Optical Resolution by Preferential Crystallization of 2-(3,4-Carbonyldioxyphenyl)-2-(phthalimidooxy)acetic Acid, a Key Intermediate of Cephalosporin Antibiotic M-14659

Kimihiro MURAKAMI,* Masayuki OHASHI,† Atsuo MATSUNAGA,†
Ichiro YAMAMOTO,† Akira TOMIGUCHI,†
and Hiroyuki NOHIRA
Department of Applied Chemistry, Faculty of Engineering, Saitama University,
Shimo-ohkubo, Urawa, Saitama 341
†Fuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd.,
722, Jimba-aza-Uenohara, Gotemba, Shizuoka 412
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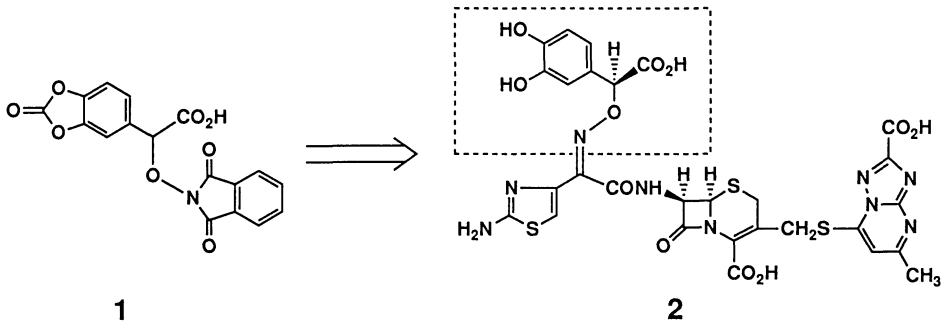
(±)-2-(3,4-Carbonyldioxyphenyl)-2-(phthalimidooxy)acetic acid [(±)-**1**] was efficiently resolved into a pair of optically active forms by preferential crystallization. Successive preferential crystallization of (±)-**1** was experimented at 15 °C under stirring in acetone, and (+)- and (−)-**1** with optical purity of 68—85% were obtained. The racemization of (−)-**1** proceeded smoothly in the presence of triethylamine to give (±)-**1** which was reusable for the preferential crystallization procedure. A potent antipseudomonal cephalosporin M-14659 (**2**) was prepared from (+)-**1**.

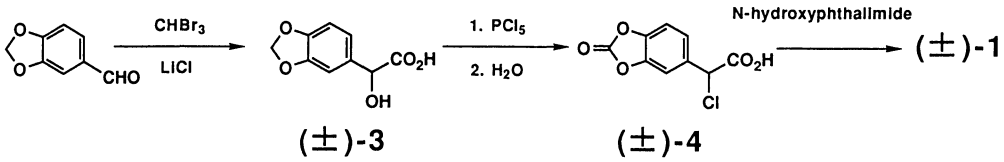
M-14659 (**2**) is a new semisynthetic cephalosporin which has a potent antibacterial activities against a variety of gram-positive and gram-negative bacteria including *Pseudomonas aeruginosa*.[1] Because the structure of **2** contains a chiral moiety, [(*S*)-carboxy(3,4-dihydroxyphenyl)methyl]oxyimino group in its side chain at the 7-position, an efficient synthetic method for an optically active intermediate of **2** is required for practical and industrial preparation of **2**. Recently, α-(phthalimidooxy)arylacetic acid as an intermediate of **2** was optically resolved by the diastereomeric procedure using quinine by Iwagami et al.[2] However, the diastereomeric procedure requires a relatively large quantity of resolving agents. On the other hand, the preferential crystallization procedure is more useful on the industrial scale than the diastereomeric one, since optical resolution can easily be achieved by providing a small amount of one enantiomer as seed crystals in a supersaturated racemic solution. We report here the optical resolution by preferential crystallization of racemic 2-(3,4-carbonyldioxyphenyl)-2-(phthalimidooxy)acetic acid [(±)-**1**] as a key intermediate of **2**.

Results and Discussion

The overall synthetic route for (±)-**1** is outlined in Scheme 2. Piperonal was treated with bromoform in aqueous dioxane in the presence of LiCl and KOH to give racemic α-hydroxy acid **3** in 63% yield.[3] The treatment of (±)-**3** with PCl_5 followed by hydrolysis



Scheme 1.



Scheme 2.

gave α-chloro acid **4** in 95% yield. Racemic α-(phthalimidooxy) acid **1** was prepared from (±)-**4** with *N*-hydroxyphthalimide in acetonitrile in the presence of triethylamine.

