Development of a synthetic route to aminofluorohexenones as precursors for novel caspase inhibitors

Ping Xi
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DEVELOPMENT OF A SYNTHETIC ROUTE TO AMINOFLUOROHEXENONES
AS PRECURSORS FOR NOVEL CASPASE INHIBITORS

by

Ping Xi

Bachelor of Science
Capital Normal University, China
1991

A thesis submitted in partial fulfillment
of the requirements for the

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University of Nevada, Las Vegas
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The Thesis prepared by

Ping Xi

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Development of a Synthetic Route to Aminofluorohexenones as Precursors for Novel Caspase Inhibitors

is approved in partial fulfillment of the requirements for the degree of

Master of Science

Examination Committee Chair

Dean of the Graduate College

Examination Committee Member

Examination Committee Member

Graduate College Faculty Representative
ABSTRACT

Development of a Synthetic Route to Aminofluorohexenones as Precursors for Novel Caspase Inhibitors

by

Ping Xi
Dr. Lydia McKinstry, Examination Committee Chair
Assistant Professor of Chemistry
University of Nevada, Las Vegas

Apoptosis is a normal biological process in which cells commit suicide. In cancer cells, a family of proteases called caspases, is involved in one or more of the signaling pathways leading to apoptosis. To better understand this cellular mechanism at the molecular level, selective inhibition of each caspase would allow scientists to determine the exact function of that caspase in the overall pathway. The long term goal of this research project is the synthesis of new, highly specific caspase inhibitors that will be used to study the mechanism of apoptosis in certain cancer cells. The first step, and the primary focus of this thesis, is successful development of a synthetic route to a group of aminofluorohexenone precursor molecules. The strategy for synthesizing these subtargets first involves investigation of a method for carbon-carbon bond formation adjacent to nitrogen, that focuses on an electrophilic substitution reaction based on charge affinity inversion of customary amine reactivity. The second part of the synthetic strategy involves generation of pentenoic acid analogs to be used as electrophiles in the overall construction of the caspase inhibitor aminofluorohexenone precursors.
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Proteolytic enzymes activate and regulate numerous cellular processes and are essential for normal biological function. In many abnormal processes such as the growth, death and migration of cancer cells, the specific roles of certain protease enzymes are not known. For instance, all cells (normal and cancerous) are known to undergo a process of cell suicide called apoptosis. In cancer cells, a family of proteases called caspases, is involved in one or more of the signaling pathways leading to apoptosis. To better understand this important mechanism at the molecular level, selective inhibition of each caspase would allow scientists to determine the exact function of that caspase in the overall pathway leading to apoptosis.

The long term goal of this research project is the synthesis of new, highly specific caspase inhibitors that will be used to study the mechanism of apoptosis in certain cancer cells. The strength, or effectiveness, of a molecule for inhibiting a certain enzyme’s activity is determined by how specifically the inhibitor interacts with that enzyme and how well the inhibitor mimics catalytic intermediates. The first property is known as inhibitor specificity. For protease inhibitors, specificity is determined by charged interactions over an extended region within the active site. One current strategy for improving inhibitor strength involves changing the molecular structure of the natural
enzyme substrate (e.g. a peptide) in order to optimize the inhibitor-enzyme binding interactions. These compounds are known as peptide based inhibitors and their binding domain can be defined as a single site or as an extended region on either side of the amide bond cleavage site. Several commercial peptide-based inhibitors are known but have limitations in terms of their specificity for caspases. Many of them only interact with the enzyme on one side of the cleavage site, reducing their specificity and potency.3

The second property determining inhibitor strength involves the mechanism by which the enzyme catalyzes the breakdown of a peptide substrate. Within the enzyme active site, a peptide bond is hydrolyzed and the substrate is cleaved into two pieces. In natural peptide hydrolysis, a catalytic intermediate called a tetrahedral intermediate is formed at the cleavage site.4 Since peptide-based inhibitors are designed to undergo the same mechanism, increasing the lifetime of this intermediate (i.e. stabilization) leads to increased inhibitor potency. Studies have shown that fluorinated inhibitors can stabilize a tetrahedral intermediate and increase the lifetime of an enzyme-inhibitor complex.6 Therefore, incorporation of fluorine into a peptide-based inhibitor molecule will result in more potent inhibitors.

1.2 Project Overview

The ultimate goal of this research project is the synthesis of new, highly specific caspase inhibitors that will be used to study the mechanism of apoptosis in certain cancer cells. As described in the following chapter and summarized in Figure 1.1, the proposed new inhibitor molecules are designed to allow for increased charged interactions throughout the entire active site by introducing functional groups on the Pn' side.
(towards the C-terminus). Increased stabilization of a tetrahedral enzyme-inhibitor intermediate will also be achieved through incorporation of fluorine.

Figure 1.1 Relationship between the proposed inhibitors (b) and the natural substrate (a)

The complete synthesis of a first generation of caspase inhibitors requires two major steps. The first step, and the primary focus of this thesis, is successful development of a synthetic route to aminohexenone subtarget molecules 2a-c as shown in Figure 1.2.
Figure 1.2 Retrosynthetic scheme for peptide-based inhibitors 1 from 2-fluoro-, 2-methyl- and 2-fluoro-2-methyl-4-pentenoyl precursors 3a-c

The strategy for synthesizing subtarget 2 first involves an investigation of methods for carbon-carbon bond formation adjacent to nitrogen. If successful, this method will find widespread application in the synthesis of a variety of amine natural products. The second step, also depicted retrosynthetically in Figure 1.2, will involve oxidation of subtarget 2 and amino acid coupling, to yield the first generation of new inhibitor molecules.
1.3 References


CHAPTER 2

BACKGROUND

2.1 Proteases, Caspases and Apoptosis

Proteases - Enzymes are protein molecules that speed up chemical reactions in all plants and animals. These molecules regulate numerous biological processes either directly or indirectly by acting on other enzymes. Without enzymes, many biological reactions would occur too slowly or not at all, and no life would be possible. Sometimes however, metabolic disorders are caused by abnormal enzyme function. Proteolytic enzymes or proteases, are defined as enzymes which break down proteins by cleaving peptide (amide) bonds. Proteases are classified into four categories according to the distinct functional groups within the enzyme active site: 1) Serine proteases (hydroxyl, imidazole and carboxyl groups), 2) Cysteine proteases (thiol, imidazole and carboxyl groups), 3) Aspartyl proteases (carboxyl groups), and 4) Metalloproteases (metal ion, often a zinc ion).\(^1\)

Caspases - Caspases are one family of cysteine proteases which have enzymatic specificity for cleaving peptide bonds at aspartic acid (Asp or D) residues.\(^2\)\(^-\)\(^5\) We are interested in caspases because of the role they play in the process of apoptosis or programmed cell death. For caspases and all cysteine proteases, the catalytic mechanism involves initial nucleophilic attack by a cysteine thiol group on a peptide amide bond, to form a tetrahedral intermediate (Figure 2.1).\(^1\)
Figure 2.1 Catalytic mechanism of cysteine proteases

A histidine bound imidazole group activates the thiol group prior to nucleophilic addition by serving as a Bronsted-Lowry base. Stabilization of the tetrahedral intermediate occurs by hydrogen-bond interactions between the anionic oxygen and amide hydrogens within the active site. Decomposition of the tetrahedral intermediate, assisted by proton transfer from the imidazole, results in an acyl-enzyme intermediate and the free amine product. Subsequent hydrolysis of the acyl-enzyme intermediate, via a second tetrahedral intermediate, releases the acid component of the substrate.

Like many proteases, caspases are formed as proenzymes (a.k.a. zymogens) which are inactive forms of the enzyme. Activation occurs when a portion of the proenzyme is
cleaved, usually by another protease. For caspases, usually another caspase will cleave the zymogen in specific regions, leading to the activated enzyme.\textsuperscript{2-4,6-10} Once activated, the caspase will then carry out its function in the mechanism of programmed cell death.

At least 15 different caspases have been identified as being associated with the process of apoptosis.\textsuperscript{2,8,9} All of these caspases have a preference for cleaving peptides at aspartic acid (D) residues, which are part of a four amino acid specificity site or recognition motif (specificity will be discussed in more detail in Section 2.2).\textsuperscript{2} Initiator caspases and effector caspases are the two general categories of caspases involved with apoptosis.\textsuperscript{2,6,8} Initiator caspases are associated with the early stages of apoptosis and preferentially cleave at specificity sites of the general form A depicted in Figure 2.2(a).\textsuperscript{2-6,8}

(a): A: N-terminus —— I —— E —— X —— D —— C-terminus

N-terminus —— L —— E —— X —— D —— C-terminus

N-terminus —— V —— E —— X —— D —— C-terminus

(b): B: N-terminus —— D —— E —— X —— D —— C-terminus

\( I = \text{isoleucine}, \ L = \text{leucine}, \ V = \text{valine}, \ E = \text{glutamic acid}, \ D = \text{aspartic acid}, \ X = \text{any amino acid residue} \)

Figure 2.2 Recognition motifs for (a) initiator caspases and (b) effector caspases

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Effector caspases are associated with later stages of apoptosis such as cell disassembly and preferentially cleave at specificity sites of the general form B depicted in Figure 2.2(b).\textsuperscript{2,4,6,8,9}

Apoptosis - Apoptosis is a normal biological process first described by Kerr, Wyllie and Currie, in which cells commit suicide.\textsuperscript{11} Apoptosis is fundamental to regulating cell growth and tissue development.\textsuperscript{2} It is a genetically programmed process designed to rapidly eliminate unwanted cells and can be triggered by either intracellular or extracellular factors. In contrast, certain metabolic diseases such as cancer and AIDS are often associated with abnormal enzyme function and/or unregulated apoptosis.\textsuperscript{7} Since one approach to disease treatment could involve controlling the process of apoptosis in unwanted cells, scientists must understand the details of the apoptotic pathway(s). We are interested in determining the specific roles of caspase enzymes in the regulation of apoptosis.

