Process Development and Pilot-Scale Synthesis of Cefotetan

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Abstract:
Strategies that were adopted during the process development of Cefotetan in order to achieve a cost-effective commercial-scale synthesis are described herein. These included replacement of the trifluoroacetic acid used for cleavage of the benzhydryl ester and the development of an alternative synthetic route. This work led to improvement of both the impurity profile and the yield of the process. The pilot-scale synthesis of Cefotetan is described in detail in the Experimental Section. The scaled-up process has been successfully used for the commercial manufacture of Cefotetan since 1983.

Introduction
The overall process development of Cefotetan synthesis was outlined previously. This paper describes the modifications that were necessary to achieve a practical pilot-plant synthesis that could be scaled up for the commercial manufacture of this substance. Cefotetan (YM09330) is a semisynthetic cephamycin antibiotic that was discovered and developed by the Yamanouchi Pharmaceutical Co., Ltd., Japan, and has been on the market since 1983. This compound shows strong wide-spectrum antibacterial activity and has high stability against β-lactamase. This active pharmaceutical ingredient has been manufactured on an industrial scale at the Takahagi plant in Ibaraki prefecture, Japan, for more than 20 years. The synthetic route to Cefotetan is shown in Scheme 1. The starting material, Oganomycin GG (1), is itself the product of a biochemical process and is converted into Cefotetan (10) after a total of eight reactions.

In general, the complex syntheses and high dosages that are required for production of an antibiotic lead to high manufacturing costs, which represent a significant hurdle that must be overcome in order to bring these products to the market. Cefotetan was no exception; however, three target areas were identified in which cost improvement could be achieved. First, as the initial pilot-plant scale-up only afforded a 60% yield for the conversion of 1 to 6 and 40% for the conversion of 6 to 10, methods for improving these yields were investigated. Second, trifluoroacetic acid, which has been traditionally used to cleave the benzhydryl ester used to protect the carboxylic acid functionality in cephalosporin chemistry, was relatively expensive; therefore, a cheaper alternative to this reagent was sought. Third, the potential for developing an alternative synthetic route, with the exception of the rearrangement reaction, was explored.

Results and Discussion
Development of the Process from 1 to 6. The esterification of 1 by diphenyldiazomethane in CH₂Cl₂ produced 2 quantitatively, with nitrogen as a byproduct of the process. The reaction mixture could be used directly in the next step. Difficulties arose at this stage because the three intermediates, 3, 4, and 5, were highly unstable with moisture or at room temperature. The overall yield of this alternative synthetic route, which did not involve any rearrangement reaction, was around 40% from 1.

Scheme 1. Synthesis of Cefotetan (10)
temperature. Compound 6 was comparatively stable and was isolated with a 60% yield from 1. HPLC revealed that the three major byproducts obtained in the CH$_2$Cl$_2$ extract of 6 were approximately 10% of the 7-epimer (6a) (Scheme 2), approximately 5% of the $\Delta^2$-isomer (6b) (Scheme 3), and 2 or 3% of the intermediate (2).

**Scheme 2. Epimerization of the 7-MACA ester (5)**

Having predicted the mechanism by which these byproducts were produced, the rates of epimerization of 5 (Scheme 2) after the methanolysis reaction of 4 were measured at three different temperatures (−10, −24, and −40 °C). Since 5 was easily epimerized in methanol at room temperature, the rates were monitored using the following method. First, after the methanolysis reaction at −45 °C for 4 h, seven samples of the reaction mixture were maintained for 0, 1, and 3 h at each temperature. Next, this allowed conversion to 6, which was then analyzed using HPLC. The results, which are shown in Figure 1, revealed that methanolysis should be conducted at below −40 °C in order to significantly reduce the rate of epimerization.

**Scheme 3. Isomerization of the GG ester (2)**

The rate of $\Delta^2$-isomerization (Scheme 3) was subsequently investigated at three different temperatures in the presence of pyridine (Py) and at one temperature with triethylamine (TEA) as the base. The results are shown in Figure 2. The required amount of amine was added to a CH$_2$Cl$_2$ solution of 2, and the rate of formation of the $\Delta^2$-isomer was measured. These data showed that a weak base, such as Py, was more effective at preventing isomerization and also revealed that the temperature was crucial. The stability of 5 in the CH$_2$Cl$_2$ solution into which it was extracted was compared at three different temperatures, to determine the most suitable temperature for the acylation reaction. The results, which are shown in Figure 3, indicated that 5 was highly sensitive to temperature and that the acylation reaction should be kept at below −20 °C.

