Synthesis of Multiply Deuterated Cephalosporins

by

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A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Pharmacy

Waterloo, Ontario, Canada, 2014

Author's Declaration

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

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

Abstract

Antibiotics are one of the greatest accomplishments in the field of science. They have been extensively used since the time of their discovery. Amongst all the antibiotics, the β-lactam class of antibiotics have been the most clinically used agents. However, the process of bacterial resistance towards the antibiotics is becoming an alarming situation in the present time. Due to the wide spread use of antibiotics, many Gram-positive and Gram-negative pathogens developed resistance against them. Various pathways are known via which the bacteria develop resistance towards the β-lactam antibiotics, among which, the production of β-lactamase enzymes is the major cause of bacterial resistance. Presently, many pharmaceutical companies and research groups are still interested in developing novel cephalosporin derivatives, which would act as antibiotic as well as β-lactamase inhibitor. The Dmitrienko research group at the University of Waterloo has been developing novel C3′ benzoylthio cephalosporin derivatives. These potential agents are proposed to possess extended spectrum activity against pathogens that have developed multidrug resistance (also called "Superbugs"). Preclinical pharmacokinetic studies of these novel C3′ benzoylthio cephalosporin derivatives will be conducted after their synthesis. The availability of core isotopic labelled cephalosporin will permit efficient synthesis of internal standards for any novel cephalosporin derivative. The main goal of this thesis work was to synthesize a multiply deuterated cephalosporin core. When this project was initiated, a survey of the literature revealed a previous attempt to perform H/D exchange at C2 position of cephalosporin sulfoxides but no attempts to incorporate deuterium at C3′ has been reported. In this study, C2 and C3′ of cephalosporin core were the proposed sites to incorporate deuterium. The first objective was to perform a H/D exchange at the C2 position of a cephalosporin core. A H/D

exchange reaction was performed with cephalosporin sulfoxide **3.3** using a mixture of benzene and D_2O at 80 ^oC to give 78% deuterium exchange at the C2 of **3.3** as determined by ^1H NMR. In order to increase the deuterium exchange another experimental condition were used in which, compound **3.16** (benzhydryl ester of 7-ACA, phenylacetylated at C7) was heated at 50 ^oC in presence of DMSO and Methanol-*OD*. NMR studies revealed that 90.2% deuterium incorporation had been achieved at each of the prochiral positions C2 of **3.16**. After the successful completion of objective 1, the second objective was to synthesize a C3′ monodeuterated derivative. Eventually after certain attempts, C3′ hydroxyl cephalosporin **3.20** was oxidized to give the corresponding aldehyde **3.21**, which was reduced by sodium borodeuteride to give the monodeuterated C3′ hydroxyl derivative **3.27**. The third objective of this thesis work was to combine the results of H/D exchange at C2 and deuterium incorporation at C3′ using functional group interconversion to synthesize a trideuterated cephalosporin analogue. The core trideuterated C3′ alcohol **3.44** was synthesized from **3.11 (**7-ACA) in 10 steps with an overall yield of 5.9%. In order to reduce the number of steps and to increase the overall yield a modified route was achieved to synthesize trideuterated cephalosporin **3.44** in 8 steps starting from **3.11** (7-ACA) with an overall yield of 19% and a isotopic purity of 87.5% (determined by NMR spectroscopy). Mass spectrometric analysis of **3.44** indicates that the ratio of trideuterated to dideuterated molecules in material is 5 to 1.

Acknowledgement

From the bottom of my heart, I would like to thank my supervisors, Professor Gary Dmitrienko and Professor Praveen Nekkar for providing me with the opportunity to pursue my graduate studies under their supervision. My supervisors have been very supportive and helpful throughout my graduate studies program. I am in debt to them for all the care and guidance they have provided me with. I am sincerely thankful to be a part of their lab and indebted for their mentorship.

I am thankful to Professors Mike Chong and Michael Beazely for being my committee members. Dr. Chong has encouraged me to study the finer nuances of organic chemistry during my thesis research proposal and committee meetings. Dr. Beazely has provided me the opportunity to pursue my core pharmacy course (PHARM 610) under his guidance.

My sincere thanks to Mrs. Jan Venne, for her assistance with NMR spectroscopy study and Dr. Richard Smith for providing detail understanding of mass spectrometry studies. I would also like to thank Dr. Jonathan Blay for providing me with the opportunity to be a part of Pharmacy graduate student. Sarah Rae, the department graduate co-ordinator, I am really very grateful for all the help she has offered during the graduate program.

I special thanks goes to Dr. Ahmad Ghavami for this constant advice and suggestion for my project. He has been almost like my guardian in Canada. I am honoured to work with him as a colleague and I have adopted several good qualities from him which I think are very necessary for becoming a successful scientist. Thanks to Dr. Anthony Krismanich for his valuable suggestions while writing my thesis. I feel his suggestions will be beneficial to me throughout my lifetime. My thanks to Dr. Nan Chen, for offering help to settle down in Canada very quickly. His suggestions for my project and NMR studies have been very valuable. Dr.

Ahmed Desoky has been a very nice lab mate as we have shared the same lab room during graduate studies. I am honoured to work with him. Dr. Glenn Abbott, a previous member in the Dmitrienko Lab, he was really very funny. With his quick wit he always had the office environment very funny. We have enjoyed watching soccer world cup game during the lab hours.

I enjoyed having the company of Mrs. Valerie Goodfellow, Dr. Geneviève Labbé and Ms. Carol Tanner. I enjoyed one of my best times in Canada when Valerie invited the lab members to her cottage farm on the lake side in Peterborough. Peizhi Qiu, or "Honeybee" as I call him started the graduate program with me. He is very honest, kind and has an amazing personality. Dr. Jarrod Johnson is a great researcher. He has motivated me to be efficient, perfect and patient.

My special thanks to all graduate students of the Department of Chemistry and the School of Pharmacy. I would like to thank my TA instructors, Josephine Orso Simon, Dr. Eric Schneider, Brett Barrett, Barbara Coulston and Dr. Cynthia Richards, for their support when I worked as a Teaching Assistant.

Very lovely thanks to my dear friend Purva Karia, who has always helped me to choose the right thing. Her constant support during my undergraduate and graduate studies has been tremendous. I would like to thank my friends with whom I traveled to Canada: Sandeep Chaudhary, Shivam Dembla, Rupinder Ghotra, Shrinu Kushagara and Rakesh Guttikonda. A special thanks to Vatsal Parikh, Sagar Patel, Dhairya Maheshwari and Niket Teckwani. Last but not the least a special thanks to Vimir Dave and Vinay Dangar, my friends since childhood. They have been more like my elder brothers to me. I am very happy to have such valuable friends.

Dedication

To the unseen force whom mankind refers as "almighty."

For my father and mother who have shown their boundless love, care, support, trust and guidance through their blessings.

For my sister and brother who have looked upon me as a role model.

For my grandfather who has always inspired me with his words.

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6-APA	6-aminopenicillanic acid	DMSO	Dimethyl sulfoxide
7-ACA	7-aminocephalosporanic acid	DCM	Dichloromethane
Å	Ångstrom	DMI	Dimethyl imidazolidinone
A. baumannii	*Acinetobacter baumannii*	EtOAc	Ethyl acetate
Ac	Acetyl	EDTA	Ethylenediamine tetra aceticacid
ACN	Acetonitrile	EtOH	Ethanol
AcCl	Acetyl chloride	ESI	Electrospray ionization
Ala	Alanine	*E. coli*	*Escherichia coli*
AMU	Atomic mass unit	ESBL	extend-spectrum β-lactamase
ADME	Absorption, distribution, metabolism and excretion	gem	geminal
AUC	Area under curve	Glu	glutamic acid
BBB	Blood brain barrier	Gln	glutamine
BSA	Bis-[trimethylsilyl]acetamide	GC/MS	Gas chromatography/ Mass spectrometry
BAIB	[bis(acetoxy)iodo] benzene	g	gram
conc.	Concentrated	HMBC	Hetero nuclear multiple bond correlation
calcd.	Calculated	H/D	Hydrogen/Deuterium
COSY	Correlation Spectroscopy	HPLC	High performance liquid chromatography
DAP	diaminopimelic acid	HMQC	Heteronuclear single quantum coherence spectroscopy
DMAP	N,N-4-dimethylamino pyridine	IC_{50}	Concentration of inhibitor that reduce the max rate of enzymatic activity by 50%
DMF	*N*,*N*-dimethylformamide	IV	Intravenous

IS	Internal standard	NAM	*N*-acetylmuramic acid
i-PrOD	*i*-Propanol-D	N.R.	no reaction
i-PrONa	Sodium *i*-propoxide	NDM	New Delhi metallo β-lactamase
KPC	*Klebsiella pneumoniae* carbapenemase	N.A.	Not available
LMW	Low molecule weight	NMR	nuclear magnetic resonance
LRMS	Low-resolution mass spectrum	OXA	Oxacillinase β-lactamase
LC/MS	Liquid chromatography/ Mass spectrometry	PBP	Penicillin binding protein
MIC	Minimum inhibitory concentration	*P.aeruginosa*	*Pseudomonas aeruginosa*
MRSA	Methicillin-resistant *Staphylococcus aureus*	PMB	*para*-methoxybenzyl
min	Minute	ppm	parts per million (spectral)
M	Molar	PK	Pharmacokinetics
MBL	Metallo β-lactamase	PD	Pharmacodynamics
Me	Methyl	Ph	Phenyl
μ	Micro	r.t.	room temperature
m	Milli	*S.pneumoniae*	*Staphylococcus pneumoniae*
MeOD	Methanol-D	*S. aureus*	*Staphylococcus aureus*
m-CPBA	*meta*-Chloroperoxybenzoic acid	*S. paratyphi*	*Staphylococcus paratyphi*
MeOH	Methanol	SBL	serine β-lactamase
MS	Mass spectrometry	temp.	temperature
MeONa	Sodium methoxide	TEMPO	2,2,6,6-Tetramethylpiperidinyloxyl
NAG	*N*-acetylglucosamine	THF	Tetrahydrofuran

TMS	Tetramethylsilane	VRE	vancomycin-resistant *Enterococcus spp*
UPLC	Ultra performance liquid chromatrography	VRSA	Vancomycin-resistant *S. aureus*
VIM	Verona integron-encoded metallo β-lactamase		

Chapter 1

Introduction

1.1 Antibiotics

The discovery of antibiotics is believed to be one of the most important accomplishments in the history of drug development.[1] Antibiotics have been derived from natural products or synthesized chemically. There has been enormous development in the field of novel antibiotic discovery in the last seven decades.[2] Based on their chemical structures, antibiotics have been classified as β-lactam antibiotics, aminoglycosides, tetracyclines, macrolides, streptogramins, quinolones, glycopeptides, polyketides and oxazolidinones (Figure 1.1).[3] The β-lactams are the most extensively used and developed classes of antibiotics, and account for more than 50% of antibiotic prescriptions yearly.[3] Figure 1.2 illustrates the timeline for discovery of antibiotics from the 1930s to the 2000s.[2]

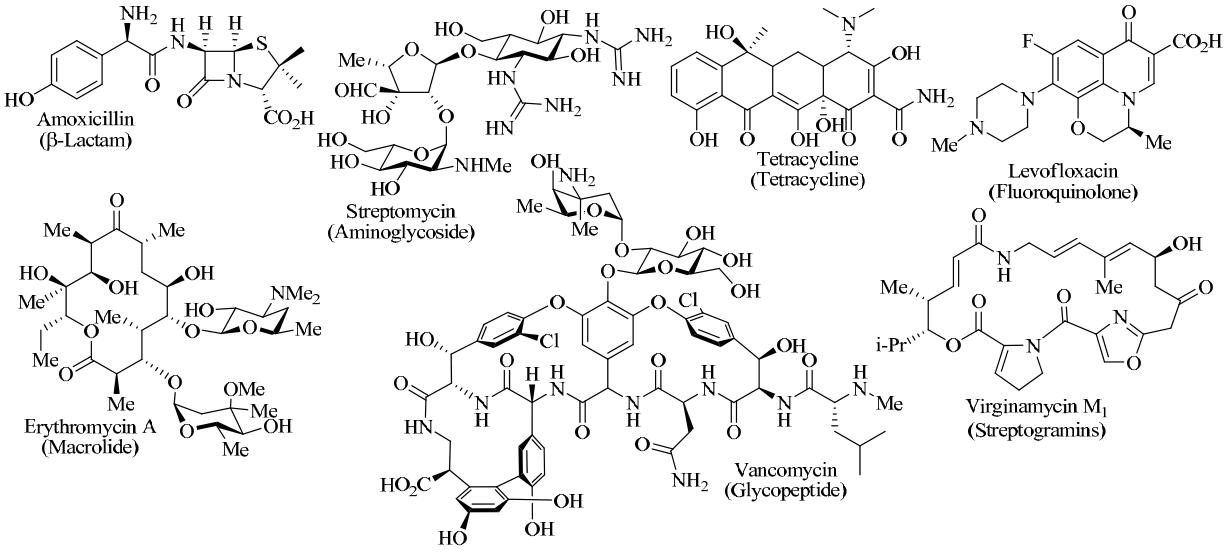


Figure 1.1: Examples from several classes of antibiotics used clinically.

Since their discovery, antibiotics they have been used extensively to cure bacterial infections.[4] It is believed that the overuse of antibiotics is the prime cause of bacterial resistance.[5] However, it has also been documented that bacterial resistance to antibiotics has existed from before the time of their discovery and is an ancient phenomenon.[6] Gram-negative pathogens are the most effective at developing resistance through various mechanisms which are listed in section 1.3.[7]

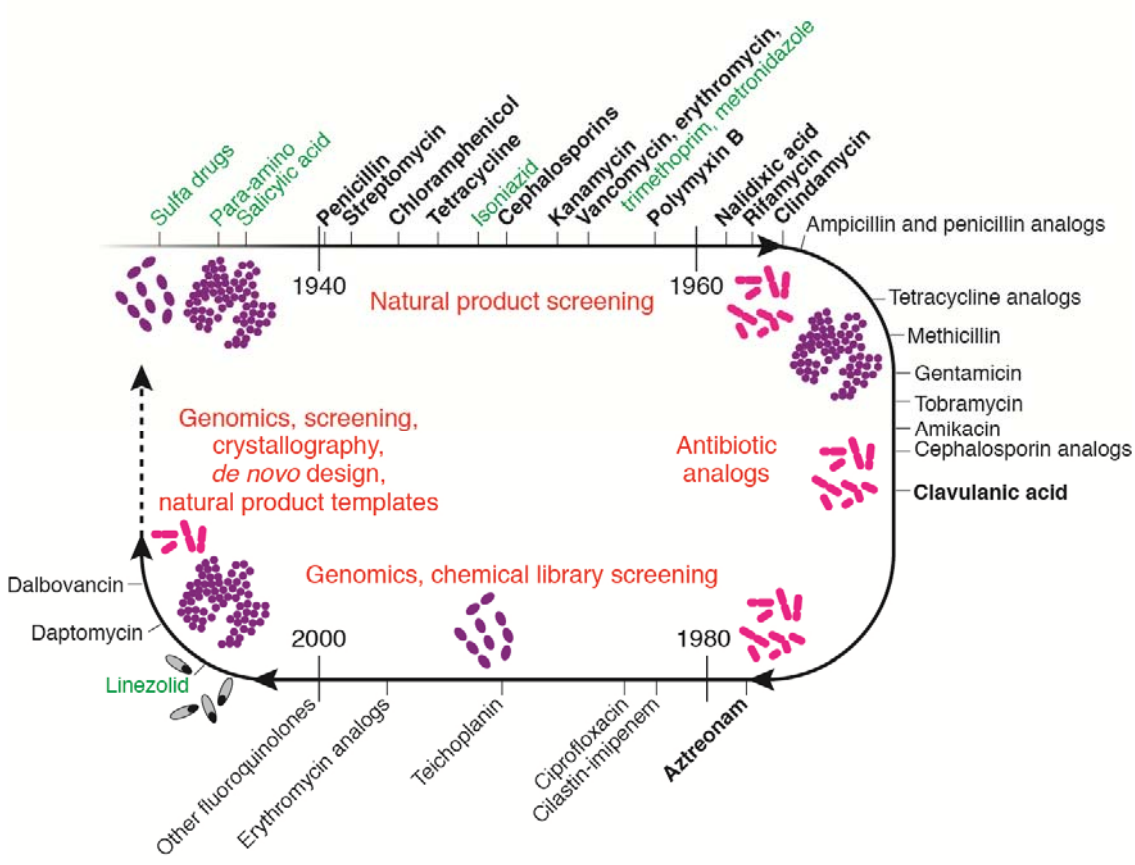


Figure 1.2: The major clinically used antibiotics and type of resistant species are shown in the timeline for decade of antibiotic evolution. Green represents synthetic derivatives; black are natural product origin derivatives; major antibiotic classes are shown in bold; red represents the technology used for their discovery; Gram-positive bacteria are shown in purple and Gram-negative bacteria in pink.[2]

1.2 β-Lactam Antibiotics

β-Lactam antibiotics have been the most used antibiotics since the time of their introduction into clinical practice.[8] All β-lactam antibiotics contain a four membered ring cyclic amide (lactam) ring. Depending on their structural difference in the non-lactam part, β-lactam antibiotics have been classified into five major classes namely: penicillins, cephalosporins, carbapenems, penems and monobactams. Figure 1.3 represents the representative structures of each class in which the rings shown in red color represent the lactam ring.[1,9]

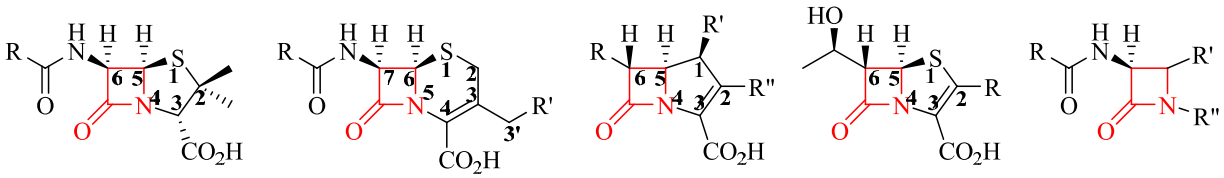


Figure 1.3: Representative structures from each class of β-lactam antibiotics.[1]

The penicillins were the first β-lactam antibiotics to be discovered in 1928. Alexander Fleming observed that a strain of *Penicillium* mould produced an agent which had antibacterial activity and which was later named penicillin.[10–12] Numerous attempts to discover novel penicillin derivatives were carried out by several research groups around the globe in the late 1940s.[13] Florey and Chain from the University of Oxford demonstrated the safe and effective use of penicillins via subcutaneous injection.[14] Only two natural sources of penicillins were known from the time of their discovery, namely penicillin G and penicillin V.[15] However, after the enzymatic hydrolysis of the C6 amido side chain of penicillins was achieved, numerous semi-synthetic derivatives were synthesized from 6-aminopenicillanic acid (6-APA) by acylation (Figure 1.4).[13] Natural penicillin G and V showed activity only against Gram-positive

bacteria. Later, semi-synthetic derivatives such as ampicillin and amoxicillin were found to be effective against both Gram-positive and Gram-negative bacteria.[16] Semi-synthetic penicillins have been used widely since World War-II to treat bacterial infections.[17] To this day, the semi-synthetic penicillin amoxicillin, manufactured by GlaxoSmithKline, is still used widely to treat bacterial infections.[18] Novel penicillin agents are still being developed; however, the main topic of interest in this study is the cephalosporin class of β-lactam antibiotics, which are discussed briefly in next section.

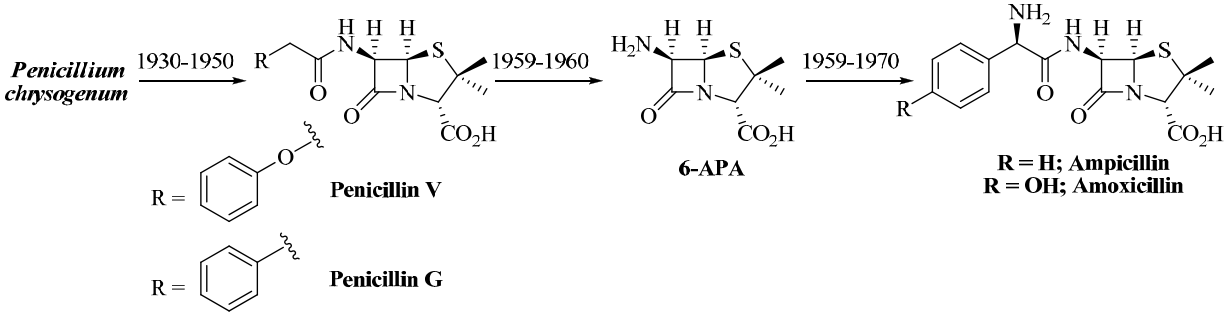
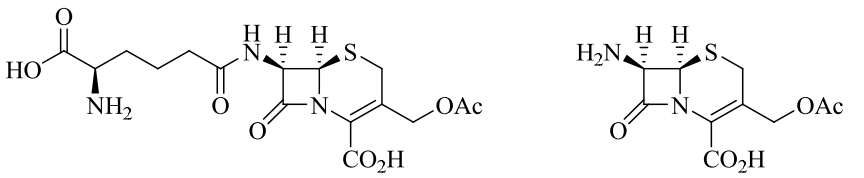


Figure 1.4: Timeline for evolution of penicillin derivatives.[13]

1.2.1 Cephalosporins

In 1945, many people suffered from an epidemic of typhoid fever caused by the Gram-negative bacterium *Salmonella enterica*.[16] Giuseppe Brotzu, an Italian pharmacologist, isolated a fungus from sewage water.[19] This fungus was later named *Cephalosporium acremonium*. The fungal broth was found to inhibit certain Gram-negative bacteria including *Salmonella typhi*, *S. paratyphi B*, *Yersinia pestis*, *Brucella melitensis*, *Vibrio cholera* and *Staphylococcus aureus*.[16,10] Three different compounds were isolated from *Cephalosporium acremonium*, namely cephalosporin P, cephalosporin N (penicillin N) and cephalosporin C. Cephalosporin C showed significantly higher antibacterial activity amongst them. Its structure was determined

(Figure 1.5) by E. P Abraham and G. C. F Newton in 1961.[19,20] All cephalosporins incorporate a β-lactam ring that is fused with a dihydrothiazine ring. Cephalosporin C on acidic treatment gave 7-amino cephalosporonic acid (7-ACA), which is a key precursor for the synthesis of major cephalosporins available in today's market (Figure 1.5).[20]

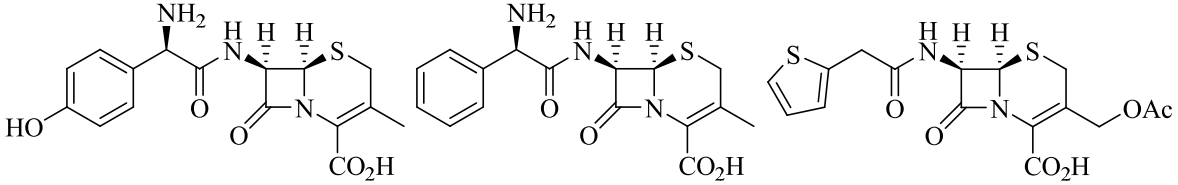


Cephalosporin C **7-Aminocephalosporanic acid**

Figure 1.5: Cephalosporin C and 7-Aminocephalosporanic acid (7-ACA) respectively.

Due to the potent antibacterial activity of cephalosporins, researchers began to study and develop derivatives with a cephalosporin core structure. Cephalosporins have been classified into five generations.[21] The classification is based on their activity towards Gram-positive and Gram-negative bacteria. However, the basis of their classification is often imprecise.

The first generation cephalosporins (Figure 1.6) show activities against most of the Gram-positive bacteria including methicillin-susceptible *Staphylococci* and *non-enterococcal Streptococci*. After the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) pathogens the first generation cephalosporins were used as first-line agents.



Cefadroxil **Cefalexin** **Cefalothin**

Figure 1.6: Examples of first generation cephalosporins.

The second generation cephalosporins (Figure 1.7) show high activities against Gram-negative bacteria such as *Haemophilus influenzae* and *Enterobacter aerogenes.* In addition, second generation cephalosporins also possess some activity against Gram-positive bacteria. Cefuroxime amongst them could penetrate the blood brain barrier (BBB), and hence was used to treat meningitis.[10]

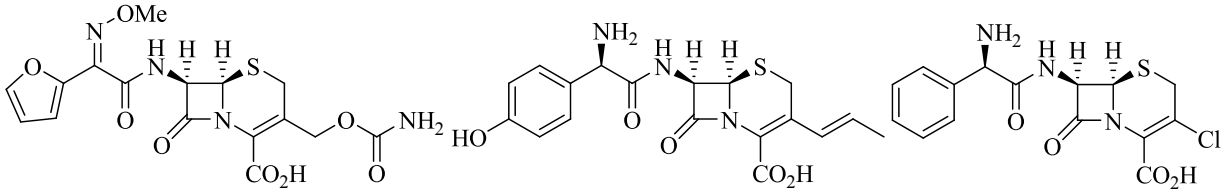


Figure 1.7: Examples of second generation cephalosporins.

The third generation cephalosporins (Figure 1.8) show broad spectrum activities against both Gram-negative as well as Gram-positive bacteria. These derivatives are less susceptible to hydrolysis by β-lactamases (later discussed briefly in section 1.3.1).

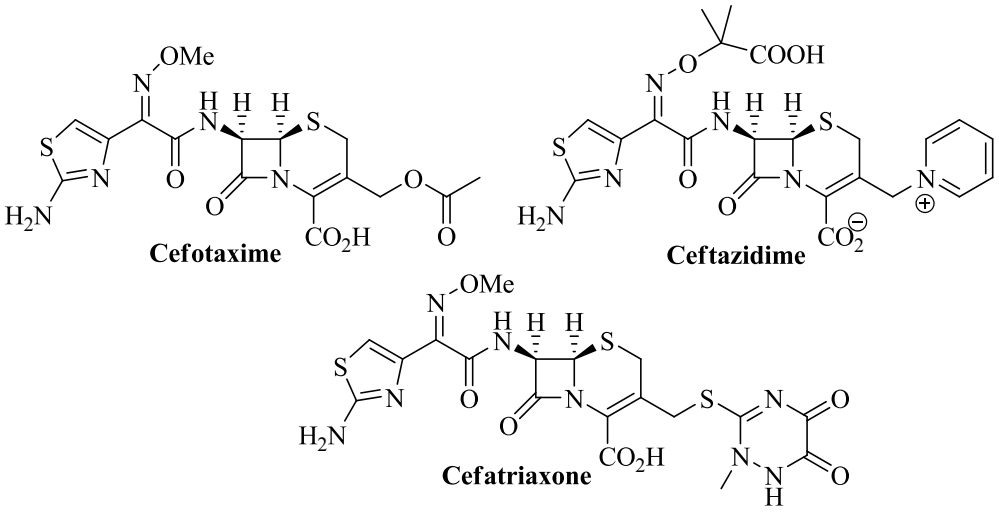
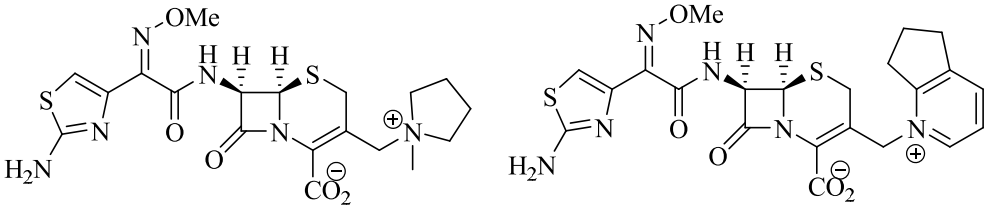


Figure 1.8: Examples of third generation cephalosporins.

Third generation cephalosporins were mainly used to treat hospital-acquired and community-acquired infections. Many compounds from this generation were also able to

penetrate the blood brain barrier (BBB) and were used to treat meningitis caused by *Pneumococci*, *Meningococci*, *Haemophilus influenza, Klebsiella* and penicillin-resistant *Neisseria gonorrhoeae*.[10]

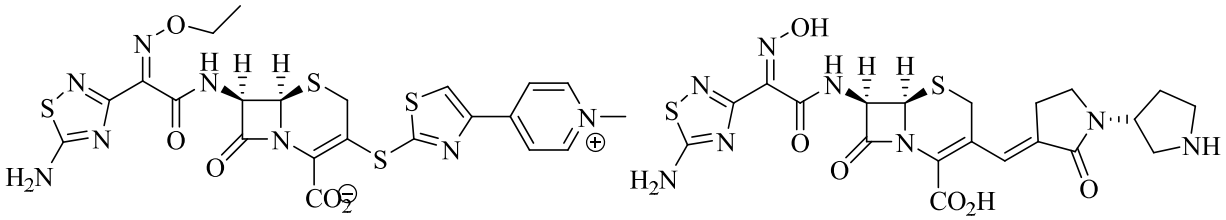
The fourth generation cephalosporins (Figure 1.9) demonstrated extended spectrum activities against *Enterobacter* spp., *Citrobacter freundii* and *Serratia marcescens.* Unfortunately, these drugs were not effective against Gram-positive bacteria which were already resistant to other β-lactams.[10]



Cefepime **Cefpirome**

Figure 1.9: Examples of fourth generation cephalosporins.

The fifth generation cephalosporins (Figure 1.10) were developed in the last decade and are still under research and clinical trials. These antibiotics show activity against multidrug-resistant *S. aureus*, such as: MRSA, VISA (Vancomycin-intermediate *S. aureus*), Vancomycin-resistant *S. aureus* and certain *S. pneumoniae*.[22]



Ceftaroline **Ceftobiprole**

Figure 1.10: Examples of fifth generation cephalosporins.

1.3 Bacterial Resistance to β-lactam Antibiotics

Widespread use and abuse of antibacterial compounds has led to antibiotic-resistant strains of Gram-positive and Gram-negative bacteria.[8,23] Bacterial resistance against β-lactam antibiotics occurs via four major pathways which include: production of β-lactamase enzymes, that cleave the key β-lactam ring of the β-lactam antibiotics hydrolytically, and which are the focus of this research; modification of the antibiotic target site, in which the binding target site of antibiotics penicillin binding protein (PBP) is altered in most resistant bacterial pathogens; expression of efflux pumps, which decrease the efflux for the drugs through the cell surface; and decreased expression of outer membrane proteins (OMPs), that are responsible for uptake of the drugs into the bacterial cell.[24,25] Among these resistance mechanisms, the production of β-lactamase enzymes is one of the major pathways and is discussed briefly in section 1.3.1. In order to explore the solution to bacterial resistance process; firstly, it is important to have an insight into the mode of action for β-lactam antibiotics.

The bacterial cell wall is made up of a tightly cross-linked peptidoglycan layer that maintains the cell rigidity, shape and protects it against environmental threats.[26] The bacterial cell comprises of a cross-linked cell wall, which is made by cell wall transpeptidase enzymes.[27] They are commonly known as penicillin binding proteins (PBPs).[28] These enzymes are responsible for the formation of the basic repeating units of *N*-acetyl glucosamine (NAG) and *N*-acetyl muramic acid (NAM) cross linked with each other by D-alanine-D-alanine amide bonds.[26] Figure 1.11 represents the cross linking process in *E. coli* and *S. aureus* cell walls.[29–31]

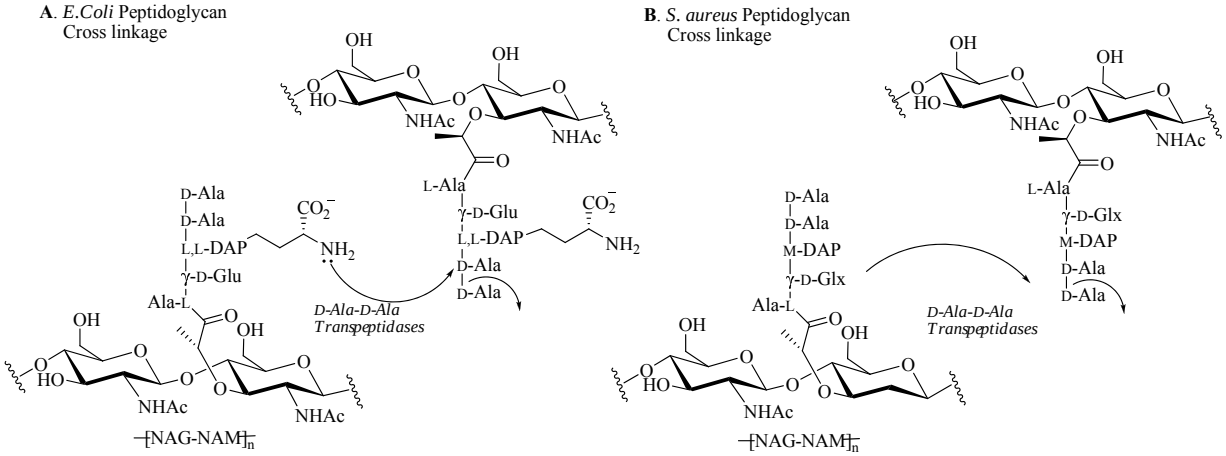


Figure 1.11: Cross linkage by D-Ala-D-Ala transpeptidase in [**A**] *E. Coli* and [**B**] *S. aureus*. (DAP = diaminopimelic acid; Glx = Gln or Glu; NAG = *N*-acetyl glucosamine; NAM = *N*-acetyl muramic acid).[29]

Tipper and Strominger in 1965 proposed a mechanism based on an analogy between the structure of penicillin and the D-Ala-D-Ala part of the trans peptidation process in formation of the peptidoglycan cell wall (Figure 1.12).[31]

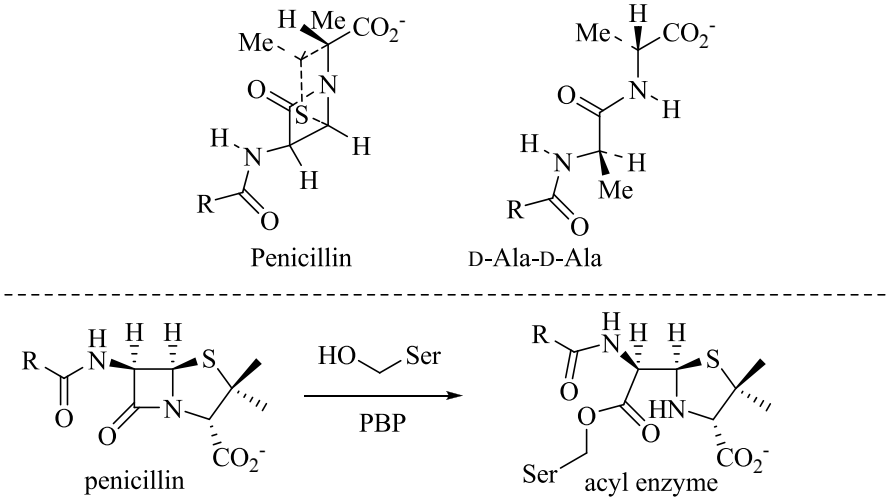


Figure 1.12: Conformational similarity between penicillin and D-Ala-D-Ala.[31]

As a result of the structural similarity of the β-lactam and of the D-Ala-D-Ala, the antibiotic forms a covalent penicilloyl-enzyme complex with the transpeptidase that is responsible for cell wall cross binding. The acyl enzyme complex hydrolyses very slowly (or not at all) to generate the free transpeptidase enzyme (Figure 1.12). The acyl enzyme linkage leads to the inhibition of bacterial transpeptidase enzyme which prevents completion of cell wall synthesis. Since the rigid cell wall is incomplete, the cell contents are subjected to severe osmotic pressure and eventually burst.[26]

1.3.1 Resistance caused by β-lactamases

The existence of resistance to β-lactam antibiotics was first observed by Abraham and Chain in 1940, who reported that some strains of *Staphylococci* developed resistance against benzylpenicillin.[32] With the clinical use of synthetic cephalosporins, resistance shown by Gram-positive organisms was decreased.[21] Gram-negative organisms, on the other hand started to develop resistance against almost all β-lactam antibiotics.[33]

Approximately 900 β-lactamase enzymes have been reported.[34] In the early stage of β-lactam antibiotic development, β-lactamases were divided into classes depending on their response to antisera, their substrate profile and on the plasmid-mediated enzymes that can be distinguished by isoelectric focusing. β-Lactamases were grouped into two classes,[35] namely Cephalosporinases (rapidly hydrolyze cephalosporins) and Penicillinases (rapidly hydrolyze penicillins).

Presently, the classification of β-lactamase is based on amino acid residues in the active site. The β-lactamases were classified into two major groups depending on their amino acid residues found in the active site, namely serine β-lactamase (SBLs) and metallo β-lactamase

(MBLs). A proposed mechanism for the hydrolysis of β-lactam antibiotics by serine β-lactamases (SBLs) and metallo β-lactamases (MBLs) is shown in figure 1.13.

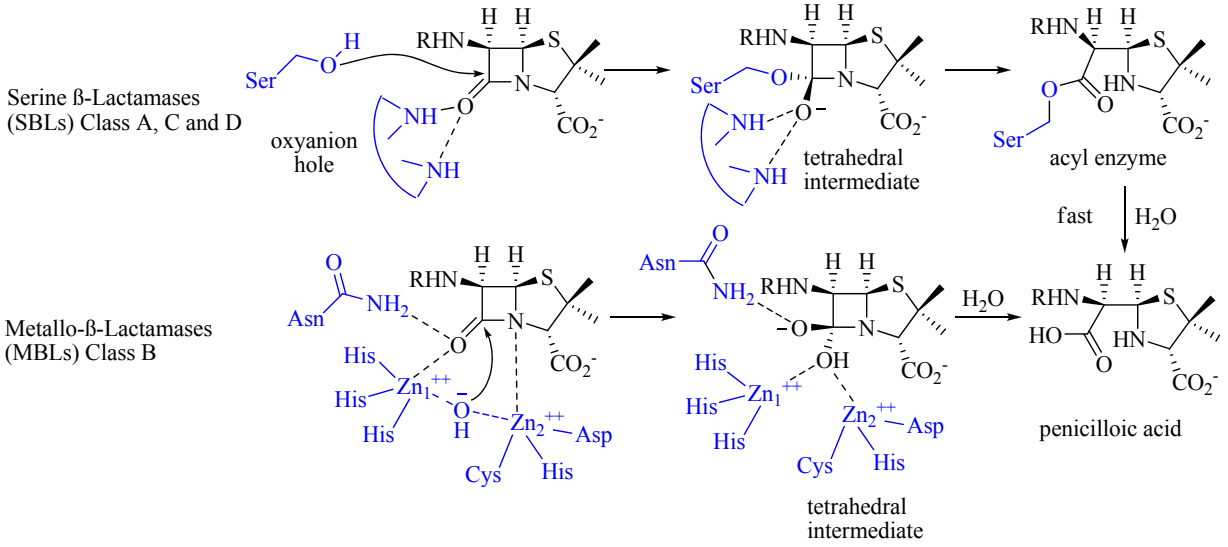
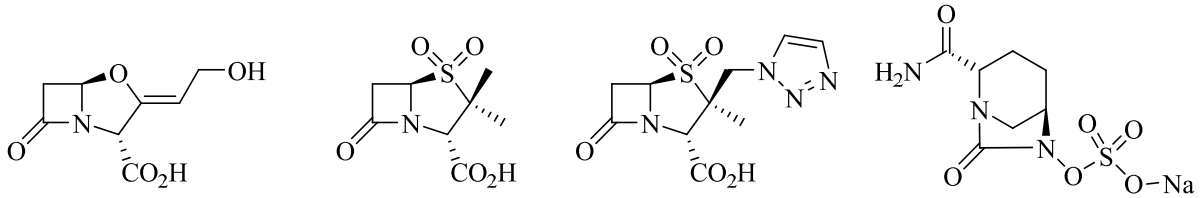


Figure 1.13: Proposed mechanism for the hydrolysis of β-lactam antibiotics.

In SBLs, the serine bound hydroxide ion attack the β-lactam bond; forming a tetrahedral intermediate which then forms a acyl enzyme complex. In presence of water in the active site, very fast hydrolysis would generate the penicilloic acid. In MBLs, the zinc bound hydroxide anion attacks the β-lactam bond; forming the tetrahedral intermediate which then undergoes hydrolysis in presence of water to give penicilloic acid. The SBLs and MBLs have been sub-classified into the following four sub-groups:[35–37]

[i] Class A β-lactamases

The enzyme active site of class A β-lactamases contains a serine residue, that acts as a nucleophile and contains a glutamate residue (Glu166) which acts as a general base in the catalysis. The most common class A β-lactamases are TEM, SHV, and CTX-M. They are resistant to penicillin derivatives and hydrolyze them easily when compared to cephalosporins and carbapenems (Figure 1.14).[35–37]



Clavulanic acid **Sulbactam** **Tazobactam** **Avibactam (Clinical trials)**

Figure 1.14: Clinically used and under clinical trial serine-β-lactamase inhibitors.

[ii] Class B β-lactamases

These enzymes use Zn^{2+}-bound water or hydroxide to hydrolyze the β-lactam antibiotics. The most wide-spread clinical threat is from IMP, VIM, SPM and NDM enzymes. Even though the metal ion chelators such as EDTA and dipicolinic acid can act as the corresponding inhibitory agents for class B β-lactamase, they are not selective and so are not suitable for clinical use. Therefore, there is an urgent need to develop class B β-lactamase inhibitors.[35–37]

[iii] Class C β-lactamases

This class was identified after class A β-lactamases in which it incorporates a tyrosine phenolate anion as a general base in catalysis**.** The class C AmpC lactamase is resistant to clavulanic acid and also to cephalosporin candidates. [35–37]

[iv] Class D β-lactamases

β-Lactamases in this group were observed to hydrolyze oxacillin derivatives particularly well. Therefore, they are also known as OXA enzymes. These were also identified as serine-containing enzymes that are usually found in *P. aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae*. They incorporate a lysine residue that reacts with CO_2 to form a

carbamate anion that acts as a general base during hydrolysis. Class D β-lactamases are inhibited by certain cephalosporin derivatives.[35–37]

As a result of the resistance of bacteria against β-lactam antibiotics, combination therapies involving the antibiotic and a β-lactamase inhibitor were developed to fight the resistance caused by ESBLs.[10] For instance, the combination therapy of ceftaroline fosamil (prodrug) and avibactam (non β-lactam β-lactamase inhibitor) was studied for phase-II clinical trials (Figure 1.15).[22] Cefotaxime and ceftazidime from the fourth generation of cephalosporin were studied for the combination therapy with β-lactamase inhibitors and prominent increase in antibiotic activity was observed against the resistant strains.[10]

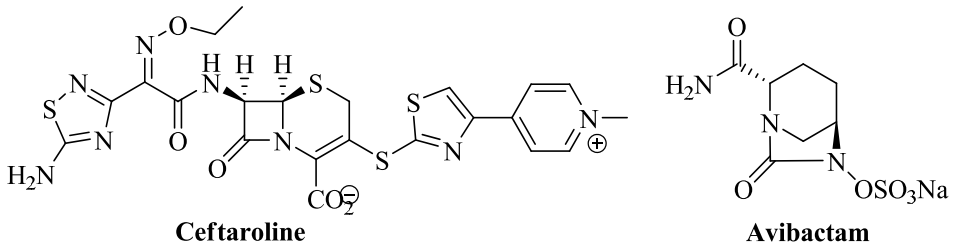


Figure 1.15: The combination therapy of cephalosporin and avibactam.[22]

Cephalosporin antibiotics are still being researched and novel cephalosporin derivatives are being developed that have the potential ability to act both as β-lactam antibiotics as well as β-lactamase inhibitors. For example, Cubist Pharmaceuticals is currently developing ceftolozane – tazobactam combination under phase-III clinical trials, which has improved activity versus *P. aeruginosa* and other Gram-negative bacteria (Figure 1.16).[38] Other pharmaceutical companies have also reported exploring new cephalosporin analogues including Shionogi & Co. Ltd., that is developing a cephalosporin called S-649266.[39] It has been reported that this cephalosporin is 10-10,000 times less susceptible to carbapenemase than

ceftazidime, cefepime and meropenem.[39] Unfortunately, the structure of S-649266 has yet to be disclosed.

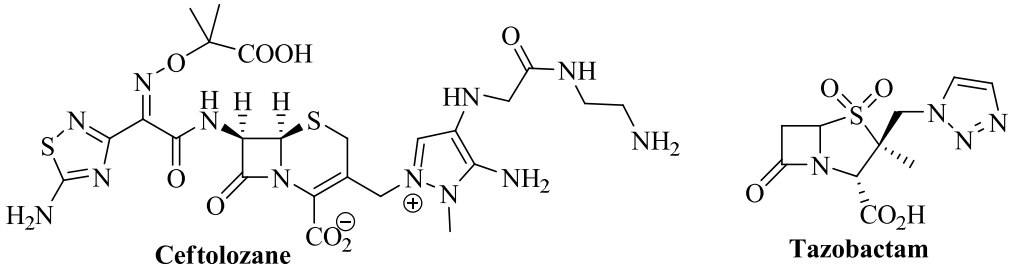


Figure 1.16: The combination therapy of ceftolozane and Tazobactam.[38]

At the University of Waterloo, the Dmitrienko group is developing novel cephalosporin derivatives which have the potential to act as metallo β-lactamase (MBLs) inhibitors. For example, cephalosporin analogues having a benzoylthio group at C3′ of a cephalosporin core structure have been developed (Figure 1.17).[34]

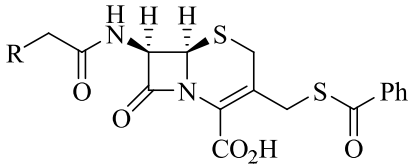


Figure 1.17: Novel cephalosporin derivative as β-lactamase inhibitors (Dmitrienko group).[34]

A possible mechanism was proposed for these C3′- benzoylthio compounds acting as metallo-β-lactamase inhibitors shown in figure 1.18. The proposed mechanism suggests that after β-lactam hydrolysis by the metallo β-lactamase enzyme the benzoylthio anion acts as a good leaving group creating an intermediate **1.5** that binds tightly to the zinc inons in the active site of the MBLs. Enzyme assay of these potential compounds were also studied against MBLs, and the corresponding IC_{50} values are shown in Table 1.1.[34] However, modifications at the R group (Figure 1.17) are currently under investigation. Extensive microbiological experiments by the Dmitrienko group have demonstrated that compounds of this type are

capable of acting in synergy with β-lactam antibiotics (e.g. meropenem Figure 1.19) resulting in a decrease in the minimum inhibitory concentration (MIC) of the antibiotic when the inhibitor and antibiotic are used in combination against resistant bacteria (e.g *Pseudomonas aeruginosa*) that produce MBLs (VIM-2).

