STUDIES TOWARD THE ASYMMETRIC SYNTHESIS OF NOVEL CHIRAL 1,4-OXAZEPAN-5-ONE DERIVATIVES

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ABSTRACT

STUDIES TOWARD THE ASYMMETRIC SYNTHESIS OF NOVEL CHIRAL 1,4-OXAZEPAN-5-ONE DERIVATIVES

Pharmacophore design to inhibit the interaction between p53 and MDM2 became a novel approach for cancer therapy. p53, known as the guardian of genome, controls the cell cycle arrest, apoptosis and DNA repair under stress. Nonetheless, when over-expressed, MDM2 causes proliferation in the cell and eventually tumorgenesis.

The feedback mechanism between p53 and MDM2 arises from the interaction of p53 through the hydrophobic cleft which consists of Phe19, Trp23 and Leu26 aminoacids in the N-terminal of MDM2.

In this study, it was aimed to synthesize a new, chiral 1,4-oxazepan-5-one derivatives by asymmetric synthesis. *(R)*-2-amino-2-(4-chlorophenyl)acetic acid was used as starting material. Amino group was protected by trityl group then the carboxylic acid part was reduced by $LiAlH_4$ to produce N-trityl-protected amino alcohol. Dess Martin periodinane was used for the oxidation of the alcohol to the aldehyde, then 3-chlorophenylmagnesium bromide was added to the aldehyde by Grignard reaction. Deprotection of N-trityl was performed with TFA then, coupling reactions of produced aminoalcohol with different α,β-unsaturated carboxylic acids were performed by HATU and DIPEA. Despite all of the attempts, cyclization to seven membered 1,4-oxazepan-5-one ring was never achieved.

ÖZET

YENİ KİRAL 1,4-OKZAZEPAN-5-ON TÜREVLERİNİN ASİMETRİK SENTEZİNE YÖNELİK ÇALIŞMALAR

p53 ve MDM2 arasındaki etkileşimi engellemek için farmakofor tasarımı kanser terapisinde yeni bir yaklaşım olmaktadır. Genomum koruyucusu olarak bilinen p53 stres altında hücre siklusunda aresti, apaptozu ve DNA tamirini kontrol eder. Bununla birlikte, MDM2 fazla eksprese edildiğinde hücrenin çabuk çoğalmasına ve neticede tümör oluşumuna neden olur.

p53 ve MDM2 arasındaki geribesleme mekanizması p53'ün, MDM2'nin N-ucundaki Phe19, Trp23 ve Leu26 aminoasitlerinden oluşan hidrofobik boşluktan etkileşir.

Bu çalışmada asimetrik sentez ile yeni kiral 1,4-oksazepan-5-on türevlerinin sentezlenmesi amaçlanmıştır. *(R)*-2-amino-2-(4-chlorophenyl)acetik asit başlangıç maddesi olarak kullanıldı. Amino grubu tritil grubu ile korundu ve daha sonra maddenin karboksilik asit kısmı $LiAlH_4$ ile N-korunmalı amino alkol elde edmek üzere indirgendi. Dess Martin periodinane alkolün aldehite oksidasyon için kullanıldı, daha sonra 3-klorofenilmagnezyum bromür Grignard reaksiyonu ile aldehite eklendi. Koruyucu tritil grubu TFA ile uzaklaştırıldı ve oluşan aminoalkolün farklı α,β-doymamış karboksilik asit ile bağlanma reaksiyonları HATU ve DIPEA kullanılarak gerçekleştirildi. Tüm denemelere rağmen yedi üyeli 1,4-oksazepan-5-on halkalaşması hiç bir zaman gerçekleştirilemedi.

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LIST OF ABBREVATIONS

BINAP	(2,2′-bis(diphenylphosphino)-1,1′-binaphthyl)
d	Doublet
dd	Doublet of doublets
dt	Doublet of triplets
ddd	Doublet of doublet of doublets
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIPEA	N,N-Diisopropylethylamine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethylsulfoxide
Et_3N	Triethylamine
Eq.	Equivalent
h	Hour
HATU	(1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HOAc	Acetic Acid
LDA	Lithium diisopropylamide
m	Multiplet
MeOH	Methanol
min	Minute
µL	Microliter
mg	Milligram
mL	Milliliter
NMR	Nuclear magnetic resonance

PCC	Pyridium chlorochromate
r-PPh_3	Resin bounded triphenyl phosphine
RT	Room temperature
s	singlet
TBDMS	*tert*-Butylmetylsilyl
Temp	Temperature
TMSCl	Trimethylsilyl chloride
THF	Tetrahydrofuran

CHAPTER 1

INTRODUCTION

1.1. p53 Gene: Guardian of Genome

The transcription factor p53, identified in 1979, is known to be the "guardian of genome".[1-2] The reason behind this name is, upon stress, it promotes the expression of genes that is responsible from cell cycle arrest, apoptosis and DNA repair.[1]

The gene p53 is a homotetramer with a domain organization of transactivation domain (TAD), proline (Pro) - rich region (PR), structural DNA binding domain (DBD), tetramerization domain (TET) and C-terminal regulatory domain (CTD) (Figure 1.1). The 1-40 residues create the region TAD1 and the remaining residues, 40-60, create TAD2. (Figure 1.1).[3]

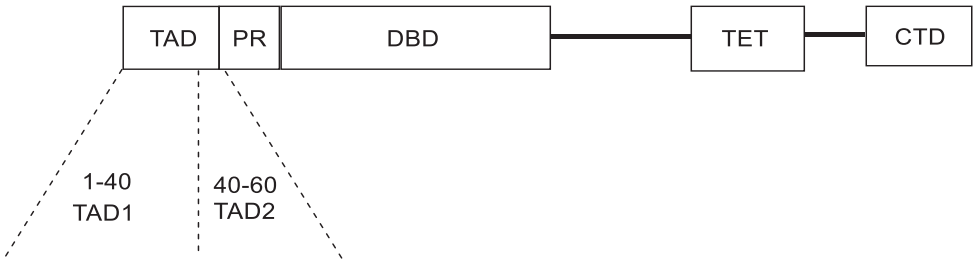


Figure 1.1. The domain organization of p53.[3]

After its identification, the gene TP53, was cloned in 1983. In 1990, p53 gene, TP53, was found to be associated with the cases of Li-Fraumeni syndrome, an inherited condition that causes infected families to get some types of cancer. As a result of series of works during the years it was concluded that alteration in p53 causes wide variety of cancers.[4] These alterations can be a mutation in p53 gene or its complete deletion, or the mutations in post-translational stage. As its activity turned off, apoptosis and cell cycle arrests cannot be arranged and eventually proliferation occurs.[1]

As 50% of human cancers caused by the genetic alterations, remaining percentage is caused by the inhibition of gene by a small protein MDM2 which is also the primary inhibitor of p53. Increased expression of MDM2 inhibits the activity of p53 and causes to cell proliferation by uncontrolled cell cycle arrest or apoptosis.[2] Expression of MDM2 is amplified mostly in bone (~16%) and soft tissue (~20%) sarcomas, and esophageal (~13%) carcinomas.[5]

1.2. MDM2: A Regulator of Cell Growth

MDM2, murine double minute 2, encodes 489 amino acids whereas its human version, HDM2, encodes 491. The expression levels of MDM2 effects the cellular transformations and cellular growth which is asspociated with the tumorgenicity.[5] The functional domains of MDM2 consist of; N-terminal (p53 binding regions) domain, nuclear localization signal (NLS), nuclear export signal (NES), an acidic domain, Zinc finger domain and RING finger domain located at C-terminal (Figure 1.2).[5-6]

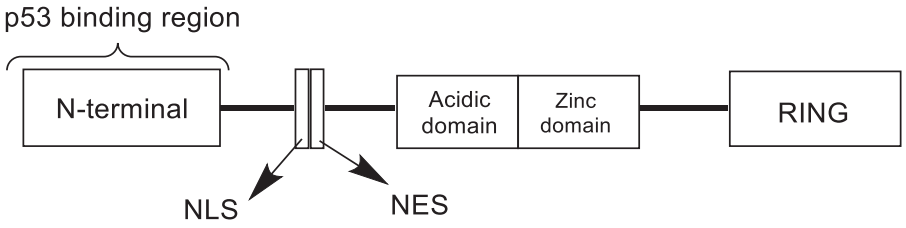


Figure 1.2. The functional domains of MDM2.[6]

The N-terminal, p53 binding region of MDM2, interacts with three important amino acid residues of p53; phenylalanine (Phe) 19, tryptophan (Trp) 23, and leucine (Leu) 26.[1-3]

The RING domain is responsible for the ubiquitin-ligase, E3, activities for the target proteins. Along with target proteins, MDM2 also has the ability of self-ubiquitination however it is incapable of controlling its expression levels in the cell.

Recent genetic works showed that, MDM2 levels are stabilized by another ubiquitin-ligase in vivo.[5-6]

1.3. p53-MDM2 Interaction: An Approach for Cancer Therapy

The vital importance between MDM2 and p53 was discovered in a research in vivo. It was seen that MDM2$^{-/-}$ mice die at very early stages of life because of p53-dependent apoptosis. Interestingly, it was also seen that knock-out mice (MDM2$^{-/-}$, p53$^{-/-}$) survive along with p53$^{-/-}$ mice, indicating that in the absence of both MDM2 and p53 development continues as normal.[6]

The activity of p53 is regulated in normal cells by a feedback mechanism with a regulatory protein MDM2 which is also the primary inhibitor of p53.[7] This mechanism between MDM2 and p53 works through the interaction between the amino acids phenylalanine (Phe) 19, tryptophan (Trp) 23, and leucine (Leu) 26 in TAD1 region of p53 and N-terminal of MDM2.[3] Upon stress the p53 gene gets activated and its expression increases inside the cell. This expression is regulated through its interaction with MDM2 by ubiqutination followed by proteasomal degredation or nuclear export (Figure 1.3).[7]

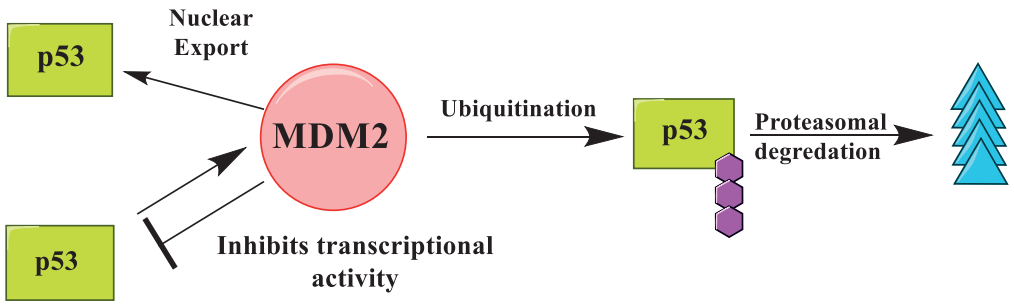


Figure 1.3. The feed-back mechanism between MDM2 and p53 in normal cells. MDM2 either provides the nuclear export of p53 or proteasomal degredation by ubiquitination.[7]

Ubiquitination is a process that labels proteins for degredation, and it consists of three steps; activation, conjugation and ligation (Figure 1.4). The mechanism works as

ubiquitin-activation enzymes, E1, targets and labels the proteins to be degraded by a proteasome, a protein complex that breaks down damaged, labeled or unnecessary proteins by proteolysis, a chemical reaction that breaks peptide bonds. The activation by E1 occurs via adenylation of C-terminus of ubiquitin and the transfer of ubiquitin on E1cysteinyl residue via thioester linkage and ATP is used during this process. The activated ubiqutin is then transferred onto ubiquitin-conjugating enzyme, E2, via thioester linkage on cystenyl residue of E2. Ubiquitin-ligase enzymes, E3, depending on its type, transfers ubiquitin from E2 to substrate either directly or after creating a thioester linkage between itself and ubiquitin protein.[8]

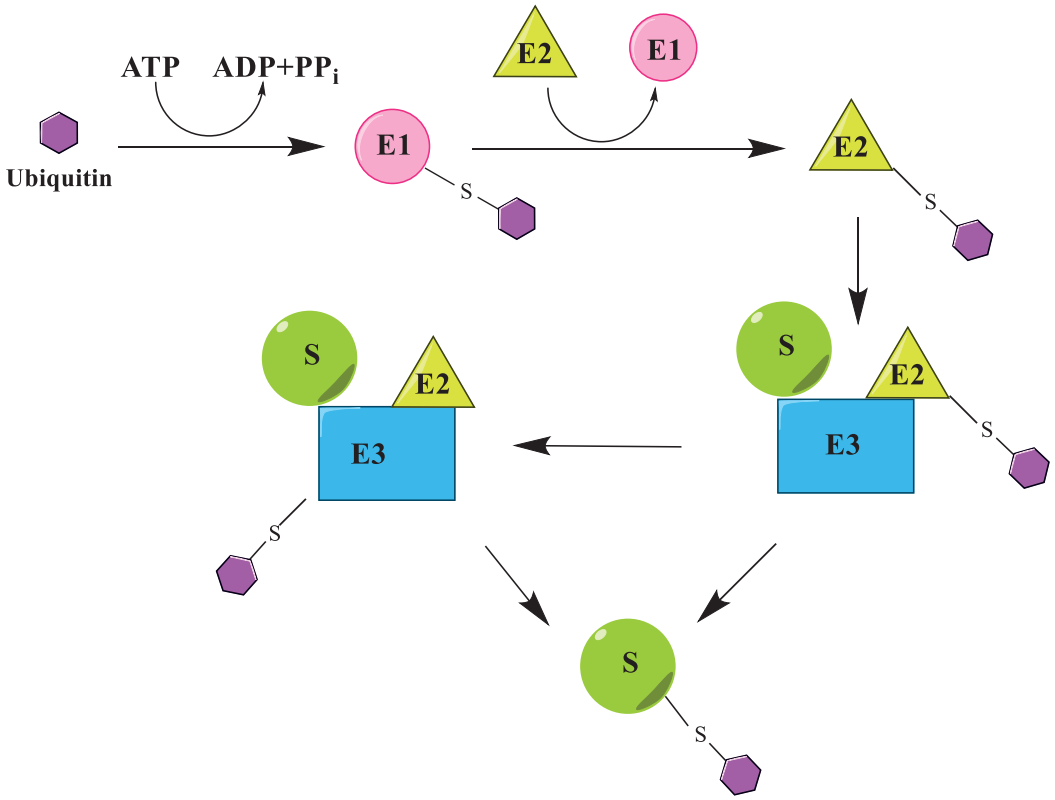


Figure 1.4. Shows ubiquitination mechanism in three steps; activation by E1, conjugation by E2 and ligation on substrate, S, by E3.[8]

MDM2 ubiquitinates p53 via its ubiquitin-ligase, E3, activity in RING domain and prevents cell cycle arrest by decreasing the levels of p53. Through understanding the

mechanism and interaction between p53-MDM2 and, regulation of the signaling pathways inside the cell, a novel approach is developed for cancer therapy.[3]

1.4. Design of a Pharmacophore for p53-MDM2 Interaction

Pharmacophore first defined as “a molecule having the essential properties which induce a biological activity” by Paul Ehrlich in 1990. The term is generated from the words pharmaco-drug and phore-carrier. [9]

Pharmacophore design can be achieved either as ligand-based or structure-based modeling. In first modeling type the aim is to determine chemical features of a ligand that is known to be interacted with the ligand and the target, in the latter the goal is to analyze the chemical features of the active site and the 3D relationship between the target and the ligand. Once the pharmacophore is designed the synthesis process begins.[10]

The high-resolution crystal structure analysis of the interaction between MDM2 and p53 showed that they bind through three hydrophobic aminoacids at i, i+4, i+7 positions, Phe19, Trp23, Leu26, in p53.[4-11] Further research in alanine scans showed that a mutation in any of those three aminoacids annihilates the binding between target proteins.[11] Pursuit of an inhibitor molecule of the MDM2-p53 interaction has arisen from those results as investigating natural compounds or new designs for the pharmacophore.[12]

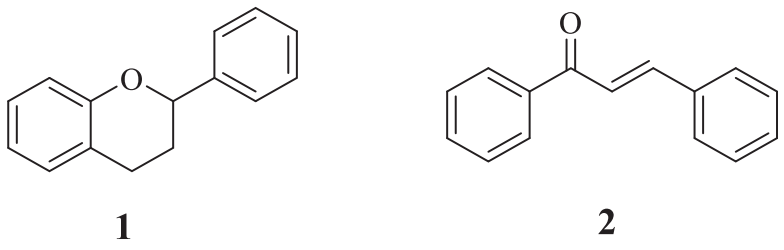
1.4.1. Natural Pharmacophores for MDM2-p53

