Cyclobutanone Analogues of β-Lactam Antibiotics as Inhibitors of Serine- and Metallo-β-Lactamases

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.

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Abstract

Bacterial resistance to antibiotics is an emerging epidemic throughout the world and there is a desperate need for new antibiotics and new strategies to maintain the effectiveness of current agents. β-Lactams, such as the penicillins and cephalosporins, have been the most important class of antibiotic for several decades and represent half of the global antibacterial market, but the continued use of β-lactams is threatened by β-lactamases, enzymes that efficiently inactivate β-lactams through hydrolysis. Class A, C, and D β-lactamases use an active-site serine residue for hydrolysis and achieve turnover through an acylenzyme intermediate while the class B metallo-β-lactamases (MBLs) use a zinc-bound hydroxide as the active-site nucleophile.

Two successful approaches to combat β-lactamase-mediated resistance have involved the development of β-lactam antibiotics which bind poorly to β-lactamases and the combination of β-lactams with β-lactamase inhibitors. These strategies have been effective for overcoming resistance due to class A β-lactamases, but the ever-increasing prevalence of extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases, and carbapenemases compromises the effectiveness of current penicillins, cephalosporins, carbapenems, and mechanism-based β-lactamase inhibitors.

Cyclobutanone analogues of β-lactam antibiotics were explored in the early 1980s as potential inhibitors of β-lactamases and D-Ala-D-Ala transpeptidases, but simple analogues showed only weak inhibitory activity and this approach was subsequently abandoned. The increasing threat of multidrug-resistant β-lactamase-producing organisms in recent years, however, has inspired a re-evaluation of these inhibitors since cyclobutanones have the potential to exhibit broad-spectrum inhibition of both serine- and metallo-β-lactamases through the formation of enzyme-bound hemiketals or hydrates.

7,7-Dichloro-2-thia-bicyclo[3.2.0]heptan-6-one-4-carboxylic acid (**65**), a dichlorocyclobutanone that had shown modest inhibition of the class B and D β-lactamases IMP-1 and OXA-10 in earlier work in this laboratory, was prepared in an efficient seven-step sequence from triethyl phosphonoacetate (**103**) with an overall yield of 28%. Initial efforts to improve upon the potency of the cyclobutanones involved functionalization at C3 and a highly stereoselective chlorination with sulfuryl chloride provided the 3α-chloro derivative **117α** in nearly quantitative yield. Elimination of HCl from **117α** was achieved under a variety of conditions and 3-alkoxy derivatives were prepared

from **117α** through diastereoselective substitution reactions with alcohols. Cyclobutanones with 3β-OR substituents were found to favour an *endo* envelope conformation while the 3α-OR derivatives adopt the *exo* envelope conformation. Evidence from X-ray crystal structures and ab initio molecular orbital calculations suggests that an anomeric effect contributes to the large conformational preference of the tetrahydrothiophene ring that favours the 3-alkoxy substituent in an axial orientation. In addition, the conformation of the bicyclic system was found to have a dramatic effect on the tendency of the cyclobutanone to undergo hemiketal formation.

Cyclobutanone analogues of penicillins, including 3-alkoxy derivatives, and cyclobutanone analogues of penems were evaluated against class A, B, C, and D β-lactamases and found to be moderate inhibitors of KPC-2, IMP-1, GC1, and OXA-10. The cyclobutanones found to be most potent were those which are hydrated to a larger extent in aqueous solution. Dichlorocyclobutanones were found to be better inhibitors than dechlorinated cyclobutanones and a 3α-methoxy derivative **152α**, which favours the *exo* envelope conformation in which the C4 carboxylate is equatorial, was found to be a better inhibitor than cyclobutanones that favour the *endo* envelope conformation. A 3,4-unsaturated penem analogue, **153**, showed comparable potency to that of **152α** and molecular models of enzyme–inhibitor complexes indicate that an equatorial carboxylate is required for binding to β-lactamases. An X-ray crystal structure of **152α** bound to the class D β-lactamase OXA-10 confirms that a serine hemiketal is formed in the active site and that the inhibitor adopts the *exo* envelope.

The biochemical data described above demonstrate that cyclobutanones can indeed act as inhibitors of serine- and metallo-β-lactamases and these cyclobutanones represent the first class of reversible inhibitors to show moderate inhibition of all four classes of β-lactamase. Although the inhibitory potency of these compounds is modest (low micromolar IC_{50} values), penem analogue **153** was able to enhance the potency of meropenem against carbapenem-resistant MBL-producing clinical isolates of *Chryseobacterium meningosepticum* and *Stenotrophomonas maltophilia* and the synergy demonstrated in these antimicrobial assays is encouraging.

Synthetic studies toward other C3-alkyl and C3-thioalkyl-substituted inhibitors are described and the design and synthesis of C7-monochloro- and 7α-hydroxymethyl-7β-chloro cyclobutanone derivatives is presented.

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Dedicated to my parents, my wife, and my son

Table of Contents

List of Figures

List of Tables

List of Schemes

List of Abbreviations

Å	angstrom(s)
Ac	acetyl
7-ACA	7-aminocephalosporanic acid
Ala	alanine
All	allyl
Alloc	allyloxycarbonyl
6-APA	6β-aminopenicillanic acid
app	apparent
aq	aqueous
Ar	aryl
Arg	arginine
Asn	asparagine
Asp	aspartic acid
bla	β-lactamase gene
Bn	benzyl
Boc	*tert*-butoxycarbonyl
bp	boiling point
br	broad (spectral)
Bu	butyl
Bz	benzoyl
B3LYP	3-parameter hybrid Becke exchange/Lee–Yang–Parr correlation functional
°C	degrees Celsius
calcd	calculated
CCDC	Cambridge Crystallographic Data Centre
CHDL	carbapenem-hydrolyzing class D β-lactamase
c-Hex	cyclohexyl
CI	chemical ionization
CIF	crystallographic information file
COSY	correlation spectroscopy
CSD	Cambridge Structural Database
Cys	cysteine
δ	chemical shift in parts per million downfield from tetramethylsilane
d	day(s); doublet (spectral)
Da	dalton
DAP	diaminopimelic acid
DCC	dicyclohexyl carbodiimide
dec	decomposition
DFT	density functional theory
DHP-I	renal dehydropeptidase I
DIC	diisopropyl carbodiimide
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
dr	diastereomeric ratio
E1	first-order elimination
E2	second-order elimination
EDTA	ethylenediaminetetraacetic acid
EI	electron impact
equiv	equivalent(s)
ESBL	extended-spectrum β-lactamase
ESI	electrospray ionization
Et	ethyl
g	gram(s)
gem	geminal
Glu	glutamic acid
Gln	glutamine
Gly	glycine
h	hour(s)
HEPES	*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid

HF	Hartree–Fock
His	histidine
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HRMS	high-resolution mass spectrum
HWE	Horner–Wadsworth–Emmons olefination
IC_{50}	concentration of inhibitor that reduces the maximum rate of enzymatic activity by 50%
Ile	isoleucine
IPNS	isopenicillin N synthase
i-Pr	isopropyl
IR	infrared
J	coupling constant (NMR)
JMOD	*J*-modulated (spectral)
Kcx	carboxylated lysine
kDa	kilodalton
K_i	inhibition constant
K_m	Michaelis constant
L	liter(s)
Leu	leucine
LRMS	low-resolution mass spectrometry
Lys	lysine
μ	micro
m	multiplet (spectral); meter(s); milli
M	molar
M^+	molecular ion
MBL	metallo-β-lactamase
Me	methyl
Mes	mesityl (2,4,6-trimethylphenyl)
MES	2-(*N*-morpholino)ethanesulfonic acid
Met	methionine
MIC	minimum inhibitory concentration
MIC_{90}	concentration of inhibitor that inhibits the growth of 90% of the bacterial strains tested
min	minute(s)
mol %	mole percent
mp	melting point
MRSA	methicillin-resistant *Staphylococcus aureus*
Ms	mesyl (methanesulfonyl)
MS	mass spectrometry, molecular sieves
m/*z*	mass-to-charge ratio
na	not available
NAG	*N*-acetyl glucosamine
NAM	*N*-acetyl muramic acid
NBA	*N*-bromoacetamide
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
nd	not determined
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser enhancement spectroscopy
obsd	observed
PAS	penicillanic acid sulfone
PBP	penicillin-binding protein
PDB	Brookhaven protein data bank (www.pdb.org/)
Ph	phenyl

Phe	phenylalanine
Phth	phthalyl
PNB	*para*-nitrobenzyl
ppm	parts per million (spectral)
Pro	proline
p-Tol	*para*-toluyl, 4-methylphenyl
Py	pyridine
q	quartet (spectral)
QM/MM	quantum mechanical/molecular mechanical
RHF	restricted Hartree–Fock
RMSD	root-mean-square deviation
rt	room temperature
s	singlet (spectral); second(s)
SAR	structure–activity relationship(s)
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Ser	serine
S_N1	unimolecular nucleophilic substitution
S_N2	bimolecular nucleophilic substitution
t	triplet (spectral)
t-Bu	*tert*-butyl
TBS	*tert*-butyldimethylsilyl
TCE	trichloroethyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFE	trifluoroethyl
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
TMS	trimethylsilyl; tetramethylsilane (spectral)
Trp	tryptophan
Ts	tosyl (toluenesulfonyl)
TS	transition state
Tyr	tyrosine
Val	valine
V_{max}	the maximum rate attainable for an enzymatic reaction
VRE	vancomycin-resistant enterococci
VRSA	vancomycin-resistant *S. aureus*
WT	wild type (enzyme)

Chapter 1
Introduction

1.1 Antibiotics and Bacterial Resistance

The development of antibiotics is perhaps the most important medical advance of the twentieth century and, since their introduction into clinical use prior to World War II, antibiotics have been used to save countless human lives.

The effectiveness of the sulfonamides and penicillin in the 1930s and 1940s for the treatment of once deadly infections triggered decades of extensive research toward other antibiotics. These efforts were rewarded with the discovery of a variety of different classes of antimicrobial agents such as the aminoglycosides, polyketides, cephalosporins, tetracyclines, macrolides, glycopeptides, streptogramins, carbapenems, quinolones, and oxazolidinones (Figure 1).[1]

The widespread use of antibiotics, however, has proven to be a major driving force for the evolution of bacteria,[2] and the enormous benefits of antibiotics to human health are necessarily coupled with the development of resistance.[3] Without exception, resistance has developed to every major class of antibiotic, both natural and synthetic, within only a few years of its introduction and it is now widely recognized that bacterial resistance to antibiotics is an inevitable consequence of antibiotic use.[4,5,6]

Unfortunately, the success of the pharmaceutical industry in the development of large numbers of antibiotics caused complacency toward antibiotic resistance and, with increasing costs for antimicrobial development, most major pharmaceutical companies withdrew from the area in the 1980s and 1990s.[7,8] As a result, the antibiotic pipeline is now ill-equipped to address the relentless increase in bacterial resistance and the current situation has been described as a "perfect storm".[9]

Antibiotic resistance was recognized as a crisis as early as 1992 with the emergence of methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), and vancomycin-resistant *Enterococcus* spp. (VRE).[10] In recent years, the list of most threatening pathogens has grown to include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter*

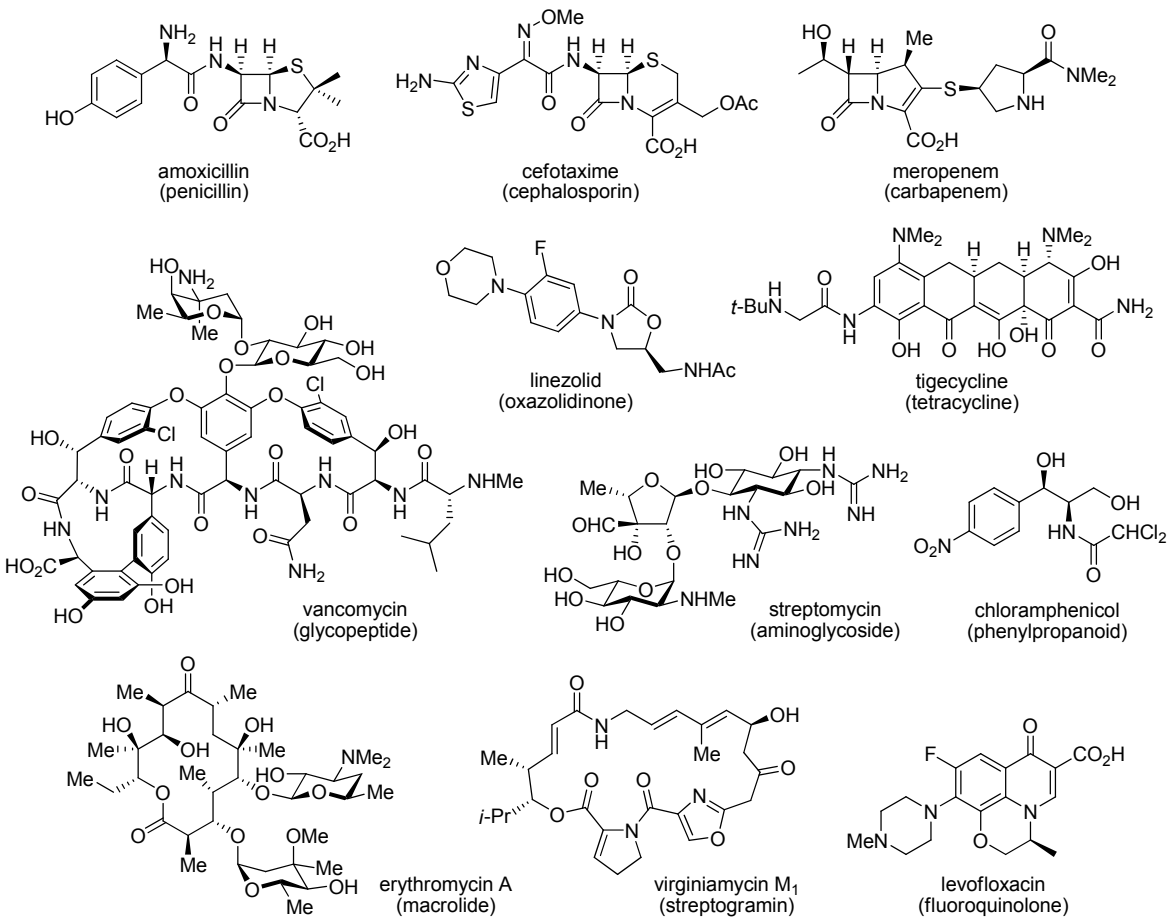


Figure 1. Representative structures of several classes of clinically used antibiotics.

baumannii, *Pseudomonas aeruginosa*, and *Enterobacter* spp., and these have been collectively dubbed the ESKAPE pathogens.[11,12] The increasing prevalence of multidrug-resistant and pan-resistant strains is also extremely concerning and has elevated fears that the era of untreatable infections has arrived and the antibiotic era is about to end.[13,14] It is clear that there is an urgent need for new antibiotics and new strategies to maintain the effectiveness of known antibiotics.[15,16,17,18]

β-Lactams are the most important antibiotics and penicillins and cephalosporins represent half of the $42 billion global antibiotic market.[19,20] In addition to obvious economic motivations, continuing efforts to preserve the usefulness of the β-lactams are justified by the unparalleled efficacy and safety of these agents.[21] Furthermore, the β-lactams are active against Gram-positive and Gram-negative bacteria and target bacterial cell wall biosynthesis, a process that is uniquely prokaryotic and more easily accessed than the intracellular targets of other antibiotics.[22,23]

Mechanisms of resistance to antibiotics include target alteration, reduced accumulation owing to

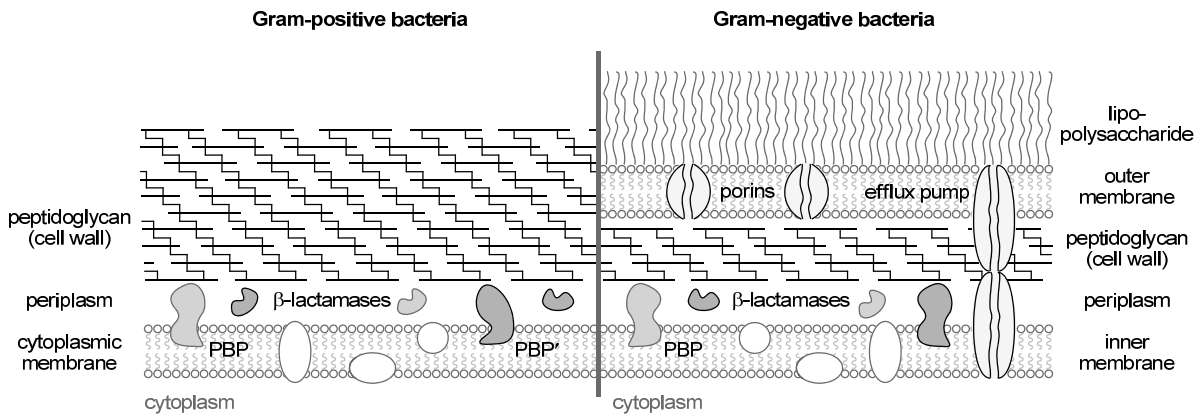


Figure 2. Comparison of Gram-positive and Gram-negative bacterial cell walls.[1,19,21]

decreased permeability or efflux,[24] and drug inactivation or modification. With respect to β-lactams, the deletion of porin proteins in the membrane, the acquisition of efflux exporter proteins, and the expression of β-lactam-sensing proteins are notable defensive measures, but penicillin-binding protein (PBP) alterations and the expression of β-lactamases are the most important mechanisms of resistance (Figure 2).[25,26]

β-Lactamases, enzymes which hydrolytically inactivate β-lactams, were first encountered by Abraham and Chain in 1940,[27] even before the widespread use of penicillin. Though not apparent at the time, β-lactamases would prove to be the largest driving force for the development of new generations of penicillins and cephalosporins in the decades to follow.[28] Initially, the most successful strategy for overcoming β-lactam resistance was to modify the sidechains of penicillins and cephalosporins in order to improve stability to β-lactamases. Another approach involves the combination of the β-lactam antibiotic with a β-lactamase inhibitor which protects the β-lactam from deactivation by β-lactamases. Such combinations have been used clinically since the mid-1980s and mechanism-based inhibitors, such as clavulanic acid, have been very successful against serine β-lactamases. However, new β-lactamases are emerging at an alarming rate (more than 850 are now known)[29] and new enzymes, such as extended-spectrum β-lactamases (ESBLs), carbapenemases, and metallo-β-lactamases (MBLs), compromise the effectiveness of current penicillins, cephalosporins, carbapenems, and mechanism-based inhibitors.

The metallo-β-lactamases are among the most threatening of the new β-lactamases since they have a spectrum of activity encompassing all β-lactam classes (except monobactams) and there are no

MBL inhibitors available for clinical use at present. As a result, there has been a longstanding interest in the Dmitrienko laboratory in the development of MBL inhibitors and this thesis describes efforts by this author in the design and synthesis of cyclobutanone analogues of β-lactams which have the potential to act as inhibitors of both serine- and metallo-β-lactamases.

This introductory chapter provides a summary of the development of β-lactam antibiotics, a brief description of penicillin-binding proteins, and a review of β-lactamases and β-lactamase inhibitors as essential context for the present study. While a comprehensive review of these topics is well beyond the scope of this thesis, efforts have been made to provide an accurate and appropriate summary of the 80 years of research in the field in the following sections of this chapter. This introduction has been written from an historical perspective with an intent to highlight the most important classical contributions to the field.

It should be noted that the evolution of the penicillins, cephalosporins, carbapenems, and other β-lactams has always been intimately interconnected with the evolution of penicillin-binding proteins (PBPs), β-lactamases, and β-lactamase inhibitors and the remainder of this chapter represents an attempt to provide a chronological yet cohesive summary of each area. The discovery and development of β-lactam antibiotics are presented in Section 1.2, followed by a discussion of penicillin-binding proteins and the β-lactam mode of action in Section 1.3. β-Lactamases and β-lactamase inhibitors are reviewed in Sections 1.4 and 1.5, respectively.

1.2 β-Lactam Antibiotics

With the prominent role that the penicillins and cephalosporins have played in the history of modern medicine, and the advancement of other antibiotics, it seems appropriate to mention some of the most important events in their early development. The following sections describe the fascinating story of the discovery of penicillin and its curative effect in infectious diseases (for which Sir Alexander Fleming, Ernst Chain, and Sir Howard Florey were awarded the Nobel Prize in medicine in 1945), the monumental collaborative effort between American and British scientists to elucidate the structure of penicillin and complete its total synthesis, the discovery of the cephalosporins, and the development of other semisynthetic penicillins and cephalosporins. In an effort to maintain historical accuracy, the

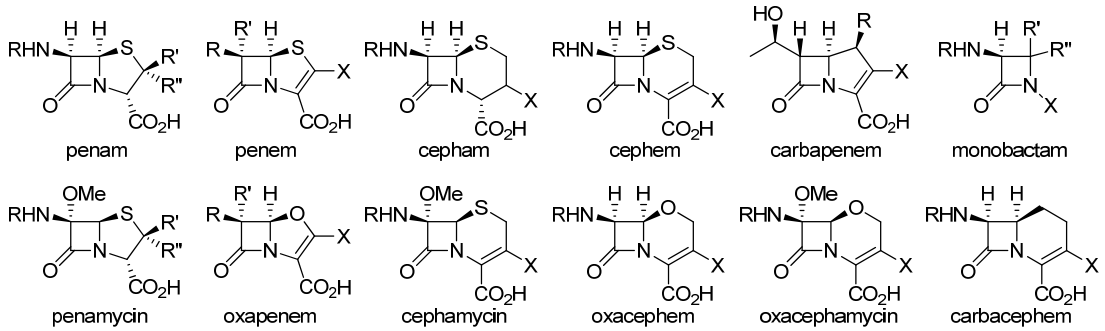


Figure 3. Families of β-lactam antibiotics.

discussion provided below of the early years of penicillin and cephalosporin research is based on several first-hand accounts from scientists directly involved in its development.[30,31,32,33,34,35,36] The following sections also describe the discovery of the cephamycins, carbapenems, penems, monobactams, and other β-lactam families (Figure 3). Structures of selected penicillins, cephalosporins, carbapenems, and monobactams are provided in Appendix A.

1.2.1 Penicillins

While Sir Alexander Fleming's name is widely recognized for the discovery of penicillin, the scientific contributions of those at Oxford and elsewhere in England and the United States are much less appreciated.[37,38] In reality, penicillin may have been forgotten if it were not for the efforts of Florey and Chain.

Alexander Fleming worked as a bacteriologist in a basement laboratory at St. Mary's hospital in London. On September 28, 1928 Fleming examined a Petri dish of *Staphylococcus* that had been left on the bench for several days and noticed that a contaminant mould, later identified as *Penicillium notatum*, had developed in a corner of it. In a fairly large circumference around the mould were 'ghosts' of the colonies of *Staphylococcus* and it was obvious to Fleming, a trained and experienced observer, that the colonies had undergone lysis under the influence of the mould. This was a striking observation as staphylococci were known to be notoriously resistant to lysis and Fleming already had a special interest in lysis through his discovery of lysozyme in 1922, six years earlier.[39]

Fleming was able to subculture the mould[40] and found that a substance was produced that passed into the liquid. This solution, which he called 'penicillin',[41] had potent antibacterial activities against many different microbes, including human pathogens,[42] and was no more toxic to mice, rabbits, or

leukocytes than the broth itself. These results were published in 1929[43] with suggestions that it could be used as an antiseptic for application to, or injection into, areas infected with penicillin-sensitive microbes. Fleming knew that more serious clinical trials would demand a large amount of active penicillin and that purification of the unstable substance would require considerable effort.[30a] It is widely believed that Fleming had lost interest in penicillin because he did not publish additional research in this area, but Wainright has argued that Fleming did continue research with penicillin throughout the 1930s.[44]

A group of natural product chemists from London reported their attempts to purify penicillin in 1932 and they had found, just as Fleming did, that the active substance was most stable between pH 5 and 6.[45] Extraction of the broth at pH 2 was much more efficient than extraction at neutral or alkaline pH, but the extracted material was considered too labile upon removal of the ether and their efforts were discontinued.

In 1935 a biochemist named Ernst Chain joined the staff of Professor Howard Florey, the newly appointed head of the Sir William Dunn School of Pathology at the University of Oxford. Chain began research into the mode of action of lysozyme and in 1938 found evidence that it acted on the bacterial cell wall with carbohydrase activity. In a search of the literature for other bacteriolytic substances, Chain came across Fleming's penicillin paper and considered the possibility that penicillin was an enzyme that had a similar carbohydrase mode of action to that of lysozyme. Chain and Florey then began their work with the *Penicillium* mould with their new colleague Norman Heatley.[31] They quickly found that penicillin was not an enzyme, but their disappointment was converted to optimism when they found that a concentrated form of the penicillin (solid but non-homogeneous) was nontoxic to mice in "enormous" doses of 10 mg. They published these results and the effectiveness of this form of penicillin in vivo against various pathogenic organisms in 1940.[46] Later that year, Abraham and Chain reported that an enzyme from *Escherichia coli*, which they called penicillinase, inactivates penicillin and that the growth of the mould and isolation of penicillin therefore require strictly sterile conditions.[27]

The group published a more refined method in 1941 for culturing the mould and for harvesting and purifying the penicillin with more precise antimicrobial activity results and the results of a small series of clinical trials with humans.[47] The new method for purification, developed by Heatley,[36]

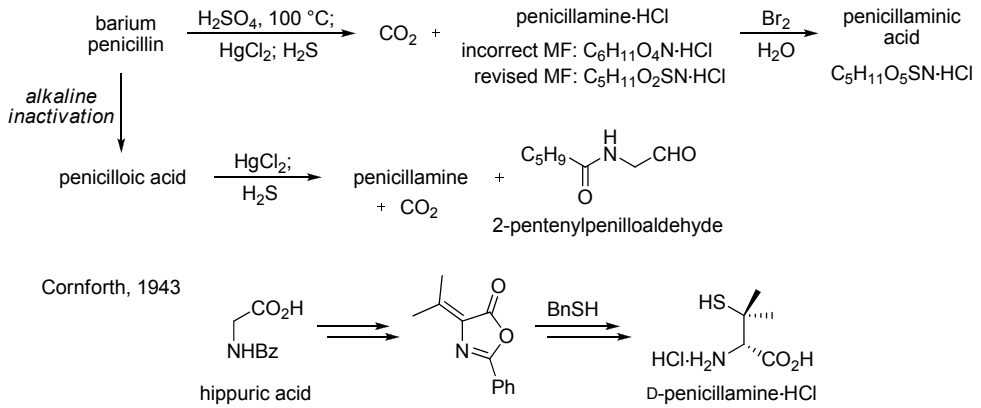
involved an extraction of the penicillin into amyl acetate, followed by a crude chromatographic purification with alumina and extraction of the penicillin back into aqueous solution at pH 6–7. In the first extraction, penicillin hydrolysis was minimized by passing the crude aqueous solution through a cooling coil and acidification to pH 2 with phosphoric acid just seconds before droplets of this solution were allowed to fall through a column of amyl acetate.

A major source of difficulty for the group was the small amount of material to work with for the structural elucidation. Cultures of the mould produced only 0.5 mg/mL and most of the pure penicillin was reserved for clinical trials. Since it was obvious that the tremendous therapeutic potential of penicillin could only be realized if its production were to be expanded beyond the means of the Oxford laboratory, pharmaceutical companies in the U.K. and the U.S. were asked to help with large-scale culture and production. Following the discovery of a deep fermentation technique in late 1941 by the U.S. Department of Agriculture (Peoria, IL), other academic and industrial laboratories in both England and the U.S. entered the collaborative effort. These included the Imperial College of Science, Imperial Chemical Industries, Wellcome, and Glaxo in England and included Harvard, Cornell, and Michigan universities and Merck, Squibb, Pfizer, Abbott, Eli Lilly, Upjohn, and Shell in the United States.[32]

The possibility that penicillin might be more easily obtained by chemical synthesis was considered and in 1942 Florey's group began to collaborate with Sir Robert Robinson and Dr. Wilson Baker of the Dyson Perrins Laboratory at Oxford. In the early stages of the collaborative chemical work at Oxford little was known about the penicillin structure except that it was a carboxylic acid, it was inactivated in weak acid and alkali to liberate new ionizable groups, and it was soluble in alcohols but readily inactivated in methanol.[48] It was most stable at pH 5–7 but was inactivated at neutral pH in the presence of heavy metal salts such as Zn^{2+}, Cu^{2+}, and Pb^{2+}. It was later found that the barium salt of penicillin was stable when dry or in organic solvents and was also highly active in vivo.[49]

The barium salt, one of the purest samples of penicillin available in 1942, was found to contain nitrogen, and the nitrogen content was correlated with antibiotic potency.[50] Analysis of this sample suggested a formula of $C_{24}H_{32}O_{10}N_2Ba$ and that sulfur was not present. This unfortunate analytical error caused confusion for several months and was not corrected until the structure of penicillamine was determined in 1943.

Scheme 1. Penicillamine and 2-Pentenylpenilloaldehyde as Degradation Products of Penicillin.



Degradation studies by Abraham in late 1942 showed that hot acid hydrolysis yielded a volatile acid and the crystalline salt of a base (called penicillamine) with the loss of CO_2 (Scheme 1).[51] Molecular weight determinations seemed to be consistent with a formula of $C_6H_{11}O_4N·HCl·H_2O$ but this formula was later found to be incorrect. The presence of sulfur was recognized after Abraham had found that penicillamine could be oxidized with bromine/water to a strongly acidic compound named penicillaminic acid, which had a molecular formula inconsistent with only C, H, N, and O. Finally, the puzzling results of earlier experiments involving penicillamine could be rationalized, and the molecular formula of penicillamine was revised to $C_5H_{11}O_2SN·HCl$. In October 1943 the structure of penicillamine was confirmed to be 3-mercapto-D-valine by John Cornforth through a synthesis from hippuric acid.[52,53]

While these advances were being made, Chain had found that hydrolysates of penicillin contained an ether-soluble compound that yielded a 2,4-dinitrophenylhydrazone in addition to the water-soluble penicillamine. They named this compound 2-pentenylpenilloaldehyde and its structure was later confirmed through synthesis.[32,36]

Meanwhile, early research in the United States had been mainly concerned with large-scale preparation of penicillin. Production had been developed to take advantage of deep-tank fermentation vessels and purification was optimized using silica gel chromatography. By 1942 Americans had also become actively involved in the chemical work and in August 1943 researchers at Squibb had successfully crystallized a sodium salt of their penicillin and found a molecular formula of $C_{16}H_{17}O_4N_2SNa$. When the Oxford group received this news, they immediately converted their

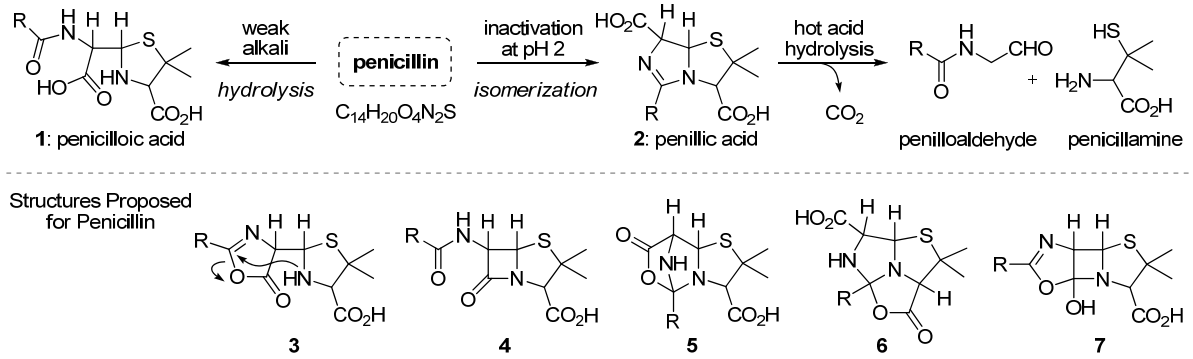


Figure 4. Structures proposed for the penicillins, penicilloic acids, and penillic acids (R = C_5H_9 or CH_2Ph).

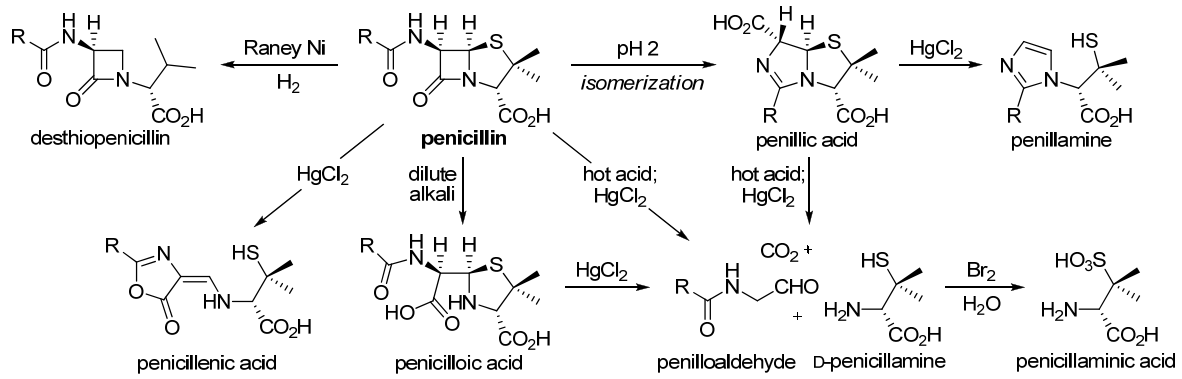
purified barium salt into the sodium salt (which they had avoided using due to its hygroscopic nature) and found that it had the formula $C_{14}H_{19}O_4N_2SNa$. It had become clear that the American penicillin was different than the British penicillin and it was later found to be due to the addition of corn steep liquor, which contained phenylacetic acid, to stimulate the growth of the *Penicillium* mould in the American fermentations. Oxford's Δ^2-pentenyl penicillin was then named penicillin F and the American benzyl penicillin was called penicillin G.[30b,32,36]

After the molecular formula for penicillin was determined and the structures of penicillamine and 2-pentenylpenilloaldehyde were confirmed, it seemed clear that penicilloic acid was a thiazolidine and that the carbon dioxide liberated upon acid-catalyzed hydrolysis came from a carboxyl group in the β-position to the formyl group of the penilloaldehyde. Structure **1** was correctly assigned to penicilloic acid, the hydrolysis product of penicillin, but it was unclear how a molecule of water had been added to the structure (Figure 4).

Robinson proposed a thiazolidine–oxazolone structure **3** for penicillin because it offered an explanation for the isomerization of penicillin to penillic acid at pH 2 (as indicated by arrows in Figure 4). Penillic acid, isolated in early 1943 by researchers at Wellcome, was known to have two acid groups and one basic group, and was correctly assigned the structure **2**.[54] Robinson's oxazolone structure appeared to have the support of the majority of those in the field until 1945.[36]

In late 1943 Abraham and Chain proposed a β-lactam structure **4** (which was independently put forward by Merck) but this ring system was not supported by Robinson and received little attention from other laboratories. The main difficulty in acceptance of the β-lactam structure was that penicillin was much more labile than all known β-lactams which, at that time, included only monocyclic β-

Scheme 2. Summary of Degradation Studies Used for the Structural Elucidation of the Penicillins.



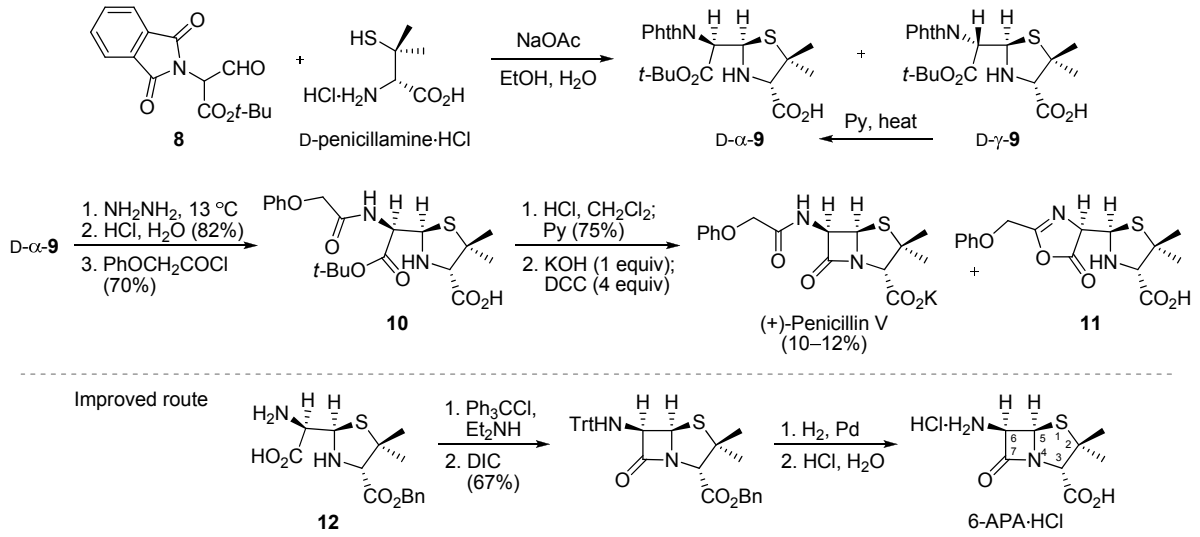
lactams.[36]

Other potential structures for penicillin were proposed by various groups, including the tricyclic structure **5** in 1944 independently by researchers at Eli Lilly and Glaxo, tri-cycle **6** by the Imperial College group, and azlactol **7** by Stodola of the U.S. Department of Agriculture. Each of these possessed a pre-formed core of penillic acid and seemed to give reasonable explanations for the formation of penillic acid at pH 2, but none of these structures was widely accepted.[32a]

A major criticism of the non-β-lactam structures and support for the β-lactam structure involved electrometric titrations done by Abraham in late 1943.[36,55] Each of the non-β-lactam structures contain a weakly basic group that could not be detected in the titration experiments and provided evidence against **5**, **6**, **7**, and Robinson's oxazolone **3**, and support for the β-lactam **4**. In addition, other evidence began to accumulate in favour of the β-lactam structure, including infrared spectroscopic studies that suggested the presence of a mono-substituted amide in penicillin, and some reasoning from Woodward regarding the labile nature of the penicillins. Woodward suggested in late 1944 that the penicillins could be less stable than monocyclic β-lactams because amide resonance would be suppressed by the non-planarity at the bridgehead nitrogen.[56] More convincing synthetic evidence provided support for the β-lactam structure when researchers at Merck treated penicillin with Raney nickel to provide desthiopenicillin, a β-lactam that behaved like other known monocyclic β-lactams (Scheme 2).[57]

Debate about the structure of penicillin continued until May 1945 when the structure was conclusively determined by Crowfoot and Low at Oxford using X-ray crystallographic analysis.[58] The β-lactam ring was shown clearly in electron density projections from crystals of the sodium,

Scheme 3. Sheehan's Total Synthesis of Penicillin V.[63,64,66]



potassium, and rubidium salts of penicillin.[59] For this and other structures of biochemical significance, Dorothy Crowfoot Hodgkin was awarded the Nobel Prize in chemistry in 1964.

Although the large-scale production of penicillin for the war effort through total synthesis was unsuccessful, fermentation methods had been become so effective by early 1944 that sufficient amounts of penicillin could be supplied to the allied soldiers for the D-Day invasion in June 1944.

At the height of the effort more than one thousand highly skilled chemists were involved in the collaborative project but research on the chemistry of penicillin had declined significantly after the war had ended.[60] Many of these researchers had come to the conclusion that the synthesis of penicillin was "an impossible problem" and even Woodward had described the molecule was "a diabolical concatenation of reactive groups".[61] Sheehan's view of the situation, however, was simply that the appropriate organic reactions for construction of the "enchanted ring" had not yet been discovered.[62] In 1957, more than a decade after Hodgkin's structure determination by X-ray crystallographic analysis, Sheehan published the first rational total synthesis of a penicillin (Scheme 3).[63,64,65]

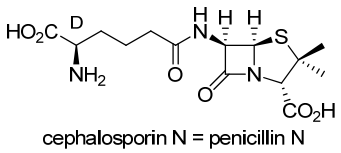
Many unsuccessful attempts were made to generate the β-lactam ring via a [2 + 2] Staudinger reaction between thiazolines and ketenes, including several studies by the Sheehan group, but the first successful synthesis involved a late-stage cyclization to form the β-lactam ring from a penicilloic acid. This was accomplished only after the development of mild conditions involving carbodiimides that are now very well-known and widely used for the coupling of amines with carboxylic acids.

The condensation of D-penicillamine with malonaldehyde **8** afforded a mixture of only two diastereomeric thiazolidines **9**, the D-γ-isomer of which could be isomerized in high yield to the natural and desired D-α-isomer. Deprotection with hydrazine, followed by acidification and acylation, provided amino ester **10** and cleavage of the *t*-butyl ester provided the penicilloic acid that was cyclized with a DCC-mediated amide coupling to provide penicillin V in 10–12% yield. Subsequent studies in 1962 demonstrated that protection of the primary amine of **12** with a trityl group prevented the undesired azlactonization in the cyclization to follow and also improved the yield of the lactamization to 67%.[66] Hydrogenolysis and acidification provided the hydrochloride salt of 6-aminopenicillanic acid (6-APA), a versatile molecule that proved to be crucial in the development of new penicillins (vide infra, Section 1.2.3).

1.2.2 Cephalosporins

In July 1945, as the therapeutic properties of penicillin were becoming widely known, a Professor of Hygiene in Sardinia named Brotzu had been looking for antibiotic-producing organisms and collected a sample of *Cephalosporium acremonium*, a seawater fungus.[60,67,68] He found that it produced antibiotic material that was active against Gram-positive and Gram-negative species and sent a culture of the organism to Oxford in 1948 with the hope that the work would be taken up by the Florey group.

The antibiotic extracted by organic solvent, named cephalosporin P, was shown to be a steroid with activity against Gram-positive bacteria but this compound alone could not be responsible for the broad-spectrum antibacterial activity observed by Brotzu. Abraham then detected an unstable hydrophilic antibiotic in the aqueous phase which was named cephalosporin N because it showed activity against both Gram-negative and Gram-positive bacteria. Cephalosporin N was inactivated by penicillinase and, after purification[69] and chemical degradation studies,[70],[71] was actually found to be a penicillin with a δ-(D-α-aminoadipoyl) sidechain.[72]

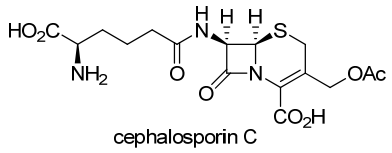


cephalosporin N = penicillin N

It was during chemical studies in 1953, to establish the molecular formula of penicillin N, that Newton and Abraham discovered a third antibiotic from the *Cephalosporium* species. Penicillin N was converted to its isomeric penillic acid at pH 3 because it was thought that purification of the penillic acid might be more straightforward than purification of the penicillin. Anion-exchange chromatography provided the desired penillic acid and an additional compound which had UV absorbance at 260 nm[73] and crystallized as a sodium salt. This compound, named cephalosporin C, was similar to penicillin N as it was found to have the aminoadipoyl sidechain and a β-lactam ring, as indicated by an IR band at 5.62 μ (1780 cm^{-1}), but was stable to dilute acid and its β-lactam was clearly fused to a ring that differed from the thiazolidine of the penicillins.[74,75,76]

Preliminary antimicrobial assays revealed cephalosporin C to be a broad-spectrum antibiotic that was resistant to hydrolysis by a penicillinase from *Bacillus cereus*. While the Oxford group's interests in this material were purely academic in nature, benzylpenicillin-resistant penicillinase-producing strains of staphylococci were becoming a serious clinical problem, and the clinical need for such a compound was obvious, even in the early-1950s.

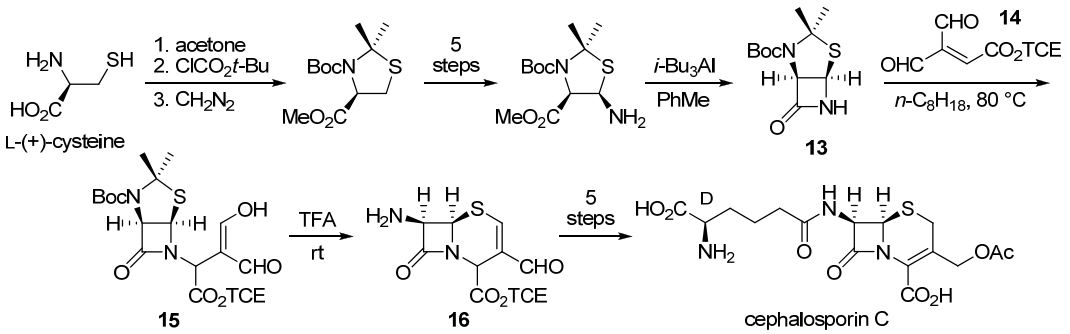
In 1957, a mutant strain of the *Cephalosporium* fungus was isolated which produced considerably more cephalosporin C than Brotzu's wild strain. This development was crucial to provide enough material for the chemical studies that led to the structural elucidation. Abraham's conclusion through chemical methods that cephalosporin C was a β-lactam fused with a dihydrothiazine ring[77] was confirmed by an X-ray crystallographic analysis by Hodgkin and Maslen and published in 1961.[78]



cephalosporin C

Woodward, who had been actively involved in the structural assignment of cephalosporin C through discussions with Abraham and Hodgkin, described the completion of its total synthesis in his Nobel lecture in 1965[79] and published the work in 1966 (Scheme 4).[80] While Sheehan's route to penicillin V involved the formation of the β-lactam ring at a late stage from a penicilloic acid (Scheme 3), an analogous route to cephalosporin C would not be viable since the cephalosporoic acid was known to be less stable than penicilloic acids and could therefore not be used as a synthetic

Scheme 4. Woodward's Total Synthesis of Cephalosporin C.[80]



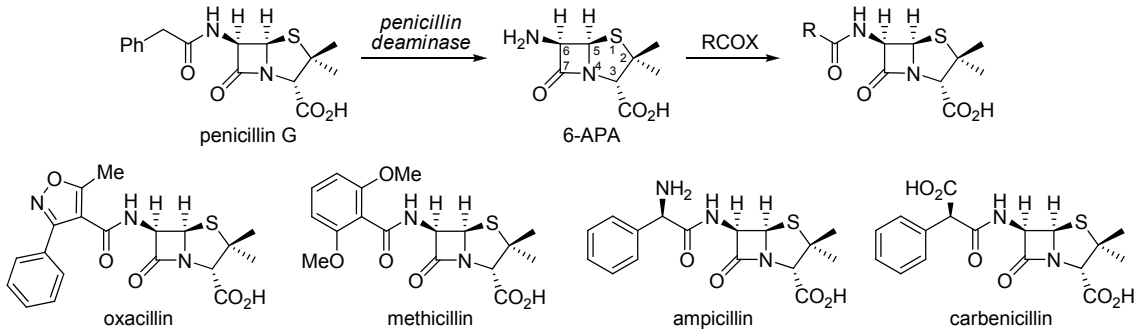
intermediate.[79] Instead, Woodward's route began with L-(+)-cysteine as a readily available and chiral building block from which the β-lactam ring could be constructed with the desired stereochemistry. Condensation of β-lactam **13** with dialdehyde **14**, followed by acid-catalyzed deprotection of **15** and cyclization provided the dihydrothiazine ring of the delicate but versatile aminoaldehyde **16** which was taken through to cephalosporin C.

1.2.3 Semisynthetic Penicillins and Cephalosporins from 6-APA and 7-ACA

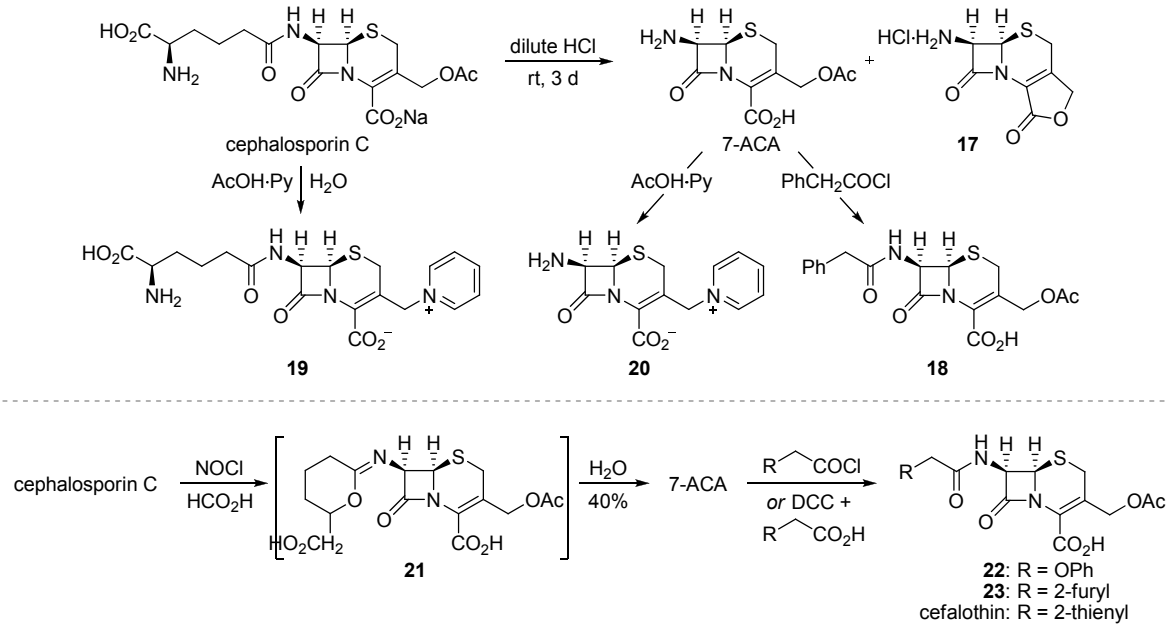
In the course of optimizing fermentation conditions for the production of penicillins, it was shown that penicillins with differing 6-acylamido sidechains were produced in different fermentation conditions. Structure–activity relationships with the penicillins had made it clear that antimicrobial activity was dependent to a great extent on the nature of the sidechain.

In 1959, Batchelor et al. reported that 6-aminopenicillanic acid (6-APA) was produced from *Penicillium chrysogenum* fermentations when no sidechain precursor was present.[81] The significant impact of this development is evident in the many semisynthetic penicillins that have been prepared

Scheme 5. 6-APA and Semisynthetic Penicillins.



Scheme 6. Conversion of Cephalosporin C to 7-ACA and other 7-Acyl Derivatives.



from 6-APA and used clinically since many of them would not have been prepared without 6-APA readily available (Scheme 5).[82] Around the same time, 6-APA was prepared by Sheehan via total synthesis[63] and methods for the conversion of benzylpenicillin to 6-APA by various microorganisms and enzyme preparations (penicillin deaminase/penicillin (de)acylase) were being studied.[60,83]

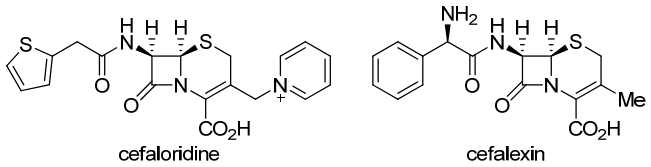
Some of the many semisynthetic penicillins that became clinically useful in the 60s and 70s include methicillin[84] and oxacillin, which were stable to staphylococcal penicillinases,[85,86] and ampicillin and carbenicillin, which showed an improved spectrum of activity including Gram-negative bacteria.[87]

After the discovery in the mid-1950s that cephalosporin C possessed the same aminoadipoyl sidechain and penicillin N, which had only 1% the antimicrobial activity of penicillin G, the Oxford group considered the logical possibility that substitution of the aminoadipoyl sidechain of the cephalosporin for a phenylacetyl group may improve its antibiotic potency. In 1961, Loder, Newton, and Abraham reported that 7-aminocephalosporanic acid (7-ACA) could be obtained in low yield by mild acid hydrolysis, although this was accompanied by formation of lactone **17** (Scheme 6). Acylation of 7-ACA with phenylacetyl chloride generated an antibiotic (**18**) 100 times more potent than cephalosporin C against *S. aureus*.[88] The group also discovered that substitution had occurred at the C3′ position when preparations of cephalosporin C were stored in aqueous pyridinium acetate and that the pyridinium salt **19** was more active than cephalosporin C.[89] 7-ACA also underwent

substitution with pyridine to give **20**.

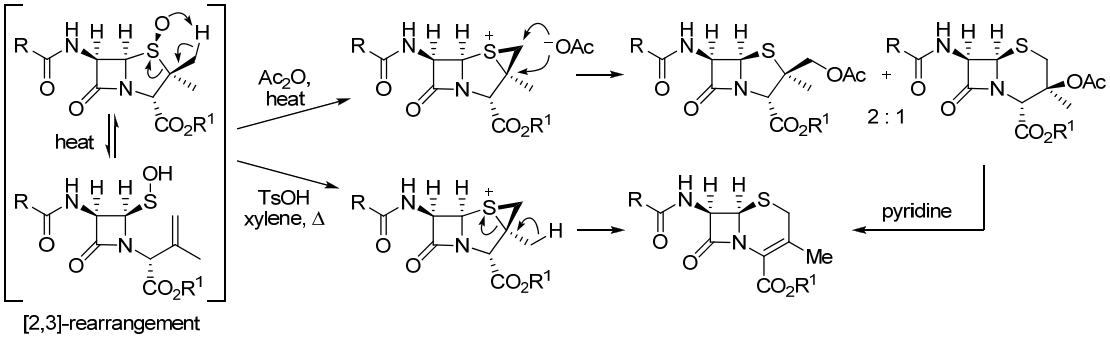
In 1962 researchers at Eli Lilly reported more practical chemical conditions for the conversion of cephalosporin C to 7-ACA (nitrosyl chloride in anhydrous formic acid) which involved an intramolecular substitution to form iminolactone **21**.[90,91] The group also prepared several 7-arylacetylamido cephalosporins from 7-ACA, including **18**, **22**, **23**, and a 2-thienylacetyl derivative they called cephalothin, which all showed better antibiotic activity than the corresponding penicillins against several organisms.[92]

Cephalothin, which became the prototype of the so-called first-generation cephalosporins, was found to be very resistant to staphylococcal penicillinase, showed activity against Gram-negative pathogens such as *E. coli* and *Klebsiella pneumoniae*, and was available for clinical use around 1964.[87] Other first-generation cephalosporins include cephaloridine and cefalexin.



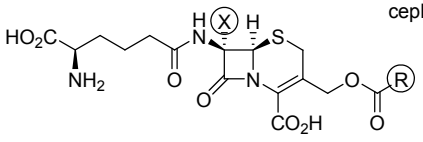
The demand for large amounts of 7-ACA in the early 1960s also prompted chemists to pursue methods for the synthesis of cephalosporins from penicillins. In 1963 Morin and coworkers at Eli Lilly discovered a process to achieve the ring-expansion through penicillin sulfoxides (Scheme 7).[93,94,95,96] This strategy may be considered to be biomimetic as the penicillins were later confirmed to be biosynthetic precursors to the cephalosporins.[97]

Scheme 7. Synthesis of Cephalosporins from Penicillin Sulfoxides.



1.2.4 Cephamycins and Expanded-Spectrum Penicillins and Cephalosporins

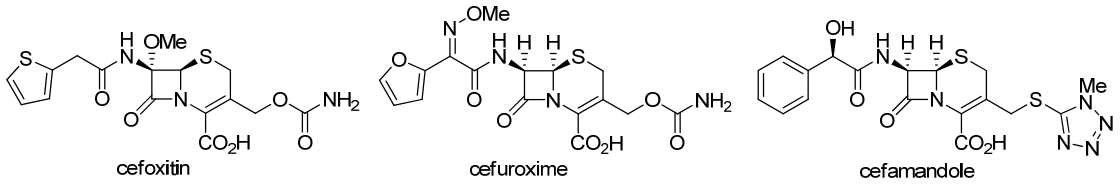
By the mid-1960s, Gram-negative bacteria had replaced *S. aureus* as the most problematic hospital pathogens and biochemical studies in the early 1970s suggested that the major mechanism for resistance to the clinically available cephalosporins was inactivation by β-lactamases.[98,99] Major pharmaceutical companies therefore directed efforts toward the design and synthesis of β-lactams with high stability to new β-lactamases and broad-spectrum antibacterial activity. These efforts were restricted to chemical modification of existing β-lactams until the discovery of the cephamycins in 1971, when researchers at Eli Lilly isolated and identified three new β-lactam antibiotics from streptomycetes.[100] 7α-Methoxycephalosporin C (**24**) was produced by a strain of *Streptomyces lipmanii* and *S. clavuligerus* produced carbamates **25** and **26**. Researchers at Merck simultaneously reported the isolation of the 7α-methoxycephalosporins **27**, **28**, and **26** from other species of *Streptomyces* (actinomycetes from soil) and named these compounds cephamycins A, B, and C, respectively.[101,102,103]



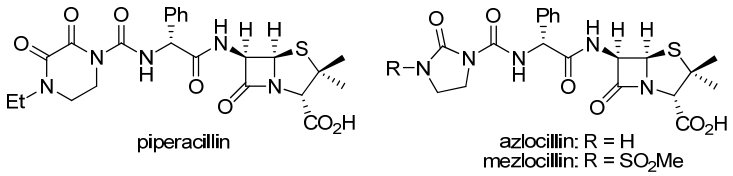
cephalosporin C: X = H, R = Me
24: X = OMe, R = Me
25: X = H, R = NH_2
26: X = OMe, R = NH_2
27: X = OMe R = C(OMe)=CH-*p*-C_6H_4-OSO_3H
28: X = OMe R = C(OMe)=CH-*p*-C_6H_4-OH

The discovery of a new family of cephalosporins among streptomycetes was considered a major breakthrough but the greatest significance of the discovery was the broad spectrum of Gram-negative activity and the high stability to hydrolysis by β-lactamases.[98] The concept of expanded-spectrum β-lactams was introduced with the discovery of the cephamycins and these new structures provided additional inspiration for the design and synthesis of new generations of cephalosporins.

Cephalosporins have been classified as belonging to the first, second, or third generation on the basis of their biological characteristics. First generation cephalosporins, including cephalothin and cephaloridine, were very potent against staphylococci and streptococci but much less active against Gram-negative bacteria.[86] The second generation cephalosporins such as cefamandole, cefoxitin, and cefuroxime, showed improved clinical utility because of their greater stability to β-lactamases and, while these compounds were more active against Gram-negative bacilli than first generation agents, they remained inactive against *Pseudomonas* organisms.[87]

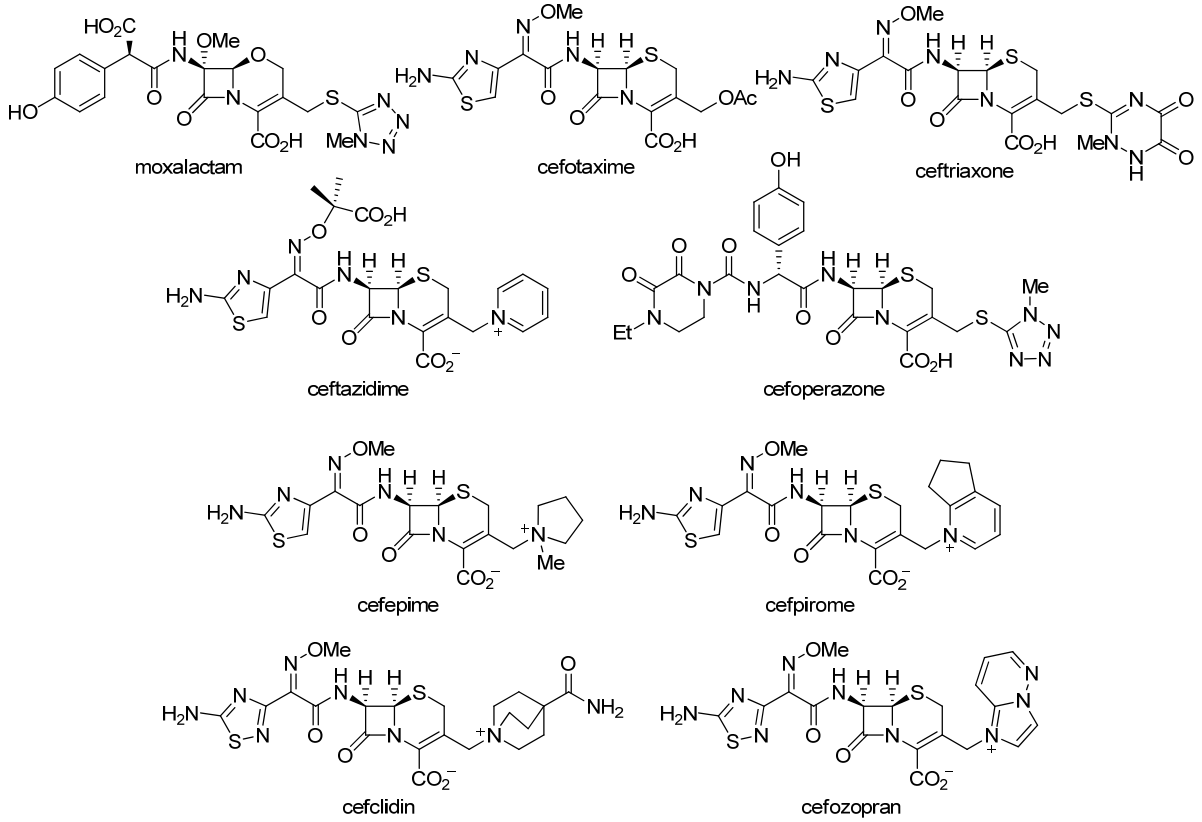


Modification of the acylamido sidechains of the penicillins was also explored extensively in the mid-1970s and led to the development of the ureidopenicillins (which are also called third-generation penicillins).[104] Piperacillin, azlocillin, and mezlocillin, which are *N*-acylated derivatives of ampicillin, were shown to have similar activity to that of ampicillin against streptococci and better activity against *P. aeruginosa* than carbenicillin. The acylureido sidechain does not significantly increase β-lactamase stability but does improve affinity for PBPs and improves penetration of the outer membrane of *P. aeruginosa* and *K. pneumoniae*.[86,87]

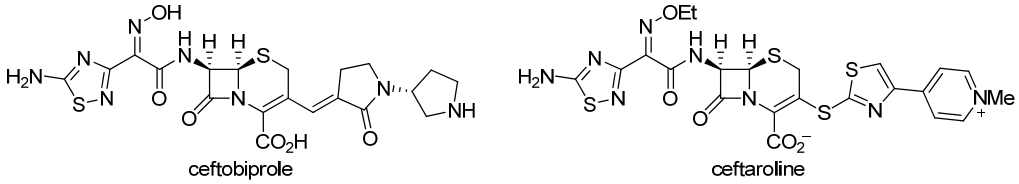


The third generation cephalosporins, which originated with cefotaxime in the early 1980s,[105] have an even broader spectrum of activity, including all or most Enterobacteriaceae and improved activity against *P. aeruginosa*. They also have improved stability to plasmid-mediated and chromosomal β-lactamases.[99] The 2-aminothiazoyloximino sidechain present in many third generation cephalosporins was found to be a major improvement. The aminothiazole heterocycle provides high affinity for PBPs and the *syn*-oximino[106] group was found to improve stability to β-lactamases.[86] Third generation cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone have very potent activity against a broad range of microorganisms, including Gram-negative aerobes, but tend to be less active than earlier generation agents against Gram-positives such as *S. aureus*.[19] Moxalactam,[107] an oxacephamycin[108] with performance superior to many other third generation cephalosporins, has suffered restricted clinical use due to side effects.[19]

In the 1990s, efforts to improve upon the spectrum and activity of the third generation cephalosporins against both Gram-negative and Gram positive organisms and the stability to β-lactamases led to the development of the fourth generation cephalosporins. The observation that ceftazidime, a third generation agent bearing a pyridinium group at C3′ position, showed improved



activity against *P. aeruginosa* prompted further exploration of other C3′ quaternary ammonium cephems, including cefepime, cefpirome, cefclidin, and cefozopran.[109] Cefipime and cefpirome, zwitterionic oximino cephalosporins,[110] indeed demonstrated reduced affinity for β-lactamases, increased outer membrane permeability, better activity than third generation agents against *P. aeruginosa*, and similar potency against *S. aureus* as first generation cephalosporins.[111]

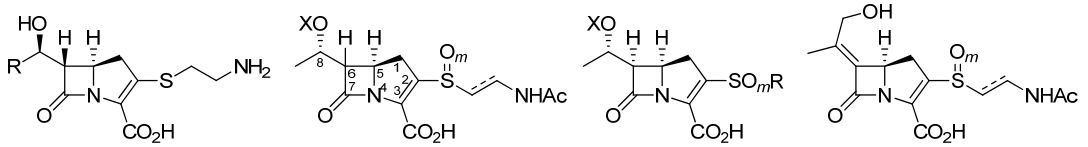


Ceftobiprole[112] and ceftaroline[113] are cephalosporins currently in development[114] which have been categorized as fifth generation because of their high activity against MRSA.[115] Ceftaroline has a spectrum of activity against Gram-negative bacteria similar to earlier broad-spectrum cephalosporins, but has poor activity against *P. aeruginosa* and is labile to hydrolysis by AmpC β-lactamases and ESBLs. Ceftobiprole has an unusually broad antibiotic spectrum, with potent activity against a wide range of Gram-positive and Gram-negative bacteria including *P. aeruginosa*, but is susceptible to

hydrolysis by class A ESBLs and class B and D β-lactamases.[19] These agents are considered the first of the fifth generation, despite their instability to β-lactamases, because of their high affinity for PBP2a,[116] the penicillin-binding protein that is largely responsible for conferring resistance to β-lactams in MRSA (see Section 1.3).

1.2.5 Carbapenems

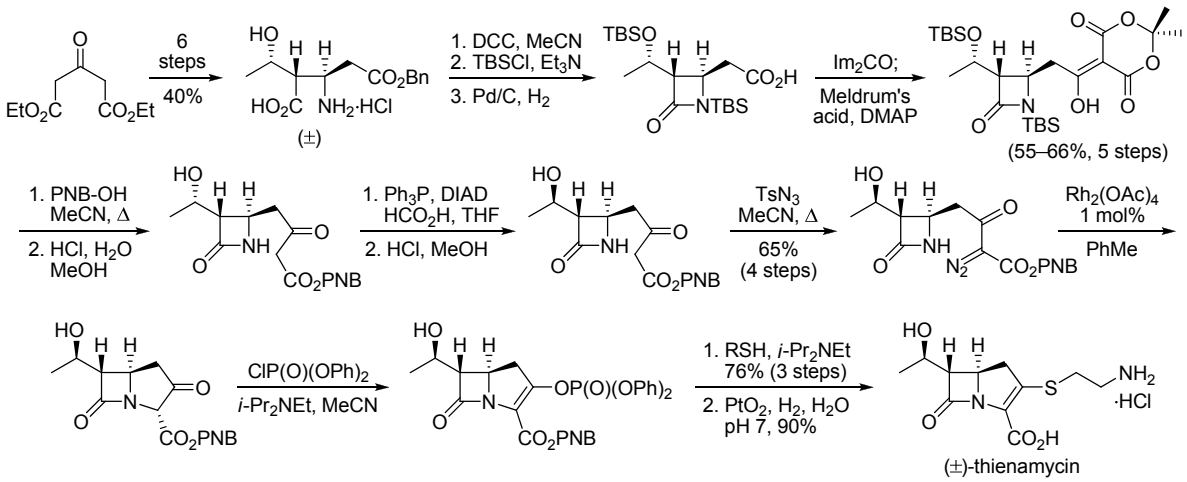
In the late 1960s and early 1970s, various pharmaceutical companies had programs to screen for inhibitors of cell wall biosynthesis and β-lactamases and the carbapenems were discovered as a direct result of these efforts.[117,118] Thienamycin was discovered at Merck in fermentation broths of the soil bacterium *Streptomyces cattleya* along with several epimeric compounds and some acetylated derivatives. Beecham independently isolated several related carbapenems from *Streptomyces olivaceus*, some of which were identical to Merck's epithienamycins, and named them olivanic acids. Some Japanese groups isolated related derivatives from *S. fulvoviridis* and *S. cremeus* including the carpetamycins and so-called *PS*- compounds (Appendix A). Other important β-lactams discovered in these screening programs include clavulanic acid,[119] monocyclic β-lactams called nocardicins,[120] and discovered later were the pluracidomycins[121,122] and asparenomycins.[123,124]



Thienamycin showed very potent antibiotic activity against both Gram-positive and Gram-negative bacteria, including *Pseudomonas* spp, and equally impressive stability to β-lactamases.[125] Its high activity was surprising at first because of the lack of acylamino functionality at C6 and because of the α stereochemistry of the hydroxyethyl substituent. Comparison of the biological activities of carbapenem stereoisomers revealed that the so-called *trans* arrangement between C5 and C6 and the (*R*)-stereochemistry at C8 of thienamycin provided maximum β-lactamase stability and antibacterial potency.[117,126]

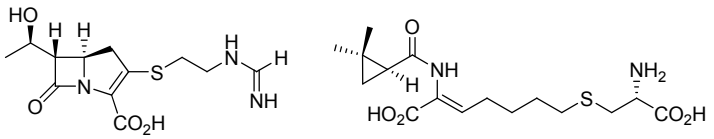
The first total synthesis of thienamycin,[127] published in 1978 by researchers at Merck, was soon

Scheme 8. Merck's Total Synthesis of Thienamycin.[128]



followed by a more practical second-generation synthesis from the process group at Merck in 1980.[128] The first synthesis relied on a late-stage cyclization to form the C2–C3 bond while the second-generation approach was based on a highly efficient rhodium-catalyzed N–H bond insertion to join C3 with N4 (Scheme 8).[65]

In the early 1980s thienamycin was thought to be the "ultimate antibiotic," both in broadness of its spectrum and in its potency against pathogenic bacteria,[87] but thienamycin itself was too unstable to be pursued clinically because of its free amine and highly reactive β-lactam.[129] Derivatization of the amine, to improve chemical stability and maintain or improve its antibacterial activity, revealed that a basic sidechain was required for antipseudomonal activity since acetylation reduced activity. Of the many derivatives prepared, the *N*-formimido derivative, now called imipenem, was the first to be selected for clinical evaluation. Imipenem showed greatly improved stability in concentrated solution, retained the antibacterial spectrum of thienamycin, and was two- to four-fold more potent against *P. aeruginosa*.[130,131]

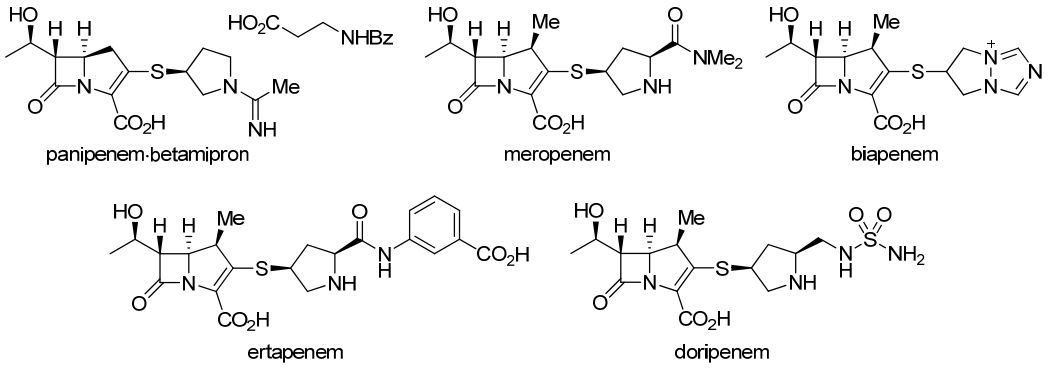


imipenem cilastatin

While imipenem showed improved antibiotic potency relative to thienamycin, a similar antibiotic spectrum, and improved pharmacokinetics in humans, imipenem retained the instability to renal

dehydropeptidase I (DHP-I) and nephrotoxicity of its metabolites.[132] The approach that was used successfully to overcome this problem has involved co-administration of imipenem with cilastatin, an inhibitor of DHP-I. This combination significantly reduces the associated nephrotoxicity and improves urinary recovery in animals. Imipenem-cilastatin (Primaxin®, Merck) has been used for more than 26 million people since it was put on the market in 1986,[133] generated $555 million in sales in 1997,[1] and is still considered one of the most important carbapenems in clinical use.[134]

In 1993, panipenem became the second carbapenem to be introduced and has been approved for use in Japan, China, and South Korea.[134] Panipenem is also unstable to DHP-I and is therefore co-administered with the DHP-1 inhibitor betamipron.



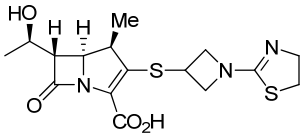
Meropenem, which was introduced in 1995, has a spectrum of antibiotic activity broadly similar to that of imipenem, but is more active against Gram-negative aerobes, active against imipenem-resistant *P. aeruginosa*, and is more stable to DHP-I.[134,135,136] A study by the Sumitomo group in 1992 revealed that the β-methyl group at C1 of meropenem provides sufficient stability to human DHP that co-administration with a DHP inhibitor is not required.[137] As a result, all carbapenems developed since the early 1990s have incorporated the 1β-methyl group or a similar substituent.

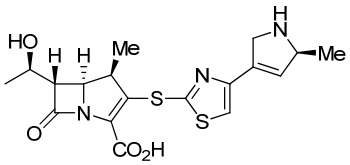
Biapenem, a zwitterionic carbapenem clinically used in Japan since 2002, is generally considered to have a spectrum of activity similar to imipenem and meropenem but is slightly more active than imipenem against Gram-positive bacteria and less active against some Gram-negative pathogens.[19]

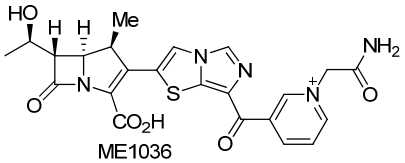
Ertapenem is an *N*-arylated analogue of meropenem which was approved in the U.S. in 2001.[138] In contrast to the zwitterionic carbapenems imipenem and meropenem, which penetrate Gram-negative outer membranes efficiently,[136] ertapenem has an overall negative charge at physiological pH and penetrates Gram-negative organisms much more slowly, but has a much longer serum half-life.[19]

Ertapenem has activity against Gram-positive and Gram-negative aerobes and anaerobes and is resistant to nearly all β-lactamases, but shows much less activity than imipenem and meropenem against nosocomial pathogens *P. aeruginosa*, *Acinetobacter*, MRSA, and enterococci. Because of its unique spectrum of activity, ertapenem represents a separate class[134] within the carbapenem group and is also unique from other carbapenems because it is considered suitable for community-acquired infections.

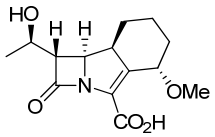
Doripenem is structurally similar to meropenem and became the most recent carbapenem to be launched in 2005.[19,134] Doripenem combines the best features of the carbapenem class as it boasts a broad antibiotic spectrum with better activity against Gram-positive organisms than meropenem and better activity against Gram-negative organisms than imipenem. It is also more potent than other β-lactams against *P. aeruginosa* and ESBL- and AmpC-producing pathogens[139] and shows the lowest rate of spontaneous resistance in vitro.[134,140]


tebipenem


razupenem (PZ-601)


ME1036

Tebipenem (as its pivaloyloxymethyl ester prodrug, L-084), is an oral carbapenem in Phase III clinical studies with high bioavailability in humans and very potent activity against *S. pneumoniae* (MIC_{90} of 0.02–0.06 μg/mL) and other bacteria that cause respiratory infections.[19,141] Razupenem, which is currently in Phase II clinical trials for complicated skin infections, features a lipophilic 2-thiazol-2-ylthio- sidechain that is thought to be important in its tight binding to PBPs. Razupenem shows activity against a wide range of Gram-positive and Gram-negative bacteria and is also very active against MRSA (MIC_{90} of 2 μg/mL) and VRE (MIC_{90} of 8 μg/mL).[142] ME1036 is a carbapenem in Phase I evaluation for the treatment of respiratory infection. While it is inactive against *P. aeruginosa*, it is very potent against community-acquired MRSA (MIC_{90} of 0.25 μg/mL),[143] *S.*


sanfetrinem

pneumoniae, and Enterobacteriaceae, and resistant to hydrolysis by ESBLs and AmpC β-lactamases.[19] Tricyclic β-lactams (trinems) represent another set of carbapenems in development. Sanfetrinem has shown very potent antimicrobial activity against Gram-positive and Gram-negative bacteria.[144,145,146]

1.2.6 Penems

The notion that the biological activities of the β-lactams were correlated with chemical reactivity of the β-lactam ring had arisen because penicillins and cephalosporins were known to be far more reactive than monocyclic β-lactams. Even before the structures of the carbapenems were published in the late 1970s, Woodward had postulated that penems, hybrid structures of penams and cephems, should be more reactive and more potent antibiotics.[147]

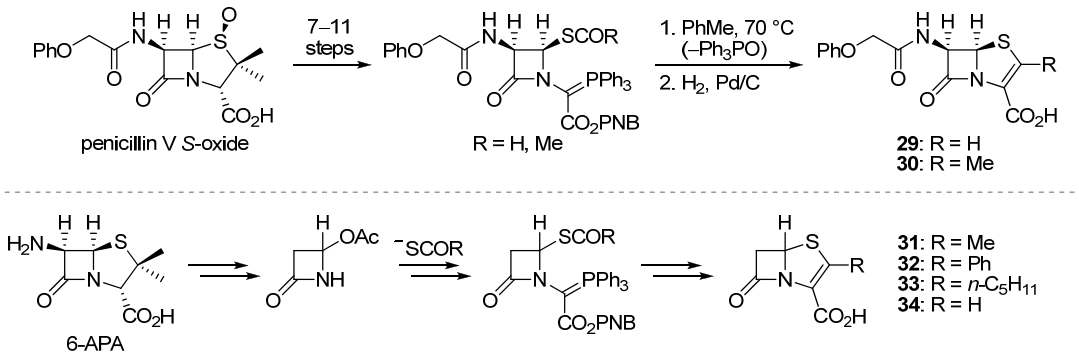
The accepted rationale for the increased reactivity of the penicillins, in comparison to monocyclic β-lactams, toward ring-opening by nucleophiles had been given by Woodward in 1944 and proposed that the pyramidal geometry of the β-lactam nitrogen limits delocalization of its unshared electrons into the carbonyl and results in a weakened amide bond.[56] The increased reactivity of the cephalosporins, however, in which the β-lactam nitrogen is nearly planar, was thought to arise from a conjugative interaction with the adjacent double bond (Δ^3) which also results in a weakened β-lactam amide bond.[148]

Woodward first targeted penems with 6-acylamino substituents, as it was clear that these sidechains were important for the activity of the penicillins and cephalosporins, and reported the synthesis of penems **29** and **30** in 1978 (Scheme 9).[149] The first penems of the series, **29** and **30**, indeed showed antibiotic activity against strains of *S. aureus* but the potency of these compounds was disappointing.

In order to study the new system in a more systematic way, a series of 6-unsubstituted penems were pursued. Even though 6-unsubstituted penams and cephems were known to have poor antibiotic activity, the appearance of clavulanic acid in the literature, which is 6-unsubstituted, encouraged the preparation of the simpler analogues.

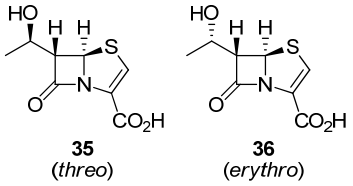
In 1979, the Woodward group published the synthesis of the racemic simplified analogues **31**–**34**.[150,151,152] Not only did each of the new penems **31**–**33** show better stability[153] and activity than **29** and **30**, but they were also more potent than cephalexin against most Gram-positive and Gram-

Scheme 9. Woodward's Syntheses of Penems.[149,150]

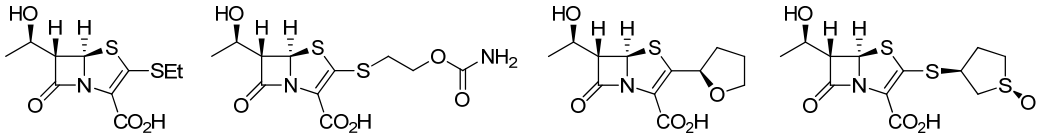


negative strains tested. These results indicate that the penems **29** and **30** were too reactive to interact specifically with their biological targets.

The recent discovery of thienamycin and other carbapenems prompted the Woodward group to incorporate the hydroxyethyl sidechain at C6 and, in 1980, the Woodward group reported the synthesis of penems **35** and **36**.[147] Penem **35** proved to be 16–64 times more potent than **36** against Gram-positive and Gram-negative bacteria and 4–32 times more potent than (±)-**34** (except against *P. aeruginosa*).[154]



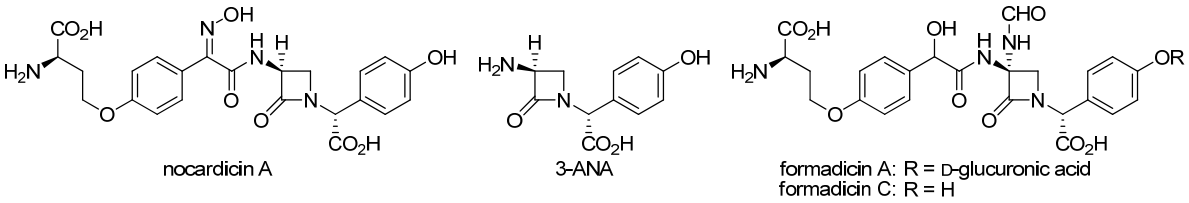
After Woodward's contributions to the field, many other penems were prepared and evaluated.[148] In general, the penems have good to excellent activity against Gram-positive bacteria but are inferior to their carbapenem analogues against Gram-negatives.[19] Of the several that were selected for clinical evaluation, including Sch 29482[155] and Sch 34343,[156] only faropenem and sulopenem remain in development. Faropenem has a broad spectrum of antibiotic activity and is stable to class A, C, and D β-lactamases. Faropenem has been available in Japan since 1997[157] and has been in Phase III development elsewhere as an oral antibiotic for community-onset infections.[158] The development of sulopenem began in the 1980s and clinical trials have recently been resumed by Pfizer. Sulopenem has potent activity against Gram-positive and Gram-negative bacteria, with MIC_{90}s less than 1 μg/mL against clinically significant bacterial species.[19]



6-Alkylidene penems represent another type of penem in development. These compounds have greater potential as broad-spectrum β-lactamase inhibitors than as antimicrobial agents and are discussed in Section 1.5.2.

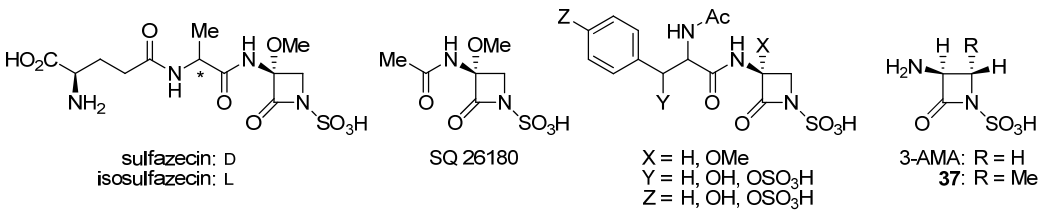
1.2.7 Monobactams

Many pharmaceutical companies had initiated large screening programs by the early 1970s to identify inhibitors of cell wall synthesis and β-lactamase inhibitors. Screening at Beecham led to the isolation of the olivanic acids and clavulanic acid,[119] screens at Merck generated thienamycin and epithienamycins,[117] and a program in Japan led to the isolation of the nocardicins from *Nocardia uniformis* in 1976.[120]

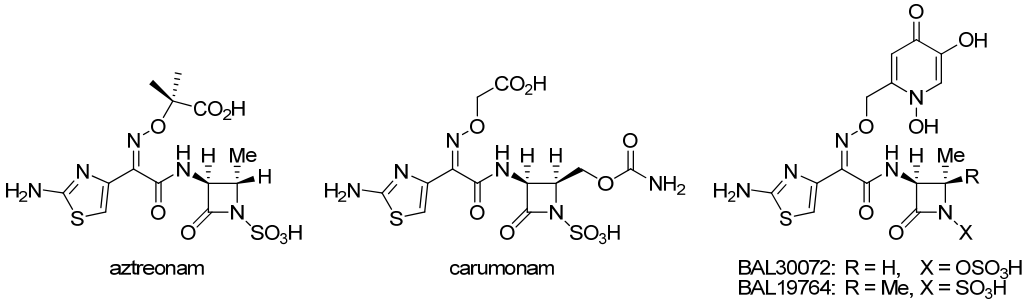


Nocardicin A shows moderate activity against some Gram-negative organisms such as *P. aeruginosa* but weak activity against *E. coli* and staphylococci. Initial efforts to improve potency focused on the synthesis of *N*-acyl derivatives of 3-amino nocardicinic acid (3-ANA) in the same way that 6-APA and 7-ACA were used for semi-synthetic penicillins and cephalosporins; however, nocardicin A proved to be the only useful antibiotic after extensive SAR with hundreds of compounds.[19] The formadicins, structural relatives of the nocardicins which have a formamido substituent at C3, were isolated later from a species of *Flexibacter*.[159,160] The formadicins have a spectrum of antibacterial activity similar to the nocardicins but the formamido substituent provides additional stability to β-lactamases.[161]

In 1981 groups at Takeda and Squibb discovered independently a new class of β-lactam produced by bacteria and Sykes named them monobactams.[162,163] The Takeda group reported the isolation and



identification of sulfazecin and isosulfazecin and the Squibb group described their discovery of sulfazecin and several other monobactams including SQ 26180. In general, these monobactams showed weak activity against *S. aureus* and only modest activity against Gram-negative aerobes, but SQ 26180 showed good activity against *P. aeruginosa* (MIC of 3.1 μg/mL). Early SAR studies showed that the *N*1-sulfonate served to increase the reactivity of the β-lactam and that the α-methoxy group was important for antibacterial activity and β-lactamase stability. With 3β-amino-3α-methoxy monobactamic acid (3-AMA) and **37** in hand, Squibb was able to prepare a series of *N*-acyl analogues and discovered SQ 26776,[164,165] an oximino-aminothaizole derivative which later became known as aztreonam.



Aztreonam showed no activity against Gram-positive organisms or anaerobes but has very good activity against Gram-negative rods including *Pseudomonas*, *Serratia*, and *Enterobacter*, bacteria typically resistant to cephalosporins.[166] Studies with PBPs indicated that it has very high affinity for PBP3 of Gram-negative organisms, moderate affinity for PBP1a, and poor interaction with all other PBPs of Gram-negatives and *S. aureus*.[164] Although aztreonam lacks the 3α-methoxy group and is slowly hydrolyzed by class A β-lactamases, its affinity for these enzymes is low and it is active against class A-producing organisms.[167,168] Aztreonam acts as a progressive inhibitor of class C enzymes and is one of the only β-lactams that is stable to hydrolysis by metallo-β-lactamases. Aztreonam was approved for clinical use in 1986 and carumonam, which shows slightly improved activity over aztreonam against Enterobacteriaceae, is also clinically available.

The renewed interest in Gram-negative therapy, against drug-resistant *A. baumannii* and *P. aeruginosa* for example, has prompted new research with the monobactams and BAL30072 and BAL19764 are two siderophore monobactams in development by Basilea.[19,169] BAL30376 is a three-component combination of BAL19764, which is stable to MBLs, with clavulanate, a class A β-lactamase inhibitor, and a bridged monobactam (BAL29880) which inhibits class C β-lactamases.[170]

1.3 Penicillin-Binding Proteins

After Florey and the Oxford group had demonstrated in the early 1940s that penicillin was effective as an antibiotic in humans, penicillin's mode of action immediately became a subject of intense research interest. Early work in the 1940s, 50s, and 60s was concerned with the elucidation of the structure of the cell wall and, in combination with morphological studies, led to the conclusion that penicillin inhibited the transpeptidase reaction, the last stage in cell wall biosynthesis.[171,172] Other research in the 1970s demonstrated that penicillin has many target enzymes, called penicillin-binding proteins (PBPs), and significant progress has been made since that time in understanding the roles that each enzyme plays in synthesis and maintenance of the cell wall.[173,174,175] Current research in this area continues to build upon the understanding of these complex processes[176] and also examines the role of PBPs in antibiotic resistance.[177]

1.3.1 Peptidoglycan and D-Ala-D-Ala Transpeptidases

Fleming noted that penicillin was not merely bacteriostatic but killed bacteria by lysis.[31] Gardner observed in 1940 that organisms do not lyse in the presence of low concentrations of penicillin but are converted to elongated forms (filaments).[178] In 1949, Park reported that uridine nucleotides accumulated in the cytoplasm of penicillin-inhibited *S. aureus*[179] and this material was later identified as a uridine pyrophosphate (UDP) linked to an *N*-acetyl-aminosugar and a peptide containing D-glutamate, L-lysine, and DL-alanine in a ratio of 1:1:3.[180] In 1957 Park and Strominger concluded that this nucleotide was a cell wall precursor since its sugar and amino acid composition was found to be similar to that of the recently discovered cell wall.[181] At the same time, Lederberg[182] and Hanh and Ciak[183] had shown that a hypertonic medium protects penicillin-treated cells from lysis. Since the cell

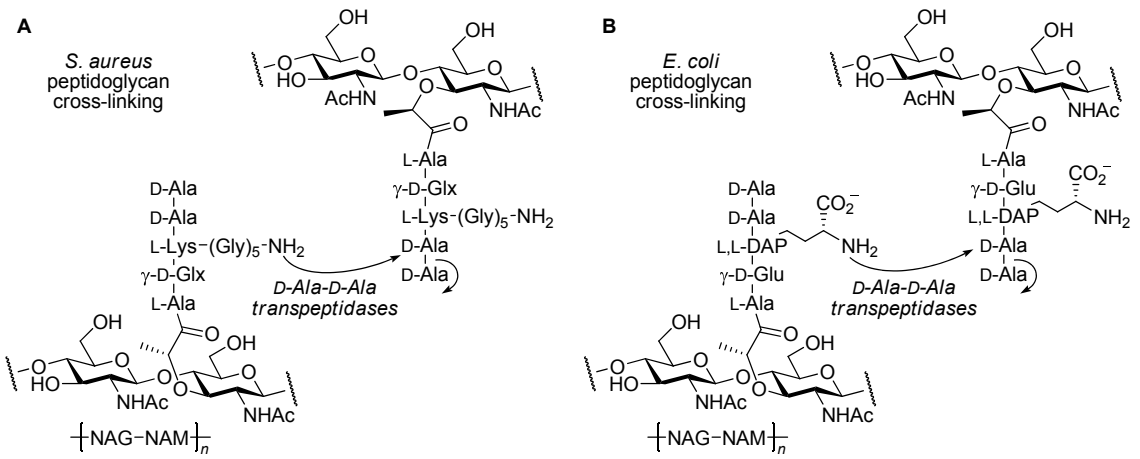


Figure 5. Peptidoglycan structure and cross-linking by D-Ala-D-Ala transpeptidases in (A) *S. aureus*, and (B) *E. coli* (DAP = diaminopimelic acid; Glx = Gln or Glu; NAG = *N*-acetyl glucosamine; NAM = *N*-acetyl muramic acid).

wall was already known to be a rigid macromolecule that determines cell shape and provides protection from osmotic rupture, each of these studies was recognized as support for the hypothesis that penicillin inhibited an essential step in synthesis of the cell wall.

Over the next few years, the complex structure of peptidoglycan and steps leading to its synthesis became more clear. By the early 1960s, the cell wall glycopeptide in all species examined was known to contain alternating units of *N*-acetylglucosamine (NAG, or GlcNAc) and *N*-acetylmuramic acid (NAM, or MurNAc) with a peptide unit attached to the D-lactyl portion of NAM (Figure 5). In Gram-negative bacteria, the pentapeptide unit is [L-Ala–γ-D-Glu–L,L-DAP–D-Ala–D-Ala]. In Gram-positive organisms, the pentapeptide unit also has a pentaglycine bridge bound to the ε-amino group of L-Lys and in *S. aureus* this peptide unit is [L-Ala–γ-D-Glx–L-Lys(ε-Gly_5)–D-Ala–D-Ala].[184]

In the early 1960s, it had become widely accepted that all reactions leading to the synthesis of the linear glycopeptide were insensitive to penicillin. Salton provided evidence that the bacterial cell wall was highly cross-linked[185] and it seemed clear that cross-linking must be the last stage in peptidoglycan synthesis and the penicillin-sensitive step. In 1965 Wise and Park confirmed that this cross-linking reaction was a transpeptidation with a loss of D-alanine and proposed that penicillin inhibits this reaction by acting as an analogue of the L-Ala–γ-D-Glu portion of the substrate.[186] Later that year, however, Tipper and Strominger proposed that penicillin was more likely to be an analogue of the D-Ala-D-Ala segment of the peptidoglycan chain and noted that the highly reactive β-lactam

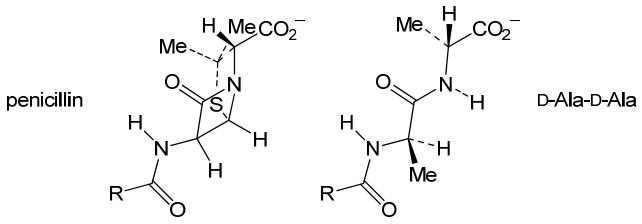


Figure 6. Structural analogy of penicillin and the D-Ala-D-Ala fragment of peptidoglycan as proposed by Tipper and Strominger in 1965.[187]

amide bond would correspond to the peptide bond cleaved during the transpeptidation process (Figure 6).[187,188] The Tipper–Strominger hypothesis is widely considered a major milestone in the understanding of the penicillin mode of action.[189]

In accord with earlier evidence from Schepartz and Johnson that penicillin binds to its target covalently,[190] Tipper and Strominger suggested that penicillin could react with the transpeptidase to form a stable penicilloyl enzyme intermediate (with the active-site serine nucleophile) which would prevent the enzyme from completing the transpeptidation (Figure 7).

