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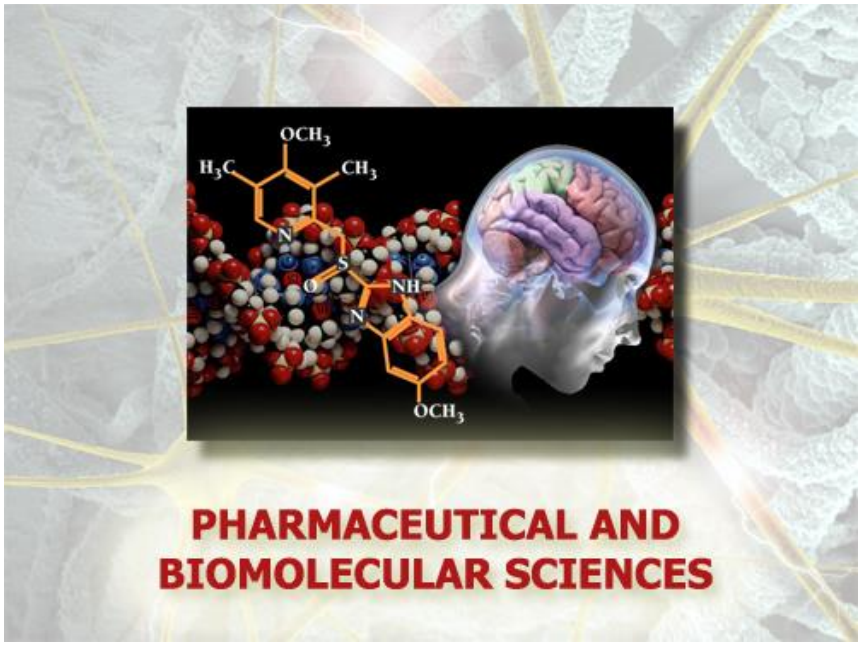

New synthetic routes for the preparation of β-lactam antibiotics

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Preface

This thesis is an original intellectual product of the author, Marziale Comito, which is submitted to apply for the degree of Doctor in Pharmaceutical and Biomolecular Sciences at the University of Turin (Italy). The present research herein was conducted under the supervision of Prof. Giancarlo Cravotto and Prof. Giovanni Palmisano at the Department of Drug Science and Technology of the University of Turin (Italy) and Research and Development Laboratory of ACS Dobfar in Tribiano (MI, Italy), between November 2020 and November 2023.

The motivation for this research stems from daily job on the β-lactam antibiotics synthesis. Although these drugs represent the most widely used therapeutic class among antimicrobial agents nowadays, their process chemistry has remained anchored to developments from many years ago. In the current context of the industrial production of Active Pharmaceuticals Ingredients (APIs), steered by streamlining, sustainability and enhancing productivity, the process innovation is the driving force to compete in the global market.

This doctoral study was funded by the ACS Dobfar and the University of Turin. This thesis has not been submitted for any other degree, diploma, or other qualification at any other university. The results of the investigations have been published in the following four peer journals: Comito, M. *et al.,* From Batch to the Semi-Continuous Flow Hydrogenation of *p*NB, *p*NZ-Protected Meropenem, *Pharmaceutics*, 2023, 15, 1322. Comito, M. *et al.,* Towards Antibiotic Synthesis in Continuous-Flow Processes, *Molecules,* 2023, 28, 1421. Comito, M. *et al.,* Cefonicid Benzathine Salt: A Convenient, Lean, and High-Performance Protocol to make an Old Cephalosporin Shine, *Antibiotics,* 2022, 11, 1095. Comito, M. *et al.,* Efficient Pilot-Scale Synthesis of the Key Cefonicid Intermediate at Room Temperature, *Green Processing and Synthesis,* 2022, 11, 96-105.

Acknowledgements

Three years ago, I didn't believe to find me here writing the doctoral thesis. After a long time from graduation, getting involved myself again with the academic world has been fascinating, engaging, and gratifying. I am enjoying this last moment.

The three years of doctorate have been a once-in-a-lifetime experience and unforgettable, brimming of emotions, commitments, and accomplishments. All results that I have reached would not have been possible without the support and guidance from people always by my side during this fantastic journey. It is my duty expressing here my sincere gratitude to the supervisor, co-tutor, committees, colleagues, girlfriend, and family.

First, I would like to express my special gratitude to my supervisor Professor Giancarlo Cravotto, for his great support, valuable guidance, and consistent encouragement during the past three years. I am also very grateful for his scientific advice, knowledge, insightful discussions, and suggestions. It is truly an honour to be his Ph.D. student.

I am also heartfelt gratitude to Professor Giovanni Palmisano. Without him, I will have never known Professor Cravotto and this collaboration would be never born. He has given me a tremendous support and guidance for Ph.D. research. His strong expertise, cautious optimistic, and patient guidance have enlightened and accelerated my research. Each time I got stuck in something, he came up with the solution and encouraged me a lot.

My sincere thanks also go to Professor Silvia Tagliapietra, who really helped me a lot in addressing the research with her scientific expertise, in the presentation's preparation, and manuscripts revision. Without her support, it would not be possible to arrive at this moment. Thanks for her kind guidance, good suggestions, and precious time.

Besides, I would like to thank Acs Dobfar to have granted me the doctoral opportunity, allowing me working, researching, and studying simultaneously.

Special mention is given to the Department of Drug Science and Technology and Doctoral School of the University of Turin for allowing me to undertake this research and all the members of Professor Cravotto's group.

Last, but not least, I would like to express my appreciation to my family. Words cannot express how grateful I am to them for all the sacrifices, love, support, and patience that they have had during these three years.

Acronyms and Abbreviations

Table A1. List of the acronyms

Materials	Acronyms
β-Lactamase inhibitors	BLIs
Active Pharmaceuticals Ingredients	APIs
Microwave	MW
Antimicrobial Resistance	AMR
Penicillin Binding Protein	PBP
Food and Drug Administration	FDA
Good Manufacturing Practice	GMP
Multi-drug Resistant	MDR
Dehydropeptidase	DHP
Extended Spectrum β-lactamases	ESBLs
Diazabicyclooctane	DBO
New Molecular Entities	NMEs
European Medicines Agency	EMA
Process Mass Intensity	PMI
Design of experiments	DOE

Table A2. List of the acronyms and abbreviations of compounds

Compounds/Solvents	Acronyms and Abbreviations
7-aminocephalosporanic acid	7-ACA
1-sulfomethyl-5-mercapto-1,2,3,4-tetrazole sodium-potassium salt	TSA
7-amino-3-[sulphomethyl-1H-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylate monosodium salt	7-SACA
N',N''-dibenzylethylene diamine diacetate	Benzathine diacetate
para-nitrobenzyl group	*p*NB
para-nitrobenzyloxycarbonyl group	*p*NZ
6-aminopenicillanic acid	6-APA
7-aminodesacetoxy-cephalosporanic acid	7-ADCA
3-chloromethyl-7-phenylacetylamino-cephalosporanic-acid-*p*-methoxybenzyl ester	GCLE
7-phenylacetamido-3-hydroxy-3-cephem-4-carboxylic acid diphenyl methyl ester	GHYH
Dimethylacetamide	DMAc
Phenylglycine Methyl Ester	PGME
Penicillin Acylase	PA

Polyethylene Glycol	PEG
Thiazolyl-7-aminocephalosporanic acid	7-TACA
Methylmercaptothiazolyl acid	MMTA
Diphenyl chlorophosphate	DPCP
N,N-diisopropylethylamine	DIPEA
1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDC
4-dimethylaminopyridine	DMAP

Extended Abstract

β-lactam antibiotics are currently the most used class of antibacterial agents in the infectious diseases' armamentarium. Since the benzylpenicillin's discovery in 1928 by Fleming to present days, they have saved millions of lives, representing a milestone in the medicinal field.

Until the 1980s, many pharmaceuticals' companies in collaboration with the universities were focused on the research and development of new synthetic protocols for these specific drugs. In the last three decades, this development has undergone a drastic fall because of investments' redirection in favour of more profitable therapeutics areas. The patents' expiry together with the globalization have brought new international competitors in the active pharmaceutical ingredients (APIs) makers panorama, triggering a costs war, and, consequently, lowering their market value. The strict regulatory guidelines have also curbed the manufacturing procedures upgrade to chemical and technological advances, making outdated the currently synthesis.

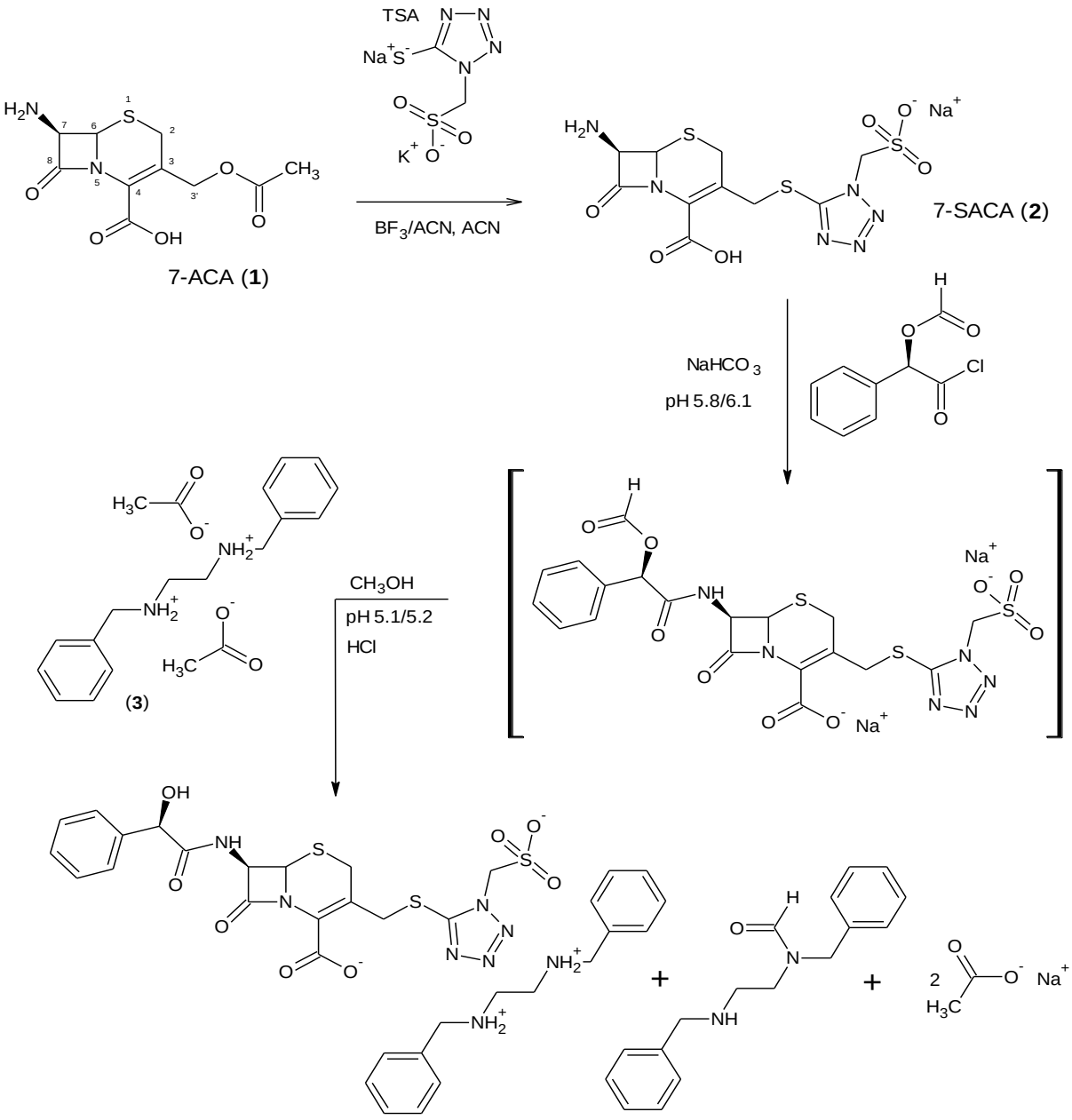
The process chemistry has made great progresses over the last few years, promoting the development and adoption of cutting-edge technologies and new synthetic schemes. These advances applied to the small-molecules synthesis has opened up new landscapes in APIs manufacturing. Being linked by a mutual interest, the pharmaceutical industry and the process chemistry are two sides of the same argument. Costs efficiency, manufacturing streamlining, productivity enhancement, sustainability, environmental footprint, and safety are paramount metrics defining and addressing the process performance, and, consequently, the product success. In the present framework of drugs industrial production, in which the competition is at the highest technological level, new protocols development is an opportunity to seize and necessary in order to compete in the global market.

The aim of doctoral thesis has been to design alternative synthetic methods for two relevant β-lactams antibiotics, exploring environmentally friendly and cost-effective technologies from an industrial point of view and of process intensification. Preserving the drugs high quality, the adaptability to great manufacturing sizes to support the massive global demands has been the driving forces for the development of new protocols.

Classical and innovative approaches have been adopted to reach our goals. With the first, we have studied technical and operational parameters able to change and improve the industrial synthesis of Cefonicid *N'*,*N''*-dibenzylethylene diamine (*aka* benzathine) salt (**4**). With the second approach, we have investigated the final step of Meropenem (**6**) synthesis applying a non-conventional technology. An in-depth bibliographic research has allowed us to obtain the results described below. The publication of a review on new technologies for the β-lactam antibiotics allows us to demonstrate the uniqueness in the world of our study on the hydrogenation of Meropenem (**6**) with microwave (MW)-assisted flow chemistry.

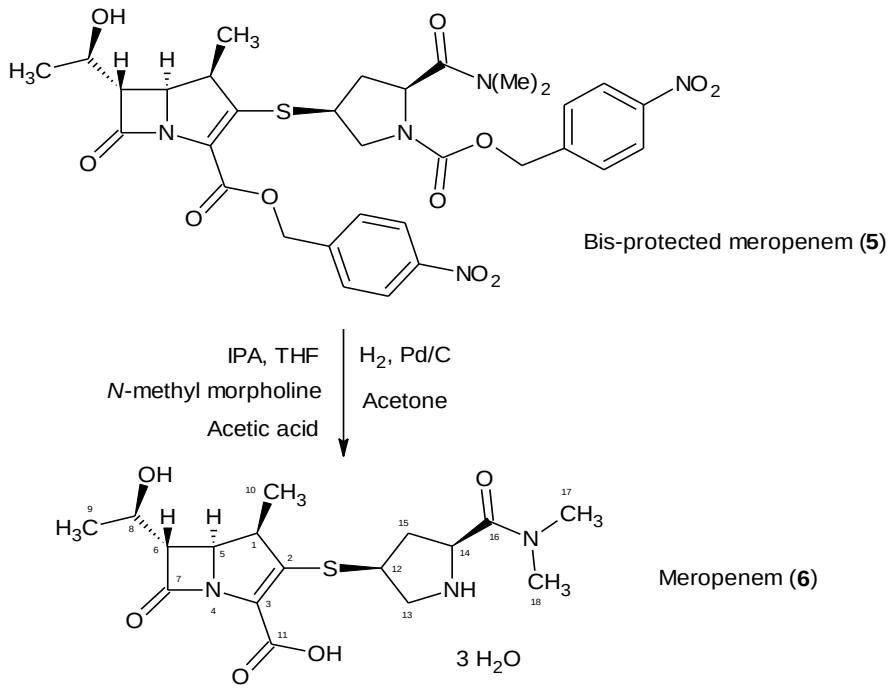
Cefonicid is a second-generation cephalosporin sold as injectable drug and obtained via freeze-drying process. The drug product is its disodium salt while the drug substance is Cefonicid benzathine salt (**4**). The drug substance preparation is characterized by cumbersome, and time-consuming steps, making the product unattractive for the international market. Starting from 7-aminocephalosporanic acid (7-ACA, **1**), the most utilized building block in the cephalosporins synthesis, all synthetic steps have been carried out. The displacement of 3'-acetoxy group, the amidation at the 7-position and the subsequent deprotection have been studied with the aim of making the process efficient, decreasing the waste and production costs, as designed in Scheme A1. The 7-amino-3-[sulphomethyl-1H-tetrazol-5-ylthiomethyl]-3-cephem-4-carboxylate monosodium salt intermediate (7-SACA, **2**) has been prepared with a telescopic route whose steps were all performed at room temperature. A simpler, scalable, cost-effective, and energy-saving protocol has been obtained to produce the cefonicid key intermediate (**2**). All process parameters have been optimized to evaluate the industrial-scale impact. The subsequent amidation with the *O*-formyl-(*R*)-mandeloyl chloride followed by removal of the *O*-protected group with the *N',N''*-dibenzylethylene diamine diacetate (**3**) have given the desired pharmaceutical molecule. Different synthetic paths for the amidation of the 7-position have been studied, but acyl chloride has proven to be the most well-performing. The double-nucleophilic and lipophilic nature of *N',N''*-dibenzylethylene diamine diacetate (**3**) has enabled the deformylation of the OH-protected group on the mandelic moiety and also enabled product crystallization to occur, allowing to obtain a new, reliable, efficient, and sustainable protocol. The high reactivity of formyl group has promoted an amidation reaction, deprotecting the hydroxy group and generating a new C-N bond in the reaction by-product. Several amines and OH-protected groups have

been studied, but none were able to replicate the excellent results of benzathine diacetate (**3**).



Scheme A1. Cefonicid benzathine salt (**4**) synthetic route.

Afterwards, Meropenem (**6**) synthesis has been investigated. Meropenem (**6**) is currently the most common carbapenem for clinical applications. It is an injectable drug administered intravenously. Industrially, the final synthetic step is characterized by a heterogeneous catalytic hydrogenation in batch mode with hydrogen and Pd/C, as reported in scheme A2. The required high-quality standard is very difficult to meet, and specific conditions are necessary for industrial production to remove both protecting groups [i.e., *p*-nitrobenzyl (*p*NB) and *p*-nitrobenzyloxycarbonyl (*p*NZ)] simultaneously. Moreover, the three-phase gas-liquid-solid system makes this step harsh and unsafe.



Scheme A2. Bis-protected meropenem (**5**) hydrogenation.

The meropenem (**6**) hydrogenolysis has been investigated using microwave (MW)-assisted flow chemistry. The reaction parameters (catalyst amount, T, P, residence time, flow rate) in the move from the batch process to semi-continuous flow has been studied under mild conditions to determine their influence on the reaction rate. The optimization of the residence time (840 s) and the number of cycles (4) has allowed to develop a novel protocol that halves the reaction time compared to

batch production (14 min *vs* 30 min), maintaining the same product quality. The increase in productivity using the semi-continuous flow technique compensates for the slightly lower yield (70% *vs* 74%) obtained via batch mode.

Given the restrictions imposed by their technological limitations and chemical behavior, the processes have been turned into sustainable route to highly efficient production. Regarding the old chemistry belonging to β-lactams, the conversion to modern, sustainable protocols has been a challenging task.

Chapter 1: Theoretical Part

1.1 Introduction

1.1.1 Antibiotics

The discovery and development of antibiotics, combined with their therapeutic use against many bacterial illnesses can be considered one of humankind's great breakthroughs, so much so that it was cited, in 2013, as one of nine ways that chemistry has changed the world [1], underling the utmost importance of these drugs. Since the salvarsan's discovery in the 1910, the antibiotics have drastically changed the modern medicine, extending the average human lifespan. From infectious disease treatment, the application area has been broadened to different and modern medical procedures, including cancer treatment, organ transplants, and open-heart surgery [2].

The penicillin G's discovery in 1928 by Sir Alexander Fleming with its clinical use since 1942 has kicked off the "golden age" of these drugs, setting the stage for the launch of the most successful drugs in the history [3,4]. Since the World War II to the end of '80s, many pharmaceuticals' companies were focused on the research and development of products treating the infectious diseases, involving even the academic world. These products were considered the most important research's pipelines and the most profitable. Several classes became available including sulfonamides, trimethoprim, β-lactams, chloramphenicol, tetracyclines, colimycins, macrolides, lincosamides, streptogramins, rifamycins, glycopeptides, aminoglycosides, fluoroquinolones, oxazolidinones, glycylglycines, lipoglycopeptides and variations of these molecules [5-11]. Since the '90s, the gradual fall in additional antibiotics discovery began, caused by the patents' expiry, to the low-profit margins due to the globalization of supply chain which decreased drastically the manufacturing costs, to the pharmaceuticals' interest towards others therapeutic classes. In the last years, the scientific community has emphasized this problem, especially since the combination between the clinical administration of the same antibiotics with the evolution of drugs resistance in many human pathogens has led to current antimicrobial resistance (AMR) [12-22].

Commonly, antibiotics are classified based on their mechanism of action, depending on chemical structure. The bactericides kill bacteria targeting the cell wall, the cell membrane or interfering with essential enzymes; the bacteriostats inhibit the protein synthesis stopping the bacterial reproduction. A further classification allows to divide these drugs on spectrum of activity relatively to specific types of target bacteria. Broad spectrum antibiotics act against gram-positive and gram-negative bacteria; those of narrow spectrum, instead, against a limited species of bacteria [23-25].

Among all compounds of this therapeutic class, β-lactams represent the most used subgroup. In the United States of America, they have accounted for 65% of all prescribed injectables antibiotics for years 2004 – 2014, as shown in Figure 1.1. Cephalosporins have represented about the half of all β-lactams, followed by penicillins (about 40%) and carbapenems (about 10%) [26].

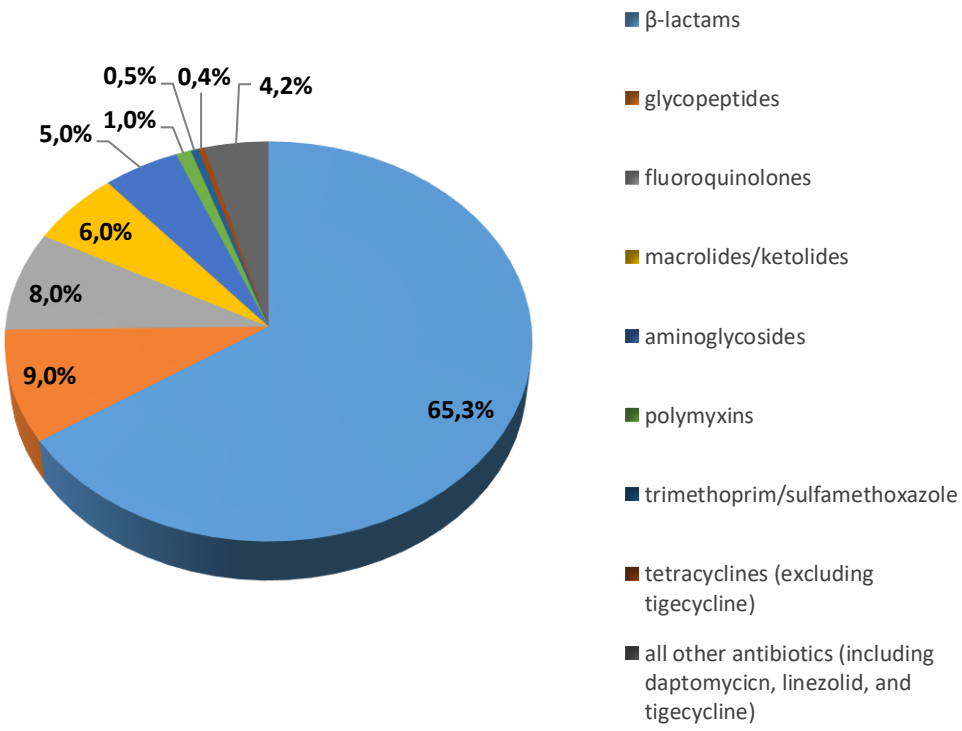


Figure 1.1. Antibiotics prescription in the United States for 2004-2014.

The effectiveness, the tolerability of human body and the low price have been the major advantages of their success.

1.1.2 β-lactam antibiotics

β-lactams are a class of broad-spectrum antibiotics containing a β-lactam ring in their molecular structure. They are bactericidal and act inhibiting the synthesis of bacteria cell wall interfering with the enzymatic transpeptidation process. The mechanism of action has been attributed to the possibility that β-lactam nucleus acts as structural analogous of terminal D-alanyl-D-alanine dipeptide of the nascent peptidoglycan, binding irreversibly to the Ser_{403} residue of the penicillin binding protein (PBP) active site. This process prevents the final crosslinking (transpeptidation) of the peptidoglycan layer, disrupting the cell wall [27] (Figure 1.2).

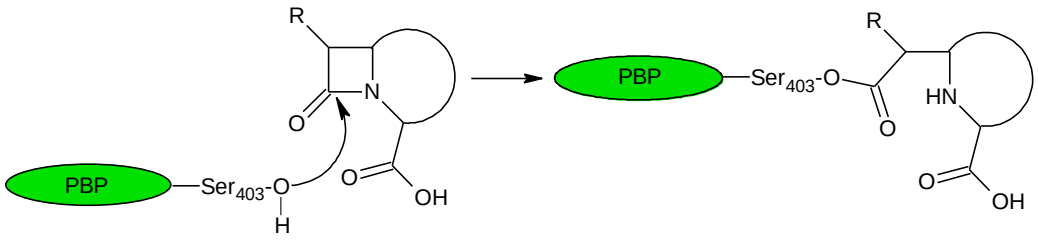


Figure 1.2. Mechanism of action of a β-lactam antibiotic.

The first β-lactam antibiotic, penicillin G, was isolated by Fleming from *Penicillium chrysogenum* [28]. After such milestone, hundreds of new penicillins derivates and related therapeutic classes of cephalosporins, penems, monobactams, carbacephems and carbapenems have been discovered. Each new subclass has been researched either to increase the activity's spectrum including additional bacterial species or to avoid specific resistance mechanisms arisen in the target bacterial population.

β-lactam antibiotics and, in particular penicillins, are known to be highly sensitizing compounds, causing severe allergic reactions in few subjects. The allergic reactions constitute the most common and significant side-effects, and they

are assumed to be the result of an antigen-antibody sensitized human cell interaction [29]. The categorization of these drugs was introduced by Food and Drug Administration (FDA), the American regulatory agencies, to address their manufacturing, emitting strict guidelines [30]. FDA guidance recommends that the manufacturing of each sensitizing β-lactams must be structurally isolated from areas in the facility in which other products or another β-lactam product are manufactured, avoiding cross-contamination, and reducing the risk of allergic reactions in the workforce.

1.1.2.1 Penicillins

As already mentioned, penicillins were the first β-lactam compounds to be discovered and used clinically as antimicrobial drugs. They are structurally constituted by a four-membered β-lactam ring fused with a five-membered thiazolidine ring, as designed in Figure 1.3.

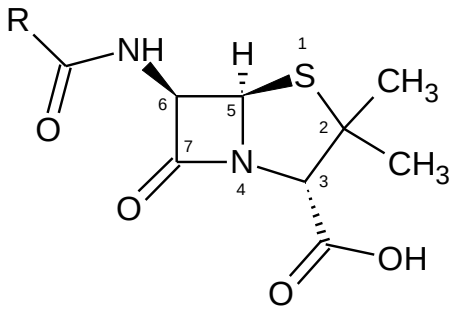
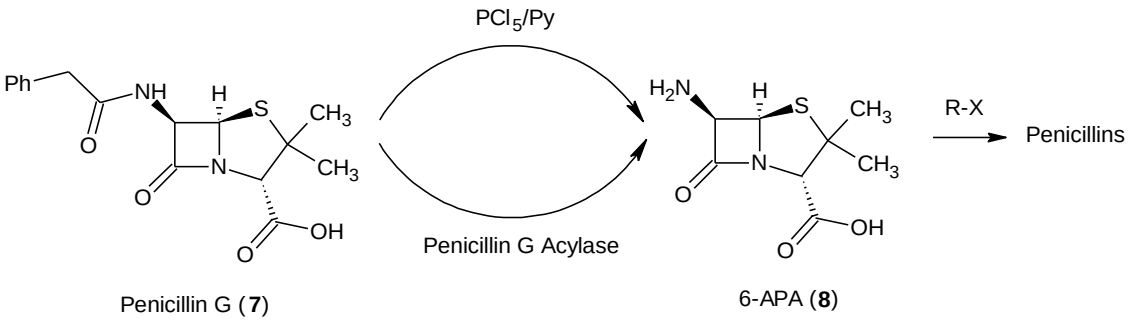


Figure 1.3. Penicillins general structure.

Penicillins differ from each other's for the nature of R acylic residue, characterizing the drug activity. They are semi-synthetic product having in 6-aminopenicillanic acid (6-APA, **8**) the principal scaffold. 6-APA (**8**) can be prepared by fermentation via the enzymatic hydrolysis of Penicillin G (**7**) [31] or chemically using phosphorus pentachloride (PCl_5). Industrially, the enzymatic synthesis is the main way to produce this nucleus for its low cost and impact on the environment. From 6-APA (**8**), all penicillins can be obtained acylating with suitable acid residue R, as depicted in Scheme 1.1.



Scheme 1.1. General penicillins synthetic pathway.

Piperacillin (**9**), Ampicillin (**10**) and Amoxicillin (**11**) represent the main blockbuster in this subclass (Figure 1.4).

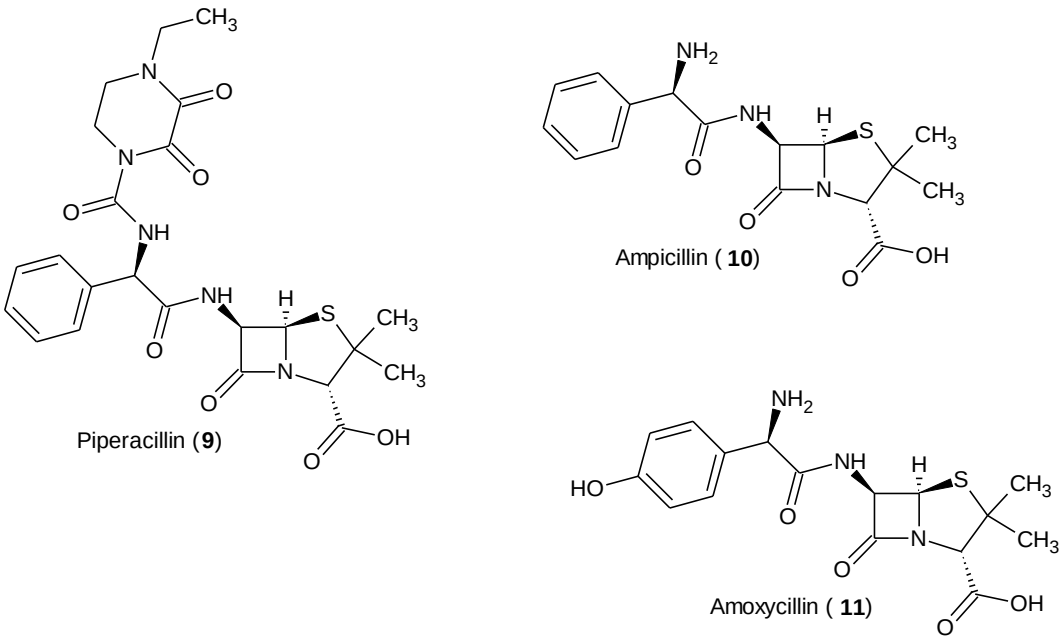


Figure 1.4. Penicillins blockbusters.

It is important emphasizing that there aren't new chemical entities under development in this subclass.

1.1.2.2 Cephalosporins

The first cephalosporin discovered was the cephalosporin C (**12**), obtained from a fungus of the genus *Acremonium* by the Italian pharmacologist Giuseppe Brotzu in 1945 [32]. Being an isolated scientist from the Italian scientific community of his age, he donated his discovery to English's that they patented it, launching a new generation of antibiotics. Cephalosporins are structurally constituted by β-lactam ring fused with a six-membered dihydrothiazine ring, as designed in Figure 1.5, differing from each other's for the nature of R_1 and R_2.

