# Orally Active Cephalosporins. IV.[1] Synthesis, Antibacterial Activity and Oral Absorption of 3-(1*H*-1,2,3-Triazol-4-yl)thiomethylthio-cephalosporins

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**The synthesis, antibacterial activity and oral absorption of 3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylic acids with various C-7 side chains (2) are described. The (1*H*-1,2,3-triazol-4-yl)thiomethylthio C-3 side chain was found to be an effective substituent for good oral absorption of cephalosporins with some C-7 side chains.**

**Keywords** cephalosporin; (1,2,3-triazol-4-yl)thiomethylthio group; synthesis; antibacterial activity; oral absorption; cefatrizine

Inspection of the structural formulas of orally absorbable cephalosporins reveals that they have a relatively small substituent at the C-3 position of the cephem nucleus, except for cefatrizine.[2] Cefatrizine has a larger C-3 side chain including 1,2,3-triazole (Fig. 1). Replacement of 1,2,3-triazole of cefatrizine with other heteroaromatic rings such as imidazole and tetrazole decreased the oral absorbability.[3] These results suggested that a 1,2,3-triazole moiety plays an important role in good oral absorption. This suggestion prompted us to conduct extensive syntheses of cephalosporins possessing 1,2,3-triazole in the C-3 side chain. We finally obtained a new orally active cephalosporin, 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylic

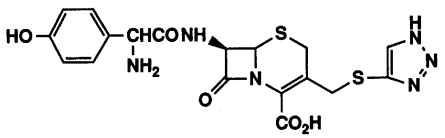


Fig. 1. Cefatrizine

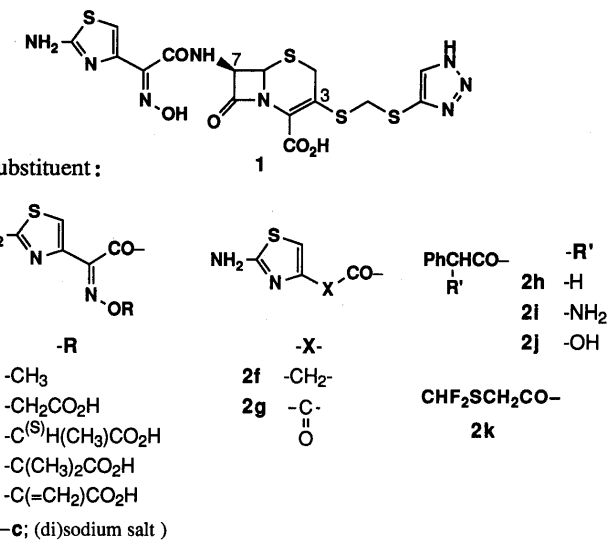
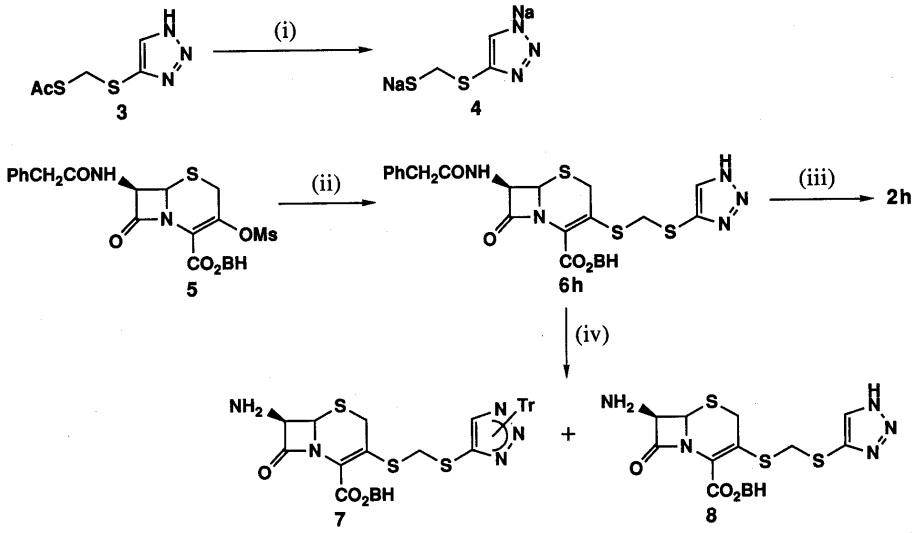


