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Synthesis and β-Lactamase Inhibitory Activity of 3-Cyano-3-cephem Derivatives

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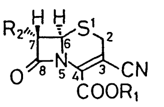
Two new 3-cyano-3-cephem derivatives, potassium 3-cyano-3-cephem-4-carboxylate (**2**) and potassium 7α-phenylacetamido-3-cyano-3-cephem-4-carboxylate (**3**), were prepared from 7β-aminocephalosporanic acid. Compounds **2** and **3** showed only weak β-lactamase inhibitory activity in contrast to sodium 7α-[(1*R*)-1-hydroxyethyl]-3-cyano-3-cephem-4-carboxylate (**1**), indicating the important role of the 7α-(1-hydroxyethyl) substituent for β-lactamase inhibitory activity.

A series of 7α-(1-hydroxyethyl)cephems and 1-oxacephems which have electron-withdrawing group at the 3 position were prepared in our recent work.[1,2] In those studies, we demonstrated that both of them have potent β-lactamase inhibitory activity and that the degree of inhibitory activity reflected the electron-withdrawing effect of the side chain at the 3 position. Among them, 3-cyanocephem **1** had especially potent inhibitory activity, but we did not elucidate the contribution of the 7α-(1-hydroxyethyl) group to their property. Thus, in this report, we describe the synthesis and biological activities of the 7-unsubstituted cephem (**2**) and the 7α-(phenylacetamido)cephem (**3**).



1 : R_1= Na, R_2= $CH_3\overset{(R)}{C}H(OH)$-

2 : R_1= K, R_2=H

3 : R_1= K, R_2= $PhCH_2CONH$-

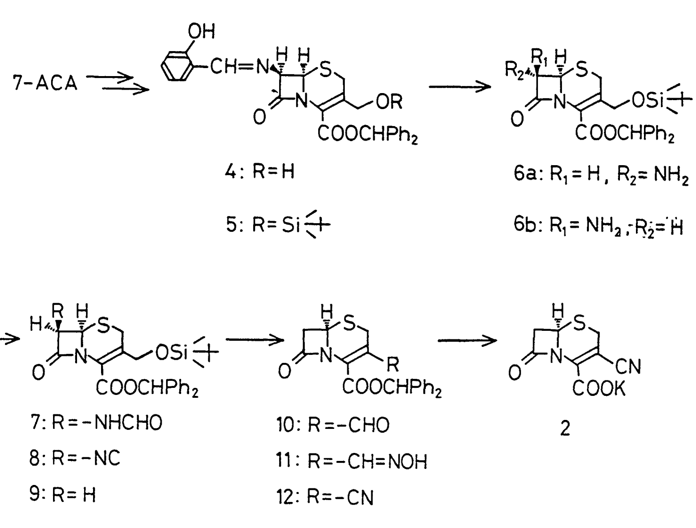
Fig. 1. Structures of **1**, **2**, and **3**.

Results and Discussion

Preparation of 3-Cyano-3-cephem Derivatives, 2 and 3. The starting compound, diphenylmethyl 7β-(*o*-hydroxybenzylideneamino)-3-hydroxymethyl-3-cephem-4-carboxylate (**4**)[3] was prepared from 7-aminocephalosporanic acid (7-ACA) in 3 steps. As shown in Scheme 1, compound **4** was protected with *t*-butyldimethylsilyl chloride and imidazole to give silyl ether **5** in 70% yield.

In order to epimerize at the 7 position, compound **5** was treated with *N*,*N*-diisopropylethylamine and then deprotected with Girard's reagent T to give a mixture of the 7α- and the 7β-aminocephem (**6a** and **6b**). The mixture was separated by silica-gel chromatography to give **6a** and **6b** in 12% and 28% yields from **5**, respectively.

