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Synthesis And Characterization Of Process-Related Impurities In Doripenem.

A prominent drug for intra-abdominal and complicated Urinary Tract Infections

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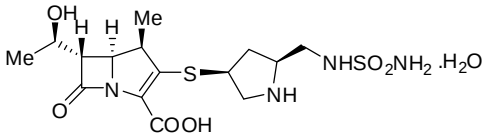
Abstract: In the production of Doripenem, process-related impurities such as tert-butyl Doripenem and its side chain derivatives, including disulfide dimers, were identified at levels of 0.2% to 2% by HPLC. These impurities were synthesized from chlorosulfonyl isocyanate, Acetylthio-hydroxymethyl-pyrrolidine nitrobenzoic anhydride and Methylvinyl phosphate. Characterization was performed using ^{1}H NMR, MS, and IR spectroscopy. The identification of these impurities is crucial to understanding their formation and impact on the quality of the Doripenem API. This study highlights the need for controlling impurity levels to ensure the efficacy and safety of the final product.

Index Terms - Carbapenems, Mitsunobu reaction, Deprotection, Disulfides.

INTRODUCTION

Carbapenem compounds like imipenem,[1] biapenem,[2] meropenem,[3] ertrapenem[4] and panipenem[5] are good for their broad and potent antibacterial activity. 1β- Methyl group introduction to the carbapenem skeleton enhances metabolic stability to renal dehydropeptidase-1(DHP-1) and leads to high antibacterial potency.[6] Doripenem monohydrate (S-466, **1**) was discovered by Shionogi Research Laboratories, Shinogi and Co., Ltd., Osaka, Japan, which is a broad spectrum non-natural 1β- Methyl carbapenem. Compound **1** (Figure 1)is optimum when compared to meropenem against Gram-positive bacteria and is also superior to imipenem against Gram-negative bacteria including pseudomonas aeruginosa.Active Pharmaceutical Ingredients (APIs) are crucial for the therapeutic effect of pharmaceutical products, but impurities and related substances can affect their safety, efficacy, and stability. In the case of Doripenem, a potent carbapenem antibiotic, strict control of impurities is essential. During its manufacture[7], tert-butyl Doripenem is formed as a process-related impurity, which can impact the final product's quality by affecting potency, stability, and pharmacokinetics. Therefore, effective detection and control measures are needed to maintain the safety and efficacy of Doripenem.

