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Orally Active Cephalosporins: Synthesis, Structure–activity Relationships and Oral Absorption of 3-[(*E*) and (*Z*)-2-Substituted Vinyl]-cephalosporins

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Abstract—A series of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)- and (*Z*)-2-substituted vinyl]-3-cephem-4-carboxylic acids was designed and synthesized using palladium-catalyzed coupling reactions of a 3-methanesulfonyloxy-3-cephem and an *E* substituted vinyl stannane or Wittig reaction of a 3-triphenylphosphoniummethyl cephem and an aldehyde as a key step. These compounds were evaluated for in vitro antibacterial activity and oral absorption in rats. A number of them exhibited excellent antibacterial activity against both Gram-positive and Gram-negative bacteria including *Haemophilus influenzae*. Among them, FR86524 (**2j**), having a (*Z*)-2-(3-pyridyl)vinyl moiety at the C-3 position, had the most well balanced activity. Although FR86254 exhibited low oral absorption, the pivaloyloxymethyl ester (**23**) of FR86524 showed improved oral absorption.

Introduction

Recently, new oral cephalosporins with broad spectra of activity and high stability against various β-lactamases, such as cefixime[1] (CFIX), cefteram pivoxil[2] (CFTM-PI), cefpodoxime proxetil[3] (CPDX-PR), cefdinir[4] (CDFN) and cefditoren pivoxil[5] (CDTR-PI) have been developed and introduced to clinical practice. In particular, CFDN, which was discovered in our laboratories, has excellent antibacterial activity against both Gram-positive and Gram-negative bacteria and high oral absorption.[4,6–8] However, CFDN exhibits relatively low efficacy against *H. influenzae*, an important pathogen that is the cause of severe respiratory infections. Thus, our next research objective was to discover a new oral cephalosporin having more potent activity against both Gram-positive and Gram-negative bacteria including *H. influenzae*.

In the last decade many cephalosporins have been synthesized and evaluated for antibacterial activity. Among these compounds, those having functional groups such as methoxy,[3] carbamoyloxy[9] or a heteroaromatic ring such as tetrazole[2] or thiazole[5] in the C-3 side chain, showed potent antibacterial activity against Gram-negative bacteria. Also, as we have previously described, the (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetyl moiety of the 3-vinylcephem series seems to be crucial for both potent activity against Gram-positive and Gram-negative bacteria and high oral absorption.[10,11] Keeping these results in mind, we then designed a series of novel 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-cephalosporins having various functional groups and/or heteroaromatic rings in the C-3 side chain connected through a vinyl bond to the cephem nucleus, expecting well balanced and more potent antibacterial activity, including *H. influenzae*, as well as high oral absorption.

We report herein the synthesis, structure–activity relationships and oral absorption of a series of novel 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido] -3-[(*E*)- and (*Z*)-2-substituted vinyl] cephalosporin derivatives (Fig. 1).

Keywords: cephalosporins; FR86524; oral absorption; *Haemophilus influenzae*.
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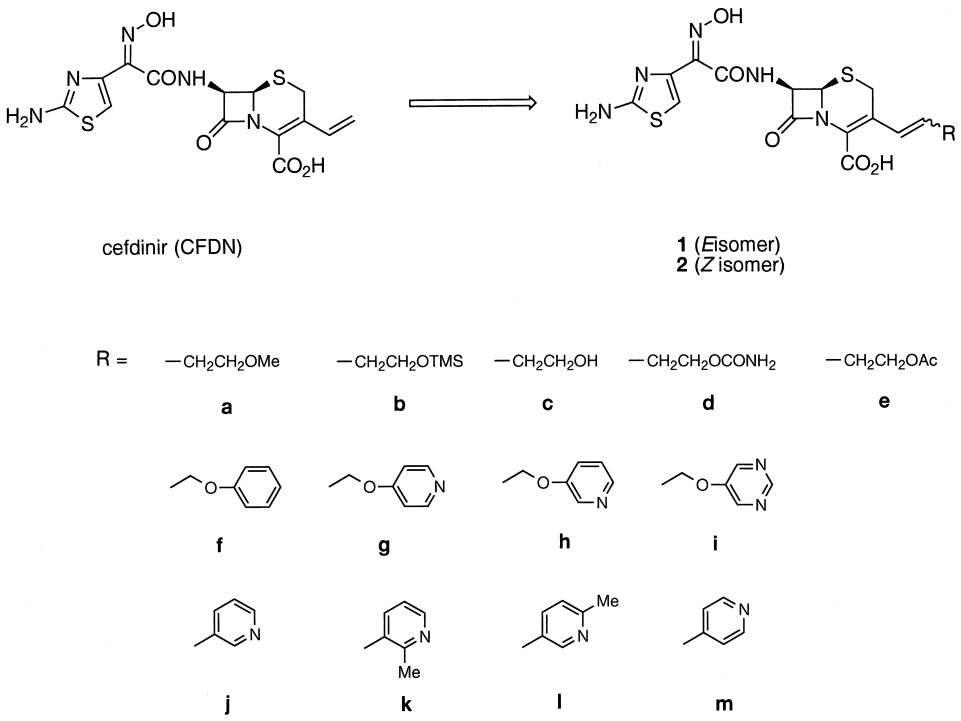


Figure 1.

Results and Discussion

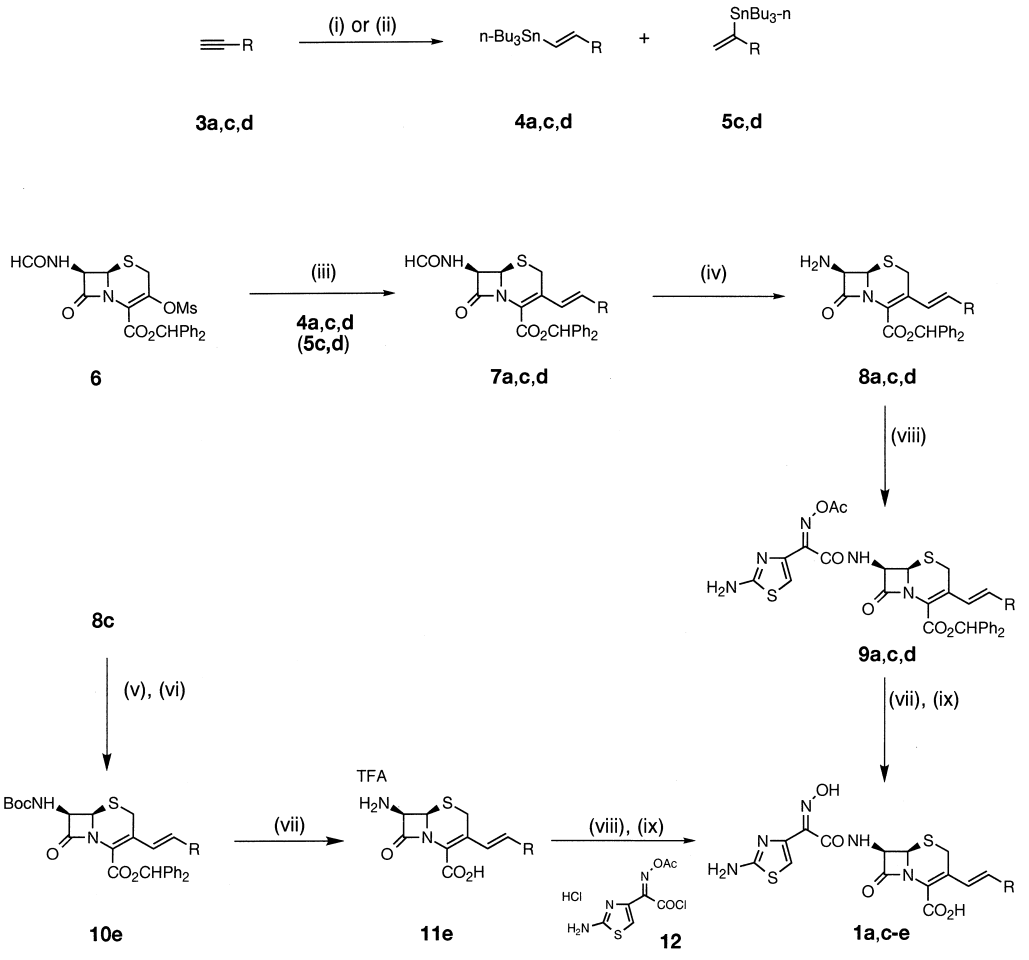
Chemistry

Schemes 1 and 2 show the two methods used for the synthesis of 3-[(*E*) and (*Z*)-2- substituted vinyl] cephems **1,2**. The preparation of **1a,c–e** was carried out according to Scheme 1 and that of **1j,m** and **2a,c,d,f–l** by the method in Scheme 2.

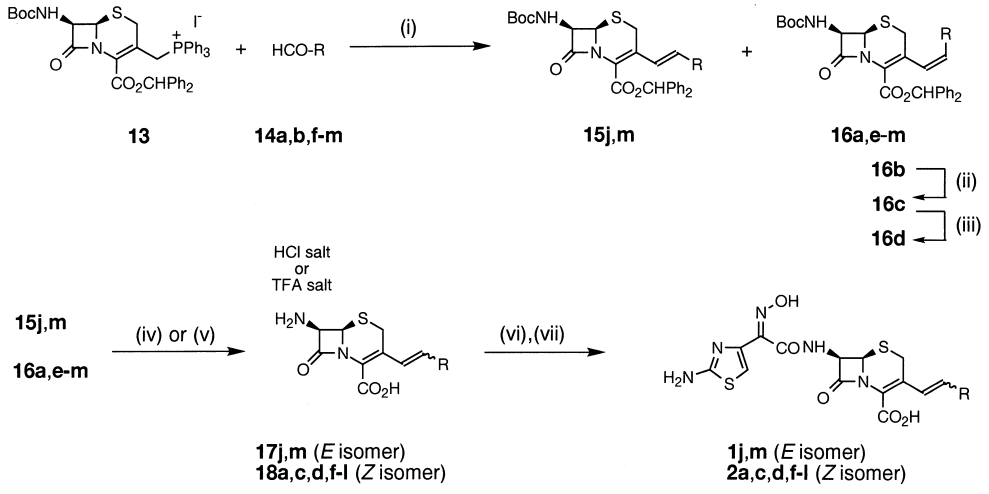
Scheme 1 involves the palladium-catalyzed coupling reaction of diphenylmethyl 7β-formamido-3-methanesulfonyloxy-3-cephem-4-carboxylate[12] (**6**) and an *E* substituted vinyl stannane (**4**) to introduce the *E* substituted double bond.[13] The vinyl stannanes (**4**) were prepared by hydrostannylation of the appropriate acetylene (**3**) with tributyltin hydride in the presence of azobisisobutyronitrile (AIBN) or dichlorobis(triphenylphosphine)palladium (II). In the palladium catalyzed hydrostannylation reaction, a mixture of *E* substituted vinyl stannane (**4**) and 1,1-disubstituted vinyl stannane (**5**) was obtained. Although the mixture of **4** and **5** was used in the next coupling reaction without separation, only **4** reacted with **6**, leading to **7** as the sole product. Subsequent deprotection of the C-7 formyl function of **7** was carried out using concentrated HCl in MeOH to give **8**, and was followed by acylation with (*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetylchloride hydrochloride[14] (**12**) in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) to give fully protected cephem **9**. Deprotection of **9** was carried out stepwise. Thus, the diphenylmethyl ester was first removed using TFA-anisole in CH_2Cl_2, followed by deacetylation of the acetoxyimino group under basic conditions, to afford the desired cephems **1a,c,d**. The synthesis of **1e** was achieved by a different route. The 7β-amino group of **8c** was protected with Boc and the hydroxy group in the C-3 side chain was acetylated (**10e**). After removal of the protecting groups, 7β-aminocephem TFA salt (**11e**) was treated with **12**, followed by deacetylation of the acetoxyimino group according to a similar procedure to that described above, to afford **1e**.

