SYNTHESIS OF A NEW SERIES OF CEPHALOSPORINS HAVING 3-SUBSTITUTED-AMMONIO-1-PROPENYL GROUP AS THE C-3 SIDE CHAIN†

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The synthesis and antimicrobial activity of eight derivatives of 7-[(*Z*)-2-(2-aminothiazol-4-yl)- and 7-[(*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]cephalosporins having an (*E*) or (*Z*)-3-ammonio-1-propenyl group in the C-3 side chain are described. The (*E*)-propenyl derivatives were more active than their corresponding *Z*-isomers and showed well-balanced, broad antibacterial activity against both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*.

Among the injectable cephalosporins having a quaternary ammoniomethyl group in the C-3 side chain, cefetazidime[1], cefepime[2], cefpirome[3] and E1040[4] are now marketed or under development. They show enhanced antibacterial activity against Gram-negative bacteria including *Pseudomonas aeruginosa*, but do not always show strong activity against Gram-positive bacteria. As a part of our research program on new injectable cephalosporins possessing improved activity against both Gram-positive and Gram-negative bacteria including *P. aeruginosa*, we have synthesized a new series of aminothiazole and aminothiadiazole cephalosporins with a 3-substituted-ammonio-1-propenyl group as the C-3 side chain. This paper describes the synthesis of these cephalosporins and their antibacterial activity.

Chemistry

The 3-(3-substituted-ammonio-1-propenyl)cephalosporins in this study were prepared by the synthetic routes shown in Schemes 1 and 2. Scheme 1 shows the introduction of a 3-chloro-1-propenyl group by the Wittig reaction into the C-3 position and displacement of the chlorine with iodine. The ylide **1**[5] was treated with freshly prepared chloroacetaldehyde[6] in dichloromethane to give the (*Z*)-3-chloro-1-propenyl derivative **2** in 30% yield after crystallization. The addition of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) improved the yield to 76%, probably because BSA scavenged HCl[7] existing in chloroacetaldehyde (although it was freshly prepared). The ^{1}H NMR spectrum of **2** showed one of the vinyl protons at 6.18 ppm as a doublet with $J=11$ Hz indicating that the product is the *Z*-isomer; the other was obscured by additional coupling with the neighboring methylene protons and also by overlap with the C-7 proton. Predominant formation of the *Z*-isomer was also reported in the Wittig reaction of **1** with acetaldehyde[5,8,9]. The benzylidene group of **2** was removed by treatment with HCl in a mixture of EtOAc and diisopropyl ether

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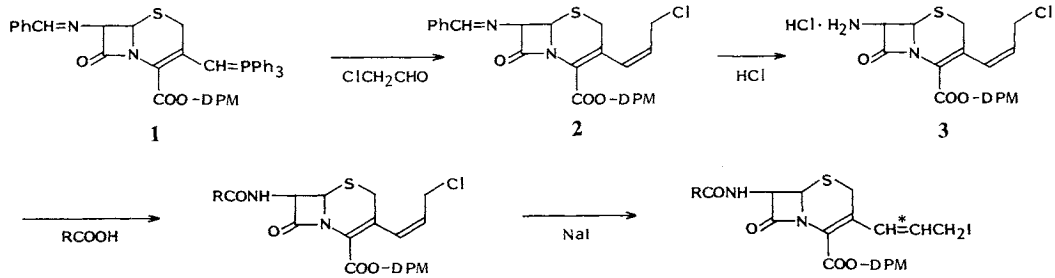
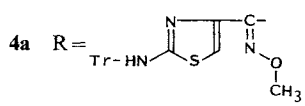
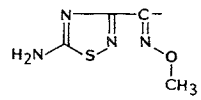
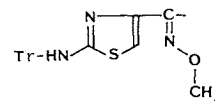
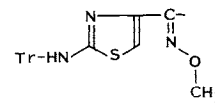
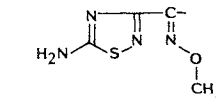
†† Deceased.

Scheme 1. Synthesis of 3-iodopropenyl derivatives **5**.



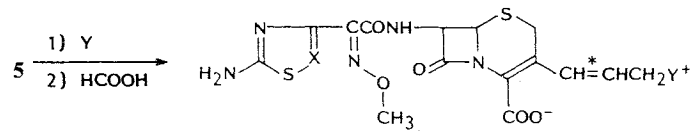
4a R = 

4b R = 

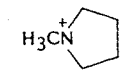
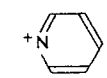
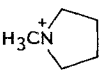
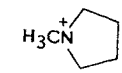
	R	*
5a		*Z*
5b		*E*
5c		*E*

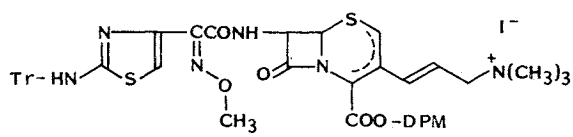
DPM : $CH(C_6H_5)_2$, Tr : $C(C_6H_5)_3$.