7-Unsubstituted cephem **2** was synthesized from **6b**. Compound **6b** was deaminated as described by Hirai and co-workers.[4] 7β-Aminocephem **6b** was converted to 7β-formamidocephem **7** with acetic formic anhydride in 93% yield. Dehydration of **7** was accomplished with phosphoryl chloride and 2,6-



Scheme 1.

lutidine in dichloromethane at 4 °C for 12 h to give 7β-isocyanocephem **8** in 52% yield [IR (Nujol) 2150 cm^{-1} (isocyano group); ^{1}H NMR ($CDCl_3$) δ=5.17 (1H, d, *J*=5 Hz, H-7)]. Radical reduction of **8** was carried out with tributyltin hydride and a catalytic amount of azobisisobutyronitrile in benzene under reflux to give 7-unsubstituted cephem **9** [^{1}H NMR ($CDCl_3$) δ=2.93 (1H, dd, *J*=2 and 16 Hz, H-7β), 3.60 (1H, dd, *J*=5 and 16 Hz, H-7α)] in 84% yield. Compound **9** was desilylated with boron trifluoride etherate in acetonitrile at −25 °C for 20 min. The 3-hydroxymethyl intermediate was so unstable that it was oxidized instantly with Collins reagent to give 3-formylcephem **10** [^{1}H NMR ($CDCl_3$) δ=9.45 (1H, s, formyl)] in 47% yield from **9**. 3-(Hydroxyiminomethyl)cephem **11** was prepared by treatment of **10** with hydroxylamine hydrochloride in aqueous tetrahydrofuran in 42% yield. Dehydration of **11** gave 3-cyanocephem **12** [IR (Nujol) 2220 cm^{-1} (cyano group)] in 68% yield. Peter and co-workers$^{5)}$ synthesized 7β-acylamino-3-cyanocephems via deprotection of the diphenylmethyl ester using trifluoroacetic acid and anisole. Additionally, Ohtani and co-workers$^{6)}$ described the mild deprotection of diphenylmethyl and *p*-methoxybenzyl esters on carbapenems using aluminum trichloride in anisole. In our case, however, these deprotection methods produced an undesirable amount of degradation products. After several trials, we found that a combination of diethylaluminum chloride and anisole deprotected the diphenylmethyl ester quite effectively. Thus, treatment of **12** with diethylaluminum chloride and anisole in dichloromethane at −20 °C for 15 min afforded the desired compound **2** [IR (Nujol) 2200, 1755 cm^{-1} (cyano group and β-lactam)] in 41% yield.

Table 1. Minimal Inhibitory Concentrations$^{a)}$ of Compounds **1**, **2**, and **3**

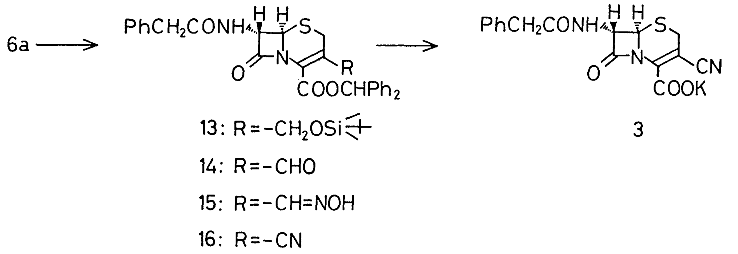
Organism	M I C/mcg ml^{-1}		
	1	**2**	**3**
Staph. aureus 209P JC-1	50	200	200
B. subtilis ATCC 6633	100	50	25
Escherichia coli 29	100	50	200
Kl. pneumoniae 12	100	50	200

a) Mueller-Hinton agar 10^{-2}: Stamp method; 37 °C, 20 h.

The other desired compound **3** was obtained from 7α-aminocephem **6a** as shown in Scheme 2. Acylation of **6a** gave 7α-(phenylacetamido)cephem **13** in 97% yield, and then **13** was converted to **3** [IR (Nujol) 2220, 1780 cm^{-1} (cyano group and β-lactam); ^{1}H NMR (DMSO-d_6) δ=4.78 (1H, d, *J*=2 Hz, H-6), 4.90 (1H, dd, *J*=2 and 8 Hz, H-7)] in a similar manner to that for **2**.

Biological Activity. The minimal inhibitory concentrations (MICs) of compounds **1**, **2**, and **3** against four test organisms are shown in Table 1. 7-Unsubstituted cephem **2** and 7α-(phenylacetamido)cephem **3** showed only weak antibacterial activity, which was similar to that of 7α-(1-hydroxyethyl)cephem **1**.

