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PIRET VILLO

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DOCTORAL DISSERTATION
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To be defended at 09:15 on 8th of November in lecture hall 121,
Institute of Technology, University of Tartu, Nooruse 1, Tartu, Estonia

Opponent:
Prof. Nina Kann

UNIVERSITY OF TARTU
PRESS
1632

Organization	Document name
UNIVERSITY OF TARTU, LUND UNIVERSITY	DOCTORAL DISSERTATION
	Date of issue
Author Piret Villo	Sponsoring organization

Title and subtitle:
Synthesis of Acetogenin Analogues. Asymmetric Transfer Hydrogenation Coupled with Dynamic Kinetic Resolution of alfa-Amido-beta-Keto Esters.

Abstract

The first part of the thesis discusses the synthesis of six analogues of annonaceous acetogenins, as well as their cytotoxicity. The acetogenins are an intriguing class of bioactive natural compounds that have shown a selective cytotoxicity against cancerous cells and as such are considered promising new drug leads. The modular synthetic route allows the construction of the analogues with a variable central part containing heteroatoms, *e.g.*, nitrogen or sulphur, and facile variation in stereochemistry with high stereoselectivity.

The second part of the thesis concerns the enantioselective synthesis of amino acid derivatives, focusing on the asymmetric transfer hydrogenation via dynamic kinetic resolution in the synthesis of *anti*-α-amido-β-hydroxy esters. The conditions found afforded both alkyl- and aryl-products with high yields and high enantio- and diastereoselectivities. These compounds are valuable building blocks for constructing complex bioactive molecules, interesting to the pharmaceutical industy, and the conditions discussed allow two adjacent stereocenters in the product to be set in one step in water emulsions.

Key words: asymmetric synthesis, kinetic resolution, transfer hydrogenation, acetogenins, amino acids

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language: English
ISSN and key title		ISBN
Recipient's notes	Number of pages	Price
	Security classification	

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Institute of Technology, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Philosophy on August 31, 2013 by the council of Institute of Technology, Faculty of Science and Technology, University of Tartu.

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Gothenburg, Sweden

Commencement: Auditorium 121, Nooruse 1, Tartu, Estonia, at 09:15 on 8th of November 2013

Publication of this dissertation is granted by University of Tartu

ISSN 2228–0855
ISBN 978–9949–32–404–0 (print)
ISBN 978–9949–32–405–7 (pdf)

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ABSTRACT

The first part of the thesis discusses the synthesis of six analogues of annonaceous acetogenins, as well as their cytotoxicity. The acetogenins are an intriguing class of bioactive natural compounds that have shown a selective cytotoxicity against cancerous cells and as such are considered promising new drug leads. The modular synthetic route allows the construction of the analogues with a variable central part containing heteroatoms, *e.g*., nitrogen or sulphur, and facile variation in stereochemistry with high stereoselectivity.

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ABBREVIATIONS

Ac	acetyl
AcOH	acetic acid
AD	asymmetric dihydroxylation
AH	asymmetric hydrogenation
AKR	aminolytic kinetic resolution
Alk	alkyl
Ar	aryl
ATH	asymmetric transfer hydrogenation
ATP	adenosine triphosphate
(*R*)-BINAP	(*R*)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthalene
Bn	benzyl
(*S,S*)-BnDPAE	(1*S*,2*S*)-2-benzylamino-1,2-diphenylethanol
Boc	*tert*-butoxycarbonyl
Bz	benzoyl
CAN	ammonium cerium (IV) nitrate
cat.	catalyst
Cbz	benzyloxycarbonyl
m-CPBA	*meta*-chloroperoxybenzoic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
L-(+)-DET	(+)-diethyl L-tartrate
$(DHQD)_2PHAL$	hydroquinidine 1,4-phthalazinediyl diether
DIBALH	diisobutylaluminium hydride
DKR	dynamic kinetic resolution
DMAP	4-(dimethylamino)pyridine
DMBA	*N,N'*-dimethylbarbituric acid
DME	dimethoxyethane
DMF	dimethylformamide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
d.r.	diastereomeric ratio
ED_{50}	effective dose, dose that produces desired effect in 50% of a population
EDG	electron donating group
ee	enantiomeric excess
ent	enantiomer
equiv.	equivalents
e.r.	enantiomeric ratio
EWG	electron withdrawing group
HeLa cell line	human epitheloid cervix carcinoma cell line
HIV-1	Human Immunodeficiency Virus, type 1
HKR	hydrolytic kinetic resolution
HMPA	hexamethylphosphoramide
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry

IR	infrared absorption spectroscopy
KR	kinetic resolution
LA	Lewis acid
LDA	lithium diisopropylamine
LiHMDS	lithium bis(trimethylsilyl)amide
Me	methyl
MDR	multidrug resistant
MOM	methoxymethyl
MW	microwave
NADH	dihydronicotinamide adenine dinucleotide
NaHMDS	sodium bis(trimethylsilyl)amide
ND1	NADH dehydrogenase subunit 1
NMR	nuclear magnetic resonance spectroscopy
Ns	nosyl, (2-nitrobenzenesulfonyl)
Nu	nucleophile
OTf	triflate
Pg	protecting group
Ph	phenyl
PKR	parallel kinetic resolution
PPTS	pyridinium *p*-toluenesulfonate
Pr	propyl
rt	room temperature
(*S,S*)-salenCo(II)	(*S,S*)-*N,N'*-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II)
SAR	structure-activity relationship
TBAF	tetrabutylammonium fluoride
TBAH	tetrabutylammonium hexafluorophosphate
TBAHS	tetrabutylammonium hydrogensulfate
TBAI	tetrabutylammonium iodide
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TEA	triethanoamine
TEAF	triethylammonium formate
TEBA	benzyltriethylammonium chloride
Teoc	2-(trimethylsilyl)ethoxycarbonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	tosyl, (4-toluenesulfonyl)
TS	transition state
(*S,S*)-TsDPEN	(1*S*,2*S*)-1,2-diphenyl-1-tosylamino-1,2-diamine-ethane
UV	ultraviolet
vt	virtual triplet

LIST OF PUBLICATIONS

This thesis is based on the following papers, referred to in the text by their Roman numerals **I–V**:

I **Synthesis of Linear Aza and Thio Analogues of Acetogenins and Evaluation of Their Cytotoxicity**
Piret Villo, Lauri Toom, Elo Eriste and Lauri Vares
Eur. J. Org. Chem. **2013**, *Published online in Early View*
(DOI: 10.1002/ejoc.201300767)

II **Hydrolytic and Aminolytic Kinetic Resolution of Terminal Bis-Epoxides**
Jevgenia Bredihhina, Piret Villo, Kārlis Andersons, Lauri Toom and Lauri Vares
J. Org. Chem. **2013**, *78*, 2379–2385.

III **Synthesis of Amphiphilic Amino Alcohols**
Lauri Toom, Piret Villo, Ilme Liblikas and Lauri Vares
Synthetic Commun. **2008**, *38*, 4295–4313.

IV **Enantioselective Synthesis of *anti*-β-Hydroxy-α-Amido Esters via Transfer Hydrogenation**
Brinton Seashore-Ludlow, Piret Villo, Christine Häcker and Peter Somfai
Org. Lett. **2010**, *12*, 5274–5277.

V **Enantioselective Synthesis of *anti*-β-Hydroxy-α-Amido Esters by Asymmetric Transfer Hydrogenation in Emulsions**
Brinton Seashore-Ludlow, Piret Villo and Peter Somfai
Chem. – Eur. J. **2012**, *18*, 7219–7223.

I have contributed to all five papers to different extent in planning, experimental and writing processes. The detailed contribution is as follows:

Paper I I conducted the majority of the research and experimental work and wrote the paper as the corresponding author.

Paper II I contributed to the paper by working on one of the substrates discussed in the paper, which was the first of its kind under study for this project. My contribution also included optimizing the reaction conditions and compiling the experimental part of the manuscript.

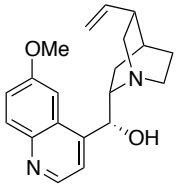
Paper III I conducted a share of the experimental work discussed in the paper.

Paper IV I conducted approximately half of the experimental work and participated in the writing of the manuscript.

Paper V I conducted a share of the experimental work, participated in the planning of the project and in writing the manuscript.

1. INTRODUCTION

We are surrounded by chiral structures – chirality plays an important role in all biological systems, and has been proven vital in the agricultural, pharmaceutical and chemical industries. Active scientific interest towards chirality and its relevancy in pharmacology and therapeutics had its beginning in 1980s and 1990s, despite the earlier pioneering work and discoveries of great minds like Pasteur, Fisher and others. Before the synthetic approach, chiral compounds were used as therapeutics unintentionally. For example, one of the earliest known cases where a chiral compound was used to cure a disease was of quinine and its antimalarial properties. Throughout the following decades and centuries, starting from the late 1620s, the story of quinine and other cinchona alkaloids can be traced. The knowledge of Peruvian Indian folk medicine that cinchona tree bark, which was unknowingly to its users rich in chiral alkaloids, could cure malarial fever was brought to Europe by returning missionaries.[1] The struggles of chemists to synthesize quinine were rewarded only in 1944 when the first formal synthesis was reported by Woodward,[2] and the first stereoselective synthesis as late as 2001.[3]



quinine

The high regard for enantiomeric compounds and the asymmetric synthesis thereof has increased with the widening knowledge of the mechanisms behind the bioactive molecules in the biological systems. A majority of the drugs manufactured industrially are chiral compounds and many of these are used as single enantiomers. Although the portion of racemate drugs has been declining over the last 30 years, they have not disappeared completely from the pharmacy scene.[4] Most of the new drugs launched now are single enantiomers, the number has almost doubled from the 1980s to the 2000s.[5] Also, the new drugs released as a single enantiomer are considerably more complex compared to the early days of industrial pharmacology. Thus, new methods for the synthesis of the bioactive compounds are always sought after. These methods also include synthesis from smaller 'molecular building blocks' or resolution of a chiral matter. Natural as well as unnatural amino acids and their derivatives are the most frequently used chirality pool. The synthesis of amino acid derivatives with *anti*-configuration is discussed in the chapter 3 of this thesis. In chapter 2, synthesis of more complex compounds is described, encompassing both new and known methods to set stereocenters.

1.1. Kinetic resolution and Asymmetric Transformation

Alongside with enantioselective synthesis, the separation of enantiomers from chiral matter has been a research area of great interest. Resolving an enantiomer from a racemate is often based on making and separating the diastereomeric derivatives and using double asymmetric induction. Here two non-enzymatic kinetic resolution methods are introduced: classical kinetic resolution (KR), parallel kinetic resolution (PKR), and an asymmetric transformation called dynamic kinetic resolution (DKR).[6] In a KR a racemate, composed of enantiomers S_S and S_R, is subjected to a chiral reagent or a catalyst R* (Figure 1). On the time scale of this selective reaction, the enantiomers are not interconvertable ($k_{inv} = 0$). One of the enantiomers is transformed into a product P_R, while the other enantiomer S_S will react considerably slower ($k_R >> k_S$), and can be recovered in a stereochemically enriched form.

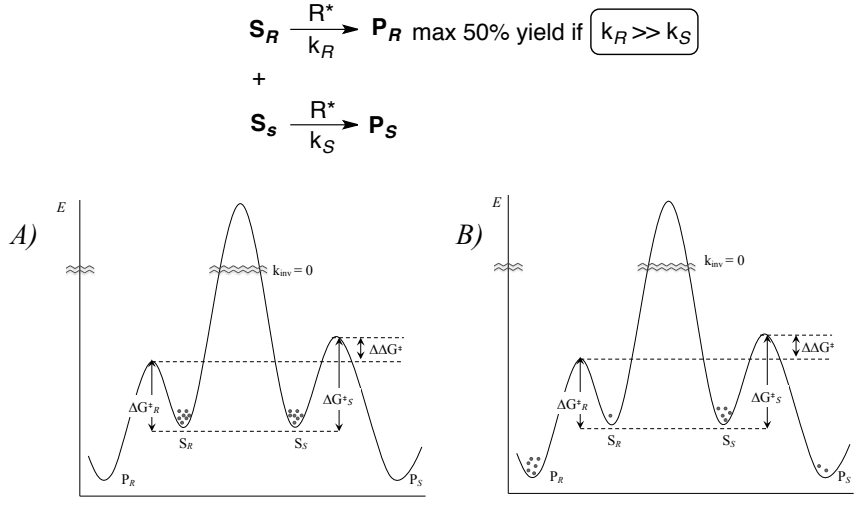


Figure 1. General reaction and energy diagrams of kinetic resolution. *A)* racemic mixture of substrates S_S and S_R, that are not interconvertable ($k_{inv} = 0$); *B)* in the presence of a chiral reagent/catalyst R*, S_R will transform into product P_R in higher rate compared to S_S ($k_R >> k_S$).

The KR method will be discussed more in chapter 2, in which a bis-epoxide is subjected to KR conditions towards the synthesis of bioactive analogues of acetogenins.

PKR is an interesting development of the KR method where two resolving agents are used to resolve enantiomers by performing two kinetic resolutions simultaneously. These resolving agents are chiral reagents or catalysts, often quasi-enantiomers – compounds relatively similar in their build-up and sometimes almost enantiomeric in their nature. The substrate S_S reacts faster with one

of the agents R_1*, and S_R reacts faster with the other agent R_2* (Figure 2), both enantiomers are converted into two different products.

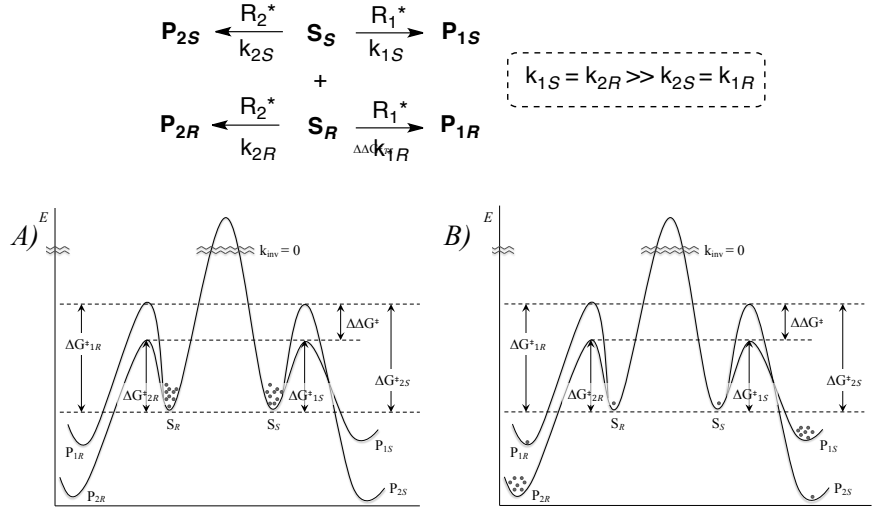


Figure 2. General reaction and energy diagrams of parallel kinetic resolution. *A)* Racemic mixture of substrates S_S and S_R, that are not interconvertable ($k_{inv} = 0$); *B)* S_S reacts faster with R_1* and forms P_{1S} ($k_{1S} > k_{2S}$), and S_R reacts faster with R_2* and forms P_{2R} ($k_{2R} > k_{1R}$).

In DKR a racemic mixture of enantiomers S_S and S_R that readily interconvert (racemize) to each other, are subjected to a chiral reagent or catalyst R* (Figure 3). The substrates react with the resolving agent at different rates, and if for the one enantiomer S_R the transformation rate to the product is considerably higher than for the other ($k_R > k_S$) and the inversion rate is $k_{inv} >> k_R$, product P_R can be afforded in up to 100% yield.

DKR will be discussed in chapter 3, where asymmetric transfer hydrogenation via DKR is shown in the synthesis of amino acid derivatives.

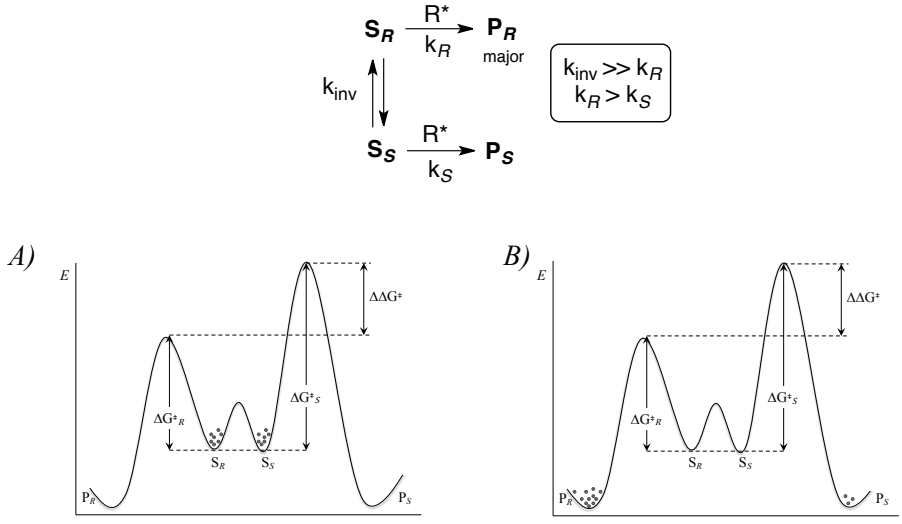
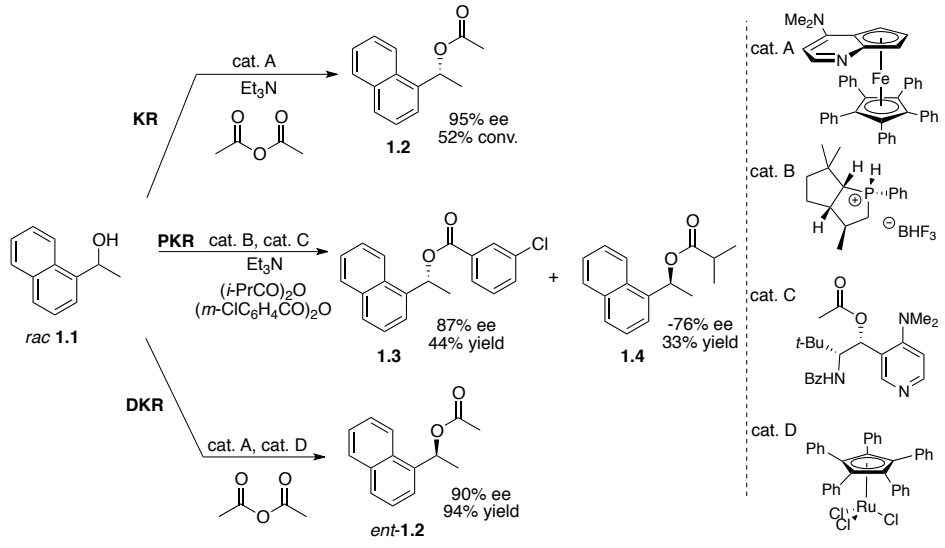


Figure 3. General reaction and energy diagrams of DKR. *A)* Racemic mixture of substrates S_S and S_R, that readily enantiomerize; *B)* S_R reacts with R* and forms P_R faster than S_S ($k_R > k_S$), while the substrates interconvert to each other ($k_{ent} >> k_R$).

A nice example to illustrate these resolution methods is of acylation of a racemic secondary alcohol **1.1** (Scheme 1). In 1998 Fu and co-workers resolved **1.1** by acylation via KR in the presence of a planar-chiral DMAP derivate catalyst (cat. A) and an achiral acyl donor. The product **1.2** was achieved in excellent enantiomeric selectivity with 95% *ee* and with 52% conversion.[7] More than a decade later the same substrate was subjected to PKR conditions by Duffey *et al*. in the presence of two chiral catalysts (phosphine cat. B and DMAP-derived cat. C) and two different achiral acyl donors.[8] Two products **1.3** and **1.4** were isolated along with some of the recovered starting material **1.1** with 8% *ee* (for ideal PKR this would be 0% *ee*). For the products enantio-selectivities 87% *ee* and 76% *ee* were determined after hydrolysis.



Scheme 1. Enantiomeric acylation of racemic alcohol **1.1** via KR, PKR and DKR.

Fu and co-workers continued their studies on resolutions of secondary alcohols and recently reported a DKR of **1.1**, where *ent*-**1.2** was afforded with a high yield of 94% and with a good enantioselectivity of 90% *ee*. They used the same acylation catalyst as earlier (cat. A) but in combination with a ruthenium complex (cat. D) as a racemization catalyst.[9] They successfully demonstrated this approach on a scope of aryl and alkyl carbinols, and showed it even to be superior to an enzymatic DKR of secondary alcohols in the case of substrates with branched substituents.

1.2. The aim of the thesis

There are two aims in this thesis.

Firstly, in chapter 2, which discusses the synthesis of analogues to natural compounds called acetogenins, the aim is to synthesize the analogues with high stereoselectivity and assess their bioactivity. A new approach of hydrolytic kinetic resolution of bis-epoxides is employed, where the afforded product has two remote stereocenters set with high enantiomeric- and diastereomeric selectivity.

In chapter 3, the work to find a general method for the asymmetric transfer hydrogenation of aryl and alkyl α-amido-β-keto esters via DKR is detailed, which would render the products *anti*-α-amido-β-hydroxy esters in high stereoselectivity and yields.

2. SYNTHESIS OF THIO- AND AZA-ANALOGUES OF ANNONACEOUS ACETOGENINS

(Papers I, II and III)

2.1. Introduction

In this work a stereoselective synthesis of six non-THF analogues of annonaceous acetogenins is reported, from which four are aza- and two thio-analogues (Figure 4). Their biological activity is assessed on a model tumorous cell line. The key steps, such as the synthesis of amino alcohols and the kinetic resolution of bis-epoxides are discussed.

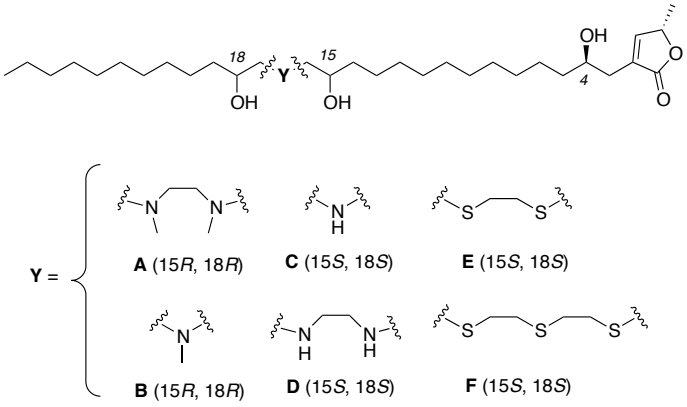
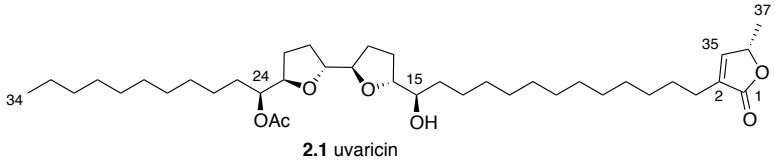


Figure 4. Aza-analogues **A–D** and thio-analogues **E** and **F** of acetogenins to be synthesized.

