STUDIES ON β-LACTAM ANTIBIOTICS

XII.† SYNTHESIS AND ACTIVITY OF NEW 3-ETHYNYLCEPHALOSPORIN

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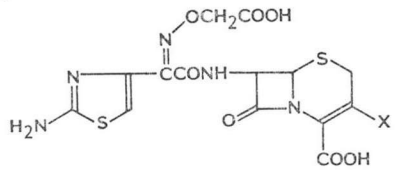
The synthesis of the orally absorbed 3-ethynylcephalosporin (**1b**) is described. In addition, the structure-activity relationships and oral absorption in rats of 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-carboxymethoxyiminoacetamido]cephalosporins (**1**) having various aliphatic hydrocarbon groups at the 3-position are discussed. Of these cephalosporins (**1**), 3-ethynylcephalosporin (**1b**) exhibited better activity against *Staphylococcus aureus* than the other cephalosporins (**1a**, **1c** and **1d**) and showed moderate oral absorption in rats.

During our extensive studies on the antibacterial activity and oral absorption of 7β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-carboxymethoxyiminoacetamido]cephalosporins[1,2] having various substituents at the 3-position of the cephem nucleus, we observed that compounds with relatively small substituents such as hydrogen, chloro, vinyl, methoxy, and methylthio exhibited higher oral absorptivity in rats. In particular, FK027[3,4] (**1c**)†† having a vinyl group at the 3-position was found to be an orally active cephalosporin with almost the same broad antimicrobial activity as the parenteral cephalosporins containing the aminothiazole ring such as ceftizoxime, cefotaxime and cefmenoxime, except against *Staphylococcus aureus*. Consequently, we directed our research efforts towards synthesizing a new cephalosporin with a sterically small substituent at the 3-position, which might exhibit higher antibacterial activity, in particular against *S. aureus*, and even better oral absorption than the 3-vinylcephalosporin (**1c**; FK027). Among possible structural changes, we were especially interested in preparing a new cephalosporin with a 3-ethynyl group, whose carbon-carbon bond length is shorter than that of the vinyl group (Fig. 1).

Fig. 1. Chemical structure of FK027 and its related compounds.



1a X=CH_3
1b X=C≡CH
1c X=CH=CH_2 (FK027)
1d X=CH_2CH_3

Thus, we report the preparation of 3-ethynylcephalosporin (**1b**) and the structure-activity relationships and oral absorption in rats of a series of cephalosporins (**1**) having various aliphatic hydrocarbon substituents at the 3-position.

Chemistry

The new 7-amino-3-ethynylcephem (**7**) was prepared by the two pathways outlined in Scheme 1.

In Pathway A, treatment of the 7-phenylacetamido-3-(2,2-dibromovinyl)-2-cephem (**2**)[2] with

† Paper XI. See ref 2).
†† Generic name: Cefixime.

Scheme 1.

