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Practical Large-Scale Synthesis of Cefmatilen, A New Cephalosporin Antibiotic

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Abstract:

A practical large-scale process for the synthesis of cefmatilen hydrochloride hydrate (1), a new oral cephalosporin antibiotic, is described. Several impurities are isolated from a bulk drug and identified. Side reactions are discussed in order to prevent them. The conditions were optimized to control the formation of impurities. The process is amenable to a multikilogram-scale preparation. Several kilograms of compound 1 for clinical trials were successfully prepared by this process from the three starting materials (7-aminocephem hydrochloride 4, triethylammonium acetate 5, and triazole 7) on a pilot scale in overall yields of 61−65% (10−14% higher than those for the previous process).

Introduction

Cefmatilen, which was discovered by Shionogi Research Laboratories, Shionogi & Co., Ltd., Osaka, Japan, is a new oral cephalosporin antibiotic.[1] Cefmatilen hydrochloride hydrate (**1**) was chosen as a candidate after screening several salts. Four protective groups (Boc for amino group, diphenylmethyl for carboxyl group, and two trityl for oxime and triazolyl groups) were used for the synthesis in the medicinal route.[1a,b] In the first-generation synthesis (Scheme 1), the trityl group as the protective group for triazolyl group was not used. The final intermediate, diphenylmethyl (−)-(6*R*,7*R*)-7-[(*Z*)-2-[2-(*N*-*tert*-butoxycarbonyl)aminothiazol-4-yl]-2-(triphenylmethyloxyimino)acetamido]-8-oxo-3-(*1H*-1,2,3-triazol-4-yl)thiomethylthio-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (**2**) was obtained as a crystal. The synthetic route for compound **1** from diphenylmethyl (−)-(6*R*,7*R*)-7-amino-8-oxo-3-methanesulfonyloxy-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride (**4**), triethylammonium (*Z*)-2-[2-(*tert*-butoxycarbonyl)aminothiazol-4-yl]-2-(triphenylmethyloxyimino)acetate (**5**), and 4-(acetylthiomethylthio)-1*H*-1,2,3-triazole (**7**) as starting materials[2] has been established. However, there were some problems in the first-generation process. The yield of coupling reaction at the 3-position of the cephem ring was still low. Some impurities in a drug substance (active pharmaceutical ingredient: API) were not identified and not controlled.

Control of impurities is significantly important for development of the manufacturing process of API since content of each impurity must be often controlled less than 0.10% in API. In addition, the structures of impurities give us valuable information on the mechanism of the expected reaction and unexpected side reactions. Therefore, it is particularly important to identify the impurities for the development of manufacturing process to increase the yield and to control the quality of the target product. In the case of the development of cefmatilen, the three impurities (Scheme 2) contained in a bulk drug of compound **1** were isolated and identified after the first-generation synthesis. In addition to them, numerous impurities in the reaction mixture, including two potential impurities (Scheme 3), were discussed. We investigated the side reactions of each step to increase the yield and to afford high quality of API by controlling the impurities, and then we developed an improved process (Scheme 4). We have already reported the isolation, identification, and preparation of impurity **9**.[3] Impurity **9** is significantly important because it acts as a habit modifier. We successfully controlled a crystal habit of compound **1** by controlling the formation of impurity **9** and established the deprotection and purification steps of an improved process to afford compound **1** from compound **2** on a bench scale.[3] In this contribution,[4] optimization of the conditions of the coupling steps at the 3- and 7-positions by controlling the impurities to give compound **2** and the large-scale synthesis of compound **1** from the three starting materials (7-aminocephem hydrochloride **4**, triethylammonium acetate **5**, and triazole **7**) on a pilot scale are described briefly.

Results and Discussion

Original Synthesis. We developed this first-generation synthesis by modification of the medicinal process for preparing biobatches and clinical batches of API at the early stages of the development. The synthetic route is described in Scheme 1. Triethylammonium acetate **5** was activated with

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(1) (a) Kubota, T.; Kume, M. U.S. Patent 5,214,037, 1993. (b) Kume, M.; Kubota, T.; Kimura, Y.; Nakashimizu, H.; Motokawa, K.; Nakano, M. *J. Antibiot.* **1993**, *46*, 177−192. (c) Kume, M.; Kubota, T.; Kimura, Y.; Nakashimizu, H.; Motokawa, K. *J. Antibiot.* **1993**, *46*, 316−330. (d) Kume, M.; Kubota, T.; Kimura, Y.; Nakashimizu, H.; Motokawa, K. *Chem. Pharm. Bull.* **1993**, *41*, 758−762.

(2) Takahashi, H.; Ide, Y. U.S. Patent 5,407,929, 1995.

(3) Masui, Y.; Kitaura, Y.; Kobayashi, T.; Goto, Y.; Ando, Okuyama, A.; Takahashi, H. *Org. Process Res. Dev.* **2003**, *7*, 334−338.

(4) A portion of this study was patented: Okuyama, A.; Masui, Y.; Kobayashi, T.; Goto, Y. Pat. Appl. No. JP 2001-223866, 2001.

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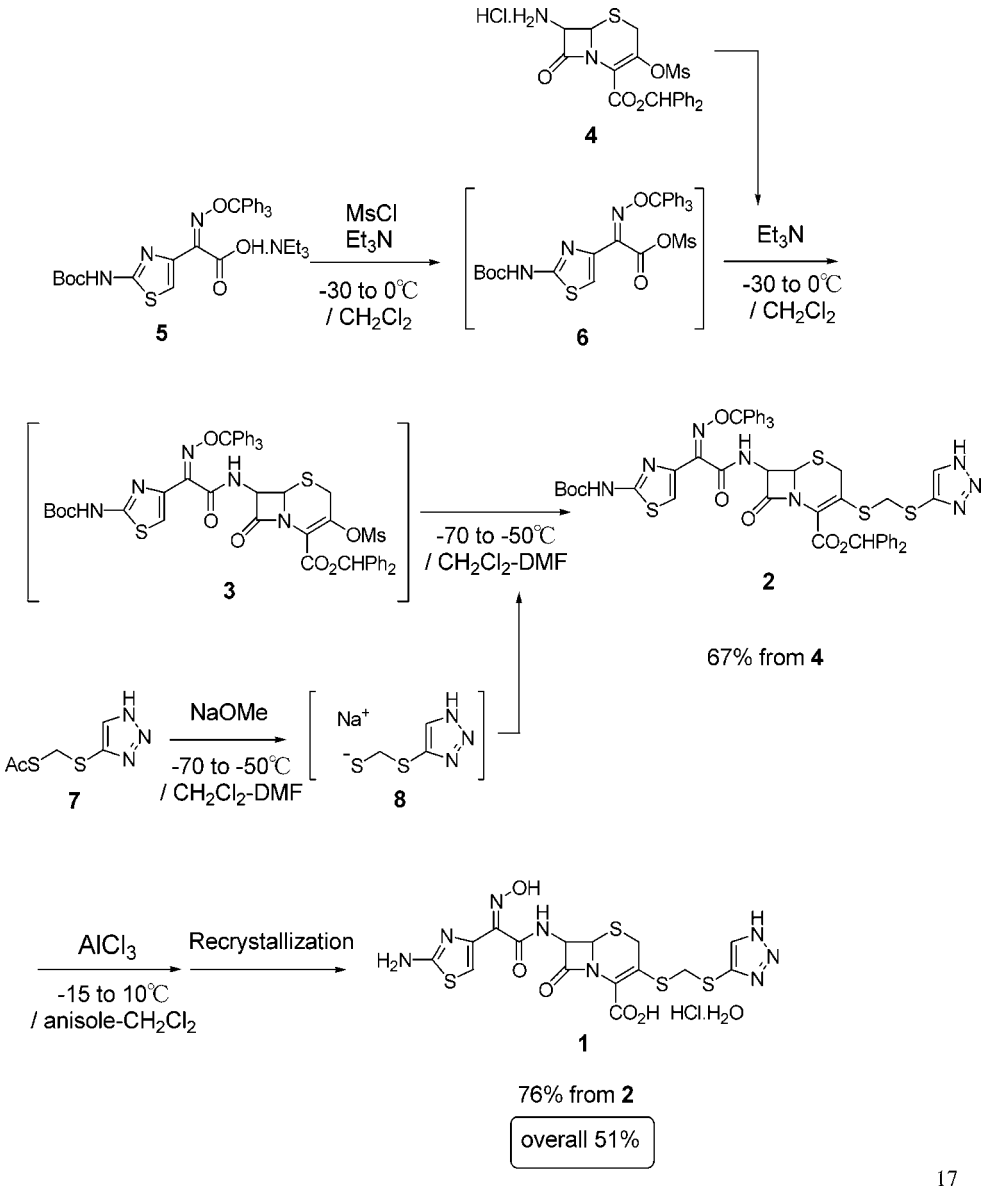
Scheme 1. **Original synthesis**



