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Studies on β-Lactam Antibiotics. XIV.[1] Synthesis and Biological Activity of the (*E*)-Isomer of FK027

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The (*E*)-isomer (**11**) of FK027 was synthesized by two methods. The effect of the configuration of the oxime in the 7-acyl side chain of FK027 (**1**) on the antimicrobial activity and oral absorbability in rats was investigated. Both FK027 (**1**) and its (*E*)-isomer (**11**) showed appreciable oral absorbability regardless of the configuration of the oxime.

Keywords——(*E*)-isomer; isomerization; (*E*)-2-alkoxyimino-4-chloro-3-oxobutyric acid; oral absorption; X-ray analysis; oral cephalosporin; FK027; antimicrobial activity

In our previous paper,[2] we reported the synthesis and biological activity of a new orally active cephalosporin, FK027 (**1**: cefixime) (Chart 1). FK027 showed a wide spectrum of antibacterial activity against Gram-negative bacteria, including β-lactamase-producing strains, and high stability to β-lactamases.[3] FK027 also had the unique pharmacological properties of oral absorbability and long-acting efficacy.[2]

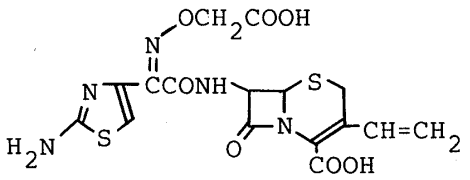

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Chart 1

With regard to the structure, FK027 is entirely distinct from the commercially available oral cephalosporins in possessing the (*Z*)-2-(2-amino-4-thiazolyl)-2-carboxymethoxyiminoacetyl side chain. The aminothiazole ring was associated with both excellent activity and oral absorbability, the amino function on the thiazole ring being essential for potent antibacterial activity.[1] Furthermore, the (*Z*)-carboxymethoxyimino group is important for oral absorbability in rats.[4] We directed our research efforts toward investigating the effect of the configuration of the oxime in the 7-acyl side chain on the antibacterial activity and oral absorbability in rats.

We here report the synthesis, antibacterial activity and oral absorbability in rats of the (*E*)-isomer (**11**) of FK027.

Chemistry

The (*E*)-isomer (**11**) of FK027 was prepared by two methods. According to method A outlined in Charts 2 and 3, *tert*-butyl (*Z*)-2-hydroxyimino-3-oxobutyrate (**2**)[2] was alkylated with *p*-nitrobenzyl chloroacetate to give the corresponding *tert*-butyl (*Z*)-2-*p*-nitrobenzyloxycarbonylmethoxyimino-3-oxobutyrate (**3**), which was converted to the desired (*Z*)-2-*p*-nitrobenzyloxycarbonylmethoxyimino-3-oxobutyric acid (**4**) by removal of the *tert*-

butyl group with trifluoroacetic acid (TFA) and anisole. The configurations of the oxime derivatives (**2**—**4**) were determined by X-ray analysis.[5)]

