Expedient Synthesis of Chiral Poly-Substituted Morpholine and Oxazepine Derivatives for the Preparation of Cyclophilin A Inhibitors

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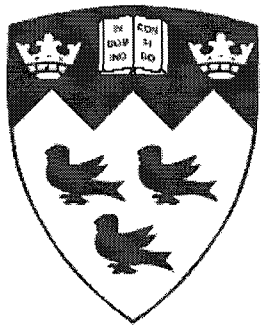

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Your file Votre référence
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This thesis is dedicated to my two best friends Hana Bilbeisi and Pierre Karam. Thank you for believing in me and supporting me till the end.

Abstract

An efficient and expedient synthetic method was developed for the preparation of chiral poly-substituted morpholine and oxazepine derivatives. The method was designed in the objective of applying the synthesis to the preparation of Cyclophilin A inhibitors.

The stereo- and regioselective method involves the reaction of enantiopure β-amino alcohols with α,β-unsaturated aldehydes. The synthesis proceeds through three steps; i) Reductive amination, ii) *N*-alkylation/ *N*-tosylation and iii) intramolecular-haloetherification. Stereoselectivity of this last step was controlled by *N*-alkyl/ *N*-tosyl groups and substitution across the double bond, and was enhanced by the addition of Brønsted acids. Substitution across the double bond of the starting material controlled the regioselectivity of the method. Morpholines were obtained through 6- *exo* cyclization and oxazepines were obtained through 7-*endo* cyclization.

A small library of morpholine-based derivatives was designed *in-silico*. Affinity and binding modes to the Cyclophilin A were investigated through a docking-based virtual screening study.

Résumé

Une stratégie de synthèse efficace et rapide de morpholine et d'oxazépine polysubstituées chirales a été développée. Ce procédé a été conçu dans l'objectif d'appliquer cette méthodologie à la préparation d'inhibiteurs de Cyclophiline A.

Cette méthode stéréo- et régiosélective implique notamment la réaction d'alcools β-aminés énantiopures avec des aldéhydes α,β-insaturés. La synthèse procède selon trois étapes; i) Amination réductrice, ii) *N*-tosylation ou *N*-alkylation iii) haloéthérification intramoléculaire. La stéréosélectivité de cette dernière étape est contrôlée par les groupes *N*-tosyl/ *N*-alkyl ainsi que par les substituants de la double liaison. De plus, il a été montré que l'addition d'acides de Brønsted permettait d'augmenter la stéréosélectivité de cette cyclisation intramoléculaire. Les substituants de la double liaison exercent également une influence remarquable sur la regiosélectivité. Ainsi, des dérivés morpholiniques sont obtenus *via* une cyclisation de type 6-*exo* alors que les correspondants oxazepines sont obtenus *via* une cyclisation de type 7-*endo*.

Une petite bibliothèque des dérivés de type morpholine a été conçue *in-silico*. L'affinité et le mode de liaison à la Cyclophiline A ont été évalués par une étude de criblage virtuel basée sur le docking.

Acknowledgements

I would like to start by thanking Dr. Nicolas Moitessier, my research supervisor. Thank you for all the guidance and help. Your ambition and motivation towards research had influenced me positively through my Master's journey.

I was very fortunate to be part of the Chemistry Department. I would like to thank the staff, especially Chantal Marotte, Fred Morin, Jean-Marc Gauthier, Badawy Sha'ath, Nadim Saade, and all the professors. Dr. Alain Lesimple's time and wonderful work are very much appreciated.

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Most importantly I would like to express my gratitude to my supporting family. My parents, my sister Razan, my brother Awn and especially my twin sister Hana. Thank you for all your prayers and encouragement.

List of Abbreviations

THF	tetrahydrofuran
EtOAc	ethyl acetate
Et2O	diethyl ether
MeCN	Acetonitrile
DCM	Dichloromethane
DBU	1,8-diazabicyclo(5.4.0)undec-7-ene
TBAF	tetrabutylammonium flouride
DMF	Dimethylformamide
MeOH	Methanol
EWG	Electron-withdrawing group
eq.	Equivalents
HMQC	Heteronuclear Multiple Quantum Coupling
HMBC	Heteronuclear Multiple-bond Coupling
NOESY	Nuclear Overhauser Effect Spectroscopy
NMR	Nulcear Magnetic Resonance
HRMS	High Resolution Mass Spectrometry
MO	Molecular Orbital
Ar	Aryl
LA	Lewis Acid
Nu^-	Nucleophile
E^+	Electrophile
de	Diastereomeric excess
dr	Diastereomeric ratio
DMSO	Dimethyl-sulfoxide
Å	Ångstrom
Bn	Benzyl
Boc	*tert*-butoxycarbonyl
Cbz	Benzyloxycarbonyl
COSY	correlation spectroscopy
δ	chemical shift
ESI-MS	electrospray ionization mass spectrometry
J	coupling constant
Kcat	turnover number
Kd	dissociation constant
m/z	mass-to-charge ratio
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
pH	negative logarithm of hydrogen ion concentration

pKa	negative logarithm of equilibrium constant for association
ppm	parts per million
PPh_3	Triphenyl phosphine
K_2CO_3	Potassium Carbonate
Py	pyridine
Rf	Retention factor
rRNA	ribosomal RNA
RT	room temperature
Bs	broad singlet
q	quartet
s	singlet
t	triplet
***t*-Bu**	*tert*-butyl
TBS	*tert*-butyldimethylsilyl
Et_3N	triethylamine
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy free radical
TsCl	toluenesulfonyl chloride
CsA	Cylosporin A
Cyp A	Cyclophilin A
AIDS	Acquired immune deficiency syndrome
HIV	Human immunodeficiency virus
WHO	World Health Organization
HAART	Highly active antiretroviral therapy

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Chapter 1: Introduction

1.1 Preface

In this chapter we are describing the design and development of synthetic methodologies towards the preparation of enzyme inhibitors. The discussion will focus on two themes; i) cyclophilin A (Cyp A) and its effect on acquired immunodeficiency syndrome (AIDS), ii) morpholines and oxazepines-based compounds.

The effect of Cyp A on AIDS will be demonstrated by reported Cyp A inhibition studies. Various Cyp A inhibitors will be presented, along with their effects as potential treatments for AIDS.

Morpholines and oxazepines are heterocyclic compounds that are of great interest to this study due to their potential wide application as scaffolds in drug design and discovery. Occurrence, synthesis and biological significance of both structures will be discussed.

Finally the interrelation between the two themes will be disclosed by the objectives of the study, which aim towards designing potential morpholine and oxazepine-based inhibitors of Cyp A.

1.2 Cyclophilin A (Cyp A): Potential therapeutic target for the treatment of acquired immunodeficiency syndrome (AIDS)

1.2.1. Cyclophilin A: Peptidyl-Prolyl Isomerase (PPIase)

Immunophilins, found in a wide range of tissue types and organisms, are cytosolic enzymes with immunosuppression and peptidyl-prolyl isomerase (PPIases) activity. The super family of immunophilins includes cyclophilins and FK-506 binding protein (FKBP). Proteins from these two families vary in their primary sequence and their three dimensional structure, but are similar in some of their functions and activities.[1-2] In this thesis, we will be focusing on cyclophilin A (Cyp A), a member of the cyclophilin family that constitutes about 0.5% of all cytosolic enzymes.[3]

Immunosuppression activity of Cyp A results from its specific strong binding affinity to cyclosporin A (CsA). CsA is an immunosuppressive cyclic peptide that is used after organ transplantation operations to prevent organ rejection. The high affinity CsA/Cyp A complex binds to calcineurin and inhibits its phosphatase activity, which in turn suppresses T cell activation.[4] Therefore, CsA/Cyp A complex prevents early immune responses.[5-6]

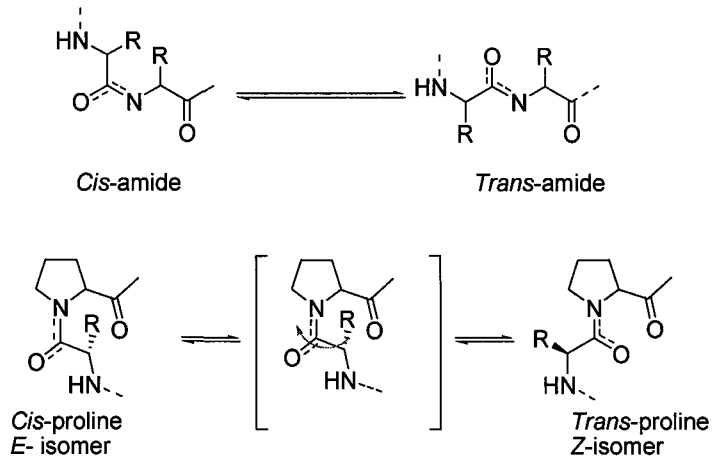


Figure 1.1: *Isomerization of cis-trans amide bonds in propyl peptides, catalyzed by cyclophilins.*[3]

At the molecular level, immunophilins assist the isomerization of amide bonds and are sometimes referred to as rotamases or peptidyl-propyl isomerases (PPlases) due to their function. This process is facilitated by reducing the rotational energy barrier about peptide bonds on the *N*-terminal side of proline residues. *Cis* to *trans* isomerization of proline residues (Figure 1.1) assist protein folding. However the isomerization process is only achieved once the energy barrier is reduced. Cyp A stabilizes the transition state (90° angle about the amide bond), hence reducing the energy barrier of this *cis-trans* isomerization. This stabilization gives access to other protein folding.[7]

1.2.1.1 Structure of Cyp A

Crystal structure of the human Cyp A bound to CsA had shown that Cyp A adopts 8 stranded β-sheets and two α-helices.[8] The active site of the protein has three pockets, where the ligand binds through a series of hydrogen-bonds, hydrophilic and hydrophobic interactions. The hydrophobic groove where

substrates like CsA usually binds, is formed of 11-13 residues. Site directed mutagenesis revealed that residues Arg55, Phe60, Phe113, and His126 are crucial for the function of the active site of Cyp A. [9]

Two water molecules exist in the crystal structure of Cyp A, located in the active site of the enzyme.[10] Water molecules create bridges between the ligand and the active site, increasing the binding of the ligands (shown in Figure 1.3 section 1.2.2.1)

1.2.1.2 Mechanism of Action

The catalytic mechanism of *cis-trans* isomerization was investigated using NMR[11] and computational studies.[12] Proline isomerization energetics were determined using potential of mean force (PMF) simulations for several mutants wild type Cyp A.[13] The contribution of some factors on proline isomerization were evaluated semi quantitatively. The positive charge of Arg55 and catalytic efficiency of Cyp A were among those factors that were studied. It was found out that both factors affect the isomerization of proline residues. The studies have expanded on the literature aiding a better understanding of the catalytic nature of the enzyme at the molecular level.[14]

The catalytic nature of the active site is dependent on the interactions of Asn102 and Arg55 with proline amide bonds during the formation of the (*TS*). As shown in Figure 1.2, Asn102 interacts with the oxygen in the carbonyl of the amide stabilizing the amide bond of the substrate with a 20° twist out-of-plane.

The guanidine group of Arg55 interacts with the lone pair of the nitrogen in the amide group, reducing the resonance with the carbonyl.

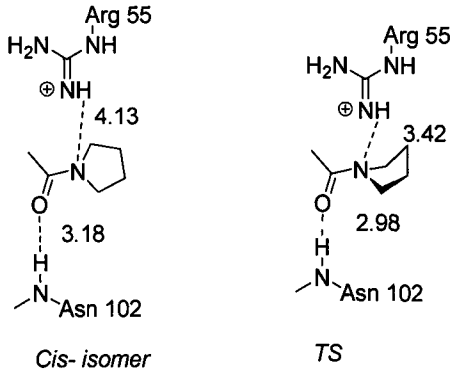


Figure 1.2: *Interactions and distances of the substrate in different forms with Asn102 and Arg55 in the active site.*[14]

In the active site, a torsion of 20° of the O=C-N-C dihedral angle (of prolyl amide bond) is stabilized through hydrogen bonding between the *cis*-prolyl amide carbonyl (O) and the backbone amide (N-H) of Asn102. As for the TS, the structure is characterized by a 90° twist of the amide bond (C-N bond), interacting more favorably with Asn102 due to the shorter distance between Asn102 (HN) and the amide carbonyl (O). The conformational change from *cis* to TS involves the pyramidalization of the amide (N), resulting in a shorter distance and higher interaction with the guanidino group of Arg55 and amide (N) (Figure 1.2). Hence it is established that Arg55 is the key residue stabilizing *cis*- TS isomerization through electrostatic interactions and hydrogen bonds.[15]

1.2.2 Acquired immunodeficiency syndrome (AIDS)

Acquired immunodeficiency syndrome (AIDS) is an epidemic life threatening disease, caused by human immunodeficiency virus (HIV).

Approximately 40 million people worldwide live with the disease. It was estimated that AIDS had killed over 25 million patients since it was discovered in June 1981, which makes it one of the most destructive diseases recorded in history.[16] The disease targets the immune system, leaving individuals prone to tumors and infections.

Many anti-retroviral agents have been introduced and are currently used for the treatment of HIV infection. The treatment consists of highly active anti-retroviral therapy (HAART), which is based on combination of drugs that can be categorized into different anti-retroviral agents; nucleoside and nucleotide reverse transcriptase inhibitors (NRTI) such as Abacavir, non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI) Atazanavir.[17] HAART stabilizes the patient's symptoms leading to a significant improvement in patients' health and life quality. However, it does not cure nor alleviate the symptoms.

HIV affects nearly every organ systems in the host. Once in the host cells, HIV takes on cellular machineries for its virion assembly, multiplication and propagation. Cyp A is one of the enzymes in the human body that is necessary for the infectious nature of HIV-1 virions. Interaction of HIV-1 with Cyp A aids the post-assembly of HIV-1 virion proteins, which enhances the infectious nature of the virus. Hence, it was established that Cyp A is a potential therapeutic target for the treatment of AIDS.[37]

1.2.2.1 Cyp A is essential to the early steps of the HIV-1 life cycle

1.2.2.1.1 Interaction of HIV residues with the active site

Cyp A is one of the most studied cyclophilins due to its involvement in the post assembly of HIV-1 virion. The enzyme is involved in the formation process of HIV-1 viral capsid. The formation of the capsid proceeds through the binding of HIV-1 gag protein to Cyp A.[18] Further explanation will be provided in the following subsection.

Crystal structure of the enzyme bound to a viral HIV-1 peptide is available in the protein data bank (PDB: 1AK4).[19] The interaction of HIV-1 gag protein with the active site of Cyp A is presented in Figure 1.3.

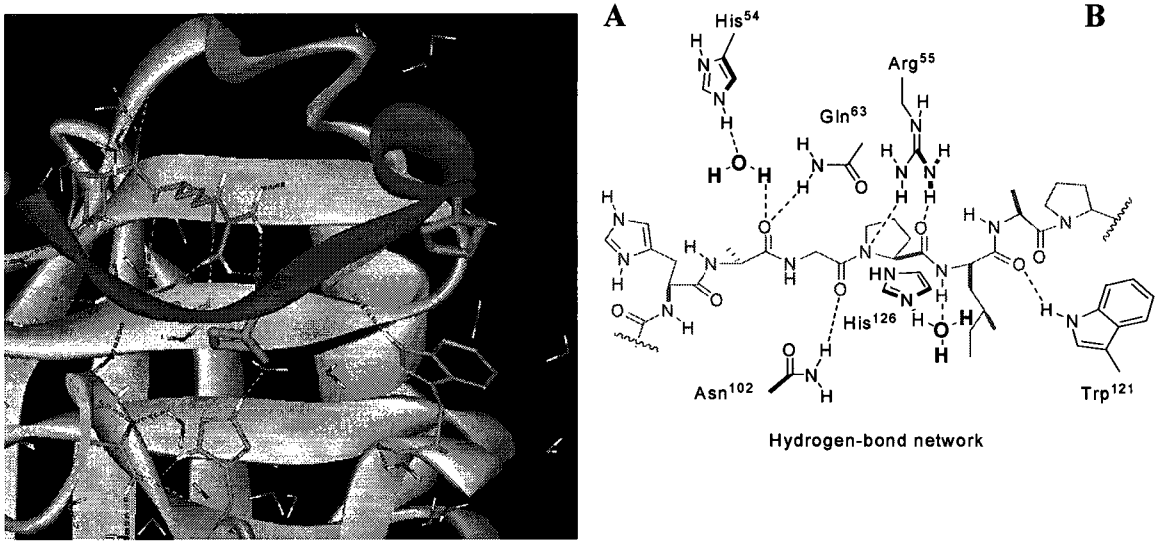


Figure 1.3: *Gag protein / CypA complex and critical interactions; **A**:crystal structure of the gag protein (red ribbon representing its main chain) bound to the active site of the Cyp A (yellow ribbon). **B**: interaction of the gag protein (red) with Arg55, Asp102 and water molecules in the active site of Cyp A.*

As illustrated in Figure 1.3, the viral peptide (red) contains two proline residues that interact with the active site of Cyp A (black) via hydrogen bonding and hydrophobic contacts. Two water molecules in the active site, form bridges between the enzyme and its substrates resulting in additional interactions.[20]

1.2.2.1.1 Role of Cyp A in AIDS infectivity

Cyp A is required for the replication and infectivity of HIV-1 virion. The enzyme is the first studied cellular protein that gets incorporated into HIV-1 virion. Cyp A gets incorporated through interactions with the viral gag polyprotein[21] and virion capsid proteins (CA), which are produced as a result of gag polyproteins cleavage.[22] CA proteins are necessary for the infection of susceptible cells since they enclose viral RNA and associated viral proteins. In the post fusion phase, uncoating takes place where CA proteins get disassembled releasing the viral nucleic acid (RNA) into the cytosol. Cyp A is responsible for the disassembly of the capsid core and liberation of viral RNA.[23] Inhibition of Cyp A using CsA or any other competitive inhibitor prevents Cyp A-CA interaction and impairs the infectivity as a result of interfering with the uncoating process.

Furthermore Cyp A is one of the first cellular factors, after cell surface receptors, found to be required for the infection of a new host cell.[24] Cyp A is not required simply to uncoat the capsid core. Binding of Cyp A to CA camouflages the target site recognized by human restriction factors (Ref-1).[25] Preventing this interaction (mutation or Cyp A inhibition) reduces infectivity of HIV-1 by

exposing the target site of CA to Ref-1 and subsequently digesting the protein. The recruitment of Cyp A by HIV-1 and the prevention of recognition by Ref-1 are shown in Figure 1.4.[26]

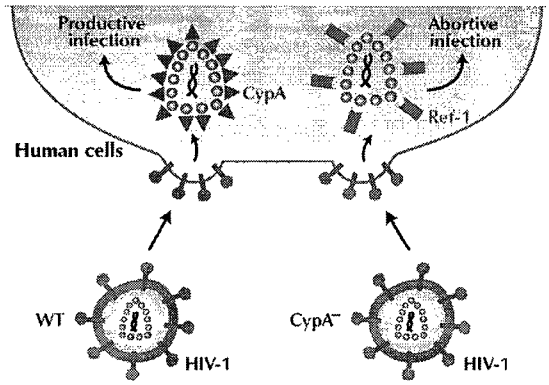


Figure 1.4: *Immunity of HIV- 1 in human cells governed by Cyp A; wild type HIV-1 recruits Cyp A and is camouflaged from restriction factors to proceed successfully, unlike mutated HIV-1 that fails to recruit Cyp A and is targeted by restriction factors to abort the infection (From Moore, J.P et al Nat. Rev.* ***2000*** *(1) 40)*

1.2.3 Cyclophilin A Inhibitors

Cyp A is an attractive target for the development of therapeutic inhibitors, due to its effect on the infectivity of AIDS. Reported inhibitors varied from natural immunosuppressive compounds (i.e., CsA) and their derivatives to small drug-like molecules. Studies illustrated the inhibition activity of various classes of Cyp A inhibitors; ground-state inhibitors, non-isomerisable analogs of proline moiety (constrained proline mimetics), and other endogenous cyclophilin inhibitors including peptidic, non-peptidic and small inhibitors. Cyp A inhibitors will be discussed in more details in the following section.

The molecular basis of the substrate specificity of Cyp A was proposed in structure-activity relationship studies (Figure 1.5).[27] It was determined that three functionally independent subsites exist; a hydrophobic pocket along with Arg55 (catalytic residue) form subsite S1'. As for subsites S2' and S3', they interact via hydrogen bond network involving Arg148 and Trp121. Other specific interactions are involved upon binding of larger peptides such as gag proteins and CsA peptides.[28]

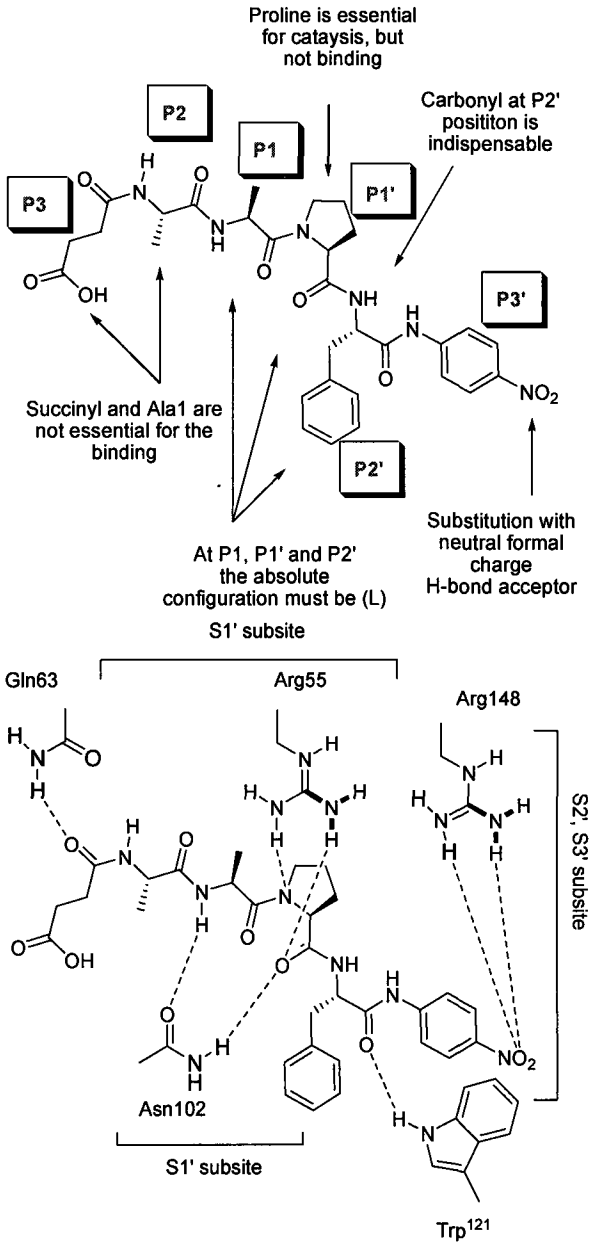


Figure 1.5: *Structural features of substrates as determined by SAR studies. Interaction of Suc-Ala-Ala-Pro-Phe-pNA with Cyp A and the delineation of the S1' and S2'-S3' subsites (From C. Dugave Current Organic Chemistry, 2002 (6), 1397).*

The enzyme is very sensitive to the absolute configuration of the substrate at each one of the pockets. The three pockets within the active site are shown in Figure 1.5. Proline at P1' interacts with Arg55, this interaction is very essential for the catalytic process (as described in section 1.2.1.2) but does not affect the binding

affinity to the enzyme. Hydrogen bond networks are involved in the binding of the substrate to the enzyme, and are necessary for the fixation of the ligand in the active site.

1.2.3.1 Natural Cyp A inhibitors_Cyclosporin A and its derivatives

CsA is a naturally occurring immunosuppressive. It is an undecapeptide with seven *N*-alkylated amides and several non-natural amino acids (Figure 1.6). Due to the high affinity of CsA for Cyp A, CsA is considered as a natural inhibitor for Cyp A. However non-immunosuppressive CsA analogues would be more suitable to be utilized as Cyp A inhibitors and potential anti-AIDS molecules, since CsA has immunosuppressive effects.

Many CsA derivatives and analogs have been synthesized via *N*-alkylation and cyclization of coupled amino acid chains.[29] In Figure 1.6, CsA fragments are numbered to delineate the role and effect of modifying each fragment. Fragments 3-9 interact with the protein, unlike fragments 4-8 that protrudes on the protein surface. *N*-Me Val11 gets buried inside the narrow hydrophobic pocket of the enzyme. *Cis* to *trans* isomerization of amide bonds in fragments 9 and 10 changes the low affinity complex to a high affinity complex. Residues 4-6 affect the binding moiety. Modifications targeting fragments 1-4 results in enhancing the binding to Cyp A.[30] However, substituting residues 2-4 with a hydrophilic side chain increased the binding affinity and decreased the immunosuppressive properties of the corresponding analogues.

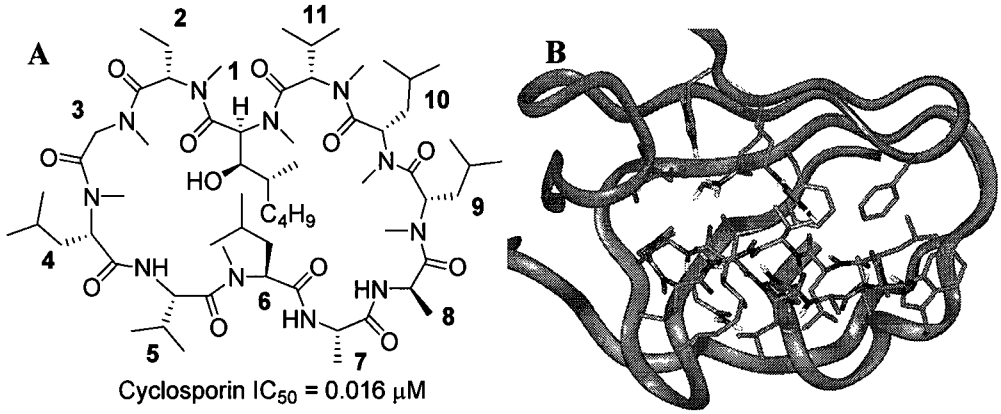


Figure 1.6: *A) Cyclosporin A (CsA) , B) CsA/Cyp A complex*

1.2.3.2 Constrained proline derivatives

Proline derivatives are attractive compounds for the inhibition of PPlase since proline is a natural substrate of the enzyme.[20] PPlase recognizes proline derivatives due to the presence of the pyrrolidine ring that is an important motif for ligand recognition by Cyp A.

Proline derivatives were used as ground state and transition state analogue inhibitors of Cyp A. Small molecular weight constrained proline peptides were synthesized and their binding affinity to Cyp A were tested.[31] Bicyclic lactams **1** shown in Figure 1.7, were synthesized to be used as ground state analogue inhibitors. Binding affinity of ground state analogue inhibitors varied with the variation of the R group. Hydrophobic groups adjacent to the lactam carbonyl showed the highest affinity (R_1: $CH_2C_{10}H_7$, R_2: Me; Kd 1.5 μM). The carbonyl of the bicyclic lactams was chemoselectively reduced to provide the *TS* analogue as **2** (Figure 1.7). Binding affinity of the transition state analogue inhibitors have

shown to be much lower (Kd of 77 μM) than those of the ground state analogues (Kd of 1.5 μM).

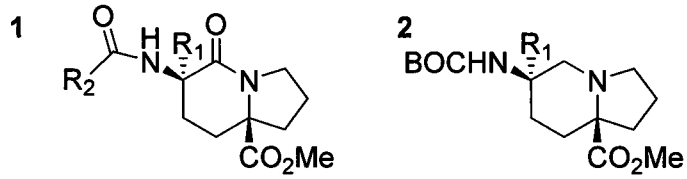


Figure 1.7: *Constrained ground-state and TS analogues as inhibitors of Cyp A.*

1.2.3.3 Other peptidic inhibitors:

Several scaffolds have been investigated for Cyp A inhibition. Functionalizing the enzyme scaffolds (ligands) with various groups to mimic key interactions of CsA is presented in the study below.

Computer aided structure based design of dimedone derivatives as Cyp A inhibitors was achieved. Scaffolds were designed to mimic key interactions between CsA and Cyp A, and binding to the hydrophobic pocket of the enzyme was explored. Peptidomimtic dimedone-based molecules have shown inhibition of Cyp A with an IC_{50} of 190 μM (compared to IC_{50} = 28 μM with CsA).[32]

1.2.3.4 Non-peptidic inhibitors

Non-peptidic small molecules' use as Cyp A inhibitors has been reported in a number of studies.[33] Among those studies was the identification of two lead compounds via computer aided structure-based design.[34] Series of molecules

were synthesized from a diarylurea scaffold and evaluated *in vitro* for their inhibition of the *cis-trans* isomerase activity of Cyp A. Compounds [1-(3-benzyloxy pyridin-2-yl)-3-(3-chlorophenyl)urea] **3** and [1-(3-benzyloxypyridin-2-yl)-3-(3-trifluoromethylphenyl)urea] **4** showed IC_{50} of ~300 nM. The diaryl moiety participates in three hydrogen bonding with Gln63, Gly72, and Asn102 of Cyp A, as well as several hydrophobic contacts anchoring the ligand into the active site.

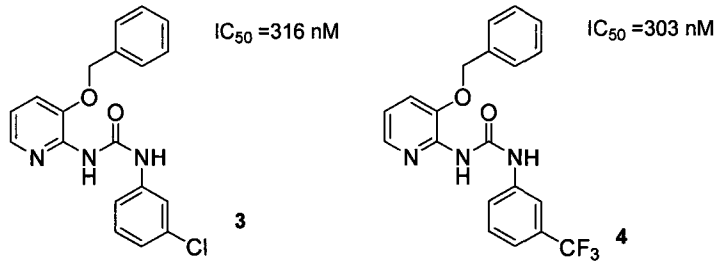
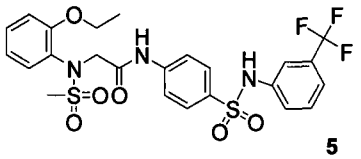
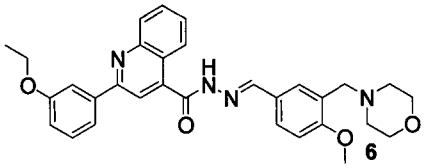
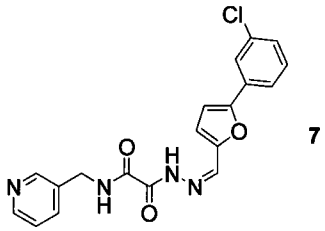
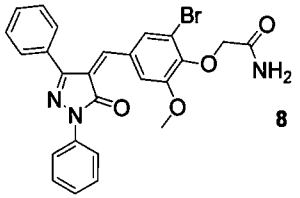
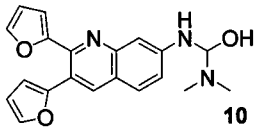


Figure 1.8: *Lead compounds* **3** *and* **4** *identified by virtual screening.*

Further enhancement of the inhibition was achieved by structural modification of the scaffolds. Five out of thirty one compounds exhibited inhibition of Cyp A. In addition, both compounds **3** and **4** have shown to be potent inhibitors of the HIV-1 replication cycle when tested on human peripheral blood mononuclear cells.

Other non-peptidic Cyp A inhibitors were identified using computer aided structure based- identification.[35] A database of 80000 small compounds were screened and scored using DOCK as the docking software. Twelve high scoring molecules were purchased consequently. Five of the 12 small molecules exhibited inhibition of Cyp A activity and HIV-1 replication. The small molecules have IC_{50} values ranging from 1 to 8 μM as shown in Table 1.

Table 1.1: *IC_{50} values for inhibition of PPIase activity of cyclophilin A and HIV-1 replication.*

Compound	IC_{50} for Cyp A inhibition (uM)	% inhibition of HIV-1 replication at 10 μM
 **5**	14.45	57.2
 **6**	18.67	62.6
 **7**	34.86	77.5
 **8**	6.98	64.2
 **10**	3.47	65.2
CsA **11**	0.45	98.2

As described in this section, HIV replication was slowed down by inhibiting Cyp A. This emphasizes the significance of Cyp A inhibition as potential treatment of AIDS.

1.3 Synthesis and biological significance of morpholine derivatives

Morpholine derivatives have shown various biological applications. They have been explored for their significance as synthetic organic tools, biological and pharmaceutical active compounds.

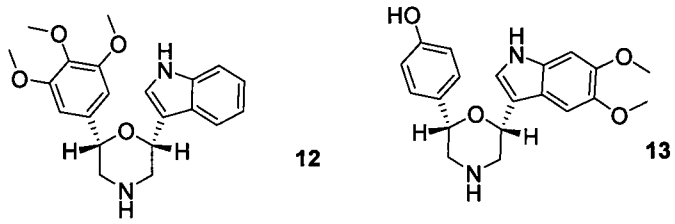
Natural occurring morpholine-based compounds have shown a wide range of biological activities; antitumor, antibiotic, anti-inflammatory and antimicrobial.[36] Therefore morpholine derivatives comprise an essential class of compounds in the constant search for new active pharmaceutical compounds. Occurrence and biological significance are described in greater details in the following sections.

1.3.1 Occurrence and biological significance of morpholine derivatives

Many morpholine derivatives were extracted from natural sources. Structures of these morpholine derivatives were elucidated by spectral analysis. Further structural confirmation was obtained through independent total synthesis.[37]

Two morpholine-based natural products among several other alkaloids were extracted from the marine sponge *Chelonaplysilla* sp. collected from a marine lake in Palau.[38] Chenolin A (**12**) and chenolin C (**13**) were the first reported 2,6-

disubstituted morpholines from natural sources (Figure 1.9). Chenolin A has shown antimicrobial activity against *Bacillus subtilis* and *in-vivo* anti-inflammatory activity.



12 13

Figure 1.9: *Chenolin A (12) and C (13) first reported 2,6-disubstituted morpholines extracted from natural sources.*

Due to their biological activity, morpholines have been intensively studied by pharmaceutical companies. Therefore efficient synthesis of chiral poly-substituted morpholine synthesis became a challenge to be resolved.

1.3.2 Synthetic approaches towards morpholine derivatives

Synthesis of chirally functionalized morpholine-based molecules has not been fully investigated and has been a long term challenge. Various synthetic approaches have been proposed to synthesize morpholine derivatives. A brief summary of different protocols for the synthesis of chiral poly-substituted morpholines is provided in Figure 1.10. Derivatized morpholines were synthesized from amino acids,[39] epoxides,[40] olefins,[41] and carboxylic acid derivatives.[42] Other synthetic protocols have utilized chiral auxiliaries[43] and organometallics,[44] to achieve enantio and diastereoselective syntheses.

Chiral substituted morpholines were obtained in most of the reported synthetic routes, through long multi-step synthetic routes that (in most cases) include protection / deprotection steps leading to reduced yields. Furthermore, three examples of different synthetic approaches are presented in Figure 1.10. The presented syntheses vary in the number of steps (1 to 8 steps), substitution sites (mono- , di- substituted), yields (32.5%- 50%) and chirality.

