SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 3-(2-PROPENYL)-CEPHALOSPORINS. I.

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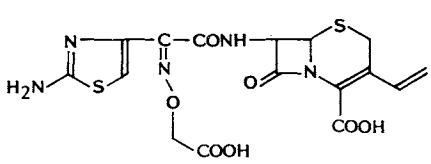
The synthesis, antibacterial activity and oral absorption of novel cephalosporins (**3a**~**3d**) having a 2-propenyl group at the C-3 position are described. Diphenylmethyl 7-amino-3-(2-propenyl)-3-cephem-4-carboxylate HCl (**4**) prepared from 7-aminocephalosporanic acid in 12 steps was acylated with various acid moieties to give cephems **3a**~**3d**. The cephems **3a**~**3c** showed similar antibacterial activities as cefixime. However, these cephems were not well absorbed orally.

Since cephaloglycin[1] was introduced as the first oral cephalosporin in 1965, other oral cephalosporins such as cephalexin[2], cefadroxil[3], cefaclor[4], cephradine[5], cefroxadine[6] have been developed for clinical use. In 1985, cefixime (**1**, FK027)[7] was prepared as a new orally active drug that showed good oral absorption. Cefixime differs in structure from the other oral cephalosporins in that it has a vinyl group in the 3-position. In 1987, BMY-28100 (**2**) was introduced, which has a (*Z*)-1-propenyl group at the C-3 position[8]. BMY-28100 reportedly shows a similar degree of antibacterial activity and oral absorption to cefixime.

In our search for new orally active cephalosporins, we prepared several novel cephems (**3**) having a 2-propenyl group at the C-3 position. This paper describes the synthesis and biological activity of 3-(2-propenyl)-cephems.

Chemistry

The synthetic route to diphenylmethyl 7-amino-3-(2-propenyl)-3-cephem-4-carboxylate hydrochloride



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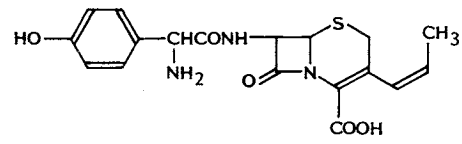

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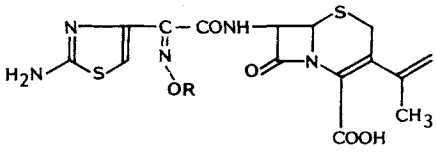
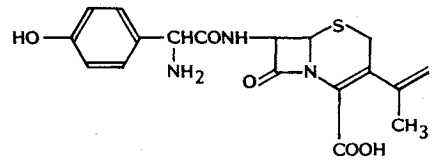


3a R=CH_3
3b R=CH_2COOH
3c R=$C(CH_3)_2COOH$



3d

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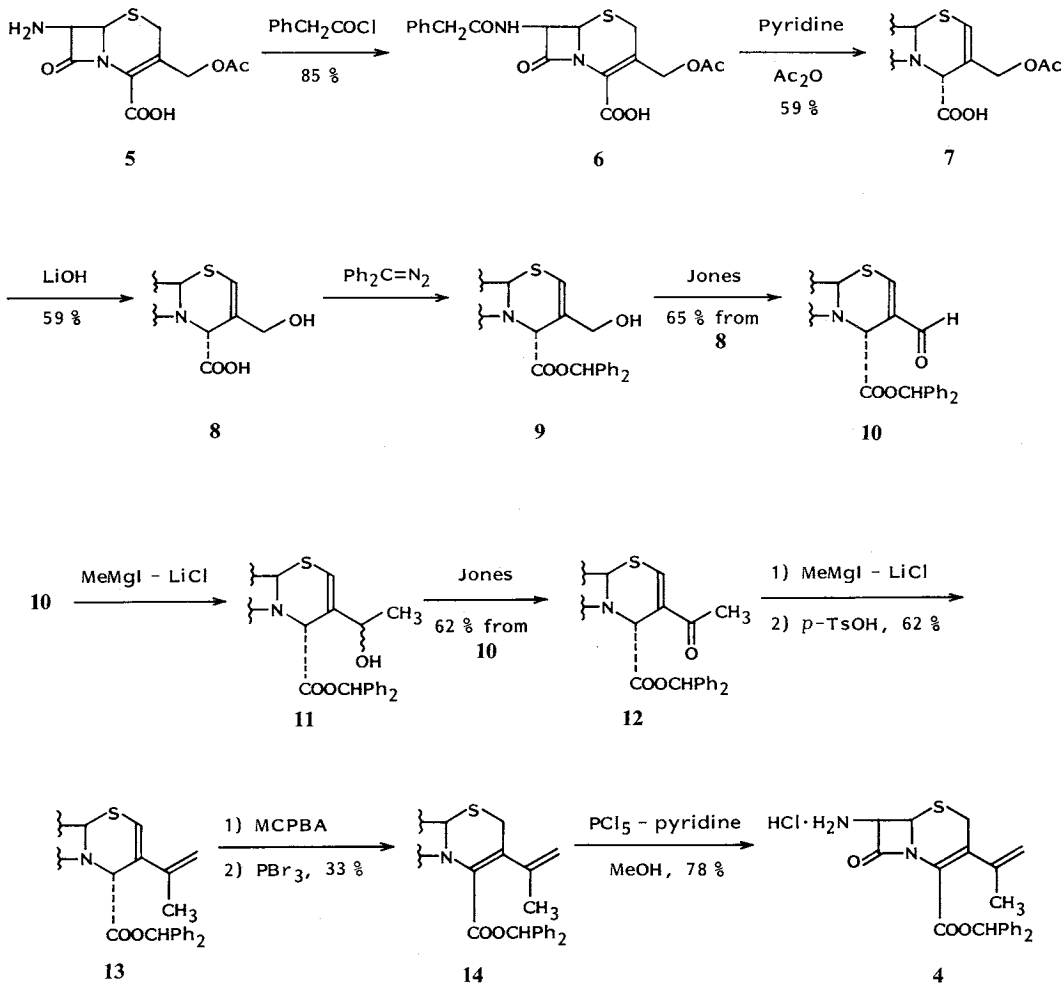
(**4**) starting from 7-aminocephalosporanic acid (**5**, 7-ACA) is shown in Scheme 1. First, the amino group of 7-ACA was protected with phenylacetyl chloride to give Δ-3-cephem (**6**) in 85% yield, which was subsequently isomerized to Δ-2-cephem (**7**) by treatment with acetic anhydride and pyridine in 59% yield[9]. The acetoxymethyl group of **7** was hydrolyzed with LiOH to give 3-hydroxymethyl-Δ-2-cephem (**8**) in 59% yield[9]. After protection of the carboxylic acid group as the diphenylmethyl ester in 76% yield, the hydroxyl group of cephem (**9**) was oxidized to the already known 3-formyl-Δ-2-cephem (**10**) in 86% yield[10]. This aldehyde **10** was converted to methyl ketone **12** by the treatment with MeMgI-LiCl in tetrahydrofuran (THF) followed by Jones oxidation of the resulting methyl carbinol (**11**) in 62% yield from **10**[11]. Then, the methyl ketone **12** was treated again with MeMgI-LiCl in THF and the resulting dimethyl carbinol was dehydrated with *p*-TsOH to give 3-(2-propenyl)cephem (**13**) in 62% yield from **12**. In a ^{1}H NMR ($CDCl_3$) spectrum signals for the vinylic protons appeared at 4.98 and 4.88 ppm as a singlet and the signal for the C-10 methyl group appeared at 1.86 ppm.

