UNIVERSITY OF LJUBLJANA
FACULTY OF PHARMACY

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MASTER'S THESIS

UNIFORM MASTER'S STUDY PROGRAMME PHARMACY

Ljubljana, 2015

UNIVERSITY OF LJUBLJANA

FACULTY OF PHARMACY

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DESIGN AND SYNTHESIS OF NOVEL LYSINE-SPECIFIC DEMETHYLASE 1 INHIBITORS

NAČRTOVANJE IN SINTEZA NOVIH ZAVIRALCEV LIZIN-SPECIFIČNE DEMETILAZE 1

UNIFORM MASTER'S STUDY PROGRAMME PHARMACY

Ljubljana, 2015

The experimental work for the master's thesis was carried out at the School of Pharmacy, University of East Anglia, under the mentorship of assoc. prof. dr. Janez Ilaš and prof. dr. Arasu Ganesan.

Acknowledgements

I would like to express my deepest of gratitude to my mentor assoc. prof. dr. Janez Ilaš for all the help, effort and useful guidance in writing this thesis. A special thanks goes to prof. dr. Arasu Ganesan for supporting me throughout this project, giving me valuable time and helpful advice. I would also like to thank my family who always stood by my side throughout the duration of the study.

Plagiarism statement

I, Jernej Marko Lobe, hereby confirm that the work submitted in this thesis is my own. Any ideas, quotations, and paraphrasing, from other peoples work and publications have been appropriately referenced.

Ljubljana, 2015

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ABSTRACT

Epigenetic factors play an important role in the development of cancer. One of these epigenetic factors are post-translational modifications of histone tails. Lysine-specific demethylase 1 (LSD1), overexpressed in various types of cancer, demethylates histone residues at Lys4 or Lys9 of histone H3. The active site of LSD1 is very similar to that of monoamine oxidase (MAO), therefore MAO inhibitors, such as tranylcypromine (TCP), also inhibit LSD1. Recently, many studies have been conducted on the synthesis of TCP analogues with the aim to produce an analogue with potent and selective LSD1 inhibitory effects. In this research thesis, the synthesis of novel LSD1 inhibitors is described with different moieties attached to the cyclopropyl ring, unlike the phenyl ring moiety in TCP. Moreover, some of the compounds have methyl groups attached to the cyclopropyl ring, which TCP analogues do not have. The syntheses start with two different compounds in five different reaction schemes but unfortunately no end products were obtained.

RAZŠIRJENI POVZETEK

Epigenetika preučuje dedovane spremembe v aktivnosti in izražanju genov, ki niso posledica sprememb nukleotidnega zaporedja. Pluripotentne celice se lahko v času njihovega razvoja diferencirajo v različne tipe celic. Razlike v izražanju genov, ki so posledica transkripcijskih dejavnikov in epigenetskih sprememb, omogočajo nastanek različnih tkiv in organov, brez spremembe naše DNK (z nekaj izjemami). Epigenetske modifikacije so pomembne pri ohranjanju celične identitete. Prisotne so pri procesih proliferacije, razvoja, diferenciacije celic in integriteti genoma. DNK, ki je dolga okoli dva metra, je v posamezni evkariontski celici zvita tako, da se prilega v celično jedro. Ta zvitost je dosežena z organizacijo DNK v kromatin. Osnovna enota kromatina je nukleosom, ki je sestavljen iz pripadajoče DNK, ovite okoli oktamera štirih osrednjih histonov (H2A, H2B, H3 in H4). Jedro histonov je predvsem kroglaste oblike, razen amino-terminalnih repov, ki se raztezajo iz kroglaste regije in so zato bolj dostopni encimom, odgovornim za histonske spremembe. V nedeleči se celici, kromatin v grobem razdelimo v dve funkcionalni obliki: evkromatin in heterokromatin. Evkromatin, ki vsebuje večino genov, predstavlja odprto obliko, v kateri je DNK dostopna za transkripcijo. Po drugi strani, heterokromatin predstavlja kondenzirano obliko kromatina, kjer DNK ni dostopna za transkripcijo. Za spremembo med funkcionalnima oblikama kromatina so odgovorne posttranslacijske modifikacije na histonskih repih. Poznamo več vrst teh modifikacij, vključno z acetilacijo, fosforilacijo, metilacijo, ubikvitinacijo, sumoilacijo in ADP-ribozilacijo.

Metilacija, ki jo izvajajo metiltransferaze, je reverzibilna. Za demetilacijo poskrbijo demetelaze, v katero spada tudi lizin-specifična demetilaza 1 (LSD1). Prva stopnja v katalitičnem mehanizmu LSD1 je oksidacija metilne skupine na lizinu do imina. Pri tem sodeluje FAD kofaktor in kisik, ki se pretvori v vodikov peroksid. V naslednji stopnji poteče hidroliza do demetiliranega lizina. Pri tem nastane tudi formaldehid kot stranski produkt. LSD1 je specifičen za mono- in di-metilirane lizine, ne pa za tri-metilirane lizine. LSD1 demetilira lizin na mestu 4 in 9 na histonu H3. Afiniteta do substrata je odvisna od kompleksa v katerem sodeluje. V kompleksu z androgenskim receptorjem demetilira lizin 9, posedica je aktivacija transkripcije. Če je v kompleksu s transkripcijskim ko-represorjem (CoREST), pa demetilira lizin 4 in utiša določene gene. LSD1 lahko deluje tudi na nehistonske proteine, na primer na tumor zaviralni protein p53. Močna izraženost LSD1 je prisotna pri raku

prostate, nevroblastomu, raku na prsih, raku sečnega mehurja, raku danke in debelega črevesa in pri akutni mieloični levkemiji.

LSD1 in monoamin-oksidaza (MAO) imata zelo podobni katalitični mesti, zato zaviralci MAO, kot je tranilcipromin (TCP), zavirajo tudi LSD1. V zadnjem času je bilo narejeno veliko raziskav na področju sinteze analogov TCP, z namenom doseči selektivnost in močno zaviranje LSD1. Selektivnost lahko dosežemo s pripenjanjem večjih hidrofobnih skupin na fenilni obroč TCP, saj tako blokiramo dostop do katalitičnega mesta MAO. Namen raziskovalnega dela je bila sinteza novih zaviralcev LSD1 z močnimi in selektivnimi lastnostmi. Kot izhodni spojini smo uporabili (±)*trans*-krizantemsko kislino in dietil *trans*-1,2-ciklopropandikarboksilat. Naše sintetizirane spojine imajo namesto fenilnega obroča, prisotnega v TCP, vezane druge skupine. Tako je mogoče oceniti pomen fenilnega obroča pri analogih TCP. Poleg tega ima nekaj naših spojin vezani dve metilni skupini na ciklopropil, medtem ko analogi TCP nimajo. Ti dve metilni skupini bi lahko vplivali na mehanizem zaviranja LSD1, kjer se ciklopropil odpre in kovalentno veže na FAD. Sinteza je potekala po petih različnih sinteznih poteh. Končnih spojin nam žal ni uspelo pridobiti.

Prvo sintezno pot smo začeli z enolončno Curtiusovo premestitvijo, v kateri smo pretvorili karboksilno kislino spojine **1** do amina zaščitenega z Boc. V drugi stopnji smo oksidirali dvojno vez do diola in dobili spojino **3**. Nato smo z $NaIO_4$ poizkusili oksidirati diol do karboksilne kisline. Po mnogih poskusih z različnimi množinskimi razmerji dodanega reagenta in različnimi časi trajanja reakcije, spojine **4** nismo uspeli sintetizirati.

Drugo sintezno pot smo začeli s selektivno hidrolizo estra spojine **5**, ki ji je sledila formacija amida. V tretji stopnji smo ester spojine **7** hidrolizirali do karboksilne kisline, v četrti pa poizkusili z enolončno Curtiusovo premestitvijo pretvoriti karboksilno kislino v aminsko skupino. Spojine **9** žal nismo uspeli sintetizirati. Razlog bi lahko bilo odprtje obroča, saj je bila na eni strani ciklopropila elektron donorska skupina, na drugi pa elektron akceptorska. V izogib razpadu molekule smo spremenili sintezno pot tako, da bi reducirali karbonilno skupino amida. Že pri prvi stopnji spremenjene sintezne poti, pa nam ni uspelo karboksilne kisline spojine **8**, pretvoriti v Boc zaščiten amin. Potencialni končni produkt **10** ni bil dovolj čist in ni ga bilo dovolj, da bi čiščenje ponovili.

Pri tretji sintezni poti smo začeli s spojino **6**, kjer smo z enolončno Curtiusovo premestitvijo pretvoriti karboksilno kislino v Boc zaščiten amin. V drugi stopnji smo izvedli redukcijo

estra z $LiAlH_4$ do alkohola, ki pa ni bila uspešna. Razlog za to bi lahko bila odščita Boc zaščitne skupine z $LiAlH_4$, ali pa ker reakcija ni bila hlajena v ledu, medtem ko je bila zaustavljena z dodatkom 1 M HCl. Zaradi pomanjkanja vhodnih materialov in časa, se preiskovanje ni nadaljevalo.

Četrto sintezno pot smo začeli z Ritterjevo reakcijo, ki je potekla v močno kislem okolju, med dvojno vezjo spojine **1** in acetonitrilom. V drugi stopnji smo poizkusili z enolončno Curtiusovo premestitvijo pretvoriti karboksilno kislino v aminsko skupino. Glede na ^{13}C NMR analizo, sta bila, kot pri prejšnji spojini, v spektru še vedno prisotna dva vrhova v regiji 150 – 200 ppm, kar pomeni, da končne spojine **14** nismo dobili.

Pri zadnji sintezni poti smo najprej zaščitili aminsko skupino na spojini **15**. V drugi stopnji je potekla Ritterjeva reakcija in dobili smo spojino **17**. V zadnji stopnji smo poizkusili z enolončno Curtiusovo premestitvijo pretvoriti karboksilno kislino v amin, vendar neuspešno. Uspešno pa smo izolirali stranski produkt **19**, ki je nastal med željenim produktom **18** in izocianatom (vmesna stopnja v reakciji).

Reakcija, kjer smo poizkusili karboksilno kislino preko enolončne Curtiusove premestitve pretvoriti v aminsko skupino, ni bila uspešna v treh različnih stopnjah (produkti **9**, **14** in **18**). Alternativna možnost za ta postopek bi bilo več zaporednih reakcij. Vsako stopnjo bi bilo bolj enostavno spremljati s TLC in nečistoče bi lahko odstranili postopoma, kar bi bilo lažje. Za aktivacijo karboksilne kisline bi lahko uporabili tionil klorid, nato bi z natrijevim azidom pretvorili kislinski klorid v kislinski azid. V naslednji stopnji bi s segrevanjem dobili izocianat in po dodatku vode bi nastal željeni amin.

LIST OF ABBREVIATIONS

2-PCPA	tranylcypromine
ADP	adenosine diphosphate
AML	acute myeloid leukaemia
AOL	amine oxidase-like domain
Boc	tert-butyloxycarbonyl protecting group
CoREST	neuronal silencer co-repressor of RE1-silencing transcription factor
CpG	regions of DNA where a cytosine occurs next to a guanine
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNMT	DNA methyltransferase
DPPA	diphenylphosphoryl azide
E2F1	a protein with a role of controlling the cell cycle
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ES MS	electrospray mass spectrometry
EtOAc	ethyl acetate
FAD	flavin adenine dinucleotide
H2A	histone 2A
H2B	histone 2B
H3	histone 3
H4	histone 4
HAT	histone acetyltransferase
HDAC	histone deacetylase
Hz	hertz
HOBt	hydroxybenzotriazole

IC_{50}	the concentration of an inhibitor that is required for 50-percent inhibition of an enzyme.
J	coupling constant
K	lysine
K_i	dissociation constant of an enzyme inhibitor complex
LRMS	low resolution mass spectra
LSD1	lysine-specific demethylase 1
LSD2	lysine-specific demethylase 2
m	multiplet
MAO	monoamine oxidase
me1/2	one methyl group or two methyl groups
MLL-AF9	fusion gene associated with leukaemia
NMR	nuclear magnetic resonance
p53	tumour suppressor gene
PE	petroleum ether
PTM	post-translational modification
q	quartet
R	arginine
R_f	retention factor
RNA	ribonucleic acid
s	singlet
SAM	S-adenosyl methionine
SWIRM	domain named after the proteins SWI3, RSC8 and MOIRA
t	triplet
TCP	tranylcypromine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
δ	chemical shift
η	yield
λ	wave length

1. INTRODUCTION

1.1. WHAT IS EPIGENETICS

Genetics is defined as the study of heritable changes in gene activity and expression due to the direct alteration of the DNA sequence. Such alterations comprise of point mutations, deletions, insertions, and translocations. In contrast, epigenetics is defined as the study of heritable changes in gene activity and expression that occur without changing the underlying DNA sequence (1). Epigenetics is a bridge between genotype and phenotype (2). In broader terms, epigenetics describes the regulation mechanisms of transcription induced by chromatin-associated proteins and post-translational modifications (PTMs) of histones (3). Cells in a pluripotent state can differentiate into many cell types during their development. Differences in gene expression caused by transcription factors and epigenetic modifications result in different tissues and organs forming without changing our DNA sequence (with a few exceptions) (4). Epigenetic modifications are important for maintaining cell identity and they are involved in essential processes like proliferation, development, differentiation, and genome integrity (3). An interesting example of epigenetics are monozygotic twins who have identical chromosomal DNA because they develop from a single fertilized egg. However, most monozygotic twins are not identical. One of the explanations why they are different is the existence of epigenetic factors (5, 6).

1.2. CHROMATIN STRUCTURE

DNA in a single human cell is about 2 meters long. This raises the question how is the chromosomal DNA packed so that it fits into the cell nucleus. This high compaction ratio is accomplished by organizing DNA into chromatin. The fundamental unit of chromatin is nucleosome which consists of ~ 147 base pairs of DNA wrapped around an octamer of four core histones (H2A, H2B, H3 and H4). The core histones are mainly globular except for their amino-terminal tails which extend from the globular region and are therefore more reachable to histone modifying enzymes (7). Another protein, linker histone H1, interacts with the nucleosome core particles. It twists and folds the chromatin fiber into higher order structures that comprise chromosomes (8). In a non-dividing cell, chromatin can be roughly divided into two functional states: euchromatin and heterochromatin. Euchromatin, which

contains the most of the genes, represents an open conformation due to the relaxed state of nucleosome arrangement, therefore DNA is accessible for transcription. On the other hand, heterochromatin represents a condensed form of chromatin where DNA is inaccessible for transcription. Heterochromatin determines the chromosomal stability and the separation of chromosomes in mitosis (9).

1.3. EPIGENETIC MODIFICATIONS

Eukaryotic DNA is wrapped around proteins called histones to form chromatin. The DNA needs to unwind from the histones to be accessible for transcription. This interaction between the DNA and histones is directed by three sets of modifiers called writers, erasers and readers. Writers set up the post-translational modifications into DNA and histones. Readers can recognise these modifications, bind to covalently modified histone tails and read the specific pattern of modifications. Erasers remove the post-translational modifications therefore return chromatin to its native state (Figure 1) (10).