Comparing melting point and infrared spectrum of optically active form with those of racemate is efficient to ensure the possibility of optical resolution by the preferential crystallization.[4] Then, in order to obtain optically active **1**, the optical resolution of (±)-**1** was initially tried using optically active α-methylbenzylamine (MBA) as a resolving agent. A treatment of (±)-**1** with an equimolar amount of (+)-MBA in acetone afforded crystals of the less soluble diastereomeric salt [(+)-**1**·(+)-MBA]. After the decomposition of the salt, (+)-**1** was obtained with 98% optical purity in 31% yield. By the same procedure, (−)-**1** was obtained from (±)-**1** and (−)-MBA.

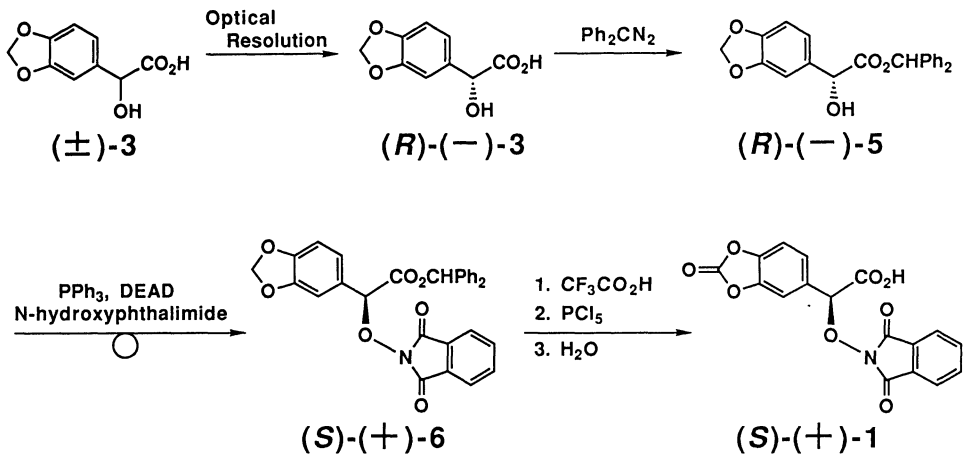
The absolute configurations of the resolved enantiomers were determined chemically by the route shown in Scheme 3. Racemic α-hydroxy acid **3** was resolved successfully using (+)-MBA to give the known (*R*)-(−)-**3** in 12% yield with 93% optical purity calculated on the specific rotation described in the literature[5] [$[\alpha]_D$ −128° (EtOH)]. Treatment of (*R*)-(−)-**3** with diphenyldiazomethane provided α-hydroxy ester [(*R*)-(−)-**5**], which was converted to (+)-α-(phthalimidooxy) ester **6** by the Mitsunobu reaction. It is known that the Mitsunobu reaction proceeds with virtually complete inversion of configuration.[6] Consequently, the absolute configuration of the obtained (+)-**6** was determined to be an (*S*)-configuration. (*S*)-(+)-**6** was deprotected with CF_3CO_2H followed by treatment with PCl_5 and then H_2O to give (+)-α-(phthalimidooxy) acid **1** with 93% optical purity. Thus, the absolute configuration of (+)-**1** was confirmed to be *S* by the transformation from the known (*R*)-(−)-**3**.

Melting point and infrared spectrum of optically active **1** obtained as above or of its salt with an achiral amine such as diethylamine (**7**), dicyclohexylamine (**8**), pyrrolidine (**9**), benzylamine (**10**), 2-hydroxyethylamine (**11**), or ammonia (**12**) were compared with those of the corresponding racemate. As shown in Table 1, only optically active **1** shows identical infrared spectrum with that of racemate and indicates higher melting point than racemate. These results suggest that crystals of (±)-**1** are deposited as conglomerate and that (±)-**1** is resolvable by preferential crystallization procedure.[4]

Actually, a supersaturated solution of (±)-**1** in acetone was seeded with the crystals of (+)-**1** and allowed to stand at −15 °C for 20 h to provide (+)-**1**. As shown in Table 2, alternate seeding of (−)- or (+)-**1** to the solution supersaturated in a similar magnitude gave (−)- or (+)-**1** in high optical purity. However, the procedure is required to improve the crystallization conditions such as concentration, cooling temperature, and cooling time for practical purposes. After several examinations on the crystallization conditions, it was found that stirring of the supersaturated solution during crystallization at 15 °C was effective to shorten the crystallization time and to increase the yield of optically active **1** as shown in Table 3. Stirring made the solution uniform and accelerated the crystal growth from seed surface.

Table 1. Properties of **1** and Its Salts

		Optically active form		Racemate
1	Mp/°C	193—196		177—179
	IR Spectrum		Identical	
1·7 Salt	Mp/°C	120—123		135—136
	IR Spectrum		Different	
1·8 Salt	Mp/°C	156—157		167—168
	IR Spectrum		Different	
1·9 Salt	Mp/°C	101—104		102—106
	IR Spectrum		Different	
1·10 Salt	Mp/°C	139—140		144—145
	IR Spectrum		Different	
1·11 Salt	Mp/°C	103—106		115—116
	IR Spectrum		Different	
1·12 Salt	Mp/°C	125—128		152—154
	IR Spectrum		Different	



Scheme 3.