Scheme 2. Synthesis of 3-quaternary-ammonio-cephalosporins.



6

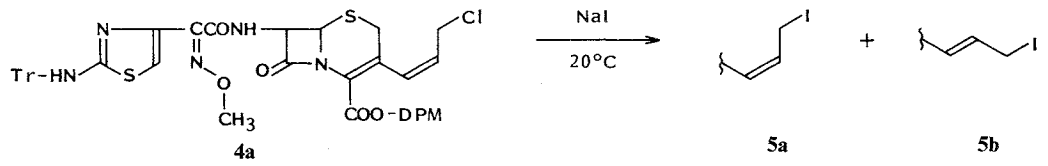
Compound	X	*	Y^+	Compound	X	*	Y^+
6a	CH	*E*		**6e**	CH	*Z*	
6b	CH	*E*	$^+N(CH_3)_3$	**6f**	N	*E*	
6c	CH	*E*		**6g**	N	*E*	$^+N(CH_3)_3$
6d	CH	*Z*	$^+N(CH_3)_3$	**6h**	N	*E*	



7a: Δ^3-Isomer
7b: Δ^2-Isomer

to afford the 7-amino derivative **3** in 81% yield as a crystalline hydrochloride. Compound **3**, pretreated with BSA[10], was acylated with (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-methoxyiminoacetic acid by the acid chloride method[2] to give the crystalline thiazole derivative **4a** in 91% yield. Also, acylation of **3** with (*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetyl chloride[11] gave the thiadiazole derivative **4b** in 98% yield. Since the reactivity of **4** was not great enough for nucleophilic displacement with pyridine or amines, the chlorides **4** were converted into the iodides **5**. Table 1 shows the time-course of the iodination reaction of **4a** with NaI. The reaction of **4a** with NaI at room temperature was monitored by HPLC. With 1 equivalent of NaI in acetone (Entry 1), the reaction was very slow, giving an unseparable mixture of the chloride **4a**, the *Z*-iodide **5a** and the *E*-iodide **5b** even after 5 hours. The iodides decomposed after 18 hours. In the presence of 3 equivalents of NaI in the same solvent (Entry 2), the reaction was fast and, after 1 hour, most of **4a** disappeared and **5b** was obtained as the major product. Prolonged reaction time resulted in decomposition of the products to give smaller peaks in HPLC and, after 18 hours, none of peaks due to **4a**, **5a** and **5b** were observed. With 1 equivalent of NaI in less polar CCl_4 containing 10% acetone (since NaI was quite insoluble in CCl_4, 10% acetone was added; Entry 3), the reaction was slower than that in Entry 2. Decomposition of the products was also slower and, even after 5 hours, the products still remained in the reaction mixture, although they disappeared after 18 hours. For the preparation of **5a** and **5b**, we selected the conditions of Entry 3 (2 hours) and Entry 2 (1 hour), respectively. Purification of the products was unsuccessful to give less pure products due to instability of the iodides. Structures of **5a** and **5b** were confirmed by predominant peaks of the ^1H NMR spectra of the products containing **5a** and **5b** as the major component, respectively. The **5a**-major product showed a doublet at 6.05 ppm with a coupling constant of 11 Hz, while the **5b**-major product showed a doublet at 6.85 ppm with a coupling constant of 16 Hz, indicating that **5a** is the *Z*-isomer and **5b** is the *E*-isomer of the C-3 side chain. The corresponding thiadiazolyl derivative was prepared from **4b** under the reaction conditions similar to Entry 2 (2 hours) to give the product containing the *E*-isomer (**5c**) predominantly. Since the iodides were unstable,

Table 1. HPLC study on iodination of the chloride **4a**.



4a **5a** **5b**

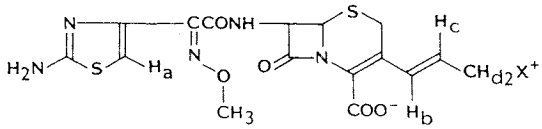
Entry	Solvent	NaI (equiv)	Component	Amount of products (%) in the reaction mixture[a]						
				0.5 hour	1 hour	2 hours	3 hours	4 hours	5 hours	18 hours
1	Acetone	1	**4a**	70	59	54	50	40	30	15
			5a	22	25	23	25	19	13	ND
			5b	8	16	23	25	24	14	ND
2	Acetone	3	**4a**	12	2	0.3	ND	ND	ND	ND
			5a	44	15	3	0.7	ND	ND	ND
			5b	44	70	44	30	25	15	ND
3	CCl_4-acetone (10:1)	1	**4a**	49	27	17	13	11	10	ND
			5a	43	60	67	68	67	62	ND
			5b	8	13	16	19	22	25	ND

[a] Estimated by HPLC: Column SSC-ODS-262, solvent MeCN-H_2O (80:20), flow rate 2 ml/minute, detection 236 nm, Rt **4a** (4.59 minutes), **5a** (5.74 minutes), **5b** (6.17 minutes).

