Design and Combinatorial Synthesis Approach of Non-peptidic Trimeric Small Molecules Mimicking i, i + 4(3), i + 7 Positions of alpha-Helices

Mingzhou Zhou
University of South Florida
Design and Combinatorial Synthesis Approach of Non-peptidic Trimeric Small Molecules Mimicking $i, i+4(3), i+7$ Positions of $\alpha$-Helices

by

Mingzhou Zhou

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
Department of Chemistry
College of Arts and Sciences
University of South Florida

Major Professor: Mark L. Mclaughlin, Ph.D.
Jianfeng Cai, Ph.D.
Wayne C. Guida, Ph.D.
Rongshi Li, Ph.D.
Roman Manetsch, Ph.D.

Date of Approval:
July 6, 2010

Keywords: protein-protein interaction, mimetics, $\alpha$-helical, non-peptidic

© Copyright 2010, Mingzhou Zhou
The search for truth has shattered most of my old beliefs and has made me commit what are probably sins where otherwise I should have kept clear of them. I do not think it has in any way made me happier; of course it has given me a deeper character, a contempt for trifles or mockery, but at the same time it has taken away cheerfulness and made it much harder to make bosom friends and, worst of all, it has debarred me from free intercourse with my people, and thus made them strangers to some of my deepest thoughts which, if by any mischance I do let them out, immediately become the subject for mockery which is inexpressively bitter to me though not unkindly meant.

-- Bertrand Arthur William Russell
Acknowledgements

First and foremost, I would like to thank Dr. Mark L. McLaughlin for his superior instruction during my Ph.D. studies. Dr. McLaughlin not only led me to solve problems during my research, but, more importantly to be a professional researcher.

I would also like to thank current and previous committee members — Dr. Wayne C. Guida, Dr. Roman Manetsch, Dr. Rongshi Li, Dr. Jianfeng Cai, and Dr. Peter Zhang for providing invaluable advice during my learning process.

I would like to thank current and previous group members — Dr. Missy Topper, Hyun Joo Kil, Dr. Priyesh Jain, David Badger, Dr. Laura Anderson, Yi Liang, Fenger Zhou, Sridhar Kaulagari, Dr. Vasudha Sharma, Dr. Umut Oguz, and Dr. Sung Wook Yi. A positive and supportive atmosphere is an important factor leading to efficient research.

I would like to thank Dr. Jiandong Chen’s group for performing the ELISA essay, Daniel N. Santiago for performing the computational study and Dr. Huiling Jiang for synthesizing some reagents for my research.

Furthermore, I would like to thank Dr. Daniele Pernazza and Dr. Roberta Pireddu for providing numerous suggestions for my dissertation. They, as well as Dr. Nick Lawrence, Dr. Harshani Lawrence, Yunting Luo, Dr. Yiyu Ge, and Dr. Binglin Wang helped me prepare for my oral defense for my dissertation.

I would like to send special thanks to Kelly M. Carper for all the grammar corrections of my dissertation.
Lastly, I would like to thank the NIH NCI P01 CA118210 for financial support of the work described in this dissertation that was carried out at Moffitt Cancer Center and University of South Florida.
# Table of Contents

List of Tables ........................................................................................................................................v

List of Figures ...................................................................................................................................... vi

List of Schemes ................................................................................................................................... ix

List of Abbreviations ........................................................................................................................ xi

Abstract ................................................................................................................................................. xiii

Chapter One: Peptidic and Non-peptidic Scaffolds to Disrupt Protein-protein Interactions that Involve \( \alpha \)-Helices

1.1 Protein-protein interactions ........................................................................................................... 1

1.2 Efforts to target the protein-protein interaction .......................................................................... 1

1.3 Efforts to target the protein-protein interactions involving \( \alpha \)-helix ...................................... 2

1.4 Peptidic \( \alpha \)-Helical mimetics ........................................................................................................ 3

1.4.1 Cyclic peptides ......................................................................................................................... 4

1.4.2 Direct covalent bonding of the side chains of the amino acids ............................................. 4

1.4.3 Metal coordinate bonding ......................................................................................................... 6

1.4.4 Connecting side chains of unnatural amino acids at \( i \) and \( i + 7 \) positions ......................... 7

1.4.5 Connecting \( i \) and \( i + 7 \) side chains with a photo-controlled azobenzene linker ................. 8

1.5 Miniproteins .................................................................................................................................. 9

1.6 Foldamers ...................................................................................................................................... 10

1.7 Synthetic non-peptidic \( \alpha \)-helical mimetics with linear scaffold ................................................. 11

1.7.1 \( \alpha \)-Helical mimetic scaffolds developed by Hamilton and coworkers .................................... 14

1.7.2 \( \alpha \)-Helical mimetic scaffolds developed by Rebek and coworkers .......................................... 16

1.7.3 Trisubstituted imidazole scaffold ............................................................................................ 17

1.7.4 Benzyldieneacetophenone scaffold ......................................................................................... 18

1.7.5 \textit{trans}-Fused polycyclic ethers scaffold ................................................................................ 19

1.8 Synthetic non-peptidic \( \alpha \)-helical mimetics with other scaffold ................................................. 20

1.8.1 Nutlin ...................................................................................................................................... 20

1.8.2 1,4-Benzodiazepine-2,5-dione scaffolds ................................................................................. 21

1.8.3 Coactivator binding inhibitors ............................................................................................... 22
Chapter Three: Convergent Approach to Synthesis 2,5-Terpyrimidinylene Based Derivatives ................................................................. 70
3.1 Introduction ............................................................................. 70
  3.1.1 Optimization of synthetic route to the 2,5-terpyrimidinylene scaffold ... 70
  3.1.2 Modifications that might lead to improvement of the bioactivities ... 70
  3.1.3 Problems existing in the developed synthetic process .................. 73
  3.1.4 Convergent synthetic strategy ............................................. 74
3.2 Results and Discussion for the synthesis of the new α,β-unsaturated ketones ........................................................................ 75
  3.2.1 Retrosynthetic to make the α,β-unsaturated α-pyrimidineketones .... 75
  3.2.2 Attempted synthesis of the α,β-unsaturated α-pyrimidineketones 3.5 76
Chapter Four: Design and Synthesis of Hybrid Scaffold Non-peptidic α-Helical Mimetics .................................................................90
4.1 Introduction ...........................................................................90
  4.1.1 General scaffold of non-peptidic α-helical mimetics ..........90
  4.1.2 New library design strategy ..............................................91
  4.1.3 Introduction of the hybrid scaffold .................................91
  4.1.4 Strategy to extend the pyrimidine rings using Huisgen Cycloaddition .................................................................92
4.2 Results and Discussion for introducing 1,2,3-triazole to the scaffold ......93
  4.2.1 Synthesis of 4-triazole pyrimidine hybrid dimer 4.6 ...............93
  4.2.2 Benefits of the 1,2,3-triazole ring ..................................98
  4.2.3 Synthesis of 2-triazole pyrimidine hybrid dimer 4.13 ............98
  4.2.4 Evaluation 1,2,3-triazole ring as a fragment in our scaffold ......101
4.3 Results and Discussion for introducing amino acids to the scaffold ......101
  4.3.1 Possibility of introducing amino acids to the scaffold ..........101
  4.3.2 Testing of the new scaffold .............................................102
  4.3.3 Retrosynthesis of the trimer ............................................103
  4.3.4 Modification of the new target scaffold and the retrosynthesis route .................................................................105
  4.3.5 Synthesis of the trimer 4.21 ............................................106
  4.3.6 Properties of the trimer 4.21 ..........................................108
4.4 Biological testing of 4.6 and 4.13 ...........................................109
4.5 Conclusion ...........................................................................110
4.6 Experimental Procedures ..................................................110
4.7 References .........................................................................128

Chapter Five: Design and Synthesis of Cyclic Urea HIV-1 Protease Inhibitor..............131
5.1 Introduction ........................................................................131
  5.1.1 AIDS .............................................................................131
  5.1.2 Efforts to treat or cure AIDS .........................................132
  5.1.3 Structure of HIV ...........................................................132
  5.1.4 HIV replication mechanism .........................................134
  5.1.5 Common targets to inhibit the HIV replication process ........135
  5.1.6 Structure of HIV protease ............................................136
  5.1.7 HIV protease function mechanism ...............................137
  5.1.8 Cyclic urea as the HIV-1 protease inhibitor ....................137
  5.1.9 Our design strategy ......................................................138
5.2 Results and Discussion ........................................................................................................139
  5.2.1 Retrosynthesis of 5-hydroxyl cyclic urea .................................................................139
  5.2.2 Synthesis of intermediate 5.3 ....................................................................................140

  5.2.3 Photochemical electrocyclization of 1,4,6-trisubstituted
      pyrimidin-2(1H)-ones 5.1 .........................................................................................142
2.3 Experimental Procedures of selected compounds .......................................................147
2.4 References ......................................................................................................................151

Appendix A: Selected \textsuperscript{1}H and \textsuperscript{13}C NMR Spectra ..............................................153
Appendix B: Selected HRMS ............................................................................................214
Appendix C: X-Ray Crystallographic Data ........................................................................233

About the Author ..................................................................................................................End Page
List of Tables

Table 1.1  Typical disease pathways that involve the interaction of α-helix ..........3
Table 2.1  Calculated logP values for terphenylene and terpyrimidinylene analogs .........................................................................................................................39
Table 2.2  Library of the synthesized pyrimidine monomers 2.4 ......................42
Table 2.3  Analogs of the synthesized pyrimidine dimers ..................................46
Table 2.4  Analogs of the synthesized pyrimidine trimers ..................................47
Table 5.1  Analogs of the synthesized 1,4,6-trisubstituted pyrimidin-2(1H)-ones 5.1 ..........................................................................................................................141
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Covalent bonding cyclic peptides</td>
</tr>
<tr>
<td>1.2</td>
<td>The smallest reported $\alpha$-helical peptide in water and its conformation</td>
</tr>
<tr>
<td>1.3</td>
<td>Metal coordinate bonding cyclic peptides</td>
</tr>
<tr>
<td>1.4</td>
<td>Cyclic peptide formed by unnatural amino acids</td>
</tr>
<tr>
<td>1.5</td>
<td>Conformational change of cyclic peptide following the change of nitrogen-nitrogen double bond conformation</td>
</tr>
<tr>
<td>1.6</td>
<td>Short chain $\alpha$-helix stabilized by mimiprotein</td>
</tr>
<tr>
<td>1.7</td>
<td>Examples of foldamers</td>
</tr>
<tr>
<td>1.8</td>
<td>Comparison of $\alpha$-helix and $\beta$-peptide helix</td>
</tr>
<tr>
<td>1.9</td>
<td>First reported synthetic non-peptidic $\alpha$-helical mimetics</td>
</tr>
<tr>
<td>1.10</td>
<td>Model of a typical $\alpha$-helix</td>
</tr>
<tr>
<td>1.11</td>
<td>Model of the common $\alpha$-helix binding residues</td>
</tr>
<tr>
<td>1.12</td>
<td>Comparison of 1,4-terphenylene scaffold with an $\alpha$-helix</td>
</tr>
<tr>
<td>1.13</td>
<td>Other $\alpha$-helical mimetic scaffolds developed by Hamilton and coworkers</td>
</tr>
<tr>
<td>1.14</td>
<td>$\alpha$-Helical mimetic scaffolds developed by Rebek and coworkers</td>
</tr>
<tr>
<td>1.15</td>
<td>a) The trisubstituted imidazole scaffolds; b) one molecule from the scaffold docking into the binding site of the Bad/Bcl-xL</td>
</tr>
<tr>
<td>1.16</td>
<td>Structure of chalcone C</td>
</tr>
</tbody>
</table>
Figure 1.17 (Left) trans-Fused polycyclic ethers scaffold; (right) its superimposition with an α-helix .................................................................19

Figure 1.18 a) Structures of the nutlins; b) nutlin-2 superimposing with Phe$^{19}$, Trp$^{23}$, and Leu$^{26}$ of p53; c) nutlin-2 docking into the binding site of p53/MDM2 ..........................................................................................21

Figure 1.19 a) Structures of 1,4-Benzodiazepine-2,5-diones; b) 1,4-Benzodiazepine-2,5-diones superimposing with Phe$^{19}$, Trp$^{23}$, and Leu$^{26}$ of p53 ........................................................................................................22

Figure 1.20 Structures of co-activator binding inhibitors .........................................................22

Figure 1.21 Analogs of 1,4-terphenylene scaffold targeting different protein-protein interactions..................................................................................................................24

Figure 1.22 p53 pathway to elicit apoptosis .............................................................................28

Figure 1.23 a) The MDM2 binding cleft; b) crystal structure of p53 binding into the MDM2 cleft .................................................................................................................................29

Figure 1.24 Bak binding with the Bcl-xL .................................................................................30

Figure 2.1 Hamilton’s most active p53-MDM2 antagonist a and its docking in MDM2 .................................................................36

Figure 2.2 Recently reported non-peptidic α-helical mimetics: Hamilton’s b–f; Rebek’s g–k ....................................................................................................................38

Figure 2.3 Geometry of phenyl ring and pyrimidine ring .....................................................40

Figure 2.4 Crystal structure of pyrimidine dimer .................................................................46

Figure 3.1 Examples of Hamilton’s 1,4-terphenylene derivatives .........................................70

Figure 3.2 Synthesized 2,5-terpyrimidinylene derivatives .........................................................71

Figure 3.3 Structure comparison of 1,4-terphenylene and 2,5-pyrimidinylene scaffold .................................................................................................................................71

Figure 4.1 Abstract structure of α-helical mimetics ...............................................................91

Figure 4.2 Trimer with an amino acid in the scaffold fitting the abstract scaffold ..............................................102
Figure 4.3  Possible conformation of the new scaffold...........................................103
Figure 4.4  Elisa results of 4.6 and 4.13.................................................................109
Figure 5.1  Estimated number of people living with HIV globally, 1990–2007.......131
Figure 5.2  Diagram of HIV .....................................................................................133
Figure 5.3  HIV replication cycle.............................................................................135
Figure 5.4  Structure of HIV PR complexed with TL-3..............................................136
Figure 5.5  Mechanism of HIV PR proteolysis.........................................................137
Figure 5.6  X-ray crystal structure of cyclic urea in the active site of HIV PR .......138
Figure 5.7  UV absorption of 5.1a.............................................................................144
List of Schemes

Scheme 2.1  Synthesis of pyrimidine rings ..................................................40
Scheme 2.2  Retrosynthesis of 1,4-terpyridinylene.................................................41
Scheme 2.3  Synthesis of the pyrimidine monomers 2.4.................................42
Scheme 2.4  Synthetic strategy to convert sterically hindered nitriles to amidines ......43
Scheme 2.5  Reaction and side reaction of hydroxylamine nucleophilic addition........44
Scheme 2.6  Proposed transition state of hydroxylamine nucleophilic addition........45
Scheme 2.7  Synthesis of the pyrimidine dimer ..................................................45
Scheme 2.8  Synthesis of the pyrimidine trimers ..................................................47
Scheme 3.1  Reaction and side reaction of hydroxylamine nucleophilic addition.......73
Scheme 3.2  Reaction and side reaction of amidine condensation reaction .............73
Scheme 3.3  Retrosynthesis using the convergent strategy .......................................74
Scheme 3.4  Retrosynthesis of the α,β-unsaturated α-pyrimidineketones..................75
Scheme 3.5  Attempted synthesis of compound 3.5.............................................76
Scheme 3.6  Hydroxylamine addition of compound 3.2 ......................................77
Scheme 3.7  Synthesis of the α,β-unsaturated α-triazoleketones .........................78
Scheme 3.8  Condensation to synthesize the hybrid trimer 3.11............................79
Scheme 4.1  Synthetic strategy to make the hybrid dimer .....................................93
Scheme 4.2  Synthesis of hybrid dimer 4.6..........................................................94
Scheme 4.3  Comparison of different substituted 1,3-diketones reacting with DMF-DMA ..................................................................................................................95

Scheme 4.4  One possible mechanism of 1,3-diketone reacting with DMF-DMA......96

Scheme 4.5  General methodology to synthesize α,β-unsaturated ketones from ketones ..........................................................................................................................99

Scheme 4.6  Synthesis of hybrid dimer 4.13 .................................................................................100

Scheme 4.7  Retrosynthesis of the hybrid trimer...........................................................................104

Scheme 4.8  Synthesis of the 2-chloro-pyrididine ring .................................................................105

Scheme 4.9  Modification of the target molecule..........................................................................106

Scheme 4.10  Synthesis of trimer 4.22 ..........................................................................................107

Scheme 5.1  Retrosynthesis of 5-hydroxyl cyclic urea .................................................................139

Scheme 5.2  Synthesis of the intermediate 5.3 .............................................................................140

Scheme 5.3  Hydrolysis of N-benzylurea under high temperature acidic conditions .................................................................141

Scheme 5.4  Synthesis of the 1,3-diazetidine-2-one intermediate .................................................143

Scheme 5.5  Side product of the oxidation of 5.2a ......................................................................145

Scheme 5.6  Equilibrium between 5.3 and its water added form 5.3’ ........................................146
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>Ac₂O</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>aq.</td>
<td>Aqueous</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
</tr>
<tr>
<td>BINAP</td>
<td>2,2'-bis(diphenylphosphino)-1,1'-binaphthyl</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butyloxycarbonyl</td>
</tr>
<tr>
<td>br</td>
<td>Broad (spectral)</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>¹³C NMR</td>
<td>Carbon-13 Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>CDI</td>
<td>N,N'-Carbonyldiimidazole</td>
</tr>
<tr>
<td>CH₃CN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Cs₂CO₃</td>
<td>Cesium carbonate</td>
</tr>
<tr>
<td>δ</td>
<td>Delta or chemical shift</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DIEA</td>
<td>Diisopropylethylamine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMF-DMA</td>
<td>N,N-dimethylformamide dimethyl acetal</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>equiv.</td>
<td>Equivalent(s)</td>
</tr>
<tr>
<td>FP</td>
<td>Fluorescence polarization</td>
</tr>
<tr>
<td>g</td>
<td>Gram(s)</td>
</tr>
<tr>
<td>¹H NMR</td>
<td>Proton Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>HR</td>
<td>High resolution</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>50% inhibitory concentration</td>
</tr>
<tr>
<td>J</td>
<td>Coupling-constant(s)</td>
</tr>
</tbody>
</table>
\( Ki \)  Inhibitor dissociation constant
KOBt  Potassium tert-butoxide
KOT-amy  Potassium tert-amylxide
Leu  Leucine
LiOH  Lithium hydroxide
M  Molar or moles per liter
MDM2  Murine double minute 2
Me  Methyl
MeOH  Methanol
mg  Milligram(s)
min  Minute(s)
mL  Milliliter(s)
mmol  Millimole(s)
m.p.  Melting point
MS  Mass spectrum
MW  Microwave
NaH  Sodium hydride
NaOEt  Sodium ethoxide
NaOH  Sodium hydroxide
nM  Nanomolar
ORTEP  Oak Ridge thermal ellipsoid plot (crystallography)
PCC  Pyridinium chlorochromate
Pd/C  Palladium on carbon
Ph  Phenyl
Phe  Phenylalanine
ppm  Parts per million
Pr  Propyl
RMSD  Root mean square deviation
rt  Room temperature
SAR  Structure activity relationship
sat.  Saturated
TFA  Trifluoroacetic acid
THF  Tetrahydrofuran
TMS-Cl  Trimethylsilyl chloride
TLC  Thin layer chromatography
Trp  Trptophan
TS  Tumor suppressor
\( \mu \)L  Microliter(s)
\( \mu \)M  Micromolar
Val  Valine
wt  Wild type
Design and Combinatorial Synthesis Approach of Non-peptidic Trimeric Small Molecules Mimicking $i, i+4(3), i+7$ Positions of $\alpha$-Helices

Mingzhou Zhou

Abstract

Protein-protein interactions are key to several biological processes that facilitate signal transduction and many other processes. These interactions are involved in pathways that are critical to many human diseases. Targeting specific protein-protein interactions is a challenging goal because protein-protein interactions are predominately through hydrophobic interactions. Antagonists of the protein-protein interactions need to be perfectly fit into the binding pockets to ensure the activity. The $\alpha$-helical domain of the proteins behaves as the recognition motifs for numerous protein-protein, and protein-nucleic acid interactions. Research has shown that pathways of many diseases contain protein-protein interactions involving $\alpha$-helical domains, e.g. neurological disorders, bacterial infections, HIV and cancer, etc. It is difficult yet very important to design small molecules to target the shallow binding areas of protein-protein interactions. So far the most successful one is Hamilton’s 1,4-terphenylene scaffold, which has been used to target the interactions between p53/MDM2, Bak/Bcl-$x_L$ etc. Inspired by this, we designed and synthesized three new scaffolds of non-peptidic $\alpha$-helical mimetics, mimicking the $i, i+4, i+7$ positions of an $\alpha$-helix. There are three basic principles that were leading our design. The side chains of our designed molecules should act as mimetics of the side chains of an $\alpha$-helix. Second, our molecules should possess
improved water solubility. Third, the molecules should be easy to synthesize to generate a focused library. Some of our molecules, including the ones whose molecular weight are as low as 294, started to show some inhibition against p53/MDM2 interactions.
Chapter One:

Peptidic and Non-peptidic Scaffolds to Disrupt Protein-protein Interactions that Involve $\alpha$-Helices

1.1 Protein-protein interactions

Protein-protein interactions are key to several biological processes that facilitate signal transduction and many other processes. These interactions are involved in pathways that are critical to many human diseases. By disrupting certain protein-protein interactions, we are able to gain an understanding of functions of individual proteins and their roles in the biological system; most importantly, this allows us to gain an understanding of the role of specific proteins in disease pathways.

1.2 Efforts to target the protein-protein interaction

Targeting specific protein-protein interactions is a challenging goal because unlike the interactions between the enzymes and their substrates which contain many hydrogen bonds, protein-protein interactions are predominately through hydrophobic interactions. Antagonists of the protein-protein interactions need to be perfectly fit into the binding pockets to ensure the activity. Unfortunately, the binding areas on the proteins are normally shallow and large (Stites, 1997), unlike that of the enzymes; this makes the design of small molecule antagonists that can disrupt protein-proteins interactions even more difficult (Ernst et al., 2003). Screening chemical libraries has not
been particularly successful in this area (Kutzki et al., 2002). However, rational design has proven to be a more suitable approach (Yin and Hamilton, 2005). Rational design is a more successful procedure because the binding sites of the protein-protein interactions are normally well-defined three-dimensional surfaces; hence, molecules with these properties have the most potential to serve as protein-protein interaction inhibitors.

Protein-protein interactions occur on shallow surface features of the protein; typically, the binding areas contain only a limited amount of crucial amino acid residues. The most rational way to disrupt these interactions is to design and synthesize molecules that are capable of mimicking the three dimensional configuration of those crucial amino acids side chains.

The two essential factors in designing small molecule protein-protein interaction antagonists are to identify the three dimensional configurations of the crucial binding amino acids of the target interactions and to design scaffolds that could hold the functional groups at specific locations.

1.3 Efforts to target the protein-protein interactions involving α-helix

One of the most important and abundant protein secondary structures is the α-helix. The α-helical domain of the proteins behaves as the recognition motifs for numerous protein-protein interactions, protein-DNA interactions, and protein-RNA interactions (Lavery, 2005); (Klug, 2005). Research has shown that pathways of many diseases contain protein-protein interactions involve α-helical domains (Table 1.1) (Davis et al., 2007).
A typical α-helix has a structural domain, which is about 15 – 25 residues in length (Garner and Harding, 2007). The α-helix residue peptides when excised from their parent proteins cannot retain their secondary structures (Kelso and Fairlie, 2003). In addition, the excised peptides, in vivo, could not bind to their target protein any more. This is the reason why we cannot use the α-helix sequence directly as the antagonist for protein-protein interactions.

Table 1.1: Typical disease pathways that involve the interaction of α-helix

<table>
<thead>
<tr>
<th>ppi</th>
<th>diseases</th>
<th>motif</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tachykinin receptors/peptide</td>
<td>neurological disorders</td>
<td>$i, i + 4$</td>
</tr>
<tr>
<td>NaIP</td>
<td>bacterial infections</td>
<td>$i, i + 4, i + 7$</td>
</tr>
<tr>
<td>gp41</td>
<td>HIV</td>
<td>$i, i + 3, i + 4, i + 7$</td>
</tr>
<tr>
<td>p53/MDM2</td>
<td>cancer</td>
<td>$i, i + 4, i + 7$</td>
</tr>
<tr>
<td>smMLCK/CaM</td>
<td>cancer</td>
<td>$i, i + 4, i + 7$</td>
</tr>
<tr>
<td>Rac1/Tiam1</td>
<td>cancer</td>
<td>$i, i + 4, i + 7$</td>
</tr>
<tr>
<td>GRIP1/Era</td>
<td>cancer</td>
<td>$i, i + 3, i + 4$</td>
</tr>
<tr>
<td>Vav</td>
<td>cancer</td>
<td>$i, i + 1, i + 4$</td>
</tr>
<tr>
<td>Bak/Bcl-xL</td>
<td>cancer</td>
<td>$i, i + 4, i + 7, i + 11$</td>
</tr>
</tbody>
</table>

Due to the importance of the α-helix in the field of protein-protein interactions, extensive research has focused on the design and synthesize of α-helical mimetics. Studies of α-helical mimetics are important because it can help us understand the roles that α-helices play in protein-protein interactions.

1.4 Peptidic α-Helical mimetics
α-Helices are the secondary structures of peptide chains; thus, it is straightforward to design their mimetics using peptides. As a matter of fact, peptidic α-helical mimetics are a successful approach toward mimicking α-helical protein-protein interactions.

1.4.1 Cyclic peptides

Despite the fact that many peptidic α-helical mimetic scaffolds have been developed, the strategy is similar. As discussed above, short peptide chains are prone to remain unfolded, even though the same primary sequences form α-helices in proteins. To make short stable peptide α-helices, the peptides could be cyclized so that their conformational flexibility is locked. In other words, cyclization of peptides is the most common strategy that peptide chemists use to stabilize short chain peptides to form α-helices.

The peptide cyclization strategy is a simple procedure. Because the side chains of the amino acid residues of an α-helix at the $i$, $i + 4$, $i + 7$, $i + 11$ positions are on the same face, if we connect two side chains among the $i$ and the $i + 4$, $i + 7$, and $i + 11$ positions of an unfolded peptide, with proper length linker, to force them be on the same face, the peptide is stabilized as an α-helical conformation. There are three major methodologies that have been developed following this strategy.

1.4.2 Direct covalent bonding of the side chains of the amino acids
There are two kinds of covalent bonds that could be directly formed between the side chains of the natural amino acids: amide bonds between lysine and aspartic acid / glutamic acid (Condon et al., 2000), and disulfide bonds between two cysteines (Jackson et al., 1991) (Figure 1.1).

![Covalent bonding cyclic peptides](image)

**Figure 1.1:** Covalent bonding cyclic peptides

The synthesis of these kinds of cyclic peptides is convenient because coupling of side chains of lysine and aspartic acid / glutamic acid forms an amide bond and the oxidation of two sulfides on cysteine side chains forms a disulfide bond.

It has been reported that this direct covalent bonding methodology has been utilized to make the smallest $\alpha$-helical peptide in water (Figure 1.2) (Shepherd et al., 2005). However, this is also the limitation of using direct covalent bonding. The side chains of the amino acid have limited length; consequently, this method could not be used to make larger $\alpha$-helical regions. When a relatively longer unfolded $\alpha$-helical peptide chain has been cyclized using this method, only the cyclized small region could be an $\alpha$-helix (Garner and Harding, 2007).
Figure 1.2: The smallest reported $\alpha$-helical peptide in water and its conformation determined by NMR

1.4.3 Metal coordinate bonding

Besides the direct covalent bonding between the side chains of two amino acids, coordinate bond was used to connect the side chains (Figure 1.3) (Kelso et al., 2003); (Kelso et al., 2004); (Beyer et al., 2004).

Figure 1.3: Metal coordinate bonding cyclic peptides

Transition metals, such as palladium, zinc, etc. were used to form the coordinated bonds with side chains of histidine / cysteine at $i, i + 4$ positions (Figure 1.3).
Like the direct covalent bonding, only small \( \alpha \)-helical regions could be formed by metal coordinate bonding. Also, these cyclic peptides are not stable in water. However, it provides one other possible way to form an \( \alpha \)-helix; especially since metal ions play important roles in biological systems.

### 1.4.4 Connecting side chains of unnatural amino acids at \( i \) and \( i + 7 \) positions.

All natural amino acids have side chains which are short in length. This limits the potential size of the cyclic peptide if we choose to directly connect the side chains. There are two ways to solve this problem; one is to use unnatural amino acids with longer side chains, or longer linkers can be used.

For the first way, unnatural amino acids were used, which have extended side chains (5 to 8 carbons-carbon single bond’s in length) introduced at the \( i \) and \( i + 7 \) positions (Figure 1.4) (Blackwell and Grubbs, 1998); (Blackwell et al., 2001).

**Figure 1.4:** Cyclic peptide formed by unnatural amino acids

Carbon-carbon double bonds were used to connect the side chains. Although stable in water, amide bonds and disulfide bonds are ruled out because they are prone to
be degraded in biological systems. In summary, two amino acids that have side chains with an alkene group at the terminal position are introduced to the peptide scaffold through the Grubbs metathesis coupling reaction.

1.4.5 Connecting $i$ and $i + 7$ side chains with a photo-controlled azobenzene linker

The second strategy to form a bigger $\alpha$-helical region is to use longer linkers to connect the $i$ and $i + 7$ positions. Azobenzene was chosen as this linker because of its length and its photo sensitive isomerization (Woolley, 2005); (Ihalainen et al., 2007).

The azobenzene linker was connected to the peptide by the help of a sulfur atom at the side chains of the cysteine. One unique property of azobenzene is that its nitrogen-nitrogen double bond isomerizes under different conditions (Figure 1.5); the distance between 4 and 4’ position of the benzene rings changes greatly between cis and trans double bonds. The azobenzene linker was designed so that, when the nitrogen-nitrogen double bond is in the cis configuration, the peptide will be stabilized as an $\alpha$-helix.

![Diagram](image1.png)

**Figure 1.5:** Conformational change of cyclic peptide following the change of nitrogen-nitrogen double bond conformation
However, when the double bond is in trans configuration, the peptide chains would be unable to maintain the α-helical secondary structure.

The azobenzene linker strategy is one method that can control the conformation of a peptide chain after the peptide has been cyclized; thus could be applied to understand the roles that α-helices play.

1.5 Miniproteins

Short peptide sequences taken from a protein tend to lose their secondary structures. This also suggests that although the other part of the protein does not participate in the bonding interaction, it helps stabilize the protein secondary structure. If a second protein was introduced to stabilize the short peptide chain, will the peptide reform its secondary structure?

Figure 1.6: Short chain α-helix stabilized by mimiprotein
Following this question, miniproteins were designed to stabilize the non-bonding face of a $\alpha$-helix through protein-protein interactions, so that a short peptide chain will form its secondary structure (Figure 1.6) (Chin and Schepartz, 2001); (Rutledge et al., 2003).

