STUDIES ON CEPHALOSPORIN ANTIBIOTICS

II. SYNTHESIS, ANTIBACTERIAL ACTIVITY AND ORAL ABSORPTION OF 3-ALKOXYCARBONYLMETHOXY-7β-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-(O-SUBSTITUTED OXYIMINO)-ACETAMIDO]CEPHALOSPORINS

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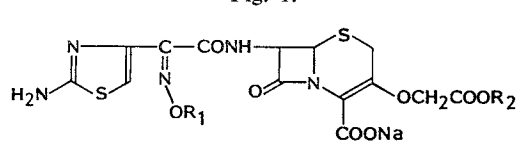
The synthesis, antibacterial activity and oral absorption in rats of 3-alkoxycarbonylmethoxy-7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(O-substituted oxyimino)acetamido]cephalosporins (**1**) are described. In this cephalosporin series, 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]cephalosporins (**1b**, **1i** and **1j**) with a lower alkoxycarbonylmethoxy group at the C-3 position of a cephem nucleus exhibited not only potent activity against Gram-negative bacteria but also good oral absorption in rats. Structure-activity relationships of **1** are also presented.

During the past decade, cephalosporins[1~5] bearing an 2-aminothiazole-oxime moiety at the C-7 position of a cephem nucleus have been developed successively. They have excellent antibacterial activities against Gram-positive and Gram-negative bacteria including β-lactamase producing strains. However, all of the new cephalosporins clinically used are not suitable for oral administration because of their low absorption from the gastro-intestinal tract. As orally active cephalosporins, only cephalexin[6] and its analogues with D-phenylglycine or closely related moieties at the C-7 position are now available in clinical use. But their activities against Gram-negative bacteria are much lower than those of the 2-aminothiazole-oxime type cephalosporins.

Therefore, much research has been undertaken aiming at obtaining a new orally active cephalosporin possessing the same antibacterial properties as those of the new parenteral cephalosporins. Recently, some orally active 2-aminothiazole-oxime type cephalosporins such as cefixime (FK027)[7] and T-2588[8] have been reported.

In the course of our research on new cephalexin analogues, we have found an interesting fact that the analogues bearing an alkoxycarbonylmethoxy group at the C-3 position exhibit much higher peak serum levels than that of cephalexin after oral administration to rats[9]. Then, we became interested in the application of the novel C-3 alkoxycarbonylmethoxy substituents into the 2-aminothiazole-oxime type cephalosporins as an attempt to improve their oral absorption. This paper describes the synthesis, antibacterial activity and oral absorption in rats of new cephalosporins (**1**) as shown in Fig. 1.

Fig. 1.



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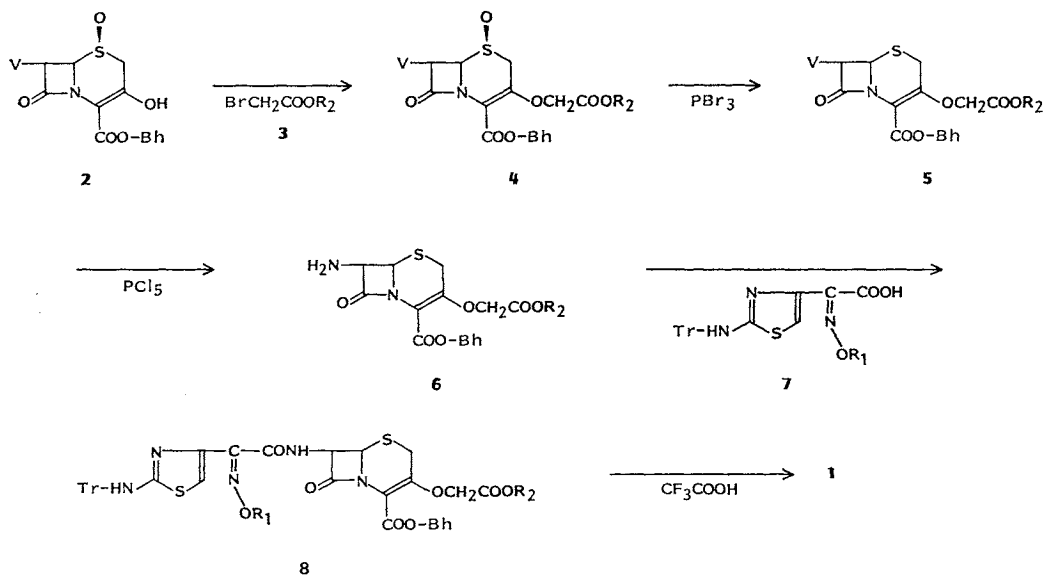
Scheme 1.