Annonaceous acetogenins belong to an intriguing class of natural bioactive compounds.[10] The most significant bioactivities include cytotoxicity, antibacterial,[11] antiproliferative,[12] insecticidal, antifungal,[13] immunosuppressive, and anti-angiogenesis[14] effects, among others reported. The acetogenins are found in a tree family *Annonaceae* from the order *Magnoliales*, which are native to mostly tropical and sub-tropical regions. Before the 1980s, investigations of the *Annonaceae* concerned other bioactive compounds found in the family, *e.g.*, isoquinoline alkaloids.[15] Since Jolad *et al.* isolated uvaricin (**2.1**, Figure 5) with anticancer activity from *Uvaria acuminata* in the 1980s,[16] the acetogenins have been under increasing interest from the scientific world. The selective cytotoxic activity against cancer cells has put them on their way towards being potential lead compounds for new anti-cancerous drugs.



2.1 uvaricin

Figure 5. Uvaricin was the first isolated acetogenin.

2.1.1. Structural characterization of annonaceous acetogenins

The typical acetogenin structure consists of a long unbranched aliphatic chain, centrally positioned one to three THF or THP rings,[17] adjacent hydroxyl groups and a terminal methyl-substituted α,β-unsaturated γ-lactone ring. There can be a variation in the stereochemistry and in the number and position of hydroxyl groups within the structure. The aliphatic chain may also contain double[18] or triple[19] bonds, and occasionally keto- or acetoxy-groups instead of hydroxyl groups. In Figure 6, a selection of acetogenins is shown to illustrate the structural variability within the family. Bullatacin (**2.2**) has two adjacent THF rings and is considered one of the most potent of the acetogenins. Annonacin (**2.3**) represents the type of acetogenin with only one THF ring and mucosin (**2.4**) a more rare variant, containing both THF and THP rings. The next three compounds **2.5–2.7** are proposed to be precursors in biosynthesis towards the final acetogenin structures and can possess lower bioactivity. To date, more than 400 different acetogenins have been identified – the structures of acetogenins can vary greatly even in an extract from a common source plant. For example, twenty structurally different acetogenins could be isolated from the seed extracts of *Annona squamosal*.[20] Also, the amount extracted depends on the region in the source plant (bark, seeds, roots or fruits) and on the season the plant samples are collected. To give an example on the laborious nature and relatively low productivity of extracting acetogenins from plant material, another research group was able to isolate 309 mg of a mixture of seven different acetogenins from 10 kg of seeds of *A. squamosa*.[21] In addition to the tedious isolation and identification of compounds being time and material consuming, some acetogenins in the mixture cannot be fully separated and remain as a mixture of isomers.

To overcome these obstacles and to have pure compound in the amounts needed for subsequent research, various synthetic routes have been developed for both mimics of natural acetogenins and their altered non-natural analogues. The latter are of great interest in elucidating the structure-activity relationship (SAR). The final consensus on SAR is yet to be established – with new approaches in studies, the world of acetogenins continues to unravel new discoveries.

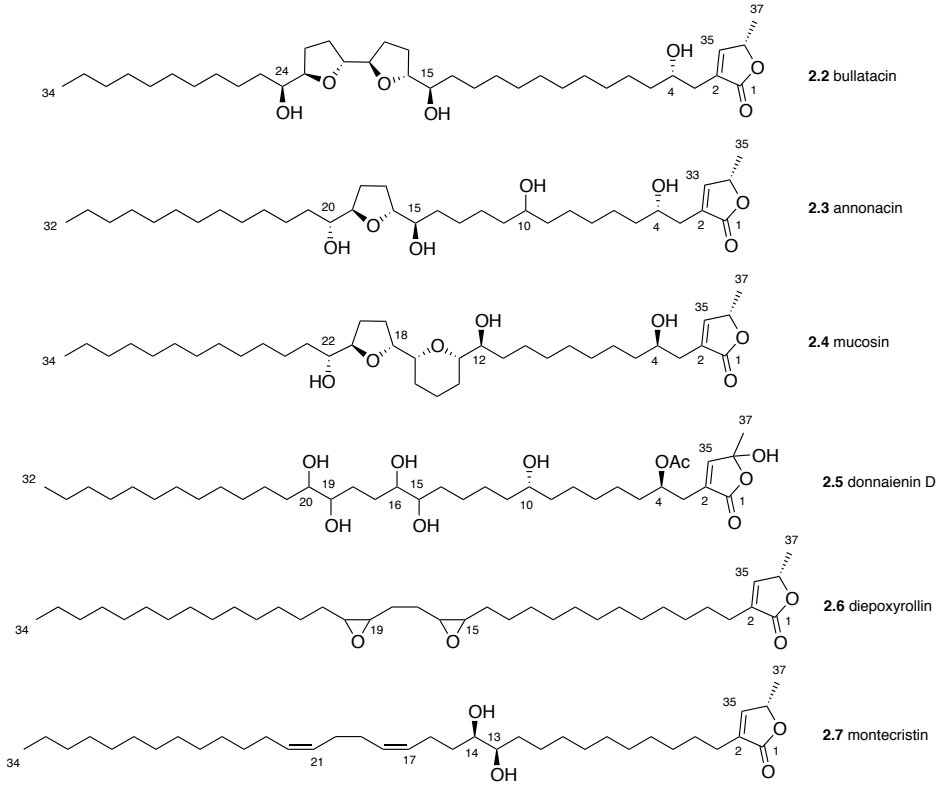


Figure 6. Illustration of the structural diversity in isolated natural acetogenins.

In the 1990s, McLaughlin and co-workers divided the isolated acetogenins into six general types (Figure 7, *a*). This tentative classification of acetogenins was based on the central fragment of the acetogenin compounds, and can branch out as many subtypes, according to differences in the remaining fragments in the structure, *e.g.*, γ-lactone ring (Figure 7, *b*). The ever-growing number and variability of structures synthesized contribute to the number of subtypes. The general classification of acetogenins according to structure would be then as follows:

1. Linear acetogenins
2. Epoxy-acetogenins
3. Mono-THF acetogenins
4. Bis-THF acetogenins
    4.1 non-adjacent bis-THF acetogenins
5. Tri-THF acetogenins
6. THP acetogenins
    6.1 THP and THF acetogenins
    6.2 non-adjacent THP-THF acetogenins

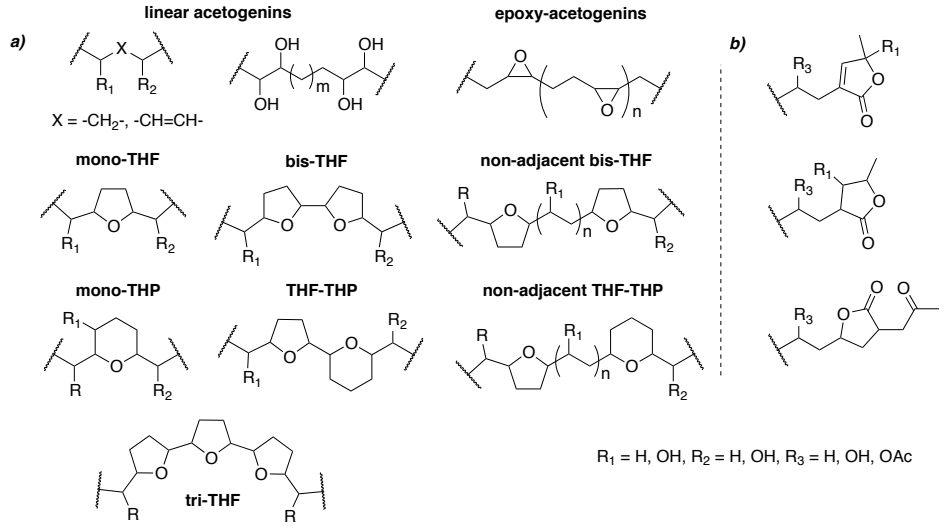


Figure 7. *a)* Central fragments of acetogenins, *b)* terminal lactone ring units.

Although the level of potency may vary depending on the cell line used for the studies, it has been shown that the adjacent bis-THF acetogenins are the most potent in the acetogenin family to date. They are followed by the non-adjacent bis-THF and then mono-THF acetogenins on a scale of decreasing potency. Therefore, a lot of attention has been focused on synthesizing THF-containing acetogenin structures to mimic the most potent acetogenins.

2.1.1.1. Unnatural analogues of non-THF acetogenins

In the search of the optimal structure for the target bioactivity, the synthesis of unnatural non-THF analogues has emerged. In these compounds heteroatom-containing fragments have substituted the THF units, eliminating at least two stereocenters (Figure 8). Compound **2.8** is a linear derivate of bullatacin, with missing ethyl bridges of THF rings, and **2.9** is a methylated amide unit bearing derivate, both with comparable bioactivities to each other.[22] Just recently the same research group reported the compound **2.10** with two lactone units.[23] This symmetric analogue was achieved by opening two equivalents of the racemic epoxide bearing the lactone ring with ethylene diamine. The *N,N'*-benzylated diamine analogue **2.11**[24] was first of its kind that incorporated an amine moiety into the alkyl chain. Unfortunately, no bioactivity was reported for the last two analogues.

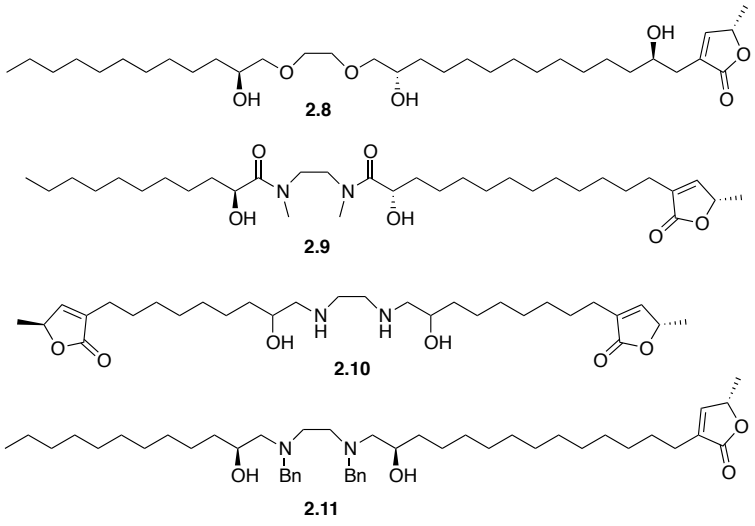


Figure 8. Selection of non-THF acetogenin analogues.

2.1.2. Bioactivity and related effects of acetogenins

The acetogenins are classified as one of the most potent NADH-ubiquinone oxidoreductase (Complex I)[25] inhibitors in mitochondrial electron transport system, as well as inhibitors of NADH oxidase of plasma membranes of cancer cells,[26] interrupting ATP synthesis. This, in turn, may lead to cell death by either apoptosis or necrosis. Although it is known that the target area for the acetogenins is the subunit ND1 in the complex I, the direct site of action is still under debate. There have been specifications in the models of binding to the active sites in the enzyme, trying to clarify the mode of action. In 1998, McLaughlin and co-workers were first to suggest a general model, which describes how the acetogenins, lateral to the complex I, diffuse into the membrane bilayer and adopt a certain conformation [Figure 9, *1)*].[27] They proposed that while the lipophilic long alkyl-chains are embedded in the membrane, the THF rings with flanking hydroxyl groups act as a hydrophilic 'anchor' in the membrane-plasma interface and the lactone ring alone interacts with the protein's active site. Any branching in the hydrocarbon chain was shown to cause a loss of activity.[28]

In the subsequent years, Miyoshi and colleagues brought forward several new approaches to the model, of which two are shown in Figure 9 [*2)* and *3)*].

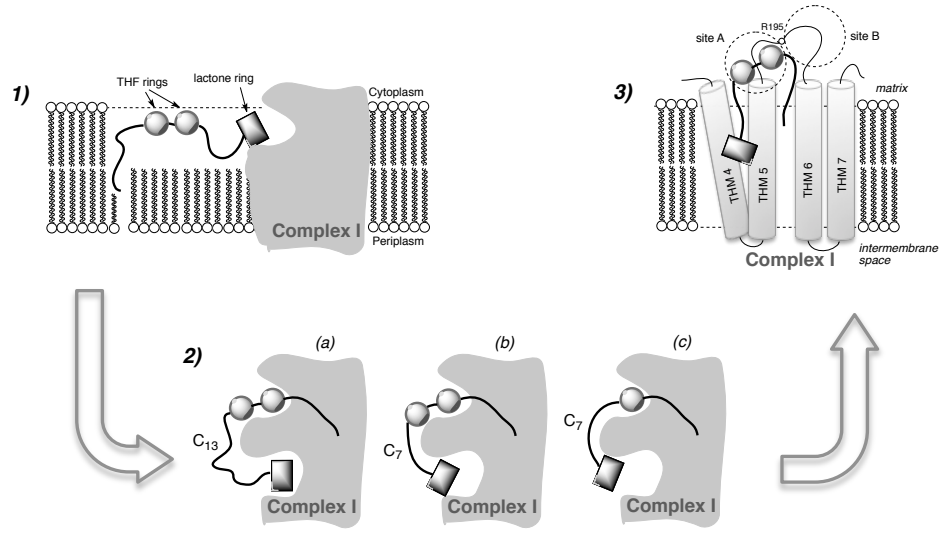


Figure 9. Evolution of the binding models of acetogenins to complex I: *1)* the first binding model by McLaughlin and co-workers in y. 1998; *2)* model showing two active sites and the relevance of the length of the linker, by Miyoshi and co-workers in y. 2008; *3)* model by Miyoshi in y. 2011 confirming the model with two active sites.

The authors made modifications to the acetogenin structure by changing the length of the alkyl linker and the number of THF rings, and proposed that there could be two binding sites in the enzyme, and that the acetogenin molecule has to occupy these simultaneously to exert inhibition on enzyme activity [Figure 9, *2)*].[29] This was confirmed a few years later by a study using a photoreactive acetogenin mimic, which showed that in the subunit ND1, there are two binding pockets [site A and B, Figure 9, *3)*] where the THF rings and the lactone ring bind.[30] Recently, a crystal structure of complex I from bacterium *Thermus thermophilus* was reported.[31] Thus, novel insights about binding of acetogenins in complex I sites may emerge.

The main focus in studying acetogenins has been their anti-cancerous effect. There is strong evidence of selectivity between normal tissue and tumorous ones. McLaughlin and co-workers have also reported the cytotoxicity of acetogenins against multidrug resistant (MDR) cells.[32] The action against MDR could be derived from the ability to lower ATP levels in the cells – the MDR cells often support ATP-fuelled plasma membrane glycoproteins, which export anti-cancerous compounds from the cell before they can execute their effect. But there is still much to be elucidated concerning the mode of action of the annonaceous acetogenins in cancer cells before any certain conclusions could be drawn.

Additionally, the acetogenins have been shown to form clusters with cations like Ca^{2+} and K^{+}, which disrupt the ionic balance in the cell.[33] This can also be linked to cell death[34] as elevated Ca^{2+} levels are reported to stimulate a cytotoxic cascade in many cell lines. It has been proposed that chelation of the acetogenins to Ca^{2+} ions may facilitate the penetration of the cell membrane or the mitochondrial membrane, and therefore may explain why some acetogenin analogues, based on their chelation ability, are more cytotoxic than others.[35]

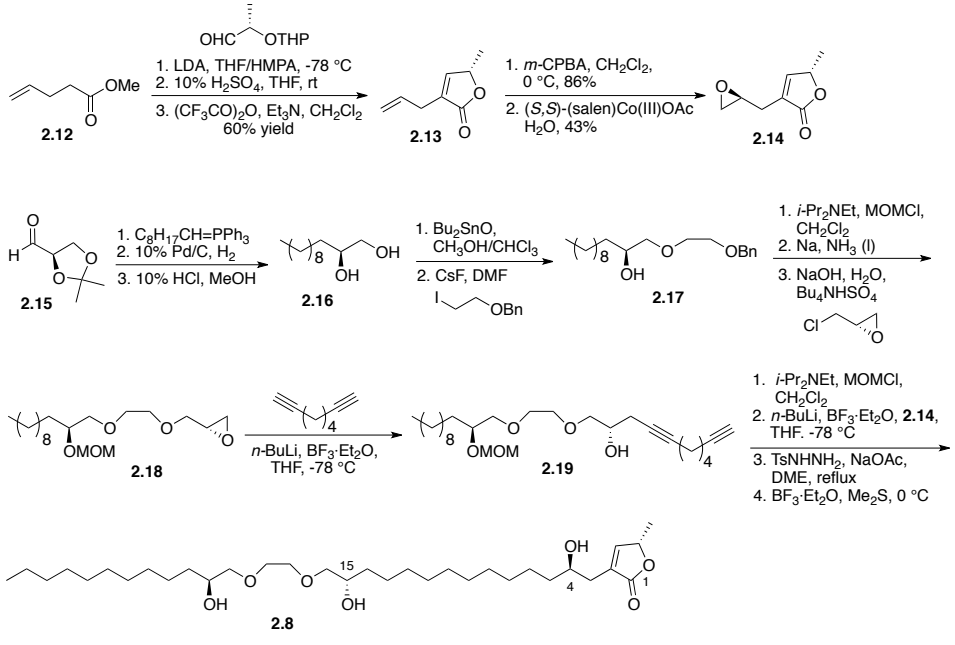
For centuries people, native to the habitat of the *Annonaceae* family, have been using these plants in their diet in various forms. Even nowadays, there are many products for internal and external use that contain annonaceous plant material in different concentrations and purity, and are claimed to have some curative potency.[36] More so, whole series of commercial products have been established on the remedial potency of annonaceous plants. Coupled with this long history of human consumption, the studies concerning the neurotoxicity of acetogenins, should be of high interest. There have been reports claiming that the acetogenins cross the blood-brain barrier and cause atypical parkinsonism.[37,38,39] This is an aspect worth examining, especially if the acetogenins are to develop into marketable drug candidates. Champy *et al.* showed a similar pattern of neurodegeneration on treated mice.[40] The mice were subjected to doses of an acetogenin called annonacin over a period of time and the observed effect was similar to the atypical parkinsonism, which is claimed to have been caused by consuming annonaceous plants. On the other hand, these plants also contain other bioactive compounds, like isoquinolic alkaloids, that can also impart neural damage. Thus, a combinatory effect of multiple toxins might be responsible for these reported effects, rather than the acetogenins alone.

2.1.3. Synthetic examples of acetogenin analogues

The interest in synthesis of acetogenins and their analogues has followed an explosive trend in the last two decades.[41] As the acetogenins have multiple stereogenic centers, the introduction of chirality into the compounds is often the key step. The first total synthesis was reported by Hoye *et al*[42] in 1991 for a stereoisomer of uvaricin (**2.1**), the acetogenin that was also the first isolated. In their synthetic route the four stereogenic centers in the central region were introduced by the chiral starting material L-(+)-diethyl tartrate, and D-(-)-diisopropyl tartrate catalyzed Sharpless asymmetric olefin epoxidation. In the 1990s, synthesized acetogenins were mostly mimics of isolated acetogenins and made often for the purpose of elucidating the stereochemistry of the parent compounds. From this high diversity of known acetogenin structures many theories concerning bioactivity have risen. For now, chemical synthesis has proven more effective in producing either mimics of the natural compounds or new unnatural analogues for the study towards drug lead compounds.

Since our synthetic targets in the current work are unnatural non-THF analogues, a handful of representative examples of the synthesis of non-THF analogues are highlighted.

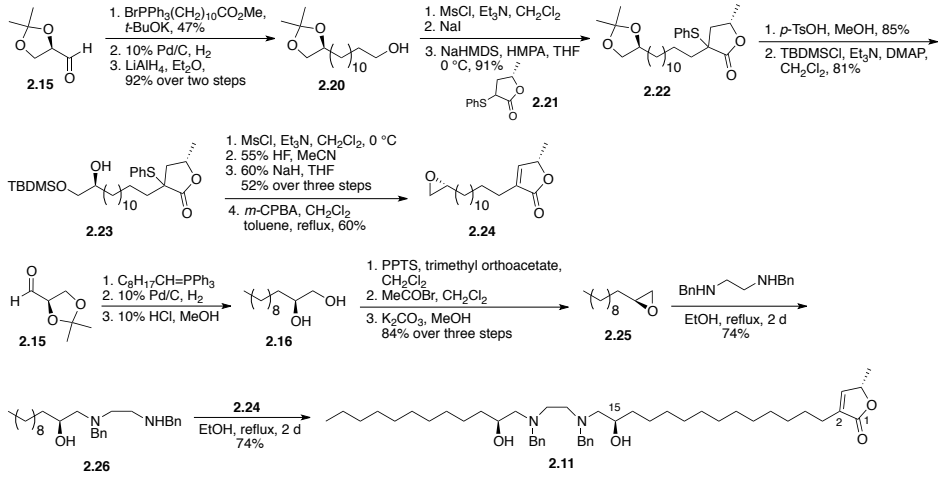
Yao and Wu with co-workers described a series of acetogenin analogues containing ethylene glycol units, where the ethylene bridges in the THF rings have been removed when compared with the bis-THF acetogenins.[43] The most bioactive analogue of the series proved to be **2.8** (Scheme 2), furnished with a hydroxyl group in position 4 like in many potent acetogenins (*e.g.,* bullatacin **2.2**).[44] One of the key fragments **2.14** was afforded after a three-step sequence starting from methyl pent-4-enoate **2.12** and (*S*)-*O*-tetrahydropyranyl lactal and a subsequent oxidation of **2.13** with *m*-CPBA, followed by Jacobsen's hydrolytic kinetic resolution of the formed racemic epoxide. Then aldehyde **2.15** was subjected to Wittig reaction, and after hydrogenation and deprotection, diol **2.16** was obtained. Diol **2.16** was regioselectively *O*-alkylated with 2-benzyloxyethyl iodide to afford compound **2.17**. After protecting the secondary alcohol as a MOM ether, the terminal benzyl-protecting group was removed by reductive cleavage. Compound **2.18** was obtained after coupling reaction with (*R*)-epichlorohydrin. Then lithium 1,7-octadiyne was treated with **2.18** in the presence of $BF_3{\cdot}Et_2O$ to afford **2.19**, which was then subsequentially lithiated and *C*-alkylated with epoxide **2.14**. The triple bonds in the product were reduced by diimide and after deprotection, end-product **2.8** was obtained.



Scheme 2. Synthesis of bis-ether analogue **2.8**.

A second example on synthesis is for *N,N'*-benzylated diamine compound **2.11** (Scheme 3).[24] This compound was first of the linear aza-analogues reported. The synthesis started with the same aldehyde **2.15** as in the previous example.

