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# Studies on Prodrugs of Cephalosporins. I.[1] Synthesis and Biological Properties of Glycyloxybenzoyloxymethyl and Glycylaminobenzoyloxymethyl Esters of 7β-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylic Acid

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*p*-Glycyloxy-, *o*-glycylamino- and *p*-glycylaminobenzoyloxymethyl esters of 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylic acid (**1**) were synthesized as prodrugs designed to improve the oral absorption of the parent cephalosporin. The esters were found to possess the desired factors for an orally active prodrug, that is, appropriate solubility, lipophilicity and lability. As predicted from these factors, the esters when administered orally to mice were well absorbed from the gastrointestinal tract and gave high blood levels of the parent compound (**1**).

**Keywords**——prodrug; cephalosporin; 7β-[2-(2-aminothiazol-4-yl-(*Z*)-2-methoxyimino-acetamido]-3-methyl-3-cephem-4-carboxylic acid ester; oral absorption; mouse

Acyloxymethyl esterification of hydrophilic β-lactam antibiotics that contain a basic amino group with $pK_a$ of 6—9 in the side chain of the molecule can give high lipophilicity without loss of aqueous solubility, and consequently can result in significant improvement in the oral absorption of the parent β-lactam antibiotics. Examples of successful penicillin esters are pivampicillin[2] and pivmecillinam,[3] and the acetoxymethyl ester of cephaloglycin[4] and other α-amino cephalosporin esters[5] illustrate the applicability of this prodrug principle to cephalosporins.

In contrast, acyloxymethyl esters of β-lactam antibiotics having a neutral or weakly basic side-chain are poorly active orally because they lack sufficient solubility in water for efficient absorption from the gastrointestinal tract to occur.[6] To overcome the low solubility of such β-lactam antibiotic esters, we prepared *p*-glycyloxy-, *o*-glycylamino- and *p*-glycylaminobenzoyloxymethyl esters (**2a—c**) containing a relatively basic amino group in the promoiety by using 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylic acid (**1**) as a parent cephalosporin. These esters might be expected to have increased aqueous solubility together with lipophilicity.

In this report we describe the synthesis of the esters (**2a—c**) of **1**, and their physicochemical and biological properties.

## Chemistry

The new esters (**2a—c**) were synthesized as outlined in Chart 1. Compounds **3a—c** were prepared by treating the corresponding *p*-hydroxy-, *o*-amino- or *p*-aminobenzoic acid with *N*-(*tert*-butoxycarbonyl) glycine in the presence of triethylamine in dichloromethane; **3a—c** thus obtained were converted to the corresponding chloromethyl esters (**4a—c**) by reaction with

chloromethyl chlorosulfate in dichloromethane–water. Treatment of **4a—c** with sodium iodide in acetone yielded the iodomethyl esters (**5a—c**). Reaction of **5a**, **b** or **c** with the parent cephalosporin **1** in dimethylformamide led to the formation of the protected intermediates (**6a—c**) which, without isolation, were hydrolyzed in HCl–dioxane to remove the amino-protecting group. The resulting esters (**2a—c**) were finally obtained as the hydrochloride salts.

