LIGANDS FOR THE SIGMA RECEPTOR AND THE μ-OPIOID RECEPTOR

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In Partial Fulfillment
Of the Requirements for the Degree

Master of Science

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by
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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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LIGANDS FOR THE SIGMA RECEPTOR AND THE μ-OPIOID RECEPTOR

Yu Lu

Dr. Susan Z. Lever, Thesis Supervisor

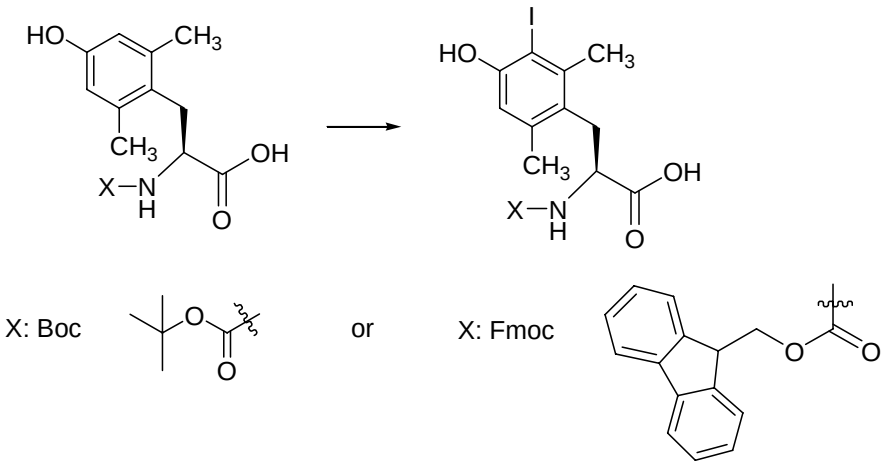
ABSTRACT

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.

R= 4-OCH_3, 3-OCH_3, 4-CH_3, 3-F, 2-Br, 3-NO_2, 2-NO_2, 4-NO_2, 3-I

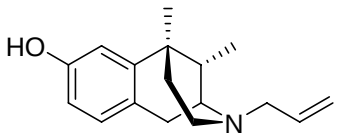
The last chapter is concerned with a different receptor family, the opioid receptors. The opioid receptors belong to the family of G-protein coupled receptors and have three subtypes: mu(μ), delta(δ), and kappa(κ). μ-opioid receptor is believed to mediate the analgesia and drug addiction by morphine and other opioids. Many synthetic peptides, such as Tyr-*D*-Arg-Phe-Lys-NH_2, Tyr-*D*-Arg-Phe-Ala-NH_2 and Dmt-*D*-Ala-Phe-Phe-NH_2 have been investigated as selective μ-opioid ligands. In order to obtain μ-opioid tetrapeptide ligand I-Dmt-*D*-Ala-Phe-Orn-NH_2 with high affinity and proper lipophilicity, mono-iodination reaction was studied on the 2,6-dimethyl-*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.[1] 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]



(+)-SKF10047

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.[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.[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.[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).[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.[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).[33] Furthermore, the sigma-1 receptor plays roles in the

regulation of gastrointestinal effects,[34] modulation of opioid analgesia,[35] attenuation of cocaine-induced locomotor activity and toxicity， and so on.[36] 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,[37] mediation of the effects of sigma ligands on K^+ channels,[38, 39] mediation of cell morphology and cell death induced by certain sigma ligands,[39] and regulation of cell proliferation and maintenance of cell viability.[40]

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.[41] 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.[42]

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.[43]

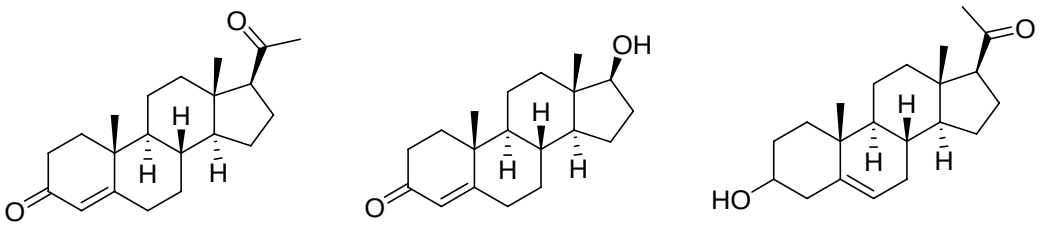


Figure 1-2. Structures of progesterone, testosterone and pregnenolone

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.[9] Because of their neuroregulative and neuroprotective functions,[44] sigma-1 receptor agents could be potentially used for the treatment of depression, schizophrenia,[45] amnesia and mental improvement,[46] cocaine abuse,[47] as well as other psychiatric disorders.[48] On the other hand, sigma-2 receptor antagonists show the ability as atypical antipsychotics without the side effects caused by classical antipsychotic agents[49] and to attenuate convulsions caused by cocaine.[50]

The sigma-2 receptor agonists could be used as novel antineoplastic agents, potentiating the cytotoxicity by reversing drug resistance in tumor cells.[27] Furthermore, because of the fact that both sigma receptors are expressed in a wide variety of tumor cell lines,[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)[51-56] and SPECT (Single Photon-Emission Computed

Tomography)[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,[53] F-18,[52, 54] and Br-76[51]. In contrast, SPECT utilizes the single mono-energetic photons emitted from the decay of I-123,[60] I-125 (in mice)[58] and Tc-99m[57] (Figure 1-3).

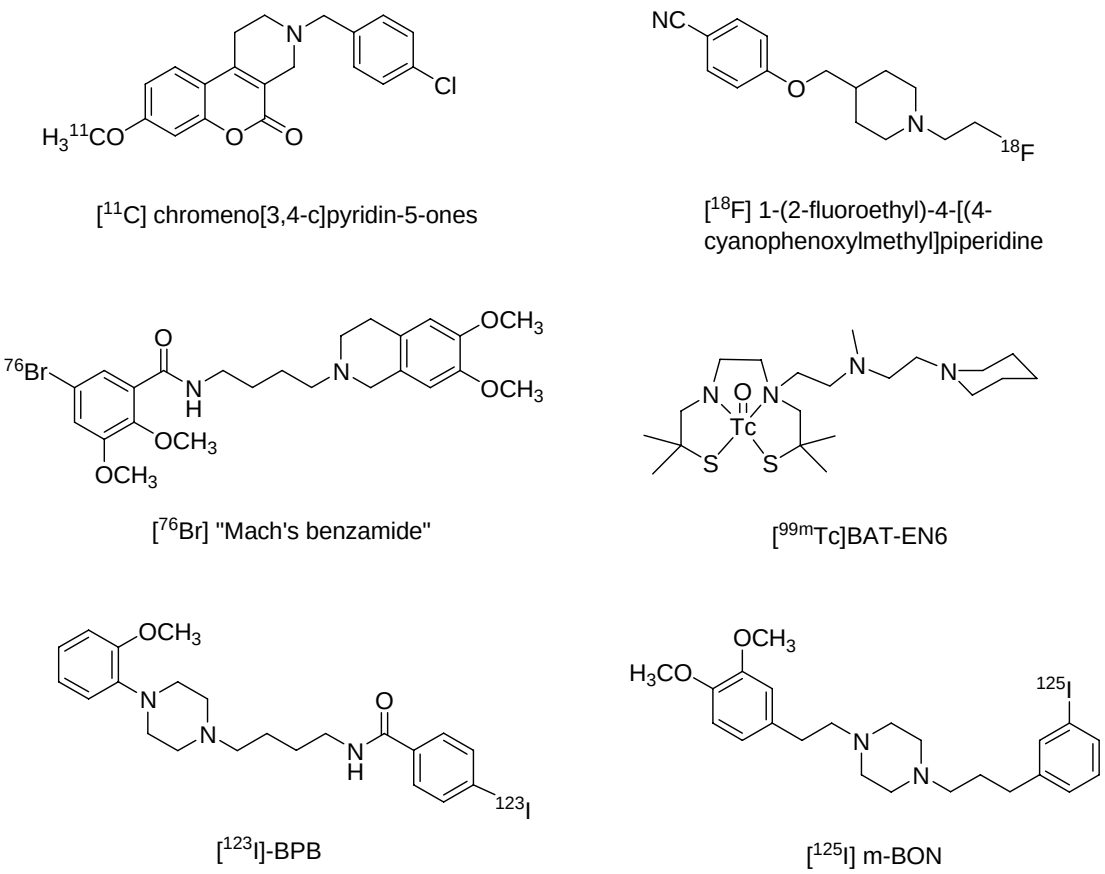


Figure 1-3. Some radiolabeled sigma receptor ligands for PET and SPECT studies[51-54, 57, 58, 60]

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[61] (Table 1-1).

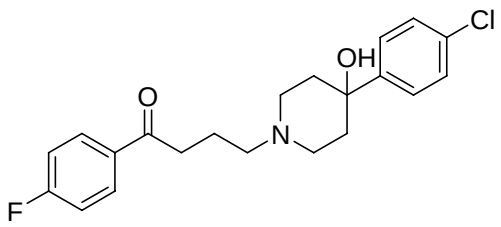
Table 1-1. Half-life of the isotopes that were used in the radio-labeled sigma receptor ligands shown in Figure 1-3.

Isotope	**I-123**	**I-125**	**Tc-99m**	**C-11**	**F-18**	**Br-76**
Half-life	13.2 hours	60.1 days	6.02 hours	20 min.	110 min.	16.2 hours
Use	SPECT	SPECT*	SPECT	PET	PET	PET

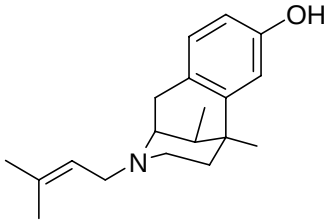
* only in mice

1.5 Sigma Receptor Ligands: Structural Diversity

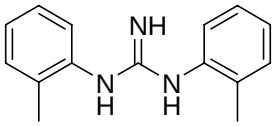
To date, a variety of sigma receptor ligands with unrelated chemical structures have been reported. Figure 1-4 shows some representative ligands with high affinity to sigma receptors. In general, in their structures, one or more substituted aromatic rings are present. Other functional groups can be piperidine,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



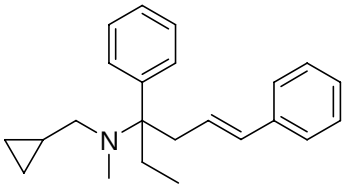
haloperidol



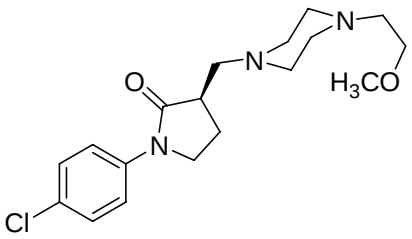
(+) pentazocine



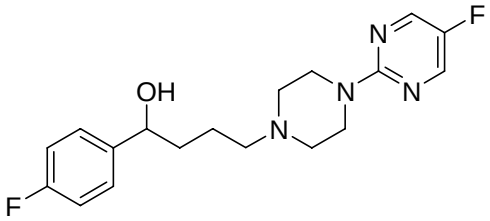
ditolylguanidine (DTG)



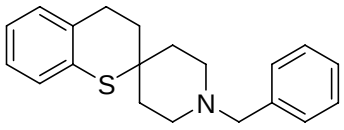
igmesine



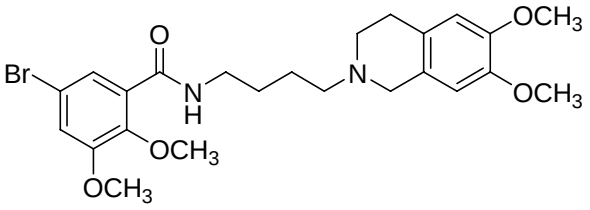
MS 377



BMY-14802



spipethiane



"Mach's benzamide"

Figure 1-4. Some representative sigma receptor ligands with high affinity[62-67]

Among these compounds, haloperidol (Ki: sigma-1, 2.20 ± 0.31 nM; sigma-2, 34.2 ± 2.3 nM)[62] exhibits the highest affinity for both sites among typical neuroleptics.[27] (+)-pentazocine (Ki: sigma-1, 3.58 ± 0.20 nM; sigma-2, 1923 nM)[62] is commonly radiolabeled as [^{3}H]-(+)-pentazocine to serve as the standard radioligand

for sigma-1 receptor in binding assays.[68] Ditolylguanidine (DTG, Ki: sigma-1, 27.7 ± 4.3 nM; sigma-2, 12.8 ± 2.1 nM)[63] is also radiolabled as [^{3}H] 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.[64] (+)-(*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.[65] 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)[69], a piperazine derivative of haloperidol, interacts non-selectively with both subtypes and has been investigated in phase II clinical trials as a potential antipsychotic.[70] Spipethiane (Ki: sigma-1, 0.5 nM; sigma-2, 416 nM) is a very potent and selective ligand for sigma-1 receptors.[66] "Mach's benzamide", 5-bromo-N-(4-(3,4-dihydro-6,7-dimethoxyisoquinoline-2(1H) -yl)butyl)-2,3-dimethoxy benzamide, (IC_{50}: sigma-1, 12,900 nM; sigma-2, 8.2 nM) belongs to the most potent and sigma-2 selective ligands described so far.[71]

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[72] (Figure 1-5).