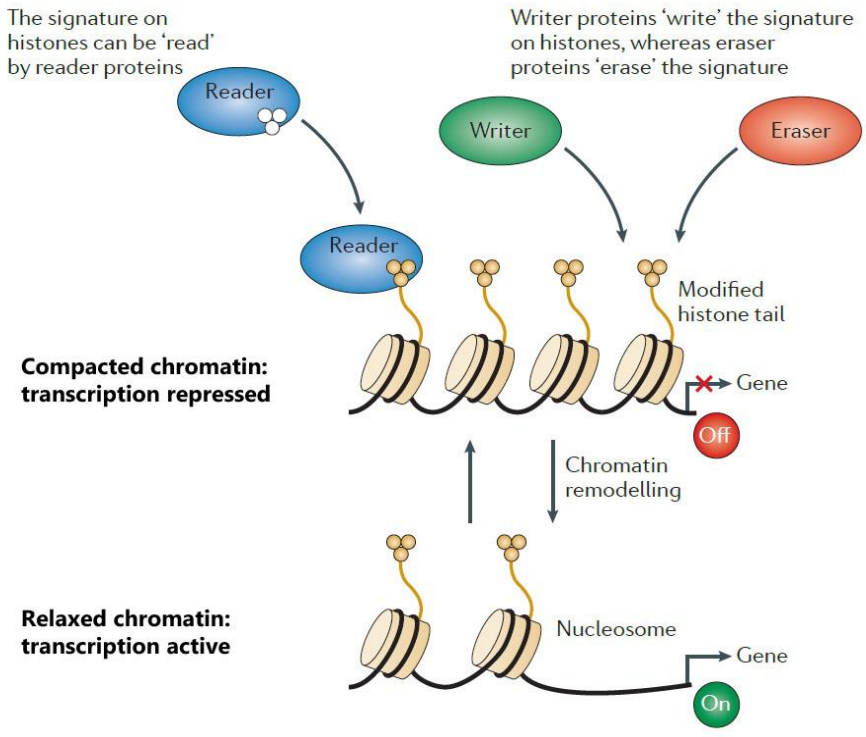


Figure 1: Writers, erasers and readers. The modification of histones and chromatin structure remodelling is presented (11).

Currently, three main epigenetic modifications are known: DNA methylation, histone modification and RNA interference (12).

1.4. DNA METHYLATION

DNA methylation is the most well-characterized epigenetic modification in humans. It mostly occurs at the cytosine ring within CpG dinucleotides of the CpG islands. CpG islands consist of more than 200 bases with a cytosine and guanine content of at least 50%. The transfer of a methyl group from a S-adenosyl methionine (SAM) to the fifth carbon of cytosine is mediated by the DNA methyltransferase enzymes (DNMTs) (13). In general, DNA methylation is associated with transcriptional silencing (14). CpG-island methylation is essential for regulating tissue-specific gene expression, genomic imprinting and X chromosome inactivation (15). DNA methylation is a reversible process. DNMT overexpression is present in many diseases, including prostate, lung and colorectal cancer (16).

1.5. HISTONE MODIFICATIONS

In the 1960s, V. Allfrey led to the understanding that histones undergo post-translational modifications. The high-resolution X-ray structure of the nucleosome gave us an idea how chromatin structure is affected by these modifications. Histone tails extend from the globular parts of the nucleosome and are in contact with nucleosomes nearby. Post-translational modifications are active on these tails hence affect the whole chromatin structure and also recruit proteins and complexes with certain enzymatic activities (17). A number of post-translational modifications can occur at the histone tails; for example: acetylation, phosphorylation, methylation, ubiquitination, sumoylation and ADP-ribosylation (Figure 2). Histone modifications have an important function in DNA repair, transcriptional regulation, DNA replication and chromosome condensation. Recently it has been discovered that we can predict gene expression by the amount of histone modifications. Histones can have more modifications at different sites. Cross-talk among the different marks can occur at the same site, in the same histone tail and among different histone tails. The outcome is determined by the combination of all histone marks (13).

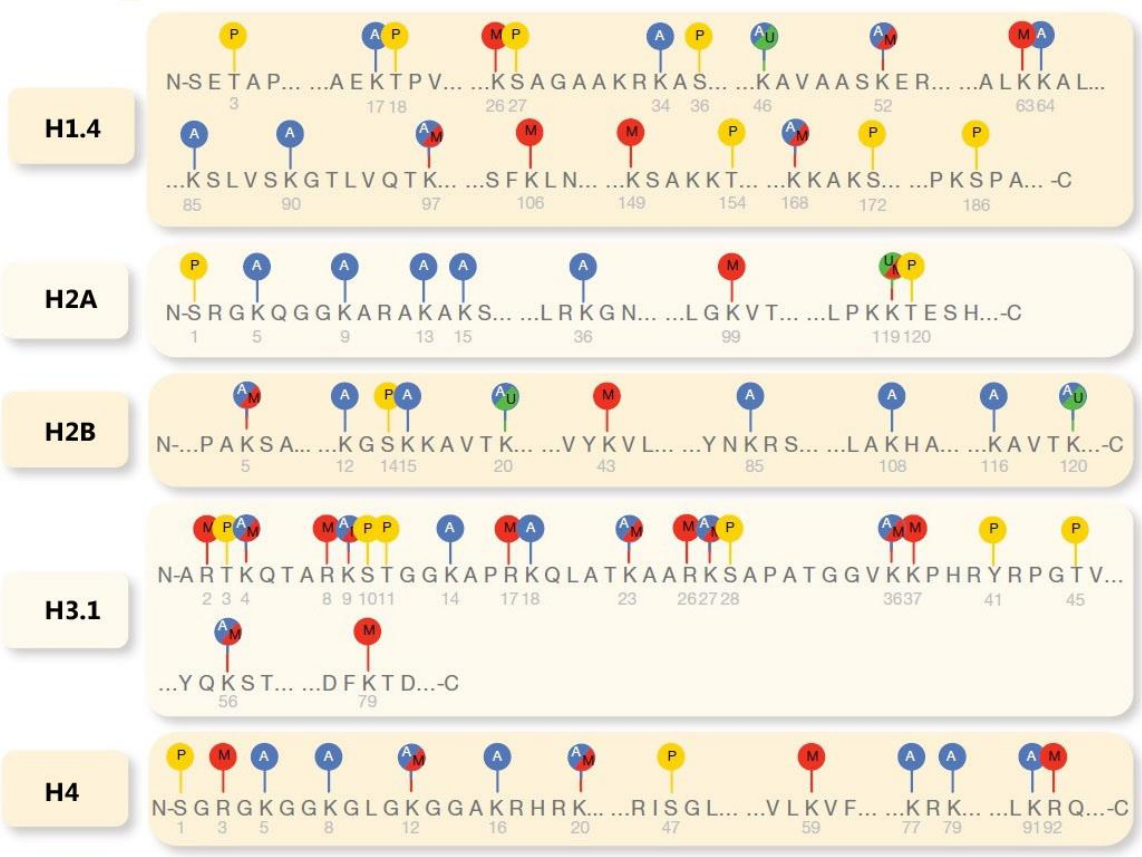


Figure 2: The main post-translational modifications are presented: methylation (red), acetylation (blue), phosphorylation (yellow) and ubiquitination (green). Under each amino acid there is a number which represents specific position of the modification in the sequence (13).

1.5.1. Histone acetylation

It has been demonstrated that acetylation of lysine residues of histone tails is a reversible process regulated by two enzyme families: histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs catalyse the transfer of acetyl group from cofactor acetyl CoA to the lysine residues. HAT enzymes have the ability to neutralize the lysine's positive charge, hence they disorganize the stabilizing influence of electrostatic interaction. Two classes of HATs have been discovered: type-A and type-B. Mainly cytoplasmic, Type-B HATs acetylate synthesized histone H4 on K5 and K12 positions and also specific sites on histone H3. This acetylation impact histone positioning. The type-A HATs acetylates marks within the N-terminal tail of the histone. On the other hand, HDACs have an opposing effect of HATs and deacetylate lysine side chains. There are four classes of HDAC enzymes and

they are commonly present in multiple complexes. Consequently, it is hard to determine which activity is responsible for a specific effect (17).

1.5.2. Histone methylation

Methylation mainly occurs on lysine (K) and arginine (R) residues of the histone tails and it is catalysed by histone methyltransferases. Furthermore, the same lysine residues may be mono-, di- or tri-methylated while arginine residues may be mono-, symmetrically or asymmetrically di-methylated. Histone lysine methylation occurs at histone H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79 and H4K20 (18). Until a decade ago, histone methylation was considered a stable modification. However, in 2004, lysine-specific demethylase 1 (LSD1) was identified and indicated that methylation is a reversible process (19). Two groups of histone demethylases are known which differ in reaction chemistry, coenzyme use and reaction product. Flavin-dependent histone demethylases use only mono- and dimethylated lysines as a substrate and the side product is hydrogen peroxide. Conversely, Jumonji-containing histone demethylases, with iron as a cofactor, act on mono-, di- and trimethylated lysine residues and on methylated arginine side chains (20).

1.6. LYSINE-SPECIFIC HISTONE DEMETHYLASE 1 (LSD1)

1.6.1. The structure of LSD1

The structure of LSD1 contains three distinct domains: an amine oxidase-like (AOL) domain, a SWIRM domain and a tower domain. The C-terminal of the AOL domain, which is the catalytic centre of the enzyme, has two functional subdomains, one for binding the substrate and another for binding an FAD cofactor. The AOL domain is homologous to flavin adenine dinucleotide (FAD)-dependent oxidases. From the oxidases it differs in its very acidic flat surface which is also an additional binding site. The second domain is the N-terminal SWIRM domain which adds to the stability of LSD1. The interaction between SWIRM domain and AOL domain could serve as an additional binding site for the histone tail. The tower domain is connected directly to the AOL domain and can allosterically modulate the activity of the LSD1. Furthermore, the tower domain interacts with the neuronal silencer co-repressor of RE1-silencing transcription factor (CoREST) which

contributes to chromatin recognition and thus enhances the ability of methylation (Figure 3) (21).

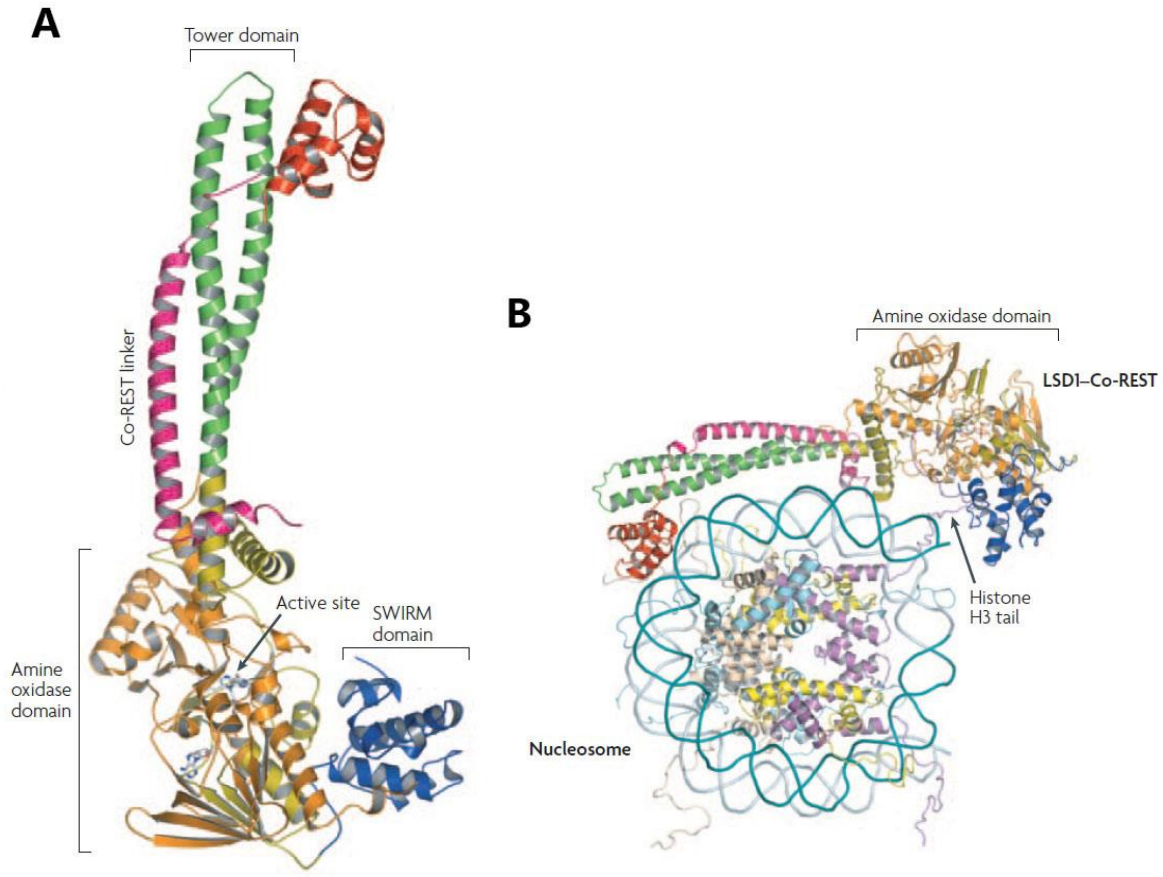


Figure 3: A | Structures of LSD1 bound to CoREST. The amine oxidase-like (AOL) domain is presented in yellow and orange, the SWIRM domain in blue, the tower domain in green and the CoREST linker region in pink. B | A potential docking of LSD1 and CoREST onto the nucleosome. The bottom half of the picture is a nucleosome with the core histones and DNA double helix. On top is the LSD1–CoREST complex (22).

1.6.2. The catalytic mechanism of LSD1

LSD1 demethylates Lys4 of histone H3 through a flavin-dependent oxidative reaction. First, the methylated Lys4 residue is oxidised by the FAD cofactor, reducing oxygen (O_2) to hydrogen peroxide (H_2O_2). Second is the hydrolyzation of the resulting imine intermediate to form the demethylated Lys4 side chain and formaldehyde (Figure 4) (20). LSD1 can act on mono- and dimethyllysine but not on trimethyllysine because the catalytic mechanism relies on a nitrogen lone pair (11).

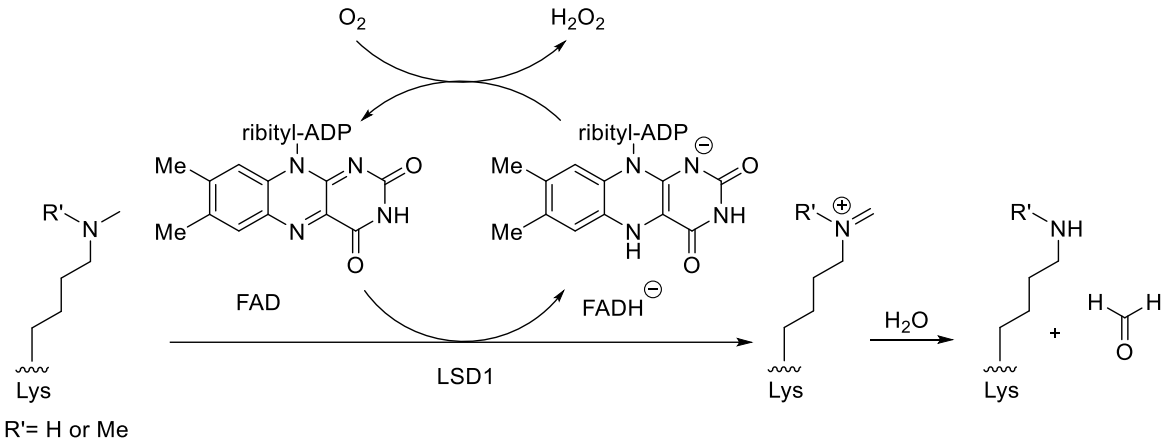


Figure 4: The catalytic mechanism of lysine-specific demethylase 1 (10).

1.6.3. A role of LSD1 in gene activation and repression

LSD1 can either activate or repress specific genes. It has different specificity towards H3K4me1/2 and H3K9me1/2 depending on which protein complex it is part of. LSD1 in association with the androgen receptor triggers demethylation of H3K9, thus have a function of transcriptional activation. In comparison, when LSD1 is a part of the transcriptional corepressor protein (CoREST), it demethylates H3K4 and represses certain genes. Furthermore, LSD1 is also a part of the cross-talk between methylated lysine side chains and acetylated side chains, since it is a member of a complex comprising of histone deacetylase 1 (HDAC1) and CoREST. Higher activity of LSD1 is present on hypoacetylated nucleosome substrate, implying that this complex first deacethylates histones and then allows LSD1 to demethylate H3K4 (Figure 5) (23).