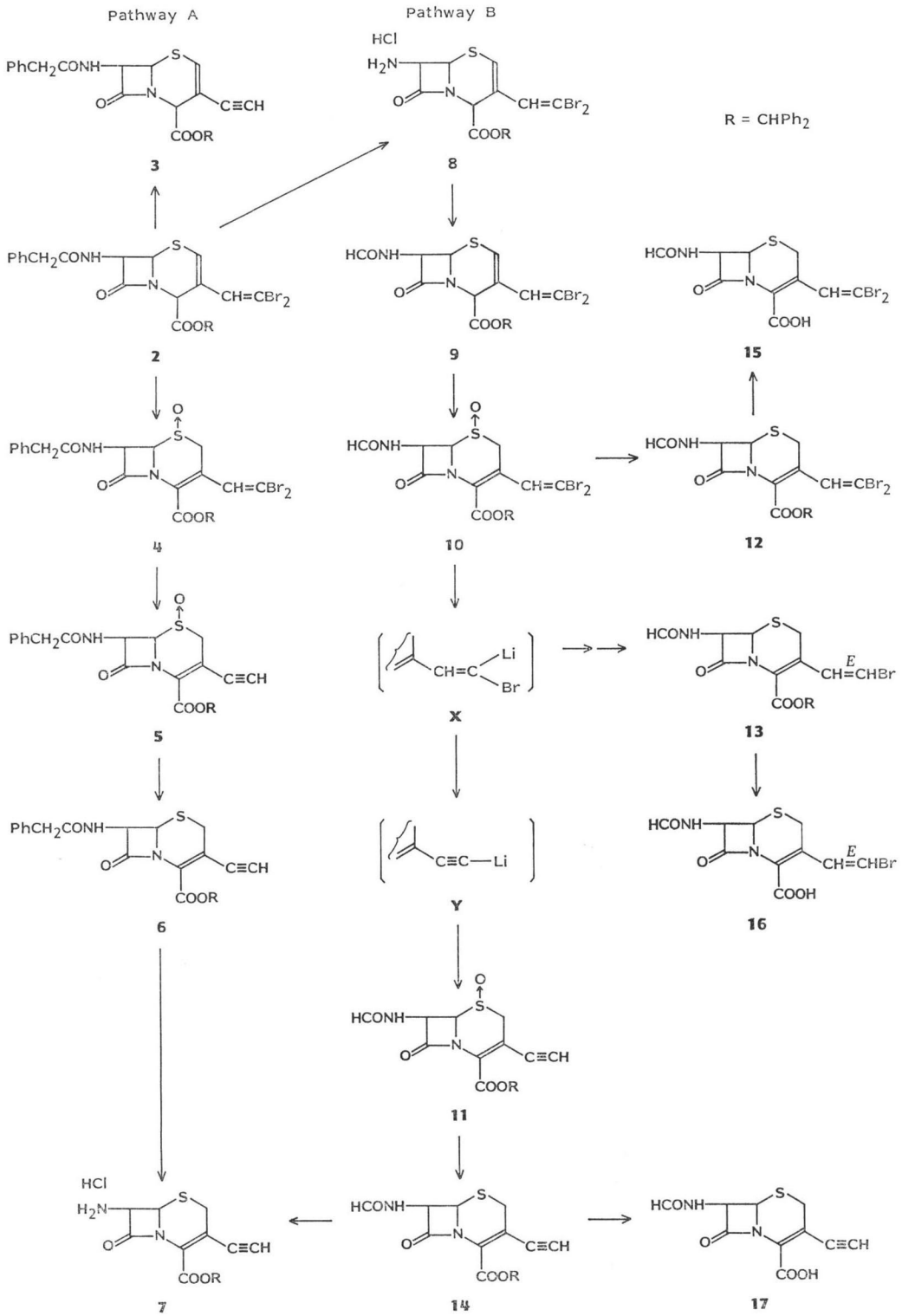
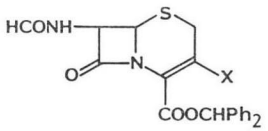
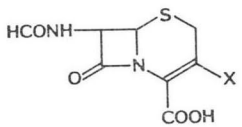


Table 1. ^{1}H NMR and IR spectral data of cephalosporins (**12**~**14**) with various substituents at the 3-position.



Compound	X	^{1}H NMR (DMSO-d_6, δ)								IR (Nujol, cm^{-1})
		C(2)-H_2 (2H, ABq, J=17 Hz)	C(6)-H_1 (1H, d, J=5 Hz)	C(7)-H_1 (1H, dd, J=5, 8 Hz)	Ph_2CH (1H, s)	Ph×2 (10H, m)	HCO (1H, s)	CONH (1H, d, J=8 Hz)	X	β-Lactam
12	CH=CBr_2	3.67 (br s)	5.21	5.89	6.90	7.53~7.20	8.14	9.10	7.36 (1H, s)	1780
13	*E* CH=CHBr	3.94, 3.63	5.20	5.86	6.95	7.55~7.20	8.13	9.09	7.27 (1H, d, J=15 Hz), 7.06 (1H, d, J=15 Hz)	1780
14	C≡CH	3.89, 3.58	5.21	5.90	6.93	7.60~7.20	8.13	9.11	4.72 (1H, s)	1800

Table 2. ^{1}H NMR and IR spectral data of cephalosporins (**15**~**17**) with various substituents at the 3-position.

