Development of synthesis pathways and characterization of cerulenin analogues as inhibitors of the fatty acid biosynthesis of *Mycobacterium tuberculosis* and of efflux pump resistant *Candida albicans*

Dissertation zur Erlangung des
naturwissenschaftlichen Doktorgrades
der Julius-Maximilians-Universität Würzburg

vorgelegt von
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Eingereicht am ……

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Tine. Für alles und alles Weitere.

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1. Introduction

1.1 New antituberculosis drugs

In the last century, tuberculosis in Europe was nearly defeated due to vaccination and excellent treatment options with several antiinfectives. However, today we have to face a new flare-up of the disease.

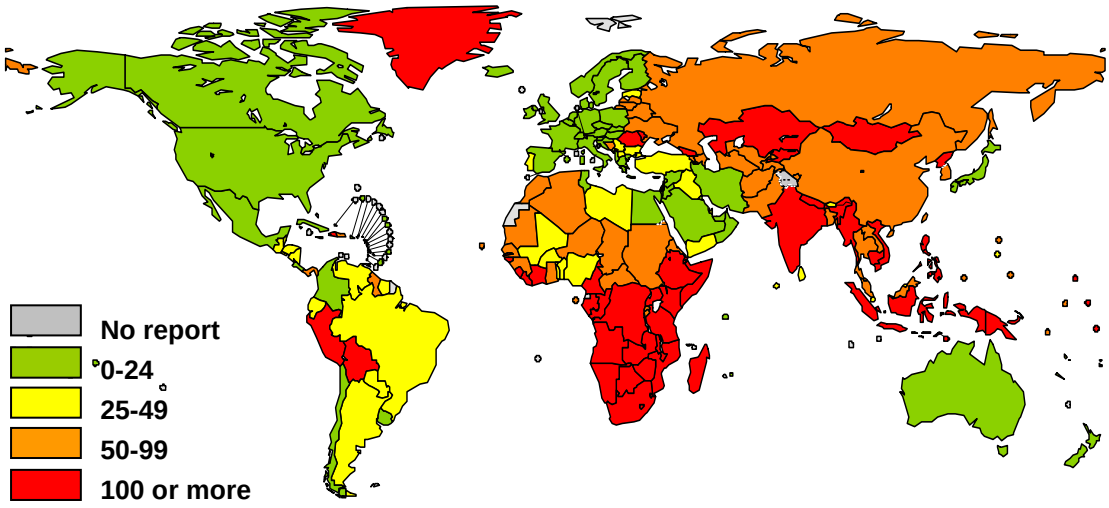


Fig. 1.1.1 Tuberculosis notification rates 2005 according to WHO 2007[1]

According to new evaluations of the World Health Organisation (WHO) one third of the world population is infected with tuberculosis and about 5-10 % of infected people develop active infections. 1.6 million people are estimated to have died of tuberculosis in 2005 and 8.8 million of new infections were reported in the same year.[2] Special problems have occurred in the last years: today we have to face a new flare-up of the disease due to the development of *Mycobacteria* resistant to antituberculosis drugs[3] and arise of tuberculosis as an opportunistic infection in HIV patients.[4] Especially the occurrence of mycobacterial resistance to several antituberculotic drugs, so called multidrug resistant (MDR) tuberculosis, reduces the efficacy of the known antituberculotics dramatically. The WHO has redefined its goal in terms of tuberculosis, because current strategies are not sufficient enough to control the disease.[5]

1.1.1 *Mycobacterium tuberculosis*

1.1.1.1 Morphology

Mycobacterium tuberculosis is the most responsible pathogen for tuberculosis. It is a gram positive, acid resistant, slowly growing, rod-like bacillus and is of 0.4 µm width and 3-4 µm length. It possesses a thick wax-like cell wall with mycolic acids (very long fatty acids containing 60-90 carbon atoms) as an essential component. This cell wall provides among other properties protection against degradation in macrophages, acid resistance and high resistance against many antibiotics that are therefore incapable of reaching the inner cell.[6]

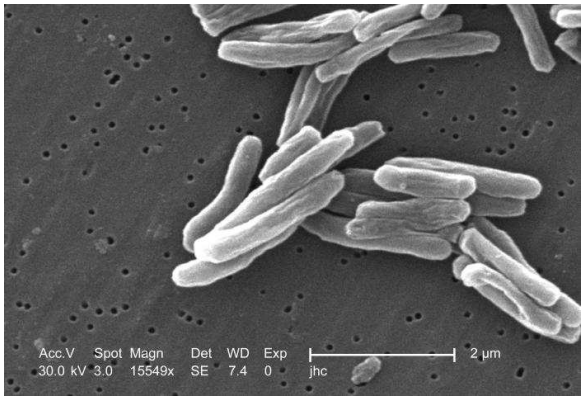


Fig. 1.1.2 SEM picture of Mycobacterium tuberculosis

1.1.1.2 Pathogenesis

Mycobacterium tuberculosis does not develop toxins; its toxicity lies in the ability to invade, survive and replicate within macrophages preventing to be eliminated. In case of a defect in the immune system, rapid replication proceeds resulting in a severe and life threatening tuberculosis infection. In case of a healthy immune sytem, a granuloma consisting of macrophages, lymphocytes, fibroblasts and epitheloid cells is formed. These granulomas develop necrosis in the center and result subsequently in healing for 90 % of incidences. However, the bacteria can survive within these granulomas and can lead to endogenous reinfection later.

Tuberculosis infections can be divided into two different types: a *primary* and a *post-primary* infection.[7] The *primary* infection is the most common in infants and is localized mostly in the lung. The course of disease takes place with very few symptoms including sweats, appetite loss, weight loss and a tendency to fatigue very easily. The *postprimary* infection results from distribution of bacteria throughout the organism from either fresh infections or older granulomas. Most of the infections proceed in the lung but can also cause infection in organs or bones etc. with life threatening consequences.

1.1.2 Antituberculosis drugs

Since vaccination and chemoprophylaxis appeared to be unsatisfactory in case of tuberculosis, treatment with antituberculosis drugs became the only option available.[8] Antituberculosis agents should rapidly kill actively metabolizing bacilli in the lung cavities, destroy less actively replicating bacilli in acidic and anoxic closed lesions and kill near-dormant bacilli that might otherwise cause a relapse of the disease.[9] In addition, it became apparent that *Mycobacterium tuberculosis* was capable of rapidly developing drug resistance soon after the discovery of streptomycin, the first effective antituberculosis agent.[10] Therefore, the DOTS (directly observed therapy, shourt course) strategy was developed by the Stop TB partnership and the WHO.[11,12] A combination of drugs is recommended for standard treatment of tuberculosis; in the initial 2-month intensive phase, isoniazid, pyrazinamide and rifampicin are administered together with a fourth agent, either ethambutol or streptomycin. These drugs are called *first line* antituberculosis agents and destroy almost all bacilli in the three physiological categories mentioned above during the initial phase of treatment. This phase is followed by a continuation phase, usually a 4-month course of rifampicin and isoniazid. The former kills any residual dormant bacilli and the latter kills any rifampicin resistant mutants that commence replication.[13] However, in cases of multidrug resistance tuberculosis (see chapter 1.1.3.1) treatment is changed to a therapy being based on the use of a *first line* drug to which strains are still susceptible and on *second line* drugs including older drugs with more adverse effects.

1.1.2.1 First line antituberculosis drugs

Aminoglycosides and cyclic peptides:

Streptomycin:

Streptomycin was the first drug discovered against tuberculosis and belongs to the aminoglycosides.[14] It has a relatively broad spectrum of antibacterial activity against many gram-positive and -negative bacteria and mycobacterial species.[15] The mechanism of action has been studied in *E. coli* and appears to be similar in mycobacteria: it is the irreversible binding of streptomycin to a single site in the bacterial 30S ribosomal subunit preventing initiation of protein synthesis and causing misreading of proteins whose translation is already under way.[16] The mycobacterial cell wall does not seem to hamper the entrance of this highly lipophilic compound, which can be explained by porins for mycobacteria intake of hydrophilic nutritients.[17] Due to the fact that resistances occur very often, streptomycin is used only in combination with other antituberculosis drugs.[18] Kanamycin, amikacin, and the cyclic peptides capreomycin and viomycin (see Fig. 1.1.4) have the same mode of action and can also be used for treatment of tuberculosis although as second line drugs (see 1.1.2.2).

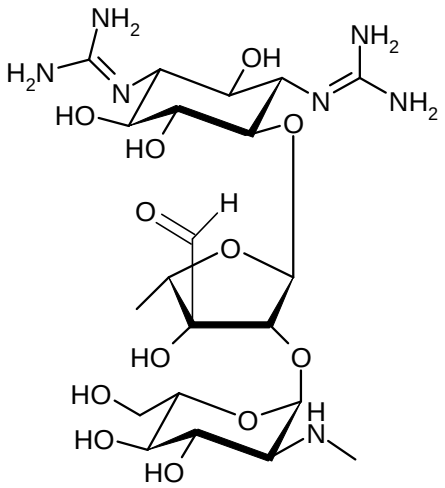
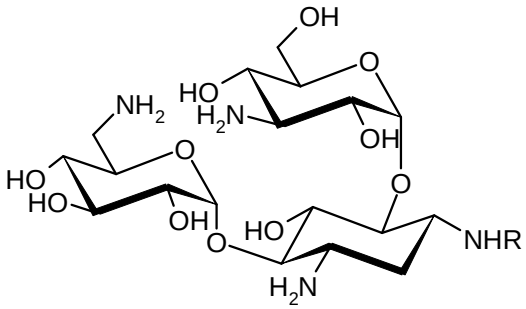
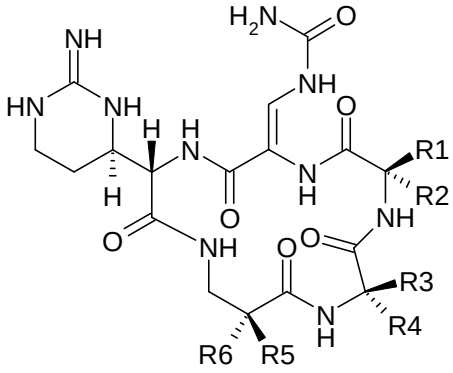


Fig. 1.1.3 Structure of streptomycin



R = H **kanamycin**
R = $COCH(OH)CH_2CH_2NH_2$ **amikacin**



R1=$CH_2NHCOCH_2CHNH_2(CH_2)_3NH_2$, R_2=H, R3=CH_2OH, R4,5=H, R6=NH_2 **capreomycin**

R1=H, R_2=CH_2OH, R3=H, R4=CH_2OH, R5=$NHCOCH_2CHNH_2(CH_2)_3NH_2$, R6=H **viomycin**

Fig. 1.1.4 Structures of kanamycin, amikacin, capreomycin and viomycin

Isoniazid:

Isoniazid represents the oldest synthetic antituberculosis drug[19] and also one of the most effective and widely used drugs against tuberculosis. It shows activity exclusively against mycobacteria, especially slow growing *M. tuberculosis* with a minimal inhibitory concentration (MIC) of 0.05 µg/ml.[20] It enters the mycobacterial cell by diffusion and targets the biosynthesis of mycolic acids, a major component of the mycobacterial cell wall, by inhibiting the fatty acid synthesis. Although the mechanism of action seems quite complex, there is evidence that InhA, the enoyl reductase in the fatty acid synthesis pathway II (FAS II, see Fig. 1.1.5), is the target for isoniazid.[21] Isoniazid represents a prodrug; it forms an adduct with NAD(H) under activation with katG, a catalase-peroxidase enzyme.[22] The resulting adduct then binds to the NAD(H) recognition site of InhA leading to the antimycobacterial effect. The predominant mechanism of resistance to isoniazid arises from mutations in katG.[23]

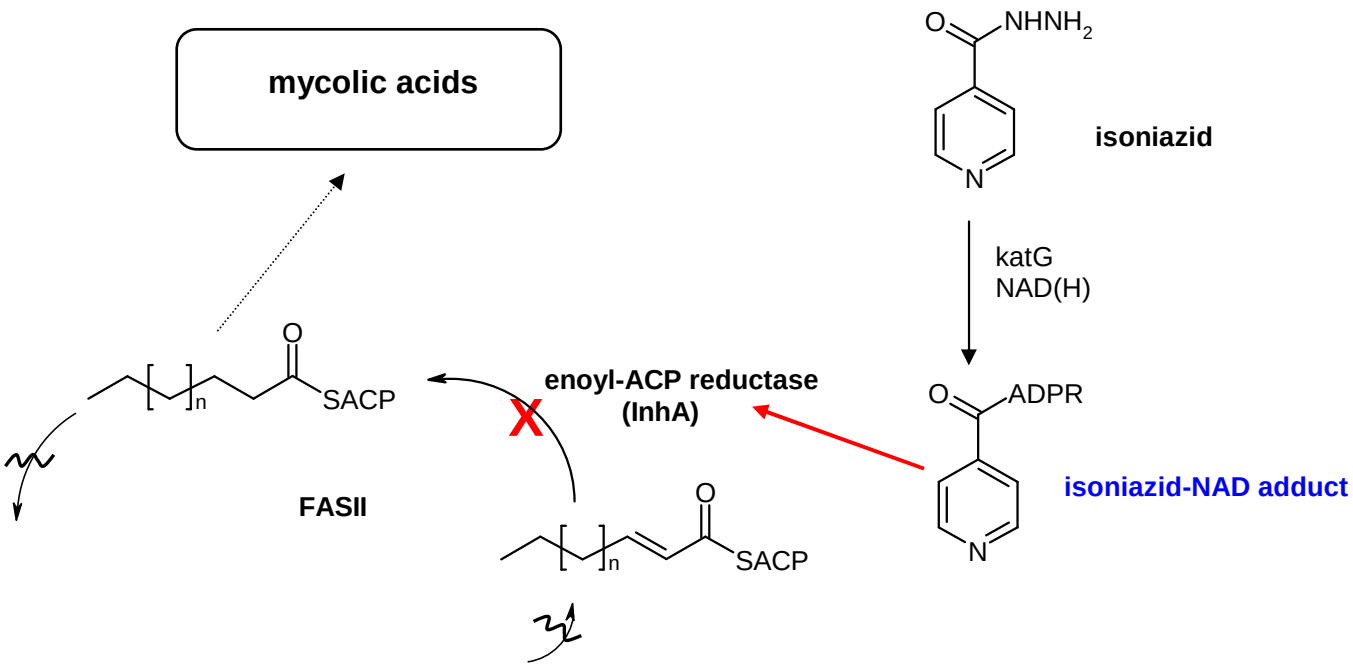
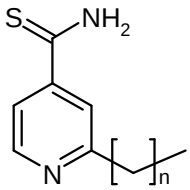


Fig. 1.1.5 Mechanism of action of isoniazid (n=8-52, ADPR=adenindinucleotide, ACP=acetyl carrier protein)

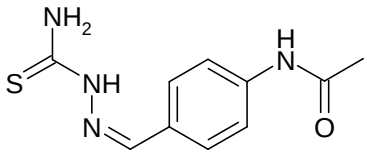
Ethionamide/prothionamide/thiacetazone:

In case of ethionamide and prothionamide a similar mechanism of action is postulated as for isoniazid although these drugs are only used as second line drugs (see 1.1.2.2). They also seem to act as a prodrug but they are not activated by katG but by another enzyme, EthA.[24] InhA seems to be the target enzyme and therefore

inhibition of mycolic acid synthesis due to the fact that some isoniazid and ethioniamide resistant *M. tuberculosis* strains were found which displayed a mutation on InhA.[25] There are indications that thiacetazone may follow a similar mode of action[24] but this still has to be elucidated. It is a relatively cheap drug which therefore has been used mostly in developing countries where the cost of medication is an limiting factor.[26]



n = 1 ethionamide
n = 2 prothionamide



thiacetazone

Fig. 1.1.6 Structures of the ethionamide, prothionamide and thiacetazone

Pyrazinamide:

Pyrazinamide plays a special role in the therapy of tuberculosis due to the fact that it addresses populations of semidormant tubercle bacilli less affected by other drugs.[27] The presumed mechanism of action for pyrazinamide is based on the release of pyrazinoic acid *in situ* inside the cell.[24] This leads to the intake of protons with a complete dysfunction of the pH balance of the cell.[28] This mode of action is supported by the observed correlation between the development of pyrazinamide resistance and loss of the pyrazinamidase (PZase) activity responsible for converting pyrazinamide to pyrazinoic acid as the bactericidal agent in *M. tuberculosis*.[29]

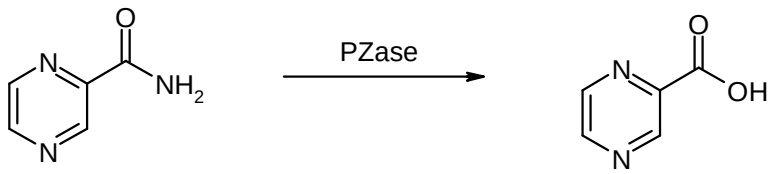


Fig. 1.1.7 Conversion of the prodrug pyrazinamid to pyrazinoic acid

Ethambutol:

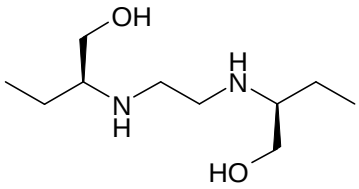
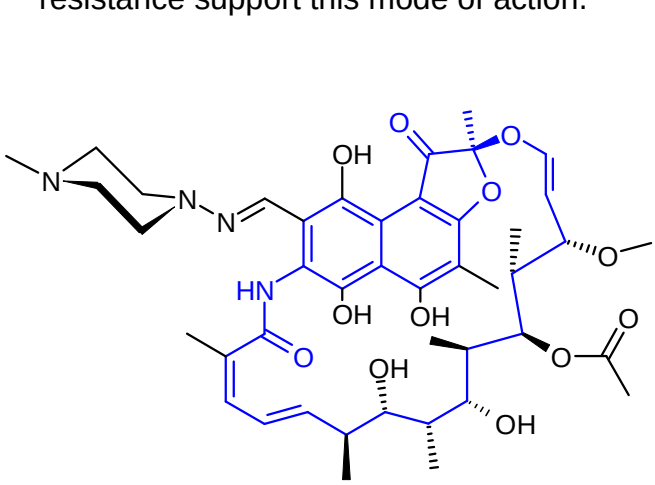


Fig. 1.1.8 Structure of S,S-ethambutol

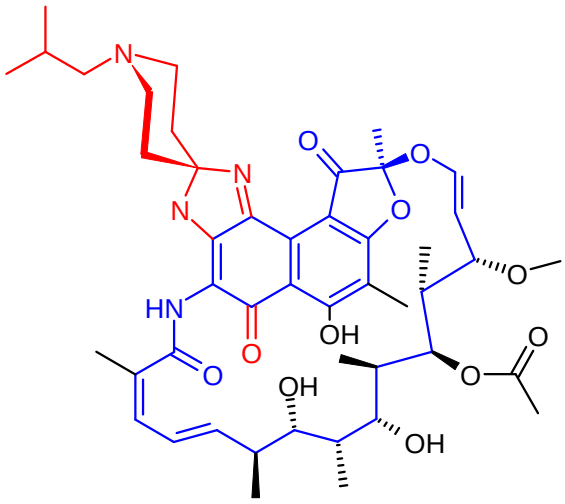
Several modes of action have been proposed for ethambutol, but due to several series of work the primary mode of action is focused on the inhibition of arabinogalactan, which acts as the intermediate binding scaffold between the mycolic acids and the inner peptido-glycan in the mycobacterial cell wall.[24] It was demonstrated that the incorporation of ^{14}C from [^{14}C] glucose into cell wall arabinan was immediately inhibited upon addition of ethambutol to young cultures.[30] Further investigations revealed that the arabinosyl transferase, responsible for the polymerization of arabinose into the arabinan of arabinogalactan, could be the target of ethambutol.[31,32] The antimicrobial effect is related to the *S*,*S*-isomer, other stereoisomers have only slight activity.[33]

Rifampicin:

Rifampicin belongs to the group of rifamycins and is a semisynthetic derivative of rifamycin B, which itself has poor antimicrobial activity. It is used as a front line drug against tuberculosis, leprosy and HIV patients with mycobacterial infections which is also true for rifabutin, a further semisynthetic derivative of rifamycin B used only as a second line drug.[15] The mechanism of action is the binding to the β-subunit of bacterial DNA-dependent RNA polymerase to avoid initiation of transcription. Several studies found evidence for inhibition of the mycobacterial RNA polymerase[34,35] and also mutations found in the β-subunit of RNA polymerase associated with acquired resistance support this mode of action.[15,36,37]



rifampicin



rifabutin

Fig. 1.1.9 Structures of the rifamycins rifampicin and rifabutin

1.1.2.2 Second line antituberculosis drugs

In cases of resistance to the *first line drugs* (see 1.1.3) the therapy is based on agents which are often more toxic, more expensive, less experienced and less active than the standard first line drugs.[13,38]

_p_-Aminosalicylic acid:

Although the mycobacterial activity against *M. tuberculosis* was found a long time ago[39] the mechanism of action of *p*-aminosalicylic acid (PAS) is still a subject of research.[24] Some inconclusive results suggest that PAS interferes with the salicylic acid metabolism and therefore with *M. tuberculosis* iron intake.[40,41] PAS is only rarely used as an antituberculosis agent today due to its limited bacteriostatic activity and frequently occurring side effects.[42]

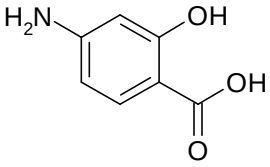
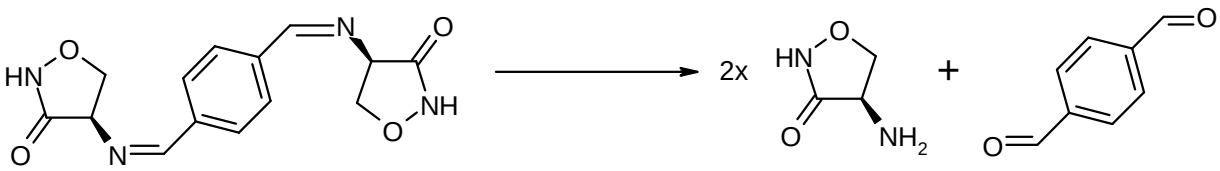


Fig. 1.1.10 Structure of p-aminosalicylic acid

Terizidon and D-cycloserine:

Terizidon is a dimer of D-cycloserine coupled via a terephtalaldehyde, which is hydrolyzed after resorption and releases D-cycloserine (see Fig. 1.1.11).[33] The target of D-cycloserine is the D-alanine racemase and ligase, which leads to inhibition of the mycobacterial cell wall biosynthesis.[43,44] Its use is however very limited due to the high incidence of neurological and psychiatric adverse effects such as headache, dizziness, depression and confusion.[13,45]



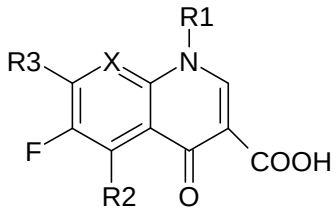
terizidone D-cycloserine

Fig. 1.1.11 Hydrolysis of terizidone into D-cycloserine

Fluoroquinolones:

Fluoroquinolones are synthetic derivatives of nalidixic acid and some of this class of compounds display broad-spectrum antibacterial activity including antimycobacterial activity.[46] Their mode of action is the interaction with DNA topoisomerase II (gyrase)

that catalyzes the negative supercoiling of the DNA. Inhibition of the gyrase prevents processes such as replication and transcription.[33] A second target for some fluoroquinolones is the topoisomerase IV, which is responsible for and its inhibition therefore prevents the resolution of replicated DNA.[33]



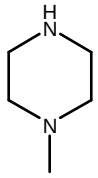
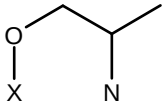
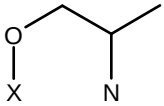
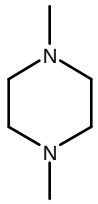
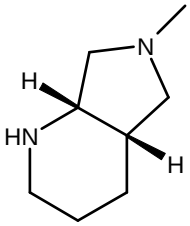
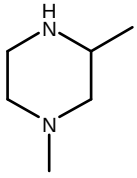
	X	R1	R2	R3
ciprofloxacin	-CH		-H	
ofloxacin / levofloxacin (only S-enantiomer)			-H	
moxifloxacin*	$-COCH_3$		-H	
gatifloxacin*	$-COCH_3$		-H	

*Fig. 1.1.12 Fluoroquinolones used as second line antituberculosis drugs; *not clearly recommended by WHO[47]*

1.1.3 Drug-resistant tuberculosis

1.1.3.1 Multidrug resistant tuberculosis

The disease tuberculosis returned during the last 20 years instead of being eradicated and represents a worldwide problem[48] not only in developing countries but also in Europe and the U.S.[49,50] The major reason for this is the occurence of multidrug resistant tuberculosis (MDR-TB), which is defined as tuberculosis in patients whose infecting isolates are resistant *in vitro* to at least isoniazid and rifampicin as the most powerful antituberculosis drugs.[47,51] The most common reasons for this MDR-TB are inadequate or poorly administered treatment (see Fig. 1.1.13) including inadequate regimens, inadequate drug supply and quality and inadequate drug intake by patients allowing a drug-resistant strain to become the dominant strain in a patient infected with TB. Further reasons can be a short-course chemotherapy for patients infected with drug-resistant strains ("amplifier effect") and transmission of established drug-resistant strains.[47,52]

Inadequate regimens	Inadequate drug supply and quality	Inadequate drug intake
• inappropriate guidelines • no monitoring of treatment • poorly organized or funded TB control programmes	• poor quality • unavailability of drugs • poor storage conditions • wrong dose or combination	• poor adherence • adverse effects • social barriers • malabsorption

Fig. 1.1.13 Causes of inadequate drug supply[47]

The third global report on antituberculosis drug resistance of the WHO has documented that in some areas occurrence of MDR-tuberculosis is extremely high:[53] The median prevalence of MDR-TB in patients *never previously treated* was 1.2 %, but included countries as the former Soviet Union exceeding 6.5 % in 2004. The report also documented that drug resistance was strongly associated with previous treatment: the median prevalence of MDR-TB in *patients previously treated* was 7.7 % ranging between 0 - 58.3 %. Early reports about the treatment of MDR-TB reported mortality rates up to 37 %.[54]

Treatment of MDR-TB is based on any of the *first line drugs* (see 1.1.2.1) to which the strains are still susceptible and on alternative or *second line drugs* (see 1.1.2.2). However, these cases of multidrug resistant tuberculosis are much more difficult to treat: the therapy is based on agents that are often more toxic and less active than the *first line drugs*.[13] Furthermore, the therapy is of longer duration being continued for 9 to 12 months and careful supervision of medication is required which leads to a tremendous rise in costs.[55]

The WHO developed a new strategy to treat MDR-TB that has been named “DOTS-Plus”.[56] Furthermore, they founded the “Green Light Committee” being responsible to establish, assess and evaluate projects under this strategy.[56]

1.1.3.2 Extensively drug-resistant tuberculosis

A further form of resistant tuberculosis has been reported to emerge in increasing numbers worldwide,[57] the extensively drug-resistant tuberculosis (XDR), which is defined as not only being resistant to at least isoniazid and rifampicin (MDR-TB), but also plus resistance to any fluoroquinolone and any one of the second line anti-TB injectable drugs amikacin, kanamycin or capreomycin.[58] According to a recent publication, 10 % of MDR-TB strains were XDR and treatment outcomes for patients with XDR-TB were poorer than for patients with MDR-TB.[59,60]

