NEW ORALLY ACTIVE CEPHALOSPORIN ESTERS

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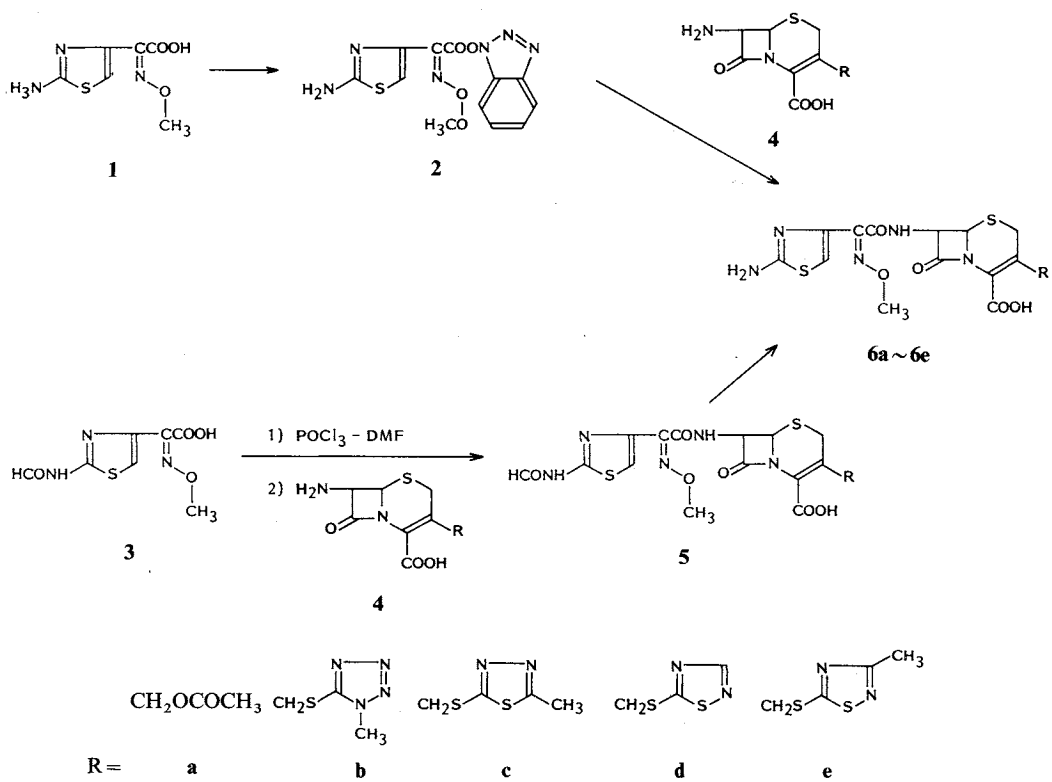
In our search for new orally active cephalosporins, we found that 7β-[2-(2-amino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetamido]-3-[(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl]-3-cephem-4-carboxylic acid (**6e**) possessed potent antibacterial activities and that the esters of **6e**, in particular the compound **9e**, were well absorbed by oral administration to rodents.

This paper describes the synthesis and the biological properties of esters of **6e** and related compounds compared with cefixime (CFIX)[1] and cefteram pivoxil (CFTM-PI)[2].

The compounds tested were prepared as shown in Schemes 1 and 2. Pivaloyloxymethyl esters, **9a** and **9b**, were prepared as reported in the ref[3]. The other pivaloyloxymethyl esters, **9c**, **9d** and **9e**, were prepared *via* another procedure, which is shown in Scheme 2. Other esters **11p**~**11w** were prepared in the manner similar to that of **9e** from the sodium salt of **5e** and the corresponding halides. The latter halides were prepared from chloromethyl chlorosulfate (or chloromethyl iodide) and the corresponding carboxylic acids.

The *in vitro* antibacterial activity of the compounds **6c**~**6e** and the reference compounds was determined by the serial 2-fold agar dilution method and the results are given as MIC (μg/ml) in Table 1. The activity of compounds **6c**, **6d** and **6e** was as potent as that of parenteral cephalosporins (cefotaxime: CTX and cefmenoxime: CMX). The activity of these compounds against *Staphylococcus aureus* was even superior to that of CTX and CMX.

