SYNTHESIS AND ACTIVITY OF 3-(ISOXAZOLIN-5-YL)- AND 3-(ISOXAZOL-4-YL)CEPHALOSPORINS†

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The 1,3-dipolar cycloaddition of nitrile oxide with 3-vinylcephalosporin provided diastereomeric isomers of 3-(isoxazolin-5-yl)cephalosporin. Cycloaddition of nitrile oxide with 3-(dimethylamino-vinyl)cephalosporin gave 3-(isoxazol-4-yl)cephalosporin. These semisynthetic cephalosporins with an aminothiazole in the C-7 side chain showed moderate antibacterial activities.

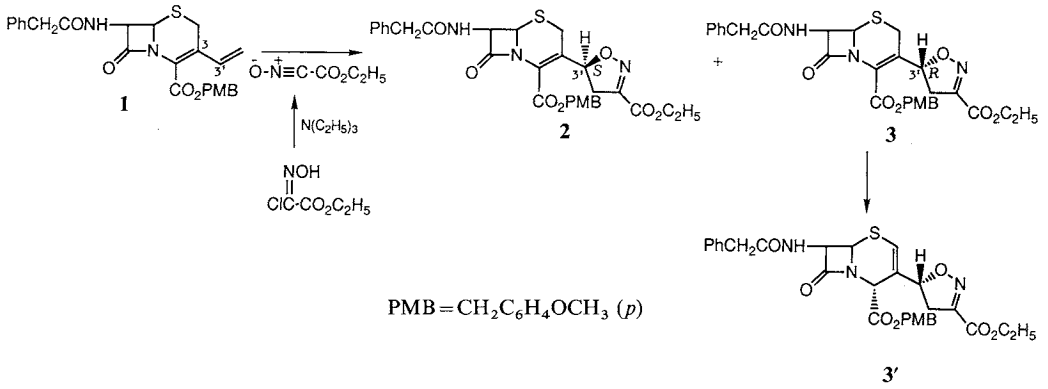
The semisynthetic cephalosporin antibiotics play a predominant role for the clinical treatment of bacterial infections. Introduction of the 2-(2-aminothiazol-4-yl)-2-(*Z*)-alkoxyiminoacetamido side chain at the C-7 position of cephem nucleus greatly enhanced the activity against Gram-negative bacteria.[1] Extensive modifications of the C-3 side chain in combination with the C-7 position have led to the highly potent antibiotics.[1] Substituents at the C-3 position strongly influence the chemical and biological reactivity of β-lactam ring. In our continuous efforts to prepare highly potent cephalosporins, we attempted to introduce a heterocyclic ring system attached directly at the C-3 position, bearing an oxygen atom at the C-3′ position of cephalosporins.[2] Few cephalosporins having 5-6 ring assemblies have been reported.[3~7] Some examples of the preparation of C-3 heterocyclic-substituted cephems by 1,3-dipolar cycloaddition were known.[3,4] These reports described the cycloaddition of 1,3-dipoles attached directly to the C-3 position of 3-cephem with dipolarophiles. We report here the synthesis and antibacterial activity of new 3-(isoxazolyl)- and 3-(isoxazolinyl)cephalosporins having 7-[2-(2-aminothiazolyl)-2-alkoxyimino)acetamido] side chain *via* 1,3-dipolar cycloaddition of 3-vinylcephem.

Chemistry

Introduction of new ring assemblies with heterocyclic ring systems at the C-3 position of cephems was achieved by the 1,3-dipolar cycloaddition reaction of nitrile oxides with 3-vinyl-3-cephem (Fig. 1). When *p*-methoxybenzyl 7-phenylacetamido-3-vinyl-3-cephem-4-carboxylate[8] (**1**) was treated with ethoxycarbonylnitrile oxide (prepared from ethyl chlorooximidoacetate and triethylamine *in situ*) in a mixture of dioxane and dichloromethane at room temperature, it was smoothly transformed into 3-[(*S*)- and (*R*)-3-ethoxycarbonyl-2-isoxazolin-5-yl]-3-cephems (**2** and **3**). The less polar isomer **2** was more soluble in ether than the more polar isomer **3**. After treatment with ether, **2** and **3** were isolated in 36% and 30% yields, respectively. Isolation of each isomer on silica gel chromatography was not suitable because of the partial isomerization of **3** to a Δ^2-cephem by silica gel. As expected, regioselectivity of the cycloaddition reaction was exclusively specific. No (isoxazolin-4-yl)cephem was detected. In ^{1}H NMR spectra, the methine proton of isoxazoline ring of **2** resonated at δ 5.89 and **3** at 6.16. Isomerization of **3** with triethyl-

† Dedicated to the late Professor HAMAO UMEZAWA on the occasion of the 30th anniversary of the Institute of Microbial Chemistry.

Fig. 1. Cycloaddition reaction with nitrile oxide.



amine in chloroform gave crystalline compound **3'**, Δ^2-isomer of **3**. X-ray crystallographic analysis of 3' confirmed the stereochemistry at the C-3' position to be *R*.† The other isomer **2**, therefore, had *S*-configuration. Thus, new cephem having isoxazoline ring at the C-3 position was constructed by 1,3-dipolar cycloaddition.

p-Methoxybenzyl 7-[2-(alkoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-vinyl-3-cephem-4-carboxylates (**4**), which were synthesized by the known method,[9] were treated with nitrile oxides in the same manner described above to afford two diastereoisomers **5** and **6** in a ratio of 1.2:1 (Fig. 2). These isomers were isolated on a silica gel chromatography. In the case of **6c** (R=CH_3), epimerization from Δ^3 to Δ^2 rapidly took place on silica gel. The treatment of **5** (and **6**) with trifluoroacetic acid (TFA) and anisole and subsequently with formic acid gave a final product **7** (and **8**). Compound **7** was also derived from **2**, whose configuration at the C-3' position as already confirmed, by removal of 7-*N*-phenylacetyl group and subsequent coupling with (aminothiazole)acetic acid.