Miniproteins are small proteins that have well defined secondary structures. They can recognize and interact with short peptide chains, forcing the peptide chains to form secondary structures, normally an $\alpha$-helix. The now well-assembled short peptides behave like the $\alpha$-helical domains in the proteins to recognize their targets.

1.6 Foldamers

![Examples of foldamers](image)

Figure 1.7: Examples of foldamers
Foldamers are oligomers have well-defined secondary structures stabilized by non-covalent bonding. The peptidic foldamers use unnatural peptidic building blocks, such as α-peptides, β-peptides, γ-peptides, peptoid, and oligourea, etc. (Figure 1.7) (Werder et al., 1999); (Cheng, 2004); (Kritzer Joshua et al., 2005).

Foldamers not only have secondary structures similar to α-helix (Figure 1.8), but are also capable of disrupting protein-protein interactions involving α-helices.

Figure 1.8: Comparison of α-helix and β-peptide helix

1.7 Synthetic non-peptidic α-helical mimetics with linear scaffold

Besides the peptidic approach to mimic the α-helices, organic chemists have built small molecular (<750 Da) scaffolds in this field. Until now, most drugs on the market are small synthetic molecules. Small molecules have several advantages to serve as the drug molecules, such as being more stable and more membrane permeable.

It is difficult for small molecules to target the shallow binding areas of protein-protein interactions; it is even more difficult for them to mimic the complicated surface
areas of α-helices. This might be the reason why the first synthetic non-peptidic α-helical mimetic small molecules (Figure 1.9) were not reported until 2001 (Garner and Harding, 2007).

![Diagram of synthetic non-peptidic α-helical mimetics](image)

**Figure 1.9:** First reported synthetic non-peptidic α-helical mimetics

Hamilton and coworkers were the first to publish synthetic small molecular non-peptidic α-helical mimetic scaffold, 1,4-terphenylene (Orner et al., 2001). Although the surface of an α-helix is complicated, its binding site is along one face of the α-helix. Therefore, we just need to design molecules to mimic that face of the α-helix. Also, an α-helix has a coil-like backbone; and its configuration is rigid, regardless of the amino acid residues. This means that only residues lined in certain primary structures sequence (e.g. \(i, i + 3, i + 4; i, i + 3, i + 7; i, i + 4, i + 7; i, i + 4, i + 7, i + 11\)) (Figure 1.10) could be on the same face with each other. Thus, the amino acid residues on one face of the α-helix should have fixed relative geometric locations to each other.
Figure 1.10: Model of a typical α-helix (NIH)

The relative location between the amino acid residues on an α-helix can be measured by two factors, the translation along the helical axis, which is 1.5 Å per two residues and the turn angle, which is 100° per residue (wiki). Figure 1.11 shows the relative location of the $i, i+3, i+4; i, i+3, i+7$; and $i, i+4, i+7$, when seen from both the helical axis (a and b) and the side of the α-helix (c). Part a) showed the relative location of the residues when seen from the helical axis. As we can see that the $i, i+3, i+4; i, i+3, i+7$; and $i, i+4, i+7$ residues are all on one face of the α-helix. Part b) eliminated the α-helix backbones, and shows only the relative location among the residues when seen from the helical axis. Part c) added the second factor into consideration – translation along the helical axis, showing their relative location when seen from the side of the α-helix.
Figure 1.11: Model of the common α-helix binding residues

1.7.1 α-Helical mimetic scaffolds developed by Hamilton and coworkers
The success of Hamilton’s 1,4-terphenylene scaffold came from the fact that the
distance between its side chains of the phenyl rings matches that of the $i, i + 4, i + 7$
(Figure 1.12); while the slightly twisted 1,4-terphenylene scaffold pointed its side chains
to similar side chain orientations of the $\alpha$-helix residues.

**Figure 1.12:** Comparison of 1,4-terphenylene scaffold with an $\alpha$-helix

The discovery of the 1,4-terphenylene scaffold has removed a lot of successive
work. Figure 1.13 shows the newer scaffolds reported by Hamilton and coworkers (Ernst
et al., 2003); (Davis et al., 2005); (Estroff et al., 2004); (Yin et al., 2005b); (Rodriguez
and Hamilton, 2006); (Kim and Hamilton, 2006). However, none of the newer scaffold
are as potent as the original 1,4-terphenylene one.
Figure 1.13: Other $\alpha$-helical mimetic scaffolds developed by Hamilton and coworkers

1.7.2 $\alpha$-Helical mimetic scaffolds developed by Rebek and coworkers

The 1,4-terphenylene series of non-peptidic $\alpha$-helical mimetics has not only inspired the Hamilton group, but also inspired chemists all over the world. Figure 1.14 showed the scaffolds developed by Rebek and coworkers (Volonterio et al., 2007); (Moisan et al., 2008).
1.7.3 Trisubstituted imidazole scaffold

One disadvantage of the 1,4-terphenylene series is that it is very challenging to synthesize. Antuch and co-workers attempted to solve this problem by modifying the 1,4-terphenylene scaffold to a trisubstituted imidazole (Figure 1.15), which could easily be synthesized by multicomponent reactions (Antuch et al., 2006).
Figure 1.15: a) the trisubstituted imidazole scaffolds; b) one molecule from the scaffold docking into the binding site of the Bad/Bcl-xL

### 1.7.4 Benzylideneacetophenone scaffold

This scaffold is based on chalcones, which have some intrinsic antitumor activities. Benzylideneacetophenones are planar molecules that mimic the $i, i + 4, i + 7$ positions of $\alpha$-helices (Stoll et al., 2001). Figure 1.16 shows the structure of chalcone C and the way it mimics the $\alpha$-helix.
1.7.5 *trans*-Fused polycyclic ethers scaffold

Polycyclic ethers are conceptually inspired by the skeletal feature of marine toxins. The distance between R₁ and R₂ group (4.8 Å) is very close to that between \( i, i + 4 \) residues (5.4 Å) (Figure 1.17) (Oguri et al., 2005).
1.8 Synthetic non-peptidic α-helical mimetics with other scaffold

We can consider α-helices as a linear backbone. There are several reported potent non-peptidic α-helical mimetics that do not have a linear backbone. An example of this type of mimetic is Nutlin which is the most potent p53/MDM2 antagonist thus far reported. The problem is that researchers have not developed a general strategy to design these kinds of α-helical mimetic scaffolds.

1.8.1 Nutlins

Nutlins were discovered while screening a diverse library of synthetic chemicals (Vassilev et al., 2004), of which, nutlin-2 demonstrated IC$_{50}$ = 140 nM against p53/MDM2 interactions. Nutlins represent a design strategy that is different from Hamilton’s scaffolds, in which all have linear backbones that mimic the coil of an α-helix. Three of the four substitutions on the imidazoline ring mimic the $i, i + 4, i + 7$ position of the p53 N-terminal α-helix. (Figure 1.18)
Figure 1.18: a) Structures of the nutlins; b) nutlin-2 superimposing with Phe$^{19}$, Trp$^{23}$, and Leu$^{26}$ of p53; c) nutlin-2 docking into the binding site of p53/MDM2

1.8.2 1,4-Benzodiazepine-2,5-dione scaffolds

1,4-Benzodiazepine-2,5-diones (Figure 1.19) was also found as a hit when screening a library (Cummings et al., 2006). 1,4-Benzodiazepine-2,5-diones have a heterocyclic backbone, which is similar to that of the nutlin molecules.
Figure 1.19: a) Structures of 1,4-Benzodiazepine-2,5-diones; b) 1,4-Benzodiazepine-2,5-diones superimposing with Phe\textsuperscript{19}, Trp\textsuperscript{23}, and Leu\textsuperscript{26} of p53

1.8.3 Co-activator binding inhibitors

Figure 1.20: Structures of co-activator binding inhibitors
Co-activator binding inhibitors also contain a heterocyclic backbone (Figure 1.20); they mimic the \( i, i+3, i+4 \) positions of an \( \alpha \)-helix (Antuch et al., 2006).

1.9 Summary of non-peptidic \( \alpha \)-helical mimetics

We have summarized the reported non-peptidic scaffolds that could mimic the \( \alpha \)-helices. Both scaffolds with linear backbones and heterocyclic backbones have proven to be potential \( \alpha \)-helical mimetics. The idea is that, the backbones should be capable to assist the hydrophobic side chains to be positioned at the right three-dimensional location. At present, the linear backbone design is more popular because this design also mimics the backbone coil of \( \alpha \)-helix making it more straightforward.

Those molecules showed potential for synthetic scaffolds to mimic the configuration of three or four critical amino acid residues on a \( \alpha \)-helix. The challenge and the joy in designing non-peptidic \( \alpha \)-helical mimetics are to understand the three dimensional structures of the molecules.

1.10 Possibility of designing general non-peptidic \( \alpha \)-helical scaffolds

Most of the reported non-peptidic \( \alpha \)-helical mimetic molecules were designed to target one specific protein-protein interaction, such interactions between p53/MDM2, Bak/Bcl-x\(_L\) etc. However, as analyzed before (1.3.5), the binding configuration should be the same, regardless of the amino acid residues. So a question emerged: is it possible to develop a general non-peptidic \( \alpha \)-helical scaffold?
Figure 1.21: Analogs of 1,4-terphenylene scaffold targeting different protein-protein interactions
This is how nature accomplishes it, as least. Nsp1P, p53/MDM2, smMLCK/CaM, and Rac1/Tiam1 protein-protein interactions all share the $i, i + 4, i + 7$ binding configuration (Table 1.1). As a matter of fact, the 1,4-terphenylene backbones have been used to target several different protein-protein interactions. By replacing the side chains of the 1,4-terphenylene scaffold, the analogs could be used to target different protein-protein interactions (Figure 1.21) (Orner et al., 2001); (Ernst et al., 2002); (Kutzki et al., 2002); (Yin et al., 2005a). This shares the same logic that nature adapted to interact with different protein-protein interactions – by changing the amino acids residues on the $\alpha$-helices.

Although sharing one scaffold, the 1,4-terphenylene scaffold molecules also showed selectivities between the different protein-protein interactions. For example, Hamilton and co-workers stated that the 1,4-terphenylene scaffold demonstrated selectivity between p53/MDM2 and and Bcl-x$_L$ by switching between one naphthyl group (Davis et al., 2007).

### 1.11 Balance between rational design and combinatorial chemistry

Due to the ease in controlling the well-defined three dimensional surface configurations of the mimetics, rational design has proven to be a more effective way to prepare $\alpha$-helical mimetic scaffolds.

One difficulty associated with most of the structurally well-defined scaffolds developed so far is they are difficult to synthesize. This has become a major factor that delays chemists from developing more potent $\alpha$-helical mimetics.
The strategy used in our research was to modify our target scaffolds so that they are suitable for combinatorial synthesis. As we were focusing on the rational design and synthesis of scaffolds with linear backbones, we divided the backbone into several modules, each of which was corresponding to one of the $i, i+4, i+7$ position side chains.

In summary, we tried to find a balance between the well-defined configuration and ease of synthesis by refining our target molecules.

1.12 Cancer: our target

Our goal was to develop $\alpha$-helical mimetic scaffolds that were easy to build, so that we could synthesize focused libraries rapidly. These libraries should be general for all protein-protein interactions that involve $\alpha$-helices. However, our group in the Moffitt Cancer Center, is interested in their anti-cancer properties, specifically, their effects in apoptosis.

Cancer has been identified as a genetic disease; it is a result of a combination of deregulated cell proliferation and suppressed apoptosis (Green and Evan, 2002).

1.12.1 Hallmarks of cancer

There are six factors that identify cancer cells from normal diseases (Hanahan and Weinberg, 2000). First, there is self-sufficiency in the growth signal. Healthy cells need extracellular growth signals, while cancer cells are capable of generating their own growth signals. Second, cancer cells are insensitive to anti-growth signals. Antigrowth
signals in normal cells can temporarily or permanently deactivate the proliferation. Cancer cells block the antigrowth signals pathways, thus are inert to the signals. Third, cancer cells evade apoptosis. Apoptosis is defined as programmed cell death. When DNA damage is sensed, apoptosis will be elicited to kill the damaged cell. Fourth, there is a limitless replicative potential. Normal cells have a finite replicative potential (60-70 doublings for normal human cell types), while cancer cells do not. The fifth factor that identifies cancer cells is that there is a sustained angiogenesis. Angiogenesis is the growth of new blood vessels which are used to supply oxygen and nutrients. This process stops once a tissue is formed. Cancer cells manage to maintain the constant growth of blood vessels so that they can continue to grow. Finally, cancer cells have the capability to invade tissue and metastasize. The primary tumor mass releases cancer cells to colonize new areas in other parts of the body. This is the cause of about 90% of human cancer death.

1.12.2 Apoptosis

As stated before, apoptosis is programmed cell death. Multicellular animals regularly need to remove excess cells and eliminate damaged cells. Avoiding apoptosis is essential for the survival of cancer cells.

Apoptosis can be divided into be two processes – sensors and effectors (Hengartner, 2000). Pro-apoptosis is initiated by the release of cytochrome C, which is regulated by the Bcl-2 family of proteins. In turn, the Bcl-2 family is, regulated by p53, which is the tumor suppressor protein.
When DNA damage is sensed, p53 will be released to upregulate the expression of Bax. Bax is a pro-apoptotic protein in Bcl-2 family; which elicits the release of cytochrome C (Figure 1.22) (SigmaAldrich). This process is called the sensor process. The released cytochrome C will then activate caspases -8 and/or -9, which are the enzymes that perform an array of proteolytic processes to kill the cell. This is the effector process.

Figure 1.22: p53 pathway to elicit apoptosis

1.12.3 p53/MDM2 interaction

p53 is a tumor suppressor protein, which helps maintain the integrity of DNA by eliciting apoptosis when DNA damage is detected. It has been found that the p53 pathway is lost in most human cancers. There are many ways that cancer cells evade apoptosis. The most common way is through the mutation of p53, which was detected in
more than 50% of human cancers (Green and Evan, 2002). For the remaining cases p53 is detected bound to MDM2, causing it to stay in its inactive form.

p53 is regulated by MDM2 by binding to it; but when DNA damage is detected, p53 should be released to initiate apoptosis. Cancer cells prevent the release of p53 by amplifying the expression of MDM2 gene, thus producing more of the MDM2 protein. If the extra MDM2 could be inhibited, the cancer cells with wild-type p53 should be killed.

The MDM2 protein is a small globular protein that has a small hydrophobic cleft at one face (Figure 1.23 a) (Kussie et al., 1996). This cleft is about 25 Å long, 10 Å at its widest place, 10 Å at its deepest location. It is formed by four helices constituting the bottom and the two sides and has two clusters of three-stranded β-sheets closing the ends.

**Figure 1.23:** a) the MDM2 binding cleft; b) crystal structure of p53 binding into the MDM2 cleft

The p53 binding site is an α-helix domain, formed from residues 18 to 26, of which Phe\(^{19}\) \((i)\), Trp\(^{23}\) \((i + 4)\), and Leu\(^{26}\) \((i + 7)\) are the key binding residues (Figure 1.23
This α-helix domain is inserted deeply into the hydrophobic cleft of MDM2, forming very strong van der Waals interaction with the hydrophobic residues of MDM2.

1.12.4 Bcl-2 family protein-protein interactions

The Bcl-2 protein family plays a central role in regulating apoptosis. The family contains both pro-apoptotic (Bax, Bak, Bid, Bim) and anti-apoptotic (Bcl-2, Bcl-xL, Bcl-W) functional proteins. Homo- and heterodimerization within the proteins are the key to modulate their functions; when pro-apoptotic proteins are released from the dimers, apoptosis will be promoted.

Figure 1.24: Bak binding with the Bcl-xL

The Bcl2 and Bcl-xL proteins contain four Bcl-2 homology (BH) domains (BH1-BH4), as well as a C-terminal hydrophobic tail. Bax and Bak contain almost the same components of Bcl2 and Bcl-xL except for the BH4 domain. Bid and Bik only contain the BH3 domain. Among the BH1-BH4 domains, the BH3 domain is involved in
eliciting the apoptosis process; all Bak, Bax, Bid and Bim share similar BH3 domains (Green and Evan, 2002).

The binding site between Bcl-xL and Bak were studied by NMR experiments (Sattler et al., 1997). The binding site of Bak is an α-helix domain (Figure 1.24) formed from residues 72 to 87, of which Val$^{74}(i)$, Leu$^{78}(i+4)$, Ile$^{81}(i+7)$ and Ile$^{85}(i+11)$ are the binding residues. This α-helix binds to the hydrophobic domain on the surface of the Bcl-xL, formed by the BH1, BH2, and BH3 domain.

1.13 References


31


Chapter Two:
Design and Synthesis of 2,5-Terpyrimidinlenes as More Drug-Like
1,4-Terphenylene Mimetics

2.1 Introduction

2.1.1 Hamilton’s 1,4-Terphenylene Scaffold and Related Work

Hamilton’s 1,4-terphenylene library produced an inhibitor that displays favorable
\textit{in vitro} activity towards p53/MDM2 heterodimerization (Figure 2.1, a)(Yin et al., 2005a).

\textbf{Figure 2.1:} Hamilton’s most active p53-MDM2 antagonist a and its docking in MDM2
The 4-, 4′-, and 4″- positions of this terphenylene scaffold are designed to mimic the i, i+4, and i+7 sites of an α-helix. The series of molecules bind to the same sites that p53 binds to MDM2; while the side chains occupy the sites that the triad of p53 (F19, W23, and L26) uses to interact with MDM2.

However, the design has one drawback which prevents it from being a drug candidate. Both the terphenyl scaffold and the 4-, 4′-, and 4″- position side chains are hydrophobic; thus the lipophilicity of this series of molecules is very high (Yin et al., 2005b). This is why the 1,4-terphenylene molecules have good bio-activities in fluorescence polarization (FP) assay, in which the 1,4-terphenylene molecule competitively replaced the p53 (residues 15–31) in interaction with MDM2 in vitro, but not in in vivo assay (Yin et al., 2005a). Further investigation on this scaffold was carried out by the Hamilton group (Ernst et al., 2003), (Davis et al., 2005), (Estroff et al., 2004), (Yin et al., 2005b), (Rodriguez and Hamilton, 2006), as well as the Rebek’s group (Moisan et al., 2008), (Volonterio et al., 2007) (Figure 2.2), but they were not as successful as the original 1,4-terphenylene a (Figure 2.1). Some were designed to mimic the interaction between Bcl-xL/Bak, but that interaction is also mediated through an α-helix, therefore the scaffold should show some similar properties.
Figure 2.2: Recently reported non-peptidic α-helical mimetics: Hamilton’s b–f; Rebek’s g–k
2.1.2 Design of the 2,5-terpyrimidinylene scaffold

Since none of the newer α-helical mimetics are as good as the original 1,4-terphenylene ones, we hypothesized that the terphenyl scaffold might be the best antagonist for the p53/MDM interaction. Our group also found that by simply replacing the phenyl rings of the scaffold with pyrimidine rings, the calculated logP value drops by an average of 4 (Table 2.1), meaning that the corresponding terpyrimidinylene molecules are expected to be more water soluble and therefore be more bio-available.

Table 2.1: Calculated logP values for terphenylene and terpyrimidinylene analogs

| R₀ | CN | CN   | CN | CN |
| R₀' | NH₂ | Ph | Ph | Ph |
| R₁ | iBu | iBu | iBu | Bn |
| R₂ | Bn | Bn | CH₂(1-Naph) | CH₂(1-Naph) |
| R₃ | iBu | iBu | iBu | iBu |
| logP(carbon) | 8.6 | 10.4 | 11.3 | 12.2 |
| logP(nitrogen) | 4.7 | 5.8 | 7 | 7.8 |
| logP(difference) | **3.9** | **4.6** | **4.3** | **4.4** |

Furthermore, due to the geometrical similarity of phenyl and pyrimidine units (Figure 2.3), we reasoned that the 2,5-terpyrimidinylene series would have similar bioactivity, to the corresponding 1,4-terphenylene scaffold.
2.2 Results and Discussion for the 1,4-pyrimidinylene scaffold

2.2.1 Retrosynthesis of the 1,4-pyrimidinylene scaffold

Our group developed a convenient methodology to synthesize pyrimidine rings with varying side chains (Scheme 2.1). We condensed commercially available formamidine/amidine/guanidine \( m \) with readily synthesized \( \alpha,\beta \)-unsaturated \( \alpha \)-cyanoketones \( l \) at elevated temperatures in the presence of base, making a library of pyrimidines \( n \) with variable 2 and 4 positions.

Scheme 2.1 Synthesis of pyrimidine rings

\[
\begin{align*}
R_1 & \quad \text{CN} \quad R_2 \\
R_0 & \quad \text{NH} \quad R_1
\end{align*}
\]

The \( \alpha,\beta \)-unsaturated \( \alpha \)-cyanoketones \( l \) contains an \( \alpha \)-cyano group because this cyano group can potentially be converted into the amidine group after the synthesis of the
monomer n. The newly generated amidine can react with another molecule of α,β-unsaturated α-cyanoketone l to form a dimer p. By repeating this process, we planned to synthesize the trimer o. The corresponding retrosynthetic strategy is shown in Scheme 2.2.

**Scheme 2.2: Retrosynthesis of 1,4-terpyrimidinylene**

\[ \begin{align*}
& \text{R}_0 = \text{H, Me, Ph, NH}_2 \\
& \text{R}_{1-3} = \text{alkyl or aromatic side chains}
\end{align*} \]

### 2.2.2 Synthesis of pyrimidine monomer 2.4 library

The synthesis of pyrimidine monomers 2.4a–g is shown in Scheme 2.3. Acetonitrile was deprotonated by potassium tert-pentoxide, and then reacted with various esters to produce α-cyanoketones 2.2 (Ji et al., 2006), which then reacted with \( N,N \)-dimethylformamide dimethyl acetal (DMF-DMA) to form the α,β-unsaturated α-cyanoketones 2.3 (Reuman et al., 2008). Most of the ester starting materials are commercially available; those that are not can be easily prepared from the corresponding acid by Fisher esterification, using methanol as the solvent, and concentrated sulfuric acid.
Scheme 2.3: Synthesis of the pyrimidine monomers 2.4

![Scheme 2.3: Synthesis of the pyrimidine monomers 2.4](image)

Table 2.2: Library of the synthesized pyrimidine monomers 2.4

<table>
<thead>
<tr>
<th></th>
<th>R₀</th>
<th>R₁</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4a</td>
<td>H</td>
<td>tBu</td>
<td>71%</td>
</tr>
<tr>
<td>2.4b</td>
<td>H</td>
<td>Bn</td>
<td>39%</td>
</tr>
<tr>
<td>2.4c</td>
<td>Me</td>
<td>CH₂(1-Naph)</td>
<td>60%</td>
</tr>
<tr>
<td>2.4d</td>
<td>Ph</td>
<td>tBu</td>
<td>60%</td>
</tr>
<tr>
<td>2.4e</td>
<td>Ph</td>
<td>Bn</td>
<td>87%</td>
</tr>
<tr>
<td>2.4f</td>
<td>Ph</td>
<td>tBu</td>
<td>83%</td>
</tr>
<tr>
<td>2.4g</td>
<td>Ph</td>
<td>CH₂(1-Naph)</td>
<td>40%</td>
</tr>
<tr>
<td>2.4h</td>
<td>NH₂</td>
<td>Bn</td>
<td>91%</td>
</tr>
<tr>
<td>2.4i</td>
<td>NH₂</td>
<td>tBu</td>
<td>75%</td>
</tr>
<tr>
<td>2.4j</td>
<td>OMe</td>
<td>Bn</td>
<td>30%</td>
</tr>
</tbody>
</table>

as the catalyst. The double bond of compound 2.3 has Z as its dominate configuration; probably due to the steric hindrance of the carbonyl group. The α,β-unsaturated α-
cyanoketones 2.3 reacted with a variety of commercially available formamidine/amidines/guanidine to form the pyrimidine monomers 2.4a–g.

This methodology has very broad application, in that a library of monomers have been synthesized (Table 2.2).

2.2.3 Conversion of the 5-cyano group to an amidine

The key step of the synthesis is the conversion of the 5-cyano to an amidine group. Several methods have been explored, including the Pinner reaction, the thio-Pinner reaction (Balo et al., 2007), ammonia in methanol (Balo et al., 2007), and NaHMDS or LiHMDS (Bruning, 1997) used as nucleophiles, followed by hydrolysis; but these methods were not successful in yielding the target compounds. Since the 4-alkyl side chain of our molecules 2.4 (Scheme 2.3) made the cyano group sterically hindered, all the regular methodologies failed. We eventually found a two-step procedure to achieve this process (Scheme 2.4) (Judkins et al., 1996). First, free hydroxylamine was the a nucleophile to attack the cyano group at elevated temperatures to form amidinoximes q; the N,O single bond was then cleaved in the second step by

Scheme 2.4: Synthetic strategy to convert sterically hindered nitriles to amidines
hydrogenation to prepare the amidine r.

This hydroxylamine nucleophilic addition reaction is very unique (Scheme 2.4, n to q), because a great amount of oxygen attacking amide byproduct (yields: 20-50%) was detected (Scheme 2.5). Typically, the neutral nitrogen atom is a lot more nucleophilic than the neutral oxygen atom.

**Scheme 2.5**: Reaction and side reaction of hydroxylamine nucleophilic addition

![Scheme 2.5](attachment:image.png)

Exact mechanistic details for this reaction have not been reported. However, a possible explanation for the diminished selectivity between the N- and O- nucleophilic species due to the steric hindrance from the ortho substituent on the pyrimidine rings.

It should be pointed out that hydroxylamine behaves not only as a nucleophile, but also as a cyano activator. In fact, we found that under the same reaction conditions without hydroxylamine, the cyano group cannot be hydrolyzed to the amide s. Further evidence in support of hydroxylamine’s properties in this reaction is that, when using O-protected hydroxylamine, (e.g. O-benzylhydroxylamine and O-methylhydroxylamine), not even nitrogen attack product q was formed; while all the starting materials remained. The mechanism proposed by Judkins et al, who originally published the hydroxylamine,
suggested that the hydrogen atom on the oxygen might behave as a Brønsted acid interacting with the nitrogen atom of the cyano group, thus activating it through a five-membered ring transition state (Scheme 2.6).

Scheme 2.6: Proposed transition state of hydroxylamine nucleophilic addition

2.2.4 Synthesis of the pyrimidine dimer

Five pyrimidinylene dimer analogs (Table 2.3) were prepared by condensing the amidines with the α,β-unsaturated α-cyanoketones (Scheme 2.7). The same reaction

Scheme 2.7: Synthesis of the pyrimidine dimer

i) NH₂OH·HCl, K₂CO₃, EtOH/H₂O, reflux, 24 h; then, Ac₂O, HOAc,K₂CO₃, HCOOH, MeOH, 10% Pd/C, rt 12 h; ii) α,β-unsaturated α-cyanoketones 2.3, Et₃N, EtOH, reflux, 24 h
conditions which were used to synthesize the pyridine monomers were also used to synthesize the dimers. This provides a good example of the broad scope of this condensation methodology to synthesize the pyrimidine rings.

Table 2.3: Analogs of the synthesized pyrimidine dimers

<table>
<thead>
<tr>
<th></th>
<th>R₀</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6a</td>
<td>Ph</td>
<td>Bn</td>
<td>CH₂(1-Naph)</td>
</tr>
<tr>
<td>2.6b</td>
<td>Ph</td>
<td>tBu</td>
<td>CH₂(1-Naph)</td>
</tr>
<tr>
<td>2.6c</td>
<td>Ph</td>
<td>CH₂(1-Naph)</td>
<td>Bn</td>
</tr>
<tr>
<td>2.6d</td>
<td>Me</td>
<td>CH₂(1-Naph)</td>
<td>Bn</td>
</tr>
<tr>
<td>2.6e</td>
<td>Me</td>
<td>CH₂(1-Naph)</td>
<td>tBu</td>
</tr>
</tbody>
</table>

2.2.5 X-Ray crystal structure of a representative dimer

The X-ray diffraction crystal structure of compound 2.6d (Figure 2.4) was obtained by Dr. Fronczek (Louisiana State University). As it can be seen, the pyrimidine scaffold is arranged in a twisted conformation. This was expected for compounds designed to mimic an α-helix.
2.2.6 Synthesis of the pyrimidine trimers

Exactly the same process used to synthesize the dimers was also applied to the synthesis of the pyrimidine trimers (Scheme 2.8). Three trimer analogs were synthesized (Table 2.4).

Scheme 2.8 Synthesis of the pyrimidine trimers

\[
\begin{align*}
\text{2.6} & \quad \text{2.7} & \quad \text{2.8} \\
\text{i) NH}_2\text{OH} \cdot \text{HCl, K}_2\text{CO}_3, \text{EtOH/H}_2\text{O, reflux, 24 h; then, Ac}_2\text{O, HOAc, K}_2\text{CO}_3,} \\
\text{HCOOH, MeOH, 10% Pd/C, rt, 12 h; ii) } & \alpha,\beta\text{-unsaturated } \alpha\text{-cyanoketones 2.3,} \\
\text{Et}_3\text{N, EtOH, reflux, 24 h} & \\
2.8 \text{a} & \quad \text{2.8b} & \quad \text{2.8c} \\
\text{R}_0 & \text{R}_1 & \text{R}_2 & \text{R}_3 \\
\text{Ph} & \text{'Bu} & \text{CH}_2(1\text{-Naph}) & \text{'Bu} \\
\text{Ph} & \text{Bn} & \text{CH}_2(1\text{-Naph}) & \text{'Bu} \\
\text{Ph} & \text{CH}_2(1\text{-Naph}) & \text{Bn} & \text{'Bu} \\
\end{align*}
\]

Table 2.4: Analogs of the synthesized pyrimidine trimers
2.3 Conclusion

We have successfully synthesized several 1,4-terpyrimidinylene analogs. The X-ray crystal structure did confirm their potential as \( \alpha \)-helical mimetics. The newly synthesized series of molecules has been the one that has the closest structural similarity to Hamilton’s most active p53/MDM2 inhibitor. However, none of our molecules are active against p53/MDM2. We reasoned that although they mimic the side chains of Hamilton’s terphenylene quite well, our molecules are unable to establish the polar interactions that are available to Hamilton’s molecules due to the polar groups present at the head and the tail position. Those hydrogen bonding/salt bridge bonding could be crucial for the molecules’ bioactivities, as well. Another drawback of our molecules is that they are still too hydrophobic to be suitable drug candidates. There is an improvement from Hamilton’s design, but it is still not enough. Third, the synthesis is a long, linear, time-consuming process; it is very hard to develop a large library of compounds with such a method.

In summary, we have successfully developed a new series of non-peptidic \( \alpha \)-helical mimetics; however, further improvement is needed for this series of molecules to be more efficient p53/MDM antagonists, and be sufficiently drug like, cell permeable compounds.