The isomerization of Δ-2-cephem (**13**) to Δ-3-cephem (**14**) was performed by a standard two-step procedure[12] using *m*-chloroperbenzoic acid oxidation (90% yield), followed by PBr_3 reduction (37% yield)

Scheme 1.



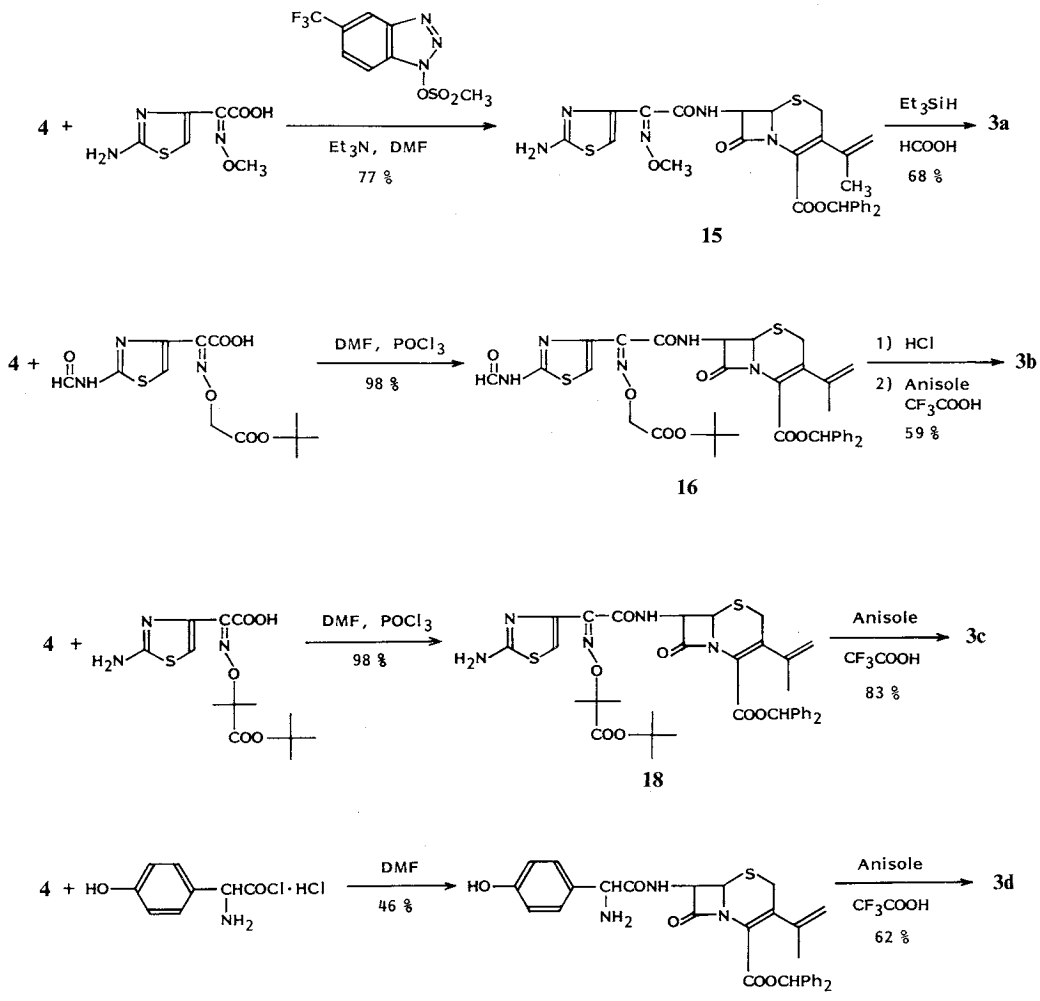
of the resulting Δ-3-cephem sulfoxide. Subsequently, deprotection of the phenylacetyl group by PCl_5-pyridine-methanol[13] gave the desired amine hydrochloride **4**, mp 170~172°C (dec) in 78% yield. In a ^1H NMR spectrum the two vinylic protons resonated at 4.74 and 4.72 ppm as a singlet and the C-10 methyl group resonated at 1.80 ppm.

