LIGANDS FOR THE SIGMA RECEPTOR AND THE $\mu$-OPIOID RECEPTOR

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by

YU LU

Dr. Susan Z. Lever, Thesis Supervisor

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

LIGANDS FOR THE SIGMA RECEPTOR AND THE μ-OPIOID RECEPTOR

presented by Yu Lu,

a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

Professor Susan Z. Lever

Professor Kent S. Gates

Professor John R. Lever
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ABSTRACT

The sigma receptor is a unique receptor family with two subtypes: sigma-1 and sigma-2. Both of the two subtype receptors are widely distributed in the central nervous system (CNS). Sigma-1 receptors are found to be related to several central nervous system diseases, while sigma-2 receptors are believed to be affiliated with the regulation of cell proliferation.

To date, a large number of ligands for both sigma subtypes have been explored. Pharmacophore models have also been defined to provide the guidance for ligand synthesis and binding studies. In order to conduct structure-activity relationship studies (SAR), a series of specific 1-phenylpropyl-4-benzylpiperidine and 1-phenylpropyl-4-benzylpiperazine analogues were synthesized. Sigma receptors binding assays were also conducted for the piperidine analogues.

\[ \text{R}= 4\text{-OCH}_3, 3\text{-OCH}_3, 4\text{-CH}_3, 3\text{-F, 2-Br, 3-NO}_2, 2\text{-NO}_2, 4\text{-NO}_2, 3\text{-I} \]
The last chapter is concerned with a different receptor family, the opioid receptors. The opioid receptors belong to the family of G-protein coupled receptors and have three subtypes: mu(μ), delta(δ), and kappa(κ). μ-opioid receptor is believed to mediate the analgesia and drug addiction by morphine and other opioids. Many synthetic peptides, such as Tyr-\textit{d}-Arg-Phe-Lys-NH₂, Tyr-\textit{d}-Arg-Phe-Ala-NH₂ and Dmt-\textit{d}-Ala-Phe-Phe-NH₂ have been investigated as selective μ-opioid ligands. In order to obtain μ-opioid tetrapeptide ligand I-Dmt-\textit{d}-Ala-Phe-Orn-NH₂ with high affinity and proper lipophilicity, mono-iodination reaction was studied on the 2,6-dimethyl-\textit{l}-tyrosine residue.
Chapter 1 Introduction of the Sigma Receptor

1.1 History and Identification of the Sigma Receptor

The sigma receptor was initially proposed as a novel subtype of opioid receptors (σ-opioid receptor) by Martin et al. in 1976. Nevertheless, the factor that none of the classical opioid antagonists was able to block the effect of sigma ligand N-allyl-normetazocine (+)-SKF-10047 (Figure 1-1) raised the question whether sigma receptor is a unique binding site.2, 3

![Structure of (+)-SKF-10047](image)

**Figure 1-1. Structure of (+)-SKF-10047**

Subsequent studies found that the sigma receptor has a high stereoselectivity for the (+)-isomer of SKF-10047 as well as other (+)-benzomorphans against their (-)-enantiomers, which was opposite to that seen with all opioid receptors subtypes in either binding assays or behavioral tests.4 The identification of sigma receptor was further confused with the binding site of phencyclidine (PCP) of the N-methyl-D-aspartate (NMDA) receptor channel, since the only available specific sigma ligand at that time, (+)SKF-10047, had an appreciable affinity for the PCP site as well.5, 6 Later on, the development of other selective sigma ligands led to more site-selective sigma ligands, among which (+)-pentazocine is one of the compounds showing no cross-reactivity with PCP sites.7 Further selective ligand binding studies
in 1992 finally led to the recognition of sigma receptor as a non-opioid, non-dopamine, non-PCP receptor.8

1.2 Sigma Receptor Subtypes: Sigma-1, Sigma-2, or More

Based on the pharmacological profile of several sigma ligands, which have heterogeneous chemical structures, sigma receptors were further divided into two subtypes: sigma-1 and sigma-2.8,9 Basically, the sigma-1 receptor is believed to have the ligand binding profile such that (+)-benzomorphans are at least 5-10 fold more potent than their counterpart (-)-isomers. On the other hand, for the sigma-2 subtype, the (-)-benzomorphans are more potent compared to their counterpart (+)-isomers in the binding assay. The existence of a third subtype, the sigma-3 receptor, was proposed by Wyrick et al.,10-13 but it has not been confirmed by others. However, the later investigation of the same group into the binding site revealed that there were no significant differences in the affinity of a series of compounds, and the CNS distribution in rodent brain of sigma-3 receptor correlated with the histamine H-1 receptor.14

Other than the different drug selectivity of these two subtypes, sigma-1 and sigma-2 receptors could be also differentiated in their distribution in tissue, biological function, as well as molecular size.6,15-17 The gene coding the sigma-1 receptor has been isolated and cloned from guinea pig,18 mouse,19 rat,20 and human.21 The protein coded from the sigma-1 receptor in rat brain consists 223 amino acid sequences (23 kDa).20 The cloned sigma-1 receptor from different sources shows 92% identical and
95% similar at the level of amino acid sequence, while it reveals no significant homology to other known mammalian receptor or protein.\textsuperscript{18, 20, 22, 23} In contrast, the sigma-2 receptor has not been cloned yet and is estimated to have a molecular weight of 18-21 kDa.\textsuperscript{16, 24}

1.3 Biological Distributions and Pharmacological Functions of Sigma Receptors

Sigma receptors are distinct from other neurotransmitter receptors. It has been recently suggested that the sigma-1 receptor is a transmembrane receptor with three hydrophobic domains, namely, the N- and the C-termini, and the center of the protein.\textsuperscript{25, 26} The structural model that was proposed suggests that both C-terminal (125 amino acids) and N-terminal (10 amino acids) are localized intracellularly, with a sequence of approximately 50 amino acids as an extracellular loop. The research on the structure characteristics of sigma-2 receptor is, however, very limited and has not yet been well characterized.

Both of the two subtypes are widely expressed in the central nervous system (CNS).\textsuperscript{9, 27} In addition to the central nervous system, both subtypes are found in endocrine, immune, and reproductive tissues, and they are found in high abundance in heart, spleen, liver and kidney.\textsuperscript{28-32}

A function of sigma receptors in psychosis is implied by the fact that both subtypes have high affinities to several typical neuroleptics (haloperidol) and anti-depressants (pentazocine).\textsuperscript{33} Furthermore, the sigma-1 receptor plays roles in the
regulation of gastrointestinal effects, modulation of opioid analgesia, attenuation of cocaine-induced locomotor activity and toxicity, and so on. In contrast, the sigma-2 receptor suffers from a lower degree of understanding because of the scarcity of ligands with high affinity and high selectivity. Nevertheless, some evidences show the involvement of sigma-2 receptor in the mediation of the motor effect of sigma ligands, mediation of the effects of sigma ligands on K\(^+\) channels, mediation of cell morphology and cell death induced by certain sigma ligands, and regulation of cell proliferation and maintenance of cell viability.

Recently, both of the two subtypes of sigma receptors were found to have very high density on the order of hundreds of thousands to millions per cell in tumor cell lines of various tissues, such as melanoma, breast cancer, small lung carcinoma and prostate cancer. The overexpression of sigma receptors in such tumors suggested that they are appropriate targets for developing tumor selective agents in cancer treatment and diagnosis. The data from the research work also suggested that the sigma receptor plays an important role in cell proliferation and adhesion of breast cancer cells.

1.4 Sigma Receptor Ligands: Pharmacological Potentials

Since the identification of sigma receptors, sigma receptor ligands like cocaine and (+)-benzomorphans have been investigated. Although it has been observed that some neurosteroids like progesterone, testosterone and pregnenolone (Figure 1-2) have considerably high affinity (nanomolar to submicromolar) at the sigma-1 receptor,
their roles as the endogenous sigma ligands still need to be further verified.\textsuperscript{43}

\textbf{Figure 1-2.} Structures of progesterone, testosterone and pregnenolone

Based on the knowledge that sigma receptors are involved in several physiological and pathophysiological processes as mentioned above, the sigma ligands are believed to modulate these physiological and pathophysiological events and influence psychiatric disorders.\textsuperscript{9} Because of their neuroregulative and neuroprotective functions,\textsuperscript{44} sigma-1 receptor agents could be potentially used for the treatment of depression, schizophrenia,\textsuperscript{45} amnesia and mental improvement,\textsuperscript{46} cocaine abuse,\textsuperscript{47} as well as other psychiatric disorders.\textsuperscript{48} On the other hand, sigma-2 receptor antagonists show the ability as atypical antipsychotics without the side effects caused by classical antipsychotic agents\textsuperscript{49} and to attenuate convulsions caused by cocaine.\textsuperscript{50}

The sigma-2 receptor agonists could be used as novel antineoplastic agents, potentiating the cytotoxicity by reversing drug resistance in tumor cells.\textsuperscript{27} Furthermore, because of the fact that both sigma receptors are expressed in a wide variety of tumor cell lines,\textsuperscript{41} radiolabeled sigma receptor ligands could serve as imaging agents for certain types of cancer in vivo. Imaging techniques such as PET (Positron Emission Tomography)\textsuperscript{51-56} and SPECT (Single Photon-Emission Computed
Tomography\textsuperscript{57-59} have been applied in imaging sigma receptors in the brain and in tumor cells to allow binding and localization studies. PET involves the imaging of a ligand with a positron-emitting atom such as C-11\textsuperscript{,}\textsuperscript{53} F-18\textsuperscript{,}\textsuperscript{52, 54} and Br-76\textsuperscript{51}. In contrast, SPECT utilizes the single mono-energetic photons emitted from the decay of I-123,\textsuperscript{60} I-125 (in mice)\textsuperscript{58} and Tc-99m\textsuperscript{57} (Figure 1-3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1-3.png}
\caption{Some radiolabeled sigma receptor ligands for PET and SPECT studies\textsuperscript{51-54, 57, 58, 60}}
\end{figure}

These two techniques are complementary to each other since the longer half-life of SPECT radioactive isotopes (I-123, Tc-99m, I-125) allow more time for radiosynthesis and observation of receptor localization; whereas, the shorter half-life
of PET radioactive isotopes (C-11, F-18, Br-76) allows multiple studies to be performed in the same subject\textsuperscript{61} (Table 1-1).

**Table 1-1.** Half-life of the isotopes that were used in the radio-labeled sigma receptor ligands shown in Figure 1-3.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>I-123</th>
<th>I-125</th>
<th>Tc-99m</th>
<th>C-11</th>
<th>F-18</th>
<th>Br-76</th>
</tr>
</thead>
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<tr>
<td>Half-life</td>
<td>13.2 hours</td>
<td>60.1 days</td>
<td>6.02 hours</td>
<td>20 min.</td>
<td>110 min.</td>
<td>16.2 hours</td>
</tr>
<tr>
<td>Use</td>
<td>SPECT</td>
<td>SPECT*</td>
<td>SPECT</td>
<td>PET</td>
<td>PET</td>
<td>PET</td>
</tr>
</tbody>
</table>

* only in mice

1.5 Sigma Receptor Ligands: Structural Diversity

To date, a variety of sigma receptor ligands with unrelated chemical structures have been reported. Figure 1-4 shows some representative ligands with high affinity to sigma receptors. In general, in their structures, one or more substituted aromatic rings are present. Other functional groups can be piperidine, guanidine, pyrrolidine, piperazine, thiochroman, and benzanide.
Figure 1-4. Some representative sigma receptor ligands with high affinity\textsuperscript{62-67}

Among these compounds, haloperidol (Ki: sigma-1, 2.20 ± 0.31 nM; sigma-2, 34.2 ± 2.3 nM)\textsuperscript{62} exhibits the highest affinity for both sites among typical neuroleptics.\textsuperscript{27} (+)-pentazocine (Ki: sigma-1, 3.58 ± 0.20 nM; sigma-2, 1923 nM)\textsuperscript{62} is commonly radiolabeled as \textsuperscript{[\textit{H}]}(+)-pentazocine to serve as the standard radioligand.
for sigma-1 receptor in binding assays. Ditolylguanidine (DTG, Ki: sigma-1, 27.7 ± 4.3 nM; sigma-2, 12.8 ± 2.1 nM) is also radiolabeled as [3H] DTG as the radioligand for sigma-2 receptor in the presence of (+)-pentazocine to mask the sigma-1 sites. A selective sigma-1 receptor agonist, igmesine (Ki: sigma-1, 39 nM; sigma-2, 390 nM), is in phase II trials for the treatment of depressive disorder. (+)-(R)-1-(4-chlorophenyl)-3-((4-(2-methoxyethyl)piperazin-1-yl)methyl)pyrrolidin-2-one (MS 377, Ki: sigma-1, 73 nM; sigma-2, 6900 nM) was suggested to be clinically active on schizophrenia without EPS (extrapyramidal symptoms) liability. 1-(4-fluorophenyl)-4-(4-(5-fluoropyrimidin-2-yl)piperazin-1-yl)butan-1-ol, (BMY-14802, Ki: sigma-1, 265 nM; sigma-2, 391 nM) is a piperazine derivative of haloperidol, interacts non-selectively with both subtypes and has been investigated in phase II clinical trials as a potential antipsychotic. Spipethiane (Ki: sigma-1, 0.5 nM; sigma-2, 416 nM) is a very potent and selective ligand for sigma-1 receptors. “Mach’s benzamide”, 5-bromo-N-(4-(3,4-dihydro-6,7-dimethoxyisoquinoline-2(1H)-yl)butyl)-2,3-dimethoxy benzamide, (IC50: sigma-1, 12,900 nM; sigma-2, 8.2 nM) belongs to the most potent and sigma-2 selective ligands described so far.

1.6 Sigma Receptor Ligands: Pharmacophore Models

Although a variety of structurally different compounds have been found to be sigma receptor ligands, many efforts have been made to define the pharmacophore for the sigma binding. In 1994, Glennon and co-workers proposed the first pharmacophore for sigma-1 receptor binding (Figure 1-5).
The structural features of this model consist of an amine site as an essential proton acceptor site, and two aromatic rings as hydrophobic sites flanking the amine site. The optimal distance between the central amine site and the primary hydrophobic site (region B) was suggested to be 6–10 Å, while the optimal distance between the amine site and the secondary binding site (region A), which is capable of tolerating bulk, was proposed to be 2.5-3.9 Å. Subsequent research revealed that the amine moiety can be embedded in a cyclic structure such as a pyrrolidine, piperidine or piperazine ring. Also, it is found that region A is associated with much more bulk tolerance so that additional bulk can be added. Furthermore, another molecular modeling study by Gund and co-workers suggested that the chains between amine site and aromatic rings need not to be simple alkyl chain. It could be bear a polar substituent such as S or O, which could be considered as the second binding site.\(^{73}\) (Figure 1-6)
The scarcity of high-affinity sigma-2 subtype selective ligands has prevented the investigation into binding modeling of sigma-2 receptor. A pharmacophore model of selective ligands for sigma-2 receptor has not been described until recently. In 2004, Cratteri and co-workers used several quantitative descriptors to summarize the sigma-2 receptor binding pharmacophore. In their study, it was suggested that the model is similar to the one of sigma-1 subtype proposed by Glennon. The difference lies in the following aspects: (1) a shorter distance between the two hydrophobic areas (aromatic rings A and B) was present (10.8-13.2 Å), as opposed to the 14-16 Å as in the sigma-1 model; (2) the distance between the primary hydrophobic site and the amine moiety varies from 11.6 Å to 13.6 Å, as compared to 6-10 Å in sigma-1 binding model; (3) lastly, other than the amine site, an additional electronic site (-O-, -O-CO-) could be incorporated into the major H-bond acceptor region (Figure 1-7).