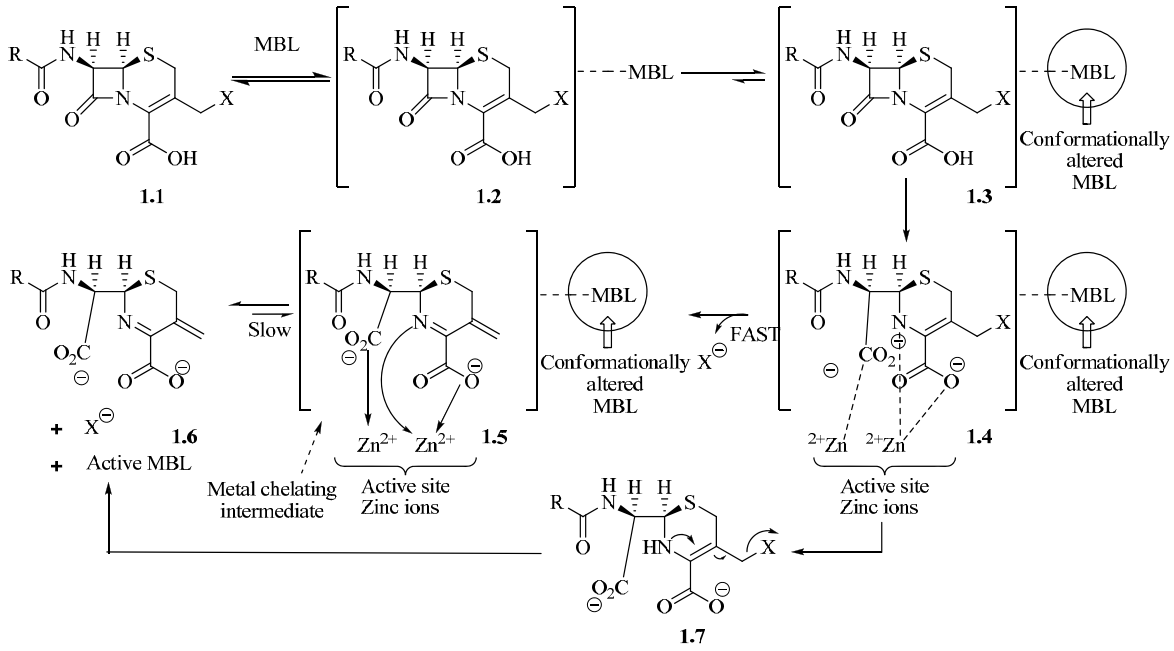
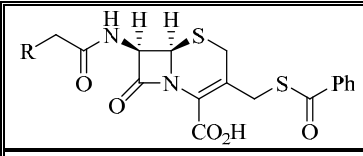
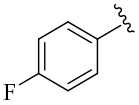


Figure 1.18: Mechanism for inhibition of metallo β-lactamases (MBLs).[34]

Table 1.1: IC_{50} values for some C3′- thiobenzoyl cephalosporin derivatives showing inhibitory activity against MBLs in vitro.[34]

	Class B IMP-1	Class B VIM-2
		
R= 	3.1 μM	1.8 μM
R= 	8.2 μM	4.2 μM

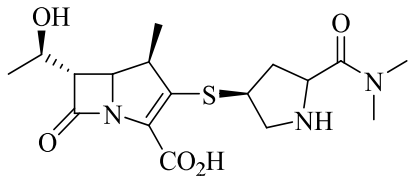


Figure 1.19: Chemical structure of meropenem.

Optimization of the potency of the inhibitors is underway in the Dmitrienko group. Once a compound with sufficient potency against MBL producing *P. aeruginosa* is achieved, the next phase of the drug development process will involve preliminary pharmacokinetic (PK) studies in mice. The animal studies will be carried at the Preclinical Drug Research Consortium (Vancouver, BC). Analysis of the concentration of the drug in serum samples will, however, be carried out in the Dmitrienko laboratory. This requires that an analytical method with the appropriate internal standards be developed, which is the overall goal of this research project.

1.4 Pharmacokinetic Parameters

Pharmacokinetic/Pharmacodynamics (PK/PD) studies are typically conducted during preclinical trial and clinical trial lasting up to phase-III for drug candidates.[40] The pharmacokinetic analysis provides insight into the time course of drug absorption, distribution, metabolism and excretion (ADME).[41,42] These PK/PD parameters are studied using various mathematical models, linear and non-linear equations, compartment models and statistical calculations.[41]

The therapeutic efficacy of antibiotics directly correlates to the time for which the antibiotic is above the minimum inhibitory concentration ($T>$ MIC).[43] Although, the β-lactam antibiotics are the first line of treatment for bacterial infection in critically ill patients, their efficacy is difficult to evaluate.[41] Most β-lactam antibiotics are available via the parenteral

route and the oral route depending on their pharmacodynamics parameters.[44] The bioavailability of β-lactam antibiotics is dependent on their half-life ($t_{1/2}$) and their serum concentration.[41] The pharmacokinetic behavior of these compounds is observed to follow a linear kinetic relation, in which the rate of reaction is directly proportional to the concentration of the drug.[43]

PK parameters are dependent on the type of the compartment model being considered. In a non-compartment modeling the whole body is considered to be one system. Non-compartment models are constructed for intravenous infusion (I.V.) of most cephalosporins during their preclinical studies.[42] The plasma drug concentration versus time profile is one of the most important PK parameters and is determined by measuring the drug concentration in plasma at various time intervals after dosing. A typical PK graph is constructed based on this data. Various PK parameters are determined from this information using mathematical formulas and statistical calculations[44] such as:

- Peak plasma concentration (C_{max}) in which the time corresponding to maximum drug concentration in plasma is determined. At this point of time, the rate of drug absorption is equivalent to the rate of drug elimination.[21,40,41]
- Time of peak concentration (t_{max}) which is the time at which the concentration of the drug in plasma achieves at its peak.[21,40,41]
- Area under curve (AUC) - The total integrated area under the plasma level versus time profile is known as the Area under the Curve (AUC). This value is used to determine the total amount of drug that is present in systemic circulation after dosing.[21,40,41]
- Apparent volume of distribution - The hypothetical volume of body fluid into which the drug is dissolved or distributed is termed as apparent volume, and a constant relation

between the concentration of drug in plasma and amount of drug in blood is proportional and represented by apparent volume of distribution.[21,40,41]

- Clearance (Cl) and Renal Clearance (Cl_R) - The hypothetical volume of body fluid containing drug from which the drug is cleared completely in a specific time period is called the clearance rate. The volume of blood or plasma completely cleared of the unchanged form of drug by the renal route per unit time is called renal clearance.[21,40,41]
- Half-life ($t_{1/2}$) - The time period required for the concentration of drug to decrease by one half is called the half-life.[21,40,41]

Determination of all the above mentioned pharmacokinetic parameters provides information about the behaviour of all therapeutic agents. The plasma concentration measurements are very useful to know the absorption behaviour of the drug, as well as its distribution in the body.[45] This helps to determine the therapeutic window. PK parameters are extremely important to decide the safe dose of new drugs. A Preclinical PK study provides an insight about the behaviour of new drugs during their clinical trial. A large number of compounds showing good activity in vitro are rejected in their clinical trials because their PK studies show that they do not possess adequate bioavailability of the drug or that they exhibit various types of toxicity. Thus, a PK study helps to optimize the lead compounds and is essential to select a new drug whose PK behaviour is within the therapeutic window.[40]

Most initial PK/PD studies are carried out in mice. To construct an accurate plasma drug concentration versus time profile, blood samples are collected at intervals before and after the dose administration of compounds which are evaluated for its PK parameters. The plasma is centrifuged and all the biomolecules are extracted using an organic solvent.[42] The organic phase is separated and the aqueous phase is dried and analyzed through liquid chromatography-

tandem mass spectrometry (LC/MS) assays. The analyte sample is mixed with an internal standard of known concentration. The concentration of analyte is measured from the relative concentration of internal standard from its mass spectrometry analysis. For example, in a pharmacokinetic study of an intravenous ceftolozane-tazobactam combination (single and multiple dose), plasma samples were collected before dosing at 30, 60, 65, 75, and 90 min and after dosing at 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours.[38] These samples were analyzed using LC/MS assays and a plasma drug concentration versus time profile was generated. All of the rest of the PK parameters are calculated from the plot of plasma drug concentration vs time profile by using WinNonlin software.[38]

Accurate determination of PK parameters is very crucial in the process of drug development.[41] Over the last decades much research has been conducted to acquire higher accuracy in these PK studies. A brief description of LC/MS and internal standards is provided in the next few sections. To conduct accurate PK studies it was very important to use internal standards which very closely resemble the analyte which is being tested.[46] The present thesis focuses on the development of these internal standards to conduct PK studies of a wide range of novel cephalosporin analogues which are in the pipeline of drug development.

1.5 Chromatography

Various forms of chromatography are used widely for purification and separation of small organic molecules which are structurally closely related.[47] Chromatographic techniques work on similar principles, in which the compounds in a mixture are distributed between a stationary phase and mobile phase.[48] The mobile phase runs in one direction and the compounds are separated as a result of their different rates of travel, which are determined by

their relative affinities for the mobile phase and the stationary phase.[47] Amongst all the chromatographic techniques high performance liquid chromatography (HPLC) is one of the most widely used methods. A more recent variant, ultra-performance liquid chromatography (UPLC) is now being widely used to conduct major analytical studies in the field of drug discovery.[49]

Mass spectrometry is an analytical technique in which small organic molecules are analyzed based on the mass-charge ratio.[50] Over the last few decades there has been an enormous development in this field. In the 1980s, John Fenn (Nobel laureate) discovered the electrospray ionization method which changed the whole scenario of mass spectrometric studies. Since then, mass spectrometers have been widely used in clinical laboratories and the pharmaceutical industry as a reliable analytical technique for compound identification and quantitative studies.[50] In order to perform mass spectrometric studies the sample to be analyzed is first prepared by mixing with suitable solvents. The compound is ionized using various ionization techniques. After the ionization of the compound, the ions are separated based on their mass to charge ratio. These ions further pass through the analyzer and are then detected with the help of a computer device. A typical MS graph shows the plot of mass to charge ratio (m/z) to its relative abundance.[47]

Hyphenated analytical techniques were discovered which showed more specificity and robustness. Gas Chromatography Mass spectrometry (GC-MS) and Liquid Chromatography Mass spectrometry (LC-MS) are widely used today for high throughput screening, selectivity, specificity and quantitative analysis of small organic molecules. Apart from simple organic molecules, MS is also used widely to analyze peptides, carbohydrates and a wide range of natural products. Owing to the nature of these compounds, especially biological species, which

cannot be vaporized for ionization in the gas phase, LC-MS is much preferred method for their analysis compared to GC-MS.[50] LC-MS plays an important role in the accurate determination of drug concentration in plasma.

In LC-MS the basic working principles of both the above mentioned analytical techniques are combined. The mixture of closely related compounds is injected on the chromatographic column.[49] Here the analyte is run through with a compatible mixture of mobile phase and separated. The solution is directly passed to the ionization chamber of the MS. After ionization, the ions are detected which helps to quantitate the analyte and analyse it. LC-MS has a wide application for the determination of PK parameters as mentioned earlier. After the collection of plasma samples during their pre-clinical and clinical studies, the analyte is extracted from plasma. In order to determine the drug concentration present at that particular time, a known amount of internal standard, which very closely resembles the analyte is mixed with it.[51] Most commonly, a known amount of isotopically-labelled compound is mixed with an unknown amount of unlabelled analyte of interest. This mixture is loaded on the chromatographic column. Since, the internal standard and analyte only differ isotopically, they cannot be separated. Once the peak for the analyte is observed, the mobile phase is introduced into the ionization chamber with the help of an automatic sample injector. The ions travel in a vacuum tube depending on their charge to mass ratio. Further, they are analyzed and reported in the mass spectrum.[47] Quantification of unlabelled compound can be carried out using the concentration of labelled compound. In this manner the plasma drug concentration is determined.[52]

1.6 Internal Standard

An internal standard is used during the quantification of analyte using mass spectrometry studies. HPLC and MS/MS (LC-MS/MS) are the most common and extensively used analytical techniques for measuring concentration of drug present in plasma, blood and urine samples. The internal standard is used as a reference substance to quantify the concentration of unknown analyte in these assays.[53,54] The choice of an internal standard is very important for accurate quantitative analysis of an analyte. The choice of internal standard (IS) is based on several considerations: IS can never be found in the untreated sample, which would result in inaccuracy; IS should always be available in pure form and should be extremely stable throughout the reaction; ideally IS should be identical to the analyte and differs only in isotopic composition or structurally similar to the analyte and is eluted after the analyte.[54]

The best internal standard is an isotope labelled analogue of the analyte, which needs to be quantified. The isotope-labeled internal standards have the same chemical structure as the analyte, only differing in their atomic mass unit (amu) due to highly enriched presence of a particular isotope. HPLC and MS/MS techniques are respectively used for purification of the analyte and detection of its mass for quantitative analysis.[55] Owing to structural identity, the analyte and its internal standard show the same retention time on the UPLC.[53] However, these are differentiated by their mass analysis giving selective, specific and quantitative results.[54] A known concentration of the isotope labeled compound is mixed in the plasma extract and the ratio of labeled to unlabeled analyte is determined using MS assay.[56] It is very important to check the purity of internal standard prior to its use.[56] Certainly, a little contamination in the

internal standard would lead to error in all the following results and hence would compromise the accuracy, reproducibility and robustness of the method.

1.7 Isotope Labeling

Synthesis of a stable isotope-labeled compound for its use as internal standard can be time consuming and costly. Stable isotopes of hydrogen (^{2}H/D), carbon (^{13}C), nitrogen (^{15}N), and oxygen (^{17}O, ^{18}O) are commonly available. However, the most cheap and easy method for synthesis of internal standard is by deuterium isotope labeling. Deuterium (D or ^{2}H) is a stable isotope of hydrogen having 1 atomic mass unit (amu) more compared to protium (H), which is the most abundant isotope of hydrogen. Deuterium has been used extensively for various studies, which includes metabolic studies for organic compounds and analytical assay using LC-MS/MS and GC-MS/MS techniques.[57] It is also useful for studies related to mechanism elucidation involved in enzymatic or chemical reactions.[58]

Cost–and time–effective reactions involving H/D (Hydrogen/Deuterium) exchange have been extensively studied.[59] Various approaches can be used that include pH-dependent exchange, exchange without the addition of acidic or basic deuterium source (these reactions might be applied for deuteration of the cephalosporin core), homogeneous metal catalysis, heterogeneous metal catalysis (e.g. palladium/carbon, platinum/carbon, rhodium/carbon and nickel/carbon). However, homogeneous and heterogeneous metal catalysis reactions are not applicable to label the core cephalosporin structure due to its instability under such conditions, since the β-lactam ring can hydrolyze at high temperature and pressure.[59]

1.8 Hypothesis and Rationale

Examination of the literature concerning deuterated analogues of cephalosporins reveals that the most common approach involves introduction of three or more deuterium atoms into one of the side chains R_1 or R_2 attached to the cephalosporin core ring structure (Figure 1.20).

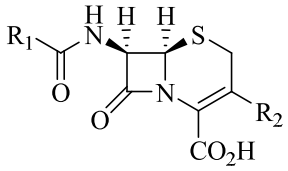
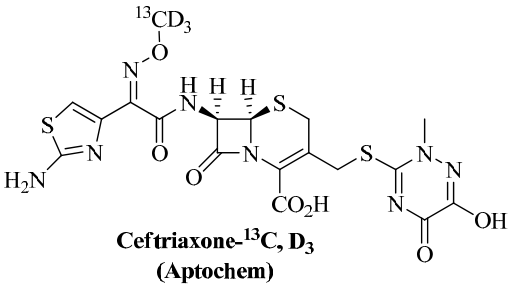
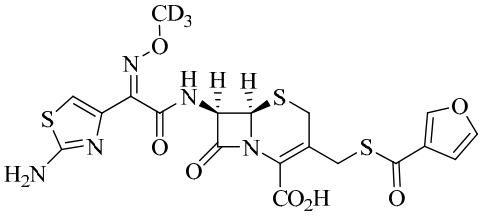


Figure 1.20: General cephalosporin structure.

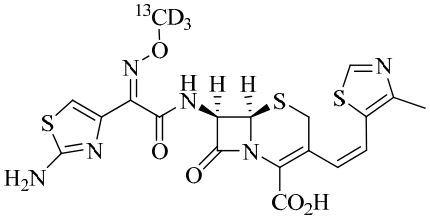
For example, Figure 1.21 illustrates a few examples of cephalosporins deuterated on their side chains along with the supplier and its cost. In all the examples, the deuterium was incorporated on the C7 side chain.



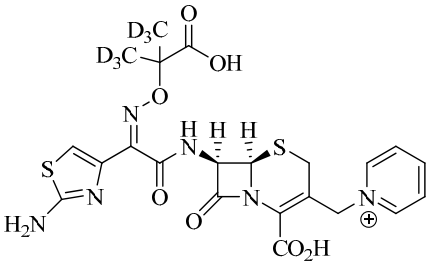
Ceftriaxone-^{13}C, D_3
(Aptochem)
1 mg - $600



Ceftiofur-D_3
(Cayman chemical)
2.5 mg - $435



Cefditoren-^{13}C, D_3
(Aptochem)
1 mg - $600



Ceftazidime-D_6
(Alsachim)

Figure 1.21: Commercially available deuterated cephalosporin analogues.

The presence of an alkoxyimino group on the C7 side chain of cephalosporin is found to be beneficial for binding to PBPs. One strategy to develop novel cephalosporin antibiotics involves modification of the alkoxyimino group. According to the current pharmaceutical and academic research, internal standards used for the assay of these cephalosporins are compounds in which this alkoxyimino side chain is isotopically labeled. This necessitates the task of preparing a standard for each promising candidate, labelled by a different synthetic approach.

Since it was proposed that in the present study, it would be possible to carry out preliminary PK/PD studies on several cephalosporin-type MBLs inhibitor, placing the deuterium isotopes in new side chains R_1 and R_2 (Figure 1.20) might require the development of several isotope labeling strategies. It occurred to this group that the PK/PD studies could be facilitated by having access to a cephalosporin intermediate in which the deuterium isotopes were incorporated in the core ring structure, since R_1 and R_2 could then be varied without the need to design new methods for deuteration. It was also thought that such a deuterated cephalosporin core structure could be of value to others who are involved in the discovery and development of novel cephalosporins with improved antibacterial potency.

Thus, this thesis aims to synthesize a core cephalosporin analogue multiply deuterated at two sites. The availability of a multiply deuterated core cephalosporin compound will facilitate screening of cephalosporin derivatives modified on the C7 and C3 side chains. In order to avoid peak overlap during quantitative analysis using mass spectrometry, a minimum of three deuteriums should be present in the internal standard. The analyte may include hydrogen, carbon, nitrogen, sulphur, oxygen, halogens etc. As a consequence of the existence of minor isotopes at natural abundance, molecules show not only molecular ion peak $[M]^+$ but

also $[M+1]^+$ and $[M+2]^+$ peaks. $[M+1]^+$ peak arise from the 1.11% ^{13}C, 0.38% ^{15}N, 0.78% ^{33}S, 0.016% ^{2}H and to a lesser extent 0.04% ^{17}O present among the sample molecules. The $[M+2]^+$ peak arises from the 4.4% ^{34}S, 0.2% ^{18}O and 32.5% ^{37}Cl (if present). In order to avoid any peak interference between the analyte and internal standard peaks, having three or more atomic mass units higher in internal standard compared to the analyte will be observed distinct from that of the analyte. The peaks for internal standard can then be differentiated from the analyte and hence an accurate concentration of the drug at that time point can be measured without interference from the analyte MS peaks due to its isotopes at natural abundance.

The primary objective of this study is to synthesize a multiply deuterated core cephalosporin compound. The core structure of the cephalosporins has hydrogens at C2, C3′, C6 and C7. The literature review discussed in Chapter 2 suggests that H/D exchange reactions have been accomplished on one of the methyl group at C2 of a penicillin sulfoxide[60] and cephalosporin sulfoxide[61,62] at the C2 position. Replacing the hydrogen with deuteration at C7 was a very time consuming exercise as mentioned by Baldwin and his co-workers.[63] No reports of incorporation of deuterium at C3′ or C6 of the cephalosporin core were found.

In this thesis research C2 and C3′ were considered as the sites for deuteration, as shown in Figure 1.22.

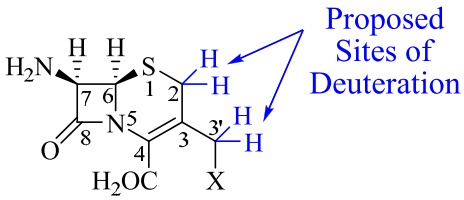


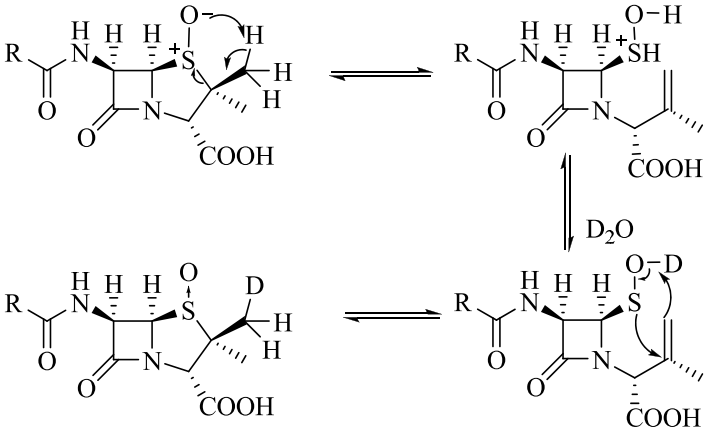
Figure 1.22: Proposed sites of deuteration in the core cephalosporin structure.

Chapter 2

Literature Review

2.1 Previous work done to Synthesize Core Deuterated Cephalosporins

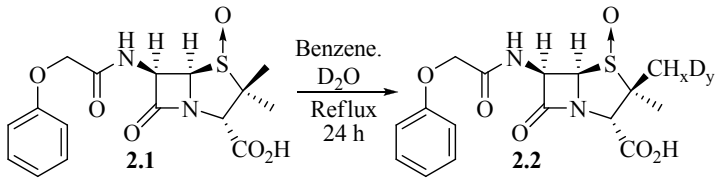
Cooper and co-workers, while studying the penicillin sulfoxide-sulfenic acid equilibrium, studies carried out deuterium incorporation at the methyl group of a penicillin sulfoxide by refluxing the sulfoxide of penicillin V in a mixture of benzene and D_2O. This reaction involves a β-elimination reaction to produce the sulfenic acid/alkene, and then deuterium exchange to generate the O-deuterated sulfenic acid. This then undergoes reversal of the elimination reaction as shown in Scheme 2.1.[60]



Scheme 2.1: The mechanism proposed by Cooper and co-workers for the deuteration of penicillin V sulfoxide.[60]

Scheme 2.2 illustrates the reaction conditions mentioned for performing H/D exchange. Benzene acts as a solvent for this reaction and D_2O is the source of deuterium incorporation at the methyl side chain. Compound **2.1** was stirred with a solution of benzene and D_2O at 80 °C for 24 hours to give compound **2.2**. The experimental detail related to the reaction was not

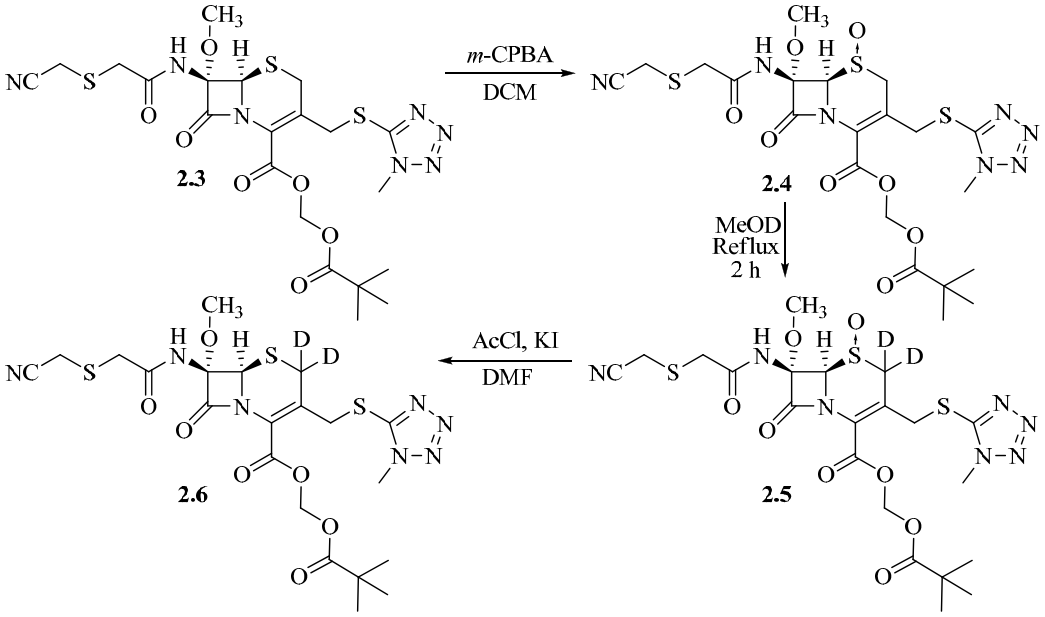
mentioned. However, the authors reported the recovery of penicillin sulfoxide **2.2** after the reaction as 100% and its ^{1}H-NMR study showed an average of one deuterium atom located only at its β-methyl group. Mass spectrometry analysis showed a mixture of 45% d_0, 43% d_1, 11% d_2, and 1% d_3 products.



X	Y	%
3	0	45
2	1	43
1	2	11
0	3	1

Scheme 2.2: Synthesis of C-2 deuterated penicillin V sulfoxide.[60]

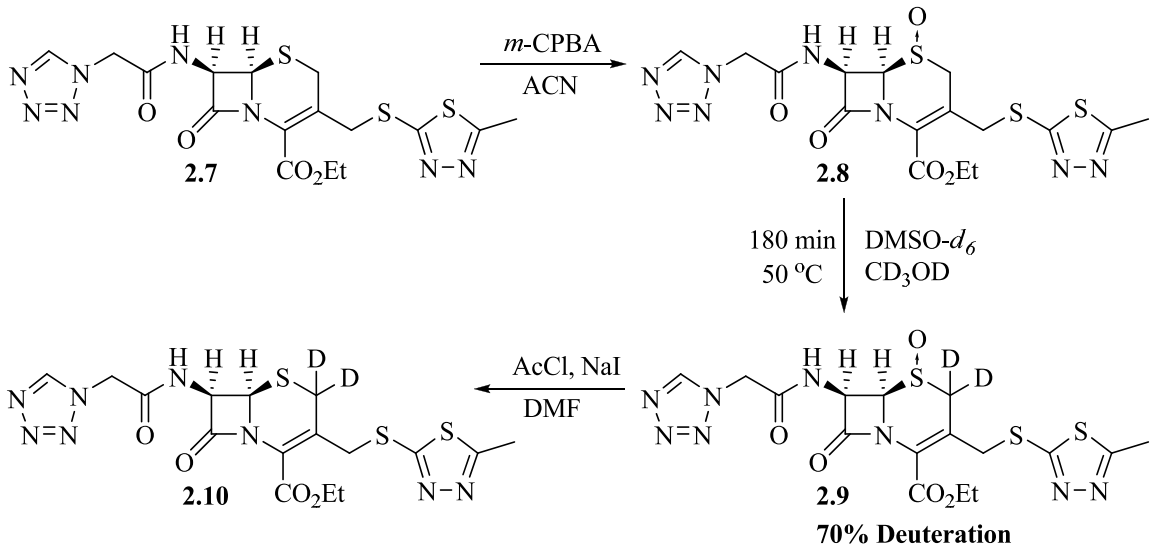
Taiichirou and his co-workers in 1981, were the first researchers to perform H/D exchange at the C2 position of a cephalosporin core.[62] However, no supporting information was given regarding the experimental conditions and the amount/percent of deuterium present at C2.



Scheme 2.3: H/D exchange at C2 of cephalosporins by Taiichirou and co-workers.[62]

Scheme 2.3 illustrates their reaction scheme in which compound **2.3** was oxidized to sulfoxide **2.4** using *m*-CPBA. The H/D exchange was carried out by refluxing compound **2.4** with MeOD for 2 hours to give compound **2.5**. This was then reduced back to the sulfide **2.6** using acetyl chloride in the presence of potassium iodide and DMF as solvent.

Later, Richter and his co-workers in 1990 performed a similar H/D exchange using DMSO-d_6 and methanol-d_4.[61] The experiment was performed to study the Δ2-Δ3 isomerization of a cephalosporin ester. When a cephalosporin ester (prodrug) is administered by an IV infusion route, it undergoes Δ2-Δ3 isomerization due to the presence of base in blood plasma. The researcher aimed to study the kinetic rate constants for the isomerization process. However, to our interest they carried out H/D exchange on C2 of a core cephalosporin analogue. The experimental procedure was not mentioned in detail. Their NMR studies show presence of 70% deuterium at C2 of the cephalosporin core.[61]

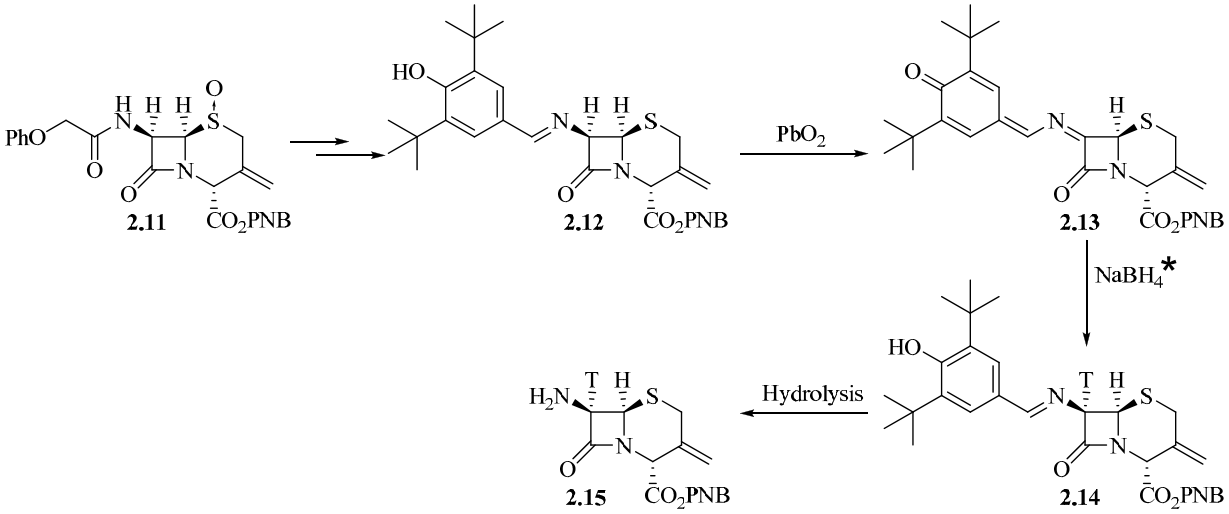


Scheme 2.4: H/D exchange on C2 of cephalosporins by Richter and co-workers.[61]

Scheme 2.4 illustrates their reaction scheme in which compound **2.7** was oxidized to sulfoxide **2.8** using *m*-CPBA. The H/D exchange was carried out by heating compound **2.8**

with methanol-d_4 and DMSO-d_6 at 50 °C for 90 minutes. Then the solvent was evaporated under reduced pressure and fresh methanol-d_4 was added and the reaction was repeated for 90 minutes to give compound **2.9**. The authors reported 70% of deuterium incorporation at C2 according to NMR studies. Compound **2.9** was reduced back to the sulfide **2.10** using acetyl chloride in the presence of sodium iodide and DMF as solvent.

Incorporation of tritium (^{3}H/T) at C7 of a cephalosporin analogue was carried out by Baldwin and co-workers as illustrated in Scheme 2.5[63] where compound **2.11** was the starting material. This was converted into the Schiff base **2.12** after several steps, which was then oxidized using lead oxide to give compound **2.13**. Compound **2.13** was reduced to the C7 tritiated Schiff base **2.14** using sodium borotritide which was then subjected to hydrolysis giving compound **2.15** as the free amine with an overall yield of 21%.



Scheme 2.5: Tritium (^{3}H/T) incorporation at C7 of cephalosporin analogue (* Tritiated).[63]

2.2 Objectives

As mentioned previously in section 1.8, deuteration at C2 and C3′ was proposed to be carried out to synthesize the desired multiply deuterated analogue as shown in Figure 1.22. Therefore the primary objectives assigned for the present thesis were as follows:

1. To perform H/D exchange at C2 of the core cephalosporin system using various approaches.
2. To incorporate deuterium at C3′ of the core cephalosporin system using various approaches.
3. After the completion of objective 1 and 2, both the strategies would be combined on one molecule to synthesize a core trideuterated cephalosporin analogue.

The deuterated core structure could then be used to synthesize isotopically labeled versions of cephalosporin derivatives. The synthesized internal standards might be used in future to determine the PK parameters for cephalosporins using HPLC and MS/MS assay.

Chapter 3

Results and Discussion

The main goal of this project is to synthesize a core-trideuterated cephalosporin. The core deuterated cephalosporin can be used to synthesize various internal standards for compounds of interest with modified C3′ and C7 side chains (Figure 1.22)

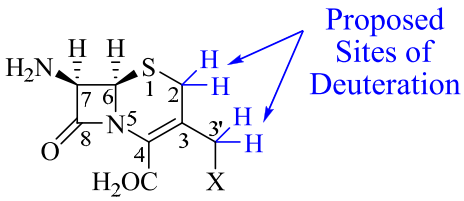


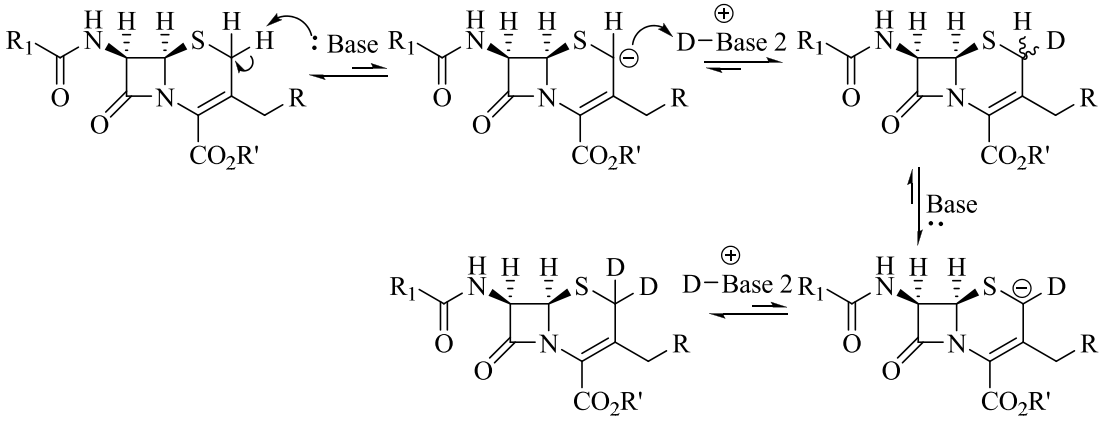
Figure 1.22: Proposed sites of deuteration in the core cephalosporin structure.

Due to the increase in antibiotic resistance, more research is being conducted to develop novel cephalosporins acting as β-lactamase inhibitors and as antibiotics. It is challenging to synthesize internal standards for each new modification on the side chains of cephalosporins. Hence, this project will be useful to synthesize internal standards for a wide range of novel cephalosporin compounds with modification on their side chains using the core trideuterated cephalosporin compound. The objectives set for this project are to perform H/D exchange on C2 and deuterium incorporation at C3′ using various approaches and then to combine the two methods to produce a trideuterated cephalosporin core compound.

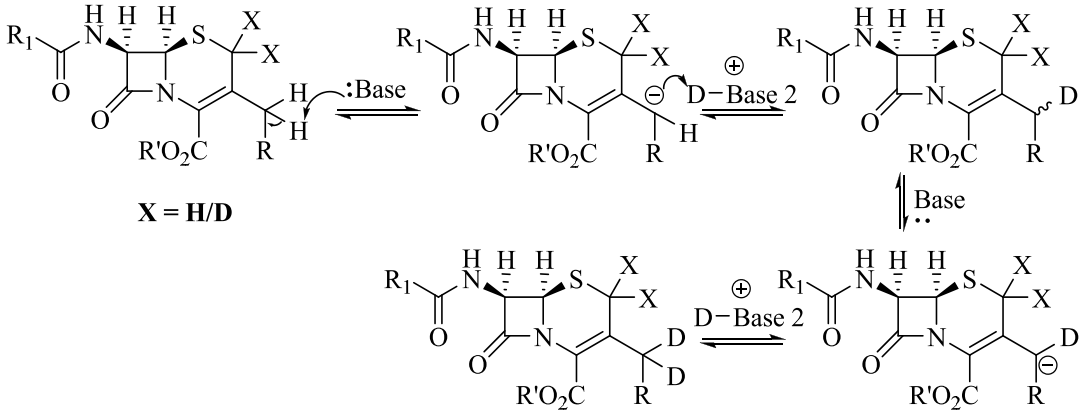
3.1 Objective 1: Hydrogen/Deuterium (H/D) Exchange at C2 of Cephalosporins

Our initial thoughts on the H/D exchange were based on the potential acidity of the hydrogens at C2 and C3'. Since the hydrogens at C2 and C3′ of the cephalosporin system are γ to the α,β-unsaturated ester, they are expected to be weakly acidic and might potentially

undergo de-protonation to form a conjugated dienolate which upon re-protonation by a weak deuterated protic acid would result in deuterium incorporation. A potential mechanism for deuteration at C2 and C3′ is proposed. Scheme 3.1 illustrates that the H/D exchange on C2 initiated with the non-deuterated base, deprotonating at C2 followed by deuteration using deuterated protic acid. This exchange is proposed to continue until it reaches an equilibrium stage. Scheme 3.2 represents the similar proposed mechanism of deuteration at C3′.



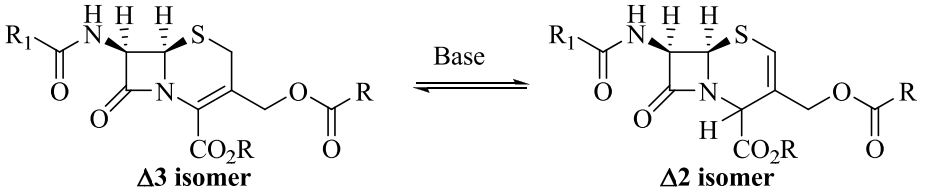
Scheme 3.1: Proposed mechanism for base-catalyzed H/D exchange at C2.



Scheme 3.2: Proposed mechanism for base-catalyzed H/D exchange at C3′.

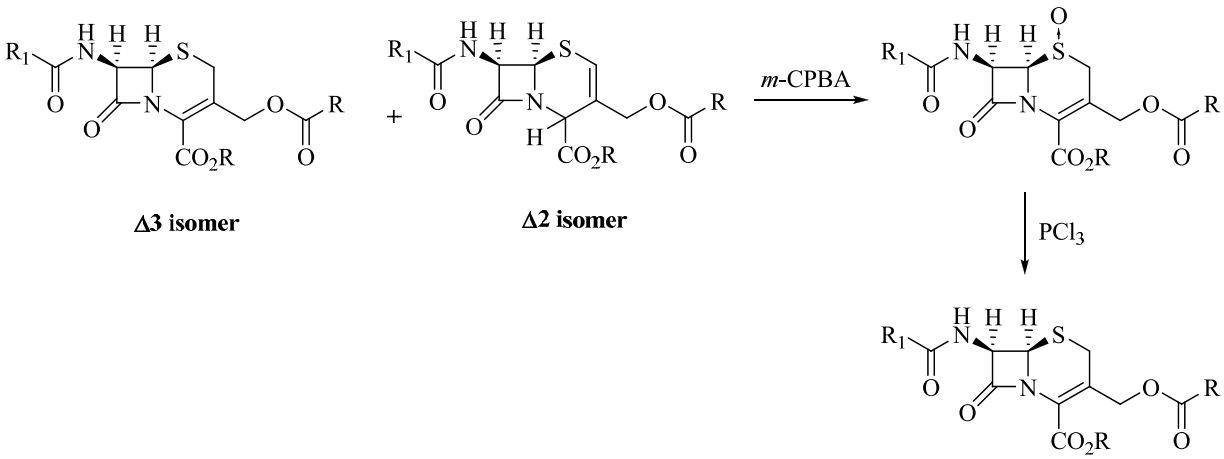
In cephalosporin esters, base catalyzed dienolate formation will compete with an isomerization reaction yielding a mixture of Δ2 and Δ3 isomers as shown in Scheme 3.3.[64] It

has been documented that the Δ2 isomer shows no biological activity and is the unwanted isomer.



Scheme 3.3: Isomerization observed for cephalosporin esters in presence of base.[64]

To convert the Δ2 isomer to the Δ3 isomer as shown in Scheme 3.4,[65] the mixture of Δ2 and Δ3 is stirred with an oxidizing agent (e.g. *m*-CPBA) to yield a sulfoxide for which the Δ2 to Δ3 equilibrium is strongly favored in the direction of the Δ3 isomer. The sulfoxide can then be reduced back to sulfide using PCl_3.[66]

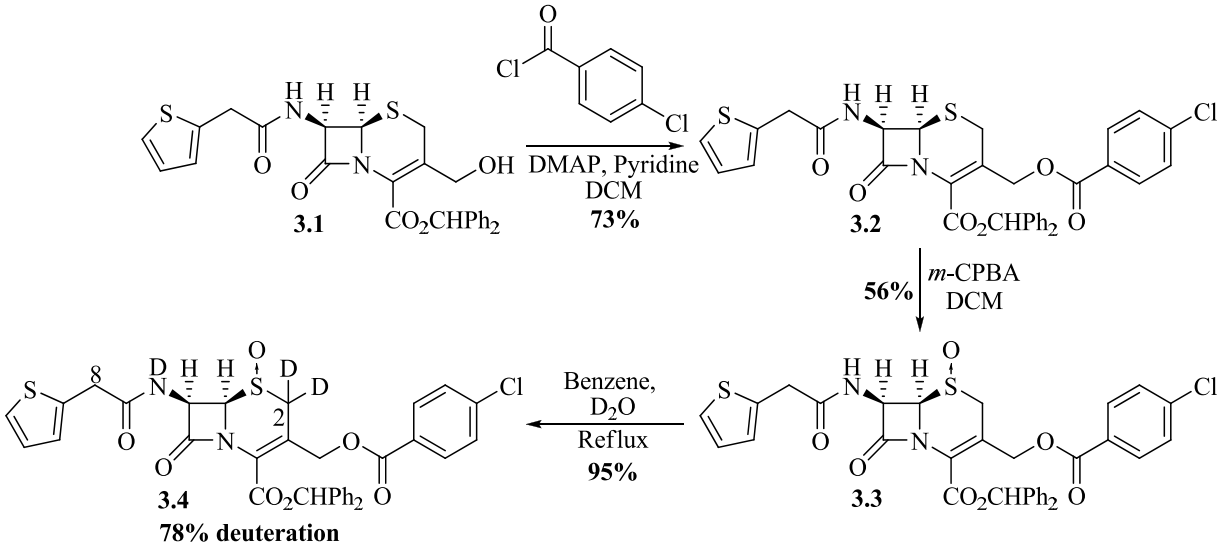


Scheme 3.4: A mixture of Δ2 and Δ3 cephalosporin isomers oxidized to corresponding Δ3 sulfoxide and its subsequent reduction back to sulfide.

Thus, if deuterium incorporation were attempted at C2, the Δ3 to Δ2 isomerization might lead to complications. The experiments described below made use of cephalosporin sulfoxide to deal with the Δ2 to Δ3 isomerization issue.

3.1.1 H/D exchange at C2 of Cephalosporin Core using Benzene/D_2O

One of the concerns about base-induced H/D exchange in the β-lactam antibiotic series is the sensitivity of the β-lactam ring towards nucleophilic attack. Thus, we sought out literature precedent for H/D exchange on β-lactams. As indicated in Chapter 2, Cooper and co-workers[60] had effected an exchange of one of the methyl groups of a penicillin sulfoxide by heating in a mixture of deuterium oxide (D_2O) and benzene without hydrolysis of the β-lactam. This indicated that such conditions might also be compatible with the β-lactam bond of cephalosporins. This led to experiments aimed to explore the synthesis of cephalosporin sulfoxide in order to make the hydrogens at C2 sufficiently more acidic so that they might undergo reversible de-protonation and deuteration in D_2O under prolonged heating conditions.[60] Thus, for the initial trial to verify the experimental conditions, the C3′ hydroxyl cephalosporin derivative **3.1** (obtained from Dr. Ahmad Ghavami, in this laboratory) was esterified at C3′ position using 4-chlorobenzoyl chloride, DMAP, and pyridine overnight at room temperature to give **3.2**. It was believed that making an ester derivative would make the hydrogens at C3′ more acidic; therefore, there was a possibility of exchanging hydrogens at C2 and C3′ in one step. In addition the free hydroxyl derivative **3.1** in presence of base is known to form C3′ lactone with the carboxylate at C4.[67] The solution of **3.2** in DCM was then oxidized to the corresponding sulfoxide at 0 °C, using *m*-CPBA to give **3.3**.[65] The H/D exchange reactions were carried out on **3.3** under various reaction conditions to give **3.4** as summarized in Table 3.1.



Scheme 3.5: H/D exchange using benzene and D_2O.