Several reports conclude that there are two general pathways for apoptosis (see Figure 2.3 below).\textsuperscript{2,5,12,13} One mechanism, known as the receptor mediated pathway is triggered by the binding of specific ligand molecules to cell surface receptors.\textsuperscript{11} Ligand binding results in transmission of a chemical signal into the cell cytoplasm which results in cleavage or activation of procaspase-8 to caspase-8 (an initiator caspase).\textsuperscript{2,12,13} Activation of caspase-10 is also believed to occur at this stage.\textsuperscript{2,12,13} Procaspase-3 is the substrate for caspase-8 and therefore caspase-3 activation occurs next in the sequence.\textsuperscript{12} Caspase-3 is an effector caspase and is the point of convergence for both apoptotic pathways.\textsuperscript{2}

The second apoptotic mechanism is initiated by signals within the cell and is called the intrinsic or mitochondrial pathway (see Figure 2.3 below).\textsuperscript{14-22} In this mechanism,
Figure 2.3 Two general pathways of apoptosis

Intracellular signals cause the mitochondria to release certain molecules into the cytoplasm resulting in the formation of an apoptosome complex. This complex contains procaspase-9 which becomes activated to caspase-9 by the apoptosome.
Caspase-9 (an initiator caspase) then activates caspase-3 by cleaving the procaspase-3 zymogen and the two apoptotic pathways converge.\textsuperscript{2,7,14} In either case, procaspase-7 is the substrate for caspase-3 (an effector caspase) and once activated, caspase-7 targets substrates within the cell nucleus leading to degradation of chromosomal DNA, digestion of structural proteins in the cytoplasm and ultimately cell death.\textsuperscript{6}

Although caspase-3 has been identified as a key mediator of apoptosis in mammalian cells, the function of all caspases (e.g. caspase-10, -6, and -4) and the mechanism of programmed cell death is not completely understood.\textsuperscript{25} By selectively blocking each caspase in the cascading mechanism(s), scientists can learn more about the overall apoptosis pathway(s). Our strategy involves constructing inhibitor molecules that will selectively inhibit the enzyme by bonding to it. By inhibiting each caspase, one at a time, we can possibly learn more about how to control the overall process of enzyme-regulated cell death. With the ability to control this process, scientists can ultimately generate new drugs that may induce programmed cell death in unwanted tissues (e.g. cancer tissue). New synthetic caspase inhibitors therefore could become tools for investigating the mechanism of caspase-dependent apoptosis.

\subsection*{2.2 Inhibitors}

Inhibitors are molecules that block enzyme function and usually are structurally similar to a natural enzyme substrate. Although such molecules are unable to react, they compete with the substrate for the active site region of the enzyme, thereby blocking its activity. By blocking catalytic activity certain cellular events might be regulated or investigated.
Enzyme substrates and many inhibitors have recognition positions within the active site (e.g. S2-S3' subsites; refer to Figure 2.4). Recognition or binding sites are numbered according to their position relative to the site of peptide bond cleavage. The specificity of binding for a substrate or inhibitor is defined as a single site (e.g. P1') or as an extended region (e.g. P2-P2') in the active site domain (Figure 2.4). An enzyme-substrate or enzyme-inhibitor complex is often stabilized by a $\beta$-pleated sheet structure consisting of hydrogen bonds and electrostatic interactions between the inhibitor (or substrate) and the amino acid residues in the active site.

![Diagram of substrate and inhibitor binding sites](image)

Figure 2.4 Substrate and inhibitor binding sites

Due to the similarity in structure and binding characteristics between natural substrates and natural inhibitors, a major strategy of drug development research involves the use of natural enzyme substrates as lead molecules for generating potent synthetic inhibitors. By this method, a myriad of inhibitors, including transition state analog inhibitors and substrate analog inhibitors have been developed. Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Substrate analog inhibitors are peptide substrates that differ from the natural enzyme substrate by one or more critical amino acid residues in the sequence. For example, an unnatural amino acid incorporated into the primary sequence (Figure 2.5).\(^{28}\)

![Figure 2.5 Substrate analog inhibitor containing an unnatural phenylalanine residue (indicated by *)](image)

Transition state analog inhibitors are peptide substrates which have been modified to resemble the tetrahedral intermediate that occurs in peptide hydrolysis (Figure 2.6 and Figure 2.1).\(^{28}\)

![Figure 2.6 Transition state analog inhibitors of cysteine proteases](image)
The strength, or effectiveness, of a molecule for inhibiting a certain enzyme's activity is determined by how well the inhibitor interacts with that enzyme and how well the inhibitor mimics catalytic intermediates. The first property is known as inhibitor specificity. For many protease inhibitors, specificity is usually determined by charged interactions over an extended region within the active site.\(^{26}\) The more extensive the binding interactions are, the more specific the inhibitor will be.\(^{26}\) Furthermore, studies have shown that binding interactions on the leaving group side of the substrate or inhibitor (P1'-P4' refer to Figure 2.4 page 12) contribute to a decrease in activation energy.\(^{29}\) Caspases are among the most specific of proteases; they have a near absolute requirement for an aspartic acid residue at the P1 subsite of the substrate.\(^1\) Caspases have an equally stringent specificity for at least four amino acid residues, called a recognition motif, on the amino terminus side of the cleavage site (P1-P4, refer to Figures 2.2 and 2.4), and it is clear that primary amino acid sequence recognition is a necessary requirement for catalysis and presumably for inhibitor binding.\(^{30}\)

The second property that determines inhibitor potency is the formation and stability of an artificial catalytic intermediate (e.g. tetrahedral intermediate) between the enzyme and the inhibitor. For caspases, an enzyme-inhibitor tetrahedral intermediate consists of a hemithioacetal complex within the active site (refer to Figures 2.1 and 2.6), which forms by a reversible addition reaction.

The formation of a hemithioacetal is one example of a reversible addition reaction to a carbonyl containing compound. The simplest form of this reaction is hydration of a carbonyl compound. In this process, water adds across the carbonyl bond (C=O) to form the corresponding hydrate. Similarly, when an alcohol or thiol adds to the carbonyl
group, the initial product is called a hemiacetal or a hemithioacetal respectively (Figure 2.7).^{31}

\[
egin{align*}
\text{Hemiacetal} & \quad \text{Hemithioacetal} \\
\text{Figure 2.7 Mechanism of hemiacetal and hemithioacetal formation}
\end{align*}
\]

In this reaction, the carbonyl group changes from a trigonal planar \(sp^2\) hybridized carbon to a tetrahedral \(sp^3\) hybridized carbon, hence the name tetrahedral intermediate. Hydrate or hemiacetal formation is an equilibrium process where the equilibrium constant \((K_{eq})\) can favor either the carbonyl compound or the tetrahedral product (e.g. hydrate, hemiacetal, etc). The position of equilibrium or the magnitude of \(K_{eq}\) depends upon the structure of each component of the reaction. In basic, acidic, or neutral water, simple carbonyl compounds are in equilibrium with their hydrates. Comparing the hydration reactions of different carbonyl compounds (e.g. trifluoroacetaldehyde, formaldehyde, chloroacetaldehyde, acetaldehyde and acetone), the \(K_{eq}\) value has a wide variation (from \(10^4\) to \(10^{-3}\)) as shown in Table 2.1.^{32}
Table 2.1 The Equilibrium Constant for Hydration of Carbonyl Compounds

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<th>Carbonyl Molecule</th>
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<td>$R_1=H, R_2=CF_3$</td>
<td>$2.9 \times 10^4$</td>
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<td>$R_1=H, R_2=H$</td>
<td>$2.3 \times 10^3$</td>
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<td>$R_1=H, R_2=CH_2Cl$</td>
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<tr>
<td>$R_1=H, R_2=CH_3$</td>
<td>1.06</td>
</tr>
<tr>
<td>$R_1=CH_3, R_2=CH_3$</td>
<td>$2 \times 10^{-3}$</td>
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</tbody>
</table>

Alkene double bonds (C=C) are stabilized by alkyl group substitution. The greater the number of attached alkyl groups, the greater is the alkene's stability. For example, the heat of formation ($\Delta H_f^\circ$) of 1-hexene is $\Delta H_f^\circ = -10$ kcal/mol, whereas 2,3-dimethylbutene is $\Delta H_f^\circ = -16$ kcal/mol. The more negative the heat of formation is for a compound, the more stable it is. Therefore, 2,3-dimethylbutene, a tetrastubstituted double bond, is more stable than 1-hexene, a monosubstituted double bond. This rule also holds for C=O double bonds (i.e. ketone vs. aldehyde). However, increasing alkyl substitution will also introduce steric destabilizations. Therefore, increased substitution leads to increased stability in the carbonyl form and decreased stability in the hydrate form (Figure 2.8). This is the reason for a decreasing trend in $K_{eq}$ for the hydration of
formaldehyde, acetaldehyde and acetone respectively (Table 2.1, entries 2, 4 and 5 respectively).\textsuperscript{32}

Electron withdrawing groups such as chlorine or fluorine destabilize carbonyl groups when attached at the $\alpha$ position (carbon directly attached to the $C=O$ group), by inductive removal of electron density.\textsuperscript{32} Inductive destabilization of the carbonyl group favors the hydrate at equilibrium as shown in Table 2.1 and Figure 2.8. Fluorine is more electronegative than chlorine, which should increase the proportion of tetrahedral intermediate (or hydrate) relative to the carbonyl species. Therefore, trifluoroacetaldehyde is more hydrated at equilibrium than other carbonyl compounds (Table 2.1, entry 1 and Figure 2.8). In summary, as depicted in Table 2.1 and Figure 2.8,

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figures/figure2.8.png}
\caption{The stability of carbonyl groups and hydrates}
\end{figure}

aldehydes and ketones $\alpha$-substituted with electron withdrawing groups (e.g. CF$_3$) favor formation of a tetrahedral intermediate ($K_{eq}$ large) and therefore could stabilize hemithioacetal formation in a cysteine protease active site (Figure 2.9).

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Several commercial enzyme inhibitors have been generated on the basis of this concept. Aldehydes have shown to be very potent reversible inhibitors \textit{in vitro}, but are easily oxidized \textit{in vivo}, reducing their effectiveness.\textsuperscript{34} Ketones are less stabilizing but allow for binding on both sides of the carbonyl group.\textsuperscript{36} When a strong electronegative group is added to the $\alpha$-carbon of a methylketone, the tetrahedral intermediate is formed more readily, which increases the potency of a methylketone inhibitor.\textsuperscript{36} However, if the electronegative group is also a good leaving group, the inhibitor will be irreversible, which has been found with chloromethylketones (Figure 2.10 (a)).\textsuperscript{33} Chlorine is a good

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2_10.png}
\caption{Reversible (b) and irreversible (a) inhibitors}
\end{figure}
leaving group in nucleophilic substitution reactions and therefore these inhibitors bind irreversibly to the enzyme. In contrast, fluorine is a weak leaving group and is more electronegative than chlorine. So it will favor rapid formation of tetrahedral enzyme-inhibitor complex (Figure 2.10 (b)). Studies have shown that fluorinated inhibitors can stabilize a tetrahedral intermediate and increase the lifetime of an enzyme-inhibitor complex, thereby blocking normal enzyme function.