![Figure 1-7. Cratteri’s Pharmacophore Model for Sigma-2 Receptor Ligands](image)

1.7 Quantitative Structure-Activity Relationship (QSAR) Studies of Sigma Receptor Ligands

Although sigma receptors and their ligands have received much attention since
1970’s, no specific sigma receptor related drug has entered the pharmaceutical market yet. A possible reason lies in the fact that the pharmacological functions of sigma receptors in biological systems have not been fully understood. To solve such a problem, the first step is that many more sigma receptor ligands need to be synthesized and tested in binding assays, and the connection between their structures and binding affinities should be studied.

Quantitative Structure-Activity Relationship (QSAR) studies are considered to be a very cost-efficient yet powerful tool for drug design. By relating biological activity with physicochemical descriptors within a mathematical equation, the biological activity could be predicted for unknown compound with similar structure.

QSAR studies in the research of sigma receptors ligands are very limited. Only a few publications have dealt with such topic. The first QSAR analysis of sigma receptor ligands seems to appear in 1995, conducted by Mascarella and co-workers. A series of substituted N-benzyl-N-normetazocines were synthesized and the relationship of the phenyl ring substituents with sigma-1 receptor binding affinities was investigated (Figure 1-8). They were able to correlate the binding affinity (Ki) to the substituent position (para-, meta-, ortho-), lipophilicity (π), as well as molar refractivity (MR).

![Figure 1-8. General structure of substituted N-benzyl-N-normetazocines](image-url)
Another QSAR study was performed by Fujimura and co-workers in 1997. For a small set of substituted derivatives of 1-[2-(3,4-dimethoxyphenyl)-ethyl]-4-(3-phenylpropyl) piperazine, the sigma-1 receptor affinity was suggested to be quantitatively dependent on the electronic nature of para-substituent $R_1$, and the resonance nature of meta-substituent $R_2$ (Figure 1-9).

$$\begin{align*}
R_1 &= H, Cl, CF_3 \\
R_2 &= H, Cl, CF_3, OH, Br, F, OCH_2Ph
\end{align*}$$

**Figure 1-9.** General structure of substituted derivatives of 1-[2-(3,4-dimethoxyphenyl)-ethyl]-4-(3-phenylpropyl) piperazine

In 1998, another QSAR study was carried out by Huang and co-workers on a series of N-(1-benzylpiperidin-4-yl)phenyl acetamide derivatives (Figure 1-10). They were able to relate both sigma-1 and sigma-2 binding affinities to the substituent parameters such as electronic ($\sigma$), hydrophobic ($\pi$), and steric bulk (MR) effects for various substituents. This study presented the first and to our knowledge the only QSAR study for sigma-2 receptor so far.

$$\begin{align*}
R_2 &= H, Cl, Br, F, CF_3, NO_2, OH, OCH_3 \\
R_3 &= H, Cl, Br, F, CF_3, NO_2, OH, OCH_3 \\
R_4 &= H, Cl, Br, F, CF_3, NO_2, OH, OCH_3, SCH_3, NH_2 \\
R_5 &= H, F, OCH_3 \\
R_6 &= H, Cl, F \\
X &= CH_2, NH, CHOH \\
Y &= CO, CH_2
\end{align*}$$

**Figure 1-10.** General structure of substituted N-(1-benzylpiperidin-4-yl)phenyl acetamide derivatives
1.8 Summary

Since its identification in 1976, the sigma receptor has been recognized as a unique intracellular receptor. At present, two sigma receptor subtypes, sigma-1 and sigma-2, are currently known. The two subtypes can be differentiated not only by their drug selectivity, but also by their distribution in tissue, biological function and molecular size. Accordingly, a variety of sigma receptor subtypes ligands have been investigated and a few pharmacophore models have been proposed. In addition, because sigma receptors are over-expressed in some tumor cells, radiolabeled sigma receptor ligands facilitate the radio imaging techniques such as PET and SPECT in the areas of cancer diagnosis and cancer therapy. To date, a few studies of quantitative structure-activity relationship (QSAR) have been carried out. In a mathematical equation, the sigma binding affinity of the sigma ligand was correlated with its physicochemical descriptors (electronic effect, hydrophobic effect, and steric bulk effect, etc.). By this means, the sigma binding affinity of unknown compound with similar structure could be predicted by applying the equation. Therefore, more compounds with a variety of structural similarities need to be synthesized to further characterize the sigma binding sites.
Chapter 2 Hypothesis and Design of Sigma Ligands

2.1 Sigma Ligands: Piperidine and Piperazine Derivatives

By conducting a search into the literature, we found that many alkylamines, especially piperidine and piperazine derivatives, have shown moderate to high affinity to sigma receptors, and strong selectivity to sigma receptors against dopamine D-2 and serotonin 5-HT\textsubscript{1A} receptors.\textsuperscript{72, 76-86} Moreover, diarylalkylpiperidine and diarylalkylpiperazine derivatives fit quite well into Glennon’s proposed model (Figure 1-5 shown on page 10),\textsuperscript{72, 78, 87} showing that they might serve as potential sigma ligands with both high affinity and high selectivity against other receptors.

In addition to the presence of amine sites and phenyl rings, the other factor we take into consideration is the length of alkyl chain that connects the amine site and the phenyl rings. Although it has been shown that the distance could be modified from one to six carbon chains,\textsuperscript{72} binding affinities of these compounds for sigma receptors are somewhat different. After comparison of their binding affinities, derivatives were chosen by a group member (Roger Nahas in Dr. Lever’s group) in which one chain contains a one carbon skeleton (benzyl moiety), and the other chain contains three carbons (phenylpropyl moiety). With these parameters, our lead compounds, Lead 1 and Lead 2 were selected (Figure 2-1).

![Lead 1 and Lead 2](Figure 2-1. Structures of Lead 1 and Lead 2)
The first appearance of the compound Lead 1 occurred in the study by Glennon and co-workers in 2002. After the establishment of their sigma-1 receptor pharmacophore, they began to investigate into the length of carbon chains between the nitrogen in the piperidine site and the hydrophobic sites. Previous research work suggests that for sigma-1 receptor affinity, the optimal spacer between the primary N atom and the phenyl-B ring is the pentyl chain. Therefore, this moiety was kept and the spacer between the nitrogen atom and phenyl-A ring was varied. While the affinity of sigma-1 receptor was retained, the sigma-2 receptor binding affinity appears to be very sensitive to changes made in the phenyl-A region. The best result for both subtype affinities comes from the compound with a benzyl moiety in the phenyl-A region, with sigma-1 Ki of 0.6 nM and sigma-2 Ki of 2.8 nM. Then, this moiety was held constant and chain length on the direction of the phenyl-B site was shortened with the expectation that if sigma-2 affinity was retained, selectivity favoring sigma-2 subtypes would be enhanced. To their surprise, the sigma-1 affinity was also retained and selectivity was not achieved (Table 2-1).

Table 2-1. Binding affinity of diarylalkylpiperidine analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\sigma_1$: Ki (nM)</th>
<th>$\sigma_2$: Ki (nM)</th>
<th>Selectivity ($\sigma_1/\sigma_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Compound" /></td>
<td>0.6</td>
<td>2.8</td>
<td>4.7</td>
</tr>
<tr>
<td><img src="image" alt="Compound" /></td>
<td>0.8</td>
<td>3.1</td>
<td>3.7</td>
</tr>
<tr>
<td><img src="image" alt="Compound" /></td>
<td>0.4</td>
<td>3.3</td>
<td>8.3</td>
</tr>
</tbody>
</table>
To explain such unexpected phenomena, they suggested that the Lead 1 might bind in a flipped mode that the binding site for phenyl-A and phenyl-B regions are switched (Figure 2-2).

![Proposed binding mode of diarylalkylpiperidines](image)

Figure 2-2. Proposed binding mode of diarylalkylpiperidines

Lead 1 also served as a lead compound in the research performed by Costantino and co-workers, which was reported in 2005, in an attempt to study the structure-activity relationships of 1-aralkyl-4-benzylpiperidine and 1-aralkyl-4-benzylpiperazine derivatives as potent sigma ligands. The affinity of Lead 1 was slightly different from the first study (Ki: sigma-1, 1.40 nM; sigma-2, 0.49 nM). Although different binding affinity values were shown and their selectivity towards sigma receptor subtypes was opposite, both studies indicates that Lead 1 can be a good point to start with in the effort of illustrating structure-affinity relationships for both sigma-1 and sigma-2 receptors.

Lead 2 compound appears in a structure-affinity relationship study of a series of
arylalkyl 4-benzyl-piperazine derivatives as sigma receptor ligands.\textsuperscript{82} Although the result from comparative binding affinity tests indicated that Lead 2 is very selective for sigma receptors ($K_i = 20 \text{ nM}$) in comparison with $5HT_{1A}$ ($K_i > 10^5 \text{ nM}$) and the $D_2$ receptors ($K_i > 10^5 \text{ nM}$), no information of sigma subtype selectivity (sigma-1 vs. sigma-2) was given. Table 2-2 shows the binding affinity values of Lead 1, Lead 2 and some potential sigma receptor ligands in Figure 1-4.

### Table 2-2. Comparison of Lead 1 and Lead 2 binding affinity with some useful and potential sigma ligands in Figure 1-4

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i: \sigma_1$ (nM)</th>
<th>$K_i: \sigma_2$ (nM)</th>
<th>Selectivity ($\sigma_2/\sigma_1$)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>2.2</td>
<td>34.2</td>
<td>15.5</td>
<td>62</td>
</tr>
<tr>
<td>(+)-Pentazocine</td>
<td>3.58</td>
<td>1923</td>
<td>537</td>
<td>62</td>
</tr>
<tr>
<td>Ditolyguanidine (DTG)</td>
<td>27.7</td>
<td>12.8</td>
<td>0.46</td>
<td>63</td>
</tr>
<tr>
<td>Igmesine</td>
<td>39</td>
<td>390</td>
<td>10</td>
<td>64</td>
</tr>
<tr>
<td>MS 377</td>
<td>73</td>
<td>6900</td>
<td>94.5</td>
<td>65</td>
</tr>
<tr>
<td>BMY-14802</td>
<td>265</td>
<td>391</td>
<td>1.48</td>
<td>69</td>
</tr>
<tr>
<td>Spipethiane</td>
<td>0.5</td>
<td>416</td>
<td>832</td>
<td>66</td>
</tr>
<tr>
<td>“Mach’s Benzamide”</td>
<td>53</td>
<td>0.90</td>
<td>0.017</td>
<td>71</td>
</tr>
<tr>
<td>Lead 1</td>
<td>0.4</td>
<td>3.3</td>
<td>8.25</td>
<td>87</td>
</tr>
<tr>
<td>Lead 1</td>
<td>1.4</td>
<td>0.49</td>
<td>0.35</td>
<td>86</td>
</tr>
<tr>
<td>Lead 2</td>
<td>20</td>
<td>ND</td>
<td></td>
<td>82</td>
</tr>
</tbody>
</table>
2.2 Phenyl Ring Substitution: Significance and Design

Investigation of the phenyl ring substitution can be very useful. Firstly, the effect of systematic phenyl ring substitution could be described by the analysis of structure-affinity relationships study. After the development of a SAR study, then for new compounds, we might be able to predict qualitatively the importance of different substituents to the binding affinity of the sigma receptors, thus contribute to a better understanding of the sigma receptor pharmacophore profile. Secondly, a set of physico-chemical parameters associated with the phenyl ring substitution could be correlated to their binding affinity in a mathematical equation. This QSAR study could predict the biological activity of compounds with similar structures and play a significant role in assisting the effective design of future sigma receptor ligands with high affinity and high selectivity. Finally, radiolabeling the derivatives containing halogenated phenyl ring substitutions with their radioactive isotopes (F-18, Br-76, I-123, etc.) suggests their application in the imaging of the distribution of sigma receptors. These ligands could be utilized in the visualization of tumor cells by PET or SPECT in vivo.

As indicated above, previous studies show that Lead 1 and Lead 2 can be regarded as very promising substrates for further study in sigma receptors. However, no study in the effect of phenyl ring substituent derivatives has yet to be reported. On the other hand, to our knowledge, QSAR studies appeared in only a few number in the research of sigma receptor ligands. Both of these two factors suggest that the systematic substitution on the phenyl rings of Lead 1 and Lead 2 should be
investigated. Therefore, the substituent group will be added to either phenyl ring in Lead 1 and Lead 2 to study the effect of substitution on the sigma binding affinity. For a total of four series, in this thesis, the focus will be on the synthesis of diarylalkylpiperidine and diarylalkylpiperazine derivatives with the substituents on the phenylpropyl moiety (Figure 2-3). Another group member (Roger Nahas in Dr. Susan Lever’s group) will include the synthesis of the other two series, which have substitution on the benzyl moiety, in his PhD dissertation.

![Figure 2-3. General structures of the substituted Lead 1 and Lead 2 derivatives](image)

Previous studies of the quantitative structure-activity relationships of sigma receptor ligands show that their biological activities are potentially dependent on general descriptors such as the electronic, hydrophobicity and steric effect parameters. These parameters determine how well the ligands could fit into the receptors and how well the ligands could interact with the receptors. The relevant physicochemical parameters, such as Hammett $\sigma$ value, $\pi_x$ value, and molar refractivity “MR”, are suitable to derive the mathematical equation in QSAR studies. Therefore, a few mathematical equations have been developed to illustrate the correlations of those physicochemical parameters and the binding affinity. A typical QSAR expression can be shown in the following form:

$$\log \left( \frac{1}{K_i} \right) = f(a\sigma, b\pi_x, cMR)$$

A Hammett $\sigma$ value represents the electronic substituent parameters. The $\pi_x$
value represents the hydrophobic contribution of the substituent. Molar refractivity “MR” represents the volume of substituent.

From the literature, we found that for a large portion of the compounds which had been investigated for sigma receptor binding affinity test, those three descriptors (Hammett $\sigma$ values, $\pi_x$ values, MR values) associated with the phenyl substituents varied in certain space ranges. In specific, Hammett $\sigma$ values are between -0.4 and 0.8, $\pi_x$ values are between -1.0 and 1.6, and MR values are between 0.1 and 2.5. Therefore, this fact was considered and the intermediate numbers were established to present a certain substituent (R: 2-OCH$_3$) (Table 2-3).