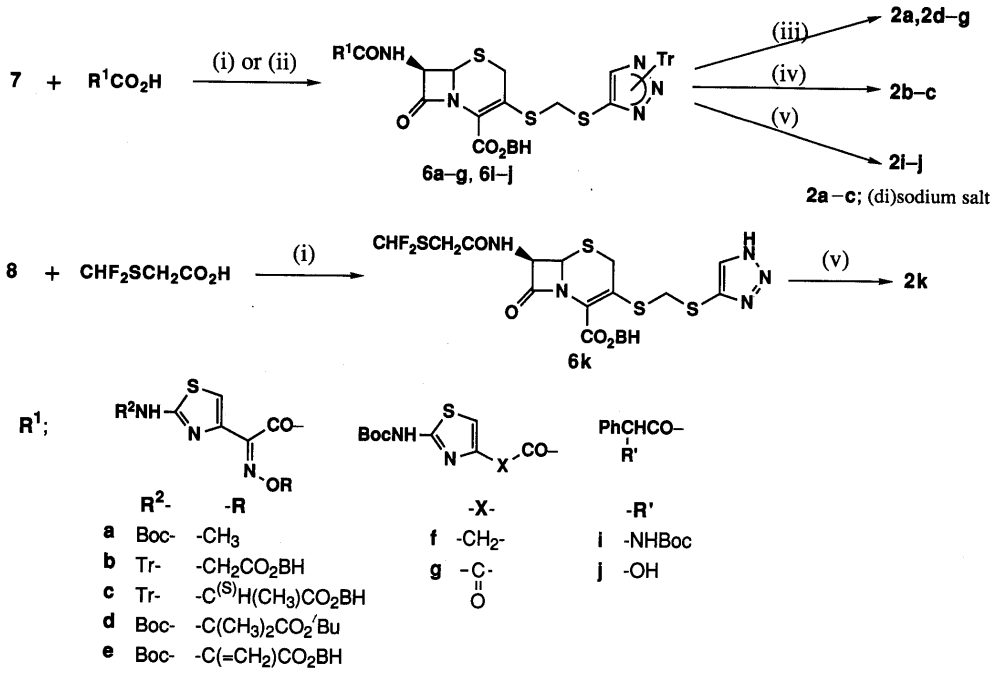

Fig. 2. 3-(1*H*-1,2,3-Triazol-4-yl)thiomethylthio-cephalosporins (**1** and **2a—k**)




(i) NaOMe / DMF-MeOH; (ii) **4** / DMF; (iii) $CF_3CO_2H$ / anisole / $CH_2Cl_2$; (iv) 1) TrCl / pyridine, 2) $PCl_5$ / pyridine, 3) 1,3-butanediol.

Tr = triphenylmethyl, BH = diphenylmethyl

Chart 1




(i) *N*-methylmorpholine / $Cl_2PO_2Ph$ / $CH_2Cl_2$; (ii) DCC / $CH_2Cl_2$; (iii) $AlCl_3$ / anisole / $CH_3NO_2$; (iv) 1) $HCO_2H$ / $H_2O$, 2) $AlCl_3$ / anisole / $CH_3NO_2$; (v) $CF_3CO_2H$ / anisole / $CH_2Cl_2$.
DCC = *N*,*N*'-dicyclohexylcarbodiimide, Boc = *tert*-butoxycarbonyl

Chart 2

TABLE I. *In Vitro* Antibacterial Activity of Cephalosporins (**2a—k** and **1**)

| Organism | MIC (μg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2a** | **2b** | **2c** | **2d** | **2e** | **2f** | **2g** | **2h** | **2i** | **2j** | **2k** | **1** |
| *Staphylococcus aureus* FDA 209P JC-1 | 0.8 | 6.3 | 12.5 | 12.5 | 3.1 | 0.1 | 0.8 | 0.05 | 25 | 0.2 | 0.1 | 0.2 |
| *S. aureus* SMITH | 1.6 | 12.5 | 12.5 | 12.5 | 3.1 | 0.1 | 1.6 | 0.05 | 50 | 0.2 | 0.1 | 0.2 |
| *S. aureus* SR3131 | 100 | >100 | >100 | >100 | 100 | 50 | 50 | 25 | 100 | 25 | 25 | 12.5 |
| *S. epidermidis* ATCC14990 | 1.6 | 6.3 | 6.3 | 6.3 | 3.1 | 0.1 | 0.8 | 0.1 | 12.5 | 0.4 | 0.1 | 0.2 |
| *Streptococcus pyogenes* C-203 | 0.006 | 0.05 | 0.1 | 0.1 | 0.1 | 0.02 | 0.02 | 0.02 | 3.1 | 0.01 | 0.02 | 0.006 |
| *S. pneumoniae* Type 1 | 0.01 | 0.2 | 0.2 | 0.2 | 0.1 | 0.05 | 0.1 | 0.02 | 6.3 | 0.05 | 0.02 | 0.02 |
| *Escherichai coli* H | 0.1 | 0.02 | 0.05 | 0.2 | 0.02 | 0.2 | 0.1 | 3.1 | 1.6 | 0.4 | 1.6 | 0.05 |
| *E. coli* NIHJ JC-2 | 0.8 | 0.4 | 0.8 | 0.8 | 0.4 | 3.1 | 0.8 | 50 | 25 | 3.1 | 12.5 | 0.2 |
| *E. coli* EC-14 | 0.4 | 0.1 | 0.2 | 0.4 | 0.1 | 0.8 | 0.4 | 12.5 | 12.5 | 1.6 | 3.1 | 0.1 |
| *E. coli* SR377 | 1.6 | 3.1 | 3.1 | 3.1 | 0.8 | >100 | 12.5 | >100 | >100 | 12.5 | >100 | 1.6 |
| *Klebsiella pneumoniae* SR1 | 0.1 | 0.02 | 0.05 | 0.1 | 0.02 | 0.4 | 0.2 | 6.3 | 6.3 | 0.8 | 1.6 | 0.1 |
| *Proteus mirabilis* PR-4 | 0.05 | 0.006 | 0.01 | 0.01 | <0.003 | 0.1 | 0.1 | 3.1 | 25 | 0.4 | 0.8 | 0.05 |
| *P. vulgaris* CN-329 | 0.02 | 0.006 | 0.006 | 0.01 | <0.003 | 0.8 | 0.4 | 12.5 | >100 | 12.5 | 25 | 0.2 |
| *Morganella morganii* SR9 | 0.1 | 0.02 | 0.1 | 0.2 | 0.01 | 12.5 | 3.1 | >100 | >100 | 25 | >100 | 0.1 |
| *Enterobacter cloacae* SR233 | 0.8 | 0.4 | 0.4 | 0.8 | 0.2 | >100 | 12.5 | >100 | >100 | 50 | >100 | 0.8 |
| *Serratia marcescens* ATCC13880 | 0.8 | 0.4 | 0.8 | 0.8 | 0.2 | >100 | 50 | >100 | >100 | >100 | >100 | 3.1 |
| *Pseudomonas aeruginosa* ATCC25619 | 100 | 50 | 3.1 | 1.6 | 3.1 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |

acid (**1**), the (1*H*-1,2,3-triazol-4-yl)thiomethylthio side chain of which was found to be the best substituent for oral absorption.[4] Our interest has now turned to the versatility of this C-3 side chain as a substituent of orally active cephalosporins. In this paper, we wish to report the synthesis, antibacterial activity and oral absorption of 3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylic acids with various C-7 side chains (**2**).

**Chemistry** The desired 3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-cephalosporins (**2**) were synthesized by the route shown in Charts 1 and 2.

Diphenylmethyl 3-methanesulfonyloxy-7β-phenylacetamido-3-cephem-4-carboxylate (**5**)[5] was allowed to react with the disodium salt (**4**), prepared *in situ* by methanolysis of (1*H*-1,2,3-triazol-4-yl)thiomethyl thioacetate (**3**),[1] to afford the protected 3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-cephalosporin (**6h**). The desired 7β-phenylacetamido-cephalosporin (**2h**) was obtained by the deprotection of **6h** with trifluoroacetic acid. For the preparation of the other desired cephalosporin derivatives (**2a—g**, **2i—k**), protection of the 1,2,3-triazole of **6h** with a triphenylmethyl (trityl) group followed by the cleavage of the C-7 phenylacetyl side chain was carried out to give 7β-amino-cephalosporin derivatives (**7** and **8**). Without tritylation, the cleavage of

TABLE II. Plasma Levels and Urinary Recovery of Cephalosporins (**2a—k**, **1**) in Mice after Oral Administration of 40 mg/kg

| Compound | Plasma level (μg/ml) | | Urinary recovery (%) |
|---|---|---|---|
| | 15 min | 120 min | |
| **2a** | 15.3 | 13.0 | 2.1 |
| **2b** | 2.05 | 4.37 | 0.6 |
| **2c** | 0.41 | 0.73 | <0.3 |
| **2d** | <0.16 | <0.16 | <0.3 |
| **2e** | 0.77 | 0.62 | 0.7 |
| **2f** | 17.1 | 2.83 | 36.3 |
| **2g** | 19.0 | 9.80 | 14.0 |
| **2h** | 15.1 | 3.96 | 22.1 |
| **2i** | 60.0 | 15.4 | 7.8 |
| **2j** | 18.5 | 7.26 | 10.7 |
| **2k** | 28.4 | 2.53 | 44.6 |
| **1** | 29.6 | 51.3 | 5.5 |

Mice: ICR-strain, 6-week-old male, $n=5$.

the phenylacetyl group gave **8** only in a poor yield. Compounds **7** and **8** were separated by column chromatography on silica gel and acylated with the corresponding carboxylic acids to afford the protected cephalosporins (**6a—g** and **6i—k**). Successive treatment of **6b** and **6c** with aqueous formic acid and aluminum chloride ($AlCl_3$) gave the desired cephalosporins (**2b** and **2c**). Cephalosporins (**2a** and **2d—g**) were obtained by the deprotection of **6a** and **6d—g** with $AlCl_3$ and cephalosporins (**2i—k**) by that of **6i—k** with trifluoroacetic acid.

