Structure-activity Relationships of Cephalosporins Having a (Dimethylisoxazolidinio)vinyl Moiety at Their 3-Position

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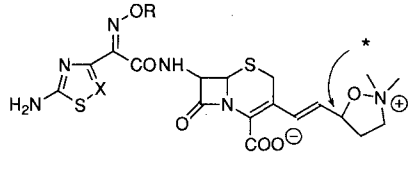
A series of cephalosporins having a (dimethylisoxazolidinio)vinyl group at their 3-position were synthesized to investigate their structure-activity relationships. With regard to the olefin geometry, the (*E*)-vinyl compound exhibited higher *in vitro* activity than the (*Z*)-compound. Regarding the C-7 substituents, the replacement of 2-aminothiazole with 5-amino-1,2,4-thiadiazole increased the anti-pseudomonal activity. Determination of the absolute configuration of the C-3 substituent is also presented. Among the compounds synthesized, we selected 7β-[(*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetamido]-3-[(*E*)-2-((*S*)-2,2-dimethyl-5-isoxazolidinio)vinyl]-3-cephem-4-carboxylate (YM-40220), which showed well-balanced *in vitro* activity and an excellent *in vivo* efficacy against *Staphylococcus aureus* Smith, as a candidate for further development.

In our preceding paper[1], we reported a cephem **1** (YM-32825) bearing a (dimethylisoxazolidinio)vinyl substituent at its 3-position (Fig. 1) which showed good *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria, except *Enterococcus faecalis* and *Pseudomonas aeruginosa*, and excellent *in vivo* efficacy against *Staphylococcus aureus*. In our continuous efforts to prepare highly potent cephalosporins which retain strong *in vivo* activity against *S. aureus* and possess a favorable broader spectrum of activity, we attempted the structural modifications of **1** (Scheme 1). Thus, the C-7 substituents as well as the olefin geometry of the C-3 side chain (*i.e.*, (*E*) or (*Z*)) were altered. Here we wish to report the structure-activity relationships of those compounds. Also the stereochemistry of an isoxazolidine ring was investigated regarding the most potent compound, **2d-II** (YM-40220).

Chemistry

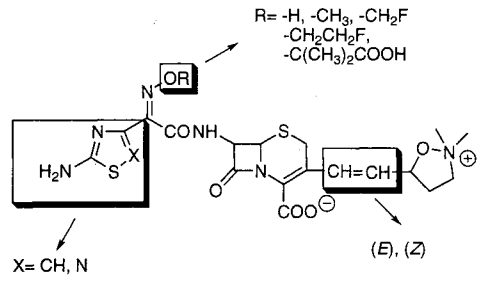
Preparation of the 7-acyl variants (**2a**, **b'**, **c**$\sim$**e**) is portrayed in Scheme 2. These compounds were synthesized using the corresponding carboxylic acids (**3a**$\sim$**e**) by a method similar to that described for **1**[1]. Acids **3a**$\sim$**e** were coupled with *p*-methoxybenzyl 7-amino-3-chloromethylcephalosporinate *via* acyl chloride. The resulting **5a**$\sim$**e** were converted to alcohols **6a**$\sim$**e** under Pd(0)-catalyzed cross-coupling conditions[2]. Elimination was achieved by conversion of the hydoxy group into phosphate and subsequent treatment with diisopropylethylamine. Cycloaddition of a nitrone with 7-acylamino-3-(1,3-butadienyl)cephems (**8a**$\sim$**e**) proceeded regiospeci-

Fig. 1. Structure of YM-32825 (**1**) and YM-40220 (**2d-II**).

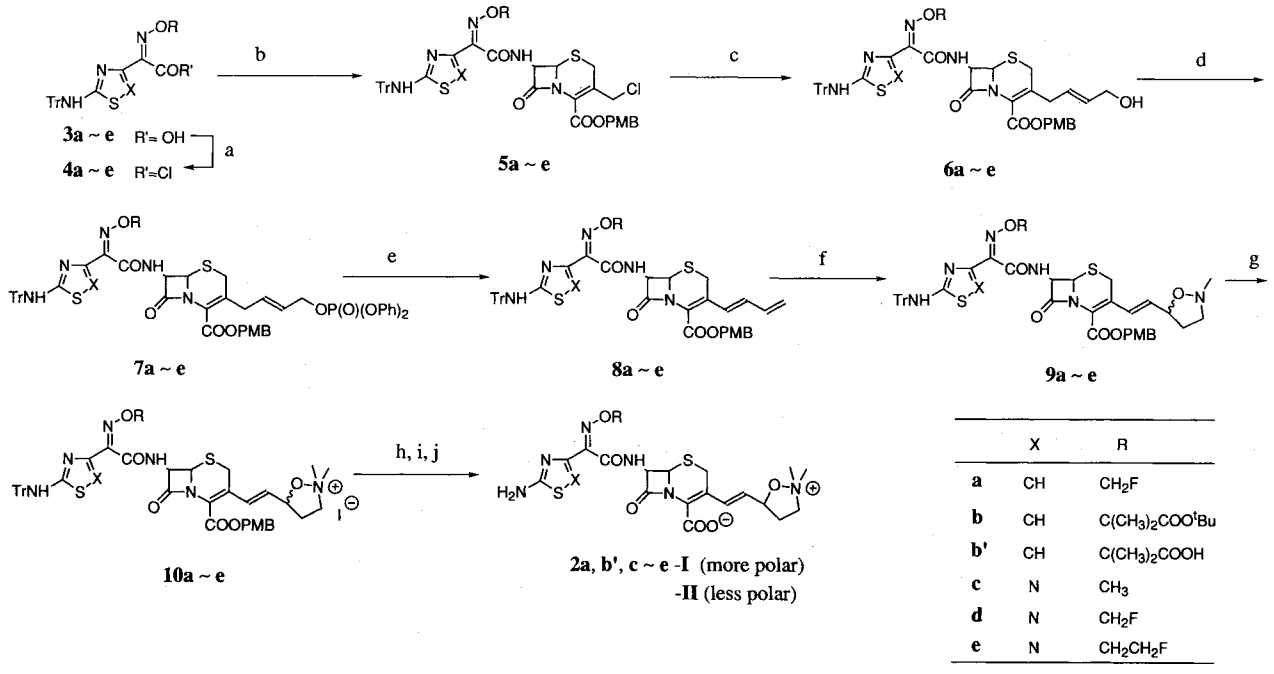


		R	X	* Configuration
1	(YM-32825)	CH_3	CH	not determined
2d-II	(YM-40220)	CH_2F	N	(*S*)

Scheme 1. Structural modification of **1**.

