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A New Route to Semisynthetic Cephalosporins from Deacetylcephalosporin C. I. Synthesis of 3-Heterocyclicthiomethyl-cephalosporins

Susumu Tsushima, Michiyuki Sendai, Mitsuru Shiraishi, Masayasu Kato, Norichika Matsumoto, Kenzo Naito, and Mitsuo Numata

Medicinal Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.[1)]

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New compounds 3-acetoacetoxymethyl-7β-acylaminoceph-3-em-4-carboxylic acids (**4**) were synthesized from deacetylcephalosporin C (**3a**), after N-protection of the amino-adipoyl group followed by acetoacetylation of the 3′-hydroxyl with diketene and acyl exchange at the 7-position. They underwent a facile nucleophilic displacement of the 3′-acetoacetoxy group with heterocyclic thiols to afford 7β-acylamino-3-heterocyclicthio-methylceph-3-em-4-carboxylic acids (**6**) including SCE-963 (**6e**) in good yields.

Keywords——cephalosporin; deacetylcephalosporin C; nucleophilic displacement; 3′-hydroxyl activation; acetoacetylation; diketene; 3-heterocyclicthiomethyl-cephalosporin; SCE-963

Many semisynthetic cephalosporins currently in wide clinical use or under development have been prepared from cephalosporin C (**1a**; CPC) by replacing the aminoadipoyl group at the 7-position with other acyl groups and the acetoxy group at the 3-methylene position or the 3′-position with nucleophiles such as pyridines and heterocyclic thiols. Thus cephaloridine, cefazolin, cefamandole, cefatrizine, cefazaflur, CS-1170, SCE-129 and SCE-963 are characterized by substituents at these positions.[2)]

The processes for the introduction of acyl groups at the 7-position have been improved considerably,[3)] however, the nucleophilic displacement reaction at the 3′-position usually requires a mild heating in an aqueous solution, which inevitably accompanies a degradation of cephalosporins and sometimes affords only a low yield of the products.[4)] In our previous study on the synthesis of SCE-963, 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethyl-aminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid (**6e**)[2d,5)] starting from CPC, the 3′-acetoxy displacement for the formation of the side chain at the 3-position afforded the desired product in relatively low yields ($\leq$45%), thus the scale-up production of SCE-963 from CPC was not feasible from the economic standpoint.

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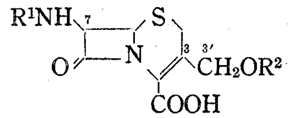
1) Location: *17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.*
2) *a*) Cephaloridine, cefazolin, cefamandole, cefatrizine and cefazaflur: J.R.E. Hoover and C.H. Nash, "Kirk-Othmer Encyclopedia of Chemical Technology," 3rd ed., Vol. 2, John Wiley and Sons, Inc., New York, 1978, p. 889 and p. 914 and ref. cited therein; *b*) CS-1170: H. Nakao, H. Yanagisawa, B. Shimizu, M. Kaneko, M. Nagano, and S. Sugawara, *J. Antibiot.* (Tokyo), **29**, 554 (1976); *c*) SCE-129: H. Nomura, T. Fugono, T. Hitaka, I. Minami, T. Azuma, S. Morimoto, and T. Masuda, *J. Med. Chem.*, **17**, 1312 (1974); *d*) SCE-963: M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, and T. Nishimura, 17th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, Oct. 1977, Abstracts, No. 44.
3) Ref. 2*a*), p. 896.
4) *a*) J.D. Cocker, B.R. Cowley, J.S.G. Cox, S. Eardley, G.I. Gregory, J.K. Lanzenby, A.G. Long, J.C.P. Sly, and G.A. Somerfield, *J. Chem. Soc.*, **1965**, 5015; *b*) E. Van Heyningen and C.N. Brown, *J. Med. Chem.*, **8**, 174 (1965); *c*) A.B. Taylor, *J. Chem. Soc.*, **1965**, 7020.
5) M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, and T. Miyawaki, Japan. Patent Provisional Publication, 50-111093 (1975); 51-56487 (1976) [*C.A.*, **84**, 74284*b* (1976)].



	R^1	R^2	Abbreviation
1a	$HOOCCH(NH_2)(CH_2)_3CO-$	CH_3CO-	CPC
1b	$HOOCCH(Ft)(CH_2)_3CO-$	CH_3CO-	Pht-CPC
2	H	CH_3CO-	7-ACA
3a	$HOOCCH(NH_2)(CH_2)_3CO-$	H	DCPC
3b	$HOOCCH(Ft)(CH_2)_3CO-$	H	Pht-DCPC

Ft=phthalimido.