**Biological Evaluation** The minimum inhibitory concentration (MIC) values of the synthesized cephalosporins (**2**) against selected strains of gram-positive and gram-negative bacteria are shown in Table I. Their plasma levels and urinary recovery after oral administration (40 mg/kg) to mice are summarized in Table II.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporin (**2a**) showed antibacterial activity comparable to that of the hydroxyimino derivative (**1**) against gram-positive and gram-negative bacteria except Staphylococci. 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-carboxyalkoxyiminoacetamido]cephalosporins (**2b—e**) were much less active against gram-positive bacteria than **1**. However, their activity against most gram-negative bacteria was much better than that of **1**. Other cephalosporins (**2f—h**, **2j** and **2k**) exhibited activity comparable to that of **1** against gram-positive bacteria but showed lower activity against gram-negative bacteria. The phenylglycyl cephalosporin (**2i**) showed only poor antibacterial activity, which seemed to be due to its instability under the conditions of measurement.

Among compounds (**2a—e**) having the C-7 side chain frequently used in so-called third generation cephalosporins, compound **2a** showed high plasma levels after oral administration, but compounds **2c—e** were almost unabsorbable. Compound **2b** exhibited much lower oral absorbability than that of **1** although **2b** was expected to be readily absorbable because of having the same C-7 side chain as the known orally active cephalosporin, cefixime.[6] All of the other cephalosporins (**2f—k**) exhibited high plasma levels and better urinary recovery as compared with **1**.

It is noteworthy that most of these cephalosporins having various C-7 side chains showed good oral absorption, considering that the C-7 side chains of orally active cephalosporins used in clinical medicine are limited to a few specific substituents, *e.g.*, arylglycine,[2] (*Z*)-2-(2-aminothiazol-4-yl)-2-carboxymethoxyiminoacetic acid,[6] and (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetic acid.[7] This (1*H*-1,2,3-triazol-4-yl)thiomethylthio side chain is potentially useful for the development of novel oral cephalosporins in combination with suitable C-7 side chains.

## Experimental

**General Procedures** Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Jasco IR-700 spectrometer. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were recorded at 200 MHz on a Varian VXR-200 NMR spectrometer using tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (in $D_2O$) as an internal standard. Mass spectra (high-resolution secondary ion (SI) MS) were measured on a Hitachi M-90 mass spectrometer. The following abbreviations are used: s, singlet; d, doublet; m, multiplet; br, broad; ABq, AB quartet. All reactions under anhydrous conditions were carried out using anhydrous solvents dried over Molecular Sieves type 4A under a nitrogen atmosphere.

**Diphenylmethyl 7β-Phenylacetamido-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylate (6h)** A solution of (1*H*-1,2,3-triazol-4-yl)thiomethyl thioacetate (**3**)[1] (6.79 g, 35.9 mmol) in *N*,*N*-dimethylformamide (DMF) (200 ml) at −70 °C was treated with sodium methoxide in MeOH (1.26 N, 56 ml). After being stirred at −60 °C for 20 min, the reaction mixture was cooled to −78 °C and a solution of diphenylmethyl 3-methanesulfonyloxy-7β-phenylacetamido-3-cephem-4-carboxylate (**5**)[5] (17.0 g, 29.4 mmol) in DMF (50 ml) was added dropwise to the mixture. The mixture was stirred at the same temperature for 40 min, then 6 ml of acetic acid and 12 ml of 10% HCl were successively added. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with brine four times, dried over anhydrous $Na_2SO_4$ and evaporated. The resulting residue was crystallized from EtOAc to give 10.3 g (56%) of **6h** as white crystals: mp 162—163 °C. *Anal.* Calcd for $C_{31}H_{27}N_5O_4S_3$: C, 59.12; H, 4.32; N, 11.12; S, 15.27. Found: C, 58.84; H, 4.36; N, 11.04; S, 15.02. The spectral data of **6h** are listed in Table III.

**Diphenylmethyl 7β-Amino-3-[1-(or 2)-triphenylmethyl-1(or 2)*H*-1,2,3-triazol-4-yl]thiomethylthio-3-cephem-4-carboxylate (7), Diphenylmethyl 7β-Amino-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylate (8)** A suspension of **6h** (10.0 g, 15.9 mmol) in $CH_2Cl_2$ (100 ml) was treated with pyridine (1.54 ml, 19.1 mmol) and trityl chloride (5.32 g, 19.1 mmol) under ice-cooling. After the mixture had been stirred at room temperature for 1 h, 2 ml of 10% HCl was added and the whole was diluted with water and extracted with EtOAc. The extract was washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated. The resulting residue was dissolved in $CH_2Cl_2$ (50 ml) and treated with pyridine (2.57 ml, 31.8 mmol) and phosphorus pentachloride (5.96 g, 28.6 mmol) under ice-cooling. After being stirred at the same temperature for 30 min, the reaction mixture was added to a solution of 1,3-butanediol (8.6 ml, 95.9 mmol) in $CH_2Cl_2$ (25 ml) at −30 °C. After being stirred at the same temperature for 10 min and at ice-bath temperature for 40 min, the reaction mixture was diluted with $CH_2Cl_2$, washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated. The resulting residue was triturated with $Et_2O$. A solution of the above powder in $CH_2Cl_2$ was washed with 5% aqueous $NaHCO_3$, dried over anhydrous $Na_2SO_4$ and evaporated. The residue was purified by column chromatography on silica gel (eluent: toluene–EtOAc, 2 : 1) to yield 6.70 g (56%) of **7** as a yellow froth and 2.72 g (33%) of **8** as white crystals.