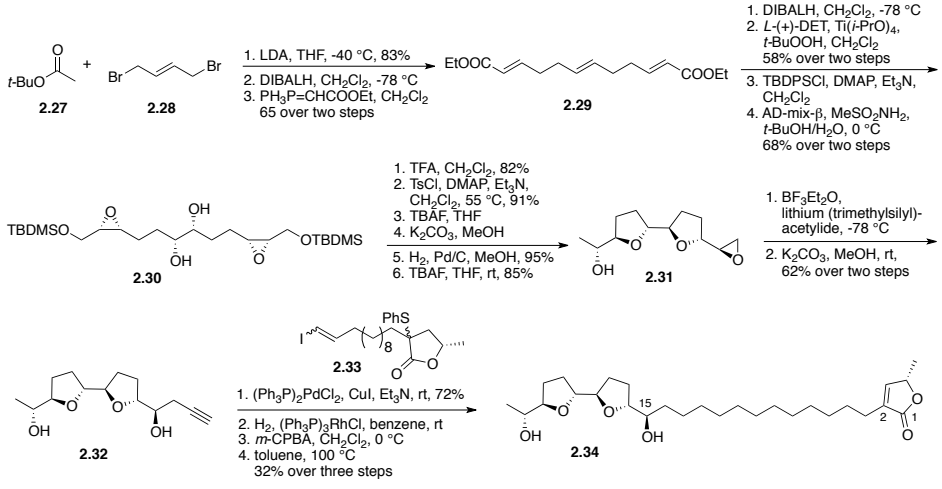
After elongation of aldehyde **2.15** with a Wittig reaction, the afforded **2.20** was used for alkylating the lactone ring **2.21** over an iodide intermediate to yield **2.22**. Then, over six steps, the diol moiety was deprotected and converted into an epoxide **2.24** over compound **2.23**. The synthesis of the amino alcohol intermediate **2.26** was also started from the aldehyde **2.15**. After Wittig reaction, hydrogenation and a subsequent deprotection, conducted similarly to the previous example,[44] diol **2.16** was afforded. Diol **2.16** was then converted into epoxide **2.25** that was subsequently opened by a *N,N'*-benzylated ethylenediamine, obtaining **2.26**. The compound **2.26** was then coupled with epoxide **2.24** to yield the end-product **2.11**. Unfortunately, the authors did not discuss debenzylation or the possible bioactivity of the synthesized analogue.



Scheme 3. Synthetic scheme for *N,N'*-benzylated analogue **2.11**.

A third example is from Miyoshi and co-workers, who mainly focused on bis-THF analogues. An interesting example of their synthesis schemes is the analogue **2.34** with a shortened alkyl 'tail' (Scheme 4).[45] The synthesis of intermediate **2.29** started by reacting *t*-butyl acetate **2.27** with *trans*-1,4-dibromo-2-butene (**2.28**) by employing a strategy developed by Hoye and Ye.[46] This was followed by a reduction with DIBALH and an extension of the formed dialdehyde by Witting olefination. After a reduction of **2.29** with DIBALH, double Sharpless asymmetric epoxidation ensued. This was followed by silylation of the hydroxyl groups and a Sharpless asymmetric dihydroxylation of the remaining double bond, yielding compound **2.30**. The THF rings were formed via a ''inside-out'' epoxide cascade reaction by treating **2.30** with trifluoroacetic acid. The remaining secondary hydroxyl groups were tosylated and epoxide was formed after treatment with TBAF and K_2CO_3. The formed epoxide was then opened by catalytic hydrogenation with Pd/C and after

another conversion to an epoxide in the presence of excess TBAF, **2.31** was obtained. The epoxide in **2.31** was opened by lithium (trimethylsilyl)acetylide, affording **2.32** after desilylation. The compound **2.32** was coupled with vinyl iodide **2.33**, catalyzed by Pd(0). The iodide **2.33** was prepared by a previously reported method.[42] After hydrogenation using a Wilkinson's catalyst and a thermal elimination of the sulphide moiety, the end-product **2.34** was obtained in a moderate yield.



Scheme 4. Synthesis of bullatacin analogue with a shortened aliphatic chain.

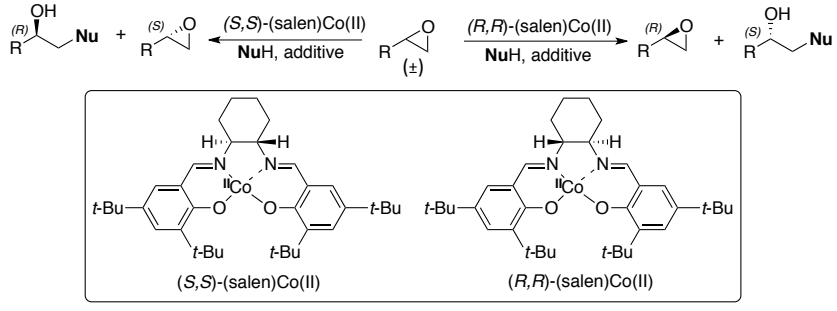
The analogue **2.34** exhibited only somewhat lower bioactivity compared to bullatacin, suggesting that the long alkyl tail might not be essentially important for the activity.

2.1.4. Introduction of remote stereocenters

The acetogenin structures often bear a pair of remote stereocenters, separated by 11 bonds or more. Although efficient methodology for remote asymmetric induction has been reported for certain compounds,[47] synthesizing a flexible acyclic compound bearing stereocenters more than four or five bond lengths apart in high stereoselectivity is still challenging.[48] Thus, in most of the synthetic routes of acetogenins, the critical fragments with remote stereocenters have been assembled from smaller chiral building blocks instead of preparing them as one unit. We sought an approach that would allow the introduction of more than one stereocenter in one step with high enantioselectivity, which shortens synthetic routes and would be cost efficient.

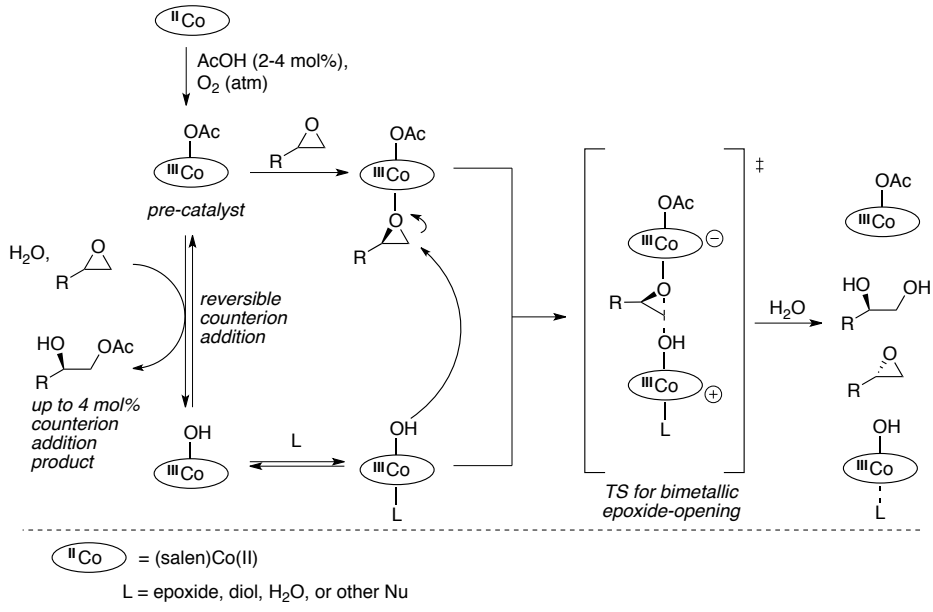
2.1.4.1. Kinetic resolution of terminal mono- and bis-epoxides

After some exploration, we turned to Jacobsen's kinetic resolution (KR) of epoxides that according to literature provides excellent stereoselectivity.[49] The hydrolytic kinetic resolution (HKR) of terminal mono-epoxides is a well-established method originally introduced by Jacobsen and co-workers (Scheme 5).[50] In the presence of an activated chiral (salen)Co(III) catalyst[51] and water, one enantiomer of the racemic epoxide is opened to afford the enantiomerically enriched diol and the unreacted epoxide. Many research groups have contributed to this method and a wide substrate array has been created. Also nucleophiles other than water have been shown to give high enantioselectivities and high yields in the kinetic resolution[52] and consequently, many synthetic routes of bioactive compounds have been utilizing this method.[53]



Scheme 5. Kinetic resolution of terminal epoxides.

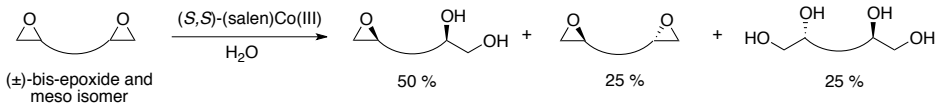
Despite of the different nucleophiles working well under HKR conditions, water has remained the most popular one, being environmentally benign, cost-effective and safe. Also, the resultant hydrolysis products are highly useful in the syntheses of more complex compounds. Both (*R,R*)-(salen)Co(II) and (*S,S*)-(salen)Co(II) are commercially available, making the synthesis of opposite enantiomers easily executable.



Scheme 6. Mechanism proposed by Jacobsen for HKR of terminal epoxides.[54]

Jacobsen's mechanistic investigations suggest a co-operative action between two (salen)Co(III) catalysts in the rate determining step (Scheme 6).[54] Firstly, the (salen)Co(II) complex is activated in the presence of air with a mild Lewis acid, *e.g.*, AcOH, to form a pre-catalyst (salen)Co(III)OAc. The HKR reaction is promoted by this complex, which has a Lewis acidic character, by binding to the epoxide. A second (salen)Co(III)OAc complex is hydrolyzed to (salen)Co(III)OH, which acts as a nucleophile delivering agent to the epoxide bound to (salen)Co(III)OAc. Negligible amount of counterion addition product can form (up to 4 mol%) when complex (salen)Co(III)OAc acts as a nucleophile instead of (salen)Co(III)OH. The epoxide is bound to the complex with increased Lewis acidic character, which depends on the counterion (here OAc). A transition state (TS) for bimetallic epoxide opening is shown in Figure 15. The hydrolysis step is relatively fast and the racemic epoxide is resolved into enantiomerically pure diol and epoxide.

Interestingly, reports of the use of bis-epoxides as KR substrates are relatively rare. Jacobsen and co-workers were one of the first to report a HKR of a racemic bis-epoxide, but lacking a meso isomer.[49*d*] The meso isomer, which allows easier asymmetric bidirectional derivatization after KR, was investigated by Kitching and co-workers in the synthesis of insect sex pheromones,[55] but unfortunately without reporting the assessment of stereoselectivity, which was presumably assumed to correlate with the literature data.

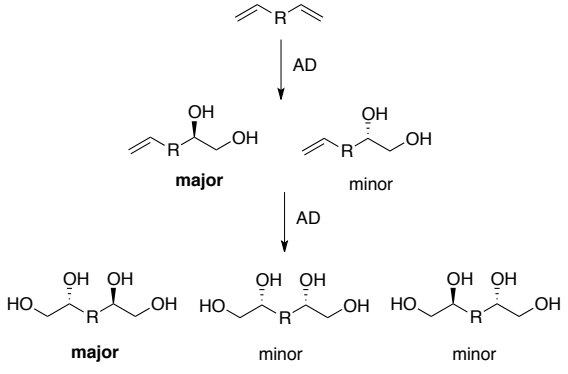


Scheme 7. HKR of bis-epoxides.

The substrate bis-epoxide (Scheme 7) consist of three isomers: 50% of (*R,S*)-meso isomer, 25% of (*S,S*)-isomer and 25% of (*R,R*)-isomer. After subjecting this isomeric mixture of the bis-epoxide to HKR conditions in the presence of the (*S,S*)-(salen)Co(III) catalyst, a statistical mixture of products is afforded – epoxy diol up to 50% theoretical yield, 25% of unreacted (*S,S*)-bis-epoxide and 25% of (*R,R*)-tetrol.

2.1.4.2. Sharpless asymmetric dihydroxylation of olefins

Sharpless asymmetric dihydroxylation (AD) of olefins[56] is also a well-known method and has often been employed in the synthesis of acetogenin analogues. Symmetrical dienes can be submitted to double AD (Scheme 8), which yields highly enantiomerically enriched tetrols for further derivatization.[57] For subsequent asymmetric bidirectional derivatizations, the second AD can be postponed to further along the synthesis route. AD, as well as KR of bis-epoxides, can be considered as establishing remote stereogenic centers with a set conformation, by synthesis that is independent of existing stereogenic centers, *i.e.* catalyst control.



Scheme 8. Double-AD of dienes.

2.1.5. Thio-compounds as bioactive molecules

Thio-moieties have not been reported in natural acetogenins. But in many other natural bioactive compounds sulfur atoms are often integrated in the structural framework.[58] For example, cyanobacterial compound largazole, which contains a thioester fragment in its structure, shows enhanced biological activity against selected cancerous cell-lines.[59]

Although, the mode of binding of the acetogenins to the active site in complex I has yet to be elucidated, the importance of hydrogen bonding in the enzymic site has been agreed upon. Recently, it has been confirmed that sulfur can also act as a considerable hydrogen bond donor.[60] It was shown that the divalent sulfur in dimethyl sulfide could form a H-bond, which, although weaker, is still comparable to H-bond with oxygen in dimethyl ether. Thus, we were intrigued to compile novel analogues of acetogenins where thio moieties are embedded into the aliphatic chain, but are otherwise structurally reminiscent of the aza-analogues. With their somewhat reduced ability to form H-bonds, the importance of the central region and the associated H-bonding could be assessed.

2.2. Results and discussion

The ethylene glycol analogue **2.8** with its retained inhibition ability and the *N,N'*-benzylated diamine analogue **2.11** inspired us to plan a series of acetogenin analogues and assess their bioactivities. We envisioned a highly stereoselective convergent synthetic route, which could be easily applicable for all analogues planned. During the course of the work, four aza-analogues **A**–**D** (Figure 10) with a hydroxyl group at position C-4 where synthesized. In addition, two thio-analogues **E** and **F** were also prepared and furthermore, the cytotoxicities of **E** and **F** were compared to the aza-analogues **A**–**D**.

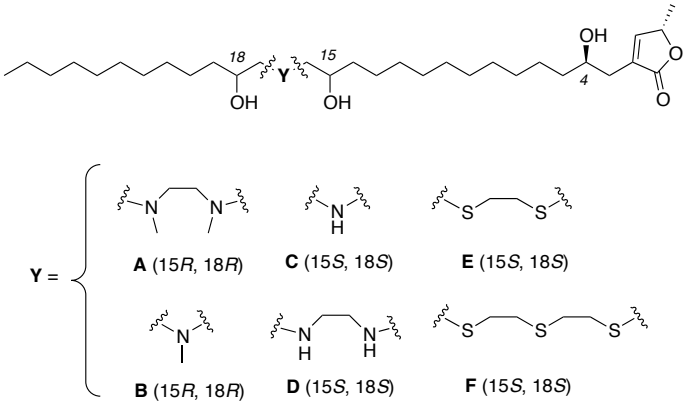
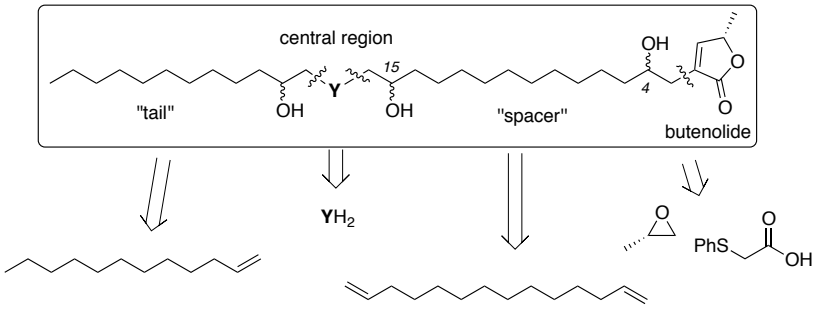


Figure 10. Aza analogues **A**–**D** and thio analogues **E** and **F** of acetogenins to be synthesized.

We began our investigations with the aza-analogues. The general retrosynthetic scheme is shown in Scheme 9. One of the key elements in the synthesis route was the installation of the relevant stereochemistry. The compilation of the structure was planned according to a convergent, modular synthetic scheme. The four modules, *i)* twelve carbons long alkyl chain termed as the ‘‘tail’’, *ii)* central region, with changeable amine fragment (or thio fragment, shown here as Y), *iii)* ‘‘spacer’’, which joins the central and the terminal region, and *iv)* terminal butenolide moiety, were planned to be synthesized separately and joined together at different stages of the route. The planned analogues contained four stereocenters – one in the ‘‘tail’’, two in the ‘‘spacer’’ region and one in the butenolide. If we were to follow through with the modular synthesis plan, a method was needed to introduce two remote stereocenters ten carbon atoms apart in the ‘‘spacer’’ region. Usually this is overcome by coupling of the two chiral fragments, or using asymmetric induction.[61] As discussed above, establishing two stereocenters that are more than three bonds apart can be challenging, let alone in one step. We considered various approaches to introduce stereochemistry, and two methods were chosen as the most suitable: Jacobsen’s hydrolytic kinetic resolution (HKR) of terminal epoxides and Sharpless asymmetric dihydroxylation (AD) of olefins. The benefits of these approaches were addressed above.

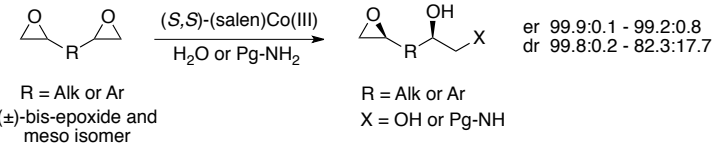


Scheme 9. General retrosynthetic plan for the analogues to be synthesized.
Y = aza- or thio-fragment.

2.2.1. Hydrolytic kinetic resolution of bis-epoxides

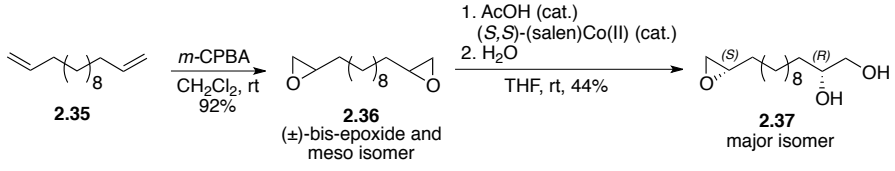
HKR of terminal bis-epoxides was employed to introduce the two remote stereocenters in the ‘’spacer’’ region. KR of bis-epoxides was investigated more closely in the Paper II. A scope of aryl and alkyl substrates was prepared and subjected to HKR and aminolytic kinetic resolution conditions (Scheme 10). The relative and absolute stereochemistry was determined by chiral HPLC and by the Mosher ester method, respectively. Excellent enantiomeric ratios were obtained for the afforded epoxy diols and epoxy amino alcohols – for most

products the er was 99.9:0.1 and only for one substrate a marginally lower er value of 99.2:0.8 was obtained.



Scheme 10. Scope of HKR of bis-epoxides.

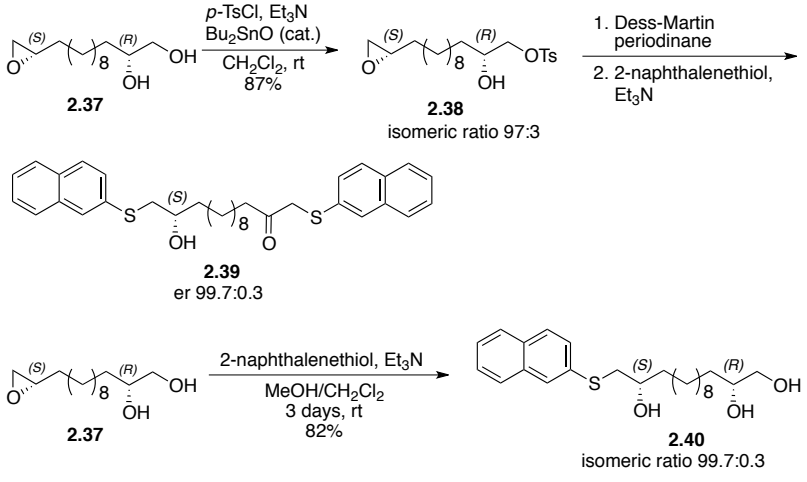
The bis-epoxide **2.36** used in the synthetic route was obtained after oxidation of diene **2.35**, and consists of three isomers: 50% of the meso isomer (*R,S*), 25% of (*S,S*)-isomer and 25% of (*R,R*)-isomer (Scheme 11). This isomeric mixture of the bis-epoxide was then subjected to HKR in the presence of the (*S,S*)-(salen)Co(III) catalyst with 1 equiv. of water as the nucleophile. Our target product was the epoxy-diol **2.37**, arising from the meso stereoisomer. The (*R,R*)-bis-epoxide is opened into a tetrol and (*S,S*)-bis-epoxide remains un-affected in the reaction. These products were easily separated on column chromatography after HKR. Various approaches were considered in order to find the best way to assess the relative stereochemistry of the epoxy-diol **2.37**. Since the two stereocenters in the epoxy-diol are separated by 11 bonds, the NMR spectra of **2.37** would be indistinguishable from possible diastereomers. Thus in the hope of assessing both enantiomeric and diastereomeric selectivity for **2.37**, HPLC analysis on a chiral column was chosen. The resolution of the remote chiral centers can also pose a problem on chiral columns. Thus, UV-active derivatives were made and compared with their enantiomers, which were achieved following identical synthetic routes, only in the presence of the HKR catalyst with an opposite stereochemistry.



Scheme 11. Synthesis of epoxy-diol **2.37** via HKR of bis-epoxide **2.36**.

Fortunately, converting the epoxy diol **2.37** into the tosylate derivative **2.38** was already included as a step in the synthesis route of the analogues. As it conveniently bears a UV-active group next to one of the chiral centers, it was analyzed on chiral HPLC affording an isomeric ratio of 97:3 by comparing it

with its enantiomer (Scheme 12). The ratio could not be interpreted as er, because we could not be sure that the chiral center incorporated in the epoxide was discriminated on the column. Thus, to have a clear resolution of that chiral center, we oxidized the secondary alcohol in tosylate **2.38** into a ketone and opened the epoxide with naphthalene thiol, a second UV derivatizing reagent, to afford **2.39**. After comparing **2.39** to its enantiomer, a gratifyingly high er 99.7:0.3 was afforded. If assuming that only the chiral center *C*-OH in **2.38** was discriminated on the chiral column, and not the one incorporated in the epoxide, the 3% of the isomer detected must have had (*S*)-orientation. The analysis of **2.39** showed negligible amount of an enantiomer, which indicates that the chiral center in the epoxide moiety in **2.37** also has (*S*)-configuration. Following this reasoning, the ratio 97:3 had to then show a presence of a diastereomer to **2.38** derived from (*S,S*)-bis-epoxide and according to the er 99.7:0.3, negligible amount of enantiomer to the epoxy-diol **2.37**. The presence of the (*R,R*)-diastereomer was reasoned to be less than 0.3%, which was indicated by the HPLC analysis of **2.39**.

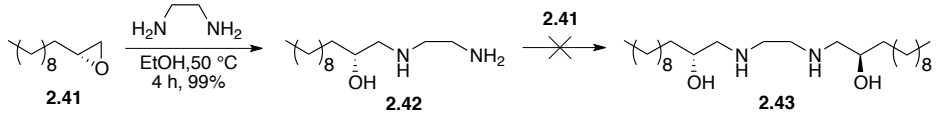


Scheme 12. Determining er and dr of epoxy diol **2.37** via derivatization.