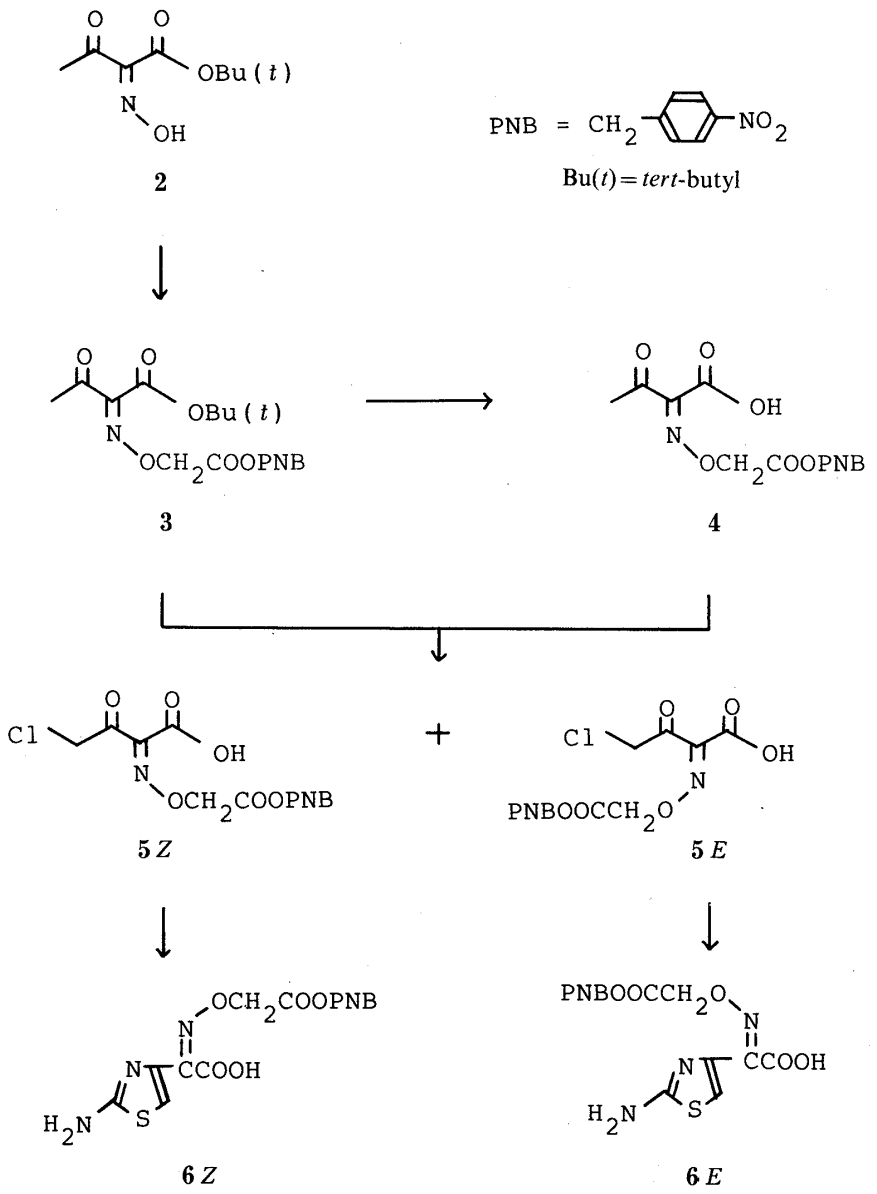


Chart 2

Chlorination of the acid (**4**) with sulfuryl chloride afforded a mixture of geometric isomers of 4-chloro-2-*p*-nitrobenzyloxycarbonylmethoxyimino-3-oxobutyric acid (**5***Z* and **5***E*) in yields of 28.2% and 12.7%, respectively. On treatment of the ester (**3**) with sulfuryl chloride, the *tert*-butyl ester of **3** was readily cleaved, in a similar way to yield a mixture of the geometric isomers (**5***Z* and **5***E*). Separation of the mixture provided the pure isomers, **5***Z* and **5***E*. Since the two isomers, **5***Z* and **5***E*, could not be characterized on the basis of their proton nuclear magnetic resonance (^{1}H-NMR) and infrared (IR) spectral data (Table I), they were separately converted to the corresponding 2-(2-amino-4-thiazolyl)-2-*p*-nitrobenzyloxycarbonylmethoxyiminoacetic acids. Reaction of **5***Z* with thiourea readily gave the (*Z*)-aminothiazolylacetic acid (**6***Z*) at room temperature. Cyclization of **5***E* with thiourea smoothly afforded the corresponding (*E*)-aminothiazolylacetic acid (**6***E*) at 40 °C in good yield, although this reaction proceeded slowly at room temperature. The configurations of the