The method outlined in Scheme 2 involved Wittig reaction of the 3-triphenylphosphoniummethyl cephem[15] (**13**) and an aldehyde (**14**) to introduce the C-3 double bond. In most cases, the *Z* substituted vinyl cephem was produced exclusively. In the case of 3-pyridinecarboxyaldehyde (**14j**) and 4-pyridinecarboxyaldehyde (**14m**), mixtures of *E,Z* isomers were produced and readily separated by column chromatography on silica-gel to give the respective *E*-isomers (**15j,m**) and *Z*-isomers (**16j,m**). Successive treatment of **15,16** with TFA-anisole or formic acid-hydrochloric acid gave the deprotected compounds **17,18**. However, 3-[(*Z*)-2-(4-pyridyl)vinyl]-cephem (**16m**) was isomerized under the TFA deprotection conditions to give the *E* isomer (**17m**) as the sole product. Introduction of the C-7 side chain according to the procedure described above afforded **9a,c,d**.

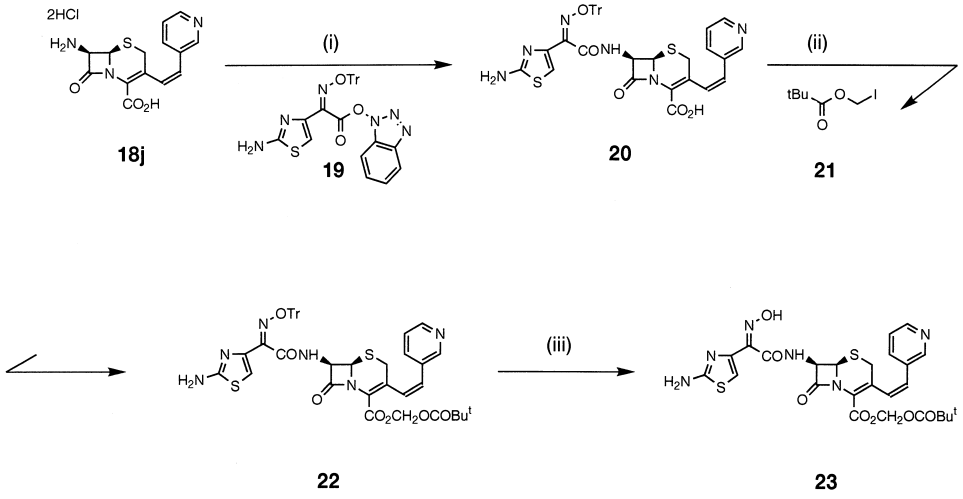
Scheme 3 shows the synthesis of the pivaloyloxymethylester of **2j**. The C-7 side chain was introduced by treating **18j** with the C-7 side chain HOBt ester[16] (**19**) in the presence of trimethylsilylchloride and Et_3N to give **20**, which was then reacted with pivaloyloxymethyliodide in the presence of potassium carbonate in DMF to afford the corresponding ester **22**. Selective deprotection of the trityl group from **22** was performed using 90% aqueous formic acid at room temperature to give pivaloyloxymethyl ester **23**.



Scheme 1. Reagents: (i) $Pd(Ph_3)_2Cl_2$, n-Bu_3SnH, THF. (ii) *n*-Bu_3SnH, AIBN, C_6H_6. (iii) $Pd(CH_3CN)_2Cl_2$, LiBr, DMF.(iv) cHCl, MeOH. (v) Boc_2O, *N*-monotrimethylsilycaetamide(MSA), THF. (vi) acetyl chloride, Et_3N, CH_2Cl_2. (vii) TFA, anisole, CH_2Cl_2. (viii) **12**, *N*,*O*-bistrimethylsilyl acetamide(BSA), CH_2Cl_2. (ix) $NaHCO_3$, NH_4Cl, MeOH-H_2O.



Scheme 2. Reagents: (i) a)1N NaOH, aq.NaCl(sat.), CH_2Cl_2, b) Separation (ii) cHCl, MeOH. (iii) a) trichloroacetyl isocyanate, CH_2Cl_2, b) SiO_2, $CHCl_3$, MeOH. (iv) TFA, anisole, CH_2Cl_2. (v) HCO_2H, cHCl. (vi) BSA or MSA, (*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacethyl chloride hydrochloride(**12**),CH_2Cl_2.(vii) $NaHCO_3$, NH_4Cl, MeOH, H_2O.



Scheme 3. Reagents: (i) $(CH_3)_3SiCl$, Et_3N, THF. (ii) K_2CO_3, DMF. (iii) 90% HCO_2Haq.

Biological results

The in vitro antibacterial activity of the new cephalosporins against Gram-positive and Gram-negative bacteria are shown in Table 1. For comparison, CFDN was employed as a reference drug. As can be seen from this data, all of the synthesized compounds exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria.

The effect of the C-3 double bond stereochemistry on the antibacterial activity is significant; the activity of the *Z* isomers against Gram-negative bacteria, including *H. influenzae* is far superior to that of the corresponding *E* isomers (**1a,c,d,j** versus **2a,c,d,j**), whereas in terms of activity against *Enterococcus faecalis*, the *E* isomers were more potent.

Compounds (**1a,c,d**) having a functional group such as methoxyethyl, hydroxyethyl, or carbamoyloxyethyl at the head of the C-3 double bond in the *E* form showed decreased antibacterial activity against all organisms tested. Although **2a,c,d** (*Z* isomers) exhibited increased antibacterial activity against *H. influenzae*, they exhibited decreased antibacterial activity against *Staphylococcus aureus*. Thus, introduction of a functional group to the C-3 double bond did not have a beneficial effect on antibacterial activity.

The aryloxymethylenevinyl cephem analogues (**2f–i**) exhibited similar potent antibacterial activity against *S. aureus*, but decreased antibacterial activity against *Morachisella catarrhalis*. Although **2f** having a phenyloxymethylenevinyl moiety showed dramatically decreased antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*, introduction of a heteroaromatic ring such as pyridine or pyrazine (**2g–i**) gave compounds that displayed excellent antibacterial activity against these organisms. In particular, **2h** having a 3-pyridyl moiety in the C-3 side chain exhibited the most well balanced activity together with potent antibacterial activity against *H. influenze*. Thus, introduction of heteroaromatic ring especially pyridine in the C-3 side chain was effective.

Table 1. Antibacterial activity of cephalosporins (**1**,**2**)[a]

Drugs	MIC (μg/ml)					
	S.a[b]	E.f.	M.c.	H.i.	E.c.	K.p
1a	0.84	17	0.46	0.93	0.57	0.57
1c	0.78	21	0.31	1.29	0.67	0.53
1d	0.78	18	0.36	0.84	0.57	0.36
1e	0.78	17	0.49	0.63	0.78	0.67
1j	0.39	1.69	1.15	0.112	0.195	0.18
1m	0.39	0.57	2.1	0.116	0.143	0.105
2a	0.98	68	0.33	0.22	0.31	0.25
2c	1.15	79	0.25	0.24	0.155	0.066
2d	0.91	50	0.25	0.26	0.143	0.071
2f	0.29	21	0.984	0.28	1.56	1.24
2g	0.53	25	0.53	0.43	0.113	0.105
2h	0.27	23	0.39	0.067	0.167	0.132
2i	0.39	40	0.49	0.133	0.18	0.097
2j	0.29	7.3	0.195	0.047	0.097	0.041
2k	0.27	3.6	0.033	0.063	0.167	0.09
2l	0.33	6.8	0.42	0.041	0.23	0.132
CFDN	0.33	13.5	0.072	0.41	0.133	0.062

[a]Müller-Hinton agar; 10^{-2}, stamp method; 37°C, 20 h.
[b]S.A., *Staphylococcus aureus* (MSSA) (9); E.f., *Enterococcus faecalis* (9); M.c., *Morachisella catarrhalis* (9); H.i., *Haemophilus influenzae* (20);E.c., *Escherichia coli* (9); K.p., *Klebsiella pneumoniae* (9). CFDN: cefdinir.

Analogues having a pyridylvinyl moiety at the C-3 position (**1j,m** and **2j–k**) also exhibited potent activity against Gram-positive bacteria. In the (*E*)-pyridylvinyl cephem series, the 3-pyridyl cephem (**1j**) showed almost the same antibacterial activity as the 4-pyridyl cephem (**1m**), and showed increased antibacterial activity against both *E. faecalis* and *H. influenzae* compared with CFDN, whereas antibacterial activity against *M. catarrhalis* was significantly decreased. The (*Z*)-2-(pyridin-3-yl)vinyl derivatives (**2j–l**) exhibited dramatically increased antibacterial activity against *H. influenzae*. Especially, **2j** showed the most well balanced, potent activity. Compared **2j** with **2k** and **2l**, methyl group

substitution at the 2- or 4-position of 3-pyridylvinyl moiety had only a marginal effect on antibacterial activity, although **2k** with a (*Z*)-2-(2-methylpyridin-3-yl)-vinyl moiety, exhibited increased antibacterial activity against *M. catarrhalis*. Consequently, aryloxymethylenevinyl cephems were superior to the series of functionalized vinyl cephems in terms of antibacterial activity against *S. aureus*, although except for **2f**, they displayed similar antibacterial activity against other organisms. Further, pyridylvinyl cephems showed more potent antibacterial activity against *E. faecalis*, *M. catarrhalis* and *H. influenzae* than aryloxymethylenevinyl cephems. Thus, (*Z*)-pyridylvinyl cephems are superior to the other two series of (*Z*)-substituted vinyl cephems with regards antibacterial activity and balance. Among all compounds prepared, FR86524 (**2j**) exhibited the most well balanced activity both Gram-positive and negative bacteria, and especially against *H. influenzae* FR86524 was 10-fold more active than CFDN. Thus, we considered FR86524 as an attractive optimal compound as far as antibacterial activity was concerned.

