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Synthesis of Acetogenin Analogues

Master thesis in organic chemistry

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List of abbreviations

Ac	–	Acetyl
ACG	–	Annonaceous acetogenins
AD	–	Asymmetric dihydroxylation
ATP	–	Adenosine-5'-triphosphate
Bn	–	Benzyl
Cat	–	Catalytic
DBTO	–	Dibutyltin oxide
DHQ	–	Dihydroquinine
DHQD	–	Dihydroquinidine
$(DHQD)_2PHAL$	–	Dihydroquinidine phthalazine-1,4-diyl diether
DMF	–	Dimethylformamide
DMSO	–	Dimethyl sulfoxide
FMN	–	Riboflavin-5'-phosphate
HCT-8	–	Human colon carcinoma cell line
HMPA	–	Hexamethylphosphoramide
HT-29	–	Human colon adenocarcinoma cell line
IC_{50}	–	50% inhibitory concentration
LDA	–	Lithium diisopropylamide
mCPBA	–	*meta*-Chloroperoxybenzoic acid
MDR	–	Multidrug-resistant
MMP^+	–	Methylphenylpyridinium

MOM	–	Methoxymethyl
MPP^+	–	1-Methyl-4-phenylpyridinium
NADH	–	The reduced form of nicotinamide adenine dinucleotide
n.c.d.	–	No conversion detected
Nosyl	–	2-Nitrobenzenesulfonyl
PD	–	Parkinson's disease
PG	–	Protecting group
ROS	–	Reactive oxygen species
rt	–	Room temperature (*ca* 20-26 °C)
SAR	–	Structure-activity relationship
SL-PTC	–	Solid-liquid phase-transfer catalysis
TB	–	Tuberculosis
TBDMS	–	*tert*-Butyldimethylsilyl
TBDPS	–	*tert*-Butyldiphenylsilyl
TBAF	–	Tetrabutylammonium fluoride
TBS	–	*tert*-Butyldimethylsilyl
TEBA	–	Benzyltriethylammonium chloride
THF	–	Tetrahydrofuran
THP	–	Tetrahydropyran
TLC	–	Thin layer chromatography

Introduction

Acetogenins are complicated natural compounds, often containing many chiral centers. Their general structure consists of a long linear aliphatic backbone of 35-37 carbons with a terminal γ-lactone, a central hydrophilic core often containing one or two tetrahydrofuran rings, and several hydroxyl groups at different positions. The chiral centers are placed mostly around the central polar core. Acetogenins are also thought as one of the strongest inhibitors of NADH-ubiquinone oxidoreductase (complex I). Contrarily to many other complex I inhibitors (rotenone), acetogenins are large and less rigid structures due the long alkyl chain. It is believed, that the two functional moieties, the polar core and terminal γ-lactone have an important role in the biological effect, but the structure-activity relationships are yet to be elucidated.

The interest of this project was to decrease the number of chiral centers of a typical acetogenin by substituting tetrahydrofuran (THF) fragment with a core containing amino- or ethylenediamine moieties, with a controlled stereochemistry around it. According to an assumption, replacing the central hydrophilic core with a simpler unit might not change the compound's bioactivity considerably. In the present study, two synthesis routes for two aza-acetogenin analogues were developed. These two synthetic routes have several important aspects, like stereoselective synthesis of the required chiral centers, possibility to easily synthesize other stereoisomers, simpler and shorter synthesis route, employing similar reaction conditions in several steps of the synthesis, and possibilities to enable easy widening of the scope of aza-acetogenin analogues by simply choosing different amino-group-bearing reagents in the key-step.

In addition to the total synthesis project, the synthesis of amphiphilic amino alcohols is discussed more deeply. This was a topic strongly related to epoxide opening problems in the acetogenin analogues synthesis. A convenient method for opening a terminal epoxide with activated amine was developed and a paper about the synthesis of symmetric amphiphilic 1,2-amino alcohols was submitted for publication.

Major goals of the study

The major goal of the present study was to develop a practically usable and versatile synthesis route of acetogenin aza-analogues that provides possibility to easily synthesize the array of products, containing hydrophilic amino-fragment, in different stereo-isomeric forms, at the same time sustaining structural features that are believed to be necessary for their biological effect.

List of papers

I. Toom, L., Villo, P., Liblikas, I., Vares, L. Synthesis of amphiphilic amino alcohols. Submitted to *Synth. Commun.* **2008-05-05**.

II. Villo, P., Toom, L., Liblikas, I., Vares, L. Synthetic studies towards the divergent synthesis of simple aza-analogs of acetogenins. Manuscript in preparation.

Chapter I

Introduction to Annonaceous acetogenins

Annonaceous acetogenins (ACG) are a wide group of bioactive molecules isolated from the plant family *Annonaceae*. The *Annonaceae*, consisting of 130 genera and 2300 species, is chemically one of the least known of the tropical plant families considering its large size. Studies on *Annonaceae* species have intensified from the year 1982, when the first annonaceous acetogenin – uvaricin – was isolated.[1] A waxy substance from root extracts of *Uvaria accuminata* was obtained, which demonstrated high antitumoral properties *in vivo* P-388 lymphocytic leukemia cells in mice. Jolad *et al.* established the structure of uvaricin (Figure 1) using IR, ^{1}H and ^{13}C NMRs and mass spectroscopic fragmentation pattern, showing uvaricin as a C_{34} fatty acid derivative with a terminal unsaturated lactone ring and two adjacent tetrahydrofuran (THF) rings flanked by hydroxyl groups along the long aliphatic chain.

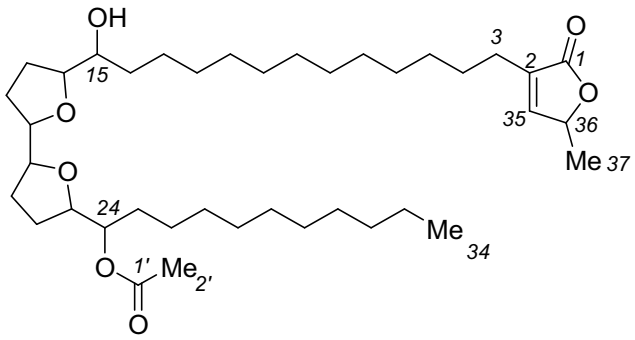


Figure 1. Uvaricin – the first acetogenin isolated by Jolan *et. al.* [1]

After uvaricin, the field of study on similar compounds has widened considerably and the natural annonaceous acetogenins found number more than 400 members.[2]

The general skeleton of acetogenins is unbranched C_{32} or C_{37} fatty acid, ending with γ-lactone. Several oxygenated functions, such as hydroxyl, ketone, epoxide, tetrahydro-

furan (THF) and tetrahydropyran (THP) may be present, as well as double and triple bonds. Thus several types of acetogenins have been characterized, based on the nature of the functional groups, which are present. Majority shows potent cytotoxicity against cancer cell lines being thus antitumoral, antimicrobial, antiparasitic, antimalarial, insecticidal, and antifeedant agents, showing also immunosuppressive activities.[2] They also have been fond to possess high toxicity towards sensitive and multidrug-resistant (MDR) cancerous cell lines.[3]

Although the metabolism of acetogenins in mammals has not yet been studied, it has been shown that acetogenins can penetrate into a cell by passive diffusion because of their lipophilic character,[4] and also cross the blood-brain barrier intact.[5]

Due to its biological activity and limited natural resources, the synthetic studies of acetogenins have been an area of active research for the last 15 years.

Classical and non-classical acetogenins

As the different acetogenins found and synthesized have exceeded the number 400 some years ago, they have been classified into 22 groups according to their structural characteristics.[2] Some of those groups share main characteristics, like the bis-THF, but differing in minor, dividing acetogeins to groups under the same name, for example the adjacent bis-THF and non-adjacent bis THF acetogenins.

General classes of acetogenins:

Linear acetogenins (Group 1)
Epoxy-acetogenins (Groups 2-4)
Mono-THF acetogenins (Groups 5-11)
Bis-THF acetogenins (Groups 12-20)
Tri-THF acetogenins (Group 21)
THP acetogenins (Group 22)

This classification is made based on mainly natural products and is rather crude even for them, considering the vast variety of acetogenins. Although, looking at the

growing number of synthesized mimics, it might be better to have them in fewer wider groups than countless detailed ones.

Family Annonaceae

The *Annonaceae*, or custard apple or annona family, is the largest family of the order Magnoliales, including among others, the cherimoya, soursap, ylang-ylang, and lancewood. Members of the *Annonaceae* grow throughout the tropics. They are particularly characteristic of lowland evergreen forests in Asia and Africa. Five of the genera contain more than one-third of the species; they are *Guatteria*, *Uvaria*, *Xylopia*, *Polyalthia*, and *Annona*. *Asimina* (the dog apple) reaches temperate regions, extending as far north as New York.[6] These plants have few and very specific pests, who are able to use the quite high and toxic amount of acetogenins by accumulating them to keep off predators of their own. One of these pests is a Zebra Swallowtail butterfly, of whose larval feedings the annonaceous acetogenins are retained and sequestered from the leaves of *Asimina triloba* (North-American pawpaw tree) and thus provide the swallowtails with a form of chemical defense against bird predation.[7] For example, one average fruit is estimated to contain 15 mg of annonacin.[8]

Biological activity and mechanism of action

Different parts of *Annonaceae* family have been used by people throughout the ages, mainly in herbal medicine.

Mechanism of action studies have shown that acetogenins are one of the most potent inhibitors of the mitochondrial respiratory chain complex I.[9] For example, acetogenins bullatacin and rolliniastatin-1 (Figure 2) are the most potent inhibitors of the bovine heart mitochondrial complex I identified to date.[10]

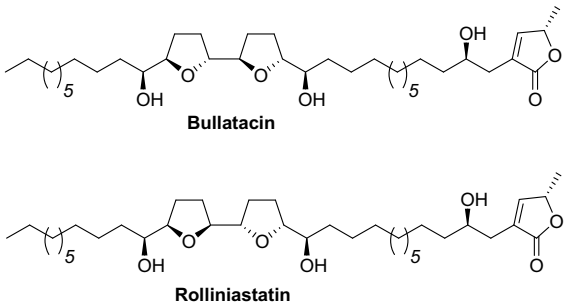


Figure 2. Acetogenins bullatacin and rolliniastatin.

Although acetogenins are thought to act at the terminal electron-transfer step of complex I (Figure 3), there is still no hard experimental evidence to verify whether they in fact bind to the ubiquinone reduction site. As inhibiting the complex I scientifically reduces the ATP levels in the cell, the cell in question goes through apoptosis.

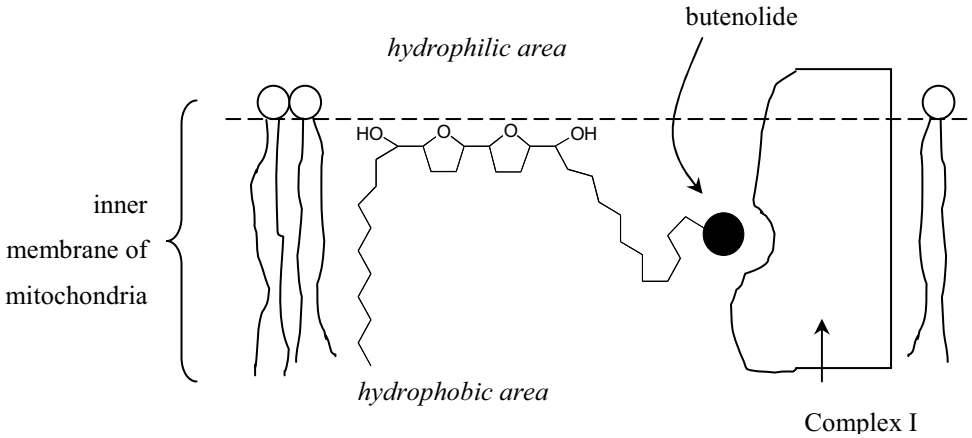


Figure 3. The proposed interaction of acetogenins and complex I.

There are few structural similarities between acetogenins and ordinary complex I inhibitors, such as rotenone, piericidin-A, fenpyroximate, and pyridaben.