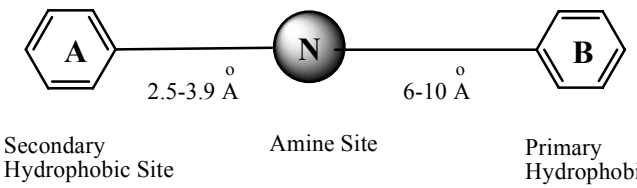


Figure 1-5. Glennon's Pharmacophore Model for Sigma-1 Receptor Ligands[72]

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)

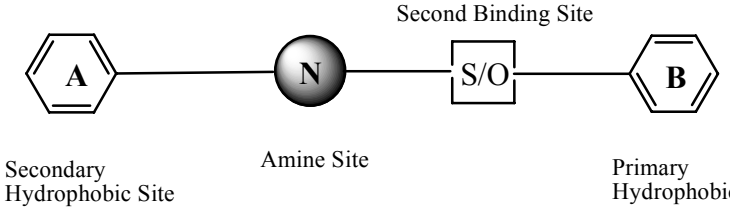


Figure 1-6. Gund's Pharmacophore Model for Sigma-1 Receptor Ligands[73]

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.[74] 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).

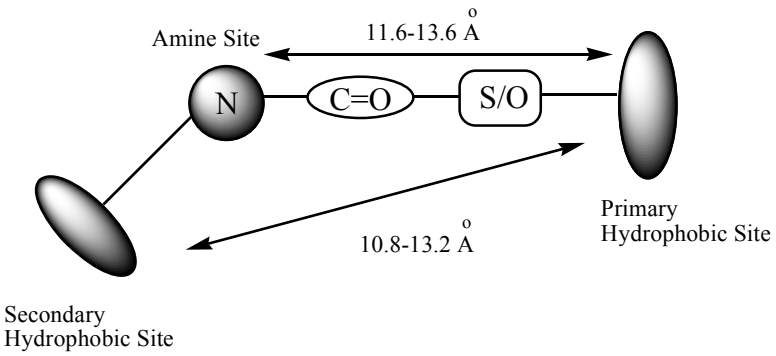


Figure 1-7. Cratteri's Pharmacophore Model for Sigma-2 Receptor Ligands[74]

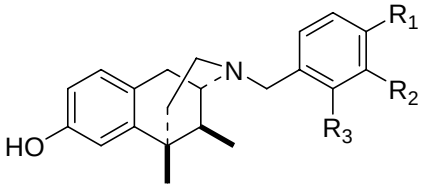
1.7 Quantitative Structure-Activity Relationship (QSAR) Studies of Sigma Receptor Ligands

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.

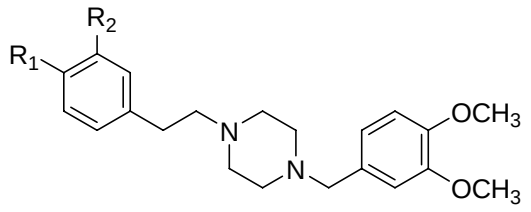
QSAR studies in the research of sigma receptors ligands are very limited. Only a few publications have dealt with such topic. The first QSAR analysis of sigma receptor ligands seems to appear in 1995, conducted by Mascarella and co-workers.[75] A series of substituted N-benzyl-N-normetazocines were synthesized 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). [75]



R_1 = H, F, CN, CH_3, NO_2, OCH_3, Cl, Br, I, NH_2, CF_3, t-Bu
R_2 = H, I, NO_2
R_3 = H, F, CH_3, I

Figure 1-8. General structure of substituted N-benzyl-N-normetazocines

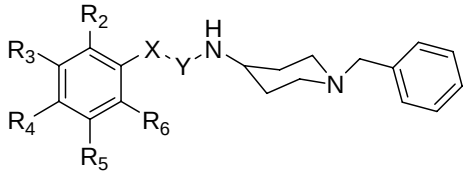
Another QSAR study was performed by Fujimura and co-workers in 1997. For a small set of substituted derivatives of 1-[2-(3,4-dimethoxyphenyl)-ethyl] -4-(3-phenylpropyl) piperazine, the sigma-1 receptor affinity was suggested to be quantitatively dependent on the electronic nature of para-substituent R_1, and the resonance nature of meta-substituent R_2 (Figure 1-9).[76]



R_1 = H, Cl, CF_3
R_2 = H, Cl, CF_3, OH, Br, F, OCH_2Ph

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)phenylacetamide derivatives (Figure 1-10). They were able to relate both sigma-1 and sigma-2 binding affinities to the substituent parameters such as electronic (σ), hydrophobic (π), and steric bulk (MR) effects for various substituents.[77] This study presented the first and to our knowledge the only QSAR study for sigma-2 receptor so far.



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

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_{1A} receptors.[72, 76-86] Moreover, diarylalkylpiperidine and diarylalkylpiperazine derivatives fit quit well into Glennon's proposed model (Figure 1-5 shown on page 10),[72, 78, 87] showing that they might serve as potential sigma ligands with both high affinity and high selectivity against other receptors.

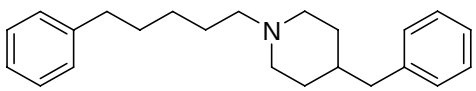
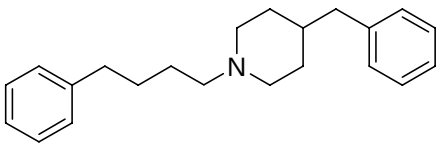
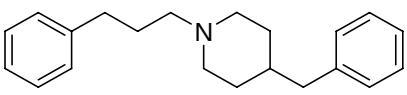
In addition to the presence of amine sites and phenyl rings, the other factor we take into consideration is the length of alkyl chain that connects the amine site and the phenyl rings. Although it has been shown that the distance could be modified from one to six carbon chains,[72] binding affinities 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 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.[87] After the establishment of their sigma-1 receptor pharmacophere, 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.[78] 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[87]

Compound	σ_1: Ki (nM)	σ_2: Ki (nM)	Selectivity (σ_1/σ_2)
	0.6	2.8	4.7
	0.8	3.1	3.7
	0.4	3.3	8.3

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).

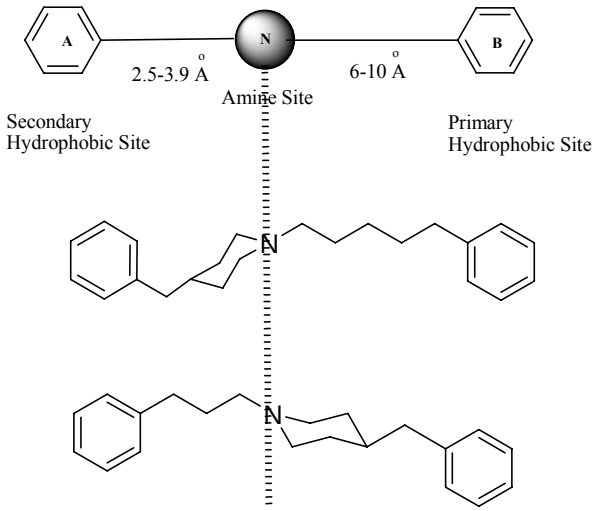


Figure 2-2. Proposed binding mode of diarylalkylpiperidines[87]

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.[86] 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.[82] Although the result from comparative binding affinity tests indicated that Lead 2 is very selective for sigma receptors (Ki = 20 nM) in comparison with $5HT_{1A}$ (Ki > 10^5 nM) and the D_2 receptors (Ki >10^5 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

Compound	Ki: σ1 (nM)	Ki: σ2 (nM)	Selectivity (σ2/σ1)	Ref.
Haloperidol	2.2	34.2	15.5	62
(+)-Pentazocine	3.58	1923	537	62
Ditolyguanidine (DTG)	27.7	12.8	0.46	63
Igmesine	39	390	10	64
MS 377	73	6900	94.5	65
BMY-14802	265	391	1.48	69
Spipethiane	0.5	416	832	66
“Mach’s Benzamide”	53	0.90	0.017	71
Lead 1	0.4	3.3	8.25	87
Lead 1	1.4	0.49	0.35	86
Lead 2	20		ND	82

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.[75-77] 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

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.[75-77] 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 **σ** value, $\boldsymbol{\pi_x}$ 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:[88]

$$\text{Log } (1/\mathbf{Ki}) = f\ (a\ \boldsymbol{\sigma},\ b\ \boldsymbol{\pi_x},\ c\ \mathbf{MR})$$

A Hammett **σ** value represents the electronic substituent parameters. The $\boldsymbol{\pi_x}$

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 decriptors (Hammett **σ** values, $\boldsymbol{\pi_x}$ values, MR values) associated with the phenyl substituents varied in certain space ranges. In specific, Hammett **σ** values are between -0.4 and 0.8, $\boldsymbol{\pi_x}$ 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 2-3. The numerical space ranges of physicochemical parameters and the intermediate values

Property	Parameter	Low Limit	High Limit	Intermediate
Electronic	σ	-0.4	0.8	0.12
Lipophilicity	π_x	-1.0	1.6	-0.02
Size	MR	0.1	2.5	0.79

Since we have three descriptors (**σ**, $\boldsymbol{\pi_x}$ 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).[89]

Table 2-4. Sets of substituents with the physicochemical parameters[90]

R	σ	π_x	MR	Level	Actual
4-CH_3	-0.17	0.56	0.57	- + -	- + -
4-OCH_3	-0.27	-0.02	0.79	- - +	- 0 0
3-OCH_3	0.12	-0.02	0.79	0 0 0	0 0 0
3-F	0.34	0.14	0.10	+ + -	+ + -
2-Br	-	0.86	0.89	- + +	- + +
3-I	0.35	1.12	1.39	+ + +	+ + +
2-NO_2	-	-0.28	0.74	- - -	- - -
3-NO_2	0.71	-0.28	0.74	+ - +	+ - -
4-NO_2	0.78	-0.28	0.74	+ - -	+ - -

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.