2.4 Experimental Section

2.4.1 Materials and Methods
Starting materials, organic and inorganic reagents (ACS grade), and solvents were obtained from commercial sources and used as received unless otherwise noted. Moisture- and air-sensitive reactions were carried out under an atmosphere of argon. Thin layer chromatography (TLC) was performed on glass plates precoated with 0.25 mm thickness of silica gel (60 F-254) with fluorescent indicator (EMD). Column chromatographic purification was performed using silica gel 60 Å, #70-230 mesh (Selecto Scientific). Automated flash chromatography was performed in a FlashMaster II system (Argonaut-Biotage) using Biotage silica cartridges. $^1$H NMR and $^{13}$C NMR spectra were obtained using a 400 MHz Varian Mercury or 250 MHz Broker plus instrument at 25 °C in chloroform-d (CDCl$_3$), unless otherwise indicated. Chemical shifts (ppm) are reported in parts per million (ppm) relative to internal tetramethylsilane (TMS) or chloroform (ppm 7.26) for $^1$H NMR and chloroform (ppm 77.0) for $^{13}$C NMR. Multiplicity is expressed as (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, or m = multiplet) and the values of coupling constants ($J$) are given in Hertz (Hz). High Resolution Mass Spectrometry (HRMS) spectra were carried out on an Agilent 1100 Series in the ESI-TOF mode. Microwave reactions were performed in a closed vessel in a Biotage Initiator I microwave reactor. Melting points (uncorrected) were determined using a Mel-Temp II®, Laboratory Devices, MA, USA.

2.4.2 Experimental Procedures

Methyl 2-(naphthalen-2-yl)acetate (2.1a)
To a solution of 2-naphthylacetic acid (5.4 mmol) in methanol (10 ml) was added concentrated sulfuric acid (0.3 mmol). The reaction mixture was stirred under reflux for 3 h. The reaction mixture was concentrated under reduced pressure, and was then extracted between dichloromethane and water. The organic layer was extracted once with saturated sodium bicarbonate solution, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford compound 2.1a as a colorless oil (1.07 g, 100%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) ppm 3.75 (s, 3H), 3.84 (s, 2H), 7.39–7.56 (m, 3H), 7.77 (s, 1H), 7.80–7.90 (m, 3H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) ppm 41.6, 52.3, 126.07, 126.4, 127.6, 127.9, 127.9, 128.2, 128.5, 131.7, 132.7, 133.7, 172.3.

3-Oxo-4-phenylbutanenitrile (2.2a)

To a solution of methyl phenylacetate (71.0 mmol) and anhydrous acetonitrile (107.2 mmol) in anhydrous THF (20 ml) at 0 ºC in an ice-bath under an argon atmosphere was added potassium tert-pentoxide (107.2 mmol). The reaction mixture was allowed to warm up to room temperature and stirred under an argon atmosphere for overnight. A precipitate was formed within a few minutes (3-5 min.) of stirring and the reaction mixture remained cloudy until completion. The mixture was filtered and the solid was washed thoroughly with hexanes. The filtered residue was then transferred to a
separatory funnel and was acidified to pH= ~2-3 with saturated aq. potassium bisulfate solution. DCM was then used to extract the desired compound. The organic layer was washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to afford pure 2.2a as a yellow oil (7.34 g, 65%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 3.46 (s, 2H), 3.86 (s, 2H), 7.21–7.40 (m, 5H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 31.1, 49.2, 113.5, 127.9, 129.3, 129.4, 131.9, 195.1.

4-(Naphthalen-1-yl)-3-oxobutanenitrile (2.2b)

![Structure of 4-(Naphthalen-1-yl)-3-oxobutanenitrile](image)

Compound 2.2b was prepared following the procedure described for 2.2a. Isolated yield: 64% yield, yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) ppm 3.41 (s, 2H), 4.31 (s, 2H), 7.43–7.66 (m, 4H), 7.81–7.98 (m, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 31.2, 47.8, 113.7, 123.3, 125.9, 126.6, 127.5, 128.7, 129.0, 129.3, 129.4, 132.0, 134.3, 196.0.

4-(Naphthalen-2-yl)-3-oxobutanenitrile (2.2c)

![Structure of 4-(Naphthalen-2-yl)-3-oxobutanenitrile](image)

Compound 2.2c was prepared following the procedure described for 2.2a. Isolated yield: 71% yield, yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) ppm 3.51 (s, 2H), 4.06 (s, 2H), 7.34 (dd, $J = 1.8$, 8.4 Hz, 1H), 7.51–7.57 (m, 2H), 7.74 (s, 1H), 7.84–7.93 (m, 3H). $^{13}$C
NMR (100 MHz, CDCl$_3$) ppm 31.4, 49.6, 113.8, 126.7, 126.9, 127.1, 127.9, 128.0, 128.8, 129.4, 129.5, 132.9, 133.7, 195.5.

5-Methyl-3-oxohexanenitrile (2.2d)

\[ \text{Compound 2.2d was prepared by the same procedure described for 2.2a. Isolated yield: 73%, yellow oil. } ^1\text{H NMR (400 MHz, CDCl}_3\text{) ppm 0.95 (d, } J = 0.85 \text{ Hz, 3H), 0.97 (d, } J = 0.85 \text{ Hz, 3H), 2.11-2.25 (m, 1H), 2.49 (d, } J = 6.88 \text{ Hz, 2H), 3.43 (s, 2H). } ^{13}\text{C NMR (100 MHz, CDCl}_3\text{) ppm 22.5, 24.7, 32.6, 51.1, 113.9, 197.2.} \]

3-((1H-Indol-3-yl)-3-oxopropanenitrile (2.2e)

\[ \text{Compound 2.2 was prepared following the procedure described by Radwan, M. A. A. et al. Isolated yield: 29%, pale orange solid, m.p. 238 °C (lit.= 240 °C). Spectral data is in agreement with literature (Bioorganic Med. Chem. Lett. 2007, 15, 1206).} \]

2-((Dimethylamino)methylene)-3-oxo-4-phenylbutanenitrile (2.3a)
To a solution of \(2.2a\) (45.8 mmol) in anhydrous THF (24 mL) was added DMF-DMA (59.8 mmol). The reaction mixture was stirred at r.t. for overnight. The reaction mixture was then concentrated under reduced pressure to afford \(2.3a\) as a yellow solid (>90%). The product was used in the next step without further purification. For characterization purpose, the product was recrystallized in ethanol to afford light yellow needle-like crystals, m.p. 134-138 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) ppm 3.20 (s, 3H), 3.38 (s, 3H), 3.95 (s, 2H), 7.21–7.34 (m, 5H), 7.80 (s, 1H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) ppm 9.0, 46.5, 48.2, 120.5, 126.9, 128.7, 129.8, 129.9, 135.0, 158.0, 192.9. HRMS (ESI) calcd. for C\(_{13}\)H\(_{14}\)N\(_2\)O \([\text{M + H}]^+\) 215.1184, found 215.1194.

2-((Dimethylamino)methylene)-4-(naphthalen-1-yl)-3-oxobutanenitrile (2.3b)

\[
\text{\begin{figure}

\end{figure}}\]

Compound \(2.3b\) was prepared following the procedure described for \(2.3a\). Isolated yield: >90%, yellow solid, m.p. 121–122 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)) ppm 3.17 (s, 3H), 3.41 (s, 3H), 4.43 (s, 2H), 7.40–7.54 (m, 4H), 7.77 (d, \(J = 7.9\) Hz, 1H), 7.80–7.86 (m, 2H), 7.99 (d, \(J = 8.2\) Hz, 1H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) ppm 39.1, 44.1, 48.1, 80.1, 120.6, 124.6, 125.7, 125.8, 126.3, 127.9, 128.7, 128.8, 131.8, 132.6, 134.4, 158.1, 192.6. HRMS (ESI) calcd. for C\(_{17}\)H\(_{16}\)N\(_2\)O \([\text{M + H}]^+\) 265.1335, found 265.1313.

2-((Dimethylamino)methylene)-4-(naphthalen-2-yl)-3-oxobutanenitrile (2.3c)
Compound 2.3c was prepared following the procedure described for 2.3a. Isolated yield: >90%, yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) ppm 3.20 (s, 3H), 3.39 (s, 3H), 4.12 (s, 2H), 7.40–7.50 (m, 3H), 7.77–7.84 (m, 5H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 39.0, 46.7, 48.2, 80.1, 120.5, 125.8, 126.2, 127.8, 128.0, 128.3, 128.5, 132.6, 133.8, 158.0, 192.9. HRMS (ESI) calcd. for C$_{17}$H$_{16}$N$_2$O [M + H]$^+$ 265.1335, found 265.1333.

2-((Dimethylamino)methylene)-5-methyl-3-oxohexanenitrile (2.3d)

Compound 2.3d was prepared following the procedure described for 2.3a. Isolated yield: >90%, yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.95 (d, $J = 1.48$ Hz, 3H), 0.96 (d, $J = 1.47$ Hz, 3H), 2.13-2.25 (m, 1H), 2.54 (dd, $J = 7.03$ Hz, 2H), 3.24 (s, 3H), 3.40 (s, 3H), 7.82 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.8, 25.8, 39.0, 48.1, 48.8, 80.8, 120.6, 157.6, 195.4. HRMS (ESI) calcd. for C$_{10}$H$_{17}$N$_2$O [M + H]$^+$ 181.1341, found 181.1333.

2-((Dimethylamino)methylene)-4,4-dimethyl-3-oxopentanenitrile (2.3e)
Compound 2.3e was prepared following the procedure described for 2.3a. Isolated yield: >90%, white solid, m.p. 48-49 °C. $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.32 (s, 9H), 3.23 (s, 2H), 3.42 (s, 2H), 7.92 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 27.0, 39.1, 43.8, 48.5, 121.5, 160.5, 200.4. HRMS (ESI) calcd. for C$_{10}$H$_{16}$N$_2$O [M + H]$^+$ 181.1341, found 181.1340.

4-(tert-Butyl)pyrimidine-5-carbonitrile (2.4a)

4-Benzylpyrimidine-5-carbonitrile (2.4b)
Compound 2.4b was prepared following the procedure described for 2.4a. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.4b as a colorless oil (39%). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) ppm 4.25 (s, 2H), 7.17–7.26 (m, 2H), 7.26–7.33 (m, 3H), 8.83 (s, 1H), 9.20 (s, 1H). \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)) ppm 43.1, 109.2, 114.8, 127.8, 129.2, 129.5, 135.7, 160.4, 160.5, 172.0. HRMS (ESI) calcd. for C\(_{12}\)H\(_9\)N\(_3\)[M + H]\(^+\) 196.0875, found 196.0868.

2-Methyl-4-(naphthalen-1-ylmethyl)pyrimidine-5-carbonitrile (2.4c)

Compound 2.4c was prepared following the procedure described for 2.4a, using commercially available acetamidine hydrochloride. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:4) affords 2.4c as an off-white solid (60%). \( ^1H \) NMR (400 MHz, CDCl\(_3\)) ppm 2.75 (s, 3H), 4.73 (s, 2H), 7.58–7.41 (m, 4H), 7.84 (dd, \( J = 8.1, 23.5 \) Hz, 2H), 8.27 (d, \( J = 8.4 \) Hz, 1H), 8.80 (s, 1H). \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)) ppm 26.9, 40.7, 106.2, 115.4, 124.4, 125.7, 126.1, 126.6, 128.6, 128.6, 129.0, 132.1, 132.2, 134.17, 160.6, 171.1, 171.5.

4-\textit{iso}-Butyl-2-phenylpyrimidine-5-carbonitrile (2.4d)
Compound 2.4d was prepared following the procedure described for 2.4a, using commercially available benzamidine hydrochloride. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.4dc as an off-white solid (60%), m.p. 68-69 ºC. $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.05 (d, $J$ = 6.7 Hz, 6H), 2.39 (m, 1H), 2.93 (d, $J$ = 7.2 Hz, 2H), 7.49–7.59 (m, 3H), 8.50–8.53 (m, 2H), 8.93 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.6, 28.9, 45.7, 106.7, 115.8, 129.0, 129.4, 132.4, 136.4, 160.4, 165.7, 173.3. HRMS (ESI) calcd. for C$_{15}$H$_{15}$N$_3$ [M + H]$^+$ 238.1344, found 238.1337.

4-Benzyl-2-phenylpyrimidine-5-carbonitrile (2.4e)

Compound 2.4e was prepared following the procedure described for 2.4a, using commercially available benzamidine hydrochloride. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:7) affords 2.4e as an off-white solid (87%), m.p. 138-141 ºC. $^1$H NMR (400 MHz, CDCl$_3$) ppm 4.37 (s, 2H), 7.27–7.30 (m, 1H), 7.35 (dd, $J$ = 10.1, 4.6 Hz, 2H), 7.45–7.59 (m, 5H), 8.49–8.54 (m, 2H), 8.92 (s, 1H).
$^{13}$C NMR (100 MHz, CDCl$_3$) ppm 43.3, 105.9, 115.7, 127.6, 129.0, 129.1, 129.5, 129.5, 132.6, 136.1, 136.2, 160.8, 166.0, 171.9. HRMS (ESI) calcd. for C$_{18}$H$_{13}$N$_3$ [M + H]$^+$ 272.1188, found 272.1196.

4-tert-Butyl-2-phenylpyrimidine-5-carbonitrile (2.4f)

![Chemical structure of 4-tert-Butyl-2-phenylpyrimidine-5-carbonitrile (2.4f)]

Compound 2.4f was prepared following the procedure described for 2.4a, using commercially available benzamidine hydrochloride. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:7) affords 2.4f as an off-white solid (83%), m.p. 101-103 ºC. $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.60 (s, 9H), 7.49–7.58 (m, 3H), 8.51–8.55 (m, 2H), 8.94 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 28.8, 40.2, 104.0, 117.2, 128.9, 129.4, 132.4, 136.5, 162.5, 164.8, 179.3. HRMS (ESI) calcd. for C$_{15}$H$_{15}$N$_3$ [M + H]$^+$ 238.1344, found 238.1347.

4-(Naphthalen-1-ylmethyl)-2-phenylpyrimidine-5-carbonitrile (2.4g)

![Chemical structure of 4-(Naphthalen-1-ylmethyl)-2-phenylpyrimidine-5-carbonitrile (2.4g)]
Compound \(2.4g\) was prepared following the procedure described for \(2.4a\), using commercially available benzamidine hydrochloride. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:7) affords \(2.4g\) as an off-white solid (40%). \(^1^H\) NMR (400 MHz, CDCl\(_3\)) ppm 4.83 (s, 2H), 7.44 – 7.63 (m, 7H), 7.82 (d, \(J = 8.2\), 1H), 7.87 (d, \(J = 8.1\), 1H), 8.37 – 8.45 (m, 3H), 8.94 (s, 1H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) ppm 40.7, 106.2, 115.8, 124.7, 125.8, 126.1, 126.5, 128.6, 128.6, 129.0, 129.0, 129.4, 132.6, 161.0, 165.8, 171.6.

**2-Amino-4-benzylpyrimidine-5-carbonitrile (2.4h)**

\[
\begin{align*}
\text{NH}_2 \\
\text{N} & \text{N} \\
\text{CN}
\end{align*}
\]

Compound \(2.4h\) was prepared following the procedure described for \(2.4a\), using commercially available guanidine hydrochloride. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:1) affords \(2.4h\) as an off-white solid (91%), m.p. 129-132 °C. \(^1^H\) NMR (400 MHz, CDCl\(_3\)) ppm 4.10 (s, 2H), 5.58 (br s, 2H), 7.24-7.38 (m, 5H), 8.45 (s, 1H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) ppm 42.9, 97.8, 116.4, 127.5, 129.0, 129.5, 136.0, 162.4, 162.8, 173.5. HRMS (ESI) calcd. for C\(_{12}\)H\(_{10}\)N\(_4\) [M + H]\(^+\) 211.0984, found 211.0972.

**2-Amino-4-\textit{tert}-butylpyrimidine-5-carbonitrile (2.4i)**
Compound **2.4i** was prepared following the procedure described for **2.4a**, using commercially available guanidine hydrochloride. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:1) affords **2.4i** as an off-white solid (75%), m.p. 89-91 °C. $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.43 (s, 9H), 5.56 (br s, 2H), 8.44 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 28.5, 39.5, 95.6, 118.3, 162.4, 164.0, 181.2. HRMS (ESI) calcd. for C$_9$H$_{12}$N$_4$ [M + H]$^+$ 177.1140, found 177.1148.

4-Benzyl-2-methoxypyrimidine-5-carbonitrile (2.4j)

Compound **2.4j** was prepared following the procedure described for **2.4a**, using commercially available O-methylisourea sulfate. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:3) affords **2.4j** as an off-white solid (30%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 4.07 (s, 3H), 4.22 (s, 2H), 7.26 (t, $J$ = 7.3 Hz, 1H), 7.29 – 7.35 (m, 2H), 7.41 (d, $J$ = 7.4 Hz, 2H), 8.68 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 43.1, 56.1, 101.9, 110.6, 115.5, 127.7, 129.1, 129.5, 135.8, 163.4, 166.2, 175.1.

4'-Benzyl-4-(naphthalen-1-ylmethyl)-2'-phenyl-[2,5'-bipyrimidine]-5-carbonitrile (2.6a)
Step 1: To a solution of compound 2.4e (4.1 mmol) in ethanol (9 mL) was added a solution of hydroxylamine hydrochloride (16.3 mmol) and potassium carbonate (8.1 mmol) in water (5 mL). The reaction mixture was stirred under reflux overnight. The reaction mixture was concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to obtain the intermediate amidoxime, which was used in the next step without further purification.

Step 2: To a solution of the intermediate from step 1 in glacial acetic acid (10 mL) was added acetic anhydride (246 µL). After 5 min. of stirring, a solution of potassium formate prepared in situ from potassium carbonate (10 mmol) and formic acid (20 mmol) in methanol (4 mL) was added, followed by 10% Pd/C (0.4 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered through Celite™ and rinsed with methanol. The filtrate was concentrated under reduced pressure to afford a yellow residue. This residue was redissolved in DCM and filtered through the Celite™ and rinsed with DCM. The filtrate was concentrated under reduced pressure to
afford the crude the carboxamidine acetate salt as a yellow solid, which was used in the next step without further purification.

Step 3: To the solution of the carboxamidine salt from step 2 in ethanol (5 mL) was added compound 2.3d (4.1 mmol) and sodium ethoxide (4.1 mmol). The reaction mixture was stirred under reflux for overnight. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed by reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:5) affords 2.4b as white solid (32%), m.p. 165–166 °C. $^1$H NMR (400 MHz, CDCl$_3$) ppm 4.45 (s, 2H), 4.84 (s, 2H), 6.88–6.95 (m, 2H), 7.06–7.11 (m, 3H), 7.42–7.56 (m, 7H), 7.84 (dd, $J = 8.1$, 19.3 Hz, 2H), 8.17 (d, $J = 8.2$ Hz, 1H), 8.45–8.52 (m, 2H), 9.01 (s, 1H), 9.33 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 40.1, 43.2, 105.0, 106.3, 115.1, 124.3, 125.5, 125.9, 126.2, 126.5, 127.3, 127.4, 127.7, 128.8, 128.9, 129.0, 129.1, 129.4, 131.6, 132.4, 134.0, 135.1, 135.6, 137.0, 160.3, 160.6, 165.1, 169.1, 172.1. HRMS (ESI) calcd. for C$_{33}$H$_{22}$N$_5$ [M + H]$^+$ 490.2026, found 490.2002.

4'-iso-Butyl-4-(naphthalen-1-ylmethyl)-2'-phenyl-[2,5'-bipyrimidine]-5-carbonitrile (2.6b)
Compound 2.6b was prepared following the procedure described for 2.6a using 2.4d. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.6c as an off-white solid (33%).

4-Benzyl-4'-{(naphthalen-1-ylmethyl)-2'-phenyl-[2,5'-bipyrimidine]-5-carbonitrile (2.6c)

Compound 2.6c was prepared following the procedure described for 2.6a using 2.4g. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.6c as an off-white solid (41%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 4.15 (s, 2H), 5.17 (s, 2H), 6.96 (d, $J = 7.1$ Hz, 1H), 7.21 (d, $J = 1.9$ Hz, 4H), 7.40–7.53 (m, 5H), 7.71 (d, $J = 8.2$ Hz, 1H), 7.83–7.88 (m, 1H), 8.10–8.15 (m, 1H), 8.39 (ddd, $J = 6.8$, 3.8, 2.0 Hz, 1H).
4-Benzyl-2'-methyl-4'-(naphthalen-1-ylmethyl)-[2,5'-bipyrimidine]-5-carbonitrile (2.6d)

Compound 2.6d was prepared following the procedure described for 2.6a using 2.4c. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.6d as an off-white solid (30%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 2.81 (s, 3H), 4.04 (s, 2H), 5.08 (s, 2H), 6.76 (d, $J = 7.10$ Hz, 1H), 7.06-7.11 (m, 2H), 7.14-7.23 (m, 4H), 7.45-7.53 (m, 2H), 7.66 (d, $J = 8.21$ Hz, 1H), 7.81 (d, $J = 8.26$ Hz, 1H), 8.06 (d, $J = 7.73$ Hz, 1H), 8.77 (s, 1H), 9.36 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 26.3, 39.7, 43.1, 106.5, 115.0, 123.9, 125.5, 126.0, 126.1, 126.3, 127.3, 127.5, 127.7, 129.0, 129.1, 129.4, 132.2, 133.9, 134.9, 135.6, 159.7, 160.6, 164.9, 169.0, 169.6, 172.0.

4-(tert-Butyl)-2'-methyl-4'-(naphthalen-1-ylmethyl)-[2,5'-bipyrimidine]-5-carbonitrile (2.6e)
Compound 2.6d was prepared following the procedure described for 2.6a using 2.4c. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.6d as an off-white solid (30%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.35 (s, 9H), 2.77 (s, 3H), 5.11 (s, 2H), 6.80 (d, $J = 7.1$ Hz, 1H), 7.20 (d, $J = 7.2$ Hz, 1H), 7.43–7.51 (m, 2H), 7.64 (d, $J = 8.3$ Hz, 1H), 7.78–7.83 (m, 1H), 8.02 (d, $J = 9.0$ Hz, 1H), 8.80 (s, 1H), 9.32 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 14.4, 21.3, 26.4, 28.6, 29.9, 39.7, 40.1, 60.6, 104.6, 116.4, 124.0, 125.4, 125.9, 126.2, 126.2, 127.3, 127.8, 128.9, 132.2, 133.9, 134.7, 159.7, 162.2, 164.1, 168.6, 169.5, 171.3, 179.6.

5’-Cyano-4,4’-diisobutyl-4’-(1-naphthylmethyl)-2-phenylterpyridine (2.8a)
Compound 2.8a was prepared following the procedure described for 2.6a using 2.6b. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.8a as an off-white solid (20%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.71 (d, $J$ = 6.7 Hz, 6H), 0.76 (d, $J$ = 6.6 Hz, 6H), 1.89–2.01 (m, 1H), 2.05-2.18 (m, 1H), 2.66 (d, $J$ = 7.3 Hz, 2H), 2.95 (d, $J$ = 7.0 Hz, 2H), 5.14 (s, 2H), 6.95 (d, $J$ = 7.0 Hz, 1H), 7.19-7.25 (m, 1H), 7.39-7.47 (m, 5H), 7.65 (d, $J$ = 8.2 Hz, 1H), 7.79 (dd, $J$ = 6.2, 3.3 Hz, 1H), 7.98 (dd, $J$ = 6.1, 3.4 Hz, 1H), 8.41-8.48 (m, 2H), 8.86 (s, 1H), 9.21 (s, 1H), 9.53 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.4, 22.7, 28.4, 29.0, 40.1, 44.5, 45.6, 107.5, 115.0, 124.0, 125.5, 126.0, 126.4, 126.7, 127.5, 127.7, 128.5, 128.8, 128.9, 129.1, 131.1, 132.3, 134.1, 134.7, 137.8, 159.5, 160.0, 160.2, 164.1, 164.5, 164.9, 169.1, 170.0, 173.8. HRMS (ESI) calcd. for C$_{38}$H$_{35}$N$_7$ [M + H]$^+$ 590.3027, found 590.2984.

$^5$"-Cyano-$^4$"-isobutyl-$^4'$-(1-naphthylmethyl)-$^4$-(2-phenylmethyl)-2-phenylterpyrimidine (2.8b):

![Structure of 2.8b]
Compound 2.8a was prepared following the procedure described for 2.6a using 2.6b. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.8b as an off-white solid (20%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.71 (d, $J = 6.7$ Hz, 6H), 0.76 (d, $J = 6.6$ Hz, 6H), 1.89–2.01 (m, 1H), 2.05-2.18 (m, 1H), 2.66 (d, $J = 7.3$ Hz, 2H), 2.95 (d, $J = 7.0$ Hz, 2H), 5.14 (s, 2H), 6.95 (d, $J = 7.0$ Hz, 1H), 7.19-7.25 (m, 1H), 7.39-7.47 (m, 5H), 7.65 (d, $J = 8.2$ Hz, 1H), 7.79 (dd, $J = 6.2$, 3.3 Hz, 1H), 7.98 (dd, $J = 6.1$, 3.4 Hz, 1H), 8.41-8.48 (m, 2H), 8.86 (s, 1H), 9.21 (s, 1H), 9.53 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.4, 22.7, 28.4, 29.0, 40.1, 44.5, 45.6, 107.5, 115.0, 124.0, 125.5, 126.0, 126.4, 126.7, 127.5, 127.7, 128.5, 128.8, 128.8, 129.1, 131.1, 132.3, 134.1, 134.7, 137.8, 159.5, 160.0, 160.2, 164.1, 164.5, 164.9, 169.1, 170.0, 173.8. HRMS (ESI) calcd. for C$_{38}$H$_{35}$N$_7$ [M + H]$^+$ 590.3027, found 590.2984.

5”-Cyano-4”-isobutyl-4’-(2-phenylmethyl)-4-(1-naphthylmethyl)-2-phenylterpyrimidine (2.8C):

![Chemical Structure](image-url)
Compound 2.8c was prepared following the procedure described for 2.6a using 2.6c. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 2.8c as an off-white solid (17%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.93 (d, $J = 6.6$ Hz, 6H), 2.13 – 2.27 (m, 1H), 2.85 (d, $J = 7.2$ Hz, 2H), 4.55 (s, 2H), 5.10 (s, 2H), 6.94 - 7.08 (m, 5H), 7.21 (t, $J = 7.6$ Hz, 1H), 7.29 - 7.40 (m, 6H), 7.62 (d, $J = 8.2$ Hz, 1H), 7.75 (dd, $J = 6.2$, 3.3 Hz, 1H), 8.06 (dd, $J = 5.9$, 3.4 Hz, 1H), 8.23 - 8.28 (m, 2H), 8.92 (d, $J = 0.5$ Hz, 1H), 9.38 (d, $J = 0.5$ Hz, 1H), 9.44 (d, $J = 0.5$ Hz, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.6, 29.2, 39.7, 42.2, 45.8, 107.6, 115.0, 124.8, 125.6, 126.0, 126.7, 127.0, 127.2, 127.5, 128.2, 128.6, 128.7, 128.7, 128.9, 129.3, 131.2, 132.7, 134.0, 135.5, 137.4, 138.1, 159.9, 160.1, 160.2, 164.2, 164.5, 164.5, 168.6, 169.1, 173.7.

2.5 References


Chapter Three:

Convergent Approach to Synthesis 2,5-Terpyrimidinylene Based Derivatives

3.1 Introduction

3.1.1 Optimization of synthetic route to the 2,5-terpyrimidinylene scaffold

We have successfully synthesized several analogs of the 2,5-terpyrimidinylene scaffold; however, the design and the synthesis need further optimizations.

3.1.2 Modifications that might lead to improvement of the bioactivities

![Diagram of Hamilton's 1,4-terphenylene derivatives]

**Figure 3.1:** Examples of Hamilton’s 1,4-terphenylene derivatives
Figure 3.2: Synthesized 2,5-terpyrimidinylene derivatives

Our strategy focused on the synthesis of novel analogs of Hamilton’s 1,4-terphenylene derivatives (Figure 3.1); however, the 2,5-terpyrimidinylene analogs (Figure 3.2) that we have synthesized did not show any activities against p53/MDM2.

Figure 3.3: Structure comparison of 1,4-terphenylene and 2,5-pyrimidinylene scaffold
We proposed the following two reasons. First, comparison of our synthesized molecules with Hamilton’s terphenylene scaffold (Figure 3.3) show that, even though the side chains of our molecules mimic the side chains of his 1,4-terphenylene derivatives, our molecules lack of the hydrogen bonding / salt bridge potential that feature at the extreme terminals in Hamilton’s molecules.

From Hamilton’s first report of the 1,4-terphenylene derivatives (Orner et al., 2001) which were used to target the interaction between calmodulin (CaM) and an R-helical domain of smooth muscle myosin light-chain kinase (smMLCK); to Hamilton’s later use of these derivatives to target the interaction between p53 and MDM2 (Yin et al., 2005), Hamilton’s group have tested the importance of the terminal carboxylic acid groups. In all cases, molecules without the terminal carboxylic acid groups did not show bio-activities. Our 2,5-terpyrimidinylene scaffold has a phenyl group, and a cyano group at the terminal positions, which cannot be involved in hydrogen bonding / salt bridge interactions at physiology pH. This lack of interaction might be one of the major reasons why our molecules did not show bioactivity. We attempted to mimic the terminal carboxyl acid groups of Hamilton’s molecules, but for the synthetic methodology that we have developed, it was very challenging. We were also trying to hydrolyze the 5’-cyano group to carboxylic acid, but because of the high steric hindrance of the 5-position, and the high hydrophobicity of the 2,5-terpyrimidinylene derivative, the hydrolysis was very challenging.

Furthermore, we suggest that more hydrophilic elements be added to our molecules. The solubility of our molecules in DMSO was not as good as we planned.
3.1.3 Problems existing in the developed synthetic process

It is difficult to make a library of molecules using the most recent synthesis process that has been developed because it is a long and tedious process. This procedure is a linear synthesis, containing 9 steps, almost all of which required at least 12 h reaction time. Typically, it took over a week to make one analog.

Scheme 3.1: Reaction and side reaction of hydroxylamine nucleophilic addition

Additionally, the overall yield is very low, normally about 1-2%. The low yield of the overall synthesis was the result of two reactions. The first is the hydroxylamine nucleophilic addition reaction, which generally produces 20-50% side product amide b (Scheme 3.1). The second yield limiting reaction is the condensation reaction to form the pyrimidine ring (Scheme 3.2). This reaction generates water in situ, which hydrolyzed

Scheme 3.2: Reaction and side reaction of amidine condensation reaction
the limiting reagent amidine d to amide f; this side product is confirmed by proton NMR. This may be the reason why we never had a yield greater than 50% when we did the condensation reaction to make the pyrimidine dimers and trimers. Unfortunately, both of these reactions need to be carried out twice during the overall synthesis, which made the overall yield of the synthesis low.

To simplify the synthesis process, we choose to make our 2,5-terpyrimidine molecules with a phenyl group at the 2-position. We could very well use more diverse groups, e.g. –NH₂, etc., but that would increase the difficulty of synthesis even more.