Chart 1

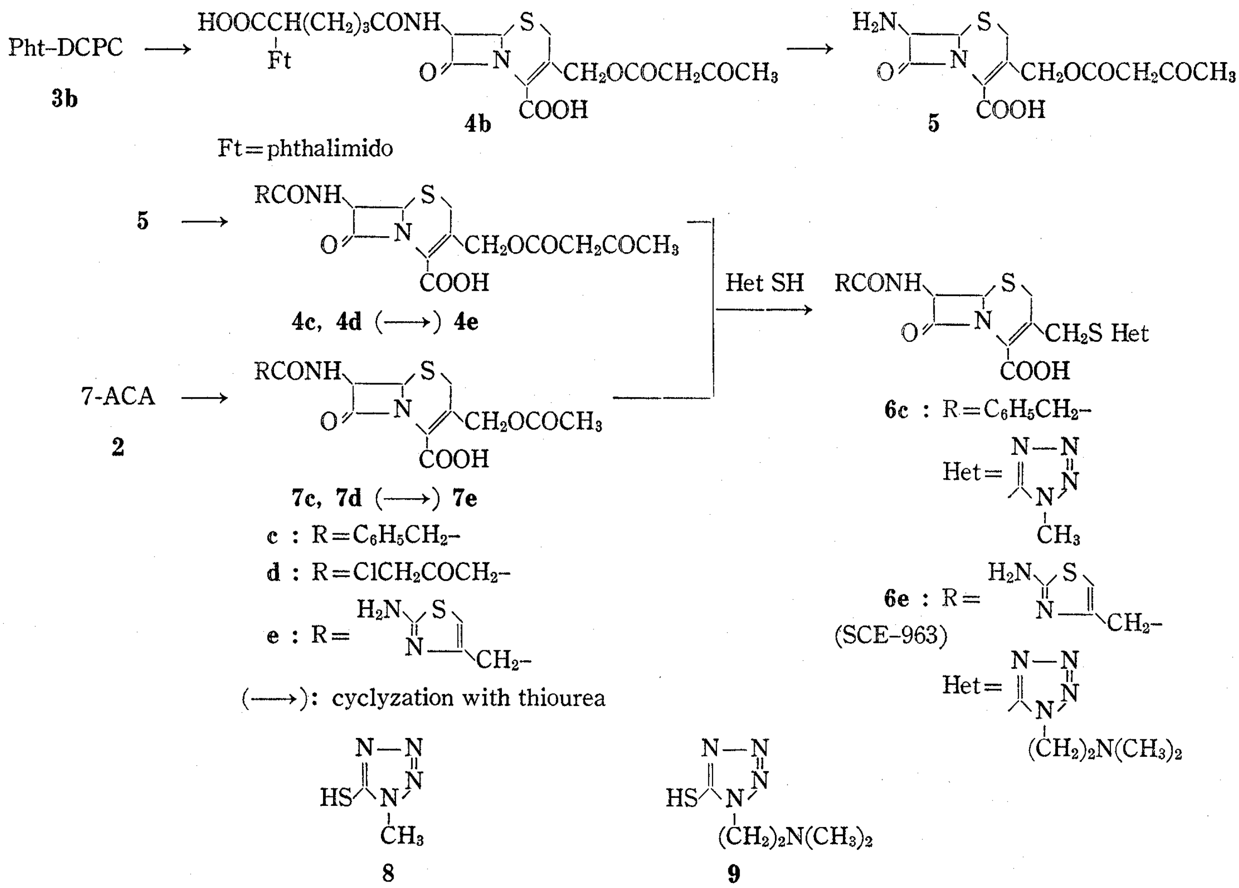


Chart 2

The situation mentioned above together with the recent development of a fermentative production of deacetylcephalosporin C (**3a**; DCPC) in this Division[6] prompted us to investigate a method to utilize DCPC instead of CPC as a starting material for the production of SCE-963.

6) Y. Fujisawa, H. Shirafuji, M. Kida, K. Nara, M. Yoneda, and T. Kanzaki, *Nature* (London), *New Biol.*, **246**, 154 (1973).

This paper deals with a new activation[7] of the 3′-hydroxyl of DCPC for nucleophilic displacements and an application of the method to the synthesis of SCE-963.

N-Protection of the aminoadipoyl group of DCPC with N-carbethoxyphthalimide followed by treatment with triethylamine afforded N-phthaloyl-DCPC (**3b**; Pht–DCPC)[8] triethylamine salt in 82% yield, which on acylation[9] with diketene gave 3′-O-acetoacetyl derivative (**4b**) in 94% yield. Removal of the aminoadipoyl side chain of **4b** by the conventional method[10] using the imino chloride-imino ether intermediates afforded 3-acetoacetoxymethyl-7β-amino-ceph-3-em-4-carboxylic acid (**5**) in 90% yield. When these steps from DCPC were carried out in one vessel, **5** was obtained in 85% over all yield.

