SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CEPHALOSPORINS HAVING A CATECHOL IN THE C3 SIDE CHAIN

TAKAAKI OKITA, KIYOTO IMAE, TOSHIFUMI HASEGAWA, SEIJI IIMURA, SHINJI MASUYOSHI, HAJIME KAMACHI and HIDEO KAMEI

Birstol-Myers Squibb Research Institute,
2-9-3, Shimo-meguro, Meguro-ku
Tokyo 153, Japan

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Cephalosporins having a catechol through a variety of linkages to the C3 position and a different C7 side chain were prepared. Among them, 3-(catechol-4-ylcarbonyloxy)methylcephalosporin (**14**) and 3-[(*E*)-3-(catechol-4-ylcarbonyloxy)-1-propen-1-yl]cephalosporin (**10**) showed excellent activity against Gram-negative activity including ceftazidime-resistant *Escherichia coli*, *Pseudomonas aeruginosa*, *Xanthomonas maltophilia* and *Pseudomonas cepacia*.

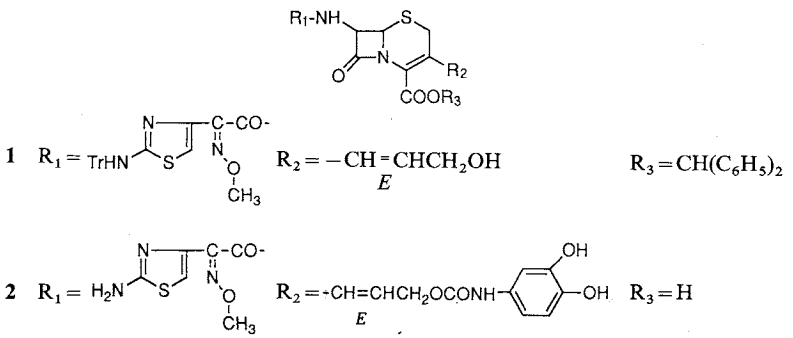
Recent reports have demonstrated that cephalosporins bearing a catechol moiety or its bioisostere exhibited imporved potency against resistant strains of Gram-negative bacteria including *Pseudomonas aeruginosa*[1~4]. Some of them proved to utilize the *tonB*-dependent iron transport system of bacteria for expression of the activity[5~7].

In a previous report[8], a new class of cephalosporins having an (*E*)-3-ammonio-1-propenyl group was found to exhibit broader and better antibacterial activity than the corresponding 3-ammoniomethylcephalosporins against both Gram-positive and Gram-negative organisms. Accordingly, our major interest is to examine the effectiveness of this propenyl linkage at the C3 position for catechol-containing cephalosporins. This report describes synthesis of cephalosporins linked with a catechol in the C3 side chain through carbonyloxymethylene, 3-carbonyloxy-1-propene and 3-carbamoyloxy-1-propene groups and their *in vitro* structure-activity relationships.

Chemistry

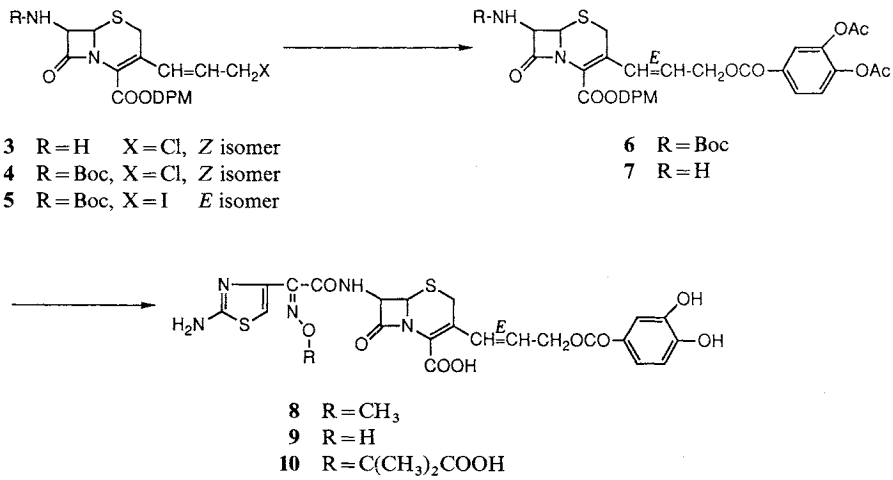
The 3-(*E*)-carbamoyloxypropenyl analog was prepared from 3-(*E*)-hydroxypropenylcephalosporin

Fig. 1. Structure of cephalosporins **1**, **2**.