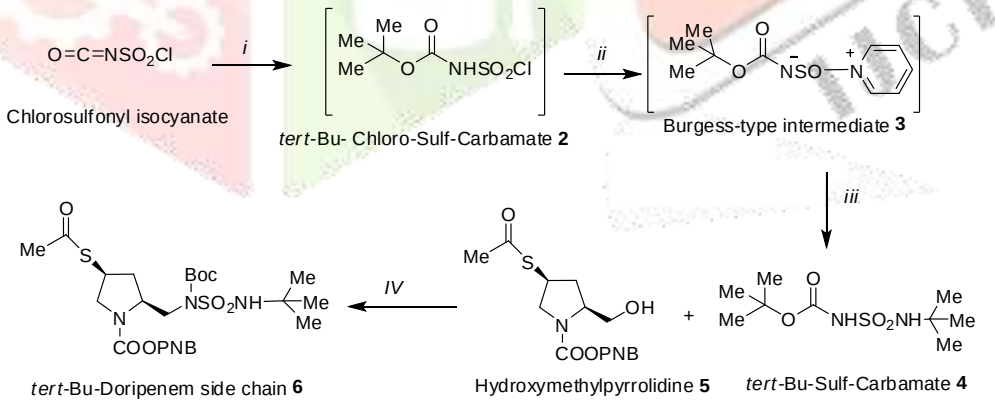


Doripenem hydrate **1**

Fig.1

RESULTS AND DISCUSSION

The synthesis of *tert*-butyl-Doripenem was achieved through a multi-step reaction sequence. The process began with the preparation of the *tert*-butyl Doripenem side chain (compound **6**). Tert-butanol was reacted with chlorosulfonyl isocyanate in the presence of toluene, yielding the moisture-sensitive intermediate tert-Bu-Sulf-Carbamate **2**. This intermediate was then reacted with pyridine to form a Burgess-type intermediate[8]**3**, which subsequently reacted with tert-butylamine, producing *tert*-butyl-*N*-*tert*-butylsulfamoylcarbamate **4**. After isolating compound **4** through acidification with sulfuric acid, it was condensed with (2S,4S)-4-(acetylthio)-2-(hydroxymethyl)pyrrolidine-1-carboxylic 4-nitrobenzoic anhydride (compound **5**) via a Mitsunobu reaction[9]. This reaction, carried out in the presence of triphenylphosphine (TPP), diethyl azodicarboxylate (DEAD), and tetrahydrofuran (THF), was monitored using thin-layer chromatography (TLC), and upon completion, *tert*-butyl-Doripenemsidechain **6** was obtained with a 70% yield, which is shown in **Scheme 1**.

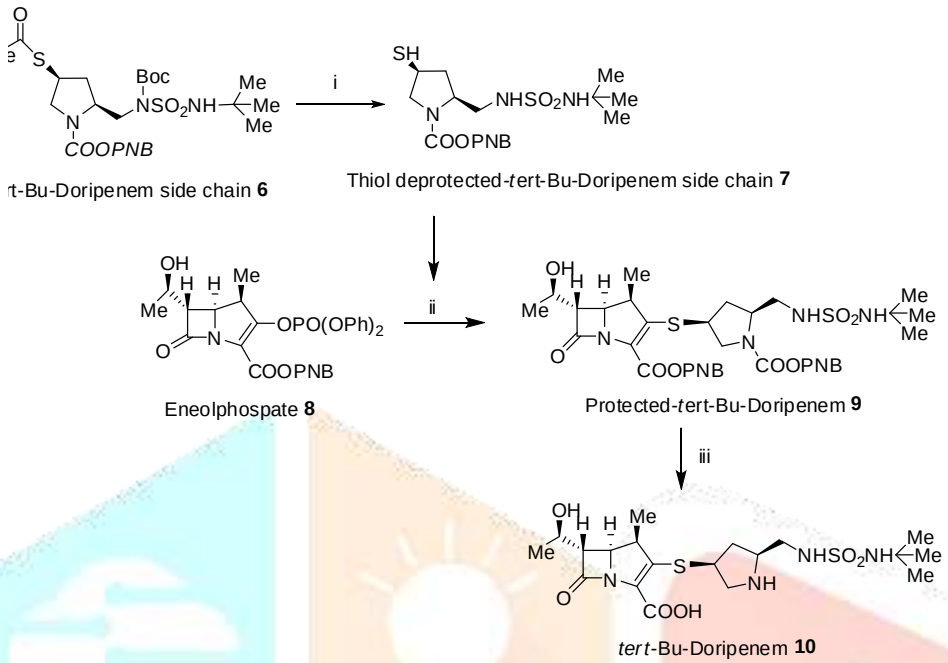


Scheme 1: Reaction conditions i)*tert*-butanol,toluene,0-5°C/1 h ii)pyridine,0-5°C/2 h iii)*tert*-butylamine, 0- 5°C/2 h,Water, H_2SO_4 iv) DEAD,TPP,THF, EtOAc20-30°C/ 3 h

Scheme 1

The next stage involved the deprotection of compound **6** using valeryl chloride in methanol at 50°C, yielding thiol-deprotected Doripenem side chain **7** after extraction and distillation. Compound **7** was then coupled with enolphosphate **8** in dimethylformamide (DMF) at -20°C, using *N*,*N*-diisopropylethylamine

(DIPEA) as a base. This reaction produced protected-*tert*-Bu- Doripenem**9**, which was purified after acidification and solvent extraction. Finally, catalytic hydrogenolysis in a morpholine-acetate buffer resulted in *tert*-Butyl-Doripenem, which was crystallized from acetone to yield compound **10**, which is shown in **Scheme 2**.