The treatment of 3-[2-(dimethylamino)vinyl]cephalosporin[10] (**9**) with ethoxycarbonylnitrile oxide, followed by the treatment with silica gel in chloroform provided 3-(3-ethoxycarbonylisoxazol-4-yl)-3-cephem (**10a**) in 63% yield (Fig. 3). On this reaction, taking place the opposite regioselectivity of the cycloaddition, isoxazoline **12a** was formed first. ^{1}H NMR spectrum of the product showed a dimethylamino group of isoxazoline ring at δ 2.29. Compound **12a** was then transformed into isoxazole **10a** by elimination of dimethylamine. In ^{1}H NMR spectrum of **10a**, a singlet proton of isoxazole ring appeared at 8.08.[11] It indicated that the C-4 position, not C-5, of isoxazole connected to the C-3 position of cephem. Removal of blocking groups of **10a** with TFA-anisole gave isoxazolecephalosporin (**11a**). By a similar procedure, **11b** was obtained from **9** and carbamoylnitrile oxide.

Antibacterial Activity

In vitro antibacterial activities of 3-(isoxazolin-5-yl)- and 3-(isoxazol-4-yl)cephalosporins synthesized here were listed in Table 1. These compounds showed moderate activities against Gram-positive and Gram-negative bacteria. Isoxazoline derivatives (**7** and **8**) were superior to isoxazole derivatives (**11**). The configuration at the C-3' position of isoxazoline derivatives did not much affect the antibacterial activities. 3'-(*S*)-Isomers (**7**) were as active as 3'-(*R*)-isomers (**8**). A carbamoyl group on the isoxazoline ring (**7b** and

† The X-ray crystallographic analysis was carried out by Mr. YOSHIO KODAMA, Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.

Fig. 2. Synthesis of 3-(isoxazolin-5-yl)cephalosporins.

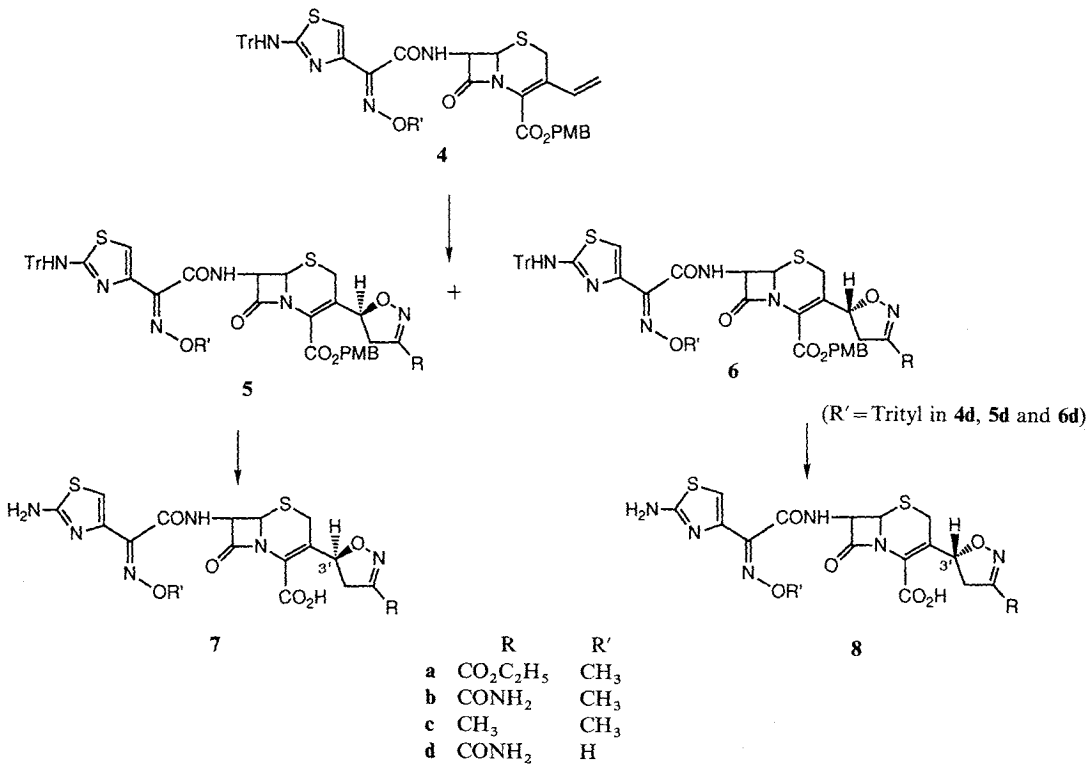


Fig. 3. Synthesis of 3-(isoxazol-4-yl)cephalosporins.

