STUDIES ON CEPHALOSPORIN ANTIBIOTICS

I. SYNTHESIS, ANTIBACTERIAL ACTIVITY AND ORAL ABSORPTION OF NEW 3-(*O*-SUBSTITUTED)-7β-[D-α-AMINO-α-(4-HYDROXYPHENYL)ACETAMIDO]CEPHALOSPORINS

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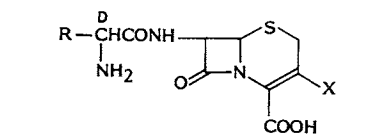
The synthesis, antibacterial activity and oral absorption of new 7β-[D-α-amino-α-(4-hydroxyphenyl)acetamido]cephalosporins (**1**) with various *O*-substituents at the C-3 position of a cephalosporin nucleus are described. Of these, the cephalosporins (**1b**~**1e**) having an alkoxycarbonylmethoxy group at the C-3 position showed good oral absorption in rats as well as potent activity against Gram-positive bacteria. The structure-activity relationships of **1** are also presented.

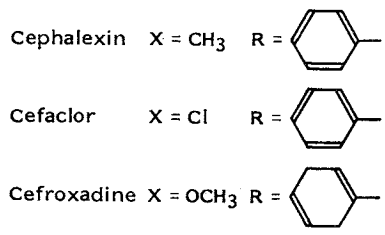
Since cephalexin[1] has been introduced into clinical medicine as an orally active cephalosporin, much research[2~7] has been reported aimed at obtaining new cephalexin analogues with improved properties. In recent years, the new analogues such as cefaclor[6] and cefroxadine[7] bearing an electron-negative hetero-atom directly attached to the C-3 position of the cephem nucleus have been developed (Fig. 1).

They are more active than cephalexin, and structurally unique among the many cephalosporins in that no carbon atom is attached to the C-3 position. In order to find more active cephalexin analogues, we also planned to prepare the new derivatives, represented by general structure **1** (Fig. 2), with various *O*-substituents directly attached to the C-3 position.

In this paper, we wish to report the synthesis of **1**, and the effects of the new substituents at the C-3 position on antibacterial activity and oral absorption in rats.

Fig. 1.

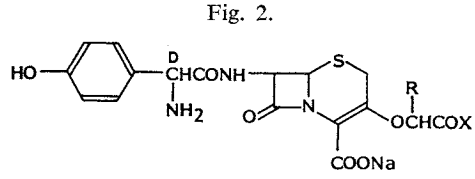




Chemistry

The new cephalosporins (**1a**~**1j**) were synthesized by the route as outlined in Scheme 1. The hydroxy group of diphenylmethyl 7-arylglycylamido-3-hydroxycephalosporinate 1-oxide (**2**) prepared by the method shown in Scheme 2

Fig. 2.



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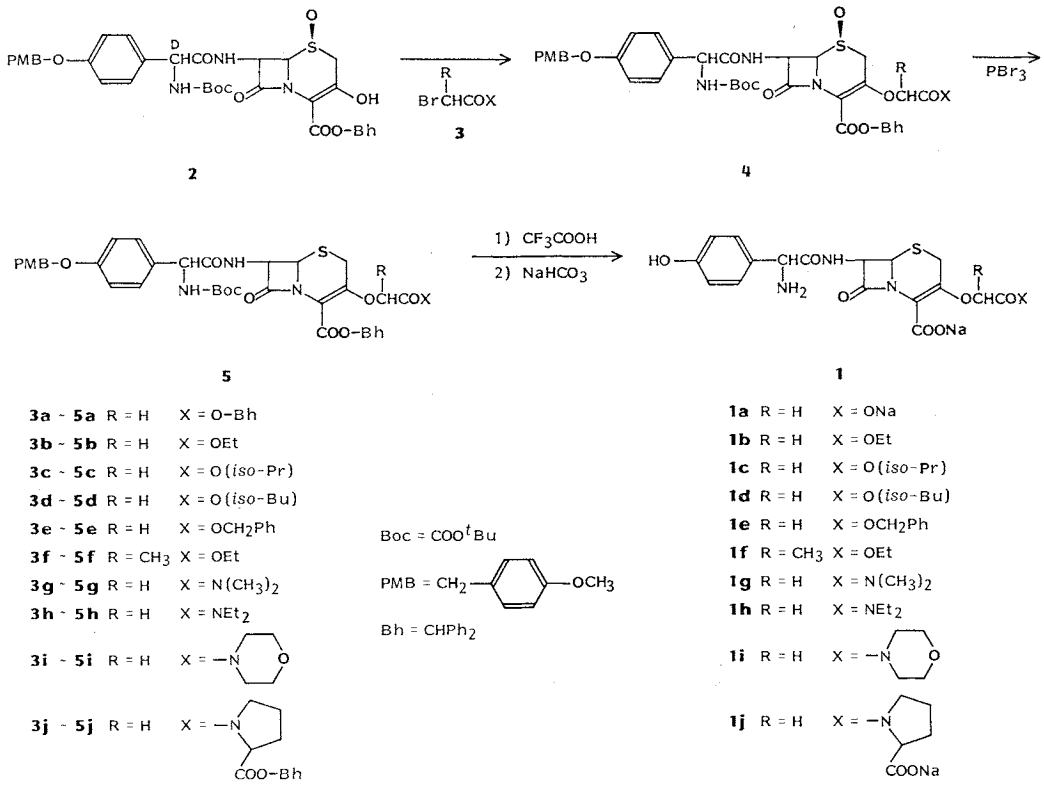
Scheme 1.