Correspondence should be addressed to JUN OKUMURA, Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan.

Scheme 1. Synthesis of cephalosporins **8**~**10**.



3 R=H X=Cl, *Z* isomer
4 R=Boc, X=Cl, *Z* isomer
5 R=Boc, X=I *E* isomer

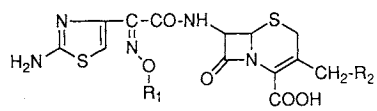
6 R=Boc
7 R=H

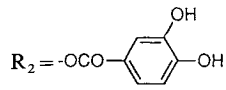
8 $R=CH_3$
9 R=H
10 $R=C(CH_3)_2COOH$

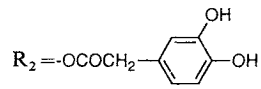
derivative (**1**)[9] by treatment of freshly prepared 3,4-diacetoxyphenyl isocyanate in 72% yield. Deprotection with trifluoroacetic acid (TFA), followed by enzymatic deblocking with acetylesterase yielded 3-(*E*)-carbamoyloxypropenylcephalosporin (**2**) (Fig. 1).

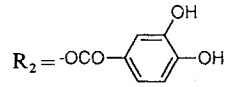
The other vinylogous cephalosporins were prepared from 3-(*Z*)-chloropropenylcephalosporin derivative (**3**)[8] in 5 steps including manipulations of protective groups (Scheme 1). The 7-amino group of cephalosporin (**3**) was protected by Boc group with treatment of di-*tert*-butyl dicarbonate in good yield. The 3-(*Z*)-chloropropenyl group of **4** was transformed into 3-(*E*)-iodopropenyl derivative (**5**) with the reaction of sodium iodide in acetone in 73% yield. Catechol group was introduced into cephem (**5**) by using cesium carbonate as a base to provide compound (**6**) in 76%. Generally, as compared with 3-(halomethyl)cephem, the corresponding propenyl analog is hard to isomerize under basic conditions[8,10]. In practice, the undesired Δ^2 isomers were not detected in the products. The Boc group was selectively removed by treatment with *p*-toluenesulfonic acid in acetonitrile to give **7** in 87% yield. The appropriate 7-side chains were introduced to the cephem (**7**) in the usual way and subsequent removal of protecting group by treatment with TFA prior to acetylesterase provided the desired products (**8**~**10**).

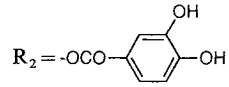
Fig. 2. Structure of cephalosporins **11**~**14**.



11 $R_1=CH_3$ $R_2=$ 

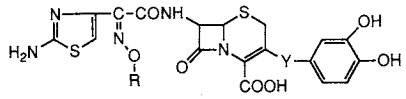
12 $R_1=CH_3$ $R_2=$ 

13 $R_1=H$ $R_2=$ 

14 $R_1=C(CH_3)_2COOH$ $R_2=$ 

Utilizing recently developed synthetic methodology[10,11], we have prepared four cephalosporins (**11**~**14**) from diphenylmethyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate hydrochloride[8] (Fig. 2). Catechol moiety was introduced by the nucleophilic displacement of the C3 halide with carboxylate ion.

Table 1. Antibacterial activity of cephalosporins.



