Synthesis and Chemistry of Carbocyclic Mimics of β-Lactams as β-Lactamase Inhibitors

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Antibiotics are very important chemical substances, which have saved millions of lives. However, bacterial resistance towards antibiotics, especially the β-lactams, is becoming increasingly serious. Currently, the β-lactam antibiotics are seriously threatened by β-lactamases that are able to efficiently hydrolyze the β-lactam. According to Ambler's classification, Class A, C and D enzymes are serine β-lactamases (SBLs), in which the hydrolysis of β-lactams is initiated by the serine residues in their active sites, whereas Class B enzymes are metallo β-lactamases (MBLs), which contain a zinc-bound hydroxide as the nucleophile to hydrolyze the β-lactams.

At present, two feasible strategies are used to relieve the threat of β-lactamases, including discovery of novel antibiotics and development of β-lactamase inhibitors. The cyclobutanone mimics of β-lactam antibiotics were first studied in the early 1980s. They are potential inhibitors of β-lactamases because they might form an enzyme-bound hemiketal or an enzyme-bound hydrate with the serine β-lactamases or metallo β-lactamases, respectively. In addition, due to the similar structure to the corresponding β-lactams, they might inhibit D-Ala-D-Ala transpeptidases, which are the targets of the β-lactam antibiotics.

An efficient procedure to prepare the parent cyclobutanone **2.5α** was successfully developed in this lab, providing 33% overall yield for 7 steps. In addition, a series of cyclobutanone analogues were prepared in this lab previously. Among them, the C3α-OMe substituted cyclobutanone **2.30α** showed considerable inhibition of common β-lactamases including KPC-2, IMP-1, VIM-2, GC1 and OXA-10.

In this thesis work, the preparation of the parent cyclobutanone **2.5α** is discussed in detail, which then was converted to the corresponding benzhydryl ester **2.44**. Synthetic studies

towards C7-modified cyclobutanone derivatives are described, involving a very essential condition for the monodechlorination of **2.44**, which is considered as a milestone for this project. This opened the door for further modification at C7 of cyclobutanones. Next, hydroxymethylation was carried out at the C7 position through a known aldol condensation procedure, which provided C7-chloro-hydroxymethyl derivative **2.60β**. Fortunately, the single crystal structure was obtained successfully, confirming the stereochemistry of **2.60β**. In addition, further dechlorination of **2.60β** was performed in order to obtain the cyclobutanone derivative **3.50β** with the alternative configuration at C7 bearing the hydroxymethyl side chain, which might be a potential β-lactamase inhibitor. Benzoylation of the C7-chloro-hydroxymethyl derivative **2.60β** was successful. This paves the way to introduce various functional groups to the side chain to interact with the active sites of β-lactamases.

Eventually, the corresponding hydrate formation and bioactivity tests of the newly prepared cyclobutanones were carried out. Monochloro acid **2.58β** generated 25% of hydrate at equilibrium and showed considerable inhibition of some Class B and Class C β-lactamases such as IMP-1, VIM-2, SPM-1, L1 and GC1, when at a high concentration (500 μM). Cyclobutanone **2.58β** combined with meropenem exhibited a synergistic inhibitory effect against a clinical isolate of the human pathogen *Stenotrophomonas maltophilia* that produces the β-lactamases L1 and L2.

Acknowledgements

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Å	angstrom	*E. coli*	*Escherichia coli*
A. baumannii	*Acinetobacter baumannii*	EDTA	ethylenediaminetetra acetic acid
Ac	acetyl		
7-ACA	7-aminocephalosporanic acid	ESBL	extend-spectrum β-lactamase
Ala	alanine	gem	geminal
6-APA	6-aminopenicillanic acid	Glu	glutamic acid
Aq.	aqueous	Gln	glutamine
Asp	aspartic acid	g	gram
B. cereus	*Bacillus cereus*	h	hour(s)
Boc	*tert*-butyloxycarbonyl	HMBC	hetero nuclear multiple bond correlation
Bp	boiling point		
br	broad	HMQC	hetero nuclear multiple quantum coherence
Bz	benzoyl		
calcd.	calculated	HPLC	high performance liquid chromatography
conc.	concentrated		
Cy	cyclohexyl	HRMS	high-resolution mass spectrum
Cys	cysteine		
DAP	diaminopimelic acid	His	histidine
DAST	diethylaminosulfur trifluoride	HMW	high molecular weight
		HOBT	hydroxybenzotriazole
DCC	*N*,*N*'-dicyclohexyl carbodiimide	H-W-E	Horner–Wadsworth–Emmons olefination
Decomp.	decomposition	IC_{50}	concentration of inhibitor that reduces the maximum rate of enzymatic activity by 50%
DHP-I	dehydropeptidase I		
DIPEA	diisopropylethylamine		
DMAP	4-dimethylaminopyridine		
DMF	*N*,*N*-dimethylformamide	*K. pneumoniae*	*Klebsiella pneumoniae*
E2	second-order elimination	Lcx	carboxylated lysine

L	liter
LDA	lithium diisopropylamide
LMW	low molecular weight
μ	micro
m	milli
M	molar
MBL	metallo β-lactamase
Me	methyl
MIC	Minimum Inhibitory Concentration
min	minute(s)
MRSA	methicillin-resistant *Staphylococcus aureus*
Ms	mesyl
MW	molecular weight
NAG	*N*-acetylglucosamine
NaHMDS	sodium bis(trimethylsilyl)amide
NAM	*N*-acetylmuramic acid
*n*Bu	normal butyl
NCS	*N*-chlorosuccinimide
NFSI	*N*-fluorobenzensulfonimide
NMR	nuclear magnetic resonance
N.R.	no reaction
OR	over reduction
P. aeruginosa	*Pseudomonas aeruginosa*
PAS	penicillanic acid sulfone
PBP	penicillin binding protein
Ph	phenyl
PMB	*para*-methoxybenzyl
ppm	parts per million (spectral)
P. putida	*Pseudomonas putida*
p-Tol	*para*-tolyl
Py.	pyridine
RO	ring-opening
r.t.	room temperature
S. aureus	*Staphylococcus aureus*
SBL	serine β-lactamase
Ser	serine
SM	starting material
S. maltophilia	*Stenotrophomonas maltophilia*
S_N1	unimolecular nucleophilic substitution
*t*Bu	*tert*-butyl
TEA	triethylamine
temp.	temperature
TFE	trifluoroethyl
TMBz	trimethoxybenzene
TMS	trimethylsilyl; tetramethylsilane (spectral)
TS	transition state
Ts	tosyl
Tyr	tyrosine
VRE	vancomycin-resistant *Enterococcus spp*
VRSA	vancomycin-resistant *S. aureus*

Chapter 1 Introduction

Antibiotics are chemical substances that have high activity against the growth of bacteria or other microbes at low concentration. Many antibiotics are commonly produced by microorganisms and other living systems while some are obtained from synthesis. This definition of an antibiotic was first proposed by Selman Waksman in 1942, who was awarded the Nobel Prize ten years later for the discovery of streptomycin, an effective antibiotic to treat tuberculosis.[1] Mankind has greatly benefited from antibiotics that have saved millions of lives. Long before modern antibiotics were discovered, ancient people around the world had been applying various substances such as bean curd, bread mould, warm soil, oil cake, beer soup and a mixture of frog bile and sour milk that have antibiotic effects to treat wounds and infections.[1, 2]

The number of natural antibiotics dramatically increased in the last six decades, starting from 30 discovered by 1945 to 16500 by 2005.[2] According to the characteristic of their chemical structures, antibiotics are generally classified into several categories including aminoglycosides, ansamacrolides, β-lactams, chloramphenicol, glycopeptides, lincomycin, lipopeptides, macrolides, polyethers, tetracyclines, and fluoroquinolones (Figure 1).[2] Among them, the β-lactam antibiotics are one of the most important classes in the world, which account for more than half of the prescriptions for antibiotics worldwide.[3, 4] The β-lactam antibiotics can be used to treat both Gram-positive and Gram-negative bacterial infections. They target bacterial enzymes called Penicillin-Binding Proteins (PBPs) that perform key functions in the biosynthesis and remodeling of the peptidoglycan structure of the bacterial cell wall,[5] which is a process that is unique to bacteria. β-Lactams are more effective than other antibiotics that aim at the intracellular substances since PBPs are more easily accessed.[6]

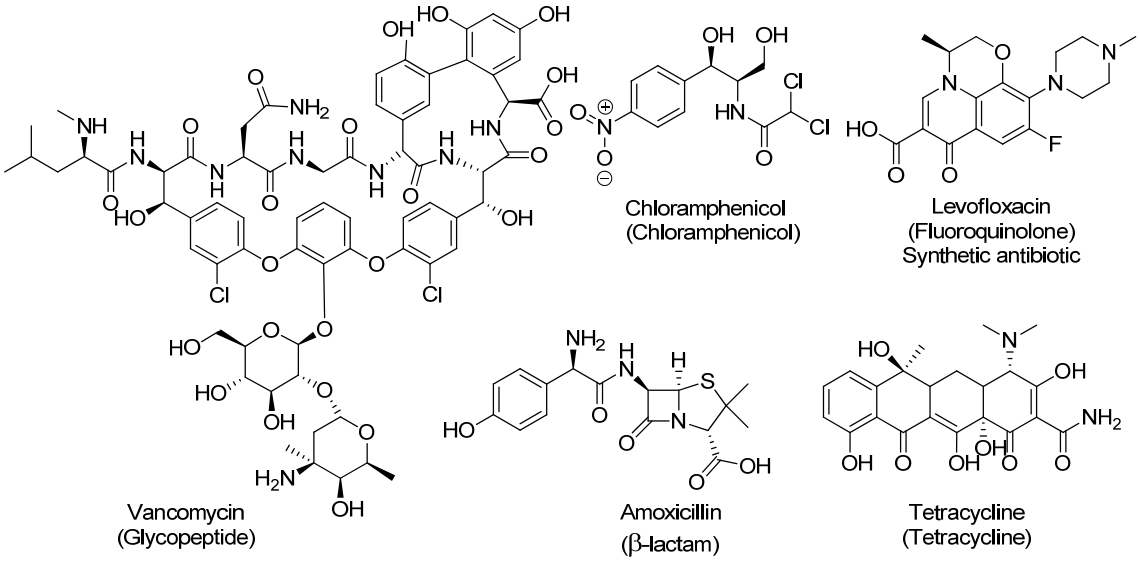


Figure 1. Chemical structures for some examples of major classes of antibiotics

The widespread use and the unfortunate abuse of antibiotics stimulate the evolution of bacteria,[7] many of which have developed resistance, by various mechanisms, towards every major class of existing antibiotics, particularly the β-lactams. As early as the 1980s, methicillin-resistant *Staphylococcus aureus* (MRSA) was noted in the United States. This resistance became a serious problem because MRSA could resist all the β-lactams. With the wide emergence of MRSA, the usage of vancomycin was significantly increased, since vancomycin was the only antibiotic available at that time to effectively treat MRSA infection. This inevitably led to vancomycin-resistant *S. aureus* (VRSA) and vancomycin-resistant *Enterococcus* spp. (VRE), and this severe vancomycin-resistance problem eventually developed into a hospital crisis by 1992.[8] Obviously, new antibiotics with improved antibiotic activity are urgently needed in order to limit bacterial resistance and maintain the effectiveness of antibiotics.[9]

Generally, there are four fundamental mechanisms of bacterial resistance to antibiotics, including enzymatic degradation of antibiotics, alteration of the antibiotic targets, decrease of

membrane permeability to antibiotics, and efflux.[10] For β-lactam antibiotics, the most significant bacterial resistance is caused by β-lactamases that can hydrolyze β-lactams, especially in Gram-negative bacteria. β-Lactamases consist of two types, the serine β-lactamases (SBLs, which are further grouped as Class A, C and D) and the metallo β-lactamases (MBLs, also known as the Class B β-lactamases). More details about the β-lactamases will be provided in Section 1.3. Another important resistance mechanism is that some penicillin-binding proteins (PBPs) could alter the β-lactam targets, particularly in Gram-positive bacteria.[11]

1.1 β-Lactam Antibiotics

All β-lactam antibiotics possess an essential four-membered amide (lactam) ring, which are commonly fused with a five or a six-membered ring (except the monobactams). In general, β-lactam antibiotics are classified into five groups depending on the difference of the non-lactam rings, including penicillins, cephalosporins, carbapenems, penems and monobactams, and some of the typical examples are shown in Figure 2.

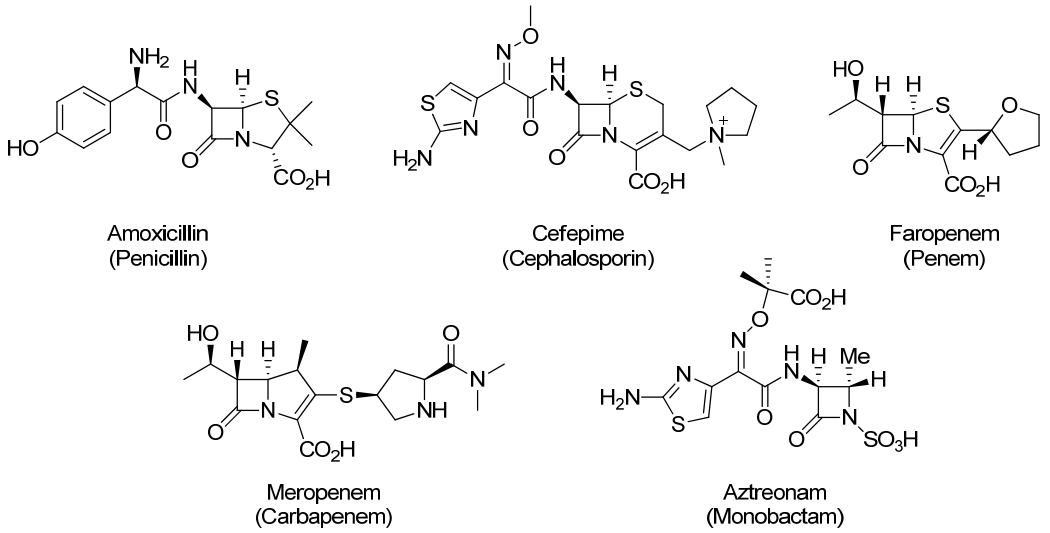


Figure 2. Structures of some typical β-lactam antibiotics

1.1.1 Penicillins

On September 28, 1928, the Scottish bacteriologist Alexander Fleming accidently discovered a bluish-greenish mould from the Petri dishes left on the bench for several days at the Saint Mary's Hospital in London. This mould, later named as *Penicillium notatum*, was found to be able to produce a liquid substance that could inhibit the growth of *Staphylococci* and it was named penicillin.[12] In 1929, Fleming reported that penicillin had potential therapeutic application, since it had great activity against several human pathogenic species including *Staphylococcus*, *Streptococcus*, *Pneumococcus* and *Gonococcus*. More importantly, penicillin showed low toxicity to experimental animals such as mice and rabbits.[13]

No significant research progress on penicillin was made until 1938, when a German-born British biochemist Ernst Chain was invited by Sir Howard Florey to join his group in the Sir William Dunn School of Pathology at the University of Oxford, where they began to study some naturally produced antibacterial substances including penicillin.[14] In 1941, an advanced procedure for purifying penicillin was developed by Norman Heatley of the Florey group. Extraction of crude penicillin by this method and further purification provided a 50% pure penicillin at the end of 1941.[15, 16]

In 1942, the Florey group started to collaborate with Sir Robert Robinson at the Dyson Perrins Lab at Oxford to determine the structure of penicillin, which was just known as a carboxylic acid at the time.[16] The structural determination focused on the degradation products of penicillin such as penicillamine and 2-pentenylpenilloaldehyde (Figure 3). Later in 1943, it was discovered by Dorothy Hodgkin and Wilson Baker that penicillin contained C, H, N, O and S through elemental analysis of the penicillaminic acid and penicillamine.[16]

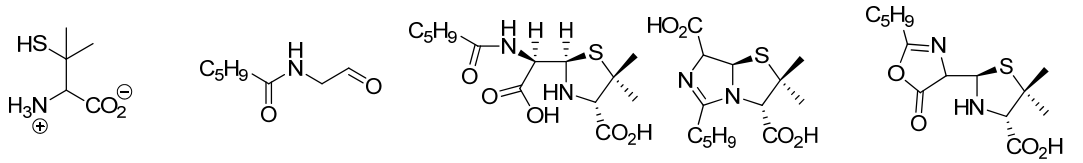
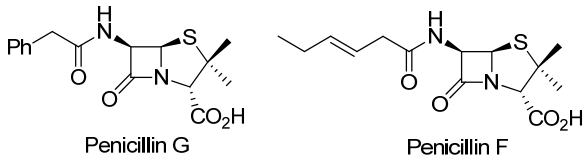


Figure 3. Structures of degradation products of penicillin F

In August 1943, US researchers Wintersteiner, MacPhillamy and Alicino at Squibb, obtained a crystal of the sodium salt of penicillin and they reported a molecular formula of $C_{16}H_{17}O_4N_2SNa$.[16] Following this discovery, the Florey group crystallized a barium salt of penicillin that provided another formula of $(C_{14}H_{19}O_4N_2S)_2Ba$.[16] Later on, the American penicillin was found to contain a benzyl group and named as penicillin G while the Oxford penicillin was named as penicillin F that contained a Δ^2-pentenyl group.[17]

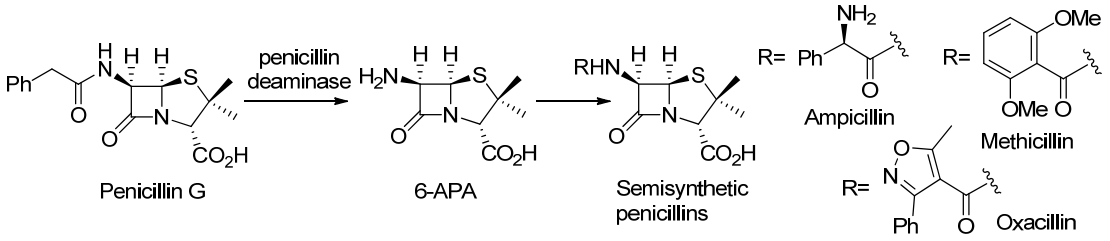


Right after the confirmation of penicillin's molecular formula, Robinson found that penicillin could be hydrolyzed to penicilloic acid under alkali conditions, and then he proposed a thiazolidine-oxazolone structure (Figure 3) for the Oxford penicillin. He also assigned a correct structure for an isomeric penillic acid possessing two acid groups and an imidazoline ring, which was formed from penicillin at pH 2 (Figure 3).[16,17] From then on, several different structures were proposed for penicillin. The β-lactam structure proposed by Abraham and Chain in 1943 was indeed the correct one. The debate on the structure of penicillin did not stop until 1945, when the structure was unambiguously confirmed by X-ray crystallographic analysis by Crowfoot and Low at Oxford, which demonstrated the presence of a β-lactam ring from the crystals of the Na, K and Rb salts of penicillin G.[18]

The discovery of penicillin created a new era for antibiotics and its clinical use has

saved numerous lives, and it became one of the most important antibiotics in human history. Sir Alexander Fleming, Ernst Chain and Howard Florey were awarded the Nobel Prize in the field of medicine in 1945 for their contributions to the discovery and application of penicillin.

The chemical synthesis of penicillin was extremely difficult even after its structure was determined. Large scale production of penicillin still relied on fermentation methods until 1957, when the first reasonable total synthesis of penicillin was reported by Sheehan that offered penicillin V in about 10% yield.[19] Nowadays, the majority of clinical penicillins such as ampicillin, methicillin, oxacillin and so forth, are obtained by semi-synthesis starting from 6-aminopenicilllanic acid (6-APA), which is produced in industrial scale fermentations of *Penicillium chrysogenum*.[20]



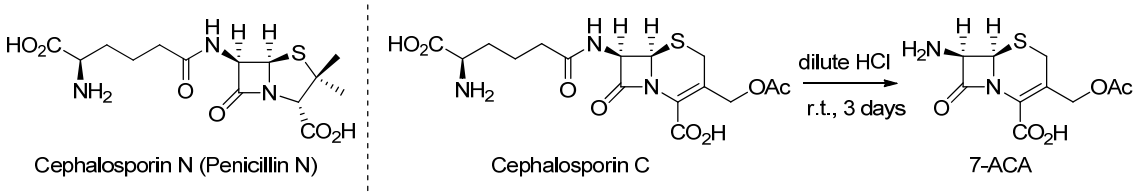
Scheme 1. Preparation of 6-APA and semi-synthetic penicillins

1.1.2 Cephalosporins

In 1945, Giuseppe Brotzu, an Italian professor studying hygiene at the University of Cagliari, noticed that the young people who regularly swam at Su Siccu Bay, near the end of the city sewer system, were less likely to suffer from typhoid fever.[12] Brotzu tested the sewer water sample of the bay and found that it could inhibit the growth of *Salmonella typhi* culture. Later, he discovered that some substance produced by a fungus in the sewer water had potent activity against Gram-negative bacteria. The fungus was isolated and named *Cephalosporium acremonium*. Further studies showed that this fungus had broad-spectrum antibacterial activity and Brotzu then sent a sample of this organism to the Florey group at Oxford.[21]

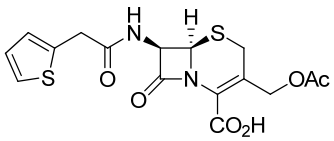
In Florey's lab, the first antibiotic extracted from *Cephalosporium acremonium* was named cephalosporin P, which showed activity against certain Gram-positive bacteria. In 1951, a second compound extracted from this organism was named as cephalosporin N (also known as penicillin N), which had broad spectrum activity against both Gram-positive and Gram-negative bacteria. Unfortunately, the bioactivity of cephalosporin N was significantly decreased in the presence of a penicillinase (a β-lactamase) that was produced by *Bacillus subtilis*.[12] In 1953, a third compound was extracted from *Cephalosporium acremonium* by Guy Newton and Edward Abraham, which was then named cephalosporin C and which possessed a broad spectrum activity against many *Staphylococus aureus* strains.[12] Later on, it was found that this compound presented greater resistance to β-lactamases and it saved mice infected by penicillin-resistant *Staphylococci*.[12,21] The structure of cephalosporin C was determined by the Oxford chemical crystallography lab of Dorothy Crowfoot Hodgkin in 1961.[22]

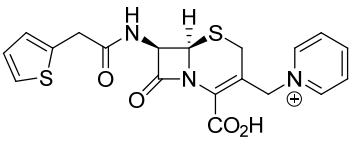
Because of the significant bioactivities of cephalosporin C, a large number of chemists were involved in its total synthesis. The first total synthesis of cephalosporin C was published by Woodward in 1966. At present, cephalosporin C is mainly produced by fermentation of *Acremonium chrysogenum*.[23,24] Upon treatment with dilute acid (Scheme 2), it is converted into 7-aminocephalosporanic acid (7-ACA),[25] which is the essential intermediate for producing various cephalosporin derivatives. Thus, cephalosporin C became the prime starting point to develop several generations of (semi-synthetic) cephalosporins.

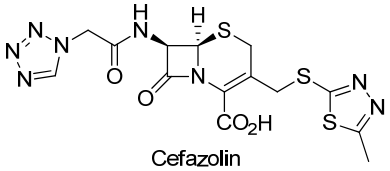


Scheme 2. Cephalosporins N, C and preparation of 7-ACA

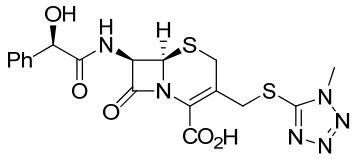
Based on their time of development and biological characteristics, cephalosporins have been classified into five generations so far. The first generation of cephalosporins was developed in the mid-1960s and included cephalothin, cephaloridine, cefazolin and others, which possess simple 7β-acylamino side chains. The antibiotics of this family have a decent capacity to eliminate Gram-positive pathogens such as *Staphylococci* and *Streptococci*. However, their ability to fight against Gram-negative bacteria is modest.[21]

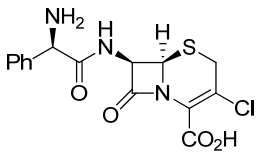

Cefalothin

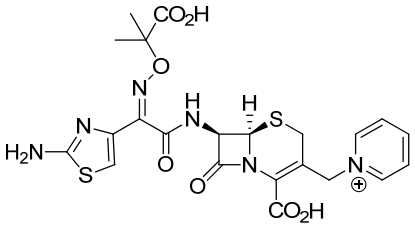

Cephaloridine


Cefazolin

The second generation cephalosporins were developed between the late 1970s and early 1980s. In these cephalosporins, the 7β-acylamino side chain was significantly modified and the 3' substituent was altered as well. This group of cephalosporins, including cefamandole, cefaclor and ceftazidime and others, have slightly less activity against Gram-positive bacteria when compared to the first generation. However, they have better stability towards β-lactamases and are more efficient at eliminating Gram-negative bacteria.[17,21]

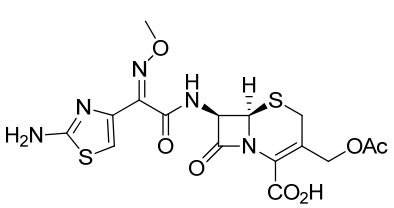

cefamandole

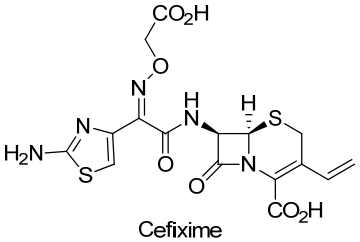

cefaclor

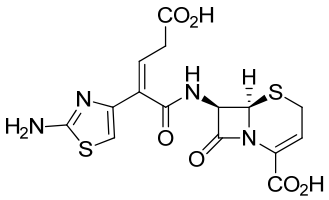

ceftazidime

In the 1980s, the third generation cephalosporins were introduced into clinical use. Generally, they are more expensive than the previous two generations, but they are extended-spectrum antibiotics with much better resistance to β-lactamases. Members of this category (e.g. cefotaxime, cefixime and ceftbuten) possess a 7β-(aminothiazoyl)-oxyiminoacetamido moiety, which plays a crucial role in their resistance to β-lactamases, especially the class A

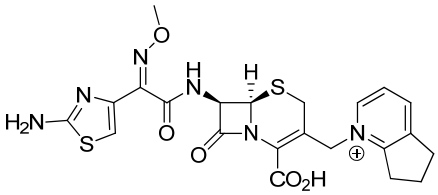
SBL. Compared to the first two generations, the third generation has slightly less activity against Gram-positive bacteria, but greater *in vitro* activity against Gram-negative aerobes.[21]

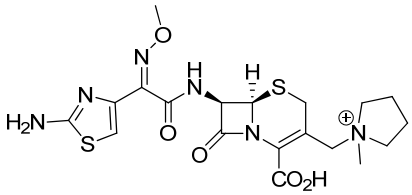

Cefotaxime


Cefixime

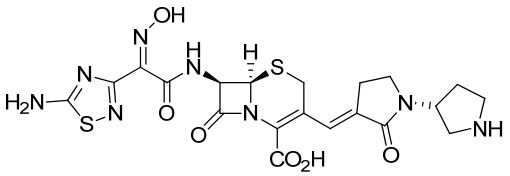

Ceftbuten

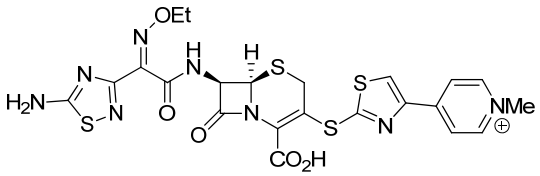
Cefepime and cefpirome are the typical examples of the fourth generation cephalosporins, which were introduced clinically in 1990s. They have extended Gram-negative coverage and increased resistance towards β-lactamases. Due to the positive charge in the 3' quaternary ammonium side chains, their ability to inhibit Class C SBL was increased.[17,21]


Cefpirome


Cefepime

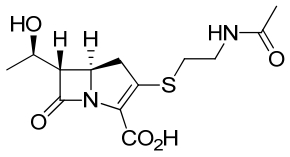
Ceftobiprole and ceftaroline are the fifth generation of cephalosporins, which are still under development, and both of them have potent activity against MRSA. Ceftaroline has extended spectrum activity against Gram-negative pathogens, while ceftobiprole possesses wide spectrum coverage of both Gram-positive and Gram-negative bacteria.[17,26]


Ceftobiprole

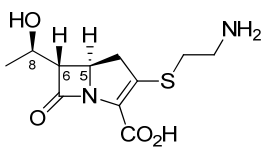

Ceftaroline

1.1.3 Carbapenems

β-Lactam antibiotics were greatly threatened by bacterial β-lactamases starting from the late 1960s. Therefore, a search for new β-lactam antibiotics and β-lactamase inhibitors became increasingly important.[27] At the moment, the Beecham Laboratory was a leader in research on β-lactamase inhibitors, and in 1976 researchers at this company discovered the first β-lactamase inhibitor, olivanic acid, which was produced by the Gram-positive bacteria *Streptomyces clavuligerus*.[27] Olivanic acid possesses a carbapenem backbone, which is a four-membered β-lactam ring fused with an unsaturated five-membered ring. Olivanic acid works as a broad-spectrum β-lactam antibiotic, however, it is not used clinically since it is not stable and cannot readily penetrate the bacterial cell.[27] In the meantime, scientists at Merck discovered thienamycin from the fermentation broth of *Streptomyces cattleya*.[21] Compared to penicillins and cephalosporins, the sulphur atom is replaced by a carbon atom in carbapenems. Thienamycin has a hydroxyethyl substituent at C6 rather than the acylamino group, which enables thienamycin to have remarkable activity against both Gram-positive and Gram-negative bacteria. In addition, the stereochemistry of C5, C6 and C8 has essential influence on the corresponding stability and bioactivity. It was found that the thienamycin with *trans* stereochemistry between C5 and C6 and a (*R*) stereochemistry at C8 presented the greatest stability and antibacterial activity.[21,28]



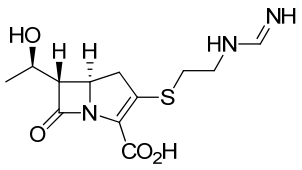
Olivanic acid



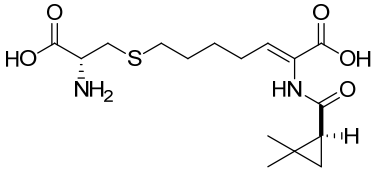
Thienamycin

In 1978, the Merck research group published the first total synthesis of thienamycin followed by a modified synthetic process developed by the same lab two years later.[21] Unfortunately, it was discovered that thienamycin is not stable in aqueous solution and is

sensitive to mild base (above pH 8). Even worse, it is highly reactive to nucleophiles including hydroxylamine and even its own primary amine.[27] In 1979, the Merck group noticed that the conversion of the primary amine to an *N*-formimidoyl group could relieve the instability of thienamycin in aqueous solution. The new antibiotic was named imipenem and showed high affinity for PBPs and higher stability towards β-lactamases. Unfortunately, imipenem was found to be the substrate for human renal dehydropeptidase I (DHP-I); therefore, the DHP-I inhibitor cilastatin was developed, to be used in combination with imipenem in clinic.[21]

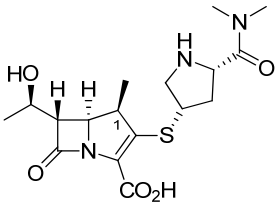


Imipenem

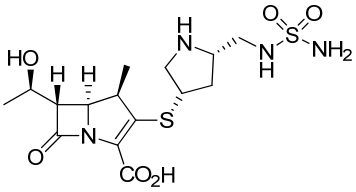


Cilastatin

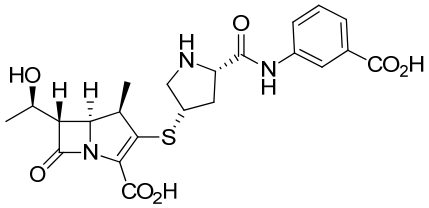
In the following 20 years, other clinically useful carbapenems, including meropenem, ertapenem and doripenem were developed. The presence of a methyl group at the C1-β position in these carbapenems makes them resistant towards DHP-I, thus eliminating the need of a DHP-I inhibitor.[27]



Meropenem



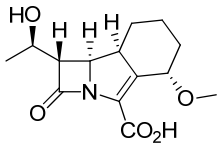
Doripenem



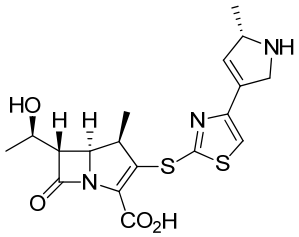
Ertapenem

Among the recently developed carbapenems, sanfetrinem by Glaxo Wellcome was an unusual one as its structure consists of three rings, and it is highly stable towards human renal DHP-I and β-lactamases while possessing broad-spectrum activity against both Gram-positive and Gram-negative bacteria.[21] Carbapenem PZ-601 was discovered at Sumitomo

Pharmaceuticals and is still in clinical trials, aimed at treatment of some serious infections caused by MRSA and extend-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*.[21]



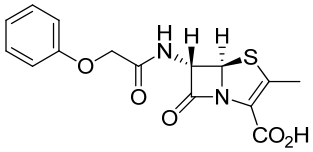
Sanfetrinem



PZ-601

1.1.4 Penems

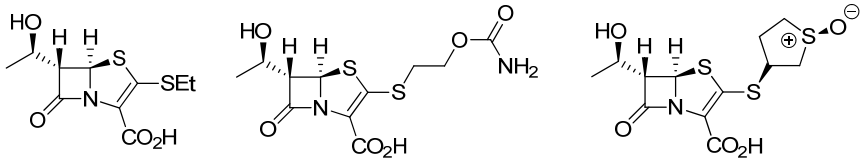
Penems were entirely obtained through total synthesis rather than bacterial fermentation.[29] The very first synthesized penem was 7-phenyl-2-methyl penem, prepared by the Woodward group in 1976. The penems possess a core skeleton that is a hybrid of that of the penams (penicillins) and cephems (cephalosporins).[30]



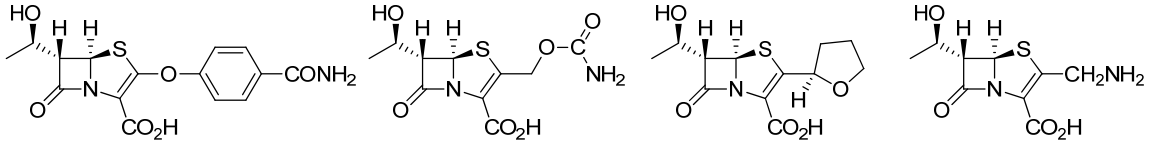
7-phenyl-2-methyl penem

Based on this core structure, a number of penems such as SCH 29482, HR 664 and sulopenem, has been synthesized. Unfortunately, only a few penems (e.g. sulopenem, ritipenem and faropenem) are used clinically due to their general instability and toxicity. In general, penems are classified into five groups (A-E) depending on the side chains at the C2 position.[30] Group A penems are thiopenems including SCH 29482, SCH 33343 and sulopenem. SCH 29482 has potent activity and SCH 33343 is undergoing Phase III clinical trials. Sulopenem, discovered in the 1980s, possesses good activity against both Gram-positive and Gram-negative excluding *Pseudomonas aeruginosa*.[29] However, sulopenem was not used

clinically until a pro-drug (PF-03709270) was discovered, which could be used for the treatment of community-acquired pneumonias.[21]



Oxypenems are Group B penems, which include HR 664 that is the most active compound in this family.[30] Alkylpenems belong to Group C and ritipenem is a typical example of this category. Ritipenem was developed and introduced by Tanabe Seiyaky Co in Japan,[21] and shows good activity against a wide range of bacteria including *Enterobacteriaceae*, *Haemophilus influenzae*, and *S. aureus*.[29] Arylpenems and faropenem are representatives of the Group D penems. The latter was developed by Daiichi Asubio Pharma in Japan and became commercially available in 1997,[21] which not only has broad spectrum antimicrobial activity but also relatively high stability towards the SBLs.[17] Group E penems are aminopenem derivatives, for example 2-aminomethyl penem, which resist hydrolysis by DHP-I due to the presence of the 2-aminomethyl side chain. The aminomethyl group reduces its activity against Gram-negative bacteria, but an unsaturated *N*-heterocyclic derivative such as one bearing an imidazole group has enhanced antibacterial activity.[30]

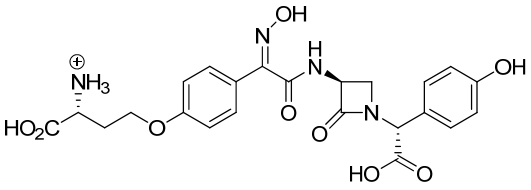


In summary, the clinically useful penems demonstrate a broad spectrum of antibacterial activity; however, they are not active against MRSA, *Enterococci* and *P. aeruginosa*.