The urinary and biliary recovery of these compounds after oral administration to rats are shown in Table 2. Compounds having aromatic ring moieties and functional groups in the C-3 side chain showed relatively low oral absorption in rats, except for carbamoyloxy cephem (**2d**), which exhibited good oral absorption. The effect of the C-3 double bond stereochemistry on oral absorption is unclear, since **2d** (*Z* isomer) showed twice as high oral absorption as **1d** (*E* isomer), whereas the other *Z* isomers (**2a,c,j**) showed similar low absorption compared with the *E* isomers (**1a,c,j**). However, the results for **2d** indicate the possibility that the linker moiety between the functional group and the cephem nucleus plays an important role in oral absorption. Additionally, methyl group substitution at the 2- or 4-position of the 3-pyridylvinyl moiety has only a marginal effect on oral absorption.

To improve oral absorption, the pivaloyloxymethyl ester (**23**) of FR86524 (**2j**) was synthesized and evaluated, and found to display improved oral absorption, thus it is potentially applicable as an orally absorbable pro-drug form of FR86524.

Table 2. 24-Hour urinary and biliary recovery after oral administration (20 mg/kg) to rats

Drugs	Recovery (%)		Drugs	Recovery (%)	
	Urine	Bile		Urine	Bile
1a	5.74	1.40	**2f**	1.53	2.55
1c	7.43	1.24	**2g**	0.30	1.28
1d	7.33	3.24	**2h**	2.26	2.80
1e	2.43	0.15	**2i**	2.62	1.97
1j	1.4	1.22	**2j**	3.76	0.62
1m*	0.15	1.1	**2k**	2.77	1.83
2a	8.50	1.01	**2l**	2.15	1.58
2c	5.37	0.64	**23**	15.1	1.51
2d	18.3	2.90	**CFDN**	32.5	1.40

*Oral administration (20 mg/kg) to mice.

Conclusion

As a result of modification of the C-3 double bond of CFDN, we discovered FR86524 (**2j**) having a (*Z*)-2-(3-pyridyl)vinyl moiety at the C-3 position, which exhibits potent antibacterial activity against both Gram-positive and Gram-negative bacteria, including *H. influenzae*. Although FR86524 showed poor oral absorption in rats, the pivaloyloxymethyl ester (**23**) of FR86524 showed improved oral absorption. Thus, FR86524 was selected as a favorable parent compound for further optimization. Our further studies to optimize the absorption and activity of compounds derived from FR86524 will be presented in subsequent papers.

Experimental

IR spectra were recorded on a Hitachi 260-10 spectrophotometer. NMR spectra were recorded at 90 MHz on a Varian EM-390 NMR spectrometer, a Hitachi R-90H NMR spectrometer or a Bruker AC200P at 200 MHz. Chemical shifts are reported in ppm downfield from TMS as internal standard. Mass spectra were obtained on a Hewlett–Packard 1100LC-MSD instrument. Elemental analyses were carried out on a Perkin–Elmer 2400 CHN Elemental Analyzer.

Hydrostannylation of acetylenes (3)

Method A. To a solution of 4-methoxy-1-butyne (17.5 g) in benzene (170 mL) was added AIBN (683 mg) under reflux. To this solution was added *n*-Bu_3SnH (56.0 mL) in benzene (50 mL) over 50 min. After refluxing for a further 1 h, the mixture was cooled to room temperature and evaporated in vacuo to give crude **4a**.

(*E*)-4-Methoxy-1-tri(*n*-butyl)stannyl-1-butene (4a). Amorphous solid. Yield: 82.7 g (quant.). ^{1}H NMR (DMSO-d_6) δ 0.8–1.0 (15H, m), 1.2–1.6 (12H, m), 2.3–2.5 (2H, m), 3.33 (3H, s), 3.44 (2H, t, $J = 7$ Hz), 5.96 (2H, m).

Method B. To a solution of 4-carbamoyloxy-1-butyne (11.31 g) in THF (100 mL) was added $Pd(PPh_3)_2Cl_2$ (702 mg). *n*-Bu_3SnH (27.11 g) was then added to the mixture over 25 min at room temperature. The mixture was stirred for 1 h at room temperature and evaporated in vacuo. The residue was chromatographed on silica-gel, eluting with ethyl acetate–hexane to give a 1:1 mixture of **4d** and **5d**.

(*E*)-1-Tri(*n*-butyl)stannyl-4-carbamoyloxy-1-butene (4d) and 2-tri(*n*-butyl)stannyl-4-carbamoyloxy-1-butene (5d). Amorphous solid. Yield: 30.9 g (76%). IR (Nujol) cm^{-1} 3300, 2900, 1700, 1585; ^{1}H NMR (DMSO-d_6) δ 0.8–1.7 (27H, m), 2.4–2.5 (2H, m), 4.1–4.2 (2H, m), 4.71 (2H, br s), 5.89 (1H, dt, $J = 18.3$, 5.6 Hz), 6.14 (1H, d, $J = 18.3$ Hz).

A 1:1 mixture of **4c** and **5c** was obtained using a method B.

(*E*)-1-Tri(*n*-butyl)stannyl-4-hydroxy-1-butene (4c) and 2-tri(*n*-butyl)stannyl-4-hydroxy-1-butene (5c). Amorphous

solid. Yield: 301.2 g (58%). IR (Nujol) cm^{-1} 3250, 2900, 1580; ^1H NMR (DMSO-d_6) δ 0.8–1.7 (27H, m), 2.3–2.5 (2H, m), 3.6–3.75 (2H, m), 5.8–6.3 (2H, m).

Coupling reaction of mesylate (6) with stannylbutene (4). To a solution of diphenylmethyl 7β-formamido-3-methanesulfonyloxy-3-cephem-4-carboxylate (**6**) (63.22 g) in DMF (630 mL) was added a 1:1 mixture of (*E*)-1-tri-(*n*-butyl)stannyl-4-carbamoyloxy-1-butene (**4d**) and 2-tri(*n*-butyl)stannyl-4-carbamoyloxy-1-butene (**5d**) (52.3 g) and dry LiBr (22.41 g) at 5 °C, and the mixture was degassed under a N_2 stream for 5 min, $Pd(CH_3CN)_2Cl_2$ (669 mg) was added to the mixture and the whole mixture was stirred at 5 °C for 1 h and at room temperature for 14 h under a N_2 stream. The mixture was poured into cold H_2O (3.5 L) and the resulting precipitate was collected by filtration and washed with cold water. The precipitate was dissolved in a mixture of THF (400 mL) and ethyl acetate (800 mL), and H_2O (400 mL) was added to the solution. The aqueous layer was separated, the organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated and the residue was chromatographed on silica-gel, eluting with ethyl acetate-IPE to give **7d**.

Diphenylmethyl 7β-formamido-3-[(*E*)-4-carbamoyloxy-1-buten-1-yl]-3-cephem-4-carboxylate (7d). Amorphous solid. Yield: 14.2 g (22%). IR (Nujol) cm^{-1} 3400, 3250, 1760, 1710, 1670; ^1H NMR (DMSO-d_6) δ 2.2–2.4 (2H, m), 3.62 and 3.85 (2H, ABq, J = 17.5 Hz), 3.90 (2H, t, J = 6.6 Hz), 5.21 (1H, d, J = 4.8 Hz), 5.82 (1H, dd, J = 9.3, 4.9 Hz), 6.12 (1H, dt, J = 15.9, 6.7 Hz), 6.47 (2H, br s), 6.57 (1H, d, J = 15.9 Hz), 6.98 (1H, s), 7.3–7.6 (10H, m), 8.16 (1H, s), 9.13 (1H, d, J = 9.3 Hz).

Diphenylmethyl 7β-formamido-3-[(*E*)-4-methoxy-1-buten-1-yl]-3-cephem-4-carboxylate (7a). Amorphous solid. Yield: 19.3 g (37%). ^1H NMR (DMSO-d_6) δ 2.24 (2H, q, J = 6 Hz), 3.18 (3H, s), 3.62 and 3.85 (2H, ABq, J = 17 Hz), 3.90 (2H, t, J = 6.6 Hz), 5.21 (1H, d, J = 5 Hz), 5.82 (1H, dd, J = 9.1, 5.1 Hz), 6.1–6.2 (1H, m), 6.55 (1H, d, J = 16 Hz), 6.98 (1H, s), 7.2–7.5 (10H, m), 8.17 (1H, s), 9.13 (1H, d, J = 9 Hz).

Diphenylmethyl 7β-formamido-3-[(*E*)-4-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylate (7c). Amorphous solid. Yield: 64.3 g (30%). IR (Nujol) cm^{-1} 3600–3100 (br), 1755, 1600; ^1H NMR (DMSO-d_6) δ 2.17 (2H, m), 3.41 (2H, q, J = 6 Hz), 3.62 and 3.85 (2H, ABq, J = 17 Hz), 4.56 (2H, t, J = 5 Hz), 5.21 (1H, d, J = 5 Hz), 5.80 (1H, dd, J = 9.3, 4.9 Hz), 6.17 (1H, dt, J = 16, 7 Hz), 6.55 (1H, s), 6.57 (1H, d, J = 16 Hz), 6.96 (1H, s), 7.2–7.5 (10H, m), 8.16 (1H, s), 9.12 (1H, d, J = 9 Hz).