The β-lactamase inhibitory activities of compounds **1**, **2**, **3** and clavulanic acid, a conventional β-lactamase inhibitor, are shown in Table 2. The inhibitory



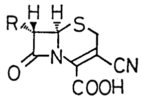
Scheme 2.

Table 2. β-Lactamase Inhibitory Activity$^{a)}$

β-Lactamase	I D$_{50}$/mcg ml^{-1}			
	1	2	3	Clavulanic acid
TEM PCase (*Escherichia coli* 18)	17	>500	>500	1.0
Ia CSase (*Enterobacter cloacae* 91)	<0.03	>500	>500	12
Ib CSase (*E. coli* HB101/pCF3)	<0.5	>500	>500	7.8
Ic CSase (*Proteus vulgaris* 9)	0.9	>500	160	0.6

a) Serial dilutions of a β-lactamase inhibitor were incubated with enzyme solution for 10 min at 37 °C. Residual β-lactamase activity was determined spectrophotometrically using the chromogenic substrate nitrocefin (50 mcg ml^{-1}) at 482 nm. ID$_{50}$ was calculated as the concentration inhibiting 50% of activity.

Table 3. CNDO/2 Calculation Data of Compound **1′**, **2′**, and **3′**



Compound	R	Charge density of β-lactam ring		
		N	C-8	O
1′	$\overset{(R)}{CH_3CH}(OH)$-	−0.1812	0.3495	−0.2810
2′	H	−0.1837	0.3537	−0.2731
3′	$PhCH_2CONH$-	−0.1912	0.3575	−0.2797

dose-fifty(ID_{50}) against both the penicillinase (PCase) and cephalosporinases (CSases) of compounds **2** and **3** showed that they have only weak β-lactamase inhibitory activity.[7] These results suggested that the 7α-(1-hydroxyethyl) group of compound **1** has an important role in the potent β-lactamase inhibitory activity as well as electron-withdrawing effect[1,8] at the 3 position. Using CNDO/2 calculation,[9,10] we investigated the effect of the 7α-substituents on the β-lactam rings of compounds **1′**, **2′**, and **3′**, which corresponded to compounds **1**, **2**, and **3**, respectively. As shown in Table 3, the atomic charge densities of the β-lactams showed that the degree of amide resonance of compounds **2′** and **3′** was lower than that of **1′**. These calculations suggested that the β-lactams of **2** and **3** are more chemically reactive than that of compound **1** in spite of their weak β-lactamase inhibitory activities. In other words, the 7α-(1-hydroxyethyl) moiety of **1** may be an important substituent in affinity to β-lactamases.

Experimental

All the melting points were measured with a Yanagimoto micro melting point apparatus without any correction. Optical rotations were measured with a Jasco DIF-140 automatic polarimeter. IR spectra were recorded with a Hitachi 260-10 spectrometer. Mass spectra were recorded with a Finnigen MAT instrument Inc. TSQ 70 spectrometer. Microanalyses were performed in our laboratory. ^{1}H NMR were recorded using a Hitachi R-90H spectrometer. Chemical shifts (δ) are recorded in ppm from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (in D_2O) or tetramethylsilane (TMS, in $CDCl_3$ and DMSO-d_6) as internal standard. In CNDO/2 calculation, molecular geometries of structures **1′**, **2′**, and **3′** were calculated by minimizing the total energy with respect to all geometrical variables, using the MNDO method.

