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AS-924, a Novel Bifunctional Prodrug of Ceftizoxime

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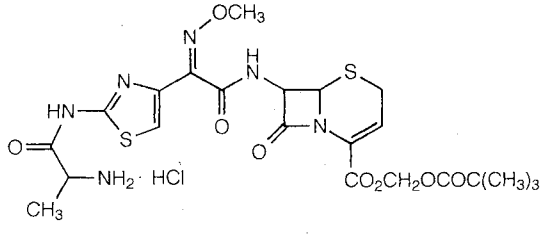
To improve the oral absorption of ceftizoxime (CZX), 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4- carboxylic acid, we synthesized and evaluated a novel series of bifunctional prodrugs, in which L-alanine was introduced into the aminothiazole-oxime moiety at the C-7 position of the various lipophilic esters of CZX. Among these prodrugs, pivaloyloxymethyl 7β-[(*Z*)-2-(2-(*S*)-alanylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate hydrochloride (ceftizoxime alapivoxil, AS-924) was well absorbed after oral administration in experimental animals and showed potent therapeutic effects in mice infected with Gram-positive and Gram-negative bacteria.

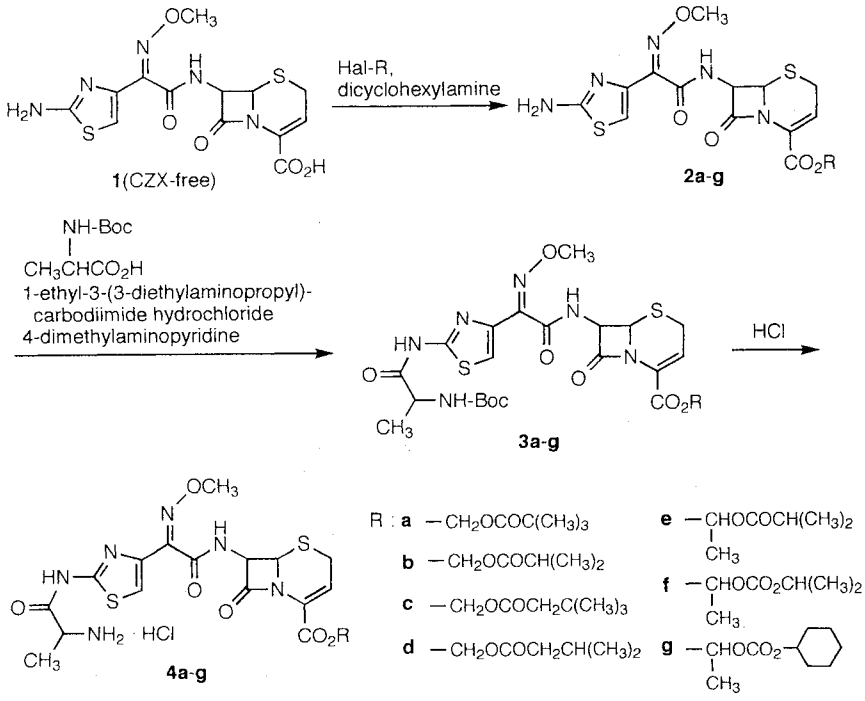
Ceftizoxime (CZX), 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylic acid is a so-called third generation cephalosporin bearing an aminothiazole-oxime moiety at the C-7 position of the cephem nucleus. CZX shows a broad spectrum of activity against Gram-positive and Gram-negative bacteria and is resistant to bacterial β-lactamase[1]. However, it has been clinically administered only by injection because of its low gastrointestinal absorption due to its low lipophilicity. To improve the oral absorption of β-lactam antibiotics which are used parenterally, there have been many attempts to increase their lipophilicity by esterification of the carboxyl group at the C-4 position. Indeed, as previously reported for ampicillin esters[2~5], pivmecillinam[6] and cefotiam hexetil[7], β-lactam antibiotics containing a basic amino group could be esterified to increase lipophilicity without loss of water-solubility, resulting in high bioavailability. In contrast, esterification of β-lactam antibiotics with a neutral or weakly basic side chain such as CZX results in an increase of lipophilicity accompanied by reduction of water-solubility, which is also an important factor for gastrointestinal absorption[8]. To resolve this problem, we previously synthesized a so-called bifunctional prodrug of cephalosporin, in which both hydrophilic and lipophilic moieties were introduced to increase oral absorption[9~11].

In the present study, we synthesized and evaluated a novel series of bifunctional prodrugs by introduction of an L-alanyl moiety into various CZX esters. Among these prodrugs, pivaloyloxymethyl 7β-[(*Z*)-2-(2-(*S*)-alanylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylate hydrochloride (ceftizoxime alapivoxil, AS-924, Fig. 1) was shown to be a promising oral anti-infectious drug.