Several studies by Cooper and others in the 1940s and 50s demonstrated that radioactive [^{35}S]- or [^{14}C]-penicillin bound specifically to a target in bacterial membranes,[191] but progress in this area was slow until the early 1970s. In 1972, the Strominger group used SDS-PAGE to study [^{14}C]-penicillin G-binding components and discovered that multiple penicillin-binding proteins were present in *B. subtilis*, *B. cereus*, *S. aureus*, and *E. coli*.[192,193]

Penicillin-binding proteins of each organism were originally numbered in order of decreasing apparent molecular weight and this historical numbering system persists to this day. The classical numbering scheme of PBPs can cause confusion, however, because PBPs from different organisms with the same number do not necessarily share any structural or functional similarities. It became apparent that PBPs did not only include D-Ala-D-Ala transpeptidases, which cross-link the glycan strands, when the Strominger group found that carboxypeptidases, which remove the terminal D-alanine through hydrolysis, were also members of the PBP group (Figure 7).[193,194] Other PBPs include endopeptidases, which hydrolyze the peptide bond connecting two strands, and transglycosylases, which catalyze the polymerization of the polysaccharide strand. PBPs vary greatly in their relative abundance, their sensitivity to different β-lactams, and regulation. It should also be noted that the structure and shape of the cell wall must be continuously modified (formation and cleavage of peptide

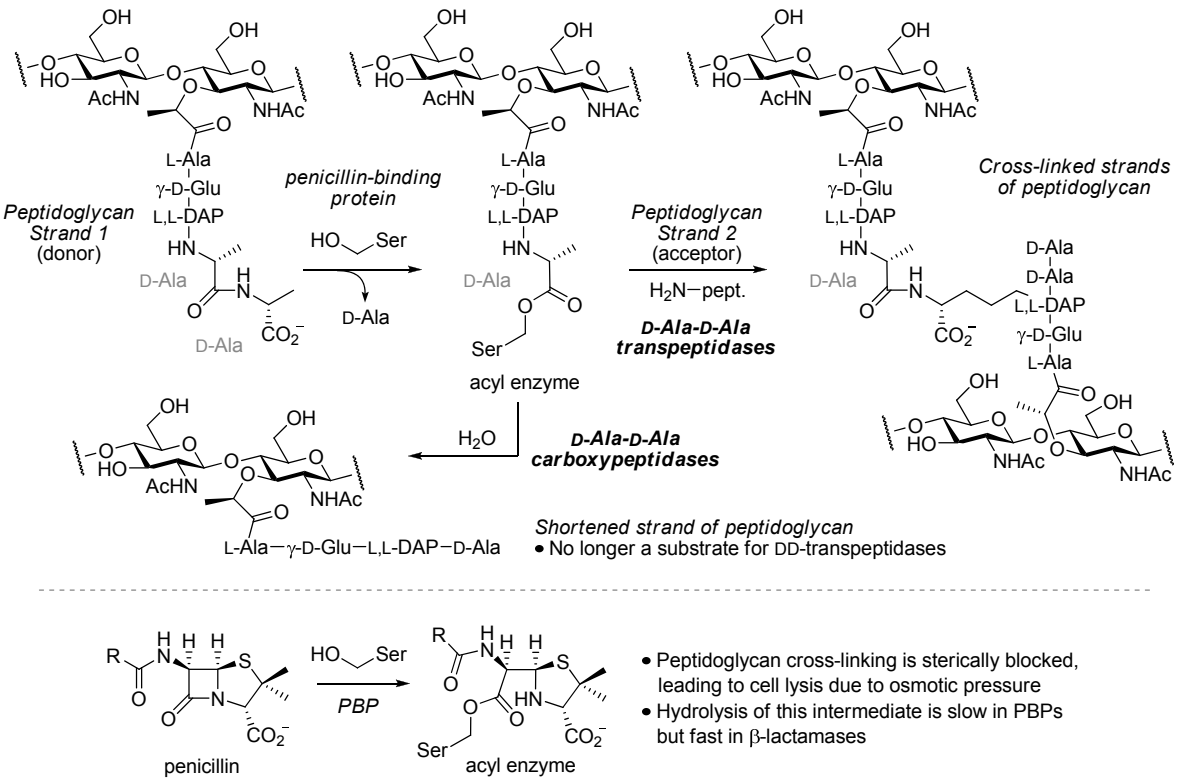


Figure 7. Reactions of penicillin-binding proteins (D-Ala-D-Ala transpeptidases and D-Ala-D-Ala carboxypeptidases) in *E. coli* and inactivation of PBPs by penicillins via a stable acyl enzyme.

cross-links) to allow for cell growth and division and that the degree of peptidoglycan cross-linking is different in each organism.[175]

PBPs are distinguished by size and have been categorized into different classes (A, B, and C) according to function.[195] High molecular weight (HMW, MW >55000) class A PBPs are multimodular proteins with a cytoplasmic tail, a transmembrane anchor, and two domains on the outer surface of the inner membrane (e.g. PBP1a and PBP1b of *E. coli*). In HMW class A PBPs, the C-terminal penicillin-binding domain has transpeptidase activity and the N-terminal domain has transglycosylase activity. HMW class B PBPs also have a C-terminal domain with transpeptidase activity but the N-terminal domain is believed to be involved with cell morphogenesis (e.g. PBP2 and PBP3 of *E. coli*). HMW class C PBPs include penicillin-sensing proteins such as BlaR and MecR which are involved in regulation of β-lactamase and PBP2a, respectively.

Low molecular weight (LMW) PBPs are also commonly divided into classes A, B, and C. LMW PBPs are not as essential for cell vitality as the HMW PBPs and have diverse functions, including

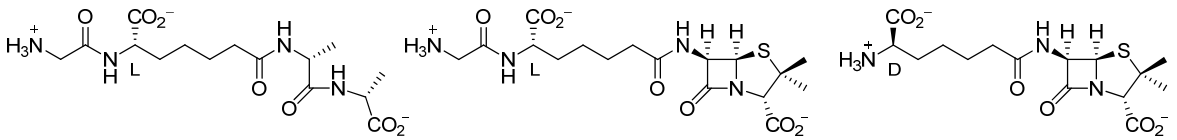


Figure 8. Peptidoglycan-mimetic peptides and penicillins prepared as substrates and inhibitors of the *Streptomyces* R61 DD-peptidase.

endopeptidase activity, carboxypeptidase activity, transpeptidase activity, or both carboxypeptidase and transpeptidase activities.[176,196]

While most physiologically important transpeptidases are membrane-bound, most of the early mechanistic information about PBPs was obtained from studies with soluble D-Ala-D-Ala carboxypeptidase–transpeptidases (often referred to as DD-peptidases) from *Actinomadura* strain R39 and *Streptomyces* strain R61, which are PBPs secreted in the extracellular medium.[175] Although these enzymes are not lethal targets in the bacteria that produce them, they have been very valuable as models.

In the last few years, however, studies by the Pratt group have demonstrated that substrate specificities of the LMW soluble R61 DD-peptidase cannot be extended to HMW PBPs and that many mechanistic uncertainties still remain. Peptide **38**, which was designed to incorporate an N-terminal glycine in order to best mimic the peptidoglycan of *Streptomyces*, was found to be the best (most specific) substrate ever found for the R61 enzyme and the corresponding peptidoglycan-mimetic penicillin **39** proved to be an excellent inhibitor of the R61 transpeptidase (Figure 8).[197,198] X-ray structures of the DD-peptidase of *Streptomyces* strain R61 were solved with this so-called "perfect penicillin" bound as a non-covalent (Michaelis) complex (PDB: 1PW1) and with **39** bound as the acylenzyme (PDB: 1PWG).[199,200] In a related study, a peptidoglycan-mimetic boronic acid was shown to be a potent inhibitor of the *Actinomadura* R39 DD-peptidase (K_i of 32 nM) and was co-crystallized with this enzyme (PDB: 2XDM).[201] Rather surprisingly, however, peptidoglycan-mimetic peptides such as **38** are not good substrates for the physiologically more important high molecular weight PBPs and peptidoglycan-mimetic β-lactams such as **40** are much poorer inhibitors of other PBPs in vitro and in vivo than generic β-lactams such as penicillin G.[202,203] It is possible that there is no specific recognition of the peptidoglycan chain by the HMW PBPs or that there is an unknown mechanism through which the active site is opened or closed.[204] Nevertheless, these studies clearly

demonstrate that cell wall biosynthesis is a complex process and give an indication of the difficulties that challenge the rational design of inhibitors and β-lactam antibiotics.[205]

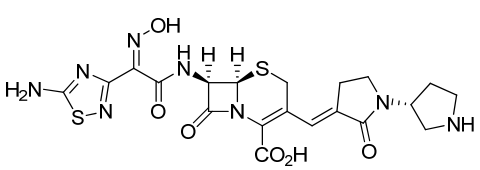
1.3.2 PBPs and Resistance to β-Lactam Antibiotics

Resistance to antibiotics is inevitable. The small proportion of mutant bacteria that escape the action of a given antibacterial agent will be selected for and amplified and various mechanisms of resistance to β-lactams have been discovered since the introduction of the penicillins in the 1940s. Aside from the restriction of the permeability of the β-lactams (porin deletion) and active export of the β-lactams from the periplasm (efflux pumps), major resistance mechanisms involve the modification of penicillin-recognizing proteins (β-lactamases, PBPs with low affinity for β-lactams, and β-lactam-sensing proteins).[177,206]

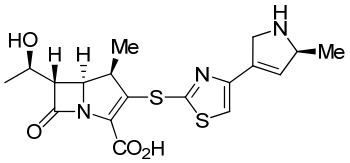
The most important mechanism of bacterial resistance to β-lactams is the expression of β-lactamases. An evolutionary link between the PBPs and β-lactamases was proposed as early as the 1960s[187] and modern gene sequence analysis has been used to describe this evolutionary relationship in detail.[207] Transpeptidases and serine β-lactamases are similar in the formation of acyl enzyme intermediates upon reaction with β-lactams (the first half-reaction), but serine β-lactamases have evolved the ability to hydrolyze the acyl enzyme intermediate much faster than transpeptidases in order to complete the second half-reaction. At the same time, however, β-lactamases have lost the ability to recognize the peptidoglycan substrate of the transpeptidases in order to be effective as enzymes that protect β-lactam-sensitive enzymes.[26,208] β-Lactamases represent the primary mode of resistance to β-lactams in Gram-negative bacteria and are discussed in more detail below (Section 1.4).

A second strategy that bacteria have used to overcome the threat of β-lactams involves PBPs which retain their main function as transpeptidases but have low-affinity for β-lactams. Some important examples of low-affinity penicillin-binding proteins include PBP1a, PBP2b, and PBP2x of *S. pneumoniae*, and PBP5 of *E. faecium*,[19,206] but the most clinically significant is PBP2a (also known as PBP2′) of MRSA.[209] *S. aureus* normally expresses four PBPs with transpeptidase activity, including three HMW PBPs (PBP1, PBP2, PBP3) and one LMW PBP (PBP4).[26] These enzymes are all unchanged in MRSA but PBP2a (which is encoded by the *mecA* gene) is expressed in addition to the

others. Surprisingly, it is not an increase in the rate of the deacylation (second half-reaction) in PBP2a, but a decrease in the rate of acylation (2–3 orders of magnitude compared to PBP2) that gives this enzyme low affinity for β-lactams.[210,211] Crystal structures indicate that a conformational change is necessary in the α-helix that contains the nucleophilic serine (Ser403) before acylation can occur.[26,212] Extensive efforts have been made to develop β-lactam antibiotics which are able to bind to PBP2a (without sacrificing activity against the other HMW PBPs) and therefore be effective against MRSA. Ceftobiprole, a new cephalosporin with impressive anti-MRSA activity,[213] is under regulatory review in the U.S. and has been approved in Canada and Switzerland for the treatment of complicated skin and skin structure infections (cSSSIs).[214] Another cephalosporin called ceftaroline and carbapenems ME1036 and razupenem are also in development as anti-MRSA β-lactams.



ceftobiprole



razupenem (PZ-601)

β-Lactam-sensing proteins represent another set of modified PBPs that contribute to β-lactam resistance. The induction of β-lactamase production in *B. cereus*,[215,216] *B. licheniformis*,[217] and other organisms has been known since the mid-1940s.[218] In the mid-1980s Salerno and Lampen reported that β-lactamase production in *B. licheniformis* 749 increased 180-fold one hour after exposure to a β-lactam and decreased slowly over the 1–2 hours that follow.[219] The induction of β-lactamase production implied that a transduction mechanism was present and a membrane-spanning protein called BlaR, which is structurally very similar to class D β-lactamases,[220] was later detected. It is believed that the reaction with the β-lactam (acylation) is accompanied by a conformational change in the protein which transmits a signal through the cell membrane to de-repress transcription of the *bla* operon and increase β-lactamase production. The *bla* operon is also present in *S. aureus* and codes for the expression of the PC1 β-lactamase.

Methicillin-resistant *S. aureus* (MRSA), however, has acquired a second mechanism for resistance with a β-lactam-sensing system coded by the *mec* operon. The sensor–transducer protein MecR1, coded by the *mecR* gene, undergoes a conformational change[221] upon reaction with β-lactams and signals the production of PBP2a, the low-affinity penicillin-binding protein mentioned above.[222]

1.4 β-Lactamases

β-Lactamases are enzymes that efficiently hydrolyze β-lactam antibiotics. Serine β-lactamases form an acyl enzyme intermediate upon reaction with β-lactams in the same way that penicillin-binding proteins do; however, β-lactamases have evolved the ability to hydrolyze this acyl enzyme intermediate much more quickly than PBPs. β-Lactamases were discovered by Abraham and Chain in 1940[27] and currently represent the most important mechanism of bacterial resistance to β-lactam antibiotics. The widespread use of penicillins and cephalosporins over the last six decades has been an enormous evolutionary driving force and, as with all types of antibiotics, has inadvertently selected for resistant bacterial strains. It should be noted, however, that β-lactamases did not evolve from PBPs *as a result of* the selective pressure exerted by humans. Instead, β-lactamases are ancient enzymes which evolved from DD-peptidases billions of years ago.[223,224] Correlation of their amino acid sequences shows that the different classes of β-lactamase are more closely related to different classes of PBPs than to each other.[207]

1.4.1 β-Lactamases and Resistance to β-Lactams

The introduction of benzylpenicillin into widespread use in the mid-1940s was quickly followed by β-lactamase-producing penicillin-resistant staphylococci. By 1948 50% of hospital isolates of *S. aureus* had become penicillin-resistant and this number had risen to 80% by the mid-1950s.[225] Methicillin, which was introduced in 1960 as the first β-lactamase-stable semisynthetic penicillin, reduced considerably the problem of penicillin-resistant staphylococci and, as a result, had also reduced the perceived clinical relevance of the β-lactamases.[226] However, these enzymes were recognized to be of crucial importance when it was realized in the mid-1960s that new broad-spectrum penicillins and cephalosporins such as ampicillin and cephaloridine (which were stable to staphylococcal β-lactamase) were not stable to β-lactamases of Gram-negative bacteria. Significant advances were made in the fight against β-lactamase-producing organisms in the 1970s and 80s with the second- and third generation cephalosporins, ureido penicillins, carbapenems, and clavulanate, but the clinical utility of these β-lactams is currently threatened by the increasingly more common extended-spectrum β-lactamases (ESBLs) and carbapenemases which are often plasmid-encoded and expressed in multidrug-resistant human pathogens.

1.4.2 β-Lactamase Classifications

Penicillinase was the original name that Abraham and Chain[27] had given to the enzyme that destroyed the growth-inhibiting activity of penicillin and this name was based simply on biochemical function. It became apparent when additional substrates became available in the years to follow (e.g. semisynthetic penicillins, cephalosporins) that enzymes from different sources exhibited different properties and enzymes were described as penicillinases or cephalosporinases based on their preferred substrates.[227] The term "β-lactamase" entered common use in the 1960s to distinguish the enzymes that hydrolyze the β-lactam amide bond from penicillin acylases or amidases, which hydrolyze the 6-acylamino sidechain of the penicillins, and cephalosporin esterases, which hydrolyze the C3′ acetoxy group.[218]

The first widely accepted classification scheme for β-lactamases was proposed by Richmond and Sykes in 1973[228] and enzymes were classified into five major groups on the basis of substrate profile and inhibition profile (cloxacillin, NaCl, and *p*-chloromercuribenzoate) and sub-grouped according to whether the enzyme was inducible or constitutive.[229] Sykes and Matthew revised and updated this classification in 1976 and divided enzymes according to whether the β-lactamase was chromosomally encoded or R-factor-mediated.[218,230]

In 1980 Ambler proposed a different classification based on the amino acid sequences of four serine β-lactamases that were available and a partial sequence for a zinc-dependent enzyme.[231,232] The four very similar serine β-lactamases (PC1, BcI, TEM-1, and *B. licheniformis* 749/C) were called class A β-lactamases and the metallo-β-lactamase (BcII) designated class B. The first of the class C β-lactamases were described in 1981 when an AmpC cephalosporinase from *E. coli* was sequenced[233] and it was suggested in 1987 that the oxacillinases OXA-1 and OXA-2 be classified as class D β-lactamases because of the lack of sequence homology with the class A and C enzymes.[234]

The Bush classification, which was proposed in 1989 as a modification of the Richmond–Sykes scheme, was the first attempt to correlate β-lactamase function (substrate and inhibitory profiles) with structure and sequence.[235,236] The Bush scheme grouped cephalosporinases into group 1 (e.g. Gram-negative AmpC enzymes), penicillinases into group 2 (e.g. Ambler class A and D enzymes), and metallo-β-lactamases into group 3. Updates to this classification were made in 1995[237] and 2010[238] to include new sub-groups for enzymes such as extended-spectrum β-lactamases and carbapenemases.[239]

A table of important β-lactamases, sorted according to the Ambler and Bush–Jacoby classifications is supplied in Appendix B. Several reviews of β-lactamases were published in the 1980s[225,226,240, 241] and 1990s[242,243] and other reviews have been written more recently.[26,244,245]

1.4.3 Class A β-Lactamases

The first enzymes to be designated as class A β-lactamases include the chromosomally-encoded penicillinases PC1 of *S. aureus*, *B. cereus* β-lactamase I (BcI), and the β-lactamase of *B. licheniformis* 749/C, and the R-factor (plasmid)-encoded broad-spectrum β-lactamase TEM-1 (R-TEM) of *E. coli*.[231] The plasmid-encoded TEM-1 quickly spread worldwide within a few years of its first isolation and its genetic location has also facilitated its transfer to other species of bacteria.[246] TEM-1 is an enzyme in Bush's functional group 2b and is able to hydrolyze penicillins and early generation cephalosporins such as cephalothin and cephaloridine. TEM-1 is the most commonly encountered β-lactamase in Gram-negative bacteria and accounts for most ampicillin resistance in *E. coli* and contributes to the increasing resistance in *H. influenzae*, and *N. gonorrhoeae*.[246] As of November 2010, more than 180 unique TEMs have been identified.[247]

Mutations to TEM-1 over the last three decades have given rise to TEM variants with the ability to hydrolyze oximino cephalosporins more efficiently. These enzymes were called extended spectrum β-lactamases (ESBLs) and have been classified by Bush as group 2be.[248] Other mutations provide resistance to clavulanic acid and these TEM derivatives were called inhibitor-resistant TEMs (IRTs) (e.g. TEM-30 and -31, group 2br). However, the mutations that increase the spectrum of activity, which generally 'open' the active site, typically also enhance the susceptibility of the enzyme to clavulanate and are usually distinct from mutations that lead to inhibitor resistance.[244] Complex mutant TEMs (CMTs) such as TEM-50 and TEM-158 represent exceptions to this trend since they both have ESBL activity and a modest increase in resistance to clavulanate (group 2ber).[238]

The SHV β-lactamases, which have high sequence homology and similar overall structures with the TEM enzymes, is the second largest among Ambler class A β-lactamases with more than 130 members.[247] Most mutants of SHV-1 (a group 2b enzyme) have ESBL activity (Bush's group 2be), a small number have an inhibitor-resistant phenotype (e.g. SHV-10, group 2br), but none of the SHV enzymes characterized to date display both phenotypes (functional group 2ber). SHV β-lactamases

are most commonly found in *K. pneumoniae* but have also been found in *E. coli* and *P. aeruginosa*.[246]

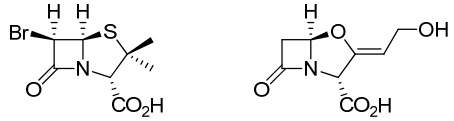
Over the last decade, the CTX-M family of ESBLs has emerged as the largest group of class A β-lactamases not related to the TEM or SHV enzymes.[249] Most (but not all) CTX-Ms have greater activity against cefotaxime than ceftazidime, they hydrolyze cephaloridine and cephalothin better than benzylpenicillin and, in contrast to TEMs and SHVs, they are inhibited more effectively by tazobactam than clavulanate.[238] Currently, more than 100 CTX-Ms are known and all belong to functional group 2be.[247] TOHO-1 and TOHO-2 are ESBLs that were later found to be part of the CTX-M group and are now also called CTX-M-44 and -45, respectively. The PER and VEB families are other ESBLs in group 2be that are unrelated to the TEMs, SHVs, or CTX-Ms.

β-Lactamases with carbapenemase activity have also arisen from class A and these group 2f enzymes include the SME, IMI, NMC-A, KPC, and GES families. The SME, IMI, and NMC-A families are closely related as IMI-1 and NMC-A share 97% amino acid identity and SME has approximately 70%. The SME, IMI, and NMC-A β-lactamases are all chromosomally located (except for IMI-2) and are isolated relatively rarely. These enzymes have a broad spectrum of activity which includes penicillins, early cephalosporins, aztreonam, and carbapenems.

The KPC and GES families, however, are plasmid-encoded β-lactamases which are able to hydrolyze oximino cephalosporins in addition to penicillins and carbapenems. While GES enzymes have been isolated from *E. cloacae*, *K. pneumoniae*, and *P. aeruginosa* from cities scattered throughout the world, they have not been associated with large outbreaks. KPCs, on the other hand, have the greatest potential to spread as they are most commonly found in *K. pneumoniae*, an organism known for its ability to transfer resistance determinants. Several reports of KPC-producing *K. pneumoniae* have come from New York and the east coast of the U.S. since the early 2000s and, more recently, from other countries worldwide.

The TEM family of class A β-lactamases were the most abundant and clinically significant group of β-lactamase in the 1980s and the class A enzymes have been the best studied of the four Ambler classes since that time. The mechanisms of β-lactam hydrolysis and β-lactamase inactivation have been topics of interest since the β-lactamases were discovered. Various chemical methods were used in the 1970s in an effort to define active site amino acids and sequencing of various β-lactamases was also undertaken with the hope that conserved residues might be identified. Speculation that the active

site nucleophile in β-lactamases was a serine was confirmed by studies in 1979 with the irreversible β-lactamase inhibitor 6β-bromopenicillanic acid (see section 1.5.2).[250] Inactivation of BcI with [^{3}H]-6β-bromopenicillanic acid followed by a tryptic digest demonstrated that the ^{3}H label was bound to the Ser44-containing peptide[251] and these results provided the first strong evidence that β-lactamases act through a serine acylenzyme mechanism.[225]



6β-bromopenicillanic acid clavulanic acid

The conclusion that an acylenzyme intermediate is formed during catalysis was supported by subsequent well-known studies in the Knowles group of the mechanism of inactivation of RTEM by clavulanate[252,253] and interaction with cefoxitin.[254] (The mechanisms of inactivation by clavulanate are discussed in more detail in Section 1.5.2). By the early 1990s X-ray crystallography and site-directed mutagenesis had become important tools for the study of β-lactamases and indicated that functional class A β-lactamase active sites included Ser70, Glu166, Lys73, Lys234, and an oxyanion hole analogous to that of serine proteases.[255] Protein crystallography continues to be very valuable for the study of β-lactamases since an understanding of the three-dimensional relationship of these residues is crucial for understanding their evolution as resistance enzymes (Figure 9).

When Glu166 was found to be near Ser70 it was naturally suspected to act as a general base both in the acylation process, by activating Ser70, and in the deacylation process, by activating the hydrolytic water molecule, but it was recognized that these conclusions were not unambiguous (Figure 10). Glu166 mutants (e.g. Glu166Asp, -Asn, -Ala, and -Arg), for example, were unable to undergo deacylation but retained the ability to undergo acylation (albeit more slowly) and these mutagenesis studies indicated that Lys73 was the general base for the acylation half-reaction.[256] In 1992 Strynadka et al. reported the X-ray crystal structure of benzylpenicillin bound to the TEM-1 deacylation-defective mutant Glu166Asn (PDB: 1FQG) as the acyl enzyme intermediate and the group supported the hypothesis that Lys73 activates Ser70.[257]

While there has been consensus since the late 1980s that Glu166 acts as the general base in the deacylation half-reaction, the identity of the general base in the acylation process has been debated

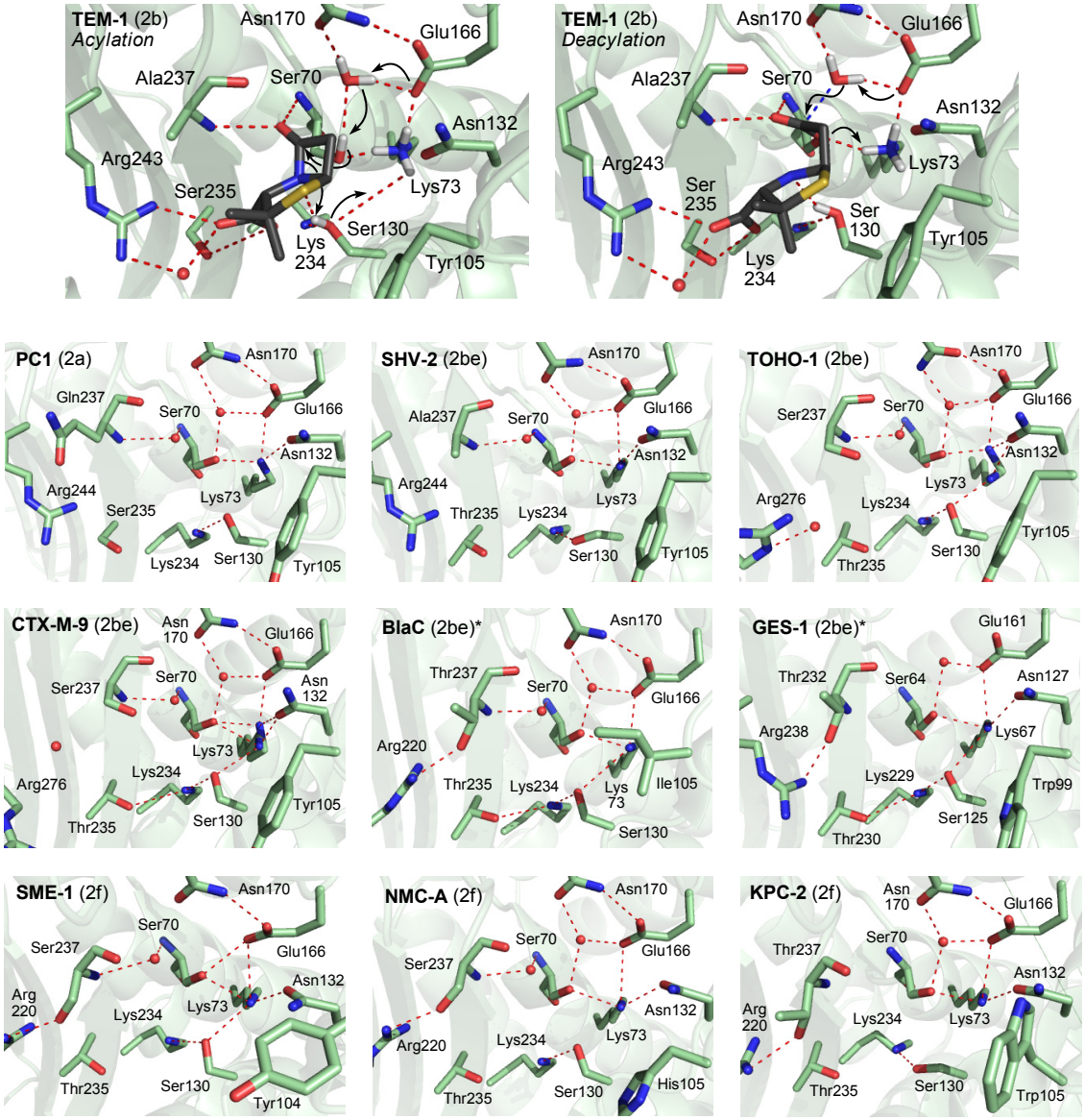


Figure 9. Class A β-lactamase active sites and the mechanism of penicillin turnover by TEM-1. The modeled Michaelis complex of TEM-1 with penicillanic acid was generated by docking the β-lactam into an X-ray structure of the wild type enzyme (PDB: 1ZG4)[258] and one of the two reasonable mechanisms[266] for acylation is indicated with arrows (albeit in a simplified manner, wherein the formation and collapse of the tetrahedral intermediate are not shown). The mechanism of deacylation is shown with arrows in a model of an acylenzyme which was generated from an X-ray structure of a penicillin-acylated TEM-1 mutant (PDB: 1FQG).[257] Figures of PC1 (3BLM), SHV-2 (1N9B), TOHO-1 (1IYS), CTX-M-9 (1YLJ), BlaC (2GDN), GES-1 (2QPN), SME-1 (1DY6), NMC-A (1BUE), and KPC-2 (3DW0) were created from X-ray coordinates deposited in the PDB. Note that the Gly170Asn mutation of GES-1 (as in GES-2 and GES-4–6) is associated with carbapenemase activity (group 2f).[259] The Bush–Jacoby functional group designations (Appendix B) for each enzyme are shown in brackets (asterisks indicate where designations are unofficial and deduced by this author). For Figure 9–Figure 14, small red spheres represent water molecules and hydrogen, nitrogen, oxygen, and sulfur atoms are coloured white, blue, red, and yellow, respectively.

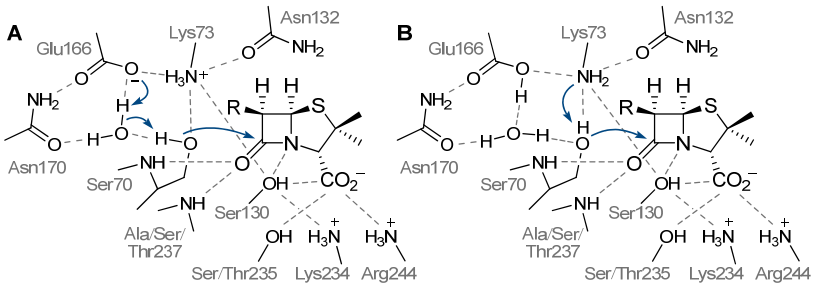


Figure 10. Mechanisms for penicillin acylation in class A β-lactamases in which Glu166 (A) or Lys73 (B) acts as the general base to activate Ser70 for nucleophilic attack. See text and ref 266.

since the early 1990s. In 2002, the Shoichet group solved an ultrahigh-resolution (0.85 Å) X-ray structure of a boronic acid transition state analogue bound to TEM-1.[260] The structure, which represents a snapshot of the complex mid-way through the acylation, exhibited clear enough electron density to view a proton bound to Glu166 H-bonding with the water molecule that bridges Glu166 and Ser70. This X-ray study of TEM-1 provides compelling evidence in favour of a mechanism in which Glu166 acts as the general base via the water molecule and Lys73 functions as a Lewis acid that increases the acidity of the Ser70 proton and facilitates its activation. The Glu166 pathway is also supported by other high-resolution X-ray studies with CTX-M enzymes[261,262] and computational work by Díaz et al.[263] and Hermann et al.[264]

Recent work by the Mobashery group, however, indicating that the pK_a of Lys73 in TEM-1 is 8.0–8.5, has led the group to suggest that the acylation step involves deprotonation of Lys73 by Glu166, followed by activation of Ser70 by a neutral Lys73.[265] Subsequent high-level ab initio QM/MM calculations from the same group indicate that both pathways exist in TEM-1 and Ser70 is activated by dual participation of Glu166 and Lys73.[266] The energy barrier calculated for the pathway in which Glu166 activates Ser70 through a water molecule is 26 kcal/mol (Figure 10A) while the pathway involving Lys73 (neutral) activating Ser70 directly has a barrier of 22 kcal/mol (Figure 10B).[267]

The formation of the tetrahedral intermediate (not shown in Figure 9 for clarity) is often called a "proton shuttle" mechanism in which the proton of Ser70 is shuttled to Glu166. The collapse of the tetrahedral intermediate is referred to in the same way as the proton of Lys73 is shuttled to the nitrogen of the substrate via Ser130 as the β-lactam ring is opened.[268] Lys234 serves to enhance the acidity of Ser130 in the breakdown of the tetrahedral intermediate. As for the second half-reaction, it is widely accepted that hydrolysis of the acylenzyme intermediate occurs through activation of the

hydrolytic water molecule by Glu166.

The roles of important residues in the active sites of class A β-lactamases have been studied in detail over the past few decades. Several common mutations in TEM- and SHV β-lactamases are apparent upon inspection of the TEM and SHV tables provided by Bush and Jacoby (http://www.lahey.org/studies/). The effects of several mutations on substrate and inhibition profiles of class A ESBLs and IRTs have been reviewed by Fisher et al.[26] and Drawz and Bonomo.[29] While the study of class A carbapenemases is less advanced, it is clear that the introduction of a new disulfide bond (Cys69–Cys238) is important for the acquisition of carbapenemase activity. This cystine serves to enlarge the binding cavity for the hydroxyethyl sidechain of the carbapenems by causing a change in conformation of the S3 strand, which contains residues 237–240, and indirectly altering the orientation of Asn132.[259]

1.4.4 Class C β-Lactamases

The penicillin-destroying "penicillinase" first reported by Abraham and Chain in 1940 was actually an *E. coli* cephalosporinase.[269] In the 1960s and 1970s, many Gram-negative bacteria were found to produce chromosomal cephalosporinases (encoded by the *ampC* gene) and many of these were normally expressed at low levels but inducible in the presence of β-lactams.[218,270] The AmpC β-lactamase of *E. coli* strain K-12 was sequenced in 1981 and became the first enzyme of the class C β-lactamases because of its lack of sequence homology with known class A and B enzymes.[233]

Plasmid-mediated AmpC enzymes have been known since the late 1980s and include the MIR, CMY, BIL, FOX, DHA, ACC, and LAT β-lactamase families.[271] These plasmid-encoded enzymes, and the very closely related chromosomal AmpC β-lactamases of Bush functional group 1, generally provide resistance to penicillins, cephamycins, oximino cephalosporins, and monobactams, and are resistant to inhibition by clavulanate. Plasmid-mediated AmpCs have been found worldwide but are less often a cause of cefoxitin resistance than an increased production of chromosomal AmpCs. Hyperproduction of chromosomal AmpC β-lactamases is often a result of mutations in the regulatory gene (*ampR*) and the development of resistance in enteric organisms upon therapy is a concern.[269] Production of group 1 β-lactamases in large amounts can even provide resistance to carbapenems.[238]

In 1995 Nukaga et al. described the isolation of the first extended-spectrum class C β-lactamase

(functional group 1e) from a clinical isolate of *E. cloacae* (strain GC1) in Japan.[272] The kinetic parameters of the new GC1 β-lactamase differed significantly from the well-known class C β-lactamase P99 (the AmpC enzyme from *E. cloacae* strain P99) and its substrate profile included oximino cephalosporins normally considered unfavourable substrates. The expanded spectrum of GC1 is attributed to a duplication of three amino acid residues (Ala208–Val209–Arg210) after position 210, which increases the flexibility of the Ω loop and allows the enzyme to accommodate the bulky sidechains of oximino cephalosporins.[273] Several other group 1e β-lactamases are now known and all have amino acid insertions or alterations in the Ω loop, the R2 loop, or the H-9 helix which increase the accessibility for bulky substrates.[270]

The class C β-lactamases share with the class A β-lactamases a similar acyl enzyme mechanism of β-lactam hydrolysis, but there are several important points of difference. While the class A and C β-lactamases have both reached "catalytic perfection" for their favoured substrates (penicillins and cephalosporins, respectively), the rate-limiting step for class A enzymes is the acylation step and for class C enzymes is the deacylation.[26] Another difference, which was revealed by an X-ray structure of P99 in 1993,[274] is that the approach of the hydrolytic water molecule in the class C β-lactamases occurs from the "β" face while the approach in class A enzymes is from the "α" face.

The first X-ray crystal structure of a class C enzyme was that of the chromosomal β-lactamase of *Citrobacter freundii* in 1990 (PDB: 1FR1).[275] Comparison of the structure of this AmpC enzyme with the class A enzyme TEM-1 revealed that they shared similar folds and active site residues. The residues of the class C β-lactamases Ser64, Lys67, Lys315, and Tyr150 occupy similar positions as the catalytically important residues Ser70, Lys73, Lys234, and Ser130 of the class A enzymes. Since the class C enzymes lack a residue equivalent to Glu166 of the class A β-lactamases, Oefner et al. suggested that Tyr150 (as its anion) might act as a general base in both the acylation and deacylation steps of β-lactam hydrolysis.[275]

The anionic Tyr150 was proposed to activate Ser64 directly in the formation of the tetrahedral intermediate, to shuttle the proton of Ser64 to the nitrogen of the β-lactam substrate in the breakdown of the tetrahedral intermediate, and to activate the hydrolytic water molecule for hydrolysis of the acyl enzyme intermediate. This hypothesis is inconsistent, however, with NMR experiments which indicate that the pK_a of Tyr150 is >11 (in the apo enzyme)[276] and site-directed mutagenesis studies by

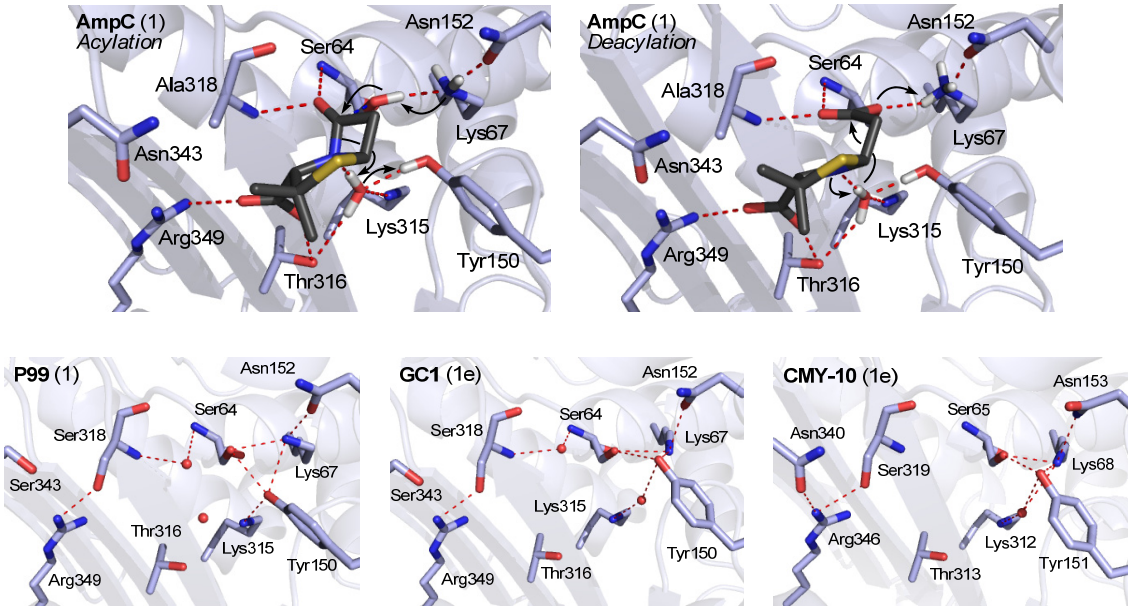


Figure 11. Class C β-lactamases and the mechanism of penicillin turnover by *E. coli* AmpC. The modeled Michaelis complex with penicillanic acid was generated by docking the β-lactam into an X-ray structure of AmpC (PDB: 3GSG)[277] and a simplified mechanism of acylation (wherein the formation and collapse of the tetrahedral intermediate are not shown) is indicated with arrows. The mechanism of deacylation is shown with arrows in a modeled acylenzyme that was generated using X-ray structures of a penicillin-acylated AmpC (PDB: 1LL9)[278] and a boronic acid-inhibited AmpC (2FFY). Figures of the P99 (1XX2), GC1 (1GCE), and CMY-10 (1ZKJ) active sites were created from X-ray structures downloaded from the PDB. Bush–Jacoby functional group designations are shown in brackets.

Dubus et al.[279] Mutation of Tyr150 to Phe, Ser, and Glu produced much smaller changes in k_{cat} (200- to 2000-fold) than would be expected for substitution of a catalytically important residue. These results implicate Lys67 (as the free base) as the general base in the acylation process, either by activating Ser64 directly or via Tyr150 (Figure 11).

The mechanism of deacylation in the class C β-lactamases has also been debated. The original proposal that Tyr150 (as its anion) activates the hydrolytic water are clearly challenged by the NMR studies of the Ishiguro group which indicate that its pK_a is >11.[276] A related model, supported by Gherman et al.,[280] involves activation of the hydrolytic water molecule by the basic form of Lys67 through Tyr150 (neutral). This hypothesis is disfavoured, however, by a recent high-resolution X-ray structure of AmpC (PDB:2FFY) from the Shoichet group which resolves a proton on Tyr150 (H-bonding with an oxygen of the boronic acid transition state analogue) and shows that Lys67 is fully protonated.[281]

Given that the approach of the hydrolytic water occurs from the β-face in class C enzymes, the Mobashery group proposed a mechanism for deacylation involving activation of the water molecule by the thiazolidine nitrogen of the substrate itself.[282] The evaluation of a pyrrolidine-containing non-β-lactam substrate and a cyclopentane (amine-lacking) analogue supported this proposal as the amine-containing analogue was turned over (underwent acylation and deacylation) and the amine-lacking substrate acted as an irreversible inhibitor (acylation only). This type of activation has been called substrate-assisted catalysis and this mechanism is consistent with available X-ray crystal structures of AmpC.[281,283]

1.4.5 Class D β-Lactamases

Among the first types of β-lactamases known to be encoded on Gram-negative plasmids were the TEM, OXA, SHV, and PSE families.[284] The OXAs were originally set apart from the TEMs by their ability to hydrolyze isoxazoyl β-lactams such as oxacillin and cloxacillin. In the late 1980s OXA-1, OXA-2, and PSE-2 (now known as OXA-10) became the first class D β-lactamases when their sequences were found to be very similar to each other but very different from the class A and C serine β-lactamases that had been sequenced.[234,285]

Over the last few years, the number of enzymes in the OXA family has increased drastically. While only 20 class D enzymes were known in 2000[286] and 50 in 2005,[26] nearly 200 OXA variants have been described to date.[247] The class D β-lactamases have expanded from a series of narrow spectrum enzymes (functional group 2d) to become the most diverse of the four Ambler classes, with increasing numbers of OXAs with ESBL activity against third generation cephalosporins (group 2de) and carbapenemase activity (group 2df). The OXAs have also expanded from their historical host of *P. aeruginosa* to other Gram-negative organisms including *E. coli*, *K. pneumoniae*, and most importantly, *A. baumannii*.[287]

The OXAs were originally defined as β-lactamases that hydrolyze oxacillin faster than benzylpenicillin but many of the recently characterized OXAs hydrolyze oxacillin only poorly. Most class D β-lactamases, however, can hydrolyze amino- and ureidopenicillins well, most are resistant to inhibition by clavulanate, tazobactam, and sulbactam, and most are inhibited by NaCl.[288]

Several OXA subgroups have been identified within the Ambler class D enzymes and the most

important of the narrow spectrum families are the OXA-1, OXA-2, and OXA-10 subgroups. Whereas OXA-1 and OXA-2 variants mostly retain a narrow spectrum of activity, many OXA-10 variants, including OXA-11, OXA-13, OXA-16, OXA-28, OXA-35, and OXA-74, have increased activities toward expanded-spectrum cephalosporins. OXAs with ESBL activities which are not variants of narrow spectrum OXAs include OXA-18, OXA-45, and OXA-53.[288]

The most clinically important OXAs are the carbapenem-hydrolyzing class D β-lactamases (CHDLs), which include those of the OXA-23, OXA-40 (OXA-24 is now known as OXA-40), OXA-48, OXA-51, and OXA-58 subgroups and others.[289] The importance of the CHDLs to carbapenem resistance has been debated because of the relatively low level of hydrolysis of carbapenems by these enzymes, but knockout and complementation experiments demonstrate that OXA-23, OXA-40, and OXA-58 (to a lesser extent) contribute significantly to carbapenem resistance in *A. baumannii*.[290] So far, none of the class D β-lactamases have both ESBL and carbapenemase activity.

Structural and mechanistic information about the class D β-lactamases was extremely limited until 2000, when two X-ray crystal structures of OXA-10 were published independently by the Strynadka[291] and Mobashery[286] groups. Both groups noted that the overall folds of the class D β-lactamase are more similar to class A enzymes than to class C enzymes and that several residues important for catalysis in TEM-1 aligned very well with residues in OXA-10. An important difference, however, was that OXA-10 appeared to lack an acidic residue equivalent to Glu166, the residue that functions as a general base in TEM-1. Lys70 was proposed as the best candidate for the general base in OXA-10 by both Strynadka and Mobashery, since it was thought that its pK_a might be lowered significantly by its hydrophobic environment. This explanation was found to be incomplete later that year, when Mobashery and coworkers published an X-ray crystal structure of OXA-10 in which Lys70 was carboxylated (Figure 12).[292] As an unusual functional group with some precedent, this carboxylated lysine was proposed to act as the general base in the hydrolysis of β-lactams, through direct activation of Ser67 in the acylation process and through activation of the hydrolytic water molecule in the deacylation half-reaction (Figure 13).

The discovery of lysine carboxylation in OXA-10, which is reversible, pH dependent, and critical for enzymatic activity,[293] was a major breakthrough in the study of class D β-lactamases because these enzymes were notorious for poorly reproducible behaviour in enzyme assays (biphasic burst-

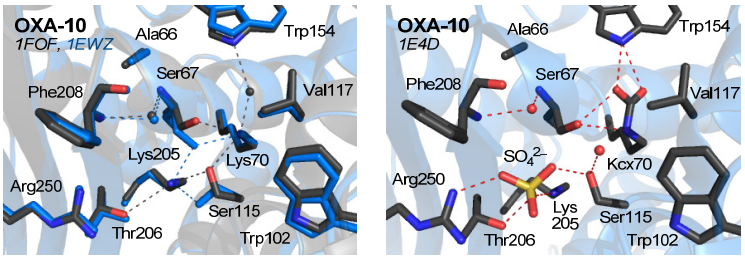


Figure 12. The discovery of Lys70 carboxylation in class D β-lactamases. The first X-ray structures of OXA-10 by Strynadka (PDB: 1FOF) and Mobashery (1EWZ) are shown in black and blue, respectively. A subsequent structure (1E4D) showed that Lys70 is *N*-carboxylated (Kcx70).

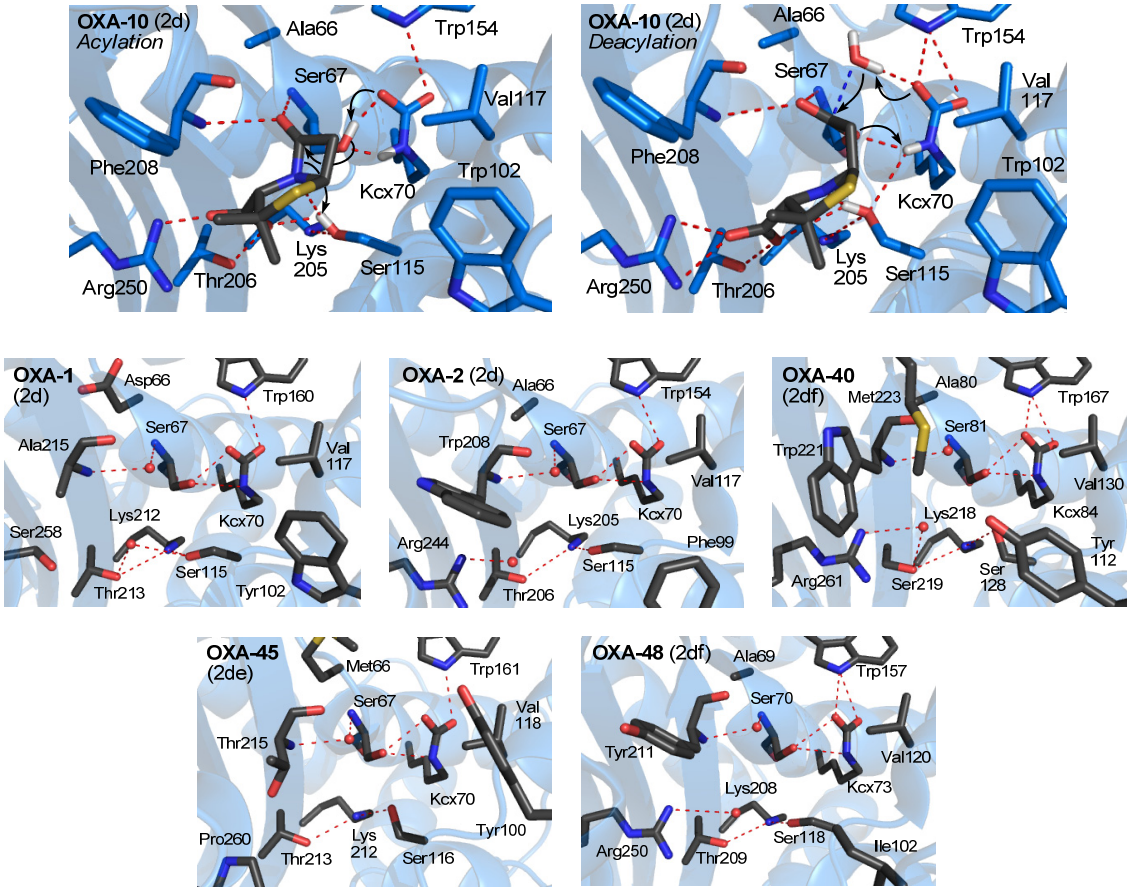


Figure 13. Class D β-lactamase active sites and the mechanism of penicillin turnover by OXA-10. The Michaelis complex shown was generated by docking penicillanic acid into an X-ray structure of OXA-10 (3LCE) and arrows indicate a simplified version (without formation and collapse of the tetrahedral intermediate) of one possible mechanism of acylation.[298] The acylenzyme structure shown is a model created from the PDB files 2WG1 and 1K54 and a possible mechanism for deacylation indicated with arrows. Images of OXA-1 (1M6K), OXA-2 (1K38), OXA-40 (3G4P), and OXA-48 (3HBR) were created from X-ray structures retrieved from the PDB. The X-ray structure of OXA-45 was kindly provided by Prof. J. Spencer (U. Bristol, U.K.) prior to publication. Bush–Jacoby functional group designations are shown in brackets.

type progress curves).[26] Supplementation of the medium with bicarbonate to mimic the in vivo concentration of CO_2 (ca. 1.3 mM)[294] restores the kinetic profile for most enzymes, but some OXAs retain biphasic profiles with some substrates. Evidence that OXA-10 exists predominantly as a dimer and that the dimer is the more active form,[295,296,297] led to the proposal that a monomer–dimer equilibrium might be responsible for the complex biphasic kinetics (of OXA-10 and other OXAs); however, this rationale is not applicable to in vitro biochemical assays conducted with nanomolar concentrations of enzyme since dissociation constants for these enzymes are typically in the micromolar range.[293]

It is widely accepted that the carboxylated lysine functions as the general base in the acylation and deacylation half-reactions, but the exact mechanisms of these processes have not yet been elucidated. Some of the most detailed mechanistic information currently available on the OXAs includes the results of advanced computational work done in 2005.[298] Li et al. used QM/MM methods to investigate the mechanism of lysine carboxylation in OXA-10 and found that carboxylation is exothermic in the enzyme and that carboxylation of Lys70 (neutral) by CO_2 (and not bicarbonate) is mediated by a water molecule. It is possible that this water molecule, which is the hydrolytic water involved in deacylation, is also involved in the acylation process as a bridge between Kcx70 and Ser67. In addition, the authors suggest the possibility that a decarboxylation event partway through β-lactam turnover may be the main source of the biphasic kinetics observed with various OXAs since it is known that Lys70 must be carboxylated for catalysis to resume.

Additional mechanistic insights have been gained from a number of X-ray structures of OXAs with and without inhibitors but a recent focus of research in this area has involved structural studies with OXA carbapenemases. The Romero group, which reported the crystal structure of OXA-24 (now OXA-40) in 2007, postulated that the specificity of OXA-40 for carbapenems is a result of the hydrophobic barrier formed by residues Met223 and Tyr112 (Figure 13).[299] The importance of these residues was demonstrated by site-directed mutagenesis but an explanation for the improved activity toward carbapenems, compared to other OXAs, was not provided. In 2009 Docquier et al. published the structure of OXA-48, a carbapenemase which is more similar in sequence and 3D structure to the narrow-spectrum enzyme OXA-10 than to the carbapenemase OXA-40.[300] With support from molecular dynamics simulations, the authors proposed that the orientation and flexibility of the β5/β6

loop of OXA-40 and OXA-48 better accommodates the hydroxyethyl sidechain of carbapenems than the corresponding loop of the narrow-spectrum enzymes OXA-10 and OXA-13.

1.4.6 Class B β-Lactamases

The first metallo-β-lactamase, BcII of *Bacillus cereus*, was discovered indirectly by Newton and Abraham during the structural elucidations of penicillin N and cephalosporin C in the mid-1950s.[75,216] Crude preparations of β-lactamase from *B. cereus* showed a selective loss of cephalosporinase activity upon purification, and a loss of penicillinase activity in aqueous solution at 60 °C, indicating that the preparations were mixtures of two enzymes. The metal dependence of the cephalosporinase was confirmed with experiments in the mid-1960s which showed that activity was lost upon treatment with EDTA but restored with the addition of $ZnSO_4$.[301] After purification of the penicillinase (called BcI) and cephalosporinase (called BcII) by fractional precipitation with a ammonium sulfate, BcII was shown to hydrolyze semisynthetic penicillins and cephalosporins at a rate comparable to benzylpenicillin.[302]

BcII was the only known Ambler class B[231] (Bush group 3) metallo-β-lactamase (MBL) for many years until the identification of L1 from *Pseudomonas* (now *Stenotrophomonas*) *maltophilia* in 1982[303] and the imipenem-hydrolyzing MBLs CphA and CcrA (also called CfiA) from *Aeromonas hydrophila* and *Bacteroides fragilis*, respectively, in 1986.[304,305] Despite the ability of these enzymes to hydrolyze carbapenems and their resistance to inhibitors, the MBLs were not widely considered an immediate threat in the 1980s because these enzymes were chromosomally encoded. A plasmid-encoded metallo-β-lactamase was discovered in 1991, however, when imipenem resistance due to the production of the MBL was found to be transferable in a Japanese isolate of *P. aeruginosa*.[306] This enzyme, which was later identified as IMP-1, has a broad spectrum activity that includes penicillins, cephalosporins, and carbapenems (but not aztreonam).[307] IMP-1 was subsequently isolated from *Serratia marcescens*[308] and members of the IMP family, now known to be mobilized by integrons, were found in other strains of *Pseudomonas* spp., *Acinetobacter* spp., and Enterobacteriaceae in Japan.[309] Since the mid-1990s the IMPs have spread worldwide[259,310,311] and 24 IMP variants have been reported to date.[247]

The VIM family of metallo-β-lactamases was first discovered in Verona from a strain of *P.*

aeruginosa isolated in 1997,[312] and VIM-1, like IMP-1, hydrolyzes efficiently most β-lactams except the monobactam aztreonam. The VIMs, which are mostly integron-borne, are the dominant MBLs in Europe and have quickly spread across the globe, with VIM-2 being the MBL most reported in the world.[309] The VIMs have become the second largest family of MBLs with 21 variants.[247]

The third type of acquired metallo-β-lactamase discovered was SPM-1, an enzyme first isolated from a highly resistant strain of *P. aeruginosa* in 2001 from São Paulo, Brazil.[313] GIM-1, which has some similarity to IMPs and VIMs, was identified from an isolate of *P. aeruginosa* in Germany in 2002[314] but GIM-1 has not spread as quickly as the other MBLs.[310] Other MBLs identified recently include SIM-1 from a strain of *A. baumannii* isolated in Seoul, Korea,[315] KHM-1 from a *C. freundii* strain isolated in Tokyo,[316] DIM-1 from a Dutch strain of *Pseudomonas stutzeri*,[317] and AIM-1 from *P. aeruginosa* in Australia.[318]

The recent discovery of a new MBL called NDM-1, which originated from New Delhi, India, represents a very concerning development. NDM-1 was found on a class 1 integron in a strain of *K. pneumoniae* which also expressed the ESBL CMY-4 and an erythromycin esterase and was resistant to all antibiotics tested (MICs > 32 μg/mL) except colistin.[319] Transferability of the MBL gene in vivo was inferred as NDM-1 was found in a strain of *E. coli* that was later isolated from the same patient. Since its first isolation in 2006, NDM-1 has spread rapidly and has been detected in India, Pakistan, the U.K.,[320] the Netherlands, Australia,[321] the U.S., Canada,[322] Kenya,[323] Norway,[324] and Germany[325] in isolates of *K. pneumoniae*, *E. coli*, *C. freundii*, *M. morganii*, *Providencia* spp., and *E. clocae*. NDM-1 is most similar to VIM-2 (32% amino acid identity) and has an activity profile similar to the IMPs and VIMs, hydrolyzing most β-lactams well except aztreonam.

As a result of the increasing number and diversity of the metallo-β-lactamases in the late 1990s, Bush proposed[326,327] that the MBLs could be subclassified according to structure (molecular subclasses B1, B2, and B3) or subgrouped according to substrate specificities (functional groups 3a, 3b, and 3c). With additional biochemical characterization, however, the functional groupings were updated in 2010[238] to include only two functional groups (groups 3a and 3b). Subgroup 3a is comprised of enzymes which hydrolyze penicillins and cephalosporins at rates of at least 60% of the rate of imipenem hydrolysis while group 3b enzymes hydrolyze carbapenems preferentially. With respect to the molecular/structural classification scheme, most known MBLs, including BcII, CcrA

(CfiA), BlaB, and the IMP, VIM, SPM, GIM families, belong to subclass B1. Subclass B2 includes CphA, ImiS, and Sfh-I, and subclass B3 contains L1, FEZ-1, THIN-B, BJP-1, and the GOB family. Structural subclasses B1 and B3 are part of Bush's functional group 3a and subclass B2 aligns with functional group 3b.[328,329,330] In 2002 Galleni et al. proposed a standardized numbering scheme for metallo-β-lactamases based on sequence homology and structural information.[331,332]

Although MBLs have now been characterized extensively by X-ray crystallography, with nearly 100 entries for MBLs in the PDB, the mechanisms of β-lactam hydrolysis have not been completely resolved. Elucidation of these mechanisms has been complicated by the fact that Zn^{II} is silent in most spectroscopic techniques[333] and, with the exception of two structures (PDB: 1X8I, 2AIO), the lack of enzyme–substrate complexes. Furthermore, the metal content of the MBLs in vivo (mono- vs di-zinc) and the site of the zinc atom (in mono-zinc enzymes), which are issues of central importance to inhibitor design, remain controversial despite extensive study.

The earliest data regarding the metal content of the MBLs and mechanism of action was obtained by chemical methods in the mid-1970s in the study of the metal-dependent β-lactamase II of *B. cereus* (BcII). A cysteine residue became the first amino acid to be implicated in zinc binding as the presence of a thiol group in the enzyme was only detected (by derivatization with iodo-[2-^{14}C]-acetate, for example) in the presence of EDTA.[216,334] Replacement of Zn^{2+} with Co^{2+}, Cd^{2+}, Mn^{2+}, and Hg^{2+} gave enzymes with significant activity (albeit much lower) and, with respect to metal content, equilibrium dialysis revealed that at least two zinc binding sites were present.[335] The enzyme bound one zinc with high affinity and the other with much lower affinity and, although BcII was active against benzylpenicillin with only one zinc (80% V_{max} at 10 μM $ZnSO_4$), the rate of hydrolysis increased with increasing occupation of the second zinc site (full activity at 1 mM $ZnSO_4$). NMR studies by Baldwin et al. implicated three histidines as ligands for one zinc and a fourth histidine ligand as a ligand in the other zinc-binding site.[336]

In the 1980s and 90s it became apparent that the metallo-β-lactamases were quite diverse in their metal requirements for activity. The class B2 MBL of *Aeromonas hydrophila* (CphA), for example, binds one zinc tightly (nM) and the binding of a second zinc is inhibitory to enzymatic activity (K_i = 46 μM).[337] The class B1 and B3 MBLs BcII, CcrA, BlaB, IMP-1, VIM-4, and L1 are known to require two zincs for maximal activity but each of these enzymes has also been shown to be active (at

a reduced level) in its mono-zinc form.[335,338,339,340,341,342,343] The metal requirements for the activity of SPM-1 are uncertain, however, since the native form of the enzyme was found to contain 1.5 mol Zn per mol protein[344] and the X-ray crystal structure of SPM-1 (PDB: 2FHX) shows only one zinc bound in the active site.[345]

With the free zinc ion concentration in the cell estimated to be in the picomolar range and the low affinity of the MBLs for a second zinc atom, Wommer et al. suggested in 2002 that the physiologically relevant form of an MBL is the mono-zinc state and that the di-zinc form is unattainable under physiological conditions.[341] The group also proposed that the apo (metal-free) form of the enzyme exists under normal conditions as they had found experimentally that the presence of a β-lactam substrate activated (increased affinity) apo MBLs for binding zinc. In this model, the use of a second zinc atom is not necessary for catalysis but is regarded as catalytic augmentation since an incremental improvement in activity is achieved. Despite the possibility that the mono-zinc form of the MBLs may be the physiologically relevant one, kinetic assays with MBLs in vitro require the addition of Zn^{2+} in order to improve enzyme stability and reproducibility.

As stated above, the metallo-β-lactamases have now been studied extensively by X-ray crystallography (Figure 14) and the first of these structures were reported in the mid-1990s. The structure of a mono-zinc form of BcII was published in 1995 by Carfi et al. (PDB: 1BMC),[346,347] followed by the structure of the dinuclear MBL CcrA by Concha et al. in 1996[348] and a di-zinc form of BcII by Fabiane et al. in 1998.[349] The common topology of these (and subsequent) metallo-β-lactamases is an αβ/βα sandwich fold and MLBs appear to be members of a large and ancient superfamily of metalloenzymes.[350] In the structure of the mono-Zn BcII, the zinc atom was coordinated in a tetrahedral geometry to three histidines and a water molecule. In the di-zinc structures of CcrA and BcII (and all other di-zinc B1 MBLs), one zinc binds to the same site (called the Zn1 site or 3H site) and the other is coordinated by an aspartate, a histidine, a cysteine, and a water molecule (called the Zn2 or DHC site) in a trigonal bipyramidal geometry. The remaining ligand for both zincs, which are 3.5 Å apart in the CcrA structure, is a bridging water molecule or hydroxide molecule that was proposed to act as the nucleophile in the hydrolysis. The structure of SPM-1 indicates that the unusual orientation of Asp120 may be responsible for the low affinity for zinc at the Zn2 site.[345]

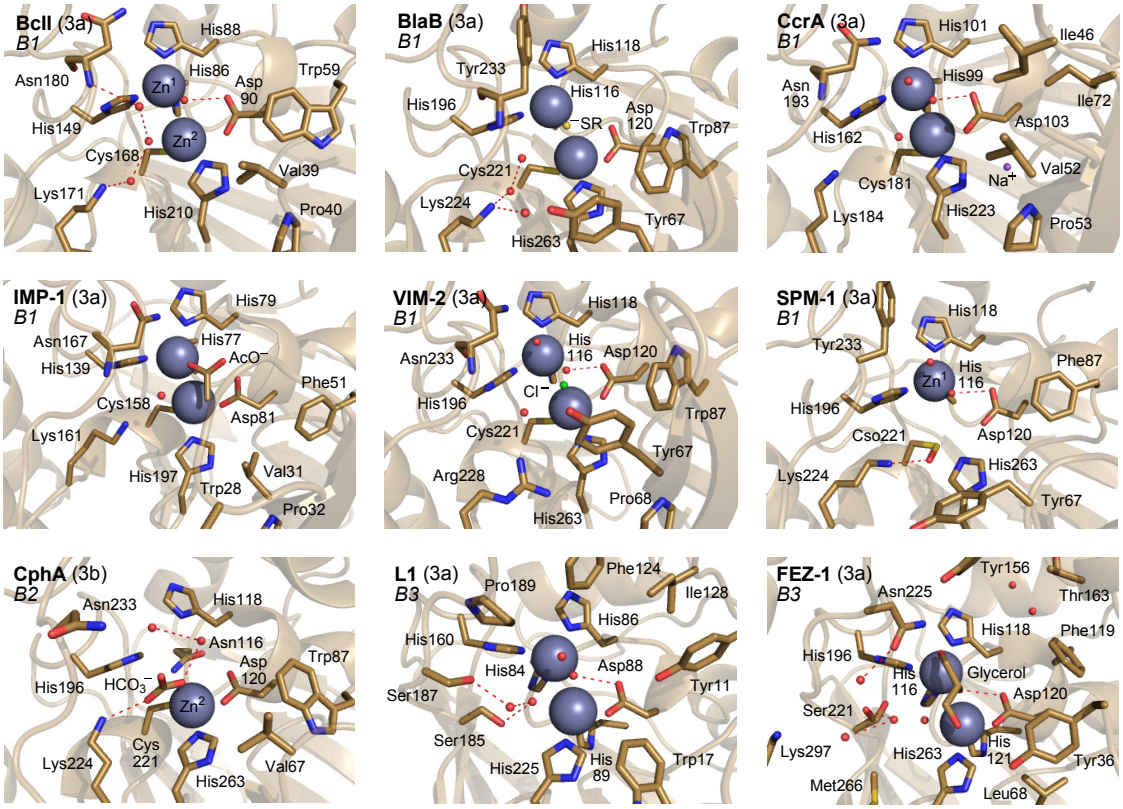


Figure 14. Class B metallo-β-lactamase active sites. Figures were generated from X-ray structures of BcII (1BC2), BlaB (1M2X), CcrA (1ZNB), IMP-1 (1DDK), VIM-2 (1KO3), SPM-1 (2FHX), CphA (1X8G), L1 (1SML), and FEZ-1 (1K07) retrieved from the PDB. All structures shown were crystallized without inhibitors except BlaB which was co-crystallized with the thiol D-captopril (not shown for clarity, except for a small yellow sphere which represents the sulfur atom that displaced the zinc-bridging hydroxide). Bush–Jacoby functional group designations are shown in brackets and molecular subclasses also indicated. It should be noted that amino acid residues have been numbered as in each PDB file and not according to the standardized (BBL) numbering schemes of Galleni[331] and Garau.[332]

The structure of L1, a di-zinc class B3 MBL from *S. maltophilia*,[351] shows that the Zn1 site is comprised of three histidines as with the class B1 enzymes but, in contrast to class B1 MBLs, the cysteine of the Zn2 site is replaced by a histidine in the class B3 enzymes and the site is therefore called the DHH site. This substitution is also present in FEZ-1,[352] indicating that the DHH site for Zn2 is a common feature of subclass B3 MBLs. The narrow-spectrum carbapenemases of subclass B2, such as CphA of *Aeromonas hydrophila*, possess the same Zn2 (DCH) site of the B1 enzymes, but the Zn1 site is comprised of two histidines and an asparagine.[353] The His-to-Asn substitution causes a substantial decrease in the affinity for zinc at the Zn1 site and class B2 MBLs function as mono-zinc

enzymes.

One of the first proposals for the mechanism of the metallo-β-lactamases was suggested by Carfi et al. in 1995 with the publication of the X-ray structure of a mono-zinc form of BcII.[346] Based on molecular models and mechanistic studies with other zinc peptidases, the zinc atom was proposed to serve as a Lewis acid toward the substrate, to polarize and activate the β-lactam carbonyl for attack, and as a Lewis acid toward the hydrolytic water, to increase its acidity. Asp90(120) was implicated as the general base that would be able to deprotonate the hydrolytic water and shuttle a proton to the nitrogen of the β-lactam. Some aspects of this early proposal are supported by recent computational work by Dal Peraro et al.[354,355] (Figure 15D) but it is thought that the pK_a of the zinc-bound water in the mono-zinc form of BcII is less than 4.9 so that it exists as a hydroxide in vivo.[356]

In one of the first proposals for the mechanism of β-lactam hydrolysis by di-zinc (class B1 and B3) MBLs, Concha et al. suggested in 1996 that nucleophilic attack at the β-lactam carbonyl is carried out by the bridging hydroxide, which is oriented appropriately through a hydrogen bond with Asp103(120), and that Zn1 and Asn193 together serve as an oxyanion hole to stabilize the tetrahedral intermediate.[348] The pK_a of the bridging hydroxide has been estimated to be between 4.9 and 5.6.[356,357] A large amount of research has been focused on the di-zinc MBLs since Concha's X-ray structure and mechanistic studies remains ongoing. A detailed summary of this research is beyond the scope of this review but several classical mechanistic studies should be mentioned. Early work in the late 1990s with BcII by Paul-Soto et al.[339,340] and Bounaga et al.,[357] for example, provided a basis for the understanding of the pH dependence of enzymatic activity, zinc binding, and inhibition by thiols. The Benkovic group proposed a minimum kinetic mechanism for the *B. fragilis* MBL (CcrA) based on the direct observation of an enzyme-bound intermediate in nitrocefin hydrolysis and was also able to rule out a mechanism involving nucleophilic catalysis by Asp103(120).[358,359,360,361] Since these reports from Benkovic, the role of Asp120 in catalysis has been studied in detail[362,363,364,365,366] and other enzyme-bound intermediates have also been detected.[333,367,368] Other recent experimental research with MBLs, which includes several publications from the Crowder and Vila groups, has been focused on elucidating the relationships of metal content, localization, and movement with enzymatic activity[356,369,370,371,372,373] and the roles of each metal in β-lactam hydrolysis.[333,372,374,375] Recent computational studies by Dal Peraro and coworkers have also made important contributions to the

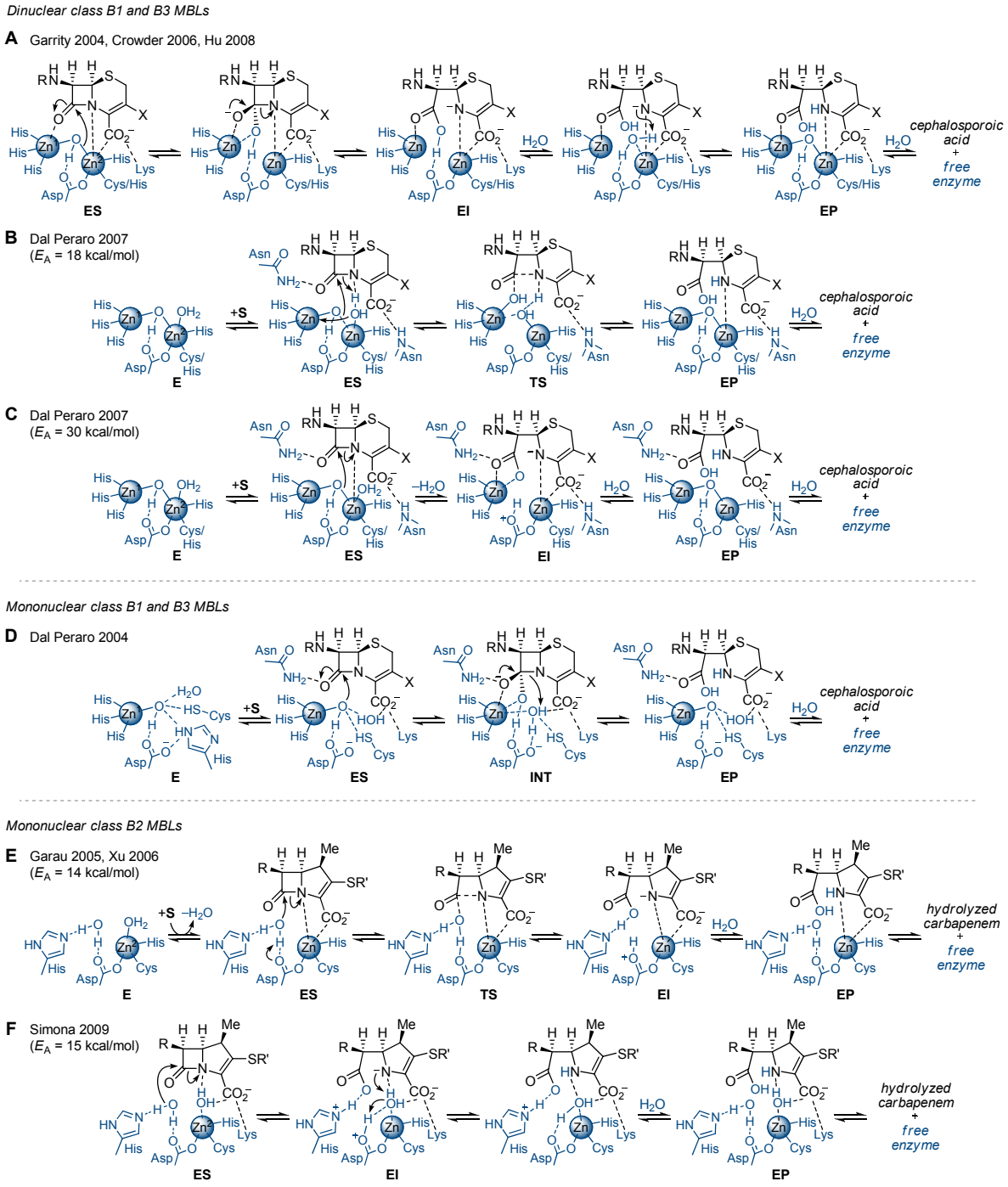


Figure 15. Proposed mechanisms of β-lactam hydrolysis by metallo-β-lactamases. Hydrolysis of cephalosporins by dinuclear class B1 and B3 enzymes (A) as adapted from Garrity et al.,[362] Crowder et al.,[350] Hu et al.[375] and (B,C) as calculated by Dal Peraro et al.[376] (D) The "water-assisted" mechanism of cephalosporin hydrolysis catalyzed by mono-zinc class B1 and B3 MBLs as studied computationally by Dal Peraro et al.[355] (E) Mechanisms of carbapenem hydrolysis by class B2 MBLs as proposed by Garau et al.[353] and calculated by Xu et al.[389] and (F) Simona et al.[390]

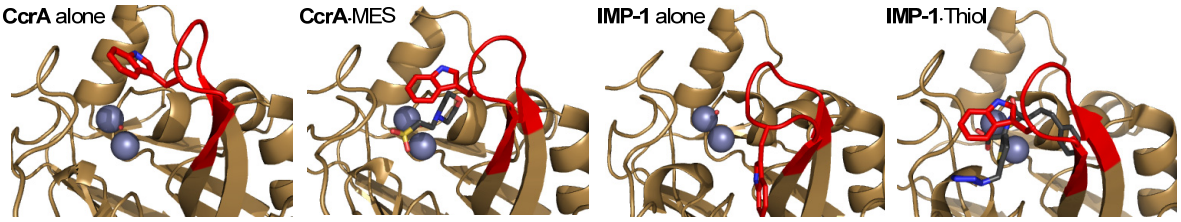


Figure 16. The flexible loop region (red) of class B1 metallo-β-lactamases. Figures were generated from X-ray structures of CcrA (2BMI), CcrA·MES (1A7T), IMP-1 (1DDK), and IMP-1·thiol (1DD6) retrieved from the PDB. (MES = 2-(*N*-morpholino)ethanesulfonic acid).

understanding of di-zinc MBL mechanisms (Figure 15B,C).[376]

An interesting characteristic of many B1 MBLs is the presence of a flexible loop near the active site. NMR characterization by Dyson and coworkers,[377,378,379] mutagenesis studies,[379,380] molecular dynamics (MD) simulations,[354,381,382,383] and X-ray crystal structures[348,384] have shown that this loop is flexible in the absence of substrate but closes down on the active site in the presence of a substrate or inhibitor (Figure 16). The tryptophan residue present in the loops of CcrA and IMP-1 interacts non-specifically with a variety of substrates and is thought to have a role in promoting catalysis. Substitution of Trp for other residues or deletion of the loop resulted in increased K_m values and reduced k_{cat} and k_{cat}/K_m values.[379,380]

While the mechanisms of the B1 and B3 MBLs have been investigated heavily since the mid-1990s, the mechanisms of the class B2 MBLs had remained poorly understood until the mid-2000s. Class B2 MBLs, which have a narrow spectrum of activity including only carbapenems, were known to bind only one zinc tightly and be inhibited by binding a second zinc,[337] but the three dimensional structure of the class B2 enzymes was unknown until the X-ray crystal structure of CphA was reported by Garau et al. in 2005.[353] In contrast to mono-zinc B1 enzymes, in which the zinc binds to the Zn1 site, the zinc of CphA was observed in the Zn2 (DCH) site as a result of the substitution of a histidine for an asparagine in the Zn1 site of the class B2 enzyme that causes a significant decrease in the affinity for a zinc atom. Although spectroscopic studies with ImiS indicate that, even in the presence of added Zn^{2+}, a second zinc atom does not binds in the active site,[385,386] recent X-ray crystal structures (PDB: 3F9O, 3FAI) of di-zinc CphA show that the second zinc binds in the Zn1 site with a distorted geometry.[387] Mutagenesis studies by Bebrone et al. demonstrate that the Asn116His/Asn220Gly double mutation significantly increases the affinity for a second zinc and also

broadens the substrate profile of CphA to include penicillins and cephalosporins.[388]

With the X-ray crystal structure of CphA alone (PDB: 1X8G) and a complex of CphA with biapenem (PDB: 1X8I), Garau and coworkers proposed a mechanism for β-lactam hydrolysis that involves activation of the hydrolytic water molecule by His118 and not by coordination to the zinc (Figure 15E).[353] Xu et al. later examined this proposal computationally and found that a similar mechanism (for the first part of the reaction), which involves activation of the hydrolytic water by Asp120 (rather than His118), has an estimated activation energy of 14 kcal/mol;[389] however, the second step of this reaction may have an energy barrier larger than experimental data.[390] Simona et. al. have advanced a mechanism in which an additional water molecule in the active site allows the nucleophilic addition and nitrogen protonation to occur in a concerted manner with a calculated activation energy of 15 kcal/mol (Figure 15F).[390] Very recently, however, Wu et al.[391] have pointed out that the Simona mechanism is inconsistent with experiments that suggest direct substrate–metal contact and refinement of mechanistic proposals for the class B2 MBLs (which should also account for recent experimental data)[392,393] appears to be an ongoing process.

1.5 β-Lactamase Inhibitors

Two approaches have been used to overcome bacterial resistance to β-lactam antibiotics due to the production of β-lactamases. The first involves alteration of the structure of the antibiotic to reduce its susceptibility to β-lactamases while its antibiotic potency is maintained. The second strategy uses a β-lactamase inhibitor in combination with a β-lactam antibiotic. While the first approach avoids the β-lactamase, the second neutralizes it.[394]

Although the concept that β-lactamase inhibitors might be used to extend the action of β-lactams against β-lactam-resistant organisms was proposed as early as 1950,[395] the first strategy that was employed clinically for circumventing bacterial resistance involved the use of semisynthetic β-lactams, such as methicillin, which retained potent antibiotic activity but were poorly recognized as substrates by β-lactamases. Early screening programs for the discovery of β-lactamase inhibitors in the 1940s and 50s, which involved miscellaneous organic, inorganic, and serine-reactive compounds, showed limited success[396] and subsequent screens of semisynthetic penicillins showed moderate

success,[397] but the screening of natural products in the late 1960s and early 1970s proved to be much more rewarding and led to the discovery of potent β-lactamase inhibitors the olivanic acids and clavulanic acid.[398] β-Lactam–β-lactamase inhibitor combinations (e.g. Augmentin, Timentin, Unasyn, Zosyn) have been used clinically for three decades and have been very successful since their introduction, with combined revenues of ca. $2.5 billion, for example, in 1997.[1]

Several excellent reviews of β-lactamase inhibition have been published, including the reviews of Cole,[396,399] Sykes and Bush,[400] and Cartwright and Waley,[401] which describe the earliest β-lactamase inhibition studies, and the reviews of Bush and Sykes,[402] Knowles,[394] Bush,[403] Pratt,[255] Rolinson,[404] and Mobashery,[405] which summarize the development of the mechanism-based inhibitors. Since 2000, several updates of these reviews have been published[406,407,408,409,410,411] and other more in-depth reviews have become available more recently.[29,412,413,414] It should be noted, however, that each of these reviews offers a different perspective on a vast subject of research that has been active for more than 70 years and, since no single article claims to be completely comprehensive, a thorough review of the work in the field requires the combination of all of the articles cited above. While a thorough review of β-lactamase inhibitors is also beyond the scope of this thesis, the summary provided herein discusses several aspects of inhibition that are often overlooked in other reviews of this topic. In addition, this summary is presented from an historical perspective in order to highlight the earliest studies that have played major roles in the development of this field of research. Some important classes of β-lactamase inhibitors are discussed only briefly but a more detailed list of known β-lactamase inhibitors is supplied in Appendix C.

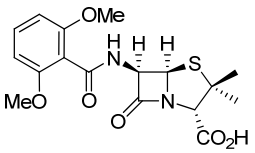
1.5.1 β-Lactams as β-Lactamase Inhibitors

Screening for β-lactamase inhibitors began in the 1940s, as noted above, but the first breakthrough came with the discovery of inhibition of penicillinase by cephalosporin C in 1956.[415] Cephalosporin C, which was insensitive to hydrolysis by the *S. aureus* penicillinase, was found to competitively inhibit the hydrolysis of penicillin G and penicillin N.

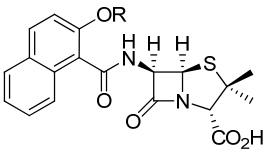
While the observation that a β-lactamase can be inhibited by a β-lactam may initially seem counterintuitive, it should be noted that each β-lactamase has a unique substrate profile and hydrolyzes one class of β-lactam more rapidly than another. β-Lactams which act as inhibitors of β-

lactamases can be considered to be substrate analogues (analogues of the enzyme's preferred substrate) and are often called poor substrates or slow substrates to emphasize this point.

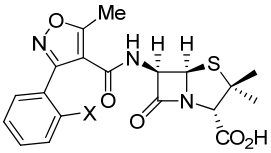
In 1960 researchers from Beecham showed that methicillin was slowly inactivated by the penicillinase of *B. cereus* (BcI) but also acted as a competitive inhibitor of benzylpenicillin hydrolysis.[84] Methicillin did not inhibit the activity of staphylococcal penicillinase (PC1) but was not hydrolyzed by this enzyme either and showed antibiotic activity against β-lactamase-producing strains of *S. aureus*. β-Lactamase inhibition gained further interest in 1964 with the demonstration of synergistic activity of methicillin and cloxacillin with benzylpenicillin and ampicillin against β-lactamase-producing Gram negative organisms.[416] Other semisynthetic penicillins and cephalosporins were evaluated for β-lactamase inhibition,[396] but synergy was found to be significant in only a small proportion of clinical isolates and levels of cloxacillin required were too high be reached in vivo.[404] Although such combinations failed to find clinical utility, these studies did encourage the continued pursuit for other β-lactamase inhibitors. While the bulky nature of the 6-acylamino sidechains decreases the affinity of β-lactamases for the aforementioned penicillins, these agents form inert acyl enzyme adducts upon reaction with the enzyme. Studies in the 1970s and 80s indicate that the substrate-induced deactivation observed is a result of conformational changes in the enzyme caused by steric interactions with the bulky aromatic sidechains.[417,418,419]



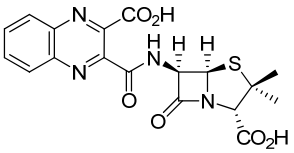
methicillin



nafcillin: R = Et
BRL 1437: R = *i*-Pr



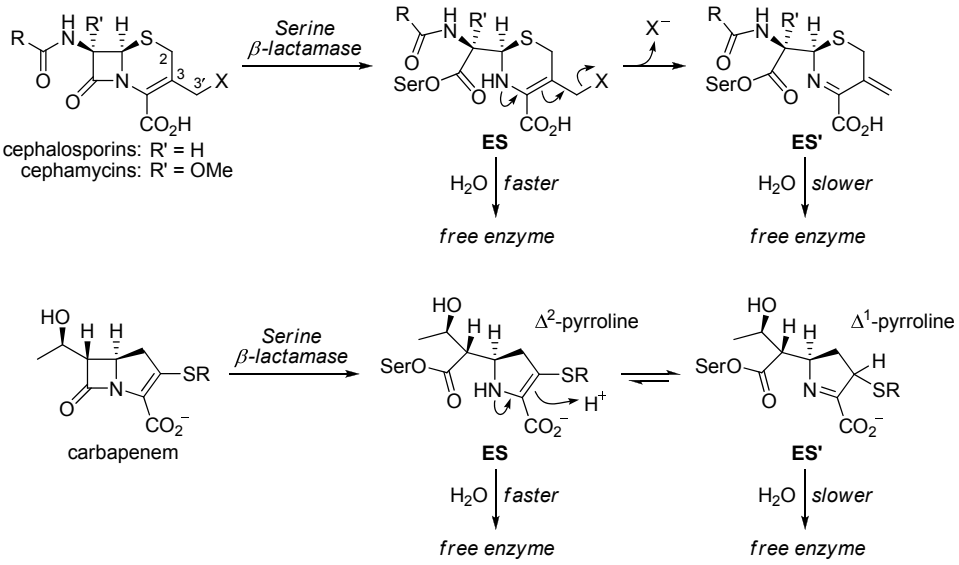
oxacillin: X = H
cloxacillin: X = Cl



quinacillin

With cephalosporins, carbapenems, and penems the formation of inert acyl enzyme species with serine β-lactamases is a result of a rearrangement of the position or conformation of the inhibitor itself within the active site. For example, the acyl enzyme complex (**ES**) formed with a cephalosporin may undergo deacylation or, if X is a sufficiently good leaving group, may undergo elimination to form **ES′** which is hydrolyzed much less readily (Scheme 10). The exact reasons for the slower hydrolysis of **ES′** are unclear but cephems which lack a C3′ leaving group are generally hydrolyzed much more rapidly and the **ES′** species has been observed as an accumulating species in class A[420] and C β-lactamases and R61 transpeptidase.[255] A similar (non-covalent) **ES′** complex is observed in

Scheme 10. Inhibition of Serine β-Lactamases by Cephalosporins and Carbapenems.

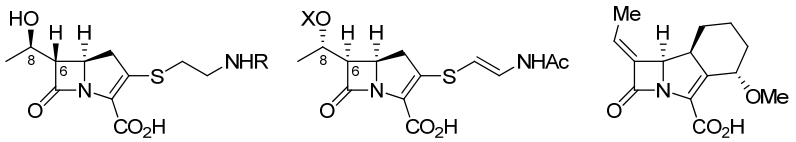


the X-ray crystal structure of a complex of the metallo-β-lactamase L1 with moxalactam (PDB: 2AIO[368]); however, to the knowledge of this author, the kinetics of elimination and importance of the C3′ leaving group with MBLs have not been studied.

The effect of the C3′ leaving group and the effect of the 7α-methoxy group of the cephamycins can reinforce each other to improve inhibition.[421] The 7α-methoxy substitution not only serves to slow the acylation process, through steric interactions which decreases the affinity of β-lactamases for cephamycins, but also slows the deacylation reaction. In acylenzyme complexes with class A β-lactamases, the methoxy group displaces the hydrolytic water molecule[422] and perturbs the conformation of Asn132 and the loop that contains Glu166.[423] In contrast, an X-ray crystal structure of a complex of the class C β-lactamase AmpC with moxalactam shows that the α-methoxy group does not displace the hydrolytic water molecule directly, but steric interactions force the oxacephem ring into a position which disfavours formation of the tetrahedral intermediate.[283]

A similar situation applies to the carbapenems which generally bind poorly to β-lactamases but also act as potent inhibitors of a broad range of β-lactamases.[255,424,425] Mechanistic studies by the Knowles group in the early 1980s indicated that the biphasic profile for carbapenem hydrolysis by TEM-1, which involved an initial fast phase for turnover followed by a slower phase, was a consequence of an isomerization of the acylenzyme to a Δ^1-pyrroline (Scheme 10).[426,427,428] While the conformations of the Δ^2- and Δ^1-acylenzyme species were found to be similar in computational work by the Mobashery

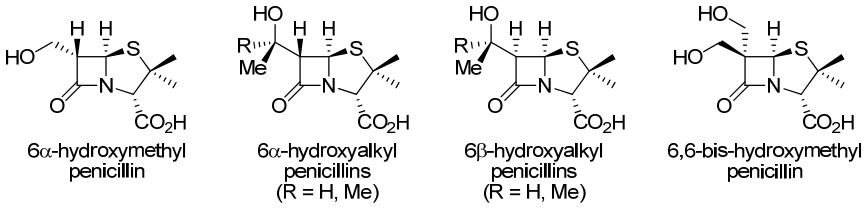
group, the studies suggested that the 6α-1*R*-hydroxyethyl substituent slowed the hydrolysis of the acylenzyme through the formation of a hydrogen bond between the hydroxyl group and Asn132 and through displacement of the hydrolytic water by the methyl group.[429] However, in 1998 an X-ray crystal structure of imipenem-acylated TEM-1 (PDB: 1BT5) showed a hydrogen bonding interaction between the hydroxyl group of the hydroxyethyl moiety and the hydrolytic water, an interaction which is thought to decrease the nucleophilicity of the water molecule.[430] In addition, the structure revealed that the carbonyl of the acylenzyme was flipped out of the oxyanion hole, as a result of a steric interaction with the adjacent methyl group, and other conformational changes in the enzyme. Rotation of the ester carbonyl out of the oxyanion hole has been observed in crystal structures of other carbapenem-acylated class A β-lactamases (e.g. SHV-1)[431] and class C β-lactamases as well.[432]



In some of the earliest studies of β-lactamase inhibition by carbapenems, the Knowles group found that the olivanic acid MM22382 behaved simply as a good substrate for TEM-2 while the sulfate ester analogue MM13902 was a poor substrate and potent inhibitor.[426] Olivanic acids, pluracidomycins, and asparenomycins were found to be potent inhibitors of a wide range of β-lactamases[406] but were not suitable for clinical use as a result of poor penetration through bacterial cell walls and rapid metabolism in humans.[404] Tricyclic carbapenems (trinems) represent another class of carbapenems which act as potent inhibitors and LK-157, one of the most potent in the series, exhibits nanomolar IC_{50} values against TEM-1, SHV-1, and AmpC, and synergy with penicillins and cephalosporins against β-lactamase-producing organisms.[433,434,435,436]

With the discovery that the hydroxyethyl group of imipenem contributes to β-lactamase inhibition, by either sterically displacing or hydrogen bonding to the hydrolytic water molecule, the Mobashery group considered the possibility that 6α-(hydroxymethyl)penam could acylate a β-lactamase but resist deacylation.[437] The molecule inhibited TEM-1 rapidly with a long-lived acylenzyme species and a partition ratio (k_{cat}/k_{inact} = 28) that is lower than those of clinically used mechanism-based inhibitors. An X-ray crystal structure of the 6-hydroxymethylated penicillin bound to TEM-1 (PDB: 1TEM) was

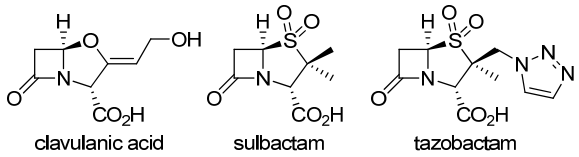
reported the following year and showed the hydroxymethyl substituent hydrogen bonded with the hydrolytic water molecule and Asn132.[438]



The strategy of incorporating 6-hydroxyalkyl substituents into penicillins for inhibition was later applied to β-lactamases such as the class A carbapenemase NMC-A[439] and the class D enzyme OXA-10[286,293,440] and X-ray crystal structures of enzyme–inhibitor complexes were solved (PDB: 1BUL, 1K54). In addition, a 6,6-bis-hydroxymethyl penicillin proved to be a moderate inhibitor of class A and C enzymes, with K_i values of 480 μM and 700 μM against TEM-1 and P99, respectively.[441]

1.5.2 Mechanism-Based Inhibitors

Each of the three clinically used β-lactamase inhibitors, clavulanate, sulbactam, and tazobactam, are called "mechanism-based" inhibitors or "suicide" inhibitors since they are recognized by the enzyme as potential substrates but, in a diversion from the normal hydrolytic process, lead to irreversible inactivation of the β-lactamase.[394,442]



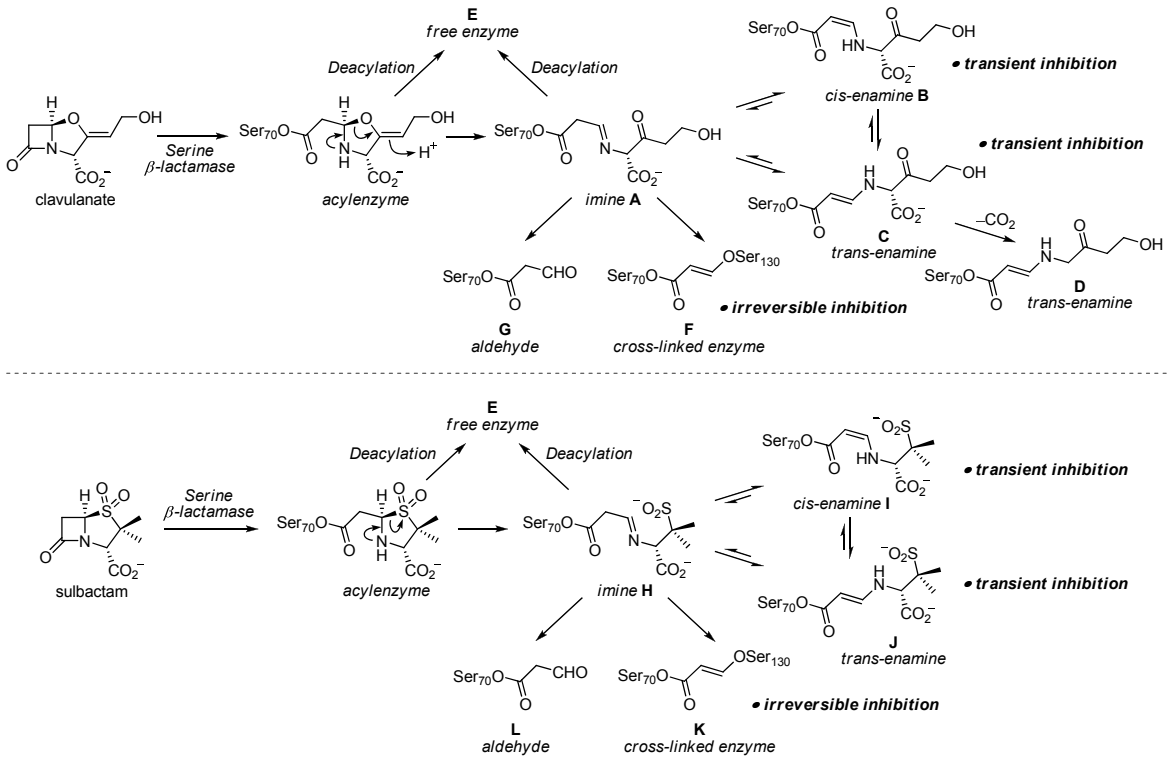
Clavulanic acid was discovered in a program begun by Beecham in the late 1960s in which microorganisms were screened for naturally occurring β-lactamase inhibitors.[398,443] Clavulanate was isolated from a strain of *Streptomyces clavuligerus* and, although it was found have only weak antibacterial activity (MICs: 31–125 μg/mL),[119] it showed synergy with ampicillin and cephaloridine against Gram-positive and Gram-negative organisms.[398] Clavulanate was found to be a potent inhibitor of penicillinases (IC_{50} values <0.1 μg/mL) but much less effective against chromosomal cephalosporinases. According to the Ambler classification of β-lactamases, clavulanic acid has potent activity against class A enzymes and some class D enzymes, but shows poorer activity against most

class C β-lactamases, and does not inhibit class B metallo-β-lactamases. Beecham's formulation of clavulanate with amoxicillin, called Augmentin, was released in the U.K. in 1981[444] and was soon followed by a combination of clavulanate with ticarcillin called Timentin.[404] Augmentin has been used in more than 819 million patient courses worldwide since its introduction[445] and generated a revenue of $1.4 billion in 1997.[5]

In 1978, shortly after the disclosure of clavulanic acid, researchers at Pfizer reported the discovery of potent β-lactamase inhibition by a semisynthetic derivative, penicillanic acid sulfone (PAS).[446] This compound showed weak antibacterial activity on its own but inhibited penicillinases with comparable potency to clavulanic acid. Penicillanic acid sulfone was also able to expand the spectrum of activity of ampicillin, penicillin G, carbenicillin, and cefazolin against Gram-positive and Gram-negative organisms, although PAS was slightly less effective than clavulanate in a head-to-head comparison. The combination of PAS (later called sulbactam) with ampicillin[447] was introduced as Unasyn in the U.S. by Pfizer in 1987[448] and its revenue of $619 million in 1997[1] indicates its clinical success. Tazobactam, a triazole-substituted analogue of sulbactam, was found to have improved activity against class C β-lactamases[449] and its combination with piperacillin is marketed by Wyeth as Zosyn.