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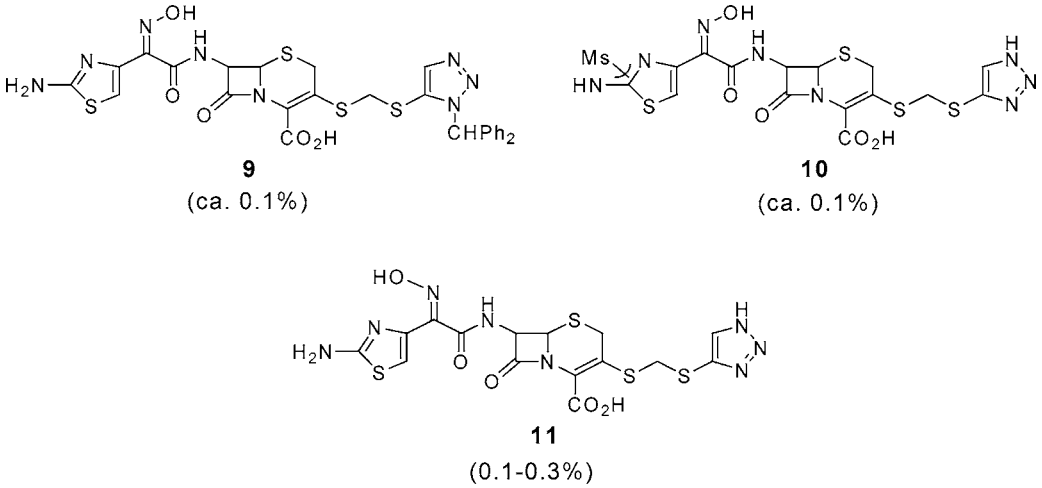
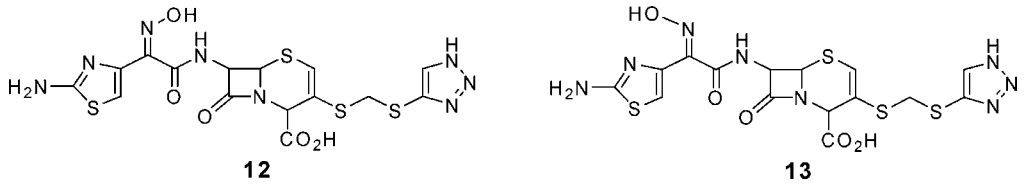
Scheme 2. **Impurities isolated from a bulk drug of cefmatilen hydrochloride hydrate (1)**



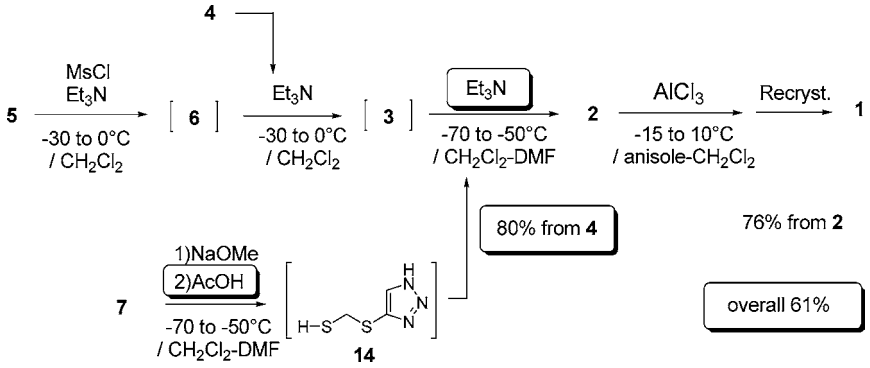
methanesulfonyl chloride (MsCl) to give mixed anhydride **6**. 7-Aminocephem hydrochloride **4** reacted with mixed anhydride **6** in the presence of triethylamine (Et_3N) to afford in situ intermediate **3**. Triazole **7** was deprotected with sodium methoxide (NaOMe) at cryogenic temperatures to give sodium thiolate **8**. The coupling reaction between

Scheme 3. **Potential impurities (usually not existing in a bulk drug)**



12 **13**

Scheme 4. **Improved synthesis (Method A)**



intermediate **3** and sodium thiolate **8** at cryogenic temperatures afforded final intermediate **2** in 67% isolated yield based on 7-aminocephem hydrochloride **4**. Deprotection of intermediate **2** followed by recrystallization gave compound **1** in 76% yield (51% overall yield from 7-aminocephem hydrochloride **4**).

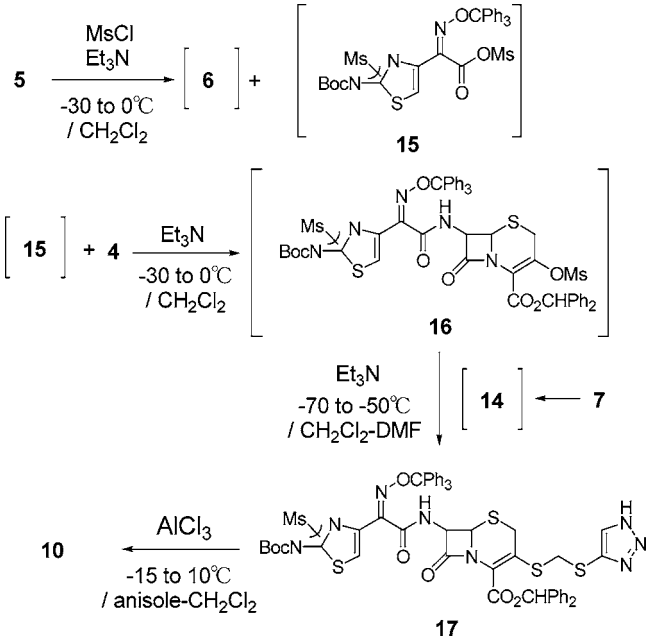
Investigation of Impurities. The following three impurities **9**, **10**, and **11** in a bulk drug of compound **1** were isolated and identified (Scheme 2).

