Studies on the Stability of Δ^2 and Δ^3 Cephem Esters. I. Marked Difference in Stability between Δ^2 and Δ^3 Cephem Prodrug Esters and Application to the Preparation of Key Intermediates for Oral Cephem Synthesis

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The esterification of Δ^3-cephem-4-carboxylic acid sodium salt (1) with 1-iodoethyl isopropyl carbonate always afforded the Δ^2 cephem ester (3) as an inseparable minor component. However, in the course of formamido cleavage reaction, the 7-amino-Δ^2-cephem ester (5) was observed to be less stable than the Δ^3 cephem ester (4), which led us to develop a practical synthetic process for Δ^3 cephem esters, including a key intermediate of E1101, a new oral cephalosporin.

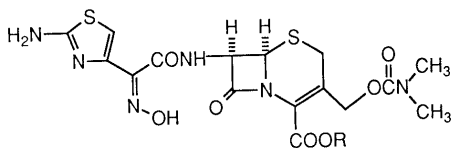
Key words antibacterial agent; oral cephalosporin; practical synthesis

The discovery of cefteram pivoxil[1] and cefixime[2] prompted many researchers in this field to look for a better oral cephalosporin with a broader antibacterial spectrum, more potent activity and better oral absorbability. As we reported in a previous paper,[3] extensive modification at the *N*-position at the C-3 carbamoyloxymethyl substituent resulted in the discovery of a well-balanced antibacterial agent (E1100, Fig. 1), and the further transformation of E1100 to several prodrug-type esters led us to obtain an optimum compound (E1101, Fig. 1) bearing an (*N*,*N*-dimethylcarbamoyloxy)methyl substituent at the C-3 position and a 1-(isopropoxycarbonyloxy)ethyl (PR) substituent at the C-4 carboxylic acid of the cephem nucleus.

One of the major problems in the synthesis of cephem esters such as E1101 is how to minimize the amount of incidentally produced Δ^2 cephem esters in the reaction between cephem carboxylate and the esterifying reagents. The only available report[4] on the exclusive preparation of Δ^3 cephem esters claimed that the solvent significantly affected the ratio of Δ^2/Δ^3, and the dioxane–*N*,*N*-dimethylformamide (DOX–DMF) system was the best solvent to afford Δ^3 cephem esters selectively. However, in our hands, these reaction conditions were not so effective. In the course of C-7 formamide cleavage reaction, the Δ^2 cephem ester (**5**) was noticed to be less stable than the corresponding Δ^3 cephem ester (**4**), and we could obtain the desired Δ^3 cephem ester in purer form by making use of this different stability. We report here this practical and useful method for the preparation of Δ^3 cephem esters.

Chart 1 shows an outline of the synthetic route to E1101 from 7-aminocephalosporanic acid (7-ACA). Our previous report[5] described a practical synthetic method for manufacturing 7-formamido-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^3-cephem acid sodium salt (**1**) without generating the corresponding Δ^2 cephem. We emphasized therein that exclusive Δ^3 cephem synthesis was the key point in the production of high-quality E1101, because Δ^2 and Δ^3 cephems have quite similar physical properties.

As shown in Chart 2, in the next step, the cephem acid sodium salt **1** was esterified with 1-iodoethyl isopropyl carbonate (I-PR)[6] to give the Δ^3 cephem PR ester (**2**), though the Δ^2 cephem ester (**3**) was found to be produced as a concomitant minor component. In order to improve the ratio of **2**/**3**, this reaction was examined in several kinds of solvents and typical results are shown in Table 1. DMF or *N*,*N*-dimethylacetamide (DMA) was a good solvent with respect to conversion from **1** to **2** (entries 1 and 2); however, the isolated products were found to contain 0.8—3.0% of the corresponding Δ^2 cephem ester **3**. In the case of dimethylsulfoxide (DMSO), the esterifica-



