STUDIES TOWARD THE SYNTHESIS OF NOVEL MDM2 INHIBITOR CANDIDATES

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İZMİR

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ABSTRACT

STUDIES TOWARD THE SYNTHESIS OF NOVEL MDM2 INHIBITORS CANDIDATES

Protein protein interactions are valuable targets to discover novel anticancer agents. One of these is the p53-MDM2 interaction. In one of these interaction MDM2 protein inhibits p53 protein and may cause cancer. New drugs that inhibit this interaction are important for the treatment of cancer. One class of these anticancer agents are morpholinone derivative.

In this study, it is aimed to synthesize new morpholinone derivatives. *(R)*-2-amino-2-(4-chlorophenyl)acetic acid was used as starting material for the synthesis. The first step was a trityl protection of amine with trityl chloride. Trityl protected amino acid was first reduced to N-Trt amino alcohol with $LiAlH_4$ then oxidized to aldehyde by using Dess-Martin periodinane. The resulting aldehyde was reacted with 3-chlorophenylmagnesium bromide. This part of the synthesis was performed successfully. Then addition of methyl fumarate to this Grignard product was studied by a coupling reagents such as HATU. All attempts were failed. Then trityl group was removed by TFA and successfully coupled with methyl fumarate by using HATU. All cyclization reactions in the presence of a base like hydroxide, alkoxide or NaH to form morpholinone skeleton was failed. The cyclization reaction with the potassim carbonate in alcohol was successful and the morpholinone skeleton was formed

ÖZET

YENİ MDM2 İNHİBİTÖRÜ ADAYLARIN SENTEZİNE YÖNELİK ÇALIŞMALAR

Protein protein etkileşimleri yeni antikanser ajanları keşfetmek için önemli etkileşimlerdir. Bu etkileşimlerden bir tanesi de p53-MDM2 etkileşimidir. Bu etkileşimde MDM2 proteini, p53 proteinini inhibe eder ve kansere neden olur. Kanser tedavisinde bu etkileşimi durduran yeni ilaçlar önemlidir. Bu antikanser ajan sınıfından biri morfolinon türevleridir.

Bu çalışmada yeni morfolinon türevlerinin sentezlenmesi amaçlanmıştır. Sentez için başlangıç maddesi olarak *(R)*-2-Amino-2-(4-klorofenil) asetik asit kullanıldı. İlk adım, tritil klorür ile aminin tepkimesinden N-Trt korunmuş amin eldesidir. Tritil korumalı amino asit ilk önce $LiAlH_4$ kullanılarak N-Trt amino alkolüne indirgenmiş, ardından ve Dess-Martin periodinan reaktifi kullanılarak aldehite yükseltgenmiştir. Elde edilen aldehit, 3-klorofenilmagnezyum bromür ile reaksiyona sokuldu. Sentezin bu kısmı başarıyla gerçekleştirildi. Daha sonra bu Grignard ürününe metil fumarat ilave edilmesi, HATU gibi bir birleştirme reaktifleri tarafından incelenmiştir. Bütün bu girişimler başarısızlıkla sonuçlandı. Daha sonra tritil grubu TFA ile ayrıldı ve HATU kullanılarak metil fumarat ile başarılı bir şekilde birleştirildi. Morfolinon iskeletini oluşturmak için hidroksit, alkoksit veya NaH gibi bir bazın varlığında bütün siklizasyon reaksiyonları başarısız olmuştur. Fakat methanol içerisinde potasyum karbonat ile oluşturulan siklizasyon reaksiyonu başarıyla gerçekleştirildi ve morfolinon iskeleti oluşturuldu.

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LIST OF ABBREVIATIONS

d	Doublet
dd	Doublet of doublets
dt	Doublet of triplets
ddd	Doublet of doublet of doublets
DMP	Dess-Martin periodinane
DCM	Dichloromethane
DMF	Dimethylformamide
Et_3N	Triethylamine
Eq.	Equivalent
m	Multiplet
mL	Millilider
TMSCI	Trimethylsilylchloride
THF	Tetrahydrofuran

CHAPTER 1

INTRODUCTION

1.1. p53 as a Therapeutic Target

p53 is a well-known tumor suppressor molecule which has a critical role in human healthy and cancerous cells. In the last 26 years, more than 40.000 articles have been published all over the world.[1-2] p53 functions in cell defense in opposition to transformation of normal cells to cancer cells. This is quite a complicated process, in which, p53 organizes a signal transduction network, which has gone through an evolution process to minimize the results of oncogenic stress.[3-4] In addition, p53 is responsible for causing cell cycle arrest, apoptosis or senescence through its activation in the case of the emergence of diverse stresses (Figure 1). Also, transcription-independent functions of p53 can mostly increase and transform cellular reactions to respond to stress. These reactions are appropriately regulated by p53 so as to ensure that different cells can decide to take the irreversible path of self-annihilation.[5]

Despite the fact that the organization of the p53 pathway is not totally comprehended at the molecular level, it is now clear that activation of wild type p53 is harmful for cancer progression. For this reason, cancer cells have put into practice various mechanisms for the inhibition of p53 function. Actually, p53 is one of the most commonly transformed proteins in the case of human cancer. 50% of all human tumors have or inactive transcription factor because of the deletion or mutation of the TP53 gene.[6] When mutant p53 function is reactivated in cancer cells, expression of the mutant p53 has been observed to lead to tumor cell death. However, proper pharmacological compounds that function in vivo have not yet been identified.[7-8] Another interesting therapeutic strategy is thought to be the activation of p53 which has been kept in its wild type structure.[9] In spite of the fact that 50% of all tumors contain a typical form of p53, many of them are supposed to have deficient p53 function because of irregularities in p53 regulation or faulty signaling in the pathway of p53.[1] Murine double minute 2 gene product (MDM2) is a negative regulator mechanism to inhibit p53 protein.[10] In human tumors, MDM2 is overexpressed because of an enhancement of a chromosome segment that has MDM2 gene or overproduction of the protein lacking gene

enhancement.[11-13] Consequently, function of p53 is potentially blocked by MDM2 overexpression and activity. In fact, cancer tumors that have MDM2 gene enhancement nearly completely produce the typical form of p53.[13] As a result, inhibition of MDM2 might restore p53 function in cancer cells. On the other hand, using p53 activation as a therapeutic agent requires several significant factors. Firstly, MDM2 is not the unique negative regulator of p53 and disruption of MDM2-p53 interaction by small molecule inhibitor still may not reactivate p53 totally. Secondly, in cancer cells with typical form of p53, abnormal p53 signaling could deactivate or prevent the response to MDM2 antagonist. Thirdly, potential growth suppressive apoptotic effect of p53 in healthy tissues could attenuate or completely remove the therapeutic function of p53 proteins. In this study, the latest advances in new therapeutic strategies to will be argued with a highlight on the use of small molecules for the inhibition of MDM2.[14]

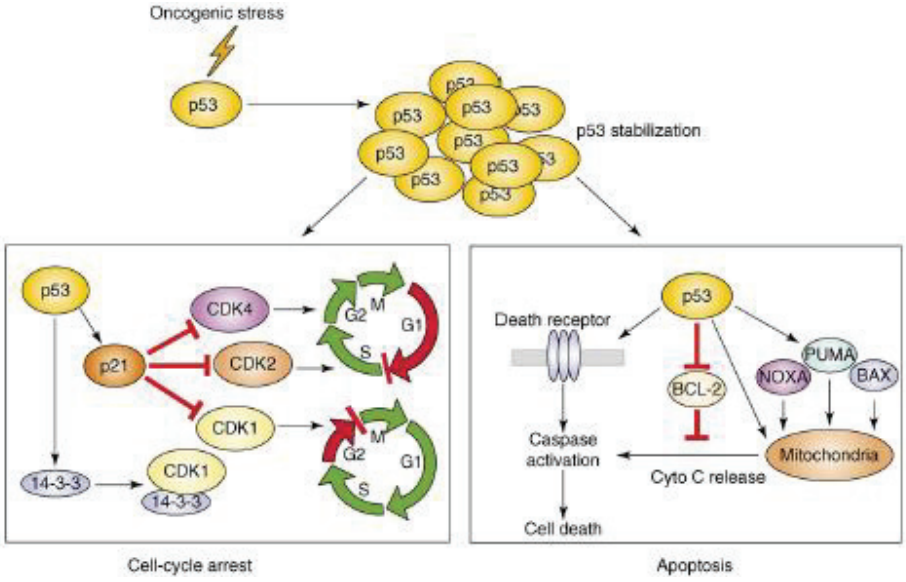


Figure 1.1. P53 is a tumor suppressor protein and can respond to oncogenic stress by either cell cycle arrest or cell death by apoptosis. [14]

1.2. P53-MDM2 Interaction as a Therapeutic Target

High levels of p53 repressor MDM2 can be observed in most of the cancer cells basically due to activity loss of MDM2 inhibitor ARF or amplification of MDM2 gene expression. Hence, a new cancer therapeutic strategy has been revealed by targeting the p53-MDM2 interaction in order to restore p53 activity.[14-32] Regulation of MDM2 and

p53 happens via an autoregulatory feedback loop between p53 and MDM2.[33] When p53 is activated, it triggers the transcription of MDM2. MDM2 has a role on nuclear export and proteasome-mediated degradation of p53 by means of its E3 ubiquitin ligase activity with ubiquitination at several lysine residues. All these reactions end up with decreased p53 levels that will decrease expression of MDM2, which permits the potential reactivation of p53 protein.[31-34]

The nature of binding of a short peptide of the p53 transactivation domain (15 residues) to the binding domain of MDM2 was studied by cocrystallization and published, which enabled researchers to understand the relationship between these two proteins.[35] This X-ray study indicated that Phe19, Trp23 and Leu26 residues of p53 peptide play a critical role by interacting the deep hydrophobic cavity of the MDM2 protein (Figure 1.2.). Phe19 and Trp23 line up in the deeper part of the cleft in the crystal structure by building hydrophobic interactions with Gly58 and Ile61 of MDM2, Phe19 has a backbone amide group and this group forms one hydrogen bond with the carbonyl of Gln72 at the access point of the cavity. Trp23 covers the deepest portion of the binding cavity through by making a hydrogen bond with Leu54 and hydrophobic interactions with Gly58 and Ile61 of MDM2. The final residue Leu26 also placed at the end of the hydrophobic cavity. Moreover, extra Van Der Waals interaction created Leu 22 of p53 enhances the interaction.[26, 35]

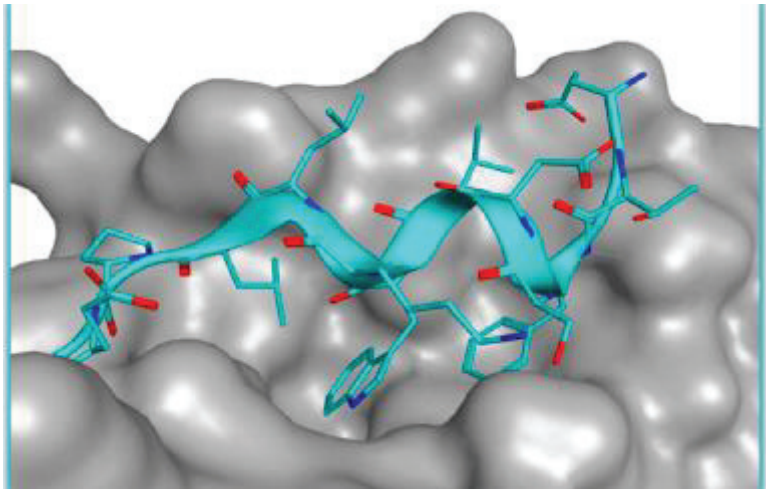


Figure 1.2. Short peptide of p53 binds to MDM2 protein.[36]

Following the publications of the crystal structure of p53 and MDM2 interaction, many studies have focused on designing more small molecules and peptide

derivatives for the inhibition of this interaction. Lately, several types of compounds has been named to be p53-MDM2 interaction inhibitors and some of them are being tested as anticancer drug candidate in clinical trials.[32]

1.3. Inhibition of the p53-MDM2 Binding as Novel Strategy to Fight with Cancer

MDM2 is well-known as an inhibitor of p53 tumor suppressor protein, and because of this, agents targeting MDM2-p53 binding could reactivate the wild type p53 protein. MDM2 can inhibit p53 by different mechanisms, depending on their direct interaction.

Consequently, small molecules and peptides can be designed to inhibit p53 and MDM2 protein–protein interaction. Because of this inhibition, stability and transcriptional activation of p53 can be increased. With the control of strong powerful tumor suppressor function of p53, compounds like this can provide a therapeutic use in the treatment for human cancer expressing wild-type p53.

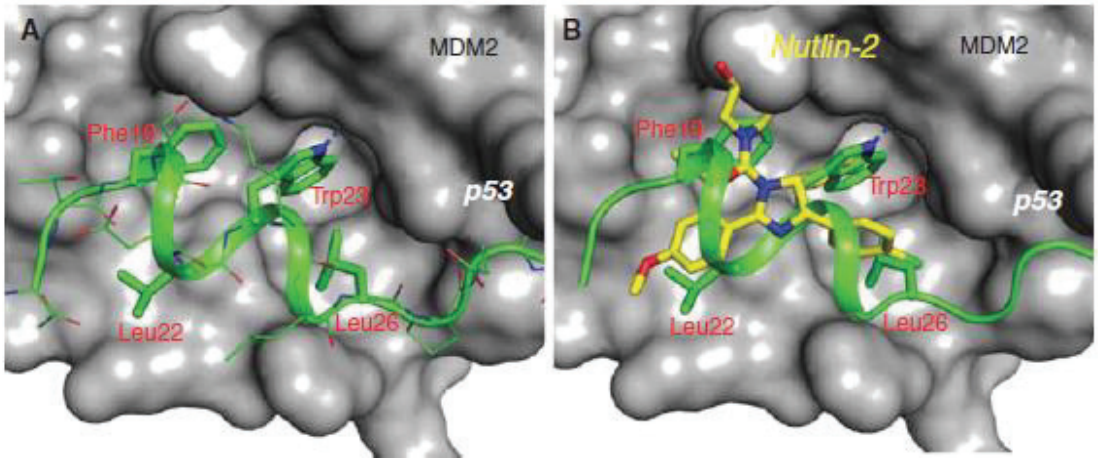


Figure1.3. Cocrystal structures. (A) Cocrystal structure of MDM2 (surface rendering) and p53 (stick model). p53 protein uses primarily residues Phe19, Trp23, and Leu26 to interact with a well-defined, surface hydrophobic pocket in MDM2. (B) Superposition of the cocrystal structures of nutlin-2/MDM2 complex and p53/MDM2 complex (PDBIDs: 1YCR and 4HG7). Nutlin-2 is shown by yellow sticks and the three key p53-binding residues are shown by green sticks with the MDM2 protein shown in the surface rendering.[40]

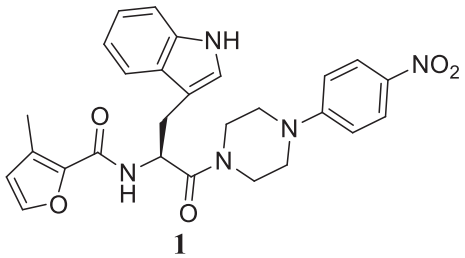
It was decoded that the first 30 amino-terminal residues of p53 interact with the first 120 amino-terminal residues of MDM2.[37-38] MDM2 can interact with some specific residues 15-29 of a p53 peptide as seen in the Figure 1.3. showing high resolution co-crystal structure of MDM2 with p53 peptide.[35] After the discover of molecular level interactions many studies were devoted to design and synthesis of small non-peptide molecules to inhibit p53-MDM2 interaction. Particularly, with the cocrystal structure, it is observed that p53 has an a-helical conformation and interacts with MDM2 by means of three hydrophobic residues, Phe19, Trp23, and Leu26. Even though the binding affinities of natural p53 peptides is a at a micromolar level, peptides designed with unnatural amino acids may provide low nanomolar binding affinities,[39] which in turn supports the possibility of creating high-affinity, non-peptide small-molecule inhibitor of MDM2-p53 interaction (Figure 1.3).