A second approach was to open the epoxy-diol **2.37** directly with 2-naphthalenethiol (Scheme 12). A ratio 99.7:0.3 was achieved for **2.40** after HPLC analysis, which matches the er for **2.39**.

2.2.2. Synthesis of amino-alcohols

We then focused on the epoxide opening reaction with various amine-moieties as nucleophiles. The epoxide **2.41** (Scheme 13) was smoothly opened by a 1,2-ethylenediamine fragment but attempts to use the afforded amino alcohol **2.42** as a nucleophile in a second epoxide opening to obtain **2.43** proved to be inefficient under the same reaction conditions.



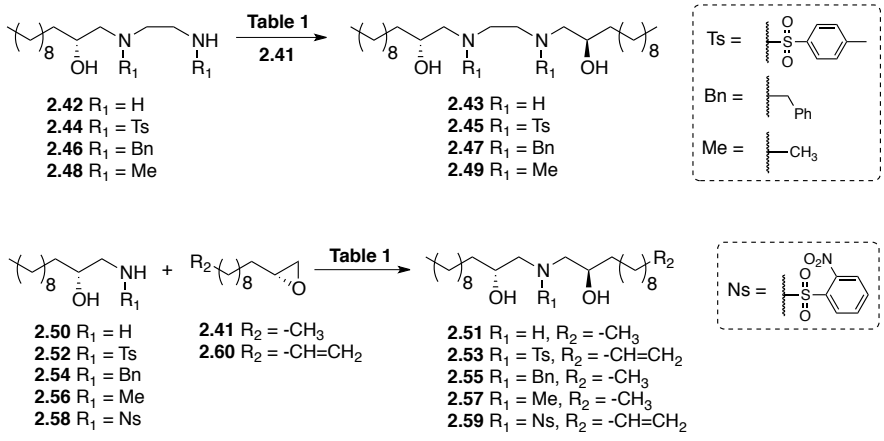
Scheme 13. Synthesis of amino alcohol **2.42** and an attempt towards symmetric product **2.43**.

The transformation was then investigated more closely. We reasoned that, either the amine moiety with the long alkyl chain was not nucleophilic enough, or the hydrophilic amino alcohol moiety with lipophilic chain contributes to the formation of micelles, thus obstructing the reaction from proceeding. In order to enhance the reactivity of the amine moiety, different *N*-protecting groups (*N*-Pg) were studied. For this tosyl (Ts), benzyl (Bn), methyl (Me) and nosyl (Ns) groups were chosen. Also, different reaction conditions were explored, including phase transfer catalysis.[62] At first, the epoxide **2.41** was opened with either monoamine or ethylene diamine moiety, furnished with appropriate *N*-Pgs, to afford amino alcohols **2.44**, **2.46**, **2.48**, additionally to aforementioned **2.42**, and **2.50**, **2.52**, **2.54**, **2.56**, and **2.58**, respectively (see Paper III and Experimental section 2.4 in the thesis for details). Then the formed amino alcohols were used as nucleophiles in the second epoxide opening reaction with **2.41** or **2.60** (Table 1).

For the unprotected amino alcohol **2.42**, the acidic conditions gave no detectable changes in the reaction (Table 1, entry 1). The presence of Lewis acid $CoCl_2$ or $FeCl_3$ in MeCN (entry 2) gave only traces of the product **2.43**. Also refluxing monoamine alcohol **2.50** with epoxide **2.41** in *i*-PrOH up to a week (entry 6) showed only traces of the desired product **2.51**. On the other hand, in the presence of Lewis acids (metal chlorides and triflates)[63] in either CH_2Cl_2 or *i*-PrOH gave *ca* 30% of **2.43** after 2–3 days at room temperature (entry 3). Warming the reaction to 40 °C almost doubled the yield (entry 4). Under microwave conditions 76% yield was achieved by irradiating the reaction at 110 °C for 30 minutes (entry 5).[64] The tosyl-protected amino alcohol **2.44** was converted into the product **2.45** with 80% yield by refluxing in *i*-PrOH for 6 days in the presence of K_2CO_3 (entry 10). Other bases, like NaOMe and NaH yielded no detectable product (entries 7 and 8). Refluxing the tosyl-amino alcohol in *i*-PrOH in the absence of additives, did not afford the product (entry 11) but interestingly, the benzyl-protected amino alcohols in the same

conditions afforded the desired products **2.47** in 74% and **2.55** in 87% yield (entries 12 and 13).

Table 1. Conditions for aminolysis of epoxides.



Nr	R_1	amino alcohol[a]	epoxide	product	Reaction conditions				yield
					addative	solvent	temp.[b]	time	
1	H	**2.42**	**2.41**	**2.43**	Triflic acid	MeCN	0 °C	24 h	ncd[c]
2					Lewis acid[d]	MeCN	82 °C	24 h	traces
3					Lewis acid[e]	CH_2Cl_2 or *i*-PrOH	rt	2–3 days	*ca* 30%
4					$Yb(OTf)_3$	CH_2Cl_2	40 °C	2 days	57 %
5					$Bi(OTf)_3$	CH_2Cl_2	110 °C[f]	30 min	76%
6		**2.50**		**2.51**	-	*i*-PrOH,	82 °C	7 days	traces
7	Ts	**2.44**	**2.41**	**2.45**	NaOMe	MeOH	rt	2 days	ncd[c]
8					NaH	benzene	80 °C	24 h	ncd[c]
9					K_2CO_3, TBAHS	DMF	100 °C	7 days	52%
10					K_2CO_3	*i*-PrOH,	82 °C	6 days	80%
11		**2.52**	**2.60**	**2.53**	-	*i*-PrOH,	82 °C	4 days	ncd[c]
12	Bn	**2.46**	**2.41**	**2.47**	-	*i*-PrOH	82 °C	24 h	74%
13		**2.54**	**2.60**	**2.55**	-	*i*-PrOH	82 °C	48 h	87%
14	Me	**2.48**	**2.41**	**2.49**	-	*i*-PrOH	82 °C	6 days	<99%
15		**2.56**		**2.57**	-	*i*-PrOH,	82 °C	3 days	96%
16	Ns	**2.58**	**2.60**	**2.59**	K_2CO_3 TBAH	dioxane	90 °C	10 days	54%

[a] For the formation of protected amino alcohols see Paper III and Experimental section 2.4 in the thesis.
[b] The reactions were run in reflux, except for entries 1, 3, 4, 5, and 7.
[c] ncd – no conversion detected
[d] Lewis acids $CoCl_2$ and $FeCl_3$ were tested.
[e] Lewis acids: $Sc(OTf)_3$, $La(OTf)_3$, $Yb(OTf)_3$, $Bi(OTf)_3$, $ZrCl_4$, $ZnCl_2$, $InCl_3$.
[f] The reaction was run under microwave conditions.

The best performing nucleophiles were the methyl-protected amino alcohols **2.48** and **2.56**, with isolated yields <99% and 96% for the formed products **2.49** and **2.57**, respectively (entries 14 and 15). The nosyl-protected amino alcohol **2.58** gave 54% of the desired product **2.59**, after refluxing 10 days in dioxane under phase transfer conditions (entry 16).

2.2.2.1. Deprotection strategies for *N*-protected amino alcohols.

In order to incorporate the protected amino alcohol moiety into the synthesis route, a suitable deprotecting strategy was needed, which would not affect other structural units in the compound, *e.g*., the lactone ring. Thus, different de-protections of *N*-Ts, *N*-Bn and *N*-Ns were explored.

For detosylation three methods were explored: *(1)* deprotection in the presence of sodium naphthalene anion radical;[65] *(2)* sonication in the presence of *N*-arenesulfonylcarbamate and magnesium turnings; [66,67] and *(3)* deprotection in the presence of samarium diiodide, triethyl amine and water, a mild reducing environment for deprotecting aryl sulfonate esters and amides,[68] which could also tolerate the presence of a α,β-unsaturated lactone ring.[69] Although with test-substrates like **2.45** (Table 1) the deprotection was observed, in the presence of the lactone moiety a mixture of products was obtained, with either the lactone decomposed or the thiosulfonyl group cleaved.

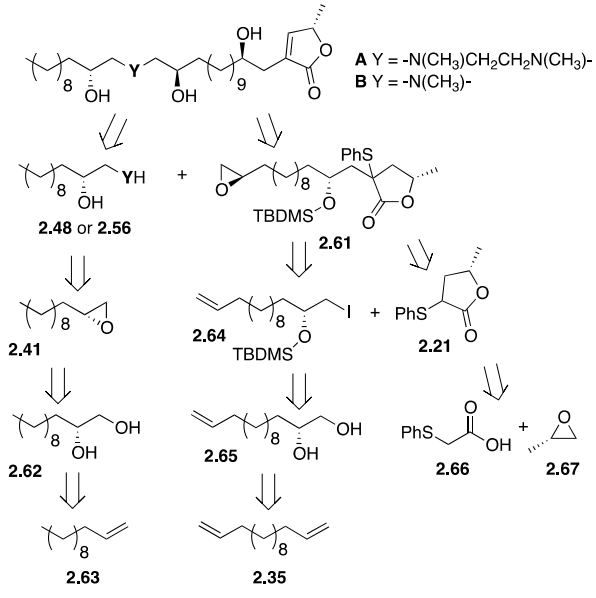
For the debenzylation two methods were tested, *(1)* oxidation with cerium (IV) ammonium nitrate (CAN), [70] and *(2)* hydrogenolysis with H_2 and palladium catalyst on carbon, reported both for alcohols[71] and amines.[72]. The deben-zylation by CAN did not yield the desired product, even when the equivalence of CAN was increased from 1 to 2.5. The hydrogenolysis with H_2 catalyzed by Pd occurred with high yield for the test-substrate, but when phenylsulfide lactone ring was also present, the catalyst was poisoned[73] and no de-benzylation occurred.

For denosylation a method via Meisenheimer complex, employing a thio-compound and base was used.[74] Thio-compounds, like β-mercaptoethanol and 1-decanethiol were tested in the presence of bases K_2CO_3 and DBU. The complete deprotection, indicated by ^{1}H NMR analysis, occurred with 1-decanethiol and DBU. When α,β-unsaturated lactone ring was introduced to the conditions, no expected product was afforded. The reason could have been because of a Michael addition of the 1-decanethiol to the double bond.[75] Thus, the denosylation reaction should be conducted before formation of the butenolide moiety in the structure.

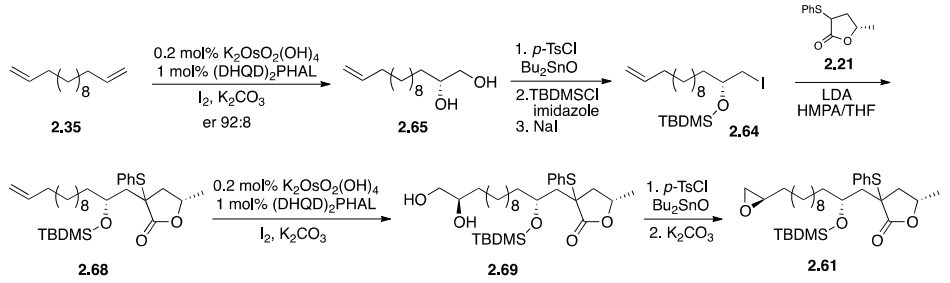
Since no suitable deprotecting methods were found for the *N*-Ts and *N*-Bn groups, the third best protecting group, *N*-Ns, for which also a good deprotecting method was worked out, was incorporated in the synthetic route for the aza-analogue with the secondary amine moiety. Also two methyl-protected aza-analogues were to be synthesized.

2.2.3. Synthesis of aza-analogues A and B via AD.

We started our synthesis with the methylated aza-analogues **A** and **B** that required no extra focus on *N*-deprotection strategies. We envisioned using the Sharpless' AD method in order to introduce three stereocenters in the analogue. The stereocenters would be inserted one at a time and the stereochemistry would depend on which of the two chiral ligands was chosen. According to the convergent synthesis route devised, there are two main intermediates, which are coupled by an epoxide opening in the last stages of the route (Scheme 14). These intermediates are termed a left-hand fragments (here amino alcohols **2.48** or **2.56**) and a right-hand fragment (here **2.61**), furnished with the lactone and epoxide rings. The amino alcohols were to be achieved by the method described above (Table 1) by opening the chiral epoxide **2.41** by an appropriate amine moiety. The epoxide, in turn, would be converted from a chiral diol **2.62**, afforded after AD of commercially available 1-dodecene **2.63**. The right-hand fragment **2.61** was to be prepared by subjecting the alkylation product of the iodide **2.64** and the lactone ring **2.21** to AD conditions, and converting the afforded diol into the epoxide in **2.61**. The iodide **2.64** is a derivate of diol **2.65**, which was to be obtained after asymmetric dihydroxylation of 1,13-tetradecadiene **2.35**. The lactone ring **2.21** would be achieved from (phenylthio)-acetic acid **2.66** and (*S*)-propyleneoxide **2.67** according to a known procedure by White *et al*.[76]. Due to the possibility of epimerization of the lactone ring under basic conditions,[77] mild reaction conditions were favoured where possible, and the insertion of the double bond into the lactone ring was planned as one of the last steps.



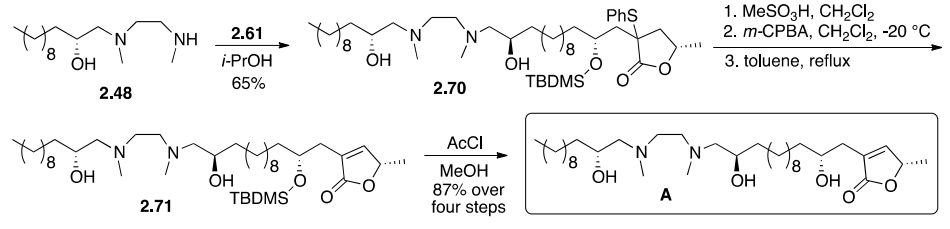
Scheme 14. Retrosynthetic scheme of acetogenin analogues **A** and **B** employing Sharpless' AD of olefins.



Scheme 15. Synthesis of right-hand fragment **2.61** via double-AD.

The AD of 1,13-tetradecadiene **2.35** was terminated after *ca* 50% conversion (monitored by TLC analysis) to yield diol **2.65** with er 92:8[78] (Scheme 15). The primary hydroxyl in **2.65** was regioselectively tosylated[79] by *p*-TsCl in the presence of catalytic amount of Bu_2SnO, and after protecting the secondary hydroxyl as silyl ether, the compound was converted into the iodide **2.64**. Next, the alkylation of the lithium enolate derived from lactone **2.21**[80] with **2.64** was investigated. In an attempt to replace HMPA as an additive, which has often been used in similar alkylations,[81] DMPU[82] and Et_3B-promoted processes[83] were investigated but without success. We returned back to using HMPA as co-solvent and achieved **2.68** in 78% yield by using LDA and 10 equiv. of HMPA. The remaining double bond was then subjected to a second Sharpless AD and the consequent diol **2.69** was converted into an epoxide **2.61** over tosyl-intermediate.

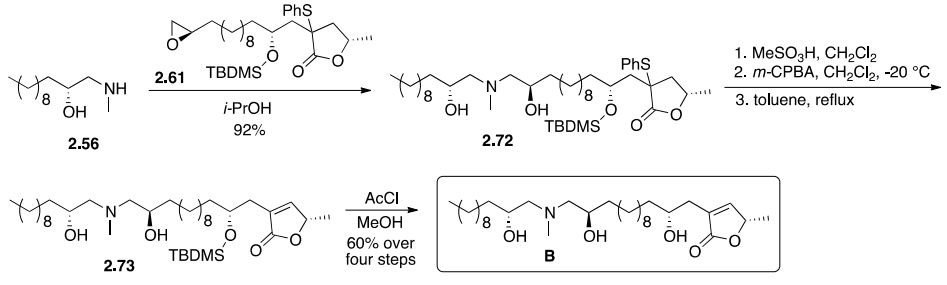
Next we had to couple the amino alcohol **2.48** with the epoxide **2.61**. In the test reaction (Table 1, entry 10) we had achieved an excellent yield for the symmetrical product after almost a week in reflux. Due to the lability of the compound in hand, we opted for a slightly lower temperature and a shorter reaction time, affording **2.70** with a moderate yield of 65% (Scheme 16).



Scheme 16. Synthesis of aza-analogue **A**.

The double bond in the lactone ring was to be achieved by the thermal elimination of phenylsulfoxide. We hoped that at low temperatures the oxidation of sulfide to sulfoxide would proceed selectively over nitrogen oxidation to *N*-

oxide due to their electronic characteristics. After subjecting **2.70** to 1.1 equivalents of *m*-CPBA in CH_2Cl_2 at –78 °C, we noticed that the tertiary amines were oxidized as readily as the sulfide, giving a mixture of products. Two options were considered – either to reduce *N*-oxides back to tertiary amines after the sulfoxide elimination, or to avoid the *N*-oxidation completely. A facile reduction of *N*-oxides has been reported by Gleave *et al.*,[84] where after oxidation the reaction mixture was treated with 10% aqueous $NaHSO_4$ during workup to recover the tertiary amine in the starting material. Unfortunately in the case of **2.70** an excess of the oxidation agent would be necessary to ensure the oxidation of all possible sites, but at the same time over-oxidation of the sulfide to sulfone should be avoided. This could be difficult to control and any over-oxidation would mean loss in the final product. Thus, we sought for an option to avoid the *N*-oxidation completely during the sulfoxide formation. A method to protect the tertiary amines from oxidation by forming quaternary amine salts[85] was investigated more closely. We conducted a series of test-reactions, where after forming the quaternary amine salt with methane sulfonic acid, the salt was subjected to the oxidation conditions: at –50 °C 1.1 equiv. of *m*-CPBA was added and after 20 min the reaction was quenched by 20% $Na_2S_2O_3$ (aq.). After washing with $NaHCO_3$ (aq. satd.), the starting material, the tertiary amine, was recoverable. Our investigations also showed that temperature around –20 °C was sufficient for the oxidation of sulfide to proceed and to avoid *N*-oxidation. Additionally, after the sulfoxide had formed, according to ^{1}H NMR analysis the elimination reaction occurred already at 60°C, which could make refluxing the final compound at higher temperatures (110°C in dioxane) redundant. It was concluded, that by converting the amine moiety to a corresponding quaternary ammonium salt and subjecting it to equimolar amounts of *m*-CPBA at –20 °C, the *N*-oxidation could be prevented during sulfoxide formation. The α,β-unsaturated lactone derivate **2.71** was desilylated in acidic conditions without prior purification to yield **A** with 87% yield over the last steps.



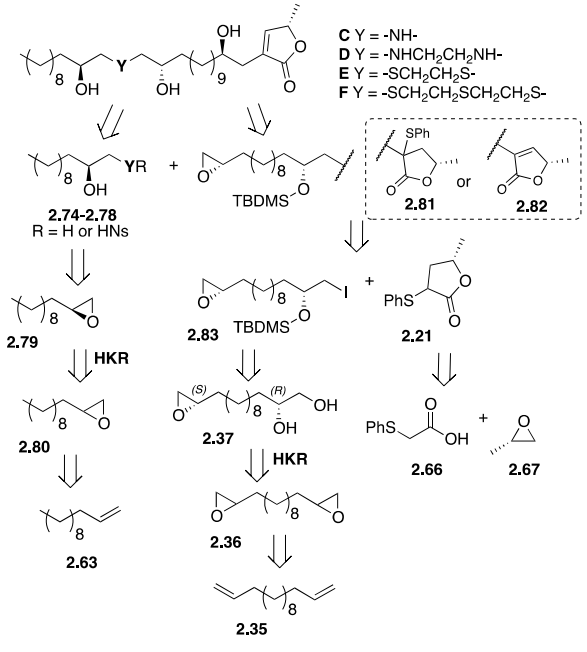
Scheme 17. Synthesis of aza-analogue **B**.

The synthesis of analogue **B** (Scheme 17) followed identical steps as described for analogue **A**. The methylated amino alcohol **2.56** (Table 1, entry 11) opened

the epoxide **2.61** with a pleasingly high yield of 92% and delivered **2.72** in much shorter time than was needed for the corresponding diamine derivate. After the quaternary amine salt formation, the phenylsulfide was oxidized by *m*-CPBA and the subsequent sulfoxide moiety was eliminated. The afforded **2.73** was desilylated and the aza-analogue **B** was afforded with a good yield of 60% over the last steps.

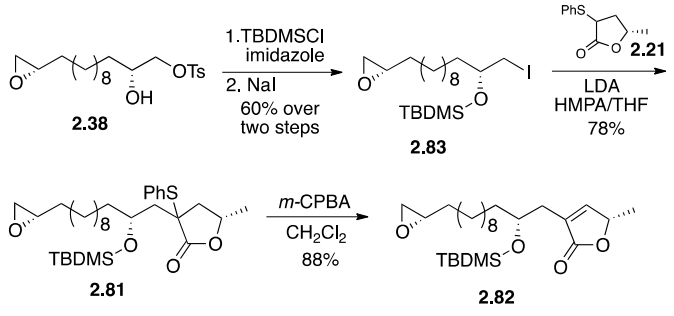
2.2.4. Synthesis of aza-analogues C and D via HKR

Similarly to the previous route, there are two main intermediates, which are coupled in the last stages of the synthesis of the aza-analogues **C** and **D** (Scheme 18). Here we envisioned that the left-hand fragment (here **2.74–2.78**) could be derived from epoxide **2.79**, after it is opened by the amino- or thio-nucleophiles. Compound **2.79** would be obtained by subjecting the racemic mono-epoxide **2.80** to Jacobsen's HKR conditions. Epoxide **2.80** can be afforded from commercial sources or via oxidation of 1-dodecene **2.63** by *m*-CPBA. The right-hand fragments **2.81** and **2.82** could be obtained by alkylating the lactone ring **2.21** with iodide **2.83**, which is a functionalized derivate of the epoxy-diol **2.37**. Compound **2.37**, in turn, could be achieved by subjecting bis-epoxide **2.36** to the HKR conditions, where two remote stereocenters are set as shown earlier in Scheme 11. The bis-epoxide **2.36** can be obtained by oxidizing commercially available 1,13-tetradecadiene **2.35** with *m*-CPBA.



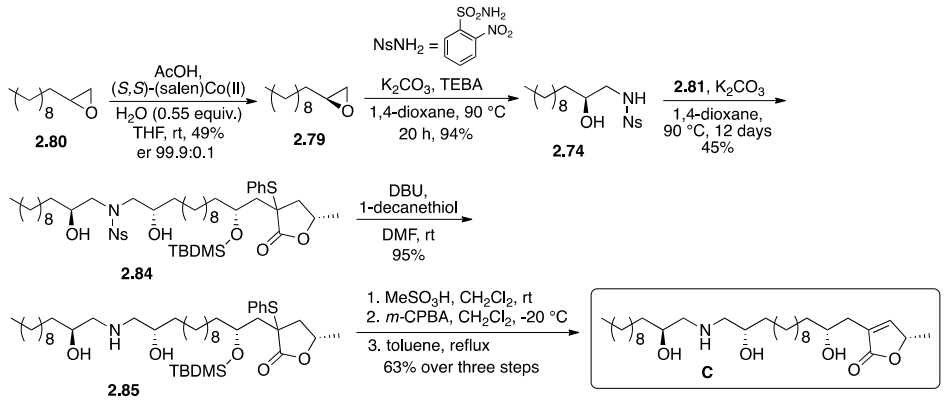
Scheme 18. Retrosynthetic scheme for acetogenin analogues **C**-**F** employing Jacobsen's HKR of mono- and bis-epoxides.

