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Semisynthetic β-Lactam Antibiotics. IV. Synthesis and Antibacterial Activity of New Ureidocephalosporin and Ureidocephamycin Derivatives Containing a Catechol Moiety or Its Acetate

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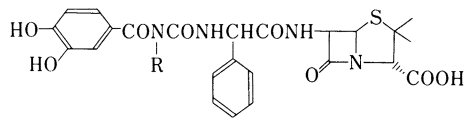
New ureidocephalosporin and ureidocephamycin derivatives containing a catechol moiety or its acetate were prepared and their minimum inhibitory concentration values against various microorganisms were determined. Among these compounds, the ureidocephalosporins (**2**, **3Aa**, **3Ba**) and ureidocephamycins (**4**, **5**) carrying a methyl group on the nitrogen atom of the ureido bond showed strong activities against *Pseudomonas aeruginosa*. 7β-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-methylureido]-2-phenylacetamido]-7α-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (**5**) had the most potent activity *in vitro* against gram-negative bacteria, its activity being 8- to 32-fold and 4-fold greater than those of cefoperazone and ceftazidime, respectively, against two strains of *P. aeruginosa*. The structure–activity relationship is discussed.

Keywords——ceftazidime; anti-*Pseudomonas* activity; ureidocephalosporin; ureidocephamycin; catechol moiety; catechol acetate moiety; minimum inhibitory concentration; structure–activity relationship

In recent years, new cephalosporin and cephamycin derivatives with an expanded antibacterial spectrum and greater β-lactamase stability have been reported,[1-10] and ceftazidime[4] was proved to have potent activity against *Pseudomonas aeruginosa* strains, which are generally less sensitive to β-lactam antibiotics. Aiming at a further improvement, we have been continuing the research effort.

In our previous papers,[11,12] we reported the synthesis, antibacterial activity and structure–activity relationship of new ureidopenicillins having a catechol moiety, which we had synthesized in the expectation that penicillins carrying an iron-sequestering moiety would be readily incorporated into bacterial cells. In the course of our studies, we found that 6-[(*R*)-2-[3-(3,4-dihydroxybenzoyl)-3-methylureido]-2-phenylacetamido]penicillanic acid (**1a**) had the highest *in vitro* activity against gram-negative bacteria, including *P. aeruginosa*, and its derivative (**1b**) showed remarkable activity against *P. aeruginosa in vivo* as well as *in vitro*.



1a : R=Me
1b : R=$(CH_2)_3OH$

Chart 1

These findings encouraged us to synthesize ureidocephalosporins and ureidocephamycins having a catechol moiety to investigate whether these compounds also have strong anti-

bacterial activity and a broad spectrum. In the course of synthesizing the ureidocephalosporins (**3**) with a catechol moiety, various substituents (R_2), *e.g.*, methyl, 1-hydroxypropyl, cyanoethyl, methoxyethyl, or ethoxycarbonylmethyl were introduced on the nitrogen atom of the ureido bond in combination with an acetoxymethyl or 1-methyl-1*H*-tetrazole-5-thiomethyl group at the C-3 position. Encouraged by the result that cephalosporins (**2**, **3Aa**, **3Ba**; R_2=Me) showed high *in vitro* activity, we introduced a 3-(3,4-diacetoxybenzoyl)-3-methyl-1-ureido group or a 3-(3,4-dihydroxybenzoyl)-3-methyl-1-ureido group into the C-7 side chain of 7α-methoxy-3-cephem derivatives carrying an acetoxymethyl or a 1-methyl-1*H*-

