Organic Process Research & Development **2003**, *7*, 846−850

Practical Large-Scale Synthesis of Doripenem: A Novel 1β-Methylcarbapenem Antibiotic

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Abstract:

A practical large-scale process for the synthesis of doripenem hydrate (1), a novel parenteral 1β-methylcarbapenem antibiotic, from *p*-nitrobenzyl-protected enolphosphate 2b and *N*-(*p*-nitrobenzyloxycarbonyl)-protected aminomethylpyrrolidine 3c is described. We found effective extraction conditions to remove *p*-toluidine and most other organic impurities using a THF/water system containing an inorganic salt. Significant improvements have been made to the previous synthesis using a medicinal chemical procedure. The new process requires no chromatographic purification and affords the target compound 1 as a sterile crystalline powder. Several kilograms of compound 1 were successfully prepared by this process.

Introduction

Carbapenem compounds are noted for their broad and potent antibacterial activity.[1] Imipenem,[2] panipenem,[3] meropenem,[4] biapenem[5] and ertapenem[6] are marketed products. In the cases of meropenem, biapenem and ertapenem, introduction of a 1β-methyl group to the carbapenem skeleton enhances metabolic stability to renal dehydropeptidase-1 (DHP-1) and leads to high antibacterial potency.[7] Doripenem hydrate (S-4661, **1**), which was discovered by Shionogi Research Laboratories, Shionogi & Co., Ltd., Osaka, Japan, is a novel parenteral 1β-methylcarbapenem antibiotic.[8] In our previous reports,[8,9] its synthesis, biology, and structure−activity relationships (SAR) have been reported. Compound **1** exhibits potent, broad, and well-balanced antibacterial activity against a wide range of both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*.

According to the conventional retrosynthetic analysis of a carbapenem, doripenem can be assembled from enolphosphate **2** and the 2-aminomethylpyrrolidin-4-ylthio-containing side chain **3** (Scheme 1). In the medicinal chemical route (Scheme 2),[8,9] compound **1** was prepared by deprotection of compound **5a** or **5b** with $AlCl_3$-anisole.[10] Compound **5a** or **5b** was synthesized from the diphenylmethyl-protected enolphosphate **2a** and *N*-*p*-methoxybenzyl(=PMZ)-protected aminomethylpyrrolidine **3a** or *N*-BOC-protected aminomethylpyrrolidine **3b**, respectively. Although this route facilitated the SAR studies and led to rapid optimization of lead derivatives, it had several drawbacks for a multikilogram-scale preparation of compound **1**. The two most serious problems resided in the isolation in the deprotection step. Compound **1** was isolated as a foam. The original route required chromatographic purification on Diaion HP-20. In the first-generation process (Scheme 3), we succeeded in obtaining compound **1** as a crystalline monohydrate. However, the process still required chromatographic purification, and the yield (49%) of compound **1** from compound **5b** through the deprotection, purification, and crystallization steps on a pilot scale was lower than that (72%) through the deprotection and purification step on a bench scale. We investigated the reason for this as follows: The step yields of the deprotection reaction were the same on bench and pilot scales. The yield of crystallization including sterilization on pilot scale was 88%. However, the yields of purified compound **1** before crystallization on bench and pilot scales were 72 and 56%, respectively. During chromatography and concentration of the eluents, decomposition of the target comound **1** was observed, resulting in a 16% yield decrease due to longer operating times on scale-up. To increase the yield and to avoid the chromatographic purification, we then developed an improved process which requires no chromatographic purification. In this contribution,[11a] we describe

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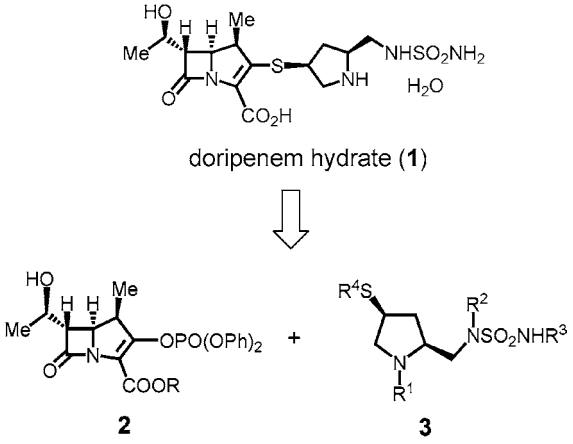
Published on Web 09/24/2003