ND: Not detected.

the products were used without purification for the next step. Scheme 2 shows the synthesis of the 3-ammonio-cephalosporins by quaternization of **5** with pyridine or aliphatic amines followed by deblocking. The *E*-iodide **5b** was quaternized with pyridine in DMF and the reaction proceeded homogeneously to give a quaternized product, which was deblocked with formic acid (40°C, 1 hour) and subsequently purified by column chromatography to give the (*E*)-3-pyridinio-1-propenyl derivative of aminothiazolyl cephalosporin **6a** in 18% overall yield from **5b**. In a similar way, the aminothiadiazolyl cephalosporin **6f** was synthesized in 17% overall yield. Quaternization of the iodide with more basic aliphatic amines resulted in some formation of the corresponding Δ^2-cephems. The reaction of **5b** with trimethylamine in DMF gave a 1:1 mixture of the desired quaternary salt **7a** and its unwanted Δ^2-isomer **7b**, based on the ^{1}H NMR spectrum. However, when the reaction was carried out in toluene or ether, the desired **7a** was precipitated in high yield from the reaction mixture. Presumably, employment of the solvents in which **7a** is hardly soluble prevents the trimethylamine induced migration of the double bond of **7a**, by immediate precipitation of **7a** from the reaction mixture as it is formed. The quaternary salt **7a** was deblocked to give the (*E*)-3-trimethylammonio-1-propenyl derivative **6b** in 27% overall yield. In a similar way, the *N*-methylpyrrolidiniocephalosporin **6c** and the *Z*-cephalosporins, **6d** and **6e**, were synthesized in 3~20% overall yield. Since solubility of the thiadiazole iodide **5c** in toluene was very low, quaternizations of **5c** with trimethylamine and *N*-methylpyrrolidine were carried out in EtOAc. The desired quaternized products, free from the Δ^2 isomer, precipitated from the reaction mixture. The quaternized products were deblocked to give the aminothiadiazolyl cephalosporins **6g** and **6h** in 35 and 20% overall yields, respectively. The UV, ^{1}H NMR and mass spectral data of **6a**~**6h** are summarized in Tables 2~5. ^{1}H NMR spectra of **6a**~**6c** and **6f**~**6h** showed a doublet at 6.94~7.08 ppm with a coupling constant of 16 Hz indicating that these cephalosporins have an (*E*)-3-ammonio-1-propenyl group, whereas those of **6d** and **6e** showed a doublet at 6.63~6.64 ppm with a coupling constant of 11 Hz characteristic of the *Z*-configuration. Their UV spectra showed the longest-wave absorption maximum at 292~293 nm for the *E*-isomers and at 285~286 nm for the *Z*-isomers.

Table 2. ^{1}H NMR of the thiazole *E*-propenyl cephalosporins.



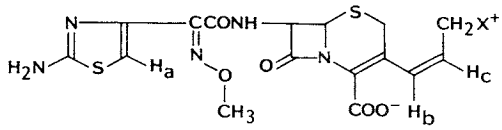
6a~**6c**

Compound	X^+	^{1}H NMR (80 MHz, D_2O, ppm)								
		H_a (s)	OCH_3 (s)	7-H (d, J=5 Hz)	6-H (d, J=5 Hz)	2-H (s)	H_b (d, J=16 Hz)	H_c[a] (dt, J=16, 7 Hz)	H_d[b] (d, J=7 Hz)	X^+
6a		7.04	4.06	5.86	5.28	3.70	6.94	6.14	5.40	8.0~9.0 (5H, m)
6b	$^+N(CH_3)_3$	7.15	4.14	5.88	5.35	3.80	7.00	6.08	4.07	3.15 (9H, s)
6c		7.06	4.08	5.87	5.33	3.75	6.95	6.00	4.10	2.25 (4H, m), 3.10 (3H, s), 3.58 (4H, m)

[a] Partially overlapped with 7-H.

[b] **6a**, partially overlapped with 6-H; **6b** and **6c**, partially overlapped with OCH_3.

Table 3. ^{1}H NMR of the thiazole *Z*-propenyl cephalosporins.

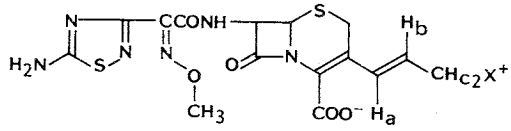


6d, 6e

Compound	X^+	^{1}H NMR (80 MHz, D_2O, ppm)						
		H_a (s)	OCH_3 (s)	7-H (d, J=5 Hz)	6-H (d, J=5 Hz)	2-H (ABq, J=18 Hz)	H_b (d, J=11 Hz)	X^+
6d	$^+N(CH_3)_3$	7.08	4.08	5.93	5.35	3.55	6.64	3.15 (9H, s)
6e		7.08	4.10	5.92	5.35	[a]	6.63	2.25 (4H, m), 3.10 (3H, s), 3.58 (4H, m)

[a] Overlapped with pyrrolidine.
H_c: Overlapped with 7-H.

Table 4. ^{1}H NMR of the thiadiazole cephalosporins.