1.3. MDM2 Inhibitors

In recent years, several small molecule inhibitors of MDM2 have been discovered. Some of them are listed briefly in the following section of this study.

1.4.1. 4-Phenyl-piperazine derivatives

4-phenyl-piperazine derivatives are the first p53-MDM2 inhibitor discovered in history. The structure of the most effective derivative is in this class of compounds was 0.5 μM IC_{50} value shown below (Figure 1.4).[21]



1

Figure 1.4. Structure of 4-phenyl-piperazine derivative inhibit p53-MDM2 by 0.5 μM IC_{50} value.

1.4.2. Chalcone derivatives

A list of chloro and acetic acid derivatized chalcone derivatives were found to be MDM2 antagonist. These chalcones bind to MDM2 and inhibit its interaction with p53.[41] Chalcones are bound to the space of the tryptophan binding site in the MDM2 protein (Figure 1.5).[21]

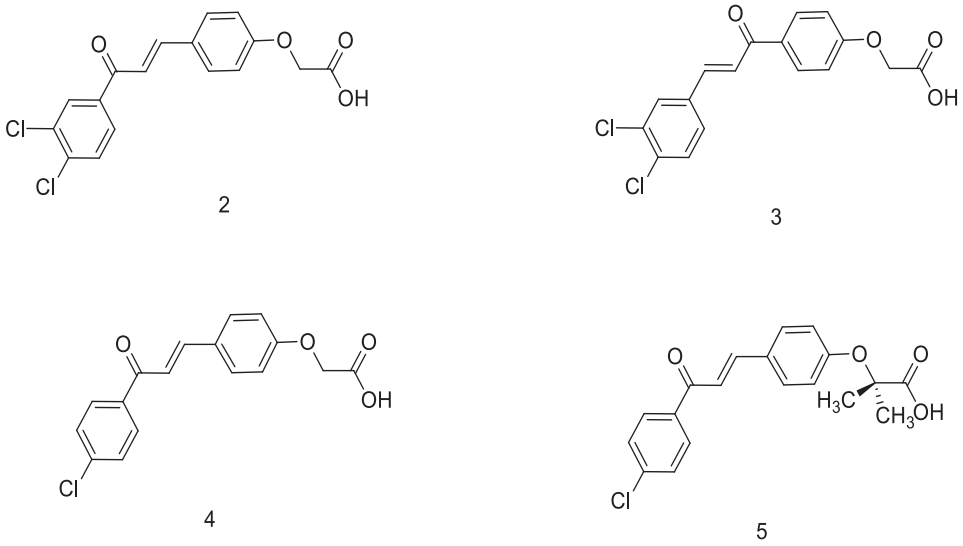


Figure 1.5. Structure of chalcone derivatives as p53-MDM2 inhibitors.

1.4.3. Sulfonamides

Compound 6 is an sulfonamide derivative and show potent MDM2 inhibition activity. These sulfonamide base MDM2 inhibitors have been discovered by the National Cancer Institute through 3D pharmacophore research (Figure 1.6).[42]

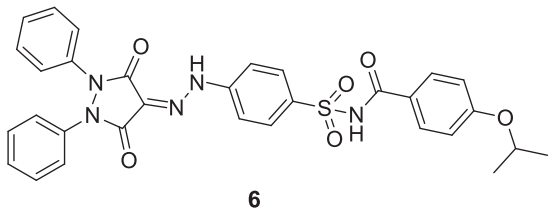


Figure 1.6. Structure of sulfonamide compound NSC 279287 having IC_{50}: 31.8 μM for p53-MDM2 inhibition.

1.4.4. Bis-aryl sulfonamides

Using a molecular modeling program, a new group of p53-MDM2 inhibitors named thiophene-2-sulphonic acid phenylamides modeled on the N-terminus of MDM2, were found.[21] These molecules have been shown to be highly potent p53-MDM2 inhibitor (Figure 1.7). Compund 8 was found to be the most active derivative.

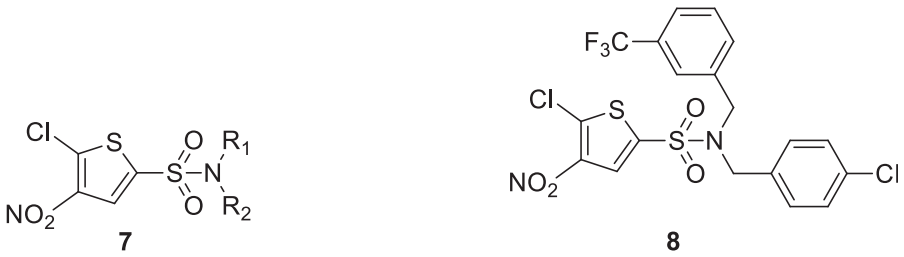


Figure 1.7. General structure of bis-aryl sulfonamides derivatives **7** and structure of the most active MDM2 inhibitor compound **8**.

1.4.5. 1,4-Benzodiazepine-2,5-dione

Compounds having MDM2 inhibitor properties with 1,4-benzodiazepine-2,5-dione structure have been developed by Johnson&Johnson pharmaceutical company[43] and the compound TDP222669 is the most effective derivative in this group with an IC_{50} value of 30 μM shown in Figure 1.8.[44]

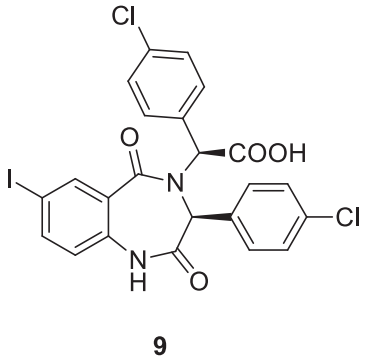


Figure 1.8. Structure of 1,4-benzodiazepine-2,5-dione derived MDM2 inhibitor TDP222669 (**9**).

Compound **9** is found to have low solubility as a result of in vivo experiments despite its promising activity in in vitro assays. It has also been observed that it can not pass through the cell membrane. It has been thought that this is due to the ionization of the carboxyl group. Therefore, the carboxyl group is replaced by the methyl group. As a result of the optimization studies, compounds **10-12** were developed with enhanced solubility.[45]

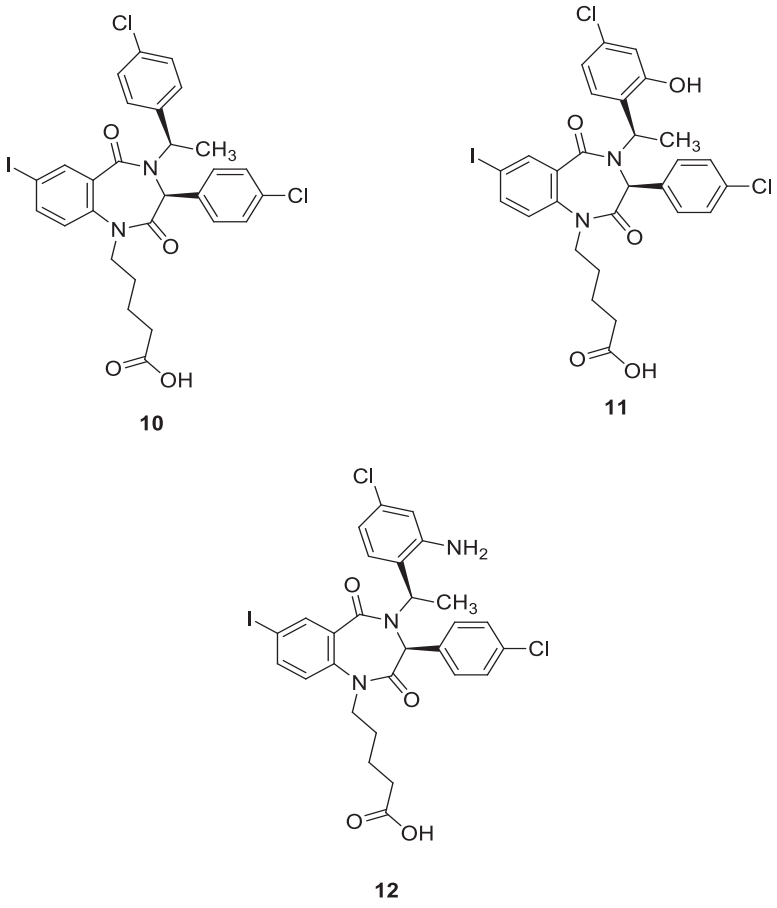


Figure 1.9. Structure of 1,4-benzodiazepine-2,5-dione derivatives having p53-MDM2 inhibitor properties with good solubility.

1.4.6. Spiro(oxindole-3,3'-pyrrolidine)

Compounds carrying the spiro (oxyindole-3,3'-pyrrolidine) parent structure may be used to design a new class of MDM2 inhibitors.[46] The oxyindole structure mimics the tryptophan side chain very well and interacts with the region to which the tryptophan binds on MDM2. The NH group at the core establishes a hydrogen bond with the

carbonyl group on MDM2 and the spiro (oxyindole-3,3'-pyrrolidine) derivative compounds interact with the hydrophobic MDM2 binding site.[46] High MDM2 inhibition activity was observed in the spiro (oxyindole-3,3'-pyrrolidine) compound **13**. However, no activity was observed for compound **14**. These results indicate that stereochemistry of spiro (oxyindole-3,3'-pyrrolidine) derivatives play a critical role during MDM2 inhibition (Figure 1.10). [47]

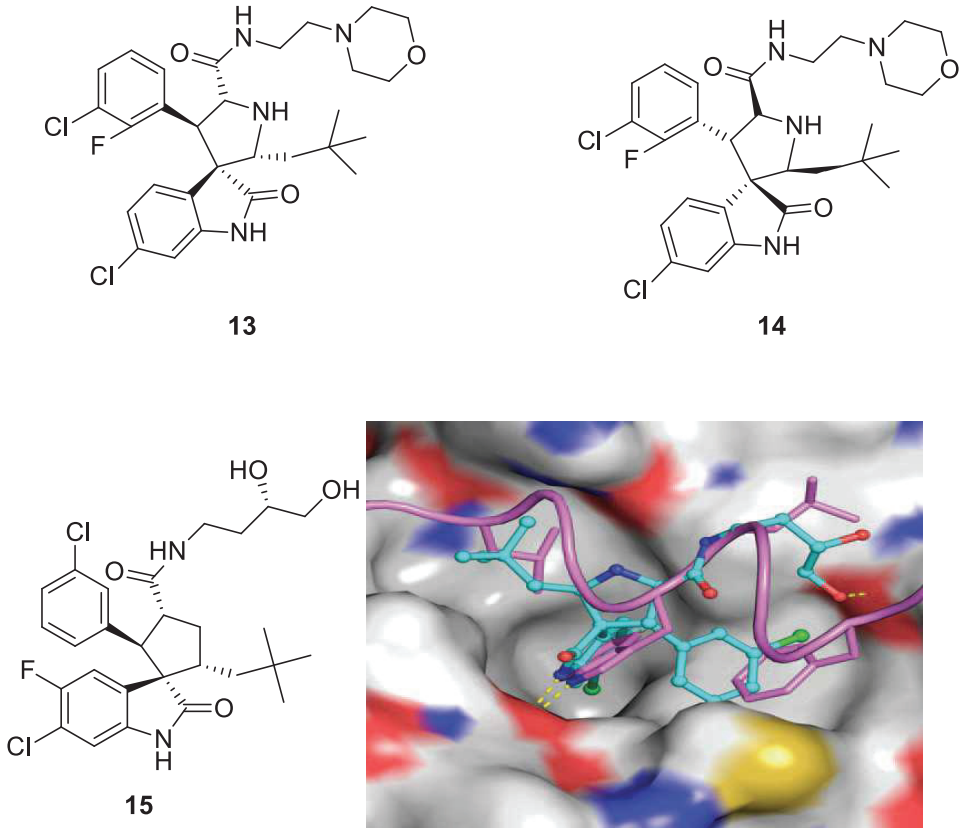
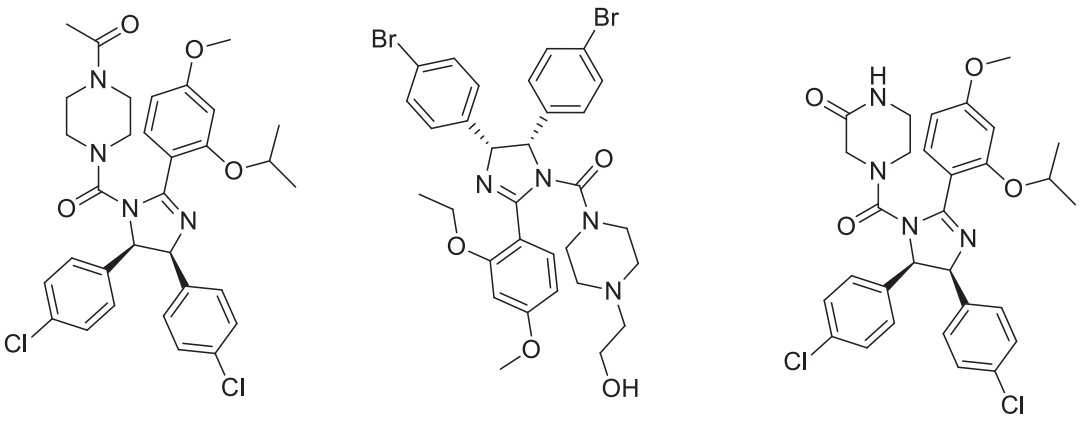


Figure 1.10. Structure of Spiro(oxindole-3,3'-pyrrolidine) derivatives (**13 and 14**) and MI-219 poses with MDM2.[48]

1.4.7. Imidazole derivatives

2,4,5-Triphenyl imidazoline compounds are referred to as nutliners. These compounds are few of the most potent p53-MDM2 inhibitors.[49-51] Docking images of MDM2 and nutlin-2 complex are shown below (Figure 1.11).