Table 2. Preferential Crystallization of **1** on Standing

Run	(±)-**1** added/g	Seed	Yield/g	Optical purity[a]/%
1	—	(+)	8.9	78.2
2	10.0	(−)	8.9	84.8
3	10.0	(+)	8.9	83.4
4	10.0	(−)	13.7	87.5
5	13.2	(+)	15.0	84.4
6	14.5	(−)	10.6	86.9
7	10.2	(+)	9.8	87.2

The initial composition of the mother liquor: (±)-**1** (100 g) in acetone (1100 ml). The crystallization conditions: standing at −15 °C for 20 h. In all runs, 0.5 g of seed was added.
a) The optical purity was determined on the basis of the specific rotation ($[\alpha]_D^{30}$+ and −268.5°).

Table 3. Preferential Crystallization of **1** under Stirring

Run	(±)-**1** added/g	Seed	Yield/g	Optical purity[a]/%
1	—	(+)	17.4	68.0
2	15.0	(−)	10.6	85.4
3	15.0	(+)	7.9	70.8
4	15.0	(−)	12.9	82.3
5	15.0	(+)	11.6	70.8
6	15.0	(−)	13.6	76.4
7	15.0	(+)	16.4	75.7
8	15.0	(−)	11.6	74.6
9	15.0	(+)	12.5	68.4
10	15.0	(−)	11.2	79.9

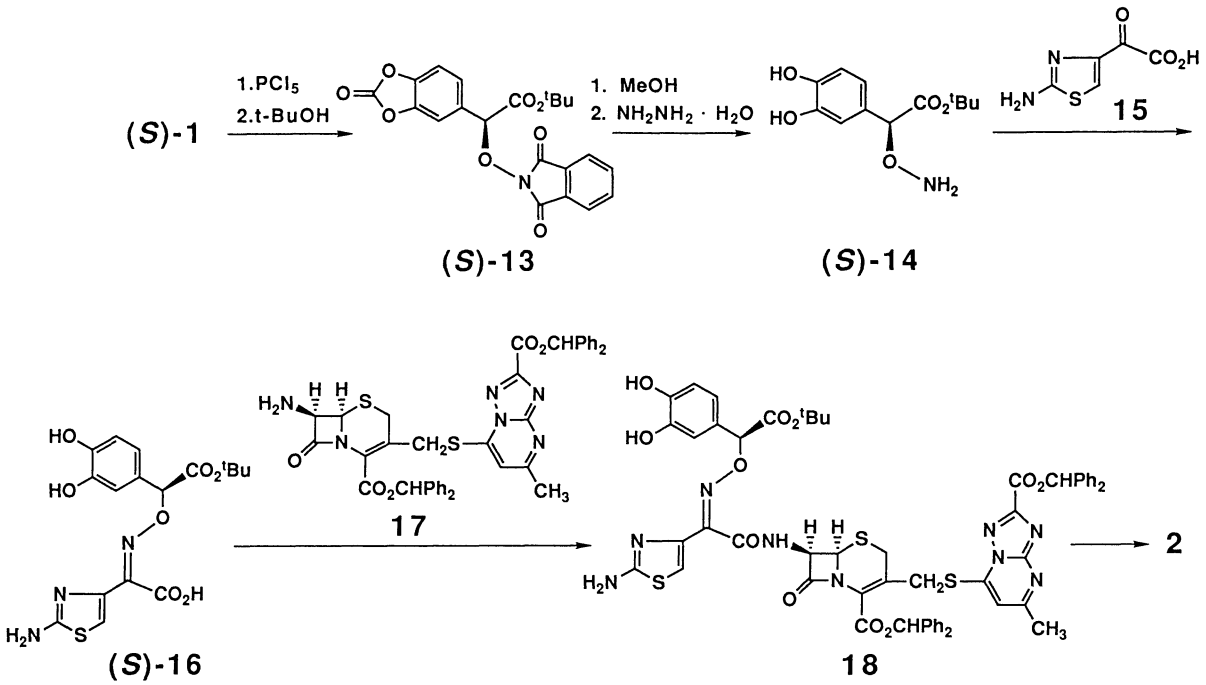
The initial composition of the mother liquor: (±)-**1** (100 g) in acetone (800 ml). The crystallization conditions: stirring at 15 °C for 1 h. In all runs, 0.5 g of seed was added.
a) The optical purity was determined on the basis of the specific rotation ($[\alpha]_D^{30}$+ and −268.5°).