<table>
<thead>
<tr>
<th>Property</th>
<th>Parameter</th>
<th>Low Limit</th>
<th>High Limit</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic</td>
<td>$\sigma$</td>
<td>-0.4</td>
<td>0.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Lipophilicity</td>
<td>$\pi_x$</td>
<td>-1.0</td>
<td>1.6</td>
<td>-0.02</td>
</tr>
<tr>
<td>Size</td>
<td>MR</td>
<td>0.1</td>
<td>2.5</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Since we have three descriptors ($\sigma$, $\pi_x$ and MR) in the QSAR expression, the minimum number of compounds needed to build the correlation equation is 8 ($2^3=8$). The “intermediate” helped to control that each single compound represents a distinctive area of the descriptors numerical space. Finally, 9 different substituents were chosen by Roger Nahas to represent the distinctive area of the parameter spaces (Table 2-4).
Table 2-4. Sets of substituents with the physicochemical parameters$^{90}$

<table>
<thead>
<tr>
<th>R</th>
<th>$\sigma$</th>
<th>$\pi_x$</th>
<th>MR</th>
<th>Level</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-CH$_3$</td>
<td>-0.17</td>
<td>0.56</td>
<td>0.57</td>
<td>- + -</td>
<td>- + -</td>
</tr>
<tr>
<td>4-OCH$_3$</td>
<td>-0.27</td>
<td>-0.02</td>
<td>0.79</td>
<td>- - +</td>
<td>- 0 0</td>
</tr>
<tr>
<td>3-OCH$_3$</td>
<td>0.12</td>
<td>-0.02</td>
<td>0.79</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>3-F</td>
<td>0.34</td>
<td>0.14</td>
<td>0.10</td>
<td>+ + -</td>
<td>+ + -</td>
</tr>
<tr>
<td>2-Br</td>
<td>-</td>
<td>0.86</td>
<td>0.89</td>
<td>- + +</td>
<td>- + +</td>
</tr>
<tr>
<td>3-I</td>
<td>0.35</td>
<td>1.12</td>
<td>1.39</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>2-NO$_2$</td>
<td>-</td>
<td>-0.28</td>
<td>0.74</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>3-NO$_2$</td>
<td>0.71</td>
<td>-0.28</td>
<td>0.74</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>4-NO$_2$</td>
<td>0.78</td>
<td>-0.28</td>
<td>0.74</td>
<td>+ - -</td>
<td>+ - -</td>
</tr>
</tbody>
</table>

Therefore, a total of 18 compounds (Lead 1 derivatives $1a$-$1i$ and Lead 2 derivatives $2a$-$2i$) will be synthesized (Figure 2-4). Then, the sigma binding assay of Lead 1 derivatives $1a$-$1i$ will be conducted and the qualitative structure-affinity relationships will be discussed. The quantitative structure-activity relationship studies will be included in Roger’s (Lever research group) PhD dissertation.
Figure 2-4. Structures of substituted diarylalkylpiperidine (1a-1i) derivatives and substituted diarylalkylpiperazine (2a-2i) to be synthesized.
2.3 Synthetic Approach to the Derivatives of Lead 1 and Lead 2

Applying the process of retrosynthetic analysis, the series of compounds 1a-1i and 2a-2i can be dissected into two moieties, 4-benzylpiperidine (or 4-benzylpiperazine) and the substituted phenylpropyl halide. The 4-benzylpiperidine is commercially available, but 4-benzylpiperazine needs to be obtained from the commercially available benzyl bromide and t-Boc piperazine. The substituted phenylpropyl halide can be prepared from the corresponding substituted cinnamic acids. Scheme 2-1 represents the retrosynthesis of the compounds 1a-1i and 2a-2i.

Scheme 2-1. Retrosynthesis of the compounds 1a-1i and 2a-2i

The preparation of 4-benzylpiperazine can be conducted by the alkylation of Boc piperazine and benzyl bromide, followed by the deprotection of the Boc group.
with hydrochloric acid in dioxane (Scheme 2-2). This product is used immediately in
the next reaction.

![Scheme 2-2. Synthetic approach to 4-benzylpiperazine](image)

For compounds 1a-1e and 2a-2e, starting from the substituted cinnamic acid,
LiAlH₄ can be used to reduce the α,β-unsaturated carboxylic acid to give the
corresponding alcohol. Then, chlorination of the alcohol by thionyl chloride (SOCl₂)
can yield the substituted phenylpropyl chloride.² In the last step, direct alkylation of
the resulting chloride with 4-benzylpiperidine (or 4-benzylpiperazine) gives the Lead
1 derivatives 1a-1e and Lead 2 derivatives 2a-2e (Scheme 2-3).

![Scheme 2-3. Synthetic approach to the Lead 1 derivatives 1a-1e and Lead 2
derivatives 2a-2e](image)
The synthesis of the compounds 1f-1h and 2f-2h (R=3-NO2, 4-NO2, 2-NO2) is more complex. Since the nitro substituent on the phenyl ring is very sensitive to strong reducing agents, LiAlH4 is not a proper reagent to reduce the starting materials. Therefore, the double bond and the carboxylic acid need to be reduced in two subsequent steps. The olefin is reduced to single bond first and then the acid is subsequently reduced with borane-tetrahydrofuran (BH3-THF) to afford the alcohol (Scheme 2-4). More details about these methods will be discussed in Chapter 4 “Results and Discussion” part. With the requisite alcohol in hand, the following steps of chlorination and alkylation are the same as the 1a-1e and 2a-2e analogues.

Scheme 2-4. Reduction of compounds 7f-7h with LiAlH4 and mild reducing agent

Compound 1i and 2i (R=3-I) can be obtained from their analogues 1f and 2f (R=3-NO2) by two steps.93 Hydrogenation of nitro substituent compound 1f (or 2f) with 5% (weight percentage) Pt/C catalyst formed the aniline, which was converted
directly to the iodo substituent 1i (or 2i) via a Sandmeyer iodination reaction (Scheme 2-5).

Scheme 2-5. Synthetic approach to the Lead 1 derivative 1i and Lead 2 derivative 2i
Chapter 3 Experimental Section

3.1 Materials and Methods

All chemicals were purchased from the Aldrich Chemical Co. and used as received unless otherwise noted. Tetrahydrofuran (THF) was freshly distilled over sodium benzophenone ketyl before used as a solvent. All the other solvents were used directly from their original bottles in HPLC grade. Moisture sensitive and hygroscopic materials (LiAlH₄, BH₃/THF, SOCl₂, etc.) were stored in a dessicator. The powder of dipotassium azodicarboxylate (PADA) was stored in a sealed container in the refrigerator prior to use. All air and moisture sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere.

Column chromatographic separations were carried out on Silicycle ultra pure silica gel (230-400 mesh) with ACS reagent grade solvents. Analytical thin layer chromatography was performed on plastic-backed silica gel plates produced by Macherey-Nagel Co. Melting points were determined with the Mel-Temp apparatus and were not corrected.

¹H NMR spectra were obtained on a Bruker AMX-250 (250 MHz), Bruker AMX-300 (300 MHz) or Bruker AMX-500 (500 MHz), respectively, in CDCl₃ solutions with tetramethylsilane (δ = 0 ppm) as the internal standard. ¹³C NMR spectra was obtained on the same instruments with CDCl₃ (δ = 77 ppm) as the internal reference. Analytical HPLC were performed on the Waters HPLC Pumps (Model: 1525EF) with Waters Dual λ Ultraviolet Absorbance Detector (Model: 2487).
Elemental analyses were determined on final target compounds by Atlantic Microlab Inc. (Norcross, GA).

### 3.2 Experiment Procedure

**tert-butyl 4-benzylpiperazine-1-carboxylate (5)**

The following procedure has been adapted from the previously reported work with appropriate modification.\(^{91}\)  

**tert-butyl piperazine-1-carboxylate, 3,** (2.50 g, 13.4 mmol) and **1-(bromomethyl) benzene, 4,** (2.30 g, 13.4 mmol) was reacted in the presence of **K\(_2\)CO\(_3\)** (5.50 g, 40 mmol) and **NaI** (2.10 g, 14 mmol) in acetonitrile (60 mL) at reflux overnight. The solvent was removed under reduced pressure, and the residue was diluted with water (20 mL) and extracted with EtOAc (3×20 mL). The organic layer was washed with brine, dried over MgSO\(_4\), and concentrated under reduced pressure. The crude residue was purified by column chromatography (hexane/ethyl acetate, 4:1 as eluent) to give 5 as a white solid (3.26 g, 88%), m.p. 72-75 °C (literature m.p. 72-74 °C).\(^{1}\)H NMR (CDCl\(_3\)): δ 1.47 (s, 9H), 2.38-2.41 (t, 4H), 3.42-3.45 (t, 4H), 3.52 (s, 2H), 7.27-7.34 (m, 5H).

**1-benzylpiperazine (6)**

The following procedure has been adapted from the previously reported work with appropriate modification.\(^{91}\) **4N HCl** (30 mL) was added dropwise to 5 (2.69 g, 9.75 mmol) in 1,4-dioxane (30 mL) at 0 °C, and was diluted
with methanol (30 mL). The solution was stirred overnight at room temperature, and concentrated under reduced pressure. The residue was basified by 2N NaOH to pH 11, and then extracted with CHCl₃. The organic layer was washed with brine, dried over MgSO₄, and concentrated under reduced pressure to yield 6 as a pale yellow oil (1.57 g, 92%). ¹H NMR (CDCl₃): δ 1.47 (s, 1H), 2.38-2.42 (t, 4H), 2.84-2.88 (t, 4H), 3.47 (s, 2H), 7.20-7.37 (m, 5H). This compound was used immediately in the subsequent reactions.

**Dipotassium Azodicarboxylate (PADA)**

The following procedure has been adapted from the previously reported work with appropriate modification.⁹⁴ In an external ice/acetone bath, azodicarbonamide (2.32 g, 20 mmol) was added to a stirred 40% aqueous KOH solution (6.96 mL) at a rate such that the temperature of the reaction mixture did not exceed 10 °C (15 min). After the addition, the mixture was stirred for 1 h at this temperature, and then filtered and washed with cold methanol (60 mL) to afford PADA as a yellow powder (3.87 g, 100%). This compound was used without further purification.

**3-(4-methoxyphenyl)propan-1-ol (9a)**

The reduction procedure has been adapted from the
previously reported work with appropriate modification. The chlorination procedure has been adapted from the previously reported work with appropriate modification. To a stirred solution of 9a (514 mg, 3.10 mmol) in CHCl3 (10 mL) was added thionyl chloride without fresh distillation (0.563 mL, 7.74 mmol) and a drop of pyridine at room temperature. The reaction mixture was refluxed for 4 h. The solution was evaporated to dryness and purified by column chromatography (hexane/ethyl acetate, 4:1 as eluent) to give 10a as a colorless oil (424 mg, 75%). H NMR (CDCl3): δ 1.99-2.10 (m, 2H), 2.68-2.74 (t, 2H), 3.48-3.53 (t, 2H), 3.78 (s, 3H), 6.82-6.84 (d, 2H), 7.10-7.12 (d, 2H).
4-benzyl-1-(3-(4-methoxyphenyl)propyl)piperidine (1a)

The alkylation procedure has been adapted from the previously reported work with appropriate modification. A stirred mixture of 10a (116.2 mg, 0.63 mmol), 4-benzylpiperidine, 11 (132.5 mg, 0.76 mmol), NaI (94 mg, 0.63 mmol), and K₂CO₃ (260 mg, 4.89 mmol) in acetonitrile (5 mL) was heated under reflux overnight and allowed to cool to room temperature. The solvent was removed under reduced pressure, and the residue was diluted with 10% aqueous NaOH solution and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄, and concentrated under reduced pressure. The crude residue was purified by column chromatography (hexane/ethyl acetate, 3:2 as eluent) to yield 1a as a pale yellow oil (183 mg, 100%). The HCl salt of 1a was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 212-214 °C. ¹H NMR (free amine in CDCl₃): δ 1.28-1.36 (m, 2H), 1.47-1.51 (m, 1H), 1.59-1.64 (d, 2H), 1.72-1.86 (m, 4H), 2.28-2.33 (t, 2H), 2.51-2.57 (m, 4H), 2.86-2.89 (d, 2H), 3.76 (s, 3H), 6.79-6.83 (d, 2H), 7.01-7.19 (m, 5H), 7.23-7.26 (d, 2H). ¹³C NMR (free amine in CDCl₃): δ 24.38, 27.65, 30.04, 33.81, 40.49, 52.88, 54.17, 55.90, 114.76, 127.54, 128.39, 128.60, 129.21, 130.58, 138.73, 158.02.

Elemental Analysis: (C₂₂H₂₉NO•HCl)

Calculated: C, 73.41; H, 8.40; N, 3.89;

Found: C, 73.16; H, 8.52; N, 3.86.
1-benzyl-4-(3-(4-methoxyphenyl)propyl)piperazine (2a)

The alkylation procedure has been adapted from the previously reported work with appropriate modification.87 A stirred mixture of 10a (34.0 mg, 0.184 mmol), 4 (48.7 mg, 0.276 mmol), NaI (30 mg, 0.184 mmol), and K₂CO₃ (80 mg, 0.580 mmol) in acetonitrile (2.5 mL) was heated under reflux overnight and allowed to cool to room temperature. The solvent was removed under reduced pressure, and the residue was diluted with 10% aqueous NaOH solution and extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄, and concentrated under reduced pressure. The crude residue was purified by column chromatography (ethyl acetate as eluent) to yield 2a as a pale yellow oil (47.8 mg, 80%). The HCl salt of 2a was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 251-253 ºC. ¹H NMR (free amine in CDCl₃): δ 1.71-1.83 (m, 2H), 2.32-2.59 (m, 12H), 3.50 (s, 2H), 3.76 (s, 3H), 6.78-6.83 (d, 2H), 7.06-7.10 (d, 2H), 7.21-7.30 (m, 5H). ¹³C NMR (free amine in CDCl₃): δ 28.85, 32.81, 53.11, 53.21, 55.21, 58.01, 63.09, 113.69, 126.97, 128.16, 129.19, 129.22, 134.21, 138.14, 157.69.

Elemental Analysis: (C₂₁H₂₈N₂O•2HCl•1/₄H₂O)

Calculated: C, 62.76; H, 7.65; N, 6.97;

Found: C, 62.76; H, 7.61; N, 6.88.
3-(3-methoxyphenyl)propan-1-ol (9b)

In a similar fashion as described for the preparation of 9a, (2E)-3-(3-methoxyphenyl) acrylic acid, 7b, (891 mg, 5 mmol) was converted to 9b as a colorless oil (753 mg, 91%). ¹H NMR (CDCl₃): δ 1.82-1.90 (m, 3H), 2.64-2.69 (t, 2H), 3.62-3.66 (t, 2H), 3.77 (s, 3H), 6.72-6.79 (m, 3H), 7.16-7.21 (t, 1H).

1-(3-chloropropyl)-3-methoxybenzene (10b)

In a similar fashion as described for the preparation of 10a, 3-(3-methoxyphenyl)propan-1-ol, 9b, (514 mg, 3.10 mmol) was converted to 10b as a colorless oil (490 mg, 86%). ¹H NMR (CDCl₃): δ 2.02-2.11 (m, 2H), 2.72-2.77 (t, 2H), 3.49-3.53 (t, 2H), 3.78 (s, 3H), 6.73-6.79 (m, 3H), 7.17-7.23 (t, 1H).

