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An improved, scalable and robust process for the synthesis of cilastatin sodium: A renal dehydropeptidase inhibitor

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ABSTRACT

An improved process developed for the synthesis of API, Cilastatin Sodium with more than 99.5% purity and all impurities well below the regulatory limits. The process was specially modified to control the epimeric impurities; solvent purification methods have been developed to achieve desired quality of API without any use of either column chromatography or ion-exchange chromatography.

Keywords: Cilastatin Sodium, Epimeric Impurities, Solvent Purification, Column Chromatography, Ion-exchange Chromatography

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INTRODUCTION

Thienamycin **(3a)** which in its form of N-formimidoyl derivative viz. Imipenem **(3b)** is one of the most universal antibiotics.[1] Meanwhile it is readily metabolized by the enzyme renal dihydropeptidase and its pharmacological activity is thus decreased. In order to prevent the decrease in the concentration of the antibiotic, a dehydroamino acid that inhibits the enzyme is added to its pharmaceutical formulation.

Among the many amino acids tested for their inhibiting activity [2] Cilastatin **(2)** was found to be practical for the use in the form of its mono sodium salt. [2, 3] Cilastatin belongs to a class of chemical compounds which inhibit the human enzyme Dehydropeptidase. Dehydropeptidase, an enzyme found in the brush borders of kidney and is responsible for degrading the antibiotic imipenem. Cilastatin is therefore combined intravenously with imipenem in order to reduce the effect of dehydropeptidase and prolong its antibacterial effect. Since the antibiotic, imipenem, is broken down by dehydropeptidase-I, which resides in the brush border of the renal tubule, cilastatin is co-administered with imipenem to increase its effectiveness. Cilastatin blocks the metabolism of imipenem in the kidney, so that co-administration of imipenem and cilastatin allows antibacterial levels of imipenem to be attained in the urine. Imipenem and Cilastatin sodium (PRIMAXIN) is used to treat infections like lower respiratory tract infections, urinary tract infections, gynecological infections, septicemia, endocarditis caused by Staphylococcus aureus, bone and joint infections and skin structure infections.[4]

According to the original route developed by Merck,[5] the key intermediate in the synthesis of Cilastatin is 7-bromo- or 7-chloro-2-oxoheptanoic acid, which is condensed with dimethyl cyclopropane carboxamide [6] with the further replacement of the halogen atom with the L-cysteine moiety. Racemic dimethyl cyclopropane carboxylic acid is the starting material for the production of compound **(2)** which is a part of the pharmaceutical formulation of antibiotic Primaxin.[7] The synthesis of compound **(2)** through ketoacid has serious disadvantages. One of them is the instability of aliphatic a-ketocarboxylic acids with more than 5 carbon atoms resulting from their tendency to undergo decarboxylation.[8] To produce acid, one has to use substances like 1,3-propane dithiol and 5-chloro-1-pentyl magnesium bromide which is inconvenient. [5] In addition to the condensation of acid with amide affords N-acylated dehydroamino acid in a low yield (30-35%). [2-5]

Synthesis of Cilastatin has also been achieved by the use of 2-amino-7-chloro heptanoic acid as the key intermediate. [9] Acid is synthesized from cycloheptanone. Further, acid is converted to acid chloride followed by bromination and replacement of bromine with amino group to form amino acid. Further esterification of acid and reaction with dimethyl cyclopropyl amide leads to formation of amide. This amide when reacted with L-cysteine leads to synthesis of Cilastatin. This process suffers from multiple steps and difficulties in handling of materials like sodium metal. Further resin purification decreases the industrial applicability of this process. [5]

Graham D.W et al [9] discloses the process for the preparation of Cilastatin sodium, Example 19A.A, more particularly describes the process. As per this process the 7-chloro-2-oxoheptanoic acid ethyl ester is condensed with (S)-2,2'- di-methyl cyclopropanecarboxamide in refluxing toluene in presence of catalytic p-toluene sulfonic acid (PTSA) by azeotropic removal of water to give (S)-7-chloro-2-(2,2'-di-methyl cyclopropane carboxamido)-2-heptanoic acid ethyl ester**,** which is hydrolyzed in aqueous caustic solution to give acid compound**.** L-cysteine hydrochloride is finally condensed with (S)-7-chloro-2-(2,2'-di-methyl cyclopropane carboxamido)-2-heptanoic acid in aqueous caustic solution to afford Cilastatin acid compound **(2)**. It is treated with aq. hydrochloric acid at pH-3.0 for isomerization of E isomer to Z isomer. Pure material is then isolated by ion exchange chromatography and crystallization. Further U.S. Patent 2004/0152780 also discloses process of Cilastatin sodium **(1),** it gives Cilastatin is dissolved in water and pH is adjusted to neutral and product is isolated by lyophylization.

