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Determination of Optimal Operation Conditions for Production of Cephalosporin G from Penicillin G Potassium

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ABSTRACT: Cephalosporin G (Ceph-G) is one of the most important components for producing β-lactam antibiotics. It is also a starting material for the synthesis of cephalosporin antibiotics. Ceph-G was produced from penicillin G potassium (Pen-G.K.) during multiple steps consisting of conversion of Pen-G.K. to penicillin G sulfoxide followed by dehydration and ring expansion to produce Ceph-G. In this work, the amount of all starting materials and stoichiometric ratios were balanced, and the optimum values were determined. In addition, the process was optimized to evaluate the parameters affecting the scale-up of our test apparatus from laboratory to pilot scale. Difficulties for the industrial production of Ceph-G have been considered, and the production cost has been considerably decreased. In this research, Ceph-G with high purity more than 98% and high yield more than 94% was obtained.

INTRODUCTION

Cephalosporin compounds were first produced from *Cephalosporium acremonium* from a sewer in Sardinia in 1948 by an Italian scientist, Giuseppe Brotzu.[1] The chemical conversion of penicillin to deacetoxycephalosporin via a cyclic sulfoxide rearrangement was also reported.[2−4]

Cephalosporins are widely used as antibiotics and are similar to penicillin in the structure and mechanism of action.[5,6] They are bactericidal and belong to the same group of β-lactam antibiotics as penicillins.[7] Similar to other β-lactam antibiotics, cephalosporins inhibit and stop the formation of the peptidoglycan layer of the bacterial cell walls.[8−10] Cephalosporins are much more expensive than penicillin because they are made from penicillin following a number of chemical and enzymatic conversions.[11] Since cephalosporin C or G is widely used as precursor in the synthesis of cefradine, cephalexin, cefadroxyl, and 4-methoxybenzyl-3-chloromethyl-1-7(2 phenylacetamido)-3-cephem-4-carboxylate,[12−14] it is necessary to produce it in high quality and large quantity. The latter compound is an important intermediate of cephalosporin widely known by the name of GCLE, which is a new mother nuclide of cephem antibiotics in the synthesis of new antibiotics. Figure 1, Table 1, and Figures 2−3 show the chemical structures of parental cephalosporin, cephalosporin G (Ceph-G) and cephalosporine C (Ceph-C), penicillin G potassium (Pen-G.K.) and GCLE, respectively. The most important step involves the expansion of the five-membered penicillin ring structure to a six-membered cephalosporin ring. The cost of these processes is too high, and the industrial production faced various operational issues such as low yield and low purity.[15,16] There are several synthesis methods published for ring expansion of penicillin sulfoxides to cephalosporins.[17] In addition, in some other articles, synthesis

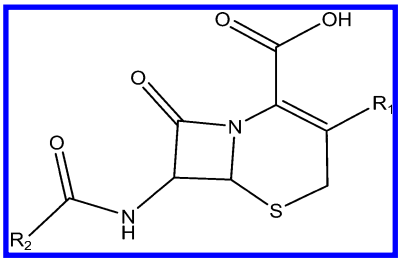
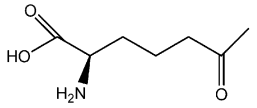
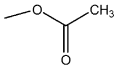
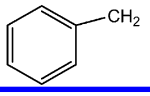


Figure 1. Parental cephalosporin.

Table 1. Chemical structures of the cephalosporin antibiotics

R2	R1	Name
		Cephalosporin C
		Cephalosporin G

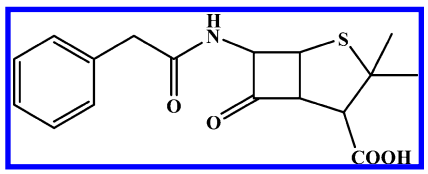


Figure 2. Chemical structure of penicillin G potassium (Pen G.K.).

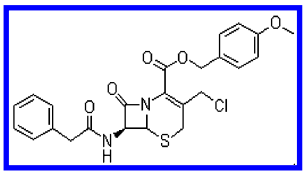


Figure 3. Chemical structure of GCLE.