4-benzyl-1-(3-(3-methoxyphenyl)propyl)piperidine (1b)

In a similar fashion as described for the preparation of 1a, 1-(3-chloropropyl)-3-methoxybenzene, 10b, (115.4 mg, 0.625 mmol), was converted to 1b as a pale yellow oil (150 mg, 100%). The HCl salt of 1b was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 138-140 °C. ¹H NMR (free amine in CDCl₃): δ 1.27-1.36 (m, 2H), 1.47-1.52 (m, 1H), 1.60-1.64 (d, 2H), 1.75-1.87 (m, 4H), 2.29-2.34 (t, 2H), 2.51-2.58 (m, 4H), 2.86-2.90 (d, 2H), 3.77 (s, 3H), 6.70-6.78 (m, 3H), 7.10-7.27 (m, 6H). ¹³C NMR (free amine in CDCl₃): δ 28.52,
32.07, 33.88, 37.93, 43.20, 53.89, 54.12, 55.08, 58.34, 111.10, 114.13, 120.81, 125.79, 128.17, 129.12, 129.26, 140.66, 143.77.

Elemental Analysis: \((C_{22}H_{29}NO)\)

Calculated: C, 81.69; H, 9.04; N, 4.33;

Found: C, 81.58; H, 9.03; N, 4.37.

1-benzyl-4-(3-(3-methoxyphenyl)propyl)piperazine (2b)

In a similar fashion as described for the preparation of 2a, 4-benzyl-1-(3-(3-methoxyphenyl)propyl)piperidine, 10b, (29.7 mg, 0.161 mmol) was converted to 2b as a pale yellow oil (47.8 mg, 80%). The HCl salt of 2b was obtained by passing HCl gas into the ethanol solution of the free amine.

Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 252-255 °C.

\(^1\)H NMR (free amine in CDCl\(_3\)): \(\delta\) 1.75-1.85 (m, 2H), 2.33-2.62 (m, 12H), 3.50 (s, 2H), 3.77 (s, 3H), 6.70-6.78 (m, 3H), 7.15-7.32 (m, 6H). \(^13\)C NMR (free amine in CDCl\(_3\)): \(\delta\) 28.52, 33.78, 53.12, 53.20, 55.08, 58.00, 63.09, 111.04, 114.12, 120.79, 126.97, 128.16, 129.19, 138.15, 143.81, 159.58.

Elemental Analysis: \((C_{21}H_{28}N_2O\cdot2HCl)\)

Calculated: C, 63.47; H, 7.61; N, 7.05;

Found: C, 63.53; H, 7.47; N, 6.99.
3-(4-methylphenyl)propan-1-ol (9c)

In a similar fashion as described for the preparation of 9a, (2E)-3-(4-methylphenyl) acrylic acid, 7c, (1.62 g, 10 mmol) was converted to 9c as a colorless oil (873 mg, 58%). $^1$H NMR (CDCl$_3$): $\delta$ 1.81-1.91 (m, 2H), 2.31 (s, 3H), 2.63-2.68 (t, 2H), 3.62-3.67 (t, 2H), 7.15 (s, 4H).

1-(3-chloropropyl)-4-methylbenzene (10c)

In a similar fashion as described for the preparation of 10a, 3-(4-methylphenyl)propan-1-ol, 9c, (873 mg, 5.82 mmol) was converted to 10c as a colorless oil (600 mg, 61.2%). $^1$H NMR (CDCl$_3$): $\delta$ 2.04-2.14 (m, 2H), 2.35 (s, 3H), 2.74-2.79 (t, 2H), 3.53-3.57 (t, 2H), 7.12 (s, 4H).

4-benzyl-1-(3-(4-methylphenyl)propyl)piperidine (1c)

In a similar fashion as described for the preparation of 1a, 1-(3-chloropropyl)-4-methylbenzene, 10c, (153 mg, 0.89 mmol) was converted to 1c as a pale yellow oil (250 mg, 92%). The HCl salt of 1c was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 170-172 °C. $^1$H NMR (free amine in CDCl$_3$): $\delta$ 1.28-1.42 (m, 2H), 1.47-1.62 (m, 1H), 1.62-1.70 (d, 2H), 1.78-1.92 (m, 4H), 2.35 (s, 3H), 2.56-2.61 (m, 4H), 2.88-2.97 (d, 2H), 7.11 (s, 4H), 7.17-7.31 (m, 5H). $^{13}$C NMR (free amine in CDCl$_3$): 24.35, 24.63, 27.41, 29.90, 33.57,
Elemental Analysis: (C$_{22}$H$_{29}$N•HCl)

Calculated: C, 76.83; H, 8.79; N, 4.07;

Found: C, 76.36; H, 8.78; N, 4.07.

1-benzyl-4-(3-(4-methylphenyl)propyl)piperazine (2c)

In a similar fashion as described for the preparation of 2a, 1-(3-chloropropyl)-4-methylbenzene, 10c (95.7 mg, 0.567 mmol) was converted to 2c as a pale yellow oil (144 mg, 82%). The HCl salt of 2c was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 258-260 °C. $^1$H NMR (free amine in CDCl$_3$): $\delta$ 1.73-1.83 (m, 2H), 2.29 (s, 3H), 2.32-2.59 (m, 12H), 3.49 (s, 2H), 7.05 (s, 4H), 7.19-7.32 (m, 5H). $^{13}$C NMR (free amine in CDCl$_3$): 24.45, 27.38, 33.72, 52.66, 53.10, 53.83, 60.19, 127.23, 128.13, 128.38, 128.64, 129.15, 135.61, 135.53, 135.78.

Elemental Analysis: (C$_{21}$H$_{28}$N$_2$•2HCl)

Calculated: C, 66.13; H, 7.93; N, 7.35;

Found: C, 66.16; H, 7.92; N, 7.22.

3-(3-fluorophenyl)propan-1-ol (9d)

In a similar fashion as described for the preparation of 9a,
(2E)-3-(3-fluorophenyl) acrylic acid, 7d, (831 mg, 5 mmol) was converted to 9d as a colorless oil (480 mg, 62%). $^{1}$H NMR (CDCl$_3$): $\delta$ 1.79-1.89 (m, 3H), 2.63-2.69 (t, 2H), 3.59-3.63 (t, 2H), 6.82-6.95 (m, 3H), 7.16-7.23 (m, 1H).

1-(3-fluoropropyl)-3-methoxybenzene (10d)

In a similar fashion as described for the preparation of 10a, 3-(3-fluorophenyl)propan-1-ol, 9d (330 mg, 2.14 mmol) was converted to 10d as a colorless oil (307 mg, 83%). $^{1}$H NMR (CDCl$_3$): $\delta$ 2.01-2.10 (m, 2H), 2.73-2.78 (t, 2H), 3.48-3.52 (m, 2H), 6.86-6.97 (m, 3H), 7.19-7.27 (t, 1H).

4-benzyl-1-(3-(3-fluorophenyl)propyl)piperidine (1d)

In a similar fashion as described for the preparation of 1a, 1-(3-fluoropropyl)-3-methoxybenzene, 10d (52.5 mg, 0.304 mmol) was converted to 1d as a pale yellow oil (75.9 mg, 96%). The HCl salt of 1d was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 138-140 °C. $^{1}$H NMR (free amine in CDCl$_3$): $\delta$ 1.24-1.37 (m, 2H), 1.43-1.56 (m, 1H), 1.60-1.64 (d, 2H), 1.74-1.87 (m, 4H), 2.28-2.30 (t, 2H), 2.51-2.62 (m, 4H), 2.85-2.89 (d, 2H), 6.81-6.94 (m, 3H), 7.11-7.28 (m, 6H). $^{13}$C NMR (free amine in CDCl$_3$): 28.52, 32.23, 33.55, 37.98, 43.23, 53.93, 58.21, 112.38, 112.66, 115.03, 115.31, 123.98, 124.01, 125.72, 128.11, 129.09, 129.53, 129.64, 140.72, 144.81, 144.90, 161.23, 164.47.
Elemental Analysis: \((C_{21}H_{27}ClFN\cdot HCl\cdot 1.5H_2O)\)

Calculated: C, 67.27; H, 8.07; N, 3.74;

Found: C, 67.58; H, 7.92; N, 3.80.

1-benzyl-4-(3-(3-fluorophenyl)propyl)piperazine (2d)

In a similar fashion as described for the preparation of 2a, 1-(3-fluoropropyl)-3-methoxybenzene, 10d (24.7 mg, 0.143 mmol) was converted to 2d as a pale yellow oil (38.6 mg, 86%). The HCl salt of 2d was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 252-255 °C. \(^1\)H NMR (free amine in CDCl\(_3\)): \(\delta 1.75-1.85 (m, 2H), 2.32-2.64 (m, 12H), 3.51 (s, 2H), 6.83-6.95 (m, 3H), 7.12-7.32 (m, 6H).\) \(^13\)C NMR (free amine in CDCl\(_3\)): 28.30, 33.40, 53.09, 53.17, 57.75, 63.07, 112.42, 112.70, 115.04, 115.32, 123.98, 124.02, 126.97, 128.15, 129.18, 129.55, 129.66, 138.11, 144.69, 144.79, 161.23, 164.47.

Elemental Analysis: \((C_{20}H_{25}FN_2\cdot 2HCl\cdot 0.75H_2O)\)

Calculated: C, 60.23; H, 7.20; N, 7.02;

Found: C, 60.09; H, 6.86; N, 7.31.

3-(2-bromophenyl)propan-1-ol (9e)

In a similar fashion as described for the preparation of 9a, (2E)-3-(2-bromophenyl) acrylic acid, 7e, (0.52 g, 2.3 mmol) was converted to 9e as a
colorless oil (156 mg, 32%). $^1$H NMR (CDCl$_3$): $\delta$ 1.75-1.91 (m, 2H), 2.70-2.80 (t, 2H), 3.18 (s, 1H), 3.59-3.69 (t, 2H), 6.96-7.01 (m, 1H), 7.18-7.25 (m, 2H), 7.47-7.52 (d, 1H).

1-(3-chloropropyl)-2-bromobenzene (10e)

In a similar fashion as described for the preparation of 10a, 3-(2-bromophenyl)propan-1-ol, 9e (136 mg, 0.63 mmol) was converted to 10e as a colorless oil (129 mg, 87%). $^1$H NMR (CDCl$_3$): $\delta$ 2.04-2.15 (m, 2H), 2.87-2.93 (t, 2H), 3.52-3.57 (t, 2H), 7.03-7.11 (m, 1H), 7.19-7.29 (m, 2H), 7.51-7.54 (d, 1H).

4-benzyl-1-(3-(2-bromophenyl)propyl)piperidine (1e)

In a similar fashion as described for the preparation of 1a, 1-(3-chloropropyl)-2-bromobenzene, 10e (43.5 mg, 0.186 mmol) was converted to 1e as a pale yellow oil (49.2 mg, 85%). The HCl salt of 1e was obtained by passing HCl gas into the ether solution of the free amine. Then, the salt was recrystallized from ethanol and ether. m.p. (HCl salt): 170-172 °C. $^1$H NMR (free amine in CDCl$_3$): $\delta$ 1.44-1.60 (m, 3H), 1.64-1.68 (d, 2H), 1.84-2.05 (m, 4H), 2.46-2.56 (m, 4H), 2.70-2.77 (m, 2H), 3.00-3.04 (d, 2H), 7.00-7.08 (m, 1H), 7.12-7.30 (m, 7H), 7.49-7.52 (d, 1H). $^{13}$C NMR (free amine in CDCl$_3$): $\delta$ 27.13, 31.92, 34.48, 37.50, 43.74, 53.32, 57.61, 124.06, 125.87, 127.49, 127.76, 128.24, 129.18, 130.21, 132.64, 140.25, 141.01.
Elemental Analysis: \((C_{21}H_{26}BrN\cdot HCl)\)

Calculated: C, 61.70; H, 6.66; N, 3.43;

Found: C, 61.50; H, 6.80; N, 3.25.

1-benzyl-4-(3-(2-bromophenyl)propyl)piperazine (2e)

In a similar fashion as described for the preparation of 2a, 1-(3-chloropropyl)-2-bromobenzene, 10e (21.0 mg, 0.09 mmol) was converted to 2e as a pale yellow oil (26.8 mg, 80%). The HCl salt of 2e was obtained by passing HCl gas into the ether solution of the free amine. Then, the salt was recrystallized from ethanol and ether. m.p. (HCl salt): 258-260 °C. \(^1\)H NMR (free amine in CDCl\(_3\)): δ 1.75-1.85 (m, 2H), 2.26-2.76 (m, 12H), 3.51 (s, 2H), 7.00-7.07 (m, 1H), 7.20-7.31 (m, 7H), 7.49-7.52 (d, 1H). \(^{13}\)C NMR (free amine in CDCl\(_3\)): δ 27.07, 29.68, 34.01, 53.10, 53.16, 57.90, 63.07, 124.41, 126.96, 127.31, 127.46, 128.15, 129.18, 130.33, 132.72, 138.15, 141.48.

Elemental Analysis: \((C_{20}H_{25}BrN_2\cdot 2HCl)\)

Calculated: C, 53.83; H, 6.10; N, 6.28;

Found: C, 54.41; H, 6.20; N, 6.39.

3-(3-nitrophenyl)propanoic acid (8f)

\[ \text{Approach A.} \] The following procedure has been adapted from the previously reported work with appropriate modification.\(^{95}\)
(E)-3-(3-nitrophenyl)acrylic acid, 7f, (386 mg, 2 mmol) was dissolved in degassed THF (14 mL) at room temperature. 4% mol. Wilkinson’s catalyst (0.074g) was added to the solution. Then the solution was saturated by H₂ and constant H₂ flow at 1 atm was kept through the reaction. After 96 h, ¹H NMR showed approximately a 4:6 ratio of 7f and 8f existed in the mixture. Both of the preparative TLC and column chromatography failed to separate 8f from 7f. Recrystallization in EtOH/H₂O failed to give pure crystal of 8f.

**Approach B.** The following procedure has been adapted from the previously reported work with appropriate modification.⁹⁶ (E)-3-(3-nitrophenyl)acrylic acid, 7f, (386 mg, 2 mmol) was added to 15 mL H₂O and cooled to 0 °C. Hydroxylamine sulfate, (H₂NOH)₂-H₂SO₄, (544.9 mg, 3.32 mmol), and hydroxylamine-O-sulfonic acid, H₂NOSO₃H, (1289.2mg, 11.40 mmol) was added to the mixture and the pH was adjusted to 6-7 with conc. NaOH solution (1.5 mL). the pH and temperature were kept constant as the solution was stirred for 5.5 h. Additional conc. NaOH solution (4.6 mL) was required to maintain the pH at 6-7. The reaction mixture was filtered and acidified to pH 2 with 2N H₂SO₄. The filtrate was refrigerated overnight and the product was collected by filtration. Recrystallization from ethanol and water produced off-white crystal. ¹H NMR showed approximately 6:4 ratio of 7f and 8f in the crystal. Further separation by either preparative TLC or column chromatography failed to separate 8f from 7f.
**Approach C.** The following procedure has been adapted from the previously reported work with appropriate modification. A solution of acetic acid (4.60 mL, 80 mmol) in dimethoxyethane (DME) (60 mL) was added to a suspension of PADA (3.88 g, 20 mmol) and (E)-3-(3-nitrophenyl)acrylic acid, 7f, (772.6 mg, 4 mmol) in DME (35 mL) dropwise at 50 °C. The mixture was stirred for 4 days at this temperature and then it was cooled to room temperature and filtered. The eluent was concentrated under reduced pressure and the residue was purified by column chromatography (hexane/ethyl acetate, 2:1 as eluent) to afford 8f as a white solid (417.3 mg, 54%). ¹H NMR (free amine in CDCl₃): δ 2.73-2.82 (t, 2H), 3.05-3.14 (t, 2H), 7.45-7.50 (m, 1H), 7.55-7.61 (m, 1H), 8.08-8.14 (m, 2H).