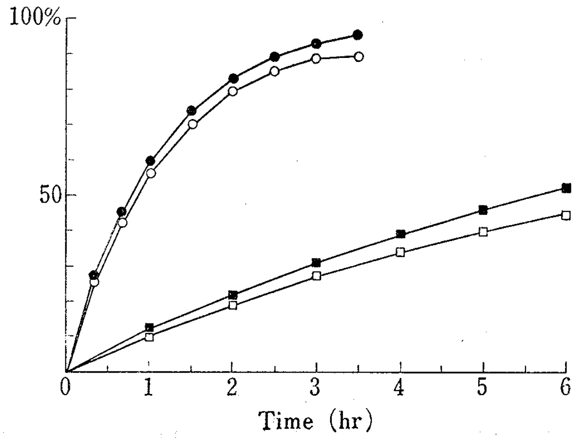


Fig. 1. Reaction of **4c** or **7c** with **8** Affording **6c**

Solvent; 0.24 M phosphate buffer. Temperature; 47°. Initial concentration of reactants; each 0.125 M. (○) quantity of **6c** formed from **4c**; (●) quantity of **4c** consumed; (□) quantity of **6c** formed from **7c**; (■) quantity of **7c** consumed.

To compare the reactivity of the 3′-acetoacetoxy group with that of the 3′-acetoxy group in simple systems, each of 3-acetoacetoxymethyl-7β-phenylacetamido-ceph-3-em-4-carboxylic acid (**4c**) and 7β-phenylacetamidocephalosporanic acid (**7c**),[11] which were prepared by acylation of **5** and 7β-aminocephalosporanic acid (**2**; 7-ACA) with phenylacetyl chloride respectively, was heated with 1-methyl-1H-tetrazole-5-thiol (**8**) in a phosphate buffer of pH 6 at 47°. The formation of a derivative incorporating the 1-methyl-1H-tetrazolylthio group into the 3′-position (**6c**)[12] was monitored by high performance liquid chromatography (HPLC) (Fig. 1).

The rate of the conversion of **4c** to **6c** was much faster than that of **7c** to **6c** as shown in Fig. 1. It apparently indicates that the 3′-acetoacetoxy group is a far easier group to replace than the 3′-acetoxy group with the nucleophile.

The superior reactivity of the 3′-acetoacetoxy group of 3-acetoacetoxymethyl-7β-acyl-aminoceph-3-em-4-carboxylic acids (**4**) to the 3′-acetoxy group of cephalosporanic acids (**7**) is also seen in the following two new modified syntheses of SCE-963, one of which was carried out using **5** in place of 7-ACA adopted in the former synthesis[2d,5] (Chart 2) and the other using 7-amino nucleus compound (**10**) as a key intermediate (Chart 3).

Acylation of **5** with 4-chloro-3-oxobutyryl chloride resulted in **4d**, which on cyclization with thiourea afforded 3-acetoacetoxymethyl-7β-[2-(2-aminothiazol-4-yl)acetamido]ceph-3-em-4-carboxylic acid (**4e**) in 85% yield. Displacement of the 3′-acetoacetoxy group of **4e** with

7) DCPC was reported to be inactive for nucleophilic displacements in ref. 4*a*).

8) C.F. Murphy, R.E. Koehler, and J.A. Webber, *Tetrahedron Lett.*, **1972**, 1585.

9) Although in the literature have been reported several methods of acylations of deacetylcephalosporanic acids, they are not suitable for the purpose of this study because of multi-steps, low yields or requirement of not easily available reagents: *a*) E. Van Heyningen, *J. Med. Chem.*, **8**, 22 (1965); *b*) G.A. Somerfield, H. Wycombe, and D. Chagouri, U.S. Patent 3532694 (1970) [*C.A.*, **65**, 15385*h* (1966)]; *c*) H. Bickel, R. Bosshardt, B. Fechtig, E. Menard, J. Mueller, and H. Peter, U.S. Patent 3639396 (1972) [*C.A.*, **74**, 31763*h* (1971)]; *d*) S. Eardley, J. Kennedy, and A.G. Long, U.S. Patent 3658799 (1972) [*C.A.*, **73**, 131016*q* (1970)]; *e*) S. Kukolja, *J. Med. Chem.*, **13**, 1114 (1970); *f*) D.A. Berges, *ibid.*, **18**, 1264 (1975).

10) *a*) B. Fechtig, H. Peter, H. Bickel, and E. Vischer, *Helv. Chim. Acta*, **51**, 1108 (1968); *b*) H.W.O. Weissenburger and M.G. Van der Hoeven, U.S. Patent 3575970 (1971) [*C.A.*, **71**, 61403*w* (1969)].

11) B. Loder, G.G.F. Newton, and E.P. Abraham, *Biochem. J.*, **79**, 408 (1961).

12) K. Harada, M. Hashimoto, and R. Nakagawa, Japan. Patent 2255 (1971) [*C.A.*, **75**, 5918*p* (1971)].

