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Synthesis of 2α-Methyl- and 2β-Methyl-3-(substituted methyl)cephalosporins, and 2,3-Diexomethylenecepham[1]

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The stereoselective synthesis of 2α-methyl- and 2β-methyl-3-(substituted methyl)-cephalosporins *via* 2-methyl-3-formyloxymethylceph-2-em (**5**) and 2-methyl-3-acetoxymethylceph-2-em (**16**) from 2-methylene-3-acetoxymethylcephalosporin (**1**) is described. Reduction of **1** with zinc in acetic acid gave 2,3-dimethylenecepham (**2**), while reduction with zinc in formic acid gave **5** and reduction with sodium borohydride gave **16**. Hydrolysis of **5** gave 2-methyl-3-hydroxymethylceph-2-em (**6**), which was stereoselectively converted *via* 2-methyl-3-(heterocyclic thiomethyl)ceph-2-em (**8**) and 2-methyl-3-formylceph-2-em (**12**) into the corresponding 2α-methyl-3-(substituted methyl)ceph-3-ems by oxidation with peracid. On the other hand, isomerization of **16** to the corresponding ceph-3-em by oxidation with peracid gave mainly the 2β-methyl isomer (55: 1 ratio). Ozonolysis of **2** followed by treatment with diazomethane gave 2-oxo-3-methoxyceph-3-em (**24**).

Keywords——2α-methylcephalosporin; 2β-methylcephalosporin; 2-methyl-3-formyloxymethylcephem; 2-methyl-3-acetoxymethylcephem; 2-methyl-3-(substituted methyl)-cephem; 2,3-diexomethylenecepham; 3-methoxy-2-oxocephem; reduction; zinc in formic acid; sodium borohydride

Although quite a number of C-2 substituted cephalosporins have been reported, only a few 2-methylcephalosporins have been reported so far[2] and 2,3-dimethylceph-3-em derivatives[3] have been the only 2-methylceph-3-em derivatives bearing C-3 substituents. As regards antimicrobial activity, it was reported that a 2β,3-dimethylceph-3-em derivative showed somewhat stronger activity than the 2α isomer, but both of them showed considerably less antimicrobial activity than the parent C-2 unsubstituted 3-methylceph-3-em derivative.[3] However, it was also reported that 2α-methylceph-3-em derivatives bearing no C-3 substituent showed greater antimicrobial activity than the 2β-methyl isomers and C-2 unsubstituted 3-methylceph-3-em derivatives.[4] Furthermore, it is also well-known that some 3-(substituted methyl)ceph-3-em derivatives show improved antimicrobial activity as compared to the corresponding 3-methylceph-3-em derivatives in the case of conventional C-2 unsubstituted cephalosporins. These preceding studies encouraged us to prepare 2α-methyl- and 2β-methyl-3-(substituted methyl)ceph-3-em derivatives.

In this paper, stereoselective synthesis of 2α- and 2β-methylceph-3-em derivatives bearing C-3 substituents from 2-exomethylenecephalosporin (**1**) is described. Some of the products showed considerable antimicrobial activities. The synthesis of a 2,3-diexomethylenecepham derivative (**2**) and a 3-methoxy-2-oxoceph-3-em derivative (**24**) from **1** is also described.

Wright and co-workers reported that reduction of a 2-methylene-3-methylceph-3-em 1-oxide derivative by catalytic hydrogenation with Rh-carbon gave the corresponding 2β-methyl-3-methylceph-3-em derivative as one of the products and, on the other hand, reduction of the same compound with disiamylborane gave the 2α-methyl-3-methylceph-3-em derivative.[3] They also reported that reduction of trichloroethyl 2-methylene-3-methylceph-3-em-4-carboxylate and trichloroethyl 2-methylene-3-acetoxymethylceph-3-em-4-carboxylate with zinc in acetic acid gave the 2-methylene-3-methylcepham-4-carboxylate and the 2-methylene-3-acetoxymethylcepham-4-carboxylate, respectively.[3]

When this reduction procedure was applied to the reduction of diphenylmethyl 7-(2-

thienylacetamido)-2-methylene-3-methylceph-3-em-4-carboxylate and its 3-acetoxymethyl analog (**1**), which were synthesized from diphenylmethyl 7-(2-thienylacetamido)-3-methylceph-3-em-4-carboxylate 1-oxide and its 3-acetoxymethyl analog, respectively, by Wright's method,[3] the 3-methyl analog gave the 2-methylene-3-methylcepham-4-carboxylate derivative,[5] but the 3-acetoxymethyl analog (**1**) gave the 2,3-diexomethylenecepham derivative (**2**)[5] instead of the 2-methylene-3-acetoxymethylcepham derivative which was expected to be produced from the literature.[3] The structure of **2** was confirmed by converting **2** to the 2-methylene-3-methylceph-3-em derivative (**3**) with Et_3N and then to the 2-methylene-3-methylcepham derivative (**4**) with zinc in acetic acid, and by direct comparison with **3** and **4** which were synthesized from 7-(2-thienylacetamido)cephalosporanic acid by Wright's method.[3]

Further investigation on the reduction of **1** has revealed that **1** was reduced to the 2-methyl-3-formyloxymethylceph-2-em derivative (**5**)[5] by treatment with zinc in formic acid instead of zinc in acetic acid, and to the 2-methyl-3-acetoxymethylceph-2-em derivative (**16**)[5] by treatment with sodium borohydride.

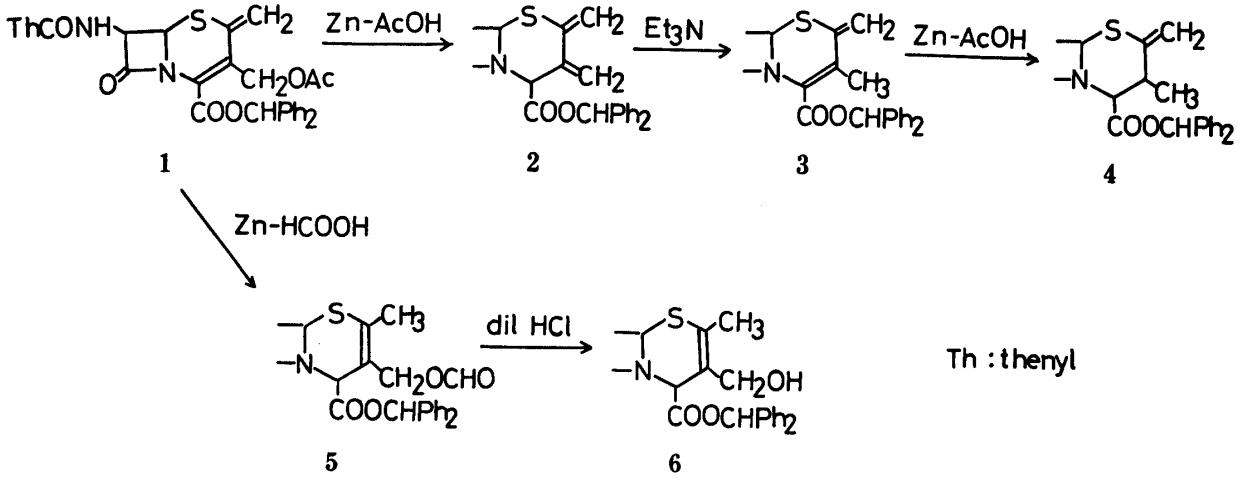
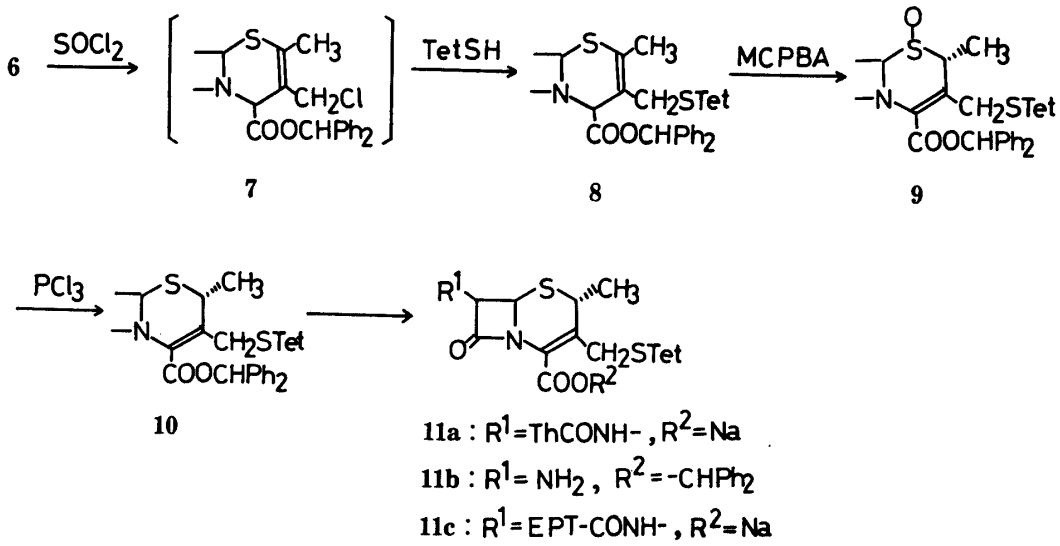


Chart 1



Tet: 1-methyl-1*H*-tetrazol-5-yl
Th: thenyl
EPT–CO–: D-(−)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(2-thienyl)acetyl

Chart 2

In the case of the reduction of **1** with zinc in formic acid, it is preferable to use 99% formic acid, and the use of formic acid which contains more than 30% water must be avoided, because **2** is formed as a by-product in such a case. An attempt to convert **2** to **5** by treatment with zinc in 99% formic acid was unsuccessful.

Hydrolysis of **5** with 2 N HCl in *N*,*N*-dimethylformamide (DMF) gave the 2-methyl-3-hydroxymethylceph-2-em derivative (**6**), which is a useful intermediate for the synthesis of 2α-methyl-3-(substituted methyl)cephalosporins.