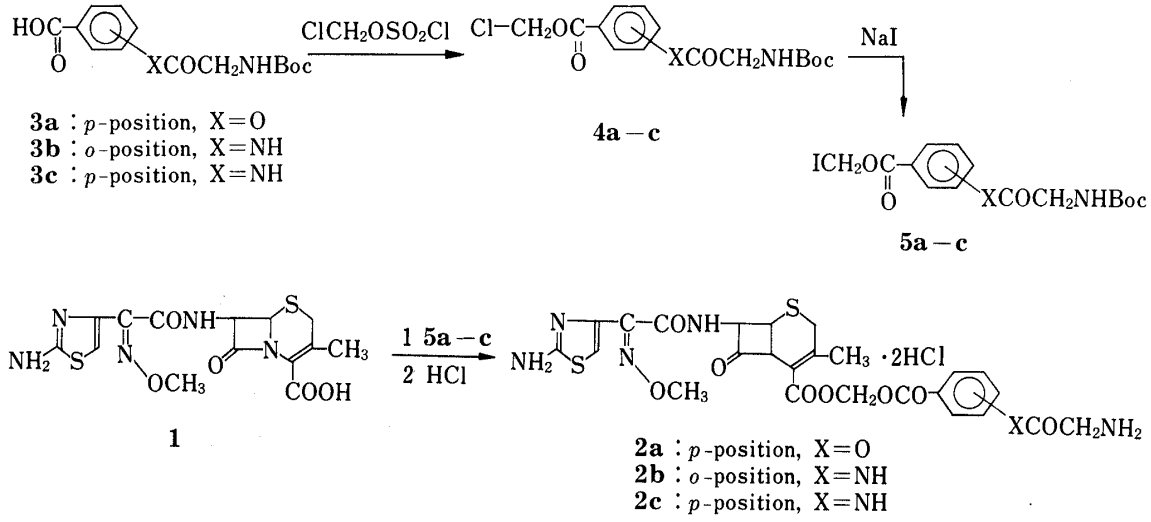



Chart 1

## Results

### MIC of Parent Cephalosporin 1

The spectrum of antibacterial activity of the parent cephalosporin **1** against standard Gram-positive and Gram-negative organisms is shown in Table I. As compared with widely used oral cephalosporins, *e.g.* cephalexin (CEX) and cefaclor (CCL), **1** had higher antibacterial activity particularly against Gram-negative organisms such as *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Serratia marcescens*, but lower activity against the Gram-positive organisms such as *Staphylococcus aureus, Micrococcus luteus* and *Bacillus subtilis*.

### Aqueous Solubility, Lipophilicity and Hydrolysis

The aqueous solubility, lipophilicity and susceptibility to hydrolysis of the esters **2a—c** are shown in Table II. The esters were all soluble in water to the extent of 25 mg/ml or more. The lipophilicity of the esters, expressed as the partition coefficient between 1-octanol and pH 6.5 phosphate buffer, was about 100 times or more greater than that of the parent cephalosporin. The esters **2a—c** were relatively stable in artificial gastric juice (pH 1.2), their half-lives being 178, 495 and 242 min, respectively. In the presence of intestinal mucosa homogenate, **2a—c** were hydrolyzed rapidly to the prarent cephalosporin, with half-lives of 1.5, 9.8 and 1.7 min, respectively.

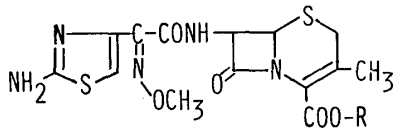
### Absorption in Mice

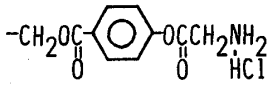
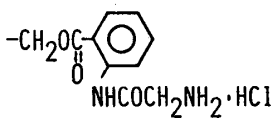
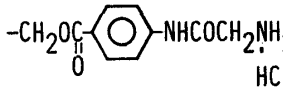
The absorption of the esters **2a—c** after oral administration was compared with that of parent cephalosporin **1** after subcutaneous and oral administration (Table III, Fig. 1). The parent cephalosporin **1**, when administered subcutaneously to mice at a single dose of 100 mg/kg gave high plasma levels with a peak level of 106.2 $\mu$g/ml at 15 min, whereas the peak plasma level at 30 min after oral administration of the same dose of **1** was 1.8 $\mu$g/ml, which is markedly inferior to that after the subcutaneous administration. In contrast to **1**, all