The acylation of **4** is shown in Scheme 2. For the preparation of **3a**, **4** was treated with (*Z*)-2-(2-amino-4-thiazole)-2-methoxyiminoacetic acid in the presence of 1-methanesulfonyloxy-6-trifluoromethylbenzotriazole[14] to give ester **15** in 77% yield, which was then deprotected with Et_3SiH in formic acid to give the final product **3a** in 68% yield.

For the preparation of **3b**, (*Z*)-2-(2-*N*-formylamino-4-thiazole)-2-(*tert*-butoxycarbonylmethoxy)-iminoacetic acid was activated using DMF-$POCl_3$[15] and then treated with **4** to give the protected cephem **16** in 98% yield. Finally, the protected cephem was deprotected to **3b** using concentrated HCl in methanol (*N*-formyl group deprotection, 66%), followed by anisole and CF_3COOH (acid group deprotection, 89%).

For the synthesis of **3c**, **4** was allowed to react with (*Z*)-2-(2-amino-4-thiazole)-2-(*tert*-butoxycarbonyl-

Scheme 2.



1-methyl)ethoxyiminoacetic acid activated by $POCl_3$-DMF to give **18** in 98% yield, which was deprotected using anisole and CF_3COOH to afford the final cephem **3c** in 83% yield.

For the preparation of **3d**, **4** was treated with D-*p*-hydroxyphenylaminoacetyl chloride HCl salt in *N*,*N*-dimethylformamide (DMF) to give **19** in 46% yield, which was then deprotected with anisole in formic acid to give **3d** in 62% yield.

Table 1. Antibacterial activity of **3a**~**3c** in comparison to cefixime against 20 aerobic test strains (agar-dilution test).

	MIC (mg/liter)			
	3a	**3b**	**3c**	Cefixime
Staphylococcus aureus SG 511	12.500	>100.000	>100.000	25.000
S. aureus 282	25.000	>100.000	>100.000	100.000
S. aureus 503	25.000	>100.000	>100.000	50.000
Streptococcus pyogenes 308 A	0.013	0.195	0.391	0.098
S. pyogenes A77	0.013	0.195	0.391	0.098
S. faecium D	>100.000	>100.000	>100.000	>100.000
Pseudomonas aeruginosa ATCC 9027	>100.000	25.000	>100.000	>100.000
P. aeruginosa 1592E	>100.000	50.000	>100.000	50.000
P. aeruginosa 1592E	>100.000	12.000	>100.000	50.000
P. aeruginosa 1771M	3.130	0.098	0.781	0.195
Escherichia coli DC 0	1.560	1.560	1.560	0.195
E. coli DC 2	0.391	0.391	0.781	0.098
E. coli O-55	0.391	0.781	0.781	0.195
E. coli TEM	1.560	0.781	0.781	0.781
E. coli 1507E	1.560	0.781	1.560	0.391
Salmonella typhimurium	0.781	1.560	0.391	0.098
Klebsiella aerogenes 1082E	1.560	0.781	1.560	0.781
K. aerogenes 1522E	0.391	0.391	0.098	0.049
Enterobacter cloacae P99	>100.000	>100.000	>100.000	>100.000
E. cloacae 1321E	0.195	0.195	0.098	0.025

Fig. 1. Mouse pharmacokinetics, 40 mg/kg (sc).

□ Cefixime, ● **3a**, ■ **3b**, ○ **3c**.

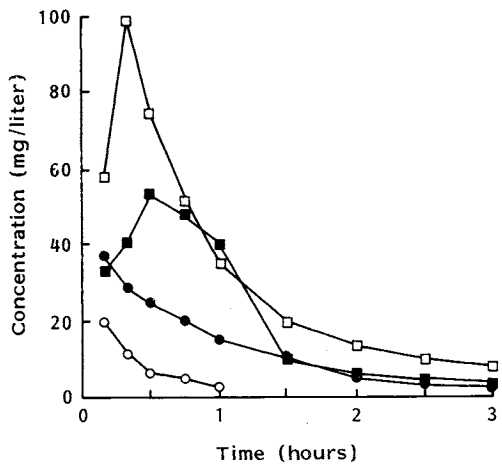
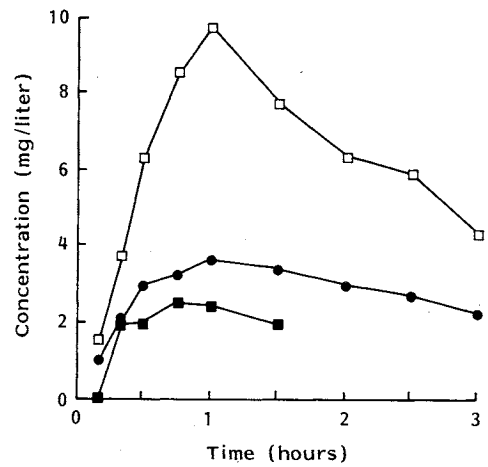


Fig. 2. Mouse pharmacokinetics, 40 mg/kg (po).