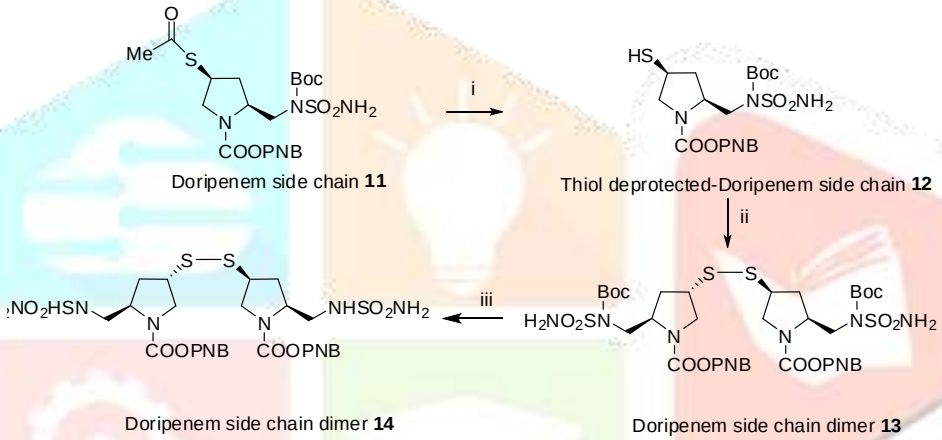
Scheme 2: *Reaction conditions i) valeryl chloride, methanol and MDC; ii) DIPA, DMF, EtOAc, HCl; iii) Pd/C, DM water, THF, Acetone.*

This synthetic pathway successfully produced tert-Butyl-Doripenem, demonstrating the effectiveness of the Mitsunobu reaction for side chain formation and catalytic hydrogenolysis for the final deprotection step. The process provided a high-purity product, showcasing the utility of this method in the synthesis of Doripenem derivatives.

Doripenem, a carbapenem antibiotic, contains a disulfide bond that forms during synthesis from thiol-containing precursors. The thiol group of the intermediate (4R,5S,6S)-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid reacts with another thiol molecule, leading to the formation of a disulfide compound. This compound is a critical impurity in Doripenem side chain and can significantly affect product quality, stability, and potency. Disulfides can arise through oxidative coupling or aerial oxidation of thiol precursors. To minimize disulfide formation and ensure compliance with ICH Q3A(R2)

and Q3B(R2) guidelines, effective control measure sincluding optimizing reaction conditions and purification processes are essential.

The Doripenem side chain (compound **11**) features thiol protection via an acetyl group, along with amine protection through Boc (tert-butoxy carbonyl) and PNZ (p-nitrobenzyloxycarbonyl) groups. The acetyl group was selectively removed using a hydrazine hydrate and methanol mixture, yielding compound **12**. This intermediate was then converted in situ into the Doripenem side chain dimer (compound **13**) using hydrogen peroxide. Reaction completion was verified by thin-layer chromatography (TLC), and the product was isolated by extracting the organic layer with aqueous sodium bisulfate and ethyl acetate. After drying and partial distillation, compound **13** was precipitated with hexanes. Finally, it was deprotected with valeryl chloride in methanol to yield compound **14**, as illustrated in **Scheme 3**.



Scheme 3: *Reaction condition: i)Acetonitrile, hydrazine hydrate,20-30°C/4h; ii)Hydrogen peroxide, sodium bisulfite /2 h; iii)Valeryl chloride, 20-30°C/6h*

CONCLUSION

In summary, the successful synthesis of tert-Butyl-Doripenemillustrates the effectiveness of multi-step reaction strategies in producing high-purity pharmaceutical compounds. The process highlights the importance of managing reaction conditions to minimize impurities, particularly disulfide bonds from thiol precursors. This work underscores the critical need for robust impurity control to ensure product quality and regulatory compliance, paving the way for future advancements in antibiotic development.

EXPERMENTAL SECTION

^{1}H NMR, spectral data were obtained in dimethyl sulfoxide (DMSO-d6) at 300 MHz.Spectrophotometers The chemical shift values were reported on the ð scale in parts per million (ppm), downfield from tetramethylsilane(TMS,ð=0.0) as an internal standard. IR spectra were recorded in the solid state as KBr dispersion using a Perkin-Elmer Spectrum one Forier transform (FT)-IR spectrophotometer. The mass spectrum was recorded using a Perkin-Elmer PE SCIEX-API 2000, equipped with an ESI source used online with a HPLC system after the ultraviolet (UV) detector. HPLC chromatographic purity was determined by using the area normalization method.