6f ~ 6h

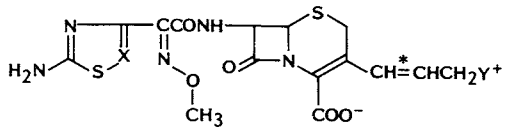
Compound	X^+	^{1}N NMR (80 MHz, D_2O, ppm)							
		OCH_3 (s)	7-H (d, J=5 Hz)	6-H (d, J=5 Hz)	2-H (s)	H_a (d, J=16 Hz)	H_b[a] (dt, J=16, 7 Hz)	H_c[b] (d, J=7 Hz)	X^+
6f		4.22	5.96	5.37	3.78	7.04	6.22	5.47	8.0 ~ 9.1 (5H, m)
6g	$^+N(CH_3)_3$	4.25	5.99	5.42	3.85	7.06	6.09	4.14	3.22 (9H, s)
6h		4.25	6.00	5.42	3.85	7.08	6.10	4.16	2.36 (4H, m), 3.18 (3H, s), 3.63 (4H, m)

[a] Partially overlapped with 7-H.
[b] **6f**, partially overlapped with 6-H; **6g** and **6h**, partially overlapped with OCH_3.

Table 5. Yield, mp, UV and mass data.

Compound	Yield from iodide (%)	MP (°C, dec)	UV $\lambda_{max}^{pH\,7\ buffer}$ nm (ε)	Mass (SI-MS) (m/z), $(M+H)^+$
6a	18	150	234 (16,400), 259 (16,600), 266 (16,300), 293 (23,100)	501
6b	27	190	227 (15,800), 292 (25,200)	481
6c	20	160	232 (16,700), 292 (25,600)	507
6d	17	150	233 (14,500), 286 (15,600)	481
6e	3	130	233 (12,000), 285 (12,400)	507
6f	17	160	239 (20,300), 259 (18,600), 261 (18,100), 292 (22,700)	502
6g	35	165	237 (18,900), 293 (22,000)	482
6h	20	185	236 (19,500), 293 (22,500)	508

Table 6. *In vitro* activity.



Compound	Structure			MIC (μg/ml)							
	X	*	Y^+	*S.a.* A9537	*S.a.* BX-1633	*E.c.* Juhl	*K.p.* D11	*P.m.* A9544	*E.cl.* A9656	*S.m.* A20019	*P.a.* A9843A
6a	CH	*E*		0.2	0.4	0.013	<0.0063	0.025	0.025	0.05	3.1
6b	CH	*E*	$^+N(CH_3)_3$	0.2	0.4	0.025	<0.0063	0.025	0.025	0.025	1.6
6c	CH	*E*		0.2	0.4	0.025	<0.0063	0.025	0.05	0.05	1.6
6d	CH	*Z*	$^+N(CH_3)_3$	0.8	1.6	0.05	0.013	0.05	0.05	0.10	3.1
6e	CH	*Z*		0.4	0.8	0.05	0.013	0.05	0.10	0.10	3.1
6f	N	*E*		0.2	0.4	0.025	0.013	0.025	0.025	0.05	1.6
6g	N	*E*	$^+N(CH_3)_3$	0.4	0.4	0.013	0.013	0.025	0.025	0.05	1.6
6h	N	*E*		0.2	0.4	0.025	0.025	0.05	0.05	0.10	1.6
Ceftazidime				6.3	12.5	0.10	0.025	0.025	0.10	0.05	1.6

Abbreviations: *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *E.cl.*, *Enterobacter cloacae*; *S.m.*, *Serratia marcescens*; *P.a.*, *Pseudomonas aeruginosa*.

Biological Activity

Table 6 shows the *in vitro* activity of the cephalosporins prepared in this study. MICs of the cephalosporins against 8 test organisms selected from Gram-positive and Gram-negative bacteria including *P. aeruginosa*, were determined by 2-fold serial agar dilution in Mueller-Hinton agar. In the case of cephalosporins having non-substituted propenyl group as the C-3 side chain such as BMY-28100[9] and BMY-28232[5], the *Z*-propenyl derivatives were more active than the corresponding *E*-propenyl derivatives especially against Gram-negative organisms. In the present series of cephalosporins, however, the (*Z*)-3-ammonio-1-propenyl cephalosporins **6d** and **6e** were 2~4 times less active against all of the organisms tested than the corresponding *E*-isomers **6b** and **6c**, respectively. At this time, we have no reasonable explanation on the reversed structure-activity relationships. Both the aminothiazolyl (**6a**, **6b** and **6c**) and the aminothiadiazolyl cephalosporins (**6f**, **6g** and **6h**) having the *E* configuration in the C-3 side chain showed very similar activity. They were 16~32 times more active than ceftazidime against two strains of *Staphylococcus aureus*, 4~8 times more active against *Escherichia coli* Juhl and showed nearly equal or slightly superior activity against other Gram-negative strains including *P. aeruginosa*.