Table I. Antibacterial Spectra of Cephalosporin (**1**), CEX and CCL

| Test organism | MIC ($\mu$g/ml) | | |
|---|---|---|---|
| | Cephalosporin (**1**) | CEX | CCL |
| *S. aureus* 209-P | 12.5 | 3.13 | 0.78 |
| *S. aureus* No. 80 | 50 | 3.13 | 1.56 |
| *M. luteus* ATCC 9341 | 1.56 | 0.20 | 0.20 |
| *B. subtilis* ATCC 6633 | 6.25 | 0.39 | 0.20 |
| *E. coli* NIHJ JC-2 | 0.39 | 6.25 | 3.13 |
| *E. coli* K-12 | 0.20 | 6.25 | 1.56 |
| *E. coli* NIH | 0.39 | 6.25 | 1.56 |
| *E. coli* No. 8 | 0.20 | 25 | 6.25 |
| *E. coli* No. 24 | 0.39 | 3.13 | 1.56 |
| *K. pneumoniae* KC-1 | 0.20 | 6.25 | 0.39 |
| *K. pneumoniae* NCTC 9632 | 0.20 | 6.25 | 0.78 |
| *P. vulgaris* OX-19 | 0.20 | 25 | 12.5 |
| *P. mirabilis* 1287 | 0.20 | 6.25 | 1.56 |
| *P. morganii* Kono | 50 | >100 | >100 |
| *S. marcescens* IFO 3736 | 0.78 | >100 | >100 |
| *P. aeruginosa* E-2 | >100 | >100 | >100 |
| *P. aeruginosa* Nc-5 | >100 | >100 | >100 |
| *P. aeruginosa* NCTC 10490 | 6.25 | >100 | >100 |

Table II. Physicochemical Properties Influencing Oral Absorption of a Prodrug




| R | | Aqueous solubility (mg/ml) | Lipophilicity *n*-octanol / water (pH 6.5) | Hydrolysis, $t_{1/2}$ (min) | |
|---|---|---|---|---|---|
| | | | | In artificial gastric juice (pH 1.2) | In mouse intestine homogenate |
| Na (Parent compd.) | (**1**) | >25 | >0.01 | — | — |
|  | (**2a**) | >25 | 9.09 | 178 | 1.5 |
|  | (**2b**) | >25 | 1.39 | 495 | 9.8 |
|  | (**2c**) | >25 | 1.10 | 242 | 1.7 |

three esters (**2a**—**c**) were well absorbed after oral administration of a dose equivalent to 100 mg of **1** per kg, producing peak plasma levels of **1** in the range from 9.8 to 26.7 $\mu$g/ml at 30—60 min. The highest plasma level was obtained with **2a**, followed by **2b** and **c**. Bioavailabilities of **2a**—**c**, calculated on the basis of the area under the curve in the plasma

TABLE III. Plasma Levels and *AUC* after Administration of the Parent Cephalosporin (**1**) and the Esters (**2a**—**c**) to Mice ($n=3$)

| Compound | Dose route | Plasma levels (μg/ml) | | | | | $AUC_{0\to4}$ (μg·h/ml) |
|---|---|---|---|---|---|---|---|
| | | 1/4 | 1/2 | 1 | 2 | 4 h | |
| **1** | *s.c.* | 106.2±4.6 | 101.6±2.9 | 45.0±5.0 | 6.6±0.9 | 0.6±0.6 | 108.3 |
| | Oral | 1.1±0.4 | 1.8±0.3 | 1.5±0.3 | 0.7±0.2 | — | 2.8 |
| **2a** | Oral | 16.1±1.8 | 23.2±1.8 | 26.7±5.2 | 7.2±1.5 | 1.1±0.2 | 44.7 |
| **2b** | Oral | 12.3±3.3 | 15.4±1.6 | 17.2±1.6 | 8.7±1.5 | 2.2±0.8 | 37.0 |
| **2c** | Oral | 4.0±0.6 | 9.8±0.5 | 7.1±1.9 | 3.3±0.4 | 1.3±0.3 | 16.3 |

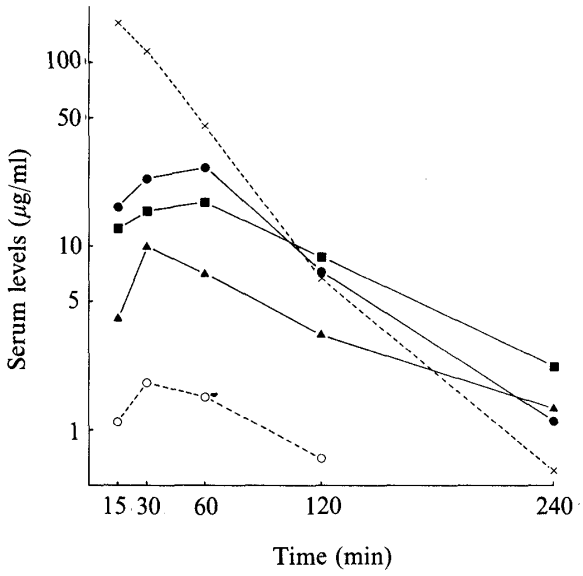



Fig. 1. Plasma Levels of the Parent Cephalosporin (**1**) after Administration of **1** or the Esters (**2a**—**c**) to Mice