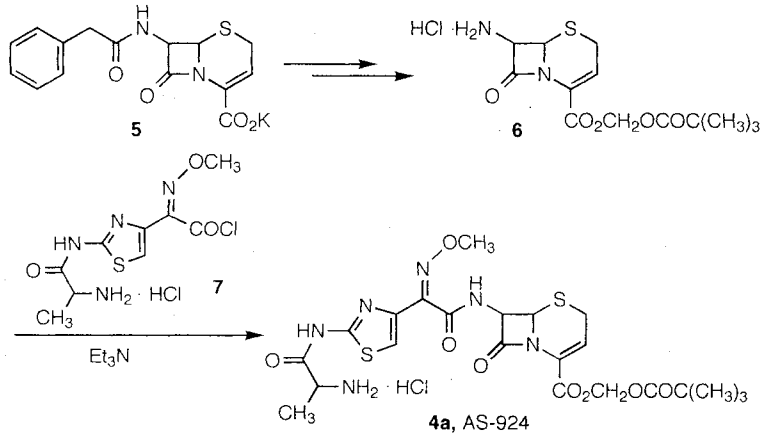
Fig. 1. Chemical structure of AS-924.



Scheme 1. Synthesis of bifunctional prodrugs of CZX.



Scheme 2. Synthesis of AS-924.



Chemistry

The general synthetic route of the bifunctional prodrug compounds **4a**~**4g** is shown in Scheme 1. The free acid compound of CZX (**1**) was treated with iodomethyl pivalate in *N*,*N*-dimethylacetamide (DMAc) in the presence of dicyclohexylamine to afford the ester compound **2a**[12]. The other ester prodrugs **2b**~**2g** were prepared by esterification of CZX with the corresponding halides under the same reaction conditions. The ester compound **2a** was treated with *N*-*tert*-butoxy-carbonyl-(*N*-Boc)-L-alanine in dichloromethane in the presence of 4-dimethylaminopyridine, using 1-ethyl-3-(3-diethyl-aminopropyl)carbodiimide hydrochloride (EDC·HCl) as a condensing agent, to afford compound **3a**. Finally, the *N*-Boc group of compound **3a** was removed by

Table 1. Antibacterial activities of CZX and other oral cephalosporins.

Organism	MIC(μg/ml)					
	CZX	CPDX	CFTM	CFIX	CFDN	CCL
S.aureus 209P JC-1	1.56	0.78	1.56	12.5	0.10	0.78
S.aureus Terajima	1.56	0.78	3.13	1.56	0.39	6.25
S.pyogenes C-203	0.012	0.012	≦0.006	0.10	≦0.006	0.20
S.pneumoniae type III	0.10	0.025	0.05	0.39	0.10	1.56
E.coli NIHJJC-2	0.05	0.78	0.20	0.78	0.39	1.56
E.coli K-12	0.10	0.78	0.39	0.78	0.39	1.56
C.freundii NIH10018-68	0.20	1.56	0.39	1.56	0.39	6.25
S.typhi 0-901	0.025	0.10	0.10	0.05	0.10	0.78
S.paratyphi A	0.025	0.20	0.10	0.05	0.10	1.56
S.enteritidis	0.05	0.39	0.20	0.10	0.20	0.78
S.flexneri 2aEW-10	0.05	0.39	0.10	0.39	0.20	1.56
K.pneumoniae KC-1	0.012	0.20	0.20	0.05	0.10	0.78
K.pneumoniae NCTC 9632	0.025	0.39	0.20	0.05	0.20	0.78
E.cloacae NCTC 9394	0.20	1.56	0.78	3.13	12.5	>100
E.aerogenes NCTC 10006	0.20	1.56	0.78	3.13	6.25	>100
P.vulgaris OX-19	0.012	0.10	0.012	0.025	0.20	3.13
P.mirabilis 1287	0.012	0.05	0.025	0.025	0.10	3.13
M.morganii Kono	0.20	0.78	0.20	0.39	6.25	>100
S.marcescens IFO3736	0.10	1.56	0.78	0.39	6.25	>100
H.influenzae N-17	0.012	0.05	0.025	0.025	0.39	3.13
P.aeruginosa Nc-5	3.13	>100	25	50	>100	>100
P.aeruginosa No.12	50	>100	>100	100	>100	>100

Agar dilution method, 10^6 CFU/ml

treatment with hydrogen chloride in formic acid to afford compound **4a** (AS-924). The other bifunctional prodrugs **4b**～**4g** were prepared by the same procedure from esters **2b**～**2g**.

Another synthetic route of AS-924 is shown in Scheme 2. Compound **6** was prepared from compound **5** by esterification followed by deacylation of the 7-subsutituent *via* imino ether[12]. Compound **6** was acylated with compound **7** in DMAc in the presence of triethylamine to afford AS-924.