E1100 R=H

E1101 (HCl salt) R=CH(CH$_3$)OCOCH(CH$_3$)$_2$

Fig. 1. The Structures of E1100 and E1101

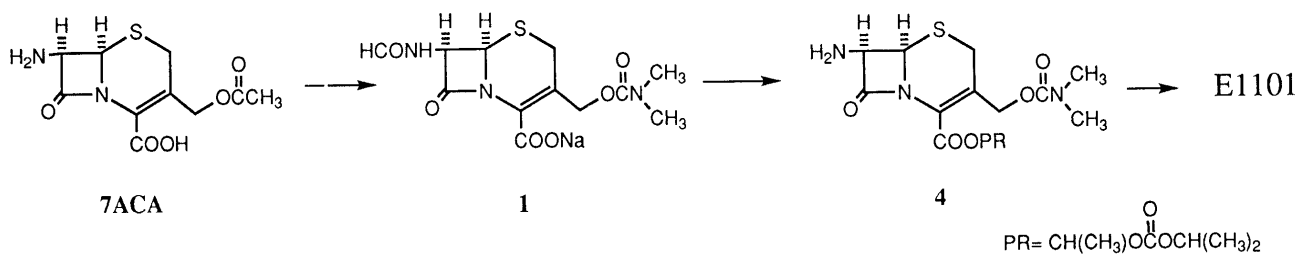


Chart 1

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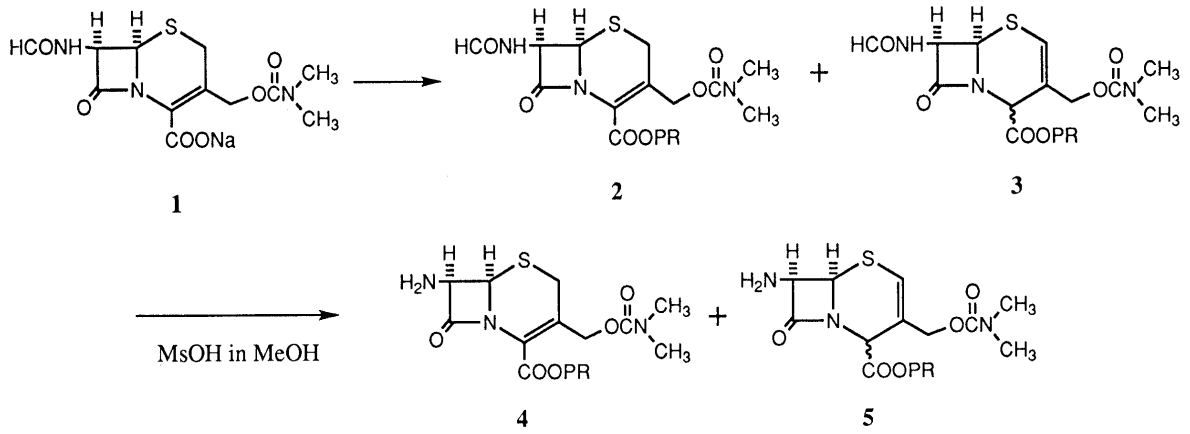

1 2 3 4 5

Chart 2

tion reagent (I-PR) decomposed and the reaction did not proceed smoothly (entry 3). In CH_3CN, the conversion was low (40%) even when the reaction time was extended (entry 4). The DOX–DMF system, which was Mobashery's best solvent system,[4] was found to be ineffective from the viewpoints of conversion (34%) and the isomer ratio (7.8%) (entry 5). An extensive investigation (including unpublished data) led to the following conclusion; slight Δ^3 to Δ^2 isomerization was unavoidable because unreacted starting material **1** acted as a base to attack the C-2 position of the product **2**, affording a mixture of the Δ^2 and Δ^3 cephem esters.[7] The amount of **3** in the product of entry 1 or 2 was not so large. However, it was extraordinarily difficult to isolate the Δ^3 cephem exclusively in the form of a 1:1 diastereomer mixture by ordinary purification methods such as crystallization or practical column chromatography due to the similar physical properties of the Δ^2 and Δ^3 cephems and the diastereomeric mixture arising from the asymmetric carbon at the ester moiety.

This problem was overcome by virtue of a fortuitous discovery in the course of the next C-7 formamido cleavage reaction of **2**. This reaction was carried out in the presence of 2 molar eq of methanesulfonic acid in methanol at room temperature and the reaction itself proceeded smoothly within 5 h. We happened to notice that the amount of 7-amino-Δ^2-cephem ester **5** was decreased in the isolated 7-amino-Δ^3-cephem **4** in the case of prolonged reaction time. We were then prompted to investigate the relative stability of **4** and **5** under this deprotection condition.