Diphenylmethyl 7β-(*o*-Hydroxybenzylideneamino)-3-*t*-butyldimethylsilyloxymethyl-3-cephem-4-carboxylate (5). To a solution of diphenylmethyl 7β-(*o*-hydroxybenzylideneamino)-3-hydroxymethyl-3-cephem-4-carboxylate (**4**)[3] (5.02 g, 10 mmol) and imidazole (1.36 g, 20 mmol) in *N,N*-dimethylformamide (30 mL) was added gradually *t*-butyldimethylsilyl chloride (1.66 g, 11 mmol) at −20 °C. The mixture was stirred at −20 °C for 30 min and poured into a mixture of ethyl acetate (300 mL) and ice-cooled 0.5 M hydrochloric acid (300 mL; 1 M=1 mol dm^{-3}). The organic layer was separated, washed with 5% aqueous sodium hydrogencarbonate (200 mL) and saturated aqueous sodium chloride (200 mL×3), dried over magnesium sulfate and evaporated in vacuo. The residue was chromatographed on silica-gel column with dichloromethane-ethyl acetate (10 : 1) to give a solid of **5** (4.30 g, 70%): mp 68—69 °C; $[\alpha]_D^{25}$ −32.0° (*c* 1, $CHCl_3$); IR (Nujol) 1780, 1700, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.10 (6H, s), 0.93 (9H, s), 3.65 (2H, m), 4.55 and 4.75 (2H, ABq, *J*=15 Hz), 5.17 (1H, d, *J*=5 Hz), 5.28 (1H, d, *J*=5 Hz), 6.90—7.10 (2H, m), 7.00 (1H, s), 7.25—7.60 (12H, m), 8.70 (1H, s), 12.30 (1H, s); FAB-MS *m/z* 615 (M^+). Found: C, 66.34; H, 6.38; N, 4.39; S, 5.16%. Calcd for $C_{34}H_{38}N_2O_5SSi$: C, 66.42; H, 6.23; N, 4.56; S, 5.22%.

Diphenylmethyl 7α-Amino-3-*t*-butyldimethylsilyloxymethyl-3-cephem-4-carboxylate (6a) and 7β-Isomer (6b). To a solution of **5** (58.0 g, 94.3 mmol) in tetrahydrofuran (1.14 L) was added dropwise *N,N*-diisopropylethylamine (16.4 mL, 94.3 mmol) at 0 °C. The mixture was stood at 4 °C for 12.5 h, poured into ice-cooled 0.1 M aqueous tartaric acid (1.5 L), and extracted with dichloromethane (2 L). The organic layer was separated, washed with water, dried over magnesium sulfate and evaporated in vacuo. The residue was dissolved in a mixture of methanol (570 mL) and ethyl acetate (280 mL). To the mixture was added Girard's reagent T (48.8 g, 219 mmol) gradually at 0 °C under stirring for an hour, and the precipitate was filtered off. The filtrate was diluted with ethyl acetate (3 L) washed with water (1 L×3), dried over magnesium sulfate, and evaporated. The residue was chromatographed on silica-gel column (2.5 kg) with dichloromethane-ethyl acetate (20 : 1) to give 7α-amino compound **6a** (5.8 g, 12%) and 7β-isomer **6b** (13.3 g, 28%) as colorless crystals, respectively. 7α-Amino compound **6a**: mp 116—117 °C; IR (Nujol) 1760, 1735, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.10 (6H, s), 0.90 (9H s), 1.60—2.00 (2H, br s), 3.40 and 3.70 (2H, ABq, *J*=18 Hz), 4.20 (1H, m), 4.40—4.60 (3H, m), 7.03 (1H, s), 7.20—7.60 (10H, m); FAB-MS *m/z* 511 (M^+). Found: C, 63.52; H, 6.83; N, 5.37; S, 6.44%. Calcd for $C_{27}H_{34}N_2O_4SSi$: C, 63.50; H, 6.71; N, 5.49; S, 6.28%. 7β-Isomer **6b**: mp 117—118 °C; $[\alpha]_D^{25}$ +31.9° (*c* 1, $CHCl_3$); IR (Nujol) 1770, 1730, 1615 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.10 (6H, s), 0.90 (9H, s), 1.60—2.00 (2H, br s), 3.50 and 3.70 (2H, ABq, *J*=18 Hz), 4.47 and 4.67 (2H ABq, *J*=15 Hz), 4.75 (1H, m), 4.95 (1H, d, *J*=5 Hz), 6.97 (1H, s), 7.20—7.60 (10H, m); FAB-MS *m/z* 511 (M^+). Found: C, 63.30; H, 6.46; N, 5.43; S, 6.52%. Calcd for $C_{27}H_{34}N_2O_4SSi$: C, 63.50; H, 6.71; N, 5.49; S, 6.28%.

