SYNTHESIS AND BIOLOGICAL ACTIVITY OF 7α-HYDROXYETHYL-1-OXACEPHEM DERIVATIVES

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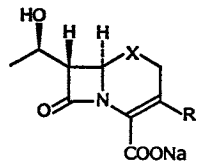
A series of 7α-hydroxyethyl-1-oxacephems (**1**) was synthesized. The main focus of this study was to investigate biological activity relationships between 1-oxacephems (**1**) and the corresponding cephems (**2**). Replacement of the sulfur atom of **2** by the oxygen atom caused an enhancement of antibacterial activity, although the antibacterial activity of **1** was not high enough. Additionally **1** showed β-lactamase inhibitory activity, especially against cephalosporinase. However, the potency was lower than that of **2**.

Our recent work[1] demonstrated the synthesis and biological activity of 7α-hydroxyethyl cephem derivatives (**2**). Cephems of type **2** have potent β-lactamase inhibitory activity against both of penicillinase and cephalosporinase, but exhibit poor antibacterial activity. Having been encouraged by the biological characteristics of **2**, we were interested in studying on the properties of other nucleus analogue of **2**.

It is well-known that the enhancement of antibacterial activity is induced by replacement of the sulfur atom of cephalosporin by the oxygen atom[2]. Thus, the question has been raised as to whether or not **1** will have more potent biological activity than **2**. Using CNDO/2 calculations[3,4], we investigated the influence of the 1 position atom on the β-lactam ring of model compounds **3** and **4** which have an electron-withdrawing group (EWG) at the 3 position as shown in Table 1.

Fig. 1. Structure of **1** and **2**.



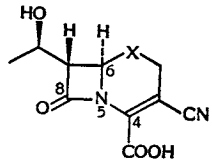
1 X=O R=EWG
2 X=S R=EWG

The altitude of a pyramid (Δh^5) of **4** is higher than that of **3**, and the atomic charge densities of the β-lactam show that the degree of amide resonance of **4** is lower than that of **3**. These calculations suggest that **4** is more reactive than **3** and encouraged us to investigate 1-oxacephems (**1**).

Chemistry

1-Oxacephem (**1**) was synthesized according to the method of synthesis of **2**[1]. As shown in Scheme 1, azetidinone **5**[6] was treated with allyl alcohol in the presence of zinc acetate to give **6**. Oxidation of **6** with $KMnO_4$ in aqueous *tert*-butanol gave diol **7**. **9** was afforded by selective protection of **7** followed by oxidation with DMSO - trifluoroacetic anhydride. Phosphorane **12** was obtained by the condensation of allyl glyoxylate with **9**, conversion to the chloride with $SOCl_2$ and subsequent reaction with Ph_3P. An intramolecular Wittig reaction of **12** in the presence of hydroquinone[7] gave

Table 1. CNDO/2 calculation[a] data of 7-hydroxyethyl cephem **3** and 1-oxacephem **4**.

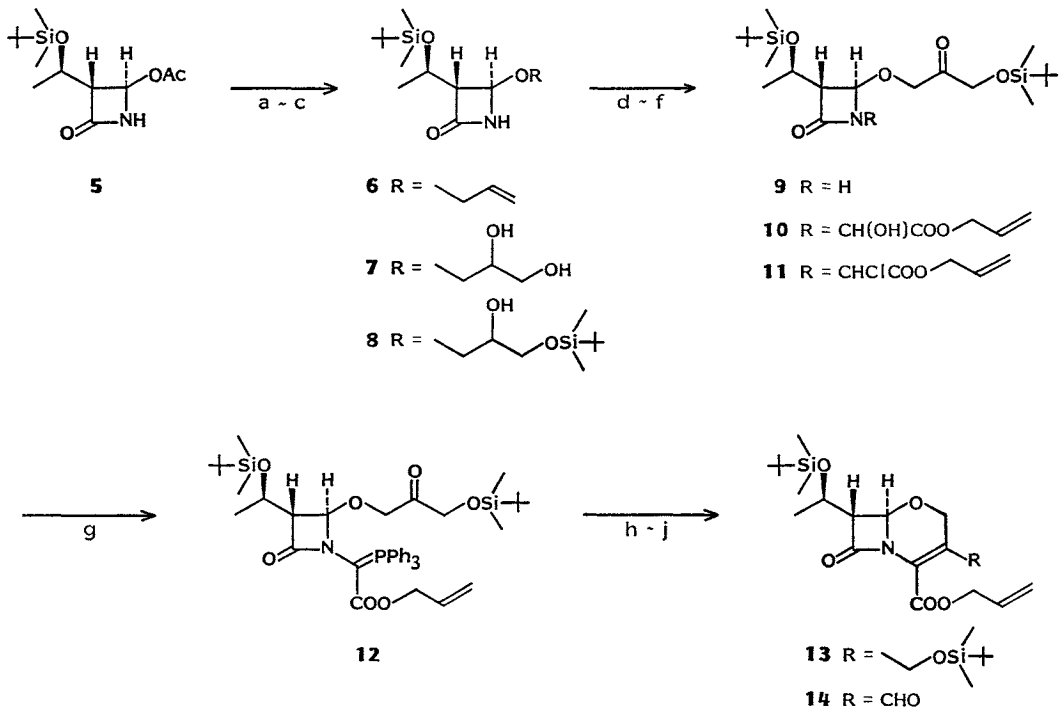


Compound No.	X	Δh^b (Å)	Charge density of β-lactam ring		
			N	C-8	O
3	S	0.23	−0.1812	0.3495	−0.2810
4	O	0.29	−0.2009	0.3509	−0.2786

[a] Molecular geometries of structures **3** and **4** were calculated by minimizing the total energy with respect to all geometrical variables, using the MNDO method.