Many of the drugs that have been designed are derived from a natural origin. Natural compounds that are discovered for inhibition of MDM2-p53 interaction can be arranged into three categories.[12] First one is the inhibitors that block the binding between MDM2 and p53 proteins. Second category is the inhibitors of E3 ligase activity of MDM2 and last one is the inhibitors that block only the activity of MDM2, not p53.[13]

1.4.1.1. Chalcone Derivatives

Flavonoids (Figure 1.5), compounds that are secondary metabolites of plants have beneficial effects on health.[14] Chalcone **(2)** is a member of flavonoid family have the effect of an antioxidant, antimicrobial, anti-imflammatory and anti-tumor for human body, also it is the first natural compound that is reported as anticancer agent.[12-15]

Chalcones can be investigated under the first category in which the compounds that block binding between two target proteins.[13] They break down the MDM2-p53 complex and causes p53 releasing into the cell. It was observed that released p53 is not able to bind to DNA and conclusions were made as chalcones causes an additional effect on p53 protein.[12]



1 **2**

Figure 1.5. Compoud **1** is the backbone of the flavonoid family, **2** is Chalcone.[14]

Boronic chalcones were designed and synthesized as an alternative and higher potency was observed. Examples of boronic chalcones, compounds **3** and **4** (Figure 1.6) showed antiproliferative activity with IC_{50} values, a measurement of the concentration of a drug at which inhibition of 50% of cell population is achieved, of 3.5 and 4.0 μM for cell line, MDA-MB-435, human breast cancer cell line, respectively.[11-16] In order to increase the binding affinity the linkage pattern was observed as the boronic acid of chalcone interacts with MDM2 by a salt bridge with amino acid Lys51. The developments on enhancing the biological activity of boronic chalcones lead to increased toxicity in human breast cancer cell lines caused by the inhibiton of other enzymes.[11-12]Most potent chalcone derivatives are compounds **5** (GI_{50} value of 2.1 – 3.2 μM range depending on

the type of tumor cell) and **6** (GI_{50} of 2.6 – 4.5 μM depending on the type of tumor cell) (Figure 1.6).[15]

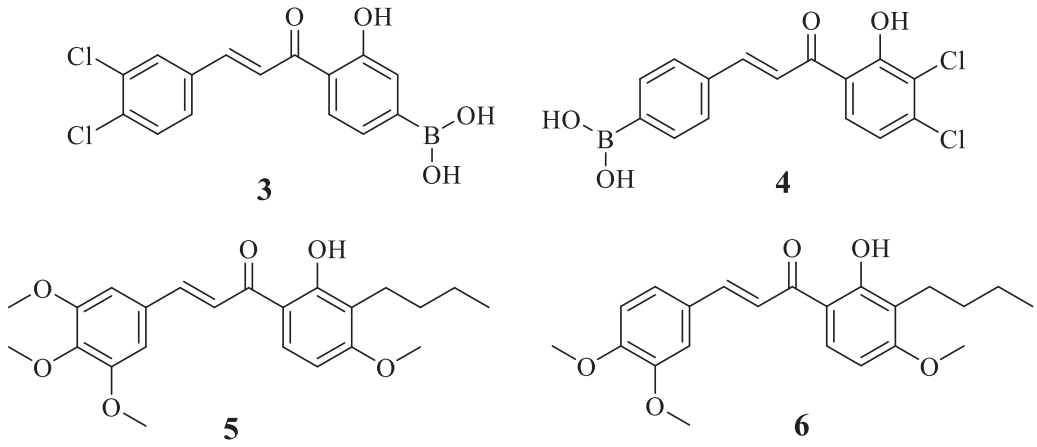


Figure 1.6. Structures of potent boronic chalcones (compounds **3** and **4**) and most potent chalcones examples (compounds **5** and **6**).[15]

Another example for chalcone derivatives is the compound isoliquiritigenin (4,2',4'-trihydroxychalcone) (Figure 1.7), compound **7**, showed potency (IC_{50} = 10.51 μg/mL) in Hep G2 cells by causing apoptosis in liver cancer cells. However the interaction between the compound and MDM2 was never discovered, therefore, it is not a candidate compound for cancer therapy.[12-17]

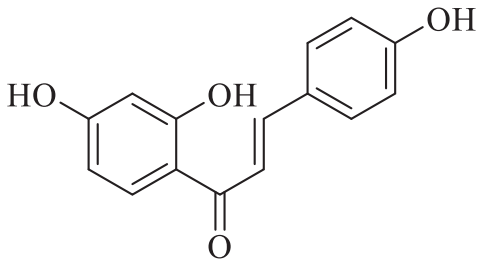
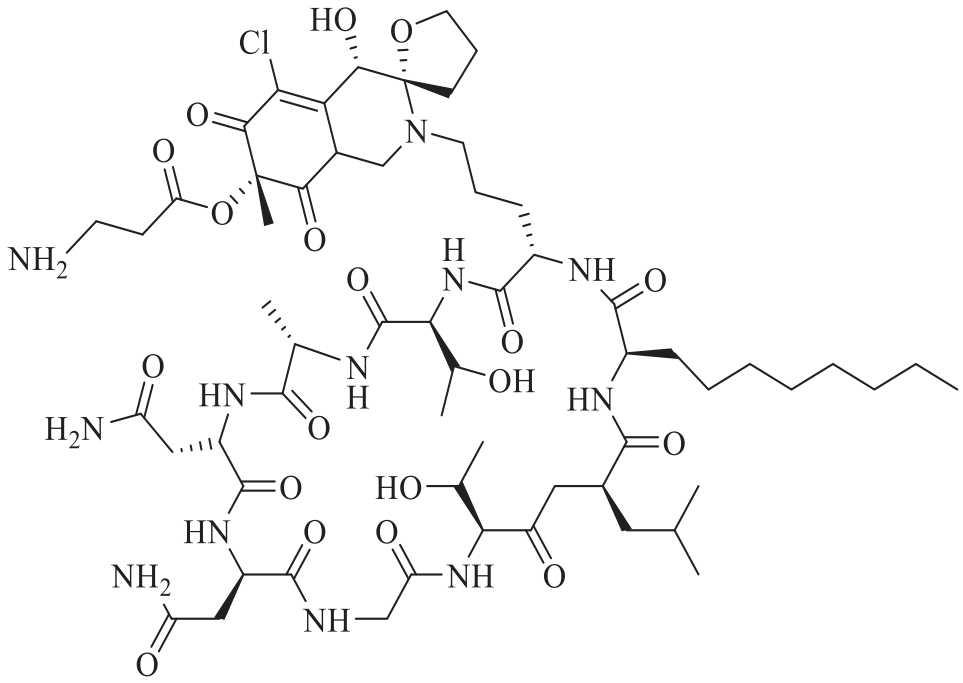


Figure 1.7. The structure of isoliquiritigenin (4,2',4'-trihydroxychalcone).[18]

1.4.1.2. Chlorofusin

Another example for natural pharmacophores is chlorofusin, compound **8**, (Figure 1.8), a natural peptide that is isolated from fungus. Chlorofousin is reported for the inhibition of MDM2-p53 interaction as a second natural compound.[11]



8

Figure 1.8. Structure of chlorofusin.[18]

Chlorofusin showed low potency (IC_{50} = 4.6 μM) in ELISA (enzyme-linked immunosorbent assay), a quantitave test that uses antibody-antigen relationship to measure the concentration of either antibody or antigen.[11-19] Although good binding affinity constant value, K_D, was obtained as 4.7 μM, the MDM2-inhibitor interaction could not be understood in some cases. [11-12]

Since the potency is low in this group of natural inhibitors and the binding pattern was not understood completely, they are not used as drugs in the today's market.[11]

1.4.2. Synthetic Pharmacophores for MDM2-p53

1.4.2.1. Nutlins

The first synthetic compound that has high potency and selectivity for the blocking the interaction between MDM2 and p53 is called as nutlins also known as *cis*-imidazoline.[20-21] Nutlin derivatives, compounds **9, 10, 11** and **12** (Figure 1.9) have IC_{50} value between 1-3 μM. Although racemic mixture of compound **11** was used at the beginning, it was then observed that compound **12** shows higher potency than its enantiomer (Figure 1.8).[21]

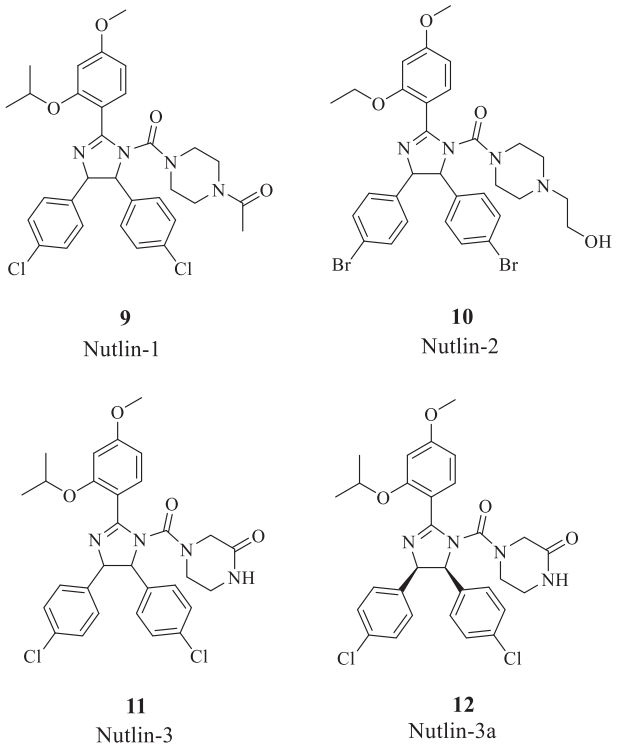


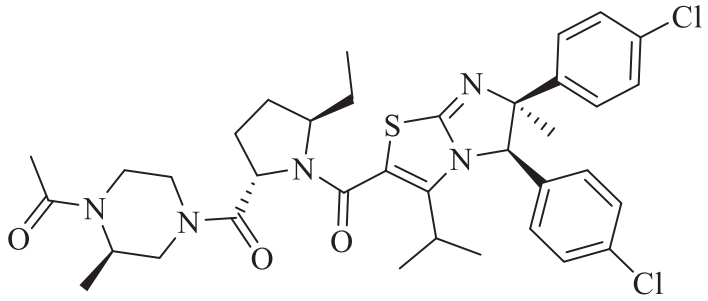
Figure 1.9. The structures of nutlin derivatives; nutlin-1, nutlin-2, nutlin-3 and the latter is more potent enantiomer nutlin-3a.[2-22]

The selectivity of compound **12** comes from its two chloro-substituted phenyl ring interacts with the Trp23 and Leu26 residues. The remaining amino acid, Phe19, interacts

with iso-proxy group on the third phenyl group. One other benefit of compound **12** is its resemblance to the α-helix on p53 with its imidazoline core.[23] Derivatives of nutlins have managed to enter clinical trials and being considered as potent inhibitors.[11]

1.4.2.2. Dihydroimidazothiazole Derivatives

After the success of nutlin-3a, a molecule with a binding resemblance to nutlins is designed. Dihydroimidazothiazole cored derivatives were designed with this approach and one derivative, compound **13** (Figure 1.10), showed desired potency (IC_{50} = 0.058 μM), metabolic stability and solubility along with enhanced antitumor activity compared to nutlin-3.



13

Figure 1.10. Structure of a dihydroimidazothiazole derivative.[24]

The increased stability of this derivative comes from the methyl linked piperazine moiety. Also, the binding of the molecule into MDM2 hydrophobic cleft was enhanced by (*2R*)-ethyl group on the pyrolidine moiety along with the interactions between chlorinated phenyl rings and Phe19, Trp23 and Leu26 residues on p53. Through these series of tests, it was also understood that the isopropyl side chain was too increased the effectiveness of binding through hydrophobic cleft.[24]

1.4.2.3. Thio-benzodiazepine Derivatives

Additional interaction, other than the three important aminoacids in hydrophobic cleft, between MDM2 and the inhibitor molecule, was seen in thio-benzodiazepine derivatives (Figure 1.11) with their ester group binds to Gly16 residue on MDM2. For compounds **14** and **15** the IC_{50} values are 5.69 μM and 7.53 μM in U2OS cell line with the binding affinities of 0.0910 μM and 0.890 μM, respectively.[25] The problem with these derivatives was the indetermination of active stereoisomer.[11]

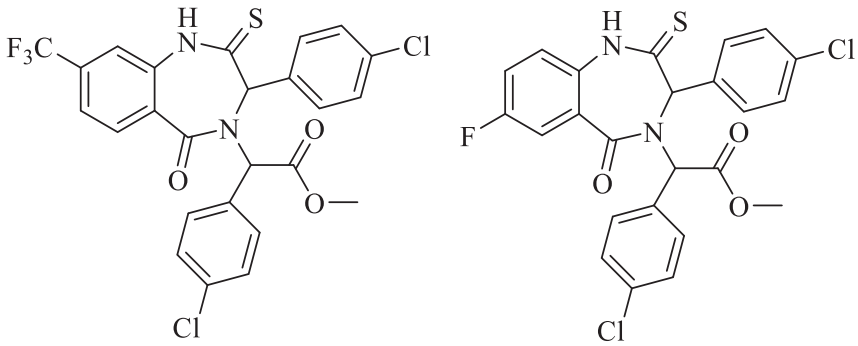


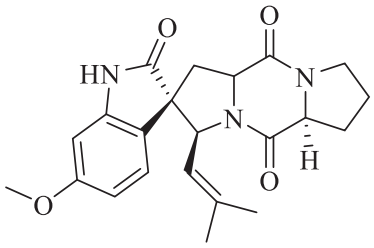
Figure 1.11. Structures of highly potent thio-benzodiazepine molecules.[25]

1.4.2.4. Spiro-oxindole Derivatives

In the interaction between MDM2 and p53, the residue Trp23 of p53 have a special feature having its indole ring buried inside the hydrophobic cleft which puts the aminoacid in a critical position for binding. From this point, a search for a molecule that can mimic this binding interaction was started and it was found that along with indole ring, oxindole is also capable of binding.

Further structure search led to spiro-oxindole cored natural compounds such as compound **16**, (Figure 1.12), however never used for trial since the molecules are highly

steric to fit in hydrophobic cleft of MDM2. Instead, compounds with the same core structure were designed.[25]



16
Spirotryprostatin A

Figure 1.12. The structure of spirotryprostatin A.[25]

The first potent inhibitor among spiro-oxindole derivatives (Figure 1.13) is compound **17** is found to fit into hydrophobic cleft (K_i = 86 nM) with a selective antiproliferative activity (IC_{50} = 0.83 μM for LNCaP cell line, IC_{50} = 10.5 μM for normal human prostate epithelial cells).[26]

The new modifications led to compound MI-219, compound **18**, which has a higher binding affinity (K_i = 5 nM) than p53 peptide and showed binding selectivity to MDM2 and apoptosis in cancer cells with low potency. Improvements were done on MI-219 to increase binding affinity and they were led to synthesis of MI-147, compound **19**, with higher binding affinity (K_i = 0.6 nM) and potency. Both MI-219 and MI-147 was used for the further optimizations led the synthesis of compound **20** which is used for clinical trials and now being used as a promising therapeutic drug.[11] The high binding affinity behind compound **20** comes from its perfect fit into hydrophobic cleft along with the additional π-π stacking between phenyl ring of 2-fluoro-3-chlorophenyl and His96. Another interaction with His96 is the hydrogen bonding of carbonyl group to the nitrogen of the imidazole side chain of the target residue. Extensive interactions with new amino acids were achieved with this molecule as N-terminal of MDM2 refolds and amino acids Val14 and Thr16 became exposed.[11-27]

A recent work also showed that another spiro-oxindole derivative, compound **21**, can act as p53 stabilizer, transactivator and an inducer of apoptosis. Compound **21** is the one having the most antiproliferative activity among other derivatives.[11]

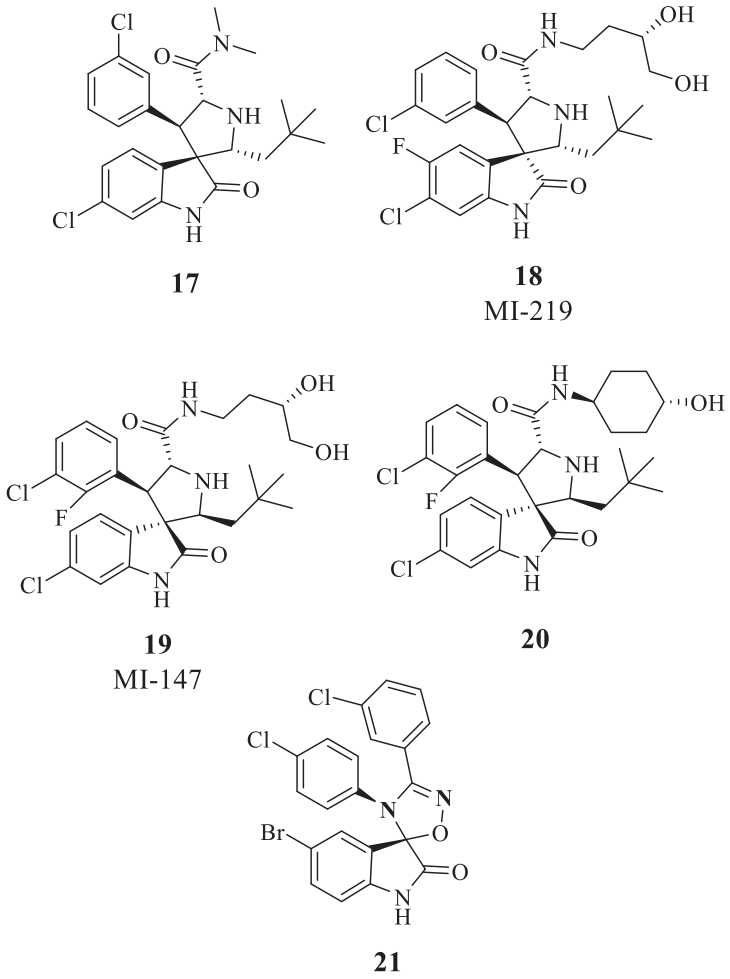


Figure 1.13. The structures of well known spiro-oxindole derivatives.[11]

1.4.2.5. Piperidinone Derivatives

Piperidinone derivatives (Figure 1.14) have the interaction between hydrophobic cleft and chlorophenyl substituents on the molecule. While chlorophenyl groups interact with Trp23 and Leu26 residues on p53, cyclopropyl group on compound **22** (IC_{50} = 34

nM) fits in Phe19 pocket. The sulfone group in compounds **25** and **26** directs ethyl group to Phe19 residue.

