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Ceftibuten:† Development of a Commercial Process Based on Cephalosporin C. Part I. Process for the Manufacture of 3-Acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic Acid 1(*S*)-oxide

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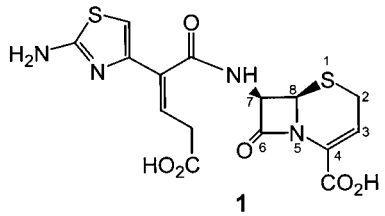
Abstract:

The manufacture of low-cost, orally active cephalosporin drugs has, until now, been achieved using intermediates prepared by the ring expansion of penicillin sulfoxides rather than fermented cephalosporin C. This report describes the preparation of 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid 1(*S*)-oxide (8) from cephalosporin C broths. This new intermediate has been shown (see the following contributions) to be a superior starting material for the low-cost preparation of the orally active cephalosporin, Ceftibuten.

Introduction

Most parenteral cephalosporin drugs are produced from 7(*R*)-aminocephalosporanic acid (7-ACA), itself derived from fermented cephalosporin C **(4)**. Orally active cephalosporin drugs emerged from the vigorous programs of work to broadly exploit the superior properties of the cephalosporins in general, particularly their enhanced spectrum of activity, their greater stability to β-lactamases, and their improved safety profiles, versus the penicillins.[1] Early on, efforts to understand the biological mechanism involved in cephalosporin formation led to the discovery[2] of the ring-expansion of low-cost penicillin sulfoxides to 3-methylcephalosporins, a few of which were manipulated to create oral activity. The ring expansion discovery led to the identification and development of several relatively low-cost orally active cephalosporins, for example, cephalexin,[3] cephradine,[4] cefadroxil,[5] and cefaclor.[6] The continued search for orally active cephalosporins with broader biological spectrum led to new compounds, most based on cephalosporin C, for example, cefroxadine,[7] cefatrizine,[8] cefixime,[9] and cefprozil.[10] The syntheses of these last four compounds were all inefficient, often requiring protection and deprotection steps, which added to their costs. Indeed the high cost of goods of the oral cephalosporin drugs derived from cephalosporin C significantly offset the attractiveness of their improved biological spectrum versus the above penicillin-based oral cephalosporins.

The discovery of the parenteral cephalosporin ceftizoxime[11] by Fujisawa Pharmaceutical Co. Ltd., and the oral cephalosporin Ceftibuten[12] (**1**) by Shionogi and Co. Ltd., only exacerbated the cost-of-goods dilemma, since both of these cephalosporins are unique in carrying an H-substituent at the 3-position of the cephalosporin nucleus.



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Introducing the 3-H substituent requires additional manipulations of the cephalosporin molecule for the conversion of the 3-acetoxymethyl group in cephalosporin C to 3-H. This has been achieved by converting acetoxymethyl to hydroxymethyl followed by oxidation of the hydroxymethyl group to the 3-aldehyde and finally decarbonylation and also by the Cr(II)-mediated reduction of the 3-acetoxymethyl

† Ceftibuten was discovered by Shionogi and Co. Ltd., Osaka, Japan, and licensed to Schering-Plough Corporation, Kenilworth, New Jersey. The drug is manufactured by Shionogi.

‡ Chemical Development, Antibioticos, S.p.A.

§ Chemical Development, Schering-Plough Research Institute.