[b] Δh: The altitude of a pyramid, having N-5 as apex and C-4, C-6 and C-8 as base.

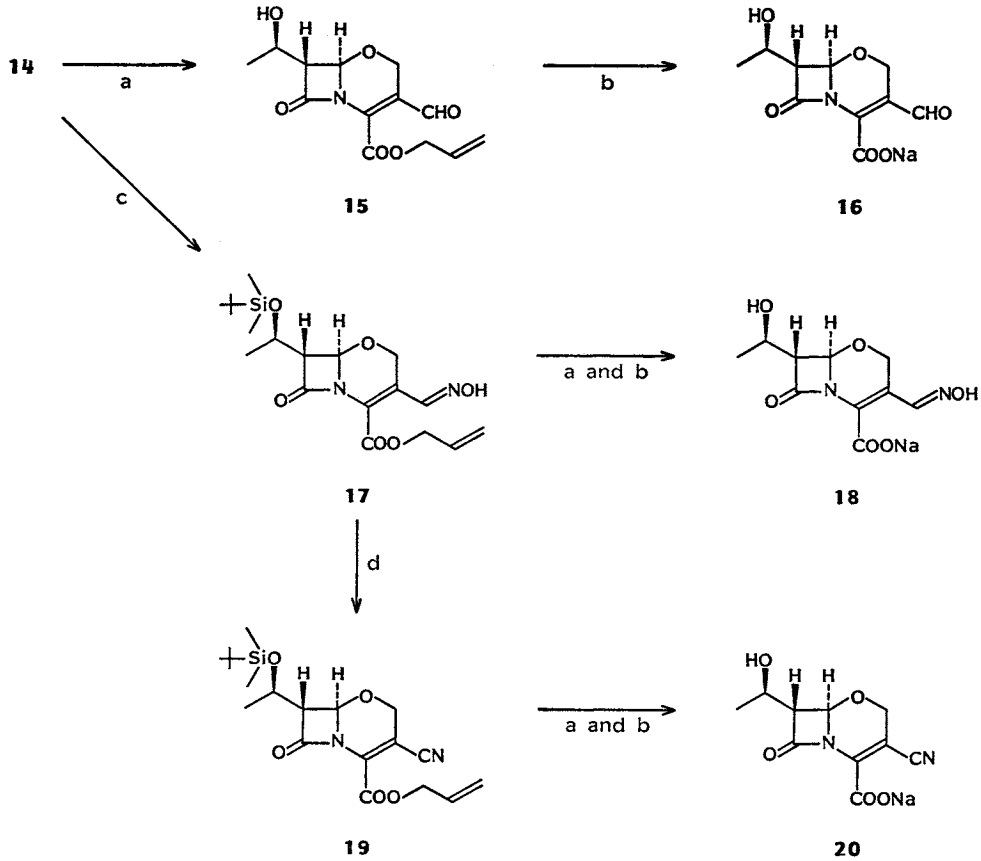
Scheme 1.



a) Allyl alcohol, $Zn(OAc)_2$, b) $KMnO_4$, NaOH, c) *tert*-$BuMe_2SiCl$, imidazole, d) DMSO, $(CF_3CO)_2O$, Et_3N, e) CH_2=$CHCH_2OOCCHO \cdot H_2O$, f) $SOCl_2$, 2,6-lutidine, g) Ph_3P, KI, 2,6-lutidine, h) hydroquinone - toluene, Δ, i) 1 N HCl, j) pyridinium dichromate.

1-oxacephem **13**, from the following characteristics: Field desorption (FD)-MS *m/z* 512 (M^+); IR ($CHCl_3$) cm^{-1} 1770; the *R* assignment of the 6 position was based upon the observed coupling constant ($J_{6,7}$=1 Hz) in the NMR spectrum. Selective desilylation of **13** was carried out with 1 N HCl in MeOH - THF and subsequent oxidation with PDC gave the key compound **14** (FD-MS *m/z* 395 (M^+); ^1H NMR ($CDCl_3$) δ 10.0 (1H, s, formyl)).

Scheme 2.