Impurity **9**, which has a diphenylmethyl group at the *N*(3)-position of the triazolyl group of compound **1**, is one of impurities (commonly ca. 0.1% by area) in API. Impurity **9** acts as a habit modifier. We previously reported the isolation, identification, preparation, and roles of impurity **9**.[3]

Impurity **10**, which has mesyl group at the *N*-position of the aminothiazolyl group of compound **1**, is one of impurities (commonly lower than 0.1% by area) of API. We could not assign which *N*-position the mesyl group attaches to. Formation of impurity **10** is described in Scheme 5. Triethylammonium acetate **5** reacts with MsCl in the presence of Et_3N to give a mixture of mixed anhydrides **6** and **15**. A coupling reaction between mixed anhydride **15** and 7-aminocephem hydrochloride **4** gives compound **16**, which reacts with thiol **14** in the presence of Et_3N to afford compound **17** in the same way as compound **3**. Deprotection of compound **17** gives impurity **10**.

Impurity **11**, a major impurity (0.1−0.3% by area) of API, is the *E*-oxime isomer of cefmatilen. There are two paths to form impurity **11** as shown in Scheme 6. Isomerization of compound **1** gives impurity **11** under acidic conditions in the crystallizing system of compound **1** (Path 1). In the case of Path 2, compound **2** was hydrolyzed under acidic conditions in the presence of water during crystallization to afford compound **18**. Compounds **18** and **20** were observed as by-products in the process, but compound **19** was not. No isomerization of compound **2** to give *O*-tritylated derivative **19** was observed. Compound **18** can be easily isomerized to give isomer **20**, which is deprotected by a

Scheme 5. **Formation of impurity 10**

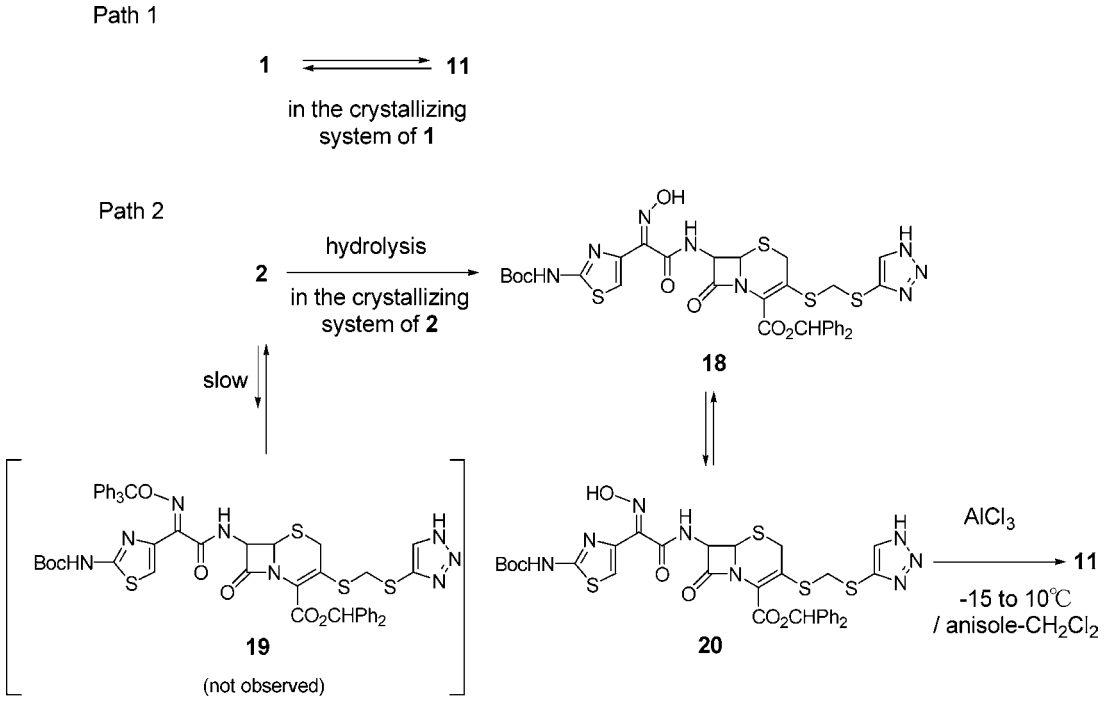


Lewis acid to give isomer **11**. A residual strong mineral acid which was used for quenching the coupling reaction at the 3-position should be removed by extraction before crystallization of compound **2** to prevent deprotection of trityl group.

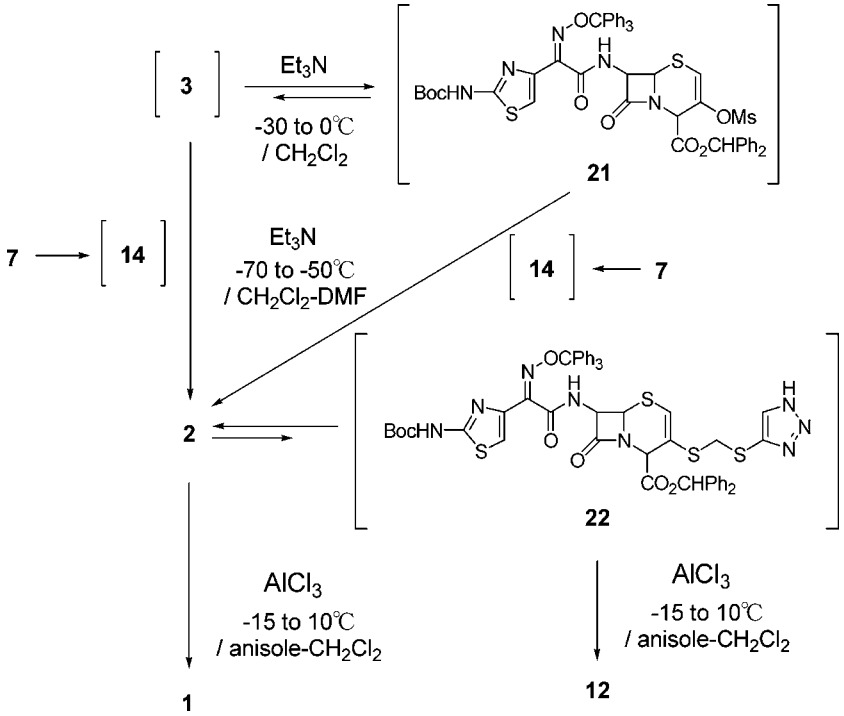
The following two compounds **12** and **13** are potential impurities of API (Scheme 3). There is no impact on the quality of API; however, there is significant impact on the yield of compound **1**.