16
Nutlin-1

17
Nutlin-2

18
Nutlin-3

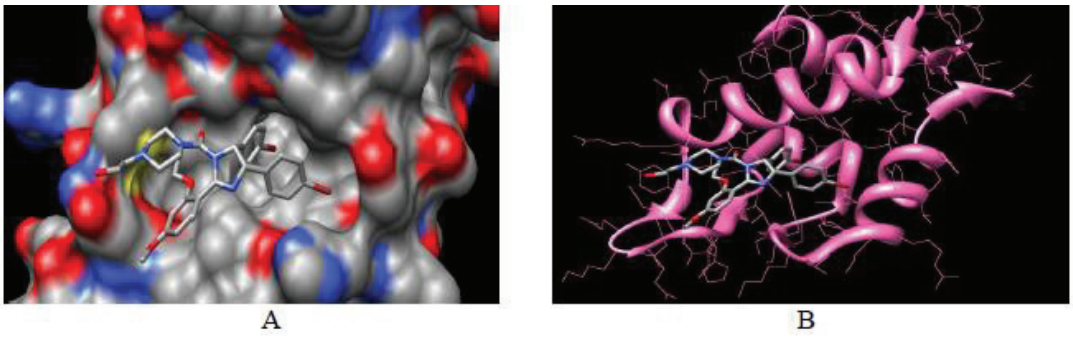
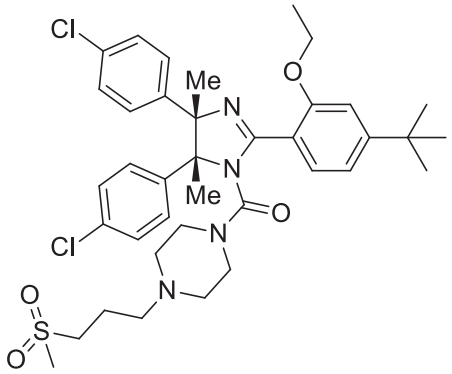


Figure 1.11. (A) Surface representation of MDM2. MDM2 and Nutlin 2 complex (the carbon atoms are gray, the nitrogen atoms are blue, the oxygen atoms are red, and the bromine atoms are burgundy). (b) Cartoon-like representation of MDM2 with Nutlin-2 (pdb code:1RV1)[49]

Nutlin-2 complexes with the binding site of p53 by taking advantage of hydrophobic interactions on MDM2. In cell line experiments with nutlins, it was found that they increase the accumulation of p53. It has been shown that functional p53 in cancer cell lines cause cell cycle arrest or cell apoptosis. It has also been found that mutant p53 causes inactivation. Studies on mice with Nutlin-3 have shown that 90% of the cases have suppressed tumor growth. Roche has made optimization studies for Nutlines.[46, 49] One of the compounds they optimize as a result of these studies is called RG7112 with an IC_{50} value of 18 nM. Clinical trials for this compound was also started (Figure 1.12).[52]



19
RG7112

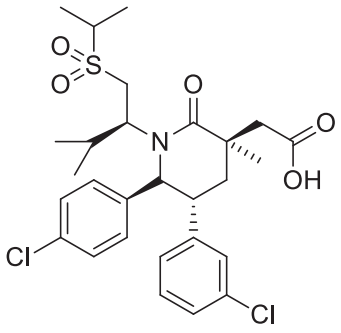
Figure 1.12. RG7112 a small-molecule inhibitor of MDM2.

1.4.8. Piperidinone Derivatives

The molecules bearing the piperidine-2-one structural group were found by Amgen scientists. They have synthesized the AMG-232 molecule. This molecule is a p53-MDM2 inhibitors. The Kd value of the AMG-232 molecule is found to be 0.045 nM and the IC_{50} values for antiproliferative activity of this molecule are 9.1 nM and 10 nM in SJSA-1 and HCT-116 cell lines, respectively. The best p53-MDM2 inhibitor in this class of molecules was found to be AM-6761 after optimization studies. (Figure 1.13)

1.4.8. Morpholinone Derivatives

Amgen researchers also discovered new morpholinone derived MDM2 inhibitors in 2014. AM-8735 emerged as an inhibitor with remarkable biochemical potency (HTRF IC_{50} = 0.4 nM) and cellular potency (SJSA-1 EdU IC_{50} = 25 nM), in addition to pharmacokinetic properties. AM-8735 also shows excellent antitumor activity in the SJSA-1 osteosarcoma xenograft model with an ED_{50} of 41 mg/kg (Figure 1.13).



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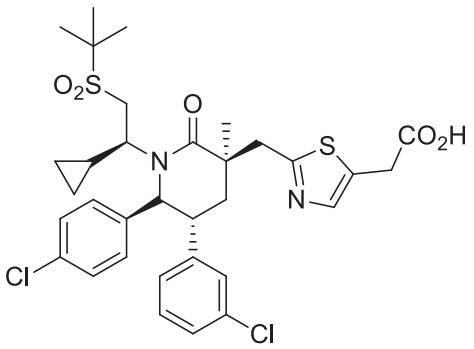
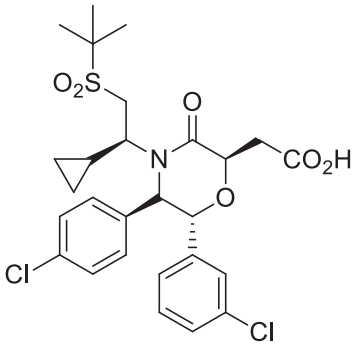
AMG 232



21 (AM 6761)
IC_{50}= 0.1 nM



22 (AM 8735)
IC_{50}= 0.4 nM

Figure 1.13. Piperidine (**20, 21**) and morpholinone (**22**) derived potent MDM2 inhibitiors.

Since, Amgen's study was a model study for our works, key points for the synthesis of AM-8735 will also be discussed in this part of the thesis. Enantioselective synthesis of (*R*,*R*) amino alcohol (**29**) is essential for the preparation of morpholinone derivatives Gonzalez and coworkers produced this precursor starting from (*R*)-4-chlorophenylglycine. That was reduced to aminoalcohol (**24**) by $LiAlH_4$ and then amino group was protected by Boc group. Then alcohol was oxidized to aldehyde by using DMP in high yields. At this point stereoselective addition of 3-chlorophenylmagnesium bromide to aldehyde **26** gave N-Boc protected amino alcohols **27**. Deprotection and neutralization reactions furnished the target aminoalcohols **29** in moderate yields. (figure 1.14) Then that is reacted with chloroacetylchloride to give compound **30** which is then reacted with ethyl

2-bromo-2-cyclopropylacetate (**31**) under basic condition to produce the compound **32**. This is converted to the AM-8735 in four steps.

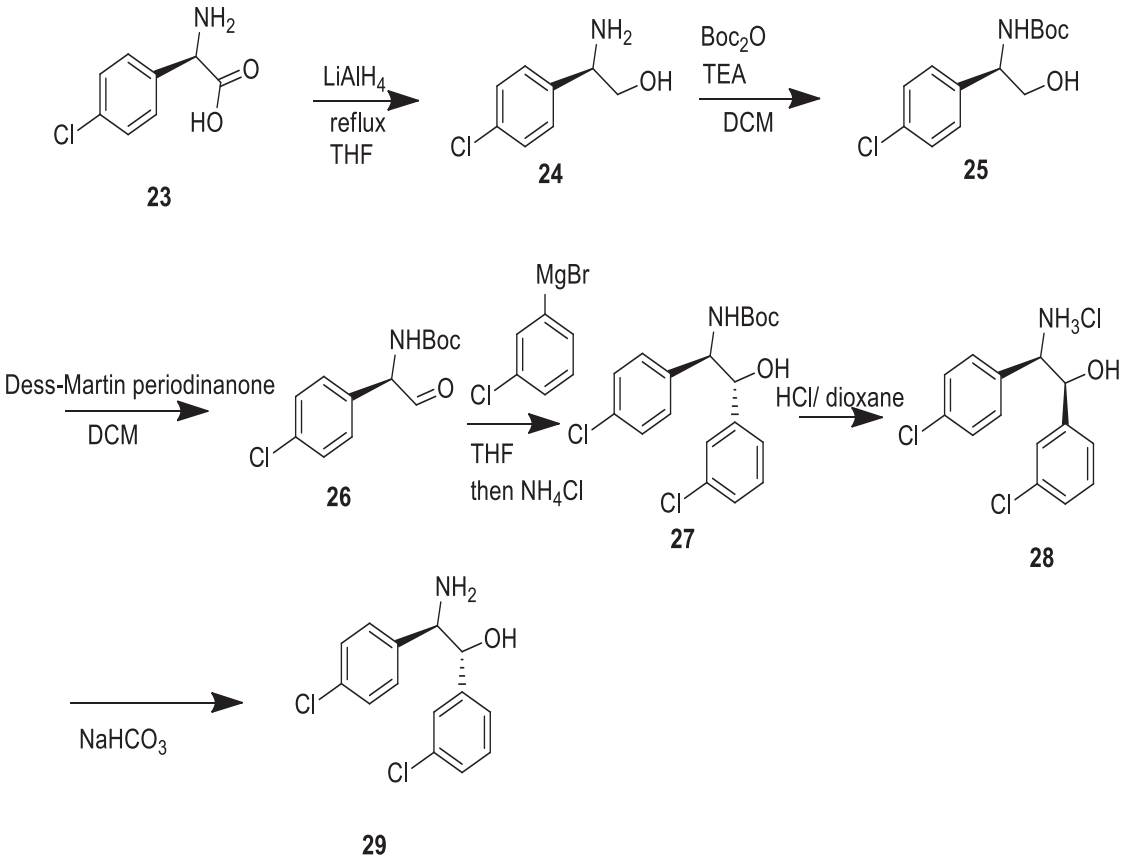


Figure 1.14. Preparation of (*R,R*)-2-amino-1-(3-chlorophenyl)-2-(4-chlorophenyl)-ethanol ((*R,R*-)36).

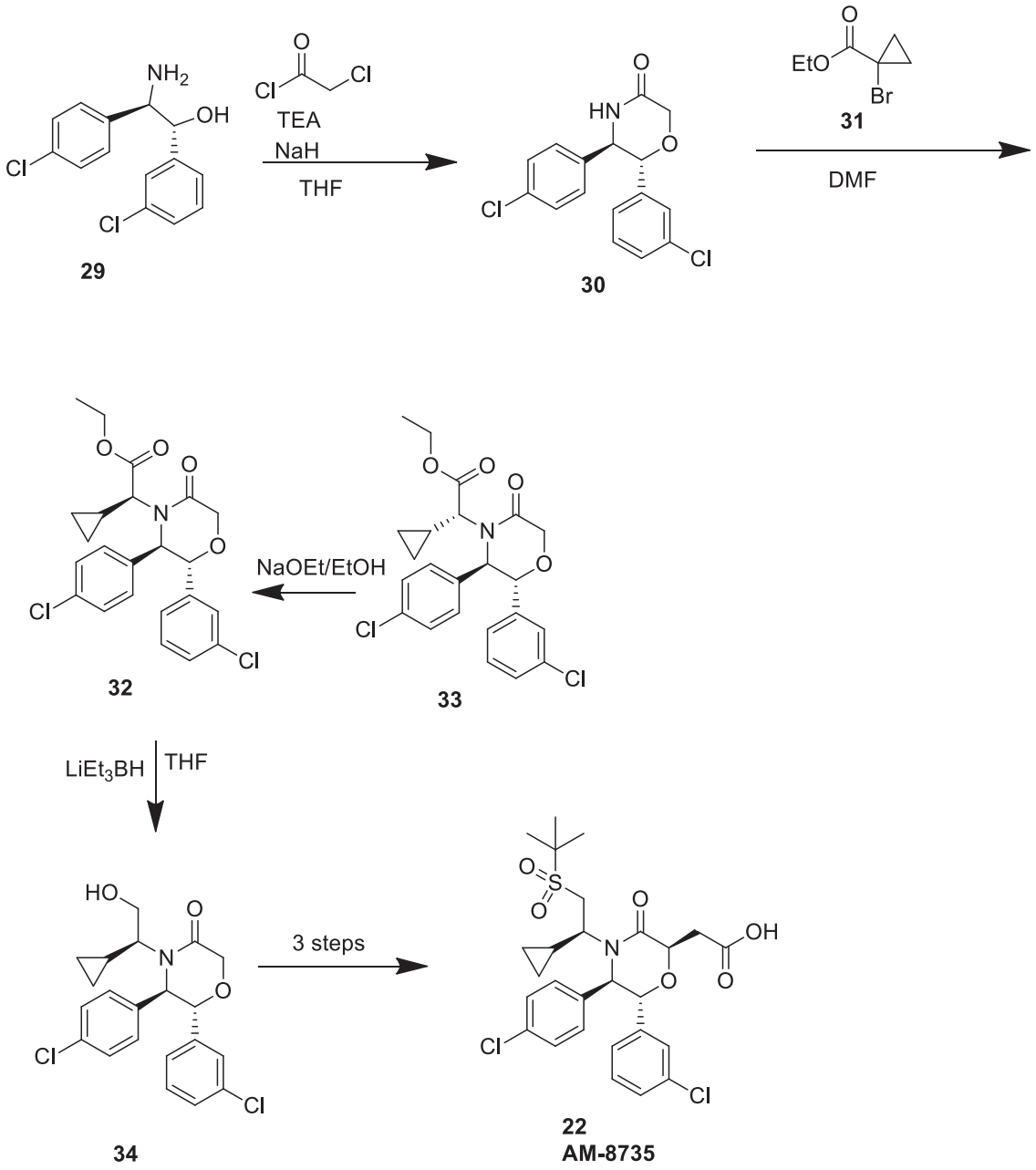


Figure 1.15. Synthesis of best morpholinone derived MDM2 inhibitor (AM-8735).