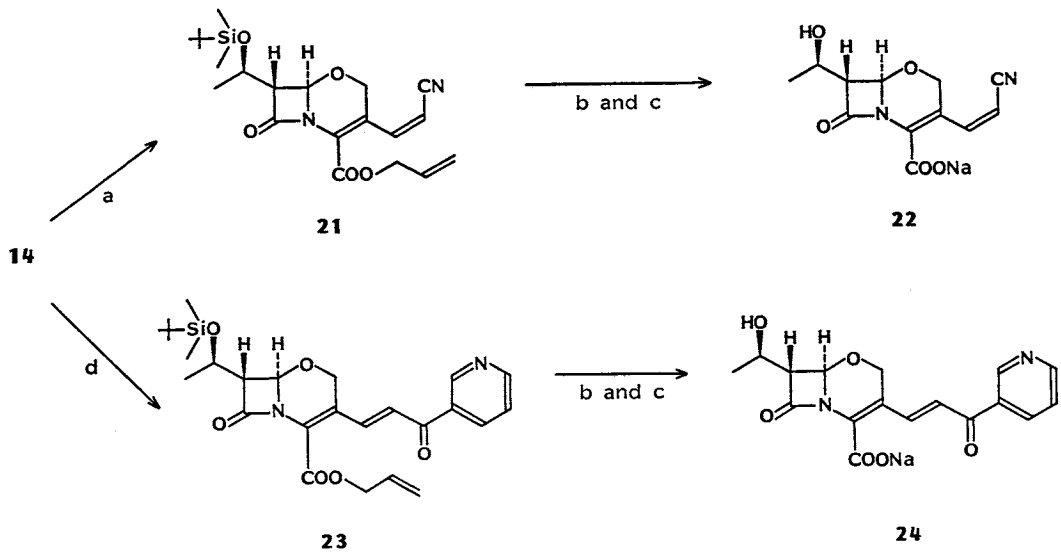


a) 2 N HCl, b) $Pd(Ph_3P)_4$, Ph_3P, sodium 2-ethylhexanoate, c) $NH_2OH \cdot HCl$, d) $SOCl_2$.

The introduction of EWG's at the 3 position was accomplished with key compound **14** and desired compounds were prepared as shown in Schemes 2 and 3. Desilylation of **14** and subsequent deprotection with palladium(0)-catalyzed exchange[6] gave 3-formyl compound **16** (IR (Nujol) cm^{-1} 1750; ^1H NMR (D_2O) δ 9.53 (1H, s, formyl)). 3-Hydroxyiminomethyl compound **18** (IR (Nujol) cm^{-1} 1740; ^1H NMR (DMSO-d_6) δ 8.20 (1H, s, CH=NO)) was also obtained from **14** by the treatment with hydroxylamine hydrochloride in isopropyl alcohol and subsequent deprotection as in the preparation of **16**. Compound **17** was dehydrated with $SOCl_2$ and then deprotected to give **20** (IR (Nujol) cm^{-1} 2220, 1760).

As shown in Scheme 3, 3-substituted vinyl derivatives were synthesized by Wittig reaction. Compound **14** was treated with (triphenylphosphoranylidene)acetonitrile in CH_2Cl_2 to give (*Z*)-cyanovinyl compound **21** (IR (CH_2Cl_2) cm^{-1} 2210, 1790; the assignment of *Z* configuration at the 3 position was based upon the observed coupling constant (J_{cis}=12 Hz)). Compound **22** was obtained by deprotection of **21** in the similar manner to that of **16**. Then **14** was converted to **23** with 3-(1-triphenylphosphoranylideneacetyl)pyridine in 1,2-dichloroethane at reflux for 30 hours. The assignment of *E* configuration was determined by the observed coupling constant (J_{trans}=16 Hz). Compound **24** was prepared by the similar manner to that of **16**.

Scheme 3.



a) $Ph_3P{=}CHCN$, b) 2 N HCl, c) $Pd(Ph_3P)_4$, Ph_3P, sodium 2-ethylhexanoate, d) $Ph_3P{=}CHCO$–N

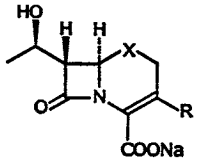
Biological Activity and Discussion

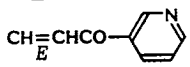
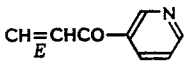
The MICs of 1-oxacephem (**16**, **18**, **20**, **22** and **24**) and cephem derivatives (**25**, **26** and **27**)[1] against four test organisms were shown in Table 2. 1-Oxacephem derivatives (**20**, **22** and **24**) showed more potent antibacterial activity than the corresponding cephem derivatives (**25**, **26** and **27**). The replacement of sulfur atom of cephem nuclei brought about 4~8-fold decrease of MIC values against *Staphylococcus aureus* 209P JC-1 in particular. But the antibacterial activity of 1-oxacephem derivatives was still low compared with a conventional cephem (cefazolin). Compound **24** had the most potent antibacterial activity among these compounds and the structure-activity relationship concerning the side chain at the 3 position of 1-oxacephem derivatives seems to be common to that of cephem derivatives[1].