**7**: $^1$H-NMR ($CDCl_3$) δ: 7.45 (1H, s), 7.5—7.05 (27H, m), 6.90 (1H, s), 4.87 (1H, d, $J$=5.0 Hz), 4.66 (1H, d, $J$=5.0 Hz), 4.16, 4.05 (2H, ABq, $J$=13.4 Hz), 3.82, 3.62 (2H, ABq, $J$=17.6 Hz). IR ($CHCl_3$): 3410, 1782, 1731, 1605, 1496, 1450, 1390, 1368 cm$^{-1}$.

**8**: mp 112—115 °C. $^1$H-NMR ($CDCl_3$) δ: 7.56 (1H, s), 7.5—7.25 (10H, m), 6.97 (1H, s), 4.94 (1H, d, $J$=5.0 Hz), 4.73 (1H, d, $J$=5.0 Hz), 4.14, 4.05 (2H, ABq, $J$=13.7 Hz), 3.73, 3.58 (2H, ABq, $J$=17.5 Hz). IR ($CHCl_3$): 3430, 1780, 1730, 1602, 1495, 1451, 1388, 1362 cm$^{-1}$. HRMS Calcd for $C_{23}H_{22}N_5O_3S_3$ ($MH^+$): 512.0885. Found *m/z*: 512.0880 ($MH^+$).

**Diphenylmethyl 7β-[(*Z*)-2-(2-Triphenylmethylaminothiazol-4-yl)-2-diphenylmethoxycarbonylmethoxyiminoacetamido]-3-[1(or 2)-triphenylmethyl-1(or 2)*H*-1,2,3-triazol-4-yl]thiomethylthio-3-cephem-4-carboxylate (6b)** A solution of **7** (800 mg, 1.06 mmol) and (*Z*)-2-(2-triphenylmethylaminothiazol-4-yl)-2-diphenylmethoxycarbonylmethoxyiminoacetic acid[8]

(728 mg, 1.11 mmol) in $CH_2Cl_2$ (8 ml) was cooled to −30 °C. *N*-Methylmorpholine (0.27 ml, 2.46 mmol) and phenylphosphoryl dichloride (0.19 ml, 1.27 mmol) were added to the mixture. After being stirred at −30 °C for 30 min, the reaction mixture was neutralized with 10% HCl, diluted with water and extracted with EtOAc. The extract was washed with brine twice, dried over anhydrous $Na_2SO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (eluent: toluene–EtOAc, 10 : 1) to yield 1.07 g (73%) of **6b** as a pale yellow froth.

Compounds **6a**, **6c—g**, **6i** and **6k** were similarly prepared from **7** or **8** with the corresponding carboxylic acids according to the procedure described for the preparation of **6b**.