As shown in Chart 2, **6** was converted to the 2-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)-thiomethylceph-2-em derivative (**8**) *via* the 2-methyl-3-chloromethylceph-2-em derivative (**7**) by treatment with $SOCl_2$–pyridine and then with 1-methyl-5-mercaptotetrazole. The sequence of oxidation of **8** to the sulfoxide (**9**) with *m*-chloroperbenzoic acid (MCPBA) and reduction of **9** with PCl_3 gave the 2-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em derivative (**10**). The stereochemistry of C-2 methyl of **10** was assigned as α-methyl on the basis of the nuclear Overhauser effect (NOE) between the C-2 methyl protons and H-6 (about 20% increase of the H-6 signal). The ester protecting group of **10** was removed by acid hydrolysis (CF_3COOH–anisole) to give the free acid (**11a**) as the Na salt.

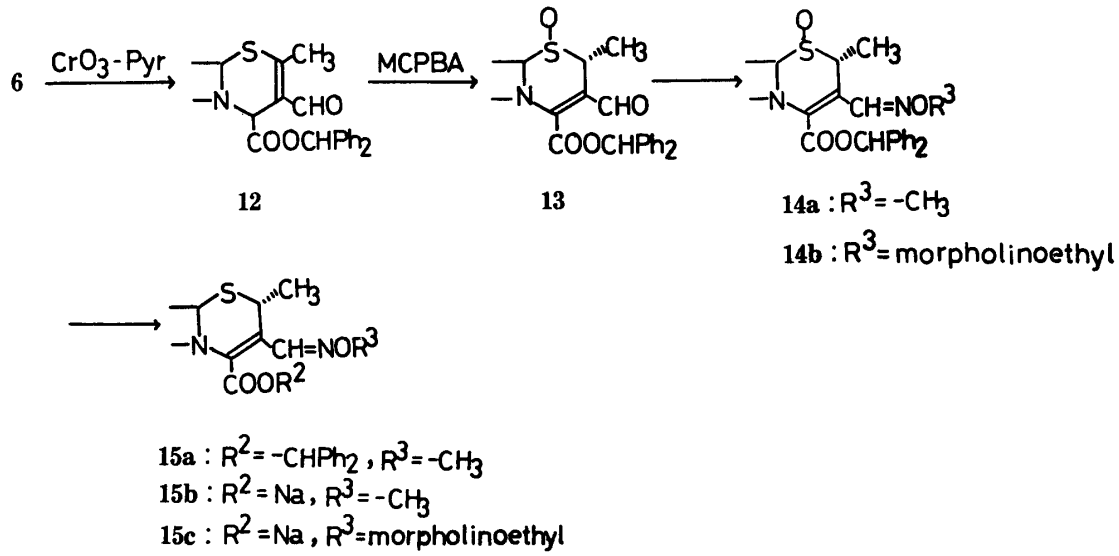
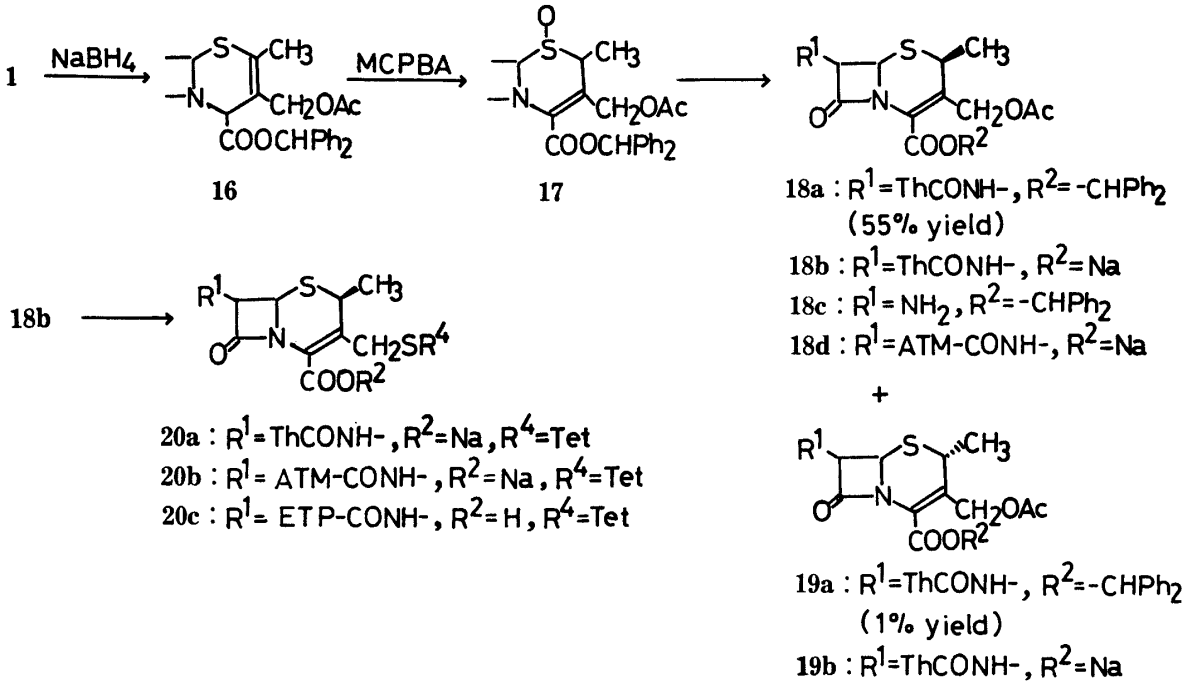


Chart 3

Compound **6** is also useful as an intermediate for the preparation of 2α-methyl-3-formylceph-3-em derivatives which can be converted to 2α-methyl-3-alkoxyiminom thylceph-3-em derivatives, such as the 3-methoxyiminomethyl and 3-morpholinoethoxyiminomethyl derivatives. As shown in Chart 3, oxidation of **6** with CrO_3–pyridine gave the 2-methyl-3-formylceph-2-em derivative (**12**). After isomerization of **12** by oxidation with MCPBA, the resulting 2-methyl-3-formylceph-3-em 1-oxide (**13**) was converted to 2-methyl-3-methoxyiminomethylceph-3-em 1-oxide (**14a**) by treatment with methoxyamine hydrochloride–pyridine, followed by reduction of **14a** with PCl_3 to give the sulfide (**15a**). The stereochemistry of C-2 methyl of **15a** was assigned as α-methyl from the NOE between the C-2 methyl protons and H-6 (about 21% signal increase for H-6). Removal of the ester protecting group with CF_3COOH–anisole gave the free acid (**15b**) as the Na salt. Similarly, the 3-morpholinoethoxyiminomethylceph-3-em derivative (**15c**) was prepared *via* **14b** starting from **13** by using morpholinoethoxyamine hydrochloride instead of methoxyamine hydrochloride.

2β-Methylceph-3-em derivatives bearing acetoxymethyl or a heterocyclic thiomethyl substituent at C-3 were synthesized from **16** as shown in Chart 4.



Th: thenyl
Tet: 1-methyl-1*H*-tetrazol-5-yl
EPT-CO-: D(−)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(2-thienyl)acetyl
ATM-CO-: 2-(2-aminothiazol-4-yl-)-(*Z*)-2-methoxyiminoacetyl

Chart 4

Compound **16** was isomerized to give the ceph-3-em 1-oxide (**17**) as a mixture of stereoisomers by oxidation with MCPBA, followed by reduction with PCl_3 to give the corresponding sulfide. The sulfide was crystallized from AcOEt–Et_2O (1 : 3) to give the 2β-methyl-3-acetoxymethylceph-3-em derivative (**18a**) in 55% yield. The mother liquor of crystallization was subjected to silica gel chromatography (toluene–AcOEt 15: 1) to give the 2α-methyl isomer (**19a**) in about 1% yield. The stereochemistry of the C-2 methyls (2β-methyl for **18a** and 2α-methyl for **19a**) was determined from the NOE between the C-2 methyl protons and H-6 (about 32% signal increase in **19a** and no signal increase in **18a** for H-6). After the removal (CF_3COOH–anisole) of the ester protecting group of **18a**, the corresponding 2β-methyl-4-carboxylic acid (**18b**) was obtained as the Na salt; **18b** was then converted to 2β-methyl-3-

TABLE I. MICs of 7-(Thienylacetamido)-2-methyl-3-(substituted methyl)cephalosporins (μg/ml)[a]

Organism	Compound			
	19b	**18b**	**11a**	**20a**
S. aureus FDA 209P	0.78	0.39	0.39	0.2
S. aureus 308 A-1	0.78	0.39	0.39	0.2
S. aureus 1840	1.56	1.56	0.78	1.56
E. coli NIHJ JC-2	>100	>100	50	25
E. coli O-111	50	50	12.5	6.25
K. pneumoniae DT	100	50	25	12.5
P. vulgaris IFO 2988	25	50	50	12.5
P. mirabilis IFO 3849	>100	>100	>100	50

a) The MICs were determined by a standard agar dilution method in trypticase soy agar (BBL).

TABLE II. MICs of 7-Acylamino-2-methyl-3-(substituted methyl) cephalosporins (μg/ml)[a]

Organism	Compound			
	11c	**20c**	**18d**	**20b**
S. aureus FDA 209P	1.56	0.78	3.13	1.56
S. aureus 308 A-1	1.56	0.78	3.13	0.78
S. aureus 1840	3.13	6.25	6.25	3.13
E. coli NIHJ JC-20	12.5	3.13	0.78	0.78
E. coli C-111	3.13	0.78	≦0.1	0.2
K. pneumoniae DT	6.25	1.56	0.2	0.39
P. vulgaris IFO 3988	6.25	0.78	≦0.1	0.2
P. mirabilis IFO 3849	25	3.13	0.39	0.78

a) The MICs were determined by a standard agar dilution method in trypticase soy agar (BBL).

(heterocyclic thiomethyl)ceph-3-em derivatives, such as the 3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl derivative (**20a**), the 3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl derivative (**20d**), and the 3-(1-carboxymethyl-1*H*-tetrazol-5-yl)thiomethyl derivative (**20e**), by conventional nucleophilic displacement of the acetoxy function with heterocyclic thiols, such as 1-methyl-5-mercaptotetrazole, 5-methyl-2-mercapto-1,3,4-thiadiazole, and 1-carboxymethyl-5-mercaptotetrazole, respectively.