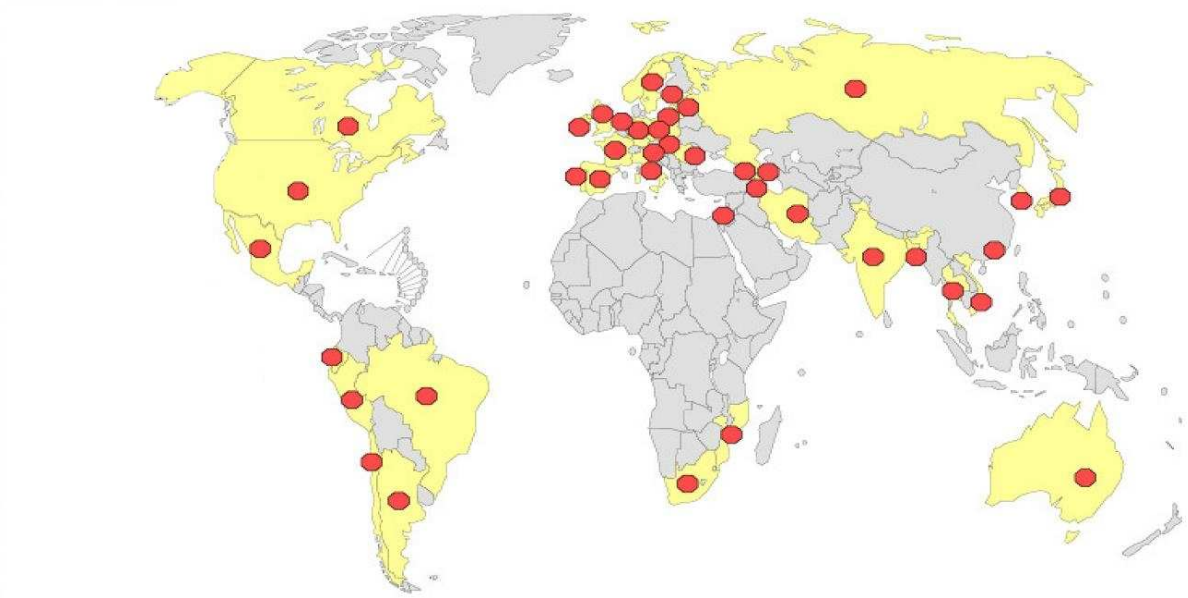


Fig. 1.1.14 Countries with known XDR-tuberculosis cases until 2007[47]

According to the WHO, XDR-TB raises concerns of a future TB epidemic with restricted treatment options.[58] With the "MDR-TB and XDR-TB response plan 2007-2008" the WHO wants to save 84 % of the treated MDR- and XDR-TB cases with estimated costs of 2.1 billion US$ for these 2 years.[58]

1.1.4 The development of new antituberculosis drugs

The occurrence of more and more multidrug resistant and even extensively drug resistant tuberculosis, the related high costs and the few drugs for a corresponding treatment (see 1.1.3) causes the urgent need to develop new antituberculosis drugs for the treatment of resistant but also the "classic" tuberculosis.
Many antituberculosis agents exert their effects on bacterial cell wall synthesis, protein synthesis and DNA replication (see 1.1.2). A further pathway, the fatty acid synthase (FAS) pathway is an especially attractive target for future drugs against tuberculosis:

- targeting the FAS pathway leads to inhibition of the biosynthesis of mycolic acids, an essential and indispensable part of the mycobacterial cell envelope
- FAS has been validated as an antituberculotic target through the demonstrated effectiveness of isoniazid and triclosan
- Extant resistance mechanisms should be relatively ineffective against new antituberculosis drugs targeting FAS system due to being largely unexploited
- The organisation of components of the fatty acid biosynthesis pathway is different in mammals (type I FAS) and in bacteria (type II FAS)
- The availability of structures of many enzymes in the fatty acid biosynthesis pathway provides a basis for rational drug design

1.1.4.1 Fatty acid synthase pathway

The pathway for fatty acid biosynthesis in *E. coli* serves as a model to understand type II FAS systems in other bacteria and plants due to the fact of being well established. The procedure of fatty acid biosynthesis in *E. coli* is outlined in Fig. 1.1.15:[61] the acyl carrier protein (ACP) is a key component in type II systems and carries the fatty acid intermediates from enzyme to enzyme through the cytosol.

Normally, C_{16} - C_{18} fatty acids are the principal end products of the pathway; in case of mycobacteria, the pathway is more complicated and consists of a FAS I and FAS II part to result in mycolic acids that contain 70-80 carbon atoms (see 1.1.4.3).

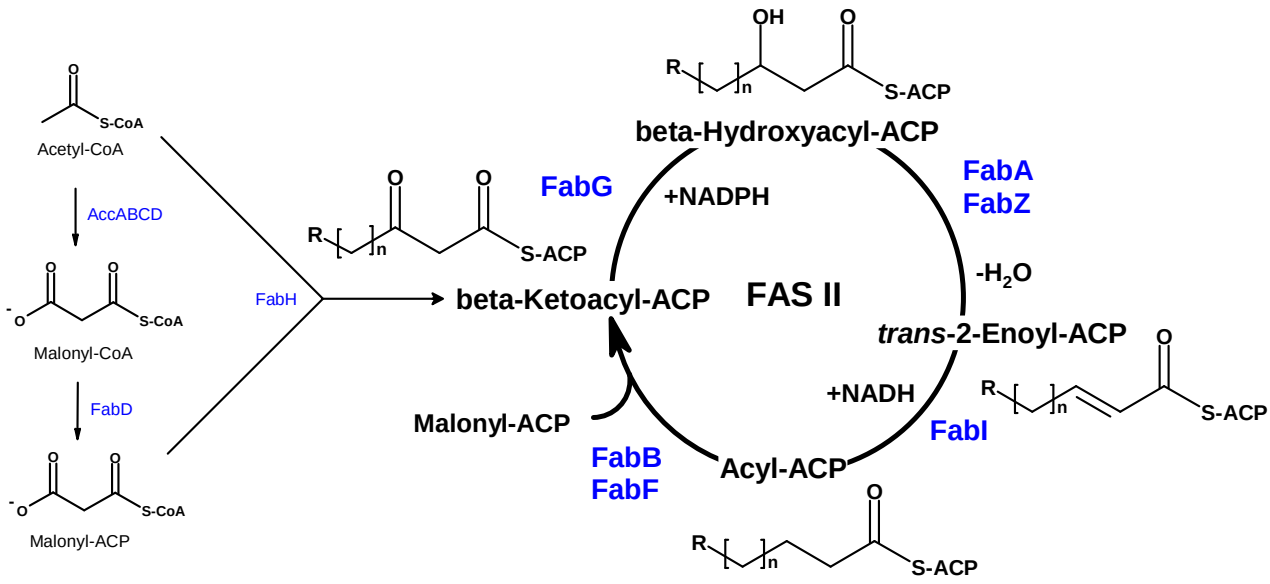


Fig. 1.1.15 Fatty acid biosynthesis pathway in E. coli. n = number of carbon atoms; n+2 per cycle

The first enzyme of the fatty acid biosynthesis is the acetyl-CoA carboxylase (accABCD) that transfers acetyl-CoA to malonyl-CoA, which subsequently connects to ACP by malonyl-CoA:ACP transacylase (FabD) to form malonyl-ACP.

The original fatty acid cycle is initiated by the condensation of malonyl-ACP with acetyl-CoA by the β-ketoacyl-ACP synthase III (FabH) to form β-ketobutyryl-ACP and CO_2. The following steps will be repeated until the desired chain length of the fatty acid is achieved: the first is the NADPH dependent reduction of the keto group to the hydroxyl group to give a β-hydroxyacyl-ACP intermediate by the β-ketoacyl-ACP reductase (FabG). In the next step water is removed by either a FabA or a FabZ isoform of the β-hydroxyacyl-ACP dehydratase to give a *trans*-2-enoyl-ACP. The third step involves the reduction of the produced double bond by the NADH dependent *trans*-2-enoyl-ACP reductase I (FabI) to form an acyl-ACP. The formed acyl-ACP is then condensed with a new malonyl-ACP by either β-ketoacyl-ACP synthase I or II (FabB and FabF) to start a subsequent cycle.

So far, the only enzyme of this pathway successfully inhibited by antibiotics (isoniazid and triclosan) in clinical use is FabI. Further inhibitors of enzymes in the fatty acid

biosynthesis have been elucidated, among them two natural inhibitors of the condensing enzymes, thiolactomycin (FabH, FabB/F) and cerulenin (FabB, F; see chapter 3).

1.1.4.2 β-Ketoacyl-ACP synthase inhibitors

The β-ketoacyl-ACP synthases I-III (FabH, FabB and FabF) perform the elongation step in fatty acid biosynthesis. They all use malonyl-CoA in a Claisen condensation to add two carbon units to the growing acyl chain. FabB and FabF operate in the elongation while FabH is responsible for the initiation step with acetyl-CoA. All three enzymes share an identical fold, the major differences are near the active site.[62,63]
The condensing enzymes operate via a ping-pong mechanism: the growing fatty acid intermediate is transferred from ACP to a sulphur of a cysteine in the active site of Fab and F, respectively. This is followed by the entry and decarboxylation of a malonyl-ACP that also binds to the acyl-enzyme intermediate. The fatty acid intermediate is then coupled to the malonyl-ACP-substrate, which still remains at the active site of the enzyme and thus elongates the growing fatty acid intermediate. The active site is divided into two halves, one for acyl formation and the second for malonyl binding.[64]
The two most thoroughly characterized inhibitors are thiolactomycin and cerulenin that have been used as templates to obtain more effective inhibitors of FabB and FabF. Cerulenin is described in detail in chapter 3.

Thiolactomycin and analogues:

Thiolactomycin (TLM) is a metabolite of the soil fungi *Nocardia* species 2-200.[65] It displays antibacterial activity against gram-negative and gram-positive bacteria and *M. tuberculosis*[66,67] and is specific for type II FAS.[68] Although thiolactomycin displays *in vivo* efficacy in mice, issues of synthesis and stability have prevented it from becoming a therapeutic agent.[61] Relatively high

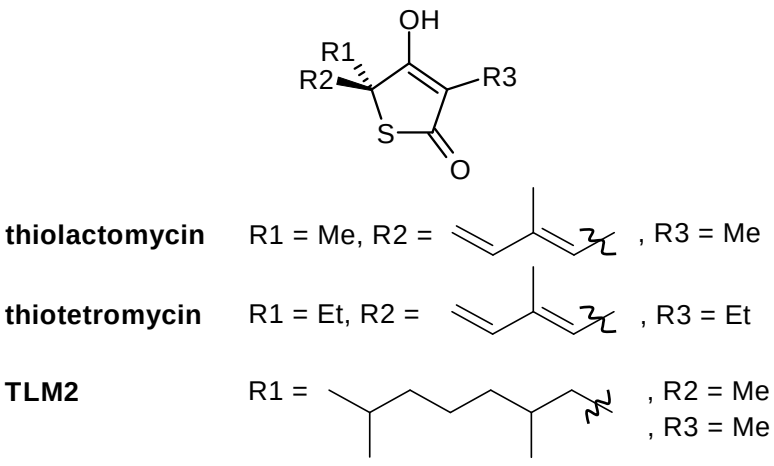


Fig. 1.1.16 Structure of thiolactomycin and analogues

concentrations are needed for an inhibition of *E. coli* growth (~50 µg/ml), while only 2 µg/ml are needed for *in vitro* inhibition of FAS activity.[68] However, it was shown that TLM binds more tightly to the β-ketoacyl-ACP synthase KasA acyl-enzyme intermediate (KasA in *M. tuberculosis* corresponds to FabB in *E. coli*, see 1.1.4.3) than to the free enzyme.[69] Thiolactomycin inhibition is reversible; it is non-competitive for acetyl-CoA and competitive for malonyl-CoA suggesting that TLM mimics the malonyl-ACP binding,[70] which is supported by the crystal structure of the FabB-thiolactomycin complex.[63] Several analogues of TLM have been synthesized (Fig. 1.1.16). Analogues with longer chains seem to be better inhibitors while shorter chains are generally less effective.[71] TLM2 seems to be more potent than TLM in terms of MIC_{90} for *M. tuberculosis* of 29 µM (TLM: 125 µM) and mycolate inhibition in extracts of *M. smegmatis* of 92 % (TLM: 54 %).[72] Thiotetromycin[73] showed similar antibacterial activity and IC_{50} values for FAS II inhibition as Thiolactomycin.[77]

Natural product inhibitors:

Screening of libraries with several thousand natural product extracts revealed further inhibitors[74] (see Fig. 1.1.17).

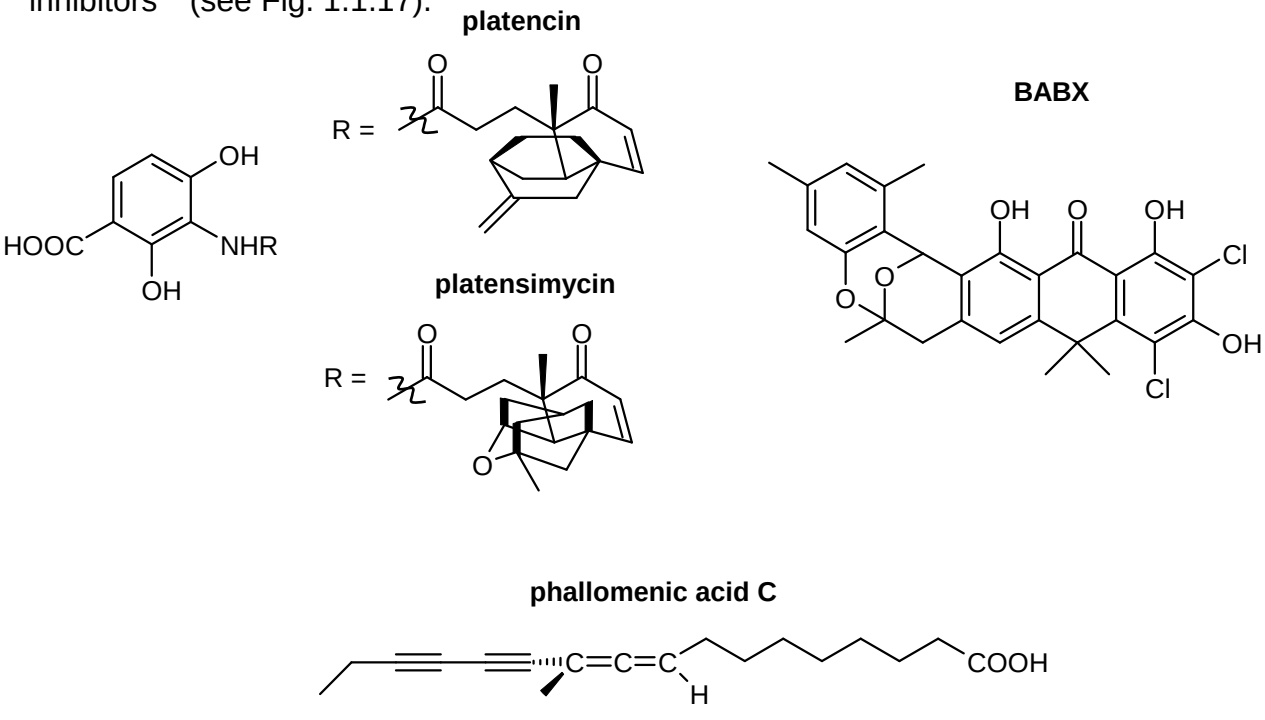


Fig. 1.1.17 Natural product β-ketoacyl-ACP synthase inhibitors

Platensimycin is a natural product from *Streptomyces platensis* and shows broad-spectrum activity against gram-positive bacteria as well as resistant *S. aureus* and *E.*

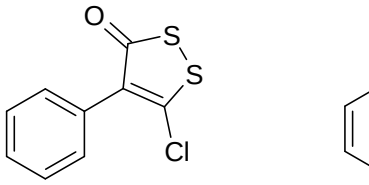
faecalis. It represents a selective FabF inhibitor with an IC_{50} of 48 nM and 160 nM for *S. aureus* and *E. coli*, respectively, in cell-free single enzyme assays with only weak inhibitory activity towards FabH (IC_{50} = 67 µM).[75] Platensimycin was found to bind to the acyl-enzyme intermediate of the target enzyme in the malonate binding site preventing the malonyl-ACP substrate from binding.

A similar analogue, *platencin*, was found by screening of natural products. It displays less selectivity and inhibits both enzymes, FabH and FabF, with comparable values (IC_{50} = 1.95 and 3.91 µg/ml, respectively) and therefore is a better FabH inhibitor but poorer FabF inhibitor than platensimycin. Platencin also displays comparable *in vitro* activity to a range of gram-positive bacteria but with better properties against vancomycin-resistant *E. faecalis* and efflux-negative *E. coli.*[76]

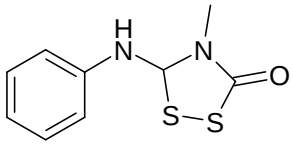
Phallomenic acid C was obtained by natural product screening as the most potent of the series of phallomenic acids.[77] Phallomenic acid C was identified to inhibit FabH/F enzymes in FAS II with MICs between 0.77-3.9 µg/ml against *S. aureus* and *H. influenzae*.

BABX was identified by screening methods and was found to be effective against cell-free *S. aureus* and *E. coli* (IC_{50} = 11.4 and 35.3 µg/ml).[78] Using cerulenin and triclosan as a reference, BABX was inferred to inhibit FabB and F.

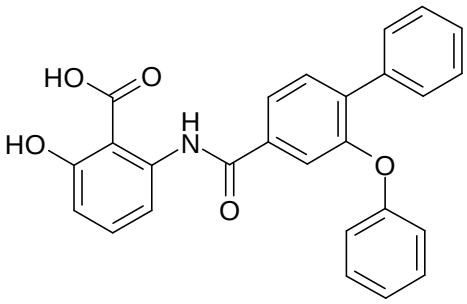
Synthetically obtained inhibitors:



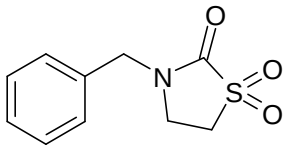
HR-12



HR-19



benzoylaminobenzoic acid



cyclic sulfone

Fig. 1.1.18 Synthetically obtained β-ketoacyl-ACP synthase inhibitors

The compounds *HR12* and *HR19* were identified by screening of a chemical library and found to inhibit FabH of *S. aureus*, which shows 40 % similarity to FabH of *E. coli*. HR12 and 19 showed a more efficient inhibition of saFabH (IC_{50} = 1.87 and 0.78 µM, respectively) than thiolactomycin in this assay. Also the antibacterial activity against *S. aureus* and MRSA was more potent for HR12 (MIC = 8.7 and 8.7 µg/ml) and for HR19 (MIC = 35.0 and 17.5 µg/ml) than for thiolactomycin (MIC = 300 and >300 µg/ml).[79] From the same group, several cyclic sulfones were identified that are inhibitors of *E. coli* FabH, the best of which (cyclic sulfone) displayed an IC_{50} value of 1.1 µM and antibacterial activity against *E. coli* (MIC = 6.6 µg/ml).[80] However, only weak activity against FabH of *M. tuberculosis* and *P. falciparum* was observed.
Screening of a chemical library also revealed a series of benzoylaminobenzoic acids that were further improved structurally. The most potent candidate (benzoylaminobenzoic acid) of this group of compounds had good to moderate inhibitory activity against FabH of different pathogens (0.004 µM/*E. faecalis*, 0.004 µM/*S. pyogenes*, 3.8 µM/*S. aureus*, 2.4 µM/*H. influenzae*). However, antimicrobial activity was only moderate due to lack of penetration or efflux (MIC = >45 µg/ml; *E. faecalis*, MIC = 22 µg/ml; *S. aureus*).[81]

1.1.4.3 β-Ketoacyl-ACP synthases in M. tuberculosis

Fatty acid synthesis of *M. tuberculosis* leads to the production of mycolic acids, which are a series of C_{60}-C_{90} α-alkyl-β-hydroxy fatty acids found in the mycobacterial cell as esters of the arabinan terminus of arabinoglactan or as extractable “free” lipids within the cell wall.[82] They consist of a meromycolate chain (up to C_{56}) and a long saturated α-branch (C_{26}) and are responsible for resistance to chemical injury, low permeability to antibiotics, virulence, the ability to persist within the host and are essential for mycobacterial survival.[83,84] The biosynthetic pathway consists of two types of fatty acid synthesis systems, FAS I and II (see Fig. 1.1.19). The eukaryotic type FAS I is responsible for *de novo* synthesis of fatty acids from acetyl-CoA whereas FAS II is analogous to other bacterial FAS II systems except that it is not capable of *de novo* synthesis and elongates acyl-CoA primers generated by FAS I.
The biosynthesis of mycolic acids involves 5 steps (Fig. 1.1.19):[85]

(1) Synthesis of C_{26} saturated straight chain fatty acids by FAS I to provide the α-branch of mycolic acids

(2) Synthesis of C_{56} fatty acids by FAS II to provide the meromycolate residue

(3) Introduction of functional groups to the meromycolate chain

(4) Condensation reaction between the α-branch and the meromycolate chain catalysed by Pks13

(5) Transfer of the mycolic acids to arabinoglactan or other receptors (not shown)

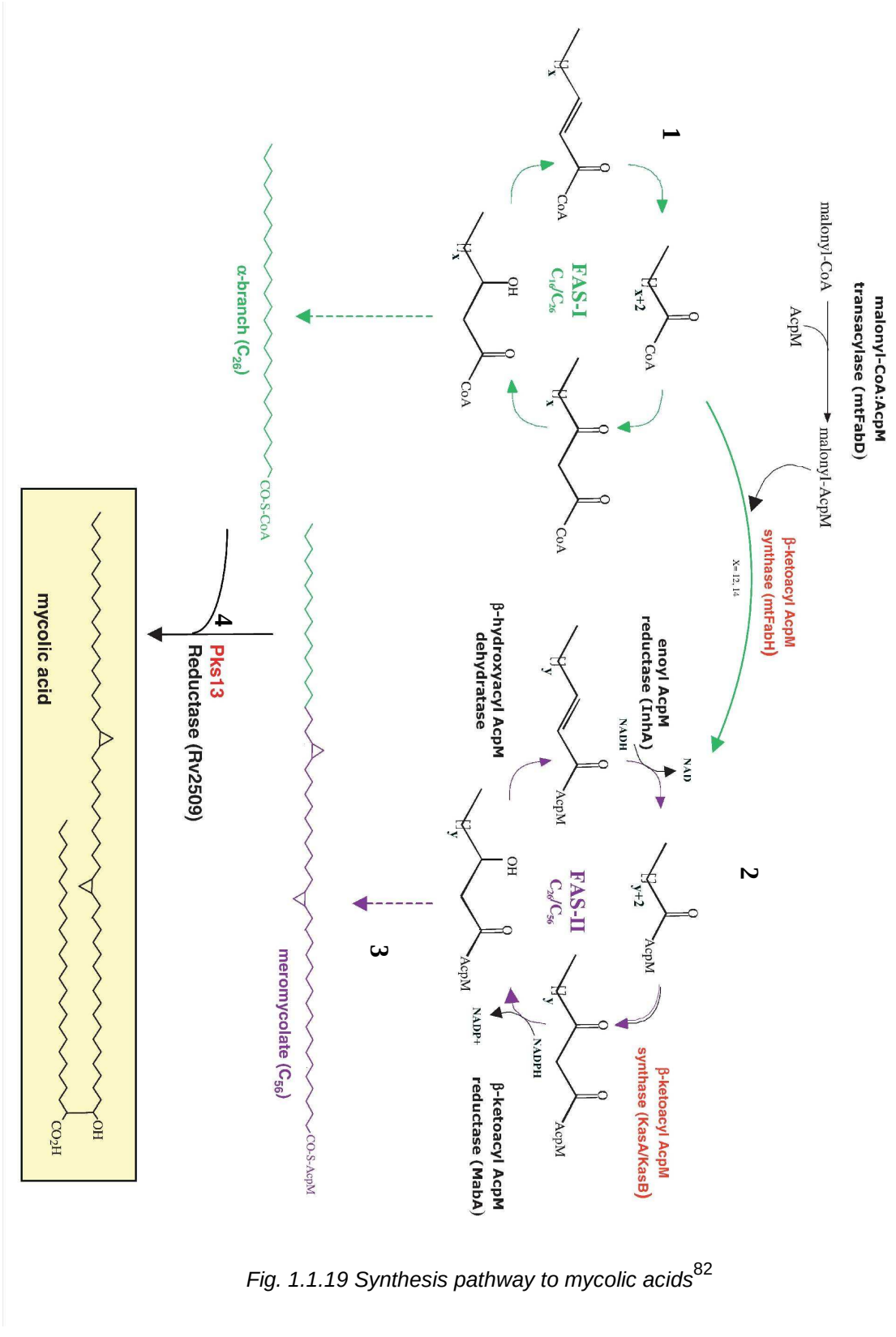


Fig. 1.1.19 Synthesis pathway to mycolic acids[82]

The condensation reaction of the elongating enzymes of *M. tuberculosis* KasA and KasB (FabB and F in *E. coli*) is similar to that in the FAS II of *E. coli* (see Fig. 1.1.15): At first, an acyl-AcpM is transferred to the active site cysteine attaching covalently to the enzyme. Then malonyl-AcpM binds to the active site and is decarboxylated so condensation can be carried out. The final product, a β-ketoacyl-AcpM with two additional carbons, is subsequently released. KasA seems to catalyse the initial elongation reactions while KasB seems to extend to full length mycolates.[82]

KasA and B share a substantial sequence similarity of ~67 % to each other and of ~40 % to FabB and F of *E. coli*. [86] Furthermore, the crystal structure of KasB has been determined and due to their similarity KasA was modelled and an almost identical structure was predicted except for a larger entrance to the active site tunnel for KasA.[87] Also a three dimensional model of KasA based on the crystallographic coordinates of the *E. coli* protein was built revealing the catalytic triad in the active site.[86]

Due to their essential role for *M. tuberculosis* and their absence in humans, the FAS II enzymes KasA and B represent ideal drug targets for future antituberculosis drug development. Furthermore, the similarity of KasA and B to the corresponding *E. coli* enzymes FabB and F, the knowledge of enzyme-inhibitor crystal structures for FabB and F with inhibitors such as cerulenin and thiolactomycin can be used for the development of new antituberculosis drugs.

1.2 Candida albicans and resistances

The number of fungal infections has increased in the last years and therefore their medical importance has risen.[88] They can generate a broad spectrum of infections from superficial over subcutaneous to invasive infections with considerable morbidity and mortality.[89] Especially the incidence of nosocomial fungal infections has highly increased, mainly due to increasing numbers of immunocompromised patients, longer hospital stays and advanced treatments.[90] The most responsible pathogens for these infections are *Candida* species, which are accountable for about 78 % of nosocomial fungal infections, the sixth most common nosocomial pathogen and the fourth most common microorganism in nosocomial sepsis cases in the U.S.[91] Especially the latter bloodstream infections are associated with attributable mortality rates that have ranged from 38 % between 1983 and 1986 to 49 % in the same institutions between 1997 and 2001.[92]

Relatively few of the more than 100 *Candida* species identified have been isolated from humans; the most common *Candida* species causing infections by far is *Candida albicans*.[93]

1.2.1 *Candida albicans*

1.2.1.1 Morphology

Candida albicans is a commensal and normally a harmless colonizer in humans.