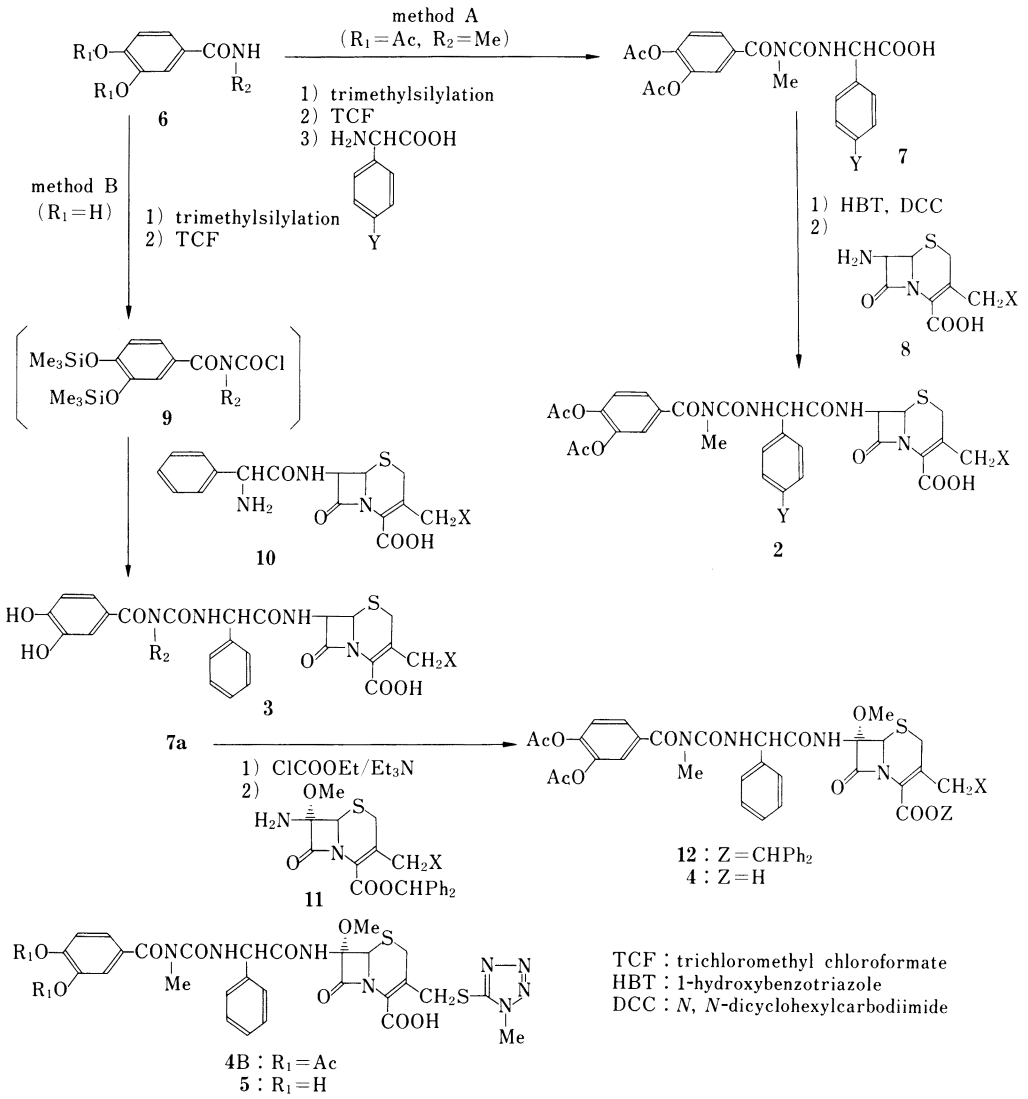
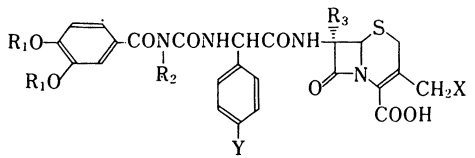


Chart 2

TABLE I. Antibacterial Activities of Cephalosporins (**2**, **3**) and Cephamycins (**4**, **5**)



