A Practical Synthetic Method for 3-(*N*,*N*-Disubstituted Carbamoyloxy)-methyl Cephems without Generating the Δ^2-Isomers

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Received November 28, 1994; accepted January 21, 1995

E1101, a new oral cephalosporin, has a (*N*,*N*-dimethylcarbamoyloxy)methyl group at the C-3 position of the cephem nucleus. The previous methods for manufacturing 3-(*N*,*N*-disubstituted carbamoyloxy)methyl cephems generate various amounts of intractable Δ^2 isomers as by-products. In this report, we describe a new, practical synthetic method for cephems of this type without generating Δ^2 isomers, *via* 7-acylamino-3-(4-nitrophenoxycarbonyloxy)methyl-Δ^3-cephem-4-carboxylic acid (5) as a key intermediate.

Key words antibacterial agent; oral cephalosporin; carbamoyloxylation

E1101, 2-(isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^3-cephem-4-carboxylate hydrochloride (Fig. 1), has been developed as a new oral cephalosporin, with a well-balanced antibacterial spectrum, good oral absorbability, and prolonged half-life in plasma.[1] One of its characteristic structural features is the substituent at the C-3 position of the cephem nucleus. Two methods have been reported for the preparation of 3-(*N*,*N*-disubstituted carbamoyloxy)methyl cephems (**6**), *i.e.*, the *N*,*N*′-carbonyldiimidazole (CDI) method[2] and the 4-nitrophenyl carbonate (PNP) method,[3] shown below as a general scheme (Chart 1). In the CDI method, 3-hydroxymethyl-Δ^3-cephem (**1a**), which is protected with a diphenylmethyl group at the C-4 carboxylic acid, is allowed to react with CDI, followed by treatment with dimethylamine to afford a 2:3 mixture of Δ^3 and Δ^2 **3a**. On the other hand, in the PNP method, which was developed in the course of our process research, **1a** was converted with 4-nitrophenyl chloroformate to afford activated esters **2b**, and subsequently allowed to react with dimethylamine to give **3a**. Some isomerization to Δ^2 occurred, and Δ^2 **3a** amounted to more than 5% in the reaction mixture. The Δ^2 and Δ^3 cephems have similar properties, and consequently the efficient separation of Δ^2 cephems and Δ^3 cephems in the mixture is difficult. Therefore, these two methods were considered unsuitable for scale-up and manufacture of a high-quality product. We have recently developed a new method for the synthesis of 3-(*N*,*N*-disubstituted carbamoyloxy)-methyl cephems without generating Δ^2 isomers, and this should be applicable to large-scale manufacturing.

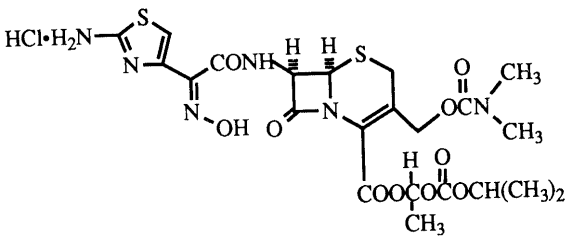
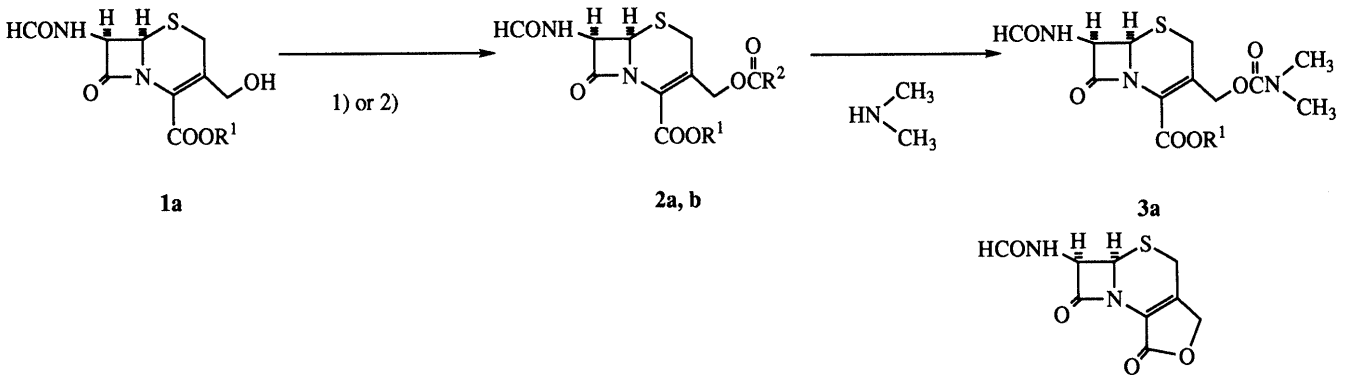