Results

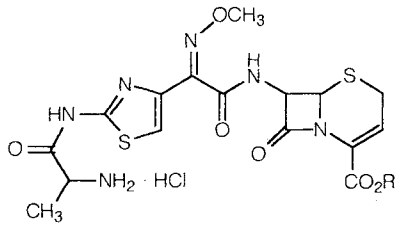
Antibacterial Spectrum of CZX

The *in vitro* antibacterial activity of CZX was examined in comparison with five orally active cephalosporins; cefpodoxime (CPDX), cefteram (CFTM), cefixime (CFIX), cefdinir (CFDN) and cefaclor (CCL). CZX showed potent and broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (Table 1). CZX was the most potent against Gram-negative bacteria among the drugs tested. CZX was about 4- to 16-fold more active than CPDX and CFTM against *Escherichia coli* and *Klebsiella pneumoniae*. CZX showed superior activity against Gram-positive bacteria as compared to CCL and CFIX. Its activity was comparable to that of CFTM, but CZX was less active than CPDX and CFDN.

Urinary Recovery and Taste of Bifunctional Prodrugs of CZX

We evaluated the oral absorption of bifunctional prodrugs of CZX, in which L-alanine was introduced into the amino group on the thiazole ring of CZX esters. Taste of prodrugs was also examined. The prodrugs of CZX were administered orally to rats at a dose of 20 mg/kg equivalent to CZX. The values of urinary recovery (UR) as an indication of intestinal absorption together with the results of taste tests are shown in Table 2. The urinary recoveries after oral administration of the prodrugs were higher than that of CZX (4.0～8.9% *vs.* 2.8%). The prodrug with pivaloyloxymethyl (POM) ester (AS-924) and 1-isobutyryloxyethyl ester (**4e**) showed good intestinal absorption. The taste of AS-924 was

Table 2. Urinary recovery and taste of CZX prodrugs.



Compounds	R	UR[1] (%)	Sweetness[2] (n=3[4])	Bitterness[3] (n=3[4])
CZX		2.8±0.4	0	−
4a(AS-924)	$-CH_2OCOC(CH_3)_3$	8.0±1.5	333	±
4b	$-CH_2OCOCH(CH_3)_2$	4.0±0.4	120	+
4c	$-CH_2OCOCH_2C(CH_3)_3$	6.5±0.4	8	+
4d	$-CH_2OCOCH_2CH(CH_3)_2$	5.7±2.0	13	+ +
4e	$-CHOCOCH(CH_3)_2$ CH_3	8.9±0.8	67	+
4f	$-CHOCO_2CH(CH_3)_2$ CH_3	4.9±0.8	27	+
4g	$-CHOCO_2$(cyclohexyl) CH_3	7.1±1.1	0	+ +

1) UR : urinary recovery in rats (20mg/kg equivalent to CZX po, 0-24hours, n=3, mean±SD)
2) Sweetness of sucrose was taken as 1
3) Bitterness : − (none), + (slight), + + (moderate)
4) Three persons

about 300 times sweeter than that of sucrose and was hardly bitter.

Oral Absorption of AS-924

AS-924 and cefteram pivoxil (CFTM-PI) were administered orally to male mice, rats, rabbits and dogs at a dose of 20 mg/kg equivalent to CZX and CFTM, respectively. The pharmacokinetic parameters of AS-924 and CFTM-PI are summarized in Table 3. After oral administration of AS-924, the values of AUC, Cmax and UR were higher and Tmax and $T_{1/2}$ longer in rabbits and dogs than those in mice and rats. The values of AUC, Cmax and UR for CFTM-PI were higher in rodents than those in rabbits and dogs. The values of AUC, Cmax and UR of AS-924 were higher than those of CFTM-PI in dogs.

Therapeutic Efficacy in Mice

The therapeutic efficacy of AS-924 against systemic infection with Gram-positive and Gram-negative bacteria was examined in comparison with those of CFTM-PI, CFIX and CFDN. The ED_{50} were shown in Table 4. The *in vivo* efficacy of AS-924 were superior to that of CFDN against all the strains used, that of CFIX against *Streptococcus pneumoniae* type III, *E. coli* 444 and *E. coli* KC-14. Against *K. pneumoniae* KC-1 and *Proteus mirabilis* XR-001, the efficacy of AS-924 was similar to that of CFIX. Against *S. pneumoniae* type III and *E. coli* 444, AS-924 was less effective than CFTM-PI.

Discussion

The present study demonstrated that CZX shows more potent antibacterial activity against Gram-negative bacteria than known orally active cephalosporins. CZX also showed potent antibacterial activity against Gram-positive bacteria, which was comparable to those of other oral cephalosporins. Thus, an orally active prodrug of CZX would be excellent as an anti-infectious agent. However, to our knowledge there have been no reports of synthesis of an orally active prodrug of CZX

Table 3. Pharmacokinetic parameters and urinary recovery after oral administration of AS-924.

