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An improved synthesis of Tazobactam and its related impurities

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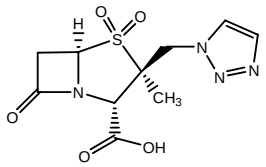
ABSTRACT

*An efficient improved synthesis of Tazobactam **1** (a β-lactamase inhibitor), is described here. This efficient process provides 40-45% higher yield of **1** in comparison to the first generation manufacturing process and which is concise and amenable for large scale synthesis. Process related impurities of Tazobactam diphenylmethyl ester **8** have been identified by LC-MS. Further these related impurities are synthesized, isolated and characterized by spectral studies.*

Key words; Tazobactam, Mercaptobenzothiazole, [3+2]cycloaddition, 1*H*-1,2,3-Triazole, Thiiranium intermediate, Acetylene gas.

INTRODUCTION

Tazobactam **1**, a penicillanic acid sulfone derivative with β-lactamase inhibitory properties [1], is chemically known as (2S,3S,5R) 3-methyl-7-oxo-3-(1*H*-1,2,3-triazol-1-yl-methyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4, 4-dioxide and has potential to enhance the activity of β-lactam antibacterials [2].



1

Several synthetic methods have been reported in the literature for the preparation of **1** that involve condensation of commercially available (2S,5R) 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 4-oxide, diphenylmethyl ester **2** with 2-mercaptobenzothiazole in toluene under reflux condition and azeotropically removal of water, which leads to the formation of azetidinonedisulfide diphenylmethyl ester **3**. Further it reacts with anhydrous copper (II) chloride to form corresponding chloromethylpenam diphenylmethyl ester **5,** which on treatment with sodium azide gave azidomethylpenam diphenylmethyl ester **6.** *In situ* potassium permanganate

oxidation of **6** yields azidomethylpenam sulfone diphenylmethyl ester **7**[3]. Compound **7,** on reaction with acetylene gas at 80-85^{O}C or with vinyl acetate at 110^{o}C in autoclave, yields Tazobactam diphenylmethyl ester **8** by [3+2] cycloaddition reaction. Finally, **8** undergoes deprotection using palladium on charcoal and molecular hydrogen gas and using protic acids, e.g., m-cresol, trifluoroacetic acid/anisole, formic acid, or aluminum chloride /anisole to produce active pharmaceutical ingredient **1**[4,5]. But these reported processes involve cryogenic reaction temperatures and column chromatographic purifications.

In another route of synthesis of **1** from **5** was reported with silver salt of 1, 2, 3-triazole followed by oxidation of the resultant compound with potassium permanganate. Compound **8** thus obtained was deprotected using palladium on charcoal and hydrogen gas [6]. Synthesis of **1** from Sulbactam was also reported in literature, which involves the introduction of azide group on methyl function followed by protection of carboxylic acid. Thereafter, the formation of 1, 2, 3-triazole ring was carried out by [3+2] cycloaddition to yield **8**, which on deprotection produced **1**[7]. These both processes provide lower yields and which are not feasible for large scale synthesis.

Many of these reported protocols suffer from drawbacks of the formation of isomeric product **4** that may be attributed due to higher temperature, longer reaction times, and lower yield of **1**. To circumvent these difficulties, some attempts have been made to perform reactions with minimum formation of isomeric product, *viz*., shorter reaction time, easier workup procedures and economic viability. These observations prompted us to undertake a thorough investigation to develop a process, which is cost effective and convenient for commercialization. In the present communication, we report an improved process for preparation of **1** with simple operations that are feasible on a commercial scale.

MATERIALS AND METHODS

Experimental

Infrared spectra were recorded in Perkin Elmer FT-IR spectrometer. ^{1}H-NMR, ^{13}C-NMR and DEPT (distortion less enhancement by polarization transfer) spectra were recorded on a Bruker 300 spectrometer. Mass spectra were measured on Perkin Elmer PE SCIEX-API 2000 mass spectrometer. All melting points were determined with polmon apparatus. Elemental analyses were performed using a Heraeus CHN-O-Rapid instrument. HPLC analysis was carried out using shimadzu with symmetry C18, 5µ (150mm x 4.6mm) column at 220nm [8]. LCMS analysis was measured using on Perkin Elmer PE SCIEX-API 2000 mass spectrometer and with shimadzu HPLC run with ZORBAX RX C8, 5µ (150mm x 4.6mm) column at 220nm [9].

Preparation of benzhydryl 2-((R)-2-(2-(benzo[d]thiazol-2-yl)disulfanyl)-4-oxoazetidin-1-yl)-3-methylbut-3-enoate (3).