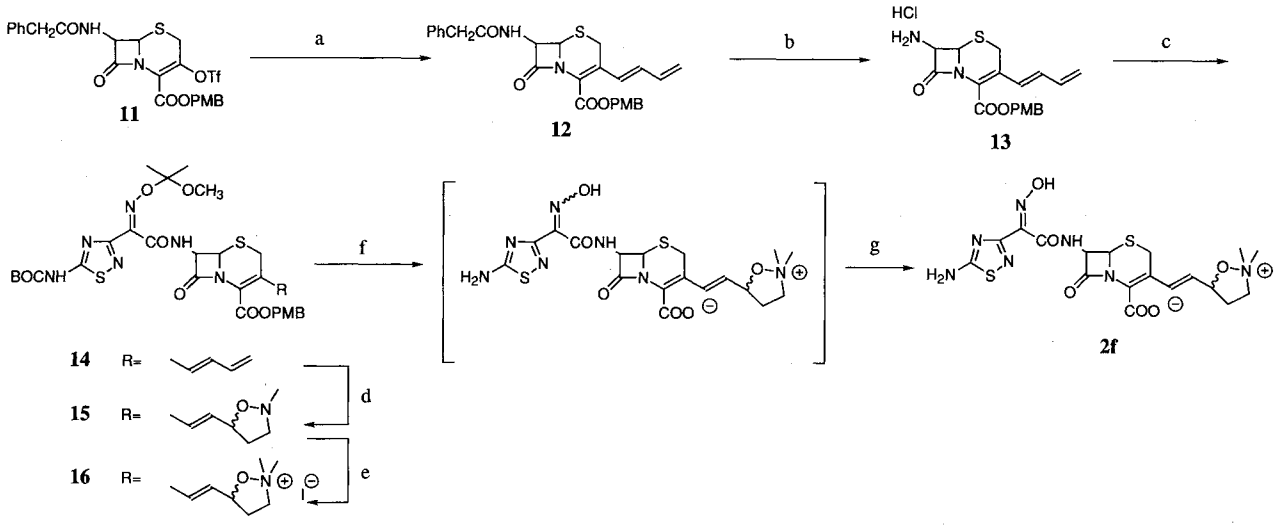


Scheme 2. Synthesis of cephalosporins from 3-chloromethyl derivatives (**5a**~**5e**).



(a) PCl_5; (b) *p*-Methoxybenzyl 7-amino-3-chloromethylcephalosporinate; (c) (*E*)-nBu$_3$SnCH=CHCH$_2$OH, Pd(dba)$_2$, tri(2-furyl)phosphine, CH_3CN; (d) P(O) $(OPh)_2Cl$, DMAP; (e) iPr$_2$NEt, 4~25°C; (f) CH_3NHOH HCl, 38% aq. HCHO, AcONa; (g) CH_3I, DMF; (h) TFA, $PhOCH_3$; (i) TFA, H_2O; (j) preparative HPLC. Tr=trityl, PMB=*p*-methoxybenzyl.

Scheme 3. Synthesis of cephalosporins from a 3-triflate (**11**).



(a) nBu$_3$SnCH=CHCH=CH$_2$, Pd(dba)$_2$, $ZnCl_2$; (b) 1) PCl_5-Pyr 2) MeOH 3) H_2O; (c) 2-(5-*tert*-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-methoxy-1-methylethoxyimino)acetyl chloride, BSA, Pyr; (d) CH_3NHOH HCl, 38% aq. HCHO, AcONa; (e) CH_3I, DMF; (f) TFA, $PhOCH_3$; (g) preparative HPLC. Tf=trifluoromethanesulfonyl, BOC=*tert*-butoxycarbonyl.

fically. After quaternization by methyl iodide, trityl (Tr) and *p*-methoxybenzyl (PMB) groups were removed using TFA/H_2O and TFA/anisole systems. Each diastereomer was separated by preparative HPLC: More polar, (**2a**, **b'**, **c**, **d** and **e**)-**I**; less polar, (**2a**, **b'**, **c**, **d** and **e**)-**II**.

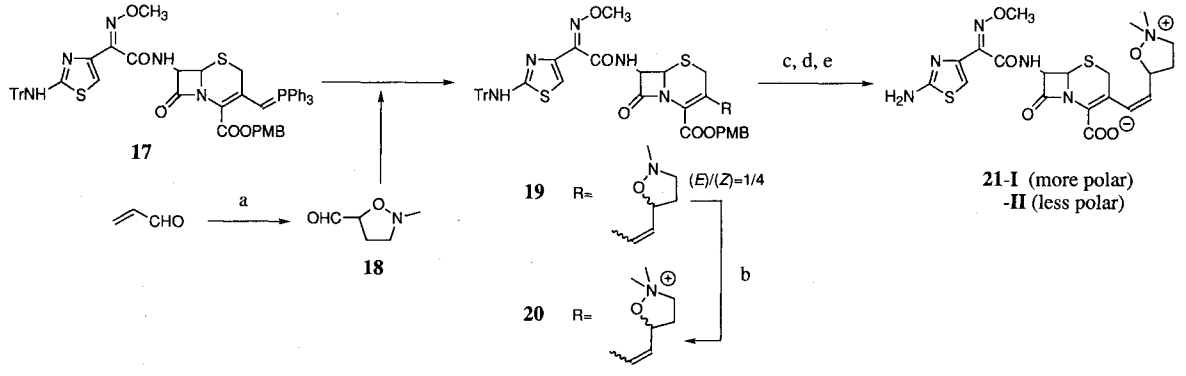
An alternative approach from commercially available *p*-methoxybenzyl 3-hydroxy-7-phenylacetylcephalosporinate is depicted in Scheme 3. The 3-hydroxy cephem was converted to the known triflate **11**[3], which was subjected to Pd(0)-catalyzed coupling reaction with

tributyl(1,3-butadienyl)stannane[4] in the presence of $ZnCl_2$[2]. Acyl exchange was achieved by deacylation of the 7-acylamino group *via* an imino chloride, followed by reacylation with a protected hydroxyimino carboxylic acid by the standard procedure. The butadienyl intermediate **14** was converted to the final product using a set of conditions similar to that in Scheme 2: 1,3-Dipolarcycloaddition, quaternization and removal of *t*-butoxycarbonyl (BOC), PMB, and 1-methyl-1-methoxyethyl groups. During the deprotection process, isomerization of a hydroxime group occurred. The final ratio was (*E*):(*Z*)=1:1 by HPLC analysis. The (*E*)-compound **2f** was isolated by preparative HPLC as an inseparable mixture of two diastereomers at the C-5'.

Synthesis of a geometric isomer at the C-3 is outlined in Scheme 4. 2-Methyl-5-isoxazolidiniocarbaldehyde was reacted with a cephem ylide to afford an (*E*), (*Z*) mixture of 3-vinyl cephems **19**. Treatment of **19** with methyl iodide and removal of the protective groups gave a mixture of crude ammonio cephems, which were separated by preparative HPLC (more polar: **21-I**, less polar: **21-II**).