---×---, *s.c.* **1**; ---○---, oral **1**; —●—, oral **2a**; —■—, oral **2b**; —▲—, oral **2c**.
All doses were 100 mg (potency)/kg, $n=3$.

after subcutaneous administration of **1**, were about 41.3, 34.2 and 15.1%, respectively, compared to 2.6% after oral administration of **1**.

## Discussion

We investigated new ester prodrugs of cephalosporin **1** having high bacterial activity, particularly against Gram-negative organisms. For the oral absorption of a prodrug as well as analogs, aqueous solubility and lipophilicity are known to be important factors.[7] In addition to having suitable lipophilicity and solubility, the prodrug must possess an optimal lability. The prodrug must be stable in the gastrointestinal lumen but should, upon systemic absorption, be rapidly hydrolyzed.

In this study, as a result of masking the strongly acidic 4-carboxy group by esterification with glycylaminobenzoyloxymethyl or glycyloxybenzoyloxymethyl fragments, the lipophilicity expressed as the partition coefficient of the compound between 1-octanol and water at pH 6.5 (about equal to the pH of the intestine) was markedly increased from less than 0.01 for parent cephalosporin **1** to 1.10—9.09 for the esters **2a**—**c** without loss of aqueous solubility (Table II). The esters were also found to be relatively stable in artificial gastric juice, and were rapidly hydrolyzed to the parent cephalosporin in the intestinal mucosa of mice (Table II). Thus, the esters **2a**—**c** were shown to possess the desired properties for an orally active prodrug, that is, appropriate lipophilicity, aqueous solubility and lability.

As we would expect from the three factors described above, the esters **2a**—**c**, when administered orally to mice, were well absorbed from the gastrointestinal tract and gave high blood levels of the parent compound (Table III, Fig. 1). Compound **2a** produced the highest bioavailability, the value being 41.3%, which was about 15 times higher than that after oral administration of the parent cephalosporin.

Although the exact mechanism of absorption of the esters has not been elucidated, it is reasonable to assume that it takes place as shown in Chart 2. During the absorption of the ester from the gastrointestinal tract, glycyloxy- or glycylaminobenzoic acid is split off enzymatically with formation of the unstable hydroxymethyl ester of **1**, which subsequently decomposes spontaneously into the parent cephalosporin and formaldehyde. Thereafter, the parent cephalosporin passes into the systemic circulation through the portal vein and liver.