The acylamino side-chain of the 2α-methyl- and 2β-methyl-3-(substituted methyl)cephalosporins described above was removed by the conventional imino-ether cleavage method to give the corresponding 7-amino derivatives (**11b**, **18c**), which were reacylated with various acyl groups, including the 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl (ATM-CO-) group and the D(−)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(2-thienyl)acetyl (EPT-CO-) group, for the synthesis of biologically more interesting derivatives (**11c**, **18d**, **20b**, **20c**). Some of them showed quite strong antimicrobial activities as shown in Table II.

Although the relative microbiological activity of 2α-methyl and 2β-methyl-3-(substituted methyl)cephalosphorins may vary with the combination of 7-acylamino side-chain and 3-substituted group, our experimental data show that 7-(2-thienylacetamido)-2β-methyl-3-(substituted methyl)cephalosporins are somewhat more active than the coresponding 2α-methyl derivatives, as shown in Table I.

In the course of this study, ozonolysis of the 2,3-diexomethylenecepham derivative (**2**) was

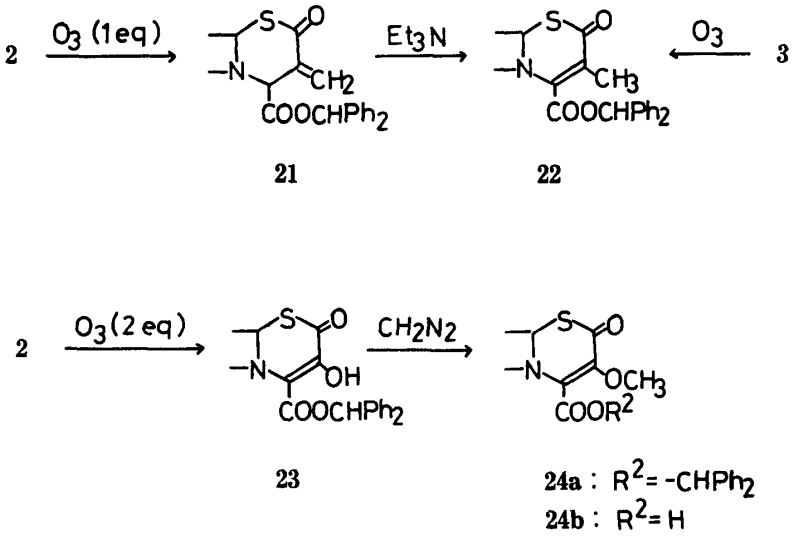


Chart 5

also investigated. As outlined in Chart 5, when ozonolysis of **2** was carried out with about an equimolecular quantity of ozone at low temperature (in an acetone-dry ice bath), the exomethylene group at C-2 was preferentially ozonized to give the 3-exomethylene-2-oxocepham derivative (**21**). Isomerization of **21** to the 3-methyl-2-oxoceph-3-em derivative (**22**) occurred on treatment with Et_3N, and the structure of **22** was confirmed by comparison with the sample obtained by ozonolysis of **3**. On the other hand, the ozonolysis of **2** with about 2 eq of ozone at the same temperature gave the 3-hydroxy-2-oxoceph-3-em derivative (**23**), which was treated with diazomethane without further purification to give the 3-methoxy-2-oxoceph-3-em derivative (**24a**). Removal of the ester-protecting group of **24a** with CF_3COOH–anisole gave the free acid (**24b**). As expected, the β-lactam carbonyl peak of **24b** in the infrared spectrum was shifted to higher wave number (1800 cm^{-1}) than in the cases of normal cephalosporins, for example 7-(2-thienylacetamido)cephalosporanic acid (around 1775 cm^{-1}). However, **24b** is labile under basic conditions and attempts to prepare the sodium salt were not successful, as was the case with the known 2-oxocephalosporins.[6]

The antimicrobial activity of **24b** against Gram-positive and -negative bacteria, disappointingly, was much weaker than those of normal cephalosporins, but **24b** shows activity (MIC 65 μg/ml) against *Cryptococcus neoformans* (IFO 0410).

Experimental

General Procedure——Melting points were measured on a Mettler FP-5 apparatus and are uncorrected, infrared (IR) spectra were recorded on a JASCO IRA-1 infrared spectrometer, mass spectra (MS) on a Hitachi RMU-6D spectrometer, and nuclear magnetic resonance (NMR) on a Varian A-60A or a Varian EM-390 spectrometer with tetramethylsilane (TMS) as a standard. Thin-layer chromatography (TLC) was performed on pre-coated Kieselgel F_{254} plates (Merck). Chromatography columns of silica gel were prepared with Kieselgel 60 (70—230 mesh ATCM; Merck). Solvents were removed in a rotary evaporator under reduced pressure.

Diphenylmethyl 7-(2-Thienylacetamido)-2,3-dimethylenecepham-4-carboxylate (**2**)——Zn powder (14 g) was added to a solution of diphenylmethyl 7-(2-thienylacetamido)-2-methylene-3-acetoxymethylceph-3-em-4-carboxylate (**1**, 4 g) in AcOH (160 ml), followed by stirring at room temperature for 45 min. AcOEt and H_2O were added to the mixture with stirring, and the whole was filtered. The AcOEt layer was washed with sat. aqueous $NaHCO_3$ and sat. aqueous NaCl, then dried over Na_2SO_4. The solvent was removed and the residue was chromatographed on a silica gel column. The fractions eluted with benzene–AcOEt (10: 1) were collected and concentrated. Et_2O was added to the residue to give crystals (2.3 g, 64%) of **2**. mp127°C (dec.), (AcOEt–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1770, 1735, 1660. NMR (dimethyl sulfoxide-d_6 (DMSO-d_6)) δ: 3.74 (2H,s, $-C\underline{H}_2CO-$), 5.18 (1H, d, J=4.5 Hz, 6-H), 5.35 (1H, s, one proton of 2-methylene), 5.40 (1H, dd, J=4.5 and 8.5 Hz, 7-H), 5.45 (1H, s, 4-H), 5.51 (2H, s, 3-methylene), 5.59 (1H, s, one proton of 2-methylene), 6.80—7.00 (3H, m, thiophene 3-H, 4-H, $-C\underline{H}(C_6H_5)_2$), 7.20—7.60 (11H, m, thiophene 5-H, $C_6\underline{H}_5\times2$), 9.11 (1H, d, J= 8.5 Hz, 7-NH). MS *m/e* 516 (M^+). *Anal.* Calcd for $C_{28}H_{24}N_2O_4S_2$: C, 65.09; H, 4.68; N, 5.42; S, 12.41. Found: C, 65.02; H, 4.71; N, 5.30; S, 12.14.

Diphenylmethyl 7-(2-Thienylacetamido)-2-methylene-3-methylceph-3-em-4-carboxylate (**3**) **from 2**——Et_3N (0.137 ml) was added to a solution of **2** (516 mg) in CH_2Cl_2 (10 ml). After being stirred for 30 min, the mixture was washed with 5% H_3PO_4 and sat. aqueous NaCl, dried over $MgSO_4$, and concentrated to give **3** (506 mg, 98%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1780, 1730, 1670. NMR (DMSO-d_6) δ: 2.04 (3H, s, 3-$C\underline{H}_3$), 3.74 (2H, s, $-C\underline{H}_2CO-$), 5.20 (1H, d, J=4.5 Hz, 6-H), 5.60 (1H, s, one proton of 2-methylene), 5.72 (1H, dd, J=4.5 and 8.5Hz, 7-H), 5.80 (1H, s, one proton of 2-methylene), 6.80—7.00 (3H, m, thiophene 3-H, 4-H, $-C\underline{H}(C_6H_5)_2$), 7.15—7.60 (11H, thiophene 5-H, $C_6\underline{H}_5\times2$), 9.22 (1H, d, J=8.5 Hz, 7-NH). *Anal.* Calcd for $C_{28}H_{24}N_2O_4S_2$: C,65.09; H,4.68; N, 5.42; S, 12.41. Found: C,65.02; H, 4.55; N, 5.35; S, 12.40.

Diphenylmethyl 7-(2-Thienylacetamido)-2-methylene-3-methylcepham-4-carboxylate (**4**)——Zn powder (1.0 g) was added to a solution of **3** (300 mg) in AcOH (30 ml) containing a few drops of DMF, and the mixture was stirred for 1.5 h at room temperature, then AcOEt was added. After filtration, the AcOEt solution was washed with sat. aqueous $NaHCO_3$ and sat. aqueous NaCl, and dried over Na_2SO_4. After removal of the organic solvent, Et_2O was added to the residue to give **4** (221 mg, 74%) as crystals. mp 150°C (dec.), (AcOEt–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1765, 1735, 1660, 1160. NMR (DMSO-d_6) δ: 1.05 (3H, d, J=7.5 Hz, 3-$C\underline{H}_3$), 2.80—3.15 (1H, m, 3-H), 3.77 (2H, s, $-C\underline{H}_2CO-$), 4.78 (1H, d, J=6.0 Hz, 4-H), 5.28 (1H, d, J=4.0 Hz, 6-H), 5.33 (2H, s, 2-methylene), 5.38 (1H, dd, J=4.0 and 7.5 Hz, 7-H), 6.80—7.00 (3H, m, thiophene 3-H and 4-H, $-C\underline{H}(C_6H_5)_2$), 7.15—7.55 (11H, m, thiophene 5-H, $C_6\underline{H}_5\times2$), 9.12 (1H, d, J=7.5 Hz, 7-NH). MS *m/e*:

518 (M^+). *Anal.* Calcd for $C_{28}H_{26}N_2O_4S_2$: C, 64.84; H, 5.05; N, 5.40; S, 12.36. Found: C, 64.68; H, 4.95; N, 5.53; S, 12.26.