Preparationof*tert*-butyl-*N*-*tert*-butylsulfamoylcarbamate 4.

To a solution of chlorosulfonyl isocyanate (40 g, 283 mmol) in toluene (400 mL), tert-butyl alcohol (21 g, 1.0 equiv.) was added dropwise at 0°C and stirred for 30 minutes. Pyridine (49.2 g, 2.2 equiv.) was then added dropwise at 0°C for30 minutes and the mixture was stirred for an additional 2 hours at 0°C. tert-Butylamine (41.4 g, 1.1 equiv.) was added dropwise over 30 minutes, and the reaction was stirred for 2 hours at 0–5°C. The reaction mixture was diluted with a mixture of toluene (200 mL) and deionized water (400 mL). The layers were separated, and the aqueous layer was washed with toluene (200 mL). The aqueous solution was then acidified with diluted aqueous sulfuric acid (140 mL, 20% w/w) and stirred for 1 hour at 0–5°C. The product was filtered, washed with deionized water (40 mL), and dried to yield 37 g of the compound **4**. The reaction gave a yield of 55%.

^{1}H NMR (500 MHz, DMSO-d6): 1.27 (s, 9H), 1.38 (m, 9H), 2.0 (ddd, 1H), 8.0 (dt, 1H). ESI-MS: 253.11 $[M + H]^+$. FT-IR (KBr cm^{-1}): 3534, 1714, 1631, 1567, 1539, 1455, 1162, and 1144.

Preparation of(2S,4S)-4-(acetylthio)-2-((*N*-tert-butylsulfamoylamino)methyl)pyrrolidine-1-carboxylic 4-nitrobenzoic anhydride (*tert-Butyl-Doripenem* side chain, compound **6**)

To a solution of Compound **5** (10g, 28 mmol),Compound **4** (11.35g, 45 mmol)and triphenylphosphine (11.1g, 42 mmol)in tetrahydrofuran (60 mL), diethyl azodicarboxylate(7.37g, 42 mmol) was added over 45 minutes, and the mixture was stirred for 3 hours at 20–30°C. After the reaction, the THF was distilled off, and the residue was dissolved in ethyl acetate. The solution was then washed with aqueous ammonia solution (30 ml) followed by deionized water (30 mL). The organic layer was concentrated under vacuum to afford 6g of the title compound**6.**

Preparation of (4R,5S,6S)-3-((3S,5S)-5-((*N*-tert-butylsulfamoylamino)methyl)pyrrolidin-3-ylthio)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid(*tert*-butyl-Doripenem**,** Compound **10**)

A mixture of the tert-Butyl-Doripenem side chain (6.08g, 10 mmol), valeryl chloride (1.21g, mmol not specified), and methanol (30mL) was stirred at 50°C for 6 hours. After the reaction was complete, the reaction mass was diluted with methylene chloride (60mL) and deionized water (60mL) at 25°C. The layers were separated, and the organic layer was washed with deionized water (60mL), followed by 1% w/v aqueous sodium chloride (NaCl) solution (60 mL). The organic layer was concentrated to an oil-like mass and dissolved in *N*,*N*-dimethylformamide(30 mL). This solution was added to a mixture of methyl vinyl phosphate (5g, 8 mmol)and DMF (15 mL) at -40°C. *N*-ethyldiisopropylamine(1.6 g, 13mmol) was added dropwise to the reaction mixture at -40°C, and the reaction was stirred for 3 hours at the same temperature. The reaction mass was then warmed to -20°C and stirred for 4 hours.