The synthesis of right-hand fragments **2.81** and **2.82** is shown in Scheme 19. The synthesis begun with kinetic resolution of bis-epoxide **2.36**, which afforded epoxy-diol **2.37** and was discussed in chapter 2.2.1 (Scheme 11). The tosylate **2.38** was prepared from epoxy-diol **2.37** as was shown in Scheme 12. After protecting the secondary hydroxyl in the tosylate **2.38** as a silyl ether (Scheme 19), the compound was converted into iodide **2.83** and used for alkylating the lithium enolate derived from lactone **2.21**. Oxidation of the sulfide moiety in **2.81** followed by elimination, gave the α,β-unsaturated lactone **2.82**. It was noted that the elimination of phenylsulfenic acid occurred during the oxidation step of the sulfide moiety and for convenience these two steps were conducted in the same pot.



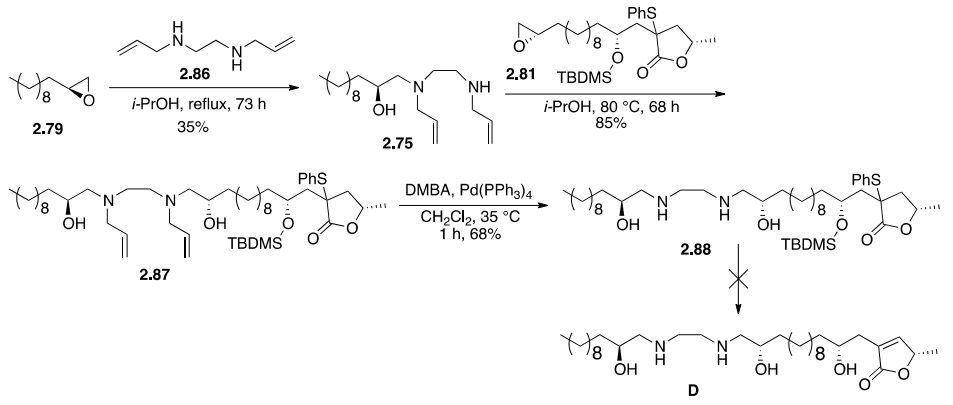
Scheme 19. Right-hand fragments **2.81** and **2.82** via HKR.

The synthesis of analogue **C** is detailed in Scheme 20. Due to the usefulness of *N*-nosyl protected amino alcohol as a nucleophile in epoxide opening (as show in Table 1, entry 16) and facile denosylation, it was employed in the synthetic route. The racemic epoxide **2.80** was subjected to HKR conditions with (*S,S*)-(salen)Co(II) catalyst. The obtained epoxide **2.79** was subjected to 2-nitro-benzenesulfonamide ($NsNH_2$) at elevated temperatures over 4 days to give amino alcohol **2.74** in high yield. Somewhat surprisingly, coupling this material with epoxide **2.81** proceeded slowly, requiring 12 days to reach acceptable conversion. As it is difficult to remove the *N*-nosyl group in the presence of α,β-unsaturated lactone moiety, the protecting group had to be removed before the sulfide was oxidized. Thus, subjecting compound **2.84** to 1-decanethiol and DBU,[86] yielded the secondary amine functionality in **2.85**, followed by elimination, analogue **C** was afforded. Gratifyingly, the silyl protecting group was also removed in the salt formation/oxidation/elimination sequence. Analogue **C** was isolated in 63% yield over three steps.



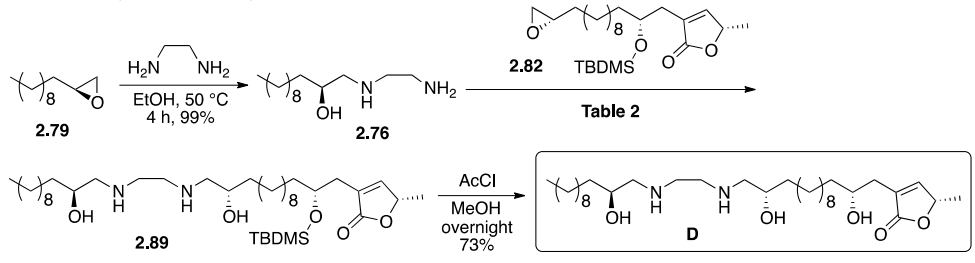
Scheme 20. Synthesis of aza-analogue **C**.

For the aza-analogue **D** two different synthetic approaches were attempted. Firstly, an approach similar to analogue **C** was followed, where a protected diamine moiety was introduced into the structure in order to improve the yield of the coupling of amino alcohol and the epoxide. A protecting group different from those tested in Table 1 was chosen – an *N*-allylamine protecting group. This was chosen because the allyl group, electronically similar to the Bn protecting group, would preform similarly well in the coupling, while allowing facile deprotection compatible with the other functional groups present in the compound. Epoxide **2.79** was opened by *N,N'*-diallylethylenediamine **2.86** (Scheme 21) delivering amino alcohol **2.75** in a somewhat surprisingly low yield after being refluxing for 3 days. Thus, it was very pleasing when the coupling of **2.75** and epoxide **2.81** proceeded with a good yield of 85% at 80 °C just under 3 days. Deallylation of **2.87** under palladium-catalyzed conditions,[87] using $Pd(PPh_3)_4$ and *N,N'*-dimethylbarbituric acid (DMBA) as an allyl group scavenger, also proceeded in high yields. The starting material was consumed within one hour as indicated by TLC analysis and the product **2.88** was obtained in a good yield, although the difficult isolation on silica-column afforded the product in lower yield than was expected. Next, the final three-step procedure of salt formation, oxidation and elimination was performed in a similar manner to the other aza-analogues. The compound **2.88** was treated with 5 equiv of methanesulfonic acid and after 50 minutes of stirring, the solution was concentrated to dryness. The formed salt-protected compound was then subjected to 1.5 equiv of *m*-CPBA at -20 °C, and then after re-dissolving in toluene, to thermal elimination. Unfortunately the desired end-product **D** was not obtained and a mixture of unidentified compounds was isolated after the three-step sequence.



Scheme 21. An attempt to synthesize aza-analogue **D** by using *N*-allylamine protecting group.

We then revisited epoxide opening with the primary amino alcohol as was shown in Table 1 and selected milder conditions, in the presence of Lewis acids, to open epoxide **2.82** with amino alcohol **2.76** (Scheme 22).[23] At room temperature both $Bi(OTf)_3$ and $FeCl_3$ gave yields around 30% for the coupled product **2.89** (Table 2, entries 1 and 2). Using microwave conditions and stirring the reaction at 110 °C for 30 minutes (entry 3) gave some recovered starting material and unidentified byproducts. A good yield of 65% for **2.89** was achieved when warming the reaction at 40 °C for 2 days in the presence of $La(OTf)_3$ (entry 4). After desilylation in acidic conditions, aza-product **D** was afforded (Scheme 22).



Scheme 22. Synthesis of aza-analogue **D**.

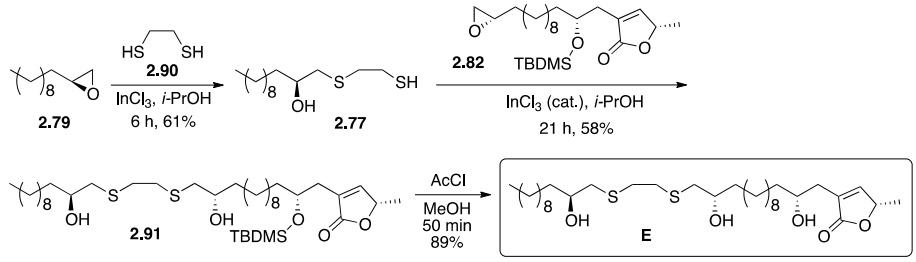
Table 2. Coupling of amino alcohol **2.76** and epoxide **2.82**.

Entry	LA	solvent	temperature	time	yield
1	$Bi(OTf)_3$	*i*-PrOH	rt	3 days	39%[a]
2	$FeCl_3$	*i*-PrOH	rt	3 days	32%[a]
3	$Bi(OTf)_3$	CH_2Cl_2	110 °C (MW)	30 min	nd[b]
4	$La(OTf)_3$	*i*-PrOH	40 °C	2 days	65%[a]

[a]Yield of isolated product **2.89**
[b]nd – not determined.

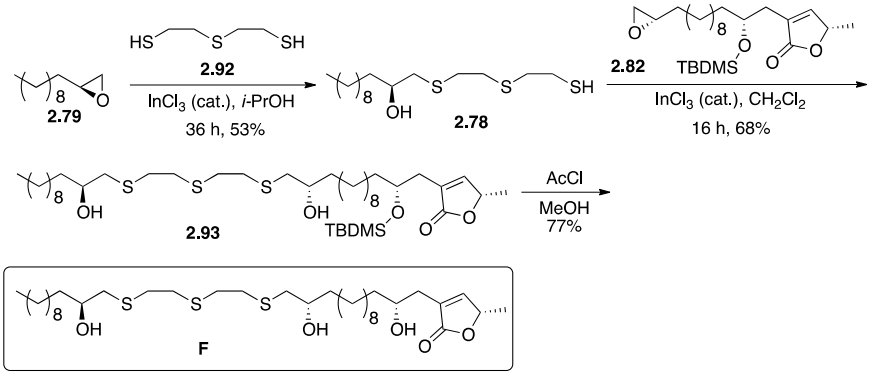
2.2.5. Synthesis of thio-analogues E and F via HKR

For the thio-analogues **E** and **F** a similar synthetic scheme as for aza-analogues **C** and **D** was followed, however, thio-fragments were used instead of amine fragments (Scheme 18). The epoxide **2.79** was opened by 1,2-ethanedithiol **2.90** (Scheme 23). Different reaction conditions were explored and the best yield for **2.77** was obtained in the presence of $InCl_3$ in *i*-PrOH at room temperature.[88] Next, **2.77** was coupled with epoxide **2.82** under the same conditions, to give **2.91**. After desilylation, thio-analogue **E** was afforded.



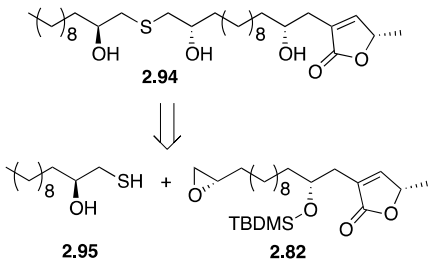
Scheme 23. Synthesis of thio-analogue **E**.

The final analogue, compound **F**, was prepared following an identical route with comparable yields, except for using 2,2'-thiodiethanethiol **2.92** as the nucleophile (Scheme 24). Epoxide **2.79** was opened by the β-hydroxy-sulfide **2.92** with a moderate yield in the presence of $InCl_3$. The obtained compound **2.78** was used to open epoxide **2.82** and after desilylation, end-product **F** was obtained.



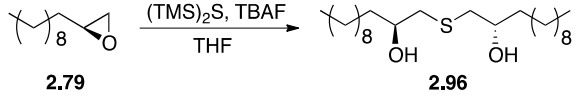
Scheme 24. Synthesis of thio-analogue **F**.

Several attempts were made to prepare a thio-analogue **2.94** (Scheme 25). The synthesis was planned according to a similar route of the previous thio-analogues, where a β-hydroxy-sulfide **2.95** would be coupled with epoxide **2.82**.



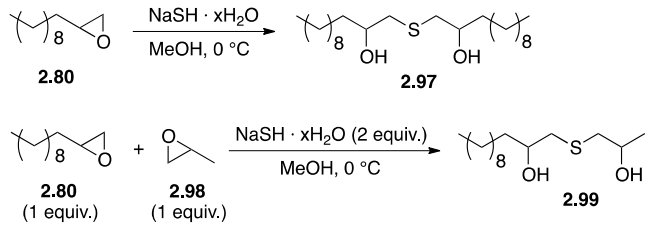
Scheme 25. Retrosynthetic scheme for attempted thio-analogue **2.94**.

Firstly, the epoxide **2.79** was reacted with hexamethyldisilathiane [$(TMS)_2S$], catalysed by tetrabutylammonium fluoride (TBAF) (Scheme 26).[89] The reaction was monitored by TLC analysis, which showed the formation of a single product. Next to a TLC stain of phosphomolybdic acid solution also Ellman's reagent stain[90] was used. Ellman's reagent helps to determine the presence of free sulfhydryl groups and is often used to determine sulfhydryl or disulphide groups in peptides.[91] Unfortunately, based on NMR and HRMS analysis, we reasoned it to be the symmetric product **2.96** not the expected product **2.95**.



Scheme 26. Thiolysis of epoxide **2.79**.

Then some test reactions were conducted following a different approach: the racemic epoxide **2.80** was reacted with sodium hydrosulfide hydrate in MeOH at 0 °C (Scheme 27). The reaction proceeded sluggishly, taking two days for a product to form (monitored by TLC analysis). Unfortunately, the symmetrical product **2.97**, same as **2.96**, was detected with some unreacted starting material. Increasing the amount of NaSH from 1 equiv. to 2 equiv., only led to faster conversion into **2.97** and dilution of the reaction made the reaction more sluggish. To gain further insight of the reactivity of the epoxides and sulfides formed, the reaction with NaSH was repeated in the 1:1 mixture of two epoxides, **2.80** and propylene oxide **2.98** (Scheme 27). This gave **2.99** as a major product – equivalent to the symmetrical products in the previous test reactions. The possible formation of a disulfide bond was eliminated by subjecting the thiolysis product to reductive conditions in the presence of $NaBH_4$, after which only starting material was detectable.

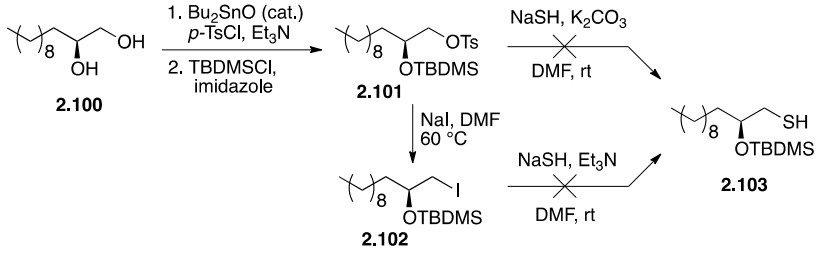


Scheme 27. Thiolysis of epoxides **2.80** and **2.97**.

It was concluded that the epoxide opening by the sulfide formed through ring opening is more reactive compared to the ring opening by NaSH.

A formation of a thiirane intermediate was also taken into account. To test this hypothesis, the reaction product was subjected to other nucleophiles, like 2-naphthalenethiol, diethylamine, NaSH in the presence of Et_3N, and benzylamine, but only starting material was recovered.

Additional approaches were explored in order to afford the β-hydroxy-sulfide **2.95** either via tosylate[92] **2.101** or iodide[93] **2.102** (Scheme 28), but unfortunately the subsequent substitution did not yield the desired compound **2.103**.



Scheme 28. Attempted synthesis of intermediate **2.103**.

2.2.6. Epimerization of the lactone ring.

The stereochemical lability of lactone ring in the acetogenins under basic conditions has been described previously[77] and the biological function of this moiety has been discussed.[94,95] As the consensus has not been reached concerning the effect of varying structure or the epimerization of the lactone ring on the bioactivity of the compound, the issue of epimerization should be addressed. The epimerization of the methyl moiety in the lactone ring was assessed by ^1H and ^{13}C NMR analysis. For some analogues, minor contamination was detected in the spectra, which was attributed to diastereomers derived from epimerization in the lactone ring. Epimerization was detected in the aza-analogues **A**, **B** and **C**, but not seen in **D** or in the thio-analogues **E** and

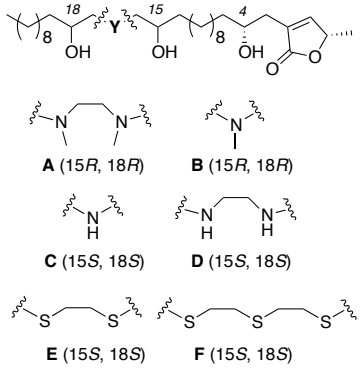
F. We reasoned that the cause for this could be the high temperature, which was imposed on the first three structures during elimination of phenylsulfoxide and the more basic nature of the aza-analogues themselves. The analogue **D** was consecutively achieved after coupling at room temperature, as were the two thio-analogues **E** and **F**.

2.2.7. Biological evaluation

The antiproliferative activity of **A-F** was assessed in comparison to rotenone in the HeLa cell line. Rotenone is another complex I inhibitor, although claimed to act via different pathway.[96] The results are shown in Table 3 as the concentration of compound necessary to give one-half the maximum response (ED_{50}). The cells were incubated with a series of dilutions of the synthesized analogues and of rotenone for 24 hours. The cell viability was evaluated by CellTiter 96®AQueous One Solution Cell Proliferation Assay. This assay uses tetrazolium salt TMS[†] that is bioreduced by cells to coloured formazan, which then can be measured colorimetrically. All four of the aza-analogues **A**-**D** exhibited cytotoxicity under 10 μM, and performed more effectively than rotenone (ED_{50} = 77.5 μM). The thio-analogues **E** and **F** had less effect on cell growth than aza-analogues, needing concentrations around 100 μM or higher to reach the effective dose.

Table 3. Antiproliferative activity of compounds **A-F** in HeLa cell line.[a]

Entry	Compound	ED_{50} (μM)
1	**A**	3.1
2	**B**	7.7
3	**C**	8.2
4	**D**	5.7
5	**E**	91.0
6	**F**	> 100
7	Rotenone[b]	77.5



[a] HeLa cell line, human epitheloid cervix carcinoma.
[b] Rotenone, as a known complex I inhibitor, was used as a positive control.

On the Chart 1, dose-dependent antiproliferation effects are plotted as relative cell viability (%) against compound concentrations 0.1 μM – 100 μM. For

[†] TMS stands for 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt

compounds **A-E** concentration 5 μM is added to illustrate the steepness of the cytotoxicity curve. This was omitted for **F** and rotenone, which followed a shallower slope, suggesting lower potency. The best result was achieved by the *N,N'*-dimethylated diamine analogue **A**, which at the concentration 3 μM had a cytotoxicity on 34.7 ± 4.4% of cells, while at 5 μM on 91.9±3.3% of cells (ED_{50} = 3.1 μM). Based on our findings, the epimerization of the butenolide in **A** did not seem to have a great effect on the bioactivity as the antiproliferative activity of **A** falls in the same range with its non-methylated counterpart **D** (ED_{50} = 5.7 μM), where epimerization was not detected. The *N*-methylated analogue **B** and the non-methylated analogue **C** had similar effective doses of 7.7 μM and 8.2 μM, respectively. It seems that the diamine containing aza-analogues, whether methylated or not, are more cytotoxic than their monoamine counterparts.

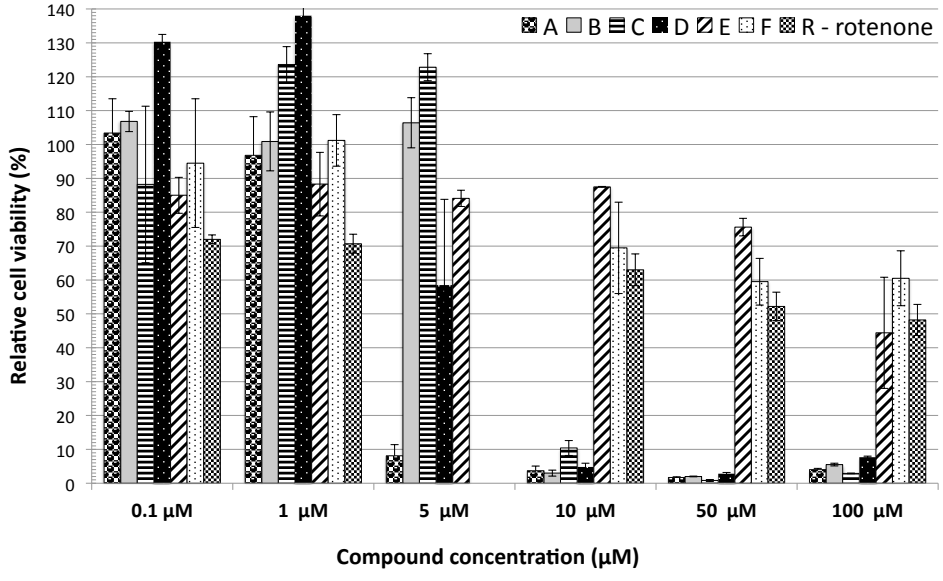


Chart 1. Dose-dependent effects of analogues **A–F** and rotenone on cell viability in HeLa cell line. The relative cell viability, represented as percentage, was determined by MTS assay at 24 hours after treatment.

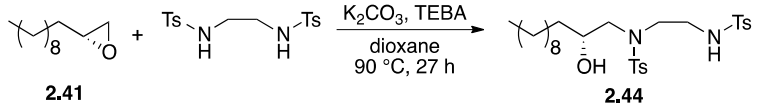
Many of the *in vitro* studies have been conducted over lengthier period of time, around 2 to 7 days. It has been shown that both the natural acetogenins (bullatacin)[97] and the polyether-analogue[98] effect the proliferation of cells also in time-dependent manner. We made a survey of dose-dependent cytotoxicity by incubating the cells over 24 h with different concentrations of our analogues synthesized. Although the incubation time interval was much shorter compared with the literature, a good dose-dependent curve was observable with the aza-analogues synthesized.

2.3. Conclusions

Four aza-analogues **A–D** and two thio-analogues **E** and **F** were synthesized according to the convergent synthetic scheme, which allows facile variability in the central part of the molecule containing heteroatoms. When there are examples of nitrogen containing analogues, the thio-analogues of acetogenins have not been reported to our knowledge. Two approaches to set the stereochemistry were compared – Sharpless asymmetric dihydroxylation of olefins and hydrolytic kinetic resolution of epoxides using the Jacobsen's catalyst. The best results in obtaining a crucial intermediate were achieved by the hydrolytic kinetic resolution of bis-epoxides. This useful and easily operative method established two remote stereocenters with high enantio- and diastereoselectivity, and allowed further two directional derivatization of the intermediate towards the end product. All the analogues synthesized underwent preliminary *in vitro* studies in the HeLa cell line. Aza-analogues showed 50% cell viability reduction at concentrations under 10 μM, while thio-analogues had less effect, needing concentrations around 100 μM and above to reach effective dose. Although we observed epimerization in the butenolide for aza-analogues **A–C**, the bioassay studies did not reflect significant activity differences between analogues **A–C** and the non-epimerized analogue **D**. To gain more understanding about the cytotoxicity of our synthesized analogues, different tumorous cell lines next to normal cell tissues have to be assessed with the compounds. Also, a more effective positive control would be useful.

