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Ceftibuten:† Development of a Commercial Process Based on Cephalosporin C. Part II. Process for the Manufacture of 3-Exomethylene-7(*R*)-glutaroylaminocepham-4-carboxylic Acid 1(*S*)-Oxide

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Abstract:

Analysis of several options for the synthesis of Ceftibuten from cephalosporin C-derived starting materials led to the conclusion that the most practical option, leading to the lowest costs, would be realized by trying to resurrect the previously discarded electrochemical reduction process. This contribution describes the preparation of 3-exomethylene-7(*R*)-glutaroylaminocepham-4-carboxylic acid 1(*S*)-oxide (10,1(*S*)-oxide) in almost quantitative yield by the electrochemical reduction of 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid 1(*S*)-oxide (9,1(*S*)-oxide) using a high-surface area tin mesh cathode. The new product has been shown (see Bernasconi, E.; Lee, J.; Sogli, L.; Walker, D. *Org. Process Res. Dev.* 2002, *6*, 169) to be a superior new intermediate for the preparation of orally active cephalosporins such as Ceftibuten.

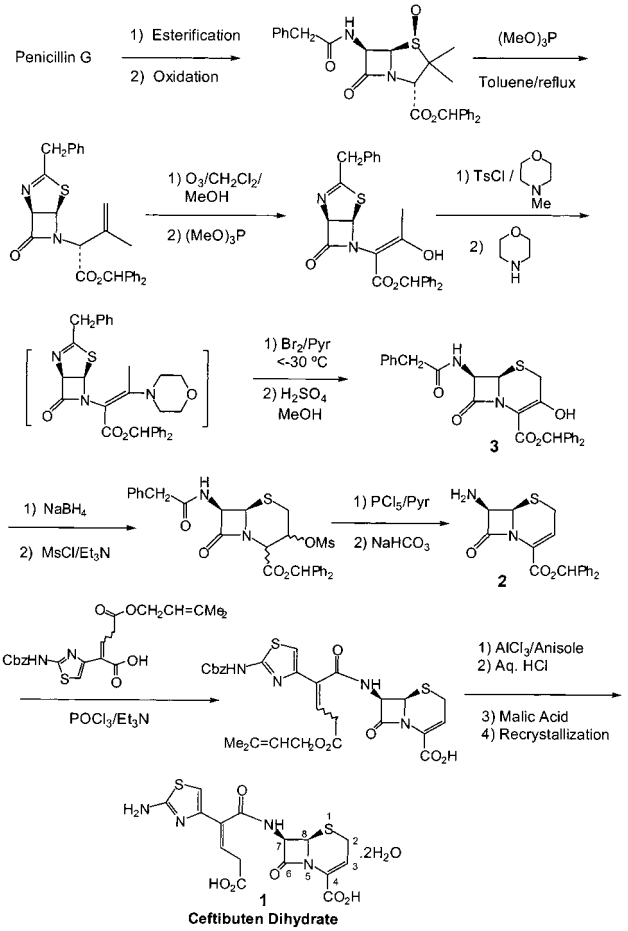
Introduction

The orally active third generation cephalosporin antibiotic, Ceftibuten (**1**) has been manufactured using a long process based on producing the key intermediate diphenylmethyl **7**(*R*)-amino-3-cephem-4-carboxylate (**2**) from a penicillin starting material via diphenylmethyl 3-hydroxy-7(*R*)-phenylacetamidoceph-3-em-4-carboxylate (**3**)[1] (Scheme 1).

This situation developed largely because no low cost route was known, or could be found, which started with commercially available, albeit fairly expensive, cephalosporins such as cephalosporin C (**4**), or 3-acetoxymethyl-7(*R*)-aminoceph-3-em-4-carboxylic acid (7-ACA, **5**). In short, until now, it has proved more economical to expand the five-membered ring of a penicillin to a six-membered cephalosporin ring[2] rather than use a molecule already containing a six-membered ring.

As a result, most oral cephalosporins, that is, cephalexin,[3] cefadroxil,[4] cephradine,[5] cefaclor,[6] and Ceftibuten[1,7] are manufactured from a penicillin starting material. The penicillin route continues to be favored, despite the fall in cost of cephalosporin C and 7-ACA, simply because the prices of these two intermediates are still too high. Also, in the case

Scheme 1. **Shionogi synthesis of Ceftibuten**



† 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.

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(1) Yoshioka, M. *Pure Appl. Chem.* **1987**, *59*, 1041 and references therein.

(2) (a) Morin, R. B.; Jackson, B. G.; Mueller, R. A.; Lavagnino, E. R.; Scanlon, W. B; Andrews, S. L. *J. Am. Chem. Soc.* **1963**, *85*, 1896. (b) 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. (c) Morin, R. B; Jackson, B. G. (Eli Lilly). U.S. Patent 3,275,626, 1966.

(3) Ryan, C. W.; Simon, R. L; Van Heyningen, E. M. *J. Med. Chem.* **1969**, *12*, 310.

(4) Crast, L. B., Jr. (Bristol-Myers). U.S. Patent 3,489,752, 1970.

(5) Dolfini, J. E.; Applegate, H. E.; Bach, G.; Basch, H.; Bernstein, J.; Schwartz, J; Weisenborn, F. L. *J. Med. Chem.* **1971**, *14*, 117.

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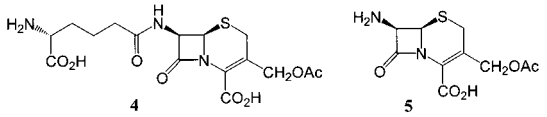
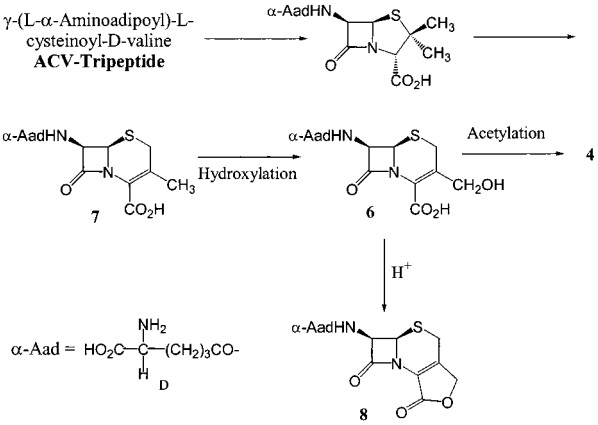
of Ceftibuten no viable route has been discovered for converting the 3-CH_2OAc group of cephalosporin C or 7-ACA (**5**) to a 3-H cephem. In addition, in practical terms, it is generally slow and difficult to displace an existing manufacturing process without an overwhelming financial justification and an absolute guarantee that the quality of the product from the new synthesis will be the same or better.



4 **5**

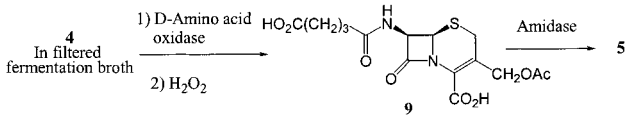
In reopening the idea of starting with a cephalosporin, we considered two old prospects.[8] The first is that desacetylcephalosporin C (**6**) may be a better fermentation target than cephalosporin C. This is because in the biosynthesis of cephalosporin C (Scheme 2), the sequence of conversions is from the aminoadipoylcysteinylvaline tripeptide (ACV) to desacetoxycephalosporin C (**7**), to **6**, to **4**.[9] In commerce, the *Cephalosporium acremonium* organism is manipulated to maximize the production of cephalosporin C. If manipulation were in the direction of disfavoring acetylation, a much higher cephalosporin titre, in the form of (**6**), would result. A corollary to the use of desacetylcephalosporin C is that lactone (**8**), which is readily obtained from desacetylcephalosporin C, might also be considered as a starting substrate. Thus, fermenting desacetylcephalosporin C instead of cephalosporin C was thought likely to introduce savings provided that simple and economical methods could be found for processing the 3-CH_2OH (or lactone) group to 3-H.