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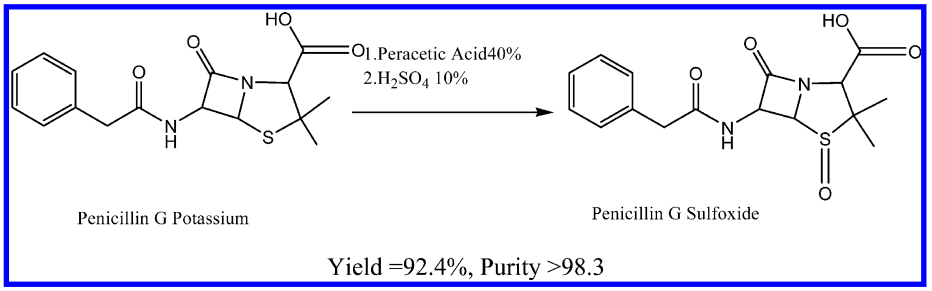
Scheme 1. Synthesis of penicillin G sulfoxide



methods have been published for converting penicillin G sulfoxide to deacetoxy cephalosporanic acid using silyl protection.[18−20] However, the proposed process was not practical because of the excess of starting material used, which would result in further problems in downstream processes, especially on pilot scales. The reported yield was about 90.7%, which was not sufficient. Some different processes were also proposed, but the maximum reported yield was about 87%, lower than the one obtained from other methods.[21]

Bovenberg and et al. (1998)[22] have published a multistep enzymatic method for ring expansion.[23−25] Although their results were promising, the process was fully enzymatic, resulting in higher operational costs. Recently, silylation has been considered as a regular chemical method for the production of cephalosporins from penicillins.[23,26,27]

In this work, Pen-G.K., as a starting material, is converted to penicillin G sulfoxide by oxidation using peracetic acid as a strong oxidizing agent; after dehydration, the process is followed by the ring-expansion process employing *N*,*N*′-bis(trimethylsilyl)urea (BSU) and pyridine hydrobromide (pyridine-HBr) to produce Ceph-G.[28,19] The main aim of this work is to optimize the consumed values of chemical agents and operational conditions to improve industrial-scale requirements. Since the excess amount of BSU and pyridine-HBr usually result in gel formation in the ring-expansion process, especially in large-scale productions,[29] optimization helps to overcome the operational issues. The operational cost was also considered as key point in process optimization.

RESULTS AND DISCUSSION

Optimization of the Synthesis of Penicillin G Sulfoxide from Pen-G.K. Strong organic acids are required for the oxidation of Pen-G.K. to penicillin G sulfoxide.[31] Sodium metaperiodate and peracetic acid are mainly used in practical cases.[18]

In our case, peracetic acid was a good candidate, since it is much cheaper than sodium metaperiodate and also easy to prepare in various concentrations (see Scheme 1). As reported in some articles, an excess amount of peracetic acid increases the possibility of the oxidation of sulfur to form sulfone derivatives.[19] In the first step, the amount of peracetic acid, the reaction time, and the pH of the solution were selected as main influential parameters on the reaction yield. In order to control the droplet rate, iodide paper was utilized as an indicator. With the increment of reaction time, the pH of the solution decreased to 3.5−3.8, and after about an hour, a suspension was formed. Several experiments were carried out, the excess amount of peracetic acid was controlled, and finally the pH of the solution decreased to 1.7 using 10% (w/w) sulfuric acid to initiate crystallization.[32]

These optimized conditions were successfully achieved with the control of peracetic acid droplet speed. The best results for the amount of peracetic acid, reaction time, and pH of crystallization were 27.5 mL (5.115 mol), 3 h, and pH 1.6, respectively. All experiments were repeated twice and average values were reported. Obtained results are shown in Tables 2, 3 and 4.