When mitochondrial complex I is *fully* inhibited, cells suffer an extensive failure of the mitochondrial oxidative metabolism that yields ATP depletion, and thus cause energetic collapse of the cellular functions: cells die by necrosis. It can occur even under slight complex I inhibition in a time-dependent manner. Resistance of the cells to this energetic collapse mainly depends on their ability to obtain energy from the anaerobiotic metabolism. Contrarily, the *apoptotic process* involves a complex sequence of events that produce selective elimination of the damaged cell in which the

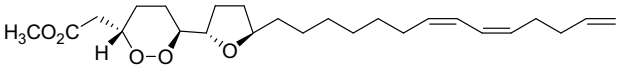
cell exerts some relative control, and it can activate more complicated defensive mechanisms. One of the main apoptotic pathways can be initiated by mitochondrial complex I inhibition.[2] Derived from this scheme, it has been found that different acetogenins, natural or synthesized as described later, possess some important biological activities, like inhibition of tumor cells.[11,12] Tumor cells are under intrinsic increased oxidative stress and vulnerable to free radical-induced apoptosis.[13]

It is also a hypothesis that the mechanism of action may also be related to ionophoric abilities based on observed complexations with cations. Although acetogenins present no ionophoric effects in biological studies with living cells[14], it is shown that structurally related analogues of acetogenins present strong selective abilities to complex bivalent cations, such as Ca^{2+} , that depend both on the nature of the studied cation and the stereochemical relationship of the stereogenic centers in the molecules. It is proposed, that when acetogenins are complexed with Ca^{2+}, they adopt spatial structures different from those of free molecules.[15] These may be considered as active conformations, when molecules interact with their binding sites on the cell membranes.

Other proposed acetogenins

It is supposed that other plant families next to *Annonaceae* might also contain bioactive acetogenin-like molecules which act as pesti- and fungicides, and are located in the low numbers of idioblast oil cells distributed all over the plant. But little research is done in that field.[16]

The Mediterranean tunicate *Stolonica socialis* contains a new class of powerful cytotoxic acetogenins, generally named *stolonoxides* with a potent inhibitory activity IC_{50}<1μM on mitochondrial electron transfer.

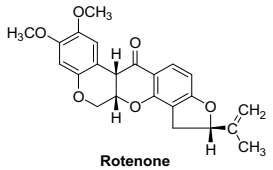


Stolonoxide A

The compounds affect specifically the functionality of complex II (succinate:ubiquinone oxidoreductase) and complex III (ubiquinol:cytochrome C oxido-reductase) in mammalian cells, making the protein target different of acetogenins. But it is still thought that, although acetogenins and stolonoxides are very similar in their molecular structure, their mode of actions as inhibitors might be coincidental.[17]

Rotenone

Lannuzel *et al*. showed that annonacin, one of the natural acetogenins, promotes dopaminergic neuronal death by impairing energy production, and suggested that acetogenins in tropical plants of *Annonaceae* family play a role in some forms of Parkinsonism.[8,10] High prevalence of the speculated acetogenin-connected atypical Parkinsonism has been reported in five different geographic isolates over the last 60 years.[18] However, experimental studies in animals demonstrated that *Annonaceae* acetogenins neurotoxicity is similar to rotenone neurotoxicity.[19] Rotenone is a lipophilic natural complex I inhibitor from the plant family *Fabaceae* or Pea family. The family consists of trees to annual herbs, which can be found in the same greographical regions as *Annonaceae*.



Rotenone

Inhibition of mitochondrial respiratory chain complex I by rotenone had been found to induce programmed cell death in a variety of cells. Rotenone-induced apoptosis was inhibited by treatment with antioxidants, for it was induced via enhancing the amount of mitochondrial reactive oxygen species (ROS) production.[20]

Structure-activity relationships

The bioactive annonaceous acetogenins have attracted a large amount of interest because of their strong inhibition effect on complex I. The plant extracts of Annonaceae contain fairly small amounts of acetogenins, also the extracts contain mix of various compounds and need long purification processes. Thus effort towards semi- and total synthesis with biological studies of these compounds appear more and more in the literature.

To elucidate the structure-activity relationship (SAR), and the mechanism of action, it is necessary to synthesize the acetogenins in various analogues. It would be very desirable to develop the simplified synthesis for mimics of natural acetogenins while maintaining all of their essential functionalities needed for the inhibitory effect.

There are many important reasons for structural simplification. Firstly, to facilitate the synthesis path, especially concerning the multiple chiral centers. It is shown, that the mimic will sustain its activity even with considerably less chiral centers. Secondly, to find out which functional features are critical retaining the inhibition effects of the compound. Thirdly, how does the compound bind to the complex and effects the organism.

There are some points, in which some researchers generally agree upon concerning SAR (Figure 4):

1) the presence of polar functional groups like OH-groups in the spacer, number or existence of THF rings in the core and stereochemistry around the hydroxylated THF rings are not essential structure factors for potent activity;

2) natural γ-lactone ring can be substituted with ubiquinone ring;

3) alkyl spacer between the core and ring is needed, with the optimal length of 13 carbon;

4) long alkyl tail helps with activity.

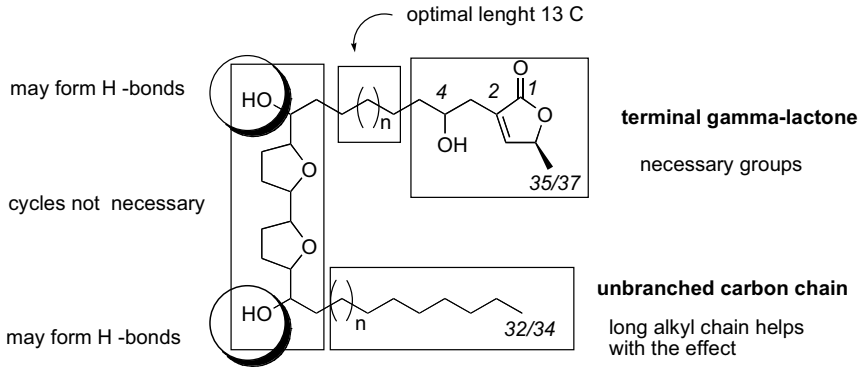


Figure 4. General structure of acetogenins.

Most studies suggest that the terminal γ-lactone is essential for the potency of acetogenins as complex I inhibitors, although some reports propose that the alkyl chain plays more crucial role. However, for the roles of other ring moieties have not been sufficiently emphasized. The α,β-unsaturated γ-methylbutyrolactone ring is common to a large number of natural acetogenins.[13] The Miyoshi group reported that the inhibitory potency was almost completely retained even by the saturation of the double bond in the γ-lactone ring.[21] But it is also said the ring can be substituted or the acetogenin mimic can show considerable inhibition even without the ring moiety. Nevertheless, there is consensus on the proposition that the ring moiety has to be directly linked to the compound core by the alkyl spacer.[22]

Regarding the core of the compound, neither the number of THF rings nor the stereochemistry around them is essential for the inhibition effect.[23]

As the structural factors can be variable in acetogenins, the complex I may be recognized by the inhibitor in a fairly loose way.

THF ring moiety seems to serve a role as an anchor in the lipid membranes. This may be related to the uniquely potent bioactivities that *Annonaceous* acetogenins exhibit at their enzyme-inhibitory sites within mitochondrial and plasma membranes.[24] For maximum potency of the inhibitor, it is recommended to have hydroxyl groups somewhere in the chain, as the acetylation decreased slightly (6-fold) the inhibition. It

was proposed, that rather than the H-bond donating ability of OH-groups, the high polarity of them is more vital for the active confirmation.

Besides the important structures, the factors deciding the potency of acetogenins included dissolubility in cells and mitochondria are the conformation of mitochondrial membrane, transportation, and the metabolism of energy conservation.[28]

Some reported total synthesis of the analogues of acetogenins

The primary objective of total and semi- synthesis of acetogenins is to establish structure-activity relationship, find facile and stereoselective synthesis routes and finally to find an optimal structure for the inhibitor.

Aza-solamin

The aza-solamin, an amino-analogue solamin, was synthesized in order to investigate the chelation properties of acetogenin mimics. A stereoselective preparation of C_1-C_{32} skeleton of aza-solamin (Figure 5), the pyrrolidino-analogue of the mono-tetrahydrofuranic solamin, isolated from several species of *Annonaceae,* which has shown interesting cytotoxic activity, was reported.[25] The synthesis started from (+)-*N*-Boc-aza-muricatacin and proceeded in a linear fashion, using *C*-glycosylation reaction with cyclic *N*-acyliminium ion and 2-trimethylsiloxyfurane to afford pyrrolidine analogues with good stereoselectivity and high yields. Unfortunately, no further experiments on structure-activity relationship were reported.

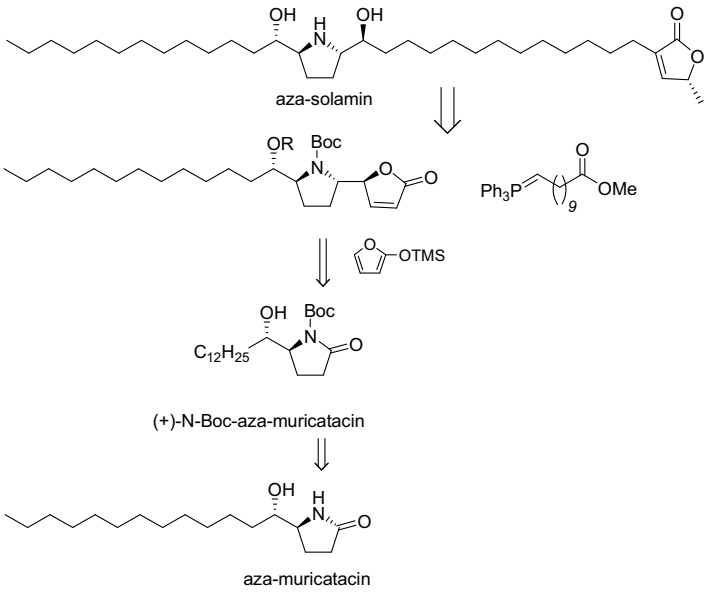


Figure 5. General synthesis of aza-solamine

Aza-analogue with N,N'-dibenzylethylene-1,2-diamine core

Wang *et. al*. published recently a synthesis of a single acetonenin aza-analogue.[26] The synthesis starts for both sides of the analogue from D-mannitol (Figure 6), which gives the two stereocenters in the amino core. This means that the chirality is fixed in this kind of synthetic route, and does not allow synthesis of enantiomers for the aza-analogue. Also, they lack a stereocenter for the hydroxyl group in the position 4, which most of the natural and synthetic acetogenins possess. They use *N,N'*-dibenzylethylene-1,2-diamine to obtain an amino alcohol over epoxide opening. Their final product still contains the benzyl protecting groups they also do not propose a method to cleave them.

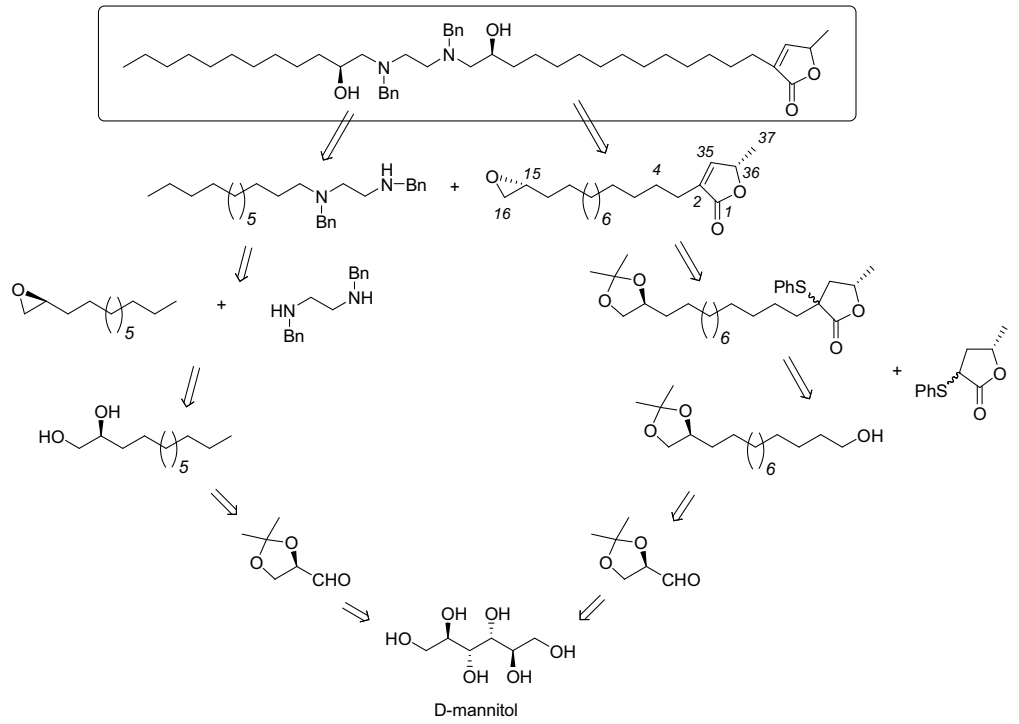


Figure 6. General synthesis of an analogue with *N*,*N*'-dibenzylethylene-1,2-diamine core.