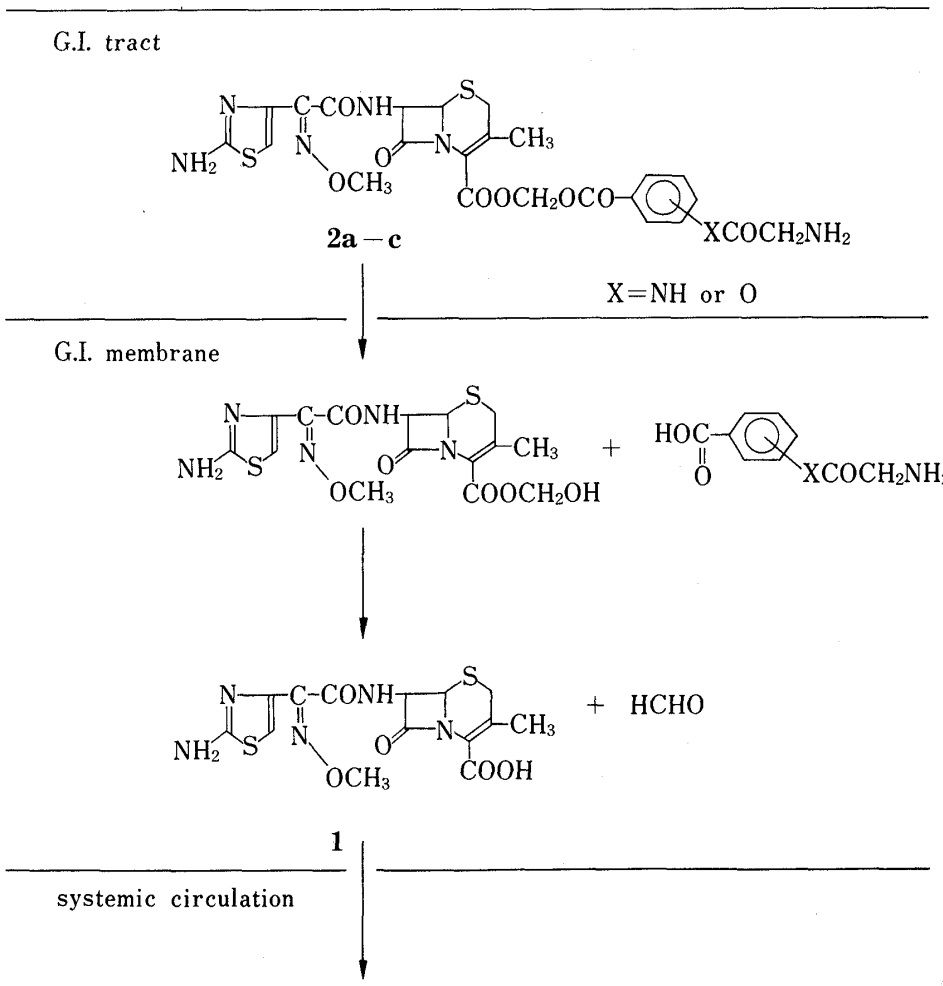



Chart 2

In conclusion, the evidence presented in this paper provides useful guidelines for the design of prodrugs of β-lactam antibiotics lacking a relatively basic amino group in the side-chain to improve oral absorbability.

## Experimental

The nuclear magnetic resonance (NMR) spectra were obtained on a Hitachi R-600 spectrometer. Infrared (IR) spectra were obtained in KBr on a Shimadzu R-600 spectrophotometer, and the main absorptions are given in $cm^{-1}$. The chemical analysis was performed by the microanalytical group of Kyoto University. Where analyses are

indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

**4-[*N*-(*tert*-Butoxycarbonyl)glycyloxy]benzoic Acid (3a)**——Triethylamine (4 ml) was added to a solution of *N*-(*tert*-butoxycarbonyl)glycine (5 g) in dichloromethane (50 ml) at −20 °C, then ethyl chlorocarbonate (2.75 g) was added. The mixture was stirred for 20 min at −10—−15 °C, then a solution of *p*-hydroxybenzoic acid (3.94 g) and triethylamine (4 ml) in dichloromethane (50 ml) was added dropwise, and stirring was continued at −5—0 °C for 1—2 h. The dichloromethane solution was washed with 10% aqueous citric acid and saturated aqueous sodium chloride, then dried ($Na_2SO_4$), and evaporated *in vacuo*. The residue was crystallized from benzene to give 4.7 g (80%) of **3a**.

IR (Nujol): 3420, 3340 and 1770 $cm^{-1}$. NMR ($(CD_3)_2CO$) $\delta$ ppm: 1.43 (s, 9H, $CH_3 \times 3$), 4.13 (d, $J$=6.5 Hz, 2H, $-CH_2-$), 6.15—6.65 (br, 1H, –NH–), 7.26, 8.08 (dd, $J$=9 Hz, 4H, $-C_6H_4-$), and 8.15 (br s, 1H, –COOH).