1-(2-dimethylaminoethyl)-1H-tetrazole-5-thiol (**9**)[13] by heating at 55° for 60 min in an aqueous solution gave SCE-963 in 83% yield determined by HPLC of the reaction mixture and 71% yield after isolation. However, the parallel nucleophilic displacement of the acetoxy group of 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporanic acid (**7e**), which was an intermediate in the former synthesis,[2d,5] with **9** by heating at 65° for 100 min afforded SCE-963 in 45% yield determined by HPLC of the reaction mixture and 22% yield after isolation.

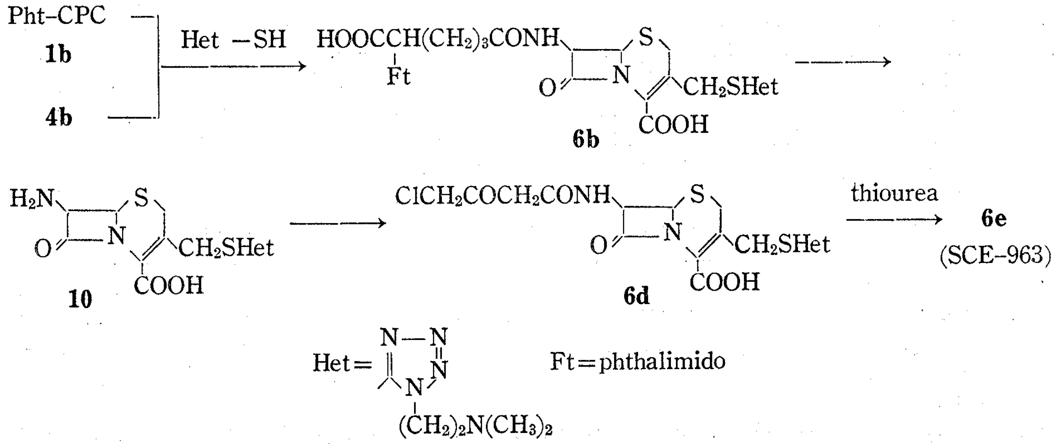


Chart 3

The nucleophilic displacement of the 3′-acetoacetoxy group of **4b** with **9** by heating at 55° for 75 min afforded **6b**, which was subjected to removal of the aminoadipoyl side chain *in situ* to give 7β-amino-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid (**10**)[14] in 81% yield. In a parallel study, N-phthaloyl-CPC (**1b**)[10a] reacted with **9** by heating at 55° for 7.5 hr gave **6b**, which was transformed into **10** in 50% yield. The compound **10** on acylation with 4-chloro-3-oxobutyryl chloride yielded **6d** and subsequent cyclization of **6d** with thiourea according to the method of Numata *et al.*[2d,5] gave SCE-963 in 72% yield.

The conditions applied to the above displacement reactions were selected to give the maximum yields. As already indicated in the results of three pairs of nucleophilic displacements, the higher activity of the 3′-acetoacetoxy group of **4** over that of the 3′-acetoxy group of **7** was clearly demonstrated. Therefore, it is obviously reasonable to state that the conditions for the conversion of **4** to **6** are milder than those for the conversion of **7** to **6** and eventually the better yields of **6** with less undesirable by-products will be achieved.

The results in this paper demonstrate that the 3′-O-acetoacetylation of deacetylcephalosporanic acids with diketene followed by the nucleophilic displacement reactions furnishes a feasible method for the production of semisynthetic cephalosporins of formula **6** including SCE-963. This method would make DCPC a valuable starting material for the production of cephalosporins.

Experimental

All melting points are uncorrected. Nuclear magnetic resonance (NMR) spectra were measured on a Hitachi Perkin-Elmer R-20 or a Varian XL-100 spectrometer using tetramethylsilane as an internal standard in DMSO-d_6 or $CDCl_3$, or as an external standard in D_2O. Infrared (IR) spectra were recorded on a Hitachi

13) *a*) M. Numata, I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, H. Akimoto, K. Naito, and M. Kida, *J. Antibiot.* (Tokyo), Ser A, **31**, 1262 (1978); *b*) T. Tsujikawa, H. Akimoto, M. Tatsuta, and N. Tokai, Japan. Patent Provisional Publication, 53-50169 (1978).

14) The compound prepared by a different method was disclosed in ref. 5.

EPI-S2 spectrometer. High performance liquid chromatography (HPLC) was performed using Hitachi Liquid Chromatograph Model 634 equipped with ultraviolet (UV) detector (254 nm).

