SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 6315-S, A NEW MEMBER OF THE OXACEPHEM ANTIBIOTIC

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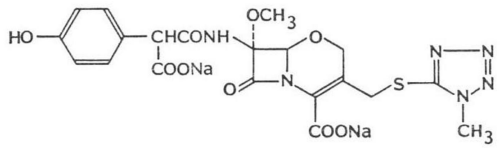
The synthesis and *in vitro* activity of 7β-difluoromethylthioacetamido-7α-methoxy-3-[[1-(hydroxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-1-oxa-3-cephem-4-carboxylic acid sodium salt, 6315-S, are described. 6315-S shows good antibacterial activity against Gram-positive and Gram-negative bacteria, being especially highly active against clinical isolates of *Staphylococcus aureus* resistant to either ampicillin or methicillin. The structure-activity relationship of related 1-oxa and 1-thia cephems is also presented.

The successful use of 1-oxacephalosporins obtainable from penicillins on an industrial scale[1] has brought on to the market latamoxef (moxalactam) (**1**). Though exhibiting four to eight-fold higher antibacterial activity than the corresponding 1-thia congener[2], **1** shows a rather weak activity against Gram-positive bacteria and, in addition, has a disulfiram-like action likely to be due to the presence of 1-methyltetrazol-5-ylthio group at the 3′-position. In fact, several β-lactam antibiotics have been reported to induce disulfiram (antabuse)-like actions, which typically consist of marked flushing, vomiting and other symptoms[3]. The reaction is believed to result from an accumulation of acetaldehyde caused by an inhibition of aldehyde dehydrogenase.

In order to overcome these biological disadvantages, we have made an extensive study of chemical modifications of the 7β-acyl moiety as well as the 3′-substituent in 7α-methoxy-1-oxacephalosporins. As a result, 6315-S (**2**), 7β-difluoromethylthioacetamido-7α-methoxy-3-[[1-(hydroxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-1-oxa-3-cephem-4-carboxylic acid sodium salt[4], has been selected as a new member of the oxacephem antibiotics, which has a broad and balanced antibacterial spectrum from aerobic to anaerobic bacteria and no disulfiram-like actions.

Chemistry

Difluorocarbene is readily generated *in situ* by base treatment of chlorodifluoromethane (Freon 22), a non-toxic and inexpensive gas, and captured effectively with mercaptanes giving the corresponding difluoromethylsulfides[5]. Thus, difluoromethylthioacetic acid (**4**) necessary for preparation of the 7β-substituent of **2** was obtained in good yield by the reaction of Freon 22 with ethyl thioglycolate in the presence of ethanolic sodium ethoxide, followed by basic hydrolysis.



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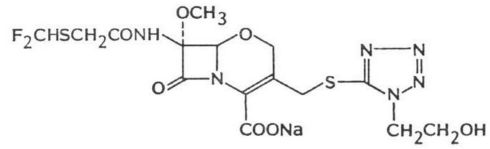

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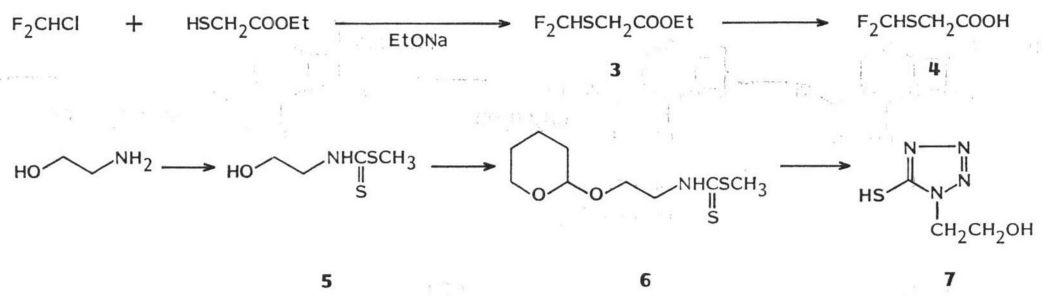
Scheme 1.



While the preparation of 1-hydroxyethyl-1*H*-tetrazole-5-thiol (**7**), necessary for substitution at C-3′, has been already reported in the patent literature[6], the yield was found not to be satisfactory to us. We have found that the yield is improved by *O*-protection of hydroxyethyldithiocarbamate (**5**), which was prepared by reaction of ethanolamine with carbon disulfide in the presence of triethylamine and subsequent methylation with methyl iodide. The tetrahydropyranyl ether (**6**), prepared by the usual acid-catalyzed etherification, was subjected to ring-closure reaction with aqueous sodium azide and acidic hydrolysis affording the tetrazolyl mercaptan (**7**) in an overall yield of 85% from ethanolamine.