**2-[*N*-(*tert*-Butoxycarbonyl)glycylamino]benzoic Acid (3b)**——Triethylamine (4 ml) was added to a solution of *N*-(*tert*-butoxycarbonyl)glycine (5 g) in dichloromethane (50 ml) at −20 °C, then ethyl chlorocarbonate (2.75 ml) was added. The mixture was stirred at −10—−15 °C for 20 min, then a solution of *o*-aminobenzoic acid (3.92 g) and triethylamine (4 ml) in dimethylformamide (50 ml) was added dropwise at the same temperature. After being stirred for 1—2 h below 0 °C, the reaction mixture was left at room temperature overnight, and evaporated *in vacuo*. Ethyl acetate (50 ml) was added to the residue, and the solution was washed with 10% aqueous citric acid and saturated aqueous sodium chloride, then dried ($Na_2SO_4$), and evaporated *in vacuo*. The residue was washed with dichloromethane and dried to yield 3.6 g (63%) of **3b**.

IR (Nujol): 3280, 1710, 1680, 1660 and 1605 $cm^{-1}$. NMR ($CD_3OD$) $\delta$ ppm: 1.45 (s, 9H, $CH_3 \times 3$), 3.88 (s, 2H, $-CH_2-$), 7.05—8.63 (4H, $-C_6H_4-$).

**4-[*N*-(*tert*-Butoxycarbonyl)glycylamino]benzoic Acid (3c)**——**3c** was prepared in a manner similar to that described for **3b**; yield 2.4 g (43%).

IR (Nujol): 3360, 3260, 1680 and 1600 $cm^{-1}$. NMR ($(CD_3)_2SO$) $\delta$ ppm: 1.40 (s, 9H, $CH_3 \times 3$), 1.36 (d, $J$=6.5 Hz, 2H, $-CH_2-$), 6.30—7.05 (br, 2H, –NH–, –COOH), 7.62, 7.88 (dd, $J$=9 Hz, 4H, $-C_6H_4-$), 11.0 (br s, 1H, –NH–).

**Chloromethyl 4-[*N*-(*tert*-Butoxycarbonyl)glycyloxy]benzoate (4a)**——A solution of chloromethyl chlorosulfate (3.8 g) in dichloromethane (20 ml) was added dropwise to a stirred mixture of **3a** (5.6 g), sodium bicarbonate (6.3 g) and tetrabutylammonium hydrogen sulfate (0.7 g) in dichloromethane–water 1 : 1 (100 ml). The mixture was stirred at room temperature for 2—3 h, then the organic phase was separated and the aqueous phase was extracted with dichloromethane (160 ml). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was dissolved in ethyl acetate (100 ml), washed with saturated aqueous sodium chloride and dried ($MgSO_4$) to yield 4.0 g (60%) of **4a**.

IR (film): 3380, 1770 and 1740 $cm^{-1}$. NMR ($(CD_3)_2CO$) $\delta$ ppm: 1.43 (s, 9H, $CH_3 \times 3$), 4.11 (d, $J$=6.5 Hz, 2H, $-CH_2-$), 6.02 (s, 2H, $-CH_2Cl-$), 6.05—6.70 (br, 1H, –NH–), 7.27, 8.06 (dd, $J$=9 Hz, 4H, $-C_6H_4-$).

**Chloromethyl 2-[*N*-(*tert*-Butoxycarbonyl)glycylamino]benzoate (4b)**——A reaction similar to that described for **4a** but using **3b** (5.5 g) and chloromethyl chlorosulfate (3.8 g) afforded **4b**, as a oil, 4.2 g.

IR (Nujol): 3375, 3340, 1765, 1700, 1680 and 1640 $cm^{-1}$. NMR ($CDCl_3$) $\delta$ ppm: 1.48 (s, 9H, $CH_3 \times 3$), 3.87 (d, $J$=6.5 Hz, 2H, $-CH_2-$), 5.10—5.50 (br, 1H, –NH–), 5.87 (s, 2H, $-CH_2Cl$), 7.07—8.69 (4H, $-C_6H_4-$), 11.20 (br, 1H, –NH–).

**Chloromethyl 4-[*N*-(*tert*-Butoxycarbonyl)glycylamino]benzoate (4c)**——A reaction similar to that described for **3a** but using **2b** (5.5 g) and chloromethyl sulfate (3.8 g) afforded **4c**, as a oil, 3.0 g.