It is a polymorphic fungus and shows a dimorphism: usually, *Candida albicans* is a gram-positive, round to oval formed yeast with a diameter of 2-6 µm (see Fig. 1.2.1), but it can switch to hyphae or pseudo-hyphae as a response to certain environmental factors. This ability to change between the yeast-like and the filamentous form is considered to be the essential factor of virulence for *Candida albicans*:[88]

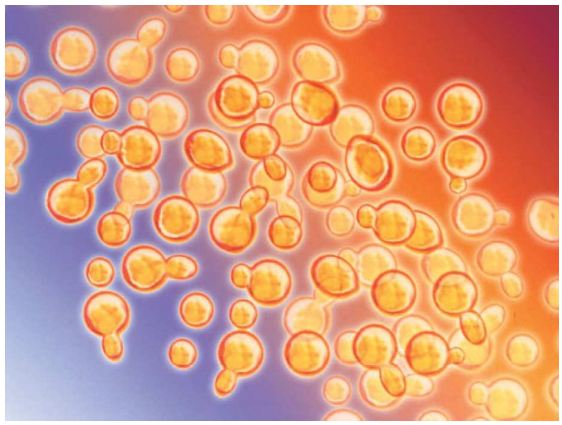


Fig. 1.2.1 Native preparation of Candida albicans[88]

in case of superficial or invasive mycoses the yeast cells transform into their filamentous form (Fig. 1.2.2). *Candida albicans* therefore is a facultative pathogen for humans and also an opportunistic fungus, because only in special environmental situations (e.g. in immunocompromised patients) the fungus causes infections, which therefore are mostly observed as endogenous infections.[94]

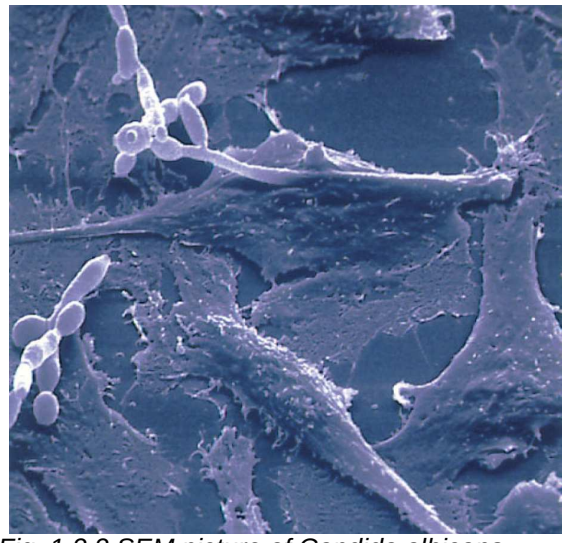


Fig. 1.2.2 SEM picture of Candida albicans hyphae intruding into human endothelial cells[95]

1.2.1.2 Systemic infections

Fungal infections can be divided into two parts: a local manifestation on skin and mucous membranes and systemic infections.[96]

Candida species can cause both, but the more dangerous and life threatening types are the systemic mycoses. These invasive infections have increased: not only in the U.S.(see 1.2),[91,97] but also in Europe[98], Canada[99] and Taiwan[100] a rising number of infections has been confirmed where candidemia turned out to be one of the most common cause of nosocomial bloodstream infections. Mortality rates of these systemic infections are extremely high (see 1.2).

The risk factors for invasive candidiasis are based on the site of infection and on the disease process (table 1.2.1): the most important are antibiotic administration followed by central venous catheters, prolonged stay in an intensive care unit and abdominal surgery.

Primary risk factors	Associated conditions
Prolonged antibiotic use	Solid organ transplant
Central venous catheter	Bone marrow transplant
Prolonged length of stay in an ICU	Hematologic malignancy
Abdominal surgery	Very low birth weight neonate
Heavy colonization	Acid-suppressing medications
immunosuppression	Extensive burns
	Acute renal failure
	Hemodialysis
	Severe pancreatitis

Table 1.2.1 Risk factors and conditions associated with invasive candidiasis[100]

The frequency distribution of *Candida* species causing invasive mycoses in humans is listed in table 1.2.2.

Species	Frequency (%)
C. albicans	48.0-59.0
C. glabrata	12.0-24.0
C. parapsilosis	7.0-11.0
C. tropicalis	5.8-19.0
C. krusei	1.0-5.0
C. lusitaniae	<1
Other *Candida* spp.	2.0-6.9

Table 1.2.2 Commonly reported species of distribution (%) of Candida bloodstream isolates[100]

Candida albicans clearly is the most common isolate responsible for candidemia.
The kind of infection of *Candida* occurs endogenous or exogenous, but the endogenous type is the most important one. The infections predominantly result from the gastrointestinal tract and skin.[101,102] However, the infection always requires defects in the normal host immunity like in immunocompromised patients with medication after transplantations, HIV patients etc.[103]

1.2.2 Systemic antimycotics

Although the number of incidences of invasive mycoses is growing, there are only few antimycotics available to treat these infections.[104]

Different substances from different classes of systemic antifungal drugs are existent like the polyenes, fluoropyrimidines, allylamines, azoles and echinocandins (Fig. 1.2.3).

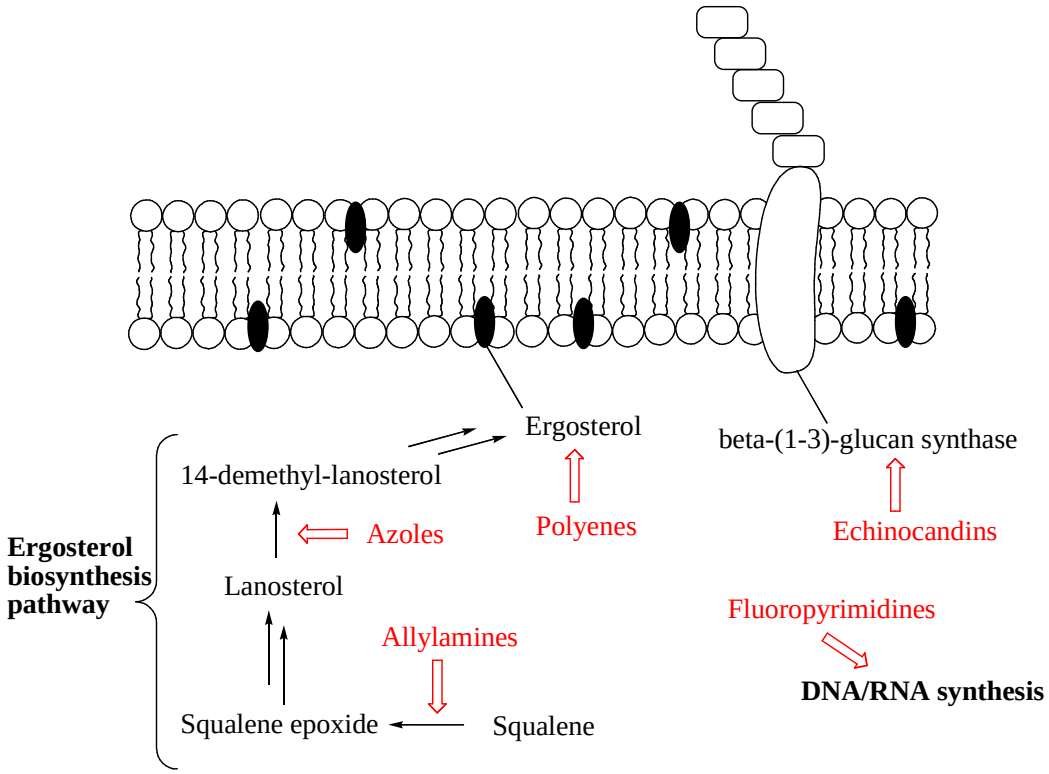


Fig. 1.2.3 Targets of systemic antifungal agents[104]

1.2.2.1 Polyene antimycotics

Amphotericin B:

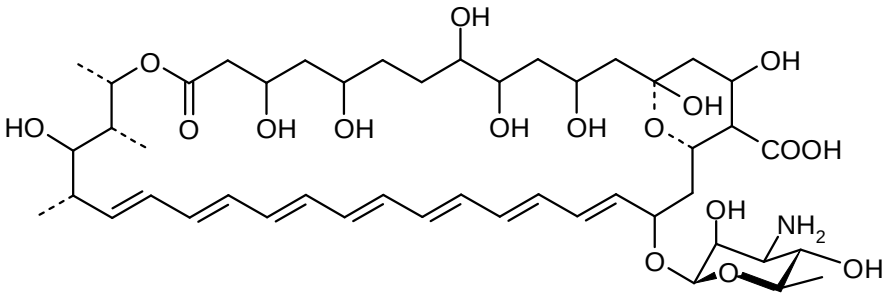


Fig. 1.2.4 Structure of amphotericin B [96]

Amphotericin B was the most commonly used drug in antifungal therapy for a long time since its approval in the 1950s.[105] Its antimycotic activity relies on its ability to

bind with ergosterol (the major sterol in the fungal plasma membrane) to the fungal plasma membrane, to form pores and thereby to change the membrane fluidity. This results in disruption of the cell permeability and rapid cell death.[106]

Amphotericin B still remains to be the antimycotic with the broadest spectrum of fungi: it is mainly used against invasive infections of *Candida* and *Aspergillus* species and also *Cryptococcus neoformans*. It has to be administered intravenously, because it is orally not bioavailable due to a lack of absorption. Therapy has to be carried out between 4 and 8 weeks.[96]

A disadvantage of amphotericin B is the enormous number of side effects. The major drug toxicity represents the nephrotoxicity, which depends on the overall administered drug concentration: up to 2.0 g nearly no renal damage is observed, up to 4.0 g 50 % of the patients show reversible damages and higher overall concentrations result in irreversible renal damages for the majority of patients.[96]

In the 1990s newer preparations of amphotericin B were developed to alleviate the drug toxicity (e.g. amphotericin B lipid complex, amphotericin B colloidal dispersion).[107] These formulations seem to decrease the neprotoxicity at least to some extent.[108]

1.2.2.2 Fluoropyrimidines

Flucytosine:

Flucytosine (5-fluorocytosine) exerts its antifungal activity via a biochemical distinctiveness of some fungi: mainly in yeasts the enzyme cytosine deaminase is present that converts cytosine via deamination to uracil which is included in RNA-synthesis. Analogous to that flucytosine is converted to 5-fluorouracil, which then competes with uracil and leads to defective RNA and inhibition of cell growth. In addition, 5-fluorouracil leads to inhibition of DNA synthesis and therefore cell division (Fig. 1.2.6). Hence, the fungicidal effect of flucytosine lies within its converted products and the low toxicity on the fact that the cytosine deaminase is either not present or merely active in mammalian cells.[109]

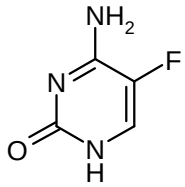


Fig. 1.2.5 Structure of flucytosine

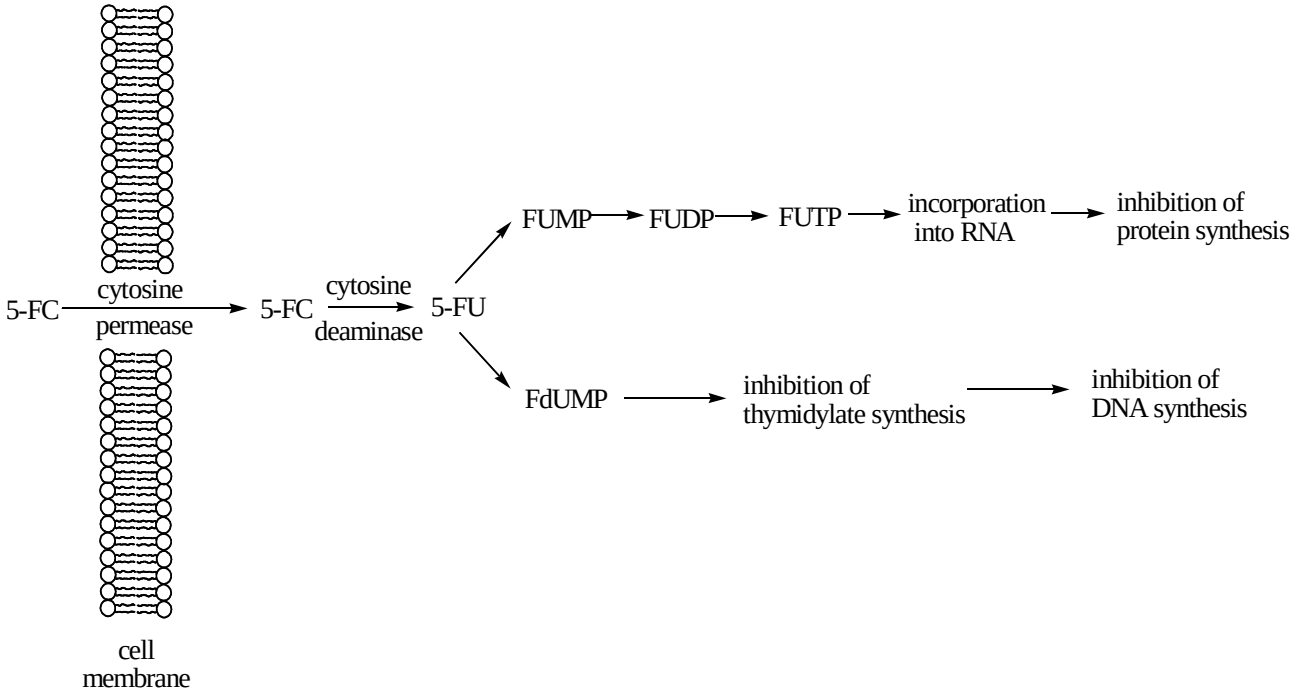


Fig. 1.2.6 Mode of action of flucytosine. Abbreviations: 5-FC: flucytosine, 5-FU: 5-fluorouracil, FUMP: 5-fluorouridine monophosphate, FUDP: 5-fluorouridine diphosphate, FUTP: 5-fluorouridine triphosphate, FdUMP: 5-fluorodeoxyuridine monophosphate[110]

The indication for flucytosine are infections with *Candida* species and *Cryptococcus neoformans* and it shows a moderate profile in terms of side effects. However, often occurring resistances have precluded its routine use and it is now mostly applied in combination with amphotericin B.[95,111]

1.2.2.3 Allylamines

Terbinafine:

Terbinafine stops fungal growth by affecting the biosynthesis of ergosterol, the major sterol in the fungal plasma membrane and equivalent to cholesterol in mammalian cells. It inhibits the enzyme squalene epoxidase (see Fig. 1.2.8) causing a poisonous amount of squalene in the cytoplasm.[112] In addition to that, the decrease of ergosterol production weakens the fungal cell wall.[113]

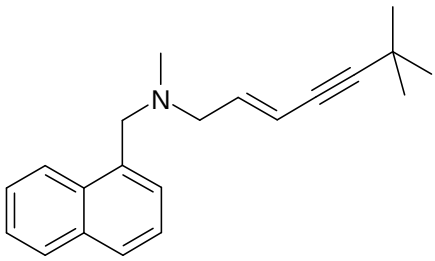


Fig. 1.2.7 Structure of terbinafine

Despite being a broad spectrum antifungal agent[112] terbinafine is only effective against dermatophytes when administered systemically and is indicated for dermatophytes that do not respond to local administration.[114] Even in cases of onychomycoses good results can be achieved.

Terbinafine shows mostly only slight side effects and does not interfere with the metabolism of drugs due to its small affinity to cytochrome P450.

1.2.2.4 Azoles

Antimycotics of the azole class are usually fungistatic and are broad spectrum drugs. They exert their antifungal activity via the inhibition of an enzyme, the 14-α-demethylase, in the biosynthesis pathway of ergosterol (Fig. 1.2.8). Azoles prevent the conversion of lanosterol to ergosterol by binding to the heme iron in the active site of 14-α-demethylase (a P450 enzyme), competing with substrate binding.[115]

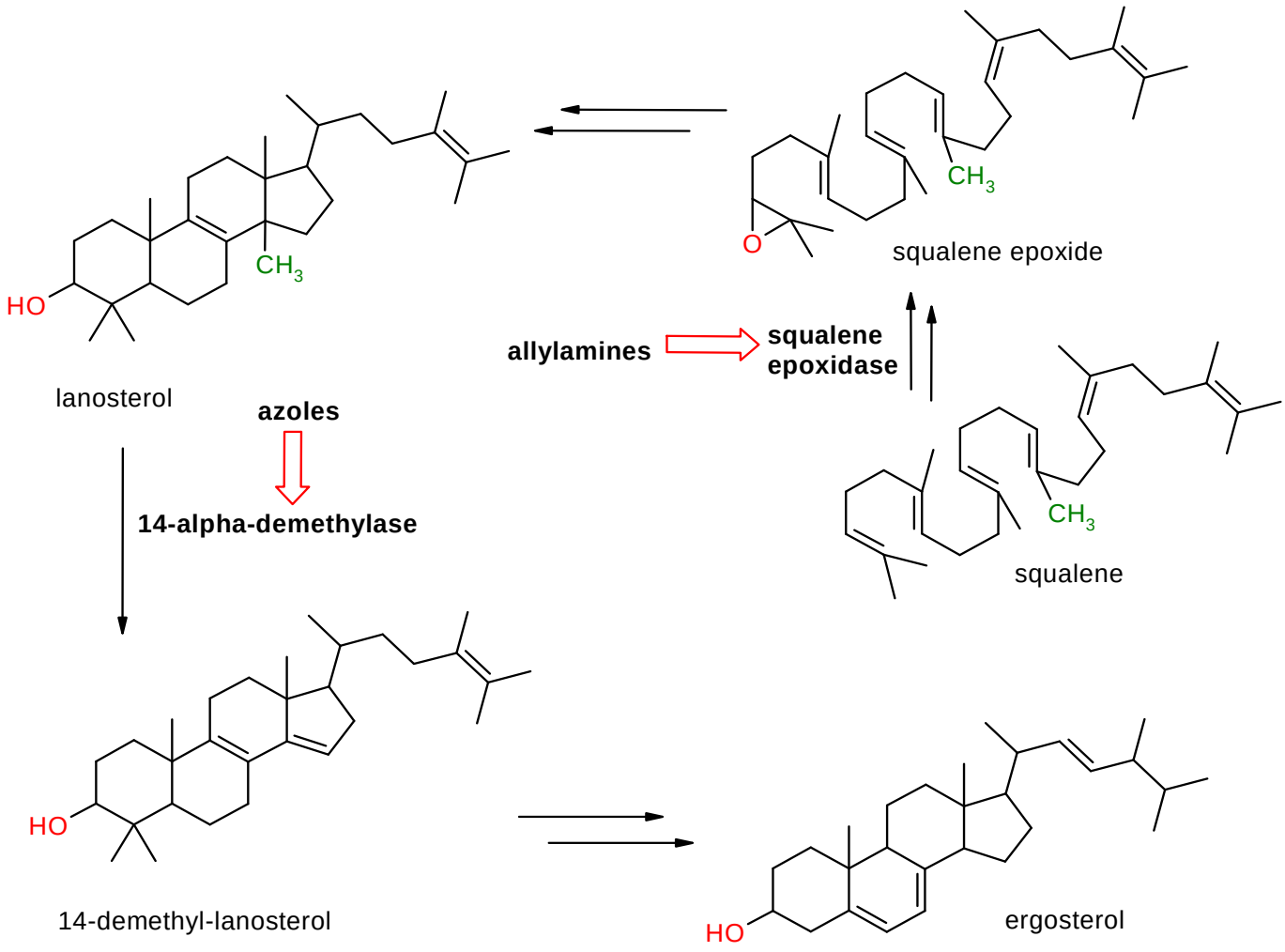


Fig. 1.2.8 Inhibition of ergosterol biosynthesis by allylamine and azole antimycotics

Azole antimycotics can be divided into two classes: the imidazole and triazole class. However, most of the systemically applied azoles belong to the triazoles.

Imidazoles:

Ketoconazole:

Ketoconazole was introduced as the first systemic azole antifungal drug.[106] In some cases severe hepatotoxicity could be observed[116] and also different drug-drug interactions lead to the use of azoles with better side effect profiles in terms of systemic application (triazoles, see below). Therefore, ketoconazole is applied mostly for local use.

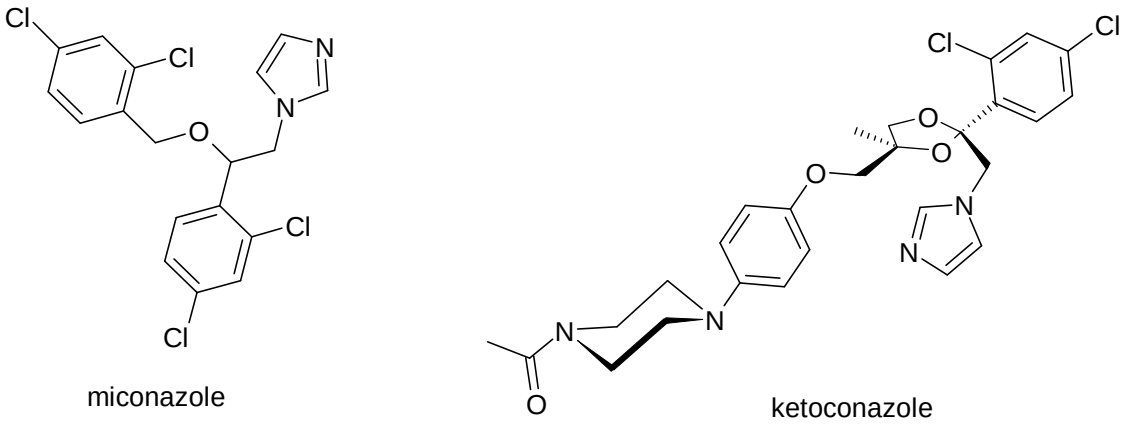


Fig. 1.2.9 Structures of miconazole and ketoconazole

Miconazole:

Miconazole can be used locally and intravenously, but due to its slow absorption and the availability of more efficient azoles, it is applied only locally today.

Triazoles:

Triazoles contain a triazole instead of an imidazole ring, which leads to much better properties in terms of hepatotoxicity and drug-drug interactions compared to the imidazoles.[114]

Itraconazole:

Itraconazole shows structural similarity to ketoconazole (Fig. 1.2.9).[117] It possesses good antimycotic properties against dermatophytes and *Aspergillus* species with lower effects also against yeasts like *Candida* species and *Cryptococci*.
Itraconazole has moderate oral bioavailability and a relatively good profile in terms of side effects. These are mostly of gastrointestinal nature but also an inhibition of the elimination of other drugs due to its interference with cytochrome P450 dependent monooxygenases can occur.[118] Itraconazole cannot be coadministered with agents that raise the gastric pH[119] and should be administered after a full meal.[120]

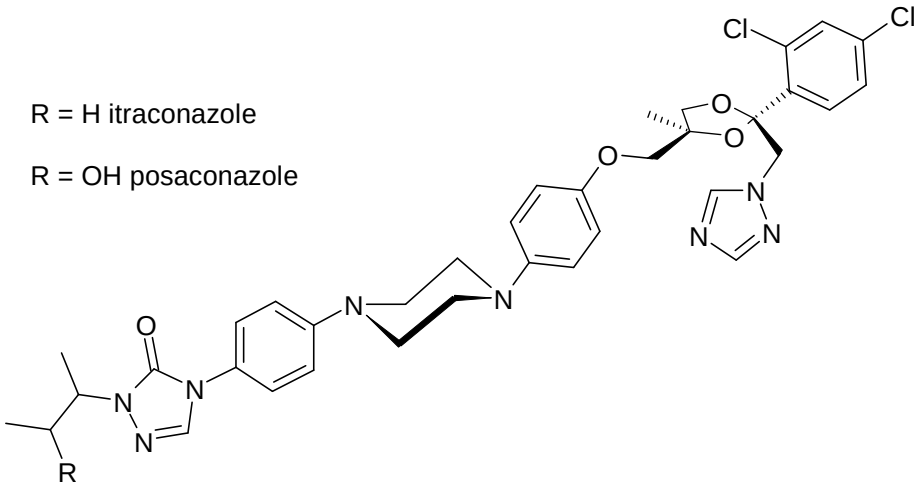


Fig. 1.2.10 Structures of itraconazole and posaconazole

Posaconazole:

Posaconazole is structurally related to itraconazole (Fig. 1.2.10). It became available in Europe in 2005 and was approved by the FDA in 2006 against invasive *Aspergillus* and *Candida* infections.[130] It has a wide range of antifungal activity including *Candida* species resistant to older azoles.[121] A major difference to voriconazole is the ability of posaconazole to exhibit activity against *Zygomycetes* and therefore is the antifungal agent besides amphotericin B that is active against these fungi.[122] Posaconazole was shown to be superior to fluconazole with systemic infections in highly

immunocompromised patients[123] and equal in the treatment of oropharyngeal candidiasis.[124] Posaconazole posseses few side effects, mostly nausea, vomiting, headache, abdominal pain and diarrhoea.[125] The absorption of posaconazol is optimized when administered with a high fat meal.[126]

Fluconazole:

Fluconazole contains two triazole ring substituents and a hydroxyl function which leads to a good solubility in water (Fig. 1.2.11). It was approved in 1990[127] and shows fungistatic activity against invasive cryptococcal and *Candida* infections, but not to filamentous fungi.[128] Fluconazole displays an excellent profile of tolerance in the range of doses recommended for invasive candidiasis. Side effects occur especially with doses >400 mg/day.[129] Common side effects include headache, nausea and abdominal pain.[128] Fluconazole is readily absorbed with a bioavailability achieving concentrations equal to 90 %, similar to those achieved by intravenous administration.[104] Absorption is not effected by food consumption or gastric pH. Due to its good pharmacokinetic and safety profile, but also due to its current low cost it remains a leading antifungal drug especially against susceptible *Candida* infections.[128]

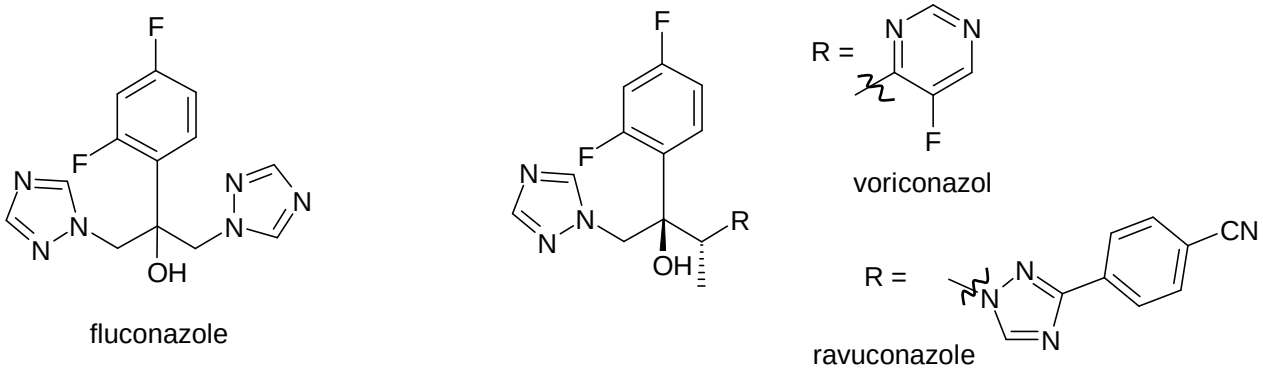


Fig. 1.2.11 Structures of fluconazole, voriconazole and ravuconazole

Voriconazole:

Voriconazole was first available in 2002. The spectrum of pathogens was expanded compared to fluconazole: it shows enhanced fungistatic activity even to fluconazole-resistant *Candida albicans*[130] and also fungicidal activity against moulds[131] even to amphotericin B resistant *Aspergillus*[132] species. Voriconazole has become the drug of choice for primary therapy of invasive aspergillosis.[133] Similar to fluconazole,

voriconazole is also quite well tolerated. The most common side effects are visual disturbances, rash and gastrointestinal symptoms.[134] The oral bioavailability of voriconazole is >90 % with an empty stomach, but decreases with food intake.[135]

Ravuconazole:

Ravuconazole is structurally related to fluconazole. It is currently in clinical trials and is highly active against a wide range of *Candida* species including some that are resistant to fluconazole.[136] It is also active against *Aspergillus* species and zygomycetes.[137] Few trials in humans have been published; ravuconazole proved to be superior to fluconazole in HIV patients with oesophageal candidiasis.[130]

1.2.2.5 Echinocandins

Echinocandins represent the newest class of antifungals. The mechanism of action is based on their inhibition of the production of (1-3)-β-D-glucan, which is an essential compound of the fungal cell wall, resulting in cell death (see Fig. 1.2.12).[138] The spectrum of activity is therefore limited to pathogens relying on these glucan polymers and is not as broad as that of polyene or azole agents. However, they are fungicidal against many *Candida* species, therefore being a good alternative to the fungistatic azole agents although they can only be administered as intravenous preparations. Due to the fact that mammalian cells have no cell wall, echinocandins display few toxic effects in humans.[139]

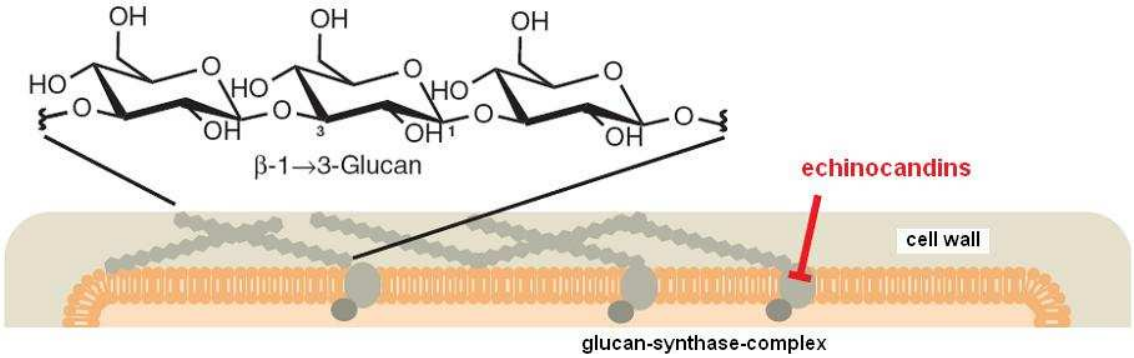


Fig. 1.2.12 Inhibition of glucan synthesis by echinocandins[140]

Caspofungin:

Caspofungin was released in 2001.[104] It exhibits high fungicidal activity *in vitro* against most isolates of *Candida* species. It has also potent inhibitory activity against *Aspergillus* species and moderate activity against other moulds. In contrast, no activity is observed for some other fungi, e.g. zygomycetes.[141] In comparison to amphotericin B, caspofungin was equally efficient and better tolerated in the treatment of invasive *Candida* infections.[142] Caspofungin has few side effects, the most common are headache, fever, nausea, rash and reversible elevation of hepatic enzyme levels. Furthermore, caspofungin concentrations are decreased with CYP450 inducers.[143]

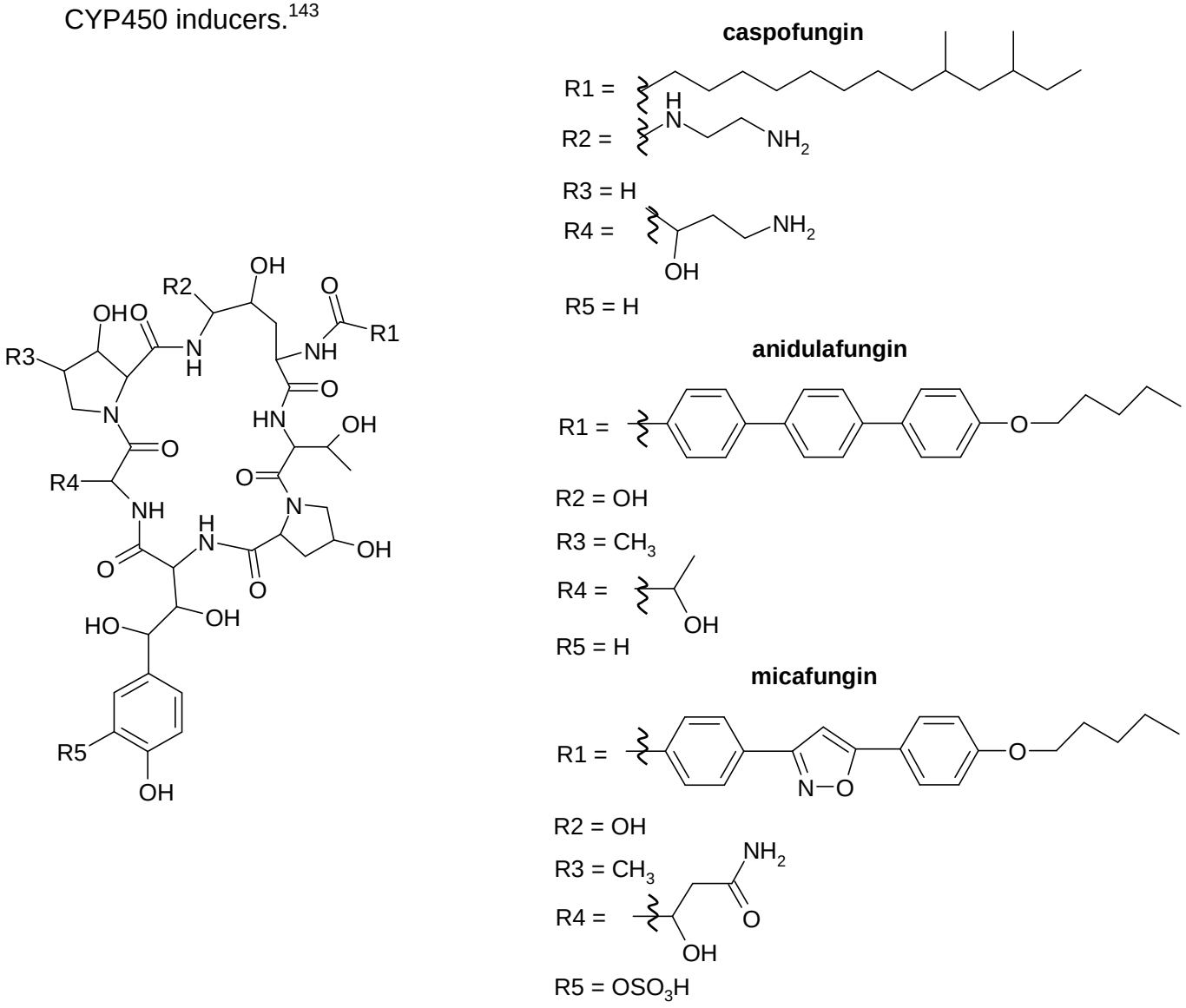


Fig. 1.2.13 Structures of caspofungin, anidulafungin and micafungin[144]

Micafungin:

Micafungin was approved in 2005[104] and has a similar spectrum of antifungal activity as caspofungin. Its efficacy has been shown in treatment of oesophageal candidiasis[145] and in severely compromised hosts with refractory aspergillosis.[146] It was shown to be superior to fluconazole when given as prophylaxis in stem cell transplantation.[147] Micafungin is well tolerated with similar side effects as caspofungin, but seems to have fewer drug interactions.[147]

Anidulafungin:

Anidulafungin obtained its FDA approval in 2006.[148] It is highly active against *Candida* species including species that are resistant to azoles, amphotericin B or other echinocandins[149,150] as well as against *Aspergillus* species.[150] It is well tolerated and seems to have fewer drug interactions than caspofungin.[130]

1.2.3 Resistances to antifungal agents

While the appearance of invasive fungal infections increases (see 1.2), the choice of antifungal agents remains relatively limited although the emergence of the new echinocandin class is a welcome development as is the expansion of members in the triazole class (see 1.2.2). However, due to this limited number but excessive use of antimycotics an increasing amount of resistances can be observed not only of intrinsically resistant but also normally sensitive fungal isolates.

Most *flucytosin* resistances occur due to mutations in the enzymes that are involved in the salvage pathway namely cytosine deaminase or UPRTase (transfers 5-FU to f-FUMP, see Fig. 1.2.6). These secondary resistances in addition to primary resistances are often observed because flucytosin is mainly used in combination with amphotericin B.[151]

Amphotericin B resistance is relatively rare and arises as a result in the decrease of ergosterol content of the cells which leads to the binding of smaller amounts of polyene than in susceptible cells.[152]

As already mentioned, *Fluconazole* represents the most commonly used drug of the azoles against *Candida* infections. Several different mechanisms can be responsible for the development of a resistance to fluconazole: (a) alterations in the biosynthesis

pathway, (b) mutations in the gene encoding the drug target enzyme, (c) overexpression of the drug target enzyme and (d) overexpression of efflux pumps.

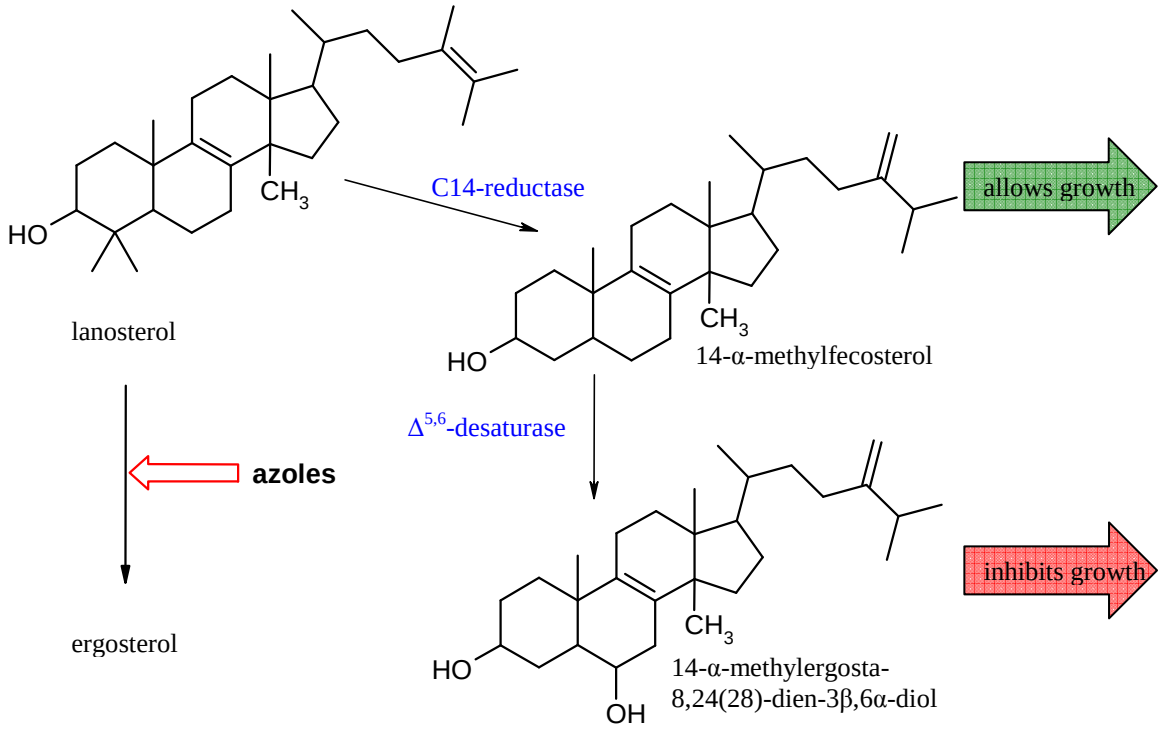


Fig. 1.2.14 Resistance mechanism (a)[95]

(a) The inhibition of the 14-α-demethylase by fluconazole (see Fig. 1.2.8) does not only lead to ergosterol depletion, but to accumulation of the methylated sterol 14-α-methylergosta-8,24(28)-dien-3β,6α-diol (see Fig. 1.2.14) that inhibits cell growth.[153] Alterations in the sterol biosynthesis pathway that avoid the accumulation of this growth inhibiting sterol can lead to a fluconazole resistance. This is observed in case of the inactivation of $\Delta^{5,6}$-desaturase, which leads to accumulation of 14-α-methylfecosterol that allows growth even in the presence of fluconazole.[154]

(b) Azoles bind to the heme in the active site of the 14-α-demethylase and avoid binding of the endogenous substrate (see Fig. 1.2.8). Mutations reducing the affinity of fluconazole to the enzyme while containing similar enzyme activity, lead to fluconazole resistance. It was found that the *ERG-11* gene expresses the fluconazole target enzyme 14-α-demethylase. Mutations in this gene in fluconazole resistant derivatives of originally susceptible *Candida albicans* isolates could be observed being responsible for the resistance.[115]

(c) Another resistance mechanism is the overexpression of the 14-α-demethylase. In *Candida albicans* the upregulation of the *ERG-11* gene could be observed in the

presence of fluconazole probably as a feedback mechanism to make up for ergosterol depletion.[155] In this case higher concentrations of fluconazole are needed for an efficient inhibition in these isolates.[156] This could also be demonstrated by artificial overexpression of the *ERG-11* gene in *S. cerevisiae* or *C. glabrata* which lead to significantly reduced fluconazole susceptibility.[157]

(d) One of the most important and most frequent fluconazole resistance mechanisms is the overexpression of efflux pumps.[115] A reduced intracellular drug concentration is observed for this type of resistance due to an active transport of fluconazole out of the cell (see Fig. 1.2.15). In *Candida albicans* and in other *Candida* species several membrane transport proteins were found that cause resistance:

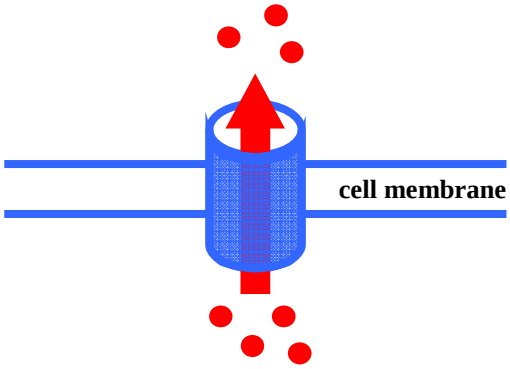


Fig. 1.2.15 Efflux mechanism (d)

CDR1p and CDR2p (*Candida* drug resistance) are "ATP-binding cassette" (ABC) transporters that use adenosine triphosphate (ATP) as the energy source, whereas the MDR1p (multidrug resistance) efflux pump is a so called "major facilitator", which uses the proton gradient across the membrane as the driving force for the transport.[115,158] These efflux pumps are present in all *Candida albicans* isolates, but are overexpressed in resistant isolates due to mutations in these strains.[159] Deletion of the corresponding genes of the efflux transporters in these efflux pump resistant isolates resulted therefore again in enhanced fluconazole susceptibility.[160]

The efflux pump resistance mechanism was also observed for other azoles.[161]

Although the *echinocandin antimycotics* represent a relatively new class of compounds (see 1.2.2), several cases of reduced susceptibility have been reported, mostly for caspofungin[162,163,164] but also for other members of the echinocandin group.[165]

The mostly observed resistance mechanism are mutations in a subunit of the 1,3-β-glucan synthase, the target of the echinocandins.[166]

1.2.4 Fungal efflux pump inhibitors

Due to the increasing number of resistance cases (see 1.2.3) and the small number of antimycotic drugs new antifungal drugs with different targets in the fungal cell are urgently needed. The development of echinocandins was a successful step, but although they represent a new class of compounds with a totally different inhibition mechanism resistances have already emerged. Therefore, other strategies against infections with resistant *Candida albicans* have to be developed. The inhibition of the above described resistance mechanisms (1.2.3) maintaining their antifungal effect could be such a strategy. This could be achieved via blocking of the efflux pumps, a strategy known for bacteria.[167] However, in case of resistant fungi there are only very few of these efflux pump inhibitors at hand. *Milbemycin* α_9 was extracted from a library of 85,000 microbial fermentation extracts and was found to enhance the activity of azoles and terbinafine against *Candida albicans* and *Candida glabrata* overexpressing ABC transporters (CDR1p and cgCDRp) up to 16fold.[168] However, milbemycin α_9 and similar derivatives display high cytotoxicity and poor water solubility.[169]

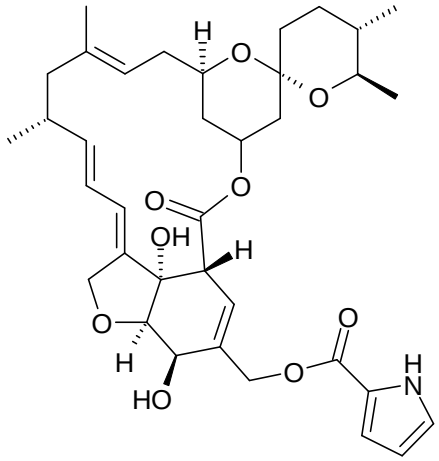


Fig. 1.2.16 Structure of milbemycin α_9

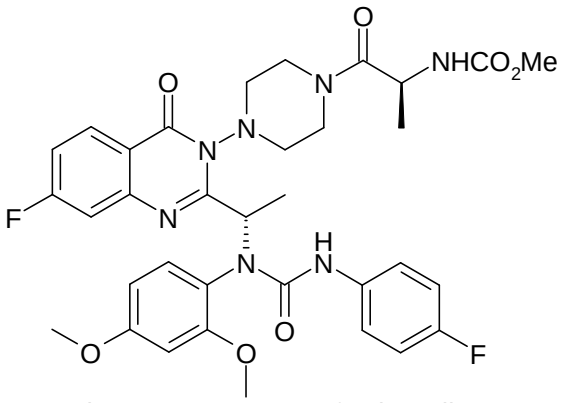


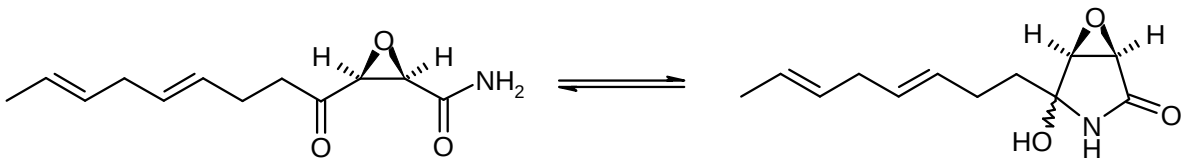
Fig. 1.2.17 Structure of quinazolinone derivative ***32***

The same research group identified a series of quinazolinones also via high throughput screening.[170] These quinazolinones were optimized by synthesizing a library of derivatives[171] resulting in a compound (Fig. 1.2.17) reducing the MIC of fluconazole in CDR1 and CDR2 overexpressing resistant *Candida albicans* strains 8fold at a concentration of 0.5 µg/ml *in vitro*.[172] Better results were even obtained for *Candida glabrata*. Due to the fact that there are so few inhibitors against efflux pump resistant *Candida albicans* strains, the development of further efflux pump inhibitors that could be used in combination with fluconazole is a promising strategy to abolish resistance of *Candida albicans* strains overexpressing these transporters.

1.3 Cerulenin

Cerulenin [(2S)(3R)2,3-epoxy-4-oxo-7,10-dodecadienoylamide; Fig. 1.3.1] was discovered because of its antifungal properties isolated from the fungus *Cephalosporium caerulens*.[173] Shortly after it was found to be an inhibitor of the fatty acid synthases of many bacteria (FAS II) and the corresponding type I FAS in many yeast, fungi and animals.[174,175,176] It also inhibits polyketide synthases,[174] has some efficacy in animal models against *Candida* infections[177] and reduces food intake and body weight in mice.[178]

A number of syntheses in the racemic as well as in the homochiral form have been published;[179,180,181] among the distinguishing features of this compound is the 1,4-diene containing side chain and the *cis*-epoxy amide function. Furthermore, in protic solvents the ring-open form of cerulenin exists in an equilibrium with its hydroxylactam form (Fig. 1.3.1).[182]



ring-opened cerulenin **hydroxylactam form**

Fig. 1.3.1 Equilibration between cerulenin and its hydroxylactam form

1.3.1 Cerulenin as a substrate of fungal efflux pumps

The overexpression of efflux pumps represents one major mechanism of resistance developed by *Candida albicans* upon presence of the antifungal agent fluconazole (see chapter 1.2). Although little is known about the *Candida albicans* efflux pumps, the mutations responsible for overexpression of genes encoding efflux pumps presumably involve alterations in regulatory proteins.[159]

It was observed that clinical *Candida albicans* isolates overexpressing the MDR1 efflux pump were also resistant to other drugs in addition to fluconazole such as brefeldin A, 4-nitroquinoline-*N*-oxide and cerulenin, as compared with matched isolates that did not detectably express *MDR1*p *in vitro*.[183] The increased resistance vanished when the MDR1 gene was deleted from the genome of these isolates (see Fig. 1.3.2). F2/F5 and G2/5 represented clinical *Candida albicans* isolates. Isolates

F5 and G5 showed increased resistance due to the overexpression of the efflux pump *MDR1p*. The *mdr1* null mutants F5M432 and G5M432 derived from the resistant isolates F5 and G5 after deletion of the *MDR1* gene.

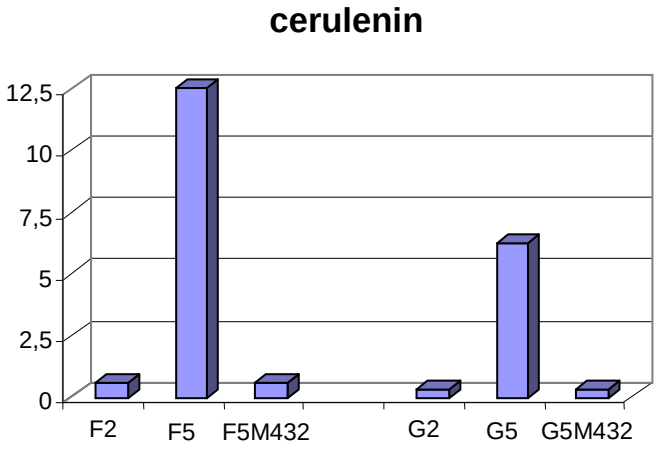


Fig. 1.3.2 Susceptibility of matched fluconazole susceptible and resistant clinical C. albicans isolates[115]

The phenomenon of abolishing the resistance after deletion of the *MDR1* gene in resistant isolates F5 and G5 does not only provide evidence that *MDR1p* overexpression in clinical *Candida albicans* isolates confers resistance to various, structurally unrelated drugs. It also reveals the corresponding resistance to be based on the overexpression of *MDR1p*. Furthermore, the substances showing this effect (e.g. cerulenin) are substrates of this efflux pump.[115]

1.3.2 Cerulenin as a fatty acid synthase inhibitor

FAS I and II systems from many different prokaryotes and eukaryotes are inhibited irreversibly by cerulenin (details on FAS see 1.1).[174,184] It targets the β-ketoacyl-ACP synthases by becoming covalently bound to the cysteine within the active site.[174] The active site of the three β-ketoacyl-ACP synthases (FabH, FabB, FabF) is very similar: it consists of a cysteine and two hydrogen bond donating side chains to form a catalytic triad. In FabB and FabF this triad contains Cys163/His298/His333 and Cys163/His303/His340, respectively,[63] whereas FabH shows a difference with Cys112/His298/Asn274.[185] The crystal structures of FabB and F from *E. coli* complexed with cerulenin have now been determined:[62,63]

In FabB, the hydrophobic cerulenin side chain is located in a hydrophobic tunnel mimicking the acyl chain of the natural substrate (Fig. 1.3.3). The sulfur of the Cys163 in the active site of FabB is covalently bound to C2 of the former epoxide ring of cerulenin. The O3 is situated in the “oxyanion hole” formed by Phe392 and Cys 163. The O2 of cerulenin interacts with nitrogens of two His in the active site where the carbonyl of an incoming malonyl group would be located.[63]

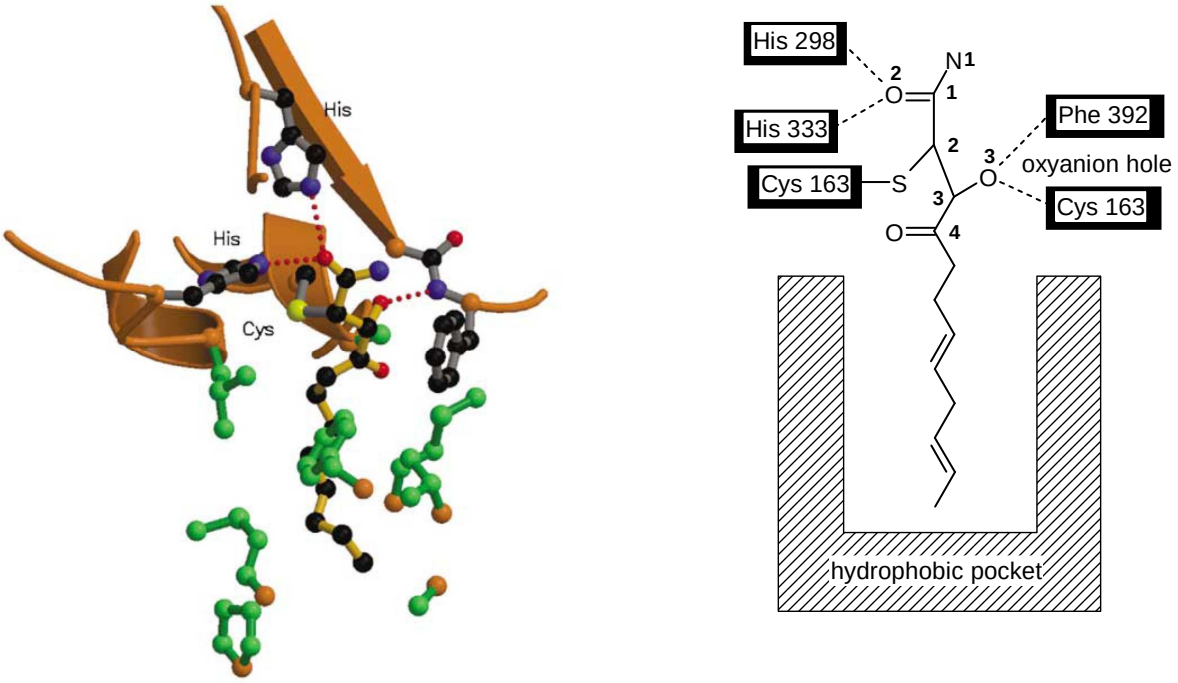


Fig. 1.3.3 Mechanism of cerulenin inhibition of FabB[61]

The active site of the condensing enzymes is divided into two halves, one for acyl-enzyme formation and one for malonyl binding. Cerulenin binds to the acyl-enzyme intermediate half of the active site and overlaps only with O2 into the malonyl half. This can be seen by a superimposition of cerulenin and thiolactomycin (Fig. 1.3.4), the latter binding to the malonyl half of the active site. Overlapping occurs only at O2 of cerulenin and O1 of thiolactomycin.[63]

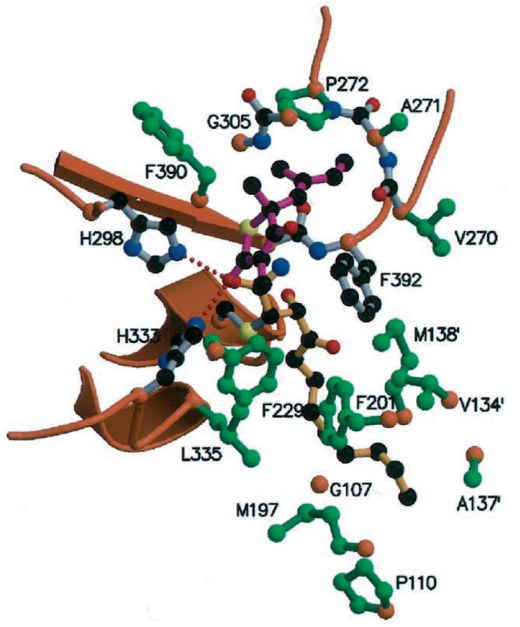


Fig. 1.3.4 Superimposition of cerulenin (orange bonds) and thiolactomycin (magenta bonds) in the FabB active site[63]

FabB binds cerulenin more tightly than FabF whereas FabH is resistant to the substance. This can also be explained by the differences in the active site between each enzyme: in FabH the His333 is replaced by an Asn leading to cerulenin resistance.[63] In FabF the side chain of Ile108 blocks partially the access to the active site tunnel whereas this is replaced by a Gly in FabB leaving more space.[61]

The IC_{50} values determined for cerulenin with the condensing enzymes are 20 µM (FabF), 3 µM (FabB) and >700 µM (FabH).[63]

Although a crystal structure has not been reported for either KasA (the corresponding FabB enzyme in *M. tuberculosis* (see chapter 1.1)) or for a cerulenin-KasA complex, the IC_{50} value for cerulenin with KasA *in vitro* was found to be 0.15 µg/ml (0.67 µM).[186]

Only a crystal structure for KasB (the corresponding FabF enzyme in *M. tuberculosis*) could be determined so far. The structure of the *mt*KasA (*mt = M. tuberculosis*) enzyme was modelled on the basis of this crystal structure of *mt*KasB due to their high sequence identity:[87] the positions of the catalytic site residues of *mt*KasA (Cys167, His307 and His341) and the active site environment were found to be conserved as for *mt*KasB and therefore also similar to that in *E. coli* (see Fig. 1.3.3). The main difference between *mt*KasA and *mt*KasB was explored at the entrance to the active site tunnel being smaller for the former enzyme. However, differences between *mt*KasB and *ec*FabF have been found especially in the capping region and the acyl binding channel. Also a crystal structure of a *mt*KasB-inhibitor complex could not be obtained.