Diphenylmethyl 7β-amino-3-[(*E*)-4-carbamoyloxy-1-buten-1-yl]-3-cephem-4-carboxylate (8d). To a solution of **7d** (14.12 g) in MeOH (140 mL) was added dropwise concd HCl (23.2 mL) at 10 °C. The mixture was stirred at room temperature for 4 h, then poured into a mixture of ethyl acetate and cold H_2O, and neutralized with sat.$NaHCO_3$. The separated organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated in vacuo to give **8d**. Amorphous solid. Yield: 12.0 g (90%). ^1H NMR (DMSO-d_6) δ 2.2–2.4 (2H, m), 2.34 (2H, br s), 3.54 and 3.80 (2H, ABq, J = 17.7 Hz), 3.89 (2H, t, J = 6.7 Hz), 4.82 (1H, d, J = 5.0 Hz), 5.05 (1H, d, J = 5.0 Hz), 6.04 (1H, dt, J = 15.8, 6.9 Hz), 6.47 (1H, d, J = 15.8 Hz), 6.47 (2H, br s), 6.96 (1H, s), 7.3–7.6 (10H, m).

The following compounds were obtained using a method similar to that used for **8d**.

Diphenylmethyl 7β-amino-3-[(*E*)-4-methoxy-1-buten-1-yl]-3-cephem-4-carboxylate (8a). Amorphous solid. Yield: 11.2 g (62%). ^1H NMR (DMSO-d_6) δ 2.21 (2H, q, J = 7 Hz), 2.41 (2H, br s), 3.18 (3H, s), 3.54 and 3.81 (2H, ABq, J = 18 Hz), 4.82 (1H, d, J = 5 Hz), 5.05 (1H, d, J = 5.0 Hz), 6.0–6.2 (1H, m), 6.45 (1H, d, J = 16 Hz), 6.56 (1H, s), 7.2–7.5 (10H, m).

Diphenylmethyl 7β-amino-3-[(*E*)-4-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylate (8c). Amorphous solid. Yield: 1.7 g (51%). IR (Nujol) cm^{-1} 3300, 1745, 1690; ^1H NMR (DMSO-d_6) δ 2.15 (2H, q, J = 7 Hz), 2.32 (2H, br s), 3.39 (1H, dt, J = 6.7 Hz), 3.54 and 3.81 (2H, ABq, J = 18 Hz), 4.56 (1H, t, J = 5 Hz), 4.81 (1H, br s), 5.05 (1H, d, J = 5.0 Hz), 6.09 (1H, dt, J = 16, 7.1 Hz), 6.47 (1H, d, J = 16 Hz), 6.96 (1H, s), 7.2–7.6 (10H, m).

Diphenylmethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetamido]-3-[(*E*)-4-carbamoyloxy-1-buten-1-yl]-3-cephem-4-carboxylate (9d). To a solution of **8d** (8.02 g) in CH_2Cl_2 (240 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (7.01 g) and the mixture was stirred for 25 min at room temperature. The mixture was cooled to 5 °C with ice and (*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetyl chloride hydrochloride (**12**) (5.90 g) was added. After stirring for 4 h at the same temperature, the mixture was poured into a mixture of ethyl acetate and cold H_2O and neutralized with sat.$NaHCO_3$. The aqueous layer was separated and the organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated in vacuo to give **9d**. Amorphous solid. Yield: 10.7 g (92%). IR (Nujol) cm^{-1} 3300, 1745, 1690; ^1H NMR (DMSO-d_6) δ 2.17 (3H, s), 2.2–2.4 (2H, m), 3.59 and 3.83 (2H, ABq, J = 17.8 Hz), 3.89 (2H, t, J = 6.6 Hz), 5.28 (1H, d, J = 4.7 Hz), 5.87 (1H, dd, J = 8.1, 4.7 Hz), 6.11 (1H, dt, J = 16.0, 6.7 Hz), 6.46 (2H, br s), 6.56 (1H, d, J = 16.0 Hz), 6.96 (1H, s), 7.11 (1H, s), 7.3–7.6 (12H, m), 9.93 (1H, d, J = 8.1 Hz).

The following compounds were obtained using a method similar to that used for **9d**.

Diphenylmethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetamido]-3-[(*E*)-4-methoxy-1-buten-1-yl]-3-cephem-4-carboxylate (9a). Amorphous solid. Yield: 15.3 g (95%). ^1H NMR (DMSO-d_6) δ 2.17 (3H, s), 2.2–2.4 (2H, m), 3.16 (3H, s), 3.3–3.4 (2H, m), 3.62 and 3.79 (2H, ABq, J = 18 Hz), 5.27 (1H, d, J = 5 Hz), 5.87 (1H, dd, J = 8.1, 5.1 Hz), 6.0–6.2 (1H, m), 6.54 (1H, d, J = 16 Hz), 7.10 (1H, s), 7.2–7.6 (12H, m), 9.93 (1H, d, J = 8 Hz).

Diphenylmethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetamido]-3-[(*E*)-4-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylate (9c). Amorphous solid. Yield: 2.9 g (quant.). IR (Nujol) cm^{-1} 3500–3000, 1750, 1680, 1620, 1510; ^1H

NMR (DMSO-d_6) δ 2.0–2.1 (2H, m), 2.13 (3H, s), 3.35 (2H, t, $J=6$ Hz), 3.59 and 3.81 (2H, ABq, $J=17$ Hz), 5.23 (1H, d, $J=5$ Hz), 5.81 (1H, dd, $J=8.1$, 5.1 Hz), 6.13 (1H, dt, $J=16.0$, 7 Hz), 6.52 (1H, d, $J=16$ Hz), 6.91 (1H, s), 7.07 (1H, s), 7.2–7.5 (12H, m), 9.89 (1H, d, $J=8$ Hz).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)-4-carbamoyloxy-1-buten-1-yl]-3-cephem-4-carboxylic acid (1d). To a solution of **9d** (0.71 g) in CH_2Cl_2 (50 mL) and anisole (10 mL) was added TFA (20 mL) under ice-cooling. After stirring at 5 °C for 2 h, the mixture was poured into IPE (800 mL). The resulting precipitate was collected by filtration and dried under reduced pressure. The precipitate was suspended in a mixture of H_2O (700 mL) and MeOH (35 mL) and NH_4Cl (2.54 g) was added and the mixture adjusted to pH 8 with aq $NaHCO_3$ solution. The mixture was stirred for 30 min at room temperature maintaining pH 8 with aq $NaHCO_3$ solution. The solution was adjusted to pH 6 with 1N HCl, and evaporated in vacuo to remove MeOH. The solution was subjected to column chromatography on HP-20 and eluted with 5% aqueous isopropyl alcohol. Fractions containing the object compound were collected and lyophilized to give crude product, which was purified by preparative HPLC utilizing a C18 μ Bondapak resin to afford **1d**. Amorphous solid. Yield: 727 mg (10%). IR (Nujol) cm^{-1} 1720, 1640; ^1H NMR (DMSO-d_6) δ 2.4–2.55 (2H, m), 3.55 and 3.75 (2H, ABq, $J=17.4$ Hz), 3.96 (2H, t, $J=6.6$ Hz), 5.16 (1H, d, $J=4.8$ Hz), 5.74 (1H, dd, $J=8.1$, 4.8 Hz), 6.01 (1H, dt, $J=15.9$, 6.9 Hz), 6.46 (2H, br s), 6.67 (1H, s), 6.75 (1H, d, $J=15.9$ Hz), 7.12 (2H, br s), 9.47 (1H, d, $J=8.1$ Hz), 11.31 (1H, s). ESIMS (neg.) *m/z* 481 [$(M-H)^+$]. Anal. calcd for $C_{17}H_{18}N_6O_7S_2\cdot 2.8H_2O$: C, 38.31; H, 4.46; N, 15.77; found: C, 38.07; H, 4.17; N, 15.58.

The following compounds were obtained using a method similar to that used for **1d**.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)-4-methoxy-1-buten-1-yl]-3-cephem-4-carboxylic acid (1a). Amorphous solid. Yield: 326 mg (3%). IR (Nujol) cm^{-1} 3300–3100, 1750, 1620–1500; ^1H NMR (DMSO-d_6) δ 2.37 (2H, q, $J=6$ Hz), 3.23 (3H, s), 3.39 (2H, t, $J=6$ Hz), 3.55 and 3.77 (2H, ABq, $J=18$ Hz), 5.16 (1H, d, $J=5$ Hz), 5.74 (1H, dd, $J=8.1$, 5.1 Hz), 6.06 (1H, dt, $J=16$, 7 Hz), 6.67 (1H, s), 6.73 (1H, d, $J=16$ Hz), 7.12 (2H, br s), 9.47 (1H, d, $J=8.1$ Hz), 11.3 (1H, s). ESIMS (neg.) *m/e* 452[$(M-H)^+$]. Anal. calcd for $C_{17}H_{19}N_5O_6S_2\cdot 2.28H_2O$: C, 41.29; H, 4.80; N, 14.16; found: C, 41.62; H, 4.67; N, 13.82.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)-4-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylic acid (1c). Amorphous solid. Yield: 515 mg (35%). IR (Nujol) cm^{-1} 3300–3000, 1740, 1620; ^1H NMR (DMSO-d_6) δ 2.28–2.55 (2H, q, $J=7$ Hz), 3.46 (2H, t, $J=7$ Hz), 3.55 and 3.75 (2H, ABq, $J=17$ Hz), 5.15 (1H, d, $J=5$ Hz), 5.73 (1H, dd, $J=8.1$, 5.1 Hz), 6.07 (1H, dt, $J=16$, 7.1 Hz), 6.67 (1H, s), 6.74 (1H, d, $J=16$ Hz), 7.13 (2H, br s), 9.47 (1H, d, $J=8.1$ Hz), 11.3 (1H, s).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-4-acetoxy-1-buten-1-yl]-3-cephem-4-carboxylate (10e). To a solution of **8c** (8.45 g) in THF (100 mL) was added MSA (11.72 g) at 45°C. After 10 min, di(*tert*-butyl)dicarbonate (5.86 g) was added to the mixture and the whole mixture was stirred for 7 h at the same temperature. The mixture was concentrated in vacuo and the residue was chromatographed on silica-gel, eluting with ethyl acetate:hexane to give diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-4-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylate (6.95 g). IR (Nujol) cm^{-1} 3510, 3320, 1740, 1705, 1670; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 2.18 (2H, td, $J=4.4$, 6.6 Hz), 3.42 (2H, td, $J=4.4$, 5.3 Hz), 3.58 and 3.79 (2H, ABq, $J=17.2$ Hz), 4.57 (1H, t, $J=5.3$ Hz), 5.14 (1H, t, $J=4.7$ Hz), 5.48 (1H, dd, $J=8.9$, 4.7 Hz), 6.19 (1H, dd, $J=15.9$, 7.0 Hz), 6.63 (1H, d, $J=15.9$ Hz), 6.94 (1H, s), 7.3–7.6 (10H, m), 8.04 (1H, d, $J=8.9$ Hz). To a solution of this compound (6.94 g) in CH_2Cl_2 was added Et_3N (1.31 mL) and acetyl chloride (1.22 g) in CH_2Cl_2 (10 mL) successively at −20°C. The mixture was stirred for 2.5 h at the same temperature and poured into cold H_2O. The aqueous layer was separated and the organic layer was washed with 1N HCl and brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on silica-gel, eluting with ethyl acetate–hexane to give **10e**. Amorphous solid. Yield: 4.0 g (54%). ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 1.95 (1H, s), 2.25–2.4 (2H, m), 3.58 and 3.81 (2H, ABq, $J=17.4$ Hz), 3.99 (2H, t, $J=6.4$ Hz), 5.14 (1H, t, $J=4.7$ Hz), 5.51 (1H, dd, $J=9.1$, 4.7 Hz), 6.14 (1H, dt, $J=15.9$, 6.8 Hz), 6.59 (1H, d, $J=15.9$ Hz), 6.95 (1H, s), 7.3–7.5 (10H, m), 8.05 (1H, d, $J=9.1$ Hz).

