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3-Trifluoromethylcephalosporins. II.[1] Synthesis and *in Vitro* Antibacterial Activities of 3-Trifluoromethylcephalosporin Derivatives

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Various acylamido derivatives of 3-trifluoromethyl-3-cephem-4-carboxylic acid were synthesized and tested for *in vitro* antibacterial activities. The 3-trifluoromethylcephalosporin derivatives synthesized herein are 7α- and 7β-acylamido-3-cephem-, 7α- methoxy-7β-acylamido-3-cephem- and 7β-acylamido-2-cephem-4-carboxylic acids. Among these compounds, the R-mandelamido derivative (IIIb) showed the highest antibacterial activity against gram-negative bacteria at pH 7.0. The minimum inhibitory concentration values of the 2-thienylacetamido- and phenylglycylamido derivatives are compared with those of the 3-methyl- and 3-chloro analogs, and structure–activity relationships are discussed.

Keywords——3-trifluoromethylcephalosporins; antibacterial activities; 7-methoxycephalosporins; structure–activity relationship; phenylglycylamidocephalosporins; MIC values

In molecular modifications of biologically active substances, the trifluoromethyl group is of great importance to medicinal chemists, since its electron-withdrawing power or lipophilicity have often been observed to affect biological activities. Interestingly, it appears that there is some similarity between the CF_3 group and the chlorine atom from a pharmacological point of view. Success by the Eli Lilly group in obtaining antibacterial 3-chlorocephalosporin derivatives, especially orally active cefachlor,[3] prompted us to synthesize 3-trifluoromethylcephalosporin derivatives. We hoped that these cephalosporin derivatives might display antibacterial activities similar to or higher than those of the corresponding chlorine analogs in view of the greater electron-withdrawing ability of the CF_3 group. An electron-withdrawing group attached to the 3-position makes the β-lactam amide bond more susceptible to nucleophilic reactions[4] and as a result enhances its antibacterial activity.[5] Table I shows that the CF_3 group has a greater Hammett σ value and higher π value (lipophilic constant)[6] than the chlorine atom. The latter parameter of CF_3 might be less favorable, since the antibacterial activity of β-lactam antibiotics against gram-negative bacteria decreases with increasing

Table I. Values of σ and π for Aromatic Substituents

	σ (*para*)	π
CF_3	0.54	0.88
Cl	0.23	0.71
CH_3	−0.17	0.56

1) Part I: T. Watanabe, Y. Kawano, T. Tanaka, T. Hashimoto, and T. Miyadera, *Chem. Pharm. Bull.*, **28**, 62 (1980).
2) Location: *1-2-58 Hiromachi, Shinagawa-ku, Tokyo, 140, Japan.*
3) R.R. Chauvette and P.A. Pennington, *J. Med. Chem.*, **18**, 403 (1975).
4) J.M. Indelicato, T.T. Norvilas, R.R. Pfeiffer, W.J. Wheeler, and W.L. Wilham, *J. Med. Chem.*, **17**, 523 (1974).
5) R.B. Hermann, *J. Antibiot.*, **26**, 223 (1973).
6) C. Hansch, A. Leo, S.H. Unger, K.H. Kim, D. Nikaitani, and E.J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).

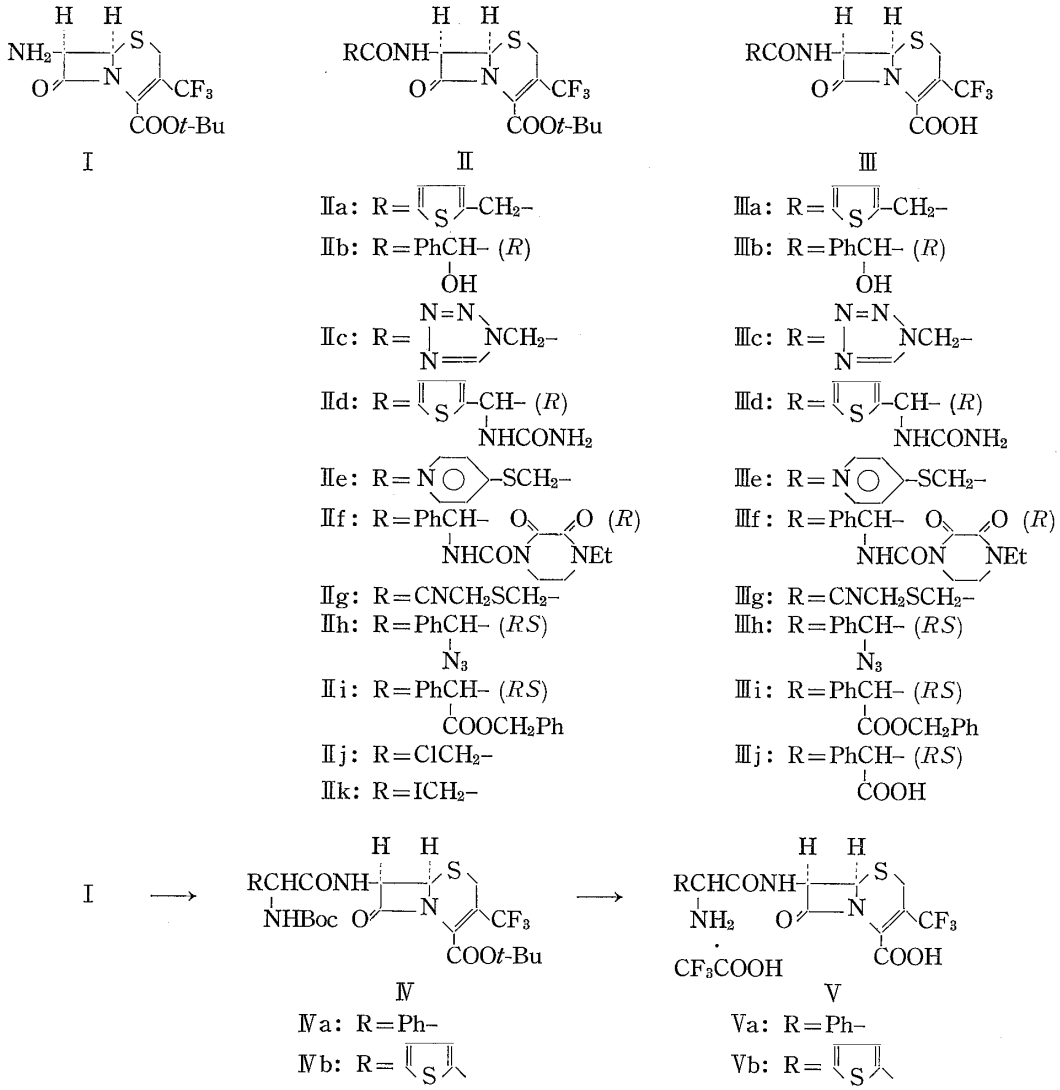


Chart 1

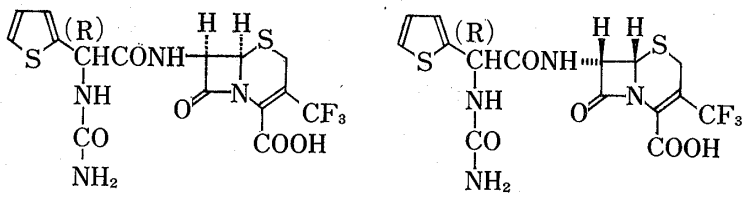
lipophilicity. The σ and π values of the methyl group are also listed in Table I for comparison.