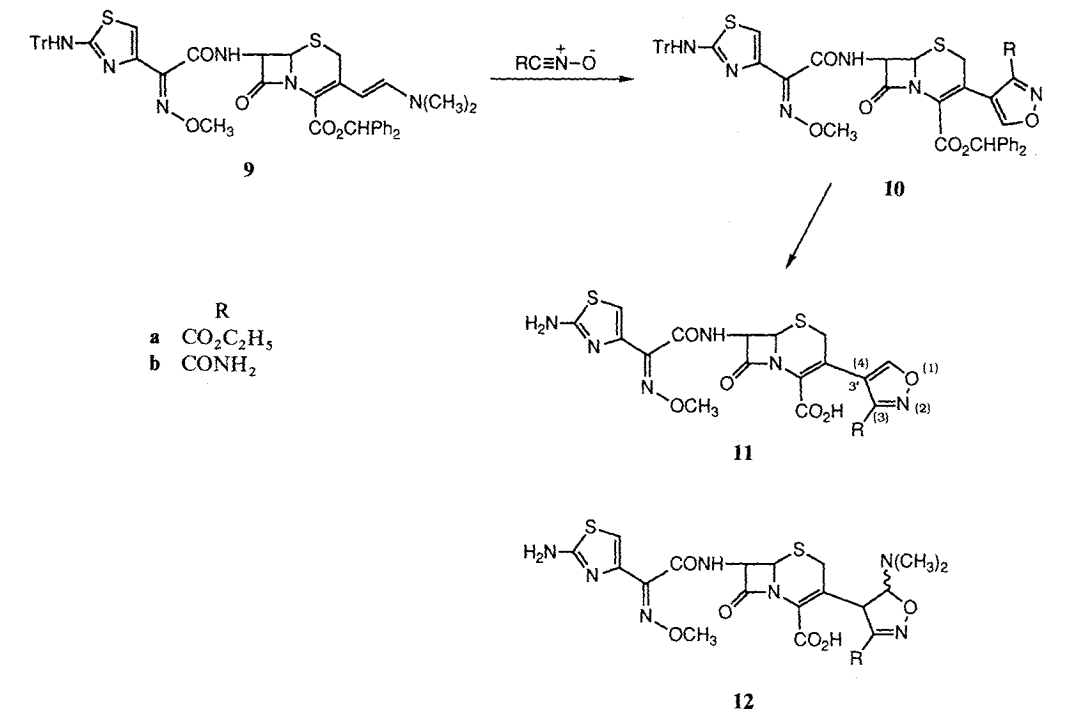


Table 1. Antibacterial activities of 3-(isoxazolin-5-yl)- and 3-(isoxazol-4-yl)cephalosporins.

Test organism	MIC (μg/ml)							
	7a	**8a**	**7b**	**8b**	**7c**	**7d**	**11a**	**11b**
Staphylococcus aureus FDA209P	6.25	6.25	6.25	3.13	12.5	0.78	6.25	6.25
S. aureus Terajima	3.13	3.13	1.56	1.56	3.13	0.20	6.25	6.25
S. aureus MS353	12.5	12.5	6.25	6.25	25	1.56	12.5	12.5
Bacillus subtilis ATCC 6633	0.39	0.39	0.39	0.39	0.78	0.10	0.78	1.56
Micrococcus luteus ATCC 9341	0.20	0.39	0.39	0.39	0.20	0.39	0.39	0.78
Escherichia coli NIHJ JC-2	0.39	0.39	0.20	0.20	1.56	0.20	3.13	3.13
E. coli K-12 C600	0.10	0.05	0.05	0.05	0.20	0.20	0.20	0.39
E. coli K-12 W3630 Rms212[a]	0.78	0.78	0.39	0.39	3.13	1.56	3.13	3.13
E. coli K-12 W3630 Rms213[a]	0.20	0.10	0.05	0.78	0.39	0.10	1.56	1.56
E. coli K-12 W3630 Rms823[a]	3.13	6.25	1.56	3.13	6.25	12.5	6.25	3.13
E. coli K-12 W3630 Rte16[a]	6.25	3.13	6.25	3.13	12.5	0.78	6.25	6.25
E. coli GN5482[b]	6.25	1.56	3.13	0.78	25	12.5	50	100
Klebsiella pneumoniae PCI602	0.10	0.05	0.025	0.025	0.20	0.20	0.025	0.10
K. oxytoca GN10560[c]	12.5	100	25	50	25	>100	12.5	3.13
Citrobacter freundii GN7391[b]	>100	100	>100	>100	>100	>100	>100	>100
Salmonella typhimurium IID971	1.56	0.39	0.78	0.20	3.13	1.56	12.5	6.25
S. typhi 901	0.78	0.39	0.39	0.10	1.56	0.39	6.25	3.13
S. paratyphi 1015	0.20	0.10	0.05	0.05	0.20	0.20	0.78	0.78
S. schottmuelleri 8006	0.10	0.025	0.05	0.025	0.20	0.10	0.20	0.20
S. enteritidis G14	0.39	0.39	0.20	0.20	1.56	0.10	6.25	3.13
Serratia marcescens IAM1184	0.78	0.20	0.78	0.10	3.13	12.5	1.56	1.56
Enterobacter cloacae 963	0.39	0.20	0.20	0.78	3.13	0.78	12.5	12.5
E. cloacae GN5795	12.5	3.13	12.5	1.56	25	>100	50	100
E. cloacae GN7471[b]	100	6.25	50	25	>100	>100	>100	>100
E. aerogenes ATCC 13048	1.56	0.78	0.39	0.20	3.13	3.13	3.13	3.13
Morganella morganii IFO3848	0.006	<0.003	0.012	<0.003	0.05	0.10	0.05	0.05
M. morganii GN5407[b]	0.78	0.20	1.56	0.05	3.13	3.13	6.25	3.13
Providencia rettgeri IFO3850	0.025	0.006	0.012	0.006	0.012	0.025	0.10	0.025
P. rettgeri GN4430[b]	0.10	0.025	0.05	0.012	0.05	0.025	0.39	1.56
Proteus vulgaris OX-19	0.39	0.39	0.78	0.39	0.20	25	0.10	0.20
P. vulgaris HX-19	0.05	0.025	0.05	0.025	0.10	3.13	0.05	0.10
P. vulgaris GN7919[c]	50	50	100	25	100	>100	25	6.25
P. mirabilis IFO3849	0.10	0.025	0.05	0.025	0.20	0.20	3.13	0.39
Pseudomonas aeruginosa IFO3345	>100	50	>100	50	>100	>100	>100	>100
P. aeruginosa NCTC10490	100	6.25	>100	6.25	>100	>100	100	>100
P. aeruginosa PAO1	>100	100	>100	100	>100	>100	>100	>100
P. aeruginosa Rms139/M1[a]	100	100	>100	50	>100	>100	100	>100
P. aeruginosa GN10362[b]	>100	>100	>100	>100	>100	>100	>100	>100