Drug (Dose)		Dog (n=3)	Rabbit (n=3)	Rat (n=5)	Mouse (n=5, * n=10)
AS-924 (20 mg/kg[1])	AUC (μg · hours/ml)	14.51	10.38	1.43	2.77
	Cmax (μg/ml)	5.81	6.07	1.47	4.00
	Tmax (hours)	1.00	0.71	0.45	0.25
	T1/2 (hours)	0.78	0.57	0.25	0.21
	UR[3] (%)	19.1	33.8	7.8	6.6*
CFTM-PI (20 mg/kg[2])	AUC (μg · hours/ml)	4.98	26.77	38.81	30.73
	Cmax (μg/ml)	2.03	11.21	14.46	13.27
	Tmax (hours)	0.83	1.01	0.83	0.62
	T1/2 (hours)	1.03	0.70	1.23	1.08
	UR[3] (%)	10.3	23.4	23.5	27.1*

1) equivalent to CZX
2) equivalent to CFTM
3) UR : urinary recovery (0-24hours)

Table 4. Therapeutic efficacy on systemic infection in mice.

Organism	Challenge dose (CFU/mouse)	Mucin	Drug	ED_{50} (mg/kg)	MIC[1] (μg/ml)
S.pneumoniae type III	1.3×10^{1}	-	AS-924	6.25	0.05[2]
			CFTM-PI	0.56	0.025[3]
			CFIX	11.0	0.20
			CFDN	8.07	0.012
E.coli 444	1.8×10^{3}	+	AS-924	0.73	0.025[2]
			CFTM-PI	0.29	0.025[3]
			CFIX	1.21	0.39
			CFDN	2.26	0.10
E.coli KC-14	1.2×10^{6}	+	AS-924	0.52	0.025[2]
			CFTM-PI	1.18	0.20[3]
			CFIX	1.54	0.20
			CFDN	1.54	0.10
K.pneumoniae KC-1	1.3×10^{2}	+	AS-924	0.52	0.012[2]
			CFTM-PI	2.33	0.20[3]
			CFIX	0.50	0.025
			CFDN	4.58	0.10
P.mirabilis XR-001	1.3×10^{6}	+	AS-924	0.19	0.012[2]
			CFTM-PI	0.25	0.05[3]
			CFIX	0.20	0.012
			CFDN	2.14	0.10
S.marcescens T-55	1.3×10^{6}	+	AS-924	2.31	0.05[2]
			CFTM-PI	7.56	0.78[3]
			CFIX	1.39	0.20
			CFDN	19.2	0.78

1) Inoculum size : 10^{6} CFU/ml, 2) MIC of CZX, 3) MIC of CFTM

for clinical use.

In general, β-lactam antibiotics are not absorbed from the gastrointestinal tract by passive transport because of low p*K*a value of the carboxyl group, due to which the antibiotics exist in an ionic form unfavorable for passive absorption. Therefore, it is possible to improve orally absorption of some β-lactam antibiotics containing a basic amino group by esterification of the carboxylic

acid[2~7]. However, in the case of cephalosporins bearing a neutral or weakly basic group at the C-7 position, esterification results in low water-solubility insufficient for dissolution into the gastrointestinal fluid. Since CZX has a weakly basic amino-thiazole moiety on its side chain at the C-7 position (p*Ka* 2.95), its esters may be hardly soluble in water. To resolve this problem, we previously synthesized a so-called bifunctional prodrug, in which a hydrophilic moiety in addition to a lipophilic moiety are introduced to improve water-solubility. In the case of cefcanel daloxate, the hydroxyl group on the side chain at C-7 position was esterified with L-alanine, resulting in marked improvement of oral absorption due to an increase in water-solubility[8,9].

In the present study, we introduced an L-alanyl group as a basic moiety into the amino group on the thiazole ring of CZX to improve water-solubility. Indeed, the water-solubility of AS-924 was 19.9 mg/ml in 1/15 M phosphate buffer of pH 4.5, which was about 50 times higher than that of POM ester of CZX (unpublished data). Among the compounds synthesized, L-alanyl derivatives of POM and 1-isobutyryloxyethyl esters of CZX (AS-924 and **4e**) showed the highest UR after oral administration in rats. Differences in UR among the esters may be related to lipophilicity and/or hydrolysis of the ester residues used. Unlike the 1-isobutyryloxyethyl moiety, POM has been used in clinically available prodrugs of β-lactam antibiotics such as pivampicillin, pivmecillinam, cefetamet pivoxil, CFTM-PI and cefditoren pivoxil. Furthermore, AS-924 was found to taste sweet but hardly bitter. This would be a beneficial property for improving compliance in children. Therefore, AS-924 was chosen for further evaluation.

AS-924 showed high plasma levels of CZX after oral administration in dogs and rabbits. The pharmacokinetic parameters were better than those of CFTM-PI in dogs but not in rodents. In the systemic infection model of mice with six strains of bacteria, AS-924 showed more potent therapeutic efficacy than CFIX and CFDN.

In conclusion, AS-924, a bifunctional prodrug of CZX was successfully synthesized by introduction of L-alanine into the side chain at the C-7 position of POM ester of CZX (monofunctional prodrug). This prodrug showed a good pharmacokinetic profile and potent anti-infectious activity.