Compd. No.	R_1	R_2	R_3	X	Y	MIC (μg/ml)								
						S. aureus		*E. coli* NIHJ	*K. Pneumoniae*	*P. morganii*	*S. marcescens*	*E. cloacae*	*P. aeruginosa*	
						209P JC-1	JU-5[a]	JC-2	JU-90	JU-241	FU-104	FU-250	Ps-6	Ps-33
2Aa	Ac	CH_3	H	OAc	H	0.78	3.13	0.20 (1.56)[b]	0.10	3.13	1.56 (100)[b]	0.39	1.56	1.56
2Ab	Ac	CH_3	H	OAc	OH	1.56	3.13	0.20 (1.56)	0.05	3.13	1.56 (100)	0.39	1.56	1.56
2Ba	Ac	CH_3	H	MTT[c]	H	0.78	1.56	0.20 (1.56)	0.10	3.13	1.56 (100)	0.39	1.56	1.56
2Bb	Ac	CH_3	H	MTT	OH	1.56	3.13	0.20 (1.56)	0.05	1.56	0.78 (50)	0.20	0.78	3.13
3Aa	H	CH_3	H	OAc	H	0.78	3.13	0.20 (12.5)	0.05	3.13	1.56 (50)	0.39	0.78	0.78
3Ab	H	$(CH_2)_3OH$	H	OAc	H	0.39	1.56	3.13 (12.5)	0.10	12.5	25 (400)	12.5	6.25	6.25
3Ac	H	$(CH_2)_2CN$	H	OAc	H	0.78	1.56	3.13 (25)	0.10	12.5	6.25 (400)	6.25	25	25
3Ad	H	$(CH_2)_2OMe$	H	OAc	H	0.78	1.56	6.25 (25)	0.10	12.5	12.5 (400)	12.5	25	50
3Ae	H	CH_2COOEt	H	OAc	H	1.56	3.13	6.25 (50)	0.20	25	100 (400)	50	12.5	12.5
3Ba	H	CH_3	H	MTT	H	0.78	3.13	0.20 (6.25)	0.05	0.78	0.78 (200)	0.78	0.78	0.78
3Bb	H	$(CH_2)_3OH$	H	MTT	H	0.78	0.78	3.13 (12.5)	0.10	6.25	6.25 (100)	1.56	12.5	6.25
4A	Ac	CH_3	OMe	OAc	H	6.25	6.25	0.39 (1.56)	0.10	6.25	3.13 (50)	0.78	6.25	6.25
4B	Ac	CH_3	OMe	MTT	H	1.56	3.13	0.20 (0.78)	0.05	1.56	0.78 (25)	0.20	1.56	1.56
5	H	CH_3	OMe	MTT	H	1.56	3.13	0.10 (0.78)	0.05	0.78	0.39 (6.25)	0.20	0.78	0.78
Cefoperazone						0.78	1.56	0.20 (0.78)	0.05	1.56	3.13 (25)	0.10	6.25	25
Ceftazidime						6.25	3.13	0.39 (0.39)	0.20	0.10	0.39 (0.78)	0.39	3.13	3.13

a) Penicillinase producer. *b*) One loopful of 1×10^9/ml. *c*) MTT: 1-methyl-1*H*-tetrazole-5-thiol.

tetrazole-5-thiomethyl group at C-3.

This paper deals with the synthesis and structure–activity relationships of ureidocephalosporins (**2**, **3**) and ureidocephamycins (**4**, **5**) containing a catechol moiety or its acetate.

Chemistry

As outlined in Chart 2, we prepared the cephalosporins listed in Table I by two general methods. The cephalosporins (**2**) having two acetoxy groups on the benzene ring were prepared by acylation of the corresponding 7-aminocephalosporins (**8**) with active esters derived from 1-hydroxybenzotriazole (HBT) and the ureidocarboxylic acids (**7**)[11] (method A). An alternative method (method B) involving reaction of the carbamoyl chloride (**9**)[11,12] and silylated phenylglycylcephalosporins (**10**), was developed for the preparation of the cephalosporins (**3**) having a catechol moiety. The cephamycins (**4**) were synthesized by acylation of the diphenylmethyl 7β-amino-7α-methoxy-3-cephem-4-carboxylates (**11**)[8,9] with the mixed anhydride derived from (*R*)-2-[3-(3,4-diacetoxybenzoyl)-1-ureido]phenylacetic acid (**7a**) and ethyl chloroformate, followed by cleavage of the diphenylmethyl protecting group using trifluoroacetic acid (TFA)/anisole. Ureidocephamycin (**5**) having a catechol group was obtained by removal of the acetyl groups from cephamycin (4B) using *N*,*N*-diethylethanolamine in *N*,*N*-dimethylformamide (DMF).[11]