R_1=CH_3, CH_2COOBh, CH_2CONH_2 *et al.*
R_2=CH_3, Et, *iso*-Pr, $CH_2CH{=}CH_2$, CH_2Ph, Bh
V=$NHCOCH_2OPh$, Bh=$CHPh_2$, Tr=CPh_3

Chemistry

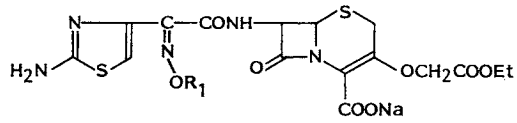
The new cephalosporin derivatives (**1a**~**1l**) listed in Tables 1 and 2 were prepared by the methods shown in Scheme 1. Diphenylmethyl (Bh) 7β-phenoxyacetamido-3-hydroxycephalosporinate 1-oxide (**2**)[10] was reacted with 2-bromoacetic acid ester derivatives (**3**) in the presence of *N*,*N*-diisopropylethylamine to afford C-3 *O*-substituted cephem compounds (**4**). Reduction of the sulfoxide of **4** using phosphorus tribromide (PBr_3) in *N*,*N*-dimethylformamide gave the corresponding sulfides (**5**). Then, the C-7 phenoxyacetyl side chain was cleaved by the known imino-chloride method[11] to yield Bh 7β-aminocephalosporinates (**6**). The coupling reaction of (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-(*O*-substituted oxyimino)acetic acid derivatives (**7**) with **6** was carried out *via* their acid chlorides (formed with phosphorus pentachloride) in dichloromethane at low temperature to give the protected cephalosporins (**8**). The protecting groups in **8** were removed with trifluoroacetic acid and anisole to afford the desired compounds (**1**).

Antibacterial Activity and Oral Absorption

The *in vitro* antibacterial activities of the new cephalosporins (**1**) against selected Gram-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 Tables 1 and 2. For comparison, the MIC values and the peak serum levels of cephalexin and cefixime are listed at the bottom of Tables 1 and 2.

Table 1 shows the effect of the oxime *O*-substituent (R_1) on antibacterial activity and oral absorption. Against the Gram-negative bacteria, all of the derivatives (**1a**~**1g**) exhibited high activity compared with cephalexin. These compounds except **1f** showed the activity comparable to that of cefixime. However, their activity against *Staphylococcus aureus* was lower than that of cephalexin

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

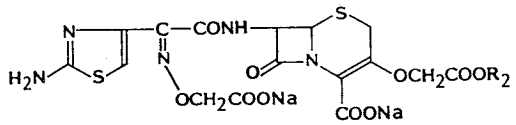


1

Compound No.	R_1	MIC (μg/ml, 10^6 cfu/ml)[a] *S.a.*	*E.c.*	*K.p.*	*M.m.*	*S.m.*	Peak serum level (μg/ml)[b] po, 50 mg/kg, rats (n=3)
1a	CH_3	50	1.56	0.20	0.20	1.56	<0.05
1b	CH_2COONa	>100	0.78	0.39	≦0.10	0.39	15.8
1c	$(CH_2)_2COONa$	>100	1.56	≦0.10	0.39	6.25	<0.4
1d	$CH(CH_3)COONa$	>100	1.56	≦0.10	0.20	1.56	0.9
1e	$C(CH_3)_2COONa$	100	1.56	≦0.10	0.20	0.39	<0.3
1f	CH_2COOEt	50	12.5	0.78	1.56	6.25	<2.2
1g	CH_2CONH_2	100	1.56	≦0.10	0.39	1.56	<2.1
Cephalexin		0.78	12.5	3.13	>100	>100	16.6
Cefixime[c]		25	0.78	≦0.10	0.20	0.78	30.2

[a] The MICs are 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 test organism.
[c] This reference compound was prepared according to the reported procedure[7].
Abbreviations: *S.a.*; *Staphylococcus aureus* 209P JC-1, *E.c.*; *Escherichia coli* NIHJ JC-2, *K.p.*; *Klebsiella pneumoniae* IFO 3317, *M.m.*; *Morganella morganii* IID 602, *S.m.*; *Serratia marcescens* IID 618.

Table 2. *In vitro* antibacterial activity and peak serum level of **1h**~**1l**.



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Compound No.	R_2	MIC (μg/ml, 10^6 cfu/ml)[a] *S.a.*	*E.c.*	*K.p.*	*M.m.*	*S.m.*	Peak serum level (μg/ml)[b] po, 50 mg/kg, rats (n=3)
1b	Et	>100	0.78	0.39	≦0.10	0.39	15.8
1h	Na	>100	12.5	1.56	6.25	50	<2.9
1i	CH_3	>100	1.56	≦0.10	0.20	1.56	24.4
1j	*iso*-Pr	>100	3.13	≦0.10	0.20	0.39	16.4
1k	$CH_2CH=CH_2$	>100	0.78	≦0.10	≦0.10	1.56	7.5
1l	CH_2Ph	>100	12.5	0.39	0.78	6.25	<3.1
Cephalexin		0.78	12.5	3.13	>100	>100	16.6
Cefixime[c]		25	0.78	≦0.10	0.20	0.78	30.2

[a~c] and organism abbreviations: See the footnote in Table 1.

and cefixime. Regarding the oral absorption of these compounds, only **1b** with a carboxymethyl group as R_1 showed good oral absorption. Its peak serum level was comparable to that of cephalexin, but was lower than that of cefixime.