The complex mechanisms by which clavulanate and penicillanic acid sulfones inhibit serine β-lactamases have been studied extensively. Initial efforts to elucidate these mechanisms in the late 1970s, when structural information on β-lactamases was not yet available and the serine acylenzyme mechanism had not yet been confirmed, indicated that clavulanate was a substrate for RTEM and inhibited the enzyme by forming two catalytically inactive forms.[252] A transiently stable form, which could decompose to free enzyme and was the major component of enzyme after short time periods, was formed three-fold faster than the irreversibly inactivated form. The Knowles group found that both species showed an increase in absorbance at 281 nm, typical of α,β-unsaturated esters, and treatment of inactivated enzyme with hydroxylamine partially restored enzymatic activity, consistent with cleavage of an acylenzyme species.[253,450] Other studies by Durkin and Viswanatha,[451] Reading and Hepburn,[452] and the Knowles group[453] were followed by spectroscopic studies with PC1 by Rizwi et al. which indicated that a slow isomerization of the initially formed *cis*-enamine **B** to the *trans*-enamine **C** was responsible for the progression of transient inhibition to inactivation.[454] Two forms of

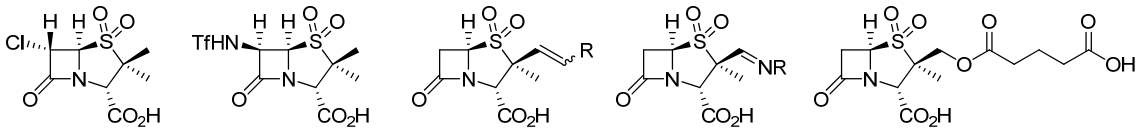
Scheme 11. Inhibition of Class A β-Lactamases by Clavulanate and Sulbactam.[29,412,467]



the inhibitor were observed in the X-ray crystal structure of clavulanate-inhibited PC1 (PDB: 1BLC) published by Chen and Herzberg in 1992 and these forms were thought to be the *cis*- and *trans*-enamines **B** and **D**.[455] Potential mechanisms for the inhibition of class A β-lactamases by clavulanate were evaluated by the Mobashery group in a detailed molecular modeling study and a refined mechanistic scheme was proposed (Scheme 11).[456] Enamines **B**–**D** are thought to give rise to transient inhibition and irreversible inhibition a result of cross-linking with Ser130 (**F**) (and not Lys73 or Lys234). Other studies of the mechanism of inhibition of class A β-lactamases by clavulanate have also been published.[457,458,459]

The discovery of potent β-lactamase inhibition by penicillanic acid sulfone in 1978[446] stimulated several studies of its interactions with class A enzymes.[460,461,462,463,464,465,466,467,468] Early indications that penicillanic acid sulfone shared a similar mechanism of inhibition with clavulanate inspired the synthesis of a number of other penam sulfones. Among the earliest PAS derivatives to show potent inhibition were 6α-chloropenicillanic acid sulfone, reported by Cartwright and Coulson,[469] and 6β-(trifluoromethanesulfonyl)amidopenicillanic acid sulfone, from the laboratories of Viswanatha and

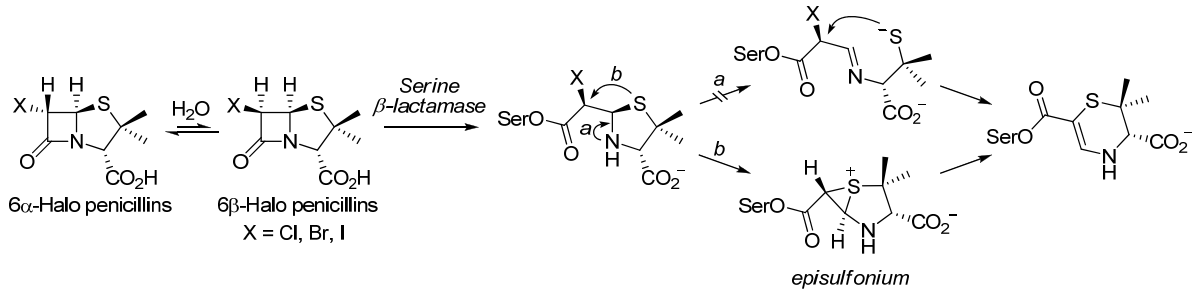
Dmitrienko.[470,471,472,473,474] Tazobactam was discovered in 1984[475] and has found widespread clinical utility, possibly as a result of its improved activity against class C β-lactamases.[449] The sulfones of other β-lactams were also evaluated[461] and more recent developments include the 2β-alkenyl PAS derivatives of Hoffman–La Roche[476] and 2β-imino penam sulfones of Phillips et al.[477] A 2β-substituted penam sulfone, SA2-13, was designed to form a stabilized *trans*-enamine intermediate in the active site of SHV-1 (PDB: 2H5S) and was found to be 10-fold more stable to hydrolysis than tazobactam.[466,478]



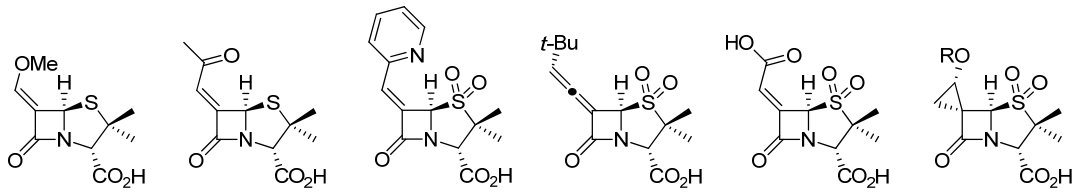
The 6-halopenicillinates represent another class of potent β-lactamase inhibitors discovered in the late 1970s. In 1978 Loosemore and Pratt discovered that 6β-bromopenicillanic acid (β-BPA) was an irreversible inhibitor of β-lactamases from *B. cereus*, *B. licheniformis*, *S. aureus*, and *E. coli*. Although 6β-BPA showed weak antibacterial activity, its inhibitory potency against β-lactamases was comparable to that of clavulanate and a study of its interactions with BcI were reported.[250,479,480,481] A ^{3}H-labelled version of this compound was used by Knott-Hunziker et al. in 1979 to covalently modify the β-lactamase of *B. cereus* and this work provided some of the first firm evidence that the active site nucleophile of β-lactamases was a serine.[251] In a detailed study of the mechanism of irreversible inhibition induced by 6β-halopenicillinates, Pratt demonstrated that a rearrangement occurs upon acylation of the β-lactamase to give an enzyme-bound dihydrothiazine and concluded that the rearrangement is more likely to proceed through an episulfonium intermediate than through thiazolidine ring-opening (Scheme 12).[255,482] The serine-bound dihydrothiazine is also observed in recently solved X-ray crystal structures of the 6β-iodopenicillinate-modified class A β-lactamase from *Bacillus licheniformis* BS3 and the 6β-iodopenicillinate-modified DD-transpeptidase from *Actinomadura* strain R39.[483]

Other potent inhibitors were discovered in the early 1980s, including 6-alkylidene penams such as 6-(methoxymethylidene)penicillanic acid **41**, which was conceived by Knowles,[484] and Hoffman–La Roche's 6-(acetylmethylidene)penicillanic acid (Ro15-1903).[485]

Scheme 12. Inhibition of Serine β-Lactamases by 6-Halopenicillins.

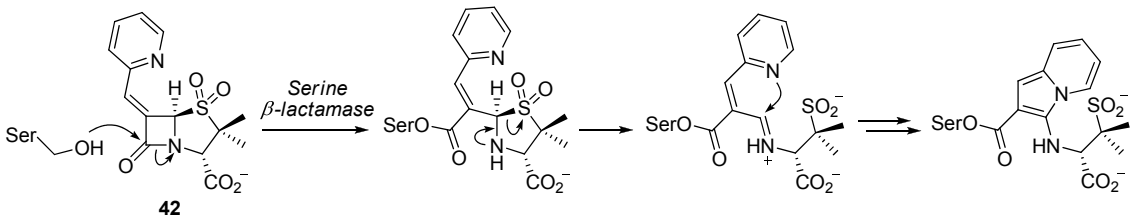


Sulfone derivatives of alkylidene penams were also found to be potent mechanism-based inhibitors and the discovery of heteroarylmethylidene PAS **42** was disclosed by researchers at Pfizer in 1986.[486,487] In 1993 6-vinylidene derivatives, including **43**, were reported by the Buynak group[488] and other alkylidenepenam sulfones (e.g. **44**) and -cephem sulfones have also been developed (Appendix C).[489,490,491,492,493] With the lack of a proton at C6, which is important in the mechanism of inhibition for clavulanate and sulbactam, inhibition by the alkylidene PASs involves an intramolecular attack of the alkylidene substituent on the imine(iminium) intermediate and subsequent tautomerization (Scheme 13). Many of the alkylidene penam sulfone inhibitors have not only shown greater potency than clavulanate and sulbactam against class A β-lactamases, but also inhibit class C and, more recently, class D β-lactamases.[494,495] Spirocyclopropylpenam sulfones **45** have been prepared by investigators at Wyeth and were shown to be potent inhibitors of TEM-1 and AmpC enzymes.[496]



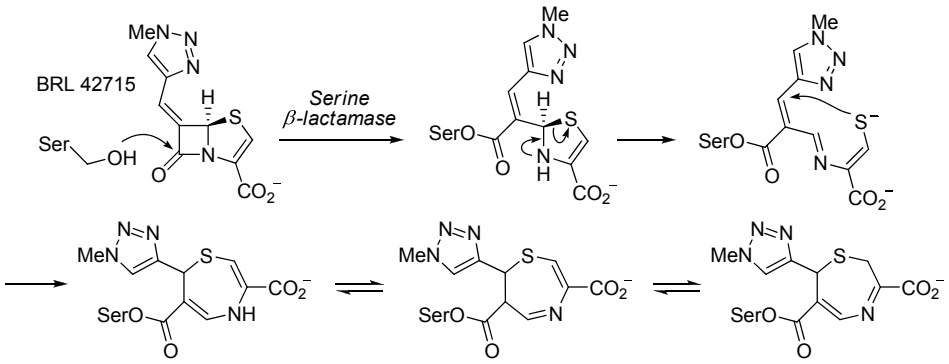
Structural modifications of penems in the late 1980s by Beecham led to the discovery of the BRL-42715, an alkylidene penem which lacked clinically useful antibiotic activity but exhibited much more potent β-lactamase inhibitory activity than clavulanate and sulbactam.[497,498,499] BRL 42715 has been shown to be a progressive inhibitor of class A, C, and D β-lactamases with nanomolar IC_{50} values against TEM-1, SHV-1, P99, GC1, OXA-1, and other enzymes. Several studies concerned with the interactions of BRL 42715 with β-lactamases[500,501,502,503,504] and transpeptidases[505] determined that the mechanism of inhibition involves the opening of the dihydrothiazole intermediate and

Scheme 13. Inhibition of β-Lactamases by 6-Heteroarylidene Penam Sulfones.[29,486]



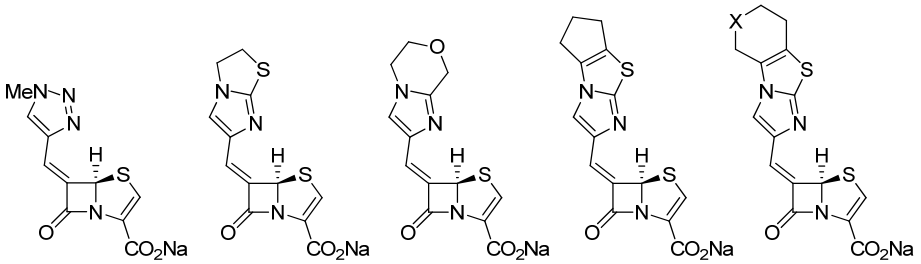
42

Scheme 14. Inhibition of β-Lactamases by Alkylidene Penems.[29,502]

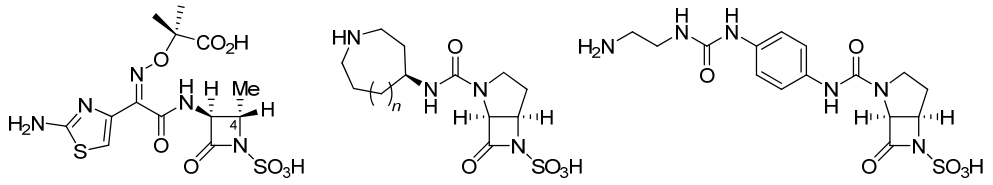


subsequent cyclization to form a dihydrothiazepine (Scheme 14). Despite the attractive inhibition profile of BRL 42715, its development has been discontinued as a result of its low serum half-life in humans and other factors.[409]

Researchers at Wyeth reported in 2004 the evaluation of new bicyclic heterocycle-bearing alkylidene penems which show improved potency against TEM-1 and AmpC enzymes and impressive synergy with piperacillin against resistant strains.[506,507,508,509] BLI-489, for example, showed IC_{50} values of 0.4 nM and 2 nM against TEM-1 and AmpC, respectively. Penems with tricyclic heterocycles, such as **46**–**48**, offer additional lipophilicity (desirable in this case for in vivo evaluation) and display potent activity against class A and C enzymes (e.g. IC_{50} for **47** vs TEM-1 = 1.9 nM; AmpC = 0.62 nM).[510] X-ray crystal structures of BRL 42715 with 908R (PDB: 1Y54)[504] and **46** with SHV-1 and GC1 have been solved (PDB: 1Q2P, 1Q2Q).[509] In addition, while BRL-42715 was found to be a substrate for the metallo-β-lactamase CcrA, many of the Wyeth penems are potent (nanomolar) inhibitors of CcrA.[507,511] More recently, potent inhibition of the class D OXA β-lactamases has also been observed.[512] Detailed reviews of the 6-alkylidene penems have been published previously by Phillips[409] and Mansour et al.[411]



The discovery of the nocardicins and monobactams as natural products in the early 1980s (Section 1.2.7) led to the development of semisynthetic derivatives such as aztreonam.[164,165] While certain monocyclic β-lactams, such as nocardicin A and desthiobenzylpenicillin, are substrates for class A and class C β-lactamases,[513] aztreonam was found to have poor affinity for broad spectrum class A β-lactamases such as TEM-2 and exhibited potent inhibition of the class C enzyme P99 (K_i = 1.9 nM).[514] Bush et al. found that the *trans* relationship between the C3-acylamino substituent and the 4-methyl substituent of aztreonam accounted for its significantly decreased affinity for TEM-2 and slower turnover relative to its 4-unsubstituted (SQ 82,402) or 4-*epi* (SQ 26,917) analogues. An X-ray crystal structure of an aztreonam-acylated AmpC enzyme (PDB: 1FR1)[515] indicates that a steric interaction between the 4-methyl group and Tyr150 causes a bond rotation to occur such that the *N*-sulfonyl group obstructs attack of the hydrolytic water. This observation prompted investigators at Hoffman–La Roche to investigate bridged monobactams which could form acylenzyme intermediates with more rigid conformations that might block hydrolysis more effectively.[516] This strategy proved effective as a series of monobactams showed low nanomolar IC_{50}s against class C enzymes, exhibited very long deacylation half-lives, and Ro-48-1256 was shown to potentiate β-lactams against *P. aeruginosa*.[517]

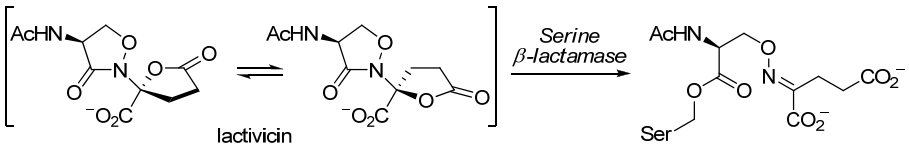


Merck researchers have recently prepared and evaluated analogues of Ro 48-1256 as inhibitors of class C β-lactamases and potential partners for combination with imipenem and the (*S*)-azepine

analogue **49** was selected for preclinical development.[518] BAL29880 is a bridged monobactam which is currently in development at Basilea as part of BAL30376, which is a three-component combination of clavulanate (for the inhibition of class A β-lactamases and ESBLs), BAL29880 (class C β-lactamase inhibitor) and BAL19764 (a siderophore monobactam with activity against Gram-negative organisms and stability to MBLs).[170]

1.5.3 Non-β-Lactam Inhibitors: β-Lactam Mimics and Transition State Analogues

The concept that analogues of β-lactams might also have biological activity was explored as early as the 1940s as researchers at Lilly prepared γ-lactam and δ-lactam analogues of penicillin.[519] The lack of antibiotic activity suggested that the amide bond of such compounds was insufficiently reactive to acylate the target enzymes or that the molecules were unable to fit appropriately into the active site and the failure of such attempts led to the belief that the β-lactam moiety was essential for antibiotic activity. With the growing threat of β-lactamases in the 1970s, researchers began to question this belief and the desire to prepare β-lactam analogues with antibiotic activity and resistance to β-lactamases was renewed. Early studies, which were mainly directed toward screening for antibacterial activity, have been reviewed[519,520] and the discussion below is limited to work related to β-lactamase inhibition.

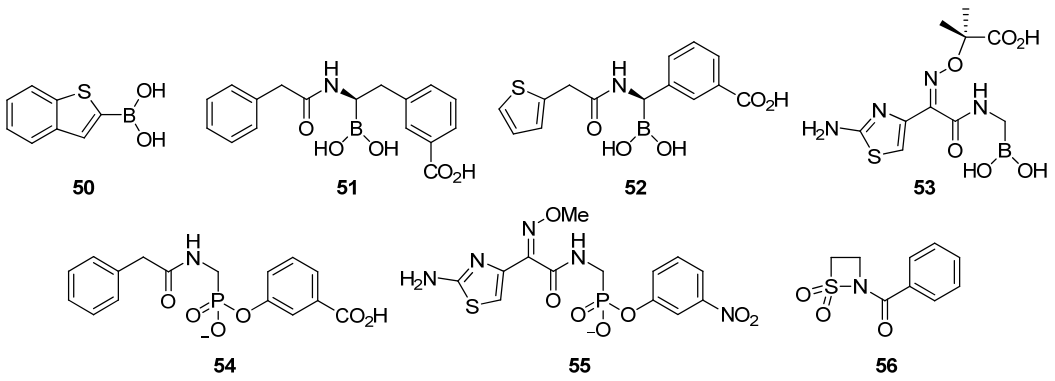


One example of a natural non-β-lactam antibiotic which inhibits PBPs and β-lactamases is lactivicin. Lactivicin was first reported in 1986 as a new antibiotic isolated from strains of *Empedobacter lactamgenus* and *Lysobacter albus* from soil samples in Japan.[521] Lactivicin shares a similar mechanism of inhibition with clavulanate and X-ray crystal structures of lactivicin bound to PBP1b (PDB: 2JE5)[522] and the class A β-lactamase BS3 has recently been solved (PDB: 2X71).[523]

Boronic acids have been known to be reversible β-lactamase inhibitors since the late 1970s.[524] Even very simple phenylboronic acids showed K_i values in the low micromolar range against class C enzymes (AmpCs) from *P. aeruginosa* and *E. coli*.[525] Since these early investigations more than 20

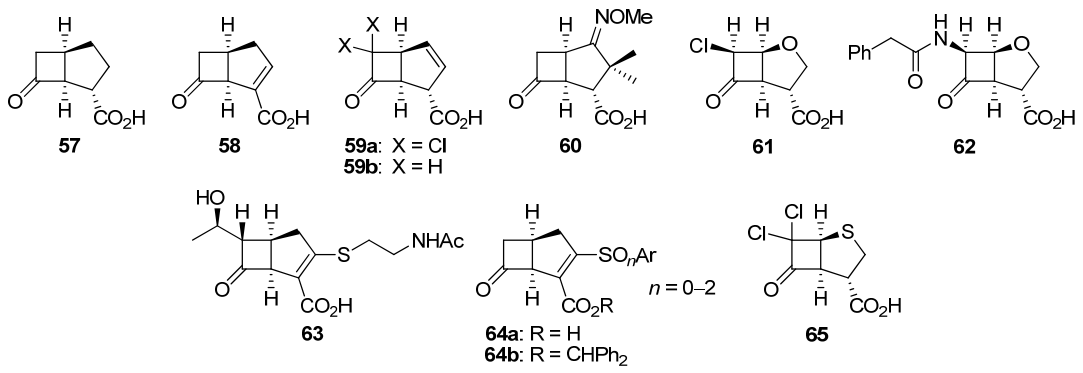
studies with boronic acids have been reported and investigators have achieved potent inhibition (low nanomolar IC_{50} values) of class A and C β-lactamases with these compounds (e.g. **50**–**53**, see Appendix C). Another series of transition state analogues that have been evaluated as β-lactamase inhibitors are the phosphonates. The phosphonate monoester **54** was first reported by Pratt in 1989 as a rapid inactivator of the P99 class C enzyme[526] and many other phosphonates (e.g. **55**) have been reported as inhibitors of class A, C, and D β-lactamases. As some of the most potent serine β-lactamase inhibitors yet discovered, boronic acids and phosphonates have proven to be extremely valuable as tools in the study of β-lactamase mechanisms and much of our knowledge in this area is derived from X-ray co-crystal structures of enzyme adducts with transition state analogues.

β-Sultams, the sulfonyl analogues of β-lactams, have been found by Page to inactivate P99 β-lactamase and ESI MS experiments indicate that the mechanism of inactivation by **56** is through sulfonation of the active-site serine rather than acylation.[527]



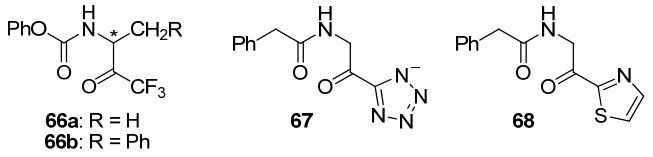
In the early 1980s several research groups proposed independently that analogues of β-lactams in which the β-lactam ring is replaced by a cyclobutanone system might inhibit serine β-lactamases and DD-transpeptidases by virtue of their ability to form an enzyme-bound hemiketal with an active-site serine residue. Gordon et al. reported the synthesis of cyclobutanones **57**–**59** for this purpose, but found no significant inhibition of either R-TEM β-lactamase or R61 transpeptidase.[528] Meth-Cohn et al. synthesized cyclobutanone **60** but did not disclose any biochemical or biological data.[529]

The 2-oxabicyclo[3.2.0]heptanones **61** and **62** prepared by Lowe and Swain, however, showed time-dependent inhibition of the class A β-lactamases R-TEM-2 and BcI and **62** demonstrated activity against R61 transpeptidase.[530,531] In a subsequent publication Cocuzza and Boswell described



the synthesis of several cyclobutanone derivatives, including the *N*-acetyl thienamycin analogue **63** and simpler analogues **64**.[532] While none of the free acids showed significant activity, several of the esters **64b** showed synergy with benzylpenicillin against penicillin-resistant *S. aureus*.[533] The Dmitrienko laboratory described the preparation of dichlorocyclobutanone **65**[534] and Kelly et al. reported that **65** was a weak competitive inhibitor of R61 transpeptidase ($K_i \approx 1$ mM).[535,536,537]

Trifluoromethyl ketones were prepared by the Schofield group in 1996 as potential inhibitors of metallo-β-lactamases.[538] Trifluoromethyl ketones **66**, for example, inhibited the MBL from *X. maltophilia* with K_i values of 1.5 and 15 μM and inhibited the MBL from *A. hydrophila* with K_i values of 44 and 6 μM. However, inhibition of the metallo-enzymes of *B. cereus* and *P. aeruginosa* was more modest with K_i values >300 μM.



A small series of α-ketoheterocycles was evaluated as inhibitors of serine β-lactamases by the Pratt group in 2001.[539] Moderate inhibition of the class C enzyme P99 was observed by tetrazole **67** (K_i = 110 μM) and thiazole **68** (K_i = 550 μM) but these compounds were not inhibitors of TEM-1.

In 2004 researchers at Aventis in France reported that a bicyclic urea derivative AVE1330A (or NXL104) was a potent inhibitor of serine β-lactamases, with IC_{50}s of 8 nM against TEM-1 (class A) and 80 nM vs P99 (class C).[540] Although AVE1330A showed weak antibacterial activity of its own, the combination of this β-lactamase inhibitor with ceftazidime showed impressive activity against a broad range of β-lactamase-producing organisms, including CTX-M-, ESBL-, and carbapenemase producers but not MBL producers.[541] NXL104 also has potent activity against the extended-spectrum

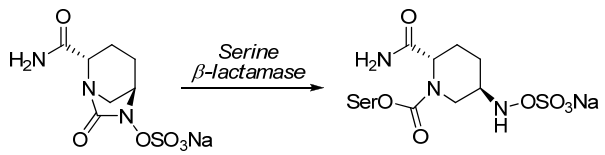


Figure 17. Reaction of a serine β-lactamase with NXL104.

β-lactamase KPC-2 (IC_{50} = 38 nM) and its combination with ceftazidime is effective against KPC-producing isolates of *K. pneumoniae*,[542,543,544] against *P. aeruginosa*,[545] and carbapenemase-producing *Enterobacteriaceae*.[546] An X-ray crystal structure of NXL104-treated CTX-M-15 confirms that the compound acylates the active-site serine (Figure 17)[547] and recent mechanistic studies indicate that the serine carbamate intermediate is more stable than the corresponding acyl enzyme intermediates of clavulanate, sulbactam, and tazobactam.[548] The combination of NXL104 with ceftazidime is currently in Phase II clinical trials and a combination of NXL104 with ceftaroline may also enter clinical trials for the treatment of MRSA infections.[549,550] Novexel, a spin-off company created by Sanofi–Aventis, was sold to AstraZeneca in March, 2010 for more than $350 million.[551]

1.5.4 Metallo-β-Lactamase Inhibitors

Metallo-β-lactamases have a broad spectrum of substrate specificity that encompasses all β-lactam classes with the exception of monobactams. The clinically available β-lactamase inhibitors, clavulanate, sulbactam, and tazobactam, are ineffective against MBL-producing organisms. A large number of MBL inhibitors has been published since the mid-1990s but none of these have been developed for clinical use. The most common strategy for MBL inhibition has involved metal-binding or -chelating functional groups. Aside from EDTA, a metal chelator used for characterization and identification of MBLs, inhibitors often contain thiol- and carboxylic acid functional groups.

Thiols have been reported as metallo-β-lactamase inhibitors by a number of research groups (Figure 18, Appendix C), including the Dmitrienko group,[552] and several thiols have been co-crystallized with MBLs. Thioesters have been investigated as thiol-releasing substrates and have also shown inhibitory activity. Mercaptophosphonates have recently been investigated as inhibitors of subclass B1, B2, and B3 MBLs.[553]

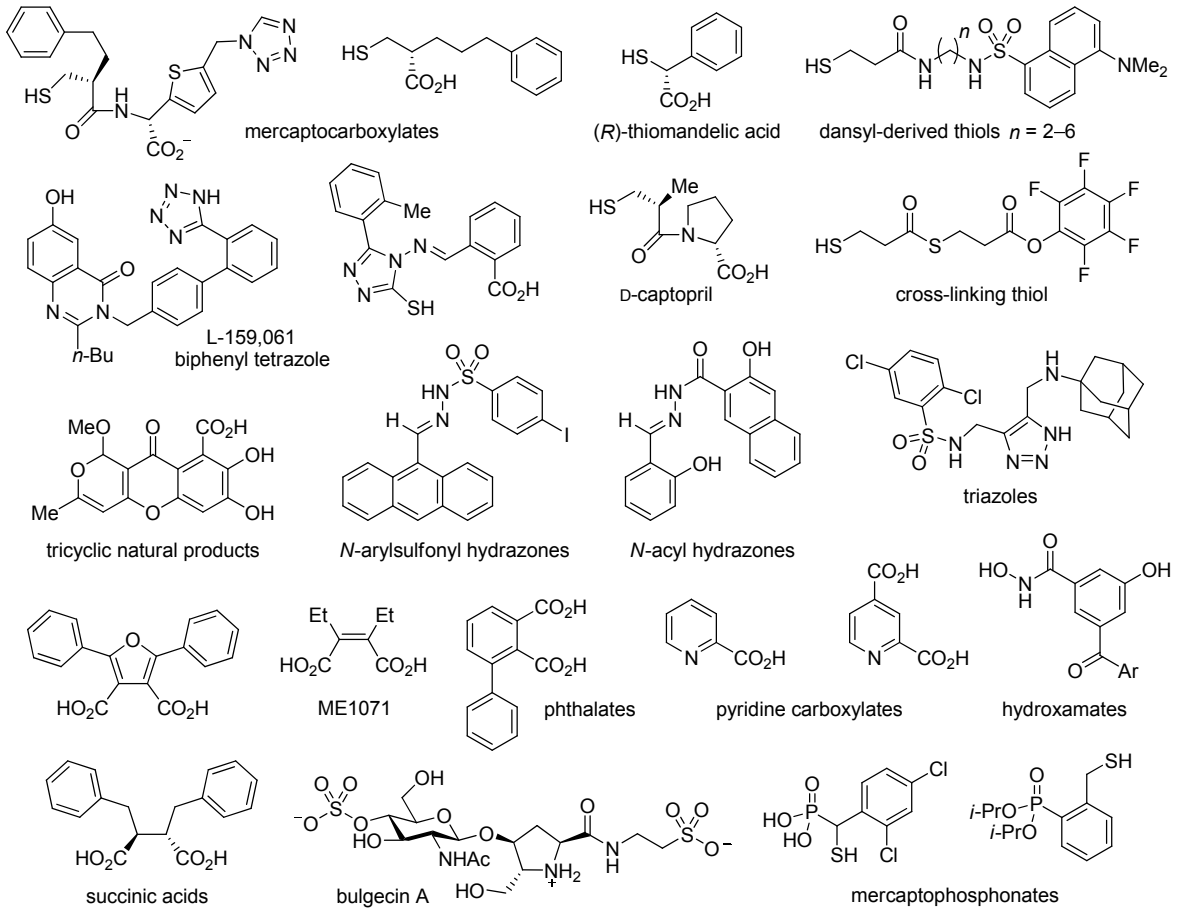
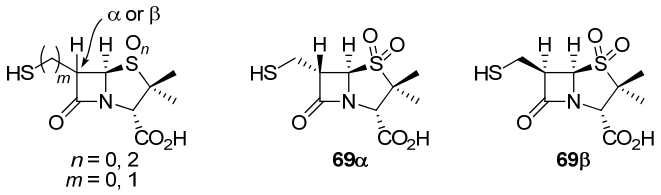


Figure 18. Inhibitors of metallo-β-lactamases.

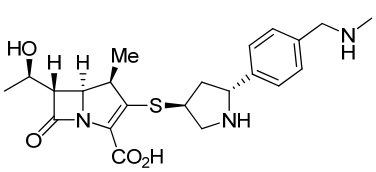
Other than thiols, pyridine carboxylates, succinic acids, maleic acids, and phthalates have been explored as metal-binding compounds, and other non-covalent inhibitors include biphenyl tetrazoles, triazoles, and tricyclic natural products (Figure 18). In this laboratory, *N*-arylsulfonyl hydrazones were found to act as competitive inhibitors of IMP-1[554] and *N*-acyl hydrazone analogues were found to be inhibitors of a more broad spectrum of enzymes, including IMP-1, VIM-2, and OXA-10, and OXA-45.[555]



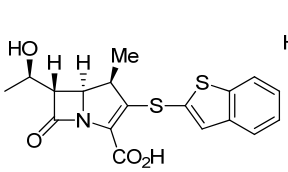
A strategy employed by the Buynak group to simultaneously target serine- and metallo-β-lactamases involved derivatization of penicillins with a thiol functionality at C6.[556] Among the series

of penams and penam sulfones prepared, the mercaptomethyl PASs **69α** and **69β** showed the most potent and broad-spectrum activity with low micromolar IC_{50} values against class A (TEM-1), class C (P99), and class B (BcII) β-lactamases and sub-micromolar IC_{50}s against the metallo-enzyme L1.

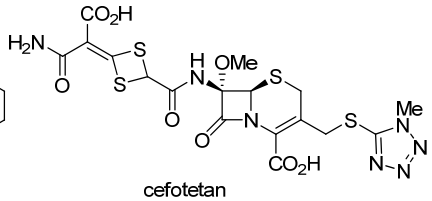
Although clinically used carbapenems can be hydrolyzed by metallo-β-lactamases, certain carbapenems have been shown to act as potent inhibitors of MBLs as a series with various sidechains at C2 was investigated by Banyu Tsukuba in the late 1990s.[557] Carbapenems J-110,441 and J-111,225, for example, inhibited IMP-1-catalyzed imipenem hydrolysis with IC_{50} values of <0.1 μM and 0.7 μM, respectively. The latter compound, which was later shown to have a K_i of 0.18 μM vs IMP-1, demonstrated better antibacterial activity than imipenem when used alone against IMP-1-producing organisms and synergy with imipenem when used in combination.[558] In addition, the enzymes IMP-1, CcrA, BcII, and L1 hydrolyzed J-111,225 more slowly than imipenem.



J-111,225



J-110,441



cefotetan

Cefotetan, a synthetic cephamycin that is considered a second generation cephalosporin, has been reported to be a poor substrate for the metallo-β-lactamases of *Aeromonas* spp. but gives rise to transient inactivation of the enzymes.[559] Inhibition was maximal after a 15 minute incubation of the inhibitor and K_i values of 12 and 40 μM against the MBLs of *A. hydrophila* and *A. schubertii*.

1.6 Clinical Significance of β-Lactamases and the Need for β-Lactamase Inhibitors

One of the most important criteria for the development of the third generation cephalosporins in the late 1970s was the requirement for stability to the class A TEM and SHV β-lactamases since TEM-1, TEM-2, and SHV-1 had emerged as the most commonly encountered plasmid-encoded β-lactamases worldwide. However, shortly after the introduction of cefotaxime and ceftazidime in the early 1980s, resistance to the third generation cephalosporins was observed as a result of mutant TEMs and SHVs.[560] These enzymes were named extended-spectrum β-lactamases (ESBLs) because of their

ability to hydrolyze extended-spectrum cephalosporins and monobactams. Class A ESBLs (Bush functional group 2be) now include the VEB and GES β-lactamases, but the most widespread ESBLs are the CTX-Ms.[249] Third generation cephalosporins, which have a broad spectrum of activity and are prescribed for a wide variety of infections,[561] are among the most widely used of all antibiotics, with revenues of $11.9 billion in 2009,[20] and ESBL-mediated resistance is deeply concerning.

Resistance also quickly emerged to β-lactamase inhibitor combinations, such as amoxicillin–clavulanate and piperacillin–tazobactam, which were also developed in the mid-1980s in order to overcome resistance to broad-spectrum, plasmid-encoded class A β-lactamases. While mutant TEMs and SHVs rarely possess both the extended-spectrum and inhibitor-resistant phenotypes (Bush group 2ber), the increased prevalence of ESBLs and inhibitor-resistant β-lactamases over the last decade has caused an increase in the use of carbapenems. Carbapenems, which have been historically considered the β-lactams of last resort, are now being used as the first line of defense against Gram-negative infections in which ESBL-mediated resistance is suspected.[562]

This increased dependence upon carbapenems adds to the concern that has already been caused by the emergence of carbapenemases in the last decade.[134,259,326,563] Among the most threatening carbapenemases are metallo-β-lactamases such as VIM-2, which has replaced IMP-1 as the dominant MBL in *P. aeruginosa*, and serine carbapenemases such as the class A KPC-2, which is increasingly encountered in Enterobacteriaceae.[564] Therapeutic options are becoming increasingly limited for carbapenem-resistant infections.

With very few new β-lactams in the antibiotic pipeline and increasing numbers of β-lactamases, there is a growing movement toward the development of combination antibiotics.[549] It is thought that single β-lactam-containing agents will be difficult to sustain in the clinical setting and that combinations of established β-lactam antibiotics with potent, broad-spectrum β-lactamase inhibitors may be better suited to contend with multi-β-lactamase-producing multidrug-resistant Gram-negative pathogens.[410,565] The ideal β-lactamase inhibitor would show potent inhibition of both serine- and metallo-β-lactamases.[311,408,549,566,567]

Chapter 2

Cyclobutanone Analogues of β-Lactam Antibiotics: Synthesis and Conformational Properties*

2.1 Previous Work with Cyclobutanones

By the early 1960s Gram-negative bacteria had replaced *S. aureus* as the most problematic hospital pathogens and biochemical studies in the early 1970s suggested that the major mechanism for resistance to the clinically available cephalosporins was inactivation by β-lactamases.[98,99] For the most part, pharmaceutical companies directed efforts toward the discovery of new cephalosporins that showed higher stability to β-lactamases, but β-lactamase inhibitors were also pursued as potential solutions to the β-lactamase problem. Clavulanic acid, for example, which was discovered in the early 1970s in a screening program by Beecham,[119] showed very potent inhibitory activity and encouraged the development of other types of inhibitors.

As noted in the previous chapter, the concept that the β-lactam ring was essential for biological activity was a widely held belief but researchers began to question this notion in the 1970s with the synthesis and evaluation of β-lactam mimics.[519] With the increasing success of aldehydes and ketones in the late 1970s as transition state analogues[569,570] for the inhibition of serine proteases,[571] the synthesis of ketone analogues of β-lactams for the inhibition of β-lactamases and/or DD-transpeptidases seemed to be a logical progression. Such compounds would be hydrolytically stable analogues of β-lactams that might inhibit these enzymes through formation of a stable hemiketal linkage in the active site. In the early 1980s it became clear that other groups had also pursued this strategy for β-lactamase inhibition.

In 1981 Gordon, Pluščec, and Ondetti reported the synthesis of simple carbocyclic cyclobutanones **57**–**59** as potential β-lactamase inhibitors through a [2 + 2] cycloaddition of dichloroketene with 6,6-

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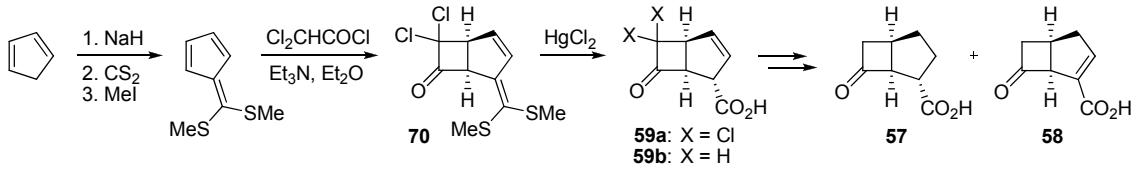
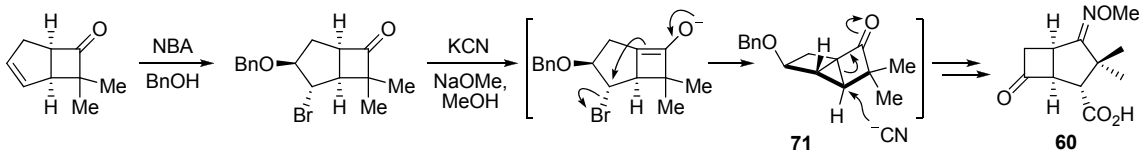
* Reproduced in part, with permission, from ref 568: Johnson, J. W.; Evanoff, D. P.; Savard, M. E.; Lange, G.; Ramadhar, T. R.; Assoud, A.; Taylor, N. J.; Dmitrienko, G. I. *J. Org. Chem.* **2008**, *73*, 6970–6982. Copyright 2008 American Chemical Society.

Scheme 15. Synthetic Routes to Cyclobutanone Analogues of β-Lactams.

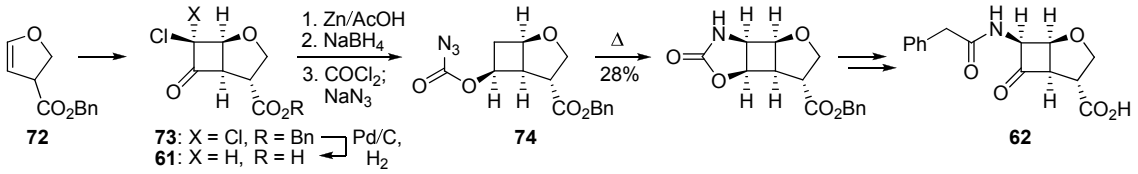
Gordon et al., 1981



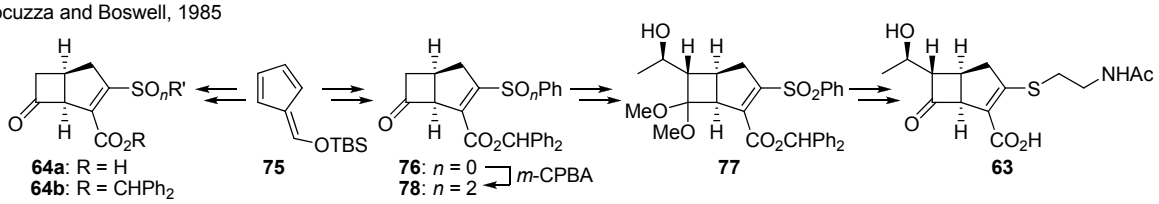
Meth-Cohn et al., 1982



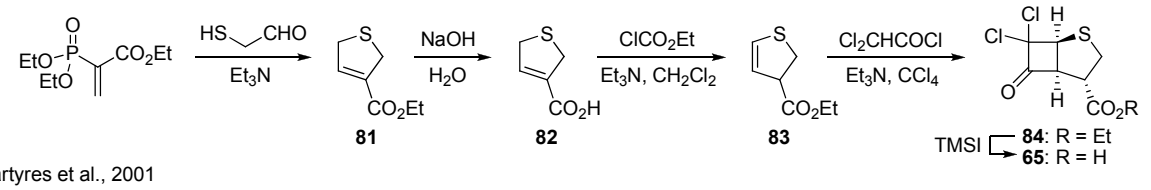
Lowe and Swain, 1983



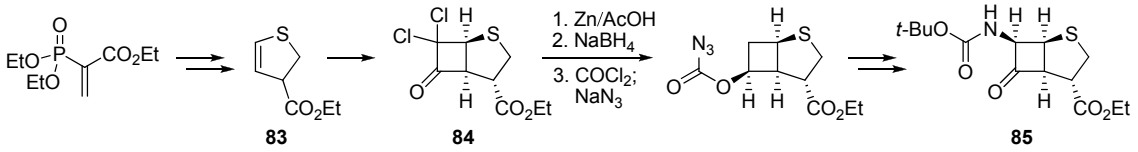
Cocuzza and Boswell, 1985



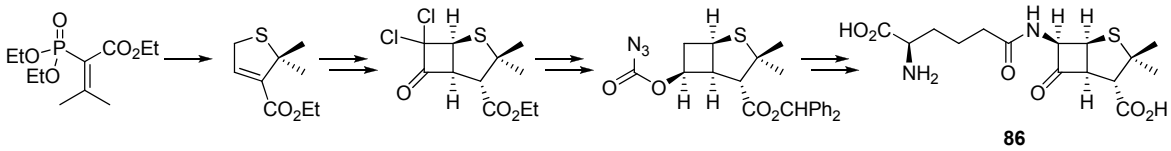
Lange, Savard, Viswanatha, Dmitrienko, 1985



Martyres et al., 2001



Ferguson et al., 2003

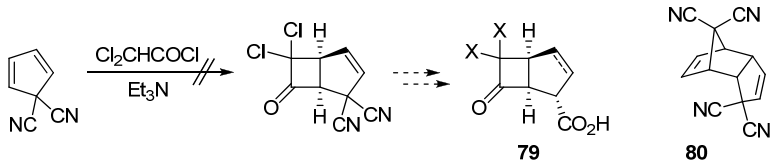


bis(methylmercapto)fulvene, followed by a low-yielding hydrolysis of dithioketeneacetal **70** with mercuric chloride (Scheme 15). These compounds did not show significant inhibition of either R-TEM β-lactamase or R61 transpeptidase.[528]

Meth-Cohn, Reason, and Roberts synthesized oxime **60**, by way of a tricyclic intermediate **71**, through interesting rearrangement chemistry developed previously by that group.[529,572] It was thought that the oxime moiety might improve the ability of cyclobutanone **60** to acylate the active-site serine but no biochemical or biological data was reported.

Lowe and Swain pursued the synthesis of 2-oxabicyclo[3.2.0]heptanone analogues of penicillins and the cycloaddition of dichloroketene with dihydrofuran **72**, prepared by Birch reduction of the parent furan, provided dichlorocyclobutanone **73**.[530,531] Catalytic hydrogenolysis of **73** gave the monochloro acid **61**, which was regarded as an analogue of 6β-bromopenicillanic acid. Attempts to introduce the 6β-acylamino sidechain directly through a cycloaddition of phthalimidoketene with dihydrofuran **72** were unsuccessful but a route involving an intramolecular nitrene insertion from **74** was devised and provided cyclobutanone **62** in low yield. Biochemical assays with the 2-oxacyclobutanones indicated that **61** and **62** showed time-dependent inhibition of the class A β-lactamases R-TEM-2 and BcI and **62** demonstrated activity against R61 transpeptidase.[530,531]

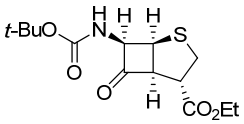
Cyclobutanone **63**, an analogue of *N*-acetyl thienamycin, was prepared by Cocuzza and Boswell in a 14-step synthesis from 6-silyloxyfulvene **75**.[532] Addition of the 7-hydroxyethyl sidechain was accomplished through treatment of a zirconium enolate of **76** with acetaldehyde and installation of the 2-aminoethylthio group at C3 was done through an addition–elimination displacement of the phenylsulfonyl group of **77** with the corresponding thiolate nucleophile. Other cyclobutanone analogues such as **64a**, which possess electron-withdrawing groups at C3 (e.g. SO_2R, COPh, CN), were prepared from **78** using a similar addition–elimination strategy. While none of the free acids **63** or **64a** showed significant anti-β-lactamase activity, several benzhydryl esters **64b** exhibited antibacterial activity against *S. aureus* (MICs: 25–50 μg/mL) and synergy with benzylpenicillin against β-lactamase-producing penicillin-resistant *S. aureus*.[533]



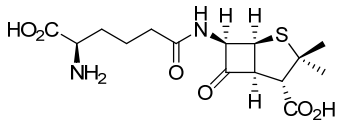
Initial attempts in this laboratory to prepare simple cyclobutanone analogues of β-lactams **79**, through a [2 + 2] cycloaddition of dichloroketene with 5,5-dicyanocyclopentadiene, were

unsuccessful since a homo-Diels–Alder reaction of the diene to give **80** was favoured.[573,574,575] As an alternative, sulfur-containing cyclobutanone derivatives were targeted and the 2-thiabicyclo-[3.2.0]heptanone **65**[534] was prepared in four steps from the known dihydrothiophene **81**.[576,577] A novel and efficient deconjugation procedure was used for the conversion of conjugated acid **82** to the deconjugated ester **83** that was required for the [2 + 2] cycloaddition to afford cyclobutanone **84** (discussed in more detail in Section 2.2). While dichlorocyclobutanone **65**[534] showed no significant inhibition of the serine β-lactamase BcI from *B. cereus* (Dr. Marc Savard, unpublished results), Kelly et al. reported that **65** was a weak competitive inhibitor (K_i = 1 mM) of R61 transpeptidase.[535] In addition, X-ray crystallographic data by Kelly et al. was consistent with the binding of **65** to the active site of the transpeptidase but the resolution of the structure was low and the data was insufficient to define a detailed structure of the enzyme-bound inhibitor.[536,537]

Other studies toward the synthesis of cyclobutanones were reported by the Page group but no biological data was reported.[578,579] It should also be noted that the Baldwin group later prepared cyclobutanones **85** and **86** as hydrolytically stable analogues of penicillins that could be used for the study of penicillin biosynthetic enzymes. Martyres et al. reported the synthesis of the model system **85** by combining the synthesis of **84**, developed in this laboratory, with the nitrene insertion methodology developed by Lowe and Swain for the installation of the 7-acylamino sidechain.[580] Ferguson et al. extended this strategy to incorporate a *gem*-dimethyl group at C3 and the aminoadipoyl sidechain at C7 and generate a cyclobutanone analogue of penicillin N (**86**) for crystallographic studies.[581] In 2007 the group published an X-ray crystal structure (PDB: 2JB4) of cyclobutanone **87** bound to isopenicillin N synthase (IPNS).[582]



85



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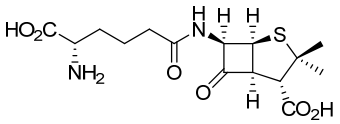


87

Although the preliminary biochemical data for **61**, **62**, **64b**, and **65** served as a proof of principle for the concept that cyclobutanones can act as β-lactamase and transpeptidase inhibitors, other more impressive advances in the inhibition of β-lactamases were being made in the early 1980s which discouraged further explorations of the cyclobutanones.

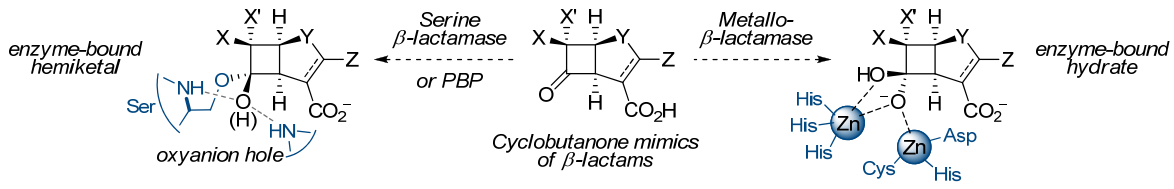


Figure 19. Cyclobutanones as potential broad-spectrum inhibitors of β-lactamases and penicillin-binding proteins (PBPs).

In the 1980s the clinically most significant β-lactamases were Ambler class A enzymes such as the plasmid-encoded TEMs of Gram-negative organisms and the introduction of the mechanism-based inhibitors clavulanic acid, sulbactam, and tazobactam, was very successful for combating such organisms. The current situation, however, is much different. Inhibitor-resistant TEMs, ESBLs, class A carbapenemases, hyper-produced AmpCs, OXA carbapenemases, and metallo-β-lactamases represent a very real threat to the continued use of penicillins and cephalosporins, the most heavily used classes of antibiotics, and carbapenems, the β-lactam antibiotics of last resort. Renewed efforts are therefore required to establish strategies with potential for overcoming this emerging epidemic.

Cyclobutanone analogues of β-lactams have the potential to form an enzyme-bound hemiketal in the active site of a serine β-lactamase and an enzyme-bound hydrate in the active site of a metallo-β-lactamases (Figure 19) and therefore represent a core structure that might form the basis for the design of broad-spectrum inhibitors. The previous work with cyclobutanones mentioned above yielded only a superficial understanding of the inhibitory properties toward class A β-lactamases and DD-transpeptidases and the interactions of such compounds with class B metallo-β-lactamases and class C and class D serine β-lactamases, which were not of clinical interest in the 1980s, were not studied. At the outset of this project, encouraging preliminary studies in the Dmitrienko group had already indicated that the dichlorocyclobutanone **65** was a moderate inhibitor of the serine β-lactamase OXA-10 and the metallo-β-lactamase IMP-1.[583] Therefore, a systematic investigation was undertaken to gain insight into the properties of the cyclobutanones and the nature of the inhibition of β-lactamases with the ultimate goal of discovering broad-spectrum β-lactamase inhibitors that might be developed for clinical use.

2.2 Synthesis of 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylates

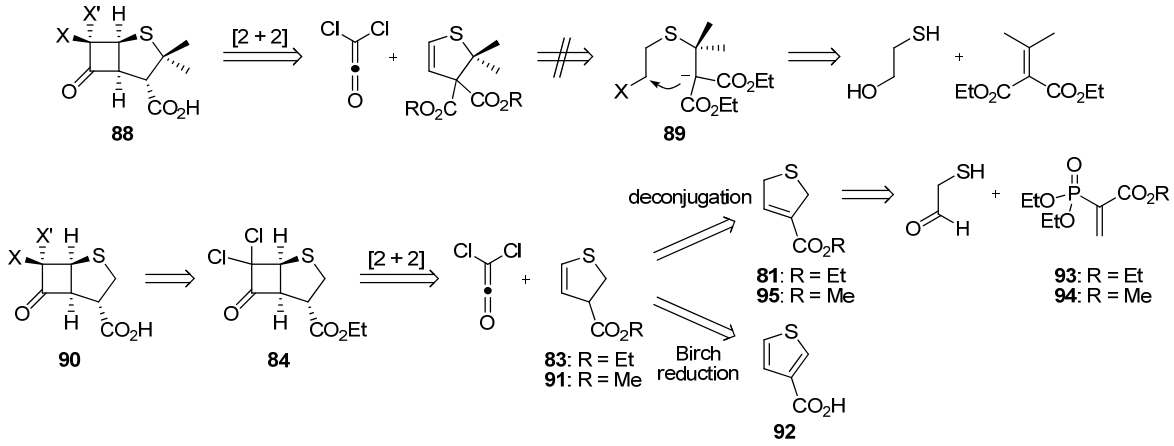
2.2.1 Early Synthetic Studies

Cyclobutanones are versatile intermediates in organic synthesis and can undergo a wide variety of transformations. Ring expansions of cyclobutanones with diazomethane, Baeyer–Villiger ring expansions with *m*-CPBA, and Favorskii[584] ring contractions are well-documented and reviewed.[585,586,587] More specifically, the bicyclo[3.2.0]heptanone system of interest to this laboratory, has been especially useful as precursors for the stereoselective synthesis of prostaglandins.[588] The most widely used method for the synthesis of cyclobutanones involves a [2 + 2] cycloaddition of an alkene with a ketene and, since such reactions are stereospecific and are often highly regioselective,[589] this strategy has been used for the synthesis of the β-lactam analogues **57**–**65** and **85**–**87** mentioned above.

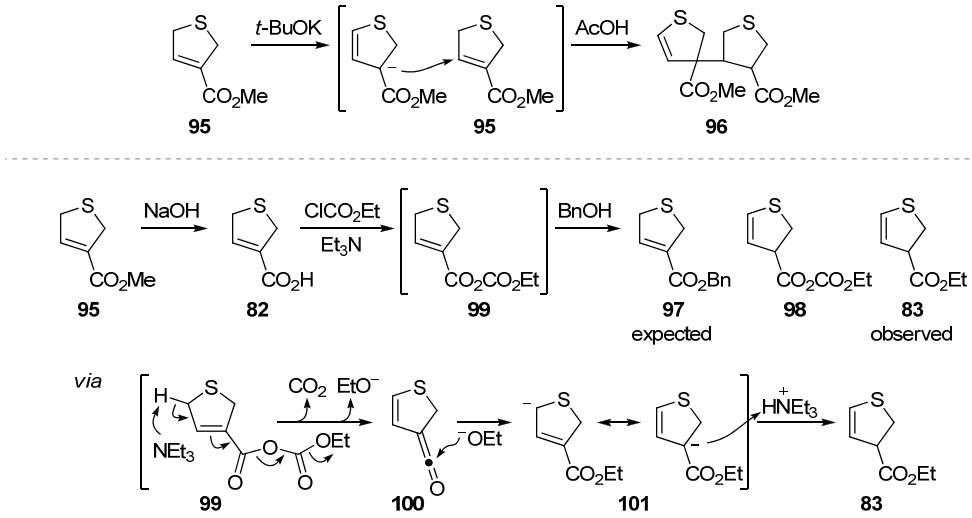
In the early 1980s the Dmitrienko group targeted cyclobutanones such as **79** and **88** as simple analogues of penicillins. The failure of 5,5-dicyanocyclopentadiene to undergo the desired [2 + 2] cycloaddition with dichloroketene to provide **79**[574] encouraged the group to pursue the synthesis of sulfur-containing analogues which might be synthetically more accessible and also act as better mimics of penicillins (Scheme 16). Early synthetic efforts in this laboratory toward **88** by Gerald Lange (M.Sc. 1984)[590] involved unsuccessful attempts to cyclize malonates such as **89** and cyclobutanone analogues such as **90**, which lack the *gem*-dimethyl group at C3, were then pursued. Dihydrothiophene **91**, which could be generated from the Birch reduction of thiophene **92** and esterification with diazomethane, indeed reacted with dichloroketene to provide cyclobutanone **84** with the desired regiochemistry, but the overall yield of 4% over three steps was considered impractical. A survey of the literature revealed that dihydrothiophene **81** was readily available from phosphonate **93** by the method of McIntosh and Sieler[576,577] and this prompted Lange to explore the possibility that **91** might be prepared by isomerization of **95**.

Initial attempts to effect the deconjugation of **95** using potassium *tert*-butoxide at –78 °C and quenching with acetic acid were unsuccessful and only the dimer **96**, the result of a Michael-type addition, was isolated (Scheme 17). It was thought that the deconjugation could be more successful with a bulkier ester that might sterically hinder the dimerization. Thus a transesterification from

Scheme 16. Retrosynthetic Analysis of 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylates.



Scheme 17. Discovery of a Deconjugation Procedure Involving Chloroformates.[590]



methyl ester **95** to benzyl ester **97** was attempted using a well-known method involving ethyl chloroformate. The saponification of **95**, followed by treatment of the conjugated acid **82** with ethyl chloroformate and benzyl alcohol generated a mixture of the conjugated benzyl ester **97** and what was thought to be the deconjugated mixed anhydride **98**. Further characterization of the undesired by-product with ^{13}C NMR and elemental analysis, however, indicated that the major product was not the mixed anhydride **98** but rather the deconjugated ethyl ester **83**.[590] The rationale for this outcome was based on the assumption that the enhanced acidity of the γ-proton of the mixed anhydride intermediate **99** would permit triethylamine to induce the elimination of carbon dioxide and ethoxide. Attack of the resulting ketene intermediate **100** by ethoxide would generate anion **101** which could

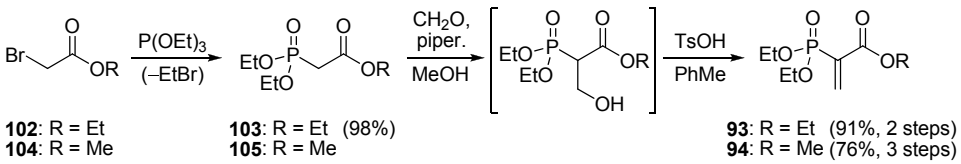
undergo kinetic protonation to provide the observed product **83**. With Lange's subsequent demonstration that the deconjugated ethyl ester **83** could be converted to the targeted cyclobutanone **84**, it became clear that the serendipitously discovered deconjugation process provided efficient access to the targeted cyclobutanones.

With the decision to revisit the cyclobutanones in the early 2000s, Darryl Evanoff (M.Sc. 2004) prepared cyclobutanone **65** according to the route developed by Lange and Savard so that the cyclobutanones could be screened for inhibitory activity against two β-lactamases that had been recently acquired. Preliminary biochemical results in this laboratory indicated that **65** was a moderate time-dependent inhibitor of the class D β-lactamase OXA-10 and, more interestingly, the class B metallo-β-lactamase IMP-1. In order to prepare additional material for more detailed biochemical studies and to synthesize other potentially more potent analogues, it seemed reasonable to continue to use the fairly efficient route developed by Lange and Savard and attempt to make improvements to the procedures where possible.

2.2.2 Synthetic Route for 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylates

As noted above, the methodology developed by McIntosh and Sieler[576,577] provided efficient access to dihydrothiophene **81** and literature procedures were used for the preparation of the substrate required for this reaction, phosphonoacrylate **93** (Scheme 18).[591] The Michaelis–Arbuzov reaction[592] of ethyl bromoacetate (**102**) with triethyl phosphite has been conducted on scales of up to 300 mmol and was found to be a very convenient and high-yielding reaction. The two reagents were combined, warmed slowly[593] without solvent to 85–95°C, and heated overnight to afford the crude phosphonoacetate **103** which could be distilled in 98% yield or used without purification in the next reaction. Triethyl phosphonoacetate (**103**), which is also available from commercial sources, was then treated with paraformaldehyde and piperidine and refluxed overnight in methanol to generate a primary alcohol that was concentrated but not purified.[594] Dehydration of the intermediate with 10 mol% *p*-toluenesulfonic acid (TsOH) in refluxing toluene under a Dean–Stark trap,[595] followed by distillation under high vacuum, provided the desired phosphonoacrylate **93** in 91% yield over two steps. As indicated in Scheme 18, the methyl ester **94** was obtained in 76% yield from methyl bromoacetate (**104**).

Scheme 18. Michaelis–Arbuzov Reactions and Synthesis of Phosphonoacrylates.



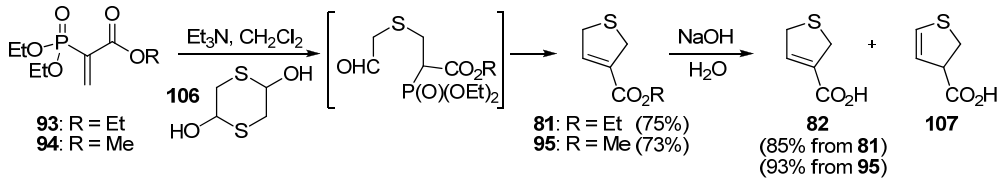
102: R = Et
104: R = Me

103: R = Et (98%)
105: R = Me

93: R = Et (91%, 2 steps)
94: R = Me (76%, 3 steps)

The method developed by McIntosh and Sieler[576,577] for the synthesis of dihydrothiophenes is a one-pot procedure involving the conjugate addition of a mercaptoaldehyde to a phosphonoacrylate and an intramolecular Horner–Wadsworth–Emmons cyclization (Scheme 19).[596] For the synthesis of dihydrothiophene **81**, phosphonate **93** was added to a suspension of triethylamine and 2,5-dihydroxydithiane (**106**), which is a dimer of mercaptoacetaldehyde, in CH_2Cl_2 and refluxed overnight. Aqueous washes of the crude product and flash chromatography provided the conjugated ethyl ester **81** in 75% yield. The methyl ester **94** was converted to dihydrothiophene **95** with a yield of 73%.

Scheme 19. Synthesis of Dihydrothiophenes by the Method of McIntosh and Sieler.



93: R = Et
94: R = Me

106

81: R = Et (75%)
95: R = Me (73%)

82
(85% from **81**)
(93% from **95**)

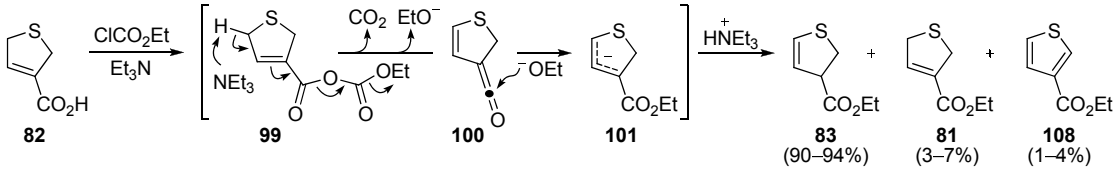
107

Hydrolysis of ester **81** with 25% aqueous NaOH at 70 °C for 6–16 hours, followed by acidification gave the conjugated acid **82** as dull yellow powder which could be recrystallized to furnish the conjugated acid **82** as a storable crystalline solid (mp: 169–170 °C (EtOAc/hexane)). Prolonged reaction times for the hydrolysis generated small amounts of the deconjugated acid **107** but isomeric mixtures of **82** and **107** could be used together in the following deconjugation reaction without issue. The acid **82** was obtained in 93% yield through hydrolysis of the methyl ester **95** under the same conditions.

As described above, the conjugated acid **82** was converted to the deconjugated ester **83** through treatment with ethyl chloroformate and triethylamine in CH_2Cl_2 at room temperature (Scheme 20). The yield of this reaction was consistently above 90% on reaction scales as large as 100 mmol and, despite a noticeable warming of the reaction vessel during the slow addition of ethyl chloroformate on

large scales, only a small proportion (typically 3–7% by ^{1}H NMR) of the undesired isomer **81** was generated. The deconjugated dihydrothiophene **83** was found to be more sensitive to oxidation than its conjugated isomer **81** and the oxidation product, thiophene **108**, was observed as a minor by-product of the reaction. The oxidation of **83** to the thiophene **108**, which occurs even at 4 °C, prevents long-term storage of the compound and the dihydrothiophene was therefore used immediately in the next step following its purification by flash chromatography.

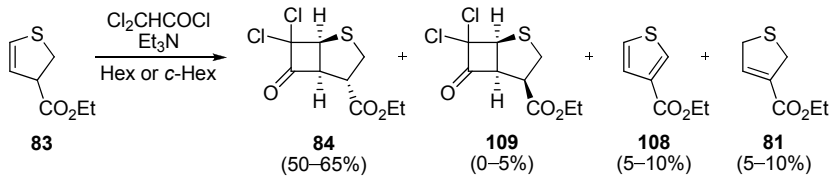
Scheme 20. Preparation of the Deconjugated Dihydrothiophene **83**.



Additional experiments in this laboratory in the mid-1980s suggest that the mixed anhydride method has some generality as straight-chain and carbocyclic α,β-unsaturated acids can also undergo deconjugation (Dr. Marc Savard, unpublished results). Related deconjugations involving α,β-unsaturated acid chlorides have also been studied.[597]

Lange's early attempts at the [2 + 2] cycloaddition of **83** with dichloroketene (generated in situ by the combination of dichloroacetyl chloride and triethylamine) revealed that the ethyl ester substituent of **83** significantly deactivates the dihydrothiophene, presumably through inductive and steric effects, relative to 2,3-dihydrothiophene itself.[590] A similar decrease in reactivity was noted by Lowe and Swain with the dihydrofuran **72**.[530,531] A slower addition of the ketene precursor dichloroacetyl chloride over 3.5 hours to a solution of triethylamine and **83** in CCl_4, in order to decrease the known polymerization of the ketene,[589] was successful for improving the yield of the dichlorocyclobutanone **84** to 33% (Scheme 21). In an effort to further improve upon the yield of the cycloaddition, Evanoff studied the effects of even slower addition times (up to 24 hours) and extended stirring times following the slow addition (up to 24 hours).[583] While the Baldwin group reported a yield of 80% for the same reaction,[580] conducted in CCl_4 with a slow addition over a 48 hour period, experiments in this laboratory indicated that an addition period of longer than 2 hours was not beneficial. The highest yield obtained from Evanoff's six experiments was 40% and was the result of a slow drop-wise addition of dichloroacetyl chloride (2.5 equiv) over 2 hours to a solution of Et_3N (2.5 equiv) and **83** in

Scheme 21. Preparation of Dichlorocyclobutanone **84** via [2 + 2] Cycloaddition.



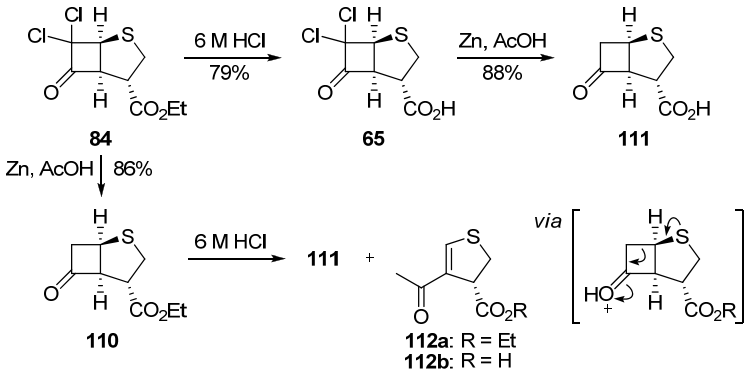
CCl_4, followed by an additional 22 hours of stirring.

With the considerable increase in the price of carbon tetrachloride, more recent attempts to improve the yield of the [2 + 2] reaction have explored the use of other solvents. Hexane and cyclohexane were found to be reasonable alternatives to CCl_4 as yields as high as 65% have been obtained. In the highest-yielding reaction, dichloroacetyl chloride (2.5 equiv) was added to a solution of Et_3N (2.5 equiv) and **83** (37 mmol) in hexane (0.1 M) at room temperature over a period of 3 hours with a motor-driven syringe pump and the solution was stirred for an additional 21 hours before workup. Attempts to improve the yield of the reaction, through slower addition times or by maintaining strictly anhydrous conditions, were unsuccessful. In addition, efforts to de-gas solvents and reagents prior to use failed to decrease the formation of the major by-product of the reaction, thiophene **108**. Other unproductive attempts to improve the yield involved a reversal of the order of addition such that Et_3N was added slowly to a solution of dihydrothiophene **83** and dichloroacetyl chloride in hexane. While only very small proportions of the C4-epimer **109** were observed in previous reactions, **109** was isolated in more significant amounts (3–5%) when Et_3N was added to the dihydrothiophene and dichloroacetyl chloride. These observations may indicate that this [2 + 2] cycloaddition is not as stereoselective as once thought to be.

As dichlorocyclobutanones are known to be sensitive to base-induced ring cleavage,[528,598] conversion of **84** to carboxylic acid **65** was accomplished with acid catalysis (Scheme 22). Ethyl ester **84** was heated to 70–80 °C in a 1:1 mixture of dioxane and 6 M HCl for 6–8 hours and extracted with CH_2Cl_2. The resulting yellow solid was recrystallized from toluene to give **65** in 79% yield.

Ester **84** was readily dechlorinated with excess zinc dust (added in portions) in hot acetic acid for 6–12 hours and purification by flash chromatography provided cyclobutanone **110** in good yield. The acid **65** could be dechlorinated with a similar procedure; however, longer reaction times were often required and chromatography was ineffective for purification. Upon completion of the reaction,

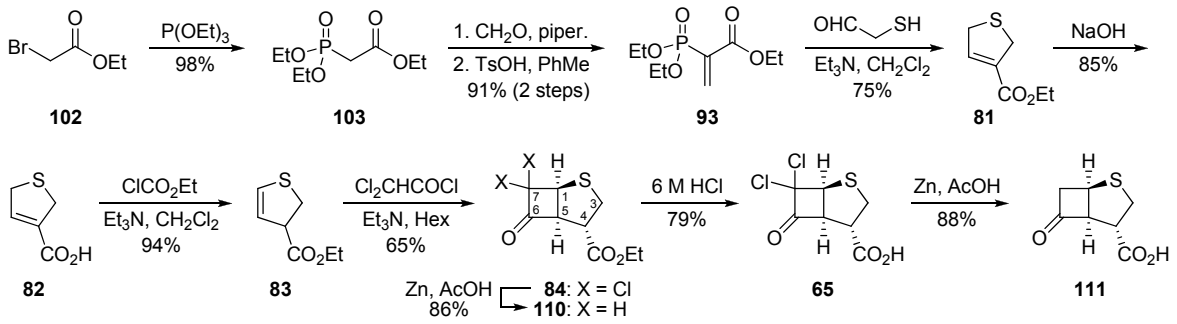
Scheme 22. Acid-Catalyzed Hydrolysis of Ethyl Ester **84** and Dechlorinations of **84** and **65**.



zinc salts were removed by filtration, the acetic acid removed under reduced pressure, and the crude product was diluted with aqueous HCl and extracted with EtOAc. In some cases, the dechlorinated acid **111** was purified by crystallization but simple trituration of the crude product was also found to be effective. The dechlorinated acid **111** could also be prepared through the acid-catalyzed hydrolysis of **110**; however, an impurity produced by these reaction conditions prevented the purification of acid **111**. This by-product, which was not purified, was identified as the methyl ketone **112** by ^{1}H NMR and is thought to be generated through the acid-catalyzed ring-opening of the cyclobutanone (Scheme 22). Cyclobutanone ring openings of this type have precedent. [599,600]

Overall, the route developed in this laboratory for the synthesis of 2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylates is very efficient as the dichlorocyclobutanone **65** was prepared in seven steps from commercially available triethyl phosphonoacetate (**103**) with an overall yield of 28% (Scheme 23). In practice, the four- or five-step synthesis of dihydrothiophene **82**, a storable solid, by this method is high-yielding, operationally simple, and these reactions have been done on scales of up to 250 mmol. The deconjugation is also high-yielding on large scale but the deconjugated dihydrothiophene **83** is unstable and must be used quickly in the next reaction. The key reaction in the sequence, the [2 + 2] cycloaddition, is low-yielding, requires careful setup and operation, and the maximum scale of this reaction (determined by practical limitations) is 75 mmol. Careful chromatography is required for purification of the [2 + 2] adduct **84** but the cyclobutanone can be stored for years at 4 °C without decomposition. The acid-catalyzed hydrolysis of **84** can be done on large scales in reasonable yield and recrystallization provides the acid **65** as a crystalline solid.

Scheme 23. Synthetic Route to 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylate Derivatives.



2.2.3 Conformations of 2-Thiabicyclo[3.2.0]heptan-6-ones

With the synthesis of cyclobutanone **84** in this laboratory in the early 1980s, Lange was able to correctly assign the stereochemistry at C4 through an examination of molecular models and ^{1}H NMR analysis.[534,590] An X-ray crystal structure of the 4-unsubstituted cyclobutanone **113** revealed that this simple analogue adopted an *endo* envelope conformation in the solid state, a preference that differed from the *exo* envelope conformation assigned to cyclobutanone **114** by Thandi (Figure 20).[574] The feature of **84** which allowed Lange to assign the stereochemistry at C4 was that H5 appeared as a doublet in the ^{1}H NMR spectrum and coupled only to H1. The lack of coupling between H4 and H5 indicated that the dihedral angle between H4 and H5 was close to 90°, according to the Karplus

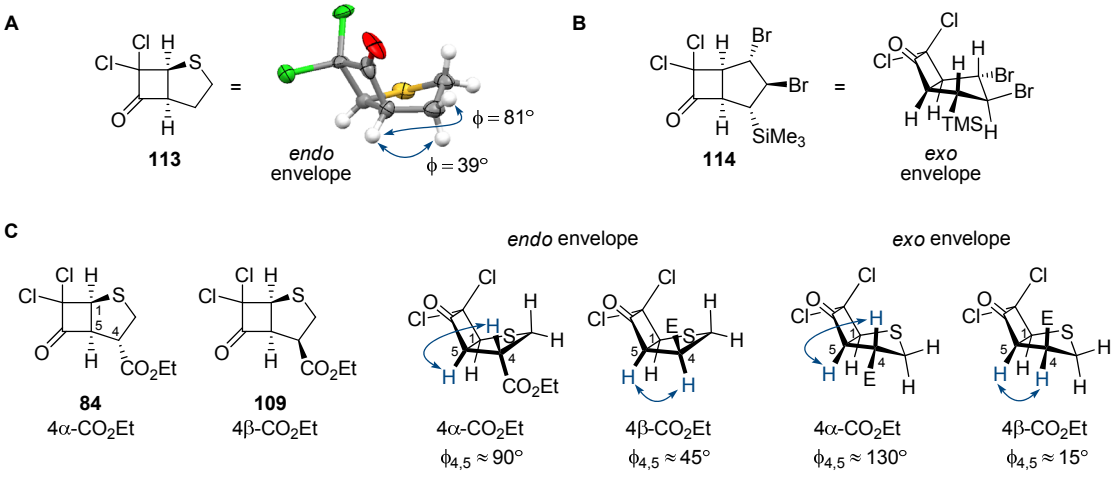


Figure 20. Conformational analysis of cyclobutanones **113**, **114**, **84**, and **109**. (A) X-ray structure of Lange's cyclobutanone **113**.[590] (B) The conformational preference of the carbocyclic cyclobutanone **114** prepared by Thandi.[574] (C) Lange's conformational analysis of cyclobutanones **84** and **109** used for assignment of the stereochemistry at C4 (ϕ = dihedral angle, E = CO_2Et).[583,590]

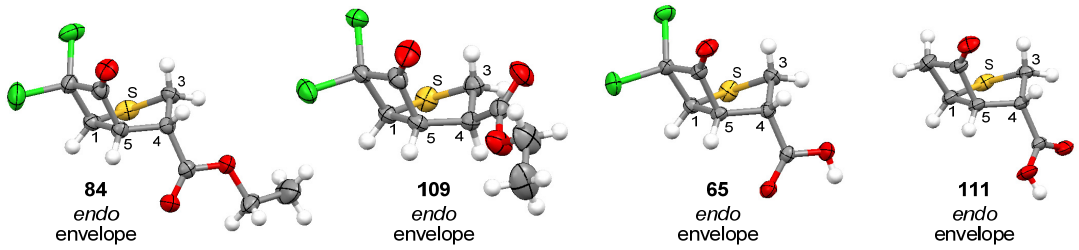


Figure 21. X-ray crystal structures of cyclobutanones **84**, **109**, **65**, and **111**. Colour scheme: white, grey, red, yellow, and green represent H, C, O, S, and Cl, respectively. See Appendix G for tables of crystallographic data.

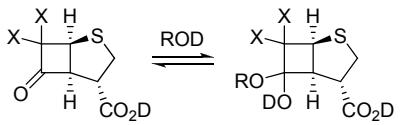
relationship,[601] and molecular models indicated that **84** possessed a 4α-CO_2Et group and favoured the *endo* envelope conformation. In the present study, X-ray crystallographic analysis (Figure 21) and ab initio molecular orbital calculations (RHF, Section 2.6) confirmed this conformational preference for ester **84** and acids **63** and **111** in the solid state and gas phase, respectively. NMR analysis of cyclobutanones **61** and **62** led Lowe and Swain to recognize that an *endo* envelope is also preferred by the 2-oxa- ring system.[530,531] The ^{1}H NMR spectrum of the recently isolated cyclobutanone **109** shows a coupling of $J_{4,5}$ = 7.6 Hz and an X-ray crystal structure confirms that this compound also favours the *endo* envelope.

2.3 β-Lactamase Inhibition with Simple Analogues 65 and 111

The first cyclobutanone analogues to be tested for inhibitory activity against the metallo-β-lactamase IMP-1 were the dichlorocyclobutanone **65** and the dechlorinated derivative **111** prepared by Evanoff.[583] These biochemical assays, which were conducted by Ms. Miriam Heynen in this laboratory in the early 2000s, indicated that the dichlorocyclobutanone **65** inhibited nitrocefin hydrolysis by IMP-1 with an IC_{50} of 526 μM but the dechlorinated cyclobutanone **111** was a very poor inhibitor with an IC_{50} of approximately 180 mM (Table 1, Figure 22).

It was thought that the dichlorocyclobutanone **65** displayed better inhibition than **111** because the electron-withdrawing chlorines could enhance the stability of an enzyme-bound hydrate. The extent to which these cyclobutanones undergo hydrate- and hemiketal formation in solution was then evaluated by NMR experiments in D_2O (with acetone-d_6 as a cosolvent to facilitate solubilization)

Table 1. Hydrate and Hemiketal Formation with Cyclobutanones **65** and **111** and Initial Inhibition Results with IMP-1 and OXA-10.[a,b]



ketone	% hydrate in D_2O/acetone-d_6 [a]	% hemiketal in CD_3OD [a]	IMP-1 IC_{50} (1 min)[b]	OXA-10 IC_{50} (10 min)[b]
65	74	88	526 μM	444 μM
111	0	24	180 mM	≥14 mM

[a] Cyclobutanone hydrate and hemiketal formation data were determined by ^{1}H NMR in the present study. [b] β-Lactamase inhibition, as reported by Evanoff,[583] was assessed by monitoring nitrocefin hydrolysis (Figure 22). The enzyme and inhibitor were allowed to preincubate prior to the addition of nitrocefin for 1 min in IMP-1 assays and for 10 min in OXA-10 assays. More recent inhibition results are reported in Chapter 3.

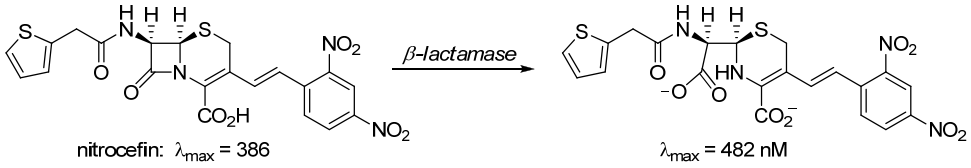


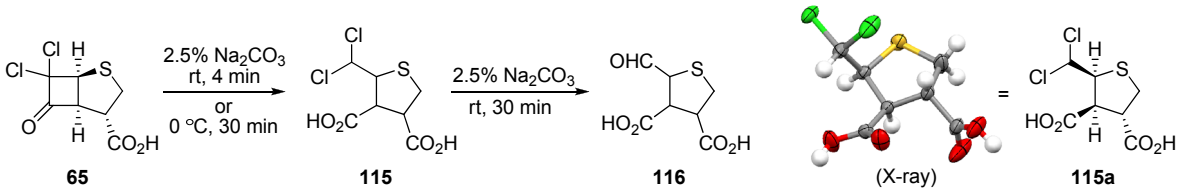
Figure 22. Enzymatic hydrolysis of nitrocefin, a chromogenic cephalosporin substrate of serine- and metallo-β-lactamases.[602]

and methanol-d_4 (Table 1). Indeed the tendencies for **65** and **111** to form tetrahedral adducts in solution were found to parallel the inhibition results described above and these data were also consistent with other studies in which di- and trifluoromethyl ketones are more potent inhibitors of serine proteases than mono- and non-fluorinated ketones.[603,604,605]

Further study of the interaction of IMP-1 with the cyclobutanones revealed that the inhibition by **65** was progressive and was related to the time allowed for preincubation of the enzyme with the inhibitor prior to the addition of nitrocefin.[583] At an inhibitor concentration of 500 μM, for example, IMP-1 activity was inhibited by 40% after 1 minute and 80% inhibited after 40 minutes. The dechlorinated cyclobutanone **111**, however, showed no significant improvement in inhibition of IMP-1 with increased preincubation time.

With the acquisition of the expression system for OXA-10 in the early 2000s, as a generous gift

Scheme 24. Ring Opening of the Dichlorocyclobutanone **65** in Aqueous Na_2CO_3.



from Prof. Mobashery (Notre Dame), cyclobutanones **65** and **111** could be evaluated as potential inhibitors of a class D serine β-lactamase. While dichlorocyclobutanone **65** showed no inhibition after a 1 minute preincubation with the enzyme, an IC_{50} value of 444 μM was obtained with a 10 minute preincubation. In a separate time course study, 500 μM of cyclobutanone **65** inhibited OXA-10 activity by 80% after 20 minutes and by 95% after 40 minutes.[583]

In addition to the possibility that the progressive inhibition of IMP-1 and OXA-10 was a result of slow binding, alternate explanations for the time dependence were also considered. As mentioned above, dichlorocyclobutanones are well-known to be susceptible to ring-opening by alkoxides but Evanoff had demonstrated that acid **65** is especially sensitive as it is fully converted to the diacid **115** in aqueous sodium carbonate in only 5 minutes and further hydrolyzed to aldehyde **116** with longer reaction times (Scheme 24). Hydrolysis to the diacid **115** was also found to occur under the assay conditions of IMP-1 (50 mM HEPES buffer, pH 7.3) and OXA-10 (100 mM Na_xPO_4, pH 7.0), with a half-life for the dichlorocyclobutanone **65** of approximately 2 hours in each case, but the aldehyde **116** was not observed.[583]

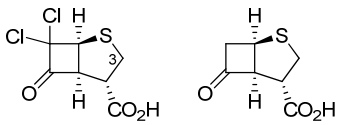
The progressive inhibition observed led to the speculation that the ring-opened diacid **115** and aldehyde **116** might be more potent inhibitors of IMP-1 and OXA-10 than the parent cyclobutanone **65**. In revisiting Evanoff's chemistry for the preparation of the ring-opened products, cyclobutanone **65** was treated with cold, dilute Na_2CO_3 to generate the diacid **115** as a mixture of diastereomers and an X-ray crystal structure of isomer **115a** was obtained (Scheme 24). Exposure of **65** to Na_2CO_3 for 30 minutes produced aldehyde **116** as a mixture of diastereomers. Biochemical evaluation of each of these diacids, however, revealed that **115** and **116** were poor inhibitors of IMP-1 and OXA-10 and ended speculation that the ring-openend products were the active inhibitory species.

Extensive efforts have been made in this laboratory by Valerie Goodfellow and, more recently, Dr. Laura Marrone to elucidate the nature of the erratic behaviour of cyclobutanones **65** and **111** with

these enzymes. For example, one source of concern in the earliest experiments with IMP-1 was the significant loss of enzyme activity over the course of the assay. While enzyme stability was improved with higher concentrations of salt in the assays (100–500 μM NaCl) inhibition of IMP-1 by the cyclobutanones decreased. Enzyme stability was also found to be sensitive to the concentration of DMSO and assays are now conducted with ≤1% DMSO for serine β-lactamases and ≤0.1% with MBLs. It should also be noted that attempts were made to detect complexes of **65** with OXA-10, as an acyl-enzyme or serine hemiketal, and IMP-1, as a non-covalent zinc-bound hydrate or zinc-bound diacid, by mass spectrometry but these studies were unsuccessful. Other variables in the assays were investigated to improve the reproducibility and reliability of the IC_{50} values but the good behaviour of other inhibitors, such as the *N*-sulfonyl hydrazones[552] and *N*-acyl hydrazones,[555] with these enzymes discouraged further study of these cyclobutanones.

2.4 Derivatization at C3: Chlorination and Substitutions

While the irregular behaviour of the cyclobutanones in biochemical assays had prevented the determination of reliable IC_{50} or K_i values for the inhibition of IMP-1 and OXA-10, it seemed clear even from the earliest assays that the dichlorocyclobutanone **65** was a much better inhibitor than the dechlorinated analogue **111**. Since the chlorines were beneficial for inhibition, synthetic efforts to improve upon the inhibitory potency focused on the functionalization of the tetrahydrothiophene ring. A sidechain at C3, for example, could increase the affinity of such inhibitors for β-lactamase active sites through favourable hydrogen bonds or other non-covalent contacts. More specifically, sidechains similar to those of carbapenem antibiotics might improve the affinity of such cyclobutanones for carbapenemases.



65 **111**

It was thought that carbapenem mimics might be accessed through a route involving chlorination at C3, followed by elimination, conjugate addition of a thiol(ate) to C3, oxidation, and deprotection

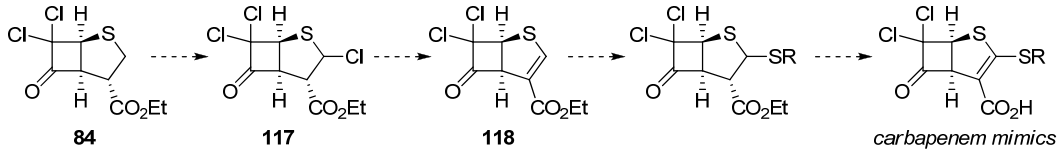
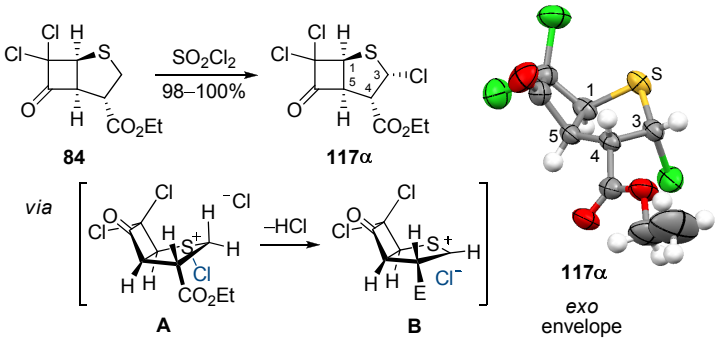


Figure 23. Initial strategy for the synthesis of carbapenem analogues.

Scheme 25. Chlorination of **84** and X-Ray Structure of **117α**.



(Figure 23). Chlorination was first considered as a possible strategy for introducing functionality at C3 because Lange had successfully chlorinated the simpler analogue **113** with sulfuryl chloride.[590]

The chlorination of **84** with SO_2Cl_2 in CH_2Cl_2 was fully regioselective and was also very stereoselective to produce **117α** in nearly quantitative yield (Scheme 25). The stereoselectivity of the process may be a consequence of the initial chlorination of **84** occurring on the *exo* face to generate the *S*-chlorosulfonium ion **A**. Elimination of HCl might then lead to the sulfur-stabilized carbocation **B** with the chloride leaving group poised for subsequent attack from the *exo* face of the ring system, which would provide the 3α-chloride **117α**.

It was clear from the ^{1}H NMR spectrum of **117α** that the conformation was different from that of **65** and **111** since the coupling constant between H4 and H5 was found to be 6 Hz ($J_{4,5} \approx 0$ Hz for **65** and **111**). The stereochemistry and *exo* envelope conformation for **117α** were confirmed by a single-crystal X-ray diffraction study (Scheme 25).

Initial attempts to effect elimination with pyridine, Et_3N, or DBU in CH_2Cl_2, THF, or MeCN were unsuccessful, however, and the use of Ag_2CO_3 resulted in a complex mixture with only a low yield of the elimination product **118**. Heating of **117α** in MeCN achieved partial elimination but the 3β-chloro epimer **117β** was observed as a major byproduct that showed even slower elimination (by NMR).

2.5 Substitutions and Eliminations at C3

As the preliminary efforts to prepare the unsaturated ester **118** indicated that **117α** was unreactive in mildly basic conditions, the potential for elimination under acidic conditions was investigated. Heating of the α-chloro derivative **117α** in AcOH, for example, generated a mixture of products with small amounts of **118** and **117β** and, more interestingly, larger proportions of substitution product. While the search for an efficient method for elimination continued, attention was also given to the exploration of substitution reactions. Hydrolysis of **117α** to generate **119**, for example, could provide another potential route toward carbapenem analogues (Figure 24).

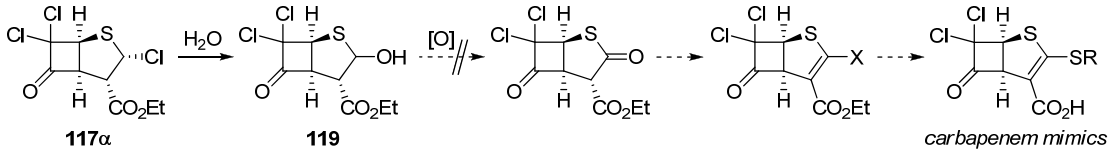


Figure 24. Revised strategy for the synthesis of carbapenem analogues.

Hydrolysis of **117α** in water and acetonitrile (1:1) generated the thiolactols **119α** and **119β**, in a 1:14 ratio, which could not be separated by flash chromatography. Interestingly, ^{1}H and ^{13}C NMR spectra of the chromatographed thiolactol mixture showed a third material (~6%) which was identified as a tricyclic hemiketal **119c** (δ_{C6} 105.9) (Figure 25). A similar oxatricyclo[3.2.1.0^{3,6}]octane **120b** (δ_{C3} 103.90) has been observed by Grudzinski and Roberts (Figure 26).[606,607,608] Since the **119α**:**119β**:**119c** ratio was consistently close to 6:88:6 in several different preparations, it is thought that the mixture may be part of an equilibrium that allows the interconversion of **119α** and **119β**. In order to gain insight into the mechanism of interconversion, an NMR experiment was conducted in which the thiolactol mixture **119** was subjected to AcCl (to generate HCl in situ) in methanol-d_4/acetonitrile-d_3 (1:1). The lack of any cross-over products from reaction with the solvent (to generate the 3-methoxy derivatives **121α** or **121β**) suggests that equilibration via ring-opened aldehyde **C** is more likely than equilibration through a sulfur-stabilized carbocation **D**.

With the 3-hydroxy derivatives **119** in hand, several attempts were made to oxidize the thiolactol to the corresponding thiolactone. Pyridinium chlorochromate (PCC),[609] pyridinium dichromate (PDC),[610] Dess–Martin periodinane (DMP),[611,612] and $(COCl)_2$/DMSO/Et_3N (Swern oxidation)[613] had each failed to react with thiolactol **119**. The 3-hydroxy derivatives **119** were also unreactive toward

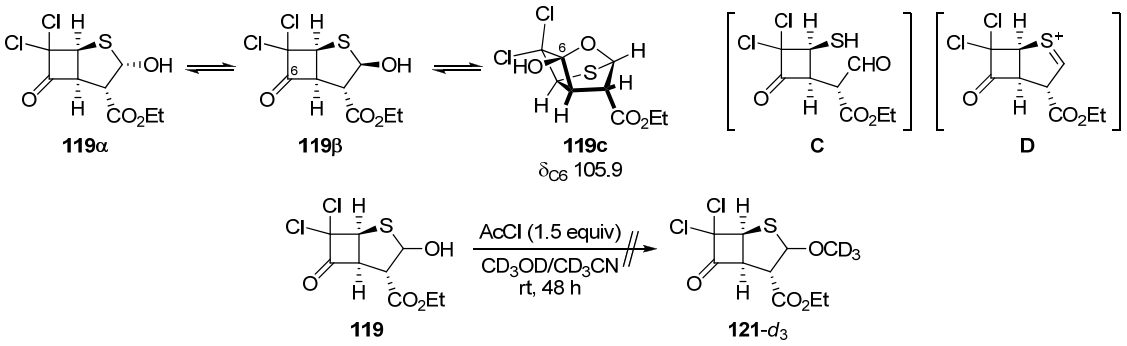


Figure 25. An equilibrium involving **119α**, **119β**, and **119c**.