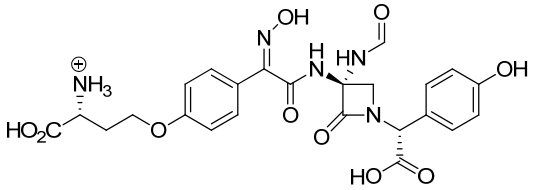
Compared to imipenem (a carbapenem), penems have similar stability towards β-lactamases, but they are more stable towards DHP-I.[21]

1.1.5 Monobactams

In the 1970s, many research groups worldwide were searching for β-lactamase inhibitors. In 1976, a Japanese group at the Tekeda Company discovered the first monobactam (i.e. a monocyclic β-lactam) called nocardicin A, which was produced by an organism later named as *Nocardia uniformis tsuyamanensis*. However, nocardicin A has only moderate *in vitro* activity against Gram-negative pathogens such as *Proteus* and *Pseudomonas*.[21] Later on, a group of modified monobactams were discovered from the fermentation broth of *Flexibacter alginoliquefaciens* YK-49, and those possessing a 3α-formylamino substituent were named as formadicins (A-D). Even though formadicins have a narrow spectrum of antimicrobial activity, they are stable towards β-lactamases due to the 3α-formylamino substituent.[21]

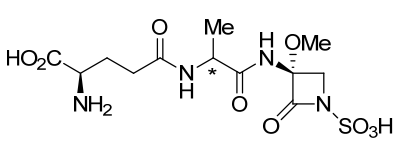


Nocardicin A

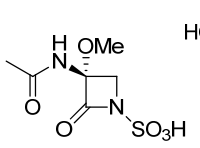


Formadicin C

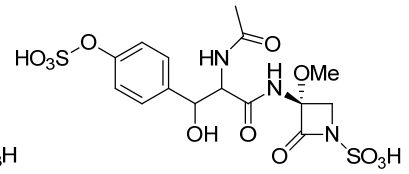
In the early 1980s, a series of monobactams including sulfazecin and isosulfazecin were found by the Tekeda group. Meanwhile, the Squibb team discovered SQ 26180 and SQ 26970 produced by *Agrobacterium radiobacter*.[21] These monobactams have activity only against Gram-negative bacteria.[31]



sulfazecin: D
isosulazecin : L

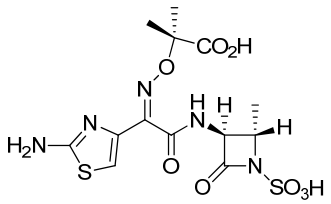


SQ 26180

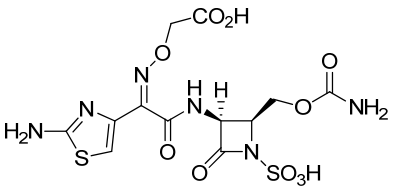


SQ 26970

In 1983, aztreonam was successfully synthesized by chemists at Squibb. It showed potent activities against Gram-negative pathogens. As a result, aztreonam has been used in clinical treatment of infection caused by Gram-negative bacteria since 1986, but it is inactivated by Gram-positive bacteria. Later on, another monobactam named carumonam was discovered by Takeda,[21] and was used for treating Gram-negative bacterial infections. Carumonam and aztreonam have similar spectra of antibacterial activity and stability towards β-lactamases. They are inhibitors of Class C SBLs, but are inactivated by Class A ESBLs. Surprisingly, although they were still hydrolyzed by MBLs, the rate was fairly slow,[21] which makes them potential MBLs inhibitors.

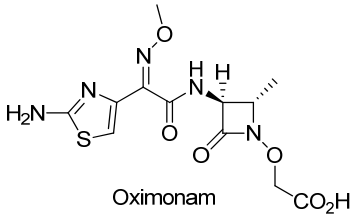


Aztreonam

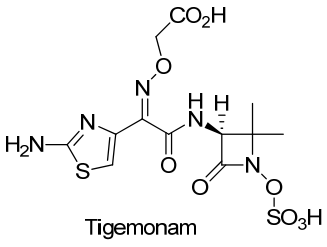


Carumonam

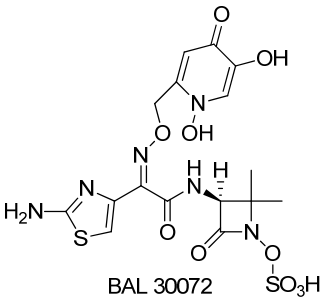
Compared to carumonam and aztreonam, oximonam and tigemonam have similar bioactivity and stability towards β-lactamases, but very little antimicrobial activity against Gram-positive bacteria.[21] BAL 30072 is an experimental monobactam with a siderophore moiety that improves uptake by *P. aeruginosa* via an active iron uptake system. BAL 30072 is stable to MBLs and has been examined against multidrug-resistant Gram-negative bacteria.[21,32]



Oximonam



Tigemonam



BAL 30072

1.2 Penicillin Binding Proteins

Between the 1940s and 1970s, much research was devoted to the analysis of the structure of the bacterial cell wall, in order to understand the mechanism-of-action of β-lactams. In the 1970s, it was discovered that penicillin interacts with several target enzymes that are involved in the synthesis and maintenance of the bacterial cell wall, which were consequently named penicillin-binding proteins (PBPs).[17]

Penicillin binding proteins could be divided into two primary groups based on their molecular weight, the high molecular weight (HMW) PBPs (MW > 55000) and the low molecular weight (LMW) PBPs.[33] HMW PBPs are multimodular and are involved in the polymerization of peptidoglycan and insertion into pre-existing cell well, while the LMW PBPs are responsible for endopeptidase, transpeptidase and carboxypeptidase activities.[34] According to their different functions, each main group of PBPs could be further categorized into three subclasses (Class A, B and C). The Class A HWM PBPs (e.g. PBP1a and PBP1b of *E.coli*) catalyze the elongation of uncross-linked glycan chains because of their *N*-terminal domain that controls their glycosyltransferase activity.[34] HMW PBPs in class B (e.g. PBP2 and PBP3 of *E. coli*) are responsible for cell morphogensis.[34] The Class C HWM PBPs are considered to be penicillin sensory proteins, such as BlaR, which is responsible for inducing the synthesis of β-lactamases.[33] In terms of cell activities, the three classes of LMW PBPs are inferior to the HMW counterparts.

1.2.1 Peptidoglycan and D-Ala-D-Ala Transpeptidases

In the 1960s, the structure of bacterial cell peptidoglycan and its biosynthesis were gradually elucidated. Schleifer and Kandler considered that bacterial cell was made up of acylated amino sugars and three to six different amino acids, which were called by a few

different names including basal structure, mucopeptide, glycopeptide, glycosaminopeptide, murein and peptidoglycan. Among these names, peptidoglycan is a more precise term to describe the polymer of the bacterial cell wall.[35] It was discovered that for both Gram-positive and Gram-negative pathogens, the glycan polymer consists of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). In addition, there is a peptide unit attached to the D-lactyl portion of NAM in order to connect the glycan polymers.[35] More specifically, the pentapeptide unit is [L-Ala–γ-D-Glu–m-DAP–D-Ala–D-Ala] in Gram-negative bacteria (Scheme 3), while in Gram-positive bacteria the corresponding unit is [L-Ala–γ-D-Glx–L-Lys(ε-Gly_5)–D-Ala–D-Ala].[35]

Slaton's experiments in 1961 suggested that the bacterial cell wall was highly cross-linked and this cross-linking process was very likely carried out during the final step of peptidoglycan biosynthesis.[36] In 1965, it was found by Wise and Park that penicillin could inhibit the synthesis of bacterial mucopeptide through a cross-link reaction, which was a transpeptidation reaction involving the loss of D-alanine. They also provided a hypothesis that during the inhibiting process, penicillin was acting as a mimic of the L-Ala–γ-D-Glu segment of the substrate.[37] Later on, Tipper and Strominger proposed that penicillin was a conformational analogue of the D-Ala-D-Ala in the linear glycopeptide instead of the L-Ala–γ-D-Glu segment (Figure 4), which acted as an inhibitor of the peptidoglycan transpeptidase to generate a stable penicilloyl enzyme intermediate (Scheme 3).[38,39] As a result, the transpeptidation process was prevented and the peptidoglycan could not be formed, leading to the incomplete generation of bacterial cell wall. This hypothesis then was widely accepted and considered as a primary milestone of understanding the penicillin mode-of-action.[17]

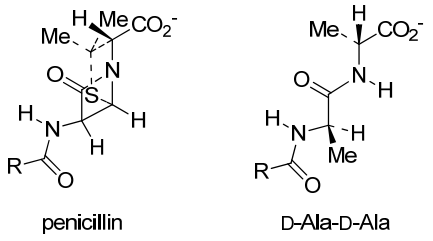
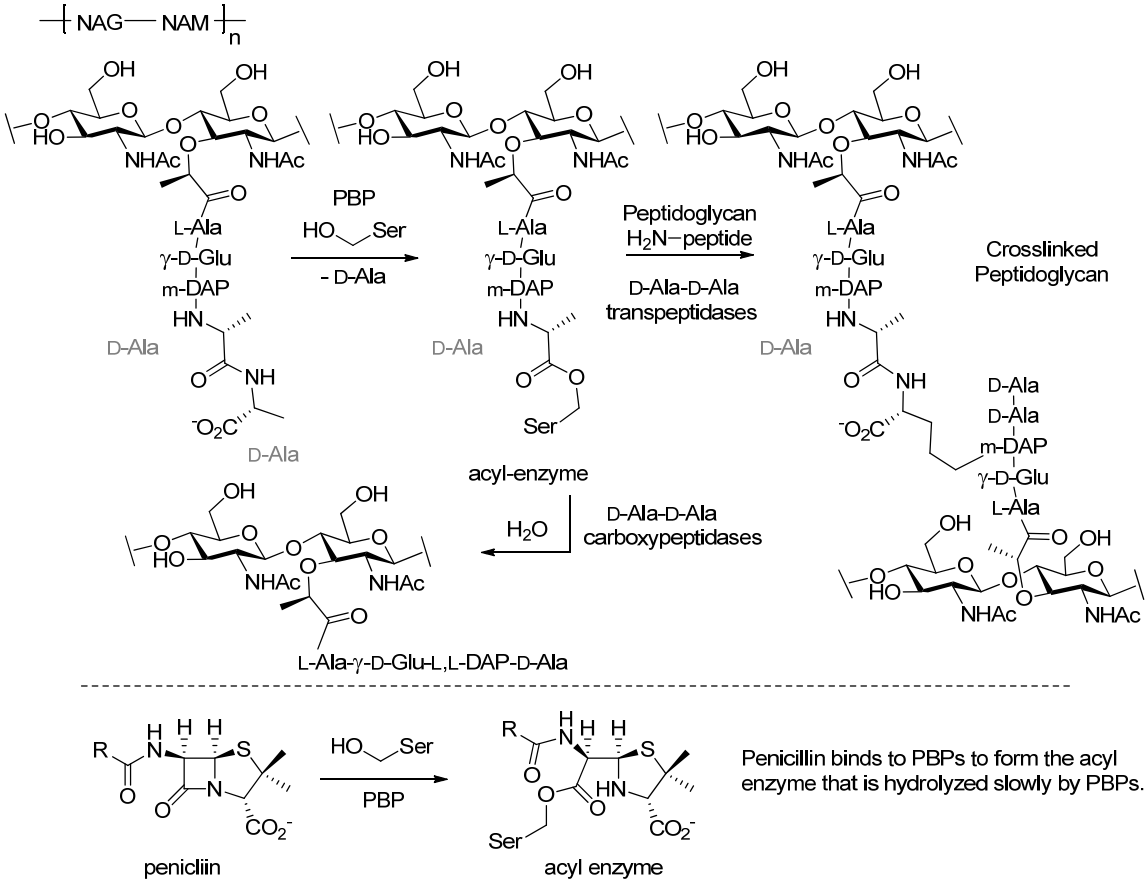


Figure 4. Structural analogy of penicillin and D-Ala-D-Ala fragment of peptidoglycan



Scheme 3. Reaction of PBPs in *E.coli* involving biosynthesis of the bacterial cell wall and the inactivation of PBPs by penicillin

1.2.2 PBP-based Resistance to β-Lactam Antibiotics

The PBPs are transpeptidases or carboxypeptidases playing an essential role in peptidoglycan metabolism. Modification of PBPs can lead to PBPs with low affinity for the antibiotics.[40] These PBPs are still functional as transpeptidases, but do not readily bind to the β-lactams (e.g. PBP1a and PBP2b of *S. pneumoniae*).[41]

1.3 β-Lactamases

β-Lactamases are enzymes produced by many bacteria, which can efficiently hydrolyze β-lactam antibiotics. The first known β-lactamase, produced by a strain of *Bacillus coli* that could inactivate penicillin was discovered by Abraham and Chain in 1940.[42] In the next six decades, with the discovery and clinical use of an increasing number of β-lactam antibiotics, bacteria evolved to produce various β-lactamases in order to defend themselves against the β-lactam antibiotics. With the appearance of ESBLs (extend-spectrum β-lactamases) and carbapenemases, the current β-lactam antibiotics suffer serious threats.[17]

In 1973, Richmond and Sykes proposed the first widely accepted classification of β-lactamases. According to their favoured substrates, β-lactamases could be divided into five groups, including Class I (cephalosporinases), Class II (penicillinases), Class III broad-spectrum enzymes that were resistant towards *p*-chloromercuribenzoate but sensitively inhibited by cloxacillin, Class IV enzymes that possessed the opposite activity as the Class III counterparts, and Class V penicillinases that could inactivate cloxacillin but were sensitive to *p*-chloromercuribenzoate.[43]

In 1980, a novel classification of the β-lactamases was developed by Ambler, which is based on their structural properties at the enzyme's active site. The Ambler rule divides the β-lactamases into serine β-lactamases (SBLs) and metallo β-lactamases (MBLs) and the serine β-lactamases are further divided into Class A, C and D while the metallo β-lactamases are designated as Class B.[21] All SBLs possess a crucial serine residue in their active sites, and the MBLs require the present of a metal ion (Zn^{2+}) in their active sites.

1.3.1 Ambler's Class A β-Lactamases

The Ambler's Class A SBLs include the penicillinases of *S. aureus* PC1, *B. licheniformis* 794/C, *B. cereus* 569/H β-lactamases and the broad-spectrum β-lactamases TEM-1 and TEM-2 and others. The active sites of penicillinases possess a serine residue that is similar to that of D-alanine carboxypeptidases.[44]

TEM-1 was discovered in the early 1960s from a single strain of *E. coli*, and after a few years it was widely found in various species including *Enterobacteriaceae*, *P. aeruginosa* and others. TEM-1 production resulted in resistance towards ampicillin, penicillin and the early cephalosporins.[45] Later on, as the first mutation of TEM-1, TEM-2 was discovered with an amino acid substitution that was potentially producing the ESBL phenotype. In 1989, it was reported that TEM-3 firstly presented the ESBL phenotype.[45] So far, more than 90 additional TEM mutations have been found, some of which are resistant to β-lactamase inhibitors and thus called inhibitor-resistant β-lactamases, while the majority of the new TEM mutations are ESBLs.[45]

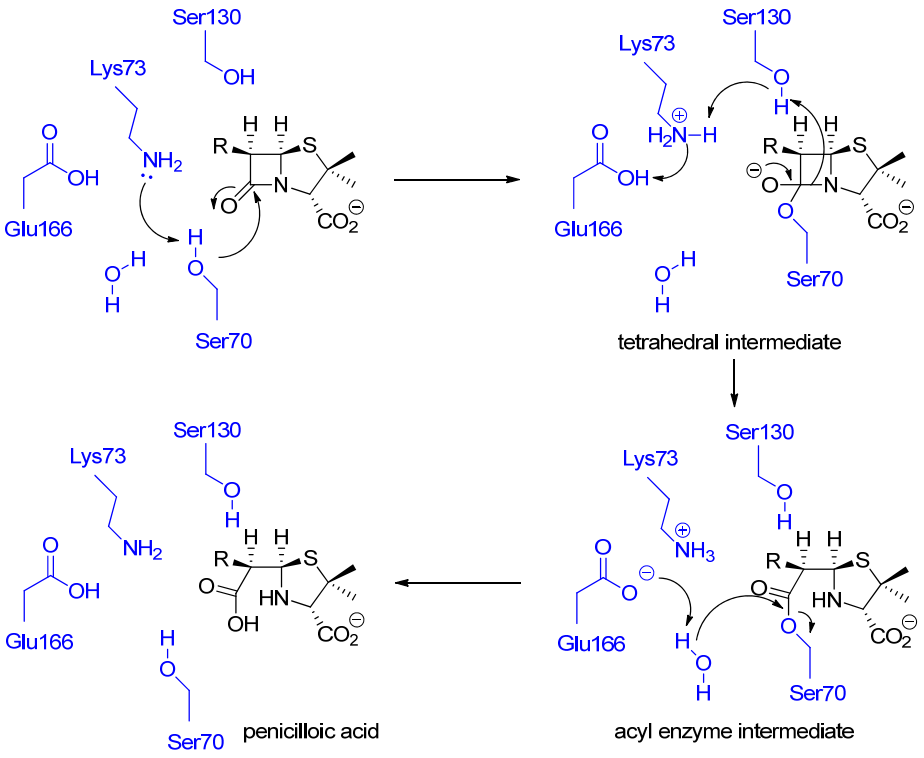
In the 1980s, oxyimino-cephalosporins were applied to treat infections caused by Gram-negative bacteria that produce the TEM family β-lactamases. Inevitably, new β-lactamases were discovered in *Enterobacteriaceae,* and *P. aeruginosa*, which are the SHV family of ESBLs with the ability to efficiently hydrolyze the oxyimino-cephalosporins.[45] The SHV family is the second largest group in the Class A SBLs, and they have similar structures to the TEM enzymes.[17]

In the mid-1980s, the most widespread β-lactamases CTX-M were initially reported. Their rate of spread has significantly increased since 1995.[46] The CTX-M family are plasmid-mediated ESBLs and mainly found in strains of *Salmonella enterica*, but they were not related

to the TEM and SHV families. The CTX-M type β-lactamases preferentially hydrolyze cephalothin, cephaloridine and cefotaxime.[45]

There are some Class A SBLs with carbapenemase activity including SME, IMI, NMC-A, KPC and GES families, which have a broad spectrum activity against penicillins, early cephalosporins, carbapenems and aztreonam.[17]

The structure of the Class A SBLs active site was determined by X-ray crystal studies and site-specific mutagenesis in the early 1990s. All Class A SBLs have similar structures at their active sites, which contain the conserved residues Ser70, Glu166 and Lys73, and an oxyanion hole.[47] For the hydrolysis mechanism by SBLs, at the beginning, it was proposed that Glu166 acted as a general base that participated not only in the acylation process, but also the deacylation process, and activated a Ser70 and hydrolytic water for each process. However, in 1992, the Strynadka group discovered, through an X-ray crystallographic study of the acyl enzyme intermediate that was generated from penicillin G bound to the TEM-1, that Lys73 acted as the general base to deprotonate Ser70 to furnish the nucleophile (Scheme 4).[48] After the nucleophilic attack, a tetrahedral intermediate was obtained and then converted into the acyl enzyme intermediate. For the deacylation process, like the initial viewpoint, it was discovered that Glu166 worked as a general base to activate a water molecule that then nucleophilically attacked the penicilloyl carbonyl group.



Scheme 4. Proposed mechanism for hydrolysis of penicillins by Class A SBLs (TEM-1)[49]

So far, this mechanism of deacylation is widely accepted, while that of acylation is still under debate. In 2002, the Shoichet team proposed that Glu166 acted as the general base to activate the water molecule and the proton of Ser70 was acidified by Lys73 and then the activated water deprotonated Ser70 to generate the nucleophile.[50] Recent work by the Mobashery lab suggested that Glu166 activated Lys73 first, Ser70 was then deprotonated by an unprotonated Lys73, which could promote the formation of the acyl enzyme intermediate.[51]

1.3.2 Ambler's Class C β-Lactamases

The class C SBLs are also called AmpC cephalosporinases. In 1981, the chromosomally mediate AmpC enzyme of *E. coli* K12 was identified by Jaruin and Grundstrom, which did not have sequence homologies as the class A SBLs, thus, it was considered as the first Class C SBLs.[44] In the late 1980s, an increasing number of plasmid-mediated AmpC β-lactamases was discovered. For instance, CMY-1, which could inactivate

cefoxitin and cefotetan as well as penicillins, was isolated from a *K. pneumoniae* in 1989. Moreover, the BIL, FOX, MOX, LAT, ACT and DHA families of β-lactamases all belong to the Class C SBLs.[52] In general, these β-lactamases are less active towards penicillins, but more active towards cephalosporins, oxyimino-cephalosporins and monobactams.[53] In the mid-1990s, the first extended-spectrum Class C SBL, which was able to hydrolyze oxyimino β-lactam antibiotics was isolated clinically and called GC1.[54]

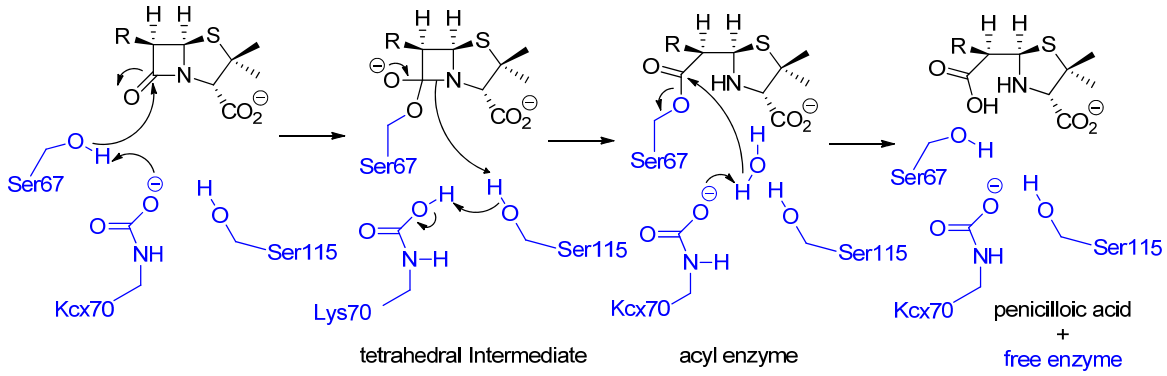
For the β-lactam hydrolysis mechanism, Class C SBLs are essentially the same as the Class A SBLs, including the active site acylation and hydrolytic deacylation.[55] However, there are still two significant differences. First of all, the rate-determining step is different. For Class A enzymes, it is the acylation step, while the rate-limiting step for Class C counterparts is the deacylation process. Second, during the deacylation step, the hydrolytic water molecule approaches the acyl intermediate through two opposite directions. In the case of Class A SBLs, water approaches the α-face, whereas in Class C enzymes it approaches the β-direction.[55] In terms of the structure of the active site, similar to that of Class A SBLs, the active site of Class C SBLs consists of Ser64, Lys67, Lys315 and Tyr150, which occupy similar positions as the corresponding amino acids of Class A SBLs active site.[17] Since the active site of Class C SBLs contains Tyr150 instead of Glu166, Tyr150 is considered to act as the general base involved in both the acylation and deacylation processes of hydrolyzing β-lactams.[56]

1.3.3 Ambler's Class D β-Lactamases

The Class D SBLs are oxacillinases (OXAs), and OXA-1, OXA-2 and OXA-10 are the first Class D enzymes, discovered in late 1980s. The OXAs possess very similar structures to each other, but are very different from that of Class A and C SBLs.[57] During the first two decades since their discovery, there were only 20 Class D SBLs. Unfortunately, this family

was expanded widely in the following ten years, and more than 250 members were found for this family by 2013.[58] The Class D SBLs are usually found among the clinically important bacteria such as *Acinetobacter baumannii*, *P. aeruginosa*, *E. coli* and *Proteus mirabilis*, which could inactivate cephalosporins, the combination of β-lactam/β-lactamase inhibitors, and carbapenems. For example, the enzymes produced by *A. baumannii* (e.g. OXA-23, OXA-24 and OXA-58) result in resistance towards carbapenems. In addition, some β-lactamases in *P. aeruginosa* (e.g. OXA-14, OXA-28 and OXA-35) can inactivate extended-spectrum cephalosporins. Recently, a new member OXA-45 was discovered in Turkey. It is a cabarpenemase produced by *K. pneumoniae*, leading to considerable death in Europe and now detected in North America.[58]

The Class D SBLs share a similar serine-initiated hydrolysis mechanism as the Class A and C SLBs.[59] In contrast to the active site of Class A enzymes, that of OXAs lacks an analogous glutamate acting as the general base.[58] In 2000, the Strynadka and Mobashery groups reported two X-ray crystal structures of OXA-10 and they proposed that the Lys70 was the general base.[60,61] Later on, the Mobashery lab hypothesized that the Lys70 was carboxylated (Lcx70) to produce a carbamate, which was considered to act as the general base in the acylation process. Lcx70 directly deprotonates Ser67 to generate the nucleophile that then attacks the carbonyl of the β-lactams.[62] In 2013, the Bonomo group confirmed Mobashery's proposal (Scheme 5).[58] For the deacylation step, Lcx70 works as the general base as well, which activates the water molecule that then underwent a nucleophilic attack on the penicilloyl carbonyl.



Scheme 5. Proposed mechanism for hydrolysis of penicillins by Class D SBLs (OXA-10)

1.3.4 Ambler's Class B β-Lactamases

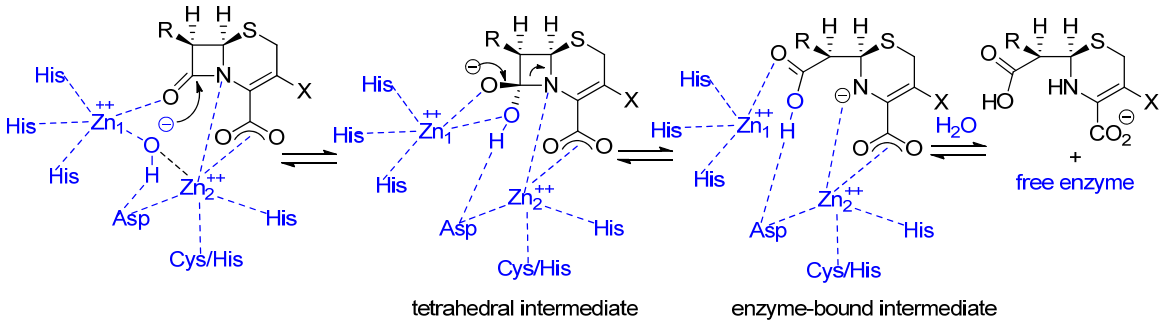
The Class B β-lactamases are metallo β-lactamases. The first Class B enzyme isolated from *Bacillus cereus* was named BcII in the mid-1950s, and was found to be capable of hydrolyzing semi-synthetic penicillins and cephalosporins.[17] MBLs were not considered a problem for the β-lactam therapy back then, since they were chromosomally encoded enzymes and were not found in clinically important bacteria. In the next decade, it was discovered that the MBLs could be inhibited by metal chelators such as EDTA, but recovered upon the addition of $ZnSO_4$,[14] indicating the essential role of Zn^{2+} (metal) with the MBLs. However, nowadays the Class B MBLs demonstrate a broad spectrum resistance to virtually all β-lactam antibiotics except the monobactams. Even worse, they can hydrolyze the mechanism-based β-lactamase inhibitors including sulbactam and tazobactam (section 1.4.1).[63]

In the late 1990s, the MBLs were divided into three subclasses (B1, B2 and B3) based on their primary amino acid sequence homology. The B1 subclass include the most common MBLs worldwide including BcII, CcrA, BlaB and IMP, VIM, SPM, GIM and NDM families.[63] CcrA produced form *Bacteroides fragilis* was discovered in the 1980s. In the next 10 years, the plasmid encoded IMP and VIM families were found in Gram-negative bacteria including *P. aeruginosa*, *Enterobacteriaceae* and *A. baumannii*.[63] Both of them showed a

broad spectrum of activity against all β-lactams except aztreonam (a monobactam). In the early 2000s, the SPMs and GIMs were isolated from *P. aeruginosa* in Brazil and Germany respectively, and possessed similar spectra of activity as IMPs and VIMs. In 2008, a new MBL named NDM-1 was detected in *K. pneumonia* and *E. coli* from a patient who was returning to Sweden from India.[63] NDM-1 then spread very rapidly and it could inactivate the majority of β-lactams except colistin.[17] Subclass B2 involves CphA, ImiS and Sfh-I, and B3 subgroup includes L1, FEZ-1 and the GOB family. In 2010, Bush proposed a new classification of MBLs depending on their functions. The MBLs could be classified into two subgroups (3a and 3b). The 3a subclass includes penicillinases and cephalosporinases while the 3b subgroup contains carbapenemases.[64]

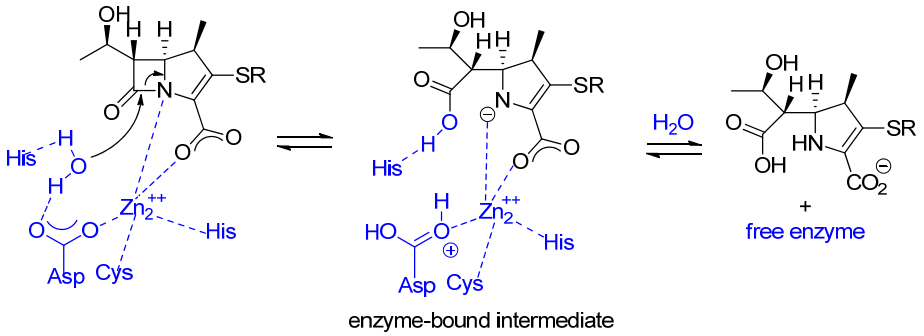
Since the mid-1970s, studies on the mechanism for hydrolysis of β-lactams by MBLs started. It was discovered that there were two zinc binding sites present in the active site of many MBLs. Between the 1980s and 1990s, it was reported that Class B1 and B3 MBLs require two zincs in order to obtain maximal activity, while Class B2 MBLs need only one zinc to bind with the β-lactams. B2 MBLs present a relative narrow spectrum activity when compared to the B1 and B3 ones.[17,63] In the mid-1990s, X-ray crystallographic studies suggested that the Zn1 binding site of B1 MBLs contains three histidines, known as the 3H site, and the Zn2 binding site called DCH site involves asparagine, cysteine, and histidine amino acid residues.[63] For B3 MBLs, their Zn1 binding sites have similar amino acids residues to those of B1 MBLs, but the corresponding Zn2 sites of B3 enzymes consist of one asparagine and two histidines, known as the DHH. As mono-zinc MBLs, B2 enzymes contain a DCH Zn2 binding site similar to that of B1/B3.[63]

The proposed mechanism for B1 and B3 MBLs involves Zn1 acting as a Lewis acid binding to the oxygen of the β-lactam moiety, which increases the electrophilicity of the carbonyl carbon for nucleophilic attack.[65] At the same time, the β-lactam ring nitrogen interacts with the Zn2 site (Scheme 6).[63] There is a bridging hydroxide that is oriented properly by the Asp through forming an H-bond between the Zn1 and Zn2 sites. Then the hydroxide between the zinc ions attacks the carbonyl to give the tetrahedral intermediate, which then further generates an enzyme-bound intermediate with a negative charge on the nitrogen atom that can be stabilized by the Zn2 site. [63, 65]



Scheme 6. Proposed mechanism for cephalosporins hydrolysis by B1 and B3 MBLs

In terms of the mechanism for B2 MBLs (Scheme 7), only the Zn2 site binds with the β-lactam and a water molecule is activated by the Asp of the Zn2 site and a His nearby, which then attacks the carbonyl. The anionic intermediate is stabilized by Zn2 as well, followed by further hydrolysis, the enzyme-bound intermediate is degraded to the hydrolyzed product and releases the free enzyme.[17, 63]



Scheme 7. Proposed mechanism for carbapenems hydrolysis by B2 MBLs

1.4 β-Lactamase Inhibitors

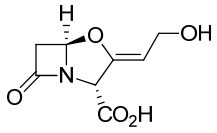
The β-lactam antibiotics are seriously threatened by the β-lactamases, which is an urgent problem that needs to be addressed. There are two feasible strategies to relieve this situation, including discovery of novel antibiotics that are not the substrate of β-lactamases and development of β-lactamase inhibitors to be used in combination with β-lactams, which could prevent the β-lactams from being hydrolyzed by the β-lactamases.[66]

1.4.1 β-Lactams as β-Lactamase Inhibitors

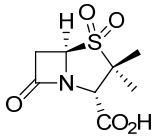
The first example of β-lactamase inhibition was reported in 1956. Cephalosporin C was resistant to penicillinase, therefore it was a potential inhibitor for certain penicillinases. Abraham and Newton found that Cephalosporin C could competitively inhibit the hydrolysis of penicillin G and N, because it was more sensitive to the *S. aureus* penicillinase.[67] It was realized that some β-lactams were potential to be substrate mimics that are analogues of the favourable substrate of the β-lactamases to inhibit the enzymes. In the next 10 years, significant efforts were made towards the development of more effective β-lactamase inhibitors. During this period, some semi-synthetic penicillins, such as methicillin and cloxacillin, demonstrated the capacity to inhibit β-lactamases produced by Gram-negative

bacteria. Certain carbapenems (e.g. olivanic acids, pluracidomycins and asparenomycins) were found to inhibit a wide range of β-lactamases as well.[17]

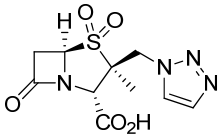
Currently, the only β-lactamase inhibitors applied to clinical practice are clavulanic acid, sulbactam and tazobactam, which are known as mechanism-based β-lactamase inhibitors. Clavulanic acid was produced by the strain of *Streptomyces clavuligerus* and discovered in the 1960s, which had potent activity against Class A and certain Class D SBLs. However, it was inactivated by most Class C SBLs and even worse it had no activity against MBLs.[17] The semi-synthetic sulbactam was discovered in 1978 and initially it was called penicillanic acid sulfone (PAS). When sulbactam was combined with β-lactam antibiotics (e.g. ampicillin and cefazolin), they showed an extended broad spectrum activity against both Gram-positive and Gram-negative pathogens. Although sulbactam could inhibit many SBLs, they were still inactivated by AmpC (a Class C SBL) and MBLs.[17] Later on, tazobactam, a C3'-triazolyl-substituted PAS, was found to effectively inhibit Class A SBLs, but they underwent reversible reaction with Class C and Class B SBLs.[68]



Clavulanic acid

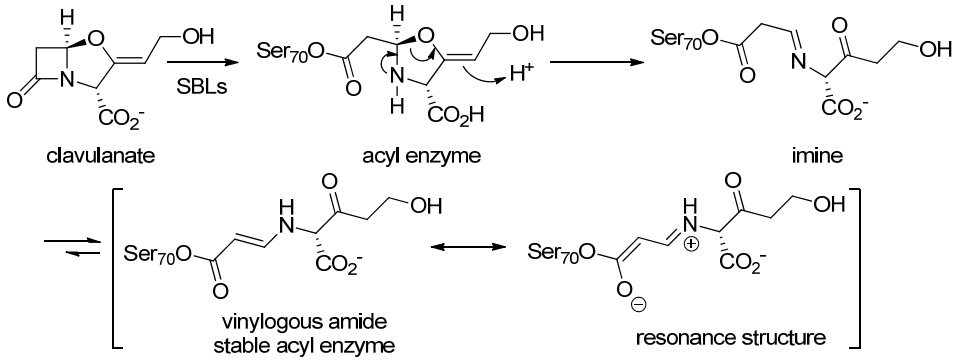


Sulbactam



Tazobactam

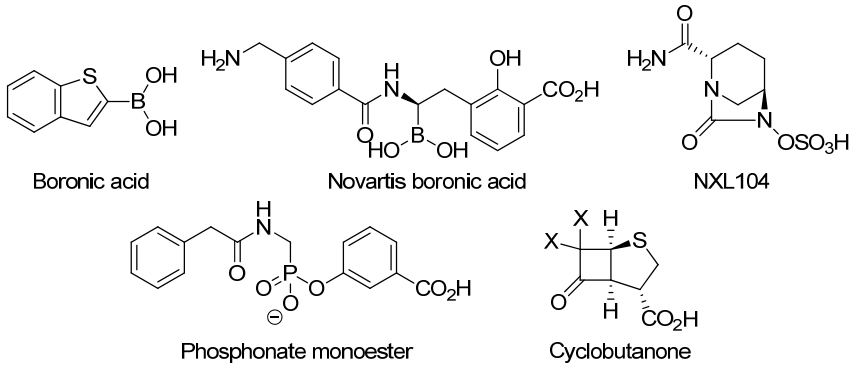
The mechanism for inhibition of Class A SBLs by clavulanate was proposed in the late 1970s (Scheme 8).[66] When the acyl enzyme intermediate is generated, a rearrangement occurs to give the corresponding imine that then isomerizes to a vinylogous amide, which is stabilized by the conjugation system (tautomer). This stabilized acyl enzyme is not readily hydrolyzed, leading to inhibition of the β-lactamases.



Scheme 8. Inhibition of Class A SBLs by clavulanate

1.4.2 Non-β-Lactam Inhibitors

As early as the 1970s, boronic acid derivatives were reported to have inhibition activity towards Class A SBL produced by *B. cereus*. Later on, further studies of the boronic acid derivatives indicated that they could inhibit *S. aureus* producing Class A SBLs and *Enterobacteriaceae*, and they could efficiently protect ampicillin and ceftazidime.[66] However, they were not used in clinic due to the toxicity of boron. Recently, some more complex boronic acid were developed by Novartis, which showed inhibition of both SBLs and MBLs.[69] Moreover, some phosphonate monoester derivatives were found to be potential β-lactamase inhibitors as well. It was reported that they could inhibit most SBLs, but they were not introduced into clinical use either due to their instability in aqueous solution.[66]



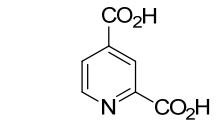
NXL104, a bridged diazabicyclo[3.2.1]octanone developed by Novexel Inc. and currently in phase II clinical trial, is a non-β-lactam inhibitor with decent activity against SBLs.

Combination of ceftazidime and NXL104 in a ratio of 4:1 presents the best activity against Class A SBLs (mainly TEM and SHV producing pathogens), Class C SBLs producing *Enterobacteriaceae,* and Class D SBLs including OXA-48.[66]

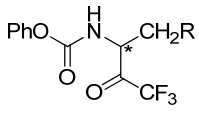
Since the 1980s, a series of cyclobutanones as carbocyclic analogues of β-lactam were reported by several groups, in which a cyclobutanone moiety replaced the β-lactam ring. They might be potential inhibitors for both the SBLs and MBLs as well as the PBPs (e.g. D-Ala-D-Ala transpeptidases). Their synthesis, properties and bioactivities will be discussed in more detail in the following chapters, which is the main subject of this thesis.