Antibacterial Activity

The minimum inhibitory concentration (MIC) values of the cephalosporins (**2**, **3**) and the cephamycins (**4**, **5**) were determined by the serial two-fold agar dilution method against two species of gram-positive and seven species of gram-negative bacteria. The *in vitro* activities of these compounds (**2**—**5**) compared to those of cefoperazone and ceftazidime are listed in Table I. The substituent (R_2) on the ureido bond influenced the antibacterial activity against gram-negative bacteria. The methyl analogs (**3Aa**, **3Ba**) showed the highest activity. The compound (**3Ab**) having a hydroxypropyl group as the substituent (R_2) was less potent against gram-negative bacteria including *P. aeruginosa* than **3Aa**, contrary to our expectation based on the case of penicillins. Enhanced antibacterial activity against gram-negative bacteria is often observed as a result of replacing the acetoxy group at C-3 with heteroaromatic thiols, such as 1-methyl-1*H*-tetrazole-5-thiol.[13,14] Again, contrary to our expectation, no significant improvement was observed when 1-methyl-1*H*-tetrazole-5-thiol was introduced into cephalosporins (**2Aa**, **2Ab**, **3Aa**). In the case of cephamycin (**4B** *vs.* **4A**), however, some improvement was attained. The cephamycin (**5**) bearing 1-methyl-1*H*-tetrazole-5-thiol as the substituent (X) was more active than the corresponding cephalosporin (**3Ba**) against *E. coli* NIHJ JC-2 and *S. marcescens* FU-104 when the inoculum size of the two strains was increased from 10^6 to 10^9 cfu/ml. Furthermore, this compound proved to be strongly active *in vitro* against *P. aeruginosa* with an 8- to 32-fold increase in activity compared with cefoperazone, and 4-fold compared with ceftazidime.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were recorded on a Hitachi EPI-G3 spectrometer. The nuclear magnetic resonance (NMR) spectra were measured on a Hitachi R-20A spectrometer using tetramethylsilane (TMS) as an internal standard. All chemical shifts are reported in δ ppm.

7-[(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methylureido]-2-phenylacetamido]cephalosporanic Acid (2Aa)——*N*,*N*-Dicyclohexylcarbodiimide (DCC) (2.1 g) in tetrahydrofuran (THF) (5 ml) was added to a stirred solution of (*R*)-2-[3-(3,4-diacetoxybenzoyl)-3-methylureido]-2-phenylacetic acid (**7a**, 5.2 g) and 1-hydroxybenzotriazole (1.4 g) in THF (50 ml) under cooling. The mixture was stirred at 0—5 °C for 1 h, and used for the following reaction.

N,*O*-Bis(trimethylsilyl) acetamide (BSA) (6.5 ml) was added to a stirred suspension of 7-aminocephalosporanic acid (7-ACA) (3.6 g) in CH_2Cl_2 (100 ml) at room temperature. Stirring was continued for 5 min, then trimethylchlorosilane (0.5 ml) was added to the mixture under ice-cooling. Stirring was continued for 1 h at 5 C. To the resulting solution was added the reaction mixture containing activated ester described above at 5—10 C, and the

whole was stirred at the same temperature for 3 h. After the organic solvents had been evaporated off, the residue was taken up in THF (150 ml). The insoluble materials were removed by filtration. Then, the filtrate was concentrated *in vacuo*, and the residue was purified by chromatography on a silica gel column using $CHCl_3$–MeOH (30 : 1) to give **2Aa** (2.0 g, 24%) as a white powder. IR (KBr): 1780, 1745—1680, 1510 cm^{-1}. NMR (acetone-d_6): 2.00 (3H, s), 2.28 (6H, s), 3.15 (3H, s), 3.5 (2H, br s), 4.78 and 5.09 (2H, AB q, J = 13 Hz), 5.03 (1H, d, J = 5 Hz), 5.5—6.0 (2H, m), 7.2—7.7 (8H, m), 8.33 (1H, d, J = 8 Hz), 9.93 (1H, d, J = 7 Hz).

7-[(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methylureido]-2-(4-hydroxyphenyl)acetamido]cephalosporanic Acid (2Ab)——Compound **2Ab** was prepared from (*R*)-2-[3-(3,4-diacetoxybenzoyl)-3-methylureido]-2-(4-hydroxyphenyl)-acetic acid (**7b**) and 7-ACA by the method described for **2Aa**. Yield 20%. IR (KBr): 1775, 1740—1670, 1510 cm^{-1}. NMR (acetone-d_6): 2.00 (3H, s), 2.28 (6H, s), 3.16 (3H, s), 3.5 (2H, br s), 4.78 and 5.11 (2H, AB q, J = 13 Hz), 5.03 (1H, d, J = 5 Hz), 5.5—6.1 (2H, m), 6.7—7.7 (7H, m), 8.29 (1H, d, J = 8 Hz), 9.87 (1H, d, J = 7 Hz).