But, the nucleation of the unseeded antipode crystals of **1** occurred when the solution was stirred too vigorously.

The crystals of (+)-**1** obtained by the preferential crystallization were combined and recrystallized from acetone to give (+)-**1**, of which the specific rotation at 589 nm in acetone was +268.5°. The purified (+)-**1** was converted to the corresponding *t*-butyl ester (+)-**13** by the usual esterification. None of the antipode was detected in obtained (+)-**13** by HPLC analysis using an optical isomer separating column. Based on the data, (+)-**1** obtained as above was considered to be almost optically pure.

On the other hand, undesirable (−)-**1** was also obtained by the preferential crystallization. Accordingly, the racemization of **1** was explored. The racemization of (−)-**1** proceeded smoothly in the presence of triethylamine. Optically active (−)-**1** was treated with triethylamine in acetonitrile for 1 h at 50 °C, and racemic **1** was recovered in 88% yield. The recovered racemic **1** could be reused as the starting material of the preferential crystallization procedure.

In order to demonstrate the availability of (*S*)-(+)-**1** obtained by the preferential crystallization method, (*S*)-(+)-**1** was converted to **2**. The overall synthetic route for **2** is outlined in Scheme 4. (*S*)-(+)-**1** was treated with PCl_5 in CH_2Cl_2 and then with *t*-BuOH to give the corresponding *t*-butyl ester (*S*)-(+)-**13**. A treatment of (*S*)-(+)-**13** with methanol followed by reaction with $NH_2NH_2 \cdot H_2O$ provided α-aminooxy ester (*S*)-(+)-**14**, which was coupled with (2-amino-4-thiazolyl)glyoxylic acid (**15**) to give (*S*)-(+)-**16** in 51% yield from (*S*)-(+)-**1**. Compound (*S*)-(+)-**16** was then reacted with cephalo-



Scheme 4.

sporin intermediate (**17**)[7] using $POCl_3$ and *N,N*-diethylaniline to give **18**, which was deprotected with anisole and CF_3CO_2H followed by chromatographic purification to give optically pure **2** in 45% yield from (*S*)-(+)-**16**. Thus, (*S*)-(+)-**1** obtained efficiently by the preferential crystallization was smoothly converted to **2**.

Experimental

General. Melting points were determined with a Mettler FP800 apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a JEOL FX-90Q (90 MHz) spectrometer. Chemical shifts are reported in ppm (δ) downfield from internal tetramethylsilane or sodium 3-trimethylsilyl-1-propanesulfonate. ^{13}C NMR spectra were obtained on a JEOL JNM-EX270 (270 MHz) spectrometer with dimethyl sulfoxide as an internal standard. Infrared (IR) spectra were obtained on a Nicolet 5DX spectrometer. Mass spectra were measured on a JEOL DX-303 mass spectrometer. Optical rotation were recorded on a JASCO DIP-181 polarimeter with a quartz cell of 1.0 dm path length. Optical purities were determined using a JASCO Trirotar-VI HPLC system under the indicated conditions.

Preparation of 2-(3,4-Methylenedioxyphenyl)-2-hydroxyacetic Acid [(±)-3]. This was prepared from piperonal and bromoform by using the literature procedure[3] (63% yield) as a yellowish powder: Mp 156—158 °C (lit, 158—160 °C[5]); ^{1}H NMR (d_6-DMSO) δ=4.93 (1H, s), 5.98 (2H, s), and 6.8—7.0 (3H, m); ^{13}C NMR (d_6-DMSO) δ=72.15, 101.03, 107.08, 108.00, 120.24, 134.25, 146.79, 147.17, and 174.25; IR (KBr) 1718 cm^{-1} (C=O); MS (EI) *m/z* 196 (M^+).

Preparation of 2-(3,4-Carbonyldioxyphenyl)-2-chloroacetic Acid [(±)-4]. To a solution of PCl_5 (800 g, 3.84 mol) in anhydrous benzene (2600 ml) was added (±)-**3** (153.5 g, 0.783 mol) in limited amounts. After heating under reflux for 13 h, additional PCl_5 (100 g, 0.48 mol) was added to the reaction and the mixture was heated under reflux for further 14 h. After concentrating in vacuo, ether (1000 ml) was added to the residue. The insoluble matters were filtered off and washed with ether (300 ml). The washings were combined with the filtrate and the combined solution was added to ice (2000 ml) by portions with care. After stirring at 0 °C for 20 min, the organic layer was separated, washed (water and then brine), dried (Na_2SO_4) and concentrated in vacuo to give (±)-**4** as a yellowish solid: Yield 341 g (95%); mp 156—158 °C; ^{1}H NMR (d_6-DMSO) δ=5.84 (1H, s), and 7.3—7.6 (3H, m); ^{13}C NMR (d_6-DMSO) δ=58.57, 110.16, 110.51, 124.82, 133.87, 143.16, 143.45, 150.96, and 169.06; IR (KBr) 1845, 1838, 1826, 1795, and 1741 cm^{-1}. Found: C, 47.14; H, 2.10%. Calcd for $C_9H_5O_5Cl$: C, 47.29; H, 2.20%.