Analogue with polyether-core

Like discussed above, the inhibition activities of acetogenins are associated with their ionophoric ability. Therefore, the hydroxyl and ethereal oxygen atoms in the acetogenins appear to be essential for the biological activity, but not the THF rings. Thus, a simplified linear analogue with (4*R*)-hydroxyl was proposed (Figure 7), where the ethylene bridge in the THF rings was removed, and eliminating four chiral centers.[27] The synthesis was done in two parts, with a two-directional *C*-alkylation of 1,7-octadiyne with the two epoxides of polyether core, and adding lactone-ring moiety in the final coupling. The synthesis for the left side of the analogue started from chiral aldehyde, prepared from D-mannitol. The extension of the carbon chain was done by Wittig reaction, and the double bond hydrogenated. The diol was attained by acid-catalyzed deprotection, followed by regioselective *O*-alkylation. The alcohol was then coupled with (*R*)-(–)-epichlorohydrin in the presence of a phase-transfer catalyst. Synthesis of other key intermediate, the lactone-ring moiety, started with an ester prepared from malonate and allyl bromide. The lactone unit was introduced trough three-step sequence, involving aldol reaction, in situ lactonisation and *β*-elimination of

hydroxyl. Regioselective epoxidation was achieved by treatment with mCPBA, after which the terminal epoxide was resolved. In the final part the key intermediates were coupled, multiple bonds reduced and hydroxyl groups deprotected, giving the target compound.

The ananalogue showed cytotoxicity in vitro against the HT-29 (IC_{50} = 1.6×10^{-3} μg/ml) and HCT-8 (IC_{50} = 8.0×10^{-2} μg/ml) cell line. The same group has published a synthesis for a similar analogue, but without the (4*R*)-hydroxyl group.[28] This showed cytotoxicity only towards the HT-29 cell line and was not as effective (IC_{50} = 2.4×10^{-2} μg/ml), showing that the (4R)-hydroxy group greatly increases the biological activity of the compound.

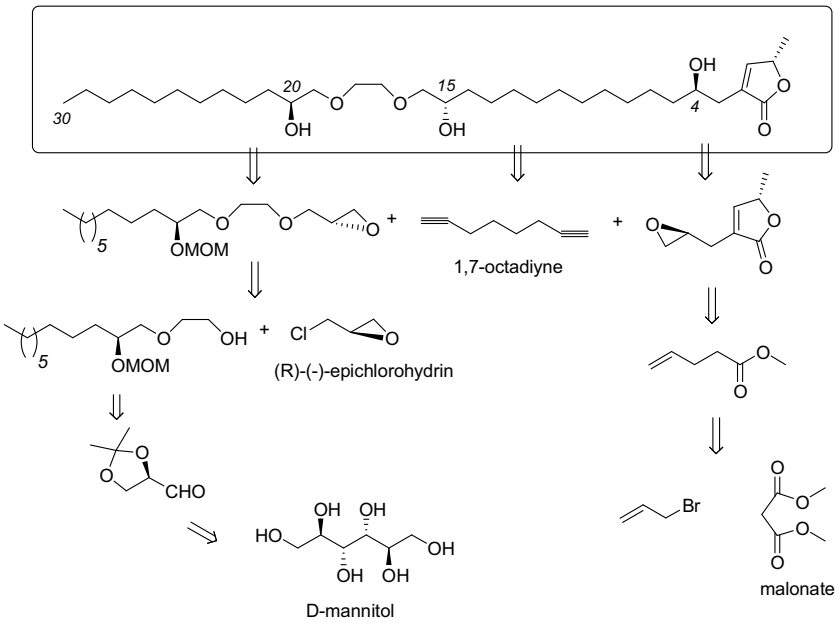


Figure 7. General synthesis of an analogue with ethylene glycol core.

Another polyether mimics were synthesized with diethylene and triethylene glycol cores[29] (Figure 8), to study if they maintain their ionophoric ability necessary for the inhibitory effect. The preliminary screening showed that the polyether mimics had compatible cytotoxicity with the corresponding natural acetogenins, bullatacin and solamin, although with decreased values. Unfortunately, no ionophoric properties were studied.

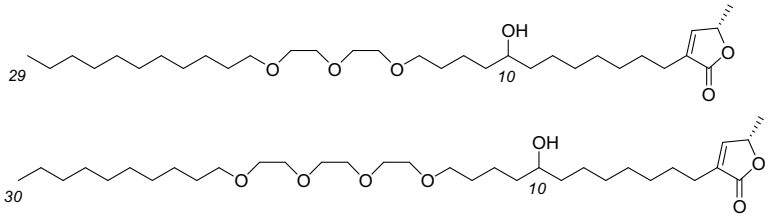


Figure 8. General synthesis of analogues with diethylene and triethylene glycol cores.

Analogue with a tetraol core

A compound named dihydroxycohibin A (Figure 9), which only has hydroxyl groups along the alkyl chain together with the terminal lactone, was proposed by Konno *et al.*[30] Authors were interested to investigate, if the hydrophilic moiety alone could compensate the lack of THF rings in the core of the compound, as the hydrophilicity in the region of THF site is essential. The synthesis begun with (+)-muricatacin. Muricatacin in a hydroxyl butanolide, originally extracted from *Annona muricata*. It also has a (–)-isomer, and other stereoisomeric analogues can be synthesized in an enantiomerically pure form.[31] The (+)-muricatacin chain was extended by Horner-Emmons reaction. Hydroxyl groups were mostly protected by MOM or by ethyl vinyl ether in different steps. Sharpless AD was used in this synthetic route to provide two other chiral centers in the core. In the epoxide forming step, they had 92% of diasteromeric excess of the desired oxirane but the undesired diasteromer could be removed by column chromatography. The cross-coupling of the two ends of the analogue was executed with $Pd(PPh_3)_4$, CuI, and pyrrolidine without solvent. After catalytic hydrogenation of multiple bonds in the alkyl linker, the sulfide moiety was eliminated by thermal elimination. Finally, the hydroxyl groups were deprotected, yielding the target compound dihydroxycohibin A.

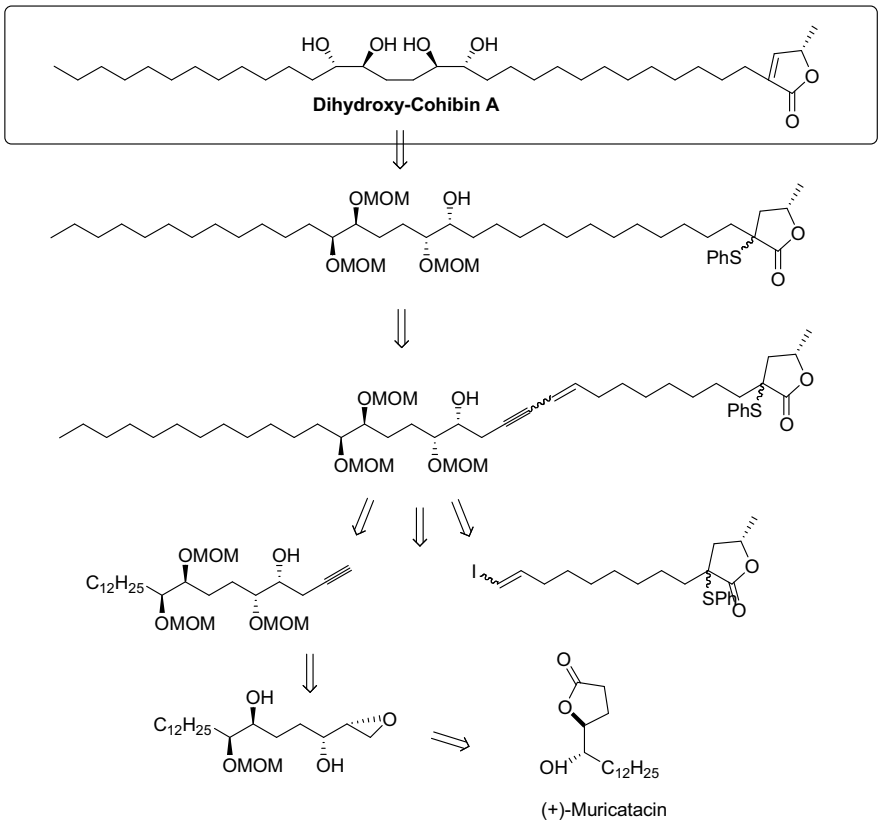


Figure 9. General synthesis of an analogue with tetrahydroxyl core.

The inhibition of complex I activity was determined by NADH oxidase assay using bovine heart submitochondrial particles. The reference was the potency of bullatacin, one of the most potent natural acetogenins (IC_{50} = 0.8 nM). Under the same experimental conditions, for dihydroxycohibin A the IC_{50} = 20 nM but with protected hydroxyl groups the activity was lost (IC_{50} = 4100 nM). Although, it was determined that the free hydroxyl groups are essential for the inhibitory effect, it is not as effective as bis-THF hydroxyl groups combination.

Chapter II

Amphiphilic compounds

Amphiphilic compounds are characterized by possessing two groups in the same molecule, which differ greatly in their polarity. These are: (i) a hydrophilic group that is typically charge-polarized and capable of hydrogen bonding and (ii) a lipophilic group that is non-polar and thus prefers neutral medium and nonpolar solvents.

According to the relative magnitudes of the hydrophilic and lipophilic parts, amphiphilic compounds may range from predominately hydrophilic to predominately hydrophobic. In solutions, molecule arranges its structure depending on its surroundings. As an example, in aqueous solutions an amphiphile may form a micelle, where the nonpolar groups (usually long hydrocarbon chains) associate together in fluid arrangement, leaving the polar groups in association with polar water molecules. The formation of micelles and their properties are complex and depending on many variables, like concentration, temperature and different solvents.

Amphiphilic amino alcohols

Amphiphilic compounds incorporate a wide variety of structurally different substances with various modes of action. In the present study, the focus is more specifically on amphiphilic 1,2-amino alcohols. Amphiphilic amino alcohols consist of a vicinal amino alcohol moiety, which is linked to one or two aliphatic alkyl chains. They are of interest because of their versatile properties, such as (i) amine-based surfactants[32], (ii) antimicrobial compounds[33], (iii) potential ionophores.[34] For example, a good example of amphiphilic amino alcohols possessing antimicrobial properties is a

series of compounds used in treatment of tuberculosis (TB). TB is an infectious disease caused by *Mycobacterium tuberculosis* and it affects millions of people world-wide. One of the main clinically used anti tuberculosis drug, for example, is ethambutol **1** (Figure 10).35 Tripathi group synthesized a galactopyranosyl amino alcohol **2** and **3** (Figure 10), and some other similar analogues, varying in the length of alkyl linker in compound **2** and the N-substituent in compound **3**. The amino alcohol analogue **3** showed an activity against Mycobacterium tuberculosis H37 Rv, but also against MDR TB (multidrug-resistant TB), which was in vitro superior to ethambutol.[36]

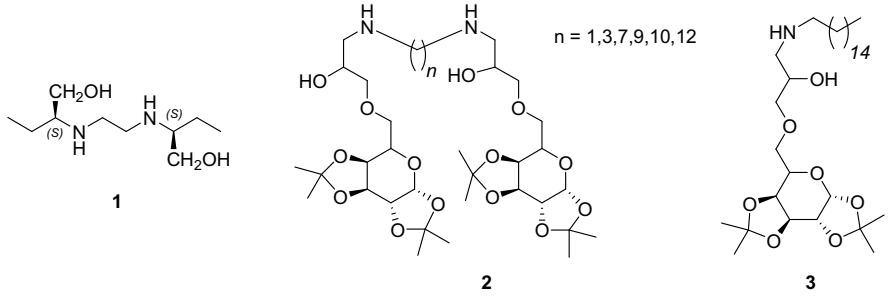


Figure 10. Ethambutol **1** and glycosyl amino alcohols **2** and **3**.