was reacted with 2-bromoacetic and 2-bromopropionic acid derivatives (**3a**~**3j**) in the presence of *N,N*-diisopropylethylamine as a base to yield the C-3 *O*-substituted derivatives (**4a**~**4j**). The alternative route to **4** with the corresponding diazo compounds such as ethyl diazoacetate in the presence of rhodium (II) acetate[8] did not proceed smoothly. Subsequently, the sulfoxides (**4a**~**4j**) were reduced using phosphorus tribromide (PBr_3) in DMF to yield the cephem compounds (**5a**~**5j**). Removal of the protecting *tert*-butoxycarbonyl (Boc), *p*-methoxybenzyl (PMB) and diphenylmethyl (Bh) groups of **5a**~**5j** by treatment with trifluoroacetic acid and anisole afforded the new cephem compounds (**1a**~**1j**).

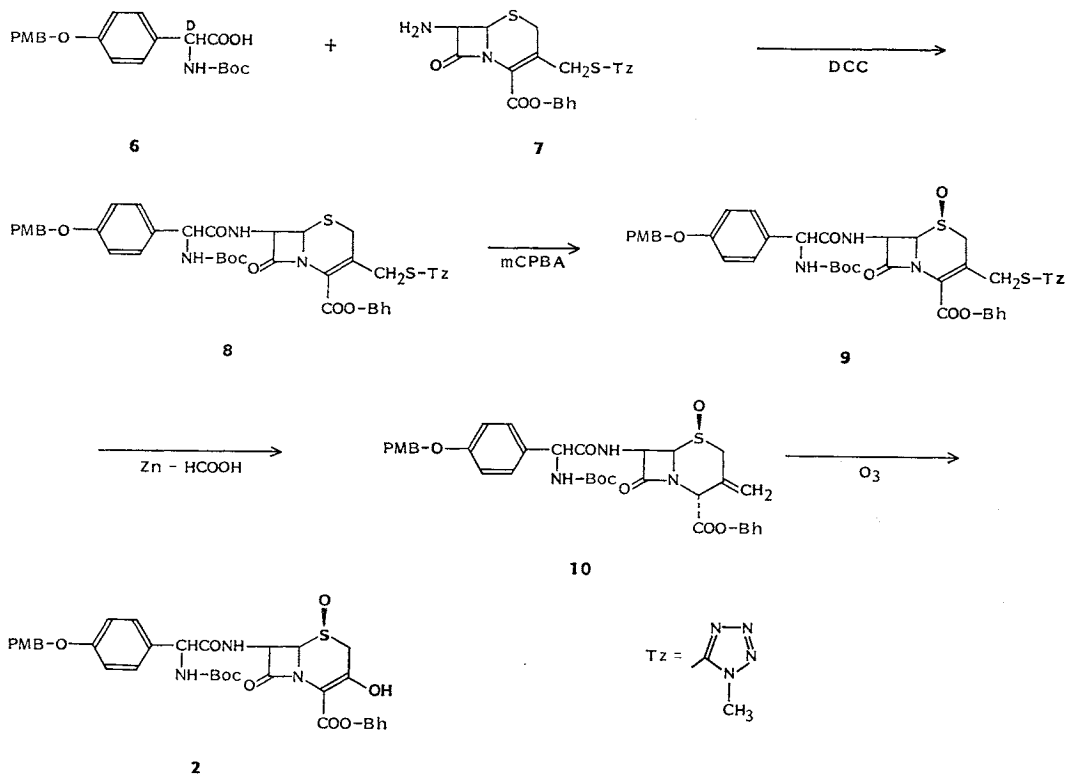
The common intermediate (**2**) for **1a**~**1j** was prepared according to the reaction sequence shown in Scheme 2. Diphenylmethyl 7β-amino-3-(1-methyltetrazol-5-yl)thiomethyl cephalosporinate (**7**)[9] was acylated with *N*-Boc-4-(4-methoxybenzyl)oxy-D-phenylglycine (**6**) by using *N,N'*-dicyclohexylcarbodiimide (DCC) as a condensing agent. The C-7 acylamino compound (**8**) was then reacted with *m*-chloroperbenzoic acid (mCPBA) to give the corresponding sulfoxide (**9**), which was treated with Zn and formic acid[10] to yield the C-3 exomethylenecepham compound (**10**).

The ozonolysis of **10** afforded the desired intermediate (**2**). The C-7 acyl moiety (**6**) was also prepared starting from *N*-Boc-4-hydroxy-D-phenylglycine (**11**) by conventional methods shown in Scheme 3. In order to prevent undesirable side-reactions in the following reactions, the hydroxy group of **11** was protected with a *p*-methoxybenzyl group[11].

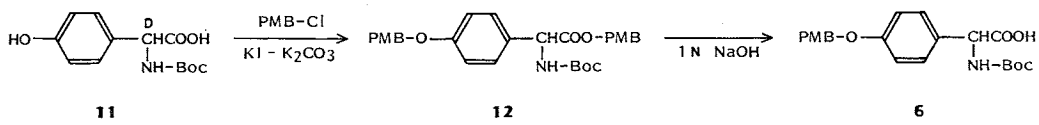
Antibacterial Activity and Oral Absorption

The *in vitro* antibacterial activities of the new cephalexin analogues (**1**) against selected Gram-

Scheme 2.