The key intermediate (I) for 3-trifluoromethylcephalosporin synthesis[7] was prepared starting from an N-benzylideneglycine ester, as described in the preceding paper.[1] Acylation of the (±)-*cis*-7-amino-3-trifluoromethylcephalosporin derivative I was carried out with acyl chloride in the presence of a tertiary amine or a mixed anhydride prepared from a carboxylic acid and isobutyl chloroformate. The phenylglycyl- and 2-thienylglycylamido-3-trifluoromethylcephalosporins (Va and Vb) were prepared by the mixed anhydride method. The *tert*-butyl group of the acylated products, together with the protecting group of the amino group, *tert*-butoxycarbonyl, was removed by treatment with trifluoroacetic acid at room temperature. The benzyl group protecting the carboxyl group of compound IIIi was removed by catalytic hydrogenolysis over palladium black. On acylation of the (±)-*cis*-7-aminocephem I with achiral acyl chlorides or racemic acylating agents, the resulting acyl derivatives

7) Several 3-trifluoromethylcephalosporin derivatives are disclosed in a Du Pont patent (US-3919204), but no physical or biological data are given therein. A Shionogi group has independently synthesized 3-trifluoromethylcephalosporin derivatives starting from a penicillin derivative (references cited in Part I[1]).

were obtained as racemic mixtures. In addition to the chemical acylations, Arai and co-workers[8] have performed the enzymic acylation of (±)-*cis*-amino-3-trifluoromethyl-3-cephem-4-carboxylic acid (obtained by treatment of I with trifluoroacetic acid[1]) with methyl 2-thienylacetate to yield IIIa. It is noteworthy that the enzymic acylation of the 4-carboxylic acid gave rise to a single optically active (6*R*,7*R*)-form having the same configuration as naturally occurring cephalosporins. To the best of our knowledge, it was not previously known that enzymes are capable of stereospecifically acylating racemic β-lactam amino compounds.

All cephem-4-carboxylic acids possessing an optically active acyl moiety, other than the 2-ureido-2-(2-thienyl)acetamido derivative (IIId), were obtained as a 1:1 diastereomeric mixture of 6*S*,7*S*- and 6*R*,7*R*-isomers. Although the two diastereomers could not be separated by preparative thick layer chromatography (TLC), the presence of the two isomers was indicated by the nuclear magnetic resonance (NMR) spectra of the acids and their *tert*-butyl esters. The acylation of I with *R*-2-ureido-2-thienylacetyl chloride,[9] followed by deprotection of the *tert*-butyl ester, permitted chromatographic separation of the binary isomeric mixture into *R*-IIId and *S*-IIId.

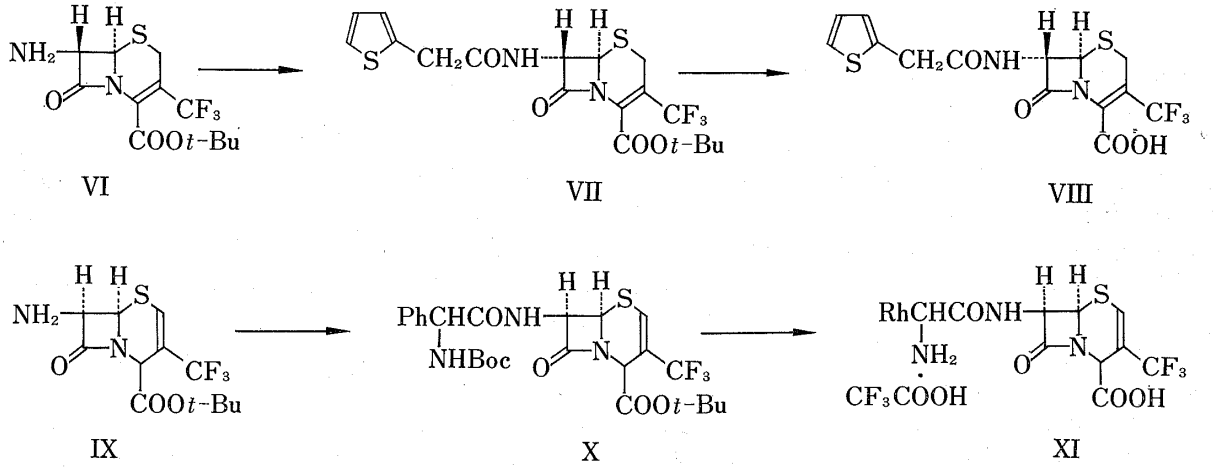


R-IIId *S*-IIId

Chart 2

The chloroacetamido derivative (IIj) was prepared by acylating I with chloroacetyl chloride as a potentially important intermediate, which might lead to a variety of acyl derivatives. The chloro derivative was converted into the more reactive iodoacetamido derivative (IIk) by treatment with sodium iodide in acetone.

Similarly, *tert*-butyl *trans*-7-amino-3-trifluoromethyl-3-cephem-4-carboxylate (VI) and *cis*-7-amino-3-trifluoromethyl-2-cephem-4-carboxylate (IX) were acylated with 2-thienylace-



VI VII VIII

IX X XI

Chart 3

8) N. Serizawa, K. Nakagawa, S. Kamimura, T. Miyadera, and M. Arai, Abstracts of Papers, Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, April, 1979, p. 362; *idem*, *J. Antibiot.*, **32**, 1016 (1979).

9) M. Nagano and S. Sugawara, Japanese patent provisional publication No. 52-122387.

tyl chloride and the phenylglycine derivative, respectively, and the acylation products (VII and X) were treated with trifluoroacetic acid to give VIII and XI. The *trans* and 2-cephem compounds were also tested for antibacterial activity and compared with the corresponding *cis* isomer IIa and 3-cephem compound Va.

7α-Methoxycephalosporin derivatives were prepared from the *trans*-7-aminocephem VI by the method of Nakao and co-workers,[10] as shown in Chart 4. The Schiff base (XII) of VI was prepared by azeotropic removal of the water formed from VI and 4-hydroxy-3, 5-di-*tert*-butylbenzaldehyde in boiling benzene. The Schiff base XII was used without purification for the subsequent oxidation with freshly prepared lead dioxide to give the imino derivative (XIII). Addition of methanol to a benzene solution containing the imino compound gave the 7β-benzylideneamino-7α-methoxycephem compound (XIV).[10] Treatment of the resulting Schiff base with Girard reagent T in methanol–benzene afforded the 7α-methoxy-7β-aminocephem derivative (XV). Acylation of XV was carried out with 2-thienylacetyl chloride and cyanomethylthioacetyl chloride in dichloroethane. The *tert*-butyl groups of these two acyl derivatives were similarly removed by treatment with trifluoroacetic acid at room temperature.