Method A: Suspended diphenylmethyl 6,6-dihydropenicillanate-1-oxide **2** (100g, 0.261moles) and 2-mercaptobenzothiazole (44.48g, 0.266moles) in toluene (1500ml) and heated to 86-89^{o}C. Stirred at this temperature under mild vacuum (300-450 mm Hg) by removing water (by-product) using a Dean Stark trap and formation of **3** was monitored by qualitative HPLC analysis. After completion of reaction, cooled the reaction mass to 25-30^{o}C, treated with carbon enoanticromos and filtered. The filtrate was concentrated at 60-70^{o}C under reduced pressure (100-20 mm Hg) to yield a light yellow foamy solid of compound **3**. Yield: ~139g, HPLC Purity: 93.52%. IR (KBr): ν =1765, 1739, 1191, 1170, 742, 726 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 1.91 (s, 3H, CH_3), 3.18-3.47 (dd, 2H, CH_2-CO), 4.90 & 5.0 (2s,2H, C=CH_2), 5.1 (s, 1H, CH-COO), 5.37-5.40 (dd, 1H, S-CH-N), 6.90 [s,1H, $(C_6H_5)_2$CH], 7.28-7.89 (m, 14H, Ar-H); MS: m/z = 532.8 (M^+).

Method B: A mixture of toluene (1000ml) and cyclohexane (1000ml) were added to **2** (100g, 0.261moles) and 2-Mercaptobenzo-thiazole (44.48g, 0.266 moles) and heated to 86-89^{o}C. Stirred under reflux while removing water using a Dean Stark trap and formation of **3** was monitored by HPLC analysis. After completion of reaction, cooled the reaction mass to 25-30^{o}C, treated with carbon and filtered. Thereafter, filtrate was concentrated at 60-70^{o}C under reduced pressure (200-20 mm Hg) to yield a light yellow foamy solid of compound **3**. Yield: 140g, HPLC Purity: 93.74%. The compound **3** was used in the preparation of **5** without further purification

Preparation of (2S,3R,5R) 3-(chloromethyl)-3-methyl)-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid, diphenylmethyl ester (5).

Anhydrous copper (II) chloride (42.46g, 0.316 moles) was added to a stirred solution of **3** (140g, 0.263 moles) in ethyl acetate (1400 ml) at 20-25^{o}C under nitrogen atmosphere. Stirred the suspension at this temperature and

progress of the reaction was monitored by HPLC analysis. After completion of reaction, by-product and copper salts were removed by filtration. Filtrate was successively washed with Demineralised water, saturated aqueous sodium bicarbonate solution and saturated aqueous sodium chloride solution. Thereafter, organic layer was concentrated at 25-35^{o}C under reduced pressure to a light yellow foam of **5**. HPLC purity: 95%, Yield: 100g ; IR (KBr): ν = 1779, 1745, 1198, 1181, 739, 699 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 1.29 (s, 3H, CH_3), 3.24 & 3.65 (dd, 2H, CH_2-CO), 3.70 & 3.92 (dd, 2H, CH_2Cl), 4.95 (s, 1H, CH-COO), 5.43 (dd, 1H, S-CH-N), 6.93 [s,1H, $(C_6H_5)_2$CH], 7.28-7.48 (m, 10H, Ar-H); MS: m/z = 419 (M^+ NH3),

Preparation of (2S,3S,5R) 3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-yl-methyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide,diphenyl methyl ester (8).

Part A: To a mixture of Demineralised water (300 ml) and *N*, *N*-dimethylformamide (300 ml), Sodium azide (38.85g, 0.597 moles) was added at 25-30^{o}C, stirred to get clear solution and cooled to 0-5^{o}C. Compound **5** (100g, 0.249 moles) in *N*,*N*-Dimethylformamide (600 ml) was added in a period of 60min at 0-5^{o}C to the above solution and raised the temperature to 25-30^{0}C. Stirring was continued for ~2hrs and azidation reaction was monitored by qualitative HPLC. After completion of reaction, diluted with demineralised water and product was extracted with ethyl acetate. Finally, organic extract was washed with aqueous sodium chloride solution and concentrated at 25-35^{o}C under reduced pressure to afford oily mass containing a mixture of **6** and **6a.** Yield: 110g. Thus obtained mixture was taken as such for oxidation reaction.