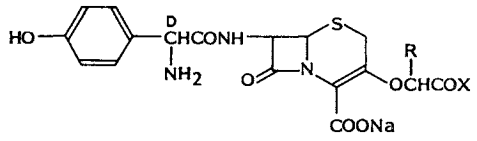
Scheme 3.



positive and Gram-negative bacteria and their peak serum levels as a measure of gastro-intestinal absorption after oral administration (50 mg/kg) to rats are summarized in Table 1. For comparison, the MIC values and the peak serum level of cephalexin are listed at the bottom of the Table 1. Against the Gram-positive bacteria, the derivatives (**1b**~**1i**) with an ester or amide group, respectively, in the C-3 substituent showed potent activity comparable to that of cephalexin. However, the derivatives (**1a** and **1j**) with a carboxy group were much less active, probably due to their high hydrophilicity. On the other hand, against the Gram-negative bacteria, the derivatives (**1b** and **1g**) with an ethyl ester or *N*,*N*-dimethylamide group, respectively, exhibited better activity than the others. Their activities were similar to cephalexin. In contrast to **1b**, its close analogue (**1f**) with a methyl substituent as R showed poor activity.

Regarding the oral absorption in rats, the ester derivatives (**1b**~**1e**) except for **1f** (R=CH_3) showed good oral absorption, and their peak serum levels were about 2 to 3 times higher than that of cephalexin. In contrast, all of the others with a carboxy group or amide group in the C-3 substituent exhibited no significant oral absorption. These results indicate that the alkoxycarbonylmethoxy group

Table 1. *In vitro* antibacterial activity and peak serum level of **1**.



1

Compound			MIC (μg/ml, 10^6 cfu/ml)[a]					Peak serum level (μg/ml)[b] po, 50 mg/kg, rats ($n=3$)
No.	X	R	*S.a.*	*S.e.*	*E.c.*	*K.p.*	*P.m.*	
1a	ONa	H	100	>100	50	50	50	<3.2
1b	OEt	H	0.78	1.56	6.25	6.25	12.5	42.4
1c	O(*iso*-Pr)	H	1.56	3.13	50	25	50	31.6
1d	O(*iso*-Bu)	H	0.78	1.56	25	12.5	100	37.8
1e	OCH_2Ph	H	1.56	1.56	50	25	100	30.9
1f	OEt	CH_3	1.56	1.56	100	50	100	3.6
1g	$N(CH_3)_2$	H	0.78	1.56	12.5	6.25	25	<4.0
1h	NEt_2	H	1.56	1.56	25	12.5	50	<3.5
1i		H	0.78	1.56	12.5	12.5	50	1.6
1j		H	100	>100	25	25	50	<2.8
Cephalexin			0.78	0.78	12.5	6.25	25	13.3

[a] The MICs were determined by a standard agar dilution method using sensitivity test agar (Eiken, Japan).

[b] The peak serum levels were measured by a disc-plate method using *Escherichia coli* SC 507 or *Micrococcus luteus* NIHJ as the test organism.

Abbreviations: *S.a.*; *Staphylococcus aureus* 209P JC-1, *S.e.*; *Staphylococcus epidermidis* sp-al-1, *E.c.*; *Escherichia coli* NIHJ JC-2, *K.p.*; *Klebsiella pneumoniae* IFO 3317, *P.m.*; *Proteus mirabilis* IFO 3849.

at the C-3 position plays an important role in the oral absorption.

In this study, we found some new cephalexin analogues with improved oral absorption in rats by the chemical modification of the C-3 position.

Experimental

MP was determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Jasco DS-701G IR spectrometer. ^1H NMR spectra were recorded on a Varian XL-200 NMR spectrometer using TMS or sodium trimethylsilyl propionate-d_4 (in D_2O) as an internal standard. MS was measured on a Jeol JMS-DX303 mass spectrometer. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet; ABq, AB quartet.

Determination of Antibacterial Activity

MIC was determined by the agar dilution method using sensitivity test agar (Eiken, Japan) after incubation at 37°C for 18 hours with inoculum size of 10^6 cfu/ml.

Oral Absorption Study

Male SLC/Wistar rats ($n=3$) weighing 180~220 g were fasted overnight and orally dosed with 50 mg/kg of the test compounds. Serum samples were collected at 0.5, 1, 2 and 4 hours respectively after dosing. The serum levels of the test compounds were measured by the disc-plate method using

Escherichia coli SC 507 or *Micrococcus luteus* NIHJ as the test organism and the sensitivity test agar (Eiken, Japan) as the test medium.

4-Methoxybenzyl *N*-(*tert*-Butoxycarbonyl)-4-(4-methoxybenzyl)oxy-D-phenylglycinate (**12**)

To a solution of *N*-(*tert*-butoxycarbonyl)-4-hydroxyphenylglycine (**11**) (25.0 g, 93.5 mM) in acetone (70 ml) were added 4-methoxybenzyl chloride (36.8 g, 235 mM), potassium iodide (31.2 g, 188 mM) and potassium carbonate (26 g, 188 mM) at room temp. After being stirred for 16 hours at the same temp, the reaction mixture was concentrated under reduced pressure to dryness. To the residue, H_2O (200 ml) was added, and extracted with EtOAc (300 ml). The extract was washed with brine (200 ml), dried ($MgSO_4$) and the solvent was evaporated.