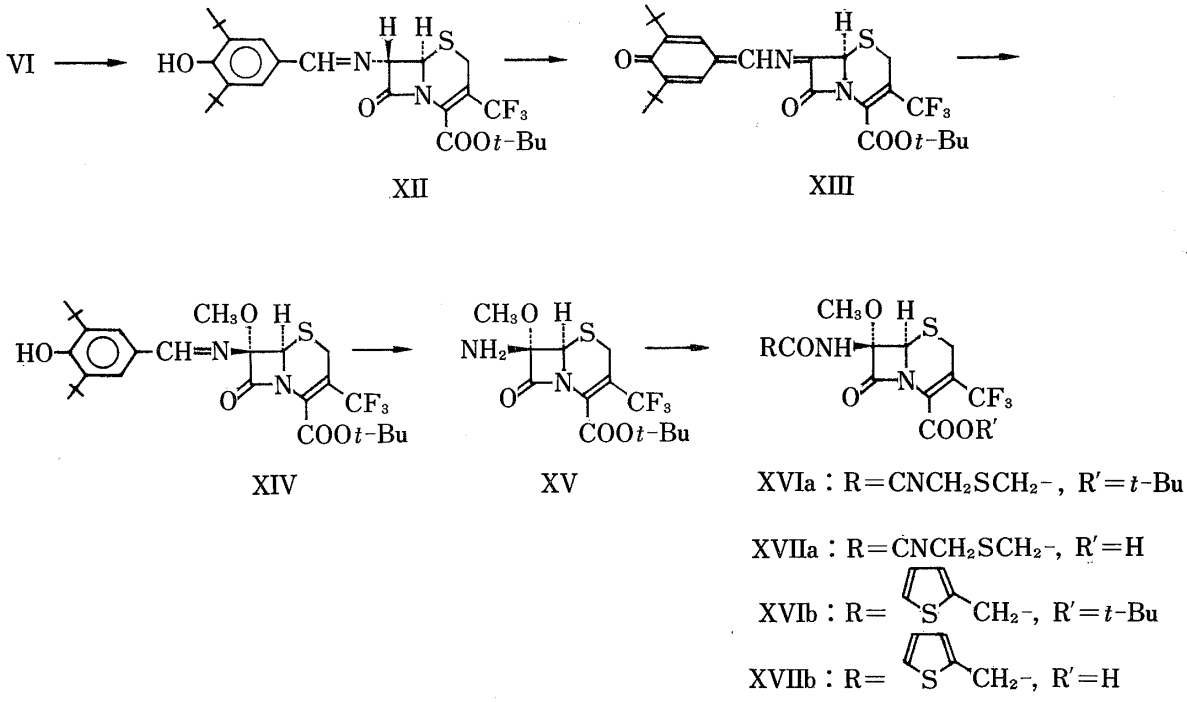


Chart 4

Antibacterial Activities and Structure-Activity Relationships

The *in vitro* antibacterial activities of the 3-trifluoromethylcephalosporin derivatives were tested by the serial agar dilution method. The minimum inhibitory concentrations (MIC) against gram-positive and gram-negative bacteria are summarized in Table II.

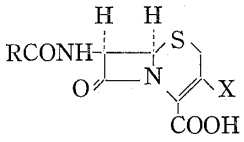
The antibacterial activity of the 2-thienylacetamido derivative IIIa prepared by chemical acylation was half that of the optically active derivative obtained by enzymic acylation.[8] Against bacteria so far examined, this compound shows higher activity than the corresponding 3-methyl analog (XVIII), and its MIC values are comparable to those of the 3-chloro analog

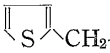
10) H. Yanagisawa, M. Fukushima, A. Ando, and H. Nakao, *Tetrahedron Lett.*, **1975**, 2705. It has been demonstrated that α-methoxylation occurs exclusively.

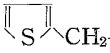
Table II. Antibacterial Activities of 3-Trifluoromethylcephalosporins
Minimum Inhibitory Concentrations (mcg/ml) at pH 7.0.

Compound	Organism								
	Staph. aureus		*Escherichia coli*		*Shigella flexneri*	*Klebsiella*		*Proteus vulgaris*	*Salmonella enteritidis* Gaertner
	209 p	56	NIHJ	609	2a	806	846		
IIIa (*RS*)	≦0.1 (≦0.1)	0.8 (0.4)	50 (6.2)	200 (>50)	50 (3.1)	6.2 (6.2)	200	3.1 (6.2)	3.1 (3.1)
IIIb (6*S*, 7*S*+6*R*, 7*R*)	0.2 (≦0.1)	1.5 (1.5)	6.2 (6.2)	>100 (>100)	6.2 (3.1)	3.1 (3.1)	100 (100)	25 (25)	0.8 (1.5)
IIIc (*RS*)	0.8 (≦0.1)	3.1 (0.8)	6.2 (1.5)	100 (25)	12.5 (3.1)	12.5 (6.2)	>100 (12.5)	100 (≦0.1)	6.2 (3.1)
R-IIId (6*R*, 7*R*)	0.4	3.1	12.5	200	6.2	3.1	>200	50	0.8
S-IIId (6*S*, 7*S*)	6.2	50	100	100	100	50	>100	>100	25
IIIe (*RS*)	≦0.1	0.8	50	>200	50	6.2	>200	12.5	1.5
IIIf (6*S*, 7*S*+6*R*, 7*R*)	0.8 (0.4)	6.2 (3.1)	12.5 (12.5)	>100 (>100)	6.2 (3.1)	25 (12.5)	6.2 (6.2)	≦0.1 (≦0.1)	12.5 (3.1)
IIIg (*RS*)	0.2 (≦0.1)	0.8 (0.2)	12.5 (3.1)	100 (50)	25 (1.5)	6.2 (3.1)	>100 (25)	25 (≦0.1)	1.5 (3.1)
IIIh (*RS*)	0.2	1.5	>200	>200	100	200	>200	50	100
IIIj (*RS*)	6.2	25	12.5	50	6.2	6.2	100	12.5	3.1
VIII (*RS*)	12.5	100	100	100	50	100	200	100	100
XI (6*S*, 7*S*+6*R*, 7*R*)	>200	>200	>200	>200	>200	>200	>200	>200	>200
XVIIa (*RS*)	50 (25)	100 (100)	50 (>100)	50 (>100)	50 (50)	25 (50)	>100 (>100)	25 (50)	50 (100)
XVIIb (*RS*)	12.5	100	200	>200	25	100	>200	12.5	100
Va (6*S*, 7*S*+6*R*, 7*R*)	12.5 (0.8)	50 (3.1) [0.8]	>200 (50) [6.2]	>200 (>100) [12.5]	>200 (50)	>200 (100) [50]	>200 (>100)	6.2 (1.5)	100 (25) [6.2]
Vb (6*S*, 7*S*+6*R*, 7*R*)	50	100	>100	>100	>100	>100	>100	>100	>100
XVIII (6*R*, 7*R*)	0.4	3.1	50	200	100	25		50	25
XIX (6*R*, 7*R*)	≦0.1	0.8	50	100	50	1.5	>100	12.5	3.1
XX (Cephalexin)	0.8 (0.2)	3.1 (1.5) [0.8]	6.2 (12.5) [25]	12.5 (25) [50]	6.2 (12.5)	6.2 (25) [100]	12.5 (50)	12.5 (50)	3.1 (12.5) [50]

Numbers in ( ) and [ ] are MIC values at pH 6.0 and 5.0, respectively.



XVIII: R=  , X=CH_3

XIX: R=  , X=Cl

XX: R=PhCH(NH$_2$)–, X=CH_3

Chart 5

(XIX). The electron-withdrawing character of the CF_3 group is apparently reflected in the MIC values, as far as the thienylacetamido derivatives are concerned. The CF_3 group appears to enhance the reactivity towards nucleophilic substances, and consequently the antibacterial activities of 3-cephem compounds, in the same way as a chlorine atom. It appears that the isomer of unnatural configuration (6*S*,7*S*) is less active than the other (6*R*,7*R*). This is also the case with 2-ureido-2-(2-thienyl)acetamido derivative; one of its isomers (possibly *R*-IIId) shows better MIC values than the other.

The glycyl derivatives (Va and Vb) prepared as potential orally active antibiotics proved to be almost inactive against bacteria at pH 7.0. These high MIC values appear to be a result of the instability of the glycyl derivatives at around pH 7.0, probably because of facile intramolecular nucleophilic attack of the amino group on the β-lactam carbonyl with ring opening. However, the phenylglycyl derivative Va is more stable in acidic media where the amino group is protonated to a large extent. The MIC values become increasingly better

as the pH of the culture media decreases. In contrast to the results obtained at pH 7.0, the 3-CF_3 derivative Va shows higher antibacterial activity than cephalexin (XX) when tested at pH 5.0. It seems probable that the 3-CF_3 substituent excessively enhances the intrinsic activity of phenylglycyl 3-cephem derivatives, making the glycyl derivatives too unstable.

The *trans*-7α-acylamido compound VIII showed less antibacterial activity than the *cis* isomer IIIa, as was anticipated, but was more active against *Escherichia coli* 609 (resistant). Introduction of a methoxy group at the 7-position of the 3-trifluoromethylcephem ring resulted in a decrease in antibacterial activity, as seen in compounds XVIIa and XVIIb. It appears that such a decrease in activity is observed on 7-methoxylation of cephalosporin derivatives bearing 3-substituents which do not make good leaving groups, such as OAc, $OCONH_2$ and (1-methyl-1H-tetrazol-5-yl)thio groups.