Table 2 shows the influence of the C-3 substituent variation on **1b**. Against the Gram-negative bacteria, the cephalosporins (**1i**, **1j** and **1k**) having a methyl, isopropyl or allyl ester group, respectively, in the C-3 substituent exhibited similar activity to that of **1b** and cefixime. However, the derivatives (**1h** and **1l**) bearing a carboxy function or benzyl ester group, respectively, were much less active. Against *S. aureus*, all of these derivatives displayed no significant activity, probably due to their high hydrophilicity. Regarding the oral absorption, compound **1i** ($R_2=CH_3$) showed the best oral absorption in this series, but its peak serum level did not reach the level of cefixime. In contrast, the other analogues such as **1h**, **1k** and **1l** exhibited low oral absorption.

The results shown in Table 2 indicate that the presence of the lower alkoxycarbonylmethoxy substituent at the C-3 position is of importance on antibacterial activity as well as oral absorption of these compounds.

In this study, we could improve the oral absorption in rats of cephalosporins carrying an 2-aminothiazole-oxime side chain by application of novel C-3 substituents which were found in a previous study on cephalexin analogues. However, further improvement of antibacterial activity against Gram-positive bacteria is desirable.

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. ^{1}H 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

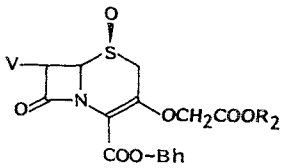
Male SLC/Wistar rats ($n=3$) weighing 180~220 g were fasted overnight and orally dosed with 50 mg/kg of the test compounds. Serum samples were collected at 0.5, 1, 2 and 4 hours respectively after dosing. The serum levels of the test compounds were measured by the disc-plate method using *Escherichia coli* SC 507 or *Micrococcus luteus* NIHJ as the test organism and the sensitivity test agar (Eiken, Japan) as the test medium.

Diphenylmethyl 7β-Phenoxyacetamido-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate 1β-Oxide (4c)

To a solution of **2** (5.32 g, 10 mM) in DMSO (50 ml) were added ethyl bromoacetate (3.54 g, 21.2 mM) and *N*,*N*-diisopropylethylamine (1.94 g, 15 mM) at room temp. After being stirred at 45°C for 1 hour, the reaction mixture was poured into 0.5% HCl (80 ml) under ice-cooling and extracted with EtOAc (150 ml). The extract was washed with brine (100 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; benzene - acetone, 5:1), and crystallized from MeOH to give 2.50 g (40.5%) of **4c**: MP 102~104°C. IR (KBr) cm^{-1} 1780, 1730, 1690; ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, $J=7$ Hz, CH_2CH_3), 3.49 (1H, dd, $J=1.5$ and 17 Hz, 2-H_α), 3.94 (1H, d, $J=17$ Hz, 2-H_β), 4.16 (2H, q, $J=7$ Hz, CH_2CH_3), 4.38 and 4.52 (2H, ABq, $J=16$ Hz, OCH_2CO), 4.54 (1H, dd, $J=1.5$ and 5 Hz, 6-H), 4.57 (2H, s, $PhOCH_2$), 6.10 (1H, dd, $J=5$ and 9 Hz, 7-H), 6.90~7.08 (4H, m, $CHPh_2$ and aromatic H), 7.26~7.50 (12H, m, aromatic H), 7.89 (1H, d, $J=9$ Hz, CONH); field desorption mass spectrum (FD-MS) m/z 618 $(M)^+$;

Anal Calcd for $C_{32}H_{30}N_2O_9S$: C 62.13, H 4.89, N 4.53.
Found: C 62.05, H 4.66, N 4.31.

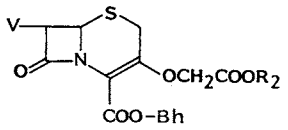
Table 3. ^{1}H NMR, MS and IR spectral data and yield of **4a**, **4b** and **4d**~**4f**.



4

Compound		^{1}H NMR, δ ($CDCl_3$)					MS (m/z)	IR (KBr) cm^{-1}	Yield (%)
No.	R_2	2-H_α (1H, dd, J=1.5, 17 Hz)	2-H_β (1H, d, J=17 Hz)	6-H (1H, dd, J=1.5, 5 Hz)	7-H (1H, dd, J=5, 9 Hz)	Other protons			
4a	Bh	3.37	3.84	4.40	6.07	4.48 and 4.62 (2H, ABq, J=16 Hz), 4.56 (2H, s), 6.88 (1H, s), 6.90~7.08 (4H, m), 7.22~7.51 (22H, m), 7.87 (1H, d, J=9 Hz)	757 $(M+1)^+$	1780, 1720	32
4b	CH_3	3.48	3.94	4.55	6.11	3.70 (3H, s), 4.40 and 4.55 (2H, ABq, J=16 Hz), 4.59 (2H, s), 6.90~7.08 (4H, m), 7.24~7.52 (12H, m), 7.89 (1H, d, J=9 Hz)	604 $(M)^+$	1780, 1750	38
4d	*iso*-Pr	3.49	3.94	4.54	6.09	1.21 (6H, d, J=7 Hz), 4.34 and 4.48 (2H, ABq, J=17 Hz), 4.57 (2H, s), 5.02 (1H, m), 6.90~7.07 (4H, m), 7.24~7.52 (12H, m), 7.88 (1H, d, J=9 Hz)	632 $(M)^+$	1780, 1720	36
4e	$CH_2CH{=}CH_2$	3.49	3.95	4.56	6.11	4.42 and 4.57 (2H, ABq, J=16 Hz), 4.49~4.66 (2H, m), 4.58 (2H, s), 5.24~5.37 (2H, m), 5.76~5.98 (1H, m), 6.90~7.08 (4H, m), 7.24~7.52 (12H, m), 7.89 (1H, d, J=9 Hz)	630 $(M)^+$	1780, 1750	39
4f	CH_2Ph	3.46	3.93	4.49	6.11	4.44 and 4.60 (2H, ABq, J=16 Hz), 4.60 (2H, s), 5.14 (2H, s), 6.90~7.10 (4H, m), 7.26~7.53 (17H, m), 7.90 (1H, d, J=9 Hz)	680 $(M)^+$	1780, 1745, 1720	33