Table 2. Optimization of $C_2H_4O_3$ quantity

no.	addition time for $C_2H_4O_3$ 40% v/v (h)	yield (%)	purity area (%)
1	1	86.5	89.8
2	2	90.5	95.5
3	3	92.4	98.3

Table 3. Optimization of the droplet rate of $C_2H_4O_3$ per 1 mol of Pen-G.K.

no.	$C_2H_4O_3$ 40% (mol)	yield (%)	purity area (%)
1	4.815 pH = 3.9	84.5	90.3
2	5.115 pH = 3.6	92.4	98.3

Table 4. Optimization of crystallization pH

no.	crystallization pH	yield (%)	purity area (%)
1	1	88.9	97.6
2	1.6	90.3	96.01
3	2	92.4	98.3

Optimization of Water Removal from Penicillin G Sulfoxide Solution. As mentioned in some published articles and patents, when the water content is kept less than 0.05% (w/w) in the penicillin G sulfoxide solution, dehydration of penicillin G sulfoxide is not observed anymore.[19,33] It has been also reported that silyl esters are easily cleaved in the ring-expansion processes in aqueous medium.[18] It is mainly caused by decarboxylation, which can occur on nonprotected carboxylic groups. To prevent this unfavorable reaction, the carboxylic group was protected as the TMS group by the use of *N*,*N*′-bis(trimethylsilyl)urea (BSU). In order to optimize the excess amount of BSU, the ratio of BSU to penicillin G sulfoxide was maintained as 1.5:1 at 60 °C.[34,3] For dehydration of penicillin G sulfoxide, the process was carried out in an organic solvent. The amount of water content was measured by using a Karl-Fischer instrument.

Various organic solvents such as cyclohexane, hexane, and toluene were selected, and the water elimination process was carried out at moderately elevated temperatures, e.g., 60−70 °C. Preliminary analysis showed that the water content in the solution in supplied cyclohexane and hexane was 0.1% (w/w), which is higher than that of toluene, 0.05% (w/w).[19,33]

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Several experiments were performed to optimize the operation temperature. Obtained results showed that the optimum temperature for the formation of an azeotropic condition which facilitates the water removal was 60 °C. At higher temperatures the β-lactam ring was easily decomposed, whereas at lower temperatures, the azeotropic conditions were not formed properly.[19,3,33] Optimized results are shown in Table 5.

Table 5. Optimization of type of solvent in dehydration of penicillin G potassium

no.	solvent	temp. (°C)	water content (%)
1	toluene	50−60	<0.05
2	cyclohexane	60−70	<0.1
3	hexane	50−60	<0.1

Optimization of the Synthesis of Ceph-G from Penicillin G Sulfoxide. Ring expansion is the most important stage in the production of Ceph-G in which five-membered penicillin ring is converted to six-membered cephalosporin ring. To perform the ring-expansion process, several strong acids have been used in industrial processes. Our results showed that, when this process was carried by pyridine-HBr, the obtained yield was considerably higher than that with other strong acids. It also prevented the gel formation, which mainly happens at industrial scales. However, it is necessary to control the amount of utilized pyridine-HBr, since the excess amount of pyridine could easily transfer to the aqueous phase and, after the hydrolysis process, increase the impurity of the final product. In the first series of experiments, several tests were performed to optimize the pyridine-HBr/BSU ratio. Achieved results revealed that, when the ratio of pyridine-HBr to BSU was about 0.26:1 (mol/mol), the maximum yield was obtained (see Scheme 2). In the second series of experiments, several parameters, such as pH, the amount of BSU, the amount of pyridine-HBr, reaction temperature, and water content in penicillin G sulfoxide were selected. Several tests were carried out to optimize the mentioned affecting parameters. Obtained results are shown in Tables 6, 7, and 8.

To eliminate the silyl group, hydrolysis using an aqueous solution of sodium bicarbonate was carried out at elevated temperatures, 50−60 °C.[34] Finally the process was followed by crystallization using 10% (w/w) sulfuric acid.

Parts a and b of Table 8 show the obtained optimized results for the synthesis of penicillin G sulfoxide from Pen-G.K. and Ceph-G from penicillin G sulfoxide, respectively.