Acetogenins is a novel class of potential anticancer agents, originally isolated from the plant family *Annonaceae*. They can be looked at as amphiphilic molecules as well, because coming in a variety of structural features; they commonly have a polar head group connected to two lipophilic tails, with one of them ending in a lactone-ring moiety.

One example of naturally occurring acetogenins is solamin (Figure 11).[37] Usually the polar head contains one or more THF rings with adjacent hydroxyl groups, thus furnishing the compound with many chiral centers. Acetogenins can also be categorized under antimicrobial and antitumoral compounds, as they have a strong inhibitory effect on complex I and show significant activity even towards multidrug-resistant cell lines. Therefore, it is of great interest to find out their structure-activity relationships and widen the scope of acetogenins by synthesizing different non-natural analogues. Due to the number of chiral centers in these compounds, the synthesis has to be carefully planned and also performed in a stereoselective manner, which makes it an interesting and challenging subject.

Recently, acetogenin analogues containing an amino alcohol structural motif have also been published, for instance aza-solamin (Figure 2)[38], which synthesis was briefly discussed in Chapter I.

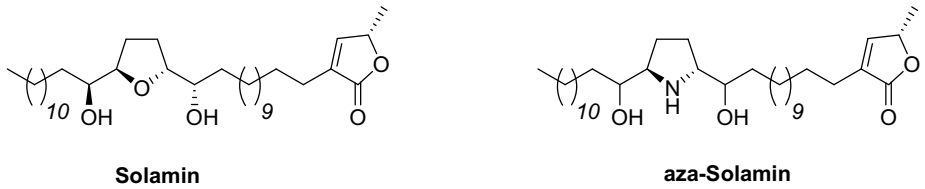


Figure 11. Naturally occurring acetogenin solamin and its synthetic non-natural analogue aza-solamin.

Chapter III

Our synthetic strategies of aza-analogues of acetogenins

In literature the dominating trend is to synthesize analogues with THF rings, mimicking the natural compound, or eliminating the ring moieties, leaving the ethereal functions. In this work synthesis of aza-analogues with amino cores are discussed. The amino functions in the analogue were chosen for several reasons. Firstly, it is hoped that the amino moiety would increase hydrophility of the compound and therefore the solubility in an organism's environment, helping it to reach its target of biological activity. Secondly, it is speculated, that acetogenins might have chelating properties in the cell promoting the biological activity. Thus the choice of diamine core, which might form an active conformation with other polar groups around ions more easily. There is also a plan to synthesize analogues with polyamine core to further contribute to these properties.

The synthesis for both **compounds I** and **II** (Figure 12) can be looked at in three sections: (i) the synthesis of the alkyl linker from diene; (ii) synthesis of lactone-ring moiety; (iii) synthesis of amino alcohol – alkyl tail in the complete compound with the core moiety.

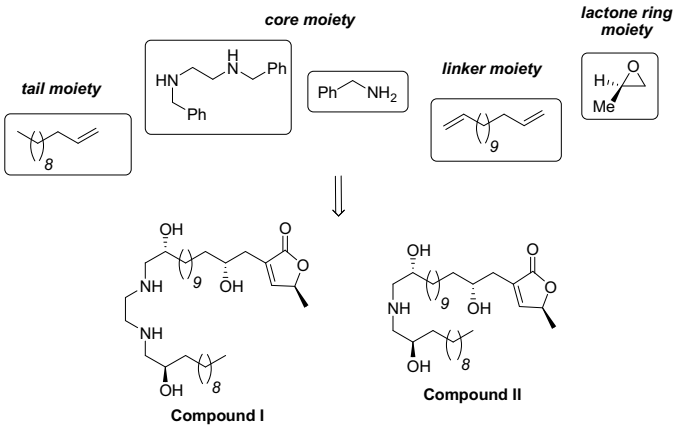


Figure 12. Compounds I and II

Synthesis of amphiphilic 1,2-amino alcohols

Synthesis

While studying the synthesis of acetogenins, an important topic arose in the form of amino alcohols. These compounds became the key intermediates in the synthesis because of a convenient and seemingly easy coupling with epoxides, and needed to be studied more closely. The amino alcohols **4** and **5** (Figure 13) in focus for the synthesis have a polar amino alcohol head group with one or two amine functionalities, and two lipophilic tail groups.

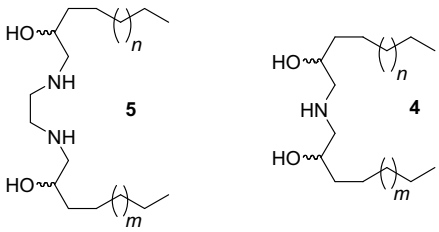


Figure 13. Amino alcohols with ethylene diamine alcohol core **4**, and amino alcohol core **5**.

The starting compounds for the synthesis of the amino alcohols were terminal olefins with varying chain lengths and number of double bonds (Figure 14). The alkenes were first dihydroxylized, the primary hydroxyl activated by tosylation and converted to epoxides. The diols synthesized in this study are enantiomerically pure (92:8), which also gives an enantioselective entry to following compounds. The epoxide **9** was opened with ammonia or ethylene-1,2-diamine (also marked as X in the Figure 14), producing amino alcohols **6** or **7**, respectively. These amino alcohols were then used to open the epoxide **8**, yielding the final products **4** or **5**, respectively. The synthetic scheme is discussed in detail further on.

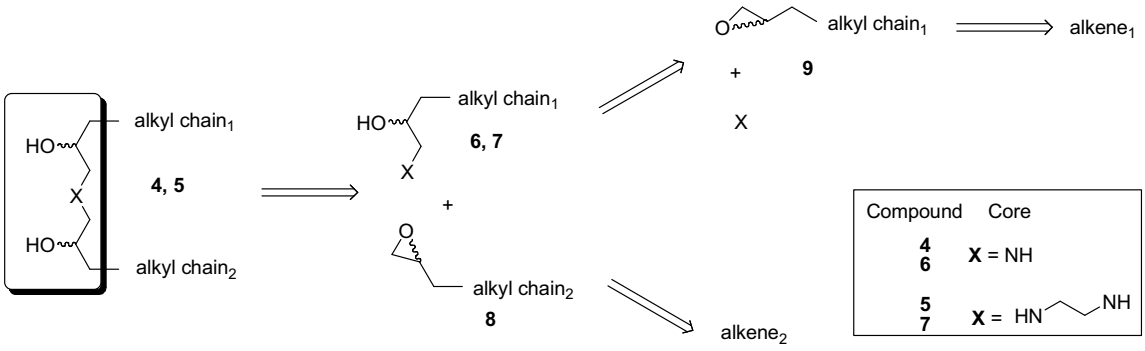


Figure 14. General synthetic route to amphiphilic amino alcohols.

Synthesizing a compound with a desired stereochemistry is an issue with an utmost importance in this project. In order to convert the alkenes to epoxides for further synthetic steps, the right stereochemistry had to be assured. Direct epoxidation of olefins depends greatly, in stereoselectivity aspect, on the geometry of the olefin and on the reaction properties. With terminal olefins lacking geometric isomerism, the direct epoxidation has thus very low stereoselectivity. Therefore, to set chiral centers before epoxidation, the olefins were asymmetrically dihydroxylated.

Asymmetric dihydroxylation of alkenes

In the present study, a Sharpless asymmetric dihydroxylation (Sharpless AD) using I_2-K_2CO_3-$K_2OsO_2(OH)_4$ catalytic system in the presence of a chiral ligand $(DHQD)_2PHAL$ was used to asymmetrically dihydroxylize olefins.[39]

The Sharpless AD takes place in basic two-phase conditions, using catalytic amount of osmium tetraoxide with a stoichiometric amount of co-oxidant (e.g. I_2) in the presence of a catalytic amount of chiral ligand (Figure 15A). The ligand used in this project was $(DHQD)_2PHAL$ (Figure 15B) to provide the desired *(R)*-enantiomer. If needed, the chirality can be inverted by simply choosing the other ligand $(DHD)_2PHAL$ (Figure 15C).

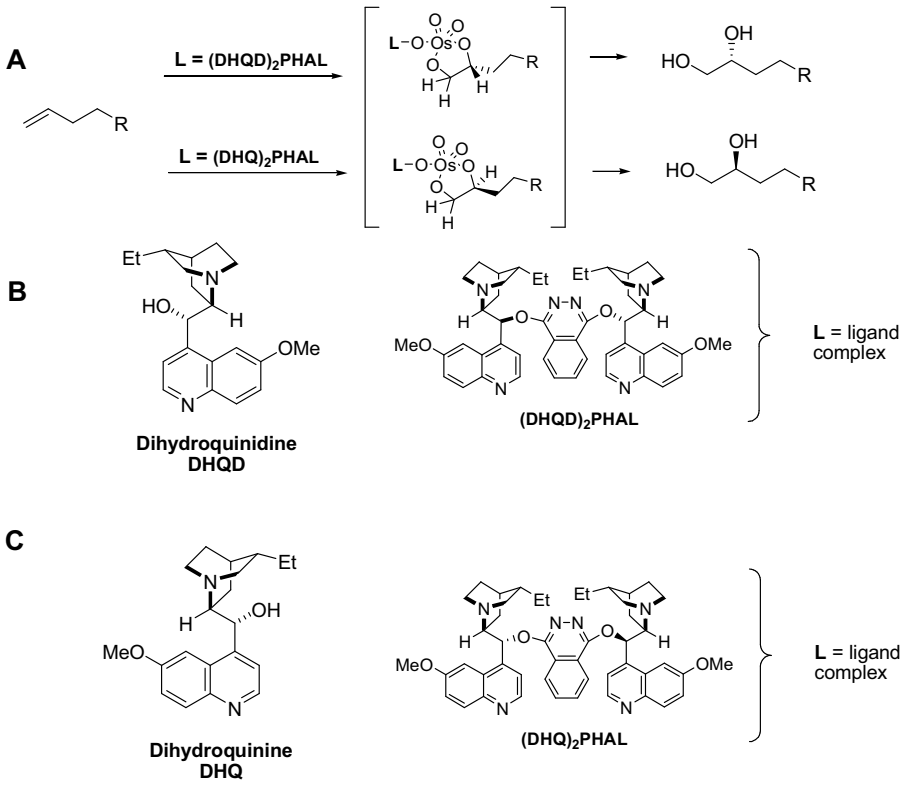
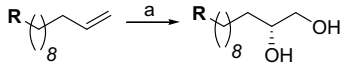


Figure 15. A) Sharpless asymmetric dihydroxylation of a terminal olefin; **B)** chiral ligand DHQD and ligand complex $(DHQD)_2PHAL$; **C)** chiral ligand DHQ and ligand complex $(DHQ)_2PHAL$.

The terminal olefins to be dihydroxylated were 1-dodecene **10a** and 1,13-tetradecadiene **10b** (Scheme 1). They were originally chosen for the synthesis of analogues of acetogenins, and thus the number of carbons in the alkyl chain were necessary.



10a R = $-CH_3$ **11a** R = $-CH_3$
10b R = $-CH_2CH{=}CH_2$ **11b** R = $-CH_2CH{=}CH_2$

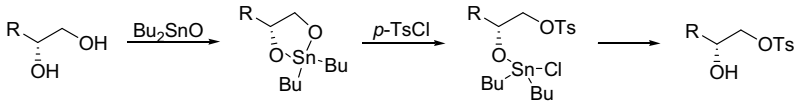
Scheme 1. Sharpless AD of **10a** and **10b**. Conditions: a) $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$, K_2CO_3, I_2, *t*-BuOH/H_2O, 0 °C, 6 h, 97% (**11a**), 3 h, 54 % (**11b**)

The procedure for the two alkenes slightly varied in the period of time because of the number of possible reaction sites in the compounds. As the diene **10b** has two potential reaction sites, the reaction must be ended when the first traces of both double bonds being dihydroxylated were observed. Nevertheless, for both cases, the olefin was added to the stirred solution of reagents and stirred at 0 °C for either 6 h or about 3 h for

the diene **10b** (the reaction was monitored by TLC), producing the diols **11a** and **11b** in high yields – 97% and 54% (95% if considering the recovered diene), respectively. The yield of **11b** was lower because some of the diene had two diol moieties, which were hydroxylated, and also some of the unreacted diene was recovered, because the early ending of the reaction. The chiral GC analysis showed enantiomeric ratio of 92:8 both for **11a** and **11b**.