Within the enzyme active site, a peptide bond is hydrolyzed and the substrate is cleaved into two pieces (see Figure 2.1). In natural peptide hydrolysis, a catalytic intermediate called a tetrahedral intermediate is formed at the cleavage site. Since peptide-based inhibitors are designed to undergo the same mechanism, increasing the lifetime of this intermediate through stronger enzyme-inhibitor interactions leads to increased inhibitor potency. Strong enzyme inhibitor interactions occur through increased specificity (extended binding) and increased stability of the tetrahedral intermediate (Figure 2.11).

Figure 2.11 Specificity and stability of proposed inhibitors

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Therefore, incorporation of fluorine into a peptide-based inhibitor molecule should lead to more potent inhibitor molecules. Several commercial peptide-based inhibitors are known but have limitations in terms of their specificity for caspases. Many of them only interact with the enzyme on one side of the cleavage site, reducing their specificity and potency.

2.3 The Aza-Allyl Anion

The aza-allyl anion is a tool used in synthesis for constructing carbon-carbon bonds adjacent to nitrogen. There are three general strategies for such amine functionalization: nucleophilic substitution, radical substitution and electrophilic substitution. Each of these strategies is described in the following paragraphs.

Most classical syntheses employ nucleophilic substitution as the key step. In this reaction, a nucleophile will attack a carbon adjacent to the nitrogen. The second step of a Mannich reaction is one example of this method (Figure 2.12).

\[
\begin{align*}
\text{Step 1:} & \\
H_2C=O + HN\text{R} & \xrightarrow{H^+} H_2C=N\text{R} + H_2O \\
& \text{Iminium ion (Mannich Base)}
\end{align*}
\]

\[
\begin{align*}
\text{Step 2:} & \\
\text{Nu}^+ + H_2C=N\text{R} & \xrightarrow{\alpha} \text{Nu}^+ \text{C-}N^\alpha \text{R} \\
& \text{New C-C bond}
\end{align*}
\]

Figure 2.12 The Mannich reaction
The first step of the Mannich reaction involves formation of an iminium ion (a.k.a. a Mannich base) from the condensation of an amine and an aldehyde or ketone. In the second step, the iminium ion is attacked by a carbon nucleophile at the α-carbon (carbon adjacent to nitrogen) to form a new carbon-carbon (C-C) bond.

Radical substitution at the α-carbon a second method for amine functionalization and the synthetic utility of this approach is at a promising stage of development. Almost all small radicals are short-lived, highly reactive species and can react or combine with a compound containing a multiple bond to produce a new larger radical and compound. The α-acylamino radical cyclization reaction depicted in Figure 2.13 is one example.\(^\text{42}\)

![Figure 2.13 The α-acylamino radical cyclization reaction](image)

In this mechanism, a carbon radical is generated adjacent to nitrogen (α-carbon) by an initiator molecule and rapidly attacks a carbon-carbon multiple bond to form a new carbon-carbon (C-C) single bond. Although this reaction generally has low regioselectivity, it is possible to guide the regiochemical course of an α-acylamino radical cyclization by controlling olefinic substitution patterns.\(^\text{42}\)
Electrophilic substitution is the third method and provides a new general strategy for amine elaboration by charge affinity inversion of customary amine reactivity. A number of amine derivatives have shown that $\alpha$-protons (on $\alpha$-carbon) which are adjacent to a nitrogen bearing an electron withdrawing group ($Z$, Figure 2.14), can be acidic ($pK_a \approx 20$). Thus $\alpha$-carbanion amine synthetic equivalents (I, Figure 2.14) can be prepared. In electrophilic substitution, this $\alpha$-carbanion serves as a nucleophile and the $\alpha$-proton becomes replaced by an electrophile ($E^+$, Figure 2.14). The general scheme of this method is shown in Figure 2.14 below.

In Step 1 of this procedure, an activating group $Z$ is added to the amine in order to increase the acidity of the $\alpha$-proton. Subsequent removal of the $\alpha$-proton (Step 2) under basic conditions gives the $\alpha$-carbanion I. In Step 3 the $\alpha$-carbanion nucleophile reacts

![Figure 2.14 General scheme of electrophilic substitution](image-url)
with a carbon electrophile (E\textsuperscript{+}) to form the new carbon-carbon (C-C) bond. Finally, the activating group (Z) is removed to yield the new unprotected amine product. The formation of an α-carbanion amine synthetic equivalent (I) by deprotonation of an amine derivative is the key to this methodology. The following paragraphs discuss each of the steps of amine electrophilic substitution in more detail.

The stabilizing group (Z) is a nitrogen protecting group but also functions as an activator by enhancing the acidity of the α-proton(s) adjacent to nitrogen. Figure 2.15 depicts several types of amine activating groups which provide inductive and/or resonance stabilization to the forming α-carbanion.\textsuperscript{43}

![Diagram of activating groups](image)

Figure 2.15 Types of activating groups

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Many activating groups also provide stabilization in the deprotonation step (Step 2, Figure 2.14), by coordinating with the metal counter-ion of the base used. Suitable Z-groups must be inert to any basic reagents used in the deprotonation step, must not interfere with the electrophilic substitution (Step 3, Figure 2.14) and must be conveniently and efficiently removed in Step 4 of the sequence.\textsuperscript{43}

The aza-allyl anion is an \( \alpha \)-carbanion generated from an amine stabilized as an imine derivative (see Figure 2.16(a) and the boxed group in Figure 2.15).\textsuperscript{43} The aza-allyl anion is named for its similarity to the conventional allyl anion depicted in Figure 2.16(b); both structures have 4 \( \pi \) electrons delocalized over three atoms.\textsuperscript{44} In these molecules, \( R_1 \) and/or \( R_2 \) can be alkyl, aryl, ether, thioether, halogen or hydrogen groups.\textsuperscript{44} The conditions for generating the aza-allyl carbanion (Step 2, Figure 2.14) depend on the nature of the \( R_3 \) substituent, which can be alkyl, hydrogen or electron withdrawing groups (e.g. ester or aryl).\textsuperscript{45} In all these molecules, the Z-group imine is usually generated in Step 1 of the sequence (Figure 2.14) by condensation of an aldehyde or ketone with the desired amine.

![Figure 2.16 Structure of (a) an aza-allyl anion and (b) a normal allyl anion](Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.)
Formation of the aza-allyl anion can occur by two general methods, as shown in Figure 2.17. Direct deprotonation usually involves proton abstraction by an alkyllithium (e.g. n-butyllithium, n-BuLi) or lithium amide base (e.g. lithium diisopropylamide, LDA). This method is suitable for imines derived from aldehydes and ketones which do not contain heteroatom substituents (i.e. $R_1$ and/or $R_2 = \text{alkyl, aryl or } H$, Figure 2.17(a)). For the more complicated imines, a tin-lithium exchange reaction has been found to work more effectively (i.e. $R_1$ and/or $R_2 = \text{OR, SR, alkyl or } H$, Figure 2.17(b)). Once formed, the $\alpha$-carbanion serves as a nucleophile for substitution and addition reactions with electrophiles such as alkyl halides and carbonyl compounds (Step 3, Figure 2.14).

![Figure 2.17](image-url)

Figure 2.17 Formation of the aza-allyl anion by (a) direct deprotonation with $n$-butyllithium ($n$-BuLi) or lithium dialkylamide (LiNR$_2$) in tetrahydrofuran (THF) and (b) tin-lithium exchange reactions

Step 4 of the overall reaction scheme (Figure 2.14, page 22) involves removal of the activating group $Z$. In the context of aza-allyl anion chemistry, several methods have been reported for the conversion of substituted imines to the corresponding amine products and most of these are simple acidic hydrolysis procedures.\footnote{44-52}
2.4 References


CHAPTER 3

RESULTS

The primary focus of this work is successful development of a synthetic route to three \( \beta \)-aminoketone molecules (2a – c, Figure 3.1); precursors for a class of novel caspase inhibitors. The synthesis of subtargets 2 can be divided into two key parts, which are described below. The first part involves a carbon-carbon bond forming reaction adjacent to nitrogen. The second part involves the preparation of 2-fluoro-, 2-methyl- and 2-fluoro-2-methyl-4-pentenoyl species (3a, 3b and 3c respectively, Figure 3.7, page 39).

3.1 \( N \)-Methyl Imine Synthesis

Currently, the most widely understood method used to form carbon-carbon bonds adjacent to the nitrogen of an amine functional group involves nucleophilic addition to
the carbon attached to nitrogen.\textsuperscript{1} In our approach, we used the electrophilic substitution reaction of aza-allyl anions (described in Chapter 2) generated from two diarylketone imines 4 and 5 (Figures 3.2 and 3.3).

The synthesis of \(N\)-methylbenzophenone imine 5 was accomplished in excellent yield (95\%) by stirring a solution of methylamine hydrochloride and benzophenone imine in dichloromethane (\(\text{CH}_2\text{Cl}_2\)) at 23 °C (>12 hr), according to a modified O'Donnell procedure (Figure 3.2).\textsuperscript{2} Following an ether extraction, the imine was isolated as a viscous yellow oil and the purity was determined to be >95\% by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. A modified Weingarten procedure\textsuperscript{3} was used to prepare \(N\)-methyl-4-methoxybenzophenone imine 4 (Figure 3.3). In this case, an ice-cold solution of 4-methoxybenzophenone and methylamine in tetrahydrofuran (THF) was treated with titanium tetrachloride (0.6 equiv, 1.25 M in pentane). After slowly warming to a reflux, the mixture was cooled to 23 °C and then extracted with ether. Imine 4 was isolated in high yield (89\%) and high purity (>95\%, determined by mass spectrometry and NMR spectroscopy) as a viscous light yellow oil (1:1.5 mixture of \(Z/E\) diastereomers).
3.2 Imine Alkylation

Using the new imine 4 and the N-methylbenzophenone imine 5, originally described by Kauffmann, Hullot and others,\(^6\) our first objective was to evaluate and optimize the reaction conditions for aza-allyl anion formation and alkylation. Although these anion nucleophiles have received a great deal of attention, several details of their reactivity, including the effect of certain reaction parameters are less understood (e.g. temperature and time of exposure to deprotonating agents).