Additional interaction exists between carboxyl group and His96 residue. What makes piperidinone derivatives good candidate for further research is high antiproliferative activity along with the suitable interaction with MDM2. Also, the stability of the molecule was increased gauche conformation by methyl group.

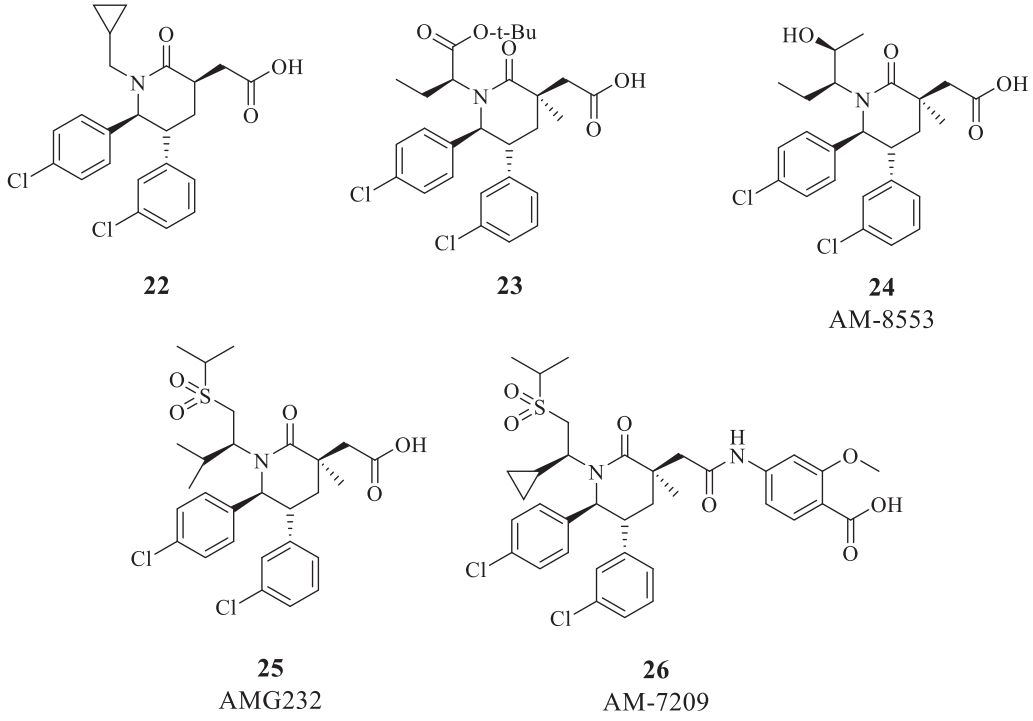


Figure 1.14. Structures of potent piperidinone derivatives.[28-29-30]

Modification on compound **22** lead to the synthesis of compound **23** that has methyl group on position 3 instead of hydrogen atom, which provides higher potency with gauche like conformation (IC_{50} = 0.22 nM). Replacement of *t*-butyl ester group with hydroxyl group led to compound **24**, AM8553, increased the binding affinity of the molecule to MDM2 by charge-charge interaction with His96 residue (IC_{50} = 11 nM).[28-29] Compound **24** was successful in trials with rats and showed high pharmacophore activity.

Further enhancement of the derivatives led the discovery of compound **25** (AMG232) which is being used in clinical trials (IC_{50} = 9.2 nM). Series of structural

modifications also led the compound **26** (AM-7209) with higher potency and pharmacokinetic profile (IC_{50} = 1.6 nM).[11-30]

1.4.2.6. Morpholinone Derivatives

Further development on the piperidinone structures based on quantum mechanical calculations, morpholinone core would provide better pharmacokinetic profile and more importantly the oxygen atom on morpholinone ring brings the molecule into more potent gauche conformation.

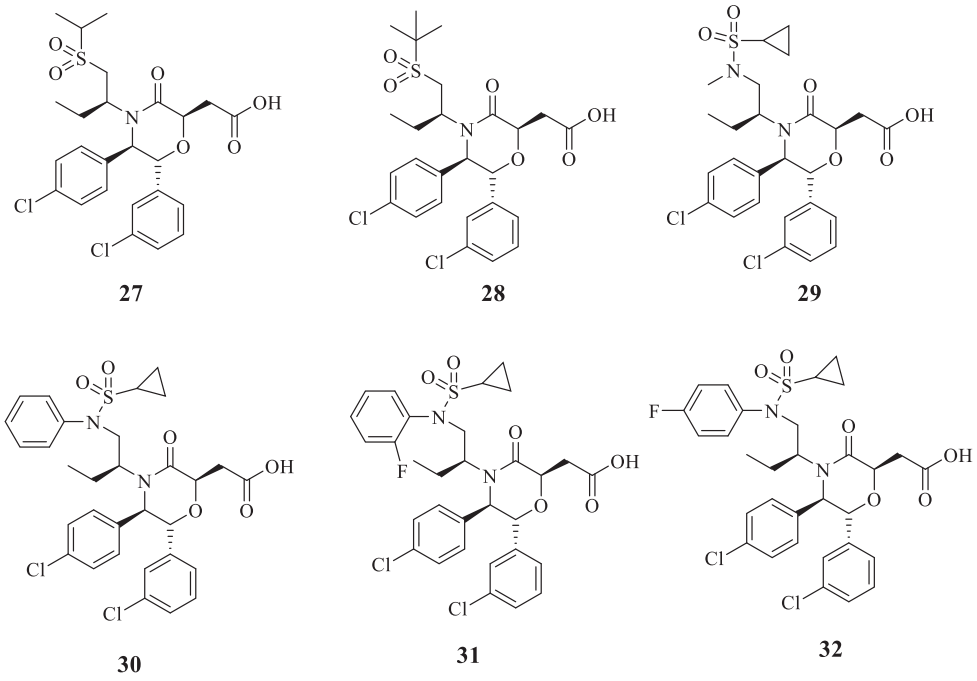


Figure 1.15. Structures of morpholinone derivatives.[1-11]

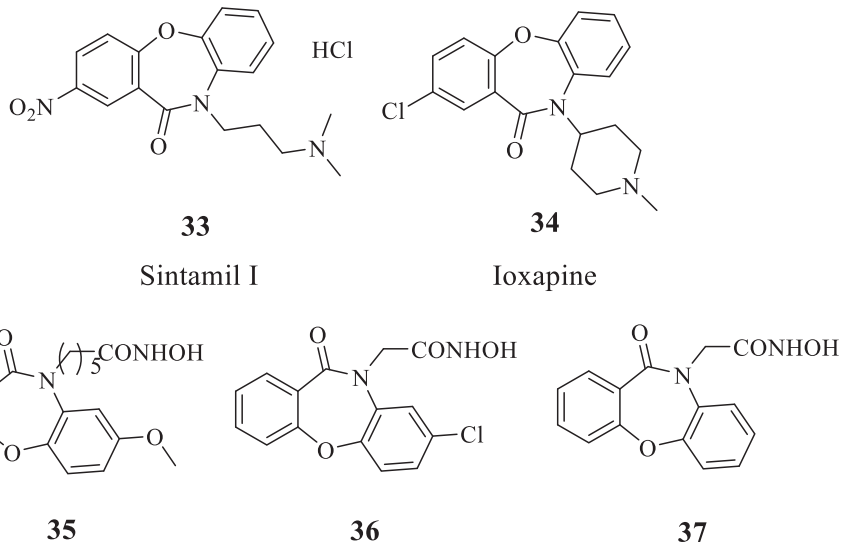
Modifications on *N*-alkyl substituent led to the synthesis of potent sulfonamides and sulfones, the examples are the compounds **27** (IC_{50} = 10.1 ± 0.1 nM) , **28** (IC_{50} = 2.0 ± 0.1 nM), **29** (IC_{50} = 11 ± 2 nM) and **30** (IC_{50}= 3.7 ± 1.5 nM) (Figure 1.15).

Most potent inhibitors were synthesized after the modifications on *N*-aryl substituent by the addition of fluorine atom on ortho position, compound **31** (IC_{50}= 2.3 ±

0.8 nM) compared to the derivative with fluorine on para position, compound **32** (IC_{50}= 6.0 ± 2.0 nM). In addition, these modifications enhanced the stability of the molecule.[1-11]

1.4.2.7. Oxazepinone Derivatives

Fused oxazepinone derivatives (Figure 1.16) show numerous biological activities such as antidepressant activity with the drugs that are being used in the market today, compound **33** and compound **34**. In addition, there are compounds among fused oxaepinone derivatives which show anticancer activity. The compounds **35**, **36** and **37** can be showed as examples.[31-32]



33 Sintamil I

34 Ioxapine

35

36

37

Figure 1.16. Structures of 1,4-oxazepan-5-one cored molecules with a known biological activity.[31-32]

1.5. Aim of the Study

The first goal of this study is to perform asymmetric synthesis of novel chiral 1,4-oxazepan-5-one derivatives as a pharmacophore for MDM2-p53 interaction, to discover

whether these derivatives can be used as inhibitors or not. The importance behind the chirality is stereoisomers might have different biological activites, therefore we aimed to perform asymmetric synthesis that is the synthesis of one stereoisomer in majority.[33]

In addition, it was aimed to enhance the content of the literature with the synthesis of derivatives of 1,4-oxazepan-5-one.

A synthetic pathway was designed in order to fulfill this goal (Figure 1.17). Pathway starts with the conversion of the amino acid *(R)*-2-amino-2-(4-chlorophenyl)acetic acid to the chiral aminoalcohol. Designed synthesis continues with the addition of benzyl ester and followed by cyclization reaction.

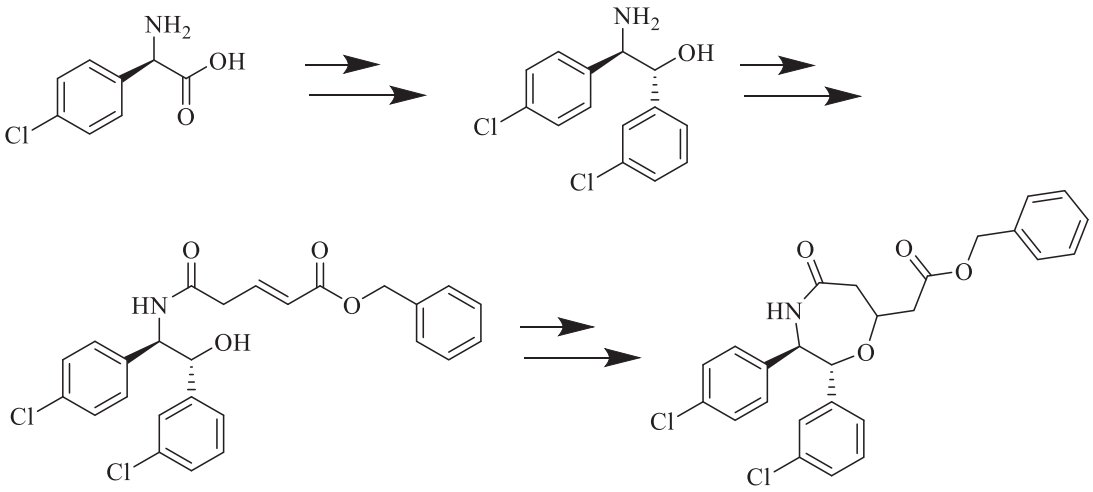


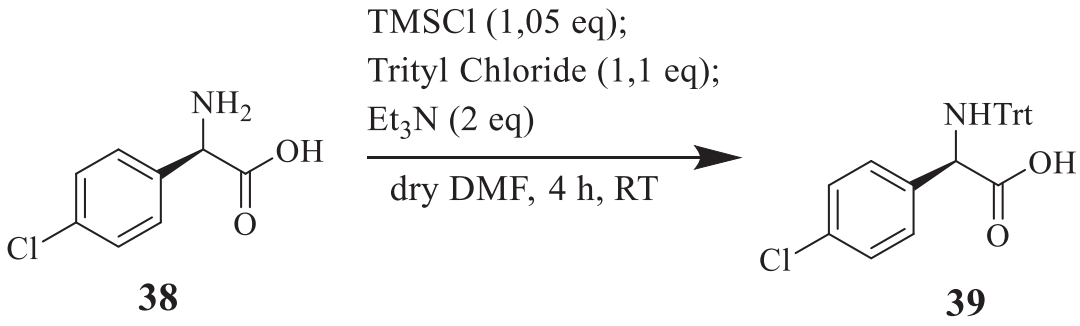
Figure 1.17. The designed synthesis pathway for the desired product.

CHAPTER 2

RESULTS AND DISCUSSION

2.1. N-Trityl Protection of (*R*)-2-amino-2-(4-chlorophenyl)acetic Acid

Synthesis was started with the protection of amino group of (*R*)-2-amino-2-(4-chlorophenyl)acetic acid **(38)** with trityl chloride (Figure 2.1). Reaction was performed in dry DMF at RT in 4 h and quenched with 1 M HCl to tune the pH of the water phase to 3 which is the optimal pH range of trityl group.



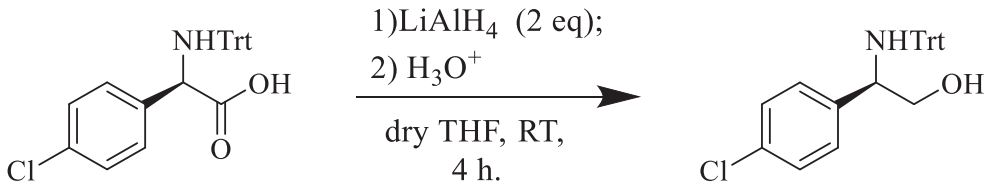
38 **39**

Figure 2.1. N-Trityl protection of (*R*)-2-amino-2-(4-chlorophenyl)acetic acid by using trityl chloride.

However, the acid used in work-up was altered from 1 M HCl to 0.05 M H_2SO_4 to minimize the interaction of trityl group with strong acid, because, according to crude ^{1}H NMR spectrum, some of the trityl group was damaged probably due to its low stability at high acidic mediums. It was thought to tune the pH by diluted acid to prevent the decomposition of the protecting group. Thus, diluted acid (0.05 M H_2SO_4) was used for further trials. The product was obtained as white foam and used without purification for the next step.[1]

2.2. Reduction of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetic Acid with $LiAlH_4$

Synthesis continued with the reduction of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetic acid **(39)** by using $LiAlH_4$ as reducing agent into (*R*)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol **(40)** in dry THF at RT in 4 hours (Figure 2.2).[1] The resultant compound was purified by column chromatography to afford compound **40** as white foam (49-73% yield).



39 **40**

Figure 2.2. The reduction of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetic acid to (*R*)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol by $LiAlH_4$.