The residue was purified by column chromatography on silica gel (eluent; benzene - acetone, 40 : 1), and crystallized from MeOH to give 41.6 g (87.7%) of **12**: MP 72~74°C. IR (KBr) cm^{-1} 1740, 1705, 1610; ^1H NMR ($CDCl_3$) δ 1.43 (9H, s, *tert*-Bu), 3.79 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.97 (2H, s, CH_2O), 5.05 and 5.13 (2H, ABq, J=11 Hz, $COOCH_2$), 5.28 (1H, d, J=8 Hz, C*H*(NH)COO), 5.50 (1H, d, J=8 Hz, N*H*$COOC_4H_9$), 6.83 (2H, d, J=9 Hz, aromatic H), 6.91 (2H, d, J=9 Hz, aromatic H), 6.93 (2H, J=9 Hz, aromatic H), 7.17 (2H, d, J=9 Hz, aromatic H), 7.25 (2H, d, J=9 Hz, aromatic H), 7.35 (2H, d, J=9 Hz, aromatic H); field desorption mass spectrum (FD-MS) m/z 508 $(M+1)^+$;

Anal Calcd for $C_{29}H_{33}NO_7$: C 68.62, H 6.55, N 2.76.
Found: C 68.66, H 6.58, N 2.66.

N-(*tert*-Butoxycarbonyl)-4-(4-methoxybenzyl)oxy-D-phenylglycine (**6**)

To a solution of **12** (39.6 g, 78.1 mM) in acetone (213 ml) was added 1 N NaOH solution (93.7 ml, 93.7 mM) under ice-cooling, and stirred for 30 minutes at room temp. After removal of acetone under reduced pressure, the resulting aqueous solution was adjusted to pH 2.0 with 0.5% HCl, and extracted with EtOAc (300 ml). The extract was washed with brine (200 ml), dried ($MgSO_4$) and evaporated to give a crystalline residue, which was collected and washed with Et_2O (100 ml) to afford 25.4 g (84.0%) of **6**. Recrystallization from MeOH gave a pure material: MP 145~146°C (dec); IR (KBr) cm^{-1} 1745, 1675, 1610; ^1H NMR (DMSO-d_6) δ 1.37 (9H, s, *tert*-Bu), 3.75 (3H, s, OCH_3), 5.01 (2H, s, CH_2O), 5.03 (1H, d, J=8 Hz, C*H*(NH)COOH), 6.95 (2H, d, J=8 Hz, aromatic H), 6.96 (2H, d, J=8 Hz, aromatic H), 7.31 (2H, d, J=8 Hz, aromatic H), 7.38 (2H, d, J=8 Hz, aromatic H), 7.48 (1H, d, J=8 Hz, N*H*$COOC_4H_9$), 12.61 (1H, br s, COOH); FD-MS m/z 387 (M^+);

Anal Calcd for $C_{21}H_{25}NO_6$: C 65.10, H 6.50, N 3.62.
Found: C 65.16, H 6.58, N 3.44.

Diphenylmethyl 7β-[D-α-(*tert*-Butoxycarbonylamino)-α-[4-(4-methoxybenzyl)oxyphenyl]acetamido]-3-(1-methyltetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate (**8**)

To a solution of diphenylmethyl 7β-amino-3-(1-methyltetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate (**7**)[9] (10.0 g, 20.2 mM) in THF (20 ml), were added **6** (8.58 g, 22.2 mM) and DCC (4.57 g, 22.2 mM) under ice-cooling. After being stirred for 3.5 hours at the same temp, the precipitate of *N*,*N*′-dicyclohexylurea formed was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent; benzene - acetone, 10 : 1), and crystallized from MeOH to give 18.0 g of **8**: MP 154~156°C; IR (KBr) cm^{-1} 1780, 1705, 1660; ^1H NMR ($CDCl_3$) δ 1.42 (9H, s, *tert*-Bu), 3.69 (2H, br s, 2-H_2), 3.82 (3H, s, OCH_3), 3.84 (3H, s, NCH_3), 4.25 and 4.39 (2H, ABq, J=14 Hz, 3′-H_2), 4.97 (1H, d, J=5 Hz, 6-H), 4.99 (2H, s, CH_2O), 5.14 (1H, d, J=6 Hz, C*H*(NH)CO), 5.52 (1H, d, J=6 Hz, N*H*$COOC_4H_9$), 5.87 (1H, dd, J=5 and 9 Hz, 7-H), 6.48 (1H, d, J=9 Hz, CONH), 6.92 (2H, d, J=8 Hz, aromatic H), 6.95 (1H, s, C*H*Ph_2), 6.97 (2H, d, J=8 Hz, aromatic H), 7.23~7.49 (14H, m, aromatic H); FD-MS m/z 863 (M^+);

Anal Calcd for $C_{44}H_{45}N_7O_8S_2$: C 61.16, H 5.25, N 11.35.
Found: C 61.25, H 5.19, N 11.42.