Fig. 1. The Structure of E1101

Chart 2 illustrates our new method. In the previous methods,[2,3] the ester **2a** or **2b** was used as a key intermediate for the reaction with secondary amines, and excess and unreacted amines acted as a base to cause isomerization of the double bond from Δ^3 to Δ^2. So, we considered that if cephalosporins **5** with the activated ester



1a **2a, b** **3a** **4**

CDI method (R^1=CH(C_6H_5)$_2$, R^2=1-imidazolyl): 1) *N*,*N*′-carbonyldiimidazole / THF at O °C, PNP method (R^1=CH(C_6H_5)$_2$, R^2= *p*-$NO_2C_6H_4O$): 2) *p*-$O_2NC_6H_4OCOCl$ / pyridine / THF

Chart 1

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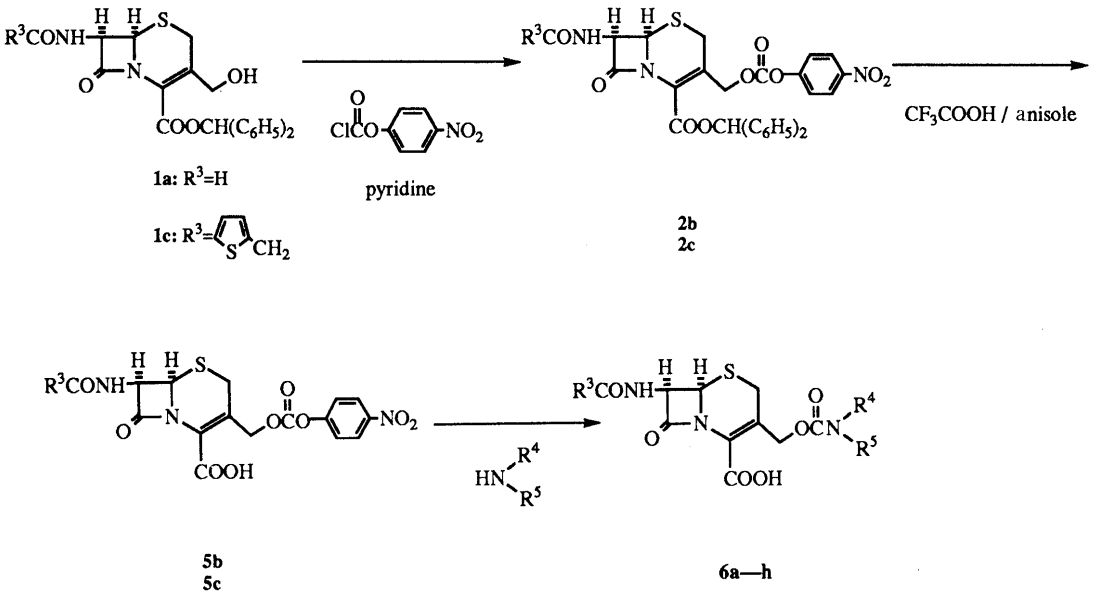


Chart 2

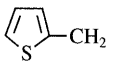
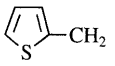
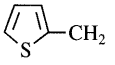
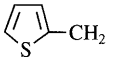
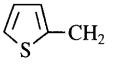
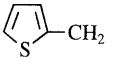
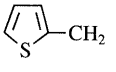
moiety at the C-3 position and carboxylic acid at the C-4 position could be obtained, the secondary amines would form a salt with the carboxylic acid, and then react with activated ester without isomerization. This concept has been described in the previous report.[2] However, the reaction of 3-(imidazolylcarbonyloxy)methyl cephem carboxylic acids ($R^1=H$, $R^2=1$-imidazolyl, in Chart 1) with dimethylamine was found to afford only the lactone (**4**), because the carboxylate anion attacked the activated ester moiety intramolecularly. This result indicated that the stability of **5** would be a crucial factor in our strategy.