2.4. Experimental

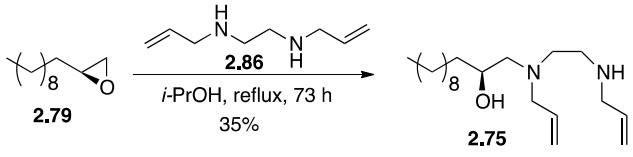
Compound 2.44



2.41 **2.44**

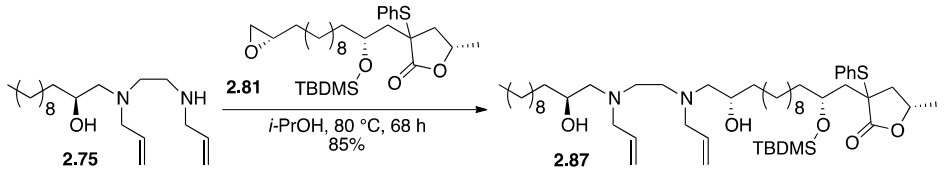
Epoxide **2.41** (369 mg, 2.00 mmol), TEBA (46 mg, 0.20 mmol), K_2CO_3 (28 mg, 0.20 mmol) and *N,N'*-bis(4-methylbenzenesulfonyl)ethane-1,2-diamine (1474 mg, 4.00 mmol) were dissolved in dioxane (3 mL) and stirred at 90 °C for 27 h. The crude was purified by column chromatography on silica (1.5% MeOH in $CHCl_3$). The product **2.44** was obtained as amorphous solid (870 mg, 79% yield). R_f = 0.5 (5% MeOH in $CHCl_3$). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ: 7.75 (m, 2H), 7.63 (m, 2H,) 7.32–7.28 (m, 4H), 6.03 (m, 1H), 3.88–3.81 (m, 1H), 3.38–3.30 (m, 1H), 3.28–3.15 (m, 2H), 3.13–3.07 (m, 1H), 3.08 (dd, *J*=14.7, 2.8 Hz, 1H), 2.88 (dd, *J*=14.7, 9.5 Hz, 1H), 2.42 (s, 6H), 1.44–1.22 (m, 18H), 0.90 (vt, 3H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 143.9, 143.3, 136.9, 134.9, 129.9, 129.7, 127.3, 127.1, 71.0, 56.9, 50.9, 43.3, 35.0, 31.9, 29.58, 29.56, 29.55, 29.52, 29.3, 25.3, 22.7, 21.5, 14.1.

Compound 2.75



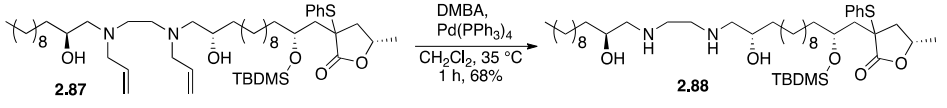
2.79 **2.75**

A solution of epoxide **2.79** (505 mg, 2.74 mmol) and *N,N'*-bis(prop-2-en-1-yl)ethane-1,2-diamine **2.86** (1050 mg, 7.49 mmol) in *i*-PrOH (1.0 mL) was heated at 100 °C for 73 h. The excess of the unreacted diamine was distilled off under vacuum (*ca* 150 °C, 0.22 mbar) and the crude containing the product was purified by flash chromatography on silica (5–15% MeOH in CH_2Cl_2). The product **2.75** was obtained as amorphous solid (311 mg, yield 35%). R_f =0.4 (10% MeOH in CH_2Cl_2). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ: 5.95–5.84 (m, 1H); 5.88–5.77 (m, 1H); 5.20–5.07 (m, 4H); 3.63–3.56 (m, 1H); 3.28–3.21 (m, 3H); 3.07 (dddd, *J*=14.2, 7.4, 1.1, 1.1 Hz, 1H); 2.84 (bs, 2H); 2.78–2.64 (m, 3H); 2.58–2.49 (m, 1H); 2.46 (dd, *J*=13.0, 2.9 Hz, 1H); 2.32 (dd, *J*=13.0, 10.1 Hz, 1H); 1.49–1.21 (m, 18H); 0.87 (vt, 3H). ^{13}C NMR (50.3 MHz, $CDCl_3$) δ: 136.5, 135.3, 117.8, 116.2, 68.1, 60.5, 58.3, 53.6, 52.1, 46.9, 34.8, 31.9, 29.8, 29.6, 29.3, 25.7, 22.7, 14.1.



Compound 2.87

Solution of allyldiamine **2.75** (39 mg, 0.121 mmol) and epoxide **2.81** (66 mg, 0.121 mmol) in *i*-PrOH (0.5 mL) was stirred at 80 °C for 68 h for *ca* 2.5 days. The course of the reaction was monitored by TLC analysis. Then the mixture was concentrated to dryness and purified by column chromatography on silica (1 % MeOH in CH_2Cl_2) obtaining product **2.87** as a beige oil (90 mg, yield 85 %). ^{1}H NMR (400.1 MHz, $CDCl_3$), δ: 7.58–7.50 (m, 2H); 7.42–7.29 (m, 3H); 5.92–5.79 (m, 2H); 5.22–5.10 (m, 4H); 4.59 (m, 0.38H, minor isomer); 4.51 (m, 1H, major isomer); 4.24 (m, 1H); 3.70–3.57 (m, 2H); 3.30–3.16 (dd, *J* = 13.9 Hz, 2H); 3.17–2.98 (dd, *J* = 13.9 Hz; dd, *J* = 14 Hz; 3H); 2.73–2.61 (m, 2H); 2.57–2.26 (m, 7H); 2.39–2.26 (m, 2H); 2.10–1.78 (m, 2H); 1.50–1.34 (m, 8H); 1.34–1.07 (m, 47 H); 0.89 (bs, 9H); 0.86 (vt, bs of minor isomer, 6H); 0.15 (bs, 3H); 0.11 (bs, 3H); 0.03 (bs, minor), 0.02 (bs, minor). ^{13}C NMR (100.6 MHz, $CDCl_3$), δ: 177.4 (major isomer); 175.0 (minor isomer); 136.9; 136.9 (minor); 136.6 (major); 134.8 (bs); 130.4 (major); 130.4 (minor); 129.8; 129.6 (minor); 129.5; 128.9; 128.8 (minor); 118.1 (bs); 73.5 (minor); 73.2 (major); 70.2 (minor); 69.5; 68.4; 61.1; 58.0; 55.3; 55.3 (minor); 52.9 (bs); 42.4 (minor); 41.6 (minor); 41.2 (major); 39.5; 38.5; 37.9 (minor); 34.7; 34.2 (minor); 31.8; 29.8–29.3 (overlapping signals); 29.2; 25.9; 25.9; 25.7; 25.7; 24.4; 24.4; 22.6; 21.2 (overlapping with minor peak); 20.3 (minor); 17.9; 14.0: -3.7 (minor); -3.8; -4.1 (minor). IR (cm^{-1}) ν_{max}: 3429.5; 2924.0; 2854.6; 1766.8; 1462.0; 1184.2; 1053.1; 1002.9; 918.1; 833.2; 775.3. HRMS (m/z) calcd for $C_{51}H_{92}N_2O_5SSi$ $(M+H)^+$ 873.6569, found 873.6552.



Compound 2.88

To a solution of *N,N'*-dimethylbarbituric acid (DMBA) (46 mg, 0.297 mmol) and $Pd(PPh_3)_4$ (1.6 mg, 1.38 μmol) in CH_2Cl_2 (0.2 mL) was added allylamine **2.87** (43 mg, 49.5 μmol) in CH_2Cl_2 (0.5 mL) and stirred at 35 °C for 1 h under argon atmosphere. Then the mixture was concentrated to dryness and re-dissolved in Et_2O. The solution was washed two times with small volumes of Na_2CO_3 (aq. satd.) and 2 N HCl until white precipitation formed in the organic phase. The organic phase was filtrated to remove solids. The filtrate was concentrated *in vacuo* and purified by column chromatography on silica (10 %

MeOH in CH_2Cl_2) obtaining product **2.88** as a beige oil (27 mg, yield 68 %). ^{1}H NMR (400.1 MHz, $CDCl_3$), δ: 7.58–7.51 (m, 2H); 7.43–7.30 (m, 3H); 4.59 (m, 0.3H, minor isomer); 4.51 (m, 1H, major isomer); 4.48 (bs, 1H); 4.25 (m, 1H); 4.00–3.88 (m, 2H); 3.37–3.08 (bs, 3H); 3.05 (dd, 1H); 2.97–2.73 (m, 4H); 2.48–2.26; 2.11–1.79 (m, 4H); 1.54–1.35 (m, 10H); 1.35–1.11 (m, 50 H); 0.89 (bs, 9H); 0.86 (vt, bs of minor isomer, 7H); 0.15 (bs, 2.3 H); 0.11 (bs, 2.5 H); 0.03 (minor); 0.02 (minor). ^{13}C NMR (100.6 MHz, $CDCl_3$), δ: 177.5 (major isomer); 175.1 (minor isomer); 137.0; 136.6; 130.4; 129.9 (minor); 129.6 (minor); 129.5; 128.9 (major); 128.9 (minor); 73.6 (minor); 73.3 (major); 70.2 (minor); 69.5 (major); 55.5 (major); 55.1 (minor); 54.0 (minor); 42.4 (minor); 41.7 (minor); 41.2 (major); 39.5; 38.5; 38.0 (minor); 35.1 (decayed); 31.9; 31.4 (minor); 30.1 (minor); 29.8–29.4 (overlapping signals); 29.3; 26.0 (overlapping signals); 25.5; 24.4; 22.6: 21.3: 20.3; 18.0; 14.1; -3.7 (minor); -3.8 (major); -4.1 (minor). IR (cm^{-1}) ν_{max}: 3336.8; 3232.7; 2924.0; 2850.7; 1766.8; 1462.0; 1438.9; 1253.7; 1184.2; 833.2; 775.3. HRMS (m/z) calcd for $C_{45}H_{84}N_2O_5SSi$ $(M+H)^+$ 793.5943, found 793.5937.

2.5. References

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but it worked well for the free sulfhydryl group detections discussed in the present work.

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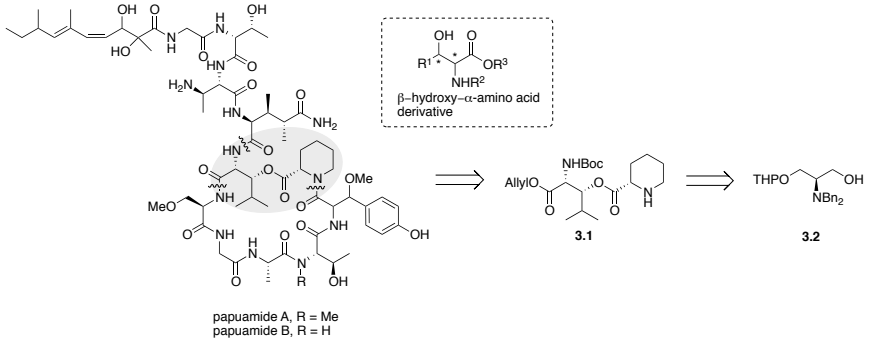
3. ASYMMETRIC TRANSFER HYDROGENATION COUPLED WITH DYNAMIC KINETIC RESOLUTION OF α-AMIDO-β-KETO ESTERS

(Paper IV and V)

3.1. Introduction

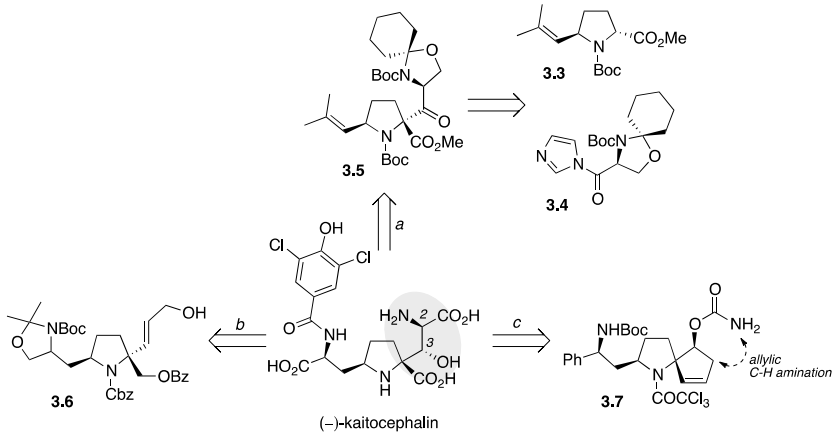
Derivatives of β-hydroxy-α-amino acids are important structural units that can be found in many complex bioactive compounds. For example, this unit can be found in papuamides A and B[99] (Scheme 29), and also in kaitocephalin[100] (Scheme 30), a relatively simple natural product compared to the aforementioned. Papuamide is a natural product with a strong inhibitory effect on the infection of human T-lymphoblastoid cells by HIV-1. The synthesis of the *anti* amino alcohol structure unit **3.1** found in its core (shaded in gray in Scheme 29) was achieved by Xie *et al*. by oxidizing the alcohol in L-serine derivate **3.2** into an aldehyde and a subsequent Grignard reaction.[101]



Scheme 29. Papuamide A and B

Kaitocephalin (Scheme 30) has become a promising lead compound towards therapeutic agents against various neuronal diseases, and thus its synthesis has gained a lot of attention over the years. For example, an 11 step synthetic route was developed by Chamberlin and co-workers using a *C*-acylation of a pyrrolidine ester **3.3** with *N*-acylimidazole **3.4** giving a single diastereomer of **3.5**,[102] and after treating with DIBALH the reduced product was afforded with dr > 30:1 (Scheme 30, *a*). This effort needed several tries and steps to reach the desired compound. Also, Sharpless asymmetric epoxidation of **3.6** and a subsequent opening by azide anion have been employed towards the end product (*b*),[103] and also rhodium-catalyzed allylic C-H amination on the cyclopentene ring of **3.7** and a subsequent oxidative cleavage of the double bond in the cyclic carbamate (*c*).[104] The examples provide the C-2 and C-3 stereocenters in

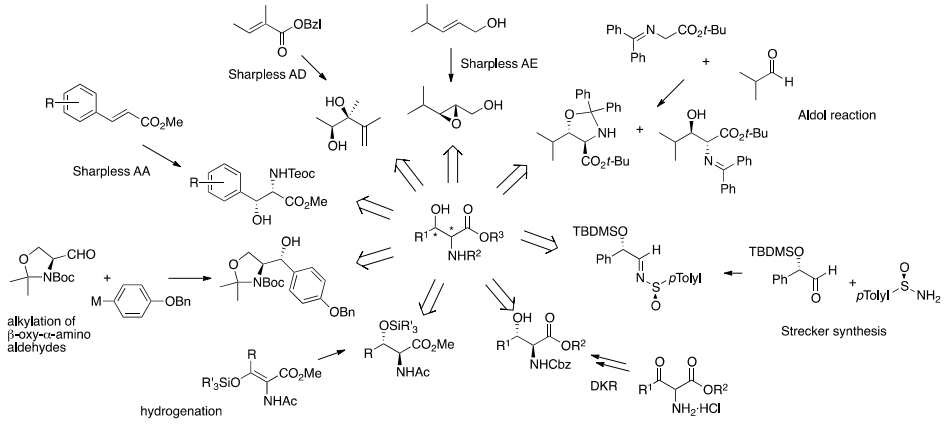
individual steps, but by employing ATH both stereogenic centers could be achieved simultaneously.



Scheme 30. Selected synthetic approaches towards (–)-kaitocephalin.

3.1.1. Synthesis of β-hydroxy-α-amino acid derivates

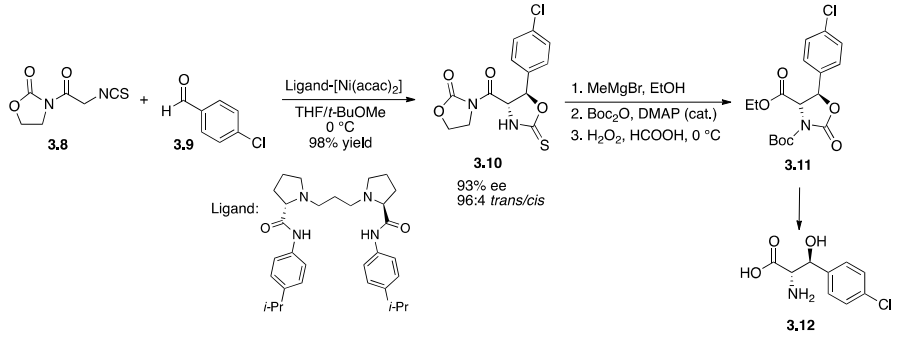
Many elegant methods have been reported for the synthesis of β-hydroxy-α-amino acid derivates, *e.g.*, aldol reaction, Sharpless asymmetric epoxidation, -dihydroxylation or -amination, Strecker reaction, and many others (Scheme 31).[105]



Scheme 31. A selection of methods for the synthesis of β-hydroxy-α-amino acid derivates.

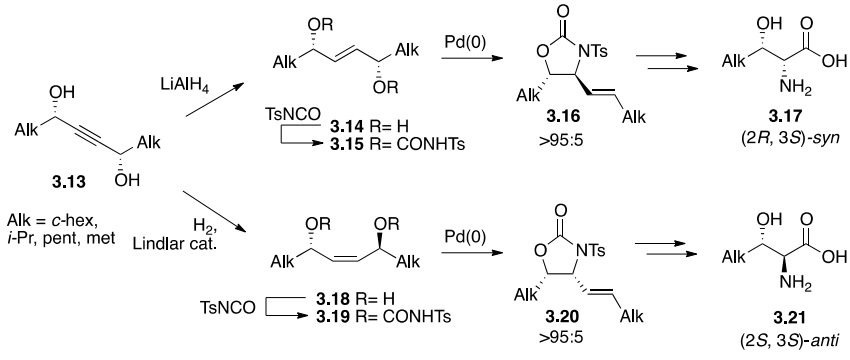
There have also been many attempts towards establishing a general and efficient method to achieve high stereoselectivity for both alkyl- and aryl sub-

strates. For example, Feng and co-workers reported the catalytic asymmetric aldol reaction in order to achieve the β-hydroxy-α-amino acid derivatives with *anti*-selectivity (Scheme 32).[106] They treated α-isothiocyanato imide **3.8** with aldehyde **3.9** (or any appropriate aldehyde depending on the end product) in the presence of a ligand-[Ni(acac)$_2$] complex (2.5 mol%) and achieved **3.10** with high stereoselectivity and yield. After reduction of **3.11**, the product **3.12** was afforded. These air-tolerant conditions were found to be compatible with a scope of aryl- and alkyl-substrates giving good to excellent enantio- and diastereoselectivities.



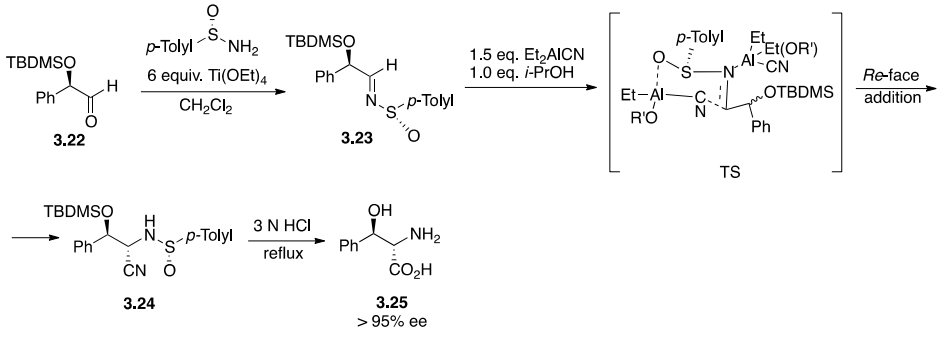
Scheme 32. Synthesis of β-hydroxy-α-amino acid derivatives via aldol reaction.

Amador *et al.* on the other hand, proposed a route according to which products with both *anti*- and *syn*-configuration could be achieved (Scheme 33), although this was demonstrated only on alkyl substrates.[107] They started with symmetrical alkyne diol **3.13**, which was transformed to corresponding (*E*)-diol **3.14** or (*Z*)-diol **3.18** by selective reductions. After Pd(0)-catalyzed allylic alkylation of **3.15** and **3.19**, the corresponding carbamates of the diols, oxazolidinines **3.16** and **3.20**, were formed with dr >95:5. These could be further elaborated to the corresponding amino acids **3.17** and **3.21**.



Scheme 33. Synthesis of alkyl β-hydroxy-α-amino acids with *anti*- and *syn*-configuration from (*E*)- or (*Z*)-diols.

Another interesting example is the route via Strecker reaction (Scheme 34), which was shown to work both with alkyl and aryl substrates and also towards both *syn*- or *anti*-selectivity.[105h] The α-substituted aldehyde **3.22** was treated with (*S*)-*p*-toluene-sulfinamide in the presence of 6 equiv. of $Ti(OEt)_4$, and the corresponding sulfinimine **3.23** was in turn treated with ethylaluminium cyanoisopropoxide generated *in situ* from *i*-PrOH and Et_2AlCN. The authors reasoned that the sulfinyl group directed the addition of the CN-group from the *Re*-face, giving rise to the compound **3.24**. After refluxing in 3 N HCl, *anti*-**3.25** was afforded with >95% ee. No diastereomers were detected by NMR analysis.



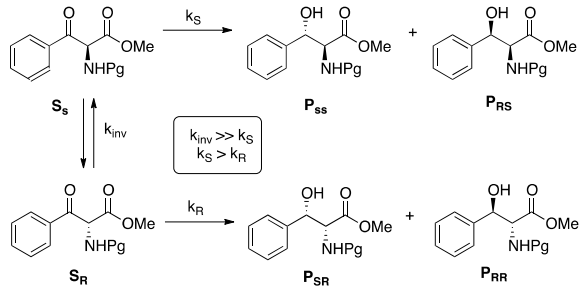
Scheme 34. Synthesis of an amino alcohol with an *anti*-selectivity via Strecker reaction.

In this light, asymmetric transfer hydrogenation via dynamic stereomutation or dynamic kinetic resolution is a relatively new approach towards the β-hydroxy-α-amino acids and has a great advantage of setting the two adjacent stereo-centers in one transformation.

3.1.2. ATH and AH via DKR

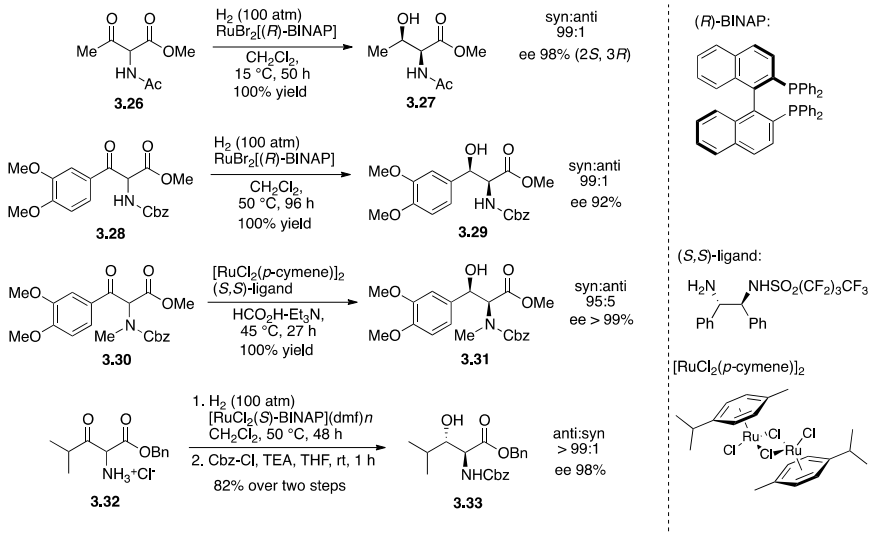
Reductions by asymmetric hydrogenation (AH) and asymmetric transfer hydrogenation (ATH) produce products in high stereoselectivities and yields.[108] In AH molecular hydrogen, and in ATH a hydrogen donor is used in the reduction process. There is tendency towards ATH becoming a good alternative to AH, which would eliminate the need for high pressures and the use of hydrogen gas.