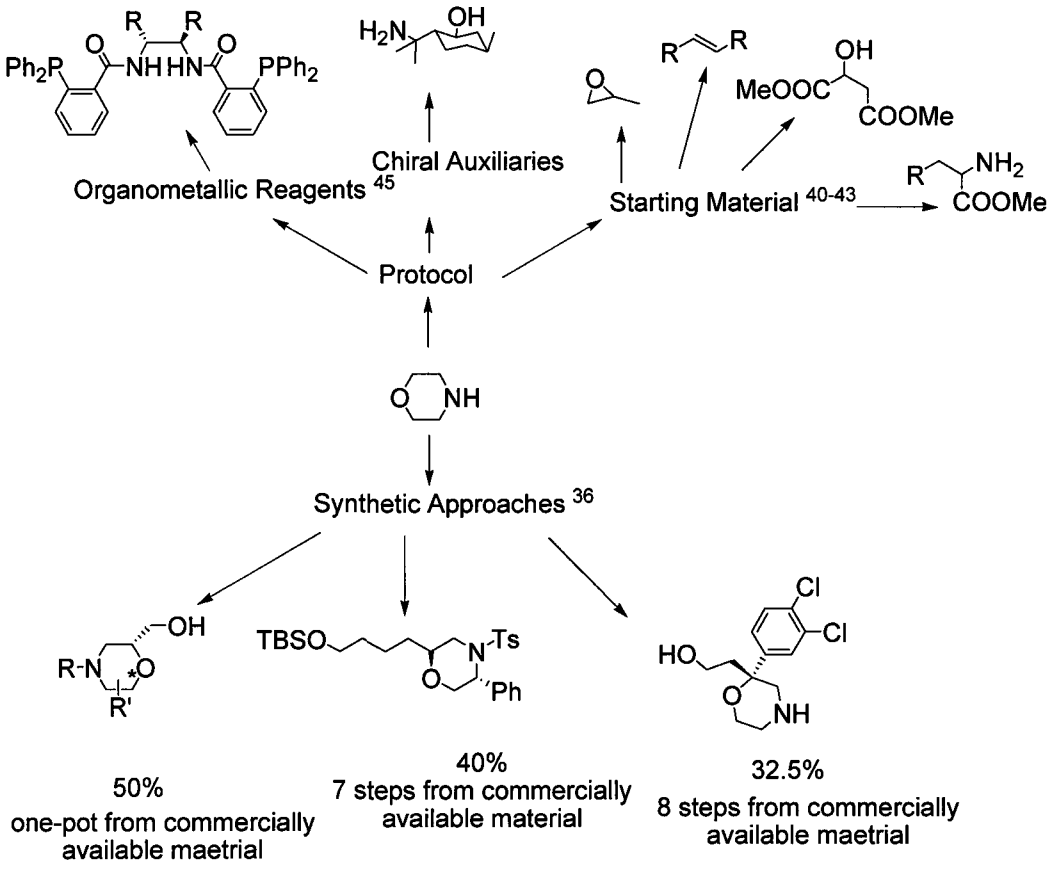
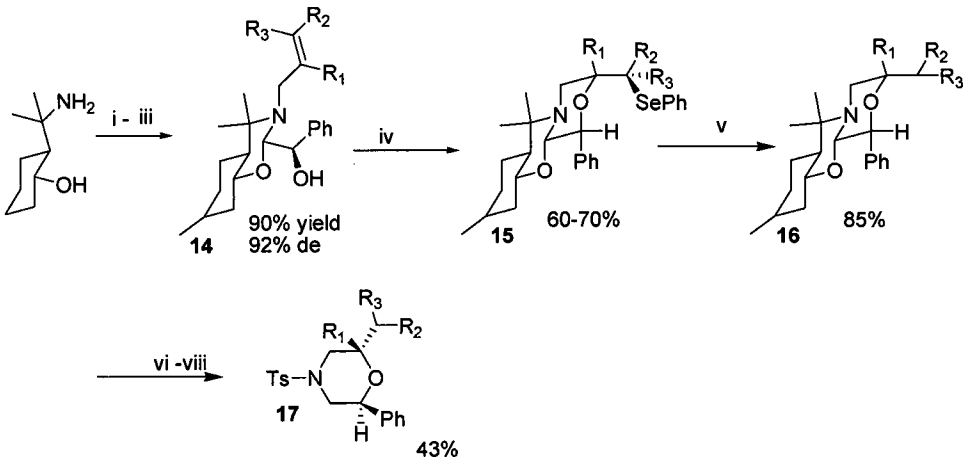


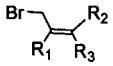
Figure 1.10: *Various reported synthetic protocols and approaches for the synthesis of chiral substituted morpholines*[30-45]

1.3.2.1 Multi-step synthesis of 2,6-disubstituted morpholines

Multi-step synthetic procedures are commonly conducted for the synthesis of morpholine derivatives. The synthesis of 2,6-disubstituted enantiopure morpholine derivatives was reported recently by Pedrosa an his coworkers.[46] Enantiopure morpholine derivatives were prepared using selenofunctionalization. Stereochemistry in the final product was induced by employing (-) 8-aminomenthol, as a chiral auxiliary. The synthetic sequence is presented in Scheme 1.1, 3-allyl-2-hydroxymethyl-substituted perhydro-1,3-benzoxazine **14** derivatives were prepared in three steps (90% yield and 92% de). This was followed by selenofunctionalization, leading to a regioselective 6- *exo* cyclization **15** with a 92% de. Cyclization was followed by oxidation of the selenium substituent and *syn* elimination of the selonoxide group (compound **16**). Finally the chiral auxiliary was removed in three steps, followed by *N*-tosylation leading to the final product **17.**



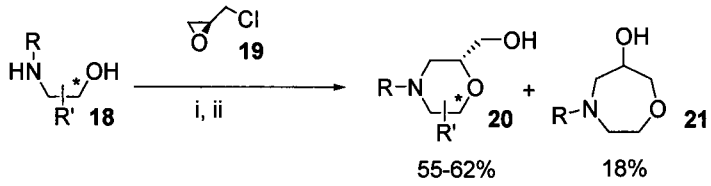
Scheme 1.1: *Synthesis of chiral 2,6-disubstituted enantiopure morpholines via selenofunctionalization*

i) PhCOCHO, CH_2Cl_2, RT; ii)  *, K_2CO_3, CH_3CN; iii) $NaBH_4$, EtOH, -10 °C iv) PhSeCl, THF, $SnCl_4$, -78°C; v) Ph_3SnH, AIBN, Toluene, reflux; vi) AlH_3, THF, 0 °C vii) PCC, CH_2Cl_2, RT; vii)KOH, TsCl, DIPEA.*

Eight steps were reported for the synthesis of 2,6-disustituted morpholines in a total yield of 19 %. The scope of the reaction was studies by variation of the substitution at the C2 position and across the double bond.

1.3.2.3 Short one-pot morpholine derivative synthetic routes

One pot synthetic approaches of morpholine derivatives are among several recent advances in the synthesis of morpholines.[29-36] A one-pot synthetic route for the preparation of enantiomerically pure 2-(hydroxymethyl)-morpholines is illustrated in Scheme 1.2.[47] Condensation of commercially available (*S*)-epichlorohydrin [(*S*)-**19**, 97% *ee*] with a selection of chiral *β*-amino alcohols **18** delivered the target molecules **20** in moderate yields of 57–62%. This is achieved through the formation of a chloro alcohol intermediate that will be treated with a base to induce the formation of an epoxide. Final ring closure of the intermediate occur in a 6-*exo-tet* fashion (attack of the OH group at the inner position of the epoxide) to give the morpholine derivative **20**. However, oxazepine **21** was formed through a 7-*endo-tet* ring closure in 18% isolated yield.



Scheme 1.2: *Enantioselective preparation of the morpholines **20** from(S)-**19** and a selection of β-amino alcohols **6**. i) (S)-**19**, $LiClO_4$, Toluene, 14 to 48 h, 50 °C; ii) NaOMe, MeOH, 17-48 h.*

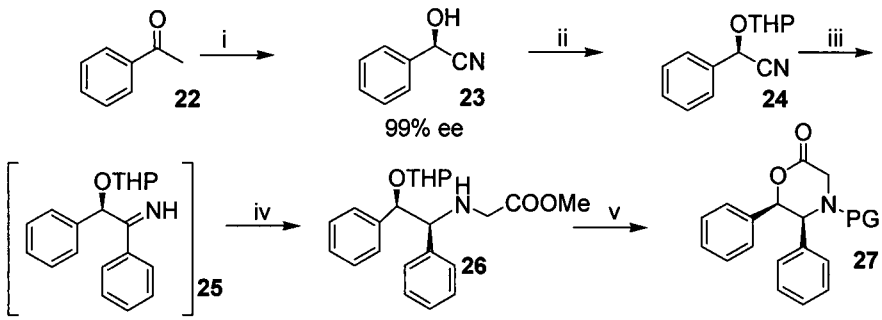
The scope of this method is limited to the synthesis of 2,5-disubtituted morpholines. Substitution at position 2 is restricted to methylalcohols, whereas position 5 was varied from Me to n-But and i-Pr.

1.3.3 Morpholines as synthetic tools

Other than the biological significance of morpholine derivatives, their comprehensive applications in organic synthesis were explored. Morpholines were used as chiral scaffolds for the synthesis of diversely poly-substituted enantiopure amino alcohols and amino acids.

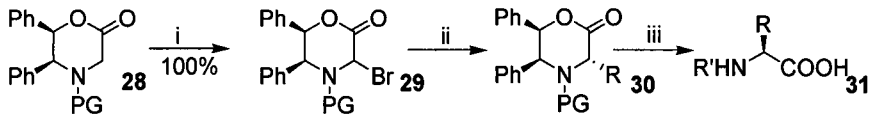
Exhaustive work has been done on orienting morpholines' chirality into chiral auxiliaries. This work is best demonstrated by the development of the William chiral auxiliary, that was first developed by Williams and co-worker, and has been widely used as an organic auxiliary ever since.[48] Different synthetic approaches were conducted for the synthesis of the auxiliaries. An efficient synthetic approach[49] yielding 99% *ee* of the Williams template in 48% total yield is outlined in Scheme 1.3. The synthetic route starts from benzaldehyde, treatment with hydrogen cyanide and an *oxynitrilase* isolated from almonds affords (*R*)-mandelonitrile (**23**) in 99% *ee*. This was followed by the protection of the

hydroxyl group giving the tetrahydropyranyl ether **24**. Precursor **24** was subsequently reacted with phenylmagnesium bromide giving intermediate **25** that reacted with glycine methyl ester, resulting in transamination, and stereoselective reduction of the imine. Finally protection of the amine, followed by ring closure yielded the chiral auxiliary without any purification throughout the synthesis. Furthermore, inverting the configuration of the stereocenter of mandelonitrile **23** would give access to the opposite enantiomer.



Scheme 1.3: *Synthetic scheme of an enantiopure Williams template.* i) *HCN, oxynitrilase; ii) p-TsOH, dihydropyran; iii) 1.PhMgBr 2.MeOH; iv) 1.glycine methylester 2. NaBH4; v) 1. N-protection 2.p-TsOH, cyclohexane*

Williams chiral auxiliaries were reported in the literature for their wide applications as enantiopure starting material for various reactions. Synthesis of natural and non-proteinogenic amino acids were achieved via the use of these auxiliaries. An example is given in Scheme 1.4 for the synthesis of amino acids through alpha bromination of the auxiliary and subsequent displacement of the bromine with various nucleophiles.[50] On the contrary, the auxiliary can be used as the nucleophile, via the formation of the enolate form. This approach was reported by several studies.[51]



Scheme 1.4: *Synthesis of enantiopure amino acids via monoalkylation of C2 position of the Williams chiral auxiliary. i) NBS, CCl_4; ii) RMgBr; iii) H_2, $PdCl_2$, EtOH, RT.*

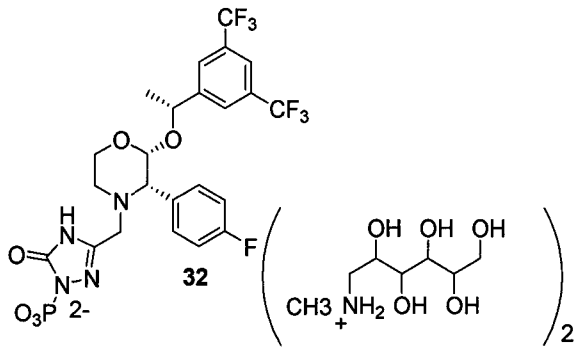
Moreover, other morpholine derivatives were used as chiral auxiliaries for the synthesis of enantiopure amino acids, pyridazines and other diverse products.[52]

In addition to their use as chiral auxiliaries, morpholines were used as protecting groups in total synthesis protocol. This was achieved by Myers and co-workers.[53] A morpholine-based methodology was developed to protect aldehyde moiety of α-amino aldehydes. The method was then applied to the total synthesis of the antitiumor agent (–)-saframycin.[54] Elimination of the morpholine protecting group was achieved by treating the precursor with trimethylsilylcyanide in the presence of a Lewis acid giving the desired product in 86% yield.

1.3.4 Morpholine based marketed drugs

This subsection illustrates two examples of the exploitation of morpholine moiety in the pharmaceutical industry. Merck and Pfizer developed the human NK-1 receptor antagonist (**32**) and Reboxetine respectively; two morpholine-based pharmacologically active compounds.

Morpholine-based hNK-1 receptor antagonist **32** [55] shown in Figure 1.11, is a non-peptidic antagonist of the human neurokinin-1 (hNK-1) receptor. Intensive search for this antagonist was induced by the central and peripheral actions of mammalian tachykinin Substance P (SP).[56] High levels of SP is usually associated with inflammatory conditions including rheumatoid arthritis, asthma, inflammatory bowel disease as well as mediation of the emetic reflex and modulation of central nervous system (CNS) disorders such as Parkinson's disease.



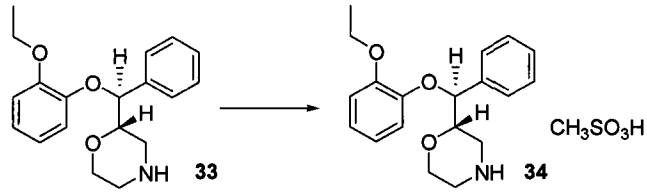
32

Figure 1.11: *Human NK-1 receptor antagonist.*

Morpholine-based hNK-1 receptor antagonist **32** had proven its potency *in-vitro* by binding to hNK-1 receptor antagonizing the effect of SP by binding to the receptors in a noncompetitive or pseudo-irreversible manner. Obtained results led to the conclusion that the morpholine-based receptor antagonist is a potential clinical candidate for treatment of pain, migraine,[57] and chemotherapy-induced emesis.[58]

Reboxetine (**33**) represents another morpholine-based inhibitor (Scheme 1.5). The racemic mixture of (2*R*,3*R*)- and (2*S*,3*S*)-2-[*R*-(2-ethoxyphenoxy)

phenylmethyl]- morpholine was proven to be a potent selective norepinephrine reuptake inhibitor (NRI).[59] Commercially sold as an antidepressant, reboxetine mesylate (**34**) is currently approved in over 60 countries as an antidepressant, and is marketed under the trade names Edronax, Norebox, Prolift, Vestra, and Integrex in Europe and Latin America.



Scheme 1.5: *Reboxetine 33, an active antidepressant drug, marketed in the mesylated form 34.*

3-Aryloxypropylamine scaffolds generally have demonstrated the potential for high affinity binding to biogenic amine transporters, leading to amine reuptake inhibition. This is an important neuropharmacological approach for the treatment of affective disorders.[60] Reboxetine specifically, has the 3-aryloxypropylamine moiety demonstrated by a constrained morpholine ring. The functionality of the aromatic substitution significantly influences the activity and selectivity of compounds as inhibitors of the serotonin transporter (SERT) and/or the norepinephrine transporter (NET).[61]

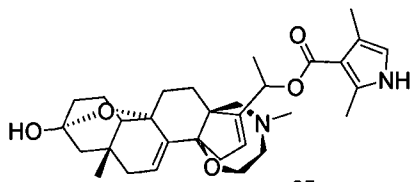
1.4 Synthesis and biological significance of oxazepine derivatives

Unlike morpholine derivatives, oxazepines are not as explored in the literature. This might be due to the synthetic challenges of 7-heterocycles. Oxazepine derivatives are present in nature as interesting natural compounds such

as the neurotoxin batrachotoxin (BTX).[62] Other synthetic oxazepine derivatives have shown biological activities, in particular antifungal activities.[63] Several oxazepines based compounds were patented as antimetics, calcium antagonists, analegestics and prostaglandin E2 antagonists.[64]

1.4.1 Occurrence and biological significance of oxazepine derivatives

Batrachotoxin (BTX Figure 1.12) is an alkaloid isolated from skin extract of the poison dart frog *Phyllobates terribilis*. The compound is composed of a steroid skeleton, dimethylpyrrole carboxylate moiety and an oxazepine ring.[65] BTX is a strong sodium channel activator. It binds to the channel at the receptor site, activating the channel to reach an extreme negative potential, preventing inactivation and promoting permanent channel opening. This allows the passage of larger ions through the pores. Sodium channels that are batrachotoxin-sensitive are expressed in peripheral and central neurons and in both cardiac and striated muscles, where they regulate excitability. The compound is of a high toxicity (lethal dose LD_{50} = 2 ug/kg in mice) leading to convulsions and death in most cases.[66]



35

Figure 1.12: *Batrachotoxin (BTX) is an oxazepine based natural product.*

Sordarin glycoside 36 (Figure 1.13 A) is a natural product discovered in 1971 as a metabolite of *Sordaria araneosa.* It was identified as a potent antifungal

compound.[67] The compound selectively stabilizes the fungal ribosome/ EF-2 complex (the transcription elongation factor EF-2) and blocks translocation. Hence it inhibits fungal protein synthesis.[68]

Sordarin glycoside analogues were explored by replacement of the glycoside with heterocyclic rings such as oxazepines. Different oxazepines derivatives were explored and it was concluded that oxazepine derivatives **37** gives the best balance of antifungal potency across different pathogens. Moreover **37** was found to be more potent than other sordarin analogues.[67]

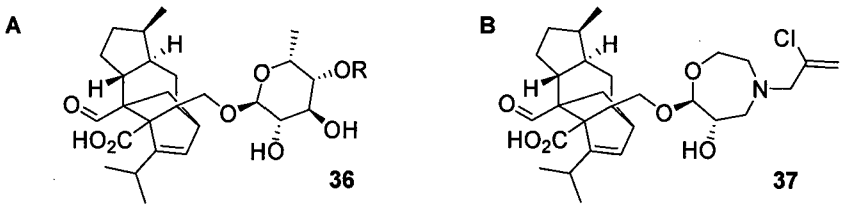
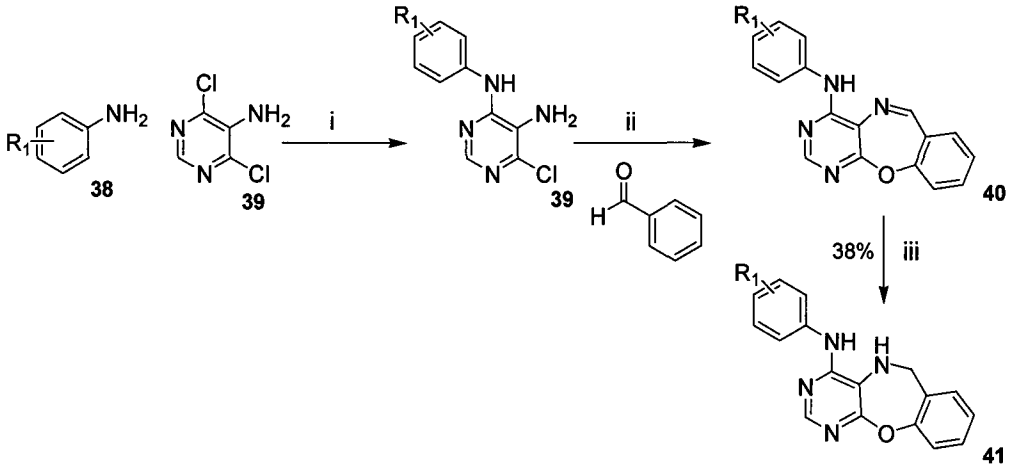


Figure 1.13: *Sordarin glycoside natural antifungal product (**36**). Sordarin oxazepine derivatives as potent antifungal analogue (**37**).*

1.4.2 Synthetic approaches of oxazepine derivatives

Synthetic procedures of chiral poly-substituted oxazepines were poorly explored in the literature. Synthetic approaches were limited to the synthesis of aromatic oxazepine derivatives as shown by the synthetic procedure below (Scheme 1.6).

The synthesis was performed in the purpose of preparing pyrimido-oxazepine analogues. This was achieved by the use of microwaves. Pyrimido-oxazepine analogues were prepared in a one pot, three components system. The final desired product **41** was obtained in 38% yield.[69]

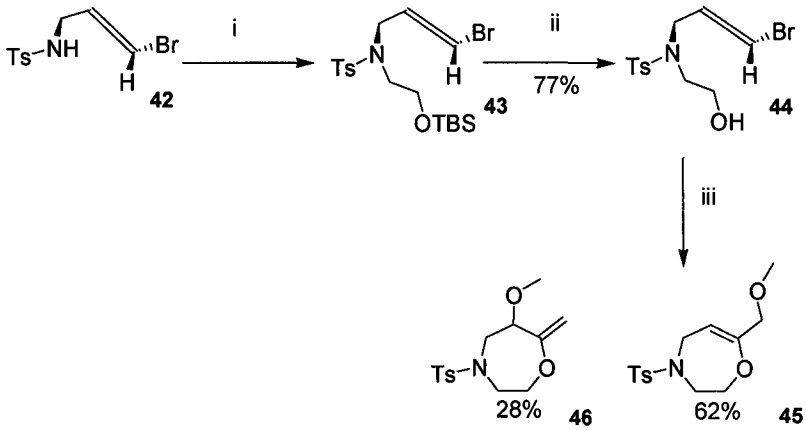


Scheme 1.6: *One pot approach for the preparation of pyrimido-oxazepine analogue.*

i)HCl, iPrOH, 150°C MW ii) benzenaldehyde, $CsCO_3$, iPrOH, 150 °C MW iii) $NaBH_4$, RT, 38%.

One of the few reported regio- and stereoselective synthetic procedures is presented in Scheme 1.7.[70] Oxazepine derivatives were obtained via palladium (0)-catalyzed cyclization of bromoallenes.[71] This reaction can be applied to a wide scope of starting material and regioselectivity can be controlled to afford seven- and eight- membered heterocycles. The cyclization step was catalyzed by palladium (0) and proceeded through intramolecular attack of a nucleophile (oxygen in the case of oxazepine formation).

The starting material was treated with Mitsunobu conditions (Scheme 1.7), to give the intermediate **43**. This was followed by deprotection of the hydroxyl group to facilitate the consequent hydroxyl attack. Cyclization takes place regioselectively to yield the major seven-membered ring **45** in 62% yield, and its regioisomer **46** in 28% yield.



Scheme 1.7:*Multi-step synthetic approach for the preparation of substituted oxazepine analogues. i) $OH(CH_2)_2OTBS$ DEAD, PPh_3, THF; ii)TBAF, THF 0°C; iii)$Pd(PPh_3)_4$ NaOMe, MeOH 6h, RT.*

Three steps were reported for the synthesis of di-substituted oxazepines, in a total yield of 47 %. The scope of the reaction was studied by variation of the substitution at the C4 position and across the double bond.

1.5 Objectives

This chapter presented the role of Cyp A in the life cycle of HIV-1 and the significance of inhibiting the enzyme to reduce the infectivity of the virion. On the other hand, significance and applicability of morpholine and oxazepine based compounds especially their biological implication were discussed.

The objective of this study is to develop efficient synthetic methodology for the preparation of poly-substituted morpholine and oxazepine derivatives. During the methodology development, the application of morpholine and oxazepine derivatives in the preparation of specific Cyp A inhibitors will be studied using computer-aided techniques.

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Chapter 2: Synthesis of poly-substituted chiral morpholines

2.1 Preface

The significance of naturally occurring or synthetic poly-substituted morpholine and oxazepine derivatives was demonstrated in Chapter 1. Studying the literature closely shows that there is a need for efficient synthetic approaches of chiral poly-substituted morpholine and oxazepine derivatives.

Two synthetic routes are presented in this chapter for the synthesis of morpholine and oxazepine derivatives, focusing on the applicability to drug discovery and process chemistry. The first proposed synthetic approach is designed for the synthesis of poly-substituted morpholine derivatives. The second synthetic route was designed to give access to both of these heterocycles through regioselective reactions; morpholine derivatives through 6-*exo* cyclization and oxazepine derivatives through 7-*endo* cyclization.

2.2 Introduction

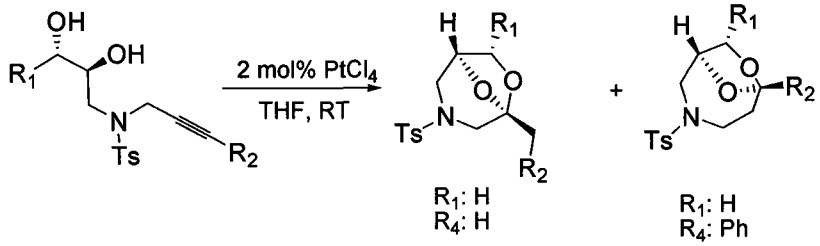
The occurrence and biological significance of morpholine and oxazepine derivatives have been discussed in Chapter 1. Mono and disubstituted morpholine-based compounds are greatly involved in the intensive search for pharmaceutical active compounds. Hence various synthetic procedures were

developed for their preparation. However, the synthesis of chiral *poly-substituted* morpholines has not been much investigated. Most reported synthetic approaches were lengthy, relatively low yielding,[1] limited in applicability and scope[2] or not applicable to large scale synthesis.

On the other hand, although benzoxazepines have been extensively studied, efficient syntheses of poly-substituted oxazepine derivatives have not been reported yet.[3,4]

Earlier this year, Ley and coworkers have reported an efficient method for the preparation of disubstituted morpholine and oxazepine derivatives built around acetal functional groups.[5] The synthesis of fused bicyclic acetals was achieved through intramolecular double alkoxylation of alkyne diols. Cyclization is affected by the substitution of the triple bond. In the case of the terminal alkyne, it would cyclize through a 6-*exo* pathway yielding the [3.2.1] bicyclic product (morpholine-based). And in the case of the aryl alkynes, cyclization would proceed through a 7-*endo* pathway giving the [4.2.1] bicycles (oxazepine-based). The reaction of the alkyne-diol derivatives in a domino cycloisomerization-hydroalkylation fashion is shown in Scheme 2.1.

Although this method was high yielding and regioselective, acetal functional groups are too labile to provide effective and stable drugs.



Scheme 2.1 : *Alkyne-diol derivatives reacting in a domino cycloisomerization-hydroalkylation fashion to give either the [3.2.1] bicyclic products through 6-exo-cyclization or [4.2.1] bicycles through 7-endo- cyclization.*

In the present work two synthetic strategies were designed for the synthesis of chiral poly-substituted morpholine and oxazepine derivatives. Synthetic strategies were designed to be applied to drug discovery.

Drug discovery is a multidisciplinary field that integrates biology, biochemistry, organic chemistry, medicinal chemistry, pharmacology and much more all together. Organic chemists are involved in many aspect of this research. First, they have to prepare potential hits, then analogues of these hits. Various factors must be considered when developing a novel methodology. Among these factors is the applicability of the approach to large scale synthesis. This implies efficiency, not only in yields but also isolation (i.e., multiple chromatography purification), reasonably priced starting material and free of harmful metals and reagents (chlorinated solvents are not likely to be used in large scale synthesis). At the lead optimization or hit generation stages, diversity in the synthesis of families of analogues is desirable. Thus, applicability of the synthetic methodology to a wide range of chemicals is also required.

Additionally, medicinal chemists contribution to the research is very significant. As soon as a potent enzyme inhibitor is found, the medicinal chemists traditionally modify the active compound structure to increase the specificity towards the targeted enzyme (like Cyp A). One way to achieve this specificity oriented structure-acitivity relationship (SAR) study is by constraining the active compound into its bioactive conformation. Cyclic scaffolds (for instance, morpholines and oxazepines) are instrumental. They restrict the conformational space accessible by the compound to the conformation necessary for binding to the target.

Our objectives are to develop efficient synthetic methodology towards the preparation of morpholine and oxazepine derivatives and their application to the preparation of specific Cyp A inhibitors.

Based on these factors two synthetic approaches were designed and are outlined below. The first synthetic procedure was designed specifically for the synthesis of morpholine derivatives. In contrast, the second synthetic procedure was designed to prepare both morpholines and oxazepines

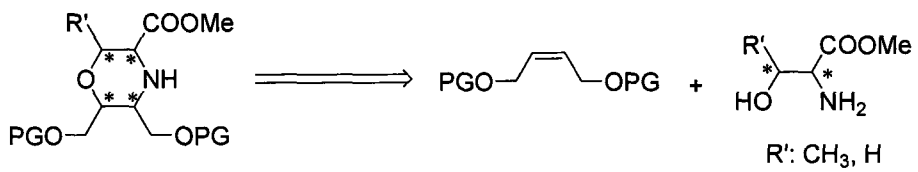
2.2.1 Methodology

2.2.1.1 Synthetic procedure I

The main focus of the study was oriented towards developing a one-pot synthetic procedure for the synthesis of chiral poly-substituted morpholines starting with enantiopure amino alcohols and functionalized olefins. The

retrosynthesis of the proposed one pot approach is presented in scheme 2.2. The synthesis involves the formation of two bonds and two stereogenic centers. This proposed method calls for an electrophilic halogen source that will add onto the olefin, forming halonium ion intermediate. This halonium ion will subsequently be attacked by each one of the nucleophilic (hydroxyl and amine) sites of the amino alcohol forming a morpholine ring.

Derivatization and stereochemistry oriented the choice of the starting material. Readily available enantiopure amino acids were used as amino alcohol sources to induce the stereoselectivity of the system. *O*-protected (*Z*)-but-2-ene-1,4-diols are inexpensive starting materials that were chosen to be the olefin source. Both starting material introduce diversity points in the final product, which enable functionalization of the scaffolds.

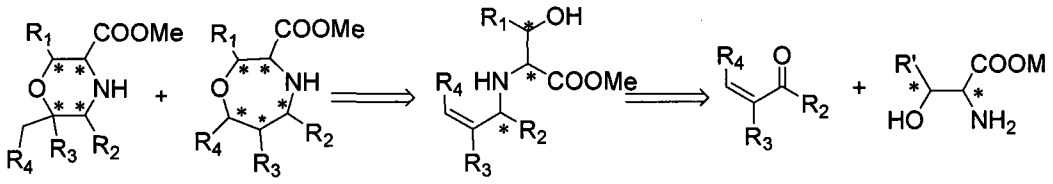


Scheme 2.2: *Proposed synthetic procedure I: One-pot retrosynthesis of poly-substituted chiral morpholines, starting from enantiopure amino acid and (Z)-but-2-ene-1,4-diol.*

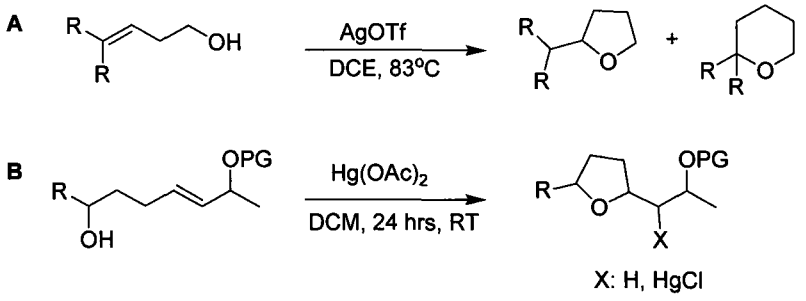
2.2.1.2 Synthetic procedure II

The second synthetic approach presented in this study varies from synthetic procedure I. Starting with an amino alcohol and an α,β-unsaturated

system, the procedure includes two steps; reductive amination of the carbonyl in the α,β-unsaturated system by the amine in the amino alcohol, followed by intramolecular hydroxyl addition to the double bond (Scheme 2.3). Intramolecular hydroxyl addition can be achieved through different metallocatalysis procedures; such as silver(I)triflate,[6] and oxymercuration[7] (Scheme 2.4).



Scheme 2.3: *Proposed synthetic procedure II: Retrosynthesis of poly-substituted chiral morpholines and oxazepines starting with an enantiopure amino acid and an α,β-unsaturated system.*



Scheme 2.4: *Intramolecular hydroxyl addition to olefins using metallocatalysis.*

In this study, intramolecular hydroxyl addition to the double bond will be achieved through haloetherification. Haloetherification is an efficient approach that was chosen to avoid the use of metals in the methodology. Few cases were found where haloetherification was conducted for the synthesis of nitrogen containing heterocycles. One of those studies was conducted by Hsung and co-workers.[8] A

stereoselective haloetherification of chiral enamides procedure was developed for the preparation of halogenated cyclic ethers including one oxazepine. However, chiral auxiliaries were used in the synthetic approach to induce stereoselectivity. Installation and removal of chiral auxiliaries add on synthetic steps, and produce additional waste.

Both introduced synthetic approaches were designed to follow a similar mechanistic principle for the formation of the ether bonds. This step implies that the formation of a halonium ion will undergo a nucleophilic attack intra/intermolecularly, forming morpholine and oxazepine derivatives. However the two approaches differ in bond formation, release of the halogen and the reactions involved in the synthesis. Additionally, both procedures focus on the preparation of poly-substituted morpholines and oxazepines starting from cheap commercially available chiral material. These starting materials and consequently the produced rings feature functional groups, which could be used as diversity points in the preparation of inhibitors.

2.3 Results and Discussion

2.3.1 Method I

A one pot synthetic approach was designed to synthesize morpholine derivatives starting from amino alcohols and functionalized olefins. Both starting materials were expected to introduce diversity points (various functionalities) to

the preparation of halogenated cyclic ethers including one oxazepine. However, chiral auxiliaries were used in the synthetic approach to induce stereoselectivity. Installation and removal of chiral auxiliaries add on synthetic steps, and produce additional waste.

Both introduced synthetic approaches were designed to follow a similar mechanistic principle for the formation of the ether bonds. This step implies that the formation of a halonium ion will undergo a nucleophilic attack intra/intermolecularly, forming morpholine and oxazepine derivatives. However the two approaches differ in bond formation, release of the halogen and the reactions involved in the synthesis. Additionally, both procedures focus on the preparation of poly-substituted morpholines and oxazepines starting from cheap commercially available chiral material. These starting materials and consequently the produced rings feature functional groups, which could be used as diversity points in the preparation of inhibitors.

2.3 Results and Discussion

2.3.1 Method I

A one pot synthetic approach was designed to synthesize morpholine derivatives starting from amino alcohols and functionalized olefins. Both starting materials were expected to introduce diversity points (various functionalities) to the produced rings. Enantiopure amino acids were used as amino alcohol sources, and were expected to induce the necessary stereoselectivity of the reaction.

This first approach is designed to form morpholine derivatives through hydroxyl amination of the double bond in the olefin. This would be facilitated through the use of an electrophilic halogen source that will be added to the olefin through electrophilic addition as shown in Scheme 2.2.