Compound	R	Y	MIC (μg/ml)		
			S.a. Smith	*S.a.* A15036 (MRSA)	*E.c.* Juhl
11	CH_3	$-CH_2OCO-$	3.1	12.5	0.1
12	CH_3	$-CH_2OCOCH_2-$	12.5	25	0.2
2	CH_3	$-CH=CHCH_2OCONH-$	6.3	50	0.8
8	CH_3	$-CH=CHCH_2OCO-$	1.6	12.5	0.05
13	H	$-CH_2OCO-$	0.4	6.3	0.2
9	H	$-CH=CHCH_2OCO-$	0.4	6.3	0.2
14	CME	$-CH_2OCO-$	12.5	100	0.2
10	CME	$-CH=CHCH_2OCO-$	12.5	>100	0.1
Ceftazidime			12.5	100	0.4

Compound	MIC (μg/ml)							
	E.c. 255	*M.m.* 1510	*C.f.* GN7391	*P.a.* A9843A	*P.a.* A20599	*P.a.* Pa-241	*X.m.* PI-19	*P.c.* Pp-105
11	0.8	12.5	100	6.3	6.3	100	100	100
12	1.6	25	100	100	50	>100	100	>100
2	3.1	50	>100	100	50	>100	>100	100
8	0.2	3.1	12.5	3.1	3.1	50	12.5	12.5
13	1.6	6.3	50	50	50	>100	>100	>100
9	1.6	12.5	50	>100	>100	>100	>100	>100
14	0.05	3.1	>100	0.05	0.2	0.1	6.3	0.8
10	0.025	6.3	50	0.1	0.2	0.8	6.3	3.1
Ceftazidime	50	25	>100	6.3	3.1	100	50	25

Medium: Mueller-Hinton agar (pH 7.2), incubation: 37°C 18 hours, inoculum size 10^6 cells/ml except for MRSA at 32°C.

S.a., *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *M.m.*, *Morganella morganii*; *C.f.*, *Citrobactor freundii*; *P.a.*, *Pseudomonas aeruginosa*; *X.m.*, *Xanthomonas maltophilia*; *P.c.*, *Pseudomonas cepacia*; CME = $-C(CH_3)_2COOH$.

During this reaction sequence, oxidation-reduction process was required to prevent the formation of the undesired Δ^2 isomers.

In Vitro Activity and Discussion

MICs of the synthesized cephalosporins against 11 test organisms were determined by the standard 2-fold serial agar dilution method in Mueller-Hinton agar after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml except for MRSA at 32°C (Table 1).

Against all the organisms tested, **11** was equal or two to eight fold more active than **12** and then **11** was selected as a lead compound. Compound **8**, which is an analog of **11** having a propenyl group as a linkage in the C3 side chain and a methoxyimino group in the 7 side chain, exhibited well-balanced activity over the above cephalosporins (**11**, **12**, and **2**).

In order to explore the further structure-activity relationships, our attention was found on substituent effect of the oxyimino group in the 7 side chain. Replacement from methoxy to hydroxy increased activity against Gram-positive organisms but decreased Gram-negative organisms. Interestingly, both compounds

9 and **13** showed good activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Unlike the methoxyimino substituent, chemical modification with a propenyl group in the C3 side chain showed no advantage over the parent compound **13**.

The 1-carboxy-1-methylethoxy (CME) analogs (**14**) showed a similar antibacterial spectrum to **12** and excellent *in vitro* activity against Gram-negative organisms including *Escherichia coli*, *P. aeruginosa*, *Xanthomonas maltophilia*, and *Pseudomonas cepacia*, but they were only poorly active against *S. aureus*.

In conclusion, a propenyl linkage in the C3 side chain, which we have developed, was also effective on the antibacterial activity of the above catechol-containing cephalosporins. Above all, cephalosporin **14** and **10** showed excellent inhibitory activity against Gram-negative organisms including ceftazidime-resistant *E. coli*, *P. aeruginosa*, *X. maltophilia* and *P. cepacia*.

Experimental

MPs were determined using a Yanagimoto micro hot-stage apparatus and were uncorrected. IR spectra were recorded on JASCO IRA-1 and a UV spectra were recorded on a Shimadzu UV-200 spectrophotometer. NMR spectra were recorded on a JEOL GX-400 (400 MHz). Mass spectra were recorded on a JEOL JMS-AX505H(FAB) mass spectrometer.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-(3,4-dihydroxyphenyl)carbamoyloxy-1-propen-1-yl]-3-cephem-4-carboxylic Acid (**2**)