Scheme 2. **Outline of cephalosporin C biosynthesis**



After reviewing this literature, and determining the fermentation/extraction and chemical processing changes which would be required to commercialize a process scheme based on intermediate **6** or **8** we were dissuaded from pursuing such a route. In brief, as is the case in utilizing **4** and **5**, evaluation of the published chemical methods[10] for converting 3-substituted cephalosporins to the desired 3-H cephalosporins from a cost standpoint revealed that we would need to undertake a great deal of exploratory work to even be in a position to determine which of the many raw chemical recipes from this research literature[10] might be improved, integrated, and developed to create an economically attractive process. In addition our evaluation indicated that a relatively large investment in new process equipment would be needed. These revelations led us to rethink our approach and particularly, to build on Antibioticos achievements in reducing the cost of **5**. Antibioticos' fermentation experts had already created alternative cost reduction opportunities by commercializing the enzyme-mediated conversion of **4** to 3-acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid (**9**) and 7-ACA (**5**) (see Scheme 3).[11a]

Scheme 3. **Outline of Antibioticos process for preparing 7-ACA**



As a result of the above appraisal we concluded that it would be better to build on the expertise already established and concentrate efforts on the utilization of **9**.

The second idea considered was to reexamine ways,[12−14] of converting the $-CH_2OAc$ group of **4**, or its simply

(6) (a) Kukolja, S. In *Recent Advances in the Chemistry of β-Lactam Antibiotics*; Elks, J. Ed.; Special Publication No. 28; The Royal Society of Chemistry: London; 1977; p 181. (b) Chauvette, R. R; Pennington, P. A. *J. Med. Chem.* **1975**, *18*, 403. (c) Kukolja, S. (Eli Lilly). U.S. Patent, 4,052,387, 1977.

(7) (a) Hamashima, Y.; Kubota, T.; Minami, K.; Ishikura, K.; Konoika, T.; Yoshioka, M.; Yoshida, T.; Nakashinizu, H; Motokawa, K. *J. Antibiot.* **1987**, *40*, 1468. (b) Hamashima, Y. (Shionogi). U.S. Patent 4,634,697, 1987.

(8) A third prospect, suggested by Dr. A. K. Ganguly, Schering-Plough Research Institute, was to exploit the reduction of 3-chlorocephem compounds, or precursors thereto, produced as intermediates in the manufacture of Cefaclor from Penicillin V.[6] Although initially unappealing, this third prospect became more attractive as our process research and development work unfolded (see Part III[11b]).

(9) (a) Arnstein, H. R. V.; Morris, D. *Biochem. J.* **1960**, *76*, 357. (b) Abraham, E. P. In *Recent Advances in the Chemistry of β-Lactam Antibiotics*; Elks, J. Ed.; Special Publication No. 28; The Royal Society of Chemistry: London; 1977; p 1. (c) Liersch, M.; Nuesch, J; Treichler, H. J. In *Second International Symposium on the Genetics of Industrial Microorganisms*; Macdonald, K. D., Ed.; Academic Press, Inc.: New York, 1976; p 179. (d) Liersch, M.; Nuesch, J; Treichler, H. J. In *Second International Symposium on the Genetics of Industrial Microorganisms*; MacDonald, K. D., Ed.; Academic Press, Inc.: New York, 1976; p 179. (e) Kohsaka, M; Demain, A. L. *Biochem. Biophys. Res. Commun.* **1976**, *70*, 465. (f) Baldwin, J. E; Schofield, C. In *The Chemistry of β-Lactams*; Page, M. I., Ed.; Blackie Academic and Professional: Glasgow, 1992; p 1.

(10) (a) Peter, H; Bickel, H. *Helv. Chim. Acta* **1974**, *57*, 2044. (b) Scartazzini, R; Bickel, H. *Helv. Chim. Acta*, **1974**, *57*, 1919. (c) Peter, H; Bickel, H. (Ciba-Geigy). U.S. Patent 4,269,977, 1981. (d) (Ciba-Geigy). Br. Patent 1,356,437, 1974. (e) See also: Chamberlin, J. W; Campbell, J. B. *J. Med. Chem.* **1967**, *10*, 966 and Webber, J. A. (Eli Lilly) for the oxidation of CH_2OH to CHO. U.S. Patent 3,674,784, 1972.

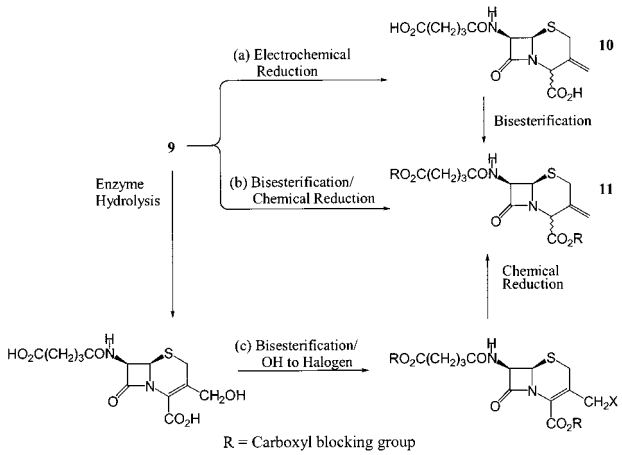
(11) (a) Part I: Bernasconi, E.; Lee, J.; Roletto, J.; Sogli, L.; Walker, D. *Org. Process Res. Dev.* **2002**, *6*, 152. (b) Part III: Bernasconi, E.; Lee, J.; Sogli, L.; Walker, D. *Org. Process Res. Dev.* **2002**, *6*, 169. (c) Part IV: Chai, D.; Genders, D.; Weinberg, N.; Zappi, G.; Bernasconi, E.; Lee, J.; Roletto, J; Sogli, L.; Walker, D.; Martin, C. R.; Menon, V.; Zelenay, P.; Zhang, H. *Org. Process Res. Dev.* **2002**, *6*, 178.

(12) (a) Chauvette, R. R; Pennington, P. A. *J. Org. Chem.* **1973**, *38*, 2994. (b) Chauvette, R. R. (Eli Lilly). U.S. Patent 3,932,393, 1976. (c) Ochiai, M.; Aki, O.; Morimoto, A.; Okada, T; Morita, K. *Tetrahedron* **1975**, *31*, 115.

(13) (a) Ochiai, M.; Aki, O.; Morimoto, A.; Okada, T.; Morimoto, A.; Shimadzu, H. *J. Chem. Soc., Chem. Commun.* **1972**, 800. (b) Ochiai, M.; Aki, O.; Okada, T.; Shinozaki, K; Asahi, Y. *J. Chem. Soc., Perkin Trans. 1* **1974**, 258. (c) Ochiai, M.; Aki, O.; Morimoto, A.; Okada, T.; Shinozaki, K; Asahi, Y. *Tetrahedron Lett.* **1972**, 2341. (d) Ochiai, M.; Aki, O.; Morimoto, A.; Okada, T.; Shinozaki, K. Asahi, Y; Masuda, K. (Takeda Chemical Industries, Ltd.). U.S. Patent 3,792,995, 1974. (e) Hall, D. A. *J. Pharm. Sci.* **1973**, *62*, 980. (f) Hall, D. A. (Eli Lilly). U.S. Patent 4,042,472, 1977. (g) Hall, D. A.; Berry, D. M; Schneider, C. J., *J. Electroanal. Chem.* **1977**, *80*, 155.

accessed derivatives such as **9**, to 3-exomethylene cephalosporins (Scheme 4).

Scheme 4. **Conversion of 3-CH_2OAc group of 9 to 3-exomethylene cephalosporins**

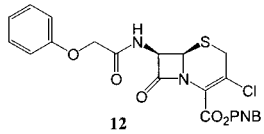


3-Exomethylene cephalosporins are particularly attractive since they are readily ozonolyzed to 3-hydroxycephems,[15] which would allow an early intersection with the existing manufacturing process for Ceftibuten (i.e., the glutaroyl analogue of compound **3** in Scheme 1). This has a "regulatory benefit" in providing several downstream purification opportunities should any new impurity be introduced by using a cephalosporin input. Additionally, the regulatory benefit would be enhanced by selecting diphenylmethyl as the carboxyl-protecting group since this selection would minimize any impurity queries which would undoubtedly occur if an alternative ester was used.