Diphenylmethyl 7β-[D-α-(*tert*-Butoxycarbonylamino)-α-[4-(4-methoxybenzyl)oxyphenyl]acetamido]-3-(1-methyltetrazol-5-yl)thiomethyl-3-cephem-4-carboxylate 1β-Oxide (**9**)

To a solution of **8** (18.0 g, 20.8 mM) in CH_2Cl_2 (200 ml) was added mCPBA (3.16 g, 20.9 mM)

under ice-cooling. After being stirred for 30 minutes at the same temp, the reaction mixture was washed with 5% $NaHCO_3$ (100 ml), brine (100 ml), and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by column chromatography on silica gel (eluent; benzene - acetone, 10 : 1 ~ 8 : 1), and then crystallized from MeOH to give 14.3 g (80.5% from **7**) of **9**. Recrystallization from MeOH - $CHCl_3$ gave a pure material: MP 139~141°C; IR (KBr) cm^{-1} 1795, 1715, 1690, 1610; ^1H NMR ($CDCl_3$) δ 1.42 (9H, s, *tert*-Bu), 3.50 (1H, d, J=18 Hz, 2-H_α), 3.82 (6H, s, NCH_3 and OCH_3), 3.69 (1H, d, J=18 Hz, 2-H_β), 4.09 and 4.55 (2H, ABq, J=13 Hz, 3'-H_2), 4.43 (1H, d, J=5 Hz, 6-H), 4.98 (2H, s, CH_2O), 5.13 (1H, d, J=5 Hz, C*H*(NH)CO), 5.58 (1H, d, J=5 Hz, N*H*$COOC_4H_9$), 6.06 (1H, dd, J=5 and 9 Hz, 7-H), 6.92 (2H, d, J=8 Hz, aromatic H), 6.94 (1H, s, C*H*Ph_2), 6.96 (2H, d, J=8 Hz, aromatic H), 7.24~7.54 (15H, m, aromatic H and CONH); FD-MS m/z 880 $(M+1)^+$;

Anal Calcd for $C_{44}H_{45}N_7O_9S_2$: C 60.05, H 5.15, N 11.14.
Found: C 59.76, H 5.10, N 11.13.

Diphenylmethyl 7β-[D-α-(*tert*-Butoxycarbonylamino)-α-[4-(4-methoxybenzyl)oxyphenyl]acetamido]-3-methylenecepham-4-carboxylate 1β-Oxide (**10**)

To a mixture of **9** (14.3 g, 16.3 mM) in THF (94.5 ml) and DMF (27.3 ml) were added Zn dust (11.6 g, 178 mM), HCOOH (27.3 ml) and H_2O (27.3 ml) under ice-cooling. After being stirred for 1 hour at the same temp, the spent Zn was filtrated and washed with EtOAc (200 ml). The separated organic layer was washed with 5% $NaHCO_3$ (100 ml×2), brine (100 ml) and dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by column chromatography on silica gel (eluent; $CHCl_3$ - MeOH, 200 : 1 ~ 100 : 1) to give 9.3 g (74.5%) of **10** as a white powder: MP 104~108°C; IR (KBr) cm^{-1} 1780, 1735, 1700, 1685; ^1H NMR ($CDCl_3$) δ 1.42 (9H, s, *tert*-Bu), 3.32 (1H, d, J=14 Hz, 2-H_α), 3.66 (1H, d, J=14 Hz, 2-H_β), 3.80 (3H, s, OCH_3), 4.72 (1H, d, J=5 Hz, 6-H), 4.96 (2H, s, CH_2O), 5.14 (1H, d, J=5 Hz, C*H*(NH)CO), 5.34 (1H, s, vinyl H), 5.43 (1H, br s, 4-H), 5.56 (1H, d, J=5 Hz, N*H*$COOC_4H_9$), 5.81 (1H, s, vinyl H), 5.86 (1H, dd, J=5 and 10 Hz, 7-H), 6.86 (2H, d, J=8 Hz, aromatic H), 6.92 (1H, s, C*H*Ph_2), 6.94 (2H, d, J=8 Hz, aromatic H), 7.22~7.38 (14H, m, aromatic H), 7.63 (1H, d, J=10 Hz, CONH); FD-MS m/z 765 (M^+);

Anal Calcd for $C_{42}H_{43}N_3O_9S$: C 65.86, H 5.66, N 5.49.
Found: C 65.81, H 5.79, N 5.65.

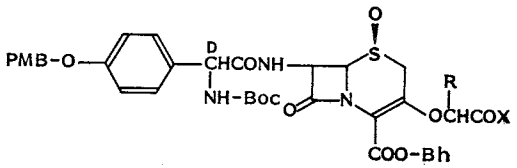
Diphenylmethyl 7β-[D-α-(*tert*-Butoxycarbonylamino)-α-[4-(4-methoxybenzyl)oxyphenyl]acetamido]-3-hydroxy-3-cephem-4-carboxylate 1β-Oxide (**2**)

Excess ozone was passed through a mixture of **10** (13.9 g, 18.2 mM) in CH_2Cl_2 (1,200 ml) and MeOH (2.5 ml) for 1.5 hours at −40~−30°C until the solution became blue. After removing excess ozone by passing dry nitrogen, dimethyl sulfide (11.2 ml) was added to the mixture at −40°C. The temp of the mixture was slowly raised to 20°C over 1 hour. The resulting solution was washed with brine (300 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; $CHCl_3$ - MeOH, 50 : 1) to give 9.82 g (70.3%) of **2** as an amorphous solid. IR (KBr) cm^{-1} 1785, 1685, 1610; ^1H NMR ($CDCl_3$) δ 1.46 (9H, s, *tert*-Bu), 3.36 (1H, d, J=18 Hz, 2-H_α), 3.69 (1H, d, J=18 Hz, 2-H_β), 3.82 (3H, s, OCH_3), 4.50 (1H, d, J=5 Hz, 6-H), 5.00 (2H, s, CH_2O), 5.14 (1H, d, J=5 Hz, C*H*(NH)CO), 5.49 (1H, d, J=5 Hz, N*H*$COOC_4H_9$), 6.01 (1H, dd, J=5 and 10 Hz, 7-H), 6.92 (1H, s, C*H*Ph_2), 6.93 (2H, d, J=9 Hz, aromatic H), 6.99 (2H, d, J=9 Hz, aromatic H), 7.28~7.48 (14H, m, aromatic H), 7.63 (1H, d, J=10 Hz, CONH); FD-MS m/z 767 (M^+).