Diphenylmethyl 7-(2-Thienylacetamido)-2-methyl-3-formyloxymethylceph-2-em-4-carboxylate (5)——HCOOH (99%; 13 ml) was added dropwise to a solution of **1** (0.7 g) in DMF (4 ml) at 0°C and then Zn powder (2.5 g)was added to the solution. The mixture was stirred for 20 h at room temperature, and then AcOEt and H_2O were added with stirring. The AcOEt layer was washed with sat. aqueous $NaHCO_3$ and sat. aqueous NaCl, then dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on a silica gel column. The fractions eluted with benzene–AcOEt (10: 1) were collected and concentrated to give **5** (381 mg, 57%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1790, 1735, 1670, 1190. NMR (DMSO-d_6) δ: 2.01 (3H, br s, 2-C$\underline{H}_3$), 3.77 (2H, s, -C$\underline{H}_2$CO-), 4.67 and 4.90 (1H each, ABq, J=13.0 Hz, -C$\underline{H}_2$O-), 5.12 (1H, d, J=4.5 Hz, 6-$\underline{H}$), 5.25 (1H, d, J=1.0 Hz, 4-$\underline{H}$), 5.46 (1H, dd, J=4.5 and 8.5 Hz, 7-$\underline{H}$), 6.78—7.00 (3H, m, thiophene 3-$\underline{H}$, 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.15—7.55 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times2$), 8.02 (1H, s, -C$\underline{H}$O), 9.14 (1H, d, J=8.5 Hz, 7-N$\underline{H}$). *Anal.* Calcd for $C_{29}H_{26}N_2O_6S_2$: C, 61.90; H, 4.66; N, 4.98; S, 11.40. Found: C, 61.79; H, 4.56; N, 4.87; S, 11.30.

Diphenylmethyl 7-(2-Thienylacetamido)-2-methyl-3-hydroxymethyl-ceph-2-em-4-carboxylate (6)——A 2 N HCl solution (22.8 ml) was added to a solution of **5** (30 g) in DMF (100 ml), and the mixture was stirred at room temperature for 20 h. Then AcOEt and H_2O were added to the solution with stirring. The AcOEt layer was separated and washed with 5% aqueous $NaHCO_3$ and sat. aqueous NaCl, and dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on a silica gel column. The column was washed with CH_2Cl_2–AcOEt (10: 1) and then eluted with acetone to give **6** (14.6 g, 62%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1780, 1745. NMR (DMSO-d_6) δ: 1.95 (3H, s, 2-C$\underline{H}_3$), 3.10—3.50 (1H, br, -CH_2O$\underline{H}$), 3.79 (2H, s, -C$\underline{H}_2$CO-), 3.85 and 4.32 (1H each, ABq, J=13.0 Hz, -C$\underline{H}_2$OH), 5.06 (1H, d, J=4.5 Hz, 6-$\underline{H}$), 5.25 (1H, s, 4-$\underline{H}$), 5.44 (1H, dd, J=4.5 and 8.5 Hz, 7-$\underline{H}$), 6.70—7.10 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$-(C_6H_5)$_2$), 7.10—7.60 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times2$), 9.10 (1H, d, J=8.5 Hz, 7-N$\underline{H}$). *Anal.* Calcd fro $C_{28}H_{26}N_2O_5S_2$: C, 62.90; H, 4.90; N, 5.24; S, 11.99. Found: C, 62.82; H, 4.76; N, 5.12; S, 11.93.

Diphenylmethyl 7-(2-Thienylacetamido)-2-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-2-em-4-carboxylate (8)——Pyridine (0.14 ml) was added to a solution of **6** (840 mg) in CH_2Cl_2 (45 ml), and then $SOCl_2$ (0.124 ml) was added to the solution at −10°C, followed by stirring at the same temperature for 30 min. H_2O was added to the mixture and the CH_2Cl_2 layer was separated. The CH_2Cl_2 layer was washed with H_2O and sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, the residue was dissolved in DMF (10 ml). 1-Methyl-5-mercaptotetrazole (180 mg) was added to the solution, and the mixture was stirred overnight at room temperature. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution of the column with CH_2Cl_2–AcOEt (20: 1) gave **8** (546 mg, 55%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1780, 1745. NMR (DMSO-d_6) δ: 1.98 (3H, s, 2-C$\underline{H}_3$), 3.77 (2H, s, -C$\underline{H}_2$CO-), 3.84 (3H, s, tetrazole 1-C$\underline{H}_3$), 4.06 and 4.47 (1H each, ABq, J=13.0 Hz, -C$\underline{H}_2$S-), 5.11 (1H, d, J=4.0 Hz, 6-$\underline{H}$), 5.41 (1H, s, 4-$\underline{H}$), 5.48 (1H, dd, J=4.0 and 7.5 Hz, 7-$\underline{H}$), 6.80—7.00 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.10—7.66 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times2$), 9.16 (1H, d, J=7.5 Hz, 7-N$\underline{H}$). *Anal.* Calcd for $C_{30}H_{28}N_6O_4S_3$: C, 56.94; H, 4.46; N, 13.28; S, 15.20. Found: C, 56.87; H, 4.42; N, 13.14; S, 14.83.

4-(1,1-Diphenylmethoxycarbonyl)-7-(2-thienylacetamido)-2α-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em 1-Oxide (9)——MCPBA (217 mg) was added to a solution of 8 (662 mg) in CH_2Cl_2 (30 ml) at −10°C, and the mixture was stirred at the same temperature for 1 h. The solution was washed with 10% $Na_2S_2O_3$ and sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution of the column with CH_2Cl_2–AcOEt (5: 1) gave **9** (321 mg, 47%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1800, 1745, 1690, 1050. NMR (DMSO-d_6) δ: 1.27 (3H, d, J=7.5 Hz, 2α-C$\underline{H}_3$), 3.84 (5H, s, tetrazole 1-C$\underline{H}_3$ and -C$\underline{H}_2$CO-), 4.01 (1H, q, J=7.5 Hz, 2β-$\underline{H}$), 4.25 (2H, s, -C$\underline{H}_2$S-), 4.96 (1H, d, J=5.0 Hz, 6-$\underline{H}$), 5.96 (1H, dd, J=5.0 and 8.5 Hz, 7-$\underline{H}$), 6.80—7.00 (3H, m, thiophene 3-$\underline{H}$, and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.10—7.70 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times2$), 8.37 (1H, d, J=8.5 Hz, 7-N$\underline{H}$). *Anal.* Calcd for $C_{30}H_{28}N_6O_5S_3$: C, 55.54; H, 4.35; N, 12.96; S, 14.83. Found: C, 55.59; H, 4.36; N, 12.98; S, 14.31.

Diphenylmethyl 7-(2-Thienylacetamido)-2α-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (10)——PCl_3 (0.38 ml) was added to a solution of the 1-oxide **9** (326 mg) in DMF (5 ml) at −10°C, and the mixture was stirred at the same temperature for 15 min, then AcOEt and 5% aqueous $NaHCO_3$ were added to the solution and partitioned. The AcOEt layer was washed with sat. aqueous NaCl and dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on a silica gel column. The column was eluted with CH_2Cl_2–AcOEt (25: 1) to give **10** (225 mg, 71%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1790, 1735, 1700. NMR (DMSO-d_6) δ: 1.53 (3H, d, J=7.0 Hz, 2α-C$\underline{H}_3$), 3.74 (2H, s, -C$\underline{H}_2$CO-), 3.84 (3H, s, tetrazole 1-C$\underline{H}_3$), 4.01 (1H, q, J=7.0 Hz, 2β-$\underline{H}$), 4.18 (2H, s, -C$\underline{H}_2$S-), 5.27 (1H, d, J=5.0 Hz, 6-$\underline{H}$), 5.85 (1H, dd, J=5.0 and 8.5 Hz, 7-$\underline{H}$), 6.80—7.00 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.15—7.60 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times2$), 9.17 (1H, d, J=8.5 Hz, 7-N$\underline{H}$). NOE: 20% signal increase for 6-$\underline{H}$ on irradiation of 2α-C$\underline{H}_3$. *Anal.* Calcd for $C_{30}H_{28}N_6O_4S_3$: C, 56.94; H, 4.46; N, 13.28; S, 15.20. Found: C, 56.94; H, 4.41; N, 13.20; S, 15.38.

Sodium 7-(2-Thienylacetamido)-2α-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (11a)——A solution of **10** (105 mg) in anisole (0.1 ml) and CF_3COOH (5 ml) was stirred at room tem-

perature for 20 min. After removal of the solvents, the residue was dissolved in AcOEt, and then transferred into 5% aqueous $NaHCO_3$. The H_2O layer was adjusted to pH 7 with 5% H_3PO_4 and applied to a column of Amberlite XAD-II. The column was washed with H_2O and then eluted with MeOH–H_2O (1: 9). Concentration of the eluate, and then lyophilization of the aqueous concentrated gave the sodium salt of **11a** (72 mg, 95%) as an amorphous solid. TLC (silica gel; AcOEt–AcOH(10: 1)): *Rf* 0.30. IR ν_{max}^{KBr} cm^{-1}: 1770, 1685. NMR (D_2O) δ: 1.52 (3H, d, J=7.5 Hz, 2α-C$\underline{H}_3$), 3.76 (1H, q, J=7.5 Hz, 2β-$\underline{H}$), 3.86 (2H, s, -C$\underline{H}_2$CO-), 3.97 (3H, s, tetrazole 1-C$\underline{H}_3$), 4.02 and 4.27 (1H each, ABq, J=13.0 Hz, -C$\underline{H}_2$S-), 5.17 (1H, J=5.0 Hz, 6-$\underline{H}$), 5.68 (1H, d, J=5.0 Hz, 7-$\underline{H}$), 6.90—7.10 (2H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$), 7.20—7.40 (1H, m, thiophene 5-$\underline{H}$). *Anal.* Calcd for $C_{17}H_{17}N_6NaO_4S_3 \cdot 3/2H_2O$: C, 39.60; H, 3.91; N, 16.30; S, 18.66. Found: C, 39.38; H, 3.72; N, 16.32; S, 18.54.