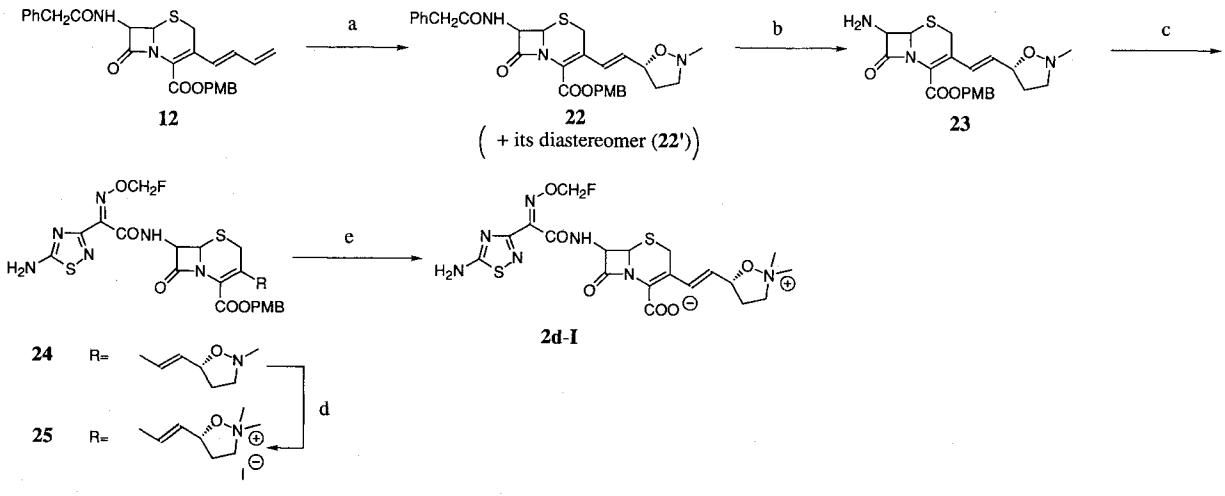
Finally, the absolute configuration of the C-3 substituent was investigated (Scheme 5). During the study, we noticed that one of the diastereomers was preferentially procured by recrystallization when the C-7 substituent was phenylacetyl. Thus, [3+2] cycloaddition of **12** gave a mixture of adducts **22** and **22'** in the ratio of *ca.* 1 to 1. Repeated recrystallizations from $CHCl_3$-MeOH provided **22**, of which the absolute configuration was established to be (*R*) by X-ray crystallography. For a correlation study, a twice-recrystallized sample (**22**:**22'**=9:1 by HPLC) was used. Deacylation by the imino chloride method gave amine

Scheme 4. Synthesis of **21-I** and **-II**.



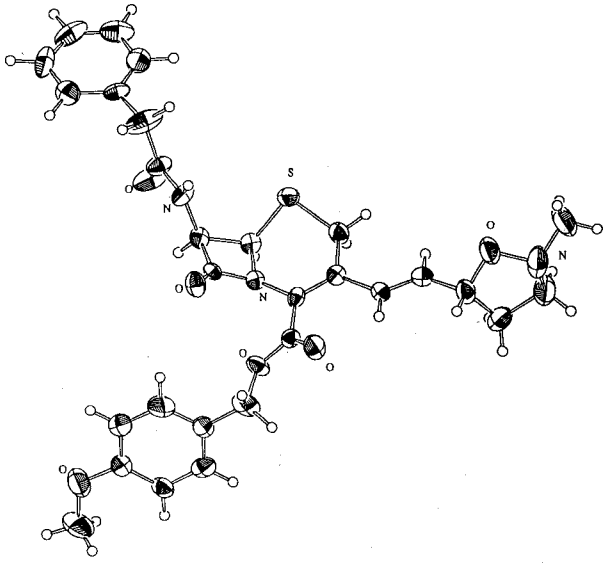
(a) CH_3NHOH HCl, 38% aq. HCHO, AcONa; (b) CH_3I, DMF; (c) TFA, $PhOCH_3$; (d) TFA, H_2O; (e) preparative HPLC.

Scheme 5. Synthesis of **2d-I**.



(a) CH_3NHOH HCl, 38% aq. HCHO, AcONa; (b) 1) PCl_5-Pyr, 2) MeOH, 3) H_2O; (c) 2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetic acid, DCC-HOBT; (d) CH_3I, DMF; (e) TFA, $PhOCH_3$, 0°C.

Fig. 2. Crystalline structure of **22**.

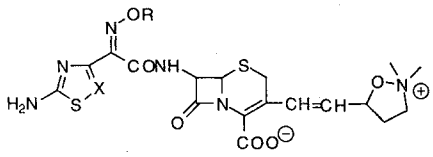


23. To avoid epimerization during the deprotection process, **23** was reacylated to **24** with 2-(5-*amino*-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetic acid by the HOBT-DCC method. Compound **24** was quaternized and the PMB group was removed using TFA/anisole below 0°C. HPLC analysis revealed that the crude product consisted of **2d-I** and **2d-II** in a ratio of 9:1. Thus, the absolute configuration of **2d-I** was (*R*) and therefore that of **2d-II** was (*S*).

Antibacterial Activity

The *in vitro* activity of the new cephalosporins (**2a**, **b'**, **c**, **d**, **e**)-**I**, -**II** and **2f**) against selected Gram-positive and Gram-negative bacteria are summarized in Table 1. For comparison, the MIC values for cefpirome (CPR), ceftazidime (CAZ), flomoxef (FMOX) and **1** (YM-32825) are also listed. MICs were determined by the standard 2-fold serial agar dilution method in Mueller-Hinton

Table 1. Comparative activity (MIC, μg/ml) of 3-(2,2-dimethyl-5-isoxazolidinio)vinylcephem compounds.



Compound	Olefin	R	X
1	(*E*)	CH_3	CH
2a-I, -II	(*E*)	CH_2F	CH
2b'-I, -II	(*E*)	$C(CH_3)_2COOH$	CH
2c-I, -II	(*E*)	CH_2	N
2d-I, -II	(*E*)	CH_2F	N
2e-I, -II	(*E*)	CH_2CH_2F	N
2f	(*E*)	H	N
21-I, -II	(*Z*)	CH_3	CH

Organism	**1**	**2a-I**	**2a-II**	**2b'-I**	**2b'-II**	**2c-I**	**2c-II**	**2d-I**	**2d-II**
S.a.1	0.39	0.39	0.39	6.25	6.25	0.39	0.39	0.2	0.2
S.a.2	12.5	25	25	>25	>25	12.5	12.5	6.25	6.25
S.p.	0.05	0.1	0.1	1.56	1.56	0.1	0.1	0.1	0.1
E.f.	25	25	25	>25	>25	25	25	12.5	12.5
E.co.	<0.006	0.013	0.013	0.025	0.025	0.05	0.025	0.025	0.013
C.f.	0.39	0.39	0.39	0.78	0.78	0.39	0.2	0.2	0.2
E.cl.	0.39	0.39	0.39	0.78	0.78	0.39	0.39	0.39	0.2
S.m.	0.78	1.56	0.78	1.56	0.78	3.13	1.56	1.56	0.78
P.a.1	>25	>25	>25	25	25	12.5	12.5	12.5	12.5
P.a.2	6.25	6.25	12.5	6.25	6.25	1.56	3.13	0.78	1.56

Organism	**2e-I**	**2e-II**	**2f**	**21-I**	**21-II**	CPR	CAZ	FMOX
S.a.1	0.39	0.2	0.2	0.78	1.56	0.39	6.25	0.39
S.a.1	12.5	6.25	6.25	>25	>25	25	100	6.25
S.p.	0.05	0.05	0.1	0.2	0.2	0.05	0.78	0.2
E.f.	12.5	12.5	3.13	>25	>25	25	>100	>100
E.co.	0.05	0.025	0.78	0.05	0.05	0.013	0.025	0.1
C.f.	0.39	0.39	1.56	1.56	1.56	0.78	12.5	12.5
E.cl.	0.39	0.39	3.13	1.56	3.13	0.78	50	>100
S.m.	3.13	1.56	>25	1.56	3.13	0.78	0.78	50
P.a.1	12.5	12.5	>25	>25	>25	50	50	>100
P.a.2	1.56	1.56	12.5	12.5	6.25	1.56	0.78	>100

Abbreviations: *S.a.1*, *Staphylococcus aureus* FDA209P JC-1; *S.a.2*, *S. aureus* CAY01-4; *S.p.*, *Streptococcus pyogenes* Cook; *E.f.*, *Enterococcus faecalis* CAY104; *E.co.*, *Escherichia coli* NIHJ; *C.f.*, *Citrobacter freundii* CAY717; *E.cl.*, *Enterobacter cloacae* CAY3207; *S.m.*, *Serratia marcescens* CAY6430; *P.a.1*, *Pseudomonas aeruginosa* ATCC 8689; *P.a.2*, *P. aeruginosa* IID5.142; CPR, cefpirome; CAZ, ceftazidime; FMOX, flomoxef.

agar after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml.

