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Synthesis of 2 α -Methyl- and 2 β -Methyl-3-(substituted methyl)cephalosporins, and 2,3-Dioxomethylenecepham¹⁾

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The stereoselective synthesis of 2 α -methyl- and 2 β -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 2 α -methyl-3-(substituted methyl)ceph-3-em by oxidation with peracid. On the other hand, isomerization of **16** to the corresponding ceph-3-em by oxidation with peracid gave mainly the 2 β -methyl isomer (55:1 ratio). Ozonolysis of **2** followed by treatment with diazomethane gave 2-oxo-3-methoxyceph-3-em (**24**).

Keywords—2 α -methylcephalosporin; 2 β -methylcephalosporin; 2-methyl-3-formyloxymethylcephem; 2-methyl-3-acetoxymethylcephem; 2-methyl-3-(substituted methyl)cephem; 2,3-dioxomethylenecepham; 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 far²⁾ and 2,3-dimethylceph-3-em derivatives³⁾ have been the only 2-methylceph-3-em derivatives bearing C-3 substituents. As regards antimicrobial activity, it was reported that a 2 β ,3-dimethylceph-3-em derivative showed somewhat stronger activity than the 2 α 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 2 α -methylceph-3-em derivatives bearing no C-3 substituent showed greater antimicrobial activity than the 2 β -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 2 α -methyl- and 2 β -methyl-3-(substituted methyl)ceph-3-em derivatives.

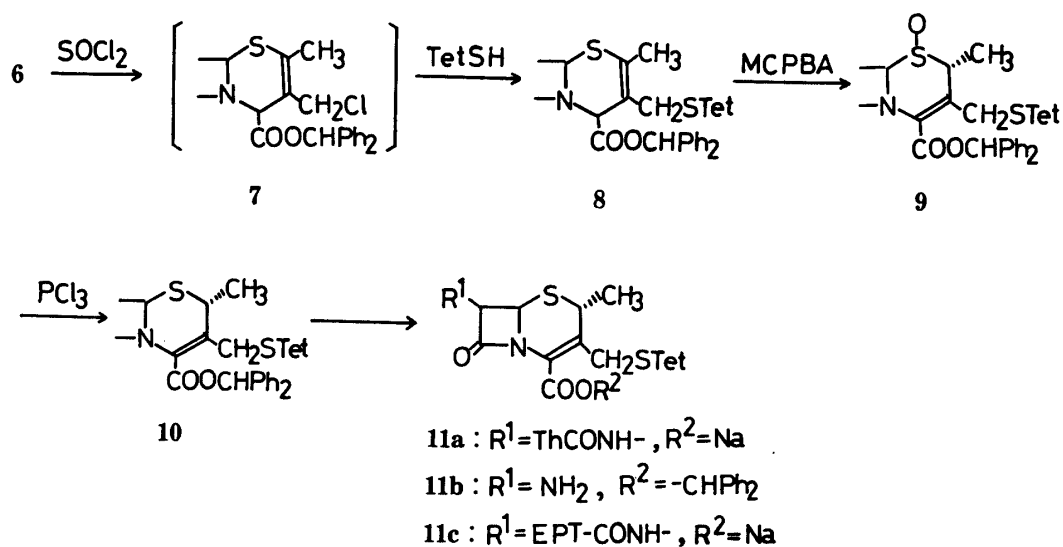
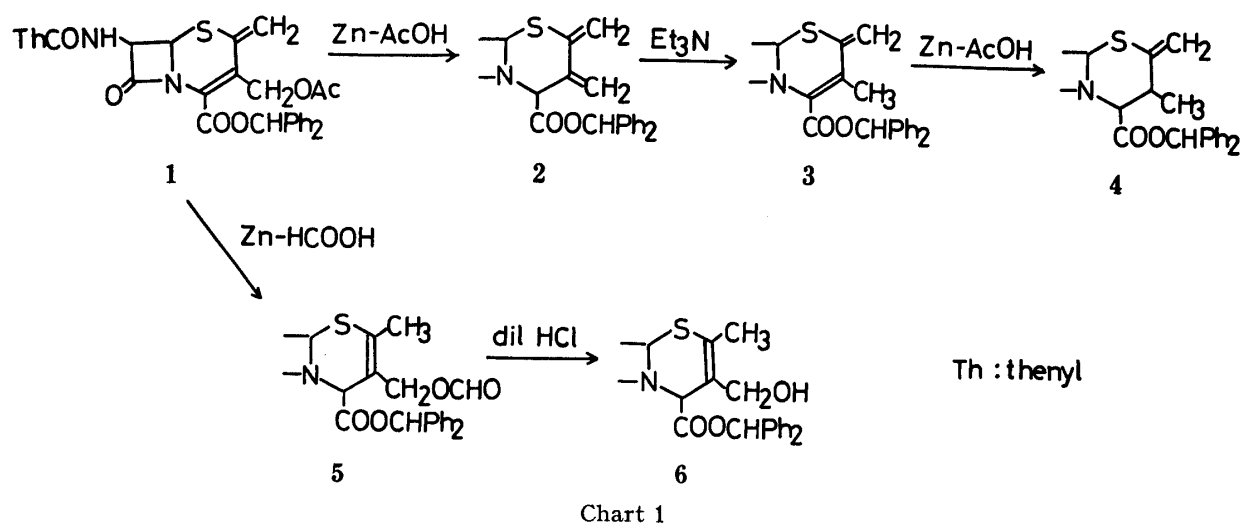
In this paper, stereoselective synthesis of 2 α - and 2 β -methylceph-3-em derivatives bearing C-3 substituents from 2-exomethylenecephalosporin (**1**) is described. Some of the products showed considerable antimicrobial activities. The synthesis of a 2,3-dioxomethylenecepham derivative (**2**) and 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 2 β -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 2 α -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 Et₃N 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.