**3-(3-nitrophenyl)propan-1-ol (9f)**

The following procedure has been adapted from the previously reported work with appropriate modification. 3-(3-nitrophenyl)propanoic acid, 8f (417.3 mg, 2.14 mmol) was dissolved in THF (2mL) in a flame-dried flask. The mixture was cooled with an ice bath under an atmosphere of nitrogen. The solution of 1M BH₃ in THF (4.28 mL, 4.28 mmol) was added by syringe slowly and the mixture was stirred at room temperature for 2 h. Ice water was added slowly and the mixture was concentrated under reduced pressure. CH₂Cl₂ was added to the residue and the resulting solution was washed with water, saturated NaHCO₃ aqueous solution, and brine. The organic layer was dried over MgSO₄ and evaporated. The oil residue was purified by column chromatography (hexane/ethyl acetate, 1:1 as eluent)
to afford 9f as a pale yellow oil (307.4 mg, 80%). \(^1\)H NMR (free amine in CDCl\(_3\)): \(\delta\)
1.89-1.98 (m, 2H), 2.18 (s, 1H), 2.82-2.87 (t, 2H), 3.68-3.72 (t, 2H), 7.43-7.48 (t, 1H),
7.54-7.57 (d, 1H), 8.03-8.07 (m, 2H).

1-(3-chloropropyl)-3-nitrobenzene (10f)

In a similar fashion as described for the preparation of 10a, 3-(3-nitrophenyl)propan-1-ol, 9f (307 mg, 1.70 mmol) was converted to 10f as a
colorless oil (339 mg, 91%). \(^1\)H NMR (CDCl\(_3\)): \(\delta\)
2.09-2.20 (m, 2H), 2.89-2.95 (t, 2H),
3.54-3.59 (t, 2H), 7.45-7.51 (m, 1H), 7.55-7.58 (d, 1H), 8.04-8.07 (m, 2H).

4-benzyl-1-(3-(3-nitrophenyl)propyl)piperidine (1f)

In a similar fashion as described for the
preparation of 1a, 1-(3-chloropropyl)-3-nitrobenzene, 10f (55.6 mg, 0.28 mmol) was
converted to 1f as a pale yellow oil (85.2 mg, 90%). \(^1\)H NMR (free amine in CDCl\(_3\)): \(\delta\)
1.23-1.36 (m, 2H), 1.48-1.56 (m, 1H), 1.60-1.65 (d, 2H), 1.80-1.87 (m, 4H),
2.28-2.33 (t, 2H), 2.51-2.54 (d, 2H), 2.70-2.75 (t, 2H), 2.84-2.87 (d, 2H), 7.12-7.19
(m, 3H), 7.24-7.29 (t, 2H), 7.38-7.43 (t, 1H), 7.48-7.51 (d, 1H), 8.01-8.05 (m, 2H) \(^{13}\)C
NMR (free amine in CDCl\(_3\)): \(\delta\)
28.43, 32.25, 33.32, 37.97, 43.23, 53.91, 57.79,
120.93, 123.30, 125.74, 128.12, 128.36, 129.09, 134.72, 140.68, 144.28, 148.26.

Elemental Analysis: \((C_{21}H_{26}N_2O_2)\)

Calculated: C, 74.52; H, 7.74; N, 8.28;
Found: C, 74.45; H, 7.98; N, 8.05.

1-benzyl-4-(3-(3-nitrophenyl)propyl)piperazine (2f)

In a similar fashion as described for the preparation of 2a, 1-(3-chloropropyl)-3-nitrobenzene, 10f (34 mg, 0.17 mmol) was converted to 2f as a pale yellow oil (58 mg, 68%). The HCl salt of 2f was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 260-261 °C. \(^1\)H NMR (free amine in CDCl\(_3\)): \(\delta\) 1.79-1.89 (m, 2H), 2.33-2.77 (m, 12H), 3.51 (s, 2H), 7.22-7.33 (m, 5H), 7.40-7.45 (t, 1H), 7.50-7.52 (d, 1H), 8.02-8.06 (m, 2H). \(^{13}\)C NMR (free amine in CDCl\(_3\)): \(\delta\) 28.18, 33.18, 53.07, 53.13, 57.34, 63.05, 120.97, 123.31, 126.98, 128.16, 129.09, 129.17, 134.71, 138.08, 144.15, 148.27.

Elemental Analysis: (C\(_{20}\)H\(_{25}\)N\(_3\)O\(_2\)•2HCl)

Calculated: C, 58.25; H, 6.60; N, 10.19;

Found: C, 58.22; H, 6.56; N, 10.03.

3-(4-nitrophenyl)propanoic acid (8g)

In a similar fashion as described for the preparation of 8f, (\(E\))-3-(3-nitrophenyl)acrylic acid, 7g, (772.6 mg, 4 mmol) was converted to 8g as a white solid (624 mg, 80%). \(^1\)H NMR (free amine in CDCl\(_3\)): \(\delta\) 2.72-2.77 (t, 2H), 3.04-3.09 (t, 2H), 7.37-7.40 (d, 2H), 8.14-8.18 (d, 2H).
3-(4-nitrophenyl)propan-1-ol (9g)

In a similar fashion as described for the preparation of 9f, 3-(4-nitrophenyl)propanoic acid, 8g (500 mg, 2.56 mmol) was converted to 9g as a pale yellow oil (360.5 mg, 78%). ¹H NMR (free amine in CDCl₃): δ 1.87-1.99 (m, 2H), 2.81-2.87 (t, 2H), 3.10 (s, 1H), 3.67-3.73 (t, 2H), 7.34-7.39 (d, 2H), 8.07-8.13 (d, 2H).

1-(3-chloropropyl)-4-nitrobenzene (10g)

In a similar fashion as described for the preparation of 10a, 3-(4-nitrophenyl)propan-1-ol, 9g (360.5 mg, 1.99 mmol) was converted to 10g as a colorless oil (354.7 mg, 89%). ¹H NMR (CDCl₃): δ 2.08-2.19 (m, 2H), 2.88-2.94 (t, 2H), 3.54-3.59 (t, 2H), 7.37-7.40 (d, 2H), 8.11-8.14 (d, 2H).

4-benzyl-1-(3-(4-nitrophenyl)propyl)piperidine (1g)

In a similar fashion as described for the preparation of 1a, 1-(3-chloropropyl)-4-nitrobenzene, 10g (90 mg, 0.45 mmol) was converted to 1g as a pale yellow oil (132.2 mg, 87%). The HCl salt of 1g was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 187-189 °C. ¹H NMR (free amine in CDCl₃): δ 1.21-1.36 (m, 2H), 1.43-1.57 (m, 1H), 1.60-1.65 (d, 2H), 1.76-1.88 (m, 4H), 2.27-2.33 (t, 2H), 2.51-2.54 (d, 2H), 2.70-2.75 (t, 2H), 2.83-2.88
(d, 2H), 7.12-7.34 (m, 7H), 8.10-8.14 (d, 2H). $^{13}$C NMR (free amine in CDCl$_3$): $\delta$
28.43, 32.25, 33.32, 37.97, 43.23, 53.91, 57.79, 120.93, 123.30, 125.74, 128.12, 128.36, 129.09, 134.72, 140.68, 144.28, 148.26.

Elemental Analysis: (C$_{21}$H$_{26}$N$_2$O$_2$•HCl)

Calculated: C, 67.28; H, 7.26; N, 7.47;
Found: C, 67.27; H, 7.28; N, 7.47.

1-benzyl-4-(3-(4-nitrophenyl)propyl)piperazine (2g)

In a similar fashion as described for the preparation of 2a, 1-(3-chloropropyl)-4-nitrobenzene, 10g (50 mg, 0.25 mmol) was converted to 2g as a pale yellow oil (72 mg, 85%). The HCl salt of 2g was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 254-256 °C. $^1$H NMR (free amine in CDCl$_3$): $\delta$ 1.78-1.88 (m, 2H), 2.33-2.47 (m, 10H), 2.71-2.76 (t, 2H), 3.51 (s, 2H), 7.22-7.34 (m, 7H), 8.10-8.13 (d, 2H). $^{13}$C NMR (free amine in CDCl$_3$): $\delta$ 28.10, 33.49, 53.07, 53.13, 57.45, 63.04, 123.55, 126.89, 128.16, 129.15, 129.18, 138.08, 146.28, 150.15.

Elemental Analysis: (C$_{20}$H$_{25}$N$_3$O$_2$•2HCl)

Calculated: C, 58.25; H, 6.60; N, 10.19;
Found: C, 58.08; H, 6.49; N, 10.08.
3-(2-nitrophenyl)propanoic acid (8h)

In a similar fashion as described for the preparation of 8f, (E)-3-(2-nitrophenyl)acrylic acid, 7h was converted to 8h as a white solid (237 mg, 30%). $^1$H NMR (free amine in CDCl₃): δ 2.75-2.81 (t, 2H), 3.18-3.30 (t, 2H), 7.36-7.43 (m, 2H), 7.52-7.58 (m, 1H), 7.93-7.98 (m, 1H).

3-(2-nitrophenyl)propan-1-ol (9h)

In a similar fashion as described for the preparation of 9f, 3-(2-nitrophenyl)propanoic acid, 8h (237 mg, 1.21 mmol) was converted to 9h as a pale yellow oil (164.5 mg, 75%). $^1$H NMR (free amine in CDCl₃): δ 1.87-1.97 (m, 2H), 2.72 (s, 1H), 2.95-3.00 (t, 2H), 3.68-3.72 (t, 2H), 7.31-7.40 (m, 2H), 7.49-7.55 (m, 1H), 7.86-7.89 (m, 1H).

1-(3-chloropropyl)-2-nitrobenzene (10h)

In a similar fashion as described for the preparation of 10a, 3-(2-nitrophenyl)propan-1-ol, 9h was converted to 10h as a colorless oil (164.1 mg, 91%). $^1$H NMR (CDCl₃): δ 2.12-2.17 (m, 2H), 3.03-3.06 (t, 2H), 3.58-3.60 (t, 2H), 7.36-7.40 (m, 2H), 7.53-7.57 (m, 1H), 7.91-7.93 (m, 1H).
4-benzyl-1-(3-(2-nitrophenyl)propyl)piperidine (1h)

In a similar fashion as described for the preparation of 1a, 1-(3-chloropropyl)-2-nitrobenzene, 10h (50 mg, 0.25 mmol) was converted to 1h as a pale yellow oil (60.3 mg, 71%). The HCl salt of 1h was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 153-155 °C. \(^1\)H NMR (free amine in CDCl\(_3\)): δ 1.22-1.35 (m, 2H), 1.43-1.56 (m, 1H), 1.59-1.64 (d, 2H), 1.78-1.88 (m, 4H), 2.32-2.37 (t, 2H), 2.51-2.53 (d, 2H), 2.85-2.91 (m, 4H), 7.12-7.19 (m, 3H), 7.24-7.35 (m, 4H), 7.43-7.51 (m, 1H), 7.84-7.86 (m, 1H). \(^1\)C NMR (free amine in CDCl\(_3\)): δ 28.06, 30.79, 32.23, 37.98, 43.23, 53.87, 58.23, 124.58, 125.72, 126.85, 128.11, 129.10, 131.92, 132.73, 137.31, 140.73, 149.38.

Elemental Analysis: (C\(_{21}\)H\(_{26}\)N\(_2\)O\(_2\)•HCl•1/4H\(_2\)O)

Calculated: C, 66.48; H, 7.31; N, 7.38;

Found: C, 66.16; H, 7.23; N, 7.29.

1-benzyl-4-(3-(2-nitrophenyl)propyl)piperazine (2h)

In a similar fashion as described for the preparation of 2a, 1-(3-chloropropyl)-2-nitrobenzene, 10h was converted to 2h as a pale yellow oil (70 mg, 83%). The HCl salt of 2h was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 250-252 °C. \(^1\)H NMR (free amine in CDCl\(_3\)): δ
1.78-1.88 (m, 2H), 2.37-2.47 (m, 10H), 2.88-2.93 (t, 2H), 3.50 (s, 2H), 7.20-7.36 (m, 7H), 7.46-7.51 (m, 1H), 7.85-7.87 (m, 1H). $^{13}$C NMR (free amine in CDCl$_3$): $\delta$ 27.83, 30.70, 53.09, 53.11, 57.78, 63.06, 124.60, 126.89, 126.95, 128.14, 129.16, 131.93, 132.73, 137.24, 138.17, 149.38.

Elemental Analysis: (C$_{20}$H$_{25}$N$_3$O$_2$•2HCl)

Calculated: C, 58.25; H, 6.60; N, 10.19;

Found: C, 58.07; H, 6.57; N, 10.05.

4-benzyl-1-(3-(3-iodophenyl)propyl)piperidine (Ii)

The following procedure has been adapted from the previously reported work with appropriate modification.$^{93}$ A solution of Ii (80 mg, 0.237 mmol) in MeOH (4 mL) was hydrogenated over 5% wt. Pt/C (80 mg) for 2.5 h at 50 psi. The suspension was filtered through celite and evaporated to dryness to yield the amine 12. Consequently, 12 was dissolved in a solution of conc. HCl (0.08 mL) and water (1 mL) and the resulting acidic solution was cooled in ice bath. NaNO$_2$ (0.02 g, 0.29 mmol) dissolved in water (0.1 mL) was added dropwise to the acidic solution to form the diazonium salt. After stirring for 15 min, excess HNO$_2$ was destroyed by the addition of urea (~10 mg). A negative starch-iodide test was obtained at this time. The solution was added in small portion to a vigorously stirred biphasic mixture of CH$_2$Cl$_2$ (1.5 mL), KI (0.09g, 2 mmol), CuI (4 mg, 0.021 mmol) and water (0.5 mL). The reaction mixture was stirred overnight at room temperature.

The brown suspension was basified by 10% NaOH solution, diluted with
CH₂Cl₂, extracted. The organic layer was washed with 10% Na₂S₂O₃ (5 mL) for three times. The resulting organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (CH₂Cl₂/EtOAc, 2:1 as eluent) to yield 1i as a pale yellow oil (42 mg, 42%). The HCl salt of 1i was obtained by passing HCl gas into the ether solution of the free amine. Then, the salt was recrystallized from ethanol and ether m.p. (HCl salt): 179-182 °C. ¹H NMR (free amine in CDCl₃): δ 1.26-1.36 (m, 2H), 1.44-1.54 (m, 1H), 1.60-1.65 (d, 2H), 1.69-1.87 (m, 4H), 2.63-2.31 (t, 2H), 2.51-2.57 (m, 4H), 2.84-2.88 (d, 2H), 6.96-7.01 (t, 1H), 7.12-7.20 (m, 4H), 7.24-7.29 (m, 2H), 7.48-7.54 (m, 2H). ¹³C NMR (free amine in CDCl₃): δ 28.58, 32.24, 33.24, 37.98, 43.24, 53.92, 58.14, 94.40, 125.72, 127.67, 128.12, 129.10, 129.99, 134.77, 137.46, 140.72, 144.75. Elemental Analysis: (C₂₁H₂₇ClIN•HCl•0.₅Et₂O)

Calculated: C, 56.05; H, 6.54; N, 2.84;
Found: C, 56.69; H, 6.14; N, 3.25.