In contrast to the antibacterial activity, it was found that β-lactamase inhibitory activity against both penicillinase and cephalosporinase of the 1-oxacephem derivatives was inferior to that of corresponding cephem derivatives, as shown in Table 3. For example, 1-oxacephem derivatives were shown to be ineffective against TEM PCase, and to have over 100-fold decreased β-lactamase inhibitory activity against Ic CSase compared with the corresponding cephem derivatives. Interestingly, of the 1-oxacephem derivatives, compound **20**, which has the strongest EWG at the 3 position, had the most potent β-lactamase inhibitory activity, while compound **24**, which had the most potent antibacterial activity, showed only poor β-lactamase inhibitory activity. The structure-activity relationships of 1-oxacephem derivatives seem to be in accord with those of the corresponding cephem derivatives[1].

It's not at all clear why the β-lactamase inhibitory activity of 1-oxacephem derivatives is inferior to that of cephem derivatives in contrast with the antibacterial activity, but it may relate to a difference in a stability of the complex formed between β-lactam and β-lactamase.

Table 2. MICs of 1-oxacephem and cephem derivatives.

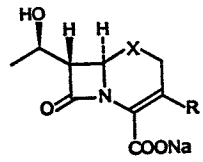


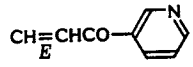
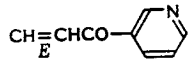
Compound No.	R	X	MIC (μg/ml)[a]			
			S.a.	*B.s.*	*E.c.*	*P.v.*
16	CHO	O	>100	>100	>100	>100
18	CH=NOH	O	50	>100	>100	>100
20	CN	O	12.5	50	>100	100
25	CN	S	50	100	>100	>100
22	CH=CHCN (*Z*)	O	12.5	50	>100	>100
26	CH=CHCN (*Z*)	S	100	>100	>100	>100
24	CH=CHCO (*E*)– 	O	6.25	6.25	12.5	12.5
27	CH=CHCO (*E*)– 	S	25	12.5	50	25
Cefazolin			0.39	0.39	1.56	6.25

Abbreviations: *S. a.*, *Staphylococcus aureus* 209P JC-1; *B.s.*, *Bacillus subtilis* ATCC 6633; *E.c.*, *Escherichia coli* 29; *P.v.*, *Proteus vulgaris* IAM 1025.

[a] Mueller-Hinton agar 10^{-2}: Stamp method; 37°C, 18 hours.

Table 3. β-Lactamase inhibitory activity[a] of 1-oxacephem and cephem derivatives.



Compound No.	R	X	ID_{50} (μg/ml)			
			TEM PCase	Ia CSase	Ib CSase	Ic CSase
16	CHO	O	>500	4.5	17	36
18	CH=NOH	O	>500	6.5	11.5	>500
20	CN	O	>500	0.66	6.5	>500
25	CN	S	17	<0.03	<0.5	0.9
22	CH=CHCN (*Z*)	O	>500	38	46	>500
26	CH=CHCN (*Z*)	S	>500	0.78	14	<7.8
24	CH=CHCO (*E*)– 	O	>500	300	>500	100
27	CH=CHCO (*E*)– 	S	30	33	450	<0.5
Sulbactam			1.2	42	14	<0.5
Clavulanic acid			1.0	12	7.8	0.6

TEM PCase: *Escherichia coli* 18, Ia CSase: *Enterobacter cloacae* 91, Ib CSase: *E. coli* HB101/pCF3, Ic CSase: *Proteus vulgaris* 9.

[a] Serial dilution of a β-lactamase inhibitor were incubated with enzyme solution for 10 minutes at 37°C. Residual β-lactamase activity was determined spectrophotometrically using the chromogenic substrate nitrocefin (50 μg/ml) at 482 nm. ID_{50} was calculated as the concentration inhibiting 50% of activity.

Experimental

MP's were measured with a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIF-140 automatic polarimeter. IR spectra were recorded with a Hitachi 260-10 spectrometer. ^{1}H NMR were recorded using a Hitachi R-90H. Chemical shifts (δ) are reported in ppm from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (in D_2O) or TMS (in $CDCl_3$ and DMSO-d_6) as internal standard. Secondary ion (SI)-MS were recorded with a Hitachi M-80 mass spectrometer and FD-MS were recorded with a Jeol D-300 mass spectrometer.

(3*R*,4*R*)-4-Allyloxy-3-[(1*R*)-1-*tert*-butyldimethylsilyloxyethyl]azetidine-2-one (**6**)

To a solution of (3*R*,4*R*)-4-acetoxy-3-[(1*R*)-1-*tert*-butyldimethylsilyloxyethyl]azetidine-2-one (**5**) (30 g) and allyl alcohol (35.5 ml) in benzene (210 ml) was added zinc acetate dihydrate (11.5 g). The mixture was refluxed for 3 hours, cooled and poured into cold aqueous solution of $NaHCO_3$. The organic layer was separated, washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated to give **6** as colorless powder (26.9 g, 90%): $[\alpha]_D^{25}$ −7.0° (*c* 1, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 1760, 1665; ^{1}H NMR ($CDCl_3$) δ 0.13 (6H, s), 0.93 (9H, s), 1.30 (3H, d, J=6 Hz), 3.07 (1H, m), 4.00~4.40 (3H, m), 5.00~5.45 (3H, m), 5.70~6.20 (1H, m), 6.63 (1H, br s); FD-MS *m/z* 286 (M^+).