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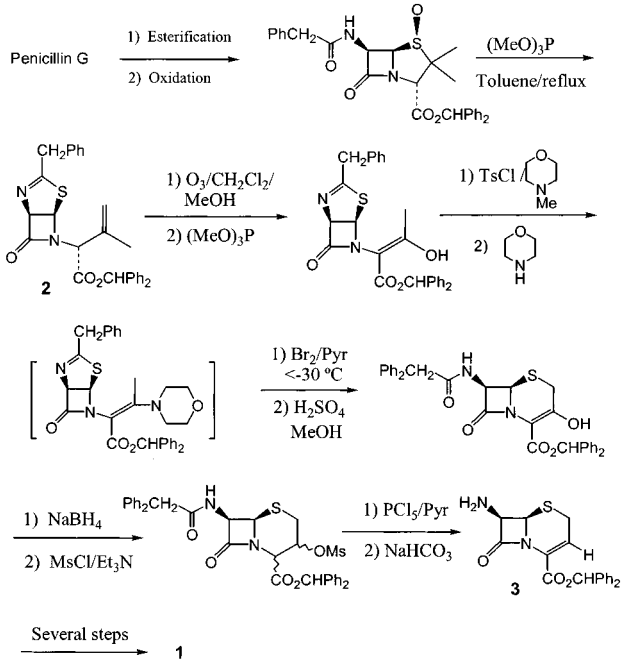
group to 3-exomethylene and thence, in a series of steps (ozonolysis to 3-hydroxycephem, reduction to 3-hydroxycepham, activation of 3-hydroxy and base treatment), to the 3-H cephem.[13] From an economic standpoint, neither cephalosporin C-based route was attractive for commercial operation and led Fujisawa workers to develop a synthesis scheme based on the intermediates generated in Lilly work on cefaclor[6] and Ciba-Geigy work on cefroxadine,[7] in short returning to intermediates derived from penicillins.[14] The added costs introduced by the molecular manipulations needed to access the 3-H compounds required for the parental drug ceftizoxime were held as justifiable since this compound offered biological advantages over the earlier parenteral cephalosporins. However, since the oral cephalosporins are generally administered at higher doses than their parenteral counterparts, what was economically acceptable for producing the parental Ceftizoxime molecule was unacceptable for producing the oral Ceftibuten molecule. Indeed the development of a practical, low-cost process for Ceftibuten posed severe challenges for the chemical development scientists and engineers in Shionogi. They evaluated cephalosporin routes to Ceftibuten[15] but quickly decided not to pursue them in light of their earlier discovery and successful exploitation[16] of a route to 3-hydroxycephems that was based on other starting materials derived from the rearrangement of penicillin sulfoxide esters, notably building on the rearrangement discovered by Cooper.[17]

The Shionogi route[18] for converting penicillin G sulfoxide to Ceftibuten (Scheme 1) thus developed quite naturally from Shionogi's position. The Cooper intermediate[19] of choice is identified as compound **2** in Scheme 1.

The step yields to diphenylmethyl 7(*R*)-aminoceph-3-em-4-carboxylate **3**, the key Ceftibuten intermediate, are good to excellent, and the quality of **3** is very high. However, not surprisingly, the large number of steps and intermediate isolations results in a high cost of **3**, and hence a high cost of Ceftibuten.

Although the use of a cephalosporin starting material, in place of the penicillin described in Scheme 1, would eliminate the several steps needed to convert the five-membered ring in the penicillin to a six-membered ring, the high cost of cephalosporin C, and the additional steps needed to introduce the 3-H substituent, have until now proved an insurmountable obstacle to the use of this starting material for Ceftibuten manufacture. The main factor responsible for

Scheme 1. **Synthesis of Ceftibuten nucleus**



the high cost of cephalosporin C is the presence of the zwitterionic α-aminoadipoyl (α-Aad) side chain which introduces water solubility and with it the need for expensive recovery procedures to extract the molecule from fermenter broths. Many attempts have been made to overcome this problem. Glaxo workers[20a,b] described the enzyme-mediated conversion of the α-Aad side chain of cephalosporin C to the glutaroyl side chain, thereby introducing possibilities for solvent extraction, but did not reduce the process to practice. Asahi[20c] patented the same transformation using a ketone and hydrogen peroxide. Several groups[21] have acylated the amino group of the aminoadipoyl side chain in fermentation broths to destroy the zwitterionic character, thereby allowing a solvent-extraction process (Bristol-Myers commercialised such a process[21c,d]). In a further variation of the derivatisation followed by solvent-extraction approach, Glaxo workers described extractive esterification of N-acylated cephalosporin C using diphenyldiazomethane[22] and the formation and solvent extraction of a Hantzsch dihydropyridine derivative of cephalosporin C.[23]

The above derivatisation/extraction processes mostly depend on isolating the functionalised cephalosporin C product, purifying it, and then converting it to the key intermediate, 7-ACA, or an ester thereof, using conventional

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(19) Cooper's original intermediate related to **2** carried a phenoxymethyl group in place of benzyl and the 2,2,2-trichloroethyl group in place of benzhydryl: ref 17, this paper.