Compound **11e** was obtained using a method similar to that used for **17m**.

7β-Amino-3-[(*E*)-4-acetoxy-1-buten-1-yl]-3-cephem-4-carboxylic acid trifluoroacetate (11e). Amorphous solid. Yield: 2.1 g (70%). ^1H NMR (DMSO-d_6) δ 2.00 (3H, s), 2.43 (2H, td, $J=6.5$, 6.9 Hz), 3.59 and 3.79 (2H, ABq, $J=17.4$ Hz), 4.06 (2H, t, $J=6.5$ Hz), 4.89 (1H, d, $J=5.0$ Hz), 5.08 (1H, d, $J=5.0$ Hz), 6.07 (1H, dt, $J=16.0$, 6.9 Hz), 6.76 (1H, d, $J=16.0$ Hz).

Compound **1e** was obtained using a method similar to that used for **2j**.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)-4-acetoxy-1-buten-1-yl]-3-cephem-4-carboxylic acid (1e). Amorphous solid. Yield: 595 mg (25%). IR (Nujol) cm^{-1} 1760, 1710; ^1H NMR (DMSO-d_6) δ 2.00 (3H, s), 2.45 (2H, td, $J=6.6$, 7.0 Hz), 3.55 and 3.78 (2H, ABq, $J=17.6$ Hz), 4.06 (2H, t, $J=6.6$ Hz), 5.17 (1H, d, $J=4.8$ Hz), 5.76 (1H, dd, $J=8.1$, 4.8 Hz), 6.04 (1H, dd, $J=16.0$, 7.0 Hz), 6.67 (1H, s), 6.74 (1H, d, $J=16.0$ Hz), 7.12 (2H, br s), 9.47 (1H, d, $J=8.1$ Hz), 11.30 (1H, s). ESIMS *m/z* 480[$(M+H)^+$]. Anal. calcd for $C_{18}H_{19}N_5O_7S_2\cdot 2.27H_2O$: C, 41.39; H, 4.54; N, 13.41; found: C, 41.70; H, 4.41; N, 13.09.

Synthesis of 15j + 16j by Wittig reaction. To a mixture of diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-triphenylphosphoniomethyl-3-cephem-4-carboxylate iodide (**13**) (100 g), CH_2Cl_2 (1000 mL) and saturated

aqueous NaCl (170 mL) was added 1N NaOH (173 mL) with stirring at room temperature. After stirring at the same temperature for 45 min, the separated organic layer was washed with brine and dried over $MgSO_4$. To this organic layer was added 3-pyridinecarboxaldehyde (**14j**) (24.6 g) at room temperature, and the mixture stirred at the same temperature for 20 h. The solvent was evaporated in vacuo and the crude product purified by column chromatography on silica-gel, eluting with ethyl acetate-hexane.

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-2-(pyridin-3-yl)vinyl]-3-cephem-4-carboxylate (15j). Amorphous solid. Yield: 7.8 g (12%). IR (Nujol) cm^{-1} 1770, 1700; ^1H NMR (DMSO-d_6) δ 1.42 (9H, s), 3.75 and 4.05 (2H, ABq, $J=17.5$ Hz), 5.21 (1H, d, $J=4.8$ Hz), 5.58 (1H, dd, $J=8.8$,4.8 Hz), 7.12 (1H, s), 7.18 (1H, d, $J=16.2$ Hz), 7.27–7.58 (13H, m), 8.12 (1H, d, $J=8.8$ Hz), 8.45 (2H, m).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-2-(pyridin-3-yl)vinyl]-3-cephem-4-carboxylate (16j). Amorphous solid. Yield: 21.0 g (32%). IR (Nujol) cm^{-1} 1765, 1700; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.30 and 3.56 (2H, ABq, $J=17.5$ Hz), 5.21 (1H, d, $J=4.8$ Hz), 5.56 (1H, dd, $J=8.3$,4.8 Hz), 6.49 (1H, d, $J=12.2$ Hz), 6.55 (1H, d, $J=12.2$ Hz), 6.79 (1H, s), 7.27–7.65 (12H, m), 8.08 (1H, d, $J=8.3$ Hz), 8.38 (1H, m), 8.43 (1H, m).

The following compounds were obtained using a method similar to that used for **15j** and **16j**.

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-4-methoxy-1-buten-1-yl]-3-cephem-4-carboxylate (16a). Amorphous solid. Yield: 1.9 g (6%). IR (Nujol) cm^{-1} 1760, 1680; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 1.8–2.3 (2H, m), 3.12 (3H, s), 3.13 (2H, t, $J=6.5$ Hz), 3.58 and 3.80 (2H, ABq, $J=17.4$ Hz), 5.17 (1H, d, $J=4.8$ Hz), 5.4–5.6 (2H, m), 6.17 (1H, d, $J=11.5$ Hz), 6.88 (1H, s), 7.2–7.5 (10H, m), 8.03 (1H, d, $J=8.9$ Hz).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-4-*tert*-butyldimethylsilyloxy-1-buten-1-yl]-3-cephem-4-carboxylate (16b). Amorphous solid. Yield: 13.6 g (14%). IR (Nujol) cm^{-1} 1760, 1700; ^1H NMR (DMSO-d_6) δ 0.02 (6H, s), 0.84 (9H, s), 1.43 (9H, s), 1.9–2.3 (2H, m), 3.46 (2H, t, $J=6.4$ Hz), 3.6–3.7 (2H, m), 5.16 (1H, d, $J=4.7$ Hz), 5.51 (1H, dd, $J=9.0$, 4.7 Hz), 5.35–5.5 (1H, m), 6.23 (1H, d, $J=11.9$ Hz), 6.88 (1H, s), 7.2–7.6 (10H, m), 8.03 (1H, d, $J=9.0$ Hz).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-3-phenoxy-1-propen-1-yl]-3-cephem-4-carboxylate (16f). Amorphous solid. Yield: 7.2 g (25%). IR (Nujol) cm^{-1} 3300, 1775, 1685; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.60 (2H, s), 4.16 (1H, d, $J=5.1$ Hz), 4.47 (1H, dd, $J=14.0$, 8.0 Hz), 5.18 (1H, d, $J=5$ Hz), 5.5–5.7 (2H, m), 6.34 (1H, d, $J=12.2$ Hz), 6.91 (1H, s), 7.2–7.5 (15H, m), 8.05 (1H, d, $J=9.0$ Hz).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-3-(4-pyridyloxy)-1-propen-1-yl]-3-cephem-4-carboxylate (16g). Amorphous solid. Yield: 10.2 g (35%). IR (Nujol) cm^{-1} 1760, 1685, 1580; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.5–3.8 (2H, m), 4.2–4.6 (2H, m), 5.19 (1H, d, $J=4.8$ Hz), 5.6–5.8 (2H, m), 6.38 (1H, d, $J=11.8$ Hz), 6.78 (2H, d, $J=6.1$ Hz), 6.92 (1H, s), 7.3–7.6 (10H, m), 8.09 (1H, d, $J=8.5$ Hz), 8.34 (2H, d, $J=6.1$ Hz).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-3-(3-pyridyloxy)-1-propen-1-yl]-3-cephem-4-carboxylate (16h). Amorphous solid. Yield: 9.0 g (40%). ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.8 (2H, m), 4.2–4.3 (1H, m), 4.5–4.6 (1H, m), 5.19 (1H, d, $J=5$ Hz), 5.6–5.8 (2H, m), 6.38 (1H, d, $J=12$ Hz), 6.91 (1H, s), 7.2–7.7 (12H, m), 8.06 (1H, d, $J=9$ Hz), 8.1–8.2 (2H, m).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-3-(pyrimidin-5-yl)oxy-1-propen-1-yl]-3-cephem-4-carboxylate (16i). Amorphous solid. Yield: 29.5 g (28%). IR (Nujol) cm^{-1} 1770, 1700, 1560, 1500; ^1H NMR (DMSO-d_6) δ 1.49 (9H, s), 3.58 and 3.68 (2H, ABq, $J=17.8$ Hz), 4.35–4.8 (2H, m), 5.18 (1H, d, $J=4.8$ Hz), 5.60 (1H, dd, $J=8.9$, 4.8 Hz), 5.69 (1H, dt, $J=11.8$, 6.0 Hz), 6.41 (1H, d, $J=11.8$ Hz), 6.89 (1H, s), 7.2–7.5 (10H, m), 8.06 (1H, d, $J=8.9$ Hz), 8.43 (2H, s), 8.80 (1H, s).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-2-(2-methylpyridin-3-yl)vinyl]-3-cephem-4-carboxylate (16k). Amorphous solid. Yield: 4.4 g (11%). IR (Nujol) cm^{-1} 1760, 1695, 1660; ^1H NMR (DMSO-d_6) δ 1.39 (9H, s), 2.40 (3H, s), 3.10 and 3.29 (2H, ABq, $J=17.3$ Hz), 5.12 (1H, d, $J=4.7$ Hz), 5.50 (1H, dd, $J=8.8$, 4.7 Hz), 6.61 (1H, d, $J=12.1$ Hz), 6.71 (1H, d, $J=12.1$ Hz), 6.83 (1H, s), 7.14–7.49 (12H, m), 8.02 (1H, d, $J=8.8$ Hz), 8.35 (1H, m).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-2-(6-methylpyridin-3-yl)vinyl]-3-cephem-4-carboxylate (16l). Amorphous solid. Yield: 8.9 g (19%). IR (Nujol) cm^{-1} 1765, 1705, 1670; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 2.43 (3H, s), 3.30 and 3.53 (2H, ABq, $J=17.7$ Hz), 5.21 (1H, d, $J=4.8$ Hz), 5.24 (1H, dd, $J=8.9$, 4.8 Hz), 6.44 (1H, d, $J=12.5$ Hz), 6.51 (1H, d, $J=12.5$ Hz), 6.83 (1H, s), 7.20–7.49 (12H, m), 8.08 (1H, d, $J=8.9$ Hz), 8.26 (1H, s).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-2-(pyridin-4-yl)vinyl]-3-cephem-4-carboxylate (15m). Amorphous solid. Yield: 3.6 g (11%). IR (Nujol) cm^{-1} 1775, 1705; ^1H NMR (DMSO-d_6) δ 1.42 (9H, s), 3.74 and 4.05 (2H, ABq, $J=17.6$ Hz), 5.22 (1H, d, $J=4.9$ Hz), 5.60 (1H, dd, $J=9.0$, 4.9 Hz), 7.01 (1H, d, $J=16.6$ Hz), 7.04 (2H, m), 7.08 (1H, s), 7.25–7.67 (11H, m), 8.13 (1H, d, $J=8.9$ Hz), 8.46 (2H, m).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-2-(pyridin-4-yl)vinyl]-3-cephem-4-carboxylate (16m). Amorphous solid. Yield: 9.0 g (28%). IR (Nujol) cm^{-1} 1770, 1720, 1695; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.28 and 3.59 (2H, ABq, $J=17.9$ Hz), 5.23 (1H, d, $J=4.8$ Hz), 5.58 (1H, dd, $J=9.0$, 4.8 Hz), 6.46 (1H, d, $J=12.1$ Hz), 6.60 (1H, d, $J=12.1$ Hz), 6.80 (1H, s), 7.11–7.50 (12H, m), 8.07 (1H, d, $J=9.0$ Hz), 8.48 (2H, m).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-4-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylate (16c). To a solution of **16b** (13.20 g) in MeOH (106 mL) was added dropwise concd HCl (8.45 mL) at room temperature. The mixture was stirred at room temperature for 1.5 h, then poured into a mixture of ethyl acetate and cold H_2O, and neutralized with sat.$NaHCO_3$. The separated organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated in vacuo and the residue was chromatographed on silica-gel, eluting with ethyl acetate–hexane to give **16c**. Amorphous solid. Yield: 7.6 g (70%). IR (Nujol) cm^{-1} 1765, 1700, 1695; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 1.8–2.25 (2H, m), 3.3–3.7 (4H, m), 4.53 (1H, t, $J=5.2$ Hz), 5.15 (1H, d, $J=4.7$ Hz), 5.53 (1H, dd, $J=9.0$, 4.7 Hz), 5.4–5.6 (1H, m), 6.18 (1H, d, $J=11.7$ Hz), 6.86 (1H, s), 7.3–7.6 (10H, m), 8.03 (1H, d, $J=9.0$ Hz).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-4-carbamoyloxy-1-buten-1-yl]-3-cephem-4-carboxylate (16d). Under a N_2 stream, trichloroacethyl isocyanate (2.37 g) in CH_2Cl_2 (8 mL) was added to a solution of **16c** (5.62 g) in CH_2Cl_2 (56 mL) at 5 °C, and stirred for 1 h. The mixture was then concentrated in vacuo, and the residue was dissolved in a mixture of $CHCl_3$ (50 mL) and MeOH (10 mL). Silica-gel (20 g) was added to this solution and the whole mixture was stirred for 24 h at room temperature. After the silica-gel was separated by filtration, the filtrate was concentrated in vacuo, and the residue was chromatographed on silica-gel, eluting with ethyl acetate-hexane to afford **16d**. Amorphous solid. Yield: 2.9 g (48%). IR (Nujol) cm^{-1} 1765, 1695; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 1.75–2.3 (2H, m), 3.5–3.6 (2H, br s), 3.75 (2H, t, $J=6.6$ Hz), 5.15 (1H, d, $J=4.8$ Hz), 5.35–5.5 (1H, m), 5.52 (1H, dd, $J=8.9$, 4.8 Hz), 6.17 (1H, d, $J=11.5$ Hz), 6.46 (2H, br s), 6.87 (1H, s), 7.3–7.5 (10H, m), 8.03 (1H, d, $J=8.9$ Hz).