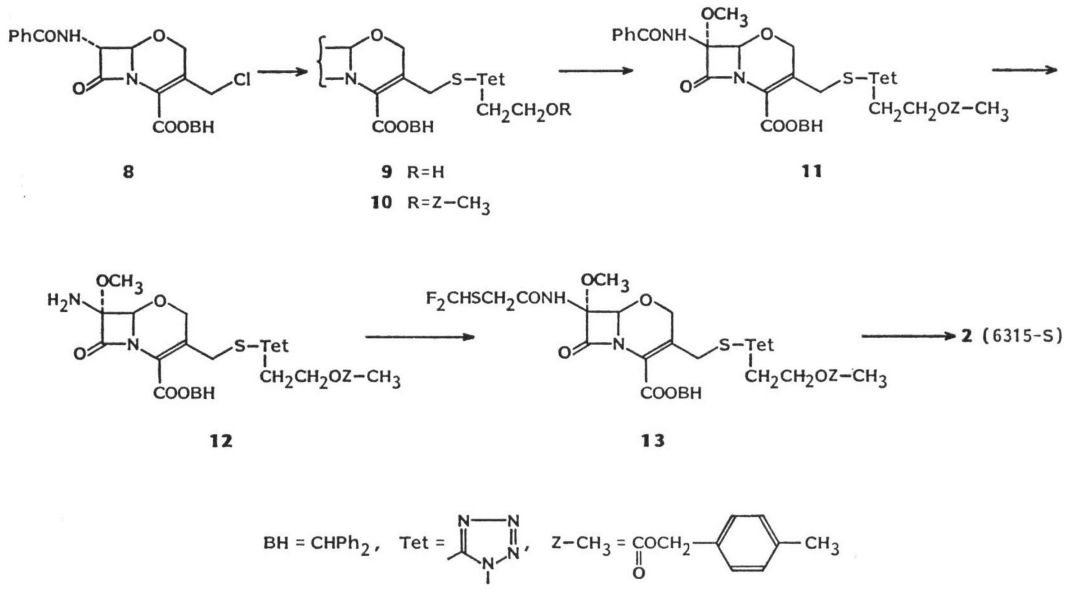
7α-Benzamido-3-chloromethyl-1-oxacephem (**8**), an important intermediate for production of **1**[7], reacted with **7** in the presence of sodium methoxide in *N*,*N*-dimethylformamide (DMF) giving the 3-[1-(hydroxyethyl)-1*H*-tetrazol-5-yl]thiomethyloxacephem (**9**) in quantitative yield. Prior to the following reaction, the hydroxyl function in the tetrazole moiety was protected with a *p*-methylbenzyloxycarbonyl group to prevent undesirable side-reactions. Compound **10** thus obtained was subjected to the usual methoxylation reaction with a combination of *tert*-butyl hypochlorite and lithium methoxide affording the inverted 7β-benzamido-1-oxacephamycin derivative (**11**) in good yield[8,9]. The side-chain cleavage with phosphorous pentachloride (PCl_5) proceeded smoothly to give the methoxy-amine (**12**) as crystals in a reasonable yield. The subsequent acylation with the acid (**4**) was achieved in the presence of phosphoryl chloride-pyridine affording **13** in good yield.

The final step for removal of the protecting groups caused a serious problem; application of our deblocking procedure using a combination of aluminum chloride ($AlCl_3$) and anisole[10] brought about predominant substitution of the fluorine atoms with anisole. This finding can be explained by the strong affinity of the fluorine atom to $AlCl_3$. Fortunately, after trials with a variety for Lewis acids, smooth deprotection of the two protecting groups was realized when stannic chloride ($SnCl_4$) was used at low temperature in place of $AlCl_3$, the fluorine atoms remaining unattacked. The free acid of **2** thus obtained was converted to the sodium salt by neutralization with aqueous sodium bicarbonate followed by freeze-drying to furnish a pure sample for biological evaluation.

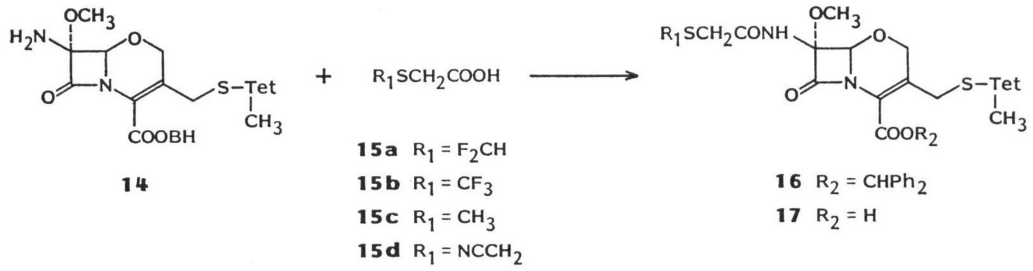
In order to elucidate the effect of the closely related alkylthio moiety on antibacterial properties, some 1-oxacephamycins possessing the 1-methyl-1*H*-tetrazol-5-ylthiomethyl substituent at C-3 position were prepared according to the known reaction sequence[7] as shown in Scheme 3, which consisted of acylation of the methoxyamine (**14**) with the respective alkylthioacetic acids (**15a**~**d**) and the final deprotection affording the acids (**17a**~**d**).

Attention was next directed to preparation of the corresponding 1-thia congener (**18**) of **2** for comparison of biological activity between the 1-oxa and 1-thia derivatives. The 1-thia congener (**18**) was prepared starting from 7β-formylamino-3-bromomethyl-3-cephem-1-oxide (**19**)[11] as shown in Scheme 4.

Scheme 2.



Scheme 3.



Replacement of the bromide **19** with the thiol **7** followed by protection of the hydroxyl function with *p*-methylbenzyloxycarbonyl group yielded the compound **20**. After reduction of the sulfoxide group and successive elimination of the formyl group by alkaline hydrolysis, the amine **21** was subjected to the modified Sankyo procedure[12] *via* a Shiff base affording the methoxy-amine **22**. Acylation with **4** and the final deprotection were achieved as described earlier to give **18**.

Antibacterial Activity

Table 1 shows the MICs of the structurally related 7β-alkylthioacetamido-7α-methoxy-1-oxacephalosporins possessing a 1-methyltetrazolylthio group at C-3′, a representative substituent in the β-lactam chemistry. Compound **17a** possesses high activity comparable to that of **17b** or **17d** having the side-chain of cefazaflur[13] or cefmetazole[14] at C-7, but is twice as active against ampicillin-resistant *Staphylococcus aureus* C-14 as **17b** and **17d**. It is worth noting that the substitution of methyl hydrogens of **17c** with electron-withdrawing and hydrophobic fluorine atoms brings about an enhancement of antibacterial activity against the Gram-positive and Gram-negative bacteria.