### 3.1.4 Convergent synthetic strategy

To improve the yield of the synthesis, and to reduce the synthesis time, we chose to use a convergent strategy (Scheme 3.3) instead of the linear synthesis. Retrosynthetic analysis revealed that the target molecule g could be derived from the amidine h and the

**Scheme 3.3:** Retrosynthesis using the convergent strategy
\[ \alpha,\beta\text{-unsaturated } \alpha\text{-pyrimidineketone } i \]. The synthesis of amidine \( h \) has been previously reported; so we focused our attention on exploring the synthesis of \( \alpha,\beta\text{-unsaturated } \alpha\text{-pyrimidineketone } i \).

A convergent synthetic approach presents several advantages. First, the amidine \( h \) and the \( \alpha,\beta\text{-unsaturated } \alpha\text{-pyrimidineketones } i \) can be simultaneously synthesized, reducing the overall synthesis time. Second, the synthesis of several analogs \( h \) and \( i \) would allow us to easily achieve the diversity of the library of the target molecules.

Last but not least, since the top amidine half \( h \) would be easily made, we can use more than 1 equiv. of the amidine \( h \) in the last condensation step to increase overall yield.

3.2 Results and Discussion for the synthesis of the new \( \alpha,\beta\text{-unsaturated ketones} \)

3.2.1 Retrosynthetic to make the \( \alpha,\beta\text{-unsaturated } \alpha\text{-pyrimidineketones} \)

The synthesis of the fragment \( i \) (Scheme 3.4) relied on our well-developed reaction: \( \alpha\text{-substituted ketone } j \) reacting with DMF-DMA. The \( \alpha\text{-pyrimidine ketone } j \), in turn, could be made by condensation of \( \alpha\text{-amidine ketone } k \) with another \( \alpha,\beta\text{-unsaturated } \alpha\text{-pyrimidineketones} \).

**Scheme 3.4:** Retrosynthesis of the \( \alpha,\beta\text{-unsaturated } \alpha\text{-pyrimidineketones} \)
\( \alpha \)-cyanoketone (Scheme 3.2 c). And the \( \alpha \)-amidine ketone k could be prepared using our extensive collection of \( \alpha \)-cyano ketone l.

3.2.2 Attempted synthesis of the \( \alpha,\beta \)-unsaturated \( \alpha \)-pyrimidineketones 3.5

With this in mind, the following steps have been carried out (Scheme 3.5). Containing a good electronphilic ketone group, starting material 3.1 was protected to prevent the side reactions. We decided to protect the ketone group by reducing it to alcohol, because the alcohol group should be inert to the following reaction conditions, which contain both acidic and basic conditions. Reduction of the starting material \( \alpha \)-cyano ketone 3.1 using sodium borohydride at 0 °C gave the \( \alpha \)-cyano alcohol 3.2 in excellent yield. To convert cyano group 3.2 to amidine 3.3, we relied on the

**Scheme 3.5: Attempted synthesis of compound 3.5**

\[
\begin{align*}
&\text{3.1} & \xrightarrow{\text{i}} & \text{3.2} & \xrightarrow{\text{ii}} & \text{3.3} \\
&\xrightarrow{\text{iii}} & \text{3.4} & \xrightarrow{\text{X}} & \text{3.5}
\end{align*}
\]

i) \( \text{NaBH}_4 \), MeOH, 0 °C, 1.5 h; ii) hydroxylamine hydrochloride, \( K_2\text{CO}_3 \), EtOH/H\text{H}_2\text{O}, reflux, 24 h; iii) \( \text{Ac}_2\text{O}, \text{HOAc}, K_2\text{CO}_3 \), HCOOH, MeOH, 10% Pd/C, rt 12 h; then 2.3, Et\text{3}N, EtOH, reflux, 24 h

76
hydroxylamine reaction. Nucleophilic addition of hydroxylamine to compound 3.2 at high temperature gave the amidoxime 3.3 in very good yield. It is worth to point out that, in this reaction (Scheme 3.6), no amide side product was observed. This shows the influence of the steric hindrance in this reaction: if the cyano group is not hindered, only the nitrogen atom of the hydroxylamine behaves as the nucleophile. After this reaction, hydrogenation of the amidoxine 3.3 gave the amidine intermediate, which condensed with an $\alpha,\beta$-unsaturated $\alpha$-cyanoketone 2.3d to give the $\alpha$-pyrimidine alcohol 3.4. However, at this stage, we could not oxidize the 2° alcohol 3.4 to the ketone 3.5. We tried Jones oxidation (Zibuck and Streiber, 1993), Swern oxidation (Dondoni and Perrone, 2000), Dess-Martin oxidation (Sniady et al., 2007), and PCC (Tu et al., 2003). However, only the Dess-Martin oxidation gave a trace mount of product, no product could be detected in the other oxidation methods. Based on the results, no starting material alcohol could be recycled after all these oxidation methods. We reasoned that the pyrimidine ring is not stable under those oxidation conditions.

**Scheme 3.6:** Hydroxylamine addition of compound 3.2

3.2.3 Attempted synthesis of $\alpha,\beta$-unsaturated ketones using 1,2,3-triazole ring
Some new results from our group showed that, besides the six-membered ring, the five-membered ring could also be used in the scaffold while retaining the \( \alpha \)-helical mimetic properties. Therefore, we modified our target molecules by replacing the pyrimidine ring with the 1,2,3-triazole ring (the detailed reason will be discussed in Chapter 4). The synthetic strategy remained unvaried (Scheme 3.7); the 1,2,3-triazole ring could be prepared using the azide alkyne Huisgen cycloaddition, also known as click chemistry.

**Scheme 3.7: Synthesis of the \( \alpha,\beta \)-unsaturated \( \alpha \)-triazoleketones**

![Scheme 3.7 diagram]

3.6

3.7

59\% for two steps

3.8 79\%

3.9 82\%

3.10 80\%

i) Bromine, aluminum chloride, diethyl ether, 0\degree C -> rt, 3 h; ii) sodium azide, DMSO, rt, 2 h; iii) 5-methyl-1-hexyn-3-ol, (+)-sodium L-ascorbate, copper sulfate, tert-butanol, water, rt, 24 h; iv) DMF-DMA, THF, rt, 16 h; v) guanidine hydrochloride, sodium ethoxide, ethanol, reflux, 15 h
First, a simple bromination reaction at the α-position of the ketone gave the α-bromine ketone 3.6. This method is not as general as the “acid chloride–CH₂N₂–HBr” strategy, but it is a simple one step high yielding reaction, perfect for the trial series. Then, azide behaved as a nucleophile to undergo a Sₙ₂ reaction to kick out the bromide as a leaving group. The resulting azide 3.7 underwent an azide alkyne Huisgen cycloaddition to form the α-triazole ketone 3.8 (Chen et al., 2007). This α-triazole ketone 3.8 reacted with DMF-DMA at room temperature resulting in compound 3.9, an analog of the α,β-unsaturated ketone i in Scheme 3.3. To check the reactivity of this series of α,β-unsaturated α-triazoleketones, 3.9 was used to condense with guanidine. As expected, a pyrimidine ring was formed, getting an 80% yield in the cyclization reaction.

**Scheme 3.8: Condensation to synthesize the hybrid trimer 3.11**

\[ \text{HN-NNH}_2 \cdot \text{AcOH} \quad \text{O} \quad \text{HO} \quad \ni \quad \text{HN-NNH}_2 \cdot \text{AcOH} \]

i) Triethylamine, ethanol, reflux, 14 h
After the proof of the reactivity of the \(\alpha,\beta\)-unsaturated \(\alpha\)-triazoleketones we condensed 3.9 with an amidine monomer 2.5d to make a hybrid pyrimidine-triazole trimer molecule 3.11 (Scheme 3.8).

### 3.2.4 Biological testing of 3.10

Compound 3.10 was tested in FP assay against p53/MDMx interaction, in which it showed 6 \(\pm\) 17\% activities at 100 \(\mu\)L. Considering 3.10 is a dimer molecule which has a molecular weight of only 324.38, this scaffold showed the potential to be an antagonist of p53/MDMx interaction.

### 3.3 Conclusion

We have successfully developed a convergent route to synthesize a new generation of non-peptidic \(\alpha\)-helix mimetics. The newer generation of the molecules has more hydrogen bonding/ionic bonding potentials at the top and bottom positions, while retaining the hydrophobic side chains that mimic the side chains of the \(\alpha\)-helical mimetics. The synthesis of this new generation of trimers has been simplified from the 2,5-terpyrimidinylene generation; it contains 7 steps from the longest route, and most of those reactions are high yielding. This strategy is more conductive in synthesizing a library of molecules.

### 3.4 Experimental Procedures

3-Hydroxy-4-phenylbutanenitrile (3.2)
To a solution of 2.2a (0.47 mmol) in methanol (2 ml) cooled to 0 °C in an ice-bath was added sodium borohydride (0.73 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. After the reaction, aq. H₂SO₄ (1 M) was added to the reaction mixture until pH = 6. The mixture was then concentrated under reduced pressure. The residue was extracted with ethyl acetate 3 times. The combined organic layer was extracted once with brine solution, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:2) to afford 3.2 as a white solid (70 mg, 92%).

\[^{1}\text{H}\] NMR (400 MHz, CDCl₃) ppm: 2.50 (qd, J = 16.7, 5.5 Hz, 2H), 2.69 (bs, 1H), 2.88 (d, J = 6.7 Hz, 2H), 4.07–4.18 (m, 1H), 7.19–7.37 (m, 5H). \[^{13}\text{C}\] NMR (100 MHz, CDCl₃) ppm: 25.3, 43.0, 68.8, 117.9, 127.4, 129.1, 129.6, 136.6.

N’,3-Dihydroxy-4-phenylbutanimidamide acetate (3.3)

To a solution of compound 3.2 (3.9 mmol) in ethanol (11 mL) was added a solution of hydroxylamine hydrochloride (19.7 mmol) and potassium carbonate (9.8 mmol) in water (4 mL). The reaction mixture was stirred under reflux for 14 h. The reaction mixture was concentrated under reduced pressure. The residue was extracted between DCM and
water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as yellow solid. Purification by flash column chromatography on silica gel (methanol/ethyl acetate, 1:20) affords the amidoxime 3.3 as a white solid (0.45 g, 59%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 2.14–2.40 (m, 2H), 2.69–2.78 (m, 2H), 4.00–4.10 (m, 1H), 4.80–5.30 (br m, 4H), 7.13–7.32 (m, 5H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 37.8, 43.7, 70.7, 126.8, 128.8, 128.8, 129.7, 138.2. HRMS (ESI) calcd. for C$_{10}$H$_{14}$N$_2$O$_2$ [M + H]$^+$ 195.1128, found 195.1203.

2-(2-Hydroxy-3-phenylpropyl)-4-isobutylpyrimidine-5-carbonitrile (3.4)

Step 1: To a solution of the 3.3 (1.5 mmol) in glacial acetic acid (3 mL) was added acetic anhydride (175 µL). After 5 min of stirring, a solution of potassium formate prepared in situ from potassium carbonate (5 mmol) and formic acid (10 mmol) in methanol (2 mL) was added followed by 10% Pd/C (0.2 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was filtered through Celite$^\text{TM}$ and rinsed with methanol. The filtrate was concentrated under reduced pressure to afford a yellow residue. This residue was redissolved in DCM and filtered through Celite$^\text{TM}$ and rinsed with DCM. The filtrate was concentrated under reduced pressure to afford the crude
amidine acetate salt as a yellow solid, which was used in the next step without further purification.

Step 2: To the solution of the amidine salt (0.8 mmol) from step 2 in ethanol (3 mL) was added compound 2.3d (0.8 mmol) and triethylamine (1.6 mmol). The reaction mixture was stirred under reflux for overnight. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed by reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:2) afforded 3.4 as white solid (32%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.98 (d, $J = 6.7$ Hz, 6H), 2.23 (tt, $J = 13.7$, 6.9 Hz, 1H), 2.84 (d, $J = 7.3$ Hz, 2H), 2.90 (dd, $J = 15.9$, 5.9 Hz, 1H), 2.98 (d, $J = 7.0$ Hz, 1H), 3.12 (dd, $J = 16.3$, 9.0 Hz, 1H), 3.23 (dd, $J = 16.3$, 2.9 Hz, 1H), 3.97 (br s, 1H), 4.43 (d, $J = 8.3$ Hz, 1H), 7.20–7.36 (m, 5H), 8.80 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.5, 22.5, 29.2, 43.5, 45.2, 45.6, 70.9, 107.2, 115.1, 126.8, 128.8, 129.8, 138.1, 160.0, 171.8, 173.2

2-Bromo-1-phenylethanone (3.6)

![2-Bromo-1-phenylethanone](image)

To an ice cooled solution of acetophenone (1.7 mmol) and catalytic amount of aluminum chloride in diethyl ether (4 ml) was added bromine (4.3 mmol). The reaction mixture was stirred at 0 °C until the red color faded. After the reaction, aq. H$_2$SO$_4$ (1 M) was added to the reaction mixture until pH = 6. The mixture was then concentrated under
reduced pressure. The residue was extracted with ethyl acetate 3 times. The combined organic layer was extracted once with brine solution, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:2) affords 3.6 as a white solid (70 mg, 92%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 2.50 (qd, $J$ = 16.7, 5.5 Hz, 2H), 2.69 (bs, 1H), 2.88 (d, $J$ = 6.7 Hz, 2H), 4.07–4.18 (m, 1H), 7.19–7.37 (m, 5H).

2-Azido-1-phenylethanone (3.7)

Sodium azide (4.3 mmol) was dissolved in DMSO (9 mL) and the solution was stirred for 12 h. To this solution was added 3.6 (2.2 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was then concentrated under reduced pressure using the Biotage V10. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:7) affords the 3.7 as a colorless oil (0.15 g, 59%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 4.56 (s, 2H), 7.46–7.54 (m, 2H), 7.59–7.66 (m, 1H), 7.88–7.92 (m, 2H).

2-(4-(1-Hydroxy-3-methylbutyl)-1H-1,2,3-triazol-1-yl)-1-phenylethanone (3.8)
To a solution of 3.7 (0.29 mmol) and 5-methyl-1-hexyn-3-ol (0.29 mmol) in tert-butanol (4 mL) was added a solution of (+)-sodium L-ascorbate (0.29 mmol) in water (2 mL) and copper(II) sulfate pentahydrate (0.14 mmol) in water (2 mL), respectively. The reaction mixture was stirred at room temperature for 24 h. The mixture was then concentrated under reduced pressure. The residue was extracted between ethyl acetate and water. The combined organic layer was extracted once with brine solution, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 2:1) affords 3.8 as a white solid (63 mg, 79%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) ppm 0.93 (d, \(J = 6.3\) Hz, 6H), 1.67 (dd, \(J = 4.2, 9.1, 13.3\) Hz, 1H), 1.81 (qd, \(J = 6.1, 13.2\) Hz, 2H), 3.36 (br s, 1H), 4.95 (dd, \(J = 5.0, 8.5\) Hz, 1H), 5.81 (s, 2H), 7.49 (dd, \(J = 4.8, 10.7\) Hz, 2H), 7.60 (s, 1H), 7.63 (t, \(J = 7.5\) Hz, 1H), 7.94 (dd, \(J = 1.1, 8.3\) Hz, 2H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) ppm 22.2, 23.4, 24.7, 46.4, 55.7, 65.3, 122.8, 128.4, 129.4, 134.1, 134.8, 152.5, 190.8. HRMS (ESI) calcd. for C\(_{15}\)H\(_{19}\)N\(_3\)O\(_2\) [M + H]\(^+\) 274.1550, found 274.1557.

3-(Dimethylamino)-2-(4-(1-hydroxy-3-methylbutyl)-1H-1,2,3-triazol-1-yl)-1-phenylprop-2-en-1-one (3.9)
To a solution of 3.8 (1.6 mmol) in THF (24 ml) under argon atmosphere was added DMF-DMA (4.3 mmol). The reaction mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure affords the crude product as brown solid. Purification by flash column chromatography on silica gel (methanol/ethyl acetate, 1:20) afforded 3.9 as a white solid (0.42 g, 82%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.90 (dd, $J$ = 6.2, 2.8 Hz, 6H), 1.55—1.80 (m, 3H), 2.32 (br s, 3H), 3.13 (br s, 3H), 4.90 (dd, $J$ = 8.1, 5.2 Hz, 1H), 7.29 (s, 1H), 7.30 (s, 1H), 7.33—7.43 (m, 4H), 7.56 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.3, 23.3, 24.7, 46.6, 65.4, 108.9, 126.6, 127.9, 128.4, 130.7, 139.2, 150.1, 152.1, 189.5. HRMS (ESI) calcd. for C$_{18}$H$_{24}$N$_4$O$_2$ [M + H]$^+$ 329.1972, found 329.1972.

1-(1-(2-Amino-4-phenylpyrimidin-5-yl)-1H-1,2,3-triazol-4-yl)-3-methylbutan-1-ol (3.10)
To a solution of 3.9 (0.2 mmol) in ethanol (3 mL) under argon atmosphere was added guanidine hydrochloride (0.5 mmol) and sodium ethoxide (0.5 mmol). The reaction mixture was stirred under reflux for 15 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed by reduced pressure affords the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 4:1) affords 3.10 as white solid (80%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.91 (dd, $J = 6.3, 1.7$ Hz, 6H), 1.47–1.78 (m, 3H), 7.11–7.49 (m, 5H), 7.76 (s, 1H), 8.37 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 21.5, 21.99, 24.4, 46.3, 64.5, 104.99, 121.1, 124.8, 128.0, 135.2, 152.6, 156.6, 162.8, 164.0. HRMS (ESI) calcd. for C$_{17}$H$_{20}$N$_6$O [M + H]$^+$ 325.1771, found 325.1763.

1-(1-(4'-isoButyl-2',4-diphenyl-[2,5'-bipyrimidin]-5-yl)-1H-1,2,3-triazol-4-yl)-3-methylbutan-1-ol (3.11)
To a solution of 2.5 (0.4 mmol), freshly made using the method described before, in ethanol (8 mL) under argon atmosphere was added 3.9 (0.2 mmol) and triethylamine (0.4 mmol). The reaction mixture was stirred under reflux for 14 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed by reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:1) affords 3.10 as white solid. $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.94–0.98 (m, 12H), 1.64–1.80 (m, 4H), 2.10–2.16 (m, 1H), 2.31–2.43 (m, 1H), 4.95–5.03 (m, 1H), 7.38–7.41 (m, 5H), 7.51–7.55 (m, 4H), 8.55–8.59 (m, 2H), 9.09 (s, 1H), 9.48 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.3, 22.9, 23.2, 24.8, 28.5, 45.0, 46.6, 65.5, 122.8, 128.1, 128.8, 131.3, 137.6, 153.1, 155.4, 159.6, 164.4, 169.9. HRMS (ESI) calcd. for C$_{31}$H$_{33}$N$_7$O [M + H]$^+$ 520.2819, found 520.2810.

3.5 References


Chapter Four:
Design and Synthesis of Hybrid Scaffold Non-peptidic α-Helical Mimetics

4.1 Introduction

4.1.1 General scaffold of non-peptidic α-helical mimetics

We have previously synthesized several analogs of 2,5-terpyrimidinylene scaffold molecules, which aimed at mimicking the most *in vitro* active non-peptidic p53-MDM2 inhibitor (Yin et al., 2005a) that has been reported so far. Nevertheless, the reported non-peptidic α-helical mimetics have more diversified structures (Figure 2.2).

A common feature of those molecules is the presence of three directly bonded rings as the backbone, positioned in a linear configuration. The ring fragments could be either regular covalent-bonded rings (Davis et al., 2005), (Volonterio et al., 2007), (Moisan et al., 2008) or rings formed by hydrogen bond (Ernst et al., 2003), (Estroff et al., 2004), (Yin et al., 2005b), (Rodriguez and Hamilton, 2006). They are preferably aromatic rings, for the benefit of the flat geometrical conformation associated. Each ring bears an alkyl or aromatic side chain designed to mimic the side chains of the original peptides. It is crucially that all alkyl or aromatic side chains point to the same face, mimicking the $i, i + 4(3), i + 7$ positions of the peptides on the same face of the helix. An
abstract model that stands for this series of non-peptide α-helical mimetics is shown in Figure 4.1.

![Abstract structure of α-helical mimetics](image)

**Figure 4.1:** Abstract structure of α-helical mimetics

### 4.1.2 New library design strategy

From previous reports and studies, it was clear that it is very challenging to find good p53/MDM2 antagonists: a favorable scaffold, favorable side chains, and a favorable order of side chains all affect the activities of the antagonists. Thus, our strategy relied on easily buildable scaffolds, amenable to library development, allowing the introduction of diverse side chains as well as terminal polar groups.

### 4.1.3 Introduction of the hybrid scaffold

The majority of non-peptidic α-helical mimetics used iterative design, e.g. a, b, c, in Figure 2.1 (Yin et al., 2005a), (Ernst et al., 2003), (Davis et al., 2005). The benefits are the following: the alkyl or aromatic side chains are ensured to point to the same
direction, and the synthesis is easy to design. However, iterative design usually involves iterative linear synthesis, which is multistep and can be low yielding. As long as we can make sure the side chains of the scaffold are pointed to the same direction, we could consider the scaffold a good choice. We decided to focus on hybrid scaffolds, featuring five-membered and six-membered aromatic rings for the simplification of synthesis. A pyrimidine core was our six-membered rings candidate of choice, for its promising properties shown in the previous projects carried out in our lab. Of the many aromatic five-membered rings, we chose to use 1,2,3-triazole rings. The 1,2,3-triazole rings have several characteristics suiting our needs. First, it could be easily synthesized from azides and acetylenes (Chen et al., 2007), by the high yielding azide-alkyne Huisgen cycloaddition, and the azides and acetylenes are readily with diverse structures to ensure the diversity of the 1,2,3-triazole rings. Second, the introduction of five-membered ring with three nitrogen atoms would lower the logP value of the molecules (see discussions of Table 2.1). In other words, the synthesized molecules have a higher water-solubility potential if the scaffold contains more nitrogen atoms; thus, 1,2,3-triazole is a better scaffold candidate than pyrimidine rings for the benefit of water solubility.

4.1.4 Strategy to extend the pyrimidine rings using Huisgen cycloaddition

1,2,3-triazole ring could be made from the reaction between azides and acetylenes. If we can attach either an azide group or an acetylene group to the pyrimidine ring, we could easily make a dimer (Scheme 4.1). With this in mind, the following dimer synthesis has been carried out as a test of the 1,2,3-triazole ring strategy.
Scheme 4.1: Synthetic strategy to make the hybrid dimer

4.2 Results and Discussion for introducing 1,2,3-triazole to the scaffold

4.2.1 Synthesis of 4-triazole pyrimidine hybrid dimer 4.6

The same procedure that was used to generate pyrimidine monomer 2.4 was applied to the synthesis of monomer 4.2 (Scheme 4.2), bearing a hydrogen in place of a cyano group at the 4-position. Further, we reacted the 4-alkyl group of compound 4.5 with an alkyl azide 4.7 to attach a 1,2,3-triazole ring to the 4-position of the pyrimidine ring.
Scheme 4.2: Synthesis of hybrid dimer 4.6

![Chemical reaction diagram]

i) DMF-DMA, THF, rt, 24 h; ii) guanidine hydrochloride, TEA, EtOH, reflux, 24 h; iii) I₂, Hg(OAc)₂, 1,4-dioxane/H₂O, 90°C, 2 h; iv) 2-methyl-3-butyln-2-ol, Pd(Ph)₄, Cui, TEA, ACN, reflux, 2 h; v) KOH, toluene, 70°C, 2 h; vi) Benzyl azide, 5-methyl-1-hexyn-3-ol, (+)-sodium L-ascorbate, CuSO₄·5H₂O, tert-butanol, water, rt, 24 h; vii) DMSO, rt, 2 h
In the first step, we reacted 1,1,1,-trifluoro-5-methyl-2,4-hexanedione with DMF-DMA to form an \( \alpha,\beta \)-unsaturated ketones \textbf{4.1}. This is an interesting reaction, because the product of the reaction is dependent on the electronegativity properties of the group adjacent \( R_1 \) (Scheme 4.3). If it is an alkyl group, \( R_1 \) will stay in the final molecule; on the other hand, if \( R_1 \) is a strong electron-withdrawing group, e.g. CF\(_3\), then the whole \( R_1\)CO group will be eliminated from the final molecules.

\textbf{Scheme 4.3}: Comparison of different substituted 1,3-diketones reacting with DMF-DMA

\[ \begin{align*}
\text{O} & \quad \text{O} & \quad \text{DMF-DMA} & \quad \text{O} & \quad \text{O} \\
\text{R} & \quad \text{R}_1 & & \quad \text{R} & \quad \text{R}_1 \\
\end{align*} \]

\( R_1 = \text{alkyl group} \)

\[ \begin{align*}
\text{O} & \quad \text{O} & \quad \text{DMF-DMA} & \quad \text{O} & \quad \text{H} \\
\text{R} & \quad \text{R}_1 & & \quad \text{R} & \quad \text{R}_1 \\
\end{align*} \]

\( R_1 = \text{CF}_3 \)

The mechanism of this reaction has not been studied, a possible mechanism is proposed in Scheme 4.4.
Scheme 4.4: One possible mechanism of 1,3-diketone reacting with DMF-DMA

The tautomer of 1,1,1-trifluoro-5-methyl-2,4-hexanedione a acts like a nucleophile to attack the iminium salt formed after DMF-DMA eliminates one methoxide. After the formation of the tetrahedral intermediate c, a second methoxide will be kicked out by the lone pair electron on the nitrogen atom to form the intermediate d. There are two possible reactions for the intermediate d. If R₁ is a regular alkyl group,
a regular E2 reaction will take place, the α-proton will be eliminated to form the product e. However, if the R₁ group is a strong enough electron-withdrawing group, the adjacent carbonyl group would be activated so it can be a good electrophile towards MeO/MeOH. After the attack of MeO/MeOH, the carbon-carbon bond on the other side of the carbonyl group will be broken, that pair of electrons will become the π electrons of a double bond. One way to prove this mechanism is that, in this case, methyl trifluoroacetate should be a side product.

After the α,β-unsaturated ketone 4.1 was prepared, it underwent a cyclization with guanidine to form a pyrimidine ring (Scheme 4.2). This reaction occurs under the same condition we utilized for the α,β-unsaturated α-cyanoketones’ cyclization, even though the α,β-unsaturated ketone 4.1 is less reactive due to the lack of the electron withdrawing cyano group. Then an iodination at the least electron deficient position of the pyrimidine ring (Shepherd and Fellows, 1948), the 5-position, gave us the iodide compound 4.3. The iodide compound 4.3 underwent Sonogashira cross-coupling reaction (Chen et al., 2007), coupling an acetone-protected acetylene to the pyrimidine ring. Deprotection of the acetylene using potassium hydroxide in toluene at elevated temperature gave us the unprotected acetylene product 4.5 (Watanabe et al., 2009). This is the synthesis of the acetylene half for the Huisgen cycloaddition.

For the azide half, we should be able to use any alkyl azides or azide derivatives of amino acids. We used benzyl azide 4.7 for it is easy synthesis (Alvarez and Alvarez, 1997).
Finally, Huisgen cycloaddition between 4.5 and 4.7 under standard condition gave the 1,2,3-triazole 4.6 in good yield.

### 4.2.2 Benefits of the 1,2,3-triazole ring

The polarity of dimer 4.6 is the highest among all the non-peptidic α-helical mimetics dimers that we have synthesized. The synthesis to attach a 1,2,3-triazole ring to the 4-position of a pyrimidine ring is easy and high yielding. The combination of the two factors makes the 1,2,3-triazole ring modified scaffold promising.

### 4.2.3 Synthesis of 2-triazole pyrimidine hybrid dimer 4.13

From the discussion above, we have successfully developed a way to attach a 1,2,3-triazole ring to the 4-position of the pyrimidine rings. We also explored the possibility of attaching a 1,2,3-triazole ring to the 2-position of the pyrimidine rings.

To form a 1,2,3-triazole ring at the 2-position of the pyrimidine ring, we can either attach an azide group or an acetylene group. Initial attempts to replace the 2-chloride on the pyrimidines with acetylde anion failed. The acetylene amidine underwent self-polymerization, therefore, cannot be used as the starting material to synthesize the pyrimidines. Therefore, a better option is to attach an azide group to the 2-position of the pyrimidine rings. Again, direct replacement of 2-chloro pyrimidine with azide derivatives did not produce any product, which was inconvenient also because of the difficulty of synthesizing the 2-chloro-4-alkyl-pyrimidines (Scheme 4.8). In the end, we
deployed the reaction of hydrazine reacting with nitrous acid to synthesis the azide; and successfully attached a 1,2,3-triazole ring to the 2-position of pyrimidine rings.

We found that DMF-DMA can react with all kinds of ketones besides the $\alpha$-cyano ketones, as long as the $\alpha$-position contains at least two hydrogen atoms (Scheme 4.5). If the $\alpha$-position is attached to some electron-withdrawing group, e.g. cyano group, nitro group, 1,2,3-triazole group, this reaction occurs at room temperature; whereas, higher temperatures are required for less activated ketones.

**Scheme 4.5:** General methodology to synthesize $\alpha,\beta$-unsaturated ketones from ketones

![Scheme 4.5](image)

4.9

i) DMF-DMA, 80°C, 12 h

4.9a: R = methyl
4.9b: R = iso-butyl
4.9c: R = cyclopropyl
4.9d: R = phenyl

The $\alpha,\beta$-unsaturated ketone 4.9d reacted with protected aminoguanidine in the microwave giving the pyrimidine ring product 4.10 (Scheme 4.6). The hydrazine group of 4.10 is protected as imine (Martins et al., 2004), which is normally acid labile. However, this imine group is very stable, as a result of the conjugated system, so even concentrated hydrochloride acid and sulfuric acid could not break down the imine group. Luckily, with a large excess of hydrazine hydrate, hydrazine product 4.11 could be formed (Gonzalez, 1988). The reaction to remove the benzylaldehyde protecting group
should be through a competition mechanism, where hydrazine competes with the hydrazine group of \(4.10\) for the benzylaldehyde. Compound \(4.11\) reacts with nitrous acid to form a 2-azido pyrimidine \(4.12\) (Lindsay and Allen, 1942). This azide compound \(4.12\) reacted with commercially available 5-methyl-1-hexyn-3-ol through Huisgen cycloaddition to form the compound \(4.13\), which has a 1,2,3-triazole ring attached at the

**Scheme 4.6: Synthesis of hybrid dimer 4.13**

\[
\begin{align*}
\text{NH}_2\text{NH}_2\text{HCl} & \quad \text{i} \quad \text{HN} \equiv \text{N} & \quad \text{HN} \equiv \text{N} \equiv \text{HCl} \quad \text{4.8} + \quad \text{iii} \quad \text{HN} \equiv \text{N} & \quad \text{N} \equiv \text{N} \\
\text{4.9} & \quad \text{ii} \quad \text{C=O} & \quad \text{4.10} & \quad \text{64\% for two steps} \\
\text{iv} & \quad \text{HN} \equiv \text{N} & \quad \text{57\% from 4.10} \\
\text{4.11} & \quad \text{v} \quad \text{5-Methyl-1-hexyn-3-ol, (+)-sodium L-ascorbate, CuSO}_4\cdot\text{5H}_2\text{O}, \text{ tert-butanol, water, rt, 24 h} \\
\text{4.12} & \quad \text{vi} \quad \text{4.13} & \quad \text{49\%}
\end{align*}
\]
2-position of the pyrimidine ring.