The first AH of α-substituted β-keto esters via DKR in the presence of a ruthenium catalyst and a chiral ligand was reported by Noyori *et al*. as a good method to achieve the product in high enantioselectivity and yield.[109] By subjecting substrates that possess a chirally labile stereogenic center prone to racemization during the hydrogenation reaction, DKR can afford a single enantiomer in up to 100% yield (Scheme 35).[110] For example, β-keto esters with α-amino groups.



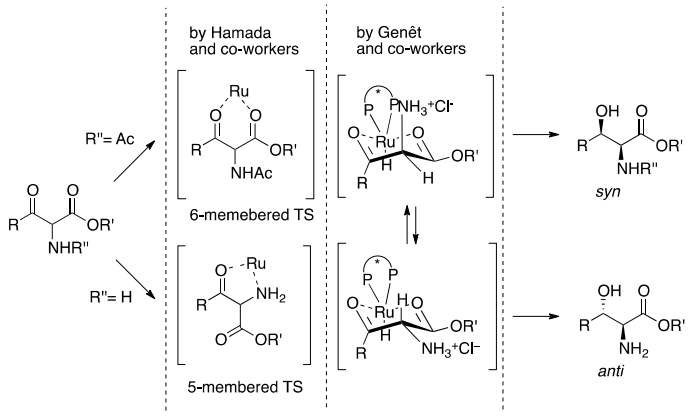
Scheme 35. Enantioselective hydrogenation of α-amino-β-keto esters via DKR.

Noyori showed that the reduction of **3.26**, **3.28** (Scheme 36) and other similar *N*-protected α-amino-β-keto esters, afforded the reduced products with a high *ee* and a *syn*-selectivity. These results were soon confirmed by other research groups, *e.g.* Mohar *et al*., who studied similar substrates as Noyori but instead of AH, subjected them to ATH via DKR with equally good results.[111] Product **3.30** was also one of the first ATH of α-amino-β-keto esters via DKR reported. The respective *anti*-product had to be achieved with other methods, often needing additional steps. By then Noyori had already demonstrated ATH on aromatic ketones in *i*-PrOH,[112] although the reduced products were achieved with lower *ee*'s compared to the AH method. In 2004 Hamada and co-workers[113] showed that using the conventional AH conditions, a product **3.33** with *anti*-selection was achievable with high *ee* and dr. They claimed that an amine hydrochloride salt led to the opposite selectivity and the reaction proceeded via five membered transition state (TS) resulting in the *anti*-product (Scheme 37).



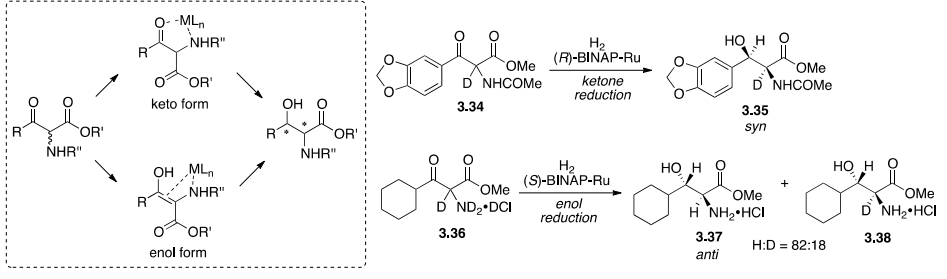
Scheme 36. AH and ATH of amino keto esters.

At this time, Genêt and co-workers had also accessed the *anti*-α-amino-β-hydroxy esters using the unprotected amines,[114] and suggested the preference towards the *anti*-configuration arises from the chair-like TS, where the NH_2·HCl group would be in the equatorial position.



Scheme 37. Transition states (TS) suggested by Hamada and Genêt for *syn*- and *anti* selectivity (P⌒*⌒P, BINAP).

A question whether the reduction of keto esters takes place via ketone reduction or enol reduction has also been under study (Scheme 38). Noyori[109] showed by the isotope labeling experiment that the anti-selection is achieved via ketone reduction. They subjected **3.34** to AH conditions. If the deuterated substrate would proceed via enolization, it would loose the α-deuterium. But the product **3.35** was afforded with 80% of the deuterium retained in the α-position and had no deuterium at β-position. Hamada and co-workers demonstrated also with the help of isotopic labeling, that the reduction of a hydrochloride salt of an α-amino-β-keto ester **3.36** proceeds via enol form under the same conditions and gives rise to the *anti*-selectivity in product **3.37**.[115]



Scheme 38. Ketone reduction and enol reduction.

Noyori proposed that when using the diamine or β-amino alcohol ligands with the Ru(arene) complexes, the transfer hydrogenation proceeds via a six-membered pericyclic TS structure (TS1, Figure 11) rather than TS2, where the metal coordinates directly to carbonyl oxygen.[116] The hypothesis was based on calculations in a gas phase and the six atoms in TS were thought to locate almost in one plane. The favored TS for aryl substrates may arise from the stabilization interaction of $C(sp^2)H/\pi$ between the Ru(arene) complex and the aryl substituent of the substrate due to the increased π-donation of arene.[117]

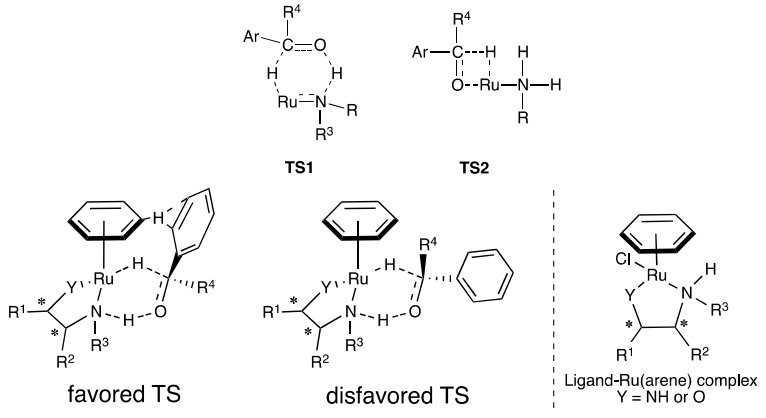


Figure 11. Metal-ligand bifunctional catalysis. Enantiodifferentiation of aryl substrates.

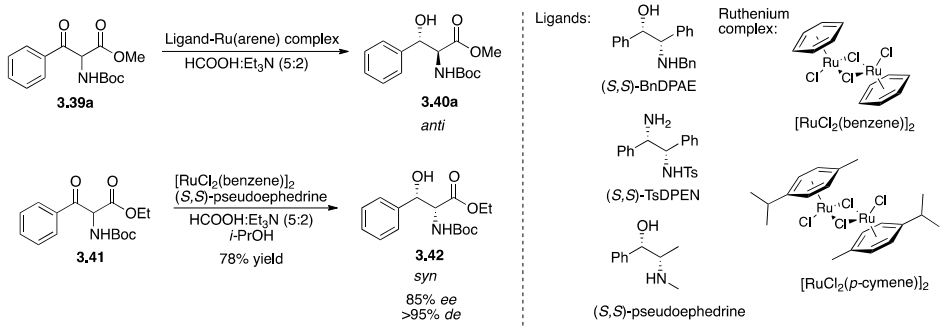
Noyori's ruthenium-ligand bifunctional complexes have found recognition, although recent calculations have suggested more complex mechanism, and a probable involvement of two catalytic cycles.[118]

3.2. Results and discussion

3.2.1. ATH via DKR using triethylformiate as hydrogen donor

This project grew out of a topic concerning the synthesis of β-hydroxy-α-amino acid derivatives using addition of azomethine ylides to aldehydes.[119] In order to prepare the β-hydroxy-α-amino acid derivatives with *syn*-configuration in higher stereoselectivity that was achieved by the method under study, additional ones, like AH and ATH were explored. ATH via DKR provided results worth further studies. There are many chiral ligands reported to accompany the metal complexes for reductions.[120] At first an array of known ligands, including (*S,S*)-BnDPAE, (*S,S*)-TsDPEN and (*S,S*)-pseudoephedrine were tested with two ruthenium complexes – $[RuCl_2(p\text{-cymene})]_2$ and $[RuCl_2(benzene)]_2$ in reducing ketone **3.39a** (Scheme 39). This is discussed in more length in Paper IV. The best results based on stereoselectivity and yield were achieved with the ligand

(*S,S*)-BnDPAE and $[RuCl_2(benzene)]_2$, which were also chosen for the following studies. Initially *syn*-selectivity was expected for **3.40a**, as was reported in the literature for **3.42** after reducing a similar compound **3.41**,[121] but somewhat surprisingly *anti*-selectivity was achieved for **3.40a**.[§]

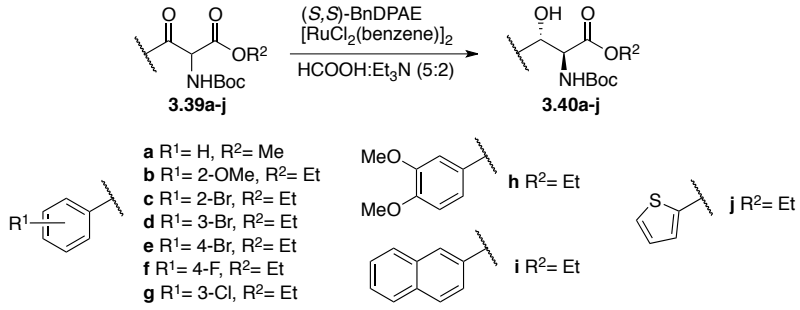


Scheme 39. Product **4.40a** with *anti*-configuration was achieved after subjecting **3.39a** to ATH via DKR. Substrate **3.41** from literature had given a *syn*-product **4.42** under similar conditions. Reaction conditions using combinations of chiral ligands and ruthenium complexes were explored.

Intrigued with the results afforded for **3.40a** a scope of aromatic substrates was planned to test the found conditions on. This constituted of substrates where the benzyl ring was furnished with either EWG or EDG, and also a naphthyl- and a thienyl-substrate. Both EDG, like *ortho*-positioned metoxy in **3.39b** (Table 4, entry 2) and bromo substrates (entries 3–5) gave high yields and excellent enantioselectivities, also the *para*-fluoro substrate **3.39f** (entry 6). Surprisingly the *meta*-chloro substrate afforded a lower yield with a rather disappointingly low stereoselectivity (entry 7). The substrate **3.39h** and a naphthyl substrate **3.39i** both gave excellent yields and enantioselectivities (entries 8 and 9). Unfortunately the thienyl-substrate afforded lower selectivities as well.

[§] The general procedure for the reduction reaction was as follows: the ruthenium-ligand complex was formed by heating $[(RuCl_2(benzene)]_2$ and the ligand (*S,S*)-BnDPAE at 80 °C for 1 hour in *i*-PrOH. After cooling to room temperature, the catalyst was added to the substrate and the reaction was stirred at room temperature in HCOOH-Et_3N (5:2) mixture until the consumption of the starting material.

Table 4. ATH or aryl substrates using triethylammonium formate as a H-donor.[a]



entry	Product **3.40a-j**	yield (%)[b]	dr[c]	er[d]
1	**a**	95	>95:5	97:3
2	**b**	83	>95:5	99:1
3	**c**	76	>95:5	99:1
4	**d**	94	>95:5	96:4
5	**e**	81	>95:5	98:2
6	**f**	89	>95:5	96:4
7	**g**	69	>95:5	66:34
8	**h**	95	>95:5	97:3
9	**i**	93	>95:5	95:5
10	**j**	75	80:20	76:24

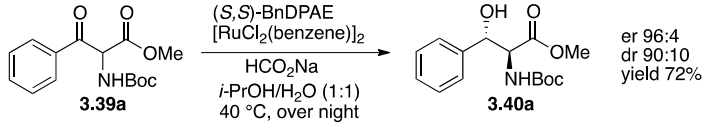
[a] General reaction conditions: the solution of $[RuCl_2(benzene)]_2$ (0.1 equiv.) and (*S,S*)-BnDPAE (0.2 equiv.) in *i*-PrOH (*c* = 0.1 M) at 80 °C for 1h. After cooling to rt, the catalyst was added to the substrate **3.39** (1.0 equiv.) with $HCOOH:Et_3N$ complex 5:2 (*c* = 0.2 M). The reaction was stirred for 5–7 days at rt.
[b] Isolated yield of product.
[c] Determined by ^1H NMR. For >95:5 only one isomer was visible.
[d] Determined by chiral HPLC.

Alongside the azeotropic mixture of $HCOOH:Et_3N$, *i*-PrOH can be a hydrogen donor in the transfer hydrogenation reaction. To be certain that in our conditions the hydrogen does not originate from the *i*-PrOH used in catalyst formation, but from the formate, a test reaction was run where using the same reaction conditions, but excluding the formate. This reaction was run over night at room temperature. However, no conversion to the product was detected and only the starting material was visible while monitoring by TLC analysis.

3.2.2. ATH via DKR in emulsions using sodium formate as hydrogen donor

Keen on widening the scope and improving the reaction conditions we explored this reaction further. Encouraging results had also been achieved by conducting

hydrogenations in water medium.[122] The reduction of ketones in water had shown increased reaction rates accompanied by excellent enantioselectivities compared to the azeotrope mixture as the reaction medium.[123] Thus, we were curious to study the aqueous conditions to see if we could shorten the reaction times for our substrates. Water as a solvent is a matter worth exploring because it possesses many good properties – it is generally non-hazardous, environmentally friendly and easily accessible, and also in many cases, acts as a reagent or an accelerator in the reaction.[124] To overcome problems with solubility a suitable surfactant can be added for some water insoluble organic compounds or the use of emulsions. Instead of TEAF, sodium formate can also be used as a hydrogen donor, especially in aqueous media. Unfortunately when submitting **3.39a** to sodium formate and water conditions with Ru-complex and either ligands BnDPAE or TsDPEN did not result the conversion of the starting material. Instead the aggregation of reagents was observed. By adding *i*-PrOH as a co-solvent, the reaction did result in the formation of the product, but with lower yield and stereoselectivity (Scheme 40).

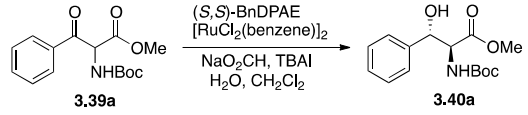


Scheme 40. ATH in *i*-PrOH/H_2O conditions.

During that time a new approach in ATH appeared by Wang *et al.*, where ATH of ketones was conducted in CH_2Cl_2-water emulsions.[125] We subjected the substrate **3.39a** in the presence of $[RuCl_2(benzene)]_2$ and ligand (*S,S*)-BnDPAE to the reported emulsion conditions (Table 5, entry 1). The product **3.40a** was isolated with a same excellent yield as in the case of using TEAF, and in high stereoselectivity in much more shorter time but unfortunately with slightly lower diastereoselectivity (Table 5, entry 1). In the search to improve the diastereoselectivity, we lowered the pH of the reaction from 7 to 5. It had been reported that transfer hydrogenation of ketones in aqueous conditions in the presence of Ru-catalyst could be pH dependent and prefer more acidic environment.[126] It was claimed that not only did the acidic media stabilize the catalyst employed but also controlled the reaction by activating the ketones through protonation, which is dependent on the Lewis acidity of the carbonyl carbon that accepts the hydride from the catalyst. But close to no conversion of the starting material **3.39a** was observed at pH 5 (entry 2). After running the ATH in basic conditions by adding 5 equivalents of Et_3N, essentially raising the pH to *ca* 9, product **3.40a** was afforded in a good yield, but with similar low dr as for reaction run in environment with pH 7 (compare entries 1 and 3). This is consistent with another reports that claim a loss of ATH reaction rate at low pH.[127] We then cooled the reaction down and run the hydrogenation at -5 °C (entry 4), which did increase the reaction times to 2–3 days, but pleasingly also

increased the dr – only one diastereomer was detectable and a high er of 97:3 was retained.

Table 5. Optimization of ATH conditions in emulsion for **3.39a**.[a]



entry	pH	t (°C)	yield (%)[b]	dr[c]	er[d]
1	pH 7	rt	97	92:8	96:4
2	pH 5	rt	traces	-	-
3	pH 9[e]	rt	95	92:8	n.d.
4	pH 7	-5 °C	92	>95:5	97:3

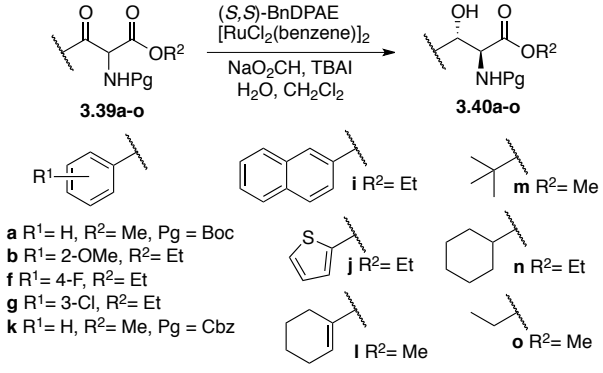
[a]General reaction conditions: the solution of $[RuCl_2(benzene)]_2$ (5 mol %), (*S,S*)-BnDPAE (1 mol %) and Et_3N (2 mol %)in CH_2Cl_2 (*c* = 0.1 M) was stirred at 40 °C for 1h. After cooling to rt, the catalyst was added to the substrate **3.39a** (1.0 equiv.) along 5M HCO_2Na (aq.) and TBAI. The mixture was sonicated for 5–10 min and then stirred at rt over night.
[b]Isolated yield of product.
[c]Determined by ^1H NMR. For >95:5 only one isomer was visible.
[d]Determined by chiral HPLC.
[e]5 equiv. of Et_3N was used

These new reaction conditions were tested against the substrate scope (Table 6). Selected aryl substrates from the TEAF scope were subjected to the emulsion conditions and the reaction was run either at room temperature or at –5 °C. After **3.39a** (Table 6, entry 1), **3.39b** with electron donating group *ortho*-MeO gave comparable results in emulsion with TEAF conditions even at room temperature while shortening the reaction time to *ca* 16 h (entry 2). But **3.39f** furnished with *para*-fluoro had to be cooled to –5 °C to increase stereo-selectivity (compare entries 3 and 4). Cooling prolonged the reaction time to 2–3 days, while at room temperature almost full conversion could be achieved over night although in slightly lower dr. Gratifyingly, **3.39g** also gave good results in the –5 °C emulsion, the same *meta*-chloro substrate that gave modest results with the TEAF method (compare Table 6, entry 5 with Table 4, entry 7). And both the naphthalene substrate **3.39i** and thiol-derivate **3.39j** afforded excellent enantio- and diastereoselectivities at –5 °C (entries 6 and 8). Satisfied with the results for aryl substrates we ventured to test the conditions on the alkyl amino keto esters. To facilitate the analysis of the alkyl products, we introduced Cbz-protecting group instead of Boc, which for the substrate **3.39k** gave slightly lower selectivity (entry 9).

Nevertheless, alkenyl substrate **3.39l** and *tert*-butyl substrate **3.39m** afforded equally good selectivities and yields as for the aryl substrates (entries 10 and 11), although the latter needed longer time to reach near full conversion (*ca* 7 days). Interestingly, *c*-hexyl substrate **3.39n** and ethyl substrate **3.39o** did not preform well under the same conditions, affording low selectivities (entries 12

and 14). When running the reduction in a different catalyst system, with the metal complex $[RuCl_2(p\text{-cymene})]_2$ and ligand (*S,S*)-TsDPEN, both substrates delivered excellent results (entries 13 and 15).

Table 6. ATH of aryl and alkyl substrates in emulsion conditions.[a]



entry	Product **3.40a-o**	Pg	t (°C)	yield (%)[b]	dr[c]	er[d]
1	**a**	Boc	-5	92	>95:5	97:3
2	**b**	Boc	rt	88	94:6	97:3
3	**f**	Boc	rt	83	92:8	96:4
4	**f**	Boc	-5	85	>95:5	98:2
5	**g**	Boc	rt	85	94:6	96:4
6	**i**	Boc	-5	87	>95:5	96:4
7	**j**	Boc	rt	79	86:14	88:12
8	**j**	Boc	-5	86	91:9	95:5
9	**k**	Cbz	-5	86	>95:5	89:11
10	**l**	Cbz	-5	90	95:5	97:3
11	**m**	Cbz	-5	95	>95:5	99:1[e]
12	**n**	Cbz	-5	93	60:40	n.d.
13[f]	**n**	Cbz	-5	92	95:5	97:3
14	**o**	Cbz	-5	72	67:33	n.d.
15[f]	**o**	Cbz	-5	97	92:8	94:6

[a]General reaction conditions: the solution of $[RuCl_2(benzene)]_2$ (5 mol %), (*S,S*)-BnDPAE (1 mol %) and Et_3N (2 mol %) in CH_2Cl_2 (c = 0.2 M) was stirred at 40 °C for 1h. After cooling to rt, the prepared catalyst was added to the substrate **3.39** (1.0 equiv.) along with 5M HCO_2Na (aq.) and TBAI. The mixture was sonicated for 5–10 min at 0 °C and then stirred over night at room temperature or 2–3 days at –5 °C.
[b]Isolated yield of product.
[c]Determined by ^1H NMR. For >95:5 only one isomer was visible.
[d]Determined by chiral HPLC.
[e]Determined by Mosher ester analysis.
[f]$[RuCl_2(p\text{-cymene})]_2$ (5 mol %) and (*S,S*)-TsDPEN (10 mol %) was used.

It is worth noting, that when the *tert*-butyl and *c*-hexyl substrates were furnished with the Boc-group instead of Cbz-group, as in **3.39r** and **3.39s** (Figure 12) and subjected to emulsion conditions, the products were afforded in much lower yields and diastereoselectivities and thus were not studied further.

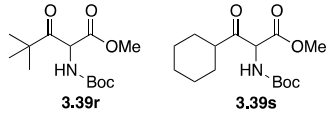
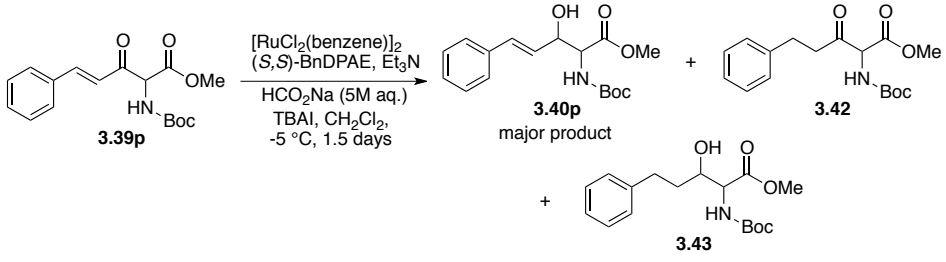


Figure 12. Additional substrates for ATH in emulsions.