2 Assignment of tasks

2.1 Cerulenin analogues as fatty acid synthase II inhibitors

Cerulenin is a known inhibitor of fatty acid synthase systems but inhibition is unselective in type I and II FAS (see 1.3). Few structure activity studies have been executed to find out which parts of the molecule are important and which parts have to be enhanced for a more efficient and more selective inhibition of FAS systems. Morisaki et al. studied the effect of the side chain structure of cerulenin analogues on inhibition of yeast FAS.[182] They found out that the double bonds at positions 7 and 10 in cerulenin analogues play an important role in specific enzyme binding. Furthermore, a chain length consisting of more than 13 carbons showed significant decrease in the IC_{50} values (see table 2.1).

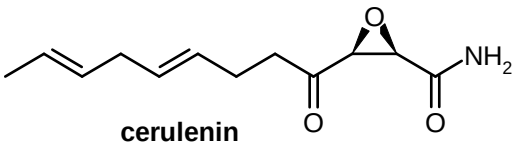
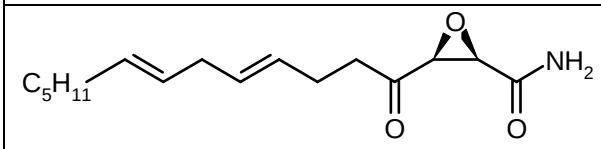
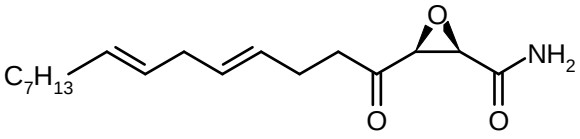
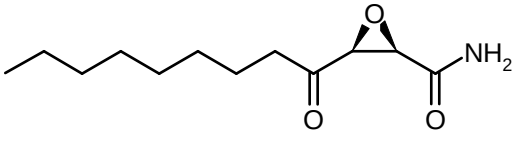
Inhibitor	IC_{50} (µM)
 **cerulenin**	4.5
	20
	1100
	360

Table 2.1 IC_{50} values of cerulenin and some analogues to baker´s yeast FAS[182]

Further SAR studies were performed with tetrahydrocerulenin analogues and aromatic cerulenin analogues.[187] These studies were carried out with human tumor cell FAS and compared tetrahydrocerulenin analogues of various chain length with unsubstituted amide and dimethylamide functions. Aromatic analogues were compared with unsubstituted amide and dimethylamide function. Analogues

substituted with a dimethylamide function showed less inhibition than free amide derivatives. For the tetrahydrocerulenin analogues a chain length of more than 11 side chain carbon atoms remained with almost none activity (see table 2.2).

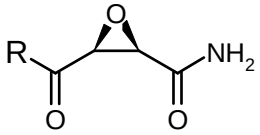
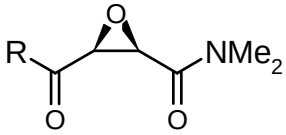
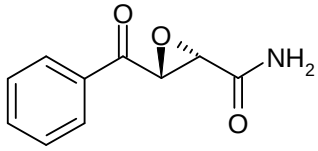
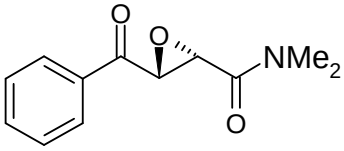
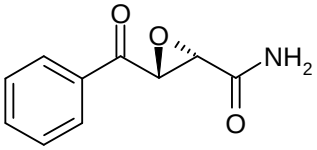
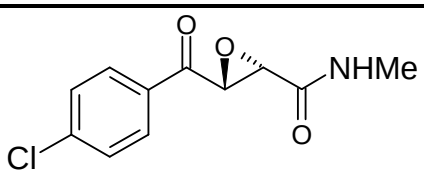
Inhibitor	**inhibition FAS (%)**	**Inhibitor**	**inhibition FAS (%)**
			
R = C_8H_{17}	74±11	R = C_8H_{17}	1±1
R = C_9H_{19}	92±6	R = C_9H_{19}	5±2
R = $C_{13}H_{27}$	0±0	R = $C_{15}H_{31}$	0±0
	85±1		3±2
cerulenin	96±3		

Table 2.2 Inhibition activities of cerulenin and analogues (c=150µM) towards human tumor cell FAS[187]

Further aromatic cerulenin analogues were tested that inhibited lipogenesis in mammals (table 2.3).[188] Also in this case, the disubstituted amide analogues showed less activity than unsubstituted amides.

Inhibitor	**inhibition lip. (%)**	**Inhibitor**	**inhibition lip. (%)**
	91		96

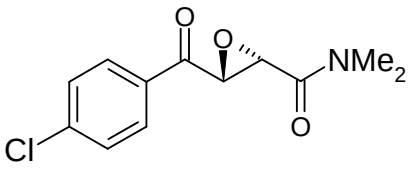
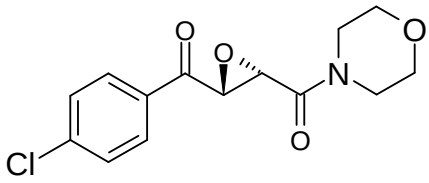
	70		77

Table 2.3 Inhibition activities of aromatic cerulenin analogues (c=100µg/ml) towards lipogenesis in mammals[188]

All of the above described compounds were tested on systems containing type I FAS. A decrease of inhibition in these FAS I systems could be observed with substituents on the amide function or in analogues lacking or with varied double bonds in the side chain. This inhibition decrease in FAS I systems could therefore be a starting point to create compounds with more inhibitory activity towards FAS II systems possessing less activity towards FAS I systems. Variation at the amide function or variation of double bonds could therefore be a possibility to gain selectivity towards type II FAS systems. The aim of this work therefore was to synthesize cerulenin analogues with different substituents at the amide function, with or without the epoxide function and/or with varying and lacking double bonds in the side chain (see Fig. 2.1).

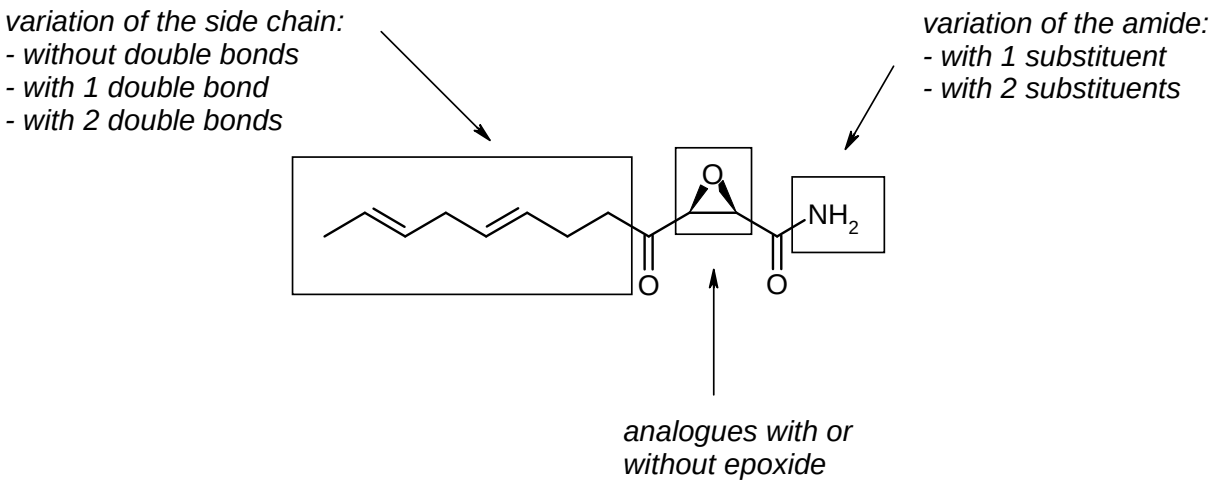
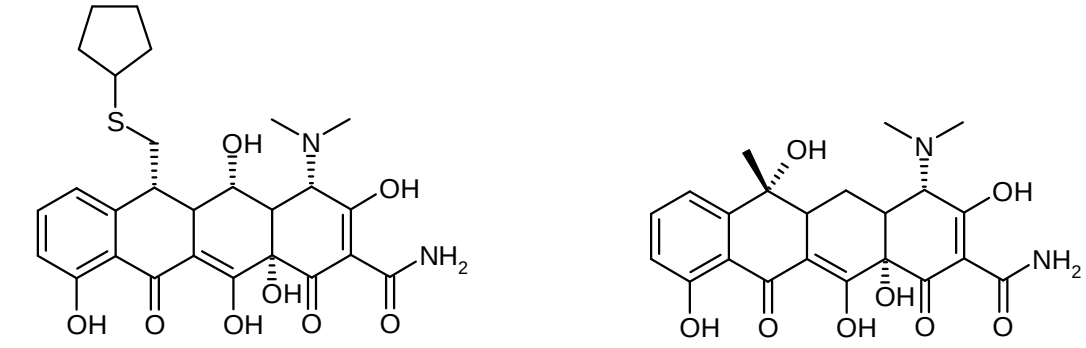


Fig. 2.1 Synthesis task with variation pattern

2.2 Cerulenin analogues as efflux pump inhibitors

Although little is known about structure, the exact mechanism how resistance is mediated or the physiological function of these membrane transport proteins in *Candida albicans*,[115] the phenomenon of efflux pumps as a cause of resistance is a more common topic in bacteria, being identified for the first time in 1980 in the context of tetracycline resistance in *E. coli*.[189] Subsequently, various semisynthetic derivatives of tetracycline have been prepared and tested for their ability to inhibit the tetracycline specific efflux pump TetB.[190] The most potent compound, 13-CPTC (Fig. 2.2), was a competitive inhibitor of the TetB efflux pump and combination with doxycyline resulted in synergistic decrease in the MIC of TetA or B resistant *E. coli*.[191]



TetB efflux pump inibitor 13-CPTC **tetracycline**

Fig. 2.2 Structure of efflux pump inhibitor 13-CPTC and tetracycline

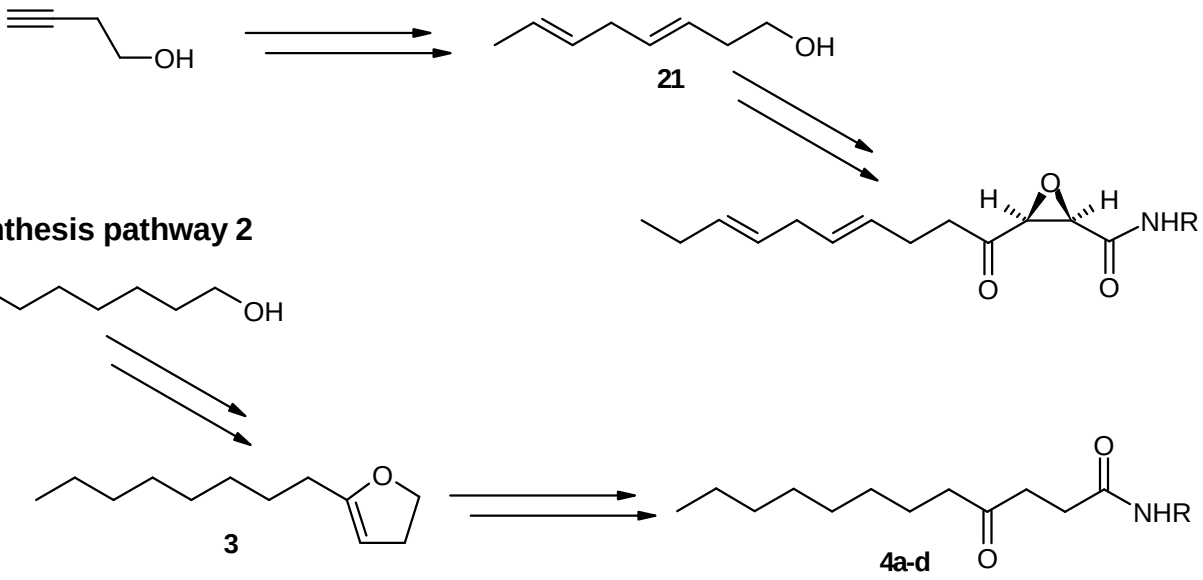
The efflux pump inhibitor 13-CPTC and the original compound tetracycline display strong structural similarity, which could be an indicator that only few structural variations can lead from a substrate to an inhibitor of the corresponding efflux pump.
Analogues of cerulenin, a substrate of the efflux pumps in *Candida albicans*, could therefore represent inhibitors of these efflux pumps. Therefore, variations concerning the hydrophobic side chain but also at the amide residue should be executed to test which of these variations would lead to a fungal efflux pump inhibitor of the *C. albicans* efflux pumps *MDR1p* and *CDR1p* and *CDR2p* (see Fig. 2.1).

3. Preparation of the cerulenin analogues

The synthesis goal of this work was to obtain cerulenin derivatives of structural diversities concerning the side chain and the chore structure. Therefore, different synthesis pathways were designed, which are summarized in Figure 3.1. *Synthesis pathway 1* should lead to *E,E*-cerulenin analogues. *Synthesis pathway 2* giving 4-oxoalkylamide analogues was applied to obtain the first derivatives. Via *Synthesis pathway 3*, the epoxide function could be introduced into the structure to gain tetrahydro- and dihydrocerulenin analogues and *synthesis pathway 4* provided both double bonds in the side chain but in a different stereochemistry compared to cerulenin. In addition, in *synthesis pathway 5* the alkyl chain was replaced by an aromatic ring.

Thus, the different pathways resulted in a variety of differently substituted compounds that represent an ideal library to find out which structural parts of the synthesised cerulenin analogues are essential for an efficient inhibition of fatty acid biosynthesis in mycobacteria and efflux pumps in *C. albicans* and which are neglectable.

Synthesis pathway 1



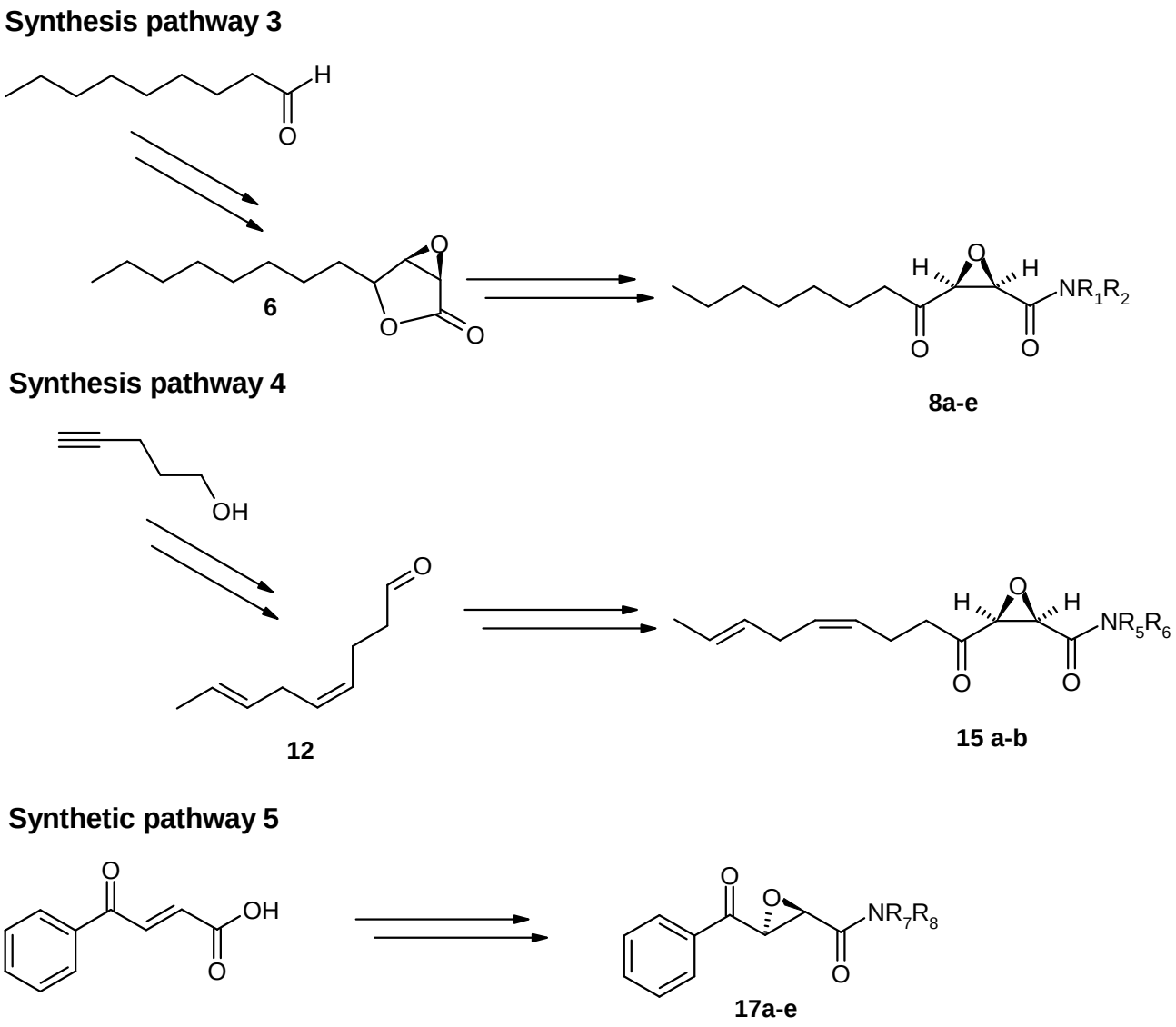


Fig. 3.1 The 5 main synthesis pathways to the cerulenin analogues

3.1 Synthesis pathway 1

3.1.1 Synthesis attempts of *E,E*-cerulenin analogues

Synthesis pathway 1 (see Fig. 3.2) leading to *E,E*-cerulenin derivatives followed a route developed by Corey et al.[192]:

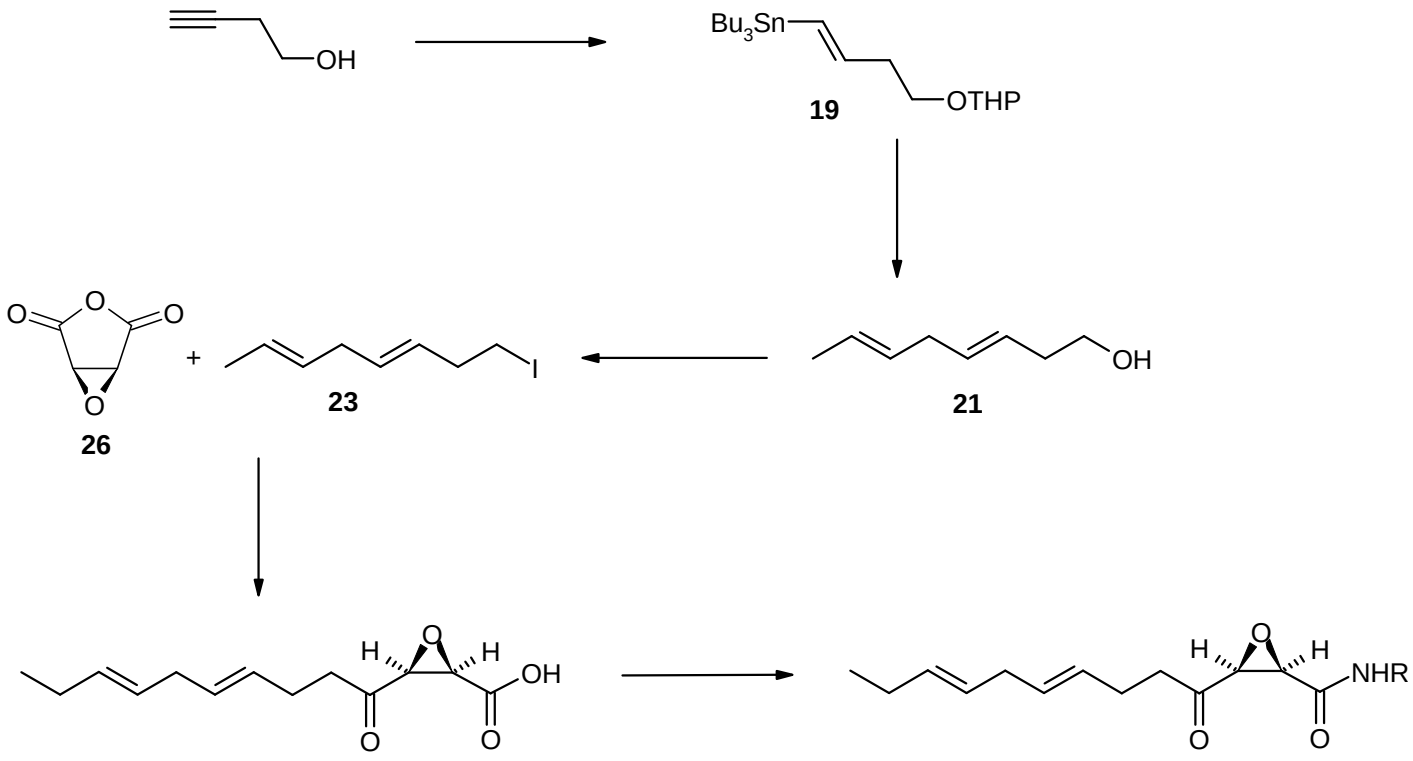


Fig. 3.2 First synthesis pathway to cerulenin analogues

The synthesis started off with the preparation of the alcohol (3*E*,6*E*)-3,6-octadien-1-ol (**21**) (see Fig. 3.2), already representing the future side chain of the cerulenin analogues. The next steps involved coupling of the side chain to the anhydride **26** and conversion of the obtained acid to corresponding amides.

The first part to the desired *E,E*-cerulenin analogues incorporated the synthesis of (3*E*,6*E*)-3,6-octadien-1-ol **21** as a key intermediate to the carbon skeleton of cerulenin.

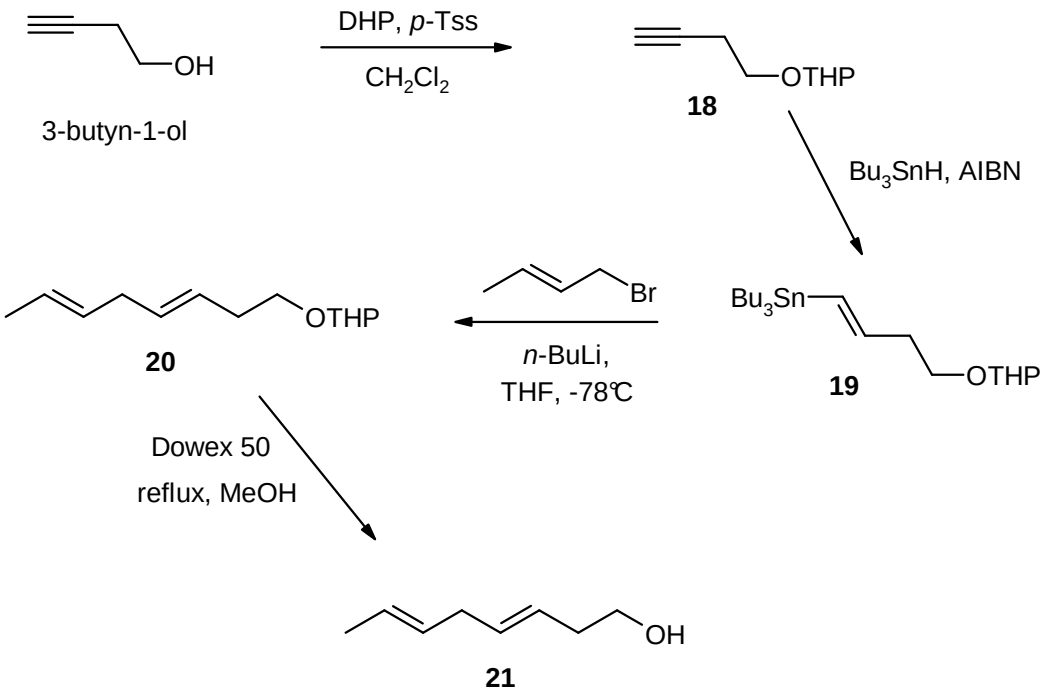


Fig. 3.3 Synthesis route to (3E,6E)-3,6-octadien-1-ol

In the synthesis scheme of Corey et al.[192], the acetylenic tetrahydropyranyl ether was prepared due to Corey et al.[193,194] from 3-butyn-1-ol by reaction with dihydropyran (DHP) in methylene chloride in presence of a catalytic amount of *p*-toluene sulfonic acid. After the working up procedure the residue was purified by distillation to give **18** as a colourless oil in 82 % yield. To obtain stannane **19**, the tetrahydropyranyl ether **18** was heated in an excess of tributyltin hydride and the radical addition of Bu_3SnH was started with a catalytic amount of the radical starter azobisisobutyronitrile (AIBN). After cooling down, the solution was distilled to gain substance **19** as a colourless oil. Substance **19** was obtained as a mixture of *E*- and *Z*- isomers in a ratio of 77:23 (*E*:*Z*). The Composition of the *Z*/*E* ratio was determined from the intensity of the ^1H NMR signal of the allylic CH_2 group (see experimental section).