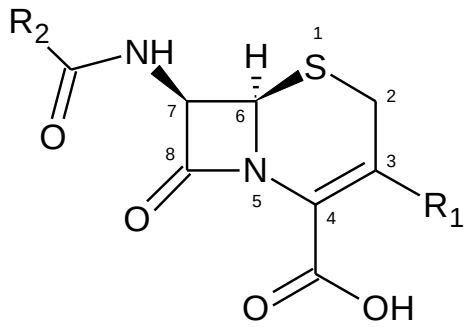


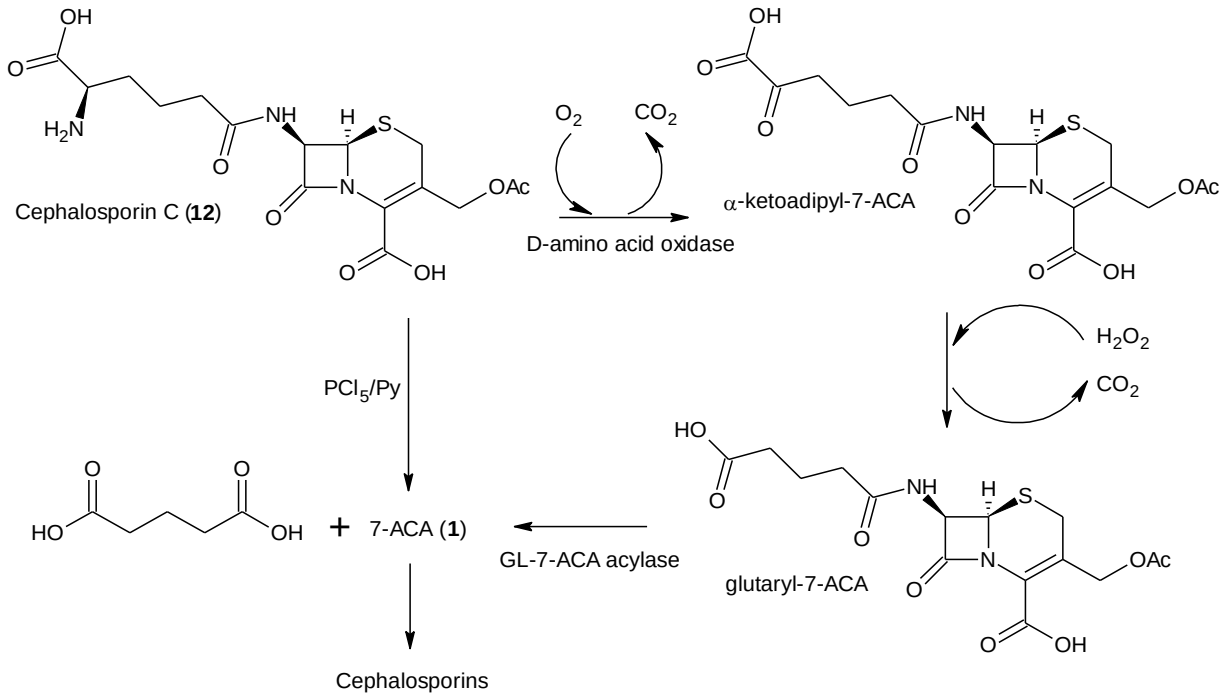
Figure 1.5. Cephalosporins general structure.

The different moieties (R_1 and R_2) linked to the cephalosporin nucleus determine their antimicrobial activity, allowing to classify these drugs into generations. Nowadays, there are five generations of cephalosporins, each new generation has been developed to significantly increase the antimicrobial activity against gram-negative bacteria.

Cephalosporins are semi-synthetic compound produced from four different cephalosporins cores [33,34]:

1. 7-ACA (**1**)
2. 7-aminodesacetoxy-cephalosporanic acid (7-ADCA, **13**)
3. 3-chloromethyl-7-phenylacetylamino-cephalosporanic-acid-*p*-methoxybenzyl ester (GCLE, **14**)
4. 7-phenylacetamido-3-hydroxy-3-cephem-4-carboxylic acid diphenyl methyl ester (GHYH, **15**)

7-ACA (**1**) represents the most used scaffold in the cephalosporins manufacturing [35]. It is produced starting from Cephalosporin C (**12**) either chemically or by two-steps enzymatic route, as depicted in Scheme 1.2. Industrially, environmentally friendly aspects and manufacturing low costs make the enzymatic route the most employed nowadays.



Scheme 1.2. General cephalosporin synthetic pathway via 7-ACA (**1**).

Starting from 7-ACA (**1**), different cephalosporins are obtained in two steps involving the introduction of two substituents R_1 and R_2. Typically, only cephalosporins bearing in 3-position a -CH_2-I substituent are produced through this strategy. Cefuroxime (second generation, **16**), Cefepime (fourth generation, **197**), Ceftazidime (third generation, **18**) and Ceftriaxone (third generation, **19**) represent the main blockbuster synthetized in this way. Ceftobiprole medocaril [34] (**20**) is a recently approved fifth-generation cephalosporin which is also produced from 7-ACA (**1**) but applying a multistep synthesis (Figure 1.6).

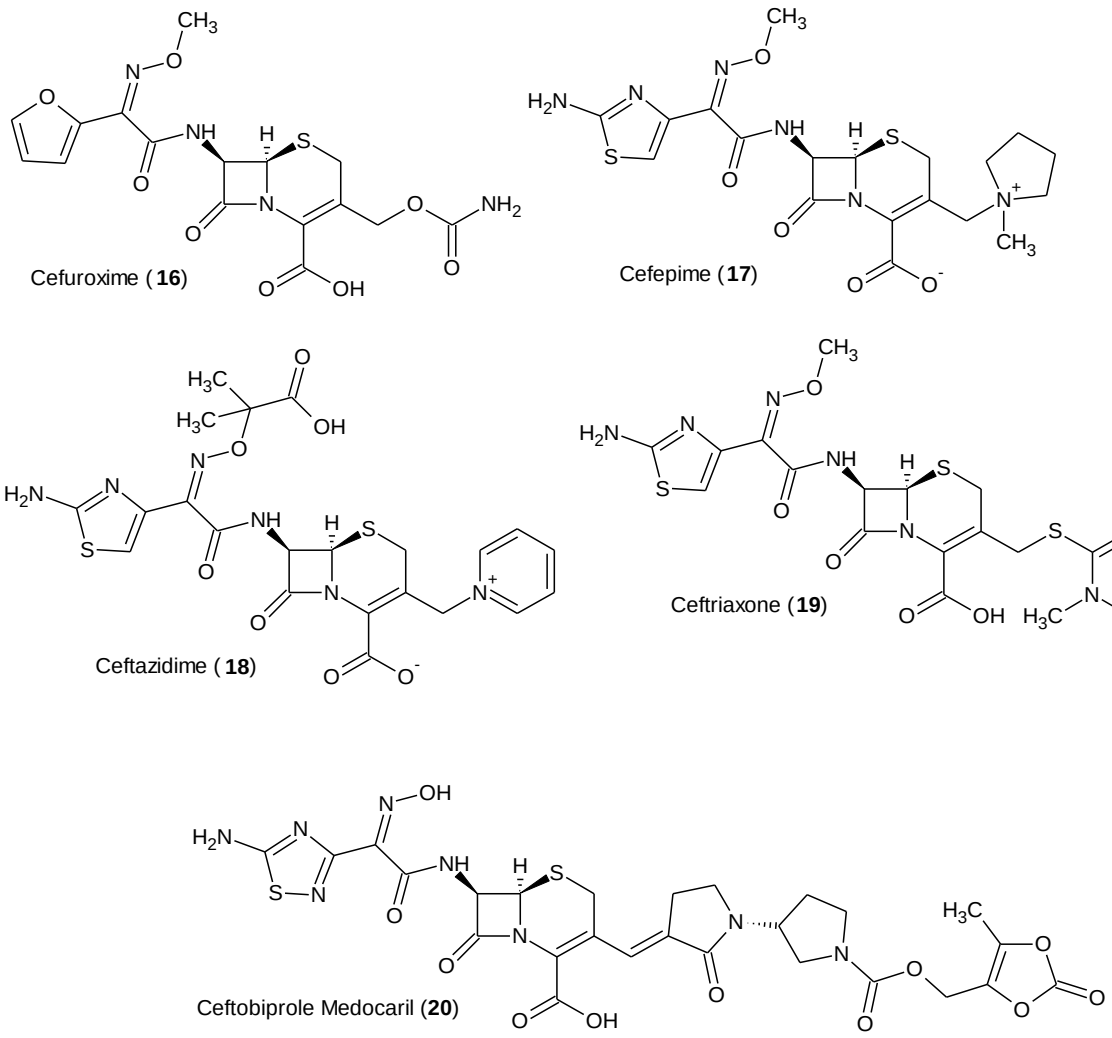
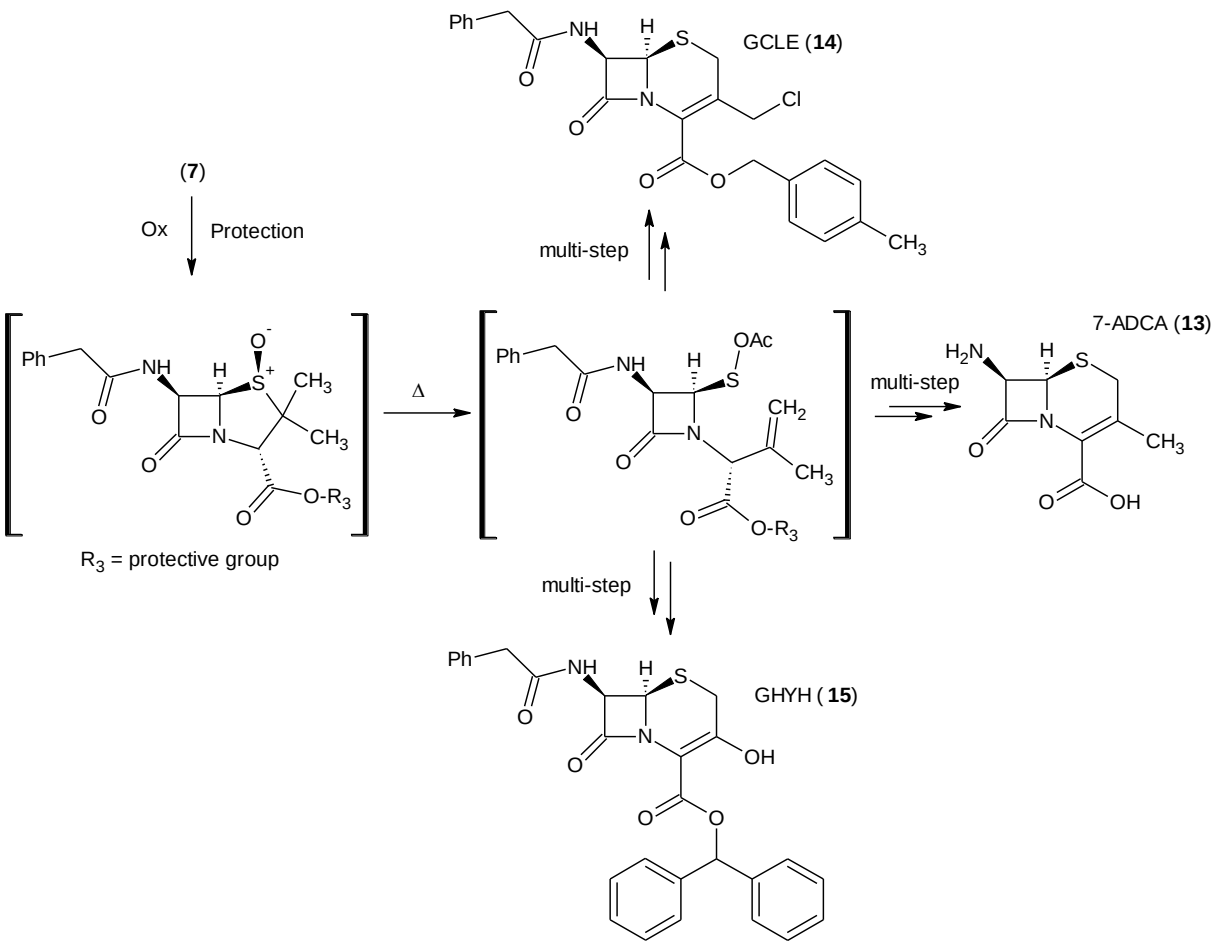


Figure 1.6. Cephalosporins blockbusters from 7-ACA (**1**).

Cephalosporins are also produced starting from penicillins, exploiting the thermal rearrangement of penicillin sulfoxides intermediate. This interesting synthetic route was discovered by Morin *et al.* at Eli Lilly [36] in 1969 and represent the starting point to produce the others cephalosporins building blocks (**13**, **14** and **15**), getting through several synthetic or enzymatic steps. The generated sulfenic derivate by heating is the key intermediate of this pathway, as depicted in Scheme 1.3.



Scheme 1.3. Penicillin sulfoxide rearrangement.

7-ADCA (**13**) is the precursor of two first generation cephalosporins, Cephalexin (**21**) and Cefadroxil (**22**), GCLE (**14**) is the precursor of the last two fifth generation cephalosporins approved by FDA, Ceftolozane [33] (**23**) and Cefiderocol [37] (**24**), instead GHYH (**15**) is the precursor of Ceftaroline fosamil [34] (fifth generation cephalosporin, **25**) and ceftibuten (third generation, **26**) (Figure 1.7).

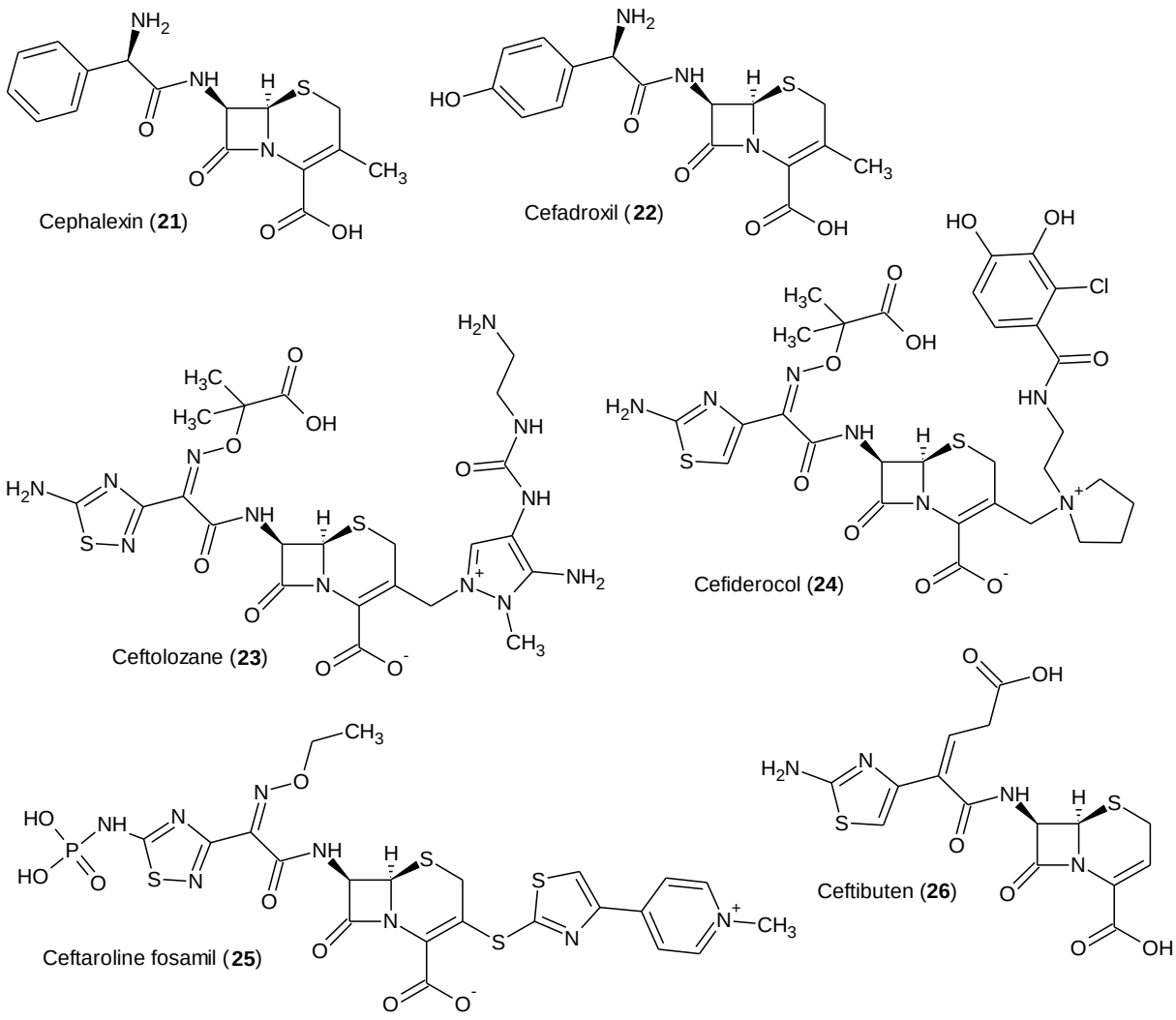


Figure 1.7. Cephalosporins blockbusters from 7-ADCA (**13**), GCLE (**14**) and GHYH (**15**).

It is necessary highlighting that the listed four fifth generation cephalosporins represent the only ones discovered in the last twenty years.

1.1.2.3 Carbacephems

Carbacephems are structurally constituted by β-lactam ring fused with a six-membered tetrahydropyridine ring, in which the sulfur atom is substituted by methylene group. It is a dead class of β-lactam antibiotics because of poor success on the market. The only one mentioning is Loracarbef (**27**) developed by Eli Lilly

[38], having the same activity of Cefaclor (second generation cephalosporin, **28**) (Figure 1.8).

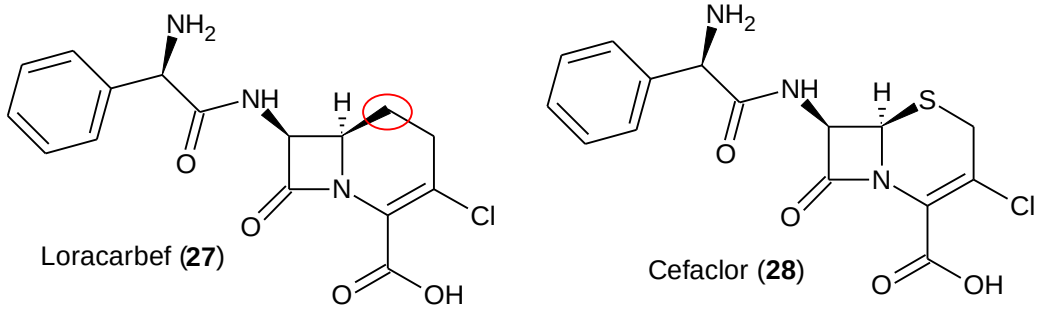


Figure 1.8. Loracarbef (**27**) as carbacephem compared to Cefaclor (**28**).

1.1.2.4 Monobactams

This subclass is structurally constituted by a monocyclic β-lactam ring, not fusing with another ring. They were discovered in 1981 [39] and Aztreonam (**29**) is the single approved drug (Figure 1.9).

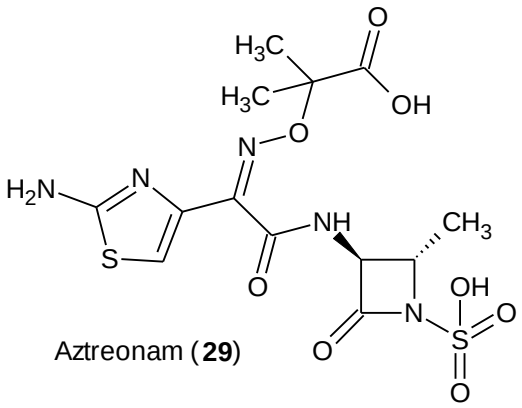


Figure 1.9. Aztreonam (**29**) as monobactam.

Some companies are developing novel monobactam antibiotics because they are only active against gram-negative bacteria. It is to be expected an expansion of this subclass in the next years. It is important to highlight that the manufacturing of this drugs is problematic due to the lack of regulated facilities under Good Manufacturing Practice (GMP) conditions.

1.1.2.5 Penems

All regulatory agencies, FDA *in primis*, incorrectly define the penems subclass the subclass in which carbapenem are categorized. In reality, penems and carbapenems have two chemically different structures. Penems are structurally constituted by β-lactam ring fused with a five-membered thiazoline ring, as designed in Figure 1.10.

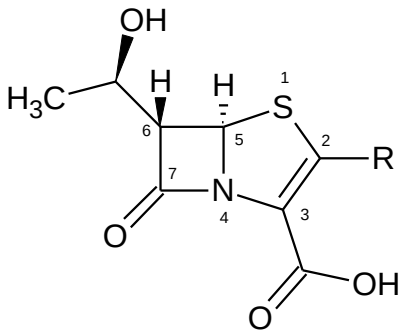
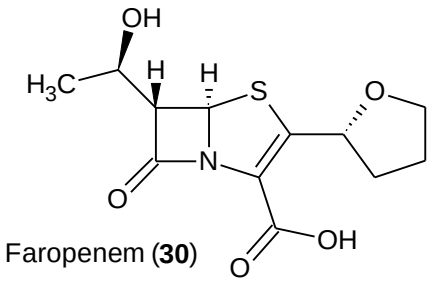
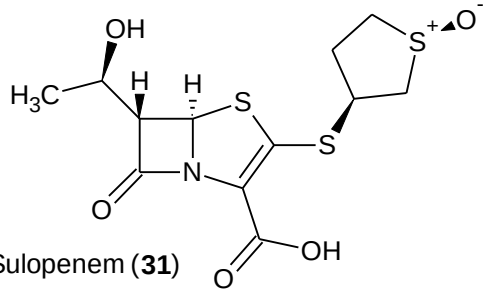


Figure 1.10. Penems general structure.

The different antimicrobial activities of penems are established by the structural moiety of R. Faropenem (**30**) is the only one approved penem on Japanese regulated market. Sulopenem (**31**) is under clinical studies by Iterum Pharmaceuticals (Figure 1.11).



Faropenem (**30**)



Sulopenem (**31**)

Figure 1.11. Approved penem and under development.

1.1.2.6 Carbapenems

Among all β-lactams, carbapenems possess the broadest spectrum of activity and exhibit the better β-lactamase stability. These exclusive properties allow to

treat serious and life-threating gram-positive and gram-negative infections known or suspected to be caused by multi-drug resistant (MDR) bacteria. For this reason, they are called the "antibiotics of last resort" [40, 41].

Carbapenems are structurally constituted by β-lactam ring fused with a five-membered pyrroline ring. They were first discovered by Brown and co-workers in 1976 [42]. Different carbapenems differ for the chemical nature of R_1 and R_2, influencing their antimicrobial activity. R_1 can be -H or -CH_3, as depicted in Figure 1.12. The stereoisomer in β position of R_1 was found to play a major role in the antimicrobial potency of these drugs.

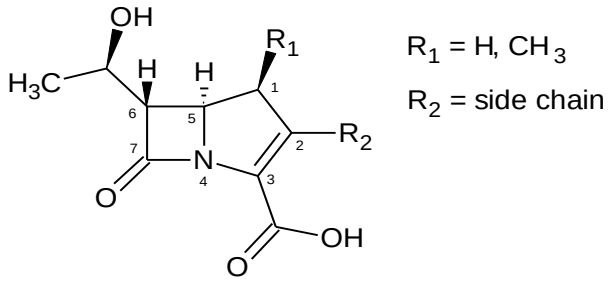


Figure 1.12. Carbapenems general structure.

The first discovered carbapenem antibiotic was thienamycin [43,44] (**32**), a naturally derived product from *Streptomyces cattleya*. Due to its poor stability, it has never been developed as drug. Imipenem (**33**) was discovered by Merck & Co. [45], and it was the first marketed carbapenem in 1985. In Imipenem (**33**) structure, the free primary amino group of thienamycin (**32**) is masked by an amidino group providing an enhanced stability than thienamycin (**32**). Imipenem is sold in combination with cilastatin, a β-lactamase inhibitor, preventing its hydrolysis by renal dehydropeptidase (DHP). In the following years, the introduction of a β-methyl group at C-1 position of carbapenem structure have made the drugs more resistant to DHP, boosting the antibiotic activity and spectrum. This useful characteristic has allowed to develop new carbapenems having a big market success. There have been six carbapenem antibiotics approved after imipenem (**33**) by worldwide regulatory agencies (Figure 1.13). Panipenem (**34**), having the same nucleus of Imipenem (**33**), was approved for Japanese market in 1993. Meropenem (**6**) was approved by FDA in 1996 for worldwide market, becoming in very short time the most effective and safe medicine in the antibiotic panorama for the health

system. In 2001, Biapenem (**35**) and Ertapenem (**36**) were approved; the first for Japanese market while the second for worldwide market. Doripenem (**37**) was approved by FDA in 2007, while Tebipenem pivoxyl (**38**) was the last carbapenem antibiotic approved by Japanese regulatory agency in 2009. All marketed drugs are produced by total synthesis, despite that thienamycin (**32**) was obtained from natural origin.

It is important emphasizing that there aren't new chemical entities under development in this subclass.

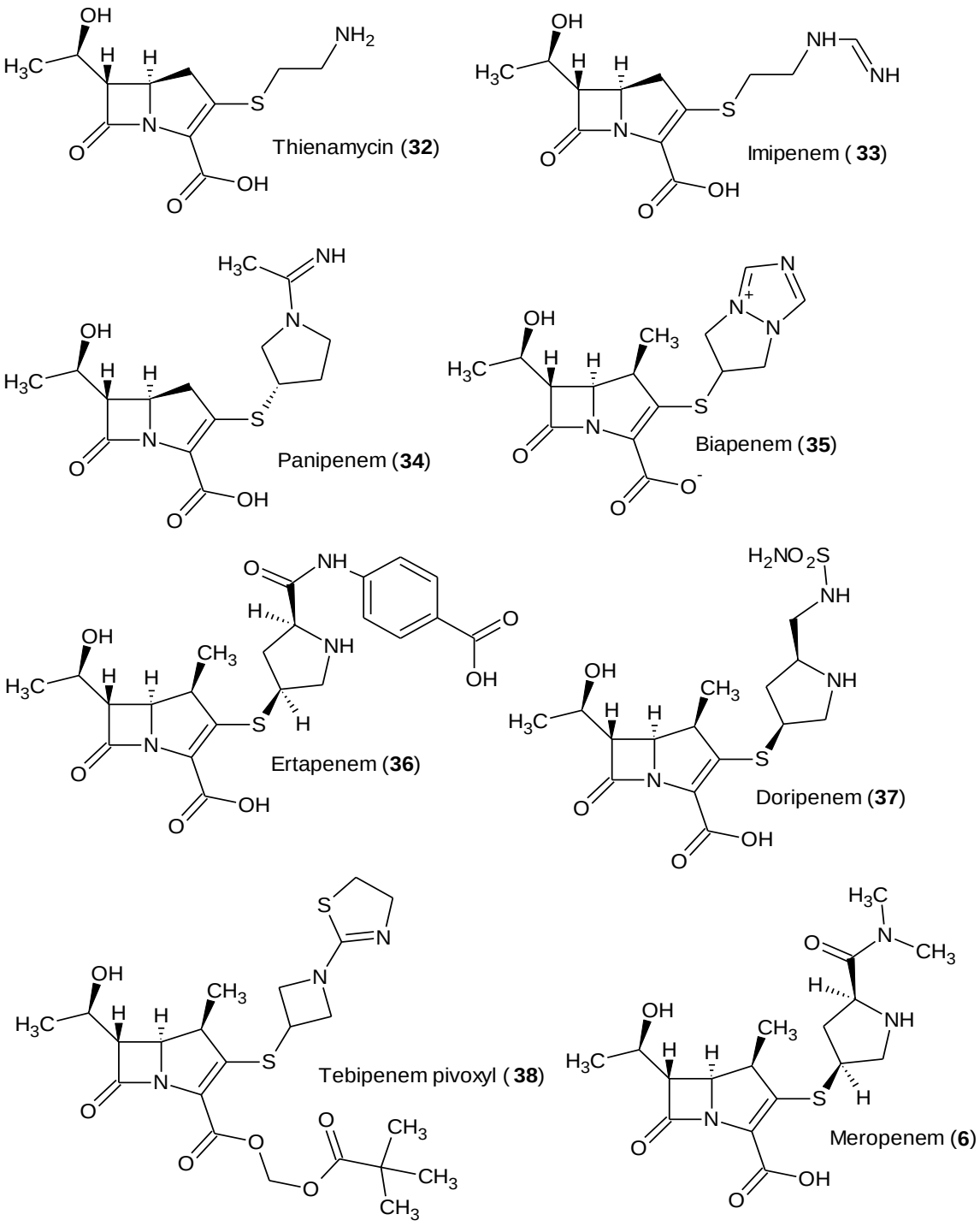


Figure 1.13. Carbapenems blockbusters.

1.1.3 β-Lactamase inhibitors (BLIs)

Since the discover of the penicillin, in parallel with the research of new and more effective antibiotics, various strategies have been investigated to overcome and fight the antimicrobial resistance. The most important strategy to cheat the resistance has been the inhibition of β-lactamases, degrading the β-lactam antibiotics [46]. β-lactamases are enzymes produced by both gram-positive and gram-negative bacteria hydrolyzing the β-lactam nucleus [47]. β-lactamase inhibitors (BLIs) are a class of compounds irreversibly blocking the activity of enzyme, binding to enzyme active site, and preventing the degradation of β-lactam antibiotic. With these compounds, the drug activity is not affected. The road to BLIs began when, as result of natural products screening, Clavulanic acid (**39**) was identified as a broad-spectrum inhibitor and isolated by *Streptomyces clavuligerus* [48]. Penicillins are particularly sensible to β-lactamases; therefore, they were the first β-lactams combined with BLIs. The first generation of BLIs used with penicillins were constituted by Clavulanic acid (**40**), Sulbactam (**41**) and Tazobactam (**42**) (Figure 1.14).

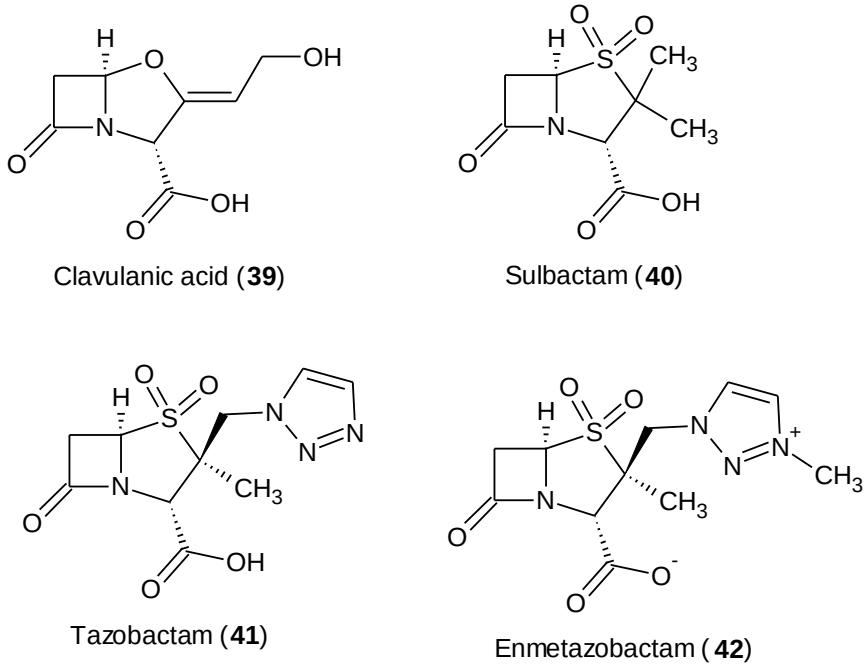


Figure 1.14. First generation of BLIs.