Again, compound 4.13 is as polar as compound 4.6, both are more polar than the pyrimidine dimers that we have synthesized.

4.2.4 Evaluation 1,2,3-triazole ring as a fragment in our scaffold

So far, we have proven the possibility of attaching 1,2,3-triazole ring to both 2-position (top) and 5-position (bottom) of a pyrimidine ring. Both compounds 4.6 and 4.13 are significantly more polar than the other pyrimidine dimers that we have previously synthesized. Unfortunately, we failed all the attempts to attach iodine at the 5-position of 4.13 (Shepherd and Fellows, 1948), (Beierlein et al., 2008), (Guillard and Viaud-Massuard, 2008), thus no trimer of this series has been synthesized yet. The failure of this reaction might be the result of the switch from the electron-donating group NH₂ to electron-withdrawing 1,2,3-triazole ring.

Nevertheless, the 1,2,3-triazole ring incorporation showed promising result toward our goal of water-soluble non-peptidic α-helical mimetic library design and synthesis.

4.3 Results and Discussion for introducing amino acids to the scaffold

4.3.1 Possibility of introducing amino acids to the scaffold

Even though most of the libraries that have been developed so far feature three flat rings (mostly aromatic) that have been attached linearly (Figure 2.1) (Yin et al., 2005a), (Davis et al., 2005), (Moisan et al., 2008), (Volonterio et al., 2007), this might
not be an essential requirement to this series of non-peptidic $\alpha$-helical mimetics. Computational studies showed that the side chain of the 2-amino acid on the pyrimidine ring is at the right position, $i+7$ (Figure 4.2).

**Figure 4.2:** Trimer with an amino acid in the scaffold fitting the abstract scaffold

There are several advantages of introducing an amino acid to the scaffold. Its side chain is in the right position; introduction of amino acid will not increase the overall lipophilicity of the molecule (it should decrease the lipophilicity for amino acids do not contain the aromatic system in the backbone), and the nitrogen carbon bond that connects the amino acid to the pyrimidine ring will not be labile like the peptide bond. Most importantly, it is very convenient to introduce an amino acid to the pyrimidine ring by aromatic substitution reactions; thus the introduction of amino acids to the scaffold fits our need to simplify the synthesis.

### 4.3.2 Testing of the new scaffold
Again, we would like to keep the second fragment of our scaffold a pyrimidine ring. This is because it is relatively easy to introduce new groups to the 2- and 5-positions of 4-alkyl pyrimidines. Between the 2- and 5-position, we decided to put the amino acids in the 2-position.

For the third fragment of our scaffold, we decided to use a 1,2,3-triazole, because of its promising properties that we have proven before. The pyrimidine ring will have an electron-donating nitrogen atom at the 2-position, so it would be possible to connect a 1,2,3-triazole ring to the 5-position.

Our new scaffold is shown in Figure 4.3.

![Possible conformation of the new scaffold](image)

**Figure 4.3:** Possible conformation of the new scaffold

4.3.3 **Retrosynthesis of the trimer**
The target molecule $g$ could be synthesized from the dimer $h$ using the method developed (Scheme 4.7). Compound $h$ could, in turn, be synthesized from the 2-chloro-4-alkyl-pyrimidine $i$.

**Scheme 4.7: Retrosynthesis of the hybrid trimer**

![Scheme 4.7: Retrosynthesis of the hybrid trimer](image)

Unfortunately, compound $i$ is relatively challenging to synthesize. For the two methods that we have tried (Scheme 4.8), neither one gave us promising results. We first tried the diazonium salt route to convert a NH$_2$ group to a chloride compound 4.14 (Dhanda et al., 1999). This method gave us about a 30% yield, with a lot of starting material remaining. In the other route, we attempted to react aminocyanogen with HCl gas to produce chloroamidine hydrochloride 4.15 in about a 30% yield (Henderson et al., 2006). Then we condensed the chloroamidine 4.15 with the $\alpha,\beta$-unsaturated $\alpha$-cyanoketone 2.3d to form the 2-chloropyrimidine 4.16. The second step has a 40% yield, so the overall yield to prepare 4.16 is around 12%.

In summary, neither one of methods that have been tried provided us promising results to pursue.
Scheme 4.8: Synthesis of the 2-chloro-pyrimidine ring

\[
\begin{align*}
\text{NH}_2 & \quad \xrightarrow{i} \quad \text{Cl} \\
\text{4.14} & \\
\text{H}_2\text{N}-\text{CN} & \quad \xrightarrow{ii} \quad \text{Cl} \quad \xrightarrow{iii} \quad \text{CN}
\end{align*}
\]

i) SbCl$_3$, isopentylnitrite, CHCl$_3$, 0°C to reflux, 7 h; ii) HCl (g), Et$_2$O, 5 h; iii) 2.3d, TEA, 1,4-dioxane, reflux, 12 h

4.3.4 Modification of the new target scaffold and the retrosynthesis route

Some of the molecules that have been developed so far used ether groups instead of alkyl group as the side chains to mimic the side chains of $\alpha$-helices (Figure 2.1) (Ernst et al., 2003), (Estroff et al., 2004), (Yin et al., 2005b). If we modify our target molecule from $g$ to $g'$ (Scheme 4.9), the overall geometry of the molecule should not be changed, but the synthesis is greatly simplified.

Target molecule $g'$ could be synthesized from the dimer $h'$, using the strategy that has been well developed (Scheme 4.2). The extra oxygen atom at the 4-position of the pyrimidine ring can further help the iodination reaction of the $h'$ molecule. The dimer $h'$ could be synthesized by nucleophilic aromatic substitution reaction one step from the monomer $i'$. Because of the reactivity difference between the two chlorine atoms on the
commercially available molecule 2,4-dichloropyrimidine, the 4-chloride could be substituted selectively at room temperature to make the monomer $i'$. 

**Scheme 4.9**: Modification of the target molecule

![Chemical diagram]

**4.3.5 Synthesis of the trimer 4.21**

The synthesis is shown in Scheme 4.10. Benzyl alcohol was deprotonated using sodium hydride, it replaced the 4-chloride of the 2,4-dichloropyrimidine at room temperature (Irie et al., 2008). Trace amounts of 2,4-dibenzyloxylylpyrimidine product could be detected, but the mono-substitution reaction product 4-benzyloxylylpyrimidine
Scheme 4.10: Synthesis of trimer 4.22

i) benzyl alcohol, NaH, DMF/THF, 0°C to r.t., 24 h; ii) L-Leucine methyl ester, hydrochloride, NaOMe, MeOH, m.w., 155°C, 60 min; iii) I₂, Hg(OAc)₂, 1,4-dioxane/H₂O, 90°C, 2 h; iv) HCl=CSi(CH₃)₃, Pd(Ph₃)₄, Cul, Et₃N, ACN, reflux, 2h; then TBAF, THF 0°C, 10 h; v) 4.18, CuSO₄·5H₂O, (+)-Sodium L-ascorbate, tert-BuOH/H₂O,r.t., 24 h; vi) K₂CO₃, CuSO₄·5H₂O, MeOH, 12 h
4.17 was the predominate product, with a yield of 78%. Then, a free amino group of an amino acid replaced the 2-chloride at 155 °C under microwave irradiation (Humphries et al., 2009). The yield of this step is relatively low; fortunately, there is no side reaction occurring, allowing us to recover most of the starting material left over. Next, an iodination reaction gave us compound 4.19 in good yield. Subsequently, Sonogashira reaction coupled an ethynyltrimethylsilane to the dimer. We did not use 2-methyl-3-butyln-2-ol here, because although we could couple it to our dimer, the potassium hydroxide that was used to deprotect the acetone group in the next step also reacted with another part of the molecule. Therefore, we chose TMS protected acetylene, and then took the TMS group off by TBAF under mild conditions to synthesize the acetylene half 4.20.

For the azide reactant, we could very well use the benzyl azide 4.7 previously synthesized. But we decided to test the compatibility of azide derivatives of amino acids with our scaffold. The azide derivative of valine 4.22 was synthesized by a reported procedure (Goddard-Borger and Stick, 2007), using 1H-imidazole-1-sulfonyl azide as the azide source, potassium carbonate as base, and copper sulfate pentahydrate as catalyst.

The Huisgen cycloaddition between acetylene 4.20 and azide 4.22 went very well, getting almost quantitative yield.

4.3.6 Properties of the timer 4.21

Trimer 4.21 has greatly improved water solubility when compared to compound 2.8, and 3.10. Also, trimer 4.17 is synthetically the easiest to prepare among the three
trimer scaffolds (2.8, 3.10, and 4.17) that we have developed; its synthesis involves seven steps, six of which are excellent yielding reactions. Due to the good water solubility as well as its amenability to library-synthesis, the scaffold of trimer series 4.17 appears to be the most promising α-helix mimetics. The bio-activity of compound 4.17 against p53/MDM and Bcl-xL are currently under investigation.

4.4 Biological testing of 4.6 and 4.13

Dimer compounds 4.6 and 4.13 was tested in Enzyme-linked immunosorbent assay (ELISA) assay against p53/MDM2 interaction (Figure 4.4, 4.6 in blue, 4.13 in yellow).

Figure 4.4: Elisa results of 4.6 and 4.13
Dimer 4.13 showed promising results at lower concentration. But its activity remained unchanged after certain concentration. This indicates that there might still be a solubility problem. Dimer 4.6 also showed some activity against p53/MDM2.

Considering the sizes of 4.6 and 4.13, we can conclude that our non-peptidic α-helical scaffolds are properly designed. The activities of trimer 4.21 are under investigation.

4.5 Conclusion

The work shown in this chapter provide a new generation of non-peptidic α-helical mimetics. There are three basic principles that are leading our design. The side chains of our designed molecules should act as mimetics of the side chains of an α-helix. Second, our molecules should possess improved water solubility. Third, the molecules should be easy to synthesize to generate a focused library. Based on those three criteria, we introduced the 1,2,3-triazole rings and amino acids into the scaffold, and those two scaffold segments showed very promising properties towards the principle. Our target molecule has been redesigned, and one analog 4.21 has synthesized. The synthesized molecule is now under biological testing to check bio-activities against p53/MDM and Bak/Bcl-xL.

4.6 Experimental Section

1-(Dimethylamino)-4-methylpent-1-en-3-one (4.1)
To a solution of commercially available 1,1,1-trifluoro-5-methyl-2,4-hexanedione (7.7 mmol) in THF (5 ml) under argon atmosphere was added DMF-DMA (10.0 mmol). The reaction mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure to afford the crude product as brown solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexanes, 4:1) affords 4.1 as a colorless oil (0.84 g, 77%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.07 (dd, $J = 6.9$, 1.7 Hz, 6H), 2.46–2.59 (m, 1H), 2.81 (br s, 3H), 3.00 (br s, 3H), 5.02 (d, $J = 12.6$ Hz, 1H), 7.54 (d, $J = 12.6$ Hz, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 19.8, 37.2, 39.8, 44.9, 93.9, 152.7, 202.5.

**4-iso-Propylpyrimidin-2-amine (4.2)**

To a solution of 4.1 (5.1 mmol) and guanidine hydrochloride (25.3 mmol) in anhydrous ethanol (10 mL) under argon atmosphere was added triethylamine (25.3 mmol). The reaction mixture was stirred in reflux for 24 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash...
column chromatography on silica gel (ethyl acetate/hexane, 2:1) affords 4.2 as a white solid (0.41 g, 58%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.22 (d, $J = 7.0$ Hz, 6H), 2.77 (hept, $J = 6.9$ Hz, 1H), 5.09 (br. s, 2H), 6.49 (d, $J = 5.2$ Hz, 1H), 8.17 (d, $J = 5.2$ Hz, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$) ppm 21.9, 36.1, 108.6, 158.3, 163.1, 177.4.

5-Iodo-4-isopropylpyrimidin-2-amine (4.3)

A suspension of 4.2 (0.4 mmol) and mercury(II) acetate (0.2 mmol) in water (4 mL), was heated to be boiling for 2 min, and a hot solution of iodine (0.4 mmol) in 1,4-dioxane (4 mL) was added. The reaction mixture was stirred at 100 ºC for 30 min. After the reaction, a mixture of potassium iodide and sodium sulfite was added to the reaction mixture, and the mixture was stirred overnight. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:3) affords 4.3 as a yellow solid (68 mg, 65%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.17 (d, $J = 6.8$ Hz, 6H), 3.19 (hept, $J = 6.7$ Hz, 1H), 5.31 (br. s, 2H), 8.37 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 21.0, 37.5, 81.1, 162.7, 164.9, 176.1. HRMS (ESI) calcd. for C$_7$H$_{10}$IN$_3$ [M + H]$^+$ 263.9992, found 264.0174.
4-(2-Amino-4-isopropylpyrimidin-5-yl)-2-methylbut-3-yn-2-ol (4.4)

To a solution of 4.3 (0.3 mmol) and 2-methyl-3-butyn-2-ol (0.3 mmol) in anhydrous acetonitrile (5 mL) under argon atmosphere was added tetrakis(triphenylphosphine)palladium(0) (0.009 mmol), copper(I) iodide (0.02 mmol) and triethylamine (1.5 mL). The reaction mixture was stirred under reflux for 20 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between ethyl acetate and water. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 2:1) affords 4.4 as a white solid (40 mg, 73%). HRMS (ESI) calcd. for C_{12}H_{17}N_3O [M + H]^+ 220.1444, found 220.1497.

5-Ethynyl-4-isopropylpyrimidin-2-amine (4.5)
To a solution of **4.4** (0.1 mmol) in toluene (5 mL) was added potassium hydroxide (0.5 mmol). The reaction mixture was stirred at 70 ºC for 3 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between dichloromethane and saturated ammonium chloride aqueous solution. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:2) affords **4.5** as a colorless oil (11 mg, 75%).

**5-(1-Benzyl-1H-1,2,3-triazol-4-yl)-4-isopropylpyrimidin-2-amine (4.6)**

![Chemical Structure](image)

To a solution of **4.4** (0.1 mmol) and benzyl azide **4.7** (0.1 mmol) in tert-butanol (2 mL) was added a solution of (+)-sodium L-ascorbate (0.1 mmol) in water (1 mL), and then a solution of copper(II) sulfate pentahydrate (0.01 mmol) in water (1 mL). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between ethyl acetate and water. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 2:1)
affords 4.6 as a white solid (14 mg, 70%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 1.19 (d, $J =$ 6.7 Hz, 6H), 3.24–3.36 (m, 1H), 5.07 (br. s, 2H), 5.59 (s, 2H), 7.30–7.44 (m, 5H), 7.47 (s, 1H), 8.33 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 21.6, 29.9, 31.8, 54.5, 121.6, 128.3, 129.4, 134.7, 144.0, 158.4, 162.8, 174.7.

**Benzyl azide (4.7)**

![Benzyl azide](image)

Sodium azide (10.0 mmol) was dissolved in DMSO (20 mL) and the solution was stirred at room temperature for 12 h. To this solution was added benzyl bromide (5.0 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was then concentrated under reduced pressure using the Biotage V10. The residue was extracted between diethyl ether and water. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a colorless oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:20) affords 3.6 as a colorless oil (0.47 g, 71%).

**2-Benzylidenehydrazinecarboximidamide hydrochloride (4.8)**

![2-Benzylidenehydrazinecarboximidamide](image)

To a solution of aminoguanidine hydrochloride (1.1 mmol) in ethanol (2 mL) was added benzaldehyde (1.0 mmol). The reaction mixture was placed in a microwave reactor for
10 min. at 160 ºC. The reaction mixture was then concentrated under reduced pressure to afford the intermediate 4.8, which was used in the next step without further purification.

**4-(Dimethylamino)but-3-en-2-one (4.9a)**

![Chemical structure of 4-(Dimethylamino)but-3-en-2-one (4.9a)](image)

The mixture of acetone (6.8 mmol) and DMF-DMA (6.8mmol) was stirred under reflux for 16 h. The reaction mixture was then concentrated under reduced pressure to afford 4.9a as a yellow solid, which was used in the next step without further purification.

**1-(Dimethylamino)-4-methylhex-1-en-3-one (4.9b)**

![Chemical structure of 1-(Dimethylamino)-4-methylhex-1-en-3-one (4.9b)](image)

The mixture of commercially available 3-methyl-2-pentanone (8.1 mmol) and DMF-DMA (10.4 mmol) was stirred at 85 ºC for 16 h. The reaction mixture was then concentrated under reduced pressure to afford 4.9b as a yellow solid, which was used in the next step without further purification. $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.81 (t, $J = 7.4$ Hz, 3H), 1.01 (d, $J = 6.9$ Hz, 3H), 1.32 (td, $J = 14.0$, 7.0 Hz, 1H), 1.60 (dq, $J = 22.2$, 7.4 Hz, 1H), 2.29 (dq, $J = 13.8$, 6.9 Hz, 1H), 2.87 (s, 6H), 4.98 (d, $J = 12.6$ Hz, 1H), 7.50 (d, $J = 12.6$ Hz, 1H).

**1-Cyclopropyl-3-(dimethylamino)prop-2-en-1-one (4.9c)**
The mixture of commercially available cyclopropyl methyl ketone (10.1 mmol) and DMF-DMA (13.4 mmol) was stirred at 85 °C for 16 h. The reaction mixture was then concentrated under reduced pressure to afford **4.9c** as a yellow solid, which was used in the next step without further purification. $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.70–0.77 (m, 2H), 0.96–1.03 (m, 2H), 1.70 (br. s, 3H), 1.74–1.84 (m, 1H), 5.19 (d, $J = 12.6$ Hz, 1H), 7.55 (d, $J = 12.6$ Hz, 1H).

**3-(Dimethylamino)-1-phenylprop-2-en-1-one (4.9d)**

The mixture of commercially available acetophenone (4.3 mmol) and DMF-DMA (5.6 mmol) was stirred at 85 °C for 16 h. The reaction mixture was then concentrated under reduced pressure to afford **4.9d** as a yellow solid, which was used in the next step without further purification. $^1$H NMR (400 MHz, CDCl$_3$) ppm 2.92 (br. s, 3H), 3.14 (br. s, 3H), 5.71 (d, $J = 12.4$ Hz, 1H), 7.34–7.49 (m, 3H), 7.80 (d, $J = 12.4$ Hz, 1H), 7.86–7.92 (m, 2H).

**2-(2-Benzylidenehydrazinyl)-4-phenylpyrimidine (4.10)**
To a solution of 4.8 (4.5 mmol) in anhydrous ethanol (3 mL) was added 4.9d (4.4 mmol). The reaction mixture was placed in a microwave reactor for 2 h at 155 °C. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between dichloromethane and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:3) affords 4.10 as a yellow solid (0.76 g, 64%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 7.20 (d, $J = 5.2$ Hz, 1H), 7.30–7.39 (m, 3H), 7.45–7.53 (m, 3H), 7.70 (d, $J = 6.7$ Hz, 2H), 7.79 (s, 1H), 8.08 (dd, $J = 6.5, 2.9$ Hz, 2H), 8.58 (d, $J = 5.1$ Hz, 1H), 9.27 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 109.6, 127.4, 127.4, 128.8, 129.1, 131.2, 134.4, 137.0, 142.7, 159.4, 160.2, 165.7.

**2-Hydrazinyl-4-phenylpyrimidine (4.11)**
To a solution of **4.10** (0.1 mmol) in ethanol (5 mL) was added hydrazine hydrate (5 mL). The reaction mixture was stirred under reflux for 12 h. The reaction mixture was then concentrated under reduced pressure to afford **4.11** as a yellow oil, which was used in the next step without further purification.

**2-Azido-4-phenylpyrimidine (4.12)**

![2-Azido-4-phenylpyrimidine](image)

To a solution of **4.11** (0.1 mmol) in acetic acid (0.5 mL) was added a solution of sodium nitrite (0.7 mmol) in water (0.5 mL). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between dichloromethane and sodium bicarbonate saturated aqueous solution. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:5) affords **4.12** as a white solid (16 mg, 57%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 7.47 (d, $J$ = 5.3 Hz, 1H), 7.49–7.57 (m, 3H), 8.11 (dd, $J$ = 7.4, 1.9 Hz, 2H), 8.63 (d, $J$ = 5.2 Hz, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 112.7, 127.5, 129.3, 131.9, 135.8, 159.8, 166.4. HRMS (ESI) calcd. for C$_{10}$H$_7$N$_5$ [M + H]$^+$ 198.0774, found 198.0808.

**3-Methyl-1-(1-(4-phenylpyrimidin-2-yl)-1H-1,2,3-triazol-4-yl)butan-1-ol (4.13)**
To a solution of 4.12 (0.1 mmol) and commercially available 5-methyl-1-hexyn-3-ol (0.1 mmol) in tert-butanol (2 mL) was added a solution of (+)-sodium L-ascorbate (0.1 mmol) in water (1 mL), and then a solution of copper(II) sulfate pentahydrate (0.03 mmol) in water (1 mL). The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between ethyl acetate and water. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 3:1) affords 4.13 as a white solid (14 mg, 70%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.95 (d, $J$ = 6.3 Hz, 6H), 1.72–1.86 (m, 3H), 5.06 (dd, $J$ = 8.3, 4.7 Hz, 1H), 7.48–7.54 (m, 3H), 7.71 (d, $J = 5.3$ Hz, 1H), 8.14 (dd, $J = 7.8$, 1.8 Hz, 2H), 8.58 (s, 1H), 8.83 (d, $J = 5.3$ Hz, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.2, 23.5, 24.8, 29.9, 45.5, 65.6, 116.2, 119.7, 127.7, 129.5, 132.37, 135.3, 160.0, 166.8. HRMS (ESI) calcd. for C$_{17}$H$_{19}$N$_5$O [M + H]$^+$ 310.1662, found 310.1688.

4-(tert-butyl)-2-chloropyrimidine-5-carbonitrile (4.14)
To a solution of 2.4i (0.6 mmol) and antimony(III) chloride (1.2 mmol) in anhydrous chloroform (5 mL) under argon atmosphere was added *iso*-pentyl nitrite (2.0 mmol) very slowly. The reaction mixture was stirred in reflux for 7 h. The reaction mixture was then poured into a saturated sodium bicarbonate aqueous solution. The mixture was filtered through Celite™. The filtrate was extracted with diethyl ether. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:10) affords 4.14 as a colorless oil (30%). ¹H NMR (400 MHz, CDCl₃) ppm 1.46 (s, 9H), 8.71 (s, 1H). ¹³C NMR (100 MHz, CDCl₃) ppm 28.7, 29.9, 40.3, 105.8, 115.6, 163.0, 164.1, 183.0.

**Carbamimidic chloride hydrochloride(4.15)**

To a solution of cyanamide (11.7 mmol) in diethyl ether (50 mL) was added HCl (g) for 30 min. The reaction mixture was stirred at room temperature for an additional 5 h. The reaction mixture was filtered through Celite™. The residue was rinsed with lots of diethyl ether to afford the crude product 4.15 as a white solid (0.40g, 30%), which was
used in the next step without further purification. $^1$H NMR (400 MHz, DMSO) ppm 6.25 (br. s, 2H), 12.17 (br. s, 2H). $^{13}$C NMR (100 MHz, DMSO) ppm 155.2.

**2-Chloro-4-isobutylpyrimidine-5-carbonitrile (4.16)**

![2-Chloro-4-isobutylpyrimidine-5-carbonitrile](image)

To a solution of 4.15 (0.5 mmol) and 2.3d (0.4 mmol) in anhydrous 1,4-dioxane (3 mL) under argon atmosphere was added triethylamine (0.5 mmol). The reaction mixture was stirred at reflux for 13 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:3) affords 4.16 as a colorless oil (40%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.98 (d, $J = 6.7$ Hz, 6H), 2.12–2.25 (m, 1H), 2.66 (d, $J = 7.3$ Hz, 2H), 5.49 (s, 2H), 8.45 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.6, 22.7, 28.8, 45.6, 116.7, 162.1, 162.9, 175.0.

**4-(Benzyloxy)-2-chloropyrimidine (4.17)**

![4-(Benzyloxy)-2-chloropyrimidine](image)
To a suspension of sodium hydride (12.6 mmol) in anhydrous \(N,N\)-dimethylformamide (5 mL) at 0 °C in an ice-bath under argon atmosphere was added benzyl alcohol (5.8 mmol) slowly. To this solution was added a solution of 2,4-dichloropyrimidine (5.8 mmol) in tetrahydrofuran. The reaction mixture was stirred at room temperature for 24 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:5) affords 4.17 as a white solid (1.0 g, 78%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) ppm 5.43 (s, 2H), 6.71 (d, \(J = 5.7\) Hz, 1H), 7.36–7.47 (m, 5H), 8.31 (d, \(J = 5.7\) Hz, 1H). \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) ppm 69.4, 107.6, 128.8, 128.9, 128.9, 135.4, 159.1, 160.4, 170.3.

**Methyl 2-((4-(benzyloxy)pyrimidin-2-yl)amino)-4-methylpentanoate (4.18)**

![Chemical structure](image)

To a solution of 4.17 (0.8 mmol) and L-leucine methyl ester hydrochloride (1.6 mmol) in anhydrous ethanol (3 mL) was added sodium methoxide (1.6 mmol). The reaction mixture was placed in a microwave reactor for 2 h at 160 °C. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between dichloromethane and water. The organic layer was dried over sodium sulfate, and the
solvent was removed under reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:3) affords 4.18 as a white solid (49 mg, 39%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.96 (dd, $J = 10.3, 6.5$ Hz, 6H), 1.58–1.87 (m, 3H), 3.70 (s, 3H), 4.55–4.69 (d, $J = 5.5$ Hz, 1H), 5.31 (s, 2H), 6.10 (d, $J = 5.6$ Hz, 1H), 7.28–7.44 (m, 4H), 8.03 (d, $J = 5.2$ Hz, 1H).

$^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.2, 23.1, 25.1, 41.8, 52.3, 53.3, 67.7, 128.3, 128.7, 136.8, 169.8, 170.4, 174.8. HRMS (ESI) calcd. for C$_{18}$H$_{23}$N$_3$O$_3$ [M + H]$^+$ 330.1812, found 330.1852.

**Methyl 2-((4-(benzyloxy)-5-iodopyrimidin-2-yl)amino)-4-methylpentanoate (4.19)**

![Methyl 2-((4-(benzyloxy)-5-iodopyrimidin-2-yl)amino)-4-methylpentanoate (4.19)](image)

To a suspension of 4.18 (0.2 mmol) and mercury(II) acetate (0.1 mmol) in water (2 mL), that was heated to boiling for 2 min, was added a hot solution of iodine (0.2 mmol) in 1,4-dioxane (2 mL). The reaction mixture was stirred at 100 °C for 2 h. After the reaction, a mixture of potassium iodide and sodium sulfite was added to the reaction mixture, and the mixture was stirred overnight. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash
column chromatography on silica gel (ethyl acetate/hexane, 1:5) affords **4.19** as a yellow solid (42 mg, 62%). $^1$H NMR (400 MHz, CDCl$_3$) 0.95 (dd, $J = 13.5, 6.3$ Hz, 6H), 1.53–1.85 (m, 3H), 3.70 (s, 3H), 4.43–4.68 (m, 1H), 5.37 (s, 2H), 7.37 (ddd, $J = 25.0, 18.5, 7.5$ Hz, 2H), 7.28–7.48 (m, 5H), 8.26 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 22.1, 23.1, 25.1, 52.4, 61.3, 68.6, 127.6, 128.2, 128.7, 136.5, 161.4, 164.8, 167.0, 173.9.

**Methyl 2-((4-(benzyloxy)-5-ethynylpyrimidin-2-yl)amino)-4-methylpentanoate (4.20)**

![Methyl 2-((4-(benzyloxy)-5-ethynylpyrimidin-2-yl)amino)-4-methylpentanoate](image)

Step 1: To a solution of **4.19** (0.1 mmol) and ethynyltrimethylsilane (0.2 mmol) in anhydrous acetonitrile (3 mL) under argon atmosphere was added tetrakis(triphenylphosphine)palladium(0) (0.003 mmol), copper(I) iodide (0.007 mmol) and triethylamine (1 mL). The reaction mixture was stirred under reflux for 20 h. The reaction mixture was then concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane, 1:5) to afford the intermediate as a white solid, which was used in the next step without further purification.

Step 2: To a solution of the intermediate from step 1 in anhydrous tetrahydrofuran (1 mL) at 0 ºC in an ice-bath under argon atmosphere was added tetrabutylammonium fluoride
(0.03 mmol). The reaction mixture was stirred at 0 °C for 10 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:5) affords 4.20 as a white solid (30 mg, 92%). $^1$H NMR (400 MHz, CDCl$_3$) ppm 0.79–1.02 (m, 6H), 1.58–1.83 (m, 3H), 3.27 (s, 1H), 3.69 (s, 3H), 4.46–4.60 (m, 1H), 5.41 (s, 2H), 6.04 (br. s, 1H), 7.31 (d, $J = 7.2$ Hz, 1H), 7.36 (t, $J = 7.3$ Hz, 2H), 7.43 (d, $J = 8.0$ Hz, 2H), 8.21 (s, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$) ppm 23.1, 25.5, 29.9, 41.5, 52.4, 53.3, 61.4, 68.1, 82.4, 127.8, 128.1, 128.7, 136.6, 160.6, 162.3, 169.5, 178.2. HRMS (ESI) calcd. for C$_{20}$H$_{23}$N$_3$O$_3$ [M + H]$^+$ 354.1812, found 354.1800.

2-(4-(4-(Benzyloxy)-2-((1-methoxy-4-methyl-1-oxopentan-2-yl)amino)pyrimidin-5-yl)-1H-1,2,3-triazol-1-yl)-3-methylbutanoic acid (4.21)

To a solution of 4.20 (0.1 mmol) and 4.22 (0.1 mmol) in tert-butanol (1 mL) was added a solution of (+)-sodium L-ascorbate (0.1 mmol) in water (0.5 mL), and then a solution of
copper(II) sulfate pentahydrate (0.05 mmol) in water (0.5 mL). The reaction mixture was stirred at room temperature for 23 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between ethyl acetate and water. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (methanol / dichloromethane, 1:20) affords 4.21 as a white solid (41 mg, 99%). \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) ppm 0.86 (dd, \(J = 32.4, 6.4\) Hz, 6H), 1.02 (dd, \(J = 20.0, 6.8\) Hz, 6H), 1.59–1.86 (m, 3H), 2.24 (dq, \(J = 13.2, 6.7\) Hz, 1H), 3.68 (s, 3H), 4.11–4.20 (m, 1H), 4.40–4.54 (m, 1H), 5.37–5.59 (m, 1H), 7.27–7.43 (m, 5H), 8.12 (s, 1H), 8.77 (s, 1H), 10.76 (br. s, 1H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) ppm 14.3, 14.5, 18.0, 19.77, 25.0, 29.3, 29.7, 31.2, 40.4, 52.5, 53.9, 61.4, 61.5, 68.7, 69.8, 70.0, 103.4, 103.4, 122.8, 128.1, 132.3, 138.5, 149.1, 159.6, 166.4, 172.9, 175.1, 179.9. HRMS (ESI) calcd. for C\(_{25}\)H\(_{32}\)N\(_6\)O\(_5\) \([M + H]^+\) 497.2507, found 497.2484.