Among these 3-trifluoromethyl compounds, the mandelamidoderivative (IIIb) showed the best MIC values against gram-negative bacteria at pH 7.0. All the compounds listed in Table II were inactive against *Pseudomonas aeruginosa* 806.

Experimental

Melting points were obtained on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra were taken using a Jasco A-2 spectrometer. Nuclear magnetic resonance spectra were recorded on a Varian A-60D or HA-100 spectrometer using tetramethylsilane as an internal standard. The chemical shifts of various compounds are given in δ units. The silica gel plates used for preparative thick layer chromatography were obtained from E. Merck, Darmstadt, West Germany.

***tert*-Butyl (±)-7β-[2-(2-Thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (IIa)**——A solution of N,N-diethylaniline (131 mg) in $ClCH_2CH_2Cl$ (1 ml) and a solution of 2-thienylacetyl chloride (87.9 mg) in THF (2 ml) were added to a stirred solution of the (±)-*cis*-7-aminocephem·HCl (I, 120 mg) in THF (2 ml) and $ClCH_2CH_2Cl$ (1 ml) with cooling at 0°. When the acylation was complete, AcOEt was added and the mixture was washed successively with aq. $KHSO_4$, 5% aq. $NaHCO_3$ and aq. NaCl, then dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by preparative TLC (benzene–AcOEt=20:1) to give 131 mg (87.8%) of a powder. NMR ($CDCl_3$) δ: 1.51 (9H, s, *t*-Bu), 3.34, 3.50 (2H, AB-q, J=19 Hz, 2×C_2–H), 3.84 (2H, s, $C\underline{H}_2CO$), 4.96 (1H, d, J=5 Hz, C_6–H), 5.85 (1H, d-d, J=5, 9 Hz, C_7–H), 6.29 (1H, d, J=9 Hz, NH), 7.0—7.3 (3H, m, thienyl protons). IR ν_{max}^{KBr} cm^{-1}: 1786 (β-lactam).

(±)-7β-[2-(2-Thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid (IIIa)——A solution of the *tert*-butyl ester (IIa, 131 mg) in CF_3COOH (3 ml) was stirred for 1.5 hr at room temperature. The CF_3COOH was evaporated off *in vacuo* and the crystalline residue (120 mg) was recrystallized from AcOEt to give colorless crystals (54 mg), mp 213—214° (dec.). IR ν_{max}^{KBr} cm^{-1}: 1785 (β-lactam). NMR (acetone-d_6) δ: 3.59, 3.73 (2H, AB-q, J=18 Hz, 2×C_2–H), 3.88 (2H, s, $C\underline{H}_2CO$), 5.26 (1H, d, J=5 Hz, C_6–H), 5.95 (1H, d-d, J=5, 9 Hz, C_7–H), 6.8—7.4 (3H, m, thienyl H), 8.16 (1H, bd, J=9 Hz, NH).

***tert*-Butyl (6*R*,7*R*)- and (6*S*,7*S*)-7-(*R*-Mandelamido)-3-trifluoromethyl-3-cephem-4-carboxylate (1:1 mixture) (IIb)**——The (±)-*cis*-7-aminocephem·HCl (I, 285 mg) was acylated with *R*-O-dichloroacetylmandeloyl chloride (333 mg) and N,N-diethylaniline (294 mg) in $ClCH_2CH_2Cl$ (15 ml) as described for IIa. The crude product was purified by preparative TLC (benzene–AcOEt=9:1) to give 182 mg of IIb (the protecting group of the OH group was removed during the preparative TLC). IR ν_{max}^{KBr} cm^{-1}: 3400 (NH, OH), 1810 (β-lactam), 1740 (ester). NMR ($CDCl_3$) δ: 1.55 (9H, s, *t*-Bu), 3.46 (3H, b, 2×C_2–H, OH), 4.93 (1H, d J=6 Hz, C_6–H), 5.80 (1H, d-d, J=6, 9 Hz, C_7–H), 7.62 (1H, d, J=9 Hz, NH), 5.12 (1H, s, $C\underline{H}CO$), 7.45 (5H, s, $C_6\underline{H}_5$).

(6*R*,7*R*)- and (6*S*,7*S*)-7-(*R*-Mandelamido)-3-trifluoromethyl-3-cephem-4-carboxylic Acid (1:1 mixture) (IIIb)——The *tert*-butyl ester (IIb, 41 mg) was treated with CF_3COOH (4.5 ml) for 1 hr at room temperature. The reaction mixture was evaporated to dryness *in vacuo*, then AcOEt and H_2O were added to the residue and the mixture was adjusted to pH 8.0 with 10% aq. K_2HPO_4, with stirring and ice-cooling. The aq. layer was saturated with NaCl, overlaid with AcOEt, acidified to pH 2.1, and extracted with AcOEt. The combined extracts were dried over Na_2SO_4 and concentrated *in vacuo* to leave 21 mg of IIIb. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 3250 (NH), 1790 (β-lactam). NMR ($CDCl_3$) δ: 3.54 (2H, bs, 2×C_2–H), 5.12 (1H, d, J=5 Hz, C_6–H), 5.77 (1H, d, J=5 Hz, C_7–H), 5.10 (1H, s, $C\underline{H}CO$), 7.2—7.6 (5H, C_6H_5).

***tert*-Butyl (±)-7β-[2-(1H-Tetrazol-1-yl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (IIc)**——The (±)-*cis*-7-aminocephem·HCl (I, 108 mg) was acylated with 1H-tetrazol-1-yl chloride (112 mg) in CH_2Cl_2 (5 ml) in the presence of N,N-diethylaniline (66 mg). The reaction mixture was worked up as described for IIa and the crude product was purified by preparative TLC (benzene–AcOEt=1:1) to give 99 mg of IIc. IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 1792 (β-lactam), 1735 (ester). NMR ($CDCl_3$+CD_3OD) δ: 1.55 (9H, s, *t*-Bu), 3.58

(2H, s, 2×C_2–H), 5.17 (1H, d, J=6 Hz, C_6–H), 5.91 (1H, s, J=6 Hz, C_7–H), 5.40 (2H, s, C$\underline{H}_2$CO), 9.17 (1H, s, tetrazolyl H).

(±)-7β-[2-(1H-Tetrazol-1-yl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid (IIIc)——The *tert*-butyl ester (IIc, 41 mg) was treated with CF_3COOH (10 ml) for 1 hr at room temperature. The CF_3COOH was removed *in vacuo* to give IIIc in quantitative yield. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1785 (β-lactam). NMR (CD_3OD) δ: 3.63 (2H, b, 2×C_2–H), 5.19 (1H, d, J=6 Hz, C_6–H), 5.87 (1H, d, J=6 Hz, C_7–H), 5.47 (2H, s, C$\underline{H}_2$CO), 9.19 (1H, s, tetrazolyl H).

***tert*-Butyl (6*R*,7*R*)- and (6*S*,7*S*)-7-[2*R*-2-Ureido-2-(2-thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (1:1 Mixture) (IId)**——The (±)-*cis*-7-aminocephem·HCl (I, 100 mg) was treated with *R*-2-ureido-(2-thienyl)acetyl chloride (121 mg) and N,N-diethylaniline (124 mg) in THF (12 ml) for 1 hr at −10°—−20° and the reaction mixture was worked up as described for IIa to give 133 mg of IId as a powder. IR ν_{max}^{Nujol} cm^{-1}: 3500, 3400, 3250 (sh) (NH, NH_2), 1800 (β-lactam), 1740 (ester). NMR (DMF-d_7) δ: 1.53 (9H, s, *t*-Bu), 3.4—3.8 (2H, b, 2×C_2–H), 5.35, 5.36 (2×0.5 H, 2×d, J=5.0 Hz, C_6–H), 5.7—6.6 (2H, C_7–H, C$\underline{H}$CO), 6.9—7.6 (3H, m, thienyl H), 9.35 (1H, d, J=8.0 Hz, amido NH), 9.35 (1H, d, J=7.0 Hz, ureido NH), 5.7—6.6 (2H, NH_2).