1.5. Aim of The Study

In this study it is aimed to convert (R)-4-chlorophenyl glycine to the corresponding novel morpholinone derived potential MDM2 inhibitors. To this end, preparation of chiral aminoalcohol **(29)** is essential. Then reaction of that with methyl fumarate would produce the corresponding cyclic structures. (Figure 1.15)

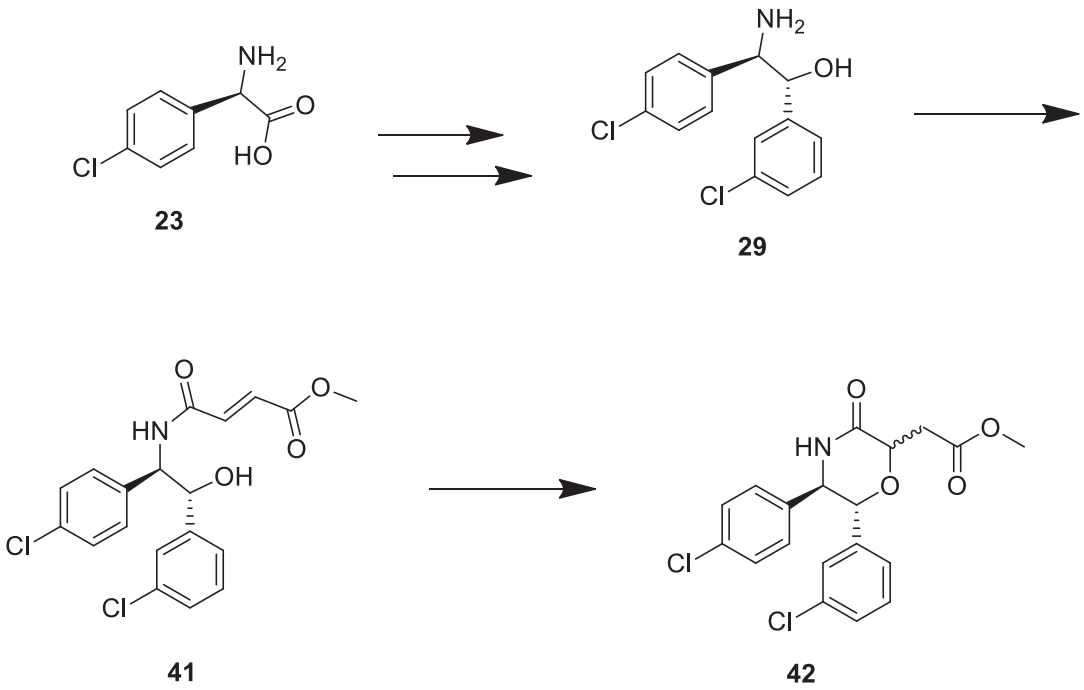


Figure 1.15 Synthesis of novel best morpholinone derived potential MDM2 inhibitors.

CHAPTER 2

RESULT AND DISCUSSION

2.1. Reduction of (*R*)-2-Amino-2-(4-chlorophenyl)acetic Acid

Synthesis was started by reduction of (*R*)-2-amino-2-(4-chlorophenyl)acetic acid **(25)** to (*R*)-2-Amino-2-(4-chlorophenyl)ethanol **(26)** by using $LiAlH_4$ in refluting THF. Reaction was completed in 4 hours and gave 77-91% yields repetedly as shown in Table 2.1. The product was used in the next step without performing any purification step.

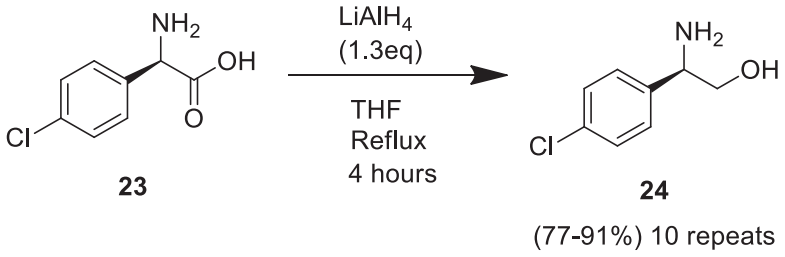
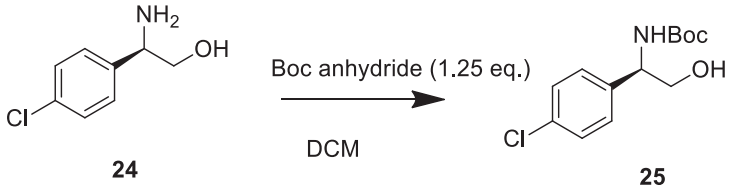


Figure 2.1. Reduction of *(R)*-2-amino-2-(4-chlorophenyl)acetic acid to (R)-2-amino-2-(4-chlorophenyl)ethanol derivatives with $LiAlH_4$

2.2. N-Boc Protection of (R)-2-Amino-2-(4-chlorophenyl)ethanol

To protect the amino group, a protection reaction with di-tert-butyl dicarbonate in DCM with (*R*)-2-amino-2-(4-chlorophenyl)ethanol was performed and the product was then purified by column chromatography the reaction gave 47-82% yields repetitively in all experiments as summarized in Table 2.2.

Table 2.1. Protection of (*R*) -2-Amino-2- (4-chlorophenyl)ethanol with Boc group



24 25

Entry	Time	Yield, %
1	overnight	48
2	2 hours	81
3	2 hours	82
4	2 hours	47
5	2 hours	59
6	2 hours	66
7	2 hours	70
8	2 hours	77
9	2 hours	76
10	2 hours	78
11	2 hours	77

2.3. Oxidation of (R)-tert-Butyl 1-(4-Chlorophenyl)-2hydroxyethlyl Carbamate with DMP

Dess-Martin periodinane is a reagent used to oxidize primary alcohols to aldehydes. This reagent is used because of milder conditions (room temperature, neutral pH), shorter reaction times, higher yields, simplified work up, higher chemoselectivity. Experiments have been carried out with very high efficiency. Due to the rapid racemization of the aldehydes, we proceeded to the next step without performing column chromatography. This step gave 65-98% yields as summarized in Table 2.3.

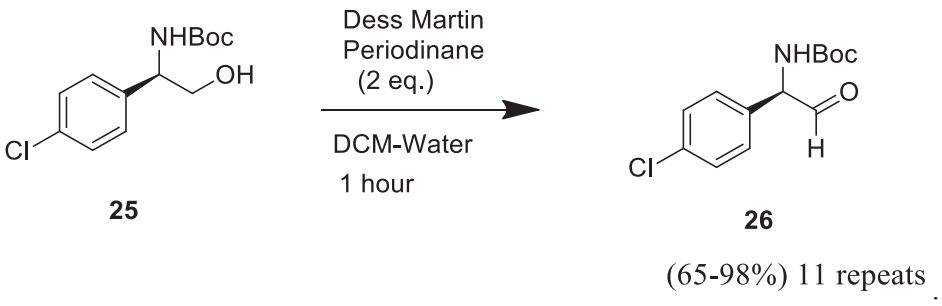
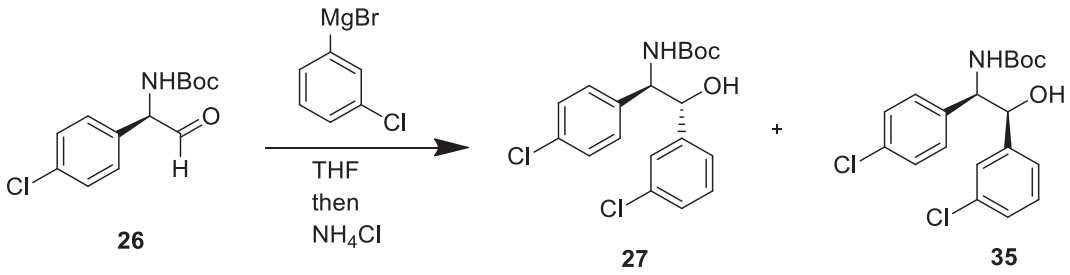


Figure 2.2. Oxidation of (R)-tert-butyl 1-(4-chlorophenyl)-2-hydroxyethylcarbamate.

2.4. Addition of 3-Chlorophenylmagnesium Bromide to (*R*)-tert-Butyl 1-(4-Chlorophenyl)-2-oxoethylcarbamate Reaction

Grignard reaction was performed by addition of commercially available 0.5 M 3-chlorophenylmagnesium bromide in THF. In this experiment, the Grignard reagent is added to carbonyl carbon by acting as a nucleophile. As a result, N-Boc protected amino alcohol (**27**) is obtained. As a result of this experiment, two diastereomers were obtained. Reaction gave many spots in TLC indicating that aldehyde is not the only reactive site for Grignard reagent. All attempts to purify the diastereomers from reaction mixture were failed. Hence yields of the reaction can not be reported (Table 2.4). Further deprotection of amino alcohol (**27**) by TFA in CH_2Cl_2 yielded amino alcohol which is reported in the literature.[53] Comparison of NMR for this amino alcohol with literature values concluded that this is not the desired product. Because of that another protective group, such as Trt, for amine will be used for further synthesis attempt.

Table 2.2 Reaction of (*R*)-tert-Butyl 1-(4-chlorophenyl)-2-oxoethylcarbamate with 3-chlorophenylmagnesium bromide



Entry	Grignard reagent Eq.	Temperature	Time	Yield, %
1	5	R.T.	Overnight	*
2	2	0 °C	4 hours	*
3	2	0 °C	4 hours	*
4	2	0 °C	2 hours	*
5	2	-40 °C	2 hours	*
6	1.1	-40 °C	2 hours	*
7	1.1	-40 °C	2 hours	*
8	1.1	-40 °C	2 hours	*
9	1.1	-40 °C	2 hours	*

*The two diastereomers could not be obtained purely.

2.5. Trityl Protection Reaction of (*R*)-2-Amino-2-(4-chlorophenyl)acetic Acid

After the failure of the protection reactions with Boc group (*R*)-2-amino-2-(4-chlorophenyl)aceticacid was reacted with first TMSCl and then reacted with tritylchloride and Et_3N. Product was used in the next step without any purification (Figure 2.3).

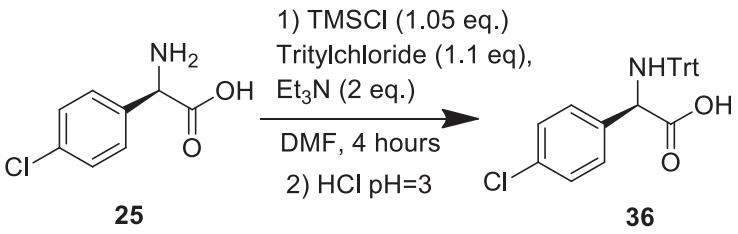
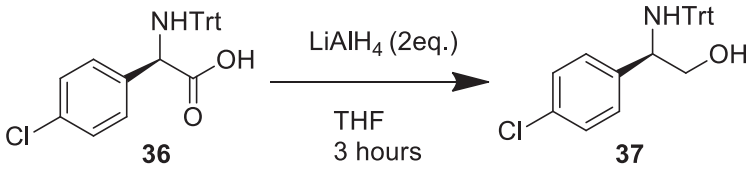


Figure 2.3. N-Trt protection of (*R*) -2-amino-2-(4-chlorophenyl)acetic acid

2.6. Reduction of (*R*)-2-(4-Chlorophenyl)-2-(tritylamino)acetic acid

Reduction of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetic acid to (*R*)-2-(4-chlorophenyl)-2-(tritylamino)ethanol was performed by using 2 eq. of $LiAlH_4$ in anhydrous THF. The product was purified by SiO_2 column chromatography and isolated yields were given in Table 2.5. for two steps.

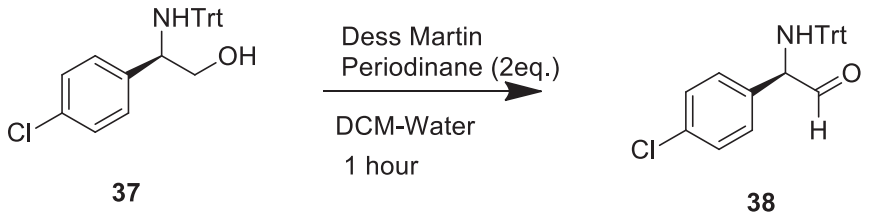


(46-68%) calculated for two steps and 9 repeats

Figure 2.4. Reduction of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)acetic acid with $LiAlH_4$

2.7. Oxidation of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)-ethane-1-ol with DMP

Oxidation of N-Trt protected aminoalcohols (**37**) to corresponding aldehyde (**38**) was performed by DMP in wet CH_2Cl_2. This reaction gave high yielded in all attempts (Table 2.6). Due to the possible racemication chiral aldehyde (**38**) was used directly in the next step without any purification.



37 38

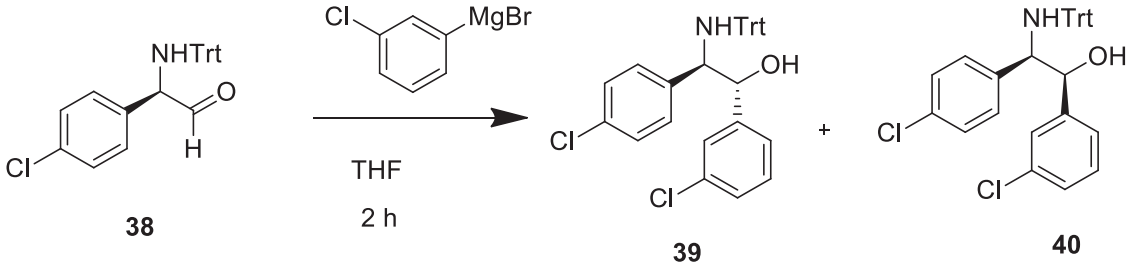
(95-100%) 10 repeats

Figure 2.5. Oxidation of (*R*)-2-(4-chlorophenyl)-2-(tritylamino)-ethane-1-ol

2.8. Addition of Grignard Reagent to (R)-2-(4-Chlorophenyl)-2-(tritylamino)-acetaldehyde

Grignard reaction was performed by addition of and commercially avalaible 0.5 M 3-Chlorophenylmagnesium bromide in THF. In this experiment, the grignard reagent was added to carbonyl carbon by acting as nucleophile. Column chromatography was used for the purification, and the mixture of diastereomers were separated from crude product.