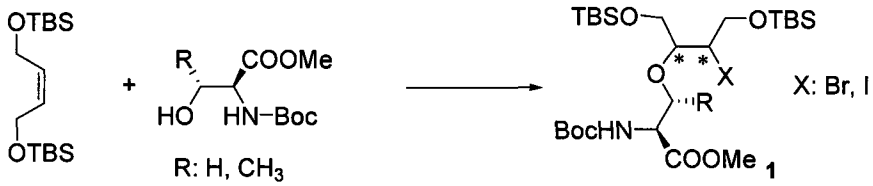
As a test case, we selected L-serine methyl-ester and (*Z*)-1,4-bis(tert-butyldimethylsilyloxy) but-2-ene to react with 2.2 equivalents of *N*-bromosuccinimide (NBS). The reaction was carried out in MeCN in the dark at 0°C-RT. MeCN was chosen as the reaction solvent since it is a commonly used solvent in haloetherification reactions,[9] and to avoid the use of protic solvent (MeOH and HOH) that might participate in side reactions. The reaction was done in the dark to reduce chances of free radical formation. As for the reaction temperature, we started at 0°C and raised it up slowly to RT to ensure that both nucleophiles would attack the intermediate (halonium ion).

The desired product was not obtained after conducting the first trial. No addition to the double bond had taken place, however a side product was obtained. It was observed that TBS ether is partially deprotected in the presence of NBS. Other protecting groups were tested (acetyl-, benzyl- and methoxy groups) however the addition to the double bond did not take place. We believe that carefully optimized reaction time and conditions could probably reduce the silyl ether cleavage.

Additionally, free amines can get brominated in the presence of NBS.[10] To avoid the formation of *N*-brominated side products we have decided to carry out the

reaction with a protected amine. The reaction was carried out sequentially starting with commercially available *N*-Boc L-Ser-OMe and (*Z*)-1,4-bis(tert-butyldimethylsilyloxy)but-2-ene. Reaction conditions were optimized (temperature and additives) and the obtained results are presented in Table 2.1. Different halogen sources were used at different conditions to obtain the desired product **1.** The product was not obtained when NIS and NBS were used (as electrophilic halogen sources) at RT. Addition of DBU to the reaction mixture in the purpose of activating the hydroxyl group did not work either. The product in trace (less than 10%) amounts was obtained upon increasing the temperature of the reaction up to 70 °C. *N*-benzyl-triethylammonium tribromide $[BnEt_3N]^+[Br]^-_3$ is a tribromide source and thus a stronger brominating agent. $[BnEt_3N]^+[Br]^-_3$ reacted to give the desired product in 30% yield, as shown by extensive spectroscopic analysis. The dibrominated form of the olefin was also produced as a major side product of the reaction. We have carried out further optimization of the reaction conditions while using $[BnEt_3N]^+[Br]^-_3$ as the brominating agent. However the yield was not enhanced and the amount of side products was not reduced.

Table 2.1: *Optimization of the reaction conditions of the intermolecular halo-etherification step.*



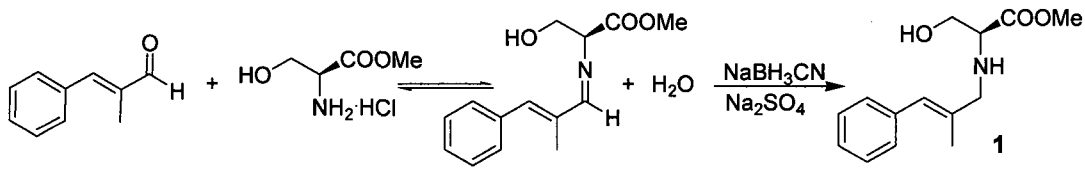
Electrophile	Conditions	Result
NIS	RT, overnight	Starting material
NIS	DBU, RT, overnight,	Starting material
NIS	70°C	Starting material
NBS	RT, overnight	Starting material
NBS	DBU, RT, overnight	Starting material
NBS	70 °C	Traces
$[BnEt_3N]^+[Br]^-_3$	0 °C, 6h	20%
$[BnEt_3N]^+[Br]^-_3$	RT, 2 h	30%
$[BnEt_3N]^+[Br]^-_3$	RT, overnight	30%
$[BnEt_3N]^+[Br]^-_3$	70 °C, 1h	30%

The obtained results demonstrate the uneasiness of intermolecular haloetherification. Even with the use of a strong brominating agent the desired product was obtained in only 30% yield. Therefore we have decided to focus our attention on developing the second synthetic approach.

2.3.2 Method II

2.3.2.1 Reductive Amination

L-serine and L-threonine methyl esters are commercially and naturally available amino acids featuring a hydroxyl side chain group. They were selected to be the first candidates in this study providing di- and tri- substituted morpholine/ oxazepine derivatives respectively upon reacting with an α,β-unsaturated aldehyde (Scheme 2.3). In addition, these are accessible in the D-forms and would potentially provide a wide set of stereoisomeric scaffolds.



Scheme 2.5: *Formation of the imine as part of an equilibrium system, followed by the reduction of the imine to the amine.*

In order to evaluate yields, confirm intermediate structures, and optimize each step, the reaction was first carried out sequentially. Reductive amination was the first step in the synthetic procedure. The reaction was carried out with careful choice of conditions. In fact, it has been shown that reductive amination is often low yielding with aminoalcohols. Our experience shows that this observation can be attributed in part to the complex formation of the aminoalcohol with boron species (reducing agent) making the isolation of the desired amine difficult.

In order to optimize the protocol, we first carefully looked at the mechanism of reductive aminations. The first step is the formation of the imine *in situ* (Scheme

2.5). Imine formation will exist in equilibrium with the starting material. According to Le Chatelier's principle, "*If a chemical system at equilibrium experiences a change in concentration, temperature, volume, or total pressure, then the equilibrium shifts to partially counter-act the imposed change*" (Henry Louis Le Chatelie 1936). In other words, a shift in the equilibrium system favoring the formation of the imine is triggered by changing the concentration of the imine. By that the change will be counter-acted by driving the equilibrium forward producing more the imine. Imine formation is increased by i) removal of water that is formed as a side product upon formation of the imine. And ii) reducing the imine to the amine using a suitable reducing agent.

Reductive amination is a common reaction in modern organic synthesis for the synthesis of secondary and tertiary amines.[11] Many studies were conducted to optimize the conditions (reducing agents, pH, solvents and others) of reactions to optimize the yields obtained. Although this chemical transformation is well established, reductive amination of amino alcohols is known to be challenging and poor yielding.[12] So the conditions were further optimized in this study to suit the use of amino alcohols in reductive amination.

α-Methyl cinnamaldehyde was the first α,β-unsaturated candidate to undergo reductive amination by L-serine methyl ester (Scheme 2.5). α-Methyl cinnamaldehyde was the first α,β-unsaturated chosen due to the substitutions across the double bond. The formed imine has two positions other than the imine that can be reduced; the double bond and the methyl ester. This automatically

excludes the use of two reducing agents; i) Metal catalysts (example: Pd/C for hydrogenation) can not be used due to the presence of the double bond. ii) $NaBH_4$ if activated would result in unwanted reduction of the ester group. Moreover reduction of the aldehyde to the corresponding alcohol is a potential side reaction. We must therefore only use chemoselective mild reducing agents. As a result, we have chosen $NaBH_3CN$ as an imine reducing agent in this study. Water removal would favor the shift of the equilibrium, thus Na_2SO_4, a drying agent, was added to the reaction mixture. Amino acids in the acidic form were used in this study, this is due to the pH effect on the reaction that will be discussed in more details below.

$NaBH_3CN$ is a commonly used reducing agent in reductive amination reactions.[13] The successful use of $NaBH_3CN$ is due to its selectivity in reducing different functionalities at different pH values.[14] Thus, the pH of the reaction mixture has a significant effect on the reaction. At pH of 3-4, $NaBH_3CN$ efficiently reduces aldehydes and ketones to the corresponding alcohols. At neutral pH, reduction of C=O functionality decreases. As for reductive amination, it is optimal at pH 6-8, where imines get reduced faster than aldehydes or ketones.[15] This can be explained in term of the reaction mechanism, species that undergo reduction are the protonated imine (ammonium ions). $NaBH_3CN$ is also stable at low pH values, and is soluble in protic solvents.

Different solvents were evaluated in this study. Reactions in THF, MeOH and DCM were carried out; yields and formation of side products were studied for

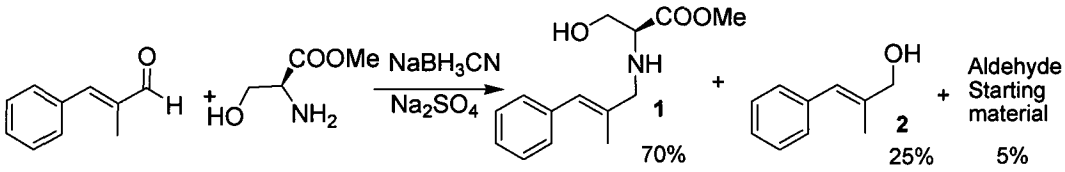
each system. It was observed that reactions in DCM and MeOH give higher yields. The reduced aldehyde was observed in higher amounts when the reaction is carried out in MeOH, [10] since MeOH activates borohydrides. However, amino acids are poorly soluble in DCM and a heterogeneous reaction would be slower (keeping the equilibrated system in mind). Finally it was determined that a 1:1 solvent system of MeOH: DCM increases the yield of the reaction and decreases the side products.

$NaBH_3CN$ is basic and would change the pH of the reaction mixture to higher than 6-8; as a result an acid is needed to lower the pH to 6-8 (optimal pH for reductive amination). In the case of L-serine methyl ester shown in Scheme 2.5, the hydrochloric salt form of the amino alcohol is used, and no additional acidic source is needed. However, in the case of other amino alcohols, acetic acid was added to the reaction.

Reductive amination was applied to explore yields and side products. The aldehyde and Na_2SO_4 were mixed with 1.5 equivalents of L-serine methyl ester hydrochloric salt in MeOH. The reaction was carried out at room temperature, for 2h. This was followed by the addition of $NaBH_3CN$ as a DCM solution. The reaction was left to stir at room temperature for 24h and was finally worked up upon completion.

The crude mixture was examined by ^1H NMR, and was purified by quick filtration on a silica gel pad to study the side products and obtain the yield of product **1**. The product was formed in around 70% yield. An alcohol resulting from the

reduction of the aldehyde was formed as a side product. And 5% of residual unreacted amino alcohol was also observed. Scheme 2.6 presents the outcome of the reaction with the NMR yields for each one of the products

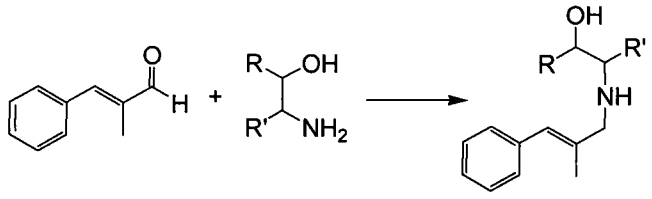


Scheme 2.6: *Reductive amination of α-methyl cinnamaldehyde by L-serine methyl ester.*

The scope of this reaction was investigated by varying both starting materials, to prepare precursors (**1- 17**) with different diversity points for cyclization after the evaluation of this step. First eight chosen commercially available amino alcohols were reacted with α-methyl cinnamaldehyde (Table 2). Second, five different aldehydes were reacted with L-serine methyl ester and L-threonine methyl ester (Table 2.3). The conditions were carefully optimized to provide a reasonable amount of the corresponding secondary amines (50- 74%).

The work-up of the reaction was optimized by Dr. Sylvestre Toumieux to obtain pure intermediates without chromatography. This was achieved through liquid / liquid extraction. This optimization was not applied in this manuscript since it was achieved after completion of my laboratory work.

Table 2.2: *Studying the scope of reductive amination of α-methyl cinnamaldehyde with various amino alcohols.*



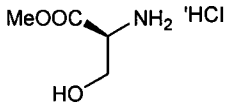
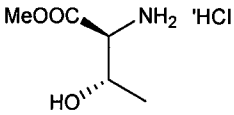
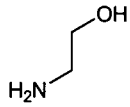
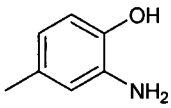
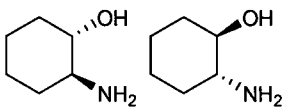
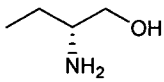
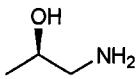
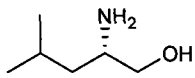
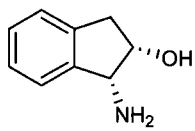
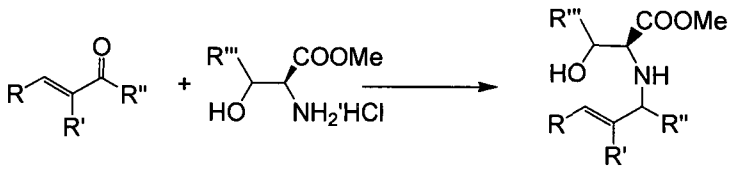
Amino Alcohols		Product	Yield (%)
L-Serine methyl ester		**1**	70
L-Threonine methyl ester		**2**	74
Ethanolamine		**3**	72
p-Methyl amino phenol		**4**	75
Trans- Amino cyclohexanol		**5**	72
R(-)2-amino-1-butanol		**6**	50
R(-)1-amino-2-propanol		**7**	60
S-(+)-Leucinol		**8**	65
(1*R*,2*S*)-(+)-*cis*-1-Amino-2-indanol		**9**	50

Table 2.3: *Studying the scope of reductive amination of various α,β-unsaturated systems with L-serine and threonine methyl ester.*



	Aldehyde		Amino alcohol	Product	Yield
R	R'	R''	R'''		(%)
Ph	Me	H	H	**10**	70
Ph	Me	H	Me	**11**	74
Ph	H	H	H	**12**	55
Ph	H	H	Me	**13**	60
Et	Me	H	H	**14**	30
Et	Me	H	Me	**15**	35
Me	H	H	H	**16**	----
Me	H	Me	H	**17**	----

2.3.2.2 *N*-alkylation and Cyclization of the intermediates

After studying the scope of reductive amination, we had the option of *N*-alkylating the precursors (**1- 17**) or proceeding with cyclization of the free amines.

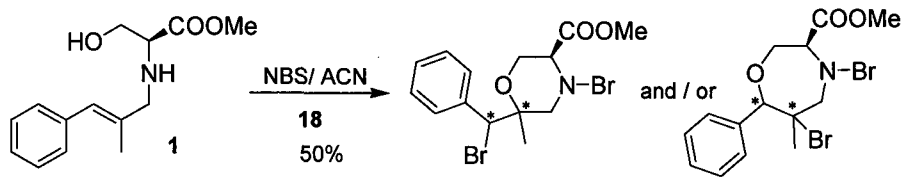
Both reactions were conducted and the obtained results are discussed in this section.

The conditions for cyclization were optimized to develop an efficient, regioselective and stereoselective method. The tri-brominating agent discussed in the first methodology is not suitable in this case, since we are using mostly aromatic systems and it is known to brominate aromatic rings. NBS was used as a brominating agent instead.

Compound **1** was the first compound to be cyclized. Cyclization was carried out by mixing compound **1** in MeCN with 1.2 equivalents of NBS in a darkened system. The reaction mixture was left to stir for 1 hr at 0 ^{o}C under argon. The temperature was then raised to room temperature, and the reaction was left to stir for 5 h (on average with continuous TLC monitoring).

Disappearance of the starting material was observed by examining the ^{1}H NMR spectrum of the crude mixture. It was determines by IR analysis that the product is missing both OH and NH stretching bands found in spectrum of compound **1**, meaning that both groups were converted into other functionalities. Additionally, mass spectroscopy (MS) generally indicates the presence of a bromine atom in the analyzed sample by giving the mass peak as a two peaks with similar intensities corresponding to each one of the bromine isotopes. This was observed when analyzing the sample with ESI-MS (LRMS, M+Na= 427.95), indicating the presence of two bromine atoms in the product. It was concluded that the cyclized product has one extra bromine atom. It was concluded that the obtained product

was potentially *N*-brominated as shown in Scheme 2.7. Further investigations were carried out to prove the formation of an *N*-Br bond.



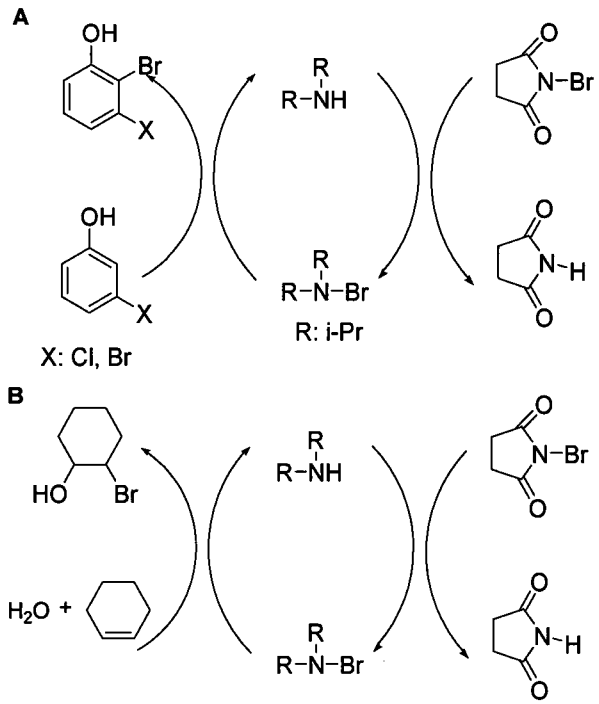
Scheme 2.7: *N-bromination of the amine during haloetherification giving product **18**.*

Before carrying out further investigations to determine the diasteromeric ratio and which regioisomer was obtained, we wished to generate the free amine. An *N*-Br bond needs to be reduced in order to generate morpholine / oxazepine-based scaffolds.

Reduction of *N*-brominated bonds is not a common reaction and is not well covered in the literature. Two approaches were found and were applied in this study. First, Fujisaki and coworkers have reported a procedure for halogenations using *N*-halogenated compounds.[16] Ortho-dibromination of phenols was achieved using NBS and a wide range of primary and secondary amines. The approach is well outlined by Scheme 2.8 A. NBS brominates an amine that will brominate the phenol. We have applied this technique to the obtained *N*-brominated cyclic product; however the reaction did not proceed and starting materials were recovered.

Similarly, Ramesh and co-workers have encountered *N*-halogenation as a result of treating an enaminone with 2.2 equivalents of NXS (X: I, Cl, Br).[17] The *N*-

brominated product was obtained in 5 min and in high yields. The authors suggested that the *N*-Br bond is more stable than NBS, due to its ease of formation and since it was isolated without the release of the bromine. They next cleaved the *N*-Br bond by using the compound as a brominating agent. The halogenated amine was treated with excess cyclohexene and water to afford *trans*-2-bromocyclohexanol as shown in Scheme 2.8 B.



Scheme 2.8: *Processing N-Br bond; A) ortho-directed bromination of phenol. B) hydrobromination of cyclohexene.*

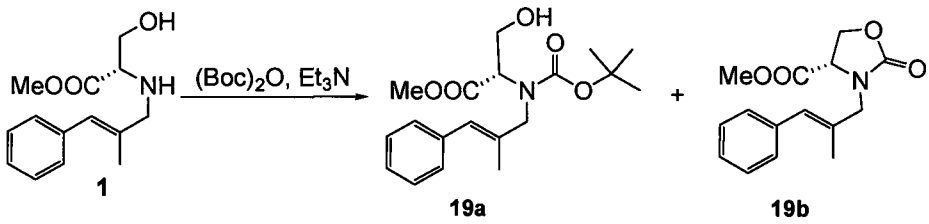
Ramesh's procedure was applied to product **18** (scheme 2.7), which was treated under the same conditions followed by Ramesh and his co-workers. While monitoring the reaction (TLC), it was concluded that compound **18** had converted into another product. ^{1}H NMR spectral shifts did not show a significant difference between the starting material and the product. On the other hand, *trans*-2-

bromocyclohexanol was observed by ^{1}H NMR, which indicated that the reaction was successful. This was further confirmed by analyzing the processed cyclized product by ESI-MS, to give a 1:1 (M+Na) peak at 350.05 m/z. Unfortunately, we were not able to isolate the product by flash chromatography even after scaling-up the product and reproducing the result. Additional attempts of *N*-acetylating the product failed as well. As a result the obtained product was not isolated over silica gel or through liquid / liquid extraction, and we were not able to process the amine, or fully characterize it. Therefore, due to time limitation we had to proceed with *N*-alkylation to be followed by cyclization.

N-alkylation is a necessary step in order to avoid the formation of *N*-Br bond. Different protecting /alkylating groups were used in this study. The choice of alkylating groups was based on chemoselectitvity among two groups prone to alkylation in the precursors; NH and OH. Other factors considered when selecting the alkylating group are: i) Ensure that the protecting group is labile to obtain the free amine after cyclization, and enable *N*-functionalization subsequently. ii) Introduce bulkiness to the intermediate to possibly enhance stereoselectivity in the cyclization step. iii) Ensure that the alkylating group would not react with the reagents (e.g. NBS) in the cyclization step resulting in reduced efficiency and formation of side products.

The first employed protecting group was *tert*-butyl carbamates ($(Boc)_2O$). $(Boc)_2O$ selectively protects amino groups in most cases. *N*-Boc protection of the amino alcohol intermediate **1** as a DCM solution, was carried out by mixing it

with 1.2 equivalents of $(Boc)_2O$ and 1.2 equivalents of Et_3N. Compounds **19a** was obtained in 65% yield, while the corresponding oxazolidinone side product **19b** was isolated in 25% yield (Scheme 2.9).



Scheme 2.9: *N-Boc protection of intermediate **1** giving the desired product **19a** in 65% and the oxazolidinone **19b** as a side product.*

A one pot sequential procedure was followed for the preparation of *N*-Boc intermediates. Starting form L-serine methyl ester and α-methyl cinnamaldehyde, amino reduction was carried out without working up the reaction. Instead the mixture was treated with $(Boc)_2O$ and Et_3N. The isolated yield of the *N*-Boc protected precursor was 50%. It was concluded that the yield obtained by conducting a one pot procedure is higher yielding than separating the two steps. Yields higher than 50% were expected, but this might be due to the borone ($NaBH_3CN$) chelation to the formed amino alcohol intermediate slowing its reaction with $(Boc)_2O$. The reaction was scaled up and the product was reproduced.

Cyclization of **19a** was achieved using the cyclization procedure discussed above in a reproducible manner. The temperature was elevated to 50 °C. Two diastereomers were obtained in a 1:1 diastereomeric ratio (dr). Characterization of

the cyclized product indicated the disappearance of the *N*-Boc protecting group which was unexpected.

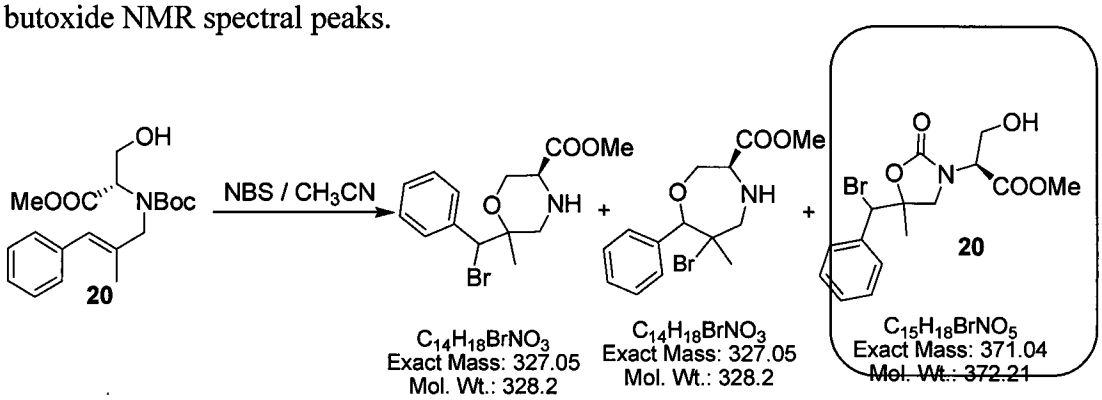
The scope of the reaction was studied to examine if the loss of the Boc protecting group will takes place upon cyclization of other *N*-Boc amino alcohol intermediates. One pot preparation of *N*-Boc intermediates and their subsequent cyclizations are summarized in Table 2.4.

Table 2.4: *Sequential approach of forming morpholines through two different steps; the formation of the intermediate (reductive amination of α-methyl cinnamaldehyde) followed by protecting the secondary amine and subsequent cyclization (haloetherification).*

Amino Alcohol	Yield (%)		
	Precursor	Cyclization	Product
L-serine methyl ester	50	70	**20**
L-threonine methyl ester	50	70	**21**
Ethanol amine	25	80	**22**
p-Methyl amino phenol	77	50	**23**
Trans- Amino cyclohexanol	25	70	**24**

High resolution mass spectroscopy (HRMS) analysis of **20** and **21** showed that the mass of each one of the products is M+CO_2 (M: is the mass corresponding to a morpholine or oxazepine-based products as the expected outcomes of haloetherification). M+CO_2 corresponds to the mass of oxazolidinone derivatives as opposed to the targeted morpholine or oxazepine derivatives. This was further

confirmed by conducting an acetylation/ deacetylation experiment demonstrating the presence of a free hydroxyl and not a free amine. Moreover, IR characterization of **22** revealed an additional C=O stretching band confirming the formation of the oxazolidinone. The obtained results clarified the loss of the *tert*-butoxide NMR spectral peaks.



Scheme 2.10: *Investigating the formation of oxazolidinone through ^1H NMR and MS.*

In fact, Guindon and coworkers have synthesized series of oxazolidinones using a similar protocol, to study the effect of subjecting them to radical-mediated hydrogen-transfer reactions.[18]

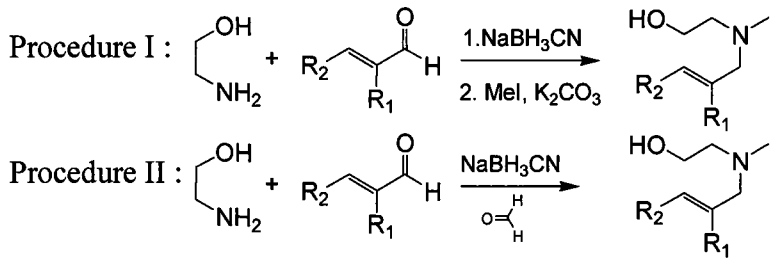
Formation of the oxazolidinone as a side product upon using NBS in the presence of *N*-Boc was reported by Smith and coworkers.[19] They have processed the side product by elimination of HBr under harsh conditions for three days. In our case it would not be feasible to do so. Alternatively we have switched protecting / alkylating groups.

We turned our attention to *N*-alkylation using a simple methyl group, to investigate the success of the cyclization step. The methyl group in this case would not participate in the cyclization reaction as in the previous case. *N*-

methylated precursors were initially prepared in two steps (two steps, one purification); i) Preparation of the free amine precursor **1** (free amine) ii) Reacting the crude mixture of precursor **1** as a solution in DMF with methyl iodide and potassium carbonate. This procedure was applied to serine and threonine methyl esters with different α-β-unsaturated systems and is referred to as *N*-Methylation I.

In the process of optimization, the two steps (formation of compound **3** *in situ* and *N*-alkylation of the crude mixture to yield **31**) were carried out in one pot. This was achieved by conducting two consecutive reductive amination steps; first reductive amination of the aldehdye of the *α,β*-unsaturated system by the primary amino alcohol. Secondly, reductive amination of formaldehyde by the secondary amine intermediate formed *in-situ.* Yields obtained from both procedures are presented in Table 2.5.

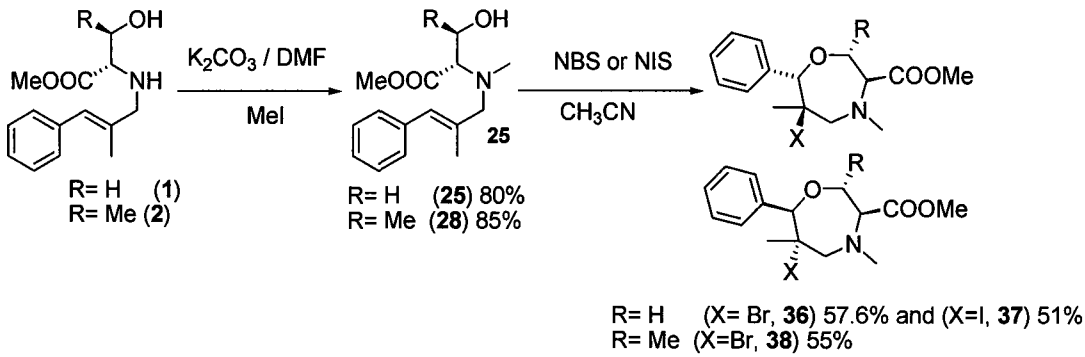
Table 2.5: *Studying the scope of the preparation of N-Me intermediates through two different procedures. I) Sequential formation of the intermediate through independent reductive amination followed by N-Methylation. II) One pot formation of the N-Me intermediates.*



Amino alcohol	R1	R2	Procedure	Product	Yield (%)
L-Serine methyl ester	Me	Ph	I	**25**	80
L-Serine methyl ester	H	Ph	I	**26**	60
L-Serine methyl ester	Me	C_2H_5	I	**27**	30
L-Threonine methyl ester	Me	Ph	I	**28**	85
L-Threonine methyl ester	H	Ph	I	**29**	75
L-Threonine methyl ester	Me	C_2H_5	I	**30**	40
Ethanol amine	Me	Ph	II	**31**	55
Trans-amino cyclohexanol	Me	Ph	II	**32**	40
p-methyl aminophenol	Me	Ph	II	**33**	85
S-(+) leucinol	Me	Ph	II	**34**	77
(1*R*,2*S*)-(+)-*cis*-1-amino-2-indanol	Me	Ph	II	**35**	90

The cyclization of these various tertiary amines was conducted using the same cyclization procedure as for the *N*-Boc precursors. Starting with precursor **25**, cyclization was achieved in 60% yield forming a 1:1 diastereomeric mixture of **36**. The diastereomeric ratio was obtained from the ^{1}H NMR spectrum of the crude mixture. Cyclization of the *N*-Me threonine **28** was carried out under the same

conditions to study the applicability of cyclization step. The cyclized product **38** was obtained in 55% yield formed in a 1:1 diastereomeric mixture. NIS was used to investigate the effect of changing the halogen source while keeping all other conditions constant. This led to a slightly lower yield when cyclizing **25**, but maintaining the same diastereomeric ratio of the product (**37**). The results of the cyclized *N*-Me precursors along with other precursors are summarized in Table 2.8.



Scheme 2.11: *Complete synthetic pathways for the formation of poly-substituted oxazepines **36- 38**. Products were formed via double reductive amination, yielding N-Me precursors that were isolated and cyclized through haloetherification.*

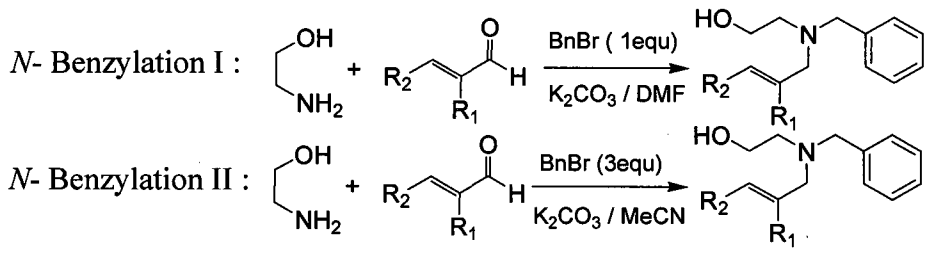
The reaction is designed to be regioselective. Cyclization in a 7-*endo* fashion was expected due to the presence of an aromatic phenyl ring at the β-position of the double bond. The β-position is more positive, the nucleophile is most likely to attack the bromonium ion on the carbon with the most carbocationic character. It was thought that morpholines would be formed as well due to the presence of the α-methyl position, which exhibits some carbocationic character. However, in the cases of compounds **36- 38** only oxazepines (7-*endo* cyclized product) were isolated. Further investigations were carried out to confirm the

formation of the oxazepine exclusively; mechanistic studied were conducted and are covered in section 2.2.2.3.

Other bulkier alkylating/ protecting groups were employed in the purpose of enhancing stereoselectivity. *N*-Benzylation of the amine introduces bulkiness to the system and was expected to induce stereochemistry in the cyclized product. *N*-benzyl group is a more labile group that can be cleaved by hydrogenation. *N*-benzylation was achieved through mixing the crude mixture **1** in DMF with 1.2 equivalents of benzylbromide and potassium carbonate (*N*-Benzylation I). The reaction was refluxed overnight. Product **39** was obtained in 70% isolated yield, ^{1}H NMR spectra indicated 100% conversion to the *N*-Bn precursor. Benzylation had also shown high chemoselectivity in protecting the amine but not the hydroxyl group.

While investigating the scope of *N*-Benzylation, we found that the formation of **44** (Table 2.6) was very challenging. Various approaches were attempted to achieve formation of **44** (such as using different solvent, different bases and even using reductive amination with benzaldehyde). Only one approach successfully afforded the tertiary amine **44**. The approach proceeded by refluxing precursor **3** (Table 2.2) with 2.5 equivalents of benzylbromide and potassium carbonate in MeCN (*N*-Benzylation II). The approach was applied to three other free amine precursors as indicated in Table 2.6. Product **44** is useful since it is a precursor for the formation of a morpholine ring that is patented and can serve us for comparison purposes (section 2.2.2.3).

Table 2.6: *Studying the scope for the preparation of N-Bn precursors through two different procedures (N- benzylations) as outlined in the text.*



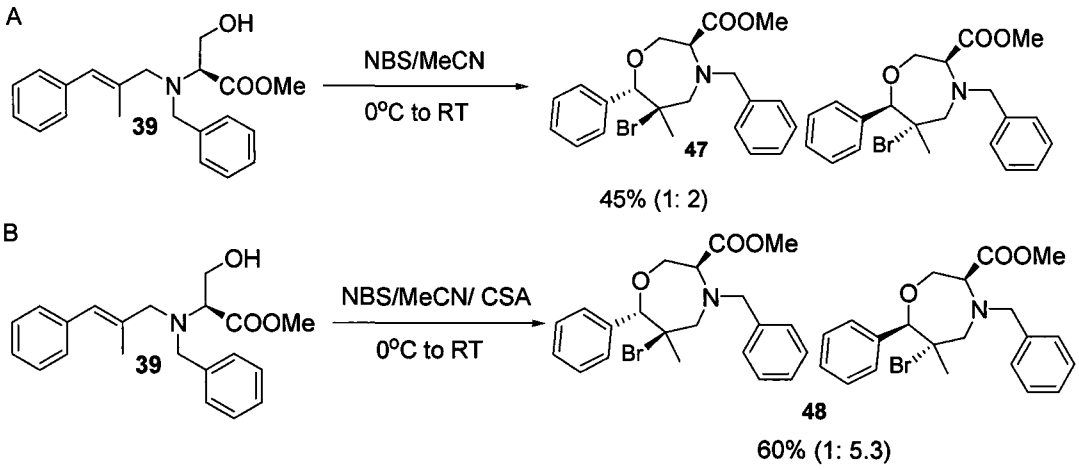
Amino Alcohol	R_1	R_2	Procedure	Product	Yield (%)
L-Serine methyl ester	Me	Ph	I	**39**	75
L-Serine methyl ester	H	Ph	I	**40**	60
L-Threonine methyl ester	Me	Ph	I	**41**	70
L-Threonine methyl ester	Me	Et	I	**42**	50
Ethanol amine	Me	Ph	II	**43**	45
Ethanol amine	H	Ph	II	**44**	30
Trans-amino cyclohexanol	Me	Ph	II	**45**	65
S-(+) leucinol	Me	Ph	II	**46**	80

Using the cyclization conditions discussed earlier, intermediate **39** was cyclized leading to the expected product **47** in 45% yield and as a 1:2.0 diastereomeric mixture. At this stage, obtaining diastereoisomeric selectivity seemed possible. Further optimizations were carried out to enhance the obtained diastereomeric excess (de). Solvents, temperature, alkylating groups and additives were

subsequently optimized. These extensive optimizations were carried out using serine and threonine methyl esters as starting material.