In our approach, we first investigated deuterium incorporation as a method for determining the extent of anion formation under the conditions of direct deprotonation (see Chapter 2, Section 2.3) with n-butyllithium (n-BuLi). Table 3.1 summarizes the results of this initial study. For each case, a solution of 5 (0.268 M in THF) was treated with n-BuLi (1.20 equiv in THF) at \(-78^\circ\text{C}\), followed by addition of a 1:1 mixture of deuterated methanol (CD\(_3\)OD) in deuterium oxide (D\(_2\)O) after the period of time indicated in Table 3.1. The metallation temperature was held at \(-78^\circ\text{C}\) in every case.
except entry 4, where the temperature was increased to 0 °C for 5 min, after 1 hr at -78 °C.

Table 3.1 Deuteration of the Aza-Allyl Anion of N-Methylbenzophenone Imine

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Product Ratio (D : H)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-78 °C, 20 min</td>
<td>1 : 3.8</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>-78 °C, 1 hr</td>
<td>1.4 : 1</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>-78 °C, 2 hr</td>
<td>1.7 : 1</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>-78 °C, 1 hr; 0 °C, 5 min</td>
<td>8.1 : 1</td>
<td>97</td>
</tr>
</tbody>
</table>

*a* Determined by $^1$H NMR integration. *b* Total mass recovered. *c* 1.10 equiv of n-BuLi

Following extraction and work-up, the products were analyzed by proton ($^1$H) NMR spectroscopy to determine the ratio of deuterated and non-deuterated imines. The results demonstrated that aza-allyl anion formation occurs efficiently at a temperature slightly higher than -78°C when the imine is directly deprotonated with n-BuLi.

Given the results of our deuterium incorporation study, we investigated the comparative alkylation of imines 4 and 5 employing four alkyl halides (Tables 3.2 and 3.3). In this approach, we developed a high yielding general procedure for aza-allyl anion alkylation using carefully controlled metallation conditions. In each case, a solution of N-methyl imine (4 or 5) in THF (0.60 M) was added dropwise to a solution of n-BuLi
(0.60 M, 1.25 equiv, in THF) at −78 °C. The resulting red-brown solution was stirred at −78 °C for one hour and then warmed to 0 °C for 5 minutes to ensure complete deprotonation. After re-cooling the mixture to −78 °C, an alkyl halide electrophile

Table 3.2 Alkylation of N-Methylbenzophenone Imine

<table>
<thead>
<tr>
<th>Entry</th>
<th>Alkyl Halide</th>
<th>Product</th>
<th>Conversion (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isolated Yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;I</td>
<td>6</td>
<td>82</td>
<td>78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;CHCH&lt;sub&gt;2&lt;/sub&gt;Br</td>
<td>7</td>
<td>99</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;=CHCH&lt;sub&gt;2&lt;/sub&gt;Br</td>
<td>8</td>
<td>93</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;Br</td>
<td>9</td>
<td>95</td>
<td>81</td>
</tr>
</tbody>
</table>

<sup>a</sup>Determined by <sup>1</sup>H NMR analysis. <sup>b</sup>Product isolated by flash chromatography.

(1.5 equiv) was added neat and stirring was continued at −78 °C for an additional 4.5 hours. Following extraction and work-up, the crude products were analyzed by <sup>1</sup>H NMR spectroscopy to determine the percent conversion of starting material to alkylated products. As shown in Tables 3.2 and 3.3, all reactions proceeded quantitatively. In addition, excellent yields of purified alkylation products were isolated following Kugel-Rohr distillation under reduced pressure (50 μTorr).
Table 3.3 Alkylation of *N*-Methyl-4-Methoxybenzophenone Imine

![Chemical structure of imine 4 and products 10-12 with reaction conditions: 1) n-BuLi, THF, -78 °C; 2) R—X, -78 °C, 4.5 hr.]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Alkyl Halide</th>
<th>Product</th>
<th>Conversion (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Isolated Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;CHCH&lt;sub&gt;2&lt;/sub&gt;Br</td>
<td>10</td>
<td>94</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;=CHCH&lt;sub&gt;2&lt;/sub&gt;Br</td>
<td>11</td>
<td>90</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;Br</td>
<td>12</td>
<td>88</td>
<td>80</td>
</tr>
</tbody>
</table>

<sup>a</sup>Determined by <sup>1</sup>H NMR analysis.

Several different procedures have been reported for removing the imine protecting group following an electrophilic substitution reaction, including concentrated HCl, ammonium hydroxide in ethanol (at pH 4-6) and aqueous citric acid. Given the range of these conditions, we ran a test experiment to confirm that a simple acid hydrolysis procedure would be a viable method for our purposes. In this experiment, a solution of *N*(2-phenylethyl)benzophenone imine (9) in diethyl ether (0.50 M) was treated with a 1N solution of aqueous HCl at 23 °C for 12 hours. Following work-up, the corresponding 2-phenylethylamine was isolated in 67% yield as a viscous yellow oil.

### 3.3 Substitution and Addition Reactions

Our next objective was to examine the reaction of imines 4 and 5 with various acylating agents including carboxylic acid chlorides, anhydrides, amides and esters.
Figure 3.4 Imine acylating agents: (a) acetyl chloride (b) benzoyl chloride (c) acetic anhydride (d) N,N-dimethyl formamide (e) methyl benzoate and (f) ethyl fluoroacetate

Figure 3.4 shows several of the acylating agents used in this study. These substitution reactions were carried out in the same manner as the alkylation reactions, where N-methylbenzophenone imine (5) was deprotonated with n-BuLi in THF at -78 °C. Following the deprotonation period (-78 °C, 1 hr then 0 °C 5 min) an acylating agent (1.5 equiv) was added neat and stirring was continued at -78 °C for 4.5 hours. No reaction was observed for acetic anhydride, acetyl chloride or benzoyl chloride, even at long reaction times (>18 hr). At higher temperatures, we observed a considerable amount of starting material decomposition. In spite of these findings, modest yields of β-iminoketone products were obtained with the methyl benzoate and ethyl fluoroacetate esters (72% and 41% respectively, Figure 3.5). For the latter reaction 1H NMR analysis indicated nearly quantitative conversion to the β-iminofluoroketone product 14. However, this compound proved to be unstable and significant yields were difficult to isolate.
As an alternative strategy to forming the desired subtargets 2a - c (refer to Figure 3.1, page 30), we also investigated aldehyde electrophiles. Nucleophilic addition of anaza-allyl anion derived from either 4 or 5 to an aldehyde, should provide a $\beta$-imino-alcohol product, which could be subsequently oxidized to the corresponding $\beta$-iminoketone as shown in Figure 3.6. In order to explore this strategy, we compared the addition reactions of imines 4 and 5 with four aldehyde electrophiles (Table 3.4). In each case, the $N$-methyl imines were deprotonated with $n$-BuLi in THF at $-78 \, ^{\circ}C$. Following the deprotonation period ($-78 \, ^{\circ}C$, 1 hr then 0 $^{\circ}C$, 5 min) an aldehyde (1.5 equiv) was added neat and...
stirring was continued at \(-78^\circ C\) for 15 - 25 hours. Following extraction and work-up, the crude products were analyzed by \(^1H\) NMR spectroscopy and gas chromatography (GC) to determine the percent conversion of starting material to addition products. As shown in Table 3.4, modest yields of \(\beta\)-imino alcohol products were obtained with both imine nucleophiles, but the isolation proved to be problematic, especially for the 4-methoxybenzophenone imine derivatives (Table 3.4, entries 2 and 4).

Table 3.4 Aldehyde Addition Reactions with \(N\)-Methyl Imines 4 and 5

<table>
<thead>
<tr>
<th>Entry</th>
<th>Imine</th>
<th>Aldehyde</th>
<th>Product</th>
<th>Conversion (%)(^a)</th>
<th>Isolated Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>CH(_3)(CH(_2))(_2)CHO</td>
<td>15</td>
<td>60</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>CH(_3)(CH(_2))(_2)CHO</td>
<td>16</td>
<td>50</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>(CH(_3))(_3)CCHO</td>
<td>17</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>(CH(_3))(_3)CCHO</td>
<td>18</td>
<td>84(^b)</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>CH(_3)CH=CHCHO</td>
<td>19</td>
<td>79</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>C(_6)H(_5)CHO</td>
<td>20</td>
<td>70</td>
<td>32</td>
</tr>
</tbody>
</table>

\(^a\) Determined by \(^1H\) NMR analysis. \(^b\) Determined by GC analysis.
3.4 Pentenoic Acid Analogs

The synthesis of β-aminoketone subtargets 2a – c (Figure 3.1, page 30) involves two key parts, the first of which has been described in the preceding sections. The second part involves the preparation of 2-fluoro-, 2-methyl- and 2-fluoro-2-methyl-4-pentenoyl species (3a, 3b and 3c respectively, Figure 3.7) and coupling of these electrophiles to an imine nucleophile.

\[
\begin{align*}
3a & \quad X = H, Cl, OR, NR_2 \\
3b & \\
3c & 
\end{align*}
\]

Figure 3.7 2-Fluoro-, 2-methyl- and 2-fluoro-2-methyl-4-pentenoic acid analogs 3a – c

Since compounds 3a – c are all 4-pentenoic acid derivatives, we synthesized the corresponding 4-pentenoic acids (22a – c, Figure 3.8) in high yields (87% – 95%) using established literature methods. In each case, a solution (0.20 M) of psuedoephedrine carboxamide (21a – c) in a 1:1 mixture of t-butyl alcohol (t-BuOH) and methanol (CH\(_3\)OH) was treated with a 2N solution of aqueous NaOH at 75 °C for 4 hours. Following work-up, the corresponding 4-pentenoic acids were isolated as colorless oils. Carboxamides 21a and 21c were synthesized from a pseudoephedrine fluoroacetamide 24 (not shown) according to the literature procedures. Alkylation of 24 with allyl iodide (lithium N,N-bis(trimethylsilyl)amide, LiCl, THF, −78 °C; allyl iodide, −78 °C) provided 21a as a viscous light-yellow oil (89%) after work-up and purification by flash column.
chromatography. Methylation of 24 by a similar procedure (lithium $N,N$-bis(trimethylsilyl)amide, LiCl, THF, $-78^\circ$C; iodomethane, $-50^\circ$C) provided a fluoropropionamide (not shown) in 97% yield. Subsequent alkylation with allyl iodide (LDA, LiCl, THF, $-78^\circ$C; allyl iodide, $-78^\circ$C) provided the 2-fluoro-2-methyl carboxamide $21c$ in 80% yield as a viscous colorless oil. Carboxamide $21b$ was prepared in the same manner (96% yield) from a pseudoephedrine propionamide (not shown) by alkylation with allyl iodide (LDA, LiCl, THF, $-78^\circ$C; allyl iodide, 0°C, 45 min). In addition, 2-fluoro-2-methyl-4-pentenal (23, Figure 3.9) was synthesized in 78% yield from carboxamide $21c$ by reduction with lithium triethoxyaluminum hydride [LiAlH(OEt)$_3$] according to the procedure of Myers.\(^{12}\)
Figure 3.9 Synthesis of 2-fluoro-2-methyl-4-pentenal 23
3.5 References


CHAPTER 4

DISCUSSION AND FUTURE WORK

The primary objective of this work was to develop a synthetic route to three \( \beta \)-aminoketone molecules \( 2a - c \); precursors for a class of novel caspase inhibitors (Figure 3.1, page 30). Although synthesis of the three subtarget molecules was not completed, several fundamental aspects of the synthetic approach were established. First, a general, high yielding procedure for carbon-carbon bond formation was developed involving aza-allyl anion alkylation, substitution and addition. Second, substituted 4-pentenoic acid molecules (\( 3a - c \), Figure 3.7, page 39) necessary for the synthesis of subtargets \( 2a - c \) were prepared in high yield.