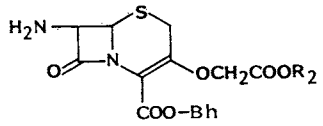
Table 4. ^{1}H NMR, MS and IR spectral data and yield of **5a**, **5b** and **5d**~**5f**.



5

Compound		^{1}H NMR, δ ($CDCl_3$)					MS (m/z)	IR (KBr) cm^{-1}	Yield (%)
No.	R_2	2-H_α (1H, d, J=17 Hz)	2-H_β (1H, d, J=17 Hz)	6-H (1H, d, J=5 Hz)	7-H (1H, dd, J=5, 9 Hz)	Other protons			
5a	Bh	3.17	3.30	4.98	5.67	4.58 (4H, br s), 6.89~7.08 (5H, m), 7.20~7.46 (22H, m), 7.52 (1H, d, J=9 Hz)	740 $(M)^+$	1770, 1760	95
5b	CH_3	3.13	3.35	5.07	5.66	3.72 (3H, s), 4.44 and 4.56 (2H, ABq, J=17 Hz), 4.60 (2H, s), 6.90~7.08 (4H, m), 7.24~7.48 (12H, m), 7.64 (1H, d, J=9 Hz)	588 $(M)^+$	1770, 1760	85
5d	*iso*-Pr	3.28	3.38	5.09	5.69	1.23 (6H, d, J=7 Hz), 4.44 and 4.52 (2H, ABq, J=16 Hz), 4.60 (2H, s), 5.06 (1H, m), 6.92~7.10 (4H, m), 7.26~7.56 (13H, m)	616 $(M)^+$	1775, 1760	88
5e	$CH_2CH{=}CH_2$	3.17	3.36	5.07	5.67	4.42~4.66 (4H, m), 4.60 (2H, s), 5.24~5.39 (2H, m), 5.79~5.99 (1H, m), 6.90~7.08 (4H, m), 7.24~7.48 (12H, m), 7.62 (1H, d, J=9 Hz)	614 $(M)^+$	1770, 1760	94
5f	CH_2Ph	3.24	3.35	5.02	5.69	4.29 and 4.59 (2H, ABq, J=17 Hz), 4.60 (2H, s), 5.16 (2H, s), 6.92~7.08 (4H, m), 7.24~7.45 (17H, m), 7.51 (1H, d, J=9 Hz)	664 $(M)^+$	1770, 1760	86

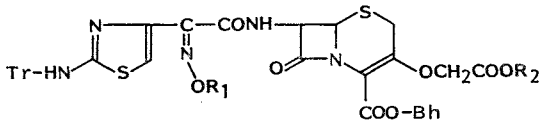
Table 5. ^{1}H NMR, MS and IR spectral data and yield of **6a**, **6b** and **6d**~**6f**.



6

Compound		^{1}H NMR, δ ($CDCl_3$)					MS (*m/z*)	IR (KBr) cm^{-1}	Yield (%)
No.	R_2	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, d, J=5 Hz)	Other protons			
6a	Bh	3.34	3.54	4.67	4.88	1.75 (2H, br s), 4.56 (2H, s), 6.94 (1H, s), 6.96 (1H, s), 7.24~7.48 (20H, m)	607 $(M+1)^+$	1780	55
6b	CH_3	3.39	3.60	4.69	4.95	1.82 (2H, br s), 3.71 (3H, s), 4.47 (2H, br s), 6.96 (1H, s), 7.24~7.48 (10H, m)	455 $(M+1)^+$	1760	56
6d	*iso*-Pr	3.40	3.59	4.69	4.97	1.22 (6H, d, J=7 Hz), 1.82 (2H, br s), 4.44 (2H, s), 5.06 (1H, m), 6.96 (1H, s), 7.22~7.50 (10H, m)	482 $(M)^+$	1760	52
6e	$CH_2CH=CH_2$	3.40	3.60	4.69	4.96	1.85 (2H, br s), 4.49 (2H, s), 4.58~4.65 (2H, m), 5.24~5.39 (2H, m), 5.78~5.99 (1H, m), 6.97 (1H, s), 7.23~7.50 (10H, m)	481 $(M+1)^+$	1760	47
6f	CH_2Ph	3.37	3.58	4.69	4.92	1.75 (2H, br s), 4.50 (2H, s), 5.15 (2H, s), 6.96 (1H, s), 7.26~7.50 (15H, m)	531 $(M+1)^+$	1760	56

Table 6. ^{1}H NMR, MS and IR spectral data and yield of **8a** and **8c**~**8l**.