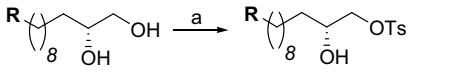
Selective tosylation of primary alcohols

In order to convert the formed vicinal diol to epoxide, the primary hydroxyl group had to be activated while assuring the conservation of the secondary hydroxyl group. This was done by selective tosylation of the primary hydroxyl-group. The tosylation was executed by following a selective sulfonylation method for primary alcohols, using dibutyltin oxide (DBTO) as a catalyst.[40] Tosylation reaction was also done without the DBTO catalyst.[41] In the literature, the DBTO-catalyzed tosylation showed selective and rapid reaction at the primary alcohol, while uncatalyzed versions proceeded slowly and with lower selectivity. The selectivity comes from the fact that the catalyst activates the primary hydroxyl group while protecting the secondary hydroxyl group (Scheme 2). The presence of a base is essential to neutralize the forming HCl in the reaction, as the catalyst performs with better regioselectivity in neutral conditions.



Scheme 2. Tosylation of vicinal diols with *p*-TsCl in the presence of cat DBTO.

The reaction mixture of **11a** and **11b** with DBTO, *p*-TsCl and triethylamine in CH_2Cl_2 was stirred at rt for 4-18 h until no unreacted diol was sited by TLC (Scheme 3). The products **12a** and **12b** had high yields and were converted to epoxides without further purifications.



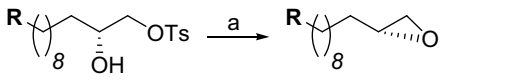
11a R = $-CH_3$ **12a** R = $-CH_3$
11b R = $-CH_2CH{=}CH_2$ **12b** R = $-CH_2CH{=}CH_2$

Scheme 3. Tosylation of **11** with DBTO as catalyst. Conditions: **a)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h, yields >95%.

Tosylation without the catalyst was also tried, using pyridine as a base instead of triethyamine, but as the yields (about 50-60 %) were lower than with the catalyst, and therefore the DBTO-catalyzed tosylation method was used in the synthesis route.

Epoxide formation

The tosylated compounds **12a** and **12b** were converted into epoxides under basic conditions in MeOH with yields of 70% (**12a**) and 96% (**12b**) over 2 steps (Scheme 4). The cyclization was made in basic conditions, where **12a** and **12 b** was stirred with K_2CO_3 in the mixture of MeOH/CH_2Cl_2 for about 30 min at rt.[42] The crude yield of both **8** and **9** exceeded 95% and they were used in the next step without further purifications.



12a R = -CH_3 **9** R = -CH_3
12b R = -$CH_2CH{=}CH_2$ **8** R = -$CH_2CH{=}CH_2$

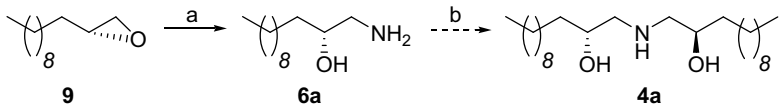
Scheme 4. Epoxide formation. Conditions: **a)** K_2CO_3 (1.5 eq), MeOH/CH_2Cl_2, 30 min.

Epoxide opening with an amine

The next step was to couple the epoxides with an amine moiety.

The epoxide opening with amine proved to be in some cases difficult. The yields and rates depended greatly on the nature of amines, thus being often low. The first epoxide opening with amine or diamine proceeded smoothly and with high yield, producing the corresponding amino alcohol. Coupling the amino alcohol with epoxides had unexpected slow rated and low yields, having often enough no conversion at all. These reactions are discussed as follows.

At first, the coupling was done with epoxide **9**, where the epoxide was opened with aqueous ammonium hydroxide, refluxing in EtOH for 16 h (Scheme 5, step a). The amino alcohol **6a** was produced in high yield of above 95% and thus used in the next step without further purifications.

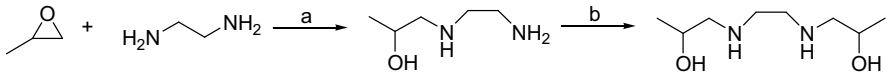


9 **6a** **4a**

Scheme 5. Epoxide opening. Conditions: **a)** NH_4OH (excess), EtOH, 60 °C, 16 h.

The coupling of amino alcohols **6a** with epoxide **9** was surprisingly sluggish, having no conversion to **4a** at all. There were several test reactions made with the second coupling. Different solvents and reaction times were tried with no success, also attempting to activate the epoxide either with some Lewis acids or a Brønsted acid did not give satisfactory results (see Table 1).

At this point, we speculated about the possibility of micelle formation, considering the amphiphilic properties of amino alcohol **6a**. It could be that the amphiphilic compounds **6a** would form a micelle in the reaction mixture, turning the long lipophilic tails out and the polar amino alcohol heads inside of the micelle moiety, thus making the reaction between amine and epoxide impossible. To test this hypothesis and the possible effect of the long alkyl chain, a new test reaction was made with an epoxide with a short alkyl tail, thus minimizing the lipophilic properties of the compound. Propylene oxide was then reacted with an excess of ethylene diamine (Scheme 6), producing a amino alcohol with a good yield as expected. The amino alcohol compound **7b** does not have the lipophilic tail like **6a** and the second step, coupling **7b** with propylene oxide proceeded smoothly to form a symmetric product **5c**. However, when propylene oxide was reacted with **6a**, no reaction took place.



7b **5c**

Scheme 6. Conditions: **a)** CH_3CN, 65 °C, 24 h, 68%; **b)** propylene oxide (3 eq), CH_3CN, 65 °C, 24 h, 87%.

It was concluded that the amino alcohol with strong lipophilic properties could form micelles and by that prohibit any reactions involving the polar head group. Minimizing the lipophilic effect would also reduce the micelle forming. Consequently, reducing the polarity of the head group in the amphiphilic compound **6a** should also reduce its

inclination to form micelles and as a result, increase its reactivity in the subsequent coupling reaction.

There was a search for suitable *N*-substituents in order to increase reactivity and to reduce polarity in the amino group side of the molecule. Three groups that were chosen: tosyl, nosyl, and benzyl groups (Figure 16), all had been used is some ways earlier in epoxide openings with *N*-protected amines. The idea of using tosyl- and nosylamides as the reagents in the opening of epoxides was driven by the fact that the acidities of the sylfonylamides are greately increased compared to the corresponding alkylamines. Tosylamide has been used to open epoxides under solid-liquid phase-trasfer catalysis (SL-PTC), and later removed in mild conditions.[43,44] Also, the nosyl and benzyl groups were used successfully in similar reactions as activating groups for amines.

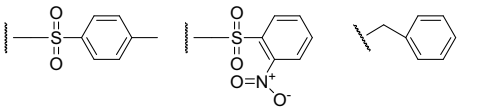
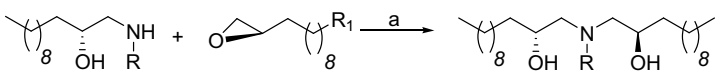


Figure 16. Protective groups.

The compounds **6b**, **6c** and **6d** were prepared from tosylamide, nosylamide and benzylamine, respectively (Scheme 7). These were coupled with epoxides **8** and **9**. When tosylamide **6b** and epoxide **9** were refluxed in 2-PrOH, no conversion was detected after five days. Coupling of **6b** with epoxide **8** gave 53% yield of compound **4b** after heating the reaction mixture for one week in dioxane at SL-PTC conditions. When **6c** was used instead, the coupling with **8** afforded **4c** in 54% yield using the same SL-PTC conditions. Therefore, the assumption of employing activating properties of tosyl and nosyl groups did not induce good conversion, but this might have also been due to decomposition of the phase-transfer catalyst under these conditions. Finally, when reacting **6d** with the epoxide **8**, the desired coupling product **4d** was obtained in 87% yield after just 48 h. The reaction conditions are shown in Table 1.



6a R = H
6b R = Ts
6c R = Ns
6d R = Bn

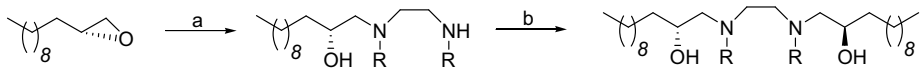
8 $R_1 = CH_2CH{=}CH_2$
9 $R_1 = CH_3$

4a R = H, $R_1 = CH_3$
4b R = Ts, $R_1 = CH_2CH{=}CH_2$
4c R = Ns, $R_1 = CH_2CH{=}CH_2$
4d R = Bn, $R_1 = CH_2CH{=}CH_2$

Scheme 7. *N*-alkylation of amino alcohols with epoxides.

a) For reaction conditions, see Table 1.

Thus, the most effective was the epoxide opening with the amino alcohol **6d** carrying the N-benzyl group. The same method was then used to prepare a symmetric compound with an ethylene-1,2-diamine core (Scheme 8). The epoxide **9** was smoothly opened with N,N'-dibenzylethylene-1,2-diamine to afford amine **7b** with very good yield as expected. The amino alcohol **7b** carrying benzyl-groups was then coupled again with epoxide **9**. The reaction took place in 24 h and the desired symmetric product **5b** was obtained in good yield (74%). Opening the same epoxide **9** with ethylene diamine, the amino alcohol **7a** was produced as predicted, but coupling **7a** again with epoxide **9**, the product **5a** was not obtained.



9 **7a** R = H **7b** R = Bn **5a** R = H **5b** R = Bn

Scheme 8. Conditions: **a)** 2-PrOH, reflux, 24 h, >95% (**7a**), 81% (**7b**); b) 2-PrOH, reflux, 24 h, 74% (**7b**), b) for reaction conditions, see Table 1, entries 11 and 12.

Table 1. *N*-Alkylation of amino alcohols with epoxides.

Entry	***Amino alcohol***	***Epoxide***	***Product***	***Reaction conditions***	***Yield (%)***
1	**6a**	**9**	**4a**	2-PrOH, reflux, one week	traces
2	**6a**	**9**	**4a**	1,4-dioxane, reflux, one week	traces
3	**6a**	**9**	**4a**	cat. $CoCl_2$, MeCN, rt up to 70 °C	n.c.d.[b]
4	**6a**	**9**	**4a**	cat. $FeSO_4$, MeCN, rt up to 70 °C	n.c.d.[b]
5	**6a**	**9**	**4a**	cat. CF_3SO_3H, MeCN, rt up to 60 °C	n.c.d.[b]
6	**6a**	**9**	**4a**	0.1 eq K_2CO_3, 0.1 eq TEBA, dioxane, 90 °C, 6 days	n.c.d.[b]
7	**6b**	**8**	**4b**	2-PrOH, reflux, 4 days	n.c.d.[b]
8	**6b**	**8**	**4b**	0.1 eq K_2CO_3, 0.1 eq TEBA, dioxane, 90 °C, 7 days	53[a]
9	**6c**	**8**	**4c**	0.1 eq K_2CO_3, 0.1 eq TEBA, dioxane, 90 °C, 10 days	54[a]
10	**6d**	**8**	**4d**	2-PrOH, reflux, 48 h	87[a]
11	**7b**	**9**	**5b**	2-PrOH, reflux, 24 h	74[a]
12	**7a**	**9**	**5a**	2-PrOH, reflux, one week	n.c.d.[b]

[a] Isolated yield.
[b] n.c.d. – no conversion detected.