Tet: 1-methyl-1H-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 2 α -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 SOCl₂-pyridine and then with 1-methyl-5-mercaptopotetrazole. The sequence of oxidation of **8** to the sulfoxide (**9**) with *m*-chloroperbenzoic acid (MCPBA) and reduction of **9** with PCl₃ 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 (CF₃COOH-anisole) to give the free acid (**11a**) as the Na salt.

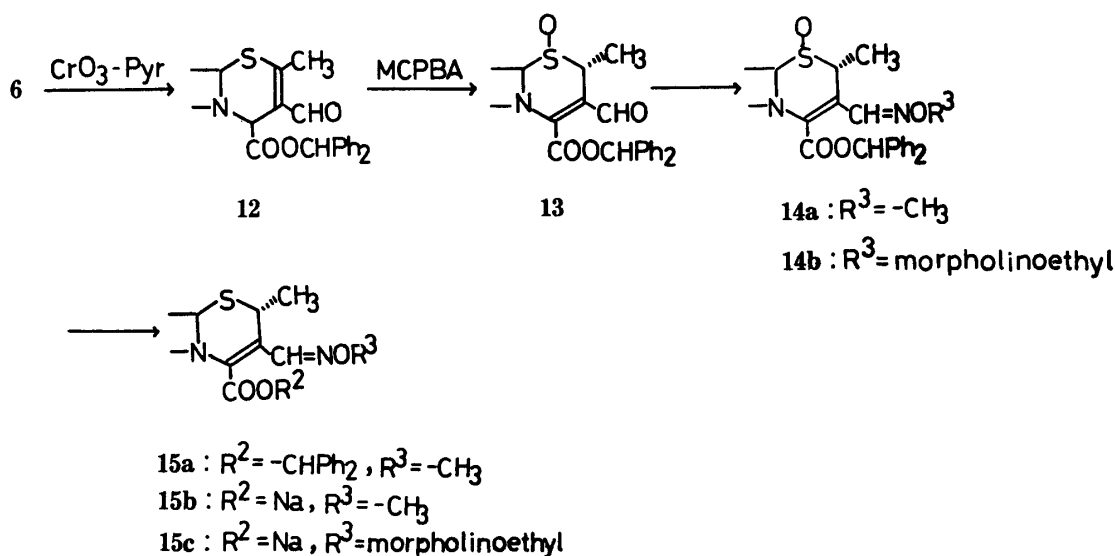
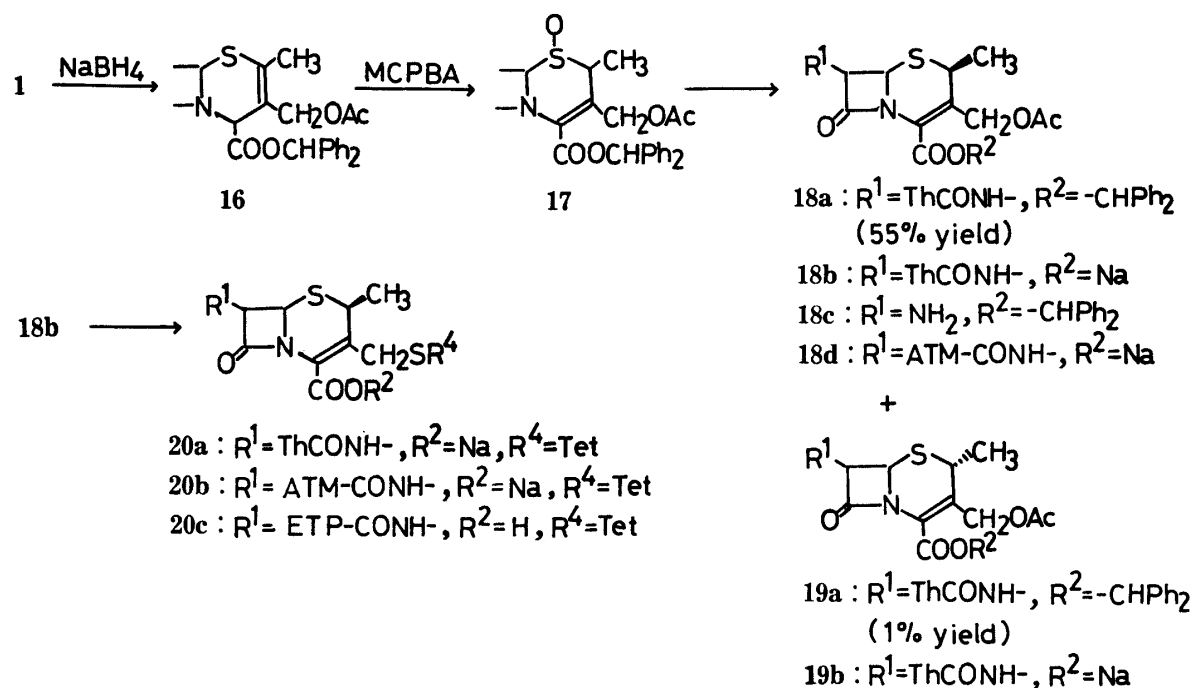


Chart 3

Compound **6** is also useful as an intermediate for the preparation of 2 α -methyl-3-formylceph-3-em derivatives which can be converted to 2 α -methyl-3-alkoxyiminomethylceph-3-em derivatives, such as the 3-methoxyiminomethyl and 3-morpholinoethoxyiminomethyl derivatives. As shown in Chart 3, oxidation of **6** with CrO₃-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 PCl₃ 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 CF₃COOH-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.