4-benzyl-1-(3-(3-iodophenyl)propyl)piperidine (2i)

In a similar fashion as described for the preparation of 1i, 2h was converted to 2i as a pale yellow oil (10 mg, 45%). The HCl salt of 2i was obtained by passing HCl gas into the ether solution of the free amine. Then, the salt was recrystallized from ethanol and ether m.p. (HCl salt): 243-245 °C. ¹H NMR (free amine in CDCl₃): δ 1.73-1.83 (m, 2H), 2.32-2.59 (m, b, 12H), 3.51 (s, 2H), 6.97-7.02 (t, 1H), 7.12-7.15 (d, 1H), 7.24-7.28 (m, 1H), 7.30-7.32 (m, 4H),
7.49-7.55 (m, 2H). $^{13}$C NMR (free amine in CDCl$_3$): $\delta$ 28.35, 33.18, 53.08, 53.14, 57.67, 63.06, 94.37, 126.96, 127.66, 128.15, 129.18, 129.99, 134.79, 137.46, 138.11, 144.63.

Elemental Analysis: (C$_{20}$H$_{27}$Cl$_2$IN$_2$•2HCl•1.5Et$_2$O)

  Calculated: C, 51.66; H, 7.00; N, 4.63;

  Found: C, 51.86; H, 6.75; N, 4.53.
Chapter 4 Result and Discussion

4.1 Synthesis of Lead 1 and Lead 2 Derivatives

4.1.1 Synthesis of 1-benzylpiperazine

The synthesis of 1-benzylpiperazine (6) was outlined in Scheme 4-1. Equivalent moles of N-Boc-piperazine (3) and benzylbromide (4) were mixed in the presence of sodium iodide and excess potassium carbonate. Iodide displaced the bromine in benzylbromide so that the resulting iodide intermediate was activated by the attack of the nucleophilic reagent 3. Potassium carbonate was added in excess amount (3 equiv.) as a base trap to deprotonate the amine formed during the reaction to give compound 5 as the free base in 88% isolated yield. Compound 5 was then treated with 4N HCl to remove the Boc protection. An overnight reaction time was required to completely deprotect compound 5, and the product 6 was obtained in a very high yield (92%). It was used directly as a starting material for alkylation with substituted Lead 1 and Lead 2 derivatives.

Scheme 4-1. Synthesis of 1-benzylpiperazine
4.1.2 Synthesis of Lead 1 derivatives 1a-1e and Lead 2 derivatives 2a-2e

When the substituent group on the phenyl ring is 4-OCH₃, 3-OCH₃, 4-CH₃, 3-F or 2-Br, the corresponding substituted cinnamic acid (7a-7e in Scheme 4-2) can be reduced with LiAlH₄ to the alcohol with the substituent group unaffected. However, reaction time and product yield were different because of the effect of substitution on the α,β-unsaturated carboxylic acid reduction. For example, it requires only 4 h in the reduction of 4-methoxycinnamic acid to obtain 84% yield of the product 9a. In comparison, the reduction of 2-bromocinnamic acid was continued for 12 h and a relatively low yield of 9e (32%) was achieved (Table 4-1). The difference in reaction time and product yield can be probably attributed to the electronic character of the substituent. The methoxy group is an electron-donating activating group on the para-position of aromatic ring. Its existence favors the addition of hydride to the double bond as well as the reduction of the carboxylic acid to alcohol. The bromo group on the ortho-position, on the other hand, is an electronegative deactivating group; thus, it hampers the addition of hydride. More information of the experimental conditions and yields is listed below in Table 4-1.
Scheme 4-2. Synthesis of Lead 1 derivatives 1a-1e and Lead 2 derivatives 2a-2e

Table 4-1. Experimental conditions and yield of 9a-9e

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Reaction Time (h)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>9a</td>
<td>4-OCH₃</td>
<td>4 h</td>
<td>84%</td>
</tr>
<tr>
<td>9b</td>
<td>3-OCH₃</td>
<td>26 h</td>
<td>91%</td>
</tr>
<tr>
<td>9c</td>
<td>4-CH₃</td>
<td>1 h</td>
<td>58%</td>
</tr>
<tr>
<td>9d</td>
<td>3-F</td>
<td>2 h</td>
<td>62%</td>
</tr>
<tr>
<td>9e</td>
<td>2-Br</td>
<td>12 h</td>
<td>32%</td>
</tr>
</tbody>
</table>

The next step is the chlorination of 9a-9e to their respective chloride 10a-10e.

Generally, moderate to high yield of the products (61% - 87%) can be achieved.

Thionyl chloride was chosen as the chlorination reagent. After the addition of thionyl chloride to the alcohol in CHCl₃, one or two drops of pyridine was added to the solution. Pyridine worked as a base to deprotonate the intermediate from the nucleophilic attack of the OH group on the thionyl chloride (Scheme 4-3).
Coupling of 10a-10e with 4-benzylpiperidine or 1-benzylpiperazine was conducted under the same condition as the synthesis of compound 5. Slightly different equivalences of 4-benzylpiperidine or 1-benzylpiperazine were added in the purpose of high yield and a better separation of product from the reagents. The ratio of the two reagents and yields are listed below in Table 4-2.

**Table 4-2. Experimental condition and yield of 1a-1e and 2a-2e**

<table>
<thead>
<tr>
<th>R</th>
<th>Compound</th>
<th>Ratio (10:11)</th>
<th>Yield</th>
<th>Compound</th>
<th>Ratio (10:6)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-OCH₃</td>
<td>1a</td>
<td>1:1.2</td>
<td>80%</td>
<td>2a</td>
<td>1:1.5</td>
<td>100%</td>
</tr>
<tr>
<td>3-OCH₃</td>
<td>1b</td>
<td>1:1</td>
<td>100%</td>
<td>2b</td>
<td>1:2</td>
<td>100%</td>
</tr>
<tr>
<td>4-CH₃</td>
<td>1c</td>
<td>1:1</td>
<td>82%</td>
<td>2c</td>
<td>1:1</td>
<td>92%</td>
</tr>
<tr>
<td>3-F</td>
<td>1d</td>
<td>1.2:1</td>
<td>86%</td>
<td>2d</td>
<td>1:1.5</td>
<td>96%</td>
</tr>
<tr>
<td>2-Br</td>
<td>1e</td>
<td>1:1</td>
<td>80%</td>
<td>2e</td>
<td>1:1.5</td>
<td>85%</td>
</tr>
</tbody>
</table>
From the table, we can conclude that both of the series 1a-1e and 2a-2e were obtained in very high isolated yield. Interestingly, from the same starting material, all piperazine derivatives (2a-2e) were obtained in an equal or higher yield than its piperidine derivatives analogues (1a-1e). Probably the presence of the tertiary amine favors the alkylation of the secondary amine with the chloride.

All of these compounds were obtained as colorless oils. They were then converted to the mono-HCl salts (1a-1e) or di-HCl salts (2a-2e) by passing HCl gas through solutions of the free base in EtOH. HPLC analyses of the compounds show >95% purity and then the samples were sent for elemental analysis, in which all gave good results.

### 4.1.3 Synthesis of Lead 1 derivatives 1f-1h and Lead 2 derivatives 2f-2h

When the nitro group is present on the phenyl ring, LiAlH₄ is such a strong reducing agent that it could reduce the nitro group as well. Three approaches (Approach A, B and C) were tried in the attempt to reduce the double bond while keeping the nitro group unaffected (See 4.3.1 to 4.3.3 for details).

Firstly, catalytic hydrogenation with Wilkinson’s catalyst was attempted. However, ¹H NMR of the crude product showed low yield of desired compounds, and the product couldn’t be separated successfully from the starting material by chromatography column. Then, the system of (H₂NOH)₂H₂SO₄/H₂NOSO₃H was applied to produce diimide to reduce the olefinic bond of the starting material.
which resulted in a low yield and a non-reproducible process for the other two starting materials. Finally, an alternative diimide source, potassium azodicarboxylate (PADA), was prepared from azodicarbonamide in aqueous KOH solution. In the reduction of the double bond, with the addition of acetic acid dropwise, HN=NH was evolved and served as a stable source of reductive H\textsuperscript{97} (Scheme 4-4) (See 4.3.1 to 4.3.3 for details).

Then the carboxylic acid was reduced to alcohol by borane solution in THF. The following reactions leading to 1f-1h and 2f-2h were similar to those for their analogues 1a-1e and 2a-2e. Compound 1f (R=3-NO\textsubscript{2}) was kept as oily free base after preparation. Its HCl salt formed as a gel and was difficult to recrystallize to give crystals (Scheme 4-4).

![Scheme 4-4. Synthesis of Lead 1 derivatives 1f-1h and Lead 2 derivatives 2g-2h](image-url)
4.1.3.1 Approach A. Catalytic hydrogenation with Wilkinson’s catalyst

Chlorotris(triphenylphosphine)rhodium(I), Wilkinson’s catalyst (Figure 4-1), has been reported as a catalyst for the selective saturation of an olefin in the presence of an aromatic nitro group.\textsuperscript{95}

![Figure 4-1. Structure of Wilkinson’s catalyst](image)

In our experiment, 4\% (mol) Wilkinson’s catalyst was added to the solution of compound 7f (3-NO\(_2\)) in degassed THF under 1 atm H\(_2\) at room temperature. The mixture was allowed to react for up to 4 d. Because 7f and 8f have a very close polarity, the decrease of 7f or the formation of 8f can hardly be shown by TLC analysis. Therefore, we took a small portion of the reaction mixture from time to time and took the \(^1\)H NMR. The starting material remained even after a long reaction time (Table 4-3). Moreover, 8f couldn’t be separated successfully from 7f by column chromatography, and we failed to recrystallize 8f in acceptable purity. The same reaction was carried out for 7g (4-NO\(_2\)) and 7h (2-NO\(_2\)), but both of them failed to give any product. The reason might be that the catalyst is readily subject to decomposition in atmosphere. Given the problems in the reaction and work-up, this approach was not further investigated.
Table 4-3. Reaction result for reduction of 7f-7h with Wilkinson’s catalyst

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Wilkinson’s Catalyst (mol.)</th>
<th>Reaction Time</th>
<th>Ratio of Reactant: Product by $^1$H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>7f</td>
<td>3-NO$_2$</td>
<td>4%</td>
<td>24h</td>
<td>56:44</td>
</tr>
<tr>
<td>7f</td>
<td>3-NO$_2$</td>
<td>4%</td>
<td>48h</td>
<td>40:60</td>
</tr>
<tr>
<td>7f</td>
<td>3-NO$_2$</td>
<td>4%</td>
<td>96h</td>
<td>37:63</td>
</tr>
<tr>
<td>7g</td>
<td>4-NO$_2$</td>
<td>4%</td>
<td>24h</td>
<td>N.R.</td>
</tr>
<tr>
<td>7h</td>
<td>2-NO$_2$</td>
<td>8%</td>
<td>24h</td>
<td>N.R.</td>
</tr>
</tbody>
</table>

4.1.3.2 Approach B. Reduction with diimide by (H$_2$NOH)$_2$-H$_2$SO$_4$/H$_2$NOSO$_3$H

Hydroxylamine sulfate, (H$_2$NOH)$_2$-H$_2$SO$_4$, together with hydroxylamine-O-sulfonic acid, H$_2$NOSO$_3$H, provides under basic conditions a source of diimide, HN=NH, which will reduce the olefinic bond via a concerted pathway$^{96}$ (Scheme 4-5).

![Scheme 4-5. Mechanism of reduction of olefinic bond with diimide](image)

However, when we tried the (H$_2$NOH)$_2$-H$_2$SO$_4$/H$_2$NOSO$_3$H system to reduce the olefinic bond in 7f-7h, the same problem as catalytic hydrogenation with
Wilkinson’s catalyst occurred. Again, the conversion ratio was low for 7f and 7g (43%, 20%, respectively), and there was no reaction for 7h. The products cannot be separated from their precursor by column. As a result, this method was also not further investigated.

Table 4-4. Reaction result for reduction of 7f-7h with \((\text{H}_2\text{NOH})_2\text{H}_2\text{SO}_4/\text{H}_2\text{NOSO}_3\text{H}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Reaction Time</th>
<th>Ratio of Reactant: Product by (^1\text{H} \text{NMR} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>7f</td>
<td>3-NO\textsubscript{2}</td>
<td>5.5h</td>
<td>57:43</td>
</tr>
<tr>
<td>7g</td>
<td>4-NO\textsubscript{2}</td>
<td>24h</td>
<td>80:20</td>
</tr>
<tr>
<td>7h</td>
<td>2-NO\textsubscript{2}</td>
<td>18h</td>
<td>N.R.</td>
</tr>
</tbody>
</table>

4.1.3.3 Approach C. Reduction with diimide by potassium azodicarboxylate (PADA)

In a recent publication, potassium azodicarboxylate, PADA, was reported as an alternative source of diimide to reduce an olefinic bond without affecting nitro substituent present on aromatic rings. Inspired by this report, we decided to try this approach for our compounds 7f-7h. PADA can be easily prepared with almost 100% yield from the reaction of azodicarbonamide, \((\text{H}_2\text{NCON})_2\), and KOH. In the reduction of double bond, acetic acid was added as a source of hydrogen to form the diimide (Scheme 4-6).
Scheme 4-6. Mechanism of production of diimide from PADA

In our experiment, we treated 7f-7h with PADA and acetic acid in DME (dimethoxyl ethane, CH₃OCH₂CH₂OCH₃) at 50 °C. The reaction was monitored by the ¹H NMR to see if the peak for H on the double bond has disappeared or not. Generally, long reaction times (2 days to 5.5 days) were required for complete reduction. However, products were easily purified and yields were sufficient to process the following reactions.

Table 4-5. Reaction result for reduction of 7f-7h with PADA and acetic acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>7: PADA: Acetic Acid</th>
<th>Reaction Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>7f</td>
<td>3-NO₂</td>
<td>1: 32: 8</td>
<td>4 d</td>
<td>54%</td>
</tr>
<tr>
<td>7g</td>
<td>4-NO₂</td>
<td>1: 3: 3</td>
<td>2 d</td>
<td>80%</td>
</tr>
<tr>
<td>7h</td>
<td>2-NO₂</td>
<td>1: 8: 8</td>
<td>5.5 d</td>
<td>30%</td>
</tr>
</tbody>
</table>
4.1.4 Synthesis of Lead 1 derivatives 1i and Lead 2 derivatives 2i

De-iodination is known to occur under reductive conditions, such as LiAlH₄ or BH₃. Therefore, we decided to introduce the iodo group in the last step of the preparation of 1i and 2i. Hydrogenation of 1f (or 2f) at 50 psi gave the aniline 12 (or 13) in a short reaction time (2.5 h). Without further work-up, the aniline was converted to 1i (or 2i) via a Sandmeyer iodination (Scheme 4-7). The overall yield of these two steps is 40%.

