Design, Synthesis & Biological Activity of Novel Protein Tyrosine Phosphatase (PTP) Mimetics

Sridhar Reddy Kaulagari

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Design, Synthesis & Biological Activity of Novel Protein Tyrosine Phosphatase (PTP) Mimetics

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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DEDICATION

To my grand father late Shri Ramreddy Kaulagari
To my mom Lalitha and dad Anjireddy
To my wife Sandhya and sisters Sukanya and Sudha
To my mentor Mark and all my teachers
To all my good friends and Well wishers
ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF FIGURES........................................................................................................ iv

LIST OF SCHEMES ..................................................................................................... vi

LIST OF TABLES .......................................................................................................... viii

LIST OF SYMBOLS AND ABBREVIATIONS............................................................. ix

ABSTRACT .................................................................................................................. xiii

CHAPTER ONE: PROTEIN PHOSPHATASE INHIBITORS ......................... 1

1.1 General introduction to phosphorylation................................. 1
1.2 Protein phosphorylation................................................................. 3
1.3 Occurrence of protein phosphorylation................................. 5
1.4 Protein phosphatases and their importance ....................... 6
1.5 Protein tyrosine phosphatases (PTPs)................................. 7
1.6 Phosphotyrosine mimetics ......................................................... 9
1.7 Phosphatase inhibitors ............................................................... 10
1.8 Challenges in designing a potent and specific phosphatase inhibitor................................. 12
1.9 PTP1B as an exciting target for therapeutic discovery .... 14
1.10 PTP1B mechanism of action ................................................... 15
CHAPTER TWO: SYNTHESIS OF NOVEL 2-AMINOPYRIMIDINE CHLORIDES, SULFONAMIDES AND ITS AMINO ACID ANALOG ................................................................. 28

2.1 General introduction ........................................................................ 28
2.2 Results and discussion .................................................................... 32
2.3 Biological activity studies ............................................................... 38
2.4 Conclusion ..................................................................................... 38
2.5 Experimental procedures ............................................................... 39
   2.5.1 General ................................................................................... 39
2.6 References ..................................................................................... 59

CHAPTER THREE: SYNTHESIS OF ARYL-1,2-EPOXY CARBOXYLATES AND PHOSPHATES ................................................................. 66

3.1 General introduction ........................................................................ 66
3.2 Phosphonates as tyrosine phosphatase inhibitors ...................... 68
3.3 Carboxylates as tyrosine phosphatase inhibitors ...................... 68
3.4 Results and discussion .................................................................... 69
   3.4.1 Synthesis of α-aryl α,β-epoxy phosphonates .............. 69
   3.4.2 Synthesis of α-aryl-α,β-epoxy carboxylates .............. 70
   3.4.3 Synthesis of α,β-aziridino carboxylates .................... 73
3.5 Biological activity studies ............................................................... 76
3.6 Conclusion and future directions ................................................... 77
CHAPTER FOUR: SYNTHESIS OF STANDARD AND CYSTEINE BASED PEPTIDE NUCLEIC ACID (CPNA) MONOMERS AND OLIGOMERS ................................................................. 102

4.1 General introduction ................................................................ 102

4.2 Results and discussion ......................................................... 107

4.2.1 Synthesis of standard PNA monomers ......................... 107

4.2.2 Synthesis of CPNA monomers ....................................... 109

4.2.3 Synthesis of novel polyether side chain ...................... 111

4.3 Solid phase synthesis of PNAs ........................................... 112

4.4 Solid phase synthesis results .............................................. 113

4.5 Conclusion ........................................................................ 114

4.6 Experimental procedures ................................................ 114

4.7 References ....................................................................... 140

CHAPTER FIVE: APPENDICES ..................................................... 136

Appendix A:-Selected $^1$H and $^{13}$C NMR spectra ................. 147

About the Author .................................................................... End Page
LIST OF FIGURES

Figure 1.1 Oxidative phosphorylation in the biosynthesis of ATP........... 2

Figure 1.2 Reversible phosphorylation on protein controlled by kinase and phosphatase enzymes ........................................ 3

Figure 1.3 Typical amino acid residues for phosphorylation............... 5

Figure 1.4 Leading causes of death in US (2009) in adults over 25 years ......................................................................................... 8

Figure 1.5 Phosphotyrosine and commonly used pTyr mimetics ..... 10

Figure 1.6 Some representative phosphatase inhibitors ............... 11

Figure 1.7 Activity and selectivity of some prominent inhibitors..... 13

Figure 1.8 PTP1B mechanism of action ....................................... 16

Figure 1.9 Proposed pyrimidine based phosphatase inhibitors .... 17

Figure 1.10 Proposed epoxy and aziridine based phosphatase inhibitors................................................................. 17

Figure 2.1 Some important drugs with sulfonamide functional groups................................................................. 30

Figure 2.2 Proposed 2-aminopyrimidine derivatives as novel PTP1B inhibitors ......................................................... 31

Figure 3.1 Conversion of pyruvate to lactate in respiratory cycle .... 67
**Figure 3.2** Some of the phosphatase inhibitors with carboxy functionality ............................................................ 68

**Figure 4.1.a** DNA double helix structure-sugar phosphate backbone-adenine, thymine, guanine, cytosine bases ........................................................................................................... 103

**Figure 4.1.b** Purines and pyrimidines of nucleic acids, DNA and RNA ........................................................................................................... 104

**Figure 4.2** Structures of achiral PNA backbone and chiral DNA backbone ........................................................................................................... 105

**Figure 4.3** Proposed structure of cysteine based PNA (CPNA) ....... 106
LIST OF SCHEMES

**Scheme 2.1** Synthesis of 5-ethynyl-2-N-[(4-methoxybenzyl)(methanesulfonyl)]-2-aminopyrimidine 2.5 .............. 33

**Scheme 2.2** Synthesis of symmetrical bipyrimidine sulfonamide 2.8 .......................................................... 34

**Scheme 2.3** Synthesis of 5-iodo-2-N,N di tert butoxycarbonyl bipyrimidine 2.10 .................................................. 35

**Scheme 2.4** Synthesis of bipyrimidine sulfonyl chloride 2.15........... 36

**Scheme 2.5** Synthesis of N-benzyl-C-chloro-N-(5-iodo-pyrimidin-2-yl)-methanesulfonamide 2.18 ................................. 37

**Scheme 2.6** Synthesis of 3-[2-(Benzyl-chloromethanesulfonyl-amino)-pyrimidin-5-yl]-2-tert-butoxycarbonylamino-propionic acid 2.24 ................................................................. 38

**Scheme 3.1** Synthesis of α,β-epoxy phosphonate 3.4 .............. 69

**Scheme 3.2** Synthesis of α,β-epoxy phosphonic acid 3.6 ............ 70

**Scheme 3.3** Synthesis of α,β-epoxy α-aryl carboxylates 3.9a-i........ 71

**Scheme 3.4** Attempts of synthesis of epoxy carboxylate with methanal equivalents .................................................... 72

**Scheme 3.5** Synthesis of α,β-aziridino carboxylate 3.14............... 74

**Scheme 3.6** Synthesis of N-alkyl α,β-aziridino carboxylate 3.19 ..... 75
**Scheme 3.7** Synthesis of *N*-benzyl aziridine carboxylate 3.20 ........ 76

**Scheme 3.8** Synthesis of α,β-diaryl α-cyclopropane carboxylate 3.22 .......................................................... 76

**Scheme 4.1** Synthesis of Fmoc protected PNA monomers ..............108

**Scheme 4.2** Synthesis of Boc protected PNA monomers ...............109

**Scheme 4.3** Synthesis of novel cysteine PNA monomers ..............110

**Scheme 4.4** Synthesis of novel polyether side chain ....................111
LIST OF TABLES

Table 4.1 Solid phase synthesis of PNAs.................................113
LIST OF SYMBOLS AND ABBREVIATIONS

1° = Primary
2° = Secondary
3° = Tertiary
3D = Three dimensional
Å = ångström
AA = Amino acid
Ac = Acetyl
Ar = Aryl
ADP = Adenosine diphosphate
ATP = Adenosine triphosphate
Boc = tert-Butoxycarbonyl
Bn = Benzyl
Bt = Benzotriazole
°C = degrees Celsius
13C = carbon 13
Calcd = calculated
Cbz = Benzyloxycarbonyl
CDI = Carbonyldiimidazole
Cys = Cysteine
DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC = 1,3-Dicyclohexylcarbodiimide
DCM = Dichloromethane
DIBAL = Diisobutyl aluminum hydride
DIEA = Diisopropylethylamine
DMAP = 4-Dimethylaminopyridine
DME or 1,2-DME = Dimethoxyethane
DMF = $N,N$-Dimethylformamide
DMS = Dimethylsulfide
DMSO = Dimethylsulfoxide
dsDNA = double strand deoxyribonucleic acid
DSPs = Dual specific phosphatases
ESI MS = Electrospray ionization mass spectrometry
Fmoc = 9-Fluorenylmethoxycarbonyl
HATU = $O$-(7-azabenzotriazol-1yl)-1,1,3,3,tetramethyluron hexafluorophosphate
HIV = Human immunodeficiency virus
HOBt = 1-Hydroxybenzotriazole
HRMS = High resolution mass spectrum
IRs = Insulin receptors
IRSs = Insulin receptor substrates
LCMS = Liquid chromatography – mass spectrometry
NBS = \textit{N}-Bromosuccinamide
NMM = \textit{N}-Methylmorpholine
NMR = Nuclear magnetic resonance
Oxone\textsuperscript{®} = Potassium peroxymonosulfate
\(\rho\text{ABSA} = 4\text{-Acetamidobenzenesulfonyl azide}\)
Ph = Phenyl
PMB = 4-methoxybenzyl
PNA = Peptide nucleic acid
PPs = Protein phosphatases
PTKs = Protein tyrosine kinases
PTP1B = Protein tyrosine phosphatase 1B
PTPs = Protein tyrosine phosphatases
PTSA/p-TsOH = \(p\)-Toluenesulfonic acid
Py = Pyridine
R = Alkyl
RNA = Ribonucleic acid
SAR = Structure activity relationship
SHP-2 = SH2 domain-containing tyrosine phosphatase 2
TBAF = Tetrabutylammonium fluoride
TBAI = Tetrabutylammonium iodide
TEA = Triethylamine
TES = Triethlysilane
Tf = Trifluoromethanesulphonyl /Triflate
TFA = Trifluoroacetic acid
TFMSA = Trifluoromethanesulfonic acid
TMEDA = N,N,N',N'-Tetramethylethylenediamine
TMSCl = Chlorotrimethylsilane
ABSTRACT

Protein phosphorylation is a post translational modification of proteins in which a serine, a threonine or a tyrosine residue is phosphorylated by an enzyme, kinase. Phosphorylation of proteins is a reversible and very important regulatory mechanism that occurs in both prokaryotes and eukaryotes. Phosphorylation turns many protein enzymes on and off, preventing or causing many diseases such as diabetes, cancer and rheumatoid arthritis. The phosphorylation on tyrosine residues of proteins is essential for transmission of signals for cell growth, proliferation and differentiation. Protein tyrosine phosphatases (PTPs) in concert with protein tyrosine kinases (PTKs) regulate many signal transduction pathways by controlling the degree of phosphorylation of tyrosine residues within the protein. While the roles and mechanisms of protein tyrosine kinases are well documented, our present understanding of protein tyrosine phosphatases is very limited. In this regard we still have much more to learn about PTPs. Here we propose the design and synthesis of novel protein tyrosine phosphatase mimetics and their activity against tyrosine phosphatases. Chapter two describes the synthesis of 2-aminopyrimidine chlorides, sulfonamides and the sequence of reactions to make its amino acid analog. Chapter three describes the synthesis of α-aryl, α,β-epoxy carboxylates, phosphonates and their
biological activity against tyrosine phosphatases. These compounds could be very helpful in significantly improving the current understandings about the roles and mechanisms of the PTPs. These proposed tyrosine phosphatase inhibitors are believed to work effectively in treating the diseases by modulating the phosphorylation in signal transductions pathways. Chapter four describes the design and the synthesis of Peptide Nucleic Acids (PNAs) both standard as well as hybrid PNAs with novel cysteine based monomers that are aimed to increase the cellular uptake by introducing positively charged or amphipathic species attached to cysteine thiol functional group.
CHAPTER ONE

PROTEIN PHOSPHATASE INHIBITORS

1.1 General introduction to phosphorylation

Phosphorylation is the addition of an inorganic phosphate group to an organic molecule or a protein. It creates adenosine triphosphate (ATP), an energy storing molecule, from adenosine diphosphate (ADP) by the addition of an inorganic phosphate group in living cells (Figure 1.1). ATP, also known as the energy currency of the cell, is the form of energy needed to sustain our cells and thereby sustain every living organism.\(^1,^2\) Protein phosphorylation in particular is an important cellular process of living organisms both prokaryotes and eukaryotes. It has immense potential in understanding and curing some of challenging diseases and this is evident from the thousands of research articles being published on this topic every year in chemistry and biochemistry disciplines. Phosphate groups on a protein were first identified by Phoebus A. Levene in vitellin in 1906\(^3\) and this observation was confirmed by Fritz Lipmann in 1933 in casein.\(^4\) Enzymatic protein phosphorylation was then described for the first time by Eugene P. Kennedy in 1954.\(^5\)
Phosphorylation plays a crucial role in many cellular processes as in biological thermodynamics, enzyme activation, enzyme inhibition, protein-protein recognition, and protein degradation. Phosphorylation of ATPase during the transport of the metal ions Na$^+$ and K$^+$ across the cell membrane in osmoregulating to maintain homeostasis of the body’s water content, phosphorylation of the enzyme GSK-3 by protein kinase B (AKT) as part of the insulin signaling pathway, phosphorylation of src tyrosine kinase by Csk, phosphorylation of NADPH oxidase are a few examples which indicate the significance of phosphorylation. It’s been recognized that phosphorylation of some proteins causes them to be degraded by the ATP-dependent ubiquitin/proteasome pathway. These target proteins will become substrates for particular E3 ubiquitin ligases only when they are phosphorylated. 

Figure 1.1. Oxidative phosphorylation in the biosynthesis of ATP
1.2 Protein phosphorylation

Enzymatic protein phosphorylation is a reversible process in most living organisms on the planet. Kinases catalyze the phosphorylation and phosphatases catalyze dephosphorylation. Reversible protein phosphorylation is the basis for the regulation of diverse cellular processes that include cellular metabolism, contractility, transport, cell division, differentiation and development, learning and memory.¹¹⁻¹³

![Figure 1.2 Reversible phosphorylation on protein controlled by kinase and phosphatase enzymes](image)

ATP: adenosine triphosphatase
ADP: adenosine diphosphate
P_i: inorganic phosphate

The extent of phosphorylation and dephosphorylation turn many enzymes on and off by changing the conformation of the corresponding enzymes and the active sites of the receptors. The usual
sites of phosphorylation on the proteins are the amino acid moieties which have hydroxyl functional groups as in serine, threonine and tyrosine. In primitive organisms the sites of the phosphorylation also include histidine, arginine, cysteine and lysine (Figure 1.3).\textsuperscript{14} Phosphorylation increases the hydrophilicity of the particular group and can sometimes occur on the side chains of the amino acids that are otherwise in hydrophobic protein patches such as occurs on the p53 tumor suppression protein.\textsuperscript{15} The sensitive cells on the retina get activated upon phosphorylation and then they process the incoming light in the signal transduction. The extraordinary diversity of both kinases (over 500) and phosphatases (over 100) that have been identified in the human genome itself speaks to the essential roles these proteins play in daily biological processes taking place in the body.\textsuperscript{16}

**Figure 1.3** Typical amino acid residues for phosphorylation (second row amino acids are phosphorylation sites in primitive organisms)\textsuperscript{17}
Phosphorylated proteins can be dephosphorylated by either specific phosphatases like serine/threonine specific or tyrosine specific phosphatases or non specific phosphatases like alkaline phosphatases.

1.3. Occurrence of protein phosphorylation

Protein phosphorylation can occur on multiple sites in the same protein at any given time and it is estimated that approximately 30% of the 10000 proteins in a typical mammalian cell are thought to be phosphorylated at any given time. This suggests that many cellular functions could be artificially altered if one could control the activity of kinases or phosphatases or both. This led to enormous interest in identifying and studying small molecules both peptidic and non-peptidic, on specific kinases and phosphatases. Since the phosphorylation is crucial for stabilizing a particular conformation of a protein in order to act as an enzyme or as signal transduction element it is very important to know the state of phosphorylation of the protein to understand the structure and function of the same.

As mentioned earlier the major sites of phosphorylation in advanced organisms, including human beings, are serine, threonine and tyrosine. Tyrosine sites are very rare sites for phosphorylation; however the corresponding proteins are easily purified by antibodies and hence well understood.
1.4 Protein phosphatases and their importance

Protein phosphatases (PPs) are an important sub family of enzymes that remove phosphate groups from proteins and work exactly opposite to the function of protein kinases which phosphorylate proteins and together these opposing enzymes maintain the equilibrium at right levels. Protein phosphatases were initially classified into families based on their dephosphorylation sites whether they dephosphorylate Threonine/Serine residues, Tyrosine residues (PTPs) or both of them (dual specific phosphatases-DSPs). But now it has been discovered that certain Ser/Thr specific enzymes can dephosphorylate Tyr, and many of the enzymes in the dual specific family based on the sequence can selectively function on Tyr, Ser, and Thr, RNA or phosphoinositides.