On the basis of the stoichiometric values of initial material, our results showed 94% yield and more than 98% purity for the

Table 6. Optimization of BSU quantity per 1 mol of penicillin G sulfoxide

no.	BSU (mol)	yield (%)	purity area (%)
1	2	80	88
2	1.5	90	98
3	1.3	88	96

Table 7. Optimization of pyridine-HBr quantity per 1 mol of Pen-G.K.

no.	pyridine-HBr (mol)	yield (%)	purity area (%)
1	0.6	88	93
2	0.4	90	98
3	0.2	89	96

produced Ceph-G which is considerably higher than the reported values.[3,18,34] The mechanism of ring expansion is shown in Scheme 3.

Economical Consideration of Modified Synthesis Processes in Large Scale. Since in this work simultaneous modifications of the conversions of Pen-G.K. to penicillin G sulfoxide and penicillin G sulfoxide to Ceph-G have been performed, in order to evaluate the cost-efficiency of the current work, the cost estimation of each modified process was compared with its previous available standard operation procedure (SOP) in DAANA Pharm. Co.

Table 9 compares the cost estimation of old and new SOPs in conversion of Pen-G.K. to penicillin G sulfoxide and Table 10 shows the estimated profit due to the process improvement. As shown in Table 10, for a batch production of penicillin G sulfoxide, a more than 12% decrease in total production cost is observed.

In the same way, Table 11 compares the cost estimation of old and new SOPs in conversion of penicillin G sulfoxide to Ceph-G, and Table 12 shows the estimated profit due to the improvement of process. As shown in Table 12, for a batch production of Ceph-G ∼22% decrease in total production is observed.

Taking into account that each production scale is designed and performed for 100 kg of Ceph-G in DAANA Pharm. Co., a total cost reduction of ∼22% would be economically valuable. It is worth mentioning that all weights are based on fully dried products.

CONCLUSIONS

The optimized results were obtained in the synthesis of cephalosporin G from penicillin G. Experiments were carried out in two steps.