Table 2 presents the antibacterial shift by S→O replacement in the 7β-difluoromethylthioacetamido derivatives. In conformity with the favorable S→O shift between latamoxef (**1**) and its 1-thia congener

Scheme 4.

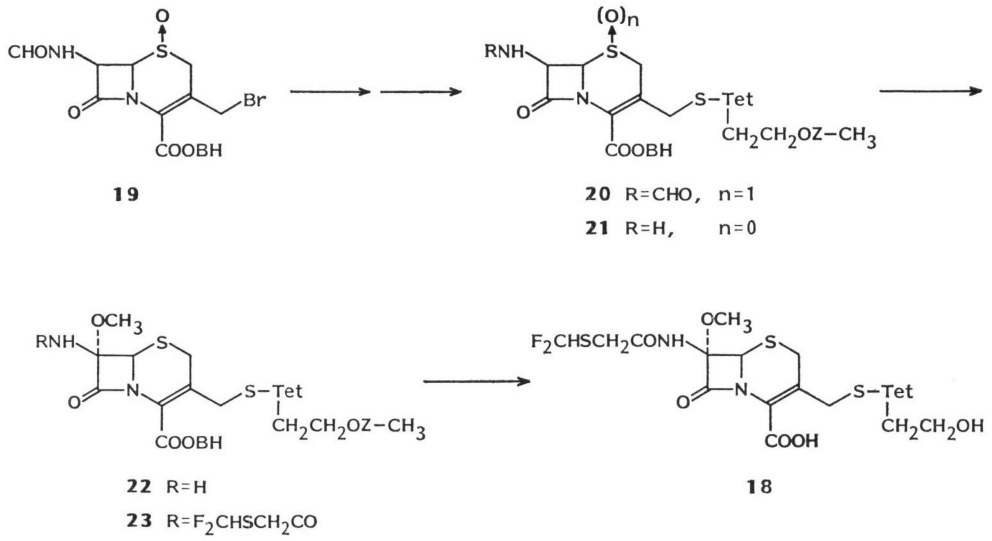
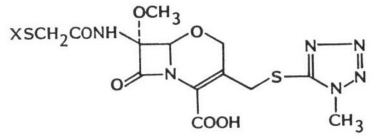


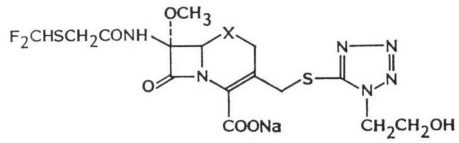
Table 1. Comparative activity (MIC μg/ml) of alkylthio-1-oxacephamycins.



17a X=F_2CH
17b X=CF_3
17c X=CH_3
17d X=$NCCH_2$

Compound	*Staphylococcus aureus* JC-1	*S. aureus* C-14 (Amp-R)	*Streptococcus pyogenes* C-203	*Escherichia coli* JC-2	*E. coli* 73 (R)	*Proteus vulgaris* CN-329	*Serratia marcescens* 13880
17a	0.2	0.2	0.2	0.05	0.1	0.4	1.6
17b	0.2	0.4	0.2	0.1	0.1	0.2	0.8
17c	0.4	0.8	0.4	0.4	0.4	1.6	6.3
17d	0.2	0.4	0.2	0.2	0.2	0.8	1.6

Table 2. S→O shift of MIC (μg/ml) in difluoromethylthioacetamido derivatives.



18 X=S
2 X=O

Compound	*S. aureus* JC-1	*S. pyogenes* C-203	*E. coli* JC-2	*E. coli* 73 (R)	*P. vulgaris* CN-329	*S. marcescens* 13880
18	0.8	0.8	0.8	1.6	1.6	6.3
2	0.2	0.4	0.1	0.1	0.4	0.8

having a phenylmalonyl side chain[2], **2** was about four times as active as the 1-thia congener (**18**).

Table 3 summarizes the *in vitro* antibacterial activity of **2** and other β-lactam antibiotics currently used clinically.

As can be seen from Table 3, 6315-S (**2**) possesses a broad antibacterial spectrum including many

Table 3. Comparative activity (μg/ml) of 6315-S (**2**) and other antibiotics in clinical use.

Organism	6315-S	LMOX	CTX	CTM	CMZ	CEZ	PIPC
Staphylococcus aureus FDA 209P JC-1	0.2	6.3	1.6	0.4	0.8	0.2	0.4
S. aureus ATCC 25923	0.2	3.1	0.8	0.4	0.8	0.2	0.4
S. aureus C-14 (Amp-R)	0.4	6.3	1.6	0.8	1.6	0.4	6.3
S. aureus 3131 (Meth-R)	1.6	25	25	6.3	6.3	12.5	>100
S. epidermidis ATCC 14990	0.4	6.3	0.4	0.4	1.6	0.2	0.2
Streptococcus pyogenes C-203	0.4	1.6	≦0.01	0.1	0.8	0.1	0.05
S. pneumoniae Type I	0.1	0.8	0.02	0.2	0.4	0.1	0.02
S. faecalis	100	>100	>100	>100	>100	50	6.3
Escherichia coli NIHJ JC-2	0.1	0.1	0.1	0.2	1.6	1.6	3.1
E. coli ATCC 25922	0.1	0.2	0.1	0.2	0.8	1.6	3.1
E. coli 73 (R)	0.1	0.4	0.2	0.8	1.6	25	>100
Klebsiella pneumoniae SRL-1	0.05	0.1	0.02	0.2	0.8	1.6	0.8
Klebsiella sp. 363 (Amp-R)	0.005	0.1	0.4	12.5	0.4	>100	>100
Proteus mirabilis PR-4	0.2	0.1	0.02	0.4	1.6	3.1	0.4
P. vulgaris CN-329	0.4	0.2	0.02	0.8	1.6	50	0.4
P. rettgeri IFO 3850	0.1	0.05	≦0.01	0.05	0.8	0.4	0.4
P. morganii IFO 3848	0.4	0.1	≦0.01	0.2	1.6	25	≦0.01
Enterobacter cloacae 233	12.5	0.1	0.2	3.1	>100	>100	1.6
Citrobacter freundii IFO 12681	0.4	0.1	0.4	1.6	1.6	25	6.3
Serratia marcescens ATCC 13880	0.8	0.2	0.2	6.3	6.3	>100	1.6
Alcaligenes faecalis NCTC 655	0.1	0.1	1.6	6.3	1.6	25	1.6
Pseudomonas aeruginosa ATCC 25619	>100	6.3	1.6	>100	>100	>100	0.8