The combinations Amoxycillin (**11**) with Clavulanic acid (**39**) (Augmentin™), Ampicillin (**10**) with Sulbactam (**41**) (Unasyn™) and Piperacillin (**9**) with Tazobactam (**41**) (Tazocin™) quickly became some of the most prescribed antibacterial agents in the world.

In the last two decades, the AMR is become an issue of great concern with a high social and economic burden, attracting the attention of health agencies, media, and global leaders. It has been estimated that the failure to treat the drug-resistant infections have had a cost of USD 20 billion in 2009 in the United States [49]. More recently, giving a response to public opinion, the interest in the discovery of new BLIs has been renewed. In this field, the research is focused to counter new β-lactamases as the extended spectrum β-lactamases (ESBLs) and carbapenemases, which are not inhibited by BLIs of first generation. ESBLs cause the resistance to penicillins, cephalosporins, and monobactams but no to carbapenems. For this reason, the prescription of carbapenems is enormously increased in the last years, driving the global spread of organisms producing carbapenemases [50-53]. This phenomenon has led to the development of two new synthetic classes of inhibitors [54], the diazabicyclooctane (DBO) series exemplified by Avibactam (**43**) (Figure 1.15) and the cyclic boronates exemplified by Vaborbactam (**48**) [55] (Figure 1.16).

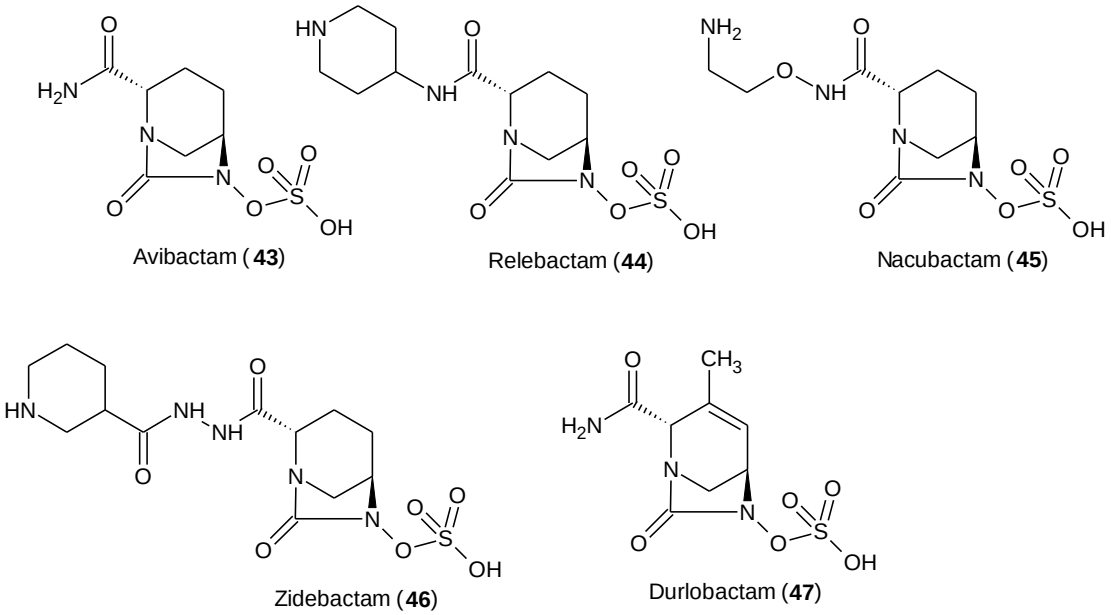
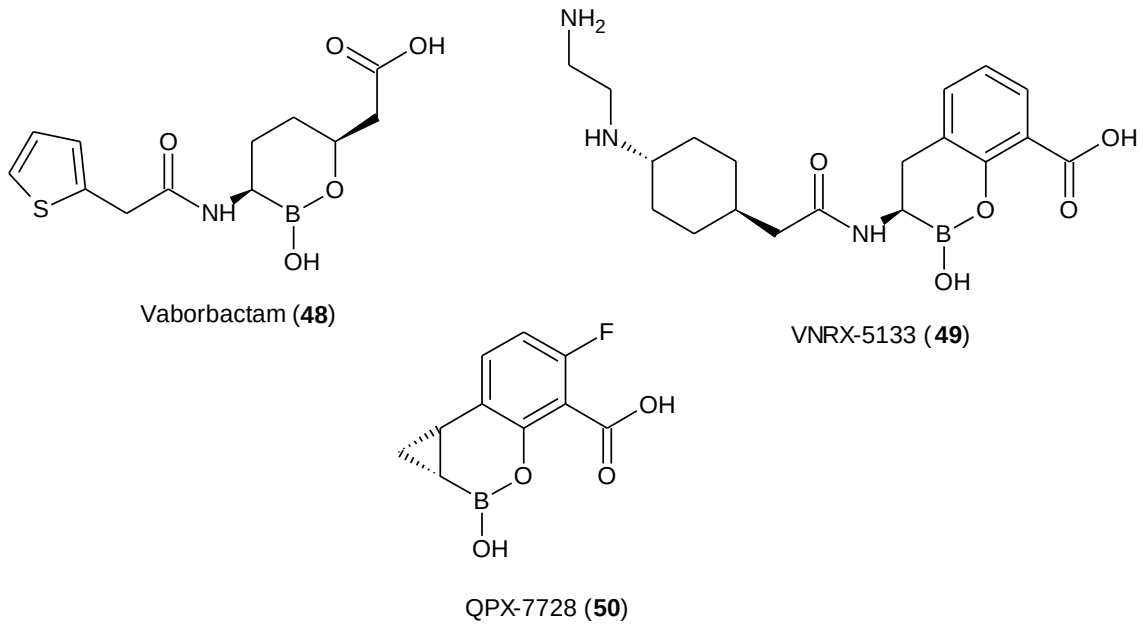


Figure 1.15. DBO generation of BLIs.



Vaborbactam (**48**)

VNRX-5133 (**49**)

QPX-7728 (**50**)

Figure 1.16. Cyclic boronates generation of BLIs.

Hereinafter, the novel combinations approved or appeared to the market in the last years:

1. Ceftolozane (**23**) with Tazobactam (**41**) approved by FDA in 2014 and sold under the brand name Zerbaxa™.
2. Ceftazidime (**18**) with Avibactam (**43**) approved by FDA in 2015 and sold under the brand name Avycaz™.
3. Meropenem (**6**) with Vaborbactam (**48**) approved by FDA in 2017 and sold under the brand name Vabomere™.
4. Imipenem (**33**) with Cilastatin and Relebactam (**44**) approved by FDA in 2019 and sold under the brand name Recarbrio™.
5. Cefepime (**17**) with Zidebactam (**46**) under clinical studies.
6. Meropenem (**6**) with Nacubactam (**45**) under clinical studies.
7. Sulbactam (**40**) with Durlobactam (**47**) under clinical studies.
8. Cefepime (**17**) with Enmetazobactam (**42**) under clinical studies.

9. Cefepime (**17**) with VNRX-5133 (**49**) under clinical studies.

10. QPX-7728 (**50**) has been recently discovered by QPEX Biopharma in 2019 and it is under clinical studies with different β-lactams antibiotics.

1.1.4 Continuous-flow processes

Advances in process chemistry are essential for the successful commercialization of innovative and efficient chemical–pharmaceutical manufacturing. Industrial and academic achievements in the last century would have been impossible without the development of cutting-edge technologies and interdisciplinary collaboration. The progress never stops and the modern trends in the synthesis of drugs and natural products are opening up opportunities on a scale previously considered unattainable in most laboratories and production lines. The use of high-throughput and breakthrough technology platforms, particularly flow chemistry and process analytics (PAT), is representative of the endless potential in the pharmaceutical field and the improvements over the current state that are possible (Figure 1.17) [56–68].

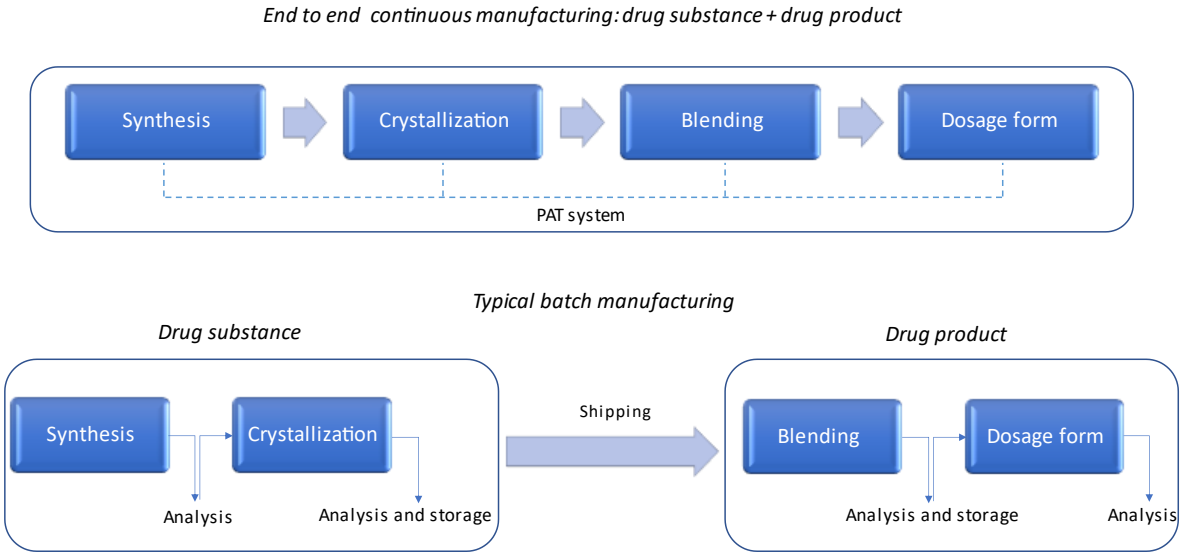


Figure 1.17. End-to-end continuous manufacturing compared to batch-manufacturing process.

In 2020, small-molecule drugs accounted for approximately USD 478 billion in sales in the global pharmaceutical markets, and this figure is expected to grow at 7% annually through to 2024 [69]. In the pharmaceutical landscape, the sustainability is driving industrialization and innovation of new processes, in accordance with environmental friendliness and green chemistry concepts [70–80]. In 2005, the American Chemical Society (ACS) Green Chemistry Institute (GCI) and the most important global pharmaceutical corporations set up the ACS GCI Pharmaceutical Roundtable. Their aim was to encourage the integration of green chemistry and green engineering into the synthesis of small molecules [81–83]. This concept has influenced all phases of drug development over the last twenty years, from preclinical to commercial stages, and has become a successful feature for new molecular entities (NMEs). At the same time, the opportunities presented by renewing old synthetic routes with new technologies have grown into a vast research field.

Of the many emerging technologies available, continuous manufacturing, which is also known as continuous processing or continuous-flow chemistry, has become the mainstream in the synthesis of APIs. Its impact on the life cycles of drugs has been so overwhelming that the FDA and European Medicines Agency (EMA) have recently drawn up guidelines for this manufacturing type [84,85]. Although publications on flow chemistry have exponentially grown in number over the last two decades, including assessments of pros and cons [61, 86-88], and despite its use being quite commonplace in many industries, the pharmaceutical world is recalcitrant to adopt it, and batch manufacturing remains king. The availability of standard reactors together with the simplicity, versatility and flexibility of their use means that “old habits die hard”. Although the positive impact of flow mode is now recognized, its application on an industrial scale is still seen as being the game changer that is too volatile to welcome. The industry’s hesitance to embrace continuous-flow processes is understandable precisely because the majority of publications derive from within the academic sphere, many processes are relatively untested and regulatory guidance is too young. In order to extend the scope of these technologies, companies must be sure of their suitability for specific

business needs, including an awareness of their operational advantages, as well as the challenges they pose [89–92].

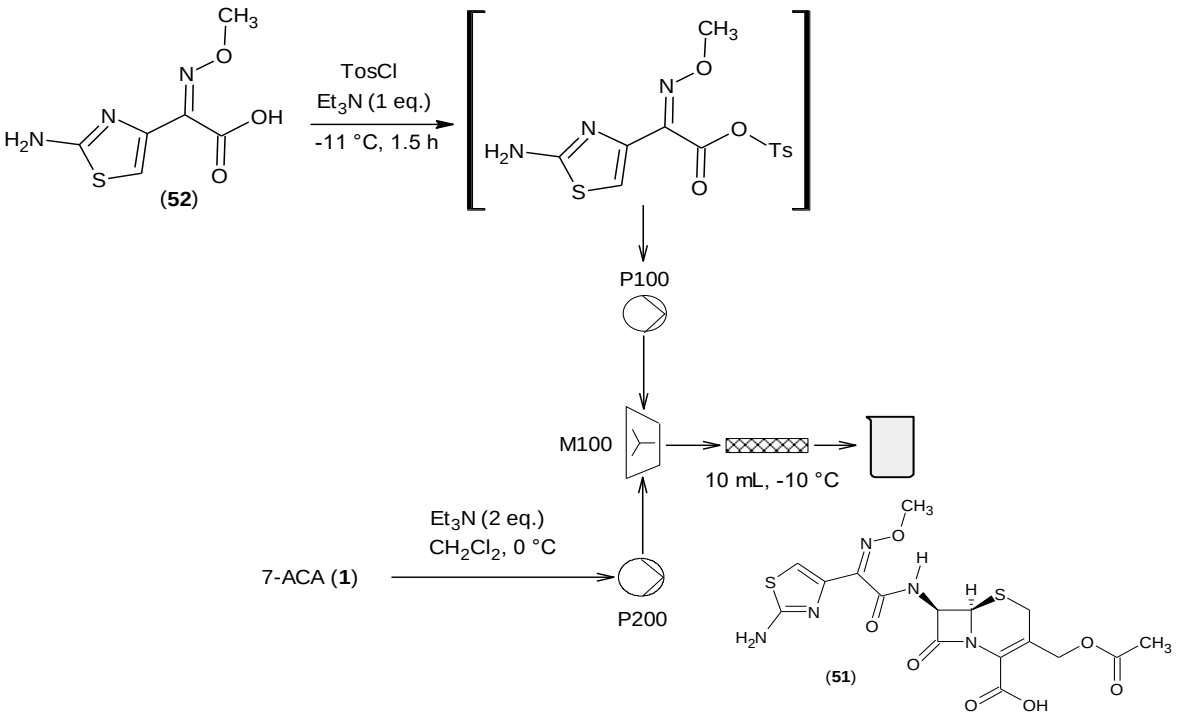
Chemical reactions in discontinuous processes occur in large vessels for a given time before the product is crystallized, discharged, analyzed and, eventually, purified. If a problem emerges during synthesis, or if the product does not comply with standard quality guidelines, production is compromised, causing undesirable losses in money and time. Continuous manufacturing runs constantly until a project is complete, slashing manufacturing times and avoiding breaks between the steps. Given that reactions take place on a much smaller scale, only small amounts of product are lost if the process fails. The automated nature of continuous processes minimizes fluctuations in reaction conditions (e.g., temperature, pressure, and reaction time) and reduces human error compared to batch manufacturing, saving assets. For the same reasons, chemists can easily control reactions in continuous flow (also combining photo- and electrochemistry), whereas this is a critical issue in batch mode because of the extreme conditions and the presence of highly reactive and unstable intermediates [93–103]. Miniaturization intrinsically improves synthesis due to the excellent mass and heat transfer that it provides, also meaning that less laboratory and industrial space is required. Modularization allows integrated synchronized operations to be performed, facilitating adaptability to different pharmaceutical processes. The closed architecture of these systems provides safer working conditions as it eliminates direct contact with hazardous chemicals and avoids production-chain incidents. Integration with process analytical technologies (PAT) and purification modules has boosted this technology's status, making the drug-production process telescopic, increasing production capability while retaining substance quality. Green chemistry concepts are met because the product does not need to be isolated and stored before use in a subsequent step, as it can directly flow into the subsequent reactor for another synthesis or into another module for another operation [104–111].

The reasons for favoring and adopting continuous manufacturing in the pharmaceutical world are several, including heavy investment to develop and implement the technology on industrial scale. Hereinafter, some examples of β-lactam antibiotics synthesis, BLIs and their intermediates in flow mode are reported. Of these, only two (key Vaborbactam intermediate (**53**) and MAP (**55**)) have become large-scale industrial processes.

1.1.4.1 Cefotaxime

Cefotaxime (**51**) is a third-generation cephalosporin, was first synthesized in 1976 and was commercialized by 1980 under the brand name Claforan™. It was approved by the FDA to treat gram-positive, gram-negative, and anaerobic bacteria. It appears on the World Health Organization's List of Essential Medicines and is available for intramuscular and intravenous administration. It is distributed in powder form in 500 mg, 1 g, 2 g, and 10 g vials or in a premixed solution for injection of 1 g and 2 g [112–116].

The processes to produce cephalosporins, including cefotaxime (**51**), were developed over thirty years ago and persist to this day. In the current context, which is characterized by strict concerns over worker safety and respect for environmental and energy savings, flow chemistry represents an attractive option for the synthesis of these drugs [117,118]. Pieper et al. have published an interesting study on the synthesis of this β-lactam antibiotic in flow mode and have made comparisons to batch mode [119]. The synthesis involved the amidation step between 7-ACA (**1**) and (Z)-(2-aminothiazol-4-yl)-methoxyimino acetic acid (**52**) under activation by 4-toluenesulfonyl chloride, as represented in Scheme 1.4.



Scheme 1.4. Cefotaxime (**51**) flow synthesis inspired by Ref. [119].

In this system, 7-ACA (**1**) was dissolved and stored in dichloromethane with triethylamine at 0 °C in a vessel. In another vessel, the mixed anhydride suspension was formed in dimethylacetamide (DMAc) at −11 °C. This vessel was constantly stirred to avoid sedimentation and was stored for a maximum of 1 h to respect the stable hold time. Peristaltic pumps (P100 and P200) transported the raw-material solution to the Y-shaped polypropylene mixer (M100) in flow towards the reactor. The flow reactor was a fluorinated ethylene propylene (FEP) tube of 10 mL with an inner diameter of 4 mm that was submerged in butyl glycol, used as a cooling material. The cooling batch was tempered with a cooling jacket through which butyl glycol was constantly pumped. The flow rate was set up at 5 mL/min to prevent sedimentation effects that may arise from the insoluble mixed anhydride. The output was collected in a vessel so that the product could be analyzed in solution. With this methodology, cefotaxime (**51**) was generated at a yield of 80.9% to 7-ACA (**1**), working at −10 °C and using a residence time of 1 min. Higher reaction temperature (+20 °C) led to higher 7-ACA (**1**) conversion, but lower product yield because of the degradation of the cephalosporin nucleus. Higher residence times decreased 7-ACA (**1**) conversion and, consequently, cefotaxime (**51**) yield. This methodology allowed much shorter reaction times (1 min) to be used, compared to the 30 min needed in the batch synthesis. Furthermore, a more convenient temperature (−10 °C), than the −30 °C used for batch mode, is possible, providing a further advantage in energy savings and costs. The space-time yield was nearly 400 times higher than when the reaction is performed in a reactor vessel and efficient heat distribution corroborated the technology's safety.

This result clearly shows the attractive features that the flow process possesses compared to the analogous batch route, although the yield was slightly lower. In order to boost productivity and overcome this shortcoming, the authors have proposed operating several identical systems in parallel [120], which would consolidate the great capabilities of flow chemistry.

1.1.4.2 Cephalexin

Cephalexin (**21**) is one of the most widely prescribed β-lactam antibiotics in the United States of America, with more than 7 million prescriptions being made in 2020 [121,122]. It is a first-generation cephalosporin discovered in 1967 and

marketed under the brand names Keflex™ and Ceporex™ since 1969. It is used against gram-positive and some gram-negative bacteria, particularly *E. coli, Proteus mirabilis and Klebsiella pneumoniae*. It is administered orally as either 250 mg or 500 mg capsules and it appears on the World Health Organization's List of Essential Medicines [116,123–126].

Vobeckà et al. have reported a continuous-flow process for the production of cephalexin (**21**), with excellent outcomes [127]. The product was synthesized from phenylglycine methyl ester (PGME) and 7-ADCA (**13**) using penicillin acylase (PA) as the enzyme in a kinetic regime (Figure 1.18). This regime was necessary to achieve a high cephalexin (**21**) yield in a water medium, as this is difficult to achieve in the thermodynamic process.

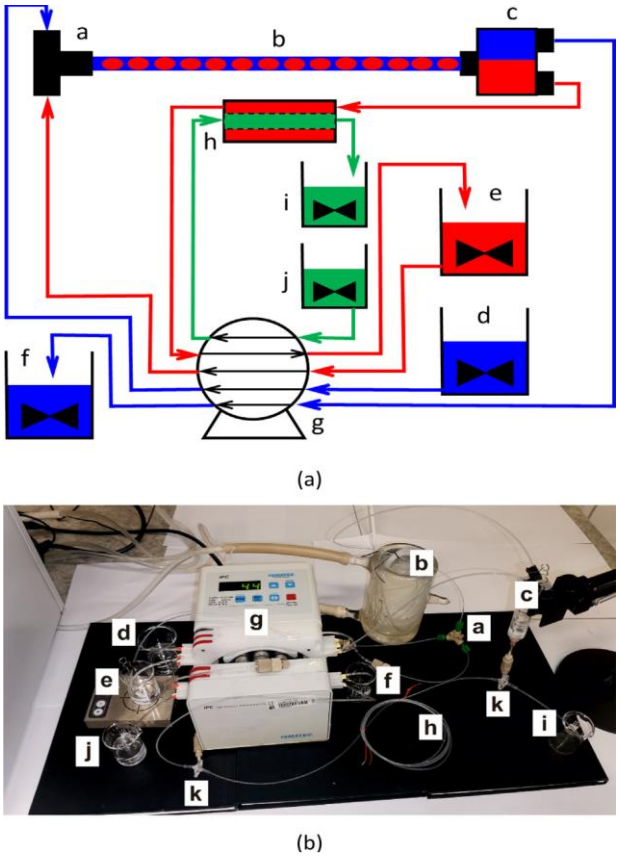
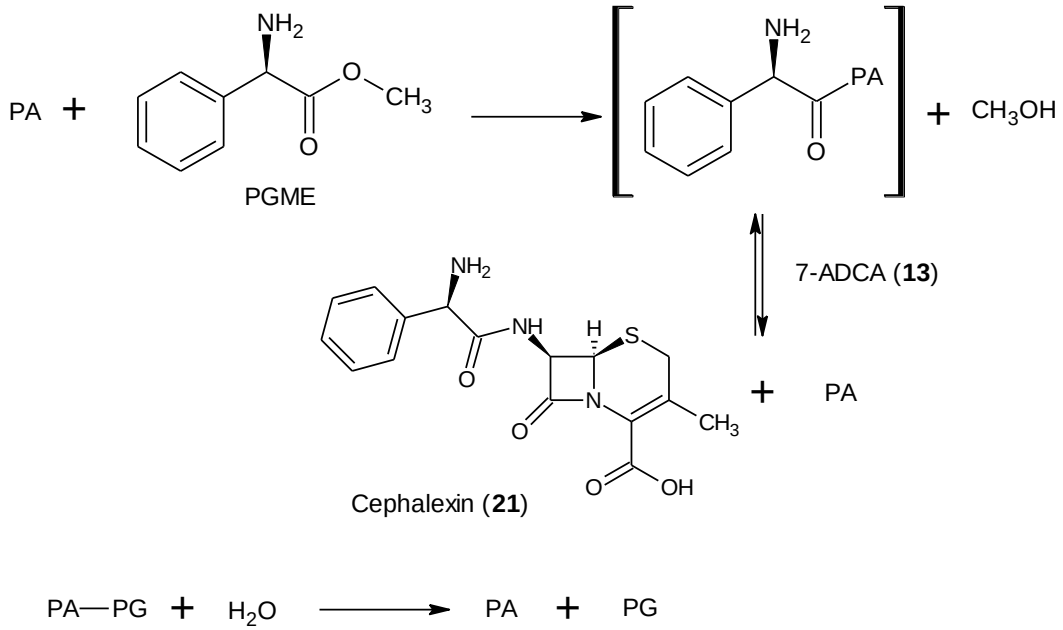


Figure 1.18. Scheme (**a**) and photo (**b**) of cephalexin (**21**) flow system with enzyme recycling. Reprinted from Ref. [127].

As depicted in Scheme 1.5, the authors employed apparatus that is characterized by an aqueous two-phase system (ATPS) that forms two phases in the flow reactor, which acts as a reaction-separation environment. This system, used in a microfluidic arrangement, guaranteed the in-situ extraction of cephalexin (**21**), as well as facilitating enzyme recycling, the addition of fresh reactants and the presence of a uniform reaction mixture. The ATPS consisted of 15 wt% of polyethylene glycol (PEG), with molecular weight ranging from 2000 g mol^{-1} to 4000 g mol^{-1}, 12 wt% of phosphates, to ensure pH 7.0, and 73 wt% of water. This composition granted cephalexin (**21**) high affinity to the top phase, which split from the PA-containing bottom phase. The reaction mixture contained PGME and 7-ADCA (**13**), in a molar ratio of 3:1, which dissolved in ATPS at concentrations of 150 mM and 50 mM, respectively. The free enzyme in water had a concentration of 10 µL for 1 mL of reaction mixture and an activity of 2.88 kU mL^{-1}. The reaction temperature was set to 30 °C by immersing the flow reactor in a water bath.



Scheme 1.5. Enzymatic cephalexin (**21**) synthesis reaction time.

Specifically, the reactants were dosed from the vessel (d) into the microcapillary reactor, (b), which has an inner diameter of 0.8 mm and a length of 87 cm, via a three-way PEEK connector (a). The PA was pumped from the recycle-

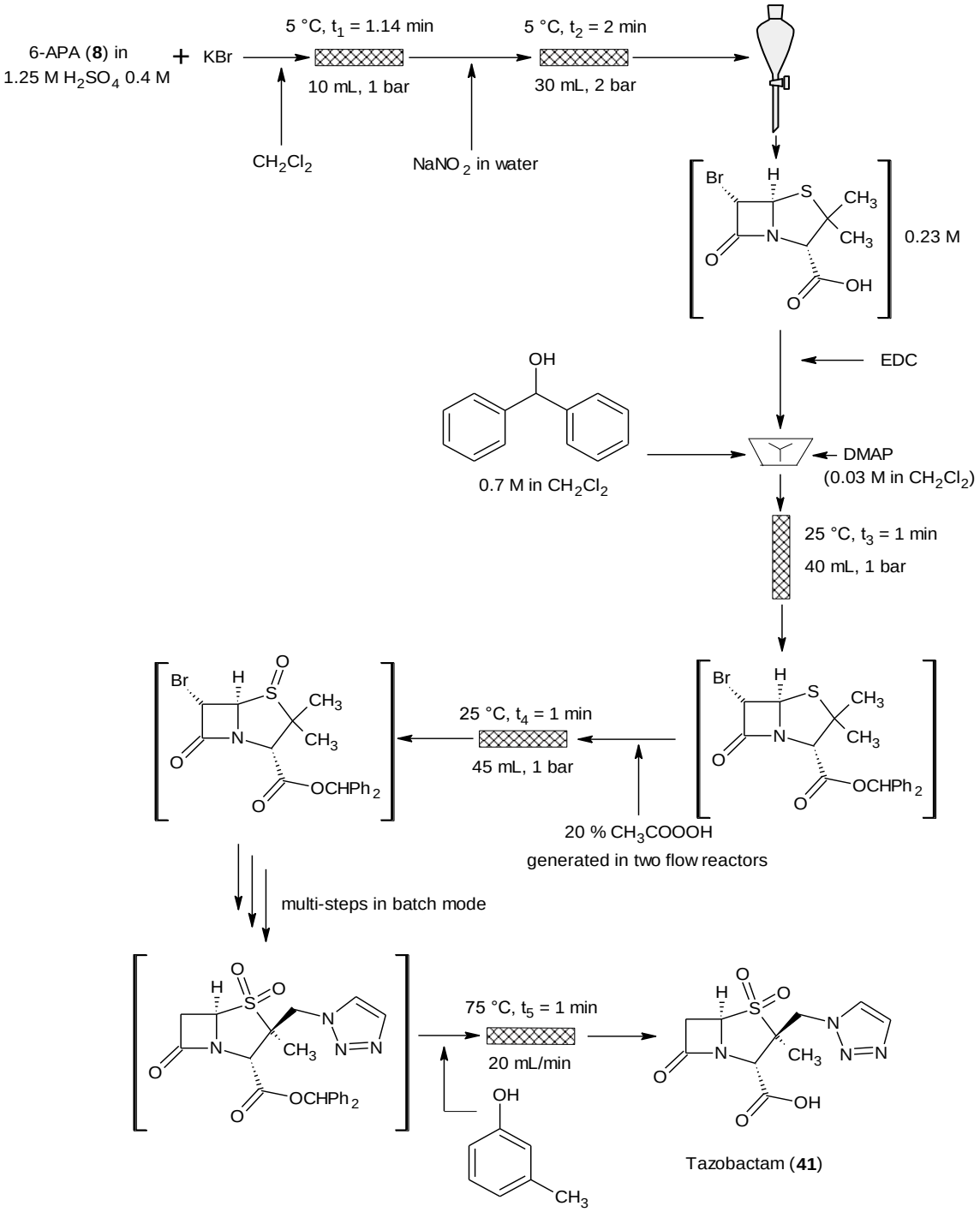
system vessel (e) into the reactor. The flow rate was set to the value that provided a residence time of 20 min. The phases were separated in a gravity settler (c) that was placed at the reactor outlet and was made of a 10 mL plastic syringe, without a vertically positioned piston and with the needle oriented downwards. Filter paper was placed at the bottom of this settler for the continuous removal of any precipitated phenyl glycine (PG). The top phase was withdrawn from the top part of the settler (c) by a peristaltic pump (g) and collected in a product vessel (f). The bottom phase was pumped from the settler (c) through the filter paper into the reservoir (e), with continuously stirred enzyme recycling. To avoid PG clogging in the reaction mixture, the authors added a microdialyzer (h), operating in counter-current flow, that was closed from both sides using ad hoc made Plexiglas ports (k). In this way, the enzyme solution was restored fresh to the reservoir (e). The dialysate solution was collected in a waste reservoir (i), while a fresh phase was pumped from the reservoir (j) into the intertubing of the microdialyzer to clean the enzyme solution.

Using this integrated microfluidic platform, the authors obtained a cephalexin (**21**) yield of 80%, with respect to 7-ADCA (**13**), whereas the yield in batch mode was 75% under the same conditions. In addition, this technology was able to operate continuously, generating new C-N bonds, for at least 5 h. The optimization of reaction conditions, performed first in batch mode and then in flow mode, was fundamental to the success of this approach. Enzyme recycling, considerable time and cost reductions and high productivity are the advantages of this protocol.

1.1.4.3 Tazobactam

Tazobactam (**41**) was first marketed in the USA in 1992. In recent years, it has been studied in combination with other β-lactam drugs due to its low toxicity and strong activity in fighting AMR. It appears on the World Health Organization's List of Essential Medicines [116].

Zhou and coworkers have reported an interesting tazobactam (**41**) synthesis that works as a combination of continuous-flow and batch conditions [128]. Flow chemistry was implemented in the first three steps and in the final step, giving a total yield of 37.1%, whereas the yield was 30.1% in batch mode (Scheme 1.6). This synthetic route was safer and more efficient, as it provided a 7% reduction in process mass intensity (PMI) while maintaining high purity (99.8%).



Scheme 1.6. Tazobactam (**41**) synthesis under combined flow and batch conditions.

The 6-monobromopenicillanic acid intermediate was obtained in a two-phase system using dichloromethane as the solvent, thus avoiding potassium bromide precipitation. The yield reached 88.2%, whereas it was 90% in batch mode. In order to ensure a high yield in the protection step with diphenylmethanol, the authors investigated the correct order in which to add the reagents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP). As reported in the Scheme, EDC was added to a rich solution of the 6-monobromopenicillanic acid intermediate, while other reagents were added to a T-shaped mixer. In this way, they obtained a yield of 92.4% of the 6-monobromopenicillanic acid intermediate in benzhydryl ester. The reaction with peroxyacetic acid to furnish the sulfoxide was carried out at room temperature, demonstrating the safety and energy savings of flow chemistry. The authors also implemented a flow process to produce, *in situ*, the peroxyacetic acid used in this system. Finally, the flow reactor was applied in the final step to deprotect the carboxylic group in the presence of m-cresol, which was used as solvent and scavenger, giving the aforementioned overall yield.