4.1 Aza-Allyl Anion Formation

We investigated two \( N \)-methyldiaryl imines in an effort to establish a general, high yielding procedure for carbon-carbon bond formation adjacent to nitrogen in protected amine synthetic equivalents. Using \( N \)-methylbenzophenone imine (5), we conducted deuterium incorporation studies to determine the approximate extent of \( \alpha \)-deprotonation with \( n \)-butyllithium (\( n \)-BuLi) at \(-78 \) °C. We chose to use \( n \)-BuLi as the deprotonating agent because of previous reports by Hullot and Kauffmann who independently investigated the alkylation of aza-allyl anions derived from

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In the only other recorded deuterium incorporation study, Kauffmann established that anion formation, from imine 5, was occurring to a reasonable extent (84% at -60 °C for 30 min) in the presence of lithium diisopropylamide (LDA). Comparing n-BuLi with lithium diisopropylamide (LDA) and lithium diethylamide (LiNEt₂) bases, Hullot and Kauffmann claimed that n-BuLi consumed >10% of the imine by direct addition to the C=N bond, yet their overall alkylation product yields were 20% higher with n-BuLi. In our preliminary deuterium incorporation study we did not observe products resulting from direct nucleophilic addition by n-BuLi and cleanly recovered >92% of combined deuterated and non-deuterated products, in each case. Although this was not intended as a classical kinetic study, it did provide a standard protocol for imine metallation in alkylation, substitution and addition reactions, which proved to be high yielding for both N-methylbenzophenone imine (5) and N-methyl-4-methoxybenzophenone imine (4). With this initial study we clearly demonstrated that aza-allyl anion formation occurs efficiently (>90%) when N-methylbenzophenone imine is directly deprotonated with n-BuLi under closely controlled metallation conditions.

4.2 N-Methyl Imine Alkylation

Our next objective involved the comparative alkylation of aza-allyl anions derived from N-methylbenzophenone imine (5) and N-methyl-4-methoxybenzophenone imine (4). First, using the direct deprotonation protocol established in our deuterium incorporation study and four alkyl halides, we obtained very high isolated yields (77% to 95%) of alkylation products (see Table 3.2, page 34) with N-methylbenzophenone imine.
In previous work, summarized in Table 4.1, Hullot and Kauffmann independently reported that the aza-allyl anion formed from 5 with lithium diisopropylamide or lithium diethylamide bases (LDA or LiNEt₂) in THF at -70 °C to -60 °C result in decent yields (58% - 81%) for primary and secondary alkyl halides, especially when the concentration of HMPA (1,1,1,3,3,3-hexamethylphosphorustriamide) is minimal (compare entry 4 and entry 5).¹ ³

Table 4.1 Reported Alkylation of N-Methylbenzophenone Imine

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions 𝑎</th>
<th>Electrophile 𝑏</th>
<th>Yield (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>CH₃CH₂CH₂Cl</td>
<td>81</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>CH₃(CH₂)₆CH₂Br</td>
<td>64</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>(CH₃)₂CHCH₂Br</td>
<td>69</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>CH₃CH₂(CH₃)CHBr  c</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>CH₃CH₂(CH₃)CHBr</td>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>CH₂=CHCH₂Br</td>
<td>58</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
<td>C₆H₅CH₂Cl</td>
<td>64</td>
<td>2</td>
</tr>
</tbody>
</table>

𝑎 = LiNEt₂ (1.12 equiv) in HMPA-benzene at -70 °C for 1.5 hr; 𝑏 = LDA (1.0 equiv) in THF at -60 °C for 30 min. b¹ 2.0 equiv dissolved in THF. c1.20 equiv dissolved in HMPA.
Compared to this earlier work, we have demonstrated that direct deprotonation of \( \textit{N}\)-methylbenzophenone imine (5) with \( \textit{n}\)-BuLi (in THF) at \(-78 \, ^\circ\text{C}\), results in alkylation product yields that are more than 30% higher than those previously reported. Due to the electron donating effect of a 4-methoxy group, we anticipated that \( \textit{N}\)-methyl imine 4 would be slightly more reactive compared to imine 5 and result in higher yields. However, as shown in Table 3.3 (page 35), the overall yields (80% – 92%) were comparable. Therefore, these results confirmed that efficient aza-allyl anion formation occurs with the deprotonating agent \( \textit{n}\)-BuLi at \(-78 \, ^\circ\text{C}\).

4.3 Substitution and Addition Reactions

Continuing our investigation of the functionalization of protected amine synthetic equivalents, we next focused on acylation reactions. This approach would involve nucleophilic substitution by aza-allyl anions on various carboxylic acid derivatives, such as acid chlorides, anhydrides and esters. Unfortunately, all of our attempts to acylate the anions derived from \( \textit{N}\)-methyl imines 4 and 5 with benzoyl chloride, acetyl chloride or acetic anhydride failed. After numerous unsuccessful reactions using the standard protocol for imine alkylation, we explored other reaction modifications, including longer reaction times (>18 hr after addition of the electrophile), higher temperatures (-60 °C to -45 °C) and complexation of the acylating agent with a Lewis acid. In nearly all attempts, we recovered unreacted starting material and unidentified decomposition products.

In contrast to the acid chlorides and anhydrides, we obtained modest yields of \( \beta\)-iminoketone products (41% - 72%) derived from methyl benzoate and ethyl fluoroacetate (Figure 3.5, page 37). Compared to acid chlorides and anhydrides, esters are...
usually less reactive acylating agents. However, the successful ester substitutions indicate that other side reactions may have been occurring with the former acylating agents. We also found the two crude β-iminoketone products (13 and 14) to be unstable for extended periods of time. In fact, the 3-fluoro-2-ketoimine 14 was extremely unstable and significant yields were difficult to isolate. In spite of this, the successful acylation of an aza-allyl anion with ethyl fluoroacetate is a very important result because subtargets 2a and 2c (Figure 3.1, page 30) are presumably available from substituted 3-fluoro-2-ketoimines derived from fluorinated acid derivatives (see Figure 3.7, page 39).

Our final objective in developing an effective procedure for carbon-carbon bond formation in protected amine equivalents, involves aza-allyl anion addition reactions. In this approach we treated aza-allyl anions derived from N-methyl imines 4 and 5 with four aldehyde electrophiles. As shown in Table 3.4 (page 38), we generally observed decent overall reactivity with aldehydes. The highest percent conversions, from starting material to product, were observed with the pivaldehyde (84% and 92%), crotonaldehyde (79%) and benzanaldehyde (70%) electrophiles. In contrast, the reaction with butanaldehyde was only 50% – 60%. Of these four compounds, only butanaldehyde contains acidic α-hydrogens. In the butanaldehyde addition reactions of anions derived from 4 and 5 (Table 3.4, entries 1 and 2, page 38), the aza-allyl anion likely competed as both a base (deprotonation at the α-carbon) and as a nucleophile, resulting in low yields (37% - 46%) of β-imino alcohol products 15 and 16. For all of our imine substitution and addition reactions we found high-vacuum (50 µTorr) Kugel-Rohr distillation to be overall the most effective method for product isolation, but as indicated in Table 3.4 only significant yields of products 17 (89%) and 19 (76%) were obtained. Hullot and Cuvigny have
reported the best known examples of N-methylbenzophenone imine addition reactions with aldehydes and ketones (Figure 4.1).²

![Figure 4.1 Addition reactions of N-methylbenzophenone imine (5) with (a) benzaldehyde and (b) benzophenone](image)

These authors reported isolated yields of ~60% as optimum using n-BuLi as the base (at -70 °C) in THF with 10% HMPA added as a co-solvent.² In contrast, they found that the use of an HMPA co-solvent was not as effective for improving yields in the substitution reactions. In fact, they reported that increasing the ratio of THF in the reaction mixture by dissolving the alkyl halide in THF (vs. HMPA) resulted in a dramatic increase in product yield (see Table 4.1 entries 4 and 5).² Overall, these results indicate that aldehyde electrophiles are comparable in yield to the previously described ester electrophiles (although they required longer reaction times).

To conclude the first part of the main objective of this thesis work, we employed deuterium labeling studies as well as alkylation, substitution and addition reactions with
aza-allyl anions derived from two N-methyl imines, to establish the framework of a synthetic procedure for preparing three \( \beta \)-aminoketone molecules. A general, high yielding method for carbon-carbon bond formation and protected amine functionalization was developed that uses a direct deprotonation protocol and carefully controlled reaction conditions. Based on our results, the metallation of non-activated diarylimines 4 and 5 with \( n \)-BuLi (in THF) at -78 °C (1 hr then 0 °C for 5 min) is overall a more effective procedure than those previously described.