Table 2.3. (R)-2-(4-chlorophenyl)-2-(tritylamino)-acetaldehyde reaction with Grignard compound

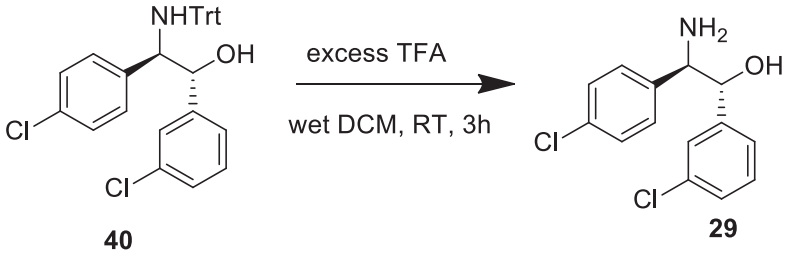


Entry	Grignard reagent	Temp.	Yield, % Fractions		DS Fractions	
1	2.5	-45 °C	58		1.00:0.58	
2	2.5	-40 °C	18	38	1.00:1.66	1.00:0.15
3	2	-40 °C	40		1.00:0.50	
4	2	-40 °C	15	22	1.00:0.10	1.00:0.60

6	2	-40 °C	11	29	1.00:1.18	1.00:0.18
8	2	-40 °C	30		1.00:0.50	
10	2	-40 °C	33	18	1.00:0.80	1.00:0.10

2.9. Deprotection of Grignard Reagent to (R)-2-(4-Chlorophenyl)-2 -(tritylamino)-acetaldehyde with TFA

Deprotection of amino alcohols to corresponding N-trt protected amino alcohols was performed by TFA in wet DCM. The product was purified by SiO_2 coloumn chromatography and isolated yields were given in Figure 2.5

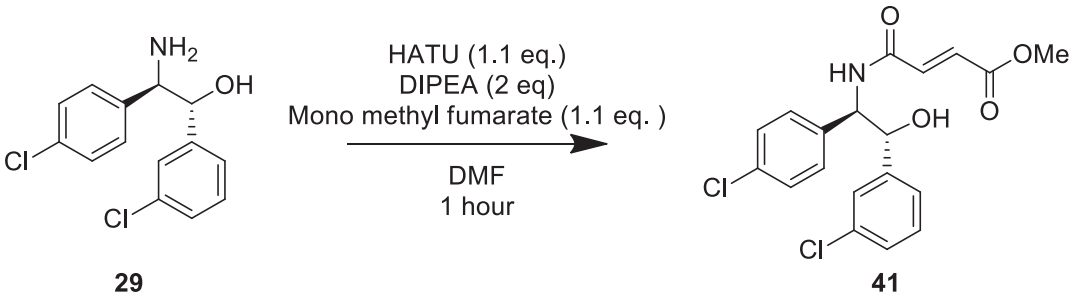


(28-62%) 4 repeats

Figure 2.6. N-Trt protected amino alcohols reaction with TFA

2.10. Coupling reaction of (1R, 2R) -2-amino-1- (3-chlorophenyl) -2-(4-chlorophenyl) ethan-1-ol with mono methyl fumarate

Copuling reaction of chiral amino alcohol **29** with monomethyl fumarate was successfully achieved by using HATU under basic condition. The product was used in the next step without any purification.



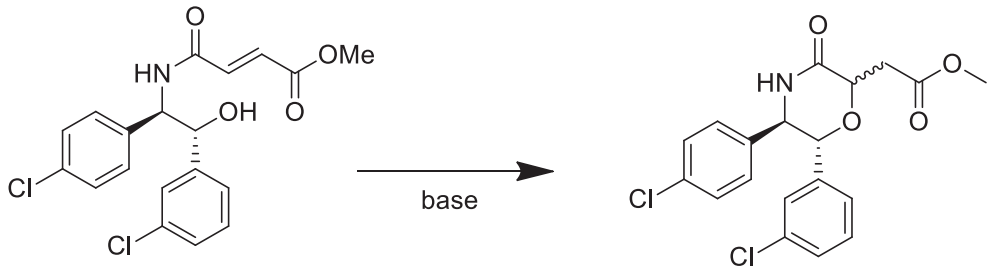
29 41

Figure 2.7. Coupling reaction of amino alcohol **29** with monomethyl fumarate by using HATU, DIPEA.

2.11. Cyclization Reaction of (E)-Methyl 3-(2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethylcarbamoyl)acrylate

In this part of the synthesis, cyclization reaction of (*E*)-methyl 3-(2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxyethylcarbamoyl)acrylate was tried in the presence of various bases, such as NaH, K_2CO_3. Among these attempts only K_2CO_3 treatment in MeOH was successful. in methanol. (Table 2.4)

Table 2.4. Amide cyclization reaction with K_2CO_3



41

Entry	NaH Eq.	K_2CO_3 Eq.	Temperature	Time	Yield, %
1	3	-	R.T.	overnight	x
2	-	2	R.T.	overnight	x
3	-	2	R.T.	3.5 hours	x
4	-	2	R.T.	3.5 hours	x

5	-	1	R.T.	3 hours	*
6	-	1	R.T.	3 hours	*

[x] product could not be obtained [*] product was obtained

CHAPTER 3

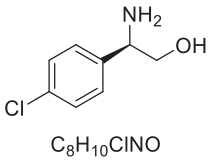
EXPERIMENTAL

3.1. General Methods

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 and ^{13}C NMR data were recorded on Varian 400-MR (400 MHz) spectrometer. Chemical shifts for ^{1}H-NMR and ^{13}C-NMR are reported in δ (ppm). $CDCl_3$ peaks were used as reference in ^{1}H-NMR (7.26 ppm), and ^{13}C-NMR (77.36 ppm) respectively.

3.1.1. (R)-2-Amino-2-(4-chlorophenyl)ethanol

In a two necked flask 62 mg $LiAlH_4$ (1.62 mmol, 1.2 eq.) is dissolved in 5.0 mL of dry THF and heated to 75 ^{o}C under was nitrogen atmosphere and reflux. Then 350 mg (R)-2-amino-2-(4-chlorophenyl) acetic acid (1.35 mmol, 1 eq.) were added in several portion into the mixture. The reaction was stirred for 3 hours, cooled to room temperature, then quenched by the addition of water (8.0 mL) and 15% NaOH solution (8.0 mL). The mixture was stirred for 30 minutes. The solid was filtered off and washed with THF. The solvent of the combined organic phase was removed in vacuo and the aqueous phase was extracted with ethyl acetate (3×30 mL) and then the combined organic phase was dried over anhydrous $MgSO_4$. Organic layer was filtered, and solvent was removed under reduced pressure to yield crude product and resulted 230 mg product with 99% yield.



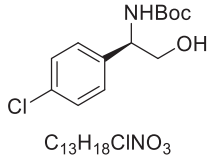
$C_8H_{10}ClNO$

R_f: 0.09 (MeOH:CH_2Cl_2, 12:1)

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 3.99 (dd, J = 8.1, 4.2 Hz, 1H), 3.66 (dd, J = 10.9, 4.2 Hz, 1H), 3.48 (dd, J = 10.9, 8.2 Hz, 1H), 2.74 (bs, 2H), 1.44 (s, 1H).

3.1.2. (R)-tert-Butyl 1-(4-Chlorophenyl)-2-hydroxyethylcarbamate

In a two necked flask 768 mg, (R)-2-amino-2-(4-chlorophenyl)ethanol (4.49 mmol, 1 eq.) was dissolved in dichloromethane (4 mL) and 1273 mg di-tert-butyldicarbonate (5.84 mmol, 1.25 eq.) dissolved in 3 mL of dichloromethane was added dropwise to this solution at 0 °C under was nitrogen atmosphere. After the addition was complete, the ice bath was removed and the reaction was stirred at room temperature for 2 hours. Then extracted with dichloromethane (3x30 mL) and the organic phase was dried over anhydrous $MgSO_4$ and filtered. After removal of solvent of the crude product, purification by column chromatography (EtOAc/Hex; 1:2) resulted desired 1.0 g product with 82% yield.



$C_{13}H_{18}ClNO_3$

R_f: 0.26 (Hexane:EtOAc, 4:1)

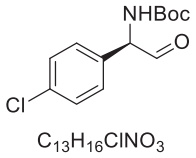
^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.36 (d, J = 8.5 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H), 4.81 (t, J = 5.8 Hz, 1H), 4.49 (dd, J = 14.1, 6.8 Hz, 1H), 3.49 – 3.41 (m, 2H), 1.35 (s, 9H).

$[\alpha]_D^{20}$ = -250.0° (c=1.00, CH_2Cl_2)

3.1.3. (R)-tert-Butyl 1-(4-Chlorophenyl)-2-oxoethylcarbamate

In a two necked flask 1.19 g Dess-Martin periodinane (3 mmol, 2 eq.), is added at room temperature to a solution of 400 mg (R)-tert-butyl 1-(4-chlorophenyl)-2-hydroxyethylcarbamate (3 mmol, 2 eq.) dissolved in wet

dichloromethane (4.0 mL of dichloromethane / 4.0 µL water) under was nitrogen atmosphere. The mixture was stirred for 1 hour, diluted with diethyl ether (50.0 mL) and quenched by the addition of a solution of $Na_2S_2O_3$ (7 eq.) dissolved in saturated aqueous $NaHCO_3$ solution (25 mL) at room temperature. The mixture was stirred for 5 minutes and the phases were separated. The water phase was extracted with diethylether (3 x 30 mL). The combined organic phase was then extracted with saturated aqueous $NaHCO_3$ solution followed by brine solution and the organic phase was dried over anhydrous $MgSO_4$ and filtered. After removal of solvent under vacuum, the product was used in the next step without purification (391 mg crude product with 98% yield).



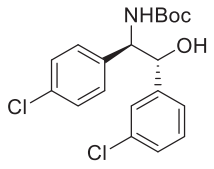
$C_{13}H_{16}ClNO_3$

R_f: 0.30 (Hexane:EtOAc, 4:1)

^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.49 (s, 1H), 7.36 (d, J = 8.5 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 5.85 (d, J = 4.6 Hz, 1H), 5.29 (d, J = 5.5 Hz, 1H), 1.41 (s, 9H).

3.1.4. Tert – Butyl (1R,2R)-2-(3-Chlorophenyl)-1-(4-chlorophenyl)-2 hydroxyethylcarbamate

In a two necked flask 193 mg (R)-tert-butyl 1-(4-chlorophenyl)-2 -oxoethylcarbamate (0.7 mmol, 1.0 eq) is dissolved in 3 mL of dry THF at -40.0 ^oC, then 2.8 mL 0.5 M 3-chlorophenylmagnesium bromide (1.4 mmol, 2 eq) added under a nitrogen atmosphere. The mixture is stirred -40.0 ^oC for a further 2 hours, the mixture is poured into 100 mL of cooled saturated aqueous NH_4Cl solution and extracted with ethyl acetate (3 x 30 mL). The combined organic phase was dried over anhydrous $MgSO_4$ and filtered. After removal of solvent of the crude product, purification by column chromatography (EtOAc/Hex; 1:8 to 1:2) resulted desired impurities 134 mg and enantiomers could not be separated.



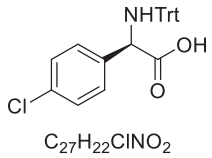
$C_{19}H_{21}Cl_2NO_3$

R_f: 0.40 (Hexane:EtOAc, 4:1)

^{1}H-NMR (400 MHz, $CDCl_3$) δ Since the substance can not be completely purified, the spectrum obtained is not fully interpreted

3.1.5. (R)-2-(4-chlorophenyl)-2-(tritylamino)acetic acid

Into a two necked flask 400 mg (R)-2-amino-2-(4-chlorophenyl) acetic acid (2.16 mmol, 1eq.) was dissolved in 4 mL of dry DMF under nitrogen and 286 µL trimethylsilyl chloride (2.26 mmol, 1.05 eq.) was added to the solution. The mixture was stirred for about 10 minutes until a clear solution was obtained, then, 660 mg trityl chloride (2.37 mmol, 1.1 eq) was added followed by 600 µL triethylamine (4.32 mmol, 2eq.). The reaction mixture was stirred under nitrogen for 2 hours, diluted with 20.0 mL of diethyl ether and 20.0 mL of water. The reaction mixture was acidified to pH 3 with 1.0 M HCl. The reaction mixture (3×30 mL) was extracted with diethyl ether, then the combined organic phase was dried over anhydrous $MgSO_4$ and filtered. The resulting product was used in the next step without purification.



$C_{27}H_{22}ClNO_2$

R_f: 0.22 (Hexzane:EtOAc, 4:1)

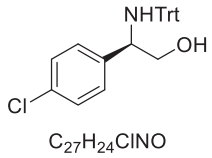
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 6.97 (m, 19H), 4.30 (s, 1H), 1.30 (s, 1H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.47, 145.55, 138.09, 133.84, 128.90, 128.84, 128.61, 128.02, 126.78, 72.09, 59.84.

3.1.6. (R)-2-(4-chlorophenyl)-2-(tritylamino)-ethane-1-ol

Into a two necked flask 1.01 g of (R)-2-(4-chlorophenyl)-2-(tritylamino)acetic acid (2.36 mmol 1.0 eq) was placed and solved in 3 mL of dry THF under nitrogen and stirred under reflux. Afterwards a solution of 175 mg of $LiAlH_4$ (0.67 mmol, 1.5 eq.) in 2 mL dry THF was added drop wise into mixture which was cooled down to 0 ^oC. The reaction was quenched with water (10 mL) followed by 20 mL of diethyl ether. 0.01 M sulfuric acid was added until the mixture's pH = 5 and extracted with diethyl ether (3 x 30 mL). The organic phase is washed with saturated NaOH and saturated brine solution. The organic phase was dried with $MgSO_4$. Organic layer was filtered and concentrated

under reduced pressure to yield crude product. Purification by column chromatography (EtOAc/Hex; 1:12 to 1:4) resulted desired 489 mg product with 55% yield.