10.1021/op034088n CCC: $25.00

Scheme 1



an efficient and practical synthesis of compound **1** from PNB-protected enolphophate **2b**[7,12] and *N*-(*p*-nitrobenzyloxycarbonyl(=PNZ))-protected aminomethylpyrrolidine **3c**.[11a,b] This process is amenable to large-scale production. Enolphosphate **2b** is also used for the synthesis of ertapenem as a starting material.[13] We have already reported an efficient and practical large-scale synthesis of aminomethylpyrrolidine **3c** in our previous paper.[11b] Both the enolphophate **2b** and aminomethylpyrrolidine **3c** are now commercially available.

Results and Discussion

Both deprotection processes, cleavage using Lewis acid ($AlCl_3$)-anisole and catalytic hydrogenolysis using Pd/C, to give compound **1** required chromatographic purification on Diaion HP-20 to remove impurities before this study. In the original process (Scheme 2)[8,9] and the first-generation process (Scheme 3), an $AlCl_3$-anisole system was used for the deprotection. The reaction mixture contained aluminum ion and numerous organic impurities (anisole, methoxybenzene derivatives and unknown water-soluble organic compounds). Anisole and methoxybenzene derivatives are easily removed by extraction. However, aluminum ion and several unknown organic impurities cannot be removed completely by extraction using acetylacetone. They were left in the API without chromatographic purification. On the other hand, after replacing the *N*-BOC or *N*-PMZ group with the *N*-PNZ group, we tried deprotection by catalytic hydrogenolysis with Pd/C. In this case, however, chromatographic purification was still necessary because the reaction mixture contained numerous kinds of impurities (*p*-toluidine and several unknown water-soluble organic compounds) which could not be removed completely by extraction because of their high solubility in water.

In the catalytic hydrogenolysis process, we found an extremely effective extraction process to remove *p*-toluidine and most of the other organic impurities using a THF/water system coupled with an inorganic salt to achieve a salting-out effect. The number of phase cuts, % recovery, and purity of compound **1** were examined by using a model system for the extraction in the presence of various inorganic salts. The aqueous layer was washed with THF (38 mL) until its amount was less than 5.5 g. The results are summarized in Table 1. The most important parameter is % purity. The theoretical water content is 4.1 wt %. With $MgCl_2$ and LiCl (entries 1 and 2), the amount of the residual inorganic salt was less than 0.1% because of their high solubility in aqueous MeOH. However, with the other salts (entries 3−7), residual salts were left in the product (8−30 wt %) because of their poor solubility in aqueous MeOH. LiCl required more phase cuts than $MgCl_2$. Therefore, we chose $MgCl_2$ as an additive. After the hydrogenolysis, $MgCl_2$ was added to the reaction mixture. As expected, after extraction the aqueous layer contained less than 0.1 wt % of *p*-toluidine. In addition, interestingly, $MgCl_2$ increased solubility of compound **1** in the aqueous layer.[14] Thus, $MgCl_2$ was used also as an additive to the biphasic reaction mixture to prevent precipitation during the hydrogenolysis and extraction. In a pilot procedure, the number of phase cuts was reduced by addition of THF after hydrogenolysis to shorten operating times (see Experimental Section).

To increase the yield and to avoid the chromatographic purification, we then developed an improved process for the synthesis of compound **1** which is shown in Scheme 4. Compound **5c** was synthesized by the coupling reaction between PNB-protected enolphosphate **2b** and in situ intermediate mercaptopyrrolidine **4c**, which was prepared from *N*-PNZ-protected aminomethylpyrrolidine **3c**, in 88% yield. Catalytic hydrogenolysis of compound **5c** with Pd/C in the presence of $MgCl_2$ in aqueous THF followed by an improved workup (removal of Pd/C by filtration, extraction, crystallization) afforded compound **1** as a nonsterile crystalline powder in 73% yield. The nonsterile crystal was sterilized and recrystallized to give compound **1** as a sterile API in 88% yield. The overall yield of compound **1** from compound **5c** was 64%, 15% higher than that by the first-generation process. The amounts of residual Pd and Mg in the API were lower than 20 ppm and 0.1%, respectively. The quality of API which was afforded by the improved procedure without chromatographic purification was satisfactory. This new process is more practical and effective than the previous process because it requires no chromatographic purification and affords the target com-