2 β -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-1H-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 $\text{AcOEt-Et}_2\text{O}$ (1 : 3) to give the 2 β -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 2 α -methyl isomer (**19a**) in about 1% yield. The stereochemistry of the C-2 methyls (2 β -methyl for **18a** and 2 α -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 2 β -methyl-4-carboxylic acid (**18b**) was obtained as the Na salt; **18b** was then converted to 2 β -methyl-3-

TABLE I. MICs of 7-(Thienylacetamido)-2-methyl-3-(substituted methyl)cephalosporins ($\mu\text{g/ml}$)^{a)}

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

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

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 $65\text{ }\mu\text{g/ml}$) 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 F₂₅₄ 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**. mp 127°C (dec.), (AcOEt- Et_2O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1770, 1735, 1660. NMR (dimethyl sulfoxide- d_6 (DMSO- d_6)) δ : 3.74 (2H, s, $-\text{CH}_2\text{CO}-$), 5.18 (1H, d, $J=4.5\text{ Hz}$, 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, $-\text{CH}(\text{C}_6\text{H}_5)_2$), 7.20—7.60 (11H, m, thiophene 5-H, $\text{C}_6\text{H}_5 \times 2$), 9.11 (1H, d, $J=8.5\text{ Hz}$, 7-NH). MS m/e 516 (M^+). Anal. Calcd for $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_4\text{S}_2$: 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1780, 1730, 1670. NMR (DMSO- d_6) δ : 2.04 (3H, s, 3- CH_3), 3.74 (2H, s, $-\text{CH}_2\text{CO}-$), 5.20 (1H, d, $J=4.5\text{ Hz}$, 6-H), 5.60 (1H, s, one proton of 2-methylene), 5.72 (1H, dd, $J=4.5$ and 8.5 Hz , 7-H), 5.80 (1H, s, one proton of 2-methylene), 6.80—7.00 (3H, m, thiophene 3-H, 4-H, $-\text{CH}(\text{C}_6\text{H}_5)_2$), 7.15—7.60 (11H, thiophene 5-H, $\text{C}_6\text{H}_5 \times 2$), 9.22 (1H, d, $J=8.5\text{ Hz}$, 7-NH). Anal. Calcd for $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_4\text{S}_2$: 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1765, 1735, 1660, 1160. NMR (DMSO- d_6) δ : 1.05 (3H, d, $J=7.5\text{ Hz}$, 3- CH_3), 2.80—3.15 (1H, m, 3-H), 3.77 (2H, s, $-\text{CH}_2\text{CO}-$), 4.78 (1H, d, $J=6.0\text{ Hz}$, 4-H), 5.28 (1H, d, $J=4.0\text{ Hz}$, 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, $-\text{CH}(\text{C}_6\text{H}_5)_2$), 7.15—7.55 (11H, m, thiophene 5-H, $\text{C}_6\text{H}_5 \times 2$), 9.12 (1H, d, $J=7.5\text{ Hz}$, 7-NH). MS m/e :