$C_{27}H_{24}ClNO$

Rf: 0.47 (Hexane:EtOAc, 4:1)

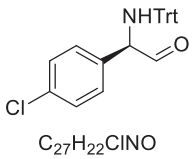
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.47 (m, 4H), 7.31 – 7.13 (m, 15H), 3.72 (t, J = 4.7 Hz, 1H), 3.20 (dd, J = 10.9, 4.4 Hz, 1H), 2.77 (dd, J = 10.9, 5.1 Hz, 1H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 146.19, 142.04, 132.14, 128.80, 128.75, 128.40, 128.25, 127.80, 126.44, 71.72, 66.78, 57.92.

$[\alpha]_D^{20}$= -315° (c= 1.n00, CH_2Cl_2)

3.1.7 (R)-2-(4-chlorophenyl)-2-(tritylamino)-acetaldehyde

In a two necked flask 1.19 g Dess-Martin periodinane (3 mmol, 2 eq.), was added at room temperature to a solution of 330 mg (R)-2-(4-chlorophenyl)-2 -(tritylamino)-ethane-1-ol (0.8 mmol, 1 eq.) dissolved in wet dichloromethane (4.0 mL of dichloromethane / 4.0 μL water) under was nitrogen atmosphere. The mixture was stirred for 1 hour, diluted with diethyl ether (50.0 mL) and quenched by the addition of a solution of $Na_2S_2O_3$ (7 eq.) dissolved in saturated aqueous $NaHCO_3$ solution (25 mL) at room temperature. The mixture is stirred for 5 minutes and the phases were separated. The water phase was extracted with diethylether (3×30 mL). The combined organic phase was then extracted with saturated aqueous $NaHCO_3$ solution followed by brine solution and the organic phase was dried over anhydrous $MgSO_4$ and filtered. After removal of the solvent under vacuum. The product was used in the next step without further purification and resulted (314 mg crude product with 95% yield).



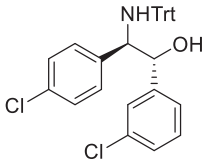
$C_{27}H_{22}ClNO$

Rf: 0.72 (Hexane:EtOAc, 4:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, J = 1,0 Hz, 1H), 7.46 – 7.40 (m, 5H), 7.35 – 7.14 (m, 14H), 4.41 (s, 1H), 3.69 (bs, 1H).

3.1.8.(1R,2R)-1-(3-chlorophenyl)-2-(4-chlorophenyl)-2-(tritylamino)-ethane-1-ol

Into a two necked flask 290 mg (R)-2-(4-chlorophenyl)-2-(tritylamino)-acetaldehyde (0.71 mmol, 1 eq.) was dissolved in 4 mL of dry THF under nitrogen at -40 °C and 3.56 mL 0.5 M 3-chlorophenylmagnesium bromide (1.78 mmol, 2.5 eq.) was added dropwise. The mixture was stirred at -40 °C under nitrogen for 2 hours. The mixture was poured into 0.01 M H_2SO_4 solution Ph=5-6 and extracted with ethyl acetate (3 x 30 mL). The combined organic phases was dried over anhydrous $MgSO_4$ and filtered. After removal of solvent of the crude product, purification by column chromatography (EtOAc/Hex; 1:16) resulted of 140 mg desired product with 38% yield.



$C_{33}H_{27}Cl_2NO$

R_f: 0,50 (Hexzane:EtOAc, 4:1)

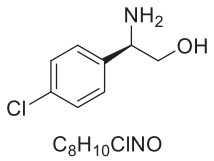
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.35 (m, 6H), 7.20 – 7.09 (m, 10H), 7.08 – 7.02 (m, 2H), 6.85 (d, J = 8.5 Hz, 2H), 6.71 (d, J = 7,6 Hz, 1H), 6.41 (d, J = 8,3 Hz, 2H), 4.42 (d, J = 7,8 Hz, 1H), 3.93 (d, J = 7,9 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 145.63, 142.84, 139.34, 133.97, 131.99, 129.16, 128.96, 127.93, 127.57, 127.29, 126.74, 125.60, 78.35, 71.52, 63.94.

$[\alpha]_D^{20}$= -416^o (c= 1,00, CH_2Cl_2)

3.1.9 (1R, 2R) -2-amino-1- (3-chlorophenyl) -2- (4-chlorophenyl) ethan-1-ol

In a two necked flask 300 mg N-Trt protected amino alcohols (0.57 mmol, 1 eq.) was dissolved in dichloromethane (4 mL) with excess TFA at room temperature 2 Hours later the solvent was evaporated. Then reaction mixtures was extraction with dichloromethane (3x30 mL) and the saturated $NaHCO_3$. Organic phase was dried over anhydrous $MgSO_4$ and filtered. After removal of solvent of the crude product, purification by column chromatography (Chloroform/Methanol; 100:8) resulted desired 100 mg product with 62% yield.



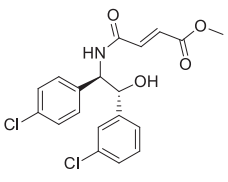
$C_8H_{10}ClNO$

Rf: 0,09 ($MeOH:CH_2Cl_2$, 12:1)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 3.99 (dd, J = 8,1, 4,2 Hz, 1H), 3.66 (dd, J = 10,9, 4,2 Hz, 1H), 3.48 (dd, J = 10,9, 8,2 Hz, 1H), 2.74 (bs, 2H), 1.44 (s, 1H).

3.1.10. (*E*)-methyl 3-(2-(3-chlorophenyl)-1-(4-chlorophenyl)-2-hydroxy ethylcarbamoyl)acrylate

In a vial flask 79.8 mg HATU (0.21 mmol, 1.1 eq.) and 66.5 µL DIPEA (0.38 mmol, 2eq.), 27mg mono methyl fumarate (0.21 mmol, 1.1 eq.) dissolved DMF (2 mL). Two necked flask 100 mg amino alcohols (0.19 mmol, 1 eq.) in DMF (2 mL) and vial mixture added two necked flasks at room temperature 1 hours. Then extraction with dichloromethane (3x30 mL) and 1M HCl. Organic phase was dried over anhydrous $MgSO_4$ and filtered. The product was used in the next step purification.

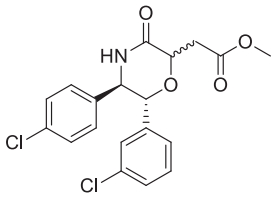


$C_{19}H_{17}Cl_2NO_4$

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 – 6.92 (m, 1H), 6.73 – 6.66 (m, 1H), 5.17 (dd, *J* = 7.8, 4.4 Hz, 1H), 4.91 (d, *J* = 4.4 Hz, 1H), 3.76 (s, 2H).

3.1.11. Methyl 2-(6-(3-chlorophenyl)-5-(4-chlorophenyl)-3-oxomorpholin-2-yl)acetate

In a two necked flask 110 mg N-Trt protected amino alcohols (0.28 mmol, 1 eq.) was dissolved in methanol (2 mL) and 20 mg K_2CO_3 at room temperature 3 hours. After extraction with ethyl acetate (3x30 mL) and water. Organic phase was dried over anhydrous $MgSO_4$ and filtered.



$C_{19}H_{17}Cl_2NO_4$

CHAPTER 4

CONCLUSION

There are many MDM2 inhibitors in the literature and morpholinone derived MDM2 inhibitors are one of the most potent inhibitors in vitro and in vivo studies. Because of this, they are attractive structures to produce novel derivatives.

For this purpose preparation of tert-butyl (*R,R*)-2-(3-chlorophenyl)-1-(chlorophenyl)-2-hydroxy ethyl carbonate was important. In this study preparation of this compound was performed by a five-step procedure reported by Amgen's scientists starting from R-4-chlorophenylglycine. Unfortunately lack of the spectroscopic data for this part of synthesis in the literature, and unexpected side reactions with Grignard reagent with (R)-tertbutyl 1-(4-chlorophenyl)-2-oxoethylcarbonate implied that this synthetic route was not so successful at our hands. Also, removal of the Boc group and comparison of the spectroscopic data of formed amino alcohol with those of literature values implies that this synthetic route has difficulties to produce the target product.

As an alternative, N-Trt protection was also used to produce amino alcohol precursor. Again, synthesis was started with (*R*)-4-chlorophenyl glycine and first protected with tritly group by addition of tritylchloride under basic condition. Then protected glycine was reduced to N-Trt protected amino alcohol by using $LiAlH_4$. Formed alcohol was successfully oxidized to aldehyde by Dess-Martin periodinane, then addition of Grignard reagent gave the target product.

To develop morpholinone derivatives coupling of N-Trt protected amino alcohol with methyl fumarate by using HATU were attempted, and all trials were failed. Interestingly, when the reactions were carried out at longer times Trt protection was removed under basic condition. Addition of methyl fumarate to unprotected amino alcohol by using HATU was successful. In the last step, the cyclization experiment with K_2CO_3 was successful and the morpholinone structure was obtained.

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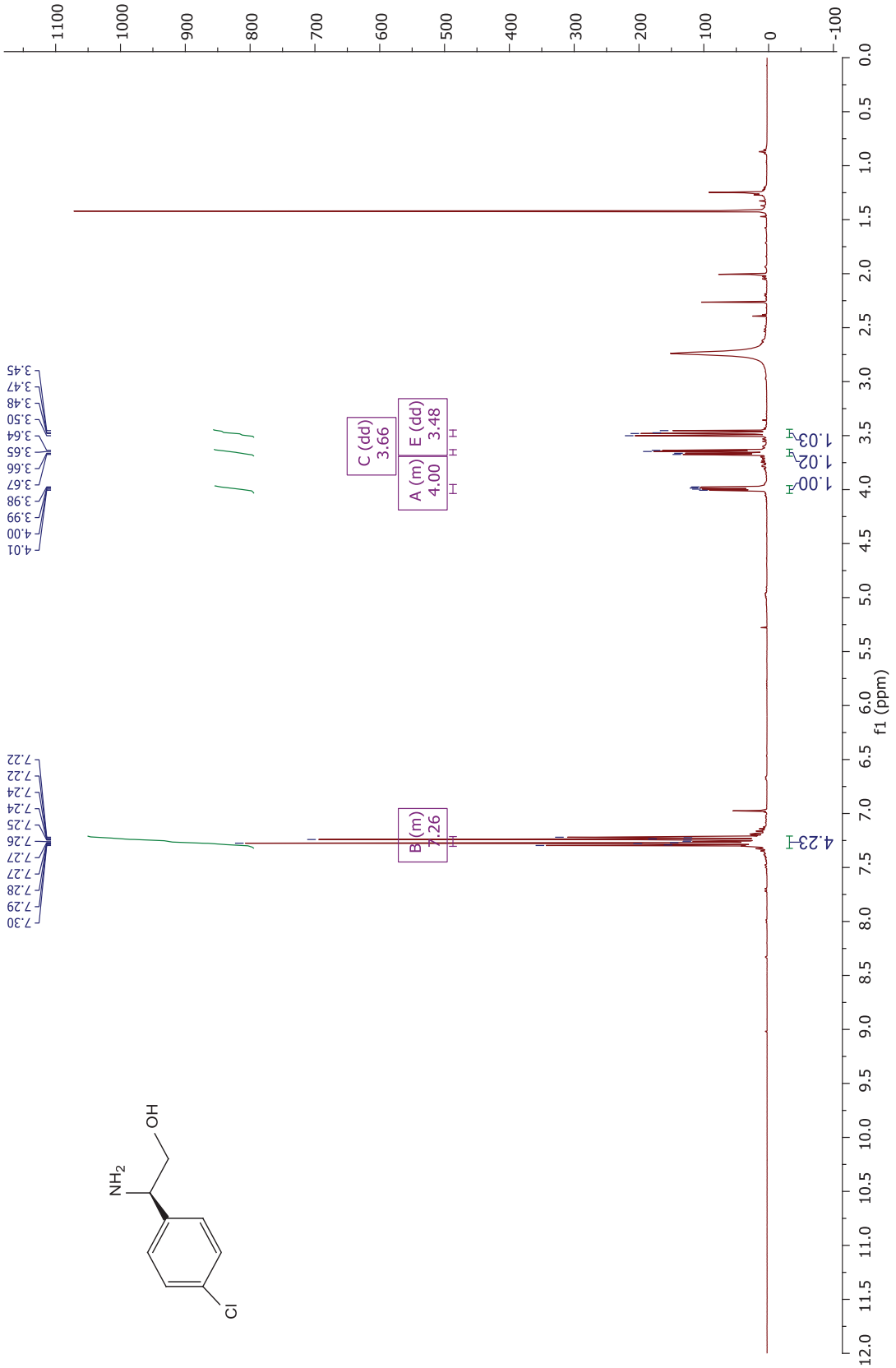
APPENDIX

