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Synthesis and Antirheumatic Activity of the Metabolites of Esonarimod

Toshiya Noguchi,* Akira Onodera, Kazuyuki Tomisawa, Miyuki Yamashita, Kimiyo Takeshita and Sadakazu Yokomori

Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama City, Saitama, 330-8530, Japan

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Abstract—We have developed esonarimod, (±)-2-acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid, as a new antirheumatic drug. Now we describe herein the preparation of the enantiomers of (±)-deacetylesonarimod, the pharmaceutically active metabolites of esonarimod, and comparison of their antirheumatic activities. No significant difference has been observed between the two enantiomers. In a pre-clinical study of esonarimod, other metabolites were detected in rat blood or urine. We also synthesized these compounds as authentic samples to analyze the human metabolites in clinical studies of esonarimod.

Introduction

We have developed of esonarimod (**1**), (±)-2-acetylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (Fig. 1), which exhibits beneficial effects in RA patients in a clinical study.[1] Compound **1** inhibits inflammatory cytokine production by human peripheral blood mononuclear cells,[2] interleukin (IL)-6 and matrix metalloproteinase (MMP) production by RA synovial fibroblast-like cells,[3,4] and the progression of this disease in various animal models of arthritis.[5–7] Compound **1** contains a thioacetyl group, which is easily deacetylated in vivo to form a pharmacologically active metabolite **2**.[2,8] The toxicity of esonarimod is significantly lower than that of penicillamine in rats. The low toxicity of esonarimod may be closely related to the low reactivity of the thiol of **2** with macromolecules in vivo.[9]

Compound **1** has been used clinically as a racemate.[10] Therefore, we investigated the pharmacological activities of its optical isomers in comparison with those of racemic **1** by characteristic tests for **1**. Although the (+)-form of (+)-**1**, strongly suppressed rat adjuvant arthritis compared to the (−)-form, (−)-**1**, no difference was detected between the two isomers with regard to the enhancement of lymphocyte transformation, an IL-1 antagonistic effect.[10]

In a pre-clinical study of **1**, metabolites **2** and **3** were detected in rat blood, and **4**–**8** were found in rat urine. The structures of these metabolites have been studied and a possible metabolic pathway has been suggested, as shown in Scheme 1.[11,12] To analyze the human metabolites in clinical studies of **1**, it is important to obtain significant quantities of these compounds as authentic samples to support the proposed metabolic pathway for **1**. However, preparation of the pharmacologically active metabolite **2** has not been reported. Although **5** and **7** have been synthesized from **3** by a biological method,[12] chemical syntheses of **5** and **7** have not been reported.

In this article, we report the formation of pharmacologically active metabolite **2** and its antirheumatic activities, as well as the syntheses of other postulated metabolites **3**–**8** as authentic samples.[13]

Results and Discussion

Preparation of the ammonium salt of 2 in racemic form (Scheme 2)

Under basic conditions, such as in aqueous ammonia or potassium hydroxide solutions, **1** was easily

*Corresponding author. Tel.: +81-48-663-1111; e-mail: t.noguchi@po.rd.taisho.co.jp

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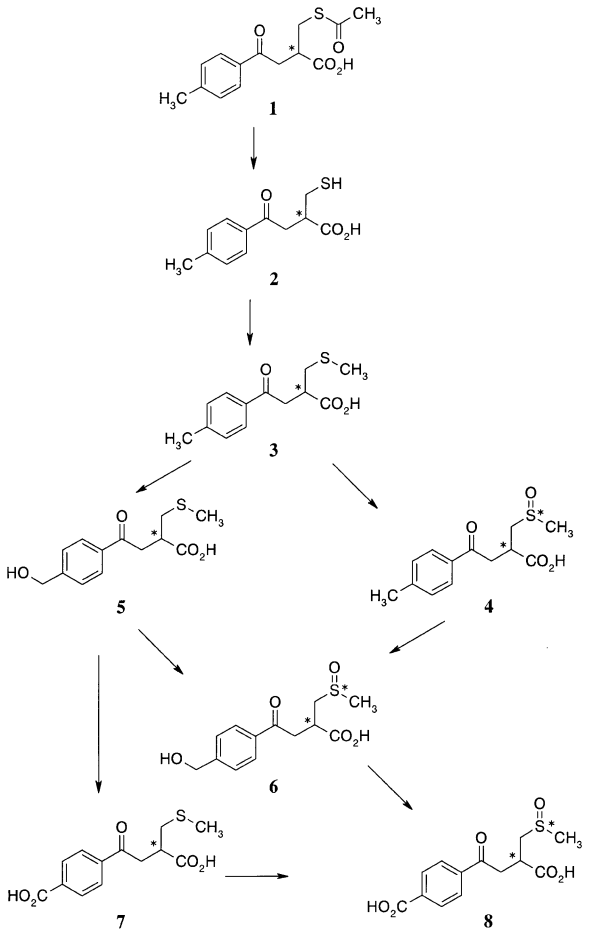
deacetylated to form thiol **2**, which was detected by ^{1}H NMR. The thiol **2** seemed to be stable in organic solution or basic conditions, but when the solvent was removed to give **2** as a solid, **2** was unstable and readily converted to dihydrothiophene **9** by cyclization and subsequent dehydration. By the usual workup (acidification and extraction with organic solvent) under alkali conditions, facile dehydration of **2** occurred to give only **9**. Therefore, we attempted to isolate **2** as an ammonium salt. Compound **1** was treated with aqueous ammonia and methanol, and the solvent was removed under reduced pressure. In this fashion, we obtained the ammonium salt of **2** as a powder (Scheme 2).



Scheme 1. A possible metabolic pathway of esonarimod (**1**) in rat.

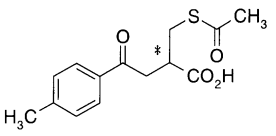


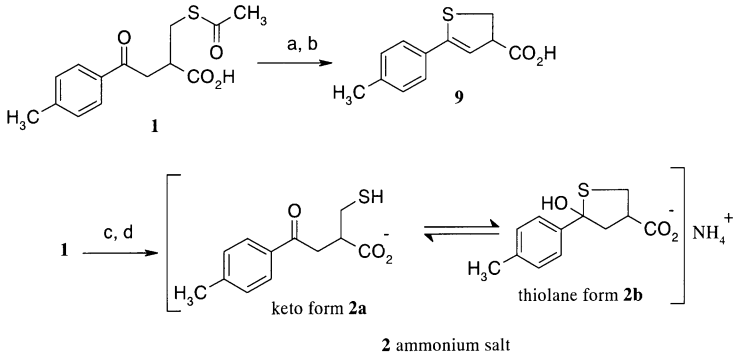
Figure 1. Esonarimod (**1**).