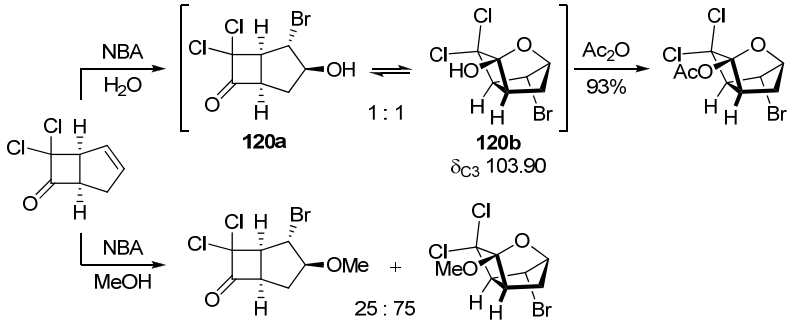


Figure 26. Tricyclic hemiketals prepared by Grudzinski and Roberts.[606]

SO_2Cl_2, *N*-chlorosuccinimide (NCS), *N*-bromosuccinimide (NBS), and *N*-methylmorpholine-*N*-oxide (NMO) at room temperature and gave complex mixtures when treated with Br_2 or I_2/K_2CO_3. Other conditions that involved aqueous alkaline conditions were not attempted due to the sensitivity of the dichlorocyclobutanone ring. While there is some literature precedent for the oxidation of simpler thiolactols,[614] Grudzinski and Roberts have reported that acetylation of a related cyclic hemiketal (**120b**) takes place at the reactive hemiketal site (Figure 26).[606]

The lack of success with the attempted oxidation of **119**, together with the relative ease with which **117α** was hydrolyzed, encouraged further exploration of substitution reactions since C3-alkoxy derivatives were thought of as penicillin analogues that would certainly be worthy of investigation.

As with the hydrolysis of **117α** in H_2O/MeCN, the solvolysis of **117α** in ROH/MeCN provided the 3-alkoxy (*S*,*O*-acetal) derivatives **121**–**125** (Figure 27).

Similar to the hydrolysis, the methanolysis of **117α** in MeOH/MeCN was complete within 48 hours and cleanly generated the substitution products **121α** and **121β** (Table 2). However, the reactions with sterically demanding alcohols, 2-propanol and *tert*-butyl alcohol, showed incomplete conversion and

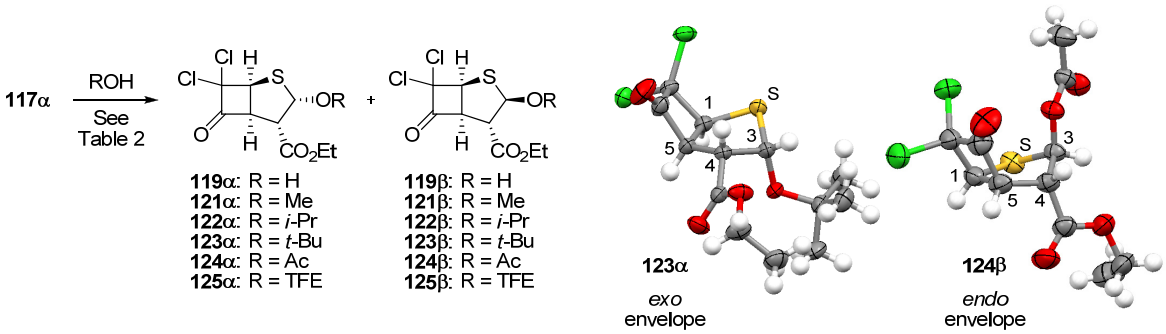
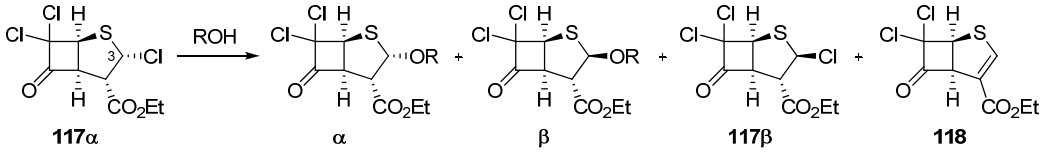


Figure 27. Solvolysis of **117α** and X-ray crystal structures of **123α** and **124β**.

Table 2. Substitutions at C3 with Alcohols and AcOH.[a]



solvent(s)	time (h)	product	OR	α[b]	β	**117β**	**118**
MeCN/H_2O 1:1	48	**119**	OH[c]	6	88	0	0
MeCN/MeOH 1:1	48	**121**	OMe	75	24	0	1
MeCN/*i*-PrOH 1:1	40	**122**	O*i*-Pr[d]	46	20	16	18
MeCN/*t*-BuOH 1:1	48	**123**	O*t*-Bu[e]	34[f]	7	44	15
AcOH	48	**124**	OAc	3	52	43	2
AcOH, 80 °C	1	**124**	OAc	4	79	6	11
CF_3CH_2OH	48	**125**	OTFE	5	76	0	19

[a] Reactions were performed at room temperature unless otherwise stated. [b] Product distributions were determined by ^{1}H NMR of the crude product. [c] Remaining 6% is attributed to **119c**. [d] 85% conversion. [e] 42% conversion. [f] An improved yield of **123α** was obtained by heating **117α** in *t*-BuOH at 80 °C. See Chapter 5 for a detailed procedure.

generated considerably more of the epimerization and elimination by-products, **117β** and **118**. The 3β-chloro isomer could not be prepared in useful quantities for further studies since it was found to be unstable during flash chromatography. However, **117β** was found to prefer an *endo* envelope conformation in solution, as indicated by the multiplicity of H3 and H4 in the ^{1}H NMR spectrum. As with **124β**, H3 and H4 in **117β** appear as singlets and $J_{3\alpha,4} = 0$ Hz.

In contrast to the hydrolysis, the substitutions in MeOH, *i*-PrOH, and *t*-BuOH selectively produced the α-OR retention products with α/β ratios of 3.1:1, 2.3:1, and 4.9:1, respectively (Table 2). Control

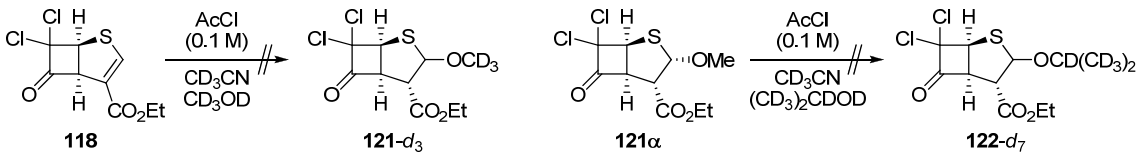


Figure 28. Control experiments which demonstrate that conjugate addition to the unsaturated ester **118** does not occur under the conditions of the substitution reactions and that equilibration of substitution products does not occur.

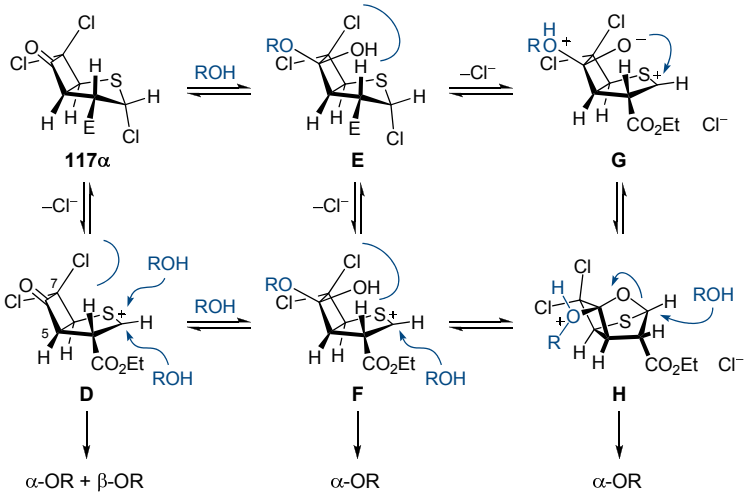


Figure 29. Possible intermediates in substitutions involving **117α** with ROH (E = CO_2Et).

experiments which involved the exposure of **118** to AcCl (1 equiv) in methanol-d_4/acetonitrile-d_3 (1:1), in order to mimic the conditions of the substitution reactions, discount the possibility that the α-OR products are the result of the conjugate addition of ROH to **118** since no reaction was observed by NMR in 48 hours (Figure 28). Similarly, the 3α-OMe derivatives **121α** and **121β** were separately exposed to AcCl in isopropanol-d_8/acetonitrile-d_3 (1:1) and the lack of reaction over a period of 4 days demonstrates that equilibration of the substitution products α-OR and β-OR does not occur. Together, these experiments imply that the α/β ratios are the consequences of kinetic and not thermodynamic control.

The high α-selectivity in the substitution with methanol led to the speculation that a cyclobutanone hemiketal could be generated that would block the *endo* face of C3 from subsequent inversion (structures **E**–**F**, Figure 29). In this way, attack from the *exo* (α) face of **F** would lead to the major isomer **121α** whereas the minor isomer **121β** would be a result of attack from the *endo* (β) face of **D**. In addition, it is possible that the hemiketal could provide higher α-selectivity through a double

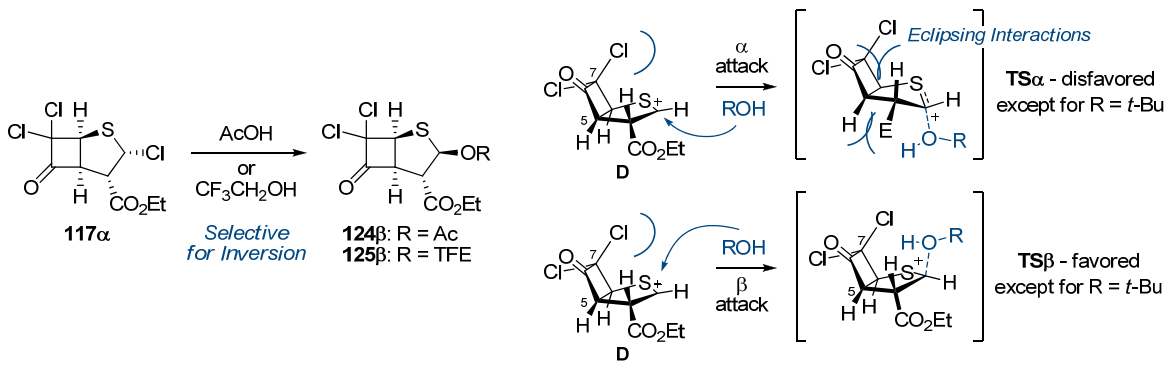


Figure 30. Solvolysis of **117α** with AcOH and TFEOH and a rationale for high β selectivity.

inversion resulting from neighbouring group participation (structures **G**–**H**). Work reported by Grudzinski and Roberts involving the bromination of similar bicyclic substrates indicates that this pathway is plausible (Figure 26). The fact that a tricyclic methyl ketal (neutral form of **H**) was not observed may indicate that either this is a minor pathway, or that **H** is very reactive since one full equivalent of HCl is generated in this solvolysis reaction while the brominations by Grudzinski and Roberts using *N*-bromoacetamide were non-acidic.

While the hypothesis involving intermediates **E**–**H** is reasonable for methanol, it was suspected that hemiketal formation with a bulkier alcohols is less significant. In order to determine the extent of hemiketal formation in alcohols other than methanol, cyclobutanone **118** was dissolved in deuterated alcohols such that hemiketal formation could be observed by NMR. In methanol-d_4, 96% hemiketal was observed after 2 hours and 96% after 4 days. In *t*-butanol-d_{10}, less than 1% hemiketal was observed after 24 hours and 5% after 18 days. In isopropanol-d_8, 7% hemiketal was observed after 5 hours and 36% after 8 days. In trifluoroethanol-d_3, less than 1% hemiketal was observed after 4 hours and 3% after 8 days.

With the confirmation that hemiketal formation in *t*-butanol occurs to a much lesser extent than in methanol, it is likely that the substitution products **123α** and **123β** are the result of attack by *t*-butanol on intermediate **D**. The five-membered ring of structure **D** is essentially flat, as indicated by a gas phase structural optimization (RHF/6-31G(d)), and the fact that the retention product **123α** is generated in preference to **123β** indicates that the *exo* face is more accessible than *endo* face as a consequence of steric shielding by the 7β-chlorine atom. The rationale that steric hindrance drives the 5:1 α:β selectivity would be consistent with the lower conversion observed with *t*-butanol.

The 2:1 α:β selectivity shown by 2-propanol could be rationalized by a combination of its capability to slowly form the hemiketal, which would lead to **122α** from **F** or **H**, and its ability to attack from the *endo* (β) face of **C** or **D**, which would yield **122β**. The high selectivity in favour of inversion demonstrated by acetic acid (**124α**:**124β** ≈ 1:17) and trifluoroethanol (**125α**:**125β** ≈ 1:15) was unexpected as it clearly contrasts with that of the aforementioned alcohols. This selectivity suggests that attack from the *endo* face must be favoured electronically since the enhanced acidity and weaker nucleophilicity of AcOH and TFEOH increase the S_N1 character of the substitution relative to reactions with ordinary alcohols (Figure 30).

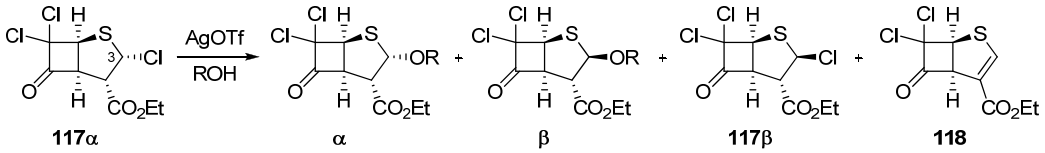
Attack on the carbocation from the α (*exo*) face may be disfavoured as a result of two destabilizing eclipsing interactions in **TSα** that are not present in **TSβ**. The high α-selectivity demonstrated by *t*-BuOH, however, indicates that the 7β-Cl imposes such a large destabilizing steric interaction in **TSβ** that **TSα** is preferred.

While the solvolysis reactions using ROH/MeCN were useful for the synthesis of **119β** and **121α**–**123α** and interesting from a mechanistic point of view, they generally required relatively long reaction times, difficult chromatographic separations, and generated large proportions of the epimerization and elimination by-products **117β** and **118**. Efforts were then made to develop a complementary method that would be more selective for inversion and improve upon the reaction times and the amount of by-products formed. The use of silver triflate was explored (Table 3) as it has been used as a promoter in substitutions at the anomeric position of chlorocarbohydrates.

Since the α/β selectivity of the hydrolysis was not a concern (based on the proposed interconversion of the products **119α** and **119β**), acetonitrile was chosen as a water-miscible solvent. However, the use of MeCN with **117α** was otherwise considered problematic due to the spontaneous epimerization to **117β** observed previously and CH_2Cl_2 was chosen for reactions with alcohols. Typically, **117α** was slowly added as a solution in CH_2Cl_2 to a suspension of AgOTf, ROH, and molecular sieves in CH_2Cl_2 at 0 °C.

Indeed, the AgOTf-promoted reactions displayed improved reaction times and an increased selectivity for inversion with ROH. Interestingly, ten equivalents of methanol showed less selectivity for inversion than ten equivalents of *t*-butanol. Since a decrease to five equivalents of MeOH improved the β selectivity, it is possible that hemiketal formation remains significant at low

Table 3. Substitutions at C3 Promoted by Silver Triflate.[a]



ROH	equiv ROH	time (h)	product	OR	α[b]	β	**117β**	**118**
H_2O [c]	excess	4	**119**	OH[d]	6	85	0	3
MeOH	5	6	**121**	OMe	22	72	2	4
MeOH	10	6	**121**	OMe	26	61	0	2
i-PrOH	5	12	**122**	O*i*-Pr	16	71	2	11
t-BuOH	10	8	**123**	O*t*-Bu[e]	5	42	30	22

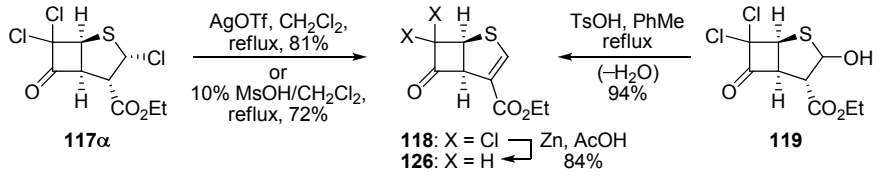
[a] Reaction conditions: AgOTf (1.2 equiv) in CH_2Cl_2 was used with 4 Å MS (3 Å MS for R = Me). Reactions were begun at 0 °C and allowed to warm to room temperature. [b] Product distributions were determined by ^{1}H NMR of the crude product. [c] Hydrolysis was performed at room temperature in MeCN/H_2O 1:1 without the use of molecular sieves. [d] Remaining 6% is attributed to **119c**. [e] 92% conversion.

concentrations of MeOH and that leakage to **121α** could be through structures **F**–**H** (and to a lesser extent **D**).

The proportion of the elimination by-product **118** remained a concern in the AgOTf-catalyzed reactions with the hindered nucleophiles *i*-PrOH and *t*-BuOH. This observation inspired an investigation into whether the elimination could be effected exclusively in the absence of a nucleophile. Gratifyingly, the addition of **117α** to AgOTf in refluxing CH_2Cl_2 furnished **118** cleanly in 81% isolated yield (Scheme 26).

It was also found that the unsaturated ester **118** could be prepared in high yield through dehydration of the thiolactols **119** with TsOH in refluxing toluene under a Dean–Stark trap,[595] although it should be noted that high yields for this reaction were achieved only when the water from TsOH·H_2O was azeotropically removed prior to the addition of **120**. A more convenient and expedient procedure for the synthesis of **118** was discovered more recently. Although one very early attempt to eliminate

Scheme 26. Methods for the Synthesis of the Unsaturated Esters **118** and **126**.



HCl from the **117α** involved methanesulfonic acid (MsOH), its use in only catalytic quantities was insufficient to promote the desired reaction. With a higher concentration of MsOH (10% in CH_2Cl_2), however, the unsaturated ester **118** was isolated in 72% yield. Cyclobutanone **118** could also be dechlorinated readily with zinc in acetic acid to give **126**.

2.6 Conformational Properties of C3 Derivatives and Anomeric Effects

During the development of the solvolysis reactions and silver-triflate promoted substitutions it became clear that cyclobutanones with 3α substituents (Z = Cl, *O*-alkyl) conformed to an *exo* envelope while those with 3β substituents (Z = H, Cl, OAc, *O*-alkyl) showed preference for the *endo* envelope. This was apparent from ^{1}H NMR spectroscopy (Figure 31) as the spectrum of each derivative displayed a pattern similar to either **117α** and **123α** or **124β**, the structures of which were solved by X-ray crystallographic studies.

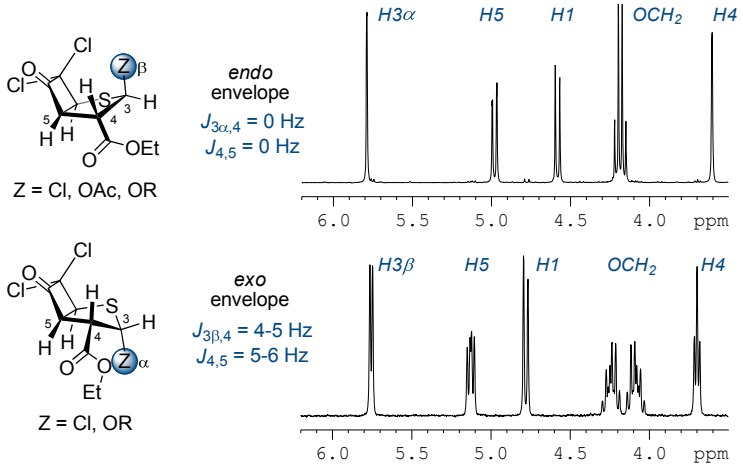


Figure 31. Conformations of C3-substituted cyclobutanones. Coupling patterns in the ^{1}H NMR spectra shown (**123β** and **123α**, Z = O*t*-Bu), are representative of derivatives **117**, **119**, and **121**–**125**.

The possibility was considered that the conformational preferences could be a result of an anomeric effect involving the sulfur and the electron-withdrawing substituents at C3.[615] Although the conformational properties of sulfur-containing six-membered rings are well known,[616] the anomeric effect has been studied relatively little in five-membered rings since the conformational consequences are much less dramatic and twisted conformations are typically favoured in which the substituents are

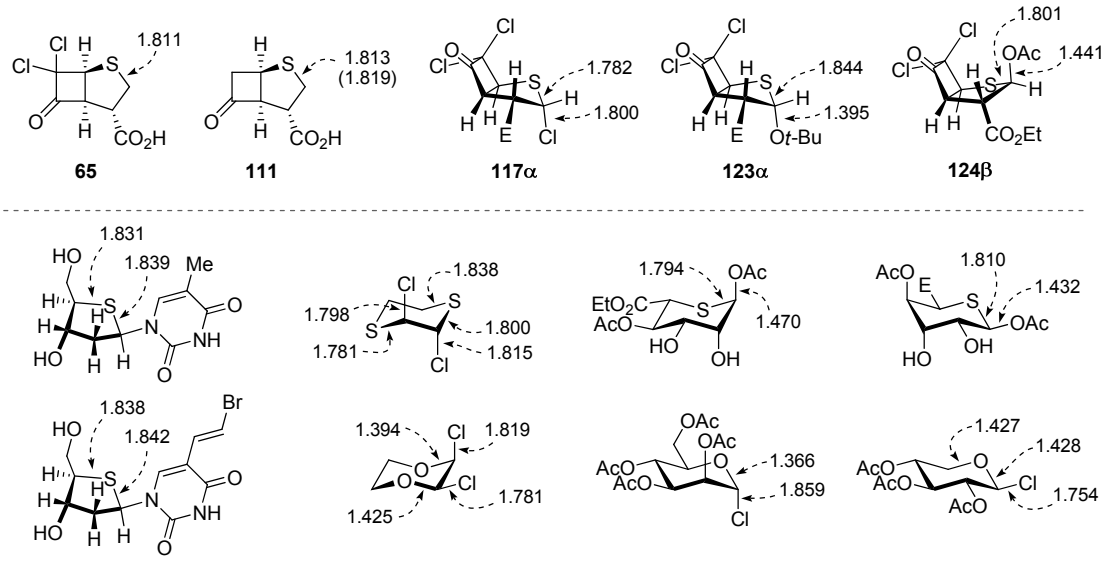


Figure 32. Comparison of bond lengths (Å) obtained from X-ray crystal structures in the present study with related compounds. The X-ray structure of **111** contains two crystallographically different molecules in the unit cell (E = CO_2Et).

in pseudo-axial or pseudo-equatorial orientations. While this is evident in the crystal structures of numerous 4′-thionucleoside derivatives (α-aza substituents),[617] anomeric effects and gauche effects have been argued previously to rationalize the conformations of 4′-thionucleosides.[617,618] In addition, it is possible that anomeric effects are responsible for the axial arrangements that have been recognized by ^{1}H NMR analysis in known α-halo and α-alkoxy tetrahydrothiophenes such as *trans*-2,3-dichlorotetrahydrothiophene,[619] 2-alkoxy-3-chlorotetrahydrothiophenes,[620] *trans*-2,3-dibromotetrahydrothiophene, and 2-alkoxy-3-bromotetrahydrothiophenes.[621]

Since the anomeric effect is often characterized by a shortening of the O–C bond and lengthening of the C–X bond in the O–C–X segment of carbohydrate derivatives, the bond lengths obtained in the X-ray crystal structures of **117α**, **123α**, and **124β** were examined closely (Figure 32). The anomeric effect is generally thought to be strongest with electron-withdrawing substituents (halogen > OR > SR > OH > NR_2)[615c] and structural evidence for the anomeric effect was indeed discovered in 2-thiabicyclo[3.2.0]heptan-6-ones which possess electron-withdrawing substituents at C3.

The X-ray crystal structure of 3α-Cl derivative **117α** clearly reveals a shortened S–C3 bond (1.782 Å) and a lengthened C3–Cl bond (1.800 Å) in comparison to structurally related tetrahydrothiophenes, dithianes and dioxanes, chlorocarbohydrates, and the unsubstituted

cyclobutanones **65** and **111** (Figure 32). Similarly, the solid-state structure of **124β** exhibits a shorter S–C3 bond (1.801 Å) relative to **65** and **111** and C3–O bond (1.441 Å) of intermediate length relative to acetoxythiopyranosides.

Interestingly, in contrast to **117α** and **124β**, the X-ray crystal structure of the 3α-O*t*-Bu derivative **123α** shows a long S–C3 bond (1.844 Å) and a short C3–O bond (1.395 Å). This is consistent with the general consensus that anomeric effects are stronger with oxygen than sulfur.[615,616,622]

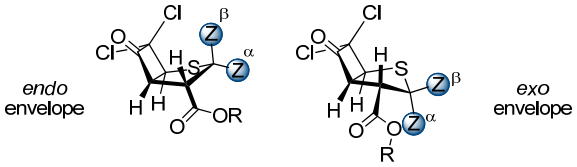
In order to probe these phenomena further, ab initio (RHF/6-31G(d)) molecular orbital (MO) calculations[623] were carried out with several of the cyclobutanone derivatives that had been prepared synthetically (Table 4). The conformational preferences observed by NMR (solution phase) and by X-ray (solid state) were also found in the gas phase and the preferred conformations are those in which C3- substituents are oriented axially.

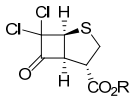
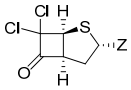
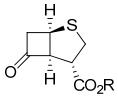
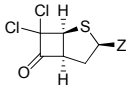
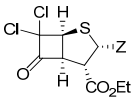
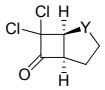
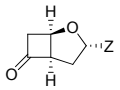
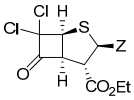
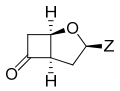
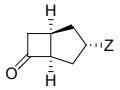
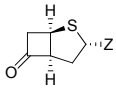
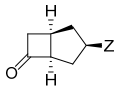
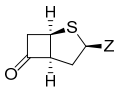
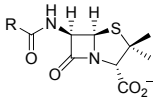
In order to gain additional insight into the origin of the conformational preferences and putative stereoelectronic stabilizations of axial arrangements, additional calculations were performed with a series of simplified cyclobutanone derivatives that lack the C4-carboxylate moiety.

These calculations show that the C4-carboxylate has a minor effect on the conformations of unsubstituted (Z = H) cyclobutanones, as each of the cyclobutanones **65**, **84**, **110**, and **111** (1.4–1.9 kcal/mol) and **112** and **130** (2 kcal/mol) have a similar preference for the *endo* envelope, and suggest that the eclipsing interactions between substituents at C4 and C5 in the *exo* envelope are significant (Figure 33). The 2-oxa and 2-carba analogues **139**–**142** also favour the *endo* envelope conformation.

Computational evidence for the anomeric effect is noted in the comparison of cyclobutanones **112** and **130** to compounds with α substituents at C3. The small preference for the *exo* envelope (0.05 kcal/mol) shown by cyclobutanone **131α**, for example, indicates that the energy gained by stereoelectronic stabilization in the *exo* envelope is enough to overcome the natural *endo* preference (1.89 kcal/mol) of the fused bicycle. Thus, comparison of **130** with **131α**–**135α** suggests that anomeric effects could be worth up to 1.9, 3.3, 3.1, 1.5, and 1.1 kcal/mol for Z = α-Cl, α-OAc, α-OMe, α-O*t*-Bu, and α-SMe, respectively. Similar trends were found with the dichlorocyclobutanones **136α**, **137α**, and **138α**, which gave values of 1.9, 3.2, and 1.0 kcal/mol for Z = α-Cl, α-OMe, and α-SMe, respectively, when compared to **112**.

Table 4. Calculated Conformational Preferences for Cyclobutanone Derivatives (kcal/mol).[a]



Cyclobutanone or β-Lactam		Relative Energy		Cyclobutanone or β-Lactam		Relative Energy	
		endo	*exo*			*endo*	*exo*
	**65**: R = H	0	+ 1.93		**136α**: Z = Cl	0	+ 0.47
	84: R = Et	0	+ 1.92		**137α**: Z = OMe	+ 0.77	0
	127: R = –	0	+ 2.28		**138α**: Z = SMe	0	+ 1.42
	**111**: R = H	0	+ 1.43		**136β**: Z = Cl	0	+ 1.59
	110: R = Et	0	+ 1.44		**137β**: Z = OMe	0	+ 4.84
	128: R = –	0	+ 1.73		**138β**: Z = SMe	0	+ 0.51
	117α: Z = Cl	+ 2.44	0		**139**: Y = O	0	+ 0.78
	119α: Z = OH	+ 3.84	0		**140**: Y = CH_2	0	+ 2.27
	**121α**: Z = OMe	+ 3.75	0				
	123α: Z = O*t*-Bu	+ 1.29	0				
	124α: Z = OAc	+ 3.41	0		**141**: Y = O	0	+ 1.40
	129α: Z = SMe	+ 0.55	0		**142**: Y = CH_2	0	+ 2.02
	117β: Z = Cl	0	+ 1.69				
	119β: Z = OH	0	+ 5.18		**143α**: Z = OMe	+ 2.10	0
	**121β**: Z = OMe	0	+ 4.85		**144α**: Z = SMe	+ 0.62	0
	123β: Z = O*t*-Bu	0	+ 3.55				
	124β: Z = OAc	0	+ 4.78				
	129β: Z = SMe	0	+ 0.64		**143β**: Z = OMe	0	+ 3.45
					144β: Z = SMe	0	+ 0.38
	**130**: X = H	0	+ 1.89				
	112: X = Cl	0	+ 2.42				
					**145α**: Z = OMe	0	+ 1.36
	131α: Z = Cl	+ 0.05	0		**146α**: Z = SMe	0	+ 2.69
	**132α**: Z = OAc	+ 1.40	0				
	133α: Z = OMe	+ 1.17	0				
	134α: Z = O*t*-Bu	0	+ 0.35		**145β**: Z = OMe	0	+ 2.41
	135α: Z = SMe	0	+ 0.75		**146β**: Z = SMe	+0.74	0
	131β: Z = Cl	0	+ 2.08				
	**132β**: Z = OAc	0	+ 4.64		**147**: R = Me	0	+ 2.05
	133β: Z = OMe	0	+ 4.46		**148**: R = CH_2Ph	0	+ 2.18
	134β: Z = O*t*-Bu	0	+ 3.33				
	135β: Z = SMe	0	+ 0.92				

[a] Comparison of the relative energy of each conformer was done following zero-point energy corrections.

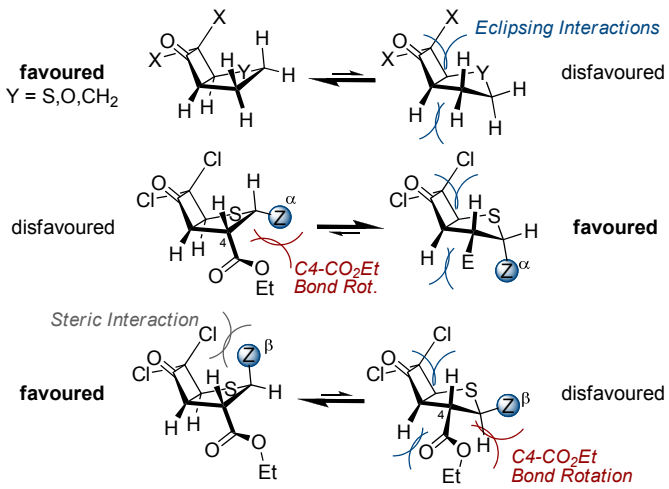


Figure 33. Favoured conformations and relevant steric interactions.

Calculations involving β-substituents at C3 also indicate that an anomeric effect is present as the β-alkoxy derivatives **132β**–**134β** show increased preferences for the *endo* envelope (2.8, 2.6, and 1.4 kcal/mol for Z = β-OAc, β-OMe, and β-O*t*-Bu) relative to **130** (Z = H). However, the β-Cl derivative **131β**, which contains a sterically larger substituent, shows only a modest increase in *endo* preference (0.2 kcal/mol) and the β-SMe analogue **135β** shows a decrease in *endo* preference (1.0 kcal/mol). Since this pattern is magnified in the 7,7-dichloro series **136β**–**138β** (–0.8, +2.4, and –1.9 kcal/mol for β-Cl, β-OMe, and β-SMe compared to **112**), it seems reasonable to conclude that large 3β-substituents experience a significant steric interaction with the 7β-H or 7β-Cl (Figure 33).[624,625]

Given the large magnitudes of the calculated conformational preferences (2–5 kcal/mol) for cyclobutanones **117**, **119**, **121**, **123**, **124**, and **129**, and the relatively small preferences with derivatives **131α**–**135α**, and **136α**–**138α**, it is clear that the C4-carboxylate moiety significantly enhances the preference for the *exo* conformation with α-substituents. It is likely that a disfavoured steric interaction between the 3α-Z function and the carboxylate in the *endo* envelope accounts for much of this energy since a significant rotation of the C4–CO_2Et bond is observed in the optimized *endo* envelope conformations of **121α**, **123α**, **124α**, and **129α**, and not in the *exo* envelope conformations. A torsional scan about the C4–CO_2Et bond of **84** revealed that the dihedral angle between the C4–C5 bond and the C=O bond of the ester was close to 0° in the lowest energy conformations, in both the *endo* envelope and *exo* envelope, and that rotation of the bond is associated with a substantial increase in potential energy (Figure 34). Comparison of the

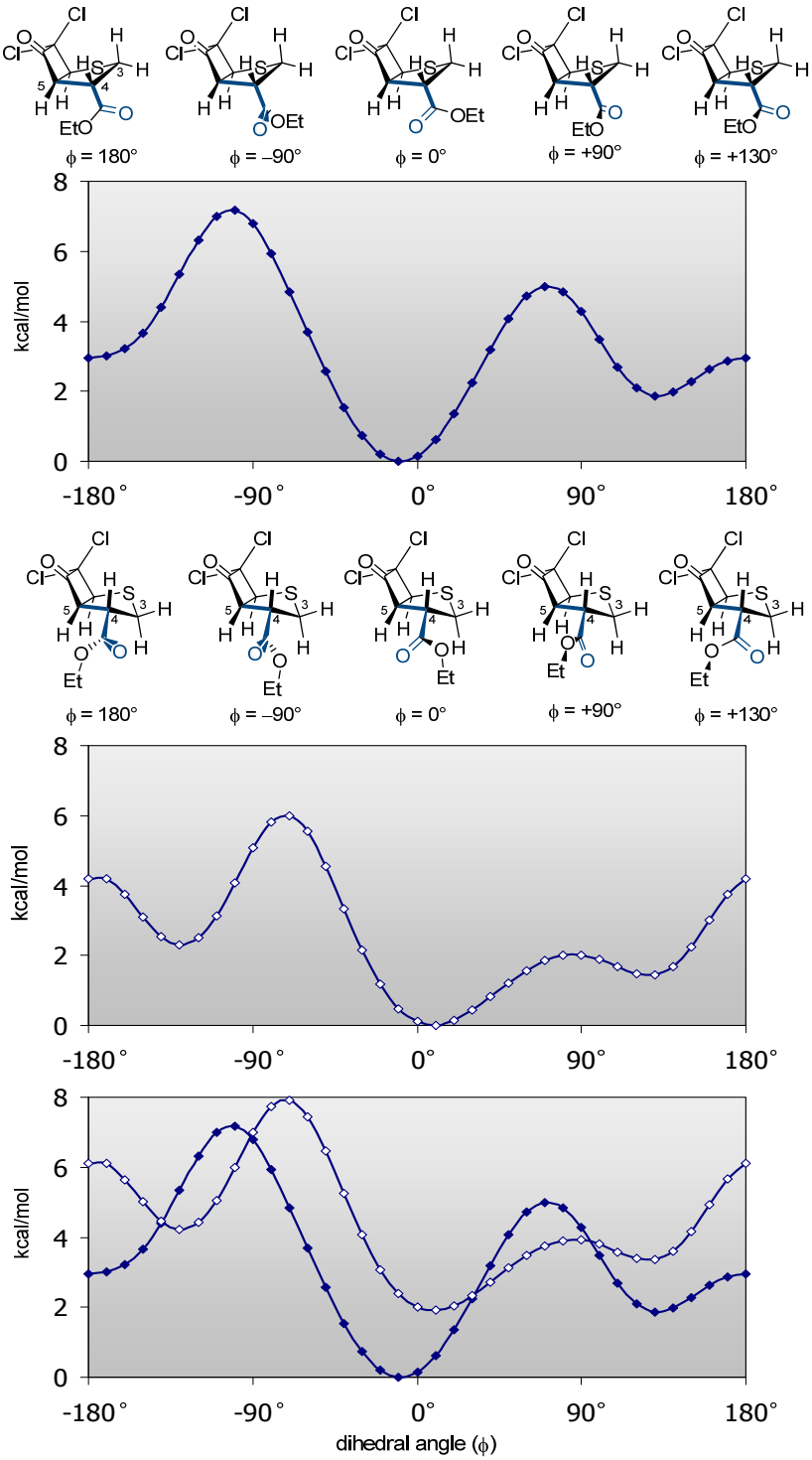


Figure 34. Rotation of the C4–CO_2Et bond in cyclobutanone **84**. Calculated (RHF/6-31G(d)) potential energies in the (A) *endo* envelope and (B) *exo* envelope conformations are superimposed and offset by 1.92 kcal/mol in (C) to reflect relative energies. ϕ represents the dihedral angle between the C4–C5 bond and the C=O bond (as coloured in blue). Determination of the potential energy at each angle involved the manual rotation of the C4-CO_2Et bond (10° increments) from the RHF/6-31G(d)-optimized geometry of **84**, followed by calculation of the RHF/6-31G(d) energy at each angle.

conformational preferences of cyclobutanones with β-substituents, however, indicates that the C4-carboxylate has a minor energetic effect as **117β**, **121β**, and **129β**, have nearly identical preferences as the de-carboxy analogues **136β**–**138β**.

In addition to the computational results described thus far, the conformational preferences of several 2-oxa and 2-carba (CH_2) analogues **139**–**146** have been calculated for the purpose of comparison. It is worth noting that the calculated conformational preferences for the 2-thia series are generally larger in magnitude than the preferences of the 2-oxa counterparts, and that this is likely a consequence of increased steric interactions in the tetrahydrofuran system due to the shorter endocyclic carbon-oxygen bond lengths.

2.7 Conformational Properties of Penicillins

The importance of the penicillins to antibiotic therapy has led to numerous studies of their three-dimensional structures by experimental and theoretical methods and particular attention has been paid to the conformational properties of the thiazolidine ring. X-ray crystal structures, which have been obtained for a variety of penicillins, show that the bicycle can adopt an *endo* envelope conformation **I** or an *exo* envelope conformation **J** (Figure 35).[626] The *endo* and *exo* envelopes are also referred to as the C3-puckered and *S*-puckered conformations, based on the atom that is most out of the plane of the ring, or the axial and equatorial conformations, respectively, based on the orientation of the 3α-carboxylate.

Attempts have been made to correlate the solid-state conformational preferences with biological activity,[627,628] but NMR studies by Dobson et al. demonstrate that these differences in conformational preference are not present in solution. The use of lanthanide ions as probes[629] and ^{13}C cross polarization magic angle spinning (CP-MAS) NMR experiments[630,631] indicate that several penicillins (with different solid-state preferences) all favour the *exo* envelope conformation **J** in aqueous solution with ratios of *endo*:*exo* conformers ranging from 45:55 to 21:79, respectively.[632]

While penicillin G has been found to favour the *endo* envelope conformation **I** in the gas phase by several computational studies[633,634] including our own (2.2 kcal/mol, Table 4), molecular dynamics (MD) studies by Díaz et al.[633] show that solvent has a significant energetic effect (~1.5 kcal/mol) in

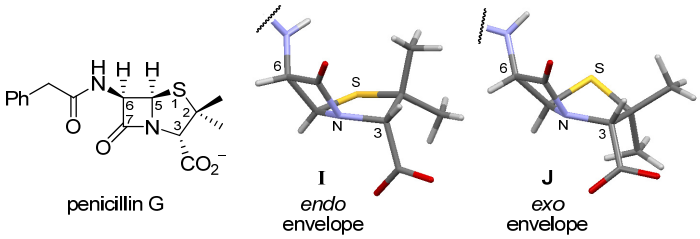


Figure 35. Two conformations of penicillin G. Figures of the *endo* envelope (C3-puckered, axial) conformation **I** and the *exo* envelope (*S*-puckered, equatorial) conformation **J** were adapted from X-ray crystal structures of the potassium salt (CCDC: BPENK01) and the procaine salt (CCDC: PRPENG), respectively.

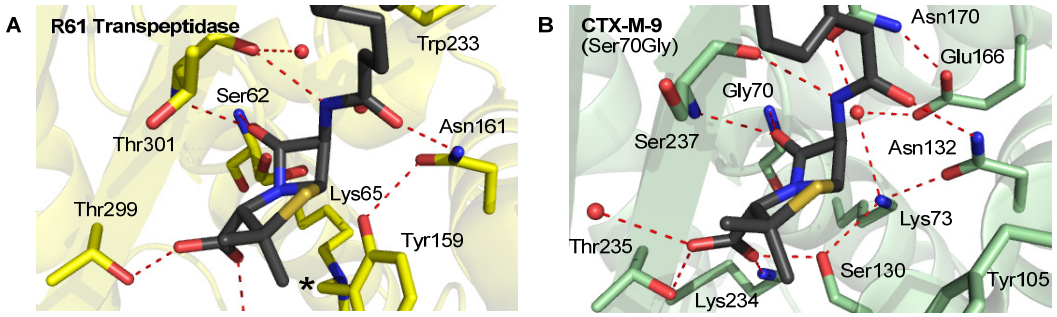


Figure 36. Non-covalent complexes of penicillins with penicillin-recognizing proteins. (A) X-ray crystal structure (PDB: 1PW1) of a peptidoglycan-mimetic penicillin bound to an acylation-deficient form of R61 DD-transpeptidase.[199] (B) X-ray crystal structure of benzylpenicillin bound to the Ser70Gly mutant of the class A β-lactamase CTX-M-9 (PDB: 3HUO).[637]

stabilizing the *exo* envelope conformation **J**, and the MD simulation which predicts an *endo*:*exo* ratio of 70:30 in aqueous solution is in reasonable agreement with Dobson's experimental results.

In the context of biological activity, it is thought that β- lactamases preferentially bind to the *exo* envelope conformer **J** of penicillins. Through detailed MD simulations of penicillin G complexed with the class A TEM-1 β-lactamase, Díaz et al. have shown that H-bonding interactions between the C3-carboxylate and Ser130, Ser235, and Arg244, are favoured with the equatorial conformer **J** and that a steric clash between the 2β-methyl group and Ala237 is also avoided.[263] Additional molecular modeling studies involving mechanisms of penicillin acylation in TEM-1 (class A)[264,266,635] and P99 (class C)[636] β-lactamases have also involved the *exo* envelope conformation **J** of the penicillins, but analogous studies with the class B and class D enzymes have yet to be reported.

Recent crystallographic work, however, could be considered the strongest evidence that the *exo* envelope is the biologically active conformation. A non-covalent complex between the R61

transpeptidase and a peptidoglycan-mimetic penicillin has been trapped crystallographically[199] and an X-ray crystal structure of benzylpenicillin bound to the Ser70Gly mutant of CTX-M-9 has been recently deposited in the PDB (Figure 36).[637] These crystal structures show that the penicillin adopts the *exo* envelope conformation **J** in the active sites of the PBP and the β-lactamase.

2.8 Conformations of Cyclobutanone Derivatives and Hemiketal Formation

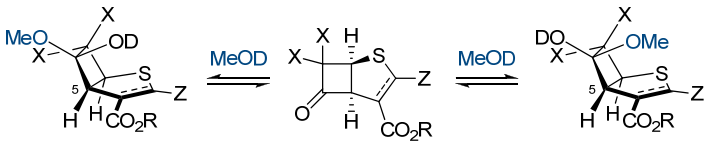
Since the tendency for the cyclobutanones to undergo hydrate and hemiketal formation is central to their ability to function as β-lactamase inhibitors, the strong evidence (discussed above) indicating that penicillins bind to PBPs and β-lactamases in the *exo* envelope conformation brought to mind the following question: Does the conformation of the tetrahydrothiophene ring have a significant effect on the tendency of the cyclobutanones to undergo hydrate and hemiketal formation?

Hemiketal formation with cyclobutanones was evaluated through simple NMR experiments in which the cyclobutanone of interest was dissolved in neat methanol-d_4 (Table 5). As mentioned above, the dichlorocyclobutanones **65** and **84** (X = Cl) underwent hemiketal formation to a much greater extent than the dechlorinated ketones **110** and **111** (X = H). As expected, this pattern was also evident in the unsaturated system as hemiketal formation occurred to a greater extent with **118** (X = Cl) than with **126** (X = H).

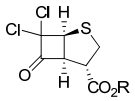
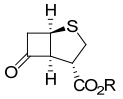
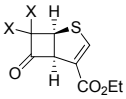
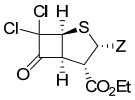
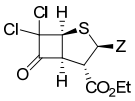
Exposure of the 3-alkoxy dichlorocyclobutanones **121α**–**123α** and **121β**–**124β** to methanol-d_4 revealed that the extent of hemiketal formation was highly dependent upon the steric environment surrounding the carbonyl group. Thus, hemiketal formation is highly favoured in cyclobutanones that prefer the *exo* envelope, **121α**–**123α** (98%), while hemiketal formation is less significant with cyclobutanones **121β**–**124β** (15–40%), which adopt the *endo* envelope. It is reasonable that the unsaturated esters **118** and **126** show larger proportions of hemiketal than **84** and **110**, respectively, since the C3 methylene unit could impose additional steric congestion in the *endo* envelope. Steric hindrance of ketone hydration has been noted previously.[605]

While it has been well established that nucleophilic attack on the carbonyl group is highly favoured from the *exo* face of bicyclo[3.2.0]heptan-6-ones, the reversible nature of hemiketal formation gave rise to a mixture of the ketone and each of the α- and β-hemiketals which often required weeks to

Table 5. Cyclobutanone Hemiketal Formation in Methanol-d_4.[a]



α-hemiketal *ketone* *β-hemiketal*

Cyclobutanone		α : β hemiketal ratio [b]	% hemiketal
	**65**: R = H	2.7 : 1	88
	84: R = Et	2.7 : 1	91
	**110**: R = Et	1.8 : 1	19
	111: R = H	1.6 : 1	24
	**118**: X = Cl	1.8 : 1	96
	126: X = H	1.5 : 1	38
	**121α**: Z = OMe	1.2 : 1	98
	122α: Z = O*i*-Pr	1.1 : 1	98
	123α: Z = O*t*-Bu	1.1 : 1	98
	**121β**: Z = OMe	4.7 : 1	15
	122β: Z = O*i*-Pr	4.2 : 1	24
	123β: Z = O*t*-Bu	1.8 : 1	40
	124β: Z = OAc	1.5 : 1	30

[a] The extent of hemiketal formation was determined by ^{1}H NMR experiments in neat CD_3OD. [b] The α/β hemiketal ratio was determined when the system had reached equilibrium.

equilibrate. Tabulation of the equilibrium ratios of α-hemiketal/β-hemiketal revealed that the relative stability of the α- and β-hemiketals is highly sensitive to steric hindrance in the same way that the extent of hemiketal formation was found to be. Namely, the cyclobutanone derivatives with the greatest steric bulk on the *endo* face of the bicycle have the highest α/β-hemiketal ratios.

Interestingly, the coupling patterns in ^{1}H NMR and the chemical shifts observed in the ^{13}C NMR indicate that the β-hemiketals of 3β-alkoxy cyclobutanones **121β**–**124β** adopt the *exo* envelope conformation (Figure 37).

In contrast to the fast hydrate formation observed with **65** (X = Cl) in D_2O and the classical study by Wiberg et al. involving carbonyl reactions,[638] hemiketal formation involving **65** and **84** was

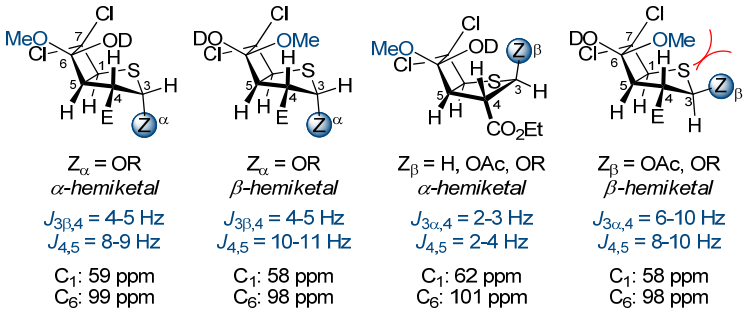


Figure 37. Conformations of cyclobutanone hemiketals as indicated by ^{1}H NMR and ^{13}C NMR (R = Me, *i*-Pr, *t*-Bu). For tables of selected ^{1}H and ^{13}C NMR data for cyclobutanones and cyclobutanone hemiketals, see Appendix D.

surprisingly slow given the high electrophilicity of the carbonyl carbon and required several hours to equilibrate (Appendix E).[639] The results reported by the Wiberg group, which show that hemiketal formation with ketones is fast, were replicated in our laboratory under our conditions for cyclobutanone itself and were also reflected in the relatively fast hemiketal formation demonstrated by the dechlorinated cyclobutanones **110** and **111**.

Although the extent of hemiketal formation is greater for the 7,7-dichlorinated systems as compared with the non-chlorinated compounds, qualitative observations of relative rates of hemiketal formation suggest that the process is somewhat impeded by the halogen substituents. The approximate trend in relative rates of hemiketal formation is: **121α–123α** > **126** ≈ **118** ≈ **110** > **84** > **123β** > **122β** > **121β**.

Hemiketal formation must involve either protonation of the carbonyl oxygen or at least substantial H-bonding to the oxygen from the solvent to activate the carbonyl for nucleophilic attack at carbon. The chlorine atoms at C7 likely diminish electron density on the carbonyl oxygen atom making it less basic and less prone to protonation by an acid catalyst or to act as an H-bond acceptor. Furthermore, the chlorines likely offer a degree of steric hindrance to the solvation which might be required for lowering the activation energy for the addition step in hemiketal formation.

2.9 Summary and Outlook

This chapter describes details of the synthetic route used for construction of the 2-thia-

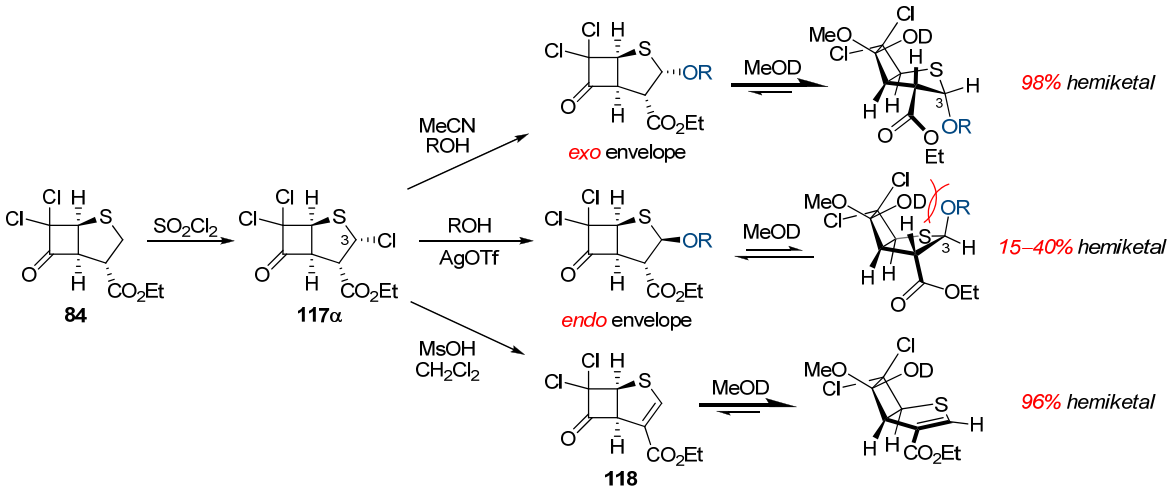


Figure 38. Synthesis of 3α-alkoxy, 3β-alkoxy, and 3,4-unsaturated cyclobutanone analogues of β-lactams and the relationship between conformation and the extent of cyclobutanone hemiketal formation in methanol-d_4.

bicyclo[3.2.0]heptan-6-one-4-carboxylate skeleton and the results of preliminary biochemical assays with cyclobutanones **65** and **111** which encouraged the synthesis of other dichlorocyclobutanone analogues of β-lactams. 3-Alkoxy derivatives were prepared through two complementary diastereoselective substitution reactions following a highly stereoselective chlorination of **84** with sulfuryl chloride (Figure 38). Cyclobutanone derivatives with 3β substituents favour an *endo* envelope conformation in solution, the solid state, and the gas phase, whereas those with 3α substituents adopt an *exo* envelope conformation. Evidence from X-ray crystal structures and ab initio calculations suggests that an anomeric effect contributes to the large conformational preference of the tetrahydrothiophene ring that favours the 3-alkoxy substituent in an axial orientation. In addition, the conformation of the bicyclic system, which is determined by the stereochemistry of the 3-alkoxy substituent, has a dramatic effect on the ability of the cyclobutanone to undergo hemiketal formation in methanol-d_4.

If the extent of hemiketal formation in alcoholic solution can be used as an estimate of the relative stability of the tetrahedral adducts, then the results presented above (Table 5, Figure 38) indicate that 3α-alkoxy cyclobutanone derivatives should also form more stable hemiketal adducts than the 3β-epimers in the active sites of serine β-lactamases. In addition, crystallographic and computational studies which indicate that penicillins bind to PBPs and β-lactamases in the *exo* envelope conformation suggest that 3α-alkoxy cyclobutanone derivatives, which strongly favour the *exo*

envelope conformation, would be better penicillin mimics than the 3β-alkoxy analogues, which strongly favour the *endo* envelope conformation. The relatively rapid and complete hemiketal formation exhibited by the 3α-alkoxy derivatives and the unsaturated cyclobutanone **118** are properties desirable for potential β-lactamase inhibitors functioning by the mechanism described above and experiments designed to exploit these observations are presented in the following chapters of this thesis.

Chapter 3

Cyclobutanones Analogues of β-Lactam Antibiotics: Inhibition of Serine- and Metallo-β-Lactamases†

3.1 A Modified Synthetic Route to the Free-Acid Form of the Cyclobutanones

The relationship between the strong conformational preferences of 3-alkoxy-substituted 2-thia-bicyclo[3.2.0]heptan-6-one-4-carboxylates and their tendency to undergo hemiketal formation led to the prediction that 3α-alkoxy cyclobutanone derivatives should be better β-lactamase inhibitors than the 3β-alkoxy counterparts. In order to test this hypothesis in enzyme inhibition assays the carboxylic acid form of these derivatives was required. Not unexpectedly, however, the conditions used for the acid-catalyzed hydrolysis of other ethyl esters (6 M HCl/dioxane, 85 °C) were found to be inappropriate for 3-alkoxy ethyl ester derivatives. Even 3 M HCl at room temperature led to the decomposition of the 3-alkoxy substrates, presumably through hydrolysis of the *S*,*O*-acetal functionality at C3. Since the sensitivity of the dichlorocyclobutanone ring to aqueous hydroxide had already been established other esters were considered for the protection of the C4-carboxylate (Figure 39).

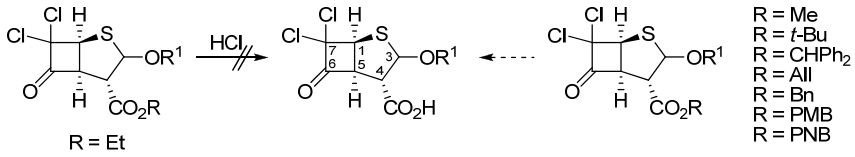
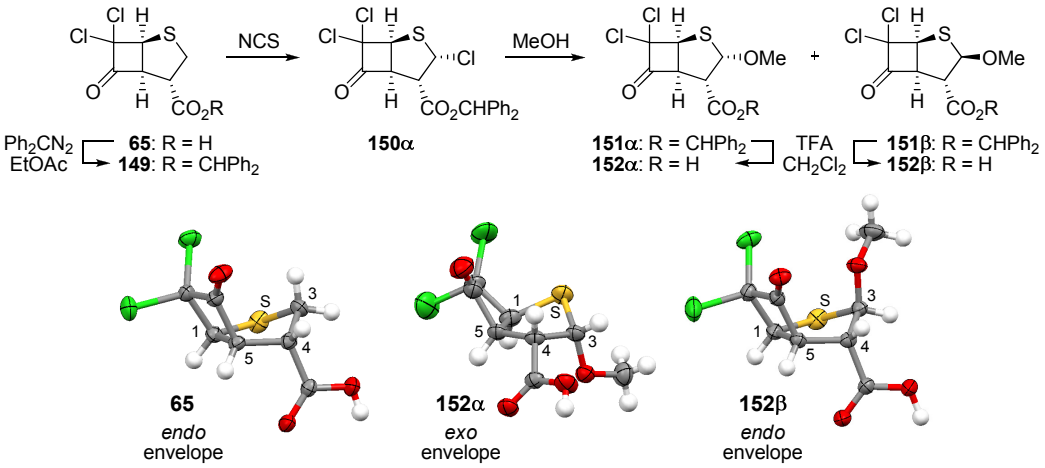


Figure 39. Protecting group strategies considered for the C4-carboxylate of 3-alkoxy cyclobutanone derivatives.

While the 3-alkoxy derivatives were unstable to concentrations of aqueous HCl required for ethyl ester hydrolysis, compounds such as **121α** and **121β** were fully stable in previous experiments with

† Reproduced in part, with permission, from ref 640. Johnson, J. W.; Gretes, M.; Goodfellow, V. J.; Marrone, L.; Heynen, M. L.; Strynadka, N. C. J.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **2010**, *132*, 2558–2560. Copyright 2010 American Chemical Society.

Scheme 27. Synthesis and X-ray Structures of 3-Methoxy Cyclobutanone Analogues of Penicillins.[a]



0.1 M HCl in *i*-PrOH. Several protecting groups were considered potentially useful for the present application (methyl, *t*-butyl, allyl, benzyl, *p*-methoxybenzyl, *p*-nitrobenzyl, etc.) but the benzhydryl ester was chosen since deprotection typically involves TFA and because diphenyldiazomethane had already been prepared by other researchers in this laboratory.

The esterification of **65** with diphenyldiazomethane in EtOAc cleanly afforded the benzhydryl ester **149** in 98% yield (Scheme 27). While previous chlorinations at C3 employed SO_2Cl_2, the chlorination of **149** used *N*-chlorosuccinimide in order to avoid the production of HCl. Chlorinations with NCS, which selectively produced the α-chloro isomer **150α**, were initially done in CH_2Cl_2 but were found to be faster in MeCN. While the succinimide byproduct could be removed with aqueous washes of **150α** in CH_2Cl_2, the washing process caused a certain amount of epimerization and elimination. As a result, the mixture of **150α** and succinimide was typically used directly in the subsequent methanolysis without purification to generate the 3-methoxy substitution products **151α** and **151β** in a 3:1 ratio. The α-chloro isomer **151α** could be selectively crystallized from the mixture prior to the difficult separation of the diastereomers with flash chromatography. Treatment of each of the 3-methoxy isomers with TFA (10% in CH_2Cl_2), followed by trituration with CH_2Cl_2/hexane, provided the free acids **152α** and **152β** without epimerization at C3.

As with the ethyl esters, the conformational preferences of the new benzhydryl- and carboxylic acid derivatives were apparent by analysis of ^{1}H NMR spectra and confirmed in some cases with single-crystal X-ray structures. Namely, derivatives which lack functionality at C3 and derivatives with β-

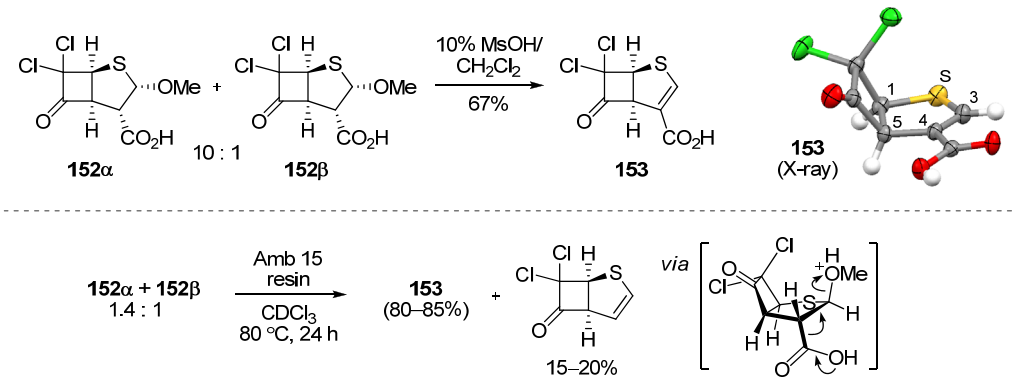
methoxy substituents adopt the *endo* envelope conformation in which $J_{3\alpha,4}$ and $J_{4,5}$ are close to 0 Hz. The α-methoxy epimers, on the other hand, favour the *exo* envelope conformation in which $J_{3\beta,4}$ = 4 Hz and $J_{4,5}$ = 6 Hz. One important consequence of the conformation of the five-membered ring is that the C4 carboxylate is oriented axially in the *endo* envelope and equatorially in the *exo* envelope.

3.2 Synthesis of Cyclobutanone Analogues of Penems

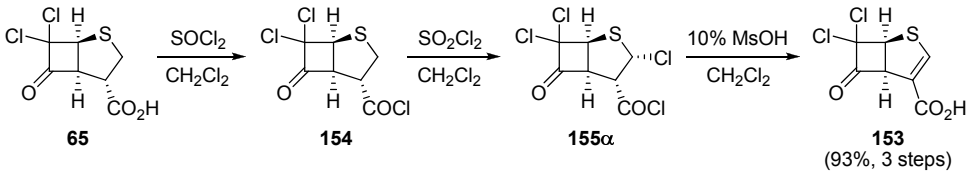
With the saturated cyclobutanone derivatives **65**, **111**, **152α**, and **152β** in hand, the potential for unsaturated cyclobutanone analogues to act as inhibitors was also considered since such compounds could be considered as analogues of penems or carbapenems.

The success of earlier elimination attempts (e.g. **119→118**) supported the notion that the unsaturated acid **153** could be obtained by elimination of methanol from **152α** or **152β** under acidic conditions. While initial experiments involving Amberlyst 15, an acidic resin, effected elimination only slowly and resulted in partial decarboxylation, the unsaturated acid **153** was obtained cleanly by subjecting mixtures of **152α** and **152β** to 10% MsOH in CH_2Cl_2 at reflux for 2 hours (Scheme 28). This method provided enough material for X-ray crystallographic analysis and preliminary studies to evaluate the extent of hydrate formation in D_2O, but this synthetic route to **153** was considered unfavourable since it consumed material (**152α** and **152β**) which could otherwise be used for biochemical and biological assays.

Scheme 28. Synthesis and X-ray Structure of the Penem Analogue **153**.

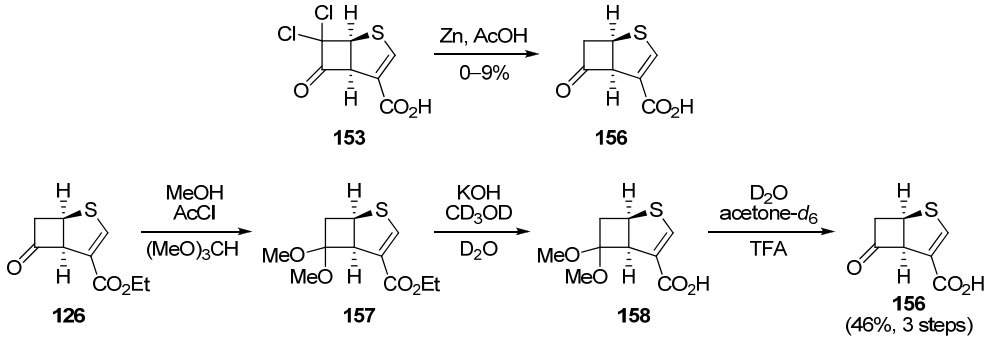


Scheme 29. Improved Three-Step One-Pot Synthesis of the Unsaturated Acid **153**.



With a view to develop a more practical synthetic route toward **153**, it was thought that the steps required for protection of the carboxylate as an ester and deprotection may be avoided if the readily available acid **65** could be used directly. Attempts to chlorinate **65** with SO_2Cl_2 directly were unsuccessful and indicated that some sort of modification of the carboxylate was necessary. As a rather unconventional solution to this problem, it was found that an acid chloride was an ideal protecting group for this purpose (Scheme 29). Thus, the saturated acid **65** was converted to the unsaturated acid **153** in three simple operations by treatment with $SOCl_2$, SO_2Cl_2, and then 10% $MsOH/CH_2Cl_2$. Since the first two steps proceed in quantitative yield (by ^1H NMR) and the by-products of $SOCl_2$ and SO_2Cl_2 are volatile, no purification of the acid chloride intermediates **154** and **155α** was required and all three steps were done in a single flask. Isolation of the final product involved an aqueous workup and provided the crude unsaturated acid (≥95% pure by ^1H NMR) in 93% yield (one-gram scale) from acid **65**.

Scheme 30. Preparation of the Dechlorinated Unsaturated Cyclobutanone **156**.



Using the typical procedure for the dechlorination of cyclobutanones, acid **153** was heated with excess zinc dust in acetic acid. While this method was effective for the synthesis of other dechlorinated cyclobutanones (**110**, **111**, **126**), this method repeatedly provided only a very low yield (0–9%) of the desired cyclobutanone **156** (Scheme 30). Variations of the dechlorination (e.g. Zn in

AcOH at rt, or Zn in *i*-PrOH/AcOH 100:1) were also unsuccessful. In a different approach toward the dechlorinated acid **156**, the direct hydrolysis of the ethyl ester **126** was attempted but the use of KOH (1 equiv) in THF or H_2O resulted in a complex mixture of products. Since the failure of the alkaline hydrolysis was suspected to be a result of the ability of the cyclobutanone to participate in undesired enolate reactions, the ketone **126** was converted to the dimethyl ketal **157** using trimethyl orthoformate in acidic methanol. Protection of the ketone permitted the alkaline hydrolysis of the ethyl ester and KOH was used in D_2O/methanol-d_4 (2:1) so that the reaction could be followed by ^1H NMR. After 36 hours at room temperature, the desired product **158** was separated from small amounts of remaining unhydrolyzed ester by washing the alkaline aqueous phase with CH_2Cl_2. Acid-catalyzed hydrolysis of the dimethyl ketal in TFA/acetone-d_6/D_2O (1:4:4) then furnished the dechlorinated acid **156** in 46% yield over three steps. TFA was used in favour of HCl because previous attempts to hydrolyze the unsaturated ester **119** with aqueous HCl led to extensive decomposition and, furthermore, HCl had been found to induce the ring-opening of cyclobutanone **110** (Scheme 22).

3.3 Inhibition of β-Lactamases with Cyclobutanone Analogues of Penams and Penems

While the synthetic studies described above were in progress, this laboratory acquired the expression systems for additional β-lactamases as generous gifts from different researchers throughout the world. In addition to IMP-1 and OXA-10, which had already been given to this group by Prof. M. Galleni (U. Liège, Belgium) and Prof. S. Mobashery (U. Notre Dame), SPM-1 and OXA-45 were provided by Prof. J. Spencer and Prof. T. Walsh (U. Bristol, U.K.), and VIM-2, KPC-2, and GC1 were obtained from Prof. P. Nordmann (U. Paris), Prof. F. van den Akker (Case Western), and Prof. M. Nukaga (Josai International University, Japan), respectively.

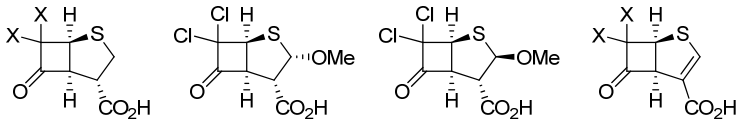
The KPCs (*Klebsiella pneumoniae* carbapenemases) are class A ESBLs that hydrolyze β-lactams of all classes and are found on transferrable plasmids. KPC-producing strains have been isolated in New York, South America, Europe, China, and very recently in Canada.[259,641,642] KPC-2 (Bush group 2f) has been characterized structurally.[643,644] IMP-1 is a class B1 metallo-β-lactamase which has a

wide spectrum of activity including all classes of β-lactam except monobactams and is found in resistant strains of *Serratia*, *Pseudomonas*, *Acinetobacter, Klebsiella, Citrobacter, Achromobacter,* and *Shigella*.[307,309,563] VIM-2 is a class B1 MBL that also has a broad spectrum of activity against penicillins, cephalosporins, and carbapenems.[645] While VIM-2 is mainly observed in *P. aeruginosa*, it has spread more rapidly than the IMP family and, since it has been found in more than 37 countries across five continents, is now considered the global MBL.[311,564] SPM-1, which was discovered from an isolate of *P. aeruginosa* in 2001,[313] is the most important metallo-β-lactamase in Brazil[564] and, unlike other class B1 MBLs, has very low affinity for the second zinc atom.[344,345] GC1 is a group 1e class C enzyme from *Enterobacter cloacae* that owes its ESBL activity to a three amino acid insertion after Arg210 which gives additional conformational flexibility to the Ω loop and allows the enzyme to accommodate bulky oximino sidechains of third-generation cephalosporins.[272,646,647] OXA-10 is a narrow-spectrum class D β-lactamase (group 2d) that has been characterized by X-ray crystallography several times.[291,293] OXA-45 is a class D enzyme with an extended-spectrum of activity (group 2de) which was discovered in a strain of *P. aeruginosa* from Texas that also expressed the metallo-enzyme VIM-7.[648] Interestingly, OXA β-lactamases employ a carbamylated lysine residue (Kcx70) as the general base in the turnover of β-lactams.[293]

With several cyclobutanone analogues of penams and penems in hand and several diverse β-lactamases available, the inhibition of class A, B, C, and D β-lactamases could be studied (Table 6). Inhibition assays were conducted by Ms. Valerie Goodfellow and Dr. Laura Marrone with some assistance from Ms. Jun Wang.

The cyclobutanone inhibitors found to be most potent against the serine β-lactamases KPC-2, GC1, and OXA-10 were those which are hydrated to a larger extent in aqueous solution (Table 6). The dichlorocyclobutanones **65** and **153** undergo hydration to a much greater extent than their dechlorinated counterparts **111** and **156** and are also more effective as inhibitors of each serine β-lactamase. Among the dichlorocyclobutanones, steric hindrance of hydration is apparent as the 3α-methoxy derivative **152α**, which adopts the *exo* envelope, undergoes hydration to a greater extent than **65** and **152β**, which favour the *endo* envelope conformation and have greater steric bulk at C3 (Figure 40). The corresponding observation that **152α** is more potent than **65** and **152β** against each enzyme is consistent with the hypothesis that each β-lactamase binds more tightly the *exo* envelope

Table 6. Inhibition of β-Lactamases by Cyclobutanone Analogues of Penams and Penems.[a,b,c]



65: X = Cl
111: X = H

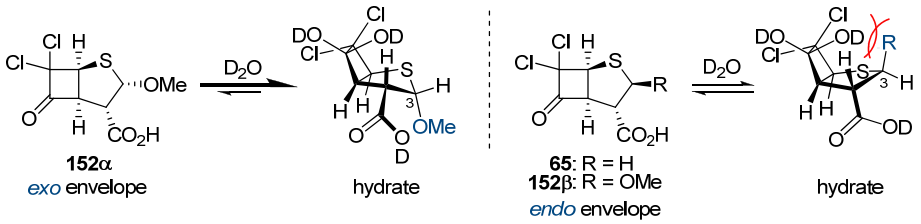
152α

152β

153: X = Cl
156: X = H

ketone inhibitor	% hydrate in D_2O[d]	class A KPC-2	class B IMP-1	class B VIM-2	class B SPM-1[e]	class C GC1	class D OXA-10	class D OXA-45[e]
65	74	76 ± 8	>1000	>1000	>500	25 ± 3	268 ± 8	194 ± 28
111	0	117 ± 13	235 ± 14	>1000		44 ± 3	1135 ± 33	689 ± 31
152α	>98	58 ± 2	122 ± 5	363 ± 9	524 ± 22	6.5 ± 1.4	156 ± 6	148 ± 21
152β	6	99 ± 5				38 ± 4	547 ± 19	392 ± 20
153	93	26 ± 2	213 ± 21	>1000		4.5 ± 0.3	370 ± 15	47 ± 6
156	<2	170 ± 2	>500			34 ± 3	>1000	

[a] IC_{50} values (μM). Inhibition was assayed by monitoring nitrocefin hydrolysis. For assay conditions see ref 640. [b] For a table of IC_{50} values corrected for the extent of hydration, see Appendix F. [c] Class A, C, and D enzymes are serine β-lactamases and class B enzymes are metallo-β-lactamases. [d] The extent of cyclobutanone hydrate formation was determined by ^{1}H NMR. Acetone-d_6 was used as a cosolvent for solubility purposes. [e] Assays for the inhibition of KPC-2, IMP-1, VIM-2, GC-1, and OXA-10 used concentrations of nitrocefin close to K_m. Concentrations of nitrocefin in SPM-1 and OXA-45 assays were >K_m.



152α
exo envelope

hydrate

65: R = H
152β: R = OMe
endo envelope

hydrate

Figure 40. Cyclobutanone hydration and steric interactions at C3.

conformation than the *endo* envelope. In addition, the 3β-methoxy derivative **152β**, which favours the *endo* envelope conformation by 5.0 kcal/mol, is a poorer inhibitor than **65**, which favours the *endo* envelope by only 1.9 kcal/mol (Table 4).

The unsaturated acid **153**, in which the carboxylate is fixed in an equatorial orientation, was found to be comparable in potency to **152α** with respect to the inhibition of GC1 and somewhat more potent than **152α** against KPC-2. On the other hand, **152α** and **65** were found to be more potent than **153** with respect to OXA-10, suggesting that the relatively subtle difference in orientation of the C4 carboxylate may be important for binding to the various serine β-lactamases.

Preliminary data indicates that cyclobutanones are also moderate inhibitors of OXA-45. While minor differences in assay conditions prevent direct comparisons with other enzymes it appears that the overall trends in inhibition for OXA-45 are similar to those of the other serine β-lactamases.

Gratifyingly, the cyclobutanones also acted as moderate inhibitors of metallo-β-lactamases. As with the serine β-lactamases, the inhibitors found to be most potent against IMP-1 were **152α** and **153**. Since the unsaturated acid **153** was more effective than its dechlorinated analogue **156** against the serine enzymes and IMP-1, it was somewhat surprising to find that the dechlorinated acid **111** was more potent than compound **65** under these assay conditions. This exception to the general trend indicates that electron-withdrawing substituents like chlorines are not necessarily beneficial for the inhibition of all β-lactamases. While the cyclobutanones were generally found to be less potent inhibitors of VIM-2 than IMP-1, the moderate inhibition exhibited by **152α** is considered encouraging. In addition, preliminary assays indicate that the α-methoxy derivative **152α** is also a moderate inhibitor of SPM-1; however, the poor supply of this enzyme in this laboratory has prevented more detailed studies thus far.

3.4 X-Ray Crystal Structure of a Cyclobutanone Bound to OXA-10 as a Serine Hemiketal

In a collaborative effort with Prof. Natalie Strynadka and Dr. Michael Gretes at the University of British Columbia, structural insight into enzyme–inhibitor interactions was pursued by solving the X-ray crystal structure of a complex of **152α** with the class D β-lactamase OXA-10 (Figure 41). The structure was obtained by soaking pre-formed crystals of OXA-10 with the inhibitor and was refined to 2.0 Å resolution.[649] Consistent with previous observations (PDB: 1K54),[293] two dimers of OXA-10 are present in the asymmetric unit and the inhibitor is bound to only two of the four monomers (Figure 41A). It is interesting to note that the use of higher concentrations of inhibitor in early crystallization trials at UBC resulted in disruption of the crystal lattice. It is possible that this is a consequence of the binding of the inhibitor to chain D and displacement the loop of chain A (which includes Lys95) from the active site of chain D. Disruption of OXA-10 crystals has been observed previously.[440] Carboxylated Lys70 (Kcx70), which has been associated with a more highly active

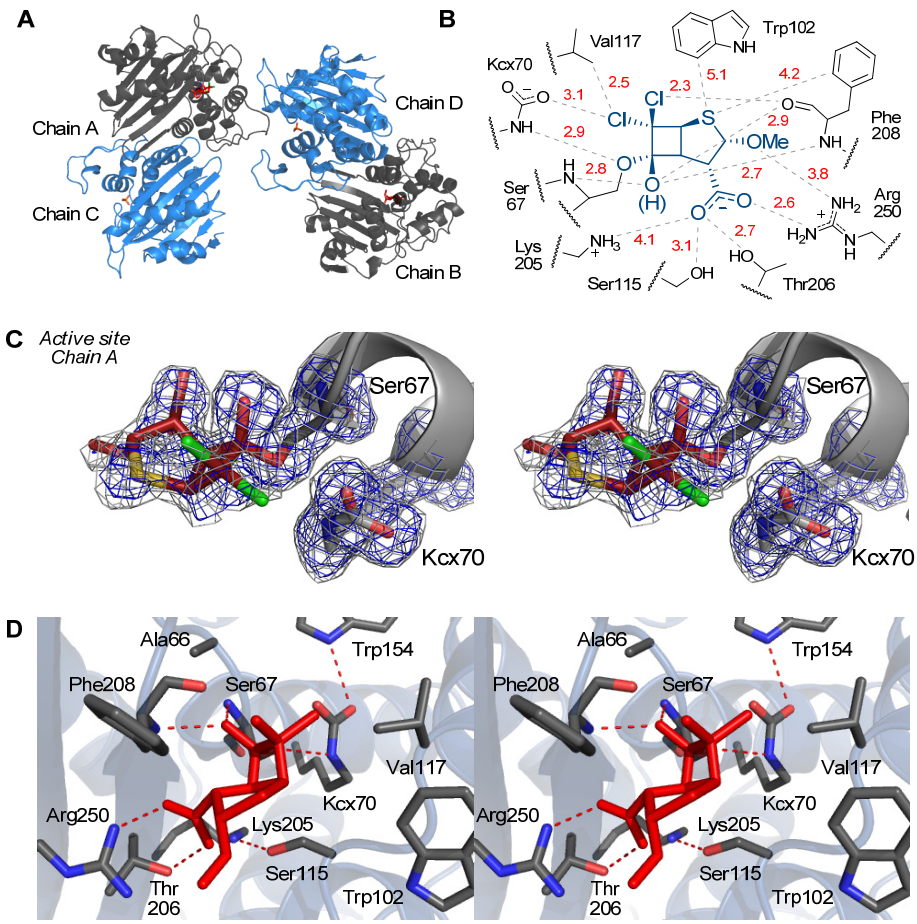


Figure 41. X-ray structure of cyclobutanone **152α** bound to OXA-10 as a serine hemiketal (PDB code: 3LCE). (A) View of the asymmetric unit cell with two OXA-10 dimers. Cyclobutanone **152α** is bound to chains A and B and a phosphate is observed in the active sites of chains C and D. (B) Interactions between OXA-10 active site residues and the bound inhibitor in chain A. (C) Stereoview of the active site of chain A with electron density. The continuous electron density from Ser67 to C6 of the inhibitor is unambiguous support for the formation of a serine-bound hemiketal. (D) Stereoview of the chain A active site from an alternate perspective.

state of OXA-10,[297] is observed in all four monomers.

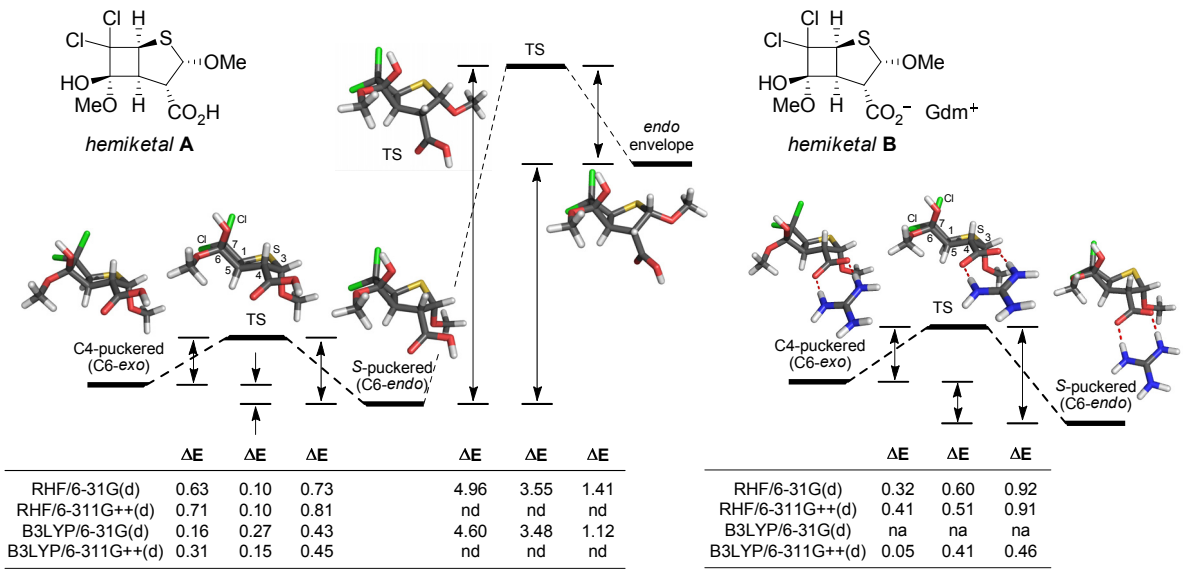
The clear continuous electron density from the active site serine through to and including the tetrahedral carbon of the hemiketal linkage (Figure 41C) provides unambiguous support for the inhibition mechanism originally envisaged for such compounds (Figure 19). Recent studies with other serine-dependent hydrolytic enzymes suggest that such a hemiketal likely exists in the alkoxide form, stabilized by the functionality that defines the oxyanion hole.[650,651,652] The structure also shows that the inhibitor adopts the *exo* envelope such that the C4 carboxylate has an equatorial orientation and the 3α-methoxy group has an axial orientation.

The electron density associated with the chlorine atoms in the structure is relatively weak, possibly indicating that X-ray radiation had caused partial dechlorination of the inhibitor. X-ray radiation has been reported to cause cleavage of disulfides, decarboxylation of aspartate and glutamate residues, and cleavage of C–Br bonds.[653] The cleavage of C–Cl bonds has also been described.[654]

Another potential contributing factor that was considered for the weak electron density surrounding the chlorines involves disorder caused by a rapid conformational change of the inhibitor. Ab initio and DFT molecular modeling studies, which were undertaken to investigate this phenomenon, indicate that model hemiketals of **152α**, acid **A** and guanidinium salt **B**, can adopt two slightly different *exo* envelope conformations in which the chlorines show the greatest differences in position (Figure 42 and Figure 43). The low energy difference (0.1–0.3 kcal/mol) between the C4-puckered and *S*-puckered conformers and the low energy barrier (0.4–0.8 kcal/mol) for interconversion support this hypothesis. The possibility of an *endo* envelope-to-*exo* envelope conformational change in the active site of OXA-10 was discounted after the structures were superimposed. In addition, the large energy difference (3.5 kcal/mol) and barrier for interconversion (>4 kcal/mol) are inconsistent with the rapid conformational change required to account for the weak electron density associated with the chlorine atoms.

Another curious aspect of the X-ray crystal structure is that close contacts are observed between the two chlorines of the inhibitor and amino acid residues of OXA-10. The contacts of 2.5 Å between the 7α-chloride and a methyl group of Val117 and 2.3 Å between the 7β-chloride and the amide oxygen of Phe208 (Figure 41B) are much closer than contacts, 3.45 Å and 3.27 Å, calculated from the sum of the atomic van der Waals radii.[625]

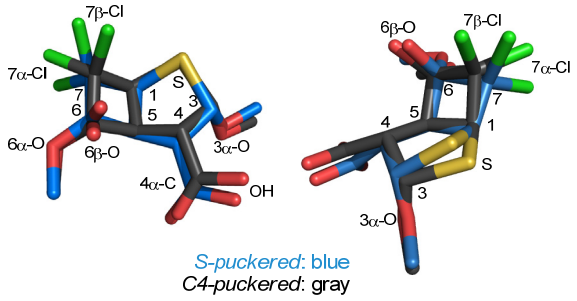
In an effort to find precedent for this sort of interaction, searches of the Cambridge structural database (CSD) and protein data bank (PDB) were conducted. In the CSD, a substructure search of the 2-chloropropane moiety returned 30 hits with Cl···O or Cl···C intermolecular contacts <3.0 Å but eight of these were disordered in some way. Of the remaining 22 structures, 18 showed Cl···O contacts[655] and five showed Cl···C contacts.[656] The structures with the shortest Cl···C contacts were CLMPMO,[657] a trichloromethyl-substituted pyrimidone, and BCARPC,[658,659] a tricyclic alkyl chloride (Figure 44). Coincidently, the structure with the shortest of the Cl···O contacts, NAFBUR, is a dichlorocyclobutanone. Each of the chlorines in this structure has a close Cl···O contact and one also



	ΔE	ΔE	ΔE	ΔE	ΔE	ΔE
RHF/6-31G(d)	0.63	0.10	0.73	4.96	3.55	1.41
RHF/6-311G++(d)	0.71	0.10	0.81	nd	nd	nd
B3LYP/6-31G(d)	0.16	0.27	0.43	4.60	3.48	1.12
B3LYP/6-311G++(d)	0.31	0.15	0.45	nd	nd	nd

	ΔE	ΔE	ΔE
RHF/6-31G(d)	0.32	0.60	0.92
RHF/6-311G++(d)	0.41	0.51	0.91
B3LYP/6-31G(d)	na	na	na
B3LYP/6-311G++(d)	0.05	0.41	0.46

Figure 42. Conformational analysis of model hemiketals **A** (left) and **B** (right). Structure optimizations and transition state calculations used *Gaussian-03*.[623] Calculated energy differences are given in kcal/mol (na = not available, nd = not determined).



Atom	Δ (Å)	Atom	Δ (Å)
C1	0.162	4α-CO_2H	0.747
S2	0.726	4α-C=O	0.960
C3	0.302	4α-C(O)OH	0.938
C4	0.401	6α-OMe	0.416
C5	0.132	3α-OCH_3	0.804
C6	0.178	6β-OH	0.752
C7	0.570	7α-Cl	**1.425**
3α-OMe	0.261	7β-Cl	**1.416**
3α-OCH_3	0.565		

Figure 43. Superimposition of two RHF-G31G(d)-optimized *exo* envelope conformations of hemiketal **A** and heavy atom displacements in the conformational change. Structure optimizations were done with *Gaussian-03*[623] and the least-squares superimposition (RMSD = 0.78 Å) done in Sybyl.[660]

shows a short Cl···C contact.

A search of the PDB for enzyme ligands that contained "chloro" (but not chlorophyll or chloride) in the ligand name returned 714 hits. Inspection of each entry with the program RCSB PDB Ligand Explorer, which is available from the protein data bank, revealed that 280 structures contained a contact between a chlorine and a carbon, oxygen, nitrogen, or sulfur of less than 3.3 Å. Of these structures, 80 contained intermolecular contacts with chlorines of less than 3.0 Å and 17 entries showed contacts shorter than 2.7 Å.[661,662,663] Aside from the structures that appeared to be disordered, Cl···O contacts as low as 2.25 Å were observed and Cl···C contacts as low as 2.40 Å were present.

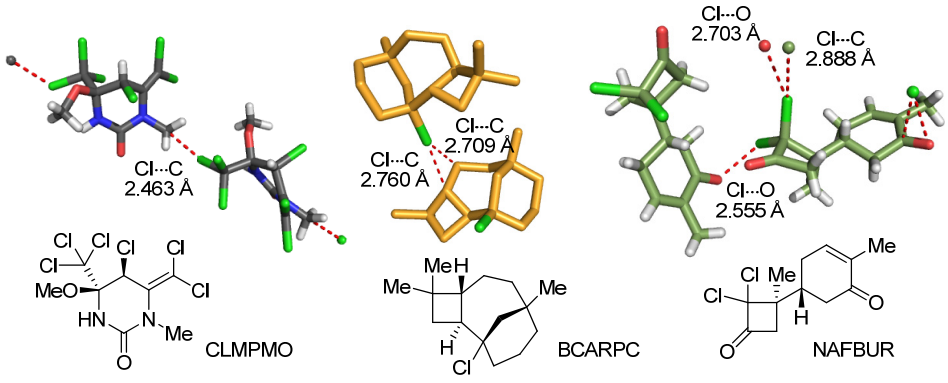


Figure 44. X-ray crystal structures of small molecules in the Cambridge structural database (CSD) that have intermolecular close contacts between chlorines and carbons or oxygens.

While most of these ligands were aryl- or heteroaryl chlorides, a dichloroacetamide (PDB: 1GRQ) and an alkyl chloride (PDB: 1XVF) were among those with the shortest contacts.

It has been noted recently that close contacts between halogens (Cl, Br, I) and oxygens are present in more than 154 PDB structures. Lu et al.[664] have studied this phenomenon computationally (ONIOM methods) in the context of halogen bonding and it is possible that halogen bonding plays a role in the present study.

3.5 Molecular Models of β-Lactamase Adducts with Cyclobutanones

Computational models of enzyme–inhibitor complexes were constructed in order to gain further insight into specific active site interactions and provide support for the inhibition observed (Figure 45). Cyclobutanone structures (obtained from optimizations in *Gaussian-03*)[623] were manually docked into X-ray crystal structures of KPC-2 (PDB: 2OV5),[643] IMP-1 (1JJE),[665] and GC1 (1RGZ)[666] using Sybyl[660] and optimized using the MMFF94 force field.

In complexes of cyclobutanones with serine β-lactamases, pre-optimized cyclobutanone hemiketals were docked such that the hemiketal moiety was positioned suitably in the oxyanion hole and the C4-carboxylate was able to interact appropriately with nearby Arg, Ser, or Thr residues. Although recent reports have concluded that tetrahedral adducts of an α-ketoheterocycle and a peptidyl trifluoromethyl ketone are not protonated in the active sites of FAAH[651] and elastase,[652] the protonation states of the cyclobutanone hemiketal adducts in β-lactamases are unknown and were

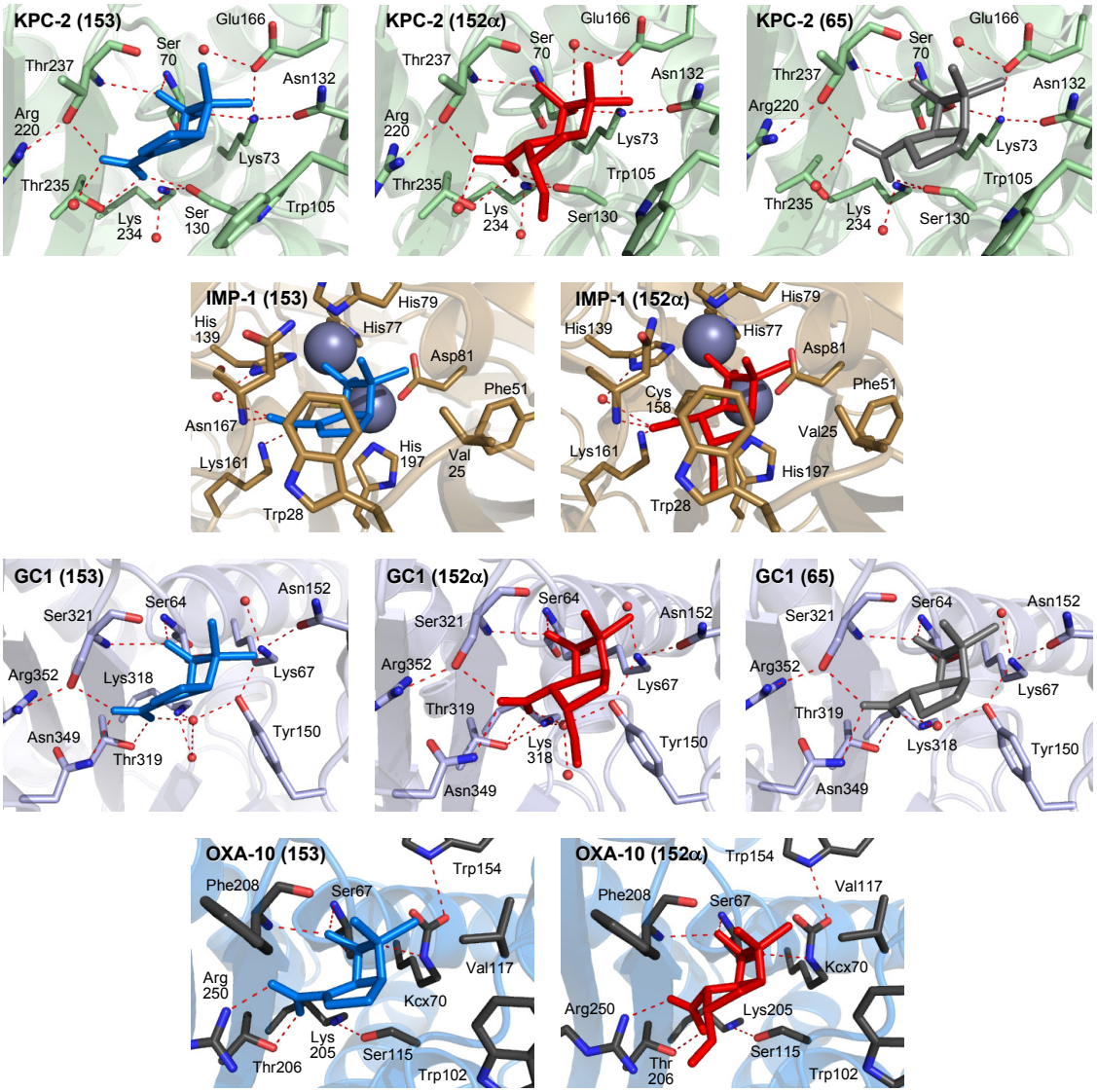


Figure 45. Structure-optimized models of cyclobutanones **153** (blue), **152α** (red), and **65** (grey) bound as tetrahedral adducts to the class A, B, and C β-lactamases KPC-2, IMP-1, and GC1. With the class D β-lactamase OXA-10, the X-ray crystal structure of **152α** bound to OXA-10 is shown (PDB: 3LCE) and this structure was also used to generate a model of **153** with OXA-10.

modeled as protonated species. Though not initially considered, it was found that the protonated hemiketal could act as a donor in a hydrogen bond with the backbone amide oxygen of the non-serine residue of the oxyanion hole (Thr237 in KPC-2, Ser321 in GC1, and Phe208 in OXA-10). Interestingly, the hemiketal of cyclobutanone **65** was initially docked in KPC-2 and GC1 in its favoured *endo* envelope conformation but the structure changed through the course of the optimization to the *exo* envelope conformation (Figure 45). The β-methoxy derivative **152β** must also

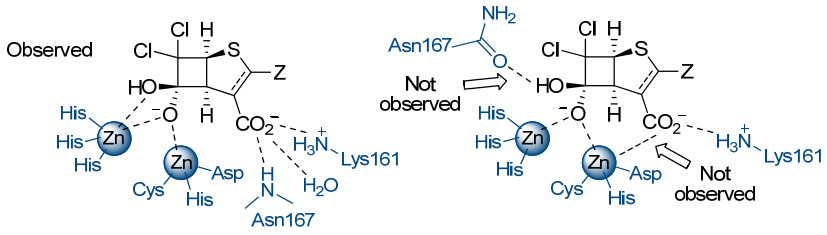


Figure 46. Interactions of cyclobutanones with IMP-1 in modeled complexes of enzyme–inhibitor adducts.