Additives like brønsted acids were used to activate NBS. Racemic camphor sulfonic acid (CSA) was used as an additive in the cyclization of precursor **39**. Stereoselectivity was indeed enhanced from 1: 2.0 dr without CSA to 1: 5.3 with the addition of (±) CSA. The yield was also significantly improved from 45% without CSA to 60% with CSA. As explained below, CSA had a great impact on the cyclization step.

We thought to evaluate the impact of each of the two enantiomers of CSA. In fact, as serine is chiral, one can postulate that one of the two enantiomers of CSA might preferentially interact with it. The increase in stereo-induction could therefore result from the addition of CSA even if in a racemic form. It was concluded from the obtained results that optical purity of CSA does not affect stereoselectivity of the reaction. Moreover, since all four reactions were stopped at the same time, the control (without CSA) reaction had shown only 50% conversion. Meaning that CSA does not only enhance stereoselectivity and the yield of the reaction, it also affects the rate of the reaction.



Scheme 2.12: *Cyclization conditions of precursor* **39***; A) using the established cyclization conditions yielding product 47 in 45% yield and 1:2 dr. B) using the optimized cyclization conditions to yield the product in 60% yield and 1: 5.3 dr.*

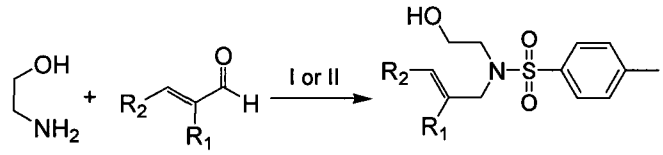
Solvents optimization was attempted by substituting MeCN with CH_2Cl_2 keeping all other conditions constant. The reaction did not 100% conversion in more than 12h at room temperature. Moreover it was observed by ^1H NMR analysis that more side products were formed. Interestingly, the diastereomeric ratio was maintained due to the presence of CSA.

The effect of temperature on the reaction was next evaluated by carrying out the cyclization reaction at 0°C or at elevated temperatures (higher than RT). It was observed that varying the temperature only affects the rate of the reaction, and not the yield, stereoselectivity or regioselectivity.

Final optimization approach was changing the functional group attached to the nitrogen. Some of the free amine precursors were *N*-tosylated to study the effect of *N*-tosylation on stereoselectivity. Various procedures were attempted to synthesize *N*-tosylated precursors. Low yields were obtained, ranging from the

highest of 55 to the lowest of 20%. This is due to the lack of chemoselectivity between the hydroxyl and the amine group. The tosylating hydroxyl group was obtained in most case as the major product. The scope of *N*-tosylation is presented in Table 2.7 using different *N*-tosylation procedures outlined in the experimental section.

Table 2.7: *Studying the scope for the preparation of N-Tosylation of intermediates through two different procedures (outlined in the experimental section).*



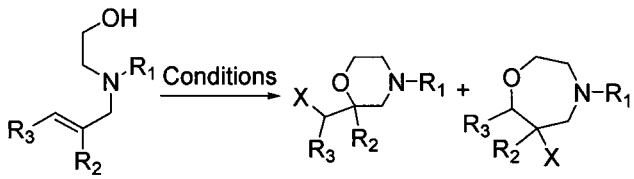
Amino Alcohol	R_1	R_2	Product	Yield (%)
L-Serine methyl ester	Me	Ph	**49**	---
L-Serine methyl ester	H	Ph	**50**	20
L-Threonine methyl ester	Me	Ph	**51**	55
L-Threonine methyl ester	H	Ph	**52**	45
L-Threonine methyl ester	Me	Et	**53**	40
Ethanol amine	H	Ph	**54**	40

Cyclization of the *N*-tosylated precursors was conducted using the optimized conditions. Despite the low yields obtained by preparing *N*-Ts precursors **49- 54**, cyclization of these precursors proceeded with high yields. Moreover, enhanced stereoselectivity was obtained. Crude mixtures have shown

clean conversion of the precursor to the cyclized products, where both the yield and the diastereomeric ratios can be easily improved.

A Summary of the obtained yields, stereoselectivity and regioselectivity of all the cyclized products is presented in Tale 2.8; including the conditions at which cyclizations were carried out.

Table 2.8: *Summary of all the cyclized products (with various N- alkylation/ tosylation groups) including the yields (%) and diastereomeric ratios (dr).*



Amino Alcoh	Conditions	R_1	R_2	R_3	X	Product	Yield (%)		Dr
							6 –*exo*	7- *endo*	
L-Threonine methyl ester	NBS, MeCN, CSA	Ts	H	Ph	Br	**55**	---	80	4:1
L-Threonine methyl ester	NBS, MeCN, CSA	Ts	Me	Et	Br	**56**	40	---	1:1
Ethanol amine	NBS, MeCN, CSA	Ts	H	Ph	Br	**57**	---	60	---
L-Serine methyl ester	NBS, MeCN, CSA	Ts	H	Ph	Br	**58**	---	60	1.5:1
L-Threonine methyl ester	NBS, MeCN	Me	Me	Ph	Br	**38**	---	55	1:1
L-Serine methyl ester	NBS, MeCN	Me	Me	Ph	Br	**36**	---	57.5	1:1

L-Serine methyl ester	NBS, MeCN	Me	Me	Ph	I	**37**	---	51	1:1
L-Serine methyl ester	NBS, MeCN,CSA	Me	Me	Ph	Br	**59**	---	60	1:1
L-Serine methyl ester	NBS, MeCN	Bn	Me	Ph	Br	**60**	---	45	2:1
L-Serine methyl ester	NBS, MeCN,CSA	Bn	Me	Ph	Br	**61**	---	60	5.3:1
L-Threonine methyl ester	NBS, MeCN,CSA	Bn	Me	Ph	Br	**62**	---	10	6:1

Regioselectivity is affected by the substituents across the double bond of the starting material (aldehyde starting material. As for yields and stereoselectivity, they are highly affected by the groups attached to the nitrogen. Higher yields were obtained when cyclizing *N*-Ts precursor (**55 -58**) and higher stereoselectivity was obtained by cyclizing *N*-Bn and *N*-Ts precursors. Finally, stereoselectivity is enhanced by the use enantiopure starting materials and the use of additives (CSA).

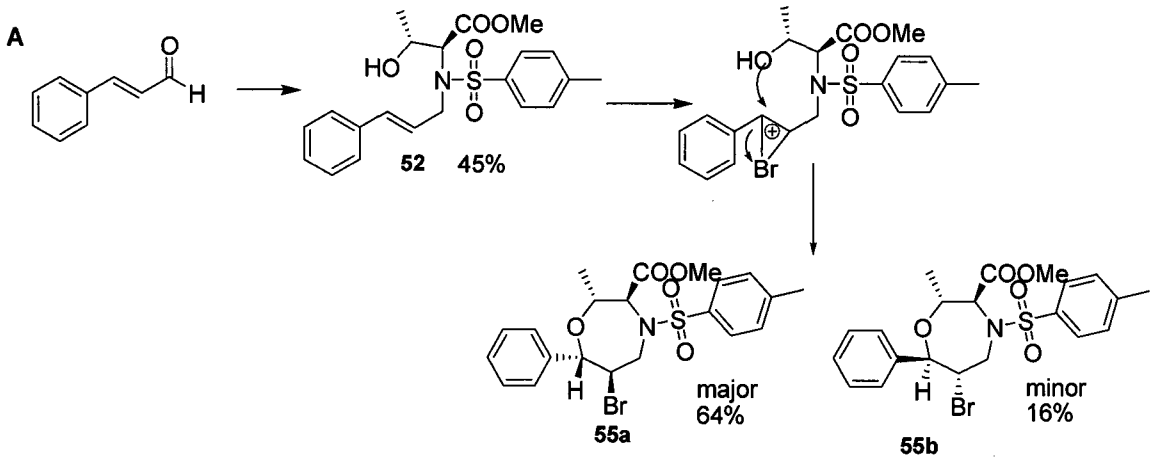
2.3.2.3 Mechanistic and structural investigations

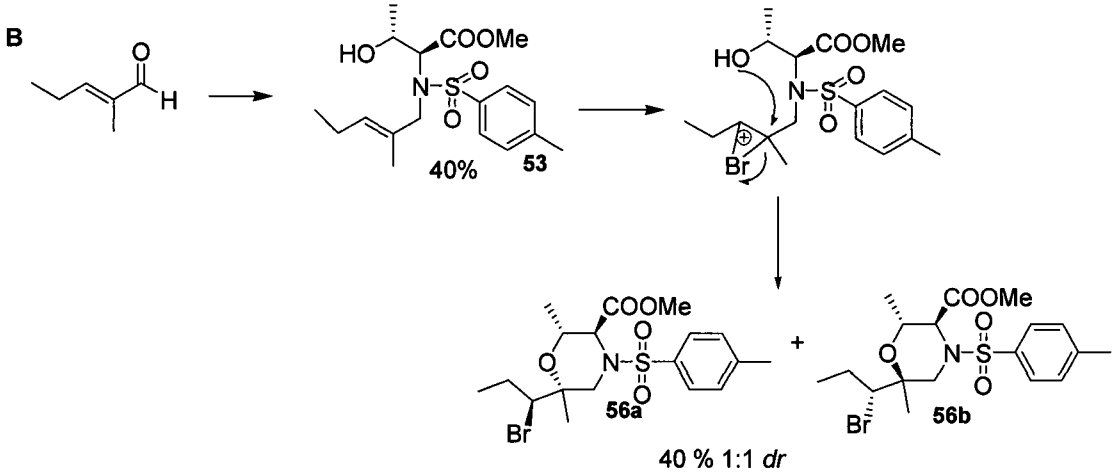
Stereoselectivity and regioselectivity of the system can be explained by the mechanism of the reaction. A bromonium ion is formed as the intermediate in the cyclization step. This cyclic intermediate is formed through electrophilic addition of bromine (Br+) to the olefin. The bromonium ion is subsequently attacked by

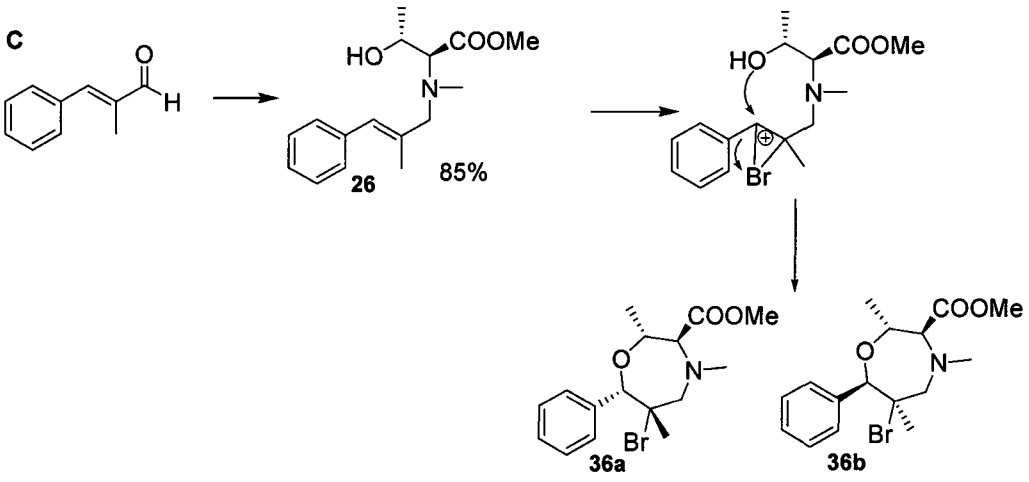
the nucleophile through an S_N2 reaction, which leads to its opening. S_N2 back side attack on the bromonium ion takes place through an anti- stereoselective pathway, and is hence responsible for the formation of the *trans* configuration of the C-Br and the ether bond in the cyclized products.

According to Baldwin rules,[20] 6-*exo-trig* and 7-*endo-trig* are favored cyclizations. The regioselectivity (six and seven membered rings) can be rationalized in terms of the stability of the carbocationic characters of the bromonium ion. In the case of *trans*-cinnamaldehye as the starting material (Scheme 2.13 A), it was expected that haloetherification would take place in a 7-*endo* fashion forming the oxazepine. This is due to presence of the aromatic β-position. When *trans*-α-methyl pentenal is used as a starting material, the nucleophile was expected attack the α- position, cyclization would take place in a 6-*exo* fashion forming a morpholine.

According to expectations, oxazepines were exclusively synthesized starting from *trans*-cinnamaldehyde and morpholines from *trans*-α-methyl pentenal (Scheme 2.13 B). However in the case of *trans*-α-methyl cinnamaldehdye, we have only isolated oxazepines (Scheme 2.13 C). Further investigations were carried out to confirm the identity of the regioisomers, since NMR spectra of both isomers are very similar, even by carrying out 2D NOESY NMR reactions.

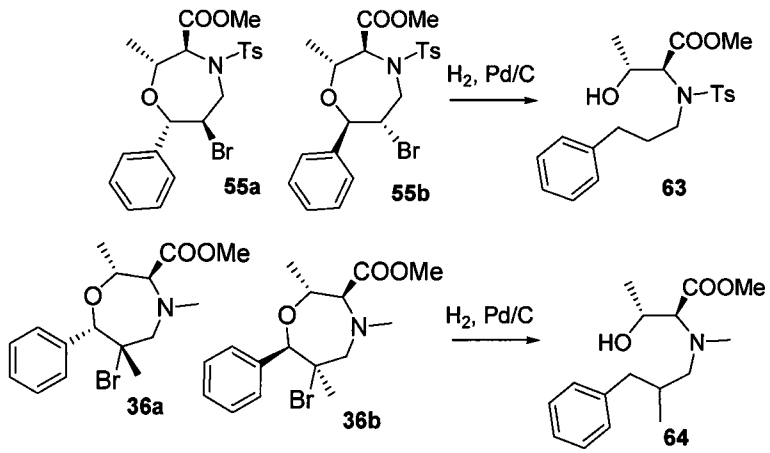

A
COOMe
HO
N
S
O
52 45%
Br
major
64%
55a
minor
16%
55b


B
COOMe
HO
N
S
O
40%
53
Br
56a
56b
40 % 1:1 dr


C
COOMe
HO
N
26
85%
Br
36a
36b
57.6% % 1:1 dr

Scheme 2.13: *Proposed mechanism for the cyclization of precursors 25,52 and 53.*

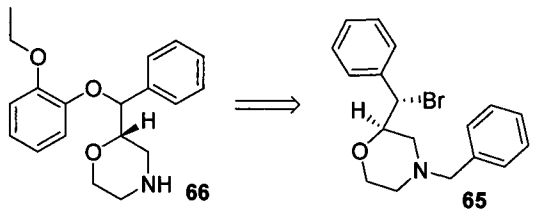
Further investigations were carried out to confirm the formation of oxazepines as major compounds. Through hydrogenation, compounds **55a** and **55b** were completely reduced to form **63**. This confirms the oxazepine's identity. Ether benzylic position in an oxazepine gets cleaved through hydrogenation, followed by elimination of the bromine, forming the double bond as an intermediate the gets hydrogenated in the presence of Pd/C. Complete hydrogenation was also achieved by hydrogenating the diastereomeric mixture **36a** and **36b.**



Scheme 2.14: *Hydrogenation confirms the formation of oxazepine as a result of cyclization*

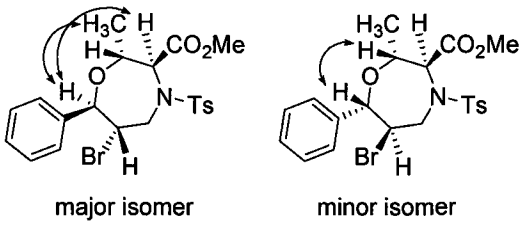
As a second validation of the structure, we used a known structure. Reboxetine is a morpholine derivative that was discussed in chapter 1 as one of the synthetic and commercial morpholine–based drugs. One of the synthetic intermediates ((*R*)-4-benzyl-2-((*S*)-bromo(phenyl)methyl)morpholine) towards Reboxetine shown in Scheme 2.15, served as a reference data. Cyclization **44** was carried out. 1H NMR shift of the crude and the purified product did not match the spectral shifts of the

patented compound **65**. The peaks were shifted significantly upfield in comparison with the spectral shift of the reference compound. This again rules out the formation of morpholine rings.



Scheme 2.15: *Retrosynthesis of Reboxetine from (R)-4-benzyl-2-((S)-bromo(phenyl)methyl)morpholine*

After extensive chromatographic work, the two diasteromers **55a** and **55b** were successfully separated. Stereocenters were assigned with the aid of clear 2D NOESY spectra. NOESY spectra of both diasteroemers are provided in the Experimental.



Scheme 2.16: *Stereochemistry assignment.*

2.4 Conclusion:

A stereo and regioselective method for the preparation of oxazepine derivatives, was developed starting from readily available materials that

introduced diversity points to the final products. Starting from enantiopure amino alcohols and α,β-unsaturated aldehydes, the method was conducted through three steps; reductive amination, *N*-alkylation/ tosylation and cyclization through haloetherification.

Yields and stereoselectivity of the cyclization step are affected by the group attached to the nitrogen atom. For instance, *N*-methylated precursors were cyclized to give the desired products in moderate yields and no diastereoselectivity. When moving to *N*-benzyl groups, the yields remained moderate but some levels of stereoselectivity were observed, ranging from 30 to 83% diastereomeric excess. Finally efficient cyclizations were obtained upon cyclizing *N*-tosylated precursors. Yields ranged from around 40% in the formation of morpholine rings to around 80% for the preparation of oxazepines. Tosyl groups also induce good diastereoselectivities with dr of up to 80% in the synthesis of oxazepines. Therefore it was concluded that higher stereoselectivity is induced by the cyclization of *N*-benzylated and *N*-tosylated precursors with aromatic substitution across the double bond. Moreover, stereoselectivity is enhanced by the use of enantiopure amino alcohols and Brønsted acids.

Regioselectivity of the cyclization reaction was controlled by the chemical nature of the substituents of the double bond to yield morpholines through 6-*exo* cyclization and oxazepines through 7-*endo* cyclization. Regioselectivity was examined by a set of experiments to confirm the identity of obtained region isomer.

2.5 Experimental

2.5.1 General Procedures

^{1}H and ^{13}C NMR spectra were recorded using Varian mercury 400 MHz, 300 MHz or Unity 500 spectrometers. The chemical shifts (δ) were reported in parts per million (ppm) relative to the $CDCl_3$ (7.260 ppm) or CD_3OD (3.31 and 4.84 ppm) solvent peak.

^{1}H and ^{13}C NMR assignments were confirmed by 2D HSQC, HMQC and NOESY experiments, recorded using the 400 MHz and 500 Unity spectrometers.

Mass spectra were obtained from the McGill University mass spectral facilities.

A Perkin Elmer Spectrum One FT-IR spectrometer was used to collect spectra of the samples at resolution of 4 cm^{-1} and 32 scans.

Freshly recrystallized NBS was used in the cyclization step. Dry purchased solvents were used for the preparation of the intermediates and cyclized products. Amino alcohols and aldehydes were purchased from Chem impex & Sigma Aldrich.

Synthetic Procedures

*- Reductive Amination_* ***typical procedure*** *used for the preparation of precursors (1- 17) demonstrated by synthetic procedure of intermediate (1):*

L-serine methyl ester hydrochloride (12.8 mmol, 2000.0 mg) was mixed with 20 mg sodium sulfate dissolved in methanol (5 mL). *Trans*-α-methyl cinnamaldehyde (0.0085 mol, 1 g) was added to the solution and the reaction mixture was left to stir at RT for 2 hrs. Solution of sodium cyano borohydride (0.0085 mol, 0.483 g) in dichloromethane (5 mL) was added drop-wise to the reaction mixture over 5 min. The reaction was left to stir at RT for 24 h (20 - 24). Upon completion (indicated the disappearance by TLC) of the reaction, a concentrated solution of sodium carbonate (10 mL) was added to the reaction mixture. The mixture was washed with 10% HCl solution to adjust the pH to 7. The product was extracted with (3 x 20 mL) chloroform and the combined organic layer was washed with brine and dried over sodium sulfate. Product was concentrated to give oily crude.

- *N-Alkylation I:*

(*E*)-Methyl 3-hydroxy-2-(2-methyl-3-phenylallylamino) propanoate (0.802 mmol, 200 mg) was mixed with methyl iodide (1.204 mmol, 170.0 mg) and K_2CO_3 (1.204 mmol, 221.5 mg) in 1:1 mixture of dimethylformamide : dichloromethane (4 mL) at 65°C overnight. Upon completion (24 h, monitored by TLC), K_2CO_3 was filtered out and the filtrate was concentrated in vacuo. Product was purified by flash chromatography (EtOAc: Hex, 6:4) to provide an oily product.

*- N-Benzylation I (**42**)*

(*E*)-methyl 3-hydroxy-2-(2-methyl-3-phenylallylamino) propanoate (0.802 mmol, 200 mg) was mixed with benzylbromide (1.204 mmol, 221.5 mg) and K_2CO_3 (1.204 mmol, 221.5 mg) in a 1:1 mixture of dimethylformamide : dichloromethane (4 mL) at 65°C overnight. Upon completion, K_2CO_3 was filtered out and the filtrate was concentrated *in vacuo*. Product was purified by flash chromatography (EtOAc: Hex, 8:2).

*- N-Benzylation II (**44**)*

(*E*)-3-(2-Methyl-3-phenylallylamino) butan-2-ol (0.911 mmol, 200 mg) was mixed with benzylbromide (2.277 mmol, 418.85 mg) and K_2CO_3 (2.277 mmol, 316.0 mg) in acetonitrile (4 mL) at 80°C overnight. Upon complete amine protection indicated by TLC, dichloromethane (20 mL) was added to the mixture. K_2CO_3 was filtered out and the filtrate was concentrated in *vacuo*. Product was purified on silica by flash chromatography (EtOAc: Hex, 8:2).

- N_Tosylation procedure I:

(2*S*,3*R*,*E*)-Methyl-3-hydroxy-2-(2-methyl-3-phenylallylamino) butanoate (3.90 mmol, 1000.0 mg) was stirred with toluenesulfonyl chloride (5.90 mmol, 1120.0 mg) in dichloromethane at 0°C, for 0.5 h. This was followed by the addition triethylamine (5.90 mmol, 590.0 mg). The reaction mixture was stirred overnight at 0°C. 10% HCl solution (15 mL) was added to the reaction mixture. The product was extracted with dichloromethane (3 x 15 mL). The combined organic layer was

washed with brine and dried over sodium sulfate. Product was concentrated to give oily crude. The crude mixture was purified by flash chromatography (EtOAc: Hex, 8:2).

*- N_Tosylation procedure II (**54**):*

(*E*)-2-(Cinnamylamino)ethanol (1.40 mmol, 250.0 mg) was stirred with triethylamine (2.90 mmol, 590.0 mg) in dichloromethane at 0°C for 20 min. This was followed by the addition of toluene sulfonyl chloride (1.47 mmol, 280 mg), the mixture was stirred at 0°C for another 20 min and then the temperature was brought to room temperature until completion (20 min, monitored by TLC). 10% HCl solution (10 mL) was added to the reaction mixture. The product was extracted with dichloromethane (3 x 10 mL). The combined organic layer was washed with brine and dried over sodium sulfate. Product was concentrated and purified by flash chromatography (EtOAc: Hex, 8:2).

*- One-pot preparation of N-alkylated intermediate (**31**):*

(*S*)-2-Amino-4-methylpentan-1-ol (1.706 mmol, 200 mg) was 100 mg sodium sulfate mixed with and in methanol (3 mL). *Trans*-α-methyl cinnamaldehyde (1.137 mmol, 182.0 mg) was added and the reaction mixture was left to stir at RT for 2 h. Solution of sodium cyano borohydride (1.137 mmol, 72.0 mg) in dichloromethane (3 mL) was added drop-wise to the reaction mixture over 5 min. This was direct followed by the addition of acetic acid (1.37 mmol, 60.0 mg) drop-wise. The reaction was left to stir at RT for 24 h. Upon completion of the reaction

(indicated by TLC), and formation of the intermediate, formaldehyde (1.706 mmol, 75.0 mg) was added to the reaction mixture at 0^{o}C, followed by the addition of formic acid (1.706 mmol, 78.5 mg). The reaction mixture was left to stir with continuous TLC monitoring until completion (24 h). Concentrated solution of sodium carbonate (20 mL) was added to the reaction mixture. The mixture was washed with 10% HCl solution to adjust the pH to 7. The product was extracted with chloroform (3 x 10 mL) and the combined organic layer was washed with brine and dried over sodium sulfate. Product was concentrated to give an oily crude. Product was purified by flash chromatography (gradient eluent system EtoAc: Hex starting from 6:4 and ending at 10:0).

- *Haloetherification: Typical cyclization procedure of N-alkylated/ protected intermediates; demonstrated by the cyclization of (2S,3R,E)-methyl 3-hydroxy-2-(4-methyl-N-(2-methyl-3-phenylallyl)phenylsulfonamido)butanoate (**36**):*

Precursor **25** (0.35 mmol, 115 mg) was dissolved in acetonitrile (5 mL) under argon gas at 0^{0}C. *N*-bromosuccinamide (0.525 mmol, 92.4 mg) and racemic 20% 10-camphor sulfonic acid (0.007 mmol, 16.26 mg) were added to the reaction mixture. The reaction mixture was left to stir for 1 h at 0^{0}C under argon. It was then brought up to RT and was left to stir for 5 h (on average) to reach completion with continuous TLC monitoring. The reaction was quenched with 10% sodium sulfite (4.0 mL), and the product was extracted with chloroform (3 x 8 mL). The organic layer was washed with brine, dried over sodium sulfate and concentrated to give oily crude.

2.5.2 Experimental

***N*-Cinnamyl-*N*-(2-hydroxyethyl)-4-methylbenzenesulfonamide (54)**: colorless oily material, isolated yield 40%. R_f=0.45 (5:5, EtOAc: Hex). IR (neat,cm^{-1}) ν 3516.3, 3029.8, 2930.65, 133.16, 1154.9, 908.55; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H), 3.28 (t, *J*=5.8 Hz, 2H), 3.74 (t, *J*=4.23, 2H), 4.00 (d, *J*=6.65 Hz, 2H), 5.96-6.00 (m, 1H), 6.43 (d, *J*=15,50 Hz, 1H), 7.24-7.32 (m, 8H), 7.73 (d, *J*=8.2, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 21.19, 49.40, 51.39, 60.85, 123.42, 126.10, 126.98, 127.73, 128.26, 129.43, 129.48, 133.88, 143.32; HRMS (M+Na) for $C_{18}H_{21}NO_3SNa$, calculated: 354.1146, found: 354.1132.

(2*S*,3*S*,*E*)-Methyl-2-(benzyl(2-methyl-3-phenylallyl)amino)-3-hydroxybutanoate (41): colorless oily product, ^{1}H NMR yield 100% of starting material, isolated yield is 70%; R_f=0.55 (8:2, Hex: EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14 (d, *J*=6.0 Hz, 3H), 1.85 (s, 3H), 3.05 (d, *J*=13.0 Hz, 1H), 3.17 (d, *J*=9.7, 1H), 336 (d, *J*=3.4Hz, 1H), 3.50 (m, 2H), 3.82 (s, 3H), 4.05 (m, 2H), 6.53 (s, 1H), 7.22-7.34 (m, 13H) ; ^{13}C NMR (400 MHz, $CDCl_3$) δ 16.96, 19.51, 51.57, 55.23, 60.51, 63.44, 67.68, 126.83, 127.77, 128.41, 128.87, 129.14, 129.53, 129.90, 134.95, 138.46, 171.00; HRMS (M+H) for $C_{22}H_{28}NO_3$, calculated: 354.2069, found: 354.2061.

(*S*,*E*)-Methyl-2-(benzyl(2-methyl-3-phenylallyl)amino)-3-hydroxypropanoate (39): colorless oily product, ^{1}H NMR yield 100% of SM, isolated yield is 75%. R_f=0.40 (8:2, Hex: EtOAc). IR (neat,cm^{-1}) *v* 738.78, 1027.8, 1729.36, 2004.82, 2848.45, 3447.91; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.85 (s, 3H), 2.49 (br, 1H), 3.26-3. 41 (dd, *J*= 13.2, 32.26 Hz, 2H), 3.63-3.68 (m, 2H), 3.74-3.81 (m+s, 5H), 3.94 (d, *J*= 13.4 Hz, 1H), 6.51 (s, 1H), 7.22-7.34 (m +solvent, 14H) ; ^{13}C NMR (300 MHz, $CDCl_3$) δ 16.46, 51.2, 54,73, 59.23, 59.98, 61.67, 126.31, 127.23, 127.95, 128.39, 128.69, 128.86, 128.89, 135.11, 137.37, 138.60, 171.62 ; HRMS (M+Na) for $C_{21}H_{26}NO_3$, calculated: 340.1913, found: 340.1903.

(2*S*,3*R*,*E*)-Methyl-3-hydroxy-2-(4-methyl-*N*-(2-methyl-3-phenylallyl) phenylsulfonamido) butanoate (51): colorless oily product, isolated yield is 55%; R_f=0.5 (6:4, Hex: EtOAc); IR (neat,cm^{-1}) ν 700.80, 1158.63, 1336.05, 1739.95, 1993,91, 2952.68, 3524.98; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (d, *J*=5.6 Hz, 3H), 1.80 (s, 3H), 2.42 (s, 3H), 3.48 (s, 3H), 4.22 (br, 2H), 4.31 (m, 2H), 6.59 (s, 1H), 7.18-7-31 (m, 10H), 7.75 (d, *J*=8.1 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 15.58, 19.95, 21.14, 51.56, 55.08, 64.76, 65.87, 127.73, 128.44, 128.992, 133.58, 136.58, 143.29, 169.31; HRMS (M+Na) for $C_{21}H_{27}NO_5SNa$, calculated: 440.1508, found: 440.1506.

(2*S*,3*R*,*E*)-Methyl-2-(*N*-cinnamyl-4-methylphenylsulfonamido)-3-hydroxybutanoate (52): colorless oily product, isolated yield is 45%; R_f =0.45 (7:3, Hex: EtOAc); IR (neat,cm^{-1}) ν 751.65, 1100, 1335.93, 1737.45, 2952.66, 3525.35; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (s, 3H), 2.49 (s, 3H), 3.53 (s, 3H), 4.17-5.0 (dd, *J*= 7.38, 8.754 Hz, 1H), 4.22 (dd, *J*= 7.38, 8.754 Hz, 1H), 4.23-4.31

(m, 1H), 4.51 (d, *J*=6.0 Hz, 1H), 6.18-6.27 (m, 1H), 6.43 (d, *J*= 15,50 Hz, 1H), 7.24-7.32 (m, 8H), 7.73 (d, *J*=8.2, 2H) ; ^{13}C NMR (300 MHz, $CDCl_3$) δ 20.09, 21.86, 49.22, 52.45, 64.81, 64.84, 67.20, 133.80, 136.47, 137.11, 143.90, 170.58; HRMS (M+Na) for $C_{21}H_{25}NO_5SNa$, calculated: 426.1351, found: 426.1347.

(2*S*,3*R*,*E*)-Methyl-2-(cinnamyl(methyl) amino)-3-hydroxybutanoate (29): colorless oily product, isolated yield is 75%; R_f= 0.58 (6:4, Hex: EtOAc). IR (neat,cm^{-1}) ν 698.3, 1100.2, 1450.3, 1730.94, 1992.06, 2804, 3442.44 ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, *J*=6.0 Hz, 3H), 1.55 (s, br 1H), 2.35 (s, 3H), 3.04 (d, *J*=9.9Hz, 2H), 3.23 (dd, *J*=7.36, 6.42 Hz. 1H), 3.42 (dd, *J*=7.36, 6.42 Hz. 1H), 3.74 (s, 3H), 3.91-3.97 (m, 1H), 6.12-6.19 (m, 1), 6.51 (d, *J*=15.8 Hz, 1H), 7.20-7.37 (m, 7H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 19.25, 37.80, 51.21, 57.70, 62.91, 71.63, 126.38, 126.53, 127.67, 128.58, 133.32, 170.40.

(2*S*,*3R*,*E*)-Methyl-3-hydroxy-2-(methyl(2-methyl-3-phenylallyl) amino) butanoate (28): colorless oily product, isolated yield 85%; R_f= 0.64 (6:4, Hex: EtOAc). IR (neat,cm^{-1}) ν 698.33, 910.46, 1731.82, 2951.67, 3451.04; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (d, *J*=6.0 Hz, 3H), 1.54 (s, br 1H), 1.90 (s, 3H), 2.78 (s, br 1H), 1.46 – 1.56 (m, 1H), 1.98 (s, 3H), 2.16 (s, 1H), 2.78- 2.88 (m, 1H), 3.02 (d, *J*=12.73 1H), 3.28 – 3.40 (dd, *J*=12.9, 22.2 Hz, 2H), 3.57 (m, 1H), 3.71(m, 1H), 3.75 (s, 3H), 3.80 (m, 1H), 6.47 (s, 1H), 7.20-7.37 (m, 7H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 16.50, 19.28, 37.62, 51.15, 63.07, 64.56, 71.86, 126.51, 128.13, 128.79, 128.85, 134.82, 171.15; HRMS (M+1) for $C_{16}H_{24}NO_3$, calculated: 278.1751, found: 278.1748.

(*S*,*E*)-Methyl-3-hydroxy-2-(methyl(2-methyl-3-phenylallyl)amino)propanoate (34): colorless oily product, isolated yield is 82%; R_f =0.38 (7:3, Hex: EtOAc); IR (neat, cm^{-1}) ν 698.6, 1036.2, 1195.3, 1729.5, 2950.8, 3441.5; ^{1}H NMR (400 MHz, $CDCl_3$):δ 1.90 (s, 3H), 2.36 (s, 3H), 2.78 (s, br 1H), 1.46 – 1.56 (m, 1H), 1.98 (s, 3H), 2.16 (s, 1H), 2.78- 2.88 (m, 1H), 3.02 (d, *J*=12.73 1H), 3.28 – 3.40 (dd, *J*=12.93, 22.15 Hz, 2H), 3.57 (m, 1H), 3.71(m, 1H), 3.75 (s, 3H), 3.80 (m, 1H), 6.47 (s, 1H), 7.20-7.37 (m, 7H); ^{13}C NMR (300 MHz, $CDCl_3$)δ 16.88, 37.57, 51.67, 59.26, 64.60, 66.18, 126.82, 128.48, 128.76, 129.22, 135.65, 137.95, 171.53; ; HRMS (M+1) for $C_{15}H_{22}NO_3$, calculated: 263.1520, found: 264.1590.