Although the above "selections" (glutaroyl side-chain and diphenylmethyl ester) limited the scope of our evaluation we did consider the commercially produced cefaclor intermediate (**12**) one more time.[8]

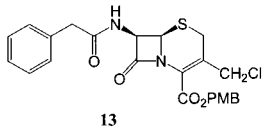
First, compound **12**, although produced on a tonnage scale for Eli Lilly is not commercially available. Second, the reductive removal of the PNB group is said to give a hazardous mixture requiring special waste disposal conditions.[16] Third, any thought that the diphenylmethyl ester, or any other acid-labile carboxyl group, such as *p*-methoxybenzyl, might be used in place of PNB in the production process for **3** is negated by the knowledge that the reactions used to produce **12** commercially will cleave acid-labile protecting groups.[17] Details of the chemistry used in the production of **12** have been published.[18]

In searching for possible commercial equivalents of **12** we found that Otsuka Kagaku Kabushiki Kaisha offered compound **13**. The electrochemical reduction of **13** to the corresponding 3-exomethylenecepham ester has also been described.[14] However, the cost and the fact that the *p*-methoxybenzyl ester was the only one Otsuka made available worked against the use of this compound. In addition we thought the electrochemical reduction process described[14b] for converting the 3-chloromethyl group of **13** to a 3-exomethylene group would not be economic as a result of its being carried out in a dilute aqueous organic solution using lithium and ammonium perchlorates as support electrolytes.



12

PNB = *p*-$NO_2C_6H_4CH_2$-

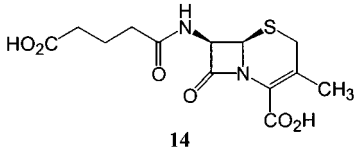


13

PMB = *p*-$CH_3OC_6H_4CH_2$-

On the basis of the above evaluations we focused all further development effort on the electrochemical reduction, option (a) in Scheme 4, since this option has the potential advantage of prolonging process operations in water, without isolating intermediates, and in addition would eliminate chemical reduction methods, and consequent hazardous waste disposal costs, associated with alternative options (b) and (c) in Scheme 4. Furthermore the capital cost projections for electrochemical reduction equipment (see Part IV of this series[11c]) are quite modest.

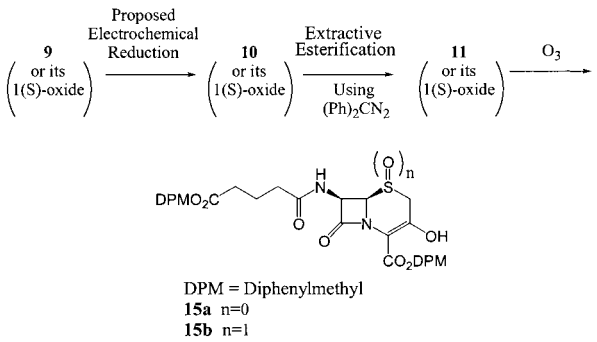
Our principal goals were to find ways of improving the yield of the desired electrochemical reduction product (**10**), to minimise the level of the most-difficult-to-remove impurity (**14**) (a target of <5% of **14** was set), and to create a practical electrochemical reduction process.



14

Practical advantage would also be realized in that the ozonolysis equipment already in place in the Shionogi manufacturing plant for their operation of Scheme 1 would serve for the conversion of **9**, or its 1(*S*)-oxide, through **10** and **11** to the glutaroylamino equivalent (**15**) of the current intermediate **3** (Scheme 5).

Scheme 5. **Proposed process for intersection with the Shionogi process for Ceftibuten manufacture**



(14) (a) Torii, S.; Tanaka, H.; Ohshima, T; Sasaoka, M. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3975. (b) Torii, S.; Tanaka, H.; Sasaoka, M; Kameyama, Y. (Otsuka Kagaku Kabushiki Kaisha). U.S. Patent 4,629,542, 1986.

(15) (a) Chauvette, R. R; Pennington, P. A. *J. Am. Chem. Soc.* **1974**, *96*, 4986. (b) Chauvette, R. R. (Eli Lilly). U. S. Patent 3,917,587, 1975**.** (c) See also ref 10b.

(16) Dr. J. Wass, private communication. Eli Lilly's Cephalexin plant in Clinton, Indiana, was built with a containment system to collect the waste from the reductive removal of the *p*-nitrobenzyl group used as a carboxyl-protecting group in the manufacture of this antibiotic. The waste is believed to contain carcinogenic compounds such as tolidines.

(17) Reference 6a, p 186.

(18) (a) Reference 6c. (b) Copp, J. D; Tharp, G. A. *J. Org. Process. Res. Dev.* **1997**, *1*, 92. (c) Kukolja, S; Lammert, S. R. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 67.

The chemistry for the conversion of **15** to Ceftibuten was thought likely to be essentially the same as in Shionogi's existing production process (Scheme 1) with the desulfoxidation step (required in utilizing **15b**) being carried out prior to reducing the cephalosporin ring double bond, thereby intersecting the Shionogi process and leading to the common intermediate **2**. The processing of **15** to Ceftibuten is detailed in Part III.[11b] Our work program was largely geared to intersection with the current Shionogi process. However, later exploration of the chemical opportunities based on our 3-hydroxycephem intermediate, **15b**, revealed that an even more attractive route to 3H-cephalosporins could result by converting **15b** to the desired 3H-cephem via a 3-chlorocephem (see Part III).[11b]

Results and Discussion

We commenced work on the electrochemical reduction of **9** to **10** using a two-compartment electrochemical cell in which the mercury cathode compartment was separated from the platinum mesh anode compartment by a Nafion 117 perfluorinated ion-exchange membrane (to prevent migration of anions from the cathode compartment to the anode compartment). In our early exploratory work we selected, from the wealth of process conditions described in the literature,[13] pH and buffer parameters that gave the best results with our substrate **9** (pH 7−10 and sodium borate buffer).

Our initial results using the above cell were comparable to those obtained by the previous workers, including the corresponding finding that unwanted 7(*R*)-glutaroylamino-3-methylceph-3-em-4-carboxylic acid (**14**) is produced in significant amounts (Table 1).

Table 1. **Electrochemical reduction of 9 using selected literature conditions**

concentration of **9** (g/L)	*T* (°C)	yield of **10** (%)	yield of **14** (%)	approximate yield of unknowns (%)	pH range
5	25	64	6	up to 30	8.3−9.3
5	0	67	5	up to 28	8.3−8.7

[a] Experiments were carried out in a 0.2 M sodium borate buffer and a current density of 5 mA cm^{-2}.

These results made it clear that the electrochemical reduction process as described in the literature was far from being practical and needed considerable development. The major deficiencies are:

(1) The use of a mercury cathode is environmentally unacceptable.

(2) The process produces significant amounts of **14** and unknown impurities. These impurities compromise the process yield and add to the cost of **10** by introducing the need for additional purification steps.

(3) The reaction concentration is much too low to be commercially attractive.

(4) There is little indication from the literature that a study of other process parameters (particularly reaction concentration, pH, temperature, current density, buffer composition, cathode material, and substrate composition) would enable us to create a viable manufacturing process.

(5) No practical isolation or extraction process for recovering the desired 3-exomethylenecepham has been described.

We reasoned that ongoing work could be continued with a mercury cathode and that work on items 2, 3, and 4 above would be more revealing in the short term. In particular we decided to determine the structure of all major impurities in the expectation that an understanding of the mechanism of their formation would enable us to devise ways of preventing unwanted transformations.

Although the use of a pH range of 7−10 appeared to give results comparable with those in the literature, we undertook a stability study at a higher pH to determine whether decomposition products of **9**, **10**, and **14** might be significant contributors to the 28−30% level of unknowns (Table 1).

We determined the rate constants of decomposition for our starting material (**9**), the exomethylene product (**10**), and the 3-methyl byproduct (**14**) at pH 11.8 and 25 °C (Table 2).