4.4 Pentenoic Acid Analogs

The second goal in developing a successful synthetic route to the three \( \beta \)-aminoketone molecules 2a – c (Figure 3.1, page 30) consists of preparing substituted 4-pentenoic acid derivatives 3a – c (Figure 3.7, page 39). Accordingly, all three of the corresponding 4-pentenoic acids (22a – c) as well as 2-fluoro-2-methyl-4-pentenal (23) were successfully synthesized in high yields from commercially available materials. In addition, we prepared acid chloride derivatives corresponding to 3a and 3b (\( X = \text{Cl} \)) in excellent yield (92% and 96% respectively). Since this group of subunit molecules 3a - c provide the structural features responsible for increased specificity and stability in the final inhibitor products (see Figures 1.1, 1.2 and Chapter 2.2), these derivatives are critical to the long range goal of caspase inhibitor synthesis. Molecules 3a and 3c are fluorinated while 3b is not and the inhibitors ultimately derived from these precursors will be used to determine whether a quaternary-, tertiary-, or non-fluorinated center affects inhibitor specificity and potency.
4.5 Future Work

The major objective of this research project is construction and bioanalysis of a homologous series of peptide-based caspase inhibitors which are designed to improve inhibitor specificity, stability and potency. Once these new inhibitors have been constructed we will perform preliminary tests with commercial recombinant caspase enzymes known to be involved in apoptosis. Successful construction of the new inhibitors requires preparation of subunits 2a - c, oxidation of the alkene moiety, and coupling on both the N-terminal and C-terminal ends with various combinations of amino acid residues. The amino acid residues selected for incorporation will be chosen on the basis of established specificities in serine proteases (similar to cysteine proteases) and in caspases known to affect apoptosis (e.g. caspases 3, 8 and 9).

Immediate future work will focus on investigating the reaction of N-methyl imine aza-allyl anions with various derivatives of the three 4-pentenoic acids (22a – c). Although we found substitution reactions with acid chlorides to be problematic, we were able to successfully form β-iminoketone products from ester derivatives. Therefore, preparation of alkyl 4-pentenoate esters (3a – c where X = OR) and their reaction with N-methyl imine 4 or 5 is a top priority. Also important is the synthesis of two other 4-pentenaldehyde derivatives (3a and 3b where X = H) and subsequent N-methyl imine addition reactions.
4.6 References


CHAPTER 5

EXPERIMENTAL

5.1 Materials and Methods

All reactions were performed in oven or flame-dried glassware under an atmosphere of dry argon unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe or cannula. Proton and carbon nuclear magnetic resonance (\(^1\)H NMR and \(^{13}\)C NMR) spectra were recorded at 400 MHz and 100 MHz respectively. Infrared (FTIR) spectra were obtained as neat films unless otherwise specified. Flash column chromatography was performed as described by Still\(^1\) employing 230 - 400 mesh silica gel.

Commercial reagents and solvents were used as received with the following exceptions. Tetrahydrofuran and diethyl ether were distilled from sodium-benzophenone ketyl. Dichloromethane, diisopropylamine, \(N,N\)-bis(trimethylsilyl)amine, ethyl acetate, xylene and hexane were distilled from calcium hydride under an atmosphere of nitrogen. Methanol and 2-butanol were distilled from magnesium under an atmosphere of argon. Pentane was purified by passage through a column of silica gel followed by distillation from sodium hydride under an atmosphere of nitrogen. Benzoyl chloride was distilled from calcium chloride under an atmosphere of nitrogen. Acetyl chloride was distilled from thionyl chloride under an atmosphere of nitrogen. Acetic anhydride was distilled
from phosphorus pentoxide under an atmosphere of nitrogen. Benzyl bromide, isobutyl bromide, allyl bromide, iodomethane, methyl benzoate, ethyl fluoroacetate and benzaldehyde were passed through neutral alumina (activity II) immediately prior to use. The molarity of n-butyllithium was determined by titration against a standard solution of 2-butanol in xylenes using 1,10-phenanthroline as an indicator (average of three determinations). A standardized solution of titanium tetrachloride in pentane (1.25 M) was prepared by dilution of commercial titanium tetrachloride in freshly distilled pentane under an atmosphere of argon.

5.2 Imine Synthesis and Alkylation

\textbf{N-Methyl-4-methoxybenzophenone imine (4):} Prepared according to a modified Weingarten procedure. An oven-dried 100-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum, was charged with 4-methoxybenzophenone (4.14 g, 19.5 mmol, 1 equiv) and methylamine (2.00 M in tetrahydrofuran, 48.8 mL, 97.6 mmol, 5.0 equiv), then purged with argon. The resulting suspension was cooled to 0 °C and titanium tetrachloride (1.25 M in pentane, 9.37 mL, 11.7 mmol, 0.6 equiv) was added dropwise by syringe. The mixture was stirred at 0 °C for an additional 1.5 hr, refluxed for 3 hr and then permitted to stir at 23 °C. After 12 hr the mixture was partitioned between ether (50 mL) and water (50 mL). The aqueous layer was separated and extracted further with three 10-mL portions of ether. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated to furnish a viscous light-yellow oil (3.90 g, 89%). $^1$H NMR (CDCl$_3$) (1:1.5 mixture of Z/E diastereomers, * denotes Z isomer peaks):
δ 7.86-6.78 (m, 18H), 3.89 (s, 3H), 3.81* (s, 3H), 3.31 (s, 3H), 3.21* (s, 3H); 13C NMR (CDCl3): δ 168.9, 163.1, 136.7, 132.6, 129.6, 128.4, 128.2, 128.1, 127.9, 113.2, 55.1, 41.1; FTIR: 3056 (m), 3001 (m, aromatic C-H), 2934 (m), 2909 (m), 2836 (m, alkane C-H), 1606 (s, C=N), 1250 (s), 1034 (m) cm⁻¹.

A-Methylbenzophenone imine (5): Prepared according to a modified O’Donnell procedure.⁴ An oven-dried 100-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum, was charged with a solution of methylamine hydrochloride (0.675 g, 10.0 mmol, 1 equiv) in dichloromethane (40 mL) and purged with argon. A solution of benzophenone imine (1.81 g, 10.0 mmol, 1 equiv) in dichloromethane (5 mL) was added dropwise via syringe and the resulting mixture was stirred at 23 °C under a dry atmosphere (argon passed through a calcium sulfate drying tube). After four days the mixture was partitioned between ether (50 mL) and water (50 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated to furnish a viscous yellow oil (1.86 g, 95%). 1H NMR (CDCl3): δ 7.60-7.15 (m, 10H), 3.26 (s, 3H); 13C NMR (CDCl3): δ 169.5, 140.0, 136.7, 129.9, 128.6, 128.4, 128.3, 128.1, 127.9, 41.6; FTIR: 3057 (m), 3024 (m, aromatic C-H), 2945 (m), 2913 (m), 2859 (m, alkane C-H), 1626 (s, C=N) cm⁻¹.

N-Ethylbenzophenone imine (6): An oven-dried 25-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum was charged with tetrahydrofuran (10 mL) and purged with argon. After cooling to −78 °C, n-butyllithium (2.21 M in hexane, 2.91 mL, 6.43 mmol, 1.20 equiv) was added by syringe. A solution of imine 5 (1.05 g, 5.36 mmol, 1 equiv) in tetrahydrofuran (20 mL) was transferred dropwise by...
syringe, over a 30 minute period. Upon completion of the addition, the red-brown suspension was stirred at \(-78^\circ\text{C}\) for 1 hr, at 0 °C for 5 min and then cooled to \(-78^\circ\text{C}\). Iodomethane (1.52 g, 10.7 mmol, 2.0 equiv) was added to the reaction flask by syringe and stirring was continued at \(-78^\circ\text{C}\) for 4.5 hr. The reddish-brown mixture was warmed to 23 °C and then partitioned between dichloromethane (6 mL) and water (6 mL). The aqueous layer was separated and further extracted with three 6-mL portions of dichloromethane. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated. The resulting orange oil was purified by flash chromatography (10% ethyl acetate in hexane) to provide a clear yellow oil (0.872g, 78%). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 7.62-7.00 (m, 10H), 3.40 (q, \(J = 7.3\) Hz, 2H), 1.25 (t, \(J = 7.2\) Hz, 3H); \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 167.6, 140.0, 136.9, 132.4, 130.0, 129.7, 128.4, 128.3, 127.9, 127.7, 48.2, 16.3; FTIR: 3056 (m), 3024 (m, aromatic C-H), 2954 (s), 2858 (m, alkane C-H), 1625 (s, C=N) cm\(^{-1}\).

\(N\)-(3-Methylbutyl)benzophenone imine (7): An oven-dried 50-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum was charged with tetrahydrofuran (18 mL) and purged with argon. After cooling to \(-78^\circ\text{C}\), \(n\)-butyllithium (2.75 M in hexane, 3.29 mL, 9.05 mmol, 1.25 equiv) was added by syringe. A solution of imine 5 (1.41 g, 7.24 mmol, 1 equiv) in tetrahydrofuran (14 mL) was transferred dropwise by syringe, over a 30 minute period. Upon completion of the addition, the red-brown suspension was stirred at \(-78^\circ\text{C}\) for 1 hr, at 0 °C for 5 min and then cooled to \(-78^\circ\text{C}\). Isobutyl bromide (1.18 g, 10.9 mmol, 1.5 equiv) was added to the reaction flask by syringe and stirring was continued at \(-78^\circ\text{C}\) for 4.5 hr. The reddish-brown mixture was warmed to 23 °C and then partitioned between dichloromethane (6 mL) and water.
(6 mL). The aqueous layer was separated and further extracted with three 6-mL portions of dichloromethane. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated. The resulting orange oil was purified by high-vacuum (50 μTorr) Kugel-Rohr distillation to provide a viscous orange oil (1.73 g, 95%). $^1$H NMR (CDCl$_3$): δ 7.82-7.0 (m, 10H), 3.39 (t, J = 7.21 Hz, 2H), 1.68 (m, 1H), 1.57 (q, J = 7.22 Hz, 2H), 0.83 (d, J = 6.50 Hz, 6H); $^{13}$C NMR (CDCl$_3$): δ 167.6, 140.0, 137.0, 129.6, 128.3, 128.2, 128.1, 52.1, 40.2, 24.9, 22.6; FTIR: 3058 (m), 3025 (m, aromatic C-H), 2954 (s), 2867 (m, alkane C-H), 1624 (s, C=N) cm$^{-1}$.

$N$-(3-Butenyl)benzophenone imine (8): The alkylation of $N$-methylbenzophenone imine (1.65 g, 8.45 mmol, 1 equiv) in tetrahydrofuran (40 mL) with allyl bromide (1.53 g, 12.7 mmol, 1.5 equiv) was carried out as described for 7. Purification of the resulting residue by high-vacuum (50 μTorr) Kugel-Rohr distillation provided a viscous yellow oil (1.53 g, 77%). $^1$H NMR (CDCl$_3$): δ 7.60-7.02 (m, 10H), 5.88-4.84 (m, 3H), 3.43 (t, J = 7.16 Hz, 2H), 2.45 (q, J = 7.03 Hz, 2H); $^{13}$C NMR (CDCl$_3$): δ 167.9, 136.9, 129.8, 128.6, 128.3, 128.0, 127.8, 127.9, 127.2, 126.0, 115.6, 53.2, 35.5; FTIR: 3057 (m), 3023 (m, aromatic C-H), 2954 (s), 2857 (m, alkane C-H), 1623 (s, C=N) cm$^{-1}$.