Anal Calcd for $C_{14}H_{27}NO_3Si$: C 58.95, H 9.54, N 4.91.
Found: C 58.66, H 9.97, N 4.89.

(3*R*,4*R*)-3-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-4-(2,3-dihydroxypropane-1-oxy)azetidine-2-one (**7**)

6 (26 g) was dissolved in the mixture solution of *tert*-BuOH (851 ml) and water (970 ml). To the mixture was added an aqueous solution (628 ml) of NaOH (5.47 g) and $KMnO_4$ (15.9 g) dropwise over 30 minutes at 5~8°C. The mixture was stirred for another 20 minutes and filtered. The filtrate was extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on a silica gel column with CH_2Cl_2-MeOH (20:1). Evaporation gave **7** as colorless oil (25 g, 86%): IR ($CHCl_3$) cm^{-1} 3400, 1750, 1450; ^{1}H NMR ($CDCl_3$) δ 0.15 (6H, s), 0.93 (9H, s), 1.30 (3H, d, J=6 Hz), 2.43 (2H, br s), 3.05 (1H, m), 3.50~4.30 (6H, m), 5.10 (1H, m), 6.73 (1H, br s); SI-MS *m/z* 320 (M^+).

Anal Calcd for $C_{14}H_{29}NO_5Si \cdot \frac{1}{2}H_2O$: C 51.19, H 9.21, N 4.26.
Found: C 51.05, H 9.02, N 4.09.

(3*R*,4*R*)-3-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-4-(3-*tert*-butyldimethylsilyloxy-2-hydroxypropane-1-oxy)azetidine-2-one (**8**)

To a mixture of **7** (25 g) and imidazole (19 g) in DMF (125 ml) was added *tert*-butyldimethylsilyl chloride (13.3 g) at 0°C. The mixture was stirred at room temperature for 1 hour and poured into a mixture of EtOAc and 1 N HCl. The organic layer was separated, washed with brine, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel column (eluent; $CHCl_3$-MeOH, 20:1), and evaporated to give 21.2 g (63%) of **8** as colorless oil: IR ($CHCl_3$) cm^{-1} 1770, 1670, 1460; ^{1}H NMR ($CDCl_3$) δ 0.13 (12H, s), 0.93 (18H, s), 1.30 (3H, d, J=6 Hz), 1.70~2.30 (1H, m), 3.03 (1H, m), 3.50~3.90 (5H, m), 4.00~4.30 (1H, m), 5.08 (1H, m), 6.40 (1H, br s); FD-MS *m/z* 434 (M^+).

(3*R*,4*R*)-3-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-4-(3-*tert*-butyldimethylsilyloxy-2-oxopropane-1-oxy)azetidine-2-one (**9**)

To a mixture of DMSO (6.63 ml) and CH_2Cl_2 (100 ml) was added trifluoroacetic anhydride (9.87 ml) at −60°C. The mixture was stirred for 30 minutes maintaining the temperature at −60°C, treated with **8** (20.2 g) and stirred for another 30 minutes, after triethylamine (18.7 ml) was added, the mixture was warmed to room temperature and poured into ice-cooled water. The organic layer was separated, washed with water, 0.5 N HCl, aqueous solution of $NaHCO_3$ and then brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel (eluent; CH_2Cl_2-

acetone, 20 : 1), and crystallized from ether to give 13 g (65%) of **9**: MP 58°C; $[\alpha]_D^{25}$ −14° (*c* 1, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 1770, 1735; ^1H NMR ($CDCl_3$) δ 0.10 (12H, s), 0.88~0.92 (18H, m), 1.25 (3H, d, J=6 Hz), 3.03 (1H, m), 4.00~4.20 (1H, m), 4.26 (2H, s), 4.42 (2H, s), 5.03 (1H, m), 6.57 (1H, s); FD-MS *m/z* 432 (M^+).

Anal Calcd for $C_{20}H_{41}NO_5Si_2 \cdot \frac{1}{2}H_2O$: C 54.50, H 9.61, N 3.17.
Found: C 54.71, H 9.30, N 2.84.

(3*R*,4*R*)-1-(1-Allyloxycarbonyl-1-hydroxymethyl)-3-[(1*R*)-1-*tert*-butyldimethylsilyloxyethyl]-4-(3-*tert*-butyldimethylsilyloxy-2-oxopropane-1-oxy)azetidine-2-one (**10**)

A mixture of **9** (13 g), allyl glyoxylate monohydrate (12 g) in toluene (1.3 liters) was stirred and heated under reflux in a Dean Stark apparatus for 3 hours in an atmosphere of nitrogen. After cooling, the mixture was evaporated. The residue was purified by column chromatography on silica gel (eluent; CH_2Cl_2 - acetone, 20 : 1) to give a colorless oil of **10** (14.5 g, 88%): IR (CH_2Cl_2) cm^{-1} 1770, 1735; ^1H NMR ($CDCl_3$) δ 0.12 (12H, s), 0.91~0.98 (18H, m), 1.30 (3H, d, J=6 Hz), 3.10 (1H, m), 4.00~5.00 (8H, m), 5.15~5.55 (3H, m), 5.70~6.20 (1H, m); FD-MS *m/z* 545 (M^+).