The NMR spectrum of the ammonium salt of **2** in methanol-d_4 showed that **2** existed as an equilibrium mixture of the keto form **2a** and thiolane form **2b** (Fig. 2). Compound **1** is racemic, as is the keto form of **2a**. However, the thiolane form **2b** has two diastereoisomers. Therefore, the ammonium salt of **2** in a solution consists of a mixture of three compounds, keto form **2a**, and *trans* and *cis* thiolane form **2b** (Fig. 2). We assigned each peak in the ^{1}H NMR and ^{13}C NMR (methanol-d_4) spectra, as shown in Table 1.

On the other hand, the IR spectrum (KBr) of the ammonium salt of **2** exhibited a strong absorption at 1558 cm^{-1} due to the carboxylate anion. No absorption peak was observed for the keto group at about 1715 cm^{-1}. Therefore, we presumed that the ammonium salt of **2** existed almost entirely as the thiolane form in the solid phase.

Synthesis of optically active 2 as 1,1-dimethyl-3-oxobutylammonium salt (Fig. 3). We next focused on the synthesis of optically active **2** from optically active **1**. Optically active **1** was obtained by a known method,[6] as illustrated in Scheme 3. The absolute configuration of (+)-**1** was determined to be S form.[6]

However, the ammonium salt of optically active **2** could not be obtained as a crystalline form. We then converted the ammonium salt to a 1,1-dimethyl-3-oxobutylammonium salt as follows: (+)-**1** was dissolved in aqueous ammonia and acetone, and the solvent was then removed under reduced pressure. We obtained (+)-**2** as a 1,1-dimethyl-3-oxobutylammonium salt, which was formed in situ by the reaction of acetone dimer with ammonia. We then obtained optically active 1,1-dimethyl-3-oxobutylammonium salt of (−)-**2**, and (±)-**2** as a powder by a similar method (Fig. 3).



Scheme 2. Synthesis of **2** ammonium salt: (a) KOH or aqueous NH_3 (b) usual workup (acidification and extraction with organic solvent); (c) aqueous NH_3–MeOH, 0–3 °C, 5 min; (d) solvent removed under reduced pressure, 65%.

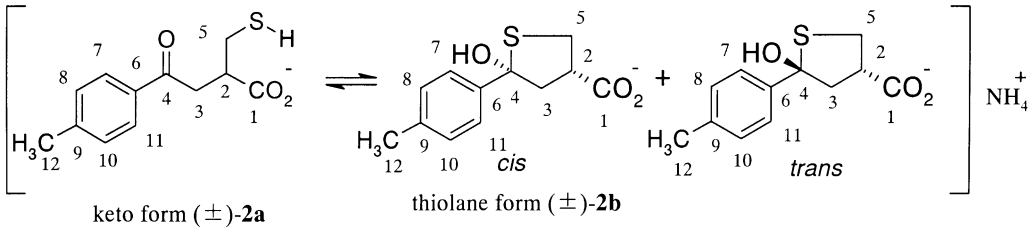


Figure 2. Keto form (±)-**2a** and thiolane form (±)-**2b**.

Table 1. Assignment of **2** ammonium salt (^{1}H NMR and ^{13}C NMR in methanol-d_4)

Carbon no.		Keto form (±)-**2a**		Thiolane form (±)-**2b**			
				cis		*trans*	
		δC	δH	δC	δH	δC	δH
1	–COO—	n.d.	—	n.d.	—	183.5	—
2	–CH—	48.3	3.02	52	3.52	52.8	3.38
3	$CHCH_2C{=}O$	41	3.20, 3.47	53.4	2.43 (2H)	52.3	2.41 or 2.60
4	$>C{=}O$	201	—	95.8	—	98.7	—
5	$–CH_2S–$	27.8	2.74, 2.81	37	3.29, +3.36	38.6	3.46,3.52
6	Ph–C	136	—	143.2	—	143.2	—
7	Aromatic	129.3	7.9	127.0 or 127.1	7.55	127.0 or 127.1	7.57
8	Aromatic	130.2	7.29	129.4	7.1	129.4	7.1
9	Aromatic	145.1	—	137.8 or 137.9	—	137.8 or 137.9	—
10	Aromatic	130.2	7.29	129.4	7.1	129.4	7.1
11	Aromatic	129.3	7.9	127.0 or 127.1	7.55	127.0 or 127.1	7.57
12	$Ph–CH_3$	21.6	2.4	21	2.3	21	2.3

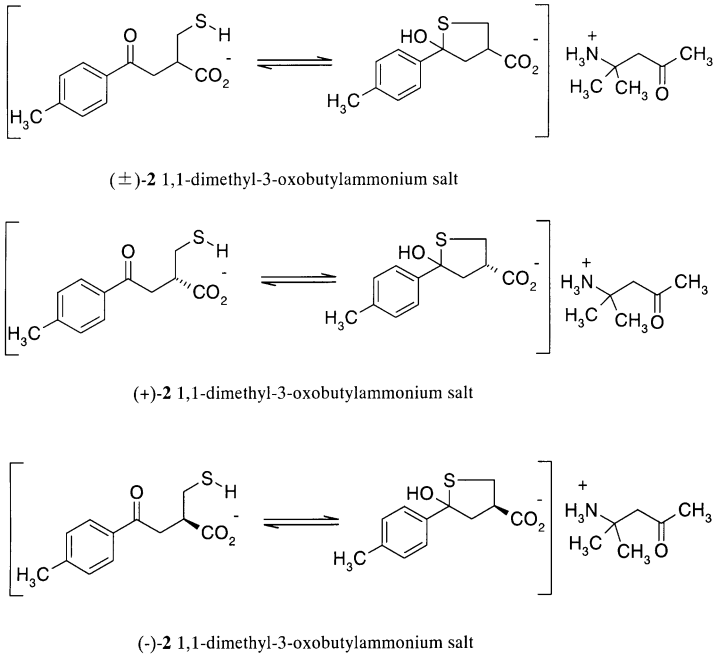
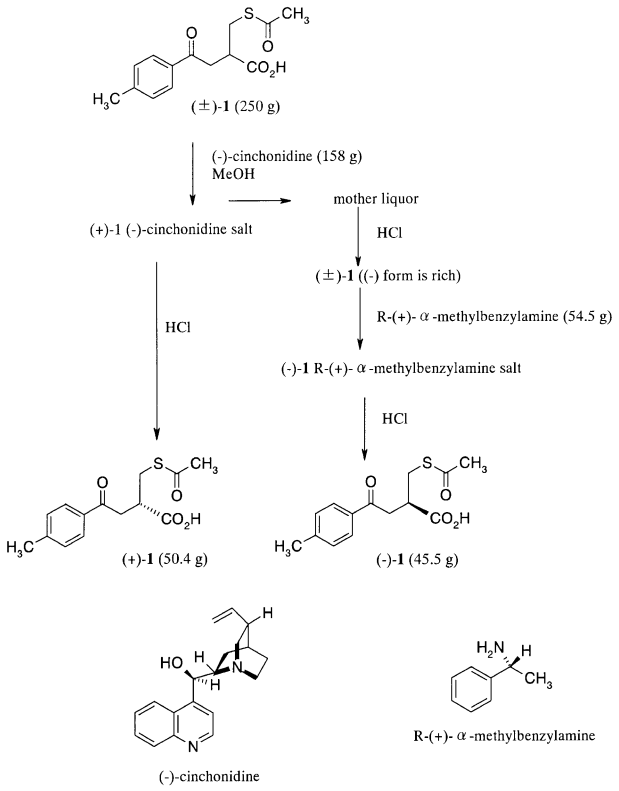