Compound **12** is a Δ^2-isomer of compound **1**. Compound **12** was not always detected in bulk quantities of API. Sometimes compound **12** existed in the mothor liquor left after filtration of compound **1**. The paths to form compound **12** are shown in Scheme 7. Intermediates **21** and **22** were observed as by-products in the process. Isomerization of the double bond occurred during the coupling reaction at the

Scheme 6. **Formation of impurity 11**



Scheme 7. **Formation of compound 12**



7-position to afford a mixture of compounds **3** and **21**. In the presence of base, compound **3** can be easily isomerized. There is an equilibrium between compounds **3** and **21**. A similar migration of the double bond to give a mixture of Δ^2- and Δ^3-isomers of acylated 7-amino-3-mesyloxycephem was reported by Scartazzini et al.[5] The ratio of compounds **3** and **21** depended on the reaction temperatures as shown in Table 1. The sum of yields of compounds **3** and **21** did not depend on reaction temperatures. Interestingly, the different ratios of compounds **3** and **21** gave compound **2** in the same yield (entries 1 and 4). This means the yield of compound **2** by the coupling reaction at the 3-position did not depend on the ratio of compounds **3** and **21**. The yield of compound **2** depended on the temperatures and bases used for the coupling reaction at the 3-position because of isomerization of compound **2** to give the Δ^2-isomer **22**. In the previous process, the coupling reaction at the 3-position was carried out under strong basic conditions in the presence

(5) Scartazzini, R.; Schneider, P.; Bickel, H. *Helv. Chim. Acta* **1975**, *58*, 2437−2450.

Scheme 8. **Mechanism of the coupling reaction at 3-position**

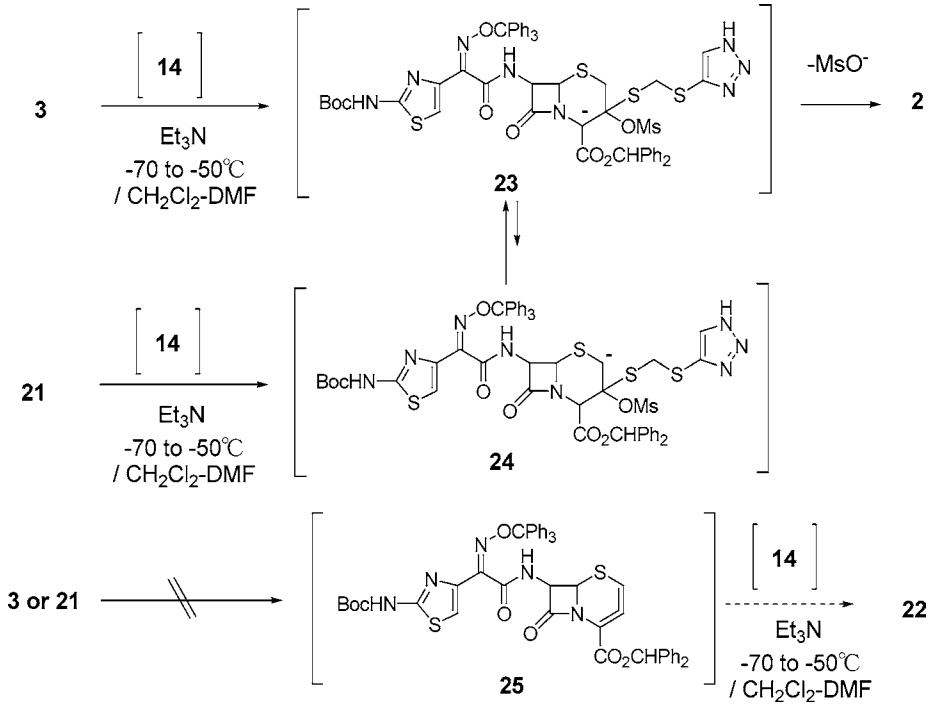


Table 1. **Formation of compound 2 via compound 3 or 21**[a]

	coupling at 7-position			coupling at 3-position[e]
entry	temp,[b] °C	ratio[c] of **3**:**21**	yield[d] of **3** and **21**, %	isolated yield of **2**, %
1	−35	1:0	98	84
2	−10	2:1	99	−
3	0	2:3	99	−
4	15	2:3	99	85

[a] By using EtOAc as a solvent: conditions similar to those of Method B described in the Experimental Section. [b] For two reactions (activation of compound **5** with MsCl and coupling between compounds **4** and **6**). [c] Determined by ^{1}H NMR. [d] Determined by HPLC. [e] Carried out below −57 °C.

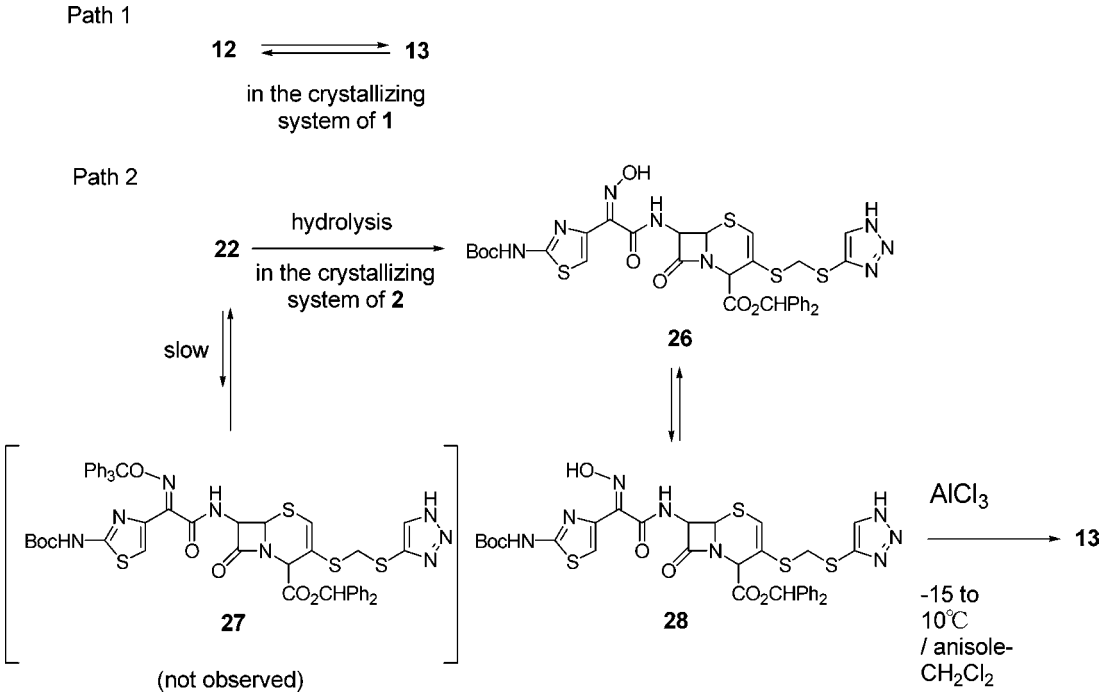
of residual NaOMe. In this case, Δ^2-isomer **22** formed in higher than 5% by area vs compound **2**. Although purification by crystallization of compound **2** can easily remove Δ^2-isomer **22**, isomerization of compound **2** is not preferred because of reducing the yield. To reduce the rate of isomerization to form Δ^2-isomer **22**, we then used a weak base such as Et_3N for the coupling reaction between compounds **3** and **14** which is afforded by neutralization of the excess strong base with a weak acid such as acetic acid (Scheme 4). As expected, the formation of Δ^2-isomer **22** was dramaically reduced, and the yield of compound **2** increased. Mechanisms of the coupling at the 3-position of cephems were discussed as shown in Scheme 8. A Michael addition−elimination on the corresponding triflate was proposed for the mechanism by Farina et al.[6] Although the other mechanism via cyclic allene intermediate generated from 3-mesyloxycephem was proposed by Cainelli et al.,[7] the Michael addition−elimination is reasonable for the mechanism of our process because of the following evidence: At the beginning of the coupling reaction at the 3-position at cryogenic reaction temperatures, compound **2** was observed as the only product by HPLC. Since the rate of isomerization of compound **2** to give Δ^2-isomer **22** was slow under the reaction conditions, if cyclic allene intermediate **25** was generated, the addition of thiol **14** to cyclic allene intermediate **25** would give Δ^2-isomer **22** as a major product.