Experimental

MP's were determined using a Yanagimoto micro hot-stage apparatus and uncorrected. IR spectra were recorded on a Jasco IRA-1 and UV spectra on a Shimadzu UV-200 spectrophotometer. NMR spectra were recorded on a Varian FT-80A (80 MHz) or a Jeol GX-400 (400 MHz) spectrometer and mass spectra on a Hitachi M-80 (SI-MS) or a Jeol JMS-AX505H (FAB) mass spectrometer.

Diphenylmethyl 7-Benzylideneamino-3-[(*Z*)-3-chloro-1-propenyl]-3-cephem-4-carboxylate (**2**)

To a chilled (−10°C) solution of **1** (36.4 g, 50 mmol) and BSA (6.82 ml, 25 mmol) in dry dichloromethane (364 ml) was added a solution of freshly prepared chloroacetaldehyde (15% solution in chloroform, 52.7 g, 0.1 mol). The mixture was stirred for 16 hours under an argon atmosphere at −10°C and then concentrated under reduced pressure. The residue was suspended in EtOAc (50 ml), filtered, and the solid was washed with EtOAc (2 × 5 ml). The filtrate and washings were combined and added dropwise into isopropyl alcohol with stirring at 5°C during a period of 20 minutes. After sitrring for 1 hour, the crystalline product (20.1 g, yield 76%) was collected by filtration. MP 85~90°C; IR ν_{max} (KBr) cm^{-1} 1770, 1720, 1630; UV $\lambda_{max}^{CHCl_3}$ nm (ε) 255 (20,800); ^1H NMR (80 MHz, $CDCl_3$) δ 3.26 (1H, d, J=18 Hz, 2-H), 3.58 (1H, d, J=18 Hz, 2-H), 3.5~4.0 (2H, m, 3-CH=CHCH_2), 5.20 (1H, d, J=5 Hz, 6-H), 5.45 (1H, dd, J=5 and 2 Hz, 7-H), 5.60 (1H, m, 2-CH=C*H*), 6.18 (1H, d, J=11 Hz, 3-C*H*=CH), 6.93 (1H, s, C*H*Ph_2), 7.1~7.6 (13H, phenyl protons), 7.75 (2H, m, phenyl protons), 8.60 (1H, d, J=2 Hz, CH=N).

Anal Calcd for $C_{30}H_{25}N_2O_3SCl$: C 68.11, H 4.76, N 5.30, S 6.06, Cl 6.70.
Found: C 68.11, H 4.79, N 5.01, S 5.93, Cl 6.87.

Diphenylmethyl 7-Amino-3-[(*Z*)-3-chloro-1-propenyl]-3-cephem-4-carboxylate Hydrochloride (**3**)

To a chilled solution of **2** (5.29 g, 10 mmol) in EtOAc (30 ml) and isopropyl ether (15 ml) was added a solution of HCl (1.5 N in MeOH, 10 ml). The mixture was allowed to stand for 4 hours at room temperature to precipitate the crystalline product which was collected by filtration and dried *in vacuo*. Yield 3.86 g (81%). MP 187~191°C (dec); IR ν_{max} (KBr) cm^{-1} 3200~2500, 1780, 1718; UV λ_{max}^{EtOH} nm (ε) 285 (8,200): ^1H NMR (80 MHz, DMSO-d_6) δ 3.66 (2H, s, 2-H), 3.90 (2H, m, 3-CH=CHCH_2), 5.16 (1H, d, J=5 Hz, 7-H), 5.34 (1H, d, J=5 Hz, 6-H), 5.60 (1H, m, 3-CH=C*H*), 6.35 (1H, d, J=12 Hz, 3-C*H*=CH), 6.85 (1H, s, C*H*Ph_2), 7.1~7.6 (10H, m, phenyl protons).

Anal Calcd for $C_{23}H_{21}N_2O_3SCl \cdot HCl$: C 57.87, H 4.65, N 5.87, S 6.72, Cl 14.85.
Found: C 57.62, H 4.53, N 5.70, S 6.64, Cl 14.89.

Diphenylmethyl 7-[(*Z*)-2-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-[(*Z*)-3-chloro-1-propenyl]-3-cephem-4-carboxylate (**4a**)