Figure 3. The structures of (±), (+), and (−)-**2** 1,1-dimethyl-3-oxobutylammonium salt.

Antirheumatic activity of 1,1-dimethyl-3-oxobutylammonium salt of 2 (Fig. 4). All forms of **2** 1,1-dimethyl-3-oxobutylammonium salt [(+) **-2**, (−) **-2** and (±) **–2**] inhibited IL-1β production,[14] as shown in Fig. 4. No difference in activity was detected between the optical isomers of **2**.

Synthesis of the metabolites 3 and 4 (Scheme 4). We synthesized 2-methylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (**3**) and 4-(4-methylphenyl)-2-methylsulfinylmethyl-4-oxobutanoic acid (**4**) from **1** (Scheme 4). The acetyl group of **1** was easily hydrolyzed in aqueous sodium hydroxide solution at room temperature to



Scheme 3. Preparation of optically active esonarimod (**1**).

give the thiol **2**. When the reaction mixture was worked up as usual (acidification and extraction with organic solvent), **2** was converted to dihydrothiophene **9**, indicating that **2** was stable compound under alkaline conditions but unstable under acidic or neutral conditions. Therefore, the reaction mixture was treated with methyl iodide immediately after hydrolysis. Since the solvent was water, the phase transfer catalyst TOMAC (trioctylmethylammonium chloride) was used for methylation, and **3** was obtained.

Oxidation of sulfide **3** with *m*-chloroperoxybenzoic acid (mCPBA) in tetrahydrofuran (THF) gave the sulfoxide **4**.

Synthesis of the metabolites 5 and 6 (Scheme 5). We synthesized 4-(4-hydroxymethylphenyl)-2-methylsulfinylmethyl-4-oxobutanoic acid (**5**) in eight steps from terephthalaldehyde monodiethylacetal (**12**), which was converted to 4-(4-hydroxymethyl-phenyl)-2-methylsulfinylmethyl-4-oxobutanoic acid (**6**) by the oxidation of **5** with mCPBA as summarized in Scheme 5.

The key step was indium-promoted coupling of 4-acetoxymethylbenzaldehyde (**13**) and 2-(bromomethyl) acrylic acid to yield 4-hydroxy-4-(4-acetoxyphenyl)-2-methylenebutanoic acid (**14**).[15] Benzylalcohol **14** was oxidized to ketone **15** by Jones reagent.[16] Michael addition of thioacetic acid with **15** gave **16** in high yield. The sulfide **5** and the sulfoxide **6** were then obtained

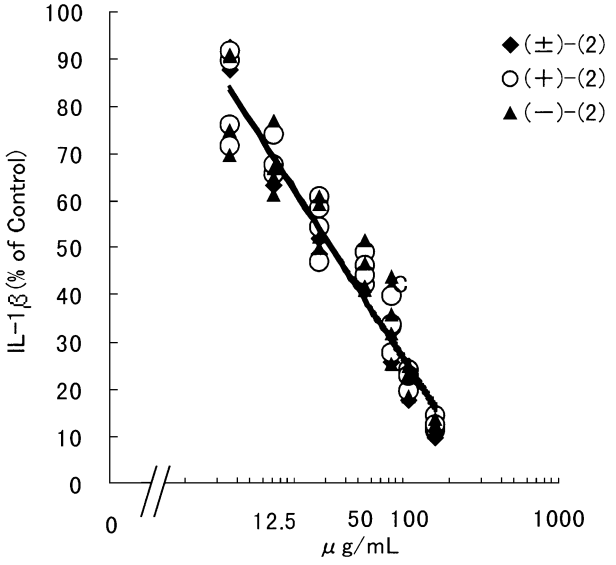
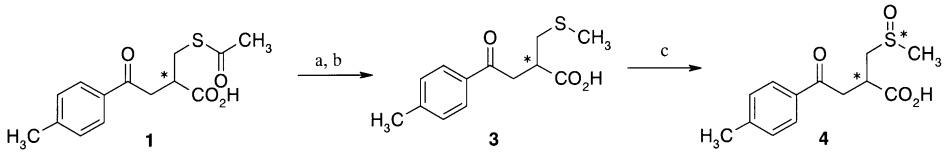
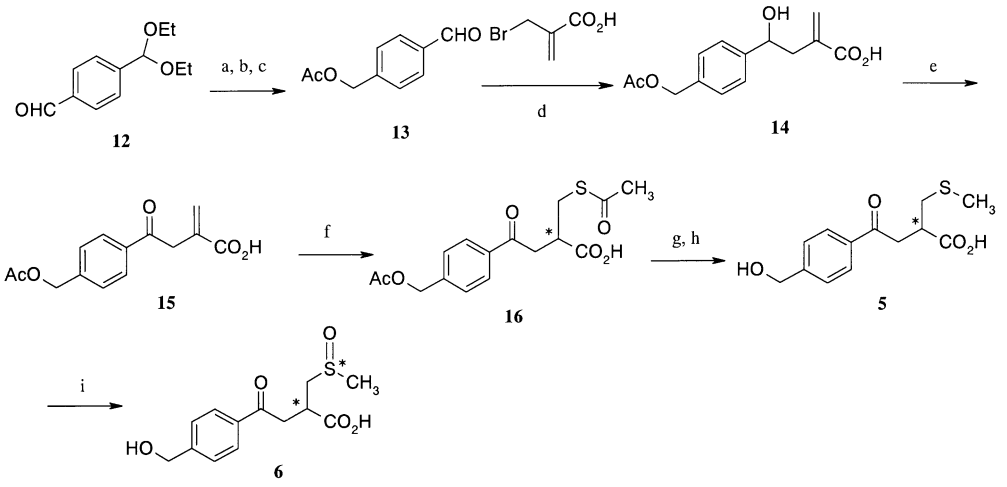