2.3. Oxidation of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol by Dess-Martin Periodinane

The reagent (*R*)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol **(40)** was oxidized to (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetaldehyde **(41)** by using Dess-Martin Periodinane as oxidizing agent in wet DCM at RT (Figure 2.3).[1] Dess-Martin Periodinane was chosen because, it gives high yields under mild reaction conditions (RT, neutral pH) in shorter reaction times with simple workups. The most important advantage of DMP is its chemoselectivity; its ability to oxidize N-protected amino alcohols without epimerization. [34-35] The reaction was completed in 1 hour, the product was obtained as

white foam and was used without purification because of the possibility of rapid racemization. The yield of the reaction was ranged from 85-95%.

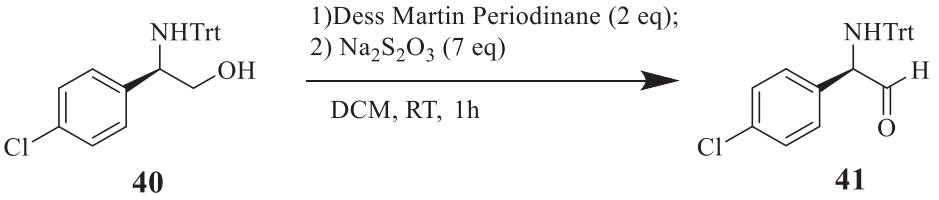


Figure 2.3. Oxidation of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol to (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetaldehyde by using Dess Martin Periodinane.

2.4. Synthesis of (1*R*,2*R*)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol by Grignard Reaction

The synthesis of (1*R*,2*R*)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol **(43)** was performed by using (3-chlorophenyl)magnesium bromide **(42)** in dry THF at -40 °C (Figure 2.4). The reaction was completed in 2 hours then, it was purified with column chromatography. [1] The resultant product was obtained as white foam. Grignard reaction was performed many times; the yield and diastereomeric ratios of the product were different in each trial. The yield of the reaction was in the range of 25-42% and the ratio of diastereomers were from 1:0.01 to 1:0.16.

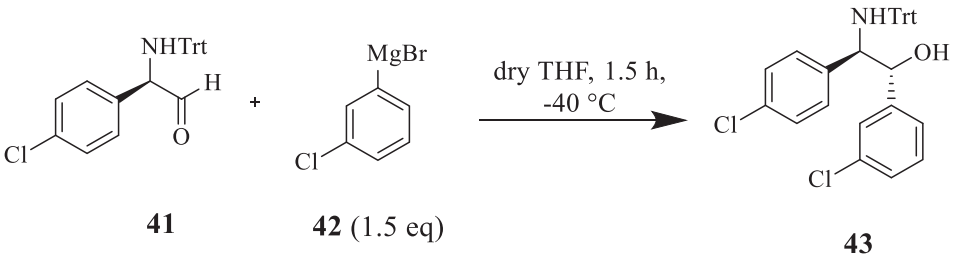
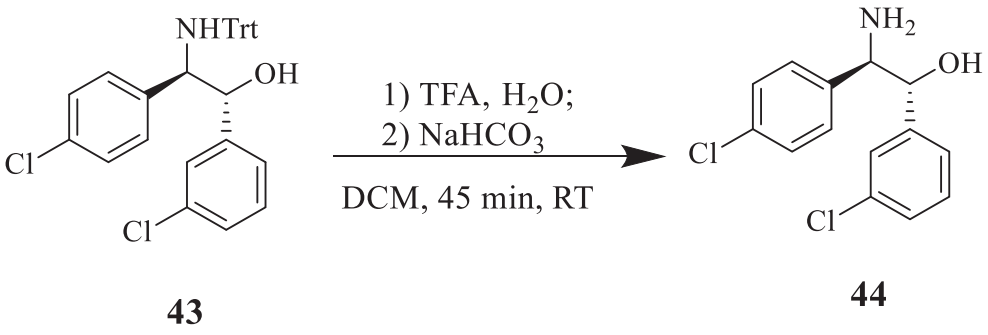


Figure 2.4. The synthesis of (1R,2R)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol.

2.5. Synthesis of (1*R*,2*R*)-2-amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethan-1-ol by Deprotection of Trityl Group with Trifluoro Acetic Acid

Deprotection of trityl group of (1*R*,2*R*)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol **(43)** was done by using TFA in DCM at RT (Figure 2.5). The product was purified with column chromatography and obtained as yellow oil. The yield of the reaction was in range of 37-56%. [1]



43 **44**

Figure 2.5. Deprotection of trityl group by trifluoroacetic acid.

2.6. Synthesis of (*E*)-5-(benzyloxy)-5-oxopent-3-enoic Acid

(*E*)-5-(benzyloxy)-5-oxopent-3-enoic acid **(47)** was tried to be synthesized from the reaction between (*E*)-pent-2-enedioic acid **(45)** and benzyl alcohol **(46)** in dry DMF at RT (Figure 2.6).[36] Reaction mixture was stirred for 6h and it was monitored with TLC control.

According to ^{1}H NMR spectrums of the products, it was concluded as benzyl alcohol is difficult to be removed during work-up. In addition, the products were not purified from benzyl alcohol in column chromatography. Due to these challenges, it was

decided to continue the synthesis with another alcohol which might afford to obtain pure product.

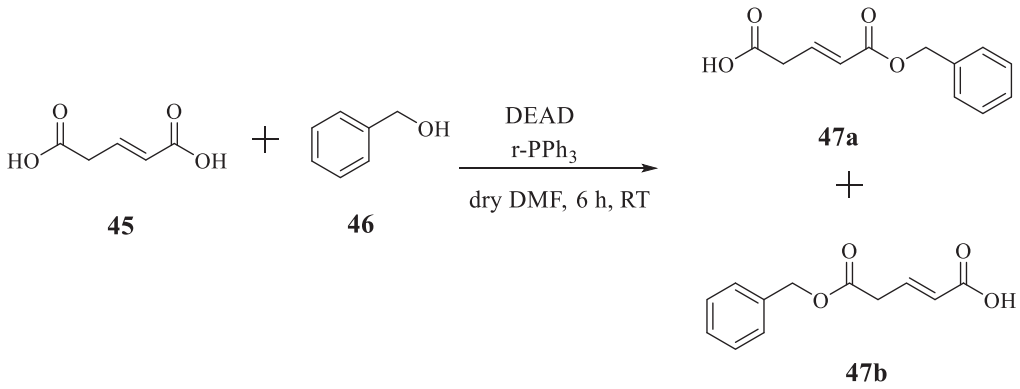


Figure 2.6. Planned synthesis of (*E*)-5-(benzyloxy)-5-oxopent-3-enoic acid occured with (*E*)-5-(benzyloxy)-5-oxopent-2-enoic acid

Thus, a new synthetic pathway was designed with MeOH instead of benzyl alcohol. MeOH having low boiling point, it would be removed by rotary evaporator process before purification step. Which would eliminate the challenges we have encountered during purification of compound **47** in column chromatography. (Figure 2.7.)

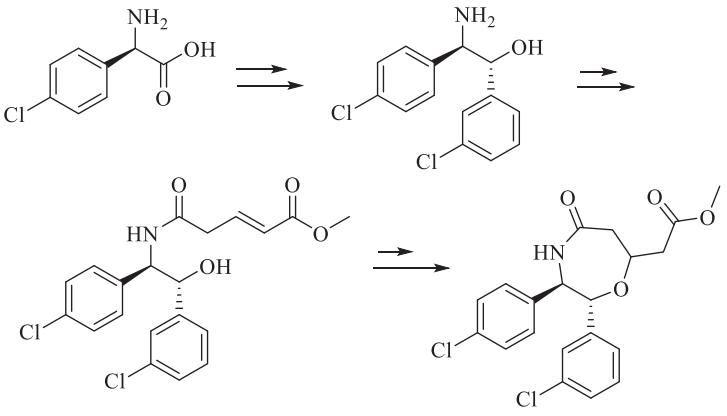
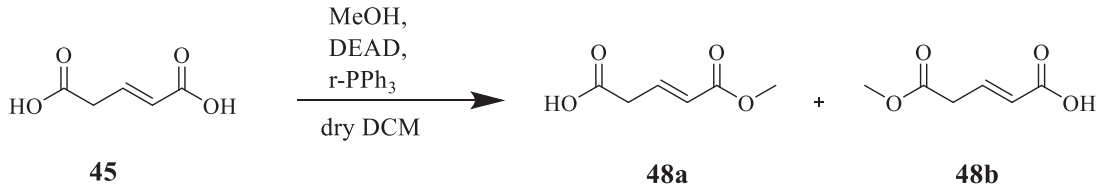


Figure 2.7. New synthesis pathway with MeOH

2.7. Synthesis of (*E*)-5-methoxy-5-oxopent-3-enoic Acid

(*E*)-5-methoxy-5-oxopent-3-enoic acid **(48a)** was tried to be synthesized in a reaction between (*E*)-pent-2-enedioic acid **(45)** and methanol in dry DCM at RT and stirred for overnight (Figure 2.8).[37] According to ^{1}H NMR signals it was concluded that two sets of products were formed. A mixture of two sets of compounds was used in the next step.



45 **48a** **48b**

Figure 2.8. Synthesis of (E)-5-methoxy-5-oxopent-3-enoic acid.

2.8. Synthesis of Methyl (*E*)-5-(((1R,2R)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)amino)-5-oxopent-2-enoate

Methyl (*E*)-5-(((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)amino)-5-oxopent-2-enoate **(49a)** was tried to be synthesized from the reaction between amino alcohol (compound **44)** and mixture of compounds **48a** and **48b** by HATU[38] and DIPEA in dry DMF (Figure 2.9). Reaction was stirred at RT and monitored by TLC control.

According to the ^{1}H NMR spectrum of the crude product, it was concluded that the desired product was not produced.

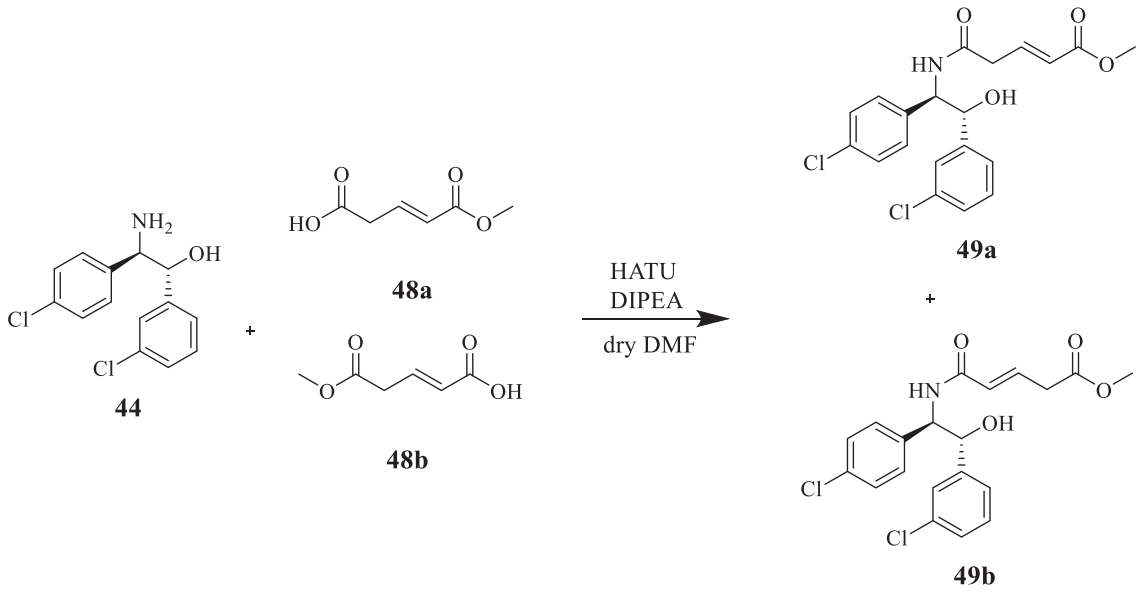


Figure 2.9. Planned synthesis of methyl (*E*)-5-(((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)amino)-5-oxopent-2-enoate.

Planned synthesis pathway was altered to prevent formation of two sets of compounds while preparing ester. For this purpose TBDMS protected alcohol was selected as starting material (Figure 2.10).

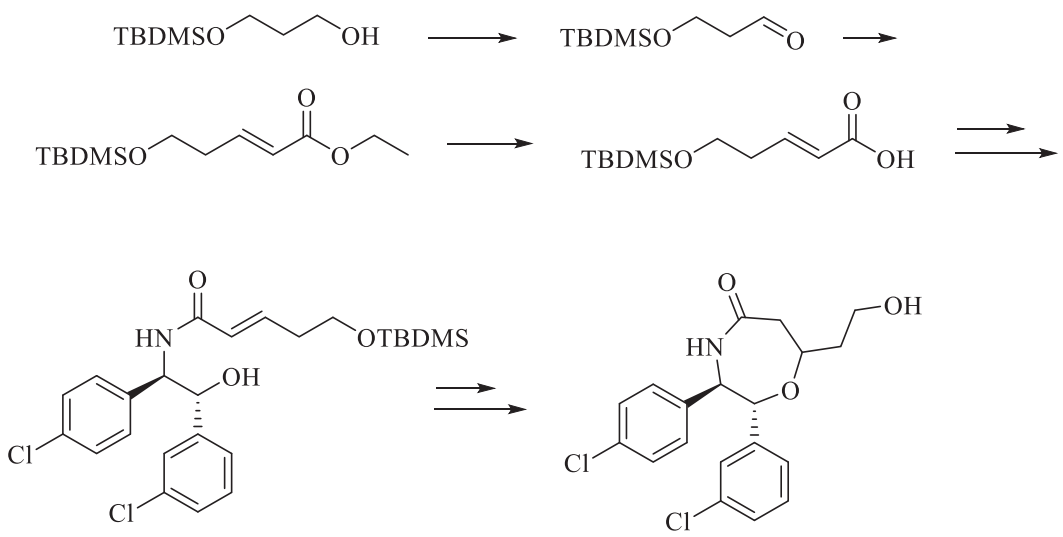
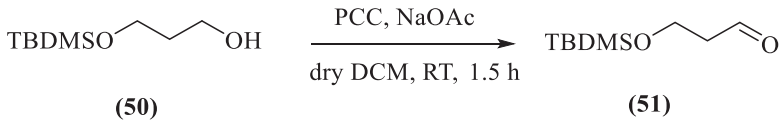


Figure 2.10. Planned synthesis pathway with TBDMS protected alcohol.

2.9. Syntheis of 3-((tert-butyldimethylsilyl)oxy)propanal from the Oxidation of 3-((tert-butyldimethylsilyl)oxy)propan-1-ol by PCC

3-((tert-butyldimethylsilyl)oxy)propanal **(51)** was synthesized by a reaction between 3-((tert-butyldimethylsilyl)oxy)propan-1-ol **(50)** and PCC in dry DCM at RT for 1.5 h (Figure 2.11).[39]



(50) **(51)**

Figure 2.11. Oxidation of 3-((tert-butyldimethylsilyl)oxy)propan-1-ol to 3-((tert-butyldimethylsilyl)oxy)propanal by PCC.

The reaction was optimized based on managing the acidity of PCC easing the filtration process due to the difficulties related with chromium salts. Many trials were performed and reaction conditions were summarized in Table 2.2.

First trial (Table 2.1. Entry 1) was performed with 1.5 eq of PCC. During filtering, SiO_2-celite mixture was used to remove chromium salts. At the end of the reaction no material was obtained. The reason might be the acidty of PCC might have effected the reaction medium. It was concluded as a buffer substance is required for the acidity of PCC.

For this reason, second trial (Table 2.1. Entry 2) was done with 1.5 g celite addition to reaction mixture as buffer. Same filtering procedure was performed and according to ^{1}H NMR spectrum the desired product was not obtained. It was concluded that celite addition does not provide any buffer effect to acidity of PCC; it was decided not to use celite in the reaction mixture. The celite was decided to be used only in filtration during work-up for the removal of the chromium salts.

Next trial (Table 2.1. Entry 3) was done with less equivalent of PCC (1.1 eq), to minimize the diverse effects of its acidity, because a buffer was not used in this reaction. The reaction was monitored with TLC and 0.3 eq of additional equivalent of PCC was

used to induce product formation. According to ^{1}H NMR spectrum, although very low amount of aldehyde was obtained, the amount of reactant was decreased. Therefore, it was decided to use a reagent other than celite, both to buffer PCC and to ease the work-up process.

Table 2.1. Optimization of PCC reaction according to filtration process.