□ Cefixime, ● **3a**, ■ **3b**.



Biological Properties

The MICs of aminothiazole cephalosporins (**3a**～**3c**) prepared in the present study are shown in Table 1. The 3-(2-propenyl)cephem (**3a**) showed better activity against *Staphylococcus aureus* and *Streptococcus*

Fig. 3. Rat pharmacokinetics, 20 mg/kg (sc).

□ Cefixime, ● **3a**, ■ **3b**, ○ **3c**.

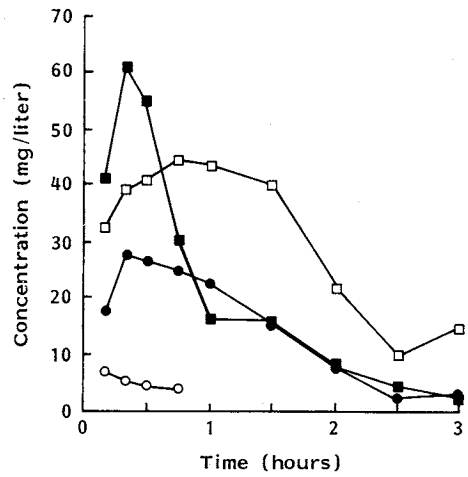


Fig. 4. Rat pharmacokinetics, 20 mg/kg (po).

□ Cefixime, ● **3a**, ■ **3b**.

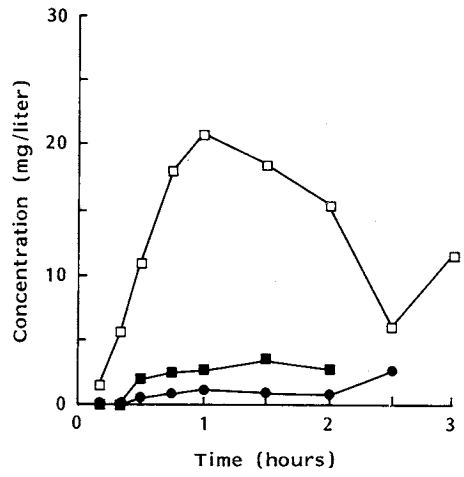


Table 2. Pharmacokinetic parameters in mice ($n=3$).

Compound		$T_{1/2}$ (hours)	Tmax (hours)	Cmax (mg/liter)	$AUC_{0\sim4\ hours}$ (mg·hour/liter)	$UR_{19\ hours}$[a] (%)
Cefixime (FK027)	sc	0.51±0.03	0.22±0.0	109.9±29.6	85.6±19.4	100
Cefixime (FK027)	po		1.97±0.24	9.3±3.6	22.4±11.1	19
3a	sc	0.64±0.10	0.12±0.12	38.7±6.9	38.8±2.7	37
3a	po		1.1±0.13	3.5±0.6	10.1±0.7	3
3b	sc	0.25±0.14	0.43±0.21	61.3±7.1	56.3±24.7	25
3b	po		0.64±0.05	2.5±0.4	6.2±0.7	2
3c	sc	0.17±0.11	0.11±0.08	24.0±7.6	8.4±2.4	4
3c	po		ND	ND	ND	3

Dose: 40 mg/kg body weight.
ND: Not determined.
[a] $UR_{19\ hours}$ is the urinary recovery during the 19 hours.

Table 3. Pharmacokinetic parameters in rats ($n=3$).

Compound		$T_{1/2}$ (hours)	Tmax (hours)	Cmax (mg/liter)	$AUC_{0\sim4\ hours}$ (mg·hour/liter)	$UR_{19\ hours}$[a] (%)
Cefixime (FK027)	sc	1.31±0.29	0.64±0.34	48.4±5.2	121.4±25.6	92
Cefixime (FK027)	po		1.20±0.33	20.6±3.3	61.9±18.3	29
3a	sc	0.63±0.27	0.47±0.23	30.7±1.6	42.2±13.5	52
3a	po		1.0±0.17	1.1±0.2	1.9±0.5	4
3b	sc	0.37±0.07	0.25±0.10	66.0±25.5	54.6±9.3	40
3b	po		0.85	3.5	4.8	5
3c	sc	0.17±0.08	0.14±0.14	7.3±5.8	5.5±3.9	7
3c	po		ND	ND	ND	ND

Dose: 20 mg/kg body weight.
ND: Not determined.
[a] $UR_{19\ hours}$ is the urinary recovery during the 19 hours.

pyogenes than cefixime, however cephems **3b** and **3c** showed slightly reduced activity. Against Gram-negative bacteria, cephems **3a**～**3c** showed similar activity as cefixime. On the other hand, cephem **3d** having a 3-hydroxyphenylglycine moiety at the 7-position showed poor activity, therefore further study was abandoned.