1.4.3 Metallo β-Lactamase Inhibitors

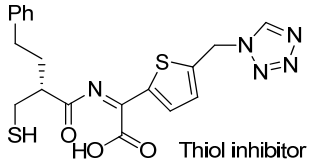
MBLs are the most threatening β-lactamases to humans currently, because they have a broad spectrum of activity to hydrolyze all β-lactams except the monobactams. The current clinical β-lactamase inhibitors, such as clavulanate, sulbactam and tazobactam, unfortunately, are not effectively against MBLs.[66] In the last decades, a series of MBL inhibitors were under development. For example, certain thiol derivatives were found to chelate the zinc ions and replace the bridging water in the active site of MBLs.[66] In addition, pyridine dicarboxylates and trifluoromethyl ketones were reported to bind to the active site Zn^{2+} ion within the MBLs as well.[66] Despite of the hydrolysis of clinically used carbapenems by MBLs, some of them with a variety of side chains at C2 have demonstrated potent inhibition of MBLs. For example, carbapenem J-110,441 shows good inhibition of IMP-1. Additionally, J-111,225 has activity against not only IMP-1, but also CcrA, L1 and BcII.[66] Succinate derivatives such as 2,3-(*S*,*S*)-disubstituted succinic acid and tricyclic natural products (e.g. SB238569) also show inhibition of IMP-1.[66]



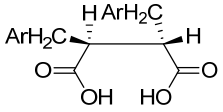
Pyridine-2,4-dicarboxylic acid



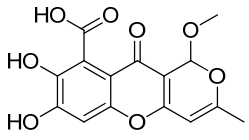
Trifluoromethyl ketone



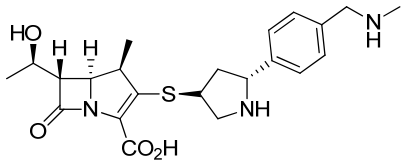
Thiol inhibitor



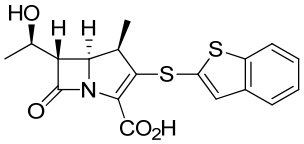
2,3-(*S*,*S*)-Disubstituted succinic acid



SB238569



J-111,225

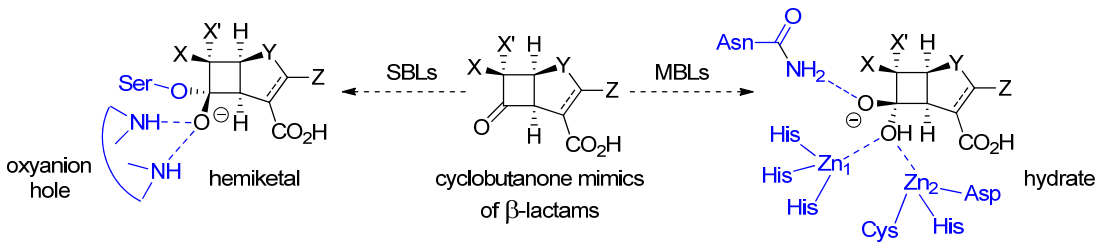


J-110,441

Chapter 2 Previous Work on Cyclobutanone Analogues of β-Lactam Antibiotics

The cyclobutanone analogues of β-lactam antibiotics are compounds in which the β-lactam nitrogen is replaced by an sp^3 carbon. They possess structures and conformations that are similar to those of the β-lactams, and thus are potential β-lactamase inhibitors to protect the β-lactams. In addition, such cyclobutanone analogues may act directly, even in the absence of a β-lactam, as effective antibiotics themselves to eliminate bacteria, if they interact with the PBPs in a fashion similar to that of β-lactams, again due to their structural and conformational similarities.

One main advantage of such cyclobutanone mimics, is that they cannot be hydrolyzed by β-lactamases in the active sites. These cyclobutanones are expected to form a tetrahedral intermediate, either an enzyme-bound hemiketal in the active site of SBLs or an enzyme-bound hydrate in that of MBLs (Scheme 9).[70] Thus, it is (theoretically) possible to develop a proper cyclobutanone as a universal and broad-spectrum β-lactamase inhibitor that can inhibit both SBLs and MBLs at the same time, which would be extremely valuable given the imminent threat from multidrug-resistant bacteria.



Scheme 9. Cyclobutanones as potential broad-spectrum inhibitors of β-lactamases

2.1 Published Cyclobutanone Mimics of β-Lactam Antibiotics

Starting from early 1980s, several research groups including this group began to study cyclobutanones as potential β-lactamase inhibitors, and the reported literature examples of such cyclobutanones are summarized in Figure 5.

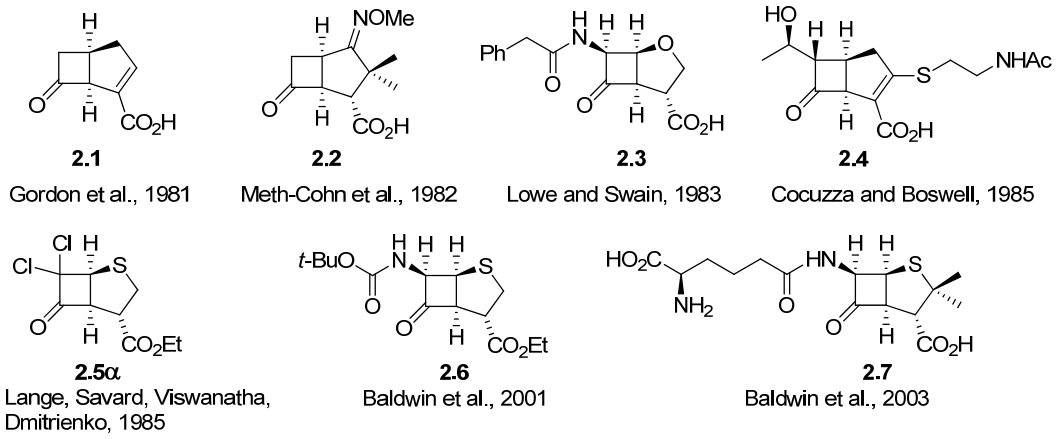


Figure 5. Reported cyclobutanone mimics of β-lactams in the literature

Cyclobutanone **2.1** made by Gordon and coworkers did not present potent inhibition of the TEM type of β-lactamase or R61 transpeptidase, but it had some activity against certain non-β-lactamase producing microorganisms.[71] Meth-Cohn's compound **2.2** with an oxime moiety was proposed to possibly promote acylation of **2.2** in the active site of SBLs, but no related biochemical data was reported.[72] The 2-oxacyclobutanone **2.3** reported by Lowe and Swain showed time-dependent activity against *E. coli* R-TEM and *B. cereus* type I β-lactamases as well as weak inhibition of R61 transpeptidase.[73,74] Compound **2.4** prepared by Cocuzza and Boswell was a mimic of *N*-acetyl thienamycin; unfortunately it did not exhibit antibiotic activity. Interestingly, the benzhydryl esters of **2.4** with the replacement of C3 aminoethylthiolate side chain with sulfoxides and sulfones, showed moderated inhibition of *S. aureus*.[75] The cyclobutanone **2.5α** was synthesized by the Dmitrienko lab, whose synthetic route and corresponding bioactivity will be discussed in detail in the next section. In the last decade, cyclobutanone **2.6** was introduced by the Baldwin lab at Oxford as a novel penicillin mimic.[76] Later on the same group synthesized cyclobutanone **2.7** and the corresponding epimer **2.8** as analogues of penicillin N.[77] This group then reported the crystal structure of **2.8** bound to isopenicillin N synthase, which suggested that cyclobutanone **2.8** is a hydrolytically

stable mimic of a penicillin (Figure 6).[78]

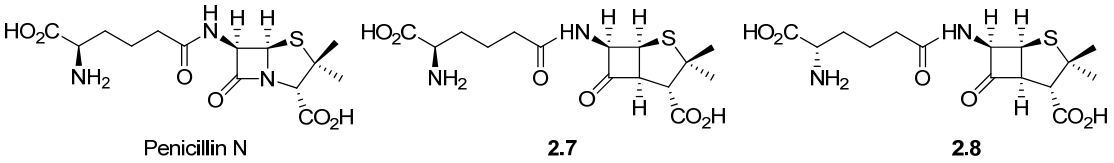
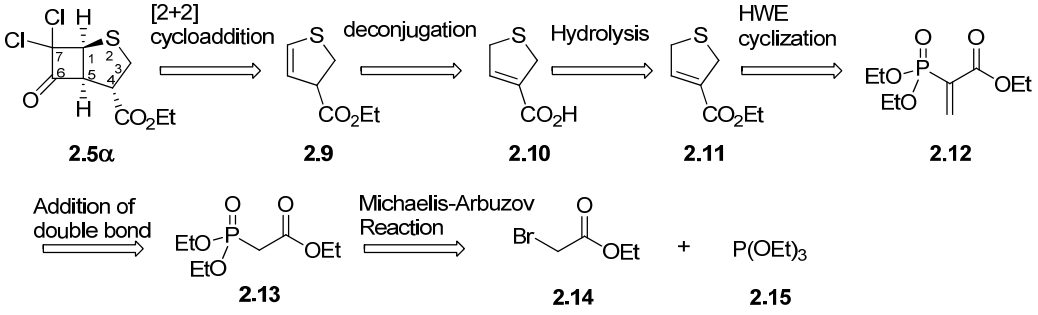


Figure 6. Cyclobutanone mimics of penicillin N

2.2 Synthesis of 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylate 2.5α [17]

In the mid-1980s, a feasible synthetic route towards the cyclobutanone **2.5α** was developed by the Dmitrienko lab at the University of Waterloo, and the corresponding retrosysntheis is showed below in Scheme 10.

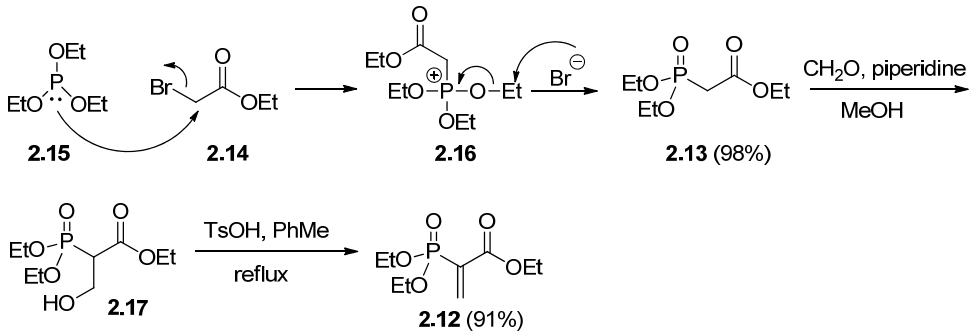


Scheme 10. Retrosynthesis of cyclobutanone **2.5α**

The target dichloroethyl ester **2.5α** could be traced back to a [2+2] cycloaddition of the dichloroketene with a decojugated dihydrothiophene ethyl ester **2.9**, and the latter could be obtained from a novel deconjugation of the corresponding conjugated dihydrothiophene acid **2.10**. Hydrolysis of ester **2.11** should give the desired acid. Compound **2.11** may be retrospected to a vinyl phosphonate **2.12** by way of a Horner-Wadsworth-Emmons cyclization with a mercaptoaldehyde. After a double bond addition step, compound **2.12** could be sought back to a phosphonate **2.13**, which might be furnished from commercially available ethyl bromoacetate **2.14** and triethyl phosphite **2.15** through a Michaelis-Arbuzov reaction.

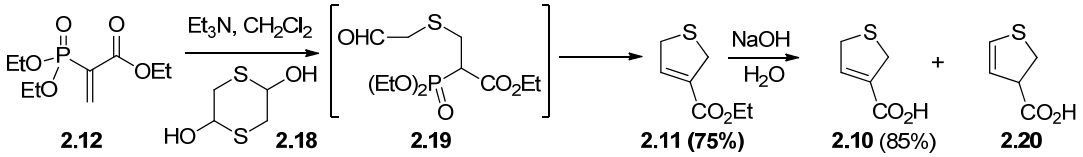
As shown in Scheme 11, the lone pair electrons on the phosphorus of triethyl phosphite

2.15 attacks the α-carbon of the ethyl bromoacetate **2.14** to displace the bromide and generate the phosphonium intermediate **2.16**, which then is attacked by the bromide ion to furnish the target phosphonate **2.13** in 98% yield.[79] The phosphonate **2.13** is deprotonated by piperidine, and the anion then undergoes an aldol condensation with paraformaldehyde in methanol to yield the intermediate alcohol **2.17**, which undergoes a dehydration upon treatment with *p*-toluenesulfonic acid in refluxing toluene using a Dean-Stark trap to provide the desired vinyl phosphonate **2.12** (91% yield, 2 steps).



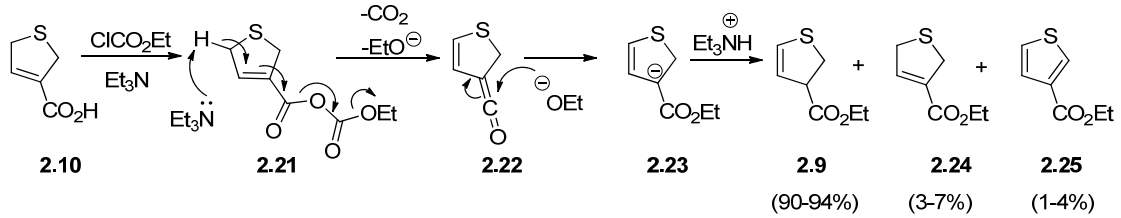
Scheme 11. Preparation of the vinyl phosphonate **2.12**

The strategy to synthesize the dihydrothiophene **2.11** was developed by McIntosh and Sieler (Scheme 12).[80] In the presence of TEA, vinyl phosphonate **2.12** reacts with 2,5-dihydrothiophene **2.18** through a 1,4-addition to give **2.19**, which then undergoes an intramolecular Horner-Wadsworth-Emmons olefination to generate the desired compound **2.11** in 75% yield. Hydrolysis of the ethyl ester **2.11** in aqueous sodium hydroxide affords the corresponding conjugated acid **2.10** in 85% yield, accompanied by a small amount of a non-conjugated acid **2.20**.



Scheme 12. Preparation of the conjugated acid **2.10**

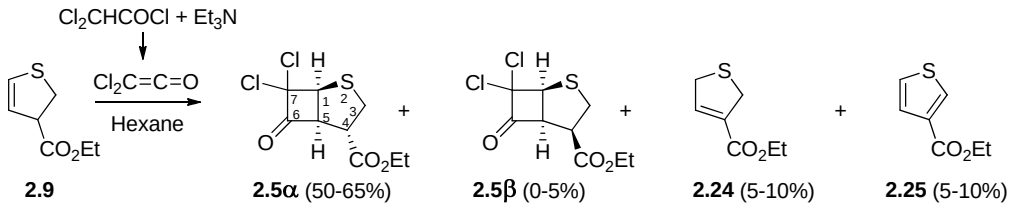
As presented in Scheme 13, the conjugated dihydrothiophene **2.10** firstly reacts with ethyl chloroformate to offer intermediate **2.21**. Deprotonation of carbon α to the sulphur of **2.21** triggers the release of carbon dioxide and ethoxide, leading to ketene **2.22**. Then the ethoxide attacks the ketene carbon of **2.22** to form the intermediate **2.23**, which is then protonated to yield the target ester **2.9** (90-94%) that is the precursor for the [2+2] cycloaddition.[81] Two possible side products might be generated in small amounts, including the conjugated ethyl ester **2.24** (3-7%) and the thiophene ester **2.25** (1-4 %).[81]



Scheme 13. Deconjugation of the conjugated acid **2.10**

The [2+2] cycloaddition of the non-conjugated dihydrothiophene **2.9** with dichloroketene (generated *in situ* from dichloroacetyl chloride and TEA) in a non-polar solvent gives the target dichlorocyclobutanone **2.5α** (Scheme 14). This step is somewhat tricky and a lot of effort has been made to increase the yield of **2.5α**. Early studies carried by Darryl Evanoff of the Dmitrienko lab indicated that slow addition of dichloroacetyl chloride (dropwise in 2 hours) to TEA and **2.9** in CCl_4 could bring the yield up to 40%.[82] However, CCl_4 is rather expensive and rare to find nowadays due to its environmental impact. Later on, a modified strategy was developed by Jarrod Johnson of this group, in which the CCl_4 was replaced by hexane or cyclohexane, and dichloroacetyl chloride (2.5 eq) was added dropwise over 3 hours through a motor-driven syringe pump to a solution of TEA (2.5 eq) and the starting material **2.9** in hexane (0.1 M) at room temperature (r.t.), and this solution then was stirred for additional 21 hours. The yield of **2.5α** was improved to 65% by this modified

condition and the C4-epimer **2.5β** might also be obtained in up to 5% yield, as well as the side products **2.24** and **2.25** in small proportion. Johnson also found that the maximum practical scale of this reaction was 75 mmol and the cyclobutanone **2.5α** was stable when stored at low temperature.[17]



Scheme 14. [2+2] Cycloaddition of the non-conjugated dihydrothiophene **2.9** and dichloroketene

2.3 Conformational Studies of Cyclobutanones 2.5

In principle, the five membered ring of the 2-thiabicyclo[3.2.0]heptan-6-ones might adopt either an *endo*envelope or *exo* envelope conformation (see Figure 7) . X-ray studies by Lange in the 1980s and later by Johnson of the Dmitrienko group indicated that, in the solid state, the *endo* envelope conformation is preferred (Figure 7).[83] In the ^{1}H-NMR spectra of **2.5α** no coupling is observed between H4 and H5. This observation, coupled with consideration of the Karplus relationship, suggests that the dihedral angle between H4 and H5 must be close to 90°.[84] Such a dihedral angle is expected for the *endo* envelope conformation in **2.5α** showing that this system has the same conformational preference in solution as it does in the solid state. In addition, the 4β-CO_2Et **2.5β** was isolated by Johnson and found to favor an *endo* envelope in solution as well. Later on, these results were further confirmed by X-ray crystallography studies (Figure 7).[17]

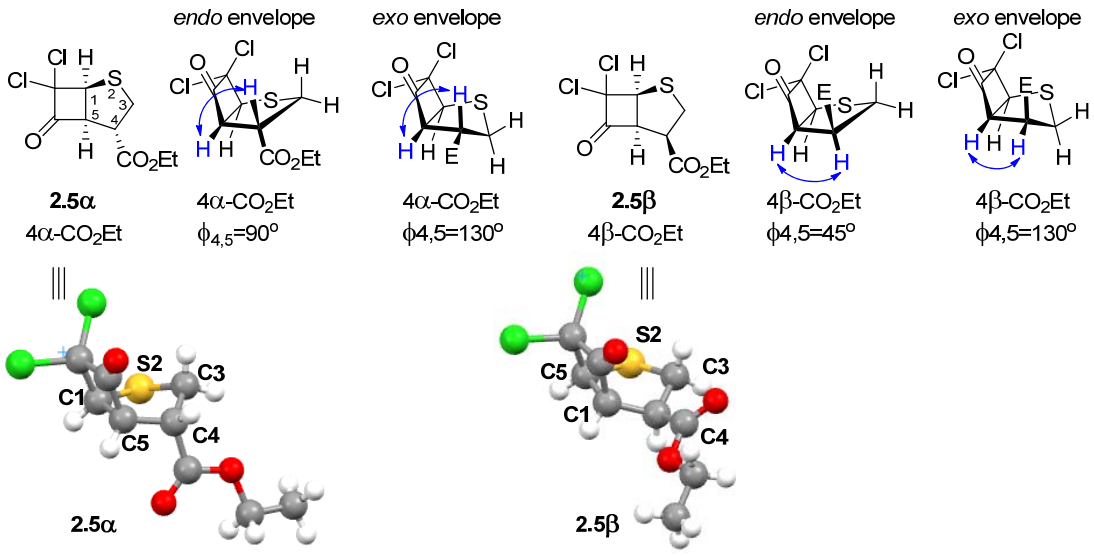


Figure 7. Conformational analysis of cyclobutanones **2.5α** and **2.5β** through molecular modeling and the corresponding X-ray crystal structures

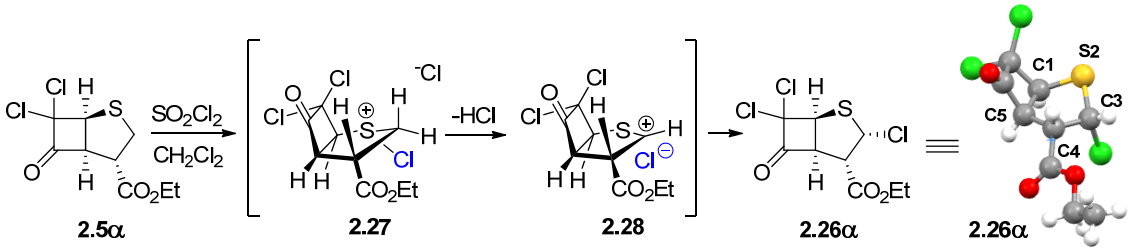
2.4 Derivatization at C3 of Cyclobutanones

Since the mid-2000s, Johnson has synthesized a series of cyclobutanone C3 derivatives that are potential β-lactamase inhibitors.

2.4.1 Chlorination at C3 of Cyclobutanones

The derivatization started with chlorination at C3 of cyclobutanone **2.5α**, which was treated with SO_2Cl_2 in CH_2Cl_2 to give a single chlorinated product **2.26α** in near quantitative yield (Scheme 15). To explain the observed stereoselectivity, it was postulated that in the first stage, an *S*-chlorosulfonium ion **2.27** is generated through the initial approach of the chlorinating agent from the *exo* face of **2.5α**, followed by elimination of HCl, to give the sulfur-stabilized carbocation **2.28**. The leaving group chloride then attacks C3 from the *exo* face, leading to the formation of product **2.26α**. In the ^{1}H-NMR spectrum, the coupling constant between H4 and H5 of **2.26α** was found to be 6 Hz. As mentioned in Section 2.3, no coupling between H4 and H5 for the cyclobutanone compounds (e.g. **2.5α**) with the *endo* conformation was observed. Therefore, **2.26α** must adopt an *exo* envelop conformation. This

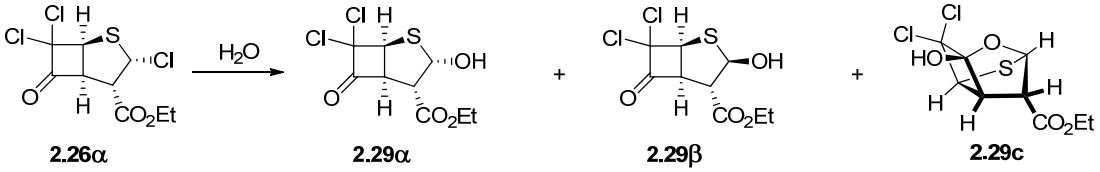
conclusion was later verified by a single crystal X-ray structure of **2.26α**.[17]



Scheme 15. Chlorination of cyclobutanone **2.5α**

2.4.2 Substitutions at C3 of Cyclobutanones

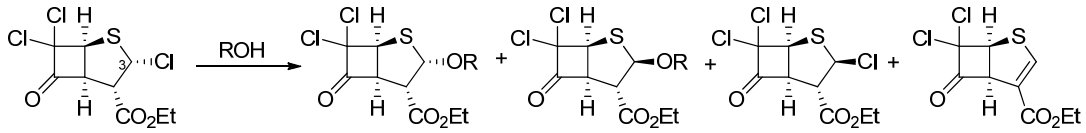
Reaction of **2.26α** in water (Scheme 16) gave a mixture of three products, the substitution product **2.29α** and its isomer **2.29β** as well as a tricyclic hemiketal **2.29c** in a ratio of 6 : 88 : 6.[17]



Scheme 16. Reaction between **2.26α** and H_2O

Furthermore, a series of solvolysis experiments of **2.26α** in various polar protic solvents were carried out by Johnson to generate the corresponding 3-alkoxy derivatives **2.30-2.34**, which are summarized in Table 1.[17]

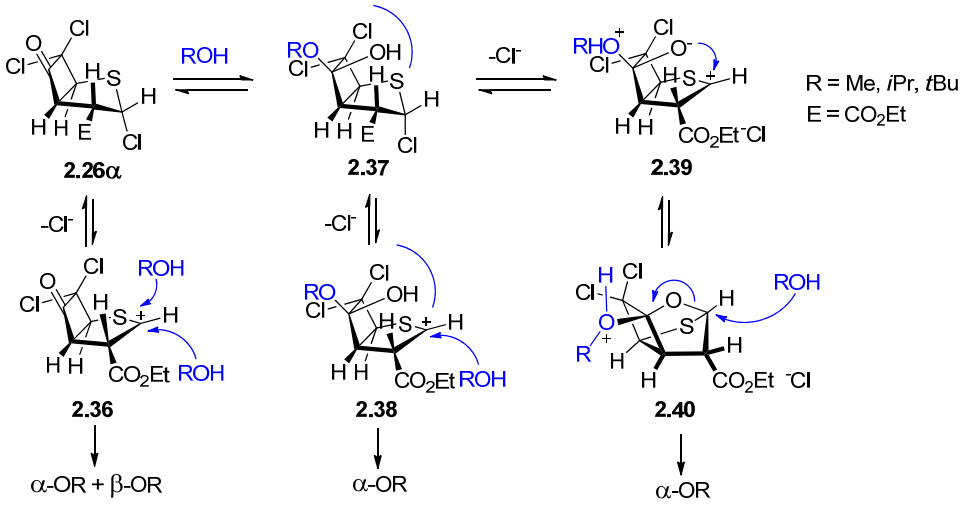
Table 1. Substitutions at C3 of **2.26α** with alcohols and acetic acid



2.26α α β **2.26β** **2.35**

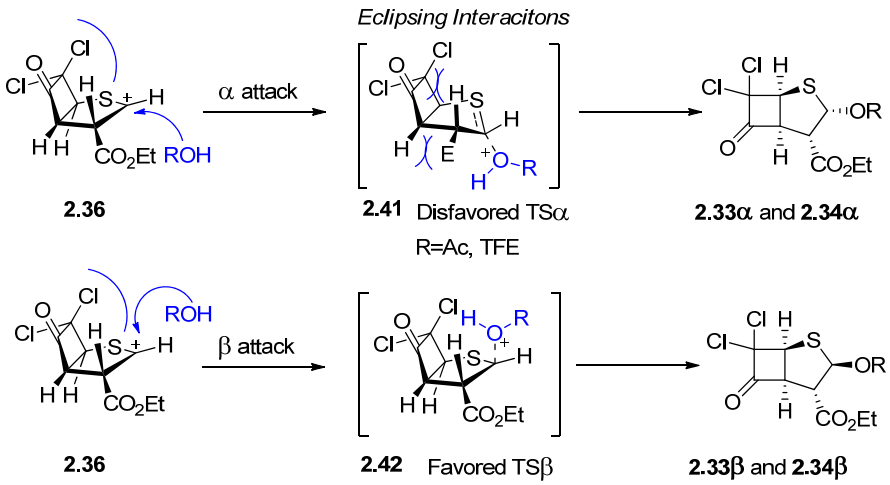
Solvent(s)	Time	Product	Yield (%)	OR	α (%)	β	**2.26β**	**2.35**
MeCN/H_2O (1:1)	48 h	**2.29**	75	OH	6	88	0	0
MeCN/MeOH (1:1)	48 h	**2.30**	73	OMe	75	24	0	1
MeCN/*i*PrOH (1:1)	40 h	**2.31**	62	O-*i*Pr	46	20	16	18
MeCN/*t*BuOH (1:1)	48 h	**2.32**	60	O-*t*Bu	34	7	44	15
AcOH	48 h	**2.33**	70	OAc	3	52	43	2
CF_3CH_2OH	48 h	**2.34**	64	OTFE	5	76	0	9

It was found that water and small alcohols provide higher yields of the corresponding α and β isomers, while bulky R (e.g. *i*Pr, *t*Bu, Ac) tend to give lower yields and favour the β isomer as well as the elimination product **2.35**. Moreover, the α isomers are the major products when the alcohols are MeOH, *i*PrOH and *t*BuOH, whereas the β isomers are the primary products when **2.26α** react with acetic acid and trifluoroethanol (TFEOH). However, the β stereoisomers were found to be unstable on silica gel during purification. Johnson also proposed a possible mechanism (Scheme 17) involving several intermediates to explain these observations.[17]



Scheme 17. Proposed mechanism for the steoroselective substitutions of **2.26α** by alcohols

On one hand, when the R groups are Me, *i*Pr and *t*Bu, **2.26α** is going to lose the chlorine to form the carbocation **2.36**, of which the alcohols could attack from either the *exo* or *endo* face to provide the corresponding α or β isomers, respectively. Moreover, **2.26α** could form a hemiketal **2.37** with the alcohols, which then converts to intermediate **2.38** that prefers attack from the *exo* face due to the steric hindrance in the *endo* face, leading to the formation of α isomers. Furthermore, **2.37** could form a tricyclic hemiketal **2.40** via intermediate **2.39**, which would also cause the generation of α isomers that are dominant under this situation. On the other hand, the more acidic solvents such as acetic acid and TFEOH are weaker nucleophiles and the corresponding reaction pathway is more likely to undergo a simple S_N1 process rather than the hemiketal formation (Scheme 18).[17] The transition state **2.41** is generated from an *exo* (α) face attack of the five-membered ring of intermediate **2.36**, but it is disfavoured due to steric interactions. In contrast, the *endo* (β) face attack of the tetrahydrothiophene gives transition state **2.42**, which is relatively more stable than **2.41**, providing the β isomers.



Scheme 18. Possible mechanism for preferential β selectivity in solvolysis of **2.26α** with AcOH and TFEOH

A modified condition using silver triflate as catalyst for substitution at C3 was examined by Johnson as well, which remarkably decreases the reaction time but the yields are lower than those under the original conditions. Interestingly, the stereoselectivity highly favored the β isomers in this case.[17]

The ^{1}H-NMR evidence of these C3 substituted products suggested that cyclobutanones with 3α substituents (Z = Cl, *O*-alkyl) prefer the *exo* conformation while the counterparts with 3β substituents (Z = H, Cl, OAc, *O*-alkyl) favour the *endo* envelope (Figure 8). It was proposed that the *J* values of H3, H4 and H4, H5 in the *endo* envelop should be zero, as the two dihedral angels are around 90°, while those in the *exo* envelop should not. According to the corresponding ^{1}H-NMR spectra, in the *endo* structure, both H3α and H4 appeared as singlets and H5 appeared as a doublet, whereas, in the *exo* conformation, a more complex splitting pattern was observed. The coupling of H3, H4 and H4, H5 could be detected clearly, which was consistent with the prediction. Later on, these were verified by the corresponding X-ray crystal structures.[17]

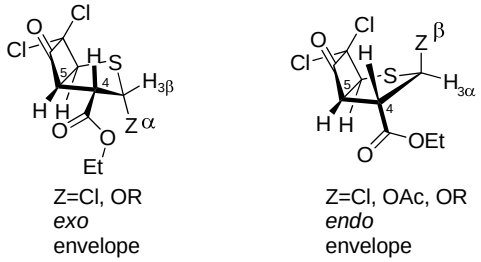
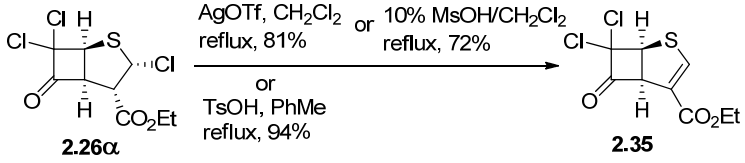


Figure 8. Conformational preference of C3-substituted cyclobutanone derivatives

In addition, substitution of the C3-chlorine of the ethyl ester **2.26α** by thiols (*i*PrSH and *p*-TolSH) and an allyl group (allylTMS) gave the corresponding products, which all strongly favor the *endo* envelop conformation.

2.4.3 Elimination at C3/C4 of Cyclobutanones

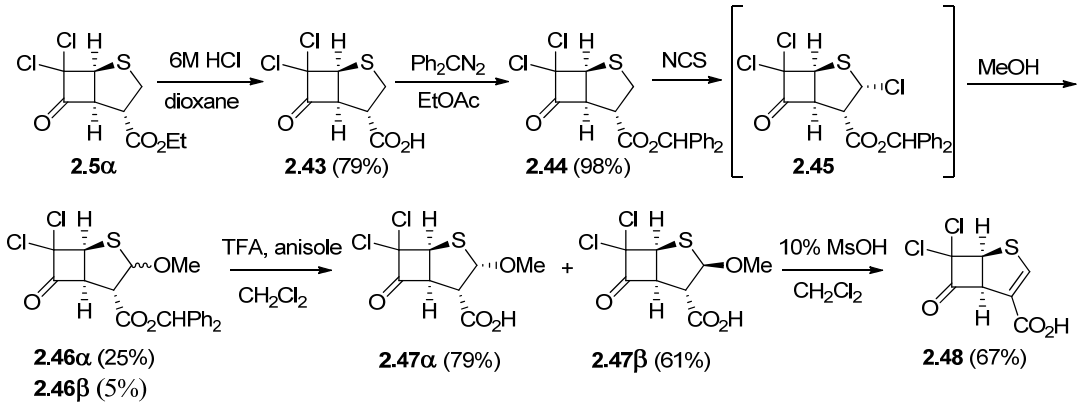
As illustrated in Scheme 19, the unsaturated ethyl ester **2.35** could be obtained in 82% yield through an elimination of **2.26α** by silver triflate in refluxing dichloromethane. Heating a solution of **2.26α** in dichloromethane with MsOH (10%) at reflux also provides the unsaturated cyclobutanone **2.35** (72% yield).[17] In addition, **2.35** could be prepared by heating **2.26α** with TsOH in toluene under reflux, giving the target **2.35** in 94% yield.



Scheme 19. Elimination of cyclobutanone **2.26α**

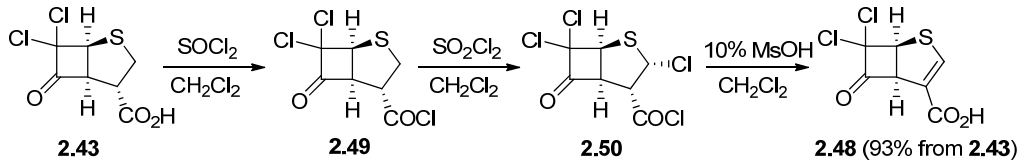
Although the ethyl ester **2.5α** can be readily hydrolyzed by 6 M HCl to the free acid **2.43**, those analogues with C3 substituents and C3/C4 unsaturation decomposed when cleavage of the ester ethyl group was attempted under the same conditions. Since the free acid forms of these cyclobutanones are better mimics of penicillins or penems, a new efficient strategy was greatly needed.

The carboxylic acid **2.43** was prepared from hydrolysis of **2.5α** with 6M HCl in dioxane (79%), which then reacted with Ph_2CN_2 to yield the benzhydryl ester **2.44** in near quantitative yield (Scheme 20). Chlorination with *N*-chlorosuccinimide (NCS), followed by methanolysis, provided the C3-OMe benzhydryl esters **2.46α** and **2.46β** in 25% and 5% yield. Then cleavage of the benzhydryl groups of **2.46α** and **2.46β** by trifluoroacetic acid (TFA) gave the corresponding free acids **2.47α** and **2.47β** in 79% and 61% yield respectively. Eventually, elimination of **2.47** with 10% MsOH afforded the unsaturated free acid **2.48** in 67% yield.[17]



Scheme 20. Preparation of C3-methoxy cyclobutanone **2.47** (mimic of penicillins) and unsaturated cyclobutanone **2.48** (mimic of penems)

Later on, an improved method for preparing the unsaturated acid **2.48** with much higher yield (93%) was developed in this lab by Johnson (Scheme 21). The acid **2.43** was converted into an acid chloride **2.49** with $SOCl_2$, which then was chlorinated by SO_2Cl_2 to give the C3-chlorinated acid chloride **2.50**. A one-pot elimination and hydrolysis with 10% MsOH gave the desired acid **2.48**.[17]



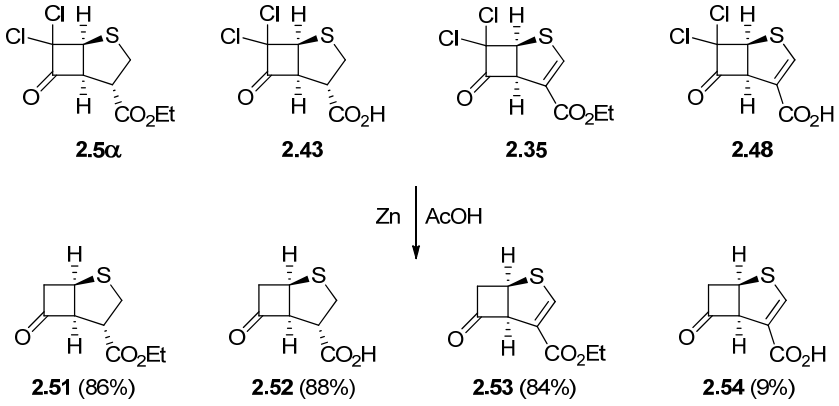
Scheme 21. Improved method for synthesizing cyclobutanone **2.48**

2.5 Reactions at C7 of Cyclobutanones

Some preliminary modifications at C7 of cyclobutanones were also carried out by Johnson to achieve more cyclobutanone mimics of carbapenems as β-lactamase inhibitors.

2.5.1 C7-Didechlorination of Cyclobutanones

The didechlorinated cyclobutanone **2.51** was obtained in 86% yield upon treating the dichloroethyl ester **2.5α** with Zn/AcOH. The free acid **2.43** and the unsaturated ones **2.35** and **2.48** could be dechlorinated using similar conditions to give the corresponding didechlorinated cyclobutanones **2.52** (88%), **2.53** (84%) and **2.54** (9%), respectively (Scheme 22).