Table 3.1: Reaction conditions for H/D exchange to obtain **3.4**

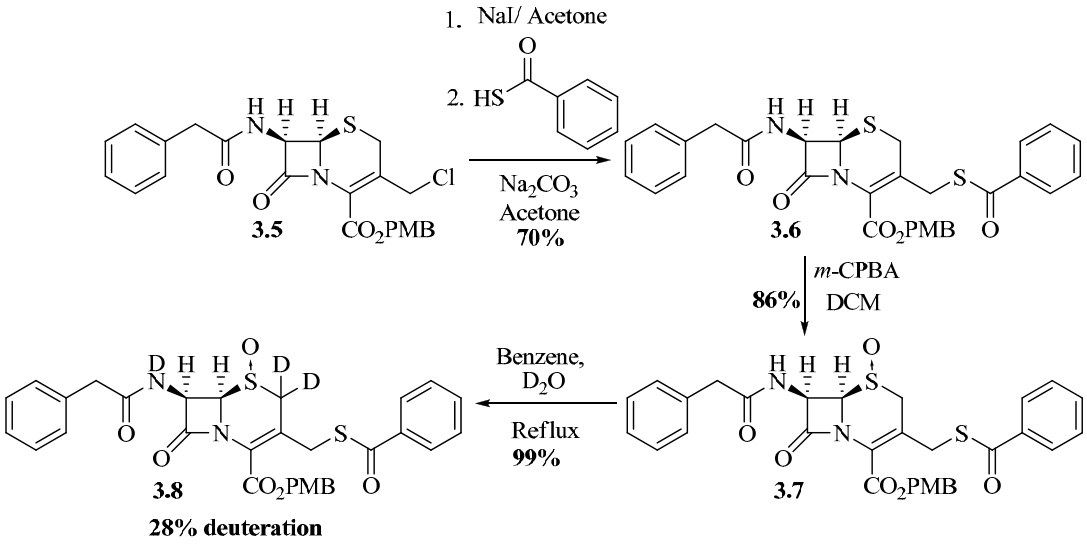
Exp. No.	Quantity of Reagent	Time (h)	(%D) by ^{1}H NMR
1.	**3.3**: 0.07 mmol; D_2O: 0.1 mL (100 eq)	19	20% D at one prochiral H of C2
2.	**3.3**: 0.07 mmol; D_2O: 1 mL (1000 eq)	93	60% D at one prochiral H of C2
3.	**3.3**: 0.15 mmol; D_2O: 10 mL (10000 eq)	136	78% D at one prochiral H of C2

The deuteration of **3.3** at C2 was monitored by ^{1}H NMR analysis. The first trial in the present thesis (entry 1) began by using 100 equivalents of D_2O relative to **3.3** and refluxing for 19 hours. The proton signal for each of the diastereotopic hydrogens at C2 is observed as a doublet. A decrease in the integration for one of these prochiral hydrogens was observed. However, the other doublet appears under the peak of hydrogens at C8 (phenyl-C*H*$_2$ side chain). Therefore the amount of deuterium enrichment was measured depending on the relative integration of only one of the prochiral hydrogen peak at C2. From the relative integration of

protons in the ^{1}H NMR spectrum, it was determined that there was 20% deuterium incorporation on one of the hydrogens at C2. In the next experiment (entry 2) in order to increase the deuteration on C2, the reaction mixture was allowed to stir for a longer period of time (93 hours). Also, the amount of D_2O was increased to 1000 equivalents relative to **3.3**, in order to favour greater deuterium incorporation at equilibrium. The ^{1}H NMR suggests the exchange of 60% deuterium with one of the hydrogen at C2. Entry 3 in table 3.1 shows that the amount of D_2O was increased from 1000 equivalents to 10,000 equivalents relative to **3.3** and the reaction time from 93 hours to 136 hours. ^{1}H NMR integration confirmed 78% of deuterium was exchanged with one of the prochiral hydrogen at C2 (Appendix B; Figure 3.1). The doublet peak for another prochiral hydrogen at C2 showed interference with the peak of hydrogens at C8 (phenyl-CH_2 side chain), therefore the deuterium enrichment cannot be measured with certainty.

Since this was the first set of reactions using these conditions, it was essential to conduct additional experiments for increasing the deuterium incorporation. Also, from the NMR spectroscopy studies there was no H/D exchange observed for the hydrogens at C3′. Since C3′ acyl thio cephalosporin derivatives are an important focus of research in this group, an H/D exchange was attempted with such a derivative. After the first attempt of H/D exchange, similar reaction conditions were used to perform H/D exchange on C3′ thioester derivatives. Compound **3.5** was used as the starting material, which was commercially available. Under Finkelstein conditions, a suspension of **3.5** in acetone was stirred with sodium iodide at 0 °C to synthesize the C3′ iodo derivative.[34] The iodine was replaced in situ with thiobenzoate anion to yield **3.6** using sodium carbonate as base. Compound **3.6** was oxidized to give the sulfoxide **3.7** using *m*-CPBA at 0 °C in 86% yield.[65] H/D exchange on **3.7** using

benzene and D_2O at 80 °C for 154 hours afforded **3.8** with 28% deuterium incorporation at one of the C2 prochiral hydrogens as determined by ^1H NMR spectroscopy (Scheme 3.6) (Appendix B; Figure 3.2). Again no H/D exchange was observed at C3′ of **3.8**. It was decided that this approach to exchange was not ideal since it required prolonged reaction and only moderate levels of deuterium incorporation.

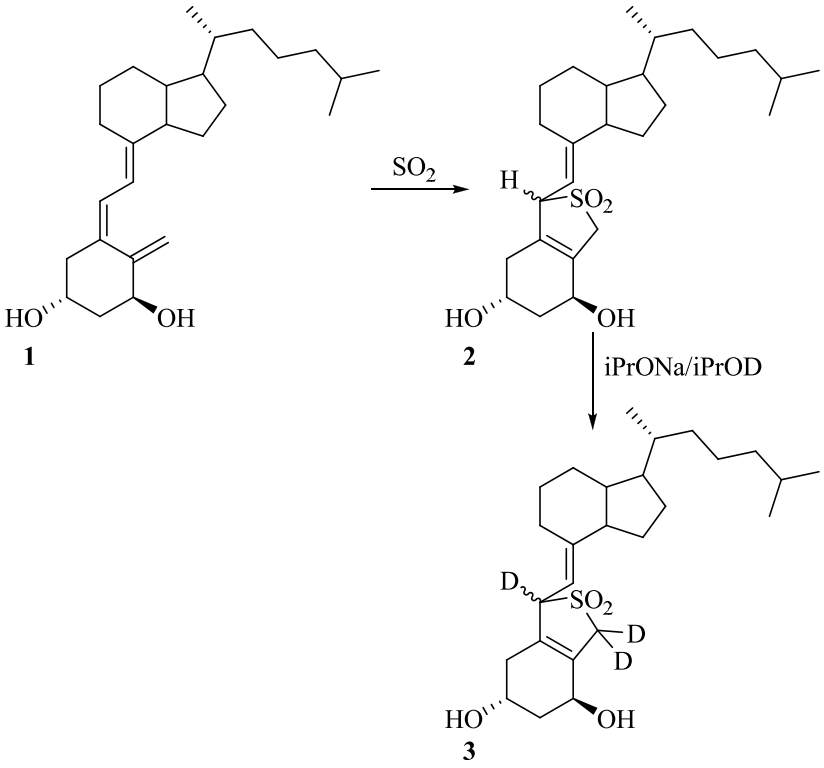


Scheme 3.6: H/D exchange on **3.7** cephalosporin derivative.

3.1.2 H/D Exchange at C2 of Cephalosporin Core using *i*PrONa/*i*PrOD

It was decided that a method of H/D exchange using a suitable base should be explored. Iwasaki et al. performed the deuteration of vitamin D_3 via formation of its sulfone adduct using a base catalyzed H/D exchange reaction.[68] Scheme 3.7 illustrates their work on base catalyzed H/D exchange in which the best result for the exchange was achieved using *i*PrONa/*i*PrOD and DMI (1,3-dimethyl-2-imidazolidinone) where DMI acts as a co-solvent, *i*PrOD is the source of deuterium and *i*PrONa acting as a base. The reaction was performed at 0 °C. Since the base is somewhat sterically hindered it was felt that the reaction with the β-lactam of a cephalosporin

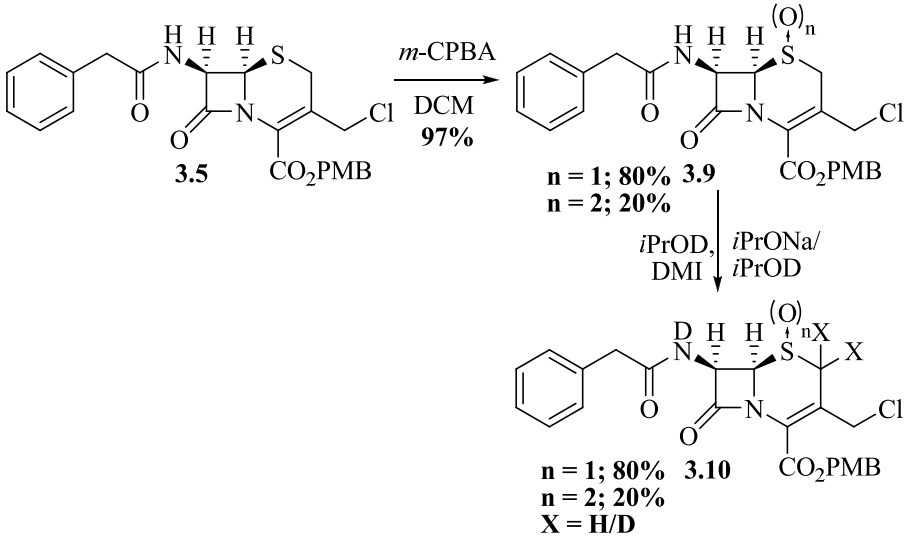
might be slow at low temperature. Therefore, in this study the base catalyzed reaction was performed at −20 °C. The reaction conditions used by Iwasaki et al. were similar to the proposed base catalyzed mechanism as mentioned previously in Schemes 3.1 and 3.2. Therefore, the same experimental condition was used on cephalosporin sulfoxide to check if the percentage of H/D exchange can be increased on C2.



Scheme 3.7: H/D exchange on vitamin D3 by Iwasaki et al.[68]

Compound **3.5** was the best starting material, as it was readily and commercially available. A suspension of **3.5** was treated with *m*-CPBA at 0 °C to give its corresponding sulfoxide **3.9**.[65] NMR studies indicated the presence of a mixture of 80% sulfoxide and 20% sulfone. The formation of the sulfoxide was very difficult to follow by TLC because the starting material **3.5** and **3.9** (sulfone) had the same R_f values. Without further purification the mixture was treated with *i*PrONa in a mixture of DMI and *i*PrOD. DMI was added as a co-

solvent and *i*PrOD was added both as a solvent and deuterium source. *i*PrONa was synthesized insitu from the reaction of *i*PrOD and NaH. The reaction was carried out at –20 °C as shown in Scheme 3.8. For all the experimental trials, one equivalent of *i*PrONa was used compared to compound **3.9**. The difference in experimental conditions was for the reaction time which is summarized in table 3.2 to obtain deuterated sulfoxide **3.10**.



Scheme 3.8: Base catalyzed H/D exchange at C2 using *i*PrONa/*i*PrOD.

Table 3.2: Reaction conditions for H/D exchange to obtain **3.10**.

Exp. No.	Reaction conditions	Time (h)	(%D) by ^{1}H NMR	% yield
1.	**3.9** stirred with *i*PrONa/*i*PrOD (one equivalent) and DMI.	1	40% D at one prochiral H of C2	70%
2.		1.5	45% D at one prochiral H of C2	60%
3.		2.0	50% D at one prochiral H of C2	60%

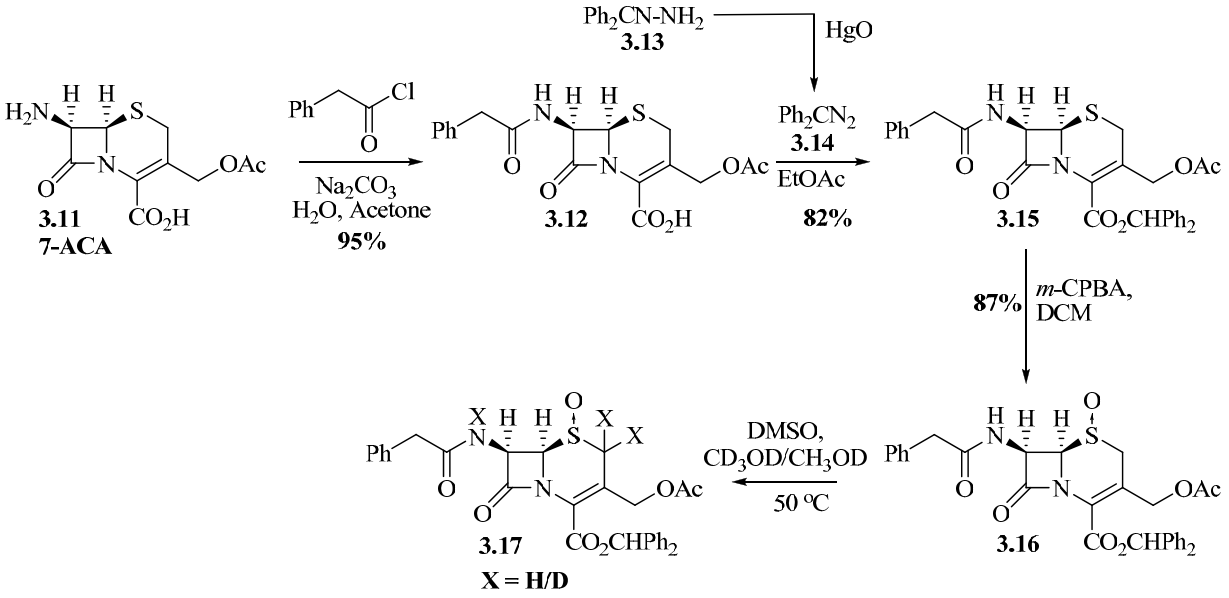
In the first attempt (entry 1), compound **3.9** was stirred with a solution of *i*PrONa/*i*PrOD and DMI at –20 °C for one hour. NMR studies showed that there was a 40% decrease in the integration of the doublet peak for one of the prochiral hydrogen at C2

(Appendix B; Figure 3.3), however the doublet peak for another prochiral hydrogen was under the peak of hydrogens at C8 (phenyl CH_2 side chain) and PMB methyl group, therefore the deuterium enrichment cannot be measured with certainty. The isolated yield for compound **3.10** was 70%. In order to increase the extent of deuterium at C2 the reaction was carried out for 1.5 hours and 2 hours as shown in entry 2 and entry 3 respectively. NMR studies for the second and third trial indicated the presence of 45% and 50% of deuterium at C2 respectively (Appendix B; Figure 3.4 and Figure 3.5). However, when the above mentioned reaction was done for prolonged time (longer than 2 hours) cleavage of the β-lactam ring became a problem. Since the amount of H/D exchange on C2 (50%) after allowable reaction time is not sufficient an alternative approach was required.

3.1.3 H/D exchange at C2 position of cephalosporin core using DMSO/Methanol

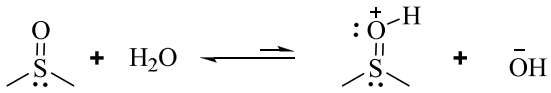
A further search of the literature revealed that Richter et al.[61] performed H/D exchange at C2 of a cephalosporin sulfoxide derivative using a mixture of DMSO-d_6 and methanol-d_4 as mentioned in Chapter 2. In the present thesis, the experimental conditions mentioned by Richter et al., were adopted to perform the H/D exchange on the compound of our interest. Therefore, compound **3.16** was prepared from 7-aminocephalosporanic acid **3.11** by first the phenyl acetylation on its amino side chain by Schotten-Baumann conditions, using phenylacetyl chloride and sodium carbonate as base to give **3.12**.[67] A suspension of **3.12** in ethyl acetate was esterified by stirring with a solution of diphenyldiazomethane **3.14** (prepared by reaction of benzophenone hydrazone **3.13** and yellow mercuric oxide) in ethyl acetate overnight to give **3.15**. This compound was further oxidized to its sulfoxide **3.16** using *m*-CPBA.[65] NMR studies of compound **3.16** confirmed absence of any sulfone adduct. H/D

exchange was performed on the sulfoxide **3.16** using DMSO as a solvent and also very weak base and methanol as source of deuterium (Scheme 3.09).



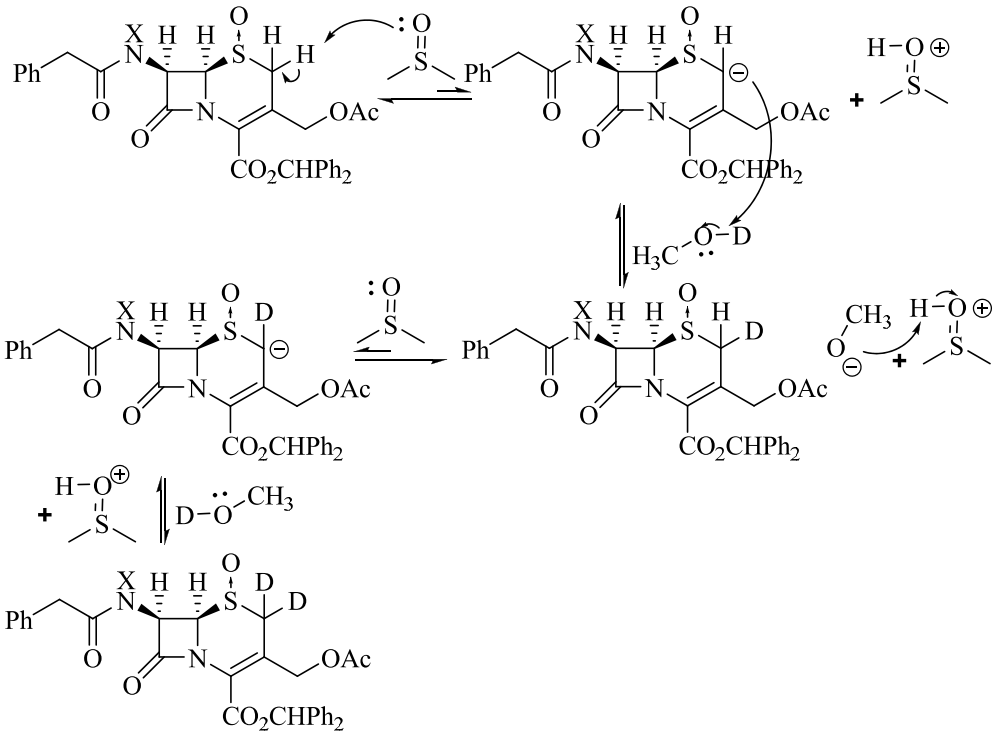
Scheme 3.9: The H/D exchange on C2 of **3.16** using DMSO/methanol under various conditions.

Although the precise mechanism involved in this H/D exchange is not known with certainty, it is worth discussing some possibilities in this regard. DMSO is not normally employed as a base. Raman spectroscopic[69] and X-ray crystallographic studies reveals that DMSO undergoes predominates on the oxygen atom rather than on sulfur.



DMSO salts are formed with very strong (super) acids.[69] The pKa for the conjugate acid of DMSO has been estimated to be -1.04.[69] The pKa's for the conjugate acid of alcohols (ROH_2^+) are reported to be approximate -2.5 as compared to -1.74 for hydronium ion.[70] So it is reasonable to suggest that DMSO is the strongest base in the reaction mixture and may affect

the deprotonation of the C2 hydrogens in the cephalosporin sulfoxide. Scheme 3.9 illustrates a proposed mechanism for the H/D exchange using DMSO and methanol.



Scheme 3.9: Proposed mechanism for H/D exchange on C2 of compound **3.16** using DMSO and methanol.

Various changes were undertaken in the experimental conditions during the course of H/D exchange (Table 3.3) to optimize the reaction conditions and increase the percentage of deuterium incorporation at C2 of compound **3.17**. Although Richter and co-workers did not mention the quantities of reactants being used for H/D exchange at C2, for the first attempt in this study, 10 eq of CD_3OD was used.

Richter and co-workers reported 70% deuterium incorporation at C2 by ^1H NMR. Whereas, from the first entry in Table 3.3, here compound **3.17** was obtained in DMSO-d_6 solution and its ^1H NMR suggested the incorporation of 75% deuterium at one of the prochiral

hydrogens C2 (Appendix B; Figure 3.6). The doublet peak for the second prochiral hydrogen at C2 showed interference with the multiplet peak of hydrogens at C8, therefore the deuterium enrichment cannot be measured with certainty. Since methanol-d_4 acts only as a source of deuterium in the reaction in entry 2 it was replaced by MeOD as deuterium source. After the reaction completion, NMR studies showed no difference in the amount of deuteration at C2 of **3.17**, compared to the entry 1; where the H/D exchange was done using methanol-d_4 (Appendix B; Figure 3.7).

Table 3.3: Modification in experimental conditions for the H/D exchange using DMSO/Methanol

Exp. No.	Reaction conditions	Time (h)	(%D) by ^{1}H NMR	% Yield
1.	**3.16**: DMSO-d_6; CD_3OD (10 eq); changed* CD_3OD every 90 min	3	75% D at one prochiral H of C2	-NA-
2.	**3.16**: DMSO-d_6; MeOD (10 eq); changed CH_3OD every 90 min	3	75% D at one prochiral H of C2	-NA-
3.	**3.16**: DMSO-d_6; MeOD (100 eq); changed CH_3OD at 24 h and 43 h	66	87.4% D in both prochiral Hs of C2	-NA-
4.	**3.16**: DMSO-d_6; MeOD (100 eq); changed CH_3OD every 2 h from t = 0 h to t = 8 h	24	90.2% D in both prochiral Hs at C2	99%

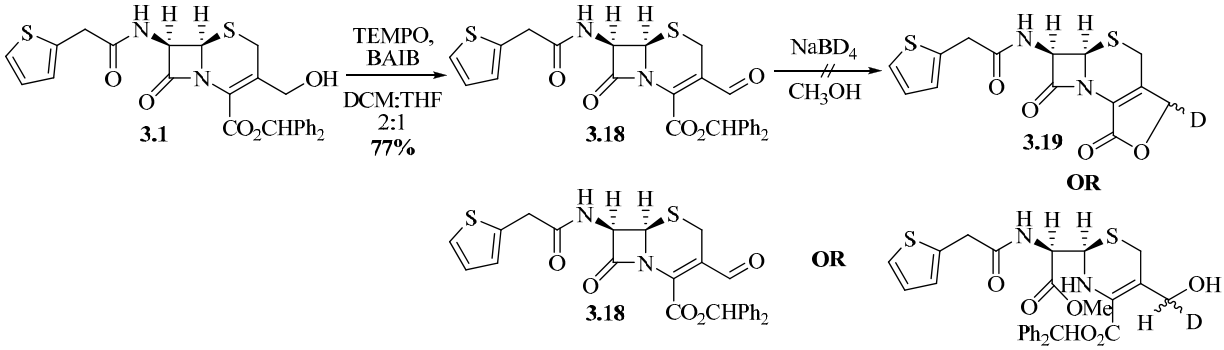
* Procedure for the change of solvent: After the reaction time of 90 minutes, methanol present in the reaction flask was evaporated under high vacuum and then, fresh methanol was introduced to the flask and reaction was continued for the above mentioned time duration. For details refer to Section 5.2 in Chapter 5 (Experimental Procedure)

Since MeOD is cheaper, it was used in the following reactions as deuterium source instead of methanol-d_4. To increase the amount of deuteration at C2, the amount of MeOD was increased from 10 equivalents to 100 equivalents and the reaction time was increased from 3 hours to 24 hours. It was believed that increasing the amount of MeOD or increasing the time of reaction would facilitate the H/D exchange. After 24 hours, NMR spectroscopy studies confirmed 92% of the hydrogen atoms were exchanged by deuterium at C2. The solvent (MeOD) was changed and new solvent was introduced to the reaction and it was continued for another 19 hours. After 43 hours NMR studies indicated deuterium incorporation of 94% at C2. The MeOD was changed and reaction was continued to 66 hours. NMR studies confirmed that 94% of one prochiral hydrogen was exchanged by deuterium at C2 of compound **3.17** and 93% of the second prochiral hydrogen was exchanged (Appendix B; Figure 3.8). Therefore, the deuterium enrichment at C2 was found to be (0.94 x 0.93) 87.4%. From the above results, it was evident that the amount of the solvent (MeOD) as well as the frequency of its change was very important in increasing the incorporation of deuterium at C2. Time was the third factor of modification in this reaction. Thus, it was confirmed that to achieve acceptable results most efficiently, the reaction should be done for 24 hours and the solvent (MeOD) should be changed frequently. Therefore, in entry 4 the amount of MeOD was kept at 100 equivalents but MeOD was changed every 2 hours from t = 0 to t = 8 hours. The reaction was carried out for 24 hours and NMR spectroscopy studies showed H/D exchange of 97% was achieved on one of the prochiral hydrogen at C2 of compound **3.17** and 93% of the second prochiral hydrogen was exchanged (Appendix B; Figure 3.9). Therefore, the deuterium enrichment at C2 was found to be (0.97 x 0.93) 90.2%.

During the NMR studies of compound **3.16**, it was observed that each of the signals for the hydrogens at C2 appears as a doublet (Appendix B; Figure 3.11 [A]). Among them, one of the doublets for one of the hydrogens at C2 was superimposed with the peak for the methylene hydrogens at Ph-CH_2 whereas; the doublet peak for the second hydrogen at C2 was isolated at down-field position. After the H/D exchange on C2 using DMSO/methanol, it was essential to determine that the doublet peak which disappears from the ^{1}H NMR of compound **3.17** (Appendix B; Figure 3.9), was in fact, for the hydrogens at C2 and not for Ph-CH_2. A detail 2D NMR for compound **3.15** (Appendix B; Figure 3.10) and **3.16** (Appendix B; Figure 3.11), was carried out. Peaks for all the hydrogen and carbon atoms were assigned using COSY, HMQC and HMBC experiments. NMR studies of compound **3.15** (Appendix B; Figure 3.10), shows that the peak for hydrogen at C2 and Ph-CH_2 are superimposed. Therefore it was difficult to assign them. However, HMBC study for compound **3.16** confirmed that, the peak which diminishes in the ^{1}H NMR of compound **3.17** (Appendix B; Figure 3.9), is for the hydrogens at C2. Thus, for compound **3.16** the peak at 3.96 ppm was for hydrogen at C2 (Appendix B; Figure 3.11) and not for the hydrogens on the phenylacetyl side chain. After the H/D exchange it was this peak at 3.96 ppm which diminishes in compound **3.17** (Appendix B; Figure 3.9). NMR studies of compound **3.17** confirmed the presence of 90.2% deuterium enrichment at C2. Hence, objective 1 in the present thesis was successfully accomplished with good yield and higher percent of deuterium incorporation at the C2 of the cephalosporin compound, compared to the previous two methods.

3.2 Objective 2: Deuteration at C3′ of Cephalosporin Core

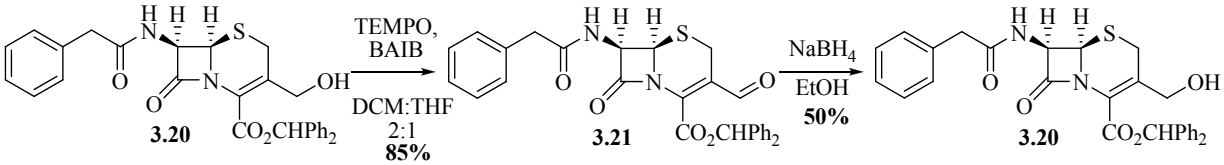
The second objective for the present study was to synthesize a C3′ monodeuterated cephalosporin compound. With a method for the deuteration of C2 in hand, the next attention was to perform deuteration of C3′ hydrogens. Initially, it was proposed that the hydrogens at C2 and C3′ might undergo a base-catalysis exchange in one pot. However, in all the different approaches to perform H/D exchange at C2, there was no exchange observed for C3′ hydrogens. Therefore, another approach to incorporate deuterium at C3' was planned. It was thought that a functional group interconversion reaction might be carried out at C3′. The oxidation of the C3′ hydroxyl cephalosporin **3.1** can give the corresponding C3′ aldehyde. This might then be reduced to an alcohol using a deuterated reducing agent. Thus, to start the first trial of the objective 2, compound **3.1** (obtained from Dr. Ahmad Ghavami, in this laboratory) was oxidized to the C3′ aldehyde **3.18** following a known procedure[71] by using [bis(acetoxy)iodo]benzene (BAIB) and a catalytic amount of 2,2,6,6-tetramethylpiperidinyloxyl (TEMPO) in 77% yield. Then, a solution of compound **3.18** was stirred with sodium borodeuteride ($NaBD_4$) at room temperature for 1 hour in methanol.



Scheme 3.11: The functional group inter-conversion reaction on C3′ hydroxyl cephalosporin.

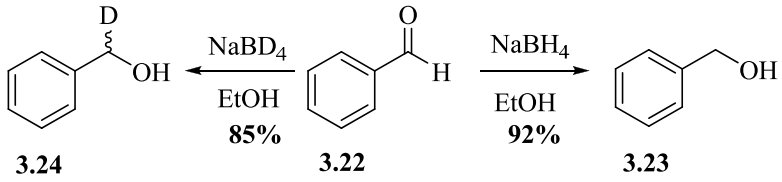
After the workup, the ^1H NMR suggested that the reaction failed and the NMR was clearly not that of the single reduction product. It was assumed that the product was either lactonized

at C3′ to give **3.19** or the β-lactam ring was hydrolyzed or both (Scheme 3.11). The reason for the failure for the reduction of compound **3.18** to give **3.19** was unknown. However, there were few possibilities for its failure which included: the possibility that sample of sodium borodeuteride was decomposed or that the β-lactam ring was cleaved by the methoxide ion formed from the reaction of methanol with sodium borodeuteride at higher temperature. The same reaction as mentioned in Scheme 3.10 was performed on compound **3.20** (obtained from Dr. Ahmad Ghavami, in this research group) since, the amount of compound **3.1** was not sufficient to go ahead with the next trials. Also, the experimental conditions used to cleave this phenylacetyl side chain were previously performed in this laboratory. Compound **3.20** was oxidized to the C3′ aldehyde derivative **3.21** using BAIB and a catalytic amount of TEMPO. In the second trial, compound **3.21** was reduced using sodium borohydride ($NaBH_4$) instead of sodium borodeuteride ($NaBD_4$). The idea was to verify if, the reaction can be performed on cephalosporin compounds. The temperature was one of the assumed reasons for the failure of first trial. Therefore, in the second trial the reaction was done at 0 °C. Reduction reactions using sodium borohydride are normally carried out in alcohol solvents; in which the rate of reaction is directly related to the structure of the alcohol (methyl > 1° > 2° > 3°).[72] A solution of compound **3.21** was stirred with $NaBH_4$ for an hour in ethanol at 0 °C to give **3.20** after acidic workup (Scheme 3.12).



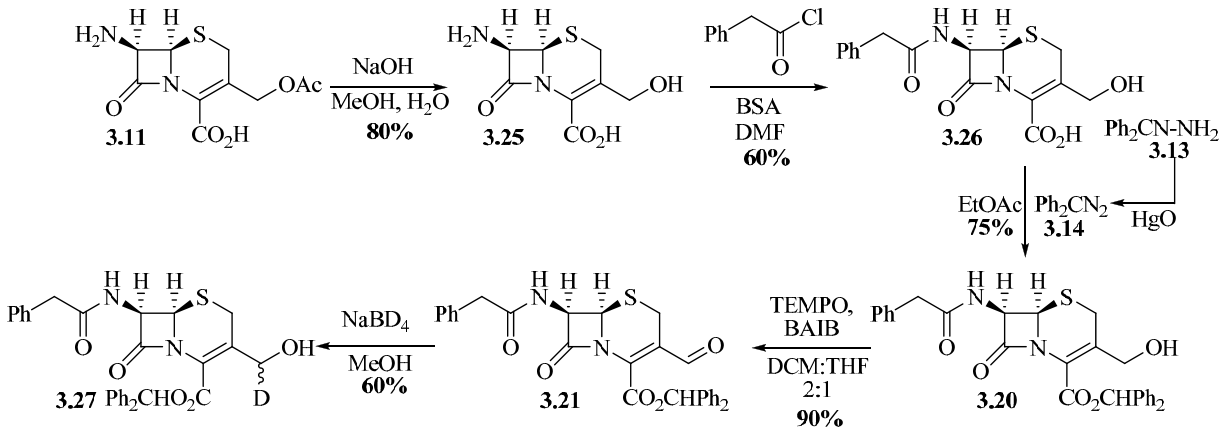
Scheme 3.12: Reduction of C3′ aldehyde using sodium borohydride.

After the second trial of reduction using sodium borohydride, it appeared that the failure of the first trial might have resulted from a sodium borodeuteride sample that had decomposed. In order to verify the purity of the $NaBD_4$, a solution of **3.22** (benzaldehyde) was reduced using $NaBH_4$ and $NaBD_4$ in ethanol at 0 °C to give **3.23** and **3.24**, respectively (Scheme 3.13)



Scheme 3.13: Reduction of benzaldehyde to **3.23** and **3.24**.

NMR studies confirmed the integrity of the $NaBD_4$ sample after the successful reduction of **3.22** to **3.23** (Appendix B; Figure 3.12) and **3.24** (Appendix B; Figure 3.13). Compound **3.20** was synthesized from 7-ACA (**3.11**). The solution of **3.11** was hydrolyzed at C3′ using sodium hydroxide solution to give compound **3.25**. Using a known procedure,[67] this was further stirred with BSA (bis[trimethylsilyl]acetamide) to protect the free hydroxyl and carboxylic acid and then phenyl acetylated at the C7 amino side chain to give compound **3.26**. The suspension of **3.26** was stirred with a solution of diphenyldiazomethane **3.14** (prepared by reaction of benzophenone hydrazone **3.13** and yellow mercuric oxide) in ethyl acetate overnight to give **3.20** in 75% yield (Scheme 3.14).

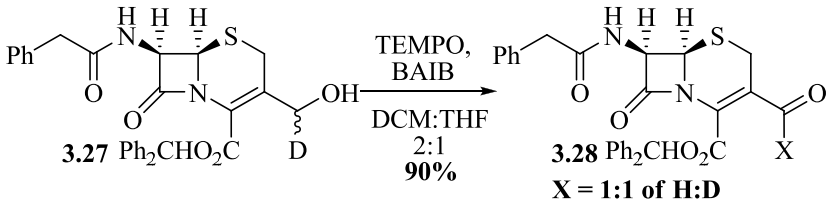


Scheme 3.14: Successful attempt of deuteration at C3′ of cephalosporin analogues.

Further, **3.20** was oxidized to the C3′ aldehyde derivative **3.21** using BAIB and a catalytic amount of TEMPO in a mixture of dichloromethane (DCM) and tetrahydrofuran (THF) in 90% yield. After the first two reduction attempts, it was known that the sample of $NaBD_4$ was of good quality and the reaction should be carried out at lower temperature to prevent reaction with the β-lactam ring. Since the reaction was carried out at low temperature, it was thought to use methanol as solvent because the rate of reaction with methanol is faster compared to ethanol.[72] Therefore, the reduction of **3.21** was done in methanol using $NaBD_4$ at −30 °C. The reaction was carried out for an hour to give **3.30** after acidic workup. A literature review suggest that, compound **3.20** in presence of acidic/basic condition tends to form the C3′ lactone.[67] It was thought that due to excess addition of an acid to quench the reaction the C3′ hydroxyl anion would attack the C4 carboxylate ester and would form a lactone with cleavage of the diphenylmethyl protecting group. In order to avoid any possibility of forming a lactone, in the present trial, saturated ammonium chloride solution (NH_4Cl) was used to do the acidic workup compared to the previous trial where hydrochloric acid was used. Since, saturated NH_4Cl is mildly acidic and would be less prone to bring about lactone formation. ^{1}H NMR of

the monodeuterated cephalosporin **3.27** confirms the presence of one deuterium at the C3′ position of the core because the integration of C3′ hydrogens was decreased by one (Appendix B; Figure 3.14 [A]). Interestingly, the difference in the multiplicity of C3′ hydrogen was observed with the change in the NMR solvent. When DMSO-d_6 was used as NMR solvent the peak for the C3′ hydrogen appears as a singlet with integration of two compared to H_7 for the non-labelled compound **3.20**. However, when the NMR experiment was performed using acetone-d_6 as solvent, the peak for C3′ hydrogen appears as a multiplet. NMR studies for the monodeuterated hydroxyl compound **3.27** suggest, when DMSO-d_6 was used as NMR solvent the peak for C3′ hydrogen appears as a singlet with integration of one compared to H_7 (Appendix B; Figure 3.14). However, when the NMR experiment was performed using acetone-d_6 as solvent, the peak for C3′ hydrogen appears as a multiplet. This indicates that the reduction gave a mixture in which one of the prochiral hydrogen positions was enriched to a greater extent (approximately 4:1) than the other.

It then occurred that possibly compound **3.27** might be oxidized to the aldehyde **3.28** which would preferably eliminate the hydrogen because of a secondary deuterium isotope effect and the subsequent reduction using sodium borodeuteride would introduce another deuterium at C3′. Thereafter, it was important to check if the re-oxidation of **3.27** to aldehyde **3.28** would show a significant primary deuterium isotope effect and react to give preferentially the deuterated aldehyde. In fact, when compound **3.27** was stirred with BAIB and TEMPO in dichloromethane and tetrahydrofuran for 24 hours gave **3.28** in 90% yield (Scheme 3.15). The ^{1}H NMR suggests presence of 1:1 ratio of H:D for its aldehyde peak (Appendix B; Figure 3.15).

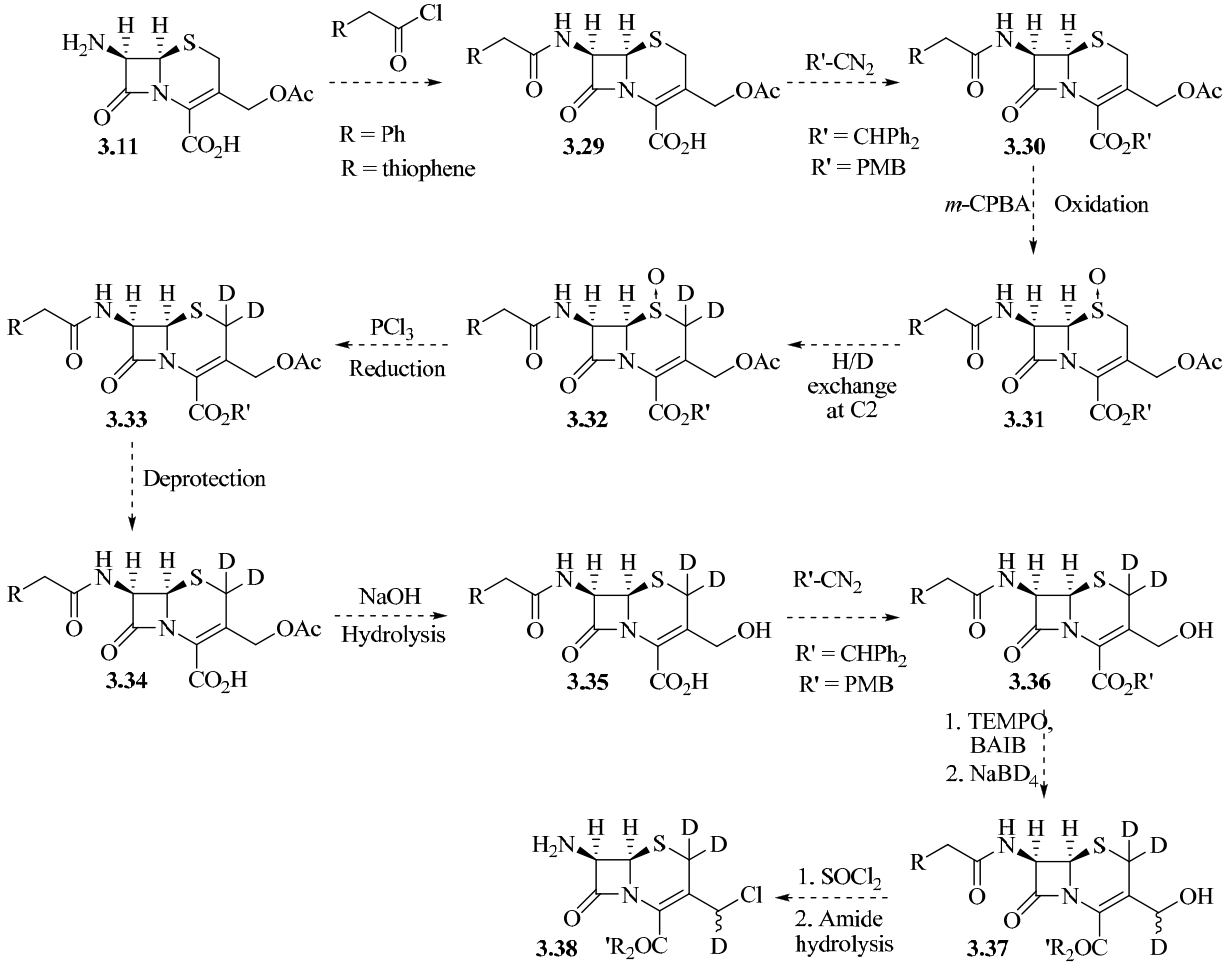


Scheme 3.15: Re-oxidation of C3′ deuterated hydroxyl derivative to aldehyde **3.28**.

3.3 Objective 3: Synthesis of a Core Trideuterated Cephalosporin Analogue

After successful accomplishment of objective 1 and objective 2, in order to synthesize the trideuterated compound both the strategies which were used to achieve objective 1 and 2 were combined and a plan was proposed as shown in scheme 3.16. The research objectives accomplished in this thesis were briefly described in this chapter earlier. A proposed approach to combine objective 1 and 2 for preparation of core trideuterated cephalosporin compound is shown in Scheme 3.16. 7-ACA was chosen as the staring material for the synthesis. Thus, **3.11** can be acylated with phenylacetyl chloride or 2-thienylacetyl chloride to give compound **3.29** under Schotten-Baumann conditions. The carboxylic acid functional group of compound **3.29** can then be protected either as a benzhydryl ester or a PMB ester to give compound **3.30** which can then be oxidized to give sulfoxide **3.31**. H/D exchange on **3.31** will give **3.32** and its subsequent reduction will yield **3.33** as a dideuterated derivative. Cephalosporins esterified at the C4 position tend to undergo Δ2 and Δ3 isomerization in presence of base. Therefore, to avoid the isomerization, compound **3.33** can be deprotected to give free acid **3.34**. The acetoxy ester functional group of compound **3.34** can be hydrolyzed using sodium hydroxide to give **3.35**. In order to oxidize compound **3.35**, it can first be protected at C4 to give **3.36** which can then be oxidized to an aldehyde and then be reduced back to a C3′ alcohol **3.37** as a core

trideuterated cephalosporin compound. Compound **3.37** can be chlorinated at the C3′ side chain using thionyl chloride and then cleaved at its C7 side chain to give the free amino derivative **3.38**. The free amino derivative could then be used as a starting material for the synthesis of internal standards for a variety of experimental cephalosporin antibiotics. These isotopically labelled compounds can then be useful as internal standards to perform pharmacokinetic analysis of their unlabeled derivatives using LC/MS methods.

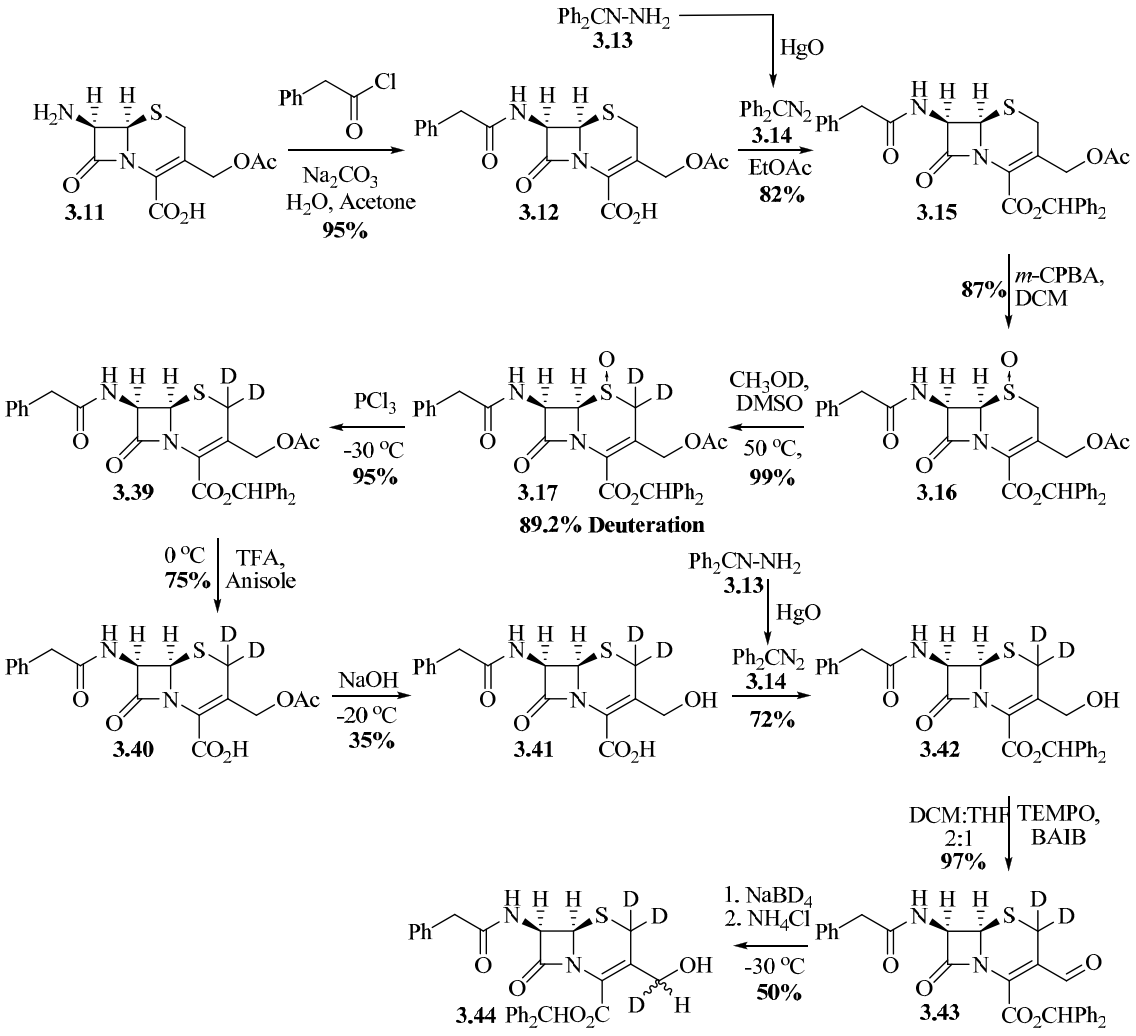


Scheme 3.16: Proposed plan for synthesis of trideuterated core cephalosporin.