The semilogarithmic plots of the mean blood concentrations in mice (40 mg/kg body weight) and rats (20 mg/kg body weight) dosed orally or subcutaneously with the present cephems **3a**～**3c** and cefixime are shown in Figs. 1～4. Also, in Tables 2 and 3 are shown the blood level parameters including half-life ($T_{1/2}$), Tmax, Cmax, urinary recovery ($UR_{19\,hours}$) and area under the drug concentration-time curve (AUC) values.

As can be seen in Figs. 1～4, the blood levels of cephems **3a**～**3c** after subcutaneous or oral administration to mice or rats were much lower than those of cefixime. In mice, the blood levels of cephem **3a** (=$NOCH_3$) after oral administration were just above the detection limit of 1 mg/liter. Even cephem **3b** (=$NOCH_2COOH$) having the same C-7 side group as cefixime was poorly absorbed orally. The blood levels of cephem **3c** (=$NOC(CH_3)_2COOH$) were too low to be detected. The results in rats paralleled those in mice. Urinary recovery values for our cephems were in the range of 4～5% and 2～3%, compared to 29% and 19% for cefixime in rats and mice, respectively (Tables 2 and 3). Also, in terms of AUC and Cmax our cephems were inferior to cefixime in oral absorption.

The 3-(2-propenyl)cephems (**3a**～**3c**) showed as good antibacterial activity as cefixime, however they were not so well absorbed orally, in spite of the structural similarity between 3-(2-propenyl)cephems and 3-vinyl cephem. Therefore, further studies including the synthesis of prodrug esters will be necessary to improve enteral absorption.

Experimental

MP's were determined using a Thomas-Hoover capillary melting point apparatus and uncorrected. ^1H NMR spectra were recorded at 300 MHz on a Bruker AM-300 NMR spectrometer using TMS as an internal standard.

Column chromatography was carried out on Merck Silica gel 60 (230～400 mesh ASTM).

All *in vitro* antibacterial activity data are given as the MICs in μg/ml. MICs were determined against 20 test organisms by the 2-fold serial dilution method in Mueller-Hinton agar (Difco) after incubation at 37°C for 18 hours with a inoculum size of about 5×10^5 cfu/spot.

Urinary Recovery

Mice: NMRI albino mice weighing 20±2 g were fasted for 16 hours and orally or subcutaneously dosed with 40 mg/kg body weight of the test drugs. At certain times after dosing, blood samples were taken from a cut at the tip of the tail using 10 μl capillary tubes. Blood clotting was prevented by rinsing the tubes with 3.8% sodium citrate solution. The blood samples were stored at 4°C until further processing. Urine samples were collected over a 19-hour period after dosing.

Rats: Wister rats weighing 100±10 g received the test compounds orally or subcutaneously at 20 mg/kg. Blood and urine samples were collected and assayed as for mice.

Bioassay

Concentrations of the antibiotics in the blood and urine samples were determined microbiologically by the agar-diffusion test using Mueller-Hinton agar.

For the determination of cefixime, **3b** and **3c** the agar was seeded with *Proteus mirabilis* 112/3 as the indicating organism. In the case of **3a** the agar was supplemented with 10% sheep's blood and seeded with *S. pyogenes* A77 as the test organism. Standard solutions with urine or whole blood from rats and

mice were prepared and 10 μl of the samples and the standard solution were pipetted into the prepared holes (4 mm) in the agar plate (layer thickness 2 mm). After a diffusion period of 1 hour (blood) or 8 hours (urine) at 4°C, the agar plates were incubated for 18 hours at 37°C. The diameters of the zones of inhibition were measured. The detection limit was about 1 mg/liter for **3a** and cefixime and 3 mg/liter for **3b** and **3c**. Blood and urine concentrations were calculated by regression analysis using the standard curves in which the logarithms of the concentrations were proportional to the areas of the inhibition zones. Blood concentration—time data were approximated to a Bateman function. Curve fitting was carried out by nonlinear regression with the PHAKOK computer program. Time and extent of the curve maximum (Tmax, Cmax), elimination ($T_{1/2}$ and the AUC) were calculated.

Diphenylmethyl 7-Phenylacetamido-3-formyl-2-cephem-4-carboxylate (**10**)

10 was prepared according to the literature procedure[10]; mp 174~175°C; ^{1}H NMR ($CDCl_3$) δ 9.30 (1H, s, CHO), 9.15 (1H, d, J=8 Hz, NH), 8.20 (1H, s, C-2), 6.75 (1H, s, $CHPh_2$), 5.45 (1H, dd, J=8 and 4 Hz, C-7), 5.35 (1H, s, C-4), 5.10 (1H, d, J=4 Hz, C-4), 3.50 (2H, s, $PhCH_2$).

Diphenylmethyl 7-Phenylacetamido-3-(1-hydroxyethyl-2-cephem-4-carboxylate (**11**)

11 was prepared as a diastereomeric mixture by the addition of methyl Grignard reagent to aldehyde (**10**), as described before[10]. This alcohol was used for the next step without further purification.