(6*R*,7*R*)- and (6*S*,7*S*)-7-[2*R*-2-Ureido-2-(2-thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid (*R*-IIId and *S*-IIId)——A solution of the *tert*-butyl ester (IId, 118 mg) in CF_3COOH (1.2 ml) was stirred for 1.5 hr at room temperature. The CF_3COOH was evaporated off *in vacuo* and the residue was purified by preparative TLC (HCOOH–AcOEt=1:20) to give *S*-IIId (41 mg, Rf=0.43) and *R*-IIId (51 mg, Rf=0.29) as a powder. More polar isomer (*S*-IIId), IR ν_{max}^{Nujol} cm^{-1}: 3500, 3400, 3280 (NH, NH_2), 1779 (β-lactam). NMR (DMF-d_7) δ: 3.72 (2H, s, 2×C_2–H), 5.26 (1H, d, J=5.0 Hz, C_6–H), 5.70 (1H, d-d, J=8.0, 5.0 Hz, C_7–H), 5.65 (1H, C$\underline{H}$CO), 6.8—7.5 (3H, m, thienyl H), 9.51 (1H, d, J=8.0 Hz, amido NH), 6.78 (1H, d, J=8.0 Hz, ureido NH), 5.5—6.0 (2H, bs, ureido NH_2). Less polar isomer (*R*-IIId), IR ν_{max}^{Nujol} cm^{-1}: 3498, 3360 (NH, NH_2), 1795 (β-lactam). NMR (DMF-d_7) δ: 3.66 (2H, s, 2×C_2–H), 5.22 (1H, d, J=5.0 Hz, C_6–H), 5.90 (1H, d-d, J=8.0, 5.0 Hz, C_7–H), 5.70 (1H, d, J=8.0 Hz, C$\underline{H}$CO), 6.8—7.5 (3H, m, thienyl H), 9.57 (1H, d, J=8.0 Hz, amido NH), 6.82 (1H, d, J=8.0 Hz, ureido NH), 5.4—6.2 (2H, bs, ureido NH_2).

***tert*-Butyl (±)-7β-[2-(4-Pyridylthio)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (IIe)**——The (±)-*cis*-7-aminocephem·HCl (I, 108 mg) was acylated with 4-pyridylthioacetyl chloride·HCl (101 mg) and N,N-diethylaniline (179 mg) in $ClCH_2CH_2Cl$ (10 ml) as described for IIa. When the reaction was complete, AcOEt was added and the mixture was washed with H_2O. The crude product was purified by preparative TLC (benzene–AcOEt=1:3) to give 120 mg of IIe. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1800 (β-lactam), 1750 (ester). NMR ($CDCl_3$+CD_3OD) δ: 1.55 (9H, s, *t*-Bu), 3.53 (2H, b, 2×C_2–H), 5.11 (1H, d, J=5.0 Hz, C_6–H), 5.86 (1H, d, J=5.0 Hz, C_7–H), 3.85 (2H, s, SC$\underline{H}_2$CO), 7.33, 9.08 (4H, A_2X_2, pyridyl H).

(±)-7β-[2-(4-Pyridylthio)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid·CF_3COOH (IIIe)——The *tert*-butyl ester (IIe, 79 mg) was treated with CF_3COOH (2 ml) for 1 hr at room temperature. Removal of the CF_3COOH afforded 60 mg of IIIe as crystals. *Anal.* Calcd for $C_{11}H_{12}F_3N_3O_3S_2$: C, 42.95; H, 2.88; N, 10.01; S, 15.29; F, 13.58. Found: C, 42.20; H, 2.83; N, 10.01; S, 15.41; F, 13.64. IR ν_{max}^{KBr} cm^{-1}: 3450 (NH), 1770 (β-lactam). NMR (DMSO-d_6+D_2O) δ: 3.56 (2H, bs, 2×C_2–H), 5.13 (1H, d, J=6.0 Hz, C_6–H), 5.70 (1H, d, J=6.0 Hz, C_7–H), 4.05 (2H, s, SC$\underline{H}_2$CO), 7.66, 8.48 (4H, A_2X_2, J_{AX}=6 Hz, pyridyl H).

***tert*-Butyl (6*R*,7*R*)- and (6*S*,7*S*)-7-[2*R*-2-(4-Ethyl-2,3-dioxo-1-piperadinyl)formamido]-2-phenylacetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (1:1 Mixture) (IIf)**——The (±)-*cis*-7-aminocephem·HCl (I, 100 mg) was acylated with *R*-2-[(4-ethyl-2,3-dioxo-1-piperadinyl)formamido] phenylacetyl chloride (187 mg) and N,N-diethylaniline (124 mg) in THF (12 ml) as described for IIa. Preparative TLC (AcOEt) of the crude product gave 138 mg of IIf as a powder. IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1800 (β-lactam). NMR (DMF-d_7) δ: 1.50 (9H, s, *t*-Bu), 3.55—4.20 (2H, 2×C_2–H), 3.55—4.20 (4H, CH_2CH_2), 5.27, 5.36 (2×0.5H, 2×d, J=0.5 Hz, C_6–H), 6.06 (1H, d-d, J=8.0, 5.0 Hz, C_7–H), 5.71, 5.75 (2×0.5H, 2×d, J=7.0 Hz, C$\underline{H}$CO), 1.13 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 3.49 (2H, q, J=7.0 Hz, $C\underline{H}_2CH_3$), 7.3—7.8 (5H, m, $C_6\underline{H}_5$), 9.46 (1H, d, J=8.0 Hz, amido NH), 10.06 (1H, d, J=7.0 Hz, NCON$\underline{H}$).

(6*R*,7*R*)- and (6*S*,7*S*)-7-[2*R*-2-[(4-Ethyl-2,3-dioxo-1-piperadinyl)formamido]-2-phenylacetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid (1:1 Mixture) (IIIf)——The *tert*-butyl ester (IIf, 120 mg) was treated with CF_3COOH (1.2 ml) for 1.5 hr at room temperature. The CF_3COOH was removed *in vacuo* and the residue was washed with ether to give 101 mg of IIIf as a powder. IR ν_{max}^{Nujol} cm^{-1}: 3700—2400 (NH, COOH), 1800 (β-lactam). NMR (DMF-d_7) δ: 1.08 (3H, t, J=7.0 Hz, $CH_2C\underline{H}_3$), 3.46 (2H, q, J=7.0 Hz, $C\underline{H}_2CH_3$), 3.57 (2H, bs, 2×C_2–H), 5.16, 5.25 (2×0.5H, 2×d, J=5.0 Hz, C_6–H), 5.74, 5.90 (2×0.5H, 2×d-d, J=8.0, 5.0 Hz, C_7–H), 5.52, 5.58 (2×0.5H, 2×d, J=7.0 Hz, C$\underline{H}$CO), 7.2—7.8 (5H, m, $C_6\underline{H}_5$), 9.62 (1H, d, J=8.0 Hz, amido NH), 9.87 (1H, d, J=7.0 Hz, NCON$\underline{H}$).