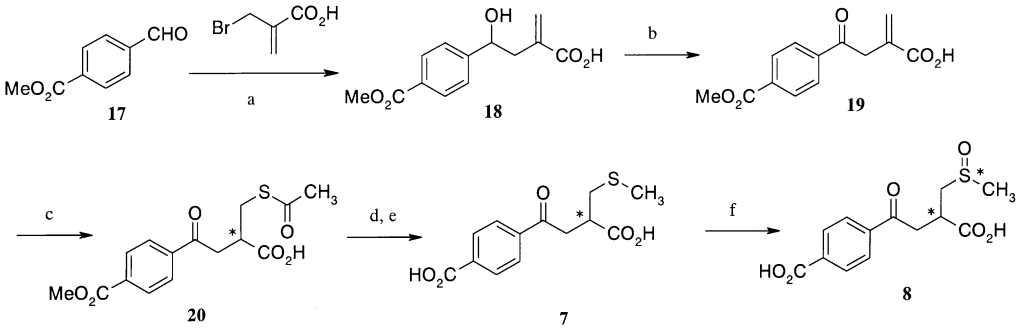
Figure 4. Suppressive effect of optical isomers of **2** 1,1-dimethyl-3-oxobutylammonium salt on IL-1β 13 production in THP-1 cells. IC_{50} (95% C.I.). (±)-2 1,1-dimethyl-3-oxobutylammonium salt: 29.6 (26.6 -32.9) μg/mL; (±)-2 1,1-dimethyl-3-oxobutylammonium salt: 30.8 (27.9-33.9) μg/mL; (-)-2 1,1-dimethyl-3-oxobutylammonium salt: 30.4 (26.8-34.3) μg/mL. Each symbol represents the production of IL-1β by THP-1 cells with various concentrations of optical isomers of 2 ($n=4$). Results were divided by the mean of the control. The mean and SE of the control were 109.4±4.4 pg/mL. IC_{50} and 95% confidence interval (CI) were determined by linear regression analysis.



Scheme 4. Synthesis of metabolites **3** and **4**: (a) KOH, H_20, rt, 30 min; (b) CH_3I, TOMAC, rt, 30 min, 80%; (c) mCPBA, THF, rt, 60 min, 37%.



Scheme 5. Synthesis of metabolites **5** and **6**; (a) $NaBH_4$, EtOH, rt, 60 min; (b) Ac_20, pyridine, rt over:night; (c) H_2SO_4, THF–H_2O, rt, 30 in, 96%; (d) indium metal, THF, 18–39 °C, 35 min, 87%; (e) CrO_3, H_2SO_4, acetone–H_2O, ice cooling, 10 min, 83%; (f) AcSH, Et_3N, toluene, 60 °C, 2 h, 92%; (g) NH_3, H_2O, rt, 60 min; (h) CH_3I, TOMAC, Et_2O, rt, 30 min, 80%; (i) mCPBA, THF, ice cooling, 60 min, 77%.



Scheme 6. Synthesis of metabolites **7** and **8**: (a) indium metal, THF, rt, overnight, 62%; (b) $(COC1)_{2'}$, DMSO, THF, Et_3N, −74 °C, 10 min, 42%; (c) AcSH, Et_3N, toluene, 60 °C, 40 min, 86%; (d) NH_3, H_2O, rt, 60 min; (e) CH_3I, TOMAC, rt, 20 min, 61%; (f) *m*CPBA, THF, ice cooling, 40 min, 80%.

from **16** in the same fashion as for the synthesis of **3** and **4**.

Synthesis of metabolites 7 and 8 (Scheme 6). We synthesized 2-methylthiomethyl-4-(4-carboxyphenyl)-4-oxobutanoic acid (**7**) in five steps from terephthalaldehydic acid methyl ester **17**, and 4-(4-carboxyphenyl)-2-methylsulfinyl-methyl-4-oxobutanoic acid (**8**) was obtained via the oxidation of **7** by the method similar to that for **3** and **4** (Scheme 6).

Conclusion

We prepared the pharmacologically active metabolite **2** of esonarimod **1** as an ammonium salt. We confirmed that the ammonium salt of **2** existed as an equilibrium of the keto form **2a** and thiolane form **2b** in basic solution, while **2** existed as the thiolane form **2b** in the solid phase. We also prepared the optically active **2** 1,1-dimethyl-2-oxopropylammonium salt, and confirmed that there was no difference in activity between the optical isomers of **2**.

We also synthesized other postulated metabolites **3**–**8** in racemic form. Indium -promoted coupling between benzaldehydes [4-acetoxymethylbenzaldehyde (**13**) or terephthalaldehydic acid methyl ester (**17**)] and 2-(bromomethyl)acrylic acid gave the corresponding benzylalcohol derivatives **14** and **18**. Using compounds **3**–**8** as authentic samples, a possible metabolic route of esonarimod **1** in rats was demonstrated.[17]

Experimental

Melting points were determined by a Buchi 535 melting point apparatus and were uncorrected. IR spectra were obtained on a Perkin–Elmer 1760 spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian VXL-200 spectrometer. Chemical shifts are reported in ppm (δ) values, as determined using a JEOL JMS-SIX102 spectrometer. Elemental analyses were performed on a Perkin–Elmer 2400. TLC was performed on silica gel pre-coated plates (Merck, Kieselgel 60F254). Column chromatography was performed over silica gel (Wako, Wako gel C-200). (±)-Esonarimod (**1**) was prepared by the known procedure.[10,18]