Table 2. **Rate constants of decomposition for 9, 10, and 14 at pH 11.8 and 25 °C**

compound	K (10^{-4}/min)
9	113
10	4.5
14	21.6

Although **9** is the least stable compound, we found that, in practice, little loss of **9** occurred in the time frame of a typical electrochemical reduction (ca. 12 h at 0−5 °C at a pH of 8.5−10). In passing, we also observed that the ratio of 3-exomethylenecepham to 3-methylcephem was somewhat greater in electrochemical reductions carried out at the higher pH levels. This observation also coincides with our earlier selection of sodium borate as the best buffer (pK_a value of $H_3BO_3/H_2BO_3^-$ is 9.27).[19]

The HPLC chromatogram of a typical solution obtained by the electrochemical reduction of **9** (Figure 1) illustrates

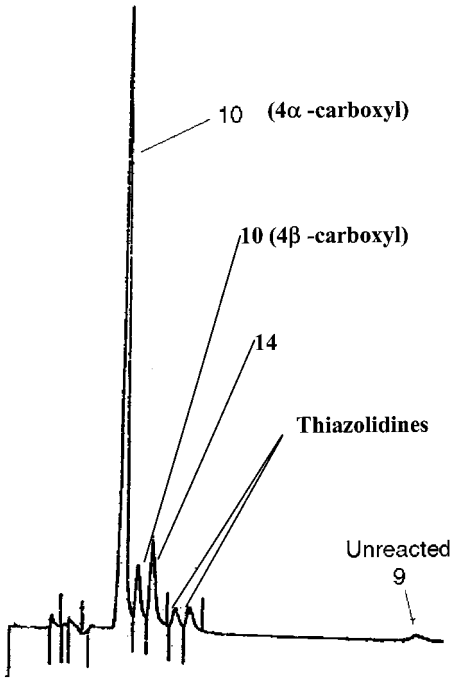
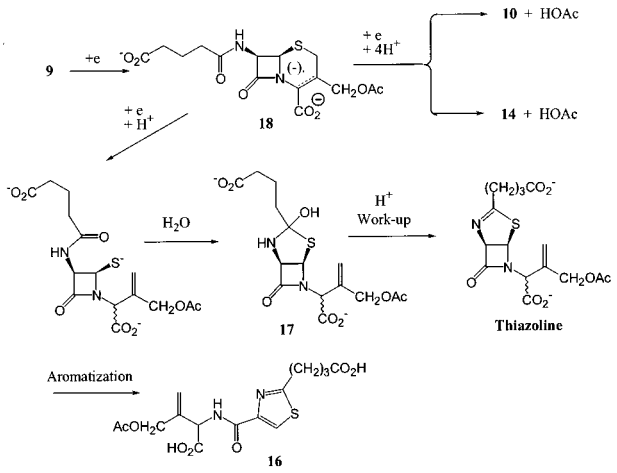


Figure 1. **HPLC chromatogram of a typical solution from the electrochemical reduction of 9.**

the impurity profile seen by HPLC. The processing of the electrochemical reduction solution to give a pure sample of **10** caused degradation of the major impurity to a new compound, identified as **16**, analogous to the thiazole identified by Hall and co-workers[13f] (Scheme 6).

Scheme 6. **Pathways for the electrochemical reduction of 9**



The HPLC/MS and NMR analysis of the original electrochemical reduction solution provided evidence that compound **17** was an intermediate to **16**.[27] Thus, the electrochemical reduction probably proceeds according to Scheme 6, in general agreement with the proposals of earlier workers.[13]

Clearly, partially reduced anion radical intermediates, such as either the crudely drawn structure **18** or products from the further addition of an electron and protons or both, are energetically positioned to break down in several ways. Previous workers were unable to find process conditions that overcame unwanted byproducts. In addition, the cost of fermented cephalosporins at the time and the fact that previous workers did not find a practical alternative to their mercury cathode discouraged their further interest in the route. Despite these earlier failures, we were spurred to explore the effect of changing process parameters by the potential of the lower costs and environmental advantages inherent in electrochemical reduction technology and also by the appeal of starting with an aqueous solution of the low-cost substrate **9**. Having established that a pH range of 7−10 and temperature in the 0−10 °C range were close to optimum, we went on to examine reaction concentration, current density, buffer composition, substrate composition, and cathode material.

Concentration of 9. Previous workers employed concentrations of only ca. 5 g/L in electrochemically reducing a variety of cephalosporanic acids. From a practical standpoint we were interested in using concentrations close to those obtained from the enzymatic process for producing **9**, that is, 35−40 g/L (see Part I[11a]). We found, in preliminary experiments (Table 3) that some loss of product **10** is incurred by increasing the concentration of **9**.

Iterative evaluation of this parameter was continued with the optimization of other parameters (see below).

Current Density. Preliminary experiments (Table 4) on the effect of current density on the conversion of **9** to **10** indicated that increasing the current density from 5 mA/cm^2 to 15 mA/cm^2 had little effect on the yield of **10**.

Table 3. **Effect of concentration in the electrochemical reduction of 9 to 10**

concentration of **9** (g/L)	yield of **10**(%) (HPLC)	ratio **10**/**14**
5	67.0	13.3
10	65.0	12.8
15	73.9	11.1
30	62.4	13.2

[a] Experiments were carried out in 0.2 M sodium borate buffer in a Nafion separated cell (Hg cathode) at a pH range of 8.4−9.2, a temperature of 5−10 °C, and a current density of 5 mA cm^{-2}.

Table 4. **Effect of current density in the electrochemical reduction of 9 to 10**

current density mA cm^{-2}	yield of **10** (%) (HPLC)	ratio **10**/**14**
5	66.9	13.3
7	66.6	11.9
10	67.3	13.2
15	67.0	10.7

[a] Experiments were carried out at an initial concentration of **9** of 5 g/L in a 0.2M sodium borate buffer at a pH of 8.8 to 9.5 and a temperature of ca. 5 °C.

Since, for commercial operation, higher current densities are desired to increase the reaction rate, increase plant capacity, and reduce capital costs, the current density parameter was revisited as process development work continued (see below).

Buffer Composition. In working with sodium borate buffer we observed that a gray film (amalgam related?) was produced on the surface of the mercury cathode. When we ran the electrochemical reduction to preform amalgam, switched off the current, and added **9**, a smooth reduction to **10** occurred. This led us to examine the reduction potentials of amalgams with other alkali metals[20] (Table 5).

Table 5. **Standard reduction potentials of alkali metal amalgams at 25 °C**

half reaction	standard potential V
$Na^+ + Hg + e^-$ Na(Hg)	−1.84
$K^+ + Hg + e^-$ K(Hg)	−1.90
$Cs^+ + Hg + e^-$ Cs(Hg)	−1.78
$Li^+ + Hg + e^-$ Li(Hg)	−2.00

It can be seen that lithium amalgam is the strongest reducing agent and cesium is the weakest.

Comparison of the different metal borates as buffer was undertaken. The results are summarized in Table 6.

Table 6. **Comparison of alkali borates in the electrochemical reduction of 9 to 10**

alkali metal borate	yield of **10** (%) (HPLC)	ratio **10**/**14**
sodium	72	40
potassium	71.5	38
cesium	79	22
lithium	80	24

[a] Electrochemical reductions were carried out on 10 g/L solution of **9** using a current density of 16 mA cm^{-2}. Buffer solutions were obtained by adding the alkali metal hydroxide to 0.2 M boric acid. The pH was in the range of 8.8−9.5 in all reactions. The temperature was held at ca. 5 °C.

(19) Lide, D. R. *Handbook of Chemistry and Physics*, 76th ed.; CRC Press Inc.: New York, 1995; pp 8−43.

As a result of the significant yield enhancement observed with lithium borate buffer, all further exploratory work on the electrochemical reduction of **9** was carried out using this buffer. The effect of using higher substrate concentrations was reexamined. The results are summarized in Table 7.

Table 7. **Effect of concentration in the electrochemical reduction of 9 in lithium borate buffer**

concentration of **9** (g/L)	yield of **10** (%) (HPLC)	ratio **10**/**14**
10	80	23
30	77	26
40	75	25
50	75	30

[a] Experiments carried out in 0.05M lithium borate buffer at a pH of ca. 9.5, a temperature of ca. 5 °C and a current density of 15 mA cm^{-2}.

As was the case in earlier experiments (Table 3) some loss of product **10** is incurred by increasing the concentration of **9**. Interestingly, the use of higher concentrations of **9** improved the ratio of **10** to **14**, somewhat offsetting the adverse ratio effects in switching from sodium borate buffer to lithium borate buffer (Table 6). We did not investigate the differences in the ratio of **10** to **14** between Tables 3 and 6, only noting that the results in Table 3 were obtained at a current density of 5 mA cm^{-2} and those in Table 6 at a current density of 16 mA cm^{-2}.