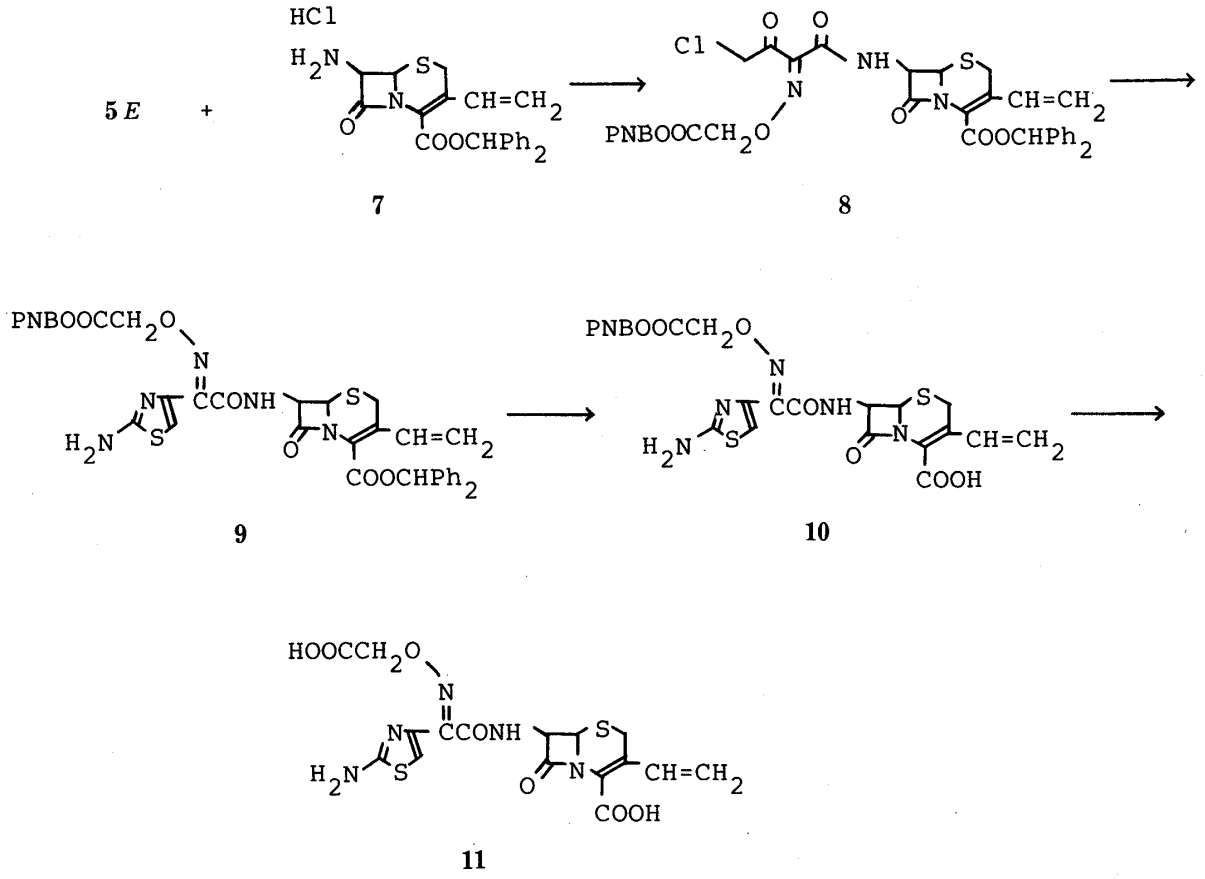


Chart 3

TABLE I. ^{1}H-NMR and IR Spectral Data for **3**—**5**

Compound No.	^{1}H-NMR (DMSO-d_6) δ						IR ν_{max}^{Nujol} (cm^{-1})
		CO_2CH_2	OCH_2CO_2	$ClCH_2$	CH_3	$C(CH_3)_3$	
	4H, A_2B_2q, J=9 Hz	2H, s	2H, s	2H, s	3H, s	9H, s	
3	7.67 8.24	5.30	5.10	—	2.31	1.47	1745, 1725
4	7.68 8.25	5.40	5.10	—	2.33	—	1755, 1725
5*Z*	7.65 8.21	5.37	5.10	4.77	—	—	1760, 1735
5*E*	7.70 8.27	5.35	5.07	4.76	—	—	1755, 1730

products, **6***Z* and **6***E*, were determined by comparison of the ^{1}H-NMR chemical shifts of the annular protons at C-5 of the thiazole ring[6] (Table II). Thus, **5***Z* and **5***E* could be assigned as the (*Z*)- and (*E*)-forms, respectively. The formation of **5***E* might be explained in terms of isomerization of the (*Z*)-alkoxyimino group to the (*E*)-alkoxyimino group under the acidic conditions (the reaction mixture contains hydrogen chloride).

The (*E*)-acid (**5***E*) was activated with Vilsmeier reagent and coupled with diphenylmethyl 7β-amino-3-vinyl-3-cephem-4-carboxylate hydrochloride (**7**) to afford the acylated compound (**8**). Cyclization of **8** with thiourea gave the aminothiazole cephem (**9**), which was successfully converted to the (*E*)-isomer (**11**) of FK027 by removal of the diphenylmethyl group, followed

TABLE II. ^{1}H-NMR and IR Spectral Data for **6**

Compound No.	^{1}H-NMR (DMSO-d_6) δ					IR ν_{max}^{Nujol} (cm^{-1})
	 4H, A_2B_2q, J=9 Hz	NH_2 2H, br s	Thiazole C_5-H 1H, s	CO_2CH_2 2H, s	OCH_2CO_2 2H, s	
6*Z*	7.63 8.21	7.20	6.86	5.33	4.83	1740
6*E*	7.67 8.24	7.25	7.63	5.37	4.97	1755

by alkaline hydrolysis of the *p*-nitrobenzyl ester.

