Studies on Orally Active Cephalosporin Esters. V.[1] A Prodrug Approach for Oral Delivery of 3-Thiazoliomethyl Cephalosporin

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Oral delivery of 3-thiazoliomethyl cephalosporin 1 was attempted through a prodrug approach by applying thiamine chemistry. The 3-thiazoliomethyl group was modified to a ring-opened structure with no ionic charge, and the 4-carboxyl group was converted to pivaloyloxymethyl ester. Lipophilicity of the resulting derivatives (8—10) was suitable for passive absorption from the intestinal tract, and chemical stability in phosphate buffer solution (pH 6.86) was moderate. When administered orally to mice, these derivatives were mainly transformed to a novel 3-spiro cephalosporin 11, and desired reconversion to the 3-thiazoliomethyl cephalosporin was minor. Isomerization to Δ^2-cephalosporin 14 was also observed. These results showed that the derivatives (8—10) tested in this study did not serve as orally active prodrugs of 3-thiazoliomethyl cephalosporin 1.

Keywords cephalosporin; prodrug; oral absorption; lipophilicity; thiazoliomethyl; spiro; antibiotic; β-lactam; thiamine

A new quarternary ammonium-type cephalosporin **1**, 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[4-methyl-5-(2-hydroxyethyl)thiazoliomethyl]-3-cephem-4-carboxylate, shows broad and potent antimicrobial activity against both gram-positive and gram-negative bacteria.[2] The cephalosporin **1** is highly hydrophilic, due to a zwitter ionic structure between the quarternary nitrogen of thiazoliomethyl group at the C-3 position and the carboxylate anion at the C-4 position, as shown in Chart 1. Therefore, its application is limited to injection, and when administered orally it is poorly absorbed from the gastrointestinal tract.[3]

Esterification at the C-4 carboxyl group of cephalosporins is known to increase their lipophilicity and improve their oral absorbability.[4-10] This approach has revealed the clinical utility of ester-type prodrugs of cephalosporin, such as CPDX-PR,[4] CXM-AX[5] and CFTM-PI.[6] Ester-type modification of the cephalosporin **1**, however, could not increase its oral bioavailability, bacause such a simple approach left a positive charge on the nitrogen atom of the thiazoliomethyl group, and it could not afford sufficient lipophilicity for oral absorption.

This paper describes chemical modification at the thiazoliomethyl moiety of cephalosporin **1**, leading to more lipophilic derivatives for oral use. Physicochemical properties and biological evaluations are also presented.

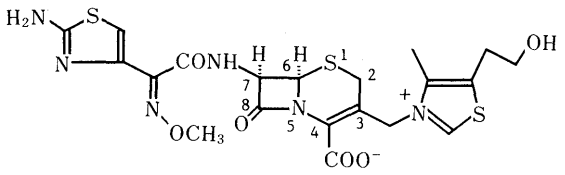

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Chart 1

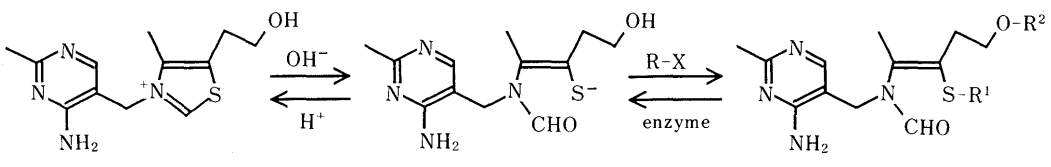
Results and Discussion

The 3-thiazoliomethyl moiety of cephalosporin **1** is the same as the side chain of thiamine (vitamin B_1). Reversible transformation is well known between the thiazolio ring structure (I) and the ring-opened thiolate anion structure (II).[11] Protection of the thiolate in II with an appropriate functional group could lead to a lipophilic structure (III) with no ionic charge, as shown in Chart 2.

Many thiamine derivatives with enhanced oral efficiency were synthesized using protective groups that could be removed under biological conditions. In this study, carbonate-type and acetate-type modifications[12] were applied to the 3-thiazoliomethyl group of cephalosporin **1**.