In a separate pair of experiments we also showed that the current efficiency of the process was more than doubled (from 20 to 45%) when the solution concentration of **9** was increased from 10 to 50 g/L. We also observed that some precipitation of **9** (free acid) occurred on the Nafion membrane at the higher concentrations, indicating a need for the cathode compartment to be stirred.

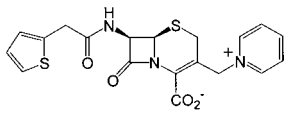
Although, from a commercial viewpoint, use of a cell employing a mercury cathode was undesirable, we did evaluate the impact of scale on the performance of the process, using a mercury cathode. The results (Table 8) on a 20-L scale, at a concentration of 30 g/L of **9**, were virtually the same as small-scale results carried out at the same concentration. At 50 g/L of **9** some precipitation of **9** free acid on the Nafion membrane occurred despite the use of vigorous stirring.

Table 8. **Electrochemical reduction of 9 on a 20 L scale**

concentration of **9** (g/L)	yield of **10** (%) (HPLC)	ratio **10**/**14**
30	79	25
50	70	not determined

[a] Experiments carried out in 0.125 M lithium borate buffer in a pH range of 8.2−9.5, at a temperature of 6−7 °C, and a current density of 16 mA cm^{-2}.

Substrate Composition. We reasoned that alternatives to the 3-acetoxymethyl group in **9**, and especially the substitution of a better leaving group than acetoxy, may be advantageous in terms of favoring formation of the 3-exomethylene group in electrochemical reduction reactions. It is known[21] that cephaloridine (**19**) undergoes a smooth two-electron electrochemical reduction. Although the products were not named, it is reasonable to assume that **19** would easily lose pyridine.



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It is also known that a halide ion is readily lost from 3-halomethylcephalosporin esters, such as **13**, in electrochemical reductions.[14]

We did not wish to lose the elegance and simplicity associated with Scheme 4a by separately preparing 3-pyridiniummethyl or 3-halomethyl derivatives of **9**, but we did study the addition of salts to buffer solutions of **9** prior to electrochemical reduction. Tetraethylammonium salts are known[22] to increase hydrogen overpotential and increase yield. The addition of tetraethylammonium hydroxide to **9** in 0.2 M lithium borate buffer (final solution pH 8.2) prior to the electrochemical reduction gave a poorer yield of **10**. Separately, the addition of potassium thiocyanate and sodium chloride to a borate buffer solution of **9** prior to electrochemical reduction of **9** did not lead to improved yields. In the case of these salts the electrochemical reduction solutions were devoid of brown color when higher current densities were used; however, this observation was not followed up. The addition of mercaptoacetic acid in place of the above two salts did not give any better result.

We also reasoned that if the sulfur atom of **9** were in a sulfoxide form the pathway for breakdown of radical anion intermediates (Scheme 6) would probably change significantly. Sulfoxidation was considered quite practical inasmuch as it can be carried out cheaply in situ and was thought likely to be quite tolerable in later steps of the process. The sulfoxide form of **9** was prepared according to the procedure described in the preceding paper.[11a]

Our first indication that the sulfoxide form of **9** was a superior substrate came from an experiment in which **9** 1(*S*)-oxide was added to electrochemically prepared lithium amalgam. Lithium amalgam was prepared by the electrochemical reduction of lithium hydroxide using a glass cell based on the design of other workers.[23] Comparison of the reduction of both **9** and its sulfoxide was made by adding solutions of the substrates in pH 9 lithium borate buffer to a lithium amalgam layer and monitoring the reduction using HPLC (Figures 2 and 3). As can be seen the yield of **10** 1(*S*)-oxide appears to be nearly quantitative. No traces of either **16** or **14** 1(*S*)-oxide were observed.

This finding was immediately integrated into successful efforts going on at the time to find an alternative to mercury as the cathode material (see below). The superior results observed with **9** 1(*S*)-oxide also carried over to Cephalosporin C 1(*S*)-oxide and cephalothin 1(*S*)-oxide.

(20) Dean, J. A. *Lange's Handbook of Chemistry*, 14th ed.; McGraw-Hill, Inc.: New York, 1992; Section 8, pp 126−134.

(21) Jones, I. F.; Page, J. E; Rhodes, C. T. *J. Pharm. Pharmacol.* **1968**, *20 (suppl.)*, 45S−47S.

(22) (a) Baizer, M. M. *J. Electrochem. Soc.* **1964**, *111*, 215. (b) Danly, D. E; King, C. J. H. In *Organic Electrochemistry*, 3rd ed.; Lund, H., Baizer, M. M., Eds.; Marcel Dekker: New York, 1991; p 1322.

(23) Knunyants, I. L.; Vyazankin, N. S. *Izv. Akad. Nauk SSSR, Chem. Sci. Sec.* **1957**, *2*, 238.

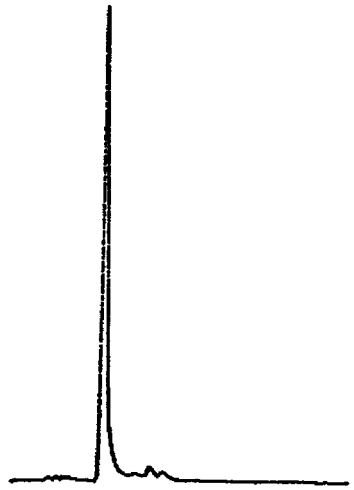

Figure 2. **HPLC trace from the reduction of 9 to 10 using lithium amalgam.**

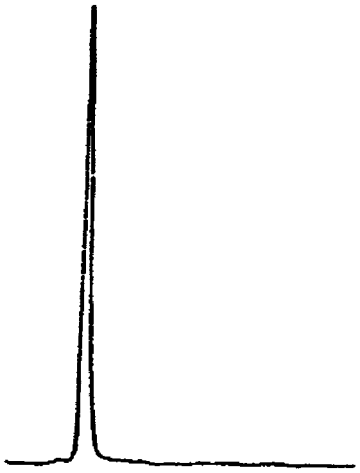

Figure 3. **HPLC trace from the reduction of 9 1(*S*)-oxide to 10 1(*S*)-oxide using lithium amalgam.**

Cathode Material. An exploratory effort to identify an alternative cathode to mercury, using the electrochemical reduction of **9** to **10** as a guide, yielded the results shown in Table 9.

Table 9. **Effect of cathode material on the electrochemical reduction of 9 to 10**

electrode	yield of **10** (HPLC %)
aluminum	<10
Zinc	<30
C (graphite)	48
lead	54
tin	62−71
mercury (Li)	85

[a] These results served only as a guide since conditions of pH, current density, and solution volumes varied from one electrode to another.

A comprehensive study of the electrochemical reduction of **9** 1(*S*)-oxide to **10** 1(*S*)-oxide was undertaken using a tin cathode. A Nafion 117 cation-exchange membrane was used as the cell divider in initial work at Colorado State University. Later work, at the Electrosynthesis Company, was carried out using a Nafion 350 cation-exchange divider.

Reevaluation of the major process parameters, that is, pH, temperature, reaction concentration, current density, and buffer composition revealed some differences versus results obtained using the mercury cathode. The pH range of 7−10 appeared to be satisfactory, with the preferred value being ca. pH 8. Somewhat higher temperatures (up to 25 °C) could be tolerated, but caution led us to prefer a lower temperature of 10 ± 5 °C. High substrate concentrations (50−100 g/L) gave similar results to those obtained with the mercury cathode; the advantage of higher current efficiencies was, however, somewhat offset by a slightly lower yield of **10** 1(*S*)-oxide. The preferred use of higher current densities (100−200 mA cm^{-2}) proved quite acceptable—only a small loss in yield was observed when solutions of **9** 1(*S*)-oxide at the higher concentrations (50−100 g/L) were electrochemically reduced at high current densities (120−150 mA cm^{-2}). The superiority of lithium borate buffer with the mercury cathode was not observed with the tin cathode, satisfactory results being obtained with potassium phosphate as buffer at approximately pH 8. Selection of the tin cathode led us to examine the form of this electrode, and to discover quite dramatic improvements in process performance by using a high-surface area tin cathode.