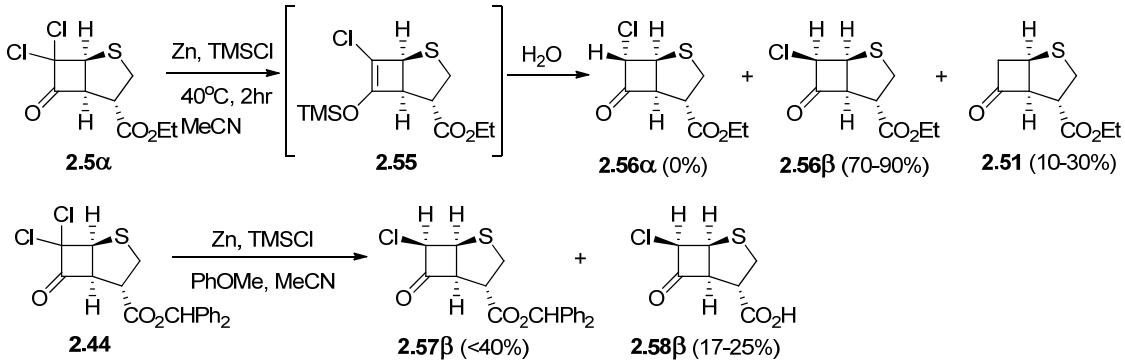


Scheme 22. Didechlorination of cyclobutanones **2.5α**, **2.35**, **2.43**, and **2.48**

2.5.2 C7-Monodechlorination of Cyclobutanones

Johnson performed some preliminary studies on the monodechlorination of the cyclobutanones **2.5α** (Scheme 23), which reacted with Zn and TMSCl in acetonitrile at 40 °C for two hours to give the TMS enol ether **2.55**. The enol ether underwent aqueous work up to provide the monochlorocyclobutanone **2.56β** in 70-90% yield and the didechlorinated compound **2.51** in 10-30% yield. However, no monochloro product **2.56α** was obtained. Following the same procedure, the dichlorobenzhydryl ester **2.44** was converted into the corresponding monochloro product **2.57β** (< 40%) and the free acid **2.58β** (17-25%), which are the precursors for the C7-hydroxymethyl cyclobutanones that are analogues of

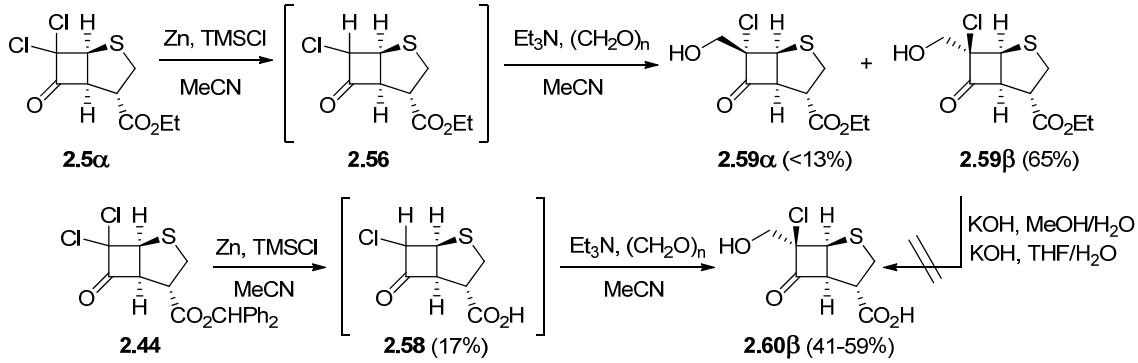
carbapenems. Some attempts had been made to generate the monochloroethyl ester **2.56** directly through the [2+2] cycloaddition between chloroketene and the deconjugated dihydrothiophene **2.9**; however, these reactions were unsuccessful.[17]



Scheme 23. Monodechlorination of ethyl ester **2.5α** and benzhydryl ester **2.44**

2.5.3 C7-Hydroxymethylation of Cyclobutanone Derivatives

With the successful monodechlorination, hydroxymethylation at C7 was carried out sequentially. As shown in Scheme 24, the mixture of **2.56** was treated with TEA in MeCN to give an enolate, which then underwent an aldol condensation with paraformaldehyde to generate the hydroxymethyl diastereomers **2.59α** (< 13%) and **2.59β** (65%). Unfortunately, they could not be hydrolyzed to the free acid under basic conditions. The C7-hydroxymethyl acid **2.60β** (41-59%) could be obtained from the benzhydryl ester **2.44** via the monochloro intermediate **2.58** (17%) through a similar monodechlorination-aldol condensation process.[17]

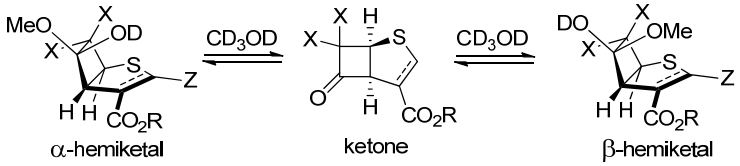


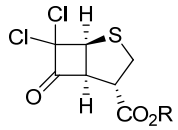
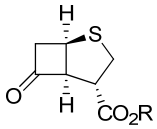
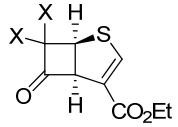
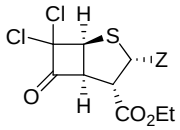
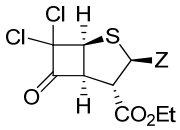
Scheme 24. C7-hydroxymethylation of cyclobutanone derivatives

2.6 Formation of Hemiketals of Cyclobutanones

It was reported by Evanoff and Johnson that cyclobutanones could generate the corresponding hydrates in D_2O (more discussion in Section 2.7) and hemiketals in MeOH-d_4, which are summarized in Table 2 below.[17, 82]

Table 2. Hemiketal formation of cyclobutanone derivatives



Cyclobutanones		α : β Hemiketal Ratio	Hemiketal (%)
	**2.43**: R = H **2.5α**: R = Et	2.7: 1 2.7: 1	88 91
	**2.51**: R = Et **2.52**: R = H	1.8:1 1.6:1	19 24
	**2.35**: X = Cl **2.53**: X = H	1.8:1 1.5:1	96 38
	**2.30α**: Z = OMe **2.31α**: Z = O*i*-Pr **2.32α**: Z = O*t*-Bu	1.2:1 1.1:1 1.1:1	98 98 98
	**2.30β**: Z = OMe **2.31β**: Z = O*i*-Pr **2.32β**: Z = O*t*-Bu **2.33β**: Z = OAc	4.7:1 4.2:1 1.8:1 1.5:1	15 24 40 30

These results indicate that the chlorocyclobutanones form a larger proportion of hemiketal in equilibrium with the corresponding keto form. For instance, the dichloro species **2.5α** generates 91% hemiketal, while its non-chlorinated counterpart **2.51** generates only 19%

of the hemiketal at equilibrium. The electronegative chlorine atoms significantly destabilize the keto form that has substantial partial positive charge on the carbon of the C=O bond. The partial positive charge on the corresponding carbon atom in the hemiketal is smaller than that in the keto form so that the ketal is less destabilized by the halogens than is the ketone.[85] For the C3 derivatives, the cyclobutanones with 3α substituents undergo greater hemiketal generation (98%), whereas those with 3β substituents form the hemiketals only to an extent of 15% to 40%.

Generally, all these tested cyclobutanones prefer generation of α-hemiketals, and the hemiketals produced by C3α-substituted compounds favor the *exo* envelope conformation while the hemiketals from C3-unsubstituted cyclobutanones prefer the *endo* envelope (Figure 9). Moreover, the α-hemiketals generated by C3β-substituted cyclobutanones have a tendency to adopt the *endo* conformation while the β-hemiketals adopt the *exo* envelope in order to avoid the steric hindrance between the OMe group and the substituents in the *endo* face of the bicyclic ring.[17]

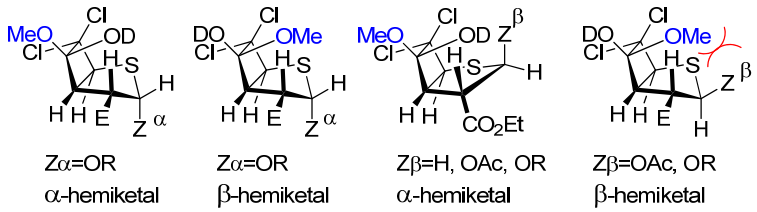
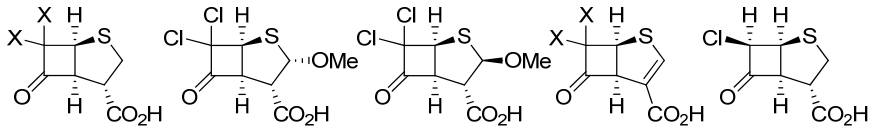


Figure 9. Conformational preference of cyclobutanone hemiketals

2.7 Bioactivities (IC_{50}) of Cyclobutanones as β-Lactamase Inhibitors

The inhibition of some common β-lactamases by the cyclobutanones prepared by Johnson has been tested by Ms. Valerie Goodfellow and Dr. Laura Marrone of the Dmitrienko group. The inhibition of the chosen β-lactamases by such cyclobutanones are summarized in Table 3 below.[17]

Table 3. IC_{50} (μM) of cyclobutanone mimics against common β-lactamases



2.43: X=Cl
2.52: X=H

2.30α

2.30β

2.35: X=Cl
2.54: X=H

2.58β

Ketone inhibitor	% hydrate in D_2O	Class A KPC-2	Class B IMP-1	Class B VIM-2	Class C GC1	Class D OXA-10
2.43	74	76±8	> 1000	> 1000	25±3	268±8
2.52	0	117±13	235±14	> 1000	44±3	1135±33
2.30α	> 98	58±2	122±5	363±9	6.5±1.4	156±6
2.30β	6	99±5	N/A	N/A	38±4	547±19
2.35	93	26±2	213±21	> 1000	4.5±0.3	370±15
2.54	< 2	170±2	> 500	N/A	34±3	> 1000
2.58β	N/A	> 500	≈ 260	> 500	> 500	> 500

According to the table, the β-lactamases KPC-2, IMP-1, GC1 and OXA-10 are mostly inhibited by cyclobutanones that are able to form a larger amount of hydrate (**2.43, 2.30α** and **2.35**). Among these β-lactamases, the SBLs are more efficiently inactivated by the cyclobutanones while the MBLs are only moderately inhibited by them. Moreover, compound **2.35** presents slightly higher activity against KPC-2 and GC1, which indicates that the stereochemistry at C4 might significantly affect the binding to the Class A and C SBLs. In addition, the C3α-OMe cyclobutanone **2.30α** shows the best inhibition towards these β-lactamases, because it was found to have a very similar structure to penicillin with an *exo* envelope conformation, which possesses higher affinity for β-lactamases. Later on, X-ray crystallographic studies provide the evidence that compound **2.30α** generate a hemiketal binding to the serine residue in the active site of OXA-10 (Figure 10).[86]

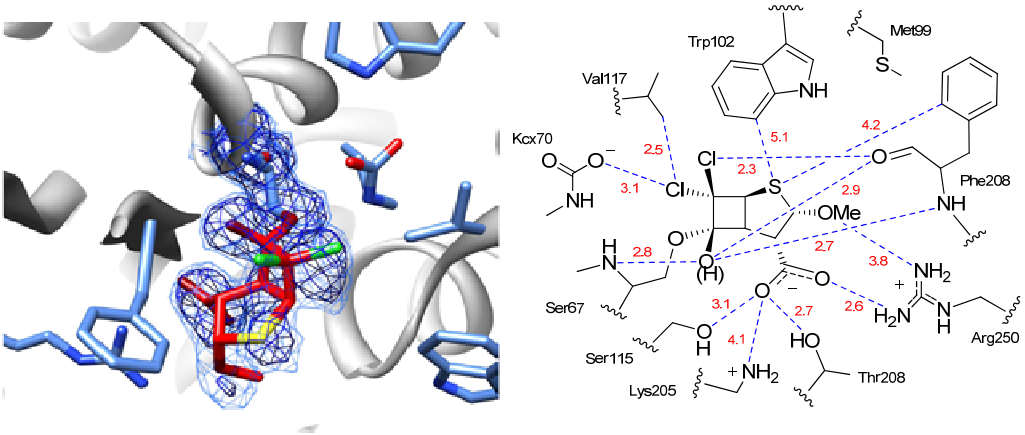
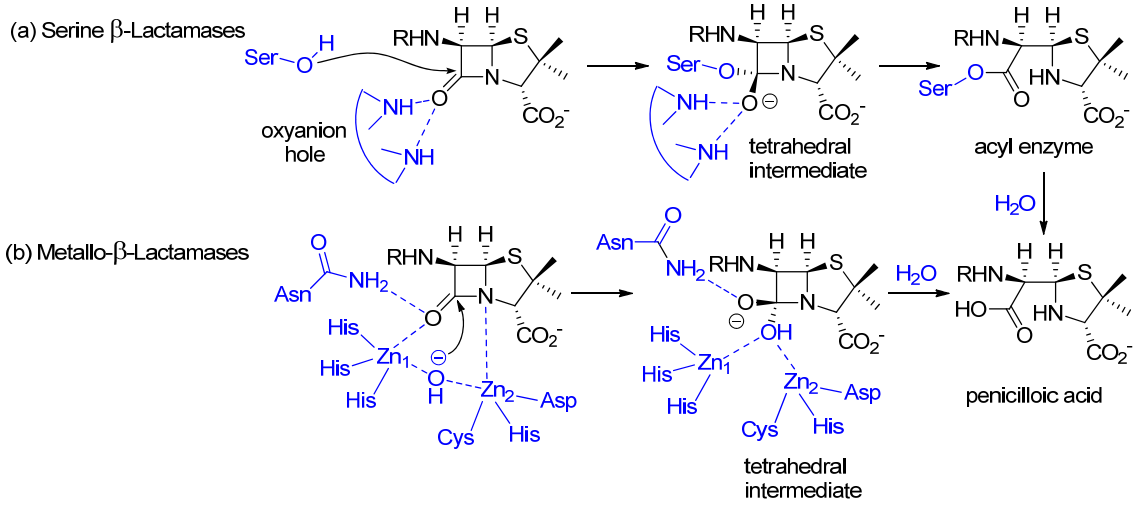


Figure 10. Interactions of cyclobutanone **2.30α** with serine residues in the active site of OXA-10 (Permission was obtained from publisher. See Appendix D for more details.)

The former members of the Dmitrienko lab made great contributions to the research on cyclobutanones. They developed some feasible conditions to prepare the key intermediate, the dichlorocyclobutanone ethyl ester **2.5α**, which was the precursor for the series of cyclobutanone derivatives, and computational and conformational studies also aided in the design newer cyclobutanone analogues as β-lactamase inhibitors. To pursue more active β-lactamase inhibitors, the cyclobutanone project in the Dmitrienko group is continued in this thesis work, which explores further chemistry at the C7 carbon of this system. More details will be discussed in the following chapters.

Chapter 3 Novel Cyclobutanone Mimics of β-Lactam Antibiotics: Synthesis and Properties

The mechanism for hydrolysis of β-lactam antibiotics by SBLs involves nucleophilic attack at the carbonyl carbon of the β-lactam by the hydroxyl group of a serine residue in the enzyme active site, which leads to the β-lactam ring-opening to form an acyl enzyme intermediate via a tetrahedral intermediate. In the next deacylation step, this ester is hydrolyzed by a water molecule that is bound nearby in the active site and activated by a glutamate residue that acts as a general base. For the MBLs, the hydrolytic mechanism involves the generation of the tetrahedral intermediate or transition state through nucleophilic attack of the carbonyl by the zinc bound water of hydroxide (Scheme 25).



Scheme 25. Hydrolysis of penicillin by β-lactamases

In terms of a cyclobutanone mimic, when it is bound to SBLs in the active site, it is expected to generate a hemiketal adduct, which would be sufficiently long-lived to prevent the β-lactams from being hydrolyzed. The cyclobutanone analogue is postulated to bind to the zinc ion(s) in the MBLs active site, and it is predicted to form a hydrate when it is attacked by the water molecule or hydroxide. Even though some cyclobutanone mimics prepared by the

Dmitrienko group showed moderate inhibition of certain MBLs, the corresponding X-ray crystallographic evidence indicating how the cyclobutanone mimics bind to the MBLs is still not available at the moment. The proposed mechanisms for cyclobutanone mimics inhibiting the β-lactamases are illustrated in Scheme 9 of Chapter 2.

3.1 Initial Cyclobutanone Targets

This thesis project initially aimed at synthetically elaborating the cyclobutanones that more closely resemble meropenem, which is one of the clinically used and last line of defence carbapenem type of β-lactam antibiotics. The other two major carbapenems currently in clinical use are imipenem and doripenem (Section 1.1.3). These carbapenems possess a broad spectrum of activity against both Gram-positive and Gram-negative pathogens, and they are also found to be resistant to hydrolysis by many SBLs. It has been discovered by several research groups that carbapenems interact with the active site serine residues of most SBLs to form an acyl intermediate that is relatively long-lived rather than hydrolyzed rapidly, when compared to β-lactams without this hydroxyethyl side chain. A crucial structural feature within the carbapenems is the hydroxyethyl side chain attached to the α position of the β-lactam carbonyl, which plays an essential role in their resistance to hydrolysis by the SBLs. This is confirmed by experimental evidence from the Bonomo group (Figure 11). After the generation of the acyl enzyme intermediate, this hydroxyl group interacts with a water molecule in the active site through H-bonding, preventing the water from acting as a nucleophile to attack the ester group of the acyl enzyme.[27]

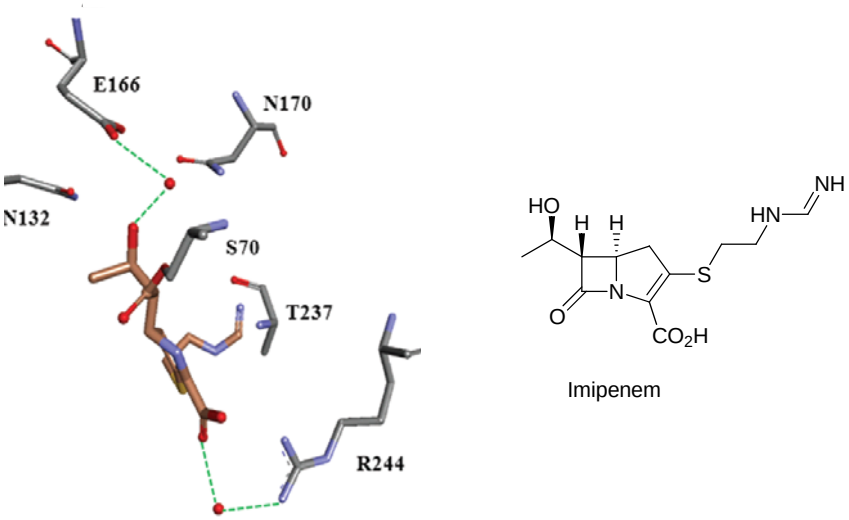


Figure 11. The hydroxyl group within the side chain of imipenem forms a hydrogen bond with the water molecule in the active site of TEM-1. (Permission was obtained from publisher. See appendix E for more details.)

Due to the evolution of bacteria, more recently, a number of SBLs including KPC-2, OXA-23 and OXA-48 were newly identified as carbapanemases and found to be able to catalyze the rate of hydrolysis of the carbapenems. Consequently, they threaten almost all the current clinical β-lactam antibiotics.[27]

The possible mechanism of the efficient hydrolysis of the carbapenems by the carbapanemases was reported by Spencer and co-workers at the University of Bristol in 2011. Some serine β-lactamases, such as SFC-1 (a Class A SBL), are very efficient in hydrolyzing carbapenems through the key residues in its active site. The active site of such carbapenemase is enlarged and induces rotation of the hydroxyl group on the hydroxyethyl side chain of the carbapenems away from the site where the nucleophilic water molecule is bound, making the hydroxyl group no longer interact with the active site water molecule by hydrogen bonding. As a result, the acyl enzymes from carbapenems undergo a rapid hydrolysis to release the free β-lactamases that totally destroys the β-lactams.[87] As demonstrated in Figure 12A, the Ser70 of an Ala mutant of SFC-1 binds to an unhydrolyzed meropenem. The distance between the water

molecule and the hydroxyl group on the side chain is 2.70 Å (d1) and an H-bond is observed. In addition, the distance between Asn132 and the OH group is 2.95 Å (d2), which also generates an H-bond. While in the acyl enzyme complex (Figure 12B, a Glu166 Ala mutant), it is observed that the hydroxyl group of meropenem is rotated by approximately 120° when compared to the former complex. As a result, the distance d2 is slightly decreased to 2.87 Å and the H-bond between Asn132 and the OH group remains. However, the distance d1 is significantly increased to 4.97 Å, which is too far to generate an H-bond between the OH group and the water molecule. Consequently, the water is able to attack the acyl enzyme to give the hydrolyzed meropenem and release free enzyme.[87]

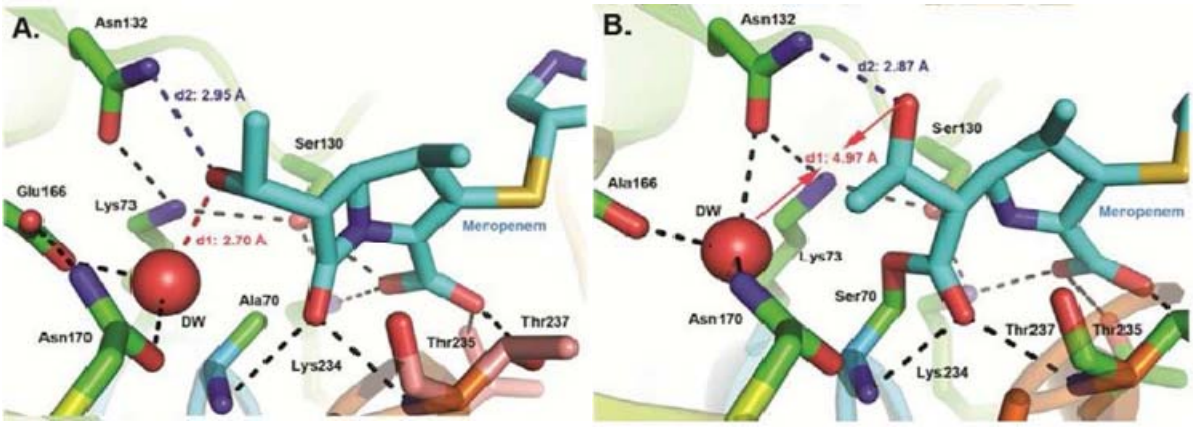
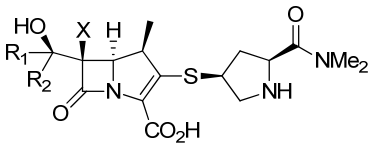


Figure 12. (A) An unhydrolyzed meropenem binding to the active site of Ser70 Ala mutant of SFC-1 (B) The acyl enzyme intermediate of meropenem binding to the active site of Glu166 Ala mutant of SFC-1 (Permission was obtained from publisher. See Appendix F for more details.)

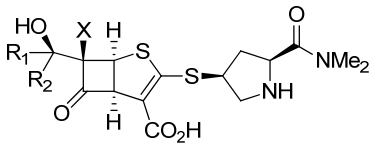
Initially, the Dmitrienko group proposed a novel idea to address Spencer's discovery. For the sake of enhancing the stability of carbapenems in the serine active site of the carbapenemases, the hydroxyethyl side chain could be modified (Figure 13). More specifically, a side chain with two hydroxyl groups was planned to replace the single hydroxyl side chain of carbapenems, leading to the formation of a germinal dihydroxyl groups compound or a hydrate

of a carbonyl group, which might solve the problem of rapid hydrolysis of carbapenems by the carbapenemases. If the modified carbapenem with a germinal diol side chain binds to the SBLs, even though these enzymes are able to orient one of the OH group of the diols away from the active site water molecule, there is another hydroxyl group remaining that may still form an H-bond with the water molecule and thus inhibit the β-lactams.

The initial research goal for this project was to synthesize cyclobutanone mimics of carbapenem with a geminal diol side chain and some related derivatives that are shown in Figure 13. Theoretically, the geminal diols are not stable as they can undergo a dehydration process, reversing to the corresponding ketone or aldehyde. Therefore, a very strong electron withdrawing group, such as trifluoromethyl group (R_2 group), is considered to be introduced into the side chain, which would favour formation of the hydrate.



R_1	R_2	X	
H	CH_3	H	meropenem
OH	CF_3	Cl	gem-diol analogue
OH	H	Cl	gem-diol analogue

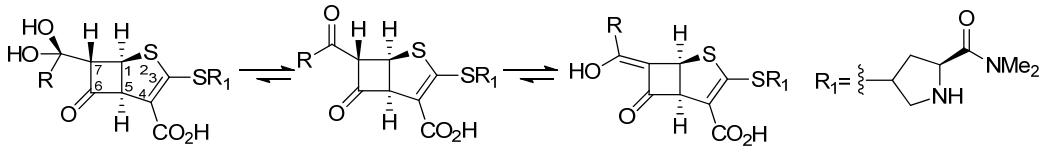


R_1	R_2	X	
H	CH_3	H	meropenem analogue
H	H	H	
H	H	Cl	
OH	CF_3	Cl	gem-diol analogue
OH	H	Cl	gem-diol analogue

Figure 13. The proposed geminal diol analogues of meropenem

It should also be noticed that the presence of a chlorine atom at C7 adjacent to the carbonyl group would prevent the unfavourable equilibrium illustrated in Scheme 26 shown below. If the chlorine atom was replaced by a hydrogen atom, as mentioned above, the geminal compounds would likely tautomerize to yield a conjugated enol that is considered to be relatively stable. The electron-negative C7 chlorine atom not only helps to stabilize the hydrate (diol) side chain, but also increases the electrophilicity of the carbonyl carbon within

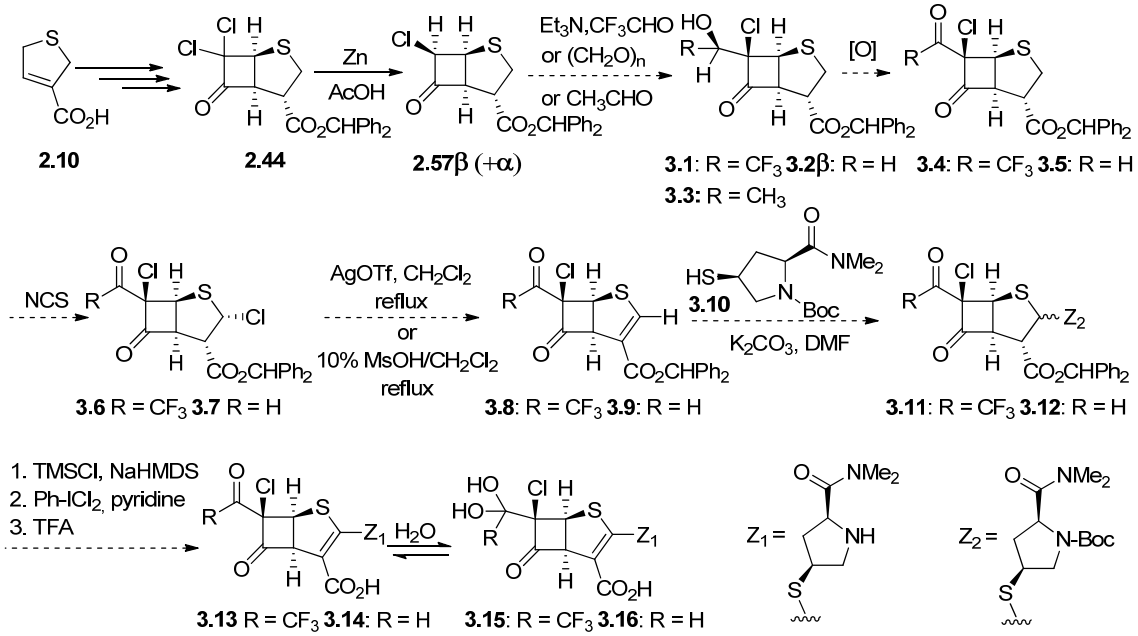
the β-lactam ring, making it more readily form a hemiketal with the active site serine residue.



Scheme 26. The potential equilibrium of the meropenem mimics

3.2 Initially Proposed Synthetic Route to Target Cyclobutanones

At the very beginning, a possible synthetic route towards the target compounds **3.15** and **3.16** was proposed as shown in Scheme 27. This project started with the unsaturated free acid **2.10** available in the Dmitrienko Lab, which then carried on several steps including deconjugation, basic hydrolysis, [2+2] cycloaddition, acidic hydrolysis and the installation of benzhydryl group to give the benzhydryl ester **2.45**. These reactions have been discussed in detail in Section 2.2.

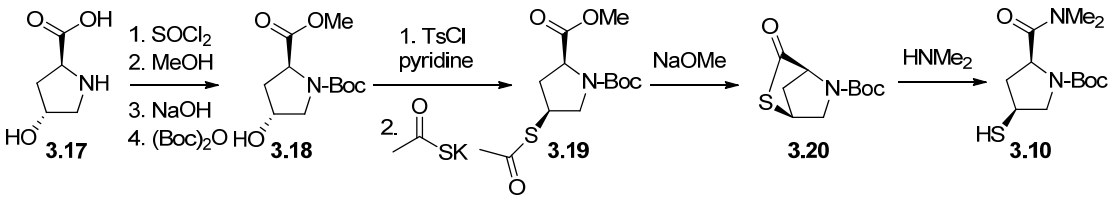


Scheme 27. Initially proposed synthetic route towards novel cyclobutanone analogues of meropenem

As shown in Scheme 27, a known monodechlorination condition could convert the

dichlorocyclobutanone precursor **2.44** to monochlorocyclobutanones **2.57**, which then might undergo aldol condensation with an aldehyde (e.g. CF_3CHO, CH_2O and CH_3CHO) to yield the corresponding C7 hydroxyalkyl cyclobutanones **3.1-3.3**. In the next step, **3.1** and **3.2** are expected to be oxidized to ketones **3.4** and **3.5**, respectively, which then may undergo a chlorination with NCS to furnish the C3-derivatives **3.6** and **3.7**, followed by elimination of HCl with either silver triflate or 10% MsOH in refluxing dichloromethane to give the corresponding unsaturated cyclobutanones **3.8** and **3.9**. The C3-chlorination and elimination method was developed by Johnson, as described in Section 2.4.3. The following step is to introduce a thioether group to the C3 position of **3.8** and **3.9**, which can take advantage of the chemistry developed in the synthesis of thienamycin analogues by the Beecham Pharmaceuticals group.[88] This proposed strategy involves a base-induced conjugate addition of a thiol **3.10** to the unsaturated ester **3.8** and **3.9**, leading to the generation of **3.11** and **3.12**, followed by an enolate formation and overall dehydrogenation with a hypervalent iodine reagent, providing the desired ketones **3.13** and **3.14**, which are expected to be able to generate spontaneously the hydrate **3.15** and **3.16** in aqueous solutions.

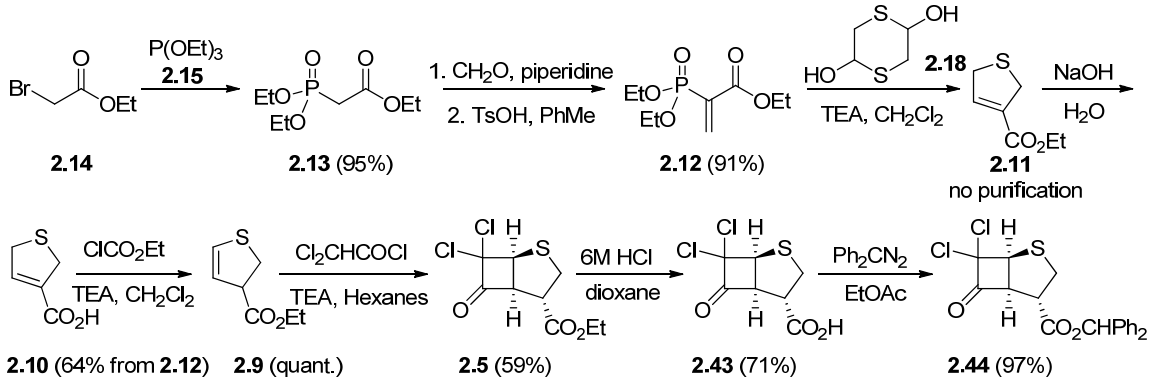
The thiol precursor **3.10** can be prepared by the reported synthetic approach in Scheme 28.[89] The commercially available starting material **3.17** reacts with thionyl chloride to turn the carboxylic acid into an acid chloride, followed by methanolysis to afford the methyl ester that then is treated with sodium hydroxide and di-*tert*-butyl dicarbonate to give compound **3.18**. In the next step, **3.18** is converted into a thiol ester **3.19** with the desire stereochemistry through reaction with TsCl and potassium thioacetate in sequence. Treatment of the thiol ester **3.19** by sodium methoxide gives the cyclized compound **3.20**, which then undergoes an aminolysis with dimethylamine to yield the target thiol **3.10**.



Scheme 28. Preparation of the thiol precursor **3.10**

3.3 Preparation of the Benzhydryl Ester 2.44

This project started with the conjugated acid **2.10** that was available in the Dmitrienko lab. Due to the large demand of the benzhydryl ester **2.44** in this project, it was necessary to go back to the very beginning, starting with the commercially available triethyl phosphite **2.15** and ethyl bromoacetate **2.14**. All the details of the related reactions have been discussed in Chapter 2 and this section just summarizes these reactions and the corresponding yields (Scheme 29), which have been carried in this thesis work. In fact, cyclobutanone **2.5** is a racemic mixture with the indicated relative stereochemistry, and all the reaction were performed with this mixture. For the sake of simplicity, only one enantiomer is shown in all the diagrams.



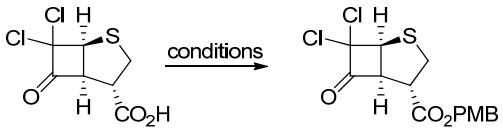
Scheme 29. Preparation of precursor **2.44** in this thesis work

3.4 Preparation of the PMB Ester 3.21

Initially, other protecting groups were considered to replace the benzhydryl group to protect the free acid **2.43**, since the PMB source (PMB-Cl or PMB-I) is much safer,

particularly at large scale, to handle than the benzhydryl source (Ph_2CN_2) that is potentially explosive. A great deal of effort was made towards the synthesis of the PMB ester **3.21**, and the results of such attempts are summarized in Table 4 below.

Table 4. Efforts towards preparation of PMB ester **3.21**

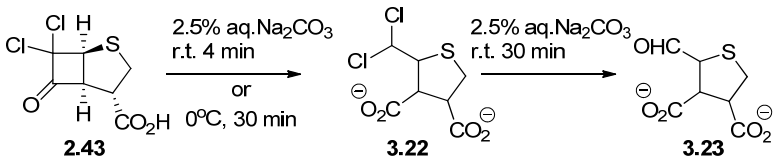


2.43 **3.21**

Trial	Reagent	Solvent	Temp.	Time	Result
1	PMB-Cl, $NaHCO_3$[90]	DMF	45°C	3 days	N.R.
2	PMB-I, *i*Pr_2NEt	DMF	r.t.	4 h	N.R.
3	PMB-I, *i*Pr_2NEt	Acetone	Reflux	23 h	31%
4	PMB-I, $NaHCO_3$	Acetone	Reflux	23 h	34%
5	DCC, DMAP, HOBT, PMB-OH	DMF	r.t.	20 h	Decomp.
6	PMB-I, K_2CO_3	Acetone	Reflux	20 h	Decomp.
7	PMB-I, K_2CO_3	Acetone	Reflux	8 h	Decomp.
8	Ph_2PCl, imidazole, PMB-OH, I_2[91]	MeCN	Reflux	22 h	Decomp.
9	Ph_2PCl, imidazole, PMB-OH, I_2	MeCN	Reflux	4 h	<29%

In the first trial, sodium bicarbonate was used as the base in order to deprotonate the acid, and the carboxylate is a potential nucleophile that could attack the benzyl carbon within the PMB-Cl to displace the chlorine, yielding the desired PMB ester **3.21**. Even when this reaction was carried out in DMF at 45 °C for 3 days, there was no PMB ester formed, as indicated by ^{1}H-NMR. One possible reason for the unsuccessful synthesis might be the decomposition of the dichlorocyclobutanone **2.43** by using sodium bicarbonate as the base. It was discovered by Evanoff that **2.43** is very sensitive to aqueous sodium carbonate. More exactly, the cyclobutanone ring of **2.43** can be easily opened in 2.5% aq. Na_2CO_3 at r.t. within

4 minutes or 0 °C within half an hour to generate a dicarboxylate **3.22** that then further degrade to an aldehyde **3.23** (Scheme 30).[82] It is possible that the acid **2.43** is partially ring opened due to the usage of $NaHCO_3$ even though the reaction mixture is non-aqueous, since $NaHCO_3$ is not strictly anhydrous.

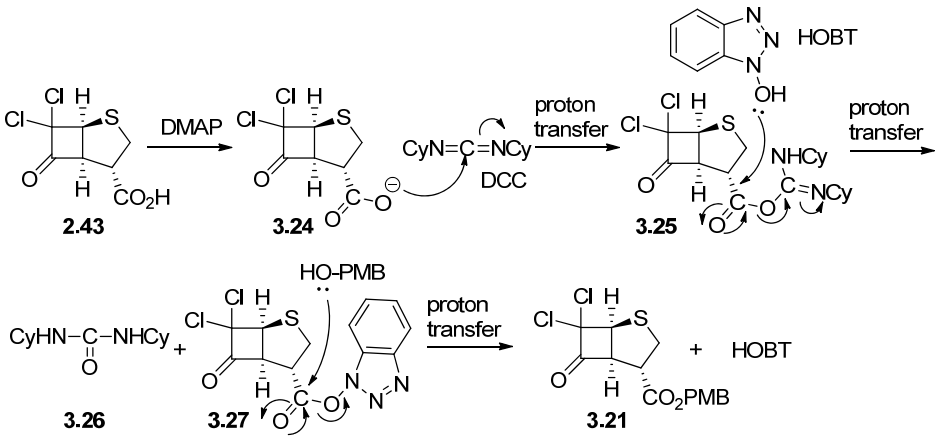


Scheme 30. Degradation of dichlorocyclobutanone **2.43** in aqueous Na_2CO_3

Another possibility is that the reactivity of the PMB source (PMB-Cl) is not high enough. Therefore, for the second trial, the base was changed to diisopropylethylamine (DIPEA), and the PMB source was changed to PMB-I that was prepared by the Finkelstein reaction of PMB-Cl and NaI.[92] This reaction was carried out at r.t. for 4 hours; however, there was still no target PMB ester obtained.

When the reaction was carried out in refluxing acetone for 23 hours (Trial 3), the desired PMB ester **3.21** was isolated by flash chromatography in 31% yield. Later on, sodium bicarbonate was used as the base again in Trial 4, because its solubility in acetone was not good, the cyclobutanone ring was expected to be stable under this condition, which eventually provided the desired **3.21** in 34% yield. The optimization continued. It was proposed that activation of the carboxylic acid might promote the esterification reaction. For Trial 5, a dicyclohexylcarbodiimide (DCC) coupling was attempted, involving the starting material treated with 4-dimethylaminopyridine (DMAP), hydroxybenzotriazole (HOBT) and PMB-OH in DMF (Scheme 31).[93] The acid **2.43** is deprotonated by DMAP to give the carboxylate **3.24** that then reacted with DCC to yield the intermediate **3.25**. Followed by nucleophilic attack by HOBT upon the carboxylate carbonyl within **3.25**, intermediate **3.27** should be obtained. The

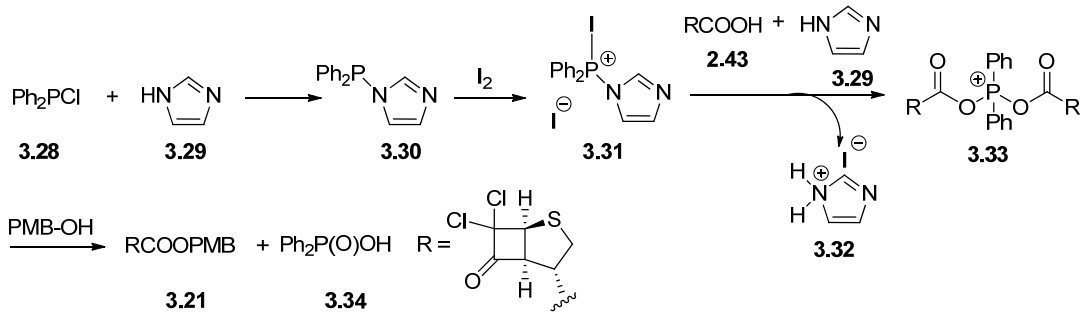
HOBT moiety within **3.27** acts as an excellent leaving group in favour of forming the PMB ester **3.21** upon PMB-OH's nucleophilic attack. However, this condition did not work and no desired product was isolated. Since this method did not seem promising, no more experiments following this strategy were performed.