2-Azido-3-methylbutanoic acid (4.22)

\[ \text{N}_3 \quad \text{COOH} \]

To a solution of L-valine (0.06 mmol), potassium carbonate (0.16 mmol), and copper(II) sulfate pentahydrate (0.006 mmol) in methanol (2 mL) was added imidazole-1-sulfonyl azide hydrochloride (0.07 mmol). The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between ethyl acetate and HCl (1M, aqueous). The organic layer was dried
over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a colorless oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:1) affords 4.21 as a colorless solid (90%). $^1H$ NMR (400 MHz, CDCl$_3$) ppm 1.01 (d, $J = 6.7$ Hz, 3H), 1.06 (d, $J = 6.8$ Hz, 3H), 2.17–2.33 (m, 1H), 3.78 (d, $J = 5.7$ Hz, 1H), 11.80 (s, 1H).

4.7 References


Chapter Five:
Design and Synthesis of Cyclic Urea HIV-1 Protease Inhibitor

5.1 Introduction

5.1.1 AIDS

Acquired immune deficiency syndrome (AIDS) was first identified on June 5th, 1981 in the USA (Gottlieb Michael, 2006). This syndrome is caused by the human immunodeficiency virus (HIV), which is believed to have originated in non-human primates in sub-Saharan Africa and then transferred to humans during the 20th century (Gao et al., 1999). While it is hard to define the beginning of the AIDS epidemic, it has

Figure 5.1: Estimated number of people living with HIV globally, 1990–2007
been estimated that during the 26 year period between 1981 and 2007 there have been more than 25 million deaths as a result of AIDS infection. This averages out to almost 1 million AIDS related deaths per year.

Statistical data shows that the number of people living with HIV is still increasing (Figure 5.1) (WHO, 2009); this implies that the number of deaths caused by AIDS is also increasing. In addition, this virus can be passed from mothers to children during pregnancy or through breast milk (Kallings, 2008). As a result, children are becoming a greater proportion of newly infected AIDS patients each year.

5.1.2 Efforts to treat or cure AIDS

Since the discovery of HIV, there have been tremendous efforts made towards targeting this deadly virus. While there are several FDA approved drugs available to help treat this disease, none of them can cure this disease.

In addition, those patients living in poor or developing countries, who account for more than 90% of all HIV infected patients, cannot afford the astronomical cost of these therapies (HIV/AIDS programme highlights, WHO report). Thus, designing synthetic routes for novel small molecule HIV inhibitors remains an important goal. This report will focus mainly on the HIV-1 virus, which accounts for about 90% of all HIV infections.

5.1.3 Structure of HIV
HIV belongs to a group of viruses called retro-viruses (Weiss, 1993), which carry their genetic information in the form of RNA. The diagram of a mature HIV virion is shown in Figure 5.2 (OracleThinkQuest). The center of the virion consists of two pieces of single stranded viral RNA, which contains all of the virus’s genetic information. All of the enzymes that are needed for the development of a new virion, such as the reverse transcriptase, integrase, ribonuclease and protease are encoded in these RNA strands. The virion is enclosed in a two layered network. The inner layer is called the conical capsid which is composed of 2000 copies of a viral protein named p24. The outer layer is called the protein matrix and is composed of a viral protein named p17. This kind of multiple-layer enclosure exists to protect the integrity of the virion particles. These layers are then surrounded by the envelope which is an outer membrane that is composed

**Figure 5.2:** Diagram of HIV
of a phospholipid bilayer and is similar to a regular eukaryotic cell membrane. Embedded in the viral envelope are about 70 copies of a viral protein known as Env which is comprised of a cap and a stem. The cap is made from a cluster of three glycoprotein gp120 molecules while the stem is made from a cluster of three glycoproteins gp41 molecules. The function of the Env protein is to identify and bind to receptor sites on the target cell’s membrane.

### 5.1.4 HIV replication mechanism

The targets of HIV are mainly the immune cells such as helper T cells (especially CD4⁺ T cells), macrophages, and dendritic cells. Figure 5.3 (wiki) shows the entire replication process for the creation of a new HIV virion (Brik and Wong, 2003). HIV’s entry to the host cell is mediated through the interaction between the gp120 protein on the HIV membrane and the CD4 molecule on the target cell. The HIV virion absorbs the glycoprotein on the host cell’s surface and then fuses the viral envelop with the cell membrane. This leads to the HIV capsid’s entry into the host cell. Once the capsid has bound to the host cell, the RNA strands and all the viral enzymes are injected into the cell. The viral RNA is then copied into double stranded viral DNA by the reverse transcriptase enzyme, which is then integrated into the host cell’s genome by another enzyme known as integrase. Afterwards, the viral DNA is transcribed into viral mRNA which is then translated into the viral polyprotein. The protease enzyme then helps to finish the maturation process by hydrolyzing the viral polyprotein into individual viral proteins. Finally, the mature viral protein, viral RNA and viral enzymes are packaged together by a portion of the host cell’s membrane to form a new HIV virion.
5.1.5 Common targets to inhibit the HIV replication process

Studying the infection mechanism has led to the development of several inhibitors, most of which target the viral enzymes. One of the most popular targets at the present time is reverse transcriptase due to the fact that this enzyme does not naturally exist in the human body thus minimizing the side effects of its inhibitors (Zheng et al.,
However, the reverse transcriptase is not actually a good target because of the high replication rate of the virus (10^8-10^9 virions per day) along with a high error rate of the HIV reverse transcriptase (~1 in 10K bases) which usually lead to a very high resistance to the current drugs. On the other hand, another critical enzyme, the protease, has become increasingly targeted because it is critical role for the development of a mature HIV virion. It has been shown that the immature HIV viral polyprotein is not infective (Kohl et al., 1988).

5.1.6 Structure of HIV protease

The HIV protease (HIV PR) is believed to belong to the family of aspartic proteases. It contains 99 amino acids which function as a homodimer with only one active site which is C_2-symmetric when the protein is in the free form (Brik and Wong, 2003). Figure 5.4 shows the cocrystal structure of HIV PR complexed with inhibitor TL-3. HIV PR’s active site is a cavity formed by the surrounding extended β-sheet region.

Figure 5.4: Structure of HIV PR complexed with TL-3
from both of the monomers. The ceiling of the cavity is Ile-50/Ile-50’, while the bottom holds the Asp-25/Asp-25’ catalytic residues. With the exception of the Asp-25/Asp-25’, Asp-29/Asp-29’, and Asp-30/Asp-30’ residues, this cavity is very hydrophobic.

5.1.7 HIV protease function mechanism

While the mechanism of this proteolysis is not very clear, it is generally accepted that a catalytic water molecule acts as a nucleophile to attack the scissile bond instead of the carboxylic group from Asp-25/Asp-25’ (Jaskolski et al., 1991). This then forms a tetrahedral diol intermediate (Figure 5.5). Many potent transition-state inhibitors are designed according to this mechanism.

![Figure 5.5: Mechanism of HIV PR proteolysis](image)

5.1.8 Cyclic urea as the HIV-1 protease inhibitor

In 1997, the DuPont Merck group found that cyclic ureas fit well in the HIV PR active site cavity (Figure 5.6) (De Lucca et al., 1997); however there has been little work
reported since. In this report, we plan to use cyclic urea as the HIV PR inhibitor basic framework and extend the substitutents on it to get better binding affinity.

![X-ray crystal structure of cyclic urea in the active site of HIV PR](image)

**Figure 5.6:** X-ray crystal structure of cyclic urea in the active site of HIV PR

### 5.1.9 Our design strategy

We would like to keep the six-membered cyclic urea ring since it sterically fits the active site cavity very well. But we would like to attach more hydrophilic functional groups (e.g. a hydroxyl group on the 4-position) to enhance the hydrogen bonding interaction between our molecule and the Asp-29/Asp-29’ or Asp-30/Asp-30’ of the HIV-1PR. Additional hydrophobic groups on the 5-position should help to increase Van der Waals bonding or hydrophobic interaction between our molecules and the hydrophobic cavity.
In this report, we would also like to develop a general synthetic procedure for making 4-hydroxyl cyclic urea. Eventually, we will be able to create a library of this type of compound in order to test their bioactivities.

5.2 Results and Discussion

5.2.1 Retrosynthesis of 5-hydroxyl cyclic urea

The creation of a 5-hydroxyl cyclic urea is challenging because of the instability of the geminal diamino functional group with an adjacent \( \text{sp}^3 \) carbon center. This kind of functional group is hard to form and easily decomposes. Our strategy in this report is to try to rearrange an even more unstable 1,3-diazetidin-2-one \( \text{b} \) to achieve our desired compound \( \text{a} \) (Scheme 5.1). As we can see, the four-membered 1,3-diazetidin-2-one \( \text{b} \) is highly strained and the nitrogen atom in the ring adjacent to the Boc group is somewhat of a good leaving group. The free amino group in the molecule could selectively open one side of the four-membered ring to form a thermodynamically more stable

**Scheme 5.1:** Retrosynthesis of 5-hydroxyl cyclic urea
six-membered ring a. This four-membered 1,3-diazetidin-2-one b could be made from a 2-oxo-1,3-diazabicyclo[2,2,0]hex-5-ene c molecule through oxidative cleavage of the alkene followed by a Henry reaction. This bicyclic intermediate c could be synthesized by photochemical electrocyclization from pyrimidin-2(1H)-ones d.

5.2.2 Synthesis of intermediate 5.3

Following this strategy, the following steps have been carried out (Scheme 5.2):

**Scheme 5.2: Synthesis of the intermediate 5.3**

\[
\begin{align*}
\text{R}_1\text{N} & \quad \text{O} \\
\text{H} & \quad \text{N} \\
\text{NH}_2 & \quad + \\
\text{R}_2\text{C}=\text{O} & \quad \text{O} \\
\text{C} & \quad \text{O} \\
\text{R}_2 & \quad \text{R}_2 \\
\text{i} & \quad \text{i} \\
\text{R}_1\text{N} & \quad \text{O} \\
\text{N} & \quad \text{N} \\
\text{R}_2 & \quad \text{R}_2 \\
\end{align*}
\]

i) TPSA, reflux, 12 - 36 h

The first major step of the synthesis is to make a variety of 1,4,6-trisubstituted pyrimidin-2(1H)-ones 5.1 as the photochemical electrocyclization precursors. Table 5.1 shows all the pyrimidinone derivatives that have been synthesized so far. Several procedures have been developed, the most successful one is the condensation between 1,3-disubstituted 1,3-propanedione and N-substituted urea in acetic acid using p-toluenesulfonic acid as the proton source (Katritzky et al., 1982). Additionally, the use of a Dean-Stark arm while refluxing has often been applied. Unfortunately, the yield of this step is only poor to fair, e.g. 32% for \( R_1 = \text{Bn}, R_2 = \text{'Pr} \).
Using the condensation reaction between 2,6-dimethyl-3,5-heptanedione and benzylurea as a model (Table 5.1 5.1a), the first step of the cyclization is believed to be the amino group of the \( N \)-benzylurea attacking one of the carbonyl groups on 2,6-dimethyl-3,5-heptanedione. One of the big problems with this reaction is that it creates a large quantity of side products (Scheme 5.3). This is due to the fact that in these high temperature and acidic reactions conditions, benzylurea is prone to hydrolysis which then generates benzylamine \( e \). Benzylamine then reacts with the protonated benzylurea to form \( N,N' \)-dibenzyurea \( f \) which then precipitates out of solution thus making this side reaction thermodynamically favorable. Additionally, this side reaction is very fast.

**Scheme 5.3:** Hydrolysis of \( N \)-benzylurea under high temperature acidic conditions
Large amounts of precipitate can be seen within 30 minutes while the reaction time of the cyclization is around 36 hours. In fact, pure \(\text{N, N}'\)-dibenzylurea \(f\) can usually be isolated in large amount after the reaction. Due to these facts, the reaction of the diketone is less favored than the reaction of benzylamine with \(\text{N}\)-benzylurea.

Several adjustments have been tried such as increasing the equivalence of benzylurea, decreasing the reaction temperature below 90 °C to slow down the formation of benzylamine, increasing the temperature to 150 °C to accelerate the cyclization, using solvent free condition, and adding a small amount of dimethylformamide (DMF) to prevent the precipitation. However, no improvement has been made so far. The good thing is that this is the very first step of the synthesis, and the two starting material are readily commercially available. Therefore, the low yield of this reaction is not a bottleneck for the whole synthesis.

It is worthwhile to mention that asymmetric diketones have been tried as well in this reaction. However, this procedure lacks regio-selectivity and would yield a mixture of products where the benzyl group on the urea would be found on either side of the parent ring. Also, the two isomers formed here have very similar polarity which makes it impractical to isolate them by chromatography on a large scale. Therefore, no further work has been carried out using asymmetric diketones.

5.2.3 Photochemical electrocyclization of 1,4,6-trisubstituted pyrimidin-2(1H)-ones

5.1
The second step, photochemical electrocyclization of the 1,4,6-trisubstituted pyrimidin-2(1H)-ones to 2-oxo-1,3-diazabicyclo[2.2.0]hex-5-enes, is one of the key steps of the synthesis (Scheme 5.4) (Nishio et al., 1981). While the photo reaction is a very demanding step, it works very efficiently for those substrates which meet the requirements of the reaction.

Scheme 5.4: Synthesis of the 1,3-diazetidine-2-one intermediate

![Scheme 5.4: Synthesis of the 1,3-diazetidine-2-one intermediate]

The light source is the first crucial factor for this reaction to make 5.2a. Several lamps such as a Xenon lamp and a mercury vapor lamp (wavelength > 253nm) have been tested, that they could not promote the reaction. We believe the failure of these reactions
is because the photochemical cyclization is an equilibrium reaction; both starting materials and products can absorb photons to go to the reverse of the reaction. The product is a lot less stable than the starting material due to the high ring strain of the bicyclic four-membered rings. Therefore, decomposition of the product is a favorable reaction. One difference between the starting material and the product is that the starting material has a conjugated double bond system while the product does not. This led us to hypothesis that if we could find a light source that can provide photons with a wavelength high enough, it is possible that only the starting material would be activated. Medium pressure mercury lamps meet this requirement. Using a medium pressure mercury lamp with a Pyrex filter provides a sufficient wavelength with a cutoff around 300 nm. This is higher than any of the absorption wavelengths that the product has (Figure 5.7) so the reaction should proceed in a highly favorable manner only towards the products.

**Figure 5.7:** UV absorption of 5.1a
The reaction is also dependent on the substituents on the pyrimidinone ring. Compounds 5.1d and 5.1f (Table 5.1) have no reaction because those compounds are too polar to be dissolved in benzene, the common solvent for the photochemical reaction. Compound 5.1e, which did not have any solubility issue, did not work because the normal photochemical cyclization is going through a triplet mechanism. The unsubstituted 6-position on the pyrimidinone ring is not able to stabilize this triplet intermediate. Compound 5.1b did not work however, is thought to be because of another reason. For this substrate, when the four-membered product is formed, the double bond in the four-membered ring would be conjugated with the phenyl group; this would cause the product to absorb photons with higher wavelength. Theoretically, if we can apply a photon generator to generate photons with wavelength in certain ranges, we should be able to get this substrate to work.

In summary, pyrimidinones 5.1 with good solubility in benzene (this can be achieved by N-benzyl substitution), and with 6-position substitutuents, activated by photons of proper wavelength (e.g. from medium pressure mercury lamp) should be able to undergo this photochemical cycloaddition.

Then we ozonized the double bond to make compound 5.3. Other conditions have been tested, e.g. NaIO₄/OsO₄, KMnO₄ on solid support; but in both cases, the double bond would not be fully cleaved, and an α-hydroxyl ketone 5.8 was isolated as the major product (Scheme 5.5).

**Scheme 5.5:** Side product of the oxidation of 5.2a
It is worthwhile to point out that compound 5.3 is very moisture sensitive. Spectroscopy normally detected both 5.3 and the molecule with one water added to form a five-membered ring (Scheme 5.6).

**Scheme 5.6: Equilibrium between 5.3 and its water added form 5.3’**

5.3’ turned out to be problematic because the five-membered ring is so stable that regular hydrolysis is not able to cleave the *iso*butyryl group on the nitrogen atom. We chose to reduce the two new generated C,O double bond to cleave that *iso*butyryl group. However, mild reductants, like sodium borohydride, could only reduce one of double bond, to synthesize compound 5.4. Later, we found that LiBH₄ is able to reductively cleave both of the C,O double bond to prepare compound 5.5.

We also performed Henry reaction on 5.3 to synthesize compound 5.6; the nitro group of 5.6 could then be easily reduced to afford compound 5.7.
5.4 Experimental Procedures of selected compounds

1-Benzyl-4,6-diisopropylpyrimidin-2(1H)-one (5.1a)

![Chemical structure of 1-Benzyl-4,6-diisopropylpyrimidin-2(1H)-one](image)

To a solution of 2,6-dimethyl-3,5-heptanedione (46.7 mmol) in a mixture of acetic acid (18 mL) and toluene (15 mL) was added *N*-benzylurea (74.2 mmol) and *p*-toluenesulfonic acid monohydrate (92.9 mmol). The reaction mixture was stirred under reflux for 12 h. The precipitate was removed by vacuum filtration, and the filtrate was concentrated under reduced pressure. The residue was then extracted between dichloromethane and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product a white solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 3:1) affords 5.1a as a white solid (4.0 g, 32%). $^1$H NMR (250 MHz, CDCl$_3$) ppm 1.16 (d, $J =$ 6.77 Hz, 6H), 1.28 (d, $J =$ 6.91 Hz, 6H), 2.83 (m, 1H), 2.95 (m, 1H), 5.38 (s, 2H), 6.15 (s, 1H), 7.11–7.41 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$) ppm 17.4, 17.6, 19.6, 19.9, 29.5, 29.7, 47.4, 82.1, 115.8, 128.1, 128.7, 128.8, 135.6, 160.8, 166.7.

1-Benzyl-4,6-diphenylpyrimidin-2(1H)-one (5.1b)

![Chemical structure of 1-Benzyl-4,6-diphenylpyrimidin-2(1H)-one](image)
Compound 5.1b was prepared following the procedure described for 5.1a. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:1) affords 5.1b as a white solid (39%). $^1$H NMR (250 MHz, CDCl$_3$) ppm 5.03 (s, 2H), 6.23 (s, 1H), 6.71–7.19 (m, 13H), 8.07 (dd, $J = 7.93, 1.68$ Hz, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) ppm 49.6, 102.0, 127.4, 127.6, 128.1, 128.3, 128.5, 128.7, 129.6, 131.6, 134.2, 136.8, 137.5, 157.1, 160.0, 169.5.

1-Benzyl-4,6-dimethylpyrimidin-2(1H)-one (5.1c)

\[ \text{O} \]
\[ \text{N} \]
\[ \text{N} \]
\[ \text{O} \]
\[ \text{C} \]

Compound 5.1c was prepared following the procedure described for 5.1a. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:1) affords 5.1c as a white solid (45%). $^1$H NMR (250 MHz, MeOD) ppm 2.38 (s, 6H), 5.37 (s, 2H), 6.45 (s, 1H), 7.14–7.44 (m, 5H).

1,4,6-trimethylpyrimidin-2(1H)-one (5.1d)

\[ \text{O} \]
\[ \text{N} \]
\[ \text{N} \]
\[ \text{O} \]
\[ \text{C} \]

Compound 5.1d was prepared following the procedure described for 5.1a. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 4:1) affords 5.1c as a
white solid (55%). $^1$H NMR (250 MHz, MeOD) ppm 2.57 (s, 3H), 2.72 (s, 3H), 3.70 (s, 3H), 6.84 (s, 1H).

3-Benzyl-4,6-diisopropyl-1,3-diazabicyclo[2.2.0]hex-5-en-2-one (5.2a)

A solution of 5.1a (3.7 mmol) in 100 mL degassed benzene was irradiated with a medium pressure mercury lamp using a Pyrex filter for 3 days. The reaction mixture was then concentrated under reduced pressure to afford the crude material as a colorless oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:7) affords 5.2a as a colorless oil (0.88 g, 88%). $^1$H NMR (250 MHz, CDCl$_3$) ppm 0.92 (d, $J = 6.75$ Hz, 6H), 1.11 (d, $J = 6.91$ Hz, 6H), 1.96–2.13 (m, 1H), 2.50–2.64 (m, 1H), 4.40 (dd, $J = 93.08$, 15.26 Hz, 2H), 5.53 (d, $J = 1.62$ Hz, 1H), 7.19–7.38 (m, 5H). $^{13}$C NMR (75 MHz, CDCl$_3$) ppm 21.3, 22.4, 30.4, 37.3, 47.8, 99.5, 126.5, 127.8, 129.1, 136.5, 158.2.

3-Benzyl-4,6-dimethyl-1,3-diazabicyclo[2.2.0]hex-5-en-2-one (5.2b)

Compound 5.2b was prepared following the procedure described for 5.2a. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:7) affords 5.1c as a
colorless oil (72%). $^1$H NMR (250 MHz, benzene-$d^6$) ppm 1.12 (s, 3H), 1.73 (d, $J = 1.68$ Hz, 3H), 3.98 (dd, $J = 63.00$, 15.16 Hz, 2H), 5.18 (d, $J = 1.68$ Hz, 1H), 6.94–7.12 (m, 5H).

1-Benzyl-3-isobutyryl-2-isopropyl-4-oxo-1,3-diazetidine-2-carbaldehyde (5.3)

![Chemical Structure](image)

Through a solution of compound 5.2a (0.5g, 1.9 mmol) in 10 mL dichloromethane cooled in acetone/dry ice bath was passed a stream of ozone until the light blue color persisted. The reaction mixture was then flushed with argon (20 min), dimethylsulfide (3 mL) was added, and stirring was continued at room temperature for 2 h. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between DCM and water. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow oil. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:2) affords 5.3 as a colorless oil (0.58 g, >90%). $^1$H NMR (250 MHz, CDCl$_3$) ppm 0.84 (d, $J = 6.92$ Hz, 3H), 0.96 (d, $J = 6.85$ Hz, 3H), 1.19 (d, $J = 6.81$ Hz, 3H), 1.25 (d, $J = 6.96$ Hz, 3H), 2.55 (td, $J = 6.80$ Hz, 1H), 3.29 (d, $J = 6.80$ Hz, 1H), 4.56 (dd, $J = 48.58$, 15.59 Hz, 2H), 7.25–7.42 (m, 5H).

1-benzyl-4-(1-hydroxy-2-nitroethyl)-3-isobutyryl-4-isopropyl-1,3-diazetidin-2-one (5.6)
To a solution of 5.3 (2.8 mmol) in ice cooled nitromethane (20 mL) was added triethylamine (3.6 mmol). The reaction mixture was stirred at room temperature for overnight. The reaction mixture was then concentrated under reduced pressure. The residue was extracted between ethyl acetate and water. The organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure to afford the crude product as a yellow solid. Purification by flash column chromatography on silica gel (ethyl acetate/hexane, 1:2) affords 5.3 as a white solid (0.58 g, 52%).

2.4 References


OracleThinkQuest


Appendix A: Selected $^1$H and $^{13}$C NMR Spectra
Methyl 2-(naphthalen-2-yl)acetate (2.1a)
3-Oxo-4-phenylbutanenitrile (2.2a)
4-(Naphthalen-1-yl)-3-oxobutanenitrile (2.2b)

![Chemical structure image]

![NMR spectrum image]
4-(Naphthalen-2-yl)-3-oxobutanenitrile (2.2c)
5-Methyl-3-oxohexanenitrile (2.2d)
2-((Dimethylamino)methylene)-3-oxo-4-phenylbutanenitrile (2.3a)
2-((Dimethylamino)methylene)-4-(naphthalen-1-yl)-3-oxobutane nitrile (2.3b)
2-((Dimethylamino)methylene)-4-(naphthalen-2-yl)-3-oxobutanenitrile (2.3c)
2-((Dimethylamino)methylene)-5-methyl-3-oxohexanenitrile (2.3d)
2-((Dimethylamino)methylene)-4,4-dimethyl-3-oxopentanenitrile (2.3e)
4-(tert-Butyl)pyrimidine-5-carbonitrile
4-Benzylpyrimidine-5-carbonitrile (2.4b)
2-Methyl-4-(naphthalen-1-ylmethyl)pyrimidine-5-carbonitrile (2.4c)
4-iso-Butyl-2-phenylpyrimidine-5-carbonitrile (2.4d)
4-Benzyl-2-phenylpyrimidine-5-carbonitrile (2.4e)
4-tert-Butyl-2-phenylpyrimidine-5-carbonitrile (2.4f)
4-(Naphthalen-1-ylmethyl)-2-phenylpyrimidine-5-carbonitrile (2.4g)
2-Amino-4-benzylpyrimidine-5-carbonitrile (2.4h)
2-Amino-4-tert-butylpyrimidine-5-carbonitrile (2.4i)
4-Benzyl-2-methoxypyrimidine-5-carbonitrile (2.4j)
4'-Benzyl-4-(naphthalen-1-ylmethyl)-2'-phenyl-[2,5'-bipyrimidine]-5-carbonitrile

(2.6a)
4-Benzyl-4'-((naphthalen-1-ylmethyl)-2'-phenyl-[2,5'-bipyrimidine]-5-carbonitrile (2.6c)
4-Benzyl-2'-methyl-4'-(naphthalen-1-ylmethyl)-[2,5'-bipyrimidine]-5-carbonitrile

(2.6d)
4-(tert-Butyl)-2'-methyl-4'-(naphthalen-1-ylmethyl)-[2,5'-bipyrimidine]-5-carbonitrile (2.6e)
5'-Cyano-4,4''-diisobutyl-4''-(1-naphthylmethyl)-2-phenylterpyridine (2.8a)
$5''$-Cyano-$4''$-isobutyl-$4'$(1-naphthylmethyl)-$4$(2-phenylmethyl)-2-phenylterpyrimidine (2.8b)
5''-Cyano-4''-isobutyl-4'-{(2-phenylmethyl)-4-(1-naphthylmethyl)-2-phenylterpyrimidine (2.8C)
3-Hydroxy-4-phenylbutanenitrile (3.2)
$N',3$-Dihydroxy-$4$-phenylbutanimidamide acetate (3.3)
2-(2-Hydroxy-3-phenylpropyl)-4-isobutylpyrimidine-5-carbonitrile (3.4)
2-Bromo-1-phenylethanone (3.6)
2-Azido-1-phenylethanone (3.7)
2-(4-(1-Hydroxy-3-methylbutyl)-1H-1,2,3-triazol-1-yl)-1-phenylethanone (3.8)
3-(Dimethylamino)-2-(4-(1-hydroxy-3-methylbutyl)-1H-1,2,3-triazol-1-yl)-1-phenylprop-2-en-1-one (3.9)
1-(1-(2-Amino-4-phenylpyrimidin-5-yl)-1H-1,2,3-triazol-4-yl)-3-methylbutan-1-ol

(3.10)
1-(1-(4'-isoButyl-2',4-diphenyl-[2,5'-bipyrimidin]-5-yl)-1H-1,2,3-triazol-4-yl)-3-methylbutan-1-ol (3.11)
1-(Dimethylamino)-4-methylpent-1-en-3-one (4.1)
4-iso-Propylpyrimidin-2-amine (4.2)
5-Iodo-4-isopropylpyrimidin-2-amine (4.3)
4-(2-Amino-4-isopropylpyrimidin-5-yl)-2-methylbut-3-yn-2-ol (4.4)
5-(1-Benzyl-1H-1,2,3-triazol-4-yl)-4-isopropylpyrimidin-2-amine (4.6)B
1-(Dimethylamino)-4-methylhex-1-en-3-one (4.9b)

1-cyclopropyl-3-(dimethylamino)prop-2-en-1-one (4.9c)
3-(Dimethylamino)-1-phenylprop-2-en-1-one (4.9c)
2-(2-Benzylidenehydrazinyl)-4-phenylpyrimidine (4.10)
2-Azido-4-phenylpyrimidine (4.12)
3-Methyl-1-(1-(4-phenylpyrimidin-2-yl)-1H-1,2,3-triazol-4-yl)butan-1-ol (4.13)
4-(tert-Butyl)-2-chloropyrimidine-5-carbonitrile (4.14)
Carbamimidic chloride hydrochloride (4.15)

\[ \text{H}_2\text{N} = \text{NH} \cdot \text{HCl} \]

\[ \text{H}_2\text{N} \equiv \text{NH} \cdot \text{HCl} \]
2-Chloro-4-isobutylpyrimidine-5-carbonitrile (4.16)
4-(Benzyloxy)-2-chloropyrimidine (4.17)
Methyl 2-((4-(benzyloxy)pyrimidin-2-yl)amino)-4-methylpentanoate (4.18)
Methyl 2-((4-(benzyloxy)-5-iodopyrimidin-2-yl)amino)-4-methylpentanoate (4.19)
Methyl 2-((4-(benzyloxy)-5-ethynylpyrimidin-2-yl)amino)-4-methylpentanoate (4.20)
2-(4-(4-(Benzyloxy)-2-((1-methoxy-4-methyl-1-oxopentan-2-yl)amino)pyrimidin-5-yl)-1H-1,2,3-triazol-1-yl)-3-methylbutanoic acid (4.21)
2-Azido-3-methylbutanoic acid (4.22)
1-Benzyl-4,6-diisopropylpyrimidin-2(1H)-one (5.1a)
1-Benzyl-4,6-diphenylpyrimidin-2(1H)-one (5.1b)
1-Benzyl-4,6-dimethylpyrimidin-2(1H)-one (5.1c)

1,4,6-trimethylpyrimidin-2(1H)-one (5.1d)
3-Benzyl-4,6-diisopropyl-1,3-diazabicyclo[2.2.0]hex-5-en-2-one (5.2a)
3-Benzyl-4,6-dimethyl-1,3-diazabicyclo[2.2.0]hex-5-en-2-one (5.2b)

![Chemical Structure of 3-Benzyl-4,6-dimethyl-1,3-diazabicyclo[2.2.0]hex-5-en-2-one](image)

1-Benzyl-3-isobutyryl-2-isopropyl-4-oxo-1,3-diazetidine-2-carbaldehyde (5.3)

![Chemical Structure of 1-Benzyl-3-isobutyryl-2-isopropyl-4-oxo-1,3-diazetidine-2-carbaldehyde](image)
Appendix B: Selected HRMS
2-((Dimethylamino)methylene)-4-(naphthalen-1-yl)-3-oxobutanenitrile (2.3b)

<table>
<thead>
<tr>
<th>Formula</th>
<th>Compound name</th>
<th>Mass</th>
<th>Peak RT (min)</th>
<th>Peak area</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C17H16N2O</td>
<td>--</td>
<td>264.12626</td>
<td>0.23</td>
<td>7.36200 E7</td>
<td>--</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance (counts)</th>
<th>Ion Mass</th>
<th>Measured Mass</th>
<th>Error (mDa)</th>
<th>Error (ppm)</th>
<th>Ret. Time Error (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H]^+</td>
<td>461589.41</td>
<td>265.13354</td>
<td>265.13128</td>
<td>-2.26019</td>
<td>-8.52</td>
<td>--</td>
</tr>
<tr>
<td>[M+Na]^+</td>
<td>20238 27</td>
<td>287.11548</td>
<td>287.11302</td>
<td>-2.45444</td>
<td>-8.56</td>
<td>--</td>
</tr>
</tbody>
</table>
2-((Dimethylamino)methylene)-4-(naphthalen-2-yl)-3-oxobutanenitrile (2.3c)
4'-Benzyl-4-(naphthalen-1-ylmethyl)-2'-phenyl-[2,5'-bipyrimidine]-5-carbonitrile

(2.6a)
5”-Cyano-4,4”-diisobutyl-4’-(1-naphthylmethyl)-2-phenylterpyrimidine (2.8a)
5"-Cyano-4"-isobutyl-4"-(1-naphthylmethyl)-4-(2-phenylmethyl)-2-phenylterpyrimidine (2.8b):
N',3-Dihydroxy-4-phenylbutanimidamide acetate (3.3)

<table>
<thead>
<tr>
<th>Formula</th>
<th>Compound Name</th>
<th>Mass</th>
<th>Peak RT (min)</th>
<th>Peak area</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10H14N2O2</td>
<td></td>
<td>194.1055</td>
<td>0.20</td>
<td>1.20343E8</td>
<td>--</td>
</tr>
</tbody>
</table>

**Species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance (counts)</th>
<th>Ion Mass</th>
<th>Measured Mass</th>
<th>Error (mDa)</th>
<th>Error (ppm)</th>
<th>Ret. Time Error (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H]^+</td>
<td>1928291.19</td>
<td>195.11283</td>
<td>195.12029</td>
<td>7.48867</td>
<td>38.38</td>
<td>--</td>
</tr>
<tr>
<td>[M+NH4]^+</td>
<td>37474.39</td>
<td>212.13635</td>
<td>212.12368</td>
<td>-15.67206</td>
<td>-73.88</td>
<td>--</td>
</tr>
</tbody>
</table>
2-(4-(1-Hydroxy-3-methylbutyl)-1H-1,2,3-triazol-1-yl)-1-phenylethanone (3.8)

Formula | Compound name | Mass | Peak RT (min) | Peak area | Description
---|---|---|---|---|---
C15H15N3O2 | -- | 273.14773 | 0.22 | 6.22444E7 | --

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance (counts)</th>
<th>Ion Mass</th>
<th>Measured Mass</th>
<th>Error (mDa)</th>
<th>Error (ppm)</th>
<th>Ret. Time Error (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+H]+</td>
<td>1570142.87</td>
<td>274.15500</td>
<td>274.15574</td>
<td>0.73944</td>
<td>2.70</td>
<td>--</td>
</tr>
<tr>
<td>M+Na]+</td>
<td>16815.45</td>
<td>296.13685</td>
<td>296.13694</td>
<td>-0.00377</td>
<td>-0.01</td>
<td>--</td>
</tr>
</tbody>
</table>
3-(Dimethylamino)-2-(4-(1-hydroxy-3-methylbutyl)-1H-1,2,3-triazol-1-yl)-1-phenylprop-2-en-1-one (3.9)
1-(1-(2-Amino-4-phenylpyrimidin-5-yl)-1H-1,2,3-triazol-4-yl)-3-methylbutan-1-ol

(3.10)
1-(1-(4'-isoButyl-2',4-diphenyl-[2,5'-bipyrimidin]-5-yl)-1H-1,2,3-triazol-4-yl)-3-methylbutan-1-ol(3.11)
5-Iodo-4-isopropylpyrimidin-2-amine (4.3)
4-(2-Amino-4-isopropylpyrimidin-5-yl)-2-methylbut-3-yn-2-ol (4.4)
2-Azido-4-phenylpyrimidine (4.12)

Sample Name: VII16one Sample Location: P1-D-06 Sample Id: VII16one Operator: Mingzhou Zhou
Data File Name: d:/PE Scies Data/Projects/chemist1/1-10/Data/VI116ONE POLITO-040110.wiff Acq Time: January 04 2010,
04:22:42 PM
Method: D: TOF_Data/damethods/EFEC1.ANMlec.xml

One or more scans have failed IRM. Review the data file for details.