IR (Nujol): 3300, 1730 and 1670 $cm^{-1}$. NMR ($(CD_3)_2CO$) $\delta$ ppm: 1.43 (s, 9H, $CH_3 \times 3$), 3.96 (d, $J$=6.5 Hz, 2H, $-CH_2-$), 6.06 (s, 2H, $-CH_2Cl$), 6.00, 6.43 (br, 1H, –NH–), 7.80, 8.04 (dd, $J$=9 Hz, 4H, $-C_6H_4-$), 9.58 (br s, 1H, –NH–).

**4-Glycylaminobenzoyloxymethyl 7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylate (2a)**——An acetone solution (30 ml) of **4a** (2.0 g) and sodium iodide (4.5 g) was stirred under $N_2$ for 16 h. The resulting sodium chloride was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was dissolved in dimethylformamide (30 ml), and added dropwise to a dimethylformamide solution (10 ml) of potassium 7$\beta$-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylate, **1**, (1.5 g) at −20 °C. After being stirred for 30 min, the reaction mixture was added to ethyl acetate (50 ml). The solution was washed with 5% aqueous sodium bicarbonate and saturated aqueous sodium chloride. The solution was dried ($Na_2SO_4$), and evaporated *in vacuo*. The residue was mixed with anisole (5 drops) to form a paste, and 0.2 N HCl–dioxane (5.0 ml) was added. The mixture was stirred at room temperature for 30—60 min, then ethyl acetate (30 ml) was added. The separated solids were collected by suction to give **2a** as its hydrochloride, 1.84 g (76.3%).

IR (KBr): 1775, 1740, 1675 and 1630 $cm^{-1}$. NMR ($(CD_3)_2SO$) $\delta$ ppm: 3.52, 3.82 (m, 2H, $C_2-H_2$), 4.07 (s, 3H, $-OCH_3$), 4.22 (s, 2H, $-CH_2-$), 5.16 (d, $J$=5 Hz, 1H, $C_6-H$), 5.90 (d, $J$=5 Hz, 1H, $C_7-H$), 6.06, 6.19 (dd, $J$=6 Hz, 2H, $-COOCH_2-$), 6.60—6.91 (m, 1H, $C_3-H$), 7.08 (s, 1H, thiazol 5-H), 7.38, 8.09 (dd, $J$=9 Hz, 4H, $-C_6H_4-$). *Anal.* ($C_{24}H_{27}Cl_2N_7O_8S_2$) C, H, N, S.

**2-Glycylaminobenzoyloxymethyl 7$\beta$-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylate (2b)**——A reaction similar to that described for **2a** but using **1** and **4b** afforded **1b**, as its hydrochloride, 0.62 g (25.7%).

IR (KBr): 1780, 1740, 1700 and 1630 $cm^{-1}$. NMR ($(CD_3)_2SO$) $\delta$ ppm: 2.18 (s, 3H, $C_3-CH_3$), 3.40, 3.70 (m, 2H,

$C_2$–$CH_3$), 4.02 (s, 2H, –$CH_2$–), 4.07 (s, 3H, –$OCH_3$), 5.18 (d, $J$=5 Hz, 1H, $C_6$–H), 5.77 (d, $J$=5 Hz, 1H, $C_7$–H), 6.06, 6.73 (dd, $J$=6 Hz, 2H, –$COOCH_2$–), 7.10 (s, 1H, thiazol, 5-H), 7.23, 7.64, 8.05, 8.33 (4H, –$C_6H_4$–). *Anal.* ($C_{24}H_{26}Cl_2N_6O_9S_2$) C, H, N, S.

**4-Glycyloxybenzoyloxymethyl 7β-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylate (2c)**——A reaction similar to that described for **2a** but using **1** and **4c** afforded **2c**, as its hydrochloride, 1.33 g (55.2%).