Scheme 2. Synthesis of cephalosporin G

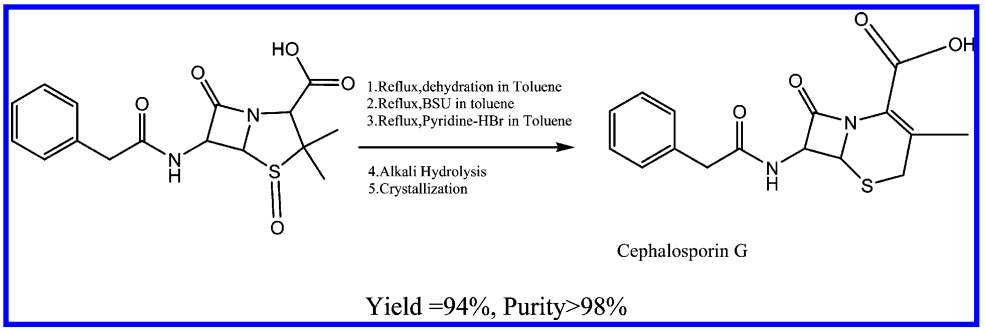

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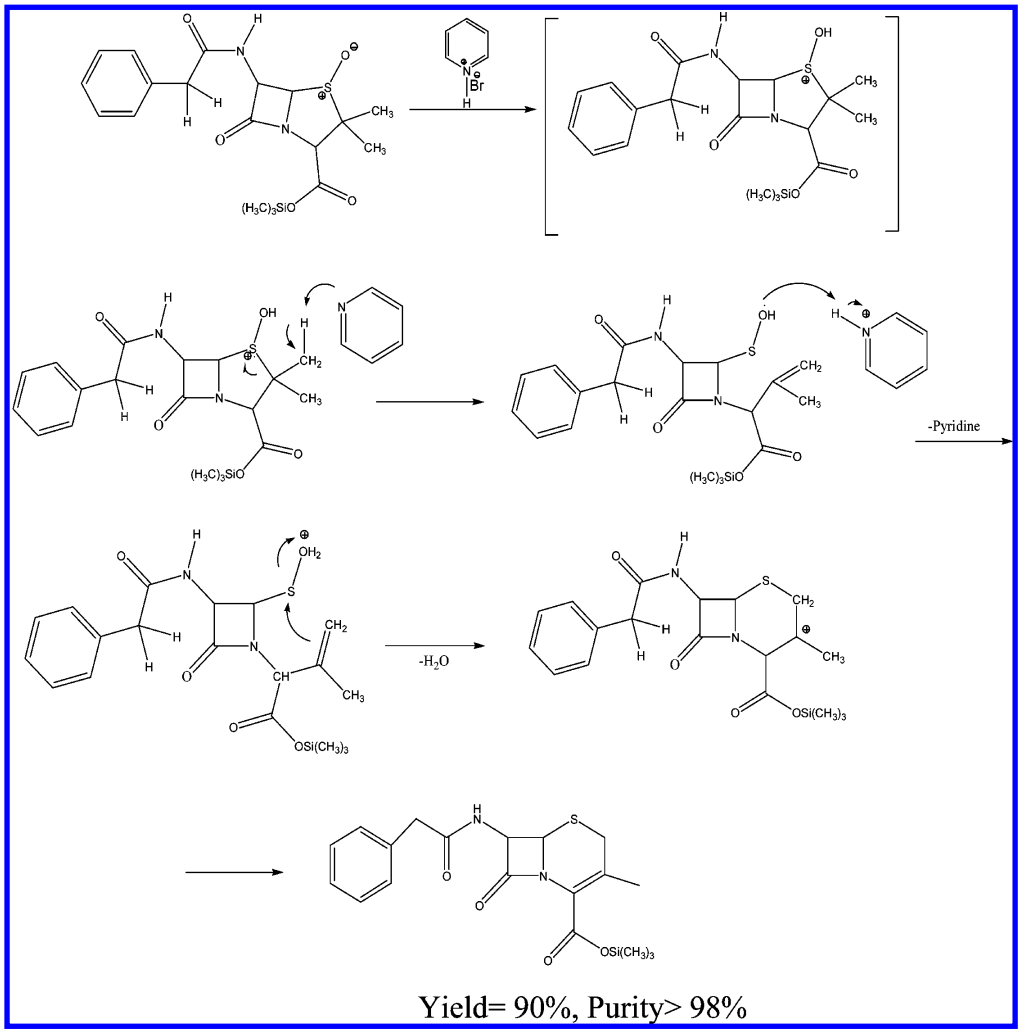
Table 8. Final optimized conditions and values

no.	step	parameters	quantity	conditions
		(a) per 1 mol of Pen G.K.		
1	penicillin G sulfoxide synthesis	peracetic acid 40%	5.115 mol	pH = 3.6
2	penicillin G sulfoxide synthesis	droplet time for peracetic acid	3 h	control with iodide paper; temp = 0−5 °C
3	penicillin G sulfoxide synthesis	crystallization pH	1.6	H_2SO_4 10%; temp = 0−5 °C
4	dehydration of penicillin G sulfoxide	toluene	1.88 mol	temp = 50−60 °C
		(b) per 1 mol of penicillin G. sulfoxide		
1	cephalosporin G synthesis	BSU	1.5 mol	reflux 1 h, temp = 60−70 °C
2	cephalosporin G synthesis	pyridine-HBr	0.4 mol	reflux 1 h; temp = 70−80 °C
3	cephalosporin G synthesis	crystallization pH	2	temp = 0−5 °C

Scheme 3. Various steps in the production of cephalosporin G from penicillin G sulfoxide



At first, penicillin G was converted to penicillin G sulfoxide, and this process was followed by ring expansion of penicillin G sulfoxide to cephalosporin G. In the first step, influential parameters such as amount of oxidation reagent, reaction time, and pH of the olution as well as the crystallization process were optimized. In the second step, several parameters, such as pH, the amount of BSU, the amount of pyridine-HBr, operation temperature, and water content in penicillin G sulfoxide were optimized. As shown in Table 8, the optimized amounts of reagents as well as operational conditions for various steps were determined. As a result, cephlosporin G was produced in good yield (>94%) and high purity (>98%). Gel formation was not observed.