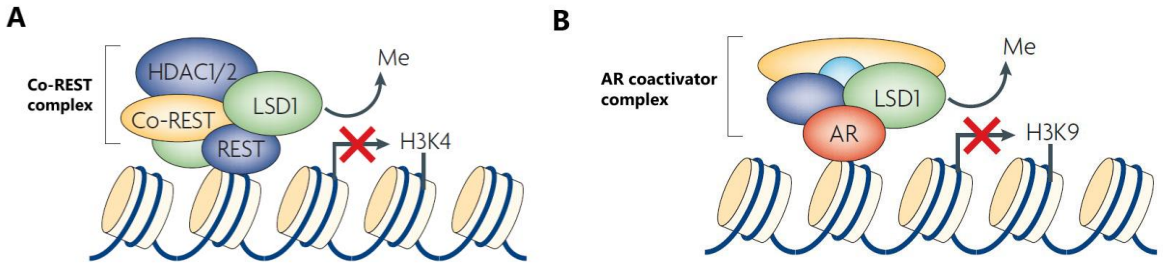


Figure 5: A | LSD1 as a part of the CoREST complex represses target genes by demethylating H3K4. B | LSD1 in the complex with an androgen receptor changes its substrate specificity and demethylates H3K9 resulting in activation of the target genes (22).

LSD1 can also act on non-histone proteins, for example p53 or E2F1 and regulate their activity (23). LSD1 controls the tumour suppressor p53 through demethylation of particular p53 lysine (Lys370me2), which is required for association with the coactivator p53-binding protein-1. Because LSD1 represses the activity of p53 it inhibits the role of p53 in apoptosis (24).

1.6.4. Features of the histone peptide-binding site in LSD1

It has been shown that LSD1 requires a sufficiently long peptide consisting of the first 20 N-terminal amino acids of the histone tail for productive binding (25). In the substrate-binding site of LSD1 there are specific pockets comprising Thr11, Lys9, Arg8 and Thr6 that interact with some H3 residues and also N-terminal pocket that interact with the N-terminal amino group of Ala1. The Arg2 residue of the histone H3 tail is essential for stabilization of this tail conformation in the binding site of LSD1, due to the formation of intrapeptide hydrogen bonds. In this way specificity of the demethylation of H3K4 is reached (Figure 6) (20).

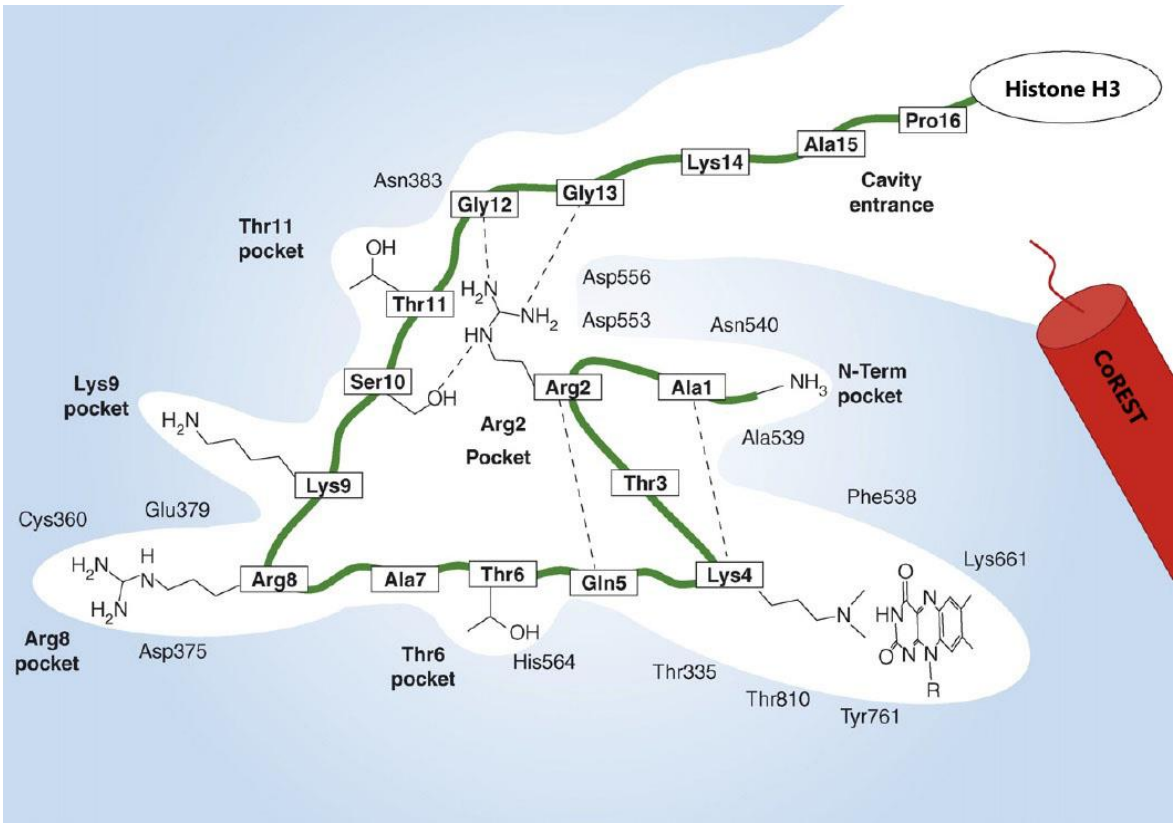


Figure 6: Characteristics of Histone H3-LSD1 binding site. Histone H3 in green and CoREST in red (20).

1.6.5. LSD1 in cancer and other diseases

Data suggest that LSD1 is highly expressed in various types of cancer, including prostate cancer, undifferentiated neuroblastoma, oestrogen-negative breast cancer, colorectal cancer and bladder cancer. The most attention is gained by the fact that LSD1 correlates with the maintenance of acute myeloid leukaemia (AML) (26). Recent data from animal models has revealed that LSD1 is required to sustain the expression of genes inducted by MLL-AF9 leukaemia stem cells (27). Although the precise mechanism of how LSD1 contributes to leukaemia is not known, LSD1 inhibitors has been shown to reduce leukaemic stem cells and one of them was already entered clinical trials. The LSD1 inhibitors are not limited to oncological diseases, they could be useful also for treating Huntington's disease and in herpes infection (26).

1.7. LSD1 INHIBITORS

Highly expressed in various diseases, LSD1 is considered as a potential drug target and because of the well-defined active site is thought to be druggable (11). LSD1 has a similar catalytic domain which is structurally homologous (45% sequence identity) with the amine oxidase group of enzymes. Well researched, human monoamine oxidases (MAOs) A and B share the same mechanism of action with the LSD1. The fact that MAOs and LSDs are so similar in the catalytic and structural properties, MAO inhibitors are suitable for investigation and design of the potential LSD1 inhibitors. In 1960s, an irreversible and poor selective MAO inhibitor tranylcypromine (TCP) was discovered (Figure 7) (28).

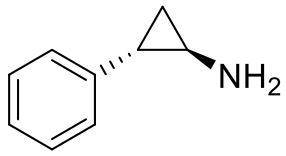


Figure 7: Structure of tranylcypromine (2-PCPA, TCP).

TCP is used to treat psychological disorders and it is approved by US Food and Drug Administration (29).

1.7.1. The mechanism of LSD1 inhibition by tranylcypromine

TCP covalently binds to FAD cofactor, forming an adduct in the MAO reactive site cavity (30). The cyclopropyl ring of the TCP opens and covalently binds to C(4a) of FAD (31). Similarly, LSD1 also uses FAD as a cofactor and it has been speculated that TCP can also form a covalent adduct with FAD of LSD1. Studies from Schmidt et al. demonstrated that TCP-FAD adduct in LSD1 is formed through carbon-carbon bond formation via single-electron transfer resulting in atropaldehyde adduct (Figure 8a) or cinnamaldehyde adduct (Figure 8b) (32).

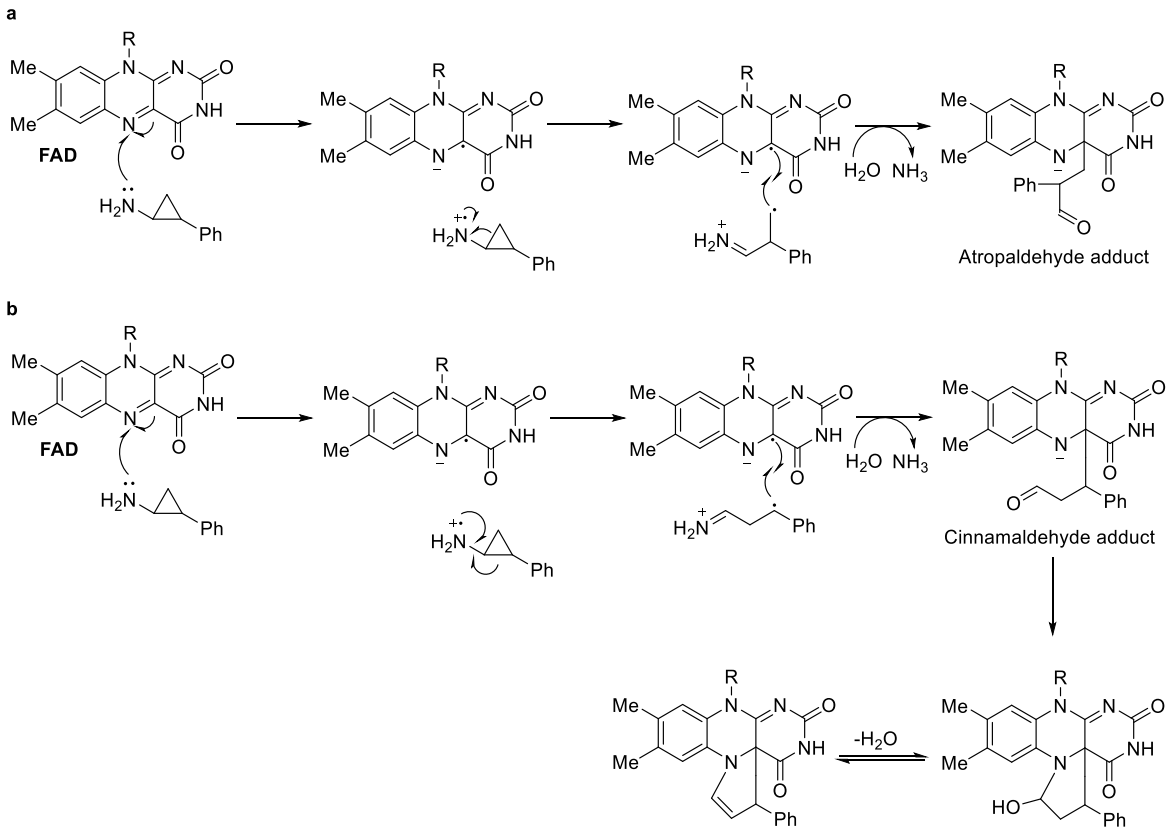


Figure 8: Possible mechanisms of inhibition of FAD by TCP to form atropaldehyde and/or cinnamaldehyde adducts and TCP-FAD five-membered adduct (33).

Yo et al. suggested a formation of the five-membered covalent ring formed by connection of C(4a) and N(5) of FAD with TCP. (Figure 8b) (28). Later, it was proposed that the five-membered ring model was not completely appropriate and that the N(5) adduct A is also formed (Figure 9) (34). TCP is a racemic mixture and recently it has been found that (+)-TCP generates N(5) adduct B and (-)-TCP generates N(5) adduct A respectively (31).

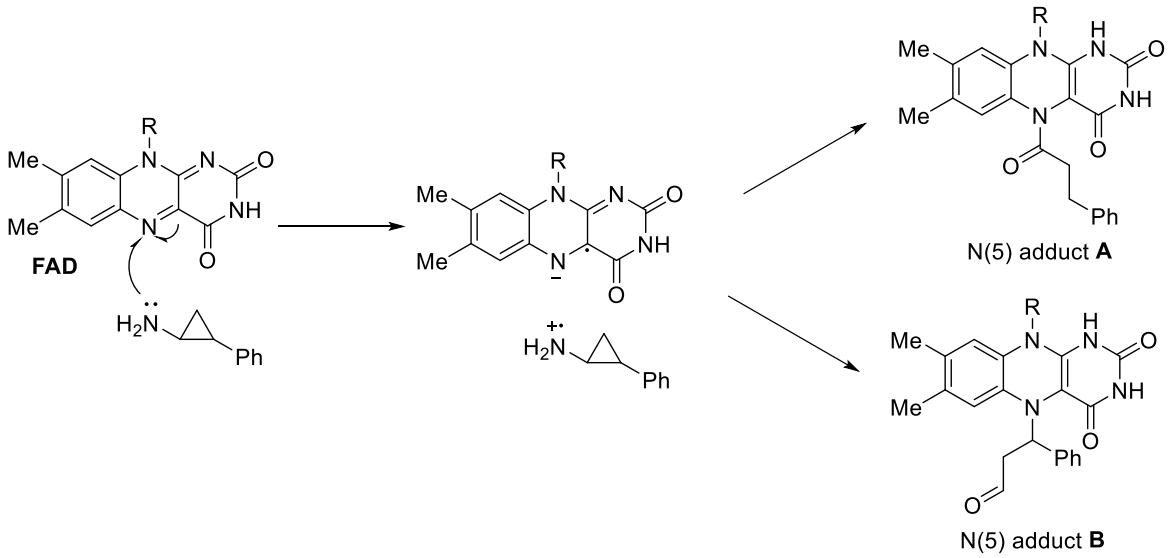


Figure 9: Inactivation mechanism of LSD1 by TCP by engaging TCP carbon in a covalent bond with the flavin N5 atom to form N(5) adduct A and N(5) adduct B (33).

1.7.2. Tranylcypromine based LSD1 inhibitors

TCP has been found to non-selectively inhibit LSD1, with a greater inhibition of MAOs. Therefore, the ultimate goal is to synthesize a more potent and selective LSD1 inhibitor. Compounds **A** and **B** have a homoserine linked with the phenyl ring of TCP through an ether bond at the meta and para positions (Figure 10). Benzylamino and benzoyl substituents on the homoserine could be recognized by hydrophobic amino acid residues at the beginning of the N-methylated lysine binding channel of LSD1. These compounds have demonstrated highly selective inhibitory activity towards LSD1 with IC_{50} values 2.5 μM in comparison with 32 μM for TCP. The selectivity is achieved by the large moiety attached to the phenyl ring of the TCP which prevents access to the active site of MAOs (33).

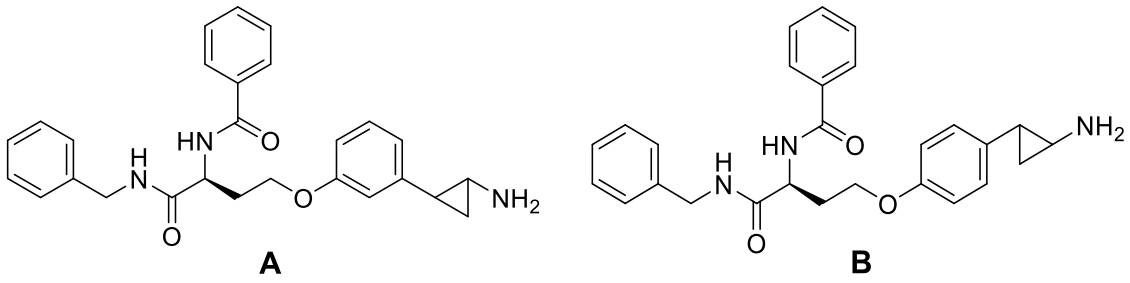


Figure 10: Potent LSD1 inhibitors (36).

Table I: In Vitro LSD1- and MAO-Inhibitory activities of compounds TCP, A and B (36).