Ammonium salt of (±)-2-mercaptomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (2). A 42.0 g (150 mmol) sample of (±)-**1** was dissolved in methanol (150 mL),

and 100 mL of 25% ammonia solution was added dropwise at 0–3 °C over 5 min. The reaction mixture was stirred at room temperature for 5 min, and then evaporated in vacuo. The resulting precipitates were collected by filtration, washed with 250 mL of acetone, and dried under reduced pressure to give 24.4 g (64%) of the ammonium salt of (±)-**2** (equilibrated with ammonium (±)-5-hydroxy-5-(4-methylphenyl)-3-thiolane-carboxylate). Mp: 166–170 °C (decomposed). ^{1}H NMR and ^{13}C NMR spectra were listed in Table 1. IR (KBr) cm^{-1}: 3204, 2996, 1558, 1426, 1407, 813, 641. FAB-Mass *m*/*z*: 239 (M^+). Anal. calcd for $C_{12}H_{17}NO_3S$: C, 56.45; H,6.71; N, 5.49; S, 12.56. Found: C, 56.60; H, 6.68; N, 5.48; S, 12.77.

1,1-Dimethyl-3-oxobutylammonium salt of (±)-2-mercaptomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (2). A 42.0 g (150 mmol) sample of (±)-**1** was dissolved in acetone (150 mL), and 100 mL of 25% ammonia solution was added dropwise at 6–15 °C over 5 min. The reaction mixture was stirred at 20–26 °C for 70 min, and then evaporated in vacuo. The resulting precipitates were collected by filtration, washed with 50 mL of acetone, and dried under reduced pressure to give 34.7 g (65%) of 1,1-dimethyl-3-oxobutylammonium salt of (±)-**2** (equilibrated with 1,1-dimethyl-3-oxobutylammonium (±)-5-hydroxy-5-(4-methylphenyl)-3-thiolane-carboxylate). Mp: 103–104 °C, ^{1}H NMR (methanol-d_4) δppm: 1.35 (s, 6H), 2.17 (s, 3H), 2.31 (s, 3H), 2.36–2.64 (m, 2.2H), 3.35–3.60 (m, 2.8H), 7.10 (d, 1.6H, J = 8.1 Hz), 7.24–7.32 (m, 0.4H), 7.54 (d, 0.8H, J = 8.1 Hz), 7.58 (d, 0.8H, J = 8.1 Hz), 7.90 (m, 0.4H); keto form/thiolane form = 1:4. IR (KBr) cm^{-1} : 3091, 2979, 2931, 1710, 1519, 1397, 614. Mass *m*/*z*: 237 (M^+). Anal. calcd for $C_{18}H_{17}NO_4S$: C, 61.16; H, 7.70; N, 3.96; S, 9.07. Found: C, 61.29; H, 7.83; N, 3.93; S, 9.07.

1,1-Dimethyl-3-oxobutylammonium salt of (+)-2-mercaptomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (2).[6] Compound (±)-**1** (250 g, 892 mmol) and (−)-cinchonidine (158 g, 537 mmol) were dissolved in methanol (1000 mL), and the solution was concentrated under reduced pressure. The residue was recrystallized from ethyl acetate (2000 mL) to give colorless needles [the mother liquor was used to give (−)-**1**], which were added to fresh ethyl acetate (1000 mL). The mixture was heated under reflux with stirring for 10 min, then allowed to cool to obtain 156 g (wet) of purified needles. The needles were partitioned between ethyl acetate (1000 mL) and concd hydrochloric acid (100 mL)–water (400 mL). The organic layer was washed concd hydrochloric acid (50 mL)– water (450 mL) and brine (500 mL), dried over $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from ether (100 mL) and hexane (100 mL) to give 50.4 g (40%) of (+)-**1**. $[\alpha]_D$ +25.1°(*c* 10.9, ethanol). Mp 87–88 °C, ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.42 (s, 3H), 3.18–3.54 (m, 5H), 7.24 (d, 2H, J = 8.0 Hz), 7.84 (d, 2H, J = 8.0 Hz). IR (KBr) cm^{-1}: 3056, 2926, 1714, 1697, 1673, 1607, 1573, 1434, 1400, 1364, 1247, 1211, 1188, 1173, 1135, 1097, 1037, 1001, 954, 842, 816, 696, 621, 568. MS *m*/*z*: 281 (MH^+). Anal. calcd for $C_{14}H_{16}O_4S$: C, 59.98; H, 5.75; S, 11.44. Found: C, 60.00; H, 5.65; S, 11.62.

A 25.0 g (89.2 mmol) sample of (+)-**1** was dissolved in acetone (90 mL), and 60 mL of 25% ammonia solution was added dropwise at 3–10 °C over 10 min. The reaction mixture was stirred at 21–25 °C for 60 min, and then evaporated in vacuo. The resulting precipitates were collected by filtration, washed with 20 mL of acetone, and dried under reduced pressure to give 10.0 g (32%) of 1,1-dimethyl-3-oxobutylammonium salt of (+)-**2** (equilibrated with 1,1-dimethyl-3-oxobutylammonium (+)-5-hydroxy-5-(4-methylphenyl)-3-thiolane-carboxylate). $[\alpha]_D$: +25.3° (*c* 10.6, ethanol). Mp 118–119 °C, ^{1}H NMR (methanol-d_4) δ ppm: 1.35 (s, 6H), 2.18 (s, 3H), 2.30 (s, 3H), 2.37–2.64 (m, 2.2H), 2.87 (s, 2H), 3.35–3.56 (m, 2.8H), 7.10 (d, 1.6H, J = 8.1 Hz), 7.29 (d, 0.4H, J = 8.1 Hz), 7.55 (d, 0.8H, J = 0.8 Hz), 7.56 (d, 0.8H, J = 0.8 Hz), 7.91 (d, 0.4H, J = 8.1 Hz). keto form/thiolane form = 1:4. IR (KBr) cm^{-1}: 2922, 2832, 1720, 1678, 1546, 1396, 1366, 810. Mass *m*/*z*: 237 (M^+). Anal. calcd for $C_{18}H_{17}NO_4S$: C, 61.16; H, 7.70; N, 3.96; S, 9.07. Found: C, 61.09; H, 7.74; N, 4.03; S, 9.06.