Part B: Mixture of **6** and **6a** (110g, 0,269 moles) were dissolved in a mixture of acetic acid (700ml), and demineralised water (200ml), at 25-30^{o}C and the resulting solution was cooled to 0-5^{o}C. Potassium permanganate solid (49.5g, 0.313 moles) was added in a period of ~60min, while the reaction was monitored by qualitative HPLC analysis. After completion of reaction, diluted the reaction mass with demineralised water, decoloured with aqueous hydrogen peroxide and extracted with ethyl acetate(1500ml) at 25-30^{o}C. Organic extract was washed with demineralised water followed by aqueous sodium metabisulfite solution. Finally, washed the organic layer with saturated aqueous sodium bicarbonate solution (1000ml). Ethyl acetate layer containing the mixture of oxidized isomers **7** and **7a** thus obtained was taken as such for acetylene reaction. **Part C:** Oxidized isomers **7** & **7a** in ethyl acetate layer (1000ml) was transferred into an autoclave at 25-30^{o}C and introduced acetylene gas upto a pressure of 6Kg/Cm^2 and raised the mass temperature to 80-85^{o}C. Stirred at this temperature for ~ 48hrs, then reaction mass was cooled to 50-60^{o}C and concentrated under reduced pressure upto ~500ml of residual volume. Thereafter concentrated mass was cooled to 0-5^{o}C, stirred for 2hrs, filtered and washed with precooled ethyl acetate (100ml) to afford a white solid **8**, which was further purified by aqueous acetone. Yield: 35g . HPLC Purity: 99.2%; Mp = 197-199^{o}C;IR (KBr) ν = 1788, 1758, 1263, 1172, 789Cm-1; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 1.12 (s, 3H, CH_3), 3.33 & 3.77 (dd, 2H, CH_2-CO), 4.94 & 5.29 (dd, 2H, CH2-N), 5.25 (s, 1H, CH-COO), 5.26 (s, 1H, N-CH-SO_2), 6.99 [s,1H, $(C_6H_5)_2$CH], 7.29-7.52 (m, 10H, Ar-H), 7.77 & 7.95 (2s, 2H, Triazole ring); ^{13}C-NMR (300 MHz, DMSO-d_6): δ= 15.4, 38.1, 49.8, 59.6, 61.9, 64.7, 78.6, 126.4, 126.8, 127.0, 128.1, 128.4, 128.6, 128.7, 133.5, 139.4, 139.5, 165.6 and 171.5; MS: m/z = 465.1 (M^-).

Preparation of (2S,3S,5R) 3-methyl-7-oxo-3-(1H-1,2,3-triazol-1-yl-methyl)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide(1).

Suspended compound **8** (20g, 0.0429moles) in ethyl acetate (100ml) and saturated aqueous sodium bicarbonate solution (100ml) 25-30^{o}C. 25-30^{o}C with hydrogen pressure of ~6Kg/Cm^2 using 10%w/w palladium on charcoal(50%water,3g) and deprotection of **8** was monitored by qualitative HPLC analysis. After completion of reaction, palladium on charcoal catalyst was filtered, aqueous filtrate was separated and aqueous layer washed with ethyl acetate. Cooled the aqueous layer to 0-5^{o}C and pH was adjusted to ~2.0 with 6N HCl, filtered the crystallized compound **1** and washed with pre-cooled water. Yield: ~12g, HPLC Purity: 99.95%. IR (KBr) ν : 2967-2801(aliphatic CH stretch),1789(β-lactam C=O stretch),1718(C=O stretch in COOH),1456,1438,1401(aliphatic CH bend), 1326,1287, 1233,1194(C-C, C-N, C-O stretching), 1142,1131(Asymmetric O=S=O stretch) Cm-1; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 1.34 (s, 3H, CH_3), 3.34 & 3.71 (dd, 2H, CH_2-CO), 4.79 (s, 1H, CH-COOH), 4.92 & 5.24 (dd, 2H, CH2-N), 5.18 (s, 1H, N-CH-SO_2), 7.79 & 8.09 (2s, 2H, Triazole ring); ^{13}C-NMR (300 MHz, DMSO-d_6): δ= 16.1, 38.3, 50.2, 60.2, 62.3, 64.9, 127.2, 133.7, 168.3 and 171.8; MS: m/z = 300.9(M^+).

Preparation of benzhydryl 2-((R)-2-(2-(benzo[d]thiazol-2-yl)disulfanyl)-4-oxoazetidin-1-yl)-3-methylbut-2-enoate (4).

2-mercaptobenzo-thiazole (10.5g, 0.063moles) and compound **2** (20g, 0.052moles) were suspended in toluene (200ml), heated to ~110^{o}C. Reaction mass was stirred under reflux by removing water (by-product) using a Dean

Stark trap. Initially the formation of **3** and which was simultaneously converted into undesired isomer **4** was monitored by qualitative HPLC analysis, after 12hrs reaction was completed. Then reaction mass was cooled to 60-70$^{o}_{C}$ and treated with carbon enoanticromos and filtered. The filtrate was concentrated at 60-70^{o}C under reduced pressure (100-20 mm Hg) to afford oily residue which was further purified by column chromatography over silica gel column with ethyl acetate: hexanes (1:9) to obtain light yellow solid **4,** Yield: 20g . Mp: 51-53^{o}C; IR (KBr): ν = 1773, 1721, 1425, 1455. Cm-1; ^{1}H-NMR (300 MHz, $CDCl_3$): δ= 1.96 & 2.14 (2s, 6H, $2CH_3$), 3.11-3.41 (dd, 2H, CH_2-CO), 5.24-5.27 (dd, 1H, S-CH-N), 6.90 [s,1H, $(C_6H_5)_2CH$], 7.26-7.87 (m, 14H, Ar-H); ^{13}C-NMR (300 MHz, $CDCl_3$): δ= 22.5, 24.6, 44.5, 63.8, 78.6, 120.0, 121.6, 122.8, 125.5, 127.0, 127.1, 127.9, 128.0, 128.4, 128.7, 128.8, 129.0, 129.2, 139.9, 140.0, 155.6, 162.6 and 164.1; MS: m/z = 533.0 (M^+). Anal. Calcd. For $C_{28}H_{24}N_2O_3S_3$ (532.1): C, 63.13; H, 4.54; N, 5.26; O, 9.01; S, 18.06. Found: C, 63.09; H, 4.46; N, 5.24; S, 18.02%.