According to method B shown in Chart 4, (*Z*)-2-*tert*-butoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)acetic acid (**12***Z*)[7] was successfully converted to the corresponding (*E*)-isomer (**12***E*) by treatment with thionyl chloride. The activated acid of **12***E* was coupled with **7** to yield the acylated compound (**13**), which was deformylated by treatment with concentrated hydrochloric acid, followed by removal of both the diphenylmethyl and *tert*-butyl groups with TFA to give **11**.

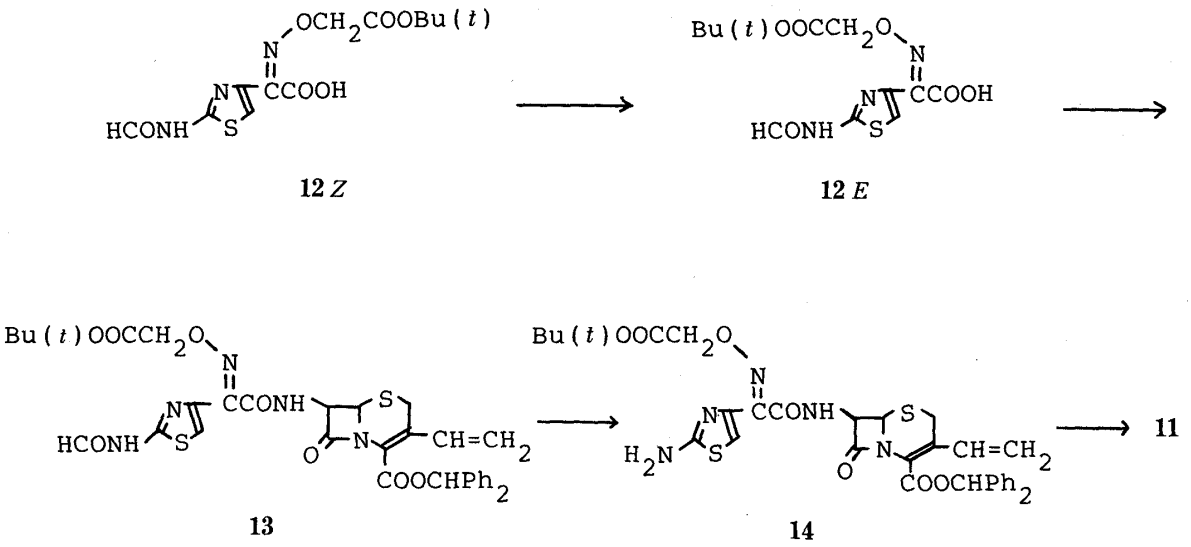


Chart 4

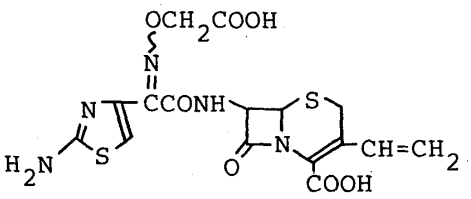
Biological Results and Discussion

The antimicrobial activity of the (*E*)-isomer (**11**) and its urinary and biliary excretions after oral administration (100 mg/kg) to rats are summarized in Table III. For comparison, the minimum inhibitory concentration (MIC) values and excretion rates of FK027 are also listed in Table III.

The (*E*)-isomer (**11**) was 2—32 times less active than FK027 against Gram-negative bacteria. However, the urinary excretion of **11** was significantly higher than that of FK027, while **11** showed almost the same recovery as FK027 in the bile.

In conclusion, the (*Z*)-configuration of the carboxymethoxyimino group in the 7-acyl side chain seems to be essential for the potent antibacterial activity of FK027 against Gram-negative bacteria, though both compounds, **1** and **11**, show appreciable oral absorbability regardless of the configuration of the oxime.

TABLE III. Antibacterial Activities of Cephalosporins (**1** and **11**) and Their Urinary and Biliary Recoveries in Rats



Compound No.	MIC (μg/ml)[a]						Recovery[c] %	
	S. aureus	*E. coli*		*K. pneumoniae*	*P. mirabilis*	*P. vulgaris*	Urine	Bile
	209P JC-1	NIHJ JC-2	28[b]	12	1	1		
1 (FK027)	25	0.2	0.39	0.1	≦0.025	≦0.025	34.0	18.2
11	>100	0.78	0.78	1.56	0.39	0.78	50.7	15.2

a) Inoculum size 10^6 C.F.U./ml. *b*) Cephalosporinase producer. *c*) Recovery within 24 h after oral administration (100 mg/kg) to rats.