1,1-Dimethyl-3-oxobutylammonium salt of (−)-2-mercaptomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (2). The mother liquor, which was given at the preparation of (+)-**1**, was washed with concd hydrochloric acid (100 mL)–water (400 mL) and brine (500 mL), dried over $MgSO_4$, and evaporated in vacuo. The residue was dissolved in ethyl acetate (2000 mL) and mixed with R-(+)-α-phenylethylamine (54.5 g, 0.500 mmol). The precipitate was recrystallized from ethyl acetate (2000 mL) to give 177 g of colorless needles. The needles were treated with ethyl acetate (1000 mL) and concd hydrochloric acid (100 mL)–water (400 mL). The organic layer was washed with concd hydrochloric acid (50 mL)–water (450 mL) and brine (500 mL), dried over $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from ether (100 mL) and hexane (200 mL). The crystal was additionally recrystallized from isopropylether to give 45.5 g (36%) of (−)-**1**. $[\alpha]_D$: −26.3°(*c* 10.5, ethanol). Mp 86–87 °C, ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H), 2.40 (s, 3H), 3.16–3.54 (m, 5H), 7.24 (d, 2H, J = 8.0 Hz), 7.84 (d, 2H, J = 8.0 Hz). IR (KBr) cm^{-1}: 3040, 1706, 1684, 1244, 1138, 808. MS *m*/*z*: 281 (MH^+). Anal. calcd for $C_{14}H_{16}O_4S$: C, 59.98; H, 5.75; S, 11.44. Found: C, 60.06; H, 5.65; S, 11.73.

A 25.0 g (89.2 mmol) sample of (−)-**1** was dissolved in acetone (90 mL), and 60 mL of 25% ammonia solution was added dropwise at 3–9 °C over 10 min. The reaction mixture was stirred at 19–23 °C for 120 min, and then evaporated in vacuo. The resulting precipitates were collected by filtration, washed with 20 mL of acetone, and dried under reduced pressure to give 13.4 g (42%) of 1,1-dimethyl-3-oxobutylammonium salt of (−)-**2** (equilibrated with 1,1-dimethyl-3-oxobutyl-ammonium (−)-5-hydroxy-5-(4-methylphenyl)-3-thiolane-carboxylate). $[\alpha]_D$−25.0°(*c* 11.1, ethanol). Mp 112–113 °C, ^{1}H NMR (methanol-d_4) δ ppm: 1.35 (s, 6H), 2.17 (s, 3H), 2.30 (s, 3H), 2.35–2.64 (m, 2.2H), 3.35–3.60 (m, 2.8H), 7.10 (d, 1.6H, J = 8.1 Hz), 7.34 (d, 0.4H, J = 8.1 Hz), 7.59 (d, 0.8H,J = 0.8 Hz), 7.60 (d, 0.8H, J = 0.8 Hz), 7.94 (d, 0.4H, J = 8.1 Hz); keto form/thiolane form = 1:4. IR

(KBr) cm^{-1} : 2920, 2830, 1720, 1678, 1546, 1396, 1366, 810. Mass *m/z*: 237 (M^+). Anal. calcd for $C_{18}H_{17}NO_4S$: C, 61.16; H, 7.70; N, 3.96; S, 9.07. Found: C, 61.19; H, 7.74; N, 3.96; S, 9.02.

IL-1β production in THP-1 cells.[14,19,20] THP-1 cells (final concentration; 5×10^5 cells/mL, Dainihon Pharmaceutical. Co. Ltd., Japan), a human monocytic leukemia cell line, were cultured in 96-well microtiterplates (Corning, USA), with each well containing 1×10^6 cells/mL in 0.1 mL of culture medium (RPMI1640 medium supplemented with 100 U/mL penicillin,100 μg/mL streptomycin, Life Technologies, Japan), 4000 units/mL IFN γ(final concentration: 1000 units/mL, Genzyme Corporation, USA) in 0.05 mL of culture medium, and various concentrations of each drug (final concentration: 6.25, 12.5, 25, 75, 100, 150 μg/mL in 0.05 mL of the culture medium or 0.05 mL of culture medium alone). Culture was performed in 95% air–5% CO_2 at 37 °C for 16 h, and 20 μg/mL LPS (final concentration; 1 μg/mL, Difco Laboratories, USA) was added in 0.01 mL of the culture medium. After 24 h of incubation, culture supernatants were removed and assayed for IL-1β concentrations using an IL-1β ELISA kit (Amersham Pharmacia Biotech, USA).

(±)-2-Methylthiomethyl-4-(4-methylphenyl)-4-oxobutanoic acid (3). A 28.0 g (99.9 mmol) sample of (±)-**1** was dissolved in 85% potassium hydroxide (20.8 g, 315 mmol) water solution (150 mL), and 0.2 g (0.50 mmol) of trioctylmethylammonium chloride (TOMAC). The reaction mixture was stirred at room temperature for 30 min. Methyliodide (7.0 mL, 112 mmol) was then added and the mixture was stirred at room temperature for 30 min and then washed with ether. The aqueous layer was acidified with 20 mL of concd hydrochloric acid, extracted with ether. The organic layer was washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from hexane-ethyl acetate to give 21.7 g (80%) of (±)-**3**. Mp: 96–97 °C. ^{1}H NMR ($CDCl_3$, 300 MHz)δ ppm: 2.14 (s, 3H), 2.42 (s, 3H), 2.77 (dd, 1H, $J=8.2$, 13.5 Hz), 2.96 (dd, 1H, $J=5.4$, 13.5 Hz), 3.28- 3.53 (m, 3H), 7.27 (d, 2H, $J=7.9$ Hz), 7.88 (d, 2H, $J=7.9$ Hz). IR (KBr) cm^{-1}: 2916, 1702, 1678, 1607, 1227, 810. Mass (ESI) *m/z*: 275 ($(M+Na)^+$). Anal. calcd for $C_{13}H_{16}O_3S$: C, 61.88; H, 6.39; S, 12.71. Found: C, 61.82; H, 6.37; S, 12.82.