TABLE III. $^1$H-NMR and IR Spectral Data for **6a—k**

| Compd. No. | $^1$H-NMR (Solvent, $\delta$) | IR ($CHCl_3$) $cm^{-1}$ (C=O) |
|---|---|---|
| **6a** | ($CDCl_3$); 8.9—8.7 (1H, br s), 7.46 (1H, s), 7.4—7.25 (20H, m), 7.15—7.05 (6H,m), 7.05 (1H, s), 6.87 (1H, s), 5.90 (1H, dd, $J$=4.8, 8.8 Hz), 5.02 (1H, d, $J$=4.8 Hz), 4.19 , 4.10 (2H, ABq, $J$=13.5 Hz), 4.09 (3H, s), 3.82, 3.64 (2H, ABq, $J$=17.4 Hz), 1.52 (9H, s) | 1780, 1720, 1680 |
| **6b** | ($CDCl_3$); 8.11 (1H, d, $J$=9.1 Hz), 7.45 (1H, s), 7.4—7.0 (51H, m), 6.93 (1H, s), 6.87 (1H, s), 6.81 (1H, s), 5.80 (1H, dd, $J$=5.0, 9.1 Hz), 5.03, 4.93 (2H, ABq, $J$=17.0 Hz), 4.90 (1H, d, $J$=5.0 Hz), 4.12, 4.07 (2H, ABq, $J$=13.2 Hz), 3.62, 3.32 (2H, ABq, $J$=17.1 Hz) | 1790, 1740, 1688 |
| **6c** | ($CDCl_3$); 8.22 (1H, d, $J$=8.7 Hz), 7.45 (1H, s), 7.4—7.0 (51H, m), 6.87 (1H, s), 6.85 (1H, s), 6.77 (1H, s), 5.82 (1H, dd, $J$=4.6, 8.7 Hz), 5.20 (1H, q, $J$=7.2 Hz), 4.91 (1H, d, $J$=4.6 Hz), 4.16, 4.12 (2H, ABq, $J$=13.4 Hz), 3.66, 3.41 (2H, ABq, $J$=16.8 Hz), 1.65 (3H, d, $J$=7.2 Hz) | 1787, 1730, 1685 |
| **6d** | ($CDCl_3$); 8.4—8.1 (1H, br s), 8.19 (1H, d, $J$=9.0 Hz), 7.46 (1H, s), 7.4—7.05 (26H, m), 6.88 (1H, s), 5.94 (1H, dd, $J$=4.9, 9.0 Hz), 5.01 (1H, d, $J$=4.9 Hz), 4.20, 4.09 (2H, ABq, $J$=13.4 Hz), 3.84, 3.63 (2H, ABq, $J$=17.3 Hz), 1.63 (3H, s), 1.61 (3H, s), 1.53 (9H, s), 1.39 (9H, s) | 1788, 1725, 1687 |
| **6e** | ($CDCl_3$); 9.1—8.7 (1H, br s), 7.68 (1H, d, $J$=9.0 Hz), 7.45 (1H, s), 7.4—7.0 (36H, m), 6.93 (1H, s), 6.84 (1H, s), 5.89 (1H, s), 5.73 (1H, dd, $J$=4.8, 9.0 Hz), 5.66 (1H, s), 4.80 (1H, d, $J$=4.8 Hz), 4.07 (2H, s), 3.51, 3.33 (2H, ABq, $J$=18.0 Hz), 1.53 (9H, s) | 1786, 1724, 1695 |
| **6f** | ($CDCl_3$); 7.76 (1H, d, $J$=8.0 Hz), 7.44 (1H, s), 7.5—7.25 (19H, m), 7.15—7.05 (6H, m), 6.76 (1H, s), 6.57 (1H, s), 5.54 (1H, dd, $J$=4.6, 8.0 Hz), 4.79 (1H, d, $J$=4.6 Hz), 4.06, 4.02 (2H, ABq, $J$=13.6 Hz), 3.75, 3.72 (2H, ABq, $J$=17.8 Hz), 3.41 (2H, s), 1.57 (9H, s) | 1780, 1718, 1672 |
| **6g** | ($CDCl_3$); 8.86 (1H, s), 8.6—8.5 (1H, br s), 8.19 (1H, d, $J$=9.2 Hz), 7.47 (1H, s), 7.45—7.2 (19H, m), 7.15—7.05 (6H, m), 6.91 (1H, s), 5.70 (1H, dd, $J$=4.7, 9.2 Hz), 5.00 (1H, d, $J$=4.7 Hz), 4.21, 4.10 (2H, ABq, $J$=13.3 Hz), 3.85, 3.67 (2H, ABq, $J$=17.1 Hz), 1.55 (9H, s) | 1788, 1725, 1672 |
| **6h** | ($CDCl_3$ + $CD_3OD$); 7.62 (1H, s), 7.5—7.25 (15H, m), 6.92 (1H, s), 5.72 (1H, d, $J$=4.8 Hz), 5.00 (1H, d, $J$=4.8 Hz), 4.14, 4.09 (2H, ABq, $J$=13.6 Hz), 3.69, 3.60 (2H, ABq, $J$=17.2 Hz), 3.63 (2H, s) | 1784, 1698[a] |
| **6i** | ($CDCl_3$); 7.59 (1H, s), 7.45—7.25 (24H, m), 7.15—7.05 (6H, m), 6.91 (1H, s), 6.50 (1H, d, $J$=9.1 Hz), 5.76 (1H, dd, $J$=4.8, 9.1 Hz), 5.62 (1H, d, $J$=6.0 Hz), 5.20 (1H, d, $J$=6.0 Hz), 4.81 (1H, d, $J$=4.8 Hz), 3.98, 3.93 (2H, ABq, $J$=13.3 Hz), 3.44, 3.32 (2H, ABq, $J$=17.5 Hz), 1.42 (9H, s) | 1788, 1710, 1697 |
| **6j** | ($CDCl_3$); 7.45 (1H, s), 7.4—7.25 (24H, m), 7.15—7.05 (6H, m), 6.98 (1H, d, $J$=9.2 Hz), 6.88 (1H, s), 5.69 (1H, dd, $J$=4.8, 9.2 Hz), 5.15 (1H, d, $J$=3.3 Hz), 4.91 (1H, d, $J$=4.8 Hz), 4.16, 4.06 (2H, ABq, $J$=13.4 Hz), 3.78, 3.57 (2H, ABq, $J$=17.4 Hz), 3.41 (1H, d, $J$=3.3 Hz) | 1788, 1725, 1692 |
| **6k** | ($CDCl_3$); 7.68 (1H, d, $J$=8.7 Hz), 7.59 (1H, s), 7.5—7.1 (10H, m), 6.91 (1H, t, $J_{HF}$=56.2 Hz), 6.90 (1H, s), 5.79 (1H, dd, $J$=4.8, 8.7 Hz), 4.99 (1H, d, $J$=4.8 Hz), 4.05 (2H, s), 3.59 (2H, s), 3.56 (2H, s) | 1785, 1690 |

*a*) KBr.