Scheme 4-7. Synthesis of Lead 1 derivative 1i and Lead 2 derivative 2i
4.2 Qualitative Analysis of Sigma binding assay results for Lead 1 derivatives 1a-1i

The sigma binding assay results of Lead 1 and Lead 1 derivatives 1a-1i were obtained (Table 4-6) (the sigma binding assay was conducted by Roger Nahas in Dr. Susan Lever’s research group). All of them show subnanomolar sigma-1 binding affinity and subnanomolar or nanomolar sigma-2 binding affinity, with moderate sigma-1 selectivity. Among these analogues, compound 1g (R: 4-NO₂) seems to be the most potent sigma-1 ligand (Ki: 0.11 nM), while compound 1a (R: 4-OCH₃) appears as the most sigma-1 selective ligand (Ki: σ₂/σ₁ = 13.45). The binding affinity of Lead 1 agrees with the literature report, with 9-fold selectivity for sigma-1. For the compounds with nitro substituent at different positions of the phenyl ring (1f, meta-; 1g, para-; 1h, ortho-), the sigma-1 and sigma-2 binding affinities have different trends (Sigma-1: 1g, 4-NO₂, 0.11 nM > 1h, 2-NO₂, 0.60 nM > 1f, 3-NO₂, 0.66 nM; Sigma-2: 1f, 3-NO₂, 0.72 nM > 1g, 4-NO₂, 1.00 nM > 1h, 2-NO₂, 2.62 nM). In particular, similar trend in meta- and para- position was confirmed from the comparison between 1a (R: 4-OCH₃) and 1b (R: 3-OCH₃), in which the para-substituent gives a better sigma-1 binding affinity (1a, 0.49 nM > 1b, 0.64 nM) while the meta-substituent gives a better sigma-2 binding affinity (1b, 3.65 nM > 1a, 6.49 nM). In the case of halogen substitution (1d, 1e, 1i), the relationship between the lipophilicity and size of the substituent and the sigma binding affinity of the compound can be related. With higher lipophilicity and larger substituent size, the higher sigma-2 binding affinities were obtained (sigma-2: 1i, 3-I, 0.95 nM > 1e, 2-Br,
1.81 nM > 1d, 3-F, 1.94 nM). However, the sigma-1 binding affinities are slightly different. Compounds 1i and 1e have similar sigma-1 binding affinity, both of which are higher than 1d (sigma-1: 1e, 2-Br, 0.32 nM > 1i, 3-I, 0.34 nM > 1d, 3-F, 0.56 nM). The comparison between 1e (2-Br) and 1h (2-NO₂) shows that at the ortho- position, higher lipophilicity results in better binding affinity for both subtypes.

Overall, although the sigma binding affinity varies with the substituent in 1a-1i analogues, it seems that their sigma binding properties are not very sensitive to the change of chosen substituent on the phenylpropyl moiety, especially for sigma-1 receptor (Ki: 0.11~0.66 nM). Therefore, we can draw a conclusion that the substitution on this phenyl ring has limited effect on the binding affinity, which means that both sigma receptor subtypes are able to tolerate the chosen substituents.
Table 4-6. Sigma receptor binding properties of Lead 1 derivatives 1a-1i
(Values: mean ± SEM, n = 3~6)

<table>
<thead>
<tr>
<th>Compound (R)</th>
<th>IC₅₀ (nM)</th>
<th>Kᵢ (nM)</th>
<th>nᵢ</th>
<th>IC₅₀ (nM)</th>
<th>Kᵢ (nM)</th>
<th>nᵢ</th>
<th>Selectivity Kᵢ Ratio σ₂ / σ₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead 1 (H)</td>
<td>0.6 ± 0.03</td>
<td><strong>0.38 ± 0.02</strong></td>
<td>1.13 ± 0.13</td>
<td>3.88 ± 0.02</td>
<td><strong>3.50 ± 0.02</strong></td>
<td>0.69 ± 0.19</td>
<td>9.21</td>
</tr>
<tr>
<td>1a (4-OCH₃)</td>
<td>0.70 ± 0.07</td>
<td><strong>0.49 ± 0.05</strong></td>
<td>1.21 ± 0.07</td>
<td>7.40 ± 0.39</td>
<td><strong>6.59 ± 0.34</strong></td>
<td>0.87 ± 0.09</td>
<td><strong>13.45</strong></td>
</tr>
<tr>
<td>1b (3-OCH₃)</td>
<td>0.94 ± 0.06</td>
<td><strong>0.64 ± 0.04</strong></td>
<td>1.12 ± 0.05</td>
<td>4.10 ± 0.15</td>
<td><strong>3.65 ± 0.14</strong></td>
<td>0.95 ± 0.14</td>
<td><strong>5.70</strong></td>
</tr>
<tr>
<td>1c (4-CH₃)</td>
<td>0.47 ± 0.01</td>
<td><strong>0.33 ± 0.01</strong></td>
<td>1.04 ± 0.02</td>
<td>3.97 ± 0.60</td>
<td><strong>3.53 ± 0.53</strong></td>
<td>1.06 ± 0.32</td>
<td><strong>10.70</strong></td>
</tr>
<tr>
<td>1d (3-F)</td>
<td>0.79 ± 0.11</td>
<td><strong>0.56 ± 0.08</strong></td>
<td>1.06 ± 0.03</td>
<td>2.18 ± 0.33</td>
<td><strong>1.94 ± 0.27</strong></td>
<td>0.98 ± 0.07</td>
<td><strong>3.50</strong></td>
</tr>
<tr>
<td>1e (2-Br)</td>
<td>0.46 ± 0.02</td>
<td><strong>0.32 ± 0.01</strong></td>
<td>1.20 ± 0.07</td>
<td>2.03 ± 0.13</td>
<td><strong>1.81 ± 0.12</strong></td>
<td>0.99 ± 0.10</td>
<td><strong>5.60</strong></td>
</tr>
<tr>
<td>1f (3-NO₂)</td>
<td>0.95 ± 0.02</td>
<td><strong>0.66 ± 0.01</strong></td>
<td>1.05 ± 0.02</td>
<td>0.81 ± 0.05</td>
<td><strong>0.72 ± 0.04</strong></td>
<td>0.88 ± 0.20</td>
<td><strong>1.09</strong></td>
</tr>
<tr>
<td>1g (4-NO₂)</td>
<td>0.16 ± 0.00</td>
<td><strong>0.11 ± 0.00</strong></td>
<td>0.81 ± 0.02</td>
<td>1.13 ± 0.12</td>
<td><strong>1.00 ± 0.10</strong></td>
<td>0.79 ± 0.11</td>
<td><strong>9.09</strong></td>
</tr>
<tr>
<td>1h (2-NO₂)</td>
<td>0.86 ± 0.03</td>
<td><strong>0.60 ± 0.02</strong></td>
<td>1.08 ± 0.02</td>
<td>2.95 ± 0.27</td>
<td><strong>2.62 ± 0.24</strong></td>
<td>0.75 ± 0.13</td>
<td><strong>4.37</strong></td>
</tr>
<tr>
<td>1i (3-I)</td>
<td>0.50 ± 0.00</td>
<td><strong>0.34 ± 0.00</strong></td>
<td>1.38 ± 0.08</td>
<td>1.07 ± 0.91</td>
<td><strong>0.95 ± 0.08</strong></td>
<td>0.92 ± 0.17</td>
<td><strong>2.79</strong></td>
</tr>
</tbody>
</table>
Chapter 5 Conclusion

Aiming at a study of quantitative structure-activity relationships of sigma receptors ligands, two series of compounds, diarylalkylpiperidine derivatives 1a-1i and diarylalkylpiperazine derivatives 2a-2i, were firstly designed and synthesized.

Compounds 1a-1e and 2a-2e were prepared from the cinnamic acids 7a-7e in the following synthetic pathways: reduction with LAH, chlorination with SOCl₂, and finally alkylation with benzylpiperidine or benzylpiperazine.

Due to the instability of the aromatic nitro group under the reductive condition of LAH, three other methods were tried in an attempt to reduce the α,β-unsaturated carboxylic acid in 7f-7h, while the nitro group was unaffected. Both catalytic hydrogenation with Wilkinson’s catalyst and the reduction with diimide produced by the system of (H₂NOH)₂H₂SO₄/H₂NOSO₃H didn’t yield good results. The reduction product was obtained with a low yield and couldn’t be successfully separated from the staring material. Finally, an alternative diimide source, potassium azodicarboxylate (PADA), was able to drive the reduction completely without affecting the nitro group. The carboxylic acid was subsequently reduced to alcohol by borane in THF. The steps of chlorination and alkylation in the same condition as the preparation of 1a-1e and 2a-2e were followed to give 1f-1h and 2f-2h.

Compounds 1i and 2i, the iodinated analogues, were obtained from their nitro analogues 1f and 2f in two steps. Hydrogenation of 1f and 2f with Pd/C followed by Sandmeyer iodination reaction finally give the iodo analogues 1i and 2i.
After the preparation of Lead 1 and Lead 2 derivatives, the sigma binding assay results of compounds 1a-1i were obtained. Qualitative analysis of the data showed that the sigma binding property was related to the chosen substitution in the phenylpropyl moiety. However, the data shown also indicated that the chosen substituents did not greatly affect the in vitro binding affinity and the sigma receptors were able to tolerate the chosen substitution in this area. Finally, mathematical equations relating the physicochemical parameters and the binding affinity will be developed to predict the biological activity of compounds with similar structures. The QSAR analysis will be included in the PhD dissertation of another group member (Roger Nahas in Dr. Lever’s group). In the future, sigma ligands which can be radiolabeled with radioactive isotopes (F-18, I-123, etc.) can be investigated to explore their use in radio-imaging via PET or SPECT.
Chapter 6. \( \mu \)-Opioid Receptor Ligand

6.1 Introduction

6.1.1 Opioid Receptors, Opiate Alkaloids and Opioid Peptides

Opioid receptors, which are known to be members of the family of G-protein coupled receptors, mediate the biological effects of opioid peptides in the central nervous system (CNS) as well as the peripheral nervous system. They have been divided into three major subtypes, namely, mu(\( \mu \)), delta(\( \delta \)), and kappa(\( \kappa \)). This subdivision is not only supported by the results of cloning, but also by the difference of their affinity for peptide and alkaloid agonists. Among the three subtypes, the \( \mu \)-opioid receptor is considered to be the one with the greatest clinical importance. It binds morphine with high affinity and is thought to be the main mediator of analgesia and drug addiction by morphine and other opioids. Moreover, it was recently suggested that \( \mu \)-opioid receptor might be an appropriate target for lung cancer imaging.

![Structure of Morphine](image)

Figure 6-1. Structure of Morphine

Morphine (Figure 6-1), known as a prototypic opiate alkaloid, is widely used as opiate analgesic to relieve severe pain in the treatment of diseases. However, other than its analgesic effect, its associated side effects, such as respiratory
depression, tolerance and physical dependence, and inhibition of gastrointestinal function, limit its utility as effective painkiller. Therefore, the major goal in opioid pharmacology is to develop new drugs with similar or even higher analgesic action than morphine, while with reduced side effects.\textsuperscript{103}

In addition to the opiate alkaloids, many endogenous opioid peptides have been identified and characterized. They were found to bind to $\mu$-opioid receptor with high affinity and selectivity against the other two opioid receptor subtypes. Among them, endomorphine-1 (Tyr-Pro-Trp-Phe-NH$_2$) and endomorphine-2 (Tyr-Pro-Phe-Phe-NH$_2$) exhibit the highest affinities and specificities for $\mu$-receptor so far in the mammalian nervous system.\textsuperscript{104} Together with other naturally-occurring opioid peptides such as enkephalins, dynorphins, and dermorphins,\textsuperscript{100} they serve as a good start for the structure-activity relationship studies to find more potent $\mu$-opioid peptides and characterize the ligand binding site.

6.1.2 Development of Synthetic Opioid Peptides

The discovery of endogenous peptides as opioid ligands initiated an intensive search for potent, selective ligands for opioid receptors, especially for the $\mu$-opioid receptor. Some structure-activity relationship studies have been investigated for the structural modification of the endogenous peptides. For example, dermorphine (Tyr-$\delta$-Ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$), which was isolated from amphibian skin in 1981,\textsuperscript{105} possesses a potent and long-lasting opioid activity. Modification of the amino acid sequences in dermorphine showed that the N-terminal tetrapeptide is the
minimum requirement for keeping the opioid reactivity.\textsuperscript{106} Derived from such tetrapeptide sequences (Tyr-\textit{d}-Ala-Phe-Gly), synthetic peptides which had Tyr in position 1 and Phe in position 3 (Tyr-\textit{d}-AA\textsuperscript{2}-Phe-AA\textsuperscript{4}, where AA\textsuperscript{2} and AA\textsuperscript{4} represent a certain amino acid) were found to show potent agonist activity for the \(\mu\)-opioid receptor.\textsuperscript{107} In particular, the Tyr residue in position 1 was found to play a key role in the interaction with opioid receptors. It satisfies a minimal pharmacophore model characterized by an appropriately spatially-oriented basic nitrogen and a hydroxylated phenyl ring.\textsuperscript{108} Among those synthetic peptides, Tyr-\textit{d}-Arg-Phe-Lys-NH\textsubscript{2}\textsuperscript{109} and Tyr-\textit{d}-Arg-Phe-Ala-NH\textsubscript{2}\textsuperscript{110} are two dermorphine analogues which are highly potent and very selective for \(\mu\)-receptor (Table 6-1).

**Table 6-1. Opioid receptor binding assay of some dermorphine analogues\textsuperscript{109-112}**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Ki (nM)</th>
<th>Ki: (\delta/\mu)</th>
<th>Ki: (\kappa/\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu)</td>
<td>(\delta)</td>
<td>(\kappa)</td>
</tr>
<tr>
<td>Tyr-\textit{d}-Arg-Phe-Lys-NH\textsubscript{2}</td>
<td>1.69 ± 0.25</td>
<td>19200 ± 2000</td>
<td>4230 ± 360</td>
</tr>
<tr>
<td>Tyr-\textit{d}-Arg-Phe-Ala-NH\textsubscript{2}</td>
<td>0.145 ± 0.040</td>
<td>385 ± 150</td>
<td>-</td>
</tr>
<tr>
<td>Dmt-\textit{d}-Arg-Phe-Lys-NH\textsubscript{2}</td>
<td>0.143 ± 0.015</td>
<td>2100 ± 310</td>
<td>22.3 ± 4.2</td>
</tr>
<tr>
<td>Dmt-\textit{d}-Arg-Phe-Ala-NH\textsubscript{2}</td>
<td>0.0021 ± 0.0007</td>
<td>1.13 ± 0.13</td>
<td>-</td>
</tr>
</tbody>
</table>

In another study which was initially aimed at making the peptide molecule more resistant to enzymatic degradation, the introduction of \textit{d}-Ala in position 2 in the general sequence of tetrapeptide was found to be vital for this goal.\textsuperscript{113} A dermorphine analogue Tyr-\textit{d}-Ala-Phe-Phe-NH\textsubscript{2} showed high \(\mu\)-receptor affinity and selectivity.\textsuperscript{109}
On the other hand, replacement of the tyrosine residue in position 1 with Dmt (2’, 6’-dimethyl-L-tyrosine) was observed to dramatically enhance μ-opioid receptor affinity or selectivity by one or two orders of magnitude. In fact, Dmt-\(D\)-Arg-Phe-Lys-NH\(_2\)\(^{111}\) and Dmt-\(D\)-Arg-Phe-Ala-NH\(_2\)\(^{112}\) are among the most potent and most selective μ-opioid receptor agonists reported to date.