8

Compound			^{1}H NMR, δ ($CDCl_3$)				MS (m/z)	IR (KBr) cm^{-1}	Yield (%)
No.	R_1	R_2	6-H (1H, d, J=5 Hz)	7-H (1H, dd, J=5, 9 Hz)	Thiazole 5-H (1H, s)	Other protons			
8a	CH_3	Et	5.11	5.76	6.80	1.25 (3H, t, J=7 Hz), 3.38 (2H, br s), 4.08 (3H, s), 4.19 (2H, q, J=7 Hz), 4.50 (2H, br s), 6.93 (1H, s), 7.02 (1H, br s), 7.20~7.46 (26H, m)	893 $(M)^+$	1775, 1720	70
8c	$(CH_2)_2COOBh$	Et	4.98	5.57	6.78	1.24 (3H, t, J=7 Hz), 2.90 (2H, t, J=6 Hz), 3.28 (2H, br s), 4.18 (2H, q, J=7 Hz), 4.26 (2H, br s), 4.62 (2H, t, J=6 Hz), 6.88 (1H, s), 6.94 (1H, s), 6.98 (1H, br s), 7.08 (1H, d, J=9 Hz), 7.20~7.48 (35H, m)	1,117 $(M)^+$	1780, 1725	43
8d	$CH(CH_3)COOBh$	Et	5.06	5.76 and 5.86	6.81 and 6.82	1.24 and 1.26 (3H, t, J=7 Hz), 2.82~3.16 (2H, m), 4.17 and 4.20 (2H, q, J=7 Hz), 4.49 and 4.55 (2H, br s), 5.15~5.30 (1H, m), 6.85 and 6.86 (1H, s), 6.92 (1H, s), 7.00 (1H, br s), 7.10~7.48 (35H, m), 8.27 (1H, d, J=9 Hz)	1,118 $(M+1)^+$	1780, 1730	45
8e	$C(CH_3)_2COOBh$	Et	5.04	5.84	6.72	1.25 (3H, t, J=7 Hz), 1.71 (3H, s), 1.74 (3H, s), 3.12 (1H, d, J=17 Hz), 3.22 (1H, d, J=17 Hz), 4.19 (2H, q, J=7 Hz), 4.51 (2H, s), 6.86 (1H, s), 6.94 (1H, br s), 6.96 (1H, s), 7.20~7.53 (36H, m)	1,131 $(M)^+$	1780, 1725	50

8f	CH_2COOEt	Et	5.12	5.77	6.87	1.25 (3H, t, J=7 Hz), 1.27 (3H, t, J=7 Hz), 3.42 (2H, br s), 4.18 (2H, q, J=7 Hz), 4.21 (2H, q, J=7 Hz), 4.56 (2H, br s), 4.88 (2H, br s), 6.92 (1H, s), 7.00 (1H, br s) 7.20~7.47 (25H, m), 8.47 (1H, d, J=9 Hz)	965 $(M)^+$	1780, 1725	60
8g	CH_2CONH_2	Et	4.98	5.75	6.80	1.26 (3H, t, J=7 Hz), 1.80 (2H, br s), 3.24 (2H, br s), 4.19 (2H, q, J=7 Hz), 4.50 (2H, br s), 4.56 and 4.68 (2H, ABq, J= 16 Hz), 6.90 (1H, s), 7.10~7.40 (27H, m)	937 $(M+1)^+$	1780, 1720	64[a]
8h	CH_2COOBh	Bh	4.95	5.75	6.83	2.94 (1H, d, J=17 Hz), 3.02 (1H, d, J= 17 Hz), 4.58 (2H, s), 4.92 and 5.05 (2H, ABq, J=17 Hz), 5.90 (2H, s), 5.92 (1H, s), 7.00 (1H, br s), 7.16~7.46 (45H, m), 8.19 (1H, d, J=9 Hz)	1,242 $(M+1)^+$	1775, 1730	49
8i	CH_2COOBh	CH_3	5.06	5.77	6.85	3.03 (1H, d, J=17 Hz), 3.13 (1H, d, J= 17 Hz), 3.71 (3H, s), 4.51 (2H, s), 4.94 and 5.07 (2H, ABq, J=17 Hz), 6.93 (1H, s,) 6.94 (1H, s), 7.00 (1H, br s), 7.24~ 7.48 (35H, m), 8.20 (1H, d, J=9 Hz)	1,090 $(M+1)^+$	1775, 1735	44
8j	CH_2COOBh	*iso*-Pr	5.05	5.75	6.84	1.23 (6H, d, J=7 Hz), 3.01 (1H, d, J=17 Hz), 3.10 (1H, d, J=17 Hz), 4.46 (2H, s), 4.94 and 5.05 (2H, ABq, J=17 Hz), 5.02 (1H, m), 6.91 (1H, s), 6.92 (1H, s), 7.00 (1H, br s), 7.20~7.48 (35H, m), 8.17 (1H, d, J=9 Hz)	1,118 $(M+1)^+$	1775, 1725	56
8k	CH_2COOBh	$CH_2CH=CH_2$	5.05	5.77	6.84	3.03 (1H, d, J=17 Hz), 3.12 (1H, d, J= 17 Hz), 4.52 (2H, s), 4.61 (2H, d, J=6 Hz), 4.94 and 5.06 (2H, ABq, J=17 Hz), 5.20~ 5.38 (2H, m), 5.77~5.99 (1H, m), 6.92 (1H, s), 6.94 (1H, s), 7.01 (1H, br s), 7.20~7.48 (35H, m), 8.19 (1H, d, J=9 Hz)	1,116 $(M+1)^+$	1770, 1730	48
8l	CH_2COOBh	CH_2Ph	4.99	5.75	6.84	2.97 (1H, d, J=17 Hz), 3.06 (1H, d, J= 17 Hz), 4.53 (2H, s), 4.94 and 5.05 (2H, ABq, J=16 Hz), 5.14 (2H, s), 6.91 (1H, s), 6.92 (1H, s), 7.00 (1H, br s), 7.22~7.46 (40H, m), 8.18 (1H, d, J=9 Hz)	1,166 $(M+1)^+$	1775, 1730	55