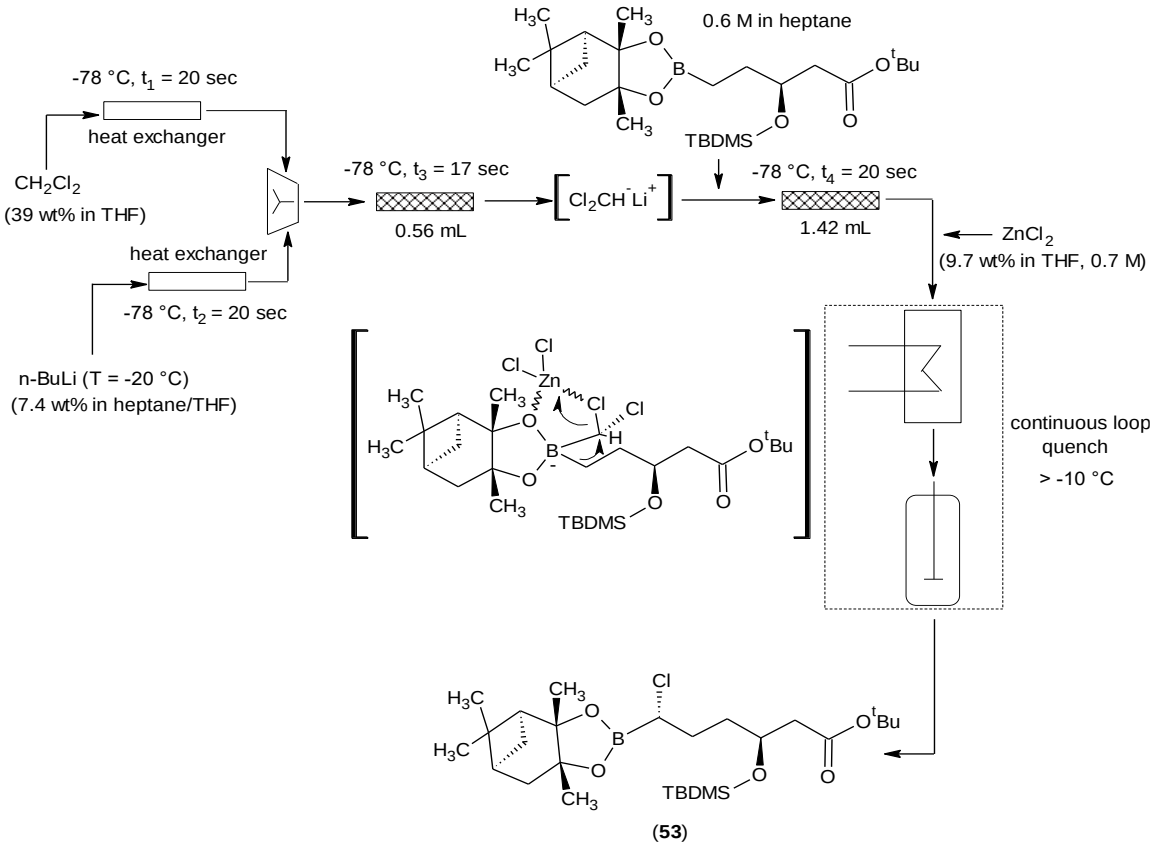
*1.1.4.4 Key Vaborbactam (**48**) intermediate*

As stated above, Vaborbactam (**48**) is part of the new generation of BLIs, classified as a non-β-lactam β-lactamase inhibitor. It is a cyclic boronic acid combined with meropenem (**6**). It acts against serine carbapenemase enzymes, including *Klebsiella pneumoniae carbapenemase* (KPC), boosting carbapenem action. This drug is administered via intravenous injection into a vein and appears on the World Health Organization's List of Essential Medicines [55,116,129–133].

Stueckler et al. have presented a flow approach for the synthesis of the key intermediate (**53**) of this inhibitor [134]. They moved the Matteson reaction from batch mode to flow mode while improving diastereoselectivity, purity, reproducibility, yield, and productivity for the key intermediate (**53**). This flow system is currently applied in the industrial-scale production of the intermediate under cGMP conditions, with several hundred kilograms being manufactured, and has been approved by FDA inspection.

In batch mode, the need to cool the process to −95 °C, the need to remove reaction heat, the high dilution and slow dosing protocols were impediments to commercial production. Moreover, byproduct formation, due to poor mixing,

limited productivity. The authors overcame these issues using the patented flow technology depicted in Scheme 1.7 [135].



Scheme 1.7. Flow process for the Matteson reaction to manufacture key Vaborbactam intermediate (**53**).

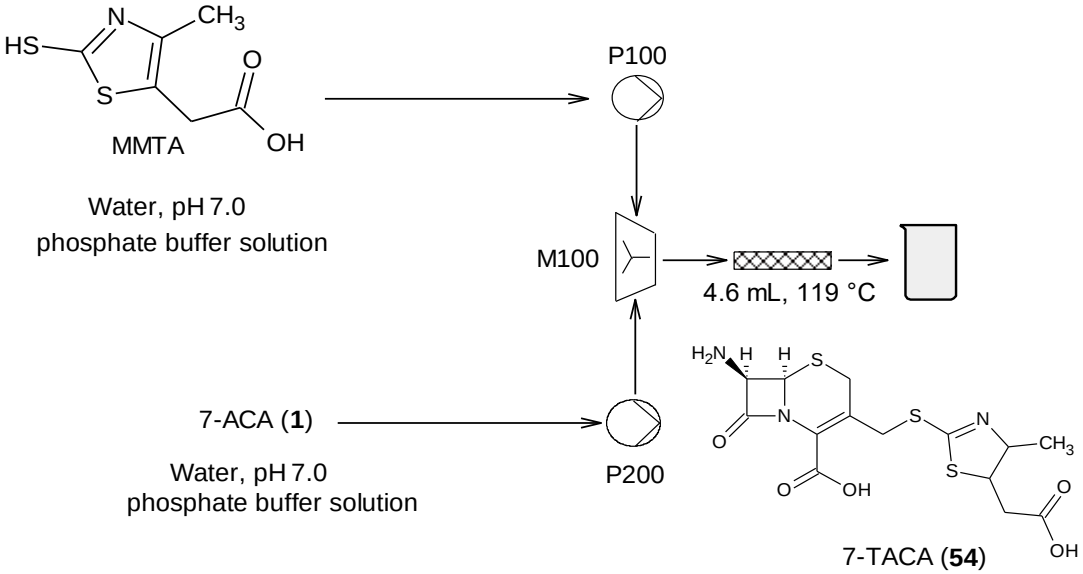
In the first heat exchanger, dichloromethane was introduced with THF and cooled to −78 °C. Similarly, n-BuLi in heptane was mixed with THF and cooled to −78 °C. The use of THF as a cosolvent was necessary to prevent the precipitation of n-BuLi at low temperature. The (dichloromethane)lithium that was formed in the first flow reactor reacted with the substrate in heptane and zinc chloride, used as a Lewis acid, to obtain the intermediate. The two Matteson homologation steps are borate-complex formation and rearrangement with the concomitant stereoselective displacement of one chlorine. The customized continuous loop quench application allowed the process to be operated at a higher temperature (T ≥ −10 °C) than in the previous conformation (T < −20 °C), facilitating industrial

production with improved economics and reduced ecological footprint. With this technology, Thermo Fisher Scientific can synthesize this intermediate (**53**) at a yield of more than 97%, a chemical purity of more than 98% area by HPLC and a diastereomer ratio of above 95:5.

1.1.4.5 Key Cefodizime intermediate

Cefodizime is a third-generation cephalosporin with broad-spectrum activity against aerobic gram-positive and gram-negative bacteria. It is administered intravenously and intramuscularly. A single dose contains 1 g or 2 g of the drug, which is used for an average of 7-to-10 days. It is not currently approved by the FDA for use in the USA [136,137].

Wirth et al. have published an interesting manuscript for the continuous synthesis of thiazolyl-7-aminocephalosporanic acid (7-TACA, **54**) [138]; the key cefodizime intermediate. This involves a 3'-modification using methylmercaptothiazolyl acid (MMTA) on 7-ACA (**1**), the antibiotic's backbone, as depicted in Scheme 1.8.



Scheme 1.8. Flow synthesis of 7-TACA (**54**).

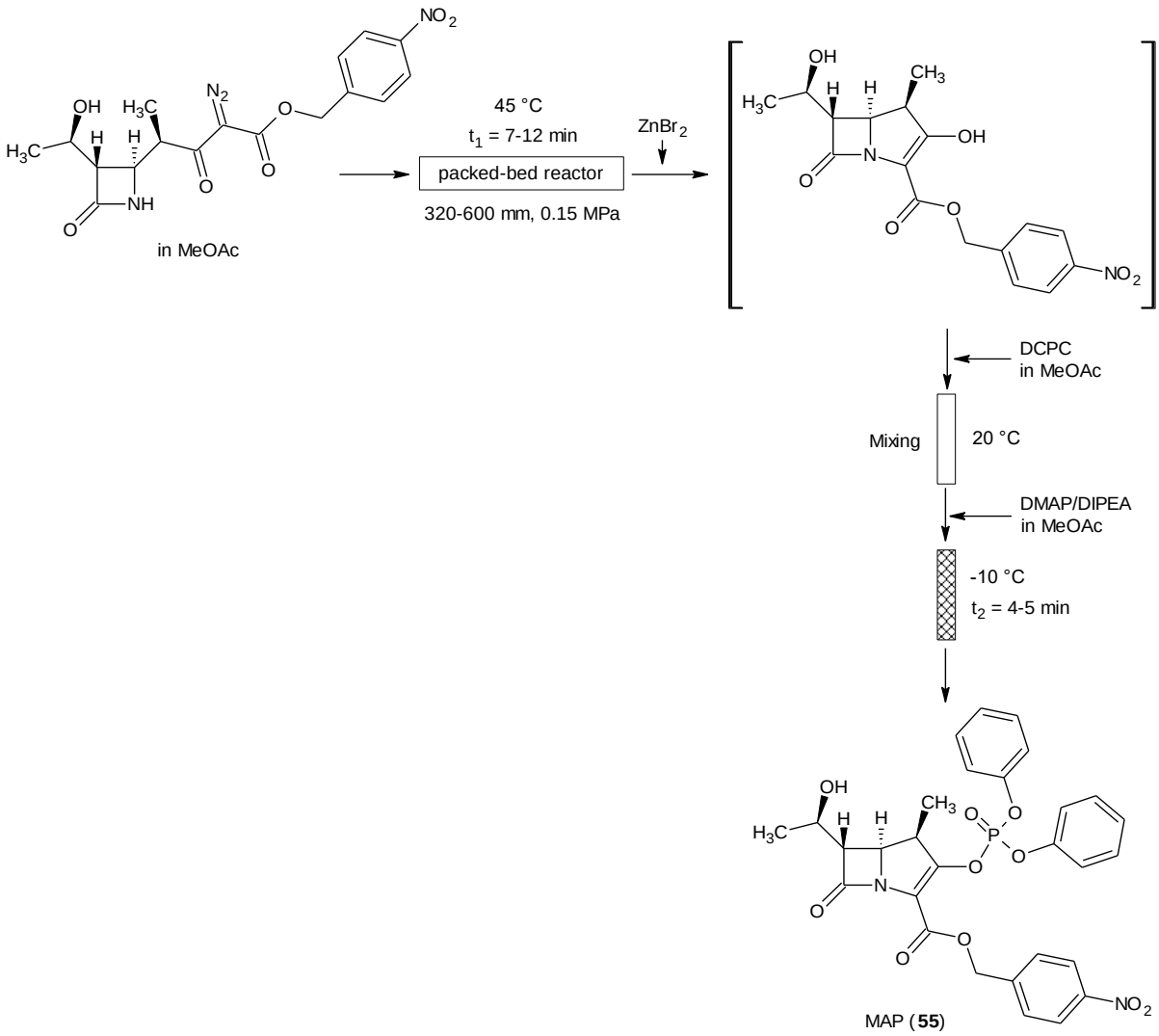
In this system, the reagents were dissolved in phosphate buffer solution at pH 7.0 and room temperature. The 7-ACA (**1**) concentration was 100 mmol/L, while 1.0

equivalent of MMTA was used. The peristaltic pumps (P100 and P200) transported the raw-material solution to the Y-shaped mixer (M100) in flow towards the reactor. The flow reactor had an inner diameter of 3.1 mm and was submerged in an oil bath, which could be heated to 180 °C. The output was collected in a vessel in order to analyze the product in solution. The common batch method runs at 60 °C for one hour. These reaction conditions led to a colored compound. Chromatographic treatment is therefore essential to achieving the requested specifications. By taking advantage of the high heat-transfer capacity and small dimensions of the system, the authors worked at higher reaction temperatures and very short times. This concept is fundamental to the application of flow chemistry in cephalosporin synthesis because of the instability of the cephalosporin nucleus and its degradation at high temperature. The authors studied the parameters using design of experiments (DOE) and obtained a yield of 85% and a selectivity of 85.3% for 7-TACA (**54**), working at 119 °C with a residence time of 3.99 min at pH 7.0–7.5. In the continuous process, the thermal stress was lower, and the results were comparable to batch production. The space-time yield increased by a factor of 130.

1.1.4.6 β-Methyl vinyl phosphate (MAP)

β-Methyl vinyl phosphate (MAP, **55**) is an advanced intermediate for the β-methyl carbapenem class, which includes meropenem (**6**), ertapenem (**36**), doripenem (**37**) and tebipenem (**38**). In the batch process, the rhodium-catalyzed insertion of a carbene generates the carbapenem fused-ring system. Subsequent reaction with diphenyl chlorophosphate (DPCP) in the presence of a base (*N,N*-diisopropylethylamine, DIPEA) and catalytic DMAP affords MAP (**55**) in 85–87% yields after crystallization. The drawbacks to commercial production include the difficulty in recovering the valuable rhodium and solvents. As the first step is performed with $Rh_2(Oct)_4$ in a homogeneous environment, recovery is typically 70%, meaning that organic-solvent incineration is required.

To overcome the shortcomings, Gage et al. have published a flow process for carbenoid N-H insertion and phosphorylation that is capable of producing 100 kg of MAP (**55**) per batch in cGMP manufacturing (Scheme 1.9) [139].



Scheme 1.9. Flow synthesis of MAP (**55**).

They initially studied several methods to immobilize the Rh(II) complex on organic polymers for use in a packed-bed reactor. A polymeric support generated from p-vinylbenzyl alcohol and sebacic acid was chosen for its excellent swelling characteristics and good mechanical stability. This support facilitated higher metal recovery (>90% versus ≈70% for batch mode) and complete solvent recovery. In addition, it was possible to use the catalyst six times without any appreciable activity loss, with Rh leaching dropping to 0.99% per run. Given that Rh-catalyzed cyclization is a gas–liquid–solid three-phase reaction, as nitrogen gas is released,

the authors investigated all the parameters in order to achieve good quality and yields. They obtained a yield of 97% for the cyclization intermediate, working at residence times in the 7-12 min range and at 45 °C. They also determined that a reactor length of 320–600 mm was capable of working at 0.15 MPa. $ZnBr_2$ was employed as the stabilizer at the onset of cyclization. Even the phosphorylation was studied, and an in-solution yield of 95% for MAP (**55**) was achieved with the cyclization substrate, DPCP, DMAP and DIPEA all well-mixed in the process, and with the same temperature as the batch process being used. This work culminated in three validation batches that produced an isolated yield of 80%, which was slightly lower than the optimized batch yield.

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Chapter 2: Cefonicid Benzathine Salt: A High-Performance Protocol to Make an Old Cephalosporin Shine

Abstract

Cefonicid is a second-generation cephalosporin sold under the brand name Sintocef™. It is an injectable drug obtained via a freeze-drying process and is also available for oral preparations. The high-quality standard required is very challenging to satisfy, and current production protocols are characterized by steps that are lengthy and cumbersome, making the product unattractive for the international market. Industrial R&D is constantly working on the process optimization for API synthesis, with the aim of increasing productivity and decreasing production costs and waste. In this thesis, we report a new and efficient method for the synthesis of the cefonicid benzathine salt (**4**) that provides a good yield and high product stability. In the first part, the synthesis of the 7-amino-3-[sulphomethyl-1-H-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt, key synthetic intermediate, has been studied. We have investigated an efficient and practical procedure for the preparation of this molecule that features a telescopic route whose synthetic steps are all performed at room temperature; from the displacement of the acetoxy group with boron trifluoride to crystallization without treatment with charcoal. A simpler, scalable, cost-effective and energy-saving protocol is herein reported as a means of moving towards commercial manufacturing. The optimization of the process parameters and the industrial scale impact assessment have paved the way for industrialization. In the second part, the double-nucleophilic and lipophilic nature of *N*',*N*''-dibenzylethylene diamine diacetate (**3**) enables the deformylation of the OH-protected group on the mandelic moiety and also enables product crystallization to occur. We demonstrated that the formyl group in the peculiar position has high reactivity, promoting an amidation reaction that deprotects a hydroxy group and generates a new C-N bond in the reaction by-product. Several amines and OH-protected groups have been studied, but none were able to replicate the excellent results of benzathine diacetate (**3**).

2.1 Introduction

The discovery and development of antibiotics, combined with their therapeutic use against many bacterial illnesses, can be considered one of

humankind's great breakthroughs, so much so that it was cited, in 2013, as one of nine ways that chemistry has changed the world [1], underlining the utmost importance of these drugs.

Although the world has observed a drastic fall in the approval of new antibacterials since the 1990s [2], the market has rewarded this therapeutic class, placing it in fifth place in sales rankings in 2018, with USD 40.6 billion being sold, putting it on a par with vaccines [3]. Of the various antibiotic types available, β-lactams are the largest class in terms of production volume and market size, with USD 27.1 billion being sold in 2018 and a growth perspective of USD 34.2 billion by 2028 [4].

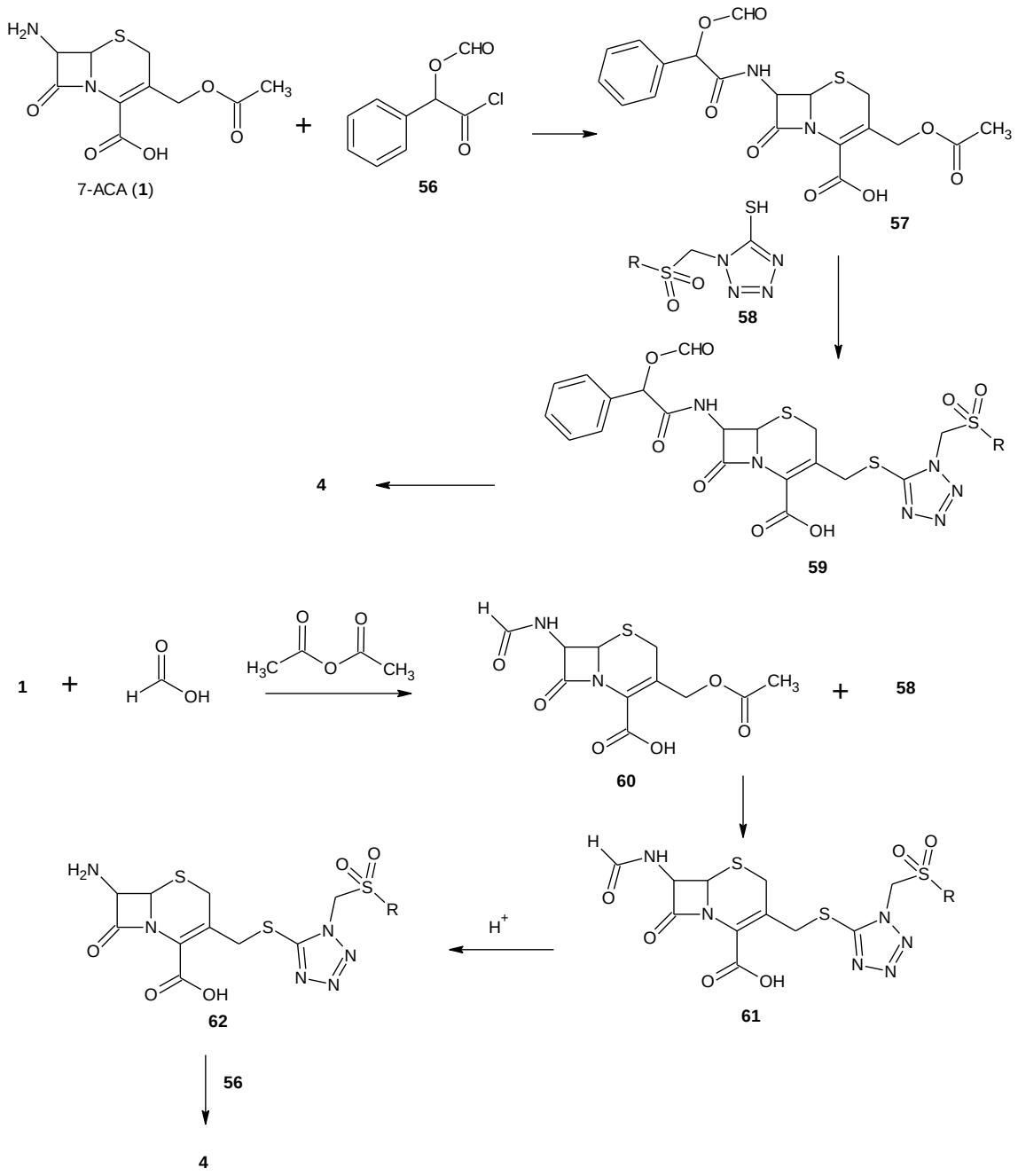
While penicillins and cephalosporins have been and continue to be the main β-lactam scaffolds, patients can fight infections caused by bacteria with other β-lactam subgroups, such as carbapenems, penems, monobactams, oxacephems, and carbacephems, that were discovered during the "golden age" of antibiotics in the 1960s, 1970s, and 1980s [5].

Over the last twenty years, except for the approval of fourth- and fifth-generation cephalosporins, ceftaroline fosamil (**25**) [6], ceftolozane (**23**) [7], ceftobiprole (**20**) [8], and cefiderocol (**24**) [9], the trend in pharmaceutical industry R&D has been to dredge up older cephalosporins and carbapenems and combine them with β-lactamase inhibitors to create more powerful medical weapons. Meropenem (**6**) /vaborbactam (**48**), sold under the brand name Vabomere™ [10], imipenem (**33**)/cilastatin/relebactam (**44**) (Recarbrio™) [11], and ceftazidime (**18**) with avibactam (**43**) (Avycaz™) [12] constitute the recent FDA approvals in the antibacterial field.

In this landscape, where new molecular entities (NMEs) are dwindling, the possibility of a cephalosporin renaissance is realistic. In this work, we have revisited the synthetic pathway of cefonicid, one of the first cephalosporins discovered, with the aim of making it appealing to new pharmaceutical research opportunities. Cefonicid is a common, second-generation cephalosporin that was patented in 1978 [13] by Glaxo Wellcome (now GlaxoSmithkline). It is parenterally administered and used for urinary tract infections, lower respiratory tract infections, and soft tissue and bone infections [14]. Although its long half-life (4.6 h) and cost-effective, once-daily dosage regimen are the major pharmacokinetic advantages found in this

generation of cephalosporins [15,16], its marketplace is currently restricted to low- and middle-income countries (LMICs), and its appeal has fallen even lower.

Chemically, the general concept behind producing these semi-synthetic molecules involves derivatization at the 3- and 7- positions of 7-aminocephalosporanic acid (7-ACA, **1**), which is the backbone of almost all cephalosporin-based antibiotics and is obtained via a modern and environmentally friendly biocatalytic process from cephalosporin C [5,17,18]. In the specific case of cefonicid, the presence of the sulfonic acid moiety on the 1-Me tetrazole ring dramatically enhances solubility in water. Industrially, this high affinity for water explains why freeze-drying is a sterile process for the injectable form, and consequently, why even cumbersome and lengthy synthetic steps, followed by purification with chromatography or ion-exchange resins, characterized the first synthetic protocols. Obtaining stable and high-purity grades of an oral drug before the lyophilization step has been the main goal of all synthetic pathways developed over the years, while at the same time, being the biggest challenge to face for process industrialization. Synthetic approaches to the target cefonicid benzathine salt (**4**), oral form of cefoncid, can be divided in two classes: those involving amidation at the 7-position, followed by the displacement of the 3’-acetoxy group of 7-ACA (**1**) by the incoming S-nucleophile; and those where the sequence is reversed (first 3’-nucleophilic substitution, then 7-amidation). Accordingly, the first path entails acylation at the 7-amino with a properly O-protected mandeloyl chloride (**56**), the subsequent displacement of the 3’-acetoxy group with sulphomethyl tetrazole thiol (**58**), and, lastly, the removal of the O-protective group to give the desired pharmaceutical molecule (**4**). In the second approach, the 7-formamidocephalosporanic acid (**60**) is prepared via the reaction of 7-aminocephalosporanic acid (7-ACA, **1**) with formic acid and acetic anhydride, followed by 3'-derivatization with a substituted tetrazole thiol (**58**) and then by deformylation of the formyl group with an acid to obtain the 3-substituted-thiometyl-7-aminocephalosporin intermediate (**62**), which is the intermediate under investigation. The subsequent deprotection of the *O*-protected mandeloyl moiety in the 7-position gives the active pharmaceutical ingredient (**4**) [19-21] (Scheme 2.1).



Scheme 2.1. Two synthetic pathways for compound (**4**).

The second has been chosen as the most straightforward of the two strategies in this work. In this context, almost all of the synthetic efforts *en route* to cefonicid

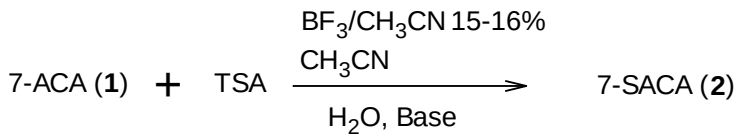
benzathine salt (**4**) have employed the pivotal intermediate 7-amino-3-[sulphomethyl-1-H-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt (7-SACA, **2**), which has led to the need for high-yielding as well as economically and environmentally sound processes. The earliest report of a 3'-OAc displacement from a cephalosporin by a S-nucleophile (i.e., thiosulfate) is probably the proposed formation of the Bunte salt in 1963 [22]. Since then, unquestionably due to the high polarizability of the sulfur atom, a plethora of S-nucleophiles (e.g., aliphatic and het (aromatic) thiols) have been observed to react smoothly, at least in aqueous medium, to give the corresponding 3'-thio substituted compounds in moderate and acceptable yields [23]. Hatfield et al., have studied the chemistry of the nucleophilic displacement of the acetoxy group as promoted by a variety of nitrogen and sulfur compounds in aqueous solution at near neutral pH, and in organic solvents with an acid as catalyst. This demonstrated that conditions in water lead to higher degradation of the cephalosporanic nucleus than non-aqueous solvents and directed academic and industrial research towards replacing the acetoxy group in the presence of strong Lewis acids [24].

The procedures of that age therefore suffered from shortcomings that ranged from long reaction times, non-ambient temperatures, strictly controlled pH-conditions, the formation of side-products arising from the hydrolysis of β-lactam, double-bond isomerization to 2-cephems and unwanted lactone formation. The age of these procedures, the intricate and cumbersome synthetic chemical steps and the purification procedures that require ionic exchange resins have been the limits to the industrialization of these types of cephalosporins using this chemistry.

The seminal paper by Saikawa et al., on the successful application of protic and Lewis-acid catalysis in non-aqueous solvents [25-27] at 30 °C, thereby avoiding undesirable by-products, was a significant breakthrough in the synthesis of 3'-modified cephems. In the first part, the authors studied a number of acids to investigate the substitution reaction between 1-methyl tetrazole thiol and 7-ACA (**1**), and it was shown that BF_3, with acetonitrile as a solvent, demonstrated the best performance in terms of yield and quality under mild conditions. In the second part, various thiols were utilized to study the previously developed reaction conditions. Moving this study to the present day, the industrial applicability of the other acids studied in the paper ($BF_3{\cdot}(C_2H_5)_2O$, $BF_3{\cdot}(C_4H_9)_2O$, $SnCl_4$, $ZnCl_2$, F_3CSO_3H, etc.) would have a severe environmental impact, which API producers are trying to avoid, and

for this reason, boron trifluoride provides the perfect blend of technical advantages and environmental-friendly features.

More recently, researchers at Farmabios [28] have shown that the BF_3-catalyzed displacement of 3'acetoxy in 7-ACA (**1**) by TSA worked well on a lab-scale. However, it had not been optimized for the production of commercial quantities of 7-SACA (**2**). Compared to the old synthetic routes, the use of a boron trifluoride complex in acetonitrile allows the 7-aminocephalosporanic acid (**1**) to be directly converted into 7-SACA (**2**) without protection on the 7-position on the amine, providing a more efficient synthesis and preserving the stability of the β-lactam ring, as indicated in Scheme 2.2.



Scheme 2.2. Boron trifluoride complex pathway to obtain (**2**).

Continuing in the cefonicid synthesis and in pursuit of the challenging goal of improving the total synthesis, we focused our attention on its final step. We retrieved an old, synthetic pathway and designed and developed a relevant improvement on the amidation of the 7-position and OH protection, ensuring a more profitable production.

The greatest challenge facing process chemistry is that of producing the required molecules in a controlled fashion, with reproducible impurity profiles, in an economic and scalable way. The rigorous inspection of batch impurity profiles over the last few years discourages any changes or adjustments in validated processes. However, the fact that the critical E-factors (environmental-factor) of the pharma industry are estimated to be in the 25 to 100 range (kg of waste per kg of drug molecule), the pharmaceutical–chemical community has recognized the urgent need for more sustainable manufacturing via the design of cost-effective and greener synthetic routes [29-31].

This situation has encouraged us to develop a new, synthetic path for the abovementioned cephalosporin, starting from the restrictions imposed by its chemical behavior and turning them into the driving force for a sustainable route

to highly efficient production. Considering the old chemistry of cefonicid preparation, the conversion to modern, sustainable processes is a challenging task. We believe that this work may be pioneering in the reevaluation of cefonicid benzathine salt (**4**) as part of the booming, β-lactam antibiotics panorama, therefore increasing its attractiveness for use in new clinicals studies in combination with β-lactamase inhibitors to fight the infinitive war against bacterial resistance.

2.2 Materials and Methods

2.2.1 Chemistry

7-ACA (**1**), TSA (Na, K) salt, (*R*)-mandelic acid (**63**), *N*',*N*''-dibenzylethane-1,2-diamine diacetate (**3**), *O*-acetyl-(*R*)-mandeloyl chloride, (*R*)-2-chloro-2-oxo-1-phenylethyl pivalate, *O*-formyl-(*R*)-mandeloyl chloride, and solvents were kindly provided by ACS Dobfar. All other reagents were purchased from Merck KGaA (Darmstadt, Germany).

The Agilent 1200 Series HPLC system, NMR (Bruker 400, Munich, Germany Munich, Germany) and Karl Fischer titration were the analytical instruments used to identify and analyze the products. Boric acid and its salts were analyzed with an ion chromatography system.