Preparation of (2S,3R,5R)-3-(Benzothiazole-2-thiomethyl)-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, diphenylmethyl ester (9).

To a solution of 2-mercaptobenzothiazole (4.16g, 0.025moles) in 20ml of N,N-dimethylformamide, sodium hydroxide (0.996g, 0.025moles) dissolved in Demineralised water (5ml) was added slowly and stirred for ~30min at 25-30^{o}C. Thereafter, compound **5** (10g, 0.025moles) dissolved in 30ml of N,N-dimethylformamide was added to above reaction mass at 25-30^{o}C and stirred for ~4hrs. Demineralised water was added and product was extracted with ethyl acetate and washed with demineralised water (2x20ml) at 25-30^{o}C. Organic layer was concentrated at 25-30^{o}C under reduced pressure to obtain an oily mass, which was purified by column chromatography over silica gel column with ethyl acetate: hexanes (1:4) to obtain light yellow oily mass **9,** IR (KBr): ν = 1778, 1742, 1130, 728cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 1.33 (s, 3H, CH_3), 3.34-3.80 (dd, 2H, CH_2-CO), 4.20-4.45 (dd, 2H, S-CH_2), 5.25 (s, 1H, CH-COO), 5.26-5.28(dd, 1H, S-CH-N), 6.97 [s,1H, $(C_6H_5)_2CH$], 7.26-7.95 (m, 14H, Ar-H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ= 16.5, 46.8, 47.8, 52.3, 54.7, 65.4, 78.6, 121.1, 122.1, 124.9, 126.1, 126.3, 126.5, 126.7, 127.0, 127.1, 127.9, 128.3, 128.5, 128.6, 128.7, 134.9, 139.5, 152.0, 165.3, 165.7 and 171.3;MS: m/z = 532.8 (M^+).

Preparation of (2S,3R,5R) 3-(Benzothiazole-2-thiomethyl)-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,4,4-dioxide, diphenylmethyl ester (10) and (2S,3R,5R) 3-(Benzothiazole-2-sulfonylmethyl)-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid, 4,4-dioxide, diphenylmethyl ester (11).

To a mixture of acetic acid (35ml), and Demineralised water (15ml), compound **9** (5g, 0.0094moles) was added, stirred for 10 min to get clear solution at 25-30^{o}C and cooled to 0-5^{o}C. potassium permanganate (5.94g,0.0376 moles) was added at this temperature, Thereafter temperature was raised to 20-25^{o}C and stirred for 4hrs to complete the reaction and worked up as mentioned in the synthesis of compound **7** and resulted mixture of **10** and **11,** which were separated by preparative HPLC [10,11]. **Compound-10:** Mp = decomposed at 201^{o}C; IR (KBr) ν = 2956, 2872, 1799, 1755, 1495, 1367, 1188, 993cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 1.33 (s, 3H, CH_3), 3.31 & 3.76 (dd, 2H, CH_2-CO), 4.22 & 4.42 (dd, 2H, S-CH_2), 5.25(s, 1H, CH-COO), 5.26-5.28(dd, 1H, N-CH-SO_2), 6.97 [s,1H, $(C_6H_5)_2CH$], 7.26-8.07 (m, 14H, Ar-H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ= 16.5, 29.0, 34.7, 60.8, 61.5, 65.4, 78.6, 121.1, 122.1, 124.9, 126.1, 126.3, 126.5, 126.7, 127.0, 127.1, 127.9, 128.3, 128.5, 128.6, 128.7, 134.9, 139.5, 152.0, 165.3, 165.7 and 171.3; MS: m/z = 565.0 (M^+). Anal. Calcd. For $C_{28}H_{24}N_2O_5S_3$ (564.1): C, 59.55; H, 4.28; N, 4.96; O, 14.17; S, 17.03. Found: C, 59.49; H, 4.24; N, 4.92; S, 17.01%. **Compound-11:** Mp = decomposed at 213^{o}C; IR (KBr) ν = 2954, 2862, 1795, 1752, 1492, 985cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 1.56 (s, 3H, CH_3); 3.32 & 3.77 (dd, 2H, CH_2-CO); 4.33 & 4.64 (dd, 2H, SO_2-CH_2); 5.18 (s, 1H, CH-COO); 5.30-5.31 (dd, 1H, N-CH-SO_2); 6.95 [s,1H, $(C_6H_5)_2CH$]; 7.28-8.39 (m, 14H, Ar-H); ^{13}C-NMR (300 MHz, DMSO-d_6): δ= 16.57, 55.67, 60.97, 61.66, 63.43, 78.75, 123.76, 125.06, 126.26, 126.89, 128.08, 128.29, 128.33, 128.58, 128.63, 128.68, 136.56, 139.28, 139.51, 152.1, 165.3, 165.7 and 171.32; MS: m/z = 596.8 (M^+). Anal. Calcd. For $C_{28}H_{24}N_2O_7S_3$ (596.1): C, 56.36; H, 4.05; N, 4.69; O, 18.77; S, 16.12. Found: C, 56.32; H, 4.01; N, 4.65; S, 16.09%.