Diphenylmethyl 7β-[D-α-(*tert*-Butoxycarbonylamino)-α-[4-(4-methoxybenzyl)oxyphenyl]acetamido]-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate 1β-Oxide (**4b**)

To a solution of **2** (1.0 g, 1.3 mM) in DMSO (8 ml) were added ethyl bromoacetate (**3b**) (435 mg, 2.61 mM) and *N*,*N*-diisopropylethylamine (252 mg, 1.96 mM) at room temp. After being stirred for 4 hours at the same temp, the reaction mixture was poured into 0.5% HCl (50 ml) under ice-cooling and extracted with EtOAc (100 ml). The extract was washed with brine (50 ml×2), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; benzene - acetone, 15 : 1 ~ 10 : 1), and crystallized from MeOH to give 420 mg (38.0%) of **4b**: MP 206~208°C;

Table 2. ^{1}H NMR and IR spectral data and yield of **4**.



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Compound			^{1}H NMR, δ ($CDCl_3$)					IR (KBr) cm^{-1}	Yield (%)
No.	X	R	2-H$_\alpha$ (1H, d, J=17 Hz)	2-H$_\beta$ (1H, d, J=17 Hz)	6-H (1H, d, J=5 Hz)	7-H (1H, dd, J=5, 9 Hz)	Other protons		
4a	OBh	H	3.25	3.70	4.29	5.95	1.43 (9H, s), 3.80 (3H, s), 4.46 and 4.59 (2H, ABq, J=16 Hz), 4.98 (2H, s), 5.12 (1H, d, J=6 Hz), 5.56 (1H, d, J=16 Hz), 6.87 (1H, s), 6.91 (2H, d, J=8 Hz), 6.92 (1H, s), 6.97 (2H, d, J=8 Hz), 7.22~7.51 (25H, m)	1790, 1705, 1610	37.9
4c	O(*iso*-Pr)	H	3.41	3.85	4.47	6.00	1.22 (6H, d, J=7 Hz), 1.44 (9H, s), 3.82 (3H, s), 4.35 and 4.48 (2H, ABq, J=16 Hz), 5.00 (2H, s), 5.02 (1H, m), 5.13 (1H, d, J=6 Hz), 5.54 (1H, d, J=6 Hz), 6.93 (2H, d, J=6 Hz), 6.95 (1H, s), 6.98 (2H, d, J=8 Hz), 7.26~7.54 (15H, m)	1785, 1705, 1610	33.0
4d	O(*iso*-Bu)	H	3.40	3.83	4.46	6.00	0.90 (6H, d, J=7 Hz), 1.44 (9H, s), 1.90 (1H, m), 3.82 (3H, s), 3.88 (2H, d, J=7 Hz), 4.39 and 4.52 (2H, ABq, J=16 Hz), 5.00 (2H, s), 5.13 (1H, d, J=6 Hz), 5.55 (1H, d, J=6 Hz), 6.92 (2H, d, J=8 Hz), 6.95 (1H, s), 6.98 (2H, d, J=8 Hz), 7.26~7.54 (15H, m)	1785, 1710, 1610	33.5
4e	OCH_2Ph	H	3.33	3.77	4.37	5.98	1.44 (9H, s), 3.82 (3H, s), 4.42 and 4.55 (2H, ABq, J=16 Hz), 5.00 (2H, s), 5.12 (2H, s), 5.13 (1H, d, J=6 Hz), 5.56 (1H, d, J=6 Hz), 6.93 (2H, d, J=8 Hz), 6.94 (1H, s), 6.98 (2H, d, J=8 Hz), 7.24~7.52 (20H, m)	1790, 1700, 1610	36.5