Some important phosphatases include CD45, PTP1B, PTP1N, TCPTP, SHP-2, LAR, PP2C, Ppz1p, Ppz2p. CD45 is present in human hematopoietic cells and its mutation may lead to dysfunction of B lymphocytes and low T cell production which can lead to immunodeficiency. PTP1B is a negative regulator of insulin and is considered as a potential therapeutic target to treat type-II diabetes. TC-PTP is prominent in hematopoietic cell types and plays critical role in bone marrow maturation.\textsuperscript{20-22}
1.5 Protein tyrosine phosphatases (PTPs)

Protein tyrosine phosphatases (PTPs) are an important super family of enzymes which remove phosphate groups from phosphorylated tyrosine residues on proteins and regulate many signal transduction pathways, including growth initiation, propagation and termination, by regulating the extent of phosphorylation by working in association with the kinases in reciprocal directions. Defects in PTPase activity can lead to aberrations in the phosphorylation of tyrosine, eventually leading to protein malfunction, which contributes to the many human diseases like cancer, diabetes, obesity and rheumatoid arthritis. In recent days, PTPases have gained importance as the drug discovery target because of its critical role in bioprocesses. There are many classes of molecules being synthesized and studied for their activity on phosphatases some of which are shown in the Figure 1.6.23

Obesity is the second largest cause of preventable deaths after smoking in the United States. Obese people are more prone to develop associated diseases than the people with normal weight like diabetes, heart diseases, strokes, high blood pressure, cancer and obstructive sleep apnea. 0.6 Million people are estimated to die because of cancer in the US alone which is alarming (Figure 1.4)
Type II diabetes alone afflicts over 200 million people worldwide and many more are unaware they are at high risk. The number of people diagnosed is expected to grow steadily over the next several years. Type II diabetes (non insulin-dependent diabetes mellitus (NIDDM) or T2DM), is characterized by a resistance to insulin which can be due to the ignorance of the body to the insulin which is necessary for the body to be able to process glucose for the required energy needs or because of inadequate beta cell activity and it accounts for 90% of all diabetic patients. Increased fatty acid oxidation contributes greatly to hyperglycemia by formation of high levels of acetyl-coenzyme A, ATP, and NADH, which increase gluconeogenesis and thus hepatic glucose production. Type I diabetes, insulin-dependent diabetes mellitus (IDDM or T1DM), results from a lack of production or under production of insulin. When we eat food, the body
processes all of the sugars and starches into glucose, which is the energy currency of the cell. Insulin signals cells to take the sugar from blood. In diabetic conditions, glucose builds up in the blood instead of going into cells.25

1.6 Phosphotyrosine mimetics

The discovery of a PTP1B knockout as a non lethal mutation with increased insulin sensitivity by Elechbly et al has lead to the realization among the scientific community that the inhibition of protein tyrosine phosphatases might be a valuable research tool for insulin biology research.26 It could also be a possible source of drug development for treating major diseases like diabetes and obesity. The research in this field can lead to treatments of infectious agents, neurological disorders, autoimmune and certain cancers. To understand the function of phosphatases and in particular tyrosine phosphatases various molecules were developed and studied to find the exact mechanism of action of various phosphatases. Some of the well studied mimetics are shown in Figure 1.5.
1.7 Phosphatase inhibitors

The initial findings of the PTP1B knock out experiments lead to the tremendous interests in both academia and industry to develop and study peptidic and non-peptidic molecules as the potential inhibitors of the protein phosphatases. The first crystal structure for PTP1B has been solved by Tonks et al in 1994. There are currently around 20 phosphatases with crystal structures that been solved. The crystal structure findings are valuable for the design of new inhibitors as they showed the secondary sites, some times called satellite sites, clearly in addition to the primary active sites. It’s been common to design molecules to interact with the secondary sites in addition to the active site increasing the specificity of the inhibitor to target desired
phosphatase enzymes. Some representative molecules are shown in the Figure 1.6.

Figure 1.6 Some representative phosphatase inhibitors$^{29-30}$
1.8 Challenges in designing a potent & specific phosphatase inhibitor

The evolution of all of the phosphatases is believed to be from the same ancestor according to some of the biologists and it explains the very close similarities among phosphatases. All protein tyrosine phosphatases carry a highly conserved active site signature motif, [(H/V)CX5R(S/T)] amino acid sequence, which adopts a unique loop structure and employ a common catalytic mechanism. They have similar core structure made of a central parallel beta sheet with flanking alpha helices containing a β-loop and α-loop surrounding signature motif. The functions and specificity of these proteins are attributed to the regulatory domains and subunits. Because of the close similarities among the PTPs, it is known to be very difficult to selectively target specific PTPase and PTP1B in particular. For example PTP1B is closely related to TC-PTP and almost 80% of the sequence is similar in both of these phosphatases. This makes it very challenging to control selectivity. Some of the best known phosphatase inhibitors are shown and their biological activities and selectivities are compared in the Figure 1.7.
Figure 1.7 Activity and selectivity of some prominent inhibitors

In addition to the similarities in structures specific PTPase may also regulate multiple signaling pathways and similarly one signaling pathway can be regulated by multiple PTPases.34

In human beings proteins are predominantly phosphorylated on three residues Ser, Thr and Tyr residues, with each accounting for approximately 86%, 12% and 2% respectively. Human DNA encodes

<table>
<thead>
<tr>
<th>IC₅₀ or Kᵢ⁺ (µM)</th>
<th>PTP1B</th>
<th>TC-PTP</th>
<th>Selectivity²</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>8.400</td>
<td>&gt;200.0</td>
<td>&gt;23</td>
</tr>
<tr>
<td>IB</td>
<td>9.000</td>
<td>182</td>
<td>20</td>
</tr>
<tr>
<td>IC</td>
<td>2.100</td>
<td>&gt;30</td>
<td>&gt;15</td>
</tr>
<tr>
<td>ID</td>
<td>0.0024</td>
<td>0.026</td>
<td>10</td>
</tr>
<tr>
<td>IE</td>
<td>0.005</td>
<td>0.036</td>
<td>7</td>
</tr>
<tr>
<td>IF</td>
<td>0.018</td>
<td>0.065</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*TC-PTP IC₅₀/PTP1B IC₅₀
~ 520 protein kinases, with nearly 428 known or predicted to phosphorylate Ser and Thr residues and 90 belonging to the tyrosine kinase family. In contrast there are only ~ 107 human phosphatases and only around 81 are predicted to be active protein phosphatases (based on human genome findings). 35-38

In addition, since the active site of PTP1B is highly hydrophilic, it remains a challenge to identify inhibitors with both excellent in vitro potency and drug-like physiochemical properties which would lead to good in vivo activities. Major breakthroughs in designing potent inhibitors have to address the following two important issues 1) Many PTPs have similar structures and sequences in their active site regions. This makes it more difficult to design inhibitors that are specific for the corresponding target. 2) Most of the small molecules that bind with high affinity in these active sites are hydrophilic leading to poor cell permeability. 39-40

1.9 PTP1B as an exciting target for therapeutic drug discovery

Since the findings of PTP1B as a target for finding new drugs to the existing problems of obesity and diabetes in biology, many research groups have come up with different functional units based on theoretical and experimental results both peptidic and non peptidic
molecules. It is worth to mention some of the molecules reached to clinical trials II and failed to reach further due to efficacy issues.\textsuperscript{41-42}

1.10 PTP1B mechanism of action

The protein tyrosine phosphatase PTP1B became an exciting target for the treatment of type II diabetes and obesity. The enzyme is a negative regulator of the insulin signaling pathway acting by dephosphorylating phosphotyrosine residues in the insulin receptors (IRs) and insulin receptor substrates (IRSs). To date more than 20 PTPs have been solved for their crystal structures.\textsuperscript{43} The PTPs are composed of $\beta$-barrels flanked by $\alpha$-helices. The catalytic site is located in a groove at the protein surface which is 9 Å deeper for classical PTPs compared to DSPs which are 6 Å deep. This difference is responsible for the higher substrate selectivity of the classical PTPs. Recognition of the substrate peptide sequence by PTP1B and binding of the phosphotyrosine deep in the catalytic site of the phosphate-binding loop (P-loop) are mediated by residues 214-221. The WPD loop consisting of tryptophan, proline and aspartic acid closes down onto the substrate and thereby positions the thiolate of cysteine 215 for nucleophilic attack at the electrophilic phosphorous on the phosphotyrosine. Aspartic acid 181 acts as a general acid catalyst. This mechanism eventually hydrolyses to give inorganic phosphate group
which diffuses from the active site and gets replaced with water molecule. (Figure 1.7)\textsuperscript{44-45}

![Figure 1.8 PTP1B mechanism of action](image)

After carefully looking at current molecules being used as inhibitors and understanding the PTP1B mechanism of action we herein propose design and synthesis of molecules which we believe could work as non covalent\textsuperscript{46} or covalent inhibitors\textsuperscript{47} for different phosphatases paying particular attention to inhibit PTP1B selectively (Figure 1.8 and Figure 1.9). Pyrimidine chloride \textbf{2.24} is particularly interesting as we will use this molecule to insert into small peptide and study its effect as it is our belief that this will increase the selectivity dramatically.\textsuperscript{48-50}
1.11 Conclusion

It’s been challenging to discover potent cell permeable and orally bioavailable PTP1B inhibitors. The highly cationic nature of the active site and the lack of adjacent hydrophobic binding sites have been obstacles in developing potent inhibitors. In this regard, we designed some of the molecules shown in Figures 1.8 and 1.9 as phosphatase inhibitors.
inhibitors and synthesized some of them and tested for their activity against protein tyrosine phosphatases (PTPs) and the activities are tabulated in the respective chapters.

1.12 References


2.1 General introduction

Ever since the introduction of synthetic sulfonamide drugs (popularly known as sulfa drugs) as antibiotics in the early 1930s, there has been significant attention being paid to the discovery of new and effective drugs with the sulfonamide functional group. There are currently over 15,000 sulfonamide derivatives, analogs and related compounds, which have been synthesized and tested for different diseases. This studies lead to the discovery of so many useful sulfonamide medicines for treating urinary, thyroid, heart diseases, malarial infection and leprosy along with many other diseases.¹

Sulfonamide drugs were the first antimicrobial agents administered in combination with antibiotics for the treatment of infection. When penicillin and streptomycin first became available they were often given in combination with sulfonamides in which cases they became less effective by themselves.² In some instances they were used as additives and in some they acted synergistically. In 1932, German chemist Gerhard Domagk discovered that a dye called
Prontosil controlled streptococcal infections in mice for which he later received the Nobel Prize in 1939 in the field of Physiology and Medicine. Prontosil was also found to control staphylococcal infections in rabbits without harming animals. Since then, sulfonamides have been clinically used for decades and have been found to have wide ranging biological activities including antiviral, antidiabetic (hypoglycemic), diuretic, and antithyroid activities and also antitumor agents. The sulfonamide functional group is present in well established thiazide diuretics, loop diuretics, sulfonyl ureas, COX-2 inhibitors, anti-inflammatory agents, anti-rheumatics, anti-ulceratives, acetazolamide and many more (Figure 2.1). Folate synthesis is the important step in the synthesis of bacterial cell wall. Antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthetase; an enzyme involved in folate synthesis, and prevents the formation of cell wall which ultimately prevents the growth of bacteria. They have been also been used in retroviral therapy as HIV protease inhibitors recently.
The compounds with sulfonamide functional group have became increasingly popular recently among medicinal chemists in part because of the ease of deprotonation at physiological $p$H in the body which makes them more water soluble, more drug-like properties, which is a fundamental requirement for any chemical entity to be used as an effective drug in treating any kind of diseases.\textsuperscript{9-12}

We, keeping in mind the fundamental principles of drug discovery low toxicity and cellular solubility, based our target molecules on commercially readily available 2-aminopyrimidine in part due to its low toxicity towards the living cells and the $p$Kb of the NH proton in the product sulfonamide.\textsuperscript{13-14}
We focused our efforts to synthesize potent, yet highly selective inhibitors for individual members of the large PTPase family of enzymes paying particular attention to PTP1B. \(^{15-17}\) In this regard, we came up with the 2-aminopyrimidine derived molecules shown in the Figure 2.2. The compound \(\text{2.8}\) is expected to work as a non covalent inhibitor. The other three compounds expected to work as either covalent or non covalent inhibitors due to the electrophilic character.\(^{18}\) To increase the specificity of the inhibition for PTP1B we were interested in making a small peptide library, to obtain structure activity relationship (SAR) data, containing the amino acid derived chloride \(\text{2.24}\) shown in Figure 2.2. For this, we will employ solid phase peptide synthesis methods.\(^{19}\)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Proposed 2-aminopyrimidine derivatives as novel PTP1B inhibitors}
\end{figure}
Biological activity is explained briefly at the end of this chapter. Some of the key synthetic reactions in this chapter are the Sonogashira cross coupling, introduction of acetylene moiety and the deprotection of tert-butoxycarbonyl groups from the 1⁰ amine group employing an environmentally friendly catalyst, Montmorillonite K-10 clay.

2.2 Results and discussion

Professor Jerry Wu at the Moffitt Cancer Center collaborated with us by studying the biological activity of the compounds synthesized in this chapter. Dr. Wayne Guida’s group supported us with the design and modeling data for the same. Feng er Zhou’s contribution in scaling up intermediate 2.5 is also appreciated.

Syntheses of all of the 2-aminopyrimidine derivatives discussed in this chapter were started from the commercially available 2-aminopyrimidine. 2-Aminopyrimidine was reacted with methanesulfonyl chloride in pyridine as solvent to afford 2-aminopyrimidine sulfonamide 2.1 in good yields. Reaction of the above sulfonamide with molecular iodine in the presence of mercuric acetate in hot 1,4-dioxane solvent afforded 5-iodo-2-N-(methanesulfonyl)aminopyrimidine 2.2 in excellent yields.
Sulfonamide 2.2 was protected as the para-methoxybenzyl sulfonamide 2.3 employing very well established reaction conditions using para-methoxybenzyl chloride, KI and K$_2$CO$_3$ in DMF at ambient temperatures in very good yields$^{23}$ Pd (0) catalyzed Sonogashira coupling conditions$^{24}$ were employed to introduce the desired alkyne functionality in the molecule using commercially available 2-methyl-3-butyln-2-ol which gave alkyne-ol 2.4 in excellent yields. The same was deprotected using NaOH in toluene as solvent at reflux temperatures to afford intermediate alkyne 2.5 in moderate yields$^{25}$ (Scheme 2.1)

![Scheme 2.1 Synthesis of 5-ethynyl-2-N-[(4-methoxybenzyl) (methanesulfonyl)]-2-aminopyrimidine 2.5](image_url)
Alkyne 2.5 and iodide 2.3 were subjected to the Sonogashira coupling conditions mentioned earlier to get the coupled product, the symmetrical alkyne 2.6, in very good yields which was subsequently hydrogenated over hydrogen gas using 10% (w/w) Pd over carbon as catalyst in EtOH: EtOAc: DCM (12:12:1) solvent system to afford the symmetrical bipyrimidine 2.7 in quantitative yields. The para-methoxy benzylamine 2.7 was deprotected to give the bipyrimidine 2.8 in good yields.27 (Scheme 2.2)

Scheme 2.2 Synthesis of symmetrical bipyrimidine sulfonamide 2.8
Next we needed 5-iodo-2-N-(di tert-butoxycarbonyl)pyrimidine as an intermediate to synthesize the chloro analogue 2.15. Commercially available 2-aminopyrimidine was subjected to aromatic electrophilic substitution reaction with molecular iodine and mercuric acetate in hot 1, 4-dioxane and water (3:1) at 70 °C to afford 5-iodo-2-aminopyrimidine 2.9 in very good yields. The obtained amine was protected as a di tert-butyl dicarbamate 2.10 using di tert-butyl dicarbonate and DMAP in DMF at ambient temperatures.28 (Scheme 2.3)

![Scheme 2.3 Synthesis of 5-iodo-2-N,N-di tert-butoxycarbonyl bipyrimidine 2.10](image)

Alkyne 2.5 and iodide 2.10 were coupled under Sonogashira coupling conditions as previously mentioned to give alkyne 2.11 in good yields, which on hydrogenation gave bipyrimidine 2.12 in excellent yields. The product was heated to reflux in acetonitrile with montmorillonite K-10 clay to deprotect tert-butoxycarbonyl groups and obtain quantitative yields of 2.13 without needing further purification. Sulfonation with chloromethanesulfonyl chloride followed by the
deprotection of PMB group gave the desired chloride 2.15 in 34% yields for two steps\textsuperscript{29} (Scheme 2.4).

![Chemical Structure](image1)

\textbf{Scheme 2.4 Synthesis of bipyrimidine sulfonyl chloride 2.15}

Suitably protected 5-iodo-pyrimidine chloride 2.18 was synthesized from 2-amino pyrimidine using the reaction conditions mentioned earlier. The PMB group was replaced by benzyl so as to make it compatible for the selective deprotection of the methyl ester\textsuperscript{30} of amino acid derivative to get amine protected amino acid 2.24 ready to use in solid phase synthesis to make small peptides and convenient deprotection of benzyl group applying Pd catalyzed hydrogenation conditions. (Scheme 2.5)

36
Scheme 2.5 Synthesis of \( N \)-Benzyl-\( N \)-(5-iodo-pyrimidin-2-yl)-chloromethanesulfonamide 2.18

Readily available L-Serine was esterified using thionyl chloride to methyl ester 2.19 and the resulting HCl salt was reacted with di-tert butyl dicarbonate in the presence of potassium carbonate in THF:H\(_2\)O (3:1) solvent to yield \( N \)-(tert-butoxycarbonyl)-L-serine methyl ester 2.20 in quantitative yields.\(^{31} \) Tosylation with p-toluenesulfonyl chloride and subsequent iodination with NaI in acetone afforded the \( N \)-(tert-butoxycarbonyl)-\( \beta \)-iodoalanine methyl ester 2.22 in excellent yields\(^ {32} \) which can be further coupled to iodide 2.18 using Pd\(_2\)dba\(_3\) as the catalyst to get chloro sulfonamide 2.23.\(^ {33} \) Selective hydrolysis of the methyl ester using sodium hydroxide in tetrahydrofuran can result in the orthogonally protected amino acid 2.24 which can be readily put into small peptides to increase the selectivity of the inhibition of selective phosphatase over closely related phosphatases. We will employ solid phase peptide synthesis to prepare a library of small peptides incorporating (Scheme 2.6) amino acid 2.24.
Scheme 2.6 Synthesis of 3-[2-(Benzyl-chloromethanesulfonyl-amino)-pyrimidin-5-yl]-2-tert-butoxycarbonylamino-propionic acid 2.24

2.3 Biological activity studies

The compounds synthesized in this chapter 2.8, 2.15 and 2.16 were tested for their activity against SHP-2 and PTP1B but showed no significant activity.