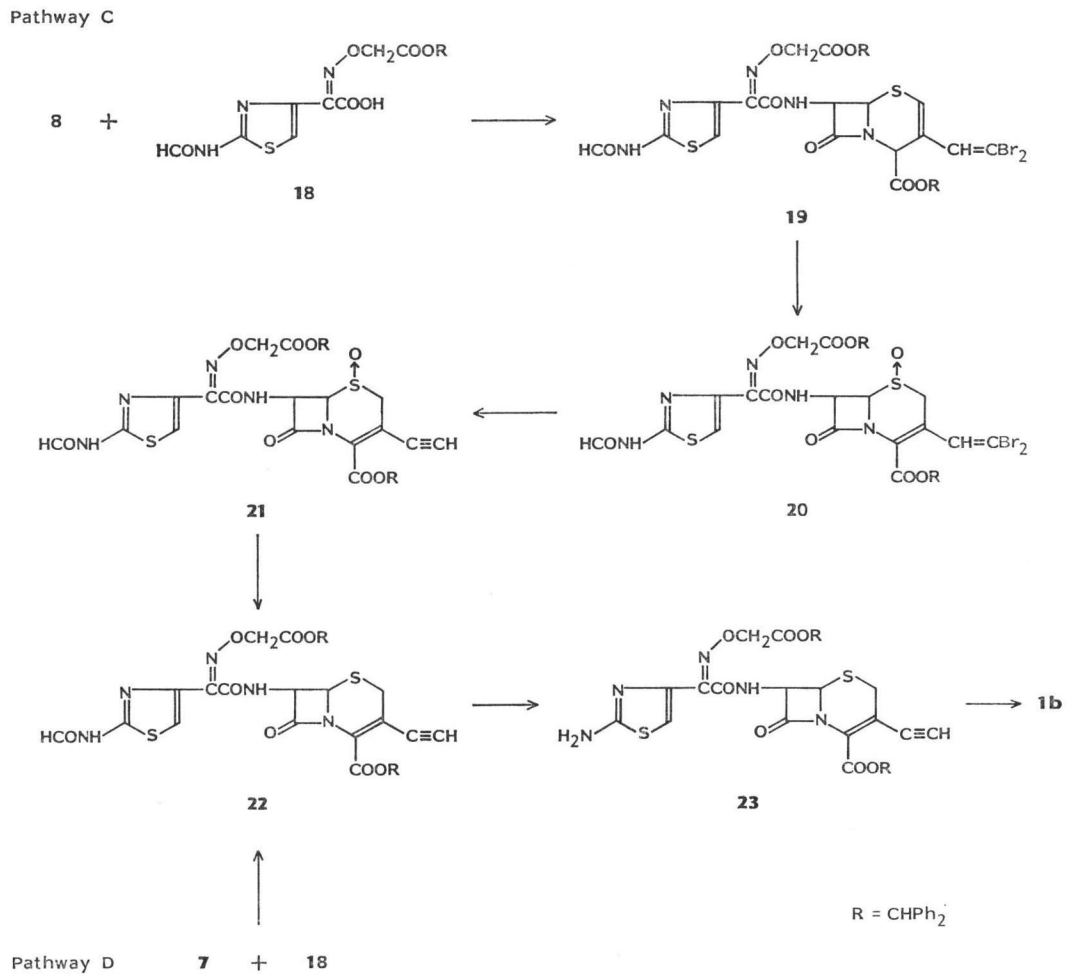


Compound	X	^{1}H NMR (DMSO-d_6, δ)						IR (Nujol, cm^{-1})
		C(2)-H_2 (2H, ABq, J=17 Hz)	C(6)-H_1 (1H, d, J=5 Hz)	C(7)-H_1 (1H, dd, J=5, 8 Hz)	HCO (1H, s)	CONH (1H, d, J=8 Hz)	X	β-Lactam
15	CH=CBr_2	3.63 (br s)	5.14	5.78	8.10	9.02	7.41 (1H, s)	1770
16	*E* CH=CHBr	3.86, 3.57	5.13	5.74	8.10	9.02	7.37 (1H, d, J=15 Hz), 7.02 (1H, d, J=15 Hz)	1760
17	C≡CH	3.78, 3.47	5.13	5.78	8.10	9.04	4.50 (1H, s)	1775

butyllithium[5] did not give the desired 3-ethynyl-2-cephem (**3**) but many unknown compounds. Therefore, we investigated an alternative approach. The 3-(2,2-dibromovinyl)-2-cephem (**2**) was oxidized with *m*-chloroperbenzoic acid to yield the corresponding sulfoxide (**4**), which was treated with two equivalents of butyllithium to recover unchanged starting material (**4**). Synthesis of the 3-ethynylcephem 1-oxide (**5**) was finally achieved by treatment of **4** with four equivalents of butyllithium (yield; 57.7%). The 3-ethynylcephem 1-oxide (**5**) was reduced with phosphorus trichloride (PCl_3) to afford the corresponding 3-ethynyl-3-cephem (**6**), whose phenylacetyl side chain was removed with phosphorus pentachloride (PCl_5) to give the 7-amino-3-ethynylcephem (**7**).

In Pathway B, the 7-formamido-3-(2,2-dibromovinyl)-2-cephem (**9**) was obtained from **2** by cleavage of the phenylacetyl side chain with PCl_5, followed by formylation with a mixture of formic acid and acetic anhydride. **9** was oxidized with *m*-chloroperbenzoic acid to afford the corresponding sulfoxide (**10**), which on treatment with four equivalents of butyllithium at −70°C immediately followed by hydrolysis and then reduction with PCl_3 gave a mixture of the corresponding 3-ethynyl-3-cephem (**14**) and 3-[(*E*)-2-bromovinyl]-3-cephem (**13**) (approximate ratio 40: 1). Deformylation of

Scheme 2.



the 3-ethynyl-3-cephem (**14**) was effected with concentrated hydrochloric acid in methanol to yield the 7-amino-3-ethynylcephem (**7**).