A comparison of tin wire with tin sheet and with a high-surface area tin mesh as the cathode material, is summarized in Table 10.

Details of the pilot-plant work, including a description of the electrochemical cell, the tin mesh cathode, operating criteria, and further results are provided in Part IV[11c] of this series (q.v.).

Before undertaking scale-up of the electrochemical reduction of **9** 1(*S*)-oxide to **10** 1(*S*)-oxide we examined a tin sheet cathode, which had been in intensive use for 4 months, to determine whether its stability would be factor in large-scale operation. This cathode showed some roughening of its surface and was subject to Energy disperse spectroscopy (EDS), scanning electron microscopy (SEM), and gravimetric analysis. As indicated by cyclic voltammetry studies (see Part IV[11c]) a large proportion of the charge passed (ca. 94%) is lost in hydrogen evolution. Large volumes of hydrogen would be expected to cause physical disruption of the tin cathode causing a roughening of the surface and accounting for the observed slight graying of the solution especially at high current densities. SEM analysis confirmed that pitting of the cathode surface had occurred. Gravimetric analysis showed that only a moderate loss (19 mg cm^{-2}, as referred to the geometric surface area of the cathode) of tin metal had occurred during the 4-month period of intensive use.

Chemical dissolution is another pathway for the loss of tin.

$$Sn^{o} + 2\ M^{I}OH \rightarrow M_2SnO_2 + H_2$$

Although such loss would be expected to be small at the relatively weak alkaline pH of the catholyte (pH 7.5) and

Table 10. **Comparison of the use of tin wire, tin sheet, and tin mesh in the electrochemical reduction of 9 1(*S*)-oxide to 10 1(*S*)-oxide[a]**

form of tin	scale of operation[b]	concentration of **9** 1(*S*)-oxide (g/L)	current density (mA $cm^{-2)}$	conversion (%)	yield (%)	current efficiency (%)	reaction time (min)
wire	lab	10	20	0	0	0	249
wire	lab	10	40	97	71.4	2.2	262
wire	lab	50	80	96	75.4	3.5	414
wire	lab	100	120	96	68.6	7.3	265
sheet	lab	50	120	88	66.8	3.6	240
sheet	lab	50	120	98	80.0	4.0	244
sheet	lab	50	200	97	75.8	3.4	170
mesh	lab	50	120	100	93.0	6.2	160
mesh[c]	pilot plant	50	120	100	92.0	6.2	160
mesh[c,d]	pilot plant	50	120	100	96.6	6.2	160
mesh[c,d]	pilot plant	50	120	100	91.1	6.2	160

[a] General conditions: catholyte 0.2 M potassium phosphate buffer pH 7−8.5 0.2 M KCl added $T = 5$ °C. [b] Laboratory runs used **9** 1(*S*)-oxide prepared by Schering-Plough, purity 77%. Pilot-plant runs used **9** 1(*S*)-oxide prepared by Antibioticos (Purity 97.6%) with yield calculated on the assumption of 100% purity. [c] The KCl was omitted from the catholyte. [d] $T = 23-25.5$ °C.

the low operating temperature, tin assays revealed that some dissolution was occurring (Table 11).

Table 11. **Tin Dissolution under open circuit[a] conditions**

time at open circuit (h)	tin concentration in catholyte (mmol/L)
0[a]	0.055[a]
6.0	0.498
24.0	0.763

[a] Measurement started immediately after shutdown of a 600 kC reduction run.

A freshly filled cell was subject to tin assays during the course of an electrochemical reduction of **9** to **10** (Table 12).

Table 12. **Tin Content in the catholyte at different stages of the electrochemical reduction of 9 to 10**

amount of electricity passed C × 10^3	concentration of tin in the catholyte (mmol/L)
0.0[a]	0.000
100	0.056
300	0.037
450	0.039
600	0.055
600[b]	0.048

[a] Before filling the cell. [b] After filtration using 1 μm glass microfiber filter.

Before the assays, catholyte samples were adjusted to pH 11 with KOH to guard against precipitation of tin hydroxide. Table 12 indicates that in an operating cell the tin content of the catholyte is practically independent of the charge passed. Slight variation of the concentration values results from change of the solution volume during electrolysis and inaccuracies in sampling (50 mL sample volume) due to hydrogen bubble evolution. The concentration of dissolved tin in steady-state operating conditions is thus about 0.05 ± 0.01 mmol/L. This value corresponds to the concentration of tin salts formed predominantly over the short period of time between the moment the cell was filled with electrolyte and the electrochemical reduction started (typically <30 s). The dissolution of tin in a filled cell under nonreducing conditions can be reduced to a negligible level by passing a "trickle" current through the cell with a current density of ca. 1 mA cm^{-2}.

We subsequently found that the tin contamination observed in the electrochemical reduction solutions of 3-exomethylene-7(*R*)-glutaroylaminocepham-4-carboxylic acid 1(*S*)-oxide did not carry over into products prepared in later stages of the Ceftibuten synthesis. By itself, the extractive esterification process step (see Part III[11b]) virtually eliminated tin as a contaminant.

Purification Considerations. In general terms column chromatography is considered to be a relatively expensive technique for purifying organic compounds, mostly because of the large volumes of solvent needed in the chromatographic separation of components and the elution of the desired product. In addition the recovery of desired product from dilute solution is often operationally unwieldy and can be problematic if product degradation occurs during the recovery step. The perception of impracticality often associated with chromatographic purification is usually based on chromatographic experience in which organic solvents are used, alone or in combination with other solvents or with water. For example, the use of preparative HPLC for purification tends only to be used as a last resort in commerce. The situation is, however, quite different for the purification of aqueous solutions of a product, especially if molecular manipulation can be carried out in water, for instance using microbiological techniques or other techniques such as electrochemical oxidation and reductions. Chromatographic purification of water-only solutions is widely practiced in industry. The disadvantages of dilution are minimized when several process steps can be efficiently carried out, one after another, in water without isolation of intermediates, as is the case in the preparation of **10** 1(*S*)-oxide. In addition the use of reverse osmosis for concentrating aqueous solutions is widely practiced in commerce,[24] as is the recovery of solid product through such as spray drying technology.[25] Table 13 illustrates some of our results in the purification of **10** 1(*S*)-oxide using macroreticular resins.

(24) Perry, R. H.; Green, D. W. *Perry's Chemical Engineers Handbook*; McGraw-Hill: New York, 1997; pp 22-48−22-52.

(25) In addition to several applications in the food industry, spray drying is employed in the recovery of a number of aminoglycoside antibiotics from aqueous solutions, notably gentamicin C, netilmicin and isepamicin sulfates, as well as in the recovery of intermediates thereto.

Table 13. **Macroreticular resin purification of 10 1(*S*)-oxide solution from the electrochemical reduction of 9 1(*S*)-oxide**

resin	source	eluent	recovery yield (approx. %)	purity of **10** 1(*S*)-oxide (approx. %)
XUS-40285	Dow Chemical	$NaHCO_3$	80	95
XAD-1600	Rohm & Haas	$NaHCO_3$	90	95
XAD-16[a]	Rohm & Haas	$NaHCO_3$	95	95
XAD-16[b]	Rohm & Haas	$NaHCO_3$	98	95

[a] Schering result. [b] Antibioticos result.

Conclusions

The electrochemical reduction of fermented cephalosporins, long regarded more as a scientific curiosity than a practical process, has been shown to be a viable option for the preparation of 3-exomethylenecephalosporins, pivotal intermediates in the synthesis of several cephalosporin antibiotics. The key steps are the use of a cephalosporin sulfoxide as substrate and the discovery that a high surface area tin cathode is an efficient replacement for mercury in the electrochemical reduction step. Process conditions for the electrochemical reduction have been defined which allow this step to be carried out in a practical way employing high substrate concentrations in water, at high current densities in conventional buffer systems. It has also been demonstrated that the electrochemical reduction process can be introduced into the existing aqueous process stream for the commercial manufacture of 7-ACA from cephalosporin C and that the chromatographic purification of the 3-exomethylenecephalosporin produced can be achieved using the same macroreticular resins as are used in purifying intermediates for 7-ACA manufacture.