At the first step of our new method, the alcohol **1a** was treated with 4-nitrophenyl chloroformate in the presence of pyridine in tetrahydrofuran (THF)–CH_3CN (7 : 1 vol) to afford the 4-nitrophenyl carbonate **2a** or **2c** in 95% yield. In fact, THF was used as a reaction solvent in the previous report.[3] However, this reaction condition was found to be inappropriate for large-scale production, because gel-like precipitates of insoluble pyridine hydrochloride made stirring of the reaction mixture impossible, and the reaction did not go to completion. In order to solve this problem, we examined this reaction in various solvent systems, and finally, the THF and CH_3CN (7 : 1) system was found to be the best, because pyridine hydrochloride could be dissolved sufficiently to allow stirring to continue.

At the next step, diphenylmethyl esters were deprotected with trifluoroacetic acid and anisole to give **5** in good yields, such as 98% for both **5b** and **5c**. As described above, the cephem carboxylic acids with a good leaving group at the C-3 position are easily transformed to lactones, so we tested the stability of **5** and found that these intermediates were unexpectedly stable under acid conditions and in several solvents including alcohols.

Table 1 shows the results of the reactions between these activated esters **5** and several secondary amines. The reaction proceeded smoothly under ice cooling to afford 3-(carbamoyloxy)methyl cephems **6a—h** at fairly good yields (around 80%). As we had anticipated, HPLC analysis[5] showed essentially no Δ^2 isomer in the reaction mixture, and exclusively furnished the Δ^3 isomer of 3-(*N*,*N*-disubstituted carbamoyloxy)methyl cephems, as was also confirmed by ^{1}H-NMR. The 7-formamido compound **6a**, a key intermediate for E1101, was isolated as the sodium salt, because it was too hydrophobic to be extracted with ethyl acetate. Thus, this method was regarded as a general route for preparing 3-(*N*,*N*-substituted carbamoyloxy)methyl Δ^3-cephems without generating the Δ^2 isomers. In a systematic investigation of this procedure, the 7-(2-thienylacetamido)cephem **5c** was treated with various types of secondary amines, and the corresponding products **6b—h** were synthesized. As shown in Table 1, **6b—d**, **f**, and **g** were obtained in good yields, though the *N*-methyl-*N*-(2-propargyl) **6e** and

Table 1. Synthesis of 3-(*N*,*N*-Disubstituted Carbamoyloxy)methyl Cephems (**6**)

Entry	R^3	R^4, R^5	Solvent	Product (Yield %)[a]
1[b,c]	H	CH_3, CH_3	MeOH	**6a** (77)
2[c]		CH_3, CH_3	DMF	**6b** (81)
3		CH_3, C_2H_5	DMF	**6c** (71)
4		C_2H_5, C_2H_5	DMF	**6d** (81)
5		CH_3, $CH_2C\equiv CH$	DMF	**6e** (50)
6[c]			DMF	**6f** (74)
7			DMF	**6g** (82)
8			DMF	**6h** (41)

a) Isolated yield, analyzed by HPLC. *b*) Isolated as the sodium salt. *c*) HPLC analysis of the reaction mixture was carried out (ref. 5).

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morpholino cephem **6h** were isolated in lower yields, because both of them were lost in the aqueous phase due to their high water solubility.

Thus, we have succeeded in developing a new method for synthesizing 3-(*N*,*N*-disubstituted carbamoyloxy)-methyl Δ^3 cephems exclusively *via* 3-(4-nitrophenoxycarbonyloxy)methyl cephem carboxylic acids (**5**) as key intermediates. This approach is amenable to scale-up. Furthermore, since 3-(substituted carbamoyloxy)methyl cephems were reported to be useful components of dual-action type drugs,[3,4] this new method should provide a practical and advantageous process for manufacturing such compounds.

Experimental

The melting points were determined on a Yamato MP21. IR spectra were recorded on either a Hitachi 260-30 or a Nicolet 205 FT-IR spectrometer. Mass spectra were determined on a JEOL JMS HX100. ^{1}H-NMR spectra were measured on a Varian UNITY 400 using tetramethylsilane as an internal standard. In general, commercially available organic solvents were used, and evaporation and concentration were carried out under reduced pressure below 30 °C.