7-[(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methylureido]-2-phenylacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic Acid (2Ba)——Compound **2Ba** was prepared from (*R*)-2-[3-(3,4-diacetoxybenzoyl)-3-methylureido]-2-phenylacetic acid (**7a**) and 7-amino-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (**8B**) by the method described for **2Aa**. Yield 22%. IR (KBr): 1775, 1740—1680, 1510 cm^{-1}. NMR (acetone-d_6): 2.27 (6H, s), 3.13 (3H, s), 3.75 (2H, br s). 3.93 (3H, s), 4.37 (2H, br s), 5.11 (1H, d, J = 5 Hz), 5.6—5.9 (2H, m), 7.2—7.7 (8H, m), 8.40 (1H, d, J = 8 Hz), 9.95 (1H, d, J = 7 Hz).

7-[(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methylureido]-2-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic Acid (2Bb)——Compound **2Bb** was prepared from (*R*)-2-[3-(3,4-diacetoxybenzoyl)-3-methylureido]-2-(4-hydroxyphenyl) acetic acid (**7b**) and **8B** by the method described for **2Aa**. Yield 20%. IR (KBr): 1775, 1720—1680, 1510 cm^{-1}. NMR (acetone-d_6): 2.29 (6H, s), 3.17 (3H, s), 3.8 (2H, br s), 3.98 (3H, s), 4.40 (2H, br s), 5.09 (1H, d, J = 5 Hz), 5.4—6.0 (2H, m), 6.7—7.7 (7H, m), 8.37 (1H, d, J = 8 Hz), 9.78 (1H, d, J = 7 Hz).

7-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-methylureido]-2-phenylacetamido]cephalosporanic Acid (3Aa)—— Triethylamine (10.1 g) was added dropwise to a stirred suspension of *N*-methyl-3,4-dihydroxybenzamide (**6a**, 5.0 g) and trimethylchlorosilane (10.9 g) in CH_2Cl_2 (70 ml) under ice-cooling. The mixture was refluxed for 1 h. After cooling of the reaction mixture, trichloromethyl chloroformate (TCF) (3.6 g) was added at 0 °C. Stirring was continued for 3 h at 20—30 C and then for 1 h at the same temperature under reduced pressure to remove excess phosgene. The residue was used for the following reaction.

Silylated cephaloglycin (**10A**) [prepared from **10A** (15.8 g), trimethylchlorosilane (12.5 g) and triethylamine (8.9 g)] in CH_2Cl_2 (200 ml) was added to the residue described above at 0—5 °C. Stirring was continued for 1.5 h at 5—10 °C. After the organic solvents had been evaporated off, the residue was taken up in mixed solvents (EtOAc : THF = 1 : 1, 300 ml) and 1 N HCl (100 ml) under ice-cooling. The organic layer was separated, washed with brine, dried over $MgSO_4$ and evaporated to dryness to give a crude product, which was dissolved in 5% $NaHCO_3$ solution (10 ml) and chromatographed over Diaion HP-20 with elution by H_2O and 30% aqueous acetone in order. The fractions containing the product were combined and adjusted to pH 2.5 with 2 N HCl after the addition of EtOAc (200 ml). The EtOAc layer was separated, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was triturated with *n*-hexane to give **3Aa** (1.4 g, 8%) as a white powder.

Compounds **3Ab**—**3Ae**, **3Ba**, **3Bb** were prepared in a manner similar to that described for **3Aa**. In the cases of **3Ba** and **3Bb**, 7-[(*R*)-2-amino-2-phenylacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid was utilized in place of **10A**. NMR and IR spectral data of these compounds and **3Aa** are given in Table II.