(*S*,*E*)-4-Methyl-2-(methyl(2-methyl-3-phenylallyl)amino)pentan-1-ol (25): colorless oily product, isolated yield is 77%, R_f = 0.40 (6:4, Hex: EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88-0.93 (m, 7H), 0.97-01.07 (m, 1H), 1.30-1.40 (m, 1H), 1.46 – 1.56 (m, 1H), 1.98 (s, 3H), 2.16 (s, 1H), 2.78- 2.88 (m, 1H), 3.02 (d, *J*=12.7 1H), 3.19 (d, *J*=12.7 Hz, 1H), 3.25 (t, *J*=10.5 Hz, 1H), 3.51(m, 2H), 6.40 (s, 1H), 7.13-7.31 (m, 8H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 16.742, 22.353, 24.124, 25.573, 33.637, 35.574, 61.269, 61.480, 63.306, 126.579, 128.076, 128.335, 128.397, 129.057, 129.357, 137.959; HRMS (M+1) for $C_{17}H_{28}NO$, calculated: 261.2092, found: 262.2160.

(*E*)-4-Methyl-2-(methyl(2-methyl-3-phenylallyl)amino)phenol (33): brown oily product, 85%; R_f= 0.7 (7:3 , Hex: EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.98 (s, 3H), 2.34 (s, 3H), 2.64 (s, 3H), 3.50 (s, 2H), 6.56 (s, 1H), 6.90 (s, 2H), 7.05 (s, 1H), 7.26 (m, 3H), 7.34 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ. 16.12, 20.51, 416, 66.42, 113.36, 122.00, 126.16, 126.19, 127.80, 127.97, 128.53, 128.84, 134.72, 137.21, 148.90; LRMS (M+H) for $C_{18}H_{21}NO$, calculated: 267.160, found: 268.28.

(2*S*,3*R*,*E*)-methyl-2-(cinnamyl(methyl)amino)-3-hydroxybutanoate (31): brown oily product, 55%; R_f 0.30 (7:3, Hex: EtOAc). IR (neat, cm^{-1}) ν 698.3, 1100.2, 1450.3, 1730.94, 1992.06, 2804, 3442.44 ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, *J*=6.0 Hz, 3H), 1.55 (s, br 1H), 2.35 (s, 3H), 3.04 (d, *J*=9.9Hz, 2H), 3.23 (dd, *J*=7.4, 6.42 Hz. 1H), 3.42 (dd, *J*=7.4, 6.42 Hz. 1H), 3.74 (s, 3H), 3.91-3.97 (m, 1H), 6.12-6.19 (m, 1), 6.51 (d, *J*=15.8 Hz, 1), 7.20-7.37 (m, 7H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 19.25, 37.80, 51.22, 57.71, 62.91, 71.63, 126.38, 126.53, 127.67, 128.58, 133.32, 170.45; HRMS (M+H) for $C_{13}H_{19}N$, calculated: 205.1460, found: 206.1540.

(2*R*,3*R*,*E*)-Methyl-3-hydroxy-2-(methyl(2-methylpent-2-enyl)amino) butanoate (30): colourless oily product, isolated yield is 40%; R_f= 0.85 (5:5, Hex: EtOAc). IR (neat, cm^{-1}) ν 698.3, 1727.94, 1990.06, 2804, 3445.44 ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.93 (t, *J*=7.51 Hz, 3H), 1.16 (d, *J*=5.98 Hz, 3H), 1.62 (s, 3H), 1.99-2.07 (m, 2H), 2.21 (s, 1H), 2.91 (m, 2 H), 3.12 (d, *J*=12.3 Hz), 3.73 (s, 3H), 3.86-3.95 (m, 1H), 5.32 (t, *J*=7.4 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.92, 14.39, 19.12, 20.94, 37.17, 50.92, 62.71, 63.74, 71.35, 76.87, 77.30, 131.26, 170.26; HRMS (M+H) for $C_{12}H_{24}NO_3$, calculated: 229.1678, found: 230.1750.

***Trans*-(*E*)-2-(benzyl(2-methyl-3-phenylallyl)amino)cyclohexanol (45):** colourless oily product, isolated yield is 65%; R_f 0.42 (7.5: 2.5, Hex: EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (m, 4H), 1.85 (s, 3H), 1.93 (s, br, 2H), 2.10-2.18 (s+ m, 3H) 2.40-2.46 (m, 2H), 2.99 (d, *J*=12.8 Hz, 1H), 3.30 (dd, *J*= 5.41, 7.91 Hz, 2H), 3.47- 3.55 (m, 1H), 3.79 (s, 1H), 3.90 (d, *J*=13.5 Hz, 1H) ; ^{13}C NMR (300 MHz, $CDCl_3$) δ 17.2, 22.0, 25.0, 25.9, 33.8, 54.0, 58.9, 64.8, 69.0, 127.28, 127.8, 129.0, 129.1, 129.1, 129.22, 129.8, 130.2, 138.6, 139.3, 141.5; HRMS (M+H) for $C_{23}H_{29}NO$, calculated: 335.2327, found: 336.2325.

(*1R*,*2S*,*E*)-1-(Methyl(2-methyl-3-phenylallyl)amino)-2,3-dihydro-1H-inden-2-ol (35): colourless oily product, isolated yield is 90%; R_f=0.85 (9:1, Hex: EtOAc) ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.93 (s, 3H), 2.04 (s, 3H), 2.78 (dd, *J*= 7.6, 8.84 Hz, 1H), 3.20 -3.30(m, 3H), 4.22 (d, *J*= 8.0 Hz, 1H), 4.42 (q, *J*=8.0 Hz, 1H), 6.43(s, 1H), 7.20-7.30 (m, 11H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 16.19, 38.41, 41.18, 65.80, 67.75, 69.32, 125.23, 125.92, 126.11, 126.19, 127.77, 128.49, 135.41, 137.15, 138.32, 141.29; HRMS (M+H) for $C_{20}H_{24}NO$, calculated: 294.1858, found: 294.1849.

***S,E*)-2-(benzyl(2-methyl-3-phenylallyl)amino)-4-methylpentan-1-ol (46):** colourless oily product, isolated yield is 80%; R_f 0.5 (7.5: 2.5, Hex: EtOAc) ; ^{1}H NMR (400 MHz, CD_3OD): δ 0.78-1.77 (dd, *J*=6.5, 36.3 Hz, 6H), 1.60- 1.24 (m, 1H), 1.47-1.53 (m, 1H), 1.81 (s, 3H), 2.87-2.90 (m, 1H), 3.21 (d, *J*=4.6 Hz, 2H), 3.50- 3.54 (m, 1H), 3.67- 3.77 (m, 3H), 6.43 (s, 1H), 7.20-7.36 (m, 11H); ^{13}C NMR (300 MHz, CD_3OD) δ 17.0, 23.2, 23.58, 26.09 37.83, 54.9, 58.1, 60.11, 62.5, 127.28, 127.8, 129.0, 129.1, 129.1, 129.22, 129.8, 130.2, 138.6, 139.3, 141.5; HRMS (M+H) for $C_{23}H_{32}NO$, calculated: 338.2476, found: 338.2483.

(2*R*,3*S*,6*S*,7*S*)-Methyl-6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate and (2*R*,3*S*,6*R*,7*R*)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate (38): (1:1 mixture) : colorless oil, isolated yield: 57.6%; R_f=0.37 (8:2, Hex: EtOAc); IR (neat, cm^{-1}) *v* 700.80, 1739.95, 2952 ; ^{1}H NMR (400 MHz, $CDCl_3$):δ 1.21 & 1.27 (d, *J*=6.0 Hz, 3H each), 1.74 & 1.81 (s, 1H each), 2.47 & 2.52 (s, 3H each), 3.11 (d, *J*=13.3 Hz, 1H), 3.14 (d, *J*=7.8, 1H), 3.20 (d, *J*= 15,7 Hz, H), 3.29 (d, *J*=9.3 Hz, 1H), 3.48 & 3.64 (2d, *J*=15.5 Hz, 1H), 3.76 & 3.78 (2s, 3H each), 3.99-4.04 (m, 2H), 5.22 &5.8 (2s, 1H each), 7.32-7.56 (m, 11H) ; ^{13}C NMR (300 MHz, $CDCl_3$) δ 19.75, 20.06, 20.07, 28.75, 29.01, 51.78, 61.33, 61.77, 64.29, 64.42, 67.51, 73.95, 74.49, 77.27, 128.18, 128.20, 128.64, 129.06, 129.09, 129.99, 130.12, 137.96, 138.12, 140.77, 170.94; HRMS (M+H) for $C_{16}H_{23}NO_3Br$, calculated: 356.0861, found: 356.0854.

(*2R,3S,6S,7S*)-methyl-6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate and (2*R,3S,6R,7R*)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate (59): colorless oil (1:1 mixture), ^{1}H NMR yield: ~70%, isolated yield: 57.6%; R_f=0.32 (8:2, Hex: EtOAc); IR (neat, cm^{-1}) *v* 700.7, 1044.9, 1425.9, 1732.31, 2952.07; ^{1}H NMR (300 MHz, $CDCl_3$):δ 1.70 &1.77 (2s, 6H), 2.47 &2.51 (2s, 6H), 3.19 (d, *J*=15.2 Hz, 1H), 3.16- 3.50 (dd, *J*=15.4, 25.6 Hz, 2H), 3.16- 3.84 (m+ s, 10H), 3.92 (m, 2H), 5.25, 5.34 (2s, 2H each), 7.30- 7.54 (m, 10H); ^{13}C NMR (300 MHz, $CDCl_3$) δ28.00, 29.24, 38.33, 38.36, 39.11, 51.24, 59.47, 59.78, 60.55, 60.88, 67.07, 67.54, 68.40, 68.57, 72.96, 73.59, 127.53, 128.33, 129.29, 129.38, 137.52, 137.60, 171.22, 171.23; HRMS (M+H) for $C_{15}H_{21}NO_3Br$, calculated: 242.0705, found: 242.17127.

(*2R,3S,6S,7S*)-methyl-6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate and (2*R,3S,6R,7R*)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate (57): colorless oil (1:1 mixture), ^{1}H NMR yield: ~70%, isolated yield: 57.6%; R_f=0.32 (8:2, Hex: EtOAc); IR (neat, cm^{-1}) *v* 700.7, 1044.9, 1425.9, 1732.31, 2952.07; ^{1}H NMR (300 MHz, $CDCl_3$):δ 1.70 &1.77 (2s, 6H), 2.47 &2.51 (2s, 6H), 3.19 (d, *J*=15.2 Hz, 1H), 3.16- 3.50 (dd, *J*=15.4, 25.6 Hz, 2H), 3.16- 3.84 (m+ s, 10H), 3.92 (m, 2H), 5.25, 5.34 (2s, 2H each), 7.30- 7.54 (m, 10H); ^{13}C NMR (300 MHz, $CDCl_3$) δ28.00, 29.24, 38.33, 38.36, 39.11, 51.24, 59.47, 59.78, 60.55, 60.88, 67.07, 67.54, 68.40, 68.57, 72.96, 73.59, 127.53, 128.33, 129.29, 129.38, 137.52, 137.60, 171.22, 171.23; HRMS (M+H) for $C_{15}H_{21}NO_3Br$, calculated: 242.0705, found: 242.17127.

(2*R*,3*S*,6*R*,7*S*)-Methyl-6-bromo-2-methyl-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate (55a): colorless oil, ^{1}HNMR yield: 80%; isolated yield: 60%; R_f=0,64 (7:3, Hex: EtOAc); IR (neat, cm^{-1}) υ 661.9, 1090.70, 1156.57, 1337.69, 1739.81, 2953.37 ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (d, J=6.7 Hz, 3H), 2.45 (s,3H), 3.62 (s,3H) 4.00-4.07 (dd, J=5.3, 10.24 Hz,1H), 4.21-4.24 (dd, J=5.3, 10.24 Hz,1H), 4.29-4.355 (m, 1H), 4.70-4.74 (m, 1H), 4.88 (d, J=8.7 Hz, 1H), 7.31-7.34 (m, 8H), 7.54 (d, J=8.2, 2H) ; ^{13}C NMR (500 MHz, $CDCl_3$) δ 20.73, 21.98, 50.58, 52.67, 65.04, 90.50, 127.59, 128.64, 130.19, 137.83, 139.87, 144.24, 171.30; HRMS (M+Na) for $C_{21}H_{24}NO_5SBrNa$, calculated: 504.0456, found: 504.0450.

(2*R*,3*S*,6*R*,7*S*)-Methyl6-bromo-2-methyl-7-phenyl-4-tosyl-1,4-oxazepane-3-arboxylate (55b): colorless oil, ^{1}H NMR yield: 15%; isolated yield: 15%; R_f= 0.60 (7:3, Hex: EtOAc); IR (neat,cm^{-1}) ν 663.12, 1091.9, 9, 1150.0, 1340.8, 1745.16, 2923.4; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (d, J=6.7 Hz, 3H), 2.46 (s,3H), 3.56 (s,3H), 3.86 (dd, J=11.83 , 3.76 Hz,1H), 4.057 (td, J=3.4, 10.97 Hz,1H), 4.30 (dd, J=3.54, 12.25 Hz, 1H), 4.48 (d, J=9.82, 1H), 7.21-7.34 (m, 8H), 7.54 (d, J=8.2, 2H) ; ^{13}C NMR (300 MHz, $CDCl_3$) δ 17.61, 21.86, 52.46, 52.79, 53.29, 65.76, 73.34, 78.53, 127.11, 128.02, 128.73, 129.82, 136.29, 140.41, 144.18, 169.75; HRMS (M+Na) for $C_{21}H_{24}NO_5SBrNa$, calculated: 504.0456, found: 504.04488.

(3*S*,6*R*,7*S*)-Methyl-6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate & (3*S*,6*S*,7*R*)-Methyl-6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate (58): (Inseparable mixture of diastereomers ~ 1.5:1); colorless oil, ^{1}H NMR yield: 80%; isolated yield: 60%; R_f= 0.55 (7:3, Hex: EtOAc) ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44, 2.46 (2s, 3.0H, 1.9H), 3.63, 370 (2s, 3.0H, 1.53H respectively), 3.92- 4.10 (m, 2H), 4.29-4.36 (m, 4H), 4.46 (d, J=6.9 Hz, 0.6H), 4.51-4.56 (m, 1.2H), 4.60 (d, J=7.8 Hz, 0.65H), 4.69 (s, br 0.6 H) , 4.96 (dd, J=7.1, 3.6 Hz, 1H), 7.31-7.34 (m, 16H), 7.76 (d, J=8.2, 4H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 21.16, 49.22, 49.78, 50.38, 52.07, 53.26, 58.752, 60.55, 69.37, 70.33, 88.50, 90.13, 126.88, 127.91, 129.39, 136.76, 138.33, 139.31, 143.53, 169.36; LRMS (M+Na) for $C_{20}H_{24}NO_5SBrNa$, calculated: 490.030, found: 490.060.

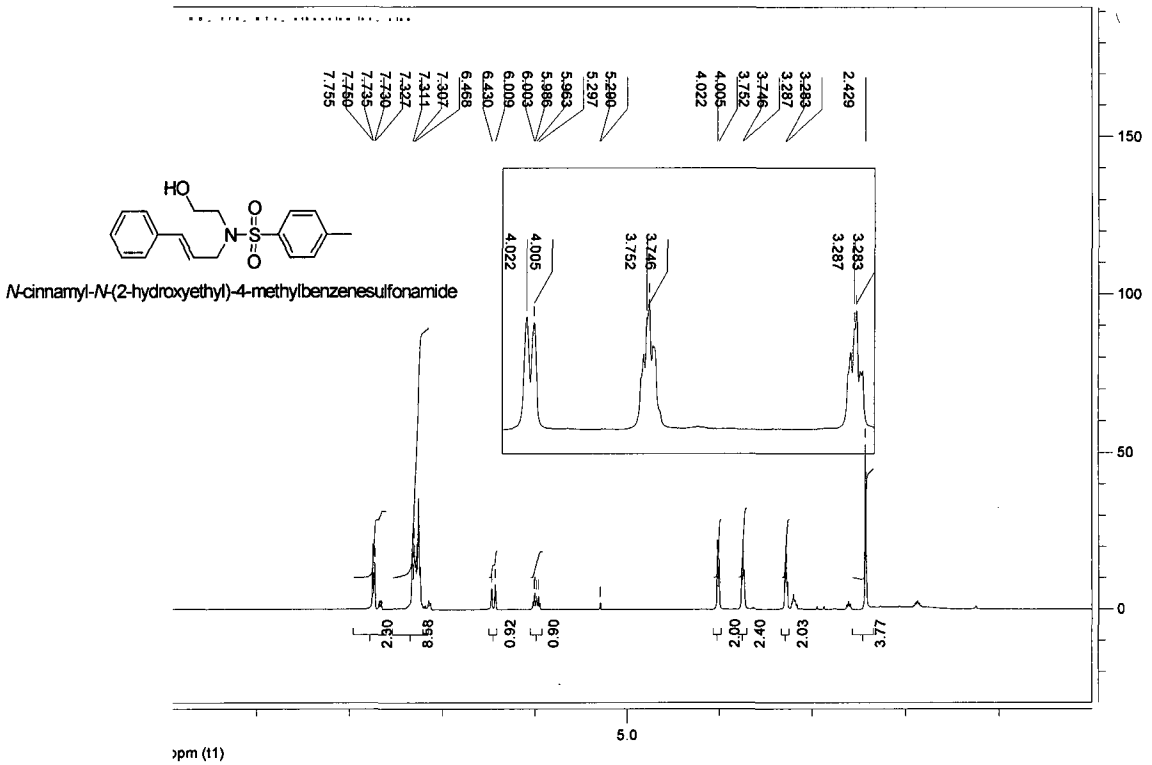
(*2R,3S,6S*)-Methyl-6-((*S*)-1-bromopropyl)-2,6-dimethyl-4-tosylmorpholine-3-carboxylate & (*2R,3S,6R*)-Methyl 6-((*R*)-1-bromopropyl)-2,6-dimethyl-4-tosylmorpholine-3-carboxylate: (inseparable mixture of diastereomers ~ 1.25 : 1) (56a and b); colorless oil, isolated yield: 40%; R_f= 0.59 (6:4, Hex: EtOAc); IR (neat, cm^{-1}) v 666.63, 1159.42, 1246.86, 1743.08, 2975.01; ^{1}H NMR (500 MHz, $CDCl_3$):δ 0.96 and 1.07(2t, J=7.1 Hz, ~3H each), 1.12 and 1.7 (2d, J=6.1 Hz, ~3H each), 1.24and 1.37 (2s, 3H each), 1.48-1.63 (m, 2H), 2.00-2.13 (m, 2H), 2.45(s, 5H), 2.77(d, J=12.7 Hz, 1H), 3.10 (d, J=13.3 Hz, 1H), 3.24 (d, J=9.3 Hz, 1H), 3.46 (d, J=13.0 Hz, 1H), 3.58 (d, J=9.1 Hz, 1H), 3.60 (s, 2H), 3.65 (d, J=13.2 Hz, 1H), 3.80 (m, 0.74 H), 3.92 (m, 1H), 4.12 (d, J=15.7, 1H), 7.31-7.34 (m, 4H), 7.68-7.76(m, 4H); ^{13}C NMR (500 MHz, $CDCl_3$)δ 13.126, 15.602, 18.742, 19.027,

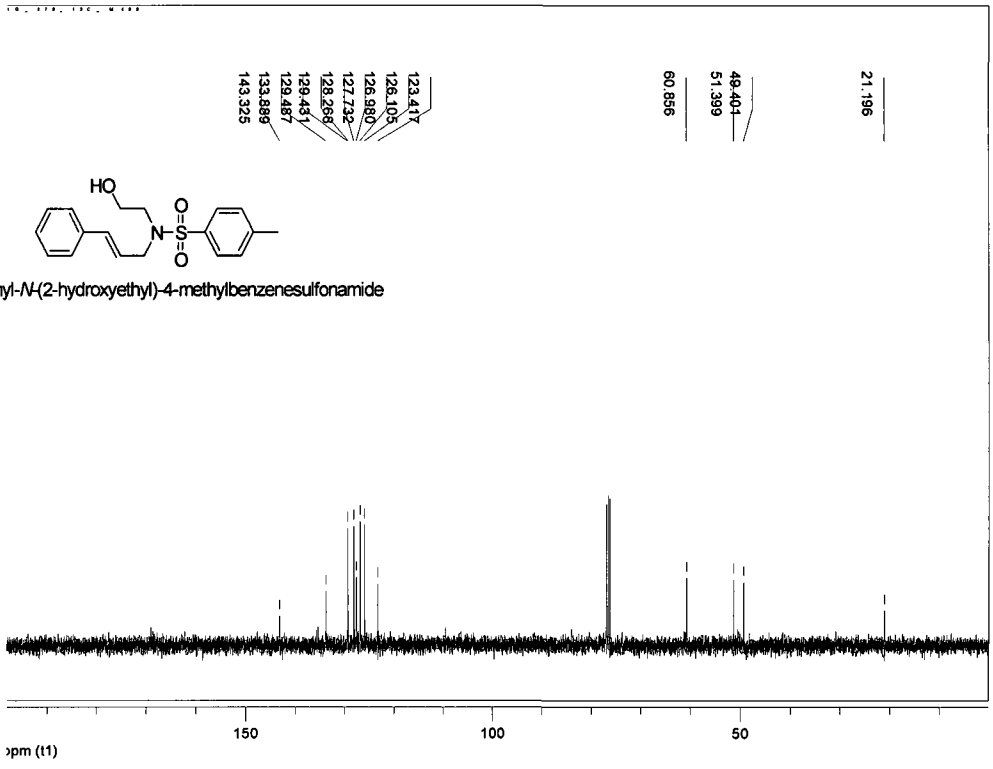
20.113, 21.809, 25.187, 25.320, 50.415, 51.556, 52.654, 52.841, 59.950, 63.271, 63.783, 64.438, 67.211, 68.081, 76.724, 76.888, 76.950, 76.992, 128.001, 128.291, 129.859, 129.954, 144.138, 144.363, 170.229, 170.369; LRMS (M+Na) for $C_{18}H_{26}NO_5SBrNa$, calculated: 470.063, found: 470.19.

(3*S*,6*R*,7*R*)-Methyl-4-benzyl-6-bromo-6-methyl-7-phenyl-1,4-oxazepane-3-carboxylate and (3*S*,6*S*,7*S*)-Methyl 4-benzyl-6-bromo-6-methyl-7-phenyl-1,4-oxazepane-3-carboxylate (61): (5.3 :1)mixture of diastereomers of: colorless oil, isolated yield: 60%; R_f= 0.93(6:4, Hex: EtOAc); IR (neat, cm^{-1}) *v* 698.8, 1101, 1738.28, 2951.01; ^1H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 5H), 2.54 (d, *J*=11.2 Hz, 1H), 2.88 (d, *J*=12.0 Hz, 1H), 3.19-3.27 (br, 1.45H), 3.46 (d, *J*=14.2 Hz, 1.35H), 3.70 (s, 4H), 3.83-3.85 (m, 2H), 3.96- 4.05 (dd, *J*=3.5, 7.9 Hz, 1H), 5.032 (s, 0.19H), 5.26 (s, 1H), 7.27-7.42(m, 20H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 21.16, 49.22, 50.38, 52.06, 53.26, 58.75, 60.55, 69.37, 70.33, 88.50, 90.13, 126.88, 127.91, 129.39, 136.76, 138.33, 139.31, 143.53, 169.36; HRMS (M+H) for $C_{21}H_{25}NO3$, calculated: 418.1018, found: 418.1009

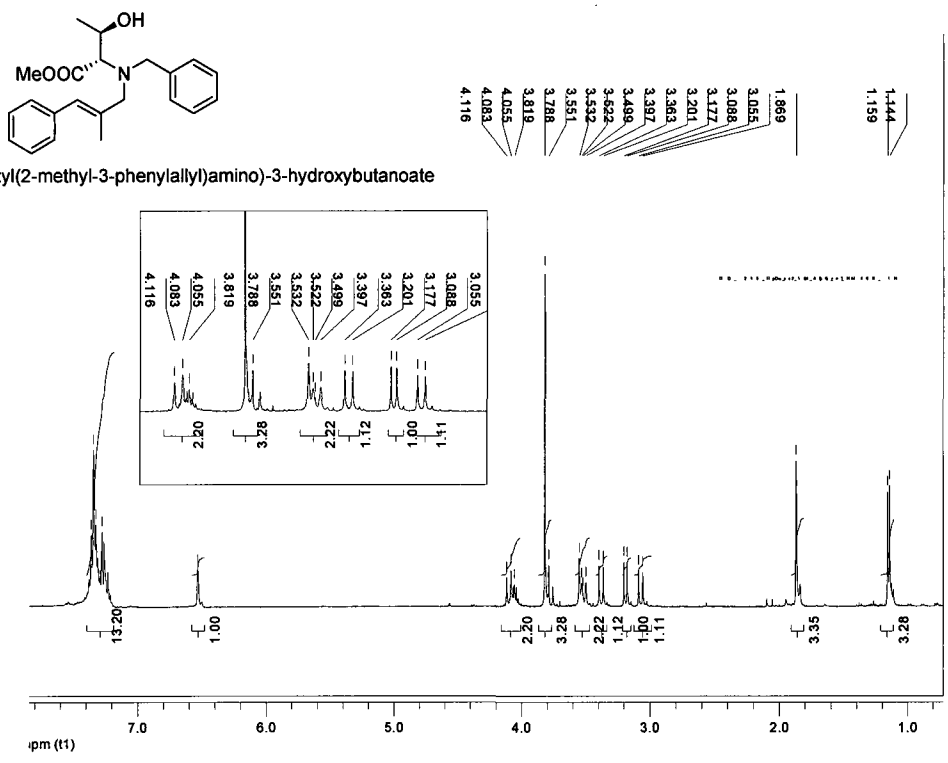
2.5.2 Spectroscopic Data

- ^1H and ^{13}C NMR

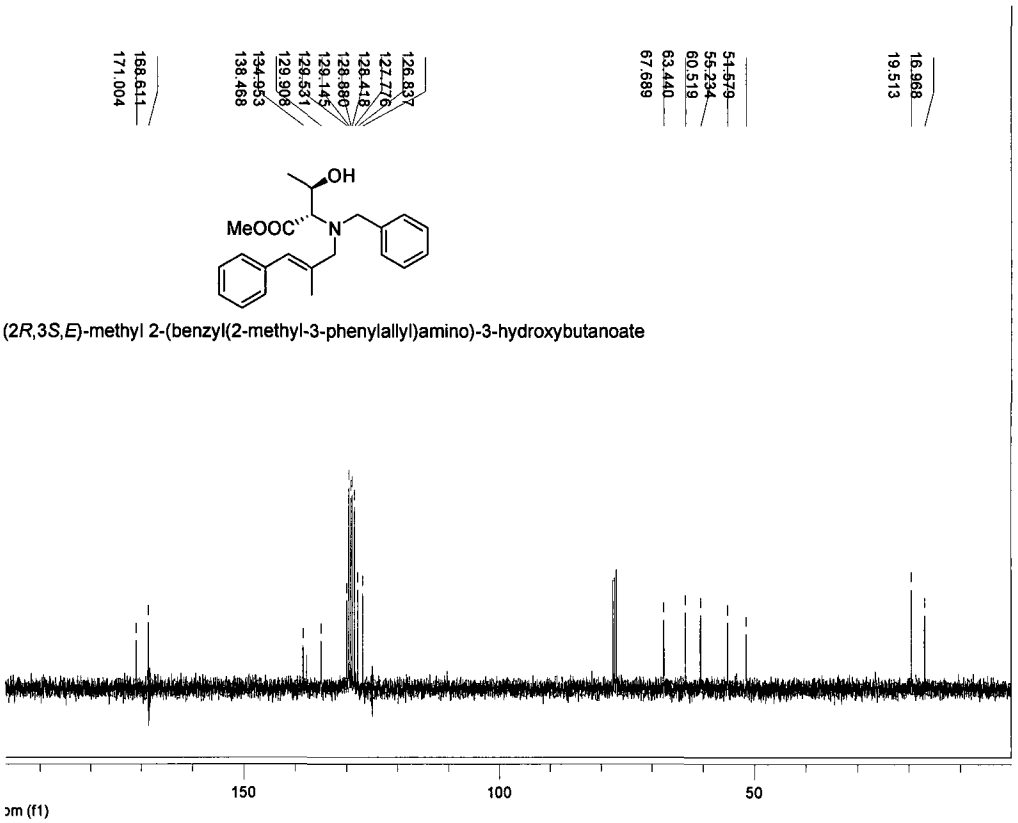




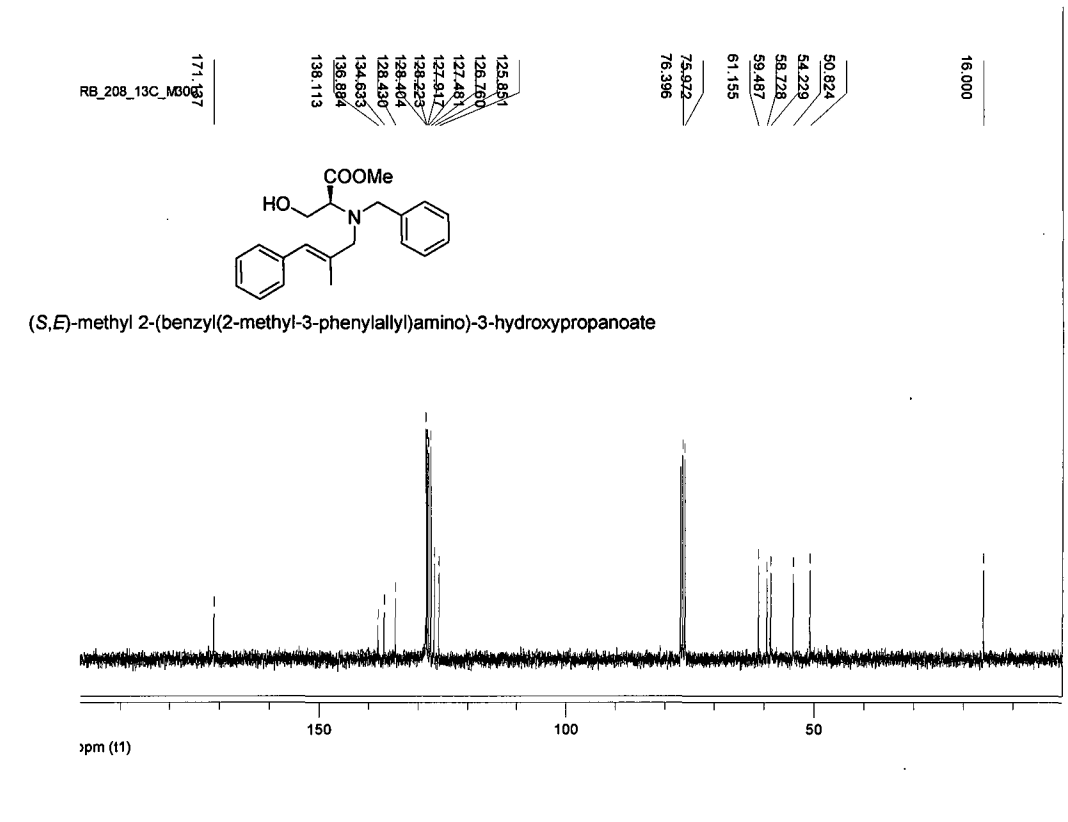
N-cinnamyl-*N*-(2-hydroxyethyl)-4-methylbenzenesulfonamide



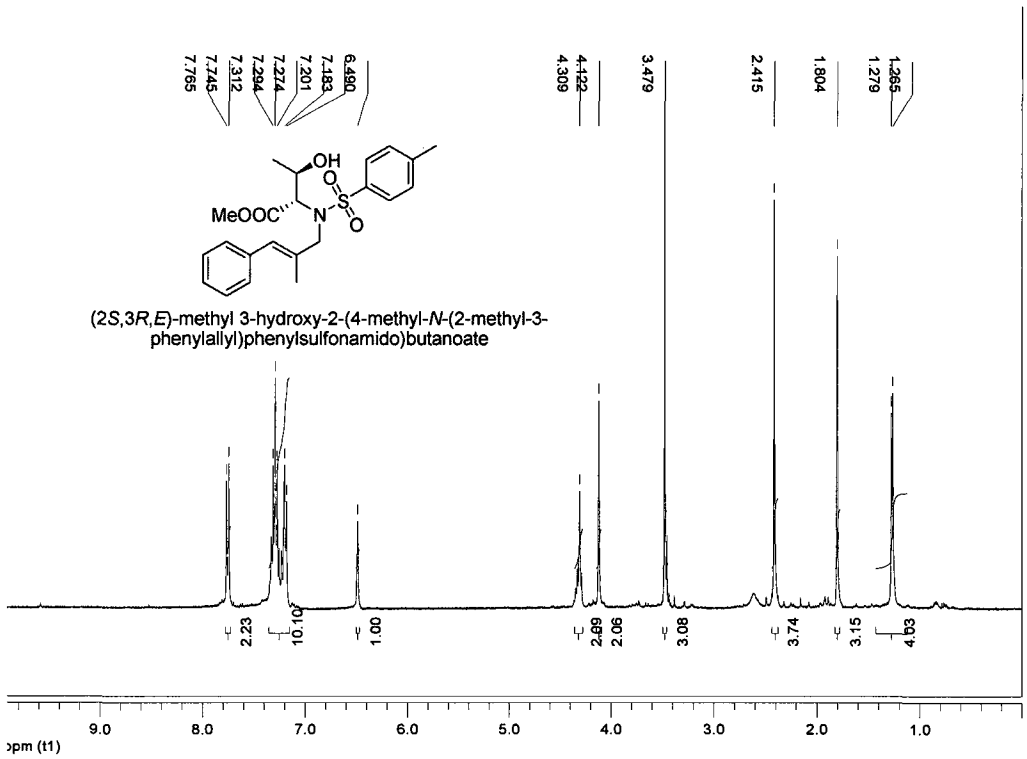
(2*R*,3*S*,*E*)-methyl 2-(benzyl(2-methyl-3-phenylallyl)amino)-3-hydroxybutanoate



(2*R*,3*S*,*E*)-methyl 2-(benzyl(2-methyl-3-phenylallyl)amino)-3-hydroxybutanoate