Kumar Y. Tyagi et al [10] describes isomerization of Cilastatin acid containing E-isomer at pH-0.5 to 1.5 (using HCl) at 90-95°C temperature. This invention is not suitable for plant scale since it involves column chromatography. It also discloses purification process for Cilastatin acid **(2)** using non-ionic resin and further crystallization.

U.S. Patent 2004/0152780 [4] claims the process for preparation of Cilastatin sodium salt of Formula-2 in amorphous form; it is prepared by use of single organic solvent or mixture of solvent or organic solvent and water mixture and or precipitation of salt in anti-solvent. It claims use of alcoholic sodium hydroxide and ketonic solvent such as acetone for precipitation of salt as anti-solvent. This is also not a suitable process since use of acetone in basic condition produces mesityl oxide impurity which is difficult to remove from Cilastatin sodium salt **(1)** moreover yield is very

low. Pandharinath K.B et al [11] declared the preparation of Cilastatin sodium salt by isolating amine Cilastatin amine salt and then conversion to sodium salt. This is not a suitable process because of the use of column chromatography for removal of inorganic salts as well as due to increase in number of steps arising out of synthesis of amine salt.

Panchapakesan. G et al [12] discloses the process for preparation of Cilastatin in aqueous or alcoholic or aqueous alcoholic solvent and extraction at pH range 2-4 with isolation of Cilastatin **(2)** in water immiscible C4-C8 alcohols preferably n-butanol. This is also not suitable process since it requires large excess (50-60 volumes) of C4-C8 alcohols and does not give repeatable results.

Experimentation using all the above processes did not yield the required regulatory passing purity nor gives a passing residue on ignition. We could not achieve the said 99.5% purity and the residue on ignition was as high as 20%.

Literature process (scheme 1) [4] lacked the ability to control the crucial epimeric impurities in the final compound **3**.

Our foremost objective is to overcome the problems associated with processes such as laborious and time consuming column and ion exchange chromatography and to achieve quality of Cilastatin sodium **(1)** according to worldwide regulatory bodies with controlled epimeric impurities; Herein we report an improved and scalable process which would be simple in operation, easily scalable and robust for the synthesis of Cilastatin sodium **(1)**, Cilastatin acid **(2)** and its intermediates.

MATERIAL AND METHODS

Shimadzu Prominence-i LC-2030 instrument with column Inertsil ODS-3v & YMC ODS AQ and UV detector at 210nm was used in recording HPLC data. The ^{1}H NMR spectra was measured in $CDCl_3$ and DMSO-d_6 on Bruker 400 MHz spectrometer. Mass spectra were recorded using AB SCIEX API 2000 LC/MS/MS System. All chemicals & solvent were purchased from the commercial suppliers & were used as such without further purification.

Synthesis of (Z)-(S)-7-chloro-2-(2,2-dimethylcyclopropane carboxamido)-2-heptenoic acid ethyl ester (6): To the solution of Ethyl-7-chloro-2-oxo heptanoate (**4;** 200 g, 0.96 mol) in toluene (1600 ml) was added (S)-2,2-Dimethyl cyclopropyl amide (**5;** 112.5 gm, 0.99 mol) and p-toluene sulphonic acid (2 g) as a catalyst. Reaction mass refluxed for ~20 hrs with azeotropic removal of water. Progress of the reaction was monitored on TLC for completion. Cooled reaction mass to RT and washed with water, pH - 7.0 was made using caustic solution and toluene layer was taken in-situ as such for the next stage. Yield ~95%, ratio of (Z):(E) isomer is 80:20 and is taken as such for the hydrolysis stage.