Preparation of (±)-2-(3,4-Carbonyldioxyphenyl)-2-(phthalimidooxy)acetic Acid [(±)-1]. To a solution of (±)-**4** (339 g, 1.48 mol) in acetonitrile (1650 ml) was added dropwise a solution of *N*-hydroxyphthalimide (242 g, 1.48 mol) and triethylamine (300 g, 2.96 mol) in acetonitrile (700 ml) over 3 h under ice-cooling. After stirring for 1 h, the resulting crystals were filtered off and washed with 300 ml of acetonitrile. The washings were combined with the filtrate. To the combined solution was added ice-cold water (1500 ml), 6 M HCl (300 ml, 1 M=1 mol dm^{-3}) and AcOEt (2500 ml). The organic layer was separated, washed (water and then brine), dried (Na_2SO_4) and concentrated in vacuo to give (±)-**1** as a white powder: Yield 422 g (80%); mp 177—179 °C; ^{1}H NMR (d_6-DMSO) δ=5.81 (1H, s), and 7.4—7.9 (7H, m); ^{13}C NMR (d_6-DMSO) δ=84.55, 110.33, 110.39, 123.42, 125.20, 128.39, 131.09, 134.93, 142.86, 143.81, 150.84, 162.82, and 168.57; IR (KBr) 1872, 1844, 1752, 1718, and 1497 cm^{-1}. Found: C, 57.22; H, 2.36; N, 3.61%. Calcd for $C_{17}H_9NO_8$: C, 57.48; H, 2.55; N, 3.94%.

Optical Resolution of (±)-1. To a solution of (±)-**1** (150 g, 0.423 mol) in acetone (3300 ml) was added a solution of (+)-MBA (51.2 g, 0.423 mol) in acetone (500 ml). After standing for 1 h under ice-cooling, formed crystals were filtered and washed with cold acetone (700 ml). The crystals were suspended in 0.5 M HCl (1500 ml) and the mixture was extracted with AcOEt (800 ml×4). The combined organic layer was washed (water and then brine), dried ($MgSO_4$) and concentrated in vacuo to give (+)-**1** as white crystals: Yield 46.2 g (31%); mp 193—196 °C; $[\alpha]_D^{25}$ +264.0° (*c* 0.4, acetone); 98.3% optical purity; The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of (±)-**1**. In a similar manner, (−)-**1** was obtained using (−)-MBA. The absolute configuration of (+)-**1** was determined chemically by the transformation from the known (*R*)-(−)-**3** as described bellow.

Determination of Absolute Configuration of (+)-1. To a solution of (±)-**3** (3.0 g, 15.3 mmol) in tetrahydrofuran (20 ml) was added a solution of (+)-MBA (1.85 g, 15.3 mmol) in tetrahydrofuran (10 ml). After standing for 3 h, formed crystals were filtered and recrystallized from tetrahydrofuran. The purified salts were suspended in 1 M HCl (50 ml) and the mixture was extracted with AcOEt (30 ml×3). The combined organic layer was washed (water and then brine), dried ($MgSO_4$), and concentrated in vacuo to give (*R*)-(−)-**3** as a yellowish powder: Yield 360 mg (12%); mp 124—127 °C (lit, 129—131 °C[5]); $[\alpha]_D^{25}$ −119.8° (*c* 0.4, EtOH) [lit, $[\alpha]_D$ −128.5° (EtOH)[5]]; The ^{1}H NMR and ^{13}C NMR spectra were identical with those of (±)-**3**.

To a solution of (*R*)-(−)-**3** (690 mg, 3.5 mmol) in acetone (20 ml) was added dropwise a solution of diphenyldiazomethane (820 mg, 4.2 mmol) in acetone (5 ml). After stirring at room temperature for 30 min, the reaction mixture was concentrated in vacuo. The residue was washed with hexane to give (*R*)-(−)-**5** as a white powder: Yield 1.1 g (86%); mp 116—119 °C; $[\alpha]_D^{25}$ −27.2° (*c* 0.4, EtOH); ^{1}H NMR (d_6-DMSO) δ=5.21 (1H, d, *J*=5 Hz), 6.00 (2H, s), 6.11 (1H, d, *J*=5 Hz), and 6.7—7.4 (14H, m); ^{13}C NMR (d_6-DMSO) δ=72.24, 76.55, 101.03, 107.12, 107.98, 120.45, 125.99, 126.58, 127.64, 127.85, 128.32, 128.52, 133.32, 140.29, 140.33, 146.94, 147.15, and 171.47.