Experimental

Chemistry

IR spectra were determined on a JASCO IR-810 spectrometer. ^{1}H-NMR spectra were recorded at 60 MHz on a Hitachi R-600 spectrometer using TMS as an internal standard. For column chromatography, silica gel (Daisogel No. 1001W, Daiso) was used.

Antibiotics

CPDX, CFTM, AS-924 and its related compounds were prepared in our Research Laboratories. CZX, CFIX, CFDN, CCL and CFTM-PI were obtained from commercial sources.

Antibacterial Activity

Antibacterial activity was determined by the standard 2-fold agar dilution method using Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA). Defibrinated horse blood (10%) was supplemented to agar for Streptococci and 5% Fildes enrichment (Difco) for *Haemophilus influenzae*. All the strains except Streptococci and *H. influenzae* were grown overnight in Mueller-Hinton broth (Difco). Streptococci and *H. influenzae* were grown overnight in Mueller-Hinton broth with 10% horse serum and with 5% Fildes enrichment, respectively. Overnight cultures were diluted to 10^6 CFU/ml with saline, and aliquots of 5 μl of the cell suspension were inoculated with a Microplanter (Sakuma Seisakusho, Tokyo, Japan) onto agar plates containing serial 2-fold dilutions of each antibiotic. The MIC was determined as the lowest concentration that inhibited visible growth after 18 hours of incubation at 37°C.

Oral Absorption Studies

Male *ddY* mice (weighing 21 to 25 g), male SD rats (182 to 243 g), male Wistar rats (190 to 230 g), male rabbits (2.0 to 2.5 kg) and male beagle dogs (10.8 to 13.4 kg) were used. The animals were fasted overnight before dosing but were given free access to water. AS-924 and CFTM-PI were dissolved and suspended in 0.5% methyl cellulose solution, for oral dosing at 20 mg/kg, respectively. Blood was collected from the jugular vein of mice, rats and dogs, and the auricular vein of rabbits at specified times after dosing, and serum was obtained after centrifugation at 3000 rpm for 10 minutes. Urine samples were collected from 0 to 6 and 6 to 24 hours (mice and rats) or 0 to 3, 3 to 6 and 6 to 24 hours (rabbits and dogs) using a metabolic cage or a ureteral catheter. The concentrations of CZX and CFTM in the serum or

urine were determined by the paper disc-agar diffusion method using *E. coli* ATCC 39188 or *Bacillus subtilis* ATCC 6633 as the test organisms. The concentration-time data after oral dosing were fitted to a two-compartment or one-compartment open model, respectively, by non-linear least-squares regression analysis (MULTI) to obtain the pharmacokinetic parameters.

Therapeutic Efficacy in Mice

The *in vivo* efficacy of the antibiotics was assessed in mice experimentally infected with six strains of Gram-positive or Gram-negative bacteria. All bacteria used except *S. pneumoniae* type III were cultured for 18 hours at 37°C in nutrient broth (Nissui, Tokyo, Japan) and suspended in 8% gastric mucin (nacalai tesque, Kyoto, Japan). *S. pneumoniae* type III was cultured in heart infusion broth (Nissui) supplemented with 10% horse serum for 18 hours at 37°C. Male *dd*Y mice weighing 20 to 24 g were infected intraperitoneally with 0.25 ml of bacterial suspension. Antibiotics were administered orally at 2 hours after infection to groups of 8 mice for each dose, and survival ratios were determined 7 days after infection. The 50% effective dose (ED_{50}) were estimated using the Litchfield-Wilcoxon method.

Evaluation of Taste

Relative sweetness of prodrugs was evaluated by matching a solution of known concentration of each prodrug with a sucrose solution of equivalent sweetness[13]. Bitterness of the prodrug solution was also estimated as follows; −: none, +: slight, + +: moderate.

Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyimino-acetamido]-3-cephem-4-carboxylate (**2a**)

To a suspension of the free acid of CZX (**1**) (9.14 g) in *N*,*N*-dimethylacetamide (45 ml) were added dicyclohexylamine (5.22 ml) and iodomethyl pivalate (7.5 g) at −5°C. After stirring for 1 hour at the same temperature,

Table 5. Yield, ^{1}H-NMR and IR spectral data (**2b**～**2g**).