Diphenylmethyl 7-Amino-2α-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (11b)——Pyridine (3.7 ml) was added to a solution of **10** (2.38 g) in CH_2Cl_2 (100 ml), followed by addition of PCl_5 (2.4 g) at −10°C. The solution was stirred at the same temperature for 30 min. MeOH (10 ml) was added to the solution, followed by stirring at −10°C for 45 min and at room temperature for 1 h, then 0.5 M K_2HPO_4 (100 ml) was added, followed by adjusting the pH to 1.5 with H_3PO_4. The mixture was stirred for 45 min, then the CH_2Cl_2 layer was separated, washed with sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, addition of Et_2O to the residue gave **11b** (1.73 g, 72%) as crystals. mp 158°C (dec.), (CH_2Cl_2–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1780, 1735. NMR (DMSO-d_6+D_2O) δ: 1.56 (3H, d, J=7.0 Hz, 2α-C$\underline{H}_3$), 3.82 (3H, s, tetrazole 1-C$\underline{H}_3$), 3.93 (1H, q, J=7.0 Hz, 2β-$\underline{H}$), 4.16 (2H, s, -C$\underline{H}_2$S-), 4.90 (1H, d, J=5.0 Hz, 6-$\underline{H}$,) 5.11 (1H, d, J=5.0 Hz, 7-$\underline{H}$), 6.84 (1H, s, -C$\underline{H}$(C_6H_5)$_2$), 7.10—7.60 (10H, m, $C_6\underline{H}_5$×2). *Anal.* Calcd for $C_{24}H_{24}N_6O_3S_2$: C, 56.67; H, 4.76; N, 16.53; S, 12.61. Found: C, 56.61; H, 4.68; N, 16.46; S, 12.40.

7-[D(−)-2-(4-Ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(2-thienyl)acetamido]-2α-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylic Acid (11c)——A mixture of **11b** (220 mg), D(−)-2-(4-ethyl-2,3-dioxo-1-piperadinecarboxamido)-2-(2-thienyl)acetic acid (169 mg) and dicyclohexylcarbodiimide (107 mg) in CH_2Cl_2 (50 ml) was stirred at −5°C for 1 h and at room temperature overnight. The resulting dicyclohexylurea was filtered off and the filtrate was washed successively with 5% H_3PO_4, 5% aqueous $NaHCO_3$ and sat. aqueous NaCl solution. The organic layer was concentrated to give the crude amido-ester (318 mg), which was dissolved in anisole (0.4 ml) and CF_3COOH (20 ml), and the solution was stirred at room temperature for 20 min. After removal of the solvents, the residue was dissolved in AcOEt, and then transferred into 5% aqueous $NaHCO_3$. The H_2O layer was adjusted to pH 7 with 5% H_3PO_4 and applied to a column of Amberlite XAD-II. The column was washed with H_2O and then eluted with a gradient of H_2O −70% MeOH. The eluate was concentrated and adjusted to pH 2.0 with 5% H_3PO_4 to give **11c** (110 mg, 34%) as crystals. mp 152°C (dec.), (AcOEt–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1785, 1710, 1690. NMR (DMSO-d_6) δ: 1.09 (3H, t, J=7.0 Hz, -$CH_2C\underline{H}_3$), 1.50 (3H, d, J=7.0 Hz, 2α-C$\underline{H}_3$), 3.39 (2H, q, J=7.0 Hz, -C$\underline{H}_2CH_3$), 3.40—4.00 (5H, m, piperazine 5-C$\underline{H}_2$, 6-C$\underline{H}_2$, 2β-$\underline{H}$), 3.90 (3H, s, tetrazole 1-C$\underline{H}_3$), 4.08 and 4.34 (1H each ABq, J=13.0 Hz, -C$\underline{H}_2$S-), 5.16 (1H, d, J=5.0 Hz, 6-$\underline{H}$), 5.79 (1H dd, J=5.0 and 9.0 Hz, 7-$\underline{H}$), 5.85 (1H, d, J=7.0 Hz, >C$\underline{H}$-NH-), 6.80—7.20 (2H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$), 7.30—7.55 (1H, m, thiophene 5-$\underline{H}$), 9.50 (1H, d, J=9.0 Hz, 7-N$\underline{H}$), 9.75 (1H, d, J=7.0 Hz, >C$\underline{H}$-N$\underline{H}$). *Anal.* Calcd for $C_{24}H_{27}N_9O_7S_3$: C, 44.36; H, 4.19; N, 19.40; S, 14.80. Found: C, 44.21; H, 4.18; N, 19.23; S, 14.75.

Diphenylmethyl 7-(2-Thienylacetamido)-2-methyl-3-formylceph-2-em-4-carboxylate (12)——CrO_3 (4.2 g) was added to CH_2Cl_2 (250 ml) containing pyridine (6.7 ml), and the mixture was stirred at 15—20°C for 1 h. A solution of **6** (2.6 g) in CH_2Cl_2 (30 ml) was then added and the whole was stirred vigorously at 15—20°C for 5 min. Next, 1 M citric acid (100 ml) was added to the reaction mixture with stirring. The CH_2Cl_2 layer was separated, washed with H_2O, and sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution of the column with CH_2Cl_2–AcOEt (20: 1) gave **12** (938 mg, 36.2%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1790, 1750. NMR (DMSO-d_6) δ: 2.50 (3H, s, 2-C$\underline{H}_3$), 3.78 (2H, s, -C$\underline{H}_2$CO-), 5.07 (1H, d, J=4.5 Hz, 6-$\underline{H}$), 5.45 (1H, s, 4-$\underline{H}$), 5.47 (1H, dd, J=4.5 and 8.5 Hz, 7-$\underline{H}$), 6.70—7.00 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.10—7.50 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5$×2), 9.16 (1H, d, J=8.0 Hz, 7-N$\underline{H}$), 9.99 (1H, s, -C$\underline{H}$O). *Anal.* Calcd for $C_{28}H_{24}N_2O_5S_2$: C, 63.14; H, 4.54; N, 5.26; S, 12.04. Found: C, 63.01; H, 4.29; N, 5.12; S, 11.98.

4-(1,1-Diphenylmethoxycarbonyl)-7-(2-thienylacetamido)-2α-methyl-3-formylceph-3-em 1-Oxide (13)——MCPBA (0.95 g) was added to a solution of 12 (2.5 g) in CH_2Cl_2 (50 ml), and the mixture was stirred at room temperature for 1 h. The solution was washed with 5% aqueous $NaHCO_3$ and sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, the crystalline residue was recrystallized from CH_2Cl_2–AcOEt to give **13** (1.27 g, 50%). mp 168°C (dec.). IR ν_{max}^{KBr} cm^{-1}: 1805, 1725, 1665, 1040. NMR (DMSO-d_6): 1.11 (3H, d, J=7.5 Hz, 2α-C$\underline{H}_3$), 3.89 (2H, s, -C$\underline{H}_2$CO-), 4.11 (1H, q, J=7.5 Hz, 2β-$\underline{H}$), 5.08 (1H, d, J=5.0 Hz, 6-$\underline{H}$), 6.20 (1H, dd, J=5.0 and 8.5 Hz, 7-$\underline{H}$), 6.84—7.20 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.20—7.60 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5$×2), 8.67 (1H, d, J=8.5 Hz, 7-N$\underline{H}$), 9.96 (1H, s, -C$\underline{H}$O). *Anal.* Calcd for $C_{28}H_{24}N_2O_6S_2$: C, 61.30; H, 4.41; N, 5.11; S, 11.69. Found: C, 61.19; H, 4.43; N, 5.14; S, 11.53.

4-(1,1-Diphenylmethoxycarbonyl)-7-(2-thienylacetamido)-2α-methyl-3-methoxyiminomethylceph-3-em 1-Oxide (14a)——Pyridine (0.12 ml) was added to a solution of **13**(300 mg) and methoxyamine hydrochloride (123 mg) in a mixture of tetrahydrofuran (THF) (4 ml) and EtOH (16 ml), and the whole was stirred at 50°C for 1 h. After removal of the organic solvents, the residue was chromatographed on a silica gel column. The

eluate with benzene–AcOEt (10: 1) was concentrated, and then AcOEt was added to the residue to give **14** (252 mg, 80%) as crystals. mp 203°C (dec.), (CH_2Cl_2–AcOEt). IR ν_{max}^{KBr} cm^{-1}: 1800, 1730, 1670. NMR ($CDCl_3$) δ: 1.25 (3H, d, J=7.5 Hz, 2α-C<u>H</u>$_3$), 3.84 (2H, s, -C<u>H</u>$_2$CO-), 3.88 (3H, s, -OC<u>H</u>$_3$), 4.50 (1H, q, J=7.5 Hz, 2β-<u>H</u>), 4.51 (1H, d, J=5.0 Hz, 6-<u>H</u>), 6.14 (1H, dd, J=5.0 and 9.0 Hz, 7-<u>H</u>), 6.80—7.05 (3H, m, thiophene 3-<u>H</u> and 4-<u>H</u>, -C<u>H</u>(C_6H_5)$_2$), 7.10—7.60 (12H, m, thiophene 5-<u>H</u>, C_6<u>H</u>$_5$×2, 7-N<u>H</u>), 8.41 (1H, s, -C<u>H</u>=N-). *Anal.* Calcd for $C_{29}H_{27}N_3O_6S_2$: C, 60.29; H, 4.71; N, 7.27 S, 11.10. Found: C, 60.17; H, 4.70; N, 7.09; S, 10.91.

Diphenylmethyl 7-(2-Thienylacetamido)-2α-methyl-3-methoxyiminomethylceph-3-em-4-carboxylate (15a)——PCl_3 (0.3 ml) was added to a solution of **14** (249 mg) in DMF (8 ml) at −20°C, and the mixture was stirred at the same temperature for 45 min. The reaction mixture was treated in a manner similar to that described for the preparation of **10**. Elution of the silica gel column with benzene–AcOEt (15: 1), removal of the solvent from the eluate, and then addition of Et_2O to the residue gave **15a** (219 mg, 90%) as crystals, mp 160°C (dec.), (AcOEt–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1790, 1725, 1665. NMR (DMSO-d_6) δ: 1.50 (3H, d, J=7.5 Hz, 2α-C<u>H</u>$_3$), 3.74 (2H, s, -C<u>H</u>$_2$CO-), 3.82 (3H, s, -OC<u>H</u>$_3$), 4.35 (1H, q, J=7.5 Hz, 2β-<u>H</u>), 5.37 (1H, d, J=4.5 Hz, 6-<u>H</u>), 5.88 (1H, dd, J=4.5 and 8.5 Hz, 7-<u>H</u>), 6.75—7.05 (3H, m, thiophene 3-<u>H</u> and 4-<u>H</u>, -C<u>H</u>-(C_6H_5)$_2$), 7.10—7.60 (11H, m, thiophene 5-<u>H</u>, C_6<u>H</u>$_5$×2), 7.90 (1H, s, -C<u>H</u>=N-), 9.18 (1H, d, J=8.5 Hz, 7-N<u>H</u>). NOE: about 21% signal increase for 6-<u>H</u> on irradiation of 2α-C<u>H</u>$_3$. *Anal.* Calcd for $C_{29}H_{27}N_3O_5S_2$: C, 62.01; H, 4.75; N, 7.47; S, 11.42. Found: C, 61.88; H, 4.75; N, 7.34; S, 11.37.