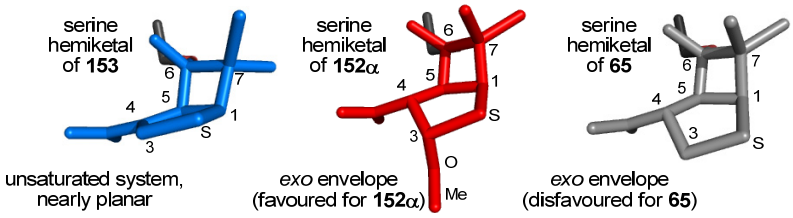


Figure 47. Conformations of enzyme-bound adducts of **153** (blue), **152α** (red), and **65** (grey) extracted from modeled complexes with GC1.

adopt the *exo* envelope conformation in order to avoid steric clashes within each active site (not shown). Changes with enzyme-bound hemiketals of **152α** and **153** were more subtle with the inhibitors adopting one of two possible conformations (twisted forms) of the four-membered ring. As shown in Figure 42, the twist of the four-membered ring in inhibitors such as **152α** causes a twist of the five-membered ring.

Cyclobutanone hydrates were initially docked in the metallo-β-lactamase IMP-1 such that the 6α-oxygen would bridge the two zinc atoms in the position normally occupied by the nucleophilic hydroxide. In accord with speculations that Asn167 plays a role similar to that of the oxyanion hole of serine β-lactamases, to polarize the β-lactam carbonyl to facilitate hydrolysis (Section 1.4.6), the cyclobutanone hydrate was initially oriented to hydrogen bond with the amide sidechain of this residue; however, such a hydrogen bond was not observed in optimized complexes (Figure 46). It has also been suggested that the C3- and C4-carboxylates of carbapenems and cephalosporins coordinate to Zn2 during catalysis. While an attractive interaction was certainly observed in optimizations of the present study, coordination led to a large amount of strain such that the outputted structures displayed highly twisted rings and unreasonably skewed conformations. Reasonable conformations of adducts of **152α** and **153**, however, were obtained when cyclobutanone hydrates were initially docked such that the C4-carboxylate could not only form a salt bridge with Lys161 but also hydrogen bond with

the backbone amide of Asn167 and, very importantly, the water molecule that also hydrogen bonds to His139.

In addition to the value that these models may have for the design of potentially more potent inhibitors, these computational studies provide support for the need for an equatorial carboxylate to interact appropriately with active-site residues and that cyclobutanones such as **65** and **152β** must adopt their normally disfavoured *exo* envelope conformation in order to bind to each serine- and metallo-β-lactamase (Figure 47).

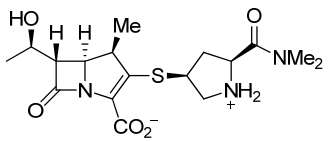
3.6 Antimicrobial Assays with Cyclobutanones

The recent acquisition of several β-lactamase-producing carbapenem-resistant bacterial strains allowed our group to test the cyclobutanones for synergy with β-lactams. These strains were collected as clinical isolates from Ontario and Calgary hospitals and were kindly provided by Prof. D. Pillai (U. Toronto) and by Prof. J. Pitout (U. Calgary), respectively. Microbiological assays were conducted in this laboratory by Valerie Goodfellow according to published procedures.[667]

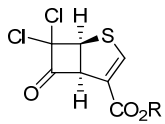
With the modest (micromolar) potency of the cyclobutanones against metallo-β-lactamases in biochemical assays, it was not surprising that cyclobutanones **65**, **111**, **152α**, and **153** were unable to enhance the potency of meropenem by greater than a factor of 2 (one 2-fold dilution) against VIM-2- and IMP-7-producing strains of *P. aeruginosa* at concentrations of up to 400 μM. It was surprising, however, to discover that cyclobutanone **153** improved the MIC of meropenem by a factor of 4 against MBL-producing strains of *Chryseobacterium meningosepticum* and *Stenotrophomonas maltophilia* at concentrations of 200 μM and 100 μM, respectively (Table 7). The MBLs expressed in these strains have not yet been identified but are thought to be BlaB[668] and L1,[368] respectively. The synergy observed with meropenem suggests that cyclobutanone **153** might have higher affinity for these enzymes than other MBLs and this encouraging possibility may be evaluated in future work.

Although the carboxylic acid form of the inhibitors is required for favourable binding to β-lactamases, Cocuzza and Boswell reported in the mid-1980s that benzhydryl ester derivatives of cyclobutanones showed synergy with penicillin G against β-lactamase-producing *S. aureus*.[532,533] Such benzhydryl esters presumably exhibited improved cell wall penetration and were converted to

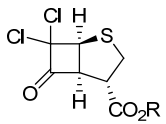
Table 7. Antimicrobial Activity of Meropenem (MIC) in the Presence of Cyclobutanones Against MBL-Producing Carbapenem-Resistant Clinical Isolates.[a,b]



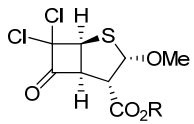
meropenem



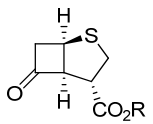
153: R = H
118: R = Et



65: R = H
149: R = $CHPh_2$



152α: R = H
151α: R = $CHPh_2$



111: R = H

ID	clinical isolate	enzyme	**153** (μg/mL)					**65** (μg/mL)				**152α** (μg/mL)				**111** (μg/mL)			
			0	25	100	200	400	0	100	200	400	0	100	200	400	0	100	200	400
24	*P. aeruginosa* C10	VIM-2	16			16	8	16		16	16	16	16	16	16	16	16		
25	*P. aeruginosa* C7	IMP-7	64			64	64	64		64	64	256	128	128	128	128	128		
26	*P. aeruginosa* IS 5563	MBL	64	64	32	32		32		64									
32	*C. meningosepticum* IS 5824	MBL [c]	64	64	32	16		32		16									
34	*S. maltophilia* IS 6081	MBL [d]	64	32	16	16		32		64									

ID	clinical isolate	enzyme	**118** (μg/mL)					**149** (μg/mL)				**151α** (μg/mL)			
			0	25	100	200	400	0	100	200	400	0	100	200	400
24	*P.aeruginosa* C10	VIM-2	16		16	16	16	16	16	16	16	16	16	16	16
25	*P.aeruginosa* C7	IMP-7	256		128	128	128	256	256	128	256 [e]	256	256	256	256

[a] The author gratefully acknowledges Ms. Valerie J. Goodfellow for the data contained in this Table. [b] Underlined MIC values indicate enhancements in the potency of meropenem relative to control values. [c] The MBL of *Chryseobacterium meningosepticum* IS 5824 is likely BlaB. [d] The MBL of *Stenotrophomonas maltophilia* IS 6081 is likely L1. [e] Precipitation was observed.

the carboxylic acid form by esterases in vivo. In order to evaluate the possibility that esters of the present study might exhibit similar behaviour, cyclobutanones **118**, **149**, and **151α** were also tested for synergy with meropenem and preliminary experiments indicate that ethyl ester **118** and benzhydryl ester **149** improved the potency of meropenem 2-fold against a strain of *P. aeruginosa*.

3.7 Summary and Outlook

Cyclobutanone analogues of penams and penems have been prepared and evaluated as inhibitors of class A, B, C, and D β-lactamases. The cyclobutanone derivatives found to be most potent as inhibitors are those that are more extensively hydrated in aqueous solution. Inhibitors which favour conformations in which the C4 carboxylate is equatorial were found to be more potent than those in which the carboxylate is axial, and molecular modeling studies with enzyme–inhibitor complexes indicate that an equatorial carboxylate is required for binding to β-lactamases. An X-ray crystal structure of the class D β-lactamase OXA-10 complexed with a cyclobutanone confirms that a serine hemiketal is formed in the active site and that the inhibitor adopts the *exo* envelope. The unsaturated penem analogue **153** was also found to enhance the potency of meropenem against MBL-producing carbapenem-resistant strains of bacteria isolated from Canadian hospitals.

β-Lactamase inhibition has been studied for several decades and, despite much research effort, the goal of a universal inhibitor that is effective against all classes of β-lactamase has remained elusive. The biochemical data described above demonstrate that cyclobutanones can indeed act as inhibitors of serine- and metallo-β-lactamases and these cyclobutanones represent the first type of reversible inhibitors to show moderate to low micromolar inhibition of all four classes of β-lactamase. It is also worth noting that the inhibition demonstrated by the cyclobutanones in this study represents, to the knowledge of this author, the most successful application of cyclobutanones as inhibitors of serine- or cysteine-active enzymes.[669] Although the activity of the compounds described herein is relatively modest, the breadth of activity and the synergy with meropenem in antimicrobial assays are particularly encouraging since it serves as a proof of principle for the concept that cyclobutanones might be effective as clinical inhibitors.

Chapter 4
Synthetic Studies Toward Other C3- and C7-Substituted Cyclobutanone Analogues of Penams and Penems

4.1 Rationale for Modifications at C3 and C7

The demonstration that cyclobutanones **152α** and **153** can act as inhibitors of serine- and metallo-β-lactamases and show synergy with meropenem against resistant bacteria is considered very encouraging. Improvement of the potency of the cyclobutanones requires increased affinity of such inhibitors for β-lactamase active sites and might be achieved with additional hydrogen bonding interactions and favourable non-covalent contacts. As indicated by molecular modeling studies with enzyme–inhibitor complexes and by direct comparison with β-lactam antibiotics (β-lactamase substrates), the most appropriate sites for modification are at C3 and C7.

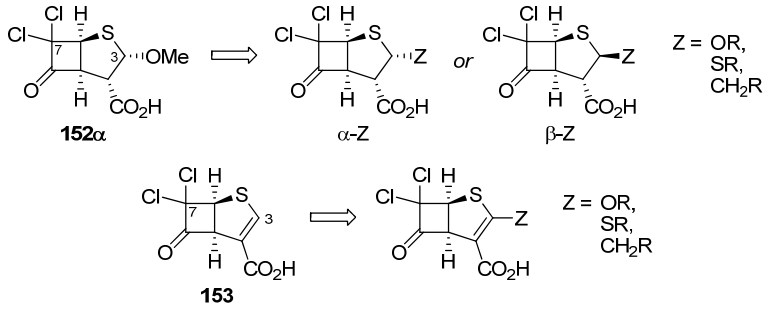
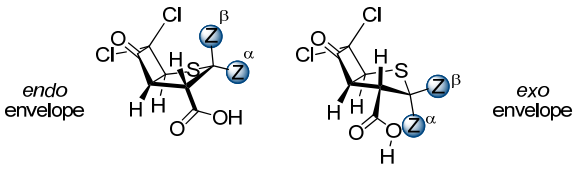


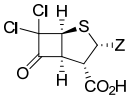
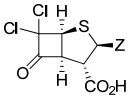
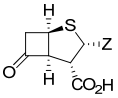
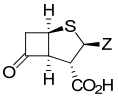
Figure 48. C3-Alkoxy, -thioalkyl, and -alkyl cyclobutanone derivatives as potentially more potent analogues of inhibitors **152α** and **153**.

In addition to 3-alkoxy cyclobutanone derivatives, 3-thioalkyl- and 3-alkyl derivatives are also considered worthy of exploration since carbapenems and mechanism-based inhibitors (e.g. tazobactam) possess thioalkyl- and alkyl sidechains at this position (Figure 48).

Ab initio molecular orbital calculations indicate that the conformational preferences of 3-thioalkyl and 3-alkyl derivatives are much weaker than those of the corresponding 3-alkoxy derivatives (Table 8). As noted in the previous chapter, the poorer inhibitory potency of the 3β-methoxy derivative **152β**, compared to its epimer **152α**, is thought to be a consequence of its large preference (5.0

Table 8. Calculated Conformational Preferences for Cyclobutanone Derivatives.[a]



Cyclobutanone		Relative Energy (kcal/mol)	
		endo envelope	*exo* envelope
	**152α**: Z = OMe	+ 3.88	0
	159α: Z = SMe	+ 0.74	0
	160α: Z = Me	0	+ 1.02
	**152β**: Z = OMe	0	+ 5.00
	159β: Z = SMe	0	+ 0.66
	160β: Z = Me	0	+ 0.12
	**161α**: Z = OMe	+ 4.12	0
	162α: Z = SMe	+ 1.42	0
	163α: Z = Me	0	+ 0.43
	**161β**: Z = OMe	0	+ 4.32
	162β: Z = SMe	0	+ 0.87
	163β: Z = Me	+ 0.11	0

[a] Structure optimizations (RHF/6-31G(d)) used *Gaussian-03*[623] and comparison of the relative energy of each conformer was done following zero-point energy corrections.

kcal/mol) for the *endo* envelope conformation since the inhibitors must bind to the β-lactamase in the *exo* envelope conformation. These computational studies therefore suggest that the weaker conformational preferences of the 3-thioalkyl and 3-alkyl derivatives might be beneficial for inhibition since the compounds could adopt the *exo* envelope conformation in the enzyme active site without a significant energetic penalty. As a result of these calculations, recent synthetic efforts have been focused more toward penam and penem analogues with thioalkyl- or alkyl sidechains at C3 than toward 3-alkoxy derivatives.

In addition to the potential alterations at C3, modifications of the cyclobutanone analogues at C7 have also been considered. Although the dichlorocyclobutanones described appear to be much better β-lactamase inhibitors than their dechlorinated counterparts, it is unlikely that a compound as reactive

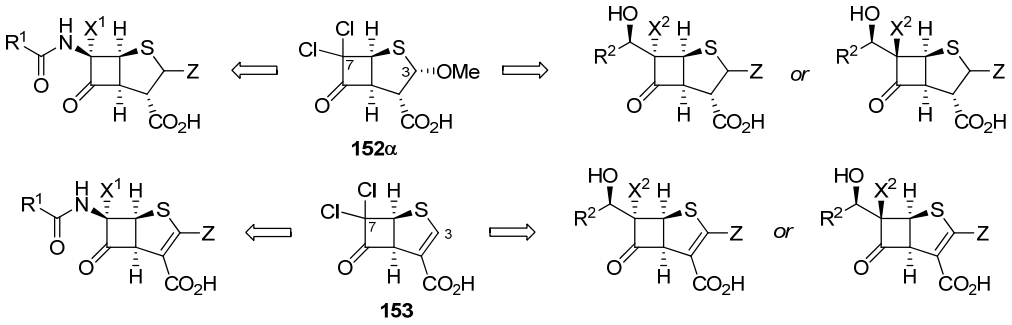


Figure 49. Penam-mimetic 7β-acylamino- and carbapenem-mimetic 7-hydroxyalkyl sidechains which may improve the inhibitory potency of cyclobutanone derivatives toward β-lactamases (X^1 = H, OMe; X^2 = H, F, Cl, OH; R^2 = H, Me; Z = OR, SR, CH_2R).

as a dichlorocyclobutanone would be appropriate for clinical evaluation. It is well known that drug candidates which rely on an electrophilic functionality may be prone to inactivation by natural electrophile scavengers such as glutathione and might exhibit toxic side effects as a result of nonspecific covalent modification of biomolecules in the host. It should also be noted, however, that these complications do not necessarily indicate that all cyclobutanones are unsuitable for clinical use since numerous ketone-containing serine protease inhibitors, which also inhibit through the formation of serine hemiketals, have been pursued as drug candidates in clinical trials.[670,671,672]

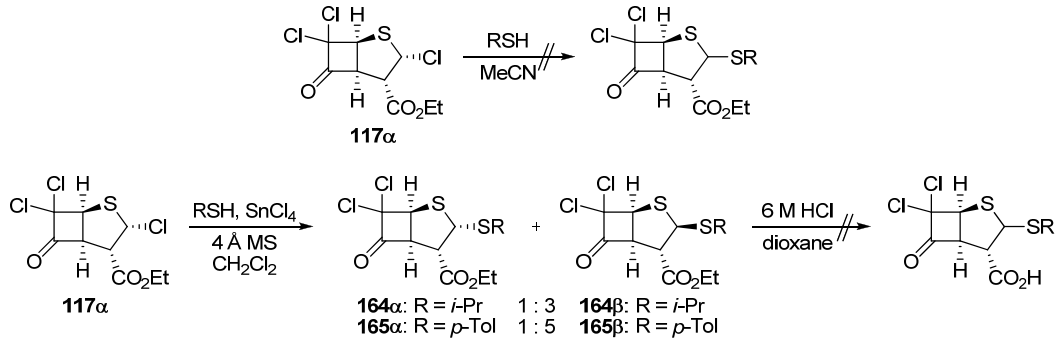
These issues indicate that cyclobutanones might be better suited for therapeutic use if the electrophilicity of the carbonyl was attenuated and if the stability of the inhibitors toward hydrolysis at neutral pH was improved. In addition, substitution of one or both of the chlorines at C7 for other functionalities could improve the affinity of the cyclobutanones for β-lactamase active sites (Figure 49). For example, the introduction of a penam-mimetic acylamino groups at C7 would provide an important hydrogen bonding interaction (Figure 36) and favourable non-covalent contacts that are important to the binding of potent (nanomolar) boronic acid-based inhibitors. Alternatively, carbapenem-mimetic hydroxymethyl or hydroxyethyl sidechains could be introduced as potential hydrogen bond donors in order to provide additional affinity for β-lactamase active sites.

The remainder of this chapter describes synthetic efforts made toward the stereoselective preparation of such C3- and C7-substituted cyclobutanone derivatives, with special focus on C3-thioalkyl and C7-hydroxymethyl derivatives.

4.2 Substitution Reactions at C3

Initial attempts to introduce sulfur-containing sidechains at C3 involved the treatment of the α-chloro derivative **117α** with thiols in MeCN. The desired substitution product was not observed, however, despite the success of similar conditions for the solvolysis of **117α** in ROH/MeCN (Figure 27). The subsequent discovery that substitutions with alcohols could also be promoted by AgOTf (Table 3) encouraged the exploration of Lewis acid-promoted substitutions with thiols and $SnCl_4$ proved to be a suitable reagent for this purpose (Scheme 31). Typically, $SnCl_4$ was added slowly to a solution of **117α** and the thiol in CH_2Cl_2 provided the 3β-SR and 3α-SR substitution products (>3:1) with very low proportions of elimination and isomerization byproducts **118** and **117β**.[673] As with the AgOTf-promoted substitutions with alcohols, the $SnCl_4$-promoted substitutions generated the inversion products in higher proportions than the retention products. ^{1}H NMR spectra of the substitution products in $CDCl_3$ indicate that the 3α-SR derivatives adopt the *exo* envelope conformation and the 3β-SR derivatives favour the *endo* envelope conformation.

Scheme 31. Substitutions at C3 with Thiols.



With the hope that the *S*,*S*-acetal moiety at C3 would show greater stability to acidic conditions than the *S*,*O*-acetal functionality of **121**–**123**, hydrolysis of the ethyl esters **164** and **165** was attempted with 6 M HCl in dioxane (Scheme 31). Unfortunately, the *S*,*S*-acetals were also found to be unstable to these conditions and it became clear that alternate protection of the C4-carboxylate would be necessary for the synthesis of the desired carboxylic acid derivatives.

Given the reactivity of the 3α-chloro derivative **117α** toward solvolysis and substitution reactions and the resemblance of the 3-position to the anomeric position of a sugar, the reactivity of **117α**

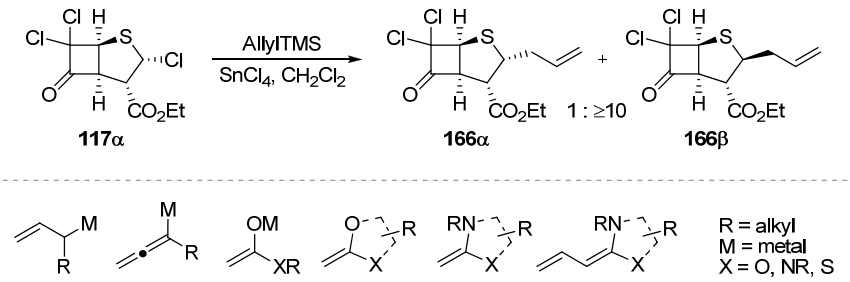
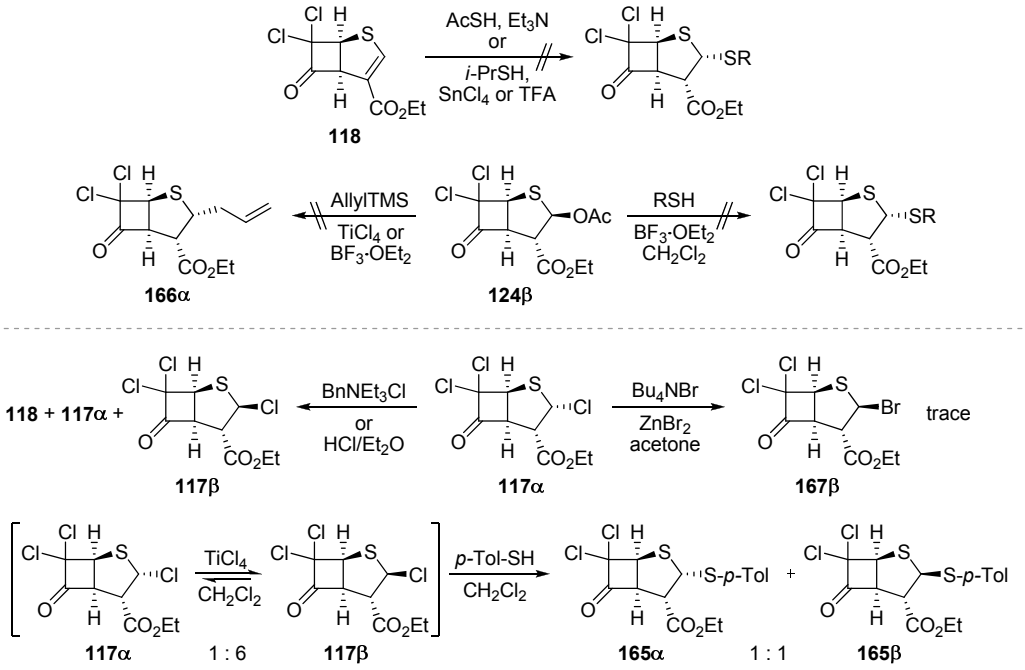


Figure 50. Allylation of **117α** with allyltrimethylsilane (*Upper panel*). Additional carbon-based nucleophiles that might be used in the synthesis of cyclobutanone inhibitors (*Lower panel*).

toward allylation was also explored (Figure 50). In the first attempt to effect this transformation, which involved the slow addition of $SnCl_4$ to a solution of **117α** and allyltrimethylsilane, the substitution products **166α** and **166β** were generated cleanly and, as with other Lewis acid-promoted substitutions, the reaction was selective toward inversion. It is possible that other latent carbon nucleophiles could be used for substitutions at C3, including allenes,[674] enolates, or imines, but such reactions have not yet been investigated.

While the $SnCl_4$-promoted substitutions provide efficient access to the 3β-thioalkyl and 3β-allyl derivatives from the α-chloro derivative **117α**, a method selective for the 3α epimers was also desired. Some of the first attempts to generate 3α-thioalkyl isomers were based on the concept that the conjugate addition of a thiol to the unsaturated ester **118** would occur preferentially from the *exo* face of the bicycle. Even weakly basic conditions, however, involving thioacetic acid and triethylamine at room temperature were found to be incompatible with **118** and decomposition of the starting material, presumably through ring-opening of the dichlorocyclobutanone ring, occurred quickly (Scheme 32). The conjugate addition of the thiol in the presence of a Lewis acid, which has some literature precedent,[675] was considered to be an attractive alternative since the dichlorocyclobutanone ring was known to be very stable to acidic conditions and because the oxidation of the thiol nucleophile would be less problematic. In order to follow the reaction conveniently by NMR, the unsaturated ester **118** was combined with *i*-PrSH and $SnCl_4$ in $CDCl_3$. Although changes in ^{13}C NMR chemical shifts had indicated that an interaction between the ethyl ester moiety and the Lewis acid was present ($\Delta\delta$ = 0.9, 0.5, 0.5 for C3, O*C*H_2, *C*O_2Et, respectively), none of the desired conjugate addition product was observed after 13 hours. The use of TFA, as a Brønsted acid substitute for the Lewis acid, was also unsuccessful and no reaction was observed.

Scheme 32. Synthetic Efforts Toward 3α-Allyl and 3α-Thioalkyl Derivatives.



While attempts to effect conjugate addition were being made, it was also considered that 3α-thioalkyl derivatives might be accessed through a Lewis acid-promoted substitution, which would be expected to favour inversion, from a 3β-substituted derivative (e.g. 3β-OAc, 3β-Cl, or 3β-Br). Perhaps the most obvious choice of 3β-X derivative would be the 3β-chloro epimer **117β**, but early attempts to prepare or isolate the 3β-chloro derivative **117β** in useful amounts were unsuccessful. While **117β** was observed as a byproduct in many different reaction conditions involving polar solvent or acidic conditions (Lewis acids, Brønsted acids, flash chromatography), efforts to prepare the β-chloride (e.g. $BnNEt_3Cl$ in MeCN; dry HCl in ether) generated mixtures of **117α**, **117β**, and the elimination byproduct **118**.

Since Lewis acid-promoted substitutions with acetoxy dihydrothiophenes are well-precedented,[618,676] the 3β-acetoxy derivative **124β** was also explored as a possible precursor to the desired 3α-thioalkyl isomers. In contrast to the high-yielding reactions reported with other acetoxy dihydrothiophenes, $BF_3{\cdot}OEt_2$ and $TiCl_4$ were not effective promoters in the present study and unreacted starting material **124β** was isolated following attempted reactions with thiols and allyltrimethylsilane.

With the apparently low reactivity of **124β**, attention had returned to the 3β-halo derivatives and

brief attempts were made to produce the β-bromo derivative **167β** from **117α**. The α-chloride **117α** was slow to react with Bu_4NBr (TBAB) in acetone-d_6 and, although the addition of $ZnBr_2$ improved the rate of conversion, the reaction remained incomplete after 21 hours at room temperature and was comprised of a mixture of compounds (**117α**:**117β**:**167β**:**118** ≈ 23:8:3:66).

While the pursuit of the 3β-bromo derivative **167β** was abandoned because of its presumed poor stability, the concept that a Lewis acid could facilitate isomerization was applied to the 3-chloro derivatives. Thus, **117α** was exposed to $TiCl_4$ in $CDCl_3$ and ^1H NMR spectra revealed that the isomerization was relatively rapid since the **117α**/**117β** ratio had reached 15:85 within 4 minutes. The ratio was unchanged after 2 hours and the fact that this result was reproduced in a second experiment in CH_2Cl_2 suggests that the two isomers had reached equilibrium under these conditions. As an important benefit, the elimination product **118** was generated only when the mixture was subjected to an aqueous workup and this observation encouraged use of the equilibrium mixture directly in substitution reactions. In such an attempt, **117α** was pretreated with $TiCl_4$ for 30 minutes before the addition of *p*-Tol-SH and the substitution products **165α** and **165β** were isolated as a mixture (1:1) with no indication that elimination had occurred (Scheme 32). While the stereoselectivity of this method is clearly lower than that desired for the synthesis of the 3α-SR derivatives, it does provide higher proportions of the 3α-SR isomers than the $SnCl_4$-promoted substitution method described above and can be considered a complementary approach.

With methods available for substitutions at C3 with thiols, efforts were focused toward the application of these reactions in the synthesis of potential inhibitors. Although 2-propyl mercaptan and 4-methylbenzenethiol were considered useful for the development of the reactions described above, the isopropyl- and *p*-toluyl substituents are thought to be poor choices for sidechains of broad-spectrum inhibitors. Instead, amine-containing acyclic and heterocyclic substituents (Figure 51) are considered more likely to provide favourable interactions within the active sites of a large number of β-lactamases. Since molecular modeling studies have revealed that this region of the active site is highly variable among class A, B, C, and D β-lactamases (and also within the same class), the design of such sidechains was based on similarity to carbapenem sidechains and the concept that hydrogen bond donors and acceptors can exhibit versatility in interactions with amino acid residues.

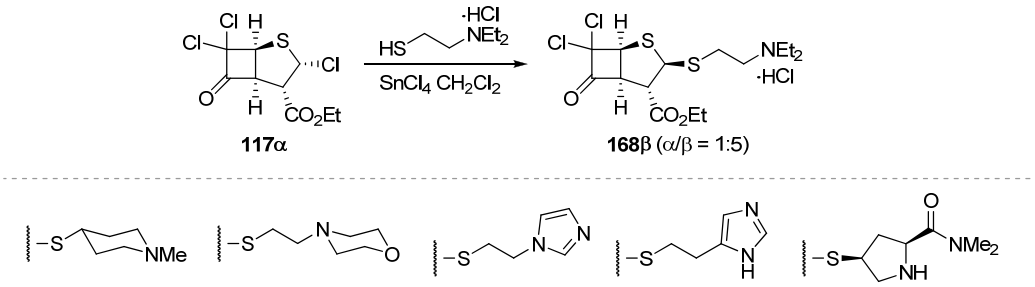
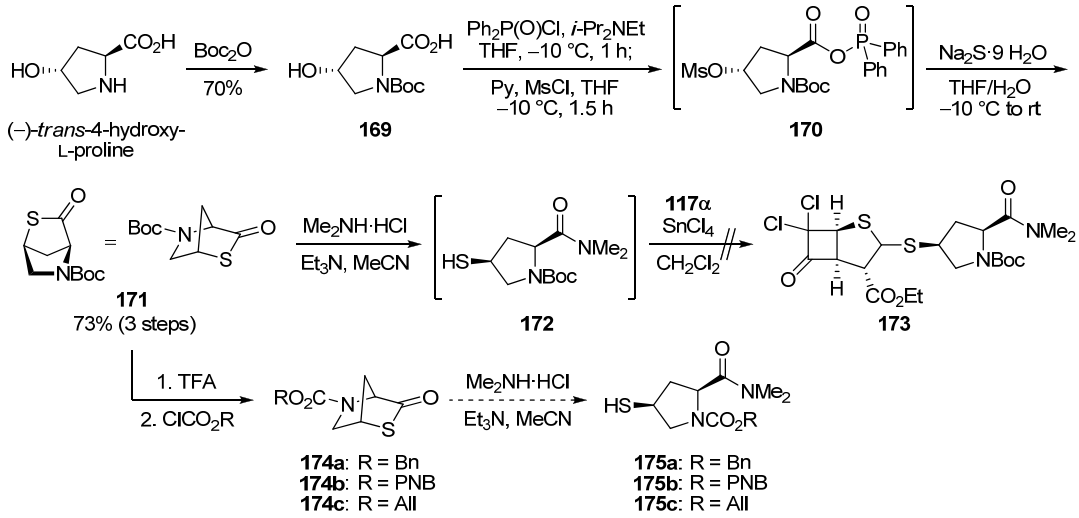


Figure 51. Installation of an imipenem-mimetic sidechain at C3 (*Upper panel*). Additional amine-containing and carbapenem-mimetic sidechains that may provide favourable hydrogen bonding interactions and non-covalent contacts in the active sites of β-lactamases (*Lower panel*).

Scheme 33. Preparation of the Mercaptoproline Sidechain of Meropenem.

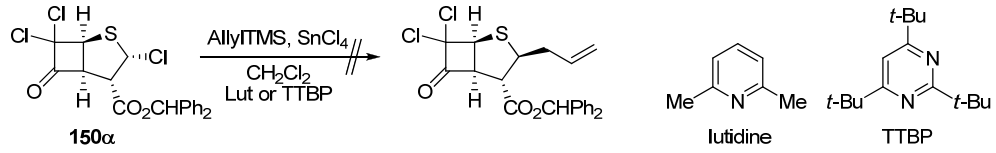


One concern that had arisen from the design of the sidechains was whether the Lewis acid-promoted substitution chemistry would be compatible with amine-containing nucleophiles. However, the reaction of **117α** with 2-diethylaminoethanethiol, as its commercially available hydrochloride salt, proceeded smoothly to produce the substitution products (**168α**/**168β** ≈ 1:5) with only small amounts of **118** (Figure 51). The success of this reaction encouraged the preparation of more complex thiols for reactions with cyclobutanones and the substituent considered most interesting was the proline-based sidechain of meropenem.

The synthesis of the meropenem sidechain in this laboratory followed the concise route published by Merck researchers in 1996 (Scheme 33).[677] Following the reaction of the amino acid (–)-*trans*-4-hydroxy-L-proline with Boc_2O under typical conditions,[678] the Boc-protected hydroxy acid **169** was

treated with diisopropylethylamine (Hünig's base) and diphenylphosphinic chloride (1 equivalent) at –10 °C and then with pyridine and methanesulfonyl chloride (1 equivalent) to generate **170** in situ. Sodium sulfide nonahydrate was allowed to react with **170** overnight to afford the [2.2.1] bicycle **171** in 73% yield over 3 steps. As noted by Brands et al.,[677] a variety of conditions can be used for the ring-opening of the cyclic thioester and $Me_2NH{\cdot}HCl$ was used with Et_3N in MeCN in this laboratory rather than a solution of Me_2NH in H_2O/THF. Since the resulting thiol **172** is known to be sensitive to oxidation, the crude product was used directly in the attempted substitution reaction with **117α** and $SnCl_4$. The desired substitution products **173α** and **173β** were not observed, however, as the *tert*-butyl carbamate was found to be unstable to the reaction conditions. While the instability of the Boc group was not completely unexpected, it indicates that a different protecting group is required. It is thought that benzyl, *p*-nitrobenzyl, or allyl carbamates (as in **174** and **175**), which have much greater stability to Lewis acids such as $SnCl_4$ and $TiCl_4$ and could be removed selectively without the use of strong base or strong acid, could be used as alternatives.

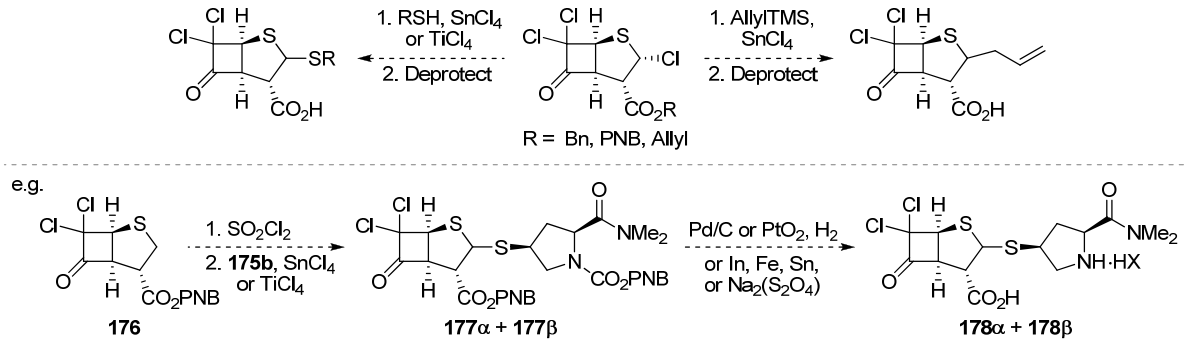
Scheme 34. Attempted Allylation of **150α** in the Presence of Weak Bases.



As noted above, the *S*,*S*-acetal moiety was found to be unstable in 6 M HCl in attempts to hydrolyze the ethyl ester functionality (Scheme 31). While the use of benzhydryl esters proved to be successful for the synthesis of the 3-alkoxy derivatives (Chapter 3), such protection would not be appropriate for the synthesis of 3-thioalkyl and 3-alkyl derivatives since benzhydryl esters are known to have similar susceptibilities to Lewis acids and Brønsted acids as *tert*-butyl esters (Scheme 34).[679] An attempted $SnCl_4$-promoted allylation of **150α**, in the presence of sterically hindered weak bases (to neutralize the HCl generated in the reaction), was unsuccessful and confirmed the suspicion that alternate protection of the C4-carboxylate is required for Lewis acid-promoted substitutions at C3.

As a result of these investigations, it is thought that the C4-carboxylate of the cyclobutanone should be protected as a benzyl-, *p*-nitrobenzyl-, or allyl ester since such derivatives would exhibit sufficient stability to Lewis acids and low concentrations of HCl and deprotection could be achieved through a

Scheme 35. Revised Protecting Group Strategy for the Synthesis of 3-Thioalkyl and 3-Alkyl Cyclobutanone Derivatives.



variety of methods that do not involve alkaline conditions or strong acid-catalyzed hydrolysis (Scheme 35).[679] The PNB ester seems particularly attractive for future work since it has been used extensively as a protecting group for β-lactam antibiotics, including sulfur-containing penams, penems, carbapenems, and oxapenems.

4.3 C3-Substituted Cyclobutanone Analogues of Penems

Although the discussion presented thus far has focused on the development of substitution reactions toward the synthesis of 3-thioalkyl and 3-thioaryl penam analogues, the synthesis of 3-thioalkyl analogues of penems and carbapenems has also been an important goal throughout this work. It is hoped that the synthetic scheme devised for the penam analogues might exhibit some versatility such that penem analogues could be generated by oxidation of the saturated precursors (Figure 52).

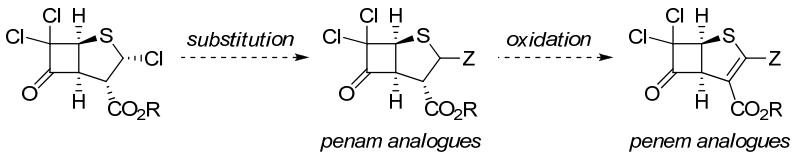
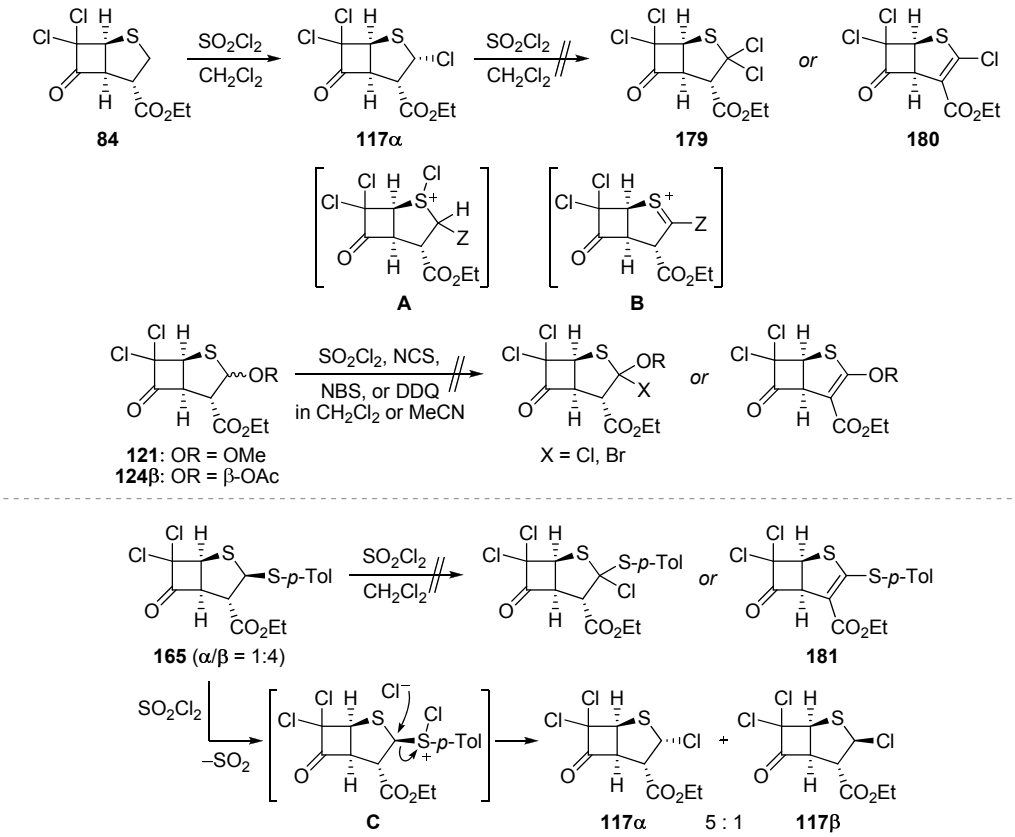


Figure 52. General synthetic strategy for the synthesis of cyclobutanone analogues of penems and carbapenems from penam analogues.

Some of the first attempts to produce unsaturated analogues involved the treatment of **117α** and **124β** with SO_2Cl_2 (Scheme 36). Interestingly, the parent cyclobutanone **84** was found to react fairly rapidly with 1 equivalent of sulfuryl chloride to generate **117α** in quantitative yield. Upon exposure

Scheme 36. Synthetic Studies Toward 3-Substituted Penem Analogues: Attempts to Oxidize 3-Alkoxy and 3-Thioalkyl Derivatives.



of **117α** to excess SO_2Cl_2 (≥5 equivalents), in experiments aimed at generating the 3,3-dichloro species **179** or the unsaturated ester **180**, **117α** reacted slowly to produce a mixture of unidentified products with spectral characteristics inconsistent with those expected for **179** and **180**. In related efforts, the β-acetoxy derivative **124β** and the 3-methoxy derivatives **121** (as mixtures of **121α** and **121β**) were found to be unreactive toward SO_2Cl_2, NCS, NBS, and DDQ in CH_2Cl_2 or MeCN. These results were somewhat unexpected since it was thought that the 3-alkoxy functionality could serve to stabilize the intermediate cation **B**; however, it seems that the electron-withdrawing properties of the C3 substituents of **117α**, **121**, and **124β** disfavour the formation of chlorosulfonium ion **A**.

An attempt to oxidize the 3-thioaryl derivative **165β** (4:1 *dr*) with SO_2Cl_2 failed to produce the desired unsaturated ester **181** and, unexpectedly, generated the 3-chloro derivatives **117α** and **117β**. Although unintended, the substitution reaction indicates that oxidations may be possible with NCS, NBS, DDQ, or other reagents and more polar solvents and higher temperatures could be explored.

4.4 7-Monochloro- and 7-Hydroxymethyl Cyclobutanone Derivatives

Although the dichlorocyclobutanones are generally much better inhibitors than their dechlorinated counterparts, dichlorocyclobutanones are susceptible to ring-opening by nucleophiles and have poor stability in aqueous solution at neutral pH. These undesirable properties indicated that modifications at C7 should be explored.

With the high electrophilicity and reactivity of the dichlorocyclobutanones and the poorer inhibition of the dechlorinated cyclobutanones, the first C7-modified derivatives to be considered were monochlorocyclobutanones. In addition to the greater stability at neutral pH expected from monochlorocyclobutanones, β-lactamase inhibition by a monochloro derivative was reported by Lowe and Swain. The 7β-chloro-2-oxacyclobutanone **61** showed time-dependent inhibition of the class A enzymes R-TEM-2 and BcI.[530,531]

Other modifications that have been considered for potential β-lactamase inhibitors are the 7β-acylamino functionality of penams and cephems and the hydroxyalkyl group of carbapenems (Figure 53). While both of these functional groups have the potential to improve inhibition through favourable hydrogen bonding interactions in β-lactamase active sites, the carbapenem-mimetic

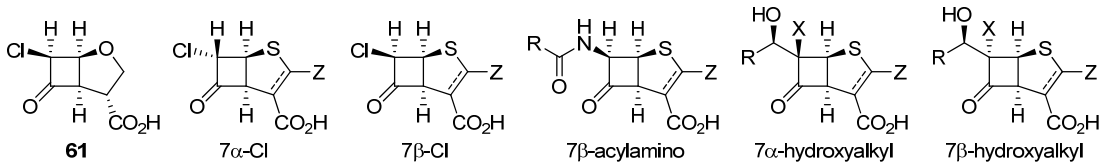


Figure 53. Modifications at C7 that could potentially improve cyclobutanone stability and affinity for β-lactamase active sites.

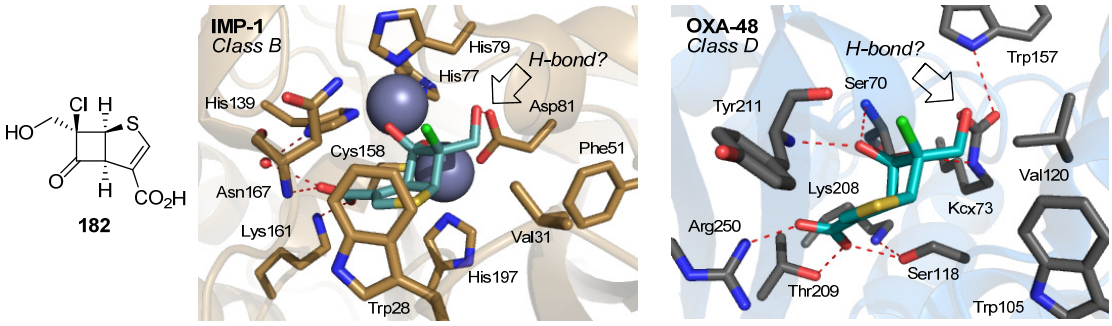
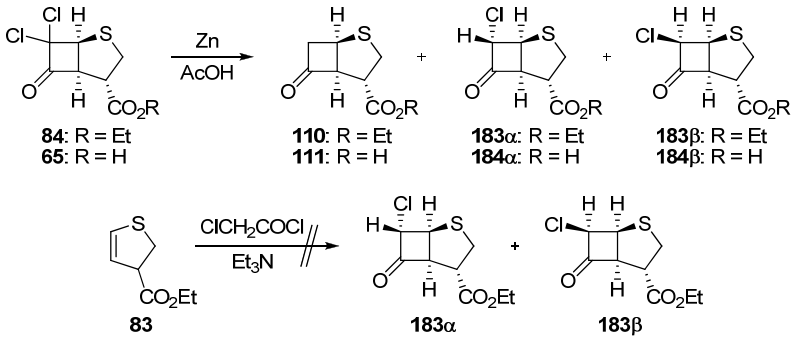


Figure 54. Structure-optimized models of a 7α-hydroxymethyl-7β-chlorocyclobutanone derivative bound to the metallo-β-lactamase IMP-1 and the class D carbapenemase OXA-48.

Scheme 37. Unsuccessful Mono-Dechlorination Attempts.

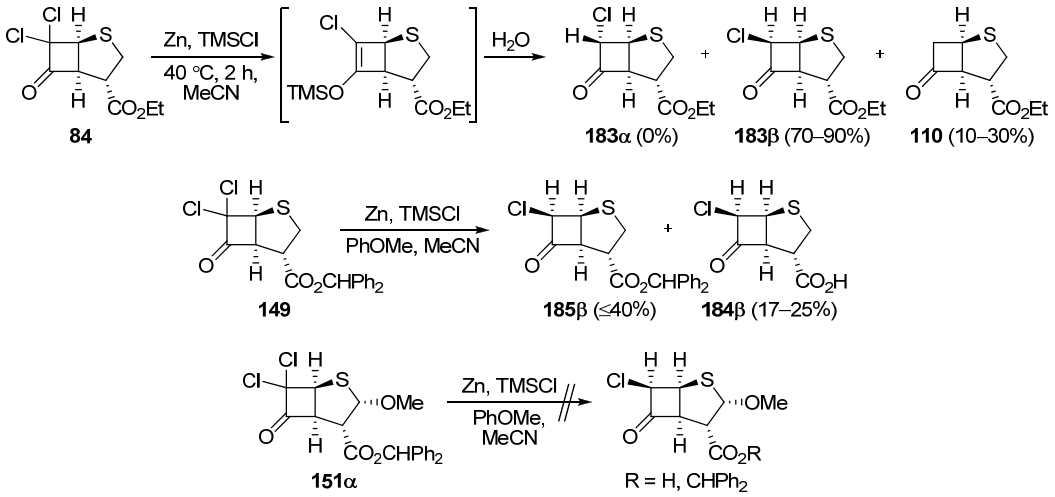


sidechain was considered especially interesting because of its potential to interact favourably with carbapenemases and its synthetic accessibility. In addition, molecular modeling studies with the 7α-hydroxymethyl derivative **182** indicate that the hydroxymethyl group could be used to interact specifically with the conserved zinc-coordinating aspartate residues in MBLs and conserved hydrolytic machinery in serine β-lactamases (Figure 54). It is thought that the 7β-chloride could be retained to improve the stability of the enzyme-bound hemiketal or hydrate and a hydroxymethyl group may be a better choice for the inhibition of β-lactamases than a hydroxyethyl substituent because it may provide improved binding to a wider range of enzymes as a result of its flexibility.

Efforts to prepare monocyclobutanones for inhibition studies were initiated in the early stages of the project but progress to this end has been made only recently. Initial efforts to effect the monodechlorination of cyclobutanone **84** with 1 equivalent of zinc dust in AcOH, at 70 °C or at room temperature, resulted in mixtures of the starting material **84** with the di-dechlorinated product **110** and each of the monochloro derivatives **183α** and **183β** and chromatographic separation of each constituent was not achieved. Since hydrolysis of the ethyl ester in alkaline conditions would likely result in Favorskii ring-contraction[588] and acid-catalyzed hydrolysis would cause epimerization, attempts were not made to convert the esters **183** into the acids **184**. Instead, efforts were made to generate the acids **184α** and/or **184β** directly through the monodechlorination of acid **65**. Not unexpectedly, a similar mixture of products **111**, **184α**, and **184β** was obtained and purification of the monochloro isomers was not possible by flash chromatography or through selective crystallizaton.

It should be noted in passing that the fairly obvious approach to monochlorocyclobutanones involving the [2 + 2] cycloaddition of **83** with chloroketene was also unsuccessul despite considerable

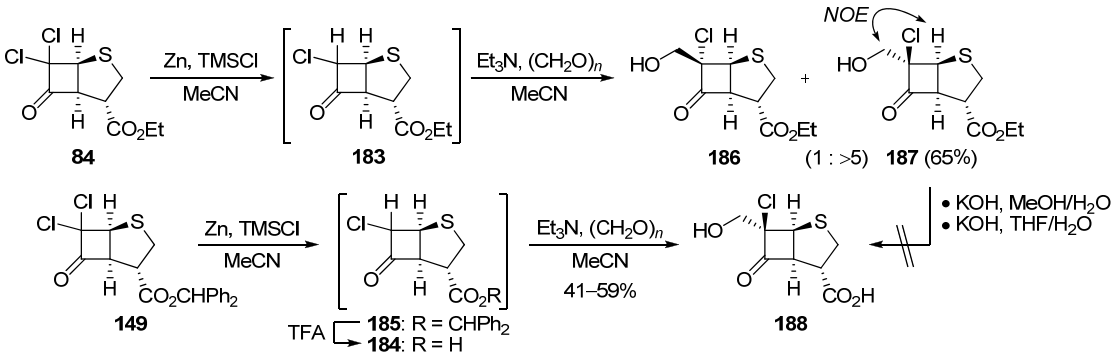
Scheme 38. Mono-Dechlorinations Using Zinc with TMSCl.



literature precedent for successful [2 + 2] cycloadditions with chloroketene. It is thought that the enolization of the cyclobutanone adduct is problematic since Lowe and Swain observed similar issues with phthalimidoketene.[530,531]

After careful consideration, it was thought that monodechlorination could be achieved efficiently if bis-dechlorination were to be prevented by the formation of a silyl enol ether intermediate. Thus, the dichlorocyclobutanone **84** was combined with zinc dust and TMSCl in MeCN and heated to 40 °C for 4 hours before the reaction mixture was concentrated and subjected to an aqueous workup. It was satisfying to discover that the monochlorocyclobutanone **183β** was produced fairly cleanly under these conditions (Scheme 38) and, while isomerization occurred upon exposure to aqueous HCl and during chromatography, the α-chloro isomer **183α** was not detected by ^{1}H NMR of the crude reaction mixture. The major byproduct of the reaction was the bis-dechlorinated cyclobutanone **110** (ca. 30%) but additional experimentation revealed that the formation of **110** could be limited with the use of more strictly anhydrous conditions. The application of this method to the benzhydryl ester **149** generated the monochloro acid **184β** in approximately 25% yield (rather than the monochloro ester **185β**) and achieved the monodechlorination and deprotection in one operation. Lower yields were obtained in subsequent experiments, however, and the relative proportions of the monochloro acid **184β** and ester **185β** were also highly variable. The addition of anisole failed to improve the yield of **184β** and **185β** and a reaction involving TMS-imidazole instead of TMSCl generated a complex mixture. An attempt to apply this method to the α-methoxy derivative **151α** was also unsuccessful.

Scheme 39. Hydroxymethylation of Cyclobutanone Derivatives at C7.

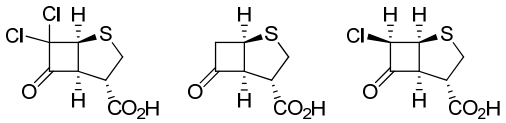


With a method for monodechlorination available, synthetic studies toward 7α-hydroxymethyl cyclobutanones were undertaken. In collaboration with Ms. Nootaree Niljianskul, an undergraduate researcher in this laboratory, the hydroxymethylation conditions first explored involved the treatment of **183** (as a mixture of **183α** and **183β**) with paraformaldehyde and Et_3N in MeCN at 70 °C (Scheme 39). This operationally simple protocol produced the hydroxymethyl diastereomers **187** and **186** in a >5:1 ratio, as indicated by ^{1}H NMR of the crude product, and flash chromatography afforded the major isomer in 65% yield. Spectral data and NOE correlations support the conclusion that the major stereoisomer was the expected 7α-hydroxymethyl derivative **187**.

Despite the realization that the hydroxymethyl derivative **186** could potentially undergo a variety of undesired base-induced reactions, including retro-aldol deformylation, Favorskii ring-contraction, and epoxidation, attempts were made to convert the ethyl ester **187** to the carboxylic acid **188** through alkaline hydrolysis. As anticipated, complex mixtures of products were observed upon exposure of **187** to KOH (1–5 equiv) in MeOH/H_2O or THF/H_2O. Efforts to hydrolyze the ethyl esters were discontinued after the pleasing discovery that the hydroxymethyl derivative **188** could be prepared directly from the monochloro acids **184**. The conditions used for the hydroxymethylation of the ethyl esters **183** were applied to a mixture of the acids **184α** and **184β** and the hydroxymethyl acid **188** was generated in modest yield. The C7-epimer of **188** was not detected by ^{1}H NMR.

Recent biochemical assays indicate that the hydroxymethyl acid **188** is a reasonable inhibitor of the recently acquired metallo-β-lactamase NDM-1 (IC_{50} = 55 μM). This result should be considered only preliminary, however, since the sample of **188** tested was of questionable purity. More detailed inhibition studies with NDM-1 and other β-lactamases are in progress.

Table 9. Inhibition of β-Lactamases by the Monochlorocyclobutanone **184β**.[a,b]



65 **111** **184β**

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
65	74	76 ± 8	>1000	>1000	25 ± 3	268 ± 8
111	0	117 ± 13	235 ± 14	>1000	44 ± 3	1135 ± 33
184β	nd [c]	>500	≈260	>500	>500	>500

[a] IC_{50} values (μM). [b] For comparisons to C3-alkoxy derivatives and penem analogues, see Table 6. [c] nd = not determined.

Preliminary biochemical assays with **184β** indicate that the monochloro derivative is a poorer β-lactamase inhibitor than the dichloro acid **65** and the dechlorinated acid **111** (Table 9). These somewhat surprising results may be an indication that the interaction of the 7β-Cl with residues in β-lactamase active sites are disfavourable and suggest that the α-chloro isomer **184α** may show greater affinity for these enzymes. Greater insight might also be gained with investigations of the tendency of **184α** and **184β** to undergo hydrate formation and the extent to which isomerization occurrs under the assay conditions.

4.5 Summary and Outlook

Ab initio molecular orbital calculations, which indicated that C3-thioalkyl and C3-alkyl cyclobutanone derivatives have relatively weak preferences for *endo* or *exo* envelope conformations, encouraged synthetic studies toward such analogues since it is possible that conformational flexibility could improve affinity for β-lactamase active sites. As an extension of the methodology developed for substitutions at C3 with alcohols, C3-thioalkyl and C3-alkyl analogues were prepared from 3α-chloro derivatives through $SnCl_4$- or $TiCl_4$-promoted substitutions with thiols or allyltrimethylsilane. Substitutions were efficient with the ethyl ester derivatives but were unsuccessful with Lewis acid-sensitive benzhydryl esters. As a result, alternate protection of the C4-carboxylate is required and further synthetic efforts toward C3-thioalkyl and C3-alkyl cyclobutanone analogues may involve the

use of benzyl-, *para*-nitrobenzyl-, or allyl esters as protective groups.

In order to explore modifications at C7 which have the potential to improve inhibition and cyclobutanone stability, a method for the monodechlorination of dichlorocyclobutanones was developed. Although the 7β-chloro cyclobutanone **184β** showed poor inhibition relative to its dichloro and dechlorinated counterparts, the monochloro derivative served as a precursor for the synthesis of a 7-hydroxymethyl cyclobutanone **188** that was designed to interact with specific residues in the active sites of both serine- and metallo-β-lactamases. Preliminary biochemical data with cyclobutanone **188** is encouraging and additional 7α-hydroxymethyl-7β-chloro analogues should be prepared and evaluated for β-lactamase inhibition and for synergy with β-lactams against resistant bacteria.

Chapter 5
Experimental Procedures

5.1 General Synthetic Experimental Procedures

Chemical shifts in ^{1}H NMR and ^{13}C NMR spectra are reported in parts per million (ppm) relative to tetramethylsilane (TMS), with calibration of the residual solvent peaks according to values reported by Gottlieb et al. (chloroform: δ_H 7.26, δ_C 77.0 (exception); acetone: δ_H 2.05, δ_C 29.84, 206.26; acetone in water: δ_H 2.22, δ_C 30.89, 215.94; acetonitrile: δ_H 1.94, δ_C 1.32, 118.26; methanol: δ_H 3.31, δ_C 49.00; DMSO: δ_H 2.50, δ_C 39.52,).[680] ^{19}F NMR chemical shifts are reported relative to $CFCl_3$ (δ 0) using a TFA external standard. ^{31}P NMR chemical shifts are reported relative to H_3PO_4 (δ 0) using an H_3PO_4 external standard. When peak multiplicities are given, the following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; sept., septet; dd, doublet of doublets; m, multiplet; br, broad; app., apparent; *gem*, geminal. ^{1}H NMR spectra were acquired at 300 MHz and 500 MHz with a digital resolution (Brüker parameter: FIDRES) of 0.245 and 0.0993 Hz/point, respectively. The coupling constants reported herein therefore have uncertainties of ±0.5 Hz and ±0.2 Hz at 300 MHz and 500 MHz, respectively. Assignments of protons and carbons relied on data from 2-dimensional NMR experiments including COSY, HMQC, and HMBC. Melting points (mp) are uncorrected. Reactions were carried out at room temperature (rt) if temperature is not specified. All reactions were done under an atmosphere of either nitrogen or argon, with the exception of selected reactions done in aqueous media. For the purification of compounds by flash chromatography, 230–400 mesh (40–63 μM) flash silica was used (Silicycle, Québec, QC). Figures of X-ray crystal structures were generated with Mercury.[681]

5.2 General Molecular Modeling Procedures

Ab initio calculations made use of GaussView[682] and *Gaussian-03*.[623] Determination of energy differences between conformations involved an optimization and frequency calculation followed by

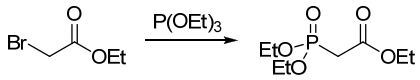
addition of the zero-point energy correction for each conformation. All optimized structures were checked for zero imaginary frequencies and all transition states for one imaginary frequency. In addition, IRC calculations were done for HF/6-31G(d)-optimized transition states. All optimizations using Gaussian were done in the gas phase.

The Sybyl 7.2 modeling package[683] was used for least-squared superimpositions of small molecules, for docking of inhibitors to enzyme active sites, and for structural optimizations of enzyme–inhibitor complexes. Structural optimizations used the MMFF94 force field and included the inhibitor and monomers (amino acid residues and waters) within a 6 Å radius of the inhibitor.

PyMOL was used to generate graphics of selected structures of small molecules and of enzyme–inhibitor complexes.[684]

5.3 Synthetic Procedures

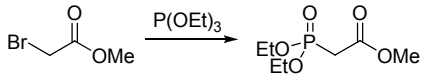
Triethyl Phosphonoacetate (103)



102 **103**

As a modification of the procedure reported by Wolinsky and Erickson,[591] ethyl bromoacetate (**102**) (35.02 g, 209.7 mmol) and triethyl phosphite (36.10 g, 217.2 mmol) were combined neat at rt. The mixture was *slowly* heated to 90 °C over 2 h[593] and stirred at this temperature for an additional 22 h. The crude product was distilled under reduced pressure to afford phosphonate **103** as a colourless oil (46.09 g, 205.6 mmol, 98%). Bp: 89–90 °C/~0.1 mmHg. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 1.35 (t, J = 7.1 Hz, 6H, $P(OCH_2CH_3)_2$), 2.97 (d, $^{2}J_{P,H}$ = 21.6 Hz, 2H, PCH_2), 4.18–4.22 (m, 6H, $P(OCH_2CH_3)_2$ and $CO_2CH_2CH_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 16.3 (d, $^{3}J_{P,C}$ = 6.2 Hz), 34.3 (d, $^{1}J_{P,C}$ = 133.4 Hz), 61.5, 62.6 (d, $^{2}J_{P,C}$ = 6.3 Hz), 165.8 (d, $^{2}J_{P,C}$ = 6.0 Hz). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 21.4. LRMS (EI) *m/z* (relative intensity): 224 (M^+, 20), 197 (100), 179 (85), 152 (50), 151 (55), 123 (65).

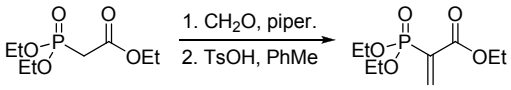
Methyl Diethylphosphonoacetate (105)



104 **105**

In a similar procedure to that described above, methyl bromoacetate (**104**) (50.04 g, 327.1 mmol) and triethyl phosphite (54.72 g, 329.3 mmol) were combined neat at rt. The mixture was *slowly* heated to 85 °C over 1.5 h[593] and stirred at this temperature for an additional 12 h. The reaction mixture was cooled to rt before volatile material was removed under reduced pressure and the crude phosphonate **105** (estimated to be ≥97% pure by ^{1}H NMR) was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, J = 7.1 Hz, 6H, $P(OCH_2CH_3)_2$), 2.95 (d, $^{2}J_{P,H}$ = 21.6 Hz, 2H, PCH_2), 3.73 (s, 3H, CO_2CH_3), 4.10–4.21 (m, 4H, $P(OCH_2CH_3)_2$).

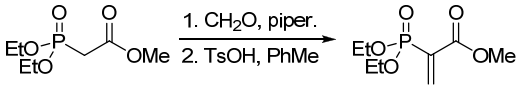
Ethyl-2-(diethoxyphosphoryl) Acrylate (93)



103 **93**

Following a published procedure,[577] phosphonate **103** (46.09 g, 205.6 mmol) was added to a solution of paraformaldehyde (12.35 g, 411.3 mmol) and piperidine (2.05 mL, 20.6 mmol) in MeOH (500 mL) over 30 min at rt using a pressure-equalized dropping funnel. The suspension was heated to reflux for 24 h and the solvent was then removed under reduced pressure to give the primary alcohol.[594] The crude oil was taken up in PhMe (500 mL), treated with $TsOH{\cdot}H_2O$ (3.918 g, 20.60 mmol), and the solution heated to reflux under a Dean–Stark trap.[595] At 16 h the solution was concentrated and distilled under reduced pressure to give the vinyl phosphonate **93** as a clear, dull yellow oil (44.00 g, 186.3 mmol, 91% over 2 steps). Bp 98–100 °C/~0.2 mmHg. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (m, 9H, $P(OCH_2CH_3)_2$ and $CO_2CH_2CH_3$), 4.08–4.25 (m, 4H, $P(OCH_2CH_3)_2$), 4.29 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 6.75 (dd, J_{gem} = 1.9 Hz, $^{3}J_{P,H\,cis}$ = 20.5 Hz, 1H, $H_{3\,cis}$), 7.00 (dd, J_{gem} = 1.9 Hz, $^{3}J_{P,H\,trans}$ = 41.9 Hz, $H_{3\,trans}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 16.3 (d, $^{3}J_{P,C}$ = 6.3 Hz), 61.5, 62.7 (d, $^{2}J_{P,C}$ = 5.9 Hz), 133.2 (d, $^{1}J_{P,C}$ = 186.2 Hz), 143.2 (d, $^{3}J_{P,C}$ = 4.8 Hz), 163.8 (d, $^{2}J_{P,C}$ = 16.2 Hz). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 13.7.

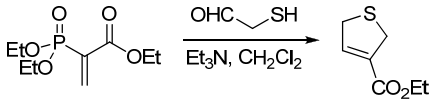
Methyl-2-(diethoxyphosphoryl) Acrylate (94)



105 94

Phosphonate **105** (25.90 g, 123.2 mmol) was added dropwise to a suspension of paraformaldehyde (7.398 g, 246.4 mmol) and piperidine (1.25 mL, 12.6 mmol) in MeOH (250 mL) at rt. The reaction mixture was slowly heated to reflux over 3 h and maintained at this temperature for an additional 12 h before the solvent was removed under reduced pressure to give the primary alcohol. The crude oil was taken up in PhMe (250 mL), treated with $TsOH \cdot H_2O$ (2.405 g, 12.61 mmol), and the solution heated to reflux under a Dean–Stark trap.[595] After 12 h at reflux the solution was concentrated and distilled under reduced pressure to give the vinyl phosphonate **94** as a clear, colourless oil (21.40 g, 96.32 mmol, 78% over 2 steps). Bp 98–100 °C/~0.2 mmHg. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (t, 6H, $P(OCH_2CH_3)_2$), 3.83 (s, 3H, CO_2CH_3), 4.13–4.21 (m, 4H, $P(OCH_2CH_3)_2$), 6.74 (dd, J_{gem} = 1.9 Hz, $^3J_{P,Hcis}$ = 20.5 Hz, 1H, H_{3cis}), 6.99 (dd, J_{gem} = 1.9 Hz, $^3J_{P,Htrans}$ = 41.9 Hz, H_{3trans}).

Ethyl 2,5-Dihydrothiophene-3-carboxylate (81)

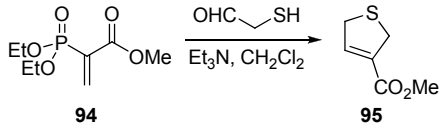


93 81

Similar to the procedures developed by McIntosh and Sieler for the synthesis of dihydrothiophenes,[576,577] a solution of the vinyl phosphonate **93** (44.00 g, 186.29 mmol) in dry CH_2Cl_2 (100 mL) was added to a suspension of *p*-dithiane-2,5-diol (14.18 g, 93.17 mmol) and Et_3N (26.4 mL, 188.64 mmol) in dry CH_2Cl_2 (200 mL) over 30 minutes using a pressure-equalized dropping funnel. The solution was stirred at reflux for 6 h before it was cooled to rt, acidified to pH 1 with 5% HCl, and extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. *Caution: the dihydrothiophene product is somewhat volatile.* Flash chromatography (20% EtOAc/hexane) gave dihydrothiophene **81** as a pale yellow oil (22.17 g, 140.12 mmol, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 3.92 (s, 4H, CH_2SCH_2), 4.22 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 6.89 (brs, 1H, H_4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.2, 37.1, 38.9, 60.8, 135.7, 140.4, 163.9. IR (film, cm^{-1}): 2982, 2908, 1715, 1645, 1334, 1263, 1211, 1079. LRMS (EI) *m/z* (relative intensity): 160 (M+2, 5), 158 (M^+, 100), 129 (50),

113 (30), 85 (80), 84 (30).

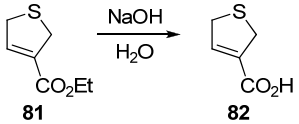
Methyl 2,5-Dihydrothiophene-3-carboxylate (95)



94 **95**

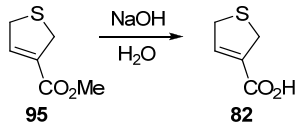
Similar to the procedures described above, a solution of vinyl phosphonate **94** (48.78 g, 208.6 mmol) in dry CH_2Cl_2 (50 mL) was added slowly to a suspension of *p*-dithiane-2,5-diol (15.89 g, 104.4 mmol) and Et_3N (32.0 mL, 229 mmol) in dry CH_2Cl_2 (200 mL) over 30 minutes using a pressure-equalized dropping funnel. The solution was stirred at reflux for 21 h before it was cooled to rt, acidified to pH 1 with 5% HCl, and extracted with CH_2Cl_2 (2 × 200 mL). The organic phase was dried over Na_2SO_4, filtered through Celite, and concentrated under reduced pressure. *Caution: the dihydrothiophene product is somewhat volatile.* Flash chromatography (10% EtOAc/hexane) gave dihydrothiophene **95** as a pale yellow oil (21.94 g, 152.2 mmol, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 3H, CO_2CH_3), 3.87–3.93 (brs, 4H, CH_2SCH_2), 6.85–6.93 (brs, 1H, H_4).

2,5-Dihydrothiophene-3-carboxylic Acid (82)



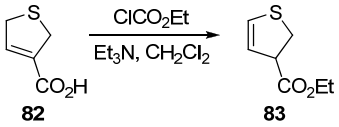
81 **82**

Ethyl ester **81** (7.699 g, 48.66 mmol) was stirred in a solution of 25% aqueous NaOH (80 mL) at 70 °C for 16 h. The reaction mixture was then cooled to rt before it was washed with CH_2Cl_2 (2 × 50 mL). The aqueous phase was acidified with concentrated HCl, extracted with CH_2Cl_2 (3 × 200 mL), dried with Na_2SO_4, and concentrated in vacuo to give the title compound **82** as a light yellow powder (5.40 g, 41.49 mmol, 85%). An analytical sample was obtained by recrystallization from EtOAc/hexane. Mp 169–170 °C (lit.[580] mp 174–176 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.94 (s, 4H, CH_2SCH_2), 7.03 (s, 1H, H_4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 36.7, 39.1, 134.9, 143.7, 169.1. IR (film, cm^{-1}): 3044, br 3200–2800, 1684, 1645, 1278. LRMS (EI) *m/z* (relative intensity): 130 (M^+, 100), 112 (10), 85 (95), 84 (40). HRMS (EI) *m/z*: 130.0089 calcd for $C_5H_6O_2S$; 130.0093 obsd.



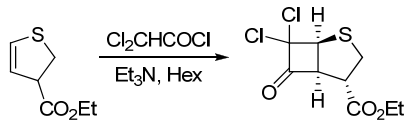
Alternatively, the acid **82** could be obtained through hydrolysis of the methyl ester **95**. The methyl ester **95** (21.94 g, 152.2 mmol) was stirred in a solution of 25% aqueous NaOH (50 mL) at 85 °C for 2.5 h. The reaction mixture was then cooled to rt before it was washed with CH_2Cl_2 (100 mL). The aqueous phase was acidified with concd HCl and extracted with CH_2Cl_2 (4 × 200 mL), dried with Na_2SO_4, and concentrated in vacuo to give the title compound **82** as an off-white powder (18.47 g, 141.8 mmol, 93%) with identical spectral characteristics as those reported above.

Ethyl 2,3-Dihydrothiophene-3-carboxylate (83)



To a stirring solution of conjugated acid **82** (992.7 mg, 7.626 mmol) and Et_3N (2.302 g, 22.75 mmol) in CH_2Cl_2 (20 mL) was added a solution of ethyl chloroformate (1.732 g, 15.96 mmol) in CH_2Cl_2 (15 mL) dropwise over 5 minutes. The solution was stirred at rt for 24 h before it was washed with 5% HCl (50 mL), sat. $NaHCO_3$ (50 mL), and brine (50 mL). The organic solvent was dried with Na_2SO_4 before it was removed under reduced pressure. *Caution: The deconjugated ester product is somewhat volatile.* The crude product was purified by flash chromatography (20% EtOAc/hexane) to give the deconjugated ester **83** (1.136 g, 7.177 mmol, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.40 (dd, J = 10.3 Hz, J_{gem} = 11.5 Hz, 1H, one of SCH_2), 3.63 (dd, J = 8.2 Hz, J_{gem} = 11.5 Hz, 1H, one of SCH_2), 3.92–3.95 (m, 1H, H_3), 4.19 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 5.63 (dd, $J_{4,3}$ = 2.8 Hz, $J_{4,5}$ = 5.9 Hz, 1H, H_4), 6.28 (dd, $J_{5,3}$ = 2.4 Hz, $J_{5,4}$ = 5.9 Hz, 1H, H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.2, 33.2, 52.9, 61.2, 120.2, 128.5, 172.0. IR (film, cm^{-1}): 3072, 2982, 1732, 1645, 1575, 1188, 1048. LRMS (EI) *m/z* (relative intensity): 160 (M+2, 2), 158 (M^+, 40), 129 (5), 111 (10), 85 (100), 84 (5). HRMS (EI) *m/z*: 158.0402 calcd for $C_7H_{10}O_2S$; 158.0401 obsd.

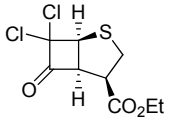
Ethyl 7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4α-carboxylate (84)



83 **84**

To a stirring solution of deconjugated ester **83** (5.986 g, 37.83 mmol) and Et_3N (13.4 mL, 95.7 mmol) in dry hexane (350 mL) was added a solution of dichloroacetyl chloride (9.10 mL, 94.6 mmol) in hexane (40 mL) over 3 h at rt using a motor-driven syringe pump. The reaction mixture was stirred for an additional 21 h before it was filtered through Celite. The filter cake was rinsed with hexane and the filtrate washed with 5% HCl (3 × 100 mL), sat. $NaHCO_3$ (3 × 100 mL), and brine (100 mL). The organic phase was dried over Na_2SO_4 before the solvent was removed in vacuo. Flash chromatography (5% Et_2O/hexane) afforded the cycloadduct **84** as a pale yellow oil which crystallized upon storage at 4 °C (6.645 g, 24.69 mmol, 65%). Mp: 32–33 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.05 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.45 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.66 (d, $J_{4,3\beta}$ = 5.8 Hz, 1H, H_4), 4.20 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.52 (d, $J_{1,5}$ = 8.3 Hz, 1H, H_1), 5.07 (d, $J_{5,1}$ = 8.3 Hz, 1H, H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.1, 35.6, 50.1, 58.9, 62.1, 67.4, 89.2, 169.8, 194.8. IR (film, cm^{-1}): 2983, 1810, 1732, 1445, 1370, 1331, 1213, 1025. LRMS (EI) *m/z* (relative intensity): 272 ([M($^{37}Cl_2$)]$^+$, 7), 270 ([M($^{37}Cl^{35}Cl$)]$^+$, 35), 268 ([M($^{35}Cl_2$)]$^+$, 50), 222 (15), 208 (45), 194 (40), 180 (20), 135 (40), 85 (100). HRMS (EI) *m/z*: 267.9728 calcd for $C_9H_{10}{}^{35}Cl_2O_3S$; 267.9726 obsd.

Ethyl 7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4β-carboxylate (109)

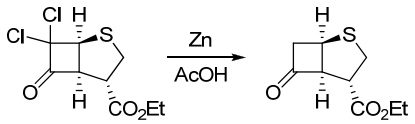


109

Small amounts of the 4β epimer were isolated by chromatography from reactions in which Et_3N was added to a solution of the dihydrothiophene with dichloroacetyl chloride. **109**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.10 (B of ABX, J_{gem} = 12.0 Hz, $J_{3\alpha,4}$ = 7.6 Hz, $H_{3\alpha}$), 3.14 (A of ABX, J_{gem} = 12.0 Hz, $J_{3\beta,4}$ = 10.5 Hz, $H_{3\beta}$), 3.28 (dt, $J_{4,3\alpha}$ = 7.6 Hz, $J_{4,3\beta}$ = 10.5 Hz, $J_{4,5}$ = 7.6 Hz, H_4), 4.23 (B of ABX_3, J_{BA} = 10.8 Hz, J_{BX} = 7.2 Hz, one of $CO_2CH_2CH_3$), 4.28 (A of ABX_3, J_{AB} = 10.8 Hz, J_{AX} = 7.2 Hz, one of $CO_2CH_2CH_3$), 4.45 (d, $J_{1,5}$ = 8.4 Hz, H_1), 4.81 (dd, $J_{5,1}$ =

8.4 Hz, $J_{5,4}$ = 7.6 Hz, H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 33.3, 51.4, 58.2, 61.8, 66.1, 89.7, 168.3, 193.3.

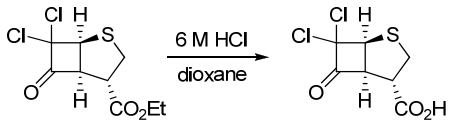
Ethyl 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (110)



84 **110**

To a stirring solution of dichlorocyclobutanone **84** (800.5 mg, 2.974 mmol) in glacial acetic acid (25 mL) was added zinc dust (972.1 mg, 14.87 mmol). The reaction mixture was stirred at 80 °C for 5 h before it was cooled, diluted with EtOAc, and filtered to remove residual zinc. The solvents were removed under reduced pressure before the crude oil taken up in EtOAc (75 mL), washed with satd Na_2CO_3 (2 × 75 mL) and brine (2 × 75 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude yellow product was purified by flash chromatography (10% EtOAc/hexane) to give the title compound **110** as a colourless oil (510.2 mg, 2.548 mmol, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.98 (dt, $J_{7\beta,5}$ = 3.3 Hz, $J_{7\beta,1}$ = 3.3 Hz, J_{gem} = 18.7 Hz, 1H, $H_{7\beta}$), 3.21 (dd, J_{gem} = 12.2 Hz, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$), 3.45 (d, J_{gem} = 12.2 Hz, 1H, $H_{3\alpha}$), 3.57 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 3.63 (ddd, J_{gem} = 18.7 Hz, $J_{7\alpha,1}$ = 8.3 Hz, $J_{7\alpha,5}$ = 3.6 Hz, 1H, $H_{7\alpha}$), 4.12–4.21 (m, 3H, H_1 and $CO_2CH_2CH_3$), 4.63 (m, 1H, H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 35.3, 36.9, 50.0, 56.5, 61.5, 71.5, 170.8, 208.4. IR (film, cm^{-1}): 2982, 1784, 1732, 1446, 1369, 1331, 1214, 1027. LRMS (EI) *m/z* (relative intensity): 200 (M^+, 12), 158 (40), 99 (10), 85 (100). HRMS (EI) *m/z*: 200.0507 calcd for $C_9H_{12}O_3S$; 200.0497 obsd.

7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic Acid (65)

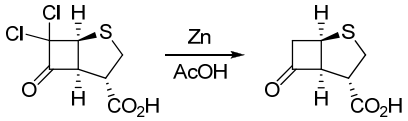


84 **65**

Ethyl ester **84** (1.323 g, 4.916 mmol) was dissolved in dioxane (10 mL) and stirred with 6 M HCl (20 mL) at 80 °C for 6 h. The reaction mixture was cooled to rt, extracted with CH_2Cl_2 (3 × 20 mL), and dried with Na_2SO_4 before the solvent was removed in vacuo. The crude beige-coloured solid was recrystallized from PhMe to give dichlorocyclobutanone **65** (936.1 mg, 3.883 mmol, 79%) as light

yellow needles. Mp 160–161 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 3.06 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$), 3.50 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.81 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 4.69 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 5.14 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5). ^{13}C NMR (75.5 MHz, acetone-d_6): δ 36.3, 50.7, 59.5, 68.6, 90.2, 171.3, 196.0. IR (film, cm^{-1}): br 3200–2600, 2951, 1810, 1700, 1453, 1417, 1262, 1190. LRMS (EI) *m/z* (relative intensity): 244 (M[(^{37}Cl$_2$)]$^+$, 5), 242 ([M(^{37}Cl^{35}Cl)]$^+$, 27), 240 ([M(^{35}Cl$_2$)]$^+$, 38), 222 (10), 194 (20), 180 (60), 141 (40), 130 (40), 85 (100). HRMS (EI) *m/z*: 239.9415 calcd for $C_7H_6{}^{35}Cl_2O_3S$; 239.9421 obsd.

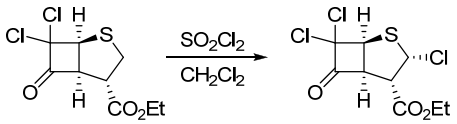
Ethyl 2-Thiabicyclo[3.2.0]heptan-6-one-4-carboxylic Acid (111)



65 **111**

Zinc dust (293.4 mg, 4.486 mmol) was added to a stirring solution of dichlorocyclobutanone **65** (212.1 mg, 0.8798 mmol) in glacial acetic acid (25 mL) at rt before it was heated to 80 °C. An additional portion of zinc dust was added after 1.5 h (293.0 mg, 4.481 mmol) and the suspension was stirred for an additional 16 h before it was cooled to rt. The solution was diluted with EtOAc (100 mL) and filtered through glass wool to remove residual solid before concentration under reduced pressure. The resulting oil was re-dissolved in EtOAc (50 mL) and washed with 10% HCl (2 × 50 ml). The organic phase was then dried over Na_2SO_4 and the solvent removed under reduced pressure. Trituration with CH_2Cl_2/hexane provided the product **111** as a white solid (134.0 mg, 0.7782 mmol, 88%). Mp 98–100 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 2.85 (ddd, J_{gem} = 18.4 Hz, $J_{7\beta,1}$ = 3.2 Hz, $J_{7\beta,5}$ = 3.2 Hz, 1H, $H_{7\beta}$), 3.22 (dd, J_{gem} = 12.1 Hz, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$), 3.44 (d, J_{gem} = 12.1 Hz, 1H, $H_{3\alpha}$), 3.56 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 3.70 (ddd, J_{gem} = 18.3 Hz, $J_{7\alpha,1}$ = 8.2 Hz, $J_{7\alpha,5}$ = 3.5 Hz, 1H, $H_{7\alpha}$) 4.17 (ddd, $J_{1,5}$ = 8 Hz, $J_{1,7\alpha}$ = 8.2 Hz, $J_{1,7\beta}$ = 3.5 Hz, 1H, H_1), 4.64 (m, 1H, H_5). ^{13}C NMR (75.5 MHz, acetone-d_6): δ 35.6, 37.5, 50.3, 56.6, 72.3, 172.4, 208.4. IR (film, cm^{-1}): br 3200–2600, 2933, 1780, 1705, 1383, 1252. LRMS (EI) *m/z* (relative intensity): 174 ([M+2]$^+$, 2.7), 173 ([M+1]$^+$, 4.4), 172 (M$^+$, 49), 130 (65), 97 (10), 85 (100). HRMS (EI) *m/z*: 172.0194 calcd for $C_7H_8O_3S$; 172.0191 obsd.

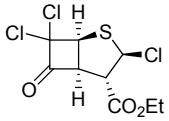
Ethyl 3α,7,7-Trichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (117α)



84 **117α**

To a stirring solution of dichlorocyclobutanone **84** (1.005 g, 3.735 mmol) in dry CH_2Cl_2 (45 mL) at 0 °C was added a solution of SO_2Cl_2 (1 M in CH_2Cl_2, 4.50 mL, 4.50 mmol). The solution was stirred at rt for 4 h before it was concentrated under reduced pressure to give **117α** as a white crystalline solid (1.132 g, 3.729 mmol, 99.8%). Mp 84–87 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.97 (dd, $J_{4,3}$ = 4.8 Hz, $J_{4,5}$ = 5.8 Hz, 1H, H_4), 4.23 (B of ABX_3, J_{AB} = 10.8 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.26 (A of ABX_3, J_{AB} = 10.8 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.94 (d, $J_{1,5}$ = 8.3 Hz, 1H, H_1), 5.20 (dd, $J_{5,1}$ = 8.3 Hz, $J_{5,4}$ = 5.8 Hz, 1H, H_5) 5.87 (d, $J_{3\beta,4}$ = 4.8 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 59.8, 60.0, 62.5, 65.5, 74.1, 85.0, 166.2, 192.0. IR (film, cm^{-1}): 2985, 1815, 1740, 1372, 1266, 1236, 1212. LRMS (EI) *m/z* (relative intensity): 306 (M[($^{37}Cl_2{}^{35}Cl$)]$^+$, 1), 304 ([M($^{37}Cl^{35}Cl_2$)]$^+$, 2), 302 ([M($^{35}Cl_3$)]$^+$, 2), 267 (10), 259 (15), 207 (100), 169 (50), 131 (55), 99 (90). HRMS (EI) *m/z*: 301.9338 calcd for $C_9H_9{}^{35}Cl_3O_4S$; 301.9336 obsd.

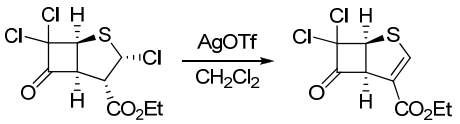
Ethyl 3β,7,7-Trichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (117β)



117β

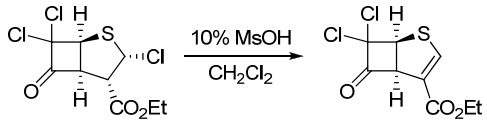
The 3β-chloro isomer **117β** was observed as a by-product in most solvolysis reactions in alcohol, substitution reactions with AgOTf, and elimination attempts involving **117α**. Although **117β** was, for the most part, unstable to chromatography, a small amount could be isolated in a nearly pure form (98%) from chromatography of the reaction mixture resulting from: **117α** in *t*-BuOH → **123α** (major) + **123β** (minor) + **117β** (minor) + **118** (minor). **117β**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 4.08 (s, 1H, H_4), 4.23 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.72 (d, $J_{1,5}$ = 8.7 Hz, 1H, H_1), 5.15 (d, $J_{5,1}$ = 8.7 Hz, 1H, H_5), 6.14 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 61.4, 62.3, 62.9, 65.9, 71.5, 89.7, 167.1, 192.7.

Ethyl 7,7-Dichloro-2-thiabicyclo[3.2.0]hept-3-ene-6-one-4-carboxylate (118)



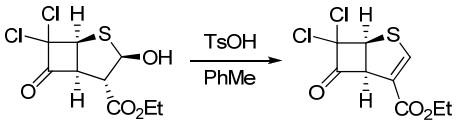
117α **118**

A solution of cyclobutanone **117α** (105.0 mg, was 0.346 mmol) in CH_2Cl_2 (2 mL) was slowly added to a stirring solution of AgOTf (104.1 mg, 0.405 mmol) in refluxing CH_2Cl_2 (20 mL) dropwise over 10 minutes. After 2 h at reflux, the solution was cooled to rt, diluted with CH_2Cl_2, filtered through Celite, and concentrated in vacuo. Purification by flash chromatography (10% EtOAc/ hexane) furnished the elimination product **118** as a colourless oil that solidified under reduced pressure (74.4 mg, 0.279 mmol, 81%). Mp 75–76 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 4.21 (B of ABX_3, J_{AB} = 10.7 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.24 (A of ABX_3, J_{AB} = 10.7 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.89 (d, $J_{1,5}$ = 10.0 Hz, 1H, H_1) 5.43 (dd, $J_{5,1}$ = 10.0 Hz, $J_{5,3}$ = 1.7 Hz, 1H, H_5), 7.43 (d, $J_{3,5}$ = 1.7 Hz, 1H, H_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.2, 59.6, 61.2, 71.3, 93.8, 122.2, 144.4, 161.1, 187.4. IR (film, cm^{-1}): 3075, 2984, 1812, 1705, 1576, 1370, 1327, 1238, 1078. LRMS (EI) *m/z* (relative intensity): 268 ([M(^{37}Cl^{35}Cl)]$^+$, 1.5), 266 ([M(^{35}Cl$_2$)]$^+$, 2.0), 238 (20), 203 (100), 175 (40). HRMS (EI) *m/z*: 265.9571 calcd for $C_9H_8{}^{35}Cl_2O_3S$; 265.9572 obsd.



117α **118**

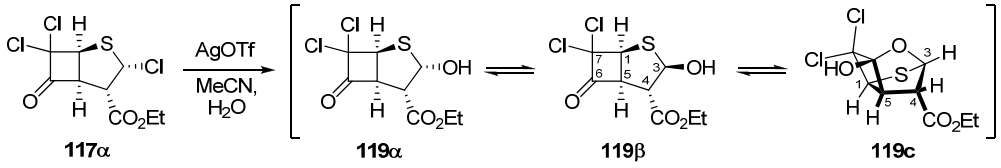
The unsaturated ester **118** could also be prepared from **117α** using MsOH. Methanesulfonic acid (2 mL) was added to a solution of the α-chloride **117α** (304.5 mg, 1.003 mmol) in CH_2Cl_2 (18 mL) and the solution was heated to reflux for 2 h. The reaction was cooled to rt, diluted with CH_2Cl_2 (70 mL), and washed with satd $NaHCO_3$ (2 × 50 mL) and brine (25 mL). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure, and filtered through silica to provide the title compound **118** as a clear light yellow oil that solidified under vacuum (194.2 mg, 0.7269 mmol, 72%).



119β **118**

Alternatively, the unsaturated ester **118** could be prepared from thiolactol **119β**. TsOH·H_2O (10.9 mg, 0.057 mmol) was stirred in PhMe (40 mL) and heated at reflux under a Dean–Stark trap[595] for 2 h. (Note: incomplete conversion was observed if the TsOH was not predried). The thiolactol **119β** (83.7 mg, 0.294 mmol) was then added as a solution in PhMe (2 mL) and stirred at reflux for an additional 18 h. The solution was concentrated under reduced pressure and purified by flash chromatography (10% EtOAc/hexane) to give a colourless oil that crystallized under vacuum (73.8 mg, 0.276 mmol, 94%).

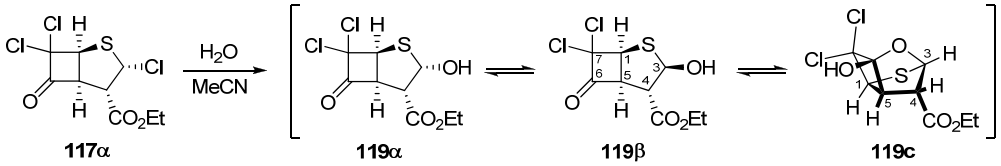
Ethyl 7,7-Dichloro-3β-hydroxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (119β)



117α **119α** **119β** **119c**

A solution of the trichlorocyclobutanone **117α** (57.5 mg, 0.189 mmol) in MeCN (2 mL) was added slowly dropwise to a stirring solution of AgOTf (60.1 mg, 0.234 mmol) in MeCN (5 mL) and H_2O (2 mL). The mixture was stirred for 4 h at rt before it was concentrated under reduced pressure, diluted with CH_2Cl_2 (25 mL), and filtered through Celite. The resulting yellow oil was subjected to flash chromatography (10% EtOAc/hexane) which provided a colourless oil (48.6 mg, 0.170 mmol, 90%) that was determined to be an 88:6:6 mixture of the thiolactol **119β**, epimer **119α**, and an oxa-thia-tricyclo-octane **119c**, respectively. **Thiolactol 119β**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.26 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.7–2.9 (brs, 1H, OH), 3.83 (s, 1H, H_4), 4.18 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.67 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1) 5.05 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5), 5.98 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 59.3, 59.8, 62.3, 65.0, 86.0, 90.4, 167.9, 193.1. IR (film, cm^{-1}): br 3600–3300, 2984, 1815, 1732, 1246, 1213, 1024. LRMS (EI) *m/z* (relative intensity): 288 (M[($^{37}Cl_2$)]$^+$, 0.4), 286 ([M(^{37}Cl^{35}Cl)]$^+$, 5), 284 ([M($^{35}Cl_2$)]$^+$, 8.0), 238 (15), 203 (50), 195 (75), 151 (85), 115 (100). HRMS (EI) *m/z*: 283.9677 calcd for $C_9H_{10}{}^{35}Cl_2O_4S$; 283.9681 obsd. **Epimer 119α**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.26 (3H, $CO_2CH_2CH_3$), 2.7–2.9 (1H, OH), 3.70 (dd, $J_{4,3\beta}$ = 4.6 Hz, $J_{4,5}$ = 4.8 Hz, 1H, H_4), 4.18 (2H, $CO_2CH_2CH_3$), 4.79 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1) 5.08 (dd, $J_{5,1}$ = 8.4 Hz, $J_{5,4}$ = 4.8 Hz, 1H, H_5),

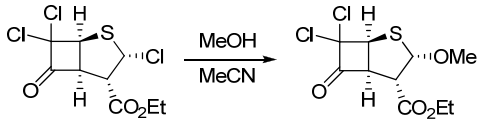
5.84 (d, $J_{3\beta,4}$ = 4.6 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 57.0, 58.7, 61.6, 65.7, 86.2, 87.0, 166.8, 192.3. **Oxa-thia-tricyclo-octane 119c**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.26 (3H, $CO_2CH_2CH_3$), 2.7–2.9 (1H, OH), 3.7 (m, 1H, H_4), 3.97 (m, 1H, H_5), 4.18 (m, 2H, $CO_2CH_2CH_3$), 4.26 (m, 1H, H_1), 5.87 (m, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 50.1, 55.2, 56.4, 62.3, 86.8, 90.6, 105.7, 168.4.