2.2.1.1 General Procedure for the synthesis of 7-SACA (2)

A solution of BF_3 in MeCN (15.2–16.8% BF_3 basis) (140 mL, 122.4 g, 288.8 mmol BF3) was added dropwise over 40 min to a stirred mixture of 1-sulphomethyl-5-mercapto-1,2,3,4-tetrazole sodium-potassium salt (7.7 g, 30.0 mmol) and 7-ACA (8.0 g, 29.3 mmol, **1**) in MeCN (60 mL), while the internal temperature was maintained at 25–28 °C. After 40 min under stirring, the reaction mixture was poured into the water (72 mL) at 25 °C, yielding a precipitate (Na, K borates). After filtration, the wet cake was washed with a (1:1) MeCN-water mixture (10 mL). The filtrates were pooled, and the precipitate was discarded. The limpid filtrate was brought to pH 3.0 ± 0.1 with 15% NaOH (45 mL) at 25 °C, and a precipitate became visible. This was aged for 2 h, filtered, and washed with acetone (32 mL) to make

the title compound (**2**) (8.6 g, 68% yield) an off-white solid. The HPLC assay was 101.0% (as sodium on the anhydrous basis).

^{1}H NMR (400 MHz, DMSO-d_6): 3.50 (1H, d, ^{2}J = 17.6 Hz), 3.74 (1H, d, ^{2}J = 17.6 Hz), 4.08 (1H, d, ^{2}J = 13.6 Hz), 4.42 (1H, d, ^{2}J = 13.6 Hz), 5.20 (1H, d, ^{3}J = 5.2 Hz), 5.40 (1H, d, ^{3}J = 5.2 Hz), 5.87 (2H, s), (Figure 2.1).

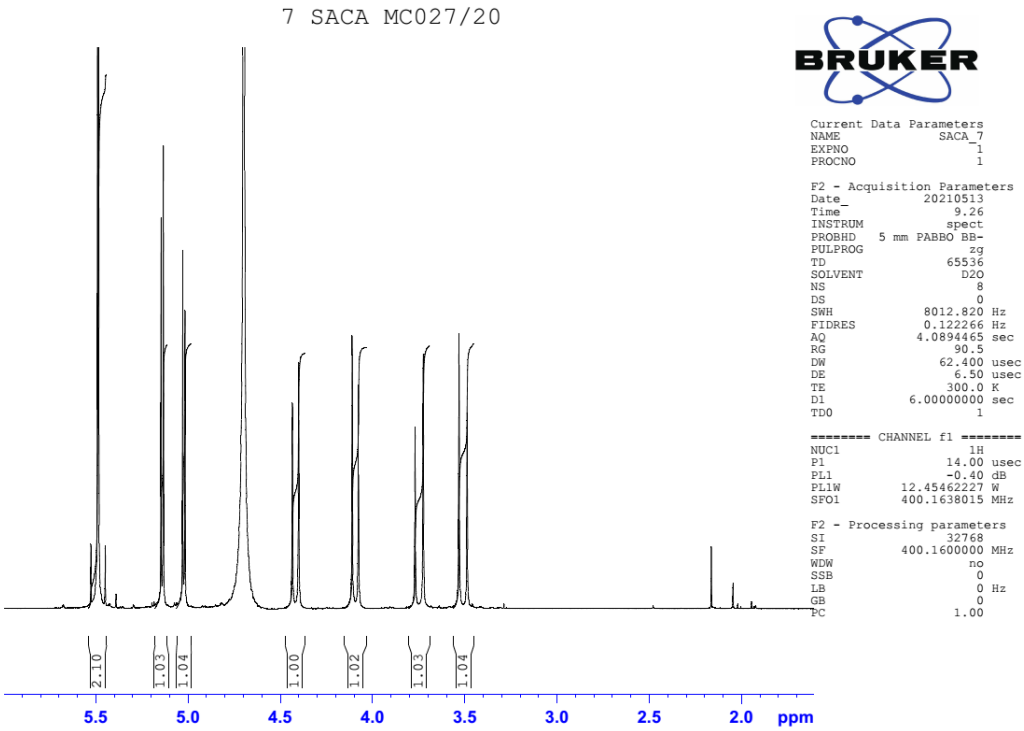


Figure 2.1. ^{1}H-NMR of 7-SACA (**2**).

^{13}C NMR (100 MHz, DMSO-d_6): 26.25 (CH_2), 36.0 (CH_2), 53.3 (CH), 57.6 (CH), 60.4 (CH_2), 119.8 (C), 130.0 (C), 154.9 (C), 159.6 (C), 161.8 (C), (Figure 2.2).

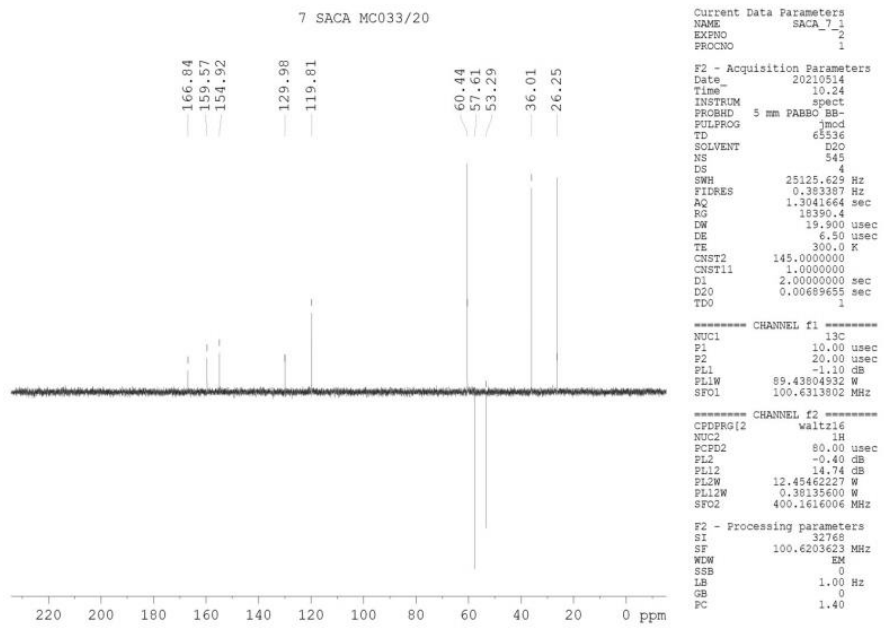
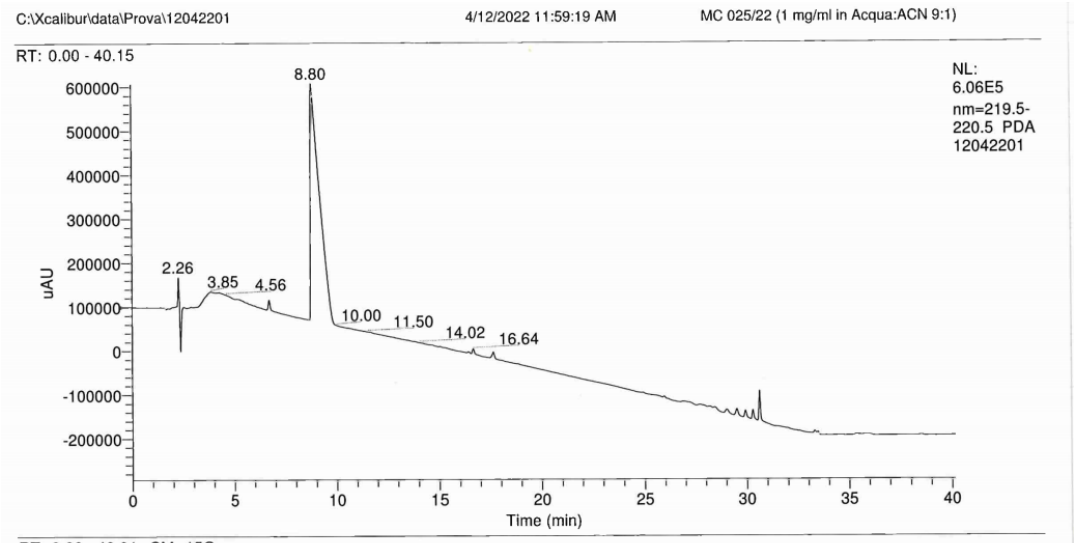


Figure 2.2. ^{13}C-NMR of 7-SACA (**2**).

2.2.1.2 General Procedure for the synthesis of O-Formyl-(R)-Mandelic Acid

A mixture of (*R*)-mandelic acid (5.0 g, 32.9 mmol, **63**) and 99% formic acid (80 mL, 2.12 mol) was heated at 80–90 °C for 12 h and concentrated to give a residue. Toluene (100 mL) was added and evaporated under reduced pressure to remove formic acid as a binary azeotrope (86 °C at 101 KPa). The residual thick oil was dissolved in toluene (200 mL) and the solution was washed with water (2 × 50 mL). The organic layer was separated, dried over sodium sulphate (0.5 g), and evaporated under reduced pressure. The residual oil was taken up in cyclohexane (100 mL) to make the title compound a colorless solid (3.40 g, 57% yield), (Figure 2.3). The crude ester was used directly for the next step without further purification.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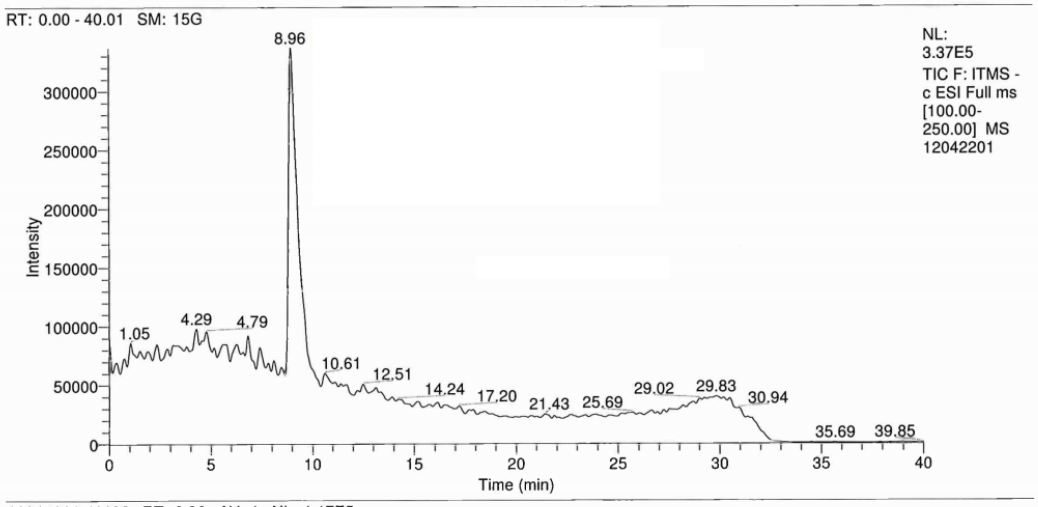
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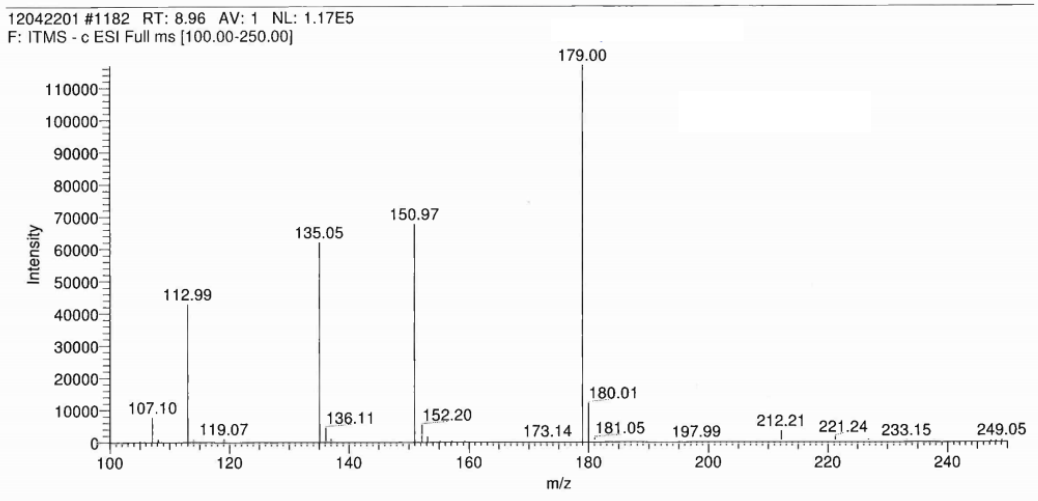
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Figure 2.3. *O*-Formyl-(*R*)-mandelic acid LC-MS analysis.

2.2.1.3 General Procedure for the synthesis of cefonicid benzathine salt (4)

A 30% (w/w) NaOH aqueous solution (24 mL) was added dropwise to a stirred suspension of 7-SACA (100.0 g, 0.23 mol, **2**) in pre-cooled (0–5 °C) water (375 mL) until complete dissolution was achieved at pH 6.9–7.1. $NaHCO_3$ (22.4 g, 0.26 mol) and *O*-formyl-(*R*)-mandeloyl chloride (52.0 g, 0.26 mol) were subsequently added to the reaction mixture, which was set aside at 0–5 °C for 45 min. The pH was adjusted to 5.8–6.1 via the addition of $NaHCO_3$ (4.5 g, 0.053 mol) and alumina (2.5 g). After 20 min, the alumina was removed by filtration and washed with water (50 mL), and the filtrate was treated with norite charcoal (5 g) for 20 min at 20–25 °C. The charcoal was removed by filtration and the filter cake was washed with water (50 mL). Methanol (500 mL) was added, at 25–28 °C, to the combined filtrate, followed by 5% HCl (5 mL) to reach pH 5.1–5.2. *N*′,*N*″-dibenzylethylene diamine diacetate (200.0 g, 0.55 mol, **3**) was added portionwise over 30 min. The reaction mixture was kept under fast stirring for 4 h to spontaneously crystallize the product. After stirring for an additional 5 h at 20–22 °C, water (600 mL) was added dropwise, and the resultant slurry was aged for 1 h. The solid was collected by filtration and subsequently washed with a (3:1) water–methanol mixture (2 L), followed by water (1700 mL) to make the cefonicid benzathine salt (**4**) (110.0 g; 63% yield) a colorless solid. M.P.: 195–198 °C; assay (HPLC, free base: 71.5% (theoretical maximum: 72.2%) for (1:1) cefonicid benzathine salt).

^{1}H NMR (400 MHz, DMSO-d_6): 8.76 (1H, d, J = 8.8 Hz), 8.0–7.0 (15H, m), 5.70 (1H, dd, J = 8.8 Hz, J = 4.8 Hz), 5.20–5.00 (4H, m), 4.27 and 4.23 (2H, AB system, J = 12.8 Hz), 4.11 (4H, s), 3.69 and 3.57 (2H, AB system, J = 17.6 Hz), 3.17 (4H, s), (Figure 2.4).

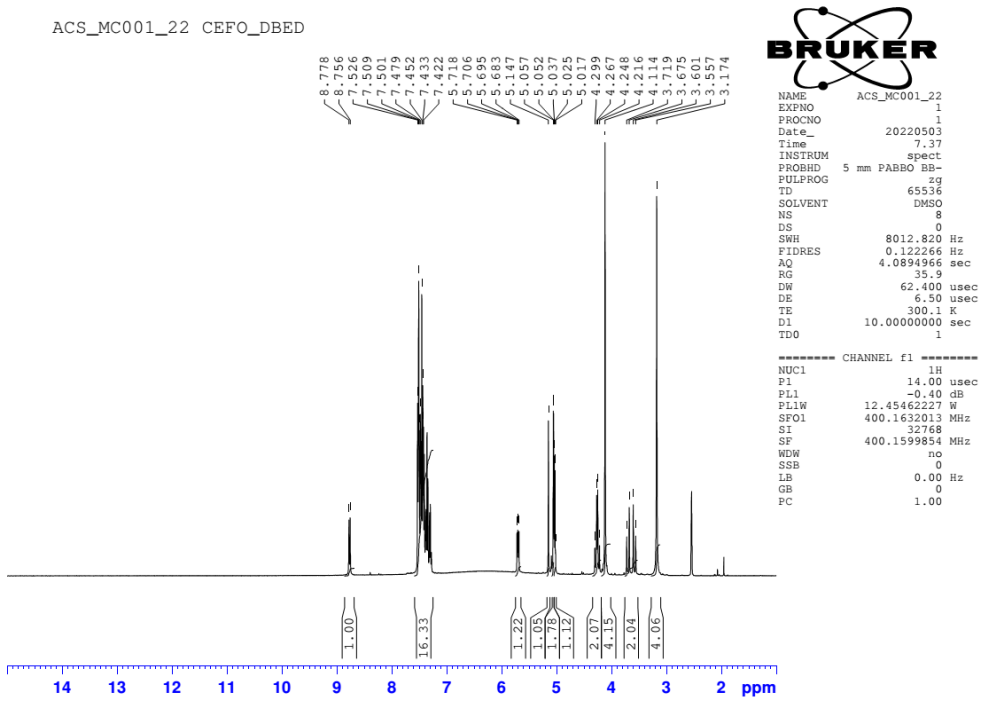


Figure 2.4. ^{1}H-NMR of cefonicid benzathine salt (**4**).

^{13}C NMR (100 MHz, DMSO-d_6): 173.54 (CONH), 165.72 (CO β-lactam), 164.94 (COO), 156.01 (C-S), 141.46 (C), 138.58 (C-4), 130.86 (CH), 129.69 (C), 129.12 (CH), 129.00 (CH), 128.42 (CH), 127.96 (CH), 126.85 (CH), 121.21 (C), 121.8 (C-3), 73.52 (C-OH), 62.25(CH_2SO_3–), 58.99 (C-7), 57.95 (C-6), 51.54 (CH_2-Ph), 44.60 (CH_2N), 37.44 (CH_2S), 27.32 (C-2), (Figure 2.5).

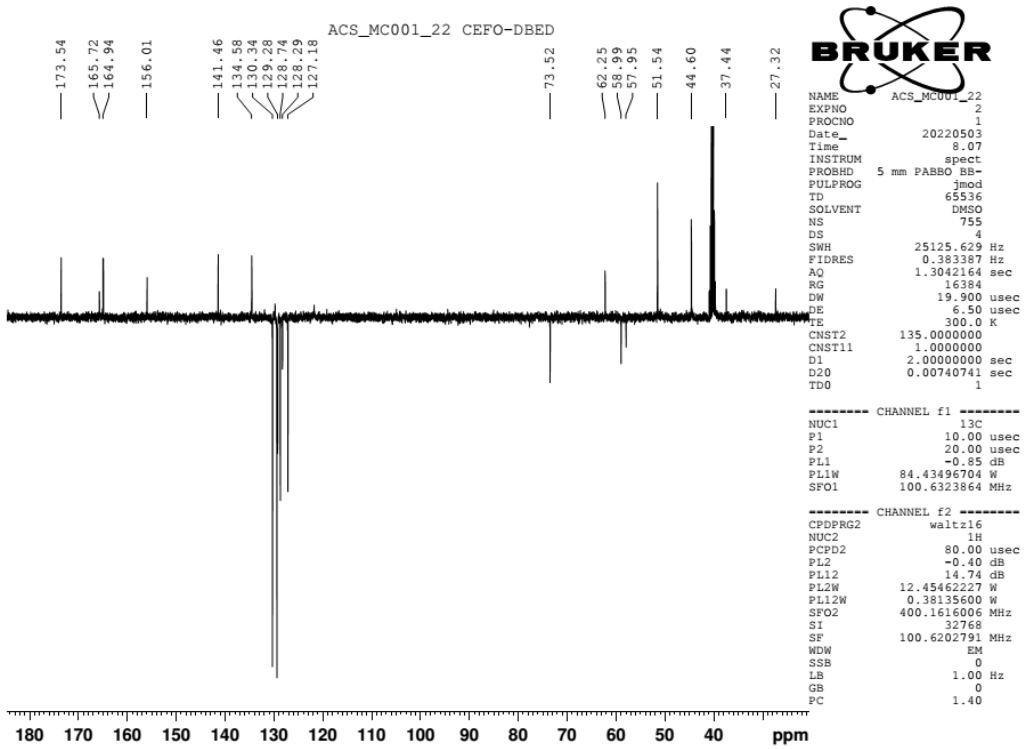


Figure 2.5. ^{13}C-NMR of cefonicid benzathine salt (**4**).

2.3 Results and discussion

First, we investigated the synthesis of 7-SACA (**2**). We defined the optimal equivalent ratio to be 16% of the boron trifluoride complex in acetonitrile to act as a Lewis acid for the synthesis, and the correct amount of acetonitrile as solvent. In the displacement reaction of the cephalosporin substrate (7-ACA, **1**), in the absence of water, BF_3 activates the OAc group at the 3'-position favouring it as leaving group, and, allowing a SN_1 reaction mechanism in the synthesis [24,32]. Moreover, it has been well established that the presence of even a tiny amount of water is leading to the opening of the β-lactam, while the desacetyl and lactone compound drop at zero [23,32,33].

The inert environment and Karl Fischer method to detect the amount of water in the solvent and avoid the destruction of 4-membered ring will be fundamental to any proposed industrial application of these concepts. In the second part, using

the double-nucleophilic and lipophilic nature of *N*',*N*''-dibenzylethylene diamine diacetate (**3**), we promoted the deformylation of the OH-protected group on the mandelic moiety and also enabled product crystallization to occur. We demonstrated that the formyl group in the peculiar position has high reactivity, promoting an amidation reaction that deprotects a hydroxy group and generates a new C-N bond in the reaction by-product. Several amines and OH-protected groups have been studied, but none were able to replicate the excellent results of benzathine diacetate (**3**).

2.3.1 Displacement of the 3'-acetoxy group of 7-ACA

Given that our substrates, 7-ACA (**1**) and 1-sulphomethyl-5-mercapto-1,2,3,4-tetrazole (Na, K) salt, are poorly soluble in highly polar solvents, such as acetonitrile, the contribution of both factors - protonation by an acid and solvolysis - is necessary to completely dissolve them and to promote SN_1 pathway. As summarized in Table 2.1, in which we present different molar ratios of the Lewis acid and substrate (**1**), using the boron trifluoride complex in acetonitrile as is, we have shown that the data for the yield and assay raise dramatically as we gradually increase the ratio and, in parallel, the volume of acetonitrile contained in the complex. This is because the substrates dissolve, facilitating the attack of the S-nucleophile.

We always used a tiny excess of (Na, K) salt, with respect to 7-ACA (**1**) (1.02 *vs* 1.00 equiv), in a 15% sodium hydroxide solution as an alkali base to crystallize the product (**2**) as a monosodium salt, and all steps were performed between 23 °C and 28 °C. To decrease the raw-material costs of the total process, we used the (Na, K) salt of sulphomethyl tetrazole thiol, as confirmed by IC analysis (found Na 8.4% and K 14.4% *vs* calcd. Na 8.9% and K 15.2%), rather than the more common disodium salt, which allowed our supplier to reduce, by one step, the complicated synthesis of this side chain in the 3'-position.

Aiming to optimize the synthetic yield of 7-SACA (**2**), the influence of BF_3-MeCN equivalents and the dilution in MeCN were investigated (Table 2.1). The experiments were carried out at room temperature and quenched after 120 min without filtration of boric acid (and/or borates).

Table 2.1: Effects of equivalents of the 16% BF_3/CH_3CN complex and volume of acetonitrile on the yield and assay of compound 2.

Entry	Mole BF_3/mole of 7-ACA	Yield (%)	Assay (in % as sodium on the anhydrous basis)
1	4.9	23	5.1
2	9.8	24	2.7
3	14.7	73	65.2
4	19.6	76	69.2

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 2.2.1.1.

In entry 1 and, to a lesser extent, in entry 2, the solution remained turbid during the reaction because of the small volume of solvent. However, working with a huge excess of complexed Lewis acid, with a greater volume of acetonitrile, as in entries 3 and 4, provided a clear solution, which indicated the activation of nucleophilic substitution. Despite the turbid appearance of the reaction mixture, owing to the residual starting materials, the boric acid salt precipitated from the clear solution after quenching with water (for all entries the amount of water was calculated by equation = 15.2 × g of 7-ACA (**1**)). This relevant point undoubtedly demonstrates that it is impossible to avoid the filtration of boric acid or its salts, as supported by the assay data in entry 9 (Table 2.2) in which we filtered the by-product after the quenching step, respect at all entries. An analysis of the filtered and dried solid using ion chromatography, also allows us to confirm the precipitation of the potassium salt (30.8%), a tiny percentage of sodium borate (0.2%) and free boric acid.

In order to cut costs and minimize the waste of boric acid salts after quenching, we investigated reducing the loading of the boron trifluoride complex. The acid-catalyzed displacement completed with an equimolar ratio of BF_3 and 7-ACA (**1**) (entries 3 and 4). We also focused our study on the use of acetonitrile as a polar solvent to reduce the Lewis acid amount. Bearing in mind the worst-cases in Table 2.1 in terms of yield and assay – entries 1 and 2 – we could only improve both by adding the minimum amount of acetonitrile to dissolve the substrates in association with the acid, as demonstrated in entries 5 and 6 of Table 2.2.

The remarkable difference in reaction time between entries 5 and 7, 8, 9, and the obtained 10% increase in yield, further supports that Lewis acid plays crucial role in cooperation with solvent.

Table 2.2: Influence of amount of acetonitrile on reaction time and equiv of BF_3.

Entry	Mole BF_3/mole of 7-ACA	volume of solvent/weight of 7-ACA (mL/g)	Time (min)	Yield (%)	Assay (in % as sodium on the anhydrous basis)
5	4.9	5.4	240	62*	69.6
6	9.8	5.4	120	72*	67.7
7	9.8	6.5	60	81*	69.9
8	9.8	7.5	60	80*	69.4
9	9.8	9.0	60	51	102.4

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 2.2.1.1.

*Without the filtration of boric acid and its salt after reaction quenching.

Considering the difference to reach the peak of concentration of 7-SACA (**2**) in solution (Figure 2.6) in association with our goal to obtain a very fast process with a high level of productivity, we permanently chose and developed the procedure with 9.8 equiv. of boron trifluoride complex.

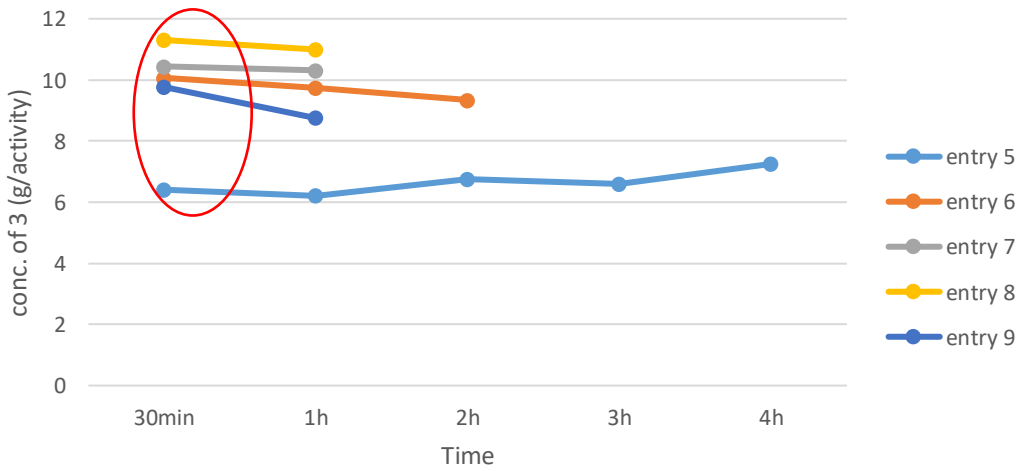


Figure 2.6: Kinetic assessment of 7-SACA (**2**) in solution.

To explain why the dual contribution of the Lewis acid and polar solvent is so crucial in this type of reaction, we have studied the kinetics of all of the entries reported in Table 2.2. We noticed that the concentration of 7-SACA (**2**) remains constant over the reaction time when working as in entry 5, while, when we employed the double equivalents of the boron trifluoride complex in acetonitrile, but with the same volume of solvent as in entry 6, the molar yield in solution increased by 43% in the first 30 min and started to decrease slightly after 1 h (Figure 2.6). Another important aspect is that the peak of concentration of 7-SACA (**2**) in the first 30 min does not change when we changed the volume of the solvent with the same equivalents of Lewis acid (as in entries 6, 7, 8, 9), allowing us to reduce the reaction time to 30-40 min.

By referring to earlier studies, we were able to set an optimal combination of factors and define the best amount of water *vs* 7-ACA (**1**) to quench the reaction mixture and remove the need for a charcoal purification step. The pre-crystallization solution provided a high quality 7-SACA sodium salt (**2**) and a little loss of product in the mother liquor. This is a relevant goal for our study in the perspective of industrializing the process of 7-SACA (**2**), as it enables us to highlight the convenience and technical feasibility of our process compared to old procedures. This telescopic downstream simplifies industrial operations and cuts the waste of the process.

In detail, we have found that a large volume of water (entry 10) corresponds to a superior loss of product in the mother liquor, while, working with a lower water volume at a selected volume of acetonitrile (entry 14), we obtained a gummy formation with water quenching, as indicated in Table 2.3.

Table 2.3: Effects of water content on quenching step and loss of product in mother liquor.

Entry	10	11	12	13	14
Volume of water/weight of 7-ACA (mL/g)	15.2	12.0	19.1	9.3	6.4
Loss in mother liquor (g/activity)	3.4	2.7	5.0	1.8	Failure
Yield (%)	59	60	46	68	
Assay (in % as sodium on the anhydrous basis)	102.4	100.6	100.9	101.0	

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 2.2.1.1.

At this point, after having successfully defined the moles of Lewis acid and the volume of acetonitrile, we were able to efficiently optimize the amount of water (entry 13), leading to high yield and quality.

We also investigated the possibility of changing the alkali base to crystallize the product, and the influence of crystallization time to evaluate, once again, the loss in the mother liquor. Crystallizing the product with 14% ammonium hydroxide, we found that the product precipitates mainly as a monoammonium salt (found 3.4% *vs* calcd. 4.2% by IC analysis), proving that the cation of the alkali base precipitates the corresponding salt. When using the 15% NaOH solution, we always obtained the monosodium salt as confirmed by IC analysis (found 4.9% *vs* calcd. 5.3%).

Performing the crystallization in 2 h or overnight does not change the amount of product loss in the mother liquor. The use of a sodium hydroxide solution, rather than an ammonia solution, makes the process safer, adding another advantage to our process. Moreover, at lower concentration of sodium hydroxide (5%) the product yield decreases due to higher amount of water that worsen the crystallization efficiency. Although our goal to industrialize the room temperature method, we noticed a slight yield increase at lower temperatures, but the product was out of specifications (Table 2.4).

Table 2.4: Crystallization temperature and product features.

Entries	Temperature of crystallization	Yield (%)	Within production specifications
15	25-28 °C	72	Yes
16	10-13 °C	74	No
17	2-5 °C	75	No

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 2.2.1.1.

After having studied and enhanced all of the operational parameters in the development of this new cost-effective and environmental-friendly process, we wish to summarize the differences between our method and the well-known standard approach [25], highlighting pros and cons (Table 2.5).

Table 2.5: Current protocol and US 5 625 058 patent comparison (features, *pros* and *cons*).

Parameters	US 5 625 058 features		Current protocol features	
Temperature	0 °C	Low productivity, long reaction time for the nucleophilic substitution and high energy costs	Room temp.	High productivity, short reaction time, energy and cost savings
Reaction Time	480 min		30-40 min	

Volume of acetonitrile as reaction solvent	3.3 × grams of 7-ACA	The low acetonitrile volume makes purification over charcoal before crystallization necessary	7.5 or 9.0 × grams of 7-ACA	The higher acetonitrile volume allows a reduction in BF_3 complex equiv, making charcoal treatment unnecessary
BF_3 complex equiv	Not reported	/	9.8	This amount gave 83% molar yield in solution in a very short time
BF_3 complex in acetonitrile Percentage (Lewis acid)	Not reported	/	9.1 - 11.6%	Lower percentage of BF_3 gas in solution leading to a safer process
1-sulphomethyl-5-mercapto-1,2,3,4-tetrazole salt	disodium salt	High raw material costs with low productivity	mono (K, Na) salts	The side chain as mono (K, Na) salts allows a reduction in the raw material costs of the entire process
Amounts of reagents used in trials lab	10.0 g of 7-ACA as limiting reagent	Such small amounts of starting materials need a second lab trial for	50.0 g of 7-ACA as limiting reagent	Reliable procedure well suited for industrialization

		scaling up		
water volume for quenching	1 × grams of 7-ACA	The tiny volume of water makes treatment with charcoal necessary to clarify the solution before crystallization	9 × grams of 7-ACA	Higher water volumes make charcoal treatment and subsequent time-consuming filtration unnecessary
Charcoal treatment step	Yes	/	No	
Quenching and crystallization temperature	0 °C	High energy costs	Room temperature	Energy saving
Alkali base	Ammonium hydroxide solution	Release of ammonia gas (toxic and irritating)	Sodium hydroxide solution	Safer work up
Molar yield	83% as monoammonium salt	Higher yield but much lower productivity	72% as monosodium salt	Lower yield but much higher productivity

2.3.2 Amidation of 7-SACA

We have investigated three different synthetic paths for the amidation of the 7-position.