Diphenylmethyl 7-Phenylacetamido-3-acetyl-2-cephem-4-carboxylate (**12**)

12 was prepared by the Jones oxidation of alcohol (**11**) in 62% purified yield after flash column chromatography (toluene-ethyl acetate, 6:1) as a white solid, mp 163~165°C; ^{1}H NMR ($CDCl_3$) δ 7.45 (1H, s, C-2), 7.20 (5H, s, Ph), 6.80 (1H, s, $CHPh_2$), 6.40 (1H, d, J=8 Hz, NH), 5.55 (1H, s, C-4), 5.40 (1H, dd, J=8 and 4 Hz), 4.95 (1H, d, J=4 Hz, C-6), 3.60 (2H, s, $PhCH_2$), 2.25 (3H, s, $COCH_3$).

Diphenylmethyl 7-Phenylacetamido-3-(2-propenyl)-2-cephem-4-carboxylate (**13**)

A mixture of 20 g (38 mmol) of methyl ketone **12** and 12.9 g (304 mmol) of LiCl in 1 liter of anhydrous THF was treated with 152 ml of 2 M methylmagnesium iodide in diethyl ether at −78°C for 5 minutes. After stirring for 30 minutes, the excess reagent was destroyed by the careful addition of 1 N HCl at −78°C. The mixture was further acidified to pH 3, then extracted with ethyl acetate. The extract was washed with $Na_2S_2O_3$ solution, dried and concentrated to give a crude dimethyl carbinol. This carbinol in 200 ml of CH_2Cl_2 was treated with a catalytic amount of *p*-toluenesulfonic acid at room temperature and the progress of the reaction was followed by TLC. After completion of the reaction, the solvent was removed and the residue was subjected to column chromatography to give 12.4 g (62%) of the product as a solid; ^{1}H NMR ($CDCl_3$) δ 6.83 (1H, s, $CHPh_2$), 6.30 (1H, s, C-2), 6.17 (1H, d, J=9 Hz, NH), 5.52 (1H, dd, J=9 and 4 Hz, C-7), 5.35 (1H, s, C-4), 5.09 (1H, d, J=4 Hz, C-6), 4.98, 4.88 (2H, s, $=CH_2$), 3.64, 3.62 (2H, ABq, J=15 Hz, $PhCH_2$), 1.86 (3H, s, CH_3).

Diphenylmethyl 7-Phenylacetamido-3-(2-propenyl)-3-cephem-4-carboxylate (**14**)

To a cooled (0°C) and well-stirred solution of 2.30 g (4.39 mmol) of Δ-2-cephem (**13**) in 20 ml of CH_2Cl_2 was added 1.23 g (30% excess) of 80% *m*-chloroperbenzoic acid in one portion. The stirring was continued for 30 minutes. Then, the mixture was diluted with 30 ml of ethyl acetate and washed with 10 ml of conc $Na_2S_2O_3$ solution. The organic phase was diluted with 50 ml of CH_2Cl_2 and again washed with 20 ml of 10% $NaHCO_3$ solution. The organic phase was dried and concentrated to give 2.13 g (90%) of the crude sulfoxide as a pale yellow solid, Rf 0.19 (toluene-ethyl acetate, 7:3, for the starting sulfide, Rf 0.69). This sulfoxide was dissolved in 30 ml of DMF and treated with 0.50 ml (1.43 g, 5.3 mmol) of PBr_3 at 0°C. The solution became cloudy. After stirring for 30 minutes, the mixture was diluted with 100 ml of water and extracted with ethyl acetate. The extract was concentrated to give 760 mg (37%) of the Δ-3-cephem as a pale yellow solid; ^{1}H NMR ($CDCl_3$) δ 6.93 (1H, s, $CHPh_2$), 6.30 (1H, s, J=9 Hz, NH), 5.82 (1H, dd, J=9 and 5 Hz, C-7), 4.96 (1H, d, J=5 Hz, C-6), 4.67, 4.65 (2H, s, $=CH_2$), 3.64, 3.60 (2H, ABq, J=16 Hz, C-2), 3.36, 3.32 (2H, ABq, J=19 Hz, $PhCH_2$), 1.73 (3H, s, CH_3).

Diphenylmethyl 7-Amino-3-(2-propenyl)-3-cephem-4-carboxylate Hydrochloride (**4**)

To a suspension of 0.62 g (2.95 mmol) of PCl_5 in 6 ml of CH_2Cl_2 was added 0.24 ml (2.97 mmol) of pyridine under ice cooling and the suspension was stirred for 1 hour. Then, 0.50 g (0.95 mmol) of cephem **14** was added. After stirring for 1.5 hours at 10°C, the mixture was cooled to −35°C, treated with 4 ml of methanol and stirred for 75 minutes below −10°C. To the resulting solution was added 0.8 ml of water and the solvent was removed. The residue was triturated with 0.2 ml of water and 2 ml of ether to give 0.33 g (78%) of the product; ^{1}H NMR ($CDCl_3$-DMSO-d_6, 1:1) δ 7.33 (10H, br s), 6.90 (1H, s, $CHPh_2$), 5.25 (1H, d, J=5 Hz), 5.12 (1H, d, J=5 Hz), 4.74, 4.72 (2H, s, $=CH_2$), 3.67, 3.50 (2H, ABq, J=18 Hz, C-2), 1.80 (3H, s); ^{13}C NMR δ 160.8, 159.5, 140.9, 139.0, 138.5, 134.5, 128.3, 128.1, 128.0, 127.8, 127.7, 127.3, 126.8, 126.5, 126.1, 122.0, 116.1, 58.1, 54.1, 27.5, 21.1.