(±)-4-(4-Methylphenyl)-2-methylsulfinylmethyl-4-oxobutanoic acid (4). An 11.1 g (44.0 mmol) sample of (±)-**3** was dissolved in tetrahydrofuran (THF) and cooled to 4 °C, and 7.97 g (46.2 mmol) of mCPBA was added. The temperature of the reaction mixture was then raised to 20 °C. The reaction mixture was stirred at 13–20 °C for 60 min, and then evaporated in vacuo. The residue was washed with 100 mL of THF and then with 250 mL of ethyl acetate. The product was recrystallized from 220 mL of chloroform and again from 170 mL of chloroform to give 4.41 g (37%) of (±)-4-(4-methylphenyl)-2-methylsulfinylmethyl-4-oxobutanoic acid (**4**). The ^{1}H NMR spectrum of **4** suggested the presence of diastereomer attached to a sulfur atom. Mp 141–142°C, ^{1}H NMR (DMSO-d_6, 300 MHz) δ ppm: 2.38 (s, 3H), 2.59 (s, 1.5H), 2.61 (s, 1.5H), 2.80–3.60 (m, 5H), 7.35 (d, 2H, $J=8.1$ Hz), 7.88 (d, 2H, $J=8.1$ Hz), 12.64 (s, 1H). IR (KBr) cm^{-1}: 3436, 2919, 2507, 1709, 1676, 1232, 1207, 994, 977, 803. Mass (CI) *m/z*: 269 ($(M+H)^+$). Anal. calcd for $C_{13}H_{16}O_4S$:C, 58.19;H, 6.01; S, 11.95. Found: C, 57.98; H, 5.94; S, 12.20.

(±)-4-(4-Hydroxymethylphenyl)-2-methylthiomethyl-4-oxobutanoic acid (5). A 53.5 g (257 mmol) sample of terephthalaldehyde mono(diethylacetal) (**12**) was dissolved in 500 mL of ethanol and 4.85 g (128 mmol) of sodium boronhydride under ice cooling. The reaction mixture was stirred at room temperature for 60 min, poured into 1500 mL of water, and extracted with 1500 mL of ether. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo to give 51.7 g of 4-hydroxymethylbenzaldehyde diethylacetal (96%).

A 51.3 g (244 mmol) sample of 4-hydroxymethylbenzaldehyde diethylacetal was dissolved in pyridine (200 mL)–acetic anhydride (50 mL), and stirred overnight. The reaction mixture was evaporated in vacuo, 500 mL of satd sodium hydrocarbonate water solution was added, and the mixture was extracted with 1500 mL of ether. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo to give 61.1 g of 4-acetoxybenzaldehyde diethylacetal (quant).

A 53.4 g (212 mmol) sample of 4-acetoxybenzaldehyde diethylacetal was dissolved in THF (267 mL)–water (107 mL). Concd sulfuric acid (2.67 g)–water (27 mL) was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was made alkaline with potassium carbonate, and extracted with 1500 mL of ethyl acetate. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo to give 37.5 g (96% from **12**) of 4-acetoxymethylbenzaldehyde (**13**).

A 5.00 g (28.1 mmol) sample of **13** was dissolved in 35 mL of THF, and 3.55 g (30.9 mmol) of indium metal (−200 mesh)[21] was added. 2-(Bromomethyl)acrylic acid (5.56 g, 33.7 mmol)–THF (25 mL) was added dropwise over 25 min under argon atmosphere. The temperature of the reaction mixture was raised from 18 to 39 °C. The reaction mixture was stirred for 35 min, 20 mL of 3 mol/L hydrochloric acid was added, and the mixture was then extracted with 300 mL of ethyl acetate. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography using ethyl acetate/hexane = 1:1–3:1 to give 6.46 g (87%) of 4-(4-acetoxymethylphenyl)-4-hydroxy-2-methylenebutanoic acid (**14**).

A 6.19 g (23.4 mmol) sample of **14** was dissolved in 468 mL of acetone, and 6.0 mL of 4 mol/L Jones reagent[16,22] was added under ice cooling. The reaction mixture was stirred for 5 min, 2.0 mL of Jones reagent was added, and stirred for 5 min. Jones reagent (2.0 mL; total 10.0 mL) was added along with 2.0 mL of isopropylalcohol. The reaction mixture was stirred for 10 min and extracted with 1000 mL of ether. The extract

was washed with water and brine, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography using ethyl acetate to give 5.80 g (83%) of 4-(4-acetoxymethylphenyl)-2-methylene-4-oxobutanoic acid (**15**).

A 5.80 g (22.1 mmol) sample of **15** was suspended in 32 mL of toluene, and 2.00 g (26.3 mmol) of thioacetic acid was added. Triethylamine (0.45 g, 4.45 mmol)–toluene (4.0 mL) was added, and the mixture was stirred at 60 °C for 2 h. The reaction mixture was poured into cooled aqueous sulfuric acid, and extracted with 200 mL of ethyl acetate. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography using ethyl acetate/hexane = 1:1–3:1 to give 6.88 g (92%) of 4-(4-acetoxymethylphenyl)-2-acetylthiomethyl-4-oxobutanoic acid (**16**).

A 6.50 g (19.2 mmol) sample **16** was dissolved in 94 mL of 8% ammonia solution, and the mixture was stirred at room temperature for 60 min. TOMAC (41 mg, 0.10 mmol) and methyliodide (1.5 mL, 24.1 mmol)–ether (7 mL) were added, in this order, and stirred at room temperature for 30 min. Subsequently, 340 mL of an aqueous 5% potassium hydroxide solution was added and the mixture was stirred at room temperature for 20 min. The reaction mixture was cooled and acidified with 3 mol/L hydrochloric acid and then extracted with 300 mL of ethyl acetate. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography using ethyl acetate/hexane = 2:1–5:1 to give 4.12 g (80%) of (±)-**5**. Mp 98–99 °C, ^{1}H NMR (DMSO-d_6 (200 MHz)δ 2.08 (s, 3H), 2.71 (dd, 1H, J = 7.5, 13.2 Hz), 2.84 (dd, 1H, J = 5.9, 13.2 Hz), 3.01–3.17 (m, 1H), 3.24 (dd, 1H, J = 4.6, 18.0 Hz), 3.45 (dd, 1H, J = 8.8, 18.0 Hz), 4.59 (s, 2H), 5.38 (br s, 1H), 7.47 (d, 2H, J = 8.3 Hz), 7.95 (d, 2H, J = 8.3 Hz), 12.42 (br s, 1H). IR (KBr) cm^{-1}: 3426, 2915, 2626, 1708, 1677, 1418, 1221, 1028, 816. FAB-MS m/z: 269 $(M+H)^+$). Anal. calcd for $C_{13}H_{16}O_4S$: C, 58.19; H, 6.01. Found: C, 58.21; H, 5.95.