Compound No	Yield (%)	^{1}H-NMR(60MHz, DMSO-d_6, δ)	IR(Nujol) cm^{-1}
2b	66	1.14 (6H, d, *J*=7.0Hz), 2.20~2.80 (1H, m), 3.50~3.90 (2H, m), 3.88 (3H, s), 5.16 (1H, d, *J* = 5.0Hz), 5.67~6.05 (3H, m), 6.46~6.70 (1H, m), 6.75 (1H, s), 7.00~7.45 (2H, br), 9.62 (1H, d, *J*=9.0Hz).	3430, 3320, 1780, 1755, 1680, 1630
2c	74	0.90 (9H, s), 2.27 (2H, s), 3.50~3.80 (2H, m), 3.87 (3H, s), 5.15 (1H, d, *J* = 5.0Hz), 5.70~6.07 (3H, m), 6.49~6.82 (1H, m), 6.73 (1H, s), 7.00~7.40 (2H, br), 9.61 (1H, d, *J*=9.0Hz).	3430, 3230, 3200, 1780, 1750, 1680
2d	84	0.92 (6H, d, *J*=7.0Hz), 1.40~2.00 (3H, m), 3.50~3.90 (2H, m), 3.85 (3H, s), 5.14 (1H, d, *J* = 5.0Hz), 5.50~6.08 (3H, m), 6.40~6.75 (1H, m), 6.73 (1H, s), 7.16 (2H, br s), 9.60 (1H, d, *J*=9.0Hz).	3420, 3320, 1780, 1750, 1675, 1630
2e	21	1.10 (6H, d, *J*=7.0Hz), 1.48 (12H, d, *J*=7.0Hz), 2.30~2.90 (1H, m), 3.40~3.80 (2H, m), 3.86 (3H, s), 5.14 (1H, d, *J* = 5.0Hz), 5.89 (1H, dd, *J* = 5.0 and 9.0Hz), 6.45~7.05 (2H, m), 6.73 (1H, s), 7.05~7.55 (2H, br), 9.61 (1H, d, *J*=9.0H).	3320, 1785, 1750, 1680, 1635
2f	34	1.27 (6H, d, *J* = 7.0Hz), 1.54 (3H, d, *J* = 7.0Hz), 3.50~3.78 (2H, m), 3.87 (3H, s), 4.60~5.06 (1H, m), 5.15 (1H, d, *J* = 5.0Hz), 5.93 (1H, dd, *J* = 5.0 and 9.0Hz), 6.50~7.04 (2H, m), 6.76 (1H, s), 7.20 (2H, br), 9.63 (1H, d, *J*=9.0H).	3430, 3320, 1760, 1680
2g	26	0.85~2.20 (10H, m), 1.52 (3H, d, *J* = 7.0Hz), 3.45~3.77 (2H, m), 3.87 (3H, s), 4.32~4.87 (1H, m), 5.14 (1H, d, *J* = 5.0Hz), 5.90 (1H, dd, *J* = 5.0 and 9.0Hz), 6.59~7.00 (2H, m), 6.75 (1H, s), 7.03~7.50 (2H, br), 9.61 (1H, d, *J*=9.0Hz).	3430, 3330, 3210, 1780, 1760, 1680

EtOAc (100 ml) was added and the resulting precipitate was filtered off. The filtrate was washed with aq $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed on a silica gel column (Daisogel No.1001W, eluent; Benzene - EtOAc, 1 : 1→1 : 2) to give **2a** (8.66 g) in 73% yield.

IR (Nujol) cm^{-1} 1785, 1750, 1680; ^{1}H-NMR (DMSO-d_6) δ 1.16 (9H, s), 3.50~3.80 (2H, m), 3.84 (3H, s), 5.13 (1H, d, J=5.0 Hz), 5.70~6.10 (3H, m), 6.50~6.70 (1H, m), 6.73 (1H, s), 7.12 (2H, br s), 9.67 (1H, d, J=9.0 Hz).

The ester compounds **2b**~**2g** were prepared by a procedure similar to that described above. IR and NMR spectral data and chemical yields are listed in Table 5.

Pivaloyloxymethyl 7β-[(*Z*)-2-[2-*N*-(tert-Butoxycarbonyl)-(*S*)-alanyl-aminothiazol-4-yl)]-2-methoxyimino-acetamido]-3-cephem-4-carboxylate (**3a**)

To a solution of **2a** (9.6 g) and *N*-(*tert*-butoxycarbonyl)-L-alanine (7.8 g) in dichloromethane (100 ml) were added EDC·HCl (7.9 g) and 4-dimethylaminopyridine (0.23 g) at room temperature. After stirring for 2 hours, the reaction mixture was washed with 10% aq citric acid, 5% aq $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was chromatographed on a silica gel column (Daisogel No.1001W, eluent; Benzene - EtOAc, 3 : 1→

Table 6. Yield, ^{1}H-NMR and IR spectral data (**3b**~**3f**).