Addendum

The electrochemical reduction work described in this paper has been patented.[26]

Experimental Section

Materials and General Methods. Electrochemical reductions were carried out in an electrochemical cell with the counter electrode (anode) separated from the working (cathode) and reference electrodes. The potential can be controlled using a constant voltage source, a Princeton Applied Research model 273 potentiostat, from −1 to −3 V. The electrochemical cells used for laboratory and medium scale work are outlined in Figures 4 and 5.

The two compartments of the cell were divided by a Nafion cation-exchange membrane. The Nafion 117 and 350 membranes used as dividers are commercially available from a number of sources, for example, DuPont, Aldrich Chemical Co., or The Electrosynthesis Company. The Nafion membrane was cleaned prior to use by boiling in 30% H_2O_2 for 30 min followed by immersion in a hot (80 °C) solution of

(26) (a) Walker, D.; Lee, J.; Martin, C. R.; Zhang, H.; Sogli, L; Bernasconi, E. (Schering Corporation and Antibioticos S.p.A.). U.S. Patent, 5, 571,910, 1996. (b) Walker, D.; Lee, J.; Martin, C. R.; Zhang, H.; Sogli, L.; Bernasconi, E; Menon, V. P. (Schering Corporation and Antibioticos S.p. A.). U.S. Patent 5,660,711, 1997. (c) Walker, D.; Lee, J.; Martin, C. R.; Zhang, H.; Sogli, L.; Bernasconi, E; Menon, V. P. (Schering Corporation and Antibioticos S.p.A.). U.S. Patent 5,847,116, 1998.

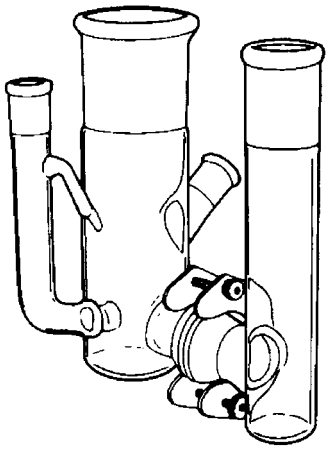

Figure 4. **Sketch of small laboratory electrochemical cell.**

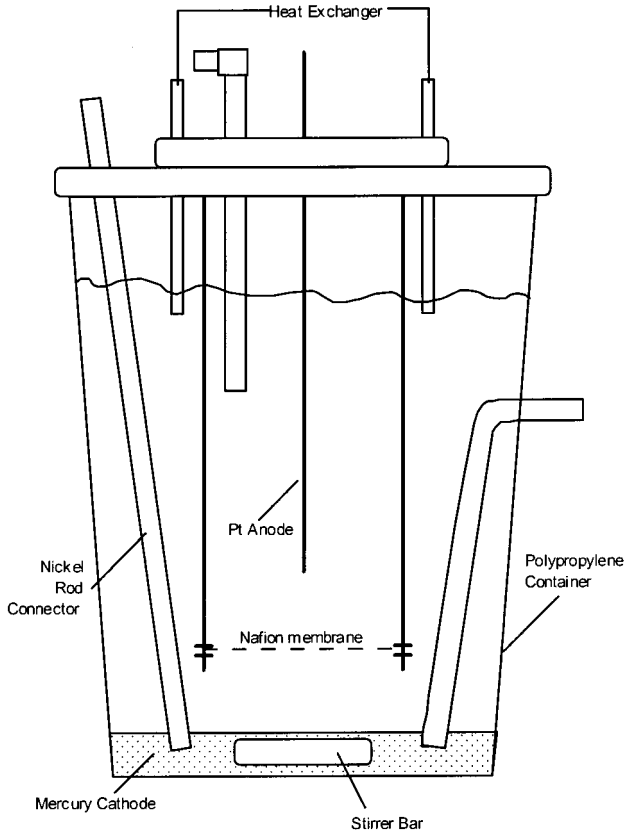

Figure 5. **Sketch of large laboratory mercury cathode cell.**

9 M nitric acid for 15 min. The membrane was then rinsed in boiling water, sonicated in several aliquots of hot (90 °C) water, and stored under distilled water until needed.

The counter electrode was a platinum mesh electrode, and the reference electrode was an Ag/AgCl electrode. The working electrode was a mercury pool (triple-distilled mercury), graphite (Johnson Matthey, 99.9995%) electrode, glassy carbon electrode, lead (Johnson Matthey, 99.9999%) electrode, tin foil electrode (Aldrich 99.9% pure, ca. 100 cm^2 surface area), or zinc (Johnson Matthey, 99.95%) rod sealed in Teflon. The tin mesh electrode was specially fabricated by the Electrosynthesis Company (see Part IV[11c]).

UV spectra were measured with a Hitachi U-3501 spectrophotometer. The HPLC analysis was carried out on

a Brownlee HPLC analytical column (RP 18 SPHER I-1. 250 mm × 4.6 mm) maintained at a temperature of 35 °C. The mobile phase was typically 6:94, CH_3CN/0.025 M K_2HPO_4 (aqueous) at a flow rate of 1 mL/min, and an UV detector (225 nm) was used.

Electrospray MS was performed in a VG Quattro SQ mass spectrometer (Fison Instruments). NMR experiments were conducted by using WP-270 TM NMR (Bruker) and AM-400 NMR (Bruker) spectrometers.

Electrochemical Reduction of 3-Acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic Acid (9) Using a Mercury Pool Working Electrode. 3-Acetoxymethyl-7(*R*)-glutaroylaminoceph-3-em-4-carboxylic acid **(9)** (600 g) was dissolved in 20 L of a 0.5 M aqueous boric acid solution. Lithium hydroxide was added to adjust the initial pH of the solution to 9.5. The solution was place in a two-chambered cell separated by a Nafion 117 divider and then cooled to 6 °C. The reduction was carried out at a current density of 16 mA cm^{-2} for 13 h. The final pH of the reaction was 8.2. The reaction was monitored by HPLC. A sample was also taken for lyophilization and used for NMR and ES-MS analysis. The yield of the reaction was 79% by HPLC and 80% by NMR. The ratio of **10** to **14** was 25:1 by HPLC and 37:1 by NMR in the lyophilized product mixture.

ES-MS analysis of the lyophilised product mixture showed mass peaks at 387.4 (monoanion of **17** or its ring-opened thiol amide?) and 193.6 (dianion of **17** or its ring-opened thiol amide?).[27] When the electrolysed solution was subjected to extractive esterification with diphenyldiazomethane (see Part III;[11b] extractive esterification includes an acidification step which would probably dehydrate **17**) followed by solvent removal and redissolution in 0.025 M K_2HPO_4 (35% v/v) and acetonitrile (65% v/v), a solution was obtained which was subject to LCMS. MH^+ peaks were found at 661 (**10** bis-DPM ester), 703 (thiazole **16** bis-DPM ester, or its β-lactam-fused precursor) and 537 (mono-DPM esters corresponding to the MH^+ 703 peak). Unassigned MH^+ peaks were also observed at MH^+ 495 and 597.

Electrochemical Reduction of 9 using a Tin Electrode. **9** (1 kg) was dissolved in 20 L of a 0.5 M boric acid. LiOH was added to adjust the initial pH of the solution to a pH = 9.5. The solution was placed in a two-chambered cell separated by a Nafion 117 divider and then cooled to 5−7 °C. The reduction was carried out at a current density of 30 mA cm^{-2} for 13 h. The reaction was monitored by HPLC. The final pH of the solution was 8.2. A sample was also taken for lyophilization and used for the NMR analysis. The yield of the reaction was 70% by HPLC. The ratio of **10** to **14** was 36:1 by HPLC.

Chromatography Column Regeneration and Preconditioning. A typical preparation of a laboratory chromatography column is as follows: A slurry of 200 mL of XAD-1600 (or XAD-16) resin (Rohm and Haas) in 1.5 L of deionized distilled water was agitated for 1 h. The water was then decanted followed by addition of 1.5 L of methanol. The methanol solution was agitated for 1 h and was then decanted. Approximately 155 mL of the resin was loaded into a chromatography column (2.4 cm × 60 cm) using 250 mL of methanol. The column was eluted with methanol (flow rate = 2 bed vols/h (BV/h) and then 7 L of deionized distilled water (flow rate = 8 BV/h). The column was then backwashed with 2 L of deionized distilled water and allowed to settle overnight. The column was eluted with 1 L of 0.5 M NaCl solution (pH = 3.0 adjusted with HCl, flow rate = 2 BV/h).