EXPERIMENTAL SECTION

Materials. Pen-G.K. with 1590 billions of unit potency (1590 BOU) was provided by Sandoz Co. in Austria. Peracetic acid, BSU (*N*,*N*-bis(trimethylsilyl)urea) (Merck Art no: 8.18101), and sulfuric acid were purchased from Merck Co. In all experimental procedures, the concentrations of penicillin G sulfoxide and Ceph-G in the reaction mixture were achieved by using high performance liquid chromatography (HPLC Waters-510) through the following working conditions. The column as a μ Bonda pak C18, 125 Å, 10 μm (4.6 mm × 250 mm) was used. Flow rate, injection volume, column temper-

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Table 9. Comparison of old and newly modified SOPs in cost estimation of Pen-G.K. conversion to penicillin G sulfoxide

material name	price per kg ($)	quantity (kg)		total price ($)	
		old	new	old	new
Pen-G.K.	22.4	100	100	2240	2240
DI water	1	340	340	340	340
peracetic acid	4.3	87.6	76.59	376.68	329.337
sulfuric acid	2	97	89.91	194	179.82
DI water for washing	1	700	700	700	700
toluene	1.6	400	400	640	640
total				4490.68	4429.157

ature, and wavelength were adjusted at 1 mL min^{-1}, 20 μ, 30 °C, and 225 nm, respectively.

Bench-Scale Synthesis of Penicillin G Sulfoxide. First, 10 g Pen-G.K. was dissolved in 27 mL deionized water. Then the solution was cooled down to 0−5 °C, and 27.5 mL (5.115 mol) peracetic acid 40% was added to the solution to increase the pH up to 3−3.5. The amount of excess oxidizing agent was monitored by iodide paper until the excess in solution disappeared. At constant pH and temperature, the mixture was stirred for an hour, and then a sufficient amount of 10% (w/w) sulfuric acid solution was added dropwise; penicillin G sulfoxide crystals appeared when pH 1.6−1.8 was reached. The mixture was stirred at this pH for 90 min and filtered, and the solid product was washed with 50 mL deionized water. The product was dried at 55−60 °C. The obtained amount of penicillin G sulfoxide was 9 g. The yield and purity were 92.5% and 98.3%, respectively.

Bench-Scale Dehydration of Penicillin G Sulfoxide. For the dehydration of penicillin G sulfoxide, a 1 L round-bottom two-necked flask connected to Dean−Stark system distillation, and 50 g penicillin G sulfoxide was added to 200 mL toluene. Then the prepared solution was heated up to 50−60 °C under vacuum, and collected water was drained out from the container. Finally, distillation continued to decrease the moisture of the solution below 0.05% (w/w). Water content was determined by Karl-Fischer instrument.[2,3,24]

Pilot-Scale Synthesis of Penicillin G Sulfoxide. The large-scale experiment was performed using 2000 L capacity, jacketed stirred-tank reactor. Pen-G.K. (100 kg) was dissolved in 275 L DI water, and the reaction was continued using the optimized procedure. After completion of the reaction, the aqueous phase was transferred to a 2000 L capacity crystallizer. Crystals start growing when 10% (w/w) sulfuric acid solution was added, dropwise. The suspension was centrifuged and washed with cold water. Then the wet cake was transferred into Ribbon dryer. The temperature was adjusted at 65−70 °C, and drying was performed under vacuum until the water content reached 6%. The process was followed by dehydration, and 90.27 kg product was obtained. Yield and purity were 92.45% and 98.97%, respectively.

Table 11. Comparison of old and newly modified SOPs in cost estimation of penicillin G sulfoxide conversion to Ceph-G

material name	price per kg ($)	quantity (kg)		total price ($)	
		old	new	old	new
penicillin G sulfoxide	59.87	100	100	5987	5987
SHS	2.2	340	340	748	748
BSU	15	60	45	900	675
pyridine-HBr	5	15	9	75	45
DI water for washing	1	1000	1000	1000	1000
toluene	1.6	200	200	320	320
$NaHCO_3$	3	270	270	810	810
sulfuric acid	2	60	40	120	80
total				9960	9665

Table 12. Profit estimation due to improvement of Ceph-G synthesis process

obtained yield in old method (kg)	obtained yield in new method (kg)	total cost in old method	total cost in new method	total cost per kg in old method	total cost per kg in new method	profit %
81.21	101.04	9960	9665	122.64	95.66	21.9

Bench-Scale Synthesis of Ceph-G. BSU (45 g) was dissolved in 200 mL toluene, and then 45 g dehydrated penicillin G sulfoxide with water content lower than 0.1% was poured into the solution. The temperature was then increased to 65−70 °C, and the solution was stirred for an hour. After that, 9 g pyridine-HBr was added, and the solution was refluxed at 80−90 °C under vacuum for about 4 h.