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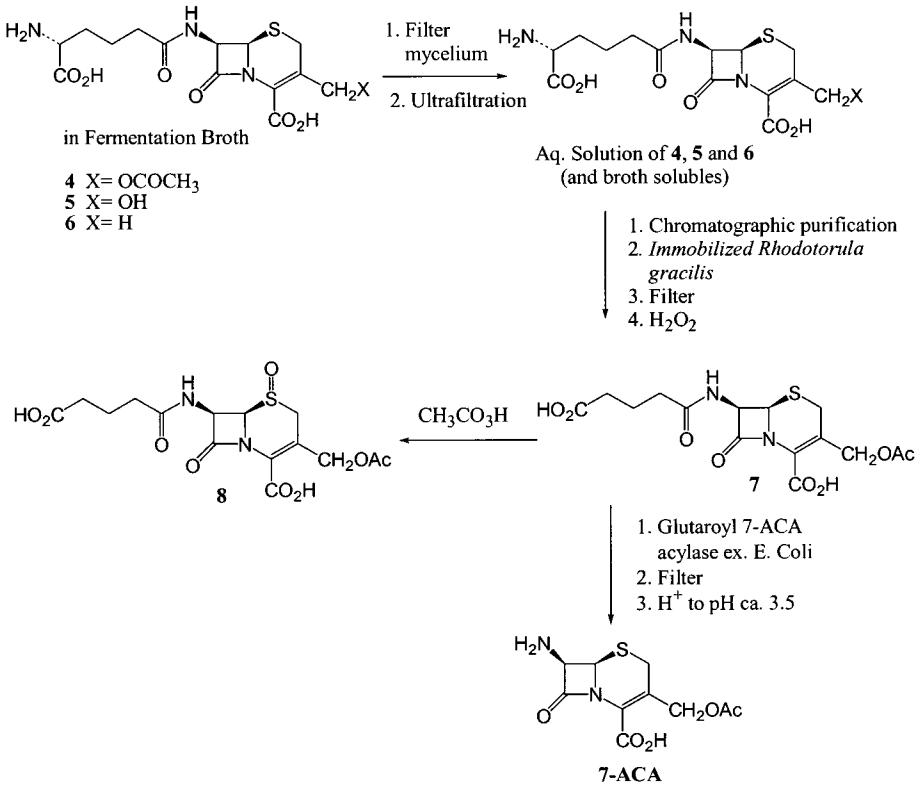
Scheme 2. **Industrial process for 7-glutaroylcephalosporanic acid and 7-ACA production**



chemistry, for example, PCl_5 cleavage of the amide group of 7-ACA or an ester thereof.[24] The several process steps needed in derivatization/extraction processes to 7-ACA involve organic solvents and require a great deal of process equipment. These operations add substantial labor, equipment depreciation, and chemical costs, which contribute significantly to the cost of 7-ACA produced via these routes. The efforts of others to find organisms which directly cleave the α-Aad side chain have met with little success, although Toyo Jozo workers[25] did show that the enzymic cleavage of the glutaroyl side chain of products produced by Glaxo,[20a,b] to give 7-ACA, was feasible.

Results and Discussion

Recognizing the disadvantages and the costs associated with the above schemes to utilize cephalosporin C, Antibioticos[26] reasoned that the use of immobilized enzymes, for both the enzyme-mediated conversion of the α-Aad side chain to glutaroyl and the enzyme-mediated cleavage of this side chain, should enable them to produce 7-ACA in an all-water system without isolating any intermediate or employing any solvent. This approach, outlined in Scheme 2, was validated and is now practiced commercially on a >100 tonne/annum scale. The process has been patented.[27]

The efficiency of the approach described in Scheme 2 is apparent from further detail of the handling of the process.