Scheme 1.



Scheme 2.

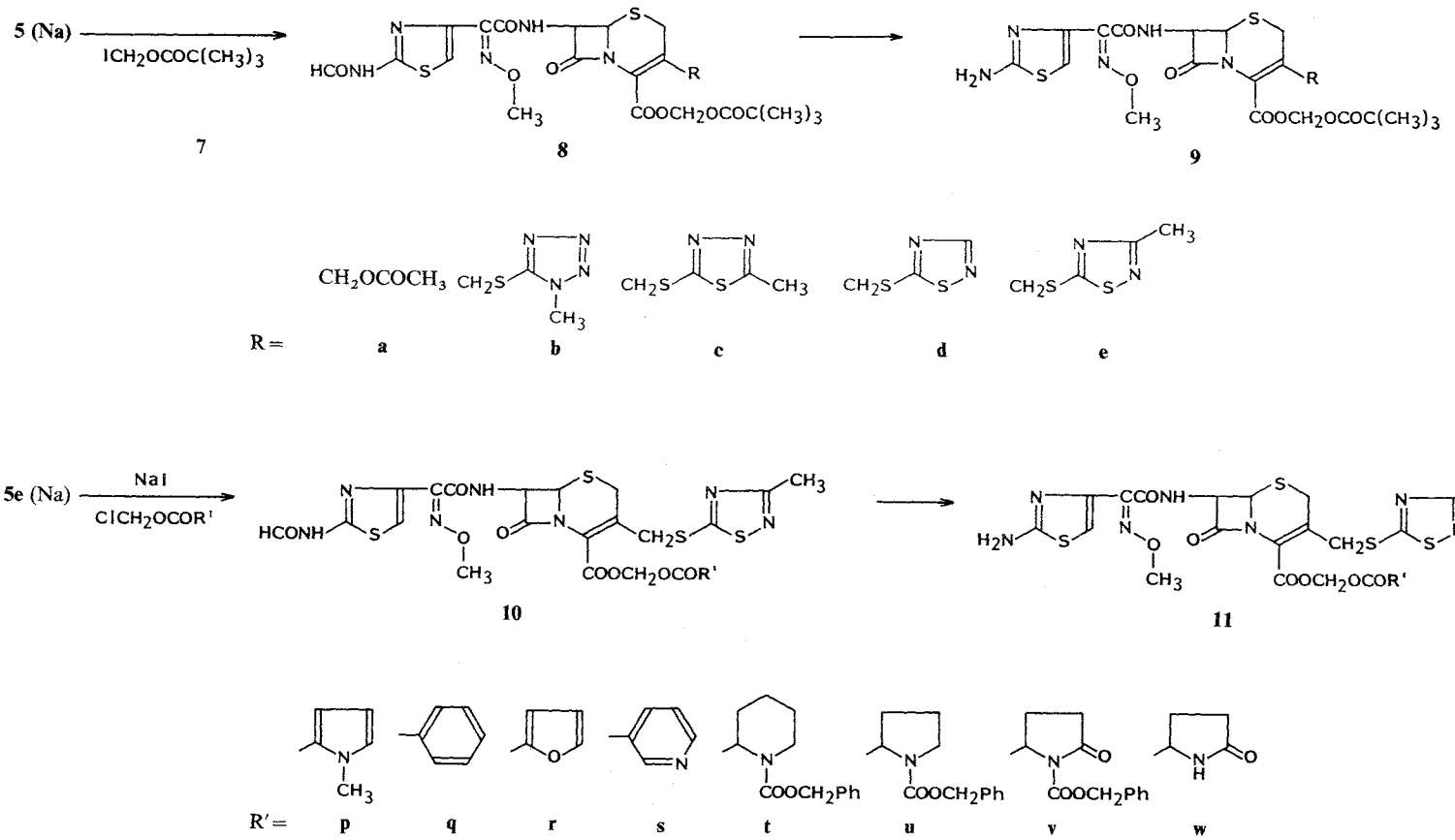
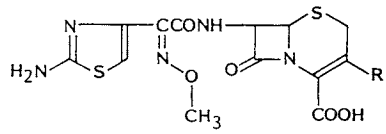
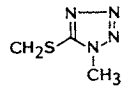
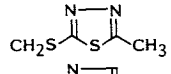
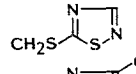
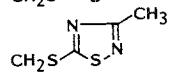
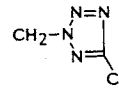

5 (Na)
ICH2OCOC(CH3)3
7
8
9
R=
a
b
c
d
e
5e (Na)
NaI
ClCH2OCOR'
10
11
R'=
p
q
r
s
t
u
v
w

Table 1. *In vitro* antibacterial activity of the cephalosporins (MIC: μg/ml).