To a solution of (*R*)-(−)-**5** (1.0 g, 2.8 mmol) and triphenylphosphine (1.45 g, 5.5 mmol) in tetrahydrofuran (15 ml) was added dropwise a solution of *N*-hydroxyphthalimide (0.68 g, 4.1 mmol) and diethyl azodicarboxylate (DEAD) (0.96 g, 5.5 mmol) in tetrahydrofuran (15 ml). After stirring at room temperature for 1 h, the reaction mixture was concentrated in vacuo. Chromatographic separation on silica gel (100 g, hexane–AcOEt, 4:1 v/v) gave (*S*)-(+)-**6** as a white powder: Yield 1.0 g (71%); $[\alpha]_D^{25}$ +121.1° (*c* 0.44, acetone); ^{1}H NMR (d_6-DMSO) δ=5.98 (1H, s), 6.05 (2H, s), and 6.9—7.9 (18H, m).

To a solution of (*S*)-(+)-**6** (400 mg, 0.8 mmol) in 1,2-dichloroethane (2 ml) were added anisole (0.3 ml) and trifluoroacetic acid (0.85 ml) under ice-cooling. After stirring for 1 h, the reaction mixture was concentrated in vacuo. The residue was washed with ether and then suspended in benzene (5 ml). To the suspension was added PCl_5 (0.43 g, 2.05

mmol) and the mixture was heated under reflux for 8 h. The reaction mixture was poured into water (100 ml) and extracted with AcOEt (200 ml). The organic layer was washed (brine), dried (Na_2SO_4), and concentrated in vacuo to give (*S*)-(+)-**1** as white crystals: Yield 330 mg (89%); mp 193—196 °C; $[\alpha]_D^{25}$ +250.4° (*c* 0.4, acetone); 93.3% optical purity. The IR, ^{1}H NMR, and ^{13}C NMR spectra were identical with those of (*S*)-(+)-**1** obtained by the optical resolution procedure described above.

Preferential Crystallization of (±)-1. A solution of (±)-**1** (100 g) in acetone (1200 ml) was concentrated at 45 °C to 800 ml and then cooled at 25 °C to give a supersaturated solution. To the solution was added (+)-**1** (0.5 g) as a seed, and the solution was stirred (about 180 rpm) by a mechanical stirrer for 1 h at 15 °C. The white precipitates deposited were collected by filtration, washed with cold acetone (15 ml), and dried to give (+)-**1**: Yield 17.4 g; $[\alpha]_D^{30}$ +182.6° (*c* 0.4, acetone); 68.0% optical purity.

To the filtrate was added (±)-**1** (15 g) and dissolved at an elevated temperature. In a similar manner, the solution was cooled, seeded with (−)-**1**, and stirred for 1 h at 15 °C to give (−)-**1**: Yield 10.6 g; $[\alpha]_D^{30}$ −229.3° (*c* 0.4, acetone); 85.4% optical purity.

After several stages of the preferential crystallization as shown in Table 3, the crystals of (+)-**1** were combined and recrystallized from acetone to give optically pure (+)-**1**. For example, recrystallization of (+)-**1** (237 g, 71% optical purity on the average) from acetone gave optically pure (+)-**1**: Yield 173.9 g (73.4%); $[\alpha]_D^{30}$ −268.5° (*c* 0.4, acetone). The optical purity of recrystallized (+)-**1** was determined by HPLC analysis of the prepared (+)-**13**. None of the (−)-enantiomer was detected in obtained (+)-**13** by HPLC analysis under conditions shown below.

Racemization of (−)-1. To a suspension of (−)-**1** (33.0 g, 87% optical purity on the average) in acetonitrile (600 ml) was added dropwise triethylamine (19.4 ml, 1.5 equiv) at 0 °C to give yellowish solution. After heating at 50 °C for 1 h, the solution was poured into 0.33 M aq citric acid (3000 ml) and extracted with AcOEt (3000 ml). The extract was washed (brine), dried (Na_2SO_4), and concentrated in vacuo to give **1** as a white powder: Yield 29.0 g (88%); $[\alpha]_D^{30}$ 0° (*c* 0.4 acetone). Physical and spectroscopic data were identical with those of (±)-**1** obtained with above procedure.