IR (KBr): 1775, 1730, 1700 and 1625 $cm^{-1}$. NMR ($(CD_3)_2SO$) $\delta$ ppm: 3.50—3.77 (m, 2H, $C_2$–$H_2$), 3.92 (s, 2H, –$CH_2$–), 4.03 (s, 3H, –$OCH_3$), 5.15 (d, $J$=5 Hz, 1H, $C_6$–H), 5.88 (d, $J$=5 Hz, 1H, $C_2$–H), 6.10 (ds, 2H, –COOCH–), 6.52—6.82 (m, 1H, $C_3$–H), 7.03 (s, 1H, thiazol, 5-H), 7.74, 7.99 (dd, $J$=9 Hz, 4H, –$C_6H_4$–). *Anal.* ($C_{24}H_{26}Cl_2N_6O_9S_2$) C, H, N, S.

**Determination of MICs**——Minimum inhibitory concentrations (MICs) were determined by the agar dilution method, using Mueller–Hinton agar (MHA). Serial 2-fold dilutions of freshly prepared antibiotic solutions were mixed with melted MHA in Petri dishes. Plates were inoculated with one loopful of $10^{-2}$ fold diluted overnight culture of organisms in Mueller–Hinton broth (MHB) unless otherwise described. The MIC values (μg/ml) were determined after 18 h of incubation at 37 °C.

**Absorption Studies in Mice**——Male ddY strain mice weighing 18—22 g were used. Before the experiment, the animals were starved overnight but were allowed to drink water. Parent cephalosporin was administered orally or subcutaneously as solutions of the sodium salt in water (2 ml/kg), at a dose of 10 mg (potency)/kg, to groups of four mice. The esters were administered orally at the same doses as solutions of the hydrochloride salts in water (2 ml/kg). Animals were killed at 5, 15, 30, 60 and 120 min after drug administration, and blood samples were collected from the cervical region of each animal. Plasma was immediately separated, and diluted 5-fold in pH 7.5 phosphate buffer. Bioassay was performed by the paper disc method with *B. subtilis* as the test organism.

**Partition Coefficient**——Solutions of $1.5 \times 10^{-2}$ mol of the esters were prepared in 100 ml of 1/15 M phosphate buffer (pH 6.5) saturated with 1-octanol. The solutions (10 ml) were put into test tubes containing 1-octanol (5 ml) saturated with pH 6.5 buffer. The test tubes were shaken vigorously at 25 °C. When equilibrium had been achieved, the aqueous layer was separated by centrifugation, and the concentrations of the esters were measured by ultraviolet (UV) spectrophotometry.

***In Vitro* Hydrolysis**——(a) In Artificial Gastric Juice: Esters **2a**—**c** were added to artificial gastric juice (pH 1.2) at 50 μg/ml, and the solutions were maintained at 37 °C. Samples were withdrawn after 60, 120, 240 and 360 min and the non-hydrolyzed esters were determined by the high performance liquid chromatography (HPLC) method (Waters ALC/GPC compact type, model 45 pump, model 440 detector, 254 nm filter, model WISP 710B injector, and reverse phase Radial Pac μ-Bondapac $C_{18}$ column). Elution was carried out with 0.3% $KH_2PO_4$ aqueous solution–$CH_3CN$ (80 : 20) at a flow rate of 3.0 ml/min.

(b) In Mouse Intestine Homogenate: The small intestines were obtained from freshly killed mice. The guts were thoroughly washed immediately with cold saline to remove any contents. The intestines were homogenized at 1.05% w/v in ice-cold saline. The esters, as hydrochlorides, were dissolved in saline at a concentration equivalent to 1 mg/ml of the parent cephalosporin. The solutions (1 ml) were rapidly added to the intestine homogenate (19 ml) so that the reaction mixtures consisted of ester at a final concentration of 1 mg (potency)/ml in 1% w/v homogenate. The reaction mixtures were incubated at 37 °C and sampled at 2, 5, 15, 30 and 60 min after mixing. Samples of 3 ml were poured into a mixture of pH 7.4 phosphate buffer (1 ml) and dichloromethane (3 ml) and shaken vigorously. By this procedure, non hydrolyzed esters pass into the organic phase, whereas the parent compound remains in the aqueous phase. The aqueous phase was separated and assayed for the parent compound by the cup method with *P. mirabilis* 1287 as the test organism.

## References and Notes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