The reaction mixture was diluted with ethyl acetate (50mL) and deionized water (30 mL), and acidified to pH 2.8 with 1 N aqueous hydrochloric acid (5 mL) at 5°C. The layers were separated, and the aqueous layer was extracted with ethyl acetate (30 L). The combined organic layers were washed with 0.25% w/v aqueous NaCl solution (2 × 150 L) at 15°C. The organic layer was concentrated to a sticky mass and dissolved in tetrahydrofuran (35 mL) at 20°C.To the reaction mixture, a buffer solution prepared by mixing *N*-methylmorpholine(0.8 kg, 8mmol), acetic acid (0.36 g, 6 mmol), and deionized water (30 mL) at 20°C was added. The solution was then added to a mixture of 10% w/w palladium on carbon (Pd/C) (9 g ~50% w/w wet) in deionized water (30 mL), and the reaction was stirred for 1 hour under a hydrogen pressure at 20°C.Tetrahydrofuran(30 mL) was added, and the reaction mixture was stirred for an additional 1 hour and 30 minutes under a hydrogen atmosphere at 105 psi and 20°C.The used Pd/C was removed by filtration, and the residue was washed with a mixture of deionized water (5 mL**)** and THF (5 mL**)**. The filtrate solution was washed with ethyl acetate (50mL) at 20°C. Added Acetone(90 mL) and distilled under vacuum at room temperature. Further added acetone(45 ml) and cooled to 0-5°C and the reaction mixture was stirred for an additional 1 h. The resulting product was filtered and dried to yield 1.5 g of the title compound, tert-Butyl Doripenem **10**with 30% yield.

^{1}H NMR (500 MHz, DMSO-d6): 1.12 (d, 3 H), 1.30 (d, 3H), 1.37 (s, 9H), 2.73-2.76 (dt, 1H), 3.36-3.51 (m, 5H), 3.94-3.74(dd, 1 H), 3.99-4.26(dd,1H), 4.67-.4.95- (m, 2H,), 8.46 (s, 1 H).^{13}C NMR (75 MHz, DMSO-

d6):15.46, 19.75, 28.48, 32.5, 38.88, 42.14, 42.84, 51.92, 55.58, 58.45, 59.4464.73, 133.8, 137.6; ESI-MS: 477.3 $[M + H]^+$.

Synthesis of doripenem side chain dimer 13

To a solution of the Doripenem side chain**11** (25g, 47mmol) in acetonitrile (150 mL), 70% w/w hydrazine hydrate (6.7g, 94 mmol) was added dropwise over 30 minutes and the reaction mixture was stirred for 4 h at 20–30°C. Afterward, 30%w/whydrogen peroxide (16g, 141mmol) was added dropwise over 15 minutes and the mixture was stirred for 2 hours at 20–30°C. Following this, sodium bisulphite (15g) was added, and the reaction was stirred for 30 minutes. The acetonitrile was then distilled off.The solid residue was dissolved in ethyl acetate (50 mL), washed with deionized water(15 mL), and dried over magnesiumsulphate(2g**)**. The resulting solution was evaporated and then dissolved in ethyl acetate at 40–45°C. To this solution, n-heptane (25 mL) was added dropwise over 30 minutes, and the mixture was cooled to 0–5°C. The precipitated product was filtered and dried, yielding 14g of the compound **13**.

^{1}H NMR (500 MHz, DMSO-d6):1.400 (m, 18 H), 1.785 (m, 2H),2.5 (d, 2H), 3.2-4.03 (br, 13H),5.2-5.26 (br, 4H),7.35 (dd, 4H), 7.63-7.64 (m, 4H), 8.2 (s, 4H).ESI-MS: 996.2596$[M + NH]^+$. FT-IR (KBr cm^{-1}): 3534, 3394, 3261, 2978, 2965, 1714, 1631, 1567, 1539, 1455, 1365, 1321, 1162, and 1144.

Synthesis of des-Boc-Doripenem side chain dimer 14

To a solution of Compound**13** (10g,10mmol) in methanol (60 mL), valeryl chloride (1.23g) was added, and the mixture was heated to 50°C. The reaction mixture was stirred for 6 hours, after which the product was extracted into methylene chloride (120 mL). The organic layer was then washed with deionized water (120 mL), evaporated, and triturated with ethyl acetate and hexanes to give 7.2 g of the product with 90% yield.

^{1}H NMR (300 MHz, DMSO-d6): 1.15-1.2. (t, 2 H), 1.97-1.98 (m, 2H), 2.39-2.51 (m, 2H), 3.01-3.05 (2H), 3.18-3.26 (dq, 2H, 1H), 3.59-3.95 (m, 2H), 4.01-4.08 (m,4H), 5.14-5.27 (dd, 4H), 6.52-6.81 (dd, 6H), 7.64-7.67 (dd, 4H), 8.21-8.24 (qd, 4H) . ESI-MS: 796.153 .

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