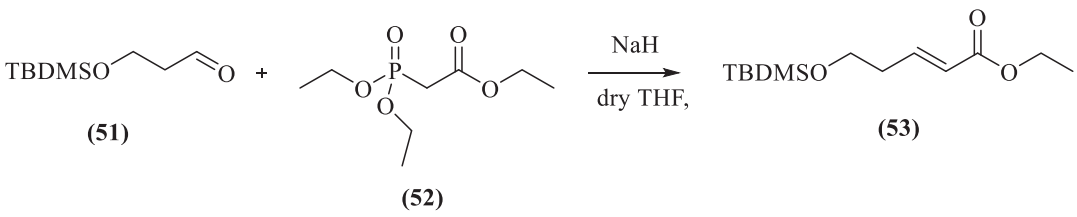
Entry	PCC (eq)	Buffer in reaction	Stationary Phase	Filtering Solvent	Result
1	1.5	-	SiO_2 (3x5 cm) & SiO_2-celite (1:1) (3x5 cm)	1:30 EtOAc:Hex	No material was obtained
2	1.5	1.5 g celite	SiO_2 (3x5 cm) & SiO_2-celite (1:1) (3x5 cm)	1:30 EtOAc:Hex	No reaction
3	1.4	-	SiO_2 (3x5 cm) & SiO_2-celite (1:1) (3x5 cm)	1:30 EtOAc:Hex	Low amount of product
4	1.1	77.5 mg NaOAc (0.9 eq)	SiO_2 (3x5 cm) & SiO_2-celite (1:1) (3x5 cm)	1:30 EtOAc:Hex	Product was formed
			SiO_2 (3x5 cm) & SiO_2-MgSO4 (1:1) (3x5 cm)	1:30 EtOAc:Hex	Product was formed
5	1.1	77.5 mg NaOAc (0.9 eq)	SiO_2 (3x5 cm) & SiO_2-MgSO4 (1:1) (3x5 cm)	Diethyl ether	Product was formed

In the next trial (Table 2.1. Entry 4), NaOAc was used as buffer in the reaction mixture. In order to test the celite during filtering in the presence of a buffer in the reaction

mixture, half of the material was filtered through a column with celite, the other half was filtered through the one with $MgSO_4$. Approximately same amount of product was obtained from both filtering procedures. It was understood that the key point was to use NaOAc to buffer the acidty of PCC. It was decided to use $MgSO_4$ due to its easier use. In this trial, it was also understood that the product **(51)** gets evaporated during rotary evaporator process because of its volatile nature. Thus, the product was only concentrated to 15 mL of diethyl ether solution under reduced pressure and used immediately.[37] The reaction was optimized as 1.1 eq of PCC, NaOAc as buffer and filtration process was optimized as $MgSO_4$ for filtration procedure and diethyl ether as filtering solvent (Table 2.1. Entry 5).

2.10. Synthesis of Ethyl (*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoate from the Wittig Reaction between 3-((tert-butyldimethylsilyl)oxy)propanal and Triethyl Phosphonoacetate.

Ethyl (*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoate **(53)** was synthesized from the esterification reaction between 3-((tert-butyldimethylsilyl)oxy)propanal **(51)** and triethyl phosphonoacetate **(52)** by NaH in dry THF (Figure 1.12). [40] Reaction was monitored by TLC control. ^{1}H NMR signals showed that the synthesis was successful with yield ranged from 20-40%.

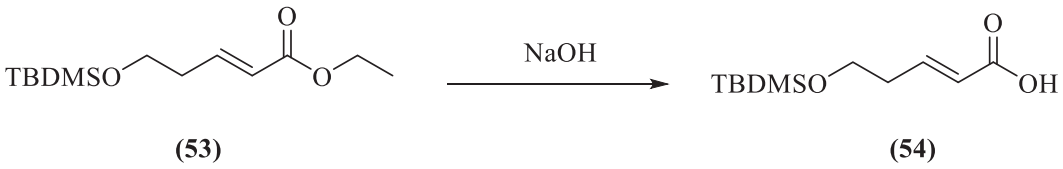


(51) (52) (53)

Figure 2.12. Synthesis of ethyl (*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoate from the esterification reaction between 3-((tert-butyldimethylsilyl)oxy)propanal and phosphonoacetate.

2.11. Synthesis of (*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoic Acid

(*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoic acid **(54)** was first tried to be synthesized through a reaction of ethyl (*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoate **(53)** with 1 M NaOH solution in THF (Figure 2.13). [41]



(53) **(54)**

Figure 2.13. Synthesis of (*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoic acid.

First trial (Table 2.2. Entry 1) was monitored with TLC and stirred for overnight According to ^{1}H NMR spectrum, it was understood that TBDMS group was cleaved. It was thought the reason behind it might be the conditions of high temperature for overnight stirring of the reaction mixture. It was decided not to use heat to test this remark.

Therefore, next trial (Table 2.2. Entry 2) was performed at 0 °C and reaction mixture was only stirred for 1.5 hour. Another changed parameter was the increment in the equivalent of NaOH to increase the probability of the success of the reaction. However, the desired product was not formed, it was concluded as although the starting material was consumed, the products might have been remained in water phase and could not be extracted to organic phase by acidification during extraction process. At this part, it was thought the problem might be the solvent used for extraction. Thus, for the next trial (Table 2.2. Entry 3), DCM was used instead of diethyl ether under the same reaction conditions. Since desired product cannot be obtained in that trial either, it was considered to change the reaction solvent.

Next trial (Table 2.2. Entry 4) was performed in DMSO. However, according to ^{1}H NMR spectrum, it was concluded that the TBDMS group was cleaved. Second trial with DMSO (Table 2.2. Entry 5) was performed with less amount of base at RT. It was

thought that other than heat, the amount of base might have caused cleavage. It was later understood that instead of formation of desired acid, transesterification took place.

Table 2.2. Trials for synthesis of (*E*)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoic acid in different solvents.

Entry	1 M NaOH	Solvent	Temp.	Time	Extraction Solvent	Result
1	4.5 eq	THF-MeOH	50 °C	Over night	Diethyl ether	TBDMS was cleaved
2	5 eq	THF-MeOH	0 °C	1.5 h	Diethyl ether	Transesterification
3	5 eq	THF-MeOH	0 °C	2 h	DCM	Transesterification
4	2 eq	DMSO	30 °C	2 h	EtOAc-Chloroform	TBDMS was cleaved
5	1 eq	DMSO	RT	2 h	EtOAc	No reaction

At the end of the trials for the formation of carboxylic acid, it was thought that TBDMS might not be a stable protecting group to work with in this reaction conditions. For this purpose, it was decided to use another protecting group. A new synthesis pathway was designed with benzyl alcohol as protecting group instead of TBDMS (Figure 2.14) to synthesize a carboxylic acid to be later coupled with the amino alcohol.

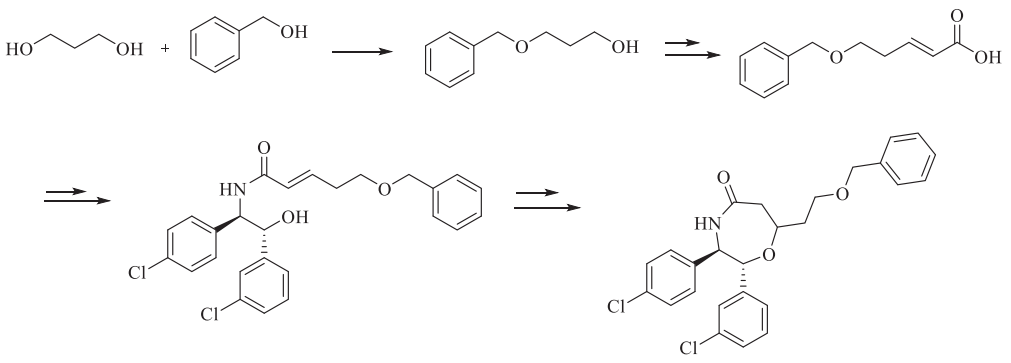


Figure 2.14. Planned synthesis pathway with benzyl alcohol as protecting group.

2.12. Synthesis of 3-(benzyloxy)propan-1-ol

Propane-1,3-diol **(55)** was reacted with benzyl bromide **(56)** with NaH in DMF at RT overnight (Figure 2.15). [42] The product was purified through SiO_2 gel column. Desired product was synthesized as oily liquid with yield ranged from 39 to 51%.

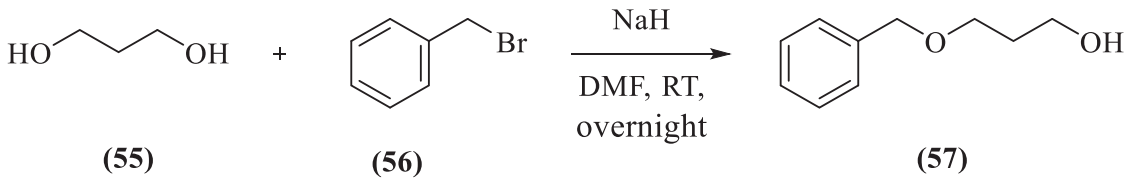


Figure 2.15. Synthesis of 3-(benzyloxy)propan-1-ol.

2.13. Oxidation of 3-(benzyloxy)propanal from 3-(benzyloxy)propan-1-ol by PCC

3-(Benzyloxy)propanal **(58)** was synthesized from 3-(benzyloxy)propan-1-ol **(57)** by PCC, using NaOAc as buffer in the reaction mixture (Figure 2.16). [43] The product was concentrated to 15 mL of diethyl ether solution and used without purification, due to low boiling point of the product.

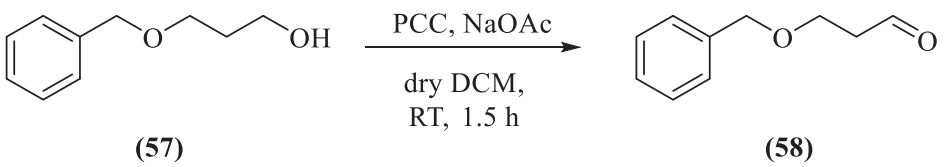


Figure 2.16. Oxidation of 3-(benzyloxy)propanal from 3-(benzyloxy)propan-1-ol by PCC.

2.14. Synthesis of ethyl (*E*)-5-(benzyloxy)pent-2-enoate from the Wittig Reaction between 3-(benzyloxy)propanal and Triethyl Phosphonoacetate

Synthesis of ethyl (*E*)-5-(benzyloxy)pent-2-enoate **(59)** was performed in a reaction between 3-(benzyloxy)propanal **(58)** and triethyl phosponoacetate **(52)** in dry THF (Figure 2.17).[44] ^{1}H NMR signals showed that the synthesis was successful with the yield ranged from 11 to 31% for two steps

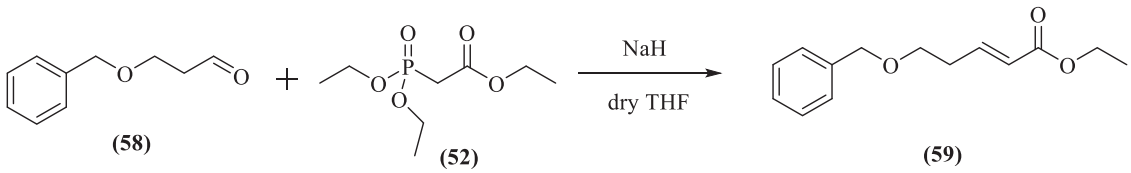


Figure 2.17. Synthesis of ethyl (*E*)-5-(benzyloxy)pent-2-enoate.

2.15. Synthesis of (*E*)-5-(benzyloxy)pent-2-enoic acid

Synthesis of (*E*)-5-(benzyloxy)pent-2-enoic acid **(60)** was performed with 1 M NaOH solution in 1,4-dioxane at RT in 3 hours (Figure 2.18). [44] As the result of the reaction, the desired product was obtained with the yield ranged from 51 to 89%.

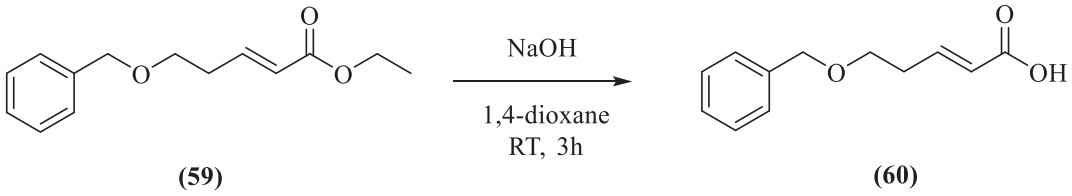


Figure 2.18. Synthesis of (*E*)-5-(benzyloxy)pent-2-enoic acid.

2.16. Coupling Reaction of (*E*)-5-(benzyloxy)-N-((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)pent-2-enamide

Synthesis of (*E*)-5-(benzyloxy)-N-((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)pent-2-enamide **(61)** was performed in a coupling reaction between (1*R*,2*R*)-2-amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethan-1-ol **(44)** and (*E*)-5-(benzyloxy)pent-2-enoic acid **(60)** in dry DMF at RT for overnight by using HATU[38] (Figure 2.19). The product was obtained with 45 % yield.

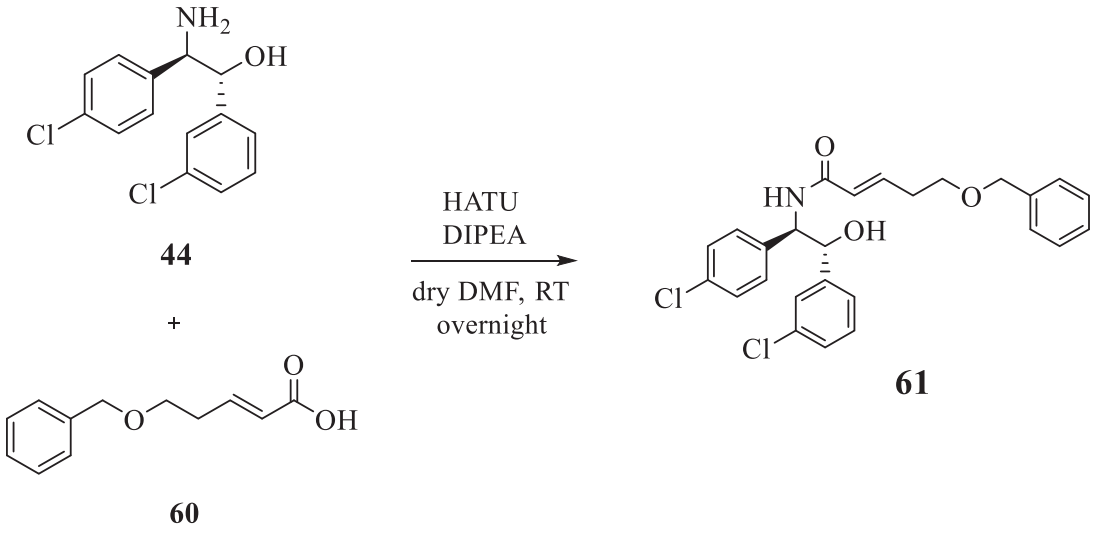


Figure 2.19. Synthesis of (*E*)-5-(benzyloxy)-N-((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)pent-2-enamide.

2.17. Cyclization Reaction of (2*R*,3*R*,7*S*)-7-(2-(benzyloxy)ethyl)-2-(3-chlorophenyl)-3-(4-chlorophenyl)-1,4-oxazepan-5-one

(2*R*,3*R*,7*S*)-7-(2-(benzyloxy)ethyl)-2-(3-chlorophenyl)-3-(4-chlorophenyl)-1,4-oxazepan-5-one **(62a)** and (2R,3R,7R)-7-(2-(benzyloxy)ethyl)-2-(3-chlorophenyl)-3-(4-chlorophenyl)-1,4-oxazepan-5-one **(62b)** were tried to be synthesized from (*E*)-5-

(benzyloxy)-N-((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)pent-2-enamide **(61)** with K_2CO_3[1] as base (Figure 2.20).

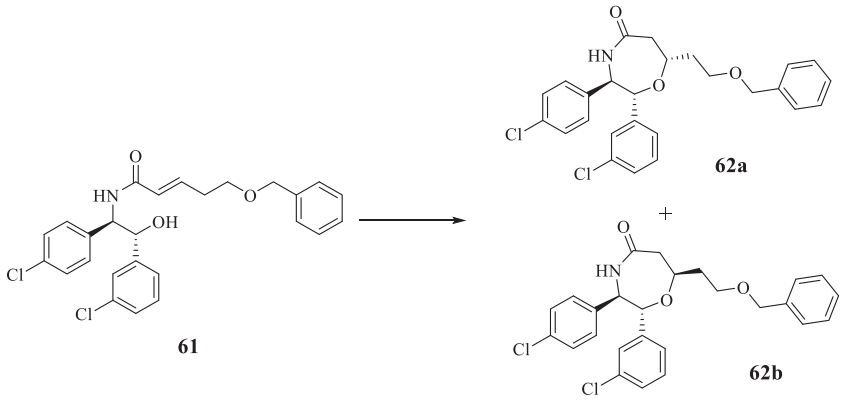


Figure 2.20. Synthesis of 7-(2-(benzyloxy)ethyl)-2-(3-chlorophenyl)-3-(4-chlorophenyl)-1,4-oxazepan-5-one isomers.