Diphenylmethyl 7β-Formamido-3-*t*-butyldimethylsilyloxymethyl-3-cephem-4-carboxylate (7). To a solution of **6b**

(5.0 g, 9.8 mmol) in dichloromethane (100 mL) was added acetic formic anhydride (0.83 mL, 10.8 mmol) dropwise at 0 °C. The mixture was stirred at 0 °C for 30 min and poured into 2% aqueous sodium hydrogencarbonate (100 mL). The organic layer was separated, washed with saturated aqueous sodium chloride (100 mL), dried over magnesium sulfate, and evaporated in vacuo to give colorless crystals of **7** (4.9 g, 93%): mp 122°C; $[\alpha]_D^{25}$ +32.9° (c 1, $CHCl_3$); IR (CH_2Cl_2) 1780, 1720, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.1 (6H, s), 0.90 (9H, s), 3.51 and 3.62 (2H, ABq, J=15 Hz), 4.50 and 4.67 (2H, ABq, J=18 Hz), 4.96 (1H, d, J=5 Hz), 5.89 (1H, dd, J=5 and 8 Hz), 6.74 (1H, d, J=8 Hz), 7.20—7.50 (10H, m), 8.20 (1H, s); FAB-MS m/z 539 (M^+). Found: C, 62.07; H, 6.41; N, 5.12; S, 5.99%. Calcd for $C_{28}H_{34}N_2O_5SSi$: C, 62.42; H, 6.36; N, 5.20; S, 5.95%.

Diphenylmethyl 7β-Isocyano-3-*t*-butyldimethylsilyloxymethyl-3-cephem-4-carboxylate (8). To a solution of **7** (1.0 g, 1.9 mmol) in dichloromethane (15 ml) were added 2,6-lutidine (0.81 ml, 7 mmol) and phosphoryl chloride (0.22 mL, 2.3 mmol) at 0 °C. After being kept at 4 °C for 12 h, the mixture was washed successively with 5% aqueous citric acid (15 mL), 5% aqueous sodium hydrogencarbonate (15 mL) and saturated aqueous sodium chloride (15 mL). The organic layer was dried over magnesium sulfate and evaporated in vacuo. The residue was chromatographed on Florisil (100—200 mesh, 20 g) with dichloromethane to give a yellow oil. This was triturated with petroleum ether and filtered to give a yellow powder of **8** (450 mg, 52%): mp 93—95 °C (decomposed); $[\alpha]_D^{25}$ +29.5° (c 1, $CHCl_3$); IR (Nujol) 2150, 1785, 1715, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.10 (6H, s), 0.90 (9H, s), 3.47 and 3.77 (2H, ABq, J=18 Hz), 4.67 (2H, m), 4.93 (1H, d, J=5 Hz), 5.17 (1H, d, J=5 Hz), 6.90 (1H, s), 7.20—7.50 (10H, m); FAB-MS m/z 521 (M^+). Found: C, 64.96; H, 6.35; N, 5.26; S, 6.40%. Calcd for $C_{28}H_{32}N_2O_4SSi$: C, 64.58; H, 6.19; N, 5.38; S, 6.16%.

Diphenylmethyl 3-*t*-Butyldimethylsilyloxymethyl-3-cephem-4-carboxylate (9). To a solution of **8** (100 mg, 0.19 mmol) in benzene (1.0 mL) were added azobisisobutyronitrile (1.0 mg) and tributyltin hydride (0.21 mL, 0.21 mmol) at room temperature. The mixture was refluxed for 50 min and evaporated in vacuo. The residue was chromatographed on silica-gel column (4.5 g) with dichloromethane to give an oil, which on recrystallization from hexane gave colorless crystals of **9** (79 mg, 84%): mp 105—110 °C (decomposed); $[\alpha]_D^{25}$ +55.8° (c 1, $CHCl_3$); IR (CH_2Cl_2) 1775, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.10 (6H, s), 0.90 (9H, s), 2.93 (1H, dd, J=2 and 16 Hz), 3.55 (2H, m), 3.60 (1H, dd, J=5 and 16 Hz), 4.47 (2H, m), 4.65 (1H, dd, J=2 and 5 Hz), 6.95 (1H, s), 7.10—7.60 (10H, m); FAB-MS m/z 496 (M^+). Found: C, 65.27; H, 6.67; N, 2.76; S, 6.59%. Calcd for $C_{27}H_{33}NO_4SSi$: C, 65.42; H, 6.71; N, 2.83; S, 6.47%.