[a] This compound was prepared from **6c** and **7** ($R_1=CH_2CONH_2$) by using *N,N'*-dicyclohexylcarbodiimide as a condensation reagent.
Tr: CPh_3.

The spectral data and yield of various derivatives (**4**) are listed in Table 3.

Diphenylmethyl 7β-Phenoxyacetamido-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate (**5c**)

To a solution of **4c** (2.85 g, 4.61 mM) in DMF (25 ml) was added dropwise phosphorus tribromide (1.25 g, 4.61 mM) under ice-cooling. After being stirred at the same temp for 30 minutes, the reaction mixture was poured into H_2O (100 ml) and extracted with EtOAc (100 ml). The extract was washed with brine (50 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; benzene - acetone, 20 : 1) to give 2.30 g (83%) of **5c** as an amorphous solid: IR (KBr) cm^{-1} 1760, 1680; ^1H NMR ($CDCl_3$) δ 1.26 (3H, t, J=7 Hz, CH_2CH_3), 3.29 (1H, d, J=17 Hz, 2-H_α), 3.40 (1H, d, J=17 Hz, 2-H_β), 4.19 (2H, q, J=7 Hz, CH_2CH_3), 4.46 and 4.54 (2H, ABq, J=17 Hz, OCH_2CO), 4.59 (2H, s, $PhOCH_2$), 5.08 (1H, d, J=5 Hz, 6-H), 5.71 (1H, dd, J=5 and 9 Hz, 7-H), 6.90~7.10 (4H, m, $CHPh_2$ and aromatic H), 7.26~7.52 (13H, m, CONH and aromatic H); FD-MS m/z 602 $(M)^+$.

The spectral data and yield of various derivatives (**5**) are listed in Table 4.

Diphenylmethyl 7β-Amino-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate (**6c**)

To a solution of **5c** (2.15 g, 3.57 mM) in CH_2Cl_2 (40 ml) were added pyridine (0.85 g, 10.8 mM) and phosphorus pentachloride (1.49 g, 7.15 mM) at −30°C. The temp of the mixture was raised to room temp over 30 minutes. After being stirred at the same temp for 1 hour, the reaction mixture was cooled to −50°C. To the cooled mixture was added MeOH (20 ml) all at once, and then stirred for 1 hour under ice-cooling. Subsequently, the reaction mixture was cooled to −30°C, and H_2O (20 ml) was added. After being stirred for 45 minutes under ice-cooling, organic solvents were removed under reduced pressure, maintaining the temp below 10°C. The resulting aqueous solution was neutralized with 5% aq $NaHCO_3$ and extracted with CH_2Cl_2 (100 ml). The extract was washed with brine (50 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; benzene - EtOAc, 1 : 1), and crystallized from Et_2O to give 1.0 g (60%) of **6c**: MP 94~96°C; IR (KBr) cm^{-1} 1765, 1750, 1720; ^1H NMR ($CDCl_3$) δ 1.24 (3H, t, J=7 Hz, CH_2CH_3), 1.80 (2H, br s, NH_2), 3.39 (1H, d, J=17 Hz, 2-H_α), 3.59 (1H, d, J=17 Hz, 2-H_β), 4.17 (2H, q, J=7 Hz, CH_2CH_3), 4.45 (2H, s, OCH_2CO), 4.68 (1H, d, J=5 Hz, 6-H), 4.95 (1H, d, J=5 Hz, 7-H), 6.95 (1H, s, $CHPh_2$), 7.26~7.50 (10H, m, aromatic H); FD-MS m/z 468 $(M)^+$;

Anal Calcd for $C_{24}H_{24}N_2O_6S$: C 61.53, H 5.16, N 5.98.
Found: C 61.54, H 5.12, N 5.93.