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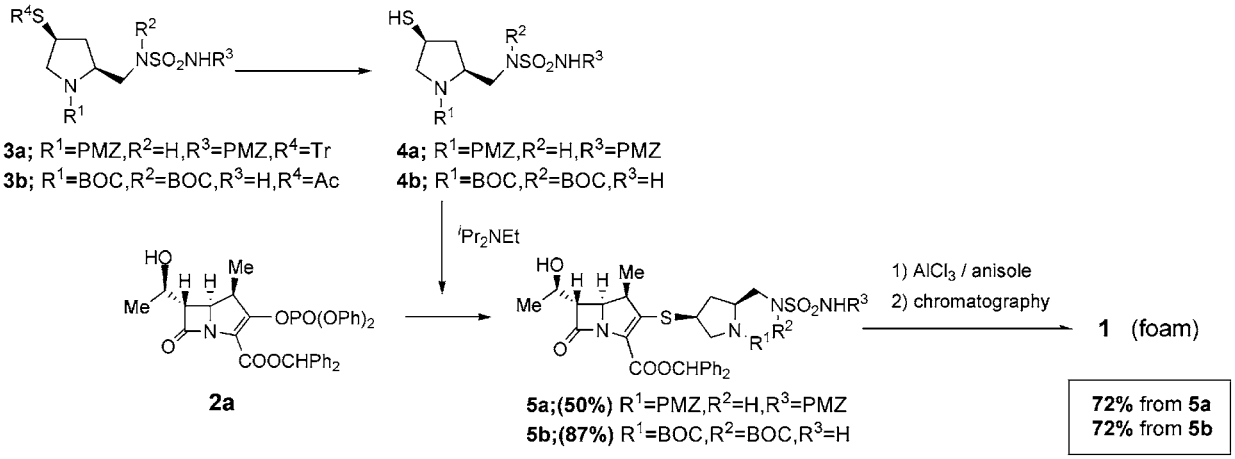
(14) $MgCl_2 \cdot 6H_2O$ was dissolved in a solution of compound **1** (0.1 g) in water (2 mL) at room temperature. Then THF (3 mL) was added to the solution at room temperature with stirring: (entry no., the amount of $MgCl_2 \cdot 6H_2O$, layer, result), (entry 1, 0 g, homogeneous, crystallized after 2 h), (entry 2, 0.06 g, homogeneous, immediately crystallized), (entry 3, 0.11 g, biphasic, slowly crystallized from aqueous layer after 0.5 h), (entry 4, 0.23 g, biphasic, no crystallization). $MgCl_2$ decreased solubility of compound **1** in homogeneous aqueous THF solution (entries 1 and 2). After appearance of aqueous and organic layers by salting-out effect (entries 3 and 4), compound **1** existed in the aqueous layer. The solubility of compound **1** with 0.23 g of $MgCl_2$ in the aqueous layer was larger than that with 0.11 g of $MgCl_2$. Thus, $MgCl_2$ increased solubility of compound **1** in the aqueous layer.

Scheme 2. **Original process for medicinal chemistry (bench scale)**



Scheme 3. **First-generation process (pilot scale)**

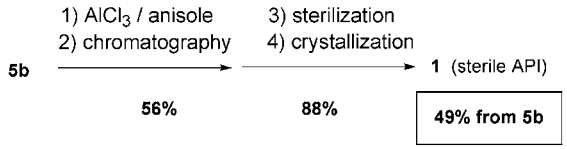


Table 1. **Screening of inorganic salts as additives in model system for extraction after deprotection of compound 5c**[a]

entry	salt	number of phase cuts, times	wt of aq layer, g	% recovery of **1**	% purity[c] of **1**
1	$MgCl_2$	4	3.5	82	97
2	LiCl	6	5.4	84	96
3	NaBr	4	3.3	84	88
4[b]	$BaCl_2$	3	3.1	38	88
5[b]	NaCl	5	3.9	90	83
6[b]	KCl	4	3.0	83	77
7	KBr	3	4.0	86	66
8	$CaCl_2$	5	4.2	no crystallization occurred	