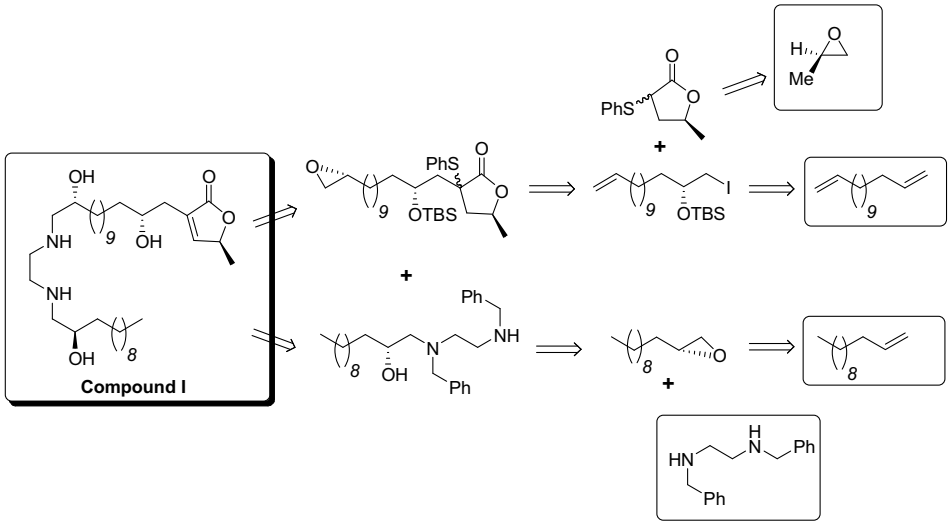
Thus, it was proven, that there is a convenient method for the synthesis of symmetric amphiphilic 1,2-amino alcohols. It grately depends on the nature of amine

moiety and affecting its polarity with *N*-benzyl protecting groups is essential for obtaining good yields in opening lipophilic epoxides with an amine.

Aza-analogue with ethylene-1,2-diamine core

General synthetic route

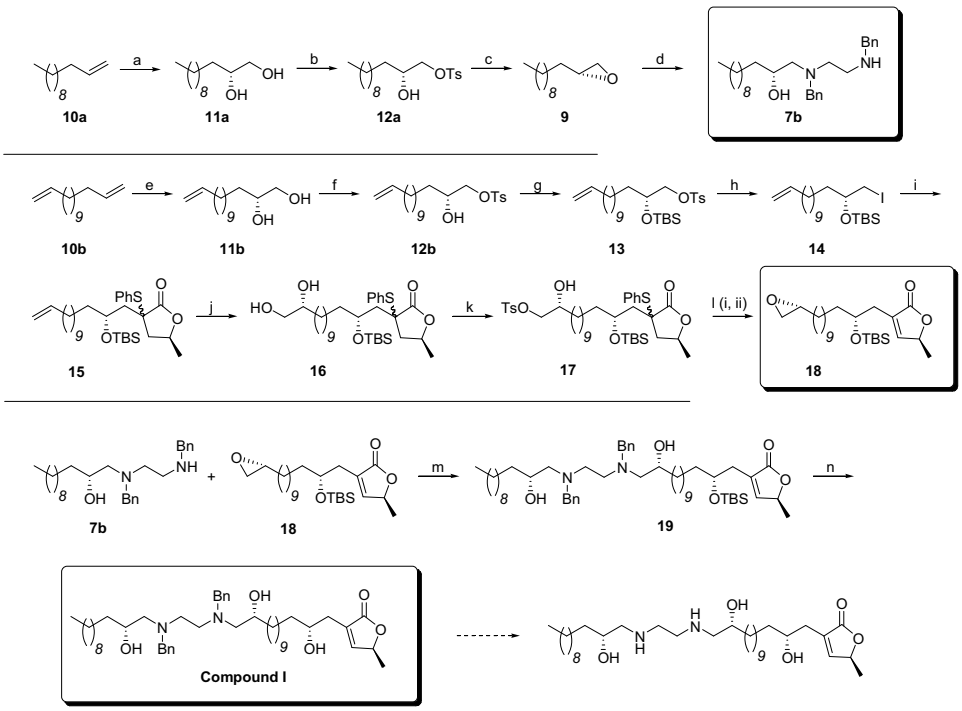
Our synthesis of aza-analogue with diamine-core (Scheme 9) started with two different olefins. One of the olefins, with one double bond, was turned into an epoxide, and opened with *N*,*N*'-dibenzylethylene-1,2-diamine compound, producing an amino alcohol. The other olefin, diene, was dihydroxylized, using Sharpless AD method, selectively tosylated, converted into iodide compound and coupled with γ-lactone ring moiety. Then the other double bond was dihydroxylated and converted into an epoxide over-tosylated product. The desired product, **compound I**, was obtained by coupling the amino alcohol and the epoxide.



Scheme 9. General synthetic route to an aza-analogue with ethylene-1,2-diamine core.

The synthesis can be looked at in three sections: (i) the synthesis of the alkyl linker from diene; (ii) synthesis of lactone-ring moiety; (iii) synthesis of amino alcohol – alkyl tail in the complete compound.

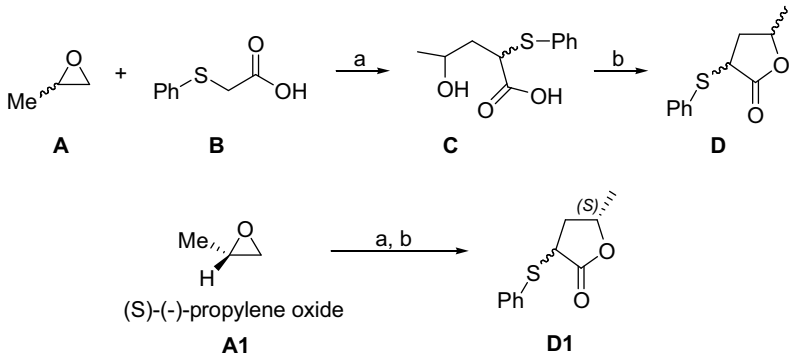
The detailed synthetic route is shown in Scheme 10. The different steps in the synthetic route are discussed in separate paragraphs below.



Scheme 10. Synthetic route; **a)** $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$, K_2CO_3, I_2, *t*-BuOH/H_2O, 0 °C, 6 h, 97%; **b)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h, >95%; **c)** K_2CO_3 (1.5 eq), MeOH/CH_2Cl_2, 30 min, >95%; **d)** *N*,*N*'-dibenzylethylene-1,2-diamine, 2-PrOH, reflux; **e)** $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$, K_2CO_3, I_2, *t*-BuOH/H_2O, 0 °C, 3 h, 54%; **f)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h, 98%; **g)** TBSCl (1.1 eq), imidazole (2.5 eq), DMF, rt, 5.5 h, 82 %; **h)** NaI (5 eq), acetone, reflux, 18 h, 91%; **i)** lactone **D1**, LDA (1.1 eq), THF, 0 °C, then **14** in HMPA (5.5 eq), 18 h, 75%; **j)** 0.2% $K_2OsO_2(OH)_4$, 1% $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/H_2O 1:1, 0 °C, 24 h; **k)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h; **l)** K_2CO_3 (1.5 eq), MeOH, rt, 3 h, 66% (from **15**); **(i)** mCPBA (1.5 eq), CH_2Cl_2, 0 °C, 40 min; **(ii)** toluene, reflux, 2 h, 95%; **m)** 2-PrOH, reflux, 24 h, 32%; **n)** AcCl, MeOH, rt, 1 h, 64%.

Preparing γ-lactone ring moiety

The γ-butyrolactone moiety was prepared following a commonly used protocol for synthesizing the 4-methyl-2-(phenylthio)-γ-butyrolactone.[45] The starting material was either (*R*)-(+)-, (*S*)-(–)-propylene oxide, or a mixture. All the lactone ring moieties in this work were prepared according to this procedure (Scheme 11).

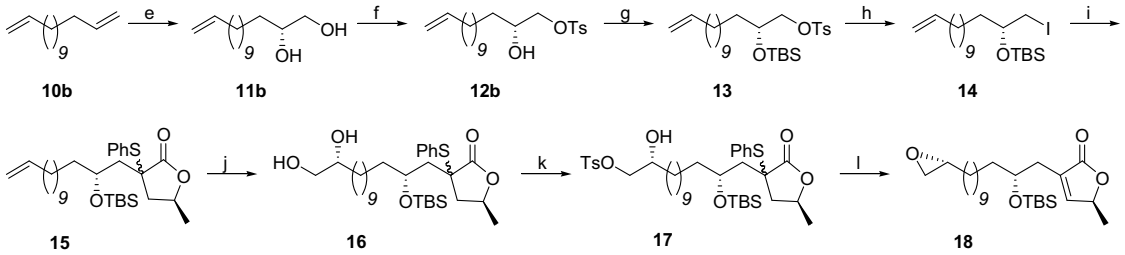


Scheme 11. Synthesis of γ-lactone **D** and **D1**. Conditions: **a)** LDA (2.1 eq), THF, -78 °C, 3 h, overnight at rt; **b)** *p*-TsOH (cat), benzene, overnight at rt.

Here the starting material was (*S*)-propylene oxide **A1**. To a stirred solution of phenylthioacetic acid **B** in THF and LDA at -78 °C was added (S)-(–)-propylene oxide. After stirring the reaction mixture at -78 °C for 3 h, it was let to warm to rt overnight. Then the solvent was substituted with benzene and a catalytic amount of *p*-toluene-sulfonic acid was added to the reaction. Product **D1** had a yield of about 52%. The reaction was also tried with racemic propylene oxide **A** under the same conditions, giving the racemic product **D** with a higher yield of 76%.

Synthesis of alkyl linker with γ-lactone-ring moiety

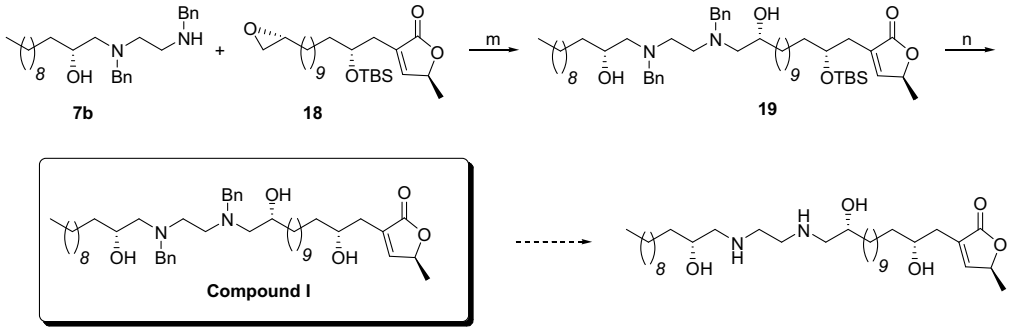
The alkyl spacer with the terminal ring moiety was synthesized in a linear synthesis route (Scheme 12). The compound **12b** was obtained in two steps as described previously in Chapter I through asymmetric dihydroxylation and selective tosylation. Then the hydroxyl group in **12b** was protected by TBS group. The reaction mixture of **12b**, TBSCl and imidazole in DMF was stirred about 5 h at rt and monitored with TLC. The protected **13** was obtained in 82% yield. The treatment of **13** with NaI in acetone in reflux, gave **14** also with a very good yield of 91%. Alkylation with lactone **D1** with iodide **14** in the presence of HMPA seemed to proceed with out problems according to controls with TLC, only yields were a bit lower, 75%. The next two steps followed the previous protocols for asymmetric dihydroxylation, and selective tosylation, producing **16** and **17** with good crude yields of >95%. Neither **16** nor **17** was further purified and conversion to epoxide fallowed the procedure described above. After purification, **18** was obtained with a yield of 66%.



Scheme 12. e) $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$, K_2CO_3, I_2, *t*-BuOH/H_2O, 0 °C, 3 h, 54%; **f)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h, 98%; **g)** TBSCl (1.1 eq), imidazole (2.5 eq), DMF, rt, 5.5 h, 82 %; **h)** NaI (5 eq), acetone, reflux, 18 h, 91%; **i)** lactone **D1**, LDA (1.1 eq), THF, 0 °C, then **14** in HMPA (5.5 eq), 18 h, 75 %; **j)** $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/H_2O 1:1, 0 °C, 24 h; **k)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h; **l)** K_2CO_3 (1.5 eq), MeOH, rt, 3 h, 66% (from **15**); **(i)** mCPBA (1.5 eq), CH_2Cl_2, 0 °C, 40 min; **(ii)** toluene, reflux, 2 h, 95%.