Freshly prepared 3,4-diacetoxyphenyl isocyanate (1.07 g, 5.3 mmol)[11] was added to a solution of diphenylmethyl 7-[(*Z*)-2-(2-tritylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-hydroxy-1-propen-1-yl]-3-cephem-4-carboxylate (**1**)[8] (452 mg, 0.53 mmol) in DMF (1 ml) and the resulting mixture was stirred for 3 hours at room temperature. The mixture was poured into water (20 ml) and extracted with EtOAc. The extract was washed with water and concentrated. The residue was purified chromatographically to afford 415 mg (72%) of diphenylmethyl 7-[(*Z*)-2-(2-tritylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-(3,4-diacetoxyphenyl)carbamoyloxy-1-propen-1-yl]-3-cephem-4-carboxylate as an amorphous powder. IR ν_{max} (KBr) cm^{-1} 1765, 1720, 1520; ^1H NMR ($CDCl_3$) δ 2.27 (3H, s, Ac), 2.28 (3H, s, Ac), 3.54 (1H, d, J=18 Hz, 2-H), 3.62 (1H, d, J=18 Hz, 2-H), 4.07 (3H, s, OCH_3), 4.64 (2H, m, 3-CH=CH-CH_2), 5.09 (1H, d, J=5 Hz, 6-H), 5.94 (1H, dd, J=5 and 9 Hz, 7-H), 6.01 (1H, dt, J=16 and 6 Hz, CH=C*H*-CH_2), 6.60 (1H, s), 6.75 (1H, s), 6.81 (1H, d, J=16 Hz, C*H*=CH-CH_2), 7.23~7.45 (25H, m, Ph-H); FAB-MS m/z 1,083 $(M+H)^+$.

A mixture of the above cephem (400 mg, 0.37 mmol) and anisole (2 ml) in TFA (3 ml) was stirred for 1 hour at room temperature. The reaction mixture was diluted with isopropyl ether. The resulting precipitate was collected by filtration and was dissolved in pH 7 phosphate buffer (0.44 M, 10 ml). The solution was treated with acetylesterase (Sigma, 0.8 ml). As the reaction proceeded, the pH was maintained at 7 by addition of $NaHCO_3$ or citiric acid. After 30 minutes at room temperature, the mixture was acidified to pH 2 by conc. HCl and absorbed on a column of HP-20 (20 ml). The column was washed with water and the product was eluted with methanol. The desired fraction was concentrated and the residue was chromatographed on a column of reverse-phase silica gel (Waters, Prep C_{18}) to give 31 mg (14%) of **2**. MP 150°C (dec); IR ν_{max} (KBr) cm^{-1} 1760, 1660, 1520; UV λ_{max} (pH 7 buffer) nm (ε) 236 (19,800), 291 (24,000); ^1H NMR ($D_2O+NaHCO_3$) δ 3.66 (1H, d, J=18 Hz, 2-H), 3.72 (1H, d, J=18 Hz, 2-H), 4.00 (3H, s, OCH_3), 4.80 (2H, m, CH=CH-CH_2), 5.26 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 6.03 (1H, dt, J=16 and 5 Hz, CH=C*H*-CH_2), 6.78 (1H, d, J=16 Hz, C*H*=CH-CH_2), 6.79 (1H, dd, J=9 and 2 Hz, Ph-H), 6.89 (1H, d, J=9 Hz, Ph-H), 6.97 (1H, d, J=2 Hz, Ph-H), 7.04 (1H, s, thiazole-H); FAB-MS m/z 591 $(M+H)^+$.

Diphenylmethyl 7-*tert*-Butoxycarbonylamino-3-[(*Z*)-3-chloro-1-propen-1-yl]-3-cephem-4-carboxylate (**4**)