Synthesis Treatment of cephalosporin **1** in water with sodium hydroxide under ice cooling gave a thiolate **4**. The resulting thiolate **4** was smoothly reacted *in situ* with ethyl chloroformate to give *O*,*S*-bisethylcarbonate **5**. Analogously, the reaction with trichloromethyl chloroformate and acetic anhydride gave *O*,*S*-cyclocarbonate **6** and *O*,*S*-diacetate **7**, respectively. The structures of these derivatives were confirmed by comparing their ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectra with those of **1**. Characteristic absorption for *N*-formyl function was observed at around 7.7 ppm. The carboxyl group at the C-4 position of these cephalosporins (**5**—**7**) was esterified with pivaloyloxymethyl (POM) iodide. Thus, the derivatives (**8**—**10**), wherein thiazolio and carboxyl groups are masked with biologically labile functions, were obtained.

On the other hand, the thiolate **4** was almost quantitatively converted to a more lipophilic product under ice-cooling. The product was a mixture of three stereoisomers of 3-spiro cephalosporin **11**. This structurally novel spiro-compound would be formed by the intra-molec-



I II III

Chart 2

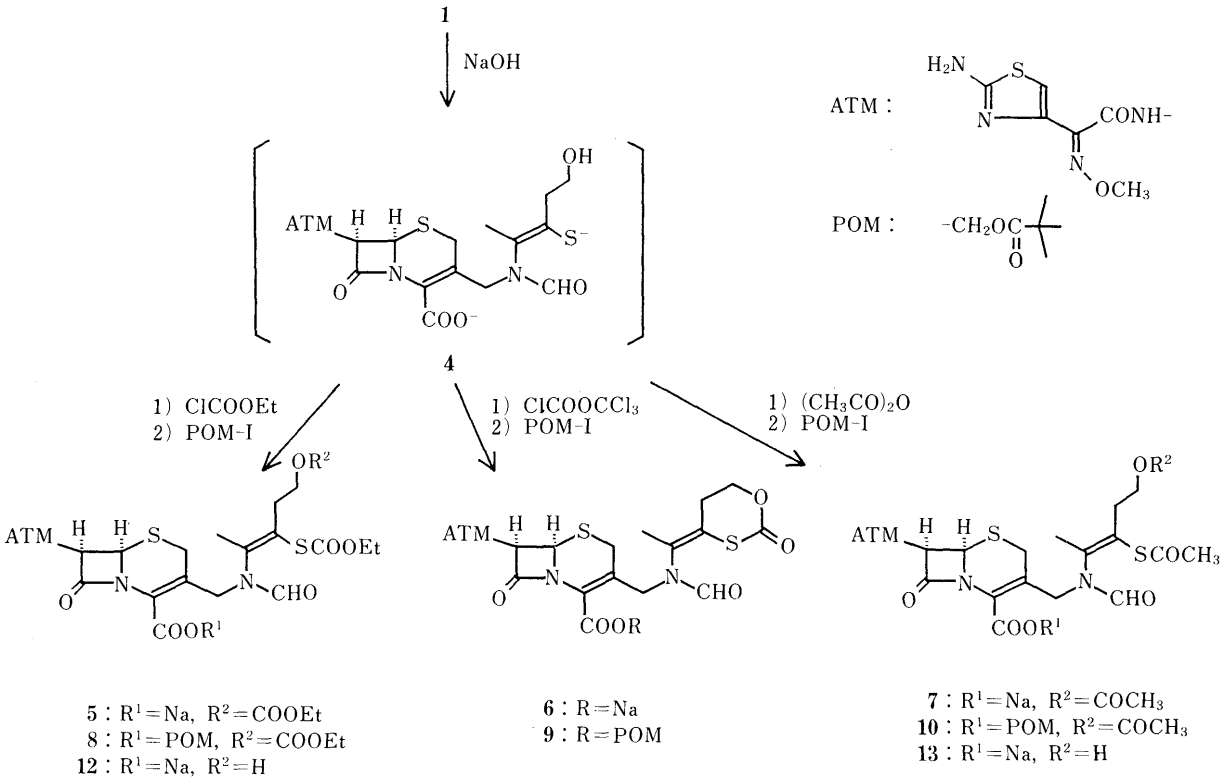
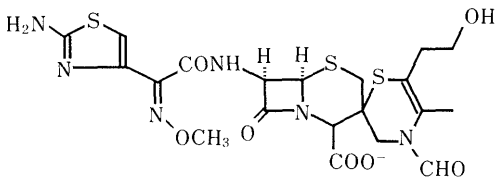