Diphenylmethyl 7-[(*Z*)-2-(2-Amino-4-thiazole)-2-methoxyiminoacetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**15**)

A solution of 0.60 g (3.0 mmol) of (*Z*)-2-(2-amino-4-thiazole)-2-methoxyiminoacetic acid in 9 ml of DMF was treated with 0.42 ml (3.0 mmol) of Et_3N at 0°C. After 10 minutes stirring, 1.01 g (3.6 mmol) of 1-methanesulfonyloxy-6-trifluoromethylbenzotriazole was added to the above solution. The mixture was stirred for 30 minutes, treated with 0.93 g (2.1 mmol) of amine hydrochloride **4** in 9 ml of DMF and stirred for 1 hour at 0°C and for 1 hour at room temperature. Then, the whole mixture was poured into a mixture of 100 ml of water and 100 ml of ethyl acetate. The pH of the aqueous phase was adjusted to 7.5 by $NaHCO_3$ solution and the organic phase was collected. The organic phase was washed with brine, dried and concentrated to give 0.93 g (77%) after column purification (toluene-ethyl acetate, 1:2); ^{1}H NMR (acetone-d_6) δ 8.58 (1H, d, J=9 Hz, 1-H), 6.93 (1H, s, $CHPh_2$), 6.82 (1H, s), 6.02 (1H, dd, J=9 and 5 Hz, C-7), 5.26 (1H, d, J=5 Hz, C-6), 4.69 (2H, br s, $=CH_2$), 3.92 (3H, s, OCH_3), 3.64, 3.55 (2H, ABq, J=18 Hz, C-2), 3.03 (2H, br s, NH_2), 1.79 (3H, s).

7-[(*Z*)-2-(2-Amino-4-thiazole)-2-methoxyiminoacetamido]-3-(2-propenyl)-3-cephem-4-carboxylic Acid (**3a**)

A solution of 1.86 g (3.23 mmol) of diphenylmethyl ester **15** in 50 ml of formic acid was allowed to react with 1.4 ml (1.02 g, 8.77 mmol) of triethylsilane at room temperature for 3 hours. After concentration, the residue was diluted with 50 ml of methanol and the solution was again concentrated. The residue was triturated with isopropyl ether to give a solid, which was dissolved in satd $NaHCO_3$ solution. The aqueous solution was washed with ethyl acetate, acidified to pH 2 with 2 N HCl and extracted with ethy acetate. Finally, the extract was washed with brine, dried and concentrated to give 0.80 g (68%) of a solid; ^{1}H NMR (acetone-d_6) δ 7.30 (1H, s, NH), 6.80 (1H, s), 5.90 (1H, d, J=5 Hz, C-7), 5.20 (1H, d, J=5 Hz), 4.94, 4.83 (2H, s, $=CH_2$), 3.90 (3H, s, OCH_3), 3.60 (2H, s, C-2).

Diphenylmethyl 7-[(*Z*)-2-(2-*N*-Formylamino-4-thiazole)-2-(*tert*-butoxycarbonylmethoxyimino)acetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**16**)

A solution of 0.04 ml (0.52 mmol) of DMF in 2 ml of THF was allowed to react with 0.05 ml (0.54 mmol) of $POCl_3$ at −50°C for 30 minutes. Then, 130 mg (0.4 mmol) of (*Z*)-2-(2-*N*-formylamino-4-thiazole)-2-(*tert*-butoxycarbonylmethoxy)iminoacetic acid was added to the above mixture and the whole solution was stirred at −50°C for 1 hour. A suspension of 50 mg (0.11 mmol) of amine hydrochloride **4** in 4 ml of ethyl acetate was treated with 0.8 ml (3.2 mmol) of bis(trimethylsilyl)acetamide. This mixture was added to the previous solution at −20°C and the whole mixture was stirred for 1 hour. Then, 5 ml of water and 10 ml of ethyl acetate were added and the organic phase was separated, which was washed with $NaHCO_3$ solution, dried and concentrated to give 80 mg (98%) of the product as a yellow solid; ^{1}H NMR ($CDCl_3$) δ12.2 (1H, br s, *H*CONH), 8.85 (1H, d, J=9 Hz, NH), 8.48 (1H, s, HCON*H*), 7.30 (1H, s), 6.65 (1H, s, $CHPh_2$), 5.83 (1H, dd, J=9 and 5 Hz, C-7), 5.00 (1H, d, J=5 Hz, C-6), 4.55 (4H, br s, OCH_2 and $=CH_2$), 3.40 (2H, s, C-2), 1.63 (3H, s), 1.31 (9H, s, *tert*-Bu).

Diphenylmethyl 7-[(*Z*)-2-(2-Amino-4-thiazole)-2-(*tert*-butoxycarbonylmethoxyimino)acetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**17**)

A solution of 80 mg (0.11 mmol) of *N*-formyl compound **16** in 10 ml of methanol was treated with

1 ml of conc HCl for 1 hour. Then, the mixture was neutralized with $NaHCO_3$ solution, concentrated and extracted with ethyl acetate. The extract was washed with brine, dried and concentrated to give 50 mg (66%) of the product.