Abbreviation: LMOX latamoxef, CTX cefotaxime, CTM cefotiam, CMZ cefmetazole, CEZ cefazolin, PIPC piperacillin.

species of important Gram-positive and Gram-negative bacteria. In particular, the most noticeable feature of **2** resides in excellent anti-staphylococcal potency to remove more or less common disadvantages of the so-called third-generation β-lactam antibiotics. Moreover, **2** was highly active against ampicillin- and methicillin-resistant *S. aureus* which are noticed as recently increasing pathogens. 6315-S (**2**) exhibited activity comparable to that of latamoxef against Gram-negative bacteria though being more active against *Escherichia coli* 73 producing β-lactamase, and not active against *Pseudomonas aeruginosa*.

In summary, 6315-S (**2**) exhibits a potent and expanded antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria*.

Experimental

Melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-10 spectrophotometer or Jasco A-702 spectrophotometer. NMR spectra were recorded on a Varian T-60A or Varian EM-390 NMR spectrometer using TMS as an internal standard. The following abbreviation are used: s singlet, d doublet, t triplet, m multiplet, ABq AB quartet, bs broad singlet. Organic solvents were dried with molecular sieves 4A.

Determination of Antibacterial Activity

MIC was determined by the agar dilution method using sensitivity test agar (Eiken, Japan). An overnight culture of bacteria in Tryptosoy broth (Eiken, Japan) was diluted to about 10^6 cells/ml with the same broth and inoculated with an inoculating device onto agar containing serial two-fold dilutions

* It has been found that 6315-S (**2**) is devoid of the disulfiram-like action in a rat model system[4]. Details will be published elsewhere.

of an antibiotic. Organisms were incubated at 37°C for 18~20 hours. The MIC of an antibiotic was defined as the lowest concentration that inhibited visible growth.

Ethyl Difluoromethylthioacetate (**3**)

To a sodium ethoxide solution prepared from sodium (25.3 g) and anhydrous ethanol (700 ml) was added ethyl thioglycolate (109.6 ml), and Freon 22 was rapidly bubbled into the solution at room temperature. When the reaction temperature rose to 60°C, introduction of the gas was made slow and continued for 2 hours at about 40°C. After neutralization of the solution with conc hydrochloric acid (9 ml), ethanol was evaporated under reduced pressure. To the residue, ethyl acetate (1,000 ml), 1 N sodium hydroxide (50 ml) and ice water were added. The separated aqueous layer was further extracted with ethyl acetate (500 ml). The combined extracts were washed with water and saturated sodium chloride solution and dried with magnesium sulfate. Removal of the solvent and vacuum distillation of the remaining liquid gave the acetate (**3**) (119.8 g, 70.5%) boiling at 46~48.5°C/3 mmHg; IR (film) cm^{-1} 1740 (br); NMR ($CDCl_3$) δ 1.30 (3H, t, J=7.0 Hz), 4.23 (2H, q, J=7.0 Hz), 3.55 (2H, s), 6.97 (1H, t, J_{HF}=56 Hz).

Difluoromethylthioacetic Acid (**4**)

A mixture of ethyl difluoromethylthioacetate (381.4 g) and 25% aqueous potassium hydroxide (491 ml) was vigorously stirred under ice-cooling over a period of 2 hours to become gradually homogeneous. The reaction solution was mixed with ethyl acetate (300 ml) and saturated aqueous sodium chloride (100 ml). The separated organic layer was washed with water (100 ml), and the aqueous layer was extracted with ethyl acetate (300 ml). The combined aqueous layers were mixed with ethyl acetate (1.6 liters), acidified with conc hydrochloric acid (255 ml) and saturated with sodium chloride (270 g). The organic layer was washed with saturated aqueous sodium chloride, and dried over magnesium sulfate. Evaporation under reduced pressure gave the acid (**4**) (309.3 g, 97.1%) which can be used in subsequent reaction without further purification; IR (film) cm^{-1} 1720 (br); NMR ($CDCl_3$) δ 3.59 (2H, s), 6.92 (1H, t, J_{HF}=56 Hz), 8.42 (1H, s).

Methyl *N*-(2-Hydroxyethyl)dithiocarbamate (**5**)

To a solution of ethanolamine (30.54 g) in ethanol (370 ml) and water (30 ml) were added triethylamine (60.6 g) and carbon disulfide (45 g) at 15°C. After stirring for 1 hour, methyl iodide (80 g) was added to the reaction solution at 15°C. After being stirred for 30 minutes, the reaction mixture was concentrated under reduced pressure, diluted with water (350 ml) and hexane and left for a while to separate the aqueous layer, which was acidified with phosphoric acid (1.5 ml). Extraction with ethyl acetate under salt-out condition and evaporation of the extract gave **5** (81.7 g) as an oil containing small amounts of solvents, which was subjected to the next reaction without further purification; NMR ($CDCl_3$) δ 2.63 (3H, s), 2.73~3.08 (1H, m), 3.60~4.17 (4H, br s), 7.50~8.17 (1H, m).