***tert*-Butyl (±)-7β-cyanomethylthioacetamido-3-trifluoromethyl-3-cephem-4-carboxylate (IIg)**——The (±)-*cis*-7-aminocephem·HCl (I, 108 mg) was acylated with cyanomethylthioacetyl chloride (67.3 mg) and N,N-diethylaniline (112 mg) in $ClCH_2CH_2Cl$ (5 ml) at −15° as described for IIa. The crude product was purified by preparative TLC (benzene–AcOEt=4:1) to give 118 mg of IIg. IR $\nu_{max}^{liq.}$ cm^{-1}: 3400 (NH), 2200 (CN), 1800 (β-lactam), 1740 (ester). NMR ($CDCl_3$) δ: 1.57 (9H, s, *t*-Bu), 3.55 (6H, s, 2×C_2–H, $CNC\underline{H}_2$-$SC\underline{H}_2$), 5.13 (1H, d, J=6 Hz, C_6–H), 5.92 (1H, d-d, J=6, 9 Hz, C_7–H), 7.82 (1H, d, J=9 Hz, NH).

(±)-7β-Cyanomethylthioacetamido-3-trifluoromethyl-3-cephem-4-carboxylic Acid (IIIg)——The *tert*-butyl ester (IIg, 100 mg) was treated with CF_3COOH (20 ml) for 1 hr at room temperature. After preparative TLC ($CHCl_3$–MeOH=2: 1), the product was treated with aq. K_2HPO_4 as described for IIIb. Yield, 86 mg. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 2240 (CN), 1790 (β-lactam). NMR (CD_3OD) δ: 3.68 (2H, s, 2×C_2–H), 5.15 (1H, d, J=5 Hz, C_6–H), 5.85 (1H, d, J=5 Hz, C_7–H), 3.83 (4H, s, $CNC\underline{H}_2SC\underline{H}_2$).

***tert*-Butyl (±)-7β-(2-Azido-2-phenylacetamido)-3-trifluoromethyl-3-cephem-4-carboxylate (IIh)**——The (±)-*cis*-7-aminocephem·HCl (I, 108 mg) was acylated with 2-azido-phenylacetyl chloride (88.0 mg) and N,N-diethylaniline (112 mg) in $ClCH_2CH_2Cl$ (5 ml) at −15°. Work-up as described for IIa followed by preparative TLC (benzene–AcOEt=7: 1) afforded 116 mg of IIh. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380 (NH), 2120 (N_3), 1800 (β-lactam), 1737 (ester). NMR ($CDCl_3$) δ: 1.51 (9H, s, *t*-Bu), 4.41 (2H, b, 2×C_2–H), 4.94 (1H, d, J=5.5 Hz, C_6–H), 5.78 (1H, d-d, J=5.5, 10 Hz, C_7–H), 5.10 (1H, s, C$\underline{H}$CO), 7.3—7.6 (6H, $C_6\underline{H}_5$, NH).

(±)-7β-(2-Azido-2-phenylacetamido)-3-trifluoromethyl-3-cephem-4-carboxylic Acid (IIIh)——The *tert*-butyl ester (IIh, 110 mg) was treated with CF_3COOH as described for IIb to give 99 mg of IIIh. IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 2100 (N_3), 1790 (β-lactam). NMR (CD_3OD) δ: 3.63 (2H, bs, 2×C_2–H), 5.22 (1H, d, J=5.0 Hz, C_6–H), 5.81 (1H, d, J=5.0 Hz, C_7–H), 5.14 (1H, s, C$\underline{H}$CO), 7.47 (5H, s, $C_6\underline{H}_5$).

***tert*-Butyl (±)-7β-(2-Benzyloxycarbonyl-2-phenylacetamido)-3-trifluoromethyl-3-cephem-4-carboxylate (IIi)**——The (±)-*cis*-7-aminocephem·HCl (I, 108 mg) was acylated with 2-benzyloxycarbonyl-phenylacetyl chloride (130 mg) and N,N-diethylaniline (112 mg) in $ClCH_2CH_2Cl$ (3 ml) as described for IIa. The crude product was purified by preparative TLC (benzene–AcOEt=7: 1) to give 113 mg of IIi. IR ν_{max}^{KBr} cm^{-1}: 3350, 3450 (NH), 1800 (β-lactam). NMR ($CDCl_3$) δ: 1.55 (9H, s, *t*-Bu), 3.43 (2H, m, 2×C_2–H), 4.95, 4.97 (2×0.5H, 2×d, J=5.0 Hz, C_6–H), 5.88 (1H, d-d, J=5.0, 9.0 Hz, C_7–H), 7.93 (1H, bd, J=9.0 Hz, NH), 4.65, 4.68 (2×0.5H, 2×s, C$\underline{H}$CO), 5.22 (2H, s, $C\underline{H}_2$Ph), 7.33 (5H, s, $C_6\underline{H}_5$), 7.40 (5H, s, $C_6\underline{H}_5$).

(±)-7β-(2-Benzyloxycarbonyl-2-phenylacetamido)-3-trifluoromethyl-3-cephem-4-carboxylic Acid (IIIi)——The *tert*-butyl ester (IIi, 113 mg) was treated with CF_3COOH to afford 102 mg of IIIi. IR ν_{max}^{KBr} cm^{-1}: 3325 (NH), 1790 (β-lactam). NMR (CD_3OD) δ: 3.53 (2H, m, 2×C_2–H), 5.09 (1H, bd, J=5.5 Hz, C_6–H), 5.79 (1H, bd, J=5.5 Hz, C_7–H), 4.84 (1H, bs, C$\underline{H}$CO), 5.21 (2H, s, $C\underline{H}_2$Ph), 7.33 (5H, s, $C_6\underline{H}_5$), 7.35 (5H, s, $C_6\underline{H}_5$).

(±)-7β-(2-Carboxy-2-phenylacetamido)-3-trifluoromethyl-3-cephem-4-carboxylic Acid (IIIj)——The benzyloxycarbonyl derivative (IIIi, 73 mg) was hydrogenated over Pd black (300 mg) in AcOEt (20 ml). When the hydrogenolysis was complete, the catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC ($CHCl_3$–MeOH=2: 1) to give 32 mg of IIIj as crystals. IR ν_{max}^{KBr} cm^{-1}: 3400 (NH), 1770 (β-lactam). NMR (CD_3OD) δ: 3.49 (2H, bs, 2×C_2–H), 5.12 (1H, d, J=5 Hz, C_6–H), 5.77 (1H, d, J=5 Hz, C_7–H), 7.2—7.6 (5H, m, $C_6\underline{H}_5$).

***tert*-Butyl (±)-7β-Chloroacetamido-3-trifluoromethyl-3-cephem-4-carboxylate (IIj)**——The (±)-*cis*-aminocephem·HCl (I, 108 mg) was acylated with chloroacetyl chloride (509 mg) and N,N-diethylaniline (112 mg) in $ClCH_2CH_2Cl$ (5 ml) as described for IIa. The crude product was purified by preparative TLC (benzene–AcOEt=4: 1) to give 90 mg of IIj. NMR ($CDCl_3$) δ: 1.55 (9H, s, *t*-Bu), 3.53 (2H, bs, 2×C_2–H), 5.09 (1H, d, J=5.5 Hz, C_6–H), 5.90 (1H, d-d, J=5.5, 9.0 Hz, C_7–H), 7.30 (1H, d, J=9.0 Hz, NH), 4.10 (2H, s, $ClCH_2$).

***tert*-Butyl (±)-7β-Iodoacetamido-3-trifluoromethyl-3-cephem-4-carboxylate (IIk)**——A solution of the chloroacetamidocephem (IIj, 79 mg) and NaI (88.6 mg) in acetone (1 ml) was stirred for 24 hr at room temperature. The solvent was removed *in vacuo* and the residue was dissolved in AcOEt and H_2O. The organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo* to leave 100 mg of IIk. IR ν_{max}^{KBr} cm^{-1}: 3250 (NH), 1790 (β-lactam). NMR ($CDCl_3$) δ: 1.55 (9H, s, *t*-Bu), 3.53 (2H, bs, 2×C_2–H), 5.09 (1H, d, J=5.0 Hz, C_6–H), 5.88 (1H, d-d, J=5.0, 9.0 Hz, C_7–H), 7.30 (1H, d, J=9.0 Hz, NH), 3.81 (2H, s, $IC\underline{H}_2$).