[a] Extraction conditions: **1** (1.0 g) in THF (15.6 mL)−water (10.4 mL); after addition of a salt (1.2 g), the aqueous layer was separated. Crystallization was carried out as described in the Experimental Section. [b] Before crystallization, inorganic salt which was precipitated from the aqueous layer was removed by filtration. [c] wt % measured on HPLC analysis.

pound **1** as a sterile API in 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

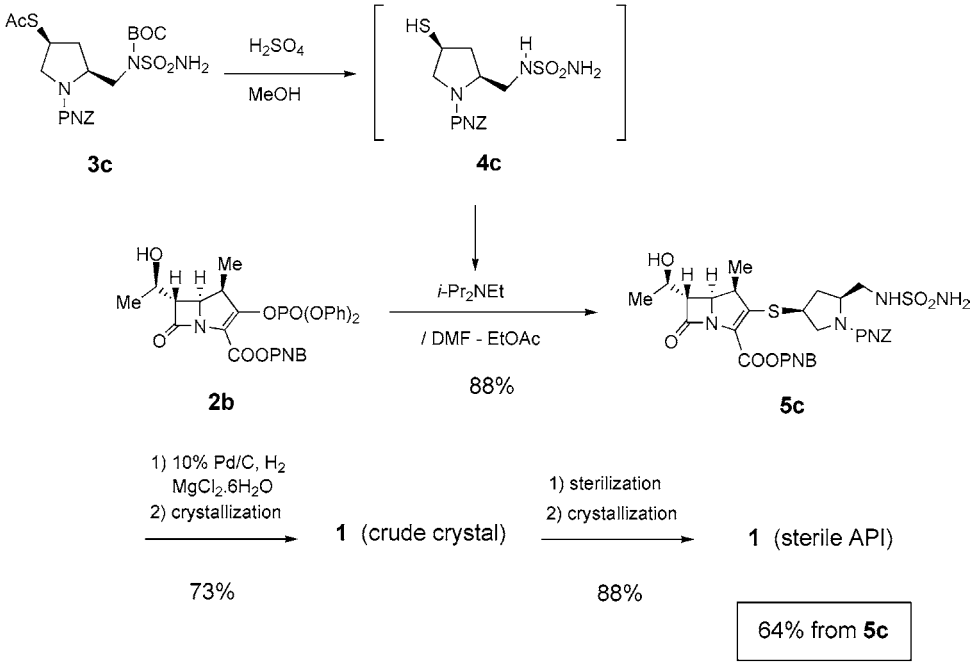
We developed and described a practical multikilogram-scale synthesis of doripenem hydrate (**1**) by deprotection of compound **5c** which was prepared from enolphosphate **2b** and *N*-PNZ-protected aminomethylpyrrolidine **3c**. We found effective extraction conditions to remove *p*-toluidine and most other organic impurities using THF/water and $MgCl_2$. The reported process requires no chromatographic purification and affords compound **1** as a sterile crystalline monohydrate in an efficient yield. This process is practical and efficient. In fact, this process has been scaled up in a pilot plant to make compound **1** for clinical studies in >10-kg scale.

Experimental Section

Materials and Instrumentations. Diphenylmethyl (4*R*,5*S*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-4-methyl-3-[[(3*S*,5*S*)-1-(*tert*-butoxycarbonyl)-5-[*N*-sulfamoyl-*N*-(*tert*-butoxycarbonyl)aminomethyl]pyrrolidin-3-yl]thio]-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (**5b**) was prepared according to the literature method.[9] 4-Nitrobenzyl (4*R*,5*S*,6*S*)-3-[(diphenylphosphono)oxy]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**2b**) and (2*S*,4*S*)-4-acetylthio-2-(*N*-sulfamoyl-*tert*-butoxycarbonylaminomethyl)-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**3c**) are commercially available. All commercially available materials and solvents were used as received. NMR experiments were conducted by using a MERCURY 300 and a INOVA 500 NMR spectrometer (Varian). IR spectra were obtained on a MAGNA 560 FT-IR spectrophotometer (Nicolet).