Experimental

Melting points were determined using a Thomas–Hoover capillary melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a JEOL-MH 100 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. IR spectra were taken on a Hitachi 260-10 or Shimadzu IR-420 spectrophotometer.

Antibiotic Activities——All the *in vitro* antibacterial activity data are given as the MIC in μg/ml. MICs were determined by the agar dilution method using heart infusion agar (Difco) after incubation at 37 °C for 20 h with an inoculum size of about 10^6 C.F.U./ml. *Escherichia coli* 28 is a cephalosporin-resistant strain.

Urinary and Biliary Excretion——Sprague Dawley rats were fasted overnight and orally dosed with 100 mg/kg of the test drugs. Urine samples were collected for 24 h after dosing. For bile collection, another group of rats underwent bile duct cannulation with polystyrene tubing, and the test drugs were given orally at a dose of 100 mg/kg. The samples were assayed by a disc-agar diffusion method using *Escherichia coli* NIHJ JC-2 as the test organism and nutrient agar (Difco) as the test medium.

Method A

***tert*-Butyl (*Z*)-2-*p*-Nitrobenzyloxycarbonylmethoxyimino-3-oxobutyrate (3)**——*p*-Nitrobenzyl chloroacetate (231 g, 1.0 mol) and K_2CO_3 (166.8 g, 1.2 mol) were added to a solution of **2** (226 g, 1.2 mol) in EtOAc (565 ml) and dimethylformamide (DMF) (340 ml) at room temperature. After being stirred at 50 °C for 5 h, the mixture was poured into a mixture of EtOAc (1 l) and H_2O (1 l). The EtOAc layer was collected, H_2O (1 l) was added to it, and the mixture was acidified to pH 3 with 10% HCl. The EtOAc layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was crystallized from diisopropyl ether to give 208.4 g (54.5%) of **3**. mp 81—82 °C. *Anal.* Calcd for $C_{17}H_{20}N_2O_8$: C, 53.68; H, 5.30; N, 7.37. Found: C, 53.72; H, 5.02; N, 7.31.

(*Z*)-2-*p*-Nitrobenzyloxycarbonylmethoxyimino-3-oxobutyric Acid (4)——TFA (240 ml) was added to a suspension of **3** (120 g, 0.315 mol) in anisole (120 ml) under ice-cooling. After being stirred at room temperature for 2 h, the resultant solution was evaporated *in vacuo*. The residue was crystallized from hexane–diisopropyl ether (1 : 1) to give 94.8 g (92.7%) of **4**. mp 104—108 °C. *Anal.* Calcd for $C_{13}H_{12}N_2O_8$: C, 48.16: H, 3.73; N, 8.64. Found: C, 48.25; H, 3.75; N, 8.69.

4-Chloro-2-*p*-nitrobenzyloxycarbonylmethoxyimino-3-oxobutyric Acid (5*Z* and 5*E*)——Sulfuryl chloride (90 ml) was added dropwise to a solution of **4** (90 g, 0.278 mol) in AcOH (270 ml) at 50—55 °C. The mixture was stirred at the same temperature for 5 h, and evaporated *in vacuo*. The residue was poured into H_2O and extracted with CH_2Cl_2. H_2O was added to the CH_2Cl_2 layer, and the mixture was adjusted to pH 6.5 with 20% K_2CO_3 solution. The resultant precipitate was collected by filtration to give the potassium salt of **5*E***. The aqueous layer of the filtrate was acidified to pH 1 with concentrated HCl, and extracted with EtOAc. The EtOAc layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was crystallized from diisopropyl ether to afford 28.11 g (28.2%) of **5*Z***. mp 82—84 °C. *Anal.* Calcd for $C_{13}H_{11}ClN_2O_8 \cdot H_2O$: C, 41.45; H, 3.48; Cl, 9.41; N, 7.44. Found: C, 41.07; H, 3.48; Cl, 9.60; N, 7.37.

The potassium salt of **5***E* was suspended in EtOAc and H_2O, and the mixture was acidified to pH 1 with concentrated HCl. The separated EtOAc layer was washed with brine, dried ($MgSO_4$), and evaporated to give 12.63 g (12.7%) of **5***E*. mp 114—117 °C. *Anal.* Calcd for $C_{13}H_{11}ClN_2O_8$: C, 43.53; H, 3.09; Cl, 9.88; N, 7.81. Found: C, 43.55; H, 3.13; Cl, 9.80; N, 7.89.

5*Z* and **5***E* were also obtained from **3** in a manner similar to that described above for the synthesis of **5***Z* and **5***E* from **4** and sulfuryl chloride.