The Δ^2 cephem ester **5** was prepared as follows. The 7-formamido-Δ^3-cephem ester **2** was treated in the presence of 2 molar eq of triethylamine in tetrahydrofuran (THF) at room temperature for 18 h to afford an approximately 2:3 mixture of **2**/**3**. After isolation of the Δ^2 cephem **3** by chromatography, the C-7 formamido substituent was removed in the presence of methanesulfonic acid at 3 °C for 6 h to give the desired compound **5** in 85% yield. The purity of **5** was calculated to be 93% by HPLC analysis.

Figure 2 compares the stability of **4** and **5** under the formamido cleavage conditions at 30 °C. The Δ^3 cephem **4** was quite stable under these conditions, whereas the Δ^2 cephem **5** was found to be very unstable. The amount of

Table 1. Esterification of **1** to **2** with I-PR

Entry	Solvent	Reaction time (h)	Conversion[a] (%)	Δ^2 Cephem (**3**)[b] (%)
1	DMF	0.5	95	0.8—3.2
2	DMA	0.5	95	0.8—3.2
3	DMSO[c]	1.0	40	0.3
4	CH_3CN	16	40	4.0
5	DOX–DMF (3:5)	24	34	7.8

a) Conversion was determined by HPLC. *b*) The amount of **3** was determined by HPLC. *c*) I-PR decomposed. All reactions were carried out under ice cooling.

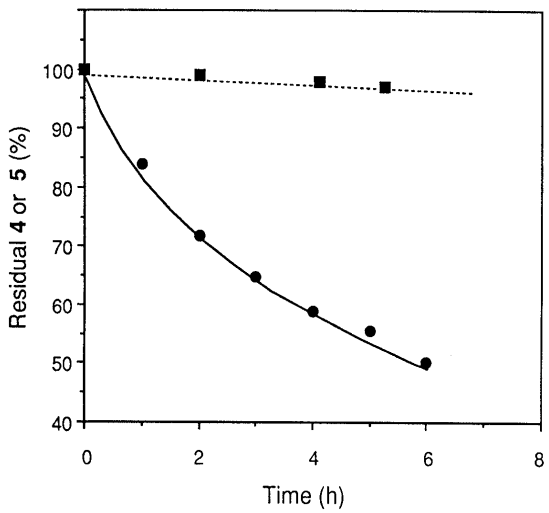

Fig. 2. Stability of **4** and **5** in the Presence of Methanesulfonic Acid in MeOH

For analytical conditions, see Experimental. Symbols: ■, **4**; ●, **5**.

5 decreased to a half of the initial concentration within 4 h. HPLC analysis indicated that the reaction process might be very complicated, because a number of peaks of polar products were observed in HPLC. These findings provided a clue to obtain a high-quality key intermediate **4**. In other words, the content of the Δ^2 cephem ester could be minimized simply by extension of the reaction time. The degraded compounds were too polar to be distributed into the extracted organic phase, and consequently, the Δ^2 content in the isolated product could be reduced from 0.8—3.0% to less than 0.4%.

A typical procedure is described below. Compound **1** was treated with I-PR (1.2 eq) in DMA under ice cooling to

give the formamido Δ^3 cephem ester **2** in 100% yield with 0.8%—3.0% of **3**. The cleavage reaction was carried out in the presence of methanesulfonic acid (2.0 eq) in MeOH for 18 h at room temperature. The reaction mixture was adjusted to pH 5.5, extracted with EtOAc and then treated with HCl to give the 7-amino-Δ^3-cephem PR ester **4** as the HCl salt form in 89% yield.

Thus, we could develop an efficient synthetic method to provide Δ^3 cephem esters preferentially by utilizing the marked difference in stability between Δ^2 and Δ^3 cephem esters in acidic conditions. β-Lactam compounds are well known to be labile under acidic and basic conditions; however, a previous report[8] disclosed that Δ^3 cephem prodrug esters are more stable under acidic conditions such as pH 4—2 than their corresponding carboxylic acids. To our knowledge, there has been no previous report on this interesting stability of Δ^2 cephem esters, which will be studied further in our laboratories.