Methyl *N*-[2-(2-Tetrahydropyranyl)oxyethyl]dithiocarbamate (**6**)

To a solution of **5** (81.7 g) in methylene chloride (300 ml) were added dihydropyran (54 g) and *p*-toluenesulfonic acid monohydrate (1.0 g). The mixture was stirred for 1 hour, washed with aqueous $NaHCO_3$ and evaporated to give **6** (129 g) as a raw product; IR ($CHCl_3$) cm^{-1} 3360; NMR ($CDCl_3$) δ 1.33~2.00 (6H, m), 2.63 (3H, s), 3.47~4.17 (6H, s), 4.33~4.67 (1H, m), 7.50~8.33 (1H, m).

1-Hydroxyethyl-1*H*-tetrazole-5-thiol (**7**)

An aqueous solution (20 ml) of sodium azide (3.40 g) was added to a solution of **6** (11.8 g) in ethanol (40 ml). After being refluxed for 2.5 hours, the reaction mixture was concentrated under reduced pressure, and the residue dissolved in water, extracted with ethyl acetate, acidified with phosphoric acid and extracted with ethyl acetate. The extract was washed with water, dried and concentrated to give the pyranyl ether of **7** (10.4 g). The pyranyl ether was dissolved in aqueous acetone and acidified with conc hydrochloric acid to pH 2. The mixture was kept at room temperature for 2 hours, concentrated and extracted with ethyl ether. The extract was dried and concentrated. The residue was crystallized from a mixture of ethyl acetate and hexane to give **7**; mp 135~137°C (6.2 g, 85%); NMR (DMSO-d_6) δ 3.82 (2H, t, J=5.8 Hz), 4.28 (2H, t, J=5.8 Hz).

Diphenylmethyl 7α-Benzamido-3-[[1-(hydroxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-1-oxa-3-cephem-4-carboxylate (**9**)

To a solution of **7** (977 mg) in DMF (5 ml) was added methanolic 5.2 N sodium methoxide solution (1.3 ml) at $-20°C$. The resulting solution was added to a solution of diphenylmethyl 7α-benzamido-3-chloromethyl-1-oxa-3-cephem-4-carboxylate (**8**) (2.80 g) in DMF (19 ml) at $-20°C$. After being stirred for 2 hours, the reaction mixture was poured into ice water and extracted with ethyl acetate. The extract was washed with water, dried with sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel to give **9** (3.7 g, 108%) as a foam containing small amounts of solvents; IR ($CHCl_3$) cm^{-1} 1788, 1720, 1668; NMR ($CDCl_3$) δ 3.10 (1H, br s, OH), 3.87 (2H, br s, C3′-H_2), 4.13 (4H, br s, N-CH_2CH_2-O), 4.49 (2H, br s, C2-H_2), 4.79 (1H, d, J=7 Hz, C7-H), 5.04 (1H, s, C6-H), 6.37 (1H, s, $CHPh_2$), 7.10~7.90 (16H, m, aromatic H and amide H).

Diphenylmethyl 7α-Benzamido-3-[[1-(*p*-methylbenzyloxycarbonyloxyethyl)-1*H*-tetrazol-5-yl]-thiomethyl]-1-oxa-3-cephem-4-carboxylate (**10**)

A solution of *p*-methylbenzyl chloroformate (3.4 g) in methylene chloride (2 ml) was added to a mixture of **9** (3.38 g), pyridine (3.6 ml) and methylene chloride (3 ml) at $-20°C$. The resulting mixture was stirred for 5 hours and poured into cooled water. The usual work-up followed by chromatography on silica gel gave **10** (3.2 g, 82.6% yield from **7**) as a foam; IR ($CHCl_3$) cm^{-1} 1785, 1745, 1672; NMR ($CDCl_3$) δ 2.31 (3H, s, CH_3), 4.20 (2H, br s, C3′-H_2), 4.40 (4H, br s, N-CH_2CH_2-O), 4.38 (1H, d, J=7 Hz, C7-H), 4.54 (2H, br s, C2-H_2), 5.07 (3H, s, C6-H and CH_2-Ph), 6.92 (1H, s, $CHPh_2$), 7.05~7.93 (20H, m, aromatic H and amide H).

Diphenylmethyl 7β-Benzamido-7α-methoxy-3-[[1-(*p*-methylbenzyloxycarbonyloxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-1-oxa-3-cephem-4-carboxylate (**11**)

A solution of **10** (1.0 g) in methylene chloride (10 ml) was cooled to $-55°C$ and successively treated with *tert*-butyl hypochlorite (192 μl) and 2 N lithium methoxide in methanol (0.92 ml). The resulting mixture was stirred for 15 minutes, quenched with acetic acid (0.8 ml), poured into ice water and extracted with ethyl acetate. The usual work-up followed by chromatography on silica-gel afforded **11** (770 mg, 74%) as a foam; IR ($CHCl_3$) cm^{-1} 1785, 1745, 1685; UV λ_{max}^{EtOH} nm (ε) 282 (10,550); NMR ($CDCl_3$) δ 2.30 (3H, s, Ph-CH_3), 3.60 (3H, s, OCH_3), 4.20 (2H, s, C3′-H_2), 4.37 (4H, br s, N-CH_2CH_2-O), 4.56 (2H, s, C2-H_2), 5.10 (2H, s, CH_2-Ph), 5.13 (1H, s, C6-H), 6.89 (1H, s, $CHPh_2$), 6.95~7.97 (20H, m, aromatic H and amide H).

Anal Calcd for $C_{41}H_{38}N_6O_6S \cdot \frac{1}{4}H_2O$: C 61.92, H 4.88, N 10.57, S 4.03.
Found: C 61.83, H 4.92, N 10.31, S 3.93.