Scheme 3.17 shows the preparation of trideuterated compound **3.44**. As mentioned earlier in Scheme 3.9, 7-ACA (**3.11**) was phenylacetylated to give **3.12** under Schotten-

Baumann conditions in 95% yield. The phenylacetyl derivative was synthesized because in the later stage its C7 hydrolysis was previously performed in the Dmitrienko group with good yield. Compound **3.12** was esterified at C4 using a solution of diphenyldiazomethane **3.14** in ethyl acetate to give **3.15** in 82% yield. The sulfoxide **3.16** was prepared again by the same experimental procedure from **3.15** using *m*-CPBA in dichloromethane in 87% yield. Compound **3.16** was stirred with DMSO and methanol-*OD* (50 equivalents) for 16 hours, then the solvent was evaporated and new methanol-*OD* was added and the reaction was continued for 24 hours to give compound **3.17** in quantitative yield. The ^{1}H NMR of **3.17** reveals that the amount or percentage of deuterium incorporation for one of the prochiral hydrogens at C2 was found to be 96% and 93% for the second prochiral hydrogen (Appendix B; Figure 3.16). Therefore, the deuterium enrichment at C2 was calculated to be (0.96 x 0.93) 89.2%. Thus, compound **3.17** was then reduced back to its sulfide **3.39** using phosphorous trichloride in dimethylformamide (DMF) at –30 °C in 95% yield. As mentioned earlier in Scheme 3.3, cephalosporins esterified at the C4 position tend to undergo Δ2 and Δ3 isomerization in presence of base. Therefore, to avoid the isomerization, compound **3.39** was deprotected at C4 position using trifluoroacetic acid and anisole at 0 °C to give the free acid **3.40** in 75% yield. This free acid **3.40** underwent deacetylation/hydrolysis at its C3′ side chain to give the compound **3.41** in 35% yield. The reason for the low yield is because, it is known from the literature that during the hydrolysis of this acetoxy group at C3′ side chain forms lactone as a side product under strong acidic and basic conditions.[67] The ^{1}H NMR for compound **3.41** shows no back exchange of deuterium at C2, nor any isomerization to the Δ2 isomer. The successful completion of the hydrolysis reaction was a important step in the present project.

Further, **3.41** was esterified using the same procedure as mentioned earlier to give compound **3.42** in 72% yields. Compound **3.42** was then oxidized using TEMPO and BAIB in a mixture of dichloromethane and tetrahydrofuran to give dideuterated aldehyde **3.43** in 97% yield. The aldehyde **3.43** was then reduced with $NaBD_4$ in methanol at −30 °C. Saturated solution of NH_4Cl was used to quench the reagents during the workup which afforded core trideuterated cephalosporin compound **3.44** in 50% yield (scheme 3.17).



Scheme 3.17: The synthesis of core trideuterated cephalosporin.

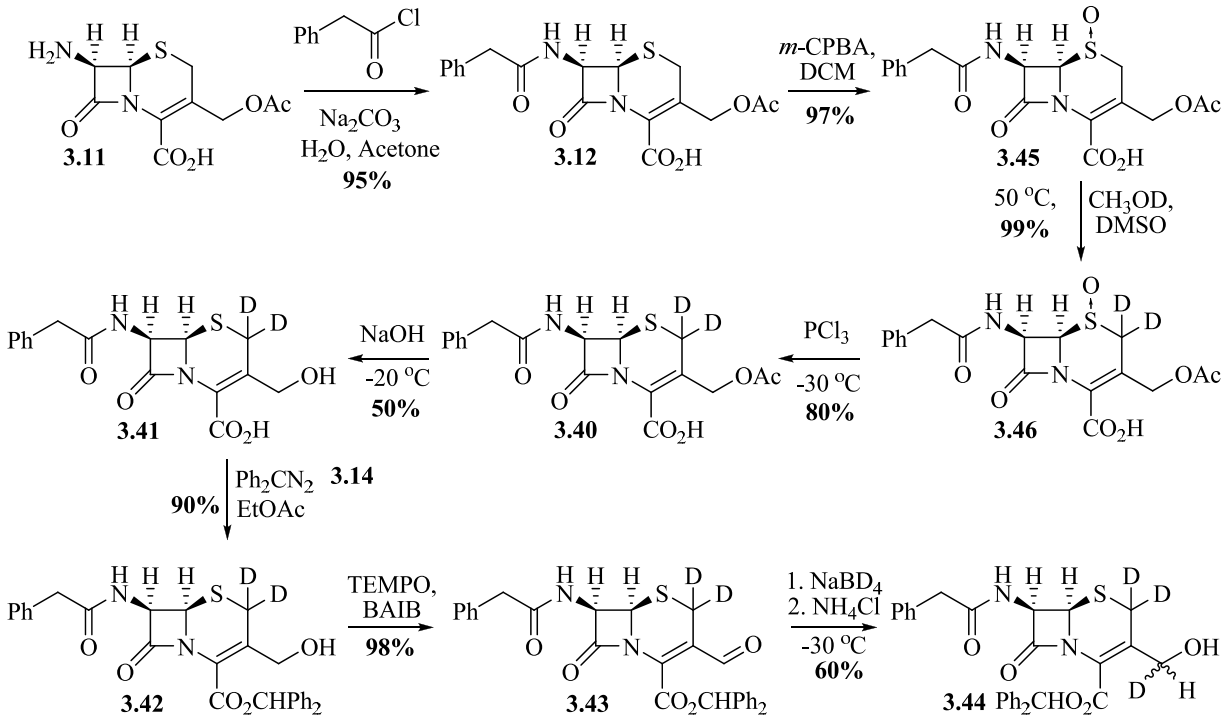
NMR studies of compound **3.44** indicated that, there was a difference in the multiplicity of C3′ hydrogen peaks with the change in the NMR solvent as mentioned earlier in Scheme 3.14. When DMSO-d_6 was used as the NMR solvent, the peak for C3′ hydrogen appears as a singlet with integration of one compared to H_7 (Appendix B; Figure 3.17 [A]). However, when the NMR was done using acetone-d_6 as solvent, the peak for C3′ hydrogen appears as a multiplet. The relative integration indicates that the reduction gave a mixture in which one of the prochiral hydrogen positions is been enriched to a greater extent (approximately 4:1) than the other (Appendix B; Figure 3.17 [B]). From the NMR studies the deuterium enrichment cannot be measured with certainty for compound **3.44**. Since the signal for hydrogens at C2 is overlapped with the peak of hydrogens at C8 (phenyl-CH_2 side chain). The deuterium enrichment for C3′ from its NMR integration of the peak was found to be 98%. Thus, with the synthesis of compound **3.44** the attempt to synthesize the core trideuterated derivate was successfully achieved.

3.4 Modified Route for the Synthesis of the Core Trideuterated Cephalosporin Analogue

Scheme 3.17 represents the synthesis of trideuterated cephalosporin **3.44** in 11 steps. After the first successful attempt for the synthesis of trideuterated analogue, the next challenge was to reduce the synthesis steps to make it more efficient and additionally, increase the overall yield for the synthesis of core trideuterated compound. It was proposed that by avoiding the C4 esterification step and deprotection of the benzhydryl group, both the above mentioned challenges might be answered. Scheme 3.18 shows the modified route of synthesis. Herein, the synthesis started from **3.11** (7-ACA) as mentioned earlier in other schemes. This compound

was phenylacetylated on the C7 amino side chain to give **3.12** in excellent yields. Further, **3.12** was directly oxidized to the sulfoxide **3.45** using *m*-CPBA in 97% yield.[73] Compound **3.45** underwent H/D exchange at C2 of using DMSO and methanol-*OD* (20 equivalents). An effort was made to reduce the duration of the H/D exchange reaction. Therefore, methanol-*OD* was changed every two hours and the reaction was done for only 6 hours. Compound **3.46** was obtained in quantitative yields and ^{1}H NMR spectroscopy of **3.46** reveals the presence of 95% deuterium incorporation at one of the prochiral hydrogens of C2 (Appendix B; Figure 3.18). The dideuterated compound **3.46** was reduced back to its sulfide form **3.40** using phosphorous trichloride at –30 °C in DMF. The mass spectrum for **3.40** shows the presence of the ratio of the dideuterated compound to the monodeuterated as 10:1 (Appendix B; Figure 3.19). Thus this infers that after the H/D exchange on compound **3.45** approximately 91% of both the prochiral hydrogens were exchanged by deuterium at C2 of core cephalosporin. Compound **3.40** was hydrolyzed at C3′ using sodium hydroxide at –20 °C to give **3.41** in 50% yield. Synthesis of **3.42** was done by esterification of **3.41** at C4 using a solution of diphenyldiazomethane **3.14** in ethyl acetate. The functional group interconversion reactions were performed on **3.42** in which oxidation to aldehyde afforded compound **3.43** in 98% yield and its reduction resulted in core trideuterated hydroxyl derivative **3.44** in 62% yield. According to the NMR studies of compound **3.44** in benzene-d_6 and a drop of DMSO-d_6 the deuterium incorporation in the core cephalosporin compound was determined to be 95% for one of the prochiral hydrogen and 94% for the second prochiral hydrogen at C2 (Appendix B; Figure 3.20).There was a difference in the multiplicity of the C3′ hydrogen with the change in the NMR solvent as mentioned earlier in Scheme 3.14. (Appendix B; Figure 3.20 [A and B]). ^{1}H NMR spectroscopy of compound **3.44** in acetone-d_6 suggests 98% of isotopic enrichment at

C3′. Also, ^{13}C NMR shows the peak for C3′ as a triplet due to the presence of deuterium (Appendix B; Figure 3.20 [C]). The mass spectrum for compound **3.44** shows a presence of trideuterated: dideuterated in a ratio of 5:1 (Appendix B; Figure 3.20[D]). Thus, the synthesis of a core trideuterated compound **3.44** having deuterium enrichment of (0.94 x 0.95 x 0.98) 87.5% by NMR spectroscopic studies. After performing the calculation from the mass spectrometric analysis it was found that the estimated ratio of the trideuterated compound to the dideuterated compound was 5 to 1.



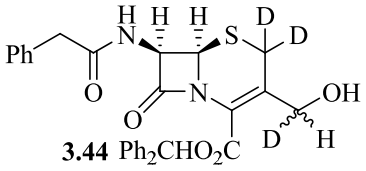
Scheme 3.18: Modified route of synthesis for core trideuterated cephalosporin analogue.

Chapter 4

Concluding statements

4.1 Summary and conclusion

The main goal of this thesis was to synthesize a core trideuterated cephalosporin compound. The isotope labelled compound can be then used to synthesize internal standards for novel cephalosporin derivatives. The proposed sites for deuteration were C2 and C3′ of the core cephalosporin structure. The synthesis involves hydrogen-deuterium exchange of the C2 hydrogens of a cephalosporin sulfoxide in a mixture of DMSO and *O*-deuterated methanol and reduction of the C3′ aldehyde group by sodium borodeuteride. Later, both the approaches were combined on one molecule and a method for synthesis of a triply deuterated cephalosporin core structure **3.44** has been devised.

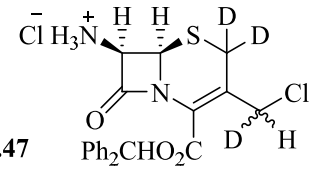


3.44 Ph_2CHO_2C

The exchange process at C2 resulted in a deuterium enrichment of 95% in each of the prochiral hydrogen positions as estimated by proton NMR signal integration. The aldehyde reduction led to a mixture with one of the prochiral hydrogen positions being enriched to a greater extent (approximately 4:1) than the other. The deuterium enrichment at C3 was approximately 98% as estimated by integration of the NMR signals for the C3′ hydrogens. This leads to the conclusion that the sample is approximately (0.95 x 0.95 x 0.98) 88.5% trideuterated.

A preliminary mass spectrometric analysis estimates the ratio of the trideuterated cephalosporin to the dideuterated variant as 5 to 1. Since there is precedent for transformation

of the unlabeled version of **3.44** into the cephalosporin synthon **3.47**, the availability of **3.44** should allow researchers in this laboratory and elsewhere to prepare internal standards for MS analysis of new cephalosporin drug candidates in pharmacokinetic studies.

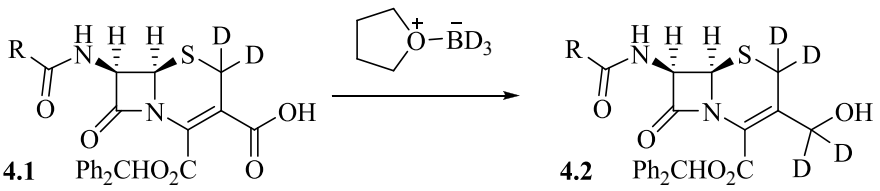


3.47

4.2 Future Work

Potential future improvements in the preparation of **3.44** could focus on increasing the level of deuterium incorporation by further variation of the methods developed in this study. The level of deuterium incorporation at C3′ is essentially that of the deuterium source $NaBD_4$ so that it is not clear how this step might be improved unless a source of more highly enriched $NaBD_4$ could be identified. On the other hand, the deuterium incorporation at C2 is 95% whereas the deuterium source methanol-*OD* (CH_3OD) is labeled to the extent of 99%. Thus, efforts might be made to improve this process by extending the reaction times and increasing the relative amount and frequency of replacement of the deuterated solvent.

Another strategy for deuterium incorporation that might be explored in future involves the creation of a tetradeuterated product through C2 hydrogen exchange as done in this work combined with a reduction of a C3′ carboxylic acid group using trideuteroborane-THF complex.



4.1 **4.2**

The deuterated compound **3.44** should be converted in to the labeled synthon **3.47** to establish if any back exchange of hydrogen for deuterium occurs in any of the steps. Also, the synthon **3.47** should be subjected to typical N-acylation, chloride displacements by typical nucleophiles and removal of the benzhydryl protecting group to determine if any back-exchange occurs in such transformations.

In future use of such a trideuterated species in LC/MS/MS studies it will be necessary to establish the separation and ionization conditions that are ideal for the specific cephalosporin derivative under study. Attention will need to be paid in such studies to any possibility that the MS ionization conditions might result in any back exchange of the deuterium by protium from the ionization medium that will likely include acid or base.

Chapter 5

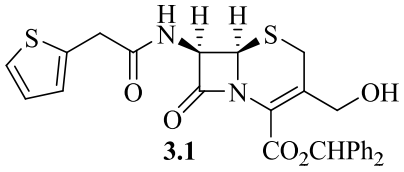
Experimental Procedure

5.1 General Procedures

All the experimental reaction were always done in flame and oven-dried glassware under nitrogen atmosphere and at room temperature (rt); where not mentioned. All the experimental reagents and chemicals were obtained from Sigma Aldrich ®, Alfa Aesar ®, Cambridge Isotopes Inc. ® etc. Solvents used in the reaction were either reagent grade and/or HPLC grade. The reactions were stirred magnetically and monitored by TLC using Merck pre-coated silica plates (silica gel 60 F_{254} on aluminium sheet). Compounds were purified by flash chromatography using SiliCycle silica gel (60 Å). The yields reported are for the isolated compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ® 300 MHz and 500 MHz spectrometer using chloroform-*d*, DMSO-d_6, acetone-d_6, D_2O and benzene-d_6. All the chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS). For ^{1}H NMR spectra, the chemical shift was calibrated relative to the signal of the proton for the isotopomer with one less deuterium than the perdeuterated solvent (Chloroform: δ_H 7.26; DMSO-d_6: δ_H 2.50; Acetone-d_6: δ_H 2.05; Benzene-d_6: δ_H 7.16; HDO: δ_H 4.79) as reported by Gottlieb et al.[74] For ^{13}C NMR spectra, the chemical shift scale was calibrated relative to that of the ^{13}C signal of the perdeuterated solvent (Chloroform: δ_C 77.16; DMSO-d_6: δ_C 39.52) as reported by Gottlieb et al.[74] Peak multiplicities were represented by abbreviations such as: s, singlet; d, doublet; t, triplet; q, quartet; qAB, AB quartet; dd, doublet of doublets; m, multiplet; gem, geminal. The coupling constants have uncertainties of ±0.5 Hz. The protons and carbons were assigned depending on their respective data from 2-dimensional NMR experiments

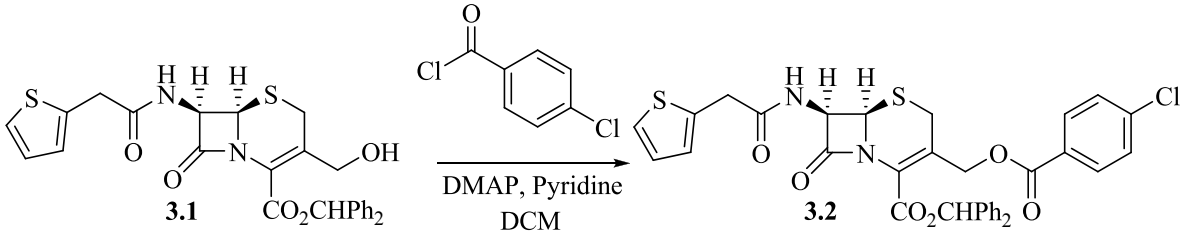
including COSY, HMQC and HMBC. Low Resolution Mass Spectra (LRMS) and High Resolution Mass Spectra (HRMS) analysis were obtained via electrospray ionization (ESI), were measured by Thermo Scientific Q Exactive™ plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometry Facility in the Department of Chemistry, University of Waterloo.

5.2 Synthetic Procedures



3.1

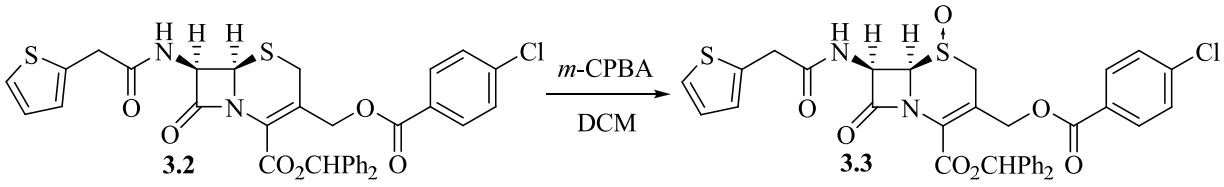
Compound **3.1** was obtained from Dr. Ahmad Ghavami. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.53 (s, 2H, SCH_2), 3.84 (s, 2H, thiophene-CH_2), 3.93 (d, J_{gem} = 13.0 Hz, 1H, CH_2OH), 4.39 (d, J_{gem} = 13.0 Hz, 1H, CH_2OH), 4.93 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.88 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 9.1 Hz, 1H, H_7), 6.51 (d, $J_{7,NH}$ = 9.1 Hz, 1H, NH), 6.89 (s, 1H, CHPh$_2$), 6.98 (m, 2H, thiophene), 7.35 (m, 11H, Ar; thiophene). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 27.71, 37.21, 57.16, 59.15, 62.16, 80.15, 124.96, 126.20, 127.18, 127.60, 127.68, 128.02, 128.38, 128.60, 128.79, 133.55, 134.80, 139.05, 139.29, 161.83, 165.14, 170.24.



3.1 **3.2**

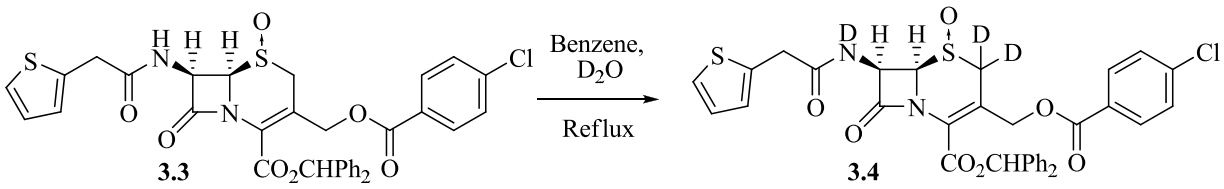
To a stirring solution of **3.1** (260 mg, 0.50 mmol) in dichloromethane (2 mL) was added a solution of DMAP (6 mg, 0.05 mmol) and pyridine (81 μL, 1.00 mmol) in dichloromethane (2 mL) at 0 °C, 4-chlorobenzoyl chloride (77 μL, 0.50 mmol) was added slowly and was allowed

to react overnight. The solvent was evaporated in vacuo. The residue was dissolved in EtOAc (20 mL), washed with H_2O (3 × 20 mL) and brine (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc:hexanes; 1:3) to give **3.2** (207 mg, 0.31 mmol, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.42 (d, J_{gem} = 18.5 Hz, 1H, SCH_2), 3.60 (d, J_{gem} = 18.5 Hz, 1H, SCH_2), 3.88 (s, 2H, thiophene-CH_2), 5.01 (m, 2H, CH_2OCO; H_6), 5.29 (d, J = 13.6 Hz, 1H, CH_2OCO), 5.89 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 9.0 Hz, 1H, H_7), 6.36 (d, $J_{7,NH}$ = 9.0 Hz, 1H, NH), 6.99 (m, 3H, CHPh$_2$; thiophene), 7.36 (m, 12H, Ar), 7.89 (d, J = 8.5 Hz, 2H, Ar), 8.03 (d, J = 8.5 Hz, 1H, thiophene). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.70, 37.24, 57.42, 59.30, 63.78, 80.02, 126.10, 126.30, 126.55, 127.17, 127.76, 127.82, 128.13, 128.28, 128.42, 128.62, 128.70, 129.02, 131.22, 131.70, 134.72, 139.03, 139.23, 140.10, 160.77, 164.80, 165.35, 170.17.



To a solution of **3.2** (89 mg, 0.13 mmol) in dichloromethane (10 mL) at 0 °C was added *m*-CPBA (35 mg, 0.20 mmol) at once and the reaction was stirred for 3 hours. The solvent was evaporated in vacuo and the residue was dissolved in EtOAc (20 mL), washed with H_2O (20 mL), sat. $NaHCO_3$ (20 mL) and brine (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.3** (51 mg, 0.07 mmol, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.20 (d, J_{gem} = 18.2 Hz, 1H, SCH_2), 3.84 (m, 3H, SCH_2; thiophene-CH_2), 4.45 (d, $J_{6,7}$ = 4.2 Hz, 1H, H_6), 4.93 (d, J_{gem} = 13.90 Hz, 1H, CH_2OCO), 5.51

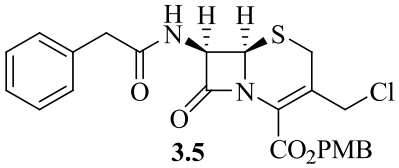
(d, J_{gem} = 13.90 Hz, 1H, CH_2OCO), 6.10 (dd, $J_{6,7}$ = 4.6 Hz, $J_{7,NH}$ = 9.5 Hz, 1H, H_7), 6.96 (m, 4H, NH; thiophene; CHPh$_2$), 7.37 (m, 13H, Ar; thiophene), 7.88 (d, J = 8.7 Hz, 2H, Ar).



3.3 **3.4**

78% deuteration

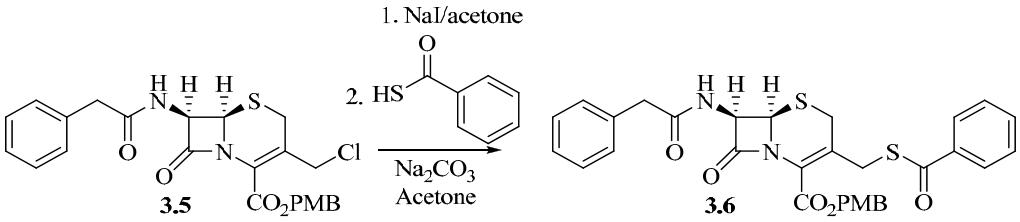
A solution of **3.3** (50 mg, 0.07 mmol) in benzene (9 mL) and D_2O (10 mL) was allowed to reflux (80 °C) for 136 hours. The solvent was evaporated in vacuo and the residue was dried under vacuum pump to give **3.4** (48 mg, 0.07 mmol, 95%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.15 (s, 0.22H and 0.78D, SCX_2, where X = H/D), 3.85 (m, 2.47H, SCX_2, where X = H/D; thiophene-CH_2), 4.47 (d, $J_{6,7}$ = 4.2 Hz, 1H, H_6), 4.94 (d, J_{gem} = 14.0 Hz, 1H, CH_2OCO), 5.5 (d, J_{gem} = 14.0 Hz, 1H, CH_2OCO), 6.08 (d, $J_{6,7}$ = 4.2 Hz, 1H, H_7), 7.00 (m, 3H; thiophene; CHPh$_2$), 7.36 (m, 13H, Ar; thiophene), 7.87 (d, J = 8.1 Hz, 2H, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 37.18, 53.56, 59.20, 63.97, 66.93, 80.57, 120.93, 125.94, 127.17, 127.48, 127.70, 127.87, 128.34, 128.42, 128.65, 128.75, 129.07, 131.21, 134.76, 138.95, 139.04, 140.22, 159.88, 164.23, 165.31, 170.58.



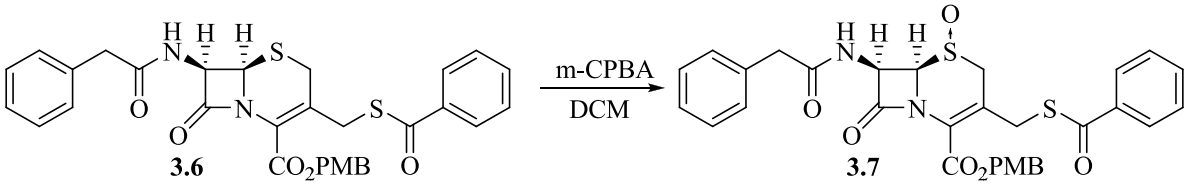
3.5

Compound **3.5** was commercially available. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.52 (m, 3H, SCH_2; Ph-CH_2), 3.73 (m, 4H, SCH_2; OCH_3), 4.49 (m, 2H, CH_2Cl), 5.20 (m, 3H, H_6; PMB-

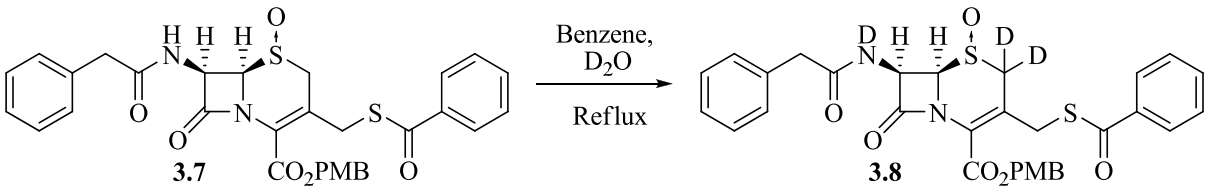
CH_2), 5.73 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.7 Hz, 1H, H_7), 6.93 (d, J = 8.6 Hz, 2H, Ar), 7.28 (m, 7H, Ar), 9.11 (d, $J_{7,NH}$ = 8.7 Hz, 1H, NH).



To the suspension of **3.5** (3000 mg, 6.16 mmol) in acetone (30 mL) at 0 °C was added NaI (1015 mg, 6.77 mmol) at once and the reaction mixture was stirred for 2 hours. Thiobenzoic acid (0.86 mL, 1029 mg, 7.39 mmol) was added to above reaction flask followed by Na_2CO_3 (979 mg, 9.24 mmol) at once and stirred overnight. The solvent was concentrated under reduced pressure and crude product was purified by flash chromatography (EtOAc:hexanes; 1:3) gave **3.6** (2500 mg, 4.25 mmol, 70%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.35 (d, J_{gem} = 18.7 Hz, 1H, SCH_2), 3.61 (m, 3H, SCH_2; Ph-CH_2), 3.74 (s, 3H, OCH_3), 3.97 (d, J_{gem} = 13.5 Hz, 1H, CH_2-SCO-), 4.30 (d, J_{gem} = 13.5 Hz, 1H, CH_2-SCO-), 4.91 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.23 (m, 2H, PMB-CH_2), 5.80 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 9.1 Hz, 1H, H_7), 5.99 (d, $J_{7,NH}$ = 9.1 Hz, 1H, NH), 6.88 (d, J = 8.6 Hz, 2H, Ar), 7.32 (m, 7H, Ar), 7.46 (t, J = 7.9 Hz, 2H, Ar), 7.6 (t, J = 7.8 Hz, 1H, Ar), 7.93 (d, J = 7.3 Hz, 1H, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 27.82, 30.65, 43.54, 55.41, 57.46, 59.26, 68.15, 66.93, 114.12, 124.96, 126.99, 127.56, 127.96, 128.67, 128.90, 129.40, 129.61, 130.90, 133.67, 134.01, 136.41, 161.75, 164.60, 171.17, 191.57.



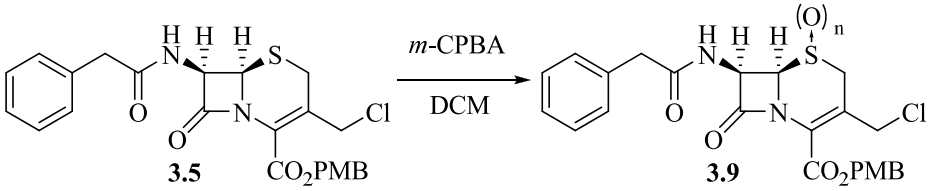
To a solution of **3.6** (588 mg, 1.00 mmol) in dichloromethane (15 mL) at 0 °C was added *m*-CPBA (292 mg, 1.30 mmol) at once and allowed to react for 3 hours gradually warm up to rt. Upon completion the solvent was evaporated in vacuo and the residue was dissolved in EtOAc (30 mL), washed with H_2O (2 × 20 mL), sat. $NaHCO_3$ (2 × 20 mL) and brine (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.7** (519 mg, 0.86 mmol, 86%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.30 (d, J_{gem} = 18.5 Hz, 1H, SCH_2), 3.61 (m, 2H, Ph-CH_2), 3.78 (m, 4H, SCH_2; OCH_3), 3.94 (d, J_{gem} = 13.9 Hz, 1H, CH_2-SCO-), 4.40 (dd†, $J_{6,7}$ = 4.8 Hz, J = 1.5 Hz, 1H, H_6), 4.47 (d, J_{gem} = 13.9 Hz, 1H, CH_2-SCO-), 5.28 (m, 2H, PMB-CH_2), 6.03 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 10.1 Hz, 1H, H_7), 6.67 (d, $J_{7,NH}$ = 10.1 Hz, 1H, NH), 6.89 (d, J = 8.7 Hz, 2H, Ar), 7.32 (m, 7H, Ar), 7.46 (t, J = 7.7 Hz, 2H, Ar), 7.6 (t, J = 7.3 Hz, 1H, Ar), 7.91 (d, J = 7.4 Hz, 1H, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 30.81, 43.64, 47.40, 55.43, 59.33, 66.98, 68.45, 114.17, 122.56, 125.07, 127.60, 127.77, 128.93, 129.21, 129.49, 130.98, 133.73, 134.16, 160.13,164.08, 171.30, 203.58.



A solution of **3.7** (50 mg, 0.08 mmol) in benzene (10 mL) and D_2O (5 mL) was allowed to reflux (80 °C) for 154 hours. The solvent was evaporated in vacuo and the residue was dried under vacuum pump to give **3.8** (49 mg, 0.08 mmol, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.31 (d, J = 14.0 Hz, 0.72H and 0.28D, SCX_2, where X= *H/D*), 3.62 (m, 2H, Ph-CH_2), 3.81

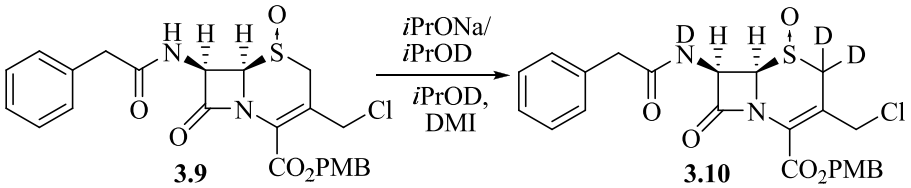
† H_6 may be showing a long range W-effect coupling with H_2.

(m, 4.11H, SCX_2, where X= *H/D*; OCH_3), 3.94 (d, J_{gem} = 13.8 Hz, 1H, CH_2-SCO-), 4.40 (dd‡, $J_{6,7}$ = 4.8 Hz, J = 2.0 Hz, 1H, H_6), 4.47 (d, J_{gem} = 13.8 Hz, 1H, CH_2-SCO-), 5.28 (m, 2H, PMB-CH_2), 6.03 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 10.3 Hz, 1H, H_7), 6.66 (d, $J_{7,NH}$ = 10.3 Hz, 0.6H, N*H*), 6.89 (d, J = 8.7 Hz, 2H, Ar), 7.32 (m, 7H, Ar), 7.46 (t, J = 8.0 Hz, 2H, Ar), 7.6 (t, J = 7.4 Hz, 1H, Ar), 7.91 (d, J = 7.2 Hz, 1H, Ar).

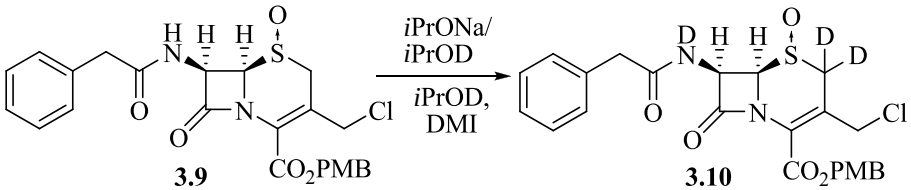


To a solution of **3.5** (487 mg, 1.00 mmol) in dichloromethane (10 mL) at 0 °C was added *m*-CPBA (224 mg, 1.30 mmol) at once and allowed to stir for 3 hours, gradually warm up to rt. Upon completion the solvent was evaporated in vacuo and the residue was dissolved in EtOAc (30 mL), washed with H_2O (2 × 20 mL), sat. $NaHCO_3$ (2 × 20 mL) and brine (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.9** (490 mg, 0.97 mmol, 97%; 80% sulfoxide and 20% sulfone) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.66 (m, 6H, SCH_2; Ph-CH_2; OCH_3), 3.93 (d, J_{gem} = 17.7 Hz, 1H, SCH_2), 3.50 (d, J_{gem} = 11.0 Hz, 1H, CH_2Cl), 4.67 (d, J_{gem} = 11.0 Hz, 1H, CH_2Cl), 4.92 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.28 (m, 2H, PMB-CH_2), 6.03 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.0 Hz, 1H, H_7), 6.94 (d, J = 8.4 Hz, 2H, Ar), 7.32 (m, 7H, Ar), 8.46 (d, $J_{7,NH}$ = 8.0 Hz, 1H, N*H*).

‡ H_6 may be showing a long range W-effect coupling with H_2.

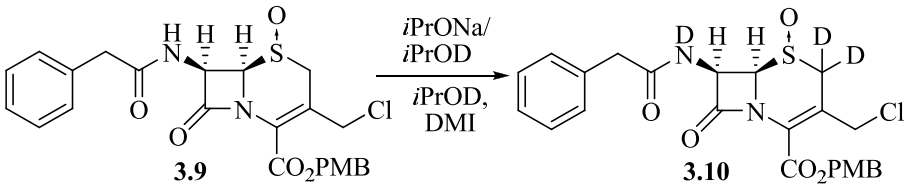


To a solution of *i*PrONa/*i*PrOD [prepared by reaction of NaH (32 mg, 0.79 mmol) and *i*PrOD (1.8 mL)] was added **3.9** (100 mg, 0.20 mmol) in *i*PrOD (3 mL) and DMI (0.8 mL) at –20 °C. The reaction was stirred for 1 hour at the same temperature. Upon completion the reaction mixture was poured into sat. NH_4Cl solution (20 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.10** (71 mg, 0.14 mmol, 70%, 80% sulfoxide and 20% sulfone) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.66 (m, 6.57H, SCX_2, where X= *H/D*; Ph-CH_2; OCH_3), 3.89 (d, J = 17.6 Hz, 0.6H and 0.4D, SCX_2, where X= *H/D*), 4.50 (d, J_{gem} = 11.7 Hz, 1H, CH_2Cl), 4.67 (d, J_{gem} = 11.7 Hz, 1H, CH_2Cl), 4.92 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.25 (m, 2H, PMB-CH_2), 5.85 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 6.94 (d, J = 8.7 Hz, 2H, Ar), 7.29 (m, 7H, Ar), 8.4 (d, $J_{7,NH}$ = 8.3 Hz, 0.5H, N*H*).

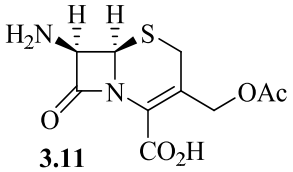


To a solution of *i*PrONa/*i*PrOD [prepared by reaction of NaH (32 mg, 0.79 mmol) and *i*PrOD (1.8 mL)] was added **3.9** (100 mg, 0.20 mmol) in *i*PrOD (3 mL) and DMI (0.8 mL) at –20 °C. The reaction stirred done for 1.5 hour at the same temperature. Upon completion the reaction mixture was poured into sat. NH_4Cl solution (20 mL) and extracted with CH_2Cl_2 (3 × 30 mL).

The combined organic phase was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.10** (60 mg, 0.11 mmol, 60%, 80% sulfoxide and 20% sulfone) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.76 (m, 6.3H, SCX_2, where X= *H/D*; Ph-CH_2; OCH_3), 3.92 (d, J = 17.8 Hz, 0.55H and 0.45D, SCX_2 where X= *H/D*), 4.50 (d, J_{gem} = 11.4 Hz, 1H, CH_2Cl), 4.67 (d, J_{gem} = 11.4 Hz, 1H, CH_2Cl), 4.92 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.25 (m, 2H, PMB-CH_2), 5.85 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 6.94 (d, J = 8.6 Hz, 2H, Ar), 7.34 (m, 7H, Ar), 8.4 (d, $J_{7,NH}$ = 8.3 Hz, 0.4H, N*H*).

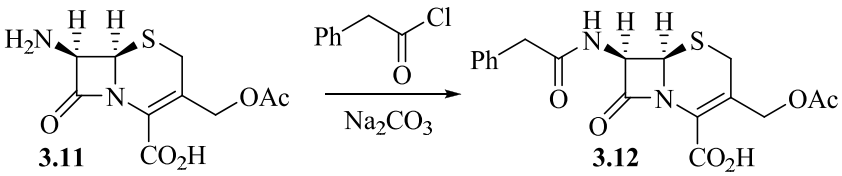


To a solution of *i*PrONa/*i*PrOD [prepared by reaction of NaH (32 mg, 0.79 mmol) and *i*PrOD (1.8 mL)] was added **3.9** (100 mg, 0.20 mmol) in *i*PrOD (3 mL) and DMI (0.8 mL) at –20 °C. The reaction was stirred for 2 hour at the same temperature. Upon completion the reaction mixture was poured into sat. NH_4Cl solution (20 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.10** (60 mg, 0.11 mmol, 60%, 80% sulfoxide and 20% sulfone) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.65 (m, 6.5H, SCX_2, where X= *H/D*; Ph-CH_2; OCH_3), 3.92 (d, J = 17.8 Hz, 0.5H and 0.5D, SCX_2, where X= *H/D*), 4.50 (d, J_{gem} = 11.4 Hz, 1H, CH_2Cl), 4.67 (d, J_{gem} = 11.4 Hz, 1H, CH_2Cl), 4.93 (d, $J_{6,7}$ = 4.6 Hz, 1H, H_6), 5.25 (m, 2H, PMB-CH_2), 5.85 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.2 Hz, 1H, H_7), 6.94 (d, J = 8.6 Hz, 2H, Ar), 7.29 (m, 7H, Ar), 8.4 (d, $J_{7,NH}$ = 8.2 Hz, 0.3H, N*H*).



3.11

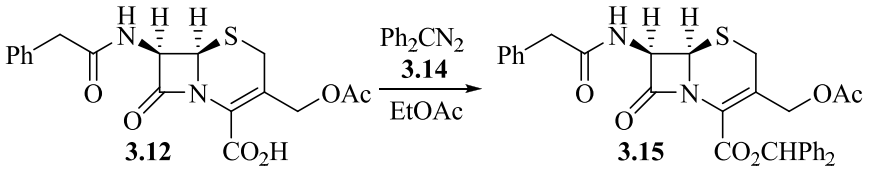
Compound **3.11** was commercially available, aka 7-aminocephalosporanic acid. ^{1}H NMR (300 MHz, D_2O): δ 1.64 (s, 3H, COC*H*$_3$), 3.20 (d, J_{gem} = 18.2 Hz, 1H, SC*H*$_2$), 3.34 (d, J_{gem} = 18.2 Hz, 1H, SC*H*$_2$), 4.47 (m, 2H, C*H*$_2$-OAc), 4.72 (d, $J_{6,7}$ = 5.0 Hz, 1H, *H*$_6$), 4.85 (d, $J_{6,7}$ = 5.0 Hz, 1H, *H*$_7$).



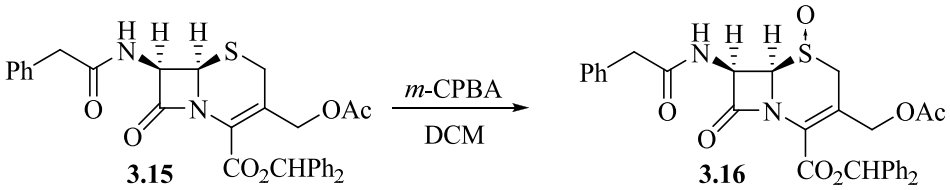
3.11 **3.12**

To a solution of Na_2CO_3 (2150 mg, 20.19 mmol) in water (30 mL) and acetone (25 mL) cooled down to −5 °C was added **3.11** (5000 mg, 18.36 mmol) and stirred for 10 minutes. A solution of phenylacetyl chloride (2.7 mL, 20.19 mmol) in acetone (6.2 mL) was added to the above reaction mixture using syringe pump over the period of 3 hours at −5 °C to 0 °C. Water (25 mL) was added to above reaction flask and was allowed to stir at the same temperature for another 2 hours. After reaction completion EtOAc (30 mL) was added and the reaction was acidified to pH of 3.0 using 10% HCl solution (10 mL). The organic layer was separated and aqueous layer was extracted with EtOAc (3 × 20 mL). The organic phase was combined and washed with brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was crushed using petroleum ether (50 mL) and the residue was filtered to give **3.12** as an off white solid (6809 mg, 17.44 mmol, 95%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.01 (s, 3H, COC*H*$_3$), 3.53 (m, 4H, SC*H*$_2$; Ph-C*H*$_2$), 4.69 (d, J_{gem} = 12.7 Hz, 1H,

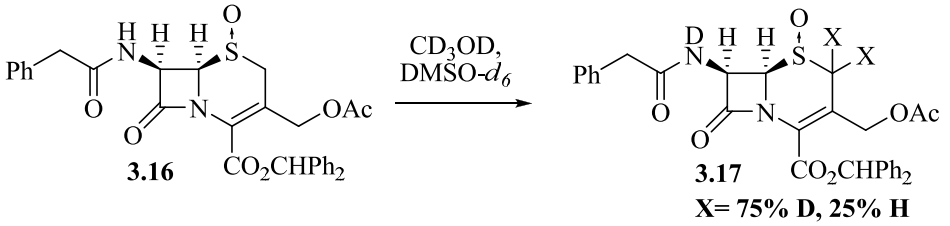
C*H*$_2$OAc), 5.04 (d, J_{gem} = 12.7 Hz, 1H, C*H*$_2$OAc), 5.68 (d, $J_{6,7}$ = 4.9 Hz, 1H, *H*$_6$), 5.68 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.2 Hz, 1H, *H*$_7$), 7.26 (m, 5H, Ar), 9.09 (d, $J_{7,NH}$ = 8.2 Hz, 1H, N*H*). LRMS: (–ESI,) *m/z* (relative intensity): 390 ([M]$^-$; 7), 389.08 ([M–H]$^-$; 37)



To a suspension of **3.12** (5270 mg, 13.50 mmol) in EtOAc (50 mL) at 0 °C was added a solution of diphenyldiazomethane **3.14** (14.85 mL, 14.85 mmol, 1M solution in EtOAc) at once and the reaction was allowed to stir for overnight gradually warming up to rt. Then the solvent was evaporated under reduced pressure and the residue was dissolved in THF (50 mL) and filtered. The filtrate was concentrated under reduced pressure in vacuo, the residue was washed with little quantity of EtOAc and filtered to give **3.15** (6150 mg, 11.04 mmol, 82%) as a white solid residue. ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.98 (s, 3H, COC*H*$_3$), 3.58 (m, 4H, SC*H*$_2$; Ph-C*H*$_2$), 4.65 (d, J_{gem} = 13.1 Hz, 1H, C*H*$_2$OAc), 4.88 (d, J_{gem} = 13.1 Hz, 1H, C*H*$_2$OAc), 5.15 (d, $J_{6,7}$ = 4.9 Hz, 1H, *H*$_6$), 5.79 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.2 Hz, 1H, *H*$_7$), 6.94 (s, 1H, *CH*Ph$_2$), 7.36 (m, 15H, Ar), 9.15 (d, $J_{7,NH}$ = 8.2 Hz, 1H, N*H*). ^{13}C NMR (75.5 MHz, DMSO-*d*$_6$): δ 20.41, 25.85, 41.60, 57.61, 59.07, 62.48, 78.78, 125.03, 126.15, 126.26, 126.51, 126.84, 127.87, 128.22, 128.38, 128.50, 129.00, 135.77, 139.68, 139.80, 160.54, 165.24, 170.03, 170.96.

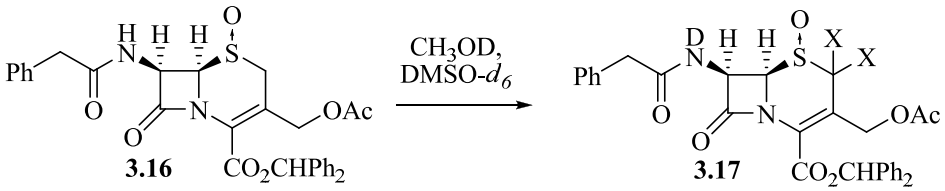


To a solution of **3.15** (2000 mg, 3.59 mmol) in dichloromethane (15 mL) at 0 °C was added *m*-CPBA (965 mg, 1.20 mmol) at once and was gradually warmed to rt in 3 hours. Upon completion the solvent was evaporated in vacuo and the residue was dissolved in EtOAc (30 mL), washed with water (2 × 30 mL), sat. $NaHCO_3$ (3 × 30 mL) and brine (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (EtOAc:hexane; 1:2) to give **3.16** (1880 mg, 3.12 mmol, 87%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.97 (s, 3H, COCH_3), 3.64 (m, 3H, SCH_2; Ph-CH_2), 3.96 (d, J_{gem} = 18.7 Hz, 1H, SCH_2), 4.61 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 4.93 (d, $J_{6,7}$ = 4.3 Hz, 1H, H_6), 5.06 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 5.93 (dd, $J_{6,7}$ = 4.3 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 6.95 (s, 1H, CHPh$_2$), 7.33 (m, 15H, Ar) and 8.44 (d, $J_{7,NH}$ = 8.3 Hz, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 20.48, 41.48, 45.47, 58.09, 62.79, 66.49, 79.08, 121.69, 124.38, 126.55, 126.81, 127.85, 127.92, 128.30, 128.38, 128.54, 129.11, 135.78, 139.78, 159.80, 164.58, 170.00, 171.12.



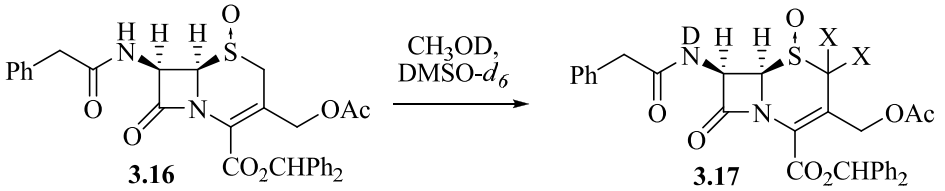
Compound **3.16** (50 mg, 0.09 mmol) was dissolved in a mixture of DMSO-d_6 (1000 µL) and methanol-d_4 (36 µL, 0.88 mmol). The mixture was stirred for 90 minutes at 50 °C. The solvent was evaporated under high vacuum and small aliquot was used to do NMR and then returned

back to the reaction flask. Fresh methanol-d_4 (36 µL, 0.88 mmol) was added and the reaction was repeated for 90 another minutes at 50 °C. Upon completion, methanol was evaporated under high vacuum to give **3.17** in DMSO-d_6. ^{1}H NMR (300 MHz, DMSO-d_6; after 90 minutes): δ 1.97 (s, 3H, COCH_3), 3.63 (m, 2.81H, SCX_2, where *X=H/D*; Ph-CH_2), 3.93 (d, 0.58H, SCX_2, where *X=H/D*), 4.61 (d, J_{gem} = 13.2 Hz, 1H, CH_2OAc), 4.93 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.06 (d, J_{gem} = 13.2 Hz, 1H, CH_2OAc), 5.93 (m, 1H, H_7), 6.95 (s, 1H, CHPh$_2$), 7.32 (m, 15H, Ar), 8.44 (d, $J_{7,NH}$ = 8.5 Hz, 0.6H, NH). ^{1}H NMR (300 MHz, DMSO-d_6; after 180 minutes): δ 1.96 (s, 3H, COCH_3), 3.63 (m, 2.47H, SCX_2, where *X=H/D*; Ph-CH_2), 3.93 (d, 0.25H, SCX_2, where *X=H/D*), 4.61 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 4.92 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.06 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 5.92 (m, 1H, H_7), 6.94 (s, 1H, CHPh$_2$), 7.31 (m, 15H, Ar) and 8.43 (d, $J_{7,NH}$ = 8.5 Hz, 0.3H, NH).



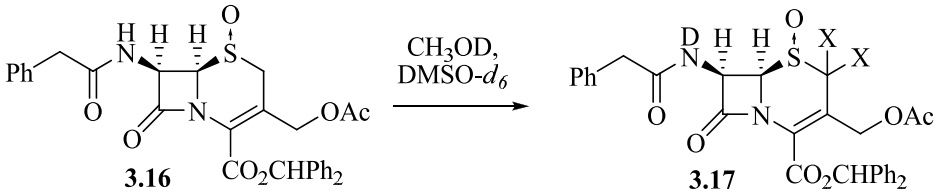
Compound **3.16** (50 mg, 0.09 mmol) was dissolved in a mixture of DMSO-d_6 (1000 µL) and methanol-*d* (36 µL, 0.88 mmol). The mixture was stirred for 90 minutes at 50 °C. The solvent was evaporated under high vacuum and small aliquot was used to do NMR and then returned back to the reaction flask. Fresh methanol-*d* (36 µL, 0.88 mmol) was added and the reaction was repeated for another 90 minutes at 50 °C. Upon completion methanol was evaporated under high vacuum to give **3.17** in DMSO-d_6. ^{1}H NMR (300 MHz, DMSO-d_6; after 90 minutes): δ 1.97 (s, 3H, COCH_3), 3.63 (m, 2.83H, SCX_2, where *X=H/D*; Ph-CH_2), 3.95 (d, 0.46H, SCX_2, where *X=H/D*), 4.61 (d, J_{gem} = 13.5 Hz, 1H, CH_2OAc), 4.93 (d, $J_{6,7}$ = 4.8 Hz,

1H, H_6), 5.06 (d, J_{gem} = 13.5 Hz, 1H, CH_2OAc), 5.92 (m, 1H, H_7), 6.95 (s, 1H, CHPh$_2$), 7.33 (m, 15H, Ar), 8.44 (d, $J_{7,NH}$ = 9.3 Hz, 0.45H, NH). ^{1}H NMR (300 MHz, DMSO-d_6; after 180 minutes): δ 1.96 (s, 3H, COCH_3), 3.64 (m, 2.49H, SCX_2, where X=H/D; Ph-CH_2), 3.95 (d, 0.25H, SCX_2, where X=H/D), 4.61 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 4.93 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.06 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 5.92 (m, 1H, H_7), 6.94 (s, 1H, CHPh$_2$), 7.31 (m, 15H, Ar), 8.43 (d, $J_{7,NH}$ = 8.5 Hz, 0.3H, NH).



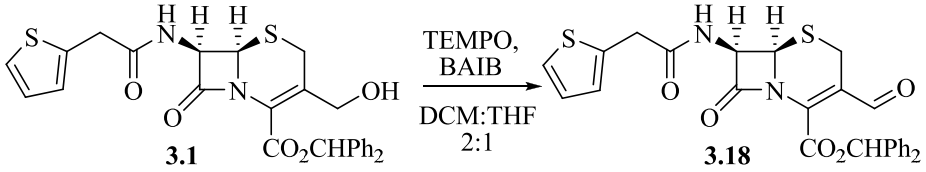
Compound **3.16** (50 mg, 0.09 mmol) was dissolved in a mixture of DMSO-d_6 (1000 µL) and methanol-d_1 (360 µL, 8.80 mmol). The mixture was stirred for 24 hours at 50 °C. The solvent was evaporated under high vacuum and small aliquot was used to do NMR and then returned back to the reaction flask. Fresh methanol-d (360 µL, 8.80 mmol) was added. The reaction was repeated for another 19 hours at 50 °C. After 19 hours, the solvent was evaporated under high vacuum and small aliquot was used to do NMR and then returned back to the reaction flask. Fresh new methanol-d (360 µL, 8.80 mmol) was added and the reaction was repeated for 23 hours more at 50 °C. After 23 hours, the solvent was evaporated under high vacuum and small aliquot was used to do NMR and then returned back to the reaction flask. Upon completion methanol was evaporated under high vacuum. The oil was dissolved in EtOAc (20 mL) and washed with water (2 × 20 mL) and brine (2 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.17** as a yellow solid. ^{1}H NMR (300 MHz,

DMSO-d_6; after 24 hours): δ 1.96 (s, 3H, COCH_3), 3.60 (m, 2.08H, SCX_2, where *X=H/D*; Ph-CH_2), 3.91 (s, 0.08H, SCX_2, where *X=H/D*), 4.61 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 4.91 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.06 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 5.93 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_7), 6.93 (s, 1H, CHPh$_2$), 7.31 (m, 15H, Ar), 8.38 (d, 0.06H, NH). ^{1}H NMR (300 MHz, DMSO-d_6; after 43 hours): δ 1.97 (s, 3H, COCH_3), 3.62 (m, 2.06H, SCX_2, where *X=H/D*; Ph-CH_2), 3.93 (s, 0.06H, SCX_2, where *X=H/D*), 4.61 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 4.92 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.06 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 5.93 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_7), 6.95 (s, 1H, CHPh$_2$), 7.38 (m, 15H, Ar), 8.45 (d, 0.05H, NH). ^{1}H NMR (300 MHz, DMSO-d_6; after 66 hours): δ 1.97 (s, 3H, COCH_3), 3.64 (m, 2.03H, SCX_2, where *X=H/D*; Ph-CH_2), 3.94 (d, 0.03H, SCX_2, where *X=H/D*), 4.61 5.06 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 4.93 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.06 (d, J_{gem} = 13.4 Hz, 1H, CH_2OAc), 5.93 (d, $J_{6,7}$ = 4.9 Hz 1H, H_7), 6.95 (s, 1H, CHPh$_2$), 7.36 (m, 15H, Ar), 8.47 (d, 0.05H, NH). ^{1}H NMR (300 MHz, $CDCl_3$; after workup): δ 2.01 (s, 3H, COCH_3), 3.14 (s, 0.06H, SCX_2, where *X=H/D*), 3.63 (m, 2H, Ph-CH_2), 3.75 (s, 0.07H, SCX_2, where *X=H/D*), 4.42 (d, $J_{6,7}$ = 4.6 Hz, 1H, H_6), 4.70 (d, J_{gem} = 14.1 Hz, 1H, CH_2OAc), 5.26 (d, J_{gem} = 14.1 Hz, 1H, CH_2OAc), 6.08 (m, 1H, H_7), 6.72 (d, $J_{7,NH}$ = 9.8 Hz 0.29H, NH), 6.94 (s, 1H, CHPh$_2$), 7.35 (m, 15H, Ar).



Compound **3.16** (200 mg, 0.36 mmol) was dissolved in a mixture of DMSO-d_6 (4 mL) and methanol-*d* (1.5 mL, 35.93 mmol). The mixture was stirred for 2 hours at 50 °C. The solvent was evaporated under high vacuum and new methanol-*d* (1.5 mL, 35.93 mmol) was added. The

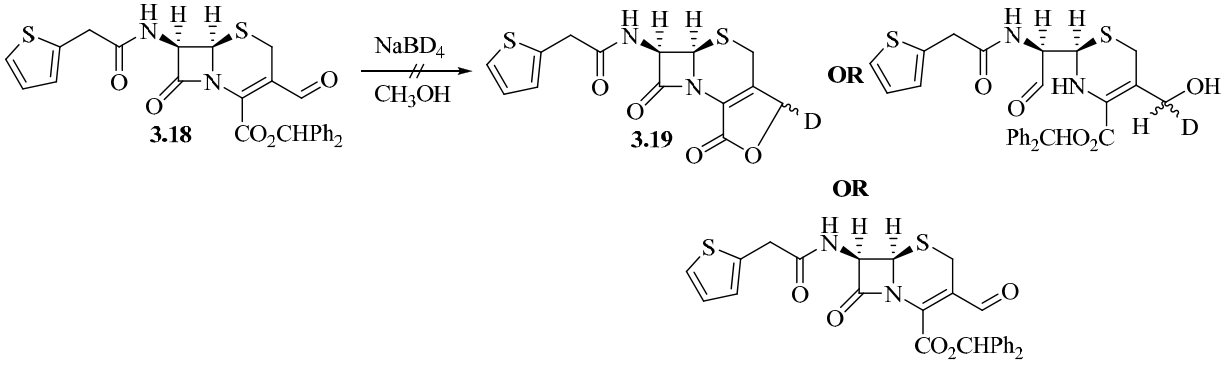
reaction was continued for a total of 24 hours at 50 °C and every 2 hours from t = 0 hours to t= 8 hours methanol-*d* was changed. After 24 hours, methanol was evaporated under high vacuum and the oil/DMSO was dissolved in EtOAc (20 mL) and washed with water (2 × 20 mL) and brine (2 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.17** (200 mg, 0.35 mmol, 99%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01 (s, 3H, COC*H*$_3$), 3.14 (s, 0.03H, SC*X*$_2$, where *X=H/D*), 3.63 (m, 2H, Ph-C*H*$_2$), 3.75 (s, 0.07H, SC*X*$_2$, where *X=H/D*), 4.43 (d, $J_{6,7}$ = 4.8 Hz, 1H, *H*$_6$), 4.70 (d, J_{gem} = 14.1 Hz, 1H, C*H*$_2$OAc), 5.26 (d, J_{gem} = 14.1 Hz, 1H, C*H*$_2$OAc), 6.07 (m, 1H, *H*$_7$), 6.68 (d, $J_{7,NH}$ = 9.9 Hz, 0.28H, N*H*), 6.94 (s, 1H, C*H*Ph$_2$), 7.33 (m, 15H, Ar). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.78, 43.50, 59.21, 63.37, 66.90, 80.49, 121.15, 125.53, 127.22, 127.71, 127.74, 128.33, 128.40, 128.65, 128.75, 129.18, 129.51, 133.77, 139.00, 139.12, 159.79, 164.23, 170.52, 171.47.



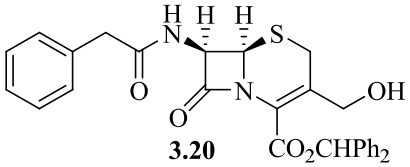
3.1 **3.18**

To a solution of **3.1** (260 mg, 0.50 mmol) in 2:1 mixture of dichloromethane:THF (4 ml:2 mL) at 0 °C was added TEMPO (8 mg, 0.05 mmol) at once and allowed to stir for 5 minutes. BAIB (177 mg, 0.55 mmol) was added at once and the reaction was allowed to gradually warm up to rt in 3 hours. Upon completion the solvent was evaporated in vacuo and the residue was dissolved in dichlormethane (4 mL), crushed out using hexanes (25 mL) and filtered to give **3.18** as a yellow solid (200 mg, 0.39 mmol, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.23 (d, J_{gem} = 18.5 Hz, 1H, SC*H*$_2$), 3.83 (s, 2H, thiophene-C*H*$_2$), 3.95 (d, J_{gem} = 18.5 Hz, 1H, SC*H*$_2$), 5.00 (d, $J_{6,7}$ = 5.3 Hz, 1H, *H*$_6$), 5.98 (dd, $J_{6,7}$ = 5.3 Hz, $J_{7,NH}$ = 8.8 Hz, 1H, *H*$_7$), 6.60 (d, $J_{7,NH}$ = 8.8

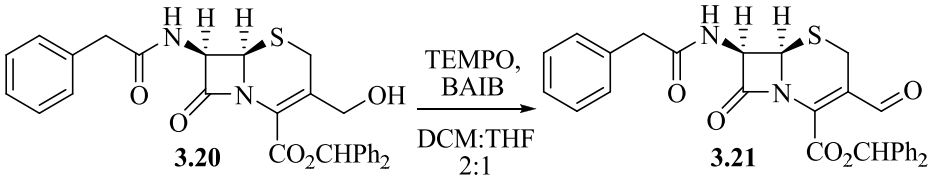
Hz, 1H, N*H*) 6.99 (m, 3H, C*H*Ph$_2$; thiophene), 7.30 (m, 11H, Ar, thiophene), 9.63 (s, 1H, C*H*O).



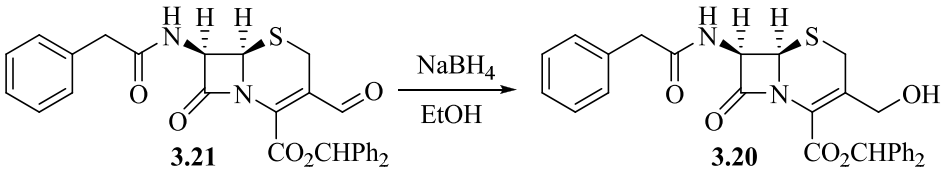
A solution of **3.18** (222 mg, 0.43 mmol) in methanol (4 mL) was cooled down to 0 °C and $NaBD_4$ (18 mg, 0.43 mmol) was added to reaction at once. The reaction was stirred for an hour. The solvent was evaporated in vacuo and the residue was dissolved in EtOAc (20 mL), washed with H_2O (3 × 20 mL) and brine (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.19** (20 mg, 0.04 mmol, 8%). The reaction yield was low, due to the formation of lactone and also cleavage of β-lactam ring.



Compound **3.20** was obtained from Dr. Ahmad Ghavami. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.53 (s, 2H, SC*H*$_2$), 3.64 (m, 2H, Ph-C*H*$_2$), 3.92 (d, J_{gem} = 12.9 Hz, 1H, C*H*$_2$OH), 4.38 (d, J_{gem} = 12.9 Hz, 1H, C*H*$_2$OH), 4.92 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.88 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 9.1 Hz, 1H, H_7), 6.16 (d, $J_{7,NH}$ = 9.1 Hz, 1H, N*H*), 6.9 (s, 1H, C*H*Ph$_2$), 7.34 (m, 15H, Ar).

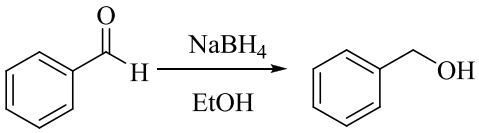


To a solution of **3.20** (520 mg, 1.00 mmol) in 2:1 mixture of dichloromethane:THF (6 ml: 3mL) at 0 °C was added TEMPO (16 mg, 0.10 mmol) at once and allowed to stir for 5 minutes. BAIB (354 mg, 1.10 mmol) was added at once and the reaction was allowed to gradually warm up to rt in 3 hours. Upon completion the solvent was evaporated in vacuo and the residue was dissolved in dichloromethane (10 mL), crushed out using hexanes (50 mL) and filtered to give **3.21** as a yellow solid (440 mg, 0.85 mmol, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.25 (d, J_{gem} = 18.4 Hz, 1H, SCH_2), 3.65 (m, 2H, Ph-CH_2), 3.96 (d, J_{gem} = 18.4 Hz, 1H, SCH_2), 5.02 (d, $J_{6,7}$ = 5.2 Hz, 1H, H_6), 5.98 (dd, $J_{6,7}$ = 5.2 Hz, $J_{7,NH}$ = 9.2 Hz, 1H, H_7), 6.09 (d, $J_{7,NH}$ = 9.2 Hz, 1H, N*H*) 7.06 (s, 1H, C*H*Ph$_2$), 7.32 (m, 15H, Ar), 9.62 (s, 1H, C*H*O).



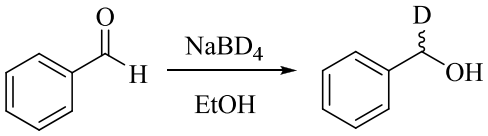
A solution of **3.21** (50 mg, 0.1 mmol) in ethanol (4 mL) was cooled down to 0 °C and $NaBH_4$ (4 mg, 0.1 mmol) was added to the reaction mixture at once. The reaction was allowed to stir for 30 minutes. Upon completion 0.1 N HCl (5 mL) was added to adjust the pH to 4.0 and then the solvent was evaporated in vacuo. The residue was extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.20** (30 mg, 0.05 mmol, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.53 (m, 4H, SCH_2; Ph-CH_2), 4.81 (m, 2H, CH_2OH), 4.98 (d, $J_{6,7}$ = 5.0 Hz,

1H, H_6), 5.91 (dd, $J_{6,7}$ = 5.0 Hz, $J_{7,NH}$ = 8.5 Hz, 1H, H_7), 6.16 (d, $J_{7,NH}$ = 8.5 Hz, 1H, N*H*), 7.28 (m, 16H, C*H*Ph_2; Ar).



3.22 **3.23**

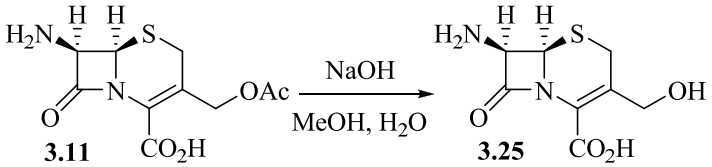
To the solution of benzaldehyde **3.22** (250 mg, 2.35 mmol) in ethanol (5 mL) was added $NaBH_4$ (89 mg, 2.35 mmol) at once and allowed to react for 15 minutes. Upon completion the reaction was acidified to pH of 3.0 using acetic acid (3 mL). The solvent was evaporated in vacuo and the residue was extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.23** (236 mg, 2.18 mmol, 92%) as colourless oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.1 (s, 2H, CH_2OH), 7.79 (m, 5H, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 65.16, 127.05, 127.65, 128.57.



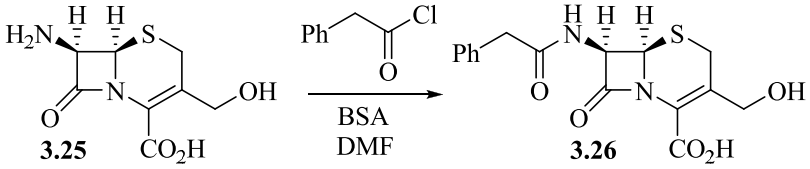
3.22 **3.24**

To the solution of benzaldehyde **3.22** (250 mg, 2.35 mmol) in ethanol (5 mL) was added $NaBD_4$ (99 mg, 2.35 mmol) at once and allowed to react for 15 minutes. Upon completion the reaction was acidified to pH of 3.0 using acetic acid (3 mL). The solvent was evaporated in vacuo and the residue was extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.24** (218 mg, 2.0 mmol, 85%) as colourless oil. ^{1}H NMR (300 MHz, DMSO-

d_6): δ 4.15 (s, 1H, CDHO*H*), 5.07 (s, 1H, CD*H*OH), 7.83 (m, 5H, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 64.30, 64.60, 64.88 (t, 1C, *C*DHOH), 127.00, 127.50, 128.45.

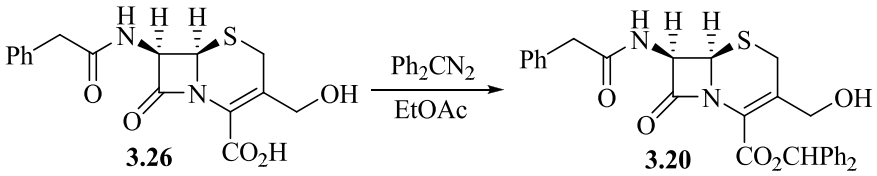


To a suspension of **3.11** (12000 mg, 44.06 mmol) in methanol (120 mL) and water (120 mL) at –30 °C was added, aqueous NaOH (2650 mg, 66.09 mmol) in water (10 mL) dropwise over a period of 30 minutes. The reaction was allowed to stir at same temperature of extra 30 minutes. The pH was adjusted to 3.0 by adding conc. HCl (10 mL). Upon acidification the solution was filtered and washed with methanol and acetone several times to give **3.25** (8000 mg, 34.74 mmol, 80%) as a yellow solid. ^{1}H NMR (300 MHz, D_2O): δ 3.53 (d, J_{gem} = 18.6 Hz, 1H, SCH_2), 3.67 (d, J_{gem} = 18.6 Hz, 1H, SCH_2), 4.98 (d, $J_{6,7}$ = 5.1 Hz, 1H, H_6), 5.04 (d, $J_{6,7}$ = 5.1 Hz, 1H, H_7), Peak for CH_2OH under solvent peak.



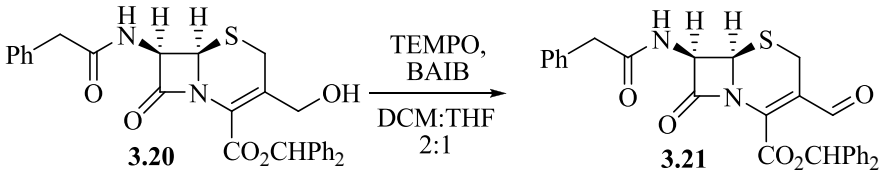
To a solution of **3.25** (7000 mg, 34.74 mmol) in DMF (80 mL) at rt was added, BSA (18.78 mL, 76 mmol) at once and was allowed to stir for 30 minutes, this was then cooled down to –40 °C. Phenylacetyl chloride (6.04 mL, 52.11 mmol) was added drop wise to above reaction mixture and it was stirred for 3 hours at –40 °C. Upon completion the reaction was poured into ice cold H_2O (50 mL) and extracted with EtOAc (4 × 80 mL). The combined organic phase was washed with brine (5 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated under

reduced pressure to give **3.26** (6376 mg, 18.30 mmol, 60%) as a yellow oil. Crushed out using diethyl ether (50 mL) and filtered to get solid residue. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.55 (m, 4H, SCH_2; Ph-CH_2), 4.25 (m, 2H, CH_2OH), 5.04 (d, $J_{6,7}$ = 4.7 Hz, 1H, H_6), 5.62 (dd, $J_{6,7}$ = 4.7 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 7.26 (m, 5H, Ar), 9.06 (d, $J_{7,NH}$ =8.3 Hz, 1H, NH).

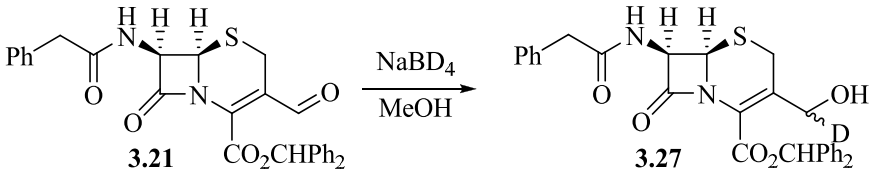


To a suspension of **3.26** (2700 mg, 7.75 mmol) in EtOAc (30 mL) at 0 °C was added a solution of diphenyldiazomethane **3.14** (9.4 mL, 9.4 mmol, 1 M solution in EtOAc) at once and it was allowed to stir overnight and gradually warmed up to rt. Upon completion the solvent was evaporated under reduced pressure, the residue was dissolved in THF (30 mL) and filtered. The filtrate was concentrated under reduced pressure in vacuo. The residue was washed with little quantity of EtOAc and filtered to give **3.20** (3000 mg, 5.82 mmol, 75%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.56 (m, 4H, SCH_2; Ph-CH_2), 4.21 (m, 2H, CH_2OH), 5.13 (m, 2H, H_6; CH$_2$OH), 5.71 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 6.90 (s, 1H, CHPh$_2$), 7.32 (m, 15H, Ar), 9.11 (d, $J_{7,NH}$ = 8.3Hz, 1H, NH). ^{1}H NMR (300 MHz, benzene-d_6): δ 3.39 (d, J_{gem} = 18.1 Hz, 1H, SCH_2), 3.58 (d, J_{gem} = 18.1 Hz, 1H, SCH_2), 3.69 (m, 2H, Ph-CH_2), 4.43 (dd, J_{gem} = 14.4 Hz, $J_{3,OH}$ = 6.2 Hz, 1H, CH_2OH), 4.55 (m, 2H, H_6; CH_2OH), 4.88 (t, J = 6.0, 1H, CH$_2$OH), 5.80 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 8.2 Hz, 1H, H_7), 7.08 (m, 10H, CHPh$_2$; Ar), 7.44 (t, J = 7.6 Hz, 4H, Ar), 7.61 (d, J = 7.6, 2H, Ar), 9.08 (d, $J_{7,NH}$ = 8.2 Hz, 1H, NH). ^{1}H NMR (500 MHz, acetone-d_6): δ 3.68 (m, 4H, SCH_2; Ph-CH_2), 4.09 (t, J = 6.0, 1H, CH$_2$OH), 4.31 (dd, J_{gem} = 13.7 Hz, $J_{3,OH}$ = 6.2 Hz, 1H, CH_2OH), 4.42 (dd, J_{gem} = 13.7 Hz, $J_{3,OH}$ = 6.2 Hz, 1H,

CH_2OH), 5.12 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.85 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 8.7 Hz, 1H, H_7), 6.91 (s, 1H, CHPh$_2$), 7.35 (m, 15H, Ar), 7.99 (d, $J_{7,NH}$ = 8.7 Hz, 1H, NH).

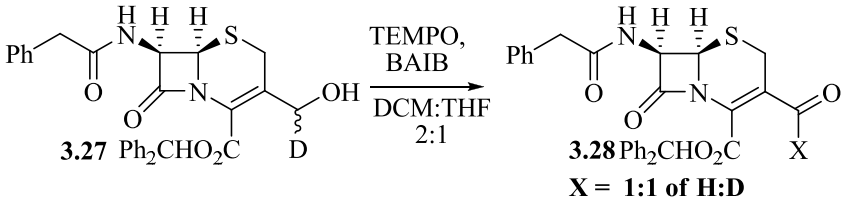


To a solution of **3.20** (1029 mg, 2.00 mmol) in 2:1 mixture of dichloromethane:THF (14 ml:7 mL) at 0 °C was added TEMPO (63 mg, 0.40 mmol) at once and allowed to stir for 5 minutes. BAIB (709 mg, 2.20 mmol) was added at once and the reaction was allowed to gradually warm to rt in 4 hours. Upon completion the solvent was evaporated in vacuo, the residue was dissolved in dichloromethane (10 mL), crushed out using hexanes (50 mL) and filtered to give **3.21** as a yellow solid (923 mg, 1.80 mmol, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.26 (d, J_{gem} = 18.4 Hz, 1H, SCH_2), 3.96 (d, J_{gem} = 18.4 Hz, 1H, SCH_2), 3.67 (qAB, J = 16.3 Hz, 2H, Ph-CH_2), 5.03 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 6.0 (m, 2H, H_7; NH), 7.06 (s, 1H, CHPh$_2$), 7.32 (m, 15H, Ar), 9.62 (s, 1H, CHO).

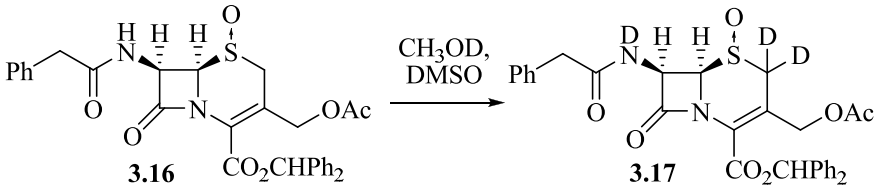


To a solution of **3.21** (500 mg, 1.08 mmol) in methanol (15 mL) at –30 °C was added $NaBD_4$ (45 mg, 1.08 mmol) at once and it was allowed to stir at the same temperature for 30 minutes. Upon completion sat. NH_4Cl solution (5 mL) was added to make it acidic and then the solvent was evaporated under high vacuum very carefully. The suspension was extracted with ethyl acetate (3 × 10 mL). The combined organic phase was washed with brine (20 mL), dried over

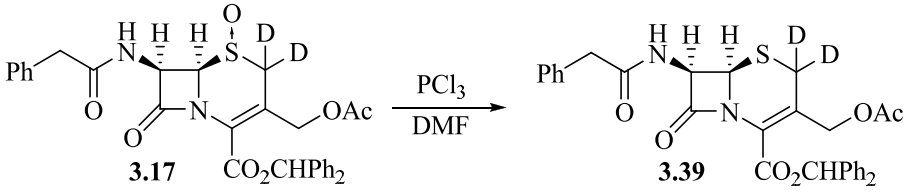
anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was washed with little quantity of EtOAc and filtered to give **3.27** (300 mg, 0.58 mmol, 60%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.55 (m, 4H, SCH_2; Ph-CH_2), 4.18 (m, 1H, C*H*DOH), 5.13 (m, 2H, H_6; CDH*OH*), 5.91 (dd, $J_{6,7}$ = 4.7 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 6.9 (s, 1H, C*H*Ph$_2$), 7.32 (m, 15H, Ar), 9.12 (d, $J_{7,NH}$ = 8.3 Hz, 1H, N*H*). ^{13}C NMR (75.5 MHz, DMSO-d$_6$): δ 25.55, 41.57, 57.70, 58.90, 78.32, 122.00, 126.47, 126.55, 126.59, 126.76, 127.76, 127.82, 128.21, 128.37, 128.50, 129.00, 134.36, 135.81, 139.96, 140.03, 160.86, 165.21, 170.95.



To a solution of **3.27** (50 mg, 0.10 mmol) in 2:1 mixture of dichloromethane:THF (4 ml:2 mL) at 0 °C was added TEMPO (3 mg, 0.01 mmol) at once and allowed to stir for 5 minutes. BAIB (34 mg, 0.10 mmol) was added at once and the reaction was allowed to gradually warm to rt in 24 hours. Upon completion the solvent was evaporated in vacuo, the residue was dissolved in dichloromethane (10 mL), crushed out using hexanes (50 mL) and filtered to give **3.28** (45 mg, 0.09 mmol, 90%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (d, J_{gem} = 18.6 Hz, 1H, SCH_2), 3.64 (m, 2H, Ph-CH_2), 3.94 (d, J_{gem} = 18.6 Hz, 1H, SCH_2), 4.99 (d, $J_{6,7}$ = 5.3 Hz, 1H, H_6), 5.97 (dd, $J_{6,7}$ = 5.3 Hz, $J_{7,NH}$ = 9.1 Hz, 1H, H_7), 6.38 (d, $J_{7,NH}$ = 9.1 Hz, 1H, N*H*), 7.07 (s, 1H, C*H*Ph$_2$), 7.32 (m, 15H, Ar), 9.62 (s, 0.5H and 0.5D, C*X*O, where *X* = *H/D*). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 22.32, 43.28, 59.09, 59.97, 80.92, 124.04, 127.16, 127.92, 127.97, 128.58, 128.69, 128.74, 128.81, 129.32, 129.52, 133.58, 138.21, 138.48, 138.53, 159.75, 165.34, 171.33, 187.84.

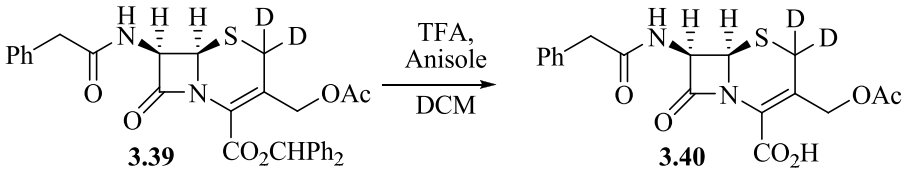


3.16 (1145.2 mg, 2.00 mmol) was dissolved in a mixture of DMSO (20 mL) and methanol-*d* (4 mL, 100.00 mmol). The mixture was stirred for 16 hours at 50 °C. The solvent was evaporated under high vacuum and new methanol-*d* (4 mL, 100.00 mmol) was added. The reaction was repeated for 8 hours at 50 °C. After 24 hours, methanol was evaporated under high vacuum. The oil was dissolved in EtOAc (20 mL) and washed with water (2 × 20 mL) and brine (2 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.17** (1137 mg, 1.97 mmol, 99%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01 (s, 3H, COC*H*$_3$), 3.15 (s, 0.04H, SC*X*$_2$, where *X=H/D*), 3.64 (m, 2H, Ph-C*H*$_2$), 3.76 (s, 0.07H, SC*X*$_2$, where *X=H/D*), 4.43 (d, $J_{6,7}$ = 4.6 Hz, 1H, *H*$_6$), 4.70 (d, J_{gem} = 14.3 Hz, 1H, C*H*$_2$OAc), 5.27 (d, J_{gem} = 14.3 Hz, 1H, C*H*$_2$OAc), 6.10 (m, 1H, *H*$_7$), 6.72 (d, $J_{7,NH}$ = 9.9 Hz, 0.49H, N*H*), 6.93 (s, 1H, C*H*Ph$_2$), 7.36 (m, 15H, Ar).



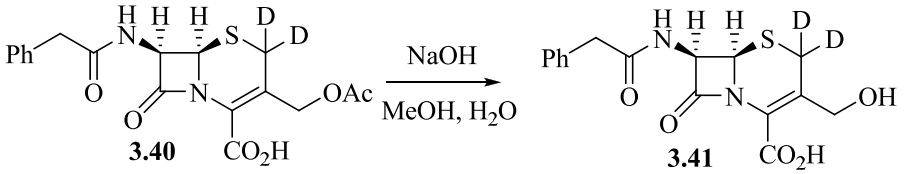
To a solution of **3.17** (970 mg, 1.69 mmol) in DMF (40 mL) at −30 °C was added PCl_3 (310 μL, 3.55 mmol) drop wise and allowed to react for 30 minutes. The reaction mixture was poured into ice cold H_2O (30 mL) and the compound was extracted with EtOAc (3 × 40 mL). The combined organic phase was washed with brine (4 × 30 mL), dried over anhydrous

Na_2SO_4 and concentrated under reduced pressure. The residue was crushed using diethyl ether (40 mL) and filtered to give **3.39** (890 mg, 1.59 mmol, 95%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01 (s, 3H, COCH_3), 3.30 (s, 0.04H, SCX_2, where *X=H/D*), 3.49 (s, 0.04H, SCX_2, where *X=H/D*), 3.64 (m, 2H, Ph-CH_2), 4.94 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 4.76 (d, J_{gem} = 13.6 Hz, 1H, CH_2OAc), 5.00 (d, J_{gem} = 13.6 Hz, 1H, CH_2OAc), 5.86 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 9.1 Hz, 1H, H_7), 6.12 (d, $J_{7,NH}$ = 9.1 Hz, 1H, N*H*), 6.92 (s, 1H, C*H*Ph$_2$), 7.32 (m, 15H, Ar).



3.39 **3.40**

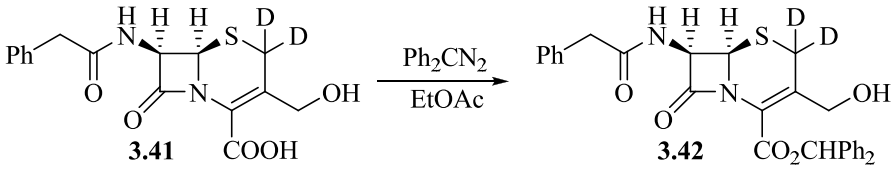
To a solution of **3.39** (890 mg, 1.59 mmol) in dichloromethane (20 mL) at 0 °C, trifluoroacetic acid (2.40 mL, 31.86 mmol) and anisole (0.70 mL, 6.37 mmol) was added to the reaction at once and it was stirred for 30 minutes at same temperature. Upon completion all the solvent was concentrated under reduced pressure and the residue was triturated with diethyl ether 5-6 times to give **3.40** (469 mg, 1.19 mmol, 75%) as a yellow solid. ^{1}H NMR (300 MHz, Acetone-d_6): δ 2.03 (s, 3H, COCH_3), 3.67 (m, 2H, Ph-CH_2), 5.12 (d, $J_{6\text{-}7}$ = 4.9 Hz, 1H, H_6), 4.81 (d, *J* = 13.1 Hz, 1H, CH_2OAc), 5.1 (d, *J* = 13.1 Hz, 1H, CH_2OAc), 5.84 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.4 Hz, 1H, H_7), 7.28 (m, 5H, Ar), 8.04 (d, $J_{7,NH}$ = 8.4 Hz, 1H, N*H*).



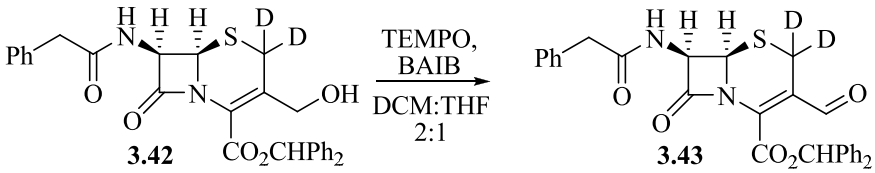
3.40 **3.41**

To a mixture of water (2.5 mL) and methanol (1.8 mL) cooled to –20 °C was added **3.40** (460 mg, 1.17 mmol). Cooled solution of sodium hydroxide (70 mg, 1.76 mmol) in water (0.5 mL)

was added to above reaction drop wise using syringe pump in 40 minutes at –20 °C. After addition the reaction was stirred for another 40 minutes and then it was diluted with EtOAc (10 mL) and then acidified to pH of 2.0 using 10% HCl (2 mL). The organic layer was separated and aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic phase was washed with brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. EtOAc was added to the residue and it was filtered to give **3.41** (124 mg, 0.35 mmol, 35%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.54 (m, 2H, Ph-CH_2), 4.25 (m, 2H, CH_2OH), 5.04 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.62 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 7.25 (m, 5H, Ar), 9.09 (d, $J_{7,NH}$ = 8.3 Hz, 1H, NH).

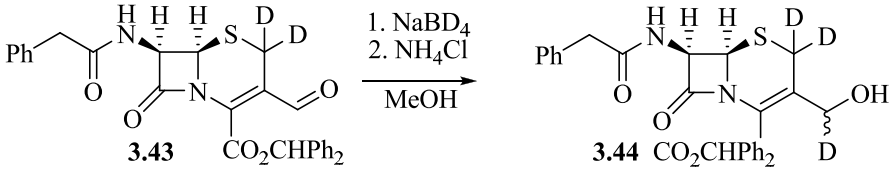


To a suspension of **3.41** (119 mg, 0.34 mmol) in EtOAc (10 mL) at 0 °C. was added solution of diphenyldiazomethane (0.40 mL, 0.40 mmol, 1M solution in EtOAc) at once and the reaction was allowed to stir for overnight gradually warming up to rt. Upon completion the solvent was evaporated under reduced pressure and the residue was dissolved in THF (20 mL) and filtered. The filtrate was concentrated under reduced pressure, the residue was washed with little quantity of EtOAc and filtered to give **3.42** (125 mg, 0.24 mmol, 72%) as a white solid. ^{1}H NMR (300 MHz, Acetone-d_6): δ 3.65 (m, 2H, Ph-CH_2), 4.11 (t, J = 6.2 Hz, 1H, CH$_2$$OH$), 4.37 (m, 2H, C$H_2$OH), 5.12 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.85 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 8.8 Hz, 1H, H_7), 6.91 (s, 1H, CHPh$_2$), 7.32 (m, 15H, Ar), 8.02 (d, $J_{7,NH}$ = 8.8 Hz, 1H, NH).



3.42 **3.43**

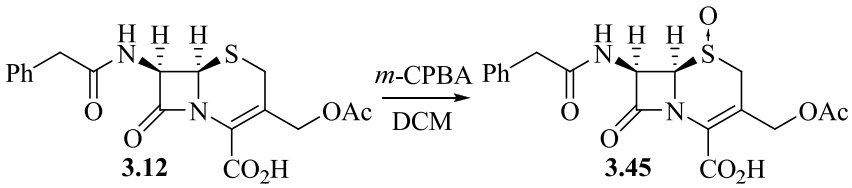
To a solution of **3.42** (119 mg, 0.23 mmol) in 2:1 mixture of dichloromethane:THF (4 ml:2 mL) at 0 °C was added TEMPO (8 mg, 0.05 mmol) at once and allowed to stir for 5 minutes. BAIB (82 mg, 0.25 mmol) was added at once and the reaction was allowed to gradually warm to rt in 4 hours. Upon completion the solvent was evaporated in vacuo, the residue was dissolved in dichloromethane (5 mL), crushed out using hexanes (20 mL) and filtered to give **3.43** as a yellow solid (115 mg, 0.22 mmol, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.24 (s, 0.05H, SC*X*$_2$, where *X=H/D*), 3.66 (m, 2H, Ph-C*H*$_2$), 3.94 (s, 0.05H, SC*X*$_2$, where *X=H/D*), 5.03 (d, $J_{6,7}$ = 4.5 Hz, 1H, *H*$_6$), 6.1 (m, 2H, *H*$_6$; N*H*), 7.06 (s, 1H, C*H*Ph$_2$), 7.30 (m, 15H, Ar), 9.62 (s, 1H, C*H*O).



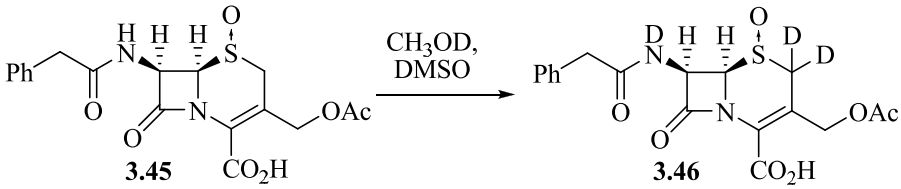
3.43 **3.44**

To a solution of **3.43** (110 mg, 0.21 mmol) in methanol (5 mL) at −30 °C was added $NaBD_4$ (9 mg, 0.21 mmol) to the reaction at once and it was allowed to react at same temperature for 30 minutes. Upon completion sat. NH_4Cl solution (5 mL) was added to make it acidic and then the solvent was evaporated under vacuum pump very carefully. The suspension was extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with brine (2 × 10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was suspended in EtOAc and filtered to give **3.44** (60 mg, 0.12 mmol, 50%) as a white solid residue. ^{1}H NMR (300 MHz, Acetone-*d*$_6$): δ 3.67 (m, 2H, Ph-C*H*$_2$), 4.09 (d, *J* = 6.1 Hz, 1H, CH$_2$O*H*), 4.37 (m,

1H, CD*H*OH), 5.12 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.86 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.7 Hz, 1H, H_7), 6.91 (s, 1H, C*H*Ph$_2$), 7.37 (m, 15H, Ar), 8.02 (d, $J_{7,NH}$ = 8.7 Hz, 1H, N*H*).

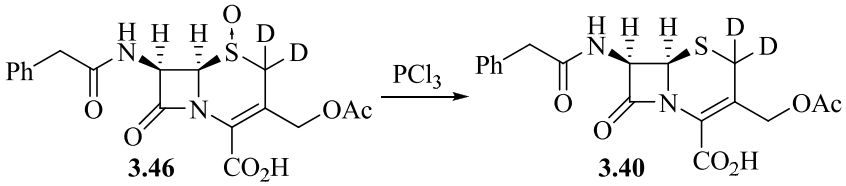


To a suspension of **3.12** (7800 mg, 20 mmol) in dichloromethane (400 mL) at 0 °C, *m*-CPBA (4500 mg, 26 mmol) was added to the reaction at once and was gradually warmed to rt in 3 hours. Upon completion the reaction was filtered and residue was washed with dichloromethane (2 × 100 mL), dried under vacuum pump to give **3.45** (7910 mg, 19.46 mmol, 97%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.02 (s, 3H, COCH_3), 3.54 (m, 3H, SCH_2; Ph-CH_2), 3.89 (d, J_{gem} = 18.2 Hz, 1H, SCH_2), 4.59 (d, J_{gem} = 13.3 Hz, 1H, CH_2OAc), 5.2 (d, J_{gem} = 13.3 Hz, 1H, CH_2OAc), 4.85 (d, $J_{6,7}$ = 4.4 Hz, 1H, H_6), 5.8 (dd, $J_{6,7}$ = 4.4 Hz, $J_{7,NH}$ = 8.2 Hz, 1H, H_7), 7.27 (m, 5H, Ar), 8.43 (d, $J_{7,NH}$ = 8.2 Hz, 1H, N*H*). LRMS: (–ESI) *m/z* (relative intensity): 406.08 ([M]$^-$, 20), 405.07 ([M–H]$^-$, 100).



3.45 (5000 mg, 12.30 mmol) was dissolved in a mixture of DMSO (60 mL) and methanol-*d* (10.0 mL, 246.00 mmol). The mixture was stirred for 2 hours at 50 °C. The solvent was evaporated under high vacuum and new methanol-*d* (10.0 mL, 246.00 mmol) was added. The reaction was repeated for 2 hours at 50 °C and the same procedure was repeated for the third set of 2 hours. After 6 hours, methanol was evaporated under high vacuum. EtOAc was added

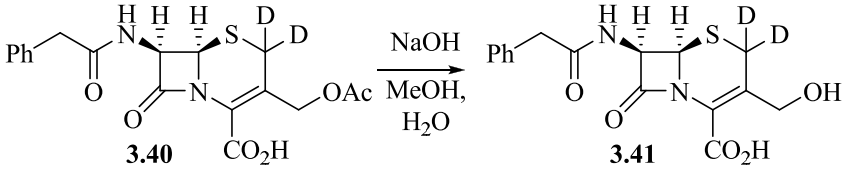
to this solution and acidified to pH of 2.0 with 10% HCl (20 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **3.46** (4974 mg, 12.17 mmol, 99%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.02 (s, 3H, COCH_3), 3.17 (s, 0.03H, SCX_2, where *X=H/D*), 3.62 (qAB, *J* = 14.1 Hz, 2H, Ph-CH_2), 3.86 (s, 0.03H, SCX_2, where *X=H/D*), 4.59 (d, J_{gem} = 13.3 Hz, 1H, CH_2OAc), 5.2 (d, J_{gem} = 13.3 Hz, 1H, CH_2OAc), 4.86 (d, $J_{6,7}$ = 4.8 Hz, 1H, H_6), 5.8 (dd, $J_{6,7}$ = 4.8 Hz, $J_{7,NH}$ = 8.2 Hz, 1H, H_7), 7.26 (m, 5H, Ar), 8.43 (d, $J_{7,NH}$ = 8.2 Hz, 0.49H, N*H*). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 20.66, 41.47, 58.19, 63.08, 66.27, 118.55, 125.99, 126.58, 128.32, 129.14, 135.86, 162.13, 164.23, 170.18, 171.10. HRMS: (–ESI) *m/z*: 406.0799 obsd; 406.0835 calcd for $C_{18}H_{16}N_2O_7S$;. (Back exchange during ionization).



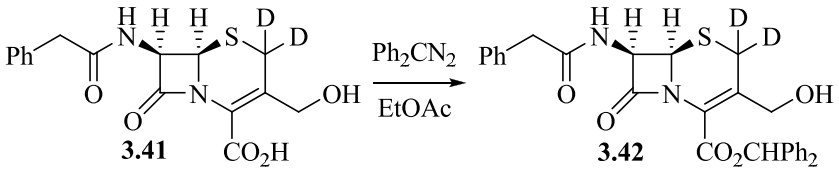
3.46 **3.40**

To a solution of **3.46** (3400 mg, 8.32 mmol) in DMF (50 mL) at –30 °C, phosphorus trichloride (1.5 mL, 17.48 mmol) was added dropwise to the above reaction and it was allowed to react for 30 minutes. The reaction mixture was poured into ice cold H_2O (40 mL) and the compound was extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine (4 × 30 mL) to remove all left over DMF, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was crushed using diethyl ether (50 mL) and filtered to give **3.40** (2600 mg, 6.62 mmol, 80%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.03 (s, 3H, COCH_3), 3.52 (d, *J* = 13.7 Hz, 2H, Ph-CH_2), 4.68 (d, J_{gem} = 12.7 Hz, 1H,

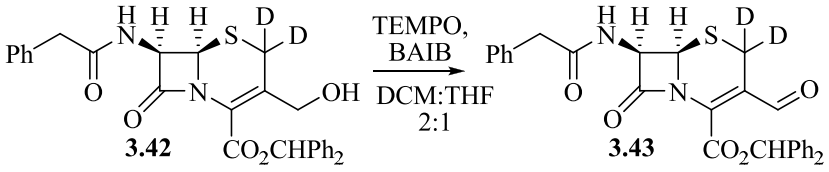
CH_2OAc), 5.01 (d, J_{gem} = 12.7 Hz, 1H, CH_2OAc), 5.09 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.69 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 7.26 (m, 5H, Ar), 9.11 (d, $J_{7,NH}$ = 8.3 Hz, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 20.60, 41.60, 57.43, 59.09, 62.70, 123.32, 126.40, 126.52, 128.26, 129.04, 135.83, 162.87, 164.83, 170.24, 170.98. HRMS: (–ESI) *m/z*: 391.0916 obsd; 391.0938 calcd for $C_{18}H_{15}D_2N_2O_6S$



To a mixture of water (11.7 mL) and methanol (8.8 mL) cooled to –20 °C was added **3.40** (2300 mg, 5.86 mmol). Cooled solution of NaOH (500 mg, 12.30 mmol) in water (2.4 mL) was added to above reaction using syringe pump in 40 minutes at –20 °C. After addition the reaction was stirred for another 40 minutes, diluted with EtOAc (30 mL) and then acidified to pH of 2.0 using 10% HCl (10 mL). The organic layer was separated and aqueous layer was extracted with EtOAc (2 × 30 mL). The combined organic phase was washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. EtOAc was added to the residue and it was filtered to give **3.41** (1000 mg, 2.85 mmol, 50%) as white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.54 (m, 2H, Ph-CH_2), 4.25 (m, 2H, CH_2OH), 5.04 (d, $J_{6,7}$ = 4.5 Hz, 1H, H_6), 5.62 (dd, $J_{6,7}$ = 4.5 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 7.25 (m, 5H, Ar), 9.09 (d, $J_{7,NH}$ = 8.25 Hz, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 41.58, 57.47, 58.93, 59.85, 123.40, 126.51, 128.25, 129.04, 131.10, 135.87, 163.28, 164.80, 170.99. HRMS: (–ESI) *m/z*: 349.0810 obsd; 349.0833 calcd for $C_{16}H_{13}D_2N_2O_5S$.

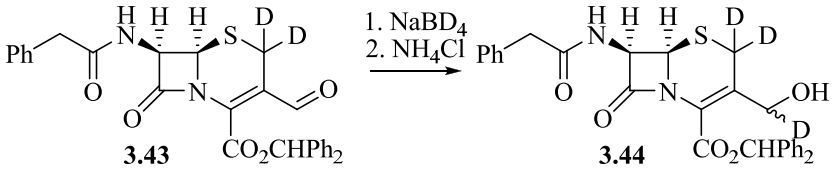


To a suspension of **3.41** (980 mg, 2.79 mmol) in EtOAc (30 mL) at 0 °C was added a solution of diphenyldiazomethane (3.4 mL, 3.40 mmol, 1M solution in EtOAc) at once and the reaction was allowed to stir overnight, gradually warming up to rt. Upon completion the solvent was evaporated under reduced pressure and the residue was dissolved in THF (30 mL) and filtered. The filtrate was concentrated under reduced pressure in vacuo. The residue was washed with little quantity of EtOAc and filtered to give **3.42** (1300 mg, 2.51 mmol, 90%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.53 (m, 2H, Ph-CH_2), 4.21 (d, J_{gem} = 4.5 Hz, 2H, CH_2OH), 5.14 (m, 2H, H_6; CH$_2$*OH*)), 5.71 (dd, $J_{6,7}$ = 5.1 Hz, $J_{7,NH}$ = 7.9 Hz, 1H, H_7), 6.91 (s, 1H, C*H*Ph$_2$), 7.38 (m, 15H, Ar), 9.12 (d, $J_{7,NH}$ = 7.9 Hz, 1H, N*H*).



To a solution of **3.42** (1290 mg, 2.50 mmol) in 2:1 mixture of dichloromethane:THF (20 ml:10 mL) at 0 °C was added TEMPO (78 mg, 0.50 mmol) at once and allowed to stir for 5 minutes. BAIB (965 mg, 2.99 mmol) was added at once and the reaction was allowed to gradually warm to rt in 4 hours. Upon completion the solvent was evaporated in vacuo, the residue was dissolved in dichloromethane (10 mL), crushed out using hexanes (50 mL) and filtered to give **3.43** as a yellow solid (1258 mg, 2.44 mmol, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.22 (s, 0.05H, SCX_2, where *X=H/D*), 3.64 (m, 2H, Ph-CH_2), 3.93 (s, 0.05H, SCX_2, where *X=H/D*),

5.01 (d, $J_{6,7}$ = 5.0 Hz, 1H, H_6), 5.98 (m, 1H, H_7), 6.21 (d, $J_{7,NH}$ = 8.5 Hz, 1H, N*H*), 7.05 (s, 1H, C*H*Ph$_2$), 7.30 (m, 15H, Ar), 9.62 (s, 1H, C*H*O).



To a solution of **3.43** (1100 mg, 2.45 mmol) in methanol (30 mL) at –30 °C was added $NaBD_4$ (103 mg, 2.45 mmol) at once and it was allowed to react at same temperature for 30 minutes. Upon completion sat. NH_4Cl solution (20 mL) was added to make it acidic and then the solvent was evaporated under high vacuum very carefully. The suspension was extracted with EtOAc (3 × 10 mL) and the combined organic phase was washed with brine (30 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The residue was suspended in EtOAc and filtered to give **3.44** (680 mg, 2.12 mmol, 62%) as a white solid residue. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.55 (m, 2H, Ph-CH_2), 4.22 (m, 1H, CD*H*OH), 5.11 (d, J = 4.7 Hz, 1H, CH$_2$O*H*), 5.2 (d, $J_{6,7}$ = 5.2 Hz, 1H, H_6), 5.71 (dd, $J_{6,7}$ = 5.2 Hz, $J_{7,NH}$ = 8.3 Hz, 1H, H_7), 6.90 (s, 1H, C*H*Ph$_2$), 7.34 (m, 15H, Ar), 9.14 (d, $J_{7,NH}$ = 8.3 Hz, 1H, N*H*). ^{1}H NMR (300 MHz, benzene-d_6): δ 3.39 (s, 0.05H, SCH_2), 3.55 (d, 0.06H, SCH_2), 3.69 (m, 2H, Ph-CH_2), 4.44 (m, 0.31H, C*H*DOH), 4.56 (m, 1.80H, H_6; CD*H*OH), 4.88 (d, J = 5.8, 1H, CHDO*H*), 5.80 (dd, $J_{6,7}$ = 4.7 Hz, $J_{7,NH}$ = 8.2 Hz, 1H, H_7), 7.09 (m, 10H, C*H*Ph$_2$; Ar), 7.44 (t, J = 7.9 Hz, 4H, Ar), 7.61 (d, J = 7.5, 2H, Ar), 9.10(d, $J_{7,NH}$ = 8.2 Hz, 1H, N*H*). ^{1}H NMR (500 MHz, acetone-d_6): δ 3.67 (m, 2H, Ph-CH_2), 4.07 (d, J = 6.0, 1H, CDHO*H*), 4.28 (m, 0.31H, CD*H*OH), 4.39 (m, 0.8H, CD*H*OH), 5.12 (d, $J_{6,7}$ = 4.9 Hz, 1H, H_6), 5.85 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,NH}$ = 8.7 Hz, 1H, H_7), 6.91 (s, 1H, C*H*Ph$_2$), 7.35 (m, 15H, Ar), 7.99 (d, $J_{7,NH}$ = 8.7 Hz, 1H, N*H*). ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.11, 41.59, 57.69, 58.90, *C*DHOH (m, 59.1-59.60),

78.33, 122.02, 126.47, 128.57, 126.76, 127.77, 127.84, 128.21, 128.38, 128.51, 129.02, 134.61, 135.83, 139.98, 140.05, 160.87, 165.22, 170.98. HRMS: (+ESI) *m/z*: 535.2087 obsd; 535.2094 calcd for $C_{29}H_{27}D_3N_3O_5S$ $[M + NH_4]^+$

Appendices

Appendix A

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Appendix B

NMR Spectrum and Mass Spectrum Images for Certain Compounds

Figure 3.1: ^{1}H NMR spectrum of 3.4

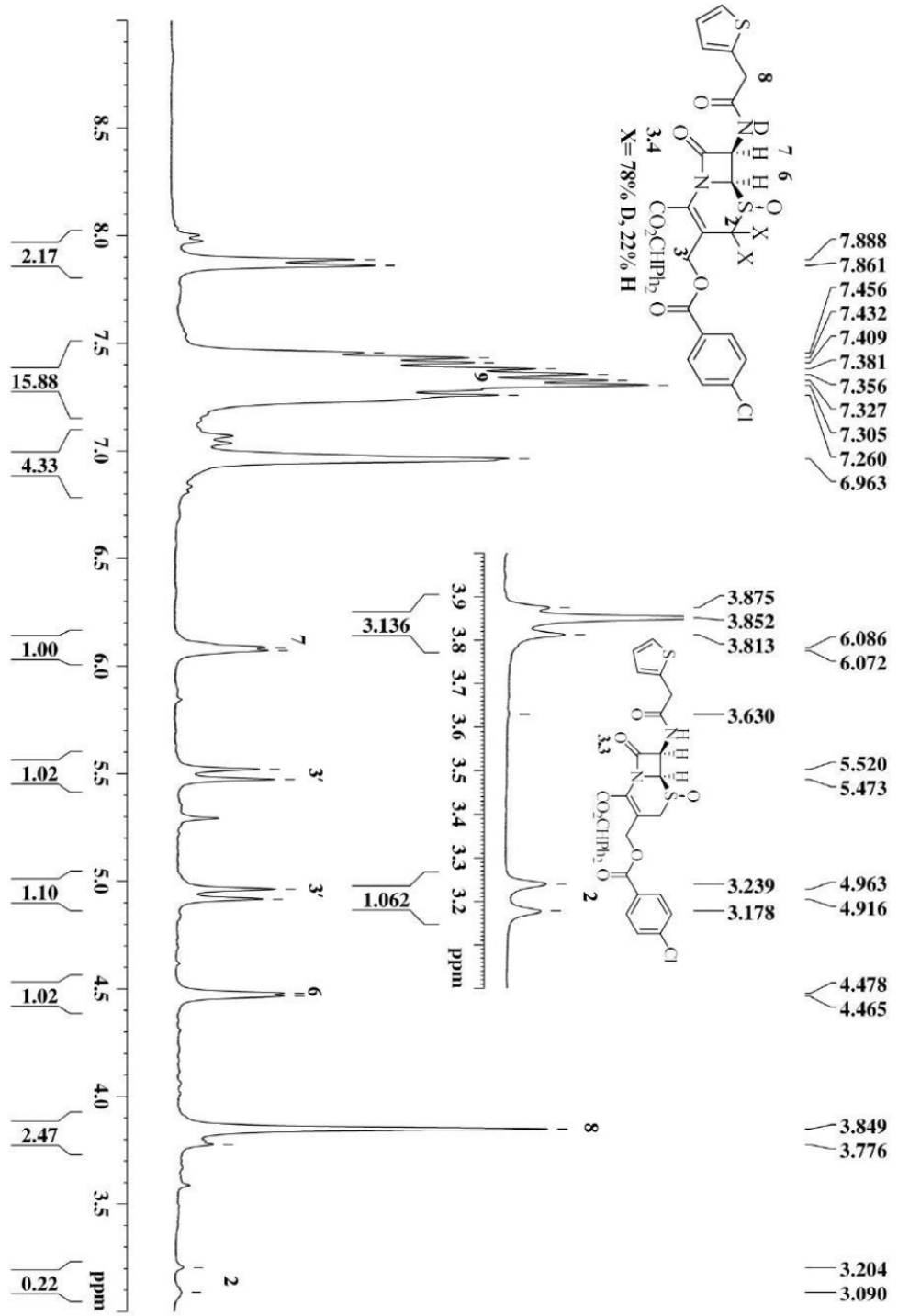


Figure 3.2: ^{1}H NMR spectrum of 3.8

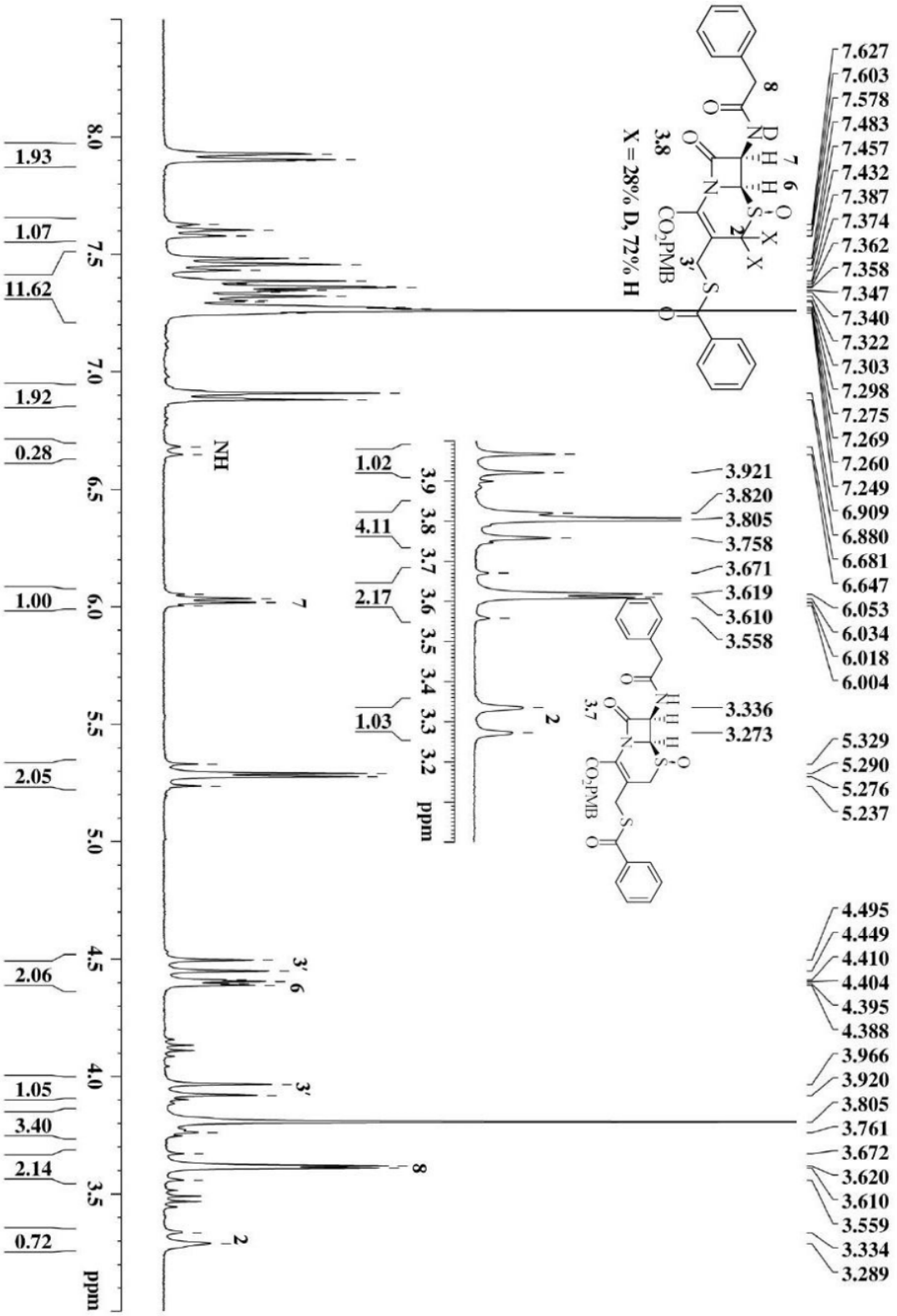


Figure 3.3: ^{1}H NMR spectrum of 3.10 (entry 1)

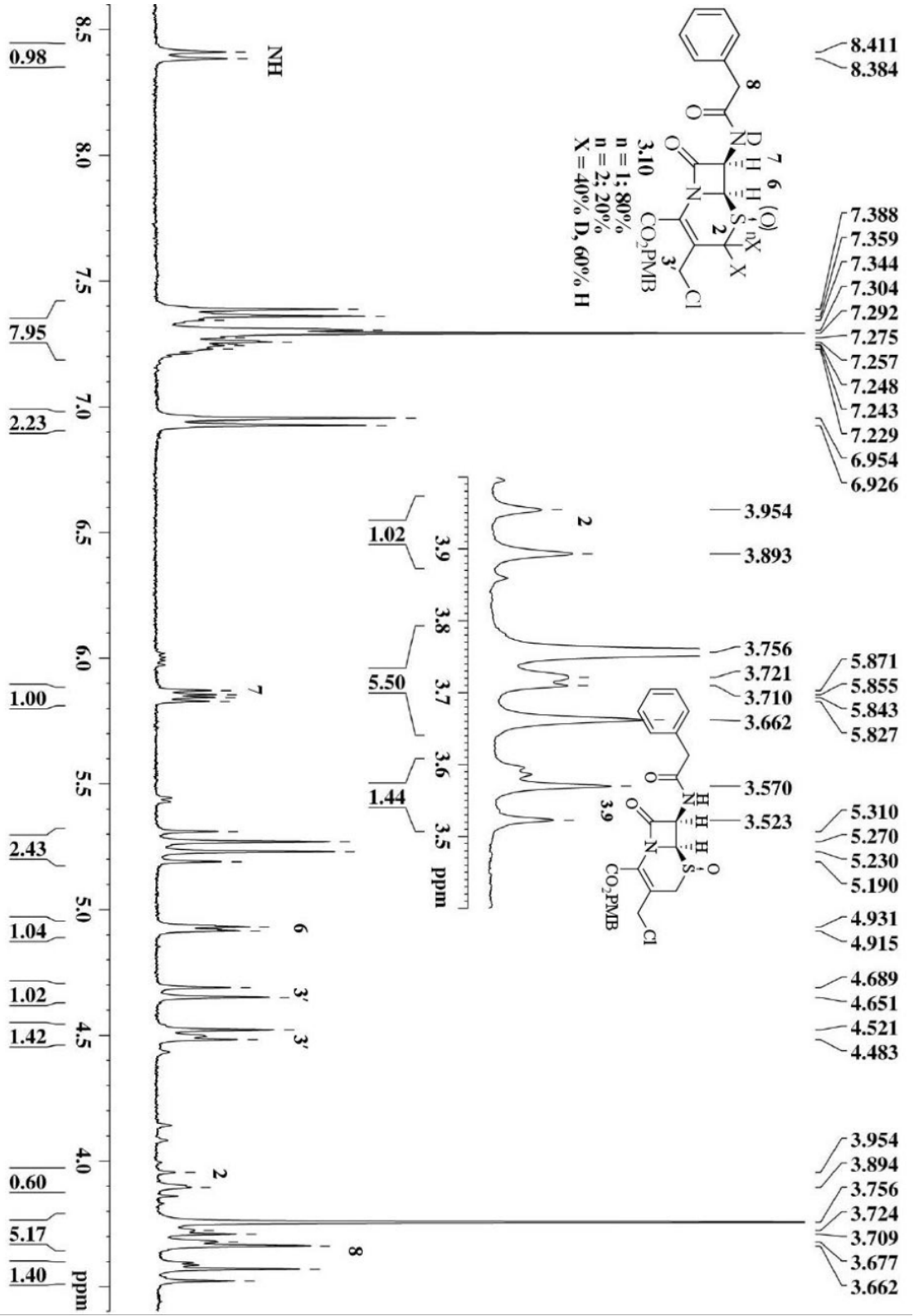


Figure 3.4: ^{1}H NMR spectrum of 3.10 (entry 2)

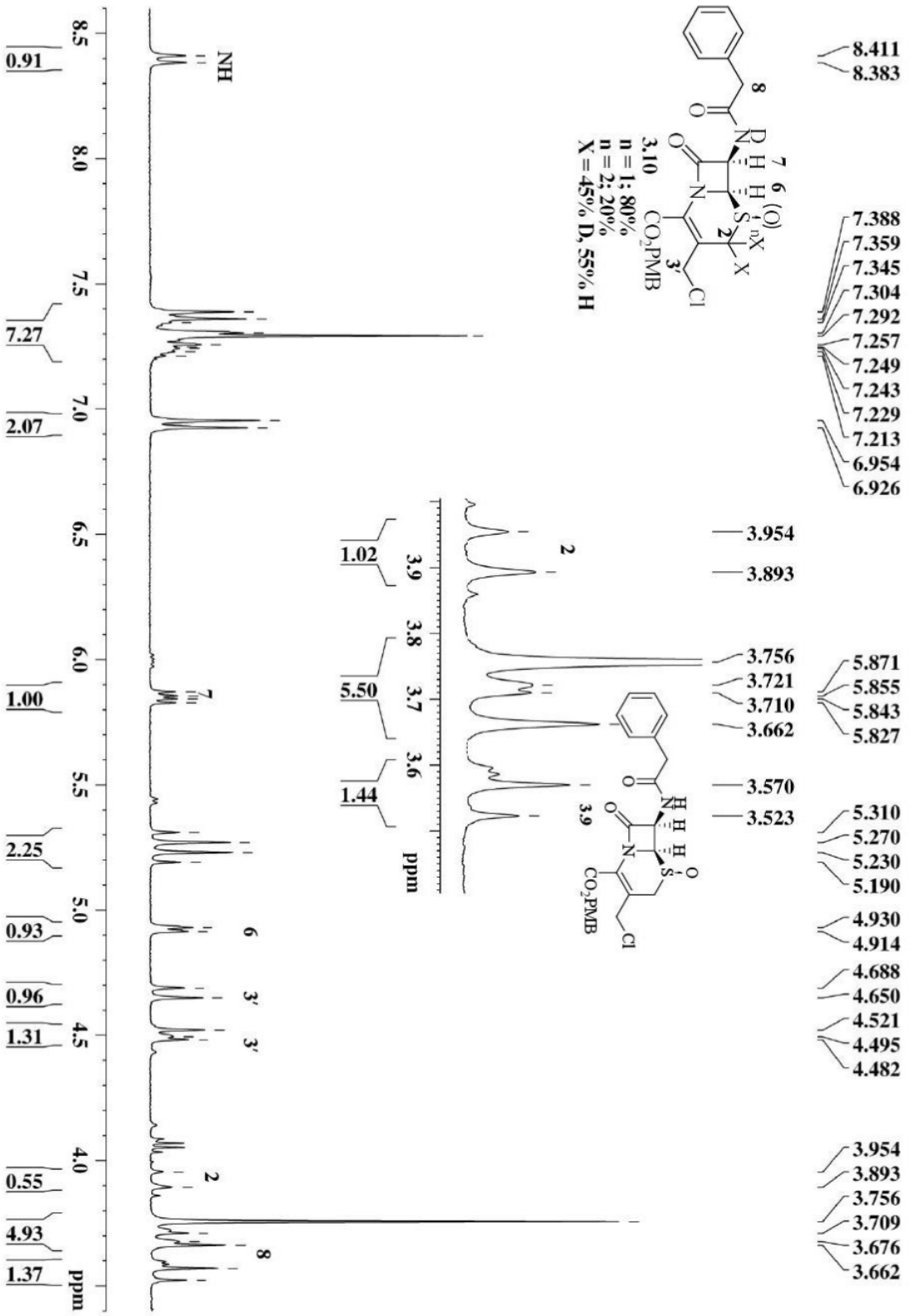


Figure 3.5: ^{1}H NMR spectrum of 3.10 (entry 3)

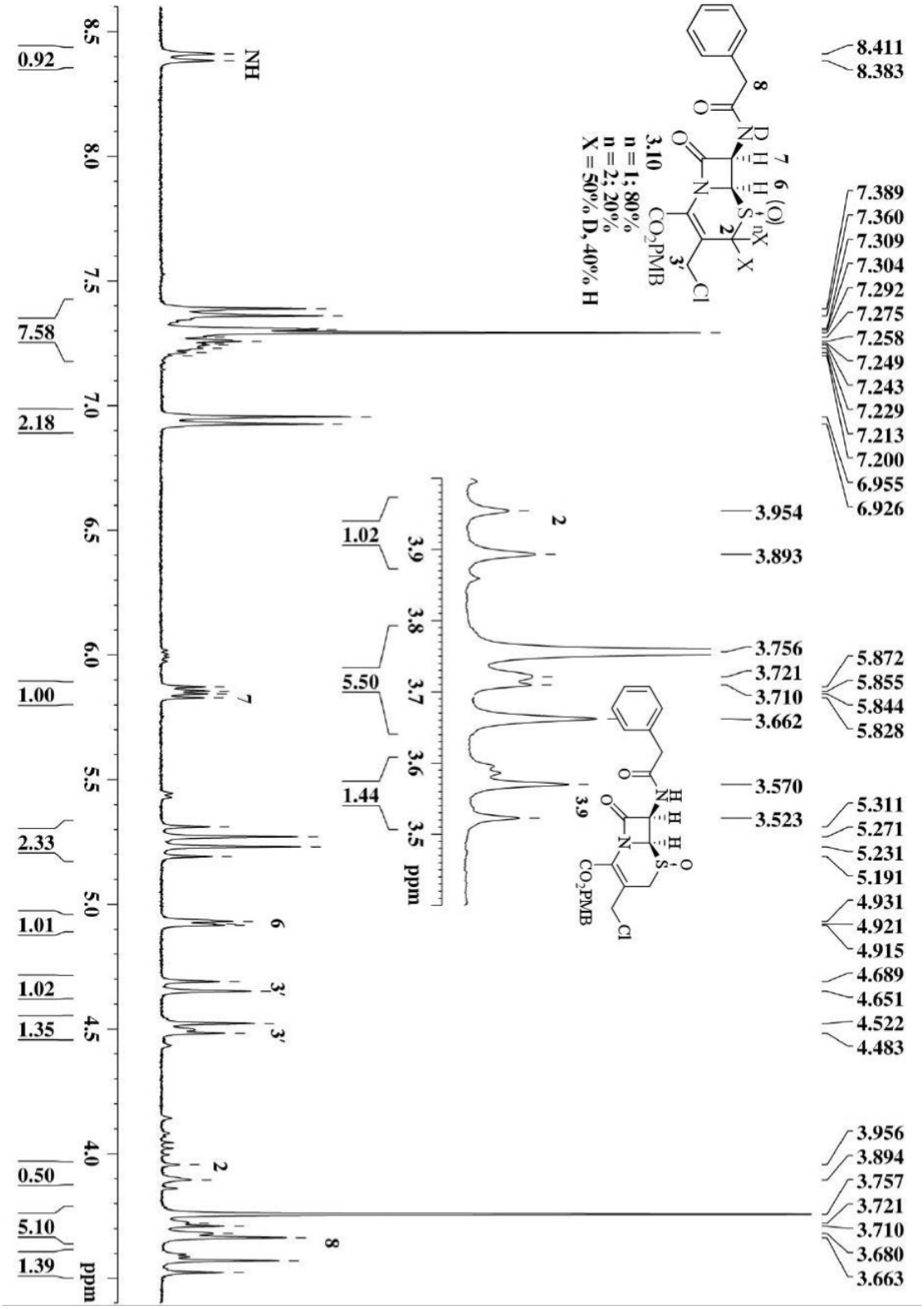


Figure 3.6: ^{1}H NMR spectrum of 3.17 (entry 1)

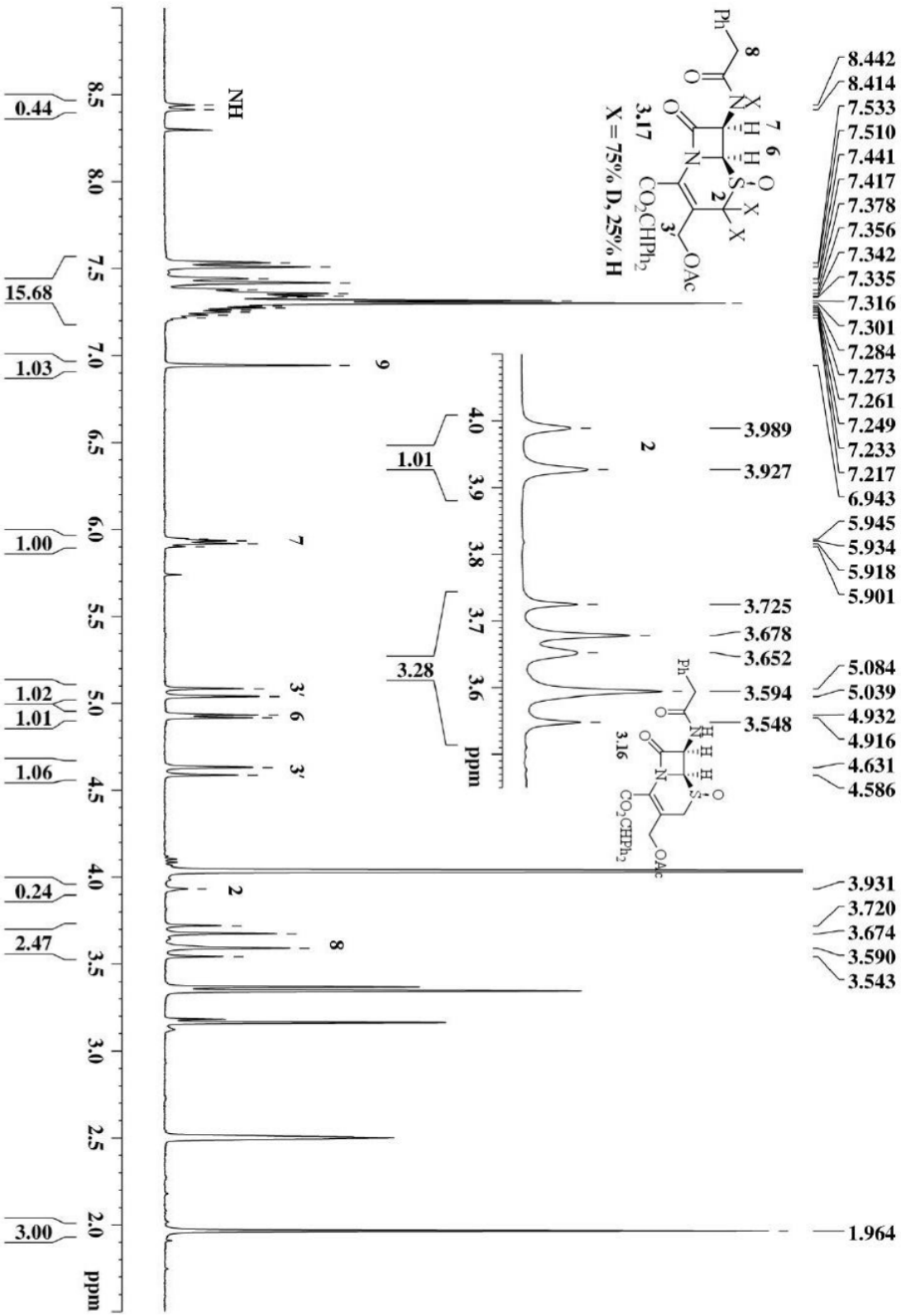


Figure 3.7: ^{1}H NMR spectrum of 3.17 (entry 2)

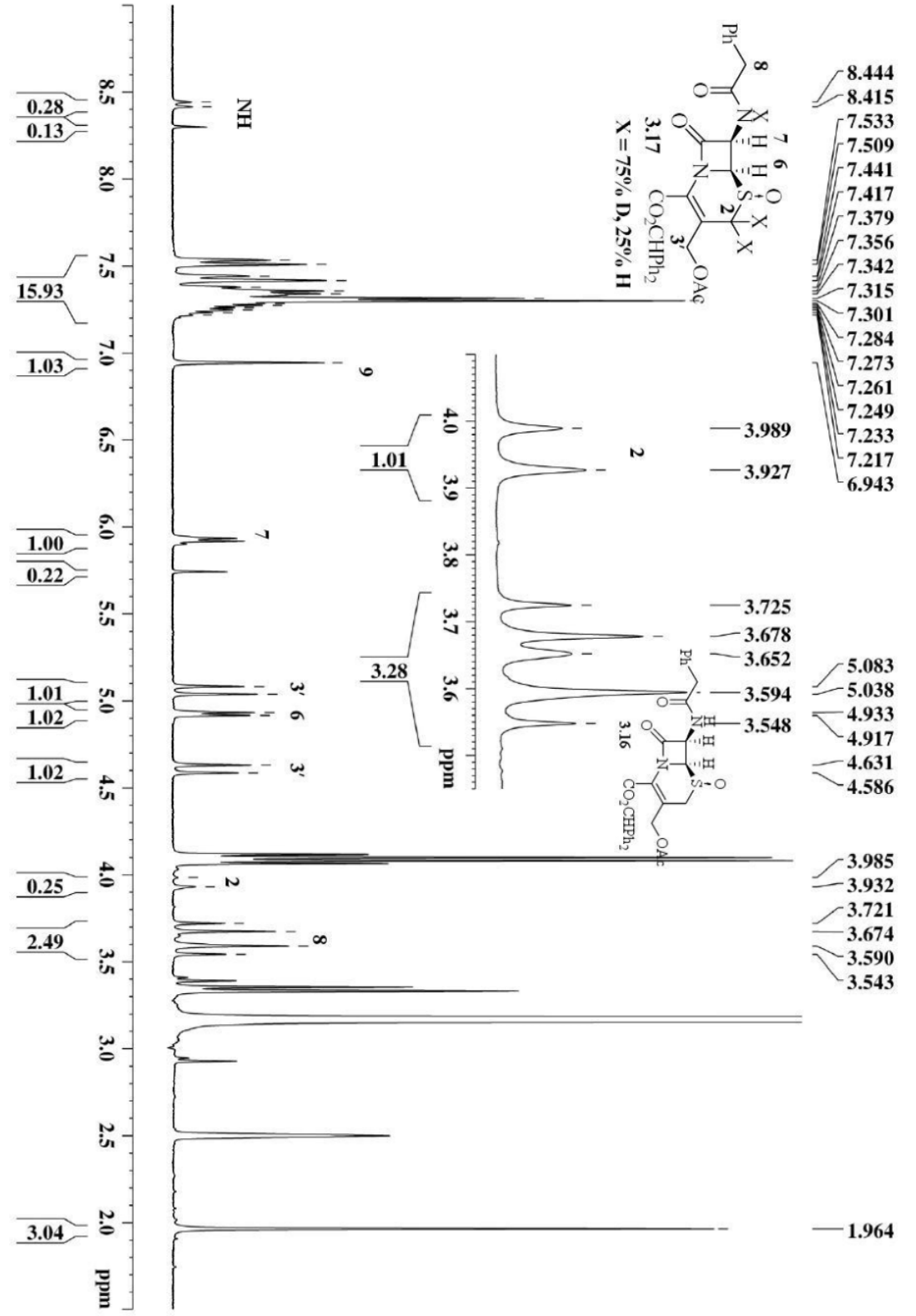


Figure 3.8: ^{1}H NMR spectrum of 3.17 (entry 3)

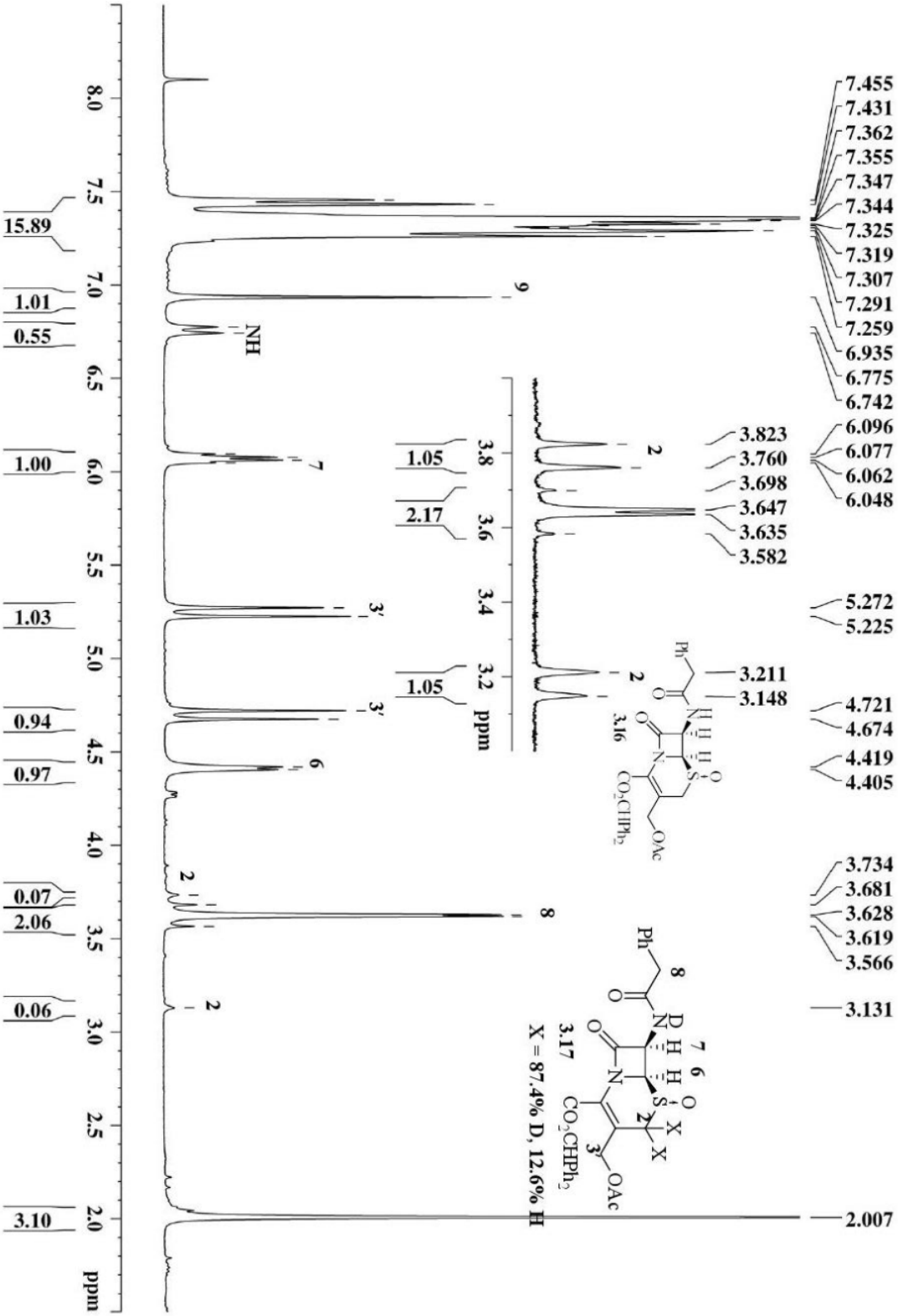


Figure 3.9: ^{1}H NMR spectrum of 3.17 (entry 4)

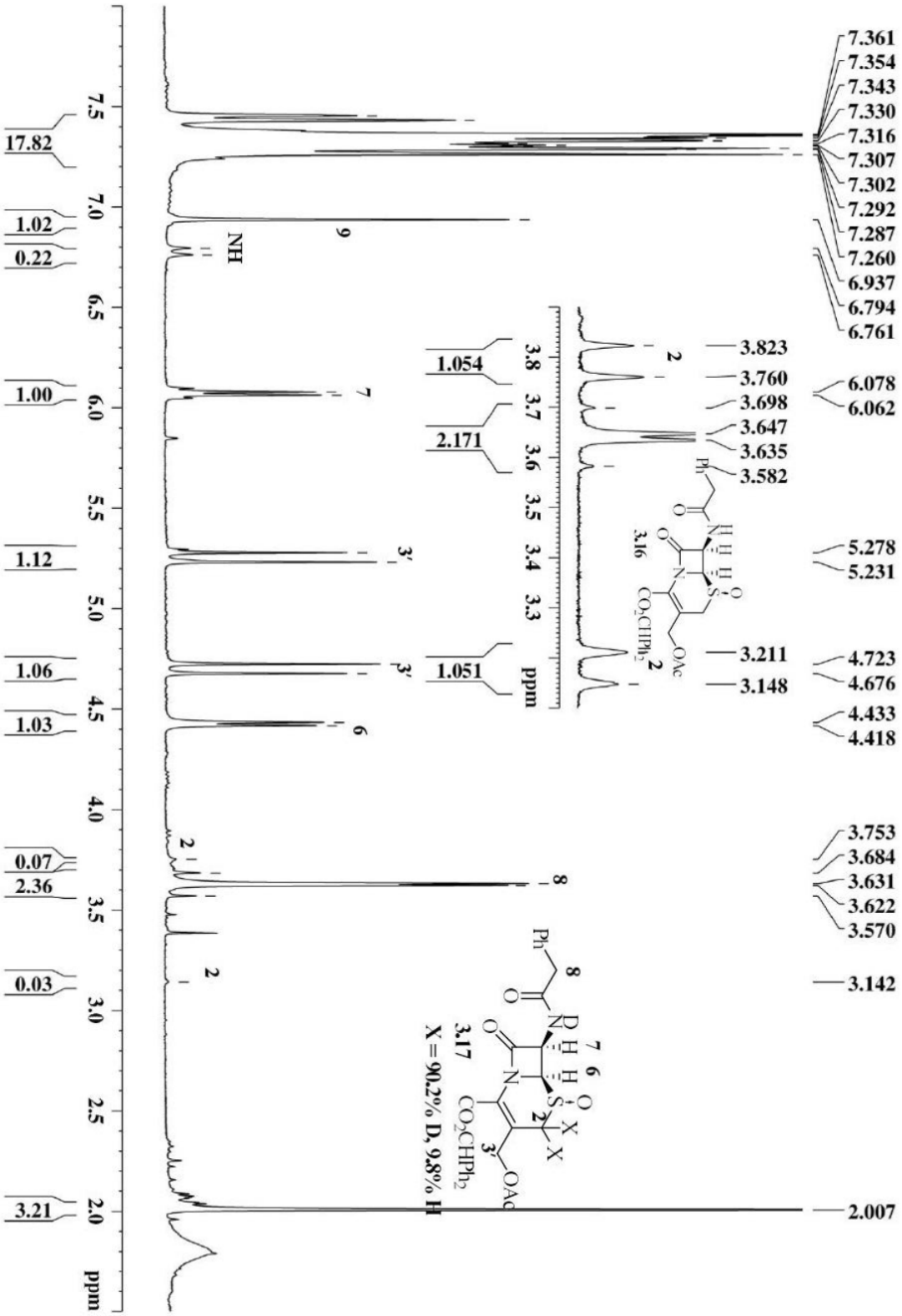


Figure 3.10 [A]: ^{1}H NMR spectrum of 3.15

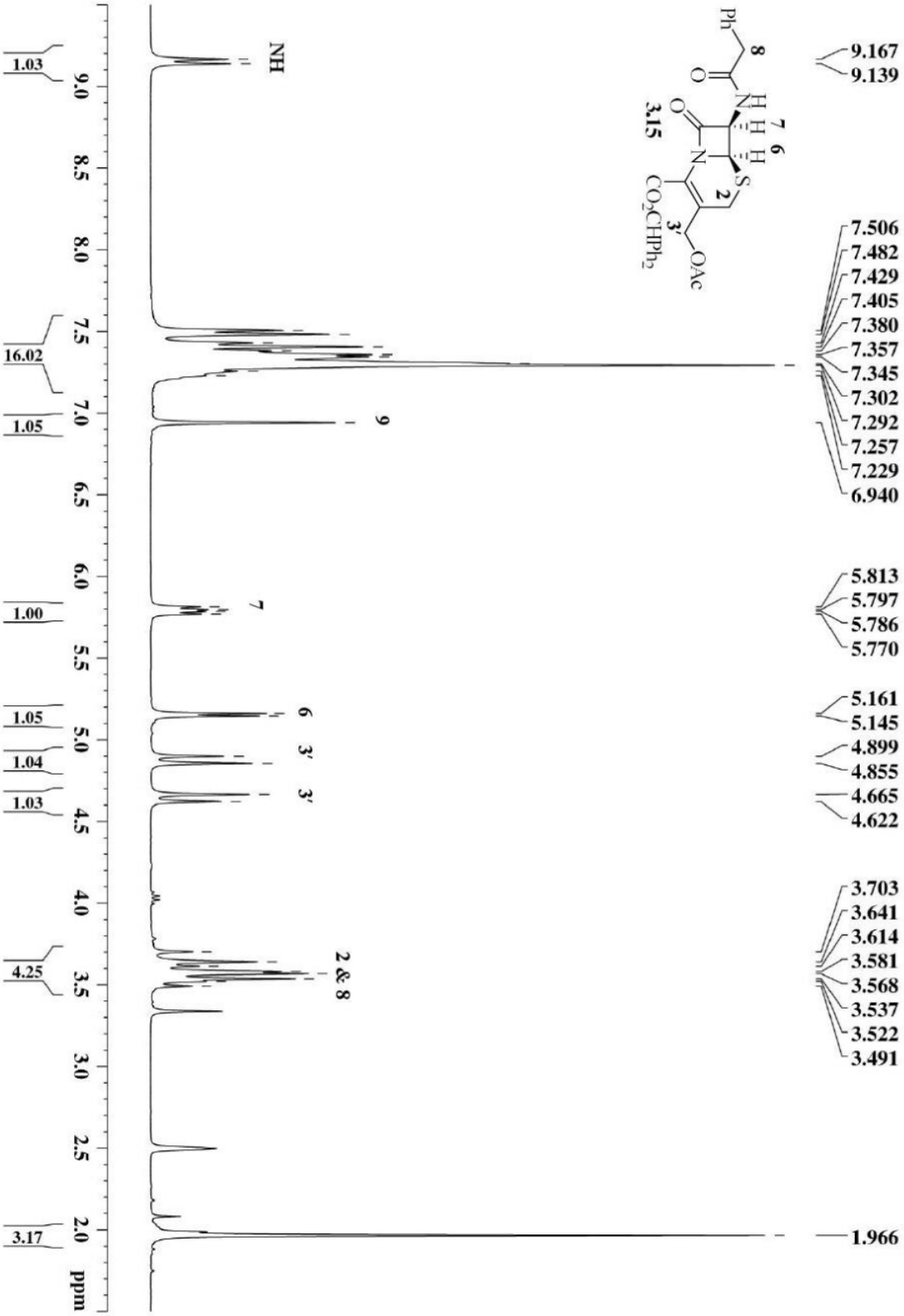


Figure 3.10 [B]: ^{13}C NMR spectrum of 3.15

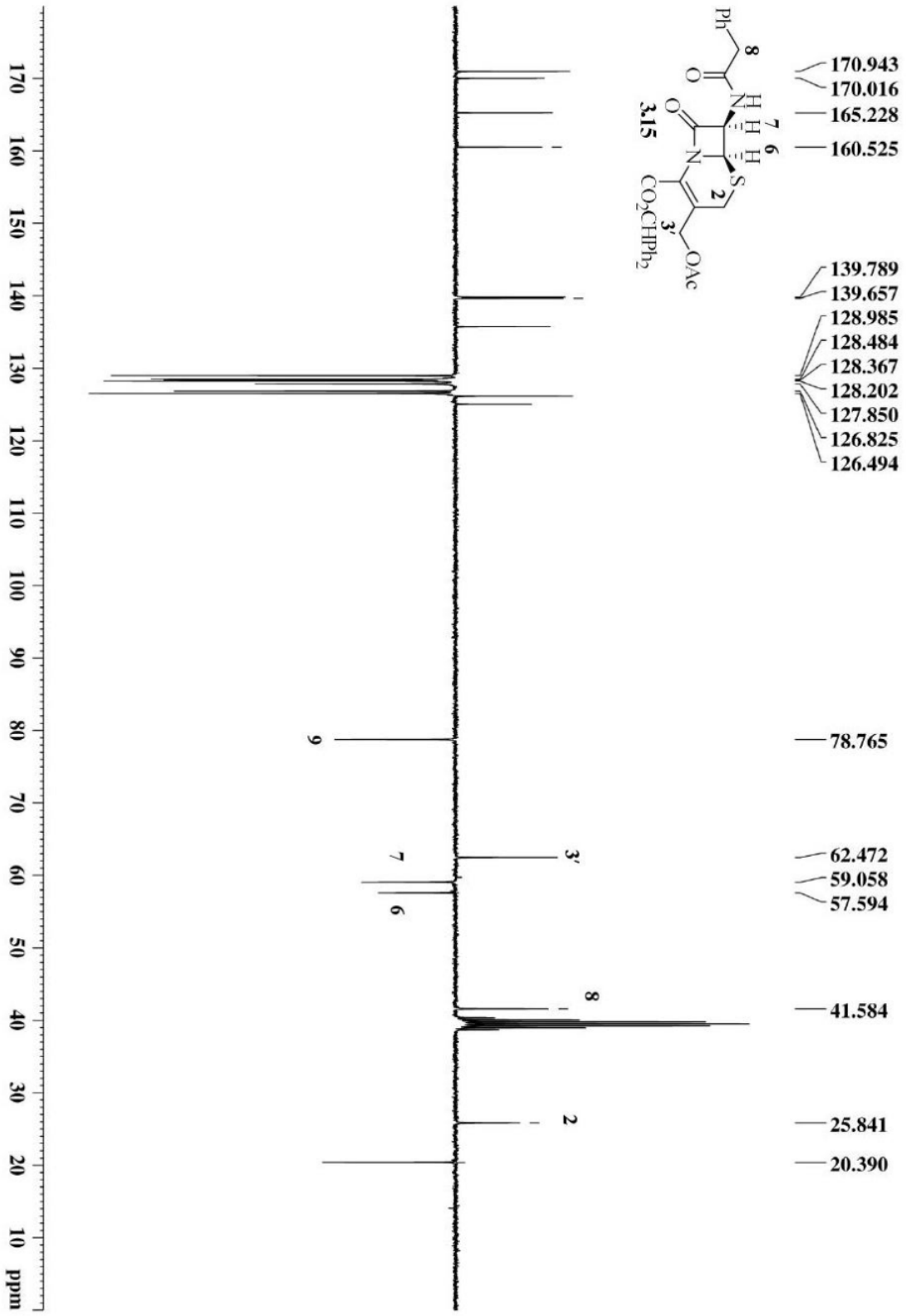


Figure 3.10 [C]: COSY spectrum of 3.15

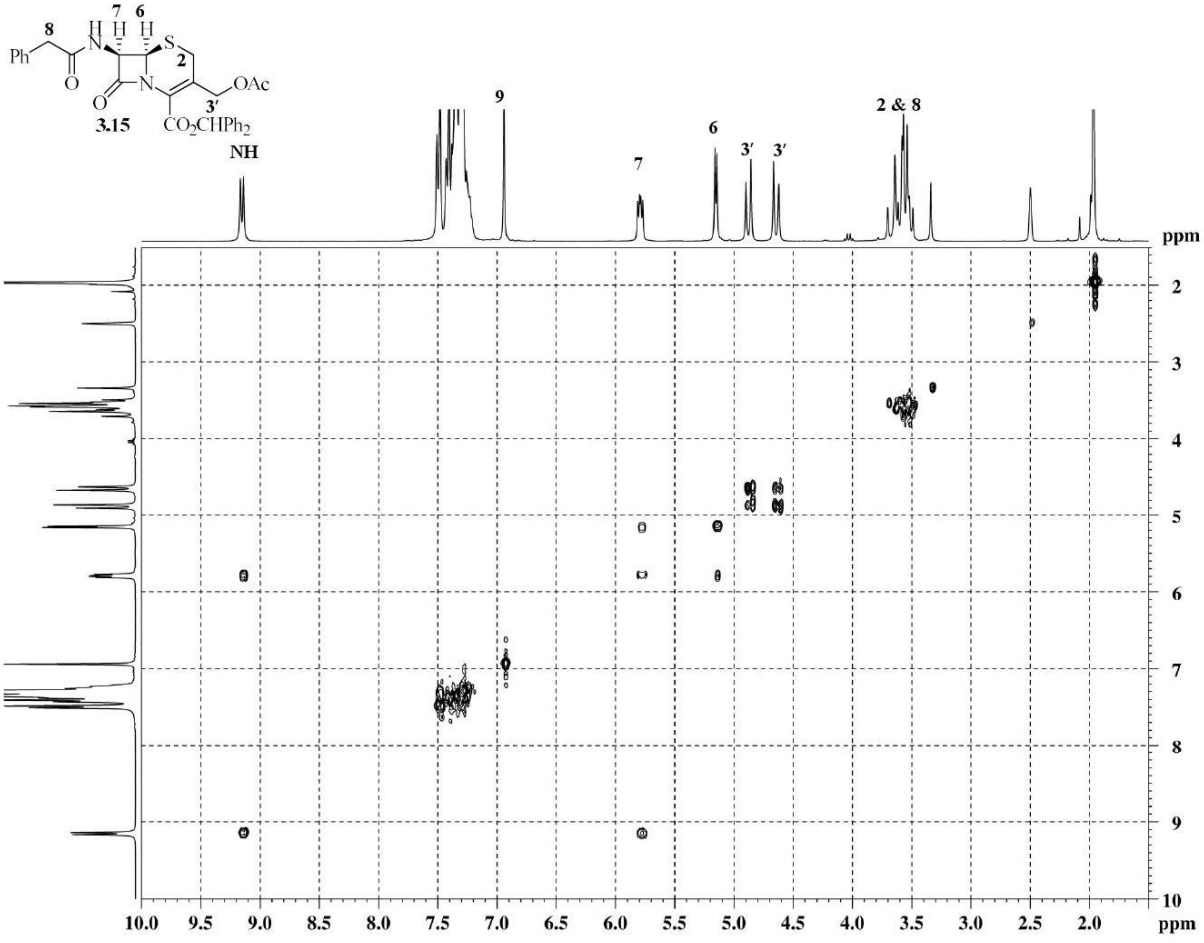


Figure 3.10 [D]: HMBC spectrum of 3.15

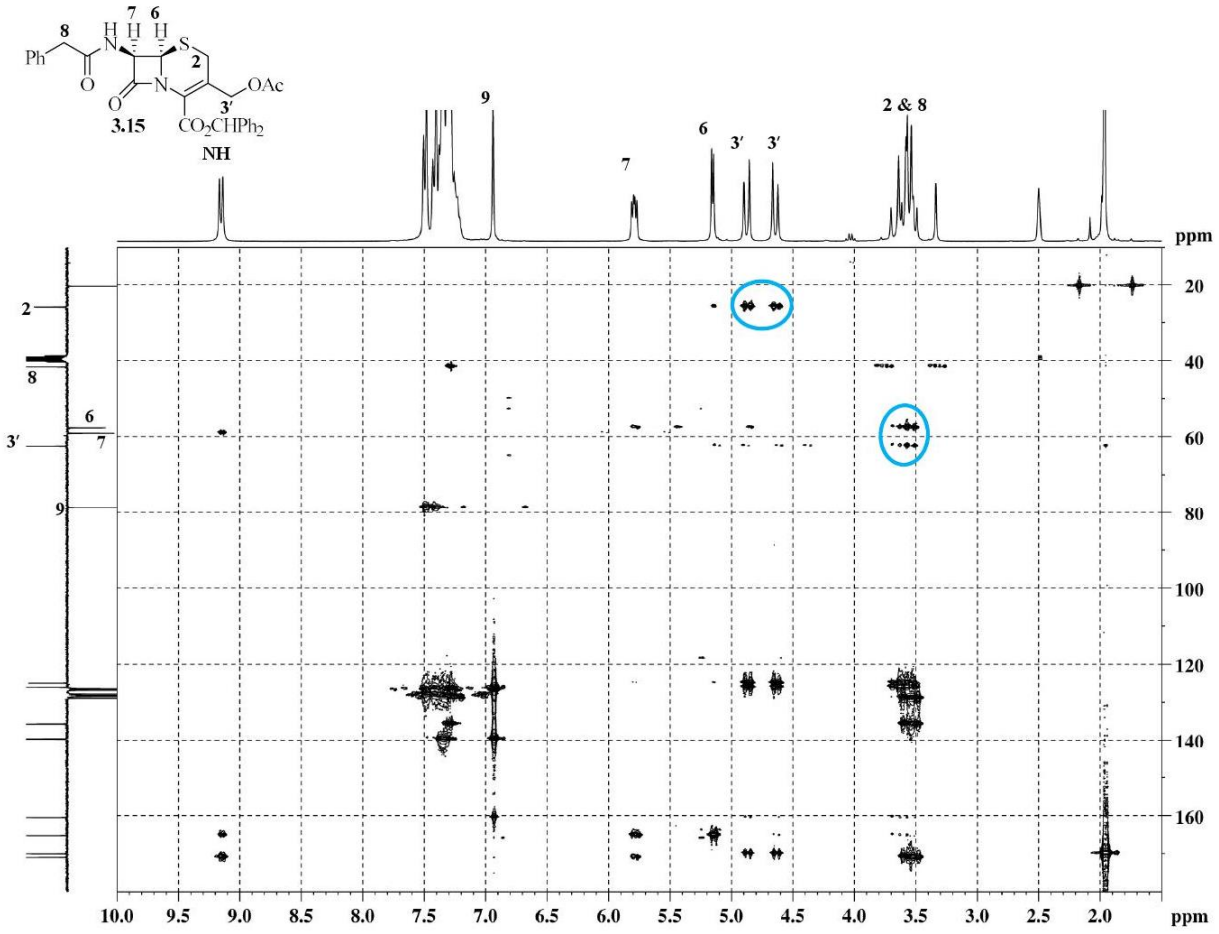


Figure 3.11 [A]: ^{1}H NMR spectrum of 3.16

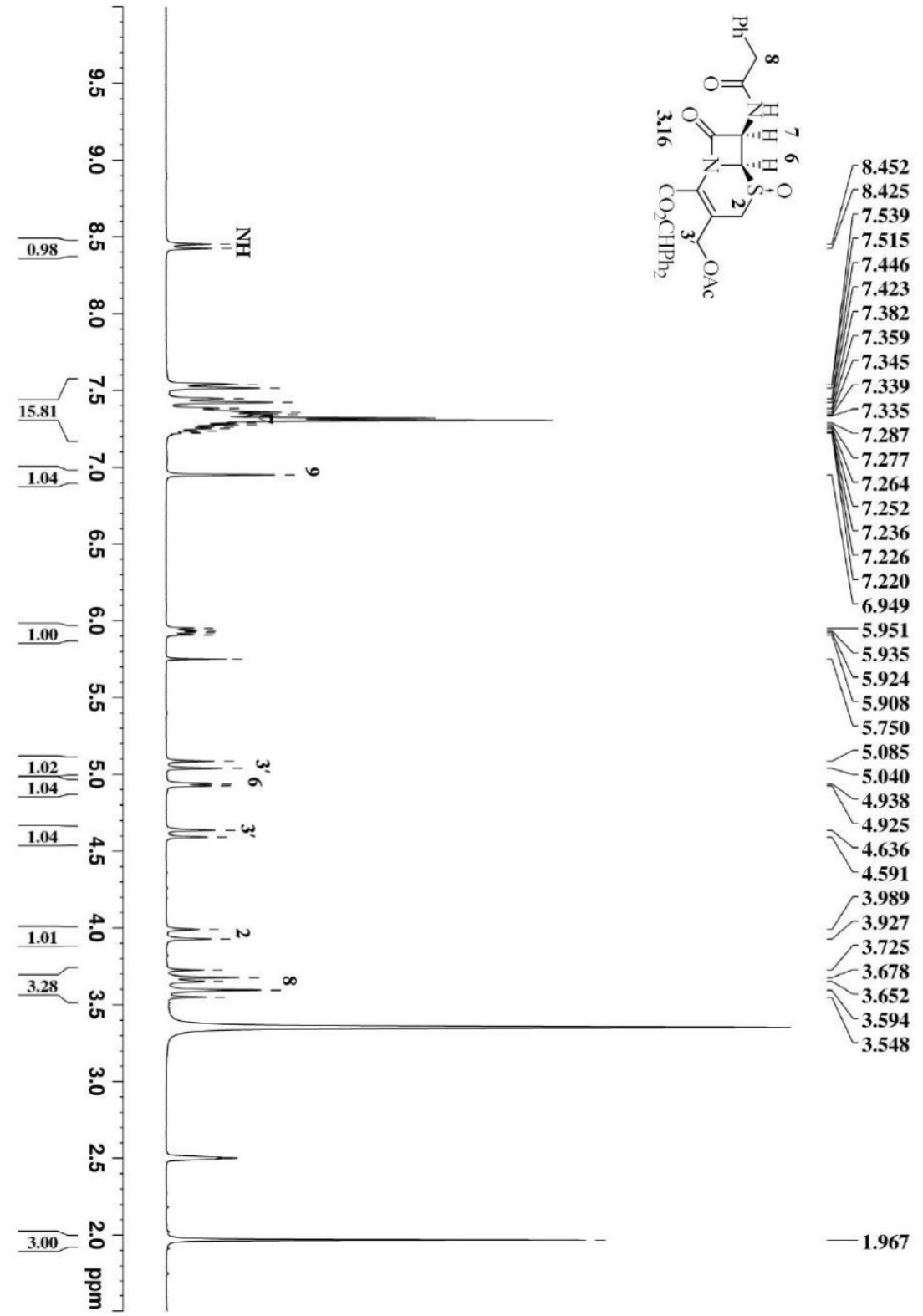


Figure 3.11 [B]: ^{13}C NMR spectrum of 3.16

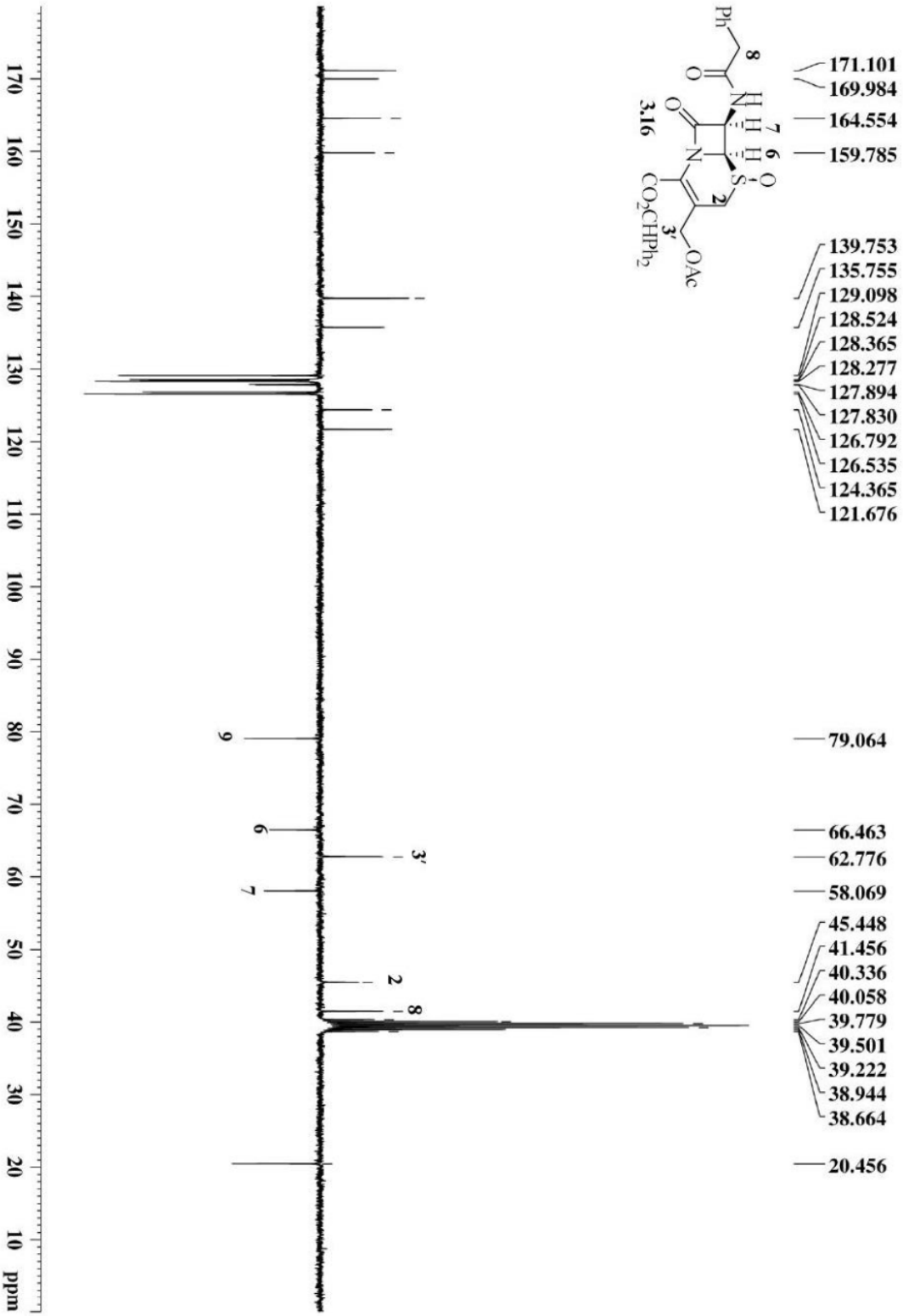


Figure 3.11 [C]: COSY spectrum of 3.16

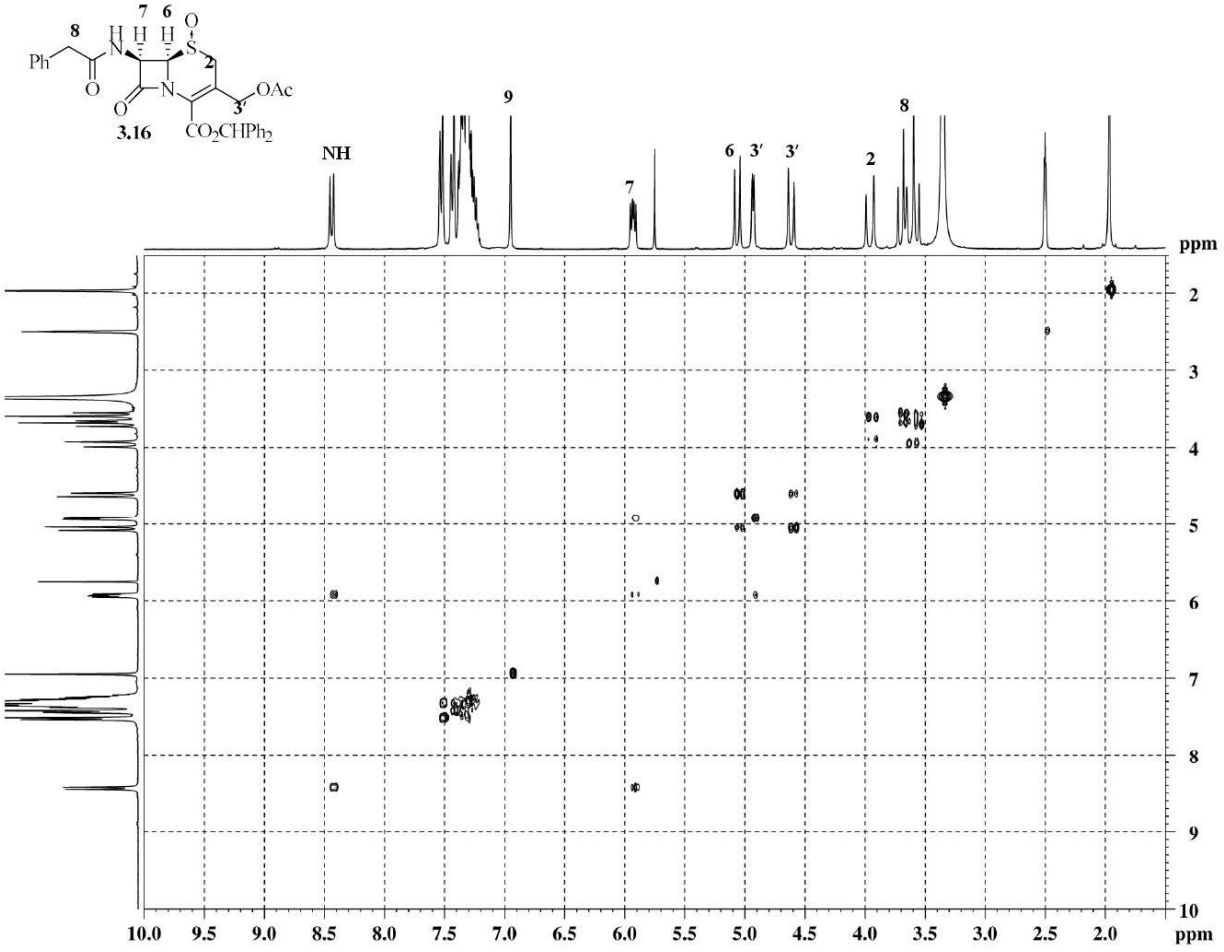


Figure 3.11 [D]: HMBC spectrum of 3.16

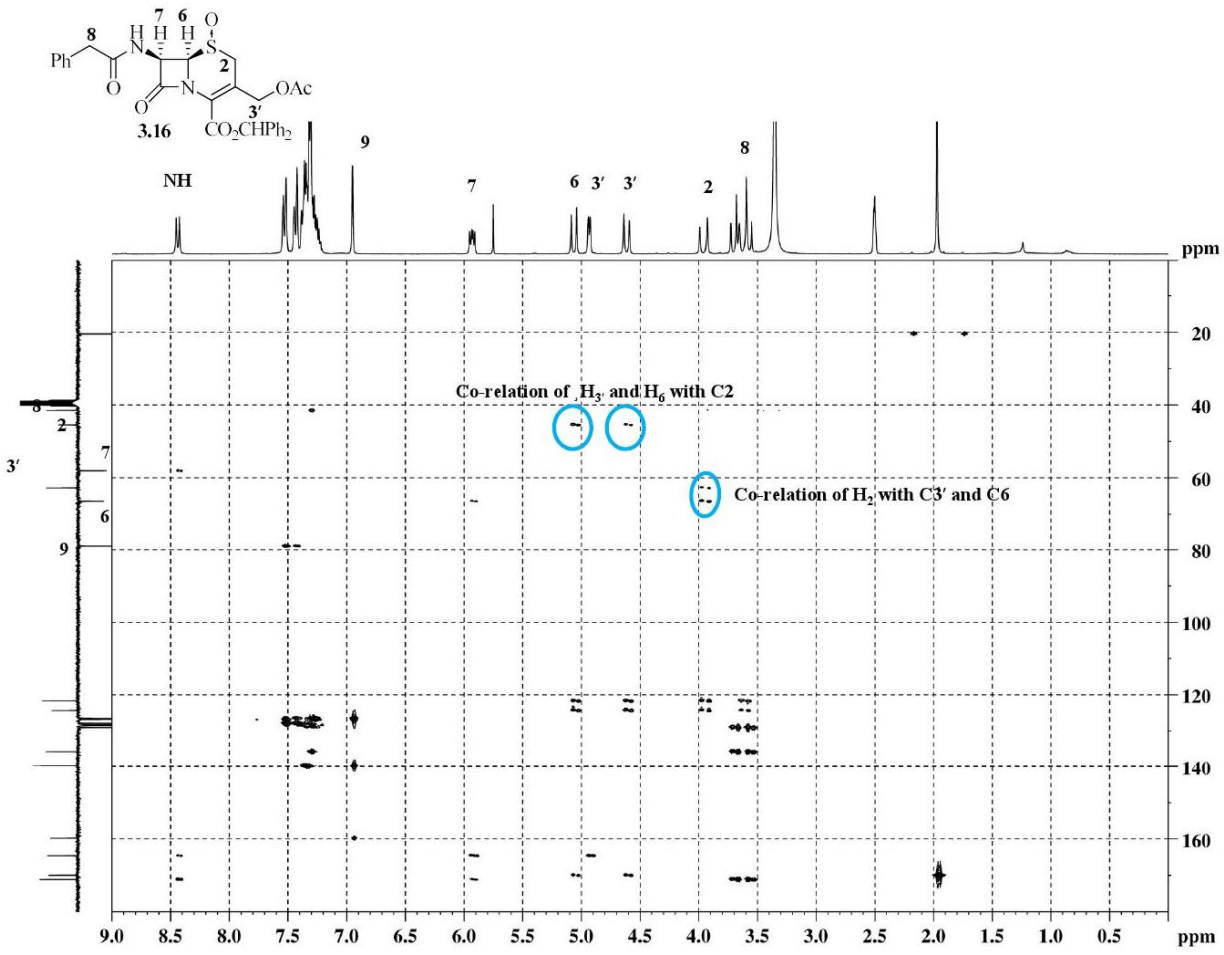


Figure 3.12: ^{1}H NMR spectrum of 3.23

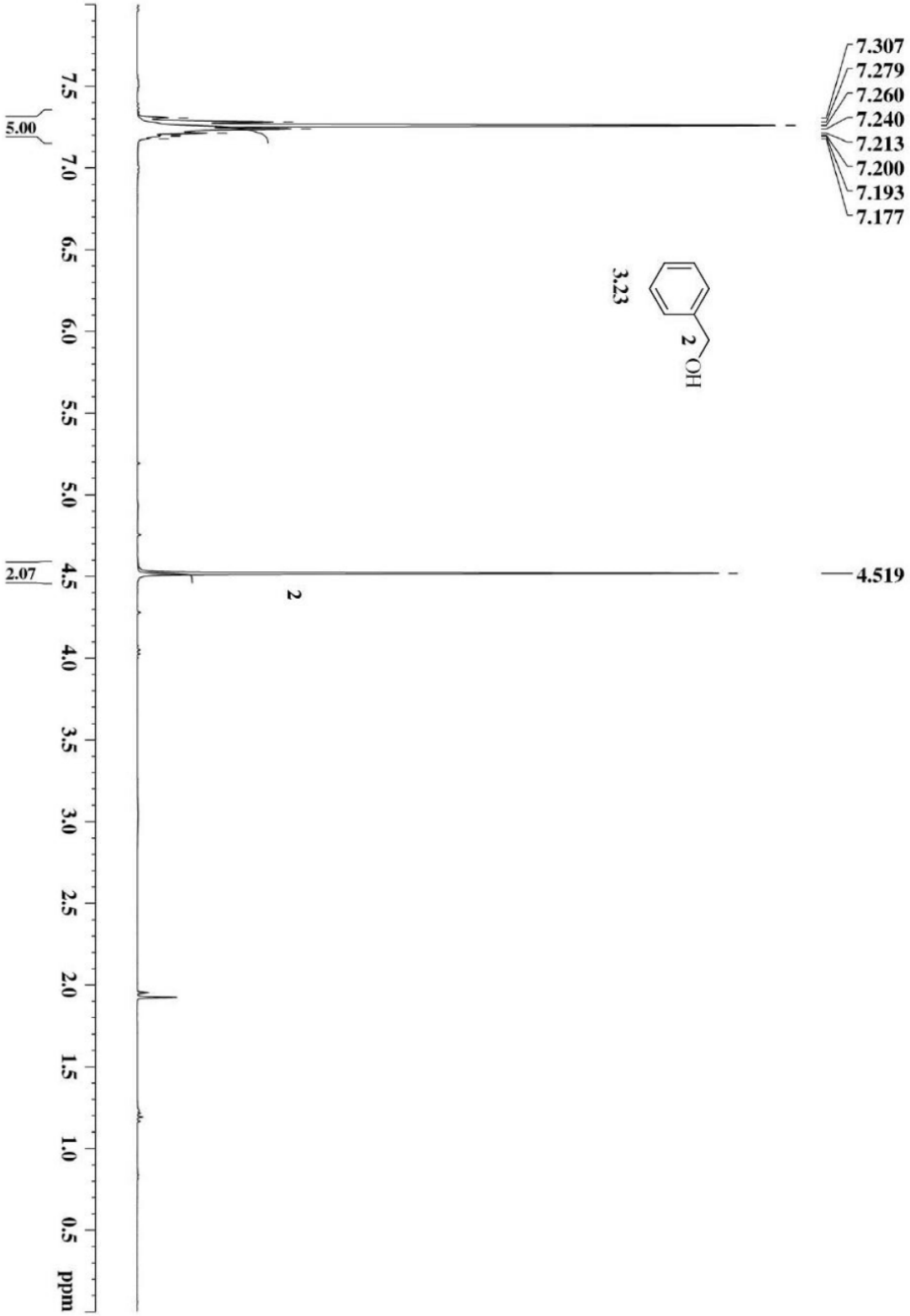


Figure 3.13 [A]: ^{1}H NMR spectrum of 3.24

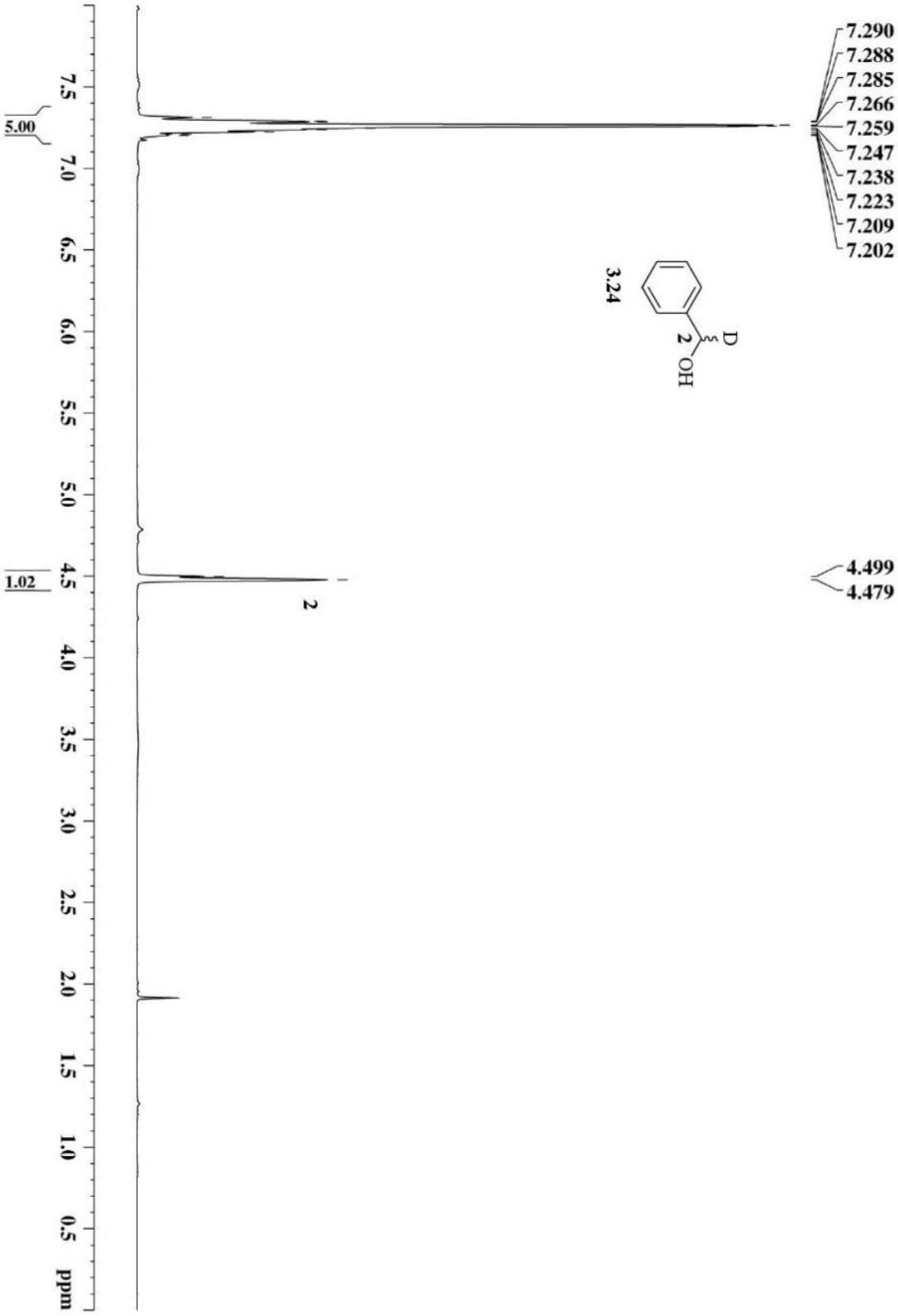


Figure 3.13 [B]: ^{13}C NMR spectrum of 3.24

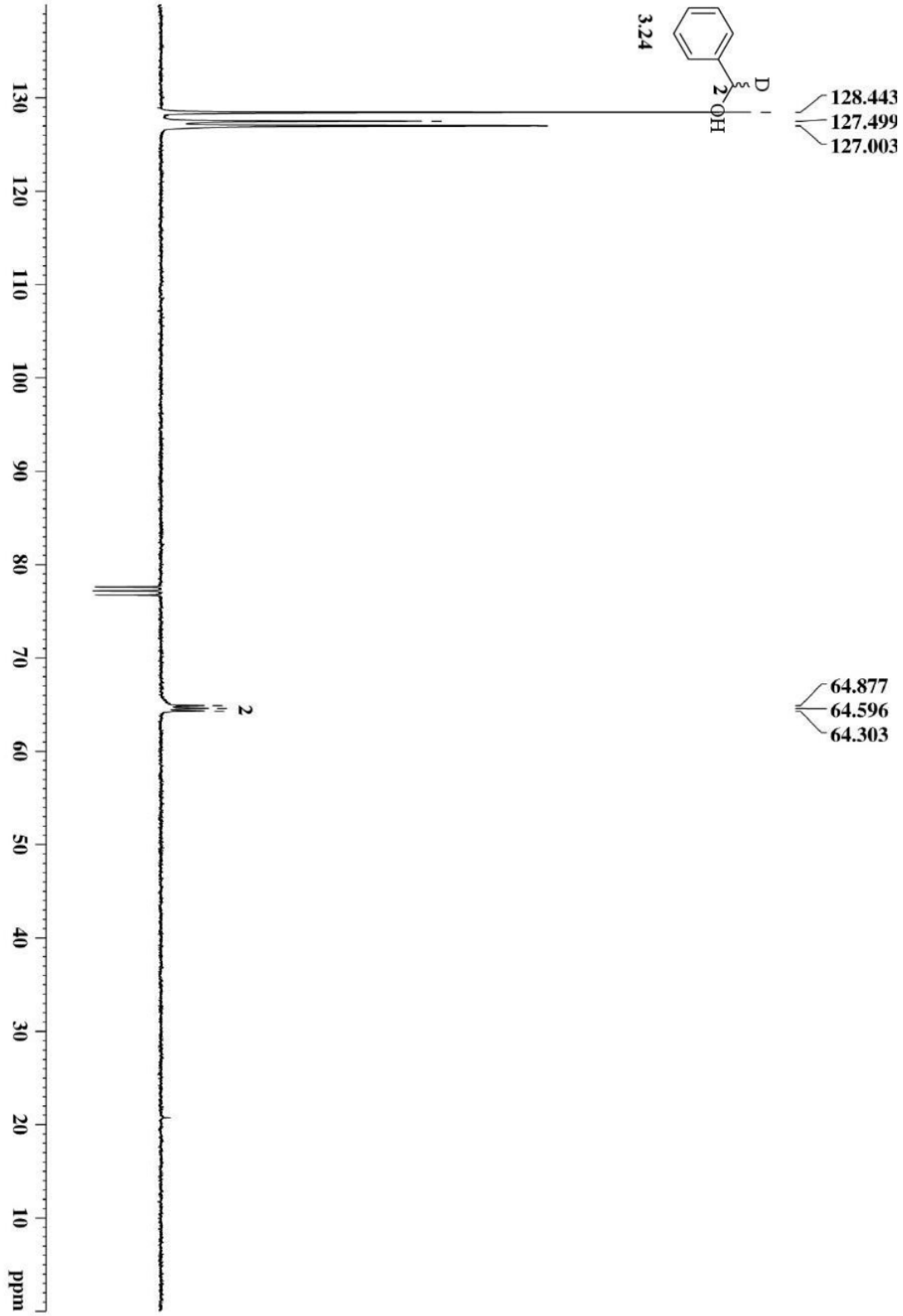


Figure 3.14: ^{1}H NMR spectrum of 3.27

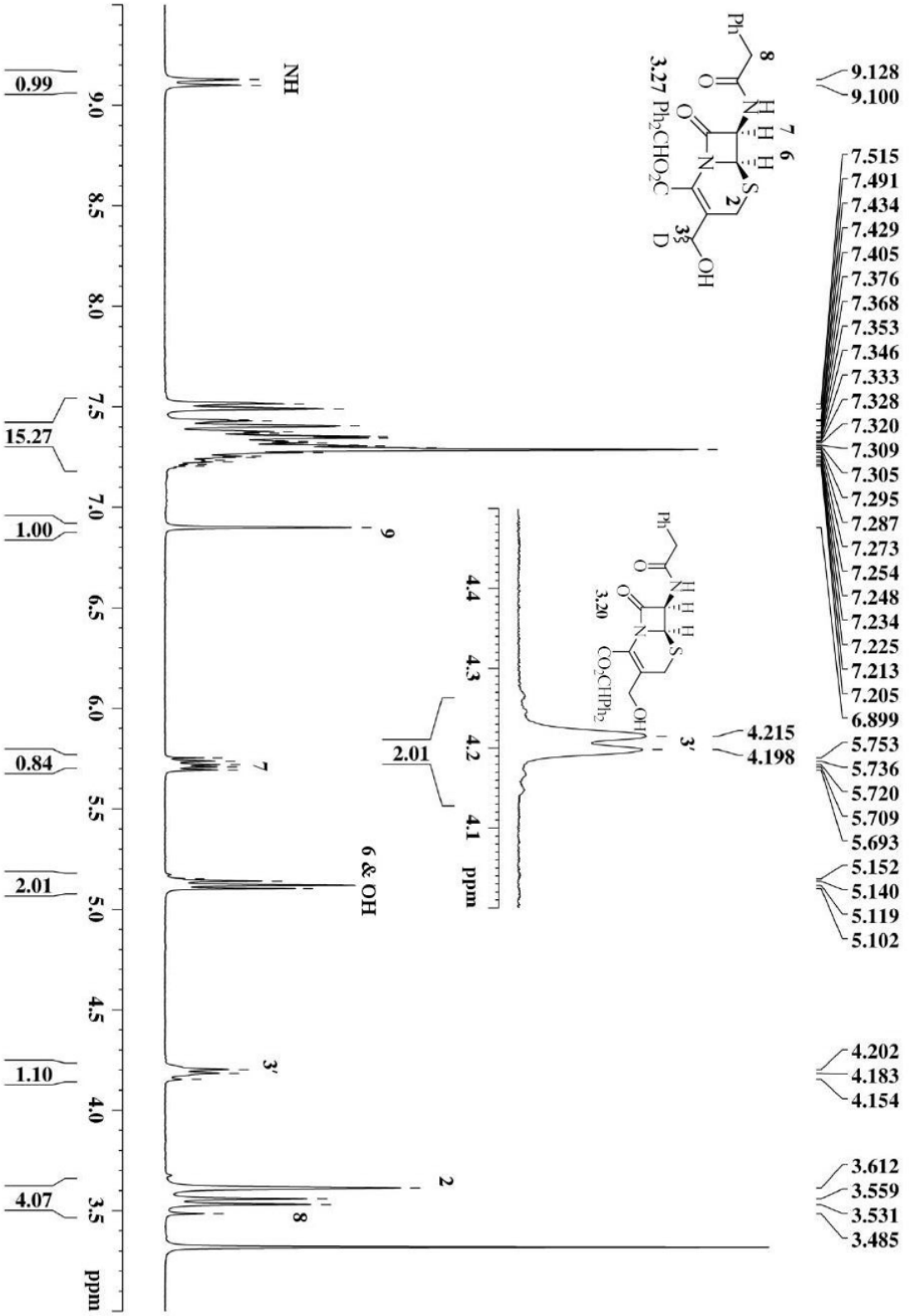


Figure 3.15 [A]: ^{1}H NMR spectrum of 3.28

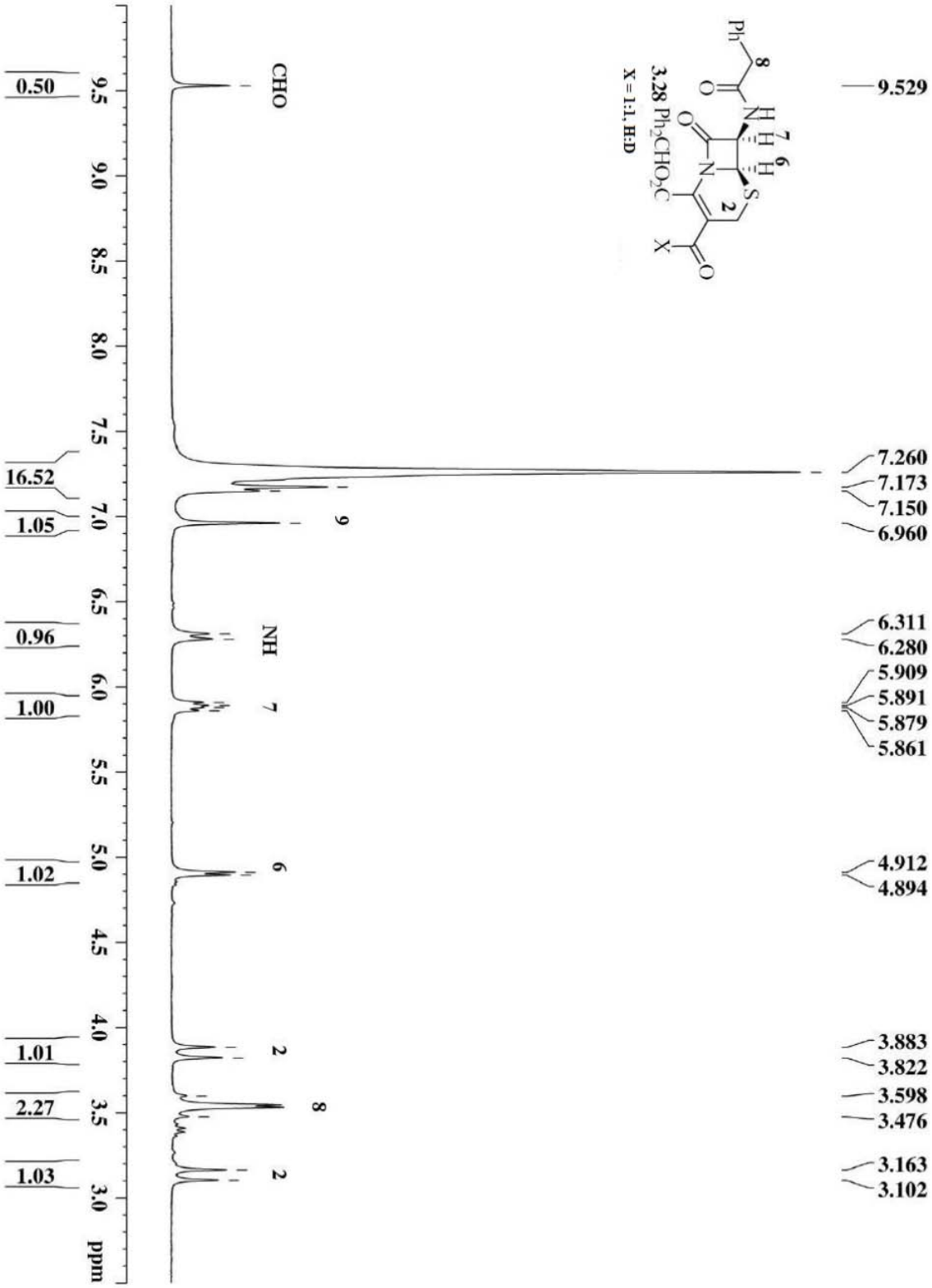


Figure 3.15 [B]: ^{13}C NMR spectrum of 3.28

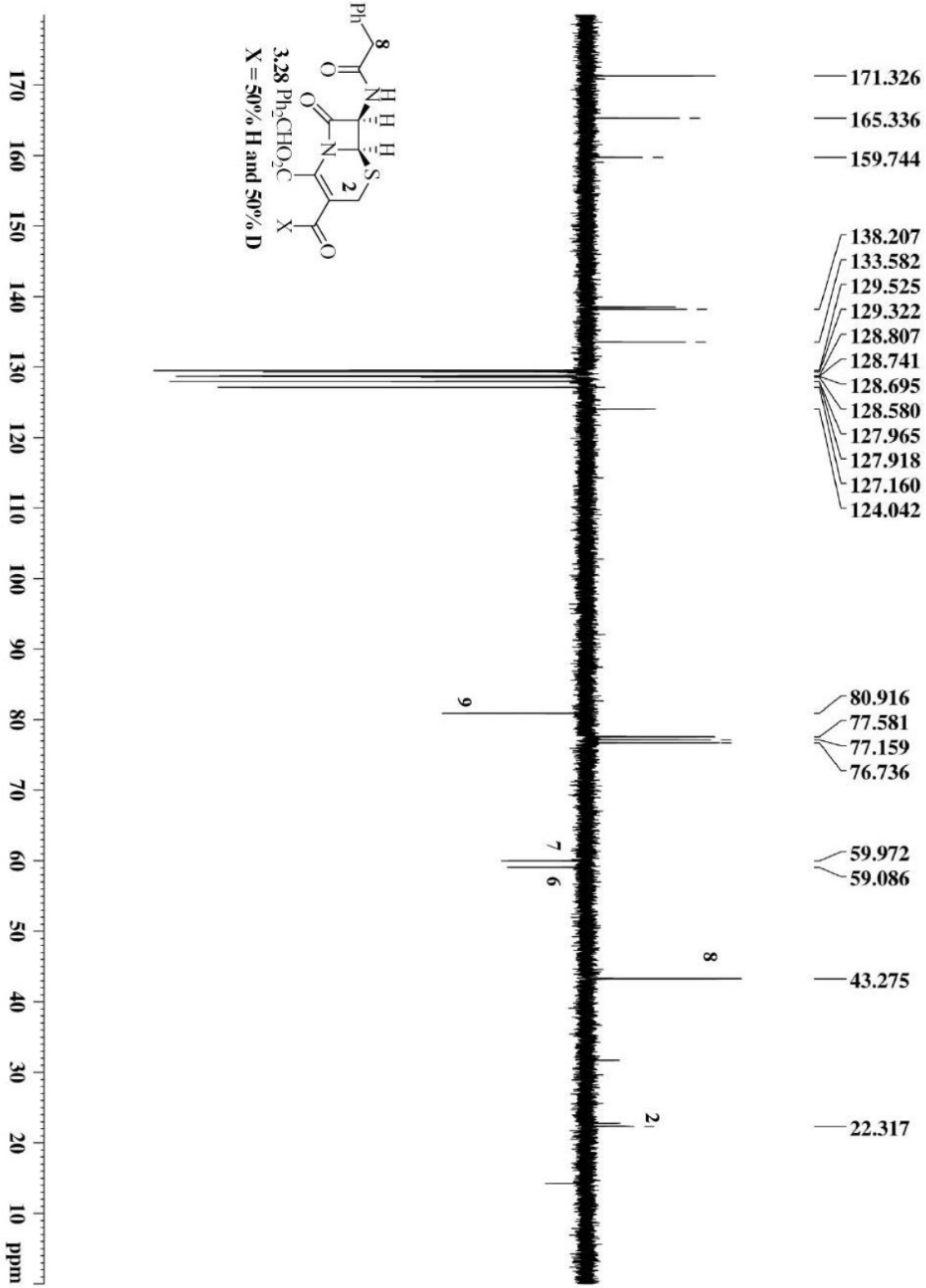


Figure 3.16: ^{1}H NMR spectrum of 3.17 (Scheme 3.17)

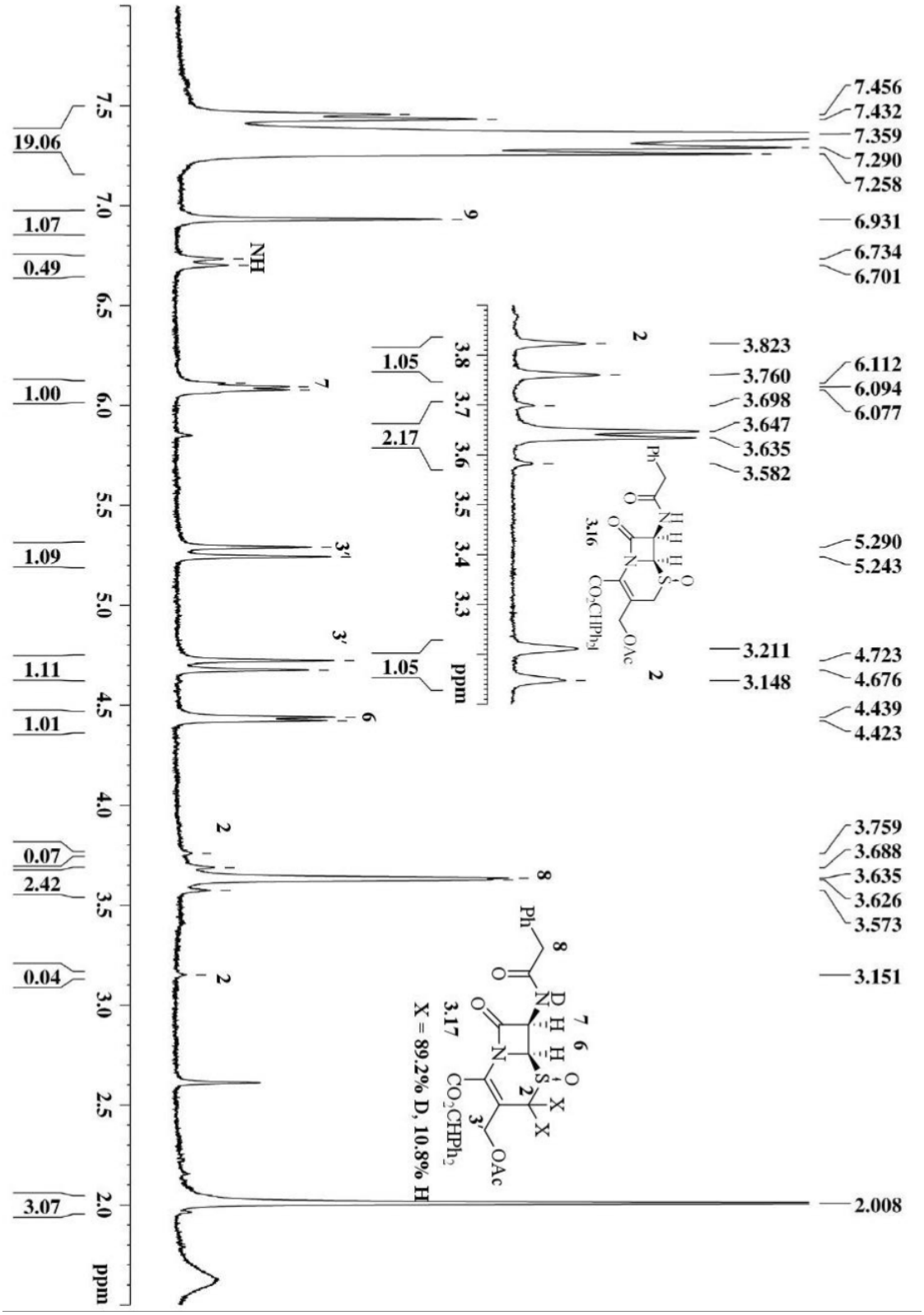


Figure 3.17 [A]: ^{1}H NMR spectrum of 3.44 in DMSO - d_6 (Scheme 3.17)

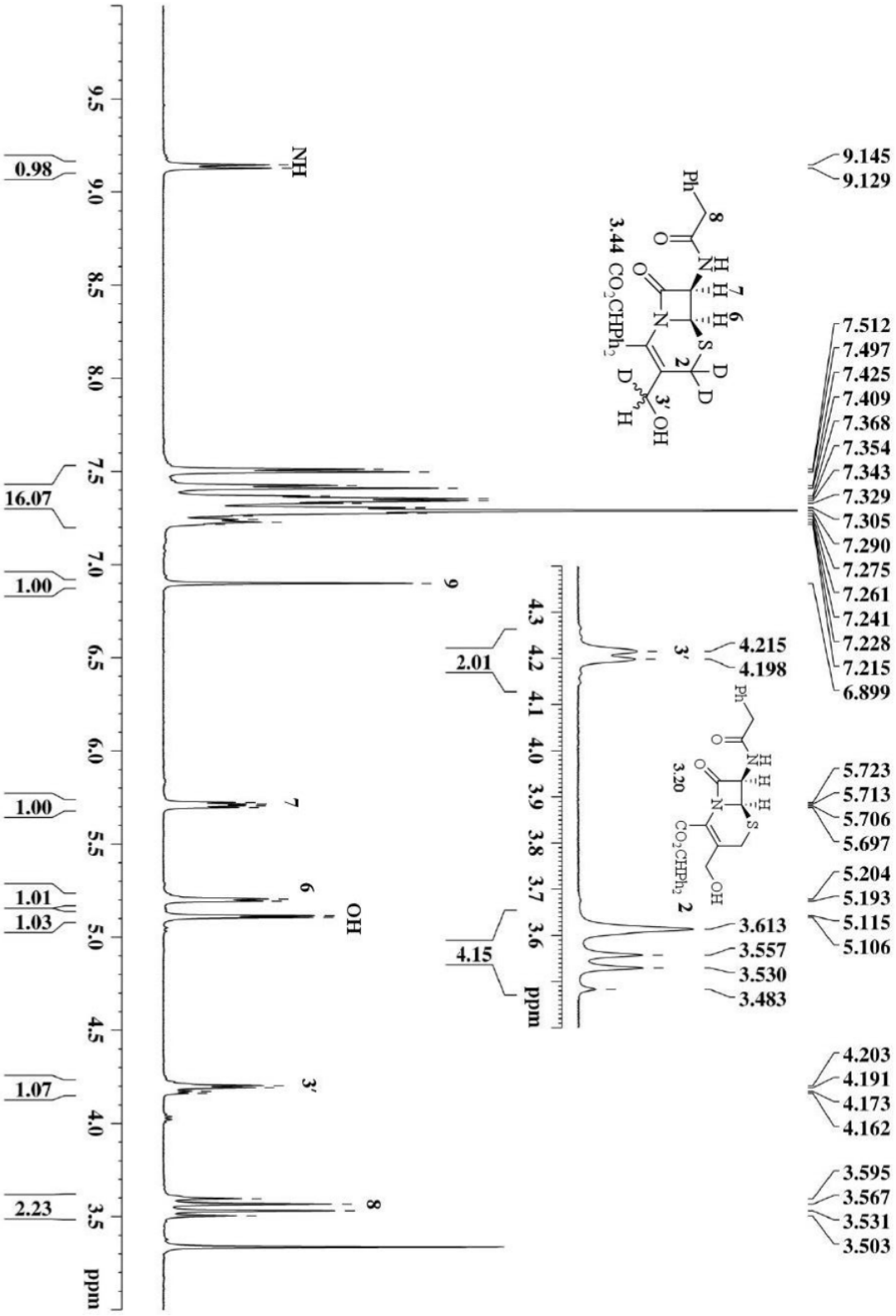


Figure 3.17 [B]: ^{1}H NMR spectrum of 3.44 in Acetone - d_6 (Scheme 3.17)

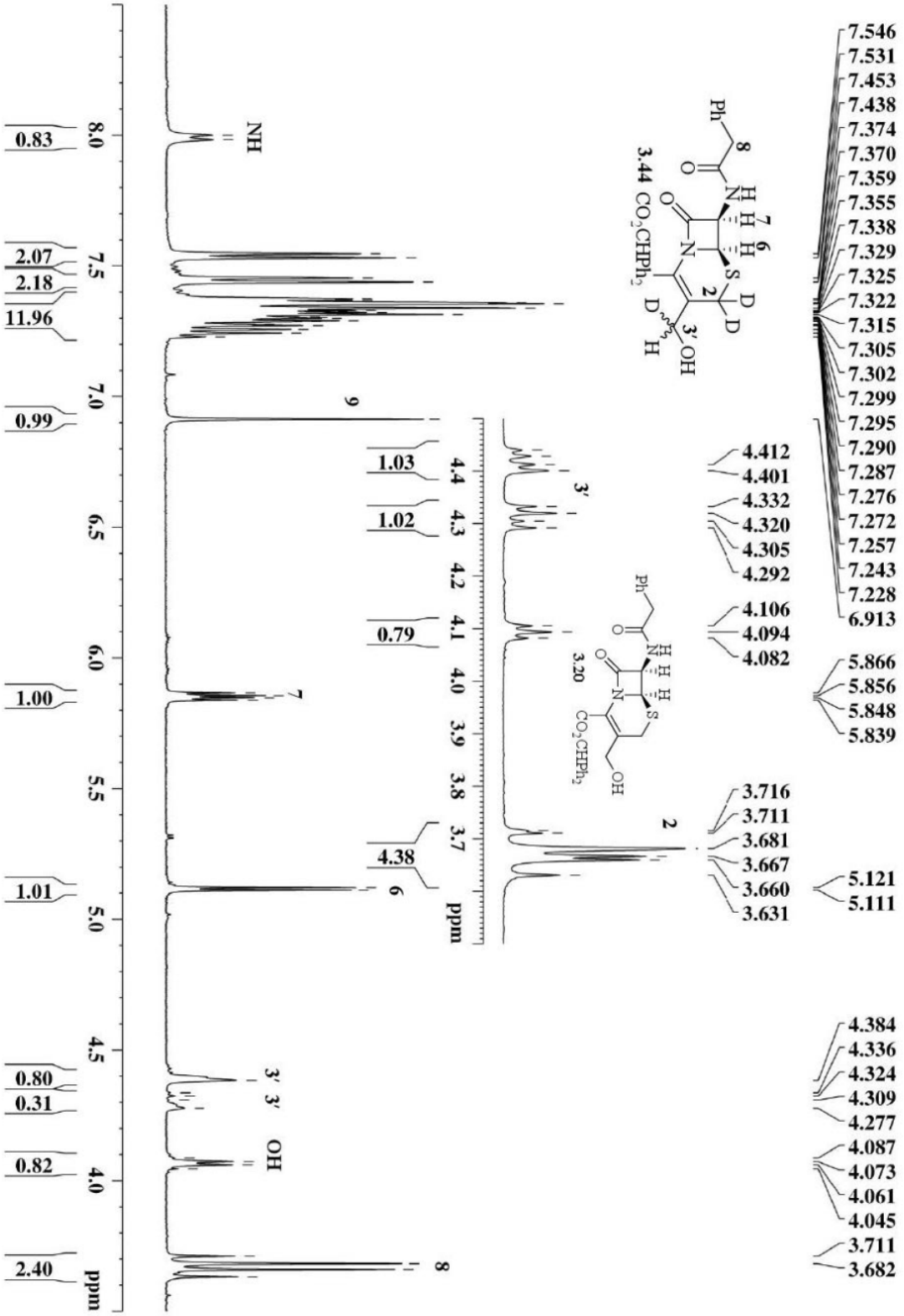


Figure 3.18 [A]: ^{1}H NMR spectrum of 3.46

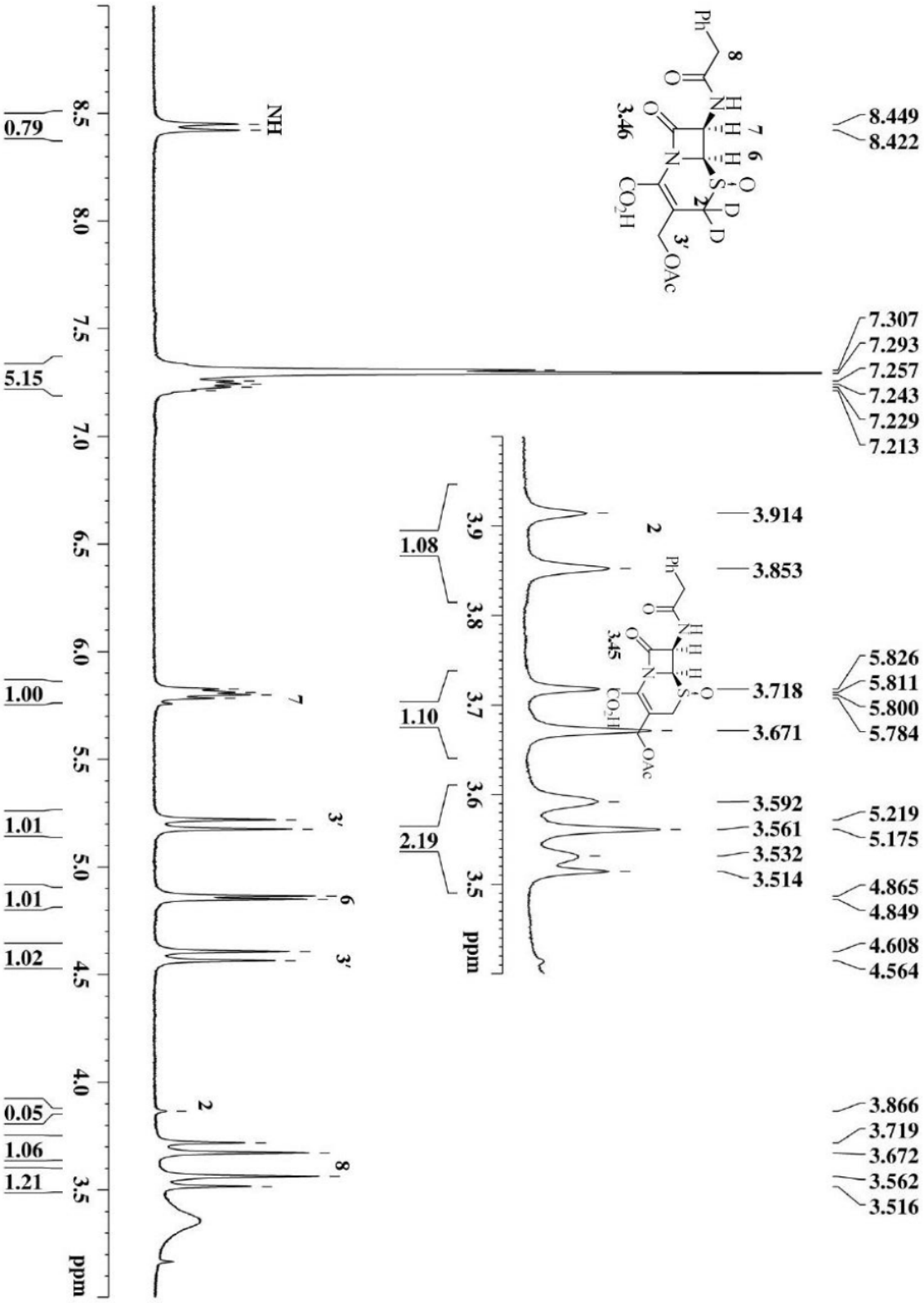


Figure 3.18 [B]: ^{13}C NMR spectrum of 3.46

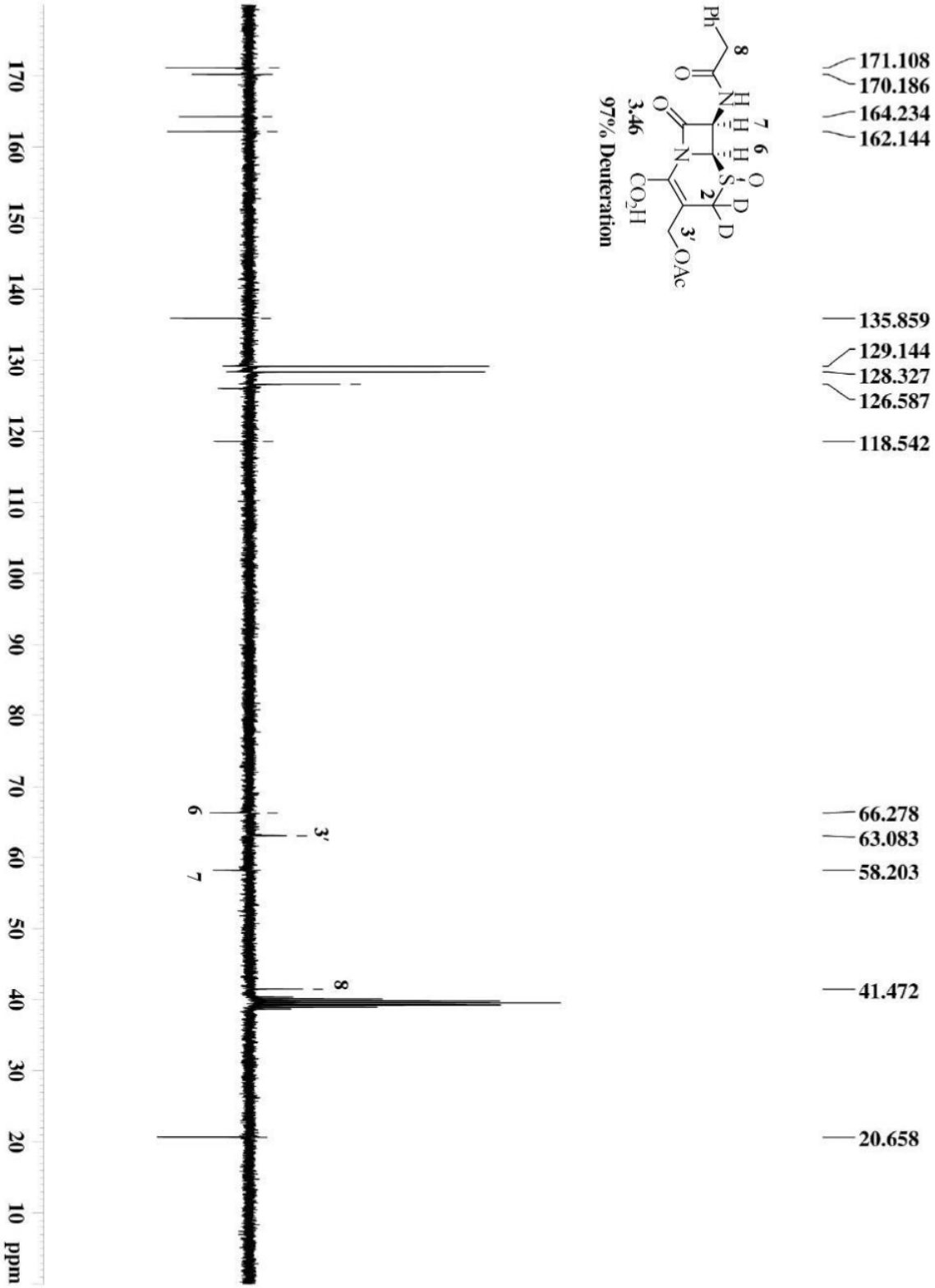


Figure 3.19 [A]: ^{1}H NMR spectrum of 3.40 (Scheme 3.18)

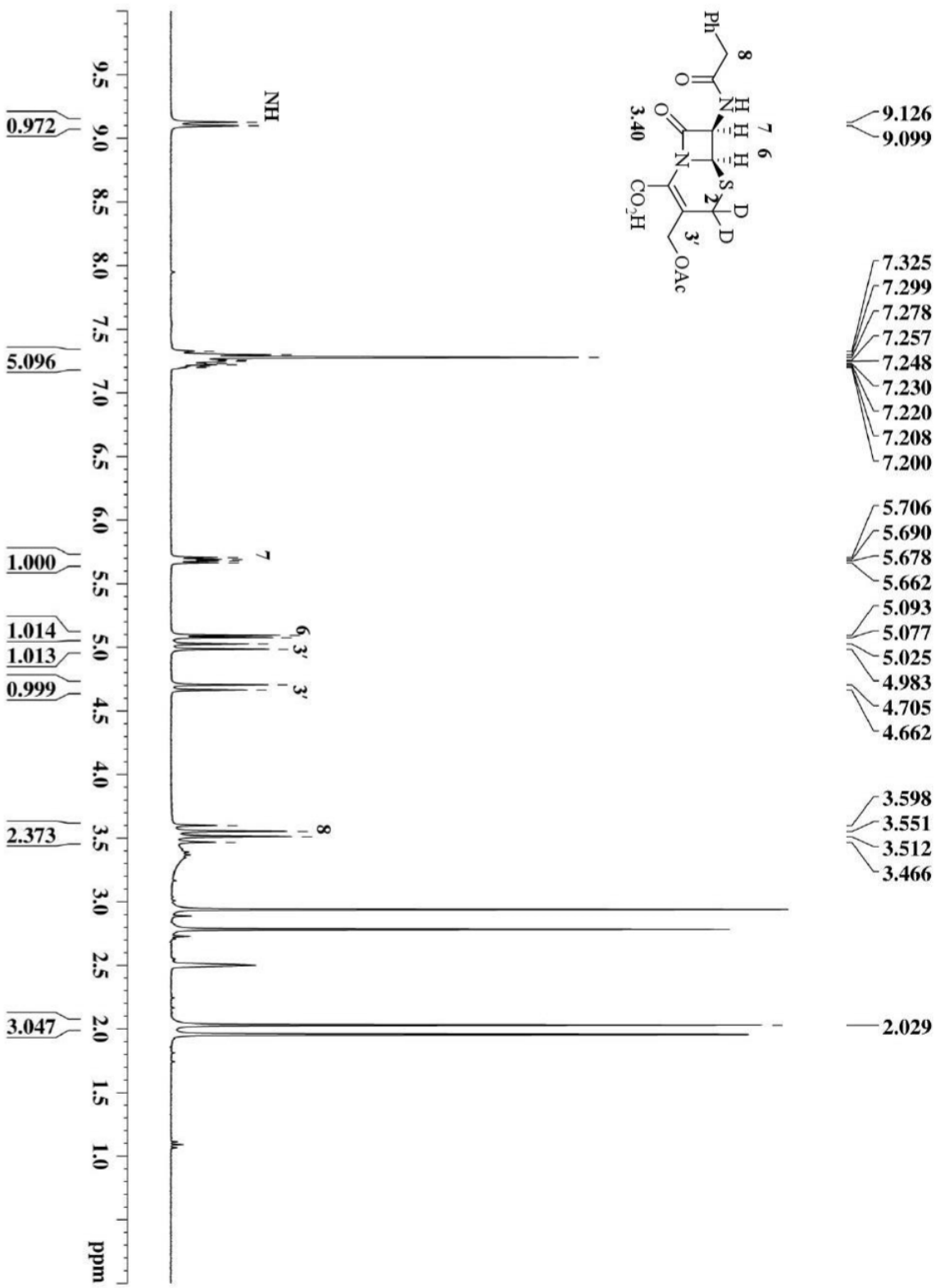


Figure 3.19 [B]: Mass spectrum of 3.40

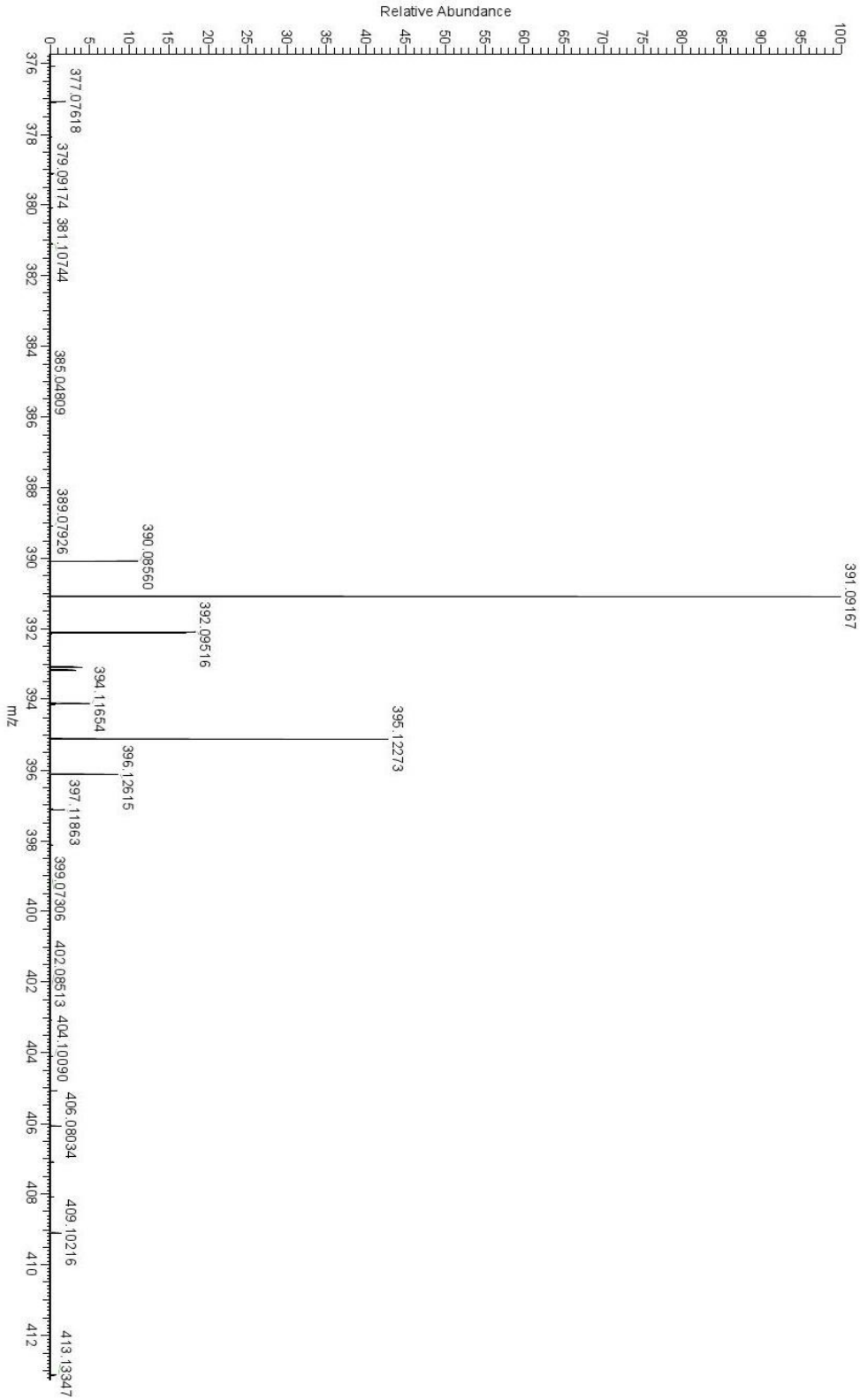


Figure 3.20 [A]: ^{1}H NMR spectrum of 3.44 in Benzene-d_6 and drop of DMSO - d_6 (Scheme 3.18)

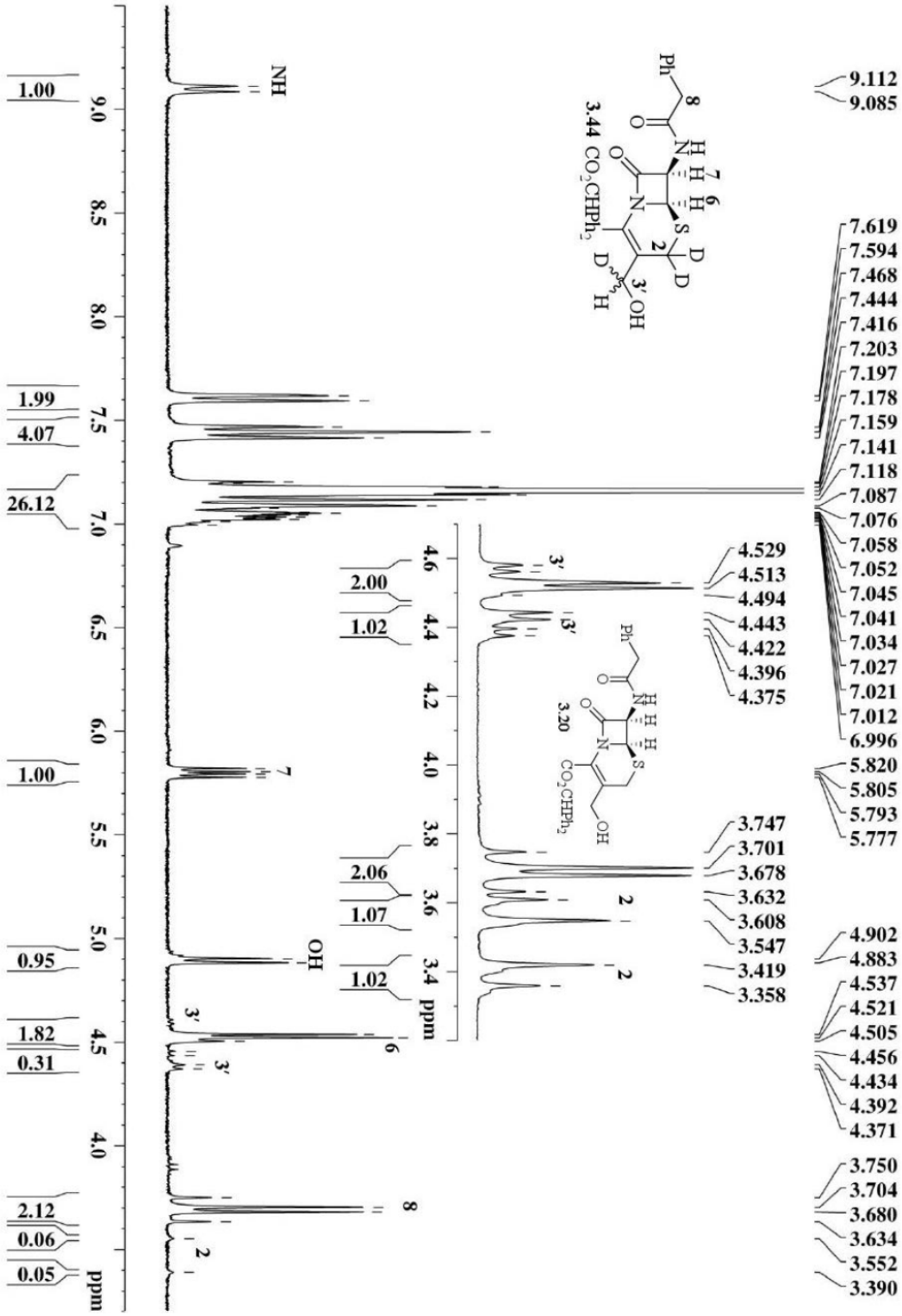


Figure 3.20 [B]: ^{1}H NMR spectrum of 3.44 in Acetone - d_6 (Scheme 3.18)

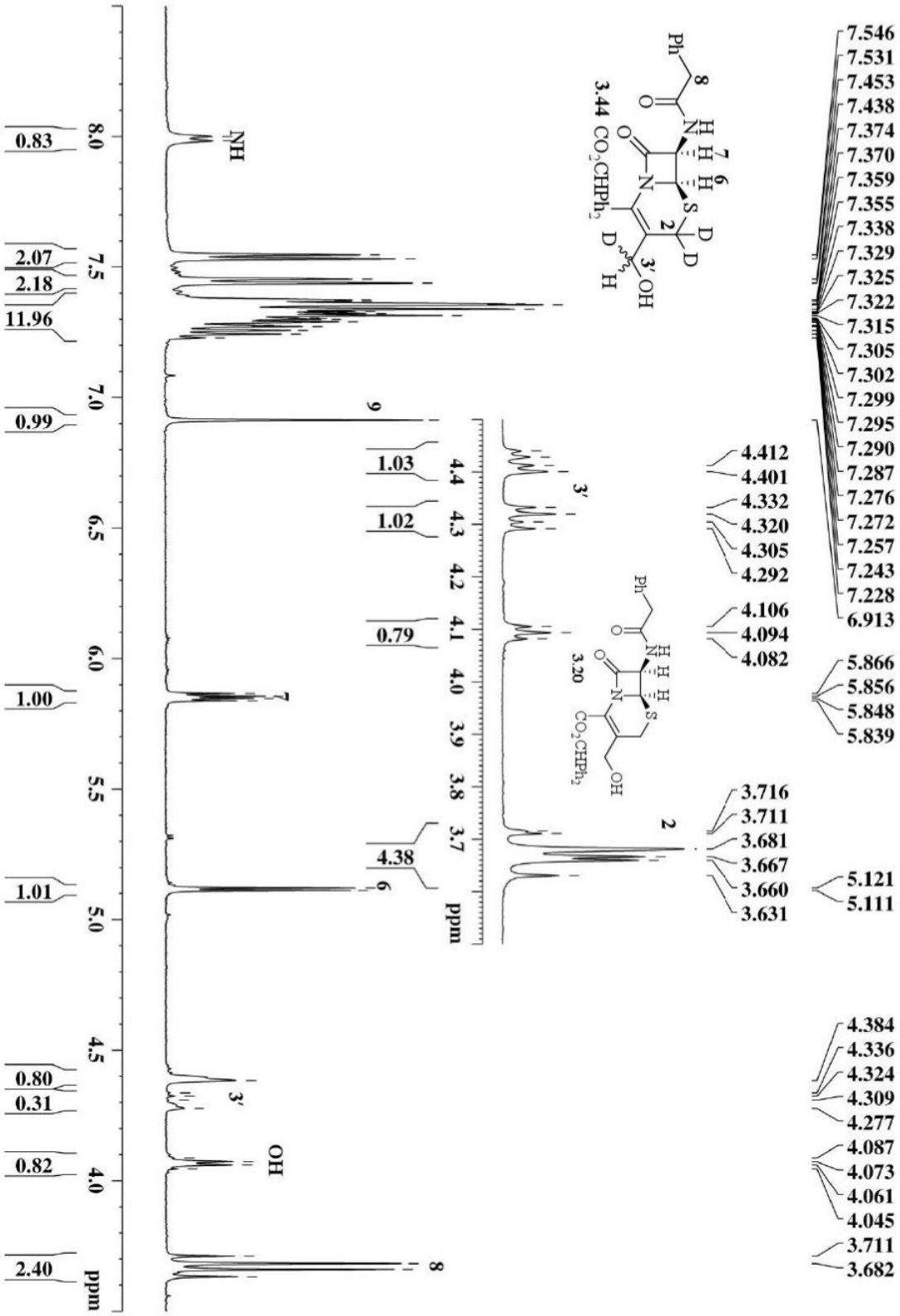


Figure 3.20 [C]: ^{13}C NMR spectrum of 3.44 (Scheme 3.18)

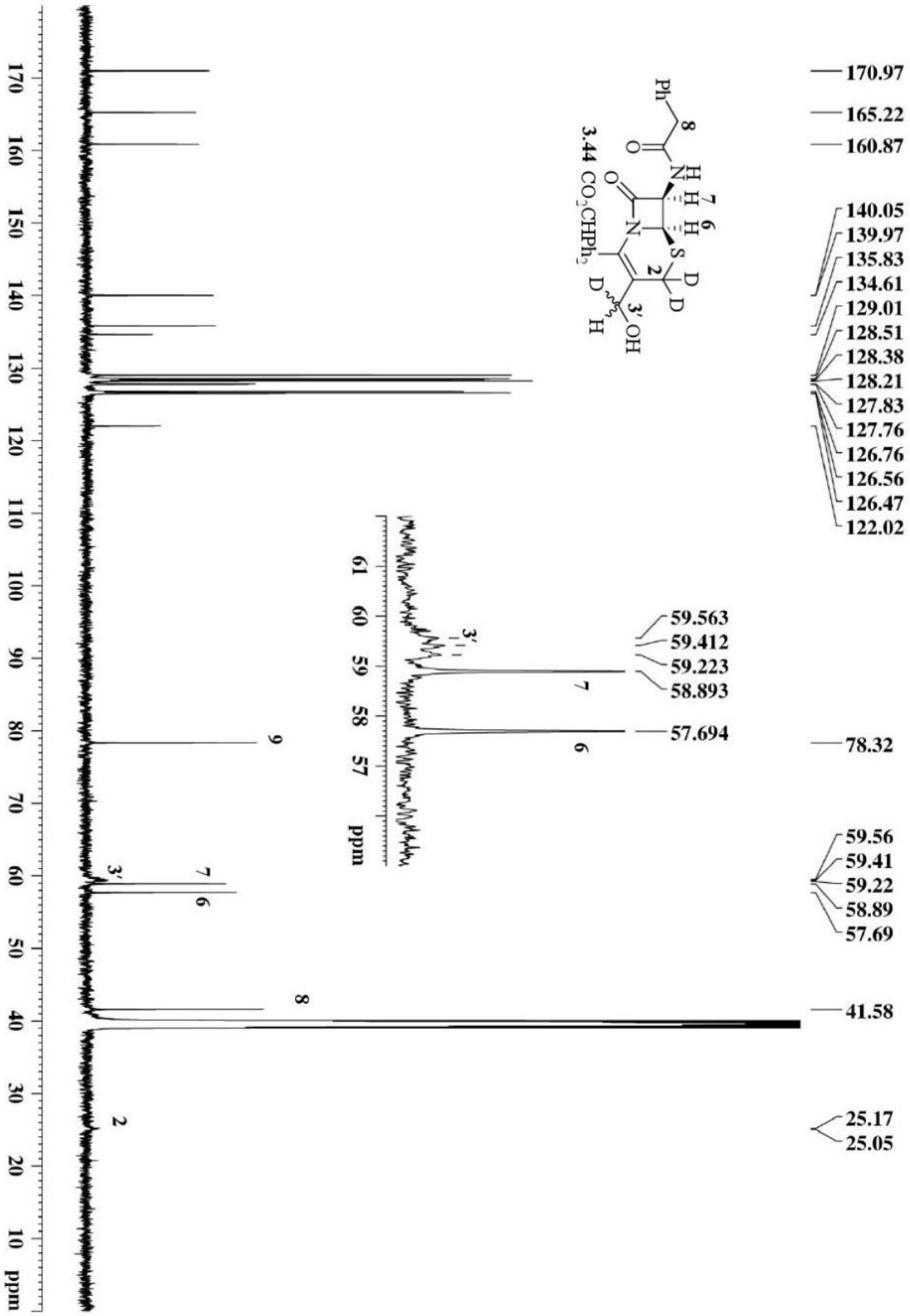
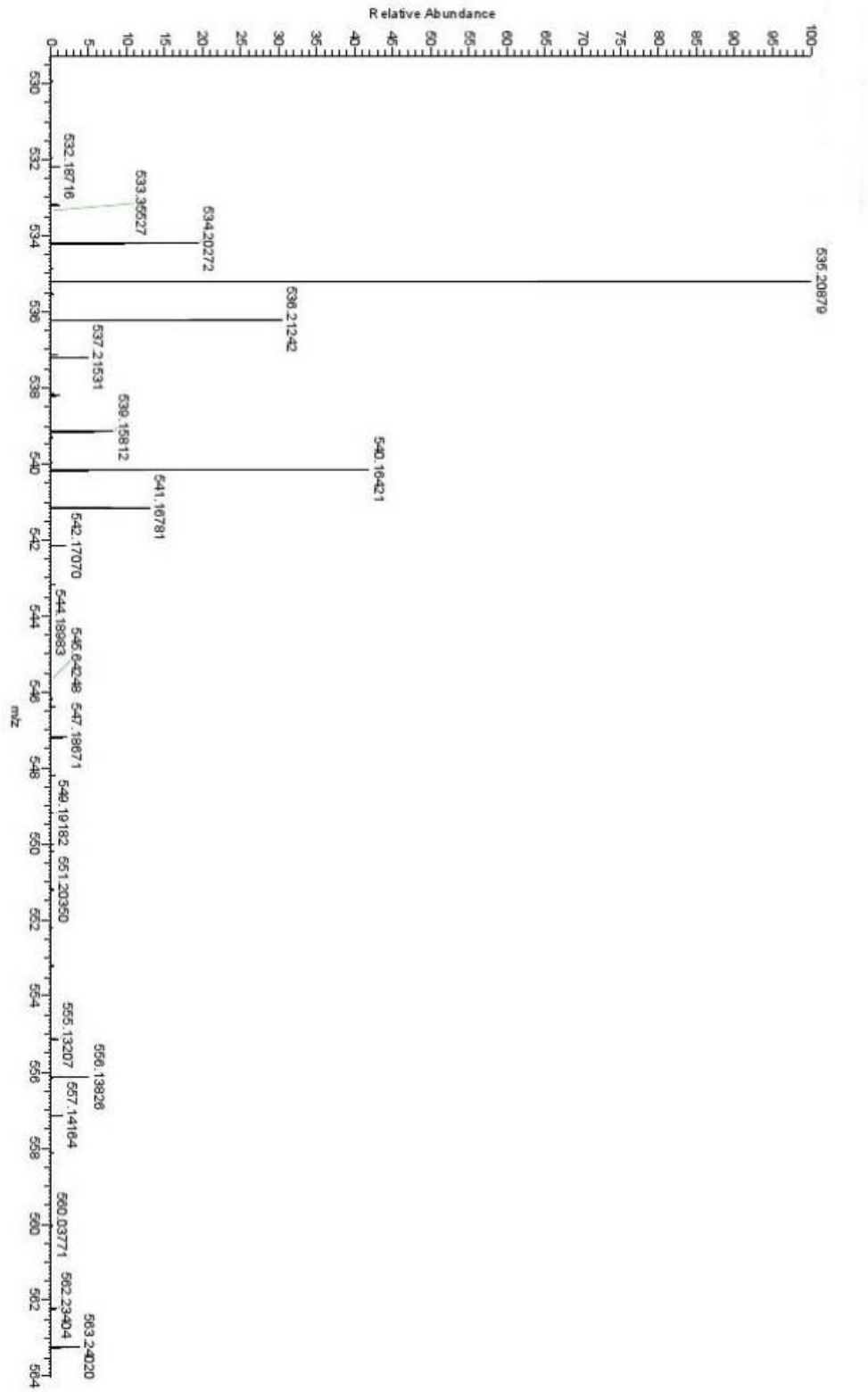


Figure 3.20 [D]: Mass spectrum of 3.44 (Scheme 3.18)



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