^{1}H NMR ($CDCl_3$): δ 7.06(s, 1H) 6.31(t, 1H) 4.22(q, 2H) 3.55(t, 2H) 2.20(q, 2H) 1.82(p, 2H) 1.65(p,2H) 1.45(t, 1H) 0.63(d, 1H) 1.33(t, 3H) 1.27(s, 6H) 1.25(s, 1H) 0.82(t, 1H) Mass-302.3 [M+H]+

Synthesis of (Z)-(S)-7-chloro-2-(2, 2-dimethyl cyclopropane carboxamido)-2-heptenoic acid (7): Compound **6** was cooled to 5-10° C and solution of sodium hydroxide (136g, 3.4 mol) in water (2000 ml) was added and the resulting biphasic solution was stirred for 8 hrs at 25-30° C up to the complete disappearance of ester on TLC. The toluene layer was separated and the aqueous layer was washed with toluene. The pH of the aqueous layer was adjusted to 4.0 to 4.5 and extracted with toluene (1Lt) and treated with dil. HCl and stirred for 4-6 hrs at 25-30°c, followed by washed with 10% sodium bi-sulfite solution. Toluene layer is concentrated under reduced pressure to the residual level; n- hexane was added and stirred for 4-6 hrs, filtered and dried to obtain compound (**7;** 175g) with about 95% purity and E isomer content about 5.0%. Above compound **(7;** 175 g) further treated with aqueous HCl-(250 ml) at 25-30°C for 5-6 hrs under stirring filtered, washed with water, dried to obtain compound **(7)** with purity of 98.0% with E isomer about 0.5%.

Purification of compound (7): Compound **(7)** is dissolved in ethyl acetate (2.5 volumes), filtered through a charcoal bed and added to 10 volume cyclohexane and stirred for 6-8 hrs, filtered, dried, washed with cyclohexane and dried to obtain pure compound **(7 ;** 128 g) with purity above 99.5% with E isomer content below 0.2%. Overall yield ~65%.

^{1}H NMR (DMSO): δ 12.3(s, 1H) 9.1(s, 1H) 6.3(t, 1H) 3.6(t, 2H) 2.1(q, 2H) 1.63(p, 2H) 1.61(d, 1H) 1.4(p, 2H) 1.2(s, 1H) 1.09(s, 3H) 0.8(t, 1H) 0.6(q, 1H)
Mass- 272.0 [M+H]+

Synthesis of (Z)-7-((S)-2-Amino-2-carboxy-ethylsulfanyl)-2-[((S)-2,2-dimethyl- cyclopropane carbonyl)-amino]-hept-2-enoic acid (Cilastatin acid) (2): To the stirring mixture of L-cysteine HCl **(8;** 113.0 g, 0.71 mol) & potassium carbonate (388.2 g, 2.80 mol) in methanol 2000 ml at 60-65°C, added compound **(7;** 128.0 g, 0.46 mol) in

methanol 560 ml, under stirring. Progress of the reaction was monitored by HPLC. Cool, filtered and flushed with fresh methanol. Adjust pH of solution to 3.8 to 4.2 using methanolic H_2SO_4. Reaction mass filtered to remove inorganic salts. Concentrated to maximum under reduced pressure, added ethanol (192 ml) and ethyl acetate (1280 ml), stirred and filtered, washed with ethyl acetate, dried to obtain cilastatin acid **2** with purity above 98.5%.

Purification of compound (2): Cilastatin acid **(2;** 77 g) was stirred in a 2:8 mixture of ethanol 154 ml and ethyl acetate 616 ml at 45-50°C. Filtered, dried to obtain pure Cilastatin acid **(2;** 65.4 g) with purity above 99.5%. Overall yield ~52%

^{1}H NMR (DMSO): δ 9.23(s, 3H) 6.32(t, 1H) 3.76(t, 2H) 2.98(dq, 2H) 2.53(d, 2H) 2.07(dd, 2H) 1.65(q, 1H) 1.47(m, 4H) 1.10(s, 6H) 0.87(t, 1H) 0.69(q, 1H)

Mass- 358.8 [M + H]+

Synthesis of Cilastatin sodium (1): In methanol (390 ml), dissolve sodium hydroxide (7.02 g, 0.17 M) under stirring at 25-30°C. Cilastatin acid **(2;** 65 g, 0.18mol) added to above solution and stirred to obtain clear solution. Add this clear solution to acetonitrile (2600 ml) under stirring at 25-30°C. Filter the solid, immediately transfer to desiccators to obtain compound **1** (57 g).Yield ~88%. Purity ~99.5%.