Alternately, when the sulfoxide (**10**) was treated with three equivalents of butyllithium at −70°C and the reaction mixture kept at this temperature for 30 minutes before hydrolysis, a mixture of 3-ethynylcephem 1-oxide (**11**) and the starting material (**10**) (approximate ratio 1:1) was obtained. Treatment of the sulfoxide (**10**) with four equivalents of butyllithium by the same procedure afforded exclusively the corresponding 3-ethynylcephem 1-oxide (**11**) (yield; 98.5%), which was then converted to the 3-ethynyl-3-cephem (**14**) by reduction with PCl_3. On the basis of the above results, these reactions seem to involve the intermediates **X** and **Y**[6~8].

In order to confirm the structure, the sulfoxide (**10**) was reduced with PCl_3 to afford the 3-(2,2-dibromovinyl)-3-cephem (**12**). The diphenylmethyl esters (**12**, **13** and **14**) were converted to the corresponding carboxylic acids (**15**, **16** and **17**) by removal of the diphenylmethyl group with trifluoroacetic acid (TFA) and anisole. The structures of all compounds (**12**~**17**) were supported by their ^1H NMR and IR spectra summarized in Tables 1 and 2.

Both methods leading to the 7-amino-3-ethynylcephem (**7**) appeared to be feasible, but the yields obtained *via* Pathway B turned out to be higher than those *via* Pathway A.

The cephalosporin (**1b**) was prepared by the two pathways outlined in Scheme 2. According to Pathway C, **8** was acylated with an activated acid which was obtained by treatment of the acid (**18**) with Vilsmeier reagent prepared from phosphoryl chloride and *N*,*N*-dimethylformamide, followed by oxidation to give the sulfoxide (**20**). **20** was successfully converted to the corresponding 3-ethynylcephem 1-oxide (**21**) with four equivalents of butyllithium and then reduced to the acylated 3-ethynyl-3-cephem (**22**) with PCl_3.

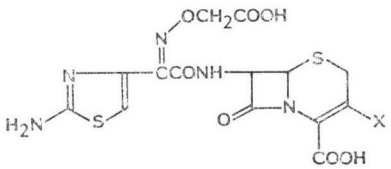
For the preparation of a variety of semisynthetic cephalosporins containing various 7-acyl side chains, Pathway D was more suitable. According to Pathway D, the acylated 3-ethynyl-3-cephem (**22**) was prepared by coupling the 7-amino-3-ethynylcephem (**7**) with the activated acid of **18**.

Cephalosporin (**1b**) was obtained by deformylation of **22** with concentrated hydrochloric acid, followed by deprotection of the diphenylmethyl groups with TFA and anisole.

Antibacterial Activity and Oral Absorption

The minimum inhibitory concentrations (MICs) of the cephalosporins (**1**) having various aliphatic

Table 3. Antibacterial activity of cephalosporins (**1**).



Inoculum size 10^6 cfu/ml

Compound	X	MIC (μg/ml)		
		Staphylococcus aureus 209P JC-1	*Escherichia coli* 31	*Proteus vulgaris* 1
1a	CH_3	>100	0.1	0.2
1b	C≡CH	6.25	0.2	0.05
1c	$CH=CH_2$	25	0.1	≦0.025
1d	CH_2CH_3	>100	0.2	0.39

Table 4. 24-Hour urinary recovery (%) of cephalosporins (**1**) after oral administration (100 mg/kg) to rats.

Compound	X	Urinary recovery (%)
1a	CH_3	25.7
1b	$C\equiv CH$	20.0
1c	$CH=CH_2$	34.0
1d	CH_2CH_3	31.5

hydrocarbon substituents at the 3-position are shown in Table 3.

The 3-ethynylcephalosporin (**1b**) exhibited better activity against *S. aureus* than any of the other cephalosporins (**1a**, **1c** and **1d**). The cephalosporins (**1a** and **1d**) with the alkyl radicals at the 3-position showed no significant activity against *S. aureus*.

Against Gram-negative bacteria, the activity of FK027 (**1c**) was highest while the 3-ethynylcephalosporin (**1b**) showed slightly reduced activity. The cephalosporins (**1a** and **1d**) with the alkyl chains were found to be 8~16 times less active against *Proteus vulgaris* 1 than FK027 (**1c**).

The urinary excretion of the cephalosporins (**1**) after oral administration (100 mg/kg) to rats can be seen in Table 4. The 3-ethynylcephalosporin (**1b**) was well absorbed, but its urinary recovery was less than that of FK027 (**1c**). Consequently, we could not find a correlation between the size of the substituent at the 3-position and the oral absorptivity in rats.

In this series, the influence of various substituents at the 3-position of the cephem nucleus on the antibacterial activity and oral absorption in rats was investigated by keeping the 7-acyl chain constant as the (*Z*)-2-(2-amino-4-thiazolyl)-2-carboxymethoxyiminoacetyl radical. The cephalosporins with the 2,2-dihalovinyl groups[2] and the ethynyl group (**1b**) showed improved activity against *S. aureus* but reduced urinary recovery after oral administration to rats in comparison with FK027 (**1c**). FK027 (**1c**) with the vinyl group at the 3-position was found to be a cephalosporin exhibiting not only higher activity against Gram-negative bacteria but also better oral absorptivity in rats.