**Figure 1. Comparison of the epimerization rate of the 7-MACA ester (5) at different temperatures.**

**Figure 2. Comparison of the isomerization rate of GG ester (2) under different conditions.**

**Figure 3. Comparison of the stability of the 7-MACA ester (5) in CH$_2$Cl$_2$ solution at different temperatures.**

It was necessary to optimize the methods used to monitor the PCl$_5$ reaction, as the intermediate 3 reacted readily with water to give 2. Since the reaction was complete within 1 to 2 h, its progress was therefore monitored by measuring the amount of residual 2 using TLC within 10 min. The procedure involved adding 1 mL of the reaction mixture of 3 to methanol, to give 5 via the intermediate 4. Hydrochloric acid was then added to decompose 5, as its $R_f$ value was similar to that of 2, and the organic layer was spotted onto small TLC plates.

Experiments were then performed to determine the most suitable conditions for these processes with respect to temperature, reaction time, and the reagents used. This resulted in an optimization of the overall yield for converting 1 to 6 to 90%. In addition, the production of each of the three major byproducts 6a, 6b, and 2 was reduced to <1%.

**Cleavage of the Protected Carboxylic Acid 6.** Although several alternative reagents such as chloromethylmethyl ether, chloromethylbenzyl ether, diphenylbromomethane, and tert-butylidimethyl silyl chloride were investigated, none compared well in yield with the performance of diphenylidiazomethane for protection of the carboxylic acid. The difficulty with the esterification was due to the $\Delta^2$-isomer-
of 9 under basic conditions. Regarding the cleavage of the benzhydryl ester, trifluoroacetic acid, which has been generally chosen for the cleavage, was considered to be unsuitable for the commercial production because of its high price. A cheaper alternative was sought, and the phenomenon that HCl gas dissolves in CH$_2$Cl$_2$ at low temperatures$^6$ was exploited to convert 6 to 7 almost quantitatively at temperatures $< -30$ °C. On the other hand, the AlCl$_3$/nitromethane cleavage of benzhydryl ester derivatives was published$^7$ around the same time. The performance of the two methods was compared. The results indicated that the HCl/CH$_2$Cl$_2$ method performed better than the AlCl$_3$/nitromethane method on a large scale. A reliable supplier of diphenylidiazomethane was able to be sourced, and so the protection and cleavage issue was concluded.

**Development of the process from 6 to Cefotetan (10).** After 6 was converted to 7 using the method described above, 7 was extracted into aqueous NaHCO$_3$ solution and subsequently reacted with 8, still in aqueous solution, to give 9 quantitatively. In the final step, Cefotetan (10) was formed from its isomer (9) via an interesting rearrangement reaction. As shown in Figure 4, Cefotetan (10) was converted to 9 via an interesting rearrangement reaction. As shown in Figure 4, 9 remained mainly in the isothiazole form at pH 10 but was mostly rearranged in the dithietane form (10) at pH 9. Although the time required to reach equilibrium was shorter at higher pH and increased temperatures, the degradation of 9 and 10 was also accelerated under these conditions.

![Figure 4. Equilibrium between 9 and 10 in 0.1 M phosphate buffer at 37 °C.](image)

The equilibrium shifted towards 10 at pH 7.0 to 7.5, and the presence of an anion$^2$ of a weak acid, such as HCO$_3^-$, accelerated the rearrangement of 9 to 10. Therefore, the rearrangement of 9 was carried out in aqueous NaHCO$_3$, with the pH adjusted several times using an acid. When HCl or phosphoric acid was used in the pH adjustment, both 9 and 10 partially precipitated from the solution and the pH could not be accurately measured until the precipitate had redissolved. As an alternative, the introduction of CO$_2$ was found to be useful in the adjustment of pH and for acceleration of the rearrangement, because it did not produce any precipitate. Furthermore, both 9 and 10 were comparatively stable around pH 7 at 1 °C. The most suitable conditions for the rearrangement reaction were determined on the basis of this information, as shown in Figure 5. The pH was lowered using a stepwise process from 8.5 to 8.0 to 7.5, over a 45 h reaction period. The final equilibrium of 9/10 was 5:95 at 1 °C.

![Figure 5. Rearrangement of 9 at 1 °C.](image)

In the early stages of the process development, the mixture of 9 and 10 was extracted into ethyl acetate/2-butanol in the ratio 3:1. Treatment of the resulting mixture with ethanol afforded 10. However, the mixed solvent showed a low efficiency for dissolving 10, and the purification using ethanol was not particularly effective. Although absolute methylethyl ketone also dissolved 10 inefficiently, hydrated methylethyl ketone produced significantly better results. Therefore methylethyl ketone was chosen as the extraction solvent, and after concentration, methanol was added to precipitate (10) as an amorphous solid. Purification with methanol proved to be highly effective at removing impurities; however, the precipitate had the appearance of cotton wool, and filtration by centrifuge was extremely slow. It was therefore filtered using a filter press with a wide filtration area. The wet cake from the filter press was then converted into a suspension in ethanol, filtered again using the filter press, and dried to give high purity 10. The overall yield of 10 from 6 was about 68%.