(*S*,*E*)-methyl 2-(benzyl(2-methyl-3-phenylallyl)amino)-3-hydroxypropanoate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1.804
1.279
1.265
OH
MeOOC
N
O
S
O
(2S,3R,E)-methyl 3-hydroxy-2-(4-methyl-N-(2-methyl-3-phenylallyl)phenylsulfonamido)butanoate
2.23
10.10
1.00
2.09
2.06
3.08
3.74
3.15
4.03
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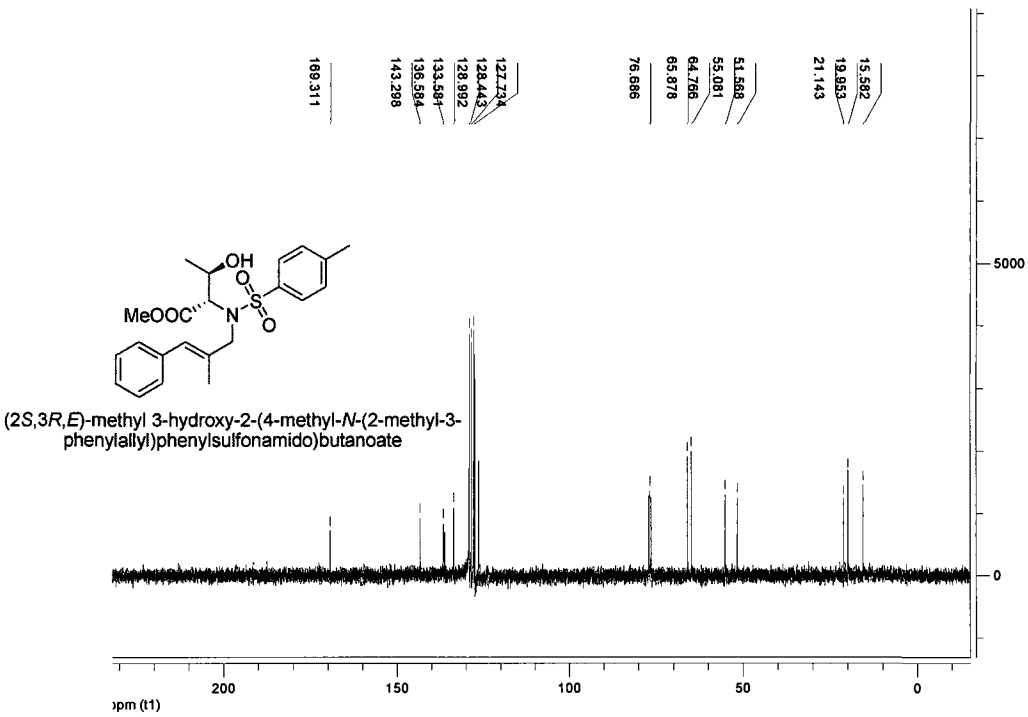
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)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64.766
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21.143
19.853
15.582
OH
MeOOC
N
O
S
O
(2S,3R,E)-methyl 3-hydroxy-2-(4-methyl-N-(2-methyl-3-phenylallyl)phenylsulfonamido)butanoate
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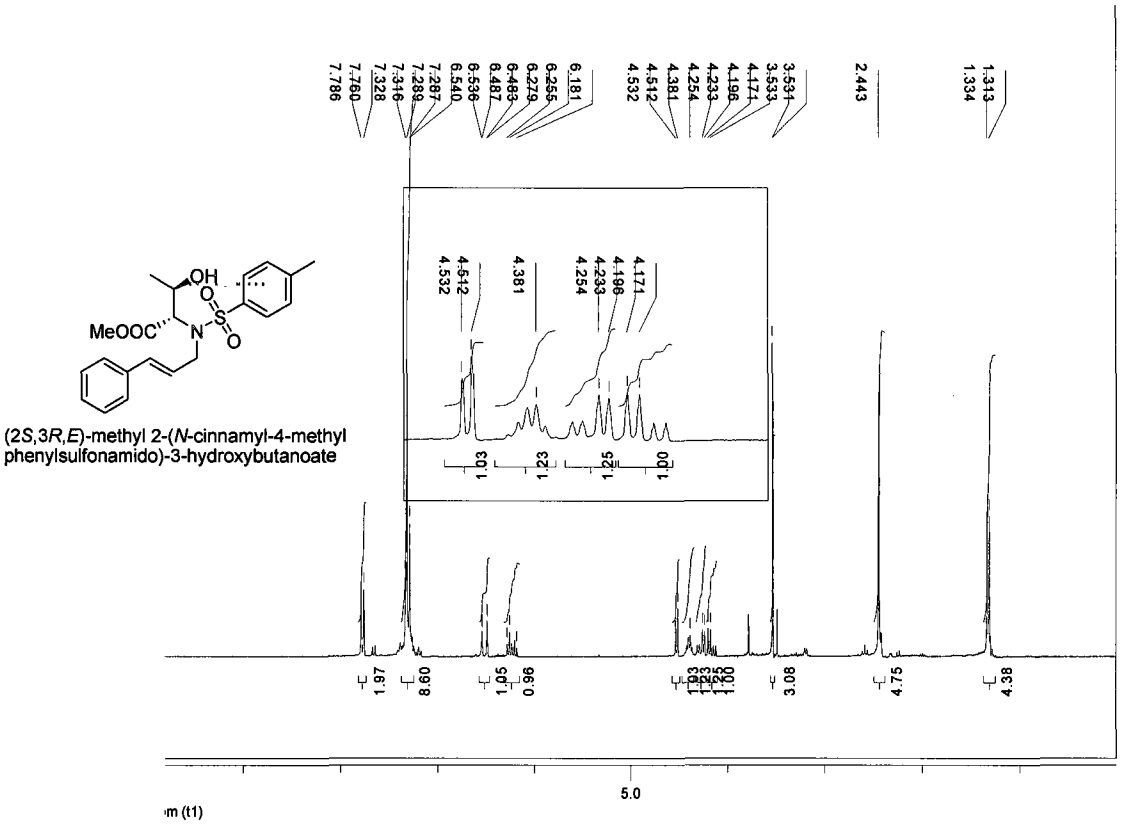
100
50
0
ppm (t1)


OH
MeOOC
N
O
S
O
(2S,3R,E)-methyl 2-(N-cinnamyl-4-methyl phenylsulfonamido)-3-hydroxybutanoate
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7.328
7.316
7.289
7.287
6.540
6.536
6.487
6.483
6.279
6.255
6.181
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4.381
4.254
4.233
4.196
4.171
3.533
3.531
2.443
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1.97
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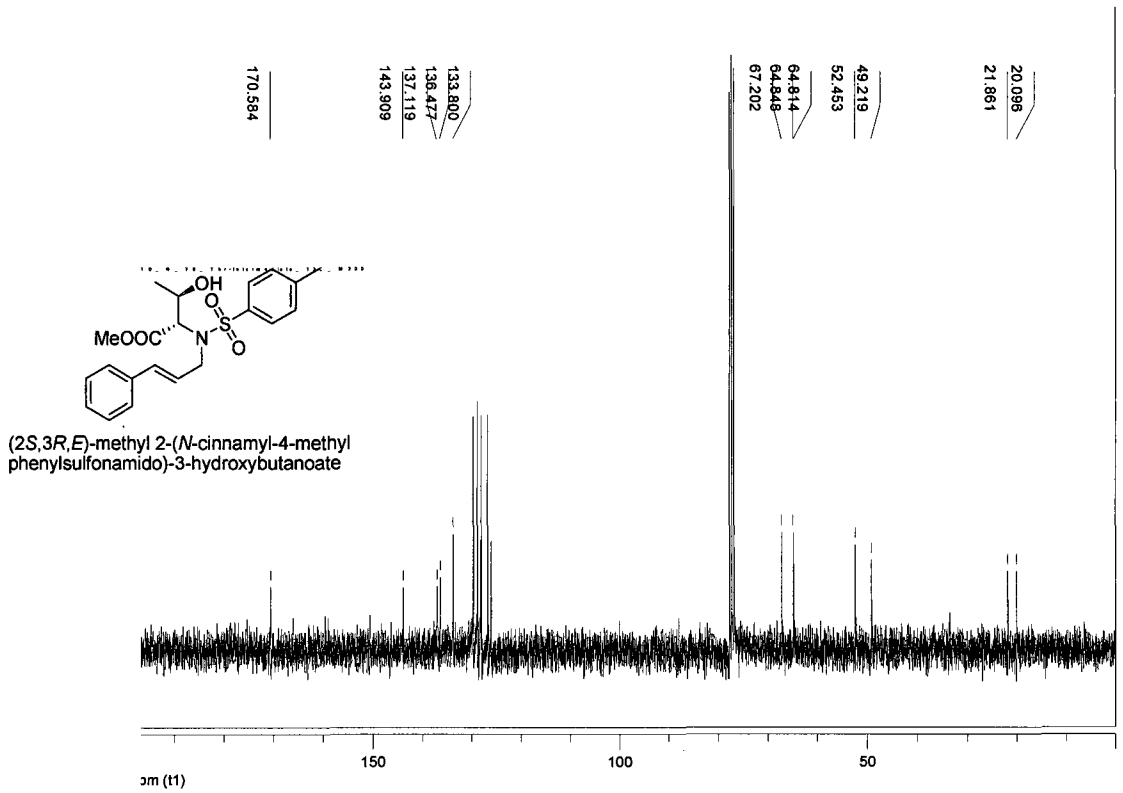
1.05
0.96
3.08
4.75
4.38
5.0
m (t1)


OH
MeOOC
N
O
S
O
(2S,3R,E)-methyl 2-(N-cinnamyl-4-methyl phenylsulfonamido)-3-hydroxybutanoate
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143.909
137.119
136.477
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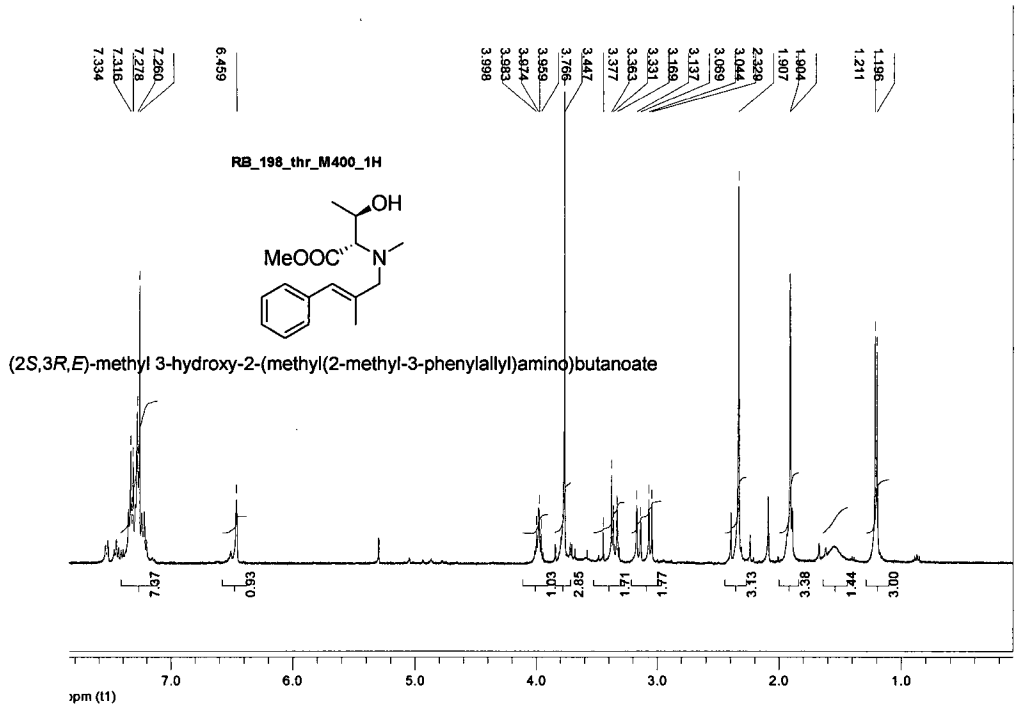
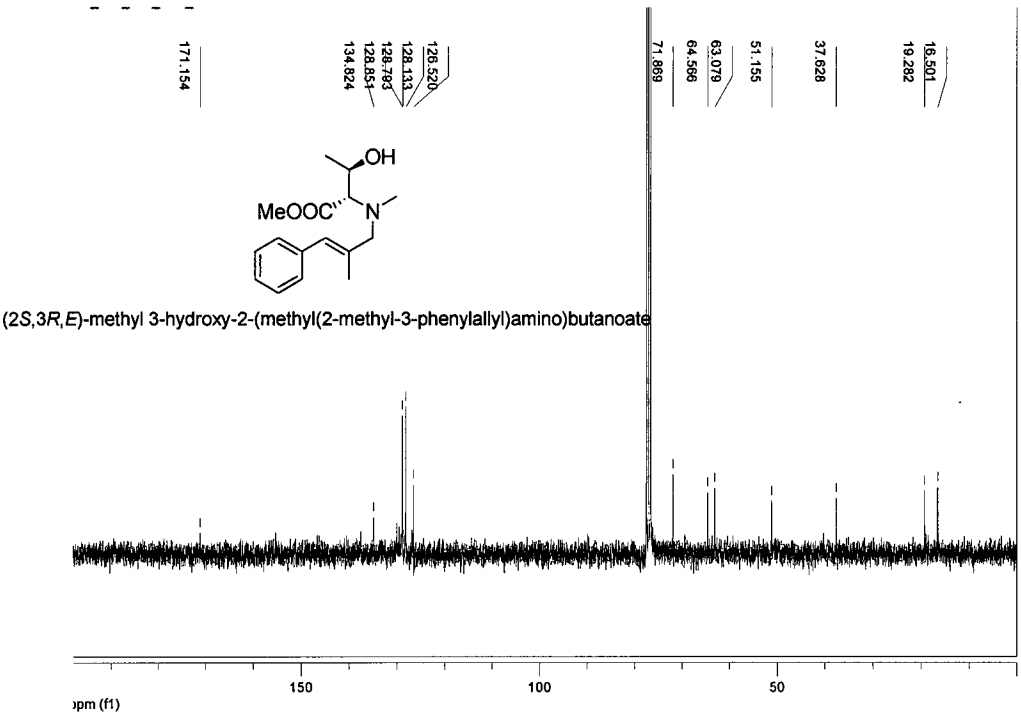
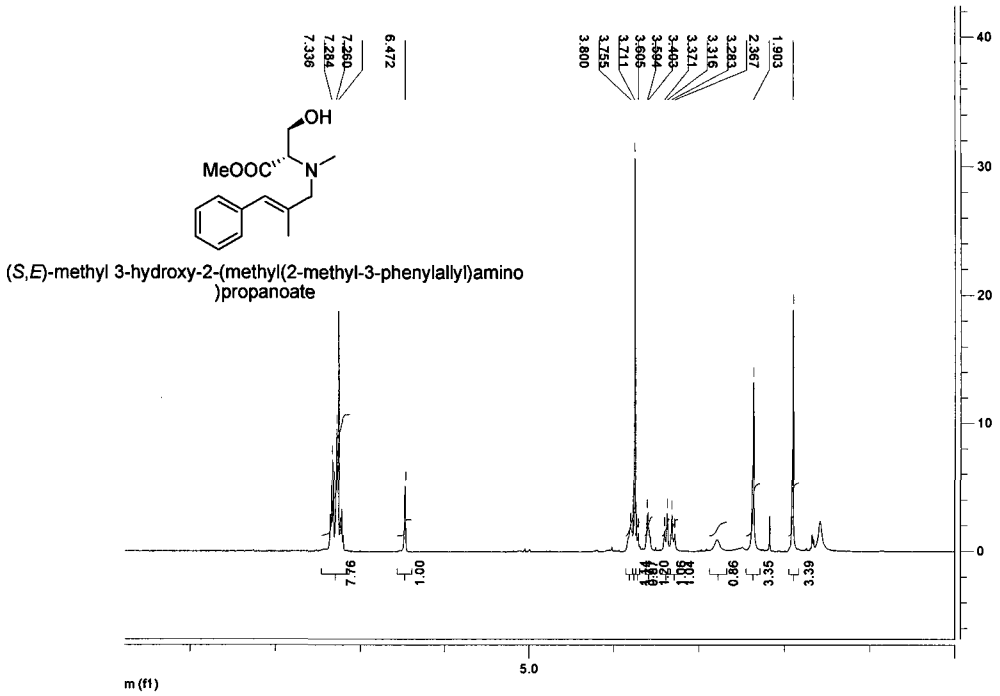
64.848
64.814
52.453
49.219
21.861
20.096
150
100
50
m (t1)



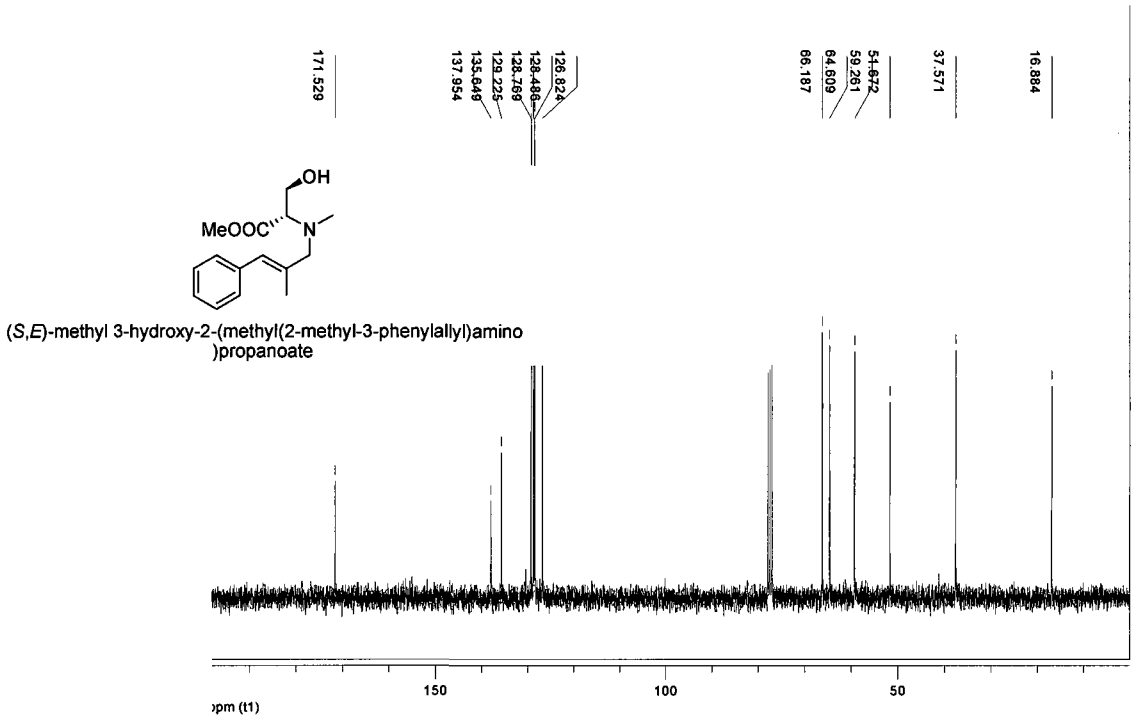
(2*S*,3*R*,*E*)-methyl 3-hydroxy-2-(methyl(2-methyl-3-phenylallyl)amino)butanoate



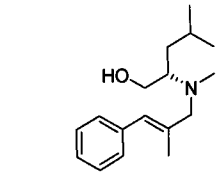
(2*S*,3*R*,*E*)-methyl 3-hydroxy-2-(methyl(2-methyl-3-phenylallyl)amino)butanoate



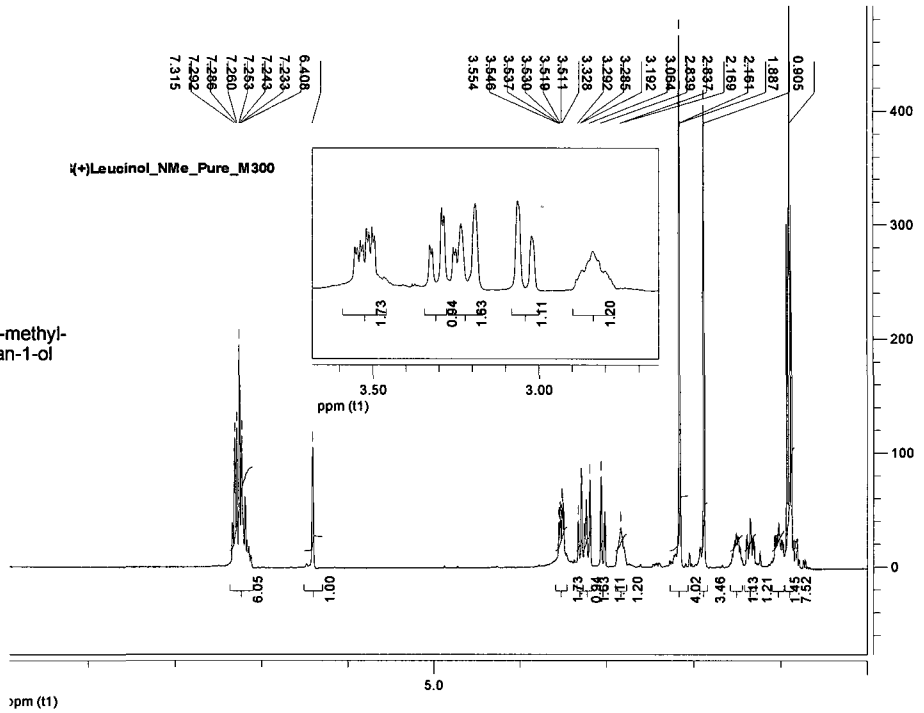
(*S*,*E*)-methyl 3-hydroxy-2-(methyl(2-methyl-3-phenylallyl)amino)propanoate

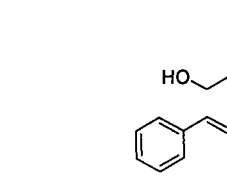


(*S*,*E*)-methyl 3-hydroxy-2-(methyl(2-methyl-3-phenylallyl)amino)propanoate

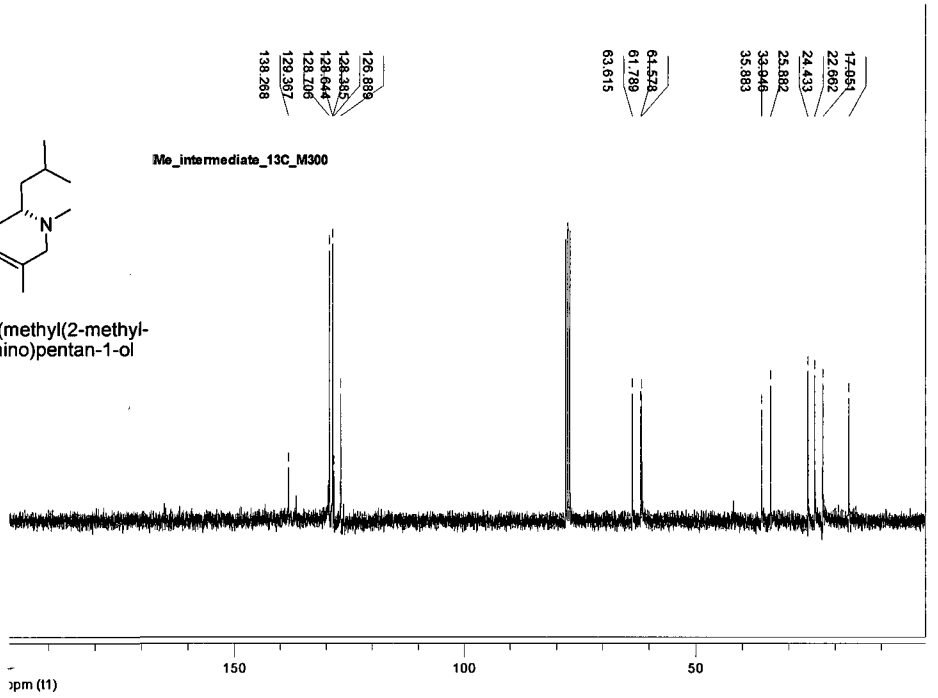


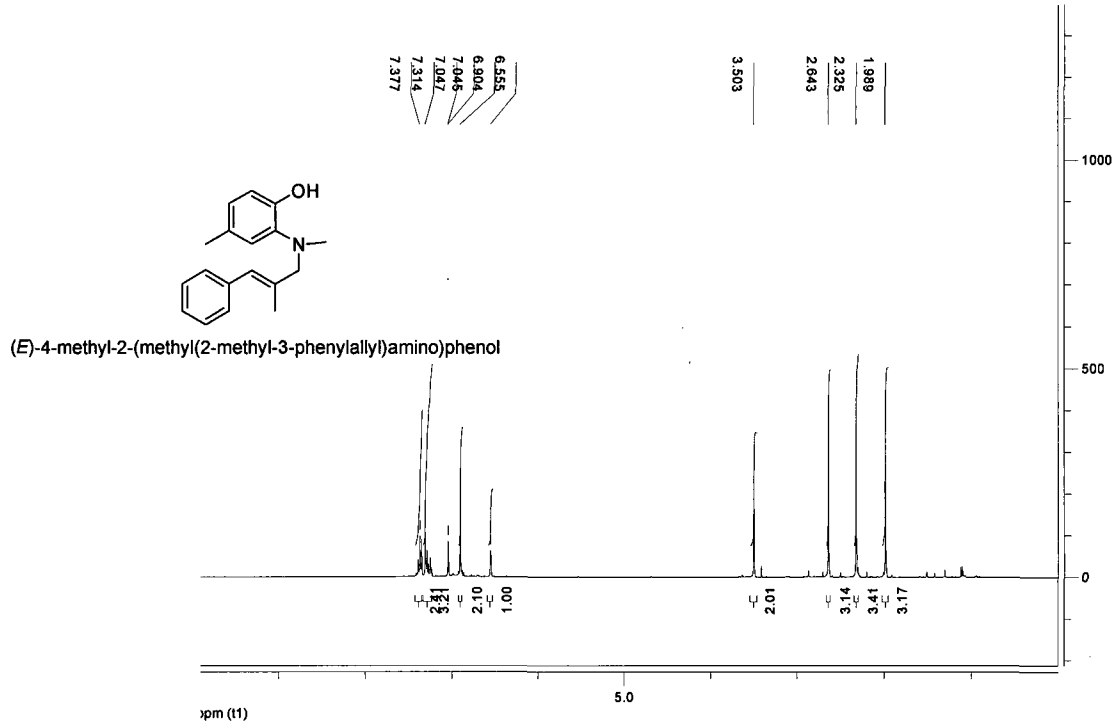
(*S*,*E*)-4-methyl-2-(methyl(2-methyl-3-phenylallyl)amino)pentan-1-ol



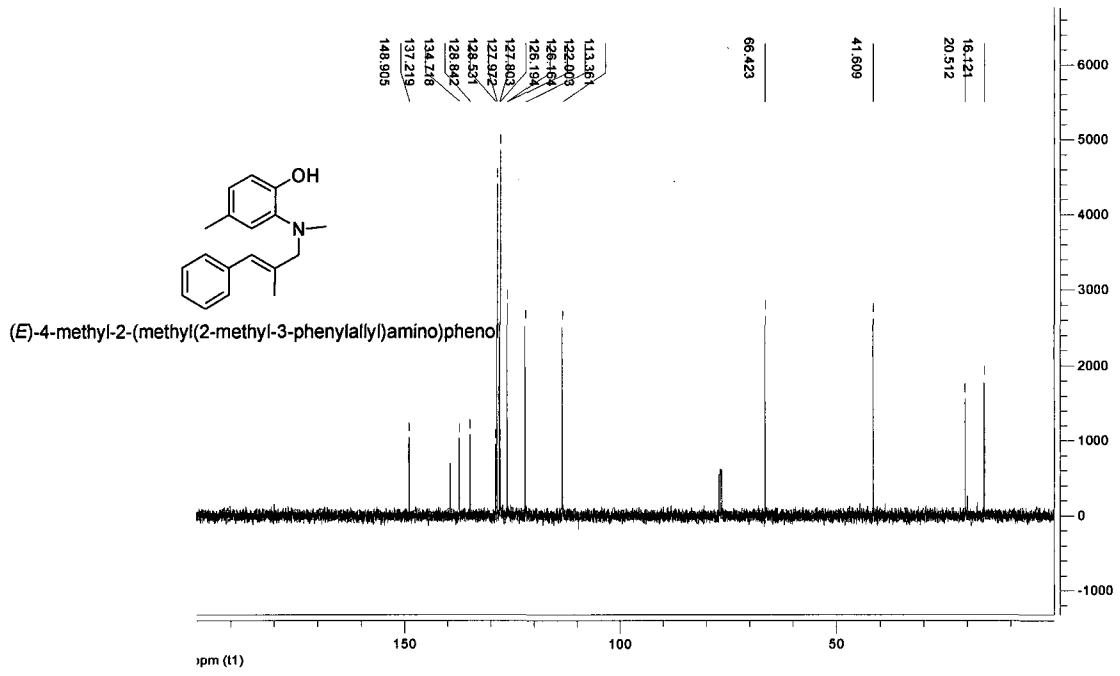


(*S*,*E*)-4-methyl-2-(methyl(2-methyl-3-phenylallyl)amino)pentan-1-ol

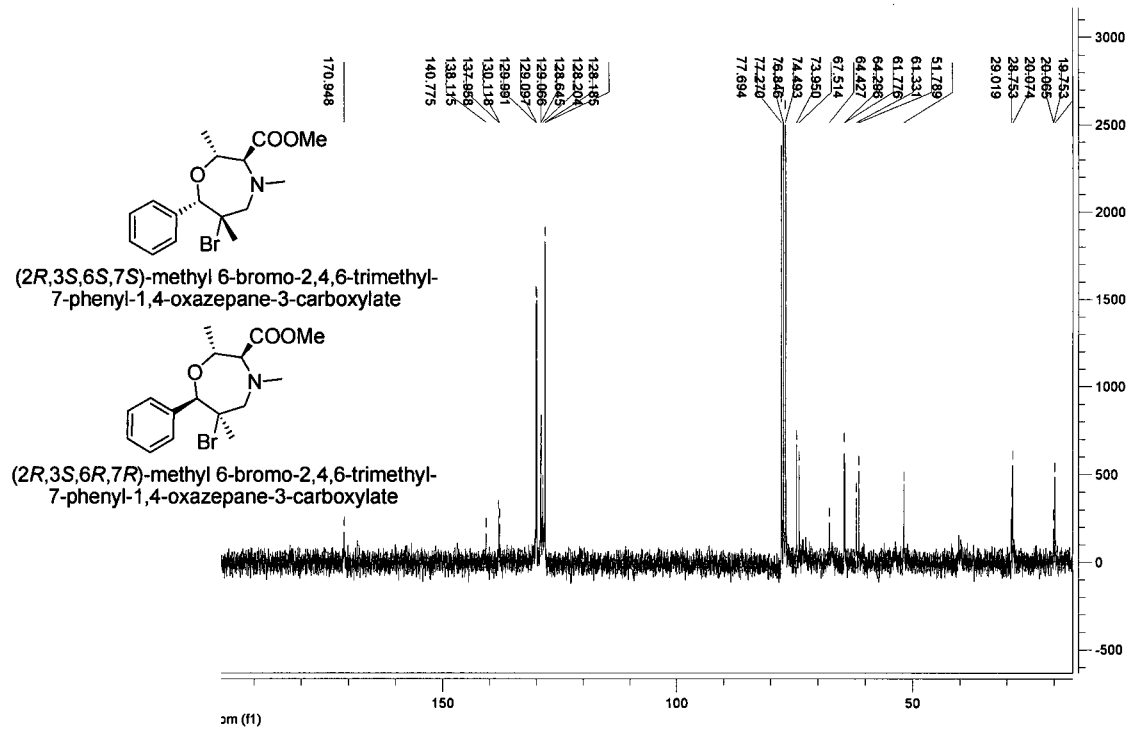


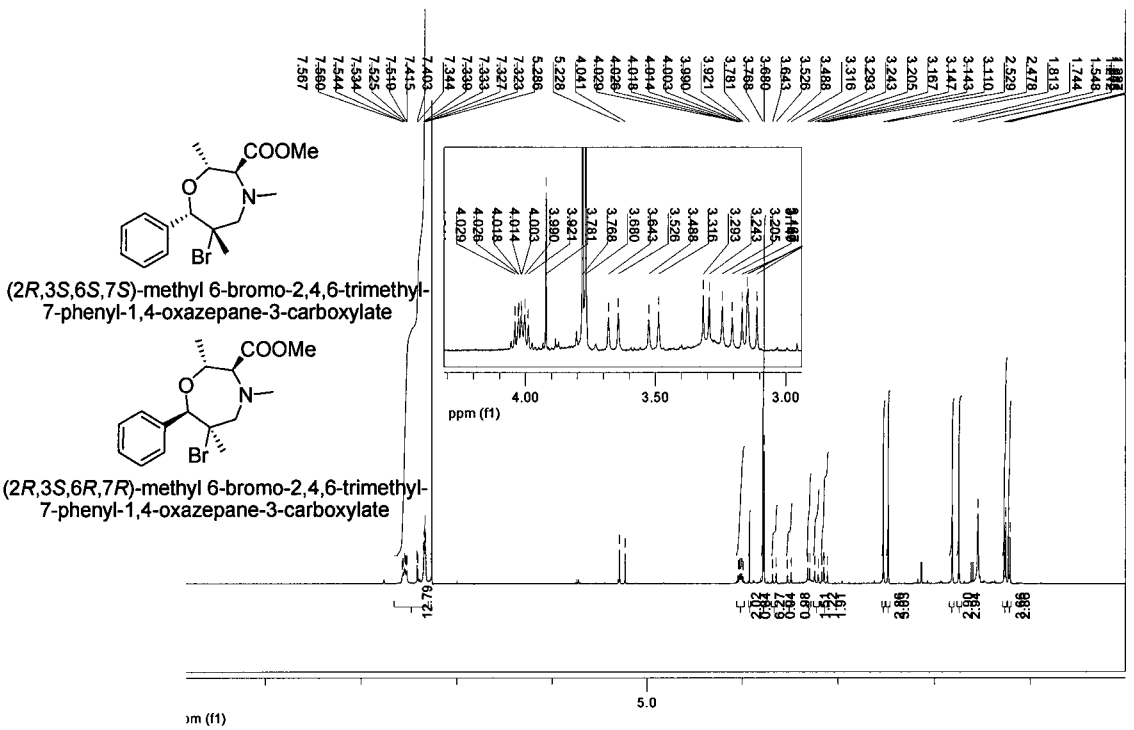


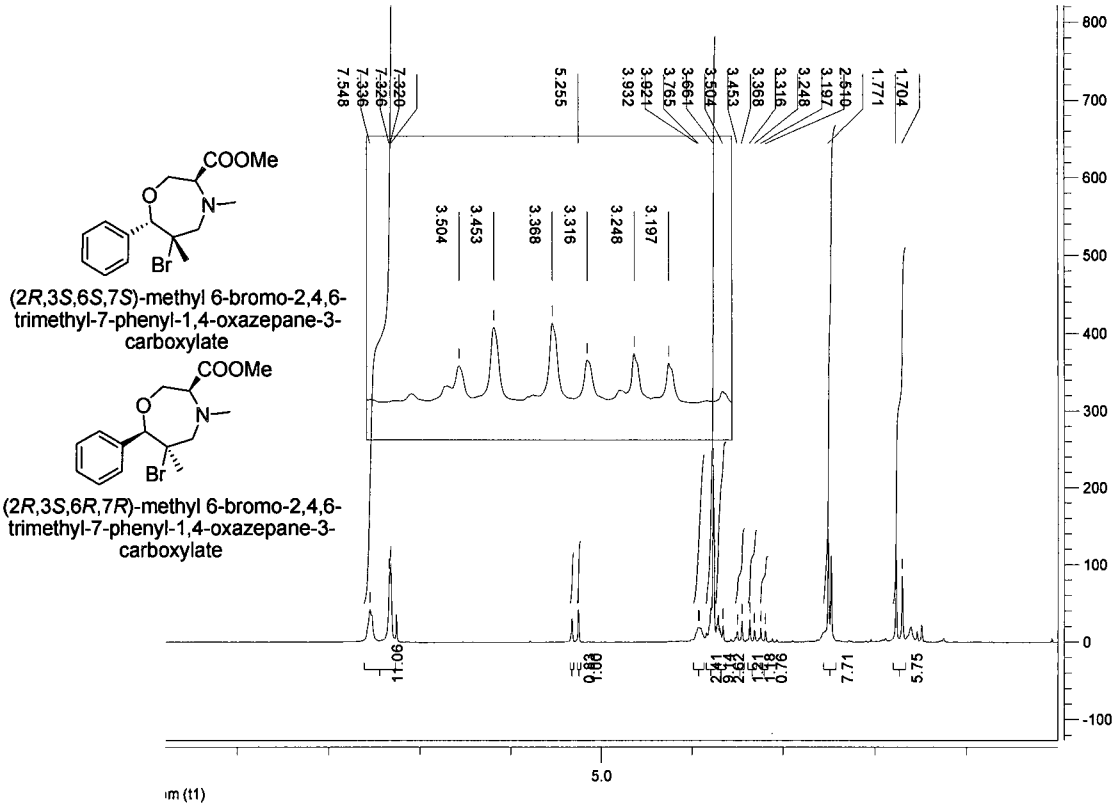
(*E*)-4-methyl-2-(methyl(2-methyl-3-phenylallyl)amino)phenol