A suspension of diphenylmethyl 7-amino-3-[(*Z*)-3-chloro-1-propen-1-yl]-3-cephen-4-carboxylate hydrochloride (**3**)[8] (7.2 g, 15 mmol) inn CH_2Cl_2 (80 ml) and aq $NaHCO_3$ (3%, 50 ml) was stirred at room

temperature for 30 minutes. The organic layer was separated, dried and concentrated. The oily residue was diluted with CH_2Cl_2 (80 ml) di-*tert*-butyl dicarbonate (11.4 g, 52 mmol) was added to the solution in three portions. The mixture was stirred at room temperature for 2 days, and concentrated. Chromatography of the residue on silica gel eluting with toluene-EtOAc afforded 5.7 g (70%) of **4** as a crystalline solid. MP 165°C (dec); IR ν_{max} (KBr) cm^{-1} 1780, 1715, 1690; UV λ_{max} (CH_2Cl_2) nm (ε) 298 (9,800); ^1H NMR ($CDCl_3$) δ 1.46 (9H, s, CH_3), 3.50 (2H, ABq, 2-H), 3.73 (2H, ABq, CH=CH–C*H*$_2$), 5.06 (1H, d, J=4.5 Hz, 6-H), 5.21 (1H, d, J=10 Hz, CONH), 5.60 (1H, dd, J=4.5 and 10 Hz, 7-H), 5.65 (1H, m, CH=C*H*–CH_2), 6.29 (1H, d, J=12 Hz, C*H*=CH–CH_2), 6.98 (1H, s, C*H*Ph_2), 7.33 (10H, s, Ph-H)

Anal Calcd for $C_{28}H_{29}N_2O_5SCl$: C 62.16, H 5.40, N 5.18.
Found: C 62.65, H 5.52, N 4.96.

Diphenylmethyl 7-*tert*-Butoxycarbonylamino-3-[(*E*)-3-iodo-1-propen-1-yl]-3-cephem-4-carboxylate (**5**)

To an ice cooled solution of sodium iodide (900 mg, 6 mmol) in acetone (7.5 ml) was added a solution of **4** (1.08 g, 2 mmol) in acetone (2.5 ml). The mixture was stirred on an ice bath for 3.5 hours and concentrated. The residue was diluted with EtOAc and aqueous thiosulfate solution. The organic layer was dried over $MgSO_4$ and concentrated. The oily residue was dissolved in a small amount of ethanol to give 923 mg (73%) of **5** as yellow powder. IR ν_{max} (KBr) cm^{-1} 1785, 1720, 1685, 1520, UV λ_{max} (CH_2Cl_2) nm (ε) 318 (19,000); ^1H NMR ($CDCl_3$) δ 1.46 (9H, s), 3.54 (2H, s), 3.83 (2H, d, J=8 Hz, CH=CH–C*H*$_2$), 4.96 (1H, d, J=5 Hz, 6-H), 5.15 (1H, d, J=10 Hz, CONH), 5.6 (1H, dd, J=5 and 10 Hz, 7-H), 6.06 (1H, dt, J=16 and 8 Hz, CH=C*H*–CH_2), 6.82 (1H, d, J=16 Hz, C*H*=CH–CH_2), 6.98 (1H, s, C*H*Ph_2), 7.33 (10H, s, Ph-H).

Diphenylmethyl 7-*tert*-Butoxycarbonylamino-3-[(*E*)-3-(3,4-diacetoxybenzoyloxy)-1-propen-1-yl]-3-cephem-4-carboxylate (**6**)

A mixture of 3,4-diacetoxybenzoic acid (240 mg, 1 mmol) and cesium carbonate (160 mg, 0.49 mmol) in DMF (3 ml) was stirred at room temperature overnight. The resulting solution was chilled on an ice-water bath and **5** (600 mg, 0.95 mmol) was added to the solution. The mixture was stirred for 30 minutes at 0°C and partitioned between EtOAc and aqueous sodium thiosulfate. The organic layer was washed, dried and concentrated to afford 815 mg of amorphous powder, which was purified by silca gel chromatograpy to give 536 mg (76%) of **6**. IR (KBr) ν_{max} cm^{-1} 1775, 1710, 1520, 1490; ^1H NMR ($CDCl_3$) δ 1.45 (9H, s, CH_3), 2.27 (6H, s, OAc), 3.55 (2H, s, 2-H), 4.46 (2H, d, J=7 Hz, CH=CH–C*H*$_2$), 4.96 (1H, d, J=5 Hz, 6-H), 5.15 (1H, d, J=10 Hz, CONH), 5.58 (1H, dd, J=5 and 10 Hz, 7-H), 6.00 (1H, dt, J=16 and 7 Hz, CH=C*H*–CH_2), 6.95 (1H, d, J=16 Hz, C*H*=CH–CH_2), 6.96 (1H, s, C*H*Ph_2), 7.28 (11H, br s, Ph-H), 7.8~7.95 (2H, m, Ph-H).