Diphenylmethyl (6*R*,7*R*)-7-Formamido-3-(4-nitrophenoxycarbonyloxy)methyl-Δ^3-cephem-4-carboxylate (2b) 4-Nitrophenyl chloroformate (522.3 g, 2.59 mol) was added to a solution of diphenylmethyl 7-formamido-3-hydroxymethyl-Δ^3-cephem-4-carboxylate (**1a**, 1.0 kg, 2.35 mol) in THF (7 l) and MeCN (1 l) under ice cooling, and the mixture was stirred for 10 min. Then pyridine (205 g, 2.59 mol) was added dropwise for 30 min and the reaction mixture was stirred for another 40 min. EtOAc (24 l) was added and the organic layer was washed with water and then brine, and dried over $MgSO_4$. The filtrate was evaporated *in vacuo* and the residue crystallized with the aid of diisopropyl ether was collected by filtration to afford 1.32 kg of **2b** in 95% yield, mp 151—153 °C (dec.). IR (Nujol): 1790, 1760, 1715, 1655 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.67 and 3.77 (2H, ABq, J=18 Hz), 4.91 and 5.11 (2H, ABq, J=12 Hz), 5.21 (1H, d, J=5 Hz), 5.91 (1H, dd, J=5, 9 Hz), 6.91 (1H, s), 7.20—7.50 (12H, m), 8.14 (1H, s), 8.31 (2H, d, J=9 Hz), 9.11 (1H, d, J=9 Hz). MS *m/z*: 612 $(M+2Na-H)^+$. *Anal.* Calcd for $C_{29}H_{23}N_3O_9S$: C, 59.08; H, 3.98; N, 6.94. Found: C, 58.98; H, 3.93; N, 7.13.

(6*R*,7*R*)-7-Formamido-3-(4-nitrophenoxycarbonyloxy)methyl-Δ^3-cephem-4-carboxylic Acid (5b) The ester (**2b**) (1.0 kg, 1.70 mol) was added to a solution of anisole (0.5 l, 4.6 mol) and trifluoroacetic acid (1.0 l, 13 mol) under ice cooling, and the reaction mixture was stirred for 40 min at the same temperature. A solution of isopropyl alcohol (12 l) and diisopropyl ether (12 l) was added to the reaction mixture, and the precipitates were collected by filtration to give 705 g of **5b** in 98% yield, mp 111—113 °C (dec.). IR (Nujol): 1790, 1750, 1680 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.62 and 3.72 (2H, ABq, J=18 Hz), 4.94 and 5.23 (2H, ABq, J=13 Hz), 5.15 (1H, d, J=5 Hz), 5.81 (1H, dd, J=5, 9 Hz), 7.56 (2H, d, J=9 Hz), 8.13 (1H, s), 8.30 (2H, d, J=9 Hz), 9.07 (1H, d, J=9 Hz).

Sodium (6*R*,7*R*)-7-Formamido-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^3-cephem-4-carboxylate (6a) A solution of dimethylamine (393 g, 4.49 mol) in THF (0.4 l) was added dropwise to a stirred and ice-cooled solution of **5b** (856 g, 2.04 mol) in MeOH (10 l) over 30 min, and the reaction mixture was stirred at the same temperature for 15 min. A solution of sodium acetate (201 g, 2.45 mol) was added, and the mixture was concentrated under reduced pressure. To the resulting slurry were added isopropyl alcohol (4.3 l) and then diisopropyl ether (1.0 l), and the whole was stirred for 10 min. The precipitates were collected by filtration, and dissolved in MeOH (3.6 l). When the crystals appeared, isopropyl alcohol (13.1 l) was added, and the formed crystals were collected by filtration, washed with diisopropyl ether and dried *in vacuo* to give **6a** (552 g, 77%), mp 163—165 °C (dec.). IR (Nujol): 1750, 1710, 1650, 1610 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.79 (6H, s), 3.22 and 3.46 (2H, ABq, J=17 Hz), 4.68 and 4.94 (2H, ABq, J=12 Hz), 4.96 (1H, d, J=5 Hz), 5.55 (1H, dd, J=5, 9 Hz), 8.10 (1H, s), 8.93 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{12}H_{14}N_3NaO_6S\cdot 1.1H_2O$: C, 38.84; H, 4.40; N, 11.32. Found: C, 38.81; H, 4.37; N, 11.11.

Diphenylmethyl (6*R*,7*R*)-3-(4-Nitrophenoxycarbonyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylate[3] (2c) Pyridine (6.2 g, 0.08 mol) was added dropwise over 10 min to a solution of diphenylmethyl 3-hydroxymethyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylate (**1c**, 20.4 g, 0.04 mol) and 4-nitrophenyl chloroformate (15.8 g, 0.08 mol) in THF (300 ml) and MeCN (20 ml) under ice cooling below 5 °C. The reaction mixture was stirred for 30 min at the same temperature, then treated in a similar manner to that described for **2a** to yield 26 g of **2c** (95%), mp 138—140 °C (dec.). IR (Nujol): 1790, 1760, 1712, 1658 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.43 and 3.62 (2H, ABq, J=19 Hz), 4.85 (2H, s), 4.97 and 5.24 (2H, ABq, J=13 Hz), 5.01 (1H, d, J=5 Hz), 5.91 (1H, dd, J=5, 9 Hz), 6.93 (1H, s), 6.97—7.02 (2H, m), 7.24—7.38 (11H, m), 7.42 (2H, d, J=8 Hz), 8.26 (2H, d, J=8 Hz).