The principal product of the fermentation of *Cephalosporium acremonium* is cephalosporin C (**4**), generally mixed with small amounts of desacetyl (**5**) and 3-methyl (**6**) compounds. The thick fermentation broth is ultrafiltered to remove the mycelium and high-molecular-weight substances. The clear solution is purified through an adsorption−elution process on macroreticular resins.[28] The purification step is needed to reduce the amounts of **5** and **6** and to eliminate proteinaceous and carbohydrate materials in the fermentation broth.

The purified solution, decolorized with ion-exchange resin,[29] and containing mostly cephalosporin C, is then enzymatically converted into 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid (**7**) using an immobilized D-amino acid oxidase [DAO] derived from a *Rhodotorula* microorganism, particularly *R. gracilis* (ATCC 26217). The reaction rate is monitored by HPLC. The oxidation reaction is generally rapid (1−2 h); however, to enhance production rates, shorter times are employed, and the residual intermediate, 3-acetoxymethyl-7(*R*)-(5′-ketoadipoyl)aminoceph-3-em-4-carboxylic acid, oxidized to **7** using a small excess of hydrogen peroxide.

The solution of **7** obtained from the above process is then submitted to a second enzymatic step to obtain 7-ACA using immobilized 7-glutaroyl ACA acylase [GLA]. The immobilized enzymes are recycled many times. Both the

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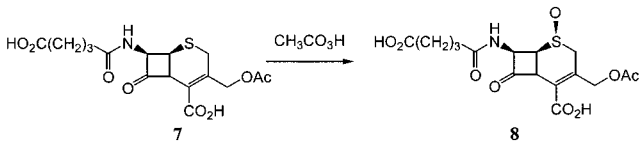
(29) IRA 68 (Rohm & Haas); WA 20 and WA 30 (Mitsubishi Chemical Corporation).

enzymes are produced by fermentation of highly selected recombinant producer strains, extracted, purified, and immobilized. 7-ACA is precipitated by adjusting the pH of the solution to 3.5−4.0, filtered, washed, and dried. The quality of purified 7-ACA obtained from the enzyme-mediated process is equivalent (containing <0.5% each of 3-methyl and 3-hydroxymethyl impurities) to that of 7-ACA produced from isolated cephalosporin C by the alternative amide cleavage process using phosphorus pentachloride.

It quickly became clear that by building on the above conceptual approach, it should be possible to produce a low-cost cephalosporin molecule suitable as an intermediate for the manufacture of Ceftibuten. Such an approach would eliminate the tedious and expensive chemical manipulations associated with the expansion of the five-membered ring of penicillin to the needed six-membered ring of a cephalosporin (Scheme 1). In addition, the all-aqueous process practiced by Antibioticos has considerable environmental merit.

As described in the following contributions we identified 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid 1(*S*)-oxide **(8)** as the substrate of choice for our new synthesis of Ceftibuten. This compound was readily obtained from the aqueous solution of **7** by oxidation with peracetic acid. A major benefit resulting from the identification of **8** as the starting material for Ceftibuten lies in the economies associated with the simple utilisation of a process stream from an existing commercial operation. This approach enabled us to capitalise on the advantage of working in water and avoiding isolations for much of the new synthesis of the key Ceftibuten intermediate, **3**.



For initial process development purposes, **8** was isolated and characterised. The solid form of **8** was used to optimise the chemistry for the later steps (see Parts II and III[30]). Once the process starting with the solid form of **8** was optimized, we proceeded to show that the process could start with the aqueous stream from the sulfoxidation step outlined in Scheme 2.

Conclusions

The conversion of cephalosporin C into 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid **(7)** utilising an immobilised D-amino acid oxidase is described. This first step in the now commercial process for 7-ACA manufacture provides a process stream which is shown to be useful for the preparation of 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid 1(*S*)-oxide **(8)**. Cephalosporin **8** is the intermediate of choice for Ceftibuten manufacture because of its ready accessibility and the unique mechanism of its electrochemical reduction (see Part II[30a]).