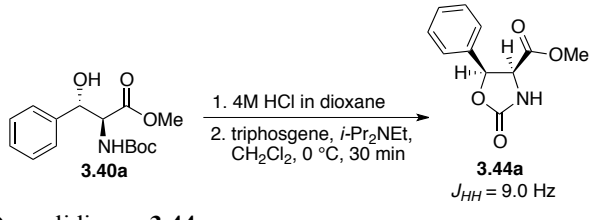
Cinnamic derivates, *e.g.* ceramide[128] and sphingosine[129] derivates and are often used in the synthesis of bioactive compounds. Thus, the substrate **3.39p** was also subjected to emulsion conditions (Scheme 41). Disappointingly, a mixture of products was afforded, a similar result also occurred under the TEAF conditions. Although the major product was identified as the reduced product **3.40p**, also products **3.42** and **3.43** formed, with reduced double bond.



Scheme 41. Cinnamic substrate **3.39p** gave a mixture of products under ATH emulsion conditions.

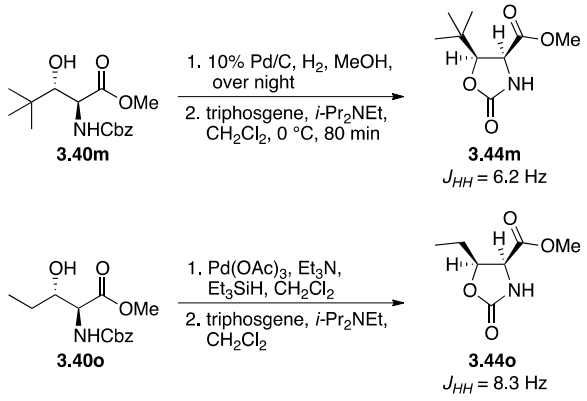
3.2.3. Determination of relative stereochemistry of ATH products.

The relative stereochemistry of selected ATH products was determined via transformation into known oxazolidinones and assessing the vicinal proton *J* coupling by ^{1}H NMR. The phenyl substrate **3.40a** was turned into oxazolidinone **3.44a** in the presence of triphosgene after deprotection of the Boc-amine in acidic conditions (Scheme 42). The J_{HH} = 9.0 Hz value indicates a *cis*-oxazolidinone, which in turn corresponds to the *anti* configuration of **3.40a**. The acquired data also matches that reported for the identical oxazolidionone.[130] Although the absolute stereochemistry of **3.40a** was not determined, the optical rotation of **3.40a** matched the data for the same compound reported in the literature.[131]



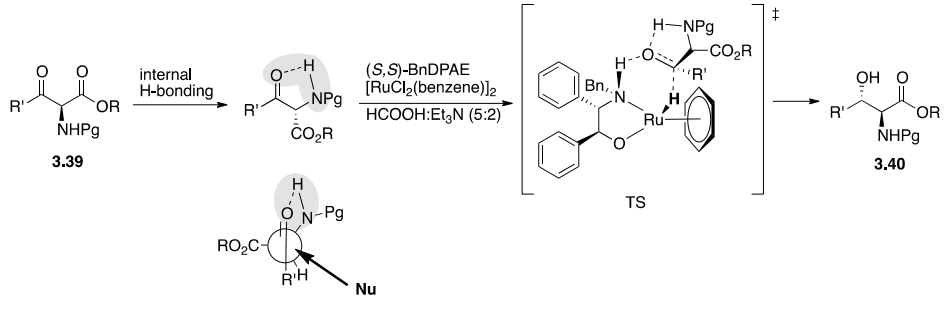
Scheme 42. Oxazolidinone **3.44a**

Similar steps were taken to confirm the relative stereochemistry of the alkyl compounds **3.40m** and **3.40o** (Scheme 43). The J_{HH} = 6.2 Hz for *tert*-butyl oxazolidinone **3.44m** and J_{HH} = 8.3 Hz for ethyl oxazolidinone **3.44o** both consistent with *cis*-stereochemistry, correspond to α-amino-β-hydroxy substituted esters **3.40m** and **3.40o** with *anti*-configuration.



Scheme 43. Oxazolidinones **3.44m** and **3.44o**.

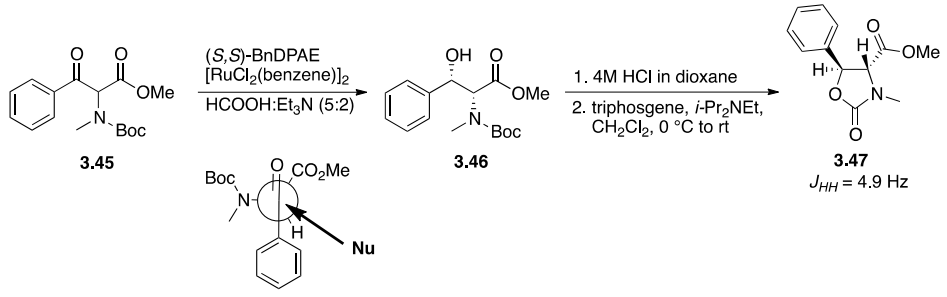
We reasoned that the relative stereochemistry could be a consequence of a intermolecular hydrogen bonding between the proton of the monoprotected amine and the carbonyl oxygen (Scheme 44, shaded in gray). This chelation action, as can be seen in the Newman projection, makes the *Re*-face open for the nucleophile attack. The transition state shows the direction of the attacking hydride and the formation of the product with the *anti*-configuration even more clearly.



Scheme 44. Rationalization of the relative stereochemistry.

The internal hydrogen bonding was confirmed when a substrate with tertiary amine **3.45** was synthesized (Scheme 45). The amine moiety contained both *N*-Me and *N*-Boc groups and was thus unable to take the same conformation as the

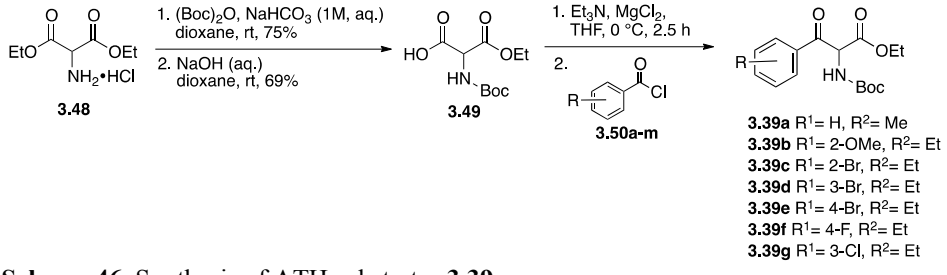
substrates with a secondary amine. The reaction was supposed to follow the polar Felkin-Ahn model, depicted via Newman projection in Scheme 45. After subjecting **3.45** to the ATH conditions, product **3.46** with *cis*-configuration was afforded, in line with our reasoning. The configuration was confirmed by formation of oxazolidinone **3.47** and assessing the vicinal proton coupling constant J_{HH} = 4.9 Hz, which is consistent with the *cis*-configuration. A similar action of mono- and diprotected amine moieties in α-amino aldehydes have been noticed, where it was reasoned that the NHBoc substrate reacted via α-chelated conformer in the presence of a Lewis acid and the doubly protected substrates did not chelate.[132]



Scheme 45. ATH of *N*-Me, *N*-Boc protected substrate. Rationalization of the relative stereochemistry.

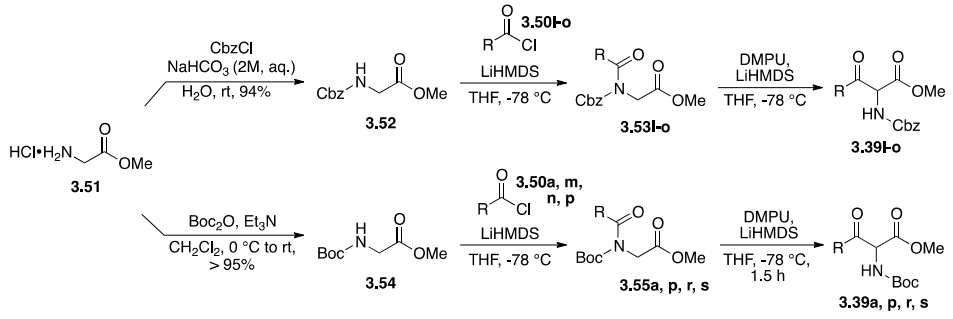
3.2.4. Substrate synthesis for ATH

Several routes were explored in order to find a straightforward, high yielding method to afford substrates for the ATH experiments. Most of the aryl substrates **3.39a–g** were made according to the route shown in Scheme 46. The commercially available diethyl aminomalonate **3.48** was treated with $(Boc)_2O$ to yield *N*-Boc protected compound, and after monosaponification the acid **3.49** was afforded.[133] This acid was then treated with acid chlorides **3.50a–m** of choice to afford the substrates **3.39a–g**.



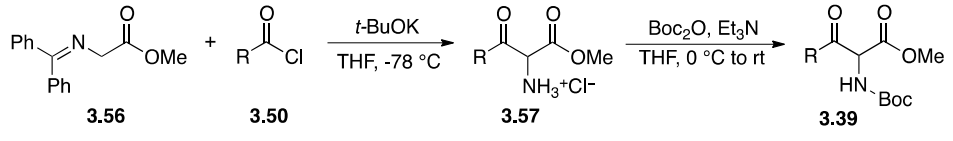
Scheme 46. Synthesis of ATH substrates **3.39a-g**.

During the course of the work a higher yielding method was found and applied for the synthesis of the alkyl substrates **3.39l-o** and **3.39a, 3.39p, r, s** and also *N*-Me, *N*-Boc substrate **3.45** (Scheme 47). The glycine-*O*-methyl ester hydrochloride **3.51** was Cbz-protected in an aqueous environment in the presence of benzyl chloroformate and 2 N $NaHCO_3$ to yield **3.52** in a good yield.[134] Then **3.52** was treated with acid chlorides **3.50l-o** of choice in the presence of LiHMDS at -78 °C in good yields. The resulting imide **3.53l-o** was subjected again to LiHMDS in the presence of DMPU for intramolecular migration of the variable carbonyl group from the nitrogen to the β-carbon to afford the products **3.39l-o**.[135] The Boc-protected substrates **3.39a, p, r, s** were synthesized in the similar fashion (Scheme 47), apart from the protection of glycine **3.51** with Boc-group.[136] Compared with the route described above in Scheme 46, these gave higher yields and should be preferred for the synthesis of all the substrates. A route where glycine **3.51** was first treated with the variable acid chloride and only then was protected with a Boc-group was also tried, but this rendered lower yields compared to when the glycine was protected first.



Scheme 47. Synthesis of Cbz-protected substrates **3.39l-o** for ATH and alternative route for Boc-protected substrates.

An acylation route where the potassium salt of Schiff base **3.56** and the variable acetyl chloride **3.50** were stirred at –78 °C was also tried for Boc-protected substrates (Scheme 48),[137] but was less effective in affording the end products in good yield compared to the aforementioned routes.



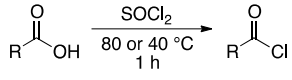
Scheme 48. Alternative synthesis route for substrates.

3.3. Conclusions

An enantioselective method was developed for the synthesis of *anti*-β-hydroxy-α-amino acid derivates by asymmetric transfer hydrogenation via dynamic kinetic resolution. The method was successfully demonstrated on a range of substrates in two different reaction media, without using high pressures or flammable gases. Firstly, a variety of aryl substrates were shown to afford excellent stereoselectivities and high yields in the azeotropic mixture of HCOOH and Et_3N. The scope of the reduction was widened to alkyl substrates in water emulsions where sodium formate acted as a hydrogen donor. The asymmetric transfer hydrogenation in water emulsions is suitable for both aryl- and alkyl substrates and affords the products in high enantio- and diastereoselectivity and in high yields.

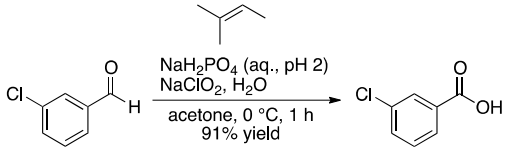
3.4. Experimental

Preparing the variable acid chlorides 3.50 for the substrates of ATH.
General procedure starting from carboxylic acid:



The acid (1.5 equiv.) was dissolved in $SOCl_2$ (1.5 equiv.) and stirred at 80 °C for 1 h. The solution was then let to cool to rt and concentrated to dryness *in vacuo*. The afforded acid chloride was directly used in the acylation reaction without prior purification.

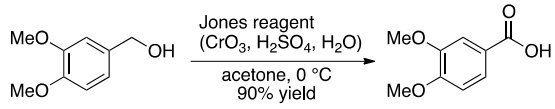
Oxidation of an aldehyde to carboxyl acid:[138]



The aldehyde (200 mg, 1.42 mmol) in acetone (30 mL) was added NaH_2PO_4 (30 mL, aq. satd., pH 2) and cooled to 0 °C. Then the solution was added 2-methyl-2-butene (500 mg, 7.12 mmol) and dropwisely a solution of $NaCl_2O_2$ (257 mg, 2.84 mmol) in H_2O (15 mL) at 0 °C. The reaction was stirred for 25 min after which it was poured onto ice cool H_2O (40 mL). The mixture was extracted three times with CH_2Cl_2. The collected organic phases were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified by column chromatography on silica (50% EtOAc in hexanes) to yield the acid as a desired product (208 mg, 91%).

This procedure was used also for preparing phenyl 3-Br and thionyl acid chlorides.

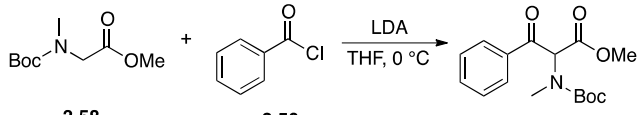
Oxidation with Jones reagent:[139]



The Jones reagent stock solution was prepared in the following way: To the stirred solution of CrO_3 (2.1 g, 21 mmol) in H_2O (5.3 mL) was added conc. H_2SO_4 (1.8 mL) and stirred at rt for 5 min.

To the solution of substrate (150 mg, 0.89 mmol) in acetone (5 mL) was added the Jones reagent stock solution (0.9 mL) at 0°C and the reaction was stirred for 2 h. Then the reaction was added *i*-PrOH (2 mL) and poured into a separatory funnel after 5 min of stirring. After the mixture was then diluted with H_2O and EtOAc, the phases were separated and the aqueous phase extracted once with EtOAc. The collected organic phases were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified by column chromatography on silica (50% EtOAc in hexanes) to yield the acid as expected product (157 mg, 90%).

Synthesis of *N*-Me, *N*-Boc protected substrate 3.45:



3.58 **3.50a** **3.45**

Compound **3.58**[140] (234 mg, 1.15 mmol) was dissolved in THF (10 mL) and the solution was cooled to 0 °C. Freshly prepared LDA solution (10 mL, 1.27 mmol) was added and the mixture was stirred for 2 h. Then the mixture was added dropwise to a benzoyl chloride **3.50a** (147 μL, 1.27 mmol) solution in THF (15 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature overnight. After quenching with H_2O (50 mL), the mixture was extracted with EtOAc (3x30 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (EtOAc in hexanes), product **3.45** was obtained as a clear oil (141.9 mg, 40%).

3.5. References

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4. CONCLUDING REMARKS

In this thesis two synthetic methods are discussed, the first incorporates kinetic resolution in a synthesis of an analogue to a natural compound, and the second focuses on a methodology study using dynamic kinetic resolution. In the first part, four aza- and two thio-analogues of the acetogenins are synthesized according to a modular route. Kinetic resolution of a terminal bis-epoxide affords a crucial intermediate and forges two remote stereocenters in high enantio- and diastereoselectivity. This intermediate forms a linker between the central heteroatom containing fragment and a terminal butenolide, which are deemed relevant in binding to the complex I in mitochondria. The preliminary cytotoxicity assays against HeLa cell line showed the higher potency of the aza-analogues compared to the thio-analogues, although the cytotoxicities of all the synthesized analogues are lower than reported for the natural compounds. We believe this study offers a more facile approach for the synthesis of the acetogenins, allowing variation in the length of the linker and the nature of the central region, accompanied by excellent stereoselectivity. By creating novel analogues with varying bioactivities the study also contributes to the elucidation of the structure-activity relationship of the acetogenins.

In the second part, the synthesis of *anti*-β-hydroxy-α-amino acid derivates is shown by asymmetric transfer hydrogenation via dynamic kinetic resolution. The method was conducted both in water emulsions using sodium formate as the hydrogen donor, and also in azeotropic mixture with triethylammonium formate as the reducing agent. The reduction in water media widened the scope to both aryl- and alkyl-substrates, and afforded the products with high enantio- and diastereoselecivity. It was found that the *anti*-selectivity in the case of monoprotected amines can be caused by the internal hydrogen bonding, absent in the substrate furnished with a dual protected amine moiety, affording the *syn*-configuration. As the method uses water as a solvent, and can be conducted at room temperatures and in air atmospere, it can be considered as a facile and environmentally friendly method for synthesising these important building blocks en route to more complex bioactive molecules.

5. SUMMARY IN ESTONIAN

Atsetogeniinide analoogide süntees. Asümmeetriline vesinikuülekanne α-amido-β-keto estritele dünaamilise kineetilise resolutsiooni teel

Selles töös käsitletakse kahte teemat, esimene kirjeldab kineetilise lahutamise meetodi kasutamist bioaktiivsete ühendite sünteesis ning teine keskendub metodoloogia uurimisele, mis kasutab dünaamilist kineetilist resolutsiooni. Töö esimseses osas näidatakse nelja asa- ja kahe tio-atsetogeniini analoogi sünteesi. Terminaalsete bis-epoksiidide kineetilise lahutamise meetodi rakendamisel saadakse kõrge enantio- ja diastereoselektiivsusega oluline vaheühend kahe kaugelasetseva kiraalse tsentriga. Sellest vaheühendist moodustub analoogis paiknev 'linker', mis ühendab heteroaatomeid sisaldavat fragmenti ja terminaalset butenoliidi. Esmased tsütotoksilisuse uuringud HeLa rakuliinis näitasid asa-analoogide suuremat rakukasvu pärssivat mõju võrreldes tio-analoogidega, samas jäid mõlemad alla looduslike atsetogeniinide tsütotoksilisusele. Tänu sünteesitee modulaarsele iseloomule saab kergelt varieerida stereokeemiat ja vahetada heteroaatomeid sisaldavat tsentraalset fragmenti vastavalt soovitavale analoogile.

Uurimistöö teises osas kirjeldatakse *anti*-α-amido-β-hüdroksü estrite sünteesi asümmeetrilise vesiniku ülekande teel läbi dünaamilise kineetilise resolutsiooni. Aminohappe derivaate kasutatakse laialdaselt keerukamate bioaktiivsete molekulide sünteesil, kus kõrge stereoselektiivsus on tihti määrava tähtsusega. Selliste molekulaarsete 'ehitusblokkide' saamiseks on alati otsitud järjest efektiivsemaid ja loodussõbralikemaid sünteesimeetodeid. Meie väljatöötatud taandamistingimustes andsid nii arüül- kui ka alküülsubstraadid kõrge enantioselektiivsusega produkte, millel on *anti*-konfiguratsioon. Leiti, et substraatidel, millel on mono-kaitstud amiin, võib *anti*-selektiivuse põhjustada molekuli-sisene vesiniksideme teke, mis kahe kaitsegrupiga tertsiaarsel amiinil puudub ja mille saadusel samadel tingimustel on *syn*-konfiguratsioon. Vesi-emulsiooni tingimustes oli võimalik saavutada kõrgete stereoselektiivsuste ja saagistega produkte nii arüül- kui alküülühendite puhul ning reaktsioonid viidi läbi toatemperatuuril ja atmosfäärirõhul, seega võib meie väljatöötatud meetodit lugeda heaks alternatiiviks olemasolevatele meetoditele antud aminohapete derivaatide sünteesiks.

ACKNOWLEDGEMENTS

About the shoulders of Giants.

I am grateful to Dr. Lauri Vares for taking me on as a master student in his newly formed research group in Tartu and later on also as a doctoral student. I have truly enjoyed the learning experience and the fact that chemistry has become increasingly more fun along the way. Although from a supervisor's perspective things might have seemed bleak at times, there have been papers at the end of those long roads. I am also very grateful to Professor Peter Somfai, who kindly allowed me to participate in the ongoing projects in his group at KTH and keeping me as a doctoral student even after moving to Lund University. I am happy to have been a part of both groups. Thank you for your patience and guidance throughout the years!

A great big 'thank you' to group members both from Tartu and KTH for your help with chemistry and good company! Ilme, Meeri, Kaija, Natalja, Alda, Kārlis, Aleksei, Jevgenia, Julia, Indrek Vi, Inderk Ve from Tartu, and from KTH – Jakob, Juho, Virendra, Yan, Morakot, and also others in the lab, coffee room and elsewhere! Special thanks goes to Dr. Brinton Seashore Ludlow and Dr. Tessie Borg from KTH, and Dr. Lauri Toom from Tartu for fruitful discussions – it was nice working with you on the projects at hand.

I am grateful to all professors and lecturers for sharing their knowledge, colleagues from other research groups for their help and a kind word, and to the staff of both in the institute and the university. Your help has not gone unnoticed!

Thanks to Dr. Elo Eriste for helping with the cell work! It was fun, especially when the experiments worked. I would also like to thank Dr. Tõnis Pehk from the National Institute of Chemical Physics and Biophysics in Tallinn for his help with some of my tricky NMR spectra. Also Lauri, Triin and Sergo from proteomics for their help with HRMS analysis.

My endless gratitude goes to my family (the best there is!) for their patience and support during these years! Suured tänud emale, isale ja kahele toredale õele kõikvõimaliku toe ja abi eest! Ly on eriti vapralt oma tugevat närvi näidanud! Aitäh! Also to my friends – new and old, including the ones made in Tartu, and the ones scattered all over Estonia and rest of the world, and of course to Wonderful Peter! You have all contributed to my growth as a person more than you can guess.

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Balticum Organicum Syntheticum (BOS), Tallinn, 01.–04.07.2012. *In Abstracts:* Kinetic Resolution of Terminal Bis-Epoxides. *Posterettekanne.*

Orgaanilise keemia päevad XXIII (Organikerdagarna XXIII), Göteborg, Rootsi, 12.–15.06.2012. *In Abstracts:* Hydrolytic Kinetic Resolution of Terminal Diepoxides in the Synthesis of Bioactive Compounds. *Suuline ettekanne.*

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XVI Euroopa orgaanilise keemia sümpoosium (ESOC), Praha, Tshehhi Vabariik, 12.–16.07.2009. *In Abstracts:* Synthesis of *N*-Analogues of Acetogenins. *Posterettekanne.*

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