7β-Amino-3-[(*Z*)-2-(pyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid dihydrochloride (18j). To a solution of **16j** (70.8 g) in formic acid (280 mL) was added dropwise concd HCl (54.8 mL) at room temperature. After stirring at room temperature for 2 h, the mixture was added dropwise to a mixture of acetone (2.1 L) and ethyl acetate (4.2 L) with ice-cooling. The resulting precipitate was collected by filtration to give **18j**. Amorphous solid. Yield: 34.3 g (74%). IR (Nujol) cm^{-1} 1760, 1700; ^1H NMR (DMSO-d_6) δ 3.54 and 3.67 (2H, ABq, $J=17.3$ Hz), 5.17 (1H, d, $J=5.0$ Hz), 5.36 (1H, d, $J=5.0$ Hz), 6.78 (2H, s), 7.93 (1H, m), 8.34 (1H, m), 8.77 (1H, m), 8.83 (1H, m).

The following compounds were obtained using a method similar to that used for **18j**.

7β-Amino-3-[(*E*)-2-(pyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid dihydrochloride (17j). Amorphous solid. Yield: 4.5 g (99%). IR (Nujol) cm^{-1} 1760, 1695; ^1H NMR (DMSO-d_6) δ 3.86 and 4.13 (2H, ABq, $J=17.2$ Hz), 5.18 (1H, d, $J=5.2$ Hz), 5.24 (1H, d, $J=5.2$ Hz), 7.30 (1H, d, $J=16.5$ Hz), 7.72 (1H, d, $J=16.5$ Hz), 7.99 (1H, m), 8.57 (1H, m), 8.77 (1H, m), 8.99 (1H, m).

7β-Amino-3-[(*Z*)-3-(4-pyridyloxy)-1-propen-1-yl]-3-cephem-4-carboxylic acid dihydrochloride (18g). Amorphous solid. Yield: 5.0 g (73%). IR (Nujol) cm^{-1} 1760, 1715, 1630, 1590, 1500; ^1H NMR (DMSO-d_6) δ 4.3–4.5 (2H, m), 4.9–5.1 (2H, m), 5.18 (1H, d, $J=4.2$ Hz), 5.27 (1H, d, $J=4.2$ Hz), 5.90 (1H, dt, $J=11.1$, 6.0 Hz), 6.59 (1H, d, $J=11.1$ Hz), 7.59 (2H, d, $J=6.7$ Hz), 8.77 (2H, d, $J=6.7$ Hz).

7β-Amino-3-[(*Z*)-3-(pyrimidin-5-yl)oxy-1-propen-1-yl]-3-cephem-4-carboxylic acid dihydrochloride (18i). Amorphous solid. Yield: 17.1 g (86%). IR (Nujol) cm^{-1} 1760, 1680, 1590, 1520; ^1H NMR (DMSO-d_6) δ 3.76 (2H, br s), 4.7–4.95 (2H, m), 5.17 (1H, d, $J=4.9$ Hz), 5.28 (1H, d, $J=4.9$ Hz), 5.89 (1H, dt, $J=11.9$, 6.1 Hz), 6.58 (1H, d, $J=11.9$ Hz), 8.57 (2H, s), 8.84 (1H, s).

7β-Amino-3-[(*Z*)-2-(2-methylpyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid dihydrochloride (18k). Amorphous solid. Yield: 2.9 g (quant.). IR (Nujol) cm^{-1} 3320, 1750, 1680; ^1H NMR (DMSO-d_6) δ 2.71 (3H, s), 3.50 and 3.60 (2H, ABq, $J=17.2$ Hz), 5.14 (1H, d, $J=5.0$ Hz), 5.26 (1H, d, $J=5.0$ Hz), 6.74 (1H, d, $J=12.0$ Hz), 6.83 (1H, d, $J=12.0$ Hz), 7.76 (1H, dd, $J=7.8$, 5.8 Hz), 8.21 (1H, d, $J=7.8$ Hz), 8.64 (1H, d, $J=5.8$ Hz).

7β-Amino-3-[(*Z*)-2-(6-methylpyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid dihydrochloride (18l). Amorphous solid. Yield: 5.9 g (quant.). IR (Nujol) cm^{-1} 3320, 1760, 1690; ^1H NMR (DMSO-d_6) δ 2.75 (3H, s), 3.52 and 3.65 (2H, ABq, $J=17.3$ Hz), 5.18 (1H, d, $J=5.1$ Hz), 5.34 (1H, d, $J=5.1$ Hz), 6.75 (2H, s), 7.81 (1H, d, $J=8.4$ Hz), 8.38 (1H, d, $J=8.4$ Hz), 8.67 (1H, s).

7β-Amino-3-[(*E*)-2-(pyridin-4-yl)vinyl]-3-cephem-4-carboxylic acid bis(trifluoroacetate) (17m). To a solution of diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*Z*)-2-(pyridin-4-yl)vinyl]-3-cephem-4-carboxylate (**16m**) (4.6 g) in CH_2Cl_2 (23 mL) and anisole (4.6 mL) was added trifluoroacetic acid (9.2 mL) under ice-cooling. After stirring at room temperature for 2 h, the mixture was poured into cold IPE. The resulting precipitate was collected by filtration to give **17m**. Amorphous solid. Yield: 4.2 g (99%). IR (Nujol) cm^{-1} 1780, 1660, 1600; ^1H NMR (DMSO-d_6) δ 3.86 and 4.15 (2H, ABq, $J=17.5$ Hz), 5.27 (1H, d, $J=5.2$ Hz), 5.34 (1H, d, $J=5.2$ Hz), 7.09 (1H, d, $J=16.6$ Hz), 7.27 (1H, d, $J=16.6$ Hz), 7.78 (2H, d, $J=6.4$ Hz), 8.73 (2H, d, $J=6.4$ Hz).

The following compounds were obtained using a method similar to that used for **17m**.