Coupling the core-unit with the terminal γ-lactone unit

In order to complete the synthesis, two halves (**7b** and **18**) were coupled by using again the opening of epoxide with an amino alcohol (discussed in detail above). Following steps included deprotection of **19** (Scheme 13). It is yet to be elucidated, what is the most convenient way to cleave the benzyl-groups to achieve unbenzylated Compound I.

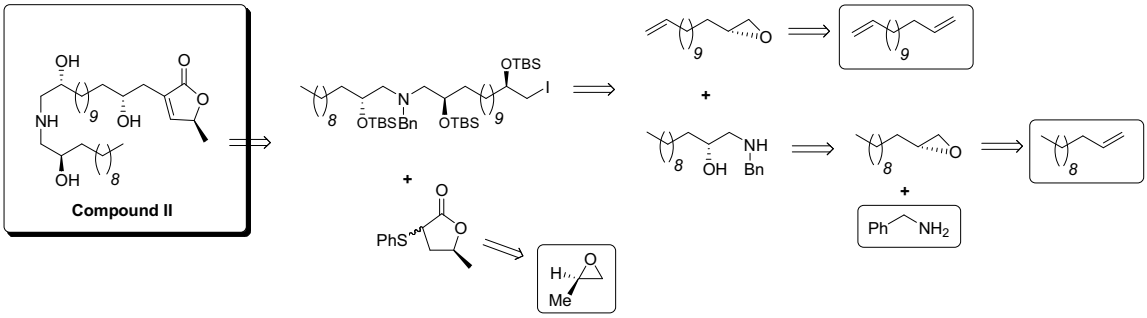


Scheme 13. Conditions: **m)** 2-PrOH, reflux, 24 h, 32%; **n)** AcCl, MeOH, rt, 1 h, 64%.

Aza-analogue with amine-core

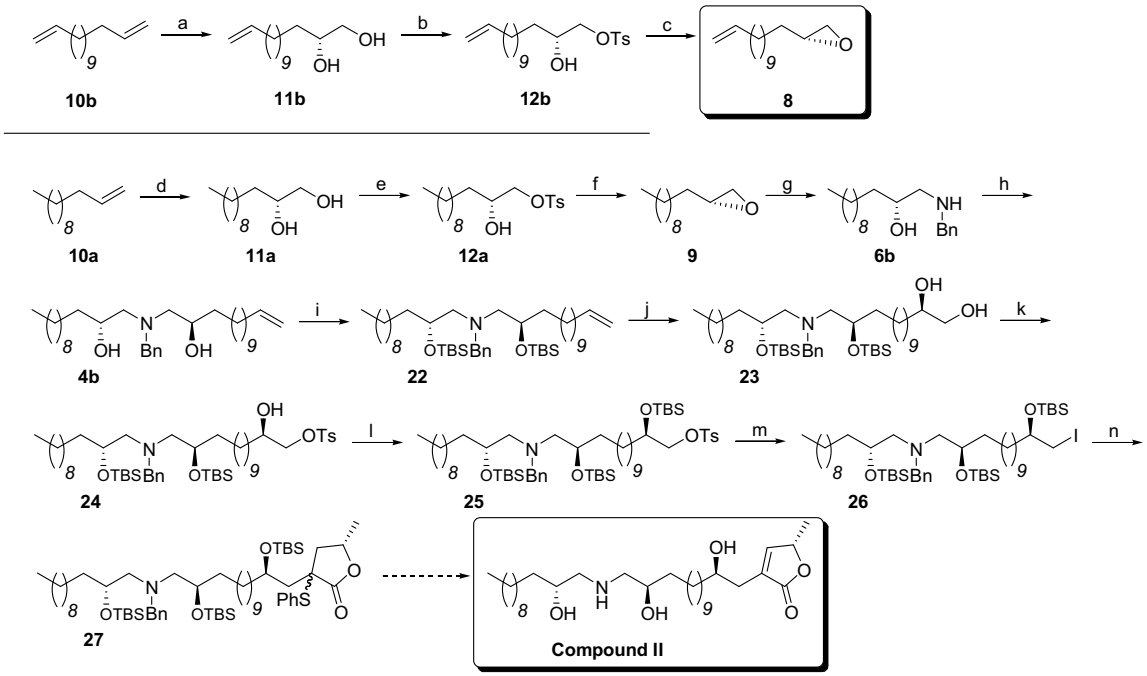
The other aza-analogue synthesized had a more linear synthetic route than the previously described analogue (Scheme 14). In this particular case, benzylamine was

used as a nitrogen source. The synthesis started by introducing two different olefins as epoxides. One of the epoxides was opened with benzylamine, producing an amino alcohol. The amino alcohol was then coupled with the other epoxide, yielding a symmetric amphiphilic product with two alkyl chains and an amino core. This was coupled with lactone-ring moiety, producing the desired product **compound II**.



Scheme 14. General synthetic route to aza-analogue with amine-core

The detailed synthetic route is shown in Scheme 15. The different steps in the synthetic route are discussed in separate paragraphs below.



Scheme 15. Synthetic route. Conditions: **a)** $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$, K_2CO_3, I_2, *t*-BuOH/H_2O, 0 °C, 3 h, 54%; **b)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h, 98%; **c)** K_2CO_3 (1.5 eq), MeOH/CH_2Cl_2, 30 min, 95%; **d)** $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$, K_2CO_3, I_2,

t-BuOH/H_2O, 0 °C, 6 h, 97%; **e)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h, >95%; **f)** K_2CO_3 (1.5 eq), MeOH/CH_2Cl_2, 30 min, >95%; **g)** benzylamine (3 eq), 2-PrOH, reflux, 3.5 h, >95%; **h)** epoxide **8** (1 eq), 2-PrOH, reflux, 48 h, 87%; **i)** TBSCl, DMF, imidazole, rt, 99%; j) 0.2% $K_2OsO_2(OH)_4$, 1% $(DHQD)_2PHAL$, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/H_2O 1:1, 0 °C, 24 h; 62%; **k)** *p*-TsCl (1 eq), Et_3N (1 eq), DBTO (cat), CH_2Cl_2, 18 h, 98%; **l)** TBSCl, DMF, imidazole, rt, 94%; **m)** NaI, acetone, reflux, 17 h, 99%; **n)** lactone **D1**, LDA (1.1 eq), THF, 0 °C, then **26** in HMPA (5.5 eq), 16 h, 64 %.

The converting olefins **10a,b** to epoxides **8** and **9**, and opening epoxide **9** with benzylamine moiety producing amino alcohol **6b**, are described in previous chapters in detail and are made here in this synthesis route according to the same methods. The following step is to couple the amino alcohol **6b** with epoxide **8** obtaining **4b**. The hydroxyl groups were protected with silyl ethers (Scheme 15) and **22** asymmetrically dihydroxylated and tosylated according to methods used before, to afford **24**. After silylating the secondary hydroxyl group in **24**, the compound **25** was treated with NaI, yielding a primary iodide **26**. The iodide was added to enolated butenolide in the presence of HMPA, affording the coupled product **27** in 64% yield. The deprotection strategies are currently under investigation, to complete the synthesis of aza-analogue **compound II**.

Summary and outlook

In this work, synthesis routes for two aza-analogues have been developed, with convenient and reliable ways to change the stereochemistry in the target compounds. The second synthetic scheme had a more convergent pathway compared to the first consecutive route. The greatest arisen problem of the study was the epoxide opening with an amine moiety, but it has been successfully solved and used in the aza-analogue synthesis.

The future prospect for this project is to complete the synthesis for the both aza-analogues, and find a suitable method for removing the benzyl protecting groups. Preliminary work on that direction has already been started. After the completion of the synthesis routes, either for the analogues having the amino or ethylenediamine core, a wider array of aza-analogues will be prepared accordingly, and their biological activities will be evaluated in collaboration with a microbiology research group. The results might give further useful information about the SAR of acetogenins and give more rational ways to design more active targets. The other analogues may contain different central cores, for instance having poly-, alkyl- or cyclic amines fragments, differ in stereo-chemistry, number of hydroxyl groups or some other structural elements.

Kokkuvõte

Selles töös töötati välja sünteesi tee kahele acetogeniinide aza-analoogile, mis võimaldab kergesti muuta analoogide stereokeemiat. Sünteesi tees on rakendatud erinevaid ja huvitavaid etappe ja katsetatud alternatiivseid meetodeid. Võrreldes esimesega, oli teine sünteesi tee rohkem koonduvat laadi. Suurim problem, mis analoogide sünteesi käigus tekkis, oli epoksiidide avamine amiiniga. See kujunes välja peamise sünteesi kõrvalt kui kõrvalharu, kuid probleem lahendati edukalt ning lahendust epoksiidide avamisel kasutati ka aza-analoogide sünteesis.

Tulevikus on kavas lõpetada sünteesi tee mõlema aza-analoogi jaoks, leides ka sobiva meetodi bensüül gruppide eemaldamiseks. Esmased katsed on selles vallas juba alanud. Pärast sünteesi tee lõpetamist, kas siis etüleen diamiini või amiini keskmega analoogi jaoks, on kavas sünteesida nende eeskujul veel erinevaid vastavaid analooge ning ka nende bioloogilisi omadusi uurida. Tulemused võivad anda väärtuslikku infot atsetogeniinide struktuur-aktiivsus suhete kohta ning paremaid lahendusi rohkem aktiivsete ühendite sünteesiks. Teised sünteesitavad analoogid võivad sisaldada erinevaid keskmeid, nagu näiteks polü-, alküül või tsüklilise amiini fragmente, erineda stereokeemias, hüdroksüülgruppide arvus või mõnes muus strukturaalses elemendis.

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References

[1] Jolad, S.D., Hoffmann, J.J., Schram, K.H., Cole, J.R, Tempesta, M.S., Kriek, G.R., Bates, R.B., Uvaricin, a New Antitumor Agent from Uvaria accuminata (Annonaceae), *J. Org. Chem.,* ***1982****, 47: 3151-53*

[2] Bermejo, A., Figadère, B., Zafra-Polo, M.C., Barrachina, I., Estornell, E., Cortes, D., Acetogenins from Annonaceae: recent progress in isolation, synthesis and mechanisms of action, *Nat. Prod. Rep.,* ***2005****, 22: 269-303*

[3] Raynaud, S., Némati, F., Miccoli, L., Michael, P., Poupon, M.F., Fourneau, Ch., Laurens, A., Hocquemiller, R., Antitumoral Effects of Squamocin on Parental and Multidrug Resistant MCF7 (Human Breast Adenocarcinoma) Cell Lines, *Life Sci.,****1999****, 65: 525-33*

[4] Lannuzel, A., Michel, P.P., Caparros-Lefebvre, D., Abaul, J., Hocquemiller, r., Ruberg, M., Toxicity of Annonaceae for Dopaminergic Neurons: Potential Role in Atypical Parkinsonism in Guanteloupe, *Movement Disorders,* ***2002****, 17(1): 84-90*

[5] Champy, P., Höglinger, G.U., Féger, J., Gleye, Ch., Hocquemiller, R., Laurens, A., Guérineau, V., Laprévote, O., Medja, F., Lombès, A., Michel, P.P., Lannuzel, A., Hirch, E.C., Ruberg, M., Annonacin, a Lipophilic Inhibitor of Mitochondrial Complex I, Induces Nigral and Striatal Neurodegeneration in Rats: Possible Relevance for Atypical Parknisonism in Guadeloupe, *J. of Neurochem.,* ***2004****, 88: 63-69*

[6] Encyclopædia Britannica, Mangoliales, http://www.britannica.com/eb/article-72771 downloaded November 2006.