Diphenylmethyl 7β-Amino-7α-methoxy-3-[[1-(*p*-methylbenzyloxycarbonyloxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-1-oxa-3-cephem-4-carboxylate (**12**)

To a solution of **11** (500 mg) in methylene chloride (10 ml) were added successively pyridine (112 μl) and PCl_5 (263 mg) at ice-bath temperature. The mixture was warmed to room temperature and stirred for 2.5 hours. After cooling to $-20°C$ and addition of methanol (5 ml), the reaction solution was further stirred for 3 hours at 2~3°C and poured into a cooled aqueous sodium bicarbonate. Extraction with ethyl acetate and the usual work-up afforded **12** (326 mg, 75%) as crystals (mp 135~138.5°C), which was somewhat sensitive to air; IR ($CHCl_3$) cm^{-1} 1780, 1745; NMR ($CDCl_3$) δ 2.17 (2H, br s, NH_2), 2.31 (3H, s, Ph-CH_3), 3.50 (3H, s, OCH_3), 4.24 (2H, s, C3′-H_2), 4.41 (4H, br s, N-CH_2CH_2-O), 4.58 (2H, d, J=3 Hz, C2-H_2), 4.84 (1H, s, C6-H), 5.05 (2H, s, CH_2-Ph), 6.92 (1H, s, $CHPh_2$), 7.05~7.7 (14H, m, aromatic H).

Diphenylmethyl 7β-Difluoromethylthioacetamido-7α-methoxy-3-[[1-(*p*-methylbenzyloxycarbonyl-oxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-1-oxa-3-cephem-4-carboxylate (**13**)

To an ice-cooled suspension of **12** (1.0 g) in methylene chloride, pyridine (0.52 g) and **3** (0.17 g) were added. After cooling to $-15°C$, phosphoryl chloride (0.25 g) was added slowly. The resulting mixture was stirred for 20 minutes at -17~$-15°C$, poured into ice water and extracted with methylene chloride. The extract was washed three times with water, dried with sodium sulfate and evaporated

under reduced pressure. Trituration of the residue with ethyl ether gave **13** (935 mg, 83%) as crystals, mp 80~82°C; IR ($CHCl_3$) cm^{-1} 1787, 1746, 1708; UV λ_{max}^{EtOH} nm (ε) 282.5 (10,500); NMR ($CDCl_3$) δ 2.30 (3H, s, Ph-CH_3), 3.53 (5H, s, OCH_3 and S-CH_2-CO), 4.20 (2H, s, C3′-H_2), 4.38 (4H, s, N-CH_2CH_2-O), 4.57 (2H, s, C2-H_2), 5.02 (3H, s, C6-H and O-CH_2-Ph), 6.87 (1H, s, $CHPh_2$), 6.87 (1H, t, J_{HF}=56 Hz), 7.03~7.63 (15H, m, aromatic H and amide H).

Anal Calcd for $C_{36}H_{34}N_6O_9S_2F_2$: C 54.81, H 4.48, N 10.36, F 4.69.
Found: C 54.69, H 4.47, N 10.36, F 4.99.

Sodium 7β-Difluoromethylthioacetamido-7α-methoxy-3-[[1-(hydroxyethyl)-1*H*-tetrazol-5-yl]-thiomethyl]-1-oxa-3-cephem-4-carboxylate (**2**, 6315-S)

To a mixture of **13** (405 mg), methylene chloride (2.5 ml) and nitromethane (0.5 ml) was added a solution of anisole (0.11 ml) and $SnCl_4$ (0.17 ml) in methylene chloride (2 ml) at −30°C. The stirred mixture was gradually warmed to −10°C over a period of 3.5 hours and poured into a mixture of 1 N hydrochloric acid, ethyl acetate and methyl ethyl ketone. The separated organic layer was mixed with aqueous sodium bicarbonate, and the aqueous layer was acidified with conc hydrochloric acid and extracted with a mixed solvent of ethyl acetate and methyl ethyl ketone. The extract was washed with brine, dried with magnesium sulfate and evaporated to give a foamy residue, which was crystallized from acetone and methylene chloride to give the acid of **2** (260 mg, 100%) as crystals (mp 82.5~87.5°C); IR (KBr) cm^{-1} 1780~1790, 1710, 1680; NMR (DMSO-d_6) δ 3.42 (3H, s, OCH_3), 3.63 (2H, s, S-CH_2-CO), 3.75 (2H, t, J=6 Hz, CH_2-CH_2-O), 4.21 (2H, s, C2-H_2), 4.33 (2H, t, J=6 Hz, N-CH_2), 4.53 (2H, s, C3′-H_2), 5.07 (1H, s, C6-H), 7.03 (1H, t, J_{HF}=56 Hz), 9.22 (1H, s). The acid was converted into the sodium salt **2** in the usual way (neutralization with aqueous sodium bicarbonate followed by freeze-drying); IR (KBr) cm^{-1} 1766, 1687, 1610; NMR (D_2O) δ 4.00 (3H, s, OCH_3), 4.18 (2H, s, S-CH_2CO), 4.47 (2H, t, J=6 Hz, CH_2CH_2-O), 4.57 and 4.74 (2H, ABq, J=7.5 Hz, C3′-H_2), 5.01 (2H, t, J=6 Hz, N-CH_2), 5.01 (2H, s, C2-H_2), 5.13 (1H, s, C6-H), 7.58 (1H, t, J_{HF}=56 Hz).

Anal Calcd for $C_{15}H_{17}O_7N_6F_2S_2Na\cdot H_2O$: C 33.58, H 3.57, N 15.68, F 7.08.
Found: C 33.56, H 3.56, N 15.62, F 6.63.