Scheme 31. Proposed mechanism for the DCC coupling

Other conditions were attempted to reach the goal as well (Trial 6 and 7). In these experiments, potassium carbonate was used as the base in refluxing acetone for 20 hours and 8 hours, respectively. Unfortunately, the starting material decomposed under such conditions.

The last few attempts were focused on the phosphine-imidazole based coupling (Scheme 32).[91] The intermediate **3.30** is generated *in situ* from the chlorodiphenylphosphine **3.28** and imidazole **3.29**, which then is treated with iodine to yield the phosphonium salt **3.31**. Followed by reaction with two moles of the acid **2.43**, the acyloxyphosphonium ion **3.33** should be generated, which then may be attacked by the PMB-OH to give the desired PMB ester **3.21** along with the side product **3.34**. For Trial 8 and 9, when the reaction was run for 22 hours, the starting material decomposed. If the reaction time was reduced to 4 hours, only a small amount of PMB ester **3.21** (< 29%) was generated. This method was not further explored because of the poor yield and relatively expensive cost of Ph_2PCl.



Scheme 32. Proposed mechanism for the phosphine-imidazole based coupling

In summary, the best yield to prepare the PMB ester **3.21** is only 34%, which is far from ideal. More importantly, the following monodechlorination on the PMB ester **3.21** was also found to be very problematic after a few attempts. Therefore, the PMB ester **3.21** was "abandoned" and the benzhydryl ester **2.44** was picked again as the key intermediate for this project, which was obtained from the acid **2.43** reacting with diphenyldiazomethane in ethyl acetate. Ph_2CN_2 is a purple solid prepared by oxidation of benzophenone hydrazone by mercury oxide in a pressure bottle, and it is usually dissolved in EtOAc and stored in the cold prior to use.

3.5 Monodechlorination of the Benzhydryl Ester 2.44

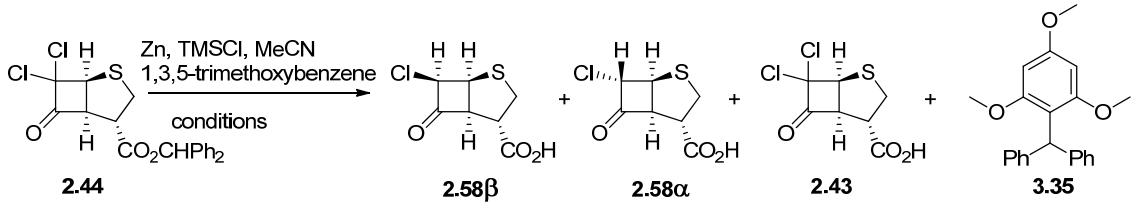
Since the monochlorocyclobutanone **2.57** is the essential precursor for the C7 derivatives, developing a convenient and efficient method to largely produce the monochloro cyclobutanone becomes essential.

3.5.1 Method Based on Zinc and TMSCl

The initial attempts towards monodechlorination of the benzhydryl ester **2.44** were based on Johnson's strategy that has been discussed in Section 2.5.2, involving treatment of the starting material with zinc dust, TMSCl and anisole in acetonitrile. During this reaction, the benzhydryl group was cleaved as a carbocation, which might have an adverse effect on the desired reaction. Therefore, the initial trapping reagent (anisole) was replaced by a much

stronger one, 1,3,5-trimethoxybenzene (TMBz). A side product **3.35** derived from alkylation of the trapping reagent TMBz by the benzhydryl carbocation was isolated by flash chromatography and characterized.[94] Moreover, several conditions were examined to optimize the reaction (Table 5).

Table 5. Efforts towards monodechlorination with Zn-TMSCl[a]



Trial	Reagents	Condition	Product Ratio[b]
1	Zn, TMSCl, TMBz	40 °C for 4 h	**2.43**/**2.58α**/**2.58β** (2:1:3, 29%)[c]
2	Zn, TMSCl, TMBz	Reflux for 4 h	**2.43**/**2.58α**/**2.58β** (4:3:10)[d]
3	Zn, TMSCl, TMBz	Reflux for 3 h, 40 °C for 1h	**2.58β** (24%)[e]
4	Zn, TMSCl, TMBz	Reflux for 2 h, r.t. for 1 h	**2.58α/β** (1: 3.5, 40%)[f]

(a) All reactions were carried on in MeCN and all reaction mixtures contained **3.35**. (b) identified by ^{1}H-NMR (c) crude yield (d) crude mixture (e) isolated yield (f) purified by flash chromatography

It is clear from the table that the yields of these reactions are noticeably increased when compared to the one (17%) previously obtained by Johnson, because of the use of a better trapping reagent. The first experiment followed the original condition (40 °C for 4 h) and provided a mixture of **2.43**, **2.58α** and **2.58β** in a ratio of 2 : 1 : 3, as indicated by ^{1}H-NMR, in approximately 29% total yield (**2.58α**/**β)**. The subsequent trials were carried first out in refluxing MeCN for a certain time (2-4 h), and then further reacted at 40 °C or room temperature. However, the best result was a mixture of **2.58α** and **2.58β** (1 : 3.5), obtained in 40% yield after flash chromatography.

These observations suggested that control of temperature and reaction time were essential for this reaction. In general, when the reaction was carried out in refluxing

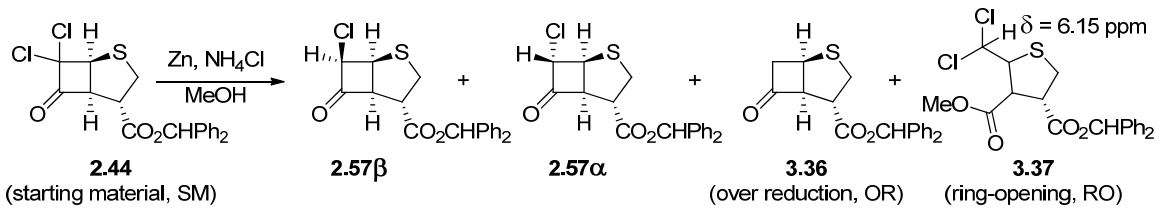
acetonitrile for a longer time, the benzhydryl group was cleaved. Heating for a shorter time led to a higher yield for the reaction. However, the maximal yield of 40% is still rather low.

3.5.2 Method Based on Zinc and Ammonium Chloride

As the Zn-TMSCl monodechlorination could not provide the desired monochloro product in a satisfactory yield, in the next phase of this project, a literature method using Zn-NH_4Cl to dechlorinate was explored on **2.44**,[95,96] which was obtained through hydrolysis of the corresponding benzhydryl ester **2.57**.

Attempts on this reaction gave a crude product that contained up to five possible compounds, including the cleaved products **2.57α/β**, the left over starting material **2.44**, a didechlorinated product **3.36** and a ring-opening product **3.37** that previously had been isolated and characterized in this group by Evanoff.[82] The results varied significantly depending on the condition used (Table 6).

Table 6. Efforts towards monodechlorination with Zn-NH_4Cl[a]



2.44 (starting material, SM) **2.57β** **2.57α** **3.36** (over reduction, OR) **3.37** (ring-opening, RO)

Trial	Reagents	Temp.	Time	**β : α : SM : 3.36 : 3.37**[b]
1	Zn (2 eq), NH_4Cl (10 eq)	0 °C	3 h	1 : 0 : 1 : trace : 0.5
2	Zn (4 eq), NH_4Cl (10 eq)	r.t.	3 h	1 : 0.6 : 0 : 2 : 1
3	Zn (10 eq), NH_4Cl (10 eq)	0 °C	45 min	1 : 0 : 0.5 : 0.1 : 0.4
4	Zn (20 eq), NH_4Cl (10 eq)	0 °C	30 min	1 : 0 : 0.3 : trace : 0.2
5	Zn (40 eq), NH_4Cl (10 eq)	0 °C	10 min	1 : 0.3 : 0.3 : 0.15 : 0.15

Table 6 (continued). Efforts towards monodechlorination with Zn-NH_4Cl

6	Zn (50 eq), NH_4Cl (50 eq)	0 °C	5 min	1 : 0 : 3 : 0.25 : 0.7
7	Zn* (10 eq)[c], NH_4Cl (10 eq)	r.t.	3 h	**3.36** only
8	Zn* (10 eq), NH_4Cl (10 eq)	r.t.	1 h	**3.36** only
9	Zn* (2.5 eq), NH_4Cl (10 eq)	0 °C	10 min	1 : 0 : 5 : 0 : 0.75
10	Zn* (5 eq), NH_4Cl (10 eq)	0 °C	20 min	1 : 0 : 2 : 0 : 1.2
11	Zn* (10 eq), NH_4Cl (10 eq)	0 °C	13 min	1 : 0 : 2.8 : trace : 0.5
12	Zn* (5 eq), NH_4Cl (10 eq)	-20 °C	3 h	1 : 0 : 2.7 : trace : 1.4

(a) All reactions were carried out in MeOH and worked up as well as examined by ^{1}H-NMR without further purification. (b) ratio in crude product (c) Zn* activated zinc (see Section 4.1)

Generally, the results based on the Zn-NH_4Cl monodechlorination were not satisfactory. Even worse, the ring-opening (RO, **3.37**) product seems inevitable. Since NH_4Cl is a proton source, the carbonyl carbon may be activated by protonation, making it more susceptible to be attacked by the solvent methanol as a nucleophile. As a consequence, the cyclobutanone ring is opened to give the compound **3.37** that shows a characteristic doublet around 6.15 ppm in ^{1}H-NMR spectra, corresponding to the proton on the carbon bearing the two chlorine atoms.[82] In addition, the monochloro compound **2.57α** was produced only in trials 2 and 5 and only in small amounts.

For the first trial, the starting material (SM) was reacted with 2 equivalents of zinc dust and 10 equivalents of NH_4Cl at 0 °C for 3 hours. The monochloro compound **2.57β** was 40% of the crude product but approximately only 40% of the SM was converted and 20% RO product was present in the crude product. The next trial doubled the amount of zinc and was carried out at room temperature in order to promote the conversion of **2.44**. The starting material in this case was completely consumed, but a significant amount of over reduction

product was obtained.

In the following experiments (Trials 3-6), the amount of zinc dust was gradually increased from 10 to 50 equivalents in order to push the reaction, while the corresponding reaction times were decreased from 45 to 5 minutes to avoid side reactions. The conversions of **2.44** ranged from 40% to 80%, but the crude products still contained some side products, which were very difficult to separate by flash chromatography. Next, activated zinc (10 eq) was used in the next two experiments (Trials 7 and 8). Surprisingly, the reactions were very clean and provided only the OR product **3.36** within 1 hour. Later on, both the amount of zinc and reaction time were reduced to prevent over reduction, but the conversions of **2.44** were still not ideal (maximal 52% in Trial 10). Trial 12 further lowered the temperature to -20 °C and extended the time to 3 hours, which provided only 47% conversion of the starting material. It was noticed that even at such low temperature, the amount of RO product still could not be ignored.

All these results indicate that the Zn-NH_4Cl monodechlorination is sensitive to temperature, the quantity of zinc, and reaction time, causing too much difficulty to optimize. The amount of NH_4Cl, however, seems to not influence the reaction much.

In order to test the solvent effect, another condition was tried involving a mixed solvent system (acetone : methanol = 50 : 1) and activated zinc (5 eq) as well as ammonium chloride (5 equivalents), which is not shown in Table 6. This condition provided a poor conversion of SM, and the crude mixture contained the β isomer, starting material and ring-opening product in a ratio of 1 : 16 : 2.7. The mixed solvent only consisted of 2% methanol, which still generated a relatively large proportion of ring-opening product, indicating that this reaction is extremely sensitive to the solvent, particularly nucleophilic ones.

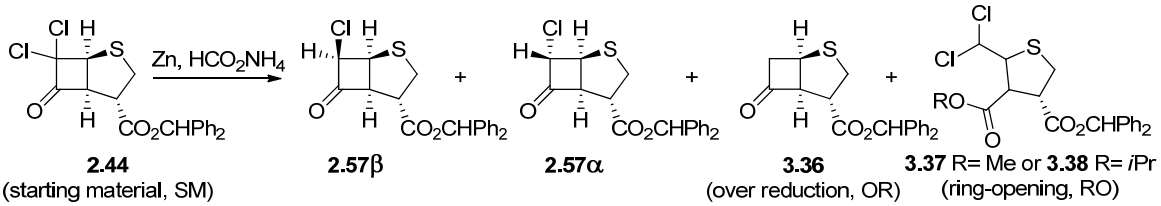
If the various side products in the crude mixture could be minimized, the difficulty to purify them might be reduced. Based on the analysis of the previous results, preventing the formation of the ring-opening side product **3.37** seemed possible. As mentioned before, the carbonyl carbon is activated by NH_4Cl, and MeOH is a relatively good nucleophile, leading to the nucleophilic opening of the cyclobutanone ring. To address this problem, a more bulky alcohol, isopropanol was considered, which is less likely to attack the carbonyl carbon for steric reasons. Unfortunately, compound **2.44** is not very soluble in isopropanol. Later on, a mixture of solvent was used to help dissolve **2.44**, which consists of *i*PrOH and acetone in a ratio of 6:1 (v/v).

The first attempt using this mixed solvent system with non-activated zinc (5 eq) and ammonium chloride (5 eq) was carried out at 0 °C for one hour, which gave a crude mixture with the β isomer **2.57β** and the starting material **2.44** in a ratio of 1 : 4.5. As predicted, under this condition, no ring-opening product was observed in the crude mixture, but the conversion of the starting material needed to be improved. Longer reaction time (5 h) with reduced quantities of both Zn and NH_4Cl (4 eq) was then attempted, since the extended time might lead to the formation of over reduction product. This gave a crude mixture containing the β isomer and SM in a ratio of 1 : 1.5, and only trace amount of ring-opening product was observed. Although the cyclobutanone ring-opening problem had been minimized, the 40% conversion percentage of **2.44** was far from ideal.

3.5.3 Method Based on Zinc and Ammonium Formate

In order to decrease the reaction time, a stronger reduction condition, involving zinc and ammonium formate that could even reduce hydroxylamine, was attempted.[97] A series of experiments has been performed and the results are shown in Table 7.

Table 7. Efforts towards monodechlorination with Zn-HCO_2NH_4[a]



Trial	Reagents	Solvent	Temp.	Time	**β : α : SM : 3.36 : RO**[b]
1	Zn* (5 eq)[c], HCO_2NH_4 (5 eq)	*i*PrOH : Acetone (6 : 1)	0 °C	1 h	1 : 0 : 7.3 : 5.5 : 1.2
2	Zn* (5 eq), HCO_2NH_4 (5 eq)	*i*PrOH : Acetone (6 : 1)	0 °C	5 min	1 : 0 : 2 : 0 : 0.4
3	Zn* (5 eq), HCO_2NH_4 (2 eq)	*i*PrOH : Acetone (6 : 1)	0 °C	30 min	1 : 0 : 1.6 : trace : 0.3
4	Zn* (5 eq), HCO_2NH_4 (2 eq)	Acetone	0 °C	1 h	1 : 0 : 11 : 0 : 0
5[d]	Zn* (4 eq), HCO_2NH_4 (4 eq)	Acetone	r.t.	5 h	1 : 0 : 6.5 : 0 : 0
6	Zn* (4 eq), HCO_2NH_4 (2 eq)	Acetone : *i*PrOH (25 : 1)	r.t.	2.5 h	1 : 0 : 6 : 0 : 0
7	Zn* (6 eq), HCO_2NH_4 (2 eq)	Acetone : MeOH (50 : 1)	0 °C to r.t.	35 min	1 : 0 : 7.5 : 0 : 0.5
8	Zn* (4 eq), HCO_2NH_4 (2 eq)	Acetone : MeOH (50 : 1)	0 °C to r.t.	1 h	1 : 0 : 7.2: 0 : 0.7
9	Zn* (6 eq), HCO_2NH_4 (2 eq)	Acetone : MeOH (50 : 1)	0 °C to r.t.	2 h	1 : 0 : 0.6 : 0 : 0.3
10	Zn* (4 eq), HCO_2NH_4 (2 eq)	Acetone : MeOH (50 : 1)	r.t.	25 min	1 : 0 : 1 : 0 : trace
11	Zn* (4 eq), HCO_2NH_4 (2 eq)	Acetone : MeOH (50 : 1)	r.t.	40 min	1 : 0 : 1.4 : 0 : 0.3

(a) All reactions were worked up and examined by ^{1}H-NMR without further purification. (b) ratio in crude mixture (c) Zn* activated zinc (d) crude product from Trial 4 used as the SM

At the beginning (Trial 1), 5 equivalents of activated zinc dust and a stoichiometric amount of ammonium formate were used in a mixture of *i*PrOH and acetone (6 : 1) at 0 °C for 60 minutes. Only around half of the starting material was consumed, and a large amount of side products such as the ring-opening and over reduction compounds were generated as well. For the next attempt, all conditions remained the same except that the reaction time was reduced to 5 minutes. The conversion of **2.44** did not change much, however, the amount of side products was significantly decreased, and the over reduction product was not formed.

In order to further eliminate the ring-opening product, pure acetone was used as the solvent. Even when the reaction was performed at higher temperature (r.t.) and a fairly long time (5 h), the maximal conversion of **2.44** was less than 13%. However, there were no side products observed in the crude mixture as predicted.

Due to the observed low reactivity in pure acetone, the solvent was shifted back to a mixed one (acetone : *i*PrOH = 25 : 1, Trial 6), but it still provided poor conversion of the starting material. When the solvent was changed to acetone : MeOH = 50 : 1, the corresponding results (Trial 7 to 11) were not ideal. Although the highest observed conversion was 68% (Trial 9), the amount of side products could not be ignored.

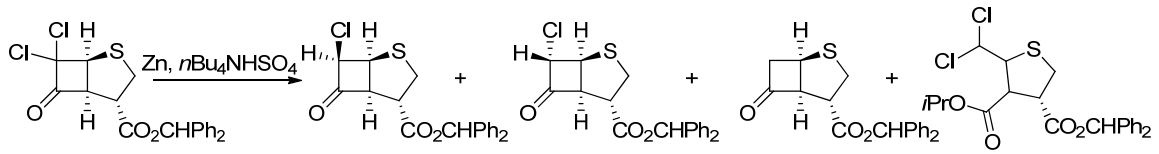
In summary, the quantity of zinc dust is not very important for this reaction, while that of ammonium formate somehow leads to over reduction. Fortunately, through careful control of the amount of ammonium formate, over reduction seems not a big problem. As for the solvent, reaction in pure acetone and acetone mixed with *i*PrOH show poor reactivity, whereas acetone mixed with MeOH gives better conversion, but leads to a small amount of ring-opening side product. When the reactions were carried on at lower temperature, the rate is relatively slow. Even though Trials 9 and 10 offered an acceptable conversion and relatively

clean crude product, the results were still not adequate.

3.5.4 Reduction with Zinc and Tetrabutylammonium Bisulfate

Since ammonium chloride and ammonium formate have very limited solubility in acetone, and even in alcohols, other more organic soluble ammonium salts were considered. Therefore, tetrabutylammonium bisulfate was chosen to perform the reactions, since it had very good solubility in organic solvents and the bisulfate was rather acidic, which may promote the reduction. Several monodechlorination attempts under such conditions are summarized in Table 8 .

Table 8. Efforts towards monodechlorination with Zn- nBu$_4$NHSO$_4$[a]



Trial	Reagents	Solvent	Temp.	Time	**β : α : SM : 3.36 : 3.38**[b]
1	Zn (5 eq), nBu$_4$NHSO$_4$ (1 eq)	Acetone	r.t.	2.5 h	1 : 0 : 5.7 : 0 : 0
2	Zn* (6 eq),[c] nBu$_4$NHSO$_4$ (6 eq)	Acetone	r.t.	2.5 h	N. R.
3	Zn* (10 eq), nBu$_4$NHSO$_4$ (5 eq)	Acetone	r.t.	3.5 h	1 : 0 : 20 : 0 : 0
4	Zn* (10 eq), nBu$_4$NHSO$_4$ (1 eq)	Acetone	r.t.	2 h	1 : 0 : 55 : 0 : 0
5	Zn* (10 eq), nBu$_4$NHSO$_4$ (2 eq)	Acetone	r.t.	4 h	N. R.
6	Zn* (4 eq), nBu$_4$NHSO$_4$ (2 eq)	Acetone : iPrOH (100 : 1)	r.t.	8 h	1 : 0 : 0 : 0 : 0.3[d]

(a) All reactions were worked up and examined by ^{1}H-NMR without further purification. (b) ratio in crude mixutre (c) Zn* activated zinc (d) with some other unidentified products accounting for 40%

Since the tetrabutylammonium bisulfate may have higher activity, therefore, only one equivalent of this salt and unactivated zinc dust (5 eq) was used in the first trial. After 2.5 hours at ambient temperature in acetone, only 15% of the β isomer was observed in the crude mixture, while 85% of **2.44** was left over. In the next experiment, the amount of nBu$_4$NHSO$_4$ was significantly increased to 6 equivalents. Surprisingly, for the same reaction time as Trial 1, only starting material was recovered. As observed from Trial 3, 10 equivalents of activated zinc dust were used and tetrabutylammonium bisulfate was kept at 5 equivalents. The reaction time was extended to 3.5 hours, but only a very small proportion of the desired β isomer was formed.

In order to understand which reagents affected the results msot, Experiment 4 kept 10 equivalents of zinc dust and dramatically decreased the ammonium salt to 1 equivalent with **2.44** stirring for 2 hours. However, no significant reaction occurred. In addition, in the next trial (No. 5), the amount of ammonium salt and reaction time were both doubled, but reaction still did not occur. When a solvent mixutre of acetone : iPrOH (100 : 1) was used, zinc dust and ammonium salt were kept 4 equivalents and 2 equivalents, respectively (Trial 6), and the mixture stirred for 8 hours at ambient temperature, ring-opening product and the β isomer were formed in a ratio of 1 : 3 and significant amounts of some unidentified products were observed.

To summarize, the unactivated zinc seems to favour the desired monodechlorination, while the activated zinc does not. On the other hand, the amount of tetrabutylammonium bisulfate does not have much influence on the reaction. Most of the reactions under the Zn-nBu$_4$NHSO$_4$ monodechlorination condition led to low conversion. A possible reason is that, due to the strong acidity of the bisulfate, it might directly react with the activated zinc dust,

resulting in insufficient reagents (either Zn or nBu$_4$NHSO$_4$) to perform the desired dechlorination. Therefore, decreasing the amount of nBu$_4$NHSO$_4$ relative to activated zinc may favour the desired reaction (Trial 6). Overall this strategy did not seem promising and was not investigated further.

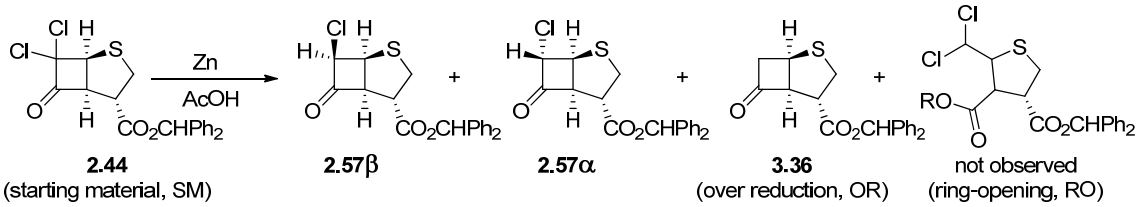
As discussed (Section 3.5.2-3.5.4), even though some monodechlorination conditions were examined in detail, the yield of the desired monochloro cyclobutanone **2.57** is still not ideal.

3.5.5 Method Based on Zinc and Acetic Acid

It was noticed that the conditions for total dechlorination of the dichloroethyl ester **2.5α** involves zinc (5 eq) and pure acetic acid as the solvent to provide the didechlorinated derivative **2.51** in 86% yield, as carried out by Johnson previously.[17] The reagents are very simple, but the conditions are somehow harsh (80 °C/5 h). It was expected that modification of this condition (e.g. decreasing the amount of zinc, temperature or reaction time) might be used to address the challenge.

The first attempt utilized a mixed solvent of AcOH and acetone in a ratio of 1 : 4 (v/v), which was supposed to reduce the reactivity of Zn. Moreover, only one equivalent of unactivated zinc dust was initially added to the reaction at room temperature. No product was observed by TLC after 30 minutes but some was observed after 3 hours. Therefore, an additional equivalent of zinc was added to promote the reaction, and the reaction mixture was stirred for another hour. After worked up, the crude product was found to contain the α-isomer, β-isomer and starting material in a ratio of 0.4 : 1 : 1.1. It was encouraging to find no over reduction or ring-opening side products were produced. This condition was further optimized, which are indicated in Table 9.

Table 9. Efforts towards monodechlorination with Zn-AcOH[a]



Trial	Reagent[b]	Solvent	Temp.	Time	**β : α : SM : 3.36 : RO**[c]
1	Zn (1 eq + 1 eq)	AcOH	r.t.	2.5 h[d] + 1 h[e]	9.7 : 1.3 : 0 : 1 : 0
2	Zn (1 eq + 1 eq)	AcOH	r.t.	2.5 h + 1 h	14: trace : 1 : 1 : 0
3	Zn (1 eq + 1 eq)	AcOH	r.t.	2.5 h + 0.5 h	1 : 0 : 3 : 0 : 0
4	Zn (2 eq + 2 eq)	AcOH	r.t.	1.5 h + 40 min	13.7 : 0 : 1 : trace : 0
5	Zn (1 eq + 0.5 eq)	AcOH	r.t.	2.5 h + 1 h	1 : 0 : 1.8 : 0 : 0

(a) All reactions were worked up and examined by ^{1}H-NMR without further purification. (b) Two aliquots of zinc were added separately. (c) ratio in crude mixture (d) for the first aliquot (e) for the second aliquot

It can be seen from the table that when acetic acid was used instead of the mixed solvent (acetic acid and acetone), the reaction was promoted. Moreover, there was no ring-opening product generated at all due to the weaker nucleophilicity of acetic acid. For Trial 1, the first aliquot of zinc was stirred with **2.44** for 2.5 hours, and then the second aliquot of zinc was added to the mixture that was further stirred for an hour. The starting material was found to be completely consumed, affording the desired monochlorocyclobutanone **2.57** and over reduction side product in a ratio of 11 : 1, and this result was found to be very reproducible.

Some more experiments were performed to examine the influences of the amount of zinc and reaction time. For Experiment 3 the quantity of zinc dust was 1 equivalent in each aliquot and the reaction time for the first aliquot of zinc was 2.5 hours, but 30 minutes for the second aliquot. As a result, only 25% of SM was converted into the β isomer. For Trial 4, the amount of zinc was doubled for both batches, but the reaction time was reduced, and a result similar to Trial 1 and 2 was obtained. Additionally, the fifth attempt maintained the condition

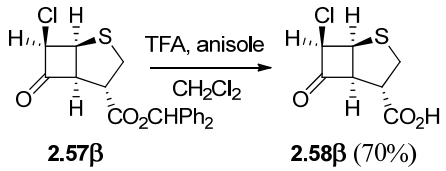
of Trial 1 expect that the second aliquot of zinc was decreased to 0.5 equivalents. Consequently, approximately only one third of **2.44** was converted into the desired product. The amount of the second aliquot of zinc dust and reaction time seem to influence the reaction outcome severely. If the reaction with the second aliquot of zinc is not long enough, such as in Trial 3, even with enough quantity of zinc, the majority of **2.44** could not be converted. If there is an insufficient amount of zinc used for the second aliquot (e.g. Trial 5), even though the reaction time is long enough, the conversion of the starting material is also not ideal.

The crude mixture mainly contained the desired β isomer, and small amount of α isomer and a minor amount of the over reduction product. This product mixture could not be purified by flash chromatography and it was noticed that an isomerization (β → α) happened during the purification attempt. Fortunately, the crude product was pure enough to be used directly as the starting material for the next step (hydroxymethylation) and the existence of the over reduction product did not affect the hydroxymethylation. The α and β isomers gave the same hydroxymethylation product and the minor over reduction product could then be easily removed upon chromatography. (More details will be given in Section 3.6.)

The monodechlorination with Zn-AcOH is very clean, but it was discovered that the quality of dichlorobenzhydryl ester **2.44** was quite essential. Some batches of the benzhydryl ester **2.44** contained a small amount of impurity (mostly benzhydryl related impurity derived from diphenyldiazomethane). Flash chromatography had to be performed to strictly purify the benzhydryl ester **2.44** before the reduction. Moreover, unlike Johnson's monodechlorination condition (Zn-TMSCl), the benzhydryl protecting group could survive under the Zn-AcOH condition, which is another advantage of this reaction.

With the monochlorobenzhydryl **2.57β** in hand, preparation of its free acid form **2.58β**

was readily achieved. As shown in Scheme 33, the free acid was furnished in 70% yield through the cleavage of the benzhydryl group by TFA in dichloromethane. The corresponding antibacterial activity test and hydrate formation experiment of the obtained cyclobutanone **2.58β** will be discussed later (Section 3.10 and 3.11). Compound **2.58β** is a new cyclobutanone mimic of β-lactams, generated in this thesis work.



2.57β **2.58β** (70%)

Scheme 33. Cleavage of benzhydryl group of **2.57β** by TFA

The successful development of a monodechlorination method paved the way for the later installation of a side chain at the C7 position of the cyclobutanone, which is considered as a milestone in this project.

3.6 C7-Hydroxymethylation of the Monochlorocyclobutanone 2.57

It was reported by Johnson that the didechlorinated cyclobutanones **2.52** and **2.54** exhibited poor inhibition of β-lactamases. It was hoped that the C7 modified derivatives might show improved inhibition by forming possible hydrogen bonds in the active site of the enzymes, which was evident from computational modeling (Figure 14).[17] Binding of the 7β-chloro-7α-hydroxymethyl cyclobutanone with IMP-1 (Class B MBL) was estimated by molecular modeling. The hydroxymethyl side chain may be able to interact with the conserved zinc-coordinating aspartate residue in the active site. Moreover, in the Class D SBLs (e.g. OXA-48) active site, the side chain might interact with the carboxylated Lys73 that acts as the general base in the deacylation process through a favorable H-bond. These hydrogen bonds might improve the affinity of the cyclobutanone towards the β-lactamases.[17]

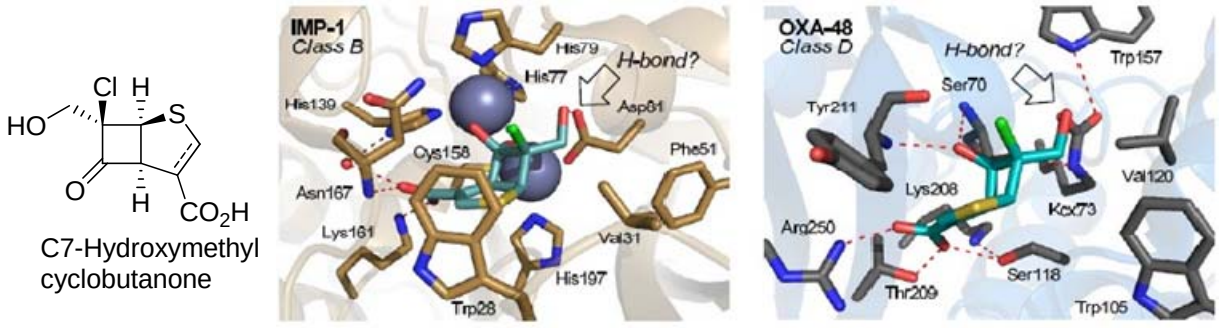
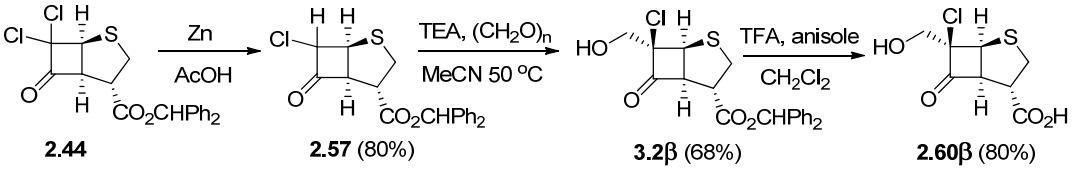


Figure 14. Modeling for C7-hydroxymethyl cyclobutanone as potential β-lactamase inhibitor binding to IMP-1 and OXA-48

As mentioned earlier (Section 2.5.3), the hydroxymethyl derivative **2.60β** was obtained by Johnson in this group, but the maximal yield was only 10% at the time due to the unsatisfactory monodechlorination, and compound **2.60β** was not tested for its bioactivity or hydrate formation back then. Fortunately, a much better monodechlorination condition was developed in the current work. As a result, the following hydroxymethylation was also quite smooth. The improved synthetic method for producing hydroxymethyl derivative **2.60β** has now been applied on gram scales in the Dmitrienko lab (Scheme 34).



Scheme 34. Preparation of hydroxymethyl cyclobutanone **2.60β** in this thesis work

The monochlorocyclobutanone **2.57** was obtained in 80% yield through the Zn-AcOH reaction, and was then treated with TEA and paraformaldehyde in acetonitrile at 50 °C to generate the 7α-hydroxymethyl benzhydryl ester **3.2β** in 68% that could be efficiently purified by flash chromatography. After cleavage of the benzhydryl group by TFA in the presence of anisole, the target 7β-chloro-7α-hydroxymethyl **2.60β** was generated. The crude product was very easily purified by simple trituration with cyclohexane, providing very pure **2.60β** in 80%

yield.

Hydrate formation and bioactivity of the hydroxymethyl derivative **2.60β** were then studied. More details will be given in Sections 3.10-3.11. Moreover, crystals of **2.60β** and **2.58β** were successfully obtained and X-ray crystal structures were determined by Dr. Assoud. It was found that cyclobutanone **2.60β** can form a tetramer in the unit cell through H-bonds, which interact between the hydroxyl group within the C7 side chain and the carboxylic acid at C4 (Figure 15A). For the cyclobutanone derivatives without the hydroxymethyl side chain at C7 such as **2.58β**, they commonly generate a dimer by the H-bond between the two carboxylic acids at C4 (Figure 15B). These crystal structures have confirmed the stereochemistry at C7 that was previously assigned by NMR experiments. In these structures, the 5-membered ring takes up an *endo* envelope conformation.

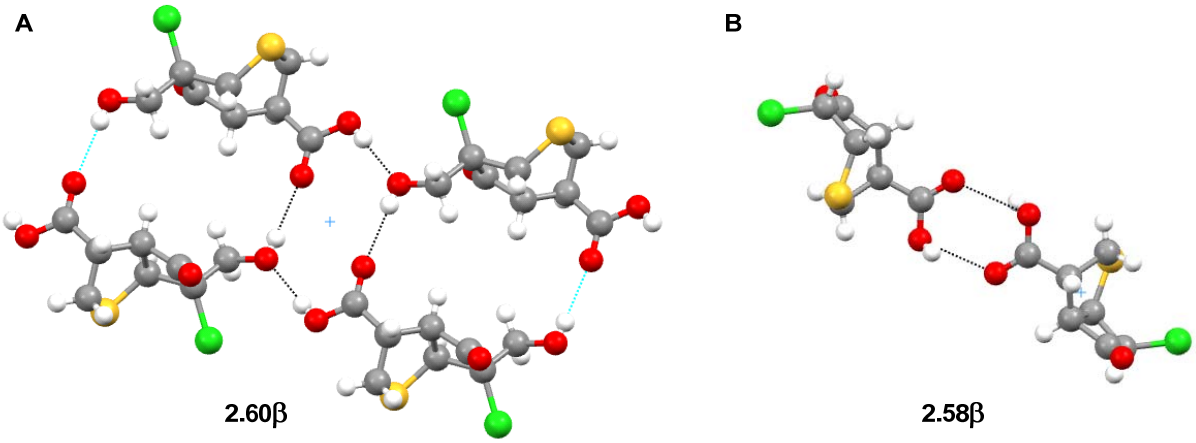
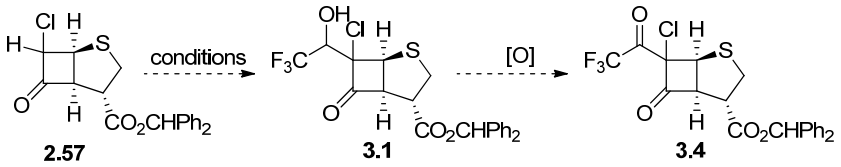


Figure 15. (A) Crystal structure of hydroxymethyl cyclobutanone **2.60β** (B) Crystal structure of monochlorocyclobutanone **2.58β**

3.7 Attempts to Install Trifluoroacetyl Side Chain at C7

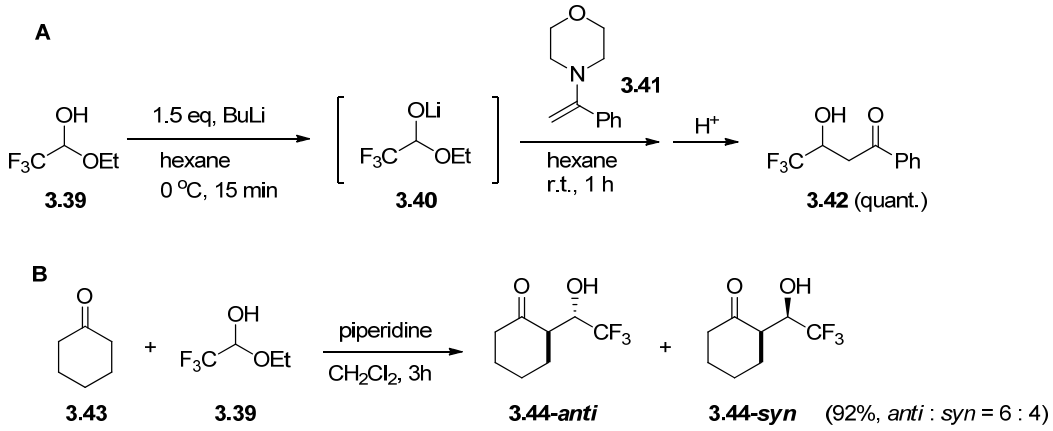
Several attempts were made to prepare the trifluoromethyl ketone **3.4** based on an initial aldol condensation the ketone **2.57** with trifluoroacetaldehyde to be followed by oxidation of the alcohol (Scheme 35).