E,Δ^2-isomer **13** is also a potential impurity (always lower than 0.05% by area) of API. There is no impact on the quality of API; however, there is significant impact on the yield of compound **1**. Sometimes compound **13** existed in the mother liquor left after filtration of compound **1**. There are two paths to form compound **13** as shown in Scheme 9. Isomerization of compound **12** gives compound **13** under acidic conditions in the crystallizing system of compound **1** (Path 1). In the case of Path 2, compound **22** was hydrolyzed under acidic conditions in the presence of water during crystallization to afford compound **26**. Intermediates **26** and **28** were observed as by-products in the process, but compound **27** was not. No isomerization of compound **22** to give compound **27** was observed. Compound **26** can be easily isomerized to give isomer **28**, which is deprotected by a Lewis acid to give compound **13**.

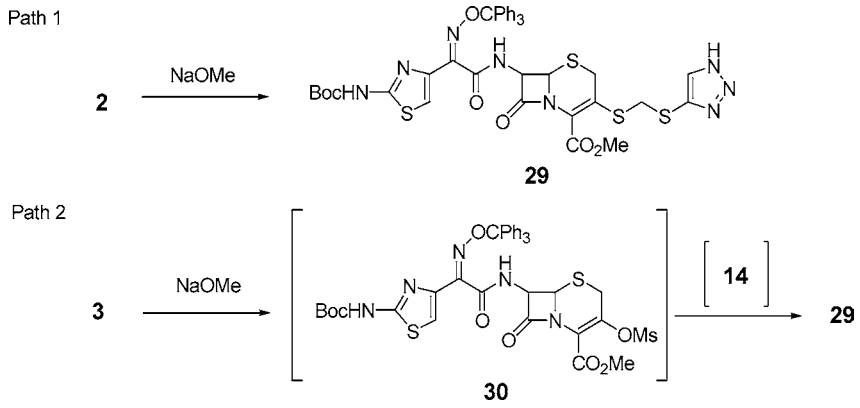
Compound **29**, one of the impurities in the bulk of final intermediate **2**, is methyl ester of carboxylate. There are two paths to form compound **29** as shown in Scheme 10. Reaction of compound **2** with NaOMe gives compound **29** (Path 1). Reaction of compound **3** with NaOMe gives compound **30**, which affords compound **29** by the coupling reaction with compound **14** (Path 2). Formation of compound **29** is not preferred because of reducing the yield of compound **2**. To reduce the formation of compound **29**, we then used a weak base such as Et_3N for the coupling reaction between

(6) Farina, V.; Baker, S. R.; Hauck, S. I. *J. Org. Chem.* **1989**, *54*, 4962−4966.
(7) Cainelli, G.; Contento, M.; Panunzio, M.; Sandri, S.; Umani-Ronchi, A.; Col, M. D. *Synlett* **1994**, 243−244.

Scheme 9. **Formation of compound 13**



Scheme 10. **Formation of compound 29**



compounds **3** and **14** which is afforded by neutralization of the excess strong base (NaOMe) with a weak acid such as acetic acid (improved process, Scheme 4). As expected, the formation of compound **29** was dramatically reduced, and the yield of compound **2** increased.

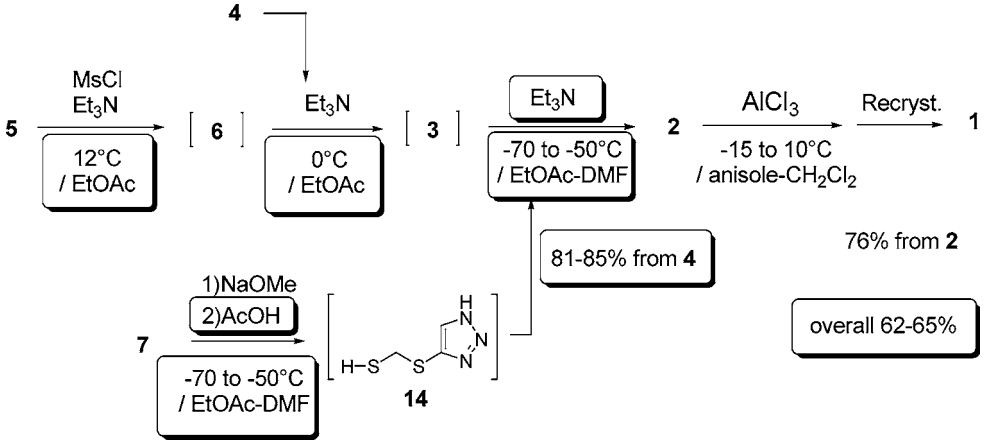
Improved Synthesis. We then developed two alternative processes as shown in Schemes 4 and 11. The formation of compounds **9**, **10**, **11**, **12**, **13**, and **29** were controlled by optimized conditions as described in the Experimental Section. The formation of compound **9** was controlled by the deprotection conditions, especially temperature when adding $AlCl_3$ according to our previous report.[3] The formation of compound **10** was controlled by the mesylation conditions, especially molar equivalents of MsCl. The formation of **11** and **13** was controlled by the work-up procedure of the 3-position coupling reaction. The reaction mixture was neutralized with glacial acetic acid below −50 °C before extraction to prevent the unexpected deprotection of trityl group. The formation of **12** and **29** was controlled by reaction conditions of the 3-position coupling reaction. After the deacetylation of compound **7**, resulting sodium thiolate **8** was neutralized with glacial acetic acid to give thiol **14**. The coupling reaction between compounds **3** and **14** in the presence of Et_3N at cryogenic temperatures gave Δ^2-isomer **22** and methyl ester **29** in smaller amounts than the reaction between compounds **3** and sodium thiolate **8** in the presence of excess NaOMe because of lower basicity. The overall yields from 7-aminocephem hydrochloride **4** were 61−65% (10−14% higher than those from the previous process).

Dichloromethane as a reaction solvent for the coupling reactions to prepare compound **2** can be replaced by EtOAc as shown in Scheme 11 (alternative process: Method B). Compound **2** can be crystallized from toluene, acetonitrile, or methyl isobutyl ketone (MIBK). MIBK is preferable because of the largest crystal size of compound **2**.

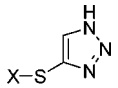
Unfortunately, cryogenic reaction temperatures are necessary because of the instability of intermediates **8** or **14** which are easily decomposed above −50 °C to give triazole thiolate **31**[8] (Scheme 12).

The new process is more practical and effective than the previous process because of well-controlled impurities and

Scheme 11. **Alternative process (Method B)**



Scheme 12



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higher yield. This process is amenable to large-scale production. In fact, several kilograms of compound **1** for clinical trials were successfully prepared by this process.