During the trials of cyclization reaction, two different bases were used; K_2CO_3 and $AgCO_3$. First trial (Table 2.3. Entry 1) was performed with K_2CO_3 in MeOH and reaction was stirred for 2 hours. The reaction was monitored with TLC control and no product formation was observed, also, reactant was not consumed. It was thought to alter the base in the reaction along with the solvent system to be able to use higher temperatures to increase the reaction kinetics.

For this purpose, next trial (Table 2.3. Entry 2) was performed with $AgCO_3$ in DMF-MeOH solvent mixture in 1:1 ratio; DMF was used in order to be able to increase the heat of the reaction. Reaction was monitored with TLC control, and it was decided to stir for overnight. According to ^{1}H NMR spectrum, it was concluded as no reaction took place.

For third trial, t-ButOH was chosen to increase the temperature of the reaction (Table 2.3. Entry 3). The base was not changed to test only the solvent effect on the reaction, and MeOH-t-ButOH was used in 1:2 ratio. According to ^{1}H NMR spectrum, the lack of expected signals indicating the cyclization took place were concluded as cyclization did not take place.

Two trials (Table 2.3. Entry 4 and 5) were performed to understand the effect of reaction time by comparing them to first trial (Table 2.2. Entry 1). One of the reactions

was stirred for 7 hours (Table 2.3. Entry 4) and the other was stirred for 5 days (Table 2.3. Entry 5). Reactions were monitored by TLC and their ^{1}H NMR spectrums were acquired. It was concluded as no reaction took place despite the longer reaction time.

Table 2.3. Attempts for synthesis of (2*R*,3*R*,7*S*)-7-(2-(benzyloxy)ethyl)-2-(3-chlorophenyl)-3-(4-chlorophenyl)-1,4-oxazepan-5-one with a base.

Entry	Catalyst	Solvent	Temp.	Time	Result
1	K_2CO_3	MeOH	RT	2 h	No reaction
2	$AgCO_3$	1:1 DMF:MeOH	80 °C	overnight	No reaction
3	K_2CO_3	1:2 MeOH:t-ButOH	70 °C	overnight	No cyclization
4	K_2CO_3	MeOH	RT	7 h	No reaction
5	K_2CO_3	MeOH	RT	5 days	No reaction

Up to this point, coupling reaction between amino alcohol **(44)** and synthesized carboxylic acid product were performed by HATU[38] reagent to form amide bond. It was thought that the unsucces of the cyclization trials might be due to the distane between acidic proton on hydroxyl group of compound **61** and Michael acceptor. As an alternative, trityl alcohol **(43)** were designed to be coupled[38] with compound **59** by oxa-michael addition reaction under base catalysis[45], and the removal of Trityl protecting group was planned to be performed after the coupling reaction.

2.18. Synthesis of Ethyl 5-(benzyloxy)-3-((1R,2R)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethoxy)pentanoate

Ethyl 5-(benzyloxy)-3-((1R,2R)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethoxy)pentanoate **(63)** was tried to be synthesized in a reaction between (1*R*,2*R*)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol **(43)** and ethyl (*E*)-5-(benzyloxy)pent-2-enoate **(59)** with reagents AgOAc, BINAP and LDA in DMF at

-20 °C overnight (Figure 2.21).[45] According to ^{1}H NMR spectrum it was concluded as, no reaction took place in this trial.

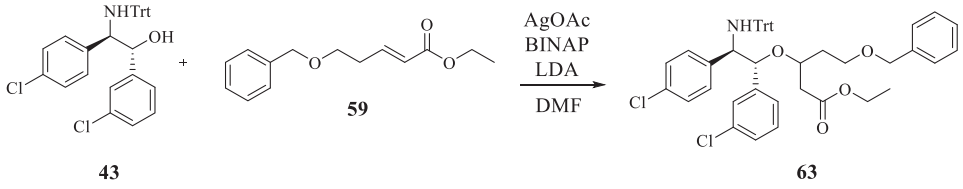


Figure 2.21. Estimated reaction for the synthesis of ethyl 5-(benzyloxy)-3-((1R,2R)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(trit-ylamino)ethoxy)pentanoate.

Due to the unsuccessful attempt of coupling reaction between Trityl alcohol **(43)** and compound **59**, a new synthesis pathway was planned (Figure 2.22) as preparing a side chain for amino group[1], and then cyclization were decided to be attemped.

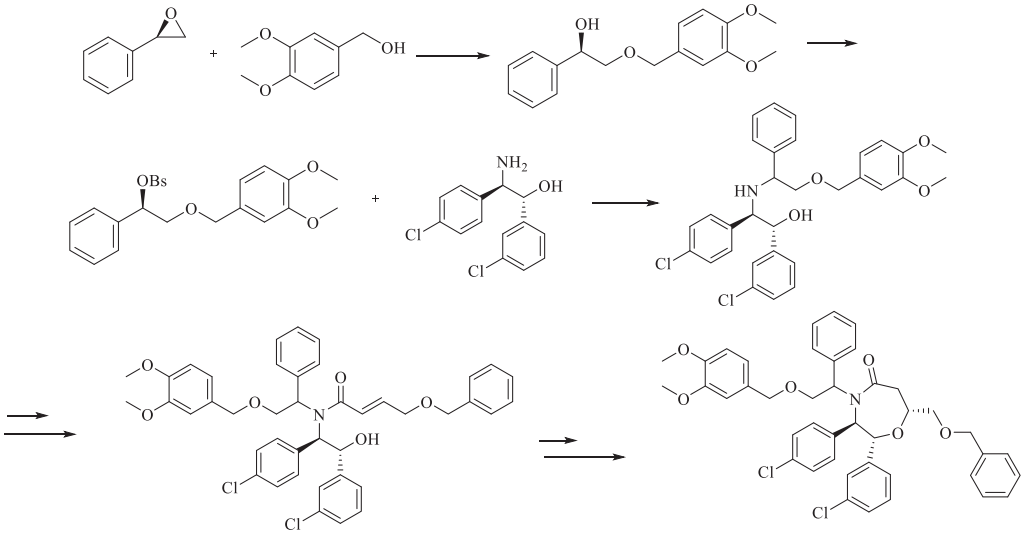


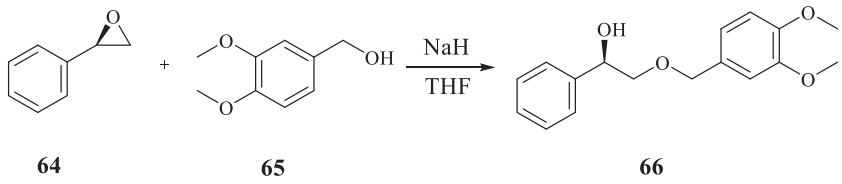
Figure 2.22. New estimated pathway for the desired derivative of 1,4-oxazepan-5-one.

For this aim, a chiral epoxide, compound **64,** was chosen for the coupling reaction under base catalysis. Sulfonation of the hydroxyl group of the resulting alcohol will be

employed as leaving group in a coupling reaction with the amino alcohol **(44)**. In this manner, it was thought to ease the cyclization process and derivative of the desired 1,4-oxazepan-5-one ring will be synthesized.

2.19. Synthesis of (*R*)-2-((3,4-dimethoxybenzyl)oxy)-1-phenylethan-1-ol

(*R*)-2-((3,4-dimethoxybenzyl)oxy)-1-phenylethan-1-ol **(66)** was tried to be synthesized in a reaction between (*R*)-2-phenyloxirane **(64)** and (3,4-dimethoxyphenyl)methanol **(65)** with NaH (Figure 2.23).



64 **65** **66**

Figure 2.23. Estimated reaction for the synthesis of (*R*)-2-((3,4-dimethoxybenzyl)oxy)-1-phenylethan-1-ol.

First trial (Table 2.4. Entry 1) was performed at 60 °C for 6 hours. The reaction was monitored with TLC control. According to H^1 NMR spectrum, the reactant was not consumed

Table 2.4: Reaction conditions for the attempted synthesis of (R)-2-((3,4-dimethoxybenzyl)oxy)-1-phenylethan-1-ol

Entry	Temperature	Time	Result
1	60 °C	6 h	Incomplete
2	60 °C	overnight	Could not be purified

2.20. Synthesis (*R*)-2-methoxy-1-phenylethan-1-ol

(*R*)-2-methoxy-1-phenylethan-1-ol **(67a)** was tried to be synthesized in a reaction between (*R*)-2-phenyloxirane **(64)** and methanol with NaH as a base (Figure 2.24).[46] The reaction took place at 60 °C, heat was appiled to enhance the reaction kinetics.

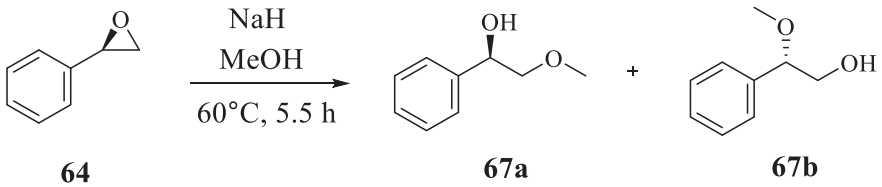


Figure 2.24: Synthesis of (*R*)-2-methoxy-1-phenylethan-1-ol.

According to ^{1}H NMR spectrum it was understood that epoxide was also opened from substituted side, probably because of the unhindered, small structure of the methanol. Thus, a bulky alcohol (t-BuOH) was decided to be used for further trials. It was thought to use t-BuOH with t-BuOK to increase the the chance of success of the reaction.

2.21. Reactions of t-BuOK with Styreneoxides

(*R*)-2-(tert-butoxy)-1-phenylethan-1-ol **(69)** was tried to be synthesized in a reaction between (*R*)-2-phenyloxirane **(64)** and t-butoxide **(68)** (Figure 2.25).

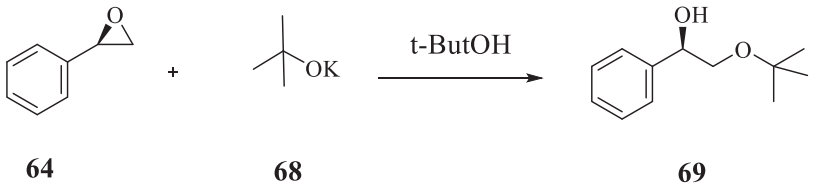
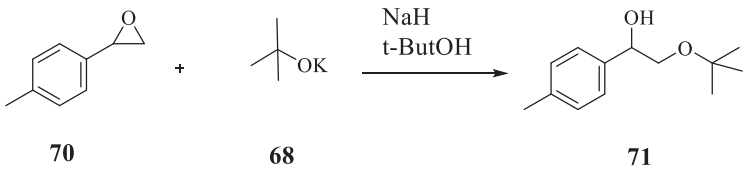


Figure 2.25: Synthesis of (*R*)-2-(tert-butoxy)-1-phenylethan-1-ol.

The reaction was performed in dry THF at RT and stirred for overnight and monitored with TLC control. It was observed that the product was not formed, therefore, reaction mixture was heated to 60 °C and stirred for overnight under reflux. Reaction mixture was monitored with TLC and it was concluded as no reaction had occurred. It was thought to use a stronger base than t-BuOK, or, additional base use might increase the chance of formation of the product. Thus, in the next trial NaH was decided to be used too.

Synthesis of 2-(tert-butoxy)-1-(p-tolyl)ethan-1-ol **(71)** was tried to be performed from the reaction between and t-ButOK **(68)** 2-(p-tolyl)oxirane **(70),** which was synthesized from 1-methyl-4-vinylbenzene with the catalysis of mCPBA in DCM at RT in 5 hours [47], NaH and t-ButOK was used as bases for this purpose (Figure 2.26).



70 **68** **71**

Figure 2.26. Synthesis of 2-(tert-butoxy)-1-(p-tolyl)ethan-1-ol.

In the first trial (Table 2.5. Entry 1) the product was formed with 16 % yield. Another trial (Table 2.5. Entry 2) was performed to increase the yield by slowly increasing the temperature up yo 100 °C, however, 5 % yield was obtained. Because of the low yield, it was decided to change the synthesis pathway.

Table 2.5: Reaction conditions for the synthesis of 2-(tert-butoxy)-1-(p-tolyl)ethan-1-ol.

Entry	Base	Solvent	Temperature	Time	Result
1	t-ButOK NaH	t-ButOH-DMF	147 °C	5.5 h	16 % yield
2	t-ButOK NaH	t-ButOH-DMF	100 °C	3 h	5 % yield

CHAPTER 3

EXPERIMENTAL

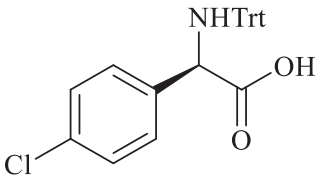
3.1. General Procedures

Reagents were used as supplied and purchased from Sigma-Aldrich and Riedel (Extra pure grade). Reactions were monitored by thin layer chromatography by using Merck TLC plates (Silica gel 60 F254). Chromatographic separations and isolations of compounds were performed by column chromatography. 70-230 mesh silica gel was used for column chromatography. Solvents were also commercial grade and were used as supplied. ^{1}H NMR data were recorded on Varian 400-MR (400 MHz) spectrometer. Chemical shifts for ^{1}H-NMR are reported in δ (ppm). $CDCl_3$ peaks were used as reference in ^{1}H-NMR (7.26 ppm).

3.2. N-Trityl Protection of (*R*)-2-amino-2-(4-chlorophenyl)acetic acid

500 mg (*R*)-2-amino-2-(4-chlorophenyl)acetic acid **(38)** (2.7 mmol) was dissolved in 6 mL dry DMF in a two necked flask under N_2 atmosphere. 360 µL trimethylsilyl chloride (307 mg, 2.82 mmol) was added, reaction mixture was stirred for approximately 15 minutes for cloudiness to disappear. Then, 828 mg tritylchloride (2.97 mmol) and 751 µL Et_3N (546 mg, 5.4 mmol) were added, respectively. The reaction mixture was stirred under N_2 atmosphere for 4 hours at RT then, it was diluted with 30 mL diethyl ether and 30 mL water. The reaction mixture was acidified to pH=3 with 0.05 M H_2SO_4 and was extracted with diethyl ether (3x30 mL). Organic phase was washed with 150 mL water and 150 mL brine solution. The combined organic phases were dried over anhydrous $MgSO_4$, then filtered. The organic solvent is evaporated under reduced pressure and the crude product was concentrated under vacuum. The resultant product **(39)** was obtained as white foam.[1]

H^1 NMR data was acquired for crude product with $CDCl_3$. The product was used in the next step without purification.



39

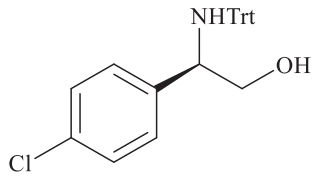
$C_{27}H_{22}ClNO_2$
427,93 g/mol

R_f: 0.22 (1:4 EtOAc:Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.08 (m, 19H), 4.32 (s, 1H).

3.3. Reduction of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetic Acid with $LiAlH_4$

In a two necked flask (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetic acid **(39)** (1 eq, 2.7 mmol) was dissolved in 4 mL dry THF. 205 mg $LiAlH_4$ (2 eq, 5.4 mmol) was dissolved in 4 mL dry THF then, it was added into reaction flask dropwise. The reaction mixture was stirred for 3 hours at RT under N_2 atmosphere then it was cooled to 0 °C. The reaction was quenched with approximately 10 mL distilled water then it was diluted with 30 mL diethyl ether. After the reaction mixture was warmed back to RT, it was acidified with 0.05 M H_2SO_4 to pH 5. Then it was extracted with 3x30 mL diethyl ether. The organic phase was washed with 150 mL 1 M NaOH and 150 mL brine solutions then dried over anhydrous $MgSO_4$ and filtered. The organic solvent was evaporated under reduced pressure and the crude product was purified in 3x20 cm SiO_2 gel column prepared with hexane [1:8 EtOAc:Hex]. The product **(40)** was obtained as white foam.[1] Collected product was concentrated under vacuum and H^1 NMR data was acquired with $CDCl_3$ (62.2% yield).