For preparing the tetrahydropyranyl ether of octadienol (**20**), a procedure similar to that of Morisaki et al.[195] was applied. The vinylstannane **19** was transferred at first into the corresponding vinyllithium compound by adding a 2.5 M solution of *n*-BuLi to a solution of **19** in dry THF at -78 ℃ under argon. Secondly, crotyl bromide was

added and allowed to react with the prepared vinyllithium compound to give the coupling product as the tetrahydropyranyl ether **20**. The reaction mixture was worked up to give an oil containing product **20** and (n-Bu)$_4$Sn. By adding methanol to this mixture, (n-Bu)$_4$Sn could be separated by extraction and **20** could be obtained in 45 % yield.

Tetrahydropyranyl ether **20** was hydrolyzed by adding the ion exchanger Dowex 50 to a solution of **20** in methanol. After 5 hours of refluxing and dismissal of the protection group, the ion exchanger and the solvent were removed and the residue was distilled for purification to give alcohol **21** and its 3-*Z*-isomer (the ratio was determined as for compound **19**) as a colourless oil and used without further purification in the next steps.

The synthesis pathway to the desired *E*,*E*-cerulenin analogues started from (3*E*,6*E*)-3,6-octadien-1-ol **21** (see Fig. 3.3).

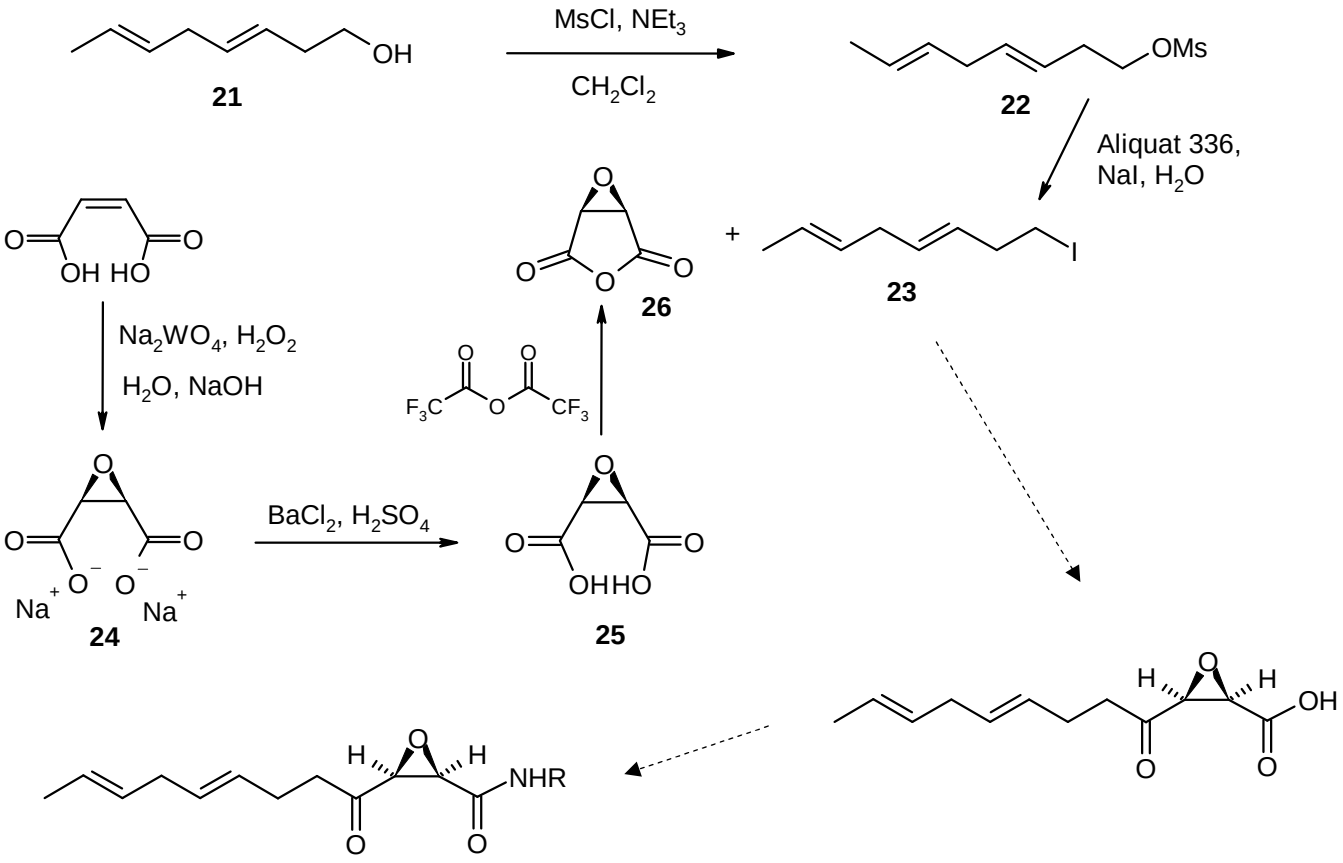


Fig. 3.4 Intended synthetic route from (3E,6E)-3,6-octadien-1-ol to E,E-cerulenin analogues

(3*E*,6*E*)-3,6-octadien-1-ol (**21**) was transferred via the mesylate **22** to the iodide **23** which was supposed to be coupled with anhydride **26**. Compound **26** was obtained starting off with maleic acid, which was epoxidized followed by ring closure.

3.1.1.1 Synthesis of (3E,6E)-3,6-octadien-1-iodide (**23**)

Mesylate **22** was synthesized according to a procedure of Crossland and Servis.[196] Alcohol **21** was deprotonated by adding triethylamine to a solution of **21** in dichloromethane. The formed octadienolate was nucleophilically attacked by the mesyl chloride to form product **22**. After the working up **22** and the corresponding 3Z-isomer were obtained fairly pure in 83 % yield in an equal ratio as **21** (determined as for **19**). The iodination of substance **22** was carried out by heating a mixture of **22,** Aliquat 336 and excess NaI in H_2O at 80 °C overnight, similar to the method of Morisaki et al.[195] The nucleophilic substitution of iodide against the mesylate was successfully executed by aid of the phase-transfer catalyst Aliquat 336. After extraction with *n*-pentane, evaporation gave fairly pure iodide **23** in 70 % yield with a ratio equal to that of **19** (determined as for **19**).

3.1.1.2 Synthesis of disodium cis-epoxysuccinic anhydride (**26**)

The synthetic procedure to substance **24** (see Fig. 3.4) followed the method of Payne and Williams[197] and commenced by adding a NaOH-solution, Na_2WO_4 and H_2O_2 to a solution of maleic acid in water. The epoxidation reaction with hydrogen peroxide was thereby catalyzed by sodium tungstate as the catalyst. The reaction mixture had to be heated to 65 - 68 °C, because otherwise a sat isfying conversion to epoxide **24** could not be achieved. The pH value also seemed to have an important influence on the whole process and had to be maintained between 4-5 throughout the reaction. After vacuum concentration of the solution, the residual liquid was poured onto acetone and pure **24** precipitated as a white solid in 99 % yield.

The free acid **25** of the disodium *cis*-epoxysuccinate (**24**) could be obtained by a procedure with a modified reaction time and work up process to that described by Payne and Williams.[197] Disodium salt **24** was converted to the barium salt by addition of 1.01 molar equivalent of barium chloride to a solution of **24** in H_2O. The barium *cis*-epoxysuccinate precipitated and was isolated by filtration. The barium salt then was suspended in diethyl ether and ice cooled. Conc. sulfuric acid was added and the

mixture was stirred overnight to complete the reaction. The crude acid **25** was filtered off, the filtrate was concentrated and *cis*-epoxysuccinic acid **25** was obtained in 63 % yield as a white solid.

The preparation of *cis*-epoxysuccinic anhydride (**26**) employed was similar to that of Allan and Noegi with modifications in the reaction and work up procedure.[198] The synthesized acid **25** was stirred in trifluoroacetic anhydride as a solvent and simultaneously as the dehydration reagent for 2 days under argon, filtered and evaporated to give pure *cis*-epoxysuccinic anhydride (**26**) as a white solid in 58 % yield.

In addition, the synthetic pathway from substance **25** to *cis*-epoxysuccinic anhydride (**26**) referring to Creighton and Mitchell was carried out,[199] but failed to form any product. Instead, only starting material could be observed.

3.1.1.3 Synthesis attempts of (4E,7E)-cis-2,3-epoxydodeca-4,7-dienoic acid

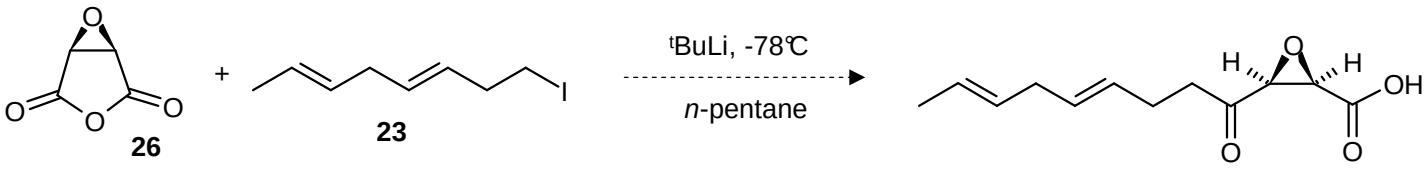


Fig. 3.5 Synthesis attempts of (4E,7E)-cis-2,3-epoxydodeca-4,7-dienoic acid

The coupling reaction between compounds **23** and **26** failed to give the desired product *(4E,7E)*-cis-2,3-epoxydodeca-4,7-dienoic acid despite several attempts to execute this reaction under several differing conditions. To achieve halide-metal exchange, substance **23** was dissolved in dry *n*-pentane, cooled down to -78 ℃ and ^{t}BuLi was added to obtain the dienyllithium intermediate. A colour change of the reaction mixture indicated lithiation of the iodide **23**. Afterwards, a solution of **26** in dry THF was added at that temperature, the mixture was allowed to warm to room temperature until TLC showed no further change, and worked up.

NMR spectra showed only a complicated mixture of compounds and after column chromatography the compounds isolated were mostly starting materials.

Although this reaction was carried out several times the desired coupling product could not be obtained. The same difficulties were encountered after having changed the above described procedure in terms of varying reaction time and temperature or

different iodides (e.g. octaniodide) and anhydrides (e.g. maleic anhydride) as starting material. A possible explanation for failing could be that, in comparison to the synthesis of Corey and Williams,[192] a mixture of *E* and *Z* isomers of compound **23** was present during the coupling reaction. Furthermore, Corey and Williams[192] used (4*E*,7*E*)-*cis*-2,3-epoxydodeca-4,7-dienoic acid without isolation in the next step for esterification with diazomethane whereas in the reaction procedure used in this work the acid was attempted to be isolated or tried to be esterified under different conditions. Furthermore, *synthesis pathways 3* and *4*, which were examined simultaneously to pathway 1, resulted successfully in cerulenin and tetrahydrocerulenin derivatives and represented easier and better alternatives to this route. Therefore, *synthesis pathway 1* (see Fig. 3.2) was no longer followed.

3.2 Synthesis pathway 2: Synthesis of 4-oxoalkylamide cerulenin analogues

Synthesis pathway 2a (see Fig. 3.6) started off with 1-octanol as the starting material and lead to 4-oxoalkanamides **4a-d**. These products showed differences concerning the side chain and epoxide function compared to the original cerulenin structure.

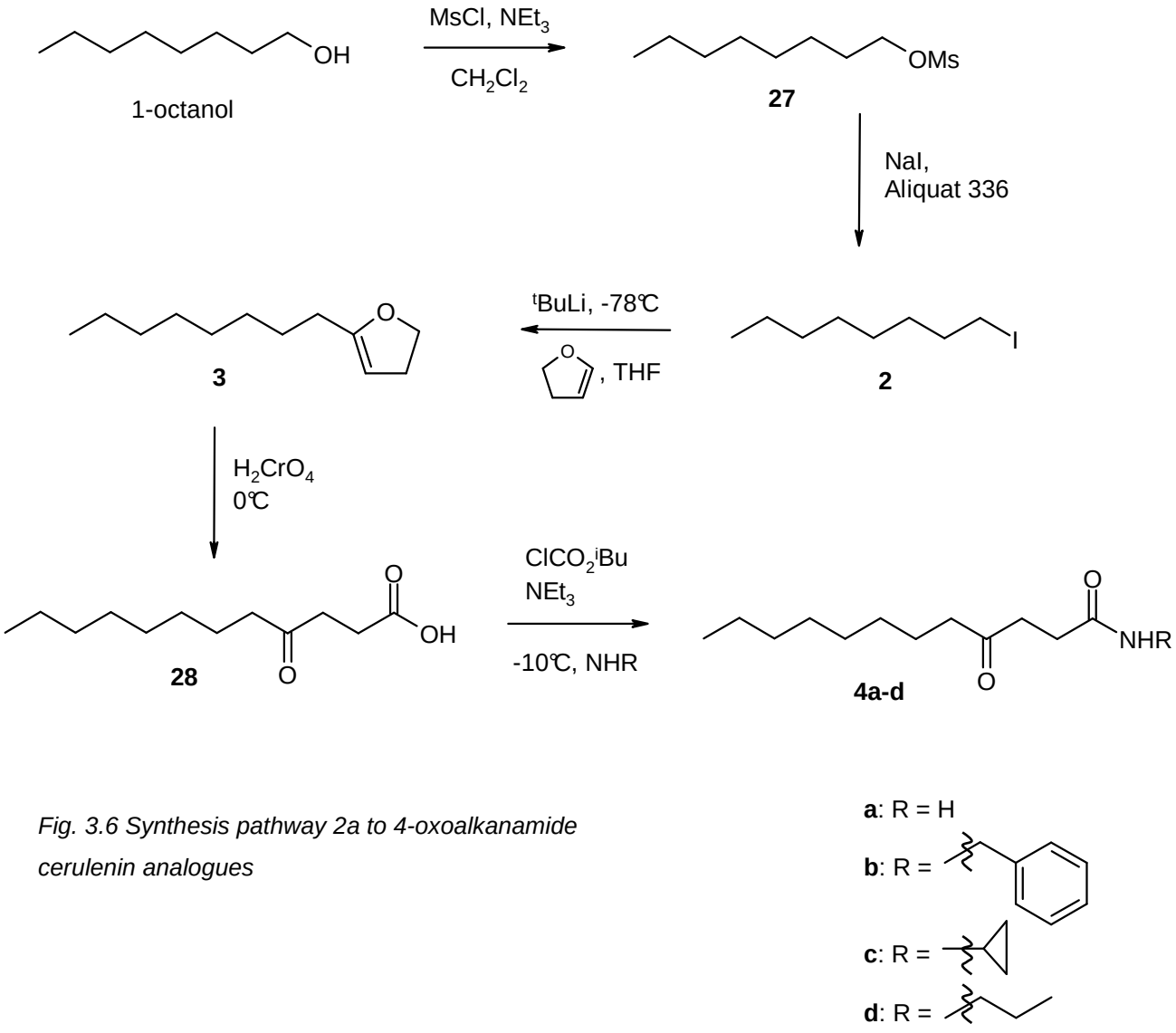


Fig. 3.6 Synthesis pathway 2a to 4-oxoalkanamide cerulenin analogues

Following this pathway, derivatives with double bond side chains were also prepared (see 3.2.2 below) to gain 4-oxoalkenamides (*Synthesis pathway 2b*).

3.2.1 Synthesis route to 4-oxoalkanamides

The preparation of **27** followed a common procedure to mesylates (see Fig. 3.6).[200] The solution of commercially available 1-octanol was dissolved in CH_2Cl_2, NEt_3 was added for deprotonation of the alcohol and mesyl chloride completed the reaction. The following workup gave the mesylate **27** fairly pure in 91 % yield.

Iodooctane **2** was synthesized similarly to the method described for substance **23**. Mesylate **27** was heated in water at 80 °C overnight in presence of sodium iodide and phase transfer catalyst Aliquat 336. After the usual workup, product **2** was gained in 86 % yield.

To obtain octyldihydrofuran **3**, 2,3-dihydrofuran was dissolved in dry THF at -78 °C under argon following a procedure similar to that of Tschantz, Burgess and Meyers.[201] For deprotonation and metallation of 2,3-dihydrofuran a solution of ^{t}BuLi in pentane was added. Alkylation of the *in situ* produced lithium reagent with 1-iodooctane **2** was achieved by adding a solution of **2** in anhydrous THF. Subsequent extraction, drying and concentration under reduced pressure without heating afforded 2,3-dihydro-5-octylfuran in 98 % yield, which was used without further purification in the next step to compound **28**.

4-Oxododecanoic acid **28** was synthesised similar to a route reported by Tschantz et al.[201] but under different reaction conditions. Substance **3** was treated with Jones reagent[202] under ice cooling to hydrolyze the enol ether and oxidize the resulting primary alcohol to the corresponding carboxylic acid. For purification, the formed acid **28** was transferred into the aqueous phase by extraction with sodium hydroxide, again protonated by addition of hydrochloric acid and retransferred into the organic layer by extraction with dichloromethane. The organic fraction was dried and evaporated, and the remaining solid was recrystallized from *n*-hexane to obtain product **28** in 41 % yield.

3.2.1.1 Synthesis attempt to 4-oxododecanamides (**4a-d**) *via methyl-4-oxododecanoate* (**29**)

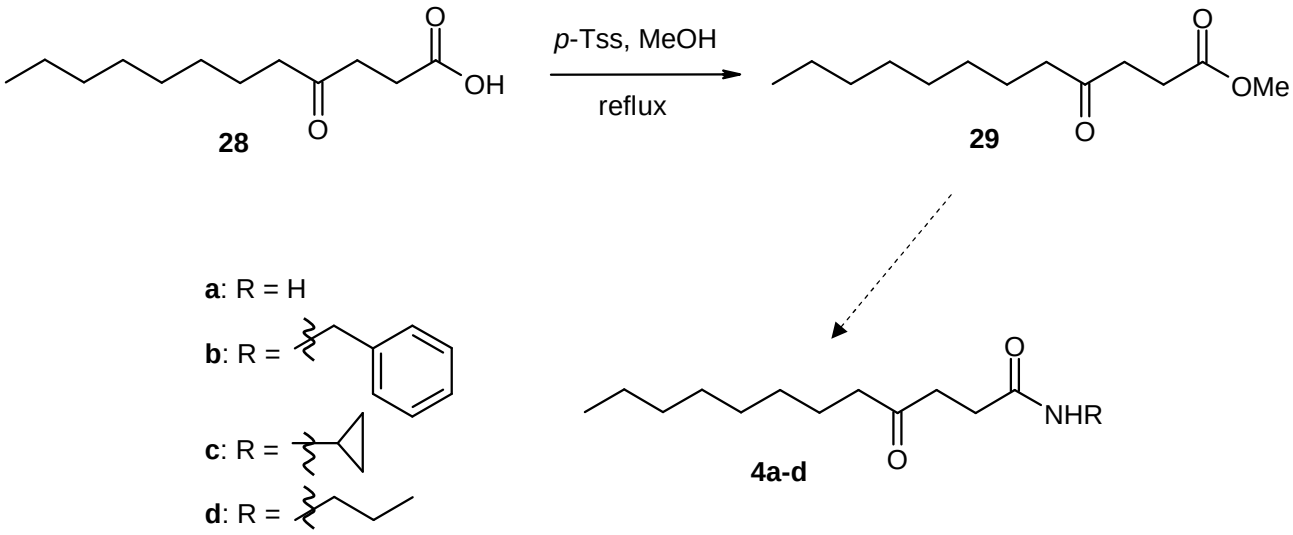


Fig. 3.7 Synthesis of 4-oxododecanamides

The attempt was made to obtain the oxododecanamides **4a-d** via a different synthetic pathway before. Therefore, methyl ester **29** was synthesized by refluxing substance **28** in methanol adding a catalytic amount of *p*-Tss. After completion of the reaction, remaining acid was removed by extracting the mixture with saturated Na_2CO_3 solution and after workup **29** was gained pure in 87 % yield.

The transfer from methyl ester **28** to **4a** was tried with either concentrated aqueous ammonia or a 7M methanolic ammonia solution at different temperatures and for different reaction times. Most of the reactions showed only a small amount of product formation and mostly starting material on the TLC plate probably because ester **29** was not activated enough; therefore attention was turned to a more successful route (see 3.2.1.2).

3.2.1.2 Synthesis of 4-oxododecanamides (**4a-d**) *via the mixed anhydride*

To convert the 4-oxododecanoic acid **28** into amides **4a-d** NEt_3 was added in the first step to a solution of **28** in THF, aiming for deprotonation to the carboxylate ion. Subsequent cooling to -10 °C and adjunct of isobutyl chloroformate resulted in the mixed anhydride (Fig. 3.8).[203]

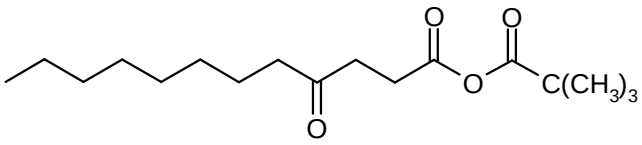


*Fig. 3.8 Mixed anhydride as an intermediate to 4-oxododecanamides **4a-d***

On the basis of Schipper and Nichols[204] a synthesis varied in reaction times and work up procedure to **4a-d** was applied: the intermediate was used without isolation and addition of either concentrated ammonia or a 0.01 M solution of the corresponding amine in THF lead to the desired amides **4a-d** in 37 - 44 % yield.

3.2.2 Synthesis route to 4-oxoalkenamides

Synthesis pathway 2 did not only include preparation of amides containing an alkyl chain, but also different octenols were used as starting materials to yield products that incorporated an alkene side chain, the 4-oxoalkenamides (Synthesis pathway 2b).

3.2.2.1 Synthesis of (E)-4-oxo-7-decenamide (**4e**)

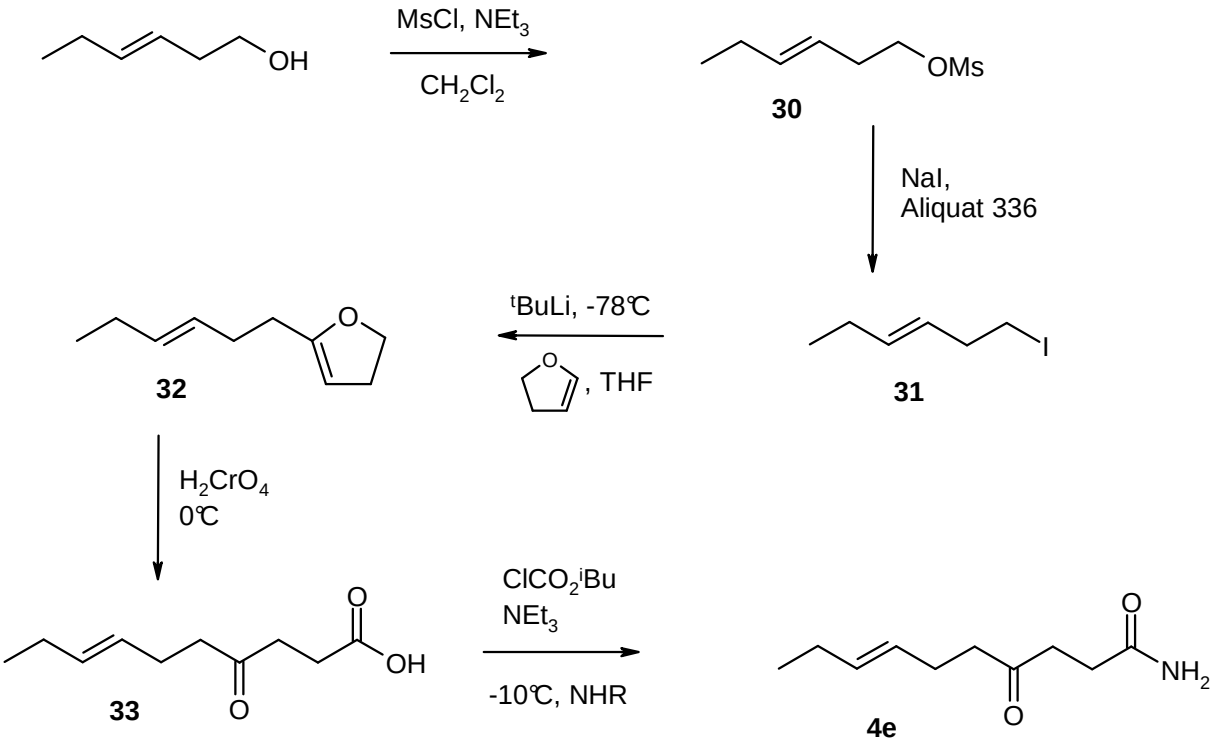


*Fig. 3.9 Synthesis pathway 2b to 4-oxoalkenamide cerulenin analogue **4e***

(*E*)-4-oxo-7-decenamide **4e** was synthesized according to the procedure described in chapter 3.2.1, starting with mesylation of commercially available (*E*)-3-hexen-1-ol in 96 % yield and subsequent iodination of mesylate **30** to the corresponding iodide **31** in 77 % yield, which was then coupled to give substance **32** in 42 % yield and ring opening and oxidation was executed to acid **33** in 31 % yield. Product **4e** was gained via the mixed anhydride (see 3.2.1.2) in 42 % yield.

3.2.2.2 Synthesis attempt of (7E,10E)-4-oxo-7,10-decadienamide

In order to yield a further 4-oxoalkenamide, iodide **23** from *synthesis pathway 1* was inserted into this pathway to gain the corresponding amide.

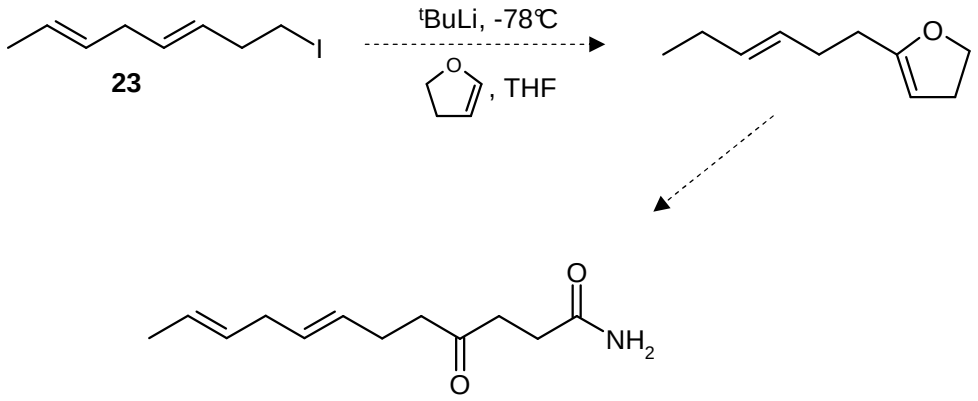


Fig. 3.10 Synthesis attempt of (7E,10E)-4-oxo-7,10-decadienamide

Coupling of **23** and 2,3-dihydrofuran to the intermediate lactone did not prove to be successful. Despite several attempts with varying conditions and work up procedures, NMR spectra only showed a complicated mixture of signals and after purification by column chromatography only starting material could be isolated.