Scheme 35. Possible synthetic approach to C7-trifluoroacetyl cyclobutanone **3.4**

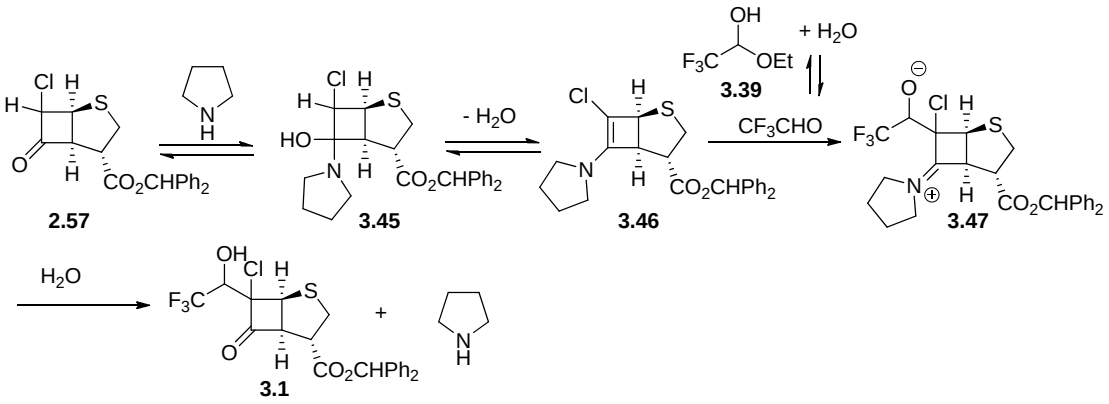
Triflouoroacetaldehyde must be generated *in situ* from the commerically available trifluoroacetaldehyde ethyl hemiacetal.[98] The first attempt at the aldol condensation used TEA as the base as in the successful hydroxymethylation reaction for this system. Unfortunately the crude product that was obtained was found to be very complicated and inseparable.

Several research groups have reported methods for the preparation of hydroxytrifluoroethylated compounds by using the hemiacetal of CF_3CHO such as the examples shown in Scheme 36.[99,100]



Scheme 36.(A) Synthesis of the hydroxyltrifluoroethyl ketone through aldol condensation between trifluoroacetaldehyde ethyl hemiacetal and enamines in the Funabiki group (B) Aldol condensation between trifluoroacetaldehyde ethyl hemiacetal **3.39** and cyclohexanone **3.43** in the Gong group

An effort was made to apply the strategy published by the Gong group to the synthesis of **3.3**. The proposed mechanism of this reaction with cyclobutanone **2.57** is shown in Scheme 37.[100]

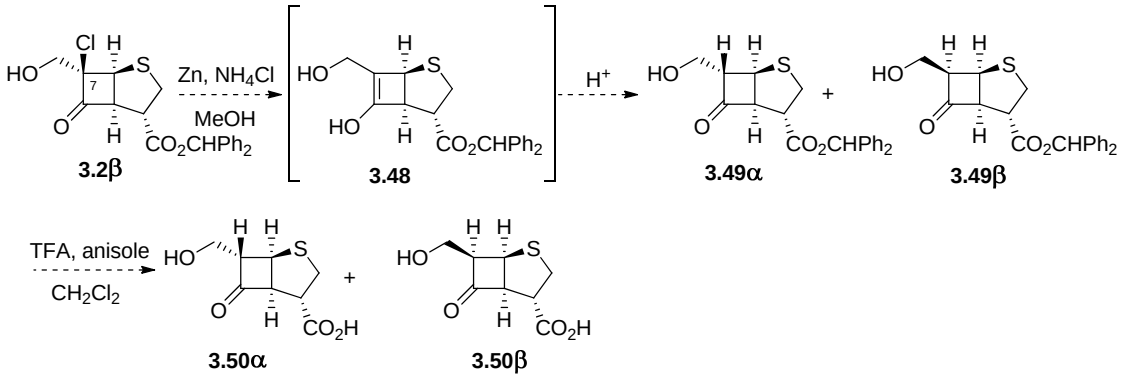


Scheme 37. Proposed mechanism for the pyrrolidine-catalyzed aldol condensation between cyclobutanone **2.57** and CF_3CHO (generated *in situ*)

Unfortunately, several attempts at this reaction yielded complex mixtures of products from which none of the desired product could be isolated. Therefore, at this stage, it was decided that this aspect of the initial proposal would not be pursued further. Instead, the following new tasks were planned to focus on the modification of the hydroxymethyl cyclobutanone **2.60β** at C7.

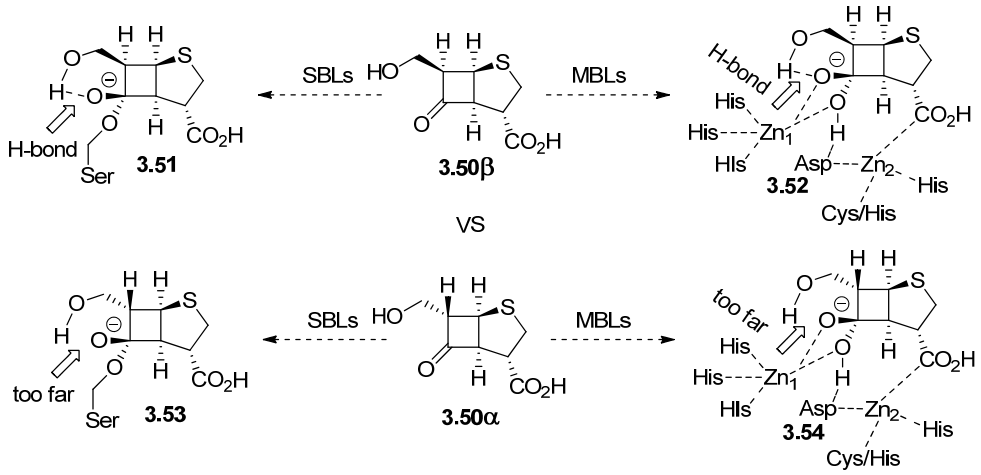
3.8 Dechlorination of the Hydroxymethyl Cyclobutanone Derivatives

Since the synthesis of the trifluoroacetyl compound **3.4** was not promising, a new research direction that involved the dechlorination of the hydroxymethyl cyclobutanone **3.2β** was proposed. The potential procedure for the dechlorination could follow that mentioned in Section 3.5.2 (Zn-NH_4Cl), the dechlorination process may involve the enol **3.48** formation, which should be then protonated to give the dechlorinated compound **3.49**. The enol has a planar conformation, allowing the proton to be added from either side, leading to a mixture of C7 epimer **3.49α** and **3.49β**. The dechlorinated compound **3.49** then could be subjected to a deprotection condition with TFA and anisole to provide the corresponding acids **3.50** (Scheme 38).



Scheme 38. Possible procedure for preparation of dechlorinated hydroxymethyl cyclobutanone derivatives

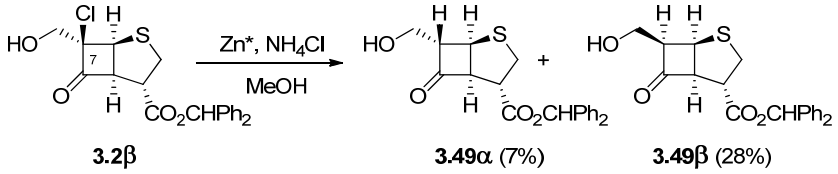
It was proposed that the hydroxymethyl cyclobutanone **3.50β** with the hydroxymethyl side chain on the β face of the cyclobutanone ring is a potential β-lactamase inhibitor. As shown in Scheme 39, **3.50β** is considered to bind to both SBLs and MBLs, providing the corresponding tetrahedral intermediates **3.51** and **3.52**. The hydroxyl group of the hydroxymethyl side of **3.50β** may interact with the tetrahedral intermediates to form a six-membered ring through favourable intramolecular hydrogen bond, which might promote the stability of the intermediates, preventing them from further generating the enzyme intermediates and releasing the free β-lactamases. The hydroxymethyl group of the cyclobutanone **3.50β** and the alkoxides in the tetrahedral intermediates orient towards the same side of the cyclobutanone ring, which might allow them to generate an intramolecular H-bond. The isomer **3.50α** might also be a β-lactamase inhibitor since it is a mimic of carbapenem. However, its inhibition may be weaker than **3.50β**, because the hydroxymethyl group and the alkoxides are on the different sides of the cyclobutanone ring, as a result, the distance and geometry between them is probably too far to form such intramolecular H-bond.



Scheme 39. Potential inhibition of β-lactamases by cyclobutanone **3.50β**

The previous exhaustively examined Zn-NH_4Cl condition (Section 3.5.2) was chosen to dechlorinate the cyclobutanone **3.2β**. Since there is only one chlorine atom at C7 to be removed in this case, so there is no over reduction issue as previously encountered. Activated zinc was used and the corresponding experimental results are summarized in Table 10.

Table 10. Efforts towards dechlorination of the hydroxymethyl cyclobutanone **3.2β**



Trial	Reagent	Solvent	Temp.	Time	**α : β : SM**[b]
1	Zn* (5 eq)[a], NH_4Cl (5 eq)	MeOH	r.t.	3 h	1 : 1 : 2
2[c]	Zn* (10 eq), NH_4Cl (10eq)	MeOH	r.t.	5 h	1 : 1 : 0.3
3	Zn* (20 eq), NH_4Cl (20 eq)	MeOH	r.t.	5 h	1 : 5 : 0
4	Zn* (20 eq), NH_4Cl (20 eq)	MeOH	r.t.	2.5 h	1 : 5 : 0
5	Zn* (20 eq), NH_4Cl (20 eq)	MeOH	r.t.	1 h	1 : 7 : 0
6	Zn* (40 eq), NH_4Cl (40 eq)	MeOH	r.t.	30 min	1 : 9 : 0
7	Zn* (40 eq), NH_4Cl (40 eq)	MeOH	r.t.	10 min	1 : 10 : 0

(a) Zn* activated zinc (b) ratio in crude mixture identified by ^{1}H-NMR (c) crude product from Trial 1 used as the SM

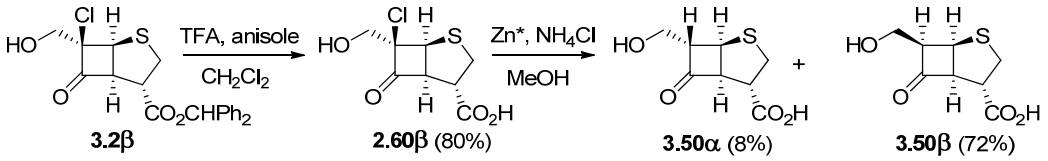
The first trial used 5 equivalents of activated zinc dust and ammonium chloride in MeOH at r.t. for 3 hours, which led to incomplete (50%) conversion of the starting material. The following trials indicated that, with the increasing amounts of zinc but ammonium chloride and decreasing reaction time, the starting material could be completely consumed. The β isomer was the dominant product and only a small proportion of α isomer was generated. If the amount of zinc dust is insufficient (less than 20 equivalents), the starting material cannot get fully dechlorinated. This reaction could be finished in a very short time (less than 10 minutes). With longer reaction time, isomerization of the β epimer to the α epimer begins to occur. The products in the crude mixture could not be purified by flash chromatography, but they could be separated by high performance liquid chromatography (HPLC) to give the minor isomer **3.49α** in 7% yield and the major isomer **3.49β** in 28% yield.

The next step was to cleave the benzhydryl group of **3.49**. The classic condition (TFA and anisole) was used to perform the task. The ^{1}H-NMR spectrum of the crude product indicated the desired free acid **3.50** was generated, but there was too much aromatic impurity that was derived from the trapping reagent (anisole) and the already cleaved benzhydryl group, which was found to be very difficult to remove completely by various methods such as trituration, flash chromatography and acid-base extraction.

When anisole was replaced by a better trapping reagent (TMBz), a very complex crude mixture was obtained. Other literature methods for cleaving the benzhydryl group were also attempted. For instance, the benzhydryl ester **3.49** was heated in formic acid[101] and it was also treated with sodium hydroxide in methanol.[102] Unfortunately, the cyclobutanone **3.49** decomposed under these conditions.

Since the deprotection of the cyclobutanone benzhydryl ester **3.2β** has no problem and

the aromatic impurity from the cleavage and the trapping agent could be easily separated by simple trituration, it was decided to convert the benzhydryl ester to the acid first and then carry out the dechlorination using the well-studied Zn-NH_4Cl method (Scheme 40).



Scheme 40. Alternative path to synthesize the dechlorinated acid **3.50**

The benzhydryl group of **3.2β** was smoothly cleaved by TFA to give the free acid **2.60β** in 80% yield, which was then treated with activated zinc dust (40 equivalents) and NH_4Cl (40 equivalents) in MeOH at r.t. for 8 minutes to provide the desire compounds **3.50**. The crude product contained **3.50α** and **3.50β** in a ratio of 1 : 5, which could be separated by HPLC, providing the minor **3.50α** and the major **3.50β** in yields of 8% and 72%, respectively. As mentioned before in this section, decreasing the amount of zinc dust and increasing the reaction time promoted the generation of more of the minor product **3.50α**. This was verified by an experiment that used 10 equivalents of activated zinc and NH_4Cl in methanol at ambient temperature for 4.5 hours, which produced a crude mixture containing the minor **3.50α** and the major **3.50β** in a ratio of 1 : 3.

With the dechlorinated acids **3.50** in hand, a series of experiments were performed including hydrate formation, bioactivity test (Sections 3.10-3.11) and single crystal X-ray study. It was possible to crystallize the minor product **3.50α** and an X-ray crystal structure was obtained, which is shown in Figure 18. Similar to the crystal structure of **2.60β**, **3.50α** can form a tetramer that is stabilized by favorable H-bonds between the hydroxyl group within the side chain at C7 and the carboxylic acid at C4. This also established the stereochemistry of the minor epimer unambiguously. The five-membered ring was found to adopt an *endo*

conformation. In addition, the crystal structure is very useful for the computational modeling of the binding of such cyclobutanone mimics to β-lactamases.

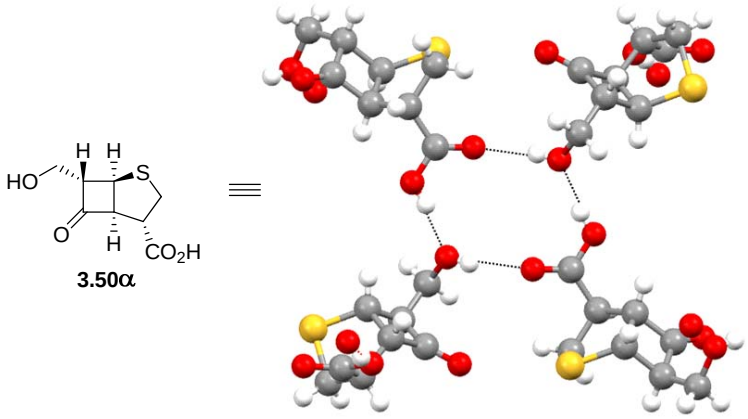
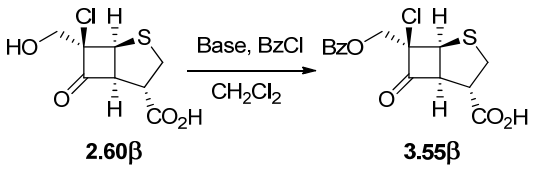


Figure 16. The crystal structure of cyclobutanone **3.50α**

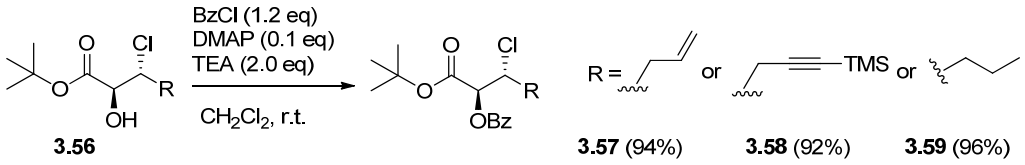
3.9 Benzoylation of the Hydroxymethyl Cyclobutanone Derivative 2.60β

It was proposed that the hydroxyl group within the side chain of cyclobutanone derivative **2.60β** might be converted into a series of esters. This would allow for the introduction of various functional groups to interact with the active sites of β-lactamases. As a proof of concept, preparation of the C7-benzoyl ester of **2.60β** was attempted (Scheme 41).



Scheme 41. Installation of benzoyl group to the hydroxymethyl side chain

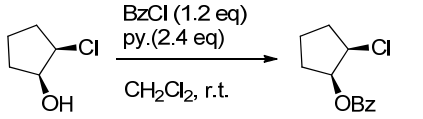
The first attempt followed a literature procedure (Scheme 42)[103], in which the starting material **2.60β** was reacted with benzoyl chloride (BzCl) with TEA as the base in dichloromethane at room temperature, in the presence of a catalytic amount of DMAP. The crude product contained too much benzoyl-based aromatic species that could not be purified by flash chromatography.



3.56 **3.57** (94%) **3.58** (92%) **3.59** (96%)

Scheme 42. Introduction of the benzoyl group in the Johnson lab

The second attempt followed a condition reported by a German group utilizing pyridine as the base (Scheme 43).[104] Although the crude product was still complex, some new signals were observed in the ^{1}H-NMR spectrum, which seemed to correspond to the desired product.

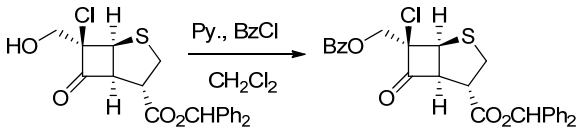


3.60 **3.61** (> 98%)

Scheme 43. Introduction of the benzoyl group in the Lassaletta lab

Since the free acid within cyclobutanone **2.60β** may interfere with the reaction, so the synthesis was traced back to its benzhydryl ester **3.2β**, on which the installation of the benzoyl group should be easier. Followed by deprotection of **3.62β**, the desired compound **3.55β** could be obtained. The corresponding results are shown in Table 11. Although the installation of a benzoyl group normally a very straight forward reaction, the first several trials were disappointing. It is presumed that, although the hydroxyl group in **3.2β** is primary, the adjacent carbon atom is a quaternary centre that makes the environment around the alcohol group sterically crowded.

Table 11. Efforts towards installation of benzoyl group to the hydroxymethyl side chain of benzhydryl ester **3.2β**



3.2β **3.62β** (67%)

Trial	Reagents	Solvent	Temp.	Time	**Product : SM**[a]
1	Py. (2.4 eq), BzCl (1.2 eq)	CH_2Cl_2	r.t.	2 h	2 : 3
2[b]	Py. (2.4 eq), BzCl (1.2 eq)	CH_2Cl_2	r.t.	19 h	7 : 3
3	Py. (4.8 eq), BzCl (2.4 eq)	CH_2Cl_2	r.t.	18 h	4 : 1
4	Py. (20 eq), BzCl (10 eq)	CH_2Cl_2	Reflux	17 h	1 : 0[c]
5	Py. (6 eq), BzCl (3 eq), DMAP (0.2 eq)	CH_2Cl_2	Reflux	18 h	1 : 2
6[d]	Py. (10 eq), BzCl (5 eq)	CH_2Cl_2	Reflux	18 h	1 : 0
7	Py. (10 eq), BzCl (5 eq)	CH_2Cl_2	Reflux	14 h	3 : 1
8	Py. (15 eq), BzCl (8 eq)	CH_2Cl_2	Reflux	21 h	1 : 0

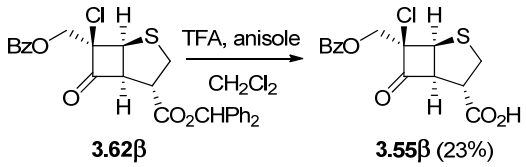
(a) ratio in crude mixture identified by ^{1}H-NMR, (b) crude product from Trial 1 used as the SM (c) Too much aromatic impurity (d) crude product from Trial 5 used as the SM

The initial trial used 2.4 equivalents of pyridine and 1.2 equivalents of benzoyl chloride in dichloromethane at r.t. for 2 hours, and the crude ^{1}H-NMR indicated that 60% of the starting material was left over. Therefore, the second trial increased the reaction time to 21 hours, but there was still about 30% of starting material unreacted. As described in Trial 3, even when the amount of pyridine and BzCl was doubled, even after 18 hours there was still 20% of unreacted cyclobutanone **3.2β** in the crude product.

In the fourth experiment, the amount of pyridine was significantly increased to 20 equivalents and BzCl to 10 equivalents in refluxing dichloromethane for 17 hours. The starting material was fully converted, but too much aromatic impurity derived from the large excess of BzCl, which made the purification very difficult. Therefore, in Experiment 5, the amount of both reagents was decreased and a catalytic quantity of DMAP was added in order to pursue a complete conversion of **3.2β**. However, only 35% of starting material was transformed. The quantity of pyridine and BzCl was increased to 10 and 5 equivalents, which were stirred with the starting material (crude product of Trial 5 to give completed conversion for Trial 6). This crude mixture was cleaner than that produced from Trial 4, and it was possible to purify the

compound by flash chromatography. The target compound **3.62β** was isolated in 62% yield. However, Trial 7 directly used 10 equivalents of pyridine and 5 equivalents of BzCl, which still afforded incomplete conversion of **3.2β**. Later on, Trial 8 with proper amounts of pyridine (15 eq) and BzCl (8 eq) finally provided a full conversion of the starting material, and the desired product **3.62β** was isolated by flash chromatography in 67% yield.

Clearly, **3.2β** is a sterically hindered alcohol that requires conditions involving larger quantities of reagents and higher temperature than those required for acylation of a simple alcohol. The next reaction was to cleave the benzhydryl group of **3.62β** to provide the desired cyclobutanone acid **3.55β** (Scheme 44).



3.62β **3.55β** (23%)

Scheme 44. Preparation of the carboxylic acid **3.55β**

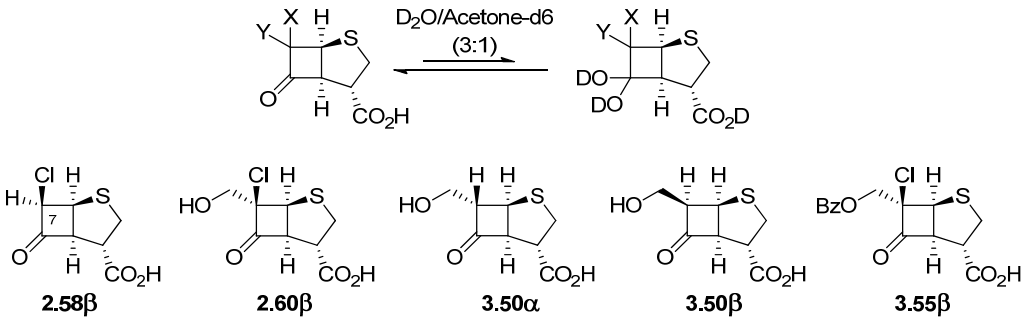
After much experimentation, it was eventually found that the best procedure involved reaction of **3.62β** with forty equivalents of TFA and seven equivalents of anisole in CH_2Cl_2 in an ice bath for 9 hours. The crude mixture was successfully purified by HPLC, providing **3.55β** in an isolated yield of 23%.

3.10 Hydrate Formation of Cyclobutanone Derivatives

The cyclobutanone derivatives are capable of generating hydrates in aqueous solution, indicating that they might form an enzyme bound hydrate in the active sites of MBLs, which may inhibit the MBLs. Based on Johnson's method, a series of cyclobutanone derivatives have been tested in a mixed solvents system (D_2O : acetone-d_6 = 3 : 1) in order to generate the corresponding hydrates, which are summarized in Table 12. Theoretically, the hydrate formation experiment should be carried out in pure D_2O, but many cyclobutanones have very

low solubility in water. Thus, acetone-d_6 was used as a co-solvent to help dissolve the substrates.

Table 12. Summary of hydrate formation of cyclobutanones prepared in this thesis work

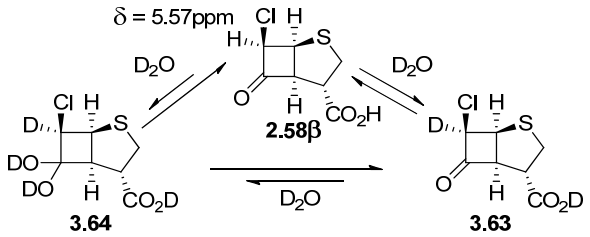


Trial	Cyclobutanone	Time	Hydrate (%)[a]
1	**2.58β**	5 min	25
2	**2.60β**	15 min	12
3	**3.50α**	5 h	< 2[b]
4	**3.50β**	489 h	< 2

(a) determined by ^{1}H-NMR (b) detection limitation of 500 MHz NMR spectrometer

For monochlorocyclobutanone **2.58β**, an equilibrium mixture containing 25% of hydrate was formed within 5 minutes and this ratio did not change in the next 18 hours. The 7β-chloro-7α-hydroxymethyl cyclobutanone **2.60β** took somewhat longer to achieve an equilibrium mixture containing 12% of the corresponding hydrate. The corresponding ^{13}C-NMR experiments were carried out as well; the related peaks for the hydrate are reported in Chapter 4.

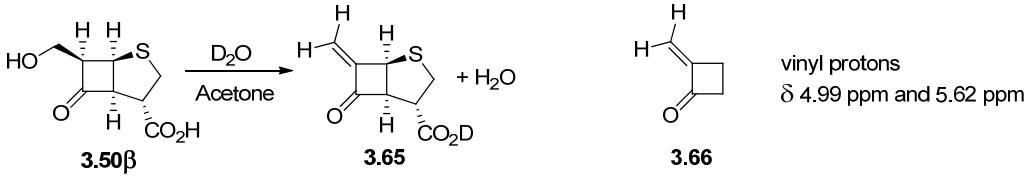
It was observed that the signal of the 7α proton within **2.58β** disappears gradually (5.57 ppm) during the hydrate formation process. As proposed in Scheme 45 below, this proton might slowly exchange with deuterium (from D_2O).



Scheme 45. Possible H/D exchange between **2.58β** and D_2O

For the 7α-hydroxymethyl derivative **3.50α**, no hydrate formation was detected by NMR under these conditions. In the case of the 7α-benzoyloxymethyl-7β-chloro derivative **3.55β**, it was not possible to carry out the NMR experiment because of poor solubility.

In the case of the 7β-hydroxymethyl cyclobutanone **3.50β**, some weak signals were seen in the ^{1}H-NMR spectrum at 8 minutes after addition of D_2O to an acetone-d_6 solution of **3.50β**. However, later it was concluded that these signals at 5.38 ppm and 5.82 ppm likely arise from the elimination product **3.65** (Scheme 46). The percentage of this side product in the reaction mixture gradually increased to a maximum of 25% after 126 hours. The chemical shifts for the vinyl protons of a compound such as **3.65** are predicted to be in the 6.2 to 6.3 ppm range based on empirical calculations.[105] After a careful search of the literature, however, it was found that Wasserman and co-workers had synthesized the parent 2-methylidenecyclobutanone **3.66** in 1980 and had found that the vinyl hydrogens had chemical shifts of 4.99 and 5.62 ppm. The reason for the anomalously low chemical shifts is not clear, they are sufficiently close to those observed in this study to allow us to speculate that they arise from the elimination product **3.65**.[106]



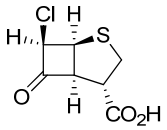
Scheme 46. Possible elimination of **3.50β** in water and acetone

In summary, the cyclobutanone derivatives with a chlorine atom at the C7 position are more likely to undergo hydration, which is consistent with Johnson's previous results (Table 3, Section 2.7). When compared to the dichlorocyclobutanones **2.43**, **2.30α** and **2.35**, the amount of hydrates produced by the monochlorocyclobutanones **2.48β** and **2.50β** are much less, because of the difference in electrophilicity of the carbonyl carbon that was affected by the nearby substituents.

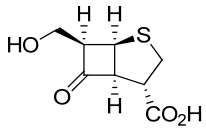
3.11 Bioactivity of Cyclobutanone Derivatives as β-lactamase Inhibitors

The inhibition of some common β-lactamases by the cyclobutanone compounds prepared in this thesis work has been tested by Dr. Geneviève Labbé and Mrs. Valerie Goodfellow of the Dmitrienko group. The corresponding biological data are shown in Table 13.

Table 13. Inhibition of some common β-lactamases by the cyclobutanones



2.58β
(25% hydrate)



3.50β
(0% hydrate)

β-Lactamases	Inhibition (%)	
	500 μM **2.58β**	500 μM **3.50β**
Class A KPC-2	1	7
Class B IMP-1	59	25
Class B VIM-2	40	0
Class B SPM-1	34	0
Class B L1	48	0
Class B NMD-1	0	0
Class C GC1	41	18
Class D OXA-10	19	0

The inhibition was assayed by Dr. Geneviève Labbé through nitrocefin hydrolysis.

In general, the cyclobutanone mimic **3.50β** has poor inhibition of these β-lactamases, even at a rather high concentration (500 μM). Even worse, **3.50β** does not show any activity against Class B and Class D enzymes that were tested, while the cyclobutanone analogue **2.58β** demonstrates moderate inhibition of these β-lactamases at 500 μM except KPC-2 and NMD-1. These observations are consistent with Johnson's previous conclusion (Table 3, Section 2.7) that the cyclobutanone capable of generating the larger amount of hydrate in aqueous solution is more likely to be a better β-lactamase inhibitor.

Some antimicrobial assays of cyclobutanones **2.58β**, **2.60β** and **3.50β** as β-lactam mimics were also carried out in this group to determine their MIC (Minimum Inhibitory Concentration), which is the lowest concentration of the drug that results in no growth of the bacterium. As seen in tables 14, none of the cyclobutanone compounds showed antibacterial activity on its own (MIC > 256 μg/mL or > 128 μg/mL) with any of the highly resistant clinical isolates tested. Each of the clinical isolates examined is resistant to the carbapenem, meropenem (MIC >> 32 μg/mL). The possibility that the cyclobutanones might act synergistically with meropenem was tested in the checkerboard synergy experiments. In these experiments the MIC for meropenem was determined at a series of concentrations of the cyclobutanone (0, 8, 16, 32, 64, 128 and 256 μg/mL). Only in the case of **2.58β** with a meropenem-resistant strain of *Stenotrophomonas maltophilia* was synergy observed, where the meropenem's MIC dropped to 32 μg/mL at a concentration of 32 μg/mL of the cyclobutanone, as compared with 128 μg/mL for meropenem in the absence of the cyclobutanone.

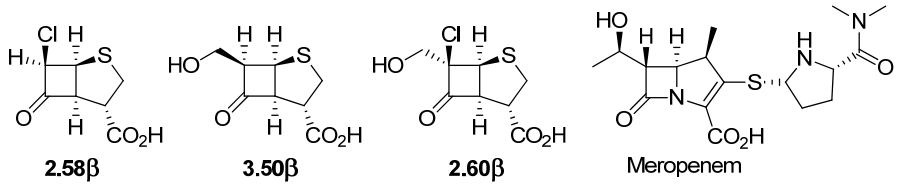


Table 14A. MIC values for meropenem in the presence of cyclobutanone **2.58β**

ID	Bacteria	Enzyme	**2.58β** (μg/mL)							MIC
			0	8	16	32	64	128	256	(**2.58β**)
26	*S. maltophilia*	L1 L2	128	64	64	32	32	32	32	> 256
31	*P. aeruginosa*	IMP-1	32	32	32	32	32	32	16	> 256
75	*E. coli*	NDM-1	128	128	128	128	128	128	64	> 256
78	*A. baumanii*	OXA-23	32	64	64	32	32	32	32	> 256

The MIC and FIC assays were carried out by Mrs. Valerie Goodfellow.

Table 14B. MIC values for meropenem in the presence of cyclobutanone **3.50β**

ID	Bacteria	Enzyme	**3.50β** (μg/mL)							MIC
			0	8	16	32	64	128	256	(**3.50β**)
26	*S. maltophilia*	L1 L2	256	256	256	256	256	256	256	> 256
31	*P. aeruginosa*	IMP-1	32	32	32	32	32	32	16	> 256
75	*E. coli*	NDM-1	128	128	128	128	128	128	128	> 256
78	*A. baumannii*	OXA-23	32	64	64	64	32	32	32	> 256

Table 14C. MIC values for meropenem in the presence of cyclobutanone **2.60β**

ID	Bacteria	Enzyme	**2.60β** (μg/mL)								MIC
			0	8	16	32	64	128	256	512	(**2.60β**)
11	*K. pneumoniae*	KPC-3	32	32	32	32	32	32			> 128
24	*P. putida*	VIM-2	64	64	64	32	32	64			> 128
26	*S. maltophilia*	L1 L2	256	256	256	256	256	256	128	128	> 128
31	*P. aeruginosa*	IMP-1	32	32	32	32	32	32	32	32	> 128
78	*P. aeruginosa*	VIM-2	64	64	64	64	32	32	32	32	> 128
94	*A. baumannii*	OXA-23	32	64	64	64	32	32	32	16	> 128

3.12 Other Effort towards New Cyclobutanone Derivatives

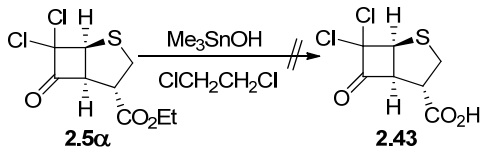
In this project, some other ideas towards making new cyclobutanone analogues and simplifying the synthesis of C7 modified cyclobutanones were proposed and synthetically examined. Unfortunately none of them was successful, but these efforts are worthy to be briefly mentioned.

3.12.1 Attempted Hydrolysis of the Ethyl Ester 2.5α by Trimethyltin Hydroxide

In 2005, based on Mascaretti's work, K. C. Nicolaou and coworkers reported that esters could be hydrolyzed under an extremely mild condition by using trimethyltin hydroxide, affording very high yields of the corresponding carboxylic acids.[107]

As mentioned in Section 2.5.2 (Scheme 24), the dichloroethyl ester **2.5α** readily undergo monodechlorination (Zn-TMSCl) to form the corresponding monochloro compound **2.56**, which then undergoes an aldol condensation with paraformaldehyde to furnish the hydroxymethyl derivatives **2.59** in acceptable yield. However, the ethyl ester **2.59** could not be hydrolyzed to the corresponding free acid under even very basic condition.

If the trimethyltin hydroxide could hydrolyze the ethyl ester derivatives of cyclobutanone **2.5α**, the preparation of benzhydryl ester **2.44** could be avoided, which requires the use of the potentially explosive Ph_2CN_2. In addition, the C7 modifications could be directly performed on the ethyl ester **2.5α**, which might simplify the synthetic route towards the C7 cyclobutanone derivatives. The attempts have been carried on ethyl ester **2.5α**, which is shown in Scheme 47 below.



2.5α **2.43**

Scheme 47. Attempted hydrolysis the ethyl ester **2.5α** by trimethyltin hydroxide

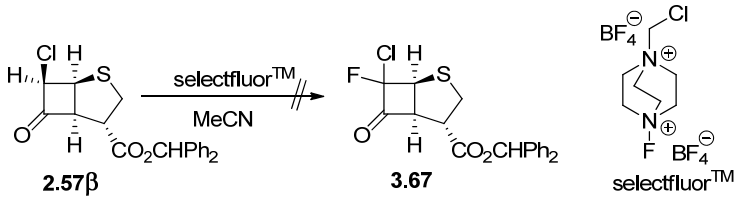
The ethyl ester **2.5α** was dissolved in 1,2-dichloroethane and stirred with trimethyltin hydroxide (5 equivalents) at 80 °C for 5 hours. However, the cyclobutanone decomposed. The second attempt kept the same amount of trimethyltin hydroxide, but decreased the temperature to 25 °C. After 18 hours, the cyclobutanone still decomposed. It seems that the cyclobutanone is incompatible with the trimethyltin hydroxide. Due to this instability of cyclobutanone when exposed to trimethyltin hydroxide, this condition was not tested again. However, future attempts based on this method might still be worthwhile, although it is probably beneficial to decrease the equivalents of the tin reagent and/or the reaction time and temperature.

3.12.2 Attempted Fluorination at C7 Position

It was proposed in this lab that if the chlorine atoms at C7 position of cyclobutanones could be replaced by fluorine atom(s), the corresponding novel cyclobutanone analogues might be better β-lactamase inhibitors. Since fluorine is more electronegative than chlorine, the tendency to form a hydrate or a hemiketal should be increased which may consequently increase the potency as an inhibitor of β-lactamases.

As reported in the literature, selectfluor^{TM}, diethylaminosulfur trifluoride (DAST) and *N*-fluorobenzenesulfonimide (NFSI) are good fluorinating reagents.[108] In this thesis work, selectfluor^{TM} was used as the fluorine source to react with the monochloro benzhydryl ester **2.57β** to prepare the corresponding monofluorocyclobutanone **3.67**. The experimental results of such attempts are illustrated in Table 15.

Table 15. Efforts towards fluorination of **2.57β** by selectfluor[TMa]



Trial	Reagents	Temp.	Time	Crude Product
1	Selectfluor[TM] (1.5 eq), TEA (3 eq)	r.t.	24 h	80% of SM left over
2	Selectfluor[TM] (1.5 eq), TEA (3 eq)	50 °C	23 h	New signals observed
3	Selectfluor[TM] (2 eq)	r.t.	21 h	Very complicated

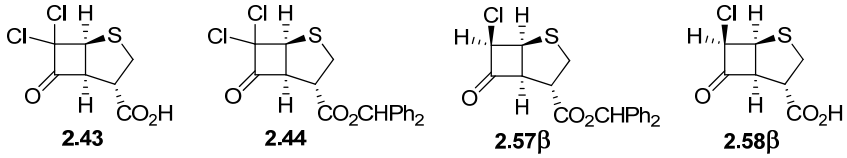
(a) All the reactions were carried out in HPLC grade MeCN and worked up as well as examined by ^{1}H-NMR.