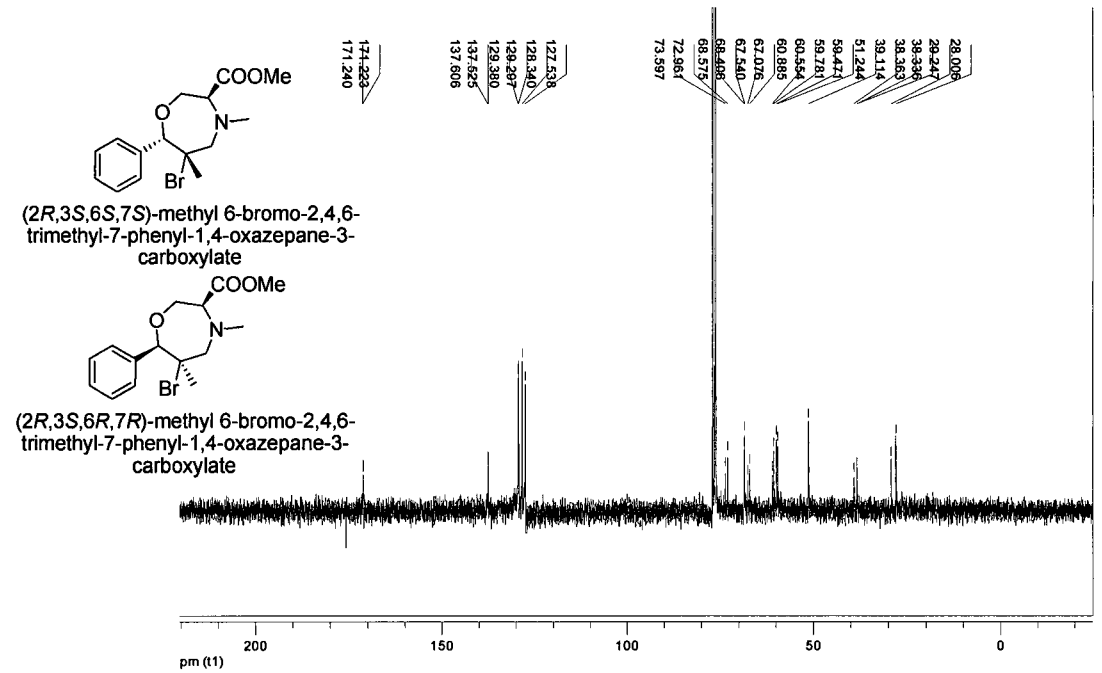


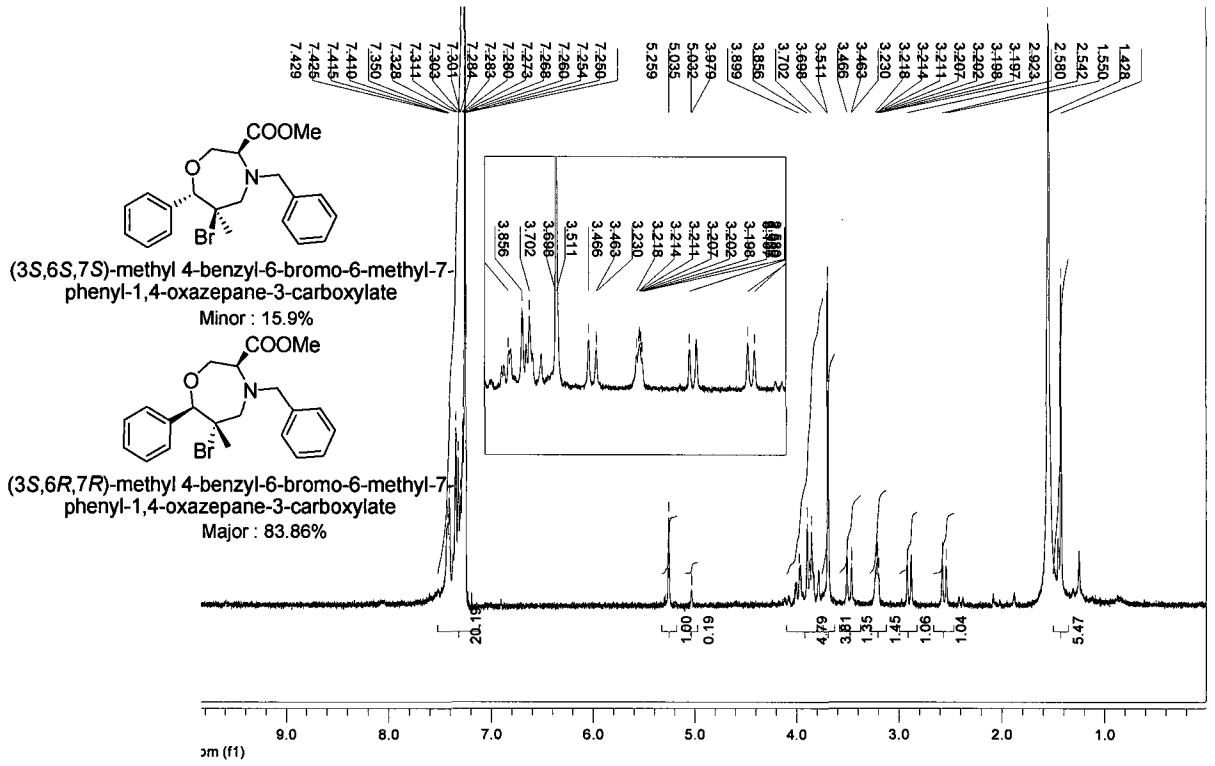
(*E*)-4-methyl-2-(methyl(2-methyl-3-phenylallyl)amino)phenol

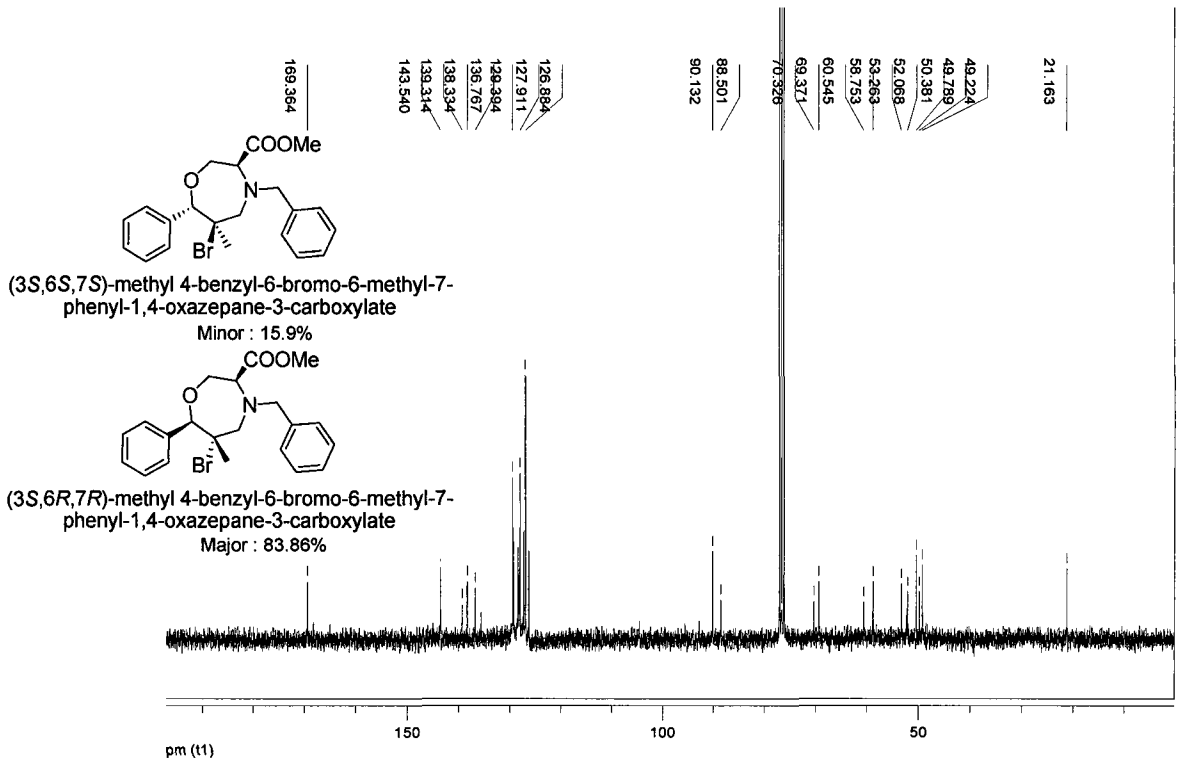

COOMe
Br
(2R,3S,6S,7S)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
COOMe
Br
(2R,3S,6R,7R)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
170.948
140.775
138.115
137.868
130.118
129.981
129.097
129.066
128.645
128.204
128.185
77.694
77.270
76.846
74.493
73.950
67.514
64.427
64.296
61.776
61.331
51.789
29.019
28.753
20.074
20.065
19.753
3000
2500
2000
1500
1000
500
0
-500
150
100
50
ppm (f1)

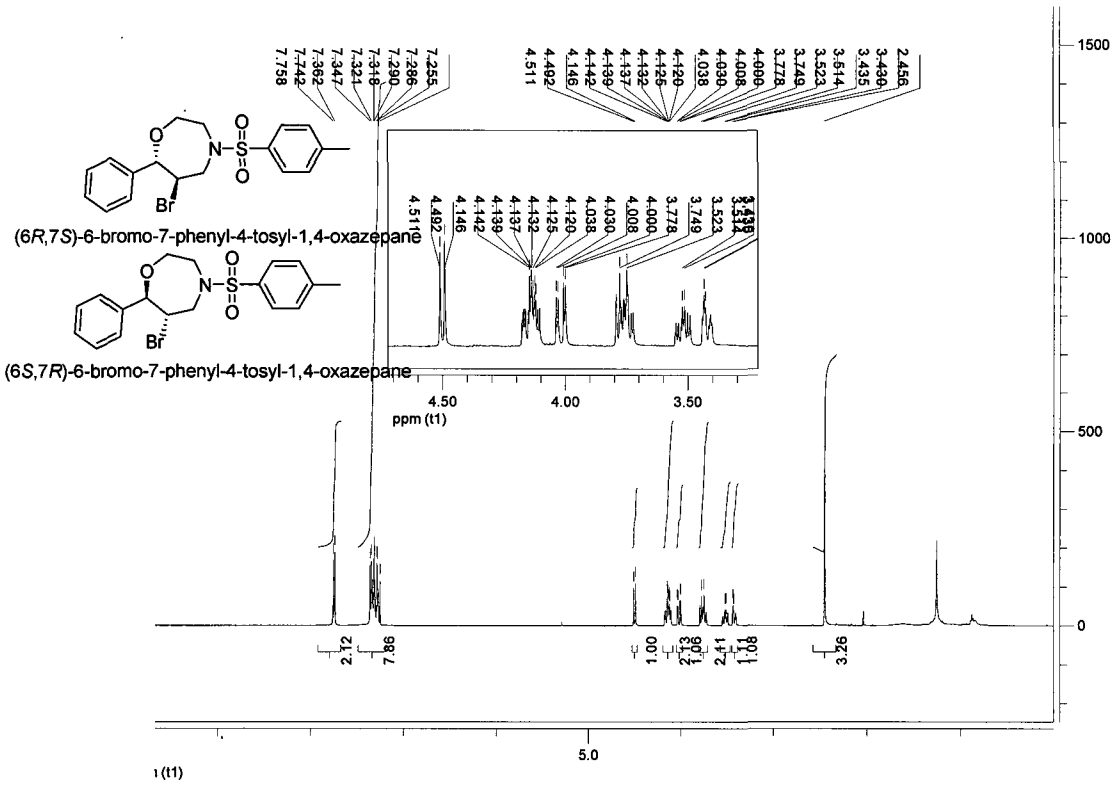

COOMe
Br
(2R,3S,6S,7S)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
COOMe
Br
(2R,3S,6R,7R)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
7.567
7.560
7.544
7.534
7.525
7.519
7.415
7.403
7.344
7.339
7.333
7.327
7.323
5.286
5.228
4.041
4.029
4.026
4.018
4.014
4.003
3.990
3.921
3.781
3.768
3.680
3.643
3.528
3.488
3.316
3.293
3.243
3.205
3.167
3.147
3.143
3.110
2.529
2.478
1.813
1.744
1.548
4.00
3.50
3.00
ppm (f1)
12.79
5.0
ppm (f1)

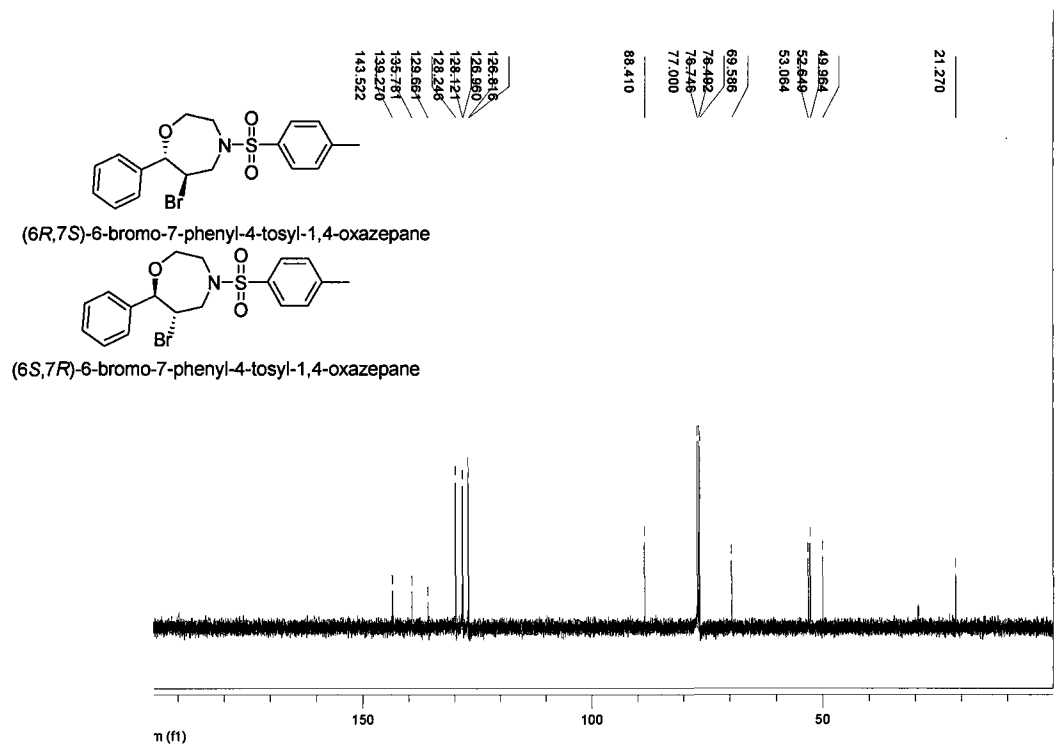

COOMe
Br
(2R,3S,6S,7S)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
COOMe
Br
(2R,3S,6R,7R)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
7.548
7.336
7.326
7.320
5.255
3.932
3.921
3.765
3.661
3.504
3.453
3.368
3.316
3.248
3.197
2.510
1.771
1.704
5.0
m (t1)

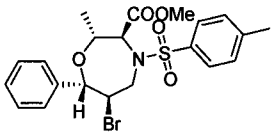

COOMe
Br
(2R,3S,6S,7S)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
COOMe
Br
(2R,3S,6R,7R)-methyl 6-bromo-2,4,6-trimethyl-7-phenyl-1,4-oxazepane-3-carboxylate
171.240
137.606
137.525
129.380
129.297
128.340
127.538
73.597
72.961
68.579
68.498
67.540
67.076
60.885
60.554
59.781
59.471
51.244
39.114
38.363
38.336
29.247
28.006
200
150
100
50
0
pm (t1)


COOMe
Br
(3S,6S,7S)-methyl 4-benzyl-6-bromo-6-methyl-7-phenyl-1,4-oxazepane-3-carboxylate
Minor : 15.9%
COOMe
Br
(3S,6R,7R)-methyl 4-benzyl-6-bromo-6-methyl-7-phenyl-1,4-oxazepane-3-carboxylate
Major : 83.86%
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
pm (f1)

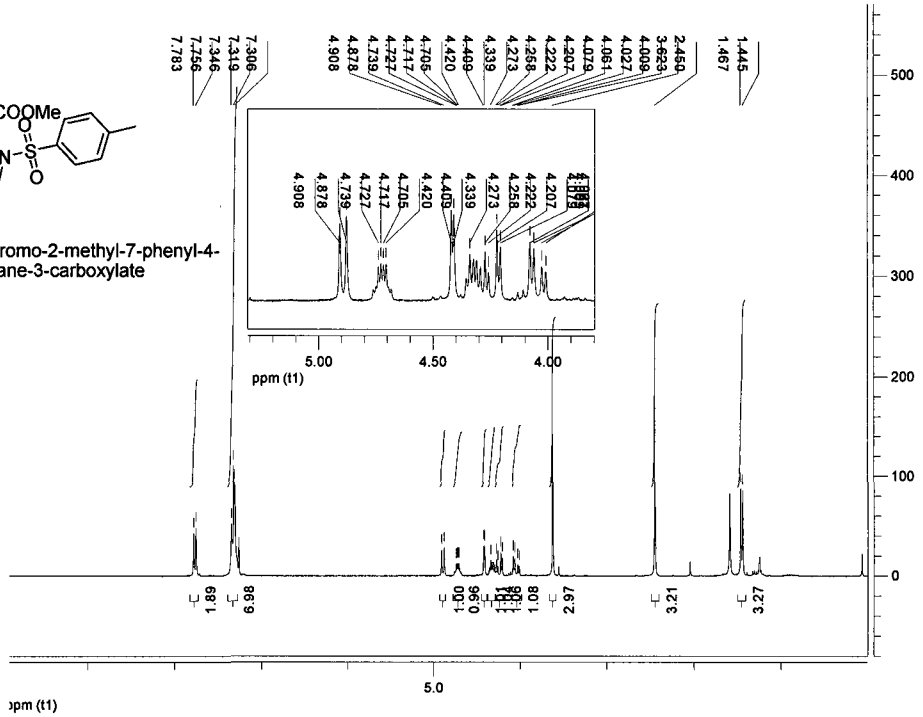

COOMe
Br
(3S,6S,7S)-methyl 4-benzyl-6-bromo-6-methyl-7-phenyl-1,4-oxazepane-3-carboxylate
Minor : 15.9%
COOMe
Br
(3S,6R,7R)-methyl 4-benzyl-6-bromo-6-methyl-7-phenyl-1,4-oxazepane-3-carboxylate
Major : 83.86%
150
100
50
pm (t1)

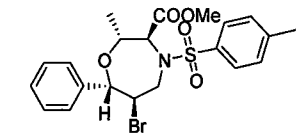

(6R,7S)-6-bromo-7-phenyl-4-tosyl-1,4-oxazepane
(6S,7R)-6-bromo-7-phenyl-4-tosyl-1,4-oxazepane
ppm (t1)
5.0


(6R,7S)-6-bromo-7-phenyl-4-tosyl-1,4-oxazepane
(6S,7R)-6-bromo-7-phenyl-4-tosyl-1,4-oxazepane
150
100
50

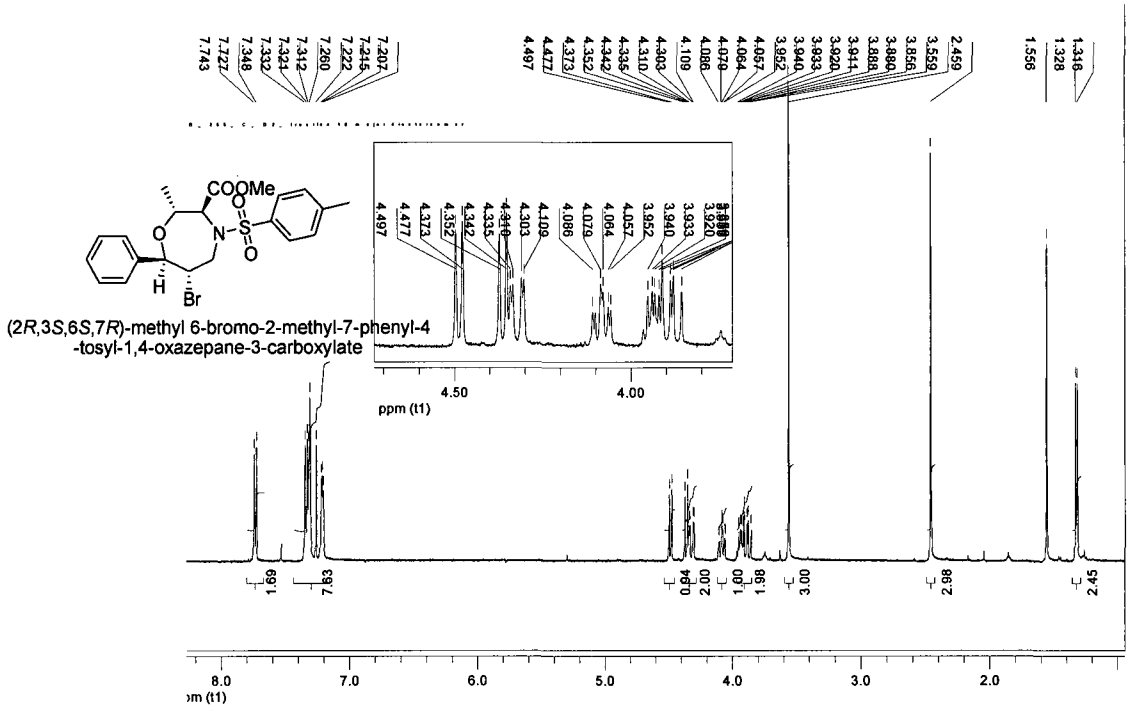


(2*R*,3*S*,6*R*,7*S*)-methyl 6-bromo-2-methyl-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate

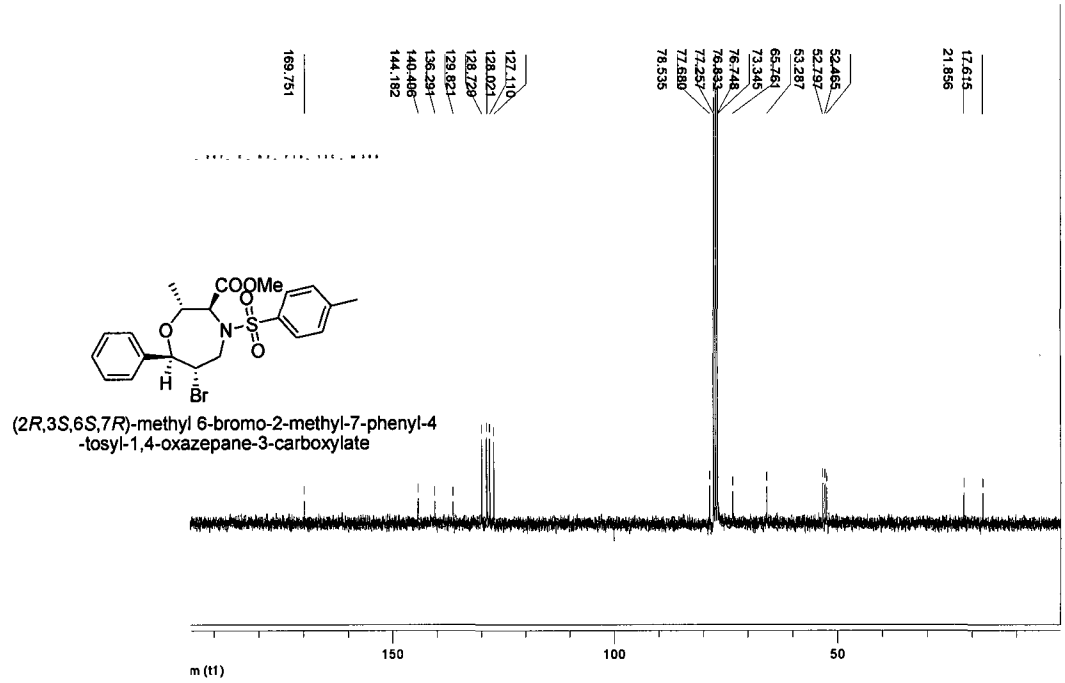




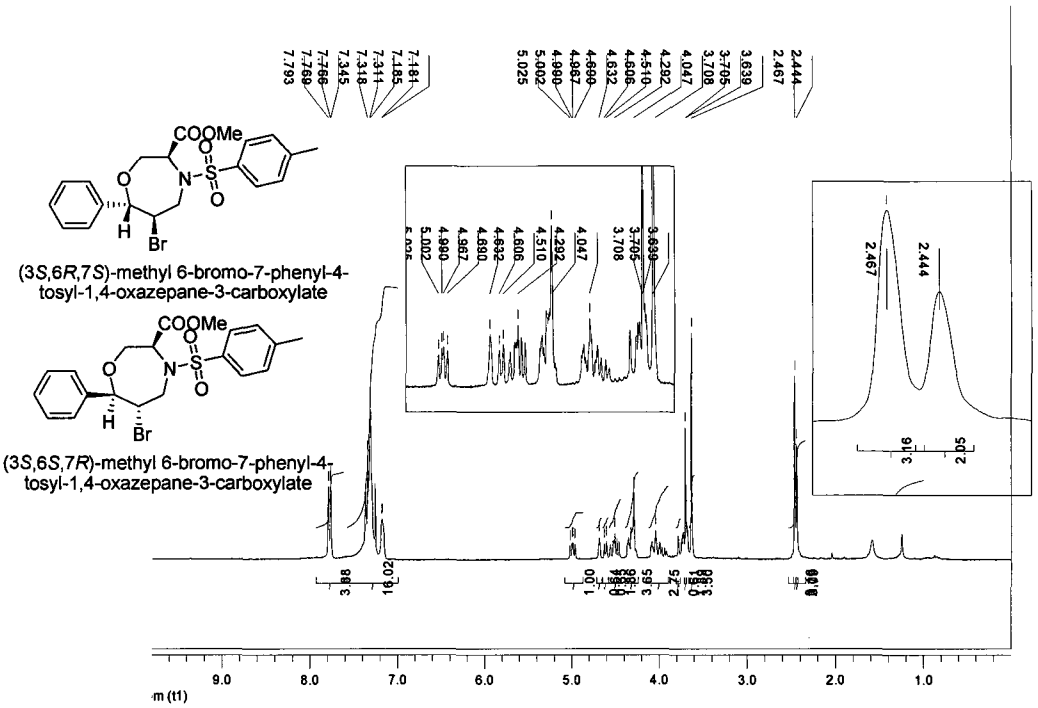
(2*R*,3*S*,6*R*,7*S*)-methyl 6-bromo-2-methyl-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate

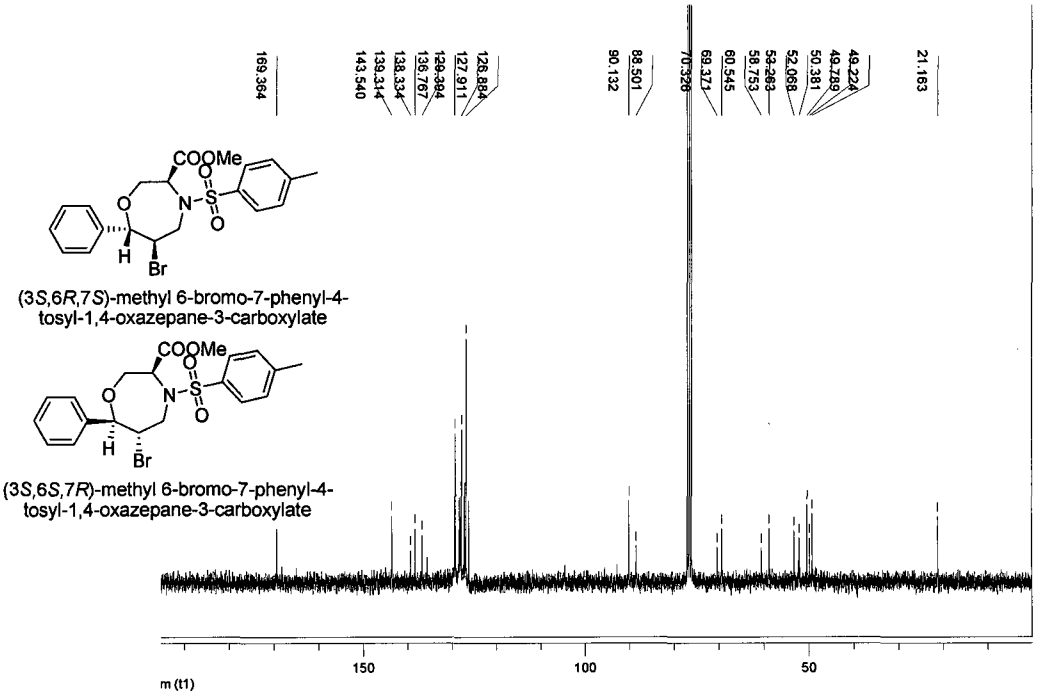


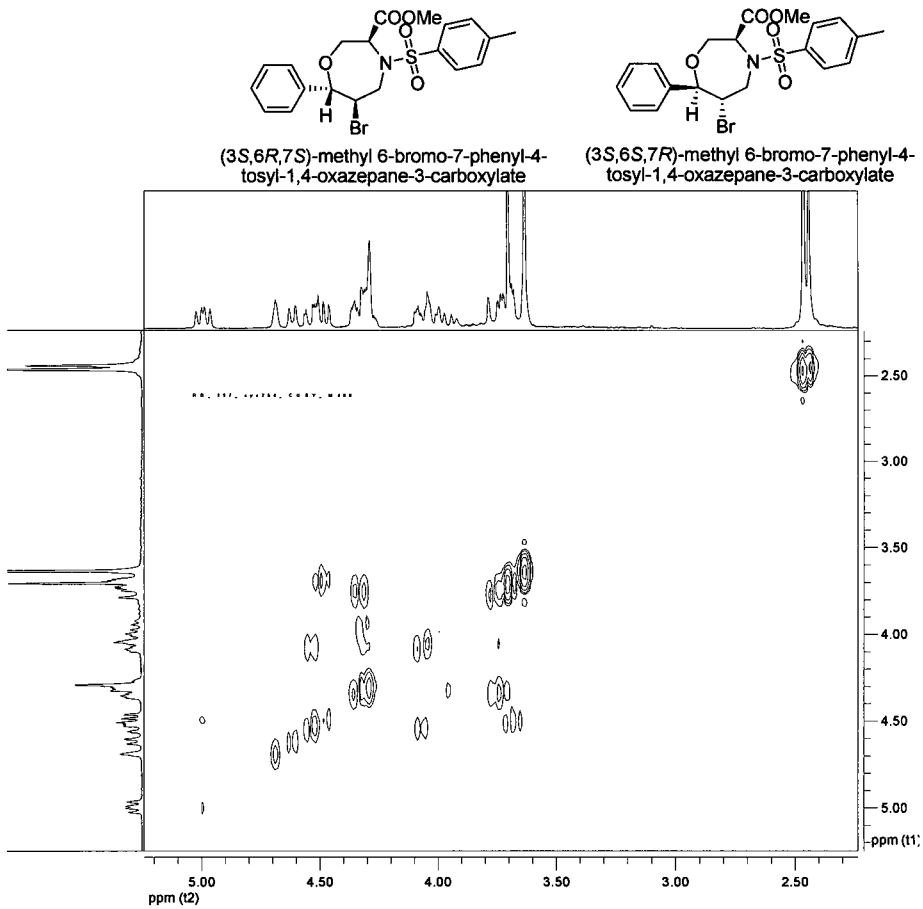
(2*R*,3*S*,6*S*,7*R*)-methyl 6-bromo-2-methyl-7-phenyl-4 -tosyl-1,4-oxazepane-3-carboxylate

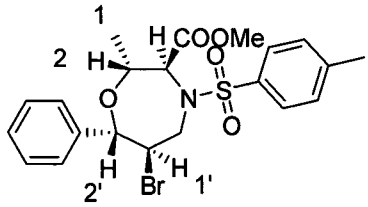


(2*R*,3*S*,6*S*,7*R*)-methyl 6-bromo-2-methyl-7-phenyl-4 -tosyl-1,4-oxazepane-3-carboxylate