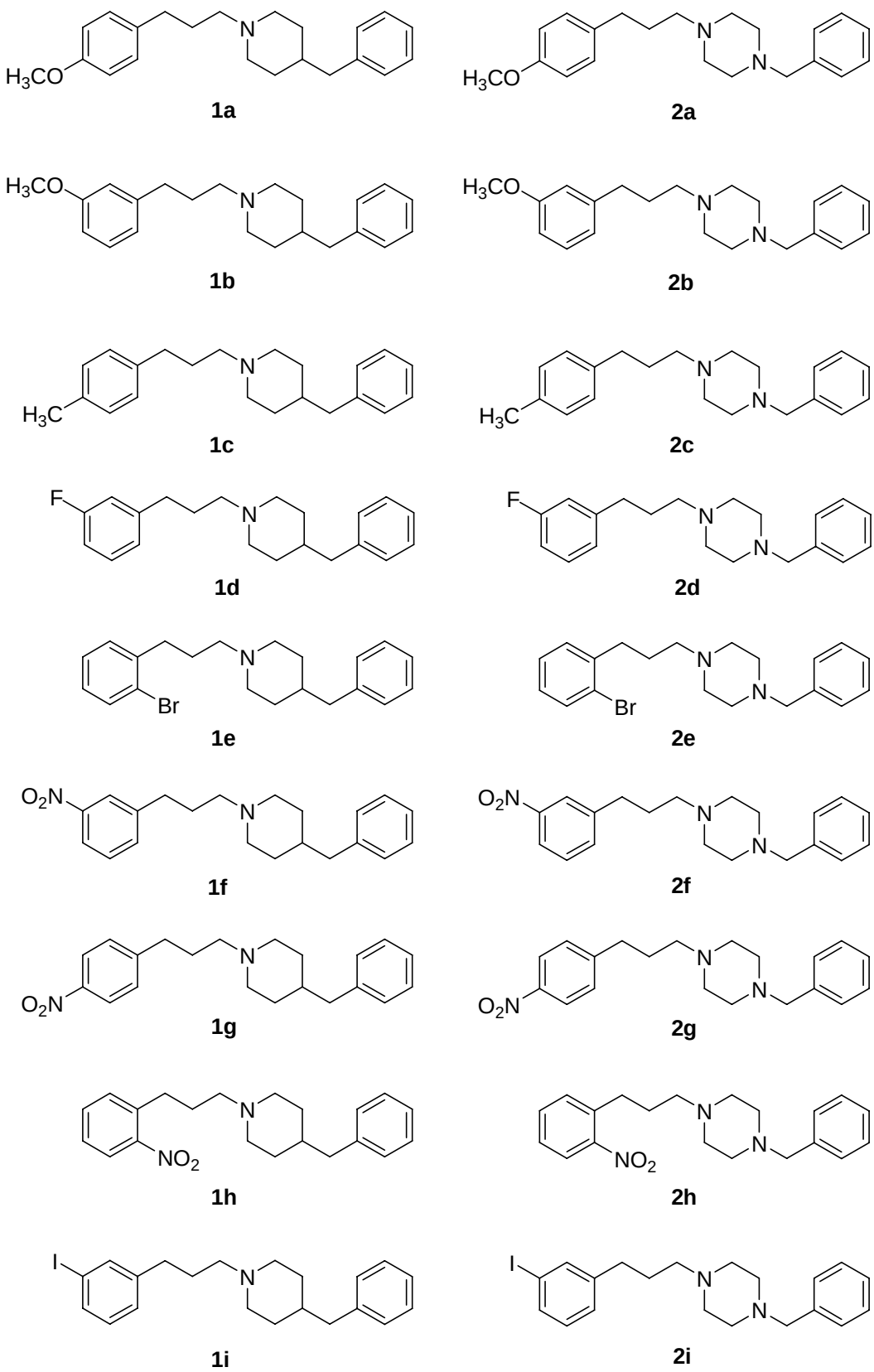
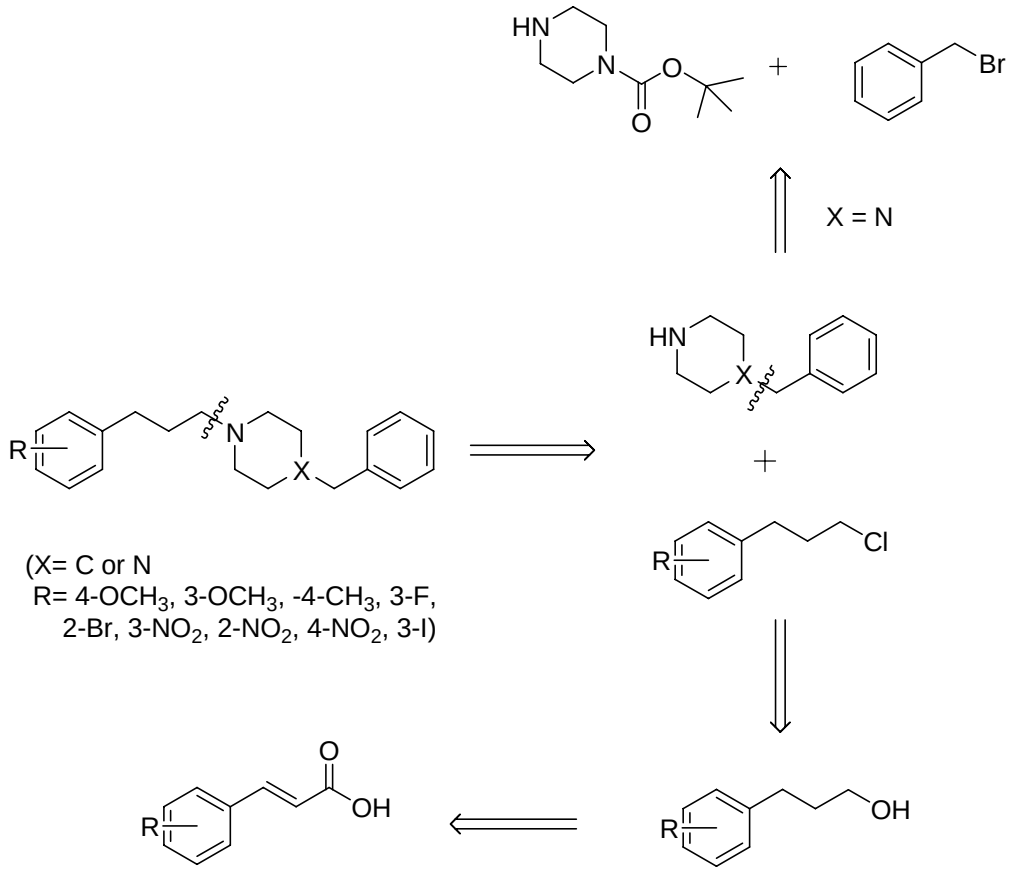


Figure 2-4. Structures of substituted diarylalkylpiperidine (**1a**-**1i**) derivatives and substituted diarylalkylpiperazine (**2a**-**2i)** to be synthesized

2.3 Synthetic Approach to the Derivatives of Lead 1 and Lead 2

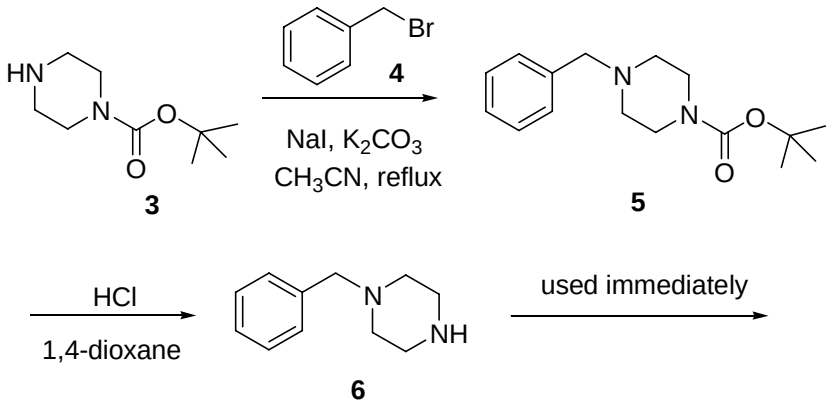
Applying the process of retrosynthetic analysis, the series of compounds **1a**-**1i** and **2a**-**2i** can be dissected into two moieties, 4-benzylpiperidine (or 4-benzylpiperazine) and the substituted phenylpropyl halide. The 4-benzylpiperidine is commercially available, but 4-benzylpiperazine needs 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.[91, 92] 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**

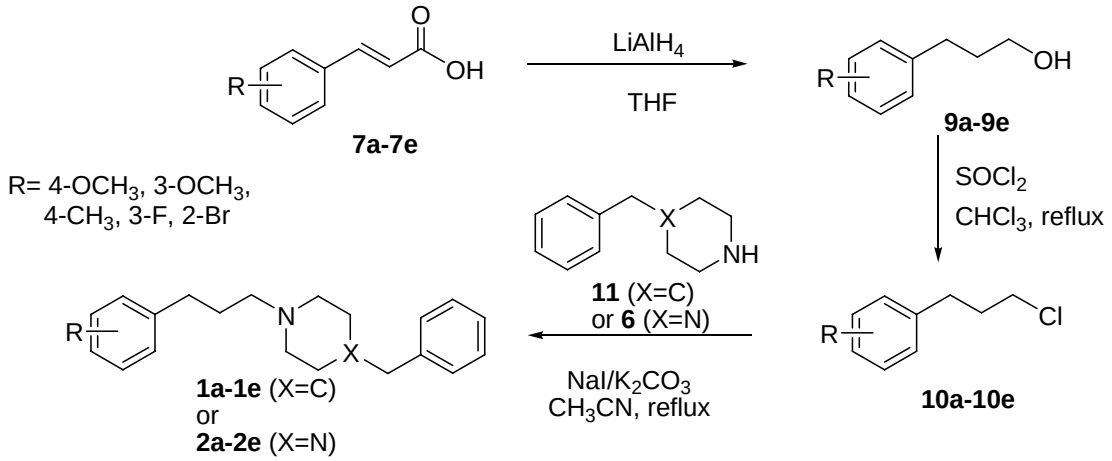
The preparation of 4-benzylpiperazine can be conducted by the alkylation of Boc piperazine and benzyl bromide, followed by the deprotection of the Boc group

with hydrochloric acid in dioxane (Scheme 2-2). This product is used immediately in the next reaction.



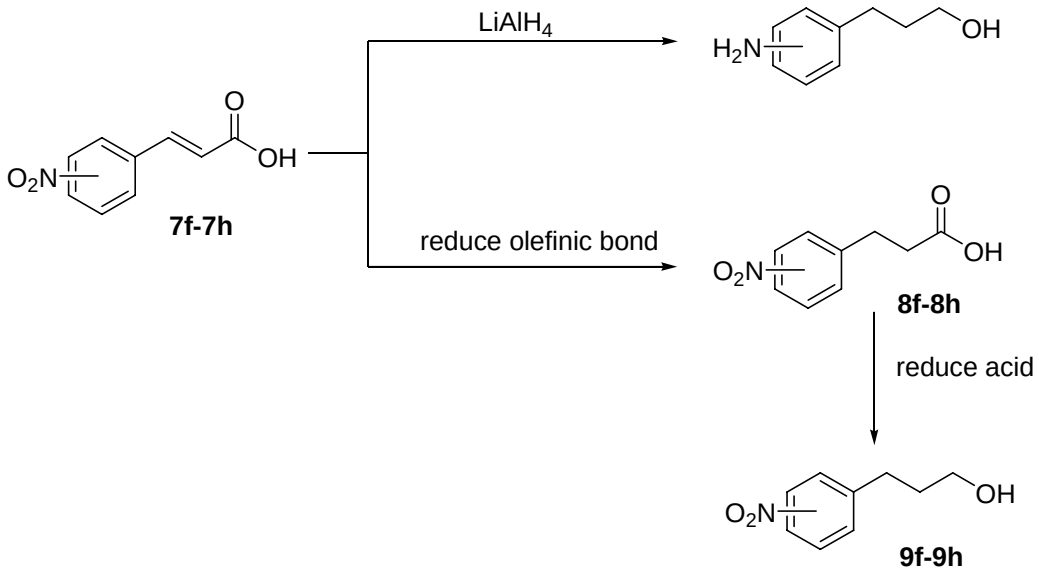
Scheme 2-2. Synthetic approach to 4-benzylpiperazine

For compounds **1a-1e** and **2a-2e**, starting from the substituted cinnamic acid, $LiAlH_4$ can be used to reduce the α,β-unsaturated carboxylic acid to give the corresponding alcohol. Then, chlorination of the alcohol by thionyl chloride ($SOCl_2$) can yield the substituted phenylpropyl chloride.[91] 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**

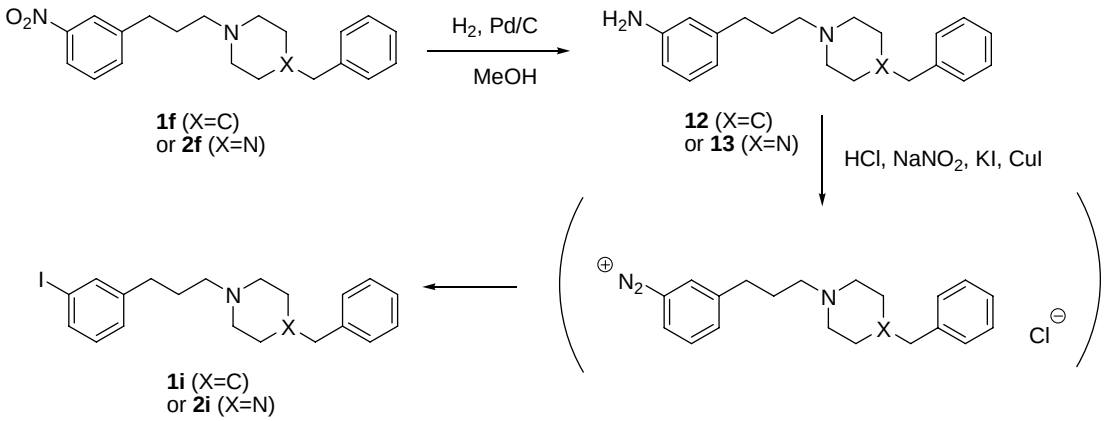
The synthesis of the compounds **1f-1h** and **2f-2h** (R=3-NO_2, 4-NO_2, 2-NO_2) is more complex. Since the nitro substituent on the phenyl ring is very sensitive to strong reducing agents, $LiAlH_4$ is not a proper reagent to reduce the starting materials. Therefore, the double bond and the carboxylic 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 (BH_3-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 $LiAlH_4$ and mild reducing agent

Compound **1i** and **2i** (R=3-I) can be obtained from their analogues **1f** and **2f** (R=3-NO_2) 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 ($LiAlH_4$, BH_3/THF, $SOCl_2$, 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.

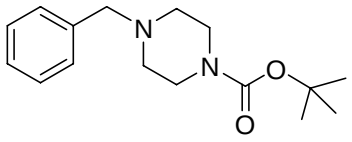
Column chromatographic separations were carried out on Silicycle ultra pure silica gel (230-400 mesh) with ACS reagent grade solvents. Analytical thin layer chromatography was performed on plastic-backed silica gel plates produced by Macherey-Nagel Co. Melting points were determined with the Mel-Temp 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 $CDCl_3$ solutions with tetramethylsilane (δ = 0 ppm) as the internal standard. ^{13}C NMR spectra was obtained on the same instruments with $CDCl_3$ (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).