Modification of the oxime substituents in the 2-aminothiazole series did not improve the anti-pseudomonal activity of YM-32825. Introduction of an acidic 1-carboxy-1-methylethoxy group resulted in lower activity against Gram-positive and even Gram-negative bacteria (**2b'-I** or **-II** *vs.* **1**). There was no meaningful difference in activity between methoxime and fluoromethoxime (**2a-I** or **-II** *vs.* **1**).

Comparing the two geometric isomers (**21-I** or **-II** *vs.* **1**), the (*Z*)-isomer was less potent than the (*E*)-isomer against both Gram-positive and Gram-negative bacteria.

Improvement of the anti-pseudomonal activity was accomplished by replacing a 2-aminothiazolyl moiety with a 5-amino-1,2,4-thiadiazolyl moiety (**2c-I** or **-II** *vs.* **1**, and **2d-I** or **-II** *vs.* **2a-I** or **-II**). Especially, **2d-I** exhibited somewhat higher anti-pseudomonal activity than that of CAZ. In the 5-amino-1,2,4-thiadiazolyl series, substitutions with methoxy, fluoromethoxy and fluoroethoxy groups showed favorable activity. Among them, a fluoromethoxyiminocephem displayed the most well-balanced spectrum activity over a wide range of Gram-positive and Gram-negative bacteria. The hydroxyimino cephem (**2f**) was the most potent against Gram-positive bacteria including *E. faecalis* among the compounds tested. However, its activity against Gram-negative bacteria was modest.

The *in vivo* efficacy of **2d-I** and **2d-II** against systemic infections by *S. aureus* Smith is shown in Table 2. As seen in the table, they maintained excellent *in vivo* efficacy as observed in YM-32825. Especially, **2d-II** was 7.2 times more potent than CPR with an ED_{50} value of 0.054 mg/kg.

From the data mentioned above, we selected **2d-II** (YM-40220), which has an (*S*) configuration at the 5-position of an isoxazoline ring, as a candidate for further development.

Table 2. *In vivo* antibacterial activity of **2d-I** and **2d-II** against a systemic infection in mice induced by *S. aureus* Smith.

Drug	MIC (μg/ml)[a]	ED_{50} (mg/kg)[b]	ED_{50}/MIC
2d-I	0.39	0.10	0.25
2d-II	0.20	0.054	0.27
Flomoxef	0.39	0.22	0.56
Cefpirome	0.39	0.39	1.0

[a] Inoculum size: 10^6 cfu/ml.
[b] Infective challenge dose: 3.3×10^6 cfu/mouse.

Experimental

NMR spectra were recorded at 90 MHz using a JEOL FX-90Q spectrometer and at 500 MHz using a JEOL GX-500 spectrometer with tetramethylsilane as an internal standard. IR spectra were taken using a Hitachi 270-30 spectrometer. Mass spectra were obtained using a JEOL DX-300 spectrometer. For column chromatography, silica gel (Merck Kieselgel 60) was used. Preparative HPLC was performed with an SCL-10A/LC-6AD (Shimadzu) apparatus set using an ODS column (YMC ODS-A SH-343-5 S5 120A; 250 × 20 mm) at 254 nm. *p*-Methoxybenzyl 7-amino-3-chloromethylcephalosporinate and *p*-methoxybenzyl 3-hydroxy-7-phenylacetylcephalosporinate were purchased from Otsuka Chemical Co., Ltd.

Biological Evaluation

MICs were determined by the 2-fold serial agar dilution method using Mueller-Hinton agar (pH 7.2) after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml. For experimental Staphylococcal infection, septicemia was induced in male Crj: CD-1 mice (4 to 5 weeks old, n=6). Mice were intraperitoneally infected with a single 0.5 ml inoculum containing a lethal dose of *S. aureus* Smith (3.3×10^6 cfu/mouse). Test compounds were administered subcutaneously two hours after the bacterial challenge. The effective dose (ED_{50}, mg/kg) was calculated based on probit analysis from survival rates 7 days after infection.

Synthesis of the Compounds

Regarding the synthesis depicted in Scheme 2, the typical procedures for **6d**, **7d**, **8d**, **9d** and **10d** are presented. Other compounds, (**6**, **7**, **8**, **9**, **10**)-**a**, **-b**, **-c** and **-e**, were prepared similarly. Yields, typical ^{1}H NMR chemical shifts, IR and mass data of these compounds are summarized in Table 3.

p-Methoxybenzyl 7β-[(*Z*)-2-(Fluoromethoxyimino)-2-(5-tritylamino-1,2,4-thiadiazol-3-yl)acetamido]-3-[(*E*)-4-hydroxy-2-butenyl]-3-cephem-4-carboxylate (**6d**)

A stirred solution of (*E*)-3-tributylstannyl-2-propen-1-ol[5] (571 mg, 1.05 mmol), tri(2-furyl)phosphine (15 mg, 0.066 mmol) and $Pd(dba)_2$ (19 mg, 0.033 mmol) in THF (20 ml) was heated at reflux under Ar for 4 hours. The solvent was removed under reduced pressure, and the residue was dissolved in CH_3CN and washed several times with *n*-hexane. Acetonitrile was removed by evaporation and the crude product was purified by silica gel chromatography ($CHCl_3$ - MeOH, 100 : 0 ~ 100 : 2) to yield the alcohol **6d** (1.235 g, 91%): IR (KBr) cm^{-1} 1780, 1724, 1692, 1520; ^{1}H NMR (DMSO-d_6) δ 2.85 ~ 2.90 (1H, m), 3.14 ~ 3.18 (1H, m), 3.32 and 3.35 (2H, ABq, J=18 Hz), 3.75 (3H, s), 3.86 (2H, m), 5.10 ~ 5.20 (3H, m), 5.50 ~ 5.64 (2H, m), 5.73 (1H, dd, J=8 and 5 Hz), 5.76 (2H, d, J=55 Hz), 6.9 ~ 7.4 (19H, m), 9.68 (1H, d, J=8 Hz), 10.08 (1H, br s); FAB-MS (positive) *m/z* 835

Table 3. Yields and spectral data of cephem intermediates.