7β-Amino-3-[(*Z*)-4-methoxy-1-buten-1-yl]-3-cephem-4-carboxylic acid trifluoroacetate (18a). Amorphous solid. Yield: 989 mg (72%). ^1H NMR (DMSO-d_6) δ 2.1–2.4 (2H, m), 3.21 (3H, s), 3.32 (2H, t, $J=6.5$ Hz), 3.45 and 3.60 (2H, ABq, $J=17.4$ Hz), 4.80 (1H, d, $J=5.0$ Hz), 5.05 (1H, d, $J=5.0$ Hz), 5.52 (1H, d, $J=11.6$, 7.1 Hz), 6.18 (1H, d, $J=11.6$ Hz).

7β-Amino-3-[(*Z*)-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylic acid trifluoroacetate (18c). Amorphous solid. Yield: 1.4 g (81%). ^1H NMR (DMSO-d_6) δ 2.3–2.6

(2H, m), 3.4–3.7 (4H, m), 4.82 (1H, d, $J=4.8$ Hz), 5.03 (1H, d, $J=4.8$ Hz), 5.03 (1H, d, $J=4.8$ Hz), 5.5–5.6 (1H, m), 6.1–6.2 (1H, m).

7β-Amino-3-[(*Z*)-4-carbamoyloxy-1-buten-1-yl]-3-cephem-4-carboxylic acid trifluoroacetate (18d). Amorphous solid. Yield: 1.4 g (quant.). ^{1}H NMR (DMSO-d_6) δ 2.2–2.5 (2H, m), 3.63 (2H, br s), 3.92 (2H, t, $J=6.7$ Hz), 5.11 (1H, d, $J=4.9$ Hz), 5.22 (1H, d, $J=4.9$ Hz), 5.5–5.7 (1H, m), 6.32 (1H, d, $J=11.5$ Hz), 6.49 (2H, br s).

7β-Amino-3-[(*Z*)-3-phenoxy-1-propen-1-yl]-3-cephem-4-carboxylic acid trifluoroacetate (18f). Amorphous solid. Yield: 4.1 g (81%). ^{1}H NMR (DMSO-d_6) δ 3.72 and 3.85 (2H, ABq, $J=17$ Hz), 4.53 (1H, ddd, $J=2$, 6, 13 Hz), 4.68 (1H, ddd, $J=2$, 6, 13 Hz), 4.95 (1H, d, $J=5$ Hz), 5.14 (1H, d, $J=5$ Hz), 5.81 (1H, p, $J=6$ Hz), 6.44 (1H, d, $J=12$ Hz), 6.8-7.4 (5H, m).

7β-Amino-3-[(*Z*)-3-(3-pyridyloxy)-1-propen-1-yl]-3-cephem-4-carboxylic acid bis(trifluoroacetate) (18h). Amorphous solid. Yield: 10.6 g (quant.). ^{1}H NMR (DMSO-d_6) δ 3.75 (2H, s), 4.7–5.3 (4H, m), 5.7–6.0 (1H, m), 6.58 (1H, d, $J=12$ Hz), 7.7–7.8 (2H, m), 8.4–8.6 (2H, m).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-2-pyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid (2j). To a solution of **18j** (12 g) and *N*-(trimethylsilyl)acetamide (49.9 g) in CH_2Cl_2 (240 mL) was added (*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetyl chloride hydrochloride (**12**) (10.9 g) under ice-cooling. After stirring for 4 h at the same temperature, the mixture was added dropwise to IPE (1.5 L). The precipitate containing 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetamido]-3-[(*Z*)-2-pyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid was collected by filtration and dried in vacuo. The precipitate was dissolved in 10% MeOH aqueous solution (280 mL), and thereto NH_4Cl (5.12 g) was added and the mixture adjusted to pH 8 with aq $NaHCO_3$ solution. The mixture was stirred for 30 min maintaining pH 8 with aq $NaHCO_3$ solution. The solution was adjusted to pH 6 with 1 N HCl, and evaporated in vacuo to remove MeOH. The solution was subjected to column chromatography on HP-20 and eluted with 15% aqueous isopropyl alcohol. The fractions containing the object compound were collected and lyophilized to give crude product, which was purified by preparative HPLC utilizing a C18 μ Bondapak resin to afford **2j**. Amorphous solid. Yield: 0.36 g (11%). IR (Nujol) cm^{-1} 1750, 1650; ^{1}H NMR (DMSO-d_6) δ 3.19 and 3.54 (2H, ABq, $J=17.7$ Hz), 5.24 (1H, d, $J=4.8$ Hz), 5.81 (1H, dd, $J=8.2$,4.8 Hz), 6.54 (1H, d, $J=12.2$ Hz), 6.61 (1H, d, $J=12.2$ Hz), 6.65 (1H, s), 7.12 (2H, br s), 7.34 (1H, m), 7.63 (1H, m), 8.43(2H, m), 9.51 (1H, d, $J=8.2$ Hz), 11.29 (1H, s). Anal. calcd for $C_{19}H_{16}N_6O_5S_2\cdot 2H_2O$: C, 44.88; H, 3.96; N, 16.53; found: C, 44.98; H, 3.73; N, 16.55.

The following compounds were obtained using a method similar to that used for **2j**.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)-2-pyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid (1j). Amorphous solid. Yield: 2.7 g (24%). IR (Nujol) cm^{-1} 3200, 1755, 1650; ^{1}H NMR (DMSO-d_6) δ 3.70 and 4.00 (2H, ABq, $J=17.5$ Hz), 5.23 (1H, d, $J=4.6$ Hz), 5.83 (1H, dd, $J=8.1$, 4.6 Hz), 6.69 (1H, s), 7.02 (1H, d, $J=16.4$ Hz), 7.14 (2H, s), 7.39 (1H, s), 7.87 (1H, d, $J=16.4$ Hz), 7.87 (1H, m), 8.45 (1H, m), 8.63 (1H, s), 9.52 (1H, d, $J=8.1$ Hz), 11.34 (1H, br s).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*E*)-2-pyridin-4-yl)vinyl]-3-cephem-4-carboxylic acid (1m). Amorphous solid. Yield: 370 mg (28%). IR (Nujol) cm^{-1} 1760, 1650.; ^{1}H NMR (DMSO-d_6) δ 3.71 and 4.04 (2H, ABq, $J=17.7$ Hz), 5.25 (1H, d, $J=4.9$ Hz), 5.85 (1H, dd, $J=8.2$, 4.9 Hz), 6.68 (1H, s), 6.98 (1H, d, $J=16.3$ Hz), 7.15 (2H, s), 7.39 (2H, d, $J=5.8$ Hz), 7.61 (1H, d, $J=16.3$ Hz), 8.54 (2H, d, $J=5.8$ Hz), 9.53 (1H, d, $J=8.2$ Hz), 11.33 (1H, s). Anal. calcd for $C_{19}H_{16}N_6O_5S_2\cdot 4.7H_2O$: C, 40.97; H, 4.59; N, 15.08; found: C, 40.97; H, 4.34; N, 14.95.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-4-methoxy-1-buten-1-yl]-3-cephem-4-carboxylic acid (2a). Amorphous solid. Yield: 120 mg (11%). IR (Nujol) cm^{-1} 1750, 1600; ^{1}H NMR (DMSO-d_6) δ 2.15–2.45 (2H, m), 3.21 (3H, s), 3.33 (2H, t, $J=6.5$ Hz), 3.46 and 3.60 (2H, ABq, $J=17.7$ Hz), 5.18 (1H, d, $J=4.8$ Hz), 5.53 (1H, dt, $J=11.6$, 7.5 Hz), 5.75 (1H, dd, $J=8.2$, 4.8 Hz), 6.23 (1H, d, $J=11.6$ Hz), 6.66 (1H, s), 7.12 (2H, br s), 9.45 (1H, d, $J=8.2$ Hz), 11.30 (1H, s). ESIMS m/z 454[(M + H)$^+$].

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-4-hydroxy-1-buten-1-yl]-3-cephem-4-carboxylic acid (2c). Amorphous solid. Yield: 195 mg (12%). IR (Nujol) cm^{-1} 1750; ^{1}H NMR (DMSO-d_6) δ 2.1–2.4 (2H, m), 3.41 (2H, t, $J=6.6$ Hz), 3.49 and 3.61 (2H, ABq, $J=17.6$ Hz), 5.18 (1H, d, $J=4.7$ Hz), 5.58 (1H, dt, $J=11.6$, 7.2 Hz), 5.76 (1H, dd, $J=8.2$, 4.7 Hz), 6.21 (1H, d, $J=11.6$ Hz), 6.67 (2H, br s), 7.13 (2H, br s), 7.13 (2H, br s), 9.46 (1H, d, $J=8.2$ Hz), 11.31 (1H, s). ESIMS m/z 440[(M + H)$^+$]. Anal. calcd for $C_{16}H_{17}N_5O_6S_2\cdot 2.6H_2O$: C, 39.52; H, 4.60; N, 14.40; found: C, 39.65; H, 4.36; N, 14.12.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-4-carbamoyloxy-1-buten-1-yl]-3-cephem-4-carboxylic acid (2d). Amorphous solid. Yield: 355 mg (22%). IR (Nujol) cm^{-1} 1750, 1640; ^{1}H NMR (DMSO-d_6) δ 2.2–2.5 (2H, m), 3.46 and 3.62 (2H, ABq, $J=17.7$ Hz), 3.90 (2H, t, $J=6.6$ Hz), 5.17 (1H, d, $J=4.8$ Hz), 5.52 (1H, dd, $J=11.6$, 5.0 Hz), 5.76 (1H, dd, $J=8.2$, 4.8 Hz), 6.24 (1H, d, $J=11.6$ Hz), 6.47 (2H, br s), 6.66 (1H, s), 7.13 (2H, br s), 9.47 (1H, d, $J=8.2$ Hz), 11.32 (1H, s).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-3-phenoxy-1-propen-1-yl]-3-cephem-4-carboxylic acid (2f). Amorphous solid. Yield: 693 g (13%). IR (Nujol) cm^{-1} 1720; ^{1}H NMR (DMSO-d_6) δ 3.54 and 3.70 (2H, ABq, $J=18$ Hz), 4.57 (1H, dd, $J=6$, 15 Hz), 5.21 (1H, d, $J=5$ Hz), 5.7–5.9 (2H, m), 6.42 (1H, d, $J=12$ Hz), 6.66 (1H, s), 6.9–7.0 (3H, m), 7.13 (2H, br s), 7.2–7.4 (2H, m), 9.48 (1H, d, $J=8$ Hz), 11.3 (1H, br s). ESIMS m/z 502[(M + H)$^+$].