N-Phthaloyl-deacetylcephalosporin C[8] (ditriethylamine salt) (3b)——To a solution of deacetylcephalosporin C (**3a**) (sodium salt, 3.5 hydrate, 165.4 g, purity:[15] 99.6%) in H_2O (400 ml) and acetone (100 ml) was added N-carbethoxyphthalimide (140 g) and the mixture was stirred maintaining the pH at 9.5—9.6 with 40% K_2CO_3 at room temperature for 90 min. The mixture was diluted with acetone (700 ml) and CH_2Cl_2 (250 ml) and adjusted to pH 2.5 with 4 N HCl at −5—0°. The organic layer was separated, washed with saturated NaCl and dried. To the solution was added H_2O (200 ml) and the mixture was adjusted to pH 5.3 with 40% K_2CO_3. The aqueous layer was separated, adjusted to pH 6.5 with 40% K_2CO_3 and mixed with acetone (750 ml). The crystals were collected and washed with 80% aq. acetone and acetone to give dipotassium salt of **3b** (5 hydrate, 202 g, 83.9%). The analytical sample was obtained by recrystallization from H_2O–acetone. mp 110—111° (dec.). IR ν_{max}^{KBr} cm^{-1}: 1770, 1750, 1710, 1600, 1540. NMR (D_2O) δ: 1.4—2.7 (6H, m, $(CH_2)_3$), 3.37 (2H, ABq, J=18 Hz, 2-CH_2), 4.28 (2H, s, 3-CH_2), 4.67 (1H, t, J=7 Hz, CH), 5.05 (1H, d, J=5 Hz, 6-H), 5.57 (1H, d, J=5 Hz, 7-H), 7.89 (4H, s, ArH). *Anal.* Calcd. for $C_{22}H_{19}N_3O_9SK_2 \cdot 5H_2O$: C, 39.45; H, 4.36; N, 6.27; S, 4.79. Found: C, 39.60; H, 4 25; N, 6 33; S, 4.97.

A solution of dipotassium salt of **3b** (134 g) in EtOAc (600 ml), THF (150 ml) and H_2O (300 ml) was adjusted to pH 2.5 with 4 N HCl at −5—0°. The organic layer was separated, washed with saturated NaCl and dried. The organic solution was added dropwise to a mixture of EtOAc (300 ml) and Et_3N (84 ml) at −10° with stirring. The precipitate was collected, washed with ether and dried to give ditriethylamine salt of **3b** (138 g, 97.7%). This salt was used without further purification in the following reaction. IR ν_{max}^{KBr} cm^{-1}: 1773, 1715, 1680, 1610, 1535. NMR ($CDCl_3$) δ: 1.26 (18H, t, J=7 Hz, $CH_3 \times 6$), 1.5—2.6 (6H, m, $(CH_2)_3$), 3.07 (12H, q, J=7 Hz, $CH_2 \times 6$), 3.39 (2H, br, 2-CH_2), 4.10 (2H, ABq, J=13 Hz, 3-CH_2), 4.66 (1H, t, J=7 Hz, CH), 4.87 (1H, d, J=5 Hz, 6-H), 5.74 (1H, q, J=5 and 8 Hz, 7-H), 7.42 (1H, d, J=8 Hz, CONH), 7.74 (4H, s, ArH).

3-Acetoacetoxymethyl-7β-(D-5-carboxy-5-phthalimidovaleramido)ceph-3-em-4-carboxylic Acid (4b)——To a solution of ditriethylamine salt of **3b** (112.8 g) in a mixture of CH_2Cl_2 (800 ml) and Et_3N (11.2 ml) was added diketene (21.5 g). The mixture was stirred at room temperature for 2 hr and evaporated *in vacuo* to leave a yellow foam, which was dissolved in EtOAc and H_2O and adjusted to pH 5.5 with 4 N HCl. The aqueous layer was separated, washed with EtOAc, layered with EtOAc and acidified to pH 2.0 with 4 N HCl. The organic layer was separated, washed with saturated NaCl, dried and evaporated *in vacuo*. The residue was triturated with ether and the powder was collected, washed with ether and dried to give **4b** (88.0 g, 93.5%). A sample for analysis was purified by chromatography on silica gel (CH_3CN–H_2O, 5:1). IR ν_{max}^{KBr} cm^{-1}: 1775, 1740, 1715, 1640, 1530. NMR (DMSO-d_6) δ: 1.30—2.40 (6H, m, $(CH_2)_3$), 2.18 (3H, s, CH_3), 3.48 (2H, ABq, J=18 Hz, 2-CH_2), 3.63 (2H, s, $COCH_2CO$), 4.73 (1H, t, J=7 Hz, CH), 4.92 (2H, ABq, J=13 Hz, 3-CH_2), 5.04 (1H, d, J=5 Hz, 6-H), 5.65 (1H, q, J=5 and 8 Hz, 7-H), 7.89 (4H, s, ArH), 8.77 (1H, d, J=8 Hz, CONH). *Anal.* Calcd. for $C_{26}H_{25}N_3O_{11}S \cdot 0.5H_2O$: C, 52.30; H, 4.31; N, 7.04. Found: C, 52.24; H, 4.27; N, 7.03.