^{1}H NMR (DMSO): δ 9.35(s, 3H) 6.2(t, 1H) 3.9(t, 2H) 2.7(dq, 2H) 2.53(d, 2H) 2.1(dd, 2H) 1.4(q, 1H) 1.32(m, 4H) 1.15(s, 6H) 0.8(t, 1H) 0.6(q, 1H)

Mass-382.6 [M+H] +

RESULTS AND DISCUSSION

In the processes reported by Kumar Y. Tyagi et al [10] and Pandharinath K.B et al [11] there was lack of clarity in the strategy to avoid epimeric impurities. These processes undertake the isomerization stage either after hydrolysis or after synthesis of Cilastatin acid, which results in heavy degradation of products and hence not recommended on the formation of active drug. With these processes E-isomer limits can't be achieved as per regulatory requirements > 0.1%. Hence we have introduced aq. HCl treatment followed by purification using ethyl acetate/cyclohexane to achieve desired quality of compound **7.**

Synthesis of compound **6** was achieved through the regular process. [4] Condensation of **4** and **5** resulted in the formation of **6** via azeotropic displacement of water in toluene. The product **6** in toluene further hydrolysed in biphasic mixture, which was carried out using aq. NaOH solution to obtain compound **7**. After hydrolysis, the percentage of E-isomer needs to be controlled below 0.5% as per EP specification and achieved through isomerization, aq. HCl treatment and purification of **7**.

Process was modified taking into consideration of several parameters in order to overcome the challenges and hurdles reported in literature [10-11]

a. Controlling the E-isomer: Isomerization stage was modified [Table no.1] to control the unwanted E-isomer content as well loss in yield. Literature suggested isomerization at 90-95°C, but we found that there was tremendous yield loss as well as poor yield. Therefore we developed an isomerization process using dilute HCl in a biphasic mixture with toluene at 25-30°C which resulted in the reduction of E-isomer below 5%. During isomerization it was observed that the selective degradation of the E isomer rather that converting it to Z isomer which is confirmed by mere raise in the percentage content of Z isomer. The degradation products of this stage are carried ahead and could be removed by newly developed ethyl acetate/cyclohexane purification process. Further aq. HCl treatment reduces the E-isomer below 1%.

b. Purification of compound 7: It was observed that the impurities in the final API are at the same relative retention times as that in **7**. This was because the impurities were epimeric in nature. So it was necessary to control these impurities at compound **7** stage in order to achieve desired purity of the final API **1.** We studied various solvent mixtures and optimized ethyl acetate/ cyclohexane (1:10) mixture was the most suitable which controlled the impurities well below the limit.

c. Controlling L-cysteine dimerization: L-cysteine. HCl was used for synthesis of compound **2**. Literature recommended excess of L-cysteine. HCl ~1.5-1.75 equivalent. L-cysteine HCl has tendency to undergo dimerization to L-cystine in highly basic conditions and in presence of water. Experiments were carried out using stronger bases and confirmed low yield of compound 2 along with the formation of dimer and hence we shifted to a milder base in the form of K_2CO_3 in methanol. This reduced the quantity of L-cysteine HCl to about 1.1-1.15 equivalent. Selection of K_2CO_3 in methanol reduced the time from conventional 15 hr to 6 hrs, formation of L-cysteine free base was crucial for the higher rate of reaction, it was also confirmed mode of addition was also very important to achieved completion of the reaction with ~99.6% purity of **2**.

d. Purification of compound 2: Another important hurdle in achieving the purity of compound **1** was the epimeric impurities of compound **2** and their salt formed. To get rid-off the impurities in compound **2,** literature suggests the use of silica column chromatography. We devised solvent mixtures ethanol/acetonitrile (1:10) for isolation of compound **2** which delivered the required purity without the requirement of column chromatography.[Table no 3].

e. Avoiding Ion Exchange Chromatography for the removal of inorganic salts: Removal of inorganic salts from the final product is a very tough task owing to similar solubility parameters of salts as well as Cilastatin acid. Literature suggested the use of ion exchange chromatography, wherein both cationic as well as non-ionic resins have been used. Use of ion exchange chromatography hinders the industrial applicability of a process. To ensure that inorganic salts are not found in the final compound, we had to try a different strategy. Inorganic salts are primarily formed in the process during the synthesis of compound **2**. As we used K_2CO_3 as a base and IPA HCl as the acid, it formed KCl as the salt. KCl has got a solubility of 0.5g/100g methanol, [13] thus resulting in inorganic content in the final product. We have done pH adjustment using methanolic H_2SO_4 forming $KHSO_4$ instead of KCl. $KHSO_4$ has a solubility of 0.005/100g methanol [13] which resolved the problem of inorganic salts in the final compound.

f. Isolation of Cilastatin sodium 1: Cilastatin sodium is a highly hygroscopic molecule making it very difficult to handle during processing. Literature suggests the use of acetone in the isolation of compound **1,** acetone has disadvantageous from two points; one being the source of a known impurity **(Impurity D)** mesityl oxide and the other owing to its high affinity for moisture. Filtration proved to be impossible with the use of acetone as the residual acetone in the upper layer of filtered solid absorbed moisture instantaneously making is difficult to handle. Acetonitrile was found to be the best suitable for the isolation of compound **1** as acetonitrile has less water affinity and thus results in no moisture absorption and gives ample time for further packing and processing.