Sodium 7-(2-Thienylacetamido)-2α-methyl-3-methoxyiminomethylceph-3-em-4-carboxylate (15b)——**15a** (160 mg) was treated with anisole (0.2 ml) and CF_3COOH (6 ml) to give **15b** (51 mg, 45%) by a procedure similar to that described above for the preparation of **11a** except that the column of Amberlite XAD-II was eluted with MeOH–H_2O (1: 4). Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1775, 1680. NMR (D_2O) δ: 1.56 (3H, d, J=7.0 Hz, 2α-C<u>H</u>$_3$), 3.91 (5H, s, -OC<u>H</u>$_3$ and -C<u>H</u>$_2$CO-), 4.22 (1H, q, J=7.0 Hz, 2β-<u>H</u>), 5.3 (1H, d, J=5.0 Hz, 6-<u>H</u>), 5.78 (1H, d, J=5.0 Hz, 7-<u>H</u>), 6.95—7.20 (2H, m, thiophene 3-<u>H</u> and 4-<u>H</u>), 7.25—7.50 (1H, m, thiophene 5-<u>H</u>). *Anal.* Calcd for $C_{16}H_{16}N_3NaO_5S_2\cdot 3/2H_2O$: C, 43.24; H, 4.31; N, 9.46; S, 14.43. Found: C, 43.22; H, 4.28; N, 9.38; S, 14.25.

Sodium 7-(2-Thienylacetamido)-2α-methyl-3-morpholinoethoxyiminomethylceph-3-em-4-carboxylate (15c)——**14b** (582 mg, 52%) prepared from **13** (946 mg) was converted to **15c** (202 mg, 42%) by a procedure similar to that described for **15a** and **15b**. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1775, 1680, 1620. NMR (D_2O) δ: 1.65 (3H, d, J=7.0 Hz, 2α-C<u>H</u>$_3$), 2.60—3.00 (6H, m, -C<u>H</u>$_2$C<u>H</u>$_2$N, morpholine 3-C<u>H</u>$_2$, 5-C<u>H</u>$_2$), 3.70—3.96 (4H, m, morpholine 2-C<u>H</u>$_2$, 6-C<u>H</u>$_2$), 4.00 (2H, s, -C<u>H</u>$_2$CO-), 4.16—4.60 (3H, m, -OC<u>H</u>$_2$CH$_2$-, 2β-<u>H</u>), 5.43 (1H, d, J=5.0 Hz, 6-<u>H</u>), 5.85 (1H, d, J=5.0 Hz, 7-<u>H</u>), 7.10—7.30 (2H, m, thiophene 3-<u>H</u> and 4-<u>H</u>), 7.35—7.55 (1H, m, thiophene 5-<u>H</u>). *Anal.* Calcd for $C_{21}H_{25}N_4NaO_6S_2\cdot 2H_2O$: C, 45.64; H, 5.29; N, 10.14; S, 11.60. Found: C, 45.83; H, 5.26; N, 9.98; S, 11.58.

Diphenylmethyl 7-(2-Thienylacetamido)-2-methyl-3-acetoxymethylceph-2-em-4-carboxylate (16)——A solution of $NaBH_4$ (4.4 g) in EtOH (250 ml) was added to a solution of **1** (45 g) in THF (150 ml) at 0°C. The mixture was stirred for 7 min, then AcOH (15 ml) was added. After removal of the solvent, AcOEt and H_2O were added to the residue and stirred. The AcOEt layer was separated, washed with 5% aqueous $NaHCO_3$ and sat. aqueous NaCl, and dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution with CH_2Cl_2–AcOEt (10: 1), concentration of the eluate, and the addition of Et_2O to the residue gave **16** (18.7 g, 41%) as crystals. mp 128°C (dec.), (AcOEt–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1770, 1730, 1650. NMR (DMSO-d_6) δ: 1.78 (3H, s, -COC<u>H</u>$_3$), 1.96 (3H, s, 2-C<u>H</u>$_3$), 3.72 (2H, s, -C<u>H</u>$_2$CO-), 4.57 and 4.73 (1H each, ABq, J=13.0 Hz, -C<u>H</u>$_2$O-), 5.06 (1H, d, J=4.5 Hz, 6-<u>H</u>), 5.20 (1H, s, 4-<u>H</u>), 5.42 (1<u>H</u>, dd, J=4.5 and 8.5 Hz, 7-<u>H</u>), 6.70—6.95 (3H, m, thiophene 3-<u>H</u> and 4-<u>H</u>, -C<u>H</u>(C_6H_5)$_2$), 7.15—7.50 (11H, m, thiophene 5-<u>H</u>, C_6<u>H</u>$_5$×2), 9.12 (1H, d, J=8.5 Hz, 7-N<u>H</u>). *Anal.* Calcd for $C_{30}H_{28}N_2O_6S_2$: C, 62.48; H, 4.89; N, 4.86; S, 11.12. Found: C, 62.55; H, 4.89; N, 4.97; S, 11.03.

4-(1,1-Diphenylmethoxycarbonyl)-7-(2-thienylacetamido)-2-methyl-3-acetoxymethylceph-3-em 1-Oxide (17)——A solution of MCPBA (13.7 g) in CH_2Cl_2 (50 ml) was added to a solution of **16** (37.3 g) in CH_2Cl_2 (300 ml) at −20°C, and the mixture was stirred at the same temperature for 30 min. The solution was washed with 5% aqueous $NaHCO_3$ and sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, addition of AcOEt–Et_2O (1: 3) to the residue gave crystalline **17** (32 g, 84%) as a mixture of stereoisomers. mp 160°C (dec.), (CH_2Cl_2–AcOEt). *Anal.* Calcd for $C_{30}H_{28}N_2O_7S_2$: C, 60.79; H, 4.76; N, 4.73; S, 10.82. Found: C, 60.83; H, 4.86; N, 4.61; S, 10.63.

Diphenylmethyl 7-(2-Thienylacetamido)-2β-methyl-3-acetoxymethylceph-3-em-4-carboxylate (18a) and Diphenylmethyl 7-(2-Thienylacetamido)-2α-methyl-3-acetoxymethylceph-3-em-4-carboxylate (19a)——PCl_3 (41.5 ml) was added to a solution of **17** (32 g) in DMF (200 ml) at −50°C. After being stirred at −30°C for 5 min, the mixture was poured into ice water, and extracted with AcOEt. The AcOEt layer was washed with 5% aqueous $NaHCO_3$ and sat. aqueous NaCl, and dried over Na_2SO_4. Removal of the solvent gave crude crystals. Recrystallization from ACOEt–Et_2O (1: 3) afforded **18a** (17 g, 55%). mp 152°C (dec.). TLC (silica gel; toluene–AcOEt (5: 1)): Rf 0.26. IR ν_{max}^{KBr} cm^{-1}: 1795, 1740, 1680, 1230. NMR (DMSO-d_6) δ: 1.45 (3H, d, J=7.0 Hz, 2β-C<u>H</u>$_3$), 1.95 (3H, s, -COC<u>H</u>$_3$), 3.79 (2H, s, -C<u>H</u>$_2$CO-), 4.04 (1H, q, J=7.0 Hz, 2α-<u>H</u>), 4.57 and 4.99 (1H each, ABq, J=13.0 Hz, -C<u>H</u>$_2$O-), 5.31 (1H, d, J=5.0 Hz, 6-H), 5.77 (1H, dd, J=5.0 and 8.0 Hz, 7-<u>H</u>), 6.90—7.05 (3H, m, thiophene 3-<u>H</u> and 4-<u>H</u>, -C<u>H</u>(C_6H_5)$_2$), 7.20—7.60 (11H, m, thiophene

5-H, $C_6H_5\times2$), 9.12 (1H, d, J=8.0 Hz, 7-NH). NOE: no signal increase for 6-H on irradiation of 2β-CH_3. *Anal.* Calcd for $C_{30}H_{28}N_2O_6S_2$: C, 62.48; H, 4.89; N, 4.86; S, 11.12. Found: C, 62.35; H, 4.71; N, 4.80; S, 11.21. The mother liquor of the crude crystals was concentrated. The residue was chromatographed on a silica gel column with toluene–AcOEt (15: 1) to give **19a** (0.3 g, 1%) as an amorphous solid. (Early fractions yielded the 2α-methyl isomer **18a** and thereafter the 2β-methyl isomer **19a** was eluted.). IR ν_{max}^{KBr} cm^{-1}: 1795, 1740, 1700, 1230. TLC (silica gel; toluene–AcOEt (5: 1)): *Rf* 0.30. NMR (DMSO-d_6) δ: 1.47 (3H, d, J= 7.0 Hz, 2α-CH_3), 1.91 (3H, s, -$COCH_3$), 3.75 (2H, s, -CH_2CO-), 3.84 (1H, q, J=7.0 Hz, 2β-H), 4.51 and 4.82 (1H each, ABq, J=13.0 Hz, -CH_2O-), 5.24 (1H, d, J=5.0 Hz, 6-H), 5.82 (1H, dd, J=5.0 and 8.0 Hz, 7-H), 6.80—7.00 (3H, m, thiophene 3-H and 4-H, -$CH(C_6H_5)_2$), 7.10—7.50 (11H, m, thiophene 5-H, $C_6H_5\times2$), 9.13 (1H, d, J=8.0 Hz, 7-NH). NOE: about 32% signal increase for 6-H on irradiation of 2α-CH_3. *Anal.* Calcd for $C_{30}H_{28}N_2O_6S_2$: C, 62.48; H, 4.89; N, 4.86; S, 11.12. Found: C, 62.73; H, 4.74; N, 4.67; S, 11.09.