Diphenylmethyl 7-Amino-3-[(*E*)-3-(3,4-diacetoxybenzoyloxy)-1-propen-1-yl]-3-cephem-4-carboxylate (**7**)

To a solution of **6** (500 mg, 0.67 mmol) in acetonitrile (10 ml) was added a solution of *p*-toluenesulfonic acid hydrate (260 mg, 1.37 mmol) in acetonitrile (10 ml) at 50°C. The mixture was stirred at the same temperature for an hour, and concentrated *in vacuo*. The residue was diluted with EtOAc, and was washed with aqueous sodium bicarbonate, brine, and dried over $MgSO_4$. The solvent was removed *in vacuo* to afford 376 mg (87%) of **7**. IR ν_{max} (film) cm^{-1} 1770, 1720, 1200; ^1H NMR ($CDCl_3$) δ 2.30 (6H, s, OAc), 3.55 (2H, br s, 2-H), 4.69 (2H, d, J=6 Hz, CH=CH–C*H*$_2$), 4.76 (1H, d, J=5 Hz, 6-H), 4.95 (1H, d, J=5 Hz, 7-H), 5.97 (1H, dt, J=16 and 6 Hz, CH=C*H*–CH_2), 6.88 (1H, d, J=16 Hz, C*H*=CH–CH_2), 7.00 (1H, s, C*H*Ph_2), 7.3 (11H, m, Ph-H), 7.8~7.95 (2H, m, Ph-H).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-(3,4-dihydroxybenzoyloxy)-1-propen-1-yl]-3-cephem-4-carboxylate (**8**)

To a stirred solution of **7** (370 mg, 0.57 mmol) in THF (2.5 ml) was added benzotriazol-1-yl (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetate (200 mg, 0.63 mmol)[8]. The mixture was stirred at room temperature for 1.5 hours, concentrated, and the residue was purified by silica gel chromatography to afford 308 mg of the product. To a cooled mixture of the acylated product (300 mg, 0.36 mmol) in methylene chloride (1.5 ml) and anisole (1 ml) was added trifluoroacetic acid (4 ml). The mixture was left at room temperature for 1 hour, concentrated and the residue was triturated with isopropyl ether (30 ml) to give 258 mg of pale yellow powder, which was dissolved with phosphate buffer (0.33 M, 25 ml). The solution

was treated with acetylesterase (Sigma, 1.5 ml) at pH 8.6~7.2. After the reaction was completed (*ca.* 1 hour), the product was isolated by a column of HP-20 (30 ml) to give 47 mg (23%) of **8** as pale yellow powder. MP>163°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 265 (sh, 21,600), 297 (28,500); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.69 (2H, ABq, 2-H), 4.00 (3H, s, OCH_3), 5.26 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 6.09 (1H, dt, J=16 and 6 Hz, CH=C*H*–CH_2), 6.86 (1H, d, J=16 Hz, C*H*=CH–CH_2), 6.96 (1H, d, J=8 Hz, Ph-H), 7.03 (1H, s, thiazole-H), 7.5~7.55 (2H, m, Ph-H); FAB-MS m/z 576 $(M+H)^+$, 598 $(M+Na)^+$.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)-3-(3,4-dihydroxybenzoyloxy)-1-propen-1-yl]-3-cephem-4-carboxylic Acid (**9**)

This compound was obtained from **7** by a procedure similar to **8**.