Diphenylmethyl 7β-Formylamino-3-[[[1-(*p*-methylbenzyloxycarbonyloxyethyl)-1*H*-tetrazol-5-yl]-thiomethyl]-3-cephem-4-carboxylate (**20**)

A solution of 3-bromomethylcephem 1-oxide (**19**) (15.8 g)[11] in DMF (80 ml) was cooled to −20°C, to which **7** (15.5 g) dissolved in DMF (26 ml) and methanolic 5.2 N sodium methoxide solution (7.26 ml) were successively added. After being stirred for 1.5 hours, the reaction mixture was poured into ice water, and the product were crystallized out from the aqueous solution. The crystals were collected, washed with water and ethyl acetate and dried to give diphenylmethyl 7β-formylamino-3-[[1-(hydroxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-3-cephem-4-carboxylate 1-oxide (14.2 g, mp 133~135°C). The hydroxyl group was protected by treatment with *p*-methylbenzyl chloroformate in a similar manner as described in the synthesis of **10** giving the corresponding carbonate (20.0 g) as a powder. To a solution of the carbonate (20.0 g) in methylene chloride (500 ml) cooled to −30°C, a solution of phosphorus tribromide (3.4 ml) in methylene chloride (150 ml) was added. After stirring for 1 hour at −30°C, the reaction mixture was treated with aqueous sodium bicarbonate. The usual work-up and trituration of the crude product with ethyl ether gave **20** (14.2 g) as a powder in overall yield of 64.6%; IR ($CHCl_3$) cm^{-1} 1783, 1740, 1700 cm^{-1}; NMR ($CDCl_3$ - acetone-d_6 - DMSO-d_6) δ 2.30 (3H, s, Ph-CH_3), 3.75 (2H, s, C3′-H_2), 4.22 and 4.41 (2H, ABq, J=14 Hz, C2-H_2), 4.55 (4H, br s, N-CH_2CH_2-O), 5.05 (2H, s, CH_2-Ph), 5.06 (1H, d, J=5 Hz, C6-H), 5.95 (1H, dd, J=10 and 5 Hz, C7-H), 6.92 (1H, s, $CHPh_2$), 7.10~7.70 (15H, m, aromatic H and amide H), 8.26 (1H, s, CHO).

Diphenylmethyl 7β-Amino-3-[[1-(*p*-methylbenzyloxycarbonyloxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-3-cephem-4-carboxylate (**21**)

A suspension of **20** (14.2 g) in methanol (150 ml) and ethyl ether (150 ml) was treated with phosphoryl chloride (8.3 ml) at ice-bath temperature giving a clear solution, which was poured into ether (2 liters) to afford the hydrochloride of **21** as crystals. Neutralization of the salt with aqueous sodium bicarbonate gave **21** (11.4 g) as a powder in 83.8% yield; IR ($CHCl_3$) cm^{-1} 1780, 1750; NMR ($CDCl_3$)

δ 1.82 (2H, br s, NH_2), 2.32 (3H, s, Ph-CH_3), 3.64 (2H, s, C3′-H_2), 4.16 and 4.38 (2H, ABq, J=13 Hz, C2-H_2), 4.43 (4H, s, N-CH_2CH_2-O), 4.72 (1H, d, J=6 Hz, C6-H), 4.88 (1H, d, J=6 Hz, C7-H), 6.93 (1H, s, $CHPh_2$), 7.05~7.57 (14H, m, aromatic H).

Diphenylmethyl 7β-Amino-7α-methoxy-3-[[1-(*p*-methylbenzyloxycarbonyloxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-3-cephem-4-carboxylate (**22**)

A mixture of **21** (9.33 g), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (3.9 g), benzene (56 ml) and methylene chloride (44 ml) was refluxed for 1.5 hours with removing water using a Dean-Stark apparatus containing molecular sieves 4A. After cooling, magnesium sulfate (5 g) was added to the reaction solution. After being stirred for 1 hour, the resulting mixture was treated with nickel peroxide (9.5 g) at −20°C for 1 hour and filtered. The filtrate was diluted with methanol (100 ml), stirred for 2 hours at room temperature, allowed to stand in a refrigerator overnight and concentrated. The residue was chromatographed on silica gel [benzene - ethyl acetate (3: 1)] yielding the Schiff base (12.9 g). To the product dissolved in THF (85 ml) and methanol (335 ml), Girard T (4.58 g) and 50% aqueous acetic acid (2 ml) were added and the mixture was stirred for 2 hours at room temperature. The usual work-up and chromatography on silica gel gave **22** (3.9 g) as a powder in 26.5% yield; IR ($CHCl_3$) cm^{-1} 1780, 1748; NMR ($CDCl_3$) δ 2.23 (2H, br s, NH_2), 2.30 (3H, s, Ph-CH_3), 3.47 (3H, s, OCH_3), 3.50 (2H, s, C3′-H_2), 4.18 and 4.44 (2H, ABq, J=13 Hz, C2-H_2), 4.40 (4H, br s, N-CH_2CH_2-O), 4.27 (1H, s, C6-H), 5.03 (2H, s, CH_2Ph), 6.89 (1H, s, $CHPh_2$), 7.00~7.56 (14H, m, aromatic H).

Diphenylmethyl 7β-Difluoromethylthioacetamido-7α-methoxy-3-[[1-(*p*-methylbenzyloxycarbonyloxyethyl)-1*H*-tetrazol-5-yl]thiomethyl]-3-cephem-4-carboxylate (**23**)

To a solution of **22** (3.0 g) in methylene chloride (25 ml), were added successively pyridine (1.55 ml), **4** (0.73 g) and phosphoryl chloride (0.47 ml) at −30°C and the mixture was stirred for 1 hour. The usual work-up and chromatography on silica gel gave **23** (2.6 g) as a powder in 73.6% yield; IR ($CHCl_3$) cm^{-1} 1780, 1745; NMR ($CDCl_3$) δ 2.33 (3H, s, Ph-CH_3), 3.55 (5H, s, OCH_3 and C3′-H_2), 4.20 and 4.34 (2H, ABq, J=14 Hz, C2-H_2), 4.83 (4H, s, N-CH_2CH_2-O), 5.04 (1H, s, C6-H), 5.08 (1H, s, CH_2-Ph), 6.93 (1H, t, J_{HF}=57 Hz), 6.93 (1H, s, $CHPh_2$), 7.13~7.60 (15H, m, aromatic H and amide H).