518 (M⁺). *Anal.* Calcd for C₂₈H₂₆N₂O₄S₂: 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 H₂O were added with stirring. The AcOEt layer was washed with sat. aqueous NaHCO₃ and sat. aqueous NaCl, then dried over Na₂SO₄. 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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1790, 1735, 1670, 1190. NMR (DMSO-*d*₆) δ : 2.01 (3H, br s, 2-CH₃), 3.77 (2H, s, -CH₂CO-), 4.67 and 4.90 (1H each, ABq, *J* = 13.0 Hz, -CH₂O-), 5.12 (1H, d, *J* = 4.5 Hz, 6-H), 5.25 (1H, d, *J* = 1.0 Hz, 4-H), 5.46 (1H, dd, *J* = 4.5 and 8.5 Hz, 7-H), 6.78–7.00 (3H, m, thiophene 3-H, 4-H, -CH(C₆H₅)₂), 7.15–7.55 (11H, m, thiophene 5-H, C₆H₅ × 2), 8.02 (1H, s, -CHO), 9.14 (1H, d, *J* = 8.5 Hz, 7-NH). *Anal.* Calcd for C₂₈H₂₆N₂O₆S₂: 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 H₂O were added to the solution with stirring. The AcOEt layer was separated and washed with 5% aqueous NaHCO₃ and sat. aqueous NaCl, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on a silica gel column. The column was washed with CH₂Cl₂–AcOEt (10:1) and then eluted with acetone to give **6** (14.6 g, 62%) as an amorphous solid. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1780, 1745. NMR (DMSO-*d*₆) δ : 1.95 (3H, s, 2-CH₃), 3.10–3.50 (1H, br, -CH₂OH), 3.79 (2H, s, -CH₂CO-), 3.85 and 4.32 (1H each, ABq, *J* = 13.0 Hz, -CH₂OH), 5.06 (1H, d, *J* = 4.5 Hz, 6-H), 5.25 (1H, s, 4-H), 5.44 (1H, dd, *J* = 4.5 and 8.5 Hz, 7-H), 6.70–7.10 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.10–7.60 (11H, m, thiophene 5-H, C₆H₅ × 2), 9.10 (1H, d, *J* = 8.5 Hz, 7-NH). *Anal.* Calcd for C₂₈H₂₆N₂O₅S₂: 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-1H-tetrazol-5-yl)thiomethylceph-2-em-4-carboxylate (8)—Pyridine (0.14 ml) was added to a solution of **6** (840 mg) in CH₂Cl₂ (45 ml), and then SOCl₂ (0.124 ml) was added to the solution at -10°C, followed by stirring at the same temperature for 30 min. H₂O was added to the mixture and the CH₂Cl₂ layer was separated. The CH₂Cl₂ layer was washed with H₂O and sat. aqueous NaCl, and dried over MgSO₄. 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 CH₂Cl₂–AcOEt (20:1) gave **8** (546 mg, 55%) as an amorphous solid. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1780, 1745. NMR (DMSO-*d*₆) δ : 1.98 (3H, s, 2-CH₃), 3.77 (2H, s, -CH₂CO-), 3.84 (3H, s, tetrazole 1-CH₃), 4.06 and 4.47 (1H each, ABq, *J* = 13.0 Hz, -CH₂S-), 5.11 (1H, d, *J* = 4.0 Hz, 6-H), 5.41 (1H, s, 4-H), 5.48 (1H, dd, *J* = 4.0 and 7.5 Hz, 7-H), 6.80–7.00 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.10–7.66 (11H, m, thiophene 5-H, C₆H₅ × 2), 9.16 (1H, d, *J* = 7.5 Hz, 7-NH). *Anal.* Calcd for C₃₀H₂₈N₆O₄S₃: 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)-2 α -methyl-3-(1-methyl-1H-tetrazol-5-yl)thiomethylceph-3-em-1-Oxide (9)—MCPBA (217 mg) was added to a solution of **8** (662 mg) in CH₂Cl₂ (30 ml) at -10°C, and the mixture was stirred at the same temperature for 1 h. The solution was washed with 10% Na₂S₂O₃ and sat. aqueous NaCl, and dried over MgSO₄. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution of the column with CH₂Cl₂–AcOEt (5:1) gave **9** (321 mg, 47%) as an amorphous solid. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1800, 1745, 1690, 1050. NMR (DMSO-*d*₆) δ : 1.27 (3H, d, *J* = 7.5 Hz, 2 α -CH₃), 3.84 (5H, s, tetrazole 1-CH₃ and -CH₂CO-), 4.01 (1H, q, *J* = 7.5 Hz, 2 β -H), 4.25 (2H, s, -CH₂S-), 4.96 (1H, d, *J* = 5.0 Hz, 6-H), 5.96 (1H, dd, *J* = 5.0 and 8.5 Hz, 7-H), 6.80–7.00 (3H, m, thiophene 3-H, and 4-H, -CH(C₆H₅)₂), 7.10–7.70 (11H, m, thiophene 5-H, C₆H₅ × 2), 8.37 (1H, d, *J* = 8.5 Hz, 7-NH). *Anal.* Calcd for C₃₀H₂₈N₆O₅S₃: 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)-2 α -methyl-3-(1-methyl-1H-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (10)—PCl₃ (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 NaHCO₃ were added to the solution and partitioned. The AcOEt layer was washed with sat. aqueous NaCl and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on a silica gel column. The column was eluted with CH₂Cl₂–AcOEt (25:1) to give **10** (225 mg, 71%) as an amorphous solid. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1790, 1735, 1700. NMR (DMSO-*d*₆) δ : 1.53 (3H, d, *J* = 7.0 Hz, 2 α -CH₃), 3.74 (2H, s, -CH₂CO-), 3.84 (3H, s, tetrazole 1-CH₃), 4.01 (1H, q, *J* = 7.0 Hz, 2 β -H), 4.18 (2H, s, -CH₂S-), 5.27 (1H, d, *J* = 5.0 Hz, 6-H), 5.85 (1H, dd, *J* = 5.0 and 8.5 Hz, 7-H), 6.80–7.00 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.15–7.60 (11H, m, thiophene 5-H, C₆H₅ × 2), 9.17 (1H, d, *J* = 8.5 Hz, 7-NH). NOE: 20% signal increase for 6-H on irradiation of 2 α -CH₃. *Anal.