[a] Penicillinase-producing strain.
[b] Cephalosporinase-producing strain.
[c] Cefuroximase-producing strain.
MICs were determined by two-fold agar dilution method at 37°C for 18 hours using Bacto Mueller-Hinton Medium (Difco).

8b) increased antibacterial activities compared with an ethoxycarbonyl group (**7a** and **8a**) and a methyl group (**7c**).

Experimental

General

Mass spectra were measured on a JEOL JMX-SX102 mass spectrometer. ^{1}H NMR spectra were

recorded on a JEOL JNM-GX400 spectrometer. IR spectra were measured on a Hitachi I-5020 FT-IR spectrometer.

p-Methoxybenzyl 3-[(*S*)-3-(Ethoxycarbonyl)isoxazolin-5-yl]-7-phenylacetamido-3-cephem-4-carboxylate (**2**) and *p*-Methoxybenzyl 3-[(*R*)-3-(Ethoxycarbonyl)isoxazolin-5-yl]-7-phenylacetamido-3-cephem-4-carboxylate (**3**)

To a solution of **1** (93 mg) in a mixture of dioxane (1 ml) and dichloromethane (1 ml) was added ethyl chlorooximidoacetate (61 mg). To this solution was carefully added a solution of triethylamine (41 mg) in dioxane (0.5 ml). After 1 hour, the insoluble material was removed by filtration and the filtrate was concentrated. The residue was dissolved in chloroform and the solution was washed with water, dried over anhydr Na_2SO_4 and concentrated. The solid was triturated with ether. The insoluble solid was isomer **3** (35 mg) and the ethereal solution was concentrated to give **2** (42 mg). **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, J=7 Hz, CH_2CH_3), 2.98 (1H, dd, J=10 and 18 Hz, 4-Hb of isoxazoline), 3.14 (1H, d, J=19 Hz, 2-Hb), 3.61 (1H, d, J=17 Hz, $COCH_2Ph$), 3.62 (1H, d, J=19 Hz, 2-Ha), 3.63 (1H, dd, J=12 and 18 Hz, 4-Ha of isoxazoline), 3.68 (1H, d, J=17 Hz, $COCH_2Ph$), 3.80 (3H, s, OCH_3), 4.35 (2H, q, J=7 Hz, CH_2CH_3), 4.91 (1H, d, J=5 Hz, 6-H), 5.17 (2H, s, CO_2CH_2Ar), 5.85 (1H, dd, J=5 and 9 Hz, 7-H), 5.89 (1H, dd, J=10 and 12 Hz, 3'-H) and 5.95 (1H, d, J=9 Hz, CONH). IR (Nujor) cm^{-1} 1790, 1725, 1690, 1520, 1245 and 1220. **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, J=7 Hz, CH_2CH_3), 2.93 (1H, dd, J=9 and 18 Hz, 4-Hb of isoxazoline), 3.32 (1H, d, J=18 Hz, 2-Hb), 3.39 (1H, dd, J=12 and 18 Hz, 4-Ha of isoxazoline), 3.44 (1H, d, J=18 Hz, 2-Ha), 3.61 and 3.68 (each 1H, d, J=16 Hz, $COCH_2Ph$), 3.81 (3H, s, OCH_3), 4.34 (2H, q, J=7 Hz, CH_2CH_3), 4.90 (1H, d, J=5 Hz, 6-H), 5.18 and 5.21 (each 1H, d, J=12 Hz, CO_2CH_2Ar), 5.80 (1H, dd, J=5 and 9 Hz, 7-H), 6.02 (1H, br d, J=9 Hz, CONH) and 6.16 (1H, dd, J=9 and 12 Hz, 3'-H). IR (Nujor) cm^{-1} 1790, 1725, 1690, 1520, 1250 and 1210.

p-Methoxybenzyl 3-[(*R*)-3-(Ethoxycarbonyl)isoxazolin-5-yl]-7-phenylacetamido-2-cephem-4-carboxylate (**3'**)