TABLE IV. $^1$H-NMR and IR Spectral Data for **2a—k**

| Compd. No. | $^1$H-NMR (Solvent, $\delta$) | IR (KBr) $cm^{-1}$ (C=O) |
|---|---|---|
| **2a** | ($D_2O$); 7.96 (1H, s), 7.01 (1H, s), 5.79 (1H, d, $J$=4.7 Hz), 5.16 (1H, d, $J$=4.7 Hz), 4.22, 4.10 (2H, ABq, $J$=13.8 Hz), 3.98 (3H, s), 3.66, 3.47 (2H, ABq, $J$=17.3 Hz) | 1753, 1659 |
| **2b** | ($D_2O$); 8.02 (1H, s), 7.04 (1H, s), 5.82 (1H, d, $J$=4.8 Hz), 5.18 (1H, d, $J$=4.8 Hz), 4.57 (2H, s), 4.24, 4.12 (2H, ABq, $J$=13.8 Hz), 3.70, 3.49 (2H, ABq, $J$=17.4 Hz) | 1760, 1655 |
| **2c** | ($D_2O$); 7.98 (1H, s), 7.02 (1H, s), 5.84 (1H, d, $J$=4.8 Hz), 5.18 (1H, d, $J$=4.8 Hz), 4.65 (1H, q, $J$=7.0 Hz), 4.23, 4.11 (2H, ABq, $J$=13.8 Hz), 3.67, 3.48 (2H, ABq, $J$=17.2 Hz), 1.46 (3H, d, $J$=7.0 Hz) | 1760, 1655 |
| **2d** | ($D_2O$ + $NaHCO_3$); 8.01 (1H, s), 6.99 (1H, s), 5.82 (1H, d, $J$=4.9 Hz), 5.19 (1H, d, $J$=4.9 Hz), 4.24, 4.12 (2H, ABq, $J$=13.9 Hz), 3.69, 3.50 (2H, ABq, $J$=17.4 Hz), 1.50 (3H, s), 1.48 (3H, s) | 1768, 1670 |
| **2e** | ($D_2O$ + $NaHCO_3$); 8.00 (1H, s), 7.21 (1H, s), 5.87 (1H, d, $J$=4.8 Hz), 5.32 (1H, d, $J$=1.7 Hz), 5.173 (1H, d, $J$=4.8 Hz), 5.168 (1H, d, $J$=1.7 Hz), 4.23, 4.11 (2H, ABq, $J$=13.9 Hz), 3.68, 3.48 (2H, ABq, $J$=17.4 Hz) | 1766, 1660 |
| **2f** | ($D_2O$ + $CD_3OD$ + $NaHCO_3$); 7.92 (1H, s), 6.49 (1H, s), 5.65 (1H, d, $J$=4.7 Hz), 5.06 (1H, d, $J$=4.7 Hz), 4.20, 4.08 (2H, ABq, $J$=13.8 Hz), 3.60, 3.43 (2H, ABq, $J$=17.4 Hz), 3.57 (2H, s) | 1759, 1664 |
| **2g** | (DMSO-$d_6$); 9.81 (1H, d, $J$=8.2 Hz), 8.1—7.9 (1H, br s), 7.87 (1H, s), 7.40 (2H, s), 5.68 (1H, dd, $J$=4.6, 8.2 Hz), 5.19 (1H, d, $J$=4.6 Hz), 4.44 (2H, s), 3.83 (2H, s) | 1764, 1660 |
| **2h** | ($D_2O$ + $NaHCO_3$); 7.89 (1H, s), 7.45—7.3 (5H, m), 5.60 (1H, d, $J$=4.7 Hz), 5.02 (1H, d, $J$=4.7 Hz), 4.19, 4.06 (2H, ABq, $J$=13.8 Hz), 3.69, 3.65 (2H, ABq, $J$=14.8 Hz), 3.55, 3.39 (2H, ABq, $J$=17.2 Hz) | 1775, 1661 |
| **2i** | ($D_2O$); 8.07 (1H, s), 7.54 (5H, s), 5.66 (1H, d, $J$=4.4 Hz), 5.27 (1H, s), 5.13 (1H, d, $J$=4.4 Hz), 4.31, 4.25 (2H, ABq, $J$=14.2 Hz), 3.67, 3.46 (2H, ABq, $J$=17.1 Hz) | 1763, 1690 |
| **2j** | ($D_2O$ + $NaHCO_3$); 8.02 (1H, s), 7.46 (5H, s), 5.62 (1H, d, $J$=4.8 Hz), 5.27 (1H, s), 5.07 (1H, d, $J$=4.8 Hz), 4.21, 4.10 (2H, ABq, $J$=13.8 Hz), 3.62, 3.39 (2H, ABq, $J$=17.4 Hz) | 1770, 1673 |
| **2k** | ($D_2O$ + $NaHCO_3$); 8.01 (1H, s), 7.09 (1H, t, $J_{HF}$=55.4 Hz), 5.66 (1H, d, $J$=4.6 Hz), 5.11 (1H, d, $J$=4.6 Hz), 4.24, 4.12 (2H, ABq, $J$=13.4 Hz), 3.68 (2H, s), 3.67, 3.47 (2H, ABq, $J$=17.4 Hz) | 1765, 1662 |