The experimental procedure was similar to the aldol condensation between **2.57β** and paraformaldehyde. In the first experiment, **2.57β** was deprotonated by triethylamine at r.t. to give the corresponding enolate that then was supposed to attack the "F^+" provided by selectfluor[TM]. However, 80% of the starting material was left over even after 24 hours and the crude product could not be purified by flash chromatography. To promote the reaction, the reaction mixture with the same amounts of reagents was stirred at 50 °C. After 23 hours, the reaction was quenched and the ^{1}H-NMR spectrum of the crude product showed some new peaks. However, these signals seemed not to belong to the protons on the cyclobutanone ring. As the reactivity of selectfluor[TM] is very high, sometimes the fluorine could replace a proton without base. Thus, only two equivalents of selectfluor[TM] were used in Experiment 3, to stir with **2.57β** at room temperature for 21 hours, but the crude mixture was found to be very complicated and inseparable.

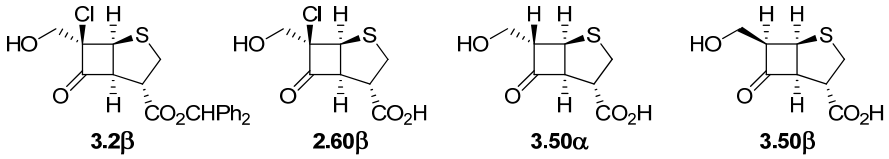
These results suggest that the future experiments should carefully control the amount of selectfluor[TM] and probably employ a longer reaction time. Moreover, attempts with other fluorine sources (DAST or NFSI) might be beneficial.

3.13 Summary and Future Work

The protection of 7,7-dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic acid **2.43** as a readily cleavable benzhydryl ester **2.44** was accomplished easily, but the corresponding PMB ester **3.25** was produced in only low yield. Monodechlorination of the dichlorobenzhydryl ester **2.44** gave predominantly the β epimer **2.57β**, which reacted with TFA to provide the free acid **2.58β**. It was found to be a weak inhibitor of the MBLs, IMP-1, VIM-2, SPM-1 and L1 and the Class C SBL GC1 and to act synergistically with meropenem against a pathogenic clinical strain of *S. maltophilia* that produces the MBL L1 and the SBL L2.

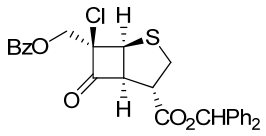


In addition, cyclobutanone **2.57β** was found to undergo an aldol condensation with formaldehyde to give the corresponding 7β-chloro-7α-hydroxymethyl derivative **3.2β**, which was deprotected with TFA to furnish the free acid **2.60β**. Unfortunately, it showed very poor activity against common β-lactamase-producing clinical bacteria. Reductive dechlorination of the acid **2.60β** afforded the 7β-hydroxymethyl cyclobutanone **3.50β** as the major product and 7α-hydroxymethyl derivative **3.50α** as the minor product. However, compound **3.50β** exhibited poor inhibition of common β-lactamases.

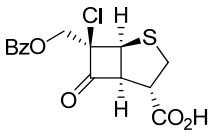


The trifluoroacetyl side chain could not be successfully installed through reaction with trifluororacetaldehyde at the C7 position of the compound **2.57β**. Even though **3.2β** is a

sterically hindered alcohol, it could be esterified with benzoyl chloride under sufficiently vigorous conditions to give the corresponding benzoyl ester **3.62β**, which reacted with TFA to generate the corresponding free acid **3.55β**.



3.62β



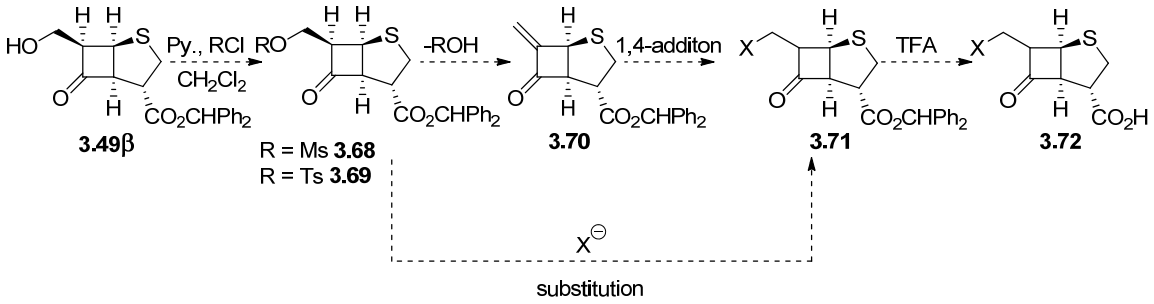
3.55β

Compounds **2.60β** and **2.58β** were found to form hydrates to the extent of 12% and 25% respectively in a mixed solvent (D_2O : acetone-d_6 = 3 : 1, v/v), whereas the dechlorinated derivatives **3.50α** and **3.50β** did not form a hydrate under comparable conditions.

Due to the time limitation, some proposed synthetic attempts were not performed. On top of that, the C7 derivatives **3.50α** and **3.55β** remain to be tested for their bioactivity. Moreover, the C7 modified cyclobutanones **2.58β**, **2.60β**, **3.50α**, **3.50β** and **3.55β** are to be examined by NMR for their tendency to form hemiketals.

In addition, as proposed at the very beginning of this project, the installation of trifluoroacetyl side chain at C7 is expected to continue, but a proper condition needs to be further examined.

As mentioned in Section 3.10, during the hydrate formation experiment of **3.50β**, an eliminated product **3.65** was observed. The corresponding elimination product is an α,β-unsaturated ketone that should be a good substrate for 1,4-addition. Therefore, compound **3.65** may be a key precursor for preparation of a series of Michael addition products and itself might be a potential cyclobutanone type of β-lactamase inhibitor, and this leads to the proposal of a more efficient synthesis of the α,β unsaturated ketone **3.70** (Scheme 48).



Scheme 48. Proposed elimination/1,4-addition or substitution reactions of cyclobutanones

Based on the successful installation of the benzoyl group, some better leaving groups such as mesyl and tosyl groups could be added on the side chain of **3.49β** by using MsCl or TsCl, yielding the corresponding esters **3.68** and **3.69** respectively. Next, elimination of a molecule of MsOH or TsOH could give the desired α,β unsaturated ketone **3.70**, which then may undergo 1,4-additions to provide a batch of new cyclobutanones **3.71**. The benzhydryl group within **3.71** should be cleaved by TFA to furnish free acids **3.72**, which are to be examined as a series of potential β-lactamase inhibitors. Alternatively, in the presence of these good leaving groups, **3.68** and **3.69** might readily undergo substitution directly to provide the cyclobutanones **3.71** as well.

The present study focused on functional group manipulation at C7 of the core system of cyclobutanones. The previous studies by Johnson in this group concentrated on chemistry at C3 and C4. These studies revealed that heteroatom substituents at C3 with the α-stereochemistry favoured the *exo*-envelope conformation of the five-membered ring and led to higher potency as β-lactamase inhibitors. In addition, introduction of a double bond between C3 and C4 improved inhibitory activity. Future work will incorporate new structural features at C7 using methods developed in the present thesis work as well as structural features at C3 and C4 that were shown by Johnson to enhance inhibition of β-lactamase by the cyclobutanones.

Chapter 4 Experimental Procedures

4.1 General Synthetic Experimental Procedures

All reactions were carried out in flame and oven-dried glassware under an argon or nitrogen atmosphere. Solvents were either reagent grade and/or HPLC grade. Dry acetonitrile was purchased from Sigma-Aldrich. Anhydrous dichloromethane was freshly distilled from calcium hydride under nitrogen prior to use. Deionized water was obtained from a Biolab vertical series reverse osmosis system. Activated zinc (Zn*) was prepared by stirring a combination the normal zinc dust with 10% HCl for 2 minutes, followed by washing with water and acetone in sequence.[109] PMB-I was obtained from a Finkelstein reaction using commercial PMB-Cl to react with sodium iodide in acetone for 2 hours.[110] Reactions were stirred magnetically and monitored by TLC with Merck pre-coated silica plates (silica gel 60 F_{254} on aluminum sheet). Flash chromatography was performed by using the SiliCycle silica gel (60 Å). All reported yields are for isolated compounds unless otherwise sepcified. HPLC separation was carried out using a Waters HPLC system (Waters 600 controller, Waters Millenum® software) equipped with a Waters 486 tunable absorbance detector and a normal phase colunm (Waters Sun Fire™ Prep Silica OBD™, 5 μm, 19 mm × 250 mm). Linear gradients of HPLC-eluents were specifically developed by Mrs. Valerie Goodfellow and Dr. Nan Chen in the Dmitrienko group for the separation of cyclobutanones, which were named as either the Method 1 or Method 2 (Table 16), were used as the separation conditions.

Table 16. HPLC gradient conditions for chromatographic separation of cyclobutanones

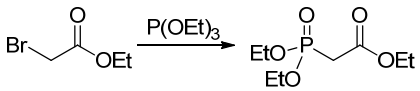
Method 1					
Time (min)	Flow Rate (mL/min)	Solvent A% (EtOAc)	Solvent B% (Hexanes)	Solvent C%	Solvent D%
0	10.00	20.0	80.0	0	0
25	10.00	33.0	67.0	0	0
40	10.00	33.0	67.0	0	0
42	10.00	20.0	80.0	0	0
60	10.00	20.0	80.0	0	0
Method 2					
Time (min)	Flow Rate (mL/min)	Solvent A% (EtOAc)	Solvent B% (Hexanes)	Solvent C%	Solvent D%
0	10.00	0	100.0	0	0
60	10.00	100.0	0.0	0	0
70	10.00	0	100.0	0	0

^{1}H-NMR spectra were recorded on a Brüker AVANCE500 (500 MHz), Brüker AVANCE300 (300 MHz) or Brüker AC300 (300 MHz) NMR spectrometers. ^{13}C-NMR spectra were broad band decoupled and recorded on a Brüker AVANCE500 (125.75 MHz), Brüker AVANCE300 (75.5 MHz) or Brüker AC300 (75.5 MHz) NMR spectrometers using the carbon signal of the deuterated solvent as the internal standard. HMQC and HMBC experiments were performed on the AVANCE500 and Brüker AC300 spectrometers. The following abbreviations are used for NMR peak multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; br, broad. Chemical shifts are reported in parts per million (ppm) relative to either TMS (δ 0.0), chloroform (δ 7.26) or acetone (δ 2.05) for ^{1}H-NMR, and either chloroform (δ 77.16) or acetone (δ 29.84) for ^{13}C-NMR. High resolution mass spectra (HRMS) and low resolution mass spectra (LRMS),

obtained via electrospray ionization (ESI), were measured on a Thermo Scientific Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometry Facility in the Department of Chemistry, University of Waterloo. X-ray crystal structures were determined by Dr. Jalil Assoud and figures of X-ray crystal structures were generated using Mercury.

4.2 Synthetic Procedures[17]

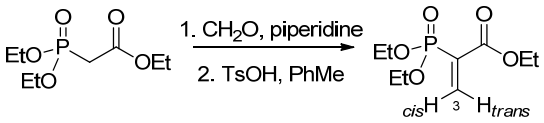
Triethyl Phosphonoacetate 2.13



2.14 **2.13**

Ethyl bromoacetate (64.79 g, 0.388 mol) and triethyl phosphite (64.47 g, 0.391 mol) were combined neat at room temperature, and then the mixture was gradually heated to 90 °C over 2 hours, and further stirred at 90 °C for an additional 23 hours. The crude product was distilled under reduced pressure to give triethyl phosphonoacetate **2.13** as a colourless oil (82.78 g, 0.369 mol, 95%). Bp: 100-106 °C/ ~ 0.1 mm Hg. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 1.20 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 1.26 (t, J = 7.2 Hz, 6H, $P(OCH_2CH_3)_2$), 2.87 (d, $^{2}J_{P,H}$ = 21.6 Hz, 2H, PCH_2), 4.04-4.15 (m, 6H, $P(OCH_2CH_3)_2$ and $CO_2CH_2CH_3$).

Ethyl-2-(diethoxyphosphoryl) Acrylate 2.12

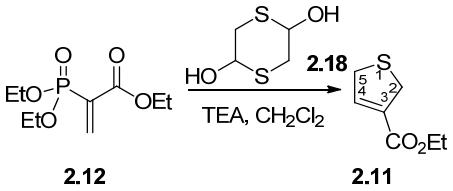


2.13 **2.12**

To a stirring suspension of paraformaldehyde (18.51 g, 0.616 mol) and piperidine (3.1 mL, 2.67 g, 0.031 mol) in 650 mL of methanol, phosphonate **2.13** (69.01 g, 0.308 mol) was added through a pressure-equalized dropping funnel over 30 minutes at room temperature. This mixture was heated and maintained at reflux for 24 hours before the solvent was evaporated to provide the primary alcohol product. The crude oil was then dissolved in toluene

(650 mL), to which was added TsOH•H_2O (5.88 g, 0.031 mmol). The solution was heated to reflux with a Dean-Stark trap for another 13 hours. The reaction mixture was concentrated and distilled under reduced pressure to provide the vinyl phosphonate **2.12** as a clear light yellow oil (66.23 g, 0.280 mol, 91%). Bp: 94-108 °C/ ~ 0.1 mmHg. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 1.30 (m, 9H, $P(OCH_2CH_3)_2$ and $CO_2CH_2CH_3$), δ 4.05-4.20 (m, 4H, $P(OCH_2CH_3)_2$) , 4.25 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 6.70 (dd, J_{gem}= 1.9 Hz, $J_{P,Hcis}$ = 20.4, 1H, H_{3cis}), 6.95 (dd, J_{gem}= 1.9 Hz, $J_{P,Htrans}$ = 42.0, 1H, H_{3trans}).

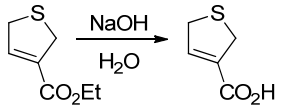
Ethyl 2,5-Dihydrothiophene-3-carboxylate 2.11



2.12 **2.11**

To a suspension of *p*-dithiane-2,5-diol **2.18** (12.92 g, 0.085 mol) and triethylamine (23.6 mL, 17.1 g, 0.169 mol) in 250 mL of dry CH_2Cl_2, a solution of vinyl phosphonate **2.12** (39.99 g, 0.169 mol) in 100 mL of dry CH_2Cl_2 was added through a pressure-equalized dropping funnel over 30 minutes at room temperature. The mixture was heated at reflux for an additional 6 hours before it was cooled to ambient temperature. After acidification to pH ~ 1 with 5% HCl, the mixture was extracted with CH_2Cl_2 (3 × 100 mL). The organic phases were combined and dried over Na_2SO_4 before concentration under reduced pressure to give the crude **2.11** as a yellow oil. This crude product was judged by ^{1}H-NMR analysis to be pure enough to be used in the next step without purification. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 1.28 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.87 (s, 4H, CH_2SCH_2), 4.17 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 6.84 (br s, 1H, H_4).

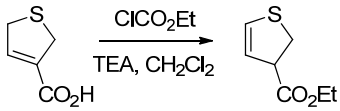
2,5- Dihydrothiophene-3-carboxylic Acid 2.10



2.11 **2.10**

The ethyl ester **2.11** (26.79 g, 0.169 mol) was stirred in 25% aqueous NaOH (w/v, 400 mL) at 70 °C for 16 hours. The reaction mixture was then cooled to room temperature and was washed with CH_2Cl_2 (2 × 200 mL). The aqueous layer was acidified with conc. HCl to pH ~ 1, and extracted with CH_2Cl_2 (6 × 200 mL). The organic phases were dried over Na_2SO_4. Concentration in vacuo gave the acid **2.10** as a light yellow solid, which was purified by recrystallization from EtOAc/hexanes (14.07 g, 0.108 mol, 64% from **2.12**). ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 3.94 (s, 4H, CH_2SCH_2), 7.02 (s, 1H, H_4).

Ethyl 2,3-Dihydrothiophene-3-carboxylate 2.9

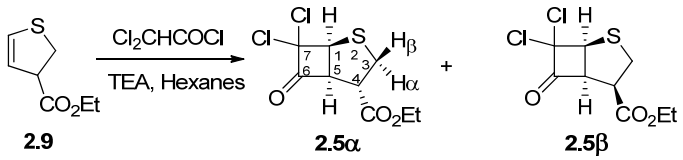


2.10 **2.9**

A solution of ethyl chloroformate (15 mL, 17.09 g, 0.157 mol) in CH_2Cl_2 (150 mL) was added to a stirring solution of the acid **2.10** (10.03 g, 0.077 mmol) and triethylamine (32.5 mL, 23.6 g, 0.233 mmol) in CH_2Cl_2 (250 mL) through a pressure-equalized dropping funnel over 5 minutes at room temperature. The reaction mixture was stirred for additional 24 hours before it was washed with 5% HCl (500 mL), sat. aq. $NaHCO_3$ (500 mL) and brine (500 mL) consecutively. The organic layer was dried over Na_2SO_4 before it was concentrated in vacuo to give the crude product **2.9** as a brown oil (12.16 g, 0.077 mol). The product was sufficiently pure to be used directly in the next step. ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 1.27 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.38 (dd, $J_{2,3}$ = 10.2, J_{gem} = 11.4 Hz, 1H, one of SCH_2), 3.61 (dd, $J_{2,3'}$ = 8.1, J_{gem} = 11.4 Hz, 1H, one of SCH_2), 3.89-3.97 (m, 1H, H_3), 4.18 (q, J = 7.2 Hz, 2H,

$CO_2CH_2CH_3$), 5.61 (dd, $J_{4,3}$ = 2.7 Hz, $J_{4,5}$ = 6.0 Hz, 1H, H_4), 6.26 (dd, $J_{5,3}$ = 2.4 Hz, $J_{5,4}$ = 6 Hz, 1H, H_5).

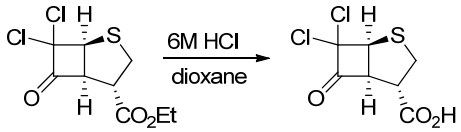
Ethyl 7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylates 2.5



A solution of dichloroacetyl chloride (3.5 mL, 5.36 g, 37.12 mmol) in hexanes (140 mL) was added to a stirring solution of the ester **2.9** (2.30 g, 14.54 mmol) and triethylamine (5.1 mL, 3.70 g, 36.56 mmol) in hexanes (15.5 mL) over 3 hours through a motor-driven syringe pump at room temperature. The reaction mixture was then stirred for another 21 hours before it was filtered through Celite. The Celite filter cake was rinsed with hexanes and the filtrate was washed with 5% HCl (3 × 70 mL), sat. aq. $NaHCO_3$ (3 × 70 mL), and brine (100 mL) consecutively. The organic phases were combined, dried over Na_2SO_4 and then concentrated under reduced pressure. Flash chromatography (CH_2Cl_2) of the crude product followed by re-chromatography of mixed fractions (5% Et_2O/hexanes, v/v) provided the cyclobutanone **2.5** (2.30 g, 8.55 mmol, 59%) and the epimer **2.5β** was obtained as yellow oil (229.5 mg, 0.85 mmol, 6%) that crystallized upon storage at 4 °C. **2.5α**: ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 1.24 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.01 (dd, J_{gem}= 12.3 Hz, $J_{3\beta,4}$= 5.7 Hz, 1H, $H_{3\beta}$), 3.40 (d, J_{gem}= 12.3 1H, $H_{3\alpha}$), 3.62 (d, $J_{4,3\beta}$= 5.7 Hz,1H, H_4), 4.16 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.48 (d, $J_{1,5}$= 8.2 Hz, 1H, H_1), 5.03 d, $J_{5,1}$=8.2 Hz, 1H, H_5). **2.5β**: ^{1}H-NMR (300 MHZ, $CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.11 (B of ABX, J_{gem}= 12.0 Hz, $J_{3\alpha,4}$= 7.2 Hz, 1H, $H_{3\alpha}$), 3.13 (A of ABX, J_{gem}= 12.0 Hz, $J_{3\beta,4}$= 10.3 Hz, 1H, $H_{3\beta}$), 3.28 (dt, $J_{4,3\alpha}$ = 7.8 Hz, $J_{4,3\beta}$ = 10.3 Hz, $J_{4,5}$ = 7.8 Hz, 1H, H_4), 4.21 (B of ABX_3, J_{BA} = 10.5 Hz, J_{BX} = 7.2 Hz, 1H,

one of $CO_2CH_2CH_3$), 4.28 (A of ABX_3, J_{AB} = 10.5 HZ, J_{AX} = 7.2 Hz, 1H, one of $CO_2CH_2CH_3$), 4.44 (d, $J_{1,5}$= 8.1 Hz, 1H, H_1), 4.81 (dd, $J_{5,1}$ = 8.1 Hz, $J_{5,4}$ = 7.8 Hz, 1H, H_5).

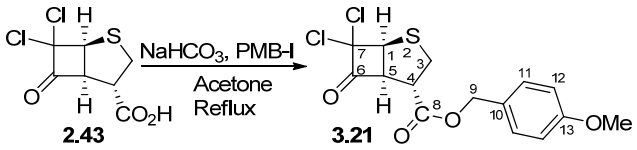
7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate Acid 2.43



2.5α **2.43**

The ethyl ester **2.5α** (902.2 mg, 3.35 mmol) was dissolved in 7 mL of dioxane and then stirred with 14 mL of 6 M HCl at 80 °C for 6 hours, before the reaction mixture was cooled to room temperature and extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to give the crude acid **2.43** as a beige solid. The crude product was recrystallized from toluene to give the pure acid **2.43** as a light grey solid (574.9 mg, 2.38 mmol, 71%). **2.43**: ^{1}H-NMR (300 MHz, acetone-d_6): δ 3.07 (dd, J_{gem}= 12.5 Hz, $J_{3\beta,4}$= 5.9 Hz, 1H, $H_{3\beta}$), 3.51 (d, J_{gem}= 12.5 Hz, 1H, $H_{3\alpha}$), 3.82(d, $J_{4,3\beta}$= 5.9 Hz, 1H, H4), 4.71 (d, $J_{1,5}$= 8.2 Hz, 1H, H_1), 5.19 (d, $J_{5,1}$=8.2 Hz, 1H, H_5)

***p*-Methoxybenzyl 7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate 3.21**

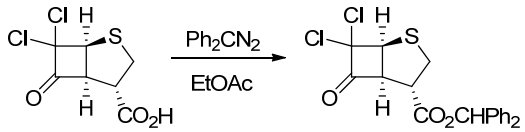


2.43 **3.21**

Sodium bicarbonate (44.4 mg, 0.529 mmol) was added to a stirring solution of the carboxylic acid **2.43** (118.8 mg, 0.493 mmol) in 3 mL of acetone at ambient temperature, and the mixture was stirred for another 30 minutes. PMB-I (128 mg, 0.516 mmol) was added to this solution and then the reaction mixture was heated at reflux for an additional 2 hours before it was cooled to room temperature and then evaporated to dryness. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with 5% HCl (3 × 10 mL), sat. $NaHCO_3$ (3 × 10 mL) and brine

(10 mL) consecutively. The organic phases were combined and dried over Na_2SO_4 before concentration under reduced pressure. Flash chromatography (CH_2Cl_2) provided the PMB ester **3.21** as a yellow solid (59.8 mg, 0.166 mmol, 34%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.02 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$), 3.42 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.66 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 3.81 (s, 3H, OC*H*$_3$), 4.49 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 5.04 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5), 5.09 (s, 2H, H_9), 6.89 (d, $J_{12,11}$ = 8.6 Hz, 2H, H_{12}), 7.27 (d, $J_{11,12}$ = 8.6 Hz, 2H, H_{11}). ^{13}C-NMR (75.5MHz, $CDCl_3$): δ35.6, 50.1, 55.3, 58.9, 67.4, 67.7, 89.2, 114.0, 127.1, 130.0, 159.9, 169.8, 194.7. LRMS (+ESI): 401.0069 (100%), 403.0016 (72%).

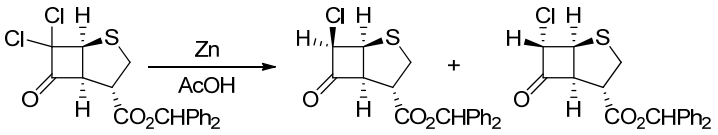
Benzhydryl 7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate 2.44



2.43 **2.44**

To a stirring solution of the acid **2.43** (604 mg, 2.51 mmol) in EtOAc (8 mL), a solution of diphenyldiazomethane (0.449 M in EtOAC, 5.6 mL) was added slowly. The reaction mixture was stirred for an additional 2 hours before it was quenched with acetic acid (1 mL), followed by stirring for another 30 minutes. The solution was then diluted to 20 mL with EtOAc and washed with H_2O (2 × 10 mL), sat. aq. $NaHCO_3$ (2 × 10 mL) and brine (2 × 10 mL) consecutively. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to give pure benzhydryl ester **2.44** (988.2 mg, 2.43 mmol, 97%) as a yellow solid, which was jugde by ^{1}H-NMR to be sufficiently pure for use in further transformations ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.10 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.52 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.78 (d, $J_{4,3\beta}$ = 5.8 Hz, 1H, H_4), 4.52 (d, $J_{1,5}$ = 8.2 Hz, 1H, H_1), 5.11 (d, $J_{5,1}$ = 8.2 Hz, 1H, H_5), 6.93 (s, 1H, C*H*Ph$_2$), 7.31-7.42 (m, 10H, Ar*H*).

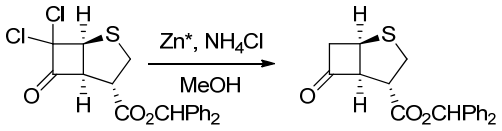
Benzhydryl 7-chloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylates 2.57



2.44 **2.57β** **2.57α**

To a stirring solution of the dichlorocyclobutanone **2.44** (51.7 mg, 0.127 mmol) in 5 mL of acetic acid was added unactivated zinc dust (8.8 mg, 0.135 mmol) at ambient temperature. After 2.5 hours, a second portion of unactivated zinc dust (8.6 mg, 0.132 mmol) was added to the solution and the reaction mixture was stirred for another 1 hour before the excess zinc was removed by filtration. The solvent was evaporated under reduced pressure and the residue was dissolved in 20 mL of EtOAc, which was then washed with 10% HCl (20 mL). The aqueous solution was extracted by EtOAc (2 × 20 mL). The organic phases were washed with brine (2 × 20 mL), which was dried over Na_2SO_4 and concentrated in vacuo to give the monochlorocyclobutanone **2.57** as a yellow oil (α : β = 1 : 10, 37.8 mg, 0.101 mmol, 80%). **2.57β**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.09 (dd, J_{gem} = 12.3 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.45 (d, J_{gem} = 12.3 Hz, 1H, $H_{3\alpha}$), 3.75 (d, $J_{4,3\beta}$ = 6.0 Hz, 1H, H_4), 4.44 (dd, $J_{1,5}$ = 8.0 Hz, $J_{1,7\alpha}$ = 8.1 Hz, 1H, H_1), 4.71 (dd, $J_{5,1}$ = 8.0 Hz, $J_{5,7\alpha}$ = 3.2 Hz, 1H, H_5), 5.18 (dd, $J_{7\alpha,1}$ = 8.1 Hz, $J_{7\alpha,5}$ = 3.2 Hz, 1H, $H_{7\alpha}$), 6.88 (s, 1H, C*H*Ph_2), 7.27-7.36 (m, 10H, Ar*H*). 13C-NMR (75.5 MHz, $CDCl_3$): δ 35.4, 46.6, 50.6, 65.6, 67.6, 78.4, 126.9, 127.1, 128.2, 128.7, 139.5, 169.6, 201.2. **2.57α**: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.26 (dd, J_{gem} = 12.3 Hz, $J_{3\beta,4}$ = 6.1 Hz, 1H, $H_{3\beta}$), 3.55 (d, J_{gem} = 12.3 Hz, 1H, $H_{3\alpha}$), 3.76 (d, $J_{4,3\beta}$ = 6.1 Hz, 1H, H_4), 4.11 (dd, $J_{1,5}$ = 8.1 Hz, $J_{1,7\beta}$ = 2.9 Hz, 1H, H_1), 4.62 (dd, $J_{7\beta,1}$ = 2.9 Hz, $J_{7\beta,5}$ = 2.5 Hz, 1H, $H_{7\beta}$), 4.99 (dd, $J_{5,1}$ = 8.1 Hz, $J_{5,7\beta}$ = 2.5 Hz, 1H, H_5), 6.90 (s, 1H, C*H*Ph_2), 7.27-7.36 (m, 10H, Ar*H*).

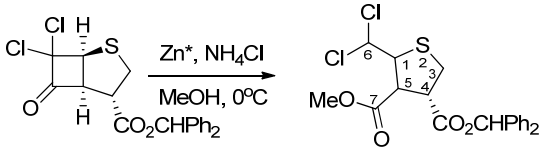
Benzhydryl 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylate 3.36



2.44 **3.36**

Activated zinc dust (77.5 mg, 1.186 mmol) and NH_4Cl (69.3 mg, 1.296 mmol) were added to a stirring solution of the dichlorocyclobutanone **2.44** (51.7 mg, 0.127 mmol) in 5 mL of methanol, and the reaction mixture was stirred at room temperature for 1 hour before the excess zinc dust and NH_4Cl were filtered off. After evaporation of the solvent, the residue was taken up by 20 mL of EtOAc, and washed with 20 mL of H_2O. The aqueous wash was further extracted with EtOAc (2 × 20 mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure, affording the didechlorinated cyclobutanone **3.36** as a yellow oil (37.1 mg, 0.110 mmol, 87%) that appeared to be pure as judged by ^{1}H-NMR. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.01 (dt, $J_{7\beta,5}$ = 3.3 Hz, $J_{7\beta,1}$ = 3.3 Hz, J_{gem} = 18.7 Hz, 1H, $H_{3\beta}$), 3.25 (dd, J_{gem} = 12.3 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.52 (d, J_{gem} = 12.3 Hz, 1H, $H_{3\alpha}$), 3.63 (ddd, J_{gem} = 18.7 Hz, $J_{7\alpha,1}$ = 8.5 Hz, $J_{7\alpha,5}$ = 3.6 Hz, 1H, $H_{7\alpha}$), 3.69 (d, $J_{4,3\beta}$ = 6.0 Hz, 1H, H4), 4.12-4.18 (m, 1H, H_1), 4.61-4.69 (m, 1H, H_5), 6.86 (s, 1H, C*H*Ph_2), 7.28-7.38 (m, 10H, Ar*H*).

6-Dichloromethyl-tetrahydrothiophene-4,5-dicarboxylic acid 4-benzhydryl ester 5-methyl ester 3.37

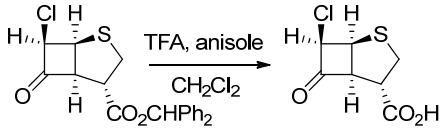


2.44 **3.37**

Activated zinc dust (81.1 mg, 1.241 mmol) and NH_4Cl (66.3 mg, 1.239 mmol) were added to a stirring solution of the dichlorocyclobutanone **2.44** (50.4 mg, 0.124 mmol) in 8 mL of methanol at 0 °C. The reaction mixture was stirred for 13 minutes before excess zinc and

NH_4Cl were filtered off and then the filtrate was concentrated. The residue was dissolved in 20 mL of EtOAc and washed with 20 mL of H_2O. The aqueous wash was further extracted with EtOAc (2 × 20mL). The organic phases were combined, dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (15% EtOAc/hexanes, v/v), followed by re-chromatography (67% CH_2Cl_2/hexanes, v/v) provided the ring-opening product **3.37** (3.3 mg, 0.008 mmol, 6%) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.10 (dd, J_{gem} = 10.7 Hz, $J_{3\beta,4}$ = 6.7 Hz, 1H, $H_{3\beta}$), 3.35 (dd, J_{gem} = 10.7 Hz, $J_{3\alpha,4}$ = 6.7 Hz, 1H, $H_{3\alpha}$), 3.63 (s, 3H, OMe), 3.79 (m, 2H, H_4, H_5), 4.33 (dd, $J_{1,5}$ = 6.8 Hz, $J_{1,6}$ = 6.1 Hz, 1H, H_1), 6.16 (d, $J_{6,1}$ = 6.1 Hz, 1H, H_6), 6.90 (s, 1H, C*H*Ph_2), 7.30-7.34 (m, 10H, Ar*H*).

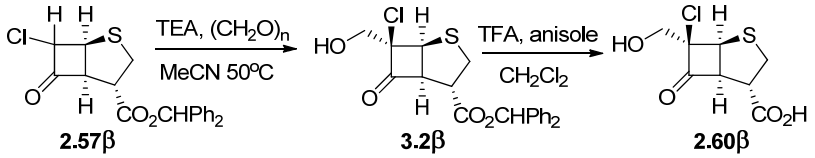
7β-Chloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic Acid 2.58β



2.57β **2.58β**

To a stirring solution of the benzhydryl ester **2.57β** (79.5 mg, 0.213 mmol) and anisole (0.07 mL, 69.5 mg, 0.643 mmol) in 10 mL of dichloromethane was added trifluoroacetic acid (0.33 mL, 491.4 mg, 4.310 mmol). The reaction mixture was stirred at room temperature for 2.5 hours before evaporating the solvent. The residue was then triturated with cyclohexane to remove anisole-derived by-products, The monochloro acid **2.58β** was obtained as a yellow solid (30.9 mg, 0.150 mmol, 70%). ^{1}H-NMR (500 MHz, acetone-d_6): δ 2.98 (dd, J_{gem} = 12.1 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.39 (d, J_{gem} = 12.1 Hz, 1H, $H_{3\alpha}$), 3.66 (d, $J_{4,3\beta}$ = 5.8 Hz, 1H, H_4), 4.58 (dd, $J_{1,5}$ = 7.9 Hz, $J_{1,7\alpha}$ = 7.9 Hz, 1H, H_1), 4.79 (dd, $J_{5,1}$ = 7.9 Hz, $J_{5,7\alpha}$ = 3.1 Hz, 1H, H_5), 5.57 (dd, $J_{7\alpha,1}$ = 7.9 Hz, $J_{7\alpha,5}$ = 3.1 Hz, 1H, $H_{7\alpha}$). ^{13}C-NMR (75.5 MHz, acetone-d_6): δ 35.1, 46.4, 49.9, 65.3, 67.8, 171.3, 205.3. HRMS (-ESI) *m/z*: 204.9716 ($[M-H]^-$), calcd. for $C_7H_6O_3ClS$: 204.9732.

7β-Chloro-7α-hydroxymethyl-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic acid 2.60β

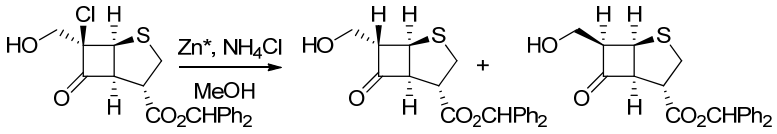


To a stirring suspension of the monochloro benzhydryl ester **2.57β** (77.5 mg, 0.208 mmol) and paraformaldehyde (13 mg, 0.433 mmol) in 16 mL of MeCN and 0.4 mL of H_2O was added triethylamine (0.12 mL, 87.1 mg, 0.861 mmol) at ambient temperature. This suspension was then heated to 50 °C for 2 hours before the reaction mixture was concentrated in vacuo. The residue was dissolved in 20 mL of CH_2Cl_2, which was washed with 10% HCl (2 × 10 mL) and brine (2 × 10 mL) consecutively. The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2 then acetone) afforded the hydroxymethyl benzhydryl ester **3.2β** as a dark yellow oil (57.2 mg, 0.142 mmol, 68%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.07 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.47 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.69 (d, $J_{4,3\beta}$ = 5.8 Hz, 1H, H_4), 3.91 (B of AB, J_{AB} = 11.7 Hz, 1H, one of CH_2OH), 4.02 (A of AB, J_{AB} = 11.7 Hz, 1H, one of CH_2OH), 4.35 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 4.72 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5), 6.86 (s, 1H, *CHPh*$_2$), 7.27-7.39 (m, 10H, Ar*H*). ^{13}C-NMR (75.5MHz, $CDCl_3$): δ 35.8, 49.4, 50.7, 65.7, 67.8, 78.5, 82.6, 127.0 , 127.1, 128.2, 128.7, 139.4, 169.7, 203.8.

Trifluoroacetic acid (0.93 mL, 1384.8 mg, 12.145 mmol) was added to a stirring solution of the benzhydryl ester **3.2β** (122.4 mg, 0.304 mmol) and anisole (0.1 mL, 99.3 mg, 0.918 mmol) in 10 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature for 2.5 hours and then concentrated under reduced pressure. The crude product was purified by trituration with cyclohexane to give the acid **2.60β** as a yellow solid (57.2 mg, 0.242 mmol, 80%). ^{1}H-NMR (500 MHz, acetone-d_6): δ 2.96 (dd, J_{gem} = 12.2 Hz, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$),

3.42 (d, J_{gem} = 12.2 Hz, 1H, $H_{3\alpha}$), 3.62 (d, $J_{4,3\beta}$ = 5.7 Hz, 1H, H4), 3.96 (B of AB, J_{AB} = 11.4 Hz, 1H, one of CH_2OH), 4.04 (A of AB, J_{AB} = 11.4 Hz, 1H, one of CH_2OH), 4.48 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 4.71 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5). ^{13}C-NMR (125.75 MHz, acetone-d_6): δ 35.6, 49.3, 50.2, 65.0, 68.4, 82.3, 171.5, 204.6. HRMS (-ESI) *m/z*: 234.9823 ([M-H]$^-$), calcd. for $C_8H_8O_4ClS$: 234.9826.