2.4 Conclusion

Novel 2-aminopyrimidine chlorides and sulfonamides were synthesized and their application to synthesize amino acid analog 2.24 with tert-butoxycarbonyl as protecting group for solid phase synthesis of small peptides were proposed and the synthesis was partially applied to achieve novel peptides as potential noncovalent inhibitors for tyrosine phosphatases. The final two steps shown in the scheme 2.6 can be easily performed by utilizing the palladium catalyzed
coupling conditions and the subsequent hydrolysis of methyl ester to get \( 2.24 \).

### 2.5 Experimental procedures

#### 2.5.1 General

\(^1\)H-NMR and \(^{13}\)C-NMR spectra were recorded on a Brucker 250 MHz and the Varian 400 MHz spectrometer in CDCl\(_3\), Methanol-d\(_4\) and DMSO-d\(_6\) with TMS as the standard. Chemical shifts are reported in ppm, spin multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), dd (doublet of doublet) and bs (broad singlet). Thin-Layer chromatography (TLC) was performed on glass plates coated with 0.25 mm thickness of silica-gel. All solvents were dried and distilled prior to use and organic solvent extracts were dried over Na\(_2\)SO\(_4\). Mass measurements were carried out on ESI LC MS system (Agilent Technologies) and High Resolution Mass measurements were done on LC MSD TOF system (Agilent Technologies). MALDI-TOF measurements were recorded on Autoflex (BRUKER) Melting points were recorded using Melt-Temp (Electrothermal) instrument and were uncorrected.
2-N(methanesulfonyl)-aminopyrimidine (2.1):- To a 250 mL two necked round bottomed flask equipped with nitrogen inlet was charged 2-aminopyrimidine (32 g, 0.34 mol) followed by the addition of pyridine (128 mL) under positive pressure of nitrogen. The mixture was brought to 0 °C using an ice bath. A solution of methanesulfonyl chloride (72.1 g, 0.63 mol) in pyridine (96 mL) was added over a period of 10 minutes after which the reaction was brought to ambient temperatures in one hour. The reaction was stirred for another 10 hours before concentrating under reduced pressure. Residual pyridine was removed azeotropically by evaporating with methanol (3×40 mL) before purifying the crude by recrystallization in methanol to get pure product 2-N-(methanesulfonyl)-aminopyrimidine 2.1 as an off-white solid. (38.3 g, 65.8%). ¹H NMR (DPX 250 MHz, CDCl₃) δ 11.32 (bs, 1H, NH), 8.64 (d, J=5.0, 2H), 7.15 (t, J=5.0), 3.38 (s, 3H, CH₃); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 158.54, 157.54, 115.72, 41.25; LCMS (ESI) m/z calcd for C₅H₇N₃O₂S 173.029 found 174.0 [M+H]⁺, mp 256.3 °C.
5-iodo-2-N (methanesulfonyl) amino pyrimidine (2.2):- To a 1L three necked flask 2-N-(methanesulfonyl)-aminopyrimidine 2.1 (13.0 g, 75.0 mmol) and glacial acetic acid (400 mL) were charged. The heterogeneous mixture was heated to 120 °C to dissolve all the solids which resulted in the light brown solution. Iodine (20.0 g, 78.8 mmol) was charged to the flask in one portion before cooling to room temperature at which Hg(OAc)$_2$ was charged in one portion. After 5 minutes of stirring at room temperature, the reaction was heated to 120 °C for an hour. Reaction was monitored by thin layer chromatography. (The disappearance of iodine color indicates the completion of the reaction). The reaction mixture was carefully poured into 15% KI solution (975 mL) and stirred for another 30 minutes. Crude product was collected by filtration and the same was recrystallized from MeOH to get pure compound, 5-iodo-2-N-(methanesulfonyl) aminopyrimidine 2.2, as an off-white solid. (19.28 g, 85.8%) $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 8.77 (s, 2H), 7.26 (s, 1H), 3.44 (s, 3H); $^{13}$C NMR (DPX 250 MHz, DMSO-d$_6$) $\delta$ 163.43, 156.19,
84.78, 41.12; LCMS (ESI) m/z calcd for C₅H₆IN₃O₂S 299.0895 found 299.9 [M+H]⁺, mp 263.3 °C.

5-iodo-2-N [(methanesulfonyl), (4-methoxybenzyl)] amino pyrimidine (2.3): To a stirred solution of the iodide 2.2 (2.0 g, 6.69 mmol), KI (0.11 g, 0.67 mmol) and potassium carbonate (1.85 g, 13.38 mmol) in 50 mL of dry DMF under nitrogen atmosphere was added p-methoxybenzyl chloride at room temperature. Upon completion of the reaction solvent was evaporated under vacuo and the residue was dissolved in ethyl acetate (80 mL) and the same was washed with water (2x15 mL) and brine (3x15 mL), successively. Organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated under vacuo. The crude was subjected to the flash chromatography on silica gel using EtOAc/Hexane (2:8) as eluent to give pure compound 2.3 as a colorless liquid (2.16 g, 77.14%). ¹H NMR (DPX 250 MHz, CDCl₃) δ 8.64 (s, 1H), 7.25 (d, 2H, J=7.50), 6.74 (d, 2H, J=7.50), 5.22 (s, 2H), 3.70(s, 3H), 3.26 (s, 3H); ¹³C NMR
(DPX 250 MHz, CDCl₃) δ 162.95, 159.12, 157.54, 129.61, 129.07, 113.89, 84.03, 55.25, 48.83, 42.91; HRMS (ESI) m/z calcd for C₁₃H₁₄IN₃O₃S 418.980 found 419.9 [M+H]⁺, mp 107.5 °C.

5-[(2-hydroxy), (2-methyl)] butynyl-2-N [(methanesulfonyl), (4-methoxynenzyl)] amino pyrimidine (2.4): To a three necked round bottomed flask were added the iodide 2.3 (1.0 g, 2.38 mmol), alkyne (0.4 g, 4.77 mmol), CuI (22.7 mg, 0.12 mmol), PPh₃ (62.5 mg, 0.24 mmol), Pd (PPh₃)₄ (27.5 mg, 0.024 mmol), TEA (2.0 mL) followed by charging with 25 mL of dry acetonitrile. The solution was brought to 60 °C. Upon completion of the reaction, in 10 hours, solvent was evaporated under vacuo. The crude was subjected to flash chromatography on silica gel using EtOAc/Hexane (2:8) as eluent to give pure compound 2.4 as a yellow fluffy solid (0.82 g, 92%). ¹H NMR (DPX 250 MHz, CDCl₃) δ 8.58 (s, 2H), 7.33 (d, 2H, J=7.50 Hz), 6.81 (d, 2H, J=7.50 Hz), 5.34 (s, 2H), 3.37 (s, 3H), 3.34 (s, 3H), 1.65 (bs, 1H), 1.62 (s, 6H); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 159.78, 159.09,
157.07, 129.62, 129.18, 113.86, 112.91, 99.48, 75.40, 65.66, 55.24, 48.71, 42.97, 31.32; LCMS (ESI) m/z calcd for C_{18}H_{21}N_{3}O_{4}S 375.1252, found, 376.1 [M+H]^+.

N-(5-Ethynyl-pyrimidin-2-yl)-N-(4-methoxy-benzyl)-methanesulfonamide (2.5):- The alkyne-ol 2.4 (0.80 g, 2.13 mmol) and sodium hydroxide (0.22 g, 5.50 mmol) were taken in toluene (50 mL) and the solution was heated to reflux for 10 hours. Upon completion of the reaction by TLC the solvent was evaporated under reduced pressure and the residue was extracted into EtOAc. The organic layer was further washed with brine solution and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude obtained was purified by flash chromatography to get pure acetylene 2.5 as a colorless solid (0.33 g, 49 %). $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 8.57 (s, 2H), 7.26 (d, 2H, $J=10.0$), 6.74 (d, 2H, $J=10.0$), 5.26 (s, 2H), 3.69 (s, 3H), 3.28 (s, 3H); $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 160.37, 159.13, 157.37, 129.61, 129.13, 113.89,
N-(5-{2-[Methanesulfonyl-(4-methoxy-benzyl)-amino]-pyrimidin-5-ylethynyl]-pyrimidin-2-yl)-N-(4-methoxy-benzyl)-methanesulfonamide (2.6):-

To a three necked round bottomed flask were added aryl iodide 2.3 (0.26 g, 0.63 mmol), alkyne 2.5 (0.20 g, 0.63 mmol), CuI (5.9 mg, 0.031 mmol), PPh₃ (16.5 mg, 0.063 mmol), Pd(PPh₃)₄ (7.2 mg, 0.006 mmol) and TEA (2 mL) followed by 25 mL of dry acetonitrile. The solution was brought to 60 °C. Upon completion of the reaction, in 10 hours, the solvent was evaporated under vacuo. The crude was subjected to flash chromatography on silica gel using EtOAc/Hexane (2:8) as eluent to give pure symmetrical alkyne 2.6 as a yellow fluffy solid (0.28 g, 74 %). ¹H NMR (DPX 250 MHz, CDCl₃) δ 8.63 (s, 4H), 7.29 (d, 4H, J=10.0), 6.76 (d, 4H, J=10.0), 5.30 (s, 4H), 3.71 (s, 6H), 3.37 (s, 6H); ¹³C NMR (DPX 250 MHz, CDCl₃) δ
MHz, CDCl₃) δ 159.75, 159.15, 157.38, 129.67, 129.05, 113.89, 112.38, 87.99, 55.26, 48.76, 43.03; HRMS (ESI) m/z calcd for C₂₈H₂₈N₆O₆S₂ 608.1512, found 609.1592 [M+H]⁺, mp 212.4 °C.

N-[5-(2-{2-[Methanesulfonyl-(4-methoxy-benzyl)-amino]-pyrimidin-5-yl}-ethyl)-pyrimidin-2-yl]-N-(4-methoxy-benzyl)-methanesulfonamide (2.7):- To a solution of alkyne 2.6 (0.27 g, 0.44 mmol) in ethanol/EtOAc/DCM (12:12:1) (25 mL), 10% (w/w) palladium on carbon (0.1 g) was added carefully. The reaction was hydrogenated over H₂ gas (45 psi) for 12 hrs. The resulting solution was filtered carefully over Celite and the Celite cake was washed with ethyl acetate (25 mL) to get most of the compound off of it. The organic filtrate was subjected to rotary evaporation to yield pure...
product 2.7 needing no further purification (~ quant %). HRMS (ESI) 
m/z calcd for C_{28}H_{32}N_{6}O_{6}S_{2} 612.1824 found 613.1929 [M+H]^+.

N-{5-[2-(2-Methanesulfonylamino-pyrimidin-5-yl)-ethyl]-pyrimidin-2-yl}-methanesulfonamide (2.8):- Compound 2.7 was taken in round bottomed flask and cooled to 0 °C in an ice bath followed by the addition of neat TFA (5 mL). The resulting solution was stirred at room temperature for 2 hours. Upon completion of the reaction, the solvent was removed under reduced pressure and the residue obtained was dissolved in DCM and subjected to rotary evaporation to azeotropically remove the residual trifluoroacetic acid (3×15 mL). The crude product obtained was precipitated using diethyl ether to get pure sulfonamide 2.8 as an off-white solid in moderate yields (0.57 g, 35 %). \(^1\)H NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 11.44 (bs, 2H),
8.5 (s, 4H), 3.36 (s, 6H), 2.50 (s, 4H), HRMS (ESI) m/z calcd for C_{12}H_{16}N_{6}O_{4}S_{2} 372.0674 found 373.0751 [M+H]^+.

5-iodo-2-amino pyrimidine (2.9): A solution of 1.02 g (10.72 mmol) of 2-aminopyrimidine in 12 mL of water was treated with 1.36 g (4.26 mmol) of mercuric acetate and the mixture was stirred for two minutes on the steam-bath. The initially formed yellow precipitate quickly turned to a thick white slurry which was treated with a solution of 1.63 g of molecular iodine, I₂, (6.44 mmol) in 12 mL of hot dioxane at 50 °C. All of the iodine reacted during 30 minutes of stirring during which time considerable evaporation was observed. The thick slurry was poured into several volumes of 15% potassium iodide solution and washed on the filter with fresh iodine solution until white. Recrystallization from absolute methanol gave pure compound, 2-amino-5-iodopyrimidine 2.9 as an off-white solid (2.07 g, 87.3 %). \(^1\)H NMR (DPX 250 MHz, DMSO-d₆) δ 8.35 (s, 2H), 6.83 (s, 2H); \(^{13}\)C NMR (DPX 250 MHz, DMSO-d₆) δ 172.04, 162.53, 98.25; HRMS (ESI) m/z calcd for C₄H₄IN₃ 220.9450, found [M+H]^+ 221.9517, mp 222.9 °C.
Di-tert-butyl (5-iodopyrimidin-2-yl)dicarbamate (2.10): To a stirred solution of 5-iodo-2-aminopyrimidine 2.9 (0.50 g, 2.3 mmol) in DMF (15 mL) were added di-tert-butyl dicarbonate (1.15 g, 5.27 mmol) and 4-dimethylaminopyridine (0.025 g, 0.11 mmol) at room temperature. Progress of the reaction was monitored by thin layer chromatography using EtOAc/Hexane (50:50) as mobile phase on silica coated TLC plates. Upon completion of the reaction, DMF was evaporated under reduced pressure. Crude product obtained was subjected to flash chromatography on silica gel using EtOAc/Hexane (50:50) as eluent to give a pure iodide 2.10 as a colorless solid (0.652g, 68.4 %). $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 8.84 (s, 2H), 1.40 (s, 18H); $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 164.09, 157.46, 150.40, 89.83, 83.91, 27.85; LCMS (ESI) m/z calcd for C$_{14}$H$_{20}$IN$_3$O$_4$, 421.049 found 444.0, [M+H]$^+$, mp 146.5 °C.
(5-{2-[Methanesulfonyl-(4-methoxy-benzyl)-amino]-pyrimidin-5-ylethynyl}-pyrimidin-2-yl)-di-carbamic acid di-tert-butyl ester (2.11):- To a three necked round bottomed flask were added alkyne 2.5 (0.500 g, 1.57 mmol), iodide 2.10 (0.69 g, 1.6 mmol), CuI (0.015 g, 0.078 mmol), PPh\(_3\) (0.041 g, 0.15 mmol), Pd(PPh\(_3\))\(_4\) (0.018 g, 0.015 mmol) and TEA (2 mL) followed by 30 mL of dry acetonitrile. The solution was brought to 60 °C. Upon completion of the reaction, in 10 hours, the solvent was evaporated under vacuo. The crude was subjected to the flash chromatography on silica gel using EtOAc/Hexane (2:8) as eluent to give pure alkyne 2.11 as a white solid (0.78 g, 82 %). \(^1\)H NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 3.59 (s, 3H), 3.19 (s, 3H), 1.30 (s, 18H); HRMS (ESI) \(m/z\) calcd for C\(_{29}\)H\(_{34}\)N\(_6\)O\(_7\)S 610.2209 found 633.2125 [M+Na]\(^+\), mp 83.2 °C.
N-{5-[2-(2-Amino-pyrimidin-5-yl)-ethyl]-pyrimidin-2-yl}-N-(4-methoxy-benzyl)-methanesulfonamide (2.13):- To a solution of compound 2.11 (0.295 g, 0.48 mmol) in ethanol was added 10 wt% Pd/C catalyst and the resultant heterogeneous solution is subjected to hydrogenation as described in the synthesis of 2.7 except using ethanol as solvent. The obtained product 2.12 was taken in acetonitrile without further purification and Montmorillonite K-10 clay (0.3 g) was added carefully. The reaction was refluxed at 82 °C overnight. The resulting solution was filtered carefully over Celite and the Celite cake was washed with ethyl acetate (25 mL). The organic filtrate was subjected to rotary evaporation to obtain pure product 2.13 as a white solid, needing no further purification, in near quantitative yields. \(^1\)H NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 8.31 (s, 2H), 8.04 (s, 2H), 7.26 (d, 2H, \(J=7.50\)), 6.74 (d, 2H, \(J=7.50\)), 5.25 (s, 2H), 5.03 (s, 2H), 3.70 (s, 3H), 3.27 (s, 3H), 2.71 (m, 4H).
**N-\{5-[2-(2-methanesulfonylamino-pyrimidin-5-yl)-ethyl]-pyrimidin-2-yl\}-chloromethanesulfonamide (2.15):** To a stirred solution of aryl amine 2.13 (0.16 g, 0.38 mmol) in pyridine (15 mL) at room temperature was added to chloromethanesulfonyl chloride dropwise over a period of 10 minutes. Progress of the reaction was monitored by thin layer chromatography using MeOH/EtOAc (2:8) as eluent. Upon completion of the reaction solvent was removed under reduced pressure to give crude compound which on flash chromatography gave pure product 2.14 as an off-white solid which was taken to deprotection. HRMS (ESI) \( m/z \) calcd for C\textsubscript{20}H\textsubscript{23}ClN\textsubscript{6}O\textsubscript{5}S\textsubscript{2} 526.0859 found 527.0939 [M+H]\(^+\).
PMB protected amine 2.14 was taken in round bottomed flask and cooled to 0 °C in an ice bath followed by the addition of neat TFA (5 mL). The resulting solution was stirred at room temperature for 2 hours. Upon completion of the reaction the solvent was removed under reduced pressure and the residue obtained was dissolved in DCM and subjected to rotary evaporation to azeotropically remove the residual trifluoroacetic acid (3×15 mL). The crude product obtained was precipitated using diethyl ether to get pure compound 2.15 as an off-white solid. (0.057g, 36.5%) ¹H NMR (DPX 250 MHz, DMSO-d₆) 8.6 (s, 2H), 8.3 (s, 1H), 8.1 (s, 2H), 4.1 (s, 3H), 2.9-2.6 (m, 4H), 2.1 (s, 3H), HRMS (ESI) m/z calcd for 406.0285 C₁₂H₁₅ClN₆O₄S₂ found 407.0489 [M+H]^+.