Sodium 7-(2-Thienylacetamido)-2β-methyl-3-acetoxymethylceph-3-em-4-carboxylate (18b)——18a (1.4 g) was treated with anisole (3 ml) and CF_3COOH (20 ml) to give **18b** (829 mg, 79%) by a procedure similar to that described above for the preparation of **11a** except that the column of Amberlite XAD-II was eluted with H_2O. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1760, 1735, 1655, 1600. NMR (D_2O) δ: 1.46 (3H, d, J=7.0 Hz, 2β-CH_3), 2.11 (3H, s, -$COCH_3$), 3.91 (2H, s, -CH_2CO-), 3.98 (1H, q, J=7.0 Hz, 2α-H), 4.69 and 5.07 (1H each, ABq, J=13.0 Hz, -CH_2O-), 5.21 (1H, d, J=5.0 Hz, 6-H), 5.60 (1H, d, J=5.0 Hz, 7-H), 6.95—7.10 (2H, m, thiophene 3-H and 4-H), 7.30—7.55 (1H, m, thiophene 5-H). *Anal.* Calcd for $C_{17}H_{17}N_2NaO_6S_2\cdot3/2$ H_2O: C, 44.44; H, 4.39; N, 6.10; S, 13.96. Found: C, 44.62; H, 4.11; N, 5.85; S, 13.75.

Sodium 7-(2-Thienylacetamido)-2α-methyl-3-acetoxymethylceph-3-em-4-carboxylate (19b)——19b (68 mg, 66%) was prepared from **19a** (144 mg) in a manner similar to that described for the preparation of the 2β-methyl isomer **18b**. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1780, 1680, 1240. NMR (D_2O) δ: 1.63 (3H, d, J=7.5 Hz, 2α-CH_3), 2.33 (3H, s, -$COCH_3$), 3.70 (1H, q, J=7.5 Hz, 2β-H), 4.00 (2H, s, -CH_2CO-), 4.78 and 5.00 (1H each, ABq, J=13.0 Hz, -CH_2O-), 5.30 (1H, d, J=5.0 Hz, 6-H), 5.82 (1H, d, J=5.0 Hz, 7-H), 7.07—7.23 (2H, m, thiophene 3-H and 4-H), 7.40—7.58 (1H, m, thiophene 5-H). *Anal.* Calcd for $C_{17}H_{17}N_2NaO_6S_2\cdot$ $3/2H_2O$: C, 44.44; H, 4.39; N, 6.10; S, 13.96. Found: C, 44.81; H, 4.48; N, 6.04; S, 13.76.

Diphenylmethyl 7-Amino-2β-methyl-3-acetoxymethylceph-3-em-4-carboxylate (18c)——18c (3.7 g, 87%) was prepared from **18a** (4 g) by a procedure similar to that described above for the preparation of **11b** except that **18c** was purified by silica gel chromatography with CH_2Cl_2–AcOEt (10: 1). mp 101°C (dec.), (CH_2Cl_2–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1760, 1730, 1220. NMR (DMSO-d_6+D_2O) δ: 1.40 (3H, d, J=7.0 Hz, 2β-CH_3), 1.89 (3H, s, -$COCH_3$), 3.94 (1H, q, J=7.0 Hz, 2α-H), 4.48 and 4.93 (1H each ABq, J=13.0 Hz, -CH_2O-), 4.76 (1H, d, J=7.0 Hz, 6-H), 5.12 (1H, d, J=5.0 Hz, 7-H), 6.85 (1H, s, -$CH(C_6H_5)_2$), 7.33 (10H, s, $C_6H_5\times2$). *Anal.* Calcd for $C_{24}H_{24}N_2O_5S$: C, 63.70; H, 5.35; N, 6.19; S, 7.08. Found: C, 63.62; H, 5.25; N, 6.11; S, 7.03.

Sodium 7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-2β-methyl-3-acetoxymethylceph-3-em-4-carboxylate (18d)——18d (305 mg, 18%) was prepared from **18c** (1.6 g) by an established method. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1770, 1670, 1610, 1240. NMR (D_2O) δ: 1.50 (3H, d, J=7.0 Hz, 2β-CH_3), 2.10 (3H, s, -$COCH_3$), 4.00 (3H, s, -OCH_3), 4.05 (1H, q, J=7.0 Hz, 2α-H), 4.69 and 5.07 (1H each, ABq, J=13.0 Hz, -CH_2O-), 5.34 (1H, d, J=4.5 Hz, 6-H), 5.78 (1H, d, J=4.5 Hz, 7-H), 7.03 (1H, s, thiazole 5-H). *Anal.* Calcd for $C_{17}H_{18}N_5NaO_7S_2\cdot2H_2O$: C, 38.70; H, 4.20; N, 13.28; S, 12.16. Found: C, 38.61; H, 4.23; N, 13.21; S, 12.06.

Sodium 7-(2-Thienylacetamido)-2β-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (20a)——A solution of **18b** (410 mg), 1-methyl-5-mercaptotetrazole (139 mg) and $NaHCO_3$ (84 mg) in 1/15 M phosphate buffer solution (pH 6.4, 20 ml) was stirred at 50°C for 6 h. The solution was maintained at pH 6.4 with 5% aqueous $NaHCO_3$. The reaction mixture was then applied to a column of Amberlite XAD-II, and the column was washed with water and eluted with a gradient of H_2O–70% MeOH. The eluate was concentrated and lyophilized to give **20a** (148 mg, 30%) as an amorphous solid. TLC (silica gel, AcOEt–AcOH (1: 1)): *Rf* 0.23. IR ν_{max}^{KBr} cm^{-1}: 1760, 1670, 1600. NMR (D_2O): 1.48 (3H, d, J=7.0 Hz, 2β-CH_3), 3.89 (2H, s, -CH_2CO-), 3.95 (1H, q, J=7.0 Hz, 2α-H), 4.03 (3H, s, tetrazole 1-CH_3), 4.43 and 4.87 (1H each, ABq, J=13.0 Hz, -CH_2S-), 5.11 (1H, d, J=4.5 Hz, 6-H), 5.47 (1H, d, J=4.5 Hz, 7-H), 6.90—7.10 (2H, m, thiophene 3-H and 4-H), 7.25—7.45 (1H, m, thiophene 5-H). *Anal.* Calcd for $C_{17}H_{17}N_6NaO_4S_3\cdot$ $3/2H_2O$: C, 39.60; H, 3.91; N, 16.30; S, 18.66. Found: C, 39.80; H, 3.84; N, 16.20; S, 18.53.

Sodium 7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-2β-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (20b)——20b (91 mg, 32%) was prepared from **18d** (264 mg) by a procedure similar to that described for **20a**. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1770, 1680, 1615. NMR (D_2O) δ: 1.53 (3H, d, J=7.0 Hz, 2β-CH_3), 4.00 (3H, s, tetrazole 1-CH_3), 4.06 (3H, s, -OCH_3), 4.17 (1H, q, J=7.0 Hz, 2α-H), 5.26 (1H, d, J=4.5 Hz, 6-H), 5.68 (1H, d, J=4.5 Hz, 7-H), 7.05 (1H, s, thiazole 5-H). *Anal.* Calcd for $C_{17}H_{18}N_9NaO_5S_3\cdot2H_2O$: C, 34.98; H, 3.80; N, 21.60; S, 16.48. Found: C, 34.84; H, 3.75; N, 21.53; S, 16.32.

7-[D(—)-2-(4-Ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(2-thienyl)acetamido]-2β-methyl-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylic Acid (20c)——20c (110 mg, 21%) was prepared from **18c** (365 mg) by a procedure similar to that described for **11c** and **20a**. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1775, 1680. NMR (DMSO-d_6) δ: 1.09 (3H, t, J=7.0 Hz, -CH_2CH_3), 1.42 (3H, d, J=7.0 Hz, 2β-CH_3), 3.39 (2H, q, J=7.0 Hz, -CH_2CH_3), 3.40—3.90 (5H, m, piperazine 5-CH_2, 6-CH_2, 2α-H), 3.92 (3H, s, tetrazole 1-CH_3),

4.08 and 4.75 (1H each, ABq, J=13.0 Hz, -C$\underline{H}_2$S-), 5.10 (1H, d, J=5.0 Hz, 6-$\underline{H}$), 5.63 (1H, dd, J=5.0 and 9.0 Hz, 7-$\underline{H}$), 5.90 (1H, d, J=7.0 Hz, >C$\underline{H}$-NH-), 6.80—7.20 (2H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$), 7.30—7.55 (1H, m, thiophene 5-$\underline{H}$), 9.43 (1H, d, J=9.0 Hz, 7-N$\underline{H}$), 9.74 (1H, d, J=7.0 Hz, >CH-N$\underline{H}$-). *Anal.* Calcd for $C_{24}H_{27}N_9O_7S_3$: C, 44.36; H, 4.19; N, 19.40; S, 14.80. Found: C, 44.32; H, 4.08; N, 19.35; S, 14.65.

Sodium 7-(2-Thienylacetamido)-2β-methyl-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethylceph-3-em-4-carboxylate (20d)——**20d** (212 mg, 42%) was prepared from **18b** (410 mg) by a procedure similar to that described for **20a** except that 5-methyl-2-mercapto-1,3,4-thiadiazole (158 mg) was employed instead of 1-methyl-5-mercaptotetrazole. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1760, 1675, 1605. NMR (D_2O) δ: 1.44 (3H, d, J=7.0 Hz, 2β-C$\underline{H}_3$), 2.73 (3H, s, thiadiazole 5-C$\underline{H}_3$), 3.89 (2H, s, -C$\underline{H}_2$CO-), 4.09 (1H, q, J=7.0 Hz, 2α-$\underline{H}$), 3.87 and 4.71 (1H each, ABq, J=14.0 Hz, -C$\underline{H}_2$S-), 5.10 (1H, d, J=4.5 Hz, 6-$\underline{H}$), 5.50 (1H, d, J=4.5 Hz, 7-$\underline{H}$), 6.96—7.10 (2H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$), 7.25—7.50 (1H, m, thiophene 5-$\underline{H}$). *Anal.* Calcd for $C_{18}H_{17}N_4NaO_4S_4\cdot H_2O$: C, 41.29; H, 3.66; N, 10.70; S, 24.49. Found: C, 41.27; H, 3.51; N, 10.98; S, 24.47.