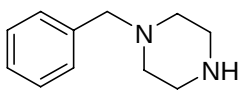
3.2 Experiment Procedure

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_2CO_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).

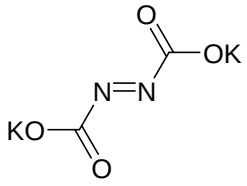
1-benzylpiperazine (6)



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

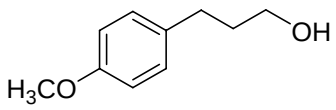
with methanol (30 mL). The solution was stirred overnight at room temperature, and concentrated under reduced pressure. The residue was basified by 2N NaOH to pH 11, and then extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to yield **6** as a pale yellow oil (1.57 g, 92%). ^{1}H NMR ($CDCl_3$): 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.

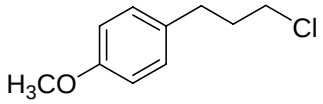
3-(4-methoxyphenyl)propan-1-ol (9a)



The reduction procedure has been adapted from the

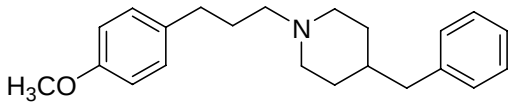
previously reported work with appropriate modification.[92] (2*E*)-3-(4-methoxyphenyl) acrylic acid, **7a**, (891 mg, 5 mmol) in 15 mL dry THF was added dropwise to $LiAlH_4$ (0.38 g, 10 mmol) in 10 mL dry THF at room temperature under N_2. After the addition (0.5 h), the solution was stirred at room temperature for 4 h. The mixture was then diluted at 0 °C with saturated $NaHCO_3$ aqueous solution and filtered, then extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give a crude oil. The crude product was purified by column chromatography (hexane/ethyl acetate, 1:1 as eluent) to give **9a** as a colorless oil (699 mg, 84%). ^{1}H NMR ($CDCl_3$): δ 1.79-1.89 (m, 3H), 2.61-2.66 (t, 2H), 3.61-3.66 (t, 2H), 3.77 (s, 3H), 6.81-6.85 (d, 2H), 7.08-7.12 (d, 2H).

1-(3-chloropropyl)-4-methoxybenzene (10a)



The chlorination procedure has been adapted from the previously reported work with appropriate modification.[92] To a stirred solution of **9a** (514 mg, 3.10 mmol) in $CHCl_3$ (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%). ^{1}H NMR ($CDCl_3$): 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)



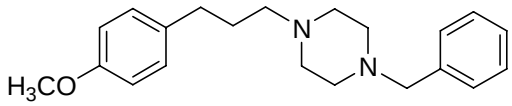
The alkylation procedure has been adapted from the previously reported work with appropriate modification.[87] A stirred mixture of **10a** (116.2 mg, 0.63 mmol), 4-benzylpiperidine, **11** (132.5 mg, 0.76 mmol), NaI (94 mg, 0.63 mmol), and K_2CO_3 (260 mg, 4.89 mmol) in acetonitrile (5 mL) was heated 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 $MgSO_4$, 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. ^{1}H NMR (free amine in $CDCl_3$): 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). ^{13}C NMR (free amine in $CDCl_3$): 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: ($C_{22}H_{29}NO$•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)



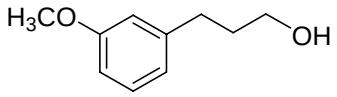
The alkylation procedure has been adapted from the previously reported work with appropriate modification.[87] A stirred mixture of **10a** (34.0 mg, 0.184 mmol), **4** (48.7 mg, 0.276 mmol), NaI (30 mg, 0.184 mmol), and K_2CO_3 (80 mg, 0.580 mmol) in acetonitrile (2.5 mL) was heated under reflux overnight 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 $MgSO_4$, 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 °C. ^{1}H NMR (free amine in $CDCl_3$): 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). ^{13}C NMR (free amine in $CDCl_3$): 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: ($C_{21}H_{28}N_2O$•2HCl•1/4H_2O)

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

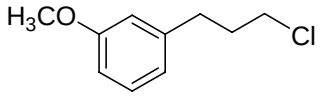
Found: C, 62.76; H, 7.61; N, 6.88.

3-(3-methoxyphenyl)propan-1-ol (9b)



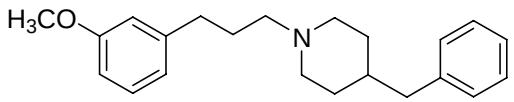
In a similar fashion as described for the preparation of **9a**, (2*E*)-3-(3-methoxyphenyl) acrylic acid, **7b**, (891 mg, 5 mmol) was converted to **9b** as a colorless oil (753 mg, 91%). ^{1}H NMR ($CDCl_3$): δ 1.82-1.90 (m, 3H), 2.64-2.69 (t, 2H), 3.62-3.66 (t, 2H), 3.77 (s, 3H), 6.72-6.79 (m, 3H), 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%). ^{1}H NMR ($CDCl_3$): 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. ^{1}H NMR (free amine in $CDCl_3$): 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). ^{13}C NMR (free amine in $CDCl_3$): δ 28.52,

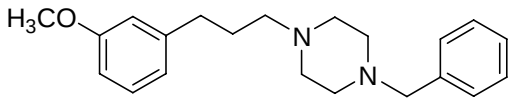
32.07, 33.88, 37.93, 43.20, 53.89, 54.12, 55.08, 58.34, 111.10, 114.13, 120.81, 125.79, 128.17, 129.12, 129.26, 140.66, 143.77.

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)



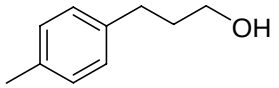
In a similar fashion as described for the preparation of **2a**, 4-benzyl-1-(3-(3-methoxyphenyl)propyl)piperidine, **10b**, (29.7 mg, 0.161 mmol) was converted to **2b** as a pale yellow oil (47.8 mg, 80%). The HCl salt of **2b** was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 252-255 °C. ^{1}H NMR (free amine in $CDCl_3$): δ 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 $CDCl_3$): δ 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: ($C_{21}H_{28}N_2O$•2HCl)

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

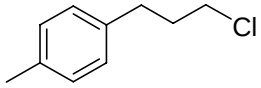
Found: C, 63.53; H, 7.47; N, 6.99.

3-(4-methylphenyl)propan-1-ol (9c)



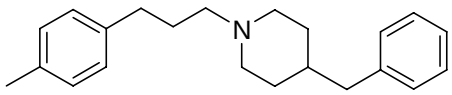
In a similar fashion as described for the preparation of **9a**, (2*E*)-3-(4-methylphenyl) acrylic acid, **7c**, (1.62 g, 10 mmol) was converted to **9c** as a colorless oil (873 mg, 58%). ^{1}H NMR ($CDCl_3$): δ 1.81-1.91 (m, 2H), 2.31 (s, 3H), 2.63-2.68 (t, 2H), 3.62-3.67 (t, 2H), 7.15 (s, 4H).

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 ($CDCl_3$): δ 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 $CDCl_3$): δ 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,

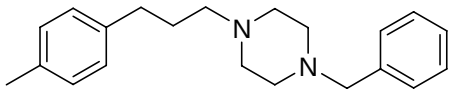
40.65, 52.48, 54.79, 126.04, 128.19, 128.67, 129.18, 129.46, 135.14, 137.81, 138.93.

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)



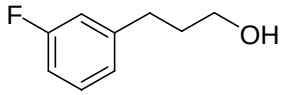
In a similar fashion as described for the preparation of **2a**, 1-(3-chloropropyl)-4-methylbenzene, **10c** (95.7 mg, 0.567 mmol) was converted to **2c** as a pale yellow oil (144 mg, 82%). The HCl salt of **2c** was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 258-260 °C. ^{1}H NMR (free amine in $CDCl_3$): δ 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.

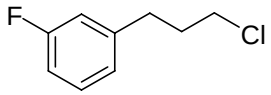
3-(3-fluorophenyl)propan-1-ol (9d)



In a similar fashion as described for the preparation of **9a**,

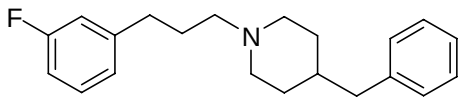
(2*E*)-3-(3-fluorophenyl) acrylic acid, **7d**, (831 mg, 5 mmol) was converted to **9d** as a colorless oil (480 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 1.79-1.89 (m, 3H), 2.63-2.69 (t, 2H), 3.59-3.63 (t, 2H), 6.82-6.95 (m, 3H), 7.16-7.23 (m, 1H).

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 ($CDCl_3$): δ 2.01-2.10 (m, 2H), 2.73-2.78 (t, 2H), 3.48-3.52 (t, 2H), 6.86-6.97 (m, 3H), 7.19-7.27 (t, 1H).

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



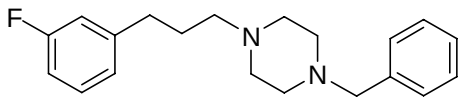
In a similar fashion as described for the preparation of **1a**, 1-(3-fluoropropyl)-3-methoxybenzene, **10d** (52.5 mg, 0.304 mmol) was converted to **1d** as a pale yellow oil (75.9 mg, 96%). The HCl salt of **1d** was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 138-140 °C. ^{1}H NMR (free amine in $CDCl_3$): δ 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: ($C_{21}H_{27}ClFN$•HCl•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)



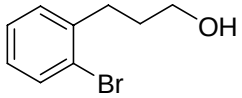
In a similar fashion as described for the preparation of **2a**, 1-(3-fluoropropyl)-3-methoxybenzene, **10d** (24.7 mg, 0.143 mmol) was converted to **2d** as a pale yellow oil (38.6 mg, 86%). The HCl salt of **2d** was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 252-255 °C. ^{1}H NMR (free amine in $CDCl_3$): δ 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: ($C_{20}H_{25}FN_2$•2HCl•0.75H_2O)

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

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

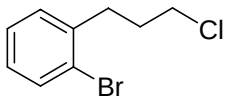
3-(2-bromophenyl)propan-1-ol (9e)



In a similar fashion as described for the preparation of **9a**, (2*E*)-3-(2-bromophenyl) acrylic acid, **7e**, (0.52 g, 2.3 mmol) was converted to **9e** as a

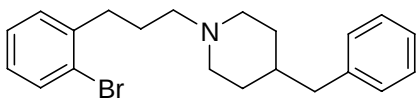
colorless oil (156 mg, 32%). ^{1}H NMR ($CDCl_3$): δ 1.75-1.91 (m, 2H), 2.70-2.80 (t, 2H), 3.18 (s, 1H), 3.59-3.69 (t, 2H), 6.96-7.01 (m, 1H), 7.18-7.25 (m, 2H), 7.47-7.52 (d, 1H).

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 ($CDCl_3$): δ 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)



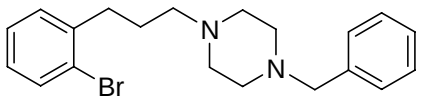
In a similar fashion as described for the preparation of **1a**, 1-(3-chloropropyl)-2-bromobenzene, **10e** (43.5 mg, 0.186 mmol) was converted to **1e** as a pale yellow oil (49.2 mg, 85%). The HCl salt of **1e** was obtained by passing HCl gas into the ether solution of the free amine. Then, the salt was recrystallized from ethanol and ether. m.p. (HCl salt): 170-172 °C. ^{1}H NMR (free amine in $CDCl_3$): δ 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 $CDCl_3$): δ 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: ($C_{21}H_{26}BrN$•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)



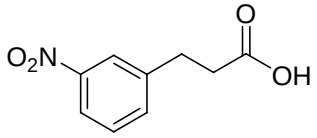
In a similar fashion as described for the preparation of **2a**, 1-(3-chloropropyl)-2-bromobenzene, **10e** (21.0 mg, 0.09 mmol) was converted to **2e** as a pale yellow oil (26.8 mg, 80%). The HCl salt of **2e** was obtained by passing HCl gas into the ether solution of the free amine. Then, the salt 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: ($C_{20}H_{25}BrN_2$•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)



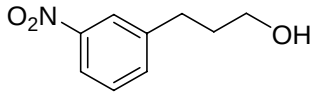
Approach A. The following procedure has been adapted from the previously reported work with appropriate modification.[95]

(*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 H_2 and constant H_2 flow at 1 atm was kept through the reaction. After 96 h, ^{1}H 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/H_2O 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 H_2O and cooled to 0 °C. Hydroxylamine sulfate, $(H_2NOH)_2$-H_2SO_4, (544.9 mg, 3.32 mmol), and hydroxylamine-O-sulfonic acid, H_2NOSO_3H, (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 H_2SO_4. The filtrate was refrigerated overnight and the product was collected by filtration. Recrystallization from ethanol and water produced off-white crystal. ^{1}H 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.[97] 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%). ^{1}H NMR (free amine in $CDCl_3$): 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).