(30) (a) Part II: Bernasconi, E.; Genders, D.; Lee, J.; Longoni, D.; Martin, C. R.; Menon, V.; Roletto, J.; Sogli, L.; Walker, D.; Zappi, G.; Zelenay, P.; Zhang, H. *Org. Process Res. Dev.* **2002**, *6*, 158. (b) Part III: Bernasconi, E.; Lee, J.; Sogli, L.; Walker, D. *Org. Process Res. Dev.* **2002**, *6*, 169.

Experimental Section

General Methods. All HPLC analyses were carried out using a Hypersil C18 column (5 μm and 2.1 mm) maintained at a temperature of 40 °C. Gradient elution was typically undertaken using solutions of A: 3.25 g/L sodium acetate at pH 5.0 and B: Eluant A (950 parts) plus acetonitrile (50 parts) at a flow rate of 0.25 mL/min and mixtures as follows:

time (min)	A (%)	B (%)
2	65	35
13	10	90
17	10	90

analysis time is generally ~20 min.

FAB MS was determined using a JEOL 110 Double Focusing magnetic sector mass spectrometer. The source was operated at an accelerating voltage of 10 kV, the FAB gun being maintained at a voltage of 6 kV.

NMR experiments were conducted using a WP-300 NMR and AM-400 NMR (both Bruker) spectrometers.

Production of D-Amino Acid Oxidase by Means of *Rhodotorula gracilis* ATCC 26217 Culture. A 100-L fermenter is charged with 70 L of broth having the following composition:

NaCl	0.5 g/L
K_2HPO_4	1.5 g/L
$MgSO_4 \cdot 7H_2O$	1 g/L
$CaCl_2$	0.25 g/L
$ZnSO_4$	0.002 g/L
$FeCl_3$	0.003 g/L
glucose	25 g/L
D-alanine	7 g/L

The medium was adjusted to pH 5.6 with 2 N H_2SO_4, sterilized at 120 °C for 20 min and cooled to 20 °C. It was inoculated with a vegetative culture of *Rhodotorula gracilis* ATCC 26217 and fermented for 28 h at 30 °C under stirring at 200 rpm and aeration at 0.5 L/L/min. During fermentation the pH is allowed to fall spontaneously to 5 at which it is maintained constant by automatic additions of 10% NaOH.

At the end of the fermentation 72 L of culture broth are obtained with $OD_{660} = 39$ and a D-amino acid oxidase activity of 4600 units/L. The broth is centrifuged at 5000*g* in a Westfalia chamber centrifuge.

Cell paste (3.1 kg) was obtained (moisture about 80%) corresponding to 320 000 units of D-amino acid oxidase.

Extraction and Purification of D-Amino Acid Oxidase. Cell paste (1 kg, 103 000 units of DAO) obtained as described above was dispersed in 3 L of 25 mM pH 8 phosphate buffer containing 0.5 g/L of sodium metabisulphite and 0.5 g/L of cetylpyridium chloride.

The suspension was cooled to 4 °C and passed through a Manton−Gaulin press at 550 bar. The homogenized product (4.3 L) was flocculated by adding 20 mL of cationic polyelectrolyte (Nymco 2045C). The flocculate was clarified by filtering through Hyflo. The clarified product (4.9 L) was concentrated by ultrafiltration at 4 °C through a polysulphonic membrane of MW 30 000.

Ammonium sulphate (262 g) was added to the concentrate obtained by the ultrafiltration (0.750 L). The precipitate was

separated from the supernatant by centrifugation and redissolved in 300 mL of 25 mM pH 8 phosphate buffer containing 0.5 g/L of sodium metabisulphite.

The solution (320 mL) was diafiltered by ultrafiltration through a membrane of MW 30 000. The diafiltrate (340 mL) contains the crude DAO in a concentration of 224 units/mL.

The D-amino acid oxidase was purified by feeding the solution of crude enzyme through a Sepharose DEAE fast-flow column (bed volume 800 mL, column height 40 cm, diameter, 5 cm) and eluting with the same 25 mM pH 8 phosphate buffer. The DAO was not retained by the resin but was only slowed in its travel and passed into the percolate.