* Calcd for C₃₀H₂₈N₆O₄S₃: 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)-2 α -methyl-3-(1-methyl-1H-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (11a)—A solution of **10** (105 mg) in anisole (0.1 ml) and CF₃COOH (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 NaHCO₃. The H₂O layer was adjusted to pH 7 with 5% H₃PO₄ and applied to a column of Amberlite XAD-II. The column was washed with H₂O and then eluted with MeOH-H₂O (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)): *R_f* 0.30. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1770, 1685. NMR (D₂O) δ : 1.52 (3H, d, *J*=7.5 Hz, 2 α -CH₃), 3.76 (1H, q, *J*=7.5 Hz, 2 β -H), 3.86 (2H, s, -CH₂CO-), 3.97 (3H, s, tetrazole 1-CH₃), 4.02 and 4.27 (1H each, ABq, *J*=13.0 Hz, -CH₂S-), 5.17 (1H, *J*=5.0 Hz, 6-H), 5.68 (1H, d, *J*=5.0 Hz, 7-H), 6.90—7.10 (2H, m, thiophene 3-H and 4-H), 7.20—7.40 (1H, m, thiophene 5-H). *Anal.* Calcd for C₁₇H₁₇N₆NaO₄S₃·3/2H₂O: 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-2 α -methyl-3-(1-methyl-1H-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (11b)—Pyridine (3.7 ml) was added to a solution of **10** (2.38 g) in CH₂Cl₂ (100 ml), followed by addition of PCl₅ (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 K₂HPO₄ (100 ml) was added, followed by adjusting the pH to 1.5 with H₃PO₄. The mixture was stirred for 45 min, then the CH₂Cl₂ layer was separated, washed with sat. aqueous NaCl, and dried over MgSO₄. After removal of the solvent, addition of Et₂O to the residue gave **11b** (1.73 g, 72%) as crystals. mp 158°C (dec.), (CH₂Cl₂-Et₂O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1780, 1735. NMR (DMSO-*d*₆+D₂O) δ : 1.56 (3H, d, *J*=7.0 Hz, 2 α -CH₃), 3.82 (3H, s, tetrazole 1-CH₃), 3.93 (1H, q, *J*=7.0 Hz, 2 β -H), 4.16 (2H, s, -CH₂S-), 4.90 (1H, d, *J*=5.0 Hz, 6-H), 5.11 (1H, d, *J*=5.0 Hz, 7-H), 6.84 (1H, s, -CH(C₆H₅)₂), 7.10—7.60 (10H, m, C₆H₅×2). *Anal.* Calcd for C₂₄H₂₄N₆O₃S₂: 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]-2 α -methyl-3-(1-methyl-1H-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylic Acid (11c)—A mixture of **11b** (220 mg), D(-)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(2-thienyl)acetic acid (169 mg) and dicyclohexylcarbodiimide (107 mg) in CH₂Cl₂ (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% H₃PO₄, 5% aqueous NaHCO₃ 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 CF₃COOH (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 NaHCO₃. The H₂O layer was adjusted to pH 7 with 5% H₃PO₄ and applied to a column of Amberlite XAD-II. The column was washed with H₂O and then eluted with a gradient of H₂O—70% MeOH. The eluate was concentrated and adjusted to pH 2.0 with 5% H₃PO₄ to give **11c** (110 mg, 34%) as crystals. mp 152°C (dec.), (AcOEt-Et₂O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1785, 1710, 1690. NMR (DMSO-*d*₆) δ : 1.09 (3H, t, *J*=7.0 Hz, -CH₂CH₃), 1.50 (3H, d, *J*=7.0 Hz, 2 α -CH₃), 3.39 (2H, q, *J*=7.0 Hz, -CH₂CH₃), 3.40—4.00 (5H, m, piperazine 5-CH₂, 6-CH₂, 2 β -H), 3.90 (3H, s, tetrazole 1-CH₃), 4.08 and 4.34 (1H each ABq, *J*=13.0 Hz, -CH₂S-), 5.16 (1H, d, *J*=5.0 Hz, 6-H), 5.79 (1H dd, *J*=5.0 and 9.0 Hz, 7-H), 5.85 (1H, d, *J*=7.0 Hz, >CH-NH-), 6.80—7.20 (2H, m, thiophene 3-H and 4-H), 7.30—7.55 (1H, m, thiophene 5-H), 9.50 (1H, d, *J*=9.0 Hz, 7-NH), 9.75 (1H, d, *J*=7.0 Hz, >CH-NH). *Anal.* Calcd for C₂₄H₂₇N₉O₇S₃: 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)—CrO₃ (4.2 g) was added to CH₂Cl₂ (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 CH₂Cl₂ (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 CH₂Cl₂ layer was separated, washed with H₂O, and sat. aqueous NaCl, and dried over MgSO₄. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution of the column with CH₂Cl₂-AcOEt (20: 1) gave **12** (938 mg, 36.2%) as an amorphous solid. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1790, 1750. NMR (DMSO-*d*₆) δ : 2.50 (3H, s, 2-CH₃), 3.78 (2H, s, -CH₂CO-), 5.07 (1H, d, *J*=4.5 Hz, 6-H), 5.45 (1H, s, 4-H), 5.47 (1H, dd, *J*=4.5 and 8.5 Hz, 7-H), 6.70—7.00 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.10—7.50 (11H, m, thiophene 5-H, C₆H₅×2), 9.16 (1H, d, *J*=8.0 Hz, 7-NH), 9.99 (1H, s, -CHO). *Anal.* Calcd for C₂₈H₂₄N₂O₅S₂: 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)-2 α -methyl-3-formylceph-3-em 1-Oxide (13)—MCPBA (0.95 g) was added to a solution of **12** (2.5 g) in CH₂Cl₂ (50 ml), and the mixture was stirred at room temperature for 1 h. The solution was washed with 5% aqueous NaHCO₃ and sat. aqueous NaCl, and dried over MgSO₄. After removal of the solvent, the crystalline residue was recrystallized from CH₂Cl₂-AcOEt to give **13** (1.27 g, 50%). mp 168°C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1805, 1725, 1665, 1040. NMR (DMSO-*d*₆) δ : 1.11 (3H, d, *J*=7.5 Hz, 2 α -CH₃), 3.89 (2H, s, -CH₂CO-), 4.11 (1H, q, *J*=7.5 Hz, 2 β -H), 5.08 (1H, d, *J*=5.0 Hz, 6-H), 6.20 (1H, dd, *J*=5.0 and 8.5 Hz, 7-H), 6.84—7.20 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.20—7.60 (11H, m, thiophene 5-H, C₆H₅×2), 8.67 (1H, d, *J*=8.5 Hz, 7-NH), 9.96 (1H, s, -CHO). *Anal.