Preparation of (4*R*,5*S*,6*S*)-6-[(1*R*)-1-Hydrozyethyl]-4-methyl-3-[[(3*S*,5*S*)-5-(sulfamoylaminomethyl)pyrrolidin-3-yl]thio]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Hydrate (Doripenem Hydrate: 1) as a Sterile Active Pharmaceutical Ingredient (API) by Deprotection of Compound 5b Followed by Purification, Sterilization, and Crystallization. A mixture of compound **5b** (11.0 kg, 14.0 mol) in CH_2Cl_2 (160 L) was added dropwise to a solution of $AlCl_3$ (11.2 kg) in anisole (110 L) at 2 °C. After the addition, the reaction mixture was cooled to −10 °C and then was added dropwise to a solution of $NaHCO_3$ (4.4 kg) in water (154 L) at 2 °C. After the aqueous layer was separated, acetylacetone (33.6 kg) was added. Aqueous 15% Na_2CO_3 (ca. 100 kg) was added dropwise to the aqueous extract at 3 °C to adjust the pH to 5.0. After stirring at 3 °C for 30 min, the mixture was washed with CH_2Cl_2 (122 L). After addition of acetylacetone (27.5 kg), the mixture was washed with CH_2Cl_2 (61 L × 2). Aqueous 15% Na_2CO_3 was added dropwise to the aqueous extract at 3 °C to adjust the pH to 5.5. After degassing in vacuo at 5 °C for 50 min, the solution (ca. 300 kg) was chromatographed on a Diaion HP-20 column. Eluent (ca. 1400 kg) of 7% EtOH was collected and concentrated by filtration through a RO membrane to give the filtrate (190 kg). The filtrate was concentrated to 50 kg and was decolorized with activated carbon (0.18 kg). The filtrate was filtered through an ultrafiltration

Scheme 4. **Improved process (pilot scale)**



membrane to remove endotoxin. MeOH (25 L) and seed crystal (16 g) were added to the filtrate (50 kg) at 7 °C. After the mixture was stirred at 15 °C for 20 min, MeOH (125 L) was added dropwise at 10 °C over 1 h. The precipitate was collected by filtration, washed with 95% MeOH and dried to give **1**[8,9] (2.98 kg, 49%) as a sterile API. ^{1}H NMR (500 MHz, D_2O) δ 1.23 (d, 3H, J = 7.2 Hz, CH_3− on 4-position), 1.30 (d, 3H, J = 6.4 Hz, CH_3CHOH−), 1.77 (ddd, 1H, J = 6.6, 9.2, and 14.9 Hz, H-4β of pyrrolidinyl), 2.75 (dt, 1H, J = 14.3 and 8.0 Hz, H-4α of pyrrolidinyl), 3.38 (dq, 1H, J = 7.2 and 9.3 Hz, H-4α), 3.44 (dd, 1H, J = 4.2 and 12.4 Hz, H-2α of pyrrolidinyl), 3.44 (dd, 1H, J = 8.3 and 15.0 Hz, one of −CH_2−$NHSO_2NH_2$), 3.48 (dd, 1H, J = 2.5 and 6.1 Hz, H-6), 3.55 (dd, 1H, J = 4.8 and 15.0 Hz, one of −CH_2−$NHSO_2NH_2$), 3.72 (dd, 1H, J = 7.0 and 12.4 Hz, H-2β of pyrrolidinyl), 3.95 (qd, 1H, J = 4.8 and 8.5 Hz, H-5α of pyrrolidinyl), 4.06 (qd, 1H, J = 7.4 and 4.2 Hz, H-3α of pyrrolidinyl), 4.24 (dd, 1H, J = 2.5 and 9.3 Hz, H-5), 4.26 (m, 1H, −CH(OH)CH_3).

Screening of Inorganic Salts as Additives in Model System for Extraction after Deprotection of Compound 5c. An inorganic salt (1.2 g) was dissolved in a solution of compound **1** (1.0 g) in THF (15.6 mL)−water (10.4 mL). The aqueous layer was separated and washed with THF (38 mL) until its weight was less than 5.5 g because a smaller amount of the aqueous extract gives smaller crystallizing volume and leads to higher throughput. If inorganic salt precipitated from the solution, it was removed by filtration. MeOH (20 mL) was added to the obtained aqueous extract. The precipitate was collected by filtration, and dried to recover compound **1**. The results are summarized in Table 1.