Isolation of (2S,3S) 2-amino-3-methyl-3-sulfino-4-(1H-1, 2, 3-triazol-1-yl) butyric acid, diphenylmethyl ester [aminosulfinic acid diphenylmethyl ester] **(12).**

Degradation impurity **12** present in aqueous acetone mother liquor of Tazobactam diphenylmethyl ester **8** (1500ml) was concentrated at 30-40^{o}C under reduced pressure (100-50 mm Hg) up to oily mass. This was dissolved in N,N-dimethylformamide (150ml) at 25-30^{o}C and added Demineralised water (150ml) in a period of 15min. Filtered the precipitated solid and again aqueous N,N-dimethylformamide filtrate was concentrated at 30-40^{o}C under reduced pressure (20-5 mm Hg) to afford semi solid. The crude product was crystallized with acetone to obtain off-white solid **12**. Mp = decomposed at 220^{o}C; IR (KBr) ν = 3410, 1741, 1496, 964, 864, 700cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 0.98 (s, 3H, CH_3); 4.26 & 4.51 (dd, 2H, CH_2-N); 4.61 (s, 1H, CH-COO); 6.98 [s,1H, $(C_6H_5)_2CH$]; 7.26-7.53 (m,10H,Ar-H); 7.66 & 7.93 (2s, 2H, Triazole ring).; ^{13}C-NMR (300 MHz, DMSO-d_6): δ= 13.3, 52.7, 54.9,

55.1, 79.8, 127.4, 127.8, 127.9, 128.9, 129.1, 129.4, 129.5, 133.5, 140.3, 140.5 and 168.0; MS: m/z = 415.0 (M^+). Anal. Calcd. For $C_{20}H_{22}N_4O_4S$ (414.1): C, 57.96; H, 5.35; N, 13.52; O, 15.44; S, 7.74. Found: C, 57.90; H, 5.32; N, 13.48; S, 7.71%.

RESULTS AND DISCUSSION

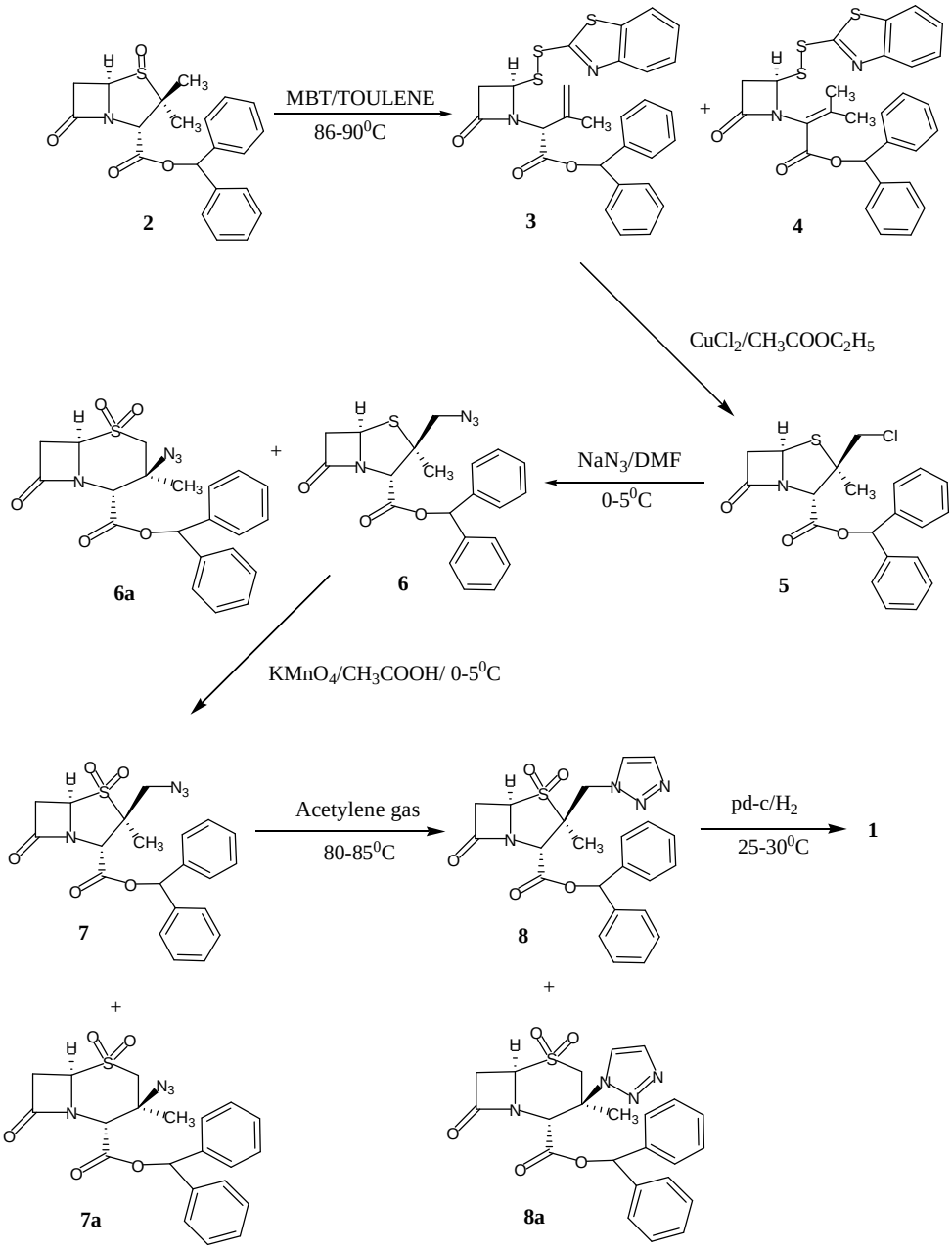
(2S,5R) 3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid, 4-oxide, diphenylmethyl ester **2** is commercially available and used as a key raw material in the synthesis of Tazobactam **1**. The overall yield of this six steps improved process for **1** starting from **2** is ~40%. Initially, **2** was condensed with 2-mercaptobenzothiazole in toluene under reflux to afford benzhydryl 2-((R)-2-(2-(benzo[d]thiazol-2-yl)disulfanyl)-4-oxoazetidin-1-yl)-3-methylbut-3-enoate desired compound **3**, along with undesired isomer **4**. This was attributed due to higher temperature / lesser dilution and also due to prolonged reaction time. But **4** could not be easily separated from **3** by any available purification methods and that would affect the quality and quantity of **3**. Therefore, some experiments were designed to avoid the formation of **4**.