As the filtrate contained approximately 22% of 10 and 5% of 9, a second recovery process was implemented. Although 10 could be recovered by concentrating the filtrate, 9 could not. Further investigation revealed that the Ca salts of both 9 and 10 could be recovered from the filtrate by the addition of triethylamine and CaCl$_2$. The recovered Ca salt comprised a mixture of both 9 and 10 in an approximately 2:8 ratio. Adding the recovered Ca salt to the next batch of the rearrangement-reaction mixture at a pH of 8.0 increased the yield of 10 from 6 to 87%.

**Conclusions**

This research program revealed that Cefotetan could be manufactured on a commercial scale using the route outlined in Scheme 1, with an overall yield for the conversion of 1 to 10 of 78%. Development was therefore scaled-up, and through collaboration with engineering specialists, a dedicated automated plant was completed in early 1983. Manufacture has subsequently continued without the need for further changes to the methods described.

**Experimental Section**

**Materials and Instrumentation.** Oganomycin GG was manufactured in the Takahagi plant of the Yamanouchi
Preparation of Diphenylmethyl 7β-Bromoacetamido-7α-methoxy-3-[[1-methyl-1H-tetrazol-5-yl]thiomethyl]-3-cephem-4-carboxylate (6) from Ogamomycin GG (1). Ogamomycin GG (1) (80.0 kg; 169 mol) and a 50% solution of DDM (72.3 kg, 372 mol) in CH₂Cl₂ were added to CH₂Cl₂ (744 L) at –5 °C. The reaction mixture was stirred for 2 d at 2 °C to produce 2. Pyridine (18.38 kg, 232 mol) and PCl₅ (44.1 kg, 211 mol) were added to the reaction mixture at 2 °C and warmed to 20 °C. The reaction mixture was stirred for 2 h at 18 to 21 °C to produce 3 and then cooled to –60 °C. Methanol, which was cooled to –45 °C (208 L), was added to the reaction mixture at –45 °C to produce 4. The reaction mixture was stirred for 4 h at –45 °C to produce 5. Next, 25% ammonia water (76.3 kg, 1120 mol) was added to water (216 L) and cooled to 2 °C. The cooled ammonia–water solution was added to the reaction mixture at –45 °C, and the mixture was stirred for 40 min at approximately 0 °C. The CH₂Cl₂ layer was separated and washed twice with cold water (280 L × 2) and cooled to –60 °C. Pyridine (22.1 kg, 279 mol) and bromoacetyl bromide (54.8 kg, 271 mol) were added to the CH₂Cl₂ layer at –60 °C, and the temperature of the reaction mixture increased to approximately –40 °C over a 10 min period. The reaction mixture was stirred for 1 h at –20 °C, then cold 5.43% H₂SO₄ (297 kg, 165 mol) was added and the mixture was stirred for 1 h at approximately 0 °C. The CH₂Cl₂ layer was separated and washed with cold water (280 L × 2) and cooled to –60 °C. Pyridine (22.1 kg, 279 mol) and bromoacetyl bromide (54.8 kg, 271 mol) were added to the CH₂Cl₂ layer at –60 °C, and the temperature of the reaction mixture increased to approximately –40 °C over a 10 min period. The reaction mixture was stirred for 1 h at –20 °C, then cold 5.43% H₂SO₄ (297 kg, 165 mol) was added and the mixture was stirred for 1 h at approximately 0 °C. The CH₂Cl₂ layer was separated and washed with water (288 L). The CH₂Cl₂ was almost completely removed by concentration under vacuum at a temperature <15 °C. Benzone (512 L) was added to the residue in order to promote the crystallization of 6. After the slurry of 6 was stirred for 15 h at 10 °C, the crystals were collected by centrifugal filtration and washed with benzene. The crystals of 6 contained a benzene molecule from the solvent of crystallization. Quantitative UV analysis showed the wet cake of 6 dissolved in CH₂Cl₂ (275 L) to be equivalent to 110 kg (156 mol) of dry crystals. The overall yield was 90%. 1H NMR (100 MHz, CDCl₃) δ 3.57 (s, 5H, –OCH₃), –3.78 (s, 3H, NCH₃), 3.88 (s, 2H, BrCH₂CO), 4.35 (q, 2H, –CH₂S–), 4.04 (s, 1H), 6.92 (s, 1H, –OCH₃), 3.62 (s, 1H, –OH, <), 7.36 (m, 17H, diphenyl, NH, CH₂).