$NaHCO_3$ solution (600 mL of 4.5% (w/v)) was poured into the solution at 70 °C, and the pH of solution was adjusted at 8. The solution was stirred for about 30 min at 50−60 °C. Sulfuric acid (10% (w/w)) solution was added to the solution, dropwise to initiate the crystallization of Ceph-G at 50 °C and the pH was adjusted at 2. The solution was cooled to 0−5 °C gently with stirring and then it was filtered. The solid was washed with 200 mL DI water and then dried at 60−65 °C. As a result, 45.1 g Ceph-G was obtained from penicillin G sulfoxide at the form of cream-colored powder with more than 98% purity in which the obtained yield was more than 94%. The water content of solid product was less than 0.05%. The obtained results were in good agreement with results reported in the literature.[28,30] This reaction is shown in Scheme 2.

Pilot-Scale Synthesis of Cephalosporin G. The large-scale experiment was performed using 2000 L capacity stirred tank reactor with jacket. 100 kg penicillin G sulfoxide was dissolved in 400 L toluene. The solution was heated up to 60−70 °C and 45 kg BSU was then added to the reactor. Reflux was continued until a clear solution was obtained. Nine kilograms of pyridine-HBr was then added to the solution, and reflux was again continued about 4 h. After adjusting the pH, the hydrolysis reaction was completed, and then the aqueous phase was transferred to a 2000 L capacity crystallizer. Crystals started growing when 10% (w/w) sulfuric acid solution was added

Table 10. Profit estimation due to improvement of penicillin G sulfoxide synthesis process

obtained yield with old method (kg)	obtained yield with new (kg)	total cost with old method (US $)	total cost with new method (US $)	total cost per kg with old method (US $)	total cost per kg with new method (US $)	profit (%)
75	84.2	4490.68	4429.157	59.875	52.602	12.14

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dropwise. The suspension was centrifuged and several times rinsed with cold water. Then the wet cake was transferred into a Ribbon dryer. The temperature was adjusted at 50−55 °C, and drying was performed under vacuum until the water content reached less than 0.05%. Finally, 101.04 kg product was obtained. Yield and purity were more than 94% and 98.5%, respectively.

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Notes

The authors declare no competing financial interest.

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NOMENCLATURE

GCLE	4-methoxybenzyl-3-chloromethyl-1-7(2 phenylacetamido)-3-cephem-4-carboxylate
Pen-G.K.	penicillin G potassium
Ceph-C	cephalosporin C
Ceph-G	cephalosporin G
BSU	*N*,*N*′-bis(trimethylsilyl)urea

REFERENCES

(1) Bortzu, G *Ricerche su di un nuovo antiobiotico*; Lavori dell'Istituto di Igiene dell' Universita di Cagillar, 1948.

(2) Morin, R. B. Penicillin conversion via sulfoxide. U.S. Patent 3,275,626, 1966.

(3) De Koning, J. J.; Kooremon, H. J.; Tan, H. S.; Verweij, J. *J. Org. Chem.* **1975**, *40*, 1346−1347.

(4) Lopez, J. L. Process for 7-aminodesacetoxycephalosporanic acid. U.S. Patent 5,521,068, 1996.

(5) Pan, J. L.; Syu, M. J *Biochem. Eng. J.* **2005**, *23*, 203−210.

(6) Dash, Ch. *J. Antimicrob. Chemother.* **1975**, *1*, 107−18.

(7) Pichichero, M. J. *Family Practice* **2006**, *55*, 106−112.

(8) Chou, T. S. Process for preparing desacetoxycephalosporins. U.S. Patent 3,944,545, 1976.

(9) Eardley, S.; Kennedy, J.; Long, A. G. Cephalosporin compounds. U.S. Patent 3,658,799, 1972.