Compound	R	*S.a.*	*S.e.*	*E.c.*	*K.p.*	*P.m.*	*P.v.*	*P.a.*	*S.m.*
CTX (**6a**)	CH_2OCOCH_3	0.78	0.78	<0.013	<0.013	<0.013	<0.013	6.25	0.10
CMX (**6b**)		1.56	0.78	0.10	<0.013	0.05	<0.013	12.5	0.20
6c		0.05	0.20	<0.013	<0.013	<0.013	<0.013	12.5	0.05
6d		0.20	0.39	0.05	<0.013	0.05	<0.013	12.5	0.20
6e		0.10	0.39	0.05	<0.013	0.10	<0.013	12.5	0.39
CFTM		1.56	1.56	0.20	<0.013	0.20	<0.013	100	1.56
CFIX		25	100	0.39	<0.013	<0.013	<0.013	25	0.10
CXM		0.78	3.13	3.13	0.05	1.56	0.39	>200	12.5
CTM		0.39	12.5	0.10	0.05	0.39	0.39	>200	0.78
CCL		1.56	6.25	3.13	0.39	1.56	6.25	>200	25

MICs were determined by the serial 2-fold agar dilution method.

Test organisms and abbreviations: *S.a.*, *Staphylococcus aureus* FDA 209P; *S.e.*, *Staphylococcus epidermidis* IAM 1296; *E.c.*, *Escherichia coli* NIHJ JC-2; *K.p.*, *Klebsiella pneumoniae* ATCC 10031; *P.m.*, *Proteus mirabilis* GN 2425; *P.v.*, *Proteus vulgaris* OX-19; *P.a.*, *Pseudomonas aeruginosa* IFO 3451; *S.m.*, *Serratia marcescens* X-100.

While the oral cephalosporins CFIX and cefteram (CFTM) were highly active against Gram-negative bacteria, they showed only weak activities against Gram-positive bacteria. Cefuroxime (CXM), cefotiam (CTM) and cefaclor (CCL) showed less potent activity than **6c**, **6d** and **6e** against both Gram-positive and Gram-negative bacteria.

The pivaloyloxymethyl esters of these compounds (**6a**~**6e**) were prepared to estimate the oral absorption in rodents. The pivaloyloxymethyl esters are generally accepted as useful prodrug esters in oral administration of β-lactam antibiotics[3,4]. The results are shown in Table 2.

It is interesting to note that **9e**, the methyl substituted analogue of **9d**, exhibited higher oral absorption than **9d** in both rats and mice. The oral absorption bioavailability of **9e** was higher than those of CFTM-PI and cefuroxime axetil (CXM-AX) in both rats and mice.

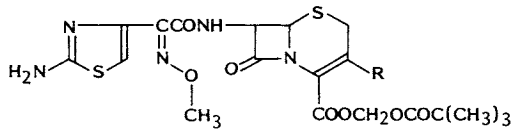
As the pivaloyloxymethyl ester of **6e** gave good urinary recovery and oral absorption, several kinds of esters other than pivaloyloxymethyl ester of **6e** were prepared and evaluated. The results are shown in Table 3. No better ester than pivaloyloxymethyl were found. The 1-methylpyrrolylcarbonyloxymethyl ester (**11p**) was moderately well absorbed orally.

A comparison test of oral absorption between **9e** and CFTM-PI was conducted. In Figs. 1 and 2, the serum levels of **6e** and CFTM after oral administration of **9e** and CFTM-PI in mice and rats are shown. The serum level of **6e** was equal to that of CFTM in mice and about two times as high as that of CFTM in rats. Moreover, $T_{1/2}$ of **6e** in serum was about two times as long as that of CFTM in both mice and rats. In consideration of these pharmacokinetic data together with the antibacterial activities of **6e**, compound **9e** was evaluated as a good oral cephalosporin.