The interfering enzymes, in particular the esterase, were not eluted with the 25 mM pH 8 buffer and were displaced only during the regeneration of the column with 0.5 M NaCl. The purified D-amino acid oxidase was collected in a volume of 1230 mL with an activity of 25 units/mL and a specific activity of 19 units/mg proteins. The total purification yield was 62%, corresponding to a total of 63 920 units. The purified D-amino acid oxidase was stable for at least 6 days at 4 °C and for at least 6 months at −20 °C.

Immobilization of D-Amino Acid Oxidase on Duolite A 365. Duolite A 365 resin with a particle size of 100−200 μm (35 g) was treated with 0.5 L of 100 mM pH 8 potassium phosphate buffer. After 15 min of stirring the pH was adjusted by sequential additions of 10% H_3PO_4 (6 mL). When the pH was constant at 8 the supernatant was removed by filtration. Glutaraldehyde (400 ml, 2%) in 25 mM pH 8 potassium phosphate buffer was added to the wet resin. It was left stirring for 30 min at a temperature of 20−25 °C, after which the supernatant was separated by filtration to obtain a wet, solid mass.

D-Amino acid oxidase solution (386 mL, 52 units/mL; 19 units/mg proteins) purified as above was added to the wet, activated resin mass. The system was kept under mild stirring for 12 h at 4 °C. The immobilization yield, calculated on the concentration of the spent supernatant, was 100%. The product was filtered and the wet mass washed with 0.5 M NaCl in 25 mM pH 8 potassium phosphate buffer and then with 25 mM pH 7.5 potassium phosphate buffer. Immobilized D-amino acid oxidase (103 g) was obtained with an activity of 48 units/g of wet product.

Production of 3-Acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid (7) from Filtered Cephalosporin C Broth. Ultrafiltered cephalosporin C broth [28 L, 265 g; 50−70% pure (area % HPLC); 0.638 mol; A_{420nm} > 2.0] was adjusted to pH 2.3 with 40% H_2SO_4. The solution was applied (at flow rate of 1.5 bed vols (BV)/h) on 14 L of adsorbent resin (XAD 16) charged into a column (height 100 cm; diameter 15 cm). The resin was previously adjusted to pH 2.3 with 10% H_2SO_4. Water (14 L) was then passed through the column (flow rate 1.5 BV/h). The elution of cephalosporin C was monitored by HPLC. The yield in the purification step was >95%. The purity of the solution of cephalosporin C was 85−90% (area % HPLC), A_{420nm} = 0.6−0.8.

Purified solution (42 L) was decolorized with 2.8 L of ion-exchange resin (IRA 68) at pH 6.0. The resin was charged into a column of height 150 cm and diameter 2 cm. The flow rate of the decolorization was 2.5 BV/h. The yield in the decolorisation step was >95%.