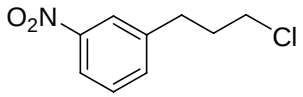
3-(3-nitrophenyl)propan-1-ol (9f)



The following procedure has been adapted from the previously reported work with appropriate modification.[96] 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 BH_3 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. CH_2Cl_2 was added to the residue and the resulting solution was washed with water, saturated $NaHCO_3$ aqueous solution, and brine. The organic layer was dried over $MgSO_4$ and evaporated. The oil residue was purified by column chromatography (hexane/ethyl acetate, 1:1 as eluent)

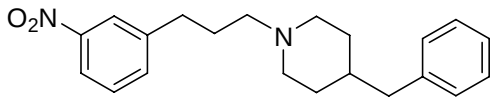
to afford **9f** as a pale yellow oil (307.4 mg, 80%). ^{1}H NMR (free amine in $CDCl_3$): δ 1.89-1.98 (m, 2H), 2.18 (s, 1H), 2.82-2.87 (t, 2H), 3.68-3.72 (t, 2H), 7.43-7.48 (t, 1H), 7.54-7.57 (d, 1H), 8.03-8.07 (m, 2H).

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 ($CDCl_3$): δ 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)



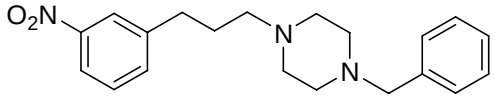
In a similar fashion as described for the preparation of **1a**, 1-(3-chloropropyl)-3-nitrobenzene, **10f** (55.6 mg, 0.28 mmol) was converted to **1f** as a pale yellow oil (85.2 mg, 90%). ^{1}H NMR (free amine in $CDCl_3$): δ 1.23-1.36 (m, 2H), 1.48-1.56 (m, 1H), 1.60-1.65 (d, 2H), 1.80-1.87 (m, 4H), 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 $CDCl_3$): δ 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)



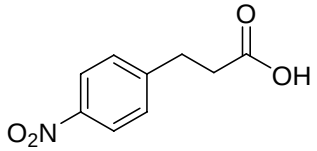
In a similar fashion as described for the preparation of **2a**, 1-(3-chloropropyl)-3-nitrobenzene, **10f** (34 mg, 0.17 mmol) was converted to **2f** as a pale yellow oil (58 mg, 68%). The HCl salt of **2f** was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 260-261 °C. ^{1}H NMR (free amine in $CDCl_3$): δ 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 $CDCl_3$): δ 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: ($C_{20}H_{25}N_3O_2$•2HCl)

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

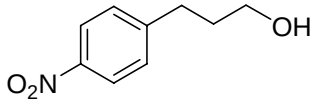
Found: C, 58.22; H, 6.56; N, 10.03.

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



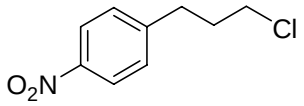
In a similar fashion as described for the preparation of **8f**, (*E*)-3-(3-nitrophenyl)acrylic acid, **7g**, (772.6 mg, 4 mmol) was converted to **8g** as a white solid (624 mg, 80%). ^{1}H NMR (free amine in $CDCl_3$): δ 2.72-2.77 (t, 2H), 3.04-3.09 (t, 2H), 7.37-7.40 (d, 2H), 8.14-8.18 (d, 2H).

3-(4-nitrophenyl)propan-1-ol (9g)



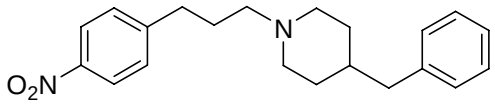
In a similar fashion as described for the preparation of **9f**, 3-(4-nitrophenyl)propanoic acid**, 8g** (500 mg, 2.56 mmol) was converted to **9g** as a pale yellow oil (360.5 mg, 78%). ^{1}H NMR (free amine in $CDCl_3$): δ 1.87-1.99 (m, 2H), 2.81-2.87 (t, 2H), 3.10 (s, 1H), 3.67-3.73 (t, 2H), 7.34-7.39 (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%). ^{1}H NMR ($CDCl_3$): 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. ^{1}H NMR (free amine in $CDCl_3$): 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

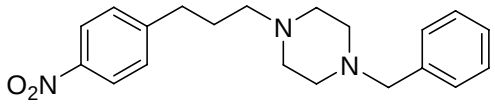
(d, 2H), 7.12-7.34 (m, 7H), 8.10-8.14 (d, 2H). ^{13}C NMR (free amine in $CDCl_3$): δ 28.43, 32.25, 33.32, 37.97, 43.23, 53.91, 57.79, 120.93, 123.30, 125.74, 128.12, 128.36, 129.09, 134.72, 140.68, 144.28, 148.26.

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



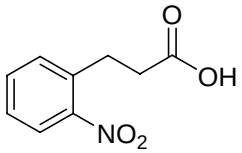
In a similar fashion as described for the preparation of **2a**, 1-(3-chloropropyl)-4-nitrobenzene, **10g** (50 mg, 0.25 mmol) was converted to **2g** as a pale yellow oil (72 mg, 85%). The HCl salt of **2g** was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt was recrystallized from ethanol and water. m.p. (HCl salt): 254-256 °C. ^{1}H NMR (free amine in $CDCl_3$): δ 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 $CDCl_3$): δ 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: ($C_{20}H_{25}N_3O_2$•2HCl)

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

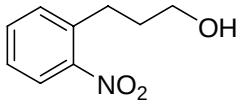
Found: C, 58.08; H, 6.49; N, 10.08.

3-(2-nitrophenyl)propanoic acid (8h)



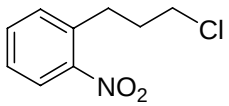
In a similar fashion as described for the preparation of **8f**, (*E*)-3-(2-nitrophenyl)acrylic acid, **7h** was converted to **8h** as a white solid (237 mg, 30%). ^{1}H NMR (free amine in $CDCl_3$): δ 2.75-2.81 (t, 2H), 3.18-3.30 (t, 2H), 7.36-7.43 (m, 2H), 7.52-7.58 (m, 1H), 7.93-7.98 (m, 1H).

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



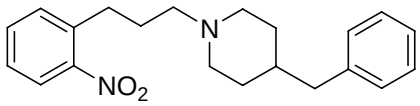
In a similar fashion as described for the preparation of **9f**, 3-(2-nitrophenyl)propanoic acid, **8h** (237 mg, 1.21 mmol) was converted to **9h** as a pale yellow oil (164.5 mg, 75%). ^{1}H NMR (free amine in $CDCl_3$): δ 1.87-1.97 (m, 2H), 2.72 (s, 1H), 2.95-3.00 (t, 2H), 3.68-3.72 (t, 2H), 7.31-7.40 (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 ($CDCl_3$): 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)



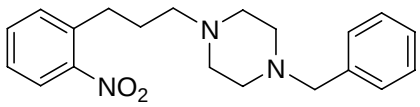
In a similar fashion as described for the preparation of **1a**, 1-(3-chloropropyl)-2-nitrobenzene, **10h** (50 mg, 0.25 mmol) was converted to **1h** as a pale yellow oil (60.3 mg, 71%). The HCl salt of **1h** was obtained by passing HCl gas into the ethanol solution of the free amine. Then, the salt 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). ^{13}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: ($C_{21}H_{26}N_2O_2$•HCl•1/4H_2O)

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

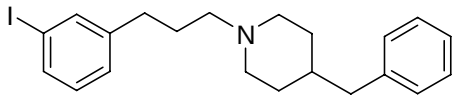
1.78-1.88 (m, 2H), 2.37-2.47 (m, 10H), 2.88-2.93 (t, 2H), 3.50 (s, 2H), 7.20-7.36 (m, 7H), 7.46-7.51 (m, 1H), 7.85-7.87 (m, 1H). ^{13}C NMR (free amine in $CDCl_3$): δ 27.83, 30.70, 53.09, 53.11, 57.78, 63.06, 124.60, 126.89, 126.95, 128.14, 129.16, 131.93, 132.73, 137.24, 138.17, 149.38.

Elemental Analysis: ($C_{20}H_{25}N_3O_2$•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 (1i)



The following procedure has been adapted from the previously reported work with appropriate modification.[93] A solution of **1f** (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_2Cl_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.

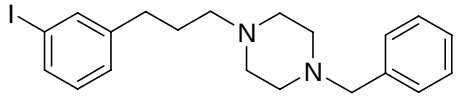
The brown suspension was basified by 10% NaOH solution, diluted with

CH_2Cl_2, extracted. The organic layer was washed with 10% $Na_2S_2O_3$ (5 mL) for three times. The resulting organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/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. ^{1}H NMR (free amine in $CDCl_3$): 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). ^{13}C NMR (free amine in $CDCl_3$): 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: ($C_{21}H_{27}ClIN$•HCl•0.5Et_2O)

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. ^{1}H NMR (free amine in $CDCl_3$): 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 $CDCl_3$): δ 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: ($C_{20}H_{27}Cl_2IN_2$•2HCl•1.5Et_2O)

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

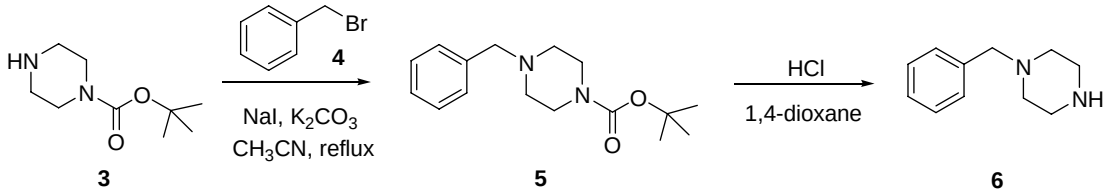
Found: C, 51.86; H, 6.75; N, 4.53.

Chapter 4 Result and Discussion

4.1 Synthesis of Lead 1 and Lead 2 Derivatives

4.1.1 Synthesis of 1-benzylpiperazine

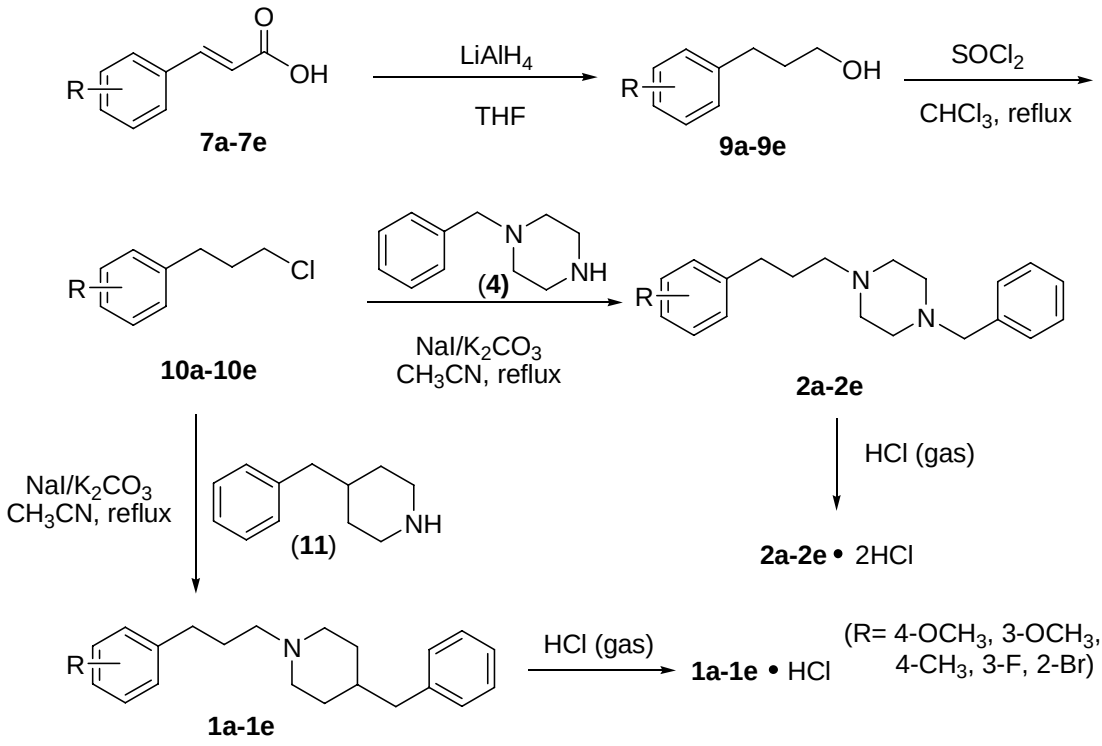
The synthesis of 1-benzylpiperazine (**6**) was outlined in Scheme 4-1. Equivalent moles of N-Boc-piperazine (**3**) and benzylbromide (**4**) were mixed in the present of sodium iodide and excess potassium carbonate. Iodide displaced the bromine in benzylbromide so that the resulting iodide intermediate 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-OCH_3, 3-OCH_3, 4-CH_3, 3-F or 2-Br, the corresponding substituted cinnamic acid (**7a**-**7e** in Scheme 4-2) can be reduced with $LiAlH_4$ 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.