(3*R*,4*R*)-1-(1-Allyloxycarbonyl-1-chloromethyl)-3-[(1*R*)-1-*tert*-butyldimethylsilyloxyethyl]-4-(3-*tert*-butyldimethylsilyloxy-2-oxopropane-1-oxy)azetidine-2-one (**11**)

To a solution of **10** (14.3 g) in THF (350 ml) was added 2,6-lutidine (5.64 ml) at −20°C. A mixture of thionyl chloride (3.53 ml) and THF (80 ml) was added dropwise to the mixture at −15°C. After 40 minutes, the mixture was poured into dry benzene (500 ml). A precipitate was removed by filtration. The filtrate was evaporated. The residue was chromatographed on silica gel (eluent; CH_2Cl_2 - acetone, 40 : 1) to give colorless oil of **11** (10.3 g, 70%): IR (CH_2Cl_2) cm^{-1} 1785, 1760; ^1H NMR ($CDCl_3$) δ 0.10 (12H, m), 0.90~1.10 (18H, m), 1.30 (3H, d, J=6 Hz), 3.10~3.30 (1H, m), 4.00~4.80 (8H, m), 5.20~5.50 (2H, m), 5.60~6.10 (2H, m); FD-MS *m/z* 563 (M^+).

(3*R*,4*R*)-1-(1-Allyloxycarbonyl-1-triphenylphosphoranylidenemethyl)-3-[(1*R*)-1-*tert*-butyldimethylsilyloxyethyl]-4-(3-*tert*-butyldimethylsilyloxy-2-oxopropane-1-oxy)azetidine-2-one (**12**)

11 (10.1 g) was dissolved in DMF (150 ml). To the solution of KI (3.66 g), 2,6-lutidine (2.57 ml) and triphenylphosphine (5.78 g) was added at room temperature. The mixture was stirred for 1 hour at room temperature and poured into a mixture of EtOAc and water. The extract was washed with ice-water, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (eluent; $CHCl_3$ - MeOH, 10 : 1). Evaporation gave colorless oil of **12** (12.6 g, 89%): IR ($CHCl_3$) cm^{-1} 1750, 1620, 1440; ^1H NMR ($CDCl_3$) δ 0.05~0.15 (12H, m), 0.90~1.00 (18H, m), 4.00~5.50 (14H, m), 5.60~6.20 (1H, m), 7.30~7.90 (15H, m); FD-MS *m/z* 790 (M^+).

Allyl 7α-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-3-*tert*-butyldimethylsilyloxymethyl-1-oxa-3-cephem-4-carboxylate (**13**)

A mixture of **12** (11.0 g) and hydroquinone (110 mg) in toluene (1.1 liters) was stirred and heated under reflux for 4 hours in an atomosphere of nitrogen. After cooling, the mixture was evaporated. The residue was purified by column chromatography on silica gel with CH_2Cl_2. Evaporation gave **13** as colorless powder (4.6 g, 62%): $[\alpha]_D^{25}$ +17° (*c* 1, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 1770, 1715; ^1H NMR ($CDCl_3$) δ 0.07 (12H, s), 0.87 (18H, s), 1.27 (3H, d, J=6 Hz), 3.00 (1H, dd, J=1 and 3 Hz), 4.22 (1H, m), 4.55 (2H, m), 4.70~4.80 (4H, m), 4.95 (1H, s), 5.15~5.50 (2H, m), 5.65~6.20 (1H, m); FD-MS *m/z* 512 (M^+).

Anal Calcd for $C_{25}H_{45}NO_6Si_2$: C 58.67, H 8.86, N 2.74.
Found: C 58.48, H 8.46, N 2.75.

Allyl 7α-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-3-formyl-1-oxa-3-cephem-4-carboxylate (**14**)

To a solution of **13** (3.40 g) in MeOH - THF (5 : 1, 82 ml) was added 1 N HCl (6.6 ml). The mixture was stirred at room temperature for 2 hours, neutralized with aqueous solution of $NaHCO_3$, and extracted with EtOAc. The extract was dried over $MgSO_4$ and evaporated. The residue was dissolved in CH_2Cl_2 (102 ml) and treated with pyridinium dichromate (3.75 g) for 3 hours at room temperature. A precipitate was removed by filtration. The filtrate was evaporated. The residue was purified by

column chromatography on silica gel with CH_2Cl_2. Evaporation gave **14** as colorless oil (1.54 g, 59%): IR (CH_2Cl_2) cm^{-1} 1800, 1730, 1665, 1610; ^1H NMR ($CDCl_3$) δ 0.10 (6H, s), 0.87~0.90 (9H, m), 1.33 (3H, d, J=6 Hz), 3.20 (1H, dd, J=1.5 and 4.0 Hz), 4.25~4.40 (1H, m), 4.33 and 4.93 (2H, ABq, J=16 Hz), 4.80~4.90 (2H, m), 5.08 (1H, d, J=1.5 Hz), 5.20~5.55 (2H, m), 5.80~6.25 (1H, m), 10.0 (1H, s); FD-MS m/z 395 (M^+).