Merged XIC, Period: 1 Experiment: 1

<table>
<thead>
<tr>
<th>Formula</th>
<th>Compound name</th>
<th>Mass</th>
<th>Peak RT (min)</th>
<th>Peak area</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10H7N5</td>
<td>--</td>
<td>197.07015</td>
<td>0.21</td>
<td>1.04766E7</td>
<td>--</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance (counts)</th>
<th>Ion Mass</th>
<th>Measured Mass</th>
<th>Error (mDa)</th>
<th>Error (ppm)</th>
<th>Ret. Time Error (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[MH]+</td>
<td>527505.31</td>
<td>198.07742</td>
<td>198.08080</td>
<td>3.37587</td>
<td>17.04</td>
<td>--</td>
</tr>
</tbody>
</table>
3-Methyl-1-(1-(4-phenylpyrimidin-2-yl)-1H-1,2,3-triazol-4-yl)butan-1-ol (4.13)

Sample Name: ZMZVII151 Sample Location: P1-D-68 Sample Id: ZMZVII151 Operator: Mingzhou Zhou
Data File Name: d:\PE_Solex Data\Projects\Chemists\011\Data\ZMZVII151-POSLOOP-120110.wiff Acq Time: January 12 2010, 03:46:17 PM
Method: D:\TOF_Data\dramethods\EFC1.ANMfetc.xml

One or more scans have failed IRM. Review the data file for details.
Methyl 2-((4-(benzyloxy)pyrimidin-2-yl)amino)-4-methylpentanoate (4.18)

<table>
<thead>
<tr>
<th>Formula</th>
<th>Compound name</th>
<th>Mass</th>
<th>Peak RT (min)</th>
<th>Peak area</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18H23N3O3</td>
<td>--</td>
<td>329.17394</td>
<td>0.22</td>
<td>3.56794E5</td>
<td>--</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Abundance (counts)</th>
<th>Ion Mass</th>
<th>Measured Mass</th>
<th>Error (mDa)</th>
<th>Error (ppm)</th>
<th>Ret. Time Error (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[M+H]⁺</td>
<td>81263.50</td>
<td>330.18122</td>
<td>330.18524</td>
<td>4.02229</td>
<td>12.16</td>
<td>--</td>
</tr>
</tbody>
</table>
Methyl 2-((4-(benzyloxy)-5-ethynylpyrimidin-2-yl)amino)-4-methylpentanoate (4.20)
2-(4-(4-(Benzyloxy)-2-((1-methoxy-4-methyl-1-oxopentan-2-yl)amino)pyrimidin-5-yl)-1H-1,2,3-triazol-1-yl)-3-methylbutanoic acid (4.21)
Appendix C: X-ray Crystallographic Data
**Crystal data**

C$_{13}$H$_{14}$N$_{3}$O

$M_r = 214.26$

Orthorhombic, $P2_12_12_1$

Hall symbol: P 2ac 2ab

$a = 5.7642$ (5) Å

$b = 9.0997$ (10) Å

$c = 21.551$ (2) Å

$V = 1130.40$ (19) Å$^3$

$Z = 4$

$F_{000} = 456$

$D_x = 1.259$ Mg m$^{-3}$

Cu Kα radiation

$\lambda = 1.54178$ Å

Cell parameters from 5949 reflections

$\theta = 4.1$–68.3°

$\mu = 0.65$ mm$^{-1}$

$T = 90$ K

Lath, colourless

$0.32 \times 0.16 \times 0.06$ mm

**Data collection**

Bruker Kappa Apex-II CCD area detector diffractometer

Radiation source: fine-focus sealed tube

Monochromator: graphite

$T = 90$ K

$\theta_{	ext{max}} = 68.3^\circ$

$\theta_{	ext{min}} = 4.1^\circ$

Absorption correction: multi-scan

SADABS (Sheldrick, 2002)

$T_{\text{min}} = 0.820$, $T_{\text{max}} = 0.962$

11346 measured reflections

2040 independent reflections

1944 reflections with $I > 2\sigma(I)$

$R_{int} = 0.002$

$\omega$ and $\phi$ scans

$h = -6$–6

$k = -10$–10

$l = -25$–24

**Refinement**

Refinement on $F^2$

H-atom parameters constrained

$w = 1/[\sigma^2(F_o^2) + (0.0383P)^2 + 0.1889P]$ where $P = (F_o^2 + 2F_c^2)/3$

$\Delta(\sigma)_{\text{max}} < 0.001$

$\Delta F_{\text{max}} = 0.12$ e Å$^{-3}$

$\Delta F_{\text{min}} = -0.16$ e Å$^{-3}$

Extinction correction: SHELXL,

$F_c^2 = kF_c[1 + 0.001x(F_c^2)^{1/2}/\sin(2\theta)]^{1/4}$

Extinction coefficient: 0.0070 (7)

Absolute structure: Flack (1983)

Primary atom site location: structure-invariant direct methods

Secondary atom site location: difference Fourier map

Flack parameter: 0.2 (2)
supplementary materials

Hydrogen site location: inferred from neighbouring sites

Special details

Geometry. All e.s.d.'s (except the e.s.d. in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell e.s.d.'s are taken into account individually in the estimation of e.s.d.'s in distances, angles and torsion angles; correlations between e.s.d.'s in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell e.s.d.'s is used for estimating e.s.d.'s involving l.s. planes.

Refinement. Refinement of $F^2$ against ALL reflections. The weighted $R$-factor wR and goodness of fit S are based on $F^2$, conventional $R$-factors $R$ are based on $F$, with $F$ set to zero for negative $F^2$. The threshold expression of $F^2 > 2\sigma(F^2)$ is used only for calculating $R$-factors(gt) etc. and is not relevant to the choice of reflections for refinement. $R$-factors based on $F^2$ are statistically about twice as large as those based on $F$, and $R$-factors based on ALL data will be even larger.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($U^2$)

<table>
<thead>
<tr>
<th></th>
<th>$x$</th>
<th>$y$</th>
<th>$z$</th>
<th>$U_{eq}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>0.40521 (16)</td>
<td>0.36064 (10)</td>
<td>0.49476 (4)</td>
<td>0.0219 (2)</td>
</tr>
<tr>
<td>N1</td>
<td>0.7009 (2)</td>
<td>0.49617 (13)</td>
<td>0.68917 (5)</td>
<td>0.0272 (3)</td>
</tr>
<tr>
<td>N2</td>
<td>0.14194 (19)</td>
<td>0.25715 (12)</td>
<td>0.66401 (5)</td>
<td>0.0203 (3)</td>
</tr>
<tr>
<td>C1</td>
<td>0.5096 (2)</td>
<td>0.41402 (13)</td>
<td>0.53857 (6)</td>
<td>0.0186 (3)</td>
</tr>
<tr>
<td>C2</td>
<td>0.4408 (2)</td>
<td>0.38695 (13)</td>
<td>0.60332 (6)</td>
<td>0.0189 (3)</td>
</tr>
<tr>
<td>C3</td>
<td>0.2510 (2)</td>
<td>0.29493 (13)</td>
<td>0.61284 (5)</td>
<td>0.0187 (3)</td>
</tr>
<tr>
<td>H3</td>
<td>0.1899</td>
<td>0.2522</td>
<td>0.5761</td>
<td>0.022*</td>
</tr>
<tr>
<td>C4</td>
<td>0.5803 (2)</td>
<td>0.44766 (13)</td>
<td>0.65145 (6)</td>
<td>0.0204 (3)</td>
</tr>
<tr>
<td>C5</td>
<td>0.1936 (3)</td>
<td>0.31522 (17)</td>
<td>0.72560 (6)</td>
<td>0.0263 (3)</td>
</tr>
<tr>
<td>H5A</td>
<td>0.2700</td>
<td>0.4110</td>
<td>0.7216</td>
<td>0.039*</td>
</tr>
<tr>
<td>H5B</td>
<td>0.2965</td>
<td>0.2471</td>
<td>0.7475</td>
<td>0.039*</td>
</tr>
<tr>
<td>H5C</td>
<td>0.0490</td>
<td>0.3266</td>
<td>0.7490</td>
<td>0.039*</td>
</tr>
<tr>
<td>C6</td>
<td>-0.0395 (3)</td>
<td>0.14517 (14)</td>
<td>0.66222 (6)</td>
<td>0.0237 (3)</td>
</tr>
<tr>
<td>H6A</td>
<td>-0.0706</td>
<td>0.1178</td>
<td>0.6190</td>
<td>0.036*</td>
</tr>
<tr>
<td>H6B</td>
<td>-0.1814</td>
<td>0.1843</td>
<td>0.6811</td>
<td>0.036*</td>
</tr>
<tr>
<td>H6C</td>
<td>0.0118</td>
<td>0.0583</td>
<td>0.6853</td>
<td>0.036*</td>
</tr>
<tr>
<td>C7</td>
<td>0.7208 (2)</td>
<td>0.51204 (15)</td>
<td>0.52952 (6)</td>
<td>0.0252 (3)</td>
</tr>
<tr>
<td>H7A</td>
<td>0.7112</td>
<td>0.5949</td>
<td>0.5592</td>
<td>0.030*</td>
</tr>
<tr>
<td>H7B</td>
<td>0.8614</td>
<td>0.4547</td>
<td>0.5400</td>
<td>0.030*</td>
</tr>
<tr>
<td>C8</td>
<td>0.7495 (2)</td>
<td>0.57392 (13)</td>
<td>0.46504 (6)</td>
<td>0.0202 (3)</td>
</tr>
<tr>
<td>C9</td>
<td>0.5858 (2)</td>
<td>0.67103 (14)</td>
<td>0.44319 (6)</td>
<td>0.0223 (3)</td>
</tr>
<tr>
<td>H9</td>
<td>0.4515</td>
<td>0.6935</td>
<td>0.4651</td>
<td>0.027*</td>
</tr>
<tr>
<td>C10</td>
<td>0.6160 (2)</td>
<td>0.73573 (14)</td>
<td>0.38349 (6)</td>
<td>0.0233 (3)</td>
</tr>
<tr>
<td>H10</td>
<td>0.5029</td>
<td>0.8019</td>
<td>0.3678</td>
<td>0.028*</td>
</tr>
<tr>
<td>C11</td>
<td>0.8115 (2)</td>
<td>0.70333 (15)</td>
<td>0.34879 (6)</td>
<td>0.0240 (3)</td>
</tr>
<tr>
<td>H11</td>
<td>0.8335</td>
<td>0.7477</td>
<td>0.3093</td>
<td>0.029*</td>
</tr>
<tr>
<td>C12</td>
<td>0.9748 (2)</td>
<td>0.60605 (15)</td>
<td>0.37176 (6)</td>
<td>0.0261 (3)</td>
</tr>
<tr>
<td>H12</td>
<td>1.1083</td>
<td>0.5831</td>
<td>0.3478</td>
<td>0.031*</td>
</tr>
<tr>
<td>C13</td>
<td>0.9447 (2)</td>
<td>0.54171 (15)</td>
<td>0.42977 (6)</td>
<td>0.0236 (3)</td>
</tr>
<tr>
<td>H13</td>
<td>1.0580</td>
<td>0.4755</td>
<td>0.4453</td>
<td>0.028*</td>
</tr>
</tbody>
</table>
supplementary materials

Atomic displacement parameters (Å²)

<table>
<thead>
<tr>
<th></th>
<th>U¹¹</th>
<th>U¹²</th>
<th>U¹³</th>
<th>U²²</th>
<th>U²³</th>
<th>U³³</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>0.0232 (5)</td>
<td>0.0244 (5)</td>
<td>0.0180 (5)</td>
<td>0.0027 (4)</td>
<td>0.0011 (4)</td>
<td>0.0005 (4)</td>
</tr>
<tr>
<td>N1</td>
<td>0.0300 (6)</td>
<td>0.0291 (6)</td>
<td>0.0226 (6)</td>
<td>0.0044 (5)</td>
<td>0.0035 (5)</td>
<td>0.0005 (5)</td>
</tr>
<tr>
<td>N2</td>
<td>0.0227 (6)</td>
<td>0.0206 (5)</td>
<td>0.0176 (5)</td>
<td>0.0007 (5)</td>
<td>0.0009 (4)</td>
<td>0.0008 (4)</td>
</tr>
<tr>
<td>C1</td>
<td>0.0190 (6)</td>
<td>0.0171 (6)</td>
<td>0.0197 (6)</td>
<td>0.0032 (5)</td>
<td>0.0019 (5)</td>
<td>0.0004 (5)</td>
</tr>
<tr>
<td>C2</td>
<td>0.0210 (7)</td>
<td>0.0171 (6)</td>
<td>0.0187 (6)</td>
<td>0.0017 (5)</td>
<td>0.0012 (5)</td>
<td>0.0005 (5)</td>
</tr>
<tr>
<td>C3</td>
<td>0.0220 (6)</td>
<td>0.0165 (6)</td>
<td>0.0175 (6)</td>
<td>0.0037 (5)</td>
<td>0.0009 (5)</td>
<td>0.0004 (5)</td>
</tr>
<tr>
<td>C4</td>
<td>0.0232 (6)</td>
<td>0.0179 (6)</td>
<td>0.0202 (6)</td>
<td>0.0015 (5)</td>
<td>0.0015 (6)</td>
<td>0.0030 (5)</td>
</tr>
<tr>
<td>C5</td>
<td>0.0238 (7)</td>
<td>0.0331 (8)</td>
<td>0.0169 (6)</td>
<td>0.0043 (6)</td>
<td>0.0012 (6)</td>
<td>0.0018 (5)</td>
</tr>
<tr>
<td>C6</td>
<td>0.0258 (7)</td>
<td>0.0219 (6)</td>
<td>0.0234 (7)</td>
<td>0.0038 (6)</td>
<td>0.0038 (5)</td>
<td>0.0009 (5)</td>
</tr>
<tr>
<td>C7</td>
<td>0.0251 (7)</td>
<td>0.0304 (7)</td>
<td>0.0200 (7)</td>
<td>0.0060 (6)</td>
<td>0.0032 (6)</td>
<td>0.0033 (5)</td>
</tr>
<tr>
<td>C8</td>
<td>0.0215 (7)</td>
<td>0.0196 (6)</td>
<td>0.0196 (6)</td>
<td>0.0044 (5)</td>
<td>0.0011 (5)</td>
<td>0.0010 (5)</td>
</tr>
<tr>
<td>C9</td>
<td>0.0222 (7)</td>
<td>0.0224 (7)</td>
<td>0.0225 (7)</td>
<td>0.0000 (6)</td>
<td>0.0026 (5)</td>
<td>0.0029 (5)</td>
</tr>
<tr>
<td>C10</td>
<td>0.0246 (6)</td>
<td>0.0198 (6)</td>
<td>0.0255 (7)</td>
<td>0.0014 (5)</td>
<td>0.0015 (5)</td>
<td>0.0012 (5)</td>
</tr>
<tr>
<td>C11</td>
<td>0.0259 (7)</td>
<td>0.0263 (7)</td>
<td>0.0197 (6)</td>
<td>0.0034 (6)</td>
<td>0.0013 (6)</td>
<td>0.0033 (5)</td>
</tr>
<tr>
<td>C12</td>
<td>0.0208 (7)</td>
<td>0.0323 (8)</td>
<td>0.0253 (7)</td>
<td>0.0006 (6)</td>
<td>0.0042 (6)</td>
<td>0.0021 (6)</td>
</tr>
<tr>
<td>C13</td>
<td>0.0196 (7)</td>
<td>0.0248 (7)</td>
<td>0.0265 (7)</td>
<td>0.0010 (6)</td>
<td>0.0016 (6)</td>
<td>0.0015 (5)</td>
</tr>
</tbody>
</table>

Geometric parameters (Å, °)

<table>
<thead>
<tr>
<th></th>
<th>O1—C2</th>
<th>1.2253 (15)</th>
<th>C6—H6C</th>
<th>0.9800</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1—C4</td>
<td>1.1573 (17)</td>
<td>C7—C8</td>
<td>1.5085 (17)</td>
<td></td>
</tr>
<tr>
<td>N2—C3</td>
<td>1.3151 (16)</td>
<td>C7—H7A</td>
<td>0.9900</td>
<td></td>
</tr>
<tr>
<td>N2—C5</td>
<td>1.4593 (16)</td>
<td>C7—H7B</td>
<td>0.9900</td>
<td></td>
</tr>
<tr>
<td>N2—C6</td>
<td>1.4607 (17)</td>
<td>C8—C13</td>
<td>1.3889 (19)</td>
<td></td>
</tr>
<tr>
<td>C1—C2</td>
<td>1.4653 (18)</td>
<td>C8—C9</td>
<td>1.3896 (19)</td>
<td></td>
</tr>
<tr>
<td>C1—C7</td>
<td>1.5225 (19)</td>
<td>C9—C10</td>
<td>1.3905 (18)</td>
<td></td>
</tr>
<tr>
<td>C2—C3</td>
<td>1.3930 (18)</td>
<td>C9—H9</td>
<td>0.9500</td>
<td></td>
</tr>
<tr>
<td>C2—C4</td>
<td>1.4238 (18)</td>
<td>C10—C11</td>
<td>1.3841 (19)</td>
<td></td>
</tr>
<tr>
<td>C3—C6</td>
<td>0.9500</td>
<td>C10—H10</td>
<td>0.9500</td>
<td></td>
</tr>
<tr>
<td>C5—H5A</td>
<td>0.9800</td>
<td>C11—C12</td>
<td>1.254 (2)</td>
<td></td>
</tr>
<tr>
<td>C5—H5B</td>
<td>0.9800</td>
<td>C11—H11</td>
<td>0.9500</td>
<td></td>
</tr>
<tr>
<td>C5—H5C</td>
<td>0.9800</td>
<td>C12—C13</td>
<td>1.3914 (19)</td>
<td></td>
</tr>
<tr>
<td>C6—H6A</td>
<td>0.9800</td>
<td>C12—H12</td>
<td>0.9500</td>
<td></td>
</tr>
<tr>
<td>C6—H6B</td>
<td>0.9800</td>
<td>C13—H13</td>
<td>0.9500</td>
<td></td>
</tr>
<tr>
<td>C3—N2—C5</td>
<td>124.78 (11)</td>
<td>C8—C7—C1</td>
<td>115.37 (11)</td>
<td></td>
</tr>
<tr>
<td>C3—N2—C6</td>
<td>120.16 (10)</td>
<td>C8—C7—H7A</td>
<td>108.4</td>
<td></td>
</tr>
<tr>
<td>C5—N2—C6</td>
<td>115.02 (10)</td>
<td>C1—C7—H7A</td>
<td>108.4</td>
<td></td>
</tr>
<tr>
<td>O1—C1—C2</td>
<td>122.40 (12)</td>
<td>C8—C7—H7B</td>
<td>108.4</td>
<td></td>
</tr>
<tr>
<td>O1—C1—C7</td>
<td>121.48 (12)</td>
<td>C1—C7—H7B</td>
<td>108.4</td>
<td></td>
</tr>
<tr>
<td>C2—C1—C7</td>
<td>116.12 (11)</td>
<td>H7A—C7—H7B</td>
<td>107.5</td>
<td></td>
</tr>
<tr>
<td>C3—C2—C4</td>
<td>124.71 (11)</td>
<td>C13—C8—C9</td>
<td>118.91 (12)</td>
<td></td>
</tr>
<tr>
<td>C3—C2—C1</td>
<td>116.95 (11)</td>
<td>C13—C8—C7</td>
<td>120.96 (12)</td>
<td></td>
</tr>
<tr>
<td>C4—C2—C1</td>
<td>118.20 (11)</td>
<td>C9—C8—C7</td>
<td>120.04 (12)</td>
<td></td>
</tr>
<tr>
<td>N2—C3—C2</td>
<td>131.01 (12)</td>
<td>C8—C9—C10</td>
<td>120.89 (15)</td>
<td></td>
</tr>
</tbody>
</table>
## supplementary materials

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
<th>Bond</th>
<th>Distance (Å)</th>
<th>Bond</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2—C3—H3</td>
<td>114.5</td>
<td>C8—C9—H9</td>
<td>119.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2—C3—H3</td>
<td>114.5</td>
<td>C10—C9—H9</td>
<td>119.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1—C4—C2</td>
<td>177.41 (14)</td>
<td>C11—C10—C9</td>
<td>119.77 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2—C5—H5A</td>
<td>109.5</td>
<td>C11—C10—H10</td>
<td>120.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2—C5—H5B</td>
<td>109.5</td>
<td>C9—C10—H10</td>
<td>120.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H5A—C5—H5B</td>
<td>109.5</td>
<td>C12—C11—C10</td>
<td>119.78 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2—C5—H5C</td>
<td>109.5</td>
<td>C12—C11—H11</td>
<td>120.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H5A—C5—H5C</td>
<td>109.5</td>
<td>C10—C11—H11</td>
<td>120.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H5B—C5—H5C</td>
<td>109.5</td>
<td>C11—C12—C13</td>
<td>120.37 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2—C6—H6A</td>
<td>109.5</td>
<td>C11—C12—H12</td>
<td>119.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2—C6—H6B</td>
<td>109.5</td>
<td>C13—C12—H12</td>
<td>119.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H6A—C6—H6B</td>
<td>109.5</td>
<td>C8—C13—C12</td>
<td>120.27 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2—C6—H6C</td>
<td>109.5</td>
<td>C8—C13—H13</td>
<td>119.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H6A—C6—H6C</td>
<td>109.5</td>
<td>C12—C13—H13</td>
<td>119.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H6B—C6—H6C</td>
<td>109.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O1—C1—C2—C3</td>
<td>0.91 (18)</td>
<td>C1—C7—C8—C13</td>
<td>−119.03 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7—C1—C2—C3</td>
<td>−178.65 (11)</td>
<td>C1—C7—C8—C9</td>
<td>64.54 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O1—C1—C2—C4</td>
<td>176.78 (12)</td>
<td>C13—C8—C9—C10</td>
<td>−0.21 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7—C1—C2—C4</td>
<td>−2.78 (17)</td>
<td>C7—C8—C9—C10</td>
<td>176.30 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5—N2—C3—C2</td>
<td>4.0 (2)</td>
<td>C8—C9—C10—C11</td>
<td>0.0 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6—N2—C3—C2</td>
<td>−173.41 (12)</td>
<td>C9—C10—C11—C12</td>
<td>0.4 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4—C2—C3—N2</td>
<td>8.7 (2)</td>
<td>C10—C11—C12—C13</td>
<td>−0.6 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1—C2—C3—N2</td>
<td>−175.68 (13)</td>
<td>C9—C8—C13—C12</td>
<td>0.04 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O1—C1—C7—C8</td>
<td>17.14 (19)</td>
<td>C7—C8—C13—C12</td>
<td>−176.44 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2—C1—C7—C8</td>
<td>−163.30 (11)</td>
<td>C11—C12—C13—C8</td>
<td>0.4 (2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
supplementary materials

Crystal data

C_{31}H_{32}N_{5}

$M_r = 489.56$

Monoclinic, $P2_1/n$

Hall symbol: -P 2yn

$a = 9.3452$ (15) Å

$b = 22.046$ (3) Å

$c = 12.0229$ (16) Å

$\beta = 102.148$ (9)°

$V = 2421.5$ (6) Å$^3$

$Z = 4$

$F_{000} = 1024$

$D_x = 1.343$ Mg m$^{-3}$

Mo Kα radiation

$\lambda = 0.71073$ Å

Cell parameters from 5308 reflections

$\theta = 2.5$ to $27.1^\circ$

$\mu = 0.08$ mm$^{-1}$

$T = 90.0$ (5) K

Plate fragment, ?

$0.30 \times 0.27 \times 0.07$ mm

Data collection

KappaCCD (with Oxford Cryosystem)
diffractometer

Radiation source: fine-focus sealed tube

Monochromator: graphite

$T = 90.0(5)$ K

$\omega$ and $\phi$ scans

Absorption correction: none

$\kappa = \text{28 to 28}$

39528 measured reflections

5294 independent reflections

Refinement

Refinement on $F^2$

Least-squares matrix: full

$R[F^2 > 2\sigma(F^2)] = 0.047$

$wR(F^2) = 0.110$

$S = 1.07$

5294 reflections

415 parameters

32 restraints

Secondary atom site location: difference Fourier map

Hydrogen site location: inferred from neighbouring sites

H-atom parameters constrained

$w = 1/\sigma^2(F^2) + (0.0427P)^2 + 0.7055P$

where $P = (F^2 + 2F_c^2)/3$

$\Delta\rho_{max} = 0.22$ e Å$^{-3}$

$\Delta\rho_{min} = -0.20$ e Å$^{-3}$

Extinction correction: SHELXL

Fc = kFc[1+0.001xFc$^2$/3$^{-\sin(2\theta)}$]$

sup-1

239
supplementary materials

Primary atom site location: structure-invariant direct methods
Extinction coefficient: 0.0059 (8)

Special details

Geometry. All e.s.d.'s (except the e.s.d. in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell e.s.d.'s are taken into account individually in the estimation of e.s.d.'s in distances, angles and torsion angles; correlations between e.s.d.'s in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell e.s.d.'s is used for estimating e.s.d.'s involving l.s. planes.