Conclusions

We identified several impurities isolated from a bulk drug of compound **1**, a new cephalosporin antibiotic, and discussed side reactions to prevent them. We described the optimization of the conditions of the coupling steps at the 3- and 7-positions by controlling the impurities to give compound **2** and the large-scale synthesis of compound **1** from the three starting materials (7-aminocephem hydrochloride **4**, triethylammonium acetate **5**, and triazole **7**) on a pilot scale in overall yield of 61−65% (based on 7-aminocephem hydrochloride **4**: 10−14% higher than the previous process).

Experimental Section

Materials and Instrumentation. Diphenylmethyl (−)-(6*R*,7*R*)-7-amino-8-oxo-3-methanesulfonyloxy-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hydrochloride (**4**) and 4-(acetylthiomethylthio)-1*H*-1,2,3-triazole (**7**) were commercially available. Triethylammonium (*Z*)-2-[2-(*tert*-butoxycarbonyl)aminothiazol-4-yl]-2-(triphenylmethyloxyimino)-acetate (**5**) was synthesized according to the literature method.[4] The HPLC analysis was carried out on a COSMOSIL column (Condition A: 150 mm × 4.6 mm, MeCN−0.02M phosphate buffer (7:3). Condition B: 100 mm × 4.6 mm, MeCN−aqueous $AcONH_4$ (0:1 to 9:1). The mobile phase was at a flow rate of 1 mL/min. NMR experiments were conducted by using a MERCURY 300 or a VXR 200 NMR spectrometer (Varian). IR spectra were obtained on a MAGNA 560 FT-IR spectrophotometer (Nicolet).

(8) The ^{1}H NMR spectra and the HPLC chromatogram of compound **31** were consistent with an authentic sample (sodium salt is commercially available from Tokyo Kasei Kogyo Co., Ltd.).

Improved Process. Method A. Preparation of Diphenylmethyl (−)-(6*R*,7*R*)-7-[(*Z*)-2-[2-(*N*-*tert*-Butoxycarbonyl)-aminothiazol-4-yl]-2-(triphenylmethyloxyimino)acetamido]-8-oxo-3-methanesulfonyloxy-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (3). Triethylammonium acetate **5** (44.2 kg, 70.1 mol) and CH_2Cl_2 (461 kg) were charged to a 600-L glass-lined reactor and dissolved. The solution was distilled under reduced pressure to remove residual water to give the concentrate (about 190 L). The distillation was continued until water content was less than 0.03%. The concentrate was transferred to a 600-L glass-lined reactor and cooled to −20 °C. After MsCl (7.6 kg, 66 mol) was added slowly to the solution below −15 °C, Et_3N (4.7 kg, 46 mol) was added dropwise to the solution below −15 °C. The reaction mixture was stirred for 1 h or more until the conversion was higher than 80% (by HPLC). 7-Aminocephem hydrochloride **4** (29.0 kg, 58.4 mol) was added to the reaction mixture, and then Et_3N (11.8 kg, 117 mol) was added dropwise below −15 °C. The reaction mixture was stirred for 1 h or more until content of thiazole **5** was less than 1% (by HPLC). After the reaction was quenched by the addition of 1.3% aqueous H_2SO_4 (178 kg), the layers were separated. The organic layer containing the product was washed with water (174 kg). Each aqueous layer was back extracted with CH_2Cl_2 (115 kg). The organic layers were combined and distilled under reduced pressure to remove residual water to give the concentrate (about 95 L). The volume of the concentrate was adjusted to about 115 L with CH_2Cl_2. This solution of diphenylmethyl ester **3** was used directly in the next step.

Preparation of Diphenylmethyl (−)-(6*R*,7*R*)-7-[(*Z*)-2-[2-(*N*-*tert*-Butoxycarbonyl)aminothiazol-4-yl]-2-(triphenylmethyloxyimino)acetamido]-8-oxo-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (2). CH_2Cl_2 (101 kg), DMF (14.6 kg), and triazole **7** (13.3 kg, 70.3 mol) were charged to a 600-L glass-lined cryogenic reactor and cooled to −60 °C. NaOMe (28%) in MeOH (27.0 kg, 140 mol) was added slowly to the solution below −57 °C. The solution was stirred for 2 h or more until the content of triazole **7** was less than 4% (by HPLC). The reaction was quenched by the slow addition of glacial AcOH (8.4 kg, 140 mol) at −57 to −67 °C, followed by stirring for 20 min. After the chilled (−10 °C) solution

of diphenylmethyl ester **3** (115 L) was added slowly to the mixture at −57 to −67 °C, Et_3N (24.2 kg, 240 mol) was added slowly at −57 to −67 °C. The reaction mixture was stirred for 2.5 h or more until content of compound **3** was less than 2% (by HPLC). The reaction was quenched by the slow addition of glacial AcOH (14.4 kg, 240 mol) at −57 to −67 °C. The mixture was added to an aqueous HCl (5.1%, 149 kg). The layers were separated. The organic layer was washed with water (116 kg). Each aqueous layer was back extracted with CH_2Cl_2 (115 kg). The organic layers were combined and MeOH (11.5 kg) was added. The extract was distilled under reduced pressure to give the concentrate (about 203 L). The concentrate was warmed to 32 °C, and then acetonitrile (137 kg) and seed crystal of compound **2** (0.10 kg) were added. After stirring for 2 h and cooling to 24 °C, the resulting suspension was distilled under reduced pressure to give the concentrate (about 230 L). The inside of vessel was washed with acetonitrile (46 kg). The resulting slurry (about 290 L) was stirred for 30 min at 22 °C, then cooled to 7 °C, and stored overnight. The precipitate was collected on a centrifuge, rinsed with acetonitrile (388 kg), and dried to give compound **2**[2] (47.8 kg, 80.0%). Mp 190−200 °C dec. t_R 11.5 min (HPLC condition A). ^{1}H NMR (300 MHz, $CDCl_3$−CD_3OD) δ 1.53 (s, 9H, −Me), 3.45, 3.63 (ABq, 2H, J = 17.2 Hz, 2-position of cephem ring), 4.12, 4.15 (ABq, 2H, J = 14.2 Hz, $-SCH_2S-$), 5.08 (d, 1H, J = 5.0 Hz, 6-position of cephem ring), 5.88 (d, 1H, J = 5.0 Hz, 7-position of cephem ring), 6.98 (s, 1H, −OCH<), 7.08 (s, 1H, 5-position of thiazolyl group), 7.20−7.50 (m, 25H, −Ph), 7.60 (s, 1H, 5-position of triazolyl group). IR (KBr) 3390, 3210, 1800, 1725, 1688, 1555, 1495, 1449, 1375, 1275, 1245, 1225, 1155 cm^{-1}.

Preparation of (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Hydrochloride Monohydrate (Cefmatilen Hydrochloride Hydrate: 1) as a Wet Crude Crystalline Powder. Anisole (45.0 kg) and anhydrous $AlCl_3$ (14.7 kg, 110 mol) were charged to a 600-L glass-lined reactor and dissolved. Anisole (45.0 kg), CH_2Cl_2 (279 kg), and compound **2** (22.5 kg, 22.0 mol) were charged into a 1000-L glass-lined reactor and cooled to −7 °C. The solution of $AlCl_3$ in anisole was added to the slurry below 4 °C, and the mixture was stirred for 1.5 h at 0 °C. The reaction was quenched with aqueous 7.2% HCl (127 kg) and MeOH (151 kg) at 13 °C or below, and the temperature was adjusted to 8 °C. The layers were separated. The aqueous layer containing the product was washed with CH_2Cl_2 (120 kg × 2). The temperature of the aqueous extract was adjusted to 11 °C, and seed crystal of compound **1** was added. The suspension was stirred for 1.5 h at 11 °C and distilled under reduced pressure to give the concentrate (about 120 L). The resulting slurry was cooled to 0 °C. The precipitate was collected on a centrifuge and rinsed with chilled water (110 kg) to give crude compound **1** (14.6 kg) as wet crystals.