	IC_{50} (μM)			Selectivity	
Compound	LSD1	MAO A	MAO B	MAO A/ LSD1	MAO B/ LSD1
TCP	32 ± 13	7.3 ± 0.45	4.3 ± 2.0	0.23	0.13
A	2.5 ± 0.5	230 ± 50	500 ± 93	92	200
B	1.9 ± 0.8	290 ± 10	> 1000	150	> 520

Compound **C**, *trans*-2-pentafluorophenylcyclopropylamine, with K_i value of 17 ± 4.9 μM, led to development of very potent TCP derivatives (Figure 11). Compounds **D** and **E**, with K_i values of 2.4 and 0.61 μM respectively, have large hydrophobic groups attached to the ortho position of TCP (Figure 11). Crystal structure revealed that these hydrophobic groups stabilize the neighbouring residues in the active site of LSD1. It is believed that fluorine substituents are enrolled in hydrophobic interactions with lipophilic or orthogonal dipolar interactions to surrounding polar groups. Molecules **D** and **E** also show better cell permeability in comparison with other LSD1 inhibitors because of their low molecular weight (33).

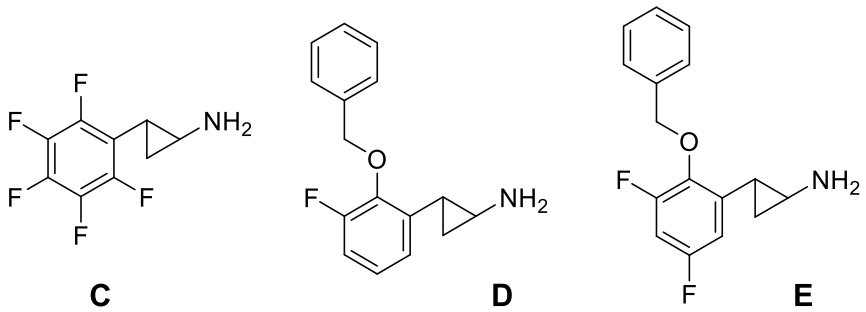
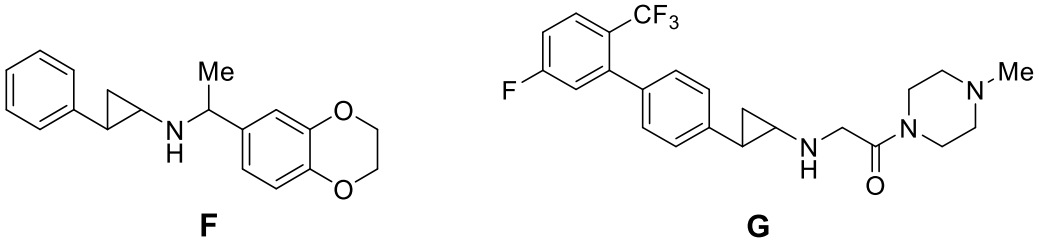


Figure 11: Fluoro derivatives of TCP as potent LSD1 inhibitors (33).

Table II: In Vitro LSD1- and MAO-Inhibitory activities of fluoro derivatives of TCP (37).

	K_i (μM)		
Compound	LSD1	MAO A	MAO B
TCP	100 ± 22	5 ± 0.70	26 ± 0.082
C	17 ± 4.9	270 ± 30	8.3 ± 0.083
D	2.4 ± 0.63	64 ± 17	42 ± 24
E	0.61 ± 0.13	110 ± 11	17 ± 2.5

Compounds **F** and **G** are members of the *N*-alkylated TCP derivatives and show high potency and selectiveness towards LSD1 inhibitors with K_i values of 5 and 9 nM (Figure 12) (33).



F **G**

Figure 12: N-alkylated analogues of TCP as potent and selective LSD1 inhibitors (33).

Table III: In Vitro LSD1- and MAO-Inhibitory activities of compounds F and G (33).

	K_i (μM)		
Compound	LSD1	MAO A	MAO B
F	0.005	16	7.4
G	0.009	15	> 40

Oryzon Genomics reported potent TCP-based analogues which are also selective for LSD1 over MAOs and LSD2 enzymes. Their drug, ORY-1001, received the grant for orphan drugs by EMA and it is in phase IIa trial studies for acute myeloid leukaemia. GlaxoSmithKline Pharmaceuticals also announced a selective, mechanism-based irreversible LSD1 inhibitor GSK 2879552, which is currently in phase I trial studies for the treatment of relapsed/refractory small cell lung carcinoma (30).

1.7.3. None-tranylcypromine based LSD1 inhibitors

Among the TCP based LSD1 inhibitors, we can find also peptide derivatives, polyamine analogues and other small molecules (30). Peptide derivative, hexapeptide, which imitates the SNAIL sequence, competitively binds to LSD1/CoREST (38).

Bisguanide and bisguanidine polyamine analogues have the same homology to FAD-dependent polyamine oxidases. Compounds **H** and **I** were found to inhibit LSD1 with inhibition kinetics at concentrations < 2.5 μM and they have the ability to inhibit growth of human colon carcinoma cells (Figure 13) (39). Furthermore, (bis)urea and (bis)thiourea

analogues (**J** - **L**), which are active in human Calu-6 lung carcinoma cells, have a similar potency as LSD1 as compounds **H** and **I** (Figure 13) (40).

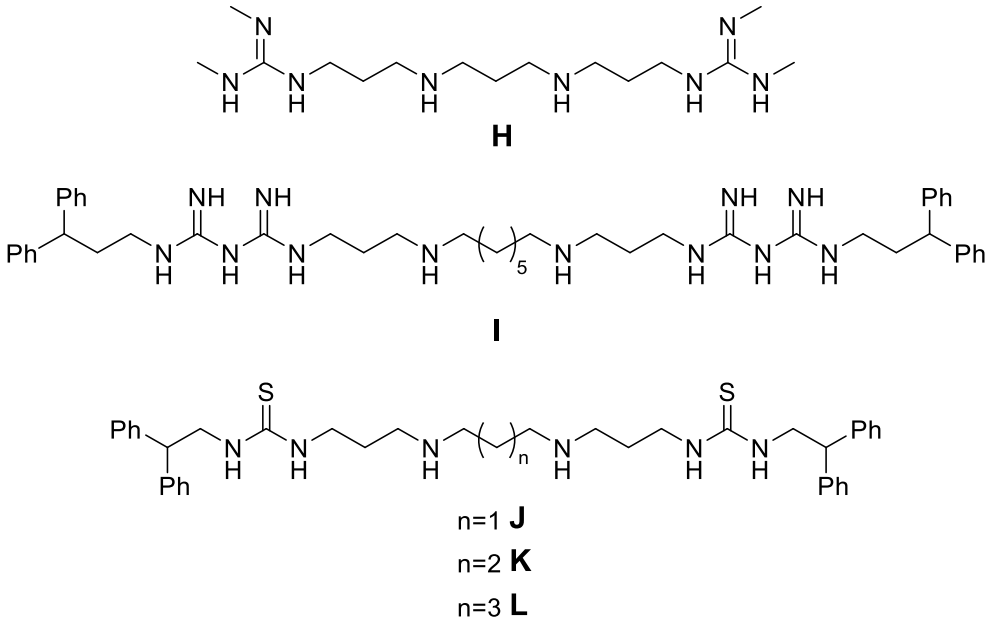


Figure 13: Bisguanide and bisguanidine polyamine LSD1 inhibitors (40).

Treatment with LSD1 inhibitors can lead to side effects because LSD1 is ubiquitously expressed. Experiments in mice show that LSD1 inhibitors can cause drug induced anaemia although it can be treated with blood transfusion. Regardless, LSD1 inhibitors display great therapeutic potential against cancer and other diseases (11).

2. AIMS

The aim of this research thesis is to synthesize novel LSD1 inhibitors with potent and selective properties. Starting material used is a (±)*trans*-chrysanthemic acid (Figure 14), which naturally occurs in pyrethrum (*Tanacetum cinerariifolium* syn. *Chrysanthemum cinerariaefolium*) in the form of pyrethrins and it is used as a pesticide (41). It is a convenient starting material because it is very inexpensive. Another starting compound is diethyl *trans*-1,2-cyclopropanedicarboxylate (Figure 14), which lacks two methyl groups attached to the cyclopropyl ring when compared with chrysanthemic acid.

Figure 14: Starting materials used in this research thesis. (±)*Trans*-chrysanthemic acid on the left and diethyl *trans*-1,2-cyclopropane dicarboxylate on the right.

LSD1 and MAOs have very similar catalytic sites. Therefore, the MAO inhibitor TCP, which is used to treat psychological disorders, also inhibits LSD1. Recently, a lot of research has been done to synthesize TCP analogues that would possess highly selective inhibitory activity against LSD1. With the influence of TCP analogues, our compounds will be synthesized (Figure 15, Figure 16). Instead of the phenyl ring attached to the cyclopropyl ring found in TCP, other moieties will be used. Therefore, it is possible to evaluate the importance of the phenyl ring in TCP analogues. Additionally, some of the final compounds will have two methyl groups attached to the cyclopropyl ring (Figure 16), which TCP analogues do not have. These methyl groups could interfere with the inhibition mechanism of LSD1, where the cyclopropyl ring opens and covalently binds to the FAD. Selectivity will be achieved using large moieties attached to one side of the cyclopropyl ring, blocking access to the catalytic site of MAOs.

Figure 15: Proposed final compounds synthesized from diethyl *trans*-1,2-cyclopropanedicarboxylate.

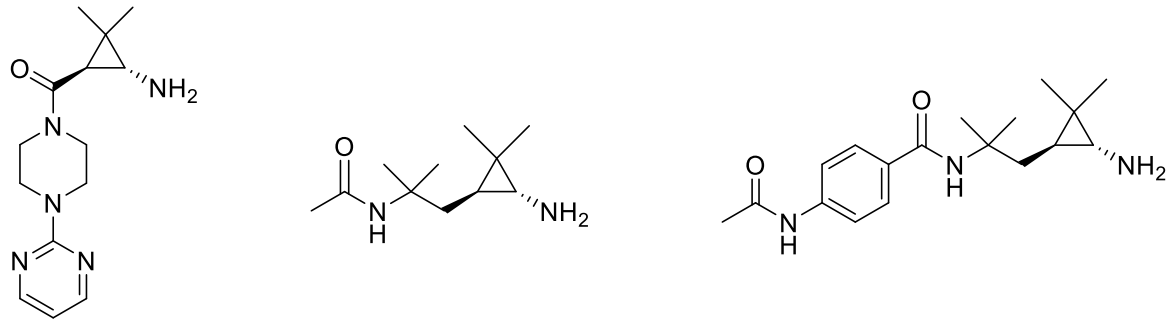


Figure 16: Proposed final compounds synthesized from (±)*trans*-chrysanthemic acid.

The synthesis pathways will be modified based on the success of completion of the particular step of the synthesis. LSD1 enzymatic activity of the compounds will be tested with fluorescence based LSD1 enzyme assay.

3. MATERIALS AND METHODS

- **Reagents and solvents**

Reagents and solvents used for synthesis and chromatography were obtained from *Fluka*, *Sigma-Aldrich*, *Merck*, *Alfa Aesar* and *Fisher Chemical*. These were used without further purification or drying unless stated.

- **Chromatographic methods**

Thin-layer chromatography (TLC): reactions were monitored by TLC on the aluminium plates Silica 60 marked with fluorescent indicator F_{254} by the manufacturer *Merck*. Spots were detected with UV light (λ=254 nm).

Column chromatography: purification of reaction mixtures was performed by flash column chromatography. Silicagel used was Silica 60, size 0.060 – 0.200 mm, from manufacturer *Acros*.

- **Software**

For drawing, naming of the structures and drawing of synthesis schemes, ChemBioOffice 11.0, from CambridgeSoft, was used.

- **Spectroscopic methods**

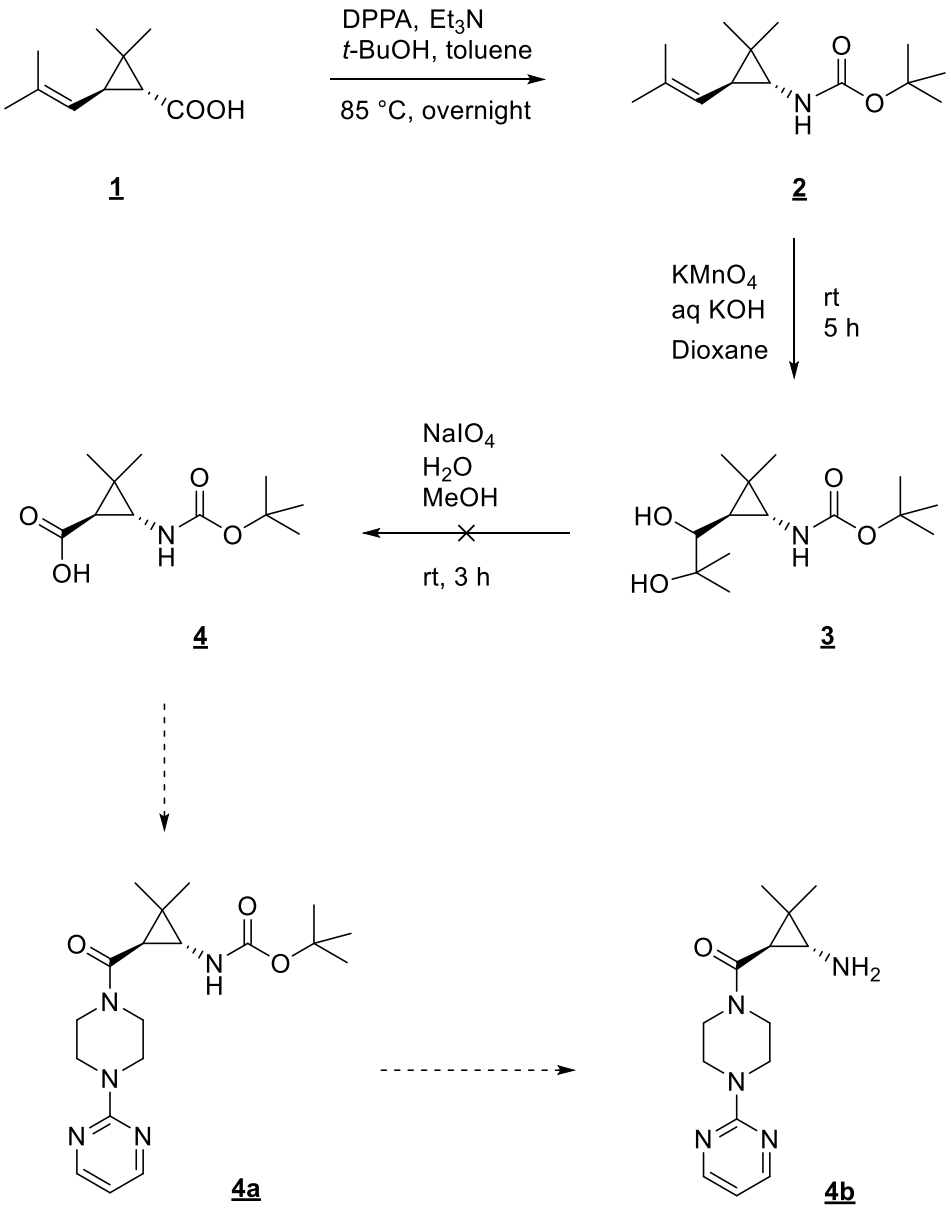
Nuclear magnetic resonance (NMR): ^{1}H and ^{13}C spectra of compounds were recorded using a Bruker Advance AM 400 (400 MHz) spectrometer from the University of East Anglia, School of Pharmacy. NMR spectra were processed using a Bruker XWIN NMR 3.5 program and with help of the predictions generated with ChemBioOffice 11.0, from CambridgeSoft. The solvents that were used included $CDCl_3$, DMSO-d_6 and CD_3OD, with tetramethylsilane (TMS) as the internal standard.