2-N(chloromethanesulfonyl)-aminopyrimidine (2.16): To a stirred solution of 2-aminopyrimidine (2.00 g, 21.0 mmol) in pyridine (25 mL) was added chloromethanesulfonyl chloride (3.29 g, 22.08 mmol) over a period of ten minutes. Progress of the reaction was monitored by TLC (10% MeOH, 90% EtOAc). Upon completion of the
reaction, solvent was evaporated under reduced pressure and the residue was taken in methanol and evaporated to azeotropically remove traces of pyridine. The same was repeated three times with 20 mL methanol each time. The crude product was subjected to flash column chromatography on silica gel using 1:9 MeOH/EtOAc as eluent to get a pure off-white solid **2.16** (2.73 g, 62.8%). $^1$H NMR (Inova 400 MHz, CDCl$_3$) $\delta$ 12.11 (s, 1H), 8.61 (d, $J$=12.63), 7.14 (t, 1H, $J$=12.63), 5.28 (s, 2H); $^{13}$C NMR (Inova 400 MHz, CDCl$_3$) $\delta$ 159.25, 157.39, 116.16, 56.06; HRMS (ESI) $m/z$ calcd for C$_5$H$_6$ClN$_3$O$_2$S 206.9869, found 207.9952, [M+H]$^+$ 229.9770 [M+Na]$^+$. 

\[
\begin{align*}
\text{N-(5-iodo-pyrimidin-2-yl)-chloromethanesulfonamide (2.17):} \\
\text{Same procedure as followed for the synthesis of 2.2 to get pure iodide 2.17 as an off white solid in good yields (86%).} \\
^1\text{H NMR (DPX 250 MHz, CDCl}_3\text{) } \delta \text{ 8.77 (s, 2H), 7.36 (s, 1H), 5.44 (s, 2H).}
\end{align*}
\]
**N-Benzyl-N-(5-iodo-pyrimidin-2-yl)-chloromethanesulfonamide (2.18)**:- To a stirred solution of above iodide 2.17 (2.0 g, 6.69 mmol), KI (0.11 g, 0.67 mmol) and potassium carbonate (1.85 g, 13.38 mmol) in 50 mL of dry DMF under nitrogen atmosphere was added benzyl bromide at room temperature. Upon completion of the reaction, solvent was evaporated under vacuo and the residue was dissolved in ethyl acetate (80 mL) and the same is washed with water (2×15 mL) and brine (2×15 mL), successively. Organic layer was dried over anhydrous sodium sulfate and the solvent was evaporated under vacuo. The crude was subjected to the flash chromatography on silica gel using EtOAc/Hexane (2:8) as eluent to give pure compound 2.18 as a colorless liquid (81% yield). $^1$H NMR (DPX 250 MHz, CDCl$_3$) δ 8.71 (s, 2H), 7.3 (m, 5H), 5.3 (s, 2H), 3.34 (s, 3H); $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) δ 163.02, 162.99, 137.05, 127.86, 127.66, 84.13, 49.39, 42.92.
L-Serine methyl ester hydrochloride (2.19): To a stirred slurry of L-serine (15.0 g, 143 mmol) in 100 mL of methanol was added SOCl₂ (12 mL) at 0 °C over a period of 1 hr. The resulting clear solution was left to come to room temperature and continued stirring for another 20 hrs before concentrating under reduced pressure. Excess HCl was azeotropically removed using methanol (3×60 mL) The compound obtained was dried under high vacuum overnight to give pure product L-Serine methyl ester hydrochloride 2.19 as white solid (22.0 g, ~ quantitative yields) ¹H NMR (DPX 250 MHz, DMSO-d₆) δ 8.60 (s, 3H), 5.63 (s, 1H), 4.07 (t, 1H), 3.82 (d, 2H), 3.72 (s, 3H); ¹³C NMR (DPX 250 MHz, DMSO-d₆) δ 168.43, 59.37, 54.32, 52.71.

N-(tert-butoxycarbonyl)-L-serine methyl ester (2.20):- To an ice cold solution of ester 2.19 (22g, 142 mmol) in THF:H₂O (3:1, 100 mL)
was added potassium carbonate (20.8g, 150 mmol) followed by Di tert butyl dicarbonate (35.9 g, 164.5 mmol). The resulting reaction was stirred vigorously for 23 hrs at room temperature. Upon completion the reaction mixture was concentrated under reduced pressure and the obtained crude was extracted into ethyl acetate (3×75 mL) from saturated brine solution. Organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The obtained crude was flash chromatographed on short column to get pure product N-(tert-butoxycarbonyl)-L-serine methyl ester 2.20 as colorless viscous oil. (30.76 g, 98.8% yield). ¹H NMR (DPX 250 MHz, CDCl₃) δ 5.72 (d, 1H), 4.49 (s, 1H), 4.04 (d, 2H), 3.91 (s, 3H), 3.19 (s, 1H), 1.58 (s, 9H); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 171.49, 155.80, 80.32, 55.69, 52.65, 28.28; LCMS (ESI) m/z calcd for C₉H₁₇NO₅ 219.11, found 242.1 [M+Na]⁺.

N-(tert-butoxycarbonyl)-O-(p-toluenesulfonyl)-L-serine methyl ester (2.21):- To an ice cold solution of N-(tert-butoxycarbonyl)-L-serine methyl ester 2.20 (1.4g, 6.38 mmol) in dry DCM (50 mL) were
added 4-dimethylaminopyridine (0.073g, 0.6 mmol) 4-toluene sulfonyl chloride (1.22g, 6.38 mmol) and triethylamine (0.64 g, 6.38 mmol) successively. The reaction was monitored for the progress by thin layer chromatography. After completion of reaction the reaction mixture was concentrated under reduced pressure and the crude was dissolved in DCM and washed with brine solution. Organic layers were mixed and dried over Na$_2$SO$_4$ before flash chromatography to get pure compound, $N$-($\text{tert}$-butoxycarbonyl)-$O$-($p$-toluenesulfonyl)-L-serine methyl ester **2.21** 1.66g (69.6 %). $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.7 (d, 2H, $J=8.0$), 7.3 (d, 2H, $J=8.0$), 5.2 (d, 1H, $J=8.0$), 4.5-4.1 (m, 2H), 3.62 (s, 3H), 2.38 (s, 3H), 1.35 (s, 9H); $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 168.98, 145.16, 132.37, 129.96, 128.03, 52.97, 28.12, 21.44.

![Chemical structure](image)

**N-(tert-Butoxycarbonyl)-β-iodoalanine methyl ester (2.22):** To a solution of $N$-(tert butoxycarbonyl)-$O$-($p$-toluenesulfonyl)-L-serine methyl ester **2.21** (0.87 g, 2.34 mmol) in acetone (25 mL) was charged NaI (0.53g, 3.52 mmol) under stirring at room temperature.
The resulting solution was stirred at same temperature for 10 hrs before concentrating under reduced pressure. The crude product obtained was subjected to flash chromatography on silica gel column using EtOAc: Hexane (1:9) as eluent to get pure product \(N\)-(tert-butoxycarbonyl)-\(\beta\)-iodoalanine methyl ester 2.22 as colorless viscous liquid (0.58g, 75.0%)

2.6 References


20 Prof. Jerry Wu at Moffitt Cancer Center collaborated with us for testing compounds


29 Shaikh, N. S.; Gajare, A. S.; Deshpande, V. H.; Bedekar, A. V. A mild procedure for the clay catalyzed selective removal of the


33 Jackson, R. W.; Perez-Gonzalez, M. Synthesis of N-(tert-butoxycarbonyl)-β-iodolanine methyl ester: a useful building block in the synthesis of nonnatural α-amino acids via palladium
CHAPTER THREE
SYNTHESIS OF α-ARYL α, β-EPOXY CARBOXYLATES,
PHOSPHONATES AND THEIR BIOLOGICAL ACTIVITY AGAINST
TYROSINE PHOSPHATASES

3.1. General introduction

Carboxylates are a very important class of compounds to both biologists and chemists as they play some very significant roles in biology. One important class of compounds with carboxylic acid functional group is amino acids which are indispensable to the living organism whether they are primitive organisms or advanced organisms.\textsuperscript{1,2} 2-Hydroxypropanoic acid also known as lactic acid plays a role in several biochemical processes and was first isolated in 1780 by Swedish chemist Carl Wilhelm Scheele. In animals, it is produced from another carboxylic acid peruvate constantly.\textsuperscript{3} (Figure 3.1). Similarly, phosphates are also very significant as they play very crucial roles in the biology as in the formation of adenosine tri-phosphate from adenosine di-phosphate and the phosphorylation of most of the enzymes to get into the right 3D conformation so as to perform the assigned function in the body.\textsuperscript{4}
Our current interest is to synthesize phosphonates and carboxylates as phosphate mimics of both epoxy and aziridine derivatives and test their activities to develop the inhibitors for tyrosine phosphatases mainly concentrating on SHP-2 and PTP1B which has been briefly explained in chapter one. Some of the important carboxylates which were tested for activity against PTP1B were shown in Figure 3.2. Ertiprotafib of Wyeth-Ayerst reached clinical trials and failed to go further because of efficacy issues.\textsuperscript{5}
Figure 3.2 Some of the phosphatase inhibitors with carboxy functionality

3.2. Phosphonates as tyrosine phosphatase inhibitors

Synthesis of phosphonate ester 3.4 was discussed and the activity is explained in the following sections of this chapter.

3.3. Carboxylates as tyrosine phosphatase inhibitors
Synthesis of carboxylates was discussed and the activity is explained in the following sections of this chapter.

3.4. Results and discussion

3.4.1 Synthesis of α-aryl α,β-epoxy phosphonates

Synthesis of the phosphonates started from commercially available benzoyl chloride which was reacted with triethyl phosphite to form α-keto phosphonate ester 3.2. Phosphonate 3.2 was epoxidized with trimethyl sulphonium iodide prepared in our lab to get α,β-epoxy phosphonate ester 3.3 in good yields. Epoxy phosphonate was subjected to mono deprotection with LiBr in 4-methyl-2-pentanone at 80 °C to get compound 3.4 in good yields. (Scheme 3.1)

Scheme 3.1 Synthesis of α,β-epoxy phosphonate 3.4
The direct di-deprotection of 3.3 failed to give the desired phosphonic acid by using the same conditions at higher temperatures. The mono deprotected compound starts to precipitate as lithium salt 3.4 and so does not give the product. We then converted the di alkyl phosphonate to di silyl phosphonate 3.5 using trimethyl silyl chloride (TMSCI) in dichloromethane as solvent which can be easily hydrolyzed to the corresponding epoxy phosphonic acid 3.6 or tested without deprotection as it can get deprotected at physiological conditions.8

\[ \text{Scheme 3.2 Synthesis of } \alpha,\beta\text{-epoxy phosphonic acid 3.6} \]

**3.4.2. Synthesis of α-aryl-α,β-epoxy carboxylates**

Synthesis of carboxylates started from the commercially available aromatic aldehydes and alpha phenyl methyl acetate. Alpha phenyl methyl acetate was converted to diaza ester 3.7 employing diazo transfer reaction with p-ABSA as diaza transfer reagent and DBU as base in excellent yields.9 Diazo-ester 3.7 was reacted with various in house aldehydes to get α,β-epoxy carboxylates in very good yields. The product with benzaldehyde was trans as reported in literature.10
We selected aromatic aldehydes with various electron donating, electron withdrawing and alkyl, halo and alkoxy groups so that we can study the effects of substituents on the activity against selected phosphatases.

Scheme 3.3 Synthesis of α,β-epoxy α-aryl carboxylates 3.9a-g

The methyl ester was deprotected using standard conditions with LiOH as base in THF: H₂O mixture to get carboxylic acid 3.9 in excellent yields.
Compound 3.9a was also prepared by using n-BuLi/TMEDA/CO$_2$ reagents from (trans)-1,2-Di-phenyl oxirane to see if it gives exclusively trans product (Boxed in Scheme 3.5). The same conditions with formaldehyde and its equivalents did not give the desired product 3.8h as shown in Scheme 3.4 instead gave the dimerized derivative of diazo-ester 3.8i which was confirmed using NMR techniques as well as crystal structure studies.$^{11}$ The directed lithiation with BuLi/TMEDA and subsequent reaction with CO$_2$ as electrophile were not reported anywhere in the literature to the best of our knowledge. Whereas different electrophiles like alkyl iodides give the corresponding derivatives in excellent yields and were reported in literature.$^{12}$

Scheme 3.4 Failed attempts to synthesis epoxy carboxylates with methanal equivalents
3.4.3 Synthesis of $\alpha,\beta$-aziridino carboxylates

The results of directed lithiation on epoxide 3.10 and trapping with carbon dioxide prompted us to apply the same methodology to make aziridine derivatives. For this approach, trans-stilbene was epoxidized using $m$-CPBA as epoxidizing agent in chloroform at 0 °C to get trans-epoxide 3.10 in very good yields. The epoxide was cleaved to get trans-azido alcohol 3.11 using sodium azide and ammonium chloride as reported in the literature. The azido alcohol was refluxed in acetonitrile in the presence of triphenylphosphine to obtain aziridine 3.12 in excellent yields. The attempted directed lithiation with n-BuLi/TMEDA followed by reaction with carbon dioxide did not give any desired product. Changing n-BuLi with s-BuLi and t-BuLi did not improve the results. Thus, we changed the strategy and the free NH group was protected as the Boc derivative using di-tert-butoxy anhydride in the presence of DMAP in dichloromethane as solvent gave excellent yields of aziridine 3.13. The product was then subjected to the same directed lithiation conditions but we were only able to run mass spectral analysis as the yields were dismal. (Scheme 3.5)
Scheme 3.5 Synthesis of α,β-aziridino carboxylate 3.14

This led us to apply the same Rhodium catalyzed reaction conditions applied above in making carboxylates 3.8a-g. For this, we needed imines with suitable groups on amine so that it is easy to remove after the insertion reaction, which will be explained in this report shortly.

Benzaldehyde was reacted with 40% methylamine in water at 0 °C to get N-methyl imine 3.16 in excellent yields. The reaction with 4-methoxybenzylamine in presence of anhydrous sodium sulfate in dichloromethane at room temperature gave very good yields of imine
3.17. The rhodium catalyzed insertion reaction with diazo-ester 3.17 did not give the desired product and we could not find any reason behind this. To find out if the free NH group is having any effect on the reaction conditions we attempted the same reaction conditions on styrene and the reaction worked perfectly to give 3.21 in very good yields as reported in the literature. So the protecting group has been changed to benzyl and for that benzaldehyde was protected as imine 3.18 and was subjected to obtain N-benzyl aziridine carboxylate 3.19 in decent yields. The ester was subjected to base hydrolysis using excess lithium hydroxide in tetrahydrofuran and water mixture as solvent to get the corresponding carboxylic acid 3.20 in moderate isolated yields. (Scheme 3.7)\(^{16}\)

**Scheme 3.6** Synthesis of N-alkyl α,β-aziridino carboxylate 3.19
Scheme 3.7 Synthesis of N-benzyl aziridine carboxylate 3.20

Scheme 3.8 Synthesis of α,β-diaryl α-cyclopropane carboxylate

3.5. Biological activity studies

IC$_{50}$ was defined as the concentration of compound that caused a decrease of 50% in magnitude in the PTP activity. 2-Aminopyrimidine chlorides, sulfonamides and α-aryl, α,β-epoxy carboxylates and phosphates synthesized were tested for activity of inhibition for IC$_{50}$ using a human recombinant GST-Shp-2 PTP domain protein. 6,8-Difluoro-4-methylumbelliferyl phosphate (DiFMUP) was used as the
substrate. Testing was performed in duplicates at room temperature in black, half area 96-well plates. Incubation was carried out at room temperature for 20 min in a 75 µL reaction mixture containing 25 mM Hepes, pH 7.0, 50 mM NaCl, 1 mM DTT, 0.01% Triton X-100, 40 µM DiFMUP, 3% DMSO or compound. Fluorescent signal was measured at excitation and emission wavelengths of 355 nm and 460 nm respectively. For IC₅₀ determination, eight concentrations of each compound at one third dilutions were tested. The ranges of compound concentrations used in PTP assay were determined from preliminary trials. According to the results obtained epoxy carboxylates exhibited good inhibitory activity among the compounds synthesized. Preliminary results show that these compounds work as noncovalent inhibitors.

Compounds 3.9d and 3.9c showed activity against Shp-2 protein phosphatase with IC₅₀ values 5.6 mM and 20.8 mM respectively. Epoxy carboxylate 3.9a without any aromatic substitutions showed good activity against the same target with IC₅₀ value of 0.0069 mM (6.9 µM).

3.6. Conclusion and future directions

Synthesis of some epoxy and aziridino carboxylates and phosphonates was performed and some of them were tested against PTPs and we were excited by some of relatively promising inhibitors
against SHP-2 phosphatase. The obtained compounds can be modified by using the same methodology mentioned in chapter-2 of this writing to make amino acid derivatives of the same carboxylates and put into small peptides which can increase the selectivity many times. Compound 3.9a shows good activity against Shp-2 phosphatase and this kind of epoxy esters are more stable than the simple epoxides and it is interesting to see if the activity can be increased simultaneously by substituting with pyrimidine units and making the second generation compounds. It can be put into small peptides and can be more selective at the same time more active against Shp-2 phosphatase.

3.7 Experimental Procedures

Diethyl-(phenylcarbonyl)-phosphonate (3.2)

\[
\text{PhCl} + \text{P(OEt)}_3 \xrightarrow{DCM, 0^\circ\text{C} - \text{rt}, 80\%} \text{Ph-PO} \quad (3.2)
\]

To an ice cold solution of benzoyl chloride (4.0 g, 28.4 mmol) in 25 mL of dichloromethane was added triethyl phosphite (4.7 g, 28.4 mmol) dropwise. Reaction was left to come to room temperature and stirred for a further 12 hours. After the complete consumption of starting material, 50 mL of dichloromethane was added, and the organic layer
was washed with saturated sodium bicarbonate solution (3×15 mL) followed by saturated brine solution (3×15 mL). The extract was dried over anhydrous MgSO₄ and evaporated in vacuo. The residue was purified by column chromatography on silica gel using EtOAc/Hexanes (1:9) as eluent to give the pure product 3.2 as a greenish yellow liquid. (4.9 g, 72 %). ¹H NMR (DPX 250 MHz, CDCl₃) δ 8.3 (m, 2H), 7.6 (t, 1H), 7.5 (m, 2H), 4.3 (q, 4H), 1.4 (t, 6H); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 200.4, 136.0, 134.7, 129.8, 128.8, 63.9, 16.3; HRMS (ESI) m/z calcd for C₁₁H₁₅O₄P is 242.070 found 243.078 [M+H]^+.