CONCLUSION

In conclusion, we have developed modified isomerization methodology which controls formation and removal of epimeric as well as isomeric impurities along with firm process control on formation and elimination of dimer impurity of L-cystine. We have also modified the process in such a way it helps to avoid column chromatography and ion exchange chromatography which otherwise hinders the industrial applicability of process. Hence process discussed and described herein is improved, scalable and a highly robust process for the synthesis of Cilastatin sodium. All the crucial parameters, hurdles and the strategies to overcome these hurdles have been demonstrated. We claim to have a better process in terms of operability and quality.

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Table no. 1- Percentage of E isomer during different stages of process

	Stages	**Percentage**
1	Condensation	10-15%
2	Hydrolysis	10-15%
3	Isomerization	5-7%
4	Aq. HCl treatment	0.5-1%
5	Purification	<0.1%

Table No. 2: Effect of solvent and Base on yield and purity

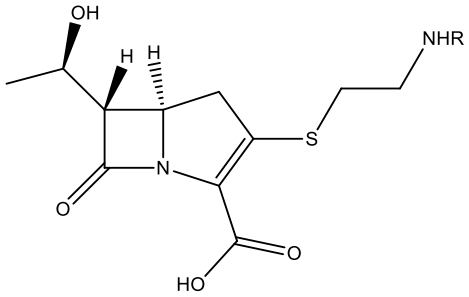
Entry No	Solvent	Base	Yield	Purity
1	Water	NaOH	30-35%	98%
2	Water	NH_3	30-35%	97%
3	Water	NaOMe	10-15%	80%
4	Water	TEA	0-5%	-
5	Water	K_2CO_3	15-20%	75%
6	Methanol	NaOH	12-15%	75%
7	Methanol	NH_3	-	-
8	Methanol	NaOMe	20-22%	65%
9	Methanol	K_2CO_3	45-50%	99.5%
10	Methanol	$Ba(OH)_2$	-	-

Table No. 3: Limits of impurities achieved in compound 2 as per EP

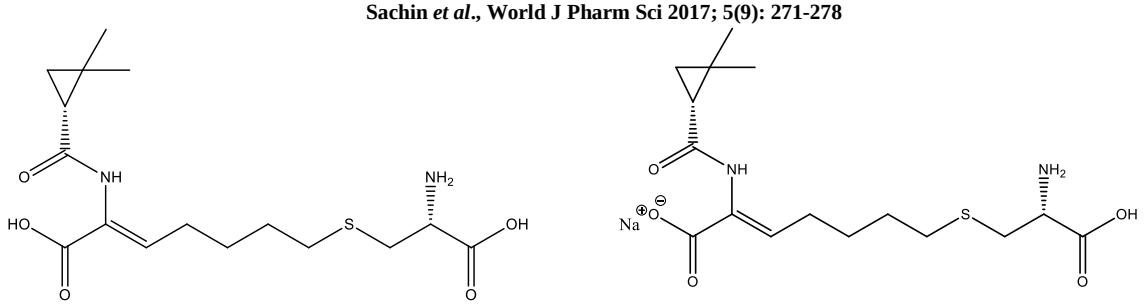
Entry no.	Impurity	Limit Specified by EP	Limit achieved
1	Impurity E	NMT 0.3%	ND
2	Impurity A(1,2)	NMT 0.5%	0.25-0.35%
3	Impurity F	NMT 0.1%	ND
4	Impurity G(1,2)	NMT 0.1%	0.04-0.05%
5	Impurity H	NMT 0.1%	0.05-0.06%
6	Impurity B	NMT 0.1%	0.05-0.06%
7	Impurity C	NMT 0.4%	ND
8	Impurity D	NMT 0.4%	ND
9	Unspecified	NMT 0.05%	0.02-0.03%
10	Total	NMT 1%	0.4-0.5%