Preparation of 4-Nitrobenzyl (4*R*,5*S*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-4-methyl-3-[[(3*S*,5*S*)-1-(4-nitrobenzyloxycarbonyl)-5-(sulfamoylaminomethyl)pyrrolidin-3-yl]thio]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (5c). A mixture of **3c**[11b] (33.6 kg, 63.1 mol) and 98% H_2SO_4 (15.8 kg) in MeOH (140 kg) was stirred at 65 °C for 2.5 h. After cooling the reaction mixture below 25 °C, the mixture was concentrated to 110 L under reduced pressure. The resultant concentrate was poured into a mixture of EtOAc (225 kg) and water (250 kg). The organic layer was separated and was washed with an aqueous 5% NaCl (175 kg × 3). Each aqueous layer was back-extracted with EtOAc (90 kg). The combined extracts were concentrated to ca. 70 kg, then EtOAc (180 kg) was added to the residue. The mixture was concentrated again to give the concentrate (ca. 70 kg) containing **4c**. Enolphosphate **2b** (30.0 kg, 50.5 mol) and DMF (143 kg) were added to the concentrate. After cooling the mixture to 0 °C, diisopropylethylamine (^{i}Pr$_2$NEt, 9.1 kg) was added. The reaction mixture was stirred at 5 °C for 18 h and then was poured into a mixture of EtOAc (200 kg) and water (225 L). The organic layer was separated and washed with aqueous 0.7% HCl (153 kg), 5% $NaHCO_3$ (63 kg), and 5% NaCl (90 kg × 2). Each aqueous layer was back-extracted with EtOAc (90 kg). The combined extracts were concentrated to ca. 104 L, and then EtOAc (180 kg × 2) was added to the residue. The mixture was concentrated again under reduced pressure to remove water. Toluene (365 kg) was added dropwise to the residue (155 L) over 0.5 h. The precipitate was collected by filtration and dried to give **5c** (32.5 kg, 88%): mp 160−180 °C dec. In the ^{1}H and ^{13}C NMR spectra, a 3:2 mixture of cis and trans C−N rotamers was observed. ^{1}H NMR (500 MHz, CD_3CN) δ 1.21 (d, 3H, J = 6.3 Hz, CH_3CHOH−), 1.22 (d, 3H, J = 7.5 Hz, CH_3− on 4-position), 1.81 (m, 1H, one of H-4 of pyrrolidinyl, major isomer), 1.91 (m, 1H, one of H-4 of pyrrolidinyl, minor isomer), 2.54 (m, 1H, one of H-4 of pyrrolidinyl), 3.17 (m, 1H, one of H-2 of pyrrolidinyl, minor isomer), 3.26 (m, 1H, H-5 of pyrrolidinyl, minor isomer), 3.26 (m, 1H, one of H-2 of pyrrolidinyl, major isomer), 3.30 (m, 1H, H-6), 3.31 (m, 1H, H-5 of pyrrolidinyl, major isomer), 3.49 (m, 1H, H-4α), 3.76 (m, 1H, H-3 of pyrrolidinyl, minor isomer), 3.80