Experimental

IR spectra were recorded on a Hitachi model EPI-G3 spectrophotometer. NMR spectra were recorded on Nihon Denshi a FX-270 (270 MHz) spectrometer using TMS as an internal standard.

Table 2. Urinary recovery and relative bioavailability of the cephalosporins after oral administration.



9a~**9e**

Compound	Dosing route	Urinary recovery (%)		Relative bioavailability[a] (%)	
		Rats[b]	Mice[c]	Rats	Mice
9a	po[d]	4.1±0.5	NT	10.3	NT
6a	iv	40.0±2.0	NT		
9b	po	9.0±0.8	NT	13.2	NT
6b	iv	68.2±12.1	NT		
9c	po	6.0±0.5	12.9	16.6	33.9
6c	iv	36.2±5.4	38.0		
9d	po	5.3±0.3	25.9	10.1	44.7
6d	iv	50.1±0.8	58.0		
9e	po	18.8±5.9	26.6	38.6	65.7
6e	iv	48.7±5.4	40.5		
CFTM-PI	po	14.3±5.7	21.0	28.7	32.1
CFTM	iv	49.8±9.0	65.5		
CXM-AX	po	17.5±0.4	38.8	30.2	46.7
CXM	iv	58.0±4.3	83.0		

[a] Relative bioavailability (%) $= \frac{\text{Urinary recovery of } \mathbf{9} \text{ (po)}}{\text{Urinary recovery of } \mathbf{6} \text{ (iv)}} \times 100.$

[b] Mean±SD ($n=3$), male JCL-SD rats.

[c] A group of eight male SLC-ICR mice was housed in each cage.

[d] As suspension in a 0.5%-methyl cellulose solution.

NT: Not tested.

Table 3. Cmax and AUC of the cephalosporins (**11p**~**11w**) after oral administration in mice (20 mg/kg).

Compound	Cmax (μg/ml)	Tmax (minutes)	AUC (0~2 hours) (μg·minute/ml)	Relative bioavailability[a] (%)
9e	44.1	15	4,350	84.6
11p	26.1	30	2,874	56.0
11q	22.3	60	2,390	46.5
11r	12.9	30	1,400	27.3
11s	8.6	60	784	15.0
11t	16.6	60	1,710	33.0
11u	7.8	60	821	16.0
11v	1.6	60	144	3.0
11w	2.7	120	209	4.1
6e (iv)			5,140	

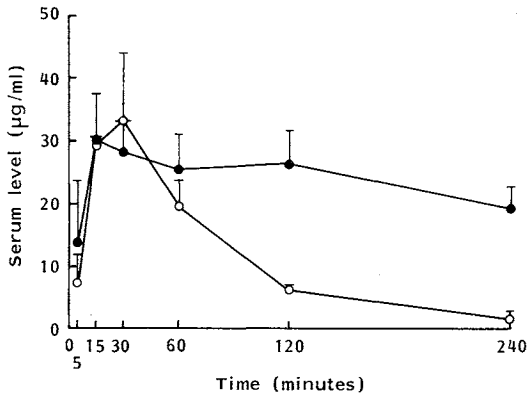
[a] Relative bioavailability (%) = 100 × (AUC **11p**~**11w**, po)/(AUC **6e**, iv).

7β-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (**6e**)

To a suspension of **2**[5] (3.05 g) and **4e** (3.00 g) in DMF (19 ml) was added triethylamine (2.64 g). The mixture was stirred at room temperature for 2 hours. To the reaction mixture was added water (135 ml) and the insoluble material was filtered off with the aid of Celite. The filtrate was adjusted to pH 2.2 by the addition of 2 N HCl. The precipitate was collected by filtration, washed with cold water and dried *in vacuo* to give a crude product (2.36 g). The filtrate was further extracted by EtOAc several times. The combined extracts were washed with saturated

Fig. 1. Serum levels of **6e** and CFTM after oral dosing of **9e** and CFTM-PI.