Table 1. The condensation of compound 2 with 2-mercaptobenzothiazole

S. No.	Solvent(s)	Time (hrs)	Temp. (^{0}C)	**2** (%, by HPLC)	**3** (%, by HPLC)	**4** (%, by HPLC)
1.	Toluene	2	110	0.46	82.31	16.25
2.	cyclohexane	24	78-81	99	Not detected	Not detected
3.	Toluene:heptane: tetrahydrofuran (9:12:4)	12	86-89	1.11	88.78	3.78
4.	Toluene: Tetrahydrofuran (5:2)	12	86-89	7.81	78.72	3.50
5.	Toluene reflux under mild vacuum	8	85-89	0.29	93.52	2.14
6.	Toluene: Cyclohexane (1:1)	8	86-89	0.57	93.74	2.08

The studies on the effect of various temperatures and different solvents such as toluene, cyclohexane, hexanes, heptanes, tetrahydrofuran and their mixture, has revealed that the use of solvents like cyclohexane, hexanes, heptanes, tetrahydrofuran were not beneficial in the formation of **3**. In fact, the good results in terms of yield, reaction time and lesser formation of **4** were obtained using toluene and its mixture with cyclohexane, heptanes at ~ 85-90^{o}C and reaction goes smoothly probably due to solubility of the reactant and reagent. These results are summarized in **Table l.**

The above experimental data clearly revealed that, when reaction was carried out in toluene under mild vacuum (300-450 mm Hg) or in a mixture of toluene and cyclohexane (1:1) and simultaneously removing water azeotropically at lower temperature (85-89^{0}C) afforded **3** in improved yield (~20%) with good quality(~2% formation of **4**), which is achieved without any additional purifications. Thereafter, the desired isomer **3** was treated with copper(II)chloride in anhydrous condition using any of the following solvents viz., ethyl acetate, methylene chloride, ethylene chloride or toluene as reaction media at 20-25^{o}C to produce (2S,3R,5R) 3-(chloromethyl)-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, diphenylmethyl ester **5**, as an yellow foamy solid. Ethyl acetate is the solvent of choice as this gives the quantitative yield. Compound **5** when treated with sodium azide in aqueous N,N-dimethylformamide (DMF) at 25-30^{o}C yielded a mixture of (2S,3S,5R) 3-(azidomethyl)-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid, diphenylmethyl ester **6** and (2S,3S,6R) 3-azido-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]octane-2-carboxylic acid, diphenylmethyl ester **6a** in 3:2 ratio, this conversion is occur us via the formation of thiiranium intermediate[3]. The mixture of **6** and **6a** on oxidation with potassium permanganate in aqueous acetic acid at 25-30^{o}C afforded mixture of (2S,3S,5R) 3-(azidomethyl)-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid, 4,4-dioxide, diphenylmethyl ester **7** and (2S,3S,6R) 3-azido-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0] octane-2-carboxylic acid, 5,5-dioxide, diphenylmethyl ester **7a.** The mole ratio of sodium azide (2 mole equivalent) and potassium permanganate (1.2 mole equivalent) were optimized for the azidation followed by oxidation of **5** to **7**. The *insitu* azidation and oxidation allowed the use of this process to produce compound **8** on a commercial scale with simple handling, greater efficiency, better quality and lower cost (**Scheme 1**).