(m, 1H, H-3 of pyrrolidinyl, major isomer), 4.05 (m, 2H, $-CH_2-NHSO_2NH_2$), 4.11 (m, 1H, $-CH(OH)CH_3$), 4.11 (m, 1H, one of H-2 of pyrrolidinyl), 4.25 (m, 1H, H-5), 5.22 (s, 2H, $-CH_2-$ of of PNZ-N<), 5.26 (d, 1H, $J = 14.3$ Hz, one of $-CH_2-$ of PNB ester), 5.43 (t, 1H, $J = 6.5$ Hz, $-SO_2NHCH_2-$ of minor isomer), 5.45 (d, 1H, $J = 14.3$ Hz, one of $-CH_2-$ of PNB ester), 5.65 (t, 1H, $J = 6.5$ Hz, $-SO_2NHCH_2-$ of major isomer), 7.61 (d, 2H, $J = 8.5$ Hz, *meta-H* of nitrophenyl of PNZ-N−), 7.69 (d, 2H, $J = 8.5$ Hz, *meta-H* of nitrophenyl of PNB ester), 8.22 (d, 4H, $J = 8.5$ Hz, *ortho-H* of nitrophenyl). ^{13}C NMR (125 MHz, CD_3CN) δ 17.7, 21.9 (minor), 22.0 (major), 35.1 (major), 35.5 (minor), 40.4 (minor), 40.9 (major), 44.4, 46.7 (minor), 46.9 (major), 55.9 (major), 56.3 (minor), 56.5, 57.5 (minor), 58.3 (major), 60.7, 65.7, 66.1, 66.4 (major), 66.5 (minor), 124.6, 124.7, 125.1 (minor), 128.9 (major), 129.1 (minor), 129.2, 129.3 (minor), 144.7, 145.6, 148.5, 148.6, 151.9 (major), 152.1 (minor), 155.0, 155.8, 161.5, 175.1. IR (KBr) 1769, 1698, 1521, 1345 cm^{-1}. MS (Ion Mode: FAB^+) *m*/*z* 735 $[M + H]^+$. Anal. Calcd for $C_{30}H_{34}N_6O_{12}S_2$: C, 49.04; H, 4.66; N, 11.44; S, 8.73. Found: C, 48.77; H, 4.79; N, 11.19; S, 8.63.

Deprotection of Compound 5c. Deionized water (40 L), 10 wt % Pd/C (5.0 kg), and $MgCl_2 \cdot 6H_2O$ (1.4 kg) were added to a solution of compound **5c** (8.8 kg, 12.0 mol) in THF (60 L). The suspension was stirred from 26 to 38 °C for 2 h under a H_2 atmosphere (0.5 MPa). The used Pd/C was removed by filtration and washed with a mixture of THF (18 L) and deionized water (12 L). $MgCl_2 \cdot 6H_2O$ (0.7 kg) was dissolved in the combined filtrates. After addition of THF (300 L) to the mixture, the aqueous layer was separated at 26 °C. After cooling the extract to 0 °C, MeOH (40 L) and seed crystals (10 g) were added to the extract. After $MgCl_2 \cdot 6H_2O$ (0.7 kg × 2) was added to the organic layer, the resulting aqueous layer was separated and added to the previous aqueous suspension of **1**. MeOH (75 L) was added dropwise to the suspension. The mixture was stirred at −10 °C for 1 h. The precipitate was collected by filtration, washed with MeOH, and dried to give **1**[8,9] (3.84 kg, 73%) as a crude nonsterile crystal.

Purification, Sterilization, and Crystallization to Give 1 as a Sterile API. Crude **1** (3.30 kg, 7.53 mol) was dissolved in water (66 L) at 55 °C. The solution was filtered through a funnel precoated with activated carbon (0.10 kg), a membrane filter (0.2 μm), a membrane for the ultrafiltration, and a filter for the sterilization. Then the filtrate was cooled to room temperature. After stirring at room temperature for 0.5 h, the mixture was stirred at 2 °C for 2 h. 2-Propanol (26.1 kg), sterilized with filtration prior to use, was added dropwise over 80 min. After stirring at −5 °C for 4 h, the mixture was stirred at −10 °C overnight. The precipitate was collected by filtration, washed with sterilized aqueous 80% 2-propanol, and dried to give **1**[8,9] (2.89 kg, 88%) as a sterile API. *p*-Toluidine and the other organic impurities were determined by HPLC: Each was less than 0.1% by area. Residual Pd: less than 20 ppm. Residual Mg: less than 0.1%.

Acknowledgment

We are grateful to Dr. K. Yoshikawa, Ms. S. Sekimoto, Mr. T. Toya, Mr. K. Hamada, Ms. M. Hiura, Dr. J. Kikuchi, and their co-workers for analytical data, to Mr. K. Kishimoto, Mr. Y. Okumura, Mr. M. Toyoda, Mr. I. Nanjo, and their co-workers for scale-up operations, and to Mr. H. Nakanishi and co-workers for process engineering data. We also thank Dr. Y. Nishitani, Dr. M. Kume, Dr. T. Irie, Mr. M. Hajima, Mr. M. Uenaka, and Mr. A. Okuyama for helpful discussion.

Received for review June 29, 2003.

OP034088N