Chart 3



11

Chart 4

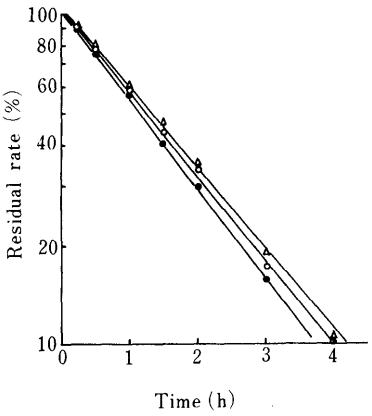


Fig. 1 Stability of POM Esters **8**, **9** and **10** in Phosphate Buffer Solution

●, *O,S*-bisethylcarbonate **8**; △, *O,S*-cyclocarbonate **9**; ○, *O,S*-diacetate **10**. Phosphate buffer; 1/20 M, pH 6.86. Temperature; 37 °C.

ular Michael-type addition of a mercapto group to the $C_3=C_4-C=O$ conjugated system of cephalosporin. The structural elucidation of **11** will be reported in detail elsewhere.[13]

Physicochemical Property Lipophilicity of these derivatives (**8**—**10**) was determined by measuring the partition coefficient (*P*) between *n*-octanol and water. log *P* values for these compounds were 3.34 for **8**, 1.37 for **9** and 2.16 for **10**, which showed that these derivatives gained sufficient lipophilicity for passive absorption from the intestinal tract.[14]

Table I. Recovery of Parent Cephalosporin **1**, 3-Spiro Cephalosporin **11** and Δ^2 Cephalosporin **14** after Oral Administration of Prodrugs **8**, **9** and **10** to Mice

Compound	1		11		14
	Urine	Feces	Urine	Feces	Feces
POM ester **8**	3.9	2.1	ND	11.7	8.6
POM ester **9**	2.1	1.3	ND	18.6	5.2
POM ester **10**	3.7	1.7	ND	26.9	5.7
Cephalosporin **1** (*p.o.*)	0.8	12.5	NT	NT	—
Cephalosporin **1** (s.c.)	94.1	0.5	NT	NT	—

POM ester (50 mg/kg as parent cephalosporin **1**) was orally administered to mice ($n=5$, ddY, male). Recovery of **1** was determined by bioassay. Recovery of **11** and **14** was determined by HPLC. ND: recovery could not be determined. NT: not tested. —: not detected.

Chemical stability of these derivatives (**8**—**10**) was examined in phosphate buffer (pH 6.86, 1/20 M, 37 °C). Degradation was monitored using high performance liquid chlomatography (HPLC). Degradation of each derivative followed the pseudo first-order kinetic, as shown in Fig. 1. Apparent degradation rate constants were 0.622 h^{-1} for **8**, 0.570 h^{-1} for **9** and 0.591 h^{-1} for **10**. These values were much larger than that (0.134 h^{-1}) for the 3-methoxymethyl derivative, and were the same level as that for the 3-tetrazolylmethyl one (CFTM-PI).[15] In the degradation products, their parents (**5**—**7**) or the grandparent (**1**) were minor constituents. Isomerization to the Δ^2 cephalosporin, which is characteristic for cephalosporin esters,[15] was also

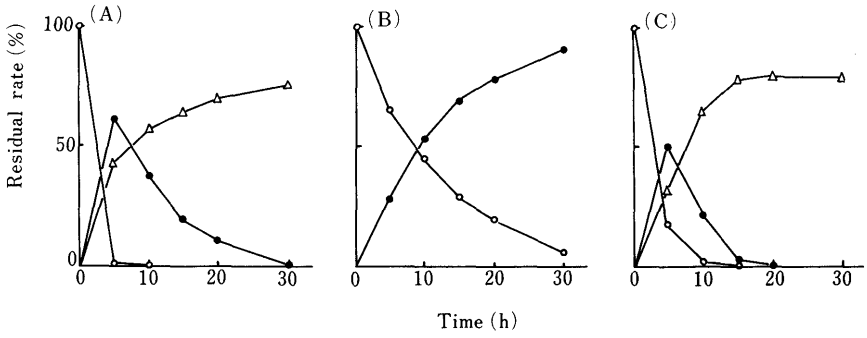


Fig. 2. Stability of POM Esters **8**, **9** and **10** in the Small Intestinal Homogenate of Mice

(A) *O*,*S*-Bisethylcarbonate (○; POM ester **8**, ●; parent acid **5**, △; *S*-ethylcarbonate **12**). (B) *O*,*S*-Cyclocarbonate (○; POM ester **9**, ●; parent acid **6**). (C) *O*,*S*-Diacetate (○; POM ester **10**, ●; parent acid **7**, △; *S*-acetate **13**).