Recrystallization of (−)-(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetamido]-8-oxo-3-(1*H*-1,2,3-triazol-4-yl)thiomethylthio-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Hydrochloride Monohydrate (Compound 1). Deionized water (140.0 kg) and wet compound **1** (14.6 kg) were charged to a 600-L glass-lined reactor and cooled to 5 °C. The slurry was dissolved at 5 °C by addition of aqueous 4% NaOH with the pH kept below 6.1. Cellulose powder (3.0 kg) was added to the solution and stirred for 20 min. The mixture was filtered through a Büchner funnel precoated with cellulose powder and activated carbon (1.2 kg), and the filter cake was rinsed with deionized water (35 kg). The pH of the filtrate was adjusted to 4.0 with 35% HCl at 5 °C. The resulting gelatinous slurry was cooled to 0 °C and then stored overnight. Deionized water (110 kg) and 35% HCl (130 kg) were combined to give 19% HCl and warmed to 40 °C. About a quarter of the gelatinous slurry was added to the aqueous 19% HCl at 37−44 °C, seed crystal (44 g) was added, and then the rest of the gelatinous slurry was added. The resulting slurry was cooled to 20 °C. The precipitate was collected on a centrifuge, rinsed with deionized water (100 kg), and dried on a fluidized bed dryer to give compound **1**[1b] (9.60 kg, 76%). t_R 17.4 min (HPLC condition B). ^{1}H NMR (300 MHz, CD_3OD) δ 3.76 (d, 1H, J = 17.2 Hz, 2-position of cephem ring), 3.92 (d, 1H, J = 17.2 Hz, 2-position of cephem ring), 4.30 (d, 1H, J = 13.6 Hz, $-SCH_2S-$), 4.38 (d, 1H, J = 13.6 Hz, $-SCH_2S-$), 5.22 (d, 1H, J = 4.8 Hz, 6-position of cephem ring), 5.80 (d, 1H, J = 4.8 Hz, 7-position of cephem ring), 7.14 (s, 1H, 5-position of thiazolyl group), 7.94 (s, 1H, 5-position of triazolyl group). ^{13}C NMR (75 MHz, CD_3OD) δ 29.7, 38.5, 59.7, 61.0, 110.1, 127.6, 131.1, 133.1, 133.9, 138.4, 143.7, 162.7, 164.8, 165.0, 172.6. IR (Nujol): 3420, 3310, 1782, 1720, 1666 cm^{-1}. MS *m*/*z* (relative intensity) 515 (100) [M + H$^+$], 499 (22), 402 (13). Anal. Calcd for $C_{15}H_{14}N_8O_5S_4.HCl\cdot 1.2H_2O$: C 31.46, H 3.06, N 19.57, S 22.39; found C 31.41, H 3.12, N 19.68, S 22.45.

Alternative Process: Method B. Preparation of Diphenylmethyl Carboxylate 3. Triethylammonium acetate **5** (41.88 g, 66.4 mmol) and EtOAc (300 mL) were charged to a 600-mL glass-lined reactor and dissolved. The solution was distilled under reduced pressure to remove residual water to give the concentrate (about 70 mL) whose water content was less than 0.03%. EtOAc was added to adjust the volume to 360 mL. The solution was cooled to 12 °C. MsCl (8.30 g, 72.4 mmol) was added slowly to the solution, Et_3N (4.89 g, 48.3 mmol) was then added dropwise at 12 °C, and the solution was stirred for 2 h or more until the conversion was higher than 95% (by HPLC). After the reaction mixture was cooled to 0 °C, 7-aminocephem hydrochloride **4** (30.00 g, 60.36 mmol) was added to the reaction mixture. After Et_3N (12.22 g, 121 mmol) was added slowly for 1 h at 0 °C, the mixture was stirred for 1 h or more until the conversion was higher than 99% (by HPLC). The reaction was quenched with aqueous 1.3% H_2SO_4 (184 g), and the layers were separated. The organic layer containing the product was washed with 5% NaCl (180 mL). Each aqueous layer was back extracted with EtOAc (90 mL). The organic layers were combined and distilled under reduced pressure to remove residual water to give the concentrate. The weight of the concentrate was adjusted to 120 g with EtOAc. This solution

of diphenylmethyl ester **3** was used directly in the next step.

Preparation of Diphenylmethyl Carboxylate 2. EtOAc (84 mL), DMF (15 g), and compound **7** (13.7 g, 72.4 mmol) were charged to a 1-L three-necked flask and cooled to −60 °C. NaOMe (23%) in MeOH (34.8 g, 145 mmol) and MeOH (6 mL) were added slowly to the solution below −57 °C, and the solution was stirred for 2 h or more until the content of compound **7** was less than 4% (by HPLC). The reaction was quenched by slow addition of glacial AcOH (8.7 g, 145 mmol) at −57 to −67 °C, followed by stirring for 20 min. Chilled solution (−10 °C) of diphenylmethyl ester **3** (120 g) was added slowly to the mixture at −57 to −67 °C. After Et_3N (33.0 g, 326 mmol) was added dropwise at −57 to −67 °C, the mixture was stirred for 2.5 h or more until the content of ester **3** was less than 2% (by HPLC). The reaction was quenched by slow addition of glacial AcOH (19.6 kg, 326 mmol) at −57 to −67 °C. The mixture was added to aqueous 8.6% HCl (200 g). The layers were separated. The organic layer was washed with 5% NaCl (180 mL). The organic layers were combined and distilled under reduced pressure to give the concentrate (about 160 g). The concentrate was warmed to 40 °C, and then MIBK (450 mL) and seed crystal of compound **2** (0.21 g) were added. After stirring for 40 min, the resulted suspension was distilled under reduced pressure to give the concentrate (348 g). The inside of the vessel was washed with MIBK (30 mL). The resulting slurry was stirred for 1 h at 0 °C. The precipitate was collected on a funnel, rinsed with MIBK (230 mL), and dried to give compound **2** (51.74 g, 80.5% yield) as a solvate which contained MIBK (4.88 wt %).

Isolation of Impurities from a Bulk Drug. The following two impurities were isolated by similar procedures as described in our previous paper.[3]

***N*-Mesyl-cefmatilen (10):** t_R 18.6 min (HPLC condition B). ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.93 (s, 3H, −Me), 3.78, 3.88 (ABq, 2H, J = 20 Hz, 2-position of cephem ring), 4.43 (s, 2H, $-SCH_2S-$), 5.20 (d, 1H, J = 4.7 Hz, 6-position of cephem ring), 5.72 (dd, 1H, J = 4.7 and 8.3 Hz, 7-position of cephem ring), 6.83 (s, 1H, 5-position of thiazolyl group), 8.03 (br s, 1H, 5-position of triazolyl group), 9.70 (d, 1H, J = 8.3 Hz, −CONH−), 12.12 (br s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.4, 35.5, 58.2, 59.0, 109.2, 125.0, 130.0, 130.5, 144.0, 161.6, 162.8, 163.1, 167.7. IR (Nujol): 1780, 1720, 1660, 1593, 1542 cm^{-1}. MS m/z 593 $[M + H]^+$.

