38}Cl_2N_2O_5S \cdot 0.5H_2O$: C, 55.66; H, 6.75; N, 4.81. Found: C, 55.50; H, 6.73; N, 4.63.

Rat Corneal Micropocket Assay The rat corneal micropocket assay was carried out as described previously.[1a]

***In Vivo* Antitumor Activity** C57BL/6 mice were inoculated s.c. on day 0 with 2×10^6 M5076 cells. Samples were dissolved in 5% arabic gum saline and administered on days 1,2,4,5,6,7,8,9, 11 and 12. Control mice were given as equal volume of 5% arabic gum saline. Tumor size and body weight were measured on day 13, and the tumor volume was

calculated by using the formula:

$$\text{volume} = A \times B^2 \times 1/2 .$$

where A is a majour axis and B is a minor axis. The antitumor effects of the samples were assessed in terms of T/C (%), or the ratio of the mean tumor volume of tested mice to that of control mice.

HPLC Analysis Solutions of **3** and **12** were analyzed by HPLC using Inertsil octadecyl silica (ODS) (Gasukuro Kogyo: 5 μm, 4.6 × 150 mm) at a flow rate of 0.7 ml/min, with a UV detector (210 nm). The developing solvents were CH_3CN–wateer (3 : 2) for **3** and CH_3CN–water (3 : 2) with PIC® reagent B-7 low UV (Waters) (5 μM) for **12**.

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