Phosphonic acid, (2-phenyloxiranyl)-, diethyl ester (3.3)

A solution of triphenylphosphine methyl bromide (1.55 g, 4.33 mmol) and DIEA (57 µL, 0.41 mmol) in THF (15 mL) was cooled to -76 °C before adding n-BuLi dropwise. After briefly stirring at -76 °C for about 15 minutes, the reaction was brought to room temperature and further stirred for an hour before cooling back to -76 °C at which temperature, the phosphate ester (1.0 g, 4.13 mmol) was added. The reaction was left to come to room temperature and further stirred for 15 hours.
before quenching with 1N HCl solution at ice cold temperature. The product was extracted into diethyl ether (3×25 mL). All organic layers were combined and washed with brine solution (3×15 mL) and dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure to get crude product which on subjecting to flash column chromatography gave pure product **3.3** as colorless liquid (0.66 g, 67 %).$^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.9-7.3 (m, 5H), 4.0 (q, 4H), 1.25 (t, 6H); $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 152.25, 134.37, 129.10, 128.40, 125.21, 97.32, 64.59, 16.07; HRMS (ESI) $m/z$ calcd for C$_{12}$H$_{17}$O$_4$P is 256.0864, found 257.0 [M+H]$^+$

(2-Phenyl-oxiranyl)-lithium phosphonate mono-ethyl ester (3.4)

![Chemical structure](image)

To a solution of diethyl phosphonate **3.3** (0.1 g, 0.39 mmol) in 4-methyl-2-pentanone (5 mL), lithium bromide (0.034 g, 0.39 mmol), was added and the mixture was stirred at 70 °C. After a few minutes lithium bromide dissolved and a white precipitate started to precipitate. Heating was continued for about 2 hours. The solvent was
removed under vacuo and ether (20 mL) was added to the residue. The product was collected by filtration and washed with another 10 mL of ether. No further purification was needed as the compound was pure on TLC and NMR studies. The pure compound 3.4 was obtained as white solid. (0.05 g, 48%).\(^{1}\)H NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 7.39-7.24 (m, 5H), 5.0 (d, 2H), 3.7 (q, 2H, J=7.50), 2.5 (s, 1H), 1.2 (t, 3H, J=7.5); \(^{13}\)C NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 153.13, 137.10, 127.95, 127.82, 124.83, 92.74, 59.99, 16.57; HRMS (ESI) \(m/z\) calcd for C\(_{10}\)H\(_{13}\)O\(_4\)P is 228.055, found 227.01 [M-H]\(^{-}\).

**Diazo-phenyl-acetic acid methyl ester (3.7):**

\[
\begin{align*}
\text{CH}_2\text{CO}_2\text{H} + \text{p-ABSA} & \xrightarrow{\text{DBU, acetonitrile, rt, 93%}} \text{CH}_2\text{CO}_2\text{N}_2 \\
3.7
\end{align*}
\]

To a stirred solution of 4-acetamidobenzenesulfonyl azide (p-ABSA, 24.8 g, 20.0 mmol) in 45 mL of dry acetonitrile was charged with methyl phenyl acetate (2.5 g, 17 mmol) followed by the dropwise addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 3.55 g, 23.3 mmol) at ambient temperatures. Reaction mixture gradually turned into reddish from yellow. After the completion of the reaction by TLC, in about 8 hours, the reaction was diluted with 20 mL of water. Product was extracted with ether (3×15 mL). Organic fractions were
combined and washed with 10% sodium bicarbonate solution (3×10 mL), followed by saturated brine solution (3×10 mL). Organic layer was dried over anhydrous sodium sulfate before evaporating under reduced pressure to give crude product. Flash chromatography on silica gel using EtOAc/Hexane (1:9) as eluent afforded pure diaza ester 3.7 as a red liquid (2.69 g, 93%). $^1$H NMR (DPX 250 MHz, CDCl$_3$) δ 7.4-7.3 (m, 2H), 7.3-7.2 (m, 2H), 7.1-7.0 (1H), 3.75 (s, 3H); $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) δ 165.58, 129.29, 128.6, 127.13, 125.50, 123.95, 52.04.

**General Procedure for Rh$_2$(OAc)$_4$ catalyzed carbene insertion reaction:**

![Diagram](image)

To a flame dried 100 mL 3 necked round bottom flask, Rh$_2$(OAc)$_4$ was charged under nitrogen flow. To the same freshly distilled dichloromethane was charged followed by the addition of aldehyde. The resulting solution was brought to 45 °C and a solution of diazo ester 3.7 in dry dichloromethane was added over a period of 1.5
hours. The reaction was monitored for the progress by thin layer chromatography. After the disappearance of the aldehyde the reaction was brought to ambient temperatures and the reaction mixture was filtered through Celite and the same was concentrated under reduced pressure. The crude product obtained was subjected to flash column chromatography on silica gel to get corresponding epoxy carboxylates (racemic) \textbf{3.8a-g} in pure form. (TLC solvent 30\% EtOAc in 70\% Hexane)

\[
\text{2,3-diphenyl-oxirane-2-carboxylic acid methyl ester (3.8a)}
\]

colorless solid, 88\% yield \textsuperscript{1}H NMR (DPX 250 MHz, CDCl\textsubscript{3}) \text{\( \delta \)} 7.6 (m, 2H), 7.4-7.3 (m, 8H), 4.1 (s, 1H), 3.5 (s, 3H); \textsuperscript{13}C (DPX 250 MHz, CDCl\textsubscript{3}) \text{\( \delta \)} 167.2, 134.7, 133.7, 128.9, 128.7, 128.6, 128.4, 126.2, 126.0, 67.1, 65.9, 52.3.

\[
\text{3.8a}
\]

\[
\text{3.8b}
\]
3-(4-chloro-phenyl)-2-phenyl-oxirane-2-carboxylic acid methyl ester (3.8b) white solid, 67% yield. $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.6-7.5 (m, 2H), 7.35-7.30 (m, 3H), 7.25-7.2 (m, 4H), 4.04 (s, 1H), 3.49 (s, 3H)

![3.8c](image)

3-(4-bromo-phenyl)-2-phenyl-oxirane-2-carboxylic acid methyl ester (3.8c) white solid, 91% yield. $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.9 (d, 2H), 7.7 (d, 2H), 7.4-7.2 (m, 5H), 4.2 (s, 1H), 3.7 (s, 3H), 3.6 (s, 3H); LCMS (ESI) m/z calcd for C$_{16}$H$_{13}$BrO$_3$ is 332.0, found 332.9 & 334.9 [M+2+H]$^+$

![3.8d](image)

3-(4-methoxy-phenyl)-2-phenyl-oxirane-2-carboxylic acid methyl ester (3.8d) white solid, 86% yield. $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.63 (d, 2H), 7.40-7.25 (m, 5H), 6.95-6.85 (d, 2H), 4.08 (s,
1H), 3.78 (s, 3H), 3.56 (s, 3H); HRMS (ESI) m/z calcd for C₁₇H₁₆O₄ is 284.10, found 285.1 [M+H]⁺.

3-(4-nitro-phenyl)-2-phenyl-oxirane-2-carboxylic acid methyl ester (3.8e) yellowish solid, 63% yield. ^1H NMR (DPX 250 MHz, CDCl₃) δ 8.4 (d, 2H), 8.1 (d, 2H), 7.8-7.1 (m, 5H), 4.1 (s, 1H), 3.4 (s, 3H); ^13C (DPX 250 MHz, CDCl₃) δ 165.3, 134.2, 130.6, 129.2, 128.5, 127.1, 126.9, 124.7, 124.5, 66.4, 63.3, 52.4,; HRMS (ESI) m/z calcd for C₁₆H₁₃NO₅ is 300.08, found 317.11 [M+NH₄]⁺

3-(2-methoxy-phenyl)-2-phenyl-oxirane-2-carboxylic acid methyl ester (3.8f) colorless solid, 87% yield. ^1H NMR (DPX 250 MHz, CDCl₃) δ 7.6 (m, 2H), 7.45-7.25 (m, 4H), 6.95-6.75 (m, 3H), 4.32 (s, 1H), 3.73 (s, 3H), 3.40 (s, 3H); HRMS (ESI) m/z calcd for C₁₆H₁₇O₄ is 284.10, found 285.10 [M+H]⁺
3-(2-nitro-phenyl)-2-phenyl-oxirane-2-carboxylic acid methyl ester (3.8g) yellowish solid, 72% yield. $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 8.2-8.1 (d, 1H), 7.8-7.6 (m, 4H), 7.4-7.2 (m, 4H), 4.6 (s, 1H), 3.3 (s, 3H); HRMS (ESI) $m/z$ calcd for C$_{16}$H$_{13}$NO$_5$ is 299.07, found 300.08 [M+H]$^+$, 317.11 [M+NH$_4$]$^+$

General procedure for the deprotection of methyl ester to carboxylic acid:-

To a solution of $\alpha,\beta$-epoxy methyl carboxylate 3.8a-g in tetrahydrofuran-water mixture (~1:1) was added excess LiOH solution
(1N) at 0 °C under stirring. Progress of the deprotection was monitored by thin layer chromatography. Upon completion of the deprotection the reaction mixture was subjected to rotary evaporation to remove organic solvent. The resulting basic solution was washed with ethyl acetate to remove any unreacted ester and the aqueous layer was cooled in an ice bath before neutralizing to pH ~7.0. The same was extracted into ethyl acetate (3×40 mL) and the organic portions were combined together and dried over anhydrous Na₂SO₄ before concentrating under reduced pressure. The product was pure enough most of the times but needed to do flash column chromatography to get the corresponding carboxylic acid 3.9a-g (racemic). (TLC solvent 10% MeOH in 90% EtOAc)

![3.9a](image)

**2,3-diphenyl-oxirane-2-carboxylic acid (3.9a)** white solid, 76% yield. ¹H NMR (DPX 250 MHz, CDCl₃) δ 11.9 (s, 1H), 7.6 (m, 2H), 7.4-7.3 (m, 3H), 4.0 (s, 1H); HRMS (ESI) m/z calcd for C₁₅H₁₂O₃ is 240.08, found 241.09 [M+H]⁺.
3-(4-chloro-phenyl)-2-phenyl-oxirane-2-carboxylic acid (3.9b)
off-white solid, 80% yield. $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 181.9, 137.8, 134.9, 132.1, 128.6, 127.8, 127.6, 127.3, 126.3; HRMS (ESI) $m/z$ calcd for C$_{15}$H$_{11}$ClO$_3$ is 274.04, found 275.04 [M+H]$^+$. 

3-(4-bromo-phenyl)-2-phenyl-oxirane-2-carboxylic acid (3.9c)
white solid 81% yield. $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.45-7.35 (m, 4H), 7.6-7.5 (m, 3H), 7.3-7.2 (m, 2H), 4.4 (s, 1H), 3.3 (bs, 1H); $^{13}$C-NMR 177.96, 133.69, 131.69, 129.41, 129.17, 128.70, 127.90, 127.44, 126.40, 65.48, 40.98.; HRMS (ESI) $m/z$ calcd for C$_{15}$H$_{11}$BrO$_3$ is 317.9, found 318.9 [M+H]$^+$
3-(4-methoxy-phenyl)-2-phenyl-oxirane-2-carboxylic acid (3.9d) white solid, 58% yield. HRMS (ESI) m/z calcd for C\textsubscript{16}H\textsubscript{14}O\textsubscript{4} is 270.09, found 271.09 [M+H]\textsuperscript{+}.

![Image](3.9e)

3-(4-nitro-phenyl)-2-phenyl-oxirane-2-carboxylic acid (3.9e) yellow solid, 62% yield. HRMS (ESI) m/z calcd for C\textsubscript{15}H\textsubscript{11}NO\textsubscript{5} is 285.06, found 286.07 [M+H]\textsuperscript{+}.

![Image](3.9f)

3-(2-methoxy-phenyl)-2-phenyl-oxirane-2-carboxylic acid (3.9f) white solid, 66% yield. HRMS (ESI) m/z calcd for C\textsubscript{16}H\textsubscript{14}O\textsubscript{4} is 270.09, found 271.09 [M+H]\textsuperscript{+}.
3-(2-nitro-phenyl)-2-phenyl-oxirane-2-carboxylic acid (3.9g)
brown solid, 74.3% yield, $^1$H NMR (DPX 250 MHz, DMSO-d$_6$) δ 8.2-8.1
(d, 1H), 7.8-7.6 (m, 4H), 7.4-7.2 (m, 3H), 7.6-7.5 (d, 1H), 4.4 (s, 1H); HRMS (ESI) m/z calcd for C$_{15}$H$_{11}$NO$_5$ is 285.06, found 286.07
[M+H]$^+$

2,3-diphenyloxirane (3.10)

To an ice cold solution of m-CPBA (65%, 2.7 g, 10.20 mmol) in
chloroform (30 mL) was added a solution of trans-stilbene (1.0 g, 5.55
mmol) in chloroform (30 mL) over a period of 30 minutes. The
reaction was left to come to ambient temperatures and stirred further
until the starting material disappeared. The reaction mixture was
diluted with chloroform (40 mL) and the same was washed with saturated NaHCO₃ (3×30 mL) and saturated brine solution (3×20 mL) successively and the organic layer was dried over anhydrous Na₂SO₄ before concentrating to get crude product which on further purification by flash column chromatography gave pure epoxide **3.10** as thick colorless oil (0.73 g, 67.59%). ¹H NMR (DPX 250 MHz, CDCl₃) δ 7.47-7.37 (m, 10H), 3.92 (s, 2H); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 137.1, 128.6, 128.4, 125.6, 62.9; HRMS (ESI) m/z calcd for C₁₄H₁₂O is 196.08, found 197.09.[M+H]⁺

**β-azido-α-phenyl benzeneethanol (3.11)**

![Chemical Reaction diagram]

Trans-stilbene oxide **3.10** (1g, 5.11 mmol), sodium azide (0.76 g, 11.73 mmol), and NH₄Cl were dissolved in 40 mL of MeOH: H₂O (4:1). The resulting clear solution was refluxed for 2.5 hrs and then allowed to stir at room temperature for additional 8 hrs. Upon completion of the reaction by TLC dichloromethane and water (25 mL each) were added to the reaction mixture. The two layers were separated and the water layer was extracted with dichloromethane (2×25 mL). The
organic layers were combined and washed with saturated brine solution (2×25 mL) before drying over anhydrous MgSO₄. Removal of solvent and further purification of the obtained crude by flash column chromatography gave pure azido-alcohol 3.11 as light yellow liquid (0.91 g, 74 %). ¹H NMR (DPX 250 MHz, CDCl₃) δ 7.3-7.2 (m, 10H), 4.8 (dd, 1H, J₁₂ = 3.00, J₁₃ = 3.50), 4.7 (d, 1H, J=6.75), 2.3 (d, 1H, J=3.00); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 139.71, 136.01, 128.74, 128.69, 128.41, 128.33, 128.11, 127.10, 77.00, 71.27.

2,3-Diphenylaziridine (3.12)

Azido-alcohol 3.11 (3.6 g, 15 mmol) and PPh₃ (4.01 g, 15.3 mmol) were dissolved in acetonitrile (40 mL) under nitrogen atmosphere and heated to reflux for 2.5 hrs. Upon completion of the reaction the solvent was removed under reduced pressure and the resultant crude was purified by flash column chromatography to get pure aziridine 3.12 as light yellow liquid (2.72 g, 92.6 %). ¹H NMR (DPX 250 MHz, CDCl₃) δ 7.32-7.12 (m, 10H), 3.00 (bs, 2H), 1.28 (bs, 1H, NH); ¹³C
NMR (DPX 250 MHz, CDCl₃) δ 139.7, 128.7, 127.5, 125.5, 43.7; HRMS (ESI) m/z calcd for C₁₄H₁₃N is 195.1048 found 196.1185 [M+H]⁺

1-Aziridinecarboxylic acid,2,3-diphenyl-, 1,1-dimethylethyl ester (3.13)

To a solution of aziridine 3.12 (0.50 g, 2.6 mmol) in dichloromethane (25 mL) at ice-cold temperature were added di-tert-butyl dicarbonate (0.67 g, 3.1 mmol) and DMAP (0.03 g, 0.25 mmol). Reaction was monitored for the progress by thin layer chromatography. Starting material completely disappeared in 40 minutes. Solvent was removed under reduced pressure and the crude was subjected to flash column chromatography to get pure compound as the colorless solid (0.69 g, 91.7 %). ¹H NMR (DPX 250 MHz, CDCl₃) δ 7.53-7.47 (m, 10H), 3.92 (s, 2H), 1.33 (s, 9H); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 159.41, 135.55, 128.52, 128.12, 127.03, 81.47, 47.63, 27.66.

2,3-diphenyl, 1-tert-butoxy carbonyl-2-aziridine carboxylic acid (3.14)
To a stirred solution of 1-tert-butoxy carbonyl-2-aziridine 3.13 in tetrahydrofuran was added TMEDA at -78 °C. To the above solution n-BuLi was added and the solution stirred at the same temperature for 2 hrs before adding dry ice. The reaction was brought to room temperature gradually and monitored for the progress by thin layer chromatography. No significant product was obtained even though HRMS and LCMS showed the presence of product. Repeated reactions with s-BuLi and t-BuLi did not result in any improvements.