A mixture of **3** (58 mg) and triethylamine (0.2 ml) in chloroform (2 ml) was stirred for 2 hours at room temperature. After evaporation, the obtained solid was crystallized from methanol to give **3'** (35 mg) as needles. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (3H, t, J=7.5 Hz, CH_2CH_3), 2.95 (1H, dd, J=10 and 18 Hz, 4-Hb of isoxazoline), 3.15 (1H, dd, J=11.5 and 18 Hz, 4-Ha of isoxazoline), 3.61 and 3.66 (each 1H, d, J=16Hz, $COCH_2Ar$), 3.82 (3H, s, OCH_3), 4.32 (2H, q, J=7.5 Hz, CH_2CH_3), 4.94 (1H, d, J=1.5 Hz, 4-H), 5.04 and 5.15 (each 1H, J=12 Hz, CO_2CH_2Ar), 5.21 (1H, dd, J=10 and 11.5 Hz, 3'-H), 5.23 (1H, d, J=4 Hz, 6-H), 5.61 (1H, dd, J=4 and 9 Hz, 7-H), 6.16 (1H, d, J=9 Hz, CONH) and 6.40 (1H, d, J=1.5 Hz, 2-H).

p-Methoxybenzyl 3-[(*S*)-3-(Ethoxycarbonyl)isoxazolin-5-yl]-7-[(*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**5a**) and *p*-Methoxybenzyl 3-[(*R*)-3-(Ethoxycarbonyl)isoxazolin-5-yl]-7-[(*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**6a**)

5a and **6a** from **4**: To a solution of **4** (193 mg) in a mixture of dioxane (1.5 ml) and dichloromethane (1.5 ml) was added ethyl chlorooximidoacetate (81 mg). To this solution was carefully added a solution of triethylamine (54 mg) in dioxane (0.7 ml). After 1 hour, the insoluble material was removed by filtration and the filtrate was concentrated. The residue was dissolved in chloroform and the solution was washed with water, dried over anhydr Na_2SO_4 and concentrated. The solid was chromatographed on silica gel with chloroform to give **5a** (81 mg) and **6a** (67 mg). **5a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, J=7 Hz, CH_2CH_3), 3.02 (1H, dd, J=10 and 19 Hz, 4-Hb of isoxazoline), 3.21 (1H, d, J=19 Hz, 2-Hb), 3.67 (1H, d, J=19 Hz, 2-Ha), 3.68 (1H, dd, J=12 and 19 Hz, 4-Ha of isoxazoline), 3.81 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.35 (2H, q, J=7 Hz, CH_2CH_3), 5.02 (1H, d, J=5 Hz, 6-H), 5.18 and 5.21 (each 1H, d, J=11 Hz, CO_2CH_2Ar), 5.93 (1H, dd, J=10 and 12 Hz, 3'-H), 5.95 (1H, dd, J=5 and 9 Hz, 7-H), 6.73 (1H, s, 5-H of thiazole), 6.73 (1H, d, J=9 Hz, CONH) and 7.00 (1H, br s, NH). **6a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, J=7 Hz, CH_2CH_3), 2.96 (1H, dd, J=9 and 18 Hz, 4-Hb of isoxazoline), 3.37 (1H, d, J=18.5 Hz, 2-Hb), 3.42 (1H, dd, J=12 and 18 Hz, 4-Ha of isoxazoline), 3.52 (1H, t, J=18.5 Hz, 2-Ha), 3.81 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.35 (2H, q, J=7 Hz, CH_2CH_3), 5.00 (1H, d, J=5 Hz, 6-H), 5.20

and 5.24 (each 1H, d, $J=12$ Hz, CO_2CH_2Ar), 5.91 (1H, dd, $J=5$ and 9 Hz, 7-H), 6.20 (1H, dd, $J=9$ and 12 Hz, 3'-H), 6.74 (1H, s, 5-H of thiazole), 6.82 (1H, d, $J=9$ Hz, CONH) and 7.00 (1H, br s, NH).

5a from **2**: Anhydr pyridine (48 mg) was added to a mixture of PCl_5 (125 mg) in dichloromethane (3 ml) at 0°C. After stirring at the temperature for 1 hour, compound **2** (116 mg) was added to above solution at 8°C. The stirring was continued for 1.5 hours at 8°C. The mixture was cooled to −30°C and methanol (0.8 ml) was added. After 1.5 hours below −15°C, it was diluted with dichloromethane (10 ml) and washed with satd NaCl solution. The organic layer was adjusted to pH 9 with aq $NaHCO_3$ solution, dried over anhydr Na_2SO_4 and evaporated to give 7-amino derivative (85 mg). To a solution of the 7-amino derivative (85 mg) in DMF (1 ml) were added (*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetic acid (82 mg), DCC (42 mg) and HOBT (28 mg). After stirring for 2 hours at room temperature, the solution was concentrated. The residue was dissolved in EtOAc and the solution was washed with satd NaCl, dried over Na_2SO_4 and concentrated. The solid was chromatographed on silica gel with chloroform to give **5a** (117 mg, 66%).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*S*)-3-(ethoxycarbonyl)isoxazolin-5-yl]-3-cephem-4-carboxylic Acid (**7a**)