Preparation of *t*-Butyl (*S*)-2-(3,4-Carbonyldioxyphenyl)-2-(phthalimidooxy)acetate [(*S*)-13]. To a suspension of (+)-**1** (10.0 g, 28 mmol) in CH_2Cl_2 (200 ml) was added PCl_5 (11.7 g, 56 mmol) under ice-cooling. After stirring for 30 min at room temperature, to the reaction mixture was added a mixture of *t*-BuOH (12.5 g, 169 mmol), pyridine (9.1 ml), and CH_2Cl_2 (50 ml) under ice-cooling. After stirring for 1 h, the reaction mixture was poured into 0.33 M aq citric acid (500 ml) and extracted with CH_2Cl_2 (500 ml). The extract was washed (0.33 M aq citric acid and then brine), dried (Na_2SO_4), and concentrated in vacuo to give (*S*)-**13** as a white powder: Yield 10.2 g (88%, >99% e.e.); $[\alpha]_D^{25}$ +160.8° (*c* 0.2, AcOEt); mp 139—147 °C; ^{1}H NMR (d_6-DMSO) δ=1.41 (9H, s), 5.79 (1H, s), and 7.5—7.9 (7H, m); ^{13}C NMR (d_6-DMSO) δ=27.43, 82.77, 85.14, 110.24, 110.42, 123.38, 125.02, 128.36, 130.66, 134.92, 142.91, 143.85, 150.75, 162.71, and 166.25; IR (KBr) 1866, 1843, 1837, 1794, 1740, and 1490 cm^{-1}. Found: C, 61.39; H, 4.04; N, 3.05%. Calcd for $C_{21}H_{17}NO_8$: C, 61.32; H, 4.17; N, 3.40%. None of the (*R*)-enantiomer was detected in obtained **13** by HPLC analysis. HPLC analysis for an optical isomer [column Daicel CHIRALPAK AD 200 mm×6 mm, eluent hexane/*i*-PrOH/H_2O (9:1:0.03), flow 1.0 ml min^{-1}, 25 °C, λ 254 nm, retention time 36.1 min for (*R*)-**13** and 39.5 min for (*S*)-**13**].

Preparation of *t*-Butyl (*S*)-2-(3,4-Dihydroxyphenyl)-2-(aminooxy)acetate [(*S*)-14]. To a suspension of (*S*)-**13** (55.5 g, 0.135 mol) and 1 M HCl (67 ml) in methanol (4400 ml) was heated at 40 °C for 4 h. The resultant solution was concentrated in vacuo and the residue was dissolved in ether (2000 ml). The organic solution was washed (water and then brine), dried (Na_2SO_4), and concentrated in vacuo. To a solution of the residue in CH_2Cl_2 (300 ml) was added dropwise a mixture of $NH_2NH_2 \cdot H_2O$ (20.2 g, 0.404 mol) and CH_2Cl_2 (600 ml) at −60 °C over 2 h. After stirring under ice-cooling for 1 h, to the reaction mixture was added 0.33 M aq citric acid (300 ml). The mixture was concentrated in vacuo and extracted with AcOEt (2000 ml) and H_2O (300 ml). The separated organic layer was washed (0.33 M aq citric acid and then brine), dried (Na_2SO_4), and concentrated in vacuo to give (*S*)-**14** as a white powder: Yield 32.0 g (93%, >99% e.e.); mp 127—129 °C; $[\alpha]_D^{25}$ +50.2° (*c* 0.23, MeOH); ^{1}H NMR (d_6-DMSO) δ=1.40 (9H, s), 6.19 (2H, s), 6.56—6.8 (3H, m), 8.91 (1H, s), and 8.94 (1H, s); ^{13}C NMR (d_6-DMSO) δ=27.71, 80.42, 85.18, 114.88, 115.28, 119.05, 126.52, 145.00, 145.68, and 170.44; IR (KBr) 1729, 1612, 1529, 1446, 1296, 1263, 1156, and 1052 cm^{-1}. Found: C, 56.84; H, 6.68; N, 5.13%. Calcd for $C_{12}H_{17}NO_5$: C, 56.46; H, 6.71; N, 5.39%. HPLC analysis for an optical isomer [column Daicel CHIRALPAK AD 200 mm×6 mm, eluent hexane/*i*-PrOH (6:1), flow 1.0 ml min^{-1}, 25 °C, λ 254 nm, retention time 18.1 min for (*S*)-**14** and 21.4 min for (*R*)-**14**].