* Calcd for C₂₈H₂₄N₂O₆S₂: 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)-2 α -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.), (CH₂Cl₂-AcOEt). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1800, 1730, 1670. NMR (CDCl₃) δ : 1.25 (3H, d, J =7.5 Hz, 2 α -CH₃), 3.84 (2H, s, -CH₂CO-), 3.88 (3H, s, -OCH₃), 4.50 (1H, q, J =7.5 Hz, 2 β -H), 4.51 (1H, d, J =5.0 Hz, 6-H), 6.14 (1H, dd, J =5.0 and 9.0 Hz, 7-H), 6.80-7.05 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.10-7.60 (12H, m, thiophene 5-H, C₆H₅ $\times$ 2, 7-NH), 8.41 (1H, s, -CH=N-). Anal. Calcd for C₂₉H₂₇N₃O₆S₂: 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)-2 α -methyl-3-methoxyiminomethylceph-3-em-4-carboxylate (15a)—PCl₃ (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 Et₂O to the residue gave **15a** (219 mg, 90%) as crystals, mp 160°C (dec.), (AcOEt-Et₂O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1790, 1725, 1665. NMR (DMSO-*d*₆) δ : 1.50 (3H, d, J =7.5 Hz, 2 α -CH₃), 3.74 (2H, s, -CH₂CO-), 3.82 (3H, s, -OCH₃), 4.35 (1H, q, J =7.5 Hz, 2 β -H), 5.37 (1H, d, J =4.5 Hz, 6-H), 5.88 (1H, dd, J =4.5 and 8.5 Hz, 7-H), 6.75-7.05 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.10-7.60 (11H, m, thiophene 5-H, C₆H₅ $\times$ 2), 7.90 (1H, s, -CH=N-), 9.18 (1H, d, J =8.5 Hz, 7-NH). NOE: about 21% signal increase for 6-H on irradiation of 2 α -CH₃. Anal. Calcd for C₂₉H₂₇N₃O₅S₂: 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)-2 α -methyl-3-methoxyiminomethylceph-3-em-4-carboxylate (15b)—**15a** (160 mg) was treated with anisole (0.2 ml) and CF₃COOH (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-H₂O (1: 4). Amorphous solid. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1775, 1680. NMR (D₂O) δ : 1.56 (3H, d, J =7.0 Hz, 2 α -CH₃), 3.91 (5H, s, -OCH₃ and -CH₂CO-), 4.22 (1H, q, J =7.0 Hz, 2 β -H), 5.3 (1H, d, J =5.0 Hz, 6-H), 5.78 (1H, d, J =5.0 Hz, 7-H), 6.95-7.20 (2H, m, thiophene 3-H and 4-H), 7.25-7.50 (1H, m, thiophene 5-H). Anal. Calcd for C₁₆H₁₆N₃NaO₅S₂·3/2H₂O: 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)-2 α -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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1775, 1680, 1620. NMR (D₂O) δ : 1.65 (3H, d, J =7.0 Hz, 2 α -CH₃), 2.60-3.00 (6H, m, -CH₂CH₂N, morpholine 3-CH₂, 5-CH₂), 3.70-3.96 (4H, m, morpholine 2-CH₂, 6-CH₂), 4.00 (2H, s, -CH₂CO-), 4.16-4.60 (3H, m, -OCH₂CH₂, 2 β -H), 5.43 (1H, d, J =5.0 Hz, 6-H), 5.85 (1H, d, J =5.0 Hz, 7-H), 7.10-7.30 (2H, m, thiophene 3-H and 4-H), 7.35-7.55 (1H, m, thiophene 5-H). Anal. Calcd for C₂₁H₂₅N₄NaO₆S₂·2H₂O: 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 NaBH₄ (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 H₂O were added to the residue and stirred. The AcOEt layer was separated, washed with 5% aqueous NaHCO₃ and sat. aqueous NaCl, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on a silica gel column. Elution with CH₂Cl₂-AcOEt (10: 1), concentration of the eluate, and the addition of Et₂O to the residue gave **16** (18.7 g, 41%) as crystals. mp 128°C (dec.), (AcOEt-Et₂O). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1770, 1730, 1650. NMR (DMSO-*d*₆) δ : 1.78 (3H, s, -COCH₃), 1.96 (3H, s, 2-CH₃), 3.72 (2H, s, -CH₂CO-), 4.57 and 4.73 (1H each, ABq, J =13.0 Hz, -CH₂O-), 5.06 (1H, d, J =4.5 Hz, 6-H), 5.20 (1H, s, 4-H), 5.42 (1H, dd, J =4.5 and 8.5 Hz, 7-H), 6.70-6.95 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.15-7.50 (11H, m, thiophene 5-H, C₆H₅ $\times$ 2), 9.12 (1H, d, J =8.5 Hz, 7-NH). Anal. Calcd for C₃₀H₂₈N₂O₆S₂: 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 CH₂Cl₂ (50 ml) was added to a solution of **16** (37.3 g) in CH₂Cl₂ (300 ml) at -20°C, and the mixture was stirred at the same temperature for 30 min. The solution was washed with 5% aqueous NaHCO₃ and sat. aqueous NaCl, and dried over MgSO₄. After removal of the solvent, addition of AcOEt-Et₂O (1: 3) to the residue gave crystalline **17** (32 g, 84%) as a mixture of stereoisomers. mp 160°C (dec.), (CH₂Cl₂-AcOEt). Anal. Calcd for C₃₀H₂₈N₂O₇S₂: 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)-2 β -methyl-3-acetoxymethylceph-3-em-4-carboxylate (18a) and Diphenylmethyl 7-(2-Thienylacetamido)-2 α -methyl-3-acetoxymethylceph-3-em-4-carboxylate (19a)—PCl₃ (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 NaHCO₃ and sat. aqueous NaCl, and dried over Na₂SO₄. Removal of the solvent gave crude crystals. Recrystallization from AcOEt-Et₂O (1: 3) afforded **18a** (17 g, 55%). mp 152°C (dec.). TLC (silica gel; toluene-AcOEt (5: 1)): *R*_f 0.26. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1795, 1740, 1680, 1230. NMR (DMSO-*d*₆) δ : 1.45 (3H, d, J =7.0 Hz, 2 β -CH₃), 1.95 (3H, s, -COCH₃), 3.79 (2H, s, -CH₂CO-), 4.04 (1H, q, J =7.0 Hz, 2 α -H), 4.57 and 4.99 (1H each, ABq, J =13.0 Hz, -CH₂O-), 5.31 (1H, d, J =5.0 Hz, 6-H), 5.77 (1H, dd, J =5.0 and 8.0 Hz, 7-H), 6.90-7.05 (3H, m, thiophene 3-H and 4-H, -CH(C₆H₅)₂), 7.20-7.60 (11H, m, thiophene