Diphenylmethyl 3-Formyl-3-cephem-4-carboxylate (10). To a solution of **9** (2.0 g, 4.1 mmol) in acetonitrile (50 mL) was added boron trifluoride etherate (0.75 mL, 6.1 mmol) at −25 °C. The mixture was stirred at −25 °C for 20 min, poured into ice-cooled water, adjusted to pH 7.0 with sodium hydrogencarbonate and extracted with dichloromethane (100 mL). The organic layer was washed with water and saturated aqueous sodium chloride and dried over magnesium sulfate. To this solution was added Collins reagent (6.3 g, 24.5 mmol); the mixture was stirred at room temperature for 30 min, and the precipitate was filtered off, washed with 2% aqueous citric acid, water and saturated aqueous sodium chloride, dried over sodium sulfate, and evaporated in vacuo. The residue was chromatographed on silica-gel column (50 g) with dichloromethane to give an amorphous powder of **10** (0.7 g, 47%): IR (Nujol) 1770, 1730, 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ=3.17 (1H, dd, J=2 and 16 Hz), 3.30 and 4.40 (2H, ABq, J=18 Hz), 3.72 (1H, dd, J=5 and 16 Hz), 4.77 (1H, dd, J=2 and 5 Hz), 7.10 (1H, s), 7.10—7.50 (10H, m); FAB-MS m/z 379 (M^+). Found: C, 66.61; H, 4.67; N, 3.55; S, 8.41%. Calcd for $C_{21}H_{17}NO_4S$: C, 66.47; H, 4.52; N, 3.69; S, 8.45%.

Diphenylmethyl 3-Hydroxyiminomethyl-3-cephem-4-carboxylate (11). To a mixture of **10** (680 mg, 1.8 mmol) in tetrahydrofuran-water (12:1, 13 ml) was added hydroxylamine hydrochloride (125 mg, 1.8 mmol) at room temperature. The mixture was stirred at 60—65 °C for 3 h, cooled and diluted with ethyl acetate (50 mL), washed with water and saturated aqueous sodium chloride, dried over magnesium sulfate, and evaporated in vacuo. The residue was chromatographed on silica-gel column (25 g) with dichloromethane to give an oil of **11** (296 mg, 42%): $[\alpha]_D^{25}$ +152.3° (c 1, $CHCl_3$); IR (CH_2Cl_2) 1780, 1620, 1490, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) δ=3.03 (1H, dd, J=2 and 16 Hz), 3.47 and 4.00 (2H, ABq, J=18 Hz), 3.65 (1H, dd, J=5 and 16 Hz), 4.73 (1H, dd, J=2 and 5 Hz), 7.00 (1H, s), 7.10—7.60 (10H, m), 8.15 (1H, s); FAB-MS m/z 394 (M^+). Found: C, 63.95; H, 4.78; N, 7.34; S, 7.95%. Calcd for $C_{21}H_{18}N_2O_4S$: C, 63.94; H, 4.60; N, 7.10; S, 8.13%.

Diphenylmethyl 3-Cyano-3-cephem-4-carboxylate (12). To a solution of *N,N*-dimethylformamide (0.065 mL, 0.84 mmol) in ethyl acetate (0.4 mL) was added phosphoryl chloride (0.088 mL, 0.84 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, and a solution of **11** (220 mg, 0.56 mmol) in ethyl acetate (4 mL) was added to the mixture at 0 °C. This mixture was stirred at room temperature for 5 h, poured into ice-cooled 3% aqueous sodium hydrogencarbonate (20 mL), and extracted with ethyl acetate (30 mL). The organic layer was washed with water and saturated aqueous sodium chloride, dried over magnesium sulfate and evaporated in vacuo. The residue was triturated with diethyl ether to give a yellow, amorphous powder of **12** (143 mg, 68%): IR (Nujol) 2220, 1785, 1730, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ=3.12 (1H, dd, J=2 and 18 Hz), 3.65 (2H, m), 3.74 (1H, dd, J=6 and 18 Hz), 4.70 (1H, dd, J=2 and 6 Hz), 7.00 (1H, s), 7.10—7.60 (10H, m); FAB-MS m/z 376 (M^+). Found: C, 67.18; H, 4.49; N, 7.39; S, 8.72%. Calcd for $C_{21}H_{16}N_2O_3S$: C, 67.00; H, 4.28; N, 7.44; S, 8.52%.