Benzhydryl 7-hydroxymethyl-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylates 3.49

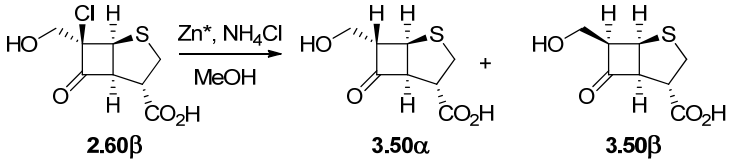


3.2β **3.49α** **3.49β**

Activated zinc dust (621.4 mg, 9.506 mmol) and NH_4Cl (501.5 mg, 9.376 mmol) were added to a stirring solution of the 7α-hydroxymethyl-7β-chlorocyclobutanone **3.2β** (190.1 mg, 0.472 mmol) in 15 mL of MeOH at ambient temperature. The reaction mixture was stirred for 1 hour before it was filtered. After evaporating the solvent from the filtrate, the residue was taken up by EtOAc (30 mL) and washed with H_2O (30 mL). The aqueous layer was then further extracted with EtOAc (3 × 30mL), and the organic layers were combined and then dried over Na_2SO_4 before concentration under reduced pressure to give a mixture of the dechlorinated cyclobutanones **3.49** as a colourless oil (β : α = 1 : 7, as determined by ^{1}H-NMR, 136.5 mg, 0.370 mmol, 78%). Flash chromatography (20% EtOAc/hexanes, v/v), followed by HPLC separation (Method 1) provided **3.49α** (9.4 mg, 0.026 mmol, 7%, retention time: 28.4 minutes) and **3.49β** (38.6 mg, 0.105 mmol, 28%, retention time: 40.1 minutes). **3.49α**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (dd, J_{gem} = 12.3 Hz, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$), 3.32 (m, 1H, $H_{7\beta}$), 3.55 (d, J_{gem} = 12.3 Hz, 1H, $H_{3\alpha}$), 3.67 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 3.88 (B of AB, J_{AB} = 11.0 Hz, $J_{B,7\beta}$ = 4.8 Hz, 1H, one of CH_2OH), 3.96 (A of AB, J_{AB} = 11.0 Hz, $J_{A,7\beta}$ = 4.8 Hz, 1H, one of CH_2OH), 4.12 (dd, $J_{1,7\beta}$ = 4.0 Hz, $J_{1,5}$ = 8.1 Hz, 1H, H_1), 4.57 (dd, $J_{5,1}$ = 8.1 Hz, $J_{5,7\beta}$ =

3.7 Hz, 1H, H_5), 6.85 (s, 1H, C*H*Ph_2), 7.31-7.37 (m, 10H, Ar*H*). ^{13}C-NMR (75.5MHz, $CDCl_3$): δ 35.7, 40.4, 50.4, 60.1, 70.4, 72.0, 78.2, 126.9, 127.1, 128.1, 128.6, 139.6, 169.9, 210.7. HRMS (+ESI) *m/z*: 369.1155 ($[M+H]^+$), calcd. for $C_{21}H_{20}O_4S$: 369.1155. **3.49β**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.08 (dd, J_{gem} = 12.2 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.47 (d, J_{gem} = 12.2 Hz, 1H, H_3), 3.66 (d, $J_{4,3\beta}$ = 6.0 Hz, 1H, H_4), 3.79 (m, 1H, $H_{7\alpha}$), 3.91-3.93 (m, 2H, C*H*$_2$OH), 4.32 (dd, $J_{1,7\alpha}$ = 8.3 Hz, $J_{1,5}$ = 8.2 Hz, 1H, H_1), 4.67 (dd, $J_{5,1}$ = 8.2 Hz, $J_{5,7\alpha}$ = 3.1 Hz, 1H, H_5), 6.86 (s, 1H, C*H*Ph_2), 7.32-7.38 (m, 10H, Ar*H*). ^{13}C-NMR (75.5MHz, $CDCl_3$): δ 35.9, 41.7, 49.0, 58.7, 64.4, 69.3, 78.3, 126.9, 127.1, 128.2, 128.4, 139.5, 170.0, 208.1. HRMS (+ESI) *m/z*: 369.1156 ($[M+H]^+$), calcd. for $C_{21}H_{20}O_4S$: 369.1155.

7-Hydroxymethyl-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic acids 3.50

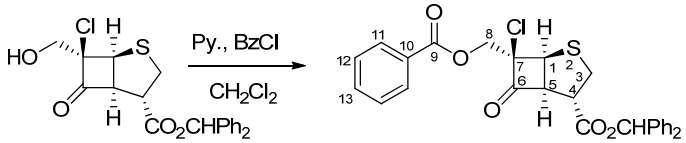


2.60β **3.50α** **3.50β**

To a stirring solution of the carboxylic acid **2.60β** (40 mg, 0.169 mmol) in 10 mL of MeOH was added activated zinc dust (439.2 mg, 6.719 mmol) and NH_4Cl (360.9 mg, 6.747 mmol) at ambient temperature. After 8 minutes, the reaction mixture was filtered and the solvent was evaporated under reduced pressure. The residue was then dissolved in EtOAc (20 mL) and washed with H_2O (20 mL). The aqueous solution was acidified by 10% HCl to pH ~ 1, which was then extracted with EtOAc (4 × 20 mL). The organic phases were combined and dried over Na_2SO_4 before concentration under reduced pressure to afford the crude mixture of dechlorinated acids **3.50** as a colourless oil (**3.50α** : **3.50β** = 1 : 6, as determined by ^{1}H-NMR, 30.1 mg, 0.149 mmol, 88%). HPLC separation (Method 2) provided **3.50α** (2.7 mg, 0.013 mmol, 8%, white solid, retention time: 37.3 minutes) and **3.50β** (24.4 mg, 0.121 mmol, 72%, colourless oil, retention time: 41.9 minutes). **3.50α**: ^{1}H-NMR (300 MHz, acetone-d_6): δ 3.12-

3.26 (m, 2H, $H_{3\beta}$, $H_{7\beta}$), 3.46 (d, J_{gem} = 12.2 Hz, 1H, $H_{3\alpha}$), 3.56 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 3.81 (B of AB, J_{AB} = 11.1 Hz, $J_{B,7\beta}$ = 4.7 Hz, 1H, one of CH_2OH), 3.87 (A of AB, J_{AB} = 11.1 Hz, $J_{A,7\beta}$ = 4.7 Hz, 1H, one of CH_2OH), 4.14 (dd, $J_{1,5}$ = 8.1 Hz, $J_{1,7\beta}$ = 3.7 Hz, 1H, H_1), 4.48 (dd, $J_{5,1}$ = 8.1 Hz, $J_{5,7\beta}$ = 3.4 Hz, 1H, H_5). ^{13}C-NMR (75.5 MHz, acetone-d_6): δ 35.1, 40.5, 49.6, 59.1, 70.5, 72.5, 171.6, 210.0. HRMS (-ESI) *m/z*: 201.0212 ([M-H]$^-$, calcd. for $C_8H_9O_4S$: 201.0216). **3.50β**: ^{1}H-NMR (300 MHz, acetone-d_6): δ 2.97 (dd, J_{gem} = 12.0 Hz, $J_{3\beta,4}$ = 6.2 Hz, 1H, $H_{3\beta}$), 3.35 (d, J_{gem} = 12.0 Hz, 1H, $H_{3\alpha}$), 3.50 (d, $J_{4,3\beta}$ = 6.2 Hz, 1H, H_4), 3.69-3.91 (m, 3H, $H_{7\alpha}$, CH_2OH), 4.34 (dd, $J_{1,5}$ = 7.8 Hz, $J_{1,7\alpha}$ = 7.6 Hz, 1H, H_1), 4.66 (d, $J_{5,1}$ = 7.8 Hz, 1H, H_5). ^{13}C-NMR (75.5MHz, acetone-d_6): δ 35.3, 48.1, 57.3, 64.9, 69.1, 171.9, 207.8. HRMS (-ESI) *m/z*: 201.0211 ([M-H]$^-$), calcd. for $C_8H_9O_4S$: 201.0216.

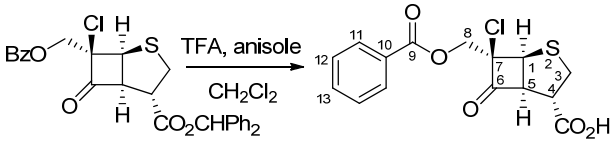
Benzhydryl 7β-chloro-7α-methylbenzoate-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate 3.62β



Pyridine (0.35 mL, 341.7 mg, 4.320 mmol) and benzoyl chloride (0.27 mL, 323.9 mg, 2.304 mmol) were added to a stirring solution of the hydroxymethyl cyclobutanone **3.2β** (116.1 mg, 0.286 mmol) in dichloromethane (10 mL). The reaction mixture was heated to reflux and stirred for another 21 hours before it was cooled to room temperature. The solution was washed with HCl (3 × 10 mL), sat. aq. $NaHCO_3$ (3 × 10 mL) and brine (3 × 10 mL) consecutively. The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (10% EtOAc/hexanes, v/v) provided the methylbenzoate **3.62β** as a white semi-solid (98.1 mg, 0.193 mmol, 67%) ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.09 (dd, J_{gem} = 12.1 Hz, $J_{3\beta,4}$ = 5.1 Hz, 1H, $H_{3\beta}$), δ 3.50 (d, J_{gem} = 12.1 Hz, 1H, $H_{3\alpha}$), 3.74 (d. $J_{4,3\beta}$ =

5.1 Hz, 1H, H_4), 4.39 (d, $J_{1,5}$ = 8.4 Hz, H1, H_1), 4.68 (B of AB, J_{AB} = 11.7 Hz, 1H, one of CH_2OBz), 4.79 (A of AB, J_{AB} = 11.7 Hz, 1H, one of CH_2OBz), 4.86 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5), 6.86 (s, 1H, CHPh$_2$), 7.28-7.35 (m, 10H, CHPh_2), 7.43 (dd, $J_{12,11}$ = 7.5 Hz , $J_{12,13}$ = 7.0 Hz, 2H, H_{12}), 7.59 (t, J = 7.0 Hz, 1H, H_{13}), 8.01 (d, $J_{11,12}$ = 7.5 Hz, 2H, H_{11}). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ 35.8, 49.7, 50.8, 65.9, 68.1, 78.7, 80.2, 126.9, 127.0, 128.2, 128.7, 129.8, 130.2, 133.8, 139.3, 165.6, 169.4, 202.0, The signal for the quaternary carbon of the benzoyl group is missing. HRMS (+ESI) *m/z*: 507.1029 ([M+H]$^+$), calcd for $C_{28}H_{24}O_5ClS$: 507.1039.

7β-Chloro-7α-methylbenzoate-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic acid 3.55β



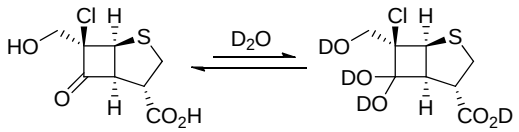
3.62β **3.55β**

Trifluoroacetic acid (0.16 mL, 237.8 mg, 2.086 mmol) was added to a stirring solution of the benzhydryl ester **3.62β** (26.4 mg, 0.052 mmol) and anisole (0.04 mL, 39.7 mg, 0.367 mmol) in dichloromethane (10 mL) at 0 °C. The reaction mixture was stirred for 9 hours before the solvent was evaporated. The residue was dissolved in EtOAc (15 mL) and washed with sat. aq. $NaHCO_3$ (2 × 10 mL). The aqueous layer was acidified to pH ~ 1 using 10% HCl and then extracted by EtOAc (4 × 15 mL). The organic phases were combined and then dried over Na_2SO_4 before concentration under reduced pressure to afford the crude product. HPLC separation (Method 2) provided the methylbenzoate acid **3.55β** as a white solid (4.1 mg, 0.012 mmol, 23%, retention time: 26.7 minutes). ^{1}H-NMR (500 MHz, acetone-d_6): δ 3.03 (dd, J_{gem} = 12.3 Hz, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$), 3.46 (d, J_{gem} = 12.3 Hz, 1H, $H_{3\alpha}$), 3.73 (d. $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 4.63 (d, $J_{1,5}$ = 8.5 Hz, H1, H_1), 4.86 (B of AB, J_{AB} = 12.2 Hz, 1H, one of CH_2OBz), 4.97 (A of AB, J_{AB} =12.2 Hz, 1H, one of CH_2OBz), 5.04 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5), 7.56 (dd, $J_{12,11}$ = 7.8 Hz, $J_{12,\ 13}$ =7.5 Hz, 2H, H_{12}), 7.69 (t, J = 7.5 Hz, 1H, H_{13}), 8.05 (d, $J_{11,12}$ = 7.8 Hz, 2H, H_{11}).

^{13}C-NMR (75.5MHz, acetone-d_6): δ 35.4, 49.5, 68.0, 81.0, 128.7, 129.4, 129.5, 133.6, 165.2, 171.0, 201.4. HRMS (-ESI) *m/z*: 339.0094 ([M-H]$^-$, calcd. for $C_{15}H_{12}O_{5Cl}S$: 339.0089).

4.3 Hydrate Formation of Cyclobutanones

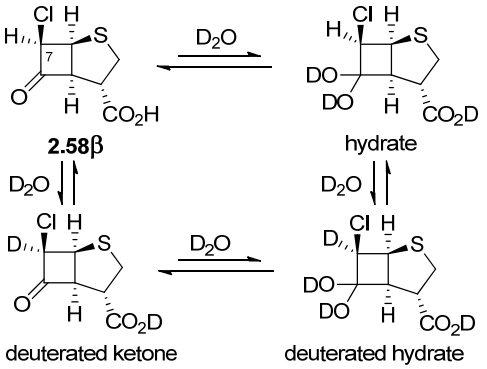
Hydrate formation of hydroxymethyl cyclobutanone 2.60β



2.60β hydrate

The hydroxymethyl cyclobutanone **2.60β** was dissolved in a mixed solvent of acetone-d_6 (0.15 mL) and D_2O (0.45 mL). The ^{1}H-NMR spectra of the mixture showed the ketone and hydrate in a ratio of 88 : 12 after 15 minutes, which did not change in the following 24 hours. **Ketone** ^{1}H-NMR (500 MHz, D_2O : acetone-d_6 = 3 : 1), δ 2.98 (dd, J_{gem} = 12.5, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.38 (d, J_{gem} = 12.5 Hz, 1H, $H_{3\alpha}$), 3.69 (d, $J_{4,3\beta}$ = 6.0 Hz, 1H, H4), 4.03 (B of AB, J_{AB} = 12.5 Hz, 1H, one of CH_2OH), 4.07 (A of AB, J_{AB} = 12.5 Hz, 1H, one of CH_2OH), 4.40 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 4.78 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5). ^{13}C-NMR (125.75 MHz, D_2O : acetone-d_6 = 3 : 1): δ 35.5, 49.1, 50.5, 64.5, 67.8, 82.7, 174.5, 206.8. **Hydrate** ^{1}H-NMR (500 MHz, D_2O : acetone-d_6 = 3 : 1), δ 3.25 (d, J_{gem} = 11.5, 1H, $H_{3\alpha}$), 3.48 (dd, J_{gem} = 11.5 Hz, $J_{3\beta,4}$ = 6.5 Hz, 1H, $H_{3\beta}$), 3.59 (d, $J_{4,3\beta}$ = 6.5 Hz, 1H, H4), 3.74 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 3.95 (B of AB, J_{AB} = 12.3 Hz, 1H, one of CH_2OH), 3.99 (A of AB, J_{AB} = 12.3 Hz, 1H, one of CH_2OH), 4.69 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5). ^{13}C-NMR (125.75 MHz, D_2O : acetone-d_6 = 3 : 1): δ 37.6, 48.8, 50.6, 57.3, 66.0, 79.8, 95.9, 176.4.

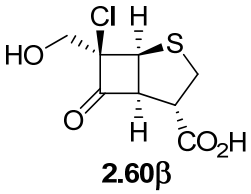
Hydrate foramtion of monochlorocyclobutanone 2.58β



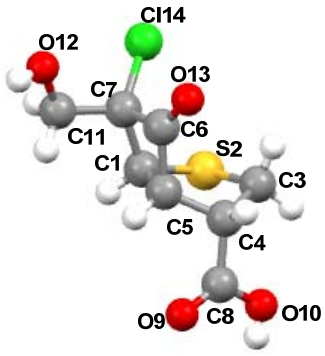
The hydroxymethyl cyclobutanone **2.58β** was dissolved in a mixed solvent of acetone-d6 (0.15 mL) and D_2O (0.45 mL). The ^{1}H-NMR spectra of the mixture showed ketone and hydrate in a ratio of 75 : 25 after 5 minutes. This ratio did not change in the following 18 hours. However, the 1H-NMR was changed because the proton at C7 was gradually exchanged with deuterium. **Ketone** (5 minutes): ^{1}H-NMR (500 MHz, D_2O : acetone-d_6 = 3 : 1), δ 2.83 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 6.1 Hz, 1H, $H_{3\beta}$), 3.21 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.58 (d, $J_{4,3\beta}$ = 6.1 Hz, 1H, H_4), 4.45 (dd, $J_{1,5}$ = 7.9 Hz, $J_{1,7\alpha}$ = 8.1 Hz, 1H, H_1), 4.64 (dd, $J_{5,1}$ = 7.9 Hz, $J_{5,7\alpha}$ = 3.3 Hz, 1H, H_5), 5.44 (dd, $J_{7\alpha,1}$ = 8.1 Hz, $J_{7\alpha,5}$ = 3.3 Hz, 1H, $H_{7\alpha}$). **Deuterated ketone** (18 hours): ^{1}H-NMR (500 MHz, D_2O : acetone-d_6 = 3 : 1), δ 2.83 (dd, J_{gem}= 12.4 Hz, $J_{3\beta,4}$= 6.1 Hz, 1H, $H_{3\beta}$), 3.21 (d, J_{gem}= 12.4 Hz, 1H, $H_{3\alpha}$), 3.58 (d, $J_{4,3\beta}$= 6.1 Hz, 1H, H_4), 4.45 (d, $J_{1,5}$= 8.0 Hz, 1H, H_1), 4.64 (d, $J_{5,1}$ = 8.0 Hz, 1H, H_5). **Hydrate** (5 minutes): ^{1}H-NMR (500 MHz, D_2O : acetone-d_6 = 3 : 1), δ 3.11 (d, J_{gem} = 11.5 Hz, 1H, $H_{3\alpha}$), 3.27 (dd, J_{gem} = 11.5 Hz, $J_{3\beta,4}$ = 6.5 Hz, 1H, $H_{3\beta}$), 3.45 (d, $J_{4,3\beta}$ = 6.5 Hz, 1H, H_4), 3.62 (d, $J_{5,1}$ = 8.2 Hz, 1H, H_5), 4.13 (dd, $J_{1,5}$ = 8.2 Hz, $J_{1,7\alpha}$ = 7.5 Hz, 1H, H_1), The peak of $H_{7\alpha}$ is overlapped with the solvent peak (HDO chemical shift around δ 4.59). **Deuterated hydrate** (18 hours): ^{1}H-NMR (500 MHz, D_2O : acetone-d_6 = 3 : 1), δ 3.19 (d, J_{gem} = 11.6 Hz, 1H, $H_{3\alpha}$), 3.27 (dd, J_{gem} = 11.6 Hz, $J_{3\beta,4}$ = 6.3 Hz, 1H, $H_{3\beta}$), 3.45 (d, $J_{4,3\beta}$ = 6.3 Hz, 1H, H_4), 3.61 (d, $J_{5,1}$ = 7.5 Hz, 1H, H_5), 4.12 (d, $J_{1,5}$ = 7.5 Hz,1H, H_1).

Appendix A

Crystal Data and Structure Refinement for Cyclobutanone 2.60β



2.60β



Empirical formula	C8H16ClO4S
Formula weight	236.66
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 5.31710(10) Å, b = 8.9724(2) Å, c = 10.5459(2) Å α = 78.3564(12)°, β = 77.0590(10)°, γ = 77.6125(10)°
Volume, Z	472.630(17) $Å^3$, 4
Density (calculated)	1.663 Mg/m^3
Absorption coefficient	0.608 mm^{-1}
F(000)	244
Crystal size	0.200 × 0.080 × 0.020 mm^3
Theta range for data collection	2.008 to 26.368°
Index ranges	-6<=h<=6, -11<=k<=11, -13<=l<=13
Reflections collected	7781
Independent reflections	1933 [R(int) = 0.0221]
Completeness to theta = 25.000°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7460 and 0.6923
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1933 / 37 / 135
Goodness-of-fit on F^2	1.038
Final R indices [I>2sigma(I)]	R1 = 0.0318, wR2 = 0.0739
R indices (all data)	R1 = 0.0413, wR2 = 0.0788
Largest diff. peak and hole	0.386 and -0.181 e.$Å^{-3}$

Atomic Coordinates ($\times 10^4$) and Equivalent Displacement Parameters ($Å^2 \times 10^3$) for **2.60β**

	x	y	z	U(eq)
C(1)	7448(3)	3519(2)	7967(2)	18(1)
S(2)	6537(1)	1920(1)	9191(1)	31(1)
C(3)	5547(4)	985(2)	8049(2)	25(1)
C(4)	7236(3)	1420(2)	6706(2)	18(1)
C(5)	7433(3)	3110(2)	6594(2)	17(1)
C(6)	4857(3)	4264(2)	6596(2)	18(1)
C(7)	5334(3)	5001(2)	7681(2)	18(1)
C(8)	9967(4)	443(2)	6478(2)	20(1)
O(9)	11776(3)	888(2)	5672(1)	26(1)
O(10)	10145(3)	-934(2)	7200(2)	34(1)
C(11)	6675(4)	6403(2)	7190(2)	22(1)
O(12)	5026(3)	7632(2)	6527(2)	29(1)
O(13)	2984(2)	4371(2)	6107(1)	24(1)
Cl(14)	2514(1)	5418(1)	8902(1)	27(1)
H(1A)	9162	3771	8019	22
H(3A)	5843	-151	8325	31
H(3B)	3665	1353	8018	31
H(4A)	6303	1328	6009	22
H(5A)	8900	3438	5887	20
H(10)	11480(60)	-1340(40)	7060(30)	62(10)
H(11A)	7083	6744	7945	27
H(11B)	8345	6119	6580	27
H(12A)	5870(50)	8040(30)	6000(30)	40(8)

Bond Lengths (Å) for Cyclobutanone **2.60β**

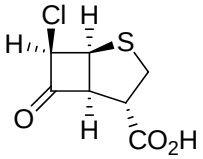
	Length [Å]		Length [Å]
C(1)-C(5)	1.566(3)	C(5)-H(5A)	1.0000
C(1)-C(7)	1.572(2)	C(6)-O(13)	1.198(2)
C(1)-S(2)	1.7974(19)	C(6)-C(7)	1.523(3)
C(1)-H(1A)	1.0000	C(7)-C(11)	1.521(3)
S(2)-C(3)	1.820(2)	C(7)-Cl(14)	1.7706(18)
C(3)-C(4)	1.524(3)	C(8)-O(9)	1.212(2)
C(3)-H(3A)	0.9900	C(8)-O(10)	1.310(2)
C(3)-H(3B)	0.9900	O(10)-H(10)	0.72(3)
C(4)-C(5)	1.521(3)	C(11)-O(12)	1.422(2)
C(4)-C(8)	1.523(2)	C(11)-H(11A)	0.9900
C(4)-H(4A)	1.0000	C(11)-H(11B)	0.9900
C(5)-C(6)	1.529(2)	O(12)-H(12A)	0.73(3)

Bond Angles (°) for cyclobutanone **2.60β**

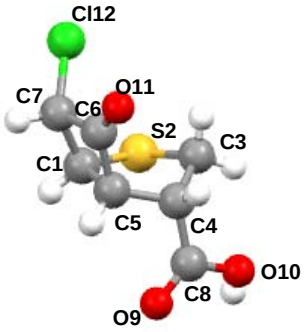
	Angle [°]		Angle[°]
C(5)-C(1)-C(7)	89.03(13)	C(6)-C(5)-H(5A)	113.3
C(5)-C(1)-S(2)	106.94(12)	C(1)-C(5)-H(5A)	113.3
C(7)-C(1)-S(2)	119.78(13)	O(13)-C(6)-C(7)	133.05(16)
C(5)-C(1)-H(1A)	112.8	O(13)-C(6)-C(5)	133.60(18)
C(7)-C(1)-H(1A)	112.8	C(7)-C(6)-C(5)	92.22(14)
S(2)-C(1)-H(1A)	112.8	C(11)-C(7)-C(6)	114.60(15)
C(1)-S(2)-C(3)	94.59(9)	C(11)-C(7)-C(1)	109.40(14)
C(4)-C(3)-S(2)	106.09(13)	C(6)-C(7)-C(1)	87.00(13)
C(4)-C(3)-H(3A)	110.5	C(11)-C(7)-Cl(14)	110.57(13)
S(2)-C(3)-H(3A)	110.5	C(6)-C(7)-Cl(14)	113.96(12)
C(4)-C(3)-H(3B)	110.5	C(1)-C(7)-Cl(14)	119.63(13)
S(2)-C(3)-H(3B)	110.5	O(9)-C(8)-O(10)	123.50(17)
H(3A)-C(3)-H(3B)	108.7	O(9)-C(8)-C(4)	122.71(17)
C(5)-C(4)-C(8)	110.04(14)	O(10)-C(8)-C(4)	113.75(16)
C(5)-C(4)-C(3)	106.42(15)	C(8)-O(10)-H(10)	109(3)
C(8)-C(4)-C(3)	114.91(16)	O(12)-C(11)-C(7)	110.39(15)
C(5)-C(4)-H(4A)	108.4	O(12)-C(11)-H(11A)	109.6
C(8)-C(4)-H(4A)	108.4	C(7)-C(11)-H(11A)	109.6
C(3)-C(4)-H(4A)	108.4	O(12)-C(11)-H(11B)	109.6
C(4)-C(5)-C(6)	115.89(14)	C(7)-C(11)-H(11B)	109.6
C(4)-C(5)-C(1)	111.30(15)	H(11A)-C(11)-H(11B)	108.1
C(6)-C(5)-C(1)	87.03(13)	C(11)-O(12)-H(12A)	107(2)
C(4)-C(5)-H(5A)	113.3		

Appendix B

Crystal Data and Structure Refinement for Cyclobutanone 2.58β



2.58β



Empirical formula	C7H7ClO3S
Formula weight	206.64
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions Å	a = 5.93510(10) Å, b = 21.2896(4) Å, c = 7.16860(10)
	α = 90°, β = 107.9793(10)°, γ = 90°
Volume, Z	861.56(3) $Å^3$, 4
Density (calculated)	1.593 Mg/m^3
Absorption coefficient	0.646 mm^{-1}
F(000)	424
Crystal size	0.170 × 0.080 × 0.020 mm^3
Theta range for data collection	1.913 to 27.999°
Index ranges	-7<=h<=7, -28<=k<=27, -9<=l<=8
Reflections collected	8836
Independent reflections	2075 [R(int) = 0.0205]
Completeness to theta = 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7460 and 0.7059
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2075 / 0 / 110
Goodness-of-fit on F^2	1.207
Final R indices [I>2sigma(I)]	R1 = 0.0463, wR2 = 0.1002
R indices (all data)	R1 = 0.0685, wR2 = 0.1105
Largest diff. peak and hole	0.390 and -0.351 e.$Å^{-3}$

Atomic Coordinates ($\times 10^4$) and Equivalent Displacement Parameters ($Å^2 \times 10^3$) for **2.58β**

	x	y	z	U(eq)
C(1)	4967(4)	5984(1)	6130(4)	44(1)
S(2)	7033(1)	5725(1)	4911(1)	46(1)
C(3)	5789(4)	6233(1)	2817(4)	40(1)
C(4)	3137(4)	6252(1)	2533(3)	37(1)
C(5)	2885(4)	6313(1)	4567(4)	40(1)
C(6)	3789(4)	6936(1)	5562(4)	42(1)
C(7)	5473(5)	6604(1)	7326(4)	51(1)
C(8)	1810(4)	5690(1)	1441(3)	38(1)
O(9)	99(3)	5471(1)	1803(3)	54(1)
O(10)	2546(3)	5489(1)	20(3)	63(1)
O(11)	3502(4)	7466(1)	5058(3)	60(1)
Cl(12)	8344(2)	6916(1)	8202(1)	88(1)
H(1A)	4439	5643	6817	53
H(3A)	6467	6651	3072	49
H(3B)	6095	6066	1660	49
H(4A)	2482	6631	1787	44
H(5A)	1317	6195	4636	48
H(7A)	4766	6585	8393	61
H(10A)	2669	5105	81	95

Bond Lengths (Å) for Cyclobutanone **2.58β**

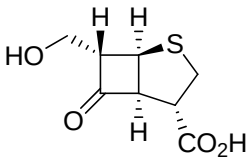
	Length [Å]		Length [Å]
C(1)-C(7)	1.553(4)	C(4)-H(4A)	0.9800
C(1)-C(5)	1.555(4)	C(5)-C(6)	1.523(3)
C(1)-S(2)	1.799(3)	C(5)-H(5A)	0.9800
C(1)-H(1A)	0.9800	C(6)-O(11)	1.180(3)
S(2)-C(3)	1.813(2)	C(6)-C(7)	1.522(4)
C(3)-C(4)	1.524(3)	C(7)-Cl(12)	1.755(3)
C(3)-H(3A)	0.9700	C(7)-H(7A)	0.9800
C(3)-H(3B)	0.9700	C(8)-O(9)	1.216(3)
C(4)-C(8)	1.510(3)	C(8)-O(10)	1.299(3)
C(4)-C(5)	1.517(3)	O(10)-H(10A)	0.8200

Bond Angles (°) for cyclobutanone **2.58β**

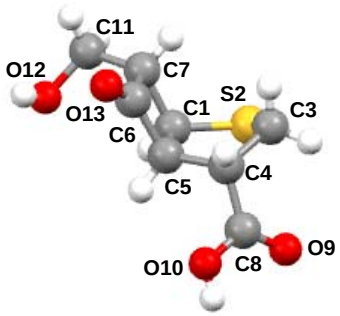
	Angle [°]		Angle[°]
C(7)-C(1)-C(5)	89.40(19)	C(4)-C(5)-C(6)	113.6(2)
C(7)-C(1)-S(2)	118.98(18)	C(4)-C(5)-C(1)	111.18(19)
C(5)-C(1)-S(2)	107.30(16)	C(6)-C(5)-C(1)	88.20(18)
C(7)-C(1)-H(1A)	112.9	C(4)-C(5)-H(5A)	113.8
C(5)-C(1)-H(1A)	112.9	C(6)-C(5)-H(5A)	113.8
S(2)-C(1)-H(1A)	112.9	C(1)-C(5)-H(5A)	113.8
C(1)-S(2)-C(3)	93.13(11)	O(11)-C(6)-C(7)	133.9(2)
C(4)-C(3)-S(2)	105.41(15)	O(11)-C(6)-C(5)	133.8(2)
C(4)-C(3)-H(3A)	110.7	C(7)-C(6)-C(5)	91.7(2)
S(2)-C(3)-H(3A)	110.7	C(6)-C(7)-C(1)	88.34(19)
C(4)-C(3)-H(3B)	110.7	C(6)-C(7)-Cl(12)	115.95(19)
S(2)-C(3)-H(3B)	110.7	C(1)-C(7)-Cl(12)	121.4(2)
H(3A)-C(3)-H(3B)	108.8	C(6)-C(7)-H(7A)	109.8
C(8)-C(4)-C(5)	112.10(19)	C(1)-C(7)-H(7A)	109.8
C(8)-C(4)-C(3)	113.64(19)	Cl(12)-C(7)-H(7A)	109.8
C(5)-C(4)-C(3)	106.16(19)	O(9)-C(8)-O(10)	123.0(2)
C(8)-C(4)-H(4A)	108.3	O(9)-C(8)-C(4)	122.0(2)
C(5)-C(4)-H(4A)	108.3	O(10)-C(8)-C(4)	114.9(2)
C(3)-C(4)-H(4A)	108.3	C(8)-O(10)-H(10A)	109.5

Appendix C

Crystal Data and Structure Refinement for Cyclobutanone 3.50α



3.50α



Empirical formula	C8H10O4S
Formula weight	202.22
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 5.5026(2) Å, b = 12.7899(6) Å, c = 12.3981(10) Å α = 90°, β = 99.047(2)°, γ = 90°
Volume, Z	861.70(6) $Å^3$, 4
Density (calculated)	1.559 Mg/m^3
Absorption coefficient	0.353 mm^{-1}
F(000)	424
Crystal size	0.314 × 0.160 × 0.060 mm^3
Theta range for data collection	2.303 to 27.986°
Index ranges	-7<=h<=7, -28<=k<=27, -9<=l<=8
Reflections collected	8652
Independent reflections	2085 [R(int) = 0.0151]
Completeness to theta = 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	N/A
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2085 / 0 / 126
Goodness-of-fit on F^2	1.081
Final R indices [I>2sigma(I)]	R1 = 0.0306, wR2 = 0.0714
R indices (all data)	R1 = 0.0360, wR2 = 0.0751
Largest diff. peak and hole	0.292 and -0.221 $e.Å^{-3}$

Atomic Coordinates ($\times 10^4$) and Equivalent Displacement Parameters ($Å^2 \times 10^3$) for **3.50α**

	x	y	z	U(eq)
C(1)	3346(2)	6338(1)	3925(1)	28(1)
S(2)	4830(1)	6353(1)	5329(1)	40(1)
C(3)	3421(3)	7591(1)	5590(1)	34(1)
C(4)	3267(2)	8235(1)	4550(1)	25(1)
C(5)	2646(2)	7485(1)	3577(1)	24(1)
C(6)	-15(2)	7113(1)	3292(1)	28(1)
C(7)	591(2)	6003(1)	3690(1)	29(1)
C(8)	5624(2)	8833(1)	4480(1)	29(1)
O(9)	7450(2)	8806(1)	5159(1)	47(1)
O(10)	5432(2)	9404(1)	3592(1)	41(1)
C(11)	-138(3)	5143(1)	2868(1)	37(1)
O(12)	701(2)	5376(1)	1859(1)	42(1)
O(13)	-1854(2)	7522(1)	2842(1)	46(1)
H(1A)	4345	5997	3439	33
H(3A)	1790	7477	5772	41
H(3B)	4409	7950	6195	41
H(4A)	1919	8739	4531	31
H(5A)	3345	7709	2934	29
H(7A)	-46	5864	4371	35
H(10)	6730(40)	9690(17)	3522(17)	63(6)
H(11A)	570	4486	3154	45
H(11B)	-1915	5069	2741	45
H(12)	-250(40)	5611(17)	1463(17)	57(6)

Bond Lengths (Å) for Cyclobutanone **3.50α**

	Length [Å]		Length [Å]
C(1)-C(7)	1.5583(18)	C(5)-H(5A)	0.9800
C(1)-C(5)	1.5602(18)	C(6)-O(13)	1.1969(16)
C(1)-S(2))	1.8031(13)	C(6)-C(7)	1.5228(19)
C(1)-H(1A)	0.9800	C(7)-C(11)	1.5098(19)
S(2)-C(3)	1.8142(15)	C(7)-H(7A)	0.9800
C(3)-C(4)	1.5222(19)	C(8)-O(9)	1.2059(17)
C(3)-H(3A)	0.9700	C(8)-O(10)	1.3112(17)
C(3)-H(3B)	0.9700	O(10)-H(10)	0.82(2)
C(4)-C(8)	1.5193(18)	C(11)-O(12)	1.431(2)
C(4)-C(5)	1.5372(17)	C(11)-H(11A)	0.9700
C(4)-H(4A)	0.9800	C(11)-H(11B)	0.9700
C(5)-C(6)	1.5273(17)	O(12)-H(12)	0.72(2)

Bond Angles (°) for cyclobutanone **3.50α**

	Angle [°]		Angle[°]
C(7)-C(1)-C(5)	90.95(9)	C(4)-C(5)-H(5A)	112.8
C(7)-C(1)-S(2))	117.61(10)	C(1)-C(5)-H(5A)	112.8
C(5)-C(1)-S(2)	108.19(8)	O(13)-C(6)-C(7)	133.26(13)
C(7)-C(1)-H(1A)	112.7	O(13)-C(6)-C(5)	132.91(14)
C(5)-C(1)-H(1A)	112.7	C(7)-C(6)-C(5)	93.59(10)
S(2)-C(1)-H(1A)	112.7	C(11)-C(7)-C(6)	116.03(11)
C(1)-S(2)-C(3)	92.28(6)	C(11)-C(7)-C(1)	118.32(12)
C(4)-C(3)-S(2)	106.85(9)	C(6)-C(7)-C(1)	87.76(10)
C(4)-C(3)-H(3A)	110.4	C(11)-C(7)-H(7A)	110.9
S(2)-C(3)-H(3A)	110.4	C(6)-C(7)-H(7A)	110.9
C(4)-C(3)-H(3B)	110.4	C(1)-C(7)-H(7A)	110.9
S(2)-C(3)-H(3B)	110.4	O(9)-C(8)-O(10)	123.40(14)
H(3A)-C(3)-H(3B)	108.6	O(9)-C(8)-C(4)	124.80(13)
C(8)-C(4)-C(3)	112.75(11)	O(10)-C(8)-C(4)	111.79(11)
C(8)-C(4)-C(5)	110.85(10)	C(8)-O(10)-H(10)	112.4(15)
C(3)-C(4)-C(5)	107.63(11)	O(12)-C(11)-C(7)	110.51(12)
C(8)-C(4)-H(4A)	108.5	O(12)-C(11)-H(11A)	109.5
C(3)-C(4)-H(4A)	108.5	C(7)-C(11)-H(11A)	109.5
C(5)-C(4)-H(4A)	108.5	O(12)-C(11)-H(11B)	109.5
C(6)-C(5)-C(4)	117.58(10)	C(7)-C(11)-H(11B)	109.5
C(6)-C(5)-C(1)	87.53(10)	H(11A)-C(11)-H(11B)	108.1
C(4)-C(5)-C(1)	110.78(10)	C(11)-O(12)-H(12)	112.5(17)
C(6)-C(5)-H(5A)	112.8		

Appendix D

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Title:	Cyclobutanone Analogues of β-Lactams Revisited: Insights into Conformational Requirements for Inhibition of Serine- and Metallo-β-Lactamases
Author:	Jarrod W. Johnson, Michael Gretes, Valerie J. Goodfellow, et al
Publication:	Journal of the American Chemical Society
Publisher:	American Chemical Society
Date:	Mar 1, 2010

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Title:	Carbapenems: Past, Present, and Future
Author:	Krisztina M. Papp-Wallace, Andrea Endimiani, Magdalena A. Taracila et al.
Publication:	Antimicrobial Agents and Chemotherapy
Publisher:	American Society for Microbiology
Date:	Nov 1, 2011

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Appendix F

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Title:	The Basis for Carbapenem Hydrolysis by Class A β-Lactamases: A Combined Investigation using Crystallography and Simulations
Author:	Fátima Fonseca, Ewa I. Chudyk, Marc W. van der Kamp, et al
Publication:	Journal of the American Chemical Society
Publisher:	American Chemical Society
Date:	Nov 1, 2012

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