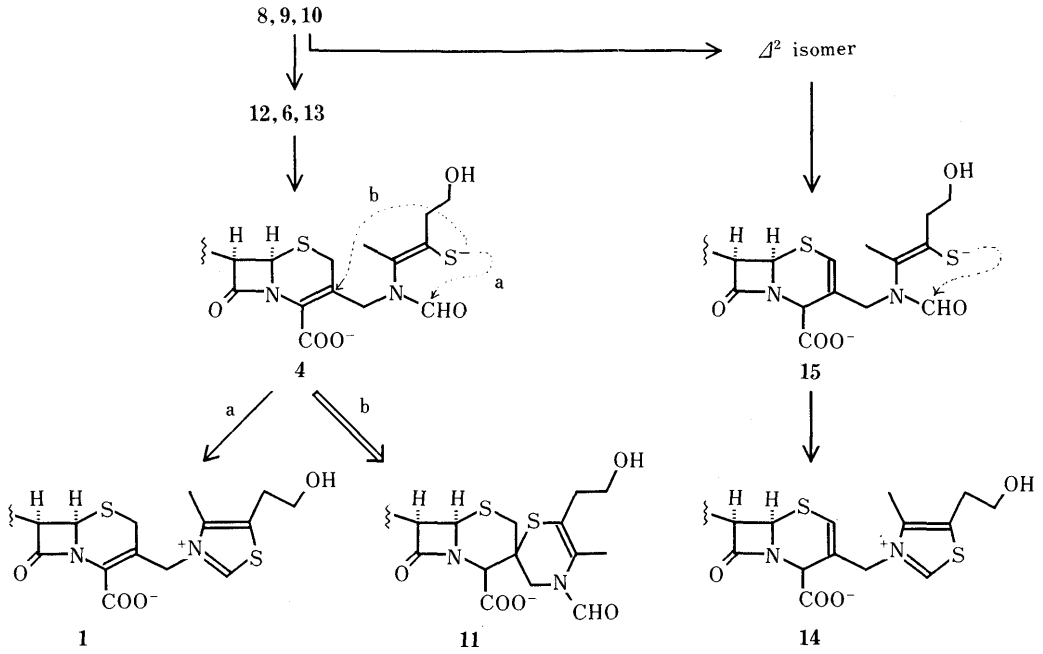


Chart 5

operative in the degradation of these derivatives.

Oral Absorption These derivatives (**8**—**10**) were administered orally to mice. Oral absorption was represented by the recovery of antimicrobial activity in urine. In each derivative, a more improved activity was recovered in urine than that in **1** itself. However, their levels were low and not adequate for the oral delivery of **1**.

Biochemical Property In order to clarify the reason for such a low oral bioavailability, the metabolism of these derivatives (**8**—**10**) was examined.

The derivatives were incubated with 1% homogenate of mouse small intestine, and the resulting hydrolysis products were analyzed by HPLC. The results are shown in Fig. 2. Pivaloyloxymethyl groups of these derivatives were hydrolyzed at first, and the corresponding parent cephalosporins (**5**—**7**) were formed. Subsequently, *O*-ethoxycarbonyl and *O*-actyl groups in the parents (**5** and **7**) were cleaved. However, *S*-ethoxycarbonyl, *S*-acetyl and *O*,*S*-cyclocarbonate groups were not hydrolyzed. Consequently, the derivatives (**8**—**10**) were not returned to the grandparent **1** in the mouse intestinal homogenate.