(*Z*)-2-(2-Amino-4-thiazolyl)-2-*p*-nitrobenzyloxycarbonylmethoxyiminoacetic Acid (6*Z*)——Thiourea (0.228 g, 3.0 mmol) was added to a solution of **5***Z* (0.359 g, 1.0 mmol) in *N*,*N*-dimethylacetamide (4 ml) at room temperature, and the mixture was stirred at the same temperature for 1 h. Then H_2O was added, and the resulting precipitate was collected by filtration to give 0.35 g (92.1%) of **6***Z*. mp 156—158 °C. *Anal.* Calcd for $C_{14}H_{12}N_4O_7S \cdot H_2O$: C, 42.21; H, 3.54; N, 14.06; S, 8.05. Found: C, 42.23; H, 3.63; N, 14.00; S, 7.84.

(*E*)-2-(2-Amino-4-thiazolyl)-2-*p*-nitrobenzyloxycarbonylmethoxyiminoacetic Acid (6*E*)——Thiourea (0.228 g, 3.0 mmol) was added to a solution of **5***E* (0.359 g, 1.0 mmol) in *N*,*N*-dimethylacetamide (4 ml) at room temperature. After being stirred at 40 °C for 3 h, the mixture was added to H_2O, and the resulting precipitate was collected by filtration to give 0.32 g (84.2%) of **6***E* mp 162—164 °C. *Anal.* Calcd for $C_{14}H_{12}N_4O_7S \cdot H_2O$: C, 42.21; H, 3.54; N, 14.06; S, 8.05. Found: C, 42.25; H, 3.83; N, 13.86; S, 8.24.

^{1}H-NMR and IR spectral data for **3**—**6** are listed in Tables I and II.

Diphenylmethyl 7β-[(*E*)-4-Chloro-2-*p*-nitrobenzyloxycarbonylmethoxyimino-3-oxobutyramido]-3-vinyl-3-cephem-4-carboxylate (8)——A mixture of DMF (0.78 ml, 10 mmol) and $POCl_3$ (0.92 ml, 10 mmol) in EtOAc (20 ml) was stirred under ice-cooling for 30 min to prepare Vilsmeier reagent. **5***E* (3.0 g, 8.4 mmol) was added to the above mixture at 0—5 °C, and the mixture was stirred at the same temperature for 30 min to produce an activated acid solution. This activated acid solution was added to a solution of **7** (2.99 g, 7.0 mmol) and *N*-(trimethylsilyl)acetamide (3.66 g, 27.9 mmol) in tetrahydrofuran (THF) (30 ml) at −30 °C, and the reaction mixture was stirred at −20—−30 °C for 30 min. The resultant mixture was poured into a mixture of EtOAc and H_2O, and adjusted to pH 7 with 5% $NaHCO_3$ solution. The separated EtOAc layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to give 4.1 g (80.2%) of **8** as an amorphous powder. mp 63—64 °C (dec.). IR (Nujol): 1770, 1740, 1715, 1670 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.62 and 3.93 (2H, ABq, J=18 Hz, C_2-H_2), 4.77 (2H, s, $ClCH_2$), 5.08 (2H, s, CH_2CO_2), 5.27 (1H, d, J=5 Hz, C_6-H), 5.32 (1H, d, J=11 Hz, $\underset{H}{\overset{H}{>}}C=C\overset{H}{\underset{H}{<}}$), 5.40 (2H, s, CO_2CH_2), 5.67 (1H, d, J=16 Hz, $\underset{H}{\overset{H}{>}}C=C\overset{H}{\underset{H}{<}}$), 5.78 (1H, dd, J=8, 5 Hz, C_7-H), 6.82 (1H, dd, J=16, 11 Hz, $-C\underline{H}=CH_2$), 6.98 (1H, s, $C\underline{H}Ph_2$), 7.22—7.62 (10H, m, Ph_2), 7.70 and 8.27 (4H, A_2B_2q, J=9 Hz, aromatic H), 9.62 (1H, d, J=8 Hz, CONH).