**N-(phenylmethylene)-methanamine (3.16)**

A mixture of benzaldehyde (12.0 g, 113 mmol) and excess of 40% methane amine in water (16 mL, 192.0 mmol) was stirred at 0 °C for 2 hrs and further stirred at ambient temperatures overnight. After the
completion of the reaction the product was extracted into ethyl acetate (3×50 mL). Organic layers were combined washed with brine solution (3×40 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and further vacuum purified to get pure product 3.16 as liquid (13.45 g, 97%) 1H NMR (250 MHz, CDCl₃) δ 8.25 (s, 1H), 7.75 (m, 2H), 7.45 (m, 3H); 13C NMR (250 MHz, CDCl₃) δ 162.50, 136.28, 130.65, 128.64, 127.01, 40.29.

**Benzenemethanamine-4-methoxy-N-(phenylmethylene) (3.17)**

To a stirred solution of benzaldehyde (1.39 g, 13.1 mmol) and 4-methoxy benzylamine (1.8 g, 57 mmol) in dichloromethane (30 mL) was added anhydrous Na₂SO₄ (1.6 g, 113 mmol) at ambient temperatures. Reaction was stirred at the same temperature for about 12 hours before filtering the solids off and concentrating under reduced pressure. The crude product obtained was subjected to flash column chromatography to get pure compound 3.17 as a colorless liquid (2.36 g, 80.0%). 1H NMR (DPX 250 MHz, CDCl₃) δ 8.21 (s, 2H), 7.68-7.58 (m, 2H), 7.30-7.20 (m, 3H), 7.17-7.08, (d, 2H), 6.80-6.70
(d, 2H); $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 192.44, 158.76, 136.30, 131.46, 130.80, 129.81, 129.08, 128.68, 128.35, 64.59, 55.32.

**Benzyl-benzylidene-amine (3.18)**

\[
\text{[Chemical Structure]}
\]

Same procedure was followed as mentioned for 3.17 benzaldehyde (6.0 g, 57 mmol) and benzylamine (6.05 g, 56.5 mmol) in dichloromethane (100 mL) with anhydrous Na$_2$SO$_4$ (16.06 g, 113.1 mmol). 3.18 is colorless liquid. (10.26 g, 92.93 %). $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 8.46 (s, 1H), 7.95-7.75 (m, 2H), 7.55-7.25 (m, 6H), 4.90 (s, 2H); $^{13}$C (DPX 250 MHz, CDCl$_3$) $\delta$ 162.08, 139.33, 136.18, 130.84, 128.67, 128.56, 128.34, 128.04, 127.06, 65.10; LCMS (ESI) $m/z$ calcd for C$_{14}$H$_{13}$N is 195.1048 found 196.10 [M+H]$^+$.  

**1-Benzyl-2,3-diphenyl-aziridine-2-carboxylicacid methyl ester (3.19)**
To a stirred solution of imine (0.886 g, 4.54 mmol) in dry dichloromethane (15 mL) was added Rh₂(OAc)₄ (0.02 g, 0.045 mmol) under nitrogen atmosphere and the reaction mixture was heated to 40 °C. A solution of diazo ester 3.7 (0.80 g, 4.54 mmol) in dry dichloromethane (10 mL) was added over a period of 40 minutes and the reaction was monitored for progress. After the completion, the reaction mixture was passed through Celite bed to remove rhodium salts. The solvent was removed and the crude was flash chromatographed to get pure compound as yellow solid (0.426 g, 30.3%). ¹H NMR (DPX 250 MHz, CDCl₃) δ 7.75 (m, 3H), 7.65-7.15 (m, 12H), 4.31 (s, 1H), 3.91 (s, 2H), 3.60 (s, 3H); ¹³C NMR (DPX 250 MHz, CDCl₃) δ 165.78, 139.47, 138.04, 132.20, 131.09, 128.89, 128.77, 128.48, 128.37, 128.18, 128.06, 127.61, 127.20, 79.86, 64.34, 52.29, 51.35. HRMS (ESI) m/z calcd for C₂₃H₂₁NO₂ 343.1572 found 344.1626 [M+H]⁺.

1-Benzyl-2,3-diphenyl-aziridine-2-carboxylicacid (3.20)
Same procedure was used as mentioned for the hydrolysis of ester 3.8a-g except increasing the THF ratio in the solvent mixture.

### 3.8. References


10. a). Lu, C. D.; Chen, Z. Y.; Liu, H.; Hu, W. H.; Mi, A. Q. Highly Chemoselective 2,4,5-Triaryl-1,3-Dioxolane Formation from Intermolecular 1,3-dipolar Addition of Carbonyl Ylide with Aryl
11. Crystal Structure Details and NMRs are available in the supporting information of this dissertation.


4.1 General introduction

The genetic information of most organisms is encoded in the sequence of double stranded DNA (dsDNA), also known as the molecule of life, which is transcribed into mRNA during transcription. The blocking of transcription has become an attractive target for therapeutic discovery.\textsuperscript{1, 2} A variety of reagents, both synthetic and natural, are capable of interacting with DNA and (or) RNA made up off purines and pyrimidines (Figure 4.1a and 4.1b). These interactions may inactivate or completely destroy the nucleic acids. This means the unwanted or disease causing genes, responsible for the production of mutated proteins and ultimately leading to the production of dysfunctional proteins, can be repaired by utilizing the specific hydrogen bonding patterns between the base pairs. Specially designed short nucleic acid sequences or oligonucleotides can selectively bind to the targeted DNA or RNA.
Oligonucleotides that selectively bind to the DNA are termed ‘antigene oligonucleotides’\(^3\) and the oligonucleotides that selectively bind to the RNA are called ‘antisense oligonucleotides’\(^4\). Peptide Nucleic Acids are hybrid structures with some special advantages.

Figure 4.1a. DNA double helix structure-sugar phosphate backbone-adenine-thymine, guanine-cytosine bases [courtesy: dearbornschools.org]

Since the discovery of Peptide Nucleic Acids (PNAs) by Nielsen et al\(^5-6\) in 1991 they have attracted significant attention as promising candidates for the gene therapeutic\(^7-11\) drug discovery. PNAs have applications in therapeutic drug discovery, diagnosis\(^12\) and biosensing\(^13\).
PNAs are achiral, neutral, unnatural DNA analogs (Figure 4.2) in which the nucleobases are attached to the pseudo peptide backbone, made of repeated units of 2-aminoethylene glycine moieties, via methylenecarbonyl linkers. PNA’s are interesting pseudo peptides because of their remarkable affinity and specificity to hybridize with complimentary nucleic acids, and their resistance to chemical and biological reactions catalyzed by proteases and nucleases. These properties are due to their uncharged and flexible polyamide backbone. They mimic DNA and/or RNA by forming hetero duplexes with complimentary nucleic acids. The complexes formed by the interaction of PNA with DNA and/or RNA generally show higher thermal stabilities than the complexes formed by the interaction of DNA with DNA or RNA.
The PNAs bind to the complimentary DNA and RNA sequences through Watson-Crick, and Hoogsteen in some cases, hydrogen bonding with higher affinity than the corresponding DNA or RNA sequences.

Even though the peptide nucleic acids are considered as promising candidates for the gene therapeutic drug discovery due to their remarkable stability towards nucleases and proteases and have many desirable properties, there are some drawbacks which have to be answered in order to have these macromolecules ready as drug candidates. Current PNAs exhibit poor cellular uptake\textsuperscript{14-15} in vitro and poor bioavailability in vivo. This inherent limitation has prevented
widespread application of PNAs as therapeutics as well as in basic research. Unmodified PNA oligomers are essentially not taken up by eukaryotic or prokaryotic cells in vitro and when delivered in animals in vivo (intravenous or intraperitoneal) they are quickly excreted through the kidneys. This is partly attributed to the uncharged property of the peptide nucleic acids. In comparison, Nucleic acid oligonucleotides are negatively charged phosphates, and can readily penetrate through the cell membrane when complexed with positively charged lipids. To solve this problem some researchers synthesized positively charged PNAs based on lysine and arginine residues\textsuperscript{16-19}. Inspired by the idea to increase cellular uptake by introducing the positively charged species into the PNA, we were interested in investigating the cellular uptake of cysteine based PNAs abbreviated as CPNAs.

![Figure 4.3. Proposed structure of cysteine based PNA (CPNA)](image)
The reason behind selecting cysteine is the presence of thiol group. This gives us an easy entry to synthesize more varied PNAs by using a variety of alkyl groups from a simple methyl group to the long poly-ether side chain as well as the guanidine-based side chain shown in Figure 4.3.

4.2 Results and discussion:

4.2.1 Synthesis of standard PNA monomers:-

The strategy of PNA synthesis is based on solid phase chemistry. For this strategy to work, protected PNA monomers were synthesized based on the ethylene diamine precursor.\textsuperscript{20} The monomers were synthesized following the standard protocols in the literature. Fmoc-based monomers are especially needed to monitor the oligomerization using UV-based probes.\textsuperscript{21}

Readily available ethylene diamine is mono protected with di-t-butyl carbonate anhydride to \textbf{4.1} and further protection of $1^\circ$ amine group with Fmoc-Cl yielded \textbf{4.2} in very good yields. Deprotection of the Boc group and subsequent alkylation with tert-butyl bromo acetate gave mono alkylated amine \textbf{4.4} as major product in very good yields.
Submonomer 4.4 is coupled with orthogonally protected nucleobase acetic acids to get compounds 4.5a-d. Deprotection of tertiary butyl ester in mild acid conditions gave desired monomers 4.6a-d in excellent yields. (Scheme 4.1)\textsuperscript{22-24}

**Scheme 4.1** Synthesis of Fmoc protected PNA monomers

Synthesis of PNA monomers for Boc based solid phase synthesis are started with the common intermediate 4.1. In this scheme, compound 4.1 is reacted with methyl bromo acetate to give submonomer 4.7 in very decent yields which is reacted with orthogonally protected nucleobase acetic acids using uranium complex HATU as coupling agent to get compounds 4.8a-d in very good yields. (Scheme 4.2).\textsuperscript{25}
4.2.2 Synthesis of CPNA monomers:

Cysteine-based PNA monomers are synthesized starting from the commercially readily available S-Trityl-N-Boc L-cysteine. (Scheme 4.3) Synthesis involves the activation of carboxylic acid with DCC followed by attack of amine nucleophile to enable the formation of amide 4.10. Upon selective reduction of amide 4.10 with etherated borane yielded amine 4.11 in very good yields. Alkylation of the amine via S$_{N}$2 reaction using DIEA as base gave the desired mono alkylated ester 4.12 in moderate yields. Selective unmasking of thiol group and subsequent alkylation with methyl iodide gave submonomer 4.14 in...
decent yields.\(^{28}\) The coupling of orthogonally protected nucleobase acetic acids to the submonomer 4.14 gave corresponding methyl esters 4.15a-d in pretty good yields. Methyl esters were hydrolyzed in alkaline conditions to get CPNA monomers 4.16a-d in very good yields.\(^{29}\)

**Scheme 4.3** Synthesis of novel cysteine PNA monomers

### 4.2.3 Synthesis of novel polyether side chain:

Oligo-ethers are known to have water solubility compared to the alkyl analogues because of the presence of oxygen group which can form hydrogen bonds with the water in biological systems.\(^{30-31}\) This property encouraged us to come up with a new alkyl group 4.21.
(Scheme 4.4) for our ongoing PNA synthesis. Synthesis of the compound 4.21 starts with the commercially available 3-bromopropanamine salt. 3-bromopropanamine hydrobromide salt was reacted with di-t-butyl dicarbonate in aqueous medium in the presence of sodium bicarbonate to afford quantitative yields of 4.17. Excess sodium bromide was used to make sure the bromide is not hydrolyzed to the corresponding hydroxide. Bromide 4.17 was alkylated onto triethylene glycol monomethyl ether 4.18 using sodium hydride to get 4.19 in decent yields. Deprotection of the Boc group and subsequent reaction with bromo acetyl bromide gave 92% of the amide 4.21.32

Scheme 4.4 Synthesis of novel polyether side chain
Novel side chain 4.21 can be alkylation onto 4.13 to achieve novel cysteine based monomers and further achieve PNAs with novel properties as to achieve the goals of this project.

4.3 Solid phase synthesis of PNAs

Standard solid phase synthesis protocol was followed for the synthesis of PNA oligomers for both normal as well as cysteine based PNA synthesis. The Symphony instrument from Protein Technologies Inc was used for the synthesis of Fmoc based methods on MBHA resin. Manual synthesis used the Boc strategy. MBHA resin was swollen in NMP for 45 minutes and was downloaded with Fmoc-Lys(Boc)-OH before growing PNA chain. Substitution level of approximately 0.15 mmol/g was maintained so as to achieve the best results as reported in the literature. Fmoc deprotection was then performed using piperidine (20%) and DBU (2%) in NMP. Coupling of monomers with protecting group was carried out with excess of reagents (at least five fold excess). After the coupling is complete the resin was washed with necessary solvents and the same cycle continued until the desired length and sequence was achieved. After the final coupling cycle PNA was global deprotected and cleaved from the resin using TFA, DMS and TFMSA.
4.4. Solid phase synthesis results

<table>
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<th>Sequence</th>
<th>MW*</th>
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</tr>
<tr>
<td>HOOC-G-G-C-T-NH₂</td>
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<tr>
<td>HOOC-Lys-T-T-T-T-NH₂</td>
<td>1742.354</td>
</tr>
<tr>
<td>H₂N-Lys-T-T-T-T-T-CO₂H</td>
<td>2026.427</td>
</tr>
<tr>
<td>HOOC-Lys-T-T-C-T-C-C-C-T-C-T-C-NH₂</td>
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<tr>
<td>COOH-C-T-T-T-C-T-A-C-A-T-T-Lys</td>
<td>3312.345**</td>
</tr>
</tbody>
</table>

* Characterization was done by MALDI-TOF on BRUKER-autoflex
** Synthesis carried out using Bts protecting group at 60 °C on Symphony Peptide Synthesizer

|Table 4.1. Solid phase synthesis of PNAs|

4.5 Conclusion

Syntheses of standard PNA monomers and novel Cysteine PNA monomers were successfully achieved. Optimizations were done to synthesize newly designed CPNA monomers with varying alkyl groups on sulphur group. Partially successful results were tabulated above and the schemes mentioned and developed for the synthesis of monomers will be utilized in the future synthesis of PNAs.
4.6 Experimental procedures

(2-Amino-ethyl)-carbamic acid tert-butyl ester (4.1): To the ice cold solution of ethylene diamine (13.48 g, 224.3 mmol) in 250 mL of tetrahydrofuran was added a solution of di-t-butyl carbonate anhydride (10.0 g, 46 mmol) in 50 mL of tetrahydrofuran over a period of 25 minutes. The reaction was continued at 0 °C for 3 hours and then brought to ambient temperatures and continued for 20 more hours. Upon complete consumption of the di-t-butyl carbonate anhydride, the reaction mixture was concentrated under reduced pressure. The residue obtained was dissolved in ethyl acetate (150 mL) and washed with water (4×45 mL). The aqueous layer was back extracted with ethyl acetate; organic portions were mixed, dried over anhydrous sodium sulphate (Na₂SO₄), and concentrated under reduced pressure. The crude product was flash chromatographed to give mono Boc protected amine 4.1 in good quantities, 5.8 g (79%). H NMR (DPX 250 MHz, CDCl₃) δ 5.35 (bs, 1H) 3.16 (m, J=5.75, 2H), 2.78 (t, J=5.5, 2H), 1.45 (s, 9H), 1.3 (s, 2H). C NMR (DPX 250 MHz, CDCl₃) δ
156.3, 78.9, 43.2, 41.7, 28.3. LCMS (ESI) m/z calcd for C_{7}H_{16}N_{2}O_{2} is 160.1, found 161.2 [M+H]^+ 

**tert-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl) amino ethyl] glycinate (4.2):** To the ice cold solution of (2-amino-ethyl)-carbamic acid *tert*-butyl ester 4.1 (0.5 g, 3.120 mmol) in 100 mL of dry dichloromethane was added diisopropylethylamine (0.443 g, 3.43 mmol). To the resulting solution of 9-fluorenylmethyl chloroformate in 7 mL of dichloromethane was added over a period of 15 minutes. The reaction was stirred at 0 °C for two hours. After the reaction was complete, the same was diluted to 150 mL with dichloromethane and washed with 45 mL of half saturated brine solution. The organic layer was dried over anhydrous Na_{2}SO_{4} and concentrated. The crude product was purified by precipitation using ethyl acetate and hexanes to get 1.19 g (90.75%) of 4.2 as a white fluffy solid. $^{1}$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.78-7.72 (d, 2H, J=7.5); 7.62-7.56 (d, 2H, J=7.5); 7.44-7.24 (m, 4H); 5.22 (bs, 1H); 4.82 (bs, 1H); 4.42 (d, 2H, J=6.75); 4.21 (t, 1H, J=5.5); 3.28 (bs, 4H); 1.44 (s, 9H).
2-(9H-Fluoren-9-ylmethoxycarbonylamino)-ethyl-ammonium chloride (4.3):- A solution of orthogonally protected ethylene diamine 4.2 (0.5 g, 1.307 mmol) in 15 mL of 1, 4-dioxane was cooled to 0 °C. HCl gas was passed through the solution for 4-5 minutes and reaction was brought to ambient temperature by removing the ice bath. The reaction was monitored by TLC for the progress. After complete consumption of the starting material, in about 4 hrs, solvent was removed under reduced pressure and the product was used in the next step after drying under high vacuum to give pure product 4.3 as white solid (~ quantitative yields). $^1$H NMR (DPX 250 MHz CDCl$_3$) $\delta$ 7.84 (d, 2H, Fmoc Ar CH); 7.65 (d, 2H, Fmoc Ar CH); 7.44-7.27 m, 5H, Fmoc Ar CH, NHFmoc); 4.42 (d, 2H, J=6.5, Fmoc CH$_2$); 4.22 (t, 1H, J=6.5, Fmoc, CH$_2$); 3.37 (t, 2H, CH$_2$-NHFmoc); 3.30 (bs, 3H, NH$_3$); 3.02 (t, 2H, CH$_2$-NH$_3$).
**tert-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl) aminoethyl] glycinate ester (4.4):** To a cold solution of 4.3 (0.5 g, 1.3 mmol) in THF (30 mL) were added diisopropylethylamine (0.432 ml, 2.6146 mmol), tetrabutylammonium iodide (0.155 g, 0.156 mmol) and tert-butyl bromoacetate (0.195 ml, 1.3073 mmol) under stirring. Reaction was continued for 10 hrs at 0 °C and then brought to ambient temperature. Reaction mixture was concentrated under reduced pressure and flash chromatographed to get the desired mono alkylated product 4.4 as white semi-solid (0.181 g, 36%). ^1H NMR (DPX 250 MHz CDCl₃) δ 7.67 (d, 2H, J=7.25, Fmoc Ar CH); 7.52 (d, 2H, J=7.25, Fmoc Ar CH); 7.35-7.16 (m, 5H, Fmoc Ar CH, NH); 5.39 (bs, 1H, NH); 4.31 (d, 2H, J=6.75, Fmoc CH₂); 4.13 (t, 1H, J=6.75, Fmoc CHO); 3.21 (bs, 1H, NH); 2.67(t, 2H, CH₂) 1.92 (s, 2H, NHCH₂CO); 1.40 (s, 9H, ^1Bu).