Metabolites in urine and feces after oral administration of **8**—**10** to mice were analyzed by HPLC. The compounds **12**, **6** and **13**, which were final metabolites in the intestinal tissue homogenate, were not observed in urine or feces. On the other hand, 3-spiro cephalosporin **11** was abundantly detected in feces, as shown in Fig. 3. Recovered rates of **11** were 11.7% from **8**, 18.6% from **9** and 26.9% from **10**. The 3-spiro cephalosporin **11** would be formed as depicted in Chart 5. That is, the administered derivatives **8**, **9** and **10** are hydrolyzed to **12**, **6** and **13** in the intestinal tract, and subsequently converted to thiolate **4** by the action of some thioesterase. Michael-type attack of the mercapto group to the $C_3=C_4-C=O$ moiety of cephalosporin leads to **11** (path b). The predicted attack on the *N*-formyl function, which leads to the 3-thiazoliomethyl cephalosporin **1**, is minor (path a). Little spiro compound was recovered in urine, and its amount could not be determined due to the overlapping of natural constituents.

The Δ^2-isomer **14** of cephalosporin **1** was also detected in feces. As reported previously, orally administered cephalosporin ester was readily isomerized to Δ^2 cephalosporin, and a significant amount of Δ^2-isomer was recovered in feces.[1] Also in the present case, a part of the administered derivatives **8**—**10** would be chemically transferred to corresponding Δ^2-isomers, and further enzymatically

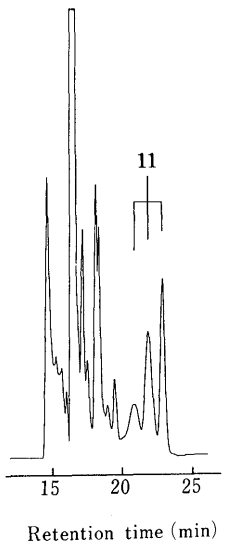


Fig. 3. HPLC Analysis of Feces after the Oral Administration of Prodrug **10** in Mice

Water extract was injected to column A. Eluate in 11.4—12.8 min was collected and injected to column B. Chromatogram was monitored at 254 nm. Column A: TOSO G-2000 SW (7.5 mm i.d. × 300 mm), potassium phosphate buffer (50 mM, pH 7.4), 1.0 ml/min. Column B: YMC ODS A-312 (6 mm i.d. × 150 mm), MeOH–water (5 mM PIC-A) (40 : 60), 1.0 ml/min.

converted to Δ^2-thiolate **15** in a similar manner to the formation of **4**. The thiolate **15**, which no longer had a Michael acceptor, would exclusively lead to 3-thiazoliomethyl-Δ^2-cephalosporin **14**, in contrast to that in **4**.[16]

Conclusion

Lipophilic derivatives (**8**—**10**) of 3-thiazoliomethyl cephalosporin **1** were successfully obtained by the application of thiamine chemistry. These derivatives were easily hydrolyzed to *S*-protected derivatives (**12**, **6** and **13**) in the small intestine of mice. Thiolate **4**, produced from them by action of thioesterase, was mainly transformed to 3-spiro cephalosporin **11**, and there was little of the desired reconversion to 3-thiazoliomethyl cephalosporin **1**. That is, the reactivity of the $C_3=C_4-C=O$ moiety of cephalosporin was higher than that of the *N*-formyl group. The spiro compound **11** was structurally novel, but had low antibacterial activity. Isomerization to Δ^2-cephalosporin, which decreases oral bioavailability of cephalosporin esters,[1] was also observed in **8**—**10**. Consequently, the presented prodrug approach for oral delivery of cephalosporin **1** was prevented by the high reactivity of the $C_3=C_4-C=O$ moiety, *i.e.* $\Delta^3 \rightarrow \Delta^2$ isomerization and Michael-type cyclization.[17]

Experimental

General ^{1}H-NMR spectra were determined on a JEOL GX-270 spectrometer using tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a Nicolet FT-IR (5SXC) spectrometer. Ultraviolet (UV) spectra were taken on a Shimadzu UV-3100 spectrometer. Fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-HX100 spectrometer. HPLC was performed using a Waters chromatography system (6000A pump, 440 absorbance detector (254 nm)) and a Shimadzu C-R3A Chromatopac.