Low resolution mass spectra (LRMS) were recorded using a Micromass Quattro Ultima mass spectrometer**.**

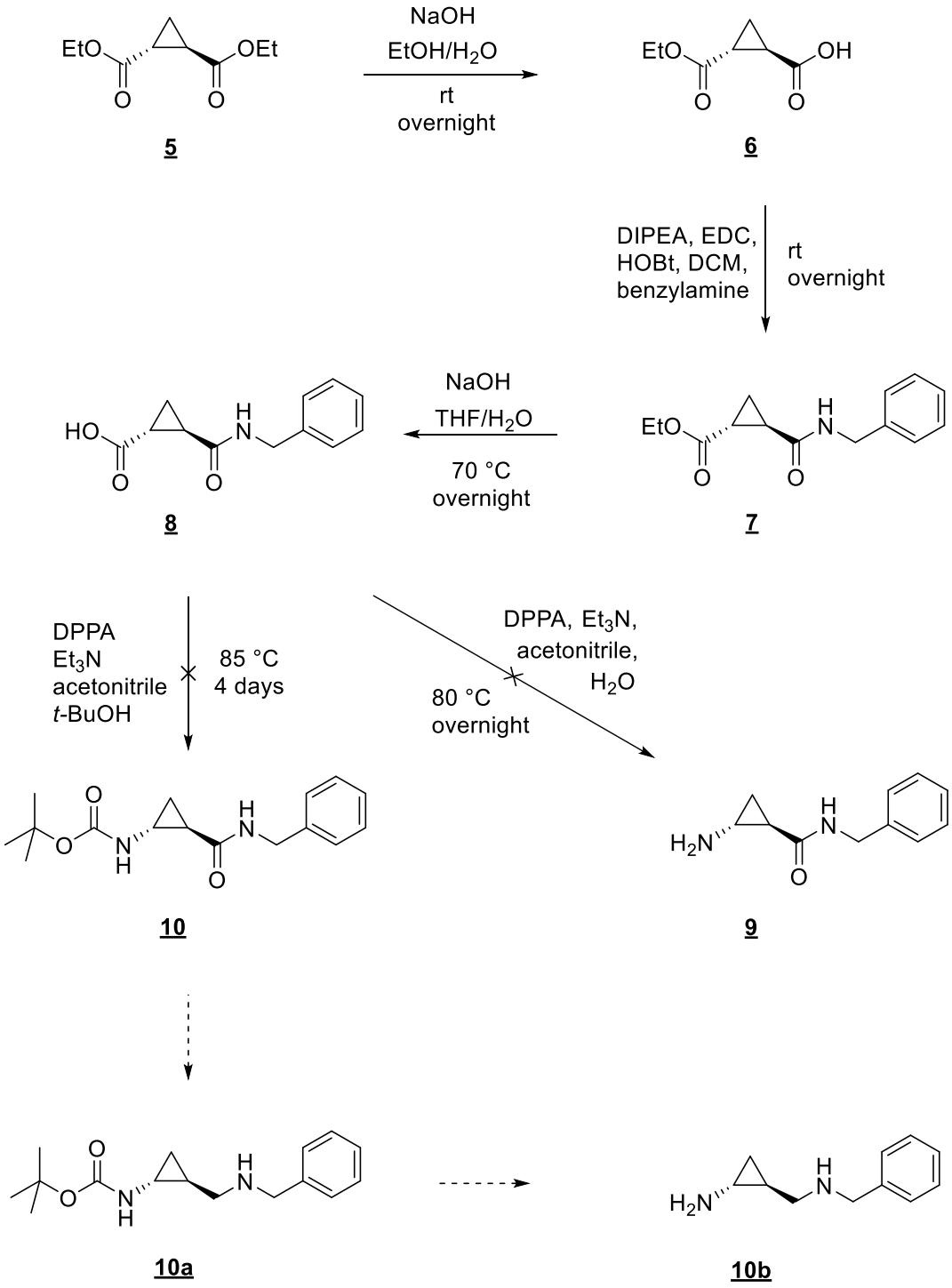
4. EXPERIMENTAL WORK

4.1. REACTION SCHEMES

4.1.1. Synthesis of (±)-*trans*-(3-amino-2,2-dimethylcyclopropyl)(4-(pyrimidin-2-yl) piperazin-1-yl)methanone (4b) from (±)-*trans* chrysanthemic acid (1)

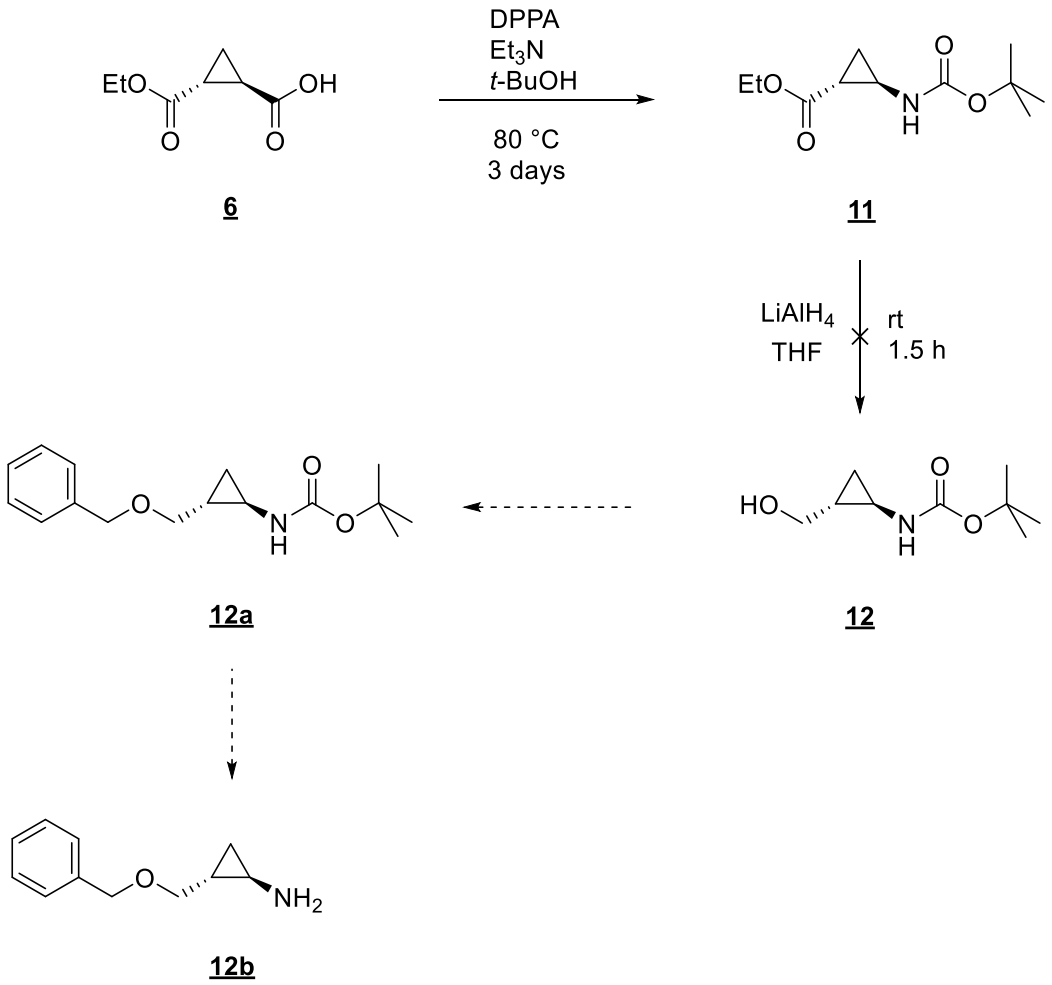


4.1.2. Synthesis of (1*R*,2*R*)-2-amino-*N*-benzylcyclopropane-1-carboxamide (9) and (1*R*,2*S*)-2-((benzylamino)methyl)cyclopropan-1-amine (10b) from diethyl (1*R*,2*R*)-cyclopropane-1,2-dicarboxylate (5)

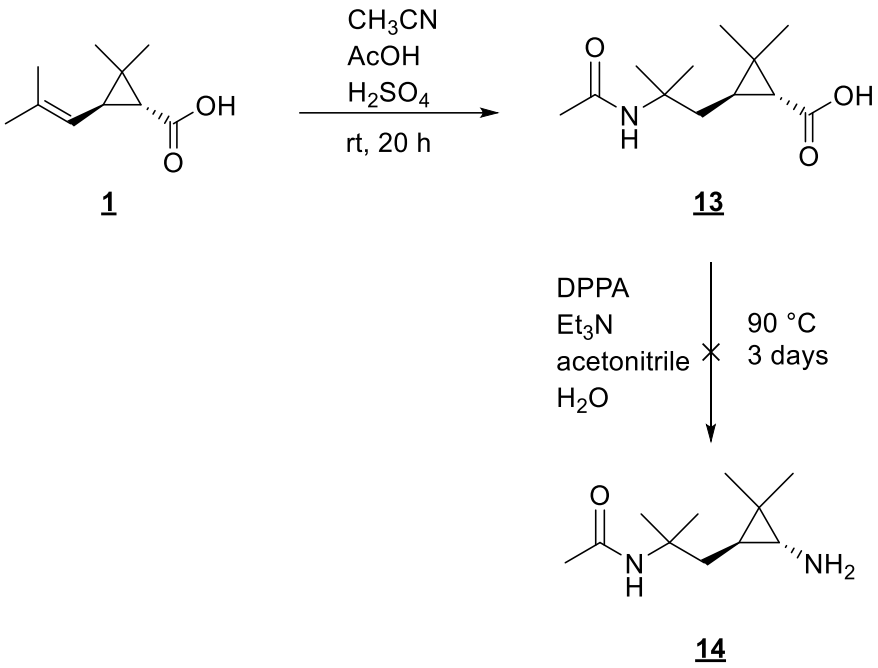


5 **6** **7** **8** **9** **10** **10a** **10b**

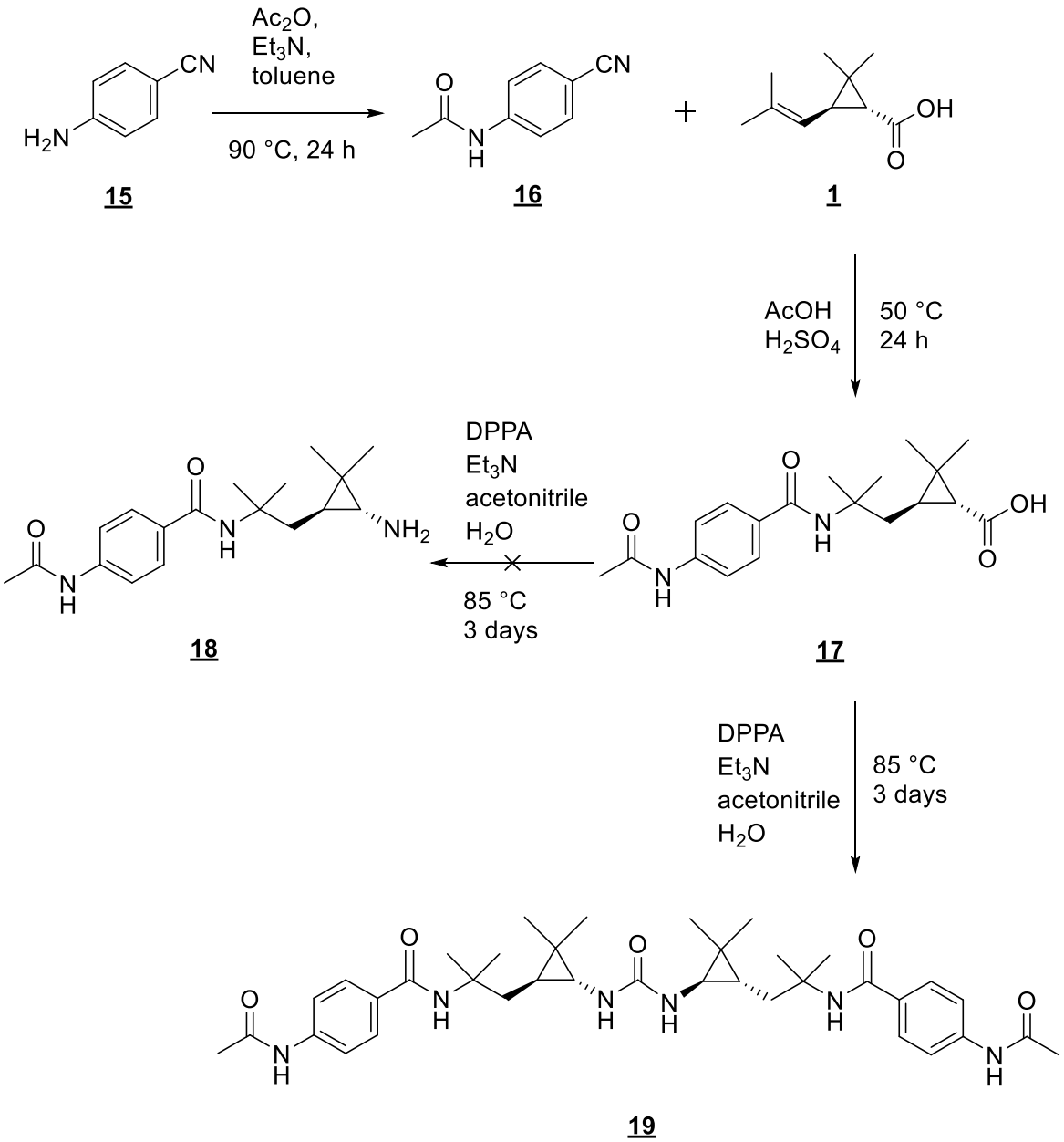
4.1.3. Synthesis of (1*R*,2*R*)-2-((benzyloxy)methyl)cyclopropan-1-amine (12b) from (1*R*,2*R*)-2-(ethoxycarbonyl)cyclopropane-1-carboxylic acid (6)



4.1.4. Synthesis of (±)-*trans*-*N*-(1-(3-amino-2,2-dimethylcyclopropyl)-2-methylpropan-2-yl) acetamide (14) from (±)-*trans* chrysanthemic acid (1)

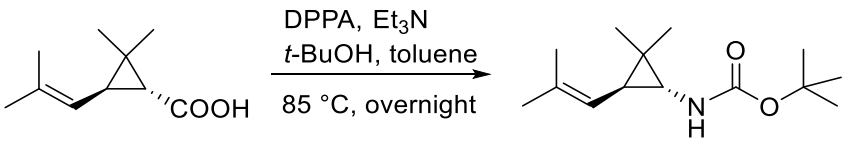


4.1.5. Synthesis of (±)-*trans*-3-(2-(4-acetamidobenzamido)-2-methylpropyl)-2,2 dimethyl cyclopropanecarboxylic acid (17) from 4-aminobenzonitrile (15) and (±)-*trans* chrysanthemic acid (1)



4.2. REACTION PROCEDURES AND RESULTS

4.2.1. Synthesis of (±)-*trans-tert*-butyl (2,2-dimethyl-3-(2-methylprop-1-en-1-yl) cyclopropyl)carbamate (2)



1 **2**

(±)-*Trans* chrysanthemic acid **1** (1 g, 5.94 mmol) was dissolved in toluene (4 mL) and triethylamine (0.59 mL, 6.54 mmol) was added. A nitrogen atmosphere was used to exclude moisture from the flask. Diphenylphosphoryl azide (1.41 mL, 6.54 mmol) was added and the resulting mixture was heated to 85 °C. After 3.5 hours *tert*-butanol (2.5 mL, 26.15 mmol) was added and stirring at 85 °C was continued overnight. The reaction mixture was diluted with EtOAc and saturated with $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/PE = 5/95), to provide desired carbamate **2** as a colourless oil.

Yield: 50 % (0.710 g, 2.97 mmol)

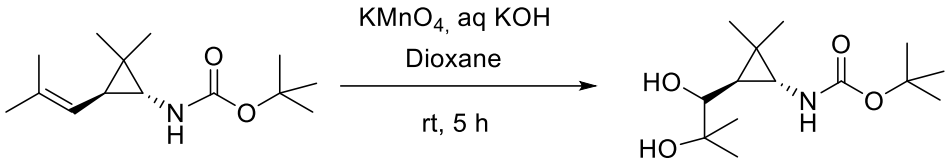
R_f (EtOAc/PE = 5/95) = 0.11

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.85 (d, J = 6.7 Hz, 1H, HC-cycloprop), 4.67 (s, 1H, NH), 2.23 (s, 1H, $C1_{cycloprop}$), 1.70 (s, 3H, H_3C-cycloprop), 1.67 (s, 3H, H_3C-cycloprop), 1.44 (s, 9H, *t*-but), 1.16 (m, 1H, $C3_{cycloprop}$), 1.12 (s, 3H, CH_3), 1.03 (s, 3H, CH_3) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 156.8, 134.5, 121.9, 79.3, 42.5, 30.8, 28.5, 25.6, 24.3, 20.6, 18.5 ppm.