Yield 37%; MP 153°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 267 (sh, 19,500), 293 (25,800); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.66 (1H, d, J=17 Hz, 2-H), 3.72 (1H, d, J=17 Hz, 2-H), 5.27 (1H, d, J=5 Hz, 6-H), 5.84 (1H, d, J=5 Hz, 7-H), 6.09 (1H, dt, J=16 and 6 Hz, CH=C*H*–CH_2), 6.85 (1H, d, J=16 Hz, C*H*=CH–CH_2), 6.88 (1H, d, J=9 Hz, Ph-H), 7.00 (1H, s, thiazole-H), 7.48 (1H, d, J=2 Hz, Ph-H), 7.52 (1H, dd, J=2 and 9 Hz, Ph-H); FAB-MS m/z 562 $(M+H)^+$.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxyl-1-methylethoxyimino)acetamido]-3-[(E)-3-(3,4-dihydroxybenzoyloxy)-1-propen-1-yl]-3-cephem-4-carboxylic Acid (**10**)

This compound was obtained from **7** by a procedure similar to **8**.

Yield 45%; MP >163°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 260 (sh, 22,000), 293 (28,500); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.53 (3H, s, CH_3), 1.54 (3H, s, CH_3), 3.72 (2H, ABq, 2-H), 5.29 (1H, d, J=5 Hz, 6-H), 5.85 (1H, d, J=5 Hz, 7-H), 6.13 (1H, dt, J=16 and 6 Hz, CH=C*H*–CH_2), 6.89 (1H, d, J=16 Hz, C*H*=CH–CH_2), 7.01 (1H, d, J=8 Hz, Ph-H), 7.04 (1H, s, thiazole-H), 7.56~7.6 (2H, m, Ph-H); FAB-MS m/z 648 $(M+H)^+$, 670 $(M+Na)^+$.

Preparation of Catechol-containing Cephalosporins (**11~14**)

These compounds were obtained from diphenylmethyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate hydrochloride[8] by a procedure similar to the reported method[11]. Physico-chemical data of each compound are as follows.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(3,4-dihydroxybenzoyloxy)methyl-3-cephem-4-carboxylic Acid (**11**)

MP >154°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 262 (19,000); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.65 (2H, ABq, 2-H), 4.00 (3H, s, OCH_3), 5.00 (2H, ABq, 3-CH_2), 5.21 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 7.01 (1H, d, J=8 Hz, Ph-H), 7.03 (1H, s, thiazole-H), 7.55~7.6 (2H, m, Ph-H); FAB-MS m/z 550 $(M+H)^+$.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2-(3,4-dihydroxyphenyl)acetoxy]-methyl-3-cephem-4-carboxylic Acid (**12**)

MP >146°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 232 (16,900), 260 (13,300); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.39 (2H, ABq, 2-H), 3.60 (2H, s, C*H*$_2$-Ph), 4.00 (3H, s, OCH_3), 4.86 (2H, ABq, 3-CH_2), 5.17 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 6.71~6.85 (2H, m, Ph-H), 6.88 (1H, d, J=8 Hz, Ph-H), 7.03 (1H, s, thiazole-H); FAB-MS m/z 564 $(M+H)^+$.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-(3,4-dihydroxybenzoyloxy)methyl-3-cephem-4-carboxylic Acid (**13**)

MP >163°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 222 (23,100), 263 (18,800), 296 (sh, 9,900); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.63 (2H, ABq, 2-H), 5.02 (2H, ABq, 3-CH_2), 5.26 (1H, d, J=5 Hz, 6-H), 5.87 (1H, d, J=5 Hz, 7-H), 6.69 (1H, d, J=8 Hz, Ph-H), 6.99 (1H, s, thiazole-H), 7.40 (1H, s, Ph-H), 7.49 (1H, d, J=8 Hz, Ph-H); FAB-MS m/z 536 $(M+H)^+$.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3,4-dihydroxybenzoyloxy)methyl-3-cephem-4-carboxylic Acid (**14**)

MP >155°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 233 (17,000), 262 (13,600); ^{1}H NMR ($D_2O+NaHCO_3$)

δ 1.50 (6H, s, CH_3), 3.38 (2H, ABq, 2-H), 3.60 (2H, s, CH_2-Ph), 5.05 (2H, ABq, 3-CH_2), 5.27 (1H, d, $J=5$ Hz, 6-H), 5.86 (1H, d, $J=5$ Hz, 7-H), 7.01 (1H, d, $J=8$ Hz, Ph-H), 7.02 (1H, s, thiazole-H), 7.56~7.6 (2H, m, Ph-H); FAB-MS m/z 622 $(M+H)^+$.

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