Diphenylmethyl 7β-[(*E*)-2-(2-Amino-4-thiazolyl)-2-*p*-nitrobenzyloxycarbonylmethoxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylate (9)——Thiourea (1.09 g, 14.3 mmol) was added to a solution of **8** (3.5 g, 4.8 mmol) in *N*,*N*-dimethylacetamide (17.5 ml) at room temperature. After being stirred at the same temperature for 12 h, the mixture was poured into a mixture of EtOAc and H_2O, and adjusted to pH 7 with 5% $NaHCO_3$ solution. The separated EtOAc layer was washed with H_2O and brine, dried ($MgSO_4$), and evaporated *in vacuo* to give 2.72 g (75.4%) of **9** as an amorphous powder. mp 107—110 °C (dec.). IR (Nujol): 1760, 1750, 1715, 1670 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.57 and 3.90 (2H, ABq, J=18 Hz, C_2-H_2), 4.96 (2H, s, OCH_2CO_2), 5.26 (1H, d, J=5 Hz, C_6-H), 5.29 (1H, d, J=11 Hz, $\underset{H}{\overset{H}{>}}C=C\overset{H}{\underset{H}{<}}$), 5.37 (2H, s, CO_2CH_2), 5.65 (1H, d, J=16 Hz, $\underset{H}{\overset{H}{>}}C=C\overset{H}{\underset{H}{<}}$), 5.92 (1H, dd, J=8, 5 Hz, C_7-H), 6.77 (1H, dd, J=16, 11 Hz, $-C\underline{H}=CH_2$), 6.97 (1H, s, $C\underline{H}Ph_2$), 7.13 (2H, br s, NH_2), 7.23—7.57 (10H, m, Ph_2), 7.60 (1H, s, thiazole C_5-H), 7.68 and 8.25 (4H, A_2B_2q, J=9 Hz, aromatic H), 9.57 (1H, d, J=8 Hz, CONH).

7β-[(*E*)-2-(2-Amino-4-thiazolyl)-2-*p*-nitrobenzyloxycarbonylmethoxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic Acid (10)——TFA (4.8 ml) was added dropwise to a suspension of **9** (2.4 g, 3.8 mmol) in CH_2Cl_2 (7.2 ml) and anisole (2.4 ml) under ice-cooling. After being stirred at the same temperature for 30 min, the mixture was added to diisopropyl ether (100 ml). The resulting precipitate was collected by filtration, and the precipitate was added to a mixture of EtOAc and H_2O. The mixture was adjusted to pH 7 with 5% $NaHCO_3$ solution, then the separated aqueous layer was acidified to pH 3 with 5% HCl, and extracted with THF–EtOAc (1 : 1). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to give 1.82 g (97.2%) of **10** as an amorphous powder. mp 132—137 °C (dec.). IR (Nujol): 1770, 1755, 1675 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.57 and 3.87 (2H, ABq, J=18 Hz, C_2-H_2), 4.99 (2H, s, OCH_2CO_2), 5.20 (1H, d, J=5 Hz, C_6-H), 5.32 (1H, d, J=11 Hz, $\underset{H}{\overset{H}{>}}C=C\overset{H}{\underset{H}{<}}$), 5.39 (2H, s, CO_2CH_2), 5.60 (1H, d, J=16 Hz, $\underset{H}{\overset{H}{>}}C=C\overset{H}{\underset{H}{<}}$), 5.81 (1H, dd, J=8, 5 Hz, C_7-H), 6.95 (1H, dd, J=16, 11 Hz, $-C\underline{H}=CH_2$), 7.63 (1H, s, thiazole C_5-H), 7.68 and 8.25 (4H, A_2B_2q, J=9 Hz, aromatic H), 9.53 (1H, d, J=8 Hz, CONH).

7β-[(*E*)-2-(2-Amino-4-thiazolyl)-2-carboxymethoxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylic Acid (11)——A suspension of **10** (0.5 g, 0.85 mmol) in H_2O (5 ml) was adjusted to pH 12.5 with 1 N NaOH solution under ice-cooling. After being stirred at the same temperature for 5 min, the mixture was adjusted to pH 6.0 with 1 N HCl, and

washed with EtOAc. The aqueous layer was subjected to column chromatography on macroporous non-ionic adsorption resin, Dianion HP-20. The desired product was eluted with H_2O, and the eluate was acidified to pH 2.2 with 1 N HCl under ice-cooling. The precipitate was collected by filtration, and dried (P_2O_5) to afford 0.239 g (62.1%) of **11** as a pale yellow solid. mp 218—225 °C (dec.). IR (Nujol): 1770, 1660 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.54 and 3.86 (2H, ABq, J=18 Hz, C_2-H_2), 4.74 (2H, s, OCH_2CO_2), 5.18 (1H, d, J=5 Hz, C_6-H), 5.32 (1H, d, J=11 Hz, $>C=C<^{H}_{\underline{H}}$), 5.61 (1H, d, J=16 Hz, $>C=C<^{\underline{H}}_{H}$), 5.79 (1H, dd, J=8, 5 Hz, C_7-H), 6.95 (1H, dd, J=16, 11 Hz, $-C\underline{H}=CH_2$), 7.11 (2H, br s, NH_2), 7.61 (1H, s, thiazole C_5-H), 9.52 (1H, d, J=8 Hz, CONH). *Anal.* Calcd for $C_{16}H_{15}N_5O_7S_2 \cdot 3H_2O$: C, 37.87; H, 4.17; N, 13.80; S, 12.63. Found: C, 38.02; H, 4.37; N, 13.97; S, 12.77.

