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Studies on Prodrugs. VIII.[1] Preparation and Characterization of (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Esters of Sulbactam and Its Analogs

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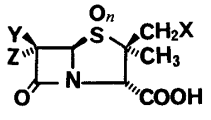
Several (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl esters of β-lactamase inhibitors were prepared and evaluated for oral absorbability. Sulbactam (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (**5a**) was found to produce a 5-fold higher serum level of sulbactam than sulbactam itself after oral administration to mice. The diester (**15**), in which ampicillin is bonded to the 5-methyl group of the above sulbactam ester (**5a**), was also prepared, but this diester (**15**) did not produce high serum levels of ampicillin and sulbactam after oral administration to mice.

Keywords——β-lactamase inhibitor; prodrug; promoiety; (5-methyl-2-oxo-1,3-dioxol-4-yl)-methyl ester; sulbactam prodrug; mutual prodrug; oral absorbability

β-Lactam antibiotics are by far the most widely used antibiotics. Recently, however, the problem of resistant bacteria has come to the fore. It is known that the resistance of bacteria to β-lactam antibiotics is due mainly to the action of β-lactamase produced by these bacteria. The discovery of a β-lactamase inhibitor, clavulanic acid,[2] has provided a new way to overcome resistant bacteria, and subsequently many semisynthetic β-lactam derivatives (Chart 1) have been explored as candidate β-lactamase inhibitors. Among these semisynthetic inhibitors, sulbactam has been studied clinically in combination with ampicillin or cefoperazone by parenteral administration. Sulbactam is very poorly absorbed upon oral administration, so the prodrug approach has been examined to overcome this difficulty, and sulbactam pivaloyloxymethyl ester has been proposed.[9] However, since the pivaloyloxymethyl ester is a diester of formaldehyde hydrate, harmful formaldehyde should be liberated after hydrolysis.[10]

Recently we reported a new promoiety, the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group, which was found to be safe in the case of lenampicillin.[11] Several applications of this promoiety have been described.[11–13] If (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl esters of β-lactamase inhibitors are superior or equal to the known prodrugs in oral absorbability, these



n	X	Y	Z		Ref.
2	H	H	H	(sulbactam)	3
2	Cl	H	H	(BL-P 2013)	4
2	Br	H	H		5
2		H	H	(YTR-830)	6
0	H	Br	H		7
0	H	I	H		8

Chart 1

esters should be useful and safe prodrugs. Therefore we attempted to use this promoiety for improving the oral absorbability of β-lactamase inhibitors, sulbactam (**5c**) and its analogs, and also to apply it to a mutual prodrug. In this paper we present the synthesis and characterization of new esters of sulbactam and its analogs.

Chemistry

We selected 6-aminopenicillanic acid (6-APA) (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (**1a**) as the starting material for β-lactamase inhibitor prodrugs, since we have already reported a convenient and practical preparation of **1a**.[14] 6-APA ester (**1a**) was diazotized with sodium nitrite in dichloromethane (CH_2Cl_2) and 2 N sulfuric acid (H_2SO_4) to give the diazo ester (**2a**) as a syrup (unstable at room temperature) in 85% yield. The bromination of **2a** with bromine or 30% hydrobromic acid/acetic acid in CH_2Cl_2 was successful, giving 6,6-dibromopenicillanate (**3a**) in 85% yield or 6α-bromopenicillanate (**8a**) in 61% yield, respectively. 6,6-Dibromopenicillanate (**3a**) also could be prepared in 80% yield through the two-phase (CH_2Cl_2/2 N H_2SO_4) diazotization/bromination of **1a**.[15] The reduction of **3a** with 5% palladium on calcium carbonate was successful to give the penicillanate (**4a**) in 72% yield, but reduction of **3a** over 5% palladium on charcoal was unsuccessful. It is interesting that the difference in the palladium support affects the reduction yield. In this reduction it is necessary to use the same weight of catalyst as that of **3a**. Compound **4a** was also obtained by the similar reduction of **8a**. An attempted reduction of **3a** with zinc powder in acetic acid was unsuccessful, but reduction of **3a** with 1.2 eq of tri-butyltin hydride gave 6β-bromopenicillanate (**6a**) in 40% yield, and reduction with 2.5 eq of tri-butyltin hydride gave **4a** in 78% yield. Compound **6a** was unstable and decomposed at room temperature.

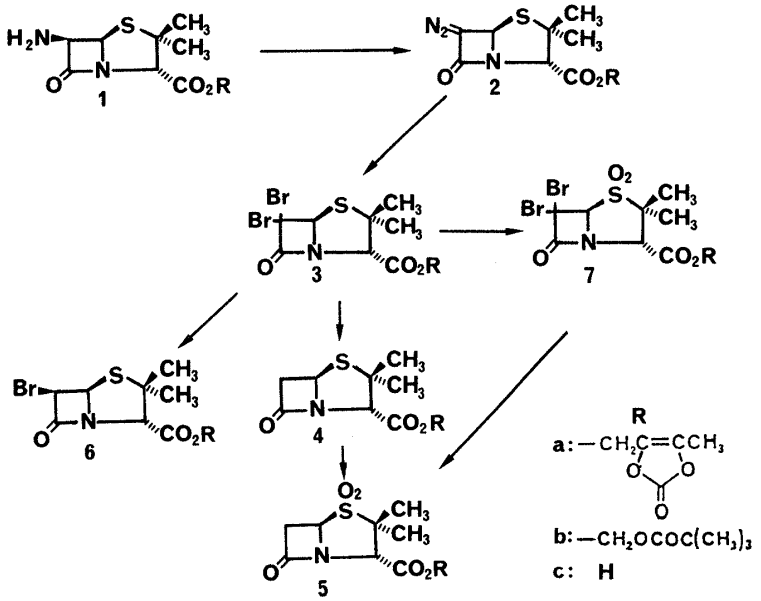


Chart 2

Oxidation of **4a** with 30% hydrogen peroxide and a catalytic amount of sodium tungstate (Na_2WO_4) in acetone gave the new sulbactam prodrug (**5a**) in 75% yield. Compound **5a** could also be obtained by the oxidation of **3a** with *m*-chloroperbenzoic acid, followed by catalytic reduction over 5% palladium on charcoal in 65% yield (from **3a**). Oxidation of **3a** to **7** with 30% hydrogen peroxide and a catalytic amount of Na_2WO_4 was unsuccessful.

As a new β-lactamase inhibitor, 2β-(chloromethyl)-2α-methylpenam-3α-carboxylic acid 1,1-dioxide (**12c**, BL-P 2013) has been reported,[4] so we prepared its (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester as shown in Chart 3. Compound **8a** was converted to the sulfoxide

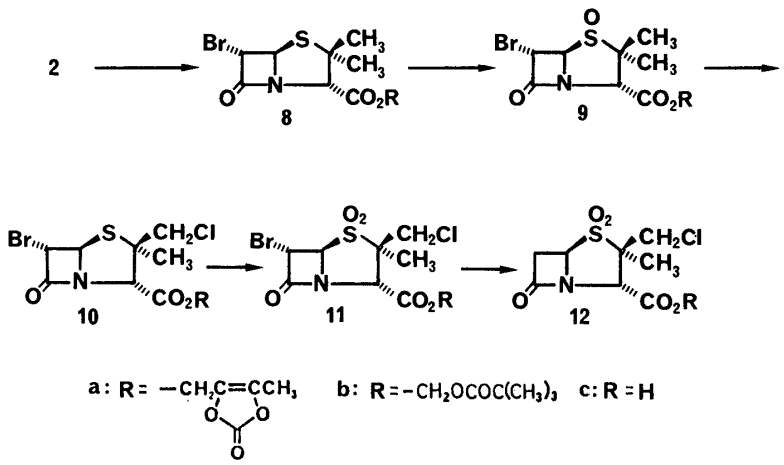


Chart 3

(**9a**) with 30% hydrogen peroxide in ethyl acetate and acetic acid in 81% yield. The rearrangement of **9a** to the 2β-(chloromethyl)penam ester (**10a**) was carried out in 69% yield with benzoyl chloride and quinoline in refluxing dioxane. This rearrangement was also carried out in refluxing tetrahydrofuran in the presence of a catalytic amount of tetramethylammonium chloride. Subsequent oxidation of **10a** with *m*-chloroperbenzoic acid in dichloromethane afforded the dioxide **11a**, reduction of which was carried out with zinc/acetic acid to give the new prodrug (**12a**).

The (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group was stable under the reaction conditions described above. The treatment of the esters **5a** and **12a** with sodium bicarbonate in 50% aqueous acetone gave the parent drugs, **5c** and **12c**, in 65% and 60% yields, respectively. Therefore the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group can be used as a protecting group of the carboxyl group in the chemical reaction.

Mutual prodrugs have been reported as another approach for improving the oral absorbability of β-lactamase inhibitors.[16] The presence of another methyl group in the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group indicates the feasibility of application of this promoiety to a mutual prodrug. Thus, we prepared the di-ester, in which ampicillin and sulbactam are linked to the 4- and 5-methyl groups of 4,5-dimethyl-1,3-dioxol-2-one. Sulbactam (**5c**) was allowed to react with excess 4,5-bis(bromomethyl)-1,3-dioxol-2-one (**16**)[17] in *N*,*N*-dimethylformamide–ethyl acetate to give the mono-ester (**13**). Reaction of **13** with the enamine-protected ampicillin in *N*,*N*-dimethylformamide–ethyl acetate led to the

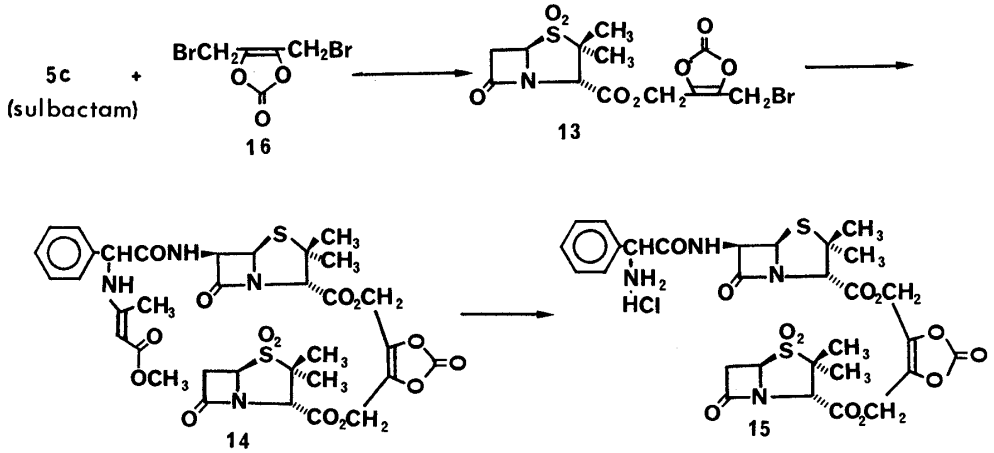


Chart 4

formation of the intermediate (**14**), which, without isolation, was hydrolyzed at pH 2.5 with 2 N hydrochloric acid (HCl) in aqueous acetone to give the di-ester (**15**).

Biological Results and Discussion

The esters (**5a**), (**12a**) and (**15**) were administered orally to mice, and the serum levels of the parent drugs were measured by microbiological assays. The results are shown in Tables I, II and III.

Sulbactam (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (**5a**) was well absorbed orally, and gave a serum level of sulbactam about a 5-fold higher than that after administration of sulbactam itself, and also higher than that after administration of sulbactam pivaloyloxymethyl ester (**5b**).[9] 2β-Chloromethylpenam (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (**12a**) gave a slightly higher serum level of **12c** than **12c** itself; the area under the blood concentration–time curve (*AUC*) value after administration of **12a** was about twice that of **12c**, and a little larger than that of **12b**. In addition, (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester (**12a**) produced a more prolonged serum level than pivaloyloxymethyl ester (**12b**).[4] Thus it has become apparent that the (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl group is a useful promoiety for improving the oral absorption of β-lactamase inhibitors, sulbactam (**5c**) and **12c** (BL-P 2013). A study on the degradation products of **5a** and **12a** *in vivo* was not performed, but it can be predicted that **5a** and **12a** would be hydrolyzed in the same way as the corresponding ampicillin ester, lenampicillin.[18] Thus, the metabolites of the prodrugs should

TABLE I. Serum Concentration (μg/ml) of Sulbactam after Oral Administration[a] of **5** to Mice

Compd.	15	30	60	120 (min)	*AUC* (μg·h/ml)
5a	39.3	23.0	11.8	4.4	29.5
5b	24.5	15.2	7.5	7.0	20.1
5c (sulbactam)	7.2	5.5	5.9	2.6	9.6

a) A dose equivalent to 50 mg/kg of sulbactam.

TABLE II. Serum Concentration (μg/ml) of BL-P2013 after Oral Administration[a] of **12** to Mice

Compd.	15	30	60	120	240 (min)	*AUC* (μg·h/ml)
12a	10.2	10.0	8.5	6.8	4.8	27.7
12b	15.4	13.5	9.2	4.3	2.1	24.4
12c (BL-P2013)	9.1	9.1	6.8	2.9	0.7	15.8

a) A dose equivalent to 100 mg/kg of BL-P2013.

TABLE III. Serum Concentrations (μg/ml) of Sulbactam and Ampicillin after Oral Administration[a] of **15** and Sultamicillin to Mice

Compd.	Concentration of sulbactam				*AUC*	Concentration of ampicillin				*AUC*
	15	30	60	120 (min)	(μg·h/ml)	15	30	60	120 (min)	(μg·h/ml)
15	2.8	2.5	2.4	0.6	3.6	3.5	2.5	1.9	0.3	3.4
Sultamicillin	29.9	14.6	3.4	0.6	15.8	48.1	27.2	8.1	1.0	28.8

a) A dose equivalent to 50 mg/kg of ampicillin.

Table IV. Hydrolysis Ratio (%)[a] of **15** and Sultamicillin in Phosphate Buffer (pH 7.2) and Mouse Serum

	Phosphate buffer		Mouse serum	
	Ampicillin	Sulbactam	Ampicillin	Sulbactam
15	62	69	105	89
Sultamicillin	74	66	99	105

a) Compound **15** and sultamicillin were incubated in phosphate buffer or mouse serum at 37 °C for 10 min, and the concentration of ampicillin and sulbactam were measured by bioassay as described in Experimental, then the hydrolysis ratio was calculated.

Table V. Serum Concentrations (μg/ml) of Sulbactam and Ampicillin after Oral Co-administration[a] of **5a** and Lenampicillin to Mice

Compd.	15	30	60	120 (min)	*AUC* (μg·h/ml)
Sulbactam	30.8	30.7	12.5	8.7	33.0
Ampicillin	42.6	21.2	5.9	1.6	23.8

a) **5a**: A dose equivalent to 50 mg/kg of sulbactam. Lenampicillin: A dose equivalent to 75 mg/kg of ampicillin.

be the parent drug, acetoin and 2,3-butanediol, which are considered to be safe, as in the case of lenampicillin.

On the other hand, the mutual-type ester (**15**) did not produce such high serum levels of ampicillin and sulbactam as sultamicillin and showed poorer bioavailability than ampicillin and sulbactam themselves. In order to study this point, we examined the stability of **15** in mouse serum and phosphate buffer. The mutual-type ester (**15**) was hydrolyzed in mouse serum immediately and liberated ampicillin and sulbactam completely as shown in Table IV. Therefore the reason why **15** did not produce high serum levels of ampicillin and sulbactam was considered to be the lack of oral absorbability of **15**.

A β-lactamase inhibitor is usually used in combination with a β-lactam antibiotic. It is naturally important that the β-lactam antibiotic and β-lactamase inhibitor are present simultaneously at the site of the infection, when the inhibitor is co-administered with the antibiotic. Thus, the sulbactam prodrug (**5a**) was co-administered orally with a new ampicillin prodrug, lenampicillin,[11] to mice, and the serum levels of sulbactam and ampicillin were measured. The results are shown in Table V. The two serum level *versus* time curves are similar. From these data it is evident that these two prodrugs show similar pharmacokinetic properties. Therefore the oral co-administration of **5a** and lenampicillin is anticipated to be effective against ampicillin-resistant strains producing β-lactamase. Detailed pharmacological studies are in progress.

Experimental

Melting points were determined with a Yamato capillary melting point apparatus, model MP-21, and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Nihon Denshi PS-100 NMR spectrometer and a Hitachi R-24A NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Infrared (IR) spectra were recorded with a Shimadzu IR-440 spectrometer. Optical rotations were determined with a JASCO DIP-181 digital polarimeter.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6-Diazopenicillanate (2a)——A solution of sodium nitrite (2.8 g) in water (20 ml) was added to a mixture of **1a** (10 g), CH_2Cl_2 (100 ml) and 5% aqueous H_2SO_4 (80 ml) at 0—5 °C. The mixture was stirred at 0—5 °C for 1 h, then the organic layer was separated and washed with 5% aqueous NaCl (70 ml) and

water (70 ml). After drying over $MgSO_4$ and evaporation *in vacuo*, 6-diazopenicillanate (**2a**) was obtained as a crude syrup (8.8 g, 85% yield). IR ν_{max}^{neat} cm^{-1}: 2090 ($N_2=C$), 1830, 1795, 1760 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.41 (3H, s, 2-CH_3), 1.62 (3H, s, 2-CH_3), 2.19 (3H, s, CH_3–C=C), 4.36 (1H, s, 3-H), 4.90 (2H, s, CH_2–C=C), 6.12 (1H, s, 5-H).

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6,6-Dibromopenicillanate (3a)——A) From 6-Diazopenicillanate (**2a**): Bromine (4.8 g) was added dropwise to a solution of **2a** (10 g) in CH_2Cl_2 (80 ml) at −10—0 °C. The mixture was stirred at 0—5 °C for 1 h, washed with cold 1% aqueous $Na_2S_2O_3$ (50 ml) and 10% aqueous NaCl (50 ml), and dried over $MgSO_4$. Removal of the solvent *in vacuo* gave a yellow syrup, which was crystallized from ethyl acetate to yield 6,6-dibromopenicillanate (**3a**) (11.8 g, 85%). mp 142—145 °C. IR ν_{max}^{KBr} cm^{-1}: 1810, 1790, 1755 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.45 (3H, s, 2-CH_3), 1.64 (3H, s, 2-CH_3), 2.23 (3H, s, CH_3–C=C), 4.56 (1H, s, 3-H), 4.95 (2H, s, –CH_2–C=C), 5.78 (1H, s, 5-H). *Anal.* Calcd for $C_{13}H_{13}Br_2NO_6S$: C, 33.14; H, 2.78; N, 2.97. Found: C, 33.37; H, 2.87; N, 3.17.

B) One Pot Reaction from **1a**: Bromine (1.2 ml) and sodium nitrite (2.8 g) were added successively to a stirred mixture of CH_2Cl_2 (100 ml) and 10% aqueous H_2SO_4 (100 ml), and the mixture was cooled to 0 °C. **1a** (6.6 g) was added portionwise to the mixture below 10 °C, and the whole was stirred at 5 °C for 1 h. Then 10% aqueous $Na_2S_2O_3$ added to the reaction mixture until a persistent brown color disappeared. The separated organic layer was washed with cold 5% aqueous NaCl (80 ml) and dried over $MgSO_4$. Removal of the solvent *in vacuo* gave a yellow syrup, which was crystallized from ethyl acetate to yield **3a** (7.6 g, 80%) as pale yellow crystals.

The physical properties were in accord with those of the product obtained in A).

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Penicillanate (4a)——A mixture of a solution of **3a** (5 g) in ethyl acetate (100 ml), 5% palladium on calcium carbonate (5 g) and a solution of K_2HPO_4 (1 g) in water (50 ml) was hydrogenated at 40 psi of hydrogen for 2 h at room temperature. The catalyst was removed by filtration, and the organic layer was separated, washed with 5% aqueous NaCl and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to give a yellow syrup, which was crystallized from ether–hexane to give **4a** (2.4 g, 72%) as pale yellow crystals. mp 77—79 °C. IR ν_{max}^{KBr} cm^{-1}: 1830, 1780, 1760 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.45 (3H, s, 2-CH_3), 1.68 (3H, s, 2-CH_3), 2.21 (3H, s, CH_3–C=C), 3.09 (1H, dd, J=2 Hz and 16 Hz, 6-H), 3.59 (1H, dd, J=4 Hz and 16 Hz, 6-H), 4.46 (1H, s, 3-H), 4.84 (1H, d, J=15 Hz, CH_2–C=C), 4.99 (1H, d, J=15 Hz, CH_2–C=C), 5.37 (1H, dd, J=2 Hz and 4 Hz, 5-H). *Anal.* Calcd for $C_{13}H_{15}NO_6S$: C, 49.83; H, 4.86; N, 4.47. Found: C, 49.95; H, 4.89; N, 4.45.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6,6-Dibromopenicillanate 1,1-Dioxide (7a)——*m*-Chloroperbenzoic acid (7.1 g) was added to a solution of **3a** (7.6 g) in CH_2Cl_2 (100 ml) under stirring at 0—5 °C. After stirring at room temperature overnight, the insoluble materials were filterd off, and the filtrate was washed with cold 2% aqueous $NaHCO_3$ (80 ml) and cold water successively, then dried over $MgSO_4$. The solvent was removed *in vacuo* to give **7a** (6.8 g, 83.7%) as a syrup. IR ν_{max}^{neat} cm^{-1}: 1820—1785, 1755 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.38 (3H, s, 2-CH_3), 1.60 (3H, s, 2-CH_3), 2.20 (3H, s, CH_3C=C), 4.52 (1H, s, 3-H), 4.98 (3H, CH_2–C=C and 5-H).

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl Penicillanate 1,1-Dioxide (5a)——A) From **4a**: A mixture of a solution of **4a** (5 g) in acetone (50 ml), 30% hydrogen peroxide (10 ml) and a catalytic amount of $Na_2WO_4 \cdot 2H_2O$ was stirred at room temperature for 48 h. After addition of ethyl acetate (80 ml) and water (40 ml), the whole was stirred vigorously for 10 min. The separated organic layer was washed with 5% aqueous NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a pale yellow syrup, which was crystallized from ethanol to yield **5a** (4.1 g, 75%) as colorless crystals. mp 140—142 °C. $[\alpha]_D^{20}$ +168° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1833, 1815, 1795, 1765 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.40 (3H, s, 2-CH_3), 1.62 (3H, s, 2-CH_3), 2.22 (3H, s, CH_3C=C), 3.50 (2H, d, J=4 Hz, 6-H), 4.43 (1H, s, 3-H), 4.62 (1H, t, J=4 Hz, 5-H), 4.97 (2H, s, CH_2–C=C). *Anal.* Calcd for $C_{13}H_{15}NO_8S$: C, 45.22; H, 4.38; N, 4.06. Found: C, 45.28; H, 4.44; N, 4.11.

B) From **7a**, i) Catalytic Hydrogenation Method: A mixture of a solution of **7a** (5 g) in ethyl acetate (100 ml), 5% palladium on charcoal (2 g), K_2HPO_4 (4 g) and water (60 ml) was hydrogenated at 50 psi of hydrogen for 1 h at room temperature. The catalyst was removed by filtration, and the organic layer was separated, washed with 5% aqueous NaCl and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to give crude crystals, which were recrystallized from ethanol to yield **5a** (2.7 g, 80%) as colorless crystals. mp, IR and ^{1}H-NMR were in accord with those of the product obtained in A). *Anal.* Calcd for $C_{13}H_{15}NO_8S$: C, 45.22; H, 4.38; N, 4.06. Found: C, 45.36; H, 4.54; N, 4.20.

ii) Zinc Metal Reduction: A solution of **7a** (2.8 g) in *N*,*N*-dimethylformamide (20 ml) was added to a stirred suspension of zinc powder (0.87 g) and acetic acid (10 ml) at 5 °C, and the mixture was stirred at room temperature for 1 h. After addition of ethyl acetate (60 ml), the insoluble materials were filtered off. The filtrate was washed with water (30 ml), 2% aqueous $NaHCO_3$ (30 ml) and 5% aqueous NaCl (30 ml) successively and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* to give crude crystals, which were recrystallized from ethanol to yield **5a** (1.5 g, 78%) as colorless crystals. mp, IR and ^{1}H-NMR were in accord with those of A). *Anal.* Calcd for $C_{13}H_{15}NO_8S$: C, 45.22; H, 4.38; N, 4.06. Found: C, 45.29; H, 4.44; N, 4.18.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6α-Bromopenicillanate (8a)——A solution of 30% HBr/AcOH (6 ml) in CH_2Cl_2 (15 ml) was added dropwise to a solution of **2a** (6.5 g) in CH_2Cl_2 (70 ml), with stirring at 0 °C. The mixture was stirred at 0—5 °C for 30 min, washed with cold 5% aqueous NaCl (80 ml × 3) and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a syrup, which was purified by column chromatography on silica gel (eluent: chloroform), and crystallized from ether to yield **8a** (4.6 g, 61%) as pale yellow crystals. mp 88—90 °C. IR ν_{max}^{KBr} cm^{-1}: 1815, 1780, 1750. ^{1}H-NMR (in $CDCl_3$) δ: 1.43 (3H, s, 2-CH_3), 1.62 (3H, s, 2-CH_3), 2.19 (3H, s, CH_3C=C), 4.58 (1H,

s, 3-H), 4.72 (1H, d, J=1.5 Hz, 5-H), 4.95 (2H, s, CH_2–C=C) 5.40 (1H, d, J=1.5 Hz, 6-H). *Anal.* Calcd for $C_{13}H_{14}BrNO_6S$: C, 39.81; H, 3.60; N, 3.57. Found: C, 40.01; H, 3.75; N, 3.58.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6α-Bromopenicillanate 1-Oxide (9a)——A mixture of a solution of **8a** (10 g) in ethyl acetate (100 ml), 30% hydrogen peroxide (10 ml), acetic acid (10 ml) and $Na_2WO_4 \cdot 2H_2O$ (10 mg) was stirred at room temperature for 6 h, and then evaporated *in vacuo* to 1/3 volume. The precipitated crystals were collected by filtration and washed with ethyl acetate to give **9a** (8.4 g, 81%) as colorless crystals. mp 120—123 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1820, 1790, 1760 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.22 (3H, s, 2-CH_3), 1.55 (3H, s, 2-CH_3), 2.20 (3H, s, CH_3C=C), 4.48 (1H, s, 3-H), 4.72—5.15 (3H, m, 5-H, CH_2–C=C), 5.38 (1H, d, J=1.5 Hz, 6-H). *Anal.* Calcd for $C_{13}H_{14}BrNO_7S$: C, 38.25; H, 3.46; N, 3.43. Found: C, 38.18; H, 3.21; N, 3.34.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6α-Bromo-2β-(chloromethyl)-2α-methylpenam-3α-carboxylate (10a)——A mixture of **9a** (6.8 g), quinoline (2 ml), benzoyl chloride (2.7 g) and tetra-methylammonium chloride (0.2 g) in tetrahydrofuran (100 ml) was refluxed for 3 h. The reaction mixture was poured into ice-water (80 ml) and extracted with ethyl acetate (100 ml × 2). The organic layer was washed with 5% aqueous $NaHCO_3$ (150 ml), 0.5 N HCl (150 ml) and water successively and dried over $MgSO_4$. After charcoal treatment, the solution was evaporated *in vacuo*. The obtained syrup was crystallized from ethyl acetate–hexane to give **10a** (4.9 g, 69%) as pale yellow needles. mp 115—118 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1820, 1770, 1755, 1740 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.53 (3H, s, 2-CH_3), 2.24 (3H, s, CH_3C=C), 3.53 (1H, d, J=15 Hz, 2-CH_2Cl), 3.68 (1H, d, J=15 Hz, 2-CH_2Cl), 4.83 (1H, d, J=1.5 Hz, 5-H), 4.97 (2H, s, CH_2–C=C), 5.12 (1H, s, 3-H), 5.48 (1H, d, J=1.5 Hz, 6-H). *Anal.* Calcd for $C_{13}H_{13}BrClNO_6S$: C, 36.60; H, 3.07; Br, 18.73; Cl, 8.31; N, 3.28. Found: C, 36.42; H, 2.90; Br, 18.68; Cl, 8.29; N, 3.02.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6α-Bromo-2β-(chloromethyl)-2α-methylpenam-3α-carboxylate 1,1-Dioxide (11a)——A solution of *m*-chloroperbenzoic acid (4 g) in ethyl acetate (30 ml) was added dropwise to a solution of **10a** (3.3 g) in dichloromethane (50 ml), with stirring at 0—5 °C. The mixture was stirred at room temperature for 20 h. After filtration, the filtrate was washed with 2% aqueous $NaHCO_3$ and 5% aqueous NaCl, and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a syrup, which was chromatographed on a column of silica gel with chloroform–methanol (100 : 1, v/v). Crystallization from ethyl acetate gave **11a** as colorless needles (1.6 g, 45%). mp 158—160 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1830, 1805, 1750 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.56 (3H, s, 2-CH_3), 2.24 (3H, s, CH_3C=C), 3.81 (1H, d, J=15 Hz, 2-CH_2Cl), 4.01 (1H, d, J=15 Hz, 2-CH_2Cl), 4.7—4.83 (2H, m, 3-H and 5-H), 5.00 (2H, s, CH_2–C=C), 5.10 (1H, d, J=1.5 Hz, 6-H). *Anal.* Calcd for $C_{13}H_{13}BrClNO_8S$: C, 34.04; H, 2.86; Br, 17.42; Cl, 7.73; N, 3.05. Found: C, 34.06; H, 2.75; Br, 17.14; Cl, 7.55; N, 2.95.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2β-(Chloromethyl)-2α-methylpenam-3α-carboxylate 1,1-Dioxide (12a)——A solution of **11a** (500 mg) in *N*,*N*-dimethylformamide (10 ml) was added dropwise to a mixture of zinc powder (100 mg) and acetic acid (3 ml), with stirring at 5 °C. The mixture was stirred at 5 °C for 3 h, then ethyl acetate (50 ml) was added, and the insoluble materials were filtered off. The filtrate was washed with 0.5 N HCl and water, and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a syrup, which was chromatographed on a column of silica gel with chloroform–methanol (100 : 1, v/v). Crystallization from ethyl acetate–ether gave **12a** as colorless crystals (300 mg, 70%). mp 70—75 °C. $[\alpha]_D^{20}$ +95° (c=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1825, 1805, 1760 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.58 (3H, s, 2-CH_3), 2.23 (3H, s, CH_3C=C), 3.50—3.61 (2H, m, 6-H), 3.87 (1H, d, J=16 Hz, 2-CH_2Cl), 4.10 (1H, d, J=16 Hz, 2-CH_2Cl), 4.64—4.73 (2H, m, 3-H and 5-H), 4.99 (2H, s, CH_2–C=C). *Anal.* Calcd for $C_{13}H_{14}ClNO_8S$: C, 41.11; H, 3.72; Cl, 9.34; N, 3.69. Found: C, 41.25; H, 3.70; Cl, 9.51; N, 3.71.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 6β-Bromopenicillanate (6a)——A solution of tri-butyltin hydride (1.48 g) in toluene (10 ml) was added to a solution of **3a** (2 g) and 2,2′-azobisisobutyronitrile (0.1 g) in toluene (70 ml) with stirring at 80 °C. The mixture was stirred at 80 °C for 2 h, and then evaporated *in vacuo* to give an oil, which was chromatographed on a silica gel using ethyl acetate–cyclohexane (1 : 1, v/v) as the eluent. **6a** (0.87 g, 53%) was obtained as a syrup. IR ν_{max}^{KBr} cm^{-1}: 1825, 1805, 1760 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.46 (3H, s, 2-CH_3), 1.67 (3H, s, 2-CH_3), 2.18 (3H, s, CH_3C=C), 4.51 (1H, s, 3-H), 4.93 (2H, s, CH_2), 5.35 (1H, d, J=5 Hz, 5-H), 5.57 (1H, d, J=5 Hz, 6-H).

(5-Bromomethyl-2-oxo-1,3-dioxol-4-yl)methyl Penicillanate 1,1-Dioxide (13)——A solution of 4,5-bis(bromomethyl)-1,3-dioxol-2-one (**16**, 10 g) in ethyl acetate (10 ml) was added dropwise to a mixture of sulbactam (3 g), $KHCO_3$ (3 g) and NaI (0.05 g) in ethyl acetate (60 ml) and *N*,*N*-dimethylformamide (20 ml) at 5 °C, and the mixture was stirred at room temperature for 8 h. After addition of cold water (30 ml), the mixture was stirred vigorously, and the organic layer was separated, washed with 5% aqueous NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a syrup, which was chromatographed on silica gel using ethyl acetate–cyclohexane (1 : 1, v/v) as the eluent to give 3.2 g of **13** (yield 61.5%). IR ν_{max}^{KBr} cm^{-1}: 1830, 1800, 1750 (C=O). ^{1}H-NMR (in $CDCl_3$) δ: 1.37 (3H, s, 2-CH_3), 1.56 (3H, s, 2-CH_3), 3.4 (2H, m, 6H), 4.27 (2H, s, $BrCH_2C$=C), 4.38 (1H, s, 3-H), 4.6 (1H, m, 5-H), 5.00 (2H, s, CO_2CH_2).

4-(6-(2-Amino-2-phenylacetamido)penicillanoyl)oxymethyl-5-(1,1-dioxopenicillanoyl)oxymethyl-1,3-dioxol-2-one Hydrochloride (15)——Enamine-protected ampicillin potassium salt[16] (4.2 g) was added to a mixture of **13** (3.2 g), $KHCO_3$ (0.2 g) and NaI (0.1 g) in ethyl acetate (60 ml) and *N*,*N*-dimethylformamide (20 ml) at 5 °C. The mixture was stirred at room temperature for 5 h. After addition of cold water (30 ml), the whole was stirred vigorously for 10 min. The organic layer was separated, washed with 5% aqueous NaCl (30 ml × 2) and concentrated *in vacuo* to give a

syrup. This syrup was dissolved in acetone (40 ml) and water (20 ml), and the pH of the solution was adjusted to 2.0 with 1 N HCl. The solution was stirred at pH 2 below 10 °C for 30 min, and then water (50 ml) was added, and the mixture was concentrated *in vacuo* to remove acetone. The aqueous layer was washed with ethyl acetate (30 ml × 2), and then saturated with NaCl, and the separated oil was extracted with ethyl acetate–acetone (40 ml–20 ml). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resultant residue was crystallized from 2-butanone to give 2.5 g of **15** as colorless crystals (45.4%). $[\alpha]_D^{20}$ +173° (c=1.0, CH_3OH). IR ν_{max}^{KBr} cm^{-1}: 1830, 1785, 1760, 1690 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.37—1.50 (12H, m, 2-CH_3, 2′-CH_3), 3.25 (1H, dd, J=1.5 Hz, 16 Hz, 6′-H), 3.72 (1H, dd, J=4 Hz, 16 Hz, 6′-H), 4.39 (1H, s, 3′-H), 4.50 (1H, s, 3-H), 5.1—5.36 (6H, m, CHPh, 5′-H, CH_2C=C), 5.4—5.64 (2H, m, 5-H, 6-H), 7.3—7.6 (5H, m, Ph), 8.9 (3H, NH_3), 9.38 (1H, d, J=7 Hz, NHCO). *Anal.* Calcd for $C_{29}H_{33}ClN_4O_{12}S_2$: C, 47.77; H, 4.56; Cl, 4.86; N, 7.68; S, 8.79. Found: C, 47.74; H, 4.81; Cl, 4.99; N, 7.44; S, 8.53.

Alkaline Hydrolysis of 5a——A solution of **5a** (0.5 g) in acetone (50 ml) was added to a solution of $NaHCO_3$ (0.12 g) in water (50 ml) at 5 °C. The solution was stirred at 5—10 °C for 4 h, while maintaining the pH at 9. Then ethyl acetate (60 ml) and NaCl (20 g) were added, and the mixture was stirred vigorously at 5 °C. The pH of the mixture was adjusted to 1.5 with 1 N HCl. The organic layer was separated, washed with cold saturated aqueous NaCl, dried over $MgSO_4$ and concentrated *in vacuo*. The resultant residue was crystallized from ethyl acetate to give 0.22 g (yield 65%) of sulbactam as colorless crystals. mp 156 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1780, 1760, 1750 (C=O). ^{1}H-NMR (in DMSO-d_6) δ: 1.40 (3H, s, 2-CH_3), 1.50 (3H, s, 2-CH_3), 3.25 (1H, dd, J=1.5 Hz, 16 Hz, 6-H), 3.68 (1H, dd, J=4.5 Hz, 16 Hz, 6-H), 4.27 (1H, s, 3-H), 5.10 (1H, dd, J=1.5 Hz, 4.5 Hz, 5-H). *Anal.* Calcd for $C_8H_{11}NO_5S$: C, 41.20; H, 4.76; N, 6.00; S, 13.75. Found: C, 41.38; H, 4.70; N, 6.06; S, 13.58.

Oral Absorption Test——A) **5**: An aqueous solution or suspension of a sulbactam ester (**5a**, **b**) or sulbactam was given to groups of five fasted male ddY mice (about 22 g body weight) at a dose of 50 mg equivalent of sulbactam per kg body weight. Blood was taken from the cut axilla region at 15, 30, 60, and 90 min after dosing, and allowed to stand for 30 min at 0 °C. The serum was obtained by centrifugation. Serum specimens obtained at the same time were combined and assayed on the day of sampling. Concentrations of sulbactam were measured by bioassay using *S. typhimurium* TA100 as a test organism on an ordinary nutrient agar medium containing ampicillin.

B) **12**: A suspension of **12** in 0.5% sodium carboxymethyl cellulose was given to a group of five fasted male ddY mice at a dose of 100 mg equivalent of BL-P 2013 per kg body weight. Concentrations of BL-P 2013 were measured in the same way as in A).

C) **15**: A solution of **15** and sultamicillin hydrochloride[16] in 0.5% sodium carboxymethyl cellulose was given to a group of three fasted male ddY mice at a dose of 50 mg equivalent of ampicillin per kg body weight. Concentrations of sulbactam were measured in the same way as in A). Those of ampicillin were measured by bioassay using *B. subtilis* ATCC 6633 as a test organism.

D) Co-administration of **5a** and Lenampicillin Hydrochloride: A suspension of **5a** (at a dose of 50 mg equivalent of sulbactam per kg body weight) and lenampicillin hydrochloride (at a dose of 75 mg equivalent of ampicillin per body weight) was given to a group of five fasted male ddY mice. Concentrations of sulbactam and ampicillin were measured in the same ways as mentioned above.

References

1) Part VII: H. Kondo, F. Sakamoto, K. Kawakami and G. Tsukamoto, *J. Med. Chem.*, **31**, (1988) in press.
2) T. T. Howarth, A. G. Brown and T. J. King, *J. Chem. Soc., Chem. Commun.*, **1976**, 266.
3) A. R. English, J. A. Retsema, A. E. Girard, J. E. Lynch and W. E. Barth, *Antimicrob. Agents Chemother.*, **14**, 414 (1978).
4) W. J. Gottstein, L. B. Crast, Jr., R. G. Graham, U. J. Haynes and D. N. McGregor, *J. Med. Chem.*, **24**, 1531 (1981).
5) W. J. Gottstein, U. J. Haynes and D. N. McGregor, *J. Med. Chem.*, **28**, 518 (1985).
6) A. G. Brown and S. M. Roberts (ed.), "Recent Advances in the Chemistry of β-Lactam Antibiotics," The Royal Society of Chemistry, London, 1985, pp. 242—254; R. G. Micetich, S. N. Maiti, P. Spevak, T. W. Hall, S. Yamabe, N. Ishida, M. Tanaka, T. Yamazaki, A. Nakai and K. Ogawa, *J. Med. Chem.*, **30**, 1469 (1987).
7) V. Knott-Hunziker, B. S. Orlek, P. G. Sammes and S. G. Waley, *Biochem. J.*, **177**, 365 (1979).
8) J. E. G. Kemp, M. D. Closier, S. Narayanaswami and M. H. Stefaniak, *Tetrahedron Lett.*, **21**, 2991 (1980).
9) G. Foulds, W. E. Barth, J. R. Bianchine, A. R. English, D. Girard, S. L. Hayes, M. M. O'Brien and P. Somani, "Current Chemotherapy and Infectious Disease," The American Society for Microbiology, Washington, D.C., 1980, p. 353.
10) H. Ferres, *Drugs of Today*, **19**, 499 (1983).
11) F. Sakamoto, S. Ikeda and G. Tsukamoto, *Chem. Pharm. Bull.*, **32**, 2241 (1984); *idem*, *Chemotherapy* (Tokyo), **32**, supplement 8 (1984).
12) F. Sakamoto, S. Ikeda, R. Hirayama, M. Moriyama, S. Sotomura and G. Tsukamoto, *Chem. Pharm. Bull.*, **35**, 642 (1987).

13) F. Sakamoto, S. Ikeda, H. Kondo and G. Tsukamoto, *Chem. Pharm. Bull.*, **33**, 4870 (1985).
14) S. Ikeda, F. Sakamoto, H. Kondo, M. Moriyama and G. Tsukamoto, *Chem. Pharm. Bull.*, **32**, 4316 (1984).
15) R. A. Volkmann, R. D. Carroll, R. B. Drolet, M. L. Elliott and B. S. Moore, *J. Org. Chem.*, **47**, 3344 (1982).
16) B. Baltzer, E. Binderup, W. von Daehne, W. O. Godtfredsen, K. Hansen, B. Nielsen, H. Sørensen and S. Vangedal, *J. Antibiot.*, **33**, 1183 (1980).
17) H. D. Scharf and H. Plum, *Justus Liebigs Ann. Chem.*, **1977**, 27.
18) N. Awata, K. Noumi, Y. Uemura, A. Takagi and T. Aoyama, *Jpn. J. Antibiot.*, **38**, 1776 (1985).