To a solution of **5a** (60 mg) in dichloromethane (0.4 ml) were added TFA (0.2 ml) and anisole (0.1 ml) at 5°C. After 1 hour, the solution was concentrated. The residue was triturated with isopropyl ether. The obtained solid was dissolved in 50% aq formic acid (1 ml). After 1 hour at 50°C, the insoluble material was removed by filtration and the filtrate was concentrated to give a solid. It was washed with EtOAc and dissolved in water. The aq solution was adjusted to pH 7.5 with aq $NaHCO_3$ solution. After filtration, the filtrate was adjusted to pH 2 with dil HCl solution and lyophilized. The residue was extracted with chloroform - MeOH (2 : 1) and evaporated to give **7a** (22 mg). FAB-MS *m/z* 525 (MH^+). ^{1}H NMR (400 MHz, CD_3OD) δ 1.33 (3H, t, $J=7.5$ Hz, CH_2CH_3), 3.07 (1H, dd, $J=10$ and 19 Hz, 4-Hb of isoxazoline), 3.40 (1H, d, $J=18.5$ Hz, 2-Hb), 3.59 (1H, dd, $J=12$ and 19 Hz, 4-Ha of isoxazoline), 3.67 (1H, d, $J=18.5$ Hz, 2-Ha), 3.97 (3H, s, OCH_3), 4.31 (2H, q, $J=7.5$ Hz, CH_2CH_3), 5.17 (1H, d, $J=5$ Hz, 6-H), 5.87 (1H, d, $J=5$ Hz, 7-H), 5.96 (1H, dd, $J=10$ and 12 Hz, 3'-H) and 6.84 (1H, s, 5-H of thiazole). IR (KBr) cm^{-1} 3413, 1779, 1721, 1676, 1628, 1530, 1381, 1250 and 1040.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*R*)-3-(ethoxycarbonyl)isoxazolin-5-yl]-3-cephem-4-carboxylic Acid (**8a**)

Compound **8a** (25 mg) was obtained from **6a** (62 mg) in the similar method used for **7a**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.32 (3H, t, $J=7.5$ Hz, CH_2CH_3), 3.17 (1H, dd, $J=9.5$ and 18 Hz, 4-Hb of isoxazoline), 3.36 (1H, d, $J=18$ Hz, 2-Hb), 3.44 (1H, dd, $J=12$ and 18 Hz, 4-Ha of isoxazoline), 3.63 (1H, d, $J=18$ Hz, 2-Ha), 3.98 (3H, s, OCH_3), 4.31 (2H, q, $J=7.5$ Hz, CH_2CH_3), 5.17 (1H, d, $J=5$ Hz, 6-H), 5.84 (1H, d, $J=5$ Hz, 7-H), 6.18 (1H, dd, $J=9.5$ and 12 Hz, 3'-H) and 6.87 (1H, s, 5-H of thiazole). IR (KBr) cm^{-1} 3295, 2940, 1775, 1723, 1659, 1539, 1379, 1252 and 1044.

p-Methoxybenzyl 3-[(*S*)-3-Carbamoylisoxazolin-5-yl]-7-[(*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**5b**) and *p*-Methoxybenzyl 3-[(*R*)-3-Carbamoylisoxazolin-5-yl]-7-[(*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**6b**)

To a solution of **4** (518 mg) in a mixture of dioxane (4.5 ml) and dichloromethane (3.5 ml) was added chlorooximidoacetamide (247 mg). To this solution was carefully added a solution of triethylamine (204 mg) in dioxane (2 ml). After 1 hour, the insoluble material was removed by filtration and the filtrate was concentrated. The residue was dissolved in chloroform and the solution was washed with water, dried over anhydr Na_2SO_4 and concentrated. The solid was chromatographed on silica gel with chloroform - MeOH (99 : 1) to give **5b** (179 mg) and **6b** (150 mg). **5b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.04 (1H, dd, $J=10$ and 19 Hz, 4-Hb of isoxazoline), 3.20 and 3.65 (each 1H, d, $J=19$ Hz, 2-H), 3.67 (1H, dd, $J=12$ and 19 Hz, 4-Ha of isoxazoline), 3.81 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 5.03 (1H, d, $J=5$ Hz, 6-H), 5.20 (2H, br s, CO_2CH_2Ar), 5.49 (1H, br s, one of $CONH_2$), 5.93 (1H, dd, $J=10$ and 12 Hz, 3'-H), 5.94 (1H, dd, $J=5$ and 9 Hz, 7-H), 6.47 (1H, br s, one of $CONH_2$), 6.73 (1H, s, 5-H of thiazole), 6.84 (1H, d, $J=9$ Hz, CONH) and 7.02 (1H, br s, NH). **6b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01 (1H, dd, $J=9$ and 19 Hz, 4-Hb of

isoxazoline), 3.38 (1H, d, J=19 Hz, 2-Hb), 3.42 (1H, dd, J=12 and 19 Hz, 4-Ha of isoxazoline), 3.48 (1H, d, J=19 Hz, 2-Ha), 3.81 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 5.01 (1H, d, J=5 Hz, 6-H), 5.20 and 5.24 (each 1H, d, J=12 Hz, CO_2CH_2Ar), 5.44 (1H, br s, one of $CONH_2$), 5.91 (1H, dd, J=5 and 9 Hz, 7-H), 6.19 (1H, dd, J=9 and 12 Hz, 3'-H), 6.49 (1H, br s, one of $CONH_2$), 6.74 (1H, br s, 5-H of thiazole), 6.84 (1H, d, J=9 Hz, CONH) and 7.01 (1H, br s, NH).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*S*)-3-carbamoylisoxazolin-5-yl]-3-cephem-4-carboxylic Acid (**7b**)