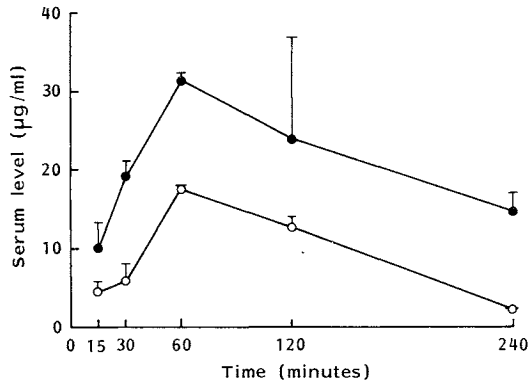
● **6e**, ○ CFTM.



Mice: 20 mg/kg. **6e**: $T_{1/2}$, 390 minutes: AUC (0~4 hours), 5,780 μg·minute/ml. CFTM: $T_{1/2}$, 43 minutes; AUC (0~4 hours), 2,700 μg·minute/ml.

Fig. 2. Serum levels of **6e** and CFTM after oral dosing of **9e** and CFTM-PI.

● **6e**, ○ CFTM.



Rats: 25 mg/kg. **6e**: $T_{1/2}$, 140 minutes; AUC (0~4 hours), 5,010 μg·minute/ml. CFTM: $T_{1/2}$, 48 minutes; AUC (0~4 hours), 2,270 μg·minute/ml.

aqueous NaCl and dried over $MgSO_4$. Concentration of dried extracts gave also the crude product (2.69 g); a total yield of crude product was 5.05 g. The crude products were combined and purified by the preparative liquid chromatography on a reverse phase column, LiChroprep RP-8, with a mobile phase consisting of 0.01 M phosphate buffer (pH 6.8), MeCN, and THF (80:10:10). The fractions containing the product were concentrated *in vacuo* to a small volume and adjusted to pH 2 by the addition of 2 N HCl at 0~5°C. The precipitate was collected, washed on a filter with cold water and dried *in vacuo* over phosphorus pentoxide.

^{1}H NMR (270 MHz, DMSO-d_6) δ 2.52 (3H, s, thiadiazole-CH_3), 3.55 and 3.76 (2H, ABq, J=18 Hz, 2-H_2), 3.83 (3H, s, =$NOCH_3$), 4.23 and 4.63 (2H, ABq, J=13 Hz, 3-CH_2), 5.14 (1H, d, J=4.6 Hz, 6-H), 5.77 (1H, dd, J=4.6 and 8 Hz), 6.73 (1H, s, thiazole-5H), 7.24 (2H, br, NH_2), 9.60 (1H, d, J=8 Hz, CONH).

7β-[2-(2-Formylamino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (**5e**)

To an ice-cold solution of DMF (1.67 g) in EtOAc (27 ml) was added $POCl_3$ (2.17 g) and the mixture was stirred at −5~0°C for 2 hours. To the resulting mixture was added 2-(2-formylamino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetic acid[6] (3.27 g) and the mixture was stirred at −5~0°C for 2 hours. The reaction mixture was added to an ice-cold solution of a silyl derivative of 7-amino-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (**4e**), prepared by adding *N,O*-bis(trimethylsilyl)acetamide (7.92 g) to a suspension of **4e** (4.34 g) in EtOAc (86 ml), and the mixture was stirred at −5~0°C for 1.5 hours. To the reaction mixture was added a solution of water (26 ml) and MeOH (5.5 ml) and the mixture was stirred for 30 minutes. The resulting crystals were collected by filtration, washed with EtOAc and dried *in vacuo* to give the product (3.49 g).

IR (KBr) cm^{-1} 1780, 1680; ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.52 (3H, s, CH_3), 3.54 and 3.76 (2H, ABq, J=18 Hz, 2-H_2), 3.89 (3H, s, =$NOCH_3$), 4.23 and 4.62 (2H, ABq, J=13.5 Hz, 3-CH_2), 5.15 (1H, d, J=5 Hz, 6-H), 5.81 (1H, dd, J=5 and 8 Hz, 7-H), 7.40 (1H, s, thiazole-5H), 8.50 (1H, s, HCO), 9.69 (1H, d, J=8 Hz, CONH), 12.62 (1H, s, HCONH).