$N$-(2-Phenylethyl)benzophenone imine (9): The alkylation of $N$-methylbenzophenone imine (1.38 g, 7.09 mmol, 1 equiv) in tetrahydrofuran (28 mL) with benzyl bromide (1.27 g, 10.6 mmol, 1.5 equiv) was carried out as described for 7. Purification of the resulting residue by high-vacuum (50 μTorr) Kugel-Rohr distillation provided a viscous yellow oil (1.63 g, 81%). $^1$H NMR (CDCl$_3$): δ 7.67-6.79 (m, 15H), 3.63 (t, J = 7.26 Hz, 2H), 2.99 (t, J = 7.26 Hz, 2H); $^{13}$C NMR (CDCl$_3$): δ 168.8, 140.3, 139.8, 136.7, 129.6,
128.9, 128.2, 128.1, 127.8, 127.5, 125.8, 55.3, 37.5; FTIR: 3057 (m), 3024 (m, aromatic C-H), 2945 (m), 2913 (m), 2859 (m, alkane C-H), 1626 (s, C=N) cm⁻¹.

*N-(3-Methylbutyl)-4-methoxybenzophenone imine* (10): An oven-dried 50-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum was charged with tetrahydrofuran (11 mL) and purged with argon. After cooling to −78 °C, *n*-butyllithium (2.42 M in hexane, 2.37 mL, 5.74 mmol, 1.25 equiv) was added by syringe. A solution of *N*-methyl-4-methoxybenzophenone imine (1.03 g, 4.59 mmol, 1 equiv) in tetrahydrofuran (10 mL) was transferred dropwise by syringe, over a 30 minute period. Upon completion of the addition, the red-brown suspension was stirred at −78 °C for 1 hr, at 0 °C for 5 min and then cooled to −78 °C. Isobutyl bromide (0.94 g, 6.89 mmol, 1.5 equiv) was added to the reaction flask by syringe and stirring was continued at −78 °C for 21 hr. The reddish-brown mixture was warmed to 23 °C and then partitioned between dichloromethane (6 mL) and water (6 mL). The aqueous layer was separated and further extracted with three 6-mL portions of dichloromethane. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated. The resulting orange oil was purified by high-vacuum (50 μTorr) Kugel-Rohr distillation to provide a viscous orange oil (1.05 g, 81%). ¹H NMR (CDCl₃) (1:1.4 diastereomer ratio, * denotes minor diastereomer peaks): δ 7.62-6.77 (m, 9H), 3.87 (s, 3H), 3.81* (s, 3H), 3.42 (t, J = 7.05 Hz, 2H), 3.35 (q, J = 7.05 Hz, 2H), 1.69-1.49 (m, 1H), 0.84 (d, J = 6.83 Hz, 3H); ¹³C NMR (CDCl₃): δ 168.0, 160.9, 137.3, 129.7, 129.5, 128.3, 128.0, 127.9, 113.3, 55.2, 40.3, 26.7, 22.8; FTIR: 3056 (m), 2998 (m, aromatic C-H), 2954 (s), 2866 (s, alkane C-H), 1603 (s, C=N), 1248 (s), 1036 (m) cm⁻¹.
N-(3-Butenyl)-4-methoxybenzophenone imine (11): The alkylation of N-methyl-4-methoxybenzophenone imine (1.38 g, 5.98 mmol, 1 equiv) in tetrahydrofuran (26 mL) with allyl bromide (1.09 g, 8.98 mmol, 1.5 equiv) was carried out as described for 10 (−78 °C for 46 hrs). Purification of the resulting residue by high-vacuum (50 μTorr) Kugel-Rohr distillation provided a viscous yellow oil (1.36 g, 86%). \(^1\)H NMR (CDCl\(_3\)) (1:1.8 diastereomer ratio, * denotes minor diastereomer peaks): \(\delta\) 7.60-6.73 (m, 9H), 5.85-4.81 (m, 3H), 3.86 (s, 3H), 3.81* (s, 3H), 3.49 (t, \(J = 7.05\) Hz, 2H), 3.41 (q, \(J = 7.05\) Hz, 2H); \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 167.6, 161.0, 136.9, 132.8, 130.2, 128.4, 128.1, 116.0, 113.7, 113.3, 55.3, 54.1, 53.2, 35.7; FTIR: 3060 (m), 3000 (m, aromatic C-H), 2954 (s), 2931 (s), 2836 (m, alkane C-H), 1603 (s, C=N), 1249 (s), 1034 (m) cm\(^{-1}\).

N-(2-Phenylethyl)-4-methoxybenzophenone imine (12): The alkylation of N-methyl-4-methoxybenzophenone imine (1.96 g, 8.69 mmol, 1 equiv) in tetrahydrofuran (36 mL) with benzyl bromide (2.23 g, 13.0 mmol, 1.5 equiv) was carried out as described for 10 (−78 °C for 19 hrs). Purification of the resulting residue by high-vacuum (50 μTorr) Kugel-Rohr distillation provided a viscous yellow oil (2.76 g, 80%). \(^1\)H NMR (CDCl\(_3\)) (1:1.2 diastereomer ratio, * denotes minor diastereomer peaks): \(\delta\) 7.64-6.79 (m, 14H), 3.84 (s, 3H), 3.81* (s, 3H), 3.68 (t, \(J = 7.05\) Hz, 2H), 3.61 (t, \(J = 7.05\) Hz, 2H); \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 168.1, 161.2, 140.3, 132.5, 129.9, 129.7, 129.2, 129.0, 128.4, 128.1, 127.8, 127.6, 125.8, 113.4, 55.2, 37.7; FTIR: 3024 (m, aromatic C-H), 2931 (s, alkane C-H), 1602 (s, C=N), 1248 (s), 1030 (m) cm\(^{-1}\).
5.3 Imine Substitution and Addition

*N-(2-Oxo-2-phenylethyl)benzophenone imine* (13): An oven-dried 25-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum was charged with tetrahydrofuran (10 mL) and purged with argon. After cooling to -78 °C, n-butyllithium (2.21 M in hexane, 2.78 mL, 6.15 mmol, 1.20 equiv) was added by syringe. A solution of imine 5 (1.06 g, 5.12 mmol, 1 equiv) in tetrahydrofuran (30 mL) was transferred dropwise by syringe, over a 30 minute period. Upon completion of the addition, the red-brown suspension was stirred at -78 °C for 1 hr, at 0 °C for 5 min and then cooled to -78 °C. Methyl benzoate (0.697 g, 5.12 mmol, 1.0 equiv) was added to the reaction flask by syringe and stirring was continued at -78 °C for 6 hr. The reddish-brown mixture was warmed to 23 °C and then partitioned between dichloromethane (5 mL) and water (5 mL). The aqueous layer was separated and further extracted with three 5-mL portions of dichloromethane. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated. The resulting orange oil was purified by flash chromatography (10% ethyl acetate in hexane) to provide a viscous yellow oil (1.10g, 72%). $^1$H NMR (CDCl$_3$): $\delta$ 8.10 -7.00 (m, 15H), 4.91 (s, 2H).

*N-(2-Oxo-3-fluoropropyl)benzophenone imine* (14): An oven-dried 25-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum was charged with tetrahydrofuran (10 mL) and purged with argon. After cooling to -78 °C, n-butyllithium (2.65 M in hexane, 4.87 mL, 12.9 mmol, 1.25 equiv) was added by syringe. A solution of imine 5 (2.02 g, 10.3 mmol, 1 equiv) in tetrahydrofuran (40 mL) was transferred dropwise by cannula, over a 30 minute period. Upon completion of the
addition, the red-brown suspension was stirred at −78 °C for 1 hr, at 0 °C for 5 min and then cooled to −78 °C. Ethyl fluoroacetate (1.31 g, 12.4 mmol, 1.2 equiv) was added to the reaction flask by syringe and stirring was continued at −78 °C for 10 hr. The orange mixture was warmed to 23 °C and then partitioned between dichloromethane (10 mL) and water (10 mL). The aqueous layer was separated and further extracted with three 10-mL portions of dichloromethane. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated. The resulting orange oil was purified by flash chromatography (10% ethyl acetate in hexane) to provide a clear yellow oil (1.07 g, 41%). \(^1\)H NMR (CDCl\(_3\)): δ 7.65-7.10 (m, 1OH), 4.80 (d, \(J_{HF} = 47.8\) Hz, 2H), 2.52 (dt, \(J_{HF} = 3.8\) Hz, \(J = 7.2\) Hz, 3H).

\(N\)-(2-Hydroxypentyl)benzophenone imine (15): An oven-dried 25-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum was charged with tetrahydrofuran (8 mL) and purged with argon. After cooling to −78 °C, \(n\)-butyllithium (2.50 M in hexane, 1.5 mL, 3.75 mmol, 1.25 equiv) was added by syringe. A solution of imine 5 (0.586 g, 3.00 mmol, 1 equiv) in tetrahydrofuran (6 mL) was transferred dropwise by syringe, over a 30 minute period. Upon completion of the addition, the red-brown suspension was stirred at −78 °C for 1 hr, at 0 °C for 5 min and then cooled to −78 °C. Butyraldehyde (0.324 g, 4.50 mmol, 1.5 equiv) was added to the reaction flask by syringe and stirring was continued at −78 °C for 16 hrs. The reddish-brown mixture was warmed to 23 °C and then partitioned between dichloromethane (6 mL) and water (6 mL). The aqueous layer was separated and further extracted with three 6-mL portions of dichloromethane. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated.
The resulting orange oil was purified by high-vacuum (50 μTorr) Kugel-Rohr distillation to provide a viscous orange oil (0.635 g, 46%). $^1$H NMR (CDCl$_3$): $\delta$ 7.82-6.98 (m, 10H), 3.90 (m, 1H), 3.43 (m, 1H), 3.21 (m, 1H), 2.57 (m, 1H), 2.38 (m, 1H), 1.40 (m, 2H), 0.98 (t, $J$ = 7.42 Hz, 3H); $^{13}$C NMR (CDCl$_3$): $\delta$169.3, 143.3, 130.0, 128.9, 128.5, 128.3, 128.0, 127.6, 127.2, 126.7, 71.2, 53.8, 20.4, 14.1; FTIR: 3409 (br, m, OH), 3057 (m, aromatic C-H), 2956 (s), 2929 (s), 2869 (s, alkane C-H), 1624 (s, C=Ν cm$^{-1}$.