Sodium 7β-Difluoromethylthioacetamido-7α-methoxy-3-[[1-(hydroxyethyl)-1*H*-tetrazol-5-yl]-thiomethyl]-3-cephem-4-carboxylate (**18**)

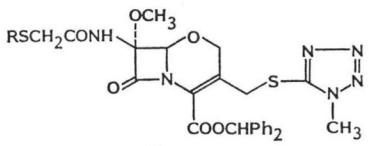
A mixture of **23** (2.56 g), methylene chloride (16 ml) and nitromethane (4 ml) was treated with anisole (670 mg) and $SnCl_4$ (1.81 ml) at −40°C. A similar work-up to that described in the deprotection of **13** gave the sodium salt **18** (1.2 g) as a powder in 72.7% yield; IR (KBr) cm^{-1} 1765, 1688; NMR (acetone-d_6) δ 3.53 (3H, s, OCH_3), 3.79 (2H, s, C3′-H_2), 3.98 (2H, s, C2-H_2), 4.45 (4H, br s, N-CH_2CH_2-O), 5.08 (1H, s, C6-H), 7.29 (1H, t, J_{HF}=57 Hz, CHF_2), 8.97 (1H, s, NH).

Anal Calcd for $C_{15}H_{17}O_6N_6S_3F_2Na \cdot H_2O$: C 32.60, H 3.46, N 15.21.
Found: C 32.47, H 3.39, N 15.18.

General Procedure for Acylation of Diphenylmethyl 7β-Amino-7α-methoxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (**14**) Followed by Deprotection

Pyridine (2.15 ml) and an alkylthioacetic acid (**15**) (6.5 mmol) were added to a suspension of the methoxyamine (**14**) (3.0 g) in methylene chloride (30 ml) under ice-cooling. To the resulting solution, phosphoryl chloride (0.6 ml) was added at −15°C. After being stirred for 20 minutes, the reaction solution was poured into ice water and extracted with methylene chloride. The extract was washed three times with water, dried with anhydrous sodium sulfate and concentrated to a small volume. Addition of an appropriate solvent (ethyl ether, methanol *etc.*) followed by filtration gave an acylated compound (**16**) as crystals or a powder in 70~85% yield. IR and ^1H NMR data of the products (**16a~d**) thus obtained are shown in Table 4. To the acylated ester (10.3 mmol) dissolved in methylene chloride (32.5 ml) were added anisole (2.2 ml) and trifluoroacetic acid (7.9 ml) under ice-cooling. The resulting solution was stirred for 1 hour, the solvent removed and the residue dissolved in 5% aqueous sodium bicarbonate. After being washed with ethyl acetate, the aqueous solution was adjusted to pH 3 with 4 N hydrochloric acid and extracted with ethyl acetate. The extract was dried and concentrated

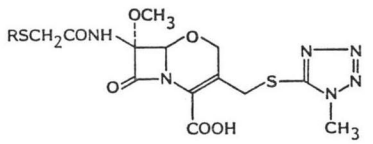
Table 4. IR and ^1H NMR data of esters **16**.



16

Compound	R	IR ($CHCl_3$) β-lactam cm^{-1}	NMR δ value ($CDCl_3$)						
			OCH_3 3H, s	NCH_3 3H, s	C3′-H_2 2H, s	C2-H_2 2H, s	C6-H 1H, s	$CHPh_2$ 1H, s	Side chain
16a	F_2CH (mp 166~167°C)	1790	3.56	3.80	4.26	4.65	5.06	6.88	3.56 (2H, s) 6.88 (1H, t, J=56 Hz)
16b	CF_3 (foam)	1790	3.55	3.80	4.25	4.63	5.07	6.90	3.70 (2H, s)
16c	CH_3 (foam)	1786	3.58	3.80	4.28	4.65	5.11	6.92	2.15 (3H, s) 3.25 (2H, s)
16d	$NCCH_2$ (powder)	1790	3.57	3.77	4.23	4.66	5.09	6.92	3.45 (4H, br s)

Table 5. IR and ^1H NMR data of acids **17**.



17

Compound	R	IR β-lactam cm^{-1}	NMR δ value						
			Solvent	OCH_3 3H, s	NCH_3 3H, s	C3′-H_2 2H, s	C2-H_2 2H, s	C6-H 1H, s	Side chain
17a	F_2CH (mp 78~85°C)	1775 (Nujol)	DMSO-d_6	3.40	3.76	4.21	4.55	5.10	3.65 (2H, s) 7.33 (1H, t, J=56 Hz)
17b	CF_3 (foam)	1784 (KBr)	CD_3OD	3.52	3.98	4.22	4.60	5.05	3.83 (2H, s)
17c	CH_3 (powder)	1783 (KBr)	CD_3OD	3.55	3.98	4.27	4.62	5.08	2.17 (3H, s) 3.22 (2H, s)
17d	$NCCH_2$ (Na salt; powder)	1781 (Nujol)	D_2O	3.49	3.98	3.97 4.23 (ABq, J=14 Hz)	4.53	5.13	3.56 (2H, s) 3.60 (2H, s)

to give crude acid **17**, which was purified by recrystallization (**17a**, from CH_2Cl_2 - acetone) or trituration with ethyl ether or CCl_4. IR and ^1H NMR data of the acids **17a~d** thus obtained are presented in Table 5.

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