Diphenylmethyl 3-Acetoxymethyl-7β-[(*R*)-2-[3-(3,4-diacetoxybenzoyl)-3-methylureido]-2-phenylacetamido]-7α-methoxy-3-cephem-4-carboxylate (12A)——*N*-Methylmorpholine (0.32 g) in CH_2Cl_2 (5 ml) was added to a stirred solution of **7a** (1.38 g) and ethyl chloroformate (0.35 g) in CH_2Cl_2 (30 ml) at −15—−10 °C. Stirring was continued at the same temperature for 1 h, then diphenylmethyl 7β-amino-7α-methoxycephalosporanate (**11A**, 1.50 g) in CH_2Cl_2 (10 ml) was added dropwise to the reaction mixture below −10 °C. The whole was stirred at −15—−10 °C for 2 h. Furthermore, stirring was continued while allowing the temperature to rise to room temperature over 2 h. Then the solvent was evaporated off and EtOAc (100 ml) was added to the residue. The EtOAc solution was washed successively with saturated $NaHCO_3$ solution (100 ml) and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by chromatography on a silica gel column. Elution with EtOAc–benzene (1 : 2) gave **12A** (0.45 g, 16%) as a pale yellow powder. IR (KBr): 3300, 1775, 1740—1680, 1510—1490 cm^{-1}. NMR ($CDCl_3$): 2.01 (3H, s), 2.28 (6H, s), 3.19 (3H, s), 3.2 (2H, br s), 3.51 (3H, s), 4.76 and 5.03 (2H, AB q, J = 13 Hz), 5.02 (1H, s), 5.62 (1H, d, J = 7 Hz), 6.90 (1H, s), 7.0—7.7 (19H, br s), 9.97 (1H, d, J = 7 Hz).

Diphenylmethyl 7β-[(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methylureido]-2-phenylacetamido]-7α-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (12B)——Compound **12B** was prepared from **7a** and diphenylmethyl 7β-amino-7α-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (**11B**) by the method described for **12A**. Yield 25%. IR (KBr): 3400, 1775, 1730—1680, 1510—1490 cm^{-1}. NMR ($CDCl_3$): 2.28 (6H, s), 3.17 (3H, s), 3.50 (3H, s), 3.74 (3H, s), 3.8 (2H, br s), 4.3 (2H, m), 5.01 (1H, s), 5.67 (1H, d, J = 7 Hz), 6.89 (1H, s), 7.1—7.7 (19H, br s), 9.99 (1H, d, J = 7 Hz).

3-Acetoxymethyl-7β-[(*R*)-2-[3-(3,4-diacetoxybenzoyl)-3-methylureido]-2-phenylacetamido]-7α-methoxy-3-

Table II. IR and NMR Spectral Data for Cephalosporins (3)

Compound No.	IR ν_{max}^{KBr} cm^{-1} (C=O)	NMR δ value (solvent)
3Aa	1775, 1730, 1675	(DMSO-d_6): 2.02 (3H, s), 3.12 (3H, s), 3.5 (2H, br s), 4.66 and 5.03 (2H, AB q, J=13 Hz), 5.5—5.9 (2H, m), 6.8—7.6 (8H, m), 9.42 (1H, d, J=8 Hz), 9.74 (1H, d, J=8 Hz)
3Ab	1775, 1715, 1675	(DMSO-d_6): 1.4—2.0 (2H, m), 2.00 (3H, s), 3.1—4.0 (6H, m), 4.65 and 5.01 (2H, AB q, J=13 Hz), 5.01 (1H, d, J=5 Hz), 5.4—5.9 (2H, m), 6.7—7.6 (8H, m), 9.22 (1H, d, J=8 Hz), 9.33 (1H, d, J=8 Hz)
3Ac	1775, 1730, 1690	(DMSO-d_6): 2.01 (3H, s), 2.6—3.0 (2H, m), 3.5 (2H, br s), 3.7—4.2 (2H, m), 4.68 and 5.03 (2H, AB q, J=13 Hz), 5.05 (1H, d, J=5 Hz), 5.4—5.9 (2H, m), 6.8—7.6 (8H, m), 9.18 (1H, d, J=8 Hz), 9.40 (1H, d, J=8 Hz)
3Ad	1775, 1725, 1680	(DMSO-d_6): 2.01 (3H, s), 3.1—4.1 (6H, m), 3.18 (3H, s), 4.67 and 5.02 (2H, AB q, J=13 Hz), 5.04 (1H, d, J=5 Hz), 5.4—5.9 (2H, m), 6.7—7.6 (8H, m), 9.18 (1H, d, J=8 Hz), 9.40 (1H, d, J=8 Hz)
3Ae	1775, 1730, 1690	(DMSO-d_6): 1.0—1.4 (3H, m), 2.00 (3H, s), 3.5 (2H, br s), 3.9—4.3 (2H, m), 4.4 (2H, br s), 4.66 and 5.03 (2H, AB q, J=13 Hz), 5.05 (1H, d, J=5 Hz), 5.5—6.0 (2H, m), 6.8—7.7 (8H, m), 9.44 (1H, d, J=8 Hz), 9.54 (1H, d, J=8 Hz)
3Ba	1775, 1680	(DMSO-d_6): 3.10 (3H, s), 3.6 (2H, br s), 3.93 (3H, s), 4.3 (2H, br s), 5.03 (1H, d, J=5 Hz), 5.4—5.8 (2H, m), 6.8—7.6 (8H, m), 9.43 (1H, d, J=8 Hz), 9.73 (1H, d, J=8 Hz)
3Bb	1775, 1680	(DMSO-d_6): 1.4—2.0 (2H, m), 3.2—4.0 (6H, m), 3.94 (3H, s), 4.3 (2H, br s), 5.02 (1H, d, J=5 Hz), 5.4—5.9 (2H, m), 6.7—7.6 (8H, m), 9.28 (1H, d, J=8 Hz), 9.41 (1H, d, J=8 Hz)