The spectral data and yield of various derivatives (**6**) are listed in Table 5.

Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate (**8b**)

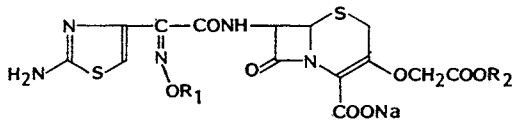
To a solution of **7** (R_1=CH_2COOBh) (627 mg, 0.96 mM) in CH_2Cl_2 (18 ml) were added pyridine (380 mg, 4.80 mM) and phosphorus pentachloride (201 mg, 0.96 mM) at −15°C, and the mixture was stirred at −15~−10°C for 15 minutes. Then, the solution of **6c** (300 mg, 0.64 mM) in CH_2Cl_2 (3 ml) was added to the above mixture at −20°C. After being stirred at −20~−10°C for 20 minutes, 0.5% HCl (20 ml) was added to the reaction mixture, and extracted with EtOAc (100 ml). The extract was washed with brine (50 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; benzene - EtOAc, 5 : 1) to give 353 mg (50%) of **8b** as an amorphous solid: IR (KBr) cm^{-1} 1780, 1730, 1680; ^1H NMR ($CDCl_3$) δ 1.26 (3H, t, J=7 Hz, CH_2CH_3), 3.04 (1H, d, J=16 Hz, 2-H_α), 3.12 (1H, d, J=16 Hz, 2-H_β), 4.19 (2H, q, J=7 Hz, CH_2CH_3), 4.51 (2H, s, OCH_2CO), 4.59 and 5.06 (2H, ABq, J=17 Hz, $NOCH_2CO$), 5.07 (1H, d, J=5 Hz, 6-H), 5.77 (1H, dd, J=5 and 9 Hz, 7-H), 6.86 (1H, s, thiazole 5-H), 6.93 (1H, s, $CHPh_2$), 6.94 (1H, s, $CHPh_2$), 7.02 (1H, br s, Ph_3CNH), 7.20~7.49 (35H, m, aromatic H), 8.19 (1H, d, J=9 Hz, CONH); FD-MS m/z 1,104 $(M+1)^+$.

The spectral data and yield of various derivatives (**8**) are listed in Table 6.

Sodium 7β-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-carboxymethoxyimino]acetamido]-3-ethoxycarbonylmethoxy-3-cephem-4-carboxylate (**1b**)

To a mixture of TFA (4 ml) and anisole (0.8 ml) was added **8b** (300 mg, 0.27 mM) under ice-cool-

Table 7. ^{1}H NMR and IR spectral data and yield of **1a** and **1c**~**1l**.



1

Compound			^{1}H NMR, δ (D_2O)						IR (KBr) cm^{-1}	Yield (%)
No.	R_1	R_2	2-H_α (1H, d, J=17 Hz)	2-H_β (1H, d, J=17 Hz)	6-H (1H, d, J=5 Hz)	7-H (1H, d, J=5 Hz)	Thiazole 5-H (1H, s)	Other protons		
1a	CH_3	Et	3.44	3.72	5.24	5.71	7.08	1.29 (3H, t, J=7 Hz), 4.01 (3H, s), 4.28 (2H, q, J=7 Hz), 4.61 and 4.72 (2H, ABq, J=16 Hz)	1755	95
1c	$(CH_2)_2COONa$	Et	3.45	3.71	5.24	5.70	7.08	1.29 (3H, t, J=7 Hz), 2.64 (2H, t, J=7 Hz), 4.28 (2H, q, J=7 Hz), 4.44 (2H, t, J=7 Hz), 4.61 and 4.73 (2H, ABq, J=16 Hz)	1750	90
1d	$CH(CH_3)COONa$	Et	3.44 and 3.46	3.69	5.26	5.72 and 5.75	7.08	1.28 (3H, t, J=7 Hz), 1.47 and 1.48 (3H, d, J=7 Hz), 4.27 (2H, q, J=7 Hz), 4.62 and 4.73 (2H, ABq, J=16 Hz)	1750	89
1e	$C(CH_3)_2COONa$	Et	3.44	3.70	5.25	5.73	7.06	1.28 (3H, t, J=7 Hz), 1.50 (3H, s), 1.52 (3H, s), 4.27 (2H, q, J=7 Hz), 4.61 and 4.73 (2H, ABq, J=17 Hz)	1755	88
1f	CH_2COOEt	Et	3.43	3.71	5.25	5.74	7.15	1.28 (6H, t, J=7 Hz), 4.28 (2H, q, J=7 Hz), 4.29 (2H, q, J=7 Hz), 4.61 and 4.72 (2H, ABq, J=17 Hz), 4.85 (2H, s)	1750	91
1g	CH_2CONH_2	Et	3.44	3.72	5.26	5.75	7.18	1.29 (3H, t, J=7 Hz), 4.28 (2H, q, J=7 Hz), 4.62 and 4.72 (2H, ABq, J=17 Hz), 4.77 (2H, s)	1750	87
1h	CH_2COONa	Na	3.40	3.62	5.28	5.69	7.15	4.36 and 4.48 (2H, ABq, J=16 Hz), 4.59 (2H, s)	1750	80
1i	CH_2COONa	CH_3	3.44	3.70	5.23	5.74	7.11	3.80 (3H, s), 4.60 (2H, s)	1750	92
1j	CH_2COONa	*iso*-Pr	3.44	3.69	5.26	5.73	7.12	1.29 (6H, d, J=7 Hz), 4.60 (2H, br s), 4.61 and 4.72 (2H, ABq, J=16 Hz), 5.12 (1H, m)	1750	90
1k	CH_2COONa	$CH_2CH=CH_2$	3.44	3.70	5.25	5.74	7.12	4.59 (2H, s), 5.26~5.46 (2H, m), 5.90~6.12 (1H, m)	1750	89
1l	CH_2COONa	CH_2Ph	3.37	3.59	5.14	5.70	7.10	4.59 (2H, s), 5.29 (2H, s), 7.48 (5H, br s)	1750	88