Experimental

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

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-3-(*N*,*N*-Dimethylcarbamoyloxy)methyl-7-formamido-Δ^3-cephem-4-carboxylate (2) 1-Iodoethyl isopropyl carbonate[6] (452 g, 1.75 mol) was added to a solution of **1**[5] (1500 g, 1.42 mol) in DMA (2.5 l) in an ice bath, and the reaction mixture was stirred for 40 min at the same temperature. The mixture was diluted with EtOAc (10 l) and washed with water (4 l), then the organic layer was separated. The aqueous layer was washed with EtOAc (4 l). The combined extracts were washed with saturated aqueous $Na_2S_2O_3$ solution and then brine. Norit A (500 g) and $MgSO_4$ (500 g) were added to the organic solution, and the mixture was stirred for 30 min. It was filtered through Celite and the filtrate was washed with EtOAc (2 l). The filtrates and washings were combined and evaporated to afford **2** (654 g, 100%). The yellow oil **2** was used for the next step without further purification. IR (Nujol): 1780, 1750, 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.29—1.38 (6H, m, $CH(C\underline{H}_3)_2$), 1.58 (3H, d, J=5 Hz, CH_3), 2.94 (6H, s, $N(CH_3)_2$), 3.49 and 3.57 (1H, ABq, J=18 Hz, H-2 of one isomer), 3.49 and 3.58 (1H, ABq, J=18 Hz, H-2 of one isomer), 4.88—5.22 (4H, m, CH_2O, $C\underline{H}(CH_3)_2$, H-6), 5.88—5.94 (1H, m, H-7), 6.58 (0.5H, d, J=8 Hz, CONH of one isomer), 6.66 (0.5H, d, J=8 Hz, CONH of one isomer), 6.91 (0.5H, q, J=5 Hz, $C\underline{H}(CH_3)$ of one isomer), 7.00 (0.5H, q, J=5 Hz, $C\underline{H}(CH_3)$ of one isomer), 9.30 (1H, s, HCO).

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^3-cephem-4-carboxylate Hydrochloride (4) Methanesulfonic acid (274 g, 2.85 mol) was added to a solution of **2** (654 g, 1.42 mol) in MeOH (2.5 l) in an ice bath, and the mixture was stirred for 18 h at room temperature. A mixture of EtOAc (10 l) and water (6.6 l) was then added under ice cooling, and the pH of the whole was adjusted to 5.5 with saturated aqueous $NaHCO_3$. The organic layer was separated. The aqueous layers was extracted with EtOAc (4 l), the extracts were combined, and washed with brine, dried over $MgSO_4$, and concentrated. A solution of 4 M HCl in EtOAc (356 ml, 1.42 ml) was added to the residue at 10 °C, and the mixture was evaporated *in vacuo*. Diisopropyl ether (3 l) was added to the resulting oil, and the mixture was stirred vigorously. The precipitate was collected by filtration to give **4** (596 g, 89.4%) as a pale yellow powder, mp 117—119 °C (dec.). *Anal.* Calcd for $C_{17}H_{26}ClN_3O_8S \cdot 0.3H_2O$: C, 43.14; H, 5.66; N, 8.88. Found: C, 43.15; H, 5.65; N, 8.67. IR (Nujol): 1790, 1860, 1710 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.29 (6H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.55 (3H, d, J=6 Hz, CH_3), 2.92 (3H, s, NCH_3), 2.94 (3H, s, NCH_3), 3.63—3.83 (2H, m, H-2), 4.80—4.94 (5H, m, 1H of CH_2O, $C\underline{H}(CH_3)_2$, $NH_2 \cdot HCl$), 5.10—5.22 (2H, m, 1H of CH_2O, H-6), 5.24 (0.5H, d, J=5 Hz, H-7 of one isomer), 5.29 (0.5H, d, J=5 Hz, H-7 of one isomer), 6.83 (0.5H, q, J=6 Hz, $C\underline{H}(CH_3)$ of one isomer), 6.95 (0.5H, q, J=6 Hz, $C\underline{H}(CH_3)$ of one isomer).