Pilot Chromatography Purification of Crude 10 Produced by the Electrochemical Reduction of 9. Two chromatography columns were prepared according to the above column regeneration and preconditioning method using XAD-1600 resin (Rohm and Haas). The first column (10 cm × 80 cm) was then connected to the second column (15 cm × 115 cm). Approximately 20 L of a 50 g/L electrolytic reduction solution at 4−5 °C containing the crude 3-exomethylene product **10** was loaded onto the first column at a flow rate of 0.5−1.0 BV/h. At the same flow rate the column was eluted with 75 L of deionized distilled water at pH of 3.0 followed by 75 L of deionized water at pH of 6.0. The column was further eluted with 120 L of 0.5 M aqueous $NaHCO_3$ solution (pH = 7.5) at a flow rate of 1.0 BV/h. The fractions collected were analyzed by HPLC. The fractions containing product **10** were combined and acidified at 4 °C to pH 3.5−4.0 using diluted HCl. HPLC assay indicated that the initial concentration of the combined fractions was approximately 12 g/L. The above solution was then subjected to reverse osmosis at 10 °C using membrane R15A (100 Dalton) with 32 bar pressure. The typical concentration after reverse osmosis was approximately 35−36 g/L. The yield over the purification and recovery steps was about 93%. The overall yield of the isolated pure product from the electrochemical reduction was 65%. The solution of purified product **10** was used directly for the extractive esterification. A sample was taken for lyophilisation and used for the NMR analysis. ^{1}H NMR (400 MHz, D_2O): δ 1.73 (m, 2H), 2.14 (t, 2H, J = 7.5 Hz), 2.36 (t, 2H, J = 7.5 Hz), 3.24 (d, 1H, J = 18 Hz), 3.48 (d, 1H, J = 18 Hz), 4.86 (s, 1H), 5.11 (s, 1H), 5.16 (s, 1H), 5.28 (m, 2H). ^{13}C NMR (100 MHz, D_2O): δ 176.1, 173.5, 172.5, 167.9, 165.7, 134.2, 116.7, 57.4, 57.5, 56.6, 34.8, 29.3, 25.1, 22.5, 20.6. ES^--MS M^+ 328.1, 282.5, 163.1, 141.3, 102.5. Fractions containing byproduct **14** were combined and lyophilized to dryness. ^{1}H NMR (270 MHz, D_2O): δ 1.89 (m, 2H), 1.95 (s, 3H), 2.14 (t, 2H, J = 7.5 Hz), 2.38 (t, 2H, J = 7.5 Hz), 3.25 (d, 1H, J = 18 Hz), 3.62 (d, 1H, J = 18 Hz), 5.11 (d, 1H, J = 4.5 Hz), 5.59 (d, 1H, J = 4.5 Hz).

Electrochemical Reduction of 9 1(*S*)-Oxide Using a Mercury Pool Working Electrode. 9 1(*S*)-oxide (1 kg) prepared according to the procedure described in Part I[11a] was dissolved in 20 L of a 0.5 M aqueous boric acid solution. Lithium hydroxide was added to adjust the initial pH of the solution to 9.5. The solution was placed in a two-chambered cell separated by a Nafion 350 divider and then cooled to 0 °C. The reduction was carried out at a current density of 15 mA cm^{-2} for 13 h. The final pH of the reaction was 8.2.

(27) In addition to the above, further ES-MS and COSY-NMR data have been generated which provide additional support for the formation of **17** during the electrochemical reduction of **9**: work of D. Dick, C. Rithner, and T. Joicel, Central Instrument Facility, Colorado State University.

The reaction was monitored by HPLC. A sample was also taken for lyophilization and used for NMR analysis. The solution yield of **10** 1(*S*)-oxide was 92% by HPLC. ^{1}H NMR (400 MHz, D_2O): δ 1.73 (m, 2H), 2.21 (m, 2H), 2.25 (m, 2H), 3.25 (d, 1H, J = 18 Hz), 3.45 (d, 1H, J = 18 Hz), 4.82 (s, 1H), 5.11 (s, 1H), 5.15 (s, 1H), 5.29 (m, 2H). HRMS calcd for $C_{13}H_{16}N_2O_7S$ (MH^+) *m*/*z*; 345.0756 found 345.0755.

Electrochemical Reduction of 9 1(*S*)-Oxide Using a Tin Mesh Electrode. 9 1**(*S*)**-oxide (1 kg) prepared according to the procedure described in Part I[11a] was dissolved in 20 L of a 0.2 M aqueous K_2HPO_4 buffer and 0.2 M KCl solution. The resulting solution was added to the catholyte reservoir. 3.3 L of 0.5 N H_2SO_4 solution was added to the anolyte reservoir. The solutions were circulated through the system, and the catholyte pH was adjusted to an initial value of pH = 8.0, by addition of 25% H_3PO_4. The temperature of the cell solution was reduced to ca. 5 °C and the power supply activated; a constant current, typically 12 mA, was passed for the length of time required to achieve the desired conversion. The reaction was monitored periodically by HPLC and was continued until full conversion was achieved. The pH was monitored continuously during the reaction, and its value was maintained at around 8 by addition of aqueous KOH. The cell was emptied and the catholyte circuit was then rinsed three times with 13 L of water. The catholyte and the rinse solutions were collected, and their pH was adjusted to a value of 4.2 by addition of 25% H_3PO_4 to increase the stability of the product. The total volume of the resulting solution was measured, and a 0.5 mL sample was diluted to 50 mL for HPLC analysis. The solution yield of **10** 1(*S*)-oxide was 95% by HPLC.

Chromatographic Purification of Crude 10 1(*S*)-Oxide Produced by the Electrochemical Reduction of 9 1(*S*)-Oxide. A solution of 450 mL of XAD-16 resin (Rohm and Haas) in 3.5 L of deionized distilled water was agitated for 1 h. The water was then decanted followed by addition of 3.5 L of methanol. The methanol solution was agitated for 1 h and was then decanted. Approximately 155 mL of the resin was loaded into a glass chromatography column (2.4 cm × 65 cm) using 550 mL of methanol. The column was eluted with methanol (flow rate = 2 BV/h) and then with 15 L of deionized distilled water (flow rate = 8 BV/h). The column was then back-washed with 5 L of deionized distilled water and allowed to settle overnight. The column was eluted with 2.25 L of 0.5M NaCl solution (pH = 3.0 adjusted with HCl, flow rate = 2 BV/h).

Approximately 180 mL of a 50 g/L electrolytic reduction solution containing 9 g of the crude 3-exomethylene product **10** 1(*S*)-oxide was cooled to 4−5 °C and loaded onto the resin column at a flow rate of 0.5−1.0 BV/h. At the same flow rate the column was eluted with 180 mL of chilled deionized water at a pH of 3.0 and then with 180 mL of deionized water at a pH of 5.0. The column was then eluted with 0.1 M aqueous $NaHCO_3$ solution (pH = 7.0) at a flow rate of 0.5−1.0 BV/h. Approximately $^1/_3$ BV of each fraction was collected and analyzed by HPLC. The fractions containing product **10** 1(*S*)-oxide were combined and acidified to pH 3.5−4.0 using diluted HCl followed by lyophilisation. The recovery yield of the isolated product (less salt) was 8.6 g, 95%. ^{1}H NMR (400 MHz, D_2O): δ 1.73 (m, 2H), 2.21 (m, 2H), 2.25 (m, 2H), 3.25 (d, 1H, J = 18 Hz), 3.45 (d, 1H, J = 18 Hz), 4.82 (s, 1H), 5.11 (s, 1H), 5.15 (s, 1H), 5.29 (m, 2H). FAB HRMS calculated for $C_{13}H_{16}N_2O_7S$ (MH^+) *m*/*z*; 345.0756 found 345.0755. Bulked fractions containing **10** 1(*S*)-oxide in sodium bicarbonate, now free of phosphates, were prepared according to this scheme and used directly in the extractive esterification process described in Part III[11b] (q.v.).

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