1H NMR AND 13C NMR SPECTRUM OF COMPOUNDS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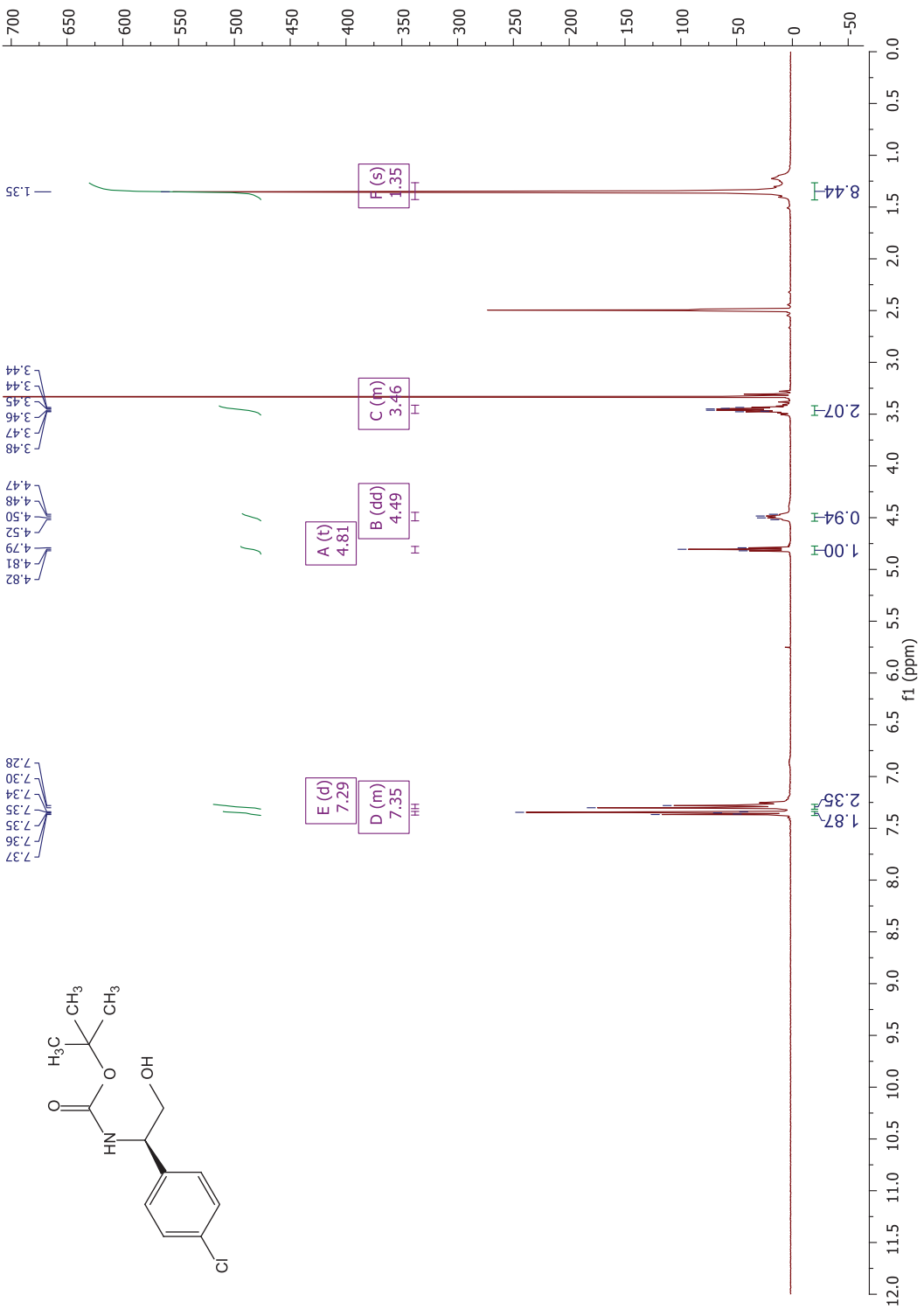
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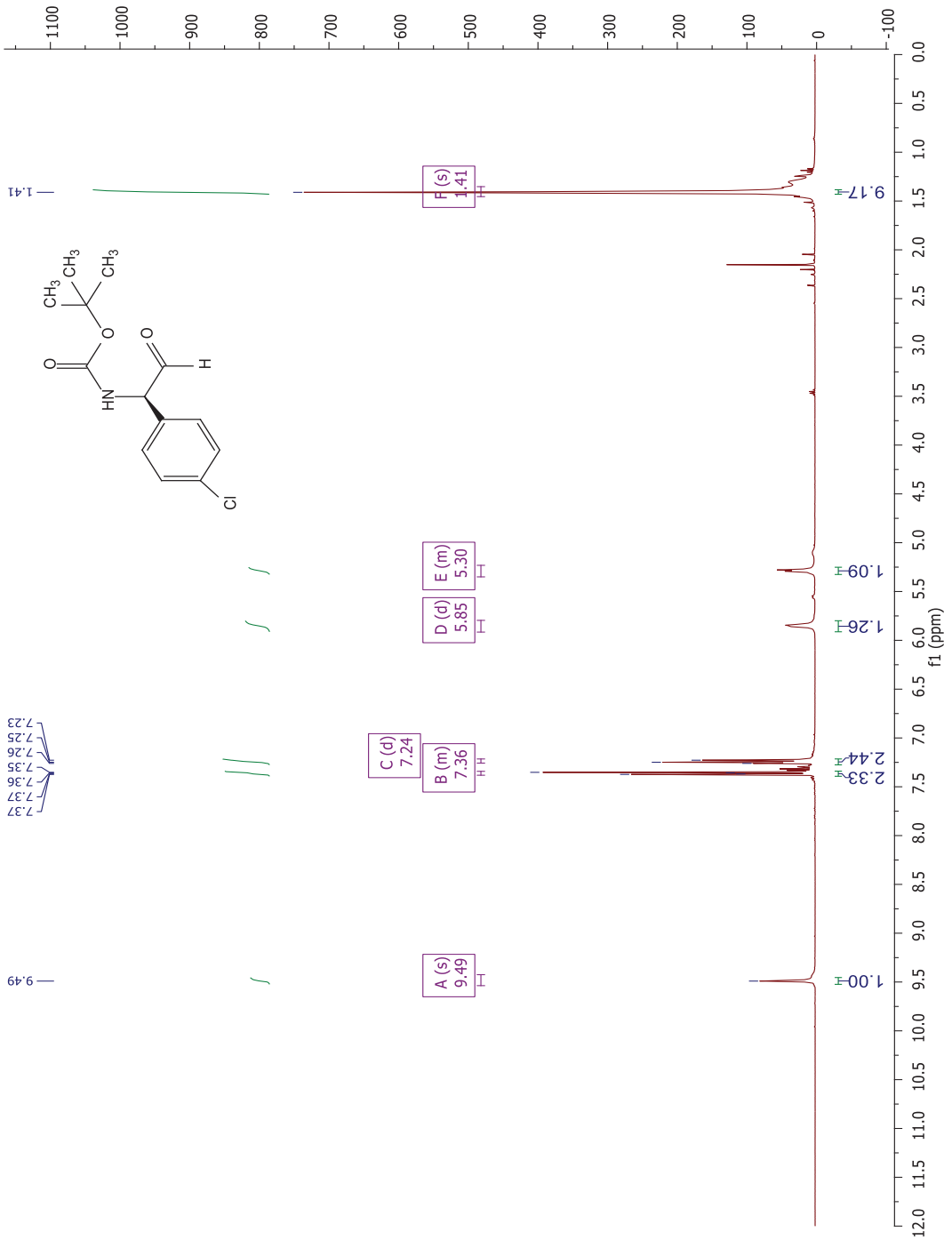
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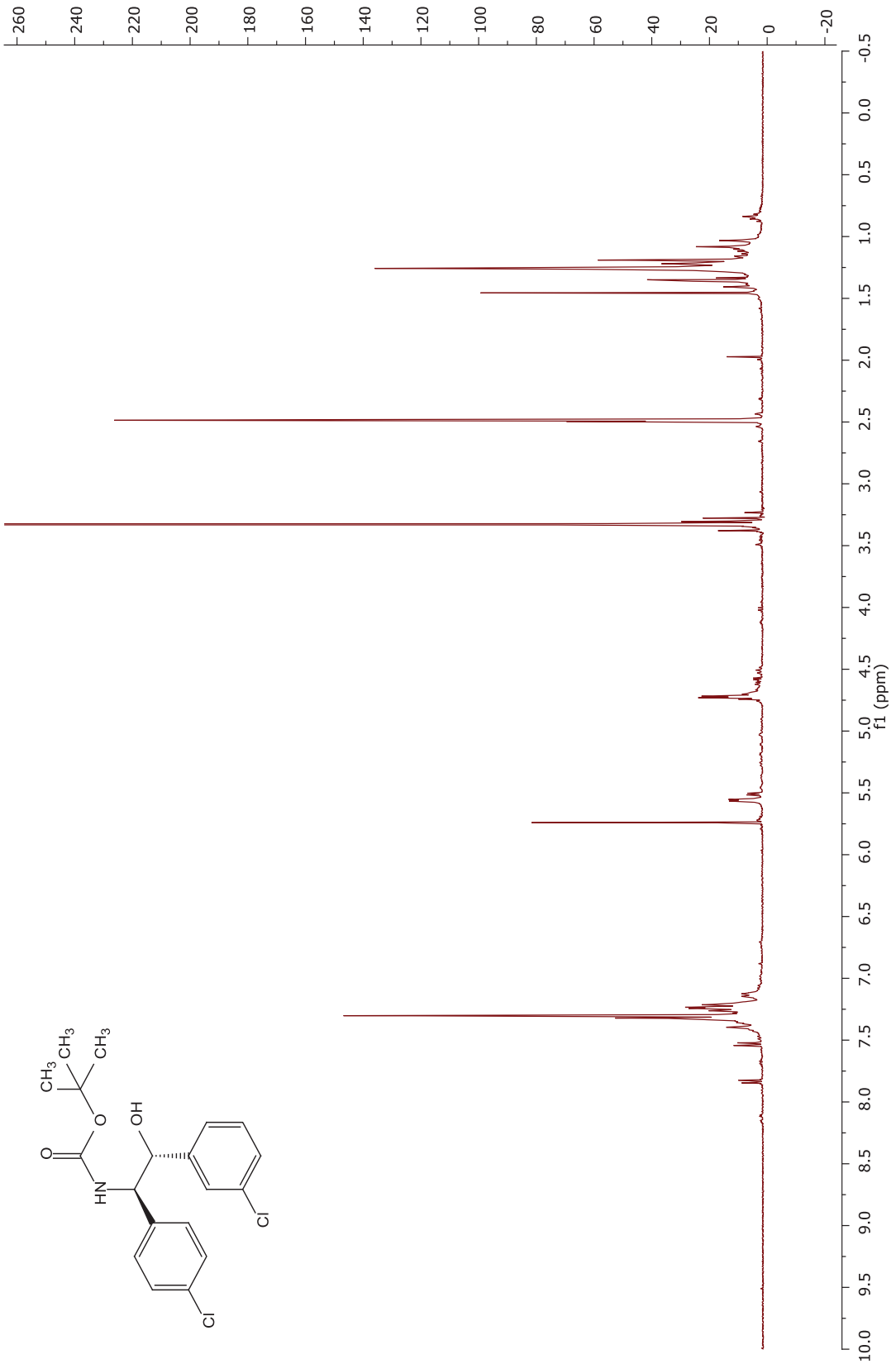
1.09
9.17
f1 (ppm)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6.5
7.0
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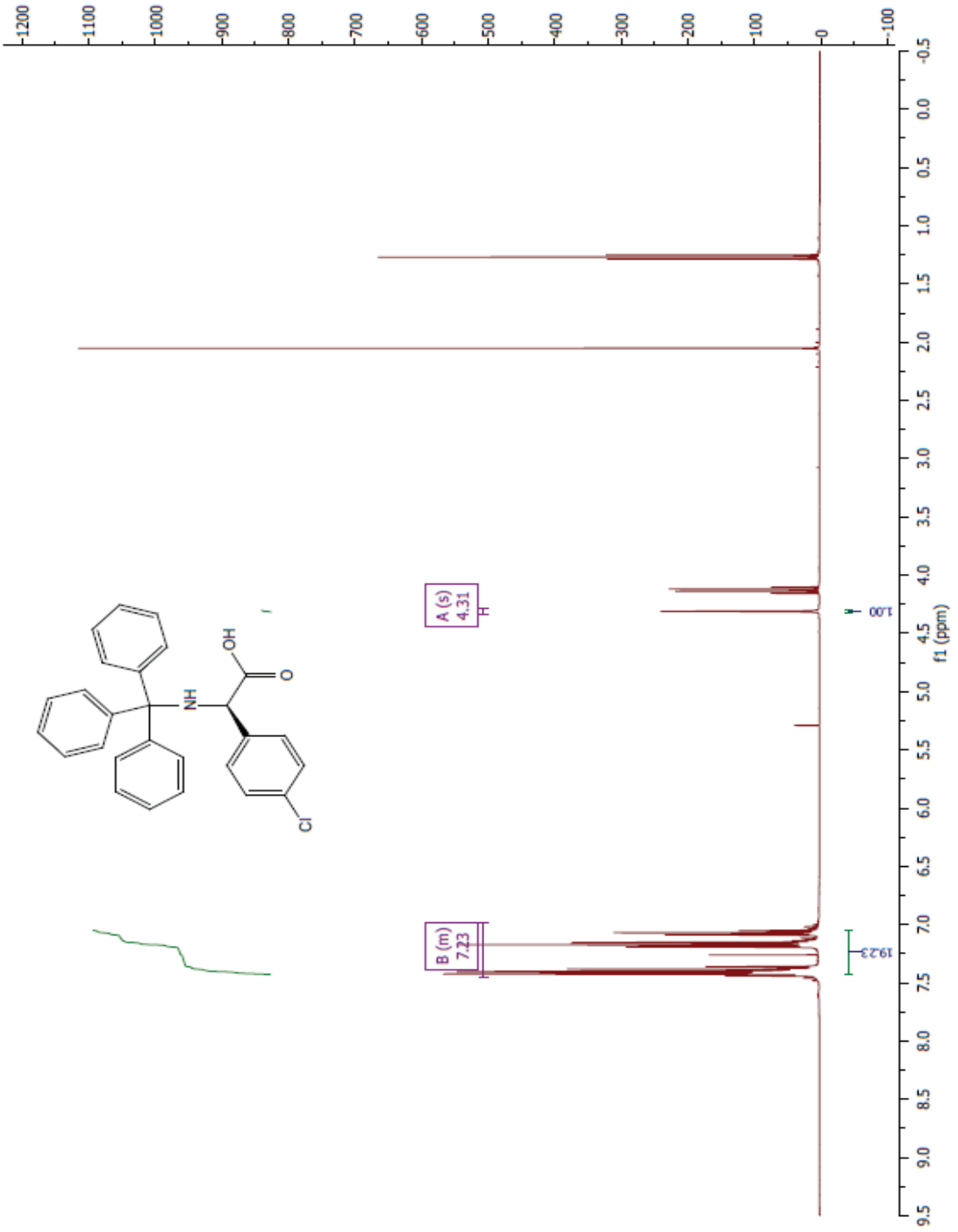
OH
Cl