Compound	Yield (%)	IR[a] (β-lactam)	^{1}H NMR δ (DMSO-d_6)			FAB-MS[b] (positive)
			C2-H (ABq)	C6-H (d)	C7-H (dd)	
6a	85	1784	3.34, 3.55	5.12	5.65	834
6b	97	1790	3.35, 3.55	5.11	5.65	944
6c	73	1778	3.30, 3.54	5.10	5.72	817
6e	67	1780	3.33, 3.64	5.11	5.73	849
7a	75	1786	3.25, 3.52	5.11	5.70	1066
7b	90	1788	3.19, 3.51	5.11	5.66	1176
7c	81	1778	3.24, 3.51	5.08	5.72	1049
7e	85	1778	3.3, 3.52	5.10	5.73	1081
8a	96	1788	3.62, 3.88	5.19	5.70	816
8b	96	1788	3.60, 3.91	5.20	5.70	926
8c	90	1778	3.58, 3.92	5.18	5.77	799
8e	93	1786	3.30, 3.89	5.18	5.79	831
9a	72	1786	3.57, 3.83	5.19	5.70	875
9b	81	1790	3.56, 3.86	5.18	5.70	985
9c	90	1784	3.53, 3.84	5.14	5.79	858
9e	76	1786	3.54, 3.84	5.15	5.79	890
10a	99	1786	3.49, 3.86	5.20	5.73	889
10b	100	1788	3.5, 3.88	5.2	5.73	999
10c	93	1784	3.48, 3.85	5.14	5.78	872
15	64	1790	3.59, 3.90	5.23	5.88	774
16	91	1788	3.63, 3.91	5.20	5.93	788

[a] KBr, cm^{-1}. [b] m/z; $(M+H)^+$ for (**6**, **7**, **8**, **9**)-**a**, -**b**, -**c**, -**e** and **15**. $(M-I)^+$ for **10**-**a**, -**b**, -**c** and **16**.

$(M+H)^+$.

p-Methoxybenzyl 3-[(*E*)-4-Diphenylphosphoryloxy-2-butenyl]-7β-[(*Z*)-2-(fluoromethoxyimino)-2-(5-tritylamino-1,2,4-thiadiazol-3-yl)acetamido]-3-cephem-4-carboxylate (**7d**)

A solution of **6d** (6.63 g, 7.94 mmol) and DMAP (1.455 g, 11.9 mmol) in CH_2Cl_2 (130 ml) was treated dropwise with diphenyl chlorophosphate (2.48 ml, 11.9 mmol) at −50°C. The cooling bath was removed and the reaction was allowed to proceed at 25°C for 1 hour. The reaction mixture was partitioned between ice-water/CH_2Cl_2. The aqueous phase was further extracted with CH_2Cl_2. The combined organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography with *n*-hexane-AcOEt (1 : 1) to afford **7d** (8.36 g, 99%): IR (KBr) cm^{-1} 1784, 1726, 1692, 1522, 1492; ^{1}H NMR (DMSO-d_6) δ 2.89~2.95 (1H, m), 3.16~3.19 (1H, m), 3.24 and 3.52 (2H, ABq, J=18 Hz), 3.73 (3H, s), 4.03 (1H, m), 4.70 (1H, m), 5.10 (1H, d, J=5 Hz), 5.13 and 5.17 (2H, ABq, J=12 Hz), 5.63~5.82 (3H, m), 5.75 (2H, d, J=55 Hz), 6.9~7.5 (29H, m), 9.69 (1H, d, J=8 Hz), 10.08 (1H, br s); FAB-MS (positive) m/z 1067 $(M+H)^+$.

p-Methoxybenzyl 3-[(*E*)-1,3-Butadienyl]-7β-[(*Z*)-2-(fluoromethoxyimino)-2-(5-tritylamino-1,2,4-thiadiazol-3-yl)acetamido]-3-cephem-4-carboxylate (**8d**)

A solution of **7d** (8.36 g, 7.83 mmol) in CH_3CN (150 ml) was treated with diisopropylethylamine (3.8 ml, 22 mmol) at 0°C. The reaction was allowed to proceed at 25°C overnight. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography with $CHCl_3$ to afford **8d** (5.42 g, 85%): IR (KBr) cm^{-1} 1780, 1698, 1520; ^{1}H NMR (DMSO-d_6) δ 3.59 and 3.90 (2H, ABq, J=18 Hz), 3.75 (3H, s), 5.1~5.3 (4H, m), 5.38 (1H, d, J=17 Hz), 5.77 (2H, d, J=55 Hz), 6.35~6.44 (1H, m), 6.65~6.76 (2H, m), 6.9~7.4 (19H, m), 9.76 (1H, d, J=8 Hz), 10.08 (1H, br s); FAB-MS (positive) m/z 817 $(M+H)^+$.

p-Methoxybenzyl 7β-[(*Z*)-2-(Fluoromethoxyimino)-2-(5-tritylamino-1,2,4-thiadiazol-3-yl)acetamido]-3-[(*E*)-2-(2-methyl-5-isoxazolidinyl)vinyl]-3-cephem-4-carboxylate (**9d**)

To a mixture of **8d** (5.42 g, 6.63 mmol), AcONa (815 mg, 9.95 mmol) and 38% aq. HCHO (1.06 ml, 13.3 mmol) in THF (30 ml) was added dropwise a solution of *N*-methylhydroxylamine hydrochloride (850 mg, 9.95 mmol) in 80% aq. EtOH. After stirring at room temperature for 0.5 hour, the mixture was heated under reflux for 3 hours, cooled, and concentrated. The residue was partitioned between water/$CHCl_3$. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. Purification of the crude product by silica gel column chromatography with $CHCl_3$-MeOH (100 : 1) gave **9d** (4.73 g, 81%): IR (KBr) cm^{-1} 1788, 1724, 1696, 1520; ^{1}H NMR (DMSO-d_6) δ 1.9 (1H, br), 2.51 (3H, br s), 2.67 (1H, br), 3.54 and 3.83 (2H, ABq, J=18 Hz), 3.75 (3H, s), 4.35 and 4.55 (1H, each br), 5.08~5.22 (3H, m), 5.77 (2H, d, J=56Hz), 5.79 (1H, m), 6.08 (1H, br), 6.64 (1H, d, J=16 Hz), 6.9~7.4 (19H, m), 9.75 (1H, d, J=8 Hz), 10.08 (1H, br s); FAB-MS (positive) m/z 876 $(M+H)^+$.

p-Methoxybenzyl 3-[(*E*)-2-(2,2-Dimethyl-5-isoxazolidinio)vinyl]-7β-[(*Z*)-2-(fluoromethoxyimino)-2-(5-tritylamino-1,2,4-thiadiazol-3-yl)acetamido]-3-cephem-4-carboxylate (**10d**)

A solution of **9d** (438 mg, 0.5 mmol) was treated with methyl iodide (355 mg, 2.5 mmol). After the solution was stirred overnight at room temperature, the volatiles were removed at the pump. The residue was triturated with Et_2O to afford **10d** (510 mg, quant.): IR (KBr) cm^{-1} 1786, 1520; ^1H NMR (DMSO-d_6) δ 2.5 (1H, m), 2.8 (1H, m), 3.46~3.65 (7H, m), 3.85 (1H, d, J=18 Hz), 4.09 (1H, m), 4.18 (1H, m), 5.11~5.25 (4H, m), 5.77 (2H, d, J=55 Hz), 5.83 (1H, m), 6.16 (1H, dd, J=16 and 8 Hz), 6.84 (1H, d, J=16 Hz), 6.9~7.4 (19H, m), 9.76 (1H, d, J=8 Hz), 10.09 (1H, br s); FAB-MS (positive) m/z 889 $(M-I)^+$.