$N$-(2-Hydroxypentyl)-4-methoxybenzophenone imine (16): An oven-dried 25-mL round-bottomed flask equipped with a magnetic stirring bar and a rubber septum was charged with tetrahydrofuran (9 mL) and purged with argon. After cooling to −78 °C, n-butyllithium (2.50 M in hexane, 1.78 mL, 4.46 mmol, 1.25 equiv) was added by syringe. A solution of $N$-methyl-4-methoxybenzophenone imine (0.91 g, 3.56 mmol, 1 equiv) in tetrahydrofuran (7 mL) was transferred dropwise by syringe, over a 30 minute period. Upon completion of the addition, the red-brown suspension was stirred at −78 °C for 1 hr, at 0 °C for 5 min and then cooled to −78 °C. Butyraldehyde (0.480 g, 5.35 mmol, 1.5 equiv) was added to the reaction flask by syringe and stirring was continued at −78 °C for 15 hr. The reddish-brown mixture was warmed to 23 °C and then partitioned between dichloromethane (6 mL) and water (6 mL). The aqueous layer was separated and further extracted with three 6-mL portions of dichloromethane. The combined organic layers were washed with a saturated aqueous sodium chloride solution, then dried over anhydrous sodium sulfate and concentrated. The resulting orange oil was purified by high-vacuum (50 μTorr) Kugel-Rohr distillation to provide a viscous orange oil (0.44 g, 37%) as a mixture of four diastereomers: $[E,2(R)]$, $[E,2(S)]$, $[Z,2(R)]$ and $[Z,2(S)]$; FTIR:
3056 (m), 3001 (w, aromatic C-H), 2955 (s), 2836 (m, alkane C-H), 1607 (s, C=N), 1253 (s), 1036 (m) cm⁻¹.

**N-(2-Hydroxy-3,3-dimethylbutyl)benzophenone imine (17):** The alkylation of *N*-methylbenzophenone imine (0.781 g, 4.00 mmol, 1 equiv) in tetrahydrofuran (18 mL) with trimethylacetaldehyde (0.648 g, 6.00 mmol, 1.5 equiv) was carried out as described for 15 (−78 °C for 20 hrs). Purification of the resulting residue by high-vacuum (50 μTorr) Kugel-Rohr distillation provided a viscous yellow oil (1.01 g, 89%). "H NMR (CDCl₃): δ 7.68-7.07 (m, 1OH), 3.58 (t, J = 8.0 Hz, 1H), 3.60 (dd, J = 8.0 Hz, 2H), 0.91 (s, 9H); "C NMR (CDCl₃): δ 167.9, 144.4, 130.1, 129.7, 128.6, 128.1, 127.8, 126.4, 126.0, 125.7, 84.4, 53.9, 26.7; FTIR: 3409 (br, m, OH), 3059 (m, aromatic C-H), 2954 (s), 2867 (s, alkane C-H), 1624 (s, C=N), 1248 (s, C-O) cm⁻¹.

**N-(2-Hydroxy-3,3-dimethylbutyl)-4-methoxybenzophenone imine (18):** The alkylation of *N*-methyl-4-methoxybenzophenone imine (1.38 g, 7.09 mmol, 1 equiv) in tetrahydrofuran (33 mL) with trimethylacetaldehyde (1.26 g, 10.6 mmol, 1.5 equiv) was carried out as described for 16 (−78 °C for 23 hrs). The crude product was isolated as a viscous yellow oil (1.79 g, 110% crude yield; 84% conversion to product by GC analysis) as a mixture of four diastereomers: [E,2(R)], [E,2(S)], [Z,2(R)] and [Z,2(S)]; FTIR: 2953 (s, aromatic C-H), 2869 (m, alkane C-H), 1609 (s, C=N), 1248 (s), 1034 (m) cm⁻¹.

**N-(3E')-N-(2-Hydroxy-3-pentenyl)benzophenone imine (19):** The alkylation of *N*-methylbenzophenone imine (0.390 g, 2.00 mmol, 1 equiv) in tetrahydrofuran (4 mL) with crotonaldehyde (0.210 g, 3.00 mmol, 1.5 equiv) was carried out as described for 15 (−78 °C for 24 hrs). Purification of the resulting residue by high-vacuum (50 μTorr) Kugel-Rohr distillation provided a viscous yellow oil (0.404 g, 76%). "H
NMR (CDCl₃): δ 7.80-6.98 (m, 10H), 5.90-5.40 (m, 2H), 3.51-2.98 (m, 3H), 1.74-1.40 (d, J = 7.0 Hz, 3H); FTIR: 3312 (br, m, OH), 3057 (m, aromatic C-H), 2954 (s, alkane C-H), 1624 (s, C=N), 1027 (m, C=O) cm⁻¹; ¹³C NMR (CDCl₃): δ 139.2, 136.9, 131.4, 129.7, 128.5, 128.4, 128.2, 128.0, 127.8, 126.0, 110.6, 72.3, 59.6, 52.3, 17.8.

N-(2-Hydroxy-2-phenylethyl)benzophenone imine (20): The alkylation of N-methylbenzophenone imine (0.781 g, 4.00 mmol, 1 equiv) in tetrahydrofuran (4 mL) with benzaldehyde (0.637 g, 6.00 mmol, 1.5 equiv) was carried out as described for 15 (−78 °C for 24 hrs). The crude product was isolated as a viscous yellow oil (1.633 g, 135% crude yield; 70% conversion to product by ¹H NMR analysis). Recrystallization from hexane yielded a white crystalline solid (0.390 g, 32%). ¹H NMR (CDCl₃): δ 7.80-6.78 (m, 15H), 3.63-3.53 (dd, J = 3.6 Hz, 1H), 3.52-3.35 (m, 2H).

5.4 Pentenoic Acid Analog Synthesis

[IS(R),2S]-N-(2-Hydroxy-1-methyl-2-phenylethyl)-N-methyl-2-methyl-4-pentenamide (21b): Prepared from (IS,2S)-N-(2-hydroxy-1-methyl-2-phenylethyl)-N-methylpropionamide (1.00 g, 4.52 mmol, 1 equiv) according to the procedure of Myers using allyl iodide (1.14 g, 6.78 mmol, 1.50 equiv) in tetrahydrofuran (0.25 M, 0 °C for 45 min). The product was obtained as a viscous colorless oil (1.13 g, 96%) after purification by flash chromatography (25% ethyl acetate in hexane). ¹H NMR (CDCl₃) (~1:4 rotamer ratio, * denotes minor rotamer peaks, C₆D₆): δ 7.45-7.30 (m, 2H), 7.30-7.00 (m, 8H), 6.00* (m, 1H), 5.72 (ddt, J = 17.1, 14.0, 7.0 Hz, 1H), 5.25* (d, J = 17.0 Hz, 1H), 5.12* (d, J = 14.0 Hz, 1H), 5.10 (d, J = 17.0 Hz, 1H), 5.00 (d, J = 14.0 Hz, 1H), 4.85* (br, s, 1H), 4.60 (dd, J = 7.0 Hz, 1H), 4.37 (br, s, 1H), 2.92* (s,
$\text{H}, 2.51 \text{ (m, 1H)}, 2.36 \text{ (m, 2H)}, 2.34 \text{ (m, 3H)}, 2.09 \text{ (m, 1H)}, 1.17^* \text{ (d, } J = 6.7 \text{ Hz, 3H)},
1.61 \text{ (two superimposed, } J = 6.7 \text{ Hz, 6H)}, 0.78^* \text{ (d, } J = 6.8 \text{ Hz, 3H); } ^{13}\text{C NMR (C}_{6}\text{D}_{6}): \delta
177.4, 176.4^*, 143.7, 142.6^*, 137.6^*, 136.7, 128.6, 127.4, 127.3, 126.8, 116.3, 76.4,
75.5^*, 59.2, 58.1, 38.6, 36.7, 35.9^*, 32.9^*, 27.1, 17.7^*, 17.1, 15.4^*, 14.3; \text{FTIR: 3363 (br,}
\text{s, OH), 2974 (s), 1606 (s, C=O), 1451 (s), 1403 (s), 1245 (m), 1022 (m), 914 (s) cm}^{-1}.$

(R)-2-Methyl-4-pentenoic acid (22b): Prepared from 21b (5.00 g, 22.6 mmol) according
to the procedure of Myers. The product was obtained as a colorless oil (2.27 g, 88%).
$^1\text{H NMR (CDCl}_3): \delta 5.79 \text{ (ddt, } J = 17.1, 14.0, 7.0 \text{ Hz, 1H)}, 5.10 \text{ (d, } J = 17.0 \text{ Hz, 1H)}, 4.95$
(d, $J = 14.0 \text{ Hz, 1H}), 2.56 \text{ (m, 2H)}, 2.43 \text{ (m, 1H)}, 2.22 \text{ (m, 1H), 1.20 \text{ (d, } J = 7.0 \text{ Hz, 3H)};}
^{13}\text{C NMR (CDCl}_3): \delta 182.0, 135.2, 116.9, 39.1, 37.4, 16.2; \text{FTIR: 3400-2600 (br, s), 2982}
\text{(s), 1709 (s, C=O), 1643 (m), 1463 (m), 1418 (m), 1286 (s), 1246 (s), 918 (s) cm}^{-1}.$

(R)-2-Fluoro-2-methyl-4-pentenal (23): Prepared from [\((S,R,2S)\]-N-(2-hydroxy-1-
 methyl-2-phenylethyl)-N-methyl-2-fluoro-2-methyl-4-pentenamide (2.40 g, 8.59 mmol)
according to the procedure of Myers. The product was obtained as a colorless oil (1.10 g,
78%). $^1\text{H NMR (CDCl}_3): \delta 7.91 \text{ (br, s, 1H)}, 5.82 \text{ (ddt, } J = 17.1, 14.0, 7.0 \text{ Hz, 1H)}, 5.22$
(m, 2H), 2.65 (m, 2H), 1.61 (d, $\text{J}_{HF} = 21.0 \text{ Hz, 3H})$. 

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5.5 References


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