2.3.2.1 (R)-5-Phenyl-1,3-Dioxolane-2,4-Dione

O-carboxyanhydride (OCA) compounds are a class of five-membered rings that is widely applied in the polymeric, peptidic, and pharmaceutical fields, thanks to the ease with which the desired epimer form is generated [34-39]. The OCA subgroup from (*R*)-mandelic acid (**63**) has been the only possible way to directly access the optically active form of the 3-methylthio-tetrazolyl-7-mandeloyl cephalosporanic derivatives (cefonicid and cefamandole) from the starting substrates, and thus, bypass the hydroxy-protection–deprotection steps and the carboxylic acid activation on the mandelic moiety, as depicted in Figure 2.7 [40-45].

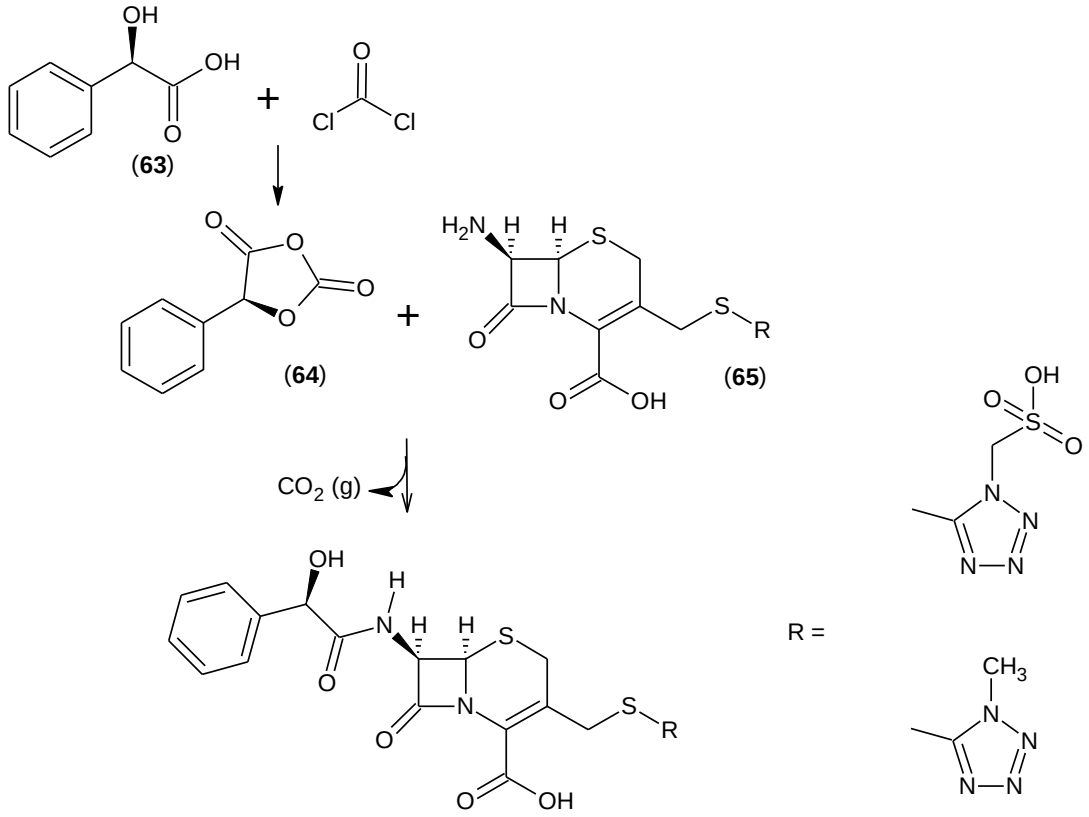


Figure 2.7: Amidation of OCA (**64**) from (*R*)-mandelic acid (**63**) and 7-SACA or 7-TACA intermediate (**65**).

The synthesis of this compound in β-lactam manufacturing (**64**), from phosgene or its derivatives (di- and triphosgene) [46-52], has always been limited by safety and environmental concerns. In addition, the racemization caused by the

hydrochloric acid that is released during mandelic cyclization means that several purification steps must be performed if the *R* form is to be obtained, making the process poorly convenient.

Over the last few years, a new family of dioxolane compounds have emerged in the fields of biocompatible and biodegradable polymers for drug delivery in tissue engineering and food packaging, as they are easily prepared from inexpensive and sustainable feedstocks. Both 5-phenyl-1,3-dioxolane-4-one (DOX) and alkyl-DOX are part of this group, and their simple, synthetic accessibility has been proven by the fact that fewer toxic resources were used as ring-closing agents for mandelic acid (**63**) in acetone or cyclohexane, giving good isolated yields. Having observed this new frontier of compounds, we have investigated the possibility of replacing phosgene-derived OCA with DOX, which was obtained from paraformaldehyde, and with methyl- and ethyl-DOX, which were synthetized from trimethyl and triethyl orthoformate (TMOF and TEOF) [53-58], as illustrated in Figure 2.8.

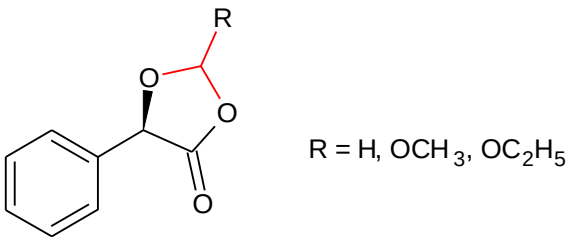


Figure 2.8: DOX, methyl-DOX, and ethyl-DOX.

Unfortunately, our efforts to obtain cefonicid using these molecules were in vain, even when we applied the same reaction conditions used for phosgene-derived OCA, having unsuccessfully studied amidation in water or in a mixture with polar aprotic solvents (acetone, ethyl acetate and acetonitrile [59]) at a neutral pH value and at weakly acidic and alkaline conditions, both at room temperature and in the 0–5 °C range. In addition, satisfactory results could not be achieved, despite the use of DMAP as an organic catalyst, such as in ring-opening polymerization (ROP) [51]. The reasons for failure lie in the fact that the irreversible decarboxylation reaction is the driving force in obtaining a new C-N bond, while formaldehyde and

methyl/ethyl formate are bad leaving groups in nucleophilic substitutions, as reported in Figure 2.9. Finally, a line was drawn under these alternative compounds.

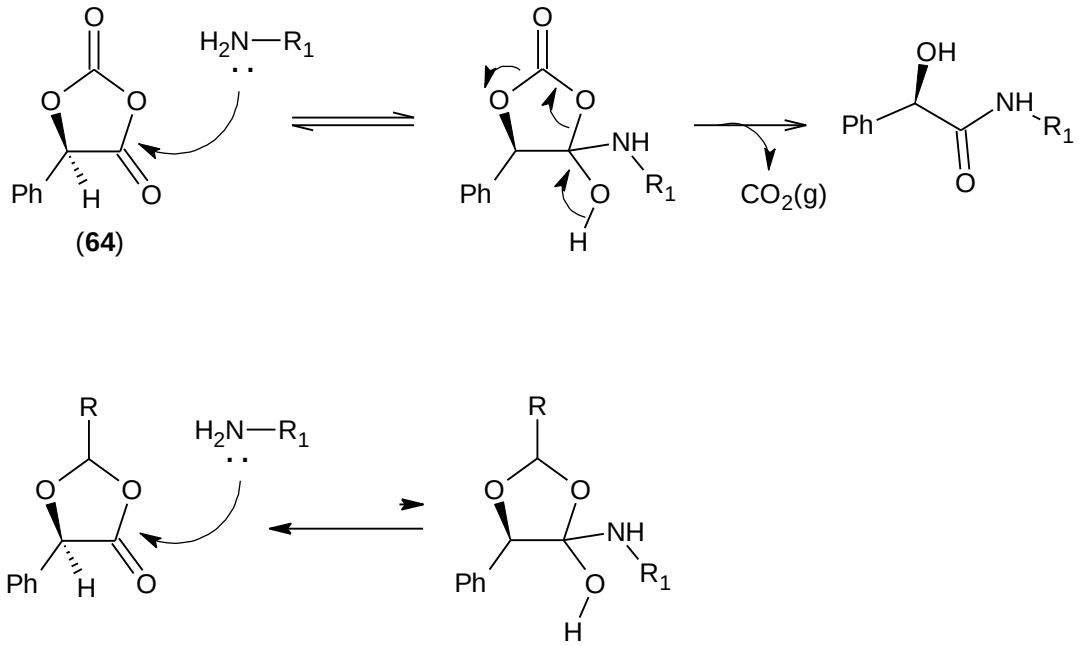


Figure 2.9: Reactivity comparison among DOX and alkyl-DOX.

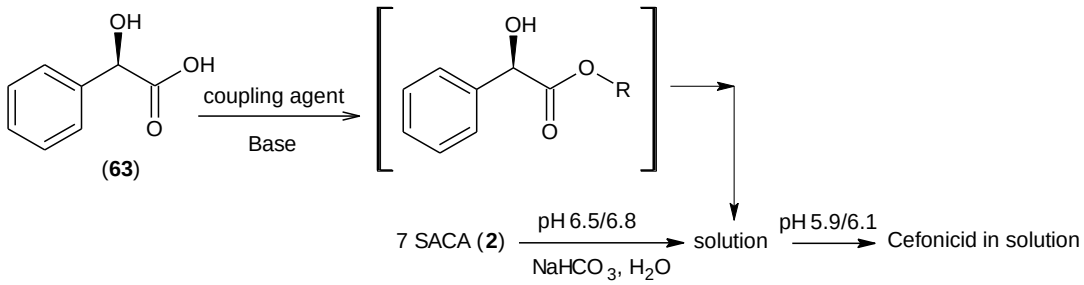
2.3.2.2 Amidation in water and organic solvents

Sustainability is and will be the key driver and guideline in the future of API manufacturing, as will the goal of applying shorter synthetic routes that do not depend on hazardous reagents and solvents. It is now well established that amidation in an organic solvent is impossible for the extremely hydrophilic 7-SACA (**2**), and the same behavior was observed with environmentally friendly organic solvents such as 2-methyl-THF, cyclopentyl methyl ether (CPME), and cyrene. Only one protocol has been developed with harsh reaction conditions, and that was forty years ago. It was never industrialized because of the production of very dangerous waste, due to the use of *N,N*-dimethylformamide and 2,2′-dithiobisbenzothiazole [40]. By contrast, the amidation of the *O*-protected mandelic moiety in water has been the technique of choice on an industrial scale over the years because it combines high-grade drug quality with green chemistry credentials and the manufacturing requirements of good yields and low-cost production [28,60-61].

In the industry, both the ultrafiltration and reverse osmosis processes required to remove the enzyme from the aqueous medium and the 20 h needed for acid OH deprotection have seriously limited large-scale production because of the ultra-low productivity and heavy plant-management costs that these processes cause. Some years ago, Terreni et al. studied an enzymatic synthesis with immobilized acylase, which fell foul to a number of undesired, parallel reactions, low stability in the native protein, and irresolvable difficulty in recovering the biocatalyst from the reaction mixture, meaning that this approach was effectively abandoned [62].

Recent developments in the synthesis of the antimuscarinic agent, mirabegron, have prompted us to replicate the linkage of the mandelic group directly to 7-SACA (**2**) in an attempt to overcome the above-mentioned shortcomings. As amidation is one of the most commonly used chemical reactions in the synthesis of these semi-synthetic compounds, and as a broad class of coupling reagents exists, we focused our selection of activators on the practical aspects of standard chemistry processes: costs, yields, safety, and waste. As indicated in Table 2.6, activation via *O*-acylisoureic ester, boron-derived mixed anhydride, and sulfonate-based mixed anhydride are the techniques used in our work [63-69].

Table 2.6: Direct amidation of (*R*)-mandelic acid (63).



Entry	Coupling agent	Base	Solvent	Activation temperature (°C)	Amidation temperature (° C)	Cefonicid yield in solution (%)
18	TsCl	TEA	CH_3CN	reflux	0-5	< 1
					r.t.	1

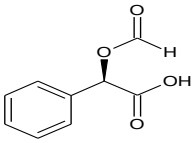
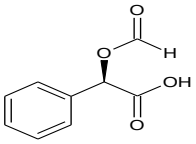
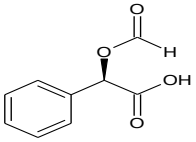
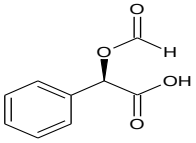
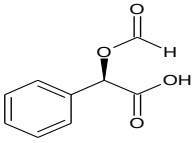
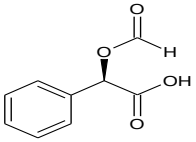
19	MsCl	TEA	CH_3CN	r.t.	0-5 r.t.	2 4
20	PivCl	TEA	CH_3CN	0-5 °C	0-5 r.t.	- -
21	$B(OMe)_3$	$NaHCO_3$[a]	CH_3CN	60 °C	0-5 r.t.	< 1 < 1
22	$B(OMe)_3$	K_2CO_3[a]	CH_3CN	60 °C	0-5 r.t.	< 1 < 1
23	EDC[(b)]	-	water	r.t.	r.t.	1
24	DIC[(b)]	-	water	r.t.	r.t.	< 1
25	TsCl	TEA	CH_2Cl_2[c] EtOAc[c]	reflux	r.t. r.t.	- -
26	MsCl	TEA	CH_2Cl_2[c] EtOAc[c]	r.t.	r.t. r.t.	< 1 1

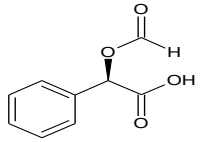
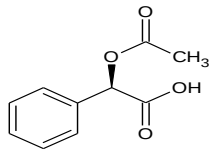
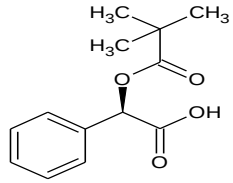
Reaction conditions: 2.0 g of 7-SACA (**2**, 1.0 eq.), (*R*)-mandelic acid (**63**, 1.2 eq.), activating agent (1.2 eq. to (*R*)-mandelic acid), base (1.3 eq. to (*R*)-mandelic acid), water volume = 7.5 mL. [a] These entries use different bases to dissolve 7-SACA (**2**). $NaHCO_3$ is the base for entry 21, while K_2CO_3 is the base for entry 22. [b] EDC and DIC were added as powders. [c] Solvent volume = 7.0 mL

These data clearly highlight that it is impossible to directly attach (*R*)-mandelic acid (**63**) to 7-SACA (**2**) due to the presence of free OH, which interferes with the coupling reagent, meaning that the carboxylic moiety is not totally activated, and to the water medium used to dissolve the cephalosporanic nucleus, which hydrolyzes the activated adduct. When the same trials were repeated and the hydroxyl group was protected via formylation [38], it appeared that coupling via carbodiimide (EDC and DIC) promoted amidation in water, although less efficiently than the corresponding acyl chloride, generating formyl cefonicid, whereas the mixed anhydride was unsuccessful. The high reactivity of the acid chloride towards the 7-SACA (**2**) amine group led to a conversion of above 99% in solution towards formyl cefonicid, demonstrating that this reaction system possesses better

adaptability in water, with the same results even being achieved when the OH-protected group was changed, as shown in Table 2.7.

Table 2.7: Amidation with OH-protected mandelic acid.

Entry	OH-protected moiety	Coupling agent	Solvent	OH-protected cefonicid conversion in solution (%)[a]
27		TsCl	CH_3CN[b]	1
28		MsCl	CH_3CN[b]	4
29		PivCl	CH_3CN[b]	< 1
30		$B(OMe)_3$	CH_3CN[b]	< 1
31		EDC	Water[c]	27
32		DIC	Water[c]	32

33		$SOCl_2$	CH_3CN[d]	> 99
34		$SOCl_2$	CH_3CN[d]	> 99
35		$SOCl_2$	CH_3CN[d]	> 99

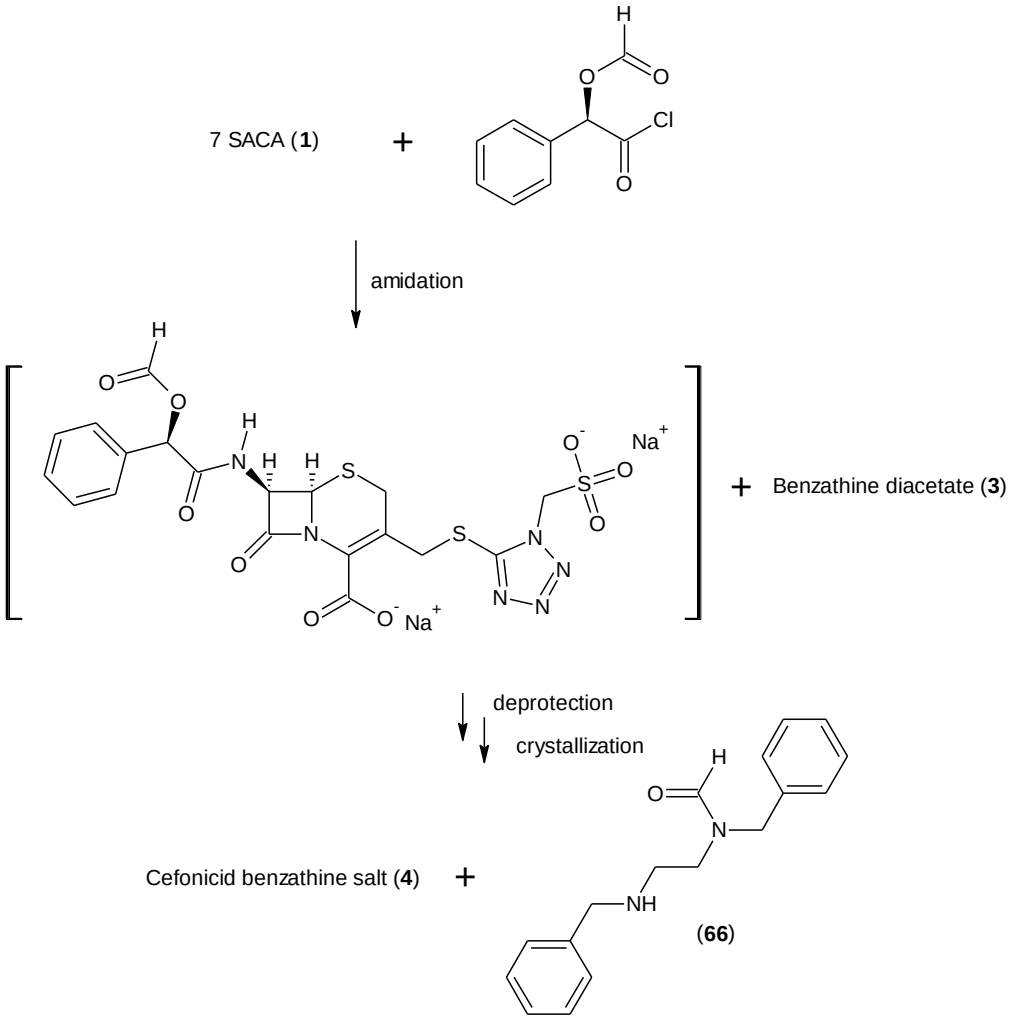
[a] The formyl-cefonicid conversion is calculated as a peak-area ratio using the formula: ((OH-protected-cefonicid area)/(OH-protected-cefonicid area + 7-SACA (**2**) area)) × 100. [b] Reaction conditions: 2.0 g of 7-SACA (**2**, 1.0 eq.), *O*-formyl-(*R*)-mandelic acid (1.2 eq.), activating agent (1.2 eq. to *O*-formyl-(*R*)-mandelic acid), and base (1.3 eq. to *O*-formyl-(*R*)-mandelic acid). [c] Reaction conditions: 2.0 g of 7-SACA (**2**, 1.0 eq.), *O*-formyl-(*R*)-mandelic acid (1.2 eq.), and activating agent (1.2 eq. to *O*-formyl-(*R*)-mandelic acid). [d] Reaction conditions: 2.0 g of 7-SACA (**2**, 1.0 eq.) and OH-protected-(*R*)-mandelic acid (1.2 eq.) as a 2.8 mol/L concentration in CH_3CN. The amidation step was always carried out at 0–5 °C.

These results placed us before an unwelcome choice because amidation via either EDC or DIC is explicitly more environmentally friendly than proceeding via acid chloride, due to the use of thionyl chloride. However, the difference in reactivity is so significant that acid chloride was chosen as the activation type.

2.3.2.3 Cefonicid benzathine salt

Our plan to develop a lean, cost-effective, and high-performance method for producing cefonicid has been achieved thanks to the simultaneous deprotection and crystallization of the drug with *N',N"*-dibenzylethylenediamine diacetate (**3**) after the amidation step. This operation allows the ultrafiltration and reverse osmosis that are necessary to remove the enzyme from the reaction mixture in enzymatic deformylation to be avoided and makes the 20 h of reaction to deprotect the OH group with acid unnecessary.

Benzathine diacetate (**3**) is a common precipitating agent in cephalosporanic production thanks to its capacity to generate a salt between the cephalosporin negative charge and two secondary amines [61,70]. The linear chain with benzylic moieties at the bottom makes the molecule lipophilic in nature, enabling the precipitation of cephalosporin from the aqueous medium. In our case, we have taken advantage of the nucleophilicity of secondary amines to deprotect formyl cefonicid, and at the same time, crystallize the drug as a benzathine salt (**4**), as described in Scheme 2.3.



Scheme 2.3. New protocol to obtain cefonicid benzathine salt (**4**).

This excellent formylic transfer surprisingly builds a new C-N bond on the reaction by-product, benzathine formylate (**66**), which is lost in the mother liquor after cake filtration, deblocks the hydroxy group on the cefonicid mandelic moiety, and all in the same step, is followed immediately by spontaneous product crystallization due to vigorous stirring. As reported in Table 2.8, the minimum amount of benzathine diacetate (**3**) required to achieve this excellent result is 2.4 equivalents, relative to 7-SACA (**2**), because the amidation/deformylation does not complete when working with lower amounts, while larger amounts do not affect the drug quality, thus meeting the theoretical assay for cefonicid (69.3%) and benzathine (30.7%).

Table 2.8. Reactivity study of formyl cefonicid with different *N',N''*-dibenzylethylenediamine diacetate (3) amounts.

Entry	Equivalents[a]	Conversion in deformylation (%)[b]	Crystallization[c]	Yields (%)	Cefonicid assay (%)	Benzathine assay (%)
36	1.3	52	A	-	-	-
37	1.7	84	A	-	-	-
38	2.0	94	B	51	72.8	25.2
39	2.4	99	B	62	71.5	27.0
40	2.8	99	B	61	72.2	26.3

[a] The equivalents were calculated as moles of benzathine diacetate (**3**)/mole of 7-SACA (**2**). [b] The cefonicid conversion is calculated as the peak-area ratio using the formula: ((cefonicid area)/(cefonicid area + formyl cefonicid area)) × 100. [c] A = no product crystallization, B = product crystallization.

We also investigated other amines (imidazole, pyridine, triethylamine, and 28% ammonia solution) in order to explore the reactivity of this OH-protected group, and we discovered that the same reactivity drove the formyl transfer, but no

crystallization occurred. These results represent a problem for our intent to streamline the process, as resins or chromatography would then be required to purify the product. However, at the same time, they also demonstrate that benzathine diacetate (**3**) shows the best performance of the amines tested.

Finally, we studied the same reaction on the *O*-acetyl- and *O*-pivaloyl-protected groups, but deprotection proved troublesome. In these cases, two subunits disconnected, with one being *O*-acetyl-mandelic acid and the others being *O*-pivaloyl-mandelic acid and 7-SACA (**2**).

As drug stability is a crucial feature when working on an industrial scale, we studied this parameter over 6 months and can confirm the high stability of the salt, as indicated in Table 2.9.

Table 2.9. Salt stability over 6 months.

Time (months)	Cefonicid assay (%)	Benzathine assay (%)	Total impurities
0	71.5	27.0	1.9
6	71.6	26.8	1.7

2.4 Conclusions

In conclusion, in the first part we have successfully developed a novel synthetic protocol for the synthesis of 7-amino-3-[sulphomethyl-1H-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt (**2**), a key intermediate in the synthesis of cefonicid benzathine salt (**4**), in a telescopic route and in about 70% overall yield from readily accessible 7-ACA (**1**). This process appears to be more compatible with the industrial scale and has some evident advantages over the existing synthetic procedures. By virtue of fine balance between equiv of 16% BF_3-MeCN and volume of MeCN (83% molar solution) the reaction occurred in only 30-40 min.

The work up avoids charcoal purification and loss in the mother liquor. A well-defined amount of water was enough to quench the reaction mixture. These improvements dramatically reduced the reaction time and increased productivity, making it more attractive for industrial production.

In the second part we have reported a new, reliable, efficient, and sustainable protocol for the production of cefonicid benzathine salt (**4**) as both oral and injectable formulations. Using the double alkaline and nucleophilic behavior of *N',N"*-dibenzylethylenediamine diacetate (**3**), we have considerably streamlined the process, producing the drug with remarkable stability and with a good yield, making it ready for industrial scale-up. The extreme reactivity of the OH-protected formyl group proved to be the winning weapon for this type of amidation, considering the bulkiness of benzathine diacetate (**3**), while its partially lipophilic nature facilitates the precipitation of the drug from the water medium and reduces waste production. In the jungle of API synthesis, where more and more companies are striving to find new ways to solve chemical-manufacturing challenges, we believe that this is a competitive process, able to be reducing waste and making it more reliable in terms of strict environmental concerns.

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Chapter 3: From Batch to the Semi-Continuous Flow Hydrogenation of *p*NB, *p*NZ-Protected Meropenem

Abstract

Meropenem (**6**) is currently the most common carbapenem in clinical applications. It is an injectable drug administered intravenously. Industrially, the final synthetic step in its preparation is characterized by a heterogeneous catalytic hydrogenation in batch mode with hydrogen and Pd/C. The required high-quality standard is very difficult to meet and specific conditions are necessary to remove both protecting groups, *p*NB and *p*NZ, simultaneously. The three-phase gas–liquid–solid system makes this step difficult and unsafe. The introduction of new technologies for small-molecule synthesis in recent years has opened up new landscapes in process chemistry. In this framework, this work has investigated meropenem (**6**) hydrogenolysis using microwave (MW)-assisted flow chemistry for use as a new technology with industrial prospects. The reaction parameters (catalyst amount, T, P, residence time, flow rate) in the move from the batch process to semi-continuous flow were investigated under mild conditions to determine their influence on the reaction rate. The optimization of the residence time (840 s) and the number of cycles (4) allowed us to develop a novel protocol that halves the reaction time compared to batch production (14 min *vs* 30 min) while maintaining the same product quality. The increase in productivity using this semi-continuous flow technique compensates for the slightly lower yield (70% *vs* 74%) obtained in batch mode. The remarkable plant miniaturization of the MW semi-continuous flow technology may be a valid alternative for the process intensification of industrial meropenem (**6**) synthesis.

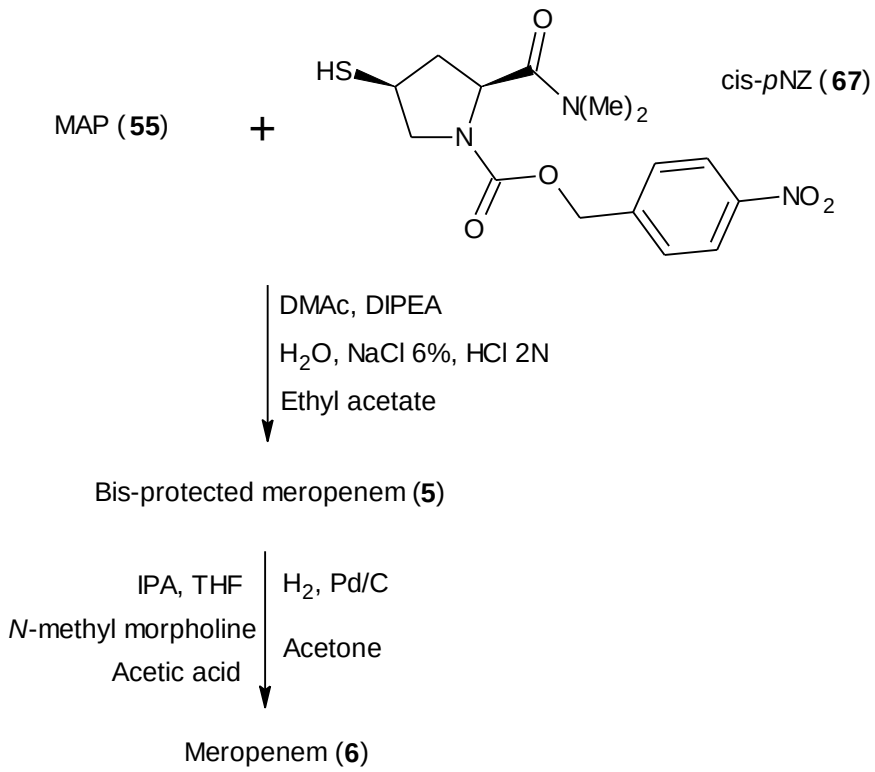
3.1 Introduction

Carbapenems are currently deemed lifesaving drugs [1], with their broad-spectrum activity meaning that they play a vital role for human health. Their medical use is so crucial and essential that they have been defined “last-line antibiotics” or “antibiotics of last resort”. They are the most effective weapon against known and suspected MDR bacterial infections [2–20].

Part of the carbapenem antibiotics subgroup, meropenem (**6**) is currently the most widely used in clinical treatment [21,22]. Its worldwide market was valued at

USD 1.7 billion in 2022 and is expected to grow to USD 2.2 billion by 2028, making this drug a blockbuster [23]. Alone, it made up 43% of the USD 3.9 billion in global sales of the carbapenem family in 2021 [24]. It was discovered by Sumitomo Dainippon Pharma, now Sumitomo Pharma, in 1983 [25–27], and was approved by the Food and Drug Administration (FDA) in 1996 [28]. The drug is sold under the brand name Merrem™, as a generic drug substance, or in combination with vaborbactam (**48**) under the brand name Vabomere™. It is administered intravenously in both forms and is listed on the World Health Organization's list of Essential Medicines thanks to its importance [29–38].

The literature reports different synthetic routes for its preparation [25-27,39–60]. The most widely used industrial synthesis includes more than twenty steps characterized by protection with *p*NB and *p*NZ on the carbapenem scaffold. This strategy, which allows the preparation of the drug with the correct stereochemical configuration (β-methyl in position 1), is still considered the most efficient and competitive in the world market. The high stability conferred to the β-lactam core during all chemical steps by these protecting groups with a high steric hindrance has been the main advantage of their use for more than 30 years. In this long synthetic route, the last two steps define the meropenem's structure (**6**). The condensation between 4-nitrobenzyl(4R,5S,6S)-3-[(diphenylphosphono)-oxy]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylate (**55**, MAP) and (2S,4S)-2-dimethylaminocarbonyl-4-mercapto-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**67**, cis-*p*NZ) gives bis-protected meropenem (**5**). Batch-mode hydrogenation with palladium over carbon (Pd/C) under pressure simultaneously removes the *p*-nitrobenzyl (*p*NB) and *p*-nitrobenzyloxycarbonyl (*p*NZ) protecting groups (Scheme 3.1).