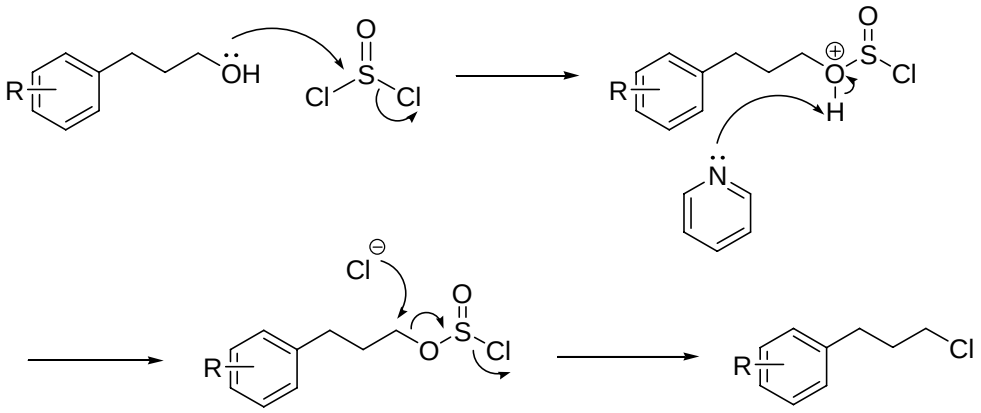


Scheme 4-2. Synthesis of Lead 1 derivatives **1a**-**1e** and Lead 2 derivatives **2a**-**2e**

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

Compound	R	Reaction Time (h)	Yield
9a	4-OCH_3	4 h	84%
9b	3-OCH_3	26 h	91%
9c	4-CH_3	1 h	58%
9d	3-F	2 h	62%
9e	2-Br	12 h	32%

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 $CHCl_3$, 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).



Scheme 4-3. Mechanism of chlorination of **9a-9e** to **10a-10e**

Coupling of **10a-10e** with 4-benzylpiperdine or 1-benzylpiperazine was conducted under the same condition as the synthesis of compound **5**. Slightly different equivalences of 4-benzylpiperdine 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**

R	Compound	Ratio (10:11)	Yield	Compound	Ratio (10:6)	Yield
4-OCH_3	**1a**	1:1.2	80%	**2a**	1:1.5	100%
3-OCH_3	**1b**	1:1	100%	**2b**	1:2	100%
4-CH_3	**1c**	1:1	82%	**2c**	1:1	92%
3-F	**1d**	1.2:1	86%	**2d**	1:1.5	96%
2-Br	**1e**	1:1	80%	**2e**	1:1.5	85%

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.

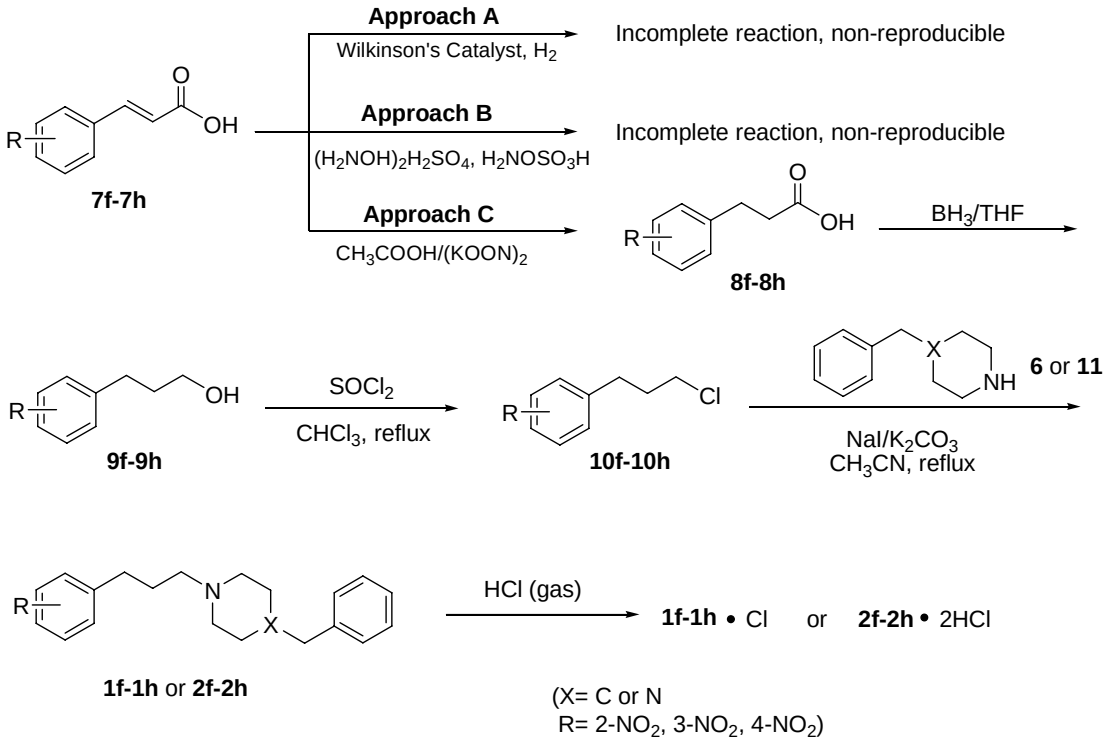
4.1.3 Synthesis of Lead 1 derivatives 1f-1h and Lead 2 derivatives 2f-2h

When the nitro group is present on the phenyl ring, $LiAlH_4$ 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.[95] 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 $(H_2NOH)_2H_2SO_4/H_2NOSO_3H$ was applied to produce diimide to reduce the olefinic bond of the starting material,[96]

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[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_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**

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.[95]

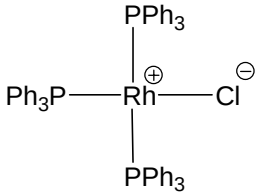


Figure 4-1. Structure of Wilkinson's catalyst

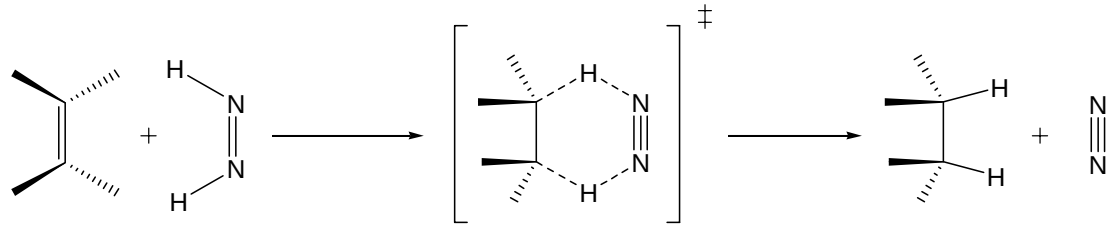
In our experiment, 4% (mol) Wilkinson's catalyst was added to the solution of compound **7f** (3-NO_2) in degassed THF under 1 atm H_2 at room temperature. The mixture was allowed to react for up to 4 d. Because **7f** and **8f** have a very close polarity, the decrease of **7f** or the formation of **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

Compound	R	Wilkinson's Catalyst (mol.)	Reaction Time	Ratio of Reactant: Product by ^{1}H NMR
7f	3-NO_2	4%	24h	56:44
7f	3-NO_2	4%	48h	40:60
7f	3-NO_2	4%	96h	37:63
7g	4-NO_2	4%	24h	N.R.
7h	2-NO_2	8%	24h	N.R.

4.1.3.2 Approach B. Reduction with diimide by $(H_2NOH)_2$-H_2SO_4/ H_2NOSO_3H

Hydroxylamine sulfate, $(H_2NOH)_2$-H_2SO_4, together with hydroxylamine-O-sulfonic acid, H_2NOSO_3H, 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

However, when we tried the $(H_2NOH)_2$-H_2SO_4/H_2NOSO_3H system to reduce the olefinic bond in **7f-7h**, the same problem as catalytic hydrogenation with

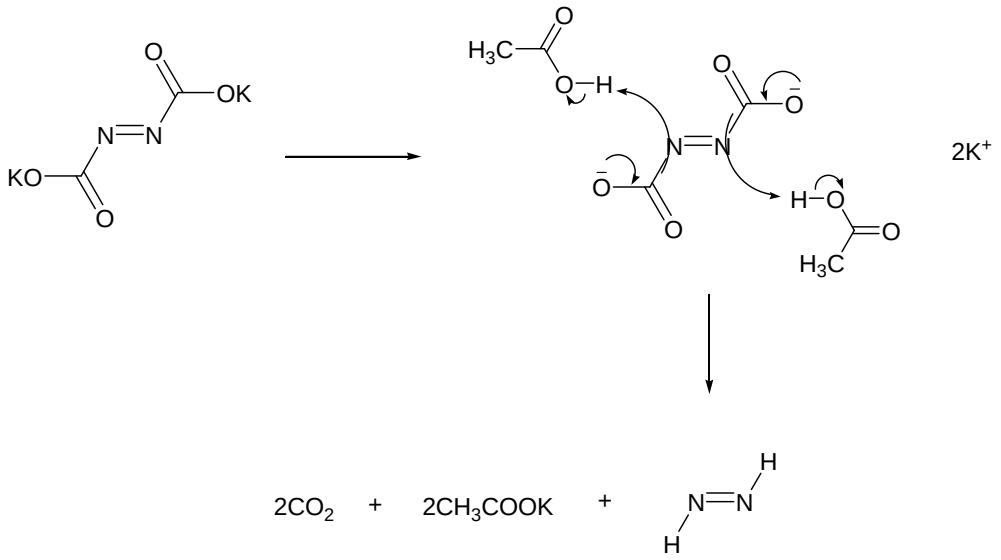
Wilkinson's catalyst occurred. Again, the conversion ratio was low for **7f** and **7g** (43%, 20%, respectively), and there was no reaction for **7h**. The products can not be separated from their precursor by column. As a result, this method was also not further investigated.

Table 4-4. Reaction result for reduction of **7f-7h** with $(H_2NOH)_2$-H_2SO_4/H_2NOSO_3H

Compound	R	Reaction Time	Ratio of Reactant: Product by ^{1}H NMR
7f	3-NO_2	5.5h	57:43
7g	4-NO_2	24h	80:20
7h	2-NO_2	18h	N.R.

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.[97] 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, $(H_2NCON)_2$, 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

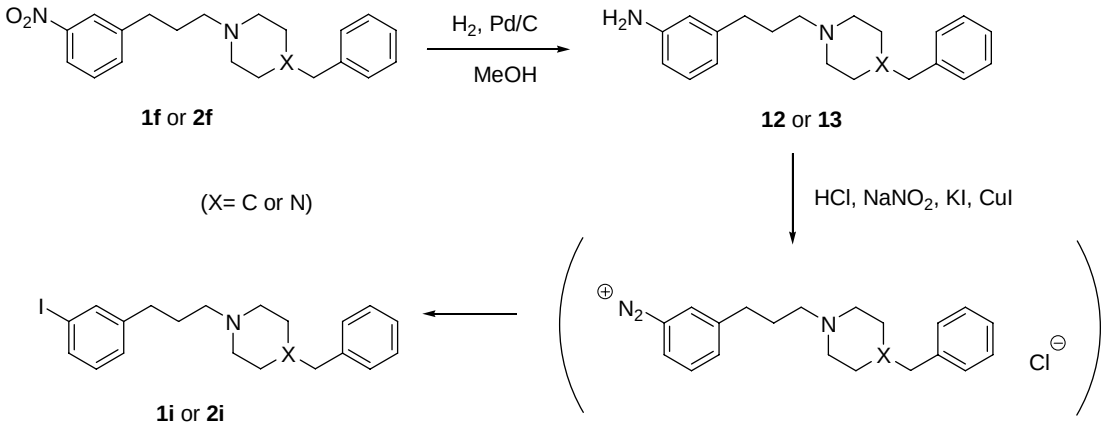
In our experiment, we treated **7f-7h** with PADA and acetic acid in DME (dimethoxyl ethane, $CH_3OCH_2CH_2OCH_3$) at 50 °C. The reaction was monitored by the ^1H NMR to see if the peak for H on the double bond has disappeared or not. Generally, long reaction times (2 days to 5.5 days) were required 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

Compound	R	7: PADA: Acetic Acid	Reaction Time	Yield
7f	3-NO_2	1: 32: 8	4 d	54%
7g	4-NO_2	1: 3: 3	2d	80%
7h	2-NO_2	1: 8: 8	5.5 d	30%

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 $LiAlH_4$ or BH_3. 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-NO_2) seems to be the most potent sigma-1 ligand (Ki: 0.11 nM), while compound **1a** (R: 4-OCH_3) appears as the most sigma-1 selective ligand (Ki: $\sigma2/\sigma1$ = 13.45). The binding affinity of Lead 1 agrees with the literature report,[87] 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-NO_2, 0.11 nM > **1h**, 2-NO_2, 0.60 nM > **1f**, 3-NO_2, 0.66 nM; Sigma-2: **1f**, 3-NO_2, 0.72 nM > **1g**, 4-NO_2, 1.00 nM > **1h**, 2-NO_2, 2.62 nM). In particular, similar trend in *meta*- and *para*- position was confirmed from the comparison between **1a** (R: 4-OCH_3) and **1b** (R: 3-OCH_3), 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-NO_2) 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)