Allyl 7α-[(1*R*)-1-Hydroxyethyl]-3-formyl-1-oxa-3-cephem-4-carboxylate (**15**)

To a solution of **14** (13.3 mg) in MeOH (0.4 ml) was added 2 N HCl (0.13 ml) at 0°C. The mixture was stirred at room temperature for 3 hours, poured into phosphate buffer (pH 7.0) and extracted with EtOAc. The extract was dried over $MgSO_4$ and evaporated to give **15** as colorless oil (11.3 mg); IR (CH_2Cl_2) cm^{-1} 1795, 1730, 1665, 1610; ^1H NMR ($CDCl_3$) δ 0.10 (6H, s), 0.90 (9H, s), 1.38 (3H, d, J=6 Hz), 1.85 (1H, m), 3.20~3.40 (1H, m), 4.20~4.65 (2H, m), 4.65~5.10 (4H, m), 5.10~5.50 (2H, m), 5.70~6.20 (1H, m).

Sodium 7α-[(1*R*)-1-Hydroxyethyl]-3-formyl-1-oxa-3-cephem-4-carboxylate (**16**)

To a solution of **15** (52 mg), triphenylphosphine (4.8 mg), sodium 2-ethylhexanoate (30.8 mg) in EtOAc (1.5 ml) was added tetrakis(triphenylphosphine)palladium(0) (4.3 mg) at 0°C under an atmosphere of nitrogen. The mixture was stirred for 1 hour at room temperature and poured into water (20 ml). The aqueous layer was separated, washed with EtOAc and lyophilized. The powder (47.2 mg) was purified by column chromatography (non-ionic adsorption resin Diaion HP-20 (10 ml), eluent; isopropyl alcohol - water (5 : 95)). Freeze drying of the product fractions gave 27 mg (55%) of **16**: IR (Nujol) cm^{-1} 1750, 1600; ^1H NMR (90 MHz, D_2O) δ 1.30 (3H, d, J=6 Hz), 3.47 (1H, dd, J=1.5 and 4 Hz), 4.10~4.40 (1H, m), 4.43 and 4.83 (2H, ABq, J=16 Hz), 5.20 (1H, d, J=1.5 Hz), 9.53 (1H, s).

Allyl 7α-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-3-hydroxyiminomethyl-1-oxa-3-cephem-4-carboxylate (**17**)

A mixture of **14** (50 mg), hydroxylamine hydrochloride (17.6 mg) and isopropyl alcohol (5 ml) was stirred, heated at 50°C for 30 minutes, and evaporated. The residue was poured into water - EtOAc (1 : 1). The organic layer was separated, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC on silica gel (eluent; $CHCl_3$) to give **17** as colorless powder (35 mg, 69%); IR ($CHCl_3$) cm^{-1} 1780, 1720, 1610, 1590; ^1H NMR ($CDCl_3$) δ 0.10 (6H, s), 0.90 (9H, s), 1.30 (3H, d, J=6 Hz), 3.15 (1H, dd, J=1.5 and 3 Hz), 4.10~4.40 (1H, m), 4.40 and 4.90 (2H, ABq, J=18 Hz), 4.70~4.80 (2H, m), 5.00 (1H, d, J=1.5 Hz), 5.10~5.50 (2H, m), 5.70~6.20 (1H, m), 8.10 (1H, s), 8.47 (1H, s); FD-MS m/z 410 (M^+).

Allyl 7α-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-3-cyano-1-oxa-3-cephem-4-carboxylate (**19**)

To a solution of **17** (500 mg) in $CHCl_3$ (228 ml) was added thionyl chloride (11.4 ml). The mixture was heated at reflux for 2 hours, poured into ice-cooled aqueous solution of $NaHCO_3$ and extracted. The extract was washed with brine, dried with $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$ to give **19** as oil (229 mg, 48%): IR ($CHCl_3$) cm^{-1} 2220, 1790, 1730, 1605; ^1H NMR ($CDCl_3$) δ 0.10 (6H, s), 0.90 (9H, s), 1.30 (3H, d, J=6 Hz), 3.25 (1H, dd, J=1.5 and 3 Hz), 4.30 (1H, m), 4.53 (2H, m), 4.83 (2H, m), 5.05 (1H, d, J=1.5 Hz), 5.20~5.60 (2H, m), 5.70~6.20 (1H, m); FD-MS m/z 393 (M^+).