40

$C_{27}H_{24}ClNO$
413,95 g/mol

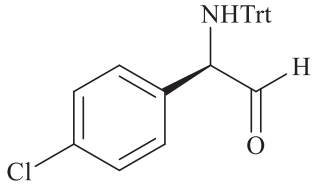
R_f: 0.46 (1:4 EtOAc:Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 1.6 Hz, 4H), 7.40 – 7.03 (m, 15H), 3.79 (t, J = 4.0 Hz, 1H), 3.21 (dd, J = 11.6, 3.6 Hz, 1H), 2.79 (dd, J = 10.9, 5.1 Hz, 1H).

3.4. Oxidation of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetaldehyde by Dess Martin Periodinane

In a two necked flask 682 mg (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetaldehyde **(40)** (1 eq, 1.65 mmol) is dissolved in 6 mL dry DCM. 1334 mg Dess-Martin periodinane (2 eq, 3.30 mmol) was added in portions. The reaction mixture was stirred for 1 hour under N_2 atmosphere at RT. 1826 mg Na_2SO_3 (7 eq, 11.55 mmol) dissolved in 50 mL 10 % $NaHCO_3$ solution, then the mixture was stirred for 10 min. It was extracted with diethyl ether (3x30 mL) and organic phase was washed with 100 mL saturated $NaHCO_3$ and 100 mL brine solutions, then, it was dried over $MgSO_4$ and filtered.[1] Organic solvent was evaporated under reduced pressure and crude product **(41)** was concentrated under vacuum and obtained as white foam.

H^1 NMR data was acquired in $CDCl_3$. 643 mg crude product with 94.5% yield was used in the next step without purification.



41

$C_{27}H_{22}ClNO$
411,93 g/mol

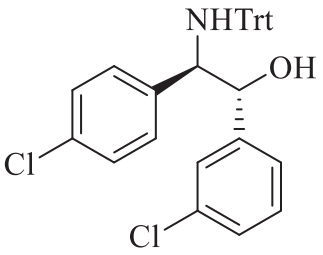
R_f: 0.79 (1:4 EtOAc:Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1H), 7.43 (dd, J = 4.7, 2.3 Hz, 4H), 7.31 – 7.20 (m, 15H), 4.41 (s, 1H).

3.5. Synthesis of (1*R*,2*R*)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol by Grignard Reaction with (3-chlorophenyl)magnesium bromide

643 mg (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetaldehyde **(41)** (1 eq, 1.36 mmol) was dissolved in a two necked flask at -40 °C with 5 mL dry THF under N_2 atmosphere. The Grignard reagent **(42)**, (3-chlorophenyl)magnesium bromide, (2 eq, 2.72 mmol, 5.44 mL) was added dropwise. The reaction mixture was stirred for 2 hours at -40 °C under N_2 atmosphere. The reaction was diluted with 30 mL EtOAc and 30 mL distilled water in portions, then, aqueous layer was acidified with 0.05 M H_2SO_4 solution to pH 5. It was extracted with EtOAc (3x30 mL). The combined organic phases were dried over $MgSO_4$ and organic solvent was evaporated under reduced pressure. The crude product is purified through 3x25 cm SiO_2 gel column prepared with hexane [1:14 EtOAc:Hex].[1] The collected substance was concentrated under vacuum and obtained as white foam **(43)**.

^{1}H NMR spectrum was acquired in $CDCl_3$. 257 mg desired product was obtained with 32 % yield and 1:0.14 diastreomeric ratio.



43

$C_{33}H_{27}Cl_2NO$

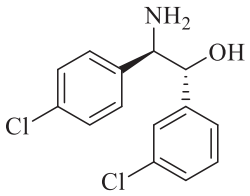
524,49 g/mol

R_f: 0.77 (1:4 EtOAc:Hex)

^{1}H NMR (400 MHz, $CDCl_3$) d 7.37 – 7.24 (m, 8H), 7.20 – 6.82 (m, 15H), 4.43 (d, J = 7.8 Hz, 1H), 3.90 (d, J = 8.2 Hz, 1H).

3.6. Synthesis of (1*R*,2*R*)-2-amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)ethan-1-ol by Deprotection of N-Trityl Group with Trifluoroacetic Acid

225 mg (1*R*,2*R*)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)ethan-1-ol **(43)** (0.43 mmol, 1 eq) was dissolved in a two necked flask in 4 mL DCM. 0.8 mL TFA and 15 µL distilled water were added on reaction mixture respectively. Reaction mixture was stirred for 45 minutes at RT under N_2 atmosphere, then, it was diluted with 10 mL DCM and 10 mL $NaHCO_3$ solution and it was extracted DCM (3x30 mL). Organic phase was dried over anhydrous $MgSO_4$ and organic solvent was evaporated under reduced pressure. Crude product was purified through 3x23 cm SiO_2 gel column prepared with chloroform [100:8 Chloroform:MeOH].[1] Desired product **(44)** is collected and concentrated under vacuum then H^1 NMR data was acquired with $CDCl_3$. The yellow oily product was synthesized in 87 mg with 74% yield.



44

$C_{14}H_{13}Cl_2NO$
282,16 g/mol

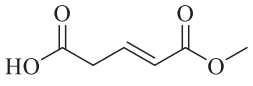
R_f: 0.28 (100:8 Chloroform:MeOH)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 – 7.06 (m, 8H), 4.56 (d, J = 7.7 Hz, 1H), 3.90 (d, J = 7.7 Hz, 1H).

3.7. Synthesis of (*E*)-5-methoxy-5-oxopent-3-enoic Acid by Dietyl azodicarboxylate and Resin Bounded Triphenyl Phosphine

100 mg (*E*)-pent-2-enedioic acid **(45)** (1 eq, 0.77 mmol) and 125 µL methanol (4 eq, 3.08 mmol) were dissolved in 8 mL dry DCM. 370 µL DEAD (1.1 eq, 0.85 mmol) and 283 mg r-PPh_3 (1.1 eq, 0.85 mmol) were added, respectively. The reaction mixture

was stirred at RT overnight under N_2 atmosphere. The reaction mixture was filtered with DCM through a filter paper, then, DCM was evaporated under reduced pressure. The product was purified through a 3x25 cm SiO_2 gel column prepared with hexane [1:1 Hex:EtOAc 1% HOAc 2% MeOH]. The purified product was collected and concentrated under vacuum, then, H^1 NMR data was acquired with $CDCl_3$.[35] The desired product, compound **48a,** was formed but was not isolated from **48b**.



48a

$C_6H_8O_4$

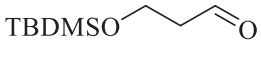
144,13 g/mol

R_f: 0.62 (1:1 EtOAc:Hex 1 % AA, 2 % MeOH)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 – 6.96 (m, 1H), 5.94 (dd, J = 15.7, 6.9 Hz, 1H), 3.72 (s, 3H), 3.27 (d, J = 6.4 Hz, 2H).

3.8. Synthesis of 3-((tert-butyldimethylsilyl)oxy)propanal from the Oxidation of 3-((tert-butyldimethylsilyl)oxy)propan-1-ol by PCC

194 mg NaOAc (2.4 mmol, 0.9 eq) was dissolved in 4 mL dry DCM. 500 mg 3-((tert-butyldimethylsilyl)oxy)propan-1-ol **(50)** (2.6 mmol, 1 eq) was added into reaction flask. Addition of 625 mg PCC (2.9 mmol, 1.1 eq) is done in two portions at 0 °C. Reaction mixture is stirred for 1.5 h at RT under N_2 atmosphere. The reaction was diluted with approximately 100 mL of diethyl ether and stirred for 5 minutes. Mixture was filtered through a short column (3x5 cm) of SiO_2 gel prepared diethyl ether, SiO_2-$MgSO_4$ gel mixture (3x5 cm) prepared with diethyl ether on top. The substance was filtered by with total of 300 mL of diethyl ether. Collected organic phase was washed with 250 mL 10% NaOH and 250 mL brine solutions, then it was dried over $MgSO_4$. The product **(51)** was concentratred to 15 mL of diethyl ether solution under reduced pressure and used immediately without purification.[37]



Rf: 0.43 (1:4 EtOAc:Hex)

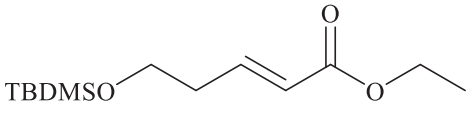
(51)

$C_9H_{20}O_2Si$

188,34 g/mol

3.9. Synthesis of ethyl (E)-5-((tert-butyldimethylsilyl)oxy)pent-2-enoate from the Esterification Reaction between 3-((tert-butyldimethylsilyl)oxy)propanal and phosphonoacetate

63.2 mg 60 % NaH (1.58 mmol, 1.5 eq) was dissolved in 3 mL dry THF at 0 °C. Triethyl phosphonoacetate **(52)** was added in 5 portions (64 µLx5=320 µL, 1.58 mmol, 1.5 eq) in 20 minutes. Once the addition is completed, reaction mixture was stirred for 40 min at 0 °C under N_2 atmosphere. After 40 minutes, reaction mixture was cooled to -78 °C and 3-((tert-butyldimethylsilyl)oxy)propanal **(51)** was added in portions in 30 minutes. As the addition is completed, reaction mixture was warmed to 0 °C and stirred for 30 minutes. Reaction was monitored with TLC and reaction mixture was diluted with EtOAc and extracted with 30 mL NH_4Cl. Organic phase was washed with 4x20 mL water and brine solutions. Then it was dried over $MgSO_4$ and filtered. The organic solvent was evaporated with rotary evaporator and the product was purified through a SiO_2 gel column prepared with hexane [1:20 EtOAc:Hex], then, collected fraction concentrated under reduced pressure. ^{1}H NMR spectrum was acquired and it was concluded that the product **(53)** was synthesized with 60 % yield. [38]



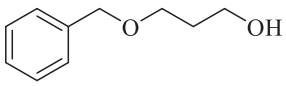
(53)

Rf: 0.69 (1:4 EtOAc:Hex)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (dt, J = 14.3, 7.1 Hz, 1H), 5.83 (d, J = 15.7 Hz, 1H), 4.15 (q, J = 7.1 Hz, 2H), 3.69 (t, J = 7.1 Hz, 2H), 2.37 (dd, J = 13.4, 6.7 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H), 0.85 (s, 9H), 0.01 (s, 6H).

3.10. Synthesis of 3-(benzyloxy)propan-1-ol through the Coupling Reaction between Propane-1,3-diol and (bromomethyl)benzene

400 mg 60 % NaH (14.5 mmol, 1.1 eq) was dissolved in 3 mL dry DMF. The reaction flask was cooled to 0 °C and 950 µL propane-1,3-diol **(55)** (13.14 mmol, 1 eq) was added on reaction mixture dropwise. Reaction was stirred for 10 minutes at 0 °C. 1.6 mL (bromomethyl)benzene (**56)** (13.14 mmol, 1 eq) was added on reaction mixture cautiously at 0 °C then reaction was warmed to RT. Reaction was stirred for 18 hours under N_2 atmosphere at RT then, it was diluted by distilled water and EtOAc and reaction mixture was extracted with 3x30 mL EtOAc. Combined organic phases were washed with approximately 1.5 L of distilled water to remove DMF, then dried over anhydrous $MgSO_4$ and filtered. The product was purified through a 3x22 cm SiO_2 gel column prepared with hexane [1:3 EtOAc:Hex]. ^{1}H NMR spectrum was acquired and it was concluded that the product was synthesized successfully with little amount of benzyl alcohol.[40]



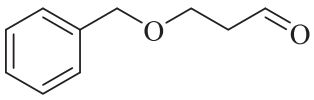
(57)

R_f: 0.2 (1:3 EtOAc:Hex)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.28 (m, 5H), 4.49 (s, 2H), 3.72 (t, J = 5.9 Hz, 2H), 3.62 (t, J = 5.9 Hz, 2H), 1.83 (p, J = 5.9 Hz, 2H).

3.11. Oxidation of 3-(benzyloxy)propanal from 3-(benzyloxy)propan-1-ol by PCC

302 mg NaOAc (3.7 mmol, 0.9 eq) was dissolved in 4 mL dry DCM. 680 mg 3-(benzyloxy)propan-1-ol **(57)** (4.1 mmol, 1 eq) was added into reaction mixture. 970 mg PCC (5 mmol, 1.1 eq) was added in portions then reaction mixture was stirred for 1.5 h at RT under N_2 atmosphere. Reaction was diluted with 100 mL diethyl ether then, stirred for 5 minutes then filtered through a short (3x5 cm) of SiO_2 gel prepared diethyl ether, SiO_2-$MgSO_4$ gel mixture (3x5 cm) prepared with diethyl ether on top. Column was washed with total of 300 mL diethyl ether, then, organic phase is washed with 250 mL

10% NaOH and 250 mL brine solutions. It was dried over $MgSO_4$, filtered and concentratred to 15 mL of diethyl ether solution under reduced pressure and the product **(58)** was used immediately without purification.[41]

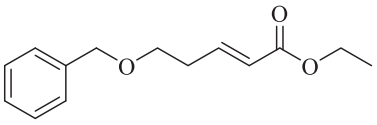


(58)

R_f: 0.46 (1:4 EtOAc:Hex)

3.12. Synthesis of ethyl (E)-5-(benzyloxy)pent-2-enoate from the Wittig Reaction between 3-(benzyloxy)propanal and Triethyl Phosphonoacetate

245 mg 60 % NaH (6 mmol, 1.5 eq) was dissolved in 4 mL dry THF at 0 °C. 1216 µL 2-(diethoxyphosphoryl)acetate **(52)** (6.14 mmol, 1.5 eq) was added in 4 portions (300 µLx4=1200 µL in 20 minutes. Once the addition is completed, reaction mixture was stirred for 40 min at 0 °C under N_2 atmosphere. After 40 minutes, reaction mixture was cooled to -78 °C and 70 mL 3-(benzyloxy)propanal **(58)** was added in portions in 30 minutes. As the addition is completed, reaction mixture was warmed to 0 °C and stirred for 30 minutes. Reaction mixture was extracted with 30 mL NH_4Cl. Organic phase was washed with 4x20 mL water and brine solutions, then, it was dried over $MgSO_4$ and filtered. The substance was purified through a SiO_2 gel column (3x25 cm) prepared with hexane [1:8 EtOAc:Hex]. The collected product was concentrated under reduced pressure and H^1 NMR spectrum was acquired, it was concluded that the product **(59)** was synthesized successfully.[42]



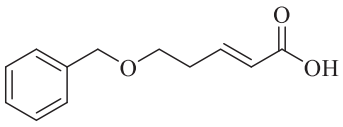
59

R_f: 0.57 (1:4 EtOAc)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 5H), 6.97 (dt, J = 15.7, 6.9 Hz, 1H), 5.86 (t, J = 1.6 Hz, 1H), 4.50 (s, 2H), 4.17 (q, J = 7.1 Hz, 2H), 3.56 (t, J = 6.5 Hz, 2H), 2.49 (qd, J = 6.5, 1.6 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H).

3.13. Synthesis of (*E*)-5-(benzyloxy)pent-2-enoic Acid

254 mg ethyl (*E*)-5-(benzyloxy)pent-2-enoate **(59)** (1.08 mmol, 1 eq) was dissolved in 1.5 mL 1,4-dioxane. Then 2.16 mL 1 M NaOH (0.43 mmol, 2 eq) was added. Reaction mixture was stirred for 3 h at RT under N_2 atmosphere. Reaction mixture was concentrated under reduced pressure, then, was diluted with 100 mL distilled water and extracted with 2x50 mL diethyl ether. The pH of the water phase was adjusted to 1.5 with 1 M HCl. Then, the pH arranged water phase was extracted with 3x30 mL EtOAc. Organic phases collected separately, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. ^{1}H NMR spectrum was acquired and it was concluded that the product was synthesized successfully.[42]



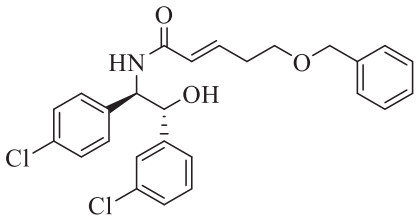
60

R_f: 0.17 (1:4 EtOac:Hex 1%AA)
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.75 (s, 1H), 7.57 – 7.21 (m, 5H), 7.10 (dt, J = 15.5, 6.9 Hz, 1H), 5.91 (d, J = 15.7 Hz, 1H), 4.52 (s, 2H), 3.59 (t, J = 6.4 Hz, 2H), 2.54 (q, J = 6.5 Hz, 2H).