***E*-Isomer of cefmatilen (11):** t_R 20.6 min (HPLC condition B). ^{1}H NMR (200 MHz, D_2O) δ 3.43, 3.56 (ABq, 2H, J = 17 Hz, 2-position of cephem ring), 4.07, 4.21 (ABq, 2H, J = 14 Hz, $-SCH_2S-$), 5.13 (d, 1H, J = 4.8 Hz, 6-position of cephem ring), 5.78 (d, 1H, J = 4.8 Hz, 7-position of cephem ring), 7.53 (s, 1H, 5-position of thiazolyl group), 7.86 (s, 1H, 5-position of triazolyl group). IR (KBr): 1760, 1620 cm^{-1}. MS m/z 515 $[M + H]^+$. Anal. Calcd for $C_{15}H_{14}N_8O_5S_4 \cdot 1.2H_2O$: C 33.60, H 3.08, N 20.90, S 23.92; found C 33.59, H 3.11, N 21.07, S 23.69.

Investigation of Side Reactions. The following compounds were identified by ^{1}H NMR or MS.

Compound 13: t_R 19.9 min (HPLC condition B). ^{1}H NMR (200 MHz, CD_3OD) δ 4.14 (d, 1H, J = 14 Hz, $-SCH_2S-$), 4.31 (d, 1H, J = 14 Hz, $-SCH_2S-$), 4.98 (d, 1H, J = 1.6 Hz, 4-position of cephem ring), 5.36 (d, 1H, J = 4.0 Hz, 6-position of cephem ring), 5.61 (d, 1H, J = 4.0 Hz, 7-position of cephem ring), 6.85 (d, 1H, J = 1.6 Hz, 2-position of cephem ring), 7.67 (s, 1H, 5-position of thiazolyl group), 7.95 (s, 1H, 5-position of triazolyl group). MS m/z 515 $[M + H]^+$.

Compound 18: t_R 2.5 min (HPLC condition A). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.50 (s, 9H, Me), 3.43 (m, 2H, 2-position of cephem ring), 4.02, 4.18 (ABq, 2H, J = 13 Hz, $-SCH_2S-$), 5.00 (d, 1H, J = 4.2 Hz, 6-position of cephem ring), 5.80 (dd, 1H, J = 4.2 and 5.3 Hz, 7-position of cephem ring), 6.91 (s, 1H, −OCH<), 7.15−7.50 (m, 11H, 5-position of thiazolyl group and −Ph), 7.56 (s, 1H, 5-position of triazolyl group), 8.86 (brs, 1H). MS m/z 781 $[M + H]^+$, 803 $[M + Na]^+$, 953 $[M + Na + NaI]^+$.

Compound 20: t_R 3.3 min (HPLC condition A). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.51 (s, 9H, −Me), 3.42, 3.55 (ABq, 2H, J = 15 Hz, 2-position of cephem ring), 4.06, 4.67 (ABq, 2H, J = 13 Hz, $-SCH_2S-$), 4.97 (d, 1H, J = 5.3 Hz, 6-position of cephem ring), 5.76 (dd, 1H, J = 5.3 and 9.5 Hz, 7-position of cephem ring), 6.95 (s, 1H, −OCH<), 7.20−7.50 (m, 14H, −Ph), 7.63 (s, 1H, 5-position of triazolyl group), 8.11 (s, 1H, 5-position of thiazolyl group), 8.56 (br d, 1H, J = 9.5 Hz, −NH−).

Compound 21 in a mixture with compound 3: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48 (s, 9H, −Me), 2.82 (s, 3H, −OMs), 5.23 (s, 1H, 4-position of cephem ring), 5.25 (d, 1H, J = 3.7 Hz, 6-position of cephem ring), 5.77 (dd, 1H, J = 3.7 and 8.7 Hz, 7-position of cephem ring), 6.21 (s, 1H, 2-position of cephem ring), 6.89 (s, 1H, −OCH<), 7.03 (s, 1H, 5-position of thiazolyl group), 7.15−7.50 (m, 25H, −Ph). cf. **Compound 3:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H, −Me), 2.78 (s, 3H, −OMs), 3.44, 3.66 (ABq, 2H, J = 18 Hz, 2-position of cephem ring), 5.04 (d, 1H, J = 4.5 Hz, 6-position of cephem ring), 6.03 (dd, 1H, J = 4.5 and 8.1 Hz, 7-position of cephem ring), 6.96 (s, 1H, −OCH<), 7.03 (s, 1H, 5-position of thiazolyl group), 7.15−7.50 (m, 25H, −Ph).

Compound 22: t_R 12.6 min (HPLC condition A). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.50 (s, 9H, -Me), 3.83 (q, 1H, J = 14 Hz, $-SCH_2S-$), 4.40 (q, 1H, J = 14 Hz, $-SCH_2S-$), 5.13 (d, 1H, J = 1.1 Hz, 4-position of cephem ring), 5.17 (d, 1H, J = 4.2 Hz, 6-position of cephem ring), 5.64 (dd, 1H, J = 4.2 and 8.4 Hz, 7-position of cephem ring), 6.59 (d, 1H, J = 1.1 Hz, 2-position of cephem ring), 6.86 (s, 1H, −OCH∠), 7.01 (s, 1H, 5-position of thiazolyl group), 7.2−7.5 (m, 25H, −Ph), 7.54 (s, 1H, 5-position of triazolyl group), 7.78 (d, 1H, J = 8.4 Hz, −CONH−). MS m/z 1045 $[M + Na]^+$.

Compound 28: t_R 3.6 min (HPLC condition A). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.54 (s, 9H, −Me), 3.70, 4.67 (ABq, 2H, J = 14 Hz, $-SCH_2S-$), 5.16 (d, 1H, J = 5.3 Hz, 6-position of cephem ring), 5.20 (d, 1H, J = 2.1 Hz, 4-position of cephem ring), 5.85 (dd, 1H, J = 5.3 and 10.5 Hz, 7-position of cephem ring), 6.68 (d, 1H, J = 2.1 Hz, 2-position of cephem ring), 6.86 (s, 1H, −OCH<), 7.25−

7.45 (m, 10H, −Ph), 7.72 (s, 1H, 5-position of triazolyl group), 8.47 (s, 1H, 5-position of thiazolyl group).

Compound 29: t_R 4.9 min (HPLC condition A). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.53 (s, 9H, −Me), 3.07 (d, 1H, J = 17 Hz, 2-position of cephem ring), 3.44 (d, 1H, J = 17 Hz, 2-position of cephem ring), 3.96 (s, 3H, MeO−), 3.99 (q, 1H, J = 15 Hz, $-SCH_2S-$), 4.34 (q, 1H, J = 15 Hz, $-SCH_2S-$), 4.93 (d, 1H, J = 5.3 Hz, 6-position of cephem ring), 5.42 (dd, 1H, J = 5.3 and 7.9 Hz, 7-position of cephem ring), 7.04 (s, 1H, 5-position of thiazolyl group), 7.23−7.40 (m, 15H, −Ph), 7.94 (s, 1H, 5-position of triazolyl group). MS m/z 871 $[M + H]^+$, 893 $[M + Na]^+$.

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