Scheme 3.1. Meropenem (**6**) synthesis from MAP (**55**) and cis-*p*NZ (**67**).

The hydrogenation mechanism involves two steps. In the first step, the nitro group is reduced to the *p*-aminobenzyl(oxycarbonyl) intermediate, which spontaneously collapses to the quinonimine methide and the deprotected compound by 1,6-electron-pair shift, as depicted in Figure 3.1 [61].

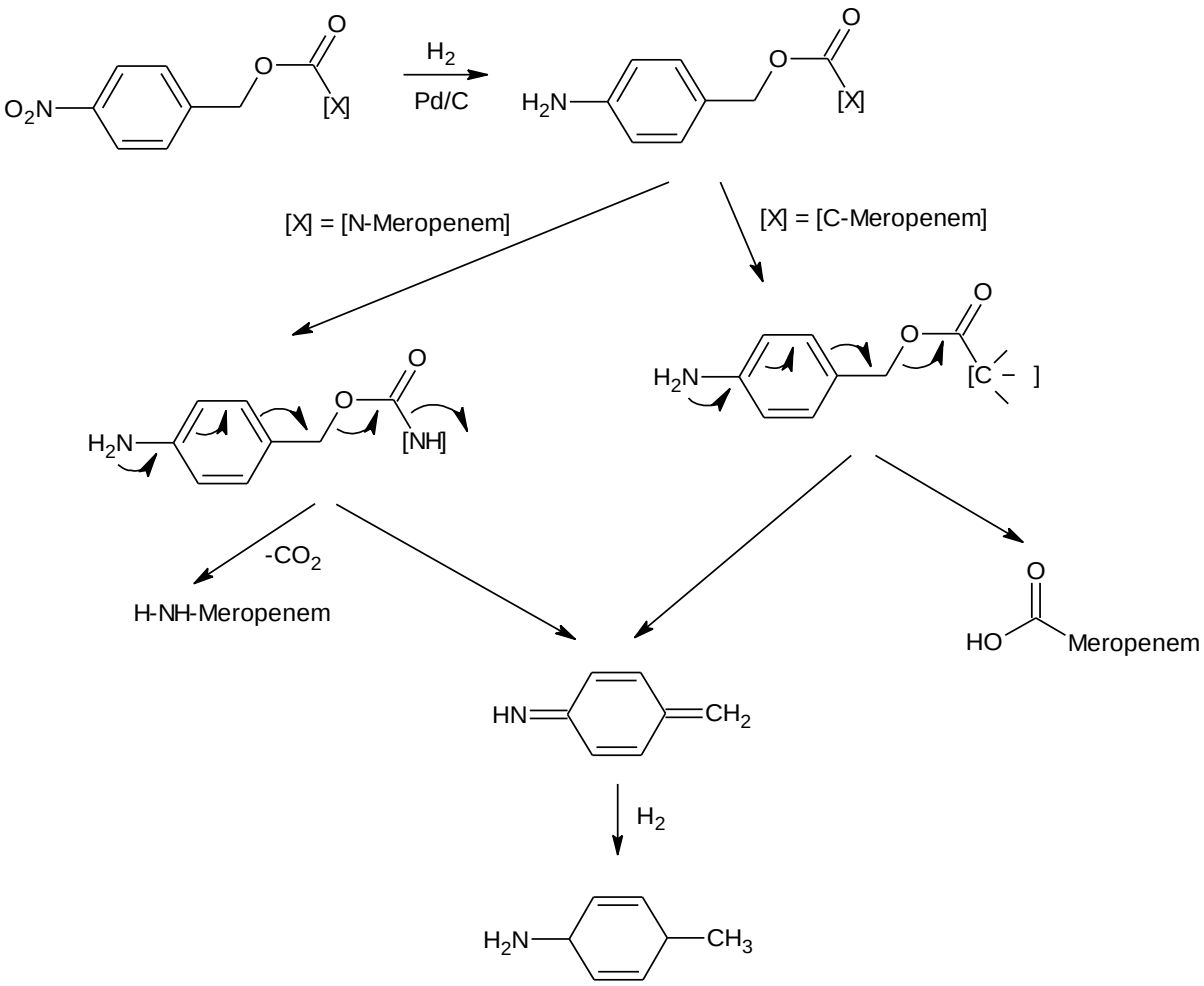


Figure 3.1. Reduction mechanism of *p*NB/*p*NZ protecting groups.

Regarding the industrial process, the subtle equilibrium between the hydrogenation operating parameters and the stability of the carbapenem skeleton has always been the key to the success of this reaction, leading all manufacturers to balance these two factors. The catalytic system was characterized by the presence of *N*-methylmorpholine and acetic acid as buffers, which allowed it to operate at pH 6.5–6.8 throughout the reaction time, ensuring the stability of both reagent and product. Water and isopropyl alcohol met the requirements of green chemistry and sustainability, and the waste was easy to handle. Catalyst filtration, workup, and subsequent first crystallization provides crude meropenem (**6**). The

product is purified by a second crystallization to reach the quality standard before it is sterilized and becomes an injectable drug.

Hydrogenation reactions pose significant risks for a production plant, and this can only be mitigated in part by heavy investment. The implementation of batch-mode manufacturing requires a bunker facility, and the engineered architecture must be designed to restrict uncontrolled events and any possible damage must be contained. The risk to people and the surrounding area has to be under control [62–66]. The considerable manufacturing capacity needed to support massive global demand, the operational accuracy needed to obtain a high-grade product and the high flammability of Pd/C are all real and routine issues that must be tackled in the commercial production of meropenem (**6**). Batch production is no longer adequate in the industrial manufacturing of pharmaceuticals, where a balance between streamlining and increased productivity is required to compete in the global active pharmaceutical ingredients (APIs) market. In addition, the high-quality standard required is very challenging to satisfy due to the instability of the carbapenem skeleton during the hydrogenation step.

In the context of the current drive towards the adoption of emerging technologies for the synthesis of APIs, functional intermediates and natural compounds, we have investigated the hydrogenation of bis-protected meropenem (**5**) under microwave (MW)-assisted flow chemistry. We have chosen this technology because of its well-known benefits to mass and heat transfer, miniaturization, safety, speed and sustainability. Semi and continuous flow chemistry have traditionally been used and developed as a safer and more efficient method when batch operations are deemed to be too dangerous. The small operational units minimize exposure to hazardous reagents and permit safe handling. The integration and modularization of operations allow for much easier study and production scale-up. Automated monitoring techniques and the reduction in the probability of human error are significant industrial advantages, making this technology flexible and agile. Although flow chemistry does not entirely eliminate safety concerns in industry, it does decrease risk factors to levels at which they are easier to manage. Furthermore, the use of MW irradiation makes this a highly efficient and green technology that can fulfil energy-saving and sustainability

requirements. Faster heating rates and uniform heat distribution mean that MW chemistry is one of the most attractive of the emerging technologies [67–78].

MW-assisted flow chemistry has been widely applied over the last two decades to synthesize myriad organic compounds, demonstrating its versatility and potential. From unsaturated organic substrates to heterocycle synthesis and metal-catalyzed chemistry, this technology has played a central role in the development of sustainable multigram preparations that meet the dual requirement of process intensification and reduced production costs. It has proven to be a promising hybrid technique that can link lab-scale investigations and pilot processes, and thus meet industrial challenges [79–86]. In addition, the FDA and the EMA have strongly encouraged the study and implementation of this innovative, sustainable and environmentally friendly technology in API synthesis [87,88]. β-lactam chemistry has remained anchored to developments from many years ago and has only recently achieved renewal in a few cases. With this revolution in place, MW-assisted flow chemistry could really upend acquired certainties in the near future.

In this work we demonstrate the effective implementation of a new emerging technology in the synthesis of a blockbuster drug. It describes a new attractive protocol that is suitable for industrial application as an alternative to batch mode. MW-assisted flow chemistry fulfills the required high-quality standard, as hydrogenation is the final step in meropenem (**6**) manufacture. The quality product is comparable to commercial drug. The parameters have been investigated with an eye to process intensification and further scale-up. The carbapenem skeleton's stability has been preserved by working under mild conditions and by exploiting its intrinsic characteristics.

3.2 Materials and methods

3.2.1 Chemistry

MAP (**55**), cis-*p*NZ (**67**), 10% Pd/C and the solvents were kindly provided by ACS Dobfar. All other reagents were purchased from Merck KGaA (Darmstadt, Germany).

The Agilent 1200 series HPLC system, NMR (Bruker 400, Munich, Germany Munich, Germany), and Karl Fischer titration were the analytical instruments used to identify and analyze the products.

3.2.1.1 General procedure for the synthesis of Bis-protected meropenem (5)

MAP (**55**, 94.0 g, 158.0 mmol) and cis-*p*NZ (**67**, 59.2 g, 167.6 mmol) were dissolved in 400 mL of dimethylacetamide and cooled to -10 °C. Over the course of 15 min, diisopropylethylamine (DIPEA) (88 mL, 505.3 mmol) was added dropwise at a temperature not exceeding -7 °C. The mixture was stirred for 90 min at -10 °C. 1200 mL of pre-cooled (0-5 °C) ethyl acetate was then added over 30 min with the temperature being maintained at -10 °C, and finally 800 mL of ice water was added, which raised the temperature to 0-5 °C. The aqueous phase was separated and extracted with 600 mL ethyl acetate at 0-5 °C. The combined organic phase was extracted twice, each time with a cold mixture of 320 mL 6% aqueous NaCl solution and 80 mL 2N hydrochloric acid. Finally, the organic phase was extracted with 400 mL phosphate buffer solution pH 7.0. The organic phase was separated, filtered to clarify, and the filter then washed with 40 mL of ethyl acetate. The filtrate was concentrated to 360 g at 40 °C and made up to 440 g with fresh ethyl acetate. The solution was stirred for 18 h at 20 °C in order to crystallize the crude bis-protected meropenem (**5**). In order to complete the crystallization, 140 mL of heptane were added dropwise, and the slurry was stirred for a further 30 min. The product was isolated by filtration, washed twice, each time with 200 mL of heptane, and dried for 16 h at 40 °C under vacuum to give the crude title compound (**5**) (101.2 g, 91.8% yield) as an off-white solid. The HPLC assay gave a result of 98.3%. Purification: crude bis-protected meropenem (**5**, 100 g, 143.3 mmol) was dissolved in 1680 mL ethyl acetate. The slurry was stirred at 20 °C for 90 min. 140 mL cyclohexane was added in order to complete the crystallization and the slurry was stirred for 2.5 h. The pure bis-protected meropenem (**5**) was isolated via filtration, washed with 200 mL cyclohexane, and dried for 16 h at 40 °C under vacuum to give the pure title compound (4) as a white solid (91.1 g, 91.1% yield). The HPLC assay gave a result of 99.6%.

Signals in the 1H- and 13C-NMR spectra are split due to the presence of *p*NZ rotamers in a ~ 56:44 ratio at 300 K.

^{1}H NMR: (400 MHz, DMSO-d_6, 300 K): δH 8.26, 8.25 (4H, 2xd, ^{3}J 8 Hz); 7.76 (2H, d, ^{3}J 8 Hz); 7.68, 7.56 (2H, 2xd, ^{3}J 8 Hz); 5.50 & 5.34 (2H, AB syst, ^{2}J 14 Hz); 5.30-5.05 (3H, m); 4.88, 4.80 (1H, 2xt, ^{3}J 8 Hz); 4.35-4.30 (1H, m); 4.28, 4.18 (1H, 2x dd, ^{3}J 10 Hz, ^{3}J 7 Hz); 4.10-4.00 (1H, m); 3.96-3.80 (1H, m); 3.70-3.60 (1H, m); 3.37-3.32 (1H, m); 3.28, 3.21 (1H, 2 x t, ^{3}J 10 Hz); 3.07, 3.01 (3H, 2 x s); 2.98-2.80 (4H, m); 1.75-1.60 (1H, m); 1.21 (3H, d, ^{3}J 7.5 Hz); 1.19 (3H, d, ^{3}J 7.5 Hz), (Figure 3.2). Underlined peaks are ascribed to the major rotamer.

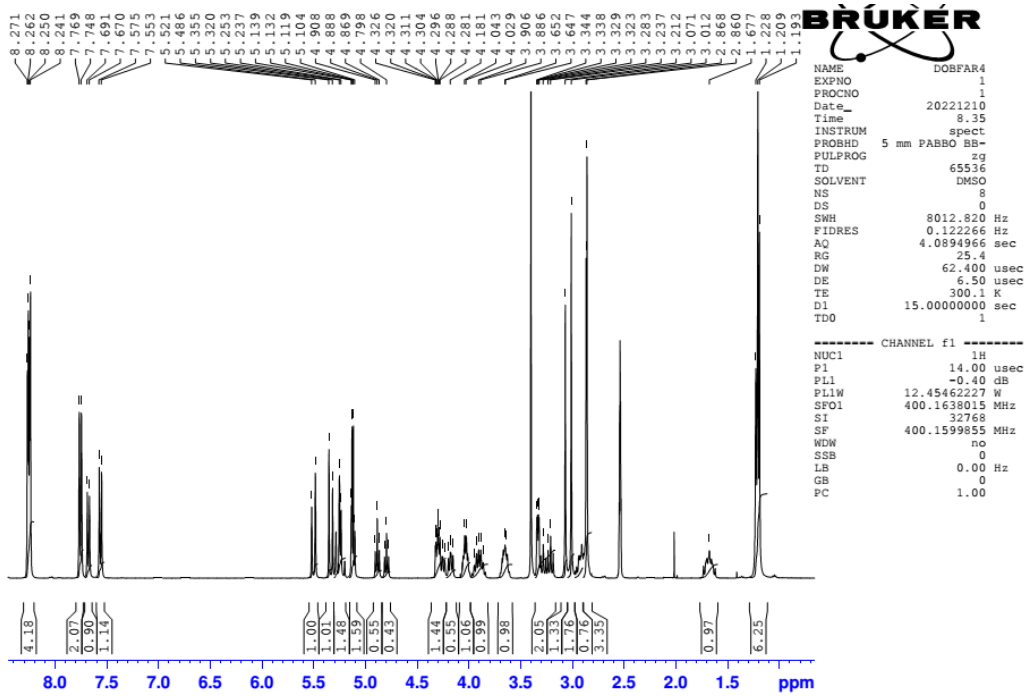


Figure 3.2. ^{1}H-NMR of bis-protected meropenem (**5**).

^{13}C NMR: (100 MHz, DMSO-d_6, 300 K): δC 175.02 (C), 174.90 (C), 171.51 (C), 171.15 (C), 160.73 (C), 153.75 (C), 153.48 (C), 151.15 (C), 151.08 (C), 147.86 (C), 147.72 (C), 145.46 (C), 145.50 (C), 145.46 (C), 145.50 (C), 129.08 (CH), 128.82 (CH), 128.56 (CH), 124.58 (C), 124.45 (C), 124.27 (CH), 124.18 (CH), 65.73 (CH_2), 65.63 (CH_2), 64.94 (CH), 64.89 (CH), 57.03 (CH), 56.67 (CH), 56.15 (CH), 43.83 (CH), 40.04 (CH), 37.15 (CH), 37.07 (CH), 36.54 (CH2), 35.82 (CH_2), 22.51 (CH3), 22.46 (CH_3), 17.84 (CH_3), (Figure 3.3).

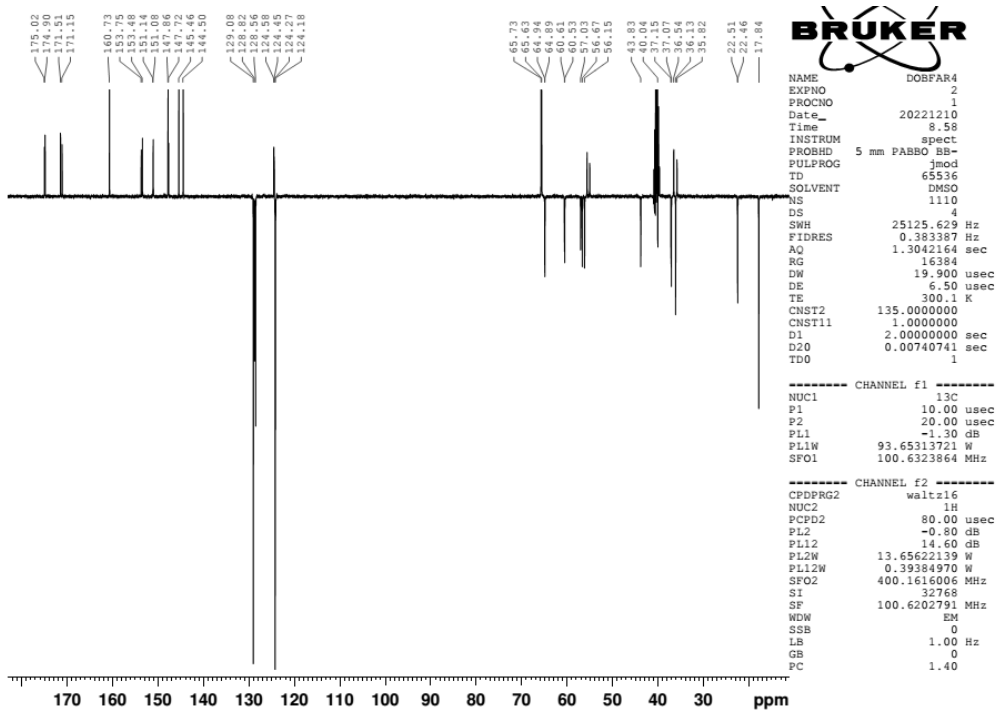


Figure 3.3. ^{13}C-NMR of bis-protected meropenem (**5**).

3.2.1.2 General procedure for the synthesis of Meropenem (6)

Bis-protected meropenem (**5**, 28.5 g, 40.8 mmol), acetic acid (1.4 g, 23.3 mmol), *N*-methyl morpholine (4.2 g, 41.5 mmol), 257 g tetrahydrofuran (THF), 282 g isopropyl alcohol and 428 g of water made up the starting solution, which was kept constant across all of the experiments. The initial concentration was about 40 mmol/L. *N*-methyl morpholine and acetic acid were used as buffers during the reaction allowing to work at pH 6.5 - 6.8. The catalyst 10% Pd/C was set as indicated in Table 1. The total volume of slurry during hydrogenation was approximately 1000 mL and had a density of 0.94 g/mL. After the hydrogenation experiments, the catalyst was filtered, and the aqueous phase was extracted with 285 mL of dichloromethane at room temperature (solution pH 5.3 - 5.5). 2850 mL of acetone was added to the aqueous phase for 3 h at 0-5 °C to crystallize the meropenem (**6**). The slurry was stirred for 60 min at 0-5 °C, filtered and washed with 30 mL of acetone. The crude product was dried for 30 min under vacuum with nitrogen flow.

The yield values are listed in Table 1 and Table 4. The HPLC assay gave a result of 98%. Purification: crude meropenem (**6**, 5.0 g, 11.4 mmol) was dissolved in 150 mL water with $NaHCO_3$ (0.1 g, 1.2 mmol) at 35 °C. After 5 min, the solution was cooled to 0-5 °C and treated with norite charcoal (0.5 g) for 1 h. The charcoal was removed by filtration and the filter cake was washed with 5 mL water. 0.5 g of seed was added, and the solution was stirred for 1 h at 0-5 °C. 450 mL acetone was added dropwise within 4 h and stirred for a further 30 min. The solid was collected by filtration, washed with 10 mL acetone and dried under vacuum for 1 h at room temperature to give pure meropenem (**6**) as a white solid (4.2 g, 84% yield). The HPLC assay gave a result of 99.5%.

^{1}H NMR (400 MHz, D_2O): δH 1.15 (3H, d, ^{3}J 7.6 Hz, 10-Me), 1.23 (3H, d, ^{3}J 6.4 Hz, 9-Me), 1.91 (1H, appt quint, J 6.8 Hz, H-15α), 2.94§ (3H, s, 17-Me), 3.02§ (3H, s, 18-Me), 3.06 (1H, m, H-15β), 3.31 (1H, dq,^{2}J 14.8 Hz, ^{3}J 7.2 Hz, H-1), 3.4-3.5 (2H, m, H-6/H-13α), 3.71 (1H, dd, ^{2}J 12.4 Hz, ^{3}J 6.4 Hz, H-13β), 4.00 (1H, appt quint, J 6.4 Hz, H-12), 4.15-4.25 (2H, m, H-5/H-8), 4.75 (1H, t, ^{3}J 8.8 Hz, H-14), (Figure 3.4).

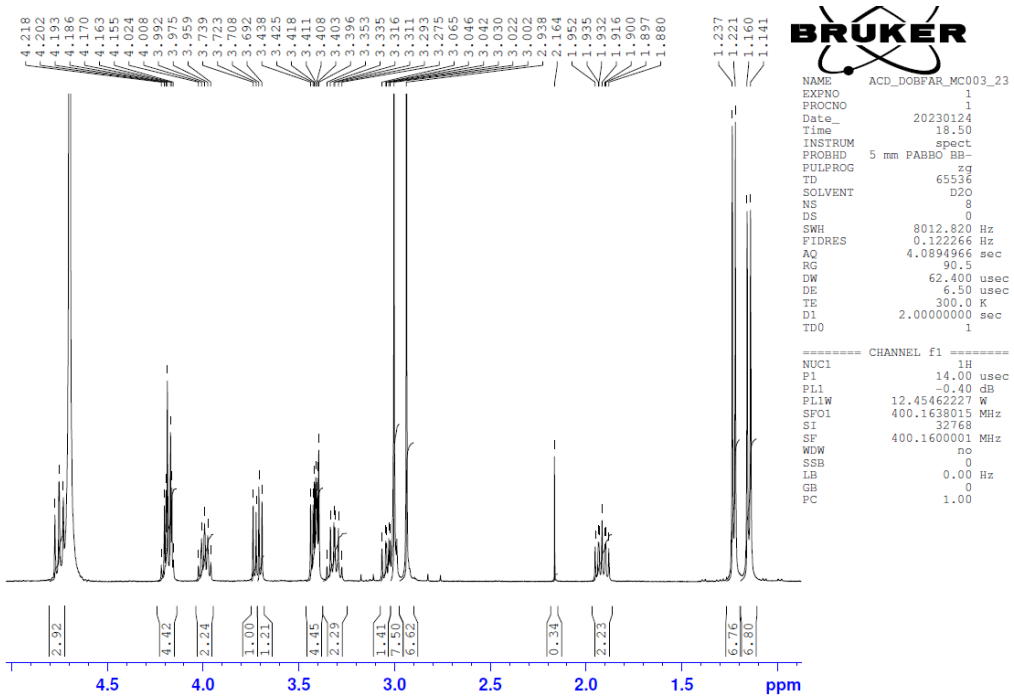


Figure 3.4. ^{1}H-NMR of meropenem (**6**).

^{13}C NMR (100 MHz, D_2O): δ_C 176.75 (C-11), 168.0 (C-16), 167.75 (C-7), 137.6 (C-2), 134.0 (C-3), 65.3 (C-8), 59.0 (C-6), 58.4 (C-14), 56.1 (C-5), 52.35 (C-13), 42.65 (C-1), 40.6 (C-12), 36.8 (C-18)#, 36.0 (C-17)#, 20.9 (C-9), 16.0 (C-10), (Figure 3.5).

§# may be interchanged, appt: apparent (first-order approximation).

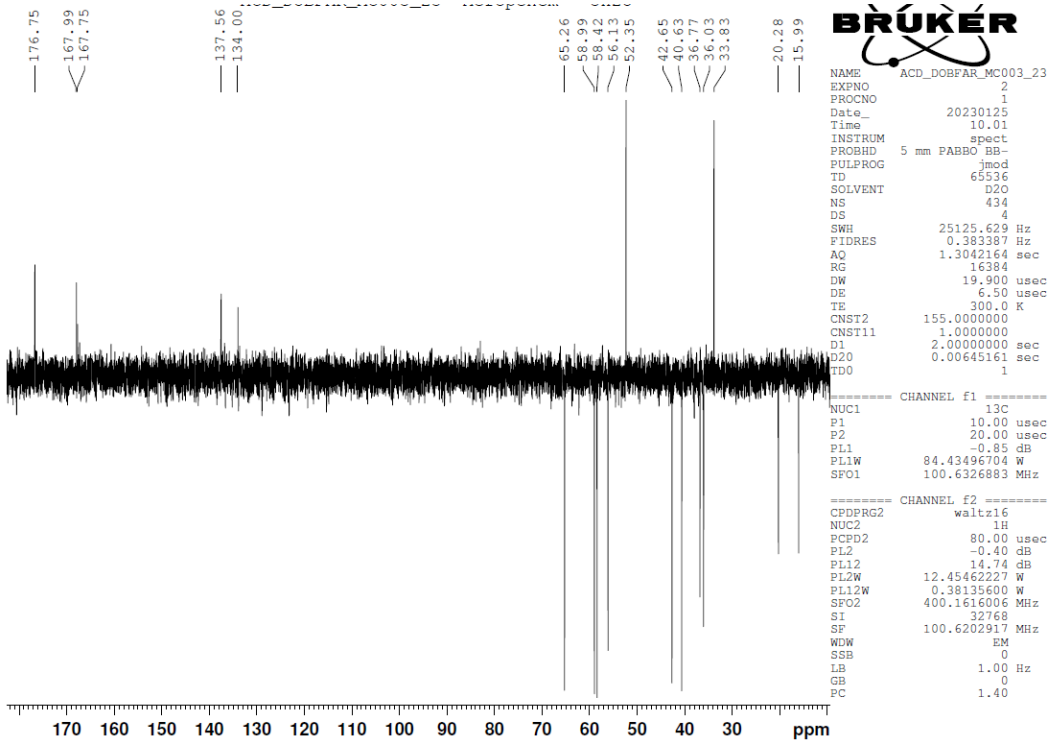


Figure 3.5. ^{13}C-NMR of meropenem (**6**).

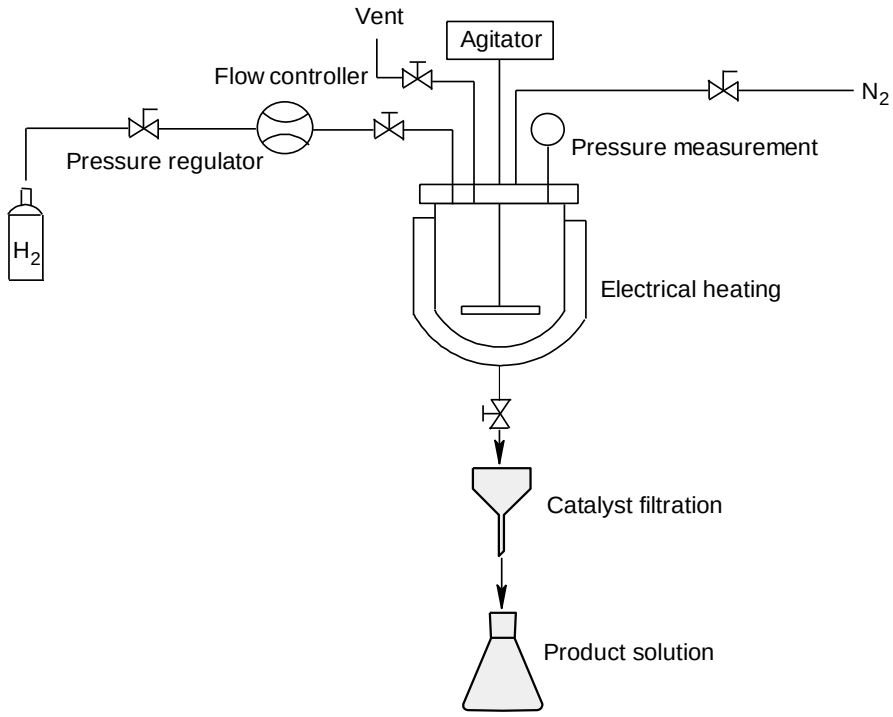
3.2.2 Hydrogenation equipment

3.2.2.1 Batch mode

A Parr reactor series 4578 High Pressure/High Temperature (Parr Instrument Company, Moline, USA) was used for the batch-mode experiments. A floor stand with a pneumatic lift mounted the fixed-head reactor, which has a volume of 1800 mL and is capable of working at up to 345 bar pressure and 500 °C temperature. A mass flowmeter placed in the hydrogen line allowed the reactor pressure, which

was measured with a pressure measurement on the top of the reactor, to be controlled. The temperature was kept constant using an electrically-driven serpentine. An impeller performed slurry stirring correctly. Scheme 3.2 depicts the reactor configuration used for batch-mode hydrogenation.

The bis-protected meropenem (**5**) solution was introduced into the reactor with the catalyst. Inertization was performed with three nitrogen purges at 5 bar, and the reactor was subsequently purged three times with hydrogen at 5 bar. The stirrer was set, and the hydrogen valve opened. A pressure ramp from 0 bar to final pressure (3 bar/min) was applied, as was a temperature ramp (2.5 °C/min). The temperature was kept constant at the final reaction temperature and the pressure was maintained using the hydrogen flowmeter (Table 3.1). The reaction was run until hydrogen uptake was completed, at which point the hydrogen flow was interrupted and the solution cooled to room temperature. The reactor was slowly vented to release the hydrogen and underwent inertization with three nitrogen purges at 5 bar. The slurry was filtered using nitrogen pressure on a Buchner funnel to remove the catalyst, allowing solution analysis to then be performed.

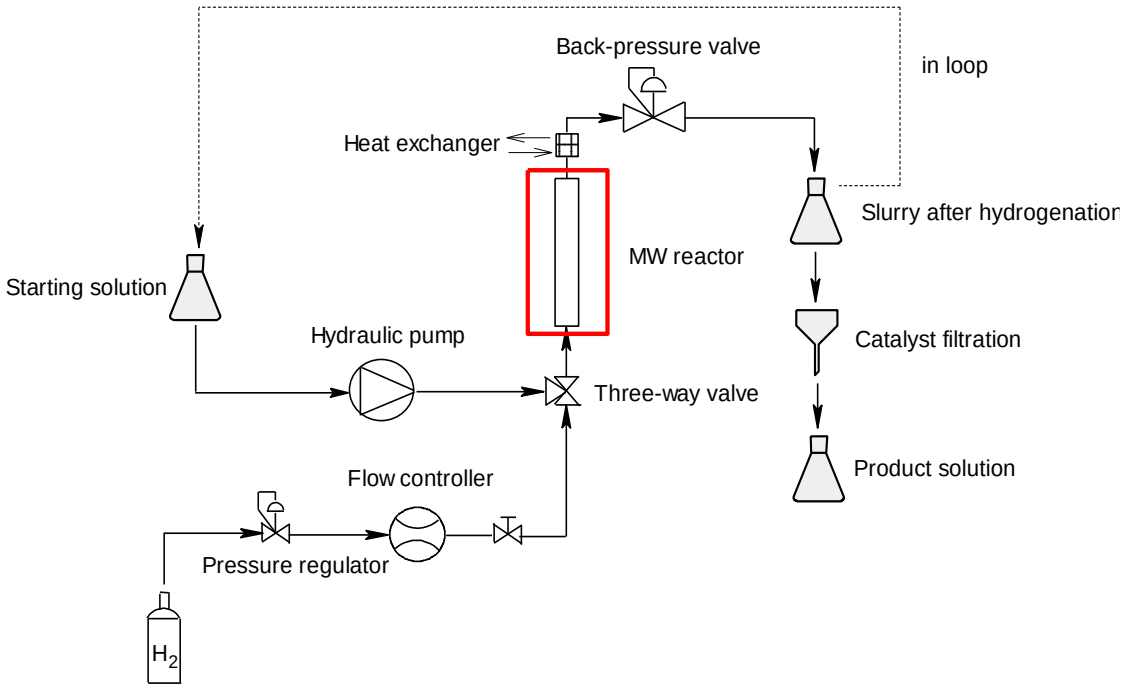


Scheme 3.2. Batch-reactor configuration.