Experimental

MP were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spectrometer and at 100 MHz on a Jeol-MH 100 NMR spectrometer using Me_4Si as an internal standard. IR spectra were taken on a Hitachi 260-10 spectrophotometer or a Shimadzu IR-420 spectrophotometer.

Antibiotic Susceptibility

All *in vitro* antibacterial activity data are given as the minimum inhibitory concentration (MIC) in μg/ml. MICs were determined by the agar dilution method using heart infusion agar (Difco) after incubation at 37°C for 20 hours with an inoculum size of about 10^6 cfu/ml.

Urinary Recovery

Sprague Dawley rats were fasted overnight and orally dosed with 100 mg/kg of the test drugs. Urine samples were collected for 24 hours after dosing. The samples were assayed by a disc-agar diffusion method using *Escherichia coli* NIHJ JC-2 or *E. coli* ATCC 33546 as the test organism and nutrient agar (Difco) as the test medium.

General Preparation of 7

Pathway A:

Diphenylmethyl 7β-Phenylacetamido-3-ethynyl-3-cephem-4-carboxylate 1-Oxide (**5**): To a soln of diphenylmethyl 7β-phenylacetamido-3-(2,2-dibromovinyl)-3-cephem-4-carboxylate 1-oxide[3] (**4**) (0.34 g, 0.5 mmol) in THF (20 ml) was added a soln of butyllithium (1.2 ml of 1.65 M soln in *n*-hexane, 2 mmol) at $-70\sim-60$°C under a nitrogen atm. After being stirred for 30 minutes at the same temp, the reaction mixture was diluted with EtOAc (20 ml) and acidified with 10% HCl. The separated organic layer was washed with H_2O and brine, and dried ($MgSO_4$). The solvent was evaporated to

afford 0.15 g (57.7%) of **5**: IR (Nujol) 1780, 1720, 1650 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.50 (1H, d, J=8 Hz), 7.70~7.20 (15H, m), 6.98 (1H, s), 5.85 (1H, dd, J=5, 8 Hz), 4.95 (1H, d, J=5 Hz), 4.70 (1H, s), 3.91 (2H, m), 3.62 (2H, s).

Diphenylmethyl 7β-Phenylacetamido-3-ethynyl-3-cephem-4-carboxylate (**6**): To a soln of **5** (3.3 g, 6.3 mmol) in DMF (30 ml) was added PCl_3 (1.17 ml, 12.6 mmol) at -30~-20°C, and the reaction mixture was stirred at the same temp for 30 minutes. The resultant mixture was poured into a mixture of EtOAc and H_2O. The separated organic layer was washed with satd sodium bicarbonate soln and brine, and dried ($MgSO_4$). The soln was evaporated *in vacuo* to afford 2.5 g (72.0%) of **6**: IR (Nujol) 1770, 1720, 1650 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.21 (1H, d, J=8 Hz), 7.70~7.20 (15H, m), 7.00 (1H, s), 5.80 (1H, dd, J=5, 8 Hz), 5.25 (1H, d, J=5 Hz), 4.72 (1H, s), 3.80 (2H, m), 3.60 (2H, s).

Diphenylmethyl 7β-Amino-3-ethynyl-3-cephem-4-carboxylate Hydrochloride (**7**): Pyridine (0.05 ml, 0.6 mmol) was added to a suspension of PCl_5 (0.125 g, 0.6 mmol) in dichlo romethane (1 ml) at -10°C, and the mixture was stirred at -15~-5°C for 30 minutes. **6** (0.153 g, 0.3 mmol) was added to the above mixture at -10°C and the reaction mixture was stirred under ice-cooling for 1 hour. Then, MeOH (0.2 ml) was added to the reaction mixture at -20°C and the resulting soln was stirred under ice-cooling for 1 hour. H_2O (0.2 ml) was added to the above mixture under ice-cooling. The resulting mixture was stirred for 30 minutes at the same temp, and then diluted with diisopropyl ether. The precipitate was collected by filtration, and washed with H_2O and diisopropyl ether to give 0.091 g (71.1%) of **7**: IR (Nujol) 1780, 1710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.60~7.07 (10H, m), 6.94 (1H, s), 5.28 (2H, s), 4.82 (1H, s), 3.92 and 3.68 (2H, ABq, J=17 Hz).

Pathway B:

Diphenylmethyl 7β-Amino-3-(2,2-dibromovinyl)-2-cephem-4-carboxylate Hydrochloride (**8**): **8** was obtained (97.8%) from **2** by a similar procedure as described for the prepn of **7**: IR (Nujol) 1775, 1730 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.63~7.13 (11H, m), 7.08 (1H, s), 6.83 (1H, s), 5.70 (1H, s), 5.20 (1H, d, J=4 Hz), 5.03 (1H, d, J=4 Hz).