Phosphorus pentachloride (4.16 g, 20 mmol) was added to an ice-cooled mixture of (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-methoxyiminoacetic acid (8.64 g, 19.5 mmol) in dichloromethane (80 ml) and the mixture was stirred for 0.5 hour at room temperature. BSA (11 ml, 45 mmol) was added to a suspension of **3** (7.11 g, 14.9 mmol) in dichloromethane (80 ml) with stirring to give a clear solution. To the above mixture was added the acid chloride solution with stirring and cooling; the mixture was stirred for 0.5 hour at room temperature. The mixture was diluted with dichloromethane, washed with water and aq $NaHCO_3$ successively and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel (Merck Kiesel gel 60, 150 g). The column was eluted with dichloromethane and the fractions containing the desired product were combined. Evaporation under reduced pressure gave 11.8 g (91%) of the product as an amorphous powder. An analytical sample was prepared by crystallization from EtOAc. Colorless prisms. MP 196~200°C (dec); IR ν_{max} (KBr) cm^{-1} 3340, 1785, 1740, 1690, 1530; UV λ_{max}^{EtOH} nm (ε) 295 (sh, 12,000); ^1H NMR (400 MHz, $CDCl_3$) δ 3.34 (1H, d, J=18 Hz, 2-H), 3.47 (1H, dd, J=12 and 8 Hz, 3-CH=CHCH_2), 3.53 (1H, d, J=18 Hz, 2-H), 3.81 (1H, dd, J=12 and 9 Hz, 3-CH=CHCH_2), 4.08 (3H, s, OCH_3), 5.12 (1H, d, J=5 Hz, 6-H), 5.63 (1H, ddd, J=11, 9 and 8 Hz, 3-CH=C*H*CH_2), 5.97 (1H, dd, J=9 and 5 Hz, 7-H), 6.22 (1H, br d, J=11 Hz, 3-C*H*=$CHCH_2$), 6.75 (1H, s, thiazole-H), 6.82 (1H, d, J=9 Hz, CONH), 6.92 (1H, s, Ph_2CH), 7.01 (1H, s, Tr-NH), 7.25~7.42 (25H, m, phenyl-H).

Anal Calcd for $C_{48}H_{40}N_5O_5S_2Cl \cdot H_2O$: C 65.18, H 4.79, N 7.92, S 7.25, Cl 4.01.
Found: C 65.54, H 4.44, N 8.05, S 7.34, Cl 4.01.

Diphenylmethyl 7-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-[(*Z*)-3-chloro-1-propenyl]-3-cephem-4-carboxylate (**4b**)

To a stirred solution of **3** (20 g, 42 mmol) in dichloromethane (420 ml) containing BSA (34 ml, 125 mmol) was added (*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetyl chloride hydrochloride (15.2 g, 59 mmol) in three portions over a period of 30 minutes at −10~0°C. The mixture was stirred for 30 minutes at 0~5°C and concentrated under reduced pressure. The residue was dissolved in EtOAc (420 ml) and the solution was washed successively with aq $NaHCO_3$, dil HCl, and water and then dried. After concentration to about 50 ml, the concentrate was diluted with *n*-heptane (200 ml) to give 25.7 g of the product as an colorless powder. Yield 98%. IR ν_{max} (KBr) cm^{-1} 3300, 1780, 1725, 1680, 1620; UV λ_{max}^{EtOH} nm (ε) 283 (12,000); ^1H NMR (80 MHz, acetone-d_6) δ 3.60 (2H, ABq, 2-H), 3.95 (3H, s, OCH_3), 4.0 (2H, m, 3-CH=CHCH_2), 5.32 (1H, d, J=5 Hz, 6-H), 5.62 (1H, m, 3-CH=C*H*CH_2), 6.03 (1H, dd, J=8 and 5 Hz, 7-H), 6.32 (1H, d, J=11 Hz, 3-C*H*=$CHCH_2$), 6.87 (1H, s, Ph_2CH), 7.2~7.6 (10H, m, phenyl protons), 8.45 (1H, d, J=8 Hz, CONH).

An analytical sample was prepared as follows; a solution of the amorphous product in EtOAc was washed with 10% HCl and aq NaCl successively and allowed to stand to precipitate the crystalline hydrochloride. MP 144°C (dec).

Anal Calcd for $C_{28}H_{25}N_6O_5S_2Cl \cdot HCl \cdot H_2O$: C 49.49, H 4.15, N 12.37, S 9.44, Cl 10.43.
Found: C 49.61, H 3.70, N 12.24, S 9.45, Cl 10.27.

Diphenylmethyl 7-[(*Z*)-2-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-[(*Z*)-3-iodo-1-propenyl]-3-cephem-4-carboxylate (**5a**)

Compound **5a** was prepared according to the conditions selected by the HPLC study (Table 1: Entry 3; 2 hours). A solution of NaI (86.5 mg, 0.58 mmol) in acetone (0.5 ml) was added to a stirred solution of **4a** (503 mg, 0.58 mmol) in CCl_4 (5 ml). The mixture was stirred for 2 hours at room temperature, washed with 10% aq $Na_2S_2O_3$ and water, and dried. Evaporation *in vacuo* below 25°C gave 475 mg of an amorphous powder containing **5a** as a major product (**4a** - **5a** - **5b**, 15 : 75 : 10, estimated by HPLC). Due to its instability, the product was used for the preparation of **6d** and **6e** without further purification. IR ν_{max} (KBr) cm^{-1} 1785, 1725, 1665: ^1H NMR (400 MHz, $CDCl_3$) δ 3.30 (1H, m, CH=CHCH_2), 3.38 (1H, d, J=18 Hz, 2-H), 3.62 (1H, d, J=18 Hz, 2-H), 3.64 (1H, m, CH=CHCH_2), 4.09 (3H, s, OCH_3), 5.14 (1H, d, J=5 Hz, 6-H), 5.76 (1H, m, CH=C*H*CH_2), 5.97 (1H, dd, J=5 and 9 Hz, 7-H), 6.05 (1H, d, J=11 Hz, C*H*=$CHCH_2$), 6.77 (1H, s, thiazole), 6.79 (1H, d, J=9 Hz, CONH), 6.93 (1H, s, Ph_2CH), 7.00 (1H, s, Tr-NH), 7.25~7.41

(25H, m, phenyl).