7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetamido]-3-[(*E*)-2-((*S* or *R*)-2,2-dimethyl-5-isoxazolidinio)vinyl]-3-cephem-4-carboxylate (**2d-I, -II**)

A DMF solution of **10d** (5.25 g, 5.16 mmol) and anisole (63 ml) in CH_2Cl_2 (100 ml) was treated with TFA (160 ml). After stirring for 1 hour at room temperature, the mixture was concentrated under reduced pressure, and the residue was triturated with Et_2O. The collected solid was dissolved in TFA (150 ml) and water (150 ml) was added with an ice-bath cooling. The reaction mixture was then warmed to room temperature and stirred for 1 hour. Evaporation and trituration with Et_2O yielded a solid. This solid was treated with TFA (150 ml) and water (70 ml) once again just as above, and the crude solid was first purified using HP-20 resin. Both diastereomers were then separated by preparative HPLC (3% CH_3CN-0.01 M aq. KH_2PO_4). The desired fractions were collected, desalted using an HP-20 column, concentrated *in vacuo*, and lyophilized to afford **2d-I** (577 mg; 21%) and **-II** (659 mg; 24%). Physical data (NMR, IR, Mass) are shown in Table 4. Other compounds (**2a**, **2b′**, **2c**, **2e**, **21)-I**, **-II** and **2f** were prepared similarly.

p-Methoxybenzyl 3-[(*E*)-1,3-Butadienyl]-7β-phenylacetamido-3-cephem-4-carboxylate (**12**)

To a solution of **11** (586 mg, 1 mmol) in *N*-methylpyrrolidinone (NMP) (2 ml) was added $ZnCl_2$ (273 mg, 2 mmol), tri(2-furyl)phosphine (12 mg, 0.05 mmol) and $Pd(dba)_2$ (15 mg, 0.025 mmol). The solution was stirred for 10 minutes, and then (1,3-butadienyl)tributylstannane (377 mg, 1.1 mmol) in NMP (1.0 ml) was added. The mixture was stirred at room temperature for 4 hours, diluted with AcOEt (30 ml) and washed with brine. The solvent was removed by evaporation. The residue was dissolved in CH_3CN (80 ml) and washed several times with *n*-hexane. The acetonitrile phase was concentrated

Table 4. Yields and spectral data of new cephem derivatives.

Compound	Yield (%)	IR[a]	MS[b]	^1H NMR δ[c]							Others
				C2-H	C6-H	C7-H	CH=CH (J[d])	Isoxazolidine 3-H	Isoxazolidine 4-H	Isoxazolidine 5-H	
2a-I	33	1770	527	3.4~3.6	5.07	5.60	5.60~5.65, 7.10 (16)	4.05~4.17	2.50, 5.74	5.21	3.53, 3.56 (each 3H, s), 5.73 (2H, d, J=56 Hz), 6.91 (1H, s)
2a-II	34	1774	527	3.4~3.6	5.08	5.61	5.60~5.65, 7.10 (16)	4.17, 4.09	2.50, 2.72	5.20	3.51, 3.55 (6H, s), 5.72 (2H, d, J=56 Hz), 6.90 (1H, s)
2b′-I	19	1774	581	3.4~3.6	5.08	5.66	5.63~5.70, 7.06 (16)	4.08~4.16	2.49, 2.74	5.18	1.44, 1.46 (each 3H, s), 3.52 (6H, s), 6.73 (1H, s)
2b′-II	14	1772	581	3.4~3.5	5.12	5.69	5.59~5.65, 7.04 (15)	4.07, 4.25	2.4~2.5	5.35	1.41, 1.47 (each 3H, s), 3.53 (6H, s), 6.72 (1H, s)
2c-I	16	1770	510	3.42	5.02	5.57	5.59~5.62, 7.11 (16)	4.08, 4.16	2.53, 2.73	5.19	3.52, 3.55 (each 3H, s), 3.91 (3H, s)
2c-II	25	1770	510	3.4~3.5	5.04	5.61	5.57~5.62, 7.10 (16)	4.08, 4.16	2.53, 2.74	5.20	3.51, 3.55 (each 3H, s), 3.91 (3H, s)
2d-I	21	1772	528	3.43	5.04	5.59	5.61~5.65, 7.10 (16)	4.08, 4.16	2.52, 2.73	5.20	3.52, 3.56 (each 3H, s), 5.79 (2H, d, J=56 Hz)
2d-II	24	1770	528	3.4~3.5	5.06	5.62	5.62~5.65, 7.09 (16)	4.08, 4.17	2.51, 2.74	5.19	3.52, 3.55 (each 3H, s), 5.79 (2H, d, J=56 Hz)
2e-I	12	1770	542	3.41	5.02	5.62	5.54~5.62, 7.10 (15)	4.05, 4.10	2.50, 2.73	5.16	3.52, 3.55 (each 3H, s), 4.34, 4.42 (each 1H, m), 4.62, 4.73 (each 1H, m)
2e-II	12	1770	542	3.45	5.04	5.63	5.56~5.60, 7.10 (16)	4.04, 4.16	2.50, 2.74	5.19	3.51, 3.55 (each 3H, s), 4.34, 4.42 (each, 1H, m), 4.61, 4.73 (each 1H, m)
3f	26	1770	496	3.4~3.5	5.02	5.5~5.6	5.5~5.7, 7.10 (16)	4.07, 4.15	2.54, 2.71	5.19	3.50, 3.55 (6H, m)
21-I	9	1770	509	3.36, 3.69	5.10	5.68	5.39, 6.70 (12)	4.07, 4.20	2.55, 2.87	5.39	3.53, 3.56 (each 3H, s), 3.90 (3H, s)
21-II	12	1776	509	3.30, 3.61	5.07	5.62	5.34, 6.62 (12)	4.03, 4.18	2.50, 2.75	5.48	3.51, 3.54 (each 3H, s), 3.83 (3H, s)

[a] KBr, cm^{-1}; [b] FAB, m/z, $(M+H)^+$; [c] DMSO-d_6 except for **21-I** (DMSO-d_6+CD_3OD); [d] Hz.

and the crude product was purified by column chromatography on silica gel with $CHCl_3$-AcOEt (8:2) to yield **12** (385 mg, 79%): IR (KBr) cm^{-1} 1780, 1718, 1520, ^1H NMR (DMSO-d_6) δ 3.40 and 3.56 (2H, ABq, $J=14$ Hz), 3.62 and 3.94 (2H, ABq, $J=18$ Hz), 3.75 (3H, s), 5.15 (1H, d, $J=5$ Hz), 5.17~5.27 (3H, m), 5.39 (1H, d, $J=17$ Hz), 5.70 (1H, dd, $J=8$ and 4 Hz), 6.66~6.77 (2H, m), 6.94 (2H, d, $J=9$ Hz), 7.21~7.32 (5H, m), 7.36 (2H, d, $J=9$ Hz), 9.16 (1H, d, $J=8$ Hz); FAB-MS (positive) m/z 491 $(M+H)^+$.

p-Methoxybenzyl 7β-Amino-3-[(*E*)-1,3-butadienyl]-3-cephem-4-carboxylate Hydrochloride (**13**)

To a suspension of PCl_5 (10 g, 48 mmol) in CH_2Cl_2 (170 ml) pyridine (3.88 ml, 48 mmol) was added at 4°C, and the mixture was stirred for 1 hour. A butadienyl cephem **12** (7.81 g, 15.9 mmol) was then added by portions and stirring was continued for a further 1.5 hours at 4°C. The mixture was cooled to −72°C and treated dropwise with MeOH (64 ml). The reaction was allowed to proceed for an additional 1 hour, during which time the temperature was raised to −25°C. The mixture was poured into water (300 ml) and extracted with CH_2Cl_2. The combined extracts were washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residual oil was washed well with *n*-pentane and triturated with AcOEt-Et_2O (1:1) to afford **13** (5.08 g, 86%): ^1H NMR (DMSO-d_6) δ 3.32 (2H, br s), 4.82 (1H, d, $J=5$ Hz), 5.04 (1H, d, $J=5$ Hz), 5.2 (2H, m), 5.23 (1H, m), 5.97 (1H, d, $J=17$ Hz), 6.38 (1H, m), 6.63~6.75 (2H, m); FAB-MS (positive) m/z 372 $(M+H)^+$.