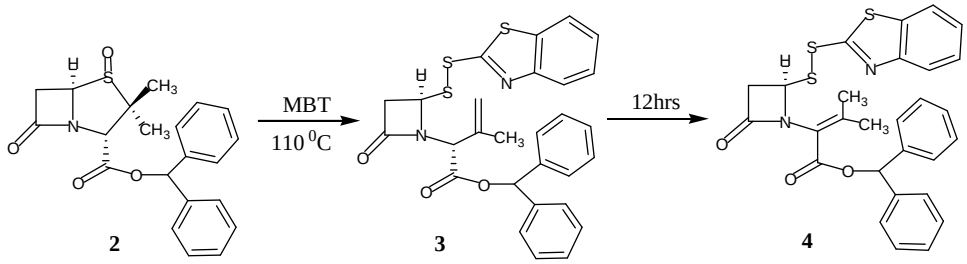


Scheme 1. Synthesis of the Tazobactam 1

Thereafter, the mixture of **7** and **7a** were converted to the mixture of (2S,3S,5R) 3-(1*H*- 1,2,3-triazol-1-yl-methyl)-3-methyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, diphenylmethyl ester **8** and (2S,3S,6R) 3-(1*H*-1,2,3-triazol-1-yl-methyl)-3-methyl-8-oxo-5-thia-1-abicyclo[4.2.0] octane-2-carboxylicacid, diphenylmethyl ester **8a** by [3+2] cycloaddition, using acetylene gas in acetone/ethyl acetate in autoclave. In this step, the effects of solvents and temperature on the reaction were also investigated. We found that the reaction proceeded well in moderately polar solvents. Ethyl acetate was a satisfactory solvent, where as more polar solvent like acetone and acetonitrile were proven to be unfavorable. Finally, the effect of temperature (40- 100°C) and internal pressure of 6-20 Kg/Cm^2 for synthesis of **8** from **7** was studied by using acetylene gas in ethyl acetate. This reaction proceeded smoothly at 80±5^{o}C and pressure of ~15Kg/Cm^2 afforded **8** with good quality and yield and further purified by aqueous acetone. While the unwanted cepham isomer **8a** remains in the mother liquor. Higher

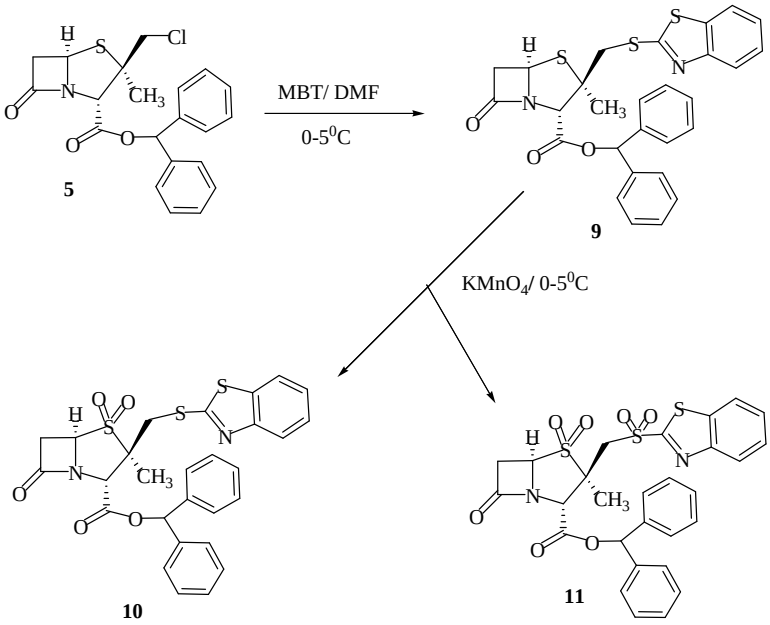
temperatures/ pressure lead to the formation of intractable mixtures, while at lower temperatures, the reaction does not progress satisfactorily. Deprotection of compound **8**, treated with palladium on charcoal in the presence of hydrogen gas (4-10 Kg/Cm^2) in Demineralised water and Ethyl acetate at 25-30^oC to produce Tazobactam **1**. The deprotection reaction proceeds slowly at lower temperatures (<20^oC) and proceeds fast at Higher temperatures (>35^oC), but which leads to the formation of degradation impurity i.e. aminosulfinic acid. After HPLC and LC-MS study, related impurities of **8** were identified, synthesized, isolated and characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT and MASS spectral data.

The formation of thermodynamically more stable undesired isomer, **4** is envisaged due to the shifting of proton at higher temperature, lesser dilution and prolonged reaction time. To establish this proposition, **3** was prepared by condensation of **2** with 2-mercaptobenzothiazole in toluene at reflux, which was concurrently converted into undesired isomer, benzhydryl 2-((R)-2-(2-(benzo[d]thiazol-2-yl) disulfanyl)-4-oxoazetidin-1-yl)-3-methylbut-2-enoate **4** in 10-12 hrs (**Scheme 2**).^{1}H-NMR of **4** showed two singlets corresponding to 3H each at 1.96 and 2.14, proving the presence of two (-CH_3) groups and also not seen three singlets of 1H each at 4.90, 5.0 and 5.10, confirming the absence of (=CH_2) and (CHCOO) groups of **3**. HPLC data also revealed that compound **3** is eluting at retention time **28.3** and isomer **4** is eluting at retention time **27.8** and both were given equal mass by LCMS.