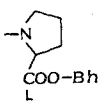
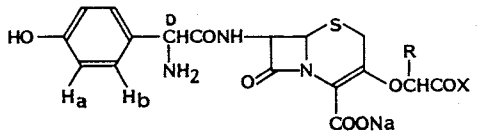
4f	OEt	CH_3	3.28	3.95	4.44	5.98	1.20 (3H, t, J=7 Hz), 1.44 (9H, s), 1.47 (3H, s), 3.81 (3H, s), 4.04~4.20 (2H, m), 4.65 (1H, q, J=7 Hz), 4.99 (2H, s), 5.11 (1H, d, J=6 Hz), 5.47 (1H, d, J=6 Hz), 6.92 (2H, d, J=8 Hz), 6.96 (2H, d, J=8 Hz), 6.97 (1H, s), 7.24~7.52 (15H, m)	1785, 1695, 1620	13.8
4g	$N(CH_3)_2$	H	3.56	3.92	4.48	6.00	1.45 (9H, s), 2.66 (3H, s), 2.87 (3H, s), 3.82 (3H, s), 4.42 and 4.62 (2H, ABq, J=16 Hz), 5.00 (2H, s), 5.14 (1H, d, J=6 Hz), 5.55 (1H, d, J=6 Hz), 6.94 (2H, d, J=8 Hz), 6.97 (1H, s), 6.99 (2H, d, J=8 Hz), 7.26~7.52 (15H, m)	1785, 1715, 1660	30.0
4h	NEt_2	H	3.59	3.94	4.47	5.99	1.01 (3H, t, J=7 Hz), 1.07 (3H, t, J=7 Hz), 1.43 (9H, s), 2.96 (2H, q, J=7 Hz), 3.29 (2H, q, J=7 Hz), 3.82 (3H, s), 4.40 and 4.59 (2H, ABq, J=14 Hz), 4.99 (2H, s), 5.11 (1H, d, J=6 Hz), 5.50 (1H, d, J=6 Hz), 6.92 (2H, d, J=8 Hz), 6.95 (1H, s), 6.98 (2H, d, J=8 Hz), 7.27~7.50 (14H, m), 7.62 (1H, d, J=9 Hz)	1790, 1725, 1675	40.4
4i		H	3.60	3.94	4.48	6.01	1.44 (9H, s), 3.04~3.68 (8H, m), 3.82 (3H, s), 4.40 and 4.59 (2H, ABq, J=16 Hz), 5.00 (2H, s), 5.13 (1H, d, J=6 Hz), 5.49 (1H, d, J=6 Hz), 6.93 (2H, d, J=8 Hz), 6.96 (1H, s), 6.98 (2H, d, J=8 Hz), 7.28~7.50 (15H, m)	1785, 1715, 1670	31.3
4j		H	3.40	3.74	4.58	5.88	1.44 (9H, s), 1.68~2.24 (4H, m), 2.98~3.22 (2H, m), 3.80 (3H, s), 4.36~4.63 (1H, m), 4.41 and 4.57 (2H, ABq, J=16 Hz), 5.00 (2H, s), 5.12 (1H, d, J=6 Hz), 5.53 (1H, d, J=6 Hz), 6.70 (1H, s), 6.92 (2H, d, J=8 Hz), 7.00 (1H, s), 7.00 (2H, d, J=8 Hz), 7.22~7.52 (25H, m)	1790, 1710, 1680	35.0

Table 3. ^{1}H NMR and IR spectral data and yield of **1**.



1

Compound			^{1}H NMR, δ (D_2O)							IR (KBr) cm^{-1} β-Lactam	Yield from **4** (%)
No.	X	R	H_a (2H, d, J=8 Hz)	H_b (2H, d, J=8 Hz)	6-H (1H, d, J=5 Hz)	7-H (1H, d, J=5 Hz)	2-H_α (1H, d, J=17 Hz)	2-H_β (1H, d, J=17 Hz)	Other protons		
1a	ONa	H	6.95	7.37	5.09	5.51	3.21	3.46	4.30 and 4.40 (2H, ABq, J=16 Hz, OCH_2CO)	1750	62.2
1c	O(*iso*-Pr)	H	6.95	7.35	5.07	5.45	3.24	3.56	1.26 (6H, d, J=7 Hz, $CH_3\times2$), 4.53 and 4.63 (2H, ABq, J=17 Hz, OCH_2CO), 5.09 (1H, m, $CH(CH_3)_2$)	1750	58.6
1d	O(*iso*-Bu)	H	6.93	7.35	5.07	5.53	3.25	3.54	0.92 (6H, d, J=7 Hz, $CH_3\times2$), 1.97 (1H, m, $CH(CH_3)_2$), 4.01 (2H, d, J=7 Hz, $CH_2CH(CH_3)_2$), 4.60 and 4.71 (2H, ABq, OCH_2CO)	1750	58.0
1e	OCH_2Ph	H	6.92	7.33	4.93	5.51	3.14	3.42	4.58 and 4.68 (2H, ABq, J=17 Hz, OCH_2CO), 5.25 (2H, br s, CH_2Ph), 7.45 (5H, br s, Ph)	1745	57.3
1f	OEt	CH_3	6.93	7.35	5.06	5.53	3.23	3.51	1.27 (3H, t, J=7 Hz, CH_2CH_3), 1.49 (3H, d, J=7 Hz, $CHCH_3$), 4.24 (2H, q, J=7 Hz, CH_2CH_3), 4.71 (1H, q, J=7 Hz, $CHCH_3$)	1745	61.5
1g	$N(CH_3)_2$	H	6.96	7.38	5.09	5.55	3.20	3.50	2.94 (3H, s, CH_3), 2.98 (3H, s, CH_3)	1760	59.4
1h	NEt_2	H	6.94	7.34	5.07	5.51	3.21	3.47	1.10 (3H, t, J=7 Hz, CH_2CH_3), 1.17 (3H, t, J=7 Hz, CH_2CH_3), 3.31 (2H, q, J=7 Hz, CH_2CH_3), 3.37 (2H, q, J=7 Hz, CH_2CH_3), 4.71 and 4.78 (2H, ABq, J=16 Hz, OCH_2CO)	1755	56.0
1i		H	6.95	7.37	5.08	5.55	3.22	3.50	3.47~3.80 (8H, m, morpholine)	1755	61.8
1j (*dl*)[a]		H	7.01	7.42	5.12, 5.13 (2×d)	5.56, 5.58 (2×d)	3.21, 3.23 (2×d)	3.55, 3.56 (2×d)	1.80~2.40 (4H, m, $CH_2\times2$), 3.45~3.62 (2H, m, NCH_2), 4.23~4.38 (1H, m, $NCHCH_2$)	1755	53.5

[a] Racemization occurred in the process from **5j** to **1j**.