Preparation of *O,S*-Bisethylcarbonate 5 To a stirred suspension of cephalosporin **1** (sulfate, 640 mg, 1 mmol) in water (10 ml) was added 1 N NaOH (*ca.* 4 ml, 4 mmol) under ice-cooling. A clear solution (pH 12.5) was given. Ethyl chloroformate (324 mg, 3 mmol) in CH_3CN (2 ml) was gradually added under the same conditions. During the addition, the pH of the mixture was maintained around 12.5—13.0 by adding 1 N NaOH. The final pH of the reaction mixture was adjusted to 7.0. The mixture was concentrated *in vacuo* to *ca.* 10 ml and subjected to CHP-20P column chromatography. A fraction eluted with 10% CH_3CN–water was concentrated *in vacuo* to *ca.* 10 ml, and then lyophilized to give sodium salt of *O,S*-bisethylcarbonate **5** (310 mg) as a colorless powder. ^{1}H-NMR (D_2O) δ: 1.07 (6H, t, J=7.0 Hz, $OCH_2C\underline{H}_3 \times 2$), 1.91 (3H, s, $=-CH_3$), 2.67—2.77 (2H, m, $=-CH_2$), 3.18 and 3.43 (2H, ABq, J=17.6 Hz, 2-CH_2), 3.79 (3H, s, OCH_3), 4.01 (4H, q, J=7.0 Hz, $OC\underline{H}_2CH_3 \times 2$), 4.07—4.52 (4H, m, OCH_2 and 3′-CH_2), 4.97 (1H, d, J=4.8 Hz, 6-CH), 5.56 (1H, d, J=4.8 Hz, 7-CH), 6.82 (1H, s, thiazol-5-H), 7.76 (1H, s, CHO). IR (KBr): 1748, 1675, 1614, 1532 cm^{-1}.

Preparation of *O,S*-Cyclocarbonate 6 Cephalosporin **1** (640 mg) was treated with trichloromethyl chloroformate (300 mg) in a similar method to that described in the preparation of **5**. The fraction eluted with 5% CH_3CN–water from a CHP-20P column was concentrated to 10 ml, and then lyophilized to give sodium salt of *O,S*-cyclocarate **6** (280 mg) as a colorless powder. ^{1}H-NMR (D_2O) δ: 1.75 (3H, s, $=-CH_3$), 2.65—2.75 (2H, m, $=-CH_2$), 3.25 and 3.54 (2H, ABq, J=18.5 Hz, 2-CH_2), 3.79 (3H, s, OCH_3), 3.96—4.45 (4H, m, OCH_2 and 3′-CH_2), 5.01 (1H, d, J=4.8 Hz, 6-CH), 5.56 (1H, d, J=4.8 Hz, 7-CH), 6.82 (1H, s, thiazol-5-H), 7.86 (1H, s, CHO). IR (KBr): 1766, 1671, 1639, 1610, 1532 cm^{-1}.

Preparation of *O,S*-Diacetate 7 Cephalosporin **1** (640 mg) was treated with acetic anhydride (250 mg) in a similar method to that described in the preparation of **5**. The fraction eluted with 8% CH_3CN–water from a CHP-20P column was concentrated to 10 ml, and then lyophilized to give sodium salt of *O,S*-diacetate **7** (290 mg) as a colorless powder. ^{1}H-NMR (D_2O) δ: 1.89 (3H, s, $=-CH_3$), 1.92 (3H, s, OAc), 2.20 (3H, s, SAc), 2.60 (2H, t, J=6.2 Hz, $=-CH_2$), 3.17 and 3.41 (2H, ABq, J=18.0 Hz, 2-CH_2), 3.80 (3H, s, OCH_3), 4.05 (2H, t, J=6.2 Hz, OCH_2), 4.28 and 4.39 (2H, ABq, J=15.0 Hz, 3′-CH_2), 4.98 (1H, d, J=4.8 Hz, 6-CH), 5.56 (1H, d, J=4.8 Hz, 7-CH), 6.84 (1H, s, thiazol-5-H), 7.67 (1H, s, CHO). IR (KBr): 1768, 1736, 1671, 1614, 1534 cm^{-1}.