Compound (R)	Sigma-1			Sigma-2			Selectivity K_i Ratio
	IC_{50} (nM)	**K_i (nM)**	n_H	IC_{50} (nM)	**K_i (nM)**	n_H	**σ_2 / σ_1**
Lead 1 (H)	0.6 ± 0.03	**0.38 ± 0.02**	1.13 ± 0.13	3.88 ± 0.02	**3.50 ± 0.02**	0.69 ± 0.19	**9.21**
1a (4-OCH_3)	0.70 ± 0.07	**0.49 ± 0.05**	1.21 ± 0.07	7.40 ± 0.39	**6.59 ± 0.34**	0.87 ± 0.09	**13.45**
1b (3-OCH_3)	0.94 ± 0.06	**0.64 ± 0.04**	1.12 ± 0.05	4.10 ± 0.15	**3.65 ± 0.14**	0.95 ± 0.14	**5.70**
1c (4-CH_3)	0.47 ± 0.01	**0.33 ± 0.01**	1.04 ± 0.02	3.97 ± 0.60	**3.53 ± 0.53**	1.06 ± 0.32	**10.70**
1d (3-F)	0.79 ± 0.11	**0.56 ± 0.08**	1.06 ± 0.03	2.18 ± 0.33	**1.94 ± 0.27**	0.98 ± 0.07	**3.50**
1e (2-Br)	0.46 ± 0.02	**0.32 ± 0.01**	1.20 ± 0.07	2.03 ± 0.13	**1.81 ± 0.12**	0.99 ± 0.10	**5.60**
1f (3-NO_2)	0.95 ± 0.02	**0.66 ± 0.01**	1.05 ± 0.02	0.81 ± 0.05	**0.72 ± 0.04**	0.88 ± 0.20	**1.09**
1g (4-NO_2)	0.16 ± 0.00	**0.11 ± 0.00**	0.81 ± 0.02	1.13 ± 0.12	**1.00 ± 0.10**	0.79 ± 0.11	**9.09**
1h (2-NO_2)	0.86 ± 0.03	**0.60 ± 0.02**	1.08 ± 0.02	2.95 ± 0.27	**2.62 ± 0.24**	0.75 ± 0.13	**4.37**
1i (3-I)	0.50 ± 0.00	**0.34 ± 0.00**	1.38 ± 0.08	1.07 ± 0.91	**0.95 ± 0.08**	0.92 ± 0.17	**2.79**

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 $SOCl_2$, 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 $(H_2NOH)_2H_2SO_4/H_2NOSO_3H$ 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. μ-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.[98] They have been divided into three major subtypes, namely, mu(μ), delta(δ), and kappa(κ). This subdivision is not only supported by the results of cloning, but also by the difference of their affinity for peptide and alkaloid agonists.[99] Among the three subtypes, the μ-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.[100] Moreover, it was recently suggested that μ-opioid receptor might be an appropriate target for lung cancer imaging.[101]

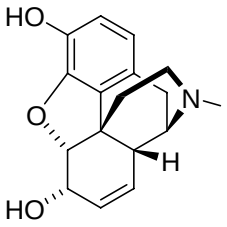


Figure 6-1. Sturcture of Morphine

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.[102] 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.[103]

In addition to the opiate alkaloids, many endogenous opioid peptides have been identified and characterized. They were found to bind to μ-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 μ-receptor so far in the mammalian nervous system.[104] Together with other naturally-occuring opioid peptides such as enkephalins, dynorphins, and dermorphins,[100] they serve as a good start for the structure-activity relationship studies to find more potent μ-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 μ-opioid receptor. Some structure-activity relationship studies have been investigated for the structural modification of the endogenous peptides. For example, dermorphine (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), which was isolated from amphibian skin in 1981,[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.[106] Derived from such tetrapeptide sequences (Tyr-*D*-Ala-Phe-Gly), synthetic peptides which had Tyr in position 1 and Phe in position 3 (Tyr-*D*-AA^2-Phe-AA^4, where AA^2 and AA^4 represent a certain amino acid) were found to show potent agonist activity for the μ-opioid receptor.[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.[108] Among those synthetic peptides, Tyr-*D*-Arg-Phe-Lys-NH_2[109] and Tyr-*D*-Arg-Phe-Ala-NH_2[110] are two dermorphine analogues which are highly potent and very selective for μ-receptor (Table 6-1).

Table 6-1. Opioid receptor binding assay of some dermorphine analogues[109-112]

Peptide	**Ki (nM)**			**Ki: δ/μ**	**Ki: κ/μ**
	μ	**δ**	**κ**		
Tyr-*D*-Arg-Phe-Lys-NH_2	1.69 ± 0.25	19200 ± 2000	4230 ± 360	11400	2500
Tyr-*D*-Arg-Phe-Ala-NH_2	0.145 ± 0.040	385 ± 150	-	2655	-
Dmt-*D*-Arg-Phe-Lys-NH_2	0.143 ± 0.015	2100 ± 310	22.3 ± 4.2	14700	156
Dmt-*D*-Arg-Phe-Ala-NH_2	0.0021 ± 0.0007	1.13 ± 0.13	-	538	-

In another study which was initially aimed at making the peptide molecule more resistant to enzymatic degradation, the introduction of *D*-Ala in position 2 in the general sequence of tetrapeptide was found to be vital for this goal.[113] A dermorphine analogue Tyr-*D*-Ala-Phe-Phe-NH_2 showed high μ-receptor affinity and selectivity.[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.[114] 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

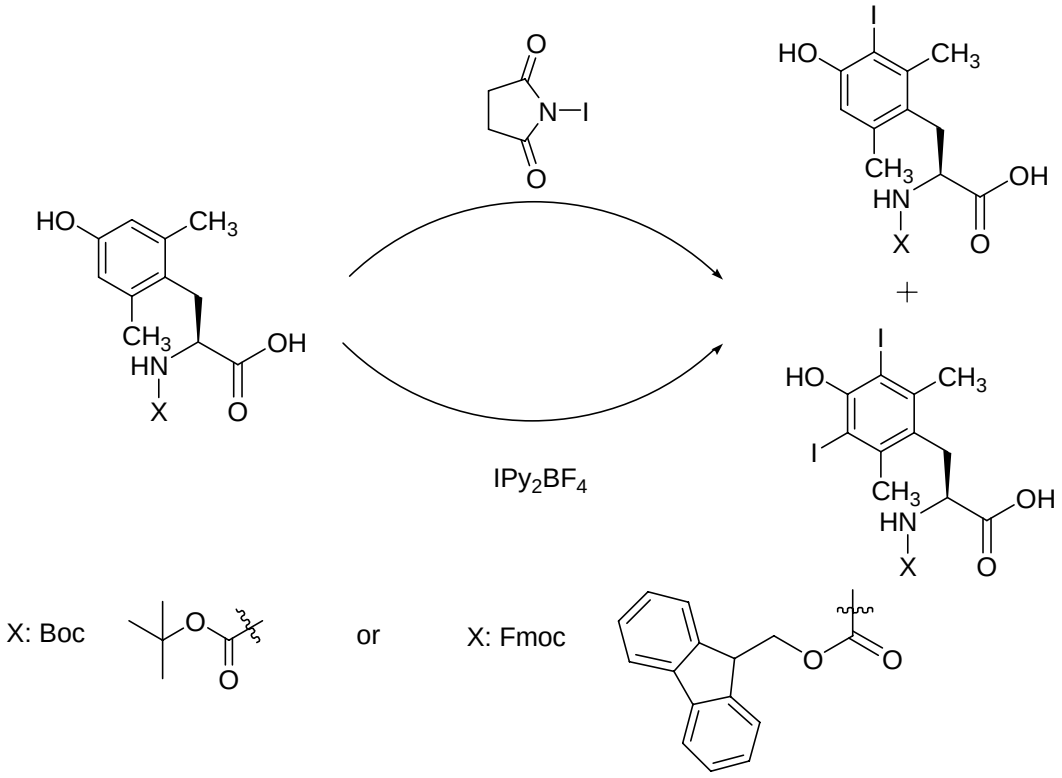
In a preliminary work in Dr. John Lever's research group, one dermorphine analogue, Dmt-*D*-Ala-Phe-Phe-NH_2, was obtained by solid phase peptide synthesis. In the subsequent *in vitro* binding assay, Dmt-*D*-Ala-Phe-Phe-NH_2 was found to have a better μ-opioid receptor binding affinity than its precursor 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 *in vivo* to cross the blood-brain barrier (BBB).

Table 6-2. *In vitro* binding affinity for Tyr-*D*-Ala-Phe-Phe-NH_2, Dmt-*D*-Ala-Phe-Phe-NH_2 and I-Dmt-*D*-Ala-Phe-Phe-NH_2

Liands	**Ki (nM)**			**δ/μ**	**κ/μ**
	μ	**δ**	**κ**		
Tyr-*D*-Ala-Phe-Phe-NH_2	3.52 ± 0.137	521 ± 72	176.7 ± 17.3	148	50
Dmt-*D*-Ala-Phe-Phe-NH_2	0.11 ± 0.010	8.32 ± 0.76	2.65 ± 0.650	76	24
I-Dmt-*D*-Ala-Phe-Phe-NH_2	2.89 ± 0.313	1090 ± 102	399.6 ± 21.5	377	138

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-NH_2. 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-NH_2: iodination of the peptide directly or preparation of the iodinated Dmt. Since –OH and $–CH_3$ 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 (IPy_2BF_4), 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.



Scheme 6-1. Iodination of Boc-Dmt or Fmoc-Dmt by NIS or IPy_2BF_4

6.2 Experimental Section

6.2.1 Methods and Materials

Nα-(9-*tert*-butoxycarbonyl)-2,6-dimethyl-L-tyrosine (Boc-Dmt) and Nα-(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 λ 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.[115] To a solution of Boc-2,6-Dimethyl -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 IPy_2BF_4

The iodination procedure with IPy_2BF_4 has been adapted from the previously reported work with appropriate modification.[116] A solution of Boc-2,6-Dimethyl -L-tyrosine (6.8 mg, 0.022 mmol) in 1.5 mL CH_3CN was added HBF_4 (30 μL, 0.022 mmol of a 5.4% solution in ether) and then IPy_2BF_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.[115] To a solution of Fmoc-2,6-Dimethyl -L-tyrosine (5.0 mg, 0.0116 mmol) in 5 mL CH_3CN 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 μL of the reaction mixture was removed to check the reaction conversion by analytical HPLC.

Iodination of Fmoc-Dmt with IPy_2BF_4

The iodination procedure with IPy_2BF_4 has been adapted from the previously reported work with appropriate modification.[116] A solution of Fmoc-2,6-Dimethyl -L-tyrosine (5.0 mg, 0.0116 mmol) in 5 mL CH_3CN was added HBF_4 (16 μL, 0.0116 mmol of a 5.4% solution in ether) and then IPy_2BF_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 μL 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).

^{1}H NMR (in d_6-DMSO): δ 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_3COOH) was used.[115] 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).

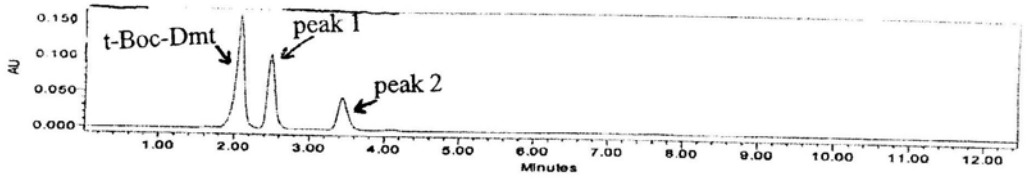


Figure 6-2. HPLC of the reaction mixture of Boc-Dmt with NIS

6.3.2 Iodination of Boc-Dmt with IPy_2BF_4

A strong acid, HBF_4, was used in the regioselective iodination in the literature.[116] 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 HBF_4 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).