Diphenylmethyl 7β-Formamido-3-(2,2-dibromovinyl)-2-cephem-4-carboxylate (**9**): **8** (5 g, 8.5 mmol) was added to a mixture of EtOAc (50 ml), THF (50 ml) and H_2O (100 ml), and the mixture was adjusted to pH 7 with satd sodium bicarbonate soln. The separated organic layer was washed with brine, and dried ($MgSO_4$). The soln was evaporated *in vacuo* and the residue was dissolved in dichloromethane (50 ml). On the other hand, a mixture of acetic anhydride (3.2 ml, 34 mmol) and formic acid (1.29 ml, 34 mmol) was stirred at 40~45°C for 30 minutes. This soln was added to the dichloromethane soln obtained above under ice-cooling, and the resultant mixture was stirred at the same temp for 1 hour. The reaction mixture was poured into a mixture of EtOAc and H_2O. The separated organic layer was washed with satd sodium bicarbonate soln and brine, and dried ($MgSO_4$). The soln was evaporated *in vacuo* to afford 4.2 g (85.4%) of **9**: IR (Nujol) 1780, 1725, 1655 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.17 (1H, d, J=8 Hz), 8.20 (1H, s), 7.73~7.23 (11H, m), 7.12 (1H, s), 6.90 (1H, s), 5.70 (1H, s), 5.60 (1H, dd, J=4, 8 Hz), 5.20 (1H, d, J=4 Hz).

Diphenylmethyl 7β-Formamido-3-(2,2-dibromovinyl)-3-cephem-4-carboxylate 1-Oxide (**10**): To a mixture of **9** (4.2 g, 7.26 mmol) in EtOAc (42 ml) was added a soln of *m*-chloroperbenzoic acid (1.72 g, 10 mmol) in EtOAc (8 ml) at -20~-10°C and the resultant mixture was stirred at -10°C for 45 minutes. The precipitate was collected by filtration to afford 1.9 g (43.7%) of **10**: IR (Nujol) 3270, 1790, 1710, 1655 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.47 (1H, d, J=8 Hz), 8.20 (1H, s), 7.77~7.20 (11H, m), 6.98 (1H, s), 6.12 (1H, dd, J=5, 8 Hz), 5.05 (1H, d, J=5 Hz), 4.17~3.67 (2H, m).

Diphenylmethyl 7β-Formamido-3-ethynyl-3-cephem-4-carboxylate 1-Oxide (**11**): **11** was obtained (98.5%) from **10** by a similar procedure as described for the prepn of **5**: IR (Nujol) 1790, 1720, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.48 (1H, d, J=8 Hz), 8.15 (1H, s), 7.78~7.12 (10H, m), 6.97 (1H, s), 6.03 (1H, dd, J=5, 8 Hz), 5.02 (1H, d, J=5 Hz), 4.72 (1H, s), 4.08~3.67 (2H, m).

Diphenylmethyl 7β-Formamido-3-ethynyl-3-cephem-4-carboxylate (**14**): **14** was obtained (55.5%) from **11** by a similar procedure as described for the prepn of **6**: mp 156~160°C;

Anal Calcd for $C_{13}H_{18}N_2O_4 \cdot \frac{1}{4}H_2O$: C 65.31, H 4.41, N 6.62, S 7.58.
Found: C 65.59, H 4.15, N 6.26, S 7.81.

Diphenylmethyl 7β-Formamido-3-[(*E*)-2-monobromovinyl]-3-cephem-4-carboxylate (**13**): To a soln of **10** (54 g, 0.091 mol) in THF (1.5 liters) was added a soln of butyllithium (235 ml of 1.55 M soln in *n*-hexane, 0.364 mol) at −70~−60°C under a nitrogen atm. Immediately after the addition, the reaction mixture was diluted with EtOAc (1 liter) and acidified with 10% HCl. The separated organic layer was washed with H_2O and brine, and dried ($MgSO_4$). The solvent was evaporated and the residue was dissolved in DMF (300 ml). To the DMF soln was added PCl_3 (15.1 ml, 0.173 mol) at −30~−20°C. The resultant mixture was stirred at the same temp for 30 minutes. The reaction mixture was poured into a mixture of EtOAc and H_2O, and the separated organic layer was washed with satd sodium bicarbonate soln and brine, and dried ($MgSO_4$). The soln was evaporated to yield a brownish powder, which was recrystallized from dichloromethane - diisopropyl ether to afford 20.03 g (55.5%) of **14**. The filtrate was purified by column chromatography on silica gel, eluting with dichloromethane - EtOAc (10: 1) to give 0.61 g (1.4%) of **13**; mp 139~142°C.