Preparation of *O,S*-Bisethylcarbonate POM Ester 8 Pivaloyloxymethyl iodide (150 mg) was added to a solution of *O,S*-bisethylcarbonate **5** (280 mg) in *N,N*-dimethylacetamide (3 ml) at −8 °C. After being stirred under the same condition for 6 min, the mixture was diluted with EtOAc (50 ml) and washed with water (30 ml × 3). The organic layer was dried with Na_2SO_4 and concentrated *in vacuo*. Chromatography of the residue with silica gel (50 g) in EtOAc–CH_3CN (5 : 1) gave **8** (190 mg) as a colorless powder. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (9H, s, *tert*-Bu), 1.29 and 1.30 (6H, t, J=7.0 Hz, $OCH_2C\underline{H}_3 \times 2$), 2.08 (3H, s, $=-CH_3$), 2.78—2.95 (2H, m, $=-CH_2$), 3.54 and 3.60 (2H, ABq, J=17.0 Hz, 2-CH_2), 4.06 (3H, s, OCH_3), 4.18 and 4.25 (4H, q, J=7.0 Hz, $OC\underline{H}_2CH_3 \times 2$), 4.13—4.26 (2H, m, OCH_2), 4.44 and 4.80 (2H, ABq, J=15.0 Hz, 3′-CH_2), 5.07 (1H, d, J=4.8 Hz, 6-CH), 5.27 (2H, br s, NH_2), 5.88 (2H, s, $COOCH_2$), 5.99 (1H, dd, J=4.8 and 9.2 Hz, 7-H), 6.87 (1H, s, thiazol-5-H), 7.44 (1H, d, J=9.2 Hz, CONH), 8.00 (1H, s, CHO). IR (KBr): 1791, 1746, 1680, 1618, 1535 cm^{-1}. UV λ^{CH_3CN} nm (ε): 233 (23082). FAB-MS *m/z*: 815 ($M+H^+$).

Preparation of *O,S*-Cyclocarbonate POM Ester 9 *O,S*-Cyclocarbonate **6** (100 mg) was treated with POM iodide (60 mg) in the same way as described above. Chromatography with silica gel in EtOAc–CH_3CN (3 : 1) gave **9** (75 mg) as a colorless powder. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (9H, s, *tert*-Bu), 1.99 (3H, s, $=-CH_3$), 2.81—2.85 (2H, m, $=-CH_2$), 3.61 and 3.65 (2H, ABq, J=19.0 Hz, 2-CH_2), 4.06 (3H, s, OCH_3), 4.36—4.52 (2H, m, OCH_2), 4.28 and 4.70 (2H, ABq, J=13.9 Hz, 3′-CH_2), 5.09 (1H, d, J=4.8 Hz, 6-CH), 5.17 (2H, br s, NH_2), 5.85 (2H, s, $COOCH_2$), 6.00 (1H, dd, J=4.8 and 9.2 Hz, 7-H), 6.92 (1H, s, thiazol-5-H), 7.31 (1H, d, J=9.2 Hz, CONH), 8.01 (1H, s, CHO). IR (KBr): 1790, 1751, 1707, 1679, 1618, 1534 cm^{-1}. UV λ^{CH_3CN} nm (ε): 234 (24910). FAB-MS *m/z*: 697 ($M+H^+$).

Preparation of *O,S*-Diacetate POM Ester 10 *O,S*-Diacetate **7** (200 mg) was treated with POM iodide (120 mg) in the same way as described above. Chromatography with silica gel in EtOAc–CH_3CN (4 : 1) gave **10** (126 mg) as a colorless powder. ^{1}H-NMR ($CDCl_3$) δ : 1.22 (9H, s, *tert*-Bu), 2.02 (3H, s, $=-CH_3$), 2.10 (3H, s, OAc), 2.26 (3H, s, SAc), 2.63—2.80 (2H, m, $=-CH_2$), 3.53 (2H, s, 2-CH_2), 4.06 (3H, s, OCH_3), 4.07—4.21 (2H, m, OCH_2), 4.38 and 4.75 (2H, ABq, J=14.6 Hz, 3′-CH_2), 5.10 (1H, d, J=4.8 Hz, 6-CH), 5.34 (2H, br s, NH_2), 5.85 and 5.88 (2H, ABq, J=5.5 Hz, $COOCH_2$), 6.04 (1H, dd, J=4.8 and 9.2 Hz, 7-CH), 6.82 (1H, s, thiazol-5-H), 7.40 (1H, d, J=9.2 Hz, CONH), 7.89 (1H, s, CHO). IR (KBr): 1790, 1740, 1679, 1622, 1536 cm^{-1}. UV λ^{CH_3CN} nm (ε): 234 (23230). FAB-MS *m/z*: 755 ($M+H^+$).