3-Acetoacetoxymethyl-7β-aminoceph-3-em-4-carboxylic Acid (5). From 4b——To a chilled solution of **4b** (58.8 g) and Et_3N (28.0 ml) in CH_2Cl_2 (700 ml) was added dichlorodimethylsilane (32.5 ml). After stirring at 15—20° for 30 min and cooling to −30°, the mixture was treated with N,N-dimethylaniline (85.0 ml) and PCl_5 (42.0 g) at −25° for 10 min and then with MeOH (250 ml) at −15—−10° for 20 min. To the reaction mixture was added H_2O (500 ml) at −5° for 5 min. The aqueous layer was separated, washed with CH_2Cl_2, diluted with MeOH (250 ml) and adjusted to pH 3.4 with 40% K_2CO_3. The precipitate was collected, and washed with H_2O and acetone. The solid was purified by dissolution in dil. HCl and following precipitation with adjustment of the pH of the solution to 3.4 to give **5** (28.4 g, 90.4%). IR ν_{max}^{KBr} cm^{-1}: 1798, 1730, 1620, 1535. NMR (D_2O+NaOD) δ: 2.29 (3H, s, CH_3), 3.48 (2H, ABq, J=18 Hz, 2-CH_2), 4.6—5.2 (4H, m, 3-CH_2, 6-H, 7-H). *Anal.* Calcd. for $C_{12}H_{14}N_2O_6S$: C, 45.86; H, 4.49; N, 8.91; S, 10.20. Found: C, 45.52; H, 4.61; N, 8.86; S, 10.19.

From DCPC (3a)——To a solution of **3a** (sodium salt, 3.5 hydrate, 16.5 g, purity:[15] 99.6%) in H_2O (40 ml) and acetone (10 ml) was added N-carbethoxyphthalimide (14.0 g) and the mixture was stirred maintaining the pH at 9.5—9.6 with 40% K_2CO_3 at room temperature for 90 min. The solution was diluted with acetone (70 ml) and CH_2Cl_2 (25 ml) and adjusted to pH 2.5 with 12 N HCl at −5—0°. The organic layer was separated, washed with saturated NaCl and dried. To the solution was added Et_3N (15 ml) and the mixture was evaporated to dryness *in vacuo*. The residue was dissolved in CH_2Cl_2 (240 ml) and treated successively with Et_3N (3.0 ml), diketene (6.54 g), dichlorodimethylsilane (14.2 ml), N,N-dimethylaniline (40.0 ml), PCl_5 (19.7 g), MeOH (100 ml) and H_2O (200 ml) and worked up in the similar manner as described above for the preparation of **4b** and **5** with omission of purification to give crude **5** (12.0 g, purity:[15] 89.1%, yield: 85.4%).

3-Acetoacetoxymethyl-7β-phenylacetamidoceph-3-em-4-carboxylic Acid (4c)——To a stirred suspension of **5** (6.28 g) in CH_2Cl_2 (30 ml) and N,N-dimethylacetamide (10 ml) was added phenylacetyl chloride (4.64 g). After stirring at room temperature for 10 hr, the mixture was evaporated *in vacuo*. The residue was dissolved

15) The purity was determined by HPLC.

in EtOAc and H_2O. The organic layer was separated, washed with saturated NaCl, dried and evaporated *in vacuo*. The residue was recrystallized from EtOAc–ether to give **4c** (7.40 g, 85.7%). mp 87—89°. IR ν_{max}^{KBr} cm^{-1}: 1785, 1745, 1718, 1655, 1537. NMR (DMSO-d_6) δ: 2.19 (3H, s, CH_3), 3.52 (4H, br, 2-CH_2, CH_2CO), 3.61 (2H, s, $COCH_2CO$), 4.95 (2H, ABq, J=13 Hz, 3-CH_2), 5.05 (1H, d, J=5 Hz, 6-H), 5.66 (1H, q, J=5 and 8 Hz, 7-H), 7.26 (5H, s, ArH), 9.04 (1H, d, J=8 Hz, CONH). *Anal.* Calcd. for $C_{20}H_{20}N_2O_7S$: C, 55.55; H, 4.66; N, 6.48. Found: C, 55.47; H, 5.00; N, 6.24.