Sodium Salt of **5e**

Compound **5e** (590 mg) was dissolved in a solution of $NaHCO_3$ (94 mg) and H_2O (17 ml), and the solution was lyophilized to give the sodium salt of **5e**.

Pivaloyloxymethyl 7β-[2-(2-Formylamino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylate (**8e**)

To an ice-cold solution of **5e** (Na salt, 0.61 g) in DMF (3 ml) was added iodomethyl pivalate (0.41 g) and the mixture was stirred at 5°C for 1 hour. The mixture was poured into a mixture of EtOAc (30 ml) and water (10 ml) with stirring, and the organic layer

was separated and washed with water (10 m × 2) and saturated brine (10 ml), then dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was triturated with Et_2O and *n*-hexane. The solid was collected by filtration and dried *in vacuo* to give **8e** (0.4 g).

IR (KBr) cm^{-1} 1790, 1755, 1690; ^1H NMR (270 MHz, DMSO-d_6) δ 1.14 (9H, s, $C(CH_3)_3$), 2.52 (3H, s, CH_3), 3.61 and 3.81 (2H, ABq, $J=18$ Hz, 2-H_2), 3.88 (3H, s, $=NOCH_3$), 4.23 and 4.59 (2H, ABq, $J=13.5$ Hz, 3-CH_2), 5.20 (1H, d, $J=5$ Hz, 6-H), 5.85~5.98 (3H, m, 7-H, $COOCH_2$), 7.42 (1H, s, thiazole-5H), 8.51 (1H, s, HCO), 9.70 (1H, d, $J=8$ Hz, CONH), 12.62 (1H, s, CONH).

Pivaloyloxymethyl 7β-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylate (**9e**)

To an ice-cold solution of **8e** (1.14 g) in a mixture of MeOH (5 ml) and THF (5 ml) was added conc HCl (1.5 ml) and the mixture was stirred at 0~5°C for 8 hours. To the reaction mixture was added water (30 ml) and the mixture was adjusted to pH 2.5 by the addition of saturated aqueous $NaHCO_3$.

The organic solvent was removed *in vacuo* and the resulting crystals were collected by filtration, washed with water and dried *in vacuo* to give **9e** (1.01 g).

^1H NMR (270 MHz, $CDCl_3$) δ 1.22 (9H, s, $(CH_3)_3$), 2.60 (3H, s, CH_3), 3.62 and 3.75 (2H, ABq, $J=18$ Hz, 2-H_2), 4.10 (3H, s, $=NOCH_3$), 4.20 and 4.68 (2H, ABq, $J=13$ Hz, 3-CH_2), 5.07 (1H, d, $J=5$ Hz, 6-H), 5.88~5.99 (3H, m, 7-H, $COOCH_2$), 6.6~7.1 (2H, br, NH_2), 7.00 (1H, s, thiazole-5H), 7.80 (1H, d, $J=9$ Hz, CONH).

Chloromethyl L-1-Benzyloxycarbonyl-2-pyrrolidinecarboxylate (**7u**)

To the mixture of L-1-benzyloxycarbonyl-2-pyrrolidinecarboxylic acid (6.0 g), $NaHCO_3$ (5.04 g) and tetrabutylammonium hydrogensulfate (0.90 g) in a mixture of CH_2Cl_2 (60 ml) and H_2O (60 ml) was added a solution of chloromethyl chlorosulfate[7] (4.75 g) in CH_2Cl_2 (30 ml) with stirring. The mixture was stirred at room temperature for 42 hours. Organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (100 ml). Extracts were combined and dried over $MgSO_4$. The solvent was removed *in vacuo* to give the product (8.35 g).

^1H NMR (270 MHz, $CDCl_3$) δ 1.88~2.32 (4H, m, pyrrolidine-3,4-H_4), 3.44~3.69 (2H, m, pyrrolidine-5H_2), 4.35~4.45 (1H, m, pyrrolidine-2H), 5.04~5.20 (2H, m, benzyl-CH_2), 5.55~5.86 (2H, m, $ClCH_2OCO$), 7.28~7.37 (5H, m, phenyl).

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