Method B

(*E*)-2-*tert*-Butoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)acetic Acid (12*E*)——Thionyl chloride (49.1 g, 0.413 mol) was added to a mixture of **12*Z*** (34 g, 0.103 mol) in $CHCl_3$ (200 ml) at room temperature, and the resultant mixture was refluxed for 2.5 h. The reaction mixture was poured into H_2O. The mixture was adjusted to pH 7.5 with 5% $NaHCO_3$ solution, and washed with Et_2O. The separated aqueous layer was acidified to pH 2 with 10% HCl and extracted with Et_2O. The Et_2O layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to give 8.1 g (23.8%) of **12*E*** as a colorless solid. mp 171—173 (dec.). IR (Nujol): 1740 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.50 (9H, s, Bu(*t*)), 4.79 (2H, s, OCH_2CO_2), 8.20 (1H, s, thiazole C_5-H), 8.60 (1H, s, HCO). *Anal.* Calcd for $C_{12}H_{15}N_3O_6S$: C, 43.77; H, 4.59; N, 12.76; S, 9.74. Found: C, 43.80; H, 4.99; N, 12.90; S, 9.90.

Diphenylmethyl 7β-[(*E*)-2-*tert*-Butoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)acetamido]-3-vinyl-3-cephem-4-carboxylate (13)——**13** was prepared (100.0%) from **7** and **12*E*** in a manner similar to that used for the synthesis of **8**. IR (Nujol): 1770, 1730, 1680 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.49 (9H, s, Bu(*t*)), 3.56 and 3.93 (2H, ABq, J=18 Hz, C_2-H_2), 4.74 (2H, s, OCH_2CO_2), 5.24 (1H, d, J=5 Hz, C_6-H), 5.25 (1H, d, J=11 Hz, $>C=C<^{H}_{\underline{H}}$), 5.61 (1H, d, J=16 Hz, $>C=C<^{\underline{H}}_{H}$), 5.90 (1H, dd, J=8, 5 Hz, C_7-H), 6.74 (1H, dd, J=16, 11 Hz, $-C\underline{H}=CH_2$), 6.92 (1H, s, $C\underline{H}Ph_2$), 7.12—7.59 (10H, m, Ph_2), 8.10 (1H, s, thiazole C_5-H), 8.45 (1H, s, HCO), 9.50 (1H, d, J=8 Hz, CONH).

Diphenylmethyl 7β-[(*E*)-2-(2-Amino-4-thiazolyl)-2-*tert*-butoxycarbonylmethoxyiminoacetamido]-3-vinyl-3-cephem-4-carboxylate (14)——Concentrated HCl (1.8 ml, 17 mmol) was added to a mixture of **13** (5.8 g, 8.53 mmol) in MeOH (35 ml) and THF (12 ml) at room temperature, and the mixture was stirred at the same temperature for 4 h. The reaction mixture was poured into a mixture of EtOAc and saturated $NaHCO_3$ solution, then the EtOAc layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo* to give 5.1 g (91.7%) of **14**. IR (Nujol): 1760, 1730, 1670 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.50 (9H, s, Bu(*t*)), 3.59 and 3.97 (2H, ABq, J=18 Hz, C_2-H_2), 4.70 (2H, s, OCH_2CO_2), 5.27 (1H, d, J=5 Hz, C_6-H), 5.30 (1H, d, J=11 Hz, $>C=C<^{H}_{\underline{H}}$), 5.67 (1H, d, J=16 Hz, $>C=C<^{\underline{H}}_{H}$), 5.93 (1H, dd, J=8, 5 Hz, C_7-H), 6.83 (1H, dd, J=16, 11 Hz, $-C\underline{H}=CH_2$), 6.99 (1H, s, $C\underline{H}Ph_2$), 7.01—7.70 (10H, m, Ph_2), 7.13 (2H, br s, NH_2), 7.63 (1H, s, thiazole C_5-H), 9.57 (1H, d, J=8 Hz, CONH).

11 was obtained (66.2%) from **14** in a manner similar to that used for the synthesis of **10**. This compound was identical with the authentic sample obtained by method A.

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References and Notes

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