3-[[(1-Methyl-1H-tetrazol-5-yl)thio]methyl]-7β-phenylacetamidoceph-3-em-4-carboxylic Acid (6c)[12]——A solution of **4c** (432 mg), 1-methyl-1H-tetrazole-5-thiol (**8**, 116 mg) and $NaHCO_3$ (170 mg) in H_2O (6.0 ml) was adjusted to pH 6.0 with 1 N HCl. After addition of 1 M phosphate buffer (pH 6.0) (2.0 ml), the mixture was heated at 47° for 210 min, cooled and chromatographed on Amberlite XAD-2 (40 ml) using H_2O and H_2O–MeOH as eluents. The eluate was concentrated and lyophilized. Recrystallization of the lyophilizate from H_2O–EtOH afforded sodium salt of **6c** (389 mg). IR ν_{max}^{KBr} cm^{-1}: 1770, 1670, 1618, 1530. NMR (D_2O) δ: 3.52 (2H, ABq, J=18 Hz, 2-CH_2), 3.65 (2H, s, CH_2CO), 3.97 (3H, s, CH_3), 4.14 (2H, ABq, J=14 Hz, 3-CH_2), 5.00 (1H, d, J=4.5 Hz, 6-H), 5.56 (1H, d, J=4.5 Hz, 7-H), 7.33 (5H, s, ArH). *Anal.* Calcd. for $C_{18}H_{17}N_6NaO_4S_2 \cdot H_2O$: C, 44.44; H, 3.94; N, 17.27. Found: C, 44.77; H, 3.88; N, 17.01.

3-Acetoacetoxymethyl-7β-[2-(2-aminothiazol-4-yl)acetamido]ceph-3-em-4-carboxylic Acid (4e)——To a solution of **5** (15.7 g) and di-*n*-butylamine (17.5 g) in CH_2Cl_2 (185 ml) was added dropwise 1.54 M solution of 4-chloro-3-oxobutyryl chloride in CH_2Cl_2 (50 ml) at −20—−15° over a period of 5 min. After stirring at −15° for 15 min, the mixture was treated with thiourea (5.7 g) and stirred at room temperature for 15 hr. The precipitate was collected, washed with CH_2Cl_2 and suspended in H_2O (100 ml). The suspension was stirred at 20° for 30 min, cooled, adjusted to pH 3.5 and allowed to stand in a refrigerator overnight. The precipitate was collected, washed with H_2O and suspended in 50% aq. acetone (600 ml). Conc. HCl was added to the suspension until the solution was obtained. After being treated with activated charcoal, the solution was adjusted to pH 3.3 with 40% K_2CO_3. The crystals formed were collected, washed with H_2O and dried to give **4e** (20.2 g, 85.4%). IR ν_{max}^{KBr} cm^{-1}: 1775, 1740, 1715, 1663, 1625, 1545. NMR (DMSO-d_6) δ: 2.19 (3H, s, CH_3), 3.40 (2H, s, CH_2CO), 3.54 (2H, br, 2-CH_2), 3.63 (2H, s, $COCH_2CO$), 4.94 (2H, ABq, J=13 Hz, 3-CH_2), 5.07 (1H, d, J=5 Hz, 6-H), 5.71 (1H, q, J=5 and 8 Hz, 7-H), 6.26 (1H, s, thiazol-H), 6.90 (2H, br, NH_2), 8.85 (1H, d, J=8 Hz, CONH). *Anal.* Calcd. for $C_{17}H_{18}N_4O_7S_2 \cdot H_2O$: C, 43.22; H, 4.27; N, 11.86; S, 13.57. Found: C, 43.06; H, 4.20; N, 11.80; S, 13.91.

7β-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic Acid (6e).[2d] **From 4e**——A mixture of **4e** (monohydrate, 4.73 g), $NaHCO_3$ (1.01 g) and 1-(2-dimethylaminoethyl)-1H-tetrazole-5-thiol[13] (**9**, 5.36 g), in H_2O (60 ml) was heated at 55° for 60 min, cooled (formation yield of **6e**, 82.9% by HPLC), adjusted to pH 3.0 with 6 N HCl and filtered. The filtrate was adjusted to pH 5.3 and chromatographed on Amberlite XAD-2 using H_2O and H_2O–MeOH as eluents. The eluate was concentrated and passed through the column packed with Al_2O_3 and then through the one with Amberlite IR-120 (H-form). The eluate was treated with activated charcoal and lyophilized to give **6e** (3.77 g, 70.5%). IR ν_{max}^{KBr} cm^{-1}: 1767, 1673, 1609, 1520. NMR (D_2O) δ: 3.03 (6H, s, $N(CH_3)_2$), 3.60 (2H, s, CH_2CO), 3.65 (2H, ABq, J=18 Hz, 2-CH_2), 3.80 (2H, t, J=6 Hz, CH_2N), 4.23 (2H, ABq, J=14 Hz, 3-CH_2), 4.87 (2H, t, J=6 Hz, CH_2N), 5.09 (1H, d, J=4.5 Hz, 6-H), 5.64 (1H, d, J=4.5 Hz, 7-H), 6.50 (1H, s, thiazol-H). *Anal.* Calcd. for $C_{18}H_{23}N_9O_4S_3 \cdot 0.5H_2O$: C, 40.44; H, 4.53; N, 23.58. Found: C, 40.19; H, 4.37; N, 23.40.