3.2.2.3 Synthesis attempt of (E)-4-oxo-6-decenamide

The synthesis for a further 4-oxoalkenamide was executed starting from commercially available (*E*)-2-hexen-1-ol.

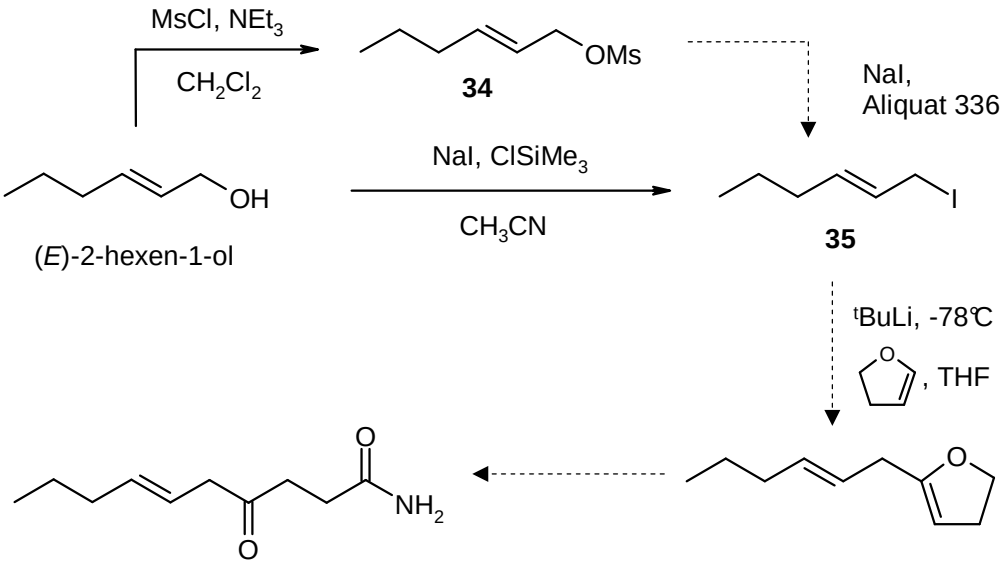


Fig. 3.11a Synthesis attempt of (E)-4-oxo-6-decenamide

The first attempts to obtain iodide **35** were made according to the pathway used before (see 3.2.2.1), that contained the preparation of mesylate **34** in 99 % yield. Difficulties were encountered when synthesizing the iodide using sodium iodide and Aliquat 336. Substance **35** could not be isolated, probably due to isomerization of the double bond and subsequent elimination of the iodide.

Another route was chosen for the synthesis of compound **35**,[205] utilizing a one step synthesis with the silylated alcohol as the *in situ* intermediate and subsequent substitution of the iodide against trimethyl silate as the leaving group. This route successfully lead to substance **35**. However, the coupling reaction of the latter with the dihydrofuran proved to be not successful and only starting materials could be isolated. Taken together, introduction of a double bond in position 6 (Fig. 3.11a) or two double bonds in position 7 and 10 (Fig. 3.10) was impossible while the introduction of only one in position 7 (**4e**) was successful. A possible explanation in case of **35** could be the isomerization of the double bond and elimination of the iodide (Fig. 3.11b).

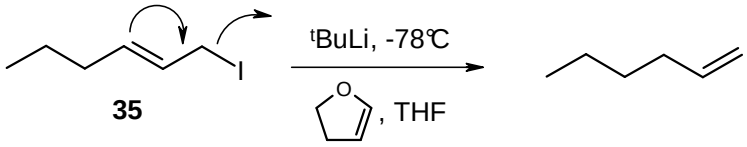


*Fig. 3.11b Possible isomerisation of compound **35***

3.3 Synthesis pathway 3: Synthesis of tetrahydro- and dihydrocerulenin analogues

Synthesis pathway 3 represents a route to cerulenin analogues that contain a side chain without or with only one double bond compared to the original cerulenin structure. However, this remains to be the only difference in structural terms to the lead compound. Compared to the 4-oxoalkylamides (see 3.2) the epoxide function was now included with regard to the mechanism of action of cerulenin (see chapter 1). The epoxide function of cerulenin is responsible for the irreversible inhibition of the elongation enzyme FabB whereas analogues missing the epoxide (as compounds **4a-e**) represent probably only competitive inhibitors.

The preparation of the tetrahydrocerulenin analogues started from 1-nonanal:

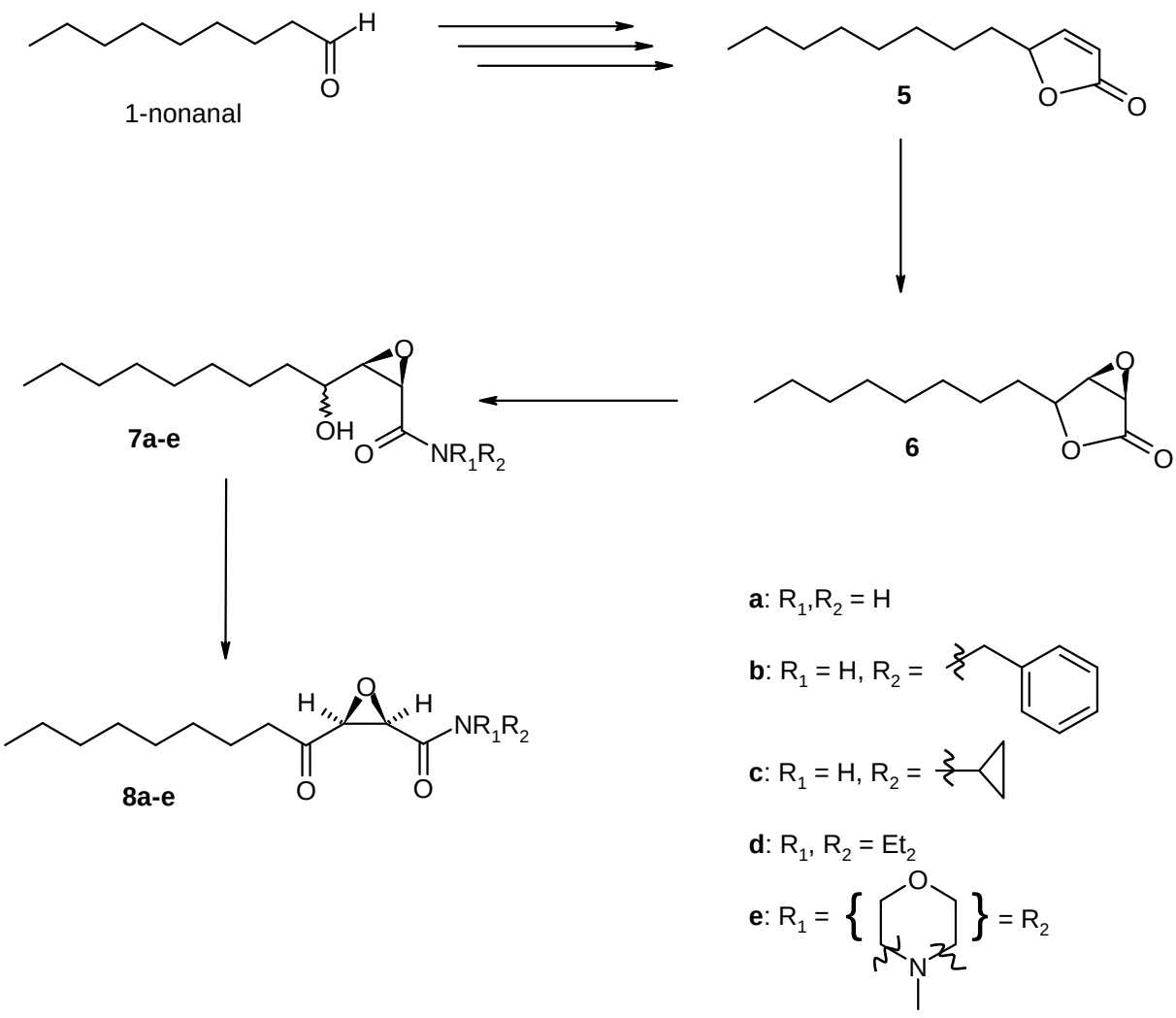


*Fig. 3.12 Synthesis pathway 3 to tetrahydrocerulenin analogues **8a-e***

3.3.1 Synthesis route to dodec-2-enoic acid γ-lactone 5

In order to prepare the key intermediate dodec-2-enoic acid γ-lactone **5** two different routes were followed.

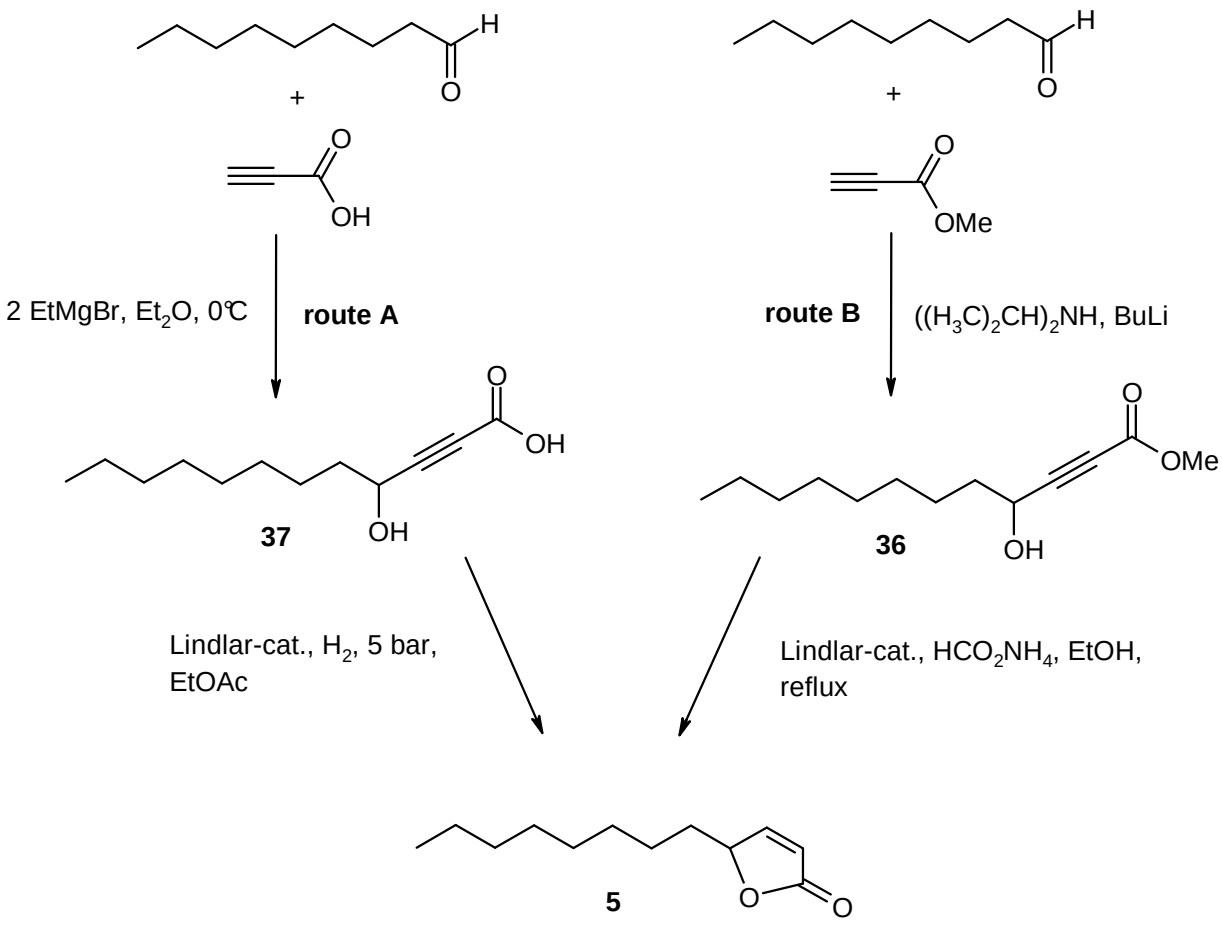


Fig. 3.13 Synthesis routes to dodec-2-enoic acid γ-lactone

Both ways started with 1-nonanal. One way (route A) coupled propiolic acid via a Grignard reaction to the aldehyde and the subsequent hydrogenation of the resulting acid **37** was carried out with the Lindlar catalyst under hydrogen pressure. The other way (route B) involved coupling of the aldehyde to the methyl ester of propiolic acid with *n*-BuLi and the hydrogenation of the intermediate ester **36** was performed with the Lindlar catalyst and ammonium formate.

3.3.1.1 Route A to dodec-2-enoic acid γ-lactone (**5**)

To obtain acid **37**, 2 equivalents of a solution of EtMgBr in Et_2O were added to propiolic acid in THF under argon at 0 °C. The formed magnesium dianion of propiolic acid was then coupled to 1-nonanal by adding the latter. The mixture was stirred for 1 h at 0 °C and was allowed to come to RT for one hour. The crude product was worked up and recrystallized from *n*-hexane to give **37** in 68 % yield.
The preparation of *γ*-lactone **5** was executed similar to the procedure of Jakubowski et al.[206] but with a different hydrogen pressure, reaction time and work up procedure. Acid **37** was dissolved in EtOAc and a catalytic amount of Lindlar catalyst was added. The hydrogenation was carried out in a sealed tube with 5 bar of hydrogen pressure for 2 h at RT. Filtration and evaporation of the filtrate at a water bath temperature of 40 °C lead to ring closure to give **5** in 57 % yield. The yield was improved by executing this reaction in a microwave oven for 2 h at a slightly higher hydrogen pressure (6 bar) to 66 % yield.

3.3.1.2 Route B to dodec-2-enoic acid γ-lactone (**5**)

To couple the aldehyde 1-nonanal to methyl propiolate, the latter was added to a solution of *n*-BuLi and diisopropylamine in dry THF under argon at 0 °C. The formed lithium salt of methyl propiolate was cooled to -78 °C and condensed with 1-nonanal by adding a solution of the aldehyde in dry THF. After warming to RT the reaction mixture was worked up and the ester **36** was obtained in 54 % yield.
The preparation of *γ*-lactone **5** from ester **36** was carried out by refluxing **36** in EtOH with a catalytic amount of Lindlar catalyst and ammonium formate as the hydrogen source for 5h. Formation of lactone **5** was monitored by TLC. After the reaction was completed, the catalyst was filtered off and the filtrate was evaporated with a water bath temperature of 40 °C leading to ring closure of the hydrogenated ester to give **5** in 40 % yield. Yield and reaction times could be improved when this reaction was executed using a microwave oven (for details see experimental section). Completion of the preparation took only 3 h and the yield was increased to 51 %.

3.3.2 Synthesis route from dodec-2-enoic acid γ-lactone 5 to tetrahydrocerulenin analogues 8a-e

The second part of *synthesis pathway 3* represented the preparation of tetrahydrocerulenin analogues from the key intermediate dodec-2-enoic acid γ-lactone **5**.

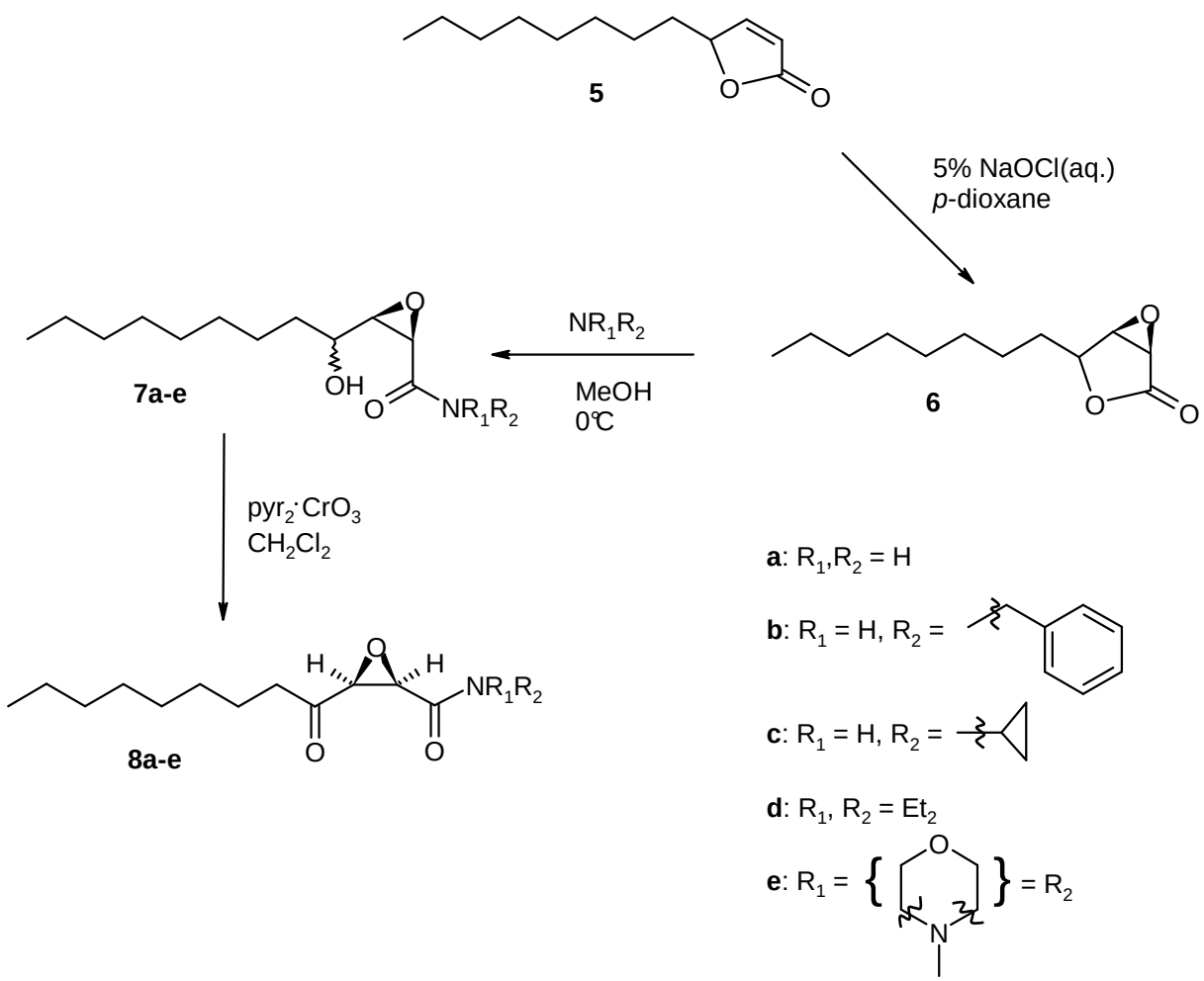


*Fig. 3.14 Synthesis routes to tetrahydrocerulenin analogues **8a-e***

Dodec-2-enoic acid γ-lactone **5** was epoxidized with NaOCl. The experimental procedure was carried out following a procedure similar to that of Lawrence et al.[187] (with a change in conditions), because Jakubowskis method[206] in pyridine with acid extraction failed to give the desired epoxide **6**, because no product was formed. Lactone **5** was dissolved in *p*-dioxane and cooled to 0 ℃. 5 % aqueous NaOCl solution was added and the mixture was stirred at 0 ℃ for 1 h and allowed to come to RT until no further change on the TLC plate could be observed. The remaining NaOCl was reduced by adding Na_2SO_3 solution and the mixture was worked up and purified to give **6** in 52 % yield. Unreacted lactone **5** could be regained and used

again for epoxidation. *Cis*-epoxidation was confirmed by the typical coupling constants (~2.5 Hz)[206] for the epoxide protons in the ^{1}H NMR spectrum. However, it is the only possible formation in this synthesis pathway. Further details are discussed in chapter 3.6.1.

Amidation of epoxylactone **6** was achieved by adding the corresponding amines to a solution of **6** in MeOH at 0 °C. The solution was stirred for 2 h at 0 °C and was allowed to warm up to RT overnight. Evaporation of the mixture and purification resulted in amides **7a-e** in 65 - 98 % yield. **7a-e** were obtained as a diastereomeric mixture concerning the hydroxy group with the *trans*-isomer as the major one in accordance with Jakubowski.[206] However, synthesis was carried out without separation of these isomers due to the oxidation in the next step leading to the ketone and therefore abolishing the stereochemistry.

Cis-2,3-epoxy-4-hydroxyalkanamides (**7a-e**) were oxidized to tetrahydrocerulenin analogues **8a-e** via a Collins oxidation similar to the procedure of Jakubowski et al.[206] In contrast to this procedure, a different temperature and work up procedure were applied and the chromium trioxide-pyridine complex was not isolated[207] but prepared *in situ*[208] to execute the oxidation. Amides **7a-e** were dissolved in a solution of dry CH_2Cl_2 and pyridine at 0 °C. Dry CrO_3 was added and the mixture was allowed to come to RT for 1h. To remove chromic salts, the mixture was filtered through Celite® and washed several times with water, worked up and purified by column chromatography using Florisil® at first to remove remaining chromic salts and secondly using a silica gel column. Tetrahydrocerulenin analogues **8a-e** were obtained in 21 - 66 % yield.

3.3.3 Synthesis route to dihydrocerulenin analogues

3.3.3.1 Synthesis of (7E)-dihydrocerulenin analogues (**10a-c**)

Dihydrocerulenin analogues **10a-c** were prepared following the same pathway as for tetrahydrocerulenin analogues starting from the unsaturated different aldehyde:

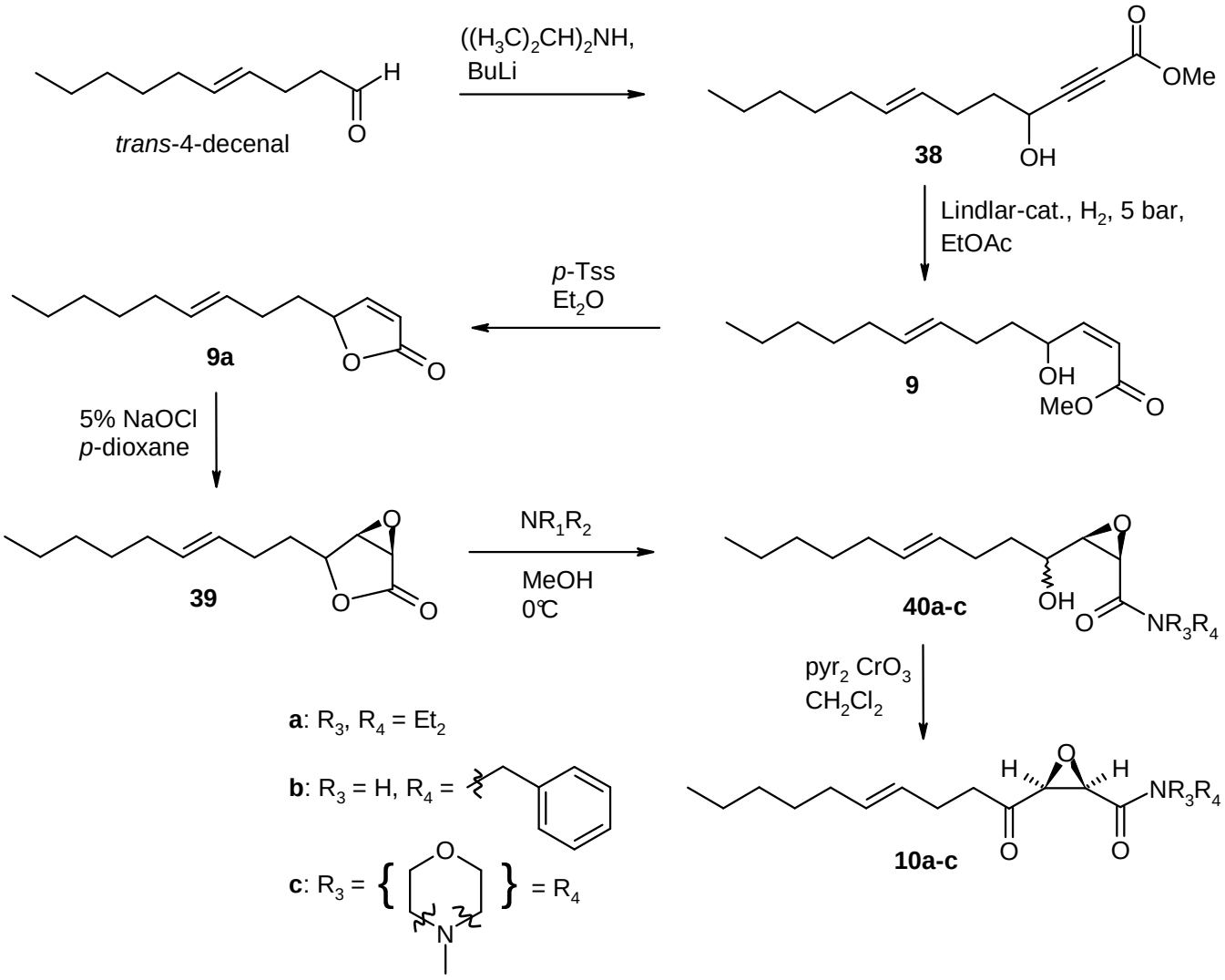


*Fig. 3.15 Synthesis route to (7E)-dihydrocerulenin analogues **10a-c***

Commercially available *trans*-4-decenal was coupled to methyl propiolate in 75 % yield and subsequently hydrogenated to ester **9** in 87 % yield. Interestingly, the ester did not cyclize during the workup procedure. In order to obtain the lactone **9a** *p*-toluene sulfonic acid had to be added to a solution of **9** in Et_2O and the mixture had to be stirred overnight. **9a** was obtained in 72 % yield. Subsequent epoxidation gave **39** in 34 % yield, addition of the corresponding amines lead to compounds **40a-c** in

34 - 72 % yield and oxidation resulted in dihydrocerulenin analogues **10a-c** in 35 - 50 % yield.