p-Methoxybenzyl 3-[(*E*)-1,3-Butadienyl]-7β-[(*Z*)-2-(5-*tert*-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-[(1-methoxy-1-methylethoxy)imino]acetamido]-3-cephem-4-carboxylate (**14**)

To a suspension of **13** (1.03 g, 4.95 mmol) in CH_2Cl_2 (20 ml) was added *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (1.12 ml), and the mixture was stirred at room temperature for 85 minutes. The resulting solution was cooled to −40°C and pyridine (1.6 ml, 20 mmol) was added. A solution of (*Z*)-2-(5-*tert*-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-methoxy-1-methylethoxyimino)acetyl chloride, prepared from the corresponding acid[6] (1.72 g, 4.73 mmol) and PCl_5 (1.03 g, 4.95 mmol) at −25°C, was subsequently added. After stirring at −40°C to −25°C for 1.5 hours, the mixture was poured into sat. NaH_2PO_4 (100 ml). The water phase was extracted with $CHCl_3$ (100 ml × 2). The combined organic fractions were dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel with AcOEt-*n*-hexane (1:1) to afford **14** (368 mg, 12%): IR (KBr) cm^{-1} 1788, 1722, 1548; ^1H NMR (DMSO-d_6) δ 1.46 (6H, s), 1.50 (9H, s), 3.17 (3H, s), 3.63 and 3.95 (2H, ABq, $J=18$ Hz), 3.76 (3H, s), 5.21 (1H, d, $J=6$ Hz), 5.23~5.27 (2H, m), 5.38 (1H, d, $J=17$ Hz), 5.88 (1H, dd, $J=8$ and 6 Hz), 6.38~6.42 (1H, m), 6.7 (1H, m), 6.94 (2H, d, $J=8$ Hz), 7.36 (2H, d, $J=8$ Hz), 9.69 (1H, d, $J=8$ Hz); FAB-MS (positive) m/z 643 $(M-C(CH_3)_2OCH_3+H)^+$.

p-Methoxybenzyl 7β-[(*Z*)-2-(5-*tert*-Butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-[(1-methoxy-1-methylethoxy)imino]acetamido]-3-[(*E*)-2-(2-methyl-5-isoxazolidinyl)vinyl]-3-cephem-4-carboxylate (**15**)

p-Methoxybenzyl 7β-[(*Z*)-2-(5-*tert*-Butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-[(1-methoxy-1-methylethoxy)imino]acetamido]-3-[(*E*)-2-(2,2-dimethyl-5-isoxazolidinio)vinyl]-3-cephem-4-carboxylate Iodide (**16**)

These compounds were prepared by a method similar to that described for **9d** and **10d**, respectively.

15: Yield 64%; IR (KBr) cm^{-1} 1790, 1722, 1520; ^1H NMR (DMSO-d_6) δ 1.46 (6H, s), 1.51 (9H, s), 1.8~2.0 (1H, m), 2.3~2.7 (3H, m), 2.5 (3H, s), 3.16 (2H, m), 3.28 and 3.31 (3H, each s), 3.58 and 3.90 (2H, ABq, $J=18$ Hz), 3.76 (3H, s), 4.2~4.6 (1H, m), 5.19~5.24 (3H, m), 5.88 (1H, dd, $J=8$ and 5 Hz), 6.1 (1H, m), 6.64 (1H, d, $J=15$ Hz), 6.94 (2H, d, $J=8$ Hz), 7.36 (2H, d, $J=8$ Hz), 9.68 (1H, m), 12.4 (1H, br s); FAB-MS (positive) m/z 774 $(M+H)^+$.

16: Yield 91%; IR (KBr) cm^{-1} 1788, 1720, 1552; ^1H NMR (DMSO-d_6) δ 1.46 (6H, s), 1.51 (9H, s), 2.5 (1H, m), 2.8 (1H, m), 3.16 (3H, s), 3.54 and 3.9 (2H, ABq, $J=18$ Hz), 3.6 (6H, m), 3.76 (3H, s), 4.0~4.3 (2H, m), 5.1~5.2 (4H, m), 5.9 (1H, m), 6.2 (1H, m), 6.84 (1H, d, $J=16$ Hz), 6.95 (2H, d, $J=8$ Hz), 7.36 (2H, d, $J=8$ Hz), 9.70 (1H, d, $J=8$ Hz); FAB-MS (positive) m/z 788 $(M-I)^+$.

7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(hydroxyimino)acetamido]-3-[(*E*)-2-((*RS*)-2,2-dimethyl-5-isoxazolidinio)vinyl]-3-cephem-4-carboxylate (**2f**)

A methylene chloride solution of compound **16** (251 mg, 0.27 mmol) and anisole (0.2 ml) was treated with TFA at room temperature for 2 hours. The mixture was concentrated under reduced pressure and triturated with Et_2O to afford the crude solid. The *syn*-isomer **2f** (35 mg, 26%) was obtained by preparative HPLC (5% CH_3CN-0.01 M aq. KH_2PO_4) as described for **2d-I**. Physical data are shown in Table 4.

2-Methyl-5-isoxazolidinecarbaldehyde (**18**)

To a mixture of acrolein (1.1 ml, 16.5 mmol), 38% aq. HCHO (2.65 ml, 33 mmol) and AcONa (2.06 g, 25 mmol) in dioxane (150 ml) was added a solution of *N*-methylhydroxylamine hydrochloride (2.10 g, 25 mmol) in 80% aq. EtOH (33.5 ml). The reaction mixture was heated under reflux for 3 hours. The mixture was then cooled to room temperature and concentrated under reduced pressure. EtOH (50 ml) was added and insoluble materials were filtered off. EtOH was removed by evaporation to obtain the crude aldehyde (4.12 g), which was used without further purification.

p-Methoxybenzyl 7β-[(*Z*)-2-(Methoxyimino)-2-(2-tritylamino-4-thiazolyl)acetamido]-3-[2-(2-methyl-5-isoxazolidinyl)vinyl]-3-cephem-4-carboxylate (**19**)

A cephem ylide, prepared[7] from 3.441 g (3 mmol) of a phosphonium salt, was treated with **18** (1.38 g, 12 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed by evaporation, and the residue was purified by silica gel column chromatography with $CHCl_3$-MeOH (99:1) to obtain **19** (517 mg, 20%): IR (KBr) cm^{-1} 1786; ^1H NMR (DMSO-d_6) δ 1.7~2.0 (1H, m), 2.3~2.7 (5H, m), 3.1 (1H, m), 3.50 (1H, m), 3.74 (3H, s), 3.80 (3H, s), 4.2~4.7 (1H, m), 5.1~5.2 (2H, m), 5.20~5.25 (1H, m), 5.48~5.57 (1H, m), 5.70 (1H, m), 6.2 (1H, m), 6.71 (1H, s), 6.9 (2H, m), 7.2~7.7 (17H, m), 8.84 (1H, s), 9.56 (1H, d, J=8 Hz); FAB-MS (positive) m/z 857 $(M+H)^+$.