Potassium 3-Cyano-3-cephem-4-carboxylate (2). To a mixture of **12** (100 mg, 0.27 mmol), anisole (0.1 mL), and dichloromethane (3 mL) was added diethylaluminum chloride (0.59 mL, in 0.9 M hexane solution) dropwise at −25 °C. The mixture was stirred at −20 °C for 15 min, poured into ice-cooled 6% aqueous sodium hydrogencarbonate (15 mL), and washed with ethyl acetate (15 mL). The aqueous layer was separated, adjusted to pH 2.0 with 1 M hydrochloric acid at 0—5 °C, and extracted with ethyl acetate (15 mL×3). The extract was washed with saturated aqueous sodium chloride, dried over magnesium sulfate and evaporated in vacuo until about 5 mL. To this solution was added potassium 2-ethylhexanoate (0.42 mL, in 0.567 M diethyl ether solution) at 0 °C. The mixture was evaporated in vacuo and the residue was triturated with diethyl ether and dried in vacuo to give an amorphous powder of **2** (27.2 mg, 41%); mp 145—150 °C (decomposed); IR (Nujol) 2200, 1755, 1620, 1580 cm^{-1}; ^{1}H NMR (D_2O) δ=3.22 (1H, dd, *J*=2 and 16 Hz), 3.48 and 3.78 (2H, ABq, *J*=18 Hz), 3.75 (1H, dd, *J*=5 and 16 Hz), 4.80—5.00 (1H, m). Found: C, 36.40; H, 2.90; N, 10.75; S, 11.90%. Calcd for $C_8H_5KN_2O_3S \cdot H_2O$: C, 36.08; H, 2.65; N, 10.52; S, 12.04%.

Diphenylmethyl 7α-Phenylacetamido-3-*t*-butyldimethylsilyloxymethyl-3-cephem-4-carboxylate (13). To a solution of **6a** (1.68 g, 3.3 mmol) in tetrahydrofuran (32 mL) was added 1,3-bis(trimethylsilyl)urea (2.0 g, 9.9 mmol) at room temperature. The mixture was stirred at room temperature for 1 h, and this was added a solution of phenylacetyl chloride (0.48 mL, 3.6 mmol) in tetrahydrofuran (8 mL) at −15 °C. The mixture was then stirred at −15 °C for 30 min, poured into 0.5% aqueous sodium hydrogencarbonate (100 mL) and extracted with ethyl acetate (60 mL×2). The organic layer was separated, washed with water and saturated aqueous sodium chloride, dried over magnesium sulfate, and evaporated in vacuo to give an amorphous powder of **13** (2.0 g, 97%): 70—75 °C (decomposed); IR (Nujol) 1785, 1735, 1685 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=0.10 (6H, s), 0.90 (9H, s), 3.32 and 3.58 (2H, ABq, *J*=15 Hz), 3.60 (2H, m), 4.40 (2H, m), 4.60 (1H, d, *J*=2 Hz), 4.80 (1H, dd, *J*=2 and 8 Hz), 6.20 (1H, d, *J*=8 Hz), 6.93 (1H, s), 7.10—7.50 (15H, m); FAB-MS *m/z* 629 (M$^+$). Found: C, 66.85; H, 6.41; N, 4.46; S, 5.10%. Calcd for $C_{35}H_{40}N_2O_5SSi$: C, 66.90; H, 6.65; N, 4.26; S, 5.02%.

Diphenylmethyl 7α-Phenylacetamido-3-formyl-3-cephem-4-carboxylate (14). Compound **13** was treated as described for compound **9** to give an amorphous powder: yield 0.52 g (32%); mp 140 °C (decomposed); IR (Nujol) 1820, 1720, 1660, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=3.27 and 3.73 (2H, ABq, *J*=18 Hz), 3.65 (2H, m), 4.67 (1H, dd, *J*=2 and 8 Hz), 4.93 (1H, d, *J*=2 Hz), 6.10 (1H, d, *J*=8 Hz), 7.10 (1H, s), 7.10—7.60 (15H, m), 9.37 (1H, s); FAB-MS *m/z* 513 (M$^+$). Found: C, 68.03; H, 4.81; N, 5.68; S, 6.04%. Calcd for $C_{29}H_{24}N_2O_5S$: C, 67.95; H, 4.72; N, 5.47; S, 6.26%.