117α **119α** **119β** **119c**

Alternatively, **119β** could be obtained by hydrolysis of **117α** without AgOTf. H_2O (15 mL) was added to a solution of the 3α-Cl cyclobutanone **117α** (769.2 mg, 2.534 mmol) in MeCN (15 mL). After stirring at rt for 48 h, the solution was concentrated in vacuo to a yellow oil. Flash chromatography (10% EtOAc/hexane) afforded a colourless oil (543.2 mg, 1.905 mmol, 75%) with identical spectral properties to material prepared in ROH/MeCN: an 88:6:6 mixture of **119β**, **119α**, and **119c**.

Ethyl 7,7-Dichloro-3α-methoxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (121α)

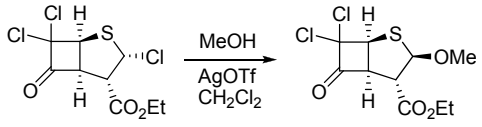


117α **121α**

The 3α-chlorocyclobutanone **117α** (102.2 mg, 0.337 mmol) was dissolved in MeCN (5 mL) and stirred with MeOH (5 mL) at rt for 48 h. The solution was concentrated under reduced pressure to give a colourless oil that partially crystallized in vacuo. ^{1}H NMR of the crude mixture showed a product distribution of 75:24:1 for **121α**:**121β**:**118**, respectively. Flash chromatography (5% EtOAc/hexane) provided **121α** (23.4 mg, 0.0782 mmol, 23%, 98% pure) and a 68:32 mixture of **121α** and **121β** (50.3 mg, 0.168 mmol, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.32 (s, 3H, OCH_3), 3.75 (app. t, $J_{4,3\beta}$ = 4.5 Hz, $J_{4,5}$ = 5.5 Hz, 1H, H_4), 4.17 (B of ABX_3, J_{AB} = 10.8 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.26 (A of ABX_3, J_{AB} = 10.8 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.66 (d, $J_{1,5}$ = 8.1 Hz, 1H, H_1), 5.17 (dd, $J_{5,4}$ = 5.5 Hz, $J_{5,1}$ = 8.1 Hz, 1H, H_5), 5.32 (d, $J_{3\beta,4}$ = 4.5 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.1, 56.7, 57.4, 58.7, 61.8,

66.4, 85.3, 97.4, 167.3, 193.4. IR (film, cm^{-1}): 2986, 2930, 2831, 1813, 1739, 1464, 1335, 1265, 1215, 1085, 1021. LRMS (EI) *m/z* (relative intensity): 302 ($M[(^{37}Cl_2)]^+$, 0.4), 300 ($[M(^{37}Cl^{35}Cl)]^+$, 1.5), 298 ($[M(^{35}Cl_2)]^+$, 2), 253 (20), 217 (10), 203 (20), 189 (20), 167 (60), 165 (100), 143 (50). HRMS (EI) *m/z*: 297.9833 calcd for $C_{10}H_{12}{}^{35}Cl_2O_4S$; 297.9827 obsd.

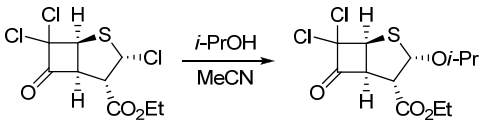
Ethyl 7,7-Dichloro-3β-methoxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (121β)



117α **121β**

The 3α-chlorocyclobutanone **117α** (104.1 mg, 0.343 mmol) in CH_2Cl_2 (1 mL) was added dropwise over 10 minutes to a suspension of AgOTf (118.3 mg, 0.460 mmol), MeOH (75 μL, 1.841 mmol), and 3 Å MS (1 g) in CH_2Cl_2 (8 mL) at 0 °C. The reaction mixture was allowed to warm to rt over 2 h and stirred for an additional 4 h at rt before dilution with CH_2Cl_2 and filtration through Celite. The filtrate was concentrated under reduced pressure and ^1H NMR of the crude mixture showed a product distribution of 22:72:2:4 for **121α**:**121β**:**117β**:**118**, respectively. Flash chromatography (5% EtOAc/hexane) provided **121β** (29.7 mg, 0.099 mmol, 29%) and a 38:62 mixture of **121α**:**121β** (32.0 mg, 0.107 mmol, 31%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.34 (s, 3H, OCH_3), 3.82 (s, 1H, H_4), 4.19 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.59 (d, $J_{1,5}$ = 8.6 Hz, 1H, H_1) 5.02 (d, $J_{5,1}$ = 8.6 Hz, 1H, H_5), 5.46 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 56.3, 57.9, 59.4, 62.2, 64.9, 90.4, 95.2, 167.8, 192.7. IR (film, cm^{-1}): 2984, 2934, 2829, 1817, 1734, 1370, 1313, 1259, 1212, 1086. LRMS (EI) *m/z* (relative intensity): 302 ($M[(^{37}Cl_2)]^+$, 1), 300 ($[M(^{37}Cl^{35}Cl)]^+$, 5), 298 ($[M(^{35}Cl_2)]^+$, 8), 263 (30), 252 (20), 217 (40), 203 (100), 189 (90), 169 (55), 165 (55). HRMS (EI) *m/z*: 297.9833 calcd for $C_{10}H_{12}{}^{35}Cl_2O_4S$; 297.9836 obsd.

Ethyl 7,7-Dichloro-3α-isopropoxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (122α)

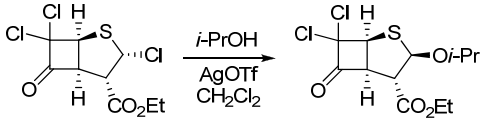


117α **122α**

The 3α-chlorocyclobutanone **117α** (197.1 mg, 0.649 mmol) was dissolved in MeCN (8 mL) and stirred with *i*-PrOH (8 mL) at rt for 40 h. The reaction mixture was concentrated in vacuo and ^1H

NMR of the crude mixture showed a product distribution of 46:20:16:18 for **122α**:**122β**:**117β**:**118**, with 85% conversion. Flash chromatography (5% EtOAc/hexane) provided **122α** (9.7 mg, 0.030 mmol, 4.6%) and a 77:23 mixture of **122α**:**122β** (121.7 mg, 0.372 mmol, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (d, J = 6.1 Hz, 3H, one of CHCH_3), 1.15 (d, J = 6.1 Hz, 3H, one of CHCH_3), 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.73 (dd, $J_{4,3\beta}$ = 4.5 Hz, $J_{4,5}$ = 5.6 Hz, 1H, H_4), 3.79 (sept., J = 6.1 Hz, 1H, CHMe$_2$), 4.18 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.69 (d, $J_{1,5}$ = 8.1 Hz, 1H, H_1), 5.20 (dd, $J_{5,4}$ = 5.6 Hz, $J_{5,1}$ = 8.1 Hz, 1H, H_5), 5.54 (d, $J_{3\beta,4}$ = 4.5 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.1, 20.3, 22.8, 57.5, 58.7, 61.7, 66.7, 70.9, 85.2, 93.3, 167.3, 193.7. IR (film, cm^{-1}): 2977, 2926, 1811, 1739, 1467, 1371, 1239. LRMS (EI) *m/z* (relative intensity): 330 (M[(^{37}Cl$_2$)]$^+$, 3), 328 ([M(^{37}Cl^{35}Cl)]$^+$, 12), 326 ([M(^{35}Cl$_2$)]$^+$, 20), 291 (12), 281 (20), 267 (30), 224 (40), 195 (90), 157 (90), 129 (100), 101 (80). HRMS (EI) *m/z*: 326.0146 calcd for $C_{12}H_{16}{}^{35}Cl_2O_4S$; 326.0152 obsd.

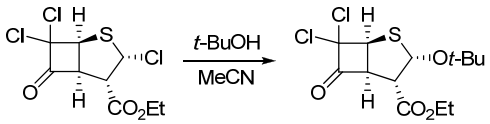
Ethyl 7,7-Dichloro-3β-isopropoxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (122β)



117α **122β**

The 3α-chlorocyclobutanone **117α** (200.4 mg, 0.660 mmol) in CH_2Cl_2 (2 mL) was added dropwise over 10 minutes to a suspension of AgOTf (206.2 mg, 0.803 mmol), *i*-PrOH (265 μL, 3.328 mmol), and 4 Å MS (1 g) in CH_2Cl_2 (8 mL) at 0 °C. The reaction mixture was allowed to warm to rt over 2 h and stirred for an additional 10 h at rt before dilution with CH_2Cl_2 and filtration through Celite. The filtrate was concentrated under reduced pressure and ^{1}H NMR of the crude mixture showed a product distribution of 16:71:2:11 for **122α**:**122β**:**117β**:**118**, respectively. Flash chromatography (2.5% EtOAc/hexane) provided **122β** (63.0 mg, 0.193 mmol, 29%) and a 1:3 mixture of **122α**:**122β** (48.5 mg, 0.148 mmol, 22%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (d, J = 6.1 Hz, 6H, CH(CH_3)$_2$), 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.75 (s, 1H, H_4), 3.98 (sept., J = 6.1 Hz, 1H, CHMe$_2$), 4.19 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.59 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1) 5.03 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5), 5.71 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.1, 19.7, 22.4, 57.9, 59.5, 62.2, 65.3, 69.4, 90.1, 90.2, 168.2, 192.3. IR (film, cm^{-1}): 2977, 1818, 1734, 1374, 1258, 1211. LRMS (EI) *m/z* (relative intensity): 330 (M[(^{37}Cl$_2$)]$^+$, 0.5), 328 ([M(^{37}Cl,^{35}Cl)]$^+$, 2.0), 326 ([M(^{35}Cl$_2$)]$^+$, 3.5), 291 (20), 267 (15), 238 (25), 205 (40), 203 (100), 175 (40). HRMS (EI) *m/z*: 326.0146 calcd for $C_{12}H_{16}{}^{35}Cl_2O_4S$; 326.0139 obsd.

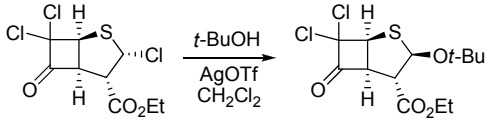
Ethyl 3α-*tert*-Butoxy-7,7-dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (123α)



117α **123α**

Solid *t*-BuOH (8 g) was combined with the 3α-chlorocyclobutanone **117α** (216.9 mg, 0.7145 mmol) at rt and the reaction mixture was slowly heated to 80 °C (with stirring) over 30 minutes. After 64 h at 80 °C, the reaction mixture was concentrated under reduced pressure and ^{1}H NMR of the crude mixture showed a product distribution of 37:26:28:9 for **123α**:**123β**:**118**:**119β**, respectively. Flash chromatography (5% EtOAc/hexane) provided **123α** (10.8 mg, 0.0316 mmol, 4.4%, 98% pure) and a 58:42 mixture of **123α**:**123β** (135.1 mg, 0.396 mmol, 55%). **123α**: Mp 97–99 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (s, 9H, C(CH_3)$_3$), 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.70 (app. t, $J_{4,3\beta}$ = 5.0 Hz, $J_{4,5}$ = 5.2 Hz, 1H, H_4), 4.09 (B of ABX_3, J_{AB} = 10.8 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.24 (A of ABX_3, J_{AB} = 10.8 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.78 (d, $J_{1,5}$ = 8.2 Hz, 1H, H_1), 5.13 (dd, $J_{5,1}$ = 8.2 Hz, $J_{5,4}$ = 5.2 Hz, 1H, H_5), 5.75 (d, $J_{3\beta,4}$ = 5.0 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.1, 27.9 (3C), 57.8, 58.8, 61.6, 66.6, 76.8, 85.9, 89.0, 167.5, 193.7. IR (film, cm^{-1}): 2983, 2946, 1808, 1739, 1469, 1370, 1235, 1210, 1179, 1060. LRMS (EI) *m/z* (relative intensity): 344 (M[($^{37}Cl_2$)]$^+$, 0.2), 342 ([M(^{37}Cl^{35}Cl)]$^+$, 1.5), 340 ([M($^{35}Cl_2$)]$^+$, 2.0), 284 (10), 267 (10), 238 (10), 203 (10), 57 (100). HRMS (EI) *m/z*: 340.0303 calcd for $C_{13}H_{18}{}^{35}Cl_2O_4S$; 340.0294 obsd.

Ethyl 3β-*tert*-Butoxy-7,7-dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (123β)

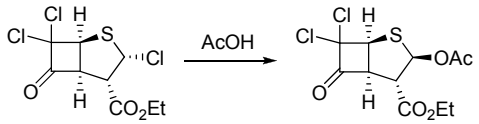


117α **123β**

The 3α-chlorocyclobutanone **117α** (201.5 mg, 0.664 mmol) in CH_2Cl_2 (2 mL) was added dropwise over 10 minutes to a suspension of AgOTf (206.0 mg, 0.802 mmol), *t*-BuOH (497.0 mg, 6.705 mmol), and 4 Å MS (1 g) in CH_2Cl_2 (8 mL) at 0 °C. The reaction mixture was allowed to warm to rt over 2 h and stirred for an additional 6 h at rt before dilution with CH_2Cl_2 and filtration through Celite. The filtrate was concentrated under reduced pressure and ^{1}H NMR of the crude mixture showed a product distribution of 5:42:30:22 for **123α**:**123β**:**117β**:**118**, respectively, with 92% conversion. Flash chromatography (5% EtOAc/hexane) provided **123β** (21.3 mg, 0.0624 mmol,

9.4%) and a 86:14 mixture of **123α**:**123β** (65.1 mg, 0.191 mmol, 29%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.23 (s, 9H, C(CH_3)$_3$), 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.61 (s, 1H, H_4), 4.19 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.58 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 4.98 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5), 5.79 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.1, 27.5 (3C), 59.6 (2C), 62.1, 65.3, 77.5, 86.6, 90.6, 168.2, 193.0. IR (film, cm^{-1}): 2980, 2934, 1818, 1735, 1369, 1252, 1209, 1188, 1064, 1027. LRMS (EI) *m/z* (relative intensity): 344 (M[(^{37}Cl$_2$)]$^+$, 0.2), 342 ([M(^{37}Cl,^{35}Cl)]$^+$, 1.2), 340 ([M(^{35}Cl$_2$)]$^+$, 1.6), 284 (10), 238 (15), 203 (30), 57 (100). HRMS (EI) *m/z*: 340.0303 calcd for $C_{13}H_{18}{}^{35}Cl_2O_4S$; 340.0302 obsd.

Ethyl 3β-Acetoxy-7,7-dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (124β)

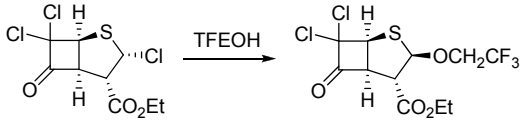


117α **124β**

The 3α-chlorocyclobutanone **117α** (53.3 mg, 0.176 mmol) was dissolved in AcOH (4 mL) at rt and stirred at 80 °C for 1 h. The solution was cooled, diluted with EtOAc (40 mL), and washed with H_2O (3 × 40 mL), dried with Na_2SO_4, and concentrated under reduced pressure to give an amber oil. ^{1}H NMR of the crude mixture showed a product distribution of 3:52:43:2 for **124α**:**124β**:**117β**:**118**, respectively.[685] Purification by flash chromatography (20% EtOAc/hexane) afforded the title compound as a dull yellow oil (40.2 mg, 0.123 mmol, 70%) that solidified under vacuum. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.02 (s, 3H, $COCH_3$), 3.81 (s, 1H, H_4), 4.21 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.64 (d, $J_{1,5}$ = 8.6 Hz, 1H, H_1) 5.13 (d, $J_{5,1}$ = 8.6 Hz, 1H, H_5), 6.55 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 20.7, 56.3, 59.6, 62.7, 65.6, 85.8, 90.1, 167.2, 169.0, 192.9. IR (film, cm^{-1}): 2999, 2956, 1813, 1747, 1740, 1369, 1213, 1022. LRMS (EI) *m/z* (relative intensity): 330 (M[(^{37}Cl$_2$)]$^+$, 0.4), 328 ([M(^{37}Cl^{35}Cl)]$^+$, 1.5), 326 ([M(^{35}Cl$_2$)]$^+$, 2.0), 266 (20), 224 (100), 220 (45), 203 (50), 195 (30). HRMS (EI) *m/z*: 325.9783 calcd for $C_{11}H_{12}{}^{35}Cl_2O_5S$; 325.9786 obsd.

The 3α-acetoxy derivative **124α** was not isolated by chromatography and could only be identified by three signals in the ^{1}H NMR of the crude reaction mixture: 2.03 (s, 3H, $COCH_3$), 3.84 (dd, H_4), 6.52 (d, 1H, $H_{3\beta}$).

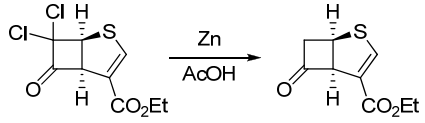
Ethyl 7,7-Dichloro-3β-(2,2,2-trifluoroethoxy)-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (125β)



117α **125β**

The 3α-chlorocyclobutanone **117α** (157.8 mg, 0.5198 mmol) was dissolved in 2,2,2-trifluoroethanol (6 mL) and stirred at rt for 48 h. The solvent was removed under reduced pressure and ^{1}H NMR of the crude mixture showed a product distribution of **125α**:**125β**:**118** of 5:76:19.[686] Flash chromatography (5% EtOAc/hexane) afforded **125β** (122.1 mg, 0.3325 mmol, 64%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.83 (B of ABF_3, J_{BA} = 12.4 Hz, J_{BF} = 8.1 Hz, 1H, one of $CO_2CH_2CF_3$), 3.93 (s, 1H, H_4), 4.04 (A of ABF_3, J_{AB} = 12.4 Hz, J_{AF} = 8.9 Hz, 1H, one of $CO_2CH_2CF_3$), 4.21 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.64 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 5.08 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5), 5.76 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 57.9, 59.7, 62.6, 65.1, 65.2 (q, $^{2}J_{C,F}$ = 35.0 Hz), 90.0, 94.2, 123.3 (q, $^{1}J_{C,F}$ = 279.1 Hz), 167.2, 191.6. ^{19}F NMR (282.4 MHz, $CDCl_3$): δ –72.5. IR (film, cm^{-1}): 2989, 1819, 738, 1371 1278, 1167, 1109. LRMS (EI) *m/z* (relative intensity): 370 (M[($^{37}Cl_2$)]$^+$, 0.5), 368 ([M($^{37}Cl^{35}Cl$)]$^+$, 2.8), 366 ([M($^{35}Cl_2$)]$^+$, 3.8), 331 (6), 320 (15), 285 (10), 257 (35), 233 (100), 203 (40), 183 (25). HRMS (EI) *m/z*: 365.9707 calcd for $C_{11}H_{11}{}^{35}Cl_2F_3O_4S$; 365.9704 obsd.

Ethyl 2-Thiabicyclo[3.2.0]hept-3-ene-6-one-4-carboxylate (126)

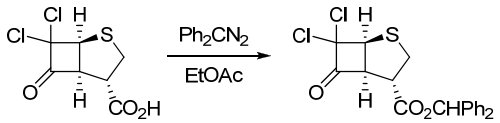


118 **126**

Dichlorocyclobutanone **118** (171.3 mg, 0.641 mmol) was dissolved in AcOH (15 mL), combined with zinc dust (216.0 mg, 3.303 mmol), and stirred at 80 °C for 5 h. The reaction mixture was cooled to rt, diluted with EtOAc, and filtered through glass wool to remove residual zinc dust. The organic solution (75 mL) was washed with 10% HCl (2 × 75 mL) and brine (50 mL) before it was dried over Na_2SO_4 and concentrated. Flash chromatography (10% EtOAc/hexane) provided the dechlorinated cyclobutanone **126** as a colourless oil (106.5 mg, 0.537 mmol, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.34 (ddd, J_{gem} = 18.7 Hz, $J_{7\beta,1}$ = 5.7 Hz, $J_{7\beta,5}$ = 3.3 Hz, 1H,

$H_{7\beta}$), 3.74 (ddd, J_{gem} = 18.7 Hz, $J_{7\alpha,1}$ = 8.3 Hz, $J_{7\alpha,5}$ = 5.1 Hz, 1H, $H_{7\alpha}$), 4.11–4.22 (m, 3H, H_1 and $CO_2CH_2CH_3$), 5.05–5.14 (m, 1H, H_5), 7.43 (s, 1H, H_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.2, 37.0, 60.7 (2C), 76.0, 121.2, 143.2, 161.8, 200.0. IR (film, cm^{-1}): 3069, 2983, 1790, 1700, 1567, 1370, 1328, 1305, 1226. LRMS (EI) *m/z* (relative intensity): 200 ($[M+2]^+$, 2), 199 ($[M+1]^+$, 5), 198 ($[M]^+$, 40), 170 (10), 156 (40), 141 (40), 128 (60), 111 (100), 97 (25). HRMS (EI) *m/z*: 198.0351 calcd for $C_9H_{10}O_3S$; 198.0349 obsd.

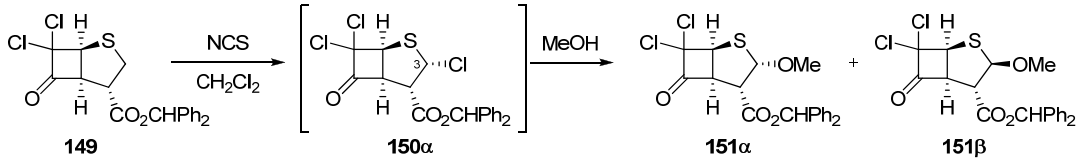
Benzhydryl 7,7-Dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (149)



65 **149**

A solution of diphenyldiazomethane[687] (1.0 M in EtOAc, 5.0 mL) was added slowly to a stirring solution of acid **65** (1207 mg, 5.004 mmol) in EtOAc (20 mL) and stirred for 2 h at rt before the reaction was quenched with acetic acid (2 mL). After stirring for 30 min, the solution was diluted with EtOAc to 50 mL and washed with H_2O (2 × 20 mL), sat. $NaHCO_3$ (2 × 20 mL), and brine (20 mL). The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to a yellow oil that partially crystallized under vacuum. Flash chromatography (10% EtOAc/hexane) provided the benzhydryl ester **149** as a white powder (2007 mg, 4.927 mmol, 98%). Mp 95–96 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.09 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.50 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.76 (d, $J_{3\beta,4}$ = 5.8 Hz, 1H, H_4), 4.50 (d, $J_{1,5}$ = 8.3 Hz, 1H, H_1), 5.07 (d, $J_{5,1}$ = 8.3 Hz, 1H, H_5), 6.87 (s, 1H, $CHPh_2$), 7.28–7.40 (m, 10H, ArH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 35.6, 50.3, 58.9, 67.4, 78.7, 89.3, 126.9 (2C), 127.1 (2C), 128.3 (2C), 128.7 (2C), 139.2 (2C), 169.0, 194.6. IR (film, cm^{-1}): 3064, 3031, 2933, 1808, 1738, 1495, 1454, 1207, 1177, 742, 699. LRMS (NH_3 CI) *m/z* (relative intensity): 428 ($[M(^{37}Cl_2) + NH_4]^+$, 1.0), 426 ($[M(^{37}Cl^{35}Cl) + NH_4]^+$, 5.5), 424 ($[M(^{35}Cl_2) + NH_4]^+$, 7.0), 167 (100).

Benzhydryl 7,7-Dichloro-3-methoxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylates (151α and 151β)

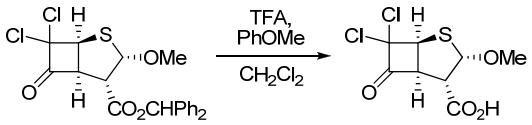


N-Chlorosuccinimide (261.9 mg, 2.015 mmol) was added as a suspension in CH_2Cl_2 (5 mL) to a stirring solution of the benzhydryl ester **149** (722.1 mg, 1.773 mmol) in CH_2Cl_2 (20 mL). After stirring the solution at rt for 24 h, the solvent was removed under reduced pressure to provide a mixture of succinimide and the 3α-chloro intermediate as a yellow oily solid. The 3β-Cl epimer **150β** was not detected by ^{1}H NMR. **150α**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.08 (app. t, $J_{4,3\beta}$ = 4.7 Hz, $J_{4,5}$ = 5.9 Hz, 1H, H_4), 4.93 (d, $J_{1,5}$ = 8.3 Hz, 1H, H_1), 5.18 (dd, $J_{5,1}$ = 8.3 Hz, $J_{5,4}$ = 5.9 Hz, 1H, H_5), 5.94 (d, $J_{3\beta,4}$ = 4.7 Hz, 1H, $H_{3\beta}$), 6.91 (s, 1H, $CHPh_2$), 7.28–7.40 (m, 10H, ArH).

The intermediate mixture of **150α** with succinimide was then dissolved in MeOH (25 mL) and stirred at rt for 24 h before concentration in vacuo. The mixture was then dissolved in CH_2Cl_2 (75 mL) and washed with H_2O (3 × 25 mL) and brine (25 mL) before the solution was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting viscous oil was then subjected to high vacuum for several hours for the removal of methanol. ^{1}H NMR spectra of the crude mixture showed a product distribution of 77:23 for **151α**:**151β**. Recrystallization from CH_2Cl_2/hexane provided a pure sample of **151α** as white needles (195.1 mg, 0.446 mmol, 25%). The mother liquor was concentrated and subjected to flash chromatography (10% EtOAc/hexane) to provide the 3β-methoxy derivative **151β** as a white solid (37.5 mg, 4.8%) and additional samples of **151α**/**151β** mixtures. **151α**: Mp 161–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.15 (s, 3H, OCH_3), 3.91 (app. t, $J_{4,3\beta}$ = 4.6 Hz, $J_{4,5}$ = 5.4 Hz, H_4), 4.66 (d, $J_{1,5}$ = 8.2 Hz, H_1), 5.21 (dd, $J_{5,1}$ = 8.2 Hz, $J_{5,4}$ = 5.4 Hz, H_5), 5.39 (d, $J_{3\beta,4}$ = 4.6 Hz, $H_{3\beta}$), 6.97 (s, 1H, $CHPh_2$), 7.30-7.41 (m, 10H, ArH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.3, 57.4, 58.6, 66.3, 78.3, 85.2, 97.3, 127.1 (2C), 127.3 (2C), 128.1, 128.2, 128.4 (2C), 128.5 (2C), 139.1, 139.4, 166.4, 193.1. IR (film, cm^{-1}): 3067, 3033, 2957, 1813, 1744, 1450, 1430, 1285, 1234, 1180, 1079, 944, 881. LRMS (NH_3 CI) *m/z* (relative intensity): 458 ([M($^{37}Cl_2$) + NH_4]$^+$, 10), 456 ([M($^{37}Cl^{35}Cl$) + NH_4]$^+$, 35), 454 ([M($^{35}Cl_2$) + NH_4]$^+$, 55), 420 (15), 184 (40), 167 (100). **151β**: Mp 136–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.35 (s, 3H, OCH_3), 3.93 (s, 1H, H_4), 4.58 (d, $J_{1,5}$ = 8.5

Hz, 1H, H_1), 5.03 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5), 5.51 (s, 1H, $H_{3\alpha}$), 6.86 (s, 1H, $CHPh_2$), 7.26–7.39 (m, 10H, ArH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 56.3, 58.1, 59.4, 64.9, 78.9, 90.5, 95.0, 127.0 (2C), 127.1 (2C), 128.3, 128.4, 128.7 (4C), 139.0 (2C), 167.0, 192.5. IR (film, cm^{-1}): 3064, 3032, 2930, 2828, 1816, 1741, 1600, 1496, 1454, 1304, 1256, 1207, 1176, 1085, 700. LRMS (NH_3 CI) *m/z* (relative intensity): 458 ($[M(^{37}Cl_2) + NH_4]^+$, 3), 456 ($[M(^{37}Cl^{35}Cl) + NH_4]^+$, 15), 454 ($[M(^{35}Cl_2) + NH_4]^+$, 20), 184 (50), 167 (100).

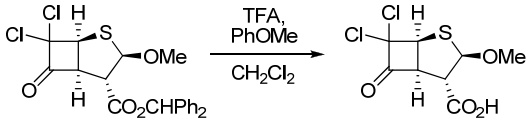
7,7-Dichloro-3α-methoxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic Acid (152α)



151α **152α**

TFA (1.0 mL) was added dropwise to a solution of the 3α-methoxy benzhydryl ester **151α** (190.5 mg, 0.436 mmol) and anisole (200 μL, 1.84 mmol) in CH_2Cl_2 (10 mL). The solution was stirred for 2 h at rt before the solvent was removed under reduced pressure. Trituration with hexane provided the acid **152α** as a white solid (93.8 mg, 0.346 mmol, 79%). Mp 144–147 °C. ^1H NMR (300 MHz, acetone-d_6): δ 3.34 (s, 1H, OCH_3), 3.84 (dd, $J_{4,3\beta}$ = 4.4 Hz, $J_{4,5}$ = 6.0 Hz, 1H, H_4), 4.77 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 5.13 (dd, $J_{5,1}$ = 8.4 Hz, $J_{5,4}$ = 6.0 Hz, 1H, H_5), 5.57 (d, $J_{3\beta,4}$ = 4.4 Hz, 1H, $H_{3\beta}$). ^{13}C NMR 75.5 MHz, acetone-d_6): δ 56.8, 58.3, 58.8, 67.7, 86.7, 98.5, 168.6, 194.7. IR (film, cm^{-1}): br 3500–2500, 2953, 2831, 1813, 1720, 1422, 1258, 1192, 1071. LRMS (EI) *m/z* (relative intensity): 274 ($[M(^{37}Cl_2)]^+$, 0.5), 272 ($[M(^{37}Cl^{35}Cl)]^+$, 1.5), 270 ($[M(^{35}Cl_2)]^+$, 2.0), 165 (100), 143 (60), 115 (40), 83 (80). HRMS (EI) *m/z*: 269.95203 calcd for $C_8H_8{}^{35}Cl_2O_4S$; 269.9530 obsd.

7,7-Dichloro-3β-methoxy-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic Acid (152β)

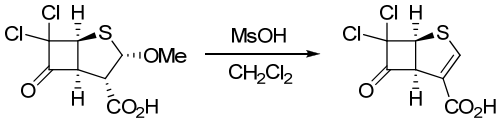


151β **152β**

TFA (1.0 mL) was added dropwise to a solution of the 3β-methoxy benzhydryl ester **151β** (95.7 mg, 0.219 mmol) and anisole (100 μL, 0.92 mmol) in CH_2Cl_2 (9 mL). The solution was stirred for 2 h at rt before the solvent was removed under reduced pressure. Trituration with hexane and recrystallization from CH_2Cl_2/hexane provided the acid **152β** as a white solid (36.1 mg, 0.133 mmol,

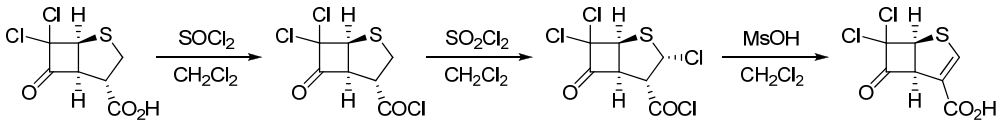
61%). Mp 190 °C (dec). ^{1}H NMR (300 MHz, acetone-d_6): δ 3.33 (s, 3H, OCH_3), 3.90 (s, 1H, H_4), 4.78 (d, $J_{1,5}$ = 8.6 Hz, 1H, H_1), 5.12 (d, $J_{5,1}$ = 8.6 Hz, 1H, H_5), 5.61 (s, 1H, $H_{3\alpha}$). ^{13}C NMR 75.5 MHz, acetone-d_6): δ 56.4, 58.6, 60.0, 66.2, 91.5, 96.4, 169.4, 193.8. IR (film, cm^{-1}): 2958, 1814, 1703, 1430, 1286, 1234, 944, 828. LRMS (EI) *m/z* (relative intensity): 274 ([M($^{37}Cl_2$)]$^+$, 2), 272 ([M($^{37}Cl^{35}Cl$)]$^+$, 2), 270 ([M($^{35}Cl_2$)]$^+$, 3), 252 (20), 217 (50), 189 (100), 175 (95), 165 (55), 128 (35), 83 (35). HRMS (EI) *m/z*: 269.9520 calcd for $C_8H_8{}^{35}Cl_2O_4S$; 269. 9512 obsd.

7,7-Dichloro-2-thiabicyclo[3.2.0]hept-3-ene-6-one-4-carboxylic Acid (153)



151α **153**

A mixture of the methoxy acids **151α** and **151β** (51.8 mg, 0.191 mmol, 10:1 *dr*) was stirred in a solution of 50% MsOH/CH_2Cl_2 (5 mL) at rt for 1 h. The solution was diluted with EtOAc (50 mL) and washed with H_2O until the aqueous washes were no longer acidic (4 × 25 mL). The combined aqueous washes were back-extracted with EtOAc (2 × 25 mL) and CH_2Cl_2 (2 × 25 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to give the unsaturated acid **153** as an off-white solid (30.4 mg, 0.127 mmol, 67%). Mp 162–163 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 5.23 (d, $J_{1,5}$ = 10.0 Hz, 1H, H_1), 5.62 (dd, $J_{5,1}$ = 10.0 Hz, $J_{5,3}$ = 1.8 Hz, 1H, H_5), 7.68 (d, $J_{3,5}$ = 1.8 Hz, 1H, H_3). ^{13}C NMR 75.5 MHz, acetone-d_6): δ 60.1, 72.8, 94.8, 123.3, 146.1, 162.3, 189.5. IR (film, cm^{-1}): br 3500–2300, 3066, 2925, 1807, 1700, br 1690–1630, 1558, 1446, 1338, 1258. LRMS (EI) *m/z* (relative intensity): 240 ([M($^{37}Cl^{35}Cl$)]$^+$, 1.8), 238 ([M($^{35}Cl_2$)]$^+$, 2.1), 210 (20), 175 (100), 165 (30), 111 (20). HRMS (EI) *m/z*: 237.9258 calcd for $C_7H_4{}^{35}Cl_2O_3S$; 237.9252 obsd.



65 **154** **155α** **153**

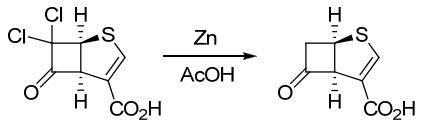
Alternatively, the unsaturated acid could be prepared in a one-pot three-step procedure from the acid **63** through the acid chloride **154**. $SOCl_2$ (4 mL, 55 mmol) was added to a solution of acid **65** (1.475 g, 6.120 mmol) in CH_2Cl_2 (30 mL) and heated to reflux for 4 h. Concentration of the solution in vacuo provided the crude acid chloride **154** as a yellow oil that was used directly in the next step

without purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.13 (dd, J_{gem} = 13.1 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.54 (d, J_{gem} = 13.1 Hz, 1H, $H_{3\alpha}$), 4.03 (d, $J_{4,3\alpha}$ = 6.0 Hz, 1H, H_4), 4.55 (d, $J_{1,5}$ = 8.2 Hz, 1H, H_1), 5.03 (d, $J_{5,1}$ = 8.2 Hz, 1H, H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 35.4, 58.3, 60.3, 66.8, 89.1, 172.0, 192.9. IR (film, cm^{-1}): 2965, 1809, 1780, 1443, 997, 927, 873. LRMS (EI) *m/z* (relative intensity): 264 ($[M(^{37}Cl_3)]^+$, 0.5), 262 ($[M(^{37}Cl_2{}^{35}Cl)]^+$, 3), 260 ($[M(^{37}Cl{}^{35}Cl_2)]^+$, 7), 258 ($[M(^{35}Cl_3)]^+$, 7), 225 (4), 223 (6), 200 (15), 198 (15), 141 (30), 131 (30), 85 (100), 55 (40). HRMS (EI) *m/z*: 257.9076 calcd for $C_7H_5{}^{35}Cl_3O_2S$; 257.9080 obsd.

SO_2Cl_2 (550 μL, 6.85 mmol) was added dropwise over 2 min to a stirring solution of the crude acid chloride **154** in CH_2Cl_2 (25 mL). After 12 h at rt, the reaction mixture was concentrated under reduced pressure to give the 3α-chloro acid chloride **155α** as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.37 (dd, $J_{4,3\beta}$ = 4.2 Hz, $J_{4,5}$ = 5.2 Hz, 1H, H_4), 4.99 (d, $J_{1,5}$ = 8.1 Hz, 1H, H_1), 5.13 (dd, $J_{5,1}$ = 8.1 Hz, $J_{5,4}$ = 5.2 Hz, 1H, H_5), 6.03 (d, $J_{3\beta,4}$ = 4.2 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 60.0, 64.1, 69.4, 72.9, 84.7, 167.2, 190.5.

MsOH (4 mL) was added to a solution of the crude acid chloride **155α** in CH_2Cl_2 (36 mL) and the solution was heated to reflux for 3 h. The reaction mixture was cooled to rt, diluted with EtOAc (100 mL), washed with H_2O (4 × 75 mL) until the aqueous washes showed a pH of 4. The combined aqueous washes were back-extracted with EtOAc (2 × 100 mL) and the combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to give the unsaturated acid **153** as a beige solid (1.368 g, 5.722 mmol, 94% over 3 steps) which was estimated to be ≥95% pure by ^{1}H NMR. Analytically pure material was obtained by recrystallization from $CHCl_3$/hexane.

2-Thiabicyclo[3.2.0]hept-3-ene-6-one-4-carboxylic Acid (156)

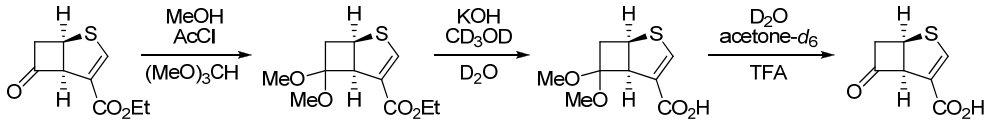


153 **156**

Following a typical procedure for the dechlorination of cyclobutanones, zinc dust (82.1 mg, 1.26 mmol) was added to a stirring solution of the dichlorocyclobutanone **153** (60.0 mg, 0.251 mmol) in AcOH (18 mL) at rt. The suspension was heated to 85 °C for 12 h before the addition of another portion of zinc dust (78.0 mg, 1.20 mmol). After stirring for an additional 2 h, the reaction mixture was cooled to rt, diluted with EtOAc, and filtered for the removal of residual zinc, and the solution

was concentrated under reduced pressure. The resulting solid was suspended in EtOAc (75 mL) and washed with 10% HCl (2 × 50 mL). The organic phase was dried over Na_2SO_4 and concentrated in vacuo to give an off-white solid (20 mg). This material was soluble only in DMSO-d_6 and ^{1}H NMR showed only a small amount of the dechlorinated acid **156** within a complex mixture. A small amount of **156** was obtained, however, from back-extraction of the combined aqueous HCl washes (100 mL) with EtOAc (50 mL) and CH_2Cl_2 (50 mL). These organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to give the acid **156** as a white solid (3.9 mg, 0.023 mmol, 9%). ^{1}H NMR (300 MHz, acetone-d_6): δ 3.30 (ddd, $J_{7\beta,1}$ = 5.4 Hz, $J_{7\beta,5}$ = 3.2 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\beta}$), 3.85 (ddd, $J_{7\alpha,1}$ = 8.4 Hz, $J_{7\alpha,5}$ = 4.9 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\alpha}$), 4.39 (ddd, $J_{1,7\alpha}$ = 8.4 Hz, $J_{1,7\beta}$ = 5.4 Hz, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 5.12–5.18 (m, 1H, H_5), 7.59 (s, 1H, H_3). ^{13}C NMR (75.5 MHz, acetone-d_6): δ 38.1, 61.6, 77.0, 122.5, 144.6, 163.0, 201.1. LRMS (EI) *m/z* (relative intensity): 172 ($[M+2]^+$, 2), 171 ($[M+1]^+$, 9), 170 (M^+, 21), 141 (20), 128 (100), 111 (100), 97 (30). HRMS (EI) *m/z*: 170.0038 calcd for $C_7H_6O_3S$; 170.0038 obsd.

With the low yield of **156** using the dechlorination procedure described above, alternate methods for the synthesis of acid **156** were pursued. Attempts to effect dechlorination of **153** were also unsuccessful with zinc in AcOH at rt and with zinc in *i*-PrOH/AcOH 100:1. While the direct hydrolysis of ethyl ester **126** using KOH (1 equiv) in THF/H_2O was also unsuccessful, hydrolysis of the ester could be accomplished through the dimethyl ketal **157**.



126 **157** **158** **156**

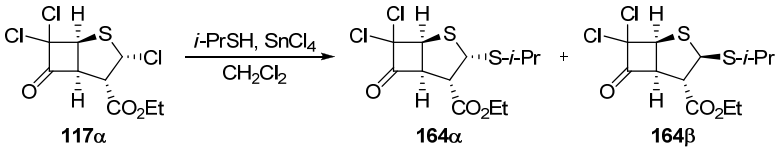
Acetyl chloride (100 μL) was added to a stirring solution of trimethyl orthoformate (1 mL) and ketone **126** (17.3 mg, 0.087 mmol) in MeOH (16 mL). After stirring at rt for 4 h, the solvents were removed under reduced pressure to give the dimethyl ketal **157** as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.49 (dd, $J_{7\beta,1}$ = 6.4 Hz, J_{gem} = 13.1 Hz, 1H, $H_{7\beta}$), 2.72 (ddd, $J_{7\alpha,1}$ = 8.3 Hz, $J_{7\alpha,5}$ = 3.2 Hz, J_{gem} = 13.1 Hz, 1H, $H_{7\alpha}$), 3.10 (s, 3H, one of OCH_3), 3.32 (s, 3H, one of OCH_3), 3.97–4.04 (m, 1H, H_1), 4.15 (B of ABX_3, J_{AB} = 10.8 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.23 (A of ABX_3, J_{BA} = 10.8 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.34–4.37 (m, 1H, H_5), 7.39 (s, 1H, H_3). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.3, 38.9, 45.2, 48.7, 49.4, 59.3, 60.2,

105.9, 126.0, 143.2, 163.3.

The crude dimethyl ketal was used in the following step without further characterization or purification. A crystal of KOH (24.0 mg, 0.429 mmol) was added to a solution of ketal **157** in CD_3OD (0.5 mL) and D_2O (1.0 mL) and the progress of the reaction was followed by NMR. After 36 h at rt, the solution was concentrated in vacuo, diluted with H_2O (25 mL), and washed with CH_2Cl_2 (25 mL). The aqueous phase (pH ~10) was then acidified with TFA (~1 mL) to pH 2 and extracted with CH_2Cl_2 (2 × 25 mL). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to provide the ketal acid **158** as a white solid. ^{1}H NMR (300 MHz, acetone-d_6): δ 2.42 (dd, $J_{7\beta,1}$ = 6.1 Hz, J_{gem} = 13.1 Hz, 1H, $H_{7\beta}$), 2.70 (ddd, $J_{7\alpha,1}$ = 8.4 Hz, $J_{7\alpha,5}$ = 2.9 Hz, J_{gem} = 13.1 Hz, 1H, $H_{7\alpha}$), 3.06 (s, 3H, one of OCH_3), 3.26 (s, 3H, one of OCH_3), 4.04–4.10 (app. br q, 1H, H_1), 4.33 (br d, $J_{5,1}$ = 8 Hz, 1H, H_5), 7.43 (s, 1H, H_3).

TFA (100 μL) was added to an NMR tube containing a solution of the ketal **158** in acetone-d_6 (400 μL) and D_2O (400 μL) and the progress of the hydrolysis was followed by NMR. After 16 h at rt the solution was diluted with H_2O (40 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to provide ketone **156** as a white solid (6.9 mg, 0.041 mmol, 46% over 3 steps) with spectral data identical to that obtained using the dechlorination procedure above.

Ethyl 7,7-Dichloro-3β-(isopropylthio)-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (164β)

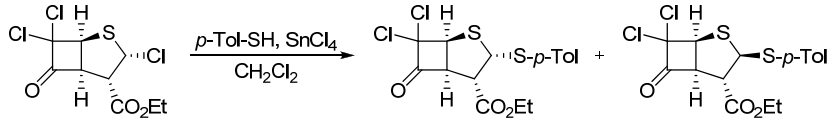


117α **164α** **164β**

A solution of $SnCl_4$ (200 μL, 1.0 M in CH_2Cl_2) was added dropwise at rt to a solution of α-chloro derivative **117α** (51 mg, 0.17 mmol) and 2-propanethiol (50 μL, 0.54 mmol) in CH_2Cl_2 (7 mL) and stirred for 16 h at rt. The reaction mixture was diluted with CH_2Cl_2 (75 mL), filtered through Celite, washed with H_2O (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The substitution products were not purified but used as a mixture in a failed attempt to effect hydrolysis of the ethyl ester. Compounds **164α** and **164β** were identified by ^{1}H NMR of the crude mixture, however, which showed a product distribution of 15:83:2 for **164α**:**164β**:**118**, respectively. **164α**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.35 (m, 9H, $CO_2CH_2CH_3$ + $SCH(CH_3)_2$), 3.06 (sept, J = 6.6 Hz, 1H, $SCH(CH_3)_2$),

3.75 (dd, $J_{4,3\beta}$ = 5.8 Hz, $J_{4,5}$ = 2.1 Hz, 1H, H_4), 4.21 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.70 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 4.90 (d, $J_{5,1}$ = 8.4 Hz, $J_{5,4}$ = 2.1 Hz, 1H, H_5), 4.67 (d, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$). **164β**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22–1.35 (m, 9H, $CO_2CH_2CH_3$ + $SCH(CH_3)_2$), 3.13 (sept, J = 6.7 Hz, 1H, $SCH(CH_3)_2$), 3.72 (s, 1H, H_4), 4.21 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.60 (d, $J_{1,5}$ = 8.6 Hz, 1H, H_1), 4.98 (d, $J_{5,1}$ = 8.6 Hz, 1H, H_5), 5.10 (s, 1H, $H_{3\alpha}$).

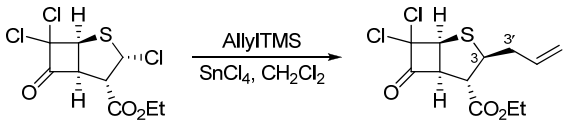
Ethyl 7,7-Dichloro-3β-(*p*-tolylthio)-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (165β)



117α **165α** **165β**

A solution of the 3α-chloride **117α** (74.9 mg, 0.247 mmol) in CH_2Cl_2 (1 mL) was added dropwise at rt to a solution of 4-methylbenzenethiol (84.7 mg, 0.682 mmol) and $SnCl_4$ (300 μL, 1.0 M in CH_2Cl_2) in CH_2Cl_2 (8 mL). After 2 h of stirring at rt, the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with H_2O (40 mL) and satd $NaHCO_3$ (30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. ^{1}H NMR spectra of the crude product showed a product distribution of 32:>67:<1 for **165α**:**165β**:**118**, respectively. Flash chromatography (10% EtOAc/Hex) provided the 3β-S-*p*-Tol isomer **165β** (33.4 mg, 0.085 mmol, 35%) and additional fractions of **165α**/**165β** mixtures. **165α**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.33 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.36 (s, 3H, $ArCH_3$), 3.85 (dd, $J_{4,3\beta}$ = 5.9 Hz, $J_{4,5}$ = 3.1 Hz, 1H, H_4), 4.24 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.57 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 4.90 (d, $J_{3\beta,4}$ = 5.9 Hz, 1H, $H_{3\beta}$), 4.95 (dd, $J_{5,4}$ = 3.1 Hz, $J_{5,1}$ = 8.5 Hz, 1H, H_5), 7.16 (d, J = 8.0 Hz, 2H, ArH), 7.40 (d, J = 8.0 Hz, 2H, ArH). **165β**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.35 (s, 3H, $ArCH_3$), 3.71 (s, 1H, H_4), 4.13 (B of ABX_3, J_{BA} = 10.7 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.16 (A of ABX_3, J_{AB} = 10.7 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.62 (d, $J_{1,5}$ = 8.6 Hz, 1H, H_1), 4.99 (d, $J_{5,1}$ = 8.6 Hz, 1H, H_5), 5.33 (s, 1H, $H_{3\alpha}$), 7.17 (d, J = 8.0 Hz, 2H, ArH), 7.35 (d, J = 8.0 Hz, 2H, ArH). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 21.2, 56.8, 60.8, 61.0, 62.4, 66.3, 89.8, 130.2 (2C), 130.5, 132.8 (2C), 139.0, 168.8, 193.9.

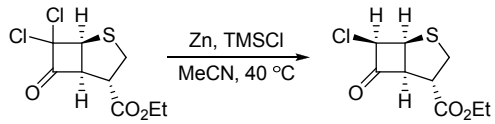
Ethyl 3β-Allyl-7,7-dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (166β)



117α **166β**

A solution of $SnCl_4$ (560 μL, 0.1 M in CH_2Cl_2) was added to a stirring solution of the 3α-chloro derivative **117α** (155 mg, 0.511 mmol) and allyltrimethylsilane (320 μL, 2.70 mmol) in CH_2Cl_2 (6 mL) and stirred for 1.5 h at rt. The solution was diluted with EtOAc (75 mL), washed with H_2O (3 × 25 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. ^{1}H NMR spectra of the crude product showed a **166β**/**166α** ratio of >10:1 and flash chromatography (5% EtOAc/Hex) provided the 3β-allyl product **166β** (47 mg, 0.151 mmol, 30%) as a colourless oil and additional fractions of **166α**/**166β** mixtures. The 3α-allyl isomer **166α** was not purified with a single column but was identified in mixtures with **166β** by ^{1}H NMR. **166α**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.12–2.28 (m, 1H, B of ABX, one of $CH_2CH{=}CH_2$), 2.52–2.66 (m, 1H, A of ABX, one of $CH_2CH{=}CH_2$), 3.55 (d, J = 3 Hz, 1H, H_4), 3.62–3.73 (m, 1H, $H_{3\beta}$), 4.10–4.35 (m, 2H, $CO_2CH_2CH_3$), 4.61 (d, $J_{1,5}$ = 8 Hz, 1H, H_1), 4.80 (d, $J_{5,1}$ = 8 Hz, 1H, H_5), 5.07–5. 18 (m, 2H, $CH{=}CH_2$), 5.60–5.85 (m, 1H, $CH{=}CH_2$). **166β**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.33–2.43 (m, 2H $CH_2CH{=}CH_2$), 3.47 (dd, $J_{4,5}$ = 2.0 Hz, $J_{4,3\alpha}$ = 3.5 Hz, 1H, H_4), 3.96 (dt, $J_{3\alpha,4}$ = 3.5 Hz, $J_{3\alpha,3'}$ = 7.8 Hz, 1H, $H_{3\alpha}$), 4.20 (B of ABX_3, J_{BA} = 10.9 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.20 (A of ABX_3, J_{AB} = 10.9 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.56 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 4.95 (dd, $J_{5,1}$ = 8.5 Hz, $J_{5,4}$ = 2.0 Hz, 1H, H_5), 5.11–5.16 (m, 2H, $CH{=}CH_2$), 5.67–5.77 (m, 1H, $CH{=}CH_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 41.4, 54.0, 55.6, 59.8, 62.1, 68.0, 88.3, 118.5, 134.4, 170.5, 194.8.

Ethyl 7β-Chloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (183β)

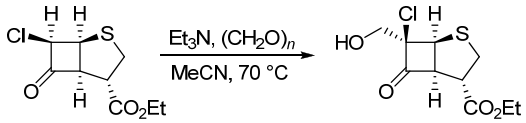


84 **183β**

Zinc dust (587 mg, 8.98 mmol) was added at rt to a solution of the dichlorocyclobutanone **84** (1.209 g, 4.491 mmol) and TMSCl (1.71 mL, 13.47 mmol) in dry MeCN (80 mL). The suspension was heated to 40 °C and stirred for an additional 8 h before the solvent was removed in vacuo. The

reaction mixture was then taken up in CH_2Cl_2, washed with H_2O, dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (20% EtOAc/Hex) provided the 7β-chlorocyclobutanone **183β** as a colourless oil (763 mg, 3.25 mmol, 72%) that was used in the following step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.03 (dd, J_{gem} = 12.3 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.38 (d, J_{gem} = 12.3 Hz, 1H, $H_{3\alpha}$), 3.62 (d, $J_{4,3\beta}$ = 6.0 Hz, 1H, H_4), 4.19 (1.29 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.47 (dd, $J_{1,5}$ = 7.9 Hz, $J_{1,7\alpha}$ = 8.1 Hz, 1H, H_1), 4.69 (dd, $J_{5,1}$ = 7.9 Hz, $J_{5,7\alpha}$ = 3.4 Hz, 1H, H_5), 5.19 (dd, $J_{7\alpha,1}$ = 8.1 Hz, $J_{7\alpha,5}$ = 3.4 Hz, 1H, $H_{7\alpha}$).

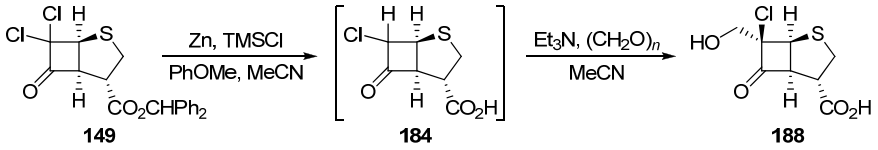
Ethyl 7-Chloro-7-hydroxymethyl-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate (187)



183β **187**

Triethylamine (0.10 mL, 0.74 mmol) was added to a suspension of the 7β-chlorocyclobutanone **183β** (115.7 mg, 0.493 mmol) and paraformaldehyde (44.4 mg, 1.48 mmol) in MeCN (20 mL) and heated to 70 °C. After 6 h at this temperature, the solution was cooled to rt and the solvent removed under reduced pressure. The reaction mixture was dissolved in CH_2Cl_2 (20 mL), washed with 5% HCl (20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (20% EtOAc/hexane) provided the title compound **187** as a dull yellow oil (85 mg, 0.32 mmol, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.50–2.58 (brs, 1H, OH), 3.02 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.38 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.58 (d, $J_{3\beta,4}$ = 5.8 Hz, 1H, H_4), 3.98 (B of AB, J_{AB} = 11.7 Hz, one of CH_2OH), 4.06 (A of AB, J_{AB} = 11.7 Hz, one of CH_2OH), 4.17 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.35 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 4.69 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 14.0, 35.7, 49.3, 50.5, 62.0, 65.6, 67.8, 82.4, 170.6, 204.1. IR (film, cm^{-1}): 3600–3200 (br), 2984, 1795, 1730, 1265, 1184, 910, 733. LRMS (EI) *m/z* (relative intensity): 266 (M[(^{37}Cl)]$^+$, 5), 264 ([M(^{35}Cl)]$^+$, 15), 246 (10), 218 (10), 173 (20), 158 (25), 99 (20), 85 (100). HRMS (EI) *m/z*: 264.0223 calcd for $C_{10}H_{13}{}^{35}ClO_4S$; 264.0226 obsd.

7-Chloro-7-hydroxymethyl-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylic Acid (188)



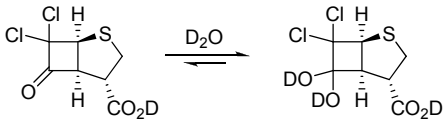
Zinc dust (323 mg, 4.97 mmol) was added at rt to a stirring solution of the dichlorocyclobutanone **149** (1003 mg, 2.46 mmol), TMSCl (935 μL, 7.37 mmol), and PhOMe (800 μL, 7.36 mmol) in dry MeCN (15 mL). The suspension was heated to 40 °C and stirred for an additional 4 h before the solvent was removed under reduced pressure. The reaction mixture was then taken up in CH_2Cl_2 (50 mL) and washed with 5% HCl (50 mL). The aqueous washes were back-extracted with CH_2Cl_2 (25 mL) before the organic extracts were combined and extracted with satd $NaHCO_3$ (2 × 20 mL). The aqueous extracts were then acidified with 6 M HCl (~4 mL) to pH 1–2 and extracted with CH_2Cl_2 (3 × 20 mL). The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to give a mixture of monochlorocyclobutanones **184α** and **184β** (87.2 mg, 0.423 mmol, 17%) that was used in the following reaction without additional purification or characterization. **184α**: ^{1}H NMR (300 MHz, acetone-d_6): δ 3.37 (dd, J_{gem} = 12.3 Hz, $J_{3\beta,4}$ = 6.2 Hz, 1H, $H_{3\beta}$), 3.55 (dd, J_{gem} = 12.3 Hz, $J_{3\alpha,4}$ = 1.9 Hz, 1H, $H_{3\alpha}$), 3.74 (dd, $J_{4,3\alpha}$ = 1.9 Hz, $J_{4,3\beta}$ = 6.2 Hz, 1H, H_4), 4.18 (dd, $J_{1,5}$ = 8.2 Hz, $J_{1,7\alpha}$ = 3.5 Hz, 1H, H_1), 4.85–4.95 (m, 2H, $H_5 + H_{7\beta}$). **184β**: ^{1}H NMR (300 MHz, acetone-d_6): δ 2.97 (dd, J_{gem} = 12.1 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.36 (d, J_{gem} = 12.1 Hz, 1H, $H_{3\alpha}$), 3.63 (d, $J_{4,3\beta}$ = 6.0 Hz, 1H, H_4), 4.55 (dd, $J_{1,5}$ = 7.9 Hz, $J_{1,7\alpha}$ = 8.0 Hz, 1H, H_1), 4.77 (dd, $J_{5,1}$ = 7.9 Hz, $J_{5,7\alpha}$ = 3.3 Hz, 1H, H_5), 5.54 (dd, $J_{7\alpha,1}$ = 8.0 Hz, $J_{7\alpha,5}$ = 3.3 Hz, 1H, $H_{7\alpha}$). LRMS (EI) *m/z* (relative intensity): 208 (M[(^{37}Cl)]$^+$, 20), 206 ([M(^{35}Cl)]$^+$, 50), 146 (20), 130 (40), 85 (100). HRMS (EI) *m/z*: 205.9804 calcd for $C_7H_7O_3{}^{35}ClS$; 205.9808 obsd.

Triethylamine (110 μL, 0.786 mmol) was added at rt to a suspension of the monochloro-cyclobutanones **184** (40.7 mg, 0.197 mmol, **184α**:**184β** ≈ 3:97) and paraformaldehyde (12 mg, 0.40 mmol) in MeCN (15 mL) and H_2O (0.4 mL). The suspension was heated to 50 °C and stirred for 1.5 h before the reaction mixture was concentrated under reduced pressure. The crude mixture was taken up in CH_2Cl_2 (25 mL) and extracted with satd $NaHCO_3$ (25 mL) before the aqueous extracts were acidified with 6 M HCl (~2 mL) to pH 2 and extracted with CH_2Cl_2 (2 × 40 mL). The organic solution was dried over Na_2SO_4 and concentrated under reduced pressure to provide hydroxymethylated

cyclobutanone derivative **188** as a white solid (23.8 mg, 0.101 mmol, 51%). ^{1}H NMR (300 MHz, acetone-d_6): δ 2.96 (dd, J_{gem} = 12.2 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.42 (d, J_{gem} = 12.2 Hz, 1H, $H_{3\alpha}$), 3.63 (d, $J_{4,3\beta}$ = 5.8 Hz, 1H, H_4), 3.96 (B of AB, J_{AB} = 11.3 Hz, 1H, one of CH_2OH), 4.04 (A of AB, J_{AB} = 11.3 Hz, 1H, one of CH_2OH), 4.47 (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 (75.5 MHz, acetone-d_6): δ 36.4, 50.2, 51.0, 66.0, 69.3, 83.2, 172.3, 205.5.

5.4 Cyclobutanone Hydrate Formation[688]

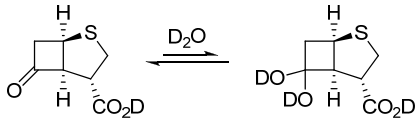
Hydrate Formation with Cyclobutanone 65



65 *hydrate*

The dichlorocyclobutanone **65** (32.5 mg, 0.135 mmol) was dissolved in acetone-d_6 (0.2 mL) and combined with D_2O (0.6 mL). ^{1}H NMR of the mixture within 5 minutes showed a ketone:hydrate ratio of 26:74 and this ratio did not change in the following 3 weeks. **Ketone**: ^{1}H NMR (300 MHz, D_2O): δ 3.08 (dd, J_{gem} = 12.6, $J_{3\beta,4}$ = 5.9, 1H, $H_{3\beta}$), 3.48 (d, J_{gem} = 12.6, 1H, $H_{3\alpha}$), 3.85 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 4.72 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 5.20 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5). ^{13}C NMR (75.5 MHz, D_2O): δ 36.9, 51.3, 59.9, 69.0, 90.3, 174.7, 198.3. **Hydrate**: ^{1}H NMR (300 MHz, D_2O): δ 3.33 (d, J_{gem} = 11.5, 1H, $H_{3\alpha}$), 3.49 (dd, J_{gem} = 11.5, $J_{3\beta,4}$ = 6.5, 1H, $H_{3\beta}$), 3.64 (d, $J_{4,3\beta}$ = 6.5 Hz, 1H, H_4), 3.97 (d, $J_{5,1}$ = 8.9 Hz, 1H, H_5), 4.41 (d, $J_{1,5}$ = 8.9 Hz, 1H, H_1). ^{13}C NMR (75.5 MHz, D_2O): δ 39.1, 49.7, 59.5, 62.1, 96.6, 98.7, 177.0.

Hydrate Formation with Cyclobutanone 111

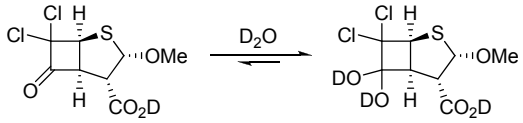


111 *hydrate*

The dechlorinated cyclobutanone **111** (21.1 mg, 0.123 mmol) was dissolved in acetone-d_6 (0.2 mL) and combined with D_2O (0.6 mL). None of the cyclobutanone hydrate could be detected by ^{1}H NMR,

even after 3 weeks in solution. **Ketone**: ^{1}H NMR (500 MHz, D_2O): δ 3.00 (ddd, $J_{7\beta,1}$ =3.4 Hz, $J_{7\beta,5}$ = 3.3 Hz, J_{gem} = 19.1 Hz, 1H, $H_{7\beta}$), 3.27 (dd, J_{gem} = 12.5 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.44 (d, J_{gem} = 12.5 Hz, 1H, $H_{3\alpha}$), 3.66 (d, $J_{4,3\beta}$ = 6.0 Hz, H_4), 3.81 (ddd, $J_{7\alpha,1}$ = 8.4 Hz, $J_{7\alpha,5}$ = 4.0 Hz, J_{gem} = 19.1 Hz, 1H, $H_{7\alpha}$), 4.27 (ddd, $J_{1,5}$ = 8.0 Hz, $J_{1,7\alpha}$ = 8.4 Hz, $J_{1,7\beta}$ = 3.4 Hz, 1H, H_1), 4.66 (m, 1H, H_5). ^{13}C NMR (125 MHz, D_2O): δ 36.3, 38.3, 51.3, 57.4, 72.6, 175.9, 214.8.

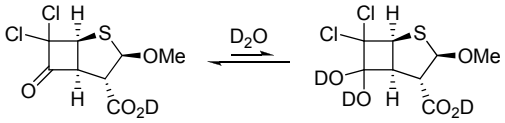
Hydrate Formation with Cyclobutanone 152α



152α *hydrate*

The 3α-methoxy dichlorocyclobutanone **152α** (23.9 mg, 0.088 mmol) was dissolved in acetone-d_6 (150 μL) and combined with D_2O (450 μL). ^{1}H NMR of the mixture within 5 minutes showed >98% as the signals corresponding to the ketone could not be identified. **Hydrate**: ^{1}H NMR (500 MHz, D_2O): δ 3.33 (s, 3H, OCH_3), 3.75 (dd, $J_{4,3\beta}$ = 4.4 Hz, $J_{4,5}$ = 9.5 Hz, 1H, H_4), 3.83 (dd, $J_{5,1}$ = 9.4 Hz, $J_{5,4}$ = 9.5 Hz, 1H, H_5), 4.44 (d, $J_{1,5}$ = 9.4 Hz, 1H, H_1), 5.52 (d, $J_{3\beta,4}$ = 4.4 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, D_2O): δ 56.4, 57.0, 57.4, 59.3, 95.3, 97.1, 97.9, 173.0.

Hydrate Formation with Cyclobutanone 152β

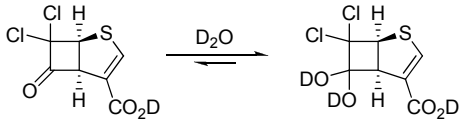


152β *hydrate*

The 3β-methoxy dichlorocyclobutanone **152β** (15 mg, 0.055 mmol) was dissolved in acetone-d_6 (150 μL) before the addition of D_2O (450 μL), as done previously with **152α** (see above). However, the addition of D_2O induced a large amount of precipitation, and additional acetone-d_6 (300 μL) was required to dissolve most of the solid. Integration of the ^{1}H NMR spectrum of the mixture within 10 minutes indicated approximately 6.5% hydration of the ketone (ketone:hydrate = 93.5:6.5) and this ratio did not change over 24 h. **Ketone**: ^{1}H NMR (500 MHz, D_2O): δ 3.37 (s, 3H, OCH_3), 3.91 (s, 1H, H_4), 4.81 (d, $J_{1,5}$ = 8.6 Hz, H_1), 5.17 (d, $J_{5,1}$ = 8.6 Hz, H_5), 5.61 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, D_2O): δ 57.2, 59.2, 60.3, 66.5, 91.5, 96.8, 171.9, 196.4. **Hydrate**: ^{1}H NMR (500 MHz, D_2O): δ 3.52 (s, 3H, OCH_3), 3.79 (br s, 1H, H_4), 4.23 (dd, $J_{5,1}$ = 9.6 Hz, $J_{5,4}$ = 1.8 Hz, H_5), 4.56 (d, $J_{1,5}$ = 9.6 Hz,

H_1), 5.75 (d, $J_{3\alpha,4}$ = 1.2 Hz, $H_{3\alpha}$). ^{13}C NMR (125 MHz, D_2O): δ 56.9, 57.7, 61.0, 62.5, 98.5.[689]

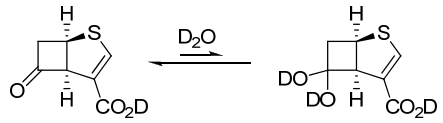
Hydrate Formation with Cyclobutanone 153



153 *hydrate*

The unsaturated dichlorocyclobutanone **153** (21.8 mg, 0.0912 mmol) was dissolved in acetone-d_6 (250 μL) and combined with D_2O (750 μL). ^{1}H NMR of the mixture within 5 minutes showed 93% hydration and the ketone:hydrate (93.3:6.7) ratio did not change over 12 h. **Ketone**: ^{1}H NMR (500 MHz, D_2O): δ 5.21 (d, $J_{5,1}$ = 10.1 Hz, 1H, H_5), 5.56 (dd, $J_{1,3}$ = 1.8 Hz, $J_{1,5}$ = 10.1 Hz, 1H, H_1), 7.73 (d, $J_{3,1}$ = 1.8 Hz, 1H, H_3). ^{13}C NMR (125 MHz, D_2O): δ 60.5, 72.5, 94.7, 123.0, 149.0, 165.4, 192.4. **Hydrate**: ^{1}H NMR (500 MHz, D_2O): δ 4.45 (dd, $J_{1,3}$ = 1.4 Hz, $J_{1,5}$ = 10.1 Hz, 3H, H_5), 4.88 (d, $J_{1,5}$ = 10.1 Hz, 1H, H_1), 7.63 (d, $J_{3,1}$ = 1.4 Hz, 1H, H_3). ^{13}C NMR (125 MHz, D_2O): δ 61.6, 62.7, 97.6, 101.5, 126.6, 149.9, 166.9.

Hydrate Formation with Cyclobutanone 156



156 *hydrate*

The unsaturated dichlorocyclobutanone **156** (6.2 mg, 0.041 mmol) was dissolved in acetone-d_6 (350 μL) and combined with D_2O (700 μL). The cyclobutanone hydrate could not be detected by ^{1}H NMR, even after 48 h in solution. Given the relatively poor solubility of **156** in this solvent mixture and the detection limit of ^{1}H NMR in this experiment, we conclude that the extent of hydration is less than 2%. **Ketone**: ^{1}H NMR (500 MHz, D_2O): δ 3.42 (ddd, $J_{7\beta,1}$ = 8.7 Hz, $J_{7\beta,5}$ = 3.4 Hz, J_{gem} = 19.1 Hz,1H, $H_{7\beta}$), 3.92 (ddd, $J_{7\alpha,1}$ = 8.4 Hz, $J_{7\alpha,5}$ = 5.3 Hz, J_{gem} = 19.1 Hz,1H, $H_{7\alpha}$), 4.39–4.45 (m, 1H, H_1), 5.14–5.18 (m, 1H, H_5), 7.71 (s, 1H, H_3). ^{13}C NMR (125 MHz, D_2O): δ 38.7, 61.6, 76.4, 122.0, 148.1, 166.1, 206.9.

5.5 Cyclobutanone Hemiketal Formation

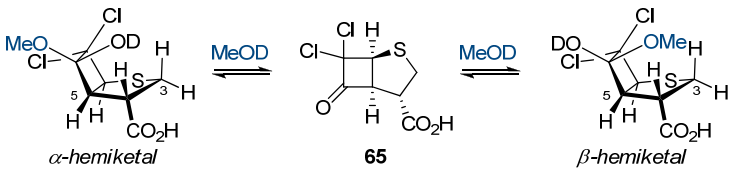
General

A neat sample of the cyclobutanone (typically 20–25 mg) was dissolved in neat methanol-d_4 (1 g) with swirling. Between periodic NMR experiments, the solution remained in the NMR tube at ambient temperature. ^{1}H NMR was done at 500 MHz, ^{13}C NMR at 125 MHz, and protons were assigned based on COSY, HMQC, and HMBC experiments.

Assignment of signals to protons of the α and β hemiketals was based on reasoning that nucleophilic attack by the alcohol on the carbonyl is kinetically much faster from the *exo* (α) face of the [3.2.0] bicycle, and would give the thermodynamically more stable α-hemiketal. Peaks of the first hemiketal to form were therefore assigned to the α-hemiketal and the peaks of the second hemiketal to form were assigned to the β-hemiketal. As expected, the ratio of α-hemiketal:β-hemiketal was >1 at equilibrium with all cyclobutanones tested. NOE and NOESY experiments were performed, but could not give an unambiguous assignment of any protons to either of the hemiketals. Ketone:α:β ratios were calculated using a weighted average of the integrated values of the most resolved peaks. In many experiments, quaternary carbons C6 and C7 could not be observed, and in some cases (in which the proportion of a compound was less than 5% of the mixture) none of the carbons could be observed.

After equilibrium had been reached, the solvent was removed in vacuo to give the ketone in all cases. For several substrates the β-hemiketal persisted, but removal of methanol could be facilitated by washing a solution of the compound in CH_2Cl_2 with water, followed by removal of the solvent under reduced pressure. With the carboxylic acids **65** and **111**, hemiketal formation was also found to be fully reversible, but after two weeks or more in methanol-d_4 a significant amount of esterification was observed (ca. 30% after two weeks by ^{1}H NMR).

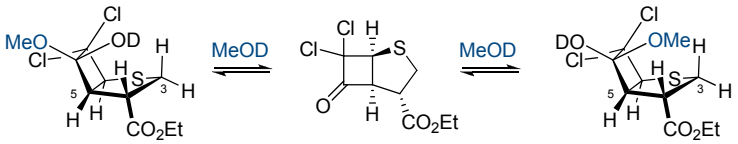
Hemiketal Formation with Cyclobutanone 65



The cyclobutanone **65** (20.6 mg, 0.085 mmol) was dissolved in methanol-d_4 (1 g). 7 d were

required for the system to reach equilibrium and the ketone:α:β ratio was 12:65:24. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 3.00 (dd, $J_{3\beta,4}$ = 5.9 Hz, J_{gem} = 12.3 Hz, 1H, $H_{3\beta}$), 3.42 (d, J_{gem} = 12.3 Hz, 1H, $H_{3\alpha}$), 3.75 (d, $J_{4,3\beta}$ = 5.9 Hz, 1H, H_4), 4.58 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 5.10 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5). ^{13}C NMR (125 MHz, CD_3OD): δ 35.2, 50.0, 58.7, 67.7, 172.9, 195.1.[690] **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 3.30 (m, 1H, $H_{3\alpha}$), 3.47–3.55 (m, 2H, $H_{3\beta}$ and H_4), 3.96 (d, $J_{5,1}$ = 8.9 Hz, H_5), 4.29 (d, $J_{1,5}$ = 8.9 Hz, H_1). ^{13}C NMR (125 MHz, CD_3OD): δ 37.3, 48.5, 55.4, 61.3, 93.9, 100.2, 174.2. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 3.30–3.35 (m, 1H, $H_{3\beta}$), 3.4–3.5 (m, 2H, $H_{3\alpha}$ and H_4), 3.89 (dd, $J_{5,4}$ = 2.6 Hz, $J_{5,1}$ = 9.2 Hz, 1H, H_5), 4.27 (d, $J_{1,5}$ = 9.2 Hz, 1H, H_1). ^{13}C NMR (500 MHz, CD_3OD): δ 37.6, 48.0, 58.6, 60.7, 94.9, 99.2, 174.3.

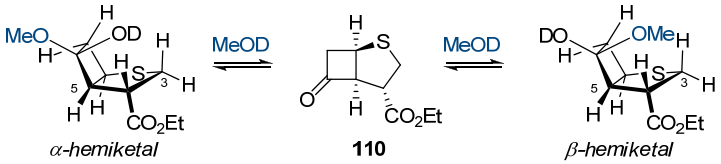
Hemiketal Formation with Cyclobutanone 84



α-hemiketal **84** *β-hemiketal*

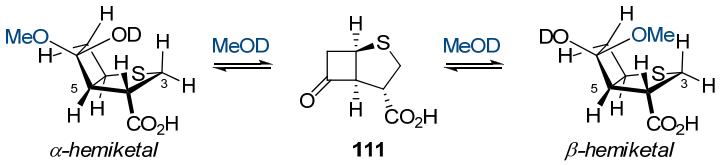
The cyclobutanone **84** (20.0 mg, 0.074 mmol) was dissolved in methanol-d_4 (1 g). 12 d were required for the system to reach equilibrium and the ketone:α:β ratio was 9:67:25. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.05 (dd, J_{gem} = 12.4 Hz, $J_{3\beta,4}$ = 5.8 Hz, 1H, $H_{3\beta}$), 3.46 (d, J_{gem} = 12.4 Hz, 1H, $H_{3\alpha}$), 3.78 (d, $J_{3\beta,4}$ = 5.8 Hz, 1H, H_4), 4.17 (app. q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.64 (d, $J_{1,5}$ = 8.4 Hz, 1H, H_1), 5.16 (d, $J_{5,1}$ = 8.4 Hz, 1H, H_5). ^{13}C NMR (125 MHz, CD_3OD): δ 13.0, 35.1, 50.1, 58.7, 61.6, 67.5, 170.2, 195.1.[690] **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.26 (m, 3H, $CO_2CH_2CH_3$), 3.29 (d, J_{gem} = 10.8 Hz, 1H, $H_{3\alpha}$), 3.50 (dd, J_{gem} = 10.8 Hz, $J_{3\beta,4}$ = 6.6 Hz, 1H, $H_{3\beta}$), 3.54 (d, $J_{4,3\beta}$ = 6.6 Hz, 1H, H_4), 3.96 (d, $J_{5,1}$ = 8.9 Hz, 1H, H_5), 4.10–4.15 (m, 2H, $CO_2CH_2CH_3$), 4.29 (d, $J_{1,5}$ = 8.9 Hz, 1H, H_1). ^{13}C NMR (500 MHz, CD_3OD): δ 13.0, 37.3, 48.5, 55.1, 60.7, 61.0, 93.9, 100.2, 174.2. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.26 (m, 3H, $CO_2CH_2CH_3$), 3.29 (dd, J_{gem} = 11.5 Hz, $J_{3\alpha,4}$ = 2.7 Hz, 1H, $H_{3\alpha}$), 3.36 (dd, J_{gem} = 11.5 Hz, $J_{3\beta,4}$ = 6.7 Hz, 1H, $H_{3\beta}$), 3.56 (app. dt, $J_{4,3\alpha}$ = 2.7 Hz, $J_{4,3\beta}$ = 6.7 Hz, $J_{4,5}$ = 2.7 Hz, 1H, H_4), 3.88 (dd, $J_{5,1}$ = 9.3 Hz, $J_{5,4}$ = 2.7 Hz, 1H, H_5), 4.10–4.15 (m, 2H, $CO_2CH_2CH_3$), 4.27 (d, $J_{1,5}$ = 9.3 Hz, 1H, H_1). ^{13}C NMR (125 MHz, CD_3OD): δ 13.0, 37.6, 48.4, 55.1, 58.7, 60.0, 60.7, 95.5, 99.2, 172.5.

Hemiketal Formation with Cyclobutanone 110



The cyclobutanone **110** (25.5 mg, 0.127 mmol) was dissolved in methanol-d_4 (1 g). Equilibrium was reached in 7 d and the ketone:α:β ratio was 81:12:6.5. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.29 (t, 3H, $CO_2CH_2CH_3$), 2.90 (app. dt, $J_{7\beta,5}$ = 3.3 Hz, $J_{7\beta,1}$ = 3.3 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\beta}$), 3.23 (dd, J_{gem} = 12.2 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.42 (d, J_{gem} = 12.2 Hz, 1H, $H_{3\alpha}$), 3.58 (d, $J_{4,3\beta}$ = 6.0 Hz, 1H, H_4), 3.70 (ddd, $J_{7\alpha,1}$ = 8.3 Hz, $J_{7\alpha,5}$ = 3.6 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\alpha}$), 4.12–4.21 (m, 3H, H_1 and $CO_2CH_2CH_3$), 4.65 (app. ddd, $J_{5,1}$ = 7.6 Hz, $J_{5,7\alpha}$ = 3.6 Hz, $J_{5,7\beta}$ = 3.3 Hz, 1H, H_5). ^{13}C NMR (125 MHz, CD_3OD): δ 13.0, 34.7, 36.8, 50.0, 55.9, 61.2, 71.5, 171.3, 208.7. **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.28 (t, 3H, $CO_2CH_2CH_3$), 2.02 (dd, $J_{7\beta,1}$ = 4.3 Hz, J_{gem} = 13.5 Hz, 1H, $H_{7\beta}$), 2.83 (ddd, $J_{7\alpha,1}$ = 7.8 Hz, $J_{7\alpha,5}$ = 3.6 Hz, J_{gem} = 13.5 Hz, 1H, $H_{7\alpha}$), 3.37 (dd, J_{gem} = 11.4 Hz, $J_{3\alpha,4}$ = 2.0 Hz, 1H, $H_{3\alpha}$), 3.43 (dd, J_{gem} = 11.4 Hz, $J_{3\beta,4}$ = 6 Hz, 1H, $H_{3\beta}$), 3.47 (dd, $J_{4,3\beta}$ = 6 Hz, $J_{4,3\alpha}$ = 2.0 Hz, 1H, H_4), 3.72–3.77 (m, 2H, H_1 and H_5), 4.15–4.19 (m, 2H, $CO_2CH_2CH_3$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 35.6, 38.0, 44.2, 49.0, 57.4, 60.7, 96.4, 173.1. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.27 (t, 3H, $CO_2CH_2CH_3$), 2.17 (dd, $J_{7\beta,1}$ = 6.0 Hz, J_{gem} = 13.4 Hz, 1H, $H_{7\beta}$), 2.62 (ddd, $J_{7\alpha,1}$ = 8.4 Hz, $J_{7\alpha,5}$ = 3.0 Hz, J_{gem} = 13.4 Hz, 1H, $H_{7\alpha}$), 3.31 (dd, J_{gem} = 11.9 Hz, $J_{3\beta,4}$ = 6.0 Hz, 1H, $H_{3\beta}$), 3.36 (dd, J_{gem} = 11.9 Hz, $J_{3\alpha,4}$ = 2.4 Hz, 1H, $H_{3\alpha}$), 3.50 (app. dt, $J_{4,3\beta}$ = 6.0 Hz, $J_{4,3\alpha}$ = 2.4 Hz, $J_{4,5}$ = 2 Hz, 1H, H_4), 3.70–3.74 (m, 1H, H_5), 3.80 (app. dt, $J_{1,5}$ = 8 Hz, $J_{1,7\alpha}$ = 8.4 Hz, $J_{1,7\beta}$ = 6.0 Hz, 1H, H_1), 4.15–4.19 (m, 2H, $CO_2CH_2CH_3$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 35.5, 37.7, 43.7, 48.5, 59.9, 60.7, 95.8, 173.2.

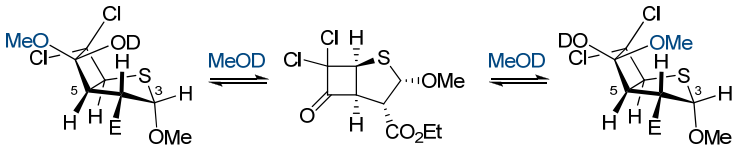
Hemiketal Formation with Cyclobutanone 111



The cyclobutanone **111** (18.3 mg, 0.1063 mmol) was dissolved in methanol-d_4 (1 g). Less than 12 h were required for the system to reach equilibrium and the ketone:α:β ratio was 76:14.5:9.2. **Ketone**:

^{1}H NMR (500 MHz, CD_3OD): δ 2.89 (dt, $J_{7\beta,5}$ = 3.3 Hz, $J_{7\beta,1}$ = 3.4 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\beta}$), 3.22 (dd, J_{gem} = 12.1 Hz, $J_{3\beta,4}$ = 6.1 Hz, 1H, $H_{3\beta}$), 3.44 (d, J_{gem} = 12.1 Hz, 1H, $H_{3\alpha}$), 3.55 (d, $J_{4,3\beta}$ = 6.1 Hz, 1H, H_4), 3.69 (ddd, $J_{7\alpha,1}$ = 8.3 Hz, $J_{7\alpha,5}$ = 3.6 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\alpha}$), 4.17 (ddd, $J_{1,5}$ = 8.0 Hz, $J_{1,7\alpha}$ = 8.3 Hz, $J_{1,7\beta}$ = 3.4 Hz, 1H, H_1), 4.64 (app. dt, $J_{5,1}$ = 8.0 Hz, $J_{5,7\alpha}$ = 3.6 Hz, $J_{5,7\beta}$ = 3.3 Hz, 1H, H_5). ^{13}C NMR (125 MHz, CD_3OD): δ 34.8, 36.8, 50.0, 55.8, 71.7, 173.2, 209.1. **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 2.02 (dd, $J_{7\beta,1}$ = 4.0 Hz, J_{gem} = 13.5 Hz, 1H, $H_{7\beta}$), 2.82 (ddd, $J_{7\alpha,1}$ = 7.4 Hz, $J_{7\alpha,5}$ = 3.9 Hz, J_{gem} = 13.5 Hz, 1H, $H_{7\alpha}$), 3.35–3.45 (m, 3H, H_4 and $H_{3\alpha}$ and $H_{3\beta}$), 3.73–3.76 (m, 2H, H_1 and H_5). ^{13}C NMR (125 MHz, CD_3OD): δ 35.6, 38.1, 44.2, 48.9, 57.5, 96.4, 175.1. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 2.17 (dd, $J_{7\beta,1}$ = 6.0 Hz, J_{gem} = 13.3 Hz, 1H, $H_{7\beta}$), 2.62 (ddd, $J_{7\alpha,1}$ = 8.4 Hz, $J_{7\alpha,5}$ = 3.0 Hz, J_{gem} = 13.3 Hz, 1H, $H_{7\alpha}$), 3.30 (dd, J_{gem} = 11.7 Hz, $J_{3\beta,4}$ = 6.1 Hz, 1H, $H_{3\beta}$), 3.41 (m, 1H, $H_{3\alpha}$), 3.48 (app. dt, $J_{4,3\beta}$ = 6.1 Hz, $J_{4,3\alpha}$ = 2 Hz, $J_{4,5}$ = 2 Hz, 1H, H_4), 3.72–3.76 (m, 1H, H_5), 3.81 (app. dt, $J_{1,5}$ = 8 Hz, $J_{1,7\alpha}$ = 8 Hz, $J_{1,7\beta}$ = 6 Hz, 1H, H_1). ^{13}C NMR (125 MHz, CD_3OD): δ 34.8, 37.8, 43.7, 48.4, 60.0, 95.4, 175.2

Hemiketal Formation with Cyclobutanone 121α

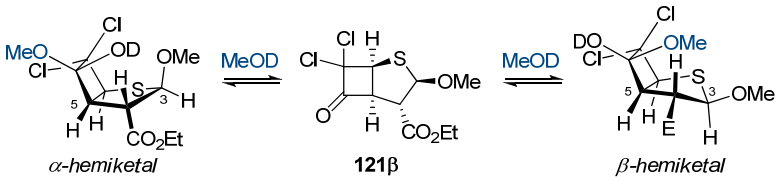


α-hemiketal **121α** *β-hemiketal*

The 3α-methoxycyclobutanone **121α** (13.5 mg, 0.045 mmol) was dissolved in methanol-d_4 (0.6 g). Equilibrium was reached within 7 d and the ketone:α:β ratio was 1.6:54:44. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.28 (t, 3H, $CO_2CH_2CH_3$), 3.34 (s, 3H, OCH_3), 3.84 (dd, $J_{4,3\beta}$ = 5.0 Hz, $J_{4,5}$ = 5.7 Hz, 1H, H_4), 4.15–4.30 (m, 2H, $CO_2CH_2CH_3$), 4.74 (d, $J_{1,5}$ = 8.3 Hz, 1H, H_1), 5.15 (dd, $J_{5,1}$ = 8.3 Hz, $J_{5,4}$ = 5.7 Hz, 1H, H_5), 5.49 (d, $J_{3\beta,4}$ = 5.0 Hz, 1H, $H_{3\beta}$). **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.28 (t, 3H, $CO_2CH_2CH_3$), 3.27 (s, 3H, OCH_3), 3.69 (dd, $J_{4,3\beta}$ = 4.4, $J_{4,5}$ = 9.1, 1H, H_4), 3.91 (dd, $J_{5,1}$ = 9.7, $J_{5,4}$ = 9.1, 1H, H_5), 4.15 (B of ABX_3, J_{AB} = 10.7 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.23 (A of ABX_3, J_{AB} = 10.7 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.31 (d, $J_{1,5}$ = 9.7, 1H, H_1), 5.39 (d, $J_{3\beta,4}$ = 4.4, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 52.2, 55.1, 55.2, 58.9, 60.7, 93.1, 96.9, 99.2, 169.1. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.27 (t, 3H, $CO_2CH_2CH_3$), 3.25 (s, 3H, OCH_3), 3.63 (dd, $J_{4,3\beta}$ = 4.4, $J_{4,5}$ = 10.5, 1H, H_4), 3.79 (dd, $J_{5,1}$ = 9.6, $J_{5,4}$ = 10.5, 1H, H_5), 4.15 (B

of ABX$_3$, J_{AB} = 10.7 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.23 (A of ABX$_3$, J_{AB} = 10.7 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.28 (d, $J_{1,5}$ = 9.6, 1H, H_1), 5.40 (d, $J_{3\beta,4}$ = 4.4, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 55.1, 55.3, 55.9, 58.2, 60.8, 93.9, 97.0, 97.8, 169.0.

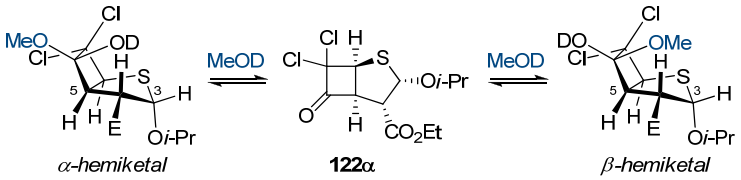
Hemiketal Formation with Cyclobutanone 121β



α-hemiketal 121β β-hemiketal

The 3β-methoxycyclobutanone **121β** (18.0 mg, 0.060 mmol) was dissolved in methanol-d_4 (1 g). Equilibrium was reached in 14 d and the ketone:α:β ratio was 85:12:2.6. **Ketone**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.34 (s, 3H, OCH_3), 3.88 (s, 1H, H_4), 4.20 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.72 (d, $J_{1,5}$ = 8.6 Hz, 1H, H_1) 5.10 (d, $J_{5,1}$ = 8.6 Hz, 1H, H_5), 5.54 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.9, 55.1, 57.8, 59.1, 61.7, 65.0, 90.4, 95.4, 168.1, 192.7. **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.30 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.49 (s, 3H, OCH_3), 3.72 (app t., $J_{4,3\alpha}$ = 1.5 Hz, $J_{4,5}$ = 1.5 Hz, 1H, H_4), 4.15 (dd, $J_{5,1}$ = 9.7 Hz, $J_{5,4}$ = 1.5 Hz, 1H, H_5), 4.20 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.47 (d, $J_{1,5}$ = 9.7 Hz, 1H, H_1), 5.70 (d, $J_{3\alpha,4}$ = 1.5 Hz, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.9, 55.5, 55.6, 57.6, 61.6 (2C), 97.2, 169.5.[691] **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.30 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.40 (s, 3H, OCH_3), 3.56 (dd, $J_{5,1}$ = 9.7 Hz, $J_{5,4}$ = 8.6 Hz, 1H, H_5), 3.64 (dd, $J_{4,3\alpha}$ = 7.0 Hz, $J_{4,5}$ = 8.6 Hz, 1H, H_4), 4.20 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.28 (d, $J_{1,5}$ = 9.7 Hz, 1H, H_1), 5.68 (d, $J_{3\alpha,4}$ = 7.0 Hz, 1H, $H_{3\alpha}$)

Hemiketal Formation with Cyclobutanone 122α

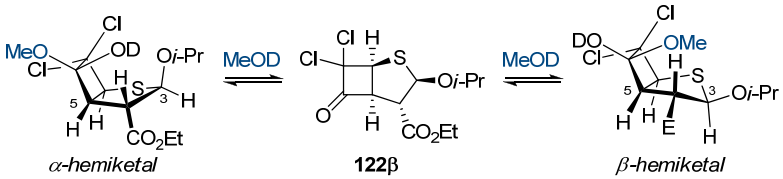


α-hemiketal 122α β-hemiketal

The 3α-isopropoxycyclobutanone **122α** (8.9 mg, 0.027 mmol) was dissolved in methanol-d_4 (0.4 g). Equilibrium was reached within 7 d and the ketone:α:β ratio was 2:51:47 by the integration of peaks in the 500 MHz ^{1}H NMR. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 4.76 (d, $J_{1,5}$ = 8.1 Hz, 1H, H_1), 5.17 (dd, $J_{5,1}$ = 8.1 Hz, $J_{5,4}$ = 5.4 Hz, 1H, H_5), 5.73 (d, $J_{3\beta,4}$ = 4.5 Hz, 1H, $H_{3\beta}$).[692] **α-Hemiketal**:

^{1}H NMR (500 MHz, CD_3OD): δ 1.09 (d, J = 6.1 Hz, 3H, one of CHCH_3), 1.13 (d, J = 6.1 Hz, 3H, one of CHCH_3), 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.68 (dd, $J_{4,3\beta}$ = 4.5 Hz, $J_{4,5}$ = 8.9 Hz, 1H, H_4), 3.76 (sept., J = 6.1 Hz, 1H, CHMe$_2$), 3.93 (app. t, $J_{5,1}$ = 9.7 Hz, $J_{5,4}$ = 8.9 Hz, 1H, H_5), 4.17 (m, 2H, $CO_2CH_2CH_3$), 4.34 (d, $J_{1,5}$ = 9.7 Hz, 1H, H_1), 5.64 (d, $J_{3\beta,4}$ = 4.5 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.2, 19.4, 21.8, 52.5, 55.2, 58.9, 60.7, 70.3, 92.8, 93.1, 99.3, 169.2. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.10 (d, J = 6.1 Hz, 3H, one of CHCH_3), 1.12 (d, J = 6.1 Hz, 3H, one of CHCH_3), 1.29 (m, 3H, $CO_2CH_2CH_3$), 3.62 (dd, $J_{4,3\beta}$ = 4.5 Hz, $J_{4,5}$ = 10.4 Hz, 1H, H_4), 3.72 (sept., J = 6.1 Hz, 1H, CHMe$_2$), 3.81 (dd, $J_{5,1}$ = 9.7 Hz, $J_{5,4}$ = 10.4 Hz, 1H, H_5), 4.15–4.19 (m, 2H, $CO_2CH_2CH_3$), 4.31 (d, $J_{1,5}$ = 9.7 Hz, 1H, H_1), 5.65 (d, $J_{3\beta,4}$ = 4.5 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.2, 19.4, 21.7, 55.4, 56.2, 58.2, 60.7, 70.4, 92.9, 94.1, 97.9, 169.1.

Hemiketal Formation with Cyclobutanone 122β

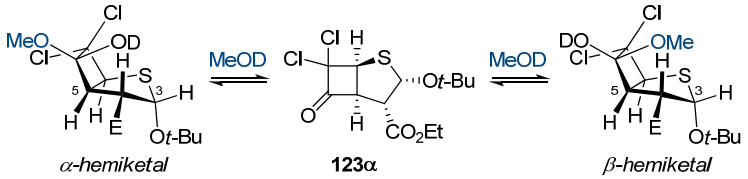


α-hemiketal **122β** *β-hemiketal*

The 3β-isopropoxycyclobutanone **122β** (21.7 mg, 0.066 mmol) was dissolved in methanol-d_4 (1 g). Equilibrium was reached in 14 d and the ketone:α:β ratio was 77:19:4. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.13 (d, J = 6.1 Hz, 1H, one of CH(CH_3)$_2$), 1.15 (d, J = 6.1 Hz, 1H, one of CH(CH_3)$_2$), 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.81 (s, 1H, H_4), 4.00 (sept., J = 6.1 Hz, 1H, CHMe$_2$), 4.20 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.71 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 5.12 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5), 5.78 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 12.9, 18.6, 21.4, 57.9, 59.3, 61.7, 65.4, 69.2, 90.1, 90.4, 168.3, 192.4. **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.15 (d, J = 6.2 Hz, 3H, one of CH(CH_3)$_2$), 1.24 (d, J = 6.2 Hz, 3H, one of CH(CH_3)$_2$), 1.29 (t, 3H, J = 7.1 Hz, $CO_2CH_2CH_3$), 3.65 (dd, $J_{4,3\alpha}$ = 1.5 Hz, $J_{4,5}$ = 1.8 Hz, 1H, H_4), 4.10 (sept., J = 6.2 Hz, 1H, CHMe$_2$), 4.13 (dd, $J_{5,1}$ = 9.6 Hz, $J_{5,4}$ = 1.8 Hz, 1H, H_5), 4.19 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.45 (d, $J_{1,5}$ = 9.6 Hz, 1H, H_1), 5.90 (d, $J_{3\alpha,4}$ = 1.5 Hz, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 12.9, 18.9, 21.3, 55.8, 57.4, 61.5, 61.6, 71.2, 92.5, 95.6, 100.8, 169.6. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.10 (d, J = 6.1 Hz, one of CH(CH_3)$_2$), 1.19 (d, J = 6.1 Hz, one of CH(CH_3)$_2$), 1.29 (m, 3H, $CO_2CH_2CH_3$), 3.42 (app. t, $J_{5,1}$ = 9.7 Hz, $J_{5,4}$ = 9.7 Hz, 1H, H_5), 3.57 (dd, $J_{4,3\alpha}$ = 9.6 Hz, $J_{4,5}$ = 9.7 Hz, 1H, H_4), 4.18 (m, 1H, H_1), 4.2

(m, 2H, $CO_2CH_2CH_3$), 5.68 (d, $J_{3\alpha,4}$ = 9.6 Hz, 1H, $H_{3\alpha}$).