(6*R*,7*R*)-3-(4-Nitrophenoxycarbonyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (5c) Compound **2c** (10 g, 0.015 mol) was added to a solution of trifluoroacetic acid (20 ml) and anisole (20 ml) under ice cooling, and the reaction mixture was stirred for 2 h. Diethyl ether (400 ml) was added to it and the resulting precipitates were collected by filtration to afford 7.65 g of **5c** (98%), mp 78—80 °C (dec.). IR (Nujol): 1770, 1720, 1670 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 3.57 and 3.73 (2H, ABq, J=19 Hz), 3.80 (2H, s), 5.06 and 5.33 (2H, ABq, J=12 Hz), 5.09 (1H, d, J=5 Hz), 5.77 (1H, d, J=5 Hz), 6.90—7.00 (2H, m), 7.23—7.28 (1H, m), 7.46 (2H, d, J=8 Hz), 8.29 (2H, d, J=8 Hz).

(6*R*,7*R*)-3-(*N*,*N*-Dimethylcarbamoyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (6b) A 50% dimethylamine aqueous solution (0.92 ml, 4.4 mmol) was added dropwise over 10 min to a solution of **5c** (1.04 g, 2 mmol) in *N*,*N*-dimethylformamide (DMF) (10 ml) under ice cooling. To the mixture, EtOAc (80 ml) and then 1 M HCl solution (5 ml) were added. The organic layer was collected, washed with water and then brine, and dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was triturated with EtOAc and diisopropyl ether. The precipitates were collected by filtration to give 688 mg of **6b** (81%), mp 144—146 °C (dec.). IR (Nujol): 1775, 1730, 1690, 1660 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.82 (3H, s), 2.84 (3H, s), 3.48 and 3.60 (2H, ABq, J=19 Hz), 2.78 (2H, s), 4.48 and 4.99 (2H, ABq, J=12 Hz), 5.09 (1H, d, J=5 Hz), 5.64 (1H, dd, J=5, 8 Hz), 6.92—7.00 (2H, m), 7.38 (1H, d, J=3 Hz), 9.12 (1H, d, J=8 Hz). MS *m/z*: 426 $(M+H)^+$. *Anal.* Calcd for $C_{17}H_{19}N_3O_6S_2$: C, 47.99; H, 4.50; N, 9.88. Found: C, 48.16; H, 4.51; N, 9.51.

All the other compounds were synthesized by a similar method to that described for **6b**.

(6*R*,7*R*)-3-(*N*-Ethyl-*N*-methylcarbamoyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (6c) mp 148—146 °C (dec.), IR (Nujol): 1780, 1720, 1690, 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.98—1.09 (3H, m), 2.77—2.84 (3H, m), 3.18—3.30 (2H, m), 3.52 and 3.62 (2H, ABq, J=18 Hz), 3.76 (2H, s), 4.65 and 4.98 (2H, ABq, J=13 Hz), 5.11 (1H, dd, J=5, 8 Hz), 5.71 (1H, dd, J=5, 8 Hz), 6.92—6.96 (2H, m), 7.36 (1H, d, J=6 Hz). MS *m/z*: 440 $(M+H)^+$. *Anal.* Calcd for $C_{18}H_{21}N_3O_6S_2$: C, 49.12; H, 4.82; N, 9.56. Found: C, 49.02; H, 4.67; N, 9.61.

(6*R*,7*R*)-3-(*N*,*N*-Diethylcarbamoyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (6d) mp 142—143 °C (dec.). IR (Nujol): 1780, 1720, 1690, 1630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.00—1.10 (6H, m), 3.20 (4H, q, J=7 Hz), 3.51 and 3.60 (2H, ABq, J=19 Hz), 3.77 (2H, s), 4.66 and 4.99 (2H, ABq, J=13 Hz), 5.12 (1H, d, J=5 Hz), 5.77 (1H, dd, J=5, 8 Hz), 6.92—6.96 (2H, m), 7.37 (1H, d, J=5 Hz), 9.15 (1H, d, J=8 Hz). MS *m/z*: 454 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{23}N_3O_6S_2$: C, 50.32; H, 5.11; N, 9.27. Found: C, 50.14; H, 5.02; N, 9.10.