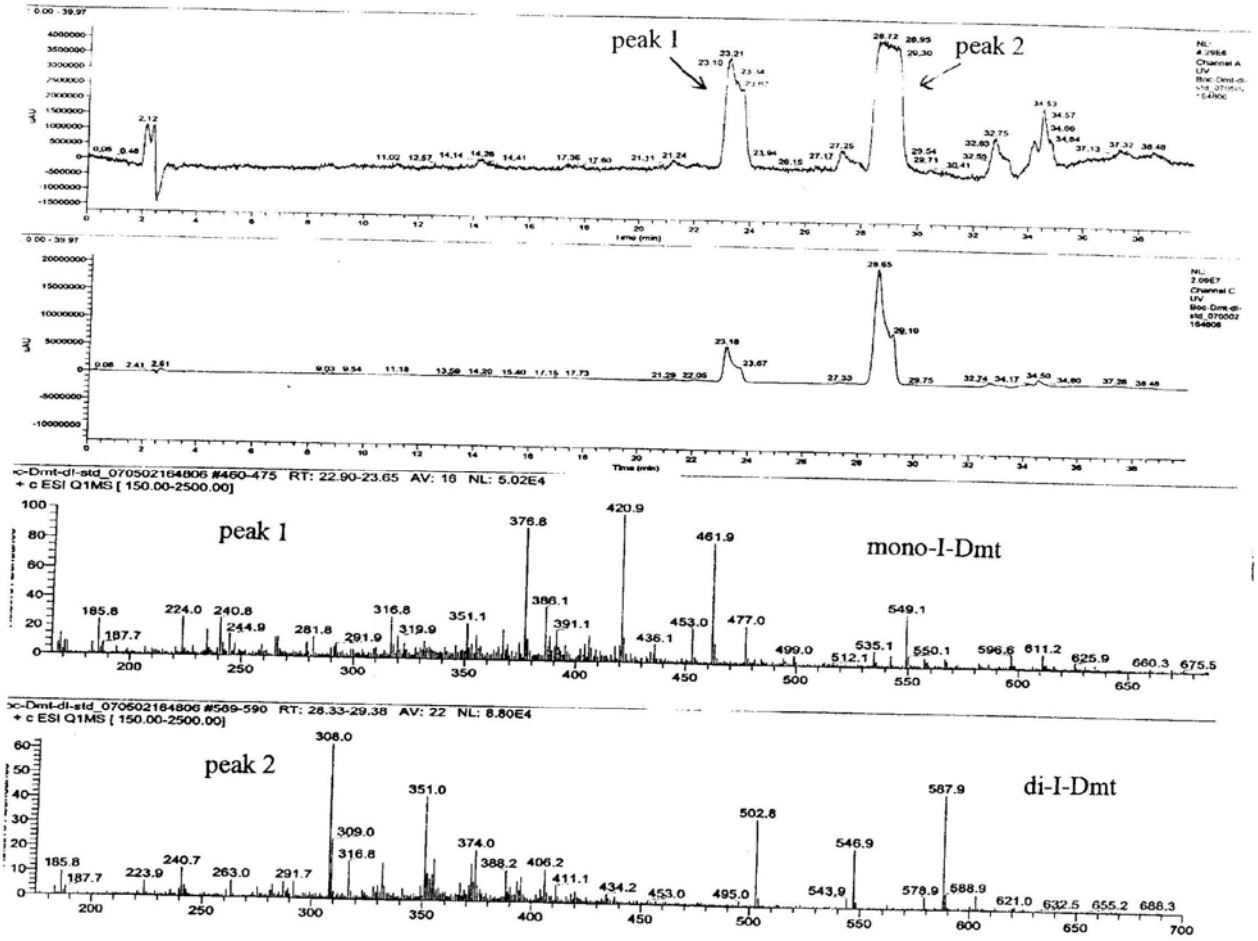


Figure 6-3. LC-MS of main products in the reaction of Boc-Dmt with IPy_2BF_4

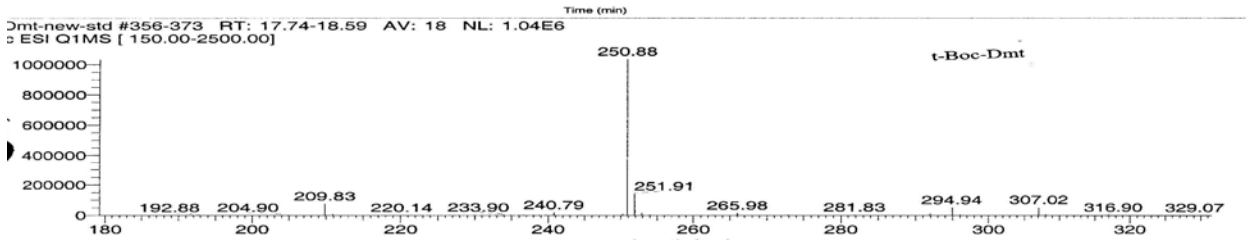


Figure 6-4. Mass spectrum of Boc-Dmt

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 (CF_3COOH) was either present or not (Figure 6-5).

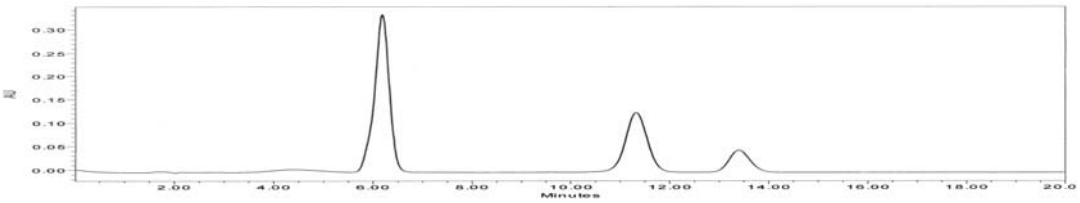


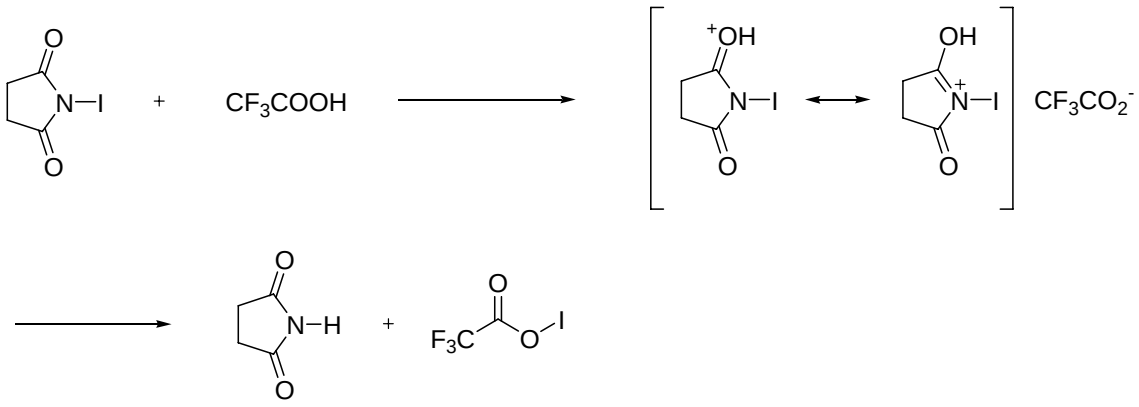
Figure 6-5. HPLC of the reaction mixture of Fmoc-Dmt with NIS

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

Reaction #	Fmoc-Dmt: CF_3COOH: NIS	Reaction Time	Fmoc-Dmt: peak 1 : peak 2 (areas of peak in HPLC)
1	1: 0: 1	10 min	5: 1: 1
		8 h	5: 1: 1
2	1: 0: 2	10 min	2.2: 1: 1.5
3	1: 1: 1	10 min	1.8: 1: 0.5

Our observation by analytical HPLC suggested that CF_3COOH 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 *in situ* from NIS and CF_3COOH (Scheme 6-2). [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.



Scheme 6-2. Proposed mechanism for the forming of iodine trifluoroacetate[115]

6.3.4 Iodination of Fmoc-Dmt with IPy_2BF_4

Similar to the iodination of Fmoc-Dmt with NIS, we also examined the iodination of Fmoc-Dmt with IPy_2BF_4 in regards of the reaction time and the equivalent of iodination reagent (Table 6-4).

Table 6-4. Optimization Table for Iodination Reaction of Fmoc-Dmt with IPy_2BF_4

Reaction #	Fmoc-Dmt: HBF_4: IPy_2BF_4	Reaction Time	Fmoc-Dmt: peak 1 :peak 2 (areas of peak in HPLC)
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.

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 IPy_2BF_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: IPy_2BF_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 ^1H NMR and ^{13}C NMR. This product was coupled with other amino acids in small batch to give I-Dmt-*D*-Ala-Phe-Orn-NH_2 by solid phase synthesis (The solid phase synthesis of I-Dmt-*D*-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).

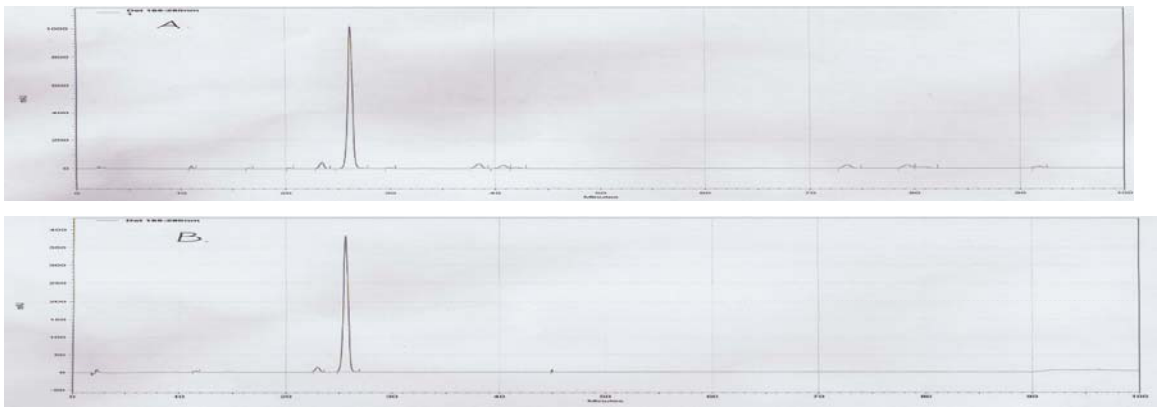


Figure 6-6. HPLC of the reaction product of Fmoc-Dmt with IPy_2BF_4
(A: crude sample; B; purified sample)

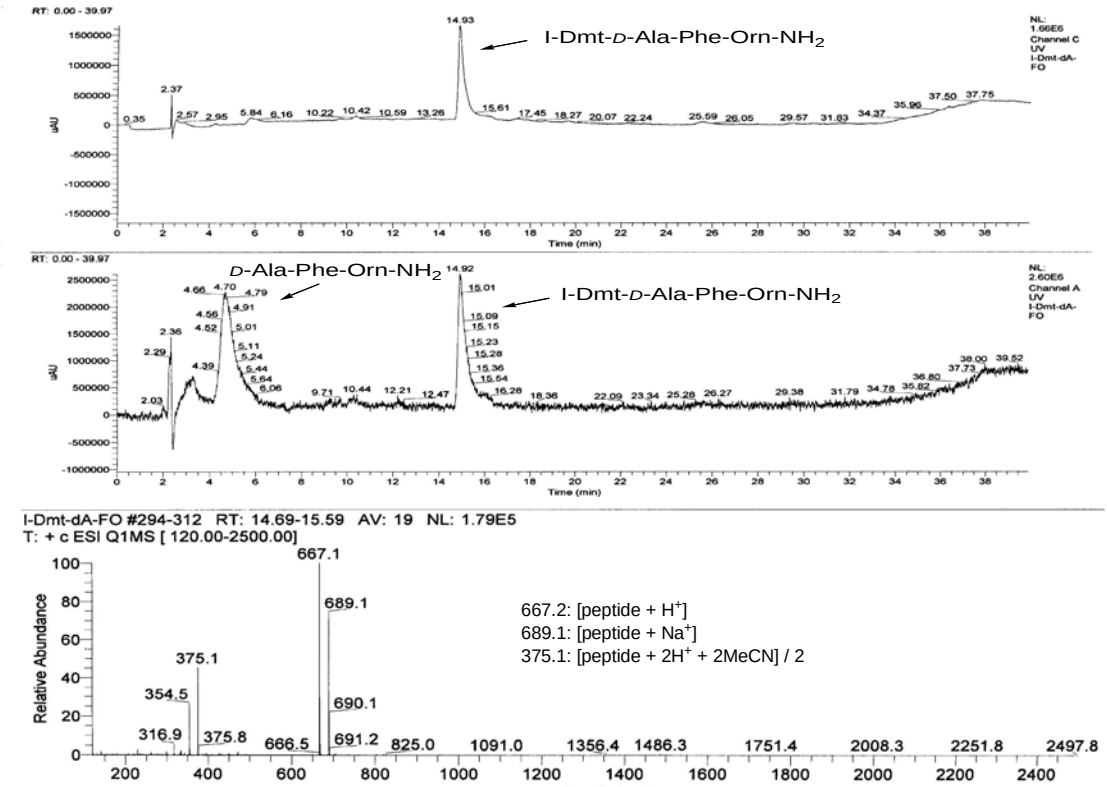


Figure 6-7. LC-MS spectrum of I-Dmt-*D*-Ala-Phe-Orn-NH_2

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 CF_3COOH or HBF_4, can be present. A large portion of di-iodinated product was shown by HPLC if NIS and CF_3COOH were used. However, under the iodination by IPy_2BF_4, a ratio of 1:1:1.1 (Fmoc-Dmt: HBF_4: IPy_2BF_4) 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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