Refinement. Refinement of $F^2$ against ALL reflections. The weighted $R$-factor wR and goodness of fit $S$ are based on $F^2$, conventional $R$-factors $R$ are based on $F$, with $F$ set to zero for negative $F^2$. The threshold expression of $F^2 > 2\sigma(F^2)$ is used only for calculating $R$-factors(gt) etc. and is not relevant to the choice of reflections for refinement. $R$-factors based on $F^2$ are statistically about twice as large as those based on $F$, and $R$-factors based on ALL data will be even larger.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($U_{eq}$)

<table>
<thead>
<tr>
<th></th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U_{eq}x^2+U_{eq}y^2+U_{eq}z^2</th>
<th>Occ. (&lt;1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>0.54827 (14)</td>
<td>0.73933 (6)</td>
<td>0.34009 (10)</td>
<td>0.0259 (3)</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>0.48108 (14)</td>
<td>0.84314 (6)</td>
<td>0.35027 (10)</td>
<td>0.0278 (3)</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>0.14753 (13)</td>
<td>0.79772 (6)</td>
<td>0.50236 (10)</td>
<td>0.0267 (3)</td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td>-0.27460 (16)</td>
<td>0.71271 (7)</td>
<td>0.61142 (12)</td>
<td>0.0394 (4)</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>0.56929 (17)</td>
<td>0.79871 (7)</td>
<td>0.32756 (12)</td>
<td>0.0250 (3)</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>0.43043 (17)</td>
<td>0.72141 (7)</td>
<td>0.37983 (12)</td>
<td>0.0260 (3)</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>0.33095 (16)</td>
<td>0.76415 (7)</td>
<td>0.40519 (12)</td>
<td>0.0258 (3)</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>0.36453 (17)</td>
<td>0.82480 (7)</td>
<td>0.38793 (13)</td>
<td>0.0283 (3)</td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>0.2994</td>
<td>0.8550</td>
<td>0.4043</td>
<td>0.034*</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>0.70276 (16)</td>
<td>0.81091 (7)</td>
<td>0.28684 (12)</td>
<td>0.0256 (3)</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>0.79186 (16)</td>
<td>0.77310 (7)</td>
<td>0.25196 (12)</td>
<td>0.0279 (3)</td>
<td></td>
</tr>
<tr>
<td>H6</td>
<td>0.7659</td>
<td>0.7315</td>
<td>0.2530</td>
<td>0.033*</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>0.91775 (17)</td>
<td>0.78961 (8)</td>
<td>0.21578 (13)</td>
<td>0.0318 (4)</td>
<td></td>
</tr>
<tr>
<td>H7</td>
<td>0.9763</td>
<td>0.7595</td>
<td>0.1905</td>
<td>0.038*</td>
<td></td>
</tr>
<tr>
<td>C8</td>
<td>0.95822 (18)</td>
<td>0.85005 (8)</td>
<td>0.21646 (13)</td>
<td>0.0357 (4)</td>
<td></td>
</tr>
<tr>
<td>H8</td>
<td>1.0456</td>
<td>0.8614</td>
<td>0.1933</td>
<td>0.043*</td>
<td></td>
</tr>
<tr>
<td>C9</td>
<td>0.8708 (2)</td>
<td>0.89399 (8)</td>
<td>0.25099 (14)</td>
<td>0.0387 (4)</td>
<td></td>
</tr>
<tr>
<td>H9</td>
<td>0.8985</td>
<td>0.9355</td>
<td>0.2515</td>
<td>0.046*</td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>0.74285 (19)</td>
<td>0.87771 (7)</td>
<td>0.28480 (13)</td>
<td>0.0333 (4)</td>
<td></td>
</tr>
<tr>
<td>H10</td>
<td>0.6823</td>
<td>0.9082</td>
<td>0.3067</td>
<td>0.040*</td>
<td></td>
</tr>
<tr>
<td>C11</td>
<td>0.42300 (19)</td>
<td>0.65354 (7)</td>
<td>0.39424 (13)</td>
<td>0.0303 (4)</td>
<td></td>
</tr>
<tr>
<td>C11A</td>
<td>0.3432</td>
<td>0.6436</td>
<td>0.4338</td>
<td>0.036*</td>
<td></td>
</tr>
<tr>
<td>C11B</td>
<td>0.5162</td>
<td>0.6389</td>
<td>0.4419</td>
<td>0.036*</td>
<td></td>
</tr>
<tr>
<td>C12</td>
<td>0.39588 (16)</td>
<td>0.62172 (6)</td>
<td>0.28001 (12)</td>
<td>0.0241 (3)</td>
<td></td>
</tr>
<tr>
<td>C13</td>
<td>0.49899 (17)</td>
<td>0.58267 (7)</td>
<td>0.25219 (13)</td>
<td>0.0289 (4)</td>
<td></td>
</tr>
<tr>
<td>H13</td>
<td>0.5872</td>
<td>0.5751</td>
<td>0.3062</td>
<td>0.035*</td>
<td></td>
</tr>
<tr>
<td>C14</td>
<td>0.4758 (2)</td>
<td>0.55434 (7)</td>
<td>0.14685 (14)</td>
<td>0.0363 (4)</td>
<td></td>
</tr>
<tr>
<td>H14</td>
<td>0.5476</td>
<td>0.5276</td>
<td>0.1292</td>
<td>0.044*</td>
<td></td>
</tr>
<tr>
<td>C15</td>
<td>0.3484 (2)</td>
<td>0.56508 (8)</td>
<td>0.06776 (14)</td>
<td>0.0379 (4)</td>
<td></td>
</tr>
<tr>
<td>H15</td>
<td>0.3320</td>
<td>0.5457</td>
<td>-0.0044</td>
<td>0.045*</td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>0.24463 (18)</td>
<td>0.60416 (8)</td>
<td>0.09382 (14)</td>
<td>0.0345 (4)</td>
<td></td>
</tr>
</tbody>
</table>
### Supplementary Materials

<table>
<thead>
<tr>
<th>Value</th>
<th>Value</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>241</td>
<td>0.1571</td>
<td>0.6118</td>
<td>0.0392</td>
</tr>
<tr>
<td>0.26739 (17)</td>
<td>0.63225 (7)</td>
<td>0.19938 (13)</td>
<td>0.0286 (3)</td>
</tr>
<tr>
<td>0.1955</td>
<td>0.6590</td>
<td>0.2168</td>
<td>0.034*</td>
</tr>
<tr>
<td>0.19644 (17)</td>
<td>0.75169 (7)</td>
<td>0.448589 (12)</td>
<td>0.0282 (3)</td>
</tr>
<tr>
<td>0.02700 (17)</td>
<td>0.78750 (7)</td>
<td>0.5413 (13)</td>
<td>0.0286 (3)</td>
</tr>
<tr>
<td>-0.0016</td>
<td>0.8194</td>
<td>0.5798</td>
<td>0.034*</td>
</tr>
<tr>
<td>-0.004401 (17)</td>
<td>0.73234 (8)</td>
<td>0.52965 (13)</td>
<td>0.0313 (4)</td>
</tr>
<tr>
<td>-0.17213 (18)</td>
<td>0.72191 (8)</td>
<td>0.55736 (13)</td>
<td>0.0302 (4)</td>
</tr>
<tr>
<td>0.1182 (3)</td>
<td>0.70051 (11)</td>
<td>0.4174 (2)</td>
<td>0.0241 (5)</td>
</tr>
<tr>
<td>-0.0007 (3)</td>
<td>0.69134 (12)</td>
<td>0.4581 (2)</td>
<td>0.0244 (6)</td>
</tr>
<tr>
<td>-0.0904 (3)</td>
<td>0.63885 (10)</td>
<td>0.4286 (2)</td>
<td>0.0301 (6)</td>
</tr>
<tr>
<td>-0.1945</td>
<td>0.6454</td>
<td>0.4031</td>
<td>0.036*</td>
</tr>
<tr>
<td>-0.0824</td>
<td>0.6099</td>
<td>0.4993</td>
<td>0.036*</td>
</tr>
<tr>
<td>-0.0501 (3)</td>
<td>0.59277 (11)</td>
<td>0.3387 (3)</td>
<td>0.0370 (6)</td>
</tr>
<tr>
<td>-0.1348 (3)</td>
<td>0.59468 (11)</td>
<td>0.2301 (3)</td>
<td>0.0307 (7)</td>
</tr>
<tr>
<td>-0.2104</td>
<td>0.6241</td>
<td>0.2121</td>
<td>0.032*</td>
</tr>
<tr>
<td>-0.1119 (3)</td>
<td>0.55393 (13)</td>
<td>0.1450 (3)</td>
<td>0.0312 (6)</td>
</tr>
<tr>
<td>-0.1712</td>
<td>0.5560</td>
<td>0.0705</td>
<td>0.037*</td>
</tr>
<tr>
<td>-0.0049 (6)</td>
<td>0.5121 (2)</td>
<td>0.1705 (4)</td>
<td>0.0349 (12)</td>
</tr>
<tr>
<td>0.0068</td>
<td>0.4835</td>
<td>0.1140</td>
<td>0.042*</td>
</tr>
<tr>
<td>0.0924 (7)</td>
<td>0.5093 (3)</td>
<td>0.2806 (5)</td>
<td>0.0250 (11)</td>
</tr>
<tr>
<td>0.2083 (5)</td>
<td>0.46869 (18)</td>
<td>0.3044 (4)</td>
<td>0.0319 (9)</td>
</tr>
<tr>
<td>0.2228</td>
<td>0.4408</td>
<td>0.2475</td>
<td>0.038*</td>
</tr>
<tr>
<td>0.3016 (3)</td>
<td>0.48812 (12)</td>
<td>0.4081 (2)</td>
<td>0.0373 (7)</td>
</tr>
<tr>
<td>0.3825</td>
<td>0.4410</td>
<td>0.4226</td>
<td>0.045*</td>
</tr>
<tr>
<td>0.2770 (3)</td>
<td>0.50802 (12)</td>
<td>0.4933 (2)</td>
<td>0.0364 (7)</td>
</tr>
<tr>
<td>0.3410</td>
<td>0.5072</td>
<td>0.5660</td>
<td>0.044*</td>
</tr>
<tr>
<td>0.1621 (3)</td>
<td>0.54815 (14)</td>
<td>0.4734 (2)</td>
<td>0.0303 (7)</td>
</tr>
<tr>
<td>0.1465</td>
<td>0.5744</td>
<td>0.5324</td>
<td>0.036*</td>
</tr>
<tr>
<td>0.0669 (3)</td>
<td>0.55057 (11)</td>
<td>0.3653 (2)</td>
<td>0.0247 (6)</td>
</tr>
<tr>
<td>0.1706 (6)</td>
<td>0.6893 (2)</td>
<td>0.4617 (5)</td>
<td>0.0231 (12)</td>
</tr>
<tr>
<td>0.0222 (7)</td>
<td>0.6775 (3)</td>
<td>0.5032 (5)</td>
<td>0.0240 (13)</td>
</tr>
<tr>
<td>0.0140 (6)</td>
<td>0.6116 (2)</td>
<td>0.5200 (4)</td>
<td>0.0303 (14)</td>
</tr>
<tr>
<td>0.0483</td>
<td>0.6013</td>
<td>0.6014</td>
<td>0.036*</td>
</tr>
<tr>
<td>-0.0939</td>
<td>0.6074</td>
<td>0.5019</td>
<td>0.036*</td>
</tr>
<tr>
<td>0.0772 (7)</td>
<td>0.5562 (2)</td>
<td>0.4590 (5)</td>
<td>0.0274 (12)</td>
</tr>
<tr>
<td>0.1932 (10)</td>
<td>0.5297 (3)</td>
<td>0.4977 (7)</td>
<td>0.032 (2)*</td>
</tr>
<tr>
<td>0.2359</td>
<td>0.5346</td>
<td>0.5761</td>
<td>0.038*</td>
</tr>
<tr>
<td>0.2513 (7)</td>
<td>0.4857 (3)</td>
<td>0.4360 (6)</td>
<td>0.0336 (15)*</td>
</tr>
<tr>
<td>0.3338</td>
<td>0.4625</td>
<td>0.4720</td>
<td>0.040*</td>
</tr>
<tr>
<td>0.1898 (13)</td>
<td>0.4761 (6)</td>
<td>0.3248 (9)</td>
<td>0.044 (4)*</td>
</tr>
<tr>
<td>0.2276</td>
<td>0.4452</td>
<td>0.2841</td>
<td>0.052*</td>
</tr>
<tr>
<td>0.0657 (16)</td>
<td>0.5129 (7)</td>
<td>0.2666 (11)</td>
<td>0.021 (3)*</td>
</tr>
<tr>
<td>0.0050 (12)</td>
<td>0.5045 (5)</td>
<td>0.1529 (8)</td>
<td>0.016 (2)*</td>
</tr>
<tr>
<td>0.0391</td>
<td>0.4730</td>
<td>0.1113</td>
<td>0.019*</td>
</tr>
<tr>
<td>-0.1067 (7)</td>
<td>0.5428 (3)</td>
<td>0.0998 (6)</td>
<td>0.0320 (17)*</td>
</tr>
<tr>
<td>-0.1903</td>
<td>0.5372</td>
<td>0.0216</td>
<td>0.038*</td>
</tr>
<tr>
<td>-0.1554 (7)</td>
<td>0.5899 (3)</td>
<td>0.1616 (6)</td>
<td>0.0323 (14)*</td>
</tr>
<tr>
<td>-0.2286</td>
<td>0.6172</td>
<td>0.1240</td>
<td>0.039*</td>
</tr>
</tbody>
</table>
242


supplementary materials

Geometric parameters (Å, °)

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
<th>Bond</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1—C1</td>
<td>1.3369 (19)</td>
<td>C22—C23</td>
<td>1.510 (3)</td>
</tr>
<tr>
<td>N1—C2</td>
<td>1.3485 (18)</td>
<td>C23—C24</td>
<td>1.517 (3)</td>
</tr>
<tr>
<td>N2—C4</td>
<td>1.3275 (19)</td>
<td>C23—H23A</td>
<td>0.9900</td>
</tr>
<tr>
<td>N2—C1</td>
<td>1.3447 (19)</td>
<td>C23—H23B</td>
<td>0.9900</td>
</tr>
<tr>
<td>N3—C19</td>
<td>1.3303 (19)</td>
<td>C24—C25</td>
<td>1.377 (4)</td>
</tr>
<tr>
<td>N3—C18</td>
<td>1.3346 (19)</td>
<td>C24—C33</td>
<td>1.420 (4)</td>
</tr>
<tr>
<td>N5—C21</td>
<td>1.149 (2)</td>
<td>C25—C26</td>
<td>1.412 (4)</td>
</tr>
<tr>
<td>C1—C5</td>
<td>1.488 (2)</td>
<td>C25—H25</td>
<td>0.9500</td>
</tr>
<tr>
<td>C2—C3</td>
<td>1.402 (2)</td>
<td>C26—C27</td>
<td>1.347 (6)</td>
</tr>
<tr>
<td>C2—C11</td>
<td>1.509 (2)</td>
<td>C26—H26</td>
<td>0.9500</td>
</tr>
<tr>
<td>C3—C4</td>
<td>1.399 (2)</td>
<td>C27—C28</td>
<td>1.441 (7)</td>
</tr>
<tr>
<td>C3—C18</td>
<td>1.485 (2)</td>
<td>C27—H27</td>
<td>0.9500</td>
</tr>
<tr>
<td>C4—H4</td>
<td>0.9500</td>
<td>C28—C29</td>
<td>1.388 (7)</td>
</tr>
<tr>
<td>C5—C10</td>
<td>1.393 (2)</td>
<td>C28—H28</td>
<td>0.9500</td>
</tr>
<tr>
<td>C5—C6</td>
<td>1.396 (2)</td>
<td>C29—C30</td>
<td>1.363 (5)</td>
</tr>
<tr>
<td>C6—C7</td>
<td>1.386 (2)</td>
<td>C29—H29</td>
<td>0.9500</td>
</tr>
<tr>
<td>C6—H6</td>
<td>0.9500</td>
<td>C30—C31</td>
<td>1.406 (4)</td>
</tr>
<tr>
<td>C7—C8</td>
<td>1.385 (2)</td>
<td>C30—H30</td>
<td>0.9500</td>
</tr>
<tr>
<td>C7—H7</td>
<td>0.9500</td>
<td>C31—C32</td>
<td>1.373 (4)</td>
</tr>
<tr>
<td>C8—C9</td>
<td>1.386 (2)</td>
<td>C31—H31</td>
<td>0.9500</td>
</tr>
<tr>
<td>C8—H8</td>
<td>0.9500</td>
<td>C32—C33</td>
<td>1.413 (4)</td>
</tr>
<tr>
<td>C9—C10</td>
<td>1.388 (2)</td>
<td>C32—H32</td>
<td>0.9500</td>
</tr>
<tr>
<td>C9—H9</td>
<td>0.9500</td>
<td>N4A—C22A</td>
<td>1.332 (7)</td>
</tr>
<tr>
<td>C10—H10</td>
<td>0.9500</td>
<td>C22A—C23A</td>
<td>1.520 (7)</td>
</tr>
<tr>
<td>C11—C12</td>
<td>1.515 (2)</td>
<td>C23A—C24A</td>
<td>1.507 (7)</td>
</tr>
<tr>
<td>C11—H11A</td>
<td>0.9900</td>
<td>C23A—H23C</td>
<td>0.9900</td>
</tr>
<tr>
<td>C11—H11B</td>
<td>0.9900</td>
<td>C23A—H23D</td>
<td>0.9900</td>
</tr>
<tr>
<td>C12—C13</td>
<td>1.385 (2)</td>
<td>C24A—C25A</td>
<td>1.375 (8)</td>
</tr>
<tr>
<td>C12—C17</td>
<td>1.395 (2)</td>
<td>C24A—C33A</td>
<td>1.419 (7)</td>
</tr>
<tr>
<td>C13—C14</td>
<td>1.388 (2)</td>
<td>C25A—C26A</td>
<td>1.398 (9)</td>
</tr>
<tr>
<td>C13—H13</td>
<td>0.9500</td>
<td>C25A—H25A</td>
<td>0.9500</td>
</tr>
<tr>
<td>C14—C15</td>
<td>1.379 (3)</td>
<td>C26A—C27A</td>
<td>1.356 (12)</td>
</tr>
<tr>
<td>C14—H14</td>
<td>0.9500</td>
<td>C26A—H26A</td>
<td>0.9500</td>
</tr>
<tr>
<td>C15—C16</td>
<td>1.381 (2)</td>
<td>C27A—C28A</td>
<td>1.466 (14)</td>
</tr>
<tr>
<td>C15—H15</td>
<td>0.9500</td>
<td>C27A—H27A</td>
<td>0.9500</td>
</tr>
<tr>
<td>C16—C17</td>
<td>1.389 (2)</td>
<td>C28A—C29A</td>
<td>1.377 (13)</td>
</tr>
<tr>
<td>C16—H16</td>
<td>0.9500</td>
<td>C28A—C33A</td>
<td>1.432 (11)</td>
</tr>
<tr>
<td>C17—H17</td>
<td>0.9500</td>
<td>C29A—C30A</td>
<td>1.389 (11)</td>
</tr>
<tr>
<td>C18—N4</td>
<td>1.354 (3)</td>
<td>C29A—H29A</td>
<td>0.9500</td>
</tr>
<tr>
<td>C18—N4A</td>
<td>1.410 (5)</td>
<td>C30A—C31A</td>
<td>1.408 (8)</td>
</tr>
<tr>
<td>C19—C20</td>
<td>1.378 (2)</td>
<td>C30A—H30A</td>
<td>0.9500</td>
</tr>
<tr>
<td>C19—H19</td>
<td>0.9500</td>
<td>C31A—C32A</td>
<td>1.383 (8)</td>
</tr>
<tr>
<td>C20—C22</td>
<td>1.358 (3)</td>
<td>C31A—H31A</td>
<td>0.9500</td>
</tr>
<tr>
<td>C20—C21</td>
<td>1.437 (2)</td>
<td>C32A—C33A</td>
<td>1.397 (9)</td>
</tr>
<tr>
<td>C20—C22A</td>
<td>1.579 (6)</td>
<td>C32A—H32A</td>
<td>0.9500</td>
</tr>
<tr>
<td>N4—C22</td>
<td>1.337 (3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
supplementary materials

C1—N1—C2  118.50 (13)  C22—C23—H23A  107.9
C4—N2—C1  115.43 (13)  C24—C23—H23A  107.9
C19—N3—C18  116.48 (13)  C22—C23—H23B  107.9
N1—C1—N2  125.41 (13)  C24—C23—H23B  107.9
N1—C1—C5  117.03 (13)  H23A—C23—H23B  107.2
N2—C1—C5  117.55 (13)  C25—C24—C33  119.7 (2)
N1—C2—C3  120.56 (13)  C25—C24—C33  118.5 (2)
N1—C2—C11  113.07 (13)  C33—C24—C33  121.7 (2)
C3—C2—C11  126.36 (13)  C24—C25—C26  121.5 (2)
C4—C3—C2  115.50 (13)  C24—C25—H25  119.2
C4—C3—C18  117.52 (13)  C26—C25—H25  119.2
C2—C3—C18  126.97 (14)  C27—C26—C25  119.3 (3)
N2—C4—C3  124.58 (14)  C27—C26—H26  120.4
N2—C4—H4  117.7  C25—C26—H26  120.4
C3—C4—H4  117.7  C26—C27—C28  122.2 (4)
C10—C5—C6  118.73 (14)  C26—C27—H27  118.9
C10—C5—C1  120.82 (14)  C28—C27—H27  118.9
C6—C5—C1  120.44 (13)  C29—C28—C33  120.4 (5)
C7—C6—C5  120.79 (15)  C29—C28—C27  122.1 (4)
C7—C6—H6  119.6  C33—C28—C27  117.5 (5)
C5—C6—H6  119.6  C30—C29—C28  121.0 (5)
C8—C7—C6  119.97 (15)  C30—C29—H29  119.5
C8—C7—H7  120.0  C28—C29—H29  119.5
C7—C8—C9  120.0  C29—C30—C31  119.3 (3)
C7—C8—H8  119.79 (15)  C29—C30—H30  120.3
C9—C8—H8  120.1  C31—C30—H30  120.3
C8—C9—C10  120.1  C32—C31—C30  121.2 (3)
C8—C9—H9  120.34 (16)  C32—C31—H31  119.4
C10—C9—H9  119.8  C30—C31—H31  119.4
C9—C10—C5  120.34 (15)  C31—C32—C33  120.2 (3)
C9—C10—H10  119.8  C31—C32—H32  119.9
C5—C10—H10  119.8  C32—C33—C24  122.5 (2)
C2—C11—C12  119.8  C32—C33—C28  117.8 (4)
C2—C11—H11A  119.8  C24—C33—C28  119.7 (4)
C2—C11—H11B  109.4  C22A—N4A—C18  114.1 (4)
C2—C11—H11B  109.4  N4A—C22A—C23A  118.3 (5)
C2—C11—H11B  109.4  N4A—C22A—C20  118.6 (4)
H11A—C11—H11B  118.53 (14)  C23A—C22A—C20  123.1 (4)
C13—C12—C17  120.97 (14)  C24A—C23A—C22A  115.5 (4)
C13—C12—C11  120.48 (14)  C24A—C23A—H23C  108.4
C17—C12—C11  121.16 (15)  C22A—C23A—H23C  108.4
C12—C13—H13  119.4  C22A—C23A—H23D  108.4
C14—C13—H13  119.4  H23C—C23A—H23D  107.5
C15—C14—C13  119.88 (16)  C25A—C24A—C33A  118.3 (6)
C15—C14—H14  120.1  C25A—C24A—C23A  121.5 (6)
C13—C14—H14  120.1  C33A—C24A—C23A  120.2 (5)
C14—C15—C16  119.75 (15)  C24A—C25A—C26A  123.0 (7)
### supplementary materials

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C14</td>
<td>C15</td>
<td>H15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>C15</td>
<td>H15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15</td>
<td>C16</td>
<td>C17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15</td>
<td>C16</td>
<td>H16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17</td>
<td>C16</td>
<td>H16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>C17</td>
<td>C12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>C17</td>
<td>H17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12</td>
<td>C17</td>
<td>H17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>C18</td>
<td>N4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>C18</td>
<td>N4A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>C18</td>
<td>C3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>C18</td>
<td>C3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4A</td>
<td>N18</td>
<td>C3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>C19</td>
<td>C20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>C19</td>
<td>H19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20</td>
<td>C19</td>
<td>H19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C22</td>
<td>C20</td>
<td>C19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C22</td>
<td>C20</td>
<td>C21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C19</td>
<td>C20</td>
<td>C21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C19</td>
<td>C20</td>
<td>C22A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C21</td>
<td>C20</td>
<td>C22A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td>C21</td>
<td>C20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>N4</td>
<td>C18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>C22</td>
<td>C20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>C22</td>
<td>C23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20</td>
<td>C22</td>
<td>C23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C22</td>
<td>C23</td>
<td>C24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>N1</td>
<td>C1</td>
<td>N2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>N1</td>
<td>C1</td>
<td>C5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>N2</td>
<td>C1</td>
<td>N1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>N2</td>
<td>C1</td>
<td>C5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>N1</td>
<td>C2</td>
<td>C3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>N1</td>
<td>C2</td>
<td>C11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C11</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>C2</td>
<td>C3</td>
<td>C18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C11</td>
<td>C2</td>
<td>C3</td>
<td>C18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>N2</td>
<td>C4</td>
<td>C3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td>N2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18</td>
<td>C3</td>
<td>C4</td>
<td>N2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>C1</td>
<td>C5</td>
<td>C10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>C1</td>
<td>C5</td>
<td>C10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>C1</td>
<td>C5</td>
<td>C6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>C1</td>
<td>C5</td>
<td>C6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>C5</td>
<td>C6</td>
<td>C7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>C5</td>
<td>C6</td>
<td>C7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>C6</td>
<td>C7</td>
<td>C8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>C7</td>
<td>C8</td>
<td>C9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>C8</td>
<td>C9</td>
<td>C10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
supplementary materials

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8---C9---C10---C5</td>
<td>1.5 (3)</td>
<td>C27---C28---C33---C32</td>
<td>−179.9 (4)</td>
</tr>
<tr>
<td>C6---C5---C10---C9</td>
<td>−1.4 (2)</td>
<td>C29---C28---C33---C24</td>
<td>178.5 (4)</td>
</tr>
<tr>
<td>C1---C5---C10---C9</td>
<td>177.40 (14)</td>
<td>C27---C28---C33---C24</td>
<td>−0.7 (7)</td>
</tr>
<tr>
<td>N1---C2---C11---C12</td>
<td>−67.24 (17)</td>
<td>N3---C18---N4A---C22A</td>
<td>22.1 (6)</td>
</tr>
<tr>
<td>C3---C2---C11---C12</td>
<td>113.86 (16)</td>
<td>N4---C18---N4A---C22A</td>
<td>−70.7 (6)</td>
</tr>
<tr>
<td>C2---C11---C12---C13</td>
<td>116.18 (16)</td>
<td>C3---C18---N4A---C22A</td>
<td>178.5 (4)</td>
</tr>
<tr>
<td>C2---C11---C12---C17</td>
<td>−62.29 (13)</td>
<td>C18---N4A---C22A---C23A</td>
<td>179.7 (5)</td>
</tr>
<tr>
<td>C17---C12---C13---C14</td>
<td>0.2 (2)</td>
<td>C18---N4A---C22A---C20</td>
<td>12.7 (7)</td>
</tr>
<tr>
<td>C11---C12---C13---C14</td>
<td>−178.75 (14)</td>
<td>C22---C20---C22A---N4A</td>
<td>82.7 (6)</td>
</tr>
<tr>
<td>C12---C13---C14---C15</td>
<td>0.2 (2)</td>
<td>C19---C20---C22A---N4A</td>
<td>−19.8 (6)</td>
</tr>
<tr>
<td>C13---C14---C15---C16</td>
<td>0.2 (2)</td>
<td>C21---C20---C22A---N4A</td>
<td>−179.5 (4)</td>
</tr>
<tr>
<td>C14---C15---C16---C17</td>
<td>−0.5 (2)</td>
<td>C22---C20---C22A---C23A</td>
<td>−95.7 (8)</td>
</tr>
<tr>
<td>C15---C16---C17---C12</td>
<td>0.4 (2)</td>
<td>C19---C20---C22A---C23A</td>
<td>161.7 (4)</td>
</tr>
<tr>
<td>C13---C12---C17---C16</td>
<td>0.0 (2)</td>
<td>C21---C20---C22A---C23A</td>
<td>2.1 (7)</td>
</tr>
<tr>
<td>C11---C12---C17---C16</td>
<td>178.48 (13)</td>
<td>N4A---C22A---C23A---C24A</td>
<td>−21.9 (8)</td>
</tr>
<tr>
<td>C19---N3---C18---N4A</td>
<td>10.9 (2)</td>
<td>C20---C22A---C23A---C24A</td>
<td>156.5 (5)</td>
</tr>
<tr>
<td>C19---N3---C18---N4A</td>
<td>179.94 (13)</td>
<td>C22A---C23A---C24A---C33A</td>
<td>−76.7 (2)</td>
</tr>
<tr>
<td>C4---C3---C18---N3</td>
<td>−21.6 (2)</td>
<td>C33A---C24A---C25A---C26A</td>
<td>0.8 (10)</td>
</tr>
<tr>
<td>C4---C3---C18---N4</td>
<td>147.73 (19)</td>
<td>C24A---C25A---C26A---C27A</td>
<td>−2.2 (12)</td>
</tr>
<tr>
<td>C2---C3---C18---N4</td>
<td>−32.8 (5)</td>
<td>C25A---C26A---C27A---C28A</td>
<td>2.2 (7)</td>
</tr>
<tr>
<td>C4---C3---C18---N4A</td>
<td>179.1 (3)</td>
<td>C26A---C27A---C28A---C29A</td>
<td>178.3 (13)</td>
</tr>
<tr>
<td>C18---N3---C19---C20</td>
<td>0.7 (2)</td>
<td>C33A---C28A---C29A---C30A</td>
<td>1(2)</td>
</tr>
<tr>
<td>C3---C19---C20---C22</td>
<td>−11.5 (3)</td>
<td>C27A---C28A---C29A---C30A</td>
<td>−176.2 (13)</td>
</tr>
<tr>
<td>N3---C19---C20---C21</td>
<td>178.51 (14)</td>
<td>C28A---C29A---C30A---C31A</td>
<td>0.8 (16)</td>
</tr>
<tr>
<td>N3---C19---C20---C22A</td>
<td>19.0 (3)</td>
<td>C29A---C30A---C31A---C32A</td>
<td>−3.0 (11)</td>
</tr>
<tr>
<td>N3---C18---N4---C22</td>
<td>−11.1 (5)</td>
<td>C30A---C31A---C32A---C33A</td>
<td>3.1 (10)</td>
</tr>
<tr>
<td>N4---C18---N4---C22</td>
<td>96.7 (6)</td>
<td>C31A---C32A---C33A---C24A</td>
<td>179.1 (6)</td>
</tr>
<tr>
<td>C3---C18---N4---C22</td>
<td>−179.6 (2)</td>
<td>C31A---C32A---C33A---C28A</td>
<td>−1.0 (13)</td>
</tr>
<tr>
<td>C18---N4---C22---C20</td>
<td>−0.6 (4)</td>
<td>C25A---C24A---C33A---C32A</td>
<td>−176.3 (6)</td>
</tr>
<tr>
<td>C18---N4---C22---C23</td>
<td>−178.0 (2)</td>
<td>C23A---C24A---C33A---C32A</td>
<td>5.1 (9)</td>
</tr>
<tr>
<td>C21---C20---C22---N4</td>
<td>−178.7 (2)</td>
<td>C23A---C24A---C33A---C28A</td>
<td>−174.8 (10)</td>
</tr>
<tr>
<td>C22A---C20---C22---N4</td>
<td>−79.8 (5)</td>
<td>C29A---C28A---C33A---C32A</td>
<td>−1(2)</td>
</tr>
<tr>
<td>C19---C20---C22---C23</td>
<td>−171.4 (2)</td>
<td>C27A---C28A---C33A---C32A</td>
<td>176.4 (11)</td>
</tr>
<tr>
<td>C21---C20---C22---C23</td>
<td>−1.3 (4)</td>
<td>C29A---C28A---C33A---C24A</td>
<td>178.7 (12)</td>
</tr>
<tr>
<td>C22A---C20---C22---C23</td>
<td>97.5 (6)</td>
<td>C27A---C28A---C33A---C24A</td>
<td>−3.7 (19)</td>
</tr>
</tbody>
</table>
About the Author

Mingzhou Zhou is currently a research assistant at the H. Lee Moffitt Cancer & Research Institute at the University of South Florida. He is pursuing his PhD with Prof. Mark L. McLaughlin from research in design and synthesis of non-peptidic α-helical mimetics and HIV-1 protease inhibitors. Prior to that, he received his BS degree in chemistry at University of Science and Technology of China.