Diphenylmethyl 7β-Amino-3-ethynyl-3-cephem-4-carboxylate Hydrochloride (**7**): Concd HCl (1.6 ml, 18.12 mmol) was added to a suspension of **14** (3 g, 7.17 mmol) in MeOH (15 ml) at room temp for 2 hours. The reaction mixture was poured into ether (100 ml) and the resulting precipitate was collected by filtration to afford 2.79 g (99.7%) of **7**.

Diphenylmethyl 7β-Formamido-3-(2,2-dibromovinyl)-3-cephem-4-carboxylate (**12**)

12 was obtained (73.5%) from **10** by a similar procedure as described for the prepn of **6**; mp 119~124°C.

Anal Calcd for $C_{23}H_{18}N_2O_4SBr_2$: C 47.77, H 3.14, N 4.84, S 5.54, Br 27.64.
Found: C 47.66, H 3.02, N 4.83, S 5.57, Br 27.48.

7β-Formamido-3-(2,2-dibromovinyl)-3-cephem-4-carboxylic Acid (**15**)

TFA (10 ml) was added to a mixture of **12** (10 g, 17.3 mmol) and anisole (10 ml) in dichloromethane (30 ml) under ice-cooling, and the reaction mixture was stirred at the same temp for 30 minutes. The resultant mixture was poured into diisopropyl ether, and the precipitate was collected by filtration. The above precipitate was added to a mixture of EtOAc and H_2O, and the mixture was adjusted to pH 7.0 with satd sodium bicarbonate soln. The separated aq layer was acidified to pH 2 with 10% HCl, and extracted with EtOAc. The EtOAc soln was washed with brine, and dried ($MgSO_4$). The soln was evaporated *in vacuo* to afford 7.15 g (73.5%) of **15**.

7β-Formamido-3-[(*E*)-2-monobromovinyl]-3-cephem-4-carboxylic Acid (**16**)

16 was obtained (87.1%) from **13** by a similar procedure as described for the prepn of **15**; mp 122~126°C.

7β-Formamido-3-ethynyl-3-cephem-4-carboxylic Acid (**17**)

17 was obtained (34.9%) from **14** by a similar procedure as described for the prepn of **15**; mp 145~153°C.

^{1}H NMR and IR data of compounds (**12**~**17**) were listed in Tables 1 and 2.

General Preparation of **1b**

Pathway C:

Diphenylmethyl 7β-[(*Z*)-2-Diphenylmethoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)-acetamido]-3-(2,2-dibromovinyl)-2-cephem-4-carboxylate (**19**): To a soln of DMF (1.89 ml, 24.4 mmol) in THF (72 ml) was added phosphoryl chloride (2.25 ml, 24.4 mmol) under ice-cooling, and the mixture was stirred at the same temp for 30 minutes to prepare the Vilsmeier reagent. To the above mixture was added the *N*-formyl acid[3] (**18**) (8.96 g, 20.4 mmol) under ice-cooling, and the resultant mixture was stirred at the same temp for 30 minutes to produce an activated acid soln of **18**. To a soln of the 7β-amino-3-ethynylcephem (**7**) (10 g, 18.2 mmol) and *N*-(trimethylsilyl)acetamide (13.4 g, 102 mmol) in EtOAc (100 ml) was added the above activated acid soln at −30°C, and the reaction mixture was stirred at −20~−10°C for 30 minutes. The reaction mixture was poured into a mixture of EtOAc and H_2O. The separated organic layer was washed with satd sodium bicarbonate soln and brine, and dried ($MgSO_4$). The soln was evaporated *in vacuo* to afford 15.3 g (92.7%) of **19**: IR (Nujol) 1760, 1740, 1680 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.77 (1H, d, J=8 Hz), 8.51 (1H, s), 7.67~

7.10 (21H, m), 7.16 (1H, s), 6.90 (1H, s), 6.87 (1H, s), 5.67 (1H, s), 5.63 (1H, dd, J=5, 8 Hz), 5.23 (1H, d, J=5 Hz), 4.98 (2H, s).

Diphenylmethyl 7β-[(*Z*)-2-Diphenylmethoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)-acetamido]-3-(2,2-dibromovinyl)-3-cephem-4-carboxylate 1-Oxide (**20**): **20** was obtained (37.9%) from **19** by a similar procedure as described for the prepn of **10**: IR (Nujol) 1790, 1710, 1680, 1650 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.10 (1H, d, J=8 Hz), 8.52 (1H, s), 7.80~7.10 (21H, m), 6.95 (1H, s), 6.90 (1H, s), 6.08 (1H, dd, J=5, 8 Hz), 5.12 (1H, d, J=5 Hz), 4.93 (2H, s), 4.07~3.67 (2H, m).