Diphenylmethyl 7-[(*Z*)-2-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-[(*E*)-3-iodo-1-propenyl]-3-cephem-4-carboxylate (**5b**)

Compound **5b** was prepared according to the conditions selected by the HPLC study (Table 1: Entry 2; 1 hour). A mixture of **4a** (1.90 g, 2.2 mmol) and NaI (1.0 g, 6.7 mmol) in dry acetone (20 ml) was stirred for 1 hour at room temperature. The mixture was concentrated *in vacuo* and the residue was dissolved in EtOAc (50 ml). The mixture was washed with aq $Na_2S_2O_3$ and aq sat NaCl successively and dried. Removal of the solvent under reduced pressure gave 2.0 g of an amorphous powder containing **5b** as a major product (**4a** - **5a** - **5b**, 2 : 15 : 83, estimated by HPLC). Due to its instability, the product was used for the preparation of **6a**, **6b** and **6c** without further purification. IR ν_{max} (KBr) cm^{-1} 1780, 1720, 1680; UV λ_{max}^{EtOH} nm (ε) 306 (17,000); ^1H NMR (400 MHz, $CDCl_3$) δ 3.52 (1H, d, J=18 Hz, 2-H), 3.58 (1H, d, J=18 Hz, 2-H), 3.86 (2H, m, CH=CHCH_2), 4.08 (3H, s, OCH_3), 5.08 (1H, d, J=5 Hz, 6-H), 5.93 (1H, dd, J=5 and 9 Hz, 7-H), 6.11 (1H, m, CH=C*H*CH_2), 6.76 (1H, s, thiazole), 6.82 (1H, d, J=9 Hz, CONH), 6.85 (1H, d, J=16 Hz, C*H*=$CHCH_2$), 7.00 (1H, s, NH), 7.02 (1H, s, Ph_2CH), 7.2~7.5 (25H, m, phenyl); FAB-MS m/z 958 $(M+H)^+$.

Diphenylmethyl 7-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-iodo-1-propenyl]-3-cephem-4-carboxylate (**5c**)

A mixture of **4b** (2.85 g, 4.56 mmol) and sodium iodide (1.9 g, 12.6 mmol) in dry acetone (40 ml) was stirred for 2 hours at room temperature. The mixture was concentrated under reduced pressure. To the residue was added EtOAc and the mixture was washed with 10% aq $Na_2S_2O_4$ and water successively and dried. Evaporation under reduced pressure gave 3.06 g of the product as an amorphous powder containing **5c** as a major product (80% pure, estimated by HPLC†). Due to its instability, the product was used for the preparation of **6f**, **6g** and **6h** without further purification. MP 120°C; IR ν_{max} (KBr) cm^{-1} 1780, 1725, 1680, 1620; UV λ_{max}^{EtOH} nm (ε) 306 (15,000); ^1H NMR (80 MHz, acetone-d_6) δ 3.71 (2H, ABq, 2-H), 3.97 (3H, s, OCH_3), 4.0 (2H, m, CH=CHCH_2), 5.26 (1H, d, J=5 Hz, 6-H), 6.03 (1H, dd, J=5 and 8 Hz, 7-H), 6.32 (1H, m, CH=C*H*CH_2), 6.79 (1H, d, J=15 Hz, C*H*=$CHCH_2$), 6.98 (1H, s, Ph_2CH), 7.35 (10H, m, phenyl-H), 7.63 (2H, br s, NH_2), 8.52 (1H, d, J=8 Hz, CON*H*).

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-pyridinio-1-propenyl]-3-cephem-4-carboxylate (**6a**)

Pyridine (0.43 ml, 5.3 mmol) was added to a chilled solution (−10°C) of **5b** (3.4 g, 3.5 mmol) in DMF (5 ml). The mixture was stirred for 4 hours at the same temperature and poured into 10% $Na_2S_2O_3$. The quaternary salt (3.3 g) was collected by filtration, washed with water and dried. A mixture of the quaternary salt and 98% formic acid (10 ml) was heated for 1 hour at 40°C, concentrated under reduced pressure and the residue was chromatographed on a column of Prep C_{18} (Waters, 100 ml). The column was eluted with water and 5~15% aq MeOH successively. The fractions were monitored by HPLC and the desired fractions were combined. After concentration followed by freeze-drying, 312 mg (18%) of **6a** was obtained as an amorphous powder.