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-3-(4-pyridyloxy)-1-propen-1-yl]-3-cephem-4-carboxylic acid (2g). Amorphous solid. Yield: 253 mg (10%). IR (Nujol) cm^{-1} 1750, 1590; ^1H NMR (DMSO-d_6) δ 3.55 and 3.73 (2H, ABq, $J=17.7$ Hz), 4.5–4.8 (2H, m), 5.21 (1H, d, $J=4.8$ Hz), 5.83 (1H, dd, $J=8.1$, 4.8 Hz), 5.7–5.85 (1H, m), 6.46 (1H, d, $J=11.6$ Hz), 6.67 (1H, s), 6.94 (2H, d, $J=6.3$ Hz), 7.14 (2H, br s), 8.40 (2H, d, $J=6.3$ Hz), 7.14 (2H, br s), 8.40 (2H, d, $J=6.3$ Hz), 9.49 (1H, d, $J=8.1$ Hz), 11.3 (1H, s). Anal. calcd for $C_{20}H_{18}N_6O_6S_2\cdot3.4H_2O$: C, 42.61; H, 4.43; N, 14.91; found: C, 42.54; H, 4.22; N, 14.67.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-3-(3-pyridyloxy)-1-propen-1-yl]-3-cephem-4-carboxylic acid (2h). Amorphous solid. Yield: 295 mg (3%). IR (Nujol) cm^{-1} 1750; ^1H NMR (DMSO-d_6) δ 3.54 and 3.71 (2H, ABq, $J=18$ Hz), 4.63 and 4.77 (2H, dABq, $J=6$, 15 Hz), 5.20 (1H, d, $J=5$ Hz), 5.7–5.8 (2H, m), 6.47 (1H, d, $J=12$ Hz), 6.66 (1H, s), 7.13 (2H, br s), 7.3–7.4 (2H, m), 8.2 (1H, m), 8.3 (1H, m), 9.49 (1H, d, $J=8$ Hz), 11.3 (1H, br s). ESIMS (neg.) m/z 501[$(M-H)^+$]. Anal. calcd for $C_{20}H_{18}N_6O_6S_2\cdot3.8H_2O$: C, 42.07; H, 4.52; N, 14.72; found: C, 41.76; H, 4.20; N, 14.67.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-3-(pyrimidin-5-yl)oxy-1-propen-1-yl]-3-cephem-4-carboxylic acid (2i). Amorphous solid. Yield: 295 mg (6%). IR (Nujol) cm^{-1} 1750, 1650, 1600; ^1H NMR (DMSO-d_6) δ 3.55 and 3.73 (2H, ABq, $J=17.9$ Hz), 4.7–4.95 (2H, m), 5.21 (1H, d, $J=4.9$ Hz), 5.79 (1H, dd, $J=8.2$, 4.9 Hz), 5.75-5.9 (1H, m), 6.50 (1H, d, $J=11.9$ Hz), 6.66 (1H, s), 7.13 (2H, br s), 8.53 (2H, s), 8.82 (1H, s), 9.49 (1H, d, $J=8.2$ Hz), 11.31 (1H, s). ESIMS (neg.) m/z 502[$(M-H)^+$]. Anal. calcd for $C_{19}H_{17}N_7O_6S_2\cdot2.95H_2O$: C, 41.00; H, 4.15; N, 17.61; found: C, 41.23; H, 3.96; N, 17.34.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-2-(2-methylpyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid (2k). Amorphous solid. Yield: 101 mg (3%). IR (Nujol) cm^{-1} 1750, 1660; ^1H NMR (DMSO-d_6) δ 2.43 (3H, s), 2.88 and 3.25 (2H, ABq, $J=17.1$ Hz), 5.10 (1H, d, $J=4.8$ Hz), 5.69 (1H, dd, $J=8.2$, 4.8 Hz), 6.54 (1H, d, $J=12.1$ Hz), 6.62 (1H, s), 6.76 (1H, d, $J=12.1$ Hz), 7.12 (2H, br s), 7.16 (1H, m), 7.45 (1H, d, $J=6.5$ Hz), 8.34 (1H, d, $J=4.8$ Hz), 9.42 (1H, d, $J=8.2$ Hz), 11.32 (1H, s). ESIMS (neg.) m/z 485[$(M-H)^+$]. Anal. calcd for $C_{20}H_{18}N_6O_5S_2\cdot2.85H_2O$: C, 44.66; H, 4.44; N, 15.62; found: C, 44.93; H, 4.34; N, 15.33.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-2-(6-methylpyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid (2l). Amorphous solid. Yield: 420 mg (12%). IR (Nujol) cm^{-1} 3260, 1740, 1655; ^1H NMR (DMSO-d_6) δ 2.45 (3H, s), 3.1 and 3.51 (2H, ABq, $J=17.7$ Hz), 5.23 (1H, d, $J=4.8$ Hz), 5.81 (1H, dd, $J=8.2$, 4.8 Hz), 6.49 (1H, d, $J=12.2$ Hz), 6.57 (1H, d, $J=12.2$ Hz), 6.65 (1H, s), 7.11 (2H, br s), 7.20 (1H, d, $J=8.1$ Hz), 7.54 (1H, d, $J=8.1$ Hz), 8.33 (1H, s), 9.50 (1H, d, $J=8.2$ Hz), 11.28 (1H, s).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-[(*Z*)-2-(pyridin-3-yl)vinyl]-3-cephem-4-carboxylic acid (20). Et_3N (1.5 mL, 10.6 mmol) was added to a suspension of **18j** (1.0 g, 2.66 mmol) in THF (20 mL). TMSCl (0.84 mL, 6.65 mmol) was added dropwise to the mixture with ice-cooling over 5 min. The mixture was stirred for 30 min at room temperature and cooled again with ice. To the mixture was added a solution of **19** (1.45 g, 2.66 mmol) in DMF (15 mL) over 10 min. The mixture was stirred overnight at room temperature and concentrated in vacuo to remove most of the THF. The concentrate was poured into ice water (100 mL) with vigorous stirring. The resulting precipitate was collected by filtration to give **20**. Amorphous solid. Yield: 955 mg (50%). IR (Nujol) cm^{-1} 1725, 1650; ^1H NMR (DMSO-d_6) δ 2.95 and 3.34 (2H, ABq, $J=17.0$ Hz), 5.21 (1H, d, $J=4.9$ Hz), 5.82 (1H, dd, $J=8.2$, 4.9 Hz), 6.43 (1H, d, $J=12.2$ Hz), 6.58 (1H, s), 6.89 (1H, d, $J=12.2$ Hz), 7.25–7.30 (18H, m), 7.65 (1H, d, $J=8.3$ Hz), 8.42–8.46 (2H, m), 9.90 (1H, d, $J=8.2$ Hz).

Pivaloyloxymethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-trityloxyiminoacetamido]-3-[(*Z*)-2-(pyridin-3-yl)vinyl]-3-cephem-4-carboxylate (22). Iodomethyl pivalate (**21**) (515 mg, 2.13 mmol) was added to a mixture of **20** (1.39 g, 1.94 mmol) and K_2CO_3 (147 mg, 1.07 mmol) in DMF (14 mL) at 0 °C. The mixture was stirred for 30 min at the same temperature, then poured into a mixture of ice-water (90 mL) and ethyl acetate (120 mL). The organic layer was separated, washed with H_2O and brine, dried over $MgSO_4$. After evaporation of the solvent, the residue was triturated with IPE. The precipitates were collected by filtration to afford **22**. Amorphous solid. Yield: 283 mg (19%). ^1H NMR (DMSO-d_6) δ 1.14 (9H, s), 3.32 and 3.65 (2H, ABq, $J=18.0$ Hz), 5.35 (1H, d, $J=4.9$ Hz), 5.64 and 5.80 (2H, ABq, $J=5.9$ Hz), 6.01 (1H, dd, $J=8.2$, 4.9 Hz), 6.51 (1H, d, $J=12.1$ Hz), 6.59 (1H, s), 6.66 (1H, d, $J=12.1$ Hz), 7.23–7.39 (18H, m), 7.64–7.68 (1H, m), 8.46–8.47 (2H, m), 9.95 (1H, d, $J=8.2$ Hz).

Pivaloyloxymethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-[(*Z*)-2-(pyridin-3-yl)vinyl]-3-cephem-4-carboxylate (23). Compound **22** (185 mg, 0.223 mmol) was dissolved in 90% aqueous formic acid (0.7 mL) and stirred for 40 min at room temperature. The reaction mixture was filtered, and the filtrate was poured into a mixture of ice-water (10 mL) and ethyl acetate (10 mL). The aqueous layer was adjusted to pH 5 with $NaHCO_3$. The organic layer was separated, washed with H_2O, with brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was triturated with IPE and collected by filtration to afford **23**. Amorphous solid. Yield: 73 mg (56%). IR (Nujol) cm^{-1} 1740, 1655.; ^1H NMR (DMSO-d_6) δ 1.12 (9H, s), 3.29 and 3.65 (2H, ABq, $J=17.9$ Hz), 5.28 (1H, d, $J=4.8$ Hz), 5.60 and 5.76 (2H, ABq, $J=5.9$ Hz), 5.86 (1H, dd, $J=8.1$, 4.8 Hz), 6.48 (1H, d, $J=12.1$ Hz), 6.59 (1H, d, $J=12.1$ Hz), 6.66 (1H, s), 7.17 (2H, br s), 7.35 (1H, dd, $J=4.8$, 8.0 Hz), 8.45–8.47 (2H, m), 9.53 (1H, d, $J=8.1$ Hz), 11.32 (1H, s).

Measurement of in vitro antibacterial activity

According to the method of the Japan Society of Chemotherapy, the MICs of compounds were determined by the 2-fold agar dilution method using heart

infusion agar (Eiken). The inoculum size was adjusted to 10^6 cfu/mL, and incubation was carried out at 37 °C for 20 h.

Urinary and biliary recovery

Sprague Dawley rats were fasted overnight and orally dosed with 20 mg/kg of the test drugs. Urine samples were collected for 24 h after dosing. For bile collection another group of rats was cannulated with a polystyrene tube into the bile duct and test drugs were given orally at doses of 20 mg/kg. The samples were assayed by a disc-agar diffusion method using *E. coli* NIHJ-JC-2 or *E. coli* ATCC 33546 as test organism and nutrient agar (Difco) as the test medium.

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