From 7e[5]——A mixture of **7e** (4.12 g), $NaHCO_3$ (1.01 g) and **9** (5.36 g) in H_2O (60 ml) was heated at 65° for 100 min (formation yield of **6e**, 44.5% by HPLC) and worked up in the same manner as described above to afford **6e** (1.15 g, 21.6%). The sample coincided with the authentic sample obtained above in NMR, IR, TLC and HPLC data.

7β-Amino-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic Acid (10)[14] **From 4b**——A solution of **4b** (5.88 g), **9** (5.19 g), $NaHCO_3$ (2.10 g) and NaCl (15.0 g) in H_2O (60 ml) was adjusted to pH 5.5 and heated at 55° for 75 min with stirring. The mixture was diluted with saturated NaCl (240 ml) and acidified to pH 1.5 with 4 N HCl. After stirring at 0—5° for 30 min, the precipitate was collected, washed with saturated NaCl and dried to give crude hydrochloride of **6b** (7.65 g). To a suspension of this salt in CH_2Cl_2 (120 ml) was added Et_3N (5.88 ml) at 0—5°. The mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in a mixture of CH_2Cl_2 (120 ml) and N,N-dimethylaniline (11.7 ml). The solution was cooled to −20° and treated with dichlorodimethylsilane (4.18 ml). After stirring at −10° for 30 min, PCl_5 (5.82 g) was added thereto. The mixture was stirred at −45—−40° for 30 min and then treated with isobutanol (30 ml) at a temperature below −30°. After stirring at −35—−30° for 60 min, H_2O (60 ml) was added and the mixture was stirred at −5° for 5 min. The aqueous layer was separated, washed with CH_2Cl_2, layered with EtOAc and adjusted to pH 6.0 with Et_3N. The aqueous layer was separated, adjusted to pH 3.2 with 4 N HCl, concentrated *in vacuo* and the residue was poured onto EtOH (300 ml) with cooling and stirring. The precipitate was collected, washed with EtOH and acetone to give hydrochloride of **10** (4.13 g, purity:[15] 82.5%, yield: 80.8%). The salt (2.0 g) was dissolved in H_2O and the solution was adjusted to pH 6.0 with 1 N NaOH. The aqueous solution was chromatographed on Amberlite XAD-2. The eluate was treated with Al_2O_3 and lyophilized to give **10** (0.9 g). IR ν_{max}^{KBr} cm^{-1}: 1760, 1613. NMR (D_2O) δ: 3.05 (6H, s, $N(CH_3)_2$), 3.70 (2H, ABq, J=18 Hz, 2-CH_2), 3.84 (2H, t, J=6 Hz,

CH_2N), 4.23 (2H, ABq, $J=13$ Hz, 3-CH_2), 4.81 (1H, d, $J=5$ Hz, 6-H), 4.91 (2H, t, $J=6$ Hz, CH_2N), 5.08 (1H, d, $J=5$ Hz, 7-H). *Anal.* Calcd. for $C_{13}H_{19}N_7O_3S_2 \cdot H_2O$: C, 38.70; H, 5.25; N, 24.30; S, 15.89. Found: C, 38.59; H, 5.25; N, 24.54; S, 15.64.

From 1b[10a]——The repetition of the same procedure as described above except **1b** (5.46 g) being used instead of **4b** and heating for 7.5 hr instead of 75 min afforded hydrochloride of **10** (2.60 g, purity:[15] 81.1%, yield: 50.0%).

Preparation of 6e from 10——To a solution of hydrochloride of **10** (2.11 g, purity:[15] 82.5%) and di-*n*-butylamine (1.94 g) in CH_2Cl_2 (40 ml) was added dropwise 1.54 M solution of 4-chloro-3-oxobutyryl chloride in CH_2Cl_2 (7.0 ml) at −20——15° over a period of 2 min and the mixture was stirred at −15° for 20 min. The precipitate was collected, washed with CH_2Cl_2 and then dissolved in N,N-dimethylacetamide (20 ml). The solution was treated with thiourea (0.42 g) and stirred at room temperature for 5 hr. After addition of EtOAc (200 ml) to the reaction mixture, the precipitate was collected, washed with EtOAc, and dissolved in H_2O. The solution was adjusted to pH 3.0 with 1 N NaOH and filtered. The filtrate was adjusted to pH 5.5 and chromatographed on Amberlite XAD-2 using H_2O and H_2O–MeOH as eluents. The eluate was concentrated and passed through the column packed with Al_2O_3 and through the one with Amberlite IR-120 (H-form). The eluate was treated with activated charcoal and lyophilized to give **6e** (1.57 g, 72.4%). The sample coincided with the authentic sample obtained above in NMR, IR, TLC and HPLC data.

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