(±)-4-(4-Hydroxymethylphenyl)-2-methylsulfinylmethyl-4-oxobutanoic acid (6). A 1.50 g (5.59 mmol) sample of (±)-**5** was dissolved in 20 mL of THF and cooled under an ice bath. MCPBA (1.06 g, 6.14 mmol) was then added. The reaction mixture was stirred under an ice bath for 60 min, and the crystal was collected by filtration to give 1.23 g (77%) of (±)-**6**. The ^{1}H NMR spectrum of **6** suggested the presence of diastereomer attached to a sulfur atom. Mp 117–118 °C, ^{1}H NMR (DMSO-d_6) (200 MHz)δ 2.58 (s, 1.5H), 2.61 (s, 1.5H), 2.80–3.64 (m, 5H), 4.58 (s, 2H), 5.38 (br s, 1H), 7.46 (d, 2H, J = 8.0 Hz), 7.94 (d, 2H, J = 8.0 Hz), 12.63 (br s, 1H). IR (KBr) cm^{-1}: 3435, 2918, 2513, 1713, 1678, 1415, 1204, 978. FAB-MS m/z: 285 $(M+H)^+$). Anal. calcd for $C_{13}H_{16}O_5S{\cdot}1/23$THF: C, 55.04; H, 5.73. Found: C, 55.01; H, 5.71.

(±)-2-Methylthiomethyl-4-(4-carboxyphenyl)-4-oxobutnanoic acid (7). A 35.0 g (213 mmol) sample of terephthalaldehydic acid methyl ester (**17**) was dissolved in 245 mL of THF, and 27.1 g (236 mmol) of indium metal (−200 mesh) was added. 2-(Bromomethyl)acrylic acid (43.1 g, 261 mmol)–THF (105 mL) was then added. The reaction mixture was heated from 21 to 55 °C, and then stirred at room temperature overnight. Hydrochloric acid (1 mol/L; 700 mL) was added and the mixture was extracted with 350 mL of ethyl acetate. The extract was washed with 1 mol/L hydrochloric acid and water, dried over $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from ethyl acetate to give 33.1 g (62%) of 4-hydroxy-4-(4-methoxycarbonylphenyl)-2-methylene-butanoic acid (**18**).

A solution of 8.55 mL (99.7 mmol) of oxalyl chloride in 222 mL of THF was cooled to −74 °C and dimethylsulfoxide (12.8 mL)–THF (38.5 mL) was added dropwise over 5 min. Compound **18** (20.4 g, 81.5 mmol)–THF (204 mL) was then added dropwise over 11 min, and 53.0 mL (382 mmol) of triethylamine was added dropwise over 4 min. After stirring for 10 min, 400 mL of concd hydrochloric acid was added over 15 min, and the mixture was extracted with ethyl acetate. The extract was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from ethyl acetate–hexane to give 8.61 g (42%) of 2-methylene-4-(4-methoxycarboxyphenyl)-4-oxobutanoic acid (**19**).

A 6.96 g (28.0 mmol) sample of **19** was suspended in 42 mL of toluene, and 2.57 g (33.8 mmol) of thioacetic acid was added. Triethylamine (0.57 g, 5.6 mmol)–toluene (5.25 mL) was added and the mixture was stirred at 60 °C for 40 min. The reaction mixture was cooled, 50 mL of 0.2 mol/L hydrochloric acid was added, and the mixture was extracted with ethyl acetate. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo. The residue was recrystallized from ethyl acetate–hexane to give 5.57 g of 2-acetylthiomethyl-4-(4-methoxycarbonylphenyl)-4-oxobutanoic acid (**20**). Its mother liquor was evaporated. The residue was purified by column chromatography using ethyl acetate/hexane = 1:1–4:1 to give 2.75 g of **20**. Overall, 8.32 g (86%) of **20** was obtained.

A 3.00 g (9.25 mmol) sample of **20** was dissolved in 43.5 mL of 8% ammonia solution, and stirred at room temperature for 60 min. TOMAC (19 mg, 0.05 mmol) and methyliodide (0.7 mL, 11.2 mmol)–ether (7 mL) were added, in this order, and the mixture was stirred at room temperature for 20 min. Subsequently, 154 mL of an aqueous 5% potassium hydroxide solution were added and the mixture was stirred at room temperature for 30 min. The reaction mixture was cooled, acidified with 3 mol/L hydrochloric acid and then extracted with 300 mL of ethyl acetate. The extract was washed with brine and water, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography using ethyl acetate/THF = 4:1–1:1 to give 1.62 g (61%) of (±)-**7**. Compound **7** (0.82 g) was recrystallized from ethyl acetate to give 0.54 g of purified **7**. Mp 169–170 °C, ^{1}H NMR (DMSO-d_6)(200 MHz)δ 2.08 (s, 3H), 2.72 (dd, 1H, J = 7.5, 13.4 Hz), 2.86 (dd, 1H, J = 5.9, 13.4 Hz), 3.04–3.20 (m, 1H), 3.31 (dd, 1H, J = 4.6,

18.2 Hz), 3.51 (dd, 1H, $J=8.4$, 18.2 Hz), 8.07 (s, 4H), 12.91 (br s, 2H). IR (KBr) cm^{-1}: 2916, 2676, 1700, 1682, 1432, 1296, 990. FAB-MS m/z: 281 ($(M-H)^+$). Anal. calcd for $C_{13}H_{14}O_5S$: C, 55.31; H, 5.00. Found: C, 55.17; H, 4.88.

(±)-4-(4-Carboxyphenyl)-2-methylsulfinyl-methyl-4-oxobutanoic acid (8). A 450 mg (1.59 mmol) sample of **7** was dissolved in 5.5 mL of THF and cooled under an ice bath. mCPBA (303 mg, 1.76 mmol) was added. The reaction mixture was stirred under an ice bath for 40 min, and the crystal was deposited. THF (2.0 mL) and ethyl acetate (2.0 mL) were added to the reaction mixture, and the resulting precipitates were collected by filtration to give 380 mg (80%) of (±)-**8**. The ^1H NMR spectrum of **8** suggested the presence of diastereomer attached to a sulfur atom. Mp 158–160 °C, ^1H NMR (DMSO-d_6)(200 MHz) δ 2.60 (s, 1.5H), 2.62 (s, 1.5H), 2.87–3.69 (m, 5H), 8.08 (s, 4H), 13.00 (br s, 2H). IR (KBr) cm^{-1}: 2924, 1726, 1700, 1683, 1408, 1220, 1014, 764. FAB-MS m/z: 299 $(M+H)^+$). Anal. calcd for $C_{13}H_{16}O_6S$ 1/3H_2O: C, 53.37; H, 5.19. Found: C, 53.17; H, 5.05.

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