Diphenylmethyl 7β-[(*Z*)-2-Diphenylmethoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)-acetamido]-3-ethynyl-3-cephem-4-carboxylate 1-Oxide (**21**): **21** was obtained (76.5%) from **20** by a similar procedure as described for the prepn of **5**: IR (Nujol) 1790, 1710, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.17 (1H, d, J=8 Hz), 8.50 (1H, s), 7.90~7.10 (21H, m), 6.97 (1H, s), 6.87 (1H, s), 6.08 (1H, dd, J=5, 8 Hz), 5.08 (1H, d, J=5 Hz), 4.90 (2H, s), 4.70 (1H, s), 4.10~3.67 (2H, m).

Diphenylmethyl 7β-[(*Z*)-2-Diphenylmethoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)-acetamido]-3-ethynyl-3-cephem-4-carboxylate (**22**): **22** was obtained (79.3%) from **21** by a similar manner as described for the prepn of **6**: IR (Nujol) 1780, 1720, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.75 (1H, d, J=8 Hz), 8.50 (1H, s), 7.75~7.07 (21H, m), 6.93 (1H, s), 6.86 (1H, s), 5.97 (1H, dd, J=5, 8 Hz), 5.25 (1H, d, J=5 Hz), 4.88 (2H, s), 4.67 (1H, s), 3.90~3.50 (2H, m).

Diphenylmethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-diphenylmethoxycarbonylmethoxyiminoacetamido]-3-ethynyl-3-cephem-4-carboxylate (**23**): To a soln of **22** (2.7 g, 3.33 mmol) in MeOH (13.5 ml) was added concd HCl (0.73 ml, 8.27 mmol) at room temp and the reaction mixture was stirred at the same temp for 2 hours. The reaction mixture was poured into a mixture of EtOAc and H_2O and the soln was adjusted to pH 7 with satd sodium bicarbonate soln. The separated organic layer was washed with brine, and dried ($MgSO_4$). The soln was evaporated *in vacuo* to afford 2.28 g (87.7%) of **23**: IR (Nujol) 1780, 1720, 1675, 1605 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.65 (1H, d, J=8 Hz), 7.77~7.00 (20H, m), 6.93 (1H, s), 6.86 (1H, s), 6.67 (1H, s), 5.93 (1H, dd, J=5, 8 Hz), 5.25 (1H, d, J=5 Hz), 4.83 (2H, s), 4.67 (1H, s), 3.77~3.57 (2H, m).

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-carboxymethoxyiminoacetamido]-3-ethynyl-3-cephem-4-carboxylic Acid (**1b**): **1b** was obtained (75.4%) from **23** by a similar procedure as described for the prepn of **15**: IR (Nujol) 3300, 1770, 1670 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.48 (1H, d, J=8 Hz), 6.75 (1H, s), 5.83 (1H, dd, J=5, 8 Hz), 5.18 (1H, d, J=5 Hz), 4.58 (2H, s), 4.48 (1H, s), 4.02~3.37 (2H, m).

Pathway D:

Diphenylmethyl 7β-[(*Z*)-2-Diphenylmethoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)-acetamido]-3-ethynyl-3-cephem-4-carboxylate (**22**): **22** was obtained (93.7%) from **7** and **18** by a similar manner as described for the prepn of **19**.

The following compounds were obtained from diphenylmethyl 7β-amino-3-ethyl-3-cephem-4-carboxylate[9] and 2-*tert*-butoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)acetic acid[10] according to Pathway D.

Diphenylmethyl 7β-[(*Z*)-2-*tert*-Butoxycarbonylmethoxyimino-2-(2-formamido-4-thiazolyl)acetamido]-3-ethyl-3-cephem-4-carboxylate: IR (Nujol) 1780, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.43 (1H, d, J=8 Hz), 7.55~7.05 (11H, m), 6.78 (1H, s), 5.73 (1H, dd, J=5, 8 Hz), 5.1 (1H, d, J=5 Hz), 4.52 (2H, s), 3.44 (2H, m), 2.7~2.0 (2H, m), 1.31 (9H, s), 0.92 (3H, m).

Diphenylmethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-*tert*-butoxycarbonylmethoxyiminoacetamido]-3-ethyl-3-cephem-4-carboxylate: IR (Nujol) 1775, 1670 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.52 (1H, d, J=8 Hz), 7.35 (10H, m), 6.89 (2H, s), 5.97 (1H, dd, J=5, 8 Hz), 5.2 (1H, d, J=5 Hz), 4.6 (2H, s), 3.54 (2H, m), 2.43 (2H, q, J=7 Hz), 1.44 (9H, s), 1.0 (3H, t, J=7 Hz).

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-carboxymethoxyiminoacetamido]-3-ethyl-3-cephem-4-carboxylic Acid (**1d**): IR (Nujol) 1760, 1670 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.37 (1H, d, J=8 Hz), 6.78 (1H, s), 5.68 (1H, dd, J=5, 8 Hz), 5.08 (1H, d, J=5 Hz), 4.56 (2H, s), 3.46 (2H, m), 2.38 (2H, q, J=7 Hz), 1.03 (3H, t, J=7 Hz).

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