ES^+ MS (m/z): 262.0 ($[M+Na]^+$)

4.2.2. Synthesis of (±)-*trans-tert*-butyl (1,2-dihydroxy-2-methylpropyl)-2,2 dimethyl cyclopropyl)carbamate (3)



2 **3**

To a solution of compound **2** (150 mg, 0.62 mmol) in 10% aqueous KOH (1.2 mL) and dioxane (4.8 mL), $KMnO_4$ (520 mg, 3.29 mmol) was added. The mixture was stirred at room temperature for 5 hours. After filtration through a Celite pad the filtrate was treated with 5 mL of distilled water. The aqueous phase was extracted with EtOAc (2 × 10 mL). Organic layers were combined, washed with saturated NaCl, dried over $MgSO_4$ and evaporated under reduced pressure. The crude material was purified by flash chromatography (EtOAc/PE = 1/1). The product **3** was obtained as colourless oil.

Yield: 45 % (0.075 g, 0.28 mmol)

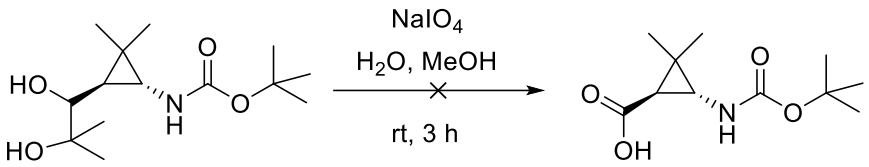
R_f (EtOAc/PE = 50/50) = 0.17

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (s, 1H, NH), 3.04 (d, J = 10.1 Hz, 1H, HC-OH), 2.13 (d, J = 4.1 Hz, 1H, $C1_{cycloprop}$), 1.43 (s, 9H, *t*-but), 1.24 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 1.13 (s, 3H, H_3C-cycloprop), 1.08 (s, 3H, H_3C-cycloprop), 0.93 (dd, J_1 = 10.1 Hz, J_2 = 4.1 Hz, 1H, $C3_{cycloprop}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1, 80.4, 77.4, 71.7, 38.4, 36.3, 28.4, 27.2, 24.1, 23.9, 20.9 ppm.

ES^+ MS (m/z): 296.1 ($[M+Na]^+$)

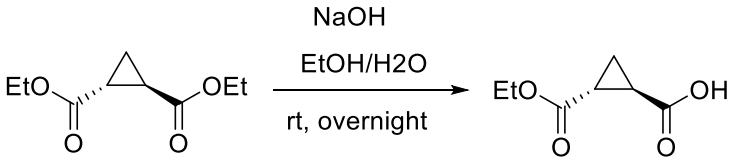
4.2.3. Synthesis of (±)-*trans*-3-((*tert*-butoxycarbonyl)amino)-2,2-dimethylcyclopropane carboxylic acid (4)



3 **4**

A solution of compound **3** (0.047 g, 0.17 mmol) and $NaIO_4$ (0.075 g, 0.35 mmol) in water/methanol (1 mL/0.1 mL) was stirred at room temperature for 3 hours. The resulting mixture was acidified to pH 4, saturated with NaCl and extracted with EtOAc (3 × 10 mL). Organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Many spots were visible on the TLC plate. After flash chromatography (MeOH/DCM = 5/95) the desired product **4** was not obtained.

4.2.4. Synthesis of (1*R*,2*R*)-2-(ethoxycarbonyl)cyclopropane-1-carboxylic acid (6)



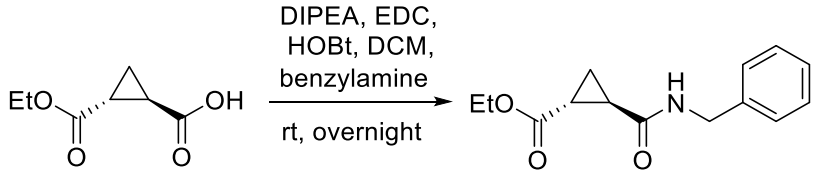
5 **6**

Compound **5** (2.12 g, 11.4 mmol), NaOH (464 mg, 11.6 mmol) in EtOH (34.5 mL) and water (2.8 mL) was stirred at room temperature overnight. The mixture was then concentrated. The residue was redissolved in water and washed with diethyl ether (20 mL) to remove any unreacted starting material. The aqueous solution was then adjusted to pH~2 with 2N HCl and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to give crude monoester monoacid **6** as a white solid.

Yield: 73 % (1.32 g, 8.28 mmol)
R_f (MeOH/DCM = 5/95) = 0.41

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (br s, 1H, OH), 4.16 (q, J_1 = 14.3 Hz, J_2 = 7.1 Hz, 2H, OCH_2), 2.24 – 2.14 (m, 2H, $C1_{cycloprop,}$ $C2_{cycloprop}$), 1.56 – 1.45 (m, 2H, $C3_{cycloprop}$), 1.27 (t, J = 7.1 Hz, 3H, CH_3) ppm.

4.2.5. Synthesis of ethyl (1*R*,2*R*)-2-(benzylcarbamoyl)cyclopropane-1-carboxylate (7)



6 **7**

A mixture of compound **6** (500 mg, 3.16 mmol), benzylamine (0.41 mL, 3.79 mmol), EDC (588 mg, 3.79 mmol), DIPEA (1.39 mL, 7.90 mmol), and HOBt (1067 mg, 7.90 mmol) in DCM (21.5 mL) was stirred at room temperature overnight. The organic layer was washed with 2M HCl (2 × 15 mL), $NaHCO_3$ (2 × 15 mL), brine (1 × 15 mL) and dried over $MgSO_4$. The crude product was purified by flash chromatography (EtOAc/PE = 2/8) to obtain **7** as a white solid.

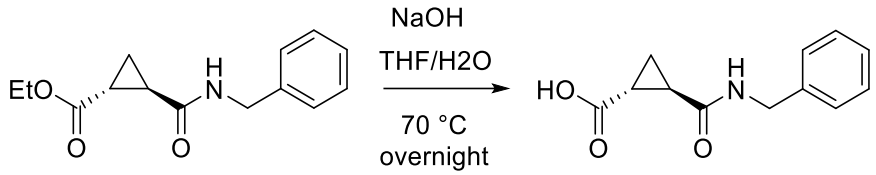
Yield: 25 % (198 mg, 0.80 mmol)

R_f (EtOAc/PE = 2/8) = 0.16

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H, Ph), 6.05 (br s, 1H, NH), 4.45 (d, J = 5.7 Hz, 2H, H_2C-Ph), 4.12 (q, J = 7.1 Hz, 2H, OCH_2), 2.22 – 2.18 (m, 1H, $C2_{cycloprop}$), 1.95 – 1.91 (m, 1H, $C1_{cycloprop}$), 1.51 – 1.47 (m, 1H, $C3_{cycloprop}$), 1.37 – 1.31 (m, 1H, $C3_{cycloprop}$), 1.26 (t, J = 7.14 Hz, 4H, CH_3) ppm.

ES$^+$ MS (m/z): 269.9 ($[M+Na]^+$)

4.2.6. Synthesis of (1*R*,2*R*)-2-(benzylcarbamoyl)cyclopropane-1-carboxylic acid (8)



7 **8**

Compound **7** (113 mg, 0.46 mmol) and NaOH (64.4 mg, 1.61 mmol) in THF (6 mL) and water (2 mL) were stirred at 70 °C overnight. The mixture was then concentrated to remove the solvent. The residue was redissolved in water, pH was adjusted to pH~2 with 2N HCl and extracted with EtOAc (4 × 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated to give crude acid **8** as a white solid.

Yield: 80 % (81 mg, 0.37 mmol)

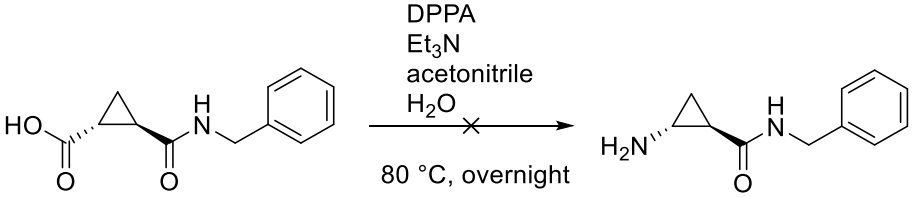
R_f (MeOH/DCM = 1/9) = 0.18

^{1}H NMR (400 MHz, CD_3OD) δ 8.77 (br s, 1H, NH), 7.34 – 7.23 (m, 5H, Ph), 4.38 (m, 2H, H_2C-Ph), 2.18 – 2.13 (m, 1H, $C2_{cycloprop}$), 2.06 – 1.98 (m, 1H, $C1_{cycloprop}$), 1.40 – 1.29 (m, 2H, $C3_{cycloprop}$) ppm.

^{13}C NMR (100 MHz, CD_3OD) δ 175.8 (C=O), 172.8 (C=O), 139.8 (C), 129.6 (CH), 128.6 (CH), 128.3 (CH), 44.4 (H_2C-Ph), 24.5 ($C2_{cycloprop}$), 22.0 ($C1_{cycloprop}$), 14.7 ($C3_{cycloprop}$) ppm.

ES$^+$ MS (m/z): 242.2 ($[M+Na]^+$)

4.2.7. Synthesis of (1*R*,2*R*)-2-amino-*N*-benzylcyclopropanecarboxamide (9)

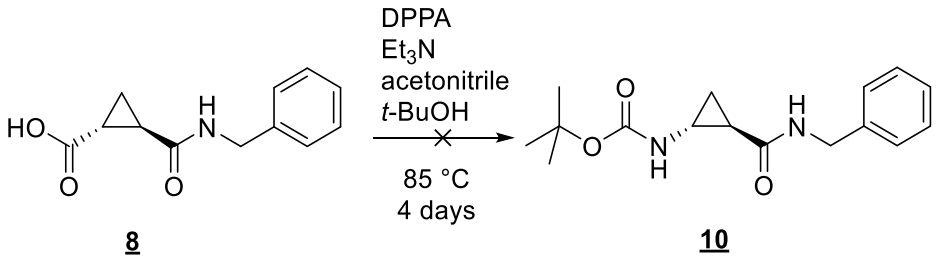


8 **9**

Compound **8** (40 mg, 0.18 mmol) was dissolved in acetonitrile (2 mL). Triethylamine (0.020 mL, 0.22 mmol) and diphenylphosphoryl azide (0.047 mL, 0.22 mmol) were added. The

resulting mixture was heated to 80 °C. After 4 hours H_2O (1 mL) was added and stirring at 80 °C was continued overnight. Acetonitrile was evaporated from the reaction mixture and water was added. Aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. Many spots were visible on the TLC plate. After flash chromatography (MeOH/DCM = 1/9) the desired product **9** was not obtained.

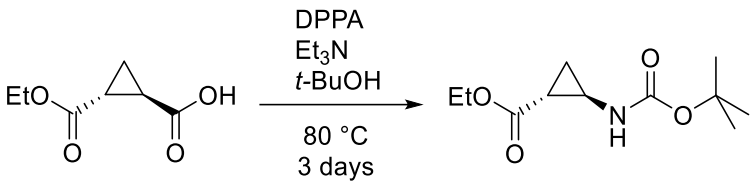
4.2.8. Synthesis of *tert*-butyl ((1*R*,2*R*)-2(benzylcarbamoyl)cyclopropyl)carbamate (10)



8 **10**

Compound **8** (40 mg, 0.18 mmol) was dissolved in acetonitrile (2 mL) and triethylamine (0.020 mL, 0.22 mmol) was added. A nitrogen atmosphere was used to exclude moisture from the flask. Diphenylphosphoryl azide (0.047 mL, 0.22 mmol) was added and the resulting mixture was heated to 85 °C. After 5 hours *tert*-butanol (1 mL) was added and stirring at 85 °C was continued for four days. The mixture was then concentrated to remove the solvent and redissolved in EtOAc and saturated with $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. Many spots were visible on the TLC plate. The crude material was purified by flash chromatography (MeOH/DCM = 3/97). The desired product **10** was not obtained.

4.2.9. Synthesis of ethyl (1*R*,2*R*)-2-((*tert*-butoxycarbonyl)amino)cyclopropane-1-carboxylate (11)



6 **11**

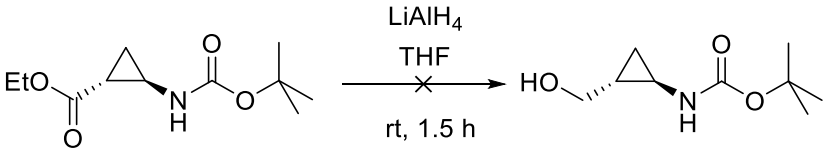
Compound **6** (100 mg, 0.63 mmol), triethylamine (0.085 mL, 0.95 mmol) and diphenylphosphoryl azide (0.16 mL, 0.76 mmol) were combined in tert-butanol (1 mL) under argon and heated at 80 °C for 3 days. The mixture was diluted with EtOAc and saturated with $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/PE = 1/4) to provide the desired carbamate **11** as a white solid.

Yield: 21 % (31 mg, 0.14 mmol)

R_f (EtOAc/PE = 1/4) = 0.54

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.72 (br s, 1H, NH), 4.19 – 4.07 (m, 2H, CH_2), 3.02 (br s, 1H, $C_{cycloprop}$), 1.74 – 1.69 (m, 1H, $C_{cycloprop}$), 1.44 (s, 9H, *t*-but), 1.40 – 1.36 (m, 1H, $C3_{cycloprop}$), 1.26 (t, J = 7.2 Hz, 3H, CH_3), 1.11 – 1.06 (m, 1H, $C3_{cycloprop}$) ppm.

4.2.10. Synthesis of *tert*-butyl ((1*R*,2*R*)-2-(hydroxymethyl)cyclopropyl)carbamate (12)

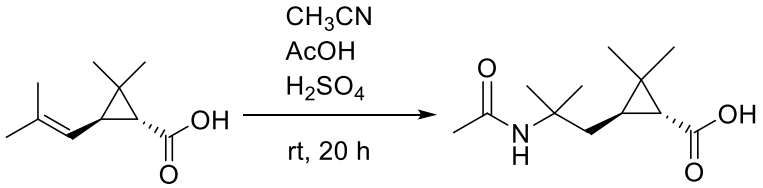


11 **12**

A solution of compound **11** (40 mg, 0.17 mmol) in THF (5 mL) was added to a solution of $LiAlH_4$ (12.9 mg, 0.34 mmol) in THF (3 mL) at 0 °C. After stirring for 90 minutes at room

temperature, the reaction was quenched by 1 M HCl (1 mL) and the whole mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. After flash chromatography (EtOAc/PE = 1/1) the desired product **12** was not obtained.

4.2.11. Synthesis of (±)-*trans*-3-(2-acetamido-2-methylpropyl)-2,2-dimethylcyclo propane -1-carboxylic acid (13)



1 **13**

A mixture of (±)-*trans* chrysanthemic acid **1** (1 g, 5.94 mmol), acetonitrile (10 mL), glacial acetic acid (10 mL), and concentrated sulfuric acid (0.2 mL) was stirred at room temperature for 20 hours. The mixture was poured into ice-water mixture and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with water, dried over $MgSO_4$ and evaporated to dryness. After flash chromatography (MeOH/DCM = 5/95) the desired product **13** was obtained as a colourless oil.