Compound **7b** sodium salt (16 mg) was obtained from **5b** (38 mg). **7b** (Na salt): FAB-MS m/z 518 (MNa^+). ^{1}H NMR (400 MHz, D_2O) δ 3.18 (1H, dd, J=9 and 18 Hz, 4-Hb of isoxazoline), 3.33 (1H, d, J=18 Hz, 2-Hb), 3.60 (1H, dd, J=11.5 and 18 Hz, 4-Ha of isoxazoline), 3.66 (1H, d, J=18 Hz, 2-Ha), 4.00 (3H, s, OCH_3), 5.28 (1H, d, J=5 Hz, 6-H), 5.81~5.88 (2H, m, 7-H and 3'-H) and 7.03 (1H, s, 5-H of thiazole).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*R*)-3-carbamoylisoxazolin-5-yl]-3-cephem-4-carboxylic Acid (**8b**)

Compound **8b** (10 mg) was obtained from **6b** (30 mg). ^{1}H NMR (400 MHz, CD_3OD) δ 3.15 (1H, dd, J=9 and 19 Hz, 4-Hb of isoxazoline), 3.40 (1H, d, J=18 Hz, 2-Hb), 3.42 (1H, dd, J=12 and 19 Hz, 4-Ha of isoxazoline), 3.62 (1H, d, J=18 Hz, 2-Ha), 3.98 (3H, s, OCH_3), 5.17 (1H, d, J=5 Hz, 6-H), 5.84 (1H, d, J=5 Hz, 7-H), 6.14 (1H, dd, J=9 and 12 Hz, 3'-H) and 6.87 (1H, s, 5-H of thiazole). IR (KBr) cm^{-1} 3146, 1773, 1680, 1406, 1204, 1134 and 1042.

p-Methoxybenzyl 3-[(*S*)-3-Methylisoxazolin-5-yl]-7-[(*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**5c**)

To a solution of **4** (308 mg) in chloroform (20 ml) was added chlorooximidoethane (468 mg). To this solution was carefully added a solution of triethylamine (505 mg) in chloroform (20 ml) in several portions for 8 hours. The solution was concentrated. The residue was dissolved in chloroform and the solution was washed with water, dried over anhydr Na_2SO_4 and concentrated. The solid was chromatographed on silica gel with chloroform to give **5c** (135 mg). The other isomer **6c** could not be isolated as a pure form because of the contamination of Δ^2. **5c**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (3H, s, CH_3), 2.76 (1H, dd, J=9 and 18 Hz, 4-Hb of isoxazoline), 3.27 (1H, d, J=19 Hz, 2-Hb), 3.39 (1H, dd, J=11.5 and 18 Hz, 4-Ha of isoxazoline), 3.69 (1H, d, J=19 Hz, 2-Ha), 3.81 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 5.01 (1H, d, J=5 Hz, 6-H), 5.16 and 5.20 (each 1H, d, J=12 Hz, CO_2CH_2Ar), 5.67 (1H, dd, J=9 and 11.5 Hz, 3'-H), 5.93 (1H, dd, J=5 and 9 Hz), 6.71 (1H, d, J=9 Hz, CONH), 6.74 (1H, br s, 5-H of thiazole) and 7.01 (1H, br s, NH).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(*S*)-3-methylisoxazolin-5-yl]-3-cephem-4-carboxylic Acid (**7c**)

Compound **7c** (45 mg) was obtained from **5c** (90 mg). FAB-MS m/z 467 (MH^+). ^{1}H NMR (400 MHz, CD_3OD) δ 1.98 (3H, s, CH_3), 2.89 (1H, dd, J=9.5 and 18 Hz, 4-Hb of isoxazoline), 3.37 (1H, dd, J=11 and 18 Hz, 4-Ha of isoxazoline), 3.37 (1H, d, J=18 Hz, 2-Hb), 3.67 (1H, d, J=18 Hz, 2-Ha), 3.97 (3H, s, OCH_3), 5.16 (1H, d, J=5 Hz, 6-H), 5.70 (1H, dd, J=9.5 and 11 Hz, 3'-H), 5.85 (1H, d, J=5 Hz, 7-H) and 6.84 (1H, s, 5-H of thiazole).

p-Methoxybenzyl 3-[(*S*)-3-Carbamoylisoxazolin-5-yl]-7-[(*Z*)-2-(trityloxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**5d**) and *p*-Methoxybenzyl 3-[(*R*)-3-Carbamoylisoxazolin-5-yl]-7-[(*Z*)-2-(trityloxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**6d**)

Compounds **5d** (72 mg) and **6d** (55 mg) were obtained from **4d** (200 mg). **5d**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 (1H, dd, J=10 and 19 Hz, 4-Hb of isoxazoline), 3.00 (1H, d, J=19 Hz, 2-Hb), 3.52 (1H, d, J=19 Hz, 2-Ha), 3.57 (1H, dd, J=12 and 19 Hz, 4-Ha of isoxazoline), 3.79 (3H, s, OCH_3), 5.00 (1H, d, J=5 Hz, 6-H), 5.21 (2H, br s, CO_2CH_2Ar), 5.84 (1H, dd, J=10 and 12 Hz, 3'-H), 5.87 (1H, br s, one of $CONH_2$), 6.06 (1H, dd, J=5 and 9 Hz, 7-H), 6.24 (1H, br s, one of $CONH_2$), 6.45 (1H, s, 5-H of thiazole), and 7.43 (1H, d, J=9 Hz, CONH). **6d**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.94 (1H, dd, J=9 and 18 Hz, 4-Hb of isoxazoline), 3.25 (2H, br s, 2-H), 3.34 (1H, dd, J=12 and 18 Hz, 4-Ha of isoxazoline), 3.80 (3H, s,