**General procedure for nucleo-base coupling (4.5a-d):** DIEA was added to a solution of Cbz protected nucleo-base acetic acid, HATU and compound 4.4 in DMF at room temperature. The reaction was monitored for its progress. Starting material was completely consumed within 20–25 minutes. Solvent was removed under high vacuum and the residue was dissolved in ethyl acetate and washed with saturated brine solution. The organic layer was dried over anhydrous Na₂SO₄ and
concentrated. Flash column chromatography of the crude gave coupled products 4.5a-4.5d in 65-95% yield.

\[ \text{FmocHN} \text{N} \text{CO}_2^\text{Bu} \quad \text{B-OH} \quad \text{FmocHN} \text{N} \text{CO}_2^\text{Bu} \]

\[ \text{HATU, DIEA, DMF} \quad \text{rt, 60-85\%} \]

\{2-(9H-Fluoren-9-ylmethoxycarbonylamino)-ethyl\}-\{2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetyl\}\-amino\}-acetic acid tert-butyl ester (4.5a) white solid, 96\% yield.

\[ ^1\text{H NMR (DPX 250 MHz, DMSO-d}_6\text{) } \delta 7.89 \text{ (d, 2H, } J=7.5 \text{ Fmoc Ar CH); 7.68 \text{ (d, 2H, } J=7.5 \text{, Fmoc Ar CH); 7.46-7.23 \text{ (m, 5H, Fmoc Ar CH, NH);}
4.64 \text{ (s, 1H, CH); 4.45 (s, 1H); 4.32 (d, 2H, } J=, \text{ Fmoc CH}_2\text{); 4.22 (t, 1H, J=} , \text{ Fmoc CH);} \text{ 1.73 (s, 3H, CH}_3\text{); 1.45-1.39 (ss, 9H, -C(CH}_3\text{)_3).} \]

\[ ^13\text{C NMR (DPX 250 MHz, DMSO-d}_6\text{) } \delta 167.9, 167.2, 164.3, 156.2, 150.9, 143.8, 142.1, 140.7, 127.6, 127.0, 125.1, 120.1, 108.1, 81.9, 80.9, 48.7, 47.6, 46.9, 27.6. \]
4.5b

{[2-(4-Benzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-acetyl]-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino}-acetic acid tert-butyl ester (4.5b) white solid, 94% yield.

$^1$H NMR (DPX 250 MHz, CDCl$_3$) δ 10.81 (s, 1H), 7.92 (m, 3H), 7.75 (d, 2H), 7.62-7.21 (m, 9H), 7.02 (d, 1H), 5.23 (s, 2H), 4.81 (s, 1H), 4.66 (s, 1H), 4.44-4.25 (m, 4H), 3.93 (s, 2H), 3.46 (2H), 3.1 (2H), 1.42 (s, 9H).

4.5c

{[2-(5-Benzyloxycarbonylamino-7-oxo-6,7-dihydro-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-acetyl]-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino}-acetic acid tert-butyl ester (4.5c) white solid, 94% yield.

$^1$H NMR (DPX 250 MHz, CDCl$_3$) δ 10.81 (s, 1H), 7.92 (m, 3H), 7.75 (d, 2H), 7.62-7.21 (m, 9H), 7.02 (d, 1H), 5.23 (s, 2H), 4.81 (s, 1H), 4.66 (s, 1H), 4.44-4.25 (m, 4H), 3.93 (s, 2H), 3.46 (2H), 3.1 (2H), 1.42 (s, 9H).
ester (4.5c) white solid, 82% yield. $^1$H NMR (DPX 250 MHz, MeOD-d$_4$)
$\delta$ 11.35 (1H, NH); 7.87 (d, 2H); 7.81 (d, 1H); 7.43-7.34 (m, 9H); 5.24 (3H); 5.08 (s, 1H); 4.43-4.18 (m, 4H); 3.96 (s, 1H); 1.53-1.34 (s, 9H).

![4.5d](image)

tert-butyl ester (4.5d) white solid, 87% yield. $^1$H NMR (DPX 250 MHz, MeOD-d$_4$) $\delta$ 10.67 (s, 1H, NH); 8.55 (s, 1H); 8.32 (s, 1H); 7.89 (d, 2H, J=7.5); 7.68 (m, 2H); 7.51-7.21 (m, 12H); 5.35 (s, 1H); 5.25 (s, 1H); 4.42-4.25 (m, 4H); 4.12-3.94 (m, 2H); 3.64 (s, 1H); 2.02 (s, 2H); 1.53-1.35 (9H).

**General procedure for the deprotection of t-butyl esters 4.5a-d:**
To a stirred solution of ester 4.5 in dichloromethane was added TFA at ice cold temperatures. After the completion of deprotection TFA was removed under reduced pressure. The residue was dissolved in DCM and subjected to rotary evaporation and the same was repeated 3-5 times so as to remove the TFA to the maximum. And the crude residue was subjected to flash column chromatography to get pure compounds 4.6a-d as solids.

\[
\text{\{[2-(9H-Fluoren-9-ylmethoxycarbonylamino)-ethyl]-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetyl]-amino\}-acetic acid (4.6a) white solid, 92\% yield.} \quad \text{\textsuperscript{1}H NMR (DPX 250 MHz, DMSO-d\textsubscript{6}) \textsuperscript{\delta} 11.02 (s, 1H), 7.35 (1H), 7.02 (1H), 4.76 (s, 2H),}
\]
4.57 (1H), 4.33 (s, 1H), 4.02 (2H), 3.74 (s, 1H), 3.63 (s, 3H), 3.44-3.03 (m, 3H), 1.75 (s, 3H), 1.4 (s, 9H).

{[2-(4-Benzoyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)acetyl]-[2-(9H-fluoren-9-y1methoxycarbonylamino)-ethyl]amino}-acetic acid (4.6b) white solid, 87% yield. ¹H NMR (DPX 250 MHz, DMSO-d₆) ð 10.93 (bs, NH, 1H), 7.94 (1H), 7.55-7.44 (5H), 7.0 (1H), 5.23 (s, 2H), 4.82 (s, 2H), 4.12 (s, 2H), 3.65 (s, 3H), 3.45-2.93 (m, 4H), 1.42 (s, 9H).
{[2-(5-Benzylloxycarbonylamino-7-oxo-6,7-dihydro-
[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-acetyl]-[2-(9H-fluoren-9-
ylethoxycarbonylamino)-ethyl]-amino}-acetic acid (4.6c)
yellowish solid, 86% yield. LCMS (ESI) m/z calcd for C_{24}H_{29}N_{7}O_{7} is
527.2, found 528.1 [M+H]^+.

{[2-(6-Benzylloxycarbonylamino-purin-9-yl)-acetyl]-[2-(9H-
fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino}-acetic acid
(4.6d) white solid, 84% yield. ^1H NMR (Inova 400 MHz, DMSO-d$_6$) $\delta$
8.56 (d, $J = 19.3$ Hz, 1H), 8.49–8.45 (m, 1H), 7.84 (d, $J = 7.5$ Hz, 2H), 7.67–7.61 (m, 2H), 7.50–7.46 (m, 1H), 7.45–7.42 (m, 2H), 7.39–7.34 (m, 4H), 7.34–7.30 (m, 1H), 7.29–7.25 (m, 2H), 5.35 (s, 1H), 5.19 (d, $J = 18.2$ Hz, 3H), 4.33 (d, $J = 7.1$ Hz, 2H), 4.28–4.14 (m, 3H), 3.97 (s, 1H), 3.55–3.50 (m, 1H), 3.33 (d, $J = 15.4$ Hz, 2H).
Methyl N-(2-Boc-aminoethyl) glycinate (4.7):

Diisopropylethylamine (DIEA) (2.47 ml, 15.0 mmol) was added to an ice cold solution of (2-amino-ethyl)-carbamic acid tert-butyl ester 4.1 (2.0 g, 12.5 mmol) in 100 ml of dry tetrahydrofuran at 0 °C under nitrogen atmosphere. To the above solution, methyl bromoacetate (1.18 ml, 12.5 mmol) was added over a period of 15 minutes followed by the addition of tetra butyl ammonium iodide, TBAI (0.23 g, 0.6245 mmol). The temperature is maintained near 0 °C for 9 hrs and then brought to ambient temperature and continued for another 10 hours. Solvent is stripped off under reduced pressure and the crude is flash column chromatographed to get (2.1 g, 72%) of the mono alkylated compound 4.7 along with unwanted di alkylated compound. $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 5.13 (bs, 1H, NH); 3.75 (s, 3H, CH$_3$); 3.44 (s, 2H, CH$_2$); 2.73 (t, 2H, CH$_2$, $J$=5.5); 2.18 (bs, 1H, NH); 1.44 (s, 9H);
Same procedure was followed for coupling of 4.7 with nucleobase acetic acids to make 4.8a-d as mentioned in the synthesis of 4.5a-d.

{(2-tert-Butoxycarbonylamino-ethyl)-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetyl]-amino}-acetic acid methyl ester (4.8a) white solid, 93% yield. \(^1\)H NMR (DPX 250 MHz, DMSO-d\(_6\)), \(\delta\) 11.0 (s, 1H), 7.3 (1H), 7.0 (1H), 4.7 (1H), 4.1 (1H), 3.7 (1H), 3.6 (s, 3H), 3.5-3.0 (m, 5H), 1.8 (s, 3H), 1.4 (s, 9H).

[[2-(4-Benzylxoycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid methyl ester (4.8b) white solid, 91% yield. \(^1\)H NMR (DPX 250 MHz, MeOD-d\(_4\)), \(\delta\) 10.8 (s, 1H), 7.8 (d, 1H), 7.4 (m, 5H), 7.0 (d, 1H), 5.2 (s, 2H), 4.6 (s, 1H), 4.5-3.5 (m, 6H), 3.4 (s, 3H), 2.5 (s, 2H), 1.4 (s, 9H).
\[
[[2-(5-Benzyloxy carbonylamino-7-oxo-6,7-dihydro-\[1,2,3\]triazolo[4,5-d]pyrimidin-3-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid methyl ester (4.8c)\] white solid, 77% yield. \(^1\)H NMR (DPX 250 MHz, DMSO-d\(_6\)), \(\delta\)

11.5 (s, 1H), 11.4 (s, 1H), 7.8 (s, 1H) 7.6-7.3 (m, 5H), 5.3 (s, 2H), 4.1-3.2 (m, 11H), 1.4 (s, 9H)

\[
[[2-(6-Benzyloxy carbonylamino-purin-9-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid methyl ester (4.8d)\] white solid, 86% yield. \(^1\)H NMR (DPX 250 MHz, DMSO-d\(_6\)), \(\delta\)

10.7 (s, 1H), 8.5 (s, 1H), 8.2 (s, 1H), 8.0 (s, 1H), 7.8-7.2 (m, 5H), 5.2 (s, 2H), 4.5-4.0 (m, 4H), 3.3 (s, 3H), 2.5 (s, 2H), 1.4 (s, 9H).
General procedure for the deprotection of methyl esters 4.8a-d:

To a stirred solution of ester 4.8a-d in THF/H$_2$O was added 1M NaOH in water at ice cold temperatures. After the completion of deprotection, THF was removed under reduced pressure. The water layer was extracted with EtOAc to remove any impurities and the water layer was acidified to pH 4.0 and the product was extracted into EtOAc and the organic solvent was dried over anhydrous Na$_2$SO$_4$ and removed under reduced pressure. The crude product was chromatographed if necessary to obtain pure products 4.9a-d as solids.

\[
\begin{align*}
\text{4.8a-d} & \quad \xrightarrow{\text{NaOH, THF/H}_2\text{O, 90-97\%}} \quad \text{4.9a-d}
\end{align*}
\]

{(2-tert-Butoxycarbonylamino-ethyl)-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetyl]-amino}-acetic acid (4.9a) white solid, 94% yield. $^1$H-NMR (DPX 250 MHz, DMSO-d$_6$) 12.1
(s, 1H), 10.2 (bs, 2H), 7.1 (1H), 4.4 (m, 4H), 3.5-3.2 (m, 4H), 1.9 (2H), 1.4 (9H). LCMS (ESI) m/z 285.1 [M+H]^+.

![4.9b](image1)

[[2-(4-Benzzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid (4.9b) white solid, 93% yield. \(^1\)H NMR (DPX 250 MHz, DMSO-d\(_6\)), \(\delta\) 11.0 (bs, 2H), 7.9 (m, 1H), 7.5-7.3 (m, 5H), 7.1-6.9 (m, 1H), 5.2 (s, 2H), 4.8 (s, 2H), 4.0 (s, 2H), 3.3-3.1(m, 4H), 1.4 (s, 9H), LCMS (ESI) m/z 504.2 [M+H]^+.

![4.9c](image2)

[[2-(5-Benzzyloxycarbonylamino-7-oxo-6,7-dihydro-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid (4.9c) white
solid, 94% yield. $^1$H-NMR (DPX 250 MHz, DMSO-d$_6$), $\delta$ 11.0 (1H), 7.8 (s, 1H), 7.5-7.3 (m, 5H), 5.2 (s, 2H), 4.0 (s, 2H), 3.9-3.7 (m, 2H), 3.6-3.2 (m, 4H), 1.4 (s, 9H); LCMS (ESI) m/z 544.2 [M+H]$^+$. 

[[2-(6-Benzylxocarbonylamino-purin-9-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid (4.9d) white solid, 82% yield. LCMS (ESI) $m/z$ calcd for C$_{24}$H$_{29}$N$_7$O$_7$ is 527.21, found 528.1 [M+H]$^+$. 

(R)-tert-butyl 1-amino-1-oxo-(tritylthio) propan-2-ylcarbamate (4.10): - To a solution of commercially available S-trityl Boc Cysteine (2.0 g, 4.31 mmol), HOBt (0.70 g, 5.17 mmol) and DCC (1.07 g, 5.18 mmol) in THF (10ml) was added 28 % NH$_4$OH (0.44 ml, 6.46 mmol) at
0 °C. After 2 hr of stirring at 0 °C, the reaction mixture was filtered through Celite and filtrate was concentrated, diluted with ethyl acetate, and washed with water and brine. Organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude is flash chromatographed to get pure compound 4.10 as a white solid in nearly quantitative yields. (1.95 g, 98%) \(^1\)H NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 7.54-7.24 (15H, C\(_6\)H\(_5\)); 6.03 (bs, 1H); 5.55 (bs, 1H); 4.87 (bs, 1H); 3.93 (bs, 1H); 2.02 (s, 2H, CH\(_2\)) 1.44 (s, 9H); \(^{13}\)C NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 173.83, 155.42, 144.42, 130.04, 129.60, 128.10, 126.93, 80.34, 67.24, 53.22, 33.80, 28.33.; LCMS (ESI) \(m/z\) calcd for C\(_{27}\)H\(_{30}\)N\(_2\)O\(_3\)S is 462.19, found 485.16 [M+Na]\(^+\).

(R)-tert-butyl-1-amino-3(tritylthio) propan-2-ylcarbamate (4.11): - To the solution of 4.10 (1 g, 2.16 mmol) in anhydrous THF (5 ml) was added 1M Borane-THF complex (5.4 ml, 5.4 mmol) at 0 °C and stirred at 0 °C for 3 hrs. The reaction was heated to 60 °C and refluxed for 12 hrs. The reaction mixture was cooled to 0 °C and
quenched by slow addition of methanol. The oil obtained upon repeated dilutions and concentrations (2-3 times) with methanol, was subjected to water work up (pH 8.5) followed by flash column chromatography to afford the corresponding amine 4.11 (0.58 mg, 60%). $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 7.40 (D, J=7.27 Hz, 6H), 7.19 (ddd, J=14.29, 5.76 Hz, 9H), 5.27-4.85 (bs, 1H), 3.55 (bs, 2H), 2.35 (tt, J=17.88, 8.99 Hz, 2H), 2.63 (d, J=5.68 Hz, 2H), 1.39 (s, 3H). $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 155.57, 144.65, 79.35, 66.79, 64.35, 60.39, 44.40, 34.37. LCMS (ESI) m/z calcd for C$_{27}$H$_{32}$N$_2$O$_2$S is 448.21, found 449.22 [M+H]$^+$. 

(2-tert-Butoxycarbonylamino-3-tritylsulfanyl-propylamino)-acetic acid methyl Ester (4.12):-To the solution of 4.11 (1.66 g, 3.7 mmol) in THF (40 ml) at 0 $^\circ$C was added methyl bromoacetate (35 ml, 3.7 mmol, dissolved in 5 ml) followed by the addition of DIEA (0.672 ml, 4.070 mmol) and stirring was continued for 7 hrs. Reaction was brought to ambient temperature and continued for another 10 hrs, after which it was concentrated to dryness. The residue obtained was dissolved in ethyl acetate and washed with saturated brine
solution. Aqueous layer was back extracted into ethyl acetate. Organic layer was dried over anhydrous sodium sulfate and concentrated to give crude product which was purified by flash column chromatographed to get pure compound 4.12 (0.721 g, 37.43 %). $^1$H NMR (DPX 250 MHz, CDCl$_3$) δ 7.54-7.12 (m, Ar 15H); 4.74 (bs, NH, 1H); 3.73 (s, CH$_3$, 3H); 3.33 (d, NHCH$_2$CO, 2H); 2.72-2.26 (m, 3H) 1.64 (s, SCH$_2$, 2H); 1.44 (s, tBu, 9H). $^{13}$C NMR (DPX 250 MHz, CDCl$_3$) δ 172.8, 155.6, 79.48, 51.8, 51.2, 50.8, 49.7, 36.9, 28.4, 16.3. HRMS (ESI) m/z calcd for C$_{30}$H$_{36}$N$_2$O$_4$S is 520.24, found 521.2 [M+H]$^+$. 