Preparation of 2-(2-Amino-4-thiazolyl)-2-[(*Z*)-[(*S*)-*t*-butoxycarbonyl(3,4-dihydroxyphenyl)methyl]oxyimino]acetic Acid [(*S*)-16]. To a solution of (*S*)-**14** (33.9 g, 0.133 mol) in DMF was added **15** (22.9 g, 0.133 mol) under ice-cooling. After stirring for 30 min at room temperature, the reaction mixture was poured into ice-cold water (1500 ml), and the pH of the mixture was adjusted to 7.0 with $NaHSO_4$. The resultant solution was washed with AcOEt (500 ml×3). After adjusting the pH to 1—2 with 1 M HCl, the aqueous solution was extracted with AcOEt (3000 ml×3). The organic layer was washed (water and then brine), dried (Na_2SO_4), and concentrated in vacuo to give (*S*)-**16** as a yellowish powder: Yield 34.0 g (62.5%); mp 129 °C (decomp); $[\alpha]_D^{25}$ +65.9° (*c* 0.5, MeOH); ^{1}H NMR (d_6-DMSO) δ=1.37 (9H, s), 5.28 (1H, s), 6.6—6.9 (4H, m), 7.24 (2H, s), and 9.06 (2H, s); ^{13}C NMR (d_6-DMSO) δ=27.73, 81.15, 83.79, 109.04, 115.13, 115.46, 119.53, 125.25, 141.33, 145.25, 146.24, 148.84, 163.61, 168.82, and 169.15; IR (KBr) 1725, 1617, 1597, 1394, 1372, and 1152 cm^{-1}. Found: C, 47.40; H, 4.68; N, 9.58%. Calcd for $C_{17}H_{19}N_3O_7S \cdot H_2O$: C, 47.77; H, 4.95; N, 9.83%.

Preparation of Diphenylmethyl (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)-2-[(*Z*)-[(*S*)-*t*-butoxycarbonyl(3,4-dihydroxyphenyl)-methyl]oxyimino]acetamido]-3-[[[2-(diphenylmethyl)-oxycarbonyl]-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl]thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (18). To a solution of **17** (5.0 g, 6.6 mmol) in CH_2Cl_2 (90 ml) was added a solution of (*S*)-**16** (2.92 g, 7.1 mmol) in THF (90 ml) and *N,N*-diethylaniline (2.69 ml). To the mixture was added dropwise over 35 min a solution of $POCl_3$ (0.77 ml) in CH_2Cl_2 (10 ml) under ice-cooling. After stirring for 10 min under ice-cooling, to the reaction mixture was added 0.1 M HCl (350 ml) and CH_2Cl_2 (200 ml). The

organic layer was separated, washed (1 M HCl, water, and brine, in order), dried (Na_2SO_4), and concentrated in vacuo to give **18** as a yellowish powder: Yield 7.74 g (100%); ^{1}H NMR (d_6-DMSO) δ=1.39 (9H, s), 2.56 (3H, s), 3.67 (2H, s), 4.30 (2H, s), 5.19 (1H, d, J=5 Hz), 5.29 (1H, s), 5.86 (1H, dd, J=5 and 9 Hz), 6.7—6.9 (31H, m), and 9.58 (1H, d, J=9 Hz); IR (KBr) 1791, 1735, 1597, 1508, 1373, 1226, and 700 cm^{-1}.

Preparation of (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)-2-[(*Z*)-[(*S*)-carboxy(3,4-dihydroxyphenyl)methyl]oxyimino]acetamido]-3-[(2-carboxy-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)thiomethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (2). To a solution of **18** (10.5 g, 9.2 mmol) in 1,2-dichloroethane (80 ml) was added anisole (7.2 ml) and CF_3CO_2H (14.2 ml) under ice-cooling, and the mixture was stirred at room temperature for 30 min. The insoluble matters were filtered off and washed with 1,2-dichloroethane (30 ml). The washings were combined with the filtrate, and Et_2O (400 ml) was added to the combined solution to give a yellowish powder. After drying, the powder was dissolved in water (50 ml) with pH adjusted to 6.5 with $NaHCO_3$. The solution was applied to chromatographic separation (Diaion HP-20, eluent water). The fractions containing **2** were combined and lyophilized to give the trisodium salt of **2** as a white powder: Yield 3.4 g (45% as trisodium salt); ^{1}H NMR (D_2O) δ=2.63 (3H, s), 3.18 (1H, d, J=17.8 Hz), 3.60 (1H, d J=17.8 Hz), 4.17 (1H, d, J=13.9 Hz), 4.52 (1H, d, J=13.9 Hz), 5.00 (1H, d, J=4.6 Hz), 5.39 (1H, s), 5.67 (1H, d, J=4.6 Hz), and 6.7—7.2 (5H, m); IR (KBr) 1763, 1653, 1624, 1616, 1598, 1515, 1406, and 1314 cm^{-1}. None of the (*R*)-diastereomer was detected in obtained **2** by HPLC analysis. HPLC analysis for a diastereomer [column YMC-PACK A-312(ODS) 150 mm×6 mm, eluent 0.01 M ammonium phosphate buffer (pH 7.0)/CH_3CN (92:8), flow 1.0 ml min^{-1}, 30 °C, λ 254 nm, retention time 5.0 min for **2** [(*S*)-diastereomer] and 5.9 min for (*R*)-diastereomer].

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