Disodium Salt of 7-(2-Thienylacetamido)-2β-methyl-3-(1-carboxymethyl-1*H*-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylic Acid (20e)——**20e** (98 mg, 20%) was prepared from **18b** (370 mg) by a procedure similar to that described for **20a** except that 1-carboxymethyl-5-mercaptotetrazole (192 mg) was employed instead of 1-methyl-5-mercaptotetrazole. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1770, 1670, 1620. NMR (D_2O) δ: 1.48 (3H, d, J=7.0 Hz, 2β-C$\underline{H}_3$), 3.92 (2H, s, -C$\underline{H}_2$CO-), 3.97 and 4.74 (1H each, ABq, J=13.0 Hz, -C$\underline{H}_2$S-), 4.07 (1H, q, J=7.0 Hz, 2α-$\underline{H}$), 5.03 (2H, s, tetrazole 1-C$\underline{H}_2$CO-), 5.16 (1H, d, J=4.5 Hz, 6-$\underline{H}$), 5.52 (1H, d, J=4.5 Hz, 7-$\underline{H}$), 7.00—7.20 (2H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$), 7.30—7.50 (1H, m, thiophene 5-$\underline{H}$). *Anal.* Calcd for $C_{18}H_{16}N_6Na_2O_6S_3\cdot 3H_2O$: C, 35.64; H, 3.64; N, 13.81; S, 15.80. Found: C, 35.92; H, 3.62; N, 13.30; S, 15.88.

Diphenylmethyl 7-(2-Thienylacetamido)-3-methylene-2-oxocepham-4-carboxylate (21)——Ozone was bubbled into a solution of **2** (4.0 g, 7.7 mmol) in CH_2Cl_2 (200 ml) at a flow rate of about 31 mmol/h at −70°C for 15 min. After nitrogen had been bubbled through the solution for 15 min, methyl sulfide (1 ml) was added to the solution at −70°C, and the mixture was stirred at the same temperature for 30 min and then room temperature for 30 min. After removal of the solvent, the residue was chromatographed on a silica gel column using benzene–AcOEt (10: 1). The eluate was concentrated, and petroleum ether was added to the residue to give **21** (1.37 g, 34.1%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1780, 1750, 1650. NMR (DMSO-d_6) δ: 3.77 (2H, s, -C$\underline{H}_2$CO-), 5.49 (1H, dd, J=5.0 and 8.0 Hz, 7-$\underline{H}$), 5.51 (1H, d, J=5.0 Hz, 6-$\underline{H}$), 5.99 (1H, s, 3-methylene), 6.12 (1H, s, 4-$\underline{H}$), 6.32 (1H, s, 3-methylene), 6.85—7.00 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.10—7.60 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times 2$), 9.18 (1H, d, J=8.0 Hz, 7-N$\underline{H}$). *Anal.* Calcd for $C_{27}H_{22}N_2O_5S_2$: C, 62.35; H, 4.28; N, 5.40; S, 12.31. Found: C, 62.39; H, 4.43; N, 5.11; S, 12.06.

Diphenylmethyl 7-(2-Thienylacetamido)-3-methyl-2-oxoceph-3-em-4-carboxylate (22)——(a) *Via* Isomerization of the 3-Exomethylene-2-oxocepham (**21**): Et_3N (0.014 ml) was added to a solution of **21** (52 mg) in CH_2Cl_2 (5 ml) at −50°C, and the mixture was stirred for 25 min at the same temperature. The reaction solution was washed with 5% H_3PO_4 and sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on a silica gel column using benzene–AcOEt (15: 1). The eluate was concentrated, and petroleum ether was added to the residue to give **22** (38 mg, 73%) as an amorphous solid. TLC (silica gel, benzene–AcOEt (5: 1)): Rf=0.52. IR ν_{max}^{KBr} cm^{-1}: 1795, 1740, 1675, 1645, 1635, 1540, 1390, 1225, 1210. NMR (DMSO-d_6) δ: 1.90 (3H, s, 3-C$\underline{H}_3$), 3.77 (2H, s, -C$\underline{H}_2$CO-), 5.80 (1H, dd, J=4.5 and 8.5 Hz, 7-$\underline{H}$), 5.98 (1H, d, J=4.5 Hz, 6-$\underline{H}$), 6.85—7.10 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.20—7.70 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times 2$), 9.42 (1H, d, J=8.5 Hz, 7-N$\underline{H}$). *Anal.* Calcd for $C_{27}H_{22}N_2O_5S_2$: C, 62.53; H, 4.28; N, 5.40; S, 12.36. Found: C, 62.48; H, 4.26; N, 5.31; S, 12.11.

b) *Via* Ozonolysis of the 2-Exomethylene-3-methylceph-3-em (**3**): Ozone was bubbled into a solution of **3** (2.1 g, 4.1 mmol) in CH_2Cl_2 (50 ml) at a flow rate of about 16 mmol/h at −70°C for 15 min. The subsequent procedure was similar to that described above for the preparation of **21** except that the silica gel column was eluted with benzene–AcOEt (15: 1) to give **22** (1.2 g, 56.9%), which was identical (TLC, IR, NMR) with a specimen prepared by route a).

Diphenylmethyl 7-(2-Thienylacetamido)-3-hydroxy-2-oxoceph-3-em-4-carboxylate (23)——Ozone was bubbled into a solution of **2** (1.4 g, 2.7 mmol) in CH_2Cl_2 (100 ml) at a flow rate of about 33 mmol/h at −70°C for 10 min. The subsequent procedure was similar to that described above for the preparation of **21** except that the silica gel column was eluted with benzene–AcOEt (20: 1) to give **23** (0.1 g, 7.1%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1785, 1670, 1650. NMR (DMSO-d_6) δ: 3.79 (2H, s, -C$\underline{H}_2$CO-), 5.69 (1H, dd, J=4.5 and 8.0 Hz, 7-$\underline{H}$), 5.95 (1H, d, J=4.5 Hz, 6-$\underline{H}$), 6.80—7.00 (3H, m, thiophene 3-$\underline{H}$ and 4-$\underline{H}$, -C$\underline{H}$(C_6H_5)$_2$), 7.15—7.60 (11H, m, thiophene 5-$\underline{H}$, $C_6\underline{H}_5\times 2$), 9.42 (1H, d, J=8.0 Hz, 7-N$\underline{H}$). *Anal.* Calcd for $C_{26}H_{20}N_2O_6S_2$: C, 59.99; H, 3.87; N, 5.38; S, 12.32. Found: C, 59.83; H, 3.81; N, 5.32; S, 12.25.

Diphenylmethyl 7-(2-Thienylacetamido)-3-methoxy-2-oxoceph-3-em-4-carboxylate (24a)——Ozone was bubbled into a solution of **2** (2.4 g, 4.6 mmol) in CH_2Cl_2 (200 ml) at a flow rate of about 28 mmol/h at −70°C for 20 min. After removal of the excess ozone, a large excess of an ethereal solution of CH_2N_2 was added to the solution at 0°C, and the mixture was stirred for 30 min. AcOH was added to decompose excess CH_2N_2, then the solution was washed with 5% $NaHCO_3$ and sat. aqueous NaCl, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on a silica gel column using benzene–AcOH (15: 1).

The eluate was concentrated, and petroleum ether was added to the residue to give **24a** (403 mg, 18.3%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1800, 1735, 1665, 1655. NMR (DMSO-d_6) δ: 3.68 (3H, s, $-OCH_3$), 3.75 (2H, s, $-CH_2CO-$), 5.75 (1H, dd, J=4.5 and 8.0 Hz, 7-H), 6.02 (1H, d, J=4.5 Hz, 6-H), 6.80—7.05 (3H, m, thiophene 3-H and 4-H, $-CH(C_6H_5)_2$), 7.10—7.60 (11H, d, J=8.0 Hz, 7-NH). *Anal.* Calcd for $C_{27}H_{22}N_2O_6S_2$: C, 60.66; H, 4.15; N, 5.24; S, 11.99. Found: C, 60.58; H, 4.19; N, 5.03; S, 11.78.

7-(2-Thienylacetamido)-3-methoxy-2-oxoceph-3-em-4-carboxylic Acid (24b)——A solution of **24a** (500 mg) in anisole (2.2 ml) and CF_3COOH (5 ml) was stirred for 20 min at room temperature. After removal of the CF_3COOH, the residue was dissolved in AcOEt, followed by extraction with 5% $NaHCO_3$. The aqueous layer was adjusted to pH 2 with 5% H_3PO_4 and extracted with AcOEt. The AcOEt layer was washed with H_2O and sat. aqueous NaCl, and dried over Na_2SO_4. After removal of the solvent, petroleum ether was added to the residue to give **24b** (298 mg, 86.5%) as an amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 1795, 1735, 1665, 1650. NMR (DMSO-d_6) δ: 3.70 (3H, s, $-OCH_3$), 3.76 (2H, s, $-CH_2CO-$), 5.69 (1H, dd, J=4.5 and 7.5 Hz, 7-H), 5.99 (1H, d, J=4.5 Hz, 6-H), 6.80—7.10 (2H, m, thiophene 3-H and 4-H), 7.15—7.45 (1H, m, thiophene 5-H), 9.41 (1H, d, J=7.5 Hz, 7-NH). *Anal.* Calcd for $C_{14}H_{12}N_6O_6S_2$: C, 44.55; H, 3.47; N, 7.42; S, 16.99. Found: C, 44.72; H, 3.25; N, 7.36; S, 16.73.

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References and Notes

1) *a*) Part of this work was presented at the 101st Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, Apr. 1981; Abstracts of papers, p. 345 (3Na2-2); *b*) Part of thisework and related experimental results were disclosed in the following patent application: Takeda Chem. Ind. Co., Ltd., Japanese Published Unexamined Patent Application 56-118085 (1981) [European Patent Application, Publication Number 35357 (1981)].
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