Yield: 60 % (0.809 g, 3.56 mmol)

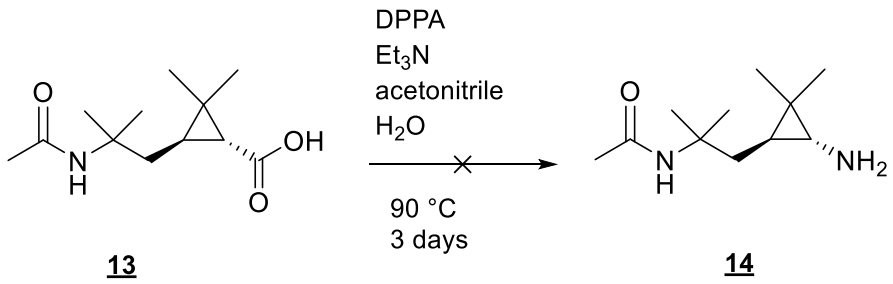
R_f (MeOH/DCM = 5/95) = 0.37

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (br s, 1H, COOH), 5.58 (NH), 1.92 (s, 3H, H_3C-CO), 1.83 (dd, J_1 = 14.3 Hz, J_2 = 6.5 Hz, 2H, (alifatic CH, CH_2)), 1.33 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.27 – 1.21 (m, 2H, (alifatic CH, CH_2)), 1.22 (s, 3H, H_3C-cycloprop), 1.11 (s, 3H, H_3C-cycloprop) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.6, 54.0, 37.2, 33.0, 30.1, 27.5, 27.3, 24.2, 21.7, 20.8 ppm.

ES^+ MS (m/z): 249.9 ($[M+Na]^+$)

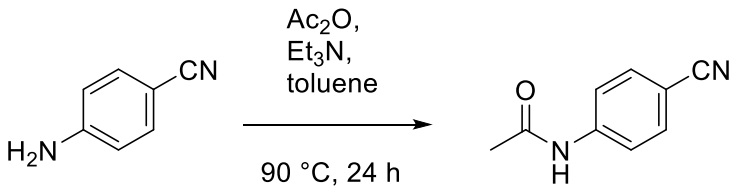
4.2.12. Synthesis of (±)-*trans*-*N*-(1-(3-amino-2,2-dimethylcyclopropyl)-2-methylpropan-2-yl)acetamide (14)



13 **14**

Compound **13** (400 mg, 1.76 mmol) was dissolved in acetonitrile (3 mL) and triethylamine (0.16 mL, 1.94 mmol) was added. A nitrogen atmosphere was used to exclude moisture from the flask. Diphenylphosphoryl azide (0.42 mL, 1.94 mmol) was added and the resulting mixture was heated to 90 °C. After 3.5 hours water (2.5 mL) was added and stirring at 90 °C was continued for three days. The reaction mixture was diluted with EtOAc (7 mL) and saturated with $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/PE = 3/2), the desired product **14** was not obtained.

4.2.13. Synthesis of *N*-(4-cyanophenyl)acetamide (16)



15 **16**

Acetic anhydride (0.44 mL, 4.64 mmol) and dry triethylamine (0.59 mL, 10.64 mmol) were added dropwise to a suspension of 4-aminobenzonitrile (500 mg, 4.24 mmol) in dry toluene (26 mL). The mixture was stirred at 90 °C for 24 h. The suspension was filtered, the precipitate was washed with acetic acid and water, and dried *in vacuo* to obtain compound **16** as a white solid.

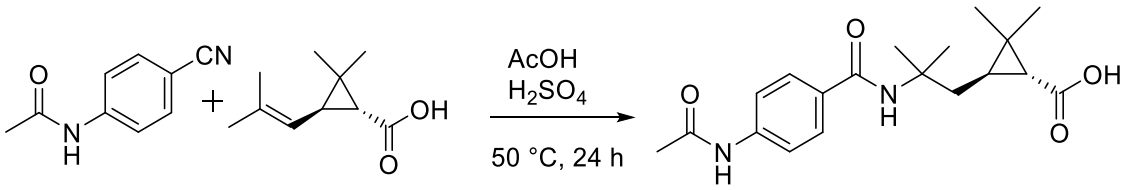
Yield: 50 % (340 mg, 2.12 mmol)

R_f (MeOH/DCM = 1/9) = 0.48

^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.37 (s, 1H, CONH), 7.75 (s, 4H, Ar), 2.09 (s, 3H, CH_3) ppm.

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 196.2 (CONH), 143.5 ($C1_{benz}$), 133.2 ($C3_{benz,}$ $C5_{benz}$), 119.1 (CN), 118.9 ($C2_{benz,}$ $C6_{benz}$), 104.7 ($C4_{benz}$), 24.2 (CH_3) ppm.

4.2.14. Synthesis of (±)-*trans*-3-(2-(4-acetamidobenzamido)-2-methylpropyl)-2,2 dimethylcyclopropanecarboxylic acid (17)



16 **1** **17**

A mixture of (±)-*trans* chrysanthemic acid **1** (300 mg, 1.85 mmol), *N*-(4-cyanophenyl)acetamide (304 mg, 1.90 mmol), glacial acetic acid (8 mL), and concentrated sulfuric acid (0.1 mL) was stirred at 50 °C for 24 hours. The mixture was poured into ice-water mixture and extracted with EtOAc (3 × 15 mL). The combined extracts were washed with water, dried over $MgSO_4$ and evaporated to dryness. After flash chromatography (MeOH/DCM = 1/9) the desired product **17** was obtained as a white solid.

Yield: 33 % (211 mg, 0.61 mmol)

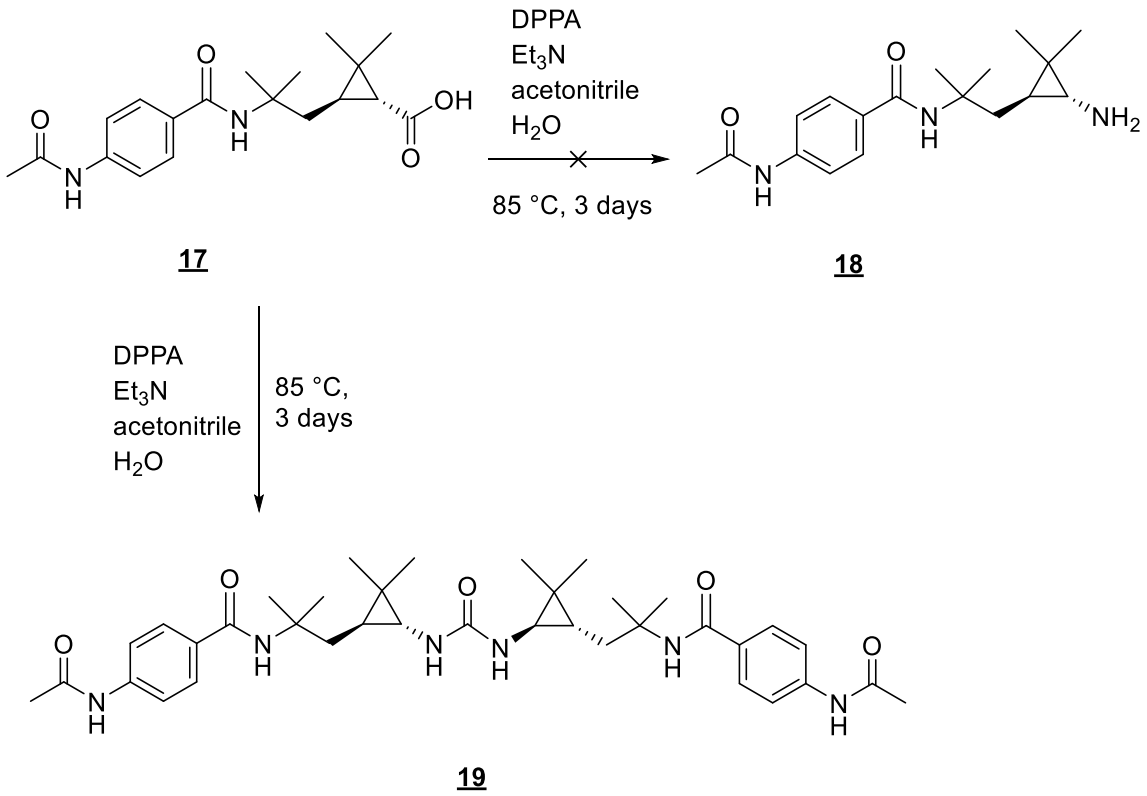
R_f (MeOH/DCM = 1/9) = 0.45

^{1}H NMR (400 MHz, CD$_3$OD) δ 7.73 – 7.61 (m, 5H, Ar, CONH), 2.14 (s, 3H, H_3C-CO), 1.99 (dd, J_1 = 14.2 Hz, J_2 = 6.5 Hz, 2H, (alifatic CH, CH_2)), 1.45 (s, 3H, H_3C-cycloprop), 1.43 (s, 3H, H_3C-cycloprop), 1.34 – 1.27 (m, 2H, (alifatic CH, CH_2)), 1.20 (s, 3H, CH_3), 1.16 (s, 3H, CH_3) ppm.

^{13}C NMR (100 MHz, CD$_3$OD) δ 176.5, 171.8, 169.8, 142.7, 132.2, 129.2, 120.1, 55.3, 38.2, 30.6, 27.6, 27.5, 27.5, 24.0, 21.9, 21.1 ppm.

ES$^-$ MS (m/z): 345.1 ($[M-H]^+$)

4.2.15. Synthesis of (±)-*trans*-4-acetamido-*N*-(1-(3-amino-2,2-dimethylcyclopropyl)-2-methylpropan-2-yl)benzamide (18)



Compound **17** (100 mg, 0.29 mmol) was dissolved in acetonitrile (3 mL) and triethylamine (0.03 mL, 0.32 mmol) was added. A nitrogen atmosphere was used to exclude moisture from the flask. Diphenylphosphoryl azide (0.07 mL, 0.32 mmol) was added and the resulting mixture was heated to 85 °C. After 3.5 hours water (0.5 ml) was added and stirring at 85 °C was continued for three days. The reaction mixture was diluted with EtOAc and saturated with $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash chromatography (EtOAc/PE = 3/2). The desired product **18** was not obtained, however a side product **19** (*N*,*N*'-(((carbonylbis(azanediyl))bis-(±)-*trans*-(3,3-dimethylcyclopropane-2,1-diyl))bis(2-methylpropane-1,2-diyl))bis(4-acetamidobenzamide)) was characterized.

Yield: 35 % (34 mg, 0.051 mmol)

R_f (MeOH/DCM = 1/9) = 0.23

^{1}H NMR (400 MHz, CD_3OD) δ 7.22 – 7.58 (m, 8H, 2 × Ar,), 2.13 (s, 6H, 2 × H_3C-CO), 1.96 – 1.80 (m, 6H, (alifatic CH, CH_2)), 1.48 (s, 6H, 2 × H_3C-cycloprop), 1.45 (s, 6H, 2 × H_3C-cycloprop), 1.03 (s, 6H, 2 × CH_3), 0.99 (s, 6H, 2 × CH_3) 0.85 – 0.53 (m, 2H, (alifatic CH, CH_2)) ppm.

^{13}C NMR (100 MHz, CD_3OD) δ 171.8, 169.8, 162.9, 142,8, 132.2, 129.1, 120.1, 55.5, 41.1, 38.5, 28.3, 27.9, 27.4, 24.0, 21.3, 20.2 ppm.

ES^+ MS (m/z): 683.2 ($[M-Na]^+$)

5. DISCUSSION

5.1. Discussion about synthesis of (±)-*trans*-(3-amino-2,2-dimethyl cyclopropyl)(4-(pyrimidin-2-yl)piperazin-1-yl)methanone (4b)

The first step of the synthesis was the formation of the carbamate from the carboxylic acid. This was done using the Curtius rearrangement in one-pot reaction. This procedure has the advantage that there is no need to isolate the normally explosive acyl azide intermediate (42). (±)-*Trans* chrysanthemic acid **1** was dissolved in toluene and trimethylamine was added. Trimethylamine deprotonates the carboxylic acid, making the acid more nucleophilic. A nitrogen atmosphere was used to exclude moisture from the flask, which would otherwise react with isocyanate intermediate and form an amine side product. Diphenylphosphoryl azide (DPPA) was added and the mixture was heated to 85 °C. DPPA forms a mixed carboxylic acid/phosphoric acid anhydride with the (±)-*trans* chrysanthemic acid and azide is eliminated. Anhydride acylates the azide ion to give acyl azide which undergoes rearrangement to form isocyanate. After 3.5 hours, when there was no more starting material according to TLC analysis, *tert*-butanol was added, reacting with isocyanate to afford Boc-protected amine **2** as a colourless oil (Figure 17).

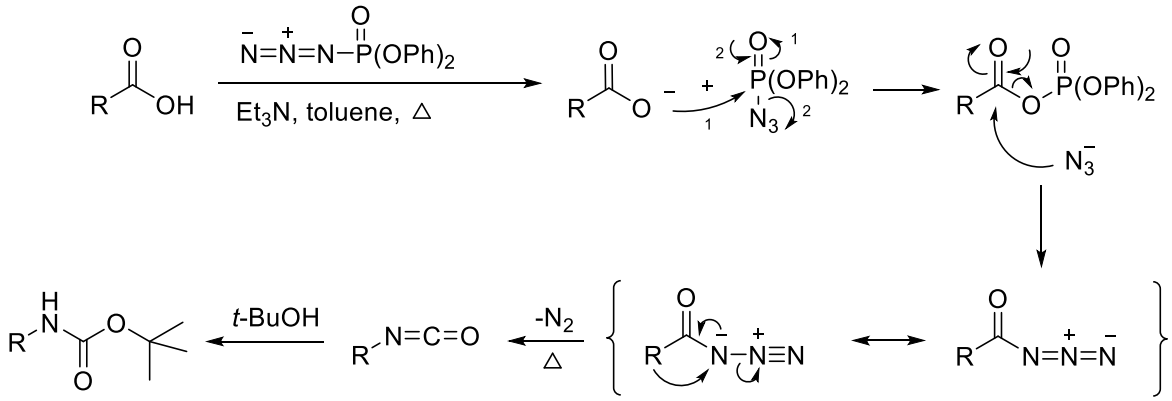


Figure 17: Mechanism of the Curtius rearrangement with DPPA.

The purification of the crude material was very challenging due to the proximity of the spots on TLC plate (similar polarity) and this was completed by flash chromatography (EtOAc/PE = 5/95). This reaction was performed under different conditions. Temperatures from 70 °C to 90 °C gave the same yield. The subsequent addition of *tert*-butanol in comparison with

initial did not seem to have a significant impact on the amount of final product. The duration of the reaction which was overnight is optimal because longer durations led to even more difficult purification.

The next step in the reaction scheme was the oxidation of the double bond of compound **2** by $KMnO_4$ to form the diol **3**. Reaction was carried out under alkaline conditions at a room temperature. $KMnO_4$, strong oxidising agent, was added in excess. After five hours, TLC analysis revealed that there is no more starting material; therefore, the reaction was stopped. By using an alkaline medium, brown precipitate of MnO_2 was produced (43). After the mixture was filtrated through a Celite pad, it was treated with a small amount of distilled water and extracted with EtOAc. The crude material was purified by flash chromatography. There was relatively low yield of the resulting product, which was obtained as colourless oil. The low yield could be due to the loss of product in the mixed fractions that were discarded. The mechanism of the reaction is still poorly understood since the reaction occurs with such high rate and the mechanism is very complex. However, the formation of a cyclic intermediate containing manganese(V) has been well accepted (Figure 18) (43).

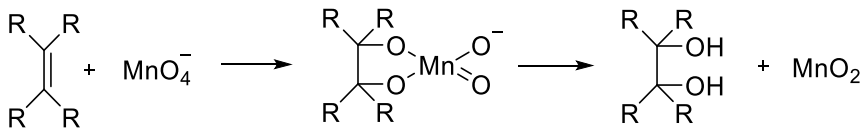


Figure 18: Proposed formation of a cyclic intermediate containing manganese(V) (44).