(10) Hatfield, L. D. Process for making desacetoxycephalosporins. U.S. Patent 3,591,585, 1971.

(11) Illanes, A.; Rodriguez, F.; Bahamondes, C.; Altamirano, C. *Biochem. Eng. J.* **2005**, *24*, 209−213.

(12) El-Shaboury, S. R.; Saleh, G. A.; Mohamed, F. A.; Rageh, A. H. J. *Pharm. Biomed.* **2007**, *45*, 1−19.

(13) Marrelli, L. P., Ed. *Analytical Profiles of Drug Substances*; Academic Press: New York, 1975; pp 21−46.

(14) Florey, K., Ed. *Analytical Profiles of Drug Substances*; Academic Press: New York, 1976; pp 21−59.

(15) Hatfield, L. D. Desacetoxycephalosporins via penicillin sulfoxide rearrangement. U.S. Patent 3,953,440, 1970.

(16) Nieuwenhuis, P. G. J. Conversion of penicillins and cephalosporins to 1-(S)-sulfoxides. U.S. Patent 5,442,058, 1995.

(17) Gruszecki, W. *11th IUPAC Int. Symp. Chem. Nat. Prod.*; **1978**, *3*, 44-46.

(18) Verweij, J.; Tan, H. S.; Kooreman, H. J. Preparation of 7-substituted amino-desacetoxycephalosporanic acid compounds. U.S. Patent 4,003,894, 1997.

(19) Chauvette, R. R.; Pennington, P. A.; Charles, W. R.; Cooper, R. D. G.; Jose, F. L.; Wright, I. G.; Van Heyningen, E. M.; Huffman, G. W. *J. Org. Chem.* **1971**, *36*, 1259−1267.

(20) Webber, J. A.; Van Heyningen, E. M. Δ-2-Cephalosporin compounds. U.S. Patent 3,637,678, 1972.

(21) Podolsky, M. L. *Cures Out of Chaos*; Harwood Academic Publishers: Amsterdam, 1998.

(22) Bovenberg, R. A. L.; et al. Process for the production of 7-ADCA via expandase activity on penicillin G. U.S. Patent 5,731,165, 1998.

(23) Bovenberg, R. A. L.; et al. Process for the production of 7-ADCA via 3-(carboxyethylthio)propionyl-7-ADCA. U.S. Patent 5,795,733, 1998.

(24) Morin, R. B.; Jackson, B. G.; Mueller, R. A.; Lavagnino, E. R.; Scanlon, W. B; Andrews, S. L. *J. Am. Chem. Soc.* **1969**, *91*, 1401−1407.

(25) Bovenberg, R. A. L.; et al. Process for the production of 7-ADCA via expandase activity on penicillin G. U.S. Patent 6,020,151, 2000.

(26) Chou, T. S. Process for preparing desacetoxycephalosporins. U.S. Patent 3,944,545, 1976.

(27) Sutherland, J. D.; et al. Process for the production of SSC's via expandase activity on penicillin G. U.S. Patent 5,919,680, 1999.

(28) Spry, D. O. *J. Am. Chem. Soc.* **1970**, *92*, 5006−5011.

(29) Alargova, R., KadiyalaI., A. Le Tiran, ZhangY., Solid forms of 1-ethyl-3-(5-(5-fluoropyridin-3-Yl)-7-(pyridin-2-Yl)-1H-benzo[*d*]imidazol-2-Yl)urea. WIPO Pat. Appl. WO/2009/076200, 2009

(30) Van Der Does, T. H.; Kapur, J. C.; De Vroom, E. Method for preparing a *β*-lactam antibiotic. U.S. 2002/0006642 A1, 1999.

(31) Mangia, A. *Synthesis* **1978**, 361−363.

(32) Ludescher, J.; Veit, W. Purification process. US 2001/0044533 A1, 2001.

(33) Yeh, J. B.; Lee, L-T; Chen, M.-H. Method for the preparation of $^{\Delta}$3-7-substituted amino deacetoxy. U.S. Patent 5,302,713, 1994.

(34) Garbrechet, W. L. Cephalexin synthesis. U.S. Patent 3,632,850, 1972.