(6*R*,7*R*)-3-(*N*-Methyl-*N*-propargylcarbamoyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (6e) mp 115—117 °C (dec.). IR (Nujol): 1780, 1720, 1690, 1660 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.73—2.91 (3H, m), 2.88 (1H, s), 3.54 and 3.62 (2H, ABq, J=18 Hz), 3.76 (2H, s), 4.06 (2H, s), 4.70 and 5.02 (2H, ABq, J=14 Hz), 5.11 (1H, d, J=5 Hz), 5.70 (1H, dd, J=5, 8 Hz), 6.92—6.97 (2H, m), 7.36—7.38 (1H, m), 9.15 (1H, d, J=8 Hz). MS *m/z*: 450 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{19}N_3O_6S_2$: C, 50.77; H, 4.26; N, 9.35. Found: C, 50.26; H, 4.52; N, 9.17.

(6*R*,7*R*)-3-(1-Pyrrolidinylcarbonyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (6f) mp 155—156 °C (dec.). IR (Nujol): 1780, 1740, 1690, 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.76—1.86 (4H, m), 3.20—3.35 (4H, m), 3.52 and 3.62 (2H, ABq, J=18 Hz), 3.76 (2H, s), 4.66 and 5.00 (2H, ABq, J=13 Hz), 5.10 (1H, d, J=5 Hz), 5.69 (1H, dd, J=5, 8 Hz), 6.92—6.96 (2H, m), 7.36 (1H, d, J=6 Hz), 9.14 (1H, d, J=8 Hz). MS *m/z*: 452 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{21}N_3O_6S_2$: C, 50.54; H, 4.69; N, 9.31. Found: C, 50.20; H, 4.75; N, 9.02.

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(6*R*,7*R*)-3-(Piperidinylcarbonyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (6g) mp 158—160 °C (dec.). IR (Nujol): 1775, 1720, 1690, 1620 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.40—1.60 (6H, m), 3.30—3.45 (4H, m), 3.51 and 3.62 (2H, ABq, J=18 Hz), 3.77 (2H, s), 4.66 and 5.00 (2H, ABq, J=13 Hz), 5.11 (1H, d, J=5 Hz), 5.93 (1H, dd, J=5, 8 Hz), 6.93—6.97 (2H, m), 7.36 (1H, d, J=6 Hz), 9.14 (1H, d, J=8 Hz). MS m/z: 454 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{23}N_3O_6S_2$: C, 51.60; H, 4.98; N, 9.03. Found: C, 51.25; H, 4.89; N, 9.01.

(6*R*,7*R*)-3-(Morpholinylcarbonyloxy)methyl-7-(2-thienylacetamido)-Δ^3-cephem-4-carboxylic Acid (6h) mp 158—160 °C (dec.). IR (Nujol): 1785, 1715, 1680, 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.25—3.50 (4H, m), 3.50—3.70 (6H, m), 3.77 (2H, s), 4.69 and 5.02 (2H, ABq, J=13 Hz), 5.11 (1H, d, J=5 Hz), 5.70 (1H, dd, J=5, 8 Hz), 6.92—6.96 (2H, m), 7.36 (1H, d, J=5 Hz), 9.14 (1H, d, J=8 Hz). MS m/z: 468 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{21}N_3O_7S_2$: C, 48.81; H, 4.53; N, 8.99. Found: C, 48.60; H, 4.65; N, 8.90.

References and Notes

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5) HPLC analytical conditions: all the compounds were detected with a column of AM312 (ODS) at UV 254 nm. Compound **6a**: mobile phase, MeOH : H_2O : ammonium acetate = 400 : 600 : 2; flow rate, 1.7 ml/min; retention time, Δ^2 **6a** 4.6 min, Δ^3 **6a** 6.0 min. Compound **6b**: mobile phase, CH_3CN : H_2O : $HClO_4$ = 400 : 600 : 5; flow rate, 2.0 ml/min; retention time, Δ^2 **6b** 5.9 min, Δ^3 **6b** 7.4 min. Compound **6f**: mobile phase, CH_3CN : H_2O : $HClO_4$ = 400 : 600 : 5; flow rate, 2.0 ml/min; retention time, Δ^2 **6f** 8.0 min, Δ^3 **6f** 10.2 min.

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