cephem-4-carboxylic Acid (4A)——Compound **12**A, (0.40 g) was dissolved in anisole (1.5 ml) and CH_2Cl_2 (3 ml), and trifluoroacetic acid (3 ml) was added to the solution under ice-cooling. The mixture was stirred for 30 min at 5°C, then the excess TFA and the organic solvents were removed *in vacuo*. The residual oil was treated with Et_2O to give **4**A (0.25 g, 78%) as a pale yellow powder. IR (KBr): 1775, 1740—1680, 1505 cm^{-1}. NMR (acetone-d_6): 2.01 (3H, s), 2.28 (6H, s), 3.17 (3H, s), 3.2 (2H, br s), 3.50 (3H, s), 4.26 and 5.07 (2H, AB q, J=13 Hz), 5.09 (1H, s), 5.72 (1H, d, J=7 Hz), 7.2—7.7 (8H, m), 8.6 (1H, br s), 9.85 (1H, d, J=7 Hz).

7β-[(*R*)-2-[3-(3,4-Diacetoxybenzoyl)-3-methylureido]-2-phenylacetamido]-7α-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic Acid (4B)——Compound **4**B was prepared in a manner similar to that described for **4**A. Yield 80%. IR (KBr): 1775, 1720—1670, 1510 cm^{-1}. NMR (acetone-d_6): 2.28 (6H, s), 3.16 (3H, s), 3.48 (3H, s), 3.8 (2H, br s), 3.94 (3H, s), 4.22 and 4.49 (2H, AB q, J=13 Hz), 5.03 (1H, s), 5.70 (1H, d, J=7 Hz), 7.2—7.6 (8H, m), 8.6 (1H, br s), 9.82 (1H, d, J=7 Hz).

7β-[(*R*)-2-[3-(3,4-Dihydroxybenzoyl)-3-methylureido]-2-phenylacetamido]-7α-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylic Acid (5)——2-Diethylaminoethanol (1 ml) was added to a solution of **4**B (0.35 g) in DMF (1 ml) under ice-cooling, and stirring was continued at room temperature for 5 h. When the reaction was over, the solution was poured into 1 N HCl (50 ml), EtOAc (50 ml) and acetone (10 ml). The aqueous layer was separated and further extracted with EtOAc (30 ml) and acetone (6 ml). The combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was treated with Et_2O to afford **5** (0.25 g, 81%) as a pale yellow powder. IR (KBr): 1775, 1680, 1515 cm^{-1}. NMR (acetone-d_6): 3.18 (3H, s), 3.49 (3H, s), 3.5 (2H, br s), 3.94 (3H, s), 4.23 and 4.51 (2H, AB q, J=13 Hz), 5.03 (1H, s), 5.70 (1H, d, J=7 Hz), 6.9—7.7 (8H, m), 8.6 (1H. br s), 9.90 (1H, d, J=7 Hz).

Determination of *in Vitro* Antibacterial Activity——All the *in vitro* antibacterial activities are given as MIC (in μg/ml) required to prevent growth of the bacterial culture. MIC's were determined by the agar dilution method using Antibiotic Medium No. 3 agar after incubation at 37°C for 20 h, with an inoculum size of about 10^6 cfu/ml.

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