3.3.3.2 Synthesis attempt of (6E)-dihydrocerulenin analogues

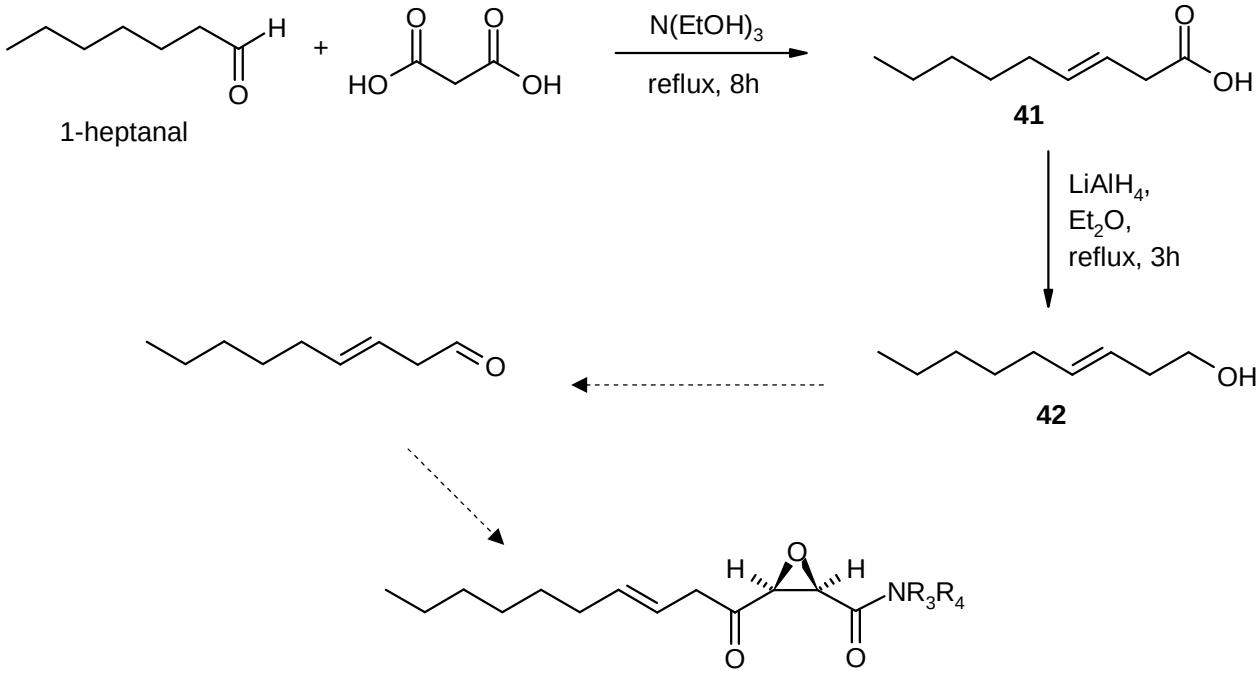


Fig. 3.16 Synthesis attempt to (6E)-dihydrocerulenin analogues

In addition to the *(7E)*-dihydrocerulenin analogues the attempt was made to synthesise *(6E)*-dihydrocerulenin analogues. Therefore, the preparation of *(E)*-3-nonenal had to be invented first before the aldehyde could be introduced into *synthesis pathway 3*. 1-Heptanal was chosen as the starting material and a Doebner condensation was executed with malonic acid to give acid **41** in 40 % yield.[209] $LiAlH_4$ reduction of the latter resulted in alcohol **42** in 61 % yield. Despite several attempts, Swern or chromium trioxide-pyridine oxidation of **42** proved to be not successful and only starting material or small amounts of rearranged *(E)*-2-nonenal could be observed in the NMR spectra.

3.3.3.3 Synthesis attempt of (5E)-dihydrocerulenin analogues

To obtain further dihydrocerulenin analogues, attempts were executed to gain (*5E*)-dihydrocerulenin analogues using (*E*)-2-nonenal as the starting material.

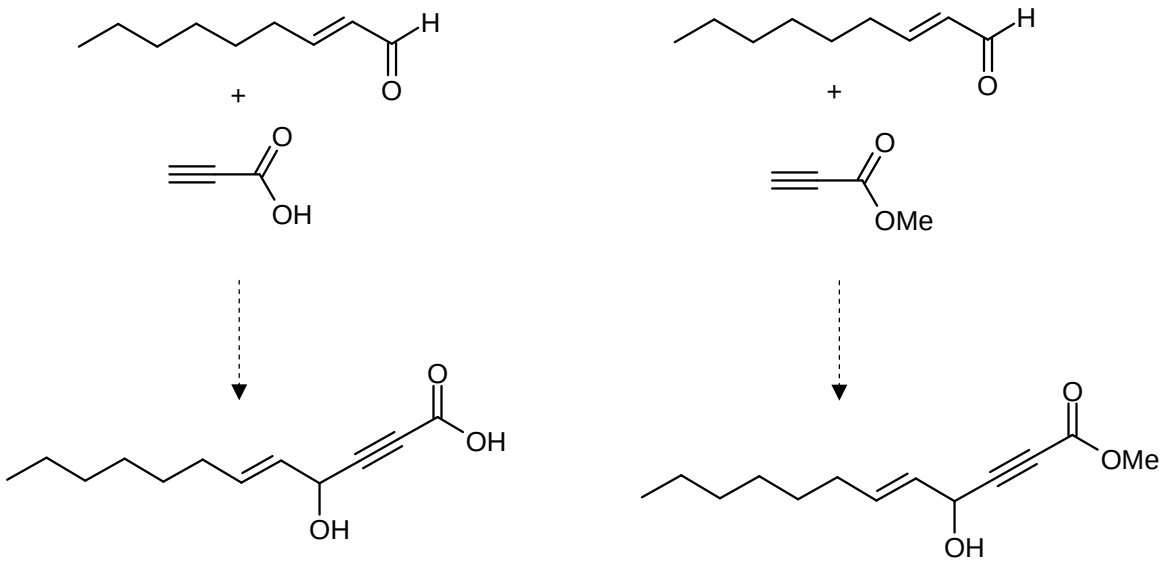


Fig. 3.17 Synthesis attempt to (5E)-dihydrocerulenin analogues

Several attempts either to couple (*E*)-2-nonenal with propiolic acid or methyl propiolate were unsuccessful, probably due to isomerization of the double bond. NMR spectra showed a complicated mixture of signals and after purification by column chromatography only starting material could be recovered.

3.4 Synthesis pathway 4: Synthesis of cerulenin analogues

By means of pathway 4 it was aimed to achieve cerulenin analogues that contained all structural features of the target including two double bonds and the epoxide function. Therefore, the compounds prepared via this pathway are most similar to the original structure of cerulenin compared to all obtained analogues so far.

However, *synthesis pathway 4* is similar to *synthesis pathway 3* because in *synthesis pathway 3* the corresponding aldehyde as the future side chain of the goal compound represents the key intermediate containing 2, 1 or no double bond. For tetrahydro- and dihydrocerulenin analogues commercially available aldehydes were used. This is not possible for the cerulenin analogues; here the aldehyde with two double bonds had to be synthesized first. Therefore *synthesis pathway 4* consists of two parts: the preparation of the aldehyde at first and secondly the insertion of the aldehyde into the scheme of *synthesis pathway 3*. Two stereoisomeric aldehydes, the (4*E*,7*E*)-4,7-nonadienal and (4*Z*,7*E*)-4,7-nonadienal, were prepared.

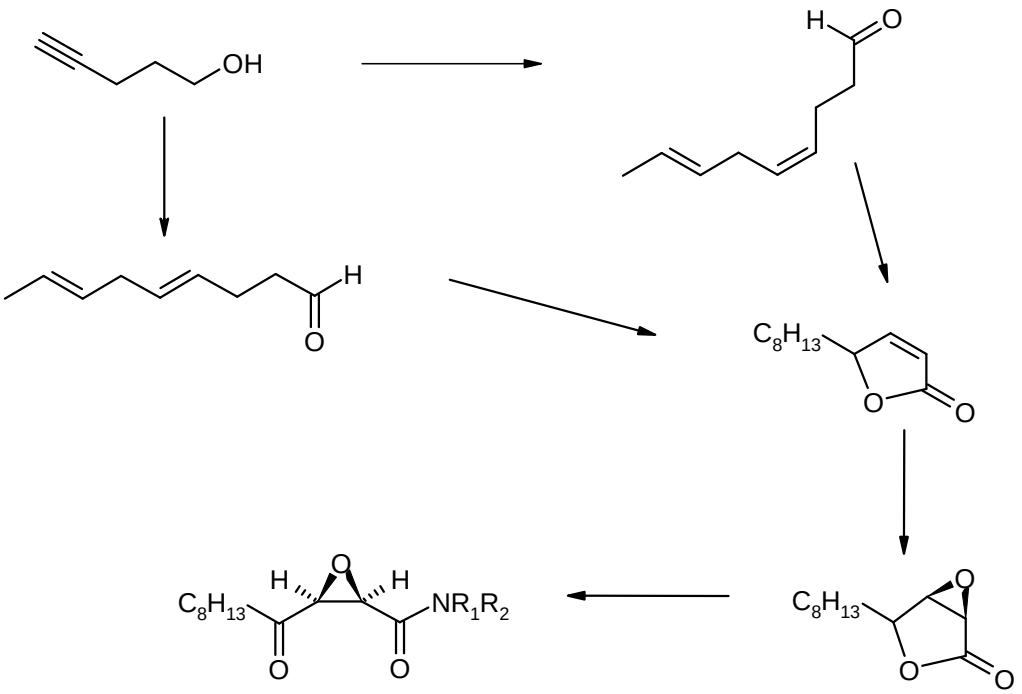


Fig. 3.18 Synthesis pathway 4 to cerulenin analogues

3.4.1 Synthesis route to *E*,*E*-cerulenin analogues

3.4.1.1 Synthesis attempt to (4E,7E)-4,7-nonadienal via the tributylstannane

In a first attempt to (4*E*,7*E*)-4,7-nonadienal the aldehyde was prepared in analogy to *synthesis pathway 1* (see chapter 3.1.1) starting from commercially available 4-pentyn-1-ol. The alcohol was protected as before to yield the tetrahydropyranyl ether **44**. Tributyltin hydride was added to give stannane **45**, which was coupled to crotyl bromide. Tetrahydropyranyl ether **46** was obtained and used without isolation for deprotection to give (4*E*,7*E*)-4,7-nonadienol **47** in 27 % yield. In addition, this alcohol was a mixture of (4*E*,7*E*)-4,7-nonadienol and (4*Z*,7*E*)-4,7-nonadienol (*E:Z*=73:27 determined by the ^{1}H NMR signals of the allylic-CH_2 between the double bonds, see exp. section), which had to be separated by means of column chromatography on silica gel impregnated with 20 % $AgNO_3$.[210] Separation was achieved successfully and pure (4*E*,7*E*)-4,7-nonadienol could be obtained, However, due to strong overlap of the fractions of both isomers only the first fractions contained pure (4*E*,7*E*)-4,7-nonadienol. This fact lead to a poor yield of 18 %, which was even slightly lower when this reaction was carried out for a second and third time. Due to this very low yield in terms of separation, a low overall yield of the crude product before and in view of a long synthesis afterwards, this pathway was condemned.

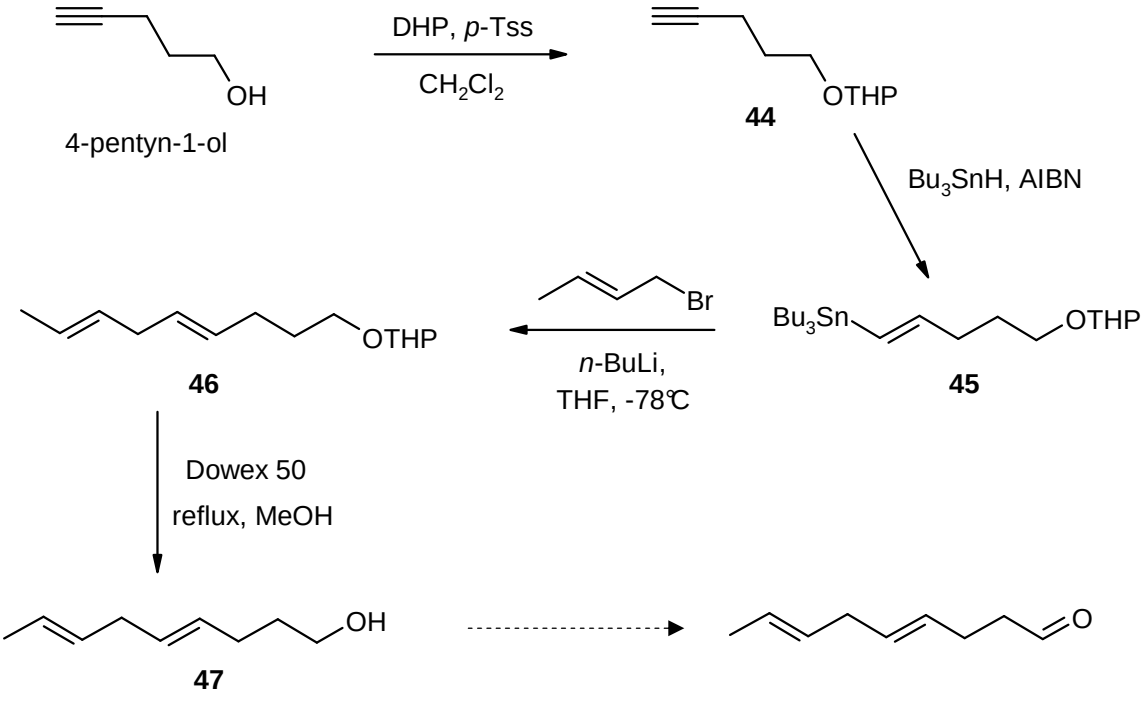


Fig. 3.19 Synthesis attempt to (4E,7E)-4,7-nonadienal

3.4.1.2 Synthesis attempt to (4E,7E)-4,7-nonadienal via (E)-7-Nonen-4-yn-1-ol (**11**)

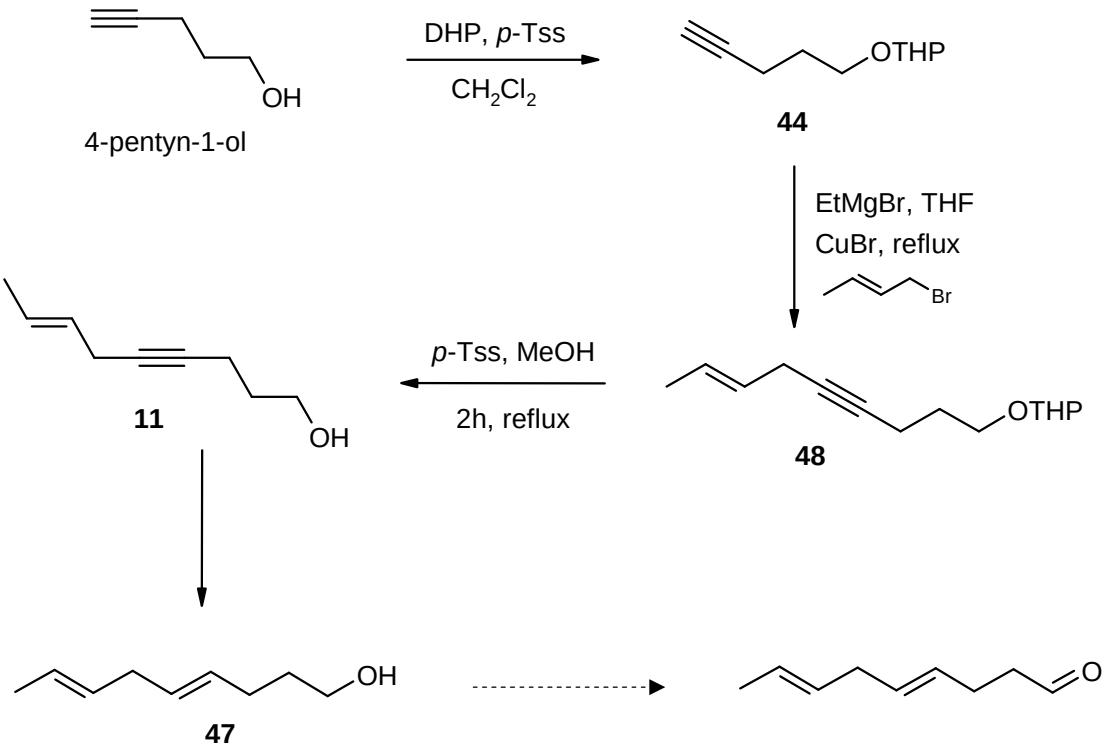


Fig. 3.20 Synthesis attempt to (4E,7E)-4,7-nonadienal

A further pathway was invented to obtain (4*E*,7*E*)-4,7-nonadienal. Starting again from 4-pentyn-1-ol to compound **44**, a coupling between the tetrahydropyranyl ether and crotyl bromide with CuBr was attained (for detailed descriptions see chapter 3.4.2). The enine **48** was deprotected to alcohol **11**. The latter substance was attempted to reduce to (4*E*,7*E*)-4,7-nonadienol with $LiAlH_4$ in diglyme similar to the procedure reported by Rossi et al.[211] Despite several attempts with variation of reaction parameters (reaction times varied from 8 - 24 hours, temperatures from 60 - 160 °C) and although reactions were monitored by TLC, reduction never stopped to yield substance **47**, but always in the reduction to single bonds even though starting material **11** was still detectable. Only a complicated mixture of different alcohols could be detected in the NMR spectra and therefore this pathway was no longer pursued.

3.4.2 Synthesis route to *Z*,*E*-cerulenin analogues

3.4.2.1 Synthesis of 2-((E)-7-Nonen-4-ynyloxy)-tetrahydro-2H-pyran (**48**)

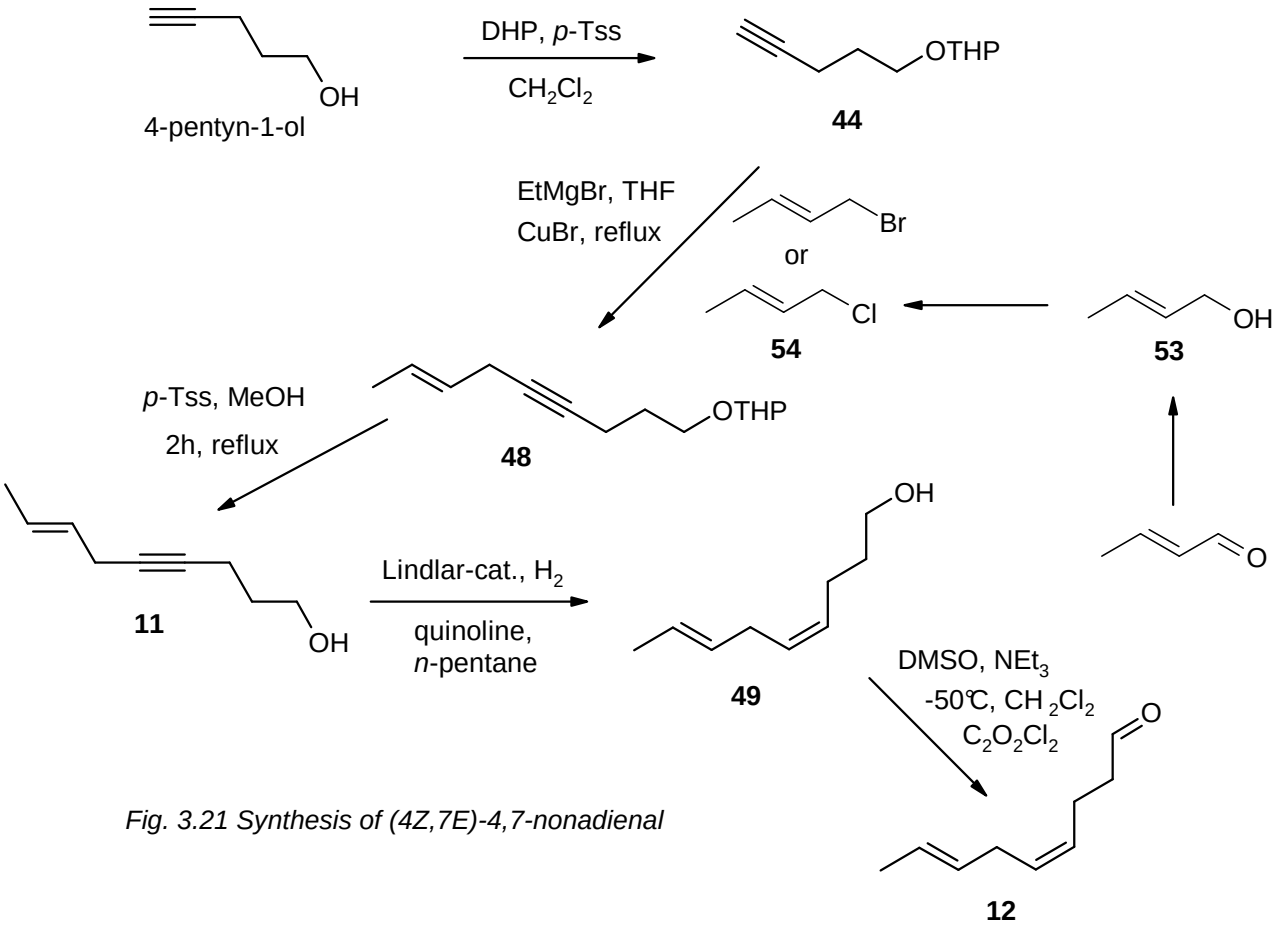


Fig. 3.21 Synthesis of (4Z,7E)-4,7-nonadienal

The synthesis of (4*Z*,7*E*)-4,7-nonadienal **12** started from the before produced tetrahydropyranyl ether **44** (see 3.4.1.1), which was coupled either with commercially available crotyl bromide or due to economical reasons with crotyl chloride that is easily prepared from croton aldehyde to **48** via a procedure similar to one reported by Rossi et al.[212]

3.4.2.2 Synthesis of crotyl chloride (**54**)

Commercially available croton aldehyde was reduced by adding the latter to a solution of $LiAlH_4$ dissolved in dry Et_2O under argon. The addition had to be carried out very carefully due to the exothermic character of this reaction. After the reduction to alcohol **53** was finished, remaining $LiAlH_4$ was carefully destroyed with ice cold water at 0 °C. Work up and purification by distilla tion gave crotyl alcohol **53** in 63 % yield. Crotyl chloride was obtained by a procedure similar to that reported by Rossi et

al.[211] but with a different work up procedure. Crotyl alcohol was dissolved in *s*-collidine and a solution of LiCl in dry DMF was added to the mixture under argon. Addition of MsCl affected mesylation of the alcohol. Subsequently, the present LiCl lead to substitution of chloride against the mesylate to give substance **54** after distillation in 81 % yield.

3.4.2.3 Synthesis of (4Z,7E)-4,7-nonadien-1-al (**12**)

The preparation of **48** afforded the coupling of commercially available crotyl bromide or synthesized crotyl chloride (see chapter 3.4.2.2) with the Grignard reagent prepared from tetrahydropyranyl ether **44** and EtMgBr in the presence of CuBr. This was executed by refluxing the mixture in dry THF for 2 h and stirring at RT for additional 24 h similar to a procedure published by Rossi et al.[212] but with differing reaction procedures. Usual workup resulted in compound **48** in 94 % yield. Alcohol **11** was obtained by refluxing substance **48** in MeOH with a catalytic amount of *p*-toluene sulfonic acid for 2h. Thus, the protection group was removed and work up of crude **11** and purification by column chromatography gave the product in 77 % yield. Compound **11** was hydrogenated at -10 °C in *n*-pentane at 7 bar hydrogen pressure in a sealed tube with a catalytic amount of quinoline. After 1 h hydrogen absorption ceased and both the low temperature and quinoline prevented product **49** from further reduction. After filtration pure (4*Z*,7*E*)-4,7-nonadien-1-ol could be obtained in 92 % yield. Aldehyde **12** was prepared by a Swern oxidation of alcohol **49** in 74 % yield. Oxalyl chloride was dissolved in dry CH_2Cl_2 at -50 °C under argon and DMSO was added to the reaction mixture. After the reactive oxidation reagent was formed alcohol **49** was added and the mixture was stirred for 15 min. NEt_3 was added to complete the reaction and the solution was warmed to RT until no further change on the TLC plate could be observed. Work up and purification by column chromatography lead to pure aldehyde **12** in 74 % yield.

3.4.2.4 Synthesis of Z,E-cerulenin analogues **15a-b**

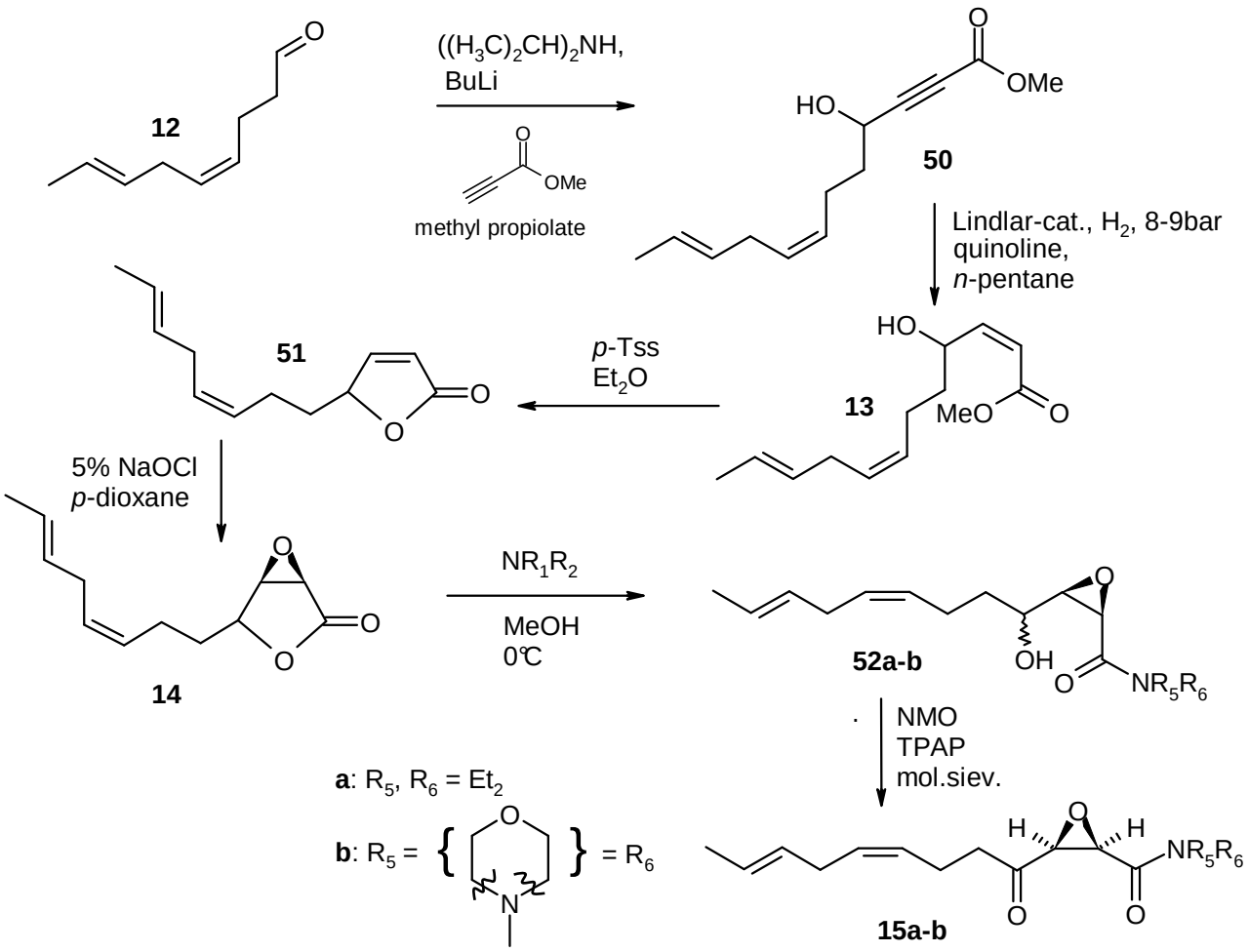


Fig. 3.22 Synthesis of Z,E-cerulenin analogues

Having successfully synthesized the aldehyde **12**, the compound could be integrated into the second part of *synthesis pathway 4*. Similar to *synthesis pathway 3* (see chapter 3.3.3), aldehyde **12** was coupled to methyl propiolate in 62 % yield. Hydrogenation was executed a little bit different compared to *synