Synthesis and Antibacterial Activity of Lactivicin Derivatives[1)]

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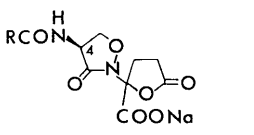
The chemical modification of the 4-acetylamino group on the cycloserine moiety of lactivicin (1a) was carried out. The lactivicin derivatives (1d, k—p and w) having heterocyclic acylamino groups which have been often utilized in β-lactam antibiotics showed potent antibacterial activities. Ester prodrugs (7a—d) of lactivicin derivatives were also prepared in order to improve the bioavailability on oral administration. The pivaloyloxymethyl (POM) esters (7a and 7b) and 1-ethoxycarbonyloxyethyl (EOE) ester (7c) were found to have slightly improved protective effect *in vivo* compared with their parent compounds 1c and 1k.

Keywords lactivicin; chemical modification; synthesis; antibacterial activity; (*S*)-cycloserine derivative; ester prodrug

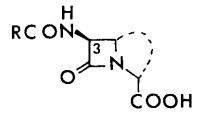
Lactivicin (**1a**) {2-[(4*S*)-acetylamino-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylic acid} is a new antibiotic isolated from *Empedobacter lactamgenus* YK-258 and *Lysobacter albus* YK-422.[2,3)] This compound has a unique structure which consists of two 5-membered rings, (*S*)-cycloserine and γ-lactone, as shown in Chart 1. Although lacking a β-lactam moiety in its structure, lactivicin showed β-lactam-like biological activities; affinity for penicillin binding proteins and susceptibility to β-lactamases.[2,3)] These observations suggested that the mode of antibacterial actions of lactivicin is similar to that of β-lactam antibiotics, *i.e.* penicillins and cephalosporins.[4)]

Thus, comparison of the chemical structures of **1a** and the β-lactams **2** (Chart 1) led us to speculate that the acetylamino group at the C-4 position of the cycloserine ring in **1a** corresponds to the acylamino group at the C-3 position of the β-lactam ring in **2**. The history of the chemical modification of β-lactam antibiotics has demonstrated that alteration of the 3-acylamino group produces dramatic changes in antibacterial activities.[5)] Therefore, we have carried out the modification of the acyl group on the C-4 amino group of lactivicin (**1a**) in the hope of obtaining new antibiotics with a better antibacterial spectrum and activity.

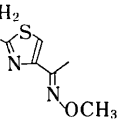
In a previous report we described the synthesis of phenylacetylamino (**1b**)[3,6)] and 2-(2-amino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetylamino (**1c**)[6)] derivatives which showed improved activities compared with **1a**. Furthermore, the cycloserine and γ-lactone moieties in **1** were both considered to play an important role in its activity on the basis of the fact that the aza analog (**3**)[7)] and γ-lactam analog (**4**)[8)] showed decreased activities.

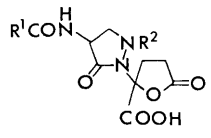


1



2

a: R = CH_3 (lactivicin)
b: R = $PhCH_2$
c: R = 



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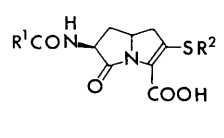


4

Chart 1

This report describes the synthesis and antibacterial activities of a wide variety of 4-modified lactivicin derivatives (**1d—y**), and some ester derivatives (**7a—d**) which were prepared with the aim of finding the orally active compounds.

Synthesis

Chart 2 shows the synthetic route to a variety of 4-acylamino derivatives (**1d—y**) starting from lactivicin (**1a**). After protection of the carboxyl group of lactivicin with a benzhydryl group, the acetyl group was removed with phosphorus pentachloride to afford compound **5**.[3,6,9)] Compound **5** is the key intermediate for the synthesis of various acylamino derivatives (**1d—y**, Table I).

The acylation of the amino group of compound **5** to give **6** was performed with carboxylic acids in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) in dimethylformamide (DMF) (method A), with acyl chloride in the presence of dimethylacetamide (DMA) in dichloromethane (method B), with acyl chloride in the presence of $NaHCO_3$ in water–ethyl ether (method C) or with benzthiazolylthioester[10)] in DMF–tetrahydrofuran (THF) (method D). The benzhydryl group of the resulting acylated compounds **6** was removed by the following methods. Method E: Hydrogenolysis in the presence of 5% palladium–charcoal in THF–phosphate buffer (pH 7.0). Method F: Hydrogenolysis in the presence of 5% (or 10%) palladium–charcoal in water–ethyl acetate. Method G: Hydrogenolysis in the presence of palladium oxide–palladium black in THF–*n*-butanol. Method H: Treatment with trifluoroacetic acid. Method I: Treatment with formic acid in dichloromethane.

The aminothiazolyl derivative (**1m**) was prepared *via* acylation of **5** with chloroacetylaminothiazolylacetic acid (method A) followed by the removal of the chloroacetyl group with sodium *N*-methyl dithiocarbamate in THF–water (method J), and subsequent deprotection (method E). The thioacylamino derivatives (**1v—x**) were synthesized *via* the three steps; chloroacetylation of **5** (method B) to **6** (R = $ClCH_2$), treatment with thiol derivatives (method K) and removal of the benzhydryl group (method H).

In addition, we were interested in the antibacterial

TABLE I. Sodium 2-(4-Acylamino-3-oxo-2-isoxazolidinyl)-5-oxo-2-tetrahydrofurancarboxylate **1**

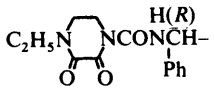
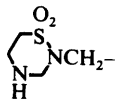
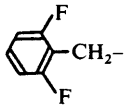
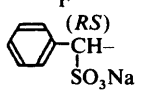
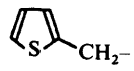
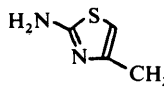
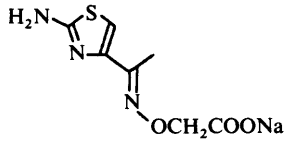
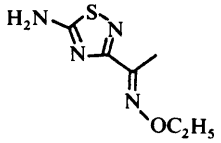
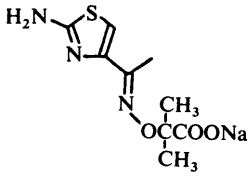
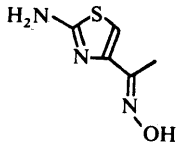
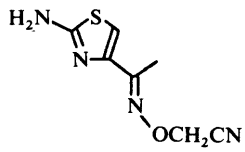
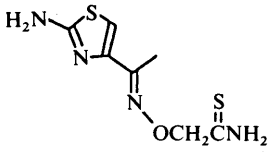
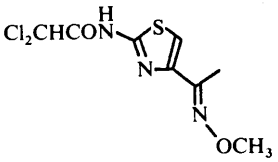
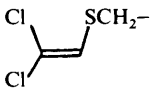
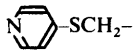
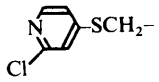
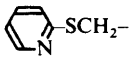
Compound	R	Analysis Calcd/Found	IR ν_{max} cm^{-1} (KBr)	^{1}H-NMR δ (ppm) (D_2O)
1d		C: 45.47/45.15 H: 4.98/ 4.76 N: 11.55/11.83 ($3H_2O$)	1780, 1720, 1690, 1650	1.14 (3H, t, J=7 Hz), 3.3—3.6 (2H, m), 3.44 (2H, q, J=7 Hz), 3.8—4.1 (2H, m), 4.2—4.6 (2H, m), 4.7—5.1 (1H, m), 5.55 (1H, d, J=7 Hz), 7.2—7.6 (5H, m), 9.26 (1H, br), 9.82 (1H, d, J=7 Hz)
1e	F_2CH–	C: 34.50/34.81 H: 3.18/ 3.42 N: 8.05/ 8.37 (H_2O)	1780, 1720, 1650	2.35—3.32 (4H, m), 4.20—4.41 (1H, m), 4.55—4.87 (1H, m), 5.07—5.21 (1H, m), 5.71, 6.25, 6.77 (1H, m)
1f	$ClCH_2CH_2$–	C: 36.63/36.36 H: 3.91/ 3.89 N: 7.77/ 7.76 (H_2O)	1780, 1720, 1650	2.22—3.31 (4H, m), 2.83 (2H, t, J=7 Hz), 3.86 (2H, t, J=7 Hz), 4.10—4.35 (1H, m), 4.51—4.82 (1H, m), 4.98—5.23 (1H, m)
1g		C: 34.29/34.49 H: 4.42/ 4.48 N: 12.30/12.60 ($1.5H_2O$)	1780, 1720, 1660	2.41—3.72 (8H, m), 4.08 (2H, s), 4.18—4.41 (1H, m), 4.48 (2H, s), 4.62—4.89 (1H, m), 5.01—5.30 (1H, m)
1h	$NCCH_2$–	C: 39.17/39.49 H: 3.59/ 3.59 N: 12.46/12.50 (H_2O)	1775, 1720, 1660	2.41—3.41 (4H, m), 4.00 (2H, s), 4.31—4.60 (1H, m), 4.81—5.09 (1H, m), 5.19—5.48 (1H, m)
1i		C: 45.29/45.08 H: 3.56/ 3.30 N: 6.60/ 6.62 (H_2O)	1780, 1720, 1650	2.44—3.28 (4H, m), 2.84 (2H, s), 4.10—4.36 (1H, m), 4.57—4.83 (1H, m), 4.98—5.22 (1H, m), 7.08—7.54 (3H, m)
1j		C: 37.14/37.21 H: 3.70/ 3.66 N: 5.41/ 5.65 ($2.5H_2O$)	1780, 1720, 1660	2.55—3.42 (4H, m), 4.32—4.59 (1H, m), 4.80—5.09 (1H, m), 5.31 (1H, s), 5.21—5.46 (1H, m), 7.61—7.92 (5H, m)
1k		C: 42.64/42.54 H: 3.83/ 3.94 N: 7.10/ 7.29 (H_2O)	1780, 1720, 1650	2.41—3.42 (4H, m), 4.11 (2H, s), 4.29—4.54 (1H, m), 4.79—5.05 (1H, m), 5.13—5.43 (1H, m), 7.24—7.81 (3H, m)
1l		C: 35.70/35.82 H: 4.14/ 4.26 N: 12.81/12.75 ($2.5H_2O$)	1780, 1720, 1660	2.10—3.50 (4H, m), 3.60—5.50 (3H, m), 6.23 (1H, s), 6.83 (2H, br), 8.45, 8.60 (each 0.5H, d, J=7 Hz)
1m		C: 32.44/32.43 H: 3.45/ 3.18 N: 12.61/12.58 ($3H_2O$)	1780, 1730, 1660, 1610	2.10—3.50 (4H, m), 3.60—5.50 (3H, m), 4.30 (2H, s), 6.85, 6.86 (each 0.5H, s), 7.20 (2H, br) (DMSO-d_6)
1n		C: 34.57/34.36 H: 3.93/ 4.02 N: 17.28/16.88 ($2H_2O$)	1780, 1720, 1650	1.53 (3H, t, J=7 Hz), 2.59—3.53 (4H, m), 4.39—4.71 (3H, m), 4.98—5.17 (1H, m), 5.32—5.61 (1H, m)
1o		C: 35.55/35.42 H: 3.86/ 3.81 N: 12.19/11.97 ($2.5H_2O$)	1780, 1730, 1650	1.71 (6H, s), 2.57—3.51 (4H, m), 4.32—4.71 (1H, m), 4.91—5.17 (1H, m), 5.31—5.59 (1H, m), 7.27 (1H, s)
1p		C: 33.48/33.41 H: 3.67/ 3.42 N: 15.02/14.73	1780, 1720, 1690, 1650	2.61—3.53 (4H, m), 4.31—4.68 (1H, m), 4.83—5.12 (1H, m), 5.31—5.62 (1H, m), 7.20 (1H, s)
1q		C: 35.39/35.09 H: 3.64/ 3.30 N: 16.51/16.23 ($2.7H_2O$)	1780, 1720, 1660	2.30—3.31 (4H, m), 4.19—4.42 (1H, m), 4.67—4.91 (1H, m), 5.01 (2H, s), 5.09—5.34 (1H, m), 7.13 (1H, s)

TABLE I. (continued)

Compound	R	Analysis Calcd/Found	IR ν_{max} cm^{-1} (KBr)	^{1}H-NMR δ (ppm) (D_2O)
1r		C: 32.32/32.65 H: 3.98/ 3.71 N: 15.07/14.78 ($3.5H_2O$)	1780, 1720, 1660	2.31—3.20 (4H, m), 4.16—4.42 (1H, m), 4.61—4.88 (1H, m), 5.0 (2H, s), 5.07—5.31 (1H, m), 7.11 (1H, s)
1s		C: 33.52/33.42 H: 2.99/ 2.75 N: 12.22/12.07 ($1.5H_2O$)	1780, 1710, 1660	2.45—3.27 (4H, m), 4.08 (3H, s), 4.27—4.54 (1H, m), 4.71—5.01 (1H, m), 5.15—5.47 (1H, m), 6.57 (1H, s), 7.69 (1H, s)
1t		C: 36.42/35.95 H: 3.31/ 3.34 N: 7.08/ 7.05 ($0.5H_2O$)	1780, 1720, 1650	2.45—3.30 (4H, m), 3.64 (2H, s), 4.02—4.33 (1H, m), 4.61—4.82 (1H, m), 4.97—5.24 (1H, m), 6.33 (1H, d, J=6 Hz), 6.58 (1H, d, J=6 Hz)
1u		C: 33.85/33.93 H: 2.72/ 2.84 N: 6.58/ 6.88 ($0.25H_2O$)	1780, 1730, 1650	2.36—3.35 (4H, m), 3.64 (2H, s), 4.07—4.35 (1H, m), 4.61—4.82 (1H, m), 4.97—5.22 (1H, m), 6.70 (1H, s)
1v		C: 41.17/41.39 H: 4.10/ 3.92 N: 9.60/ 9.30 ($1.9H_2O$)	1780, 1730, 1660	2.41—3.42 (4H, m), 4.22 (2H, s), 4.15—4.50 (1H, m), 4.73—5.02 (1H, m), 5.12—5.48 (1H, m), 7.59 (2H, m), 8.61 (2H, m)
1w		C: 39.53/39.58 H: 3.32/ 3.46 N: 9.22/ 9.49 (H_2O)	1780, 1720, 1650	2.60—3.43 (4H, m), 4.12—4.53 (1H, m), 4.20 (2H, s), 4.75—5.02 (1H, m), 5.13—5.40 (1H, m), 7.42—7.61 (2H, m), 8.40 (1H, d, J=5 Hz)
1x		C: 42.76/42.55 H: 3.83/ 3.66 N: 9.97/10.08 (H_2O)	1780, 1730, 1650	2.48—3.47 (4H, m), 4.09—4.42 (1H, m), 4.13 (2H, m), 4.63—4.93 (1H, m), 5.08—5.42 (1H, m), 7.35—8.80 (4H, m)
1y	—	Not analyzed	1785, 1730, 1695, 1660	2.7—3.8 (4H, m), 3.79 (3H, s), 4.36 (2H, s), 4.90 (1H, d, J=10 Hz), 5.28 (1H, d, J=10 Hz)

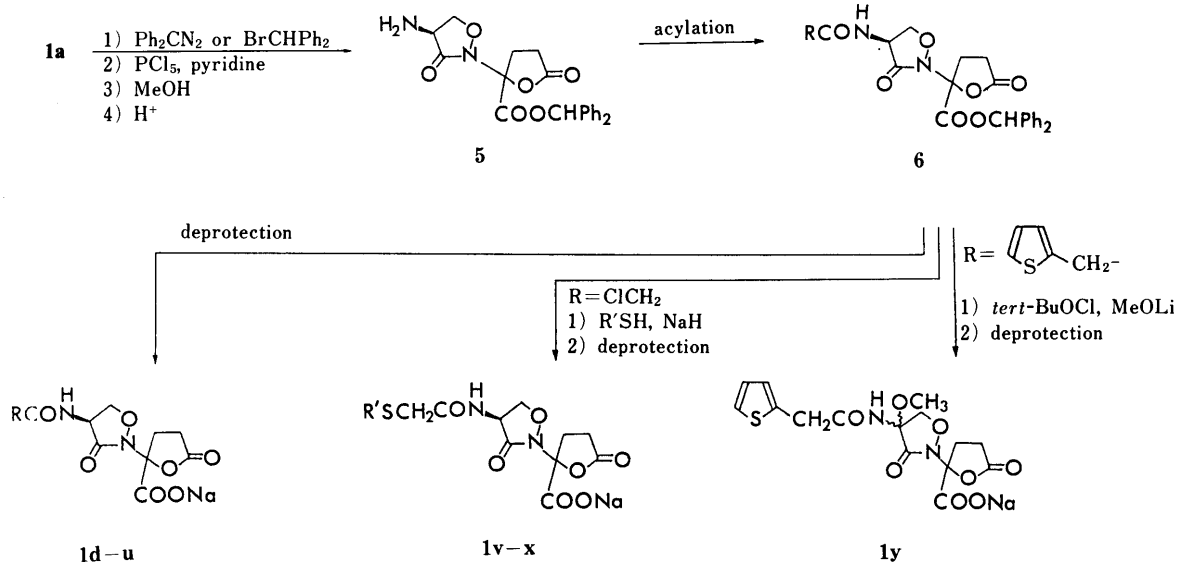
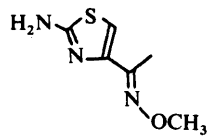
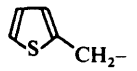
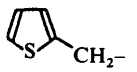
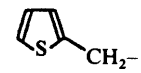


Chart 2

activity of the 4-methoxy derivative **1y** because improved stability to β-lactamases was reported in the case of the methoxy derivatives of cephalosporins[11] and monobactams.[12] Thienylacetylation of **5** (method C) followed by introduction of a methoxy group at the 4-position by reaction with *tert*-butylhypochlorite and lithium methoxide in THF, then deprotection (method G) afforded the desired **1y**.

Next, our attention was directed to synthesis of esters of lactivicin derivatives. The utility of ester prodrugs in antibiotic therapeutics has been established with bacampicillin[13] or pivmecillinam,[14] which showed improved bio-

TABLE II. Prodrug Esters of Lactivicin Derivatives 7

Compound	R	R′	Yield (%)	IR ν_{max} cm^{-1} (C=O)	^{1}H-NMR ($CDCl_3$) δ
7a		POM	55	1800, 1750, 1670	1.19 (9H, s), 2.31—3.39 (4H, m), 3.91 (3H, s), 4.05—4.40 (1H, m), 4.61—5.38 (2H, m), 5.71—6.09 (2H, br), 5.89 (2H, s), 6.82 (1H, s)
7b		POM	49	1800, 1740, 1660	1.20 (9H, s), 2.29—3.30 (4H, m), 3.78 (2H, s), 3.93—4.20 (1H, m), 4.58—5.28 (2H, m), 5.81 (2H, m), 6.60—7.29 (3H, m)
7c		EOE	21	1800, 1740, 1670	1.26 (3H, t, J=7 Hz), 1.57 (3H, d, J=7 Hz), 2.32—3.50 (4H, m), 3.83 (2H, s), 3.99—4.32 (3H, m), 4.59—5.00 (2H, m), 6.52—6.70 (1H, m), 6.76—6.88 (1H, m), 6.88—7.32 (3H, m)
7d		COE	13	1800, 1750, 1670	1.12—1.99 (16H, m), 2.32—3.51 (4H, m), 3.81 (2H, s), 3.92—4.25 (1H, m), 4.52—5.00 (2H, m), 6.71—6.89 (1H, m), 6.88—7.32 (3H, m)

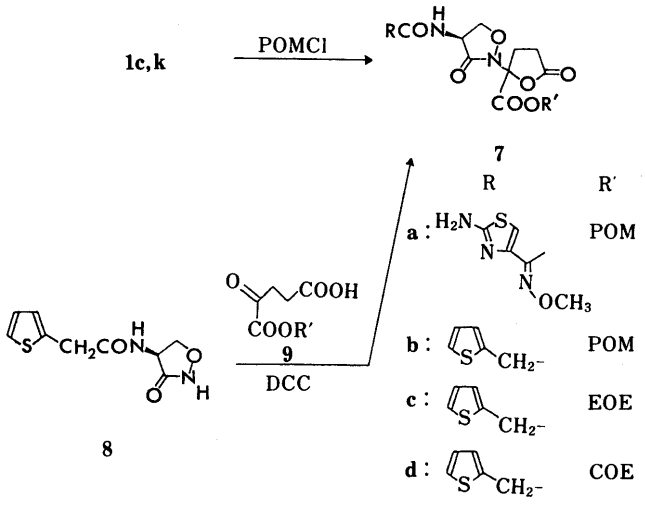


Chart 3

availability on *p.o.* administration.

Compounds **1c**[6] and **1k** having potent antibacterial activity *in vitro* (Table III) were tested for *in vivo* protective effect in mice infected with *Escherichia coli* O-111.[15] They showed potent ED_{50}s (0.44 and 16.2 mg/kg, respectively) on subcutaneous administration, but weaker activities (5.4 and > 100 mg/kg, respectively) on oral administration as shown in Table IV. Thus, we chose compounds **1c** and **1k** as the parent compounds, and prepared their pivaloyloxymethyl (POM), 1-ethoxycarbonyloxyethyl (EOE) and cyclohexyloxycarbonyloxyethyl (COE) esters (**7a—d**, Table II) in order to improve their bioavailabilities.

The POM esters **7a** and **7b** were prepared by alkylation of the parent compounds **1c** and **1k** with chloromethyl pivalate in DMF, respectively (Chart 3). The EOE ester **7c** and the COE ester **7d** were synthesized by the one-step reaction[6] of (*S*)-thienylacetylcycloserine (**8**) with the corresponding 2-oxoglutaric acid esters **9** (R′=EOE and COE) in the presence of DCC.

Biological Results

The 4-modified lactivicin derivatives (**1d—y**) prepared as described in this report were tested for *in vitro* antibacterial activities.

The 4-(2-phenylsulfoacetyl) derivative **1j** was less active than the penicillin derivative which has the same acylamino group[16] at the 6-position.

The MICs of the most potent derivatives (**1d, k—p, s** and **w**) and the related compounds (**1a** and **1c**) are presented in Table III. A series of lactivicin derivatives containing a heterocyclic group (often used in cephalosporin derivatives) showed strong antibacterial activities as seen in Table III. 4-Heteroarylacetylamino derivatives (**1k, 1l** and **1w**) had potent activities against gram-positive bacteria (*S. aureus* FDA 209 P). The antibacterial spectra of these compounds are similar to those of cephalosporin derivatives containing the same acyl groups.[17] Introduction of a methoxy group into the 4-position of **1k** to give **1y** resulted in lower activity.

The importance of the α-alkyloxyiminoaminothiazole side chain for outstanding antibacterial properties has been established in cephalosporin derivatives.[17] In a previous report,[6] the α-methoxyiminothiazolylacetyl derivative **1c** was prepared and found to show potent, broad-spectrum antibacterial activity. Further modification was done to prepare several related compounds **1m—p**. Among them, α-carboxymethoxyimino derivatives (**1m** and **1o**) showed potent activity against gram-negative bacteria at the expense of activity against gram-positive bacteria. Replacement of the aminothiazole ring with 1,2,4-thiadiazole (**1n**) enhanced the antibacterial activity compared with **1c**. Furthermore, the hydroxyimino derivative **1p** had potent activity against both gram-positive and -negative bacteria at the same level of potency. These observation indicated that the relationship between the antibacterial activity and the modification of acylamino moiety of lactivicin is similar to that in the case of the cephalosporin derivatives.[17]

Thus, the modification of the acetylamino group of **1a** led to remarkable enhancement of the antibacterial potency, as in the case of β-lactam antibiotics. These results support our speculation that the acetylamino substituent at the C-4 position of lactivicin corresponds to the acylamino moiety at the C-3 position of β-lactams.

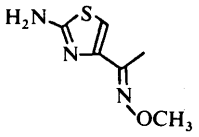
The *in vivo* antibacterial activities of the ester prodrugs (**7a—d**) in *E. coli* infected mice are listed in Table IV. The POM esters (**7a, b**) and EOE ester (**7c**) showed slightly improved protective effects after oral administration compared with their parent compounds, **1c** and **1k**.

Table III. Antibacterial Activity of 4-Acylamino Derivatives

Organism	1a	1c	1d	1k	1l	1m	1n	1o	1p	1s	1w
In vitro MIC (μg/ml)											
S. aureus FDA 209 p	3.13	12.5	6.25	0.39	0.78	100	6.25	>100	1.56	25	<0.1
E. coli NIHJ JC-2	100	1.56	25	6.25	6.25	0.78	0.39	0.78	3.13	3.13	12.5
E. coli O-111	100	0.39	3.13	3.13	6.25	<0.1	0.2	<0.1	1.56	1.56	3.13
C. freundii IFO 12681	100	3.13	50	12.5	2.5	0.78	0.78	0.78	3.13	6.25	100
K. pneumoniae DT	100	0.78	6.25	6.25	6.25	0.39	0.39	0.2	3.13	1.56	12.5
S. marcescens IFO 12648	>100	3.13	50	>100	>100	0.78	1.56	0.39	6.25	6.25	>100
P. vulgaris IFO 3988	100	0.78	3.13	6.25	12.5	0.39	0.39	<0.1	3.13	1.56	6.25

MIC, minimum inhibitory concentration.

Table IV. *In Vivo* Antibacterial Activity of Lactivicin Derivatives

Compound	R	R′	*E. coli* O-111 ED_{50} (mg/kg) s.c.	*p.o.*
1c		Na	0.438	5.42
7a		POM	—	2.21
1k		Na	16.2	>100
7b		POM	—	44.5
7c		EOE	—	60.1
7d		COE	—	>100

Experimental

Infrared (IR) spectra were measured with a Hitachi 215 spectrophotometer. Proton-nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Varian EM-390 (90 MHz) or a Varian XL-100A (100 MHz) with tetramethylsilane as an internal standard. Abbreviations are follows: s = singlet; d = doublet; t = triplet; br = broad. Extracted solutions were dried over sodium sulfate. The MICs were determined by a standard dilution method in Trypticase soy agar (BBL) as described previously.[15]

Sodium 2-[(4*S*)-[2-(4-Ethyl-2,3-dioxo-1-piperazinylcarbonylamino)-(2*R*)-2-phenylacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1d) Method A: HOBT (10 mg, 0.07 mmol) and DCC (58 mg, 0.28 mmol) were added to a solution of benzhydryl 2-[(4*S*)-4-amino-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (**5**) (100 mg, 0.25 mmol) and *N*-(4-ethyl-2,3-dioxo-1-piperazinylcarbonyl)-(2*R*)-phenylglycine (81 mg, 0.25 mmol) in DMF (2 ml), and the mixture was stirred at room temperature for 40 min. AcOEt was added to the reaction mixture and the precipitates were filtered off. The filtrate was washed successively with aqueous $NaHCO_3$ and water, and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with AcOEt gave the benzhydryl ester of **1d** (145 mg, 82%) as a colorless powder. IR ν_{max} cm^{-1} (KBr): 3300, 1805, 1760, 1720, 1685, 1500, 1180. ^{1}H-NMR ($CDCl_3$) δ: 1.09, 1.12 (each 1.5H, t, J = 7 Hz, CH_3), 3.2—3.6 (2H, m, CH_2), 3.8—4.1 (2H, m, CH_2), 5.53, 5.75 (each 0.5H, d, J = 7 Hz, CH), 6.87, 6.90 (each 0.5H, s, $CHPh_2$), 7.1—7.6 (15H, m, arom H), 7.86, 7.92 (each 0.5H, d, J = 7 Hz, NH), 9.75, 9.81 (each 0.5H, d, J = 7 Hz, NH).

Method E: A mixture of **6** (141 mg, 0.20 mmol), THF (6 ml), phosphate buffer (NaH_2PO_4–Na_2HPO_4, pH 7.0, 3 ml) and 5% palladium–charcoal (141 mg) was stirred under a hydrogen atmosphere at 0 °C for 20 min. The catalyst was filtered off, and the filtrate was washed with AcOEt and concentrated under reduced pressure. The concentrate was subjected to chromatography on XAD-2 using 20% EtOH as an eluent. The fractions were collected and lyophilized to give **1d** (89 mg, 80%) as a colorless powder.

Sodium 2-[(4*S*)-4-Difluoroacetylamino-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1e) Benzhydryl 2-[(4*S*)-difluoroacetylamino-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 62%. IR ν_{max} cm^{-1} (KBr): 3350, 2930, 1770, 1740, 1700, 1570. ^{1}H-NMR ($CDCl_3$) δ: 2.22—3.48 (4H, m, CH_2CH_2), 3.73—4.30 (1.5H, m, $CHCH_2$), 4.55—5.01 (1.5H, m, $CHCH_2$), 5.91 (1H, t, J = 51 Hz, CHF_2), 7.01 (1H, s, $CHPh_2$), 7.25—7.40 (10H, m, arom H). The ester (325 mg, 0.686 mmol) was converted into the sodium salt **1e** (97 mg, 43%; colorless powder) by method E.

Sodium 2-[(4*S*)-4-(2-Chloropropionyl)-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1f) A mixture of **5** (300 mg, 0.75 mmol) and 5% palladium–charcoal (300 mg) in THF (20 ml) and water (10 ml) was stirred at 0 °C under a hydrogen atmosphere for 40 min. The catalyst was filtered off, and the filtrate was washed with AcOEt. $NaHCO_3$ (222 mg, 2.55 mmol) and 2-chloropropionyl chloride (0.08 ml, 0.84 mmol) were added to a mixture of the aqueous phase and THF (6 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then the mixture was washed with AcOEt, and the aqueous phase was concentrated under reduced pressure. The concentrate was subjected to chromatography on XAD-2 using water as an eluent. The fractions were collected and lyophilized to give **1f** (21 mg, 8%)[18] as a colorless powder.

Sodium 2-[(4*S*)-4-[2-(Tetrahydro-1,1-dioxo-1,2,4-thiadiazin-2-yl)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1g) Benzhydryl 2-[(4*S*)-4-[2-(tetrahydro-1,1-dioxo-1,2,4-thiadiazin-2-yl)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 58%. IR ν_{max} cm^{-1} (KBr): 3340, 2930, 1800, 1700, 1520, 1180. ^{1}H-NMR ($CDCl_3$) δ: 2.20—3.31 (6H, m, $CH_2 \times 3$), 3.81—5.30 (7H, m, $CHCH_2$, CH_2CH_2), 5.01 (2H, s, CH_2), 5.15 (2H, s, CH_2), 6.95 (1H, s, $CHPh_2$), 7.20—7.39 (15H, m, arom H). The ester (166 mg, 0.24 mmol) was converted into the sodium salt **1g** (39 mg, 36%; colorless powder) by method E.

Sodium 2-[(4*S*)-4-Cyanoacetylamino-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1h) Benzhydryl 2-[(4*S*)-4-cyanoacetylamino-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 65%. IR ν_{max} cm^{-1} (KBr): 3340, 2930, 1800, 1700, 1520, 1180. ^{1}H-NMR (DMSO-d_6) δ: 2.20—3.31 (4H, m, CH_2CH_2), 3.66 (2H, s, CH_2), 3.91—4.47 (1H, m, CH), 4.55—5.09 (1H, m, CH), 5.41—5.60 (1H, m, CH), 6.91 (1H, s, $CHPh_2$), 7.3—7.4 (10H, m, arom H). The ester (100 mg, 0.22 mmol) was converted into the sodium salt **1h** (50 mg, 67%; colorless powder) by method E.

Sodium 2-[(4*S*)-4-(2,6-Difluorophenylacetylamino)-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1i) Benzhydryl 2-[(4*S*)-4-(2,6-difluorophenylacetylamino)-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 42%. IR ν_{max} cm^{-1} (KBr): 3360, 1790, 1770, 1690, 1620, 1530, 1470. ^{1}H-NMR ($CDCl_3$) δ: 2.31—3.30 (4H, m, CH_2CH_2), 3.66 (2H, s, CH_2), 3.86—4.18 (1H, m, CH), 4.51—5.01 (2H, m, CH_2), 6.19—6.38 (1H, m, CH), 6.81—7.71 (13H, m, arom H), 6.89 (1H, s, $CHPh_2$). The ester (300 mg, 0.55 mmol) was converted into the sodium salt **1i** (97 mg, 42%; colorless powder) by method H.

Disodium 2-[(4*S*)-4-(2-Phenyl-2-sulfonatoacetylamino)-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1j) Method E: Compound **5** (200 mg, 0.50 mmol) was hydrogenated in a manner similar to that described for the preparation of **1f** (method E).

Method C: 2-Phenyl-2-sulfoacetyl chloride (119 mg, 0.75 mmol) and $NaHCO_3$ (148 mg, 1.76 mmol) were added to a mixture of the aqueous phase (10 ml) and ethyl ether (10 ml), and the mixture was stirred at 0 °C for 1 h. The mixture was washed with AcOEt, and the aqueous phase was concentrated under reduced pressure. The concentrate was subjected to chromatography on XAD-2 using water as an eluent. The fractions were collected and lyophilized to give **1j** (34 mg, 13%)[19] as a colorless powder.

Sodium 2-[(4*S*)-(2-Thienylacetylamino)-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1k) Method B: DMA (0.16 ml) and 2-thienylacetyl chloride (60 mg, 0.37 mmol) were added to a solution of **5** (100 mg, 0.25 ml) in CH_2Cl_2 (3 ml) at 0 °C, and the mixture was stirred at 0 °C for 10 min, then at room temperature for 20 min. The reaction mixture was diluted with AcOEt, and the organic phase was washed

successively with dilute $NaHCO_3$ and water, and dried. After evaporation of the solvent, Et_2O was added to the residue to deposit **6** (110 mg, 84%) as a colorless powder. IR ν_{max} cm^{-1} (KBr): 3400, 1780, 1760, 1680, 1510, 1180. ^{1}H-NMR ($CDCl_3$) δ: 2.2—3.3 (4H, m, CH_2CH_2), 4.76 (2H, s, CH_2), 3.5—4.2 (1H, m, CH), 4.63—4.90 (2H, m, CH_2), 6.2—6.5 (1H, m, CH), 7.00—7.61 (13H, m, arom H, thienyl H).

Method H: Anisole (0.092 ml, 0.85 mmol) and trifluoroacetic acid (0.18 ml) were added to a solution of the benzhydryl ester (105 mg, 0.20 mmol) in CH_2Cl_2 (8 ml) at -10 °C, and the resulting mixture was stirred at -10 °C for 5 h. The reaction mixture was poured into aqueous $NaHCO_3$. The aqueous solution was washed with CH_2Cl_2 and concentrated under reduced pressure. The concentrate was subjected to chromatography on HP-20 using water as an eluent. The fractions were collected and lyophilized to give **1k** (35 mg, 47%) as a colorless powder.

Sodium 2-[(4*S*)-4-[2-(2-Amino-4-thiazolyl)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1l) Benzhydryl 2-[(4*S*)-4-[2-(2-chloroacetylamino-4-thiazolyl)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method B (99%). IR ν_{max} cm^{-1} (KBr): 3330, 1770, 1745, 1670, 1525, 1180. ^{1}H-NMR (DMSO-d_6) δ: 2.30—3.50 (4H, m, CH_2CH_2), 3.56 (2H, s, CH_2CO), 3.70—5.10 (3H, m, $CHCH_2$), 6.90 (1H, s, thiazole H), 6.97 (1H, s, $CHPh_2$), 7.20—7.55 (10H, m, arom H), 8.71 (1H, d, $J=7$ Hz, NH).

Method J: A mixture of the ester (400 mg, 0.65 mmol), sodium *N*-methyldithiocarbamate (129 mg, 0.78 mmol), THF (4 ml) and water (4 ml) was stirred at room temperature for 1 h. The mixture was extracted with AcOEt, and the organic phase was washed with water and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with AcOEt afforded the ester of **1l** (225 mg, 64%). IR ν_{max} cm^{-1} (KBr): 3350, 1770, 1670, 1540, 1185. ^{1}H-NMR (DMSO-d_6) δ: 2.30—3.50 (4H, m, CH_2CH_2), 3.80—5.10 (3H, m, $CHCH_2$), 6.26 (1H, s, thiazole H), 6.83 (1H, s, $CHPh_2$), 7.20—7.55 (10H, m, arom H), 8.57 (1H, d, $J=7$ Hz, NH). The ester (308 mg, 0.58 mmol) was converted into the sodium salt **1l** (160 mg, 63%; colorless powder) by method H.

Disodium 2-[(4*S*)-4-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(carboxylatomethyloxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1m) Benzhydryl 2-[(4*S*)-4-[2-(2-amino-4-thiazolyl)-(*Z*)-2-(4-nitrobenzyloxycarbonylmethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A (98%) followed by method J (69%). IR ν_{max} cm^{-1} (KBr): 3330, 1800, 1750, 1680, 1605, 1520. ^{1}H-NMR (DMSO-d_6) δ: 2.10—3.50 (4H, m, CH_2CH_2), 3.60—5.50 (3H, m, $CHCH_2$), 4.87 (2H, s, CH_2), 5.25 (2H, s, CH_2Ar), 6.96 (1H, s, $CHPh_2$), 7.20 (1H, s, thiazole H), 7.30—7.33 (10H, m, arom H), 7.40—8.00 (1H, m, NH). The ester (500 mg, 0.66 mmol) was converted into the sodium salt **1m** (85 mg, 25%; colorless powder) by method E.

Sodium 2-[(4*S*)-4-[2-(5-Amino-1,2,4-thiadizol-3-yl)-(*Z*)-2-(ethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1n) Benzhydryl 2-[(4*S*)-4-[2-(5-amino-1,2,4-thiadizol-3-yl)-(*Z*)-2-(ethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 71%. IR ν_{max} cm^{-1} (KBr): 3320, 1800, 1760, 1660, 1520. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, $J=7$ Hz, CH_3), 2.23—3.28 (4H, m, CH_2CH_2), 3.93—4.32 (3.5H, m, CH_2, $CHCH_2O$), 4.58—4.96 (1.5H, m, $CHCH_2O$), 6.63 (2H, br, NH), 6.91 (1H, s, $CHPh_2$), 7.25—7.40 (10H, m, arom H). The ester (299 mg, 0.51 mmol) was converted into the sodium salt **1n** (62 mg, 28%; colorless powder) by method H.

Disodium 2-[(4*S*)-4-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(1-carboxylato-1-methylethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1o) Benzhydryl 2-[(4*S*)-4-[2-(2-amino-4-thiazolyl)-(*Z*)-2-[1-methyl-1-(4-nitrobenzyloxycarbonyl)ethoxyimino]acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A (79%) followed by method J (76%). IR ν_{max} cm^{-1} (KBr): 3420, 3350, 1800, 1760, 1690, 1520, 1350. ^{1}H-NMR ($CDCl_3$) δ: 1.61 (6H, s, $CH_3\times2$), 2.28—3.32 (4H, m, CH_2CH_2), 3.97—4.36 (1.5H, m, $CHCH_2O$), 4.71—4.98 (1.5H, m, $CHCH_2O$), 5.26 (2H, s, CH_2Ar), 6.41 (2H, br, NH_2), 6.77 (1H, s, $CHPh_2$), 7.01 (1H, s, arom H), 7.21—7.51 (12H, m, arom H), 7.90—8.07 (2H, m, arom H). The ester (474 mg, 0.60 mmol) was converted into the sodium salt **1o** (132 mg; colorless powder) by method E (55%) followed by method H (74%).

Sodium 2-[(4*S*)-4-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(hydroxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1p) A mixture of 2-(2-triphenylmethylamino-4-thiazolyl)-(*Z*)-2-(triphenylmethoxyimino)acetic acid (4.0 g, 6.25 mmol), *N*-methylmorpholine (0.92 ml, 8.36 mmol), 2,2′-dithiobis(benzothiazole) (2.24 g, 6.74 mmol) and triethylphosphite (1.68 ml, 9.78 mmol) and CH_3CN (30 ml) was stirred at 0 °C for 4 h. Insoluble materials were filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was subjected to chromatography on silica gel. Elution with AcOEt–hexane (1 : 1—3 : 2, v/v) gave *S*-(2-benzothiazolyl) 2-(2-triphenylmethylamino-4-thiazolyl)-(*Z*)-2-(triphenylmethoxyimino)thioacetate (4.5 g, 92%) as a colorless powder. IR ν_{max} cm^{-1} (neat): 2990, 1700, 1500, 1320, 1250, 1030,

Method D: A solution of **5** (200 mg, 0.50 mmol) in DMF (2 ml) was added dropwise to a solution of 2-benzothiazolylthio-2-(2-triphenylmethylamino-4-thiazolyl)-(*Z*)-2-(triphenylmethoxyimino)acetate (497 mg, 0.63 mmol), and the resulting mixture was stirred at room temperature for 16 h. The mixture was diluted with AcOEt, and the AcOEt layer was washed successively with aqueous $NaHCO_3$, water and saturated aqueous NaCl, and dried. The solvent was evaporated off, and the residue was chromatographed on silica gel. Elution with AcOEt–hexane (1 : 2, v/v) afforded the benzhydryl ester (488 mg, 93%; colorless powder). IR ν_{max} cm^{-1} (KBr): 3390, 1800, 1740, 1680, 1520, 1490. ^{1}H-NMR (DMSO-d_6) δ: 2.31—3.35 (4H, m, CH_2CH_2), 3.93—4.26 (1.5H, m, $CHCH_2$), 4.67—4.95 (1.5H, m, $CHCH_2$), 6.52 (1H, s, thiazole H), 6.89 (0.5H, s, $CHPh_2$), 6.98 (0.5H, s, $CHPh_2$), 7.11—7.42 (40H, m, arom H).

Method I: Formic acid (3 ml) was added to a solution of the benzhydryl ester (488 mg, 0.48 mmol) in CH_2Cl_2 (1 ml) at 0 °C, and the mixture was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2, and the organic phase was extracted with phosphate buffer (pH 7.0). The aqueous phase was concentrated under reduced pressure, and the concentrate was subjected to chromatography on Diaion HP-20 using water as an eluent. The fractions were collected and lyophilized to give **1p** (48 mg, 25%) as a colorless powder.

Sodium 2-[(4*S*)-4-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(cyanomethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1q) Benzhydryl 2-[(4*S*)-4-[2-(2-triphenylmethylamino-4-thiazolyl)-(*Z*)-2-(cyanomethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 56%. IR ν_{max} cm^{-1} (KBr): 3340, 1800, 1750, 1680. ^{1}H-NMR ($CDCl_3$) δ: 2.32—3.36 (4H, m, CH_2CH_2), 4.17—4.92 (3H, m, $CHCH_2$), 4.81 (2H, s, CH_2CN), 6.78 (1H, s, $CHPh_2$), 6.98 (1H, s, thiazole H), 7.13—7.40 (25H, m, arom H). The benzhydryl ester (200 mg, 0.24 mmol) was converted to **1q** (35 mg, 31%) by method H.

Sodium 2-[(4*S*)-4-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(aminothiocarbonylmethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1r) Benzhydryl 2-[(4*S*)-4-[2-(2-amino-4-thiazolyl)-(*Z*)-2-(aminothiocarbonylmethoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 50%. IR ν_{max} cm^{-1} (KBr): 3340, 1800, 1750, 1680, 1530, 1180. ^{1}H-NMR ($CDCl_3$) δ: 2.2—3.3 (4H, m, CH_2CH_2), 4.0—5.3 (3H, m, $CHCH_2$), 5.02 (2H, s, CH_2), 6.75 (1H, s, $CHPh_2$), 6.91 (1H, s, thiazole H), 7.11—7.40 (25H, m, arom H). The ester (500 mg, 0.78 mmol) was converted into the sodium salt **1r** (193 mg, 44%; colorless powder) by method I.

Sodium 2-[(4*S*)-4-[2-(2-Dichloroacetylamino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1s) Dichloroacetyl chloride (0.2 ml, 2.0 mmol) was added to a solution of **1c** (1.0 g, 1.7 mmol) in DMA (8 ml) at 0 °C and the mixture was stirred for 30 min. The mixture was poured into water and extracted with AcOEt. The organic phase was washed with water and dried. After evaporation of the solvent, the residue was chromatographed on silica gel. Elution with AcOEt–hexane (4 : 1, v/v) afforded benzhydryl 2-[(4*S*)-4-[2-(2-dichloroacetylamino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (924 mg, 79%) as a colorless powder. IR ν_{max} cm^{-1} (KBr): 3250, 1800, 1760, 1680, 1550, 1450. ^{1}H-NMR ($CDCl_3$) δ: 2.34—3.59 (4H, m, CH_2CH_2), 3.98 (3H, s, OCH_3), 3.90—4.37 (1H, m, CH), 4.61—5.28 (2H, m, CH_2O), 6.08 (1H, s, $CHCl_2$), 6.96 (1H, s, thiazole H), 7.12—7.90 (11H, m, arom H, $CHPh_2$). The ester (307 mg, 0.44 mmol) was converted into the sodium salt **1s** (118 mg, 48%; colorless powder) by method H.

Sodium 2-[(4*S*)-4-[(*Z*)-(2-Chloro)vinylthioacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1t) Benzhydryl 2-[(4*S*)-4-[(*Z*)-(2-chloro)vinylthioacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method A. Yield 85%. IR ν_{max} cm^{-1} (KBr): 3340, 1780, 1720, 1680, 1520, 1180. ^{1}H-NMR ($CDCl_3$) δ: 2.32—3.40 (4H, m, CH_2CH_2), 3.37 (2H, s, CH_2S), 3.91—4.20 (1H, m, CH), 4.52—5.01 (2H, m, CH_2), 6.12 (1H, d, $J=6$ Hz, vinylic H), 6.33 (1H, d, $J=6$ Hz, vinylic H), 6.82 (1H, s, $CHPh_2$), 7.22—7.43 (10H, m, arom H). The ester (200 mg, 0.38 mmol) was converted into the sodium salt **1t** (88 mg, 59%; colorless powder) by method H.

Sodium 2-[(4*S*)-4-[(2,2-Dichloro)vinylthioacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1u) Benzhydryl 2-[(4*S*)-4-[(2,2-dichloro)vinylthioacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-

tetrahydrofurancarboxylate was prepared by method A. Yield 79%. IR ν_{max} cm^{-1} (KBr): 3340, 1770, 1720, 1680, 1520, 1180. ^{1}H-NMR ($CDCl_3$) δ: 2.15—3.52 (4H, m, CH_2CH_2), 3.67 (2H, s, CH_2S), 3.91—4.20 (1H, m, CH), 4.53—4.98 (2H, m, CH_2), 6.50 (1H, s, vinylic H), 6.99 (1H, s, $CHPh_2$), 7.21—7.45 (10H, m, arom H). The ester (240 mg, 0.42 mmol) was converted into the sodium salt **1u** (118 mg, 66%; colorless powder) by method H.

Sodium 2-[(4*S*)-4-[(4-Pyridylthio)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1v) Benzhydryl 2-[(4*S*)-4-chloroacetamido]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (750 mg, 63%) was synthesized by method B from **5** (1.0 g, 2.52 mmol) and chloroacetyl chloride (0.21 ml, 2.64 mmol). IR ν_{max} cm^{-1} (KBr): 3500, 3450, 1795, 1785, 1670, 1525. ^{1}H-NMR (DMSO-d_6) δ: 2.6—3.3 (4H, m, CH_2CH_2), 4.12 (2H, s, CH_2), 4.5—5.2 (3H, m, $CHCH_2$), 6.90 (1H, s, $CHPh_2$), 7.3—7.5 (10H, m, arom H), 8.91 (1H, d, J=7.5 Hz, NH).

Method K: A mixture of the ester (200 mg, 0.42 mmol), 4-pyridinethiol (56.4 mg, 0.50 mmol), sodium hydride (19.2 mg, 0.48 mmol), sodium iodide (100 mg, 0.67 mmol) and DMF (1 ml) was stirred at room temperature for 30 min. Water was added to the mixture and the whole was extracted with AcOEt. The organic phase was washed with water and dried. The solvent was evaporated off, and the residue was subjected to chromatography on silica gel. Elution with AcOEt–acetone (2 : 1) afforded the benzhydryl ester (163 mg, 70%) as a colorless powder. IR ν_{max} cm^{-1} (KBr): 3350, 1770, 1740, 1680, 1570. ^{1}H-NMR ($CDCl_3$) δ: 2.12—3.22 (4H, m, CH_2CH_2), 3.38—4.09 (1.5H, m, $CHCH_2$), 4.69 (2H, s, CH_2), 4.46—4.93 (1.5H, m, $CHCH_2$), 6.91, 6.93 (each 0.5H, s, $CHPh_2$), 7.02—7.40 (12H, m, arom H), 8.39 (2H, d, J=7 Hz, pyridine H). The ester (135 mg, 0.25 mmol) was converted to **1v** (21 mg, 19%; colorless powder) by method H.

Sodium 2-[(4*S*)-4-[(2-Chloro-4-pyridylthio)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1w) Benzhydryl 2-[(4*S*)-4-[(2-chloro-4-pyridylthio)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method K. Yield 46%. IR ν_{max} cm^{-1} (KBr): 3350, 1770, 1720, 1680, 1570, 1520. ^{1}H-NMR ($CDCl_3$) δ: 2.20—3.51 (4H, m, CH_2CH_2), 3.69 (2H, s, CH_2), 3.81—4.19 (1.5H, m, $CHCH_2$), 4.48—4.88 (1.5H, m, $CHCH_2$), 6.98 (1H, s, $CHPh_2$), 7.02—8.23 (13H, m, arom H). The ester (200 mg, 0.71 mmol) was converted into the sodium salt **1w** (207 mg, 64%; colorless powder) by method H.

Sodium 2-[(4*S*)-4-[(2-Pyridylthio)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1x) Benzhydryl 2-[(4*S*)-4-[(2-pyridylthio)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate was prepared by method K. Yield 68%. IR ν_{max} cm^{-1} (KBr): 3350, 1770, 1730, 1670, 1570, 1510. ^{1}H-NMR ($CDCl_3$) δ: 2.21—3.40 (4H, m, CH_2CH_2), 3.68 (2H, s, CH_2), 3.87—4.19 (1.5H, m, $CHCH_2$), 4.46—4.82 (1.5H, m, $CHCH_2$), 6.92 (1H, s, $CHPh_2$), 6.93—8.81 (14H, m, arom H). The ester (200 mg, 0.37 mmol) was converted into the sodium salt **1x** (115 mg, 74%; colorless powder) by method H.

Sodium 2-[4-Methoxy-4-(2-thienylacetamido)-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (1y) *tert*-Butylhypochlorite (0.06 ml, 0.50 mmol) and lithium methoxide (0.44 ml of 1.368 M MeOH solution) were added to a solution of the benzhydryl ester of **1k** (210 mg, 0.40 mmol) in THF (20 ml) at −78 °C, and the mixture was stirred for 5 min. After addition of one drop of acetic acid, the mixture was diluted with AcOEt. The organic phase was washed successively with aqueous $Na_2S_2O_3$ and saturated aqueous NaCl, and dried. The solvent was evaporated off, and the residue was chromatographed on silica gel. Elution with AcOEt–hexane (2 : 3, v/v) afforded the benzhydryl ester (144 mg, 65%) as a colorless oil. IR ν_{max} cm^{-1} (neat): 3350, 1800, 1795, 1770, 1690, 1175, 1055. ^{1}H-NMR (DMSO-d_6) δ: 2.30—3.20 (4H, m, CH_2CH_2), 3.17, 3.21 (1.5H each, s×2, OCH_3), 3.81 (2H, s, CH_2), 4.32—4.92 (2H, m, CH_2), 6.69, 6.72 (0.5H each, s×2, $CHPh_2$), 6.9—7.5 (3H, m, thiophene H), 7.29—7.32 (10H, m, arom H).

Method G: A mixture of the ester (144 mg, 0.27 mmol), palladium black (144 mg) and palladium oxide (72 mg), THF (10 ml) and phosphate buffer (pH 7.0, 10 ml) was stirred under a hydrogen atmosphere at room temperature for 1 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was subjected to chromatography on Diaion HP-20 using 10% ethanol as an eluent. The fractions were collected and lyophilized to give **1y** (76 mg, 69%) as a colorless powder.

Pivaloyloxymethyl 2-[(4*S*)-4-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-(methoxyimino)acetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (7a) Chloromethyl pivalate (0.13 ml, 0.86 mmol) was added to a solution of **1c** (112 mg, 0.25 mmol) in DMF (4 ml), and the mixture was stirred at room temperature for 20 h. The reaction mixture was poured into AcOEt–water and extracted with AcOEt. The organic phase was washed with water and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with AcOEt–acetone afforded **7a** (73 mg, 55%) as a colorless powder.

Pivaloyloxymethyl 2-[(4*S*)-4-(2-Thienylacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (7b) Compound **7b** was prepared in a manner similar to that described for the preparation of **7a**. Yield 49%. Colorless powder.

1-(Ethoxycarbonyloxy)ethyl 2-[(4*S*)-4-(2-Thienylacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (7c) A mixture of (4*S*)-4-(2-thienylacetylamino)-3-oxo-2-isoxazolidinone (**8**) (226 mg, 1 mmol), 1-[1-(ethoxycarbonyl)oxyethyl] 2-oxoglutarate (**9**) (262 mg, 1 mmol), DCC (230 mg, 1.1 mmol) and CH_3CN (20 ml) was stirred at room temperature for 3 h. The precipitates were filtered off, and the filtrate was washed successively with aqueous $NaHCO_3$ and water, and dried. After evaporation of the solvent, the residue was subjected to chromatography on silica gel. Elution with AcOEt–hexane (3 : 2) afforded **7c** (100 mg, 21%) as a colorless powder.

1-(Cyclohexycarbonyloxy)ethyl 2-[(4*S*)-4-(2-Thienylacetylamino]-3-oxo-2-isoxazolidinyl]-5-oxo-2-tetrahydrofurancarboxylate (7d) Compound **7d** was prepared in a manner similar to that described for the synthesis of **7c**. Yield 13%. Pale yellow powder.

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References and Notes

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