***tert*-Butyl (6*R*,7*R*)- and (6*S*,7*S*)-7-(2*R*-2-*tert*-Butoxyformamido-2-phenylacetamido)-3-trifluoromethyl-3-cephem-4-carboxylate (1: 1 Mixture) (IVa)**——A solution of isobutyl chloroformate (61.0 mg) in THF (1 ml) was cooled at −10° and a mixture of *R*-*tert*-butoxyformamido-phenylacetic acid (113 mg) and triethylamine (63 μl) in THF (1 ml) containing one drop of N,N-diethylaniline was added dropwise. After stirring for 30 min, a solution of the (±)-*cis*-7-aminocephem·HCl (I, 145 mg) in THF (2 ml) was added and the mixture was stirred for 3 hr at −10°. After removal of the solvent *in vacuo*, the residue was taken up in AcOEt and washed successively with H_2O, 5% HCl, 5% aq. $NaHCO_3$, and H_2O, then dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by preparative TLC (benzene–AcOEt=4: 1) to give IVa (193 mg). NMR ($CDCl_3$) δ: (100 MHz) 1.52 (9H, s, *t*-Bu), 1.41 (9H, s, *t*-Bu), 3.30, 3.50 (2×0.5H, AB-q, C_2–H), 3.37, 3.47 (2×0.5H, AB-q, C_2–H), 4.93, 4.99 (2×0.5H, 2×d, J=5.0 Hz, C_6–H), 5.22, 5.28 (2×0.5H, 2×d, C$\underline{H}$CO), 5.54, 5.62 (2×0.5H, N$\underline{H}$Boc), 5.82, 5.86 (2×0.5H, 2×d-d, J=5.0, 9.0 Hz, C_7–H), 6.81, 6.96 (2×0.5H, 2×d J=9.0 Hz, NH), 7.34 (5H, s, $C_6\underline{H}_5$).

(6*R*,7*R*)- and (6*S*,7*S*)-7-(*R*-2-Amino-2-phenylacetamido)-3-trifluoromethyl-3-cephem-4-carboxylic Acid·CF_3COOH (Va)——The *tert*-butyl ester (IVa, 193 mg) was dissolved in CF_3COOH (3 ml) and the solution was stirred for 1.5 hr at room temperature. The CF_3COOH was removed *in vacuo* to give Va (169 mg). IR ν_{max}^{KBr} cm^{-1}: 1783 (β-lactam). NMR (DMSO-d_6) δ: 3.55, 3.67 (2H, m, 2×C_2–H), 4.98 (1H, bs, PhC$\underline{H}$), 5.15, 5.25 (2×0.5H, 2×d, J=5.8, 5.0 Hz, respectively, C_6–H), 5.72, 5.88 (2×0.5H, C_7–H), 7.46 (5H, s, $C_6\underline{H}_5$), 8.76 (b, $\overset{+}{N}H_3$).

***tert*-Butyl (6*R*,7*R*)- and (6*S*,7*S*)-7-[2*R*-2-*tert*-Butoxyformamido-2-(2-thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (1:1 Mixture) (IVb)**——*R*-*tert*-Butoxyformamido-(2-thienyl)acetic acid (171 mg) was treated with triethylamine (67 mg) and isobutyl chloroformate (90 mg) in THF (12 ml) at −20° as described for IVa. The resulting mixed anhydride was similarly reacted with the (±)-*cis*-7-aminocephem·HCl (I, 200 mg) in the presence of N,N-diethylaniline. The reaction mixture was worked up as usual and the crude product was subjected to preparative TLC (benzene–AcOEt=4:1) to give 181 mg of IVb as a powder, mp 93—95°. IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1800 (β-lactam). NMR ($CDCl_3$) δ: 1.41 (9H, s, *t*-Bu), 1.53 (9H, s, *t*-Bu), 3.3—3.6 (2H, bs, 2×C_2–H), 5.00, 5.04 (2×0.5H, 2×d, J=5.0 Hz, C_6–H), 5.90 (1H, d-d, J=8.0, 5.0 Hz, C_7–H), 5.55, 5.82 (1H, C<u>H</u>CO), 6.85—7.45 (3H, m, thienyl H), 7.0—7.6 (1H, NH), 5.6—6.0 (1H, N<u>H</u>Boc).

(6*R*,7*R*)- and (6*S*,7*S*)-7-[*R*-2-(2-Thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid·CF_3COOH (1:1 Mixture) (Vb)——The *tert*-butyl ester (IVb, 160 mg) was treated with CF_3COOH (3.2 ml) for 2.5 hr and worked up as described for Va to give 76 mg of Vb as a powder. IR ν_{max}^{Nujol} cm^{-1}: 1788 (β-lactam). NMR (DMF-d_7) δ: 3.51, 3.64 (2H, b, 2×C_2–H), 5.26 (1H, d, J=5.0 Hz, C_6–H), 5.77 (1H, d, J=5.0 Hz, C_7–H), 5.48 (1H, s, C<u>H</u>CO), 6.9—7.8 (3H, m, thienyl H), 9.19 (1H, b, amido NH).

***tert*-Butyl (±)-7α-[2-(2-Thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (VII)**——The (±)-*trans*-7-aminocephem (VI, 148 mg) was acylated with 2-thienylacetyl chloride (81 mg) and N,N-diethylaniline (75 mg) in THF (5 ml) at −10° as described for IIa. The crude product was triturated with pet. ether and filtered to give 150 mg of VII. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1800 (β-lactam), 1745 (ester). NMR (DMF-d_7) δ: 1.52 (9H, s, *t*-Bu), 3.86, 3.67 (2H, 2×d, J=16.5 Hz, 2×C_2–H), 3.90 (2H, s, C<u>H</u>$_2$CO), 4.98—5.28 (2H, C_6–H, C_7–H), 6.9—7.6 (3H, m, thienyl H), 9.24 (1H, d, J=7.0 Hz, CON<u>H</u>).

(±)-7α-[2-(2-Thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid (VIII)——The *tert*-butyl-ester (VII, 140 mg) was treated with CF_3COOH (6 ml) as described for IIIc to give VIII as a powder. IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 1780 (β-lactam). NMR (acetone-d_6+D_2O) δ: 3.79, 3.57 (2H, AB-q, J=18.0 Hz, 2×C_2–H), 3.87 (2H, s, C<u>H</u>$_2$CO), 5.10, 4.99 (2H, 2×d, J=3.0 Hz, C_6–H, C_7–H), 6.9—7.5 (thienyl H).

***tert*-Butyl (6*R*,7*R*)- and (6*S*,7*S*)-7-[2*R*-2-(*tert*-Butoxyformamido)-2-phenylacetamido]-3-trifluoromethyl-2-cephem-4-carboxylate (1:1 Mixture) (X)**——The mixed anhydride was formed from *R*-2-(*tert*-butoxyformamido)-phenylacetic acid (81.3 mg) and isobutyl chloroformate (41.4 mg) as described for IVa. The resulting mixed anhydride was similarly reacted with the (±)-*cis*-7-amino-2-cephem (IX, 70 mg) in THF (3 ml) and the crude product was purified by preparative TLC (benzene–AcOEt=4:1) to give 113 mg of X. IR ν_{max}^{KBr} cm^{-1}: 1785 (β-lactam). NMR ($CDCl_3$) δ: 1.49 (9H, s, *t*-Bu), 1.47 (9H, s, *t*-Bu), 5.03 (1H, b, C_4–H), 5.2—5.8 (4H, m, C_6–H, C_7–H, C<u>H</u>CO, NH), 6.97, 7.10 (2×0.5H, m, C_2–H), 7.40 (5H, s, C_6–H).