Preparation of β-[4-((Carbamoyl carboxymethylene)-1,3-dithietan-2-yl)carboxamido]-7α-methoxy-3-[[1-methyl-1H-tetrazol-5-yl]thio][methyl]-3-cephem-4-carboxylic Acid (10) from 6. A mixed solution of CH₂Cl₂ (770 L) and n-butanol (8.8 L) was cooled to –60 °C, and HCl gas (55.5 kg, 1520 mol) was introduced at a temperature < –50 °C. The solution of 6 in CH₂Cl₂ was added dropwise into the HCl/CH₂Cl₂ solution at temperatures from –60 to –35 °C, and the reaction mixture was stirred for 3 h at –35 to –38 °C to produce 7. The completion of the reaction was confirmed using TLC. A mixed solution of water (330 L) and n-butanol (220 L) was added to the reaction mixture at –38 °C, and the temperature of the reaction mixture rose to –4 °C. After the mixture was stirred, the CH₂Cl₂ layer was separated and the aqueous layer was back-extracted with CH₂Cl₂ (142 L). A cold solution of water (621 L) and NaHCO₃ (28.1 kg) was added to the combined CH₂Cl₂ layers, and the mixture was stirred at 0 °C in order to extract 7 into the aqueous phase. The aqueous layer was washed twice with CH₂Cl₂ (248 L, 110 L). The pH of the aqueous phase was then adjusted to 6.7 using CO₂, and 32 kg of NaHCO₃ was added to the aqueous solution. An aqueous solution of tripotassium 4-carboxy-3-hydroxy-5-mercaptoisothiazole (8) (44.5 kg, 153 mol) in water (115 L) was cooled to 6 °C and added dropwise to the aqueous solution of 7 at 0 °C. As the aqueous solution of 8 was strongly basic, the pH of the mixture was adjusted using CO₂ to between pH 7.0 and 8.6 during the addition. The reaction of 7 and 8 was monitored using HPLC, and the addition of 8 was continued until the complete disappearance of 7 to produce 9. The pH of the reaction mixture was initially adjusted with CO₂ to pH 8.5, and stirring was continued for 3 h at 0 °C. The pH was then adjusted again, using CO₂, to a value of 8.0. The recovered Ca salt from the previous batch (39 kg) was added to the reaction mixture. This was initially stirred at pH 8.0 to 7.8 for 22 h at 0 °C and then at pH 7.5 for 24 h at 0 °C. Cold methylethyl ketone (1,260 L) and cold 20% HCl (218 kg) were added to the reaction mixture, and the product was extracted into the methylethyl ketone. The organic layer was separated, and the aqueous layer was back-extracted with methylethyl ketone (290 L). The moisture content of the combined organic layers was adjusted to 8.3% with water addition, activated charcoal (6 kg) was added to the organic layer, and the slurry was stirred for 30 min at 15 °C. The activated charcoal was then removed by filtration. The moisture of the filtrate was adjusted to 9.5% with further water addition. The organic solution was concentrated to about 250 L under vacuum at <15 °C. MeOH (1220 L) was added to the residue, and the slurry was stirred for 12 h at temperatures between 0 and –5 °C. The precipitate was filtered using a filter press at –5 °C, and the wet cake from the filter press was converted into a slurry in EtOH (850 L). The product was filtered again at –5 °C, and the wet cake was dried in vacuum at 10 °C to yield 76 kg (132 mol) of the desired product (10). The purity of 10 was 99.42% as HPLC area. The major impurities were 0.27% of Δ²-isomer of 10 and 0.20% of 9. The total yield was 87% from 6. 1H NMR (100 MHz, DMSO-d₆) δ 3.42 (s, 3H, –OCH₃), 3.62 (q, 2H, –CH₂S–), 4.29 (q, 2H, –CH₂S–), 5.12 (s, 1H), 5.16 (s, 1H, S₂=CH–), 7–8 (b, 2H, –NH₂), 9.63 (s, 1H, –NH–). HPLC was performed with UV detection at a wavelength of 254 nm using an ODS 150 mm × 4 mm column and eluting with 0.1 M phosphoric acid/acetonitrile/acetic acid/methanol, 17/1/1/1. TLC was performed with silica gel F₂₅₄ plates (Merck), eluting with ethyl acetate/n-hexane 2/1.
Recovery of the Ca Salt. A solution of CaCl₂ (0.67 kg) in water (0.67 L) and triethylamine (1.19 kg) was added to the first MeOH filtrate at 5 °C, and the mixture was stirred for 30 min at 0 °C. The resulting precipitate was removed using a filter press. The second EtOH filtrate was concentrated to 168 L under vacuum at around 20 °C. The residue and second MeOH filtrate were combined, and a solution of CaCl₂ (7.4 kg) in water (7.4 L) and triethylamine (13.43 kg) was added to the combined filtrates. The mixture was stirred for 15 h at 0 °C, and the resulting precipitate was collected using a filter press. The wet cake was dried under vacuum at 10 °C to yield 40 kg of the Ca salt.

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