6.1.3 Background and Specific Aim of This Work

In a preliminary work in Dr. John Lever’s research group, one dermorphine analogue, Dmt-\(D\)-Ala-Phe-Phe-NH\(_2\), was obtained by solid phase peptide synthesis. In the subsequent \textit{in vitro} binding assay, Dmt-\(D\)-Ala-Phe-Phe-NH\(_2\) was found to have a better μ-opioid receptor binding affinity than its precursor Tyr-\(D\)-Ala-Phe-Phe-NH\(_2\) (Table 6-2). The mono-iodinated peptide (I-Dmt-\(D\)-Ala-Phe-Phe-NH\(_2\)) was also found to have high affinity and selectivity for μ-opioid receptors. Radioiodination of this peptide could aid in imaging of tumors. However, its high lipophilicity (log D = 2.99±0.12 at pH 7.4) would not allow easy travel \textit{in vivo} to cross the blood-brain barrier (BBB).

<table>
<thead>
<tr>
<th>Liands</th>
<th>Ki (nM)</th>
<th>(\delta/\mu)</th>
<th>(\kappa/\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr-(D)-Ala-Phe-Phe-NH(_2)</td>
<td>3.52 ± 0.137</td>
<td>521 ± 72</td>
<td>176.7 ± 17.3</td>
</tr>
<tr>
<td>Dmt-(D)-Ala-Phe-Phe-NH(_2)</td>
<td>0.11 ± 0.010</td>
<td>8.32 ± 0.76</td>
<td>2.65 ± 0.650</td>
</tr>
<tr>
<td>I-Dmt-(D)-Ala-Phe-Phe-NH(_2)</td>
<td>2.89 ± 0.313</td>
<td>1090 ± 102</td>
<td>399.6 ± 21.5</td>
</tr>
</tbody>
</table>
In order to obtain a proper lipophilicity (log D = 2~3) but keep the µ-opioid receptor affinity and selectivity, an alternative peptide was synthesized in our group as control: Dmt-D-Ala-Phe-Orn-NH₂. The purpose of switching the phenylalanine in position 4 to ornithine was to replace the more lipophilic phenyl ring with more hydrophilic amino group. The non-iodinated peptide analogue can serve as control sample for the in vitro studies. Then, two routes could be adopted for the preparation of I-Dmt-D-Ala-Phe-Orn-NH₂: iodination of the peptide directly or preparation of the iodinated Dmt. Since –OH and –CH₃ groups on the aromatic ring of Dmt activate the nucleophilic reaction in the ortho- position of aromatic ring, it is highly likely that the di-iodinated reaction would compete with the mono-iodinated reaction. As a result, it would be less efficient to iodinate the peptide due to the unwanted di-iodinated peptide product and difficulty in the separation of mono-iodinated peptide product. Therefore, we decided to investigate the iodination of Dmt.

In this work, we aim at finding a practical synthetic method of mono-iodination of the Dmt residue with two different reagents (Scheme 6-1). The first reagent, N-iodosuccinimide (NIS), was reported to be an excellent reagent for the regioselective iodination of activated aromatic ring.¹¹⁵ The second reagent, Bis(pyridine)iodonium(I) Tetrafluoroborate (IPy₂BF₄), is very effective at iodinating aromatic functions of both simple organic molecules and more complex polyfunctional peptides.¹¹⁶ We’ll use Boc protected or Fmoc protected Dmt as the starting material. We’ll exam the iodination reaction under different conditions and then pick the best one to prepare the mono-iodinated Boc-Dmt or Fmoc-Dmt.
6.2 Experimental Section

6.2.1 Methods and Materials

\[ \text{Na-}\text{-(9-tert-butoxycarbonyl)-2,6-dimethyl-L-tyrosine (Boc-Dmt) and} \]
\[ \text{Na-}\text{-(9-fluorenylmethoxycarbonyl)-2,6-dimethyl-L-tyrosine (Fmoc-Dmt)} \]

were purchased from RSP Amino Acids LLC, Shirley, MA. They were stored in a

dessicator at room temperature after received. All other chemicals were purchased

from the Aldrich Chemical Co. All solvents used for reactions and analytical HPLC

were HPLC grade. All reactions were protected from light. \(^1\)H NMR and \(^13\)C NMR

spectrum were obtained on a Bruker AMX-300 (300 MHz) in \(d_6\)-DMSO solution.

Analytical HPLC was performed on the Waters HPLC Pumps (Model: 1525EF) with

Waters Dual \(\lambda\) Ultraviolet Absorbance Detector (Model: 2487). Preparative HPLC
was performed on a Beckmann Coulter System Gold 126P Solvent Module. The column used for preparative HPLC was Waters® prep. Nova-Pak C18 Kromosil, 19×300 nm, 6 μm, 60 Å.

6.2.2 Experiment Procedure

**Iodination of Boc-Dmt with NIS**

The iodination procedure with NIS has been adapted from the previously reported work with appropriate modification.\textsuperscript{115} To a solution of Boc-2,6-Dimethyl-\textit{L}-tyrosine (2.3 mg, 0.0074 mmol) in 0.5 mL phosphate buffer solution (pH 7.4) was added N-iodosuccinimide (1.7 mg, 0.0074 mmol). The reaction vial was protected from the light and was stirred for 10 min at room temperature. 10 μL of the reaction mixture was removed to check the reaction conversion by analytical HPLC.

**Iodination of Boc-Dmt with \textit{IPy}_2\textit{BF}_4**

The iodination procedure with \textit{IPy}_2\textit{BF}_4 has been adapted from the previously reported work with appropriate modification.\textsuperscript{116} A solution of Boc-2,6-Dimethyl-\textit{L}-tyrosine (6.8 mg, 0.022 mmol) in 1.5 mL CH₃CN was added HBF₄ (30 μL, 0.022 mmol of a 5.4% solution in ether) and then \textit{IPy}_2\textit{BF}_4 (8.2 mg, 0.022 mmol) was added. The reaction vial was protected from the light and was stirred for 10 min at room temperature. 10 μL of the reaction mixture was removed to check the reaction conversion by analytical HPLC and LC-MS.
Iodination of Fmoc-Dmt with NIS

The iodination procedure with NIS has been adapted from the previously reported work with appropriate modification.\textsuperscript{115} To a solution of Fmoc-2,6-Dimethyl-L-tyrosine (5.0 mg, 0.0116 mmol) in 5 mL CH\textsubscript{3}CN was added trifluoroacetic acid (1.3 mg, 0.0116 mmol) and N-iodosuccinimide (2.6 mg, 0.0116 mmol). The reaction vial was protected from the light and was stirred for 10 min at room temperature. 10 \textmu L of the reaction mixture was removed to check the reaction conversion by analytical HPLC.

Iodination of Fmoc-Dmt with IPy\textsubscript{2}BF\textsubscript{4}

The iodination procedure with IPy\textsubscript{2}BF\textsubscript{4} has been adapted from the previously reported work with appropriate modification.\textsuperscript{116} A solution of Fmoc-2,6-Dimethyl-L-tyrosine (5.0 mg, 0.0116 mmol) in 5 mL CH\textsubscript{3}CN was added HBF\textsubscript{4} (16 \textmu L, 0.0116 mmol of a 5.4% solution in ether) and then IPy\textsubscript{2}BF\textsubscript{4} (4.7 mg, 0.0128 mmol) was added. The reaction vial was protected from the light and was stirred for 10 min at room temperature. 10 \textmu L of the reaction mixture was removed to check the reaction conversion by analytical HPLC. Then, a larger scale reaction (150 mg Fmoc-Dmt) was conducted under the same conditions. The reaction mixture was concentrated to 10 mL and the preparative HPLC was applied to isolate the mono-iodinated product. After the collection by HPLC and the removal of the solvent, the mono-I-Dmt was obtained as a white solid (50 mg).

\textsuperscript{1}H NMR (in d\textsubscript{6}-DMSO): \textdelta 2.20 (s, 3H), 2.50 (s, 3H), 2.98-3.02 (dd, 1H), 3.13-3.19
(dd, 1H), 3.55 (br, 1H), 4.02-4.10 (m, 1H), 4.14-4.22 (m, 3H), 6.56 (s, 1H), 7.28-7.35 (dd, 2H), 7.39-7.43 (t, 2H), 7.65-7.80 (m, 2H), 7.87-7.89 (d, 2H), 9.96 (s, 1H), 12.83 (br, 1H). $^{13}$C NMR (in $d_6$-DMSO): δ 20.50, 26.34, 32.55, 46.99, 54.80, 66.12, 90.98, 114.65, 120.52, 125.69, 126.91, 127.50, 128.05, 138.24, 140.60, 141.11, 144.15, 144.23, 155.04, 156.34, 173.81.

6.3 Result and Discussion

6.3.1 Iodination of Boc-Dmt with NIS

The Boc group can be readily removed under acidic conditions. Therefore, we didn’t follow the same protocol as in the literature, in which the trifluoroacetic acid (CF$_3$COOH) was used. Instead, the reaction was carried out in the phosphate buffer solution at pH 7.4. However, the analytical HPLC showed the presence of two more lipophilic compounds, presumably the mono-iodinated product and the di-iodinated product (peak 1 and peak 2 in Figure 6-2 respectively).

![Figure 6-2](image)

Figure 6-2. HPLC of the reaction mixture of Boc-Dmt with NIS

6.3.2 Iodination of Boc-Dmt with IPy$_2$BF$_4$

A strong acid, HBF$_4$, was used in the regioselective iodination in the literature. We compared the results between the reaction with such acid and the reaction without such acid. As we anticipated, if HBF$_4$ was not added to the reaction
mixture, both of mono-iodinated and di-iodinated products were present. If HBF₄ was added, LC-MS indicated that the Boc protecting group was removed (Figure 6-3). However, the Boc group was also found to be cleaved when Boc-Dmt was analyzed by mass spectroscopy (expected MW: 309.87; actual MW: 209.83) (Figure 6-4).

**Figure 6-3.** LC-MS of main products in the reaction of Boc-Dmt with IPy₂BF₄

**Figure 6-4.** Mass spectrum of Boc-Dmt
6.3.3 Iodination of Fmoc-Dmt with NIS

The Fmoc protecting group is stable under acidic conditions. Therefore, the iodination reaction of Fmoc-Dmt with NIS was carried out when strong acid (CF₃COOH) was either present or not (Figure 6-5).

![Figure 6-5. HPLC of the reaction mixture of Fmoc-Dmt with NIS](image)

Table 6-3. Optimization Table for Iodination Reaction of Fmoc-Dmt with NIS

<table>
<thead>
<tr>
<th>Reaction #</th>
<th>Fmoc-Dmt: CF₃COOH: NIS</th>
<th>Time</th>
<th>Fmoc-Dmt: peak 1 : peak 2 (areas of peak in HPLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1: 0: 1</td>
<td>10 min</td>
<td>5: 1: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8 h</td>
<td>5: 1: 1</td>
</tr>
<tr>
<td>2</td>
<td>1: 0: 2</td>
<td>10 min</td>
<td>2.2: 1: 1.5</td>
</tr>
<tr>
<td>3</td>
<td>1: 1: 1</td>
<td>10 min</td>
<td>1.8: 1: 0.5</td>
</tr>
</tbody>
</table>

Our observation by analytical HPLC suggested that CF₃COOH acted in a very important role (Table 6-3). If it’s not present, the starting material Fmoc-Dmt still remained in a large ratio even after an overnight reaction time. Both mono-iodinated product and di-iodinated product were produced. However, when 1 equiv. of the acid was added, more Fmoc-Dmt converted to the products. In reaction 3, the ratio of the mono-iodinated product was twice as much as the di-iodinated product. It’s possible
that the iodine trifluoroacetate was formed \textit{in situ} from NIS and CF$_3$COOH (Scheme 6-2).\textsuperscript{115} It acted as a very reactive electrophile and reacted with Fmoc-Dmt to yield both the mono-iodinated Fmoc-Dmt and the di-iodinated Fmoc-Dmt.

\begin{equation}
\text{\textsc{Scheme 6-2. Proposed mechanism for the forming of iodine trifluoroacetate}}^{115}
\end{equation}

6.3.4 Iodination of Fmoc-Dmt with IPy$_2$BF$_4$

Similar to the iodination of Fmoc-Dmt with NIS, we also examined the iodination of Fmoc-Dmt with IPy$_2$BF$_4$ in regards of the reaction time and the equivalent of iodination reagent (Table 6-4).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Reaction #} & \textbf{Fmoc-Dmt: HBF$_4$: IPy$_2$BF$_4$} & \textbf{Reaction Time} & \textbf{Fmoc-Dmt: peak 1 : peak 2} \\
\hline
1 & 1: 0: 1 & 10 min & 0.5: 1: 0.4 \\
2 & 1: 1: 1 & 10 min & 0.24: 1: 0.07 \\
 & & 8 h & 0.24: 1: 0.07 \\
3 & 1: 1: 1.1 & 10 min & 0.07: 1: N.D. \\
\hline
\end{tabular}
\caption{Optimization Table for Iodination Reaction of Fmoc-Dmt with IPy$_2$BF$_4$}
\end{table}
As illustrated in the table, if HBF$_4$ was not added, the di-iodinated product was found in a higher portion, and more staring material was left unreacted (Reaction 1 vs. Reaction 2). We could also conclude that this reaction was also not sensitive to reaction time. From HPLC, the ratio of Fmoc-Dmt and two products almost didn’t change with respect to reaction time (Reaction 2). With the addition of 1.1 equiv. of IP$_2$BF$_4$, mono-iodinated product became predominant and the conversion was almost completed in 10 minutes (Reaction 3). Therefore, a ratio of 1:1:1.1 (Fmoc-Dmt: HBF$_4$: IP$_2$BF$_4$) was considered to be the optimal condition for this reaction. A larger scale reaction (150 mg Fmoc-Dmt) was conducted under this condition and the product was isolated by preparative HPLC (Figure 6-6). The purified mono-iodinated Dmt (50 mg) was further analyzed by $^1$H NMR and $^{13}$C NMR. This product was coupled with other amino acids in small batch to give I-Dmt-$\beta$-Ala-Phe-Orn-NH$_2$ by solid phase synthesis (The solid phase synthesis of I-Dmt-$\beta$-Ala-Phe-Orn-NH$_2$ was conducted by Dr. Fabio Gallazzi and an undergraduate student Alison Oostendorp). LC-MS spectrum of this peptide also confirmed the result of our work (Figure 6-7).

Figure 6-6. HPLC of the reaction product of Fmoc-Dmt with IP$_2$BF$_4$
(A: crude sample; B: purified sample)
6.4 Conclusion

The Boc group was easily deprotected under the acidic conditions, which complicated the iodination reaction of Boc-Dmt. Iodination reaction of Boc-Dmt in absence of acid gave both mono-iodinated product and di-iodinated product. In contrast, if Fmoc-Dmt was used as the starting material instead of Boc-Dmt, a strong acid, such as CF₃COOH or HBF₄, can be present. A large portion of di-iodinated product was shown by HPLC if NIS and CF₃COOH were used. However, under the iodination by IPy₂BF₄, a ratio of 1:1:1:1 (Fmoc-Dmt: HBF₄: IPy₂BF₄) was found to give mainly the anticipated mono-iodinated product.

Future work will include synthesis of mono-iodinated peptides, radio-iodination, test for their lipophilicity, and test for μ-opioid receptor affinity and selectivity.
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