OCH_3), 4.99 (1H, d, J=5 Hz, 6-H), 5.22 (2H, br s, CO_2CH_2Ar), 5.62 (1H, br s, one of $CONH_2$), 6.02 (1H, dd, J=5 and 9 Hz, 7-H), 6.15 (1H, dd, J=9 and 12 Hz, 3'-H), 6.42 (1H, s, 5-H of thiazole).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(*S*)-3-methylisoxazolin-5-yl]-3-cephem-4-carboxylic Acid (**7d**)

Compound **7d** sodium salt (15 mg) was obtained from **5d** (60 mg). FAB-MS m/z 504 (MNa^+). ^1H NMR (400 MHz, D_2O) δ 3.18 (1H, dd, J=9.5 and 18.5 Hz, 4-Hb of isoxazoline), 3.33 (1H, d, J=18 Hz, 2-Hb), 3.59 (1H, dd, J=11.5 and 18.5 Hz, 4-Ha of isoxazoline), 3.65 (1H, d, J=18 Hz, 2-Ha), 5.29 (1H, d, J=5 Hz, 6-H), 5.84 (1H, dd, J=9.5 and 11.5 Hz, 3'-H), 5.87 (1H, d, J=5 Hz, 7-H) and 7.01 (1H, s, 5-H of thiazole).

Diphenylmethyl 3-(3-Ethoxycarbonylisoxazol-4-yl)-7-[(*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**10a**)

To a solution of (dimethylamino)vinylcephalosporin (**9**) (451 mg) in a mixture of dioxane (4 ml) and dichloromethane (4 ml) was added ethyl chlorooximidoacetate (159 mg). To this solution was carefully added a solution of triethylamine (106 mg) in dioxane (1.5 ml). After 1 hour, the solution was concentrated. The residue was washed with hexane. ^1H NMR spectrum indicated that the residue contained mainly **12a** [(400 MHz, $CDCl_3$) δ 2.29 (6H, s, $N(CH_3)_2$), 5.02 (1H, d, J=4 Hz, 4-H of isoxazoline) and 5.32 (1H, d, J=4 Hz, 5-H of isoxazoline)] along with **10a**. To a solution of the residue in chloroform (40 ml) was added silica gel (8 g). After stirring 3 hours, silica gel was removed by filtration and the filtrate was concentrated. The solid was chromatographed on silica gel with chloroform to give **10a** (305 mg, 63%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.37 (3H, t, J=7 Hz, CH_2CH_3), 3.54 (1H, d, J=18.5 Hz, 2-Hb), 3.64 (1H, d, J=18.5 Hz, 2-Ha), 4.09 (3H, s, OCH_3), 4.35 (2H, q, J=7 Hz, CH_2CH_3), 5.18 (1H, d, J=5 Hz, 6-H), 6.04 (1H, dd, J=5 and 9 Hz, 7-H), 6.80 (1H, d, J=9 Hz, CONH) and 8.08 (1H, s, 5-H of isoxazole).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(3-ethoxycarbonylisoxazol-4-yl)-3-cephem-4-carboxylic Acid (**11a**)

Compound **10a** (126 mg) was deblocked with TFA (0.7 ml) and anisole (0.3 ml) by a usual method to give **11a** (35 mg). FAB-MS 523 (MH^+). ^1H NMR (400 MHz, CD_3OD) δ 1.35 (3H, t, J=7 Hz, CH_2CH_3), 3.65 (1H, d, J=18.5 Hz, 2-Hb), 3.81 (1H, d, J=18.5 Hz, 2-Ha), 4.04 (3H, s, OCH_3), 5.27 (1H, d, J=5 Hz, 6-H), 5.94 (1H, d, J=5 Hz, 7-H), 6.99 (1H, s, 5-H of thiazole) and 8.81 (1H, s, 5-H of isoxazole).

Diphenylmethyl 3-(3-Carbamoylisoxazol-4-yl)-7-[(*Z*)-2-(methoxyimino)-2-(2-tritylaminothiazol-4-yl)acetamido]-3-cephem-4-carboxylate (**10b**)

Compound **10b** (298 mg) was obtained from **9** (483 mg) and chlorooximidoacetamide (123 mg) and triethylamine (101 mg). ^1H NMR (400 MHz, $CDCl_3$) δ 3.64 (1H, d, J=18.5 Hz, 2-Hb), 3.70 (1H, d, J=18.5 Hz, 2-Ha), 4.07 (3H, s, OCH_3), 5.18 (1H, d, J=5 Hz, 6-H), 5.52 (1H, br s, one of $CONH_2$), 6.02 (1H, dd, J=5 and 9 Hz, 7-H), 6.46 (1H, br s, one of $CONH_2$), 6.90 (1H, d, J=9 Hz, CONH) and 8.04 (1H, s, 5-H of isoxazole).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(3-carbamoylisoxazol-4-yl)-3-cephem-4-carboxylic Acid (**11b**)

Compound **11b** (28 mg) was obtained from **10b** (90 mg). FAB-MS 494 (MH^+). ^1H NMR (400 MHz, CD_3OD) δ 3.72 (1H, d, J=18.5 Hz, 2-Hb), 3.79 (1H, d, J=18.5 Hz, 2-Ha), 4.07 (3H, s, OCH_3), 5.27 (1H, d, J=5 Hz, 6-H), 5.93 (1H, d, J=5 Hz, 7-H), 7.08 (1H, s, 5-H of thiazole) and 8.72 (1H, s, 5-H of isoxazole).

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