Cl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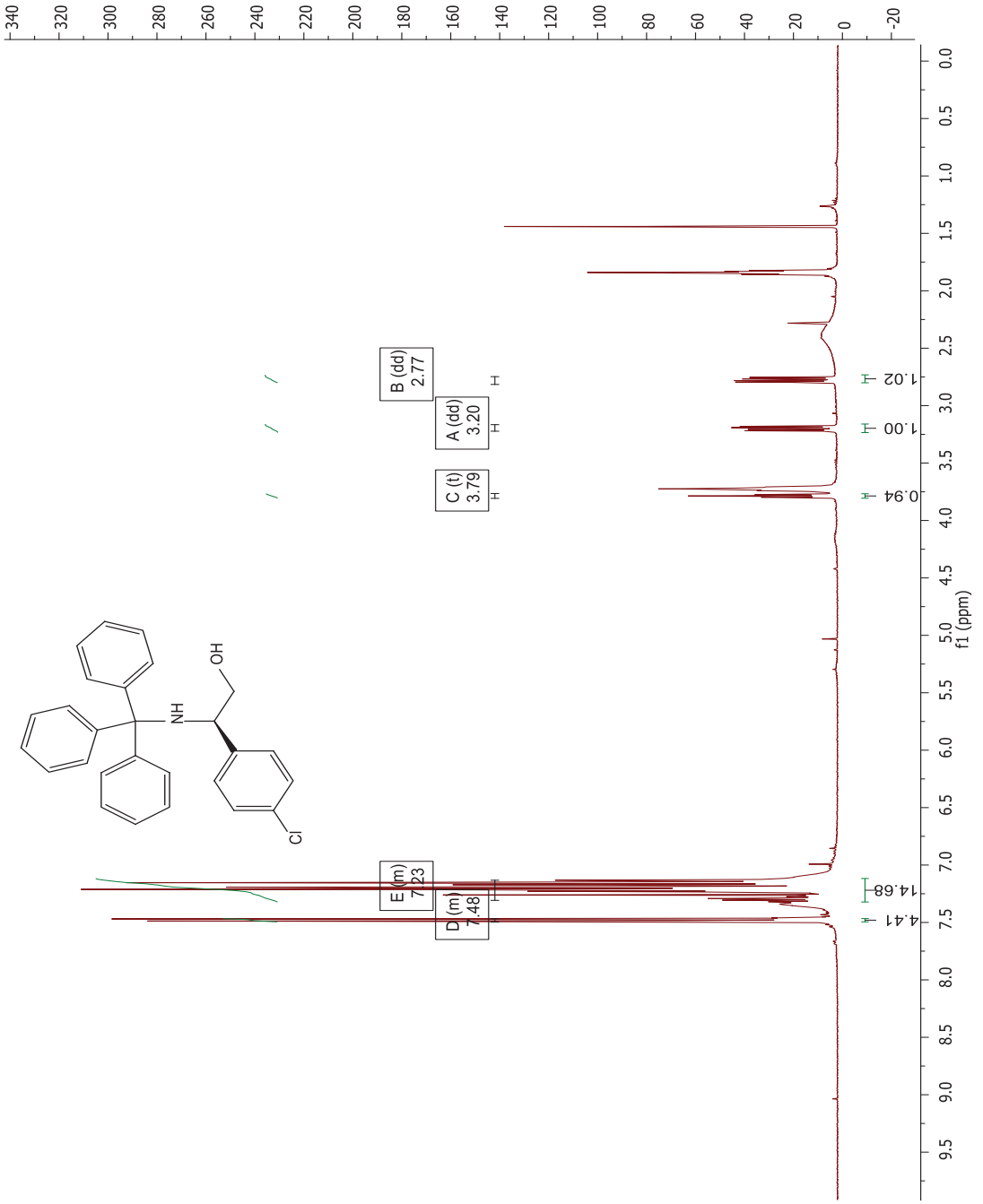
O
Cl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NH
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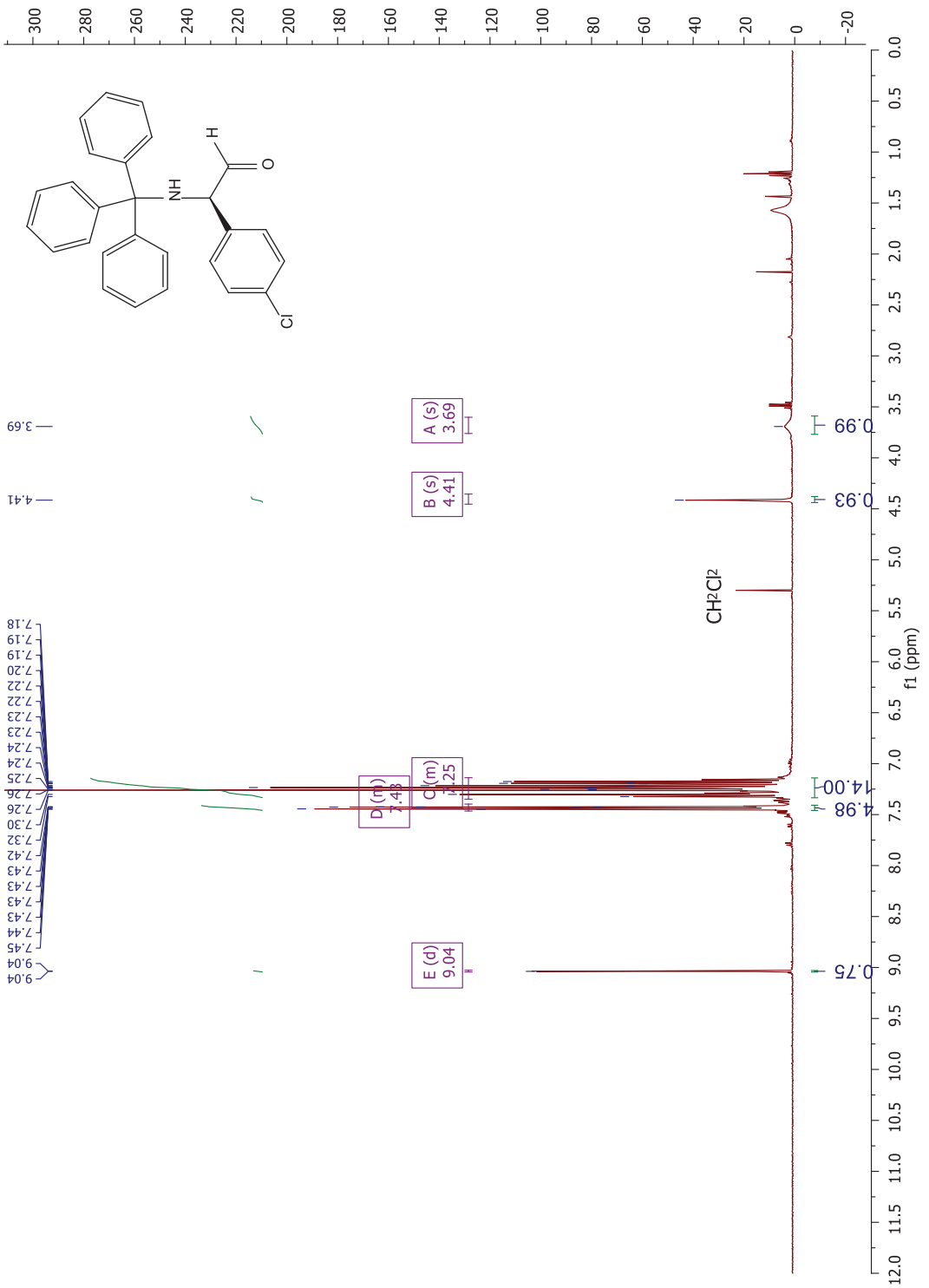
f1 (ppm)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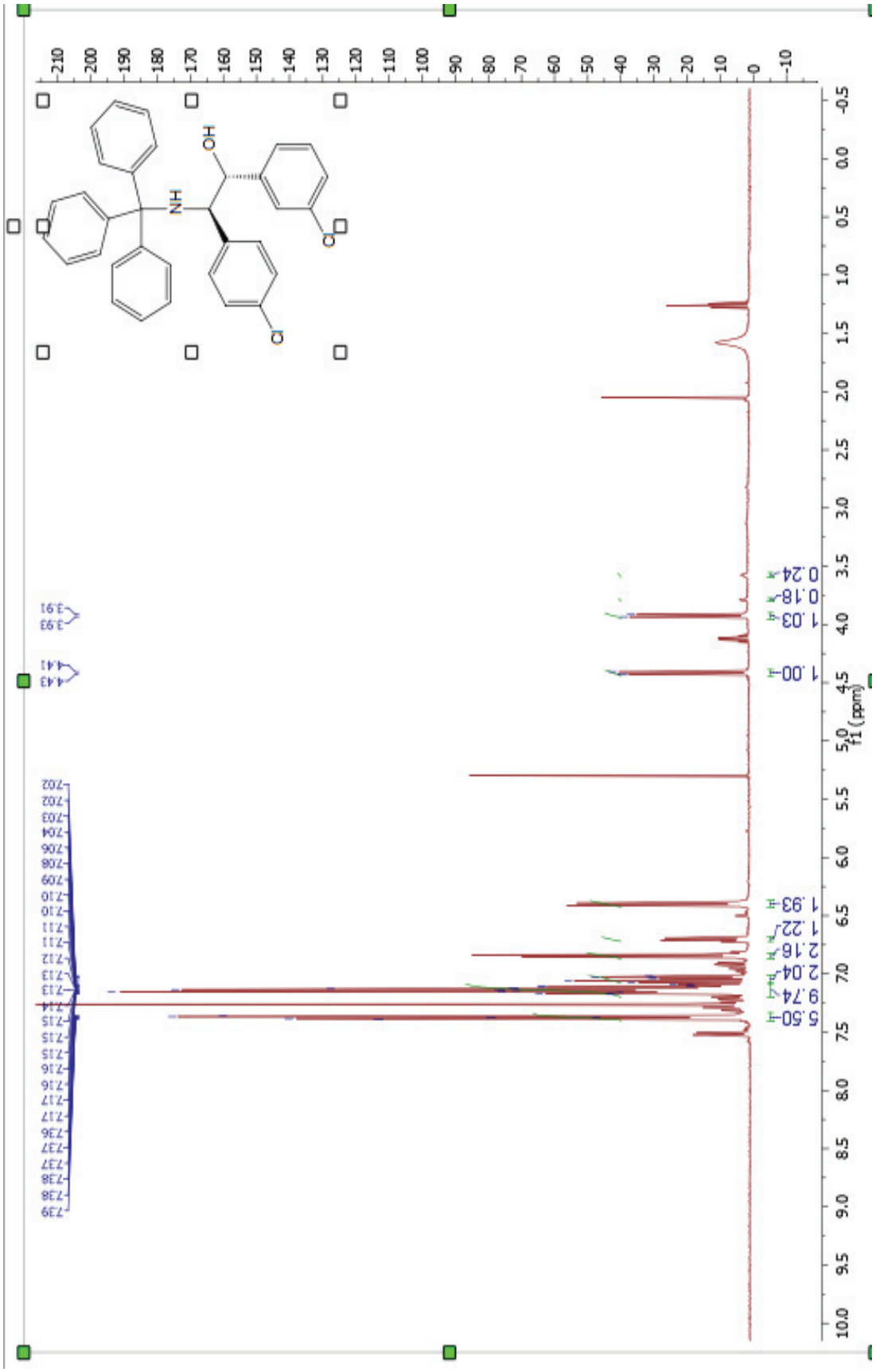
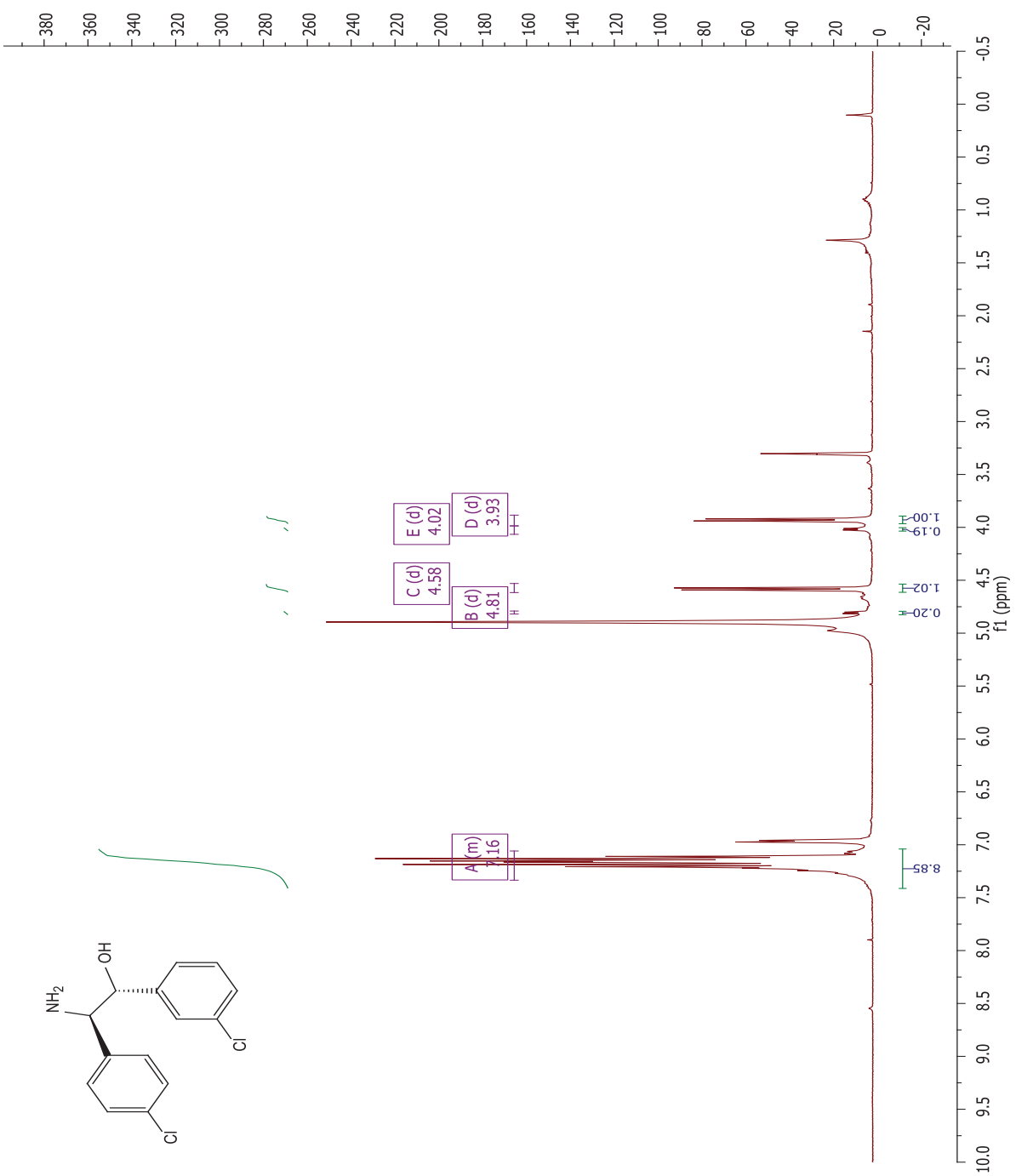
14.00
4.98
0.75
f1 (ppm)


OH
NH
Cl
Cl
f1 (ppm)


NH2
OH
Cl
Cl
A (m) 7.16
B (d) 4.81
C (d) 4.58
D (d) 3.93
E (d) 4.02
8.85
0.20
1.02
0.19
1.00
f1 (ppm)