3.2.2.2 MW-assisted flow mode

MW-assisted flow hydrogenation was carried out in the FlowSYNTH reactor (Milestone Srl, Bergamo, Italy), which is a multimode system that operates at 2.45 GHz and is equipped with a vertical PTFE-TFM flow-through reactor that can work up to a maximum temperature of 200 °C and 20 bar of pressure, in open or closed loop modes. A three-way connection fitted with a non-return valve allows the starting solution and hydrogen feeds to be pumped simultaneously. A back-pressure valve placed on the top of the reactor, after a water-cooled heat exchanger, ensures pressure control and flow-stream decompression, allowing the solution to be collected. For a single cycle run, the slurry was run in the reactor (V_R = 200 mL) using a hydraulic pump and was collected in an Erlenmeyer flask after catalyst filtration, and this was done in-loop for multiple-cycle runs. The starting solution and hydrogen flowed from the bottom to the top of the reactor, with the applied MW power being modified in real time to maintain the predefined temperature. The pressure was kept constant, using a hydrogen mass flowmeter,

at the indicated pressure. Integrated reactor sensors continuously monitored the internal pressure, temperature and applied power inside the reactor for each run. Leak tests were successfully performed first with water and then with isopropyl alcohol and nitrogen to test the pressure control. A general scheme of the FlowSYNTH reactor is depicted in Scheme 3.3.



Scheme 3.3. FlowSYNTH reactor configuration.

3.3 Results and discussion

As a first step, we focused on investigating the preferred reaction parameters for batch-mode hydrogenation in order to subsequently upload them for flow-mode use. The key factors in determining successful *p*NB/*p*NZ removal are pressure, temperature and Pd/C amount. As reported in Table 3.1, catalyst load has a decisive impact on the meropenem (**6**) yield from hydrogenation and influences how the process is run. Upon performing the deblock in the Parr reactor at 20 bar and 37 °C, we observed a drop-in yield and an increase in the reaction time when the catalyst

amount per test was halved. The same trend was observed when working at lower pressure (6.8 bar) and temperature (30 °C) but starting with a greater catalyst load. An inversely proportional relationship exists between these three parameters, and this had a significant impact on the choice of reaction conditions for the subsequent flow-mode experiments. In industry, reducing the amounts of the highly expensive catalysts would undoubtedly be impactful, but incompatible with the instability of the carbapenem nucleus under harsh conditions. We observed a reduction in yield even when the dry catalyst percentage and pressure value were kept constant, but the temperature increased (35 °C and 45 °C), demonstrating once again the need to work under moderate conditions. In all tests, the reagent conversion was complete, meaning that the reaction generated different undetectable impurities. Using a catalyst amount of 21% dry w/w for bis-protected meropenem (**5**), at 30 °C and 6.8 bar, granted a meropenem (**6**) yield in solution, after catalyst filtration, of 89%. We decided to only crystallize the hydrogenated solution formed under moderate conditions to have a comparison with the products investigated in flow mode.

Table 3.1. Screening of hydrogenation conditions in the Parr reactor.

Entry	H_2 (bar)	Temperature (°C)	Catalyst[a] (g)	Dry catalyst weight / substrate weight (%)	Time (min)	Meropenem (6) yield in solution (%) after catalyst filtration	Crude meropenem (6) yields (%)
1	20	37	5.7	10.0	30	66	-
2	20	37	2.8	5.0	60	63	-
3	20	37	1.4	2.5	120	53	-
4	6.8	30	12.0	21.0	30	89	74
5	6.8	30	6.0	10.5	60	84	71
6	6.8	35	6.0	10.5	60	75	-
7	6.8	45	6.0	10.5	60	60	-
8	6.8	30	3.0	5.3	90	71	61

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 3.2.1.2. The Parr reactor is described in Hydrogenation equipment in paragraph 3.2.2.1. [a] The 10% Pd/C is to be considered 50% wet.

The work-up of the aqueous solution together with the purifying effect of acetone, used in crystallization, led to the isolation of crude meropenem (**6**), in Table 3.1, with an assay result of 98% and a yield of 74%. Compared to the data reported in literature [40], our system has proven to be more performing. An LC-MS analysis of the product derived from Entry 4 identified two impurities: *p*-amino (**68**, 0.7%) and *p*-hydroxylamino (**69**, 0.3%) derivatives, which arose, after the removal of the *p*NZ side chain, from the stepwise reduction of the nitro group in the transient mono-protected *p*NB compound (Figure 3.6). These structures would seem to indicate that the reaction mechanism proposed in Figure 3.1. The purification of the crude product purged these impurities and gave a pure product with 99.5% assay result, comparable to commercial product.

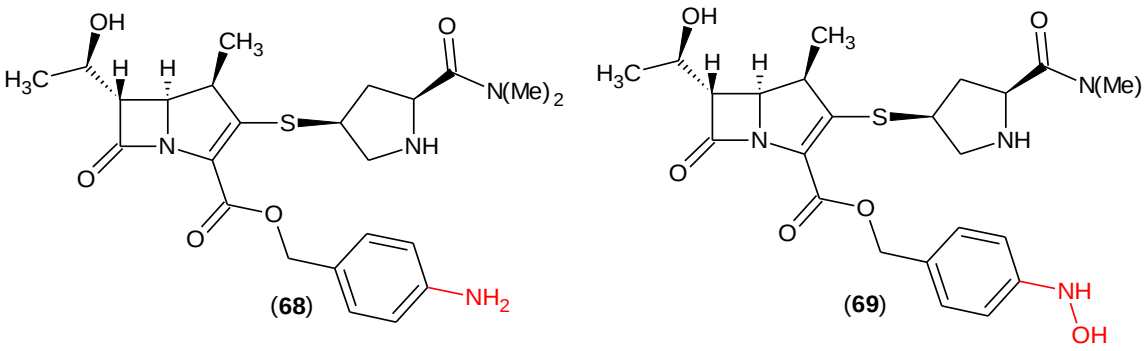


Figure 3.6. Impurities in crude meropenem (**6**), from Entry 4, investigated using LC-MS.

Meropenem (**6**) hydrogenation under MW-assisted flow chemistry has never been investigated. Moreover, this technology appears to me to be the most suitable for a study into the potential industrial production of Meropenem (**6**) as the drug is a blockbuster and manufactured on a large scale.

Having identified the best conditions for carrying out the hydrogenation in batch mode, the reaction was investigated using the FlowSYNTH reactor. The best

test (Entry 4) was taken as the reference point, and the catalyst amount for all experiments run in the MW-assisted flow reactor was not changed. As a first step, we focused on the influence of hydrogen pressure and reaction temperature, while keeping slurry-solution flow, residence time (t_r) and, consequently, run time constant. The concept of developing a quick process supported by leak tests with the slurry solution directed us towards using a short residence time. The slurry-solution flow was set at 150 NmL/min, the hydrogen flow rate between 150 – 200 NmL/min, with 80 s of residence time (considering only the liquid phase) and each run taking 7.5 min. The system was flushed with THF and nitrogen to clean the reactor and lines after each loop.

A pressure range of 4.0 bar to 8.0 bar was investigated, with MW irradiation being applied between 30 – 35 °C and the starting solution being flowed for one cycle. Under these conditions, we did not observe experimental evidence for the bis-protected meropenem (**5**) deblock in meropenem (**6**). Each analysis was performed on the aqueous solution after catalyst filtration.

Since pressure and temperature were observed to have no measurable impact, we decided to increase the residence time for the next experiments and use the same pressure conditions as Entry 4 (6.8 bar). This decision was taken to give more time to the hydrogen, with the catalyst, to react with the substrate. To ensure that the reactor, and consequently the slurry, are sufficiently pulsed with MW irradiation, the temperature was set at 35 °C, and, in order to achieve these new conditions, the slurry-solution flow was decreased and, accordingly, the run time was increased.

With the solution flow set at 105 mL/min and the hydrogen flow at 100 NmL/min, to give 115 s of residence time and 10.5 min of run time, we obtained a decrease in bis-protected meropenem (**5**) concentration in a single cycle, as indicated in Table 3.2. Compared to the batch experiments, an 88% value of raw materials was still high, and so the slurry was run until the complete disappearance of the substrate. After ten cycles and a total residence time of 1150 s (19 min), we obtained a meropenem (**6**) yield in solution of 69% with a bis-protected meropenem (**5**) residual of 3%. A progressive and constant decrease in the substrate has been observed without altering the catalyst activity.

Table 3.2. Semi-continuous hydrogenation test with t_r = 115 s for one loop at 6.8 bar.

Entry	Cycle	Bis-protected meropenem (5) residual (%)
1	1st	88
2	2nd	69
3	3rd	51
4	4th	38
5	5th	24
6	6th	16
7	7th	10
8	8th	5
9	9th	4
10	10th	3

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 3.2.1.2. The FlowSYNTH reactor is described in Materials and Methods in paragraph 3.2.2.2. The catalyst amount was 21% dry w/w with reference to bis-protected meropenem (**5**) as reported in Table 3.1, entry 4.

The same experiment was repeated with a solution flow of 57 mL/min and a run time of 19 min, maintaining the hydrogen flow rate at 100 NmL/min, in order to have a residence time of 210 s to carry out the synthesis; substrate hydrogenation was completed in four cycles, as indicated in Table 3.3. A meropenem (**6**) yield in solution of 67% was obtained with a bis-protected meropenem (**5**) residual of 3% in a total residence time of 840 s (14 min). These semi-continuous tests clearly show the considerable impact of residence time on the hydrogenation, making it a key parameter and necessary for reaction success. The synthesis was now performed in-loop, and the total residence time was increased to allow the reaction between hydrogen and the starting materials to

achieve protecting-group deblock. However, the slurry solution flow could not be reduced further to avoid the risks of clogging problems and pressure instability.

Table 3.3. In-loop hydrogenation test with t_r = 210 s for one loop and 6.8 bar.

Entry	Cycle	Bis-protected meropenem (5) residual (%)
1	1st	66
2	2nd	38
3	3rd	11
4	4th	3

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 3.2.1.2. The FlowSYNTH reactor is described in Materials and Methods in paragraph 3.2.2.2. The catalyst amount was 21% dry w/w with respect to bis-protected meropenem (**5**), as reported in Table 3.1, entry 4.

Taking the process parameters used in the Table 3.3 experiments as the best conditions, the hydrogenation was carried out at a higher temperature induced by MW irradiation. A clear difference between MW-assisted flow hydrogenation and the batch-mode process emerges in the data reported in Table 3.4. Passing from 35 °C to 45 °C, the yield was enhanced of more 10% (67% *vs* 80%), instead, a 5% drop in yield was observed from 45 °C to 55 °C. This trend represents a useful information for a future industrial application because it significantly highlights the subtle equilibrium between operating parameters with the carbapenem skeleton's instability. The combination between the better mixing in flow reactor with the uniform spread of MW irradiation have allowed to improve the meropenem (**6**) yield in solution after catalyst filtration, while the opposite result was observed in experiments in the Parr reactor upon increasing the temperature. A solution yield of 80% is slightly lower than the batch process data, as was the yield of the isolated product (70% *vs* 74%). However, the benefits of running a semi-continuous process would enhance productivity and eliminate the small yield deviation.

Table 3.4. In-loop hydrogenation test at 45 °C and 55 °C and 6.8 bar.

Entry	Cycles	Temperature (°C)	Total residence time (s)	Meropenem (6) yield in solution (%) after catalyst filtration	Crude meropenem (6) yields (%)
1	4	45	840	80	70
2	4	55	840	75	65

Reaction conditions: The reaction conditions are reported in Materials and Methods in paragraph 3.2.1.2. The FlowSYNTH reactor is described in Materials and Methods in paragraph 3.2.2.2. The catalyst amount was 21% dry w/w with respect to bis-protected meropenem (**5**), as reported in Table 3.1, entry 4.

It is worth noting that the product quality obtained using MW-assisted flow hydrogenation is equivalent to that obtained using the batch process (98%). An LC-MS investigation of the crude meropenem (**6**), from Entry 23, showed the same impurities as reported in Figure 3.6 at the same values, confirming that they are characteristic of this specific reaction applied to this carbapenem.

3.4 Conclusions

An experimental study into the MW-assisted catalytic hydrogenation of bis-protected meropenem (**5**) has been carried out. The FlowSYNTH reactor has been used to this purpose, giving a meropenem (**6**) yield in solution after catalyst filtration of 80% with an isolated-product yield of 70%. The optimization of residence time (840 s) and cycle number (4) has led to the development of a novel protocol that has halved the reaction time (14 min *vs* 30 min). Using MW irradiation, the deblock reaction was completed in only 14 min whereas the batch process required 30 min. To achieve this result, the solution underwent four cycles with a residence time of 210 s per loop, with a solution flow of 57 mL/min, at 45 °C and 6.8 bar. This technology has proven to be softer than the Parr reactor as it can achieve a similar yield result at a higher temperature. The carbapenem nucleus has been preserved and product quality is comparable to that of the batch product. Two characteristic impurities in the hydrogenation process have been found, and they are present both in the products of the MW-assisted flow process and the batch process, confirming that they are specific to this reaction. The results

obtained using this technology show, once again, its potential and adaptability, even in the synthesis of an important carbapenem, such as meropenem. It is our hope that this work can pave the way for new opportunities for this emerging technology in the pharmaceutical world.

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Chapter 4: Conclusions and Perspectives

In summary, two new and alternative synthetic protocols have been designed for two relevant β-lactams antibiotics, Cefonicid benzathine salt (**4**) and Meropenem (**6**), exploring environmentally friendly and cost-effective technologies from an industrial point of view and of process intensification.

Preserving the drugs high quality, the adaptability to great manufacturing sizes to support the massive global demands has been the driving forces for the development of new protocols.

In the context of industrial production of APIs and pharmaceutical intermediates, in which the balance between streamlining and enhancing productivity is necessary to compete in the global market, the design of new synthetic routes is the trump card.

Regarding Cefonicid benzathine salt (**4**), we have successfully developed a novel synthetic protocol for the synthesis of 7-amino-3-[sulphomethyl-1H-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt (**2**), its key intermediate, in a telescopic route and in about 70% overall yield from readily accessible 7-ACA (**1**). This process appears to be more compatible with the industrial scale and has some evident advantages over the existing synthetic procedures. By virtue of fine balance between equiv of 16% BF_3-MeCN and volume of MeCN (83% molar solution) the reaction occurred in only 30-40 min.

The work up avoids charcoal purification and loss in the mother liquor. A well-defined amount of water was enough to quench the reaction mixture. These improvements dramatically reduced the reaction time and increased productivity, making it more attractive for industrial production.

Continuing in the process, we have reported a new, reliable, efficient, and sustainable protocol for the production of cefonicid benzathine salt (**4**) as both oral formulations. Using the double alkaline and nucleophilic behavior of *N′,N″*-dibenzylethylenediamine diacetate (**3**), we have considerably streamlined the process, producing the drug with remarkable stability and with a good yield, making it ready for industrial scale-up. The extreme reactivity of the OH-protected formyl group proved to be the winning weapon for this type of amidation, considering the bulkiness of benzathine diacetate (**3**), while its partially lipophilic nature facilitates the precipitation of the drug from the water medium and reduces waste production. In the worldwide market for the production of APIs, where companies are striving

to find new ways to solve chemical-manufacturing challenges, we believe that this is a competitive process, able to be reducing waste and making it more reliable in terms of strict environmental concerns.

This new protocol has been submitted to be validated by FDA and EMA.

Regarding Meropenem (**6**), an experimental study into the MW-assisted catalytic hydrogenation of bis-protected meropenem (**5**) has been carried out. The FlowSYNTH reactor has been used to this purpose, giving a meropenem (**6**) yield in solution after catalyst filtration of 80% with an isolated-product yield of 70%. The optimization of residence time (840 s) and cycle number (4) has led to the development of a novel protocol that has halved the reaction time (14 min *vs* 30 min). Using MW irradiation, the deblock reaction was completed in only 14 min whereas the batch process required 30 min. To achieve this result, the solution underwent four cycles with a residence time of 210 s per loop, with a solution flow of 57 mL/min, at 45 °C and 6.8 bar. This technology has proven to be softer than the Parr reactor as it can achieve a similar yield result at a higher temperature. The carbapenem nucleus has been preserved and product quality is comparable to that of the batch product. Two characteristic impurities in the hydrogenation process have been found, and they are present both in the products of the MW-assisted flow process and the batch process, confirming that they are specific to this reaction. The results obtained using this technology show, once again, its potential and adaptability, even in the synthesis of an important carbapenem, such as meropenem.

The perspective for this study is the scale-up from laboratory to pilot scale.

Appendix

1. Refereed Journal Publications

[1] **M. Comito**, R. Monguzzi, S. Tagliapietra, G. Palmisano, G. Cravotto*, Efficient pilot-scale synthesis of key cefonicid intermediate at room temperature. Green Processing and Synthesis 2022, 11, 96-105.

[2] **M. Comito**, R. Monguzzi, S. Tagliapietra, G. Palmisano, G. Cravotto*, Cefonicid benzathine salt: a convenient, lean, and high-performance protocol to make an old cephalosporin shine. Antibiotics 2022, 11, 1095.

[3] **M. Comito**, R. Monguzzi, S. Tagliapietra, G. Palmisano, G. Cravotto*, Towards antibiotic synthesis in continuous-flow processes. Molecules 2023,28, 1421.

[4] **M. Comito**, R. Monguzzi, S. Tagliapietra, A. Maspero, G. Palmisano, G. Cravotto*, From batch to the semi-continuous flow hydrogenation of *p*NB, *p*NZ-protected meropenem. Pharmaceutics, 2023, 15, 1322.

M. Comito, R. Monguzzi, S. Tagliapietra, G. Palmisano, G. Cravotto*, Efficient pilot-scale synthesis of key cefonicid intermediate at room temperature. Green Processing and Synthesis 2022, 11, 96-105.

DE GRUYTER Green Processing and Synthesis 2022; 11: 96–105

Research Article

Marziale Comito, Riccardo Monguzzi, Silvia Tagliapietra, Giovanni Palmisano, and Giancarlo Cravotto*

Efficient pilot-scale synthesis of the key cefonicid intermediate at room temperature

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Abstract: Cefonicid is a common second-generation cephalosporin, and the 7-amino-3-[sulphomethyl-1-*H*-tetrazol-5-yl-thiomethyl]-3-cephem-4-carboxylate monosodium salt is a key synthetic intermediate in its preparation. Despite the considerable international demand for this antibiotic, its preparation is hampered by low synthetic yield, long reaction time, and time-consuming industrial filtration over charcoal after the purification step. In the context of the industrial production of pharmaceutical intermediates, in which the balance between streamlining and enhancing productivity is necessary in order to compete in the global active pharmaceutical ingredients (API) market, we have investigated an efficient and practical procedure for the synthesis of a key cefonicid intermediate that features a telescopic route whose synthetic steps are all performed at room temperature; from the displacement of the acetoxy group with boron trifluoride to crystallization without treatment with charcoal. In other words, a simpler, scalable, cost-effective and energy-saving protocol is herein reported as a means of moving towards commercial manufacturing. The optimization of the process parameters and the industrial-scale impact assessment should pave the way for industrialization.

Keywords: API production, cephalosporin, mild reaction conditions, pilot-scale method, energy saving

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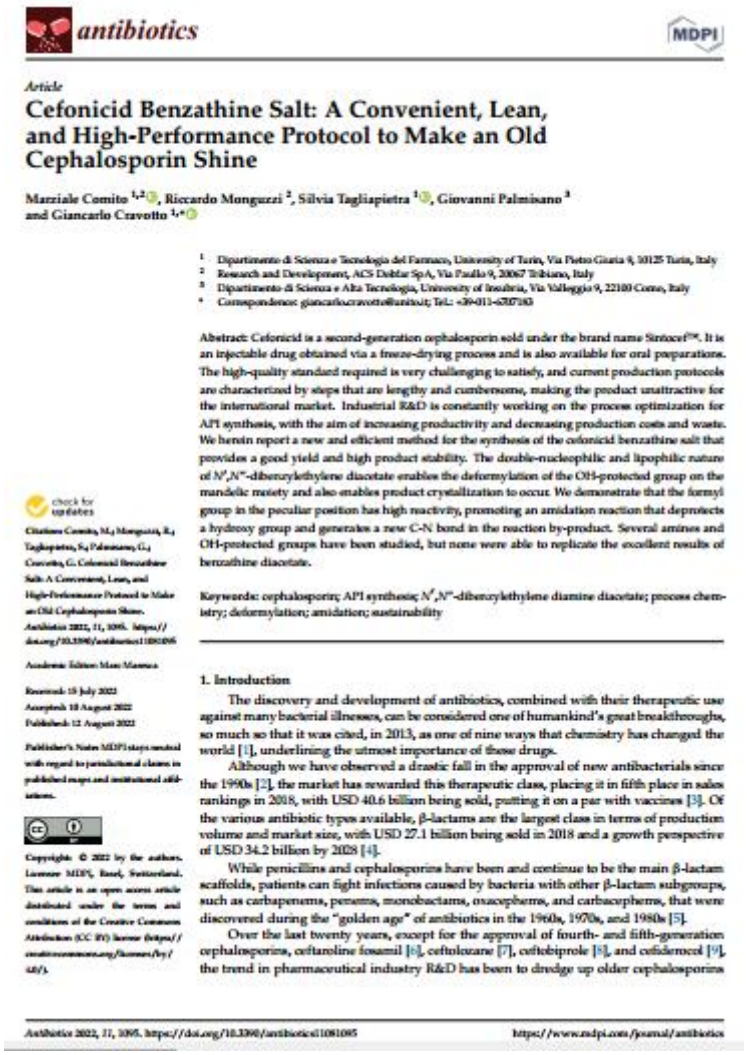
1 Introduction

β-Lactams (and β-lactamase inhibitors) are the most frequently employed class of bactericidal antibiotics, accounting for more than 60% of all antibiotics use with an annual expenditure of approx. 15 billion USD. These compounds share the 4-membered 2-azetidinone subunit as a common structural motif and can be classified into two subgroups according to their structural environment: (a) conventional β-lactams (penicillin (penams), cephalosporins, cephamycins, cephabacins) and (b) nonconventional β-lactams (clavams, carbapenems, nocardicins, monobactams) (see refs [1,2]; other relevant references are listed in the article of Southgate and Elson [3]). In the conventional β-lactams group, penams and cephalosporins deserve particular mention. Cephalosporins are reminiscent of penicillins in which the 5-membered thiazolidine ring of the penams is replaced by a 6-membered dihydrothiazine ring, and are categorized in five groups, or generations, depending on their activity spectrum and resistance to enzymatic hydrolysis.

7-Aminocephalosporanic acid (7-ACA) is the lead compound in the booming synthesis of cephalosporin, in which complex congeners (structures) are made in the hope of increasing their antibacterial activity (broad or selective) while minimizing inactivation by β-lactamase and cross-reactivity with cephalosporins and penicillins. Structure optimization is performed via the empirical (trial-and-error) systematic addition of a new chain at position 7 and/or the modification of the 3′-side chains embodied in the 7-ACA scaffold [4].

Cefonicid (CFND – [7-o-mandelamido-3-(1-sulfomethyl-tetrazol-5-yl-thiomethyl)-3-cephem-4-carboxylic acid, as disodium salt]) is a broad-spectrum second-generation cephalosporin that is resistant to β-lactamase, parenterally administered and used in the management of urinary tract infections, lower respiratory tract infections, and soft tissue and bone infections [5]. CFND (SKBF 75073) was patented by GlaxoSmithKline in 1978 [6], approved for medical use by the US Food and Drug Administration on July 26, 1993, and marketed under the brand name Monocid. CFND shares

M. Comito, R. Monguzzi, S. Tagliapietra, G. Palmisano, G. Cravotto*, Cefonicid benzathine salt: a convenient, lean, and high-performance protocol to make an old cephalosporin shine. Antibiotics 2022, 11, 1095.



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MDPI

Article

Cefonicid Benzathine Salt: A Convenient, Lean, and High-Performance Protocol to Make an Old Cephalosporin Shine

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Abstract: Cefonicid is a second-generation cephalosporin sold under the brand name Sinfocef™. It is an injectable drug obtained via a freeze-drying process and is also available for oral preparations. The high-quality standard required is very challenging to satisfy, and current production protocols are characterized by steps that are lengthy and cumbersome, making the product unattractive for the international market. Industrial R&D is constantly working on the process optimization for API synthesis, with the aim of increasing productivity and decreasing production costs and waste. We herein report a new and efficient method for the synthesis of the cefonicid benzathine salt that provides a good yield and high product stability. The double-nucleophilic and lipophilic nature of *N*′,*N*″-dibenzylethylene diacetate enables the deformylation of the OH-protected group on the mandelic moiety and also enables product crystallization to occur. We demonstrate that the formyl group in the peculiar position has high reactivity, promoting an amidation reaction that deprotects a hydroxy group and generates a new C-N bond in the reaction by-product. Several amines and OH-protected groups have been studied, but none were able to replicate the excellent results of benzathine diacetate.

Keywords: cephalosporin; API synthesis; *N*′,*N*″-dibenzylethylene diamine diacetate; process chemistry; deformylation; amidation; sustainability

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1. Introduction

The discovery and development of antibiotics, combined with their therapeutic use against many bacterial illnesses, can be considered one of humankind's great breakthroughs, so much so that it was cited, in 2013, as one of nine ways that chemistry has changed the world [1], underlining the utmost importance of these drugs.

Although we have observed a drastic fall in the approval of new antibacterials since the 1990s [2], the market has rewarded this therapeutic class, placing it in fifth place in sales rankings in 2018, with USD 40.6 billion being sold, putting it on a par with vaccines [3]. Of the various antibiotic types available, β-lactams are the largest class in terms of production volume and market size, with USD 27.1 billion being sold in 2018 and a growth perspective of USD 34.2 billion by 2028 [4].

While penicillins and cephalosporins have been and continue to be the main β-lactam scaffolds, patients can fight infections caused by bacteria with other β-lactam subgroups, such as carbapenems, penems, monobactams, oxacephems, and carbacephems, that were discovered during the "golden age" of antibiotics in the 1960s, 1970s, and 1980s [5].

Over the last twenty years, except for the approval of fourth- and fifth-generation cephalosporins, ceftaroline fosamil [6], ceftolozane [7], ceftobiprole [8], and cefiderocol [9], the trend in pharmaceutical industry R&D has been to dredge up older cephalosporins

M. Comito, R. Monguzzi, S. Tagliapietra, G. Palmisano, G. Cravotto*, Towards antibiotic synthesis in continuous-flow processes. Molecules 2023,28, 1421.

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Review

Towards Antibiotic Synthesis in Continuous-Flow Processes

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Abstract: Continuous-flow chemistry has become a mainstream process and a notable trend among emerging technologies for drug synthesis. It is routinely used in academic and industrial laboratories to generate a wide variety of molecules and building blocks. The advantages it provides, in terms of safety, speed, cost efficiency and small-equipment footprint compared to analog batch processes, have been known for some time. What has become even more important in recent years is its compliance with the quality objectives that are required by drug-development protocols that integrate inline analysis and purification tools. There can be no doubt that worldwide government agencies have strongly encouraged the study and implementation of this innovative, sustainable and environmentally friendly technology. In this brief review, we list and evaluate the development and applications of continuous-flow processes for antibiotic synthesis. This work spans the period of 2012–2022 and highlights the main cases in which either active ingredients or their intermediates were produced under continuous flow. We hope that this manuscript will provide an overview of the field and a starting point for a deeper understanding of the impact of flow chemistry on the broad panorama of antibiotic synthesis.

Keywords: flow chemistry; antibiotics; continuous process; drug synthesis; process control; industrial application; miniaturization

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1. Introduction

Advances in industrial organic synthesis are essential for the successful commercialization of innovative and efficient chemical–pharmaceutical manufacturing. Achievements, from the discovery of salvarsan to advanced therapy medicinal products (ATMPs), would have been impossible without cutting-edge technology and interdisciplinary collaboration [1–4]. The new technologies and modern trends in the synthesis of drugs and natural products that have been developed by academia and industry are opening up opportunities on a scale previously considered unattainable in most laboratories and production lines. The use of high-throughput and breakthrough technology platforms, particularly flow chemistry and process analytics (PAT), is representative of the endless potential in the pharmaceutical field and the improvements over the current state that are possible (Figure 1) [4–25].

In an era where sustainability is driving industrialization and innovation, in accordance with environmental friendliness and green chemistry concepts [26–37], the pharmaceutical industry is at the forefront of embracing and leading change. The pharmaceutical industry's mission is to provide patients with new medicines to help them live longer and healthier lives by creating small molecules in accordance with drug-development protocols. Until not so long ago, drug companies ignored risks to workers and the environment. Today, their approach has changed completely. In 2020, small-molecule drugs accounted for approximately USD 478 billion in sales in the global pharmaceutical markets, and this figure is expected to grow at 7% annually through to 2024 [38].

M. Comito, R. Monguzzi, S. Tagliapietra, A. Maspero, G. Palmisano, G. Cravotto*, From batch to the semi-continuous flow hydrogenation of pNB, pNZ-protected meropenem. Pharmaceutics, 2023, 15, 1322.

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Article

From Batch to the Semi-Continuous Flow Hydrogenation of *p*NB, *p*NZ-Protected Meropenem

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Abstract: Meropenem is currently the most common carbapenem in clinical applications. Industrially, the final synthetic step is characterized by a heterogeneous catalytic hydrogenation in batch mode with hydrogen and Pd/C. The required high-quality standard is very difficult to meet and specific conditions are required to remove both protecting groups [i.e., *p*-nitrobenzyl (pNB) and *p*-nitrobenzyloxycarbonyl (pNZ)] simultaneously. The three-phase gas–liquid–solid system makes this step difficult and unsafe. The introduction of new technologies for small-molecule synthesis in recent years has opened up new landscapes in process chemistry. In this context, we have investigated meropenem hydrogenolysis using microwave (MW)-assisted flow chemistry for use as a new technology with industrial prospects. The reaction parameters (catalyst amount, T, P, residence time, flow rate) in the move from the batch process to semi-continuous flow were investigated under mild conditions to determine their influence on the reaction rate. The optimization of the residence time (840 s) and the number of cycles (4) allowed us to develop a novel protocol that halves the reaction time compared to batch production (14 min vs. 30 min) while maintaining the same product quality. The increase in productivity using this semi-continuous flow technique compensates for the slightly lower yield (70% vs. 74%) obtained in batch mode.

Keywords: flow chemistry; microwave-assisted; semi-continuous synthesis; meropenem; hydrogenation; drug synthesis; heterogeneous catalysis; miniaturization; sustainability

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1. Introduction

Carbapenems are currently considered to be lifesaving drugs [1], with their broad-spectrum activity meaning that they play a vital role for human health. They have the greatest potency of all β-lactam antibiotics against Gram-positive and Gram-negative bacteria. Their medical use is so crucial and essential that they have been defined "last-line antibiotics" or "antibiotics of last resort", as they are the most effective weapon against known and suspected multidrug-resistant (MDR) bacterial infections [2–26].

Part of the carbapenem antibiotics subgroup, meropenem (1, Figure 1) is currently the most widely used in clinical treatment [27–29]. Its worldwide market was valued at USD 1.7 billion in 2021 and is expected to grow to USD 2.1 billion by 2027, making this drug a blockbuster [30]. Alone, it made up 43% of the USD 3.9 billion in global sales of the carbapenem family in 2021 [31]. It was discovered by Sumitomo Dainippon Pharma, now Sumitomo Pharma, in 1983 [32–34], and was approved by the Food and Drug Administration (FDA) in 1996 [35]. The drug is sold under the brand name Merrem™, as a generic drug substance, or in combination with vaborbactam, part of a new generation of β-lactamase inhibitors, under the brand name Vabomere™. It is administered intravenously

Resume

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Scientific Research Achievements

Publications

1. **M. Comito,** R. Monguzzi, S. Tagliapietra, G. Palmisano, G. Cravotto*, Efficient pilot-scale synthesis of key cefonicid intermediate at room temperature. Green Processing and Synthesis 2022, 11, 96-105.
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