The next reaction was second oxidation to afford the carboxylic acid. The compound **3** was treated with $NaIO_4$ in in the presence of water and methanol. This reaction occurs through the formation of a periodate ester (45). After the C-C bond cleavage, acetone is formed as a side product (Figure 19). The solution was stirred at a room temperature for three hours. The mixture was acidified to pH 4 to protonate the acid, which moved from the water to organic layer during the extraction. The precise adjustment of the pH was important since the Boc deprotection could occur at a lower pH. On the TLC plate there were many visible spots. After flash chromatography an NMR analysis did not show any distinct signals. This reaction was conducted many times with often TLC monitoring, varying duration of the reaction (1 and 2 hours) and different molar equivalents of the reagent (2 and 3 equivalents). However, the desired product **4** was not obtained. To synthesize the final product, the compound **4**

would undergo a coupling reaction resulting in **4a**, and followed by deprotection of an amine leading to **4b** as a final product.

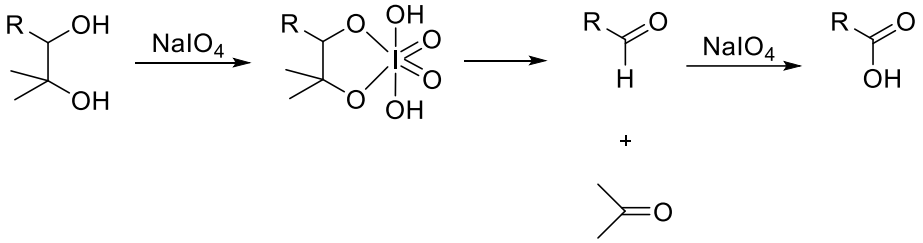


Figure 19: Oxidative cleavage of diol with $NaIO_4$ (45).

5.2. Discussion about synthesis of (1*R*,2*R*)-2-amino-*N*-benzylcyclo propane-1-carboxamide (9) and (1*R*,2*S*)-2-((benzylamino) methyl) cyclopropan-1-amine (10b)

The first reaction was a selective ester hydrolysis to remove the ethyl group from the ester substituent, allowing the coupling reaction to occur. The compound **5** was treated with NaOH in the presence of methanol and water. The selectivity of the hydrolysis was accomplished with the addition of the same molar equivalents of NaOH as of starting material. After stirring overnight at a room temperature, the mixture was concentrated to remove the ethanol, redissolved in water and washed with diethyl ether to remove any unreacted starting material. By adjusting the pH value of the water phase to 2, product **6** became protonated and was extracted with ethyl acetate. The obtained yield was 73 %.

Coupling of **6** to benzylamine was done with *N*,*N*-diisopropylethylamine (DIPEA), Hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and DCM as the solvent. The reaction occurs between the carboxylic acid and the amine to form the amide **7** at an ambient temperature. DIPEA is a non-nucleophilic base which deprotonates a carboxylic acid leading it to become more nucleophilic. Carboxyl activating agent EDC is water soluble and also forms soluble urea derivative (side product) and both can be easily removed by an extraction in an acidic aqueous phase (46). HOBt is a catalyst and it improves the efficiency of the reaction. The organic phase was washed with acidic and alkaline aqueous phases, saturated solution of salt and finally purified by flash column

chromatography. This reaction was also conducted with more molar equivalents of reagents in order to improve the low yield; however, with no success.

Ester **7** was treated with NaOH in THF and water, left at 70 °C overnight to afford free carboxylic acid **8**. The work-up was the same as by already described selective ester hydrolysis from compound **5** to **6**. An NMR analysis showed that the product was pure and yield was found to be 80 %.

The next step of the reaction scheme was the one-pot Curtius rearrangement. Compound **8** was dissolved in acetonitrile. Trimethylamine and DPPA were added and the resulting mixture was heated to 80 °C. Four hours later, when a TLC analysis showed that starting material was consumed, water was added and stirring was continued overnight. After work-up and flash chromatography dark red oil was obtained. An NMR analysis did not show any distinct signals. This could be due to instability of the molecule that could led to degradation (47). The compound **9** has a carbonyl group on one side of the cyclopropyl ring which is electron donor group and free amine on the other side which is electron accepting group. This could lead to ring opening (Figure 20).

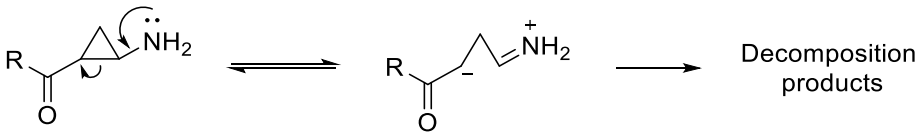


Figure 20: Proposed ring opening of the donor acceptor-substituted cyclopropanes.

Therefore, reaction scheme was altered and Curtius rearrangement with the addition of *tert*-butanol instead of water was performed. This way, the amine would be protected to form the carbamate **10**. Next, the carbonyl group of the amide would undergo reduction with $LiAlH_4$ to obtain **10a**, followed by deprotection of the amine to synthesize the **10b**. The final product **10b** would be stable because of the reduction of the electron accepting group. However, already at the Curtius rearrangement compound **10** was not obtained. The reaction mixture was left stirring for 4 days at 85 °C. On the TLC plate multiple spots were visible. After work-up and flash chromatography an NMR analysis showed that the potential product was not pure and further purification should be carried out. Unfortunately, there was not enough product nor available time, thus this synthesis pathway stopped here.

5.3. Discussion about synthesis of (1*R*,2*R*)-2-((benzyloxy)methyl) cyclopropan-1-amine (12b)

The first reaction was the one-pot Curtius rearrangement. Compound **6** was dissolved in *tert*-butanol which was used as a solvent and a reagent. Triethylamine and DPPA were added and argon atmosphere was used to exclude moisture. The mixture was heated to 80 °C and left for three days until a TLC showed complete consumption of the starting material. Desired carbamate **11** was obtained after work-up and flash chromatography as a white solid.

The next step was the reduction of the ester **11** to primary alcohol **12** with the two molar equivalents of $LiAlH_4$ in THF at an ambient temperature. The nucleophilic H^- from the $LiAlH_4$ attacks the carbonyl group of the ester and the tetrahedral intermediate is formed. This intermediate then collapses and forms an aldehyde which reacts more easily than the starting ester. Another reduction with the H^- from the $LiAlH_4$ takes place to give an alcohol (Figure 21). (45)

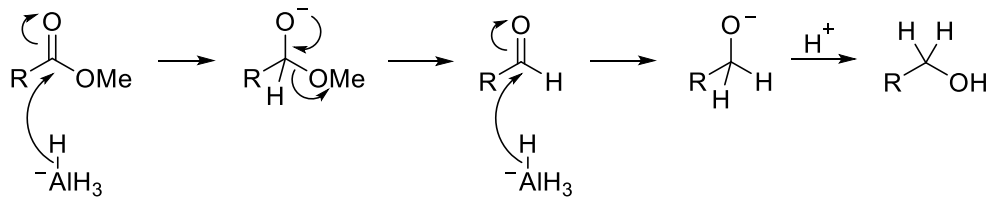


Figure 21: Reduction of esters to alcohols by $LiAlH_4$ (45).

After 1.5 hours the reaction was quenched with 1M HCl. This way, the aluminium hydroxide does not form emulsions in water and can be removed more easily (48). After work-up and flash chromatography the NMR analysis did not show any recognisable signals. The reaction scheme ended here due to absence of synthesized ester and lack of time. The reason why reaction did not work could be in $LiAlH_4$ which is a powerful reducing agent and could potentially reduce the Boc protecting group (49). It can be replaced with diisobutylaluminium hydride in DCM at -78 °C (50). Another reason could be that the reaction mixture was not cooled in ice when the reaction was quenched or that 1 M HCl was not added carefully enough. The alternative of quenching with an acid is "Fieser" work-up. 1 mL of water for each gram of $LiAlH_4$ is added to the reaction mixture. Subsequently, 1 mL of 15 % NaOH for each gram of $LiAlH_4$ is added, followed by a few minutes of stirring

and finally, 3 mL of water for each gram of $LiAlH_4$ is added. This work-up allows aluminate salts to form alumina, which could be easily removed (48).

To achieve the final product from this scheme, compound **12** would undergo etherification using NaH which is strong base and benzyl bromide to form the compound **12a**. In the next step, the amine would be deprotected to afford **12b** as a final product.

5.4. Discussion about synthesis of (±)-*trans*-*N*-(1-(3-amino-2,2-dimethyl cyclopropyl)-2-methylpropan-2-yl) acetamide (14)

First step of this synthesis was the Ritter reaction (51) where the alkene of the compound **1** reacted with acetonitrile in presence of glacial acetic acid and concentrated sulfuric acid. After the reaction mixture was stirred for 20 hours at the ambient temperature and TLC showed complete consumption of starting material, the reaction mixture was poured into an ice-water mixture. Ice was used to avoid overheating. After work-up and flash chromatography, the amide **13** was obtained as a colourless oil. The procedure in which a few changes were made was adopted from (52). In this mechanism, strongly acidic conditions were used to generate a carbenium ion. Although the nitrile is a weak nucleophile it attacks the carbenium ion to form resonance stabilized nitrilium ion, which is then subjected to hydrolysis to form corresponding amide (Figure 22) (48).

The next step in the synthesis was the one-pot Curtius rearrangement, which was described previously. After 3.5 hours, when there was no more starting material according to TLC analysis, instead of *tert*-butanol used in previous rearrangements, water was added. The desired final product **14** was not obtained. According to ^{13}C NMR analysis two peaks in 150 – 200 ppm region were still present like in spectrum of compound **13**. That suggests that there is no amine in the final product. The alternative for this one-pot procedure would be to perform more reactions which might be simpler to monitor with TLC and easier to purify with the flash chromatography since impurities would be removed gradually. For the activation of the acid, ethyl chloroformate or thionyl chloride could be used, followed by sodium azide to introduce an azide group to the molecule. With heating, the azide would undergo Curtus rearrangement to form isocyanate, which is quite reactive and would react with added water to create an amine (53, 54).

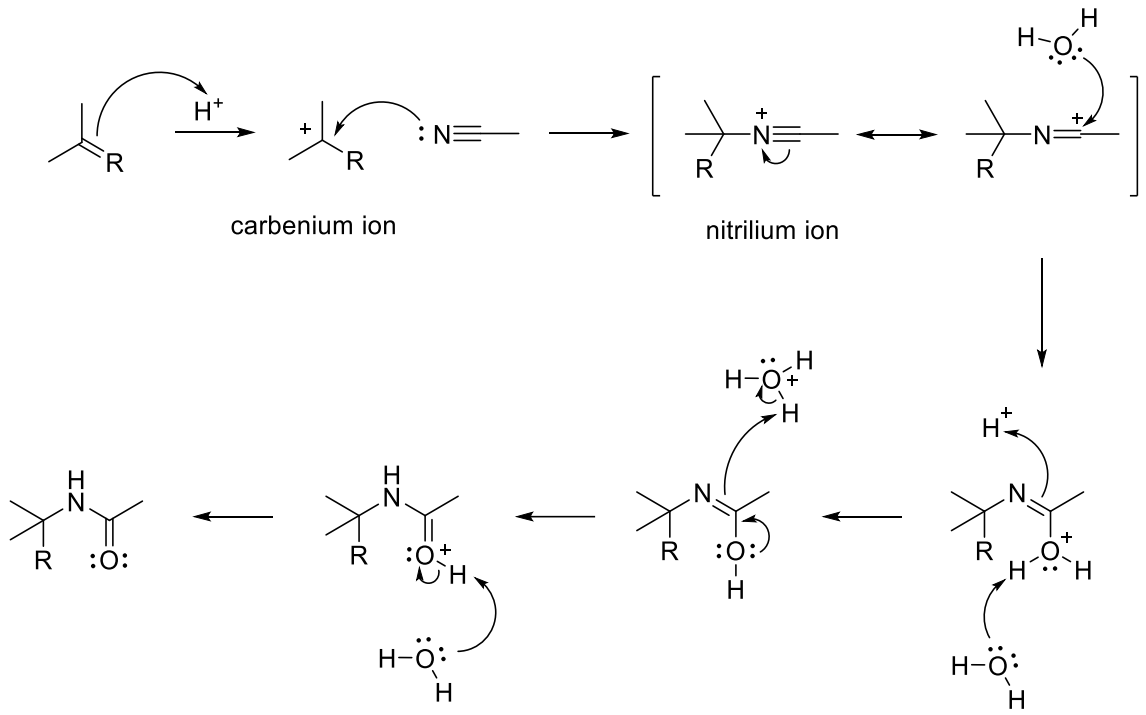


Figure 22: Mechanism of the Ritter reaction (Adopted from 55).

5.5. Discussion about synthesis of (±)-*trans*-3-(2-(4-acetamidobenzamido)-2-methyl propyl)-2,2 dimethyl cyclopropanecarboxylic acid (17)

In the first reaction, the amine group of 4-aminobenzonitrile was protected using acetic anhydride in presence of dry triethylamine and dry toluene. Dry reagent and solvent were used to prevent the potential side reaction between water and acetic anhydride. Dry triethylamine was used to neutralize the acetic acid, a side product, which would otherwise protonate the amine of 4-aminobenzonitrile, reducing the speed of the reaction. After the mixture was stirred at 90 °C for 24 hours, TLC showed complete consumption of the starting material. The suspension was filtered and precipitate was washed with acetic acid and water to remove any unreacted starting material. The yield of the product **16** was found to be 50 %.

The next step of the reaction scheme was the aforementioned Ritter reaction (synthesis of **13**). Compounds **16** and **1** were dissolved in glacial acetic acid and concentrated sulfuric acid and stirred for 24 hours. The difference here is that the mixture was heated to 50 °C; therefore, the reaction rate was increased. After work-up and flash chromatography the desired product **17** was obtained in relatively low yield.

The last synthesis was the previously mentioned one-pot Curtius rearrangement (synthesis of **14**). The final product **18** was not obtained, however a side product **19** was characterized. That suggests that the free amine of the desired product attacked isocyanate, forming compound **19**. To avoid side product formation, one-pot reaction could be replaced with more successive reactions, where reaction conditions could be better adjusted (more acidic environment in the last reaction).

6. CONCLUSION

In this research thesis, the synthesis of novel lysine-specific demethylase 1 inhibitors has been attempted. Five different synthesis pathways were followed however no final compounds were obtained.

In the first scheme, the first step was done using the Curtius rearrangement in a one-pot reaction to get the Boc protected amine from (±)*trans*-chrysanthemic acid, followed by double bond oxidation. Next, the oxidation of diol was performed with $NaIO_4$. After many attempts with different molar equivalents of the reagent and duration of the reaction we failed to create the desired free amine **4**.

The second scheme began with selective ester hydrolysis of compound **5** followed by coupling reaction. Ester of compound **7** was then hydrolysed and a one-pot Curtius rearrangement was performed to get the free amine. Unfortunately, compound **9** was not obtained. The reason for this could be due to the ring opening because of the electron donor group on the one side of the cyclopropyl ring and electron accepting group on the other. To avoid this degradation the existing reaction scheme was altered that the carbonyl group of the amide would be reduced. Another one-pot Curtius rearrangement was performed to get the Boc protected amine however the potential product **10** was not pure and in too little quantity to repeat the purification.

The next scheme started with one-pot Curtius rearrangement to get the Boc protected amine **11**. The second step, reduction with $LiAlH_4$, was not successful. This might have been because the $LiAlH_4$ could deprotect the Boc protecting group or that reaction mixture was not cooled in ice when the reaction was quenched with 1 M HCl. The investigation did not continue due to lack of starting materials and time.

The forth scheme started with the Ritter reaction followed by one-pot Curtius rearrangement to get the free amine. According to ^{13}C NMR analysis, two peaks in 150 – 200 ppm region were still present like in previous spectrum of the compound **13**, suggesting that the final product **14** was not obtained.

In the last scheme, the first step was protection of the amine group followed by the Ritter reaction to get the compound **17**. The last step was one-pot Curtius rearrangement which

was not successful, however a side product **19** was characterized. That implies the unwanted reaction between the free amine of the product **18** and isocyanate.

The one-pot Curtius rearrangement resulting in the free amine was not successful for three different reactions (products **9**, **14**, **18**). An alternative option would be to perform more successive reactions and use different reagents. Every step might be simpler to monitor and impurities would be removed gradually.

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