**Diphenylmethyl 7β-[2-(*R*)-Hydroxy-2-phenylacetamido]-3-[1(or 2)-triphenylmethyl-1(or 2)*H*-1,2,3-triazol-4-yl]thiomethylthio-3-cephem-4-carboxylate (6j)** A mixture of **7** (800 mg, 1.06 mmol), (*R*)-mandelic acid (242 mg, 1.59 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (328 mg, 1.59 mmol) was stirred at ice-bath temperature for 1 h and concentrated. EtOAc was added to the concentrate and the precipitate was filtered off. The filtrate was evaporated and purified by column chromatography on silica gel (eluent: toluene–EtOAc, 2 : 1) to yield 466 mg (50%) of **6j** as a yellow froth.

The spectral data of **6a**—**g** and **6i**—**k** are listed in Table III.

**7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)-acetamido]-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylic Acid (2d)** A solution of $AlCl_3$ (0.75 g, 5.64 mmol) in anisole (3 ml) was added dropwise to a solution of **6d** (822 mg, 0.706 mmol) in anisole (3 ml) and nitromethane (12 ml) at −30——40 °C. The mixture was stirred at the same temperature for 1 h, then 5.7 ml of 1 N HCl, water and EtOAc were added to the mixture. The aqueous layer was separated and the organic layer was re-extracted with water. The combined aqueous layer was chromatographed on a Diaion HP-20 column (eluent: methanol–water 4 : 1). After concentration, the resulting precipitate was collected by filtration, washed with EtOAc and dried *in vacuo* to give 299 mg (71%) of **2d** as a pale yellow powder.

Compounds **2a** and **2e**—**g** were similarly prepared from **6a** and **6e**—**g** according to the procedure described for the preparation of **2d**.

**Disodium 7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-carboxymethoxyiminoacetamido]-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylate (2b)** A solution of **6b** (1.04 g, 0.749 mmol) in 98% formic acid (16 ml) and water (0.8 ml) was stirred at room temperature for 3 h and evaporated. The resulting residue was triturated and rinsed with ether to give 572 mg of a yellowish white powder. A solution of $AlCl_3$ (0.50 g, 3.76 mmol) in anisole (2 ml) was added dropwise to a suspension of the above powder in anisole (2 ml) and nitromethane (8 ml) at −30——40 °C. The mixture was stirred at the same temperature for 1 h, then 3.8 ml of 1 N HCl, water and EtOAc were added. The aqueous layer was separated and the organic layer was re-extracted with water. The combined aqueous layer was chromatographed on a Diaion HP-20 column (eluent: methanol–water, 4 : 1). After concentration, the resulting precipitate was collected by filtration and washed with EtOAc to give 170 mg of a pale yellow powder. This powder was dissolved in diluted aqueous $NaHCO_3$, re-purified by column chromatography on a Diaion CHP-20P resin (eluent: water) and freeze-dried to give 121 mg (26%) of **2b** as a white powder.

Compound **2c** was prepared from **6c** as described for the preparation of **2b**.

**7β-Difluoromethylthioacetamido-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-3-cephem-4-carboxylic Acid (2k)** A suspension of **6k** (451 mg, 0.710 mmol) in $CH_2Cl_2$ (3 ml) was treated with anisole (1.2 ml) and trifluoroacetic acid (3.0 ml) under ice-cooling. After being stirred at ice-bath temperature for 1 h, the reaction mixture was evaporated. The resulting residue was triturated with $Et_2O$ to give 257 mg (77%) of **2k** as a white powder.

Compounds **2h**—**j** were similarly prepared from **6h**—**j** according to the procedure described for the preparation of **2k**.

The spectral data of **2a**—**k** are listed in Table IV.

**Determination of Antibacterial Activity** All the *in vitro* antibacterial activities are given as MIC in μg/ml required to prevent growth of the bacterial culture. MICs were determined by the serial agar dilution method (Sensitivity Disk Agar-N) after incubation at 37 °C for 18—20 h with an inoculum size of about $10^6$ cells/ml.

**Oral Absorption Study** Male ICR-strain mice aged 6 weeks, weighing 24—30 g, were used in groups of 5. The antibiotics were given to mice orally at a single dose of 40 mg/kg or subcutaneously at 20 mg/kg as an aqueous solution, or if necessary, as a solution in dilute aqueous sodium bicarbonate. Plasma samples were collected at 0.25 and 2 h after dosing and urine specimens were collected over a period of 2 h after dosing. The concentrations of the test compounds were determined by the band culture method using *Micrococcus luteus* ATCC 9341 or *Escherichia coli* 7437 as a test organism and Trypto-soy agar as the test medium.

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