[7] Martin, J.M., Madigosky, S.R., Gu, Zh-M., Zhou, D., Wu, J., McLaughlin, J.L., Chemical Defence in the Zebra Swallowtail Butterfly, *Eurytides marcellus*, Involving Annonacecous Acetogenins, *J. Nat. Prod.,* ***1999****, 62: 2-4*

[8] Lannuzel, A., Höglinger, G.U., Verhaeghe, S., Gire, L., Belson, S., Escobar-Khondiker, M., Poullain, P., Oertel., W.H., Hirsch, E.C., Dubois, B., Ruberg, M., Atypical parkinsonism in Guadeloupe: a common risk factor for two closely related phenotypes?, *Brain,* ***2007****, 130: 816-27*

[9] Miyoshi, H., Abe M., Murai, M., Ichimaru, N., et al, Dynamic Function of the Alkyl Spacer of Acetogenins in Their Inhibitory Action with Mitochondrial Complex I (NADH-Ubiquinone Oxidoreductase*), Biochem.,**2005**, 44: 14898-906*

[10] Lannuzel, A., Michel, P.P., Höglinger, G.U., Champy, P., Jousset, A., Medja, F., Lombes, A., Darios, F., Gleye, C., Laurens, A., Hocquemiller, R., Hirsch, E.C., Ruberg, M., The Mithochondrial Complex I Inhibitor Annonacin is Toxic to Mesencephalic Dopaminergic Neurons by Impairment of Energy Metabolism, *Neuroscience **2003**, 121: 287-96*

[11] Lu, M-Ch., Yang, Sh-H., Hwang, Sh-L., Lu, Y-Jh., Lin, Y-H., Wang, S-R., Wu, Y-Ch., Lin, Sh-R., Introduction of G2/M phase arrest by squamocin in chronic myeloid leukemia (K562) cells, *Life Sc., **2005***

[12] Éparvier, V., Nguyen V.H., Thoison, O., Martin, M-T., Sévenet, T., Guéritte, F., Cytotoxic Monotetrahydrofuran Acetogenins from Disepalum plagioneurum, *J. Nat. Prod. **2006**, 69: 1289-94*

[13] Pelicano, H., Feng, L., Zhou, Y., Carew, J.S., Hileman, E.O., Plunkett, W., Keating, W.J., Huanq, P., Inhibition of mitochondrial respiration: a novel strategy to enhance drug-induced apoptosis in human leukemia cells by a reactive oxygen species-mediated mechanism, *J. Biol. Chem., **2003**, 278(39): 37832-9*

[14] Araya, H., Fuijmoto, Y., Hirayama, K., Structural elucidation of tetrahydrofuranic acetogenins by means of precursor-ion scanning method, *J. Syn. Org. Chem. Jpn., **1994**, 52:765-777*

[15] Peyrat, J.F., Mahuteau, J., Figadère, B., Cavé, A., NMR Studies of Ca2+ Complexes of Annonaceous Acetogenins, *J. Org. Chem., **1997**, 62: 4811-15*

[16] Saona-Rodriguez, C., Trumble, J.T., Biologically Active Aliphatic Acetogenins from Specialized Idioblast Oil Cells, Curr. *Org. Chem., **2000**, 4: 1249-60*

[17] Fontana, A., Cimono, G., Gavagnin, M., Gonzalez, C., Estornell, E., novel Inhibitors of mitochondrial Respiratory Chain: Endoperoxides from the Marine Tunicate *Stolonica socialis*, *J. Med. Chem., **2001**, 44: 2362-65*

[18] Höglinger, G.U., Michel, P.P., Champy, P., Feger, J., Hirch, E.C., Ruberg, M., Lannuzel, A., Experimental Evidence for a Toxic Etiology of Tropical Parkinsonism, *Movement Disorders, **2005**, 20(1): 118-119*

[19] Caparros-Lefebvre, D., Steele, J., Kotake, Y., Ohta, Sh., Geographic isolates of atypical Parkinsonism and tautopathy in the tropics: Possible synergy of neurotoxins., *Movement Disorders, **2006**, 21(10): 1769-1771*

[20] Li, N., Ragheb, K., Lawler, G., Sturgis, J., Rajwa, B., Melendez, J.A., Robinson, J.P., Mitochondrial Complex I Inhibitior Rotenone Induces Apoptosis through Enhancing Mitochondrial Reactive Oxygen Species Production, *J. Biol. Chem.,* ***2003****, 10(7): 8516-25*

[21] Kuwabara, K., Takada, M., Iwata, J., Tatsumoto, K., Sakamoto, K., Iwamura, H., Miyoshi, H., Design synthesis and mitochondrial complex I inhibitory activity of novel acetogenin mimics, *Eur. J. Biochem.,* ***2000****, 267: 2538-2546*

[22] Hamada, T., Ichimaru, N., Abe, M., Fujita, D., Kenmochi, A., Nishioka, T., Zwicker, K., Brandt, U., Miyoshi, H., Synthesis and Inhibitory Action of Novel Acetogenin Mimics with Bovine Heart Mitochondrial Complex I, *Biochem.,* ***2004****, 43: 3651-3658*

[23] Abe, M., Kenmochi, A., Ichimaru, N., Hamada, T., Nishioka, T., Miyochi, H., Essential structural features of acetogenins: role of hydroxy groups adjacent to the bis-THF rings, *Bioorg. And Med. Chem. Lett.,* ***2004****, 14: 779-782*

[24] Shimada, H., Grutzner, J.B., Kozlowski, J.F., McLaughlin, J.L., Membrane Conformations and Their Relation to Cytotoxicity of Asimicin and Its Analogues, *Biochem.,* ***1998****, 37: 854-866*

[25] Pichon, M., Hocquemiller, R., Figadère, B., Synthesis of C1-C32 fragment of aza-solamin, an unnatural analogue of the annonaceous acetogenin solamin, *Tetrahedron Lett.,* ***1999****, 40: 8567-8570*

[26] Wang, M., Shen, J.K., Synthesis of simplified Analogue of Acetogenin Bearing *N,N'*-dibenzyl Ethylenediamine Moiety, *Chin. J. Synth. Chem.,* ***2006****, 14(4):337-41*

[27] Jiang, S., Liu, Z.H., Sheng G., Zeng, B.B., Cheng, X.G., Wu, Y.L., Yao, Z.J., Mimicry of Annonaceoous Acetogenins: Enantioselective Synthesis of a (4R)-Hydroxy Analogue Having Potent Antitumor Activity*, J. Org. Chem.,* ***2002****, 67: 3404-08*

[28] Zeng, B.B., Wu, Y., Yu, Q., W, Y.L., Li, Y., Chen, X.G., Enantiopure Simple Analogues of Annonaceous Acetogenins with Remarkable Selective Cytotoxicity towards Tumor Cell Lines, *Angew. Chem.,* ***2000****, 39(11): 1934-37*

[29] Yao, Z.J., Wu, H.P., Wu, Y.L., Polyether Mimics of Naturally Occurring Cytotoxic Annonaceous Acetgenins, *J. Med. Chem.,* ***2000****, 43: 2484-87*

[30] Konno, H., Hiura, N., Makabe, H., Abe, M., Miyoshi, H., Synthesis and mitochondrial complex I inhibition of dixydroxy-cohibin A, non-THF annonaceous acetogenin analogue, *Bioorg. Med. Chem. Lett.,* ***2004****, 14: 629-632*

[31] Konno, H., Hiura, N., Yanaru, M., Synthesis of (4*R*,5*S*)- and (4S,5*R*)-muricatacins, and (4*S*,5*R*)-aza-muricatacin, unnatural analogues of the annonaceous acetogenins, *Heterocycles,* ***2002****, 57: 1793-97*

[32] a) Hepworth, P., Non-ionic Surfactants. Chapter 5 in *Chemistry and Technology of Surfactants, Farm, R., Ed., Blackwell Publishing Ltd:* ***2006****;* b) Oskarsson, H., Frankenberg, M., Annerling, A.,

Holmberg, K., Adsorption of Novel Alkylaminoamide Sugar Surfactants at Tailor-made Surfaces, *J. Surfact. Deterg.,* ***2007****, 10: 41-52*

[33] a) Tewari, N., Tiwari, V.K., Mishra, R.C., Tripathi, R.P., Srivastava, A.K., Ahmad, R., Srivastava, R., Srivastava, B.S., Synthesis and Bioevaluation of Glycosyl Ureas as α-Glucosidase Inhibitors and Their Effect on Mycobacterium, *Bioorg. Med. Chem.,* ***2003****, 11: 2911-2922;* b) Tripathi, R.P., Tewari, N., Mishra, R.C, Srivastava, R., Sinha, S., Srivastava, A., Chaturvedi, V., Srivastava, B., (Council of Scientific and Industrial Research, India). Indian Pat. Appl., 2006, IN 2004DE01609,

[34] Spichiger-Keller, U.E., Ionophores, Ligands and Reactands, *Anal. Chim. Acta,* ***1999****, 400: 65-72*

[35] Lee, R.E., Mikusova, K., Brennan, P.J., Besra, G.S., Synthesis of the Mycobacterial Arabinose Donor β-D-Arabinofuranosyl-1-monophosphoryldecaprenol, Development of a Basic Arabinosyl-Transferase Assay, and Identification of Ethambutol as an Arabinosyl Transferase Inhibitor, *J. Am. Chem. Soc.,* ***1995****, 117: 11829-32*

[36] Tewari, N., Tiwari, V.K., Tripathi, R.P., Chaturvedi, V., Srivastava, A., Srivastava, R., Shukla, P.K., Chatuverdi, A.K., Gaikwad, A., Sinha, S., Srivastava, B.S., Synthesis of Galactopyranosyl Amino Alcohols as a New Class of Antitubercular and Antifungal Agents, *Bioorg. Med. Chem. Lett.,* ***2004****, 14: 329-332*

[37] Bermejo, A., Figadère, B., Zafra-Polo, M.C., Barrachina, I., Estornell, E., Cortes, D., Acetogenins from Annonaceae: recent progress in isolation, synthesis and mechanisms of action, *Nat. Prod. Rep.,* ***2005****, 22: 269-303*

[38] Wang, M., Chen, Y., Lou, L., Tang, W., Wang, X., Shen, J., Synthesis of Pyrrolidine Analogues of Solamin, *Tetrahedron Lett.,* ***2005****, 46: 5309-5312*

[39] a) Torii, Liu, P., Bhuvaneswari, N., Amatore, C., Jutand, A., Chemical and Electrochemical Asymetric Dihydrolation of Olefins in I_2-K_2CO_3-$K_2OsO_2(OH)_4$ and I_2-K_3PO_4/K_2HPO_4-$K_2OsO_2(OH)_4$ Systems with Sharpless' Ligand, *J. Org. Chem.,* ***1996****, 61: 3055-60;* b) Kolb, H.C., VanNieuwenhze, M.S., Sharpless, K.B., Catalytic Asymmetric Dihydroxylation, *Chem. Rev.,* ***1994****, 94: 2483-2547*

[40] Martinelli, M.J., Nayyar, N.K., Moher, E.D., Dhokte, U.P., Pawlak, J.M., Vaidyanathan, R., Dibutyltin Oxide Catalysed Selective Sulfonylation of α-Chelatable Primary Alcohols, *Org. Lett.,* ***1999****, 1(3): 447-450*

[41] Kurosaki, Y., Fukuda, T., Iwao, M., Asymmetric synthesis of 3-substituted 3,4-dihydroisocoumarins via stereoselective addition of laterally lithiated chiral 2-(*o*-tolyl)oxazolines to aldehydes followed by diastereomer-selective lactonization, *Tetrahedron,* ***2005****, 61: 3289-3303*

[42] Tinsley, J.M., Roush, R., Total Synthesis of Asimicin via Highly Stereoselective [3+2] Annulation Reactions of Substituted Allylsilanes, *J. Am. Chem. Soc.,* ***2005****, 127: 10818-19*

[43] Lupi, V., Albanese, D., Landini, D., Scaletti, D., Penso, M., Synthesis of 2,6-disubstituted morpholines through regioselective oxiranes ring opening by tosylamide under PTC conditions, *Tetrahedron,* ***2004****, 60: 11709-18*

[44] Lupi, V., Albanese, D., Landini, D., Scaletti, D., Penso, M., Synthesis of 2,6-disubstituted morpholines through regioselective oxiranes ring opening by tosylamide under PTC conditions, *Tetrahedron,* ***2004****, 60: 11709-18*

[45] a) White, J.D., Somers, T.C., Reddy, G.N, Degradation and Absolute Configurational Assignment to C_{34}-Botryococcene, *J. Org. Chem.,* ***1992****, 57: 4991-98;* b) Iwai, K., Kosugi, H., Uda, H., Kawai, M., New Method for Synthesis of Various Types of Substituted 2(5*H*)-Furanones*, Bull. Chem. Soc. Jpn.,* ***1977****, 50: 242-247*

Appendixes