5-H, $C_6H_5 \times 2$), 9.12 (1H, d, $J=8.0$ Hz, 7-NH). NOE: no signal increase for 6-H on irradiation of 2 β -CH₃. *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 2 α -methyl isomer **18a** and thereafter the 2 β -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, 2 α -CH₃), 1.91 (3H, s, -COCH₃), 3.75 (2H, s, -CH₂CO-), 3.84 (1H, q, $J=7.0$ Hz, 2 β -H), 4.51 and 4.82 (1H each, ABq, $J=13.0$ Hz, -CH₂O-), 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(C₆H₅)₂), 7.10–7.50 (11H, m, thiophene 5-H, $C_6H_5 \times 2$), 9.13 (1H, d, $J=8.0$ Hz, 7-NH). NOE: about 32% signal increase for 6-H on irradiation of 2 α -CH₃. *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)-2 β -methyl-3-acetoxymethylceph-3-em-4-carboxylate (18b)——18a (1.4 g) was treated with anisole (3 ml) and CF₃COOH (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 H₂O. Amorphous solid. IR ν_{max}^{KBr} cm^{-1} : 1760, 1735, 1655, 1600. NMR (D₂O) δ : 1.46 (3H, d, $J=7.0$ Hz, 2 β -CH₃), 2.11 (3H, s, -COCH₃), 3.91 (2H, s, -CH₂CO-), 3.98 (1H, q, $J=7.0$ Hz, 2 α -H), 4.69 and 5.07 (1H each, ABq, $J=13.0$ Hz, -CH₂O-), 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 $C_{17}H_{17}N_2NaO_6S_2 \cdot 3/2 H_2O$: 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)-2 α -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 2 β -methyl isomer **18b**. Amorphous solid. IR ν_{max}^{KBr} cm^{-1} : 1780, 1680, 1240. NMR (D₂O) δ : 1.63 (3H, d, $J=7.5$ Hz, 2 α -CH₃), 2.33 (3H, s, -COCH₃), 3.70 (1H, q, $J=7.5$ Hz, 2 β -H), 4.00 (2H, s, -CH₂CO-), 4.78 and 5.00 (1H each, ABq, $J=13.0$ Hz, -CH₂O-), 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 $C_{17}H_{17}N_2NaO_6S_2 \cdot 3/2 H_2O$: 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-2 β -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 CH₂Cl₂-AcOEt (10: 1). mp 101°C (dec.), (CH₂Cl₂-Et₂O). IR ν_{max}^{KBr} cm^{-1} : 1760, 1730, 1220. NMR (DMSO- d_6 + D₂O) δ : 1.40 (3H, d, $J=7.0$ Hz, 2 β -CH₃), 1.89 (3H, s, -COCH₃), 3.94 (1H, q, $J=7.0$ Hz, 2 α -H), 4.48 and 4.93 (1H each ABq, $J=13.0$ Hz, -CH₂O-), 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(C₆H₅)₂), 7.33 (10H, s, $C_6H_5 \times 2$). *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]-2 β -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 (D₂O) δ : 1.50 (3H, d, $J=7.0$ Hz, 2 β -CH₃), 2.10 (3H, s, -COCH₃), 4.00 (3H, s, -OCH₃), 4.05 (1H, q, $J=7.0$ Hz, 2 α -H), 4.69 and 5.07 (1H each, ABq, $J=13.0$ Hz, -CH₂O-), 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 $C_{17}H_{18}N_5NaO_7S_2 \cdot 2H_2O$: 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)-2 β -methyl-3-(1-methyl-1H-tetrazol-5-yl)thiomethylceph-3-em-4-carboxylate (20a)——A solution of **18b** (410 mg), 1-methyl-5-mercaptopotetrazole (139 mg) and NaHCO₃ (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 NaHCO₃. 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 H₂O–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 (D₂O) δ : 1.48 (3H, d, $J=7.0$ Hz, 2 β -CH₃), 3.89 (2H, s, -CH₂CO-), 3.95 (1H, q, $J=7.0$ Hz, 2 α -H), 4.03 (3H, s, tetrazole 1-CH₃), 4.43 and 4.87 (1H each, ABq, $J=13.0$ Hz, -CH₂S-), 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 $C_{17}H_{17}N_6NaO_4S_3 \cdot 3/2 H_2O$: 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]-2 β -methyl-3-(1-methyl-1H-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 (D₂O) δ : 1.53 (3H, d, $J=7.0$ Hz, 2 β -CH₃), 4.00 (3H, s, tetrazole 1-CH₃), 4.06 (3H, s, -OCH₃), 4.17 (1H, q, $J=7.0$ Hz, 2 α -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 $C_{17}H_{18}N_9NaO_5S_3 \cdot 2H_2O$: 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-[p(-)-2-(4-Ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(2-thienyl)acetamido]-2 β -methyl-3-(1-methyl-1H-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, -CH₂CH₃), 1.42 (3H, d, $J=7.0$ Hz, 2 β -CH₃), 3.39 (2H, q, $J=7.0$ Hz, -CH₂CH₃), 3.40–3.90 (5H, m, piperazine 5-CH₂, 6-CH₂, 2 α -H), 3.92 (3H, s, tetrazole 1-CH₃),