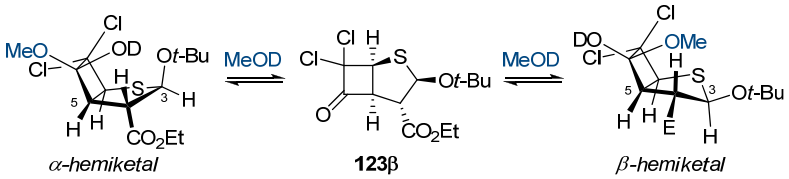
Hemiketal Formation with Cyclobutanone 123α



α-hemiketal **123α** *β-hemiketal*

The 3α-*tert*-butoxycyclobutanone **123α** (10.8 mg, 0.031 mmol) was dissolved in methanol-d_4 (0.5 g). Equilibrium was reached within 7 d and the ketone:α:β ratio was 2.1:52:46. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.25 (s, 9H, $C(CH_3)_3$), 1.30 (t, 3H, J = 7.1 Hz, $CO_2CH_2CH_3$), 3.76 (dd, $J_{4,3\beta}$ = 5.1 Hz, $J_{4,5}$ = 5.7 Hz, 1H, H_4), 4.05–4.25 (m, 2H, $CO_2CH_2CH_3$), 4.51 (d, $J_{1,5}$ = 8.3 Hz, 1H, H_1), 5.13 (dd, $J_{5,1}$ = 8.3 Hz, $J_{5,4}$ = 5.7 Hz, 1H, H_5), 5.94 (d, $J_{3\beta,4}$ = 5.1 Hz, 1H, $H_{3\beta}$). **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.21 (s, 9H, $C(CH_3)_3$), 1.30 (t, 3H, $CO_2CH_2CH_3$), 3.60 (dd, $J_{4,3\beta}$ = 5.1 Hz, $J_{4,5}$ = 8.3 Hz, 1H, H_4), 3.91 (dd, $J_{5,1}$ = 9.6 Hz, $J_{5,4}$ = 8.3 Hz, 1H, H_5), 4.10 (B of ABX_3, J_{AB} = 10.8 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.22 (A of ABX_3, J_{AB} = 10.8 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.39 (d, $J_{1,5}$ = 9.6 Hz, 1H, H_1), 5.90 (d, $J_{3\beta,4}$ = 5.1 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 26.9, 53.0, 55.5, 59.2, 60.7, 75.6, 88.5, 93.6, 99.4, 169.2. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.19 (s, 9H, $C(CH_3)_3$), 1.29 (t, 3H, J = 7.1 Hz, $CO_2CH_2CH_3$), 3.61 (dd, $J_{4,3\beta}$ = 5.3, $J_{4,5}$ = 10.6 Hz, 1H, H_4), 3.85 (dd, $J_{5,1}$ = 9.6 Hz, $J_{5,4}$ = 10.6 Hz, 1H, H_5), 4.05 (B of ABX_3, J_{AB} = 10.8 Hz, J_{BX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.24 (A of ABX_3, J_{AB} = 10.8 Hz, J_{AX} = 7.1 Hz, 1H, one of $CO_2CH_2CH_3$), 4.36 (d, $J_{1,5}$ = 9.6 Hz, 1H, H_1), 5.85 (d, $J_{3\beta,4}$ = 5.3 Hz, 1H, $H_{3\beta}$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.1, 26.9, 55.8, 56.6, 58.4, 60.8, 75.6, 88.8, 94.2, 98.1, 169.5.

Hemiketal Formation with Cyclobutanone 123β

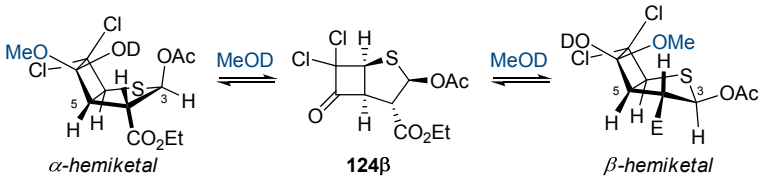


α-hemiketal **123β** *β-hemiketal*

The 3β-*tert*-butoxycyclobutanone **123β** (20.6 mg, 0.060 mmol) was dissolved in methanol-d_4 (1 g). Equilibrium was reached in 12 d and the ketone:α:β ratio was 60:26:14. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.25 (s, $C(CH_3)_3$), 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.68 (s, 1H, H_4), 4.20 (q, J = 7.1

Hz, 2H, $CO_2CH_2CH_3$), 4.68 (d, $J_{1,5}$ = 8.5 Hz, 1H, H_1), 5.07 (d, $J_{5,1}$ = 8.5 Hz, 1H, H_5), 5.89 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 26.9, 59.5, 59.6, 61.6, 65.4, 76.9, 86.9, 90.5, 168.4, 192.9. **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.29 (3H, $CO_2CH_2CH_3$), 1.32 (s, $C(CH_3)_3$), 3.58 (dd, $J_{4,3\alpha}$ = 3.6 Hz, $J_{4,5}$ = 3.7 Hz, 1H, H_4), 3.93 (dd, $J_{5,1}$ = 9.6 Hz, $J_{5,4}$ = 3.7 Hz, 1H, H_5), 4.20 (2H, $CO_2CH_2CH_3$), 4.35 (d, $J_{1,5}$ = 9.6 Hz, 1H, H_1), 5.95 (d, $J_{3\alpha,4}$ = 3.6 Hz, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 26.9, 55.8, 56.6, 60.9, 61.4, 77.6, 88.7, 95.2, 100.6, 170.3. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.19 (s, $C(CH_3)_3$), 1.29 (3H, $CO_2CH_2CH_3$), 3.37 (dd, $J_{5,1}$ = 9.8 Hz, $J_{5,4}$ = 10.3 Hz, 1H, H_5), 3.53 (dd, $J_{4,3\alpha}$ = 9.0 Hz, $J_{4,5}$ = 10.3 Hz, 1H, H_4), 4.16 (d, $J_{1,5}$ = 9.8 Hz, 1H, H_1), 4.20 (2H, $CO_2CH_2CH_3$), 5.79 (d, $J_{3\alpha,4}$ = 9.0 Hz, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 26.9, 54.9, 55.0, 58.4, 61.0, 75.3, 90.3, 94.2, 98.4, 172.2.

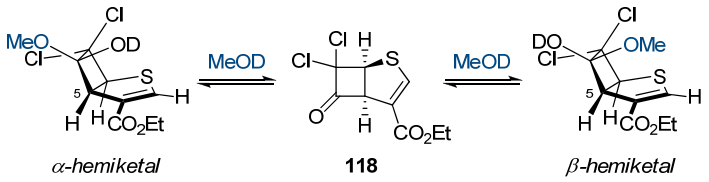
Hemiketal Formation with Cyclobutanone 124β



α-hemiketal **124β** *β-hemiketal*

The 3β-acetoxycyclobutanone **124β** (25.2 mg, 0.077 mmol) was dissolved in methanol-d_4 (1 g). Equilibrium was reached in 14 d and the ketone:α:β ratio was 70:18:12 by the average integration of the most resolved peaks in the ^{1}H NMR: H_1 and H_5 in this case. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 1.99 (s, 3H, $COCH_3$), 3.99 (s, 1H, H_4), 4.18 (app. q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.76 (d, $J_{1,5}$ = 8.6 Hz, 1H, H_1), 5.21 (d, $J_{5,1}$ = 8.6 Hz, 1H, H_5), 6.52 (s, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.0, 19.4, 56.2, 59.4, 62.1, 65.8, 85.9, 90.0, 167.5, 169.1, 193.1. **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.26 (t, 3H, $CO_2CH_2CH_3$), 2.10 (s, 3H, $COCH_3$), 3.79 (dd, $J_{4,3\alpha}$ = 3.3 Hz, $J_{4,5}$ = 4.1 Hz, 1H, H_4), 3.86 (dd, $J_{5,1}$ = 9.6 Hz, $J_{5,4}$ = 4.1 Hz, 1H, H_5), 4.2 (m, 2H, $CO_2CH_2CH_3$), 4.34 (d, $J_{1,5}$ = 9.6 Hz, 1H, H_1), 6.47 (d, $J_{3\alpha,4}$ = 3.3 Hz, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.0, 19.2, 53.9, 56.3, 61.5, 61.9, 88.6, 167.5, 170.7.[691] **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.26 (m, 3H, $CO_2CH_2CH_3$), 2.04 (s, 3H, $COCH_3$), 3.62 (dd, $J_{5,1}$ = 9.8 Hz, $J_{5,4}$ = 7.5 Hz, 1H, H_5), 3.82 (dd, $J_{4,3\alpha}$ = 5.6 Hz, $J_{4,5}$ = 7.5 Hz, 1H, H_4), 4.2 (m, 2H, $CO_2CH_2CH_3$), 4.31 (d, $J_{1,5}$ = 9.8 Hz, 1H, H_1), 6.51 (d, $J_{3\alpha,4}$ = 5.6 Hz, 1H, $H_{3\alpha}$). ^{13}C NMR (125 MHz, CD_3OD): δ 13.0, 19.2, 53.5, 58.5, 60.8, 61.5, 88.9, 167.5, 170.8.[691]

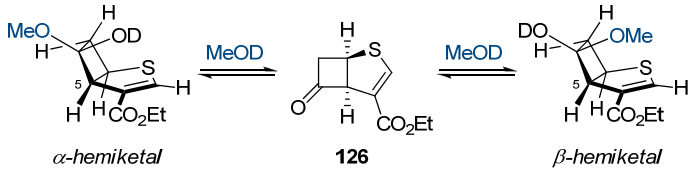
Hemiketal Formation with Cyclobutanone 118



α-hemiketal **118** *β-hemiketal*

The cyclobutanone **118** (21.2 mg, 0.079 mmol) was dissolved in methanol-d_4 (1 g). The system required 4 d to reach equilibrium and the ketone:α:β ratio was 4:62:34. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.30 (t, 3H, J = 7.1 Hz, $CO_2CH_2CH_3$), 4.20 (m, 2H, $CO_2CH_2CH_3$), 5.13 (d, $J_{1,5}$ = 10.0 Hz, 1H, H_1), 5.53 (dd, $J_{5,1}$ = 10.0 Hz, $J_{5,3}$ = 1.8 Hz, 1H, H_5), 7.64 (d, $J_{3,5}$ = 1.8 Hz, 1H, H_3). **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.30 (t, 3H, $CO_2CH_2CH_3$), 4.20 (m, 2H, $CO_2CH_2CH_3$), 4.45 (dd, $J_{5,1}$ = 10.2 Hz, $J_{5,3}$ = 1.1 Hz, 1H, H_5), 4.80 (d, $J_{1,5}$ = 10.2 Hz, 1H, H_1), 7.50 (d, $J_{3,5}$ = 1.1 Hz, 1H, H_3). ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 58.3, 60.2, 61.0, 95.1, 103.1, 125.3, 146.0, 162.9. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.30 (t, 3H, $CO_2CH_2CH_3$), 4.20 (m, 2H, $CO_2CH_2CH_3$), 4.35 (dd, $J_{5,1}$ = 10.0 Hz, $J_{5,3}$ = 1.5 Hz, 1H, H_5), 4.72 (d, $J_{1,5}$ = 10.0 Hz, 1H, H_1), 7.43 (d, $J_{3,5}$ = 1.5 Hz, 1H, H_3). ^{13}C NMR (125 MHz, CD_3OD): δ 13.1, 59.7, 60.2, 62.2 95.5, 102.6, 126.4, 145.0, 163.2

Hemiketal Formation with Cyclobutanone 126

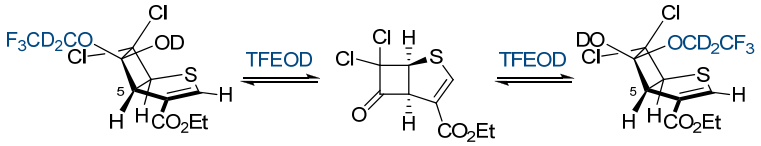


α-hemiketal **126** *β-hemiketal*

The cyclobutanone **126** (25.6 mg, 0.129 mmol) was dissolved in methanol-d_4 (1 g). 16 d were required for the system to reach equilibrium and the ketone:α:β ratio was 62:23:15. **Ketone**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.31 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.33 (ddd, $J_{7\beta,1}$ = 5.7 Hz, $J_{7\beta,5}$ = 3.3 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\beta}$), 3.83 (ddd, $J_{7\alpha,1}$ = 8.5 Hz, $J_{7\alpha,5}$ = 5.2 Hz, J_{gem} = 18.5 Hz, 1H, $H_{7\alpha}$), 4.18–4.24 (m, 2H, $CO_2CH_2CH_3$), 4.32 (app. dt, $J_{1,5}$ = 8.5 Hz, $J_{1,7\alpha}$ = 8.5 Hz, $J_{1,7\beta}$ = 5.7 Hz, 1H, H_1), 5.14 (dddd, $J_{5,1}$ = 8.5 Hz, $J_{5,3}$ = 1.7 Hz, $J_{5,7\alpha}$ = 5.2 Hz, $J_{5,7\beta}$ = 3.3 Hz, 1H, H_5), 7.62 (d, $J_{3,5}$ = 1.7 Hz, H_3). ^{13}C NMR (125 MHz, CD_3OD): δ 13.2, 37.2, 60.3, 60.4, 75.6, 120.9, 144.2, 162.3, 201.0. **α-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.30 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 2.40 (ddd, $J_{7\beta,1}$ = 6.5 Hz, $J_{7\beta,5}$ = 1.2 Hz, J_{gem} = 13.1 Hz, 1H, $H_{7\beta}$), 2.91 (ddd, $J_{7\alpha,1}$ = 8.4 Hz, $J_{7\alpha,5}$ = 3.5 Hz, J_{gem} = 13.1 Hz, 1H, $H_{7\alpha}$), 3.99 (app. dt, $J_{1,5}$ = 8 Hz, $J_{1,7\alpha}$ = 8.4 Hz, $J_{1,7\beta}$ = 6.5 Hz, 1H, H_1), 4.18–4.24 (m, 2H, $CO_2CH_2CH_3$),

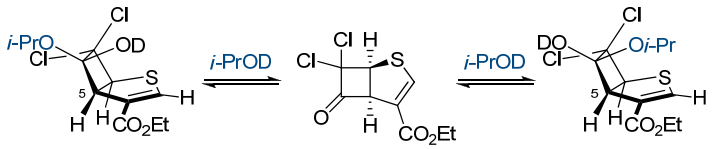
4.20–4.24 (m, 1H, H_5), 7.53 (s, 1H, H_3). ^{13}C NMR (125 MHz, CD_3OD): δ 13.2, 38.4, 47.2, 60.1, 102.7, 125.8, 144.3, 163.9. **β-Hemiketal**: ^{1}H NMR (500 MHz, CD_3OD): δ 1.26 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 3.46 (ddd, $J_{7\beta,1}$ = 6.9 Hz, $J_{7\beta,5}$ = 1.2 Hz, J_{gem} = 12.8 Hz, 1H, $H_{7\beta}$), 2.73 (ddd, $J_{7\alpha,1}$ = 8.2 Hz, $J_{7\alpha,5}$ = 3.6 Hz, J_{gem} = 12.8 Hz, 1H, $H_{7\alpha}$), 4.05 (app. dt, $J_{1,5}$ = 8 Hz, $J_{1,7\alpha}$ = 8.2 Hz, $J_{1,7\beta}$ = 6.9 Hz, 1H, H_1), 4.18–4.24 (m, 2H, $CO_2CH_2CH_3$), 4.30 (m, 1H, H_5), 7.46 (s, 1H, H_3). ^{13}C NMR (125 MHz, CD_3OD): δ 13.2, 38.6, 46.3, 60.0, 64.1, 102.2, 126.1, 143.6, 164.1

Hemiketal Formation with Cyclobutanone 118 in Trifluoroethanol-d_3.[693]



The dichlorocyclobutanone **118** (24.9 mg, 0.0932 mmol) was dissolved in trifluoroethanol-d_3 (1 g). After 8 d in solution the ketone:α:β ratio was 96.6:2.4:1.0. **Ketone**: ^{1}H NMR (500 MHz, CF_3CD_2OD): δ 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 4.20 (B of ABX_3, J_{BX} = 7.1 Hz, J_{BA} = 10.6 Hz, 2H, one of $CO_2CH_2CH_3$), 4.22 (A of ABX_3, J_{AX} = 7.1 Hz, J_{AB} = 10.6 Hz, 2H, one of $CO_2CH_2CH_3$), 4.91 (d, $J_{1,5}$ = 10.0 Hz, 1H, H_1), 5.37 (dd, $J_{5,1}$ = 10.0 Hz, $J_{5,3}$ = 1.8 Hz, 1H, H_5), 7.52 (d, $J_{3,5}$ = 1.8 Hz, 1H, H_3). ^{13}C NMR (125 MHz, CF_3CD_2OD): δ 14.4, 61.2, 63.7, 72.7, 95.3, 123.4, 148.2, 165.0, 192.0. **α-Hemiketal**: ^{1}H NMR (500 MHz, CF_3CD_2OD): δ 4.43 (dd, $J_{5,1}$ = 10.2 Hz, $J_{5,3}$ = 1.0 Hz, 1H, H_5), 4.76 (d, $J_{1,5}$ = 10.2 Hz, 1H, H_1).[694] **β-Hemiketal**: ^{1}H NMR (500 MHz, CF_3CD_2OD): δ 4.51 (d, $J_{5,1}$ = 9 Hz, 1H, H_5), 4.76 (d, $J_{1,5}$ = 9 Hz, 1H, H_1).[694]

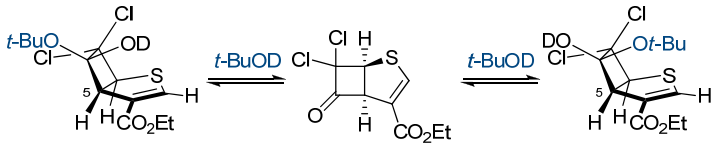
Hemiketal Formation with Cyclobutanone 118 in Isopropanol-d_8.[695]



The dichlorocyclobutanone **118** (25.0 mg, 0.0936 mmol) was dissolved in isopropanol-d_8 (0.8 mL). After 8 d in solution the ketone:α:β ratio was 63:33:3.8. **Ketone**: ^{1}H NMR (500 MHz, $(CD_3)_2CDOD$): δ 1.29 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 4.17 (B of ABX_3, J_{BX} = 7.3 Hz, J_{BA} = 10.8 Hz, 2H, one of $CO_2CH_2CH_3$), 4.22 (A of ABX_3, J_{AX} = 7.1 Hz, J_{AB} = 10.8 Hz, 2H, one of $CO_2CH_2CH_3$), 5.13 (d, $J_{1,5}$ =

10.1 Hz, 1H, H_1), 5.54 (dd, $J_{5,1}$ = 10.1 Hz, $J_{5,3}$ = 1.8 Hz, 1H, H_5), 7.58 (d, $J_{3,5}$ = 1.8 Hz, 1H, H_3). ^{13}C NMR (125 MHz, $(CD_3)_2CDOD$): δ 14.4, 60.0, 61.4, 72.2, 94.6, 122.7, 145.8, 161.7, 188.0. **α-Hemiketal**: ^{1}H NMR (500 MHz, $(CD_3)_2CDOD$): δ 1.26 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 4.12 (B of ABX_3, J_{BX} = 7.2 Hz, J_{BA} = 10.9 Hz, 2H, one of $CO_2CH_2CH_3$), 4.17 (A of ABX_3, J_{AX} = 7.2 Hz, J_{AB} = 10.9 Hz, 2H, one of $CO_2CH_2CH_3$), 4.36 (d, $J_{5,1}$ = 10.2 Hz, 1H, H_5), 4.66 (d, $J_{1,5}$ = 10.2 Hz, 1H, H_1), 7.37 (s, 1H, H_3). ^{13}C NMR (125 MHz, $(CD_3)_2CDOD$): δ 14.4, 60.1, 60.8, 63.3, 97, 104, 126.6, 146.1, 163.6. **β-Hemiketal**: ^{1}H NMR (500 MHz, $(CD_3)_2CDOD$): δ 1.26–1.28 (m, 3H, $CO_2CH_2CH_3$), 4.15–4.25 (m, 2H, $CO_2CH_2CH_3$), 4.36 (d, $J_{5,1}$ = 9.9 Hz, 1H, H_5), 4.57 (d, $J_{1,5}$ = 9.9 Hz, 1H, H_1), 7.27 (s, 1H, H_3).

Hemiketal Formation with Cyclobutanone 118 in *tert*-Butanol-d_{10}.[696]



α-hemiketal **118** *β-hemiketal*

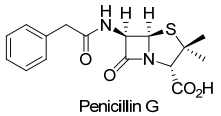
The dichlorocyclobutanone **118** (34.2 mg, 0.128 mmol) was dissolved in liquid *t*-butanol-d_{10} (1 g, mp: 11 °C). After 18 d in solution the ketone:α:β ratio was ≥94:4.7:≤0.9. **Ketone**: ^{1}H NMR (300 MHz, $(CD_3)_3COD$): δ 1.28 (t, J = 7.1 Hz, 3H, $CO_2CH_2CH_3$), 4.16 (B of ABX_3, J_{BX} = 7.1 Hz, J_{BA} = 10.9 Hz, 2H, one of $CO_2CH_2CH_3$), 4.22 (A of ABX_3, J_{AX} = 7.1 Hz, J_{AB} = 10.9 Hz, 2H, one of $CO_2CH_2CH_3$), 5.09 (d, $J_{1,5}$ = 10.0 Hz, 1H, H_1), 5.53 (d, $J_{5,1}$ = 10.0 Hz, 1H, H_5), 7.56 (s, 1H, H_3). **α-Hemiketal**: ^{1}H NMR (300 MHz, $(CD_3)_3COD$): δ 4.35 (d, $J_{5,1}$ = 10.0 Hz, 1H, H_5), 4.62 (d, $J_{1,5}$ = 10.0 Hz, 1H, H_1), 7.33 (s, 1H, H_3).[694] The β-hemiketal could not be observed by NMR.

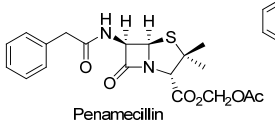
Appendix A

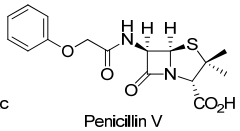
Structures of Selected β-Lactam Antibiotics [a]

Penicillins

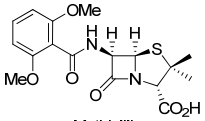
β-Lactamase-Sensitive Penicillins

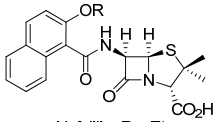

Penicillin G
(Benzyl penicillin)

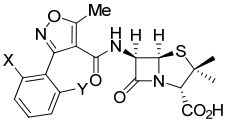

Penamecillin


Penicillin V
(Phenoxymethylpenicillin)

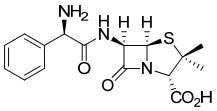
β-Lactamase-Resistant Penicillins

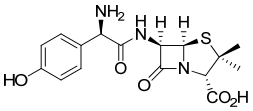

Methicillin

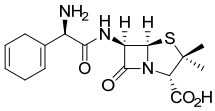

Nafcillin: R = Et
BRL 1437: R = *i*-Pr

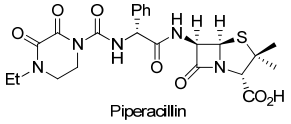

Oxacillin: X = H, Y = H
Cloxacillin: X = Cl, Y = H
Dicloxacillin: X = Cl, Y = Cl
Flucloxacillin: X = Cl, Y = F

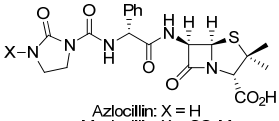
Expanded-Spectrum Penicillins [b]

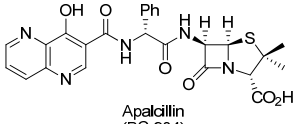

Ampicillin

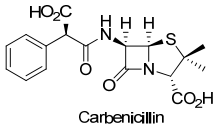

Amoxicillin

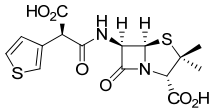

Epicillin

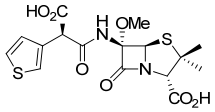

Piperacillin


Azlocillin: X = H
Mezlocillin: X = SO_2Me

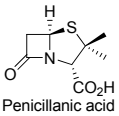

Apalcillin
(PC-904)

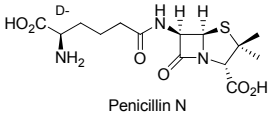

Carbenicillin

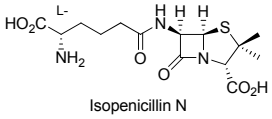

Ticarcillin

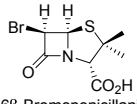

Temocillin (penamycin)

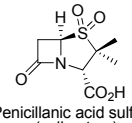
Other Penicillins of Interest

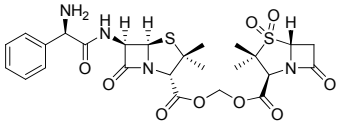

Penicillanic acid


Penicillin N


Isopenicillin N

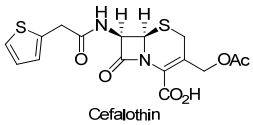

6β-Bromopenicillanic acid (Brobactam)

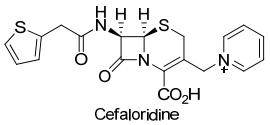

Penicillanic acid sulfone (sulbactam)

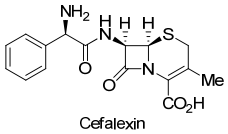

Sultamicillin

Cephalosporins

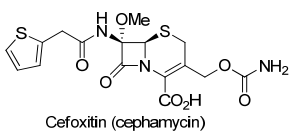
First Generation Cephalosporins

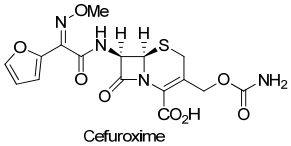

Cefalothin

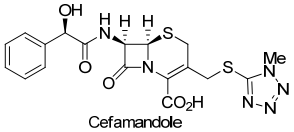

Cefaloridine


Cefalexin

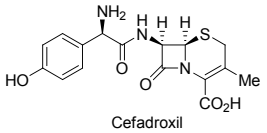
Second Generation Cephalosporins


Cefoxitin (cephamycin)

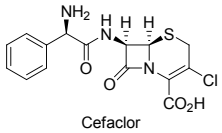

Cefuroxime


Cefamandole

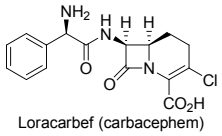
Second Generation Continued



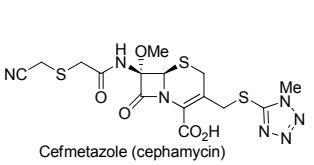
Cefadroxil



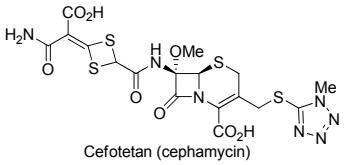
Cefaclor



Loracarbef (carbacephem)

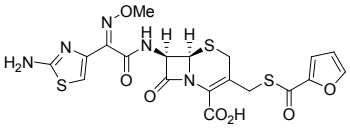


Cefmetazole (cephamycin)

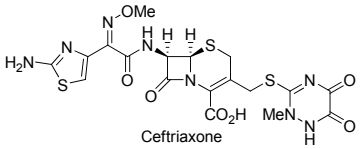


Cefotetan (cephamycin)

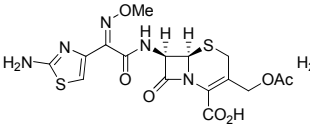
Third Generation Cephalosporins



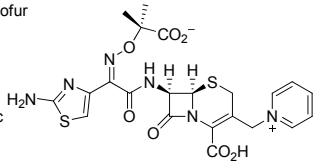
Ceftiofur



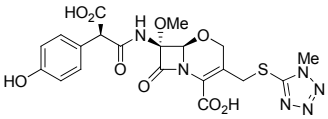
Ceftriaxone



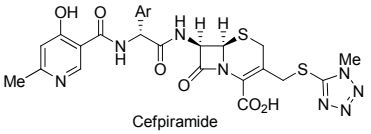
Cefotaxime



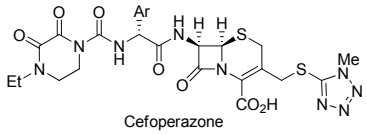
Ceftazidime



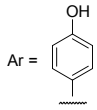
Moxalactam (oxacephamycin)



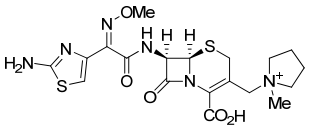
Cefpiramide



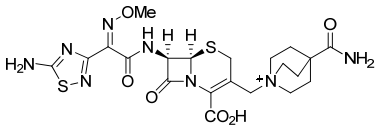
Cefoperazone



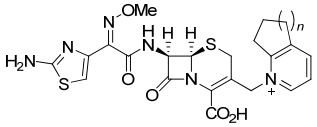
Fourth Generation Cephalosporins



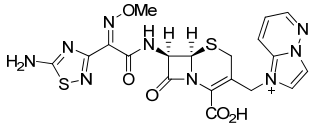
Cefepime



Cefclidin

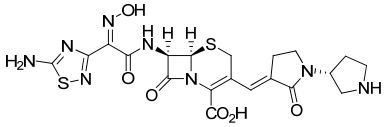


Cefpirome: $n = 1$
Cefiquinome: $n = 2$

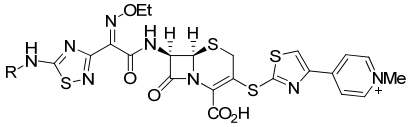


Cefozopran

Fifth Generation Cephalosporins

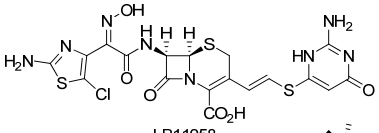


Ceftobiprole

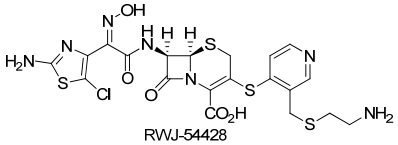


Ceftaroline: R = H
Ceftaroline fosamil: R = PO_3H_2

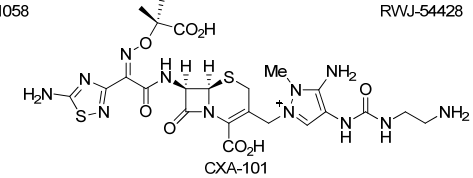
Cephalosporins in Development



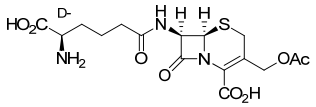
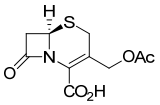
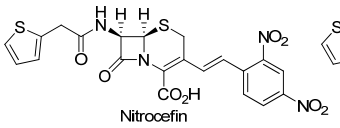
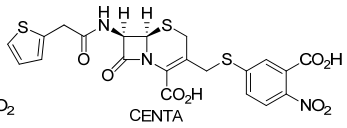
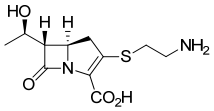
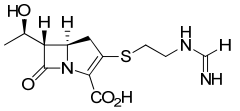
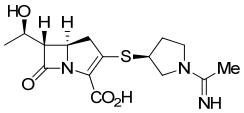
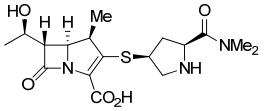
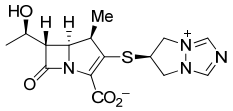
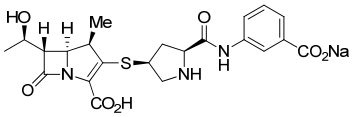
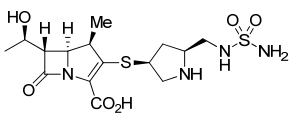
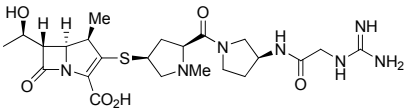
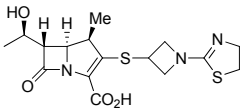
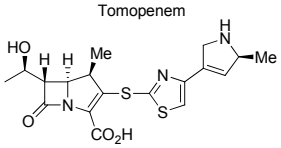
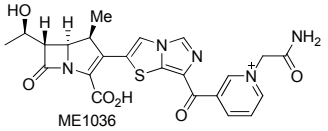
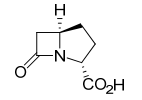
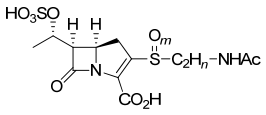
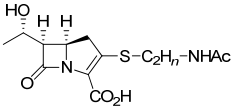
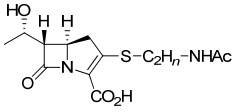
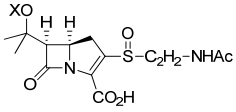
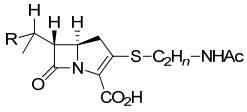
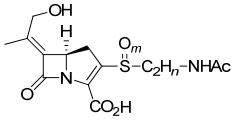
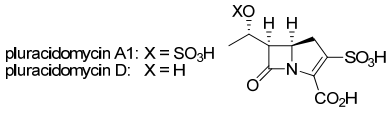
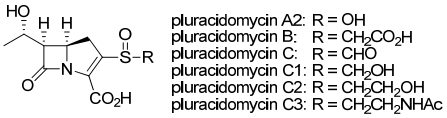
LB11058



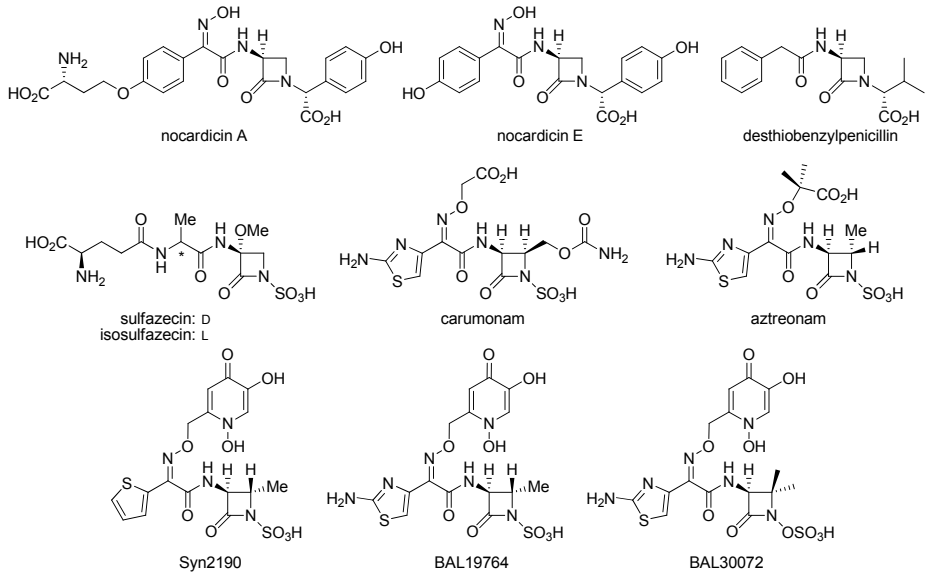
RWJ-54428



CXA-101

Other Cephalosporins of Interest	 Cephalosporin C  Cephalosporanic acid  Nitrocefin  CENTA
Carbapenems	
nor-1β-methyl Carbapenems	 Thienamycin  Imipenem  Panipenem
1β-Methyl Carbapenems	 Meropenem  Biapenem  Ertapenem  Doripenem
New Carbapenems	 Tomopenem  Tebipenem  Razupenem (PZ-601)  ME1036
Other Carbapenems of Interest	 carbapenemic acid  Olivanic acid MM 17880: *m* = 0, *n* = 4 Olivanic acid MM 13902: *m* = 0, *n* = 2 Olivanic acid MM 4550: *m* = 1, *n* = 2  Olivanic acid MM 22380: *n* = 4 Olivanic acid MM 22382: *n* = 2  Olivanic acid MM 22381: *n* = 4 Olivanic acid MM 22383: *n* = 2  carpetamycin A: X = H carpetamycin B: X = SO_3H  *PS-5*: R = H, *n* = 4 *PS-6*: R = Me, *n* = 4 *PS-7*: R = H, *n* = 2  asparenomycin A: *m* = 1, *n* = 2 asparenomycin B: *m* = 1, *n* = 4 asparenomycin C: *m* = 0, *n* = 2  pluracidomycin A1: X = SO_3H pluracidomycin D: X = H  pluracidomycin A2: R = OH pluracidomycin B: R = CH_2CO_2H pluracidomycin C: R = CHO pluracidomycin C1: R = CH_2OH pluracidomycin C2: R = CH_2CH_2OH pluracidomycin C3: R = CH_2CH_2NHAc

Monobactams



[a] This table provides the structures of representative β-lactams of each class and should not be considered to be a comprehensive list of all β-lactam antibiotics. [b] Expanded-spectrum refers to antibiotic activity and not β-lactamase inhibition.

Appendix B

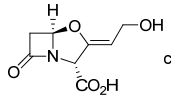
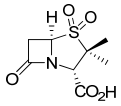
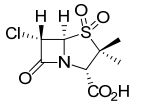
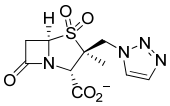
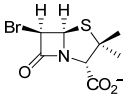
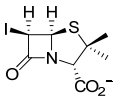
Classification Schemes for β-Lactamases [a]

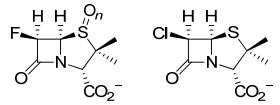
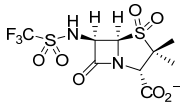
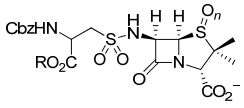
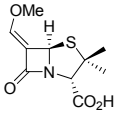
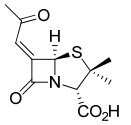
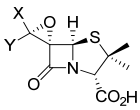
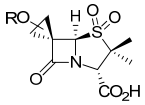
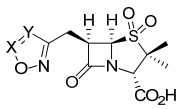
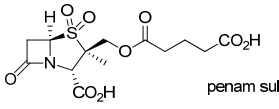
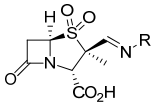
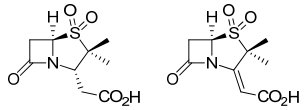
Bush–Jacoby group [a]	Ambler class [b]	Distinctive substrate(s)	Inhibited by clav or tazo [c]	Characteristics	Representative enzymes
1	C	Cephalosporins	No	Greater hydrolysis of cephalosporins than PenG; hydrolyze cephamycins	*E. coli* AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
1e	C	Cephalosporins	No	Increased hydrolysis of ceftazidime and often other oximino-β-lactams	GC1, CMY-10, CMY-19, CMY-37
2a	A	Penicillins	Yes	Greater hydrolysis of PenG than cephalosporins	PC1, BcI
2b	A	Penicillins, early cephalosporins	Yes	Similar hydrolysis of PenG and cephalosporins	TEM-1, TEM-2, SHV-1
2be	A	Extended-spectrum cephalosporins	Yes	Increased hydrolysis of oximino-β-lactams (cefotaxime, ceftazidime, ceftizxone, cefepime, aztreonam)	TEM-3, TEM-26, SHV-2, CTX-M-15, K1, PER-1, VEB-1
2br	A	Penicillins	No	Resistance to clavulanic acid, sulbactam, and tazobactam	TEM-30, TEM-31, SHV-10
2ber	A	Extended-spectrum cephalosporins, monobactams	No	Increased hydrolysis of oximino-β-lactams combined with resistance to clav/sul/tazo	TEM-50
2c	A	Carbenicillin	Yes	Increased hydrolysis of carbenicillin	PSE-1, CARB-3
2ce	A	Carbenicillin, cefepime	Yes	Increased hydrolysis of carbenicillin, cefepime, and cefpirome	RTG-4
2d	D	Cloxacillin	Variable	Increased hydrolysis of cloxacillin or oxacillin	OXA-1, OXA-2, OXA-10
2de	D	Extended-spectrum cephalosporins	Variable	Hydrolyzes cloxacillin or oxacillin and oximino-β-lactams	OXA-11, OXA-15, OXA-18,[d] OXA-45 [d]
2df	D	Carbapenems	Variable	Hydrolyzes cloxacillin or oxacillin and carbapenems	OXA-23, OXA-40, OXA-48
2e	A	Extended-spectrum cephalosporins	Yes	Hydrolyzes cephalosporins, Inhibited by clav but not aztreonam	CepA
2f	A	Carbapenems	Variable	Increased hydrolysis of carbapenems, oximino-β-lactams, cephamycins	KPC-2, IMI-1, SME-1, GES-2 to GES-15, NMC-1
3a	B (B1)	Carbapenems	No	Broad-spectrum hydrolysis including carbapenems but not monobactams	BcII, IMP-1, VIM-1, CcrA, IND-1
	B (B3)				L1, GOB-1, FEZ-1
3b	B (B2)	Carbapenems	No	Preferential hydrolysis of carbapenems	CphA, Sfh-1

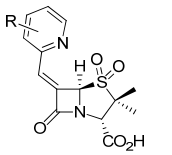
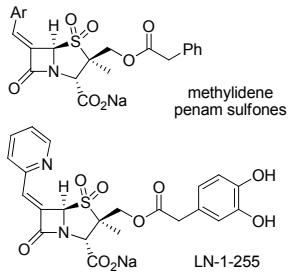
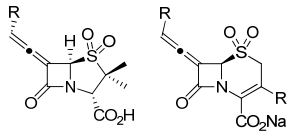
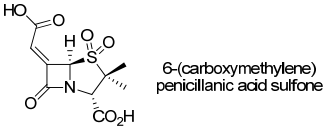
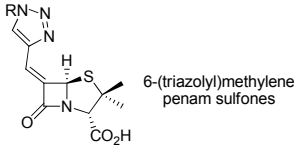
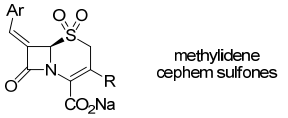
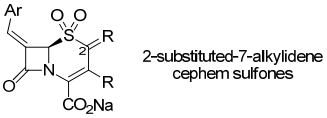
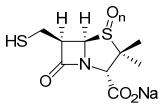
[a] Adapted from refs 235–238, 697 and tables at www.lahey.org/studies. [b] See ref 231. [c] clav = clavulanic acid, tazo = tazobactam, sul = sulbactam, PenG = penicillin G = benzyl penicillin. [d] Classifications of OXA-18 and OXA-45 were deduced by this author based on ref 648.

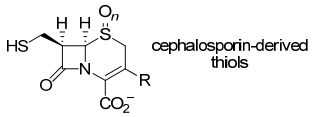
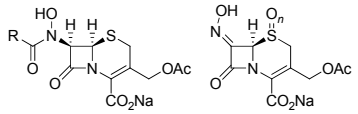
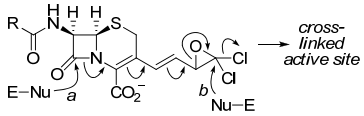
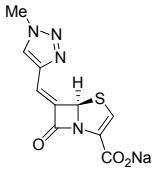
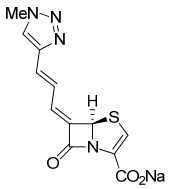
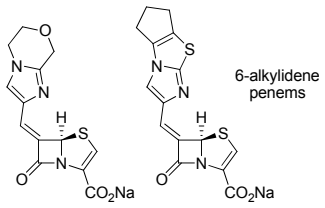
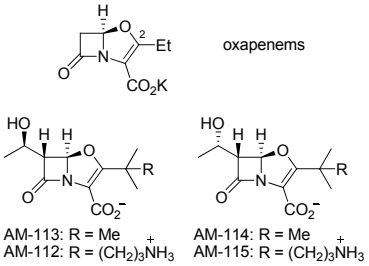
Appendix C

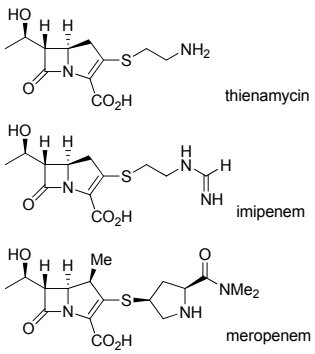
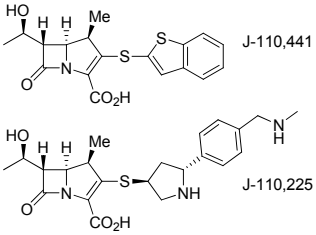
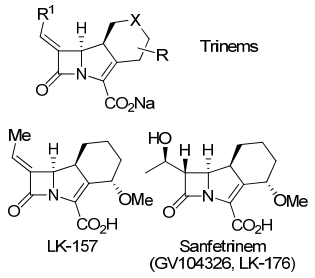
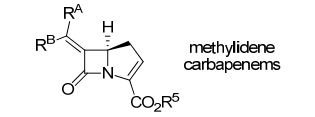
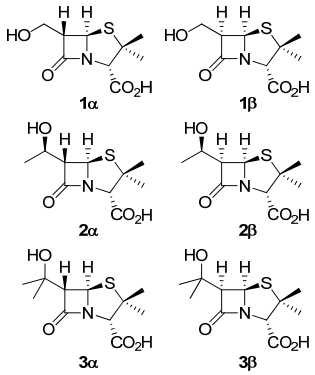
Known β-Lactamase Inhibitors [a]

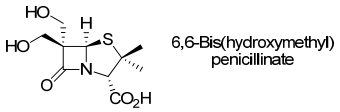
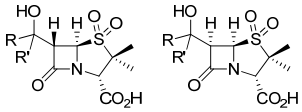
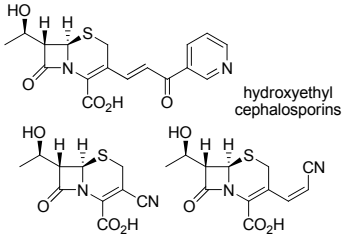
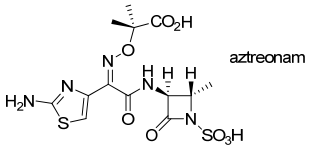
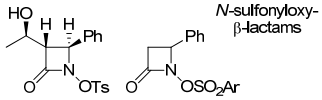
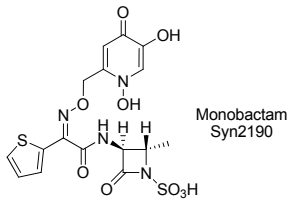
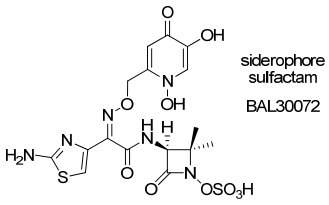
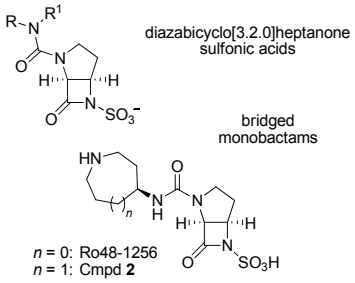
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
1	 clavulanic acid	A, (C), (D)	- Beecham, 1976: X-ray crystal structure.[443] - Weak antibiotic activity (31–125 μg/mL),[119] but a potent inhibitor of penicillinases and cephalosporinases.[398] - Potent mechanism-based inhibitor of penicillinases such as TEMs and SHVs (class A) (IC_{50} values < 0.1 μg/mL); ineffective against MBLs (class B); much less active against cephalosporinases (class C); variable efficacy against OXAs (class D).[403,414] - Herzberg, 1992: X-ray of complex with TEM-1 (class A) (1BLC).[455] - Sulton et al., 2006: Mechanistic studies of TEM-1 and SHV-1inactivation.[459] - Blanchard, 2008: X-ray of complex with BlaC (class A) (3CG5).[698] - X-ray structure of clavulanate bound to a E166A mutant of SHV-1 (2A49)[699] and a double mutant (M69V, E166A) of SHV-1 (2H0T).[700] - Review of clavulanate combinations for use against ESBLs.[701]
2	 penicillanic acid sulfone sulbactam	A	- Pfizer, 1978: Weak antibacterial activity but showed similar potency against penicillinases to that of clavulanate.[446] - Potent mechanism-based inhibitor of class A enzymes, but much less effective against class C and class D enzymes. Ineffective against MBLs.[414] - Labia,[460] Knowles,[461–464] and Mobashery[465] groups, 1981–1994: Mechanisms of inhibition of class A β-lactamases. - Poor inhibitor of OXA-10 (class D).[704] - X-ray structures of sulbactam bound to the E166A mutant of SHV-1 (2A3U)[699] and the M69V E166A double mutant of SHV-1 (2H10).[700] - Mechanism in SHV-1: Raman studies by Carey.[702] - Akova, 2008: Review of sulbactam-containing β-lactamase inhibitor combinations.[703]
3	 α-chloropenicillanic acid sulfone	A	- Cartwright, Coulson, 1979: irreversible inhibition of PC1 at pH 7.[469] - The apparent K_m is 100 μM and the k_{cat}/k_i = 100 (similar to clavulanate).
4	 tazobactam	A	- Aronoff, 1984: Synergy with Amoxicillin and Ampicillin and comparisons with clavulanate.[475] - Bush, 1993: Kinetic interactions of tazobactam with β-lactamase from all major structural classes.[449] - Effective mechanism-based inhibitor vs TEM-1. - Poor activity against MBLs but some activity against CcrA. - IC_{50} value of 48 μM vs AmpC (class C). - Poor inhibitor of OXA-10 (class D).[704] - X-ray structure of tazobactam bound to WT SHV-1 (1VM1),[705] a S130G mutant of SHV-1 (1TDG),[706] an E166A mutant of SHV-1 (1RCJ),[478] and a M69V/E166A double mutant of SHV-1 (2H10).[700]
5	 6β-bromo-penicillanic acid	A	- Loosemore and Pratt, 1978: Epimerization in solution and preparation of 6β-bromopenicillanic acid.[479] - Pratt and Loosemore, 1978: Potent irreversible inhibition of BcI and enzyme of *B. licheniformis* by 6β-BPA; less potent vs penicillinases of *S. aureus* and *E. coli*. Poor inhibition of BcII.[250] - Knott-Hunziker et al., 1979: ^{3}H-labelled 6β-BPA and labelling of Ser44 of BcI.[251] - Loosemore, Cohen, Pratt, 1980: Kinetics of inactivation of BcI.[480] - Wise, Andrews, Patel, 1981: Comparisons with clavulanate and sulbactam.[481]
6	 6β-iodo-penicillanic acid	A	- Pfizer, 1980: 6β-Iodopenicillanic acid via S_N2 reactions from penicillin-6-triflates.[707] - Daehne, 1980: Synthesis and purification of 6-halopenicillins.[708] - Sauvage (Liège), Pratt, *JACS* 2009: X-rays in the class A enzyme BS3 (2WK0) and R39 DD-transpeptidase (2WKE).[483]

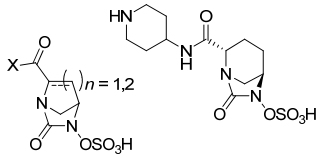
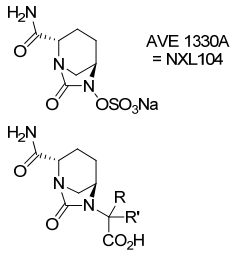
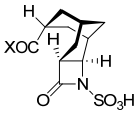
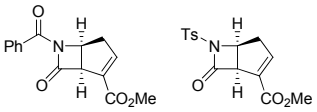
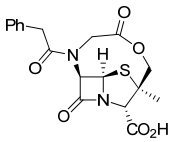
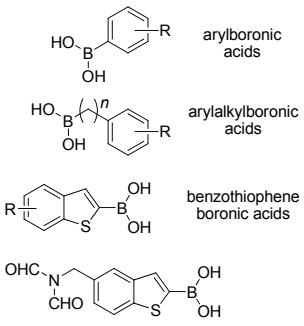
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
7	 6β-fluoro-penicillanic acids; 6β-chloro-penicillinic acid	A	- Roveri, 1993: Poor inhibition of BcI (mM IC_{50} values).[709] - The 6β-chloropenicillanic acid is less potent than the bromo- and iodo penicillinates.[708]
8	 6β-triflimido penicillanic acid sulfone	A	- Dmitrienko and Viswanatha groups, 1982–1985.[470,471,472,473,474] - Potent mechanism-based inhibitor of *B. cereus* 569/H, *B. licheniformis* 749/C, and *E. coli* R6K. But less effective against *S. aureus* PC1.
9	 6β-cysteine-sulfonamido penicillanic acids	(A)	- Changov et al., 1999 (Bulgarian group): Synergy with Ampicillin against Gram-positives; poor synergy against Gram-negatives.[710] - No direct enzyme inhibition data reported.
10	 6-(methoxymethylene) penicillinate	A	- Knowles, 1984: Designed to generate an α,β-unsaturated acylenzyme upon ring-opening of β-lactam.[484]
11	 6-(acetylmethylene) penicillinate Ro 15-1903	A, C, D	- Hoffman–La Roche, 1982: Potent irreversible inhibitor of PC1, TEMs, SHV-1, OXAs, and chromosomal β-lactamases (class C). More potent than clavulanate and sulbactam.[485a] - Hoffman–La Roche, 1982: Poor affinity for PBPs of *E. coli*. Synergy with piperacillin and ceftriaxone.[485b] - Hoffman–La Roche, 1983: Mechanism of inactivation of TEM-1.[485c] - Hoffman–La Roche, 1986 and 1992: Prodrug evaluations.[485e,485g] - Hoffman–La Roche, 1987: the geometrical isomer is less active.[485f]
12	 6-spiroepoxides	A	- Bycroft, 1988: "Exhibit significant β-lactamase inhibitory and antibacterial properties" but no biochemical details provided.[711]
13	 spirocyclopropyl penam sulfones	A, C	- Wyeth patent 2002: Improves antimicrobial activity of piperacillin.[712] - Wyeth 2003: Mechanism-based inhibition similar to that of the 6-alkylidene derivatives.[496] - IC_{50} values 0.1–13 μM against TEM-1 (class A) and 0.02–66 μM vs AmpC (class C).
14	 heterocyclic penam sulfones	A, B, C	- Wyeth group, 2000: Mid to sub-μM activities reported against TEM-1 (class A), CcrA (class B), and AmpC (class C).[713]
15	 penam sulfone SA2-13	A	- Buynak, Bonomo, Carey, van den Akker, 2006: X-ray structure of a complex of SA2-13 with SHV-1 (class A) (2H5S).[466] - Rational design of an inhibitor that could form a long-lived *trans*-enamine intermediate.
16	 oxime and imine penam sulfones	A, C	- Naeja Pharmaceuticals, 2005 (Edmonton, AB): Low to sub-micromolar IC_{50} values against TEM-1 (class A), CTX-1 (class A), and a class C enzyme.[477]
17	 carboxymethyl penam sulfones; carboxymethylidene penam sulfones	A, C	- Buynak, 2005: IC_{50} values of 34 μM against TEM-1 (class A) and 28 μM against P99 (class C) for the carboxymethyl compound; better than sulbactam.[714] - IC_{50}s for 3-methylidene analogues were >1 mM.

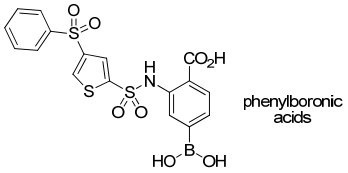
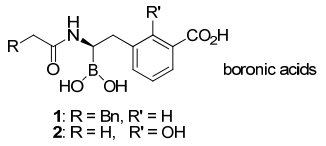
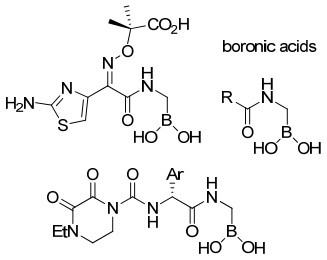
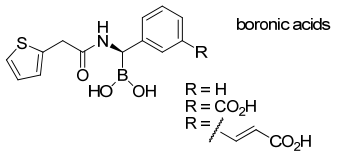
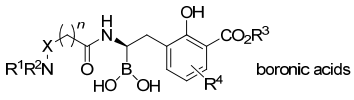
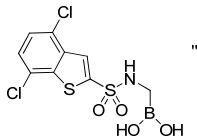
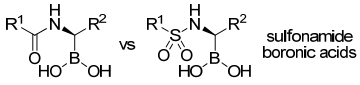
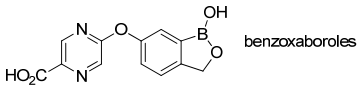
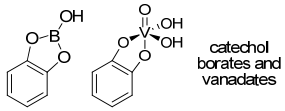
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
18	 6-(Heterocyclyl)-methylene penam sulfones	A, C, D	- Pfizer, 1986: more potent than clavulanate against β-lactamases from *S. aureus* and *E. coli*. Its reaction with methoxide provided insight into its likely mechanism of inhibition.[486] - Pfizer, 1986: SAR with heterocycle portion.[487]
19	 methylidene penam sulfones LN-1-255	A, D	- Buynak, 1999: Moderate inhibition of TEM-1 (class A), PC1 (class A), and P99 (class C). Mid-μM to mid-nM IC_{50}s.[715] - Buynak, 2004: More active against *Bacillus anthracis* class A Bla1 (low to high nM) than class B Bla 2 (low μM).[716] - Buynak, Bonomo, Carey, 2007: One analogue (SA-1-204) has a K_i value of 42 nM against SHV-1 (class A) and 1 μM against OXA-1 (class D).[494] Raman spectroscopy indicates that hey bind as Michaelis complexes. - Buynak, Bonomo, 2009: X-ray structure of a complex of LN-1-255 (Ar = 2-pyridyl) in SHV-1 (3D4F).[717] LN-I-255 has K_i values of 110 and 100 nm against SHV-1 and SHV-2; Improves pipericillin potency vs SHV-producing *E. coli*. - Buynak, Bonomo, 2010: LN-I-255 has low to sub-micromolar K_is against class D enzymes: 0.70 (OXA-1), 8.0 (OXA-10), 1.4 (OXA-14), 0.20 (OXA-17), 0.65 (OXA-24/40).[718]
20	 6-vinylidene penam sulfones 7-vinylidene cephem sulfones	C	- Buynak, 1993: The vinylidene PAS with R = *t*-Bu showed an IC_{50} of 1 μM vs P99, approximately equal in potency to clavulanate.[488] - Buynak, 1994: The cephem sulfone with R = *t*-Bu gave an IC_{50} of 0.05 μg/mL vs P99 and other derivatives sub-micromolar as well.[489]
21	 6-(carboxymethylene) penicillanic acid sulfone	A, C	- Buynak, 1995: 6-(Carboxymethylene)PAS showed potent, partially irreversible broad spectrum activity against class A and C enzymes.[490] - IC_{50} values against TEM-2, P99, and E-2 (from *E. cloacae*) were 120 nM, 45 nM, and 91 nM, respectively. - Synergy with piperacillin against several organisms.
22	 6-(triazolyl)methylene penam sulfones	A, C	- SynPhar Labs (Edmonton), 1996: IC_{50} of 0.2 and 0.7 μM vs TEM (class A) and P99 (class C) and synergy with ampicillin against β-lactamase-producing isolates.[491]
23	 methylidene cephem sulfones	A, C	- Buynak patent 2000: Antibacterial Activity of β-Lactamase-Inhibiting Cephalosporins.[719] - Buynak, 2000: Potent IC_{50} values against TEM-1 (class A), PC1 (class A), P99 (class C), and GC1 (class C).[720] - Buynak, 2000: Synthesis of alkylidene cephalosporins.[721] - Knox, 2001: X-ray structure of an alkylidene cephem sulfone in GC1 (1GA0).[722] - Buynak, 2002: Potent IC_{50} values against TEM-1 (class A) and P99 (class C): sub-micromolar to low nanomolar.[723] - Buynak patent for preparation, 2003.[724] - Buynak, 2001: Coupling reactions with 3-stannylated cephem sulfones.[725]
24	 2-substituted-7-alkylidene cephem sulfones	A, C	- Buynak, 2000: IC_{50} values: Low-μM against TEM-1 (class A), high μM vs PC1 (class A), nanomolar against P99 (class C).[493] - Buynak, Bonomo, 2010: ASR-II-292 has low-μM K_i values against OXAs: 1.40 (OXA-1), 17 (OXA-10), 4.0 (OXA-14), 0.87 (OXA-17), 2.4 (OXA-24/40).[718]
25	 penicillin-derived thiols	A, B, C	- Buynak patent 2003, *BMCL* 2004: Low μM IC_{50} values against TEM-1 (class A), L1 (class B), BcII (class B), and P99 (class C).[556,726] - Buynak, 2004: More active against *Bacillus anthracis* class B Bla2 (low μM) than class A Bla1 (high μM).[716]

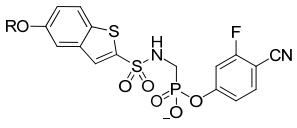
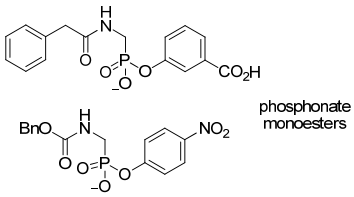
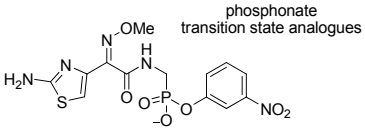
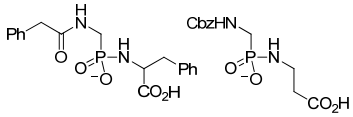
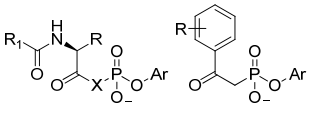
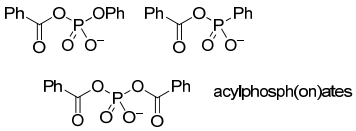
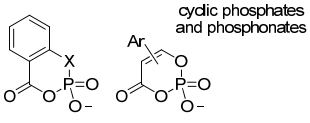
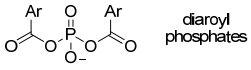
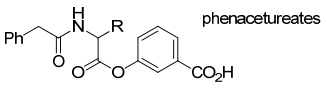
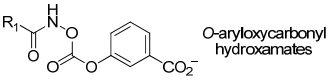
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
26	 cephalosporin-derived thiols		- Buynak, US Patent 2006.[727]
27	 *N*-hydroxy cephalosporins oximino cephalosporins	B (A, C, D)	- Pratt, Buynak, 2009: Intended for both serine- and metallo-β-lactamases, but they are turned over by TEM-1 (class A), P99 (class C), and OXA-1 (class D).[728] - Low μM IC_{50} values against class B MBLsVIM-2 and GIM-1 (2–270 μM).
28	 *cross-linked active site*		- Sutton and Yu, patents 2009, 2010: Mechanism-based inhibitors designed to release a leaving group (e.g. epoxide ring-opening) and cross-link the active site.[729]
29	 BRL 42715	A, C, D	- Beecham, 1989: Penem BRL 42715 lacks antibacterial activity but showed potent (0.001–0.01 μg/mL) inhibition of TEM-1, SHV-1, K1, P99, OXA-1, PSE-4, and others; synergy with amoxicillin.[497] - SmithKline Beecham, 1994: BRL 42715 quickly inactivates class A (BcI, TEM-1 (K_i est. 6.3 nM), K1 (K_i est. 7.3 nM)) and the C enzyme P99.[501] - Mobashery, 1995: Kinetic studies with BRL 42715. Nanomolar K_i values against TEM-1 (class A) and Q908R (class C).[502] - SmithKline Beecham and Frère groups, 1995: Kinetic studies with BRL 42715 and β-lactamases and DD-peptidases. The penem inhibits the R61 transpeptidase and does not inhibit BcII.[505]
30	 SYN-1012	A, C	- SynPhar Laboratories (Edmonton, AB), 1997: Synergy with piperacillin against Gram-positive and Gram-negative isolates was slightly inferior to that of BRL 42715.[730]
31	 6-alkylidene penems BLI-489	A, B, C, D	- Nukaga, 2003: X-ray structures of a penem bound to SHV-1 (class A) (1ONG) and GC1 (class C) (1ONH). IC_{50} values are 0.4, 9.0, and 6.2 nm against TEM-1, SHV-1, and GC1, respectively.[503] - Wyeth, 2004: Low to high nM IC_{50}s against TEM-1 (class A), Imi-1 (class A), CcrA (class B), AmpC (class C), and OXA-1 (class D).[506,508,509,510,511,512,731,732] - Frère, 2005: X-ray of BRL-42715 bound to *Enterobacter clocae* 908R (class C) (1Y54).[504] - Bonomo, 2010: Wyeth's Penem-1 shows synergy with piperacillin, aztreonam, cefotaxime, and cefepime against *bla*$_{KPC}$-possessing *K. pneumoniae* isolates.[733]
32	 oxapenems AM-113: R = Me AM-112: R = $(CH_2)_3NH_3^+$ AM-114: R = Me AM-115: R = $(CH_2)_3NH_3^+$	A, C, D	- Cherry, 1978: The 2-ethyl derivative was a potent inhibitor (superior to clavulanate) but was inactive against intact bacteria and lacked chemical stability.[734] - Wild and Metzger (Bayer), 1993: Synthesis of various 6-substituted derivatives.[735] - Pfaendler et al., 1993: Synthesis and antibacterial activity of derivatives such as AM-114, which showed low MIC values (<2 μg/mL) vs *S. aureus*, *B. fragilis*, *E. coli*, and others.[736] - Aston University and Micron Research, UK. Amura, UK. 2003: AM-112–AM-115 show low to high nanomolar activity against TEM-1, TEM-10, SHV-5 (class A enzymes), P99, S2, S+A (class C enzymes), OXA-1, and OXA-5 (class D enzymes).[737]

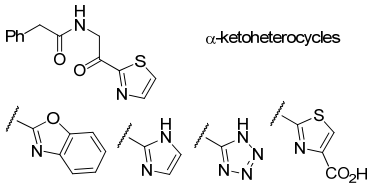
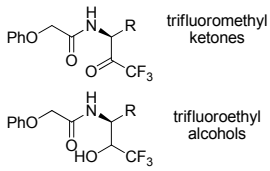
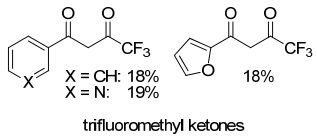
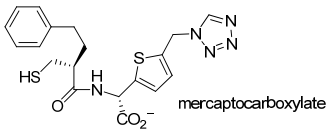
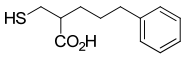
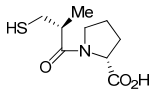
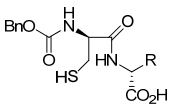
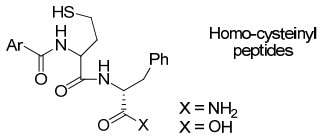
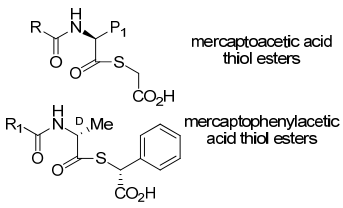
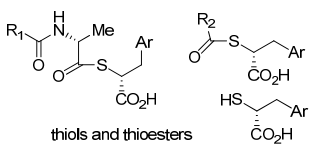
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
33		A, C, (D)	- Carbapenems are generally poor substrates for class A and C enzymes. Some OXA- enzymes are susceptible, some are carbapenemases. - Mobashery, 1995: Mechanism of turnover of imipenem by TEM-1.[429] - Mobashery, Samama 1998: X-ray structure of imipenem in TEM-1 (1BT5).[430] - Shoichet, 2002: Imipenem inhibits class C enzymes. X-ray structure of a complex with AmpC (class C) (1LL5).[432] - Nukaga, Knox, Bonomo, 2008: Carbapenems are resistant to hydrolysis by SHV-1. X-ray of meropenem in SHV-1 (2ZD8) shows two conformations of meropenem.[431] - Blanchard, 2009: Carbapenems are poor/very slow substrates for BlaC (class A), allowing the determination of the X-ray structure a complex with meropenem (3DWZ).[738] - Queenan, 2010: Hydrolysis and inhibition profiles of β-lactamases with Doripenem, Imipenem, and Meropenem.[697]
34		B	- Banyu Tsukuba Research Institute (Japan), 1999: A series of carbapenems were screened for inhibition of IMP-1 (class B MBL).[557] - J-110,441 had an IC_{50} of <0.1 μM against IMP-1 and J-110,225 showed an IC_{50} of 0.7 μM; K_i of 0.18 μM relative to imipenem. - Banyu, 2000: J-110,225 had better antibacterial activity than imipenem, and was hydrolyzed slower by CcrA, L1, and BcII.[558] - J-100, 225 was not hydrolyzed by TEM-1 or a class C enzyme.
35		A, C	- Glaxo, 1994: Antibacterial activity of GV104326.[144] - Wise, 1996: In vitro activity of GV104326.[145] - Tamura et al., 1998: In vivo antibacterial activities of sanfetrinem.[146] - Matagne, 2001: Kinetic study of the interaction of LK-156 and LK-157 with class C β-lactamases".[433] - Lek Pharmaceuticals (Slovenia), 2002: Low μM IC_{50}s against TEM-1(class A) and 908R (class C).[434,739] - Lek, 2007: Mid-nanomolar IC_{50}s against TEM-1, SHV-1 (class A), and AmpC (class C).[435] - Lek, 2009: X-ray structure of LK-157 (R = Me, R^1 = Me) and another trinem (R = *n*-Bu) in AmpC (2Q9M, 2Q9N).[436] - Lek patents 2009 (compounds with the hydroxyethyl sidechain).[740]
36			- Wyeth: US Patent, 2010.[741]
37		A, D	- Mobashery, 1995: 6α-hydroxymethyl penicillin (**1α**) inhibits TEM-1 (class A) rapidly with a partition ratio (k_{cat}/k_{inact}) of 28; K_i = 48 μM; k_{cat} = 0.029 s^{-1}; K_m = 24 μM.[437] - The low rate of hydrolysis (k_{cat}/K_m = 1200 M^{-1} s^{-1}) makes it a very poor substrate for TEM-1; No conformational change associated with inhibition. - Mobashery, 1996: X-ray structure of **1α** in TEM-1 (1TEM).[438] - Mobashery, 1998: Comparison of **1α**, **2α**, and **3α** against TEM-1 and NMC-A (class A).[439] - Hydroxypropyl moiety is too big to fit in TEM-1 but this derivative (**3α**) inactivates NMC-A with a K_i value of 390 μM. - X-ray of the 6α-hydroxypropyl derivative **3α** in NMC-A (class A) (1BUL). - The 6α-hydroxymethyl cmpd (**1α**) has a K_m of 70 μM vs NMC-A. - Mobashery, 2000: **1α**, **1β**, **2α**, and **2β** tested against OXA-10.[286] - Compounds **1β** and **2β** are substrates for OXA-10 but **1α** and **2α** are competitive inhibitors with K_i values of 1900 and 1200 μM. - Mobashery, 2002: X-ray structure of 6β-hydroxypropyl (**3β**) in OXA-10.[440] The structure was first published in *PNAS* (1K54).[293] - **3α** and **3β** had K_i values of 300 and 240 μM against OXA-10.

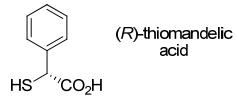
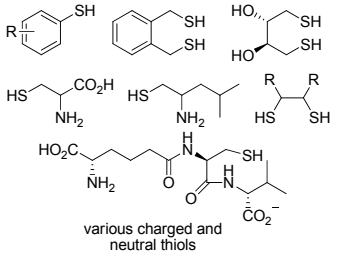
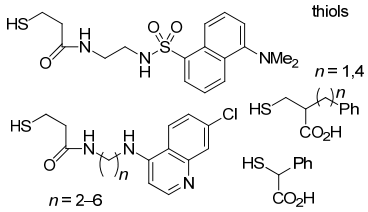
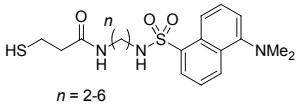
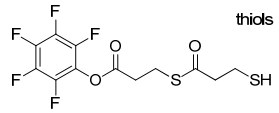
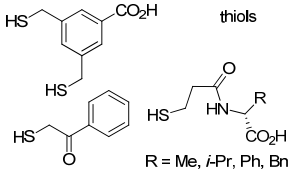
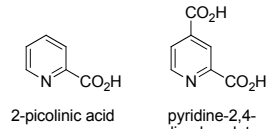
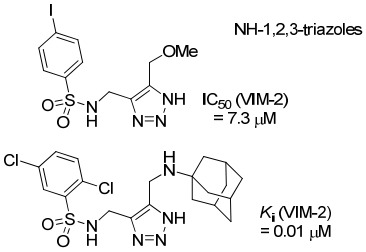
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
38	 6,6-Bis(hydroxymethyl) penicillinate	A, C	- Mobashery, 2001: Postulated that a bis(hydroxymethyl) derivative might be able to impair the approach of the hydrolytic water molecule (which approaches from different faces) in class A and class C β-lactamases.[441] - K_i values were 480 μM against TEM-1 and 700 μM against P99.
39	 hydroxyalkyl PASs	A, C	- Wyeth 1999: Synthesis of several hydroxyethyl- and hydroxymethyl penicillins and PASs and evaluation against TEM-1 and AmpC.[742] - Most showed IC_{50} values in the mid-μM range but the 6β-hydroxymethyl PAS was best with an IC_{50} of 8 nM against TEM-1 and 1.2 μM vs AmpC. - Also showed synergy with piperacillin vs β-lactamase-producing *E. coli* and *S. marcescens*. - Wyeth 1999: 2β-substituted derivatives of the hydroxymethyl compounds above.[743]
40	 hydroxyethyl cephalosporins	A, C	- Fujisawa Pharma (Japan), 1989: Poor antibacterial activity alone but reasonable inhibitors of penicillinases and cephalosporinases.[744]
41	 aztreonam	C	- Squibb, 1982: Discovery[165] and interaction with β-lactamases.[514] - Hydrolyzed slowly by TEMs (class A), but binds poorly and is active against class A-producing organisms. - Competitive, progressive inhibitor of *C. freundii* cephalosporinase (class C).[167] - Bind poorly to MBLs such as IMP-1 (class B). Not turned over.
42	 *N*-sulfonyloxy-β-lactams	A	- Miller, Mobashery, 1993,[745] 1995,[746] 1999,[747] 2000.[748] - Fast reaction with TEM-1 but activity is recovered. - X-rays in TEM-1 (not deposited in PDB). - K_i values against Q908R (class C) ranged from 5 to 680 μM.
43	 Monobactam Syn2190	(A), C	- Naeja Pharmaceuticals, 1999: IC_{50} values of 2–10 nM against AmpC (class C); modest inhibition of TEMs (class A).[749]
44	 siderophore sulfactam BAL30072	C	- Page, Basilea Pharmaceutica 2010 : Modest inhibition of class C β-lactamases, poor inhibition of MBLs and class A enzymes, but good inhibition (sub-micromolar) of PBPs.[750] - Page, Bonomo, 2011: Activity against *A. baumannii*.[751]
45	 diazabicyclo[3.2.0]heptanone sulfonic acids bridged monobactams *n* = 0: Ro48-1256 *n* = 1: Cmpd **2**	C	- Hoffman–La Roche patent, 1992.[752] - Hoffman–La Roche, *J. Med. Chem.* 1998.[516] - Miller, 1997: Synthetic route.[753] - Livermore, 1997: Synergy of Ro48-1256 with β-lactams against *P. aeruginosa*.[517] - Merck patent 2009: IC_{50} of 1.2 μM vs P99 (class C). - Merck, 2010: The (*S*)-azepine analogue **2** was better than Ro48-1256 and was selected for preclinical development.[518] - IC_{50} for cmpd **2** against *P. aeruginosa* AmpC was 1.0 μM. - X-ray structures of AmpC of *P. aeruginosa* (class C) with **2** (*S*)-(2WZX) and (*R*)-azepine-bearing inhibitors (2WZZ).

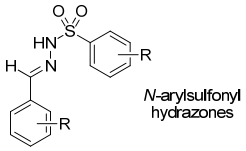
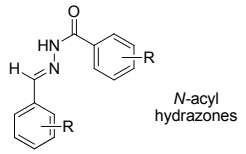
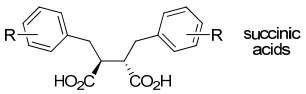
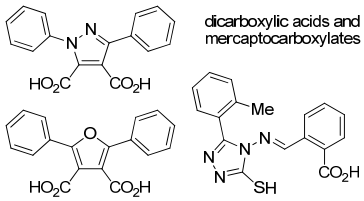
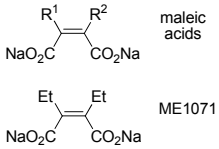
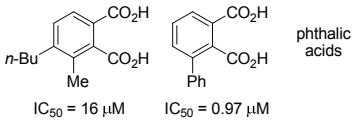
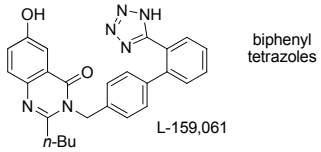
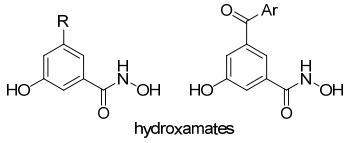
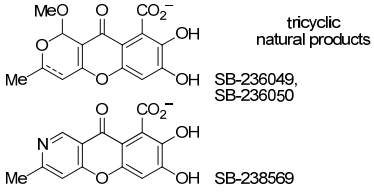
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
46		C	- Merck patents, 2009: One compound showed an IC_{50} value of 465 nM against AmpC (class C).[754]
47	 AVE 1330A = NXL104	A, C, D	- Aventis Pharma patent, 2003: Synthesis of azabicycles. - Aventis Pharma, 2004: Nanomolar IC_{50}s vs TEM-1 (8 nM) and P99 (80 nM); Improved the antibacterial activity of ceftazidime against SHV- and AmpC producers.[540] - Livermore, Woodford, Novexel (France), 2008: NXL 104 improved the potency of cephalosporins against *Enterobacteriaceae* that produced CTX-M ESBLs, AmpC (class C), KPC (class A), SME (class A), and OXA-48 (class D).[541] - Novexel, 2009: Activity against KPC-2 and KPC-2-producing Enterobacteriaceae; IC_{50}s of 170 nM and 35 nM after 5- and 30 min pre-incubations, respectively, against KPC-2.[542] - Bonomo, 2009: NXL104 effectively lowers MICs of β-lactams against KPC-2-producing clinical isolates of *K. pneumoniae*.[543] - NXL104: Currently in clinical development; Displays broad-spectrum inhibition against class A (TEM-, SHV-, and CTX-M-types), and class C (CTX-M ESBLs), and some class D (OXA...). - Patents in 2009.[755] - But not effective with cephalosporins against MBL producers (IMP or VIM MBLs).
48	 CN 1727334		- Chen, Liang. Chinese Patent 2006.[756]
49	 6-azabicyclo[3.2.0]hept-2-enes	C	- Cooper, 1994: Some analogues showed inhibition (>50%) of P99 at a fixed concentration of 200 μM but poor inhibition of TEM-1.[757] - Carboxylic acids were inactive as antibiotics and β-lactamase inhibitors.
50	 bridged penams	–	- Buynak. *JACS* 1998: No activity against TEM-1 (class A) or P99 (class C) β-lactamases.[758]
51	 arylboronic acids arylalkylboronic acids benzothiophene boronic acids	A, C	- Waley, 1978: Phenylboronic acid, *m*-aminobenzeneboronic acid, and boric acid as reversible inhibitors of BcI (class A).[524] - Waley, 1983: Inhibition of class C β-lactamases by boronic acids.[525] - Jones group (UofT), 1994: Arylalkylboronic and arylboronic acids with K_i values ranging between 13 and 3700 μM against R-TEM-1.[759] - Shoichet group, 1998: X-ray of *m*-aminophenylboronic acid (MAPB) bound to AmpC.[760] K_i values for MAPB and MNPB (nitro) were 7.3 and 1.2 μM, respectively, vs AmpC. Inhibition is not time-dependent. - Shoichet group, 1998: Benzo[*b*]thiophene-2-boronic acid was the most potent of several arylboronic acids tested with an IC_{50} of 27 nM against AmpC (class C).[761] - Shoichet, 1999: X-ray structure of benzo[*b*]thiophene-2-boronic acid bound to AmpC (1C3B).[762] - Shoichet group, 2002: Structure-based approach for binding site identification in AmpC (class C).[763] - X-ray structures of AmpC with 3-nitrophenylboronic acid (1KDS), 4-carboxyphenylboronic acid (1KDW, 3BM6), 4-(carboxyvin2-yl)-phenylboronic acid (1KE0), and 4,4′-biphenyldiboronic acid (1KE3). - Shoichet, 2007: Complex of the di-*N*-formylmethyl derivative in AmpC (2I72).[764] - X-ray structures of benzo[*b*]thiophene-2-boronic acid with WT AmpC (1C3B) and the K67R mutant (3FKV).[765]

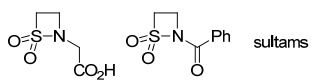
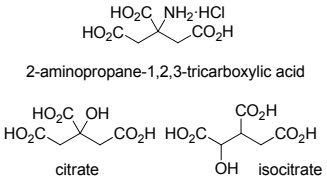
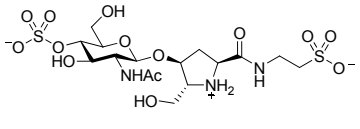
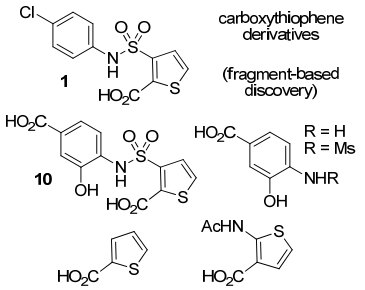
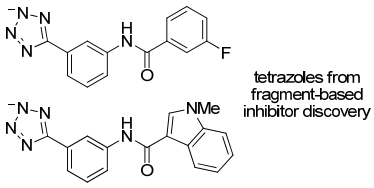
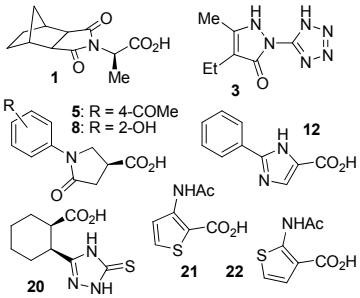
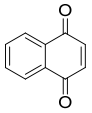
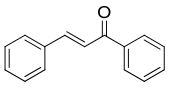
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
52	 phenylboronic acids	C	- Shoichet group 2001: X-ray structure of AmpC with a boronic acid lacking the carboxylic acid at the 4-position (1GA9). - Shoichet group and Italian group, 2010: IC_{50} values of high-μM to low-μM.[766] - X-ray structure of the compound shown in AmpC (3BM6); IC_{50} of 26 nM.
53	 boronic acids **1**: R = Bn, R' = H **2**: R = H, R' = OH	A	- Jones group (UofT), 1995.[767] - The compounds shown are very potent against TEM-1 with K_i values of 5.9 and 13 nM. - X-ray structures of these boronic acids in TEM-1 by the Strynadka group (1ERM, 1ERO, 1ERQ).[768,769]
54	 boronic acids	A, C	- Shoichet group, 2001: Several acylglycineboronic acid analogues of β-lactams with low to sub-micromolar K_i values against TEM-1 and AmpC.[770] - The analogue of ceftazidime has K_i values of 0.39 and 0.020 μM against TEM-1 and AmpC, respectively. - X-ray structures of AmpC with the cloxacillin analogue (1FSY) and the cephalothin analogue (1FSW). - Synergy observed with amoxicillin and cefotaxime against resistant strains of *E. cloacae* and *S. aereus*. - Bonomo, van den Akker, 2011: X-ray structures of boronic acids with the sidechains of cefoperazone and ceftazidime bound to SHV-1 (these inhibitors bind with only micromolar affinity).[771]
55	 boronic acids R = H R = CO_2H R =	C	- Shoichet, *Biochemistry* 2003: X-ray of carboxy (1PI5) and non-carboxy (1PI4) cmpds in N289A mutant of AmpC.[772] - Shoichet, *JACS* 2003: X-ray structures of same compounds in WT AmpC (class C) (1MXO, 1MY8).[773] - Shoichet, *JACS* 2006: Ultrahigh resolution X-ray structure of the non-carboxy cmpd in the N289A mutant of AmpC (2FFY – a higher resolution structure of 1PI4).[281] - Shoichet patent 2007.[774] - Shiochet 2008: X-ray of a carboxyvinyl derivative in AmpC (2RCX); K_i values for non-carboxy, carboxy, and carboxyvinyl compounds against AmpC: 35 nM, 1 nM, and 1 nM.[775]
56	 boronic acids	A, C	- Burns, Jackson, patents 2009 (Protez Pharma).[776] - Burns, patent 2010 (Novartis).[777]
57	 "DSABA"	A, C, D	- Merck Rahway, 2010: DSABA gave IC_{50} values of 1.1, 0.57, 1.2, 0.62, and 5.6 μM against TEM-1, SHV-5 (class A enzymes), AmpC, P99 (class C), and OXA-40 (class D).[778]
58	 sulfonamide boronic acids	C	- Shoichet, 2010: Sulfonamides compared with carboxamides vs AmpC (class C). No simple correlations observed due to differences in geometry and polarity.[779] - One compound showed a K_i value of 25 nM.
59	 benzoxaboroles	A, C	- Anacor, Naeja Pharmaceutials, 2011. Low micromolar to low nanomolar K_i values against CTX-M-9a, TEM-1, P99, and CMY-2.[780]
60	 catechol borates and vanadates	A, C, D	- Pratt, 2008: Vanadates are better inhibitors than the boronates.[781] - Low μM IC_{50} values against TEM-2 (class A), P99 (class C), and OXA-1 (class D).

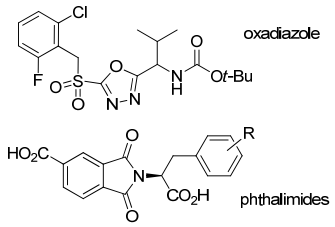
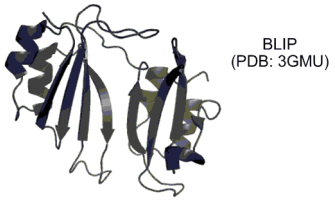
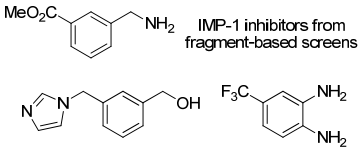
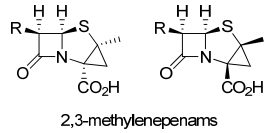
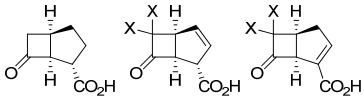
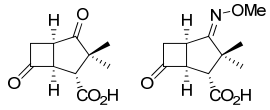
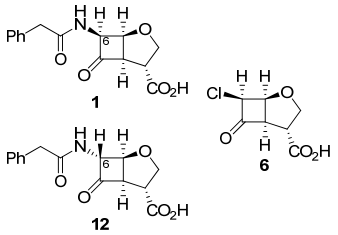
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
61	 (Hetero)arylsulfonamidomethylphosphonates	A, C, D	- Merck, Methylgene Patents 2006, 2007, and 2008.[782] - IC_{50} of 0.6 μM vs TEM-1 (class A). - Also inhibitors of AmpC enzymes from *P. aeruginosa* and *A. baumannii* (class C), and OXA-40 (class D).
62	 phosphonate monoesters	A, C	- Pratt, *Science* 1989: Rapid inactivation of P99 and very slow reactivation.[526] - Pratt, 1992: Mechanism of P99 inhibition.[783] - Pratt, 1994: Thermodynamic and kinetic data with PC1 confirms that phosphonates act as transition state analogues.[784] - Pratt and Herzberg, 1993: X-ray in PC1, the class A β-lactamase from *S. aureus* (1BLH).[785] - Pratt, Knox, 1994: X-ray structure in P99 (1BLS).[786] - Pratt, Samama, 1998: X-ray in TEM-1 (1AXB).[787]
63	 phosphonate transition state analogues	C	- Nukaga, Pratt, Knox, 2004: X-rays of the transition state analogue shown in class C β-lactamase from *Citrobacter freundii* (1RGY) and in class C ESBL GC1 (1RGZ).[666] - Silvaggi, Pratt, Kelly, 2003: Similar phosphonates have been used as transition state analogues with R61 transpeptidase (1MPL).[788]
64	 phosphonamidates	C	- SmithKline Beecham 1994: Analogues of dipeptides mostly showed IC_{50}s greater than 100 μM against TEM-1, P99, PC1, SHV-5, OXA-1, and PSE-4, but acyclic compounds showed IC_{50}s in the 10′s of μM against P99 (only).[789]
65	 ketophosphonates and ketophosphates	(A, C), D	- Pratt, *JOC* 2006: Ketophosph(on)ates were modest inhibitors of P99 (class C), OXA-1, and OXA-10 with K_i values of 70–1590 μM.[790] - Pratt, 2008: Intramolecular cooperativity between hydrophobic sites of OXA-1.[791]
66	 acylphosph(on)ates	A, C	- Pratt, *JACS* 1998: Acyl phosph(on)ates react β-lactamases P99 and TEM-1 to form phosphoryl enzymes which can hydrolyze or react further to form more inert complexes.[792]
67	 cyclic phosphates and phosphonates	A, C	- Pratt, 1998: Salicyloyl phosphate inhibits TEM-1 (class A), P99 (class C), and R61 transpeptidase.[793] - Pratt, 1998[792] and 2001: Additional studies revealed subtle differences between cyclic and acylic acyl phosphates.[794] - Silvaggi, Pratt, Kelly, 2004: X-ray structures in R61 transpeptidase (1SDE, 1SCW).[795]
68	 diaroyl phosphates	D	- Pratt, 2005: Potent inhibitors of OXA-1: K_i values from 200 nM down to <1 nM.[796] - Pratt, 2005: These compounds are less potent vs OXA-10.[797] - Pratt, 2009: Inhibition of TEM and P99 (low to sub-micromolar K_is), 2009.[798]
69	 phenacetureates	–	- Pratt, 2006: Tested against TEM-1, PC1 (class A), P99 (class C), and DD-peptidases R61, R39, and PBP3, but no inhibition of these enzymes was observed. Some derivatives were substrates, but most were not.[799]
70	 O-aryloxycarbonyl hydroxamates	C	- Pratt and Shoichet, 2007: X-ray of cross-linked AmpC (2P9V).[800] - Pratt, 2008: Irreversible inhibitors of P99 (class C).[801]

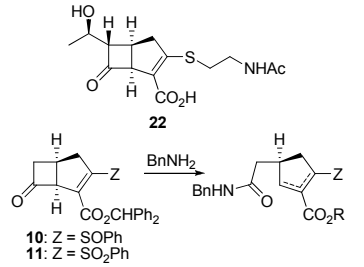
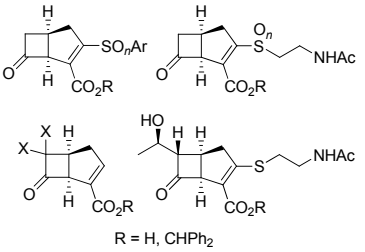
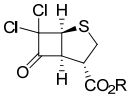
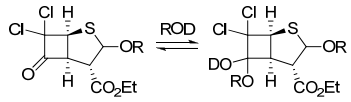
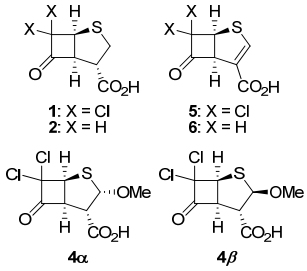
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
71	 α-ketoheterocycles	A, C	- Pratt, 2001: Concludes that specific electrophilic ketones are, at best, moderate inhibitors of β-lactamases.[539]
72	 trifluoromethyl ketones trifluoroethyl alcohols	B	- Adlington, Baldwin, Schofield groups, 1996: Some TFMKs showed low μM K_i values against the MBL from *X. maltophilia* (e.g. 1.5 and 3.0 μM).[538] - TFMKs and alcohols showed low μM K_i values against the MBL from *A. hydrophilia* (e.g. 6, 11, 44 μM); All TFMKs and alcohols had high μM K_i values against the MBLs from *B. cereus* and *P. aeruginosa*. - Synthesis, 1997[802] and 1998.[803]
73	 X = CH: 18% X = N: 19% 18% trifluoromethyl ketones	C	- Merck Rahway group, 2010 (as a small part of a paper about boronic acids).[778] - No IC_{50}s given; only % inhibition of AmpC (class C) at 2 μM.
74	 mercaptocarboxylate	B	- Concha et al., 2000: X-ray structures of IMP-1 alone (1DDK) and with the mercaptocarboxylate inhibitor (1DD6).[384] - This mercaptocarboxylate inhibits IMP-1, *B. fragilis*, and L1 enzymes with IC_{50} values between 100 and 500 nM.
75	 (±) mercaptocarboxylate	B	- Yamaguchi et al., 2007: X-ray of the thiol in VIM-2 (2YZ3).[804] - This mercaptocarboxylate has K_i values of 220 nM and 1660 nM against VIM-2 and IMP-1, respectively.
76	 D-captopril	B	- García-Sáez, 2003: X-ray of D-captopril in the dizinc MBL BlaB from *C. meningosepticum* (1M2X).[668] - K_i approx. 70–100 μM against BlaB. - Schofield, 2008: X-ray of D-captopril in CphA (2QDS).[805] Very surprisingly, the thiol does not coordinate with the zinc atom.
77	 cysteinyl peptides	B	- Page, 2001.[806] - K_i values against BcII range between 3.0 μM and 515 μM. - Inhibition is reversible and competitive.
78	 Homo-cysteinyl peptides X = NH_2 X = OH	B	- Crowder and Geysen, 2006.[807] - Homo-cysteinyl peptides more potent than cysteinyl peptides against L1. - Several compounds with low nanomolar K_i values against L1 (competitive inhibition), including one with a K_i of 2.1 nM.
79	 mercaptoacetic acid thiol esters mercaptophenylacetic acid thiol esters	B	- Payne et al., SmithKline Beecham, 1997.[808] - IC_{50} values 2–186 μM against L1, 0.5–30 against CphA, and 38–650 against BcII. IC_{50}s were >1000 μM against CfiA (*B. fragilis*). - Irreversible inhibition of BcII via mechanism-based delivery of mercaptoacetic acid and formation of a disulfide bond with the active site Cys; One compound showed uncompetitive inhibition of CphA but irreversible inhibition of L1. - Payne, 1997: With mercaptophenylacetic acid thioesters, potent IC_{50} values against L1 (as low as <1.95 μM); competitive inhibitors of BcII but not as potent (K_i of 185 μM for one compound).[809]
80	 thiols and thioesters	B	- Merck Rahway, 1999.[810,811] - Several compounds were very potent against IMP-1 (IC_{50}s as low as 0.4 nM) but few had activity against CcrA (lowest IC_{50} was 180 μM). - The parent thiols themselves were potent (20–300 nM) but less potent than thioesters. - Reversible inhibition of IMP-1.

Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
81		B	- Schofield, Galleni, Frère, 2001: The (*R*) isomer has a K_i value of 0.09 μM vs BcII and the (*S*)- isomer has a K_i value of 1.28 μM.[812] - Other simple thiols were also screened against BcII with K_i values between 0.21 μM and 346 μM. - Broad-spectrum MBL inhibition was demonstrated with racemic thiomandelic acid with the following K_i values: 0.34 (BcII), 0.80 (CfiA), 0.081 (L1), 0.029 (IMP-1), 0.059 (IMP-2), 0.23 (VIM-1), 0.56 (BlaB), 0.27 (FEZ-1), 144 (CphA). - Schofield, 2003.[813]
82		B	- Dmitrienko, 2003.[552] - Classical, slow-binding inhibitors of IMP-1 (pH dependent) with low μM IC_{50} values. Differences observed between negatively charged thiols and neutral ones. - Two compounds were similarly potent against BcII (IC_{50}s of 30 and 25 μM), but were poorer inhibitors of CcrA (IC_{50}s of 950 and 170 μM).
83		B	- Goto, 1997: Screened simple thiols against IMP-1 which showed low μM IC_{50} values.[814] - Goto, 2004: Arylalkyl thiols and dansyl thiols screened against IMP-1 and VIM-2 (low μM IC_{50}s against each enzyme).[815]
84		B	- Kurosaki, 2006: X-ray structure of a dansyl thiol in IMP-1 (2DOO).[816]
85		B	- Kurosaki, 2005: X-ray in IMP-1 (1VGN).[817] - Irreversible inhibitor of IMP-1 via thiol coordination with zinc atoms and subsequent aminolysis of the aryl ester by Lys224.
86		B	- Schofield 2008: Thiols which have low to sub-micromolar K_i values against MBLs of all three classes: B1 (IMP-1, BcII), B2 (CphA), B3 (L1, FEZ-1).[805] - The valine derivative drawn had K_i values of 0.063, 0.32, 3.6, and 0.082 μM against IMP-1, BcII, CphA, and L1, respectively. - X-ray crystal structure of alanine derivative with L1 (2QDT).
87		B	- Galleni, Schofield, 2007: Screened pyridine carboxylates for inhibition of MBLs.[818] - Little inhibition of MBLs except for 2-picolinic acid and the 2,4-isomer with CphA (K_i values of 5.7 and 4.5 μM, respectively, relative to imipenem hydrolysis). (pH dependent inhibition). - X-ray structure of pyridine-2,4-dicarboxylate in CphA (2GKL). The pyridine nitrogen and 2-carboxylate coordinate to the zinc atom.
88		B	- Hodder and Fokin at Scripps, 2009[819] and 2010.[820] - Libraries of triazoles from Click chemistry. - Inhibit VIM-2 only; no inhibition of IMP-1, TEM-1, or AmpC. - Enhance the antimicrobial activity of imipenem against VIM-2-producing *E. coli* at low concentrations of inhibitor (10, 20 μM).

Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
89	 *N*-arylsulfonyl hydrazones	B	- Dmitrienko, 2002.[554] - Low micromolar IC_{50} values against IMP-1.
90	 *N*-acyl hydrazones	B, D	- Dmitrienko group, patent 2010.[555] - Low micromolar IC_{50} values against IMP-1, VIM-2, OXA-10, and OXA-45.
91	 succinic acids	B	- Toney et al., Merck Rahway, 2001.[665] - Screened the Merck chemical collection and found some of these compounds to have IC_{50}s of 2.7, 3.7, 9, and 13 nM against IMP-1. - X-ray of two of these compounds in IMP-1 (1JJE, 1JJT). - Later, in 2005, one of these compounds was reported to enhance the activity of meropenem against a carbapenem-resistant MBL-producing strain of *E. coli*.[821]
92	 dicarboxylic acids and mercaptocarboxylates	B	- Olsen et al., 2006: "New leads" for MBL inhibitors from structure-based pharmacophore design.[822] - IC_{50}s against BcII from 7 to 100 μM (for the compounds shown) and 15–150 μM against L1.
93	 maleic acids ME1071	B	- Japanese group: Patents 2007.[823] - Patent 2008.[824] Preparation. Compound with R^1 = *i*-Pr, R^2 = *c*-Hex had IC_{50}s of 0.19 and 0.16 μM against IMP-1 and VIM-2. - Patent 2009.[825] Combination with ceftriaxone is effective against MBL-producing *P. aeruginosa*. - Ishii et al., 2010: Potentiation of carbapenems with ME0171.[826]
94	 phthalic acids IC_{50} = 16 μM IC_{50} = 0.97 μM	B	- Japanese group. Patent 2008.[827] *Bioorg Med Chem Lett.*, **2009**.[828] - High μM IC_{50}s down to sub-μM against IMP-1 (class B). - Increase the potency of biapenem against IMP-1-producing biapenem-resistant strains of *P. aeruginosa*.
95	 biphenyl tetrazoles L-159,061	B	- Toney et al., Merck, 1988: Inhibition of the di-zinc MBL from *Bacteriodes fragilis*.[829] - X-ray of L-159,061 in the *B. fragilis* MBL (1A8T). - X-ray of MES (buffer molecule) bound to this same MBL (1A7T). - Toney et al., 1999: A series of biphenyl tetrazoles were not as potent against the *B. fragilis* MBL as L-159,061 (low to high μM IC_{50}s), only a few had IC_{50}s <200 μM against IMP-1, and most had high (100–800 μM) IC_{50}s against DHP-1.[830]
96	 hydroxamates	B	- Schofield group, 2007.[831] - Selectively inhibit FEZ-1 with surprisingly little inhibition of IMP-1, BcII, CphA, and L1. - Very modest inhibitors of FEZ-1 as well, except for the benzophenone derivative shown (K_i of 6.1 μM). Several others with IC_{50} values around 100 μM.
97	 tricyclic natural products SB-236049, SB-236050 SB-238569	B	- Payne, GSK 2002: Screening of natural products for activity against BcII led to a small set of tricyclic natural products which had activity against IMP-1 and CfiA as well (low micromolar K_i values). But little inhibition of the MBL L1 and of serine β-lactamases.[832] - Improve activity of meropenem against MBL-producing strains of *B. fragilis* and *S. maltophilia*, but not *P. aeruginosa*. - X-ray of SB236050 in the MBL from *B. fragilis* CfiA (1HLK, 1KR3).

Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
98	 sultams	C	- Page group, 1996: The carboxy derivative is not an inhibitor of any of the β-lactamases tested.[833] - Page, 2003: Rates of inactivation of P99 (class C) given for the *N*-acyl sultam, but no IC_{50} values.[527]
99	 2-aminopropane-1,2,3-tricarboxylic acid; citrate; isocitrate	A, C	- Marchand-Brynaert, 2008: The amino derivative inhibit BS3 (class A enzyme from *Bacillus licheniformis*) and TEM-1 (class A) with IC_{50}s of 250 μM and 150 μM, respectively – modest, but more potent than citrate and isocitrate.[834] - X-ray of aminocitrate in BS3 (3B3X); X-ray of isocitrate in BS3 (1W7F, to be published); X-ray of citrate in the class C enzyme CMY-2 (1ZC2, to be published). - Galleni, 2010: X-ray of citrate in VIM-4 (2WHG).[343]
100	 Bulgecin A	B	- Simm, Walsh, Bennett, 2005.[835] - Competitive inhibition of the di-zinc form of BcII (K_i = 230 μM), but not the mono-zinc form (K_i >900 μM). - No inhibition of the ImiS, the mono-zinc MBL from *Aeromonas veronii* (K_i >900 μM); Partial non-competitive inhibition of L1 from *S. maltophilia* (K_i = 2.5 μM). - Models suggest that the sulfate could coordinate to the zincs.
101	 carboxythiophene derivatives (fragment-based discovery)	C	- Shoichet, 2002: Non-β-lactam inhibitors of AmpC and X-ray structure with **1** (1L2S).[836] - Shoichet 2005: Several analogues with low micromolar K_i values and X-ray structures of AmpC with **10** (1XGJ) and **11** (1XGI).[837] - Shoichet, 2006: Deconstructing fragment-based inhibitor discovery: X-ray structures of AmpC with fragments 2-carboxythiophene (2HDQ), 4-amino-3-hydroxybenzoic acid (2HDR), 4-methanesulfonylaminobenzoic acid (2HDS), 2-acetamidothiophene-3-carboxylic acid (2HDU).[838]
102	 tetrazoles from fragment-based inhibitor discovery	A,(C)	- Shoichet, 2009: Fragment-based inhibitor discovery: These 2 compounds showed K_i values of 21 and 12 μM against CTX-M (class A) and 2.8 mM vs AmpC (class C).[839] - Strongest non-covalent inhibitors of class A enzymes.
103	 **5**: R = 4-COMe; **8**: R = 2-OH; **1**; **3**; **12**; **20**; **21**; **22**	C	- Shoichet, 2009: Docking for fragment inhibitors of AmpC.[277] - Docked >137,000 fragments, 48 of the highest ranked structures were purchased and tested. 23 had K_i values from 0.7 to 9.2 mM. - X-ray structures were determined for 8 complexes with AmpC: **1** (3GSG), **3** (3GR2), **5** (3GQZ), **8** (3GVB), **12** (3GRJ), **20** (3GTC), **21** (3GV9); **22** was done previously (2HDU).
104	 naphthalene-1,4-dione	A?	- Indian group, 2010: Extracts from the plant *Holoptelea integrifolia* inhibited a 30 kD enzyme (probably a β-lactamase) isolated from amoxicillin-resistant *S. aureus*.[840] - No IC_{50} or K_i values given. - Naphthoquinone was identified by GCMS and IR only.
105	 chalcones	A	- Jaramillo et al., 2009: "K_m' 407 μM" for the best inhibitor.[841]

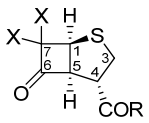
Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
106		C	- Shoichet and Italian groups, 2008: Analysis of hits from HTS and docking screens.[842] - Of the 1274 hits from a library of 70,563 compounds, 95% were found to be detergent-sensitive. Of the 70 remaining compounds, 25 were β-lactams (covalent-acting), 12 were promiscuous covalent inhibitors, and the last 33 were aggregators or irreproducible. Thus, the library produced 0 compounds that were specific reversible inhibitors (desired). - Analogues of a phthalimide hit improved K_is to 8 μM. - X-ray structures of AmpC with an oxadiazole covalently bound (2PU4) and with phthalimides non-covalently bound (2PU2, 2R9W, 2R9X).
107		A	- Miyamura, Ochiai, 1974: A macromolecular β-lactamase inhibitor (M-540) from a species of *Streptomyces* (see ref 396 and refs therein). - Strynadka, *Nature* 1994: Structure and kinetic characterization.[843] - Gretes, Strynadka, 2009: X-ray crystal structures of BLIP alone (3GMU), BLIP-I alone (3GMV), BLIP-I with TEM-1 (3GMW), BLP alone (3GMX), and a selenomethionine BLP (3GMY).[844] - Both BLIP (β-lactamase inhibitory protein) and BLP (BLIP-like-protein) have nanomolar affinities for TEM-1. - Wang et al., 2009.[845] - Bonomo, Handel groups, 2009: KPC-2 with BLIP (3E2L, 3E2K).[846]
108	single-stranded DNA	B	- Kim et al., 2009: Nanomolar K_i values against *B. cereus* 5/B/6 MBL.[847] - Rapid, reversible, non-competitive inhibition.
109		B	- Vella et al., 2011: Fragment-based screening for MBL inhibitors.[848] - K_i values as low as 400 μM against IMP-1.
110	 2,3-methylenepenams	A,C	- Hoffman La Roche, 1983: Antibacterial and β-lactamase inhibiting properties.[849] - Roche, 1988: Interactions with PBPs.[850] - Roche, 1988: Antibacterial activity.[851]
	CYCLOBUTANONES		
C1	 **2** **11**: X = Cl **13**: X = H **14**: X = Cl **4**: X = H		- Gordon, Pluščec, and Ondetti. *Tetrahedron Lett.* **1981**.[528] - Compounds **2**, **4**, **11**, and **13** were also tested for inhibition of R61 transpeptidase and R-TEM β-lactamase and "did not exhibit significant reversible or irreversible enzyme inhibition." - No comments were made concerning the activity of **14**.
C2	 **9b** **9d**		- Meth-Cohn, Reason, and Roberts. *Chem. Commun.* **1982**.[529] - The authors describe only the synthesis of these compounds in the paper and write that "It is hoped that β-lactamase inhibitory activity might be optimized in these derivatives as shown in scheme 3."
C3	 **1** **12** **6**	A	- Lowe and Swain. *Chem. Commun.* **1983** and *Perkin Trans. 1* **1985**.[530,531] - "The mixture of epimeric ketones **1** and **12** showed inhibition of *Streptomyces* R61 D,D-carboxypeptidase only at 260 μg/mL and no significant activity against a range of bacteria at 128 μg/mL. The chloroketone **6** and the epimeric mixture of ketones **1** and **12**, however, showed time-dependent inhibition of *E. coli* R-TEM and *B. cereus* I β-lactamases which may be associated with the slow formation of a tetrahedral adduct between the inhibitor and the enzyme." - Lowe and Swain. In *Recent Advances in the Chemistry of β-Lactams*, **1985**. - No antibacterial activity was observed at a maximum concentration of

Entry	β-Lactamase Inhibitor Structural Type	Target Classes	Comments
			128 mg/L. - The mixture of **1** and **27** did show weak inhibition of *Streptomyces* R61 D,D -carboxypeptidase with 50% inhibition at 260 mg/L (MW = 289.28; IC_{50} = 900 μM). - The mixture of **1** and **27** and **2** each showed slow, time-dependent inhibition of *E. coli* RTEM-2 β-lactamase and β-lactamase type I from *B. cereus* strain 568/H.
C4	 **22** **10**: Z = SOPh **11**: Z = SO_2Ph		- Cocuzza and Boswell (Du Pont). *Tetrahedron Lett.* **1985**.[532] - Synthesis of a cyclobutanone analogue of *N*-acetylthienamycin. - Derivatives with electron-withdrawing groups at C3 were able to acylate benzylamine. - "While no cyclobutanone carboxylic acids (such as **22**) of the present study demonstrated any antibacterial activity, we were pleased that sulfoxide and sulfone benzhydryl esters (such as **10**, **11**, **19**, and **20**) were active against Gram-positive bacteria (with typical MICs of 25–50 μg/mL vs *S. aureus*). Moreover, several sulfide, sulfone, and sulfone esters demonstrated anti-β-lactamase activity [as deduced from synergy with penicillin G against β-lactamase-producing *S. aureus*]."
C5	 R = H, $CHPh_2$	A	- Boswell and Cocuzza. US Patent **1985**; *Chem. Abstr.* **1985**.[533] - Synthetic and biological procedures are given with the tables of synergy data below. - None of the free acids showed synergy but many of the benzhydryl esters did show synergy with Penicillin G against *S. aureus* and *B. cereus*.
C7	 **7**: R = H **27**: R = Et	PBP	- Lange, Savard, Viswanatha, Dmitrienko. *Tetrahedron Lett.* **1985**: Synthesis of the 7,7-dichloro-2-thiabicyclo[3.2.0]heptan-6-one-4-carboxylate system described but no biochemical data given in the paper.[534] - No significant inhibition of TEM-1 or BcI was observed (unpublished results, Dmitrienko and Viswanatha groups). - Kelly, Knox, Moews, Hite, Bartolone, and Zhao. *J. Biol. Chem.* **1985**: Reported weak competitive inhibition of R61 transpeptidase (K_i = 1 mM); 4 Å resolution X-ray structure of a complex with R61 D-alanyl carboxypeptidas-transpeptidase.[535] (Synthesis of this compound was done by Tomczuk).[537]
C8			- Johnson, Evanoff, Savard, Lange, Ramadhar, Assoud, Taylor, Dmitrienko. *J. Org. Chem.* **2008**.[568] - Synthetic studies, conformational properties, and study of hydration and hemiketal formation, but no biochemical results described.
C9	 **1**: X = Cl **2**: X = H **5**: X = Cl **6**: X = H **4α** **4β**	A, B, C, D	- Johnson, Gretes, Goodfellow, Marrone, Heynen, Strynadka, Dmitrienko. *J. Am. Chem. Soc.* **2010**.[640] - Low-micromolar IC_{50}s against KPC-2 (aESBL) and GC1 (cESBL). - Mid-micromolar IC_{50}s against IMP-1 (MBL) and OXA-10 (class D). - Dichlorocyclobutanones are better inhibitors of serine enzymes than the dechlorinated compounds, but **2** was reasonable against IMP-1. - Compounds that favour the carboxylate in an equatorial orientation are better inhibitors that those with axial carboxylates. - X-ray structure of **4α** bound to OXA-10 (3LCE). - The unsaturated acid **5** enhanced the antimicrobial activity of meropenem against carbapenem-resistant clinical isolates.

Appendix D

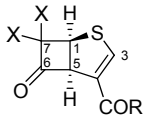
Selected Spectral Data for Cyclobutanone Derivatives

Selected ^{1}H NMR, ^{13}C NMR, and IR Data for Cyclobutanones.[a]



	X	COR	δ H_1	δ $H_{3\alpha}$	δ $H_{3\beta}$	δ H_4	δ H_5	IR ketone	IR COR
65[b]	Cl	CO_2H	4.77	3.37	2.88	3.75	5.14	1810	1700
154	Cl	COCl	4.55	3.54	3.13	4.03	5.02	1809	1780
84	Cl	CO_2Et	4.52	3.45	3.05	3.66	5.07	1810	1732
149	Cl	CO_2CHPh_2	4.50	3.50	3.09	3.76	5.07	1808	1738
110	H	CO_2Et	4.15	3.45	3.21	3.57	4.64	1784	1732
111[b]	H	CO_2H	4.17	3.44	3.19	3.55	4.64	1780	1705

	X	COR	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7	δ COR
65[b]	Cl	CO_2H	59.5	36.3	50.7	68.6	196.0	90.2	171.3
154	Cl	COCl	58.3	35.4	60.3	66.8	192.9	89.1	172.0
84	Cl	CO_2Et	58.9	35.6	50.1	67.4	194.8	89.2	169.8
149	Cl	CO_2CHPh_2	58.9	35.6	50.3	67.4	194.6	89.3	169.0
110	H	CO_2Et	36.9	35.3	50.0	71.5	208.4	56.5	170.8
111[b]	H	CO_2H	37.5	35.6	50.3	72.3	208.4	56.6	172.4

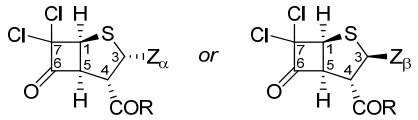


	X	COR	δ H_1	δ H_3	δ H_5	IR ketone	IR COR
118	Cl	CO_2Et	4.89	7.43	5.43	1812	1704
153[b]	Cl	CO_2H	5.23	7.68	5.62	1807	1700
126	H	CO_2Et	4.15	7.43	5.15	1790	1700
156	H	CO_2H	4.39	7.59	5.15	nd	nd

	X	COR	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7	δ COR
118	Cl	CO_2Et	59.6	144.4	122.1	71.3	187.4	93.7	161.1
153[b]	Cl	CO_2H	60.1	146.1	123.3	72.8	189.5	94.8	162.3
126	H	CO_2Et	37.0	143.2	121.2	76.0	200.0	60.7	161.8
156	H	CO_2H	38.1	144.6	122.5	77.0	201.1	61.6	163.0

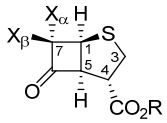
(a) NMR spectra were acquired in $CDCl_3$ except where indicated.

(b) Acetone-d_6 was the solvent used for NMR.



	α-Z	COR	δ H_1	δ $H_{3\beta}$	δ H_4	δ H_5	IR ketone	IR COR
117α	Cl	CO_2Et	4.95	5.88	3.97	5.21	1815	1740
150α	Cl	CO_2CHPh_2	4.93	5.94	4.08	5.18	nd	nd
155α	Cl	COCl	4.99	6.03	4.37	5.13	nd	nd
119α[c]	OH	CO_2Et	4.79	5.83	3.71	5.09	na	na
121α	OMe	CO_2Et	4.66	5.32	3.75	5.17	1813	1739
151α	OMe	CO_2CHPh_2	4.66	5.39	3.91	5.21	1813	1744
152α[b]	OMe	CO_2H	4.77	5.57	3.84	5.13	1813	1720
122α	O*i*-Pr	CO_2Et	4.69	5.54	3.73	5.20	1811	1739
123α	O*t*-Bu	CO_2Et	4.78	5.76	3.70	5.12	1808	1739
164α	S-*i*-Pr	CO_2Et	4.70	4.67	3.75	4.90	nd	nd
165α	S-*p*-Tol	CO_2Et	4.57	4.90	3.85	4.95	nd	nd
166α	Allyl	CO_2Et	4.61	4.70	3.56	4.82	nd	nd
	β-Z	COR	δ H_1	δ $H_{3\alpha}$	δ H_4	δ H_5	IR ketone	IR COR
117β	Cl	CO_2Et	4.72	6.14	4.08	5.15	nd	nd
119β	OH	CO_2Et	4.67	5.98	3.83	5.05	1815	1732
121β	OMe	CO_2Et	4.60	5.46	3.82	5.02	1817	1734
151β	OMe	CO_2CHPh_2	4.58	5.51	3.93	5.03	1816	1741
152β[b]	OMe	CO_2H	4.78	5.61	3.90	5.12	1814	1703
122β	O*i*-Pr	CO_2Et	4.59	5.71	3.76	5.03	1818	1734
123β	O*t*-Bu	CO_2Et	4.58	5.79	3.61	4.98	1818	1735
124β	OAc	CO_2Et	4.62	6.51	3.79	5.10	1813	1740-7
125β	OTFE	CO_2Et	4.64	5.76	3.93	5.08	1819	1738
164β	S-*i*-Pr	CO_2Et	4.60	5.10	3.72	4.98	nd	nd
165β	S-*p*-Tol	CO_2Et	4.62	5.33	3.71	4.99	nd	nd
166β	Allyl	CO_2Et	4.56	3.95	3.47	4.95	nd	nd

	α-Z	COR	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7	δ COR
117α	Cl	CO_2Et	60.0	74.1	59.8	65.5	192.0	85.0	166.2
150α	Cl	CO_2CHPh_2	59.8	73.7	60.1	65.5	191.9	85.1	165.4
155α	Cl	COCl	60.0	72.9	69.4	64.1	190.5	84.7	167.2
119α[c]	OH	CO_2Et	58.7	87.0	57.0	65.7	192.3	86.2	166.8
121α	OMe	CO_2Et	58.7	97.4	57.4	66.4	193.4	85.3	167.3
151α	OMe	CO_2CHPh_2	58.6	97.3	57.4	66.3	193.1	85.2	166.4
152α[b]	OMe	CO_2H	58.8	98.5	58.2	67.7	194.7	86.7	168.6
122α	O*i*-Pr	CO_2Et	58.7	93.4	57.6	66.7	193.7	85.3	167.3
123α	O*t*-Bu	CO_2Et	58.8	89.0	57.9	66.6	193.7	85.9	167.5
	β-Z	COR	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7	δ COR
117β	Cl	CO_2Et	61.4	71.5	62.3	65.9	192.7	89.7	167.1
119β	OH	CO_2Et	59.8	86.0	59.3	65.0	193.1	90.4	167.9
121β	OMe	CO_2Et	59.4	95.2	57.9	64.9	192.7	90.4	167.8
151β	OMe	CO_2CHPh_2	59.4	95.0	58.1	64.9	192.5	90.5	167.0
152β[b]	OMe	CO_2H	60.0	96.4	58.6	66.2	193.8	91.5	169.4
122β	O*i*-Pr	CO_2Et	59.5	90.1	57.9	65.3	192.3	90.2	168.2
123β	O*t*-Bu	CO_2Et	59.6	86.6	59.6	65.3	193.0	90.6	168.2
124β	OAc	CO_2Et	59.6	85.8	56.3	65.6	192.9	90.1	167.2
125β	OTFE	CO_2Et	59.7	94.2	57.9	65.1	191.6	90.0	167.2
165β	S-*p*-Tol	CO_2Et	60.8*	61.0*	56.8*	66.3	193.9	89.8	168.8

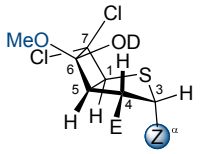


	$X_{7\alpha}$	$X_{7\beta}$	COR	δ H_1	δ $H_{3\alpha}$	δ $H_{3\beta}$	δ H_4	δ H_5
65[b]	Cl	Cl	CO_2H	4.77	3.37	2.88	3.75	5.14
84	Cl	Cl	CO_2Et	4.52	3.45	3.05	3.66	5.07
110	H	H	CO_2Et	4.15	3.45	3.21	3.57	4.64
111[b]	H	H	CO_2H	4.17	3.44	3.19	3.55	4.64
183α[c]	Cl	H	CO_2Et	4.09	3.50	3.20	3.61	4.94
184α[b,c]	Cl	H	CO_2H	4.18	3.55	3.37	3.74	4.9
183β	H	Cl	CO_2Et	4.46	3.36	3.01	3.60	4.68
184β[b]	H	Cl	CO_2H	4.57	3.38	2.97	3.65	4.79
185β[d]	H	Cl	CO_2CHPh_2	4.46	3.38	2.98	3.74	4.73
187	CH_2OH	Cl	CO_2Et	4.35	3.38	3.02	3.58	4.69
188[b]	CH_2OH	Cl	CO_2H	4.48	3.41	2.96	3.64	4.70

(c) This compound was observed by NMR only, and was not isolated.

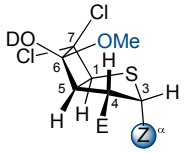
(d) CD_3CN was the solvent used for NMR.

Selected ^{1}H NMR and ^{13}C NMR Data for Cyclobutanone Hemiketals.[e]



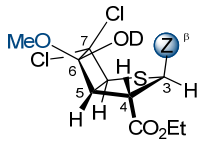
α-hemiketal

ketone	Z	δ H_1	δ $H_{3\beta}$	δ H_4	δ H_5	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7
121α	OMe	4.31	5.39	3.69	3.91	58.9	96.9	55.2	52.2	99.2	93.1
122α	O*i*-Pr	4.34	5.64	3.68	3.93	58.9	92.8	55.2	52.5	99.3	93.1
123α	O*t*-Bu	4.39	5.90	3.60	3.91	59.2	88.5	55.5	53.0	99.4	93.6



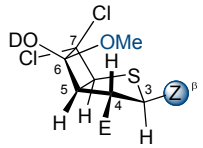
β-hemiketal

ketone	Z	δ H_1	δ $H_{3\beta}$	δ H_4	δ H_5	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7
121α	OMe	4.28	5.40	3.63	3.79	58.2	97.0	55.3	55.9	97.8	93.9
122α	O*i*-Pr	4.31	5.65	3.62	3.81	58.2	92.9	55.4	56.2	97.9	94.1
123α	O*t*-Bu	4.36	5.85	3.62	3.85	58.4	88.8	55.8	56.6	98.1	94.2



α-hemiketal

ketone	Z	δ H_1	δ $H_{3\alpha}$	δ H_4	δ H_5	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7
121β	OMe	4.47	5.70	3.72	4.15	61.6	97.2	55.6	57.6	-	-
122β	O*i*-Pr	4.45	5.90	3.65	4.13	61.5	92.5	55.8	57.4	100.8	95.6
123β	O*t*-Bu	4.35	5.95	3.58	3.93	60.9	88.7	56.6	55.8	100.6	95.2
124β	OAc	4.34	6.47	3.79	3.86	61.5	88.6	56.3	63.9	-	-



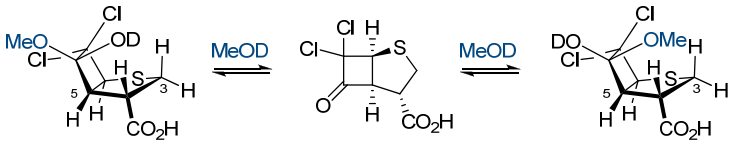
β-hemiketal

ketone	Z	δ H_1	δ $H_{3\alpha}$	δ H_4	δ H_5	δ C_1	δ C_3	δ C_4	δ C_5	δ C_6	δ C_7
121β	OMe	4.28	5.68	3.64	3.56	-	-	-	-	-	-
122β	O*i*-Pr	4.18	5.68	3.57	3.42	-	-	-	-	-	-
123β	O*t*-Bu	4.16	5.79	3.53	3.37	58.4	90.3	54.9	55.0	98.4	94.2
124β	OAc	4.31	6.45	3.82	3.62	58.5	88.9	53.5	60.8	-	-

(e) NMR spectra were acquired in methanol-d_4 (^{1}H: 500 MHz; ^{13}C: 125 MHz).

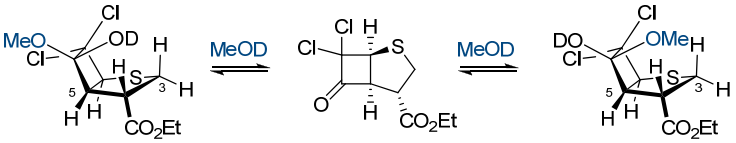
Appendix E

Hemiketal Formation with Cyclobutanones in Methanol-d_4 [f,g]



α-hemiketal **65** *β-hemiketal*

time (h)	ketone **65**[h]	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.17	1.00	0.43	0.00	69.9	30.1	0.0	30.1
1.08	0.29	1.00	0.05	21.6	74.6	3.7	78.4
25	0.18	1.00	0.16	13.4	74.6	11.9	86.6
167	0.18	1.00	0.35	11.8	65.4	22.9	88.2
284	0.18	1.00	0.37	11.6	64.5	23.9	88.4
312	0.18	1.00	0.38	11.5	64.1	24.4	88.5



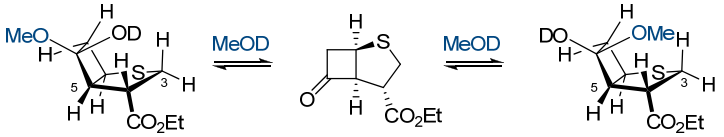
α-hemiketal **84** *β-hemiketal*

time (h)	ketone **84**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.83	1.00	2.55	0.00	28.2	71.8	0.0	71.8
14	0.11	1.00	0.06	9.4	85.5	5.1	90.6
194	0.13	1.00	0.31	9.0	69.4	21.5	91.0
284	0.13	1.00	0.37	8.7	66.7	24.7	91.3

(f) ^{1}H NMR spectra were acquired at 500 MHz as overlapping peaks in the 300 MHz spectra made integrations unreliable.

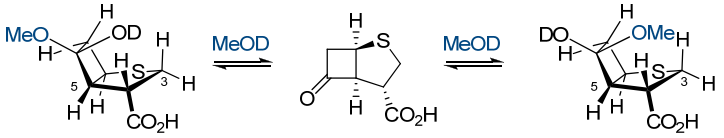
(g) The data tabulated in this Appendix is also presented graphically in the supporting information for reference 568.

(h) The values listed for ketones, α-hemiketals, and β-hemiketals are integrations that were used for the calculation of relative percentages.



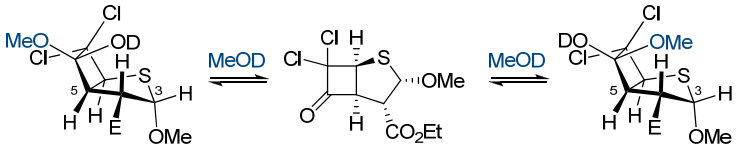
α-hemiketal **110** *β-hemiketal*

time (h)	ketone **110**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.25	1.00	0.20	0.00	83.3	16.7	0.0	16.7
16	1.00	0.18	0.03	82.6	14.9	2.5	17.4
112	1.00	0.16	0.07	81.3	13.0	5.7	18.7
142	1.00	0.16	0.09	80.0	12.8	7.2	20.0
163	1.00	0.16	0.09	80.0	12.8	7.2	20.0
324	1.00	0.16	0.09	80.3	12.9	6.8	19.7



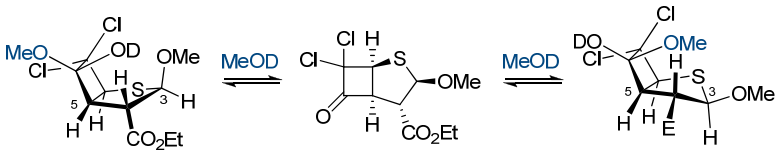
α-hemiketal **111** *β-hemiketal*

time (h)	ketone **111**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
13	1.00	0.20	0.12	75.8	15.2	9.1	24.2
25	1.00	0.20	0.12	75.8	15.2	9.1	24.2
46	1.00	0.20	0.12	75.8	15.2	9.1	24.2
192	1.00	0.20	0.12	75.8	15.2	9.1	24.2



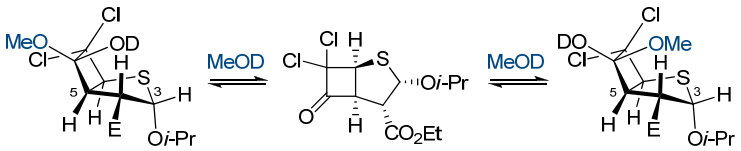
α-hemiketal **121α** *β-hemiketal*

time (h)	ketone **121α**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.08	0.25	1.00	0.04	19.4	77.5	3.1	80.6
0.17	0.06	1.00	0.04	5.5	90.9	3.6	94.5
0.50	0.04	1.00	0.05	3.2	92.2	4.6	96.8
2.4	0.03	0.96	0.08	2.8	89.7	7.5	97.2
23	0.03	0.81	0.25	2.8	74.3	22.9	97.2
163	0.03	1.00	0.82	1.6	54.1	44.3	98.4
181	0.03	1.00	0.82	1.6	54.1	44.3	98.4



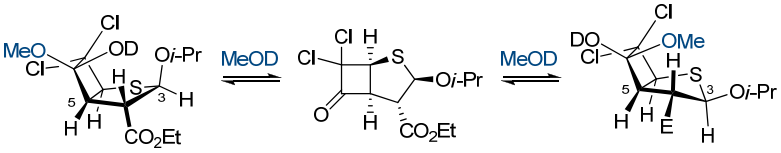
α-hemiketal **121β** *β-hemiketal*

time (h)	ketone **121β**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.17	1.00	0.00	0.00	100	0.0	0.0	0.0
3.0	1.00	0.03	0.00	97.1	2.9	0.0	2.9
18	1.00	0.09	0.00	91.7	8.3	0.0	8.3
168	1.00	0.13	0.03	86.2	11.2	2.6	13.8
408	0.98	0.14	0.03	85.2	12.2	2.6	14.8



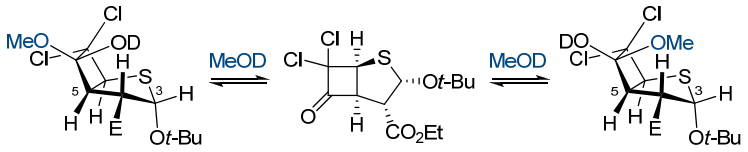
α-hemiketal **122α** *β-hemiketal*

time (h)	ketone **122α**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
1.0	0.05	0.96	0.07	4.6	88.9	6.5	95.4
3.0	0.04	0.93	0.10	3.7	86.9	9.3	96.3
18	0.04	0.80	0.25	3.7	73.4	22.9	96.3
144	0.03	0.55	0.50	2.8	50.9	46.3	97.2
168	0.025	0.55	0.50	2.3	51.2	46.5	97.7
180	0.025	0.55	0.50	2.3	51.2	46.5	97.7



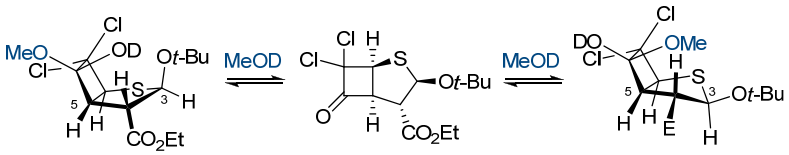
α-hemiketal **122β** *β-hemiketal*

time (h)	ketone **122β**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.50	1.00	0.00	0.00	100	0.0	0.0	0.0
7.0	1.00	0.07	0.00	93.5	6.5	0.0	6.5
24	1.00	0.18	0.00	84.7	15.3	0.0	15.3
42	0.99	0.23	0.01	80.5	18.7	0.8	19.5
168	0.96	0.23	0.03	78.7	18.9	2.5	21.3
192	0.95	0.24	0.03	77.9	19.7	2.5	22.1
2000	1.00	0.25	0.06	76.3	19.1	4.6	23.7



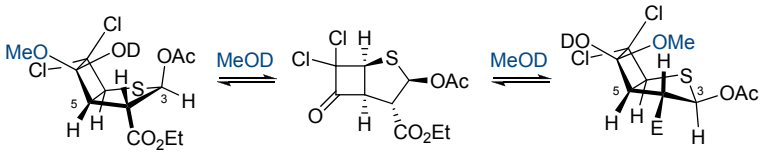
α-hemiketal **123α** *β-hemiketal*

time (h)	ketone **123α**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.33	0.06	1.00	0.06	5.4	89.3	5.4	94.6
0.50	0.04	1.00	0.06	3.6	90.9	5.5	96.4
1.5	0.04	1.00	0.07	3.6	90.1	6.3	96.4
23	0.04	1.00	0.32	2.9	73.5	23.5	97.1
168	0.04	1.00	0.87	2.1	52.4	45.5	97.9
288	0.04	1.00	0.87	2.1	52.4	45.5	97.9



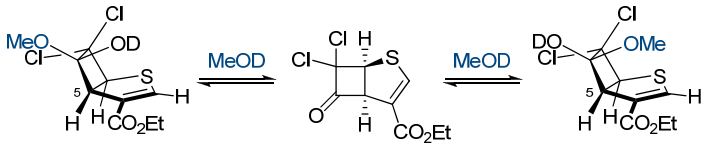
α-hemiketal **123β** *β-hemiketal*

time (h)	ketone **123β**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
0.17	1.00	0.02	0.00	98.0	2.0	0.0	2.0
1.0	1.00	0.05	0.00	95.2	4.8	0.0	4.8
2.0	1.00	0.09	0.01	90.9	8.2	0.9	9.1
24	1.00	0.41	0.12	65.4	26.8	7.8	34.6
168	1.00	0.41	0.23	61.0	25.0	14.0	39.0
360	1.00	0.42	0.24	60.2	25.3	14.5	39.8



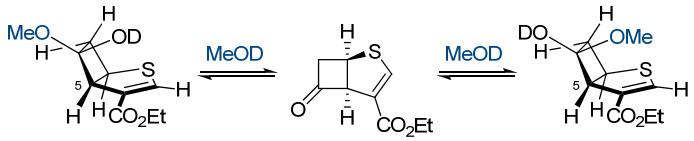
α-hemiketal **124β** *β-hemiketal*

time (h)	ketone **124β**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
1.0	0.96	0.07	0.00	93.2	6.8	0.0	6.8
7.0	0.87	0.18	0.02	81.3	16.8	1.9	18.7
119	0.98	0.22	0.14	73.1	16.4	10.4	26.9
293	0.95	0.24	0.18	69.3	17.5	13.1	30.7



α-hemiketal **118** *β-hemiketal*

time (h)	ketone **118**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
2.5	0.05	1.00	0.13	4.2	84.7	11.0	95.8
18	0.05	1.00	0.30	3.7	74.1	22.2	96.3
92	0.06	1.00	0.52	3.8	63.3	32.9	96.2
144	0.06	1.00	0.55	3.7	62.1	34.2	96.3

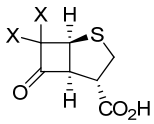


α-hemiketal **126** *β-hemiketal*

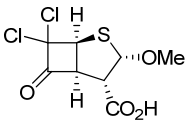
time (h)	ketone **126**	α-hemi-ketal	β-hemi-ketal	% ketone	% α-hemiketal	% β-hemiketal	total % hemiketal
0	1.00	0.00	0.00	100	0.0	0.0	0.0
1.0	1.00	0.33	0.00	75.2	24.8	0.0	24.8
2.8	1.00	0.34	0.00	74.6	25.4	0.0	25.4
17	1.00	0.33	0.03	73.5	24.3	2.2	26.5
162	1.00	0.35	0.16	66.2	23.2	10.6	33.8
336	1.00	0.36	0.24	62.5	22.5	15.0	37.5

Appendix F

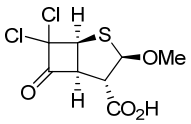
Corrected IC_{50} Values for the Inhibition of β-Lactamases by Cyclobutanones[a]



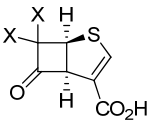
65: X = Cl
111: X = H



152α



152β



153: X = Cl
156: X = H

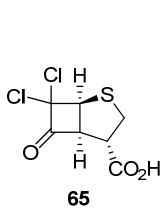
ketone inhibitor	% hydrate in D_2O[b]	KPC-2 (class A)		IMP-1 (class B)		GC1 (class C)		OXA-10 (class D)	
		obsd	(corrd)[c]	obsd	(corrd)	obsd	(corrd)	obsd	(corrd)
65	74	76	(20)	>1000	(>260)	25	(6.5)	268	(70)
111	0	117	(117)	235	(235)	44	(44)	1135	(1135)
152α	>98	58	(<1.2)	122	(<2.4)	6.5	(<0.13)	156	(<3.1)
152β	6	99	(93)	nd[d]	na[e]	38	(36)	547	(514)
153	93	26	(1.8)	213	(15)	4.5	(0.32)	370	(26)
156	<2	170	(>167)	>500	(>490)	34	(>33)	>1000	(>980)

[a] IC_{50} values (μM). Inhibition was assayed by monitoring nitrocefin hydrolysis. [b] Acetone-d_6 was used as a cosolvent for solubility purposes. [c] corrd = corrected. [d] nd = not determined. [e] na = not available.

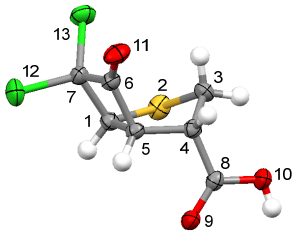
Appendix G

Tables of Crystallographic Data

Crystal Data and Structure Refinement for Cyclobutanone **65**.[852]

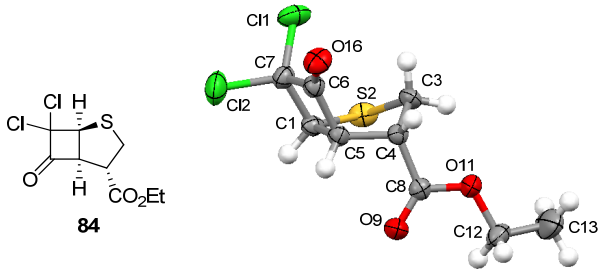


65



Empirical formula	$C_7H_6Cl_2O_3S$
Formula weight	241.09
Temperature	180(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 6.0993(3) Å, b = 8.1188(4) Å, c = 10.5373(5) Å
	α = 73.8750(10)°, β = 77.5700(10)°, γ = 71.4820(10)°
Volume, Z	470.67(4) $Å^3$, 2
Density (calculated)	1.701
Absorption coefficient	0.879 mm^{-1}
F(000)	244
Crystal size	0.44 mm × 0.30 mm × 0.15 mm
θ range for data collection	2.03 to 29.99°
Limiting indices	$-8 \le h \le 8$, $-11 \le k \le 11$, $-14 \le l \le 14$
Reflections collected	5854
Independent reflections	2745 (R_{int} = 0.0341)
Completeness to θ = 29.99	99.6 %
Absorption correction	Integration
Max. and min. transmission	0.888 and 0.671
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2745 / 0 / 123
Goodness-of-fit on F^2	1.062
Final R indices [I>2σ(I)]	R1 = 0.0299, wR2 = 0.0784
R indices (all data)	R1 = 0.0325, wR2 = 0.0800
Extinction coefficient	0.070(6)
Largest diff. peak and hole	0.320 and –0.460 e·$Å^{-3}$

Crystal Data and Structure Refinement for Cyclobutanone **84**.



Empirical formula	$C_9H_{10}Cl_2O_3S$
Formula weight	269.13
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 10.845(4) Å, b = 11.266(4) Å, c = 12.189(4) Å
	α = 107.842(4)°, β = 103.885(4)°, γ = 113.162(4)°
Volume, Z	1187.6(7) $Å^3$, 4
Density (calculated)	1.505
Absorption coefficient	0.706 mm^{-1}
F(000)	552
Crystal size	0.46 mm × 0.40 mm × 0.02 mm
θ range for data collection	3.35 to 28.00°
Limiting indices	$-14 \le h \le 14$, $-14 \le k \le 14$, $-16 \le l \le 16$
Reflections collected	14463
Independent reflections	5699 (R_{int} = 0.0259)
Completeness to θ = 28.00	99.5%
Absorption correction	Empirical
Max. and min. transmission	0.9860 and 0.7373
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5699 / 6 / 277
Goodness-of-fit on F^2	1.250
Final R indices [I>2σ(I)]	R1 = 0.0377, wR2 = 0.0772
R indices (all data)	R1 = 0.0495, wR2 = 0.0826
Largest diff. peak and hole	0.397 and –0.439 e.$Å^{-3}$

Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **84**.[a,b]

	x/a	y/b	z/c	U_{eq}
C(1A)	5983(2)	9628(2)	7031(2)	29(1)
S(2A)	4757(1)	7787(1)	5861(1)	37(1)
C(3A)	3712(2)	7420(2)	6973(2)	37(1)
C(4A)	4853(2)	8269(2)	8165(2)	32(1)
C(5A)	5908(2)	9751(2)	8327(2)	31(1)
C(6A)	5250(2)	10719(2)	8295(2)	33(1)
C(7A)	5444(2)	10735(2)	7081(2)	30(1)
C(8A)	5686(2)	7514(2)	8457(2)	35(1)
O(9A)	6994(2)	8031(2)	8864(2)	48(1)
O(11A)	4750(2)	6177(2)	8198(2)	54(1)
C(12A)	5361(3)	5276(3)	8377(3)	68(1)
C(13A)	4220(4)	3834(4)	7844(5)	76(1)
C(13C)	4375(14)	4048(13)	6898(16)	76(1)
Cl(1A)	6876(1)	12451(1)	7424(1)	44(1)
Cl(2A)	3817(1)	10208(1)	5840(1)	38(1)
O(16A)	4720(2)	11226(2)	8906(2)	50(1)
C(1B)	1266(2)	1768(2)	3153(2)	34(1)
S(2B)	1143(1)	798(1)	4079(1)	43(1)
C(3B)	–798(2)	–480(2)	3097(2)	38(1)
C(4B)	–996(2)	–730(2)	1744(2)	31(1)
C(5B)	–57(2)	746(2)	1825(2)	31(1)
C(6B)	–663(2)	1763(2)	2108(2)	35(1)
C(7B)	703(2)	2866(2)	3374(2)	39(1)
C(8B)	–549(2)	–1798(2)	1137(2)	32(1)
O(9B)	294(2)	–1595(2)	645(2)	45(1)
O(11B)	–1259(2)	–3023(2)	1211(2)	42(1)
C(12B)	–965(3)	–4170(2)	652(2)	46(1)
C(13B)	–1525(4)	–5252(3)	1106(4)	87(1)
Cl(1B)	291(1)	3333(1)	4702(1)	58(1)
Cl(2B)	1817(1)	4446(1)	3282(1)	69(1)
O(16B)	–1771(2)	1731(2)	1612(2)	49(1)

[a] Two crystallographically different molecules of cyclobutanone **84** are present in the unit cell. Atoms are labeled with either A or B to differentiate the two molecules. [b] Atoms C13A and C13C are disordered with 75% and 25% occupancy, respectively.

Bond Lengths (Å) for Cyclobutanone **84**.

	Length [Å]		Length [Å]
C(1A)–C(7A)	1.564(3)	C(6A)–C(7A)	1.548(3)
C(1A)–C(5A)	1.568(3)	C(7A)–Cl(2A)	1.7603(19)
C(1A)–S(2A)	1.792(2)	C(7A)–Cl(1A)	1.776(2)
S(2A)–C(3A)	1.813(2)	C(8A)–O(9A)	1.197(2)
C(3A)–C(4A)	1.529(3)	C(8A)–O(11A)	1.323(3)
C(4A)–C(8A)	1.517(3)	O(11A)–C(12A)	1.453(3)
C(4A)–C(5A)	1.524(3)	C(12A)–C(13A)	1.407(4)
C(5A)–C(6A)	1.525(3)	C(12A)–C(13C)	1.640(16)
C(6A)–O(16A)	1.189(2)		

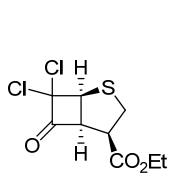
Bond Lengths (Å) for Cyclobutanone **84** (continued).

	Length [Å]		Length [Å]
C(1B)–C(7B)	1.563(3)	C(6B)–O(16B)	1.190(2)
C(1B)–C(5B)	1.565(3)	C(6B)–C(7B)	1.549(3)
C(1B)–S(2B)	1.788(2)	C(7B)–Cl(1B)	1.763(2)
S(2B)–C(3B)	1.813(2)	C(7B)–Cl(2B)	1.771(2)
C(3B)–C(4B)	1.531(3)	C(8B)–O(9B)	1.196(2)
C(4B)–C(8B)	1.521(3)	C(8B)–O(11B)	1.334(2)
C(4B)–C(5B)	1.526(3)	O(11B)–C(12B)	1.458(3)
C(5B)–C(6B)	1.524(3)	C(12B)–C(13B)	1.466(4)

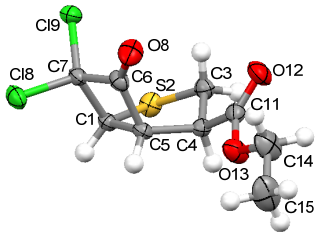
Bond Angles (°) for Cyclobutanone **84**.

	Angle [°]		Angle [°]
C(7A)–C(1A)–C(5A)	89.88(14)	C(7B)–C(1B)–C(5B)	89.97(15)
C(7A)–C(1A)–S(2A)	118.02(13)	C(7B)–C(1B)–S(2B)	118.36(15)
C(5A)–C(1A)–S(2A)	108.40(13)	C(5B)–C(1B)–S(2B)	107.91(13)
C(1A)–S(2A)–C(3A)	92.10(10)	C(1B)–S(2B)–C(3B)	92.70(10)
C(4A)–C(3A)–S(2A)	105.15(14)	C(4B)–C(3B)–S(2B)	105.68(13)
C(8A)–C(4A)–C(5A)	110.24(16)	C(8B)–C(4B)–C(5B)	111.03(16)
C(8A)–C(4A)–C(3A)	111.20(17)	C(8B)–C(4B)–C(3B)	112.43(16)
C(5A)–C(4A)–C(3A)	106.72(15)	C(5B)–C(4B)–C(3B)	106.41(16)
C(4A)–C(5A)–C(6A)	114.76(16)	C(4B)–C(5B)–C(6B)	114.46(16)
C(4A)–C(5A)–C(1A)	109.64(16)	C(4B)–C(5B)–C(1B)	110.79(16)
C(6A)–C(5A)–C(1A)	89.15(14)	C(6B)–C(5B)–C(1B)	89.20(15)
O(16A)–C(6A)–C(5A)	135.39(19)	O(16B)–C(6B)–C(5B)	135.4(2)
O(16A)–C(6A)–C(7A)	132.44(19)	O(16B)–C(6B)–C(7B)	132.4(2)
C(5A)–C(6A)–C(7A)	92.08(15)	C(5B)–C(6B)–C(7B)	92.03(15)
C(6A)–C(7A)–C(1A)	88.46(14)	C(6B)–C(7B)–C(1B)	88.39(15)
C(6A)–C(7A)–Cl(2A)	113.93(14)	C(6B)–C(7B)–Cl(1B)	114.36(15)
C(1A)–C(7A)–Cl(2A)	118.90(14)	C(1B)–C(7B)–Cl(1B)	118.46(15)
C(6A)–C(7A)–Cl(1A)	110.80(13)	C(6B)–C(7B)–Cl(2B)	111.26(15)
C(1A)–C(7A)–Cl(1A)	112.28(13)	C(1B)–C(7B)–Cl(2B)	112.86(15)
Cl(2A)–C(7A)–Cl(1A)	110.67(10)	Cl(2B)–C(7B)–Cl(1B)	109.97(12)
O(9A)–C(8A)–O(11A)	124.08(19)	O(9B)–C(8B)–O(11B)	124.26(19)
O(9A)–C(8A)–C(4A)	125.93(19)	O(9B)–C(8B)–C(4B)	125.78(18)
O(11A)–C(8A)–C(4A)	109.99(18)	O(11B)–C(8B)–C(4B)	109.95(16)
C(8A)–O(11A)–C(12A)	117.28(19)	C(8B)–O(11B)–C(12B)	116.70(16)
C(13A)–C(12A)–O(11A)	109.8(3)	C(13B)–C(12B)–O(11B)	108.3(2)
C(13A)–C(12A)–C(13C)	49.1(5)		
O(11A)–C(12A)–C(13C)	92.7(4)		

Crystal Data and Structure Refinement for Cyclobutanone **109**.



109



Empirical formula	$C_9H_{10}Cl_2O_3S$
Formula weight	269.13
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 5.316(12) Å, b = 14.52(3) Å, c = 14.90(3) Å α = 84.58(4)°, β = 89.84(4)°, γ = 88.88(4)°
Volume, Z	1145(4) $Å^3$, 4
Density (calculated)	1.562
Absorption coefficient	0.732 mm^{-1}
F(000)	552
Crystal size	0.40 mm × 0.10 mm × 0.04 mm
θ range for data collection	2.97 to 25.00°
Limiting indices	$-6 \le h \le 6$, $-17 \le k \le 17$, $-11 \le l \le 17$
Reflections collected	5769
Independent reflections	3859 (R_{int} = 0.0168)
Completeness to θ = 25.00	95.4%
Max. and min. transmission	0.9713 and 0.7583
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3859 / 0 / 272
Goodness-of-fit on F^2	1.131
Final R indices [I>2σ(I)]	R1 = 0.0586, wR2 = 0.0864
R indices (all data)	R1 = 0.0783, wR2 = 0.0930
Extinction coefficient	0.0006(3)
Largest diff. peak and hole	0.264 and –0.220 $e.Å^{-3}$

Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **109**.[a]

	x/a	y/b	z/c	U_{eq}
C(1A)	4985(7)	46(3)	1477(3)	47(1)
S(2A)	6062(2)	1012(1)	728(1)	63(1)
C(3A)	8007(8)	1452(3)	1560(3)	58(1)
C(4A)	6617(7)	1247(3)	2425(3)	48(1)
C(5A)	5569(7)	267(3)	2440(3)	46(1)
C(6A)	7504(8)	–504(3)	2499(3)	54(1)
C(7A)	6662(7)	–837(3)	1621(3)	49(1)
Cl(8A)	8974(2)	–981(1)	841(1)	68(1)
Cl(9A)	4908(2)	–1859(1)	1862(1)	72(1)
O(8A)	9191(6)	–706(2)	2990(2)	87(1)
C(11A)	8165(9)	1361(3)	3245(3)	57(1)
O(12A)	10335(6)	1527(3)	3227(3)	85(1)
O(13A)	6792(6)	1280(3)	3958(2)	79(1)
C(14A)	7969(11)	1400(5)	4813(4)	102(2)
C(15A)	6082(12)	1391(5)	5472(4)	114(2)
C(1B)	–8(7)	4955(3)	3526(3)	46(1)
S(2B)	1063(2)	3987(1)	4271(1)	64(1)
C(3B)	2998(8)	3547(3)	3450(3)	59(1)
C(4B)	1609(7)	3747(3)	2573(3)	49(1)
C(5B)	577(7)	4739(3)	2562(3)	45(1)
C(6B)	2516(8)	5497(3)	2494(3)	53(1)
C(7B)	1656(7)	5832(3)	3374(3)	50(1)
Cl(8B)	–93(2)	6859(1)	3138(1)	72(1)
Cl(9B)	3976(2)	5984(1)	4159(1)	68(1)
O(8B)	4195(6)	5705(2)	2015(2)	85(1)
C(11B)	3169(9)	3630(3)	1768(3)	58(1)
O(12B)	5338(6)	3474(3)	1771(3)	86(1)
O(13B)	1793(6)	3719(3)	1040(2)	80(1)
C(14B)	2962(11)	3593(5)	198(4)	103(2)
C(15B)	1080(13)	3617(5)	–475(4)	118(2)

[a] Two crystallographically different molecules of cyclobutanone **109** are present in the unit cell. Atoms are labeled with either A or B to differentiate the two molecules.

Bond Lengths (Å) for Cyclobutanone **109**.

	Length [Å]		Length [Å]
C(1A)–C(7A)	1.545(6)	C(6A)–O(8A)	1.173(5)
C(1A)–C(5A)	1.534(6)	C(6A)–C(7A)	1.510(6)
C(1A)–S(2A)	1.809(5)	C(7A)–Cl(8A)	1.712(5)
S(2A)–C(3A)	1.787(5)	C(7A)–Cl(9A)	1.775(5)
C(3A)–C(4A)	1.492(6)	C(11A)–O(12A)	1.183(5)
C(4A)–C(11A)	1.499(6)	C(11A)–O(13A)	1.284(6)
C(4A)–C(5A)	1.537(6)	O(13A)–C(14A)	1.448(6)
C(5A)–C(6A)	1.502(6)	C(14A)–C(15A)	1.402(8)

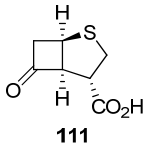
Bond Lengths (Å) for Cyclobutanone **109** (continued).

	Length [Å]		Length [Å]
C(1B)–C(7B)	1.563(6)	C(6B)–O(8B)	1.168(5)
C(1B)–C(5B)	1.529(6)	C(6B)–C(7B)	1.507(6)
C(1B)–S(2B)	1.793(5)	C(7B)–Cl(8B)	1.749(5)
S(2B)–C(3B)	1.755(5)	C(7B)–Cl(9B)	1.733(5)
C(3B)–C(4B)	1.503(6)	C(11B)–O(12B)	1.171(5)
C(4B)–C(11B)	1.478(6)	C(11B)–O(13B)	1.305(6)
C(4B)–C(5B)	1.530(6)	O(13B)–C(14B)	1.425(6)
C(5B)–C(6B)	1.519(6)	C(14B)–C(15B)	1.416(8)

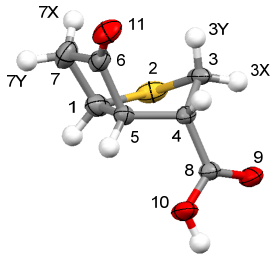
Bond Angles (°) for Cyclobutanone **109**.

	Angle [°]		Angle [°]
C(7A)–C(1A)–C(5A)	89.8(3)	C(7B)–C(1B)–C(5B)	89.2(3)
C(7A)–C(1A)–S(2A)	119.0(3)	C(7B)–C(1B)–S(2B)	119.5(3)
C(5A)–C(1A)–S(2A)	106.8(3)	C(5B)–C(1B)–S(2B)	107.7(3)
C(1A)–S(2A)–C(3A)	94.2(2)	C(1B)–S(2B)–C(3B)	93.3(2)
C(4A)–C(3A)–S(2A)	104.6(3)	C(4B)–C(3B)–S(2B)	105.7(3)
C(11A)–C(4A)–C(5A)	111.8(3)	C(11B)–C(4B)–C(5B)	111.1(3)
C(11A)–C(4A)–C(3A)	113.6(4)	C(11B)–C(4B)–C(3B)	113.9(4)
C(5A)–C(4A)–C(3A)	108.1(3)	C(5B)–C(4B)–C(3B)	106.3(3)
C(4A)–C(5A)–C(6A)	115.4(4)	C(4B)–C(5B)–C(6B)	116.1(3)
C(4A)–C(5A)–C(1A)	109.9(3)	C(4B)–C(5B)–C(1B)	109.4(3)
C(6A)–C(5A)–C(1A)	88.5(3)	C(6B)–C(5B)–C(1B)	89.2(3)
O(8A)–C(6A)–C(5A)	133.4(4)	O(8B)–C(6B)–C(5B)	135.7(4)
O(8A)–C(6A)–C(7A)	133.7(4)	O(8B)–C(6B)–C(7B)	132.2(4)
C(5A)–C(6A)–C(7A)	92.4(3)	C(5B)–C(6B)–C(7B)	91.7(3)
C(6A)–C(7A)–C(1A)	87.8(3)	C(6B)–C(7B)–C(1B)	88.4(3)
C(6A)–C(7A)–Cl(8A)	116.1(3)	C(6B)–C(7B)–Cl(8B)	108.4(3)
C(1A)–C(7A)–Cl(8A)	117.3(3)	C(1B)–C(7B)–Cl(8B)	113.3(3)
C(6A)–C(7A)–Cl(9A)	108.6(3)	C(6B)–C(7B)–Cl(9B)	116.6(3)
C(1A)–C(7A)–Cl(9A)	113.0(3)	C(1B)–C(7B)–Cl(9B)	118.2(3)
Cl(8A)–C(7A)–Cl(9A)	111.7(2)	Cl(8B)–C(7B)–Cl(9B)	110.2(3)
O(12A)–C(11A)–O(13A)	125.2(5)	O(12B)–C(11B)–O(13B)	123.9(4)
O(12A)–C(11A)–C(4A)	124.2(5)	O(12B)–C(11B)–C(4B)	125.4(5)
O(13A)–C(11A)–C(4A)	110.6(4)	O(13B)–C(11B)–C(4B)	110.7(4)
C(11A)–O(13A)–C(14A)	118.1(4)	C(11B)–O(13B)–C(14B)	118.7(4)
C(15A)–C(14A)–O(13A)	108.2(5)	C(15B)–C(14B)–O(13B)	108.8(5)

Crystal Data and Structure Refinement for Cyclobutanone **111**.[852]

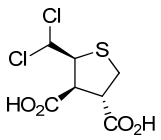


111

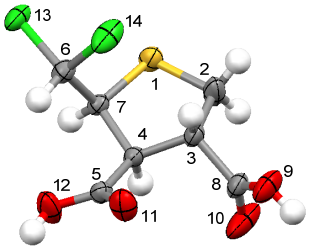


Empirical formula	$C_7H_8O_3S$
Formula weight	172.20
Temperature	180(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	C2/c
Unit cell dimensions	a = 24.0534(13) Å, b = 6.8583(4) Å, c = 19.0432(10) Å β = 102.8310(10)°
Volume, Z	3063.0(3) $Å^3$, 16
Density (calculated)	1.494
Absorption coefficient	0.373 mm^{-1}
F(000)	1440
Crystal size	0.37 mm × 0.30 mm × 0.20 mm
θ range for data collection	1.74 to 30.00
Limiting indices	$-32 \le h \le 33$, $-9 \le k \le 9$, $-26 \le l \le 26$
Reflections collected	12193
Independent reflections	4468 (R_{int} = 0.0168)
Completeness to θ = 30.00	99.8 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4468 / 0 / 208
Goodness-of-fit on F^2	1.074
Final R indices [I>2σ(I)]	R1 = 0.0405, wR2 = 0.1111
R indices (all data)	R1 = 0.0435, wR2 = 0.1135
Extinction coefficient	0.0031(4)
Largest diff. peak and hole	0.400 and -0.389 e $Å^{-3}$

Crystal Data and Structure Refinement for Cyclobutanone **115a**.



115a



Empirical formula	$C_7H_9Cl_2O_4S \cdot ½\ H_2O$
Formula weight	268.10
Temperature	295(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 5.5833(4) Å, b = 13.6105(9) Å, c = 14.7211(9) Å
	α = 96.940(1)°, β = 95.644(1)°, γ = 95.335(1)°
Volume, Z	1098.85(13) $Å^3$, 4
Density (calculated)	1.621
Absorption coefficient	0.772 mm^{-1}
F(000)	548
Crystal size	0.40 mm × 0.29 mm × 0.13 mm
θ range for data collection	1.52 to 30.01°
Limiting indices	$-7 \le h \le 7, -19 \le k \le 19, -20 \le l \le 20$
Reflections collected	13507
Independent reflections	6349 (R_{int} = 0.0327)
Completeness to 2θ = 30.01	99.1%
Max. and min. transmission	0.909 and 0.774
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6349 / 0 / 271
Goodness-of-fit on F^2	1.987
Final R indices [I>2σ(I)]	R1 = 0.0608, wR2 = 0.1592
R indices (all data)	R1 = 0.0675, wR2 = 0.1611
Largest diff. peak and hole	0.776 and –0.561 e.$Å^{-3}$

Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **115a**.

	x/a	y/b	z/c	U_{eq}
S(1)	825(1)	2180(1)	768(1)	36(1)
C(2)	3135(6)	1817(2)	1565(2)	49(1)
C(3)	4827(5)	2745(2)	2002(2)	32(1)
C(4)	3301(4)	3623(2)	1975(2)	27(1)
C(5)	4728(5)	4633(2)	2220(2)	31(1)
C(6)	2933(4)	3949(2)	283(2)	31(1)
C(7)	1731(4)	3503(2)	1043(1)	26(1)
C(8)	5749(5)	2628(2)	2984(2)	35(1)
O(9)	8029(4)	2522(2)	3108(1)	55(1)
O(10)	4416(4)	2629(2)	3590(2)	67(1)
O(11)	6918(4)	4725(1)	2463(2)	49(1)
O(12)	3467(4)	5365(1)	2414(2)	55(1)
Cl(13)	886(1)	3909(1)	–723(1)	42(1)
Cl(14)	5489(1)	3374(1)	–39(1)	53(1)
S(15)	9938(2)	–235(1)	6660(1)	49(1)
C(16)	7752(5)	384(2)	6005(2)	43(1)
C(17)	8412(4)	1524(2)	6241(2)	30(1)
C(18)	11080(4)	1685(2)	6604(2)	27(1)
C(19)	12080(4)	2731(2)	7023(2)	29(1)
C(20)	11162(5)	1141(2)	8251(2)	32(1)
C(21)	11630(5)	898(2)	7249(2)	31(1)
C(22)	7816(5)	1983(2)	5368(2)	34(1)
O(23)	9344(4)	2239(2)	4882(1)	43(1)
O(24)	5528(4)	2039(2)	5193(2)	65(1)
O(25)	14193(4)	2859(1)	7397(1)	44(1)
O(26)	10696(4)	3418(1)	6949(1)	45(1)
Cl(27)	12183(2)	192(1)	8882(1)	56(1)
Cl(28)	8103(1)	1248(1)	8400(1)	55(1)
O(29)	1290(60)	4933(17)	4653(11)	310(20)
O(30)	6040(100)	5130(14)	4833(11)	380(30)

[a] Two crystallographically different molecules of cyclobutanone **115a** are present in the unit cell. O29 and O30 represent disordered water molecules.

Bond Lengths (Å) for Cyclobutanone **115a**.

	Length [Å]		Length [Å]
S(1)–C(2)	1.802(3)	C(5)–O(11)	1.230(3)
S(1)–C(7)	1.809(2)	C(5)–O(12)	1.281(3)
C(2)–C(3)	1.537(4)	C(6)–C(7)	1.519(3)
C(3)–C(8)	1.518(3)	C(6)–Cl(13)	1.772(2)
C(3)–C(4)	1.533(3)	C(6)–Cl(14)	1.767(3)
C(4)–C(5)	1.508(3)	C(8)–O(9)	1.292(3)
C(4)–C(7)	1.537(3)	C(8)–O(10)	1.217(3)

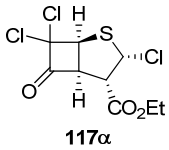
Bond Lengths (Å) for Cyclobutanone **115a** (continued).

	Length [Å]		Length [Å]
S(15)–C(16)	1.813(3)	C(19)–O(25)	1.240(3)
S(15)–C(21)	1.807(2)	C(19)–O(26)	1.275(3)
C(16)–C(17)	1.549(3)	C(20)–C(21)	1.525(3)
C(17)–C(22)	1.518(3)	C(20)–Cl(27)	1.783(2)
C(17)–C(18)	1.519(3)	C(20)–C(28)	1.760(3)
C(18)–C(19)	1.513(3)	C(22)–O(23)	1.214(3)
C(18)–C(21)	1.546(3)	C(22)–O(24)	1.290(3)

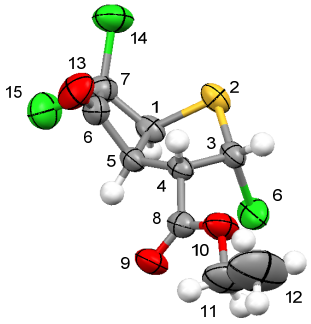
Bond Angles (°) for Cyclobutanone **115a**.

	Angle [°]		Angle [°]
C(2)–S(1)–C(7)	95.21(11)	C(21)–S(15)–C(16)	95.31(11)
C(3)–C(2)–S(1)	109.15(17)	C(17)–C(16)–S(15)	108.49(17)
C(8)–C(3)–C(4)	111.2(2)	C(22)–C(17)–C(18)	113.7(2)
C(8)–C(3)–C(2)	109.9(2)	C(22)–C(17)–C(16)	107.3(2)
C(4)–C(3)–C(2)	105.9(2)	C(18)–C(17)–C(16)	106.6(2)
C(5)–C(4)–C(3)	114.5(2)	C(19)–C(18)–C(17)	116.8(2)
C(5)–C(4)–C(7)	113.45(18)	C(19)–C(18)–C(21)	112.82(18)
C(3)–C(4)–C(7)	109.02(18)	C(17)–C(18)–C(21)	108.88(19)
O(11)–C(5)–O(12)	124.2(2)	O(25)–C(19)–O(26)	124.9(2)
O(11)–C(5)–C(4)	121.6(2)	O(25)–C(19)–C(18)	117.7(2)
O(12)–C(5)–C(4)	114.2(2)	O(26)–C(19)–C(18)	117.4(2)
C(7)–C(6)–Cl(14)	114.15(17)	C(21)–C(20)–Cl(28)	114.02(17)
C(7)–C(6)–Cl(13)	111.75(17)	C(21)–C(20)–Cl(27)	108.89(17)
Cl(13)–C(6)–Cl(14)	107.70(13)	Cl(28)–C(20)–Cl(27)	108.72(14)
C(6)–C(7)–C(4)	114.80(19)	C(20)–C(21)–C(18)	116.80(19)
C(6)–C(7)–S(1)	113.17(16)	C(21)–C(21)–S(15)	114.08(17)
C(4)–C(7)–S(1)	105.24(14)	C(18)–C(21)–S(15)	103.84(15)
O(10)–C(8)–O(9)	123.9(2)	O(23)–C(22)–O(24)	124.5(2)
O(10)–C(8)–C(3)	121.8(2)	O(23)–C(22)–C(17)	123.2(2)
O(9)–C(8)–C(3)	114.3(2)	O(24)–C(22)–C(17)	112.3(2)

Crystal Data and Structure Refinement for Cyclobutanone **117α**.[852]

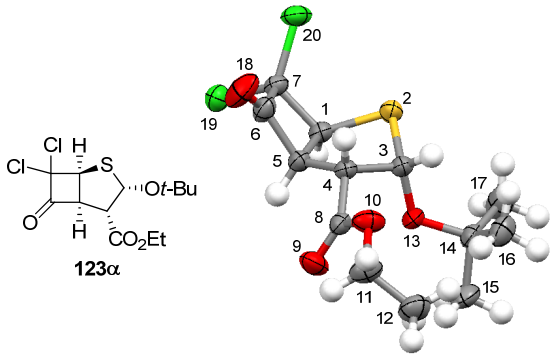


117α



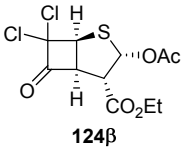
Empirical formula	$C_9H_9Cl_3O_3S$
Formula weight	303.59
Temperature	295(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 6.4915(15) Å, b = 9.542(2) Å, c = 11.396(3) Å α = 102.655(5)°, β = 103.497(5)°, γ = 103.414(5)°
Volume, Z	639.2(3) $Å^3$, 2
Density (calculated)	1.577
Absorption coefficient	0.868 mm^{-1}
F(000)	308
Crystal size	0.40 mm × 0.38 mm × 0.20 mm
θ range for data collection	1.92 to 30.00°
Limiting indices	$-9 \le h \le 9$, $-13 < k \le 13$, $-16 \le l \le 16$
Reflections collected	7767
Independent reflections	3711 (R_{int} = 0.0367)
Completeness to θ = 30.00	99.5 %
Absorption correction	Integration
Max. and min. transmission	0.869 and 0.737
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3711 / 0 / 147
Goodness-of-fit on F^2	1.378
Final R indices [I>2σ(I)]	R1 = 0.0494, wR2 = 0.0990
R indices (all data)	R1 = 0.0601, wR2 = 0.1020
Extinction coefficient	0.090(5)
Largest diff. peak and hole	0.452 and –0.407 e $Å^{-3}$

Crystal Data and Structure Refinement for Cyclobutanone **123α**.[852]

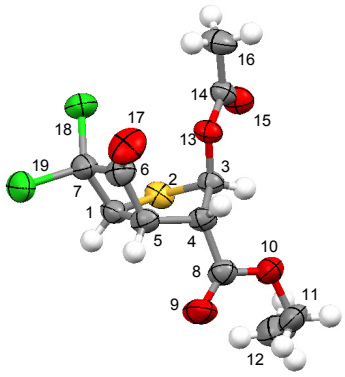


Empirical formula	$C_{13}H_{18}Cl_2O_4S$
Formula weight	341.25
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 6.3646(6) Å, b = 10.6579(10) Å, c = 11.9831(11) Å α = 92.940(2)°, β = 104.377(2)°, γ = 101.967(2)°
Volume, Z	765.74(12) $Å^3$, 2
Density (calculated)	1.480
Absorption coefficient	0.569 mm^{-1}
F(000)	356
Crystal size	0.38 mm × 0.23 mm × 0.08 mm
θ range for data collection	3.38 to 30.08
Limiting indices	$-8 \le h \le 8$, $-15 \le k \le 15$, $-16 \le l \le 16$
Reflections collected	10709
Independent reflections	4302 (R_{int} = 0.0134)
Completeness to θ = 30.08	96.2 %
Absorption correction	Empirical
Max. and min. transmission	0.9559 and 0.8128
Refinement method	Fullmatrix leastsquares on F^2
Data / restraints / parameters	4302 / 0 / 185
Goodness-of-fit on F^2	1.182
Final R indices [I>2σ(I)]	R1 = 0.0227, wR2 = 0.00653
R indices (all data)	R1 = 0.0238, wR2 = 0.0739
Largest diff. peak and hole	0.505 and 0.245 e.$Å^{-3}$

Crystal Data and Structure Refinement for Cyclobutanone **124β**.[852]

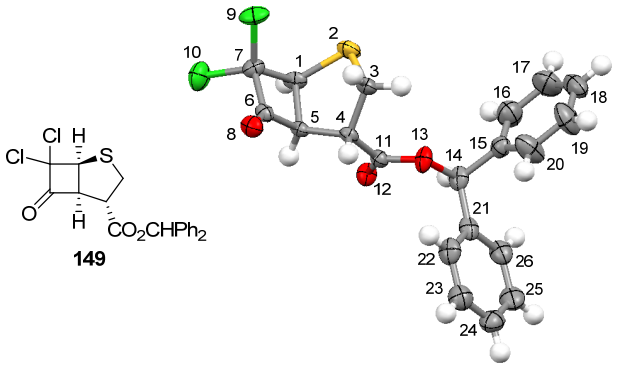


124β



Empirical formula	$C_{11}H_{12}Cl_2O_5S$
Formula weight	327.18
Temperature	295(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 6.6343(6) Å, b = 20.9581(17) Å, c = 10.6260(9) Å β = 102.039(2)°
Volume, Z	1445.0(2) $Å^3$, 4
Density (calculated)	1.504
Absorption coefficient	0.605 mm^{-1}
F(000)	672
Crystal size	0.50 mm × 0.13 mm × 0.13 mm
θ range for data collection	1.94 to 30.00
Limiting indices	$-9 \le h \le 8$, $-29 \le k \le 24$, $-14 \le l \le 14$
Reflections collected	11763
Independent reflections	4208 (R_{int} = 0.0323)
Completeness to θ = 30.00	99.9 %
Absorption correction	Integration
Max. and min. transmission	0.944 and 0.814
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4208 / 0 / 175
Goodness-of-fit on F^2	1.039
Final R indices [I>2σ(I)]	R1 = 0.0467, wR2 = 0.1153
R indices (all data)	R1 = 0.0606, wR2 = 0.1224
Extinction coefficient	0.0071(15)
Largest diff. peak and hole	0.244 and –0.276 e.$Å^{-3}$

Crystal Data and Structure Refinement for Cyclobutanone **149**.



Empirical formula	$C_{20}H_{16}Cl_2O_3S$
Formula weight	407.29
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 5.4269(11) Å, b = 11.291(2) Å, c = 16.198(3) Å α = 103.048(3)°, β = 97.757(3)°, γ = 93.275(3)°
Volume, Z	954.1(3) $Å^3$, 2
Density (calculated)	1.418
Absorption coefficient	0.466 mm^{-1}
F(000)	420
Crystal size	0.30 mm × 0.20 mm × 0.10 mm
θ range for data collection	3.55 to 26.00°
Limiting indices	$-6 \le h \le 6$, $-13 \le k \le 12$, $-19 \le l \le 18$
Reflections collected	5248
Independent reflections	3552 (R_{int} = 0.0398)
Completeness to θ = 26.00	94.5 %
Absorption correction	Empirical
Max. and min. transmission	0.9548 and 0.8727
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3552 / 0 / 236
Goodness-of-fit on F^2	1.074
Final R indices [I>2σ(I)]	R1 = 0.0681, wR2 = 0.1008
R indices (all data)	R1 = 0.1109, wR2 = 0.1214
Extinction coefficient	0.0068(10)
Largest diff. peak and hole	0.301 and –0.299 e.$Å^{-3}$

Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **149**.

	x/a	y/b	z/c	U_{eq}
C(1)	3468(8)	7645(4)	789(3)	31(1)
S(2)	2611(3)	6143(1)	119(1)	44(1)
C(3)	166(8)	6652(4)	–547(3)	32(1)
C(4)	1144(7)	7921(4)	–606(3)	24(1)
C(5)	2361(7)	8588(4)	288(3)	25(1)
C(6)	564(8)	8886(4)	933(3)	30(1)
C(7)	1879(8)	8115(4)	1512(3)	32(1)
O(8)	–1274(6)	9417(3)	967(2)	41(1)
Cl(9)	–31(2)	7075(1)	1850(1)	52(1)
Cl(10)	3745(3)	9135(1)	2402(1)	58(1)
C(11)	3008(8)	7860(4)	–1226(3)	28(1)
O(12)	5116(5)	8338(3)	–1068(2)	36(1)
O(13)	2002(5)	7165(3)	–1999(2)	40(1)
C(14)	3600(8)	6926(4)	–2669(3)	37(1)
C(15)	2969(9)	5612(5)	–3124(3)	38(1)
C(16)	4478(12)	4741(6)	–2959(4)	65(2)
C(17)	3893(14)	3512(6)	–3359(5)	78(2)
C(18)	1770(13)	3144(5)	–3920(4)	61(2)
C(19)	236(12)	3991(6)	–4092(4)	74(2)
C(20)	839(11)	5217(5)	–3694(4)	68(2)
C(21)	3154(8)	7797(4)	–3247(3)	39(1)
C(22)	1390(9)	8629(5)	–3175(3)	46(1)
C(23)	1100(10)	9407(5)	–3725(4)	52(2)
C(24)	2504(10)	9331(5)	–4378(4)	54(2)
C(25)	4264(11)	8491(5)	–4464(4)	57(2)
C(26)	4609(10)	7727(5)	–3907(3)	49(1)

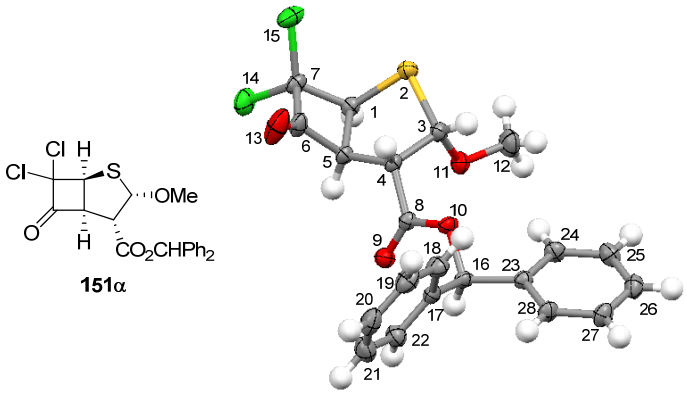
Bond Lengths (Å) for Cyclobutanone **149**.

	Length [Å]		Length [Å]
C(1)–C(7)	1.563(6)	C(14)–C(15)	1.497(6)
C(1)–C(5)	1.576(6)	C(14)–C(21)	1.512(7)
C(1)–S(2)	1.792(5)	C(15)–C(16)	1.364(7)
S(2)–C(3)	1.799(5)	C(16)–C(17)	1.390(8)
C(3)–C(4)	1.527(5)	C(17)–C(18)	1.349(9)
C(4)–C(11)	1.513(5)	C(18)–C(19)	1.354(8)
C(4)–C(5)	1.514(6)	C(19)–C(20)	1.389(7)
C(5)–C(6)	1.520(6)	C(20)–C(15)	1.362(7)
C(6)–C(7)	1.551(6)	C(21)–C(22)	1.377(7)
C(6)–O(8)	1.194(5)	C(22)–C(23)	1.386(7)
C(7)–Cl(9)	1.749(4)	C(23)–C(24)	1.375(7)
C(7)–Cl(10)	1.776(5)	C(24)–C(25)	1.383(8)
C(11)–O(12)	1.206(5)	C(25)–C(26)	1.384(7)
C(11)–O(13)	1.343(5)	C(26)–C(21)	1.404(6)
O(13)–C(14)	1.467(5)		

Bond Angles (°) for Cyclobutanone **149**.

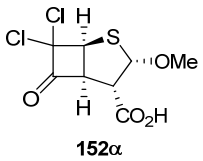
	Angle [°]		Angle [°]
C(7)–C(1)–S(2)	118.0(3)	O(12)–C(11)–O(13)	123.4(4)
C(5)–C(1)–S(2)	107.8(3)	C(11)–O(13)–C(14)	118.0(3)
C(1)–S(2)–C(3)	93.0(2)	O(13)–C(14)–C(15)	105.8(3)
S(2)–C(3)–C(4)	105.5(3)	O(13)–C(14)–C(21)	109.7(4)
C(3)–C(4)–C(5)	106.8(3)	C(15)–C(14)–C(21)	113.6(4)
C(4)–C(5)–C(6)	114.7(3)	C(20)–C(15)–C(16)	116.7(5)
C(5)–C(6)–C(7)	92.1(3)	C(20)–C(15)–C(14)	122.3(5)
C(4)–C(5)–C(1)	109.7(3)	C(14)–C(15)–C(16)	121.0(5)
C(6)–C(5)–C(1)	88.9(3)	C(15)–C(16)–C(17)	121.9(6)
C(6)–C(7)–C(1)	88.3(3)	C(16)–C(17)–C(18)	120.4(6)
C(7)–C(1)–C(5)	89.5(3)	C(17)–C(18)–C(19)	118.9(6)
C(5)–C(6)–O(8)	135.2(4)	C(18)–C(19)–C(20)	120.5(6)
C(7)–C(6)–O(8)	132.5(4)	C(19)–C(20)–C(15)	121.8(6)
C(6)–C(7)–Cl(9)	116.9(3)	C(22)–C(21)–C(26)	118.6(5)
C(6)–C(7)–Cl(10)	107.9(3)	C(14)–C(21)–C(26)	117.3(5)
C(1)–C(7)–Cl(9)	119.8(3)	C(14)–C(21)–C(22)	124.2(4)
C(1)–C(7)–Cl(10)	110.8(3)	C(21)–C(22)–C(23)	120.9(5)
Cl(9)–C(7)–Cl(10)	111.0(3)	C(22)–C(23)–C(24)	120.6(6)
C(3)–C(4)–C(11)	111.9(3)	C(23)–C(24)–C(25)	119.1(5)
C(5)–C(4)–C(11)	110.3(3)	C(24)–C(25)–C(26)	120.9(5)
C(4)–C(11)–O(12)	126.4(4)	C(25)–C(26)–C(21)	119.9(5)
C(4)–C(11)–O(13)	110.2(3)		

Crystal Data and Structure Refinement for Cyclobutanone **151α**.[853]

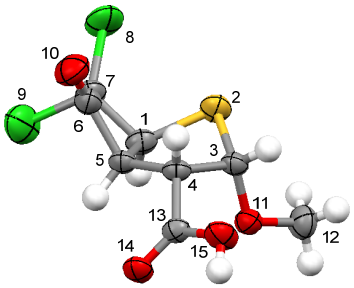


Empirical formula	$C_{21}H_{18}Cl_2O_4S$
Formula weight	437.31
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 9.6137(19) Å, b = 9.816(2) Å, c = 12.382(3) Å
	α = 72.737(4)°, β = 67.724(3)°, γ = 70.553(4)°
Volume, Z	999.9(3) $Å^3$, 2
Density (calculated)	1.453 g/cm^3
Absorption coefficient	0.454 mm^{-1}
F(000)	452
Crystal size	0.38 mm × 0.35 mm × 0.20 mm
θ range for data collection	2.82 to 29.99°
Limiting indices	$-11 \le h \le 13$, $-11 \le k \le 13$, $-17 \le l \le 17$
Reflections collected	10634
Independent reflections	5728 (R_{int} = 0.0208)
Completeness to θ = 29.99	98.0 %
Max. and min. transmission	0.9146 and 0.8463
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5728 / 0 / 253
Goodness-of-fit on F^2	1.243
Final R indices [I>2σ(I)]	R1 = 0.0361, wR2 = 0.0881
R indices (all data)	R1 = 0.0393, wR2 = 0.0900
Largest diff. peak and hole	0.444 and –0.402 e $Å^{-3}$

Crystal Data and Structure Refinement for Cyclobutanone **152α**.[853]

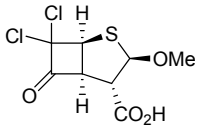


152α

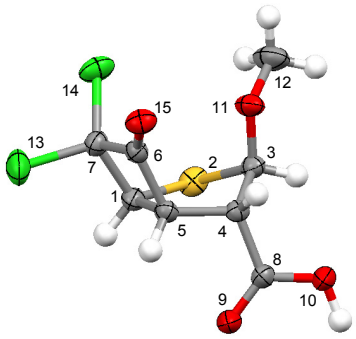


Empirical formula	$C_{21}H_{18}Cl_2O_4S$
Formula weight	437.31
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	*P*-1
Unit cell dimensions	a = 9.6137(19) Å, b = 9.816(2) Å, c = 12.382(3) Å
	α = 72.737(4)°, β = 67.724(3)°, γ = 70.553(4)°
Volume, Z	999.9(3) $Å^3$, 2
Density (calculated)	1.453 g/cm^3
Absorption coefficient	0.454 mm^{-1}
F(000)	452
Crystal size	0.38 mm × 0.35 mm × 0.20 mm
θ range for data collection	2.82 to 29.99°
Limiting indices	$-11 \le h \le 13$, $-11 \le k \le 13$, $-17 \le l \le 17$
Reflections collected	10634
Independent reflections	5728 (R_{int} = 0.0208)
Completeness to θ = 29.99	98.0 %
Max. and min. transmission	0.9146 and 0.8463
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5728 / 0 / 253
Goodness-of-fit on F^2	1.243
Final R indices [I>2σ(I)]	R1 = 0.0361, wR2 = 0.0881
R indices (all data)	R1 = 0.0393, wR2 = 0.0900
Largest diff. peak and hole	0.444 and –0.402 e $Å^{-3}$

Crystal Data and Structure Refinement for Cyclobutanone **152β**.[853]

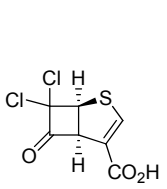


152β

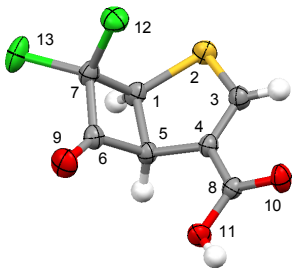


Empirical formula	$C_8H_8Cl_2O_4S$
Formula weight	271.10
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 12.0074(17) Å, b = 9.2141(13) Å, c = 10.8132(16) Å, α = 90°, β = 112.413(2)°, γ = 90°
Volume, Z	1106.0(3) Å^3, 4
Density (calculated)	1.628 g/cm^3
Absorption coefficient	0.765 mm^{-1}
F(000)	552
Crystal size	0.28 mm × 0.10 mm × 0.02 mm
θ range for data collection	2.87 to 30.00°
Limiting indices	$-16 \le h \le 15$, $-12 \le k \le 12$, $-15 \le l \le 15$
Reflections collected	11393
Unique reflections	3193 (R_{int} = 0.0370)
Completeness to θ = 30.00	99.6 %
Absorption correction	Empirical
Max. and min. transmission	0.9849 and 0.8143
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3193 / 0 / 141
Goodness-of-fit on F^2	1.165
Final R indices [I>2σ(I)]	R1 = 0.0414, wR2 = 0.0736
R indices (all data)	R1 = 0.0621, wR2 = 0.0819
Largest diff. peak and hole	0.298 and –0.286 e Å^{-3}

Crystal Data and Structure Refinement for Cyclobutanone **153**.[853]



153



Empirical formula	$C_7H_4Cl_2O_3S$
Formula weight	239.06
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	a = 7.464(3) Å, b = 10.568(4) Å, c = 11.510(4) Å β = 101.187(5) °
Volume, Z	890.6(6) $Å^3$, 4
Density (calculated)	1.783 g/cm^3
Absorption coefficient	0.929 mm^{-1}
F(000)	480
Crystal size	0.32 mm × 0.28 mm × 0.15 mm
θ range for data collection	3.39 to 27.99°
Limiting indices	$-9 \le h \le 9, -13 \le k \le 13, -14 \le l \le 15$
Reflections collected	7845
Unique reflections	2100 (R_{int} = 0.0284)
Completeness to θ = 27.99	97.7 %
Max. and min. transmission	0.8732 and 0.7553
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2100 / 0 / 123
Goodness-of-fit on F^2	1.319
Final R indices [I>2σ(I)]	R1 = 0.0316, wR2 = 0.0982
R indices (all data)	R1 = 0.0352, wR2 = 0.1012
Largest diff. peak and hole	0.482 and –0.324 e $Å^{-3}$

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> "Fleming had produced such conditions, not by design, but by accident, by a curious coincidence of external factors. He incubated his Petri dish at two different temperatures, at 37 °C overnight, which allowed the staphylococcal colonies to develop (not completely but sufficiently to form visible colonies), and then for many days at room temperature when a contaminant mould, later recognized as *Penicillium notatum*, could develop producing penicillin. These two growth conditions were produced because, thank God, Fleming was not a tidy bacteriologist. In a tidy laboratory, penicillin could not have been discovered." – Sir Ernst Chain.[34]

(41) Fleming gave then name 'penicillin' to the lytic culture fluid to avoid repetition of the "cumbersome" phrase "mould broth filtrate".[43]

(42) Growth inhibition was observed with staphylococci, streptococci, gonococci, meningococci, and *Corynebacterium diptheria*, but not with *Bacillus coli*, *Haemophilus influenzae*, *Salmonella typhi*, *Pseudomonas pyocyanea*, *Bacillus proteus*, or *Vibrio cholerae*. The growth of staphylococci was inhibited even after dilution of the penicillin 800- to 1000-fold.

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(686) The 3α-trifluoroethoxy derivative **125α** could not be isolated after chromatography but three signals could be observed in the ^{1}H NMR of the crude reaction mixture: 4.69 (d, $J_{1,5}$ = 8.2 Hz, 1H, H_1), 5.22 (dd, $J_{5,1}$ = 8.2 Hz, $J_{5,4}$ = 5.5 Hz, 1H, H_5), 5.58 (d, $J_{3\beta,4}$ = 4.5 Hz, 1H, $H_{3\beta}$).

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(689) Signals corresponding to C6, C7, and C8 of the hydrate of **152β** could not be observed in the ^{13}C spectrum or by HMBC.

(690) The signal for C7 could not be observed by either of the ^{13}C or HMBC NMR spectra.

(691) The signals for C6 and C7 could not be observed by ^{13}C or HMBC NMR experiments.

(692) The signals for the remaining protons of **122α** could not be observed in CD_3OD by ^{1}H or COSY NMR experiments.

(693) The residual solvent peaks of CF_3CD_2OD were calibrated to δ_H 3.88 (qt); δ_C 61.5 (m) and 126.3 (q).

(694) None of the remaining protons of the α- or β-(trifluoroethyl)hemiketals of **118** or the α-(*t*-butyl)hemiketal of **118** could be observed.

(695) The residual solvent peaks of $(CD_3)_2CDOD$ were calibrated to δ_H 1.10 (d) and 3.89 (m); δ_C 24.2 (m) and 62.9 (t).

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(853) For additional tables of crystallographic data for cyclobutanones **151α**, **152α**, **152β**, and **153**, see the Supporting Information for ref 640.