(6*R*,7*R*)- and (6*S*,7*S*)-7-(*R*-2-Amino-2-phenylacetamido)-3-trifluoromethyl-2-cephem-4-carboxylic Acid·CF_3COOH (1:1 mixture) (XI)——The *tert*-butyl ester (X, 99 mg) was treated with CF_3COOH (1 ml) for 1.5 hr at room temperature. Work-up as usual gave 68.2 mg of XI. IR ν_{max}^{KBr} cm^{-1}: 1780 (β-lactam).

***tert*-Butyl (±)-7α-Methoxy-7β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-3-trifluoromethyl-3-cephem-4-carboxylate (XIV)**——A solution of the (+)-*trans*-7-aminocephem (VI, 1.56 g) and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (1.13 g) in benzene (30 ml) was refluxed for 1 hr with azeotropic removal of the water formed. Removal of the solvent *in vacuo* gave *tert*-butyl (±)-*trans*-7α-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-3-trifluoromethyl-3-cephem-4-carboxylate (XII, 2.88 g). The crude Schiff base (XII, 0.50 g) was added to a suspension of PbO_2 (freshly prepared from $Pb(OAc)_4$ (1.2 g)) in benzene (20 ml) and the mixture was stirred for 2.5 hr at room temperature. The solid material was removed by filtration, and washed with benzene, then the filtrate was concentrated *in vacuo* to 10 ml, and MeOH (10 ml) was added. After stirring the mixture for 1 hr at room temperature, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel, eluting with benzene, to give 273 mg (51.7%) of XIV. NMR ($CDCl_3$) δ: 1.58 (9H, s, *t*-Bu), 1.47 (18H, s, 2×*t*-Bu), 3.38, 3.45 (2H, AB-q, 2×C_2–H), 3.56 (3H, s, OCH_3), 5.10 (1H, s, C_6–H), 5.66 (1H, s, OH), 7.76 (2H, s, aromatic H).

***tert*-Butyl (±)-7β-amino-7α-methoxy-3-trifluoromethyl-3-cephem-4-carboxylate (XV)**——A solution of the Schiff base (XIV, 136 mg) in AcOEt (4 ml) was treated with a solution of Girard reagent T (193 mg) in MeOH (3 ml) and the mixture was stirred for 7 hr at room temperature. The solvent was removed *in vacuo* and the residue was taken up in AcOEt and H_2O. The organic layer was washed with H_2O and dried over Na_2SO_4, then the solvent was evaporated off *in vacuo* to give XV as crystals (82.7 mg). NMR ($CDCl_3$) δ: 1.55 (9H, s, *t*-Bu), 2.15 (2H, bs, NH_2), 3.52, 3.37 (2H, AB-q) with further long-range coupling of H_A with CF_3. J_{AB}=18 Hz, J_{HF}=1 Hz, 2×C_2–H), 3.51 (3H, s, OCH_3), 4.80 (1H, s, C_6–H). IR $\nu_{max}^{liq.}$ cm^{-1}: 3320, 3400 (NH_2), 1790 (β-lactam), 1740 (ester).

***tert*-Butyl (±)-7α-Methoxy-7β-[2-(2-thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylate (XVIb)**——A solution of N,N-diethylaniline (53 mg) in $ClCH_2CH_2Cl$ (1 ml) and then a solution of 2-thienylacetyl chloride (58 mg) in $ClCH_2CH_2Cl$ (1 ml) were added to a solution of the (±)-7β-amino-7α-methoxycephem (XV, 82.7 mg) in $ClCH_2CH_2Cl$ (1 ml) with stirring at −10°. After stirring the mixture for 4 hr at 0—5°, AcOEt and H_2O were added. The AcOEt layer was washed successively with 5% HCl, aq. NaCl, 5% aq. $NaHCO_3$, and aq. NaCl, then dried over Na_2SO_4. The solvent was evaporated off and the residue (117 mg) was purified by preparative TLC (benzene–AcOEt=10:1) to give 75.7 mg of XVIb. IR ν_{max}^{MeOH}

cm^{-1}: 3260 (NH), 1790 (β-lactam), 1740 (ester). NMR ($CDCl_3$) δ: 1.54 (9H, s, *t*-Bu), 3.31, 3.51 (2H, AB-q, $J=18.0$ Hz, $2\times C_2$–H), 3.45 (3H, s, OCH_3), 3.89 (2H, s, $C\underline{H}_2CO$), 5.07 (1H, s, C_6–H), 6.46 (1H, s, NH), 6.9—7.4 (3H, m, thienyl H).

(±)-7α-Methoxy-7β-[2-(2-thienyl)acetamido]-3-trifluoromethyl-3-cephem-4-carboxylic Acid (XVIIb)——A solution of the *tert*-butyl ester (XVIb, 60 mg) in CF_3COOH (0.6 ml) was stirred for 3 hr at room temperature. The CF_3COOH was removed *in vacuo* and the residue was purified by preparative TLC ($CHCl_3$–MeOH=2:1) to give a solid. The solid was dissolved in H_2O by adding 10% aq. K_2HPO_4 at 0° until the pH of the solution was 8.0. The aq. solution was overlaid with AcOEt and acidified to pH 2.3 with 6 N HCl while stirring and cooling, then extracted with AcOEt. The combined extracts were washed with aq. NaCl and dried over Na_2SO_4. Removal of the solvent gave 39.8 mg of XIIb as crystals. IR ν_{max}^{KBr} cm^{-1}: 3250 (NH), 1786 (β-lactam). NMR (acetone-d_6) δ: 3.72, 3.45 (2H, AB-q, with further long-range coupling of H_A with CF_3, $J=18$ Hz, $J_{HF}=2$ Hz, $2\times C_2$–H), 3.44 (3H, s, OCH_3), 3.93 (2H, s, $C\underline{H}_2CO$), 5.22 (1H, s, C_6–H), 6.70—7.40 (3H, m, thienyl H), 8.50 (1H, s, NH).

***tert*-Butyl (±)-7β-Cyanomethylthioacetamido-7α-methoxy-3-trifluoromethyl-3-cephem-4-carboxylate (XVIa)**——The (±)-7β-amino-7α-methoxycephem (XV, 49.8 mg) was acylated with cyanomethylthioacetyl chloride (51.0 mg) and N,N-diethylaniline (50.0 mg) in $ClCH_2CH_2Cl$ (3 ml) as described for XVIb. Similarly, the reaction mixture was worked up to give 44.1 mg of XVIa as crystals. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3330 (NH), 2256 (CN), 1806 (β-lactam), 1750 (ester). NMR ($CDCl_3$) δ: 1.57 (9H, s, *t*-Bu), 3.40, 3.52 (2H, AB-q, $J=18.0$ Hz, $2\times C_2$–H), 3.55 (2H, s, $SC\underline{H}_2CO$), 3.57 (2H, s, $NCC\underline{H}_2S$), 3.59 (3H, s, OCH_3), 5.15 (1H, s, C_6–H), 7.80 (1H, s, NH).

(±)-7β-Cyanomethylthioacetamido-7α-methoxy-3-trifluoromethyl-3-cephem-4-carboxylic Acid (XVIIa)——The *tert*-butyl ester (XVIa, 42 mg) was treated with CF_3COOH (0.8 ml) and the reaction mixture was worked up as described for XVIIb to give 32.3 mg of XVIIa as a solid. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 2250 (CN), 1790 (β-lactam). NMR (acetone-d_6) δ: 3.30, 3.50 (2H, AB-q, $J=18.0$ Hz, $2\times C_2$–H), 3.57 (3H, s, OCH_3), 3.65 (2H, s, $SC\underline{H}_2CO$), 3.78 (2H, s, $NCC\underline{H}_2S$), 5.33 (1H, s, C_6–H), 8.78 (1H, s, NH).

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