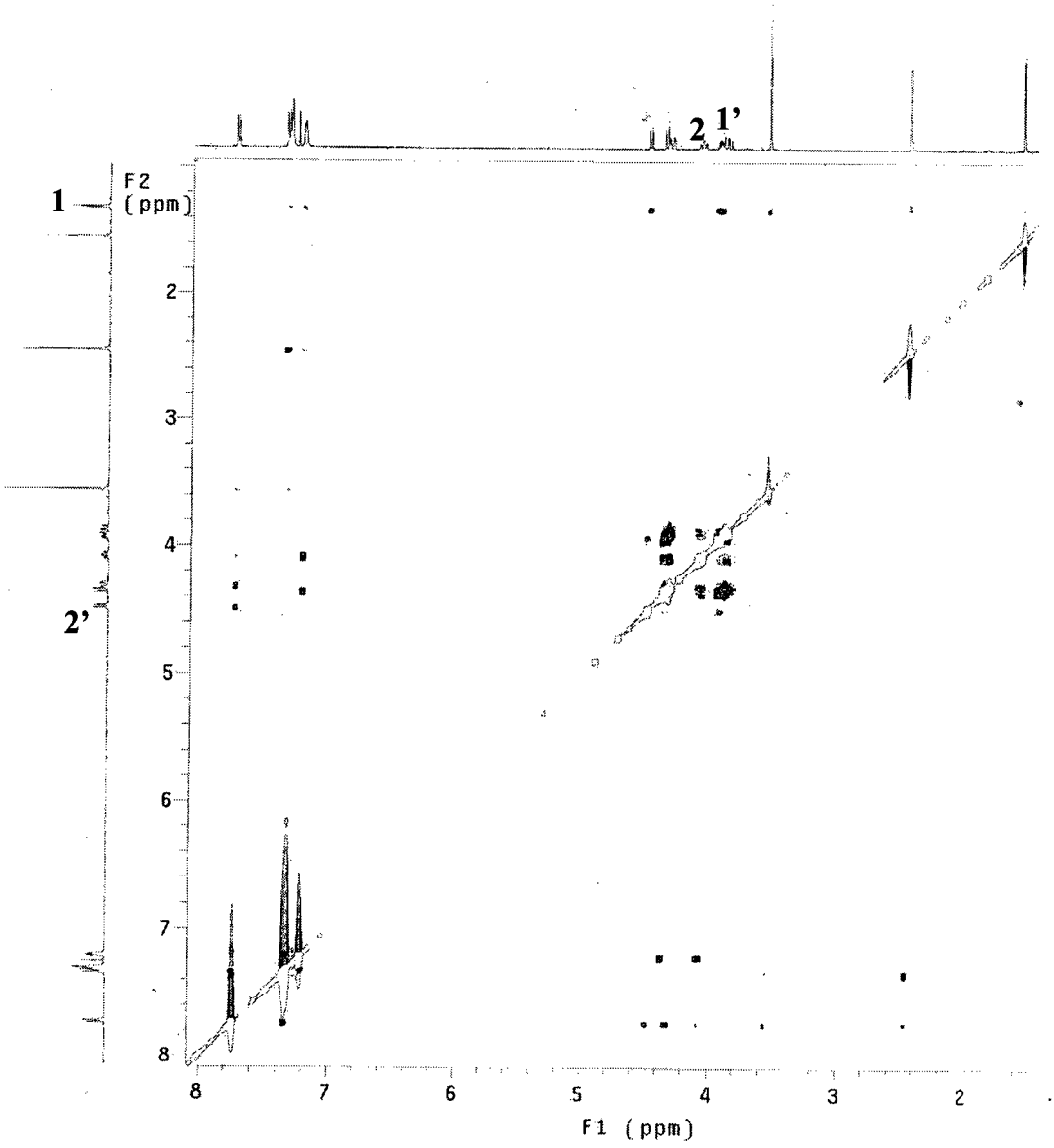

(3S,6R,7S)-methyl 6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate
(3S,6S,7R)-methyl 6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate
m (t1)

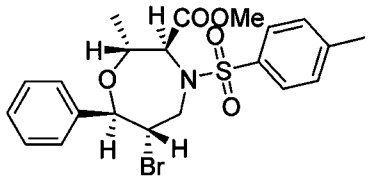

(3S,6R,7S)-methyl 6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate
(3S,6S,7R)-methyl 6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate
m (t1)


COOMe
O
N
S
O
O
H
Br
(3S,6R,7S)-methyl 6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate
(3S,6S,7R)-methyl 6-bromo-7-phenyl-4-tosyl-1,4-oxazepane-3-carboxylate
2.50
3.00
3.50
4.00
4.50
5.00
ppm (t1)
5.00
4.50
4.00
3.50
3.00
2.50
ppm (t2)

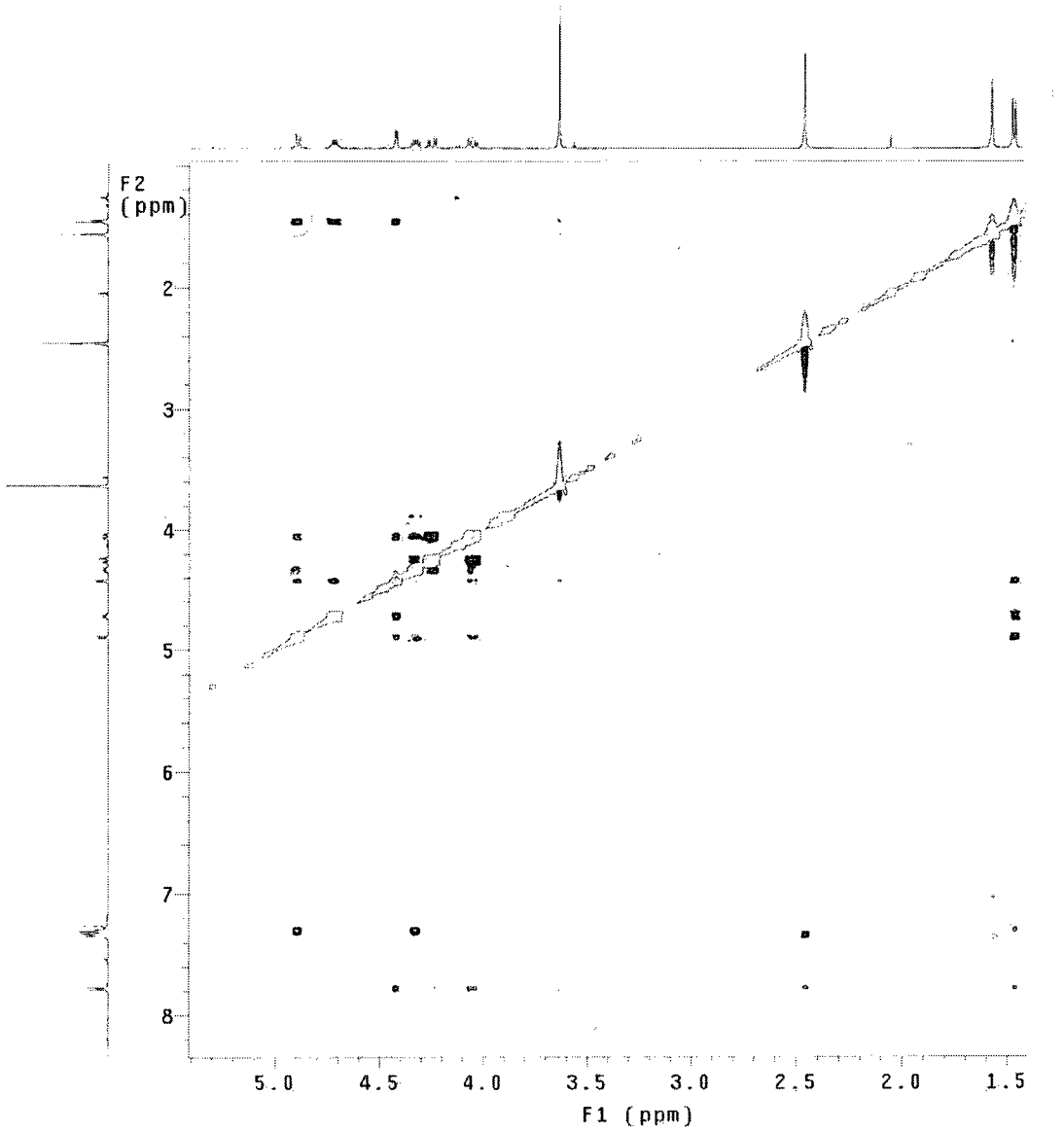


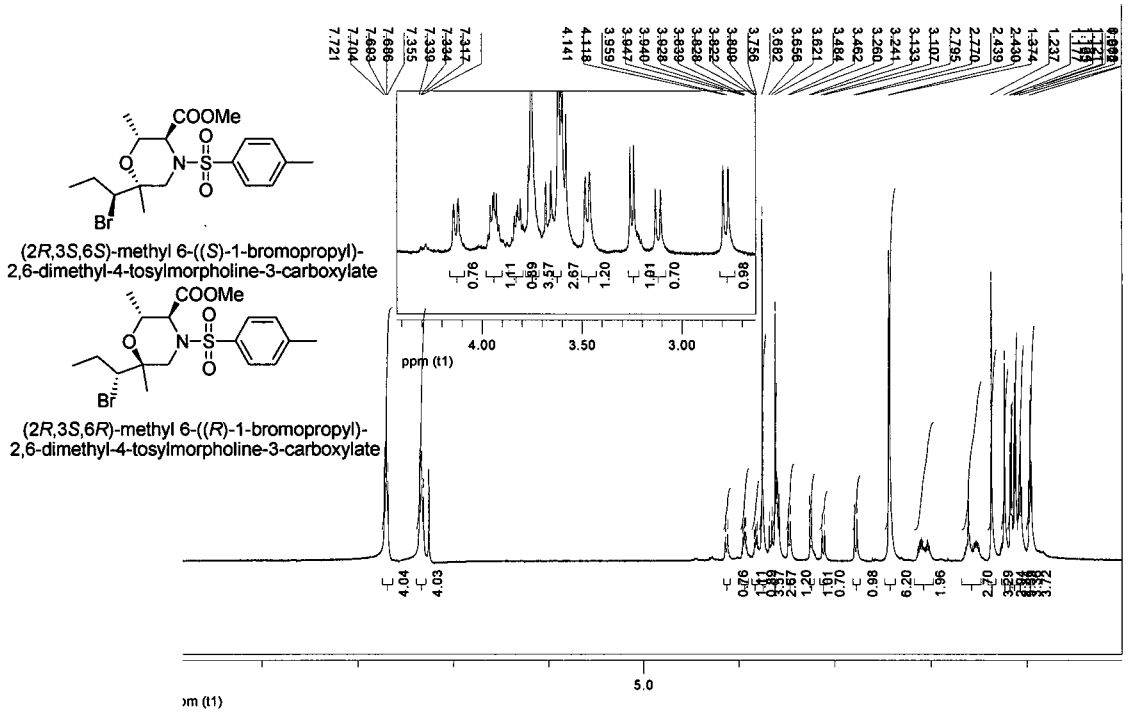
Major

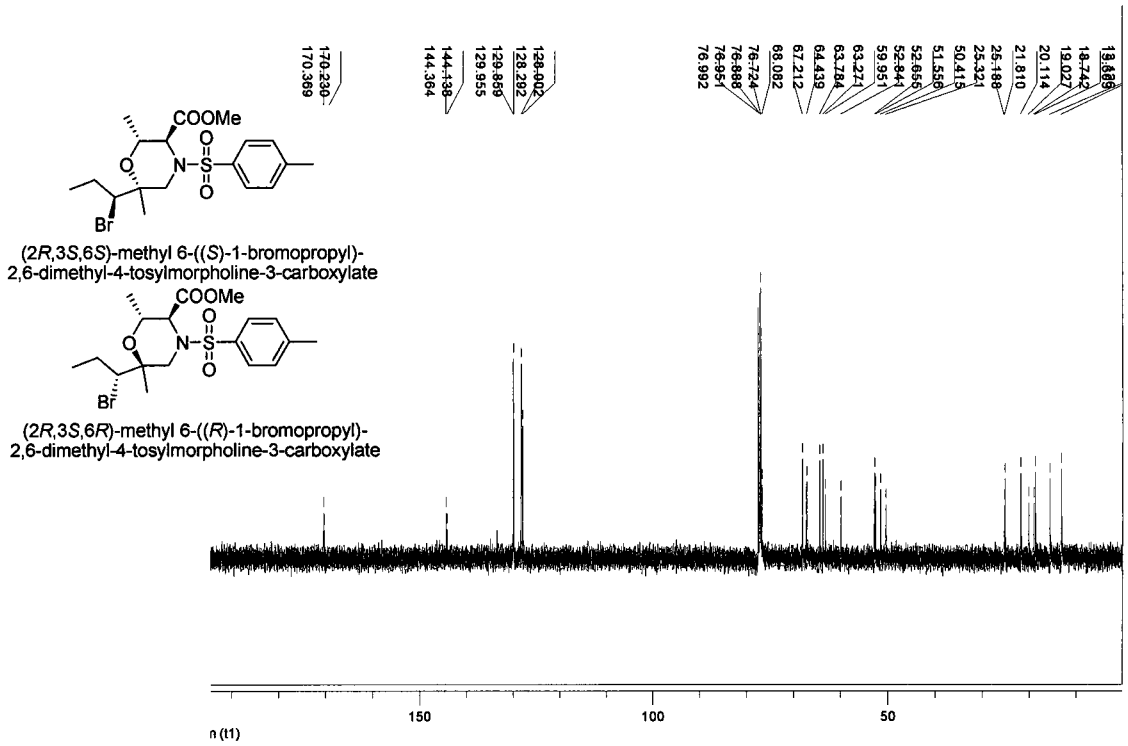




Minor




COOMe
Br
(2R,3S,6S)-methyl 6-((S)-1-bromopropyl)-2,6-dimethyl-4-tosylmorpholine-3-carboxylate
COOMe
Br
(2R,3S,6R)-methyl 6-((R)-1-bromopropyl)-2,6-dimethyl-4-tosylmorpholine-3-carboxylate
ppm (t1)
5.0


COOMe
Br
(2R,3S,6S)-methyl 6-((S)-1-bromopropyl)-2,6-dimethyl-4-tosylmorpholine-3-carboxylate
COOMe
Br
(2R,3S,6R)-methyl 6-((R)-1-bromopropyl)-2,6-dimethyl-4-tosylmorpholine-3-carboxylate
150
100
50

2.6 References:

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Chapter 3: Morpholine-base Compounds as Cyclophilin A Inhibitors

3.1 Preface

The effect of Cyclophilin A (Cyp A) on acquired immunodeficiency syndrome (AIDS) was the focus of Chapter 1 (section 1.2). Reported studies showed that inhibition of Cyp A is a potential approach for the treatment of AIDS. A virtual screening study of morpholine-based compounds as potential inhibitors for Cyp A is reported in this chapter.

3.2 Introduction

Computational approaches have shown great advances in drug discovery. *In silico* hit or lead identification through screening a library of compounds (virtual screening), is faster and cheaper than carrying out actual screening assays of large libraries of compounds (high-throughput screening).[1] Docking methods are among the most applied virtual screening techniques. Docking is a computational approach that predicts the binding mode of ligands (such as enzyme inhibitors) to their biological targets (such as target enzymes).[2] Docking is usually followed by scoring of the docked compounds using a mathematical function referred to as scoring function to rank the compounds according to their predicted binding affinity.

In this study, a small library of morpholine-based compounds was designed. The library was designed in parallel to the development of the synthetic methodology of chiral poly-substituted morpholine and oxazepine compounds. Morpholines and oxazepines are

potential proline mimetics and hence were chosen to be developed as scaffolds for Cyp A inhibitors. This preliminary docking based virtual screening study is conducted to direct the future design of a larger library of morpholine and oxazepine-based potential inhibitors of Cyp A. However, the design of only morpholine-based compounds is described in this chapter, since the design of the oxazepine-based compounds is still in progress.

Cyp A is a flexible protein with two conserved water molecules in the active site. FITTED 2.2 (developed by Chris Corbeil, Pablo Englebienne, Jeremy Schwartzentruber and Nicolas Moitessier in our lab) was used as the docking software in this study.[3] A unique FITTED feature allows docking of small molecules to flexible proteins, while another one accounts for bridging and displaceable water molecules in the active site.

3.3 Results and Discussion:

As part of a medicinal chemistry project, morpholine-based compounds were designed to participate in a virtual screening (*VS*) study for screening potential Cyp A inhibitors. Chiral poly-substituted morpholine derivatives were designed in correlation with the synthetic methodology that was being developed in parallel. In other words, if a hit compound was obtained by *VS* of this library, it would be easily accessible through the developed synthetic methodology.

Several (>20) structures of Cyp A co-crystallized with HIV capsid protein chains, cyclosporine A, cyclosporine A analogues and other peptides were obtained from the Protein Data Bank.[4] At this stage, a very careful selection of structures was carried out.

First, crystal structures with low resolutions (greater than 2.5 Å) were discarded and the others were studied closely and processed. The remaining crystal structures including the various structures extracted from dimers and hexamers were superimposed. From this superposition, seven crystal structures representative of the various conformational moves were selected. These proteins were prepared for docking by adding hydrogen atoms, setting the pH to neutral conditions, carefully assigning the protonation state and the resonance of some of the residues (e.g. histidine) and finally fixing the torsions in some residues (e.g. serine hydroxyl groups). The proteins were still not ready for docking yet. PROCESS prepares the protein for docking by FITTED. This is done by automatic manipulations such as assignments of atom types and atomic charges. PROCESS also identifies the binding site cavity and potential interaction sites. Ligands on the other hand, were prepared with a different module called SMART, which prepares the ligands (by assignment of atom types and identification of rotatable bonds).

Fifty seven morpholine-based compounds were docked to the proteins. The library contained a known potent inhibitor of Cyp A (Filename: cypAlibrary_57.out). This molecule was added to the library to evaluate the suitability of the scoring function for this study. The obtained results are presented in Table 3.1.

Table 3.1: *Scores obtained by docking morpholine based compounds Cyp A using* FITTED.

Filename	Score	RMSD (A°)	Filename	Score	RMSD (A°)
ligand_1bck_1.out	-26.536	0.68	cypAlibrary_42.out	-12.772	n/a
ligand_1awq_1.out	-24.023	1.01	cypAlibrary_49.out	-12.639	n/a
ligand_1awu_1.out	-22.564	1.05	cypAlibrary_19.out	-12.563	n/a
ligand_1cwb_1.out	-21.216	12.29	cypAlibrary_9.out	-12.556	n/a
cypAlibrary_57.out	-16.523	n/a	cypAlibrary_59.out	-12.492	n/a

cypAlibrary_53.out	-16.099	n/a	cypAlibrary_50.out	-12.484	n/a
cypAlibrary_54.out	-15.715	n/a	cypAlibrary_52.out	-12.441	n/a
cypAlibrary_22.out	-15.499	n/a	cypAlibrary_25.out	-12.382	n/a
cypAlibrary_47.out	-15.208	n/a	cypAlibrary_58.out	-12.12	n/a
cypAlibrary_31.out	-14.73	n/a	cypAlibrary_56.out	-12.072	n/a
cypAlibrary_20.out	-14.722	n/a	cypAlibrary_44.out	-12.015	n/a
cypAlibrary_35.out	-14.578	n/a	cypAlibrary_43.out	-11.956	n/a
cypAlibrary_37.out	-14.524	n/a	cypAlibrary_4.out	-11.831	n/a
cypAlibrary_26.out	-14.396	n/a	cypAlibrary_45.out	-11.783	n/a
cypAlibrary_21.out	-14.332	n/a	cypAlibrary_5.out	-11.553	n/a
cypAlibrary_55.out	-14.327	n/a	cypAlibrary_3.out	-11.401	n/a
cypAlibrary_18.out	-14.097	n/a	cypAlibrary_10.out	-11.256	n/a
cypAlibrary_36.out	-13.991	n/a	cypAlibrary_15.out	-11.126	n/a
cypAlibrary_51.out	-13.953	n/a	cypAlibrary_1.out	-10.817	n/a
cypAlibrary_28.out	-13.895	n/a	cypAlibrary_6.out	-10.55	n/a
cypAlibrary_40.out	-13.804	n/a	cypAlibrary_2.out	-10.487	n/a
cypAlibrary_24.out	-13.631	n/a	cypAlibrary_14.out	-10.393	n/a
cypAlibrary_39.out	-13.561	n/a	cypAlibrary_13.out	-10.219	n/a
cypAlibrary_30.out	-13.547	n/a	cypAlibrary_12.out	-10.161	n/a
cypAlibrary_48.out	-13.512	n/a	cypAlibrary_16.out	-10.058	n/a
cypAlibrary_27.out	-13.511	n/a	cypAlibrary_8.out	-9.968	n/a
cypAlibrary_17.out	-13.424	n/a	cypAlibrary_11.out	-9.914	n/a
cypAlibrary_29.out	-13.342	n/a	cypAlibrary_33.out	10000	1000
cypAlibrary_32.out	-13.342	n/a	cypAlibrary_34.out	10000	1000
cypAlibrary_23.out	-13.281	n/a	cypAlibrary_7.out	10000	1000
cypAlibrary_38.out	-13.171	n/a	ligand_1fgl_1.out	10000	1000
cypAlibrary_46.out	-12.828	n/a	ligand_1ynd_1_1.out	10000	1000
cypAlibrary_41.out	-12.81	n/a	ligand_1ynd_2_1.out	10000	1000

The scores of all the molecules in the library are also presented in Table 3.1. The root mean square deviation (RMSD) between the crystal structure and the docked pose of each

of the co-crystallized protein ligands is presented as well. Docking of these ligands to their crystal structure was done to make sure that FITTED is well parameterized for this protein.

The RMSD values ranged from 0.68 Å to 12.29 Å. The smaller the RMSD value means that the ligand was docked in a similar pose to its crystal structure, this was the case with 1bck (the ligand is CsA and RMSD is 0.68 Å). In the case 1cwb (the ligand is a large CsA analogue and RMSD is 12.29 Å). This RMSD value can be explained by the size of the ligand, the interactions with the protein and the flexibility of the ligand and the protein. Studying ligand 1cwb in the crystal structure and the docked pose, it was concluded that all the same hydrogen bonds existed in the docking poses. However the ligand is rotated making some additional hydrophobic interactions.

As the scores intends to predict the free energy of binding, the lower the scoring value the better the binding of the ligand to the active site. Higher scores were obtained by docking the designed ligands relative to the crystal structure ligands. This shows that the designed ligands are expected to exhibit lower affinities. The interactions of the three highest ranked compounds with the active site were further examined. The highest ranked compound was the known potent inhibitor (Filename: cypAlibrary_57.out), used to test the scoring function (Figure 3.1). The compound interacts with the active site through hydrogen bonding and hydrophobic interactions with the active site. Residues involved in the binding are Trp121, Arg55, Gln63 and Asn102. This compound is a lead compound that exhibits Cyp A inhibition with IC_{50} of 6 μM. [5] The score obtained for this compound is -16.52, meaning a lower affinity was predicted for this compound.

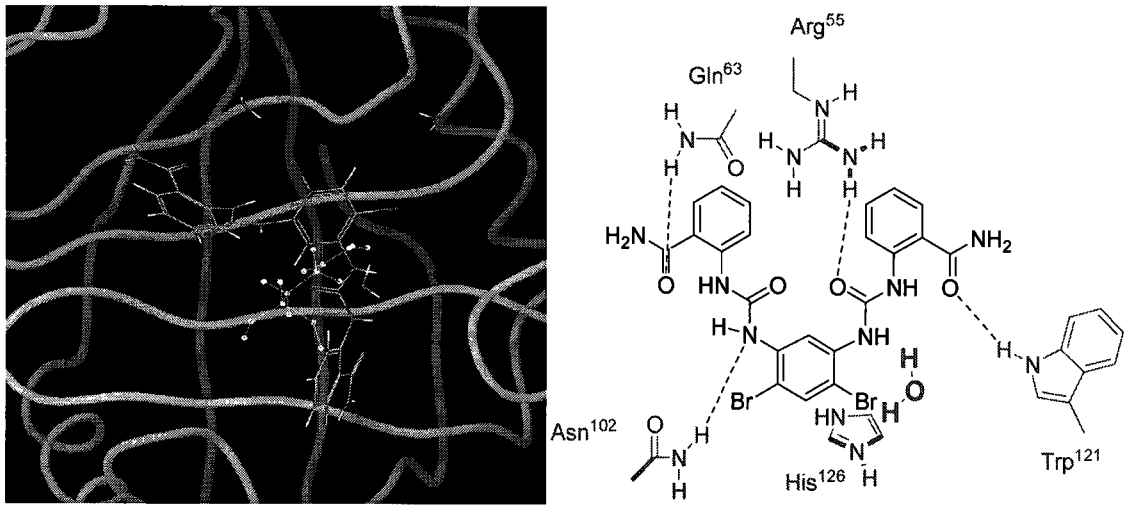
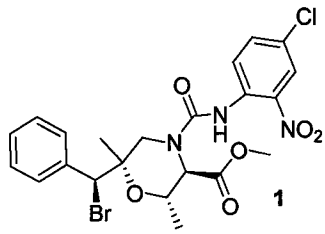
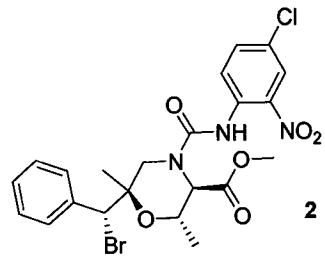


Figure 3.1: *Lead compound bound to the active site of Cyp A (1bck protein).*

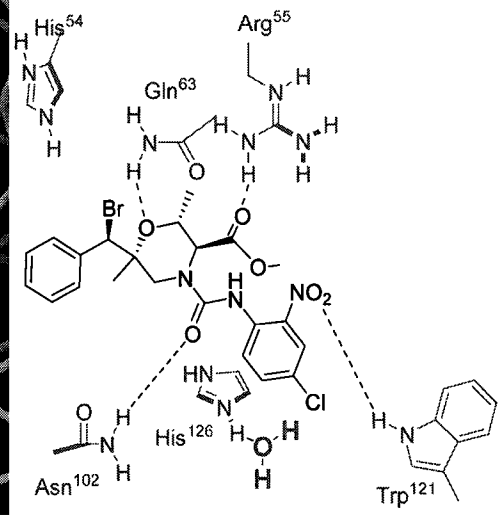
The second two highest scoring compounds (Filenames: cypAlibrary_53.out and cypAlibrary_54.out) are morpholine-based diastereomers **1** and **2**. Both compounds were predicted to show similar affinities (scores: (**2**)-15.7 and (**1**)-16.0) and were close to the ranking of the lead compound (-16.5). Scoring poses and interactions between Cyp A active site and both compounds are shown below in Figure 3.2. The two ligands are bound to 1bck protein since it was the one with lowest RMSD value. The binding mode of the each one of the ligands to the active site is different. However both compounds give second highest rankings in the library. The two diastereomers interact with the same residues, but the interactions of each residue with the specific functionalities in each compound differ. For instance interaction of the side chain (nitro group) of Gln63 with the ligand varies. The nitro side chain of Gln63 interacts with the ether oxygen in the ring of compound **1**, whereas in compound **2,** it interacts with the nitro group.

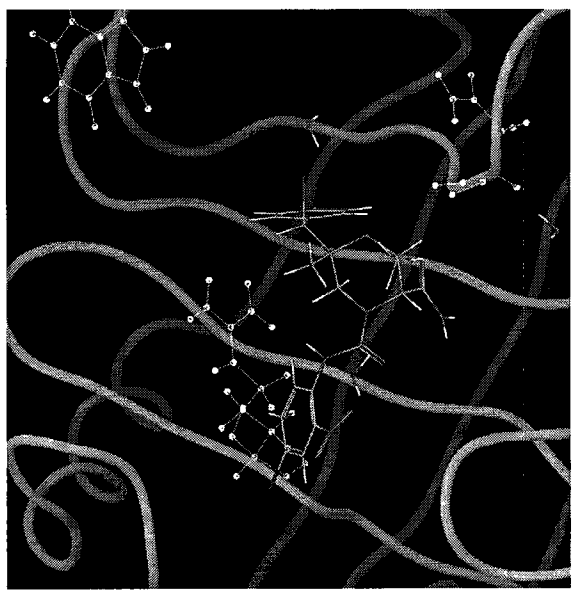


(2*R*,3*S*,6*S*)-methyl 6-((*R*)-bromo(phenyl)methyl)-4-((4-chloro-2-nitrophenyl)carbamoyl)-2,6-dimethyl morpholine-3-carboxylate



(2*S*,3*R*,6*S*)-methyl 6-((*R*)-bromo(phenyl)methyl)-4-((4-chloro-2-nitrophenyl)carbamoyl)-2,6-dimethyl morpholine-3-carboxylate





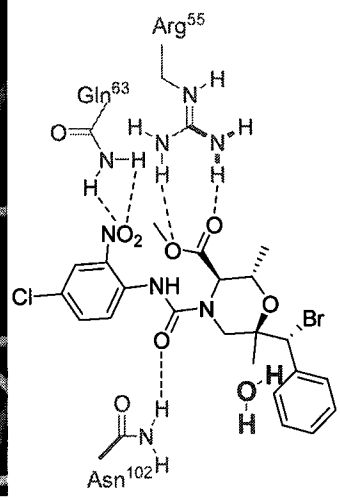


Figure 3.2: *Binding modes of compound* **1** *and* **2** *with the active site of Cyp A.*

Other compounds in the library were very similar in structure to compounds **1** and **2**, with *N*- group variation. *N*-methyl, benzyl and tosyl groups are present in the library, but diarylurea compound have shown stronger binding to the active site. This was confirmed by studying the fourth highest ranking compound (Filename: cypAlibrary_22.out) that has a diarylurea moiety as well.

As of now, the bromide derivatives were docked. Although, they are expected to be toxic, this was the first step of an *in silico* multi-step synthesis. Now that the best nitrogen functional groups are identified, we will substitute the bromide with a library of alkoxides and amines and dock this second library.

3.4 Conclusion

A docking- based virtual screening study was conducted to investigate the binding mode of morpholine-based compounds to the active site of Cyp A. The scores obtained for the designed ligands were much higher than the scores obtained for the crystal structure ligands (meaning that designed ligands are predicted to be weaker binders or non binders). The interactions between the two designed compounds (with the highest rankings) and the active site of Cyp A were described.

It was concluded that more work should be done. First, substituting the bromide and then switching to a bigger library of morpholines and oxazepines.

3.5 References

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Chapter 4: Conclusions and Future Work

4.1 Conclusion

An expedient synthetic approach has been developed for the preparation of chiral poly-substituted morpholine and oxazepine derivatives. Aiming towards its application to the preparation of potential Cyp A inhibitors and other drugs, we designed this methodology with the focus on its applicability to drug discovery.

The stereo and regioselective method was developed starting from readily available materials that introduced stereoselectivity and diversity points to the final products. Starting from enantiopure amino alcohols and α,β-unsaturated aldehydes, the method was conducted through three steps; reductive amination, *N*-alkylation/ tosylation and cyclization through haloetherification. Regioselectivity of the cyclization reaction was controlled by the chemical nature of the substituents of the double bond to yield morpholines through 6-*exo* cyclization and oxazepines through 7-*endo* cyclization.

Yields and stereoselectivity of the cyclization step were found to be significantly affected by the group attached to the nitrogen atom. For instance, *N*-methylated precursors were cyclized to give the desired products in moderate yields and no diastereoselectivity. When moving to *N*-benzyl groups, the yields remained moderate but some levels of stereoselectivity were observed, ranging from 30 to 83% diastereomeric excess. Finally efficient cyclizations were obtained upon cyclizing *N*-tosylated precursors. Yields ranged from around 40% in the formation of morpholine rings to around 80% for the preparation of oxazepines. Tosyl groups also induce good diastereoselectivities with dr of up to 80% in the synthesis of oxazepines. Therefore it was

concluded that higher stereoselectivity is induced by the cyclization of *N*-benzylated and *N*-tosylated precursors with aromatic substitution across the double bond. Moreover, stereoselectivity is enhanced by the use of enantiopure amino alcohols and Brønsted acids.

A docking-based virtual screening study was next performed to evaluated the affinity and predict the binding mode of morpholine-based derivatives to Cyp A binding site. A library of 58 morpholine based compounds was screened. Four compounds were given the highest scores, and are considered potential hits (starting points).

4.2 Future Work

4.2.1 One-pot Synthesis

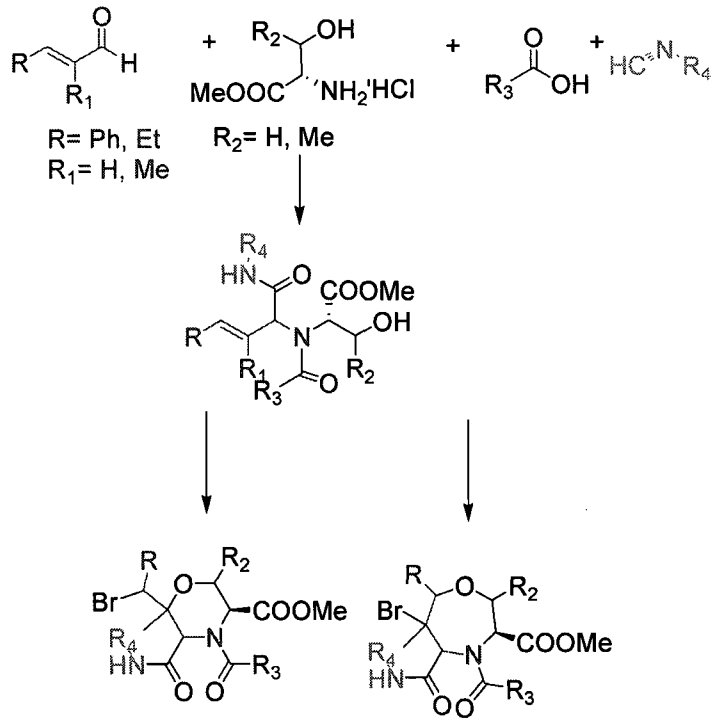
One-pot synthesis of poly-substituted morpholines and oxazepines would be ideal. Applying the developed methodology to a one-pot approach can be the next step in optimizing the developed method. This can be done by careful optimization of the reaction conditions to suit the three reactions at once. Choice of the solvent for one-pot synthesis is critical since different solvents are used for each step in the developed methodology. One-pot approach can be carried out in MeCN as a solvent, since MeCN is suitable for reductive amination,[1] *N*-alkylation/ tosylation and cyclization.

4.2.2 Multi-component approach

The developed method describes the preparation of chiral poly-substituted morpholine and oxazepine derivatives through 2-3 steps depending on the *N*-alkylating/ tosylating procedure. Challenges faced by reductive amination and *N*-alkylation/ tosylation steps can be overcome using different synthetic approaches. One-pot synthesis of *N*-alkylated/ tosylated precursors can be achieved by conducting multi-component reactions. The Ugi reaction[2] can be used for the

preparation of *N*-alkylated/ tosylated precursors leading to interesting peptidomimetic compounds,[3] with more diversity points.

The Ugi reaction consists of four-component condensations (U-4CC); an aldehyde or a ketone, an amine, a carboxylic acid and an isocyanide producing α-aminoacyl amide derivatives. In other words, starting from cheap readily available starting materials, poly-substituted *N*-alkylated/ tosylated precursors will be formed in one step and in good yields.[4,5] Proposed precursor preparation through Ugi reaction is presented in Scheme 1.



Scheme 4.1: *Ugi multicomponent approach for the preparation of N-alkylated/ tosylated cyclization precursors, followed by haloetherification.*

Upon formation of the precursor, cyclization can proceed as described in Chapter 2. A large library of scaffolds with different functionalities can be obtained by varying the starting material (wide range of reagents/ starting material are commercially available), which aids generating a large library of Cyp A inhibitors in the future. The final product bears an amide group that can undergo peptide coupling and hence generating peptidomimetic Cyp A inhibitors.

4.2.3 Library of Cyp A potential inhibitors

Preliminary work has been done towards designing a library of morpholine-based compounds *in-silico*. The small library of compounds was screened for potential Cyp A inhibitors. However, potential bioactive morpholine and oxazepine based Cyp A inhibitors must be investigated more closely. Design and synthesis of a larger library of functionalized morpholine and oxazepine derivatives can be part of the future work. Biological assays of the resulting hit compounds would be the last stage to complete the medicinal chemistry project. Arrangements for biological assays have already been made with Walkinshaw and coworker.[6] Activity and potency of hit compounds would be assessed against *Caenorhabditis elegans*.

4.3 References

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