Allyl 7α-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-3-[(*Z*)-2-cyano-1-vinyl]-1-oxa-3-cephem-4-carboxylate (**21**)

To a solution of **14** (100 mg) in CH_2Cl_2 (2 ml) was added (triphenylphosphoranylidene)acetonitrile (80 mg) at 0°C. The mixture was stirred at room temperature for 3 hours, and purified by preparative TLC on silica gel (eluent; CH_2Cl_2) to give **21** as oil (84 mg, 79%): $[\alpha]_D^{25}$ −30° (c 1, $CHCl_3$); IR (CH_2Cl_2) cm^{-1} 2210, 1790, 1720, 1600, 1565; ^1H NMR ($CDCl_3$) δ 0.10 (6H, s), 0.90 (9H, s), 1.25 (3H, d, J=6 Hz), 3.17 (1H, dd, J=1.5 and 3 Hz), 4.15~4.45 (1H, m), 4.65~4.90 (3H, m), 5.00~5.50 (5H, m), 5.70~6.20 (1H, m), 7.50 (1H, d, J=12 Hz); FD-MS m/z 418 (M^+).

Allyl 7α-[(1*R*)-1-*tert*-Butyldimethylsilyloxyethyl]-3-[(*E*)-3-(3-pyridyl)-3-oxo-1-propene-1-yl]-1-oxa-3-cephem-4-carboxylate (**23**)

To a solution of **14** (500 mg) in 1,2-dichloroethane (15 ml) was added 3-(1-triphenylphosphoranylideneacetyl)pyridine (506 mg) at room temperature. The mixture was heated at reflux for 30 hours and evaporated. The residue was purified by column chromatography on silica gel (eluent; CH_2Cl_2-acetone, 20:1). Evaporation gave **23** as powder (268 mg, 43%): IR (CH_2Cl_2) cm^{-1} 1785, 1720, 1665, 1640, 1590; ^1H NMR ($CDCl_3$) δ 0.10 (6H, s), 0.90 (9H, s), 1.30 (3H, d, J=6 Hz), 3.20 (1H, dd, J=1.5 and 4 Hz), 4.20~4.40 (1H, m), 4.53 and 4.77 (2H, ABq, J=16 Hz), 4.75 (2H, m), 5.05 (1H, m), 5.15~5.50 (2H, m), 5.70~6.20 (1H, m), 6.72 (1H, d, J=15 Hz), 7.30~7.50 (1H, m), 8.05 (1H, d, J=15 Hz), 8.10~8.30 (1H, m), 8.75 (1H, dd, J=2 and 6 Hz), 9.05 (1H, d, J=2 Hz); FD-MS m/z 498 (M^+).

Preparation of **18**, **20**, **22** and **24**

These compounds were prepared as described for **16** from **14** in 2 steps.

Sodium 7α-[(1*R*)-1-Hydroxyethyl]-3-hydroxyiminomethyl-1-oxa-3-cephem-4-carboxylate (**18**)

IR (Nujol) cm^{-1} 1740, 1600; ^1H NMR (D_2O) δ 1.32 (3H, d, J=6 Hz), 3.35 (1H, dd, J=1.5 and 4 Hz), 4.00~4.80 (2H, m), 5.12 (1H, m), 8.20 (1H, s).

Sodium 7α-[(1*R*)-1-Hydroxyethyl]-3-cyano-1-oxa-3-cephem-4-carboxylate (**20**)

IR (Nujol) cm^{-1} 2220, 1760; ^1H NMR (DMSO-d_6) δ 1.17 (3H, d, J=6 Hz), 3.10 (1H, dd, J=1.5 and 4 Hz), 3.95 (1H, m), 4.27 and 4.47 (2H, ABq, J=15 Hz), 4.95 (1H, d, J=1.5 Hz), 5.10 (1H, m).

Sodium 7α-[(1*R*)-1-Hydroxyethyl]-3-[(*Z*)-2-cyano-1-vinyl]-1-oxa-3-cephem-4-carboxylate (**22**)

IR (Nujol) cm^{-1} 2200, 1750, 1600; ^1H NMR (D_2O) δ 1.33 (3H, d, J=6 Hz), 3.40 (1H, dd, J=1.5 and 4 Hz), 4.00~4.40 (1H, m), 4.80 and 5.15 (2H, ABq, J=17 Hz), 5.15 (1H, d, J=15 Hz), 5.40 (1H, d, J=12 Hz), 7.15 (1H, d, J=12 Hz); SI-MS m/z 263 (M^+ −Na).

Sodium 7α-[(1*R*)-1-Hydroxyethyl]-3-[(*E*)-3-(3-pyridyl)-3-oxo-1-propene-1-yl]-1-oxa-3-cephem-4-carboxylate (**24**)

IR (Nujol) cm^{-1} 1760, 1650, 1610, 1585; ^1H NMR (D_2O) δ 1.35 (3H, d, J=6 Hz), 3.40 (1H, dd, J=1.5 and 6 Hz), 4.25 (1H, m), 4.60 and 4.90 (2H, ABq, J=15 Hz), 5.15 (1H, d, J=1.5 Hz), 6.83 (1H, d, J=15 Hz), 7.55 (1H, dd, J=6 and 9 Hz), 7.80 (1H, d, J=15 Hz), 8.25 (1H, dt, J=1.5 and 9 Hz), 8.65 (1H, m), 8.95 (1H, m); SI-MS m/z 366 (M^+).

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