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-3-(*N*,*N*-Dimethylcarbamoyloxy)methyl-7-formamido-Δ^2-cephem-4-carboxylate (3) A solution of **2** (7.06 g, 15.4 mmol) and triethylamine (1.6 g, 15.8 mmol) in THF (60 ml) was stirred for 18 h at room temperature to give a *ca.* 2:3 mixture of **2**/**3**. The reaction mixture was concentrated *in vacuo*, and the residue was chromatographed on silica gel (Wako C-200, 200 g) with dichloromethane–EtOAc (4:1) to afford **3** as a pale yellow oil (2.33 g, 33%). IR (Nujol): 1789, 1767, 1694 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.35 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.58 (3H, d, J=6 Hz, CH_3), 2.93 (6H, s, $N(CH_3)_2$), 4.58 and 4.70 (2H, ABq, J=13 Hz, CH_2O), 4.85—4.95 (1H, m, $C\underline{H}(CH_3)_2$), 5.04 (1H, s, H-4), 5.26—5.32 (1H, m, H-6), 5.72—5.80 (1H, m, H-7), 6.46 (1H, s, H-2), 6.43—6.49 (1H, m, CONH), 6.95 (0.5H, q, J=6 Hz, $C\underline{H}(CH_3)$ of one isomer), 6.98 (0.5H, q, J=6 Hz, $C\underline{H}(CH_3)$ of one isomer), 8.26 (1H, s, HCO). MS *m/z*: 460 $(M+H)^+$.

1-(Isopropoxycarbonyloxy)ethyl (6*R*,7*R*)-7-Amino-3-(*N*,*N*-dimethylcarbamoyloxy)methyl-Δ^2-cephem-4-carboxylate (5) Methanesulfonic acid (209 mg, 2.2 mmol) was added to a solution of **3** (500 mg, 1.09 mmol) in MeOH (2.5 ml) in an ice bath, and the mixture was stirred for 6 h at 3 °C. EtOAc (50 ml) and water (50 ml) were added to the mixture under ice cooling; the pH of the mixture was adjusted to 5.5 with saturated aqueous $NaHCO_3$, and then the organic layer was separated. The extracts were washed with water, then brine, and dried over $MgSO_4$. After removal of $MgSO_4$, the filtrate was concentrated under reduced pressure to give 400 mg of **5** as a pale yellow oil (85%). IR (Nujol): 1770, 1740, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.33 (3H, d, J=6 Hz, $CH(C\underline{H}_3)_2$), 1.56 (1.5H, d, J=5 Hz, $CH(C\underline{H}_3)$ of one isomer), 1.57 (1.5H, d, J=5 Hz, $CH(C\underline{H}_3)$ of one isomer), 2.92 (6H, s, $N(CH_3)_2$), 4.61 and 4.70 (2H, ABq, J=13 Hz, CH_2O), 4.86—4.95 (1H, m, $C\underline{H}(CH_3)_2$), 5.04 (1H, s, H-4), 5.19 (0.5H, d, J=4 Hz, H-6 of one isomer), 5.22 (0.5H, d, J=4 Hz, H-6 of one isomer), 6.46 (1H, s, H-2), 6.77 (0.5H, q, J=5 Hz, $C\underline{H}(CH_3)$ of one isomer), 6.79 (0.5H, q, J=5 Hz, $C\underline{H}(CH_3)$ of one isomer).

Kinetic Runs Substrate **4** or **5** was dissolved in a solution of methanesulfonic acid (0.66 M) and 4-bromobenzyl alcohol in MeOH (10 ml) at a concentration of 0.33 M, and the reaction mixture was stirred in a water bath equipped with a thermocontroller at 30 °C. The concentration of **4** or **5** was determined by HPLC.

HPLC Conditions The HPLC operating conditions were as follows: Apparatus; LC-10A system (Shimadzu Co., Kyoto, Japan) equipped with a UV detector (SPD-10A. Shimadzu), Rheodyne type 7125 injector valve and integrated data analyzer (C-R4A, Shimadzu). Stationary phase: AM312 5C18 packed column (YMC, Kyoto, Japan). Column temperature: 24 °C. Detection: UV 254 nm. Mobile phase: CH_3CN–0.1% aqueous ammonium acetate solution=30:70 (v/v). Flow rate: 2.0 ml/min. Retention times (min): 12.3, 13.6 (**4**), 11.0 (**5**), 8.7 (4-bromobenzyl alcohol, internal standard).

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