Decolorized solution (42 L) containing 239.0 g of cephalosporin C (A_{420nm} < 0.1) was evaporated to reach a volume of ca. 10 L and then fed into a 20-L reactor with 840 g of immobilized wet D-amino acid oxidase. Incubation was conducted at 25 °C under slight stirring and with an oxygen flow through a bottom diffuser of 1 vol/vol/min. The pH was maintained at 7.5 by automatic additions of 5% ammonia. In 75 min the cephalosporin C was completely transformed. The percentage composition of the cephalosporin C transformation products was 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid (88%), 3-acetoxymethyl-7(*R*)-(5′-ketoadipoyl)aminoceph-3-em-4-carboxylic acid (6%), and other (4%). The solution obtained after incubation was separated from the immobilized enzyme mass by filtration. For each liter of filtrate 56 mL of 3.5% hydrogen peroxide was added under stirring. The mixture was left for 15 min at 25 °C after which 2.8 g of sodium pyruvate was added. The purity of the solution of 3-acetoxymethyl-**7**(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid **7** was 93% (area % HPLC). A sample was taken and crystallised to give a solid product for characterisation. This was done by diluting the solution obtained after the sodium pyruvate treatment with deionised water to a concentration of **7** of ca. 20−25 g/L. Sodium chloride (1.5 kg) was added to a 10 L quantity of the solution of **7** and the mixture stirred until all the NaCl had dissolved. The pH was adjusted to 1.5 to 2 using 18% hydrochloric acid. The crystals of **7** were filtered and suspended in water (1.5 L), stirred for 30 min, filtered, and dried. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72 (m, 2H), 2.03 (s, 3H), 2.21 (m, 4H), 3.48 (d, 1H, J = 18 Hz), 3.62 (d, 1H, J = 18 Hz), 4.68 (d, 1H, J = 12.8 Hz), 4.99 (d, 1H, J = 12.8 Hz), 5.08 (d, 1H, J = 4.8 Hz), 5.67 (dd, 1H, J = 8, 4.8 Hz), 8.85 (d, 1H, J = 8 Hz), 12.75 (bs, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.9, 173.5, 171.1, 165.6, 163.7, 127.4, 124.0, 63.6, 59.9, 58.3, 34.7, 33.7, 26.4, 21.4; FAB HRMS calcd for $C_{15}H_{19}N_2O_8S$ (MH^+) m/z 387.0862 found 387.0850.

Oxidation of 7 to 8 in Aqueous Na_2CO_3 Solution. 7 (200 g, 93% purity, 0.48 mol), isolated as above, was suspended in 1.2 L of water and was added to a three-neck round-bottom flask equipped with agitator, thermometer, and addition funnel. The slurry was cooled to 0−5 °C. A solution of Na_2CO_3 (54.8 g, 0.518 mol) in 800 mL of water was added slowly. The mixture was agitated at 0−5 °C until dissolution was complete. Peracetic acid (32%, 100 mL, 0.48 mol) was added over 30 min while the reaction temperature was maintained at 0−5 °C. The oxidation was monitored by HPLC. After the reaction was complete, a sample was taken for lyophilisation and NMR analysis. The solution of the reaction (>95% solution yield) was used for further chemical transformations (comparative Zn/H^+ reductions; see Part III[30b]) without isolation. A sample was prepared for analysis using the method described in the previous experiment. ^{1}H

NMR (400 MHz, DMSO-d_6): δ 1.73 (m, 2H), 2.02 (s, 3H), 2.29 (m, 4H), 3.57 (d, 2H, J = 18.4 Hz), 3.82 (d, 2H, J = 18.4 Hz), 4.60 (d, 1H, J = 12.8 Hz), 4.86 (d, 1H, J = 3.8 Hz), 5.21 (d, 1H, J = 12.8 Hz), 5.76 (dd, 1H, J = 8, 4.8 Hz), 8.19 (d, 1H, J = 8.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.0, 174.6, 172.1, 165.8, 164.2, 122.7, 110.1, 67.4, 64.7, 59.3, 46.5, 34.9, 34.0, 21.7, 21.6; FAB HRMS calcd for $C_{15}H_{19}N_2O_9S$ (MH^+) m/z 403.0811; found 403.0801.

Pilot-Scale Preparation and Isolation of 3-Acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic Acid 1(*S*)-Oxide (8). 3-Acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid **(7)** wet crystals, prepared as above, (titer HPLC: 45.4%; HPLC purity: 96.1%; Water K.F.: 45.6%; 37 kg as dry product) were suspended in 925 L of anhydrous isopropyl alcohol. The suspension was stirred and cooled to a temperature of 2−4 °C. Peracetic acid (40% corresponding to a 40−45% of molar excess (referred to 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid) was added over 180 min. The suspension was stirred for 12 h at a temperature of 2−4 °C. The suspension was then filtered on a filter screen and the filter cake was washed with 370 L (0.4 vols referred to the volume of the solvent used for the reaction) of anhydrous isopropyl alcohol. The white crystals were dried in a vacuum oven, at a temperature of 28−32 °C, for 36 h. The total yield of 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid 1(*S*)-oxide **(8)** was 36.5 kg (95%), purity, 98% (area % HPLC).

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