Preparation of Δ^2 Isomer 14 To a suspension of cephalosporin **1**

TABLE II. Conditions of HPLC Analysis

Compound	Eluent (% of MeOH)	Retention time (min)
Cephalosporin **1**	15	9.96
Δ^2 Cephalosporin **14**	15	8.52
Carbonate **5**	80	3.94
Carbonate **6**	60	3.09
Acetate **7**	70	4.11
POM ester **8**	80	5.58
POM ester **9**	60	8.58
POM ester **10**	70	6.06
S-Carbonate **12**	80	3.12
S-Acetate **13**	70	3.15

Column: YMC ODS A-312 (6×150 mm). Flow rate: 1.0 ml/min. Eluent: MeOH–water (0.2% $AcONH_4$).

(200 mg) in *N,N*-dimethylacetamide (3 ml) was added pivaloyloxymethyl iodide (90 mg) under ice-cooling. After being stirred at room temperature for 10 min, the resulting solution was diluted with water (30 ml) and kept at room temperature for 18 h. The solution was charged on a CHP-20P column and eluted successively with water, 3% CH_3CN, 6% CH_3CN and 10% CH_3CN. A fraction eluted with 10% CH_3CN was concentrated and chromatographed on an RP-8 Lobar column. The column was eluted successively with 7.5% MeOH, 10% MeOH and 12.5% MeOH. The desired fractions were combined and concentrated. Lyophilization from aqueous solution gave **14** (31 mg) as a colorless powder. ^{1}H-NMR (D_2O) δ: 2.28 (3H, s, =–CH_3), 2.95 (2H, t, J=5.8 Hz, =–CH_2), 3.66 (2H, t, J=5.8 Hz, CH_2OH), 3.80 (3H, s, OCH_3), 4.46 (1H, s, 4-CH), 5.02 and 5.17 (2H, ABq, J=15.6 Hz, 3′-CH_2), 5.24 (1H, d, J=3.9 Hz, 6-CH), 5.32 (1H, d, J=3.9 Hz, 7-CH), 6.32 (1H, s, thiazole-5-H), 6.89 (1H, s, thiazolio-2-H). IR (KBr): 1762, 1667, 1625, 1533, 1359, 1036 cm^{-1}.

Lipophilicity of POM Esters POM ester was dissolved in *n*-octanol (*ca.* 500 μg/ml). This solution (1 ml) and phosphate buffer (1 ml, 1/20 M, pH 6.86) were shaken at 25 °C. The mixture was centrifuged to separate the phases. The concentration of the ester in each phase was determined by HPLC. The organic phase was diluted 100—1000 times with MeOH before analysis. The partition coefficient (P) was obtained by dividing the concentration in the organic phase by that in the aqueous phase. HPLC conditions are listed in Table II.

Stability of POM Esters i) Chemical Stability: A solution of POM ester (10 mg/ml) in *N,N*-dimethylformamide was added to phosphate buffer (1/20 M, pH 6.86) preincubated at 37 °C, and the mixture was stirred at 37 °C. The initial concentration of POM ester was about 100 μg/ml. Samples were taken at suitable intervals. The concentrations of the remaining POM ester were determined by HPLC.

(ii) Biological Stability: A solution of POM ester (5 mg/ml) in EtOH was added to 1% homogenate of mouse small intestine in phosphate buffer (1/20 M, pH 6.86) preincubated at 37 °C, and the mixture was stirred at 37 °C. The intial concentration of POM ester was about 100 μg/ml. Samples were taken at suitable intervals. The concentrations of POM ester that remained and the hydrolysis products were determined by HPLC. HPLC conditions are listed in Table II.

Oral Absorption of POM Esters POM ester was dissolved in polyethyleneglycol (PEG-400) and diluted with an equal volume of water. The solution was administered orally to slc ddY mice (male, n=5, 50 mg/kg as a parental cephalosporin). Mice were given free access to water but were fasted overnight before administration. Excretion of the parent cephalosporin **1** into urine and feces after administration was determined by bioassay using *Bacillus subtilis* ATCC 6633 as a test strain.

Detection of 3-Spiro Cephalosporin 11 in Urine and Feces Water extract from feces was injected to column A. Eluate in 11.4—12.8 min was collected and injected to column B. The chromatogram was monitored at 254 nm.

Condition of column A: TOSO G-2000 SW (7.5 mm i.d. × 300 mm), potassium phosphate buffer (50 mM, pH 7.4), 1.0 ml/min. Condition of column B: YMC ODS A-312 (6 mm i.d. × 150 mm), MeOH–Water (5 mM PIC-A) (40 : 60), 1.0 ml/min.

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