Diphenylmethyl 7α-Phenylacetamido-3-hydroxyiminomethyl-3-cephem-4-carboxylate (15). Compound **14** was treated as described for compound **10** to give an amorphous powder: yield 0.47 g (90%); mp 162—165 °C (decomposed); IR (Nujol) 1780, 1750, 1720, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$-DMSO-d_6, 1 : 1) δ=3.50 and 3.97 (2H, ABq, *J*=18 Hz), 3.60 (2H, m), 4.77 (1H, d, *J*=2 Hz), 4.90 (1H, dd, *J*=2 and 8 Hz), 6.97 (1H, s), 7.20—7.60 (15H, m), 7.90 (1H, d, *J*=8 Hz), 8.05 (1H, s); FAB-MS *m/z* 528 (M$^+$). Found: C, 65.67; H, 4.59; N, 7.69; S, 6.28%. Calcd for $C_{29}H_{25}N_3O_5S$: C, 66.02; H, 4.78; N, 7.96; S, 6.08%.

Diphenylmethyl 7α-Phenylacetamido-3-cyano-3-cephem-4-carboxylate (16). Compound **15** was treated as described for compound **11** to give an amorphous powder: yield 0.17 g (46%); mp 188—193 °C (decomposed); IR (Nujol) 2220, 1790, 1730, 1660, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ=3.55 (2H, m), 3.73 and 3.97 (2H, ABq, *J*=18 Hz), 4.90 (1H, dd, *J*=2 and 8 Hz), 5.03 (1H, d, *J*=2 Hz), 7.00 (1H, s), 7.10—7.60 (15H, m), 9.05 (1H, d, *J*=8 Hz); FAB-MS *m/z* 510 (M$^+$). Found: C, 67.18; H, 4.84; N, 7.87; S, 6.41%. Calcd for $C_{29}H_{23}N_3O_4S \cdot 1/2H_2O$: C, 67.16; H, 4.66; N, 8.10; S, 6.18%.

Potassium 7α-Phenylacetanido-3-cyano-3-cephem-4-carboxylate (3). Compound **16** was treated as described for compound **12** to give an amorphous powder: yield 17.3 mg (46%); mp 160 °C (decomposed); IR (Nujol) 2220, 1780, 1640, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ=3.37 and 3.70 (2H, ABq, *J*=18 Hz), 3.50 (2H, m), 4.78 (1H, d, *J*=2 Hz), 4.90 (1H, dd, *J*=2 and 8 Hz), 7.27 (5H, s), 9.10 (1H, d, *J*=8 Hz); Found: C, 50.63; H, 3.40; N, 10.86; S, 8.26%. Calcd for $C_{16}H_{12}KN_3O_4S$: C, 50.38; H, 3.17; N, 11.02; S, 8.14%.

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References

1) S. Nishimura, N. Yasuda, H. Sasaki, Y. Matsumoto, T. Kamimura, K. Sakane, and T. Takaya, *J. Antibiot.*, **42**, 159 (1989).

2) S. Nishimura, H. Sasaki, N. Yasuda, Y. Matsumoto, T. Kamimura, K. Sakane, and T. Takaya, *J. Antibiot.*, **42**, 1124 (1989).

3) H. Yamanaka, T. Chiba, K. Kawabata, H. Takasugi, T. Masugi, and T. Takaya, *J. Antibiot.*, **38**, 1738 (1985).

4) H. Hirai, K. Sawada, M. Aratani, and M. Hashimoto, *Tetrahedron Lett.*, **25**, 5075 (1984).

5) H. Peter, B. Müller, and H. Bickel, *Helv. Chim. Acta*, **58**, 2450 (1975).

6) M. Ohtani, F. Watanabe, and M. Narisada, *J. Org. Chem.*, **49**, 5271 (1984).

7) 7β-Isomer of **3** was synthesized as described for Peter's report,[5] and it was found to have no β-lactamase inhibitory activity.

8) R. B. Hermann, *J. Antibiot.*, **26**, 223 (1973).

9) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966).

10) M. J. S. Dewar and W. Thiel, *J. Am. Chem. Soc.*, **99**, 4899 (1977).