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(methoxyimino)acetamido]-3-[(*Z*)-2-((*R* or *S*)-2,2-dimethyl-5-isoxazolidinio)vinyl]-3-cephem-4-carboxylate (**21-I**, **-II**)

Compound **19** (227 mg, 0.265 mmol) was transformed to a quarternary ammonio cephem **20**, which was deprotected in a fashion similar to that described for **2d-I**. Preparative HPLC was performed with 7.5% CH_3CN-0.01 M aq. KH_2PO_4. Desalting and lyophilization gave **21-I** (11 mg, 9%) amd **21-II** (16 mg, 12%). Physical data are shown in Table 4.

p-Methoxybenzyl 3-[(*E*)-2-((*R*)-2-Methyl-5-isoxazolidinyl)vinyl]-7β-phenylacetamido-3-cephem-4-carboxylate (**22**)

Compound **12** (8.9 g, 8.14 mmol) was subjected to a 1,3-dipolar cycloaddition as described for **9d**. After workup, the crude product was purified by silica gel column chromatography with $CHCl_3$-AcOEt (1:0~5:1~3:1) to yield 7.36 g of 3-(isoxazolin-5-yl)vinylcephem. In this case, some (*ca.* 16%) isomerization of a double bond (Δ3→Δ2) occurred concomitantly. The Δ2 isomer was removed by trituration with AcOEt. The pure Δ3 isomer (3.5 g) was recrystallized from $CHCl_3$-MeOH twice to yield a solid (640 mg), which was analyzed to be a 9:1 mixture of the (*R*)- and (*S*)-diastereomers by HPLC using a chiral column (Chiralcel AS (45)) with *n*-hexane-EtOH (7:3): ^1H NMR (DMSO-d_6) δ 1.8~2.0 (1H, m), 2.3~2.7 (5H, m), 3.15 (1H, br), 3.50 and 3.56 (2H, ABq, J=14 Hz), 3.57 and 3.88 (2H, ABq, J=18 Hz), 3.75 (3H, s), 4.34 and 4.59 (each 0.5H, br; conformational isomers), 5.13 (1H, d, J=4 Hz), 5.17 and 5.23 (2H, ABq, J=12 Hz), 5.69 (1H, dd, J=8 and 4 Hz), 6.08 (1H, br), 6.66 (1H, d, J=15 Hz), 6.9~7.4 (9H, m), 9.14 (1H, d, J=8 Hz); FAB-MS (positive) m/z 550 $(M+H)^+$.

p-Methoxybenzyl 7β-Amino-3-[(*E*)-2-(2-methyl-5-isoxazolidinyl)vinyl]-3-cephem-4-carboxylate (**23**)

A mixture of PCl_5 (625 mg, 3 mmol) and pyridine (0.24 ml, 3 mmol) was stirred at 0°C for 1 hour. The mixture was cooled to −20°C and 7-acylaminocephem (**22**:**22'**=9:1, 550 mg, 1 mmol) was added slowly. Stirring was continued for an additional 1 hour below 0°C. The resulting pale yellow solution was cooled to −70°C, and MeOH (4 ml) was added dropwise. The temperature was raised to −15°C in 1.5 hours and water (15 ml) was added. The mixture was partitioned between CH_2Cl_2/H_2O and the aqueous layer was made basic with Na_2CO_3 to pH 9 and extracted with AcOEt. The organic extracts were combined, washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave **23** (350 mg, 81%) as a colorless caramel: ^1H NMR (DMSO-d_6) δ 1.86 (1H, m), 2.33 (1H, br), 2.4~2.6 (3H, br s), 2.69 (1H, br), 3.14 (1H, br), 3.52 and 3.82 (2H, ABq, J=18 Hz), 3.75 (3H, s), 4.58 and 4.78 (each 0.5H, br), 4.79 (1H, d, J=4 Hz), 5.01 (1H, d, J=4 Hz), 5.14 and 5.19 (2H, ABq, J=12 Hz), 6.04 (1H, br), 6.64 (1H, d, J=15 Hz), 6.92 (2H, d, J=9 Hz), 7.35 (2H, d, J=9 Hz); FAB-MS (positive) m/z 432 $(M+H)^+$.

p-Methoxybenzyl 7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetamido]-3-[(*E*)-2-(2-methyl-5-isoxazolidinyl)vinyl]-3-cephem-4-carboxylate (**24**)

To a solution of 2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetic acid (178 mg, 0.81 mmol) in DMF (2 ml) was added DCC (184 mg, 0.89 mmol) and HOBT (120 mg, 0.89 mmol). After stirring for 15 minutes at room temperature, the mixture was added to a solution of **23** (340 mg, 0.79 mmol) in DMF (3 ml). The resulting mixture was stirred at room temperature for 14.5 hours. Insoluble materials were filtered off, and the filtrate was concentrated under reduced pressure. The residue was partitioned between AcOEt/H_2O. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel column chromatography with $CHCl_3$-MeOH (20:1) to yield **24** (406 mg, 81%): IR (KBr) cm^{-1} 1784, 1724; ^1H NMR (DMSO-d_6) δ 1.8~2.0 (1H, m), 2.34 (1H, br), 2.4~2.6 (3H, br s), 2.68 (1H, br), 3.58 and 3.86 (2H, ABq, J=18 Hz), 3.76 (3H, s), 4.35 and 4.59 (each 0.5H, br), 5.2 (3H, br s), 5.79 (2H, d, J=56 Hz), 5.86 (1H, dd, J=8 and 4 Hz), 6.09 (1H, br), 6.66 (1H, d, J=16 Hz), 6.94 (2H, d, J=8 Hz), 7.36 (2H, d, J=8 Hz), 8.21 (2H, br s), 9.81 (1H, d, J=8 Hz); FAB-MS (positive) m/z 634 $(M+H)^+$.

p-Methoxybenzyl 7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetamido]-3-[(*E*)-2-(2,2-dimethyl-5-isoxazolidinyl)vinyl]-3-cephem-4-carboxylate (**25**)

Compound **24** was methylated to an ammonio cephem intermediate as described for **10d**: IR (KBr) cm^{-1} 1780, 1720, 1520; ^1H NMR (DMSO-d_6) δ 2.58 (1H, m), 2.83 (1H, m), 3.55 (3H, s), 3.58 (3H, s), 3.62 and 3.89 (2H, ABq, J=18 Hz), 3.76 (3H, s), 4.11 (1H, m), 4.20 (1H, m), 5.17~5.28 (4H, m), 5.79 (2H, d, J=56 Hz), 5.90 (1H, dd, J=8 and 5 Hz), 6.20 (1H, dd, J=16 and 8 Hz), 6.85 (1H, d, J=16 Hz), 6.95 (2H, d, J=8 Hz), 7.36 (2H, d, J=8 Hz), 8.22 (2H, br s), 9.82 (1H, d, J=8 Hz); FAB-MS

(positive) m/z 648 $(M-I)^+$.

7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(fluoromethoxyimino)acetamido]-3-[(*E*)-2-((*R*)-2,2-dimethyl-5-isoxazolidinio)vinyl]-3-cephem-4-carboxylate (**2d-I**)

To a solution of **25** (430 mg, 0.55 mmol) and anisole (7.5 ml) in CH_2Cl_2 (10 ml) TFA (12.5 ml) was added dropwise at 0°C. The mixture was stirred for 1 hour and concentrated under reduced pressure. The residue was triturated with Et_2O to yield the crude product (342 mg) as the monotrifluoroacetate. HPLC analysis of this solid revealed that the product consisted of **2d-I** and **2d-II** in the ratio of 9 : 1.

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