ing. After being stirred at the same temp for 40 minutes, the resulting solution was 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, and washed with a mixture of Et_2O and *n*-hexane (1:2, 40 ml). The above trifluoroacetate and $NaHCO_3$ (68 mg, 0.81 mM) were dissolved in H_2O (5 ml), and the solution was treated with column chromatography on Sephadex LH-20 (eluent; H_2O), and then lyophilized to give 140 mg (90%) of **1b** as a white solid: IR (KBr) cm^{-1} 1750; ^1H NMR (D_2O) δ 1.28 (3H, t, J=7 Hz, CH_2CH_3), 3.43 (1H, d, J=17 Hz, 2-H_α), 3.70 (1H, d, J=17 Hz, 2-H_β), 4.28 (2H, q, J=7 Hz, CH_2CH_3), 4.60 (2H, s, $NOCH_2CO$), 5.27 (1H, d, J=5 Hz, 6-H), 5.74 (1H, d, J=5 Hz, 7-H), 7.12 (1H, s, thiazole 5-H).

The spectral data and yield of various derivatives (**1**) are listed in Table 7.

References

1) HEYMES, R.; A. LUTZ & E. SCHRINNER: Experimental evaluation of HR 756, a new cephalosporin derivative: Pre-clinical study. Infection 5: 259~260, 1977
2) OCHIAI, M.; O. AKI, A. MORIMOTO, T. OKADA & Y. MATSUSHITA: New cephalosporin derivatives with high antibacterial activities. Chem. Pharm. Bull. 25: 3115~3117, 1977
3) REINER, R.; U. WEISS, U. BROMBACHER, P. LANZ, M. MONTAVON, A. FURLENMEIER, P. ANGEHRN & P. J. PROBST: Ro 13-9904/001, a novel potent and long-acting parenteral cephalosporin. J. Antibiotics 33: 783~786, 1980
4) WEBBER, J. A. & W. J. WHEELER: Antimicrobial and pharmacokinetic properties of newer penicillins and cephalosporins. *In* Chemistry and Biology of β-Lactam Antibiotics. Vol. 1. Penicillins and Cephalosporins. *Eds.*, R. B. MORIN & M. GORMAN, pp. 371~436, Academic Press, New York, 1982
5) ROLINSON, G. N.: β-Lactam antibiotics. J. Antimicrob. Chemother. 17: 5~36, 1986
6) RYAN, C. W.; R. L. SIMON & E. M. VAN HEYNINGEN: Chemistry of cephalosporin antibiotics. XIII. Desacetoxycephalosporins. The synthesis of cephalexin and some analogs. J. Med. Chem. 12: 310~313, 1969
7) YAMANAKA, H.; T. CHIBA, K. KAWABATA, H. TAKASUGI, T. MASUGI & T. TAKAYA: Studies on β-lactam antibiotics. IX. Synthesis and biological activity of a new orally active cephalospolin, cefixime (FK027). J. Antibiotics 38: 1738~1751, 1985
8) SADAKI, H.; H. IMAIZUMI, T. INABA, T. HIRAKAWA, Y. MUROTANI, Y. WATANABE, S. MINAMI & I. SAIKAWA: Studies on β-lactam antibiotics for medicinal purpose. XVIII. Synthesis and structure-activity relationships of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-substituted methyl-3-cephem-4-carboxylic acid derivatives. Yakugaku Zasshi 106: 129~146, 1986
9) YOKOO, C.; M. GOI, A. ONODERA, M. MURATA, T. NAGATE, Y. WATANABE & K. SOTA: 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. J. Antibiotics 41: 170~180, 1988
10) KUKOLJA, S.: Synthesis of 3-methylenecepham, a key and general intermediate in the preparation of 3-substituted cephalosporins. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Ed.*, J. ELKS, pp. 181~188, The Chemical Society, London, 1977
11) FECHTIG, B.; H. PETER, H. BICKEL & E. VISCHER: 124. Modifikationen von Antibiotika. Über die Darstellung von 7-Aminocephalosporan säure. Helv. Chim. Acta 51: 1108~1119, 1968