ND- Not detected, NMT- Not more than, EP-European pharmacopeia

Reaction Schemes:



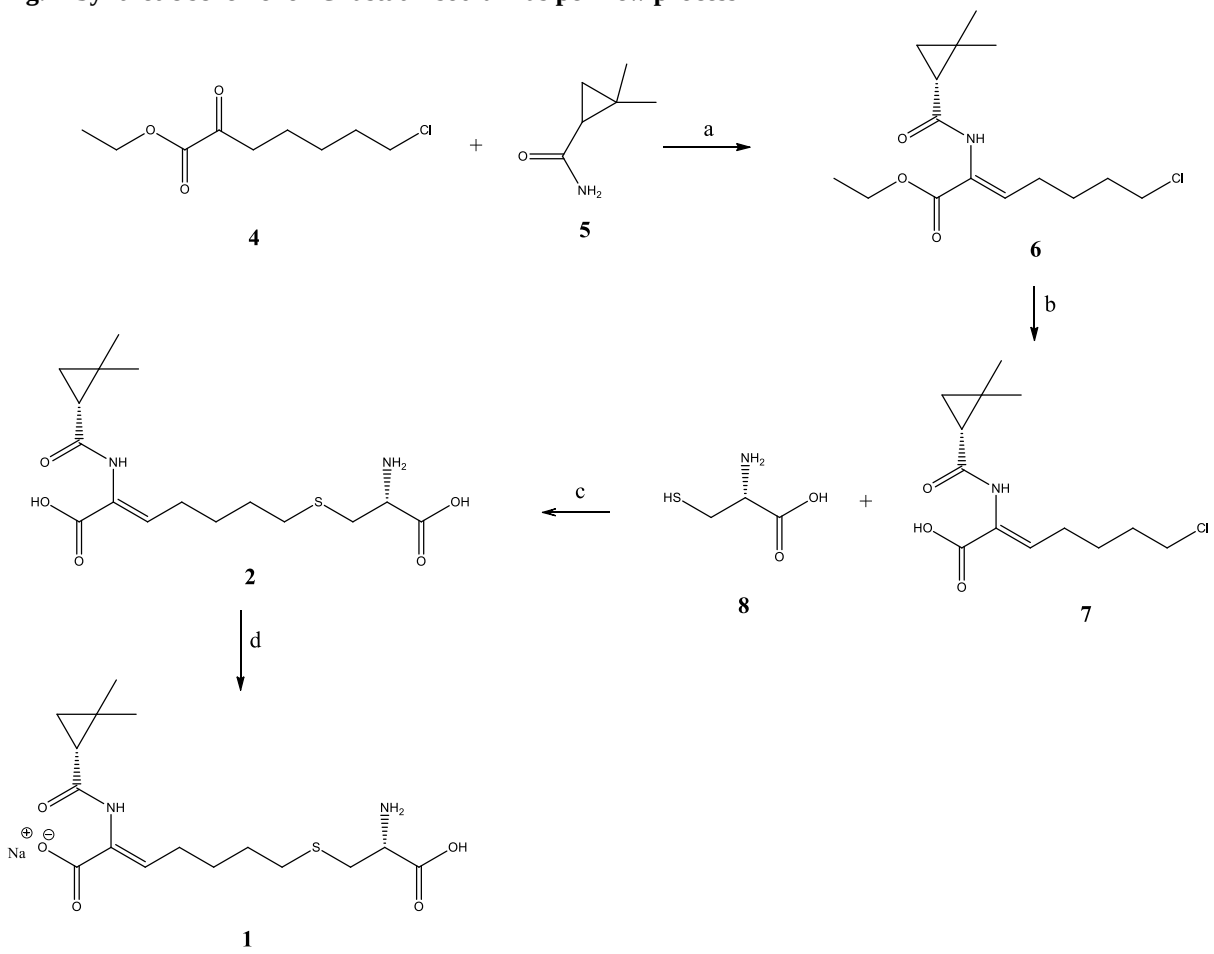
3(a-b)
(a: R=H, b: R=CH=NH)



2 **1**

Fig. 1

Fig.2- Synthetic scheme for Cilastatin sodium as per new process



Scheme.1 (Fig. 2)

Reaction conditions: a:- toluene,PTSA; b:- NaOH,RT, Conc. HCl, Aq. HCl, Ethyl acetate, Cyclohexane; c:- K_2CO_3, Methanol; d:- NaOMe, Methanol, Acetonitrile

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