4.08 and 4.75 (1H each, ABq, $J=13.0$ Hz, $-\text{CH}_2\text{S}-$), 5.10 (1H, d, $J=5.0$ Hz, 6-H), 5.63 (1H, dd, $J=5.0$ and 9.0 Hz, 7-H), 5.90 (1H, d, $J=7.0$ Hz, $\text{>CH-NH}-$), 6.80—7.20 (2H, m, thiophene 3-H and 4-H), 7.30—7.55 (1H, m, thiophene 5-H), 9.43 (1H, d, $J=9.0$ Hz, 7-NH), 9.74 (1H, d, $J=7.0$ Hz, $\text{>CH-NH}-$). *Anal.* Calcd for $\text{C}_{24}\text{H}_{27}\text{N}_9\text{O}_7\text{S}_3$: 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)-2 β -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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1760, 1675, 1605. NMR (D_2O) δ : 1.44 (3H, d, $J=7.0$ Hz, 2 β - CH_3), 2.73 (3H, s, thiadiazole 5- CH_3), 3.89 (2H, s, $-\text{CH}_2\text{CO}-$), 4.09 (1H, q, $J=7.0$ Hz, 2 α -H), 3.87 and 4.71 (1H each, ABq, $J=14.0$ Hz, $-\text{CH}_2\text{S}-$), 5.10 (1H, d, $J=4.5$ Hz, 6-H), 5.50 (1H, d, $J=4.5$ Hz, 7-H), 6.96—7.10 (2H, m, thiophene 3-H and 4-H), 7.25—7.50 (1H, m, thiophene 5-H). *Anal.* Calcd for $\text{C}_{18}\text{H}_{17}\text{N}_4\text{NaO}_4\text{S}_4 \cdot \text{H}_2\text{O}$: 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)-2 β -methyl-3-(1-carboxymethyl-1H-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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1770, 1670, 1620. NMR (D_2O) δ : 1.48 (3H, d, $J=7.0$ Hz, 2 β - CH_3), 3.92 (2H, s, $-\text{CH}_2\text{CO}-$), 3.97 and 4.74 (1H each, ABq, $J=13.0$ Hz, $-\text{CH}_2\text{S}-$), 4.07 (1H, q, $J=7.0$ Hz, 2 α -H), 5.03 (2H, s, tetrazole 1- $\text{CH}_2\text{CO}-$), 5.16 (1H, d, $J=4.5$ Hz, 6-H), 5.52 (1H, d, $J=4.5$ Hz, 7-H), 7.00—7.20 (2H, m, thiophene 3-H and 4-H), 7.30—7.50 (1H, m, thiophene 5-H). *Anal.* Calcd for $\text{C}_{18}\text{H}_{16}\text{N}_6\text{Na}_2\text{O}_6\text{S}_3 \cdot 3\text{H}_2\text{O}$: 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-oxoceph-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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1780, 1750, 1650. NMR ($\text{DMSO}-d_6$) δ : 3.77 (2H, s, $-\text{CH}_2\text{CO}-$), 5.49 (1H, dd, $J=5.0$ and 8.0 Hz, 7-H), 5.51 (1H, d, $J=5.0$ Hz, 6-H), 5.99 (1H, s, 3-methylene), 6.12 (1H, s, 4-H), 6.32 (1H, s, 3-methylene), 6.85—7.00 (3H, m, thiophene 3-H and 4-H, $-\text{CH}(\text{C}_6\text{H}_5)_2$), 7.10—7.60 (11H, m, thiophene 5-H, $\text{C}_6\text{H}_5 \times 2$), 9.18 (1H, d, $J=8.0$ Hz, 7-NH). *Anal.* Calcd for $\text{C}_{27}\text{H}_{22}\text{N}_2\text{O}_5\text{S}_2$: 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)): $R_f=0.52$. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1795, 1740, 1675, 1645, 1635, 1540, 1390, 1225, 1210. NMR ($\text{DMSO}-d_6$) δ : 1.90 (3H, s, 3- CH_3), 3.77 (2H, s, $-\text{CH}_2\text{CO}-$), 5.80 (1H, dd, $J=4.5$ and 8.5 Hz, 7-H), 5.98 (1H, d, $J=4.5$ Hz, 6-H), 6.85—7.10 (3H, m, thiophene 3-H and 4-H, $-\text{CH}(\text{C}_6\text{H}_5)_2$), 7.20—7.70 (11H, m, thiophene 5-H, $\text{C}_6\text{H}_5 \times 2$), 9.42 (1H, d, $J=8.5$ Hz, 7-NH). *Anal.* Calcd for $\text{C}_{27}\text{H}_{22}\text{N}_2\text{O}_5\text{S}_2$: 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1785, 1670, 1650. NMR ($\text{DMSO}-d_6$) δ : 3.79 (2H, s, $-\text{CH}_2\text{CO}-$), 5.69 (1H, dd, $J=4.5$ and 8.0 Hz, 7-H), 5.95 (1H, d, $J=4.5$ Hz, 6-H), 6.80—7.00 (3H, m, thiophene 3-H and 4-H, $-\text{CH}(\text{C}_6\text{H}_5)_2$), 7.15—7.60 (11H, m, thiophene 5-H, $\text{C}_6\text{H}_5 \times 2$), 9.42 (1H, d, $J=8.0$ Hz, 7-NH). *Anal.* Calcd for $\text{C}_{28}\text{H}_{20}\text{N}_2\text{O}_6\text{S}_2$: 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1800, 1735, 1665, 1655. NMR (DMSO- d_6) δ : 3.68 (3H, s, $-\text{OCH}_3$), 3.75 (2H, s, $-\text{CH}_2\text{CO}-$), 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, $-\text{CH}(\text{C}_6\text{H}_5)_2$), 7.10—7.60 (11H, d, $J=8.0$ Hz, 7-NH). Anal. Calcd for $\text{C}_{27}\text{H}_{22}\text{N}_2\text{O}_6\text{S}_2$: 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1795, 1735, 1665, 1650. NMR (DMSO- d_6) δ : 3.70 (3H, s, $-\text{OCH}_3$), 3.76 (2H, s, $-\text{CH}_2\text{CO}-$), 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 $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_6\text{S}_2$: 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 this work 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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- 5) The stereochemistry at C-3 and C-4 of **4** and at C-4 of **2**, **5**, and **16** has not been established.
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