IR (KBr) cm^{-1} 1780, 1700, 1655; ^1H NMR ($CDCl_3$) δ 1.23 (3H, t, J=7 Hz, CH_2CH_3), 1.43 (9H, s, *tert*-Bu), 3.40 (1H, d, J=17 Hz, 2-H_α), 3.82 (3H, s, OCH_3), 3.83 (1H, d, J=17 Hz, 2-H_β), 4.16 (2H, q, J=7 Hz, CH_2CH_3), 4.36 and 4.51 (2H, ABq, J=17 Hz, OCH_2COO), 4.46 (1H, d, J=5 Hz, 6-H), 4.99 (2H, s, CH_2O), 5.13 (1H, d, J=6 Hz, CH(NH)CO), 5.53 (1H, d, J=6 Hz, NHCOOC$_4$H$_9$), 6.00 (1H, dd, J=5 and 9 Hz, 7-H), 6.92 (2H, d, J=8 Hz, aromatic H), 6.95 (1H, s, $CHPh_2$), 6.98 (2H, d, J=8 Hz, aromatic H), 7.30~7.50 (15H, m, aromatic H and CONH); FD-MS m/z 853 (M^+);

Anal Calcd for $C_{45}H_{47}N_3O_{12}S$: C 63.29, H 5.55, N 4.92.
Found: C 63.35, H 5.56, N 5.10.

Similarly, compounds **4a** and **4c~4j** were prepared from **2** using the same procedure for **4b**. Their spectral data and yield are summarized in Table 2.

Diphenylmethyl 7β-[D-α-(*tert*-Butoxycarbonylamino)-α-[4-(4-methoxybenzyl)oxyphenyl]acetamido]-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate (**5b**)

To a solution of **4b** (400 mg, 0.47 mM) in DMF (3.5 ml) was added dropwise phosphorus tribromide (127 mg, 0.47 mM) under ice-cooling. After being stirred for 30 minutes at the same temp, the reaction mixture was poured into H_2O (40 ml) and extracted with EtOAc (50 ml). The extract was washed with brine (40 ml), dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent; benzene - acetone, 30:1~20:1) to give 303 mg (70.0%) of **5b** as an amorphous solid. IR (KBr) cm^{-1} 1780, 1705; ^1H NMR ($CDCl_3$) δ 1.24 (3H, t, J=7 Hz, OCH_2CH_3), 1.42 (9H, s, *tert*-Bu), 3.26 (1H, d, J=16 Hz, 2-H_α), 3.40 (1H, d, J=16 Hz, 2-H_β), 3.82 (3H, s, OCH_3), 4.18 (2H, q, J=7 Hz, OCH_2CH_3), 4.46 (2H, s, OCH_2COO), 4.98 (2H, s, CH_2O), 4.99 (1H, d, J=5 Hz, 6-H), 5.16 (1H, d, J=6 Hz, CH(NH)CO), 5.59 (1H, d, J=6 Hz, NHCOOC$_4$H$_9$), 5.68 (1H, dd, J=5 and 9 Hz, 7-H), 6.56 (1H, d, J=9 Hz, CONH), 6.92 (2H, d, J=9 Hz, aromatic H), 6.93 (1H, s, $CHPh_2$), 6.97 (2H, d, J=9 Hz, aromatic H), 7.30~7.50 (14H, m, aromatic H); FD-MS m/z 838 $(M+1)^+$.

Sodium 7β-[D-α-Amino-α-(4-hydroxyphenyl)acetamido]-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate (**1b**)

To a mixture of TFA (3.5 ml) and anisole (0.7 ml) was added **5b** (250 mg, 0.30 mM) under ice-cooling. After being stirred for 50 minutes at the same temp, the resulting solution was slowly added dropwise to a mixture of Et_2O and *n*-hexane (1:2, 30 ml). The precipitated trifluoroacetate of the desired product was collected by filtration, washed with a mixture of Et_2O and *n*-hexane (1:2, 30 ml). The above trifluoroacetate and $NaHCO_3$ (50 mg, 0.60 mM) were dissolved in H_2O (5 ml), and the solution was treated by column chromatography on Sephadex LH-20 (eluent; H_2O), and then lyophilized to give 120 mg (85.0%) of **1b** as white solid: IR (KBr) cm^{-1} 1750, 1670, 1600; ^1H NMR (D_2O) δ 1.27 (3H, t, J=7 Hz, OCH_2CH_3), 3.27 (1H, d, J=17 Hz, 2-H_α), 3.56 (1H, d, J=17 Hz, 2-H_β), 4.26 (2H, q, J=7 Hz, OCH_2CH_3), 4.56 and 4.67 (2H, ABq, J=16 Hz, OCH_2COO), 5.06 (1H, d, J=5 Hz, 6-H), 5.54 (1H, d, J=5 Hz, 7-H), 6.91 (2H, d, J=8 Hz, aromatic H), 7.34 (2H, d, J=8 Hz, aromatic H).

Compounds **1a** and **1c~1j** were similarly prepared from **4a** and **4c~4j** (Table 2) using the same procedures described for **1b**. Their spectral data and yield are summarized in Table 3.

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