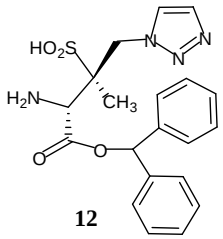
Scheme 2. Synthesis of the isomer 4.

A side reaction was noticed between **5** and residual 2-mercaptobenzothiazole leading to the formation of compound **9.**Which was also oxidized to produce compounds **10** and **11** in the range of 3.0% to 6.0 % by HPLC in the reaction mass. Isolated compound **8** was found to contain the related impurities **9**, **10, 11** and a degradation impurity **12** by HPLC in the range of 0.2% to 1.2%. These impurities were proposed based on LCMS data. Thereafter, compound **9** was synthesized from **5** with MBT in aqueous *N*, *N*-dimethylformamide and was further purified by column chromatography over silica gel. Compound **9** was subsequently oxidized with potassium permanganate in aqueous acetic acid to afford compounds **10** and **11** were isolated by preparative HPLC (**Scheme 3**). Degradation impurity **12**, aminosulfinic acid diphenylmethyl ester, was isolated from the aqueous acetone mother liquor of compound **8** and further purified. Elemental analysis data proved that $C_{28}H_{24}N_2O_3S_3$, $C_{28}H_{24}N_2O_5S_3$, $C_{28}H_{24}N_2O_7S_3$ and $C_{20}H_{22}N_4O_4S$ are the empirical formulae of compounds **9, 10, 11** and **12** respectively, and also MASS spectra of compounds **9, 10, 11** and **12** showed molecular ions at m/z 532, 564, 596 and 414 respectively. Further these compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and DEPT.



5 **9** **10** **11**

Scheme 3. Synthesis of 9,10,11 from 5



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CONCLUSION

We have developed an improved, novel scaleable and convenient procedure for the synthesis of Tazobactam diphenylmethyl ester **8**. The formation of undesired isomer **4** was controlled by using mixture of solvents for removal of water azeotropically at lower temperature (85-89^{o}C) during early stage of process development. The optimization was quite successful with much easier and more reliable operation. Therefore, this process is cost-effective and affords the product in better quality and also provides improved overall yield of 30%, when compared to the 18% which reported in literature. At the same time the experimentation lead us to identify and isolate the some of new and novel related impurities of tazobactam diphenylmethyl ester **8**.

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[8]. HPLC analyses run with Symmetry C18, 5µ (150mm x 4.6mm) column at 220nm with CH_3CN: 13g of potassium dihydrogen phosphate dissolved in 1000ml of water and pH adjusted to 2.5±0.05)for the preparation of Tazobactam diphenylmethyl ester **8** retention time (in minutes) was **12.5** and its related impurities **12**, **11**, **10**, **9** and **4** retention times (in minutes) were **2.6**(0.21RRT), **22.5**(1.80RRT), **25.2**(2.02RRT), **27.3**(2.18RRT) and **27.8**(2.22RRT) respectively.
[9]. LCMS analysis was carried out using on Perkin Elmer PE SCIEX-API **2000** mass spectrometer and shimadzu HPLC run with ZORBAX RX C8 (150 x 4.6mm) column with 5µ particle at 220nm. Mobile phase A consists 0.01M ammonium acetate. Mobile phase B consists acetonitrile. Flow rate was 1ml/min, and gradient program, time(min)/A(v/v):B(v/v): $T_{0.01}$ / 60:40, $T_{20.0}$ / 30:70, $T_{35.0}$ / 20:80, $T_{37.0}$ / 60:40, $T_{45.0}$ / 60:40.
[10]. Compound **10** isolation was carried out by preparative HPLC using PEERLESS BASIC C18 (500 x 30mm) column with 10µ particle at 220nm. Mobile phase A consists water. Mobile phase B consists acetonitrile. Flow rate was 30ml/min and gradient program, time(min)/A(v/v): B(v/v): $T_{0.01-30}$ / 85:15, T_{30-50} / 75:25, T_{50-75} / 60:40, T_{75-80} / 40:60, T_{80-100} / 20:80.
[11]. Compound **11** isolation was carried out by preparative HPLC using ZODIAC SIL SH C18 (500 x 30mm) column with 10µ particle at 220nm. Mobile phase A consists 0.2% w/w glacial acetic acid. Mobile phase B consists acetonitrile. Flow rate was 30ml/min and gradient program, time(min)/A(v/v):B(v/v): $T_{0.01-30}$ / 90:10, T_{30-45} / 80:20, T_{45-60} / 70:30, T_{60-75} / 55:45, T_{75-85} / 40:60, T_{85-95} / 35:65.