Compound No	Yield (%)	^{1}H-NMR(60MHz, DMSO-d_6, δ)	IR(Nujol) cm^{-1}
3b	77	1.15 (9H, d, J = 7.0Hz), 1.40 (9H, s), 2.40~2.90 (1H, m), 3.50~3.83 (2H, m), 3.95 (3H, s), 4.00~4.50 (1H, m), 5.20 (1H, d, J = 5.0Hz), 5.70~6.20 (3H, m), 6.45~6.85 (1H, m), 6.90~7.35 (1H, br), 7.40 (1H, s), 9.73 (1H, d, J = 9.0Hz), 12.58 (1H, br s).	3260, 1780, 1760, 1685
3c	68	1.00 (9H, s), 1.26 (3H, d, J = 7.0Hz), 1.40 (9H, s), 2.26 (2H, s), 3.50~3.80 (2H, m), 3.94 (3H, s), 4.00~4.58 (1H, m), 5.17 (1H, d, J = 5.0Hz), 5.66~6.16 (3H, m), 6.52~6.86 (1H, m), 6.87~7.54 (1H, br), 7.56 (1H, s), 9.73 (1H, d, J = 9.0Hz), 12.55 (1H, br s).	3270, 1775, 1690
3d	72	0.90 (6H, d, J = 7.0Hz), 1.40 (9H, s), 1.45 (6H, d, J = 7.0Hz), 1.80~2.50 (3H, m), 3.50~3.90 (2H, m), 3.95 (3H, s), 3.90~4.50 (1H, m), 5.18 (1H, d, J = 5.0Hz), 5.60~6.16 (3H, m), 6.50~6.90 (1H, m), 6.90~7.40 (1H, br), 7.35 (1H, s), 9.73 (1H, d, J = 9.0Hz), 12.50 (1H, br s).	3260, 3215, 1770, 1690
3e	21	1.10 (6H, d, J = 7.0Hz), 1.40 (12H, s), 1.50 (3H, d, J = 7.0Hz), 2.20~2.90 (1H, m), 3.40~3.80 (2H, m), 3.84 (3H, s), 4.00~4.40 (1H, m), 5.15 (1H, d, J = 5.0Hz), 5.95 (1H, dd, J = 5.0 and 9.0Hz), 6.50~7.00 (2H, m), 7.36 (1H, s), 9.71 (1H, d, J = 9.0H), 12.25 (1H, br s).	3260, 1780, 1755, 1685
3f	47	1.32 (6H, d, J = 7.0Hz), 1.40 (12H, s), 1.53 (3H, d, J = 7.0Hz), 3.42~3.82 (2H, m), 3.94 (3H, s), 4.00~4.44 (1H, m), 4.60~5.08 (1H, m), 5.14 (1H, d, J = 5.0Hz), 5.95 (1H, dd, J = 5.0 and 9.0Hz), 6.50~7.00 (2H, m), 7.00~7.60 (1H, br), 7.40 (1H, s), 9.74 (1H, d, J=9.0H), 12.56 (1H, br s).	3270, 1765, 1690
3g	56	1.00~2.20 (10H, m), 1.27 (3H, d, J=5.5 Hz), 1.36 (9H, s), 1.50 (3H, d, J=5.5 Hz), 3.50~3.70 (2H, m), 3.90 (3H, s), 3.90~4.30 (1H, m), 4.40~4.80 (1H, m), 5.15 (1H, d, J=5.0 Hz), 5.90 (1H, dd, J=5.0 and 8.0 Hz), 6.50~6.70 (1H, m), 6.78 (1H, q, J=5.5 Hz), 7.11 (1H, d, J=8.0 Hz), 7.34 (1H, s), 9.67 (1H, d, J=8.0 Hz), 12.50 (1H, br s).	3270, 1795, 1765, 1690

2:1) to give **3a** (6.45 g) in 50% yield.

IR (Nujol) cm^{-1} 1780, 1755, 1680; ^{1}H-NMR (DMSO-d_6) δ 1.16 (9H, s), 1.27 (3H, d, J=6.0 Hz), 1.36 (9H, s), 3.50~3.80 (2H, m), 3.89 (3H, s), 3.90~4.40 (1H, m,), 5.14 (1H, d, J=5.0 Hz), 5.70~6.10 (3H, m), 6.50~6.80 (1H, m), 7.05 (1H, d, J=5.0 Hz), 7.30 (1H, s), 9.17 (1H, d, J=8.0 Hz), 12.4 (1H, br s).

Compounds **3b~3g** were prepared by a procedure similar to that described above. IR and NMR spectral data and chemical yields are listed in Table 6.

Pivaloyloxymethyl 7β-[(*Z*)-2-[2-(*S*)-Alanylaminothiazol-4-yl)]-2-methoxyiminoacetamido]-3-cephem-4-carboxylate Hydrochloride (**4a**, AS-924)

To a solution of **3a** (4.9 g) in formic acid (25 ml) was added 9.15 N HCl-isopropanol (3.2 ml) below 7°C with stirring. After stirring for 5 minutes at 0~5°C, the reaction mixture was poured into diethylether (100 ml). The resulting precipitate was collected by filtration. The solid was dissolved in methanol and poured into diisopropyl ether, the resulting precipitate was collected by filtration to give AS-924 (4.08 g) as a powder in 92% yield.

IR (Nujol) cm^{-1} 1775,1750,1670; ^{1}H-NMR (DMSO-d_6) δ 1.20 (9H, s), 1.52 (3H, d, J=7.0 Hz), 3.50~3.90 (2H, m), 3.90 (3H, s), 3.90~4.30 (1H, m), 5.16 (1H, d, J=5.0 Hz), 5.65~6.10 (3H, m), 6.50~6.80 (1H, m), 7.44 (1H, s), 8.40~8.90 (3H, br), 9.68 (1H, d, J=9.0 Hz), 13.00 (1H, br s).

Anal Calcd for $C_{22}H_{29}ClN_6O_8S_2$:
C 43.44, H 4.86, Cl 5.83, N 13.82, S 10.54.
Found: C 43.21, H 4.82, Cl 5.80, N 13.82, S 10.48.

Compounds **4b~4g** were prepared by a procedure

Table 7. Yield, ^{1}H-NMR and IR spectral data (**4b~4g**).

Compound No	Yield (%)	^{1}H-NMR(60MHz, DMSO-d_6, δ)	IR(Nujol) cm^{-1}
4b	66	1.15 (6H, d, J = 7.0Hz), 1.55 (3H, d, J = 7.0Hz), 2.30~3.00 (1H, m), 3.60~3.85 (2H, m), 3.95 (3H, s), 3.85~4.50 (1H, m), 5.20 (1H, d, J=5.0Hz), 5.60~6.15 (3H, m), 6.40~6.90 (1H, m), 7.53 (1H, s), 8.00~9.40 (3H, br), 9.75 (1H, d, J=9.0Hz), 13.10 (1H, br).	3140, 1780, 1750, 1705, 1670
4c	68	1.00 (9H, s), 1.52 (3H, d, J= 7.0Hz), 2.28 (2H, s), 3.72 (2H, m), 3.94 (3H, s), 3.90~4.40 (1H, m), 5.18 (1H, d, J =5.0Hz), 5.60~6.18 (3H, m), 6.44~6.85 (1H, m), 7.48 (1H, s), 7.98~10.00 (3H, br), 9.72 (1H, d, J = 9.0Hz), 11.00~14.00 (1H, br).	3350, 1790, 1755, 1715, 1680
4d	48	0.90 (6H, d, J = 7.0Hz), 1.45 (3H, d, J = 7.0Hz), 1.70~2.40 (3H, m), 3.65 (2H, br s), 3.90 (3H, s), 3.80~4.30 (1H, m), 5.15 (1H, d, J=5.0Hz), 5.52~6.10 (3H, m), 6.40~6.80 (1H, m), 7.45 (1H, s), 7.60~11.00 (4H, br), 9.70 (1H, d, J=9.0Hz).	3330, 3140, 1785, 1750, 1715, 1680
4e	52	1.10 (6H, d, J = 7.0Hz), 1.50 (6H, d, J = 7.0Hz), 2.20~9.90 (1H, m), 3.30~3.80 (2H, m), 3.90 (3H, s), 3.80~4.40 (1H, m), 5.25 (1H, d, J = 5.0Hz), 5.95 (1H, dd, J = 5.0 and 9.0Hz), 6.50~7.20 (2H, m), 7.48 (1H, s), 8.20~9.00 (3H, br), 9.70 (1H, d, J = 9.0H), 13.10 (1H, br s).	3400, 3150, 1780, 1750, 1705, 1670, 1640
4f	44	1.28 (6H, d, J = 7.0Hz), 1.54 (6H, d, J = 7.0Hz), 3.50~3.84 (2H, m), 3.95 (3H, s), 4.00~4.40 (1H, m), 4.60~5.10 (1H, m), 5.17 (1H, d, J=5.0Hz), 5.97 (1H, dd, J = 5.0 and 9.0Hz), 5.49~7.10 (2H, m), 7.51 (1H, s), 7.90~10.20 (3H, br), 9.75 (1H, d, J = 9.0H), 11.80~14.00 (1H, br).	3150, 1780, 1765, 1705, 1670
4g	77	0.96~2.20 (10H, m), 1.52 (6H, d, J = 7.0Hz), 3.66 (2H, br s), 3.93 (3H, s), 4.00~4.32 (1H, m), 4.32~4.86 (1H, m), 5.16 (1H, d, J = 5.0Hz), 5.94 (1H, dd, J = 5.0 and 9.0Hz), 6.46~7.02 (2H, m), 7.46 (1H, s), 7.96~9.02 (3H, br), 9.73 (1H, d, J=9.0Hz), 12.80~13.24 (1H, br).	3250, 1790, 1760, 1690, 1660

similar to that described above. IR and NMR spectral data and chemical yields are listed in Table 7.

Another synthetic route of AS-924 was also established as follows. To a solution of **6** (465 mg) and triethylamine (0.20 ml) in *N*,*N*-dimethylacetamide (2.3 ml) was added **7** (477 mg) at −15°C. After stirring for 30 minutes at the same temperature, 2-propanol (11.5 ml) was added and the resulting crystalline precipitate was collected by filtration to give AS-924 (425 mg) in 53% yield.

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