7-[(*Z*)-2-(2-Amino-4-thiazole)-2-(carboxymethoxyimino)acetamido]-3-(2-propenyl)-3-cephem-4-carboxylic Acid (**3b**)

A solution of 50 mg (0.072 mmol) of ester **17** in 20 ml of CH_2Cl_2 was treated with 1 ml of anisole and 1 ml of TFA at 0°C for 1 hour. The solvent was removed and the residue was triturated with isopropyl ether to give 30 mg (89%) of the product; ^{1}H NMR (DMSO-d_6) δ 9.50 (1H, s, NH), 6.75 (1H, s), 5.75 (1H, dd, J=9 and 5 Hz, C-7), 5.15 (1H, d, J=5 Hz, C-6), 4.80 (2H, br s, $=CH_2$), 4.55 (2H, s, OCH_2), 3.50 (2H, ABq, C-2), 1.85 (3H, s)

Diphenylmethyl 7-[(*Z*)-2-(2-Amino-4-thiazole)-2-(*tert*-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**18**)

A solution of 0.04 ml (0.52 mmol) of DMF in 2ml of THF was allowed to react with 0.05 ml (0.54 mmol) of $POCl_3$ at -5°C for 30 minutes. Then, 130 mg (0.4 mmol) of (*Z*)-2-(2-amino-4-thiazole)-2-(*tert*-butoxycarbonyl-1-methyl)ethoxyiminoacetic acid was added to the above solution and the whole solution was stirred for 1 hour. A suspension of 50 mg (0.113 mmol) of amine hydrochloride **4** in 4 ml of ethyl acetate was treated with 0.8 ml (3.2 mmol) of bis(trimethylsilyl)acetamide. This mixture was added to the previous solution at -20°C. After 1 hour 5 ml of water and 5 ml of ethyl acetate was added. The organic phase was separated, washed with $NaHCO_3$ solution, dried and concentrated to give 80 mg (98%) of the product as a solid, mp 118~120°C; ^{1}H NMR ($CDCl_3$) δ 8.90 (1H, d, J=9 Hz, NH), 8.70 (2H, s, NH_2), 6.90 (1H, s), 6.05 (1H, dd, J=9 and 5 Hz, C-7), 5.15 (1H, d, J=5 Hz, C-6), 4.80 (2H, br s, $=CH_2$), 3.65 (2H, ABq, C-2), 1.60 (6H, s, $2CH_3$), 1.40 (9H, s, *tert*-Bu).

7-[(*Z*)-2-(2-Amino-4-thiazole)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(2-propenyl)-3-cephem-4-carboxylic Acid (**3c**)

3c was prepared by the deprotection of ester **18** using anisole and CF_3COOH in CH_2Cl_2 in 83% yield; ^{1}H NMR (DMSO-d_6) δ 9.60 (1H, d, J=9 Hz, NH), 6.60 (1H, s), 5.70 (1H, dd, J=9 and 5 Hz, C-7), 5.20 (1H, d, J=5 Hz, C-6), 4.70 (2H, s, $=CH_2$), 3.50 (2H, ABq, C-2), 1.80 (3H, s), 1.40 (6H, s).

Diphenylmethyl 7-[D-α-(*N*-*tert*-Butoxycarbonylamino)-α-(4-hydroxyphenyl)acetamido]-3-(2-propenyl)-3-cephem-4-carboxylate (**19**)

A solution of 155 mg (0.35 mmol) of amine hydrochloride **4** in 2ml of DMF was allowed to react with 87 mg (0.39 mmol) of D-*p*-hydroxyphenylaminoacetyl chloride HCl salt at 0°C for 1 hour. The solvent was evaporated and the residue was dissolved in 2 ml of water and 15 ml of ethyl acetate. The pH was adjusted to 6.5~7.0 with satd $NaHCO_3$ and the organic phase was separated. The aqueous phase was extracted with 10 ml of ethyl acetate. The combined organic phase was washed with brine, dried and concentrated to give 95 mg (46%) of the product after column purification (toluene-ethyl acetate, 1 : 1); ^{1}H NMR ($CDCl_3$) δ 7.11 (2H, d, J=8 Hz), 6.95 (1H, s, $CHPh_2$), 6.67 (2H, d, J=8 Hz), 5.78 (1H, dd, J=9 and 5 Hz, C-7), 5.13 (1H, br s), 4.94 (1H, d, J=5 Hz, C-6), 4.66, 4.64 (2H, s, $=CH_2$), 3.31 (2H, s, C-2), 1.72 (3H, s), 1.43 (9H, s, *tert*-Bu).

7-[D-α-Amino-α-(4-hydroxyphenyl)acetamido]-3-(2-propenyl)-3-cephem-4-carboxylic Acid (**3d**)

A solution of 150 mg (0.25 mmol) of ester **19** in 4 ml of formic acid and 0.1 ml of anisole was stirred at room temperature for 3 hours, then concentrated. The residue was dissolved in 5 ml of methanol and again concentrated. The residue was dissolved in ethyl acetate and extracted with 10% $NaHCO_3$ solution. The bicarbonate extract was acidified to pH 2 with 1 N HCl and extracted with ethyl acetate. The extract was concentrated and the residue was triturated with ether to give 60 mg (62%) of the product.

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