3.14. Synthesis of (*E*)-5-(benzyloxy)-N-((1*R*,2*R*)-2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethyl)pent-2-enamide

88 mg of compound **44** (0.31 mmol, 1 eq) was dissolved in 1 mL dry DMF in a two necked flask. 130 mg HATU (0.34 mmol, 1.1 eq), 107 µL DIPEA (0.62 mmol, 2 eq) and 64 mg (*E*)-5-(benzyloxy)pent-2-enoic acid (**60)** (0.31 mmol, 1 eq) was mixed in another flask and was dissolved in 1.5 mL dry DMF, then, was added over compound **44**. Reaction mixture was stirred overnight at RT under N_2 atmosphere, then, it was diluted with water and EtOAc. Aqueous layer was acidified to pH 1.5-2 with 0.05 M H_2SO_4 and it was extracted with 3 x 30 mL EtOAc. Combined organic phases were extracted with distilled water and dried over anhydrous $MgSO_4$ and filtered. The product was purified in a SiO_2 gel column (3 x 25 cm) prepared with chloroform [100ch:4MeOH]. Collected

fractions containing the product were concentrated under reduced pressure and ^{1}H NMR spectrum was acquired with $CDCl_3$. It was concluded as the product **(61)** was formed.



61

$C_{26}H_{25}Cl_2NO_3$
470,39 g/mol

R_f: 0.29 (100:4 chloroform:MeOH)

^{1}H NMR (400 MHz, $CDCl_3$) d 7.35 – 7.06 (m, 13H), 6.75 (dt, J = 14.0, 6.8 Hz, 1H), 6.28 (d, J = 7.5 Hz, 1H), 5.82 (dd, J = 15.4, 1.1 Hz, 1H), 5.13 (dd, J = 7.4, 4.6 Hz, 1H), 4.47 (s, 2H), 3.52 (t, J = 6.4 Hz, 2H), 2.44 (q, J = 6.5 Hz, 2H).

CHAPTER 4

CONCLUSION

The importance of designing a pharmacophore for the inhibition of the interaction between p53 gene and MDM2 comes from the feedback mechanism between them. p53 gene induces cell cycle arrest and apoptosis under stress. MDM2 being the main inhibitor of p53, when overexpressed in the case of mutation, cell proliferates and carcionegesis takes place.

In this study it was aimed to synthesize a new inhibitor for this interaction as an approach for cancer therapy and to extend the contents of the literature with novel chiral oxazepine derivatives.

Synthesis for the desired oxazepine derivatives started with the amino group protection of *(R)*-2-amino-2-(4-chlorophenyl)acetic acid with trityl group. Trityl protected aminoacid **(39)** was then reduced to alcohol **(40)** by $LiAlH_4$ which is followed by the oxidation to aldehyde **(41)** by Dess Martin Periodinane. Addition of 3-chlorophenylmagnesium bromide to the aldehyde by grignard reaction gave the product of trityl alcohol **(43)** then, deprotection of N-amino group was performed with TFA and amino alcohol **(44)** was synthesized.

Different carboxylic acid derivatives were planned to be coupled with amino alcohol. Compound **47** was coupled with the amino alcohol **(43)**, because of the challenges during work-up due to benzyl alcohol, a low boiling point alcohol was selected. Therefore, compound **48** was synthesized to be coupled with amino alcohol **(43),** during the coupling reaction, polymerizartion of compound **48** took place. In order to prevent it, one-side TBDMS protected diol was used to synthesize compound **54**, however the desired product was not formed. Due to low stability of TBDMS protecting group, benzyl group was selected as protecting group of an alcohol and compound **60** was synthesized then coupled with amino alcohol **(44)** to give compound **61**.

Cyclization trials were performed with compound **61** under base catalysis, however, the desired product was not formed probably due to the reaction between the base and the acidic amino proton. Therefore, trityl deprotection was designed to be performed after the coupling reaction, the compound **63** was designed to be obtained, however no reaction took place. In order to inhibit the possible reaction between acidic

proton on amide group and the base used in cyclization, a side chain was designed to be synthesized for amino group. First trials were performed with compound **66**, but due to the impurities probably related with the benzyl alcohol, MeOH was decided to be used to obtain compound **67**. Because of the small structure of MeOH, it opened the epoxide ring from both sides and compounds **67a** and **67b** was formed. The last trials were performed with t-ButOH to obtain compound **69**. Due to low yield of the reactions, t-ButOH was also not used.

In this work, a strong base was not used in cyclization reaction because of its possible reaction with the acidic proton on amino group. A side chain, that will not cause steric effect in 3D during cyclization, might be designed to be used for amino group on amino alcohol **(44)**. In the presence of a stronger base, cyclization reaction might take place.

REFERENCES

1. Gonzales A. Z.; Eksterowicz J.; Bartberger M. D.; Beck H. P.; Canon J.; Chen A.; Chow D.; Duquette J.; Fox B. M.; Fu J.; Huang X.; Houze J. B.; Jin L.; Li Y.; Li Z.; Ling Y.; Lo M.; Long . M.; McGee L.R.; McIntosh J.; McMinn D. L.; Oliner J. D.; Osgood T.; Rew Y.; Saiki A. Y.; Shaffer P.; Wortman S.; Yakowec P.; Yan X.; Ye Q.; Yu D.; Zhao X.; Zhou X.; Olson S. H.; Medina J. C.; Sun D. Selective and potent morpholinone inhibitors of the MDM2-p53 protein-protein interaction *J. Med. Chem.* **2014**, *57*, 2472-2488.
2. Zhao Y.; Bernard D.; Wang S. Small molecule inhibitors of MDM2-p53 and MDMX-p53 interactions as new cancer therapeutics *BioDiscovery* **2013**, *8*, 4.
3. Joerger A. C.; Fersht A. R. The p53 pathway: origins, inactivation in cancer, and emerging therapeutic approaches *Annu. Rev. Biochem.* **2016**, *85*, 375-404.
4. Shangary S., Wang S. Targeting the MDM2-p53 interaction for cancer therapy *Clin. Cancer. Res.* **2008**, *14* (17), 5318-5324.
5. Vargas D. A.; Takashi S,; Ronal Z.; MDM2: a regulator cell growth and death *Adv. Cancer Res.* **2003**, *89*, 1-34
6. Marine J. C. MDM2 and MDMX in Cancer and Development. In *Current Topics in Developmental Biology* **2011**, *94*, 45-75.
7. Shangary S.; Wang S.; Small-molecule inhibitors of the MDM2-p53 protein-protein interaction to reactivate p53 function: a novel approach for cancer therapy *Annu Rev. Biochem.*, **2009**, *49*, 223-241.
8. Callis J. The Ubiquitination Machinery of the Ubiquitin System. In *The Arabidopsis Book* **2014**, *12*
9. Gao Q.; Yang L.; Zhu Y. Pharmacophore based drug design approach as a practical process in drug discovery *Curr. Computer-Aided Drug Design* **2010**, *6*, 37-49.
10. Yang S. Y. Pharmacophore modeling and applications in drug discovery: challenges and recent advances *Drug Disovery Today* **2010**, *15*, 445-450.
11. Nayak S. K.; Khatik G. L.; Narang R.; Monga V.; Chopra H. K. p53-Mdm2 interaction inhibitors as novel nongenotoxic anticancer agents *Curr. Cancer Drug Targets* **2018**, *18*, 749-772.

12. Beloglazkina A,; Zyk N.; Majouga A.; Beloglazkina E. Recent small-molecule inhibitors of the p53-MDM2 protein-protein interaction *Molecules* **2020**, *25*, 1211.

13. Qin J. J.; Li X.; Hunt C.; Wang W.; Wang H.; Zhang R. Natural products targeting the p53-MDM2 pathway and mutant p53: Recent advances and implications in cancer medicine *Genes Dis* **2018**,1-16.

14. Panche A. N.; Diwan A. D.; Chandra S. R. Flavonoids: an overview **2016**, *5*, 1-15.

15. Pereira D.; Lima R. T.; Palmeira A.; Seca H.; Soares J.; Gomes S.; Raimundo L.; Maciel C.; Pinto M.; Sousa E.; Vasconselos M. H.; Saraiva L.; Cidade H. Design and synthesis of new inhibitors of p53-MDM2 interaction with a chalcone scaffold *Arabian J. Chem.* **2016**.

16. Aykul S.; Martinez-Hackert E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis *Anal. Biochem.* **2016**, *508,* 97-103.

17. Hsu Y.; Kuo P.; Lin L.; Lin C. Isoliquiritigenin inhibits cell proliferation and induces apoptosis in human hepatoma cells *Planta Med.* **2005**, *71*, 130-134

18. Peng F.; Du Q.; Peng C.; Wang N.; Tang H.; Xie X.; Shen J.; Chen J. A review: the pharmacology of isoliquiritigenin *Phytother. Res.* **2015**, *29*, 969-977.

19. Aydın S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA *Peptides* **2015**, *72*, 4-15.

20. Ding Q.; Zhang Z.; Liu J.; Jiang N.; Zhang J.; Ross T. M.; Chu X.; Bartkoviz D.; Podlaski F.; Janson C.; Tovar C.; Filipovic Z. M.; Higgins B.; Glenn K.; Packman K.; Vassilev L. T.; Graves B. Discovery of RG7388, a potent and selective p53-MDM2 inhibitor in clinical development *J. Med. Chem.* **2013**, *56*, 5979-5983.

21. Vasillev L. T.; Vu B. T.; Graves B.; Carvajal D.; Podlaski F.; Filipovic Z.; Kong N.; Kammlott U.; Lukacs C.; Klein C.; Fotouhi N.; Liu E. A. In-vivo activation of the p53 pathway by small-molecule antagonists of MDM2 *Science* **2004**, *303(5659)*, 844-848.

22. Hu C.; Li X.; Wang W.; Zhang L.; Tao L.; Dong X.; Sheng R.; Yang B.; Hu. Y. Design, synthesis, and biological evaluation of imidazoline derivatives as p53-MDM2 binding inhibitors *Bioorg. Med. Chem.* **2011**, *19*, 5454-5461.

23. Anil B.; Riedinger C.; Endicott J. A.; Noble M. E. M. The structure of an MDM2-Nutlin-3a complex solved by the use of a validated MDM2 surface-entropy reduction mutant *Acta Cryst.* **2013**, *69*, 1358-1366.

24. Miyazaki M.; Naito H.; Sugimoto Y.; Yoshida K.; Kawato H.; Okayama T.; Shimizu H.; Miyazaki M.; Kitagawa M.; Seki T.; Fukutake S.; Shiose Y.; Aonuma M.; Soga T. Synthesis and evaluation of novel orally active p53-MDM2 interaction inhibitors *Bioorg. Med. Chem.* **2013**, *21(14)*, 4319-4331.

25. Guo Z.; Zhuang C.; Zhu L.; Zhang Y.; Yao J.; Dong G.; Wang S.; Liu Y.; Chen H.; Sheng C.; Miao Z.; Zhang W. Structure activity relationship and antitumor activity of thio-benzodiazepines as p53-MDM2 protein-protein interaction inhibitors *Eur. J. Med. Chem.* **2012**, *56*, 10-16.

26. Ding K.; Lu Y.; Nikolovska-Koleska Z.; Qui S.; Ding Y.; Gao W.; Stuckey J.; Krajewski K.; Roller P. P.; Tomita Y.; Parrish D. A.; Deschamps J. R.; Wang S. Structure-based design of potent non-peptide MDM2 inhibitors *J. Am. Chem Soc.* **2005**, *127*, 10130-10131.

27. Wang S.; Sun W.; Zhao Y. SAR405838: an optimized inhibitor of MDM2-p53 interaction that induces complete and durable tumor regression *Cancer Res.* **2014**, *74(20)*, 5855-5865.

28. Zhao Y.; Aguilar A.; Bernard D.; Wang S. Small-molecule inhibitors of the MDM2-p53 protein-protein interaction (MDM2 inhibitors) in clinical trials for cancer treatment *J. Med. Chem.* **2015**, *58*, 1038-1052.

29. Bernard D.; Zhao Y.; Wang S. AM-8553: A novel MDM2 inhibitor with a promising outlook for potential clinical development *J. Med. Chem.* **2012**, *55*, 4934-4935.

30. Rew Y.; Sun D.; Yan X.; Beck H. P.; Canon J.; Chen A.; Duquette J.; Eksterowicz J.; Fox B. M.; Fu J.; Gonzales A. Z.; Houze J.; Huang X.; Jiang M.; Jin L.; Li Y.; Li Z.; Ling Y.; Lo M.; Long A. M; McGee L. R.; McIntosh J.; Oliner J. D.; Osgood T.; Saiki A. Y.; Shaffer P.; Wang Y. C.; Wortman S.; Yakowec P.; Ye Q.; Yu D.; Zhao X.; Zhou J.; Medina J. C.; Olson S.H. Discovery of AM-7209, a potent and selective 4-amidobenzoic acid inhibitor of the MDM2-p53 interaction *J. Med. Chem.* **2014**, *57*, 10499-10511.

31. Liu Y.; Chu C.; Huang A.; Zhan C.; Ma Y.; Ma C.; Regioselective synthesis of fused oxazepinone scaffolds through one-pot smiles tandem reaction *ACS Comb. Sci.* **2011**, *13*, 547-553.

32. Binaschi M.; Boldetti A; Gianni M.; Maggi C. A.; Gensini M.; Bigioni M.; Parlani M.; Giolitti A.; Fratelli M.; Valli C.; Terao M.; Garattini E. Antiproliferative and differentiating activities of a novel series of histone deacetylase inhibitors *ACS Med. Chem. Lett.* **2010**, *1*, 411-415.

33. Lin G.; Li Y.; Chan A. S. *Principles and Applications of Asymmetric Synthesis*; John Wiley and Sons: New York, 2001.

34. Dess, D. B., & Martin, J. C. Readily accessible 12-I-5 oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones *J. Org. Chem.* **1983,** *48*(22), 4155–4156.

35. Dess, D. B., & Martin, J. C. A useful 12-I-5 triacetoxyperiodinane (the Dess-Martin periodinane) for the selective oxidation of primary or secondary alcohols and a variety of related 12-I-5 species *J. Am. Chem. Soc.* **1991,** *113*(19), 7277–7287.

36. Baldwin, J. E., Cha, J. K., & Kruse, L. I. Total synthesis of antitumor agent at-125, (αS,5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid *Tetrahedron* **1985**, *41*(22), 5241–5260.

37. Gioia, C., Fini, F., Mazzanti, A., Bernardi, L., & Ricci, A. Organocatalytic Asymmetric Formal [3 + 2] Cycloaddition with in Situ-GeneratedN-Carbamoyl Nitrones *J. Am. Chem. Soc.* **2009**, *131*(28), 9614–9615.

38. Carpino, L. A. 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive *J. Am. Chem. Soc.* **1993**, *115*(10), 4397–4398.

39. Vijn, R. J., Hiemstra, H., Kok, J. J., Knotter, M., & Speckamp, W. N. Synthetic studies towards gelsemine, I The importance of the antiperiplanar effect in the highly regioselective reduction of non-symmetrical cis-hexahydrophthalimides *Tetrahedron* **1987**, *43*(21), 5019–5030.

40. Fernandes, R. A., Gholap, S. P., Chavan, V. P., Saiyed, A. S., & Bhattacharyya, S. Fischer Carbene Pentannulation with Alkynes Having Adjacent Carbonate or Acyloxy Groups: Synthesis of 3-Substituted 1-Indanones *Organic Letters* **2020**

41. Könning, D., Olbrisch, T., Sypaseuth, F. D., Tzschucke, C. C., & Christmann, M. Oxidation of allylic and benzylic alcohols to aldehydes and carboxylic acids. *Chem. Commun.* **2014,** *50*(39), 5014–5016.

42. Zhang, P., Brozek, L. A., & Morken, J. P. Pd-Catalyzed Enantioselective Allyl−Allyl Cross-Coupling *J. Am. Chem. Soc.* **2010***, 132*(31), 10686–10688.

43. Vintonyak, V. V., & Maier, M. E. Formal Total Synthesis of Neopeltolide *Org. Lett.* **2008**, *10*(6), 1239–1242

44. Chattopadhyay, S. K., & Pattenden, G. A total synthesis of the unique tris-oxazole macrolide ulapualide A produced by the marine nudibranch Hexabranchus sanguineus. *J. Chem. Soc., Perkin Trans. 1* **2000**, *15*, 2429–2454.

45. Wang, L., & Menche, D. Construction of Multisubstituted Tetrahydropyrans by a Domino Oxa-Michael/Tsuji–Trost Reaction *J. Org. Chem.* **2012**, *77*(23), 10811-10823.

46. Ferraboschi, P., Grisenti, P., Manzocchi, A., & Santaniello, E. Baker's yeast-mediated reduction of α-hydroxy ketones and derivatives: The steric course of the biotransformation *Tetrahedron* **1994**, *50*(35), 10539–10548

47. Tanaka, Y., Nishimura, K., & Tomioka, K. Chiral ligand-controlled catalytic asymmetric epoxidation of α,β-unsaturated carbonyl compounds with peroxide *Tetrahedron* **2003***, 59*(25), 4549–4556.