Compound **6f** was prepared from **5c** by a procedure similar to that described above. The ^1H NMR data of **6a** and **6f** are summarized in Tables 2 and 4, respectively. Yields, UV and mass data are summarized in Table 5.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-trimethylammonio-1-propenyl]-3-cephem-4-carboxylate (**6b**)

A solution of trimethylamine (2 M solution in toluene, 4 ml) was added dropwise to a chilled (−10°C) solution of **5b** (3.83 g, 4 mmol) in toluene (60 ml) and the mixture was stirred for 1 hour at the same temperature to precipitate the quaternary salt **7a** (3.8 g), which was collected by filtration and dried. ^1H NMR (400 MHz, $CDCl_3$) δ 3.18 (9H, s, $N(CH_3)_3$), 3.67 (1H, d, J=19 Hz, 2-H), 3.87 (1H, d, J=19 Hz,

† Column: SSC-ODS-262, solvent; MeCN - pH 7 phosphate buffer (55 : 45), flow rate; 2 ml/minute, Rt; **5c** (8.19 minutes).

2-H), 4.02 (1H, m, 3-CH=CHCH_2), 4.05 (3H, s, OCH_3), 4.19 (1H, m, 3-CH=CHCH_2), 5.12 (1H, d, J=5 Hz, 6-H), 5.92 (1H, dd, J=9 and 5 Hz, 7-H), 6.11 (1H, m, 3-CH=CHCH$_2$), 6.70 (1H, s, thiazole-H), 6.94 (1H, s, Ph_2CH), 7.02 (1H, d, J=9 Hz, CONH), 7.03 (1H, d, J=15 Hz, 3-CH=CHCH$_2$), 7.15~7.45 (25H, m, phenyl protons).

Reaction of **5b** with trimethylamine-DMF gave a 1:1 mixture of **7a** and **7b** as follows. A solution of trimethylamine (2 M solution in toluene, 0.093 ml) was added to a chilled (−10°C) solution of **5b** (89 mg, 0.093 mmol) in DMF (0.5 ml). The mixture was stirred for 1 hour at −10°C and diluted with CH_2Cl_2. The mixture was washed with water, dried and concentrated *in vacuo*. Toluene was added to afford the precipitate (45 mg), which was collected by filtration. 400 MHz ^{1}H NMR ($CDCl_3$) spectra of the precipitate showed comparable pairs of peaks due to 6-H and 7-H of **7a** and **7b**. Peaks due to **7a**: δ 5.12 (d, J=5 Hz, 6-H), 5.92 (dd, J=5 and 9 Hz, 7-H). Peaks due to **7b**: δ 5.26 (d, J=5 Hz, 6-H), 5.51 (dd, J=5 and 9 Hz, 7-H). A singlet due to 4-H of **7b** was also observed at 5.23 ppm.

The quaternary salt **7a** prepared in the above was used for the preparation of **6b** without further purification. Thus, a mixture of **7a** (3.65 g) and 98% formic acid (4 ml) was heated at 40°C for 1 hour and concentrated under reduced pressure. The residue was chromatographed on a column of Prep C_{18} (Waters, 100 ml) and the column was eluted with water, 5~20% MeOH successively. The methanolic fractions containing the desired product were combined, concentrated under reduced pressure and freeze-dried to give 515 mg (27%) of **6b** as an amorphous powder.

Compounds **6c**, **6d** and **6e** were prepared by a similar procedure. The ^{1}H NMR data of **6b** and **6c** are summarized in Table 2 and those of **6d** and **6e** in Table 3. Yields, UV and mass data are summarized in Table 5.

7-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-[(*E*)-trimethylammonio-1-propenyl]-3-cephem-4-carboxylate (**6g**)

To a chilled (−5°C) solution of **5c** (1.3 g, 1.8 mmol) in dry EtOAc (4 ml) was added a solution of trimethylamine (1.1 M in EtOAc, 1.75 ml) and the mixture was stirred for 1 hour at −5°C. The precipitated quaternary salt (1.25 g) was collected by filtration and dried. A mixture of the quaternary salt (1.04 g) and 85% formic acid (2 ml) was stirred for 3 hours at room temperature and concentrated under reduced pressure. The residue was chromatographed on a column of Diaion HP-20 (30 ml) and the column was eluted with water and 10~30% MeOH successively. The fraction containing the product was concentrated and chromatographed again on a column of Prep C_{18} (Waters, 30 ml). The column was eluted with water and then 30% MeOH successively, followed by concentration and freeze-drying to give 252 mg (35%) of the product as an amorphous powder.

Compound **6h** was prepared by a similar procedure. The ^{1}H NMR data of **6g** and **6h** are summarized in Table 4. Yields, UV and mass data are summarized in Table 5.

Determination of MICs

MICs were determined on solid medium by the standard 2-fold agar dilution method[12] in Mueller-Hinton Agar (Difco). Overnight broth cultures served as the source of inoculum. A volume of approximately 0.003 ml of the diluted culture containing 10^6 cfu/ml was applied to the surface of the antibiotic-containing agar plates with a multi-inoculator. After incubation at 37°C for 18 hours, plates were examined for colony development, and the lowest concentration of antibiotic causing no visible growth was recorded as the MIC.

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