![Chemical structures](image)

(2-tert-Butoxycarbonylamino-3-methylsulfanyl-propylamino)-acetic acid methyl ester (4.14):- To the solution of compound 4.12 (0.700, 1.34 mmol) in 100 ml of dry dichloromethane was added TFA (1.6 ml) at 0 °C over a period of 7 minutes which turned into yellow color. To the above solution, triethylsilane (0.436 ml) was added over a period of 3 minutes. The reaction mixture turned to a pale yellow from dark yellow color. Reaction was complete in 12 minutes by TLC. Reaction mixture was concentrated to half of its volume and diluted
with 50 ml of DCM. Again concentrated and repeated the same thing for four times to make sure TFA was removed as much as possible.
The crude compound 4.13 was dried under high vacuum and taken to the next step without further purification. The above residue was dissolved in 10 ml of Methanol and cooled to 0 °C to which 3.36 ml of 1N NaOH was added followed by the addition of 0.16 ml of iodomethane. The reaction was over in 15 minutes. The reaction mixture was neutralized by adding 20% citric acid solution and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate and washed with saturated brine solution. The organic layer was dried over anhydrous sodium sulphate and concentrated. Flash chromatography gave pure compound 4.14 (0.22 g, 56%). ¹H NMR (DPX 250 MHz, CDCl₃) δ 3.73 (s, CH₃, 3H); 3.53 (d, 2H); 2.91-2.62 (m, 3H); 2.38 (bs, 2H); 2.25 (s, 3H); 1.93 (s, tBu, 9H); LCMS (ESI) m/z calcd for C₁₂H₂₄N₂O₄S is 292.14, found 293.1 [M+H]+.

Representative base coupling procedure: Diisopropylethylamine (0.37 ml, 0.29 mmol) was added to a stirring solution of 4.14 (0.22 g,
0.75 mmol), thymine acetic acid (0.207 g, 1.1278 mmol) and HATU (0.343 g, 0.90 mmol) in DMF (3 ml) at ambient temperatures. The reaction mixture turned yellow. TLC showed the reaction was complete in 10 minutes. DMF was removed under high vacuum without heating. The crude was dissolved in ethyl acetate (150 ml) and washed with saturated brine (7 ml) The layers were separated and the organic layer was dried over anhydrous sodium sulphate and concentrated. Flash chromatography of the crude gave pure compound 4.15a as white solid (0.29 g, 87.8%). The same procedure is used to synthesize 4.15a-d compounds.

4.15a: product as white solid, 0.29 g, 87.8%. \(^1\)H NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 7.33 (1H), 4.42 (s, 1H), 3.85 (s, 2H), 4.34-3.96 (m, 2H), 3.74 (s, 3H), 3.33 (1H), 2.74-2.55 (m, 2H), 2.27 (s, 2H), 2.13 (2H), 1.93 (s, 3H), 1.53 (s, \(^{t}\)Bu, 9H); LCMS (ESI) \(m/z\) calcd for C\(_{19}\)H\(_{30}\)N\(_4\)O\(_7\)S is 458.18, found 458.19 [M+H]+, 481.17 [M+Na]+.
4.15b: pure product as white solid, (0.168 g, 85.27%). $^1$H NMR (DPX 250 MHz, CDCl$_3$) $\delta$ 8.71 (d, NH), 8.35 (NH), 7.85 (d, CH), 7.56-7.23 (Ar, 5H), 4.7 (1H), 4.41 (d, CH), 4.16 (CH$_2$), 3.80 (CH$_2$), 3.75 (CH$_2$), 3.70 (CH$_2$), 2.21 (CH$_2$), 2.02 (CH$_3$), 1.45 ($^t$Bu); LCMS (ESI) $m/z$ calcd for C$_{26}$H$_{35}$N$_5$O$_8$S is 577.22, found 578.2 [M+H]$^+$. 

4.15c: (0.33 g, 56.21%); 1H NMR (DPX 250 MHz, MeOD-d$_4$) $\delta$ 8.8 (s, 1H), 8.4 (1H), 7.6-7.2 (m, 5H), 5.5 (1H), 5.3 (1H), 4.0-3.6 (4H), 3.4-3.1 (m, 2H), 3.0 (2H), 2.9 (3H), 1.4 (s, 9H); LCMS (ESI) $m/z$ calcd for C$_{27}$H$_{35}$N$_7$O$_8$S is 617.22, found 618.2 [M+H]$^+$. 

135
Hydrolysis of methyl esters was performed following the same procedure employed for the hydrolysis of 4.8a-d to get pure compounds 4.16a-d.

(4.16d): colorless solid, 84% yield. $^1$H-NMR (DPX 250 MHz, MeOD-d$_4$) \(\delta 8.8\) (1H), 8.6 (1H), 8.0 (2H), 7.6-7.3 (3H), 5.5 (2H), 5.3 (2H), 4.3-3.6 (5H), 3.0 (2H), 2.8 (2H), 2.2 (3H), 1.4 (9H)
**N-tert-butoxycarbonyl-3-bromopropylamine (4.17):** A solution of sodium bicarbonate (0.39 g, 4.64 mmol) in 15 ml of water was added to 3-bromopropylamine hydrobromide (1.0 g, 4.56 mmol) in 20 ml of chloroform. To the above mixture, di tert-butyl dicarbonate anhydride (1.0 g, 4.58 mmol) was added at ambient temperature, with vigorous stirring, followed by the addition of sodium bromide (1.0 g, 9.72 mmol). The reaction mixture was refluxed for 18 hrs and then brought to ambient temperature and the layers were separated. The aqueous layer was extracted with chloroform (2x20 ml). The organic layers were mixed and dried over anhydrous sodium sulphate and concentrated to give pure compound 4.17 without any further purification (1.04 g, ~100%) \[^1\text{H}\] NMR (DPX 250 MHz, CDCl\(_3\)) \(\delta\) 4.71 (bs, 1H, NH); 3.44 (t, 2H, J=6.5); 3.27 (q, 2H, J=6.5); 2.05 (m, 2H, J= 6.5); 1.44 (s, 9H, CH\(_3\));

![Chemical structure](image)

**3-(2-(2-(2-Methoxy-ethoxy)-ethoxy)-ethoxy)-propyl)-carbamic acid tert-butyl ester (4.19):** Sodium hydride (60% in mineral oil, 58 mg, 1.461 mmol) was added to an ice cold solution of triethylene

137
glycol monomethyl ether 4.18 (0.2 g, 1.218 mmol) in tetrahydrofuran (15 ml) and stirred for five minutes. *N*-tert-Butoxycarbonyl-3-bromopropylamine 4.17 (0.29 g, 1.218 mmol) was added to the above mixture at the same temperature. The reaction was gradually brought to ambient temperature and stirred for another 8 hrs. The reaction mixture was then cooled to 0 °C and quenched by slow addition of methanol (10 ml) and concentrated to dryness. The residue was dissolved in ethyl acetate and washed with saturated brine solution. The organic layer was dried over anhydrous sodium sulphate and concentrated. Flash column chromatography yielded the pure compound 4.19 (0.20 g, 52%). $^1$H NMR (DPX 250 MHz, CDCl$_3$) δ 5.14 (bs, 1H, NH); 3.73-3.54 (m, 16H) 3.42 (s, 3H, CH$_3$); 3.26 (q, 2H, J=6); 1.76 (p, 2H, J=6.25, 6.0); 1.44 (s, 9H)

3-{2-[2-(2-Methoxy ethoxy) ethoxy]-ethoxy}-propylamine hydrochloride (4.20): - Dry HCl gas was passed into an ice cold solution of compound 4.19 (0.179 g, 0.55 mmol) dissolved in 1,4-dioxane (25 ml) for five minutes. Progress of the reaction was
monitored by TLC. The reaction was complete in 25 minutes. The solvent was removed under reduced pressure and dried under high vacuum. The compound 4.20 was used in the next step without any further purification.

3-{2-[2-(2-Methoxy ethoxy)-ethoxy]-ethoxy}-propylamine bromo acetamide (4.21):- The above hydrochloride salt (4.20) is dissolved in benzene (20 ml) and cooled to 0 °C. To the solution, was added potassium carbonate (0.153 g, 1.114 mmol) followed by the addition of bromo acetyl bromide (0.11 g, 0.55 mmol). The reaction was brought to ambient temperature and stirred for 8 hrs. TLC showed there was no starting material left. Solids were filtered and the solution was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with water and saturated brine solution. Layers were separated and the organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to give crude which was purified by flash column chromatography to give the amide compound 4.21. ¹H NMR (DPX 250 MHz, CDCl₃) δ 9.33 (bs, 1H, NH); 4.34-3.52 (16H); 3.43 (s, 3H, CH₃); 2.13 (s, 2H, CH₂);
\(^{13}\text{C} \text{NMR (250 MHz, CDCl}_3\) \(\delta \) 171.23, 71.76, 70.41, 70.35, 70.32, 70.25, 69.00, 63.55, 58.84, 38.93, 28.85, 20.85.\)

4.7 References:


**APPENDIX-A**: Selected $^1$H, $^{13}$C NMR Spectra & Mass spectra
2-\(N\)-(methanesulfonyl)-aminopyrimidine (2.1)
5-iodo-2-\(N\)-(methanesulfonyl) amino pyrimidine (2.2)
5-iodo-2-N-[(methanesulfonyl), (4-methoxybenzyl)] amino pyrimidine (2.3)
5-[(2-hydroxy), (2-methyl)] butynyl-2-N-[(methanesulfonyl), (4-methoxynenzyl)] amino pyrimidine (2.4)
$N$-(5-Ethynyl-pyrimidin-2-yl)$-N$-(4-methoxy-benzyl)$-$methanesulfonamide (2.5)
$N$-($5$-{$2$-[$\text{Methanesulfonyl-(4-methoxy-benzyl)-amino}]$}$-pyrimidin-5$-ylethynyl$}$-pyrimidin-2$-yl$}$-$N$-($4$-methoxy-benzyl$)$-$N$-methanesulfonamide (2.6)
$N$-{5-[2-(2-Methanesulfonylamino-pyrimidin-5-yl)-ethyl]-pyrimidin-2-yl}-methanesulfonamide (2.8)
5-iodo-2-amino-pyrimidine (2.9)
Di-tert-butyl (5-iodopyrimidin-2-yl) dicarbamate (2.10)
(5-{2-[Methanesulfonyl-(4-methoxy-benzyl)-amino]-pyrimidin-5-ylethynyl}-pyrimidin-2-yl)-di-carbamic acid di-tert-butyl ester (2.11)
$^1$H-NMR, $^{13}$C-NMR & $^{13}$C-DEPT135 (2.12)
$N$-{5-[2-(2-Amino-pyrimidin-5-yl)-ethyl]-pyrimidin-2-yl}-$N$-(4-methoxy-benzyl)-methanesulfonamide (2.13)
C-Chloro-N-[5-\{2-\{2-\{\text{methanesulfonyl-(4-methoxy-benzyl)-amino}\}-pyrimidin-5-yl\}-ethyl\}-pyrimidin-2-yl\}-methanesulfonamide (2.14)
C-Chloro-N-\{5-[2-(2-methanesulfonylamino-pyrimidin-5-yl)-ethyl]-pyrimidin-2-yl\}-methanesulfonamide (2.15)
2-N-(chloro methanesulfonyl)-aminopyrimidine (2.16)
L-Serine methyl ester hydrochloride (2.19)
Diethyl-(phenylcarbonyl)-phosphonate (3.2)
Phosphonic acid, (2-phenyloxiranyl)-, diethyl ester (3.3)
(2-Phenyl-oxiranyl)-lithium phosphonate mono-ethyl ester

(3.4)
Diazophenyl-acetic acid methyl ester (3.7)

$^1$H-NMR (DPX 250 MHz, CDCl$_3$)

![Chemical structure image]
2,3-diphenyl-oxirane-2-carboxylic acid methyl ester (3.8a)
3-(2-nitro-phenyl)-2-phenyl-oxirane-2-carboxylic acid methyl ester (3.8g)

$\text{H NMR (DPX 250 MHz, DMSO-d$_6$)}$

3-(2-nitro-phenyl)-2-phenyl-oxirane-2-carboxylic acid (3.9g)
[(Methoxycarbonyl-phenyl-methylene)-hydrazono]-phenyl-acetic acid methyl ester (3.8i)
2,3-diphenyloxirane (3.10)

$^1$H-NMR (250 MHz, CDCl$_3$)

$^{13}$C-NMR (DPX 250 MHz, CDCl$_3$)
$\beta$-azido-$\alpha$-phenyl benzeneethanol (3.11)
2,3-Diphenylaziridine (3.12)

\[^1\text{H-NMR ( DPX 250 MHz, CDCl}_3\)]

![NMR Spectrum](image-url)
1-Aziridinecarboxylic acid, 2,3-diphenyl-, 1,1-dimethylethyl ester (3.13)
N-(phenylmethylene)-methanamine (3.16)
Benzenemethanamine-4-methoxy-N-(phenylmethylene) (3.17)
Benzyl-benzylidene-amine (3.18)

$^1$H-NMR (DPX 250 MHz, CDCl$_3$)

$^{13}$C-NMR (DPX 250, CDCl$_3$)
1,2-diphenyl-cyclopropanecarboxylic acid methyl ester (3.21)

$^1$H-NMR (DPX 250, CDCl₃)

$^{13}$C-NMR (DPX 250, CDCl₃)
1,2-diphenyl-cyclopropanecarboxylic acid (3.22)

$^1$H-NMR (DPX 250, CDCl$_3$)

$^{13}$C-NMR (DPX 250, CDCl$_3$)
(2-Amino-ethyl)-carbamic acid tert-butyl ester (4.1)
*tert*-Butyl N-[2-(N-9-fluorenlymethoxycarbonyl) amino ethyl] glycinate (4.2)

$^1$H-NMR (DPX 250 MHz, CDCl$_3$)

![Chemical structure](image-url)
2-(9H-Fluoren-9-ylmethoxycarbonylamino)-ethyl-ammonium chloride (4.3)
tert-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl) aminoethyl] glycinate ester (4.4)
[2-(4-Benzylxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-acetyl]-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino]-acetic acid tert-butyl ester (4.5b)

$^1$H-NMR (DPX 250 MHz, DMSO-d$_6$)
[2-(5-Benzylxycarbonylamino-7-oxo-6,7-dihydro-1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-acetyl]-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino]-acetic acid tert-butyler ester (4.5c)

$^1$H-NMR (DPX 250 MHz, DMSO-$d_6$)
[2-(6-Benzylxycarbonylamino-purin-9-yl)-acetyl]-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino]-acetic acid tert-butyl ester (4.5d)

$^1$H-NMR (DPX 250 MHz, MeOD-d$_4$)
2-(9H-Fluoren-9-ylmethoxycarbonylamino)-ethyl]-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetyl]-amino\}-acetic acid (4.6a)
[2-(4-Benzoyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-acetyl]-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino]-acetic acid (4.6b)

$^1$H-NMR (INOVA 400 MHz, DMSO-d$_6$)
[2-(6-Benzylxycarbonylamino-purin-9-yl)-acetyl]-[2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl]-amino]-acetic acid (4.6d)

Methyl N-(2-Boc-aminoethyl) glycinate (4.7)
(2-tert-Butoxycarbonylamino-ethyl)-[2-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-acetyl]-amino]-acetic acid methyl ester (4.8a)
[2-(4-Benzylxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid methyl ester (4.8b)
2-(5-Benzoyloxycarbonylamino-7-oxo-6,7-dihydro-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid methyl ester (4.8c)
2-(6-Benzylloxycarbonylamino-purin-9-yl)-acetyl]-[2-tert-butoxycarbonylamino-ethyl]-amino]-acetic acid methyl ester (4.8d)
2-(4-Benzylloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-acetyl]-\[2\text{-tert-butoxycarbonylamino-ethyl}-amino\]-acetic acid (4.9b)

$^1$H-NMR (DPX 250 MHz, CDCl$_3$)
[[2-(2-Benzoxycarbonylamino-6-oxo-1,6-dihydro-purin-9-yl)-acetyl]-(2-tert-butoxycarbonylamino-ethyl)-amino]-acetic acid (4.9c)
Data Acquired By: sridhar reddy kaulagari

Date: 6/14/2007

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N-tert-butoxycarbonyl-3-bromopropylamine (4.17)

3-(2-(2-(2-Methoxy-ethoxy)-ethoxy)-ethoxy)-propyl)-carbamic acid tert-butyl ester (4.19)
3-{2-[2-(2-Methoxy ethoxy) ethoxy]-ethoxy}-propylamine bromo acetamide (4.21)
About the Author

Sridhar Reddy Kaulagari was born in Korpole, Andhra Pradesh and grew up in a neighboring small town. After graduating from Junior college he attended Tara Degree College, Osmania University, Sangareddy, where he majored in natural sciences and earned his B.Sc and then went to the Osmania University main campus at Hyderabad where he earned his B.Ed in biological sciences. In the summer of 2000 he began his graduate studies at the University of Hyderabad, Hyderabad where he joined the lab of Professor. Ashwini Nangia to do project as part of the requirements to earn degree in the School of Chemistry and received his Master’s degree in Chemistry in 2002. He worked as lecturer at Narayana Junior College and MJ College in Hyderabad for one year. He then moved to Bangalore and worked as a junior research associate at Cadila Pharmaceuticals Ltd. In the Fall of 2004, he began his research at the University of South Florida, Tampa, where he joined the lab of Professor Mark L. McLaughlin in the Department of Chemistry and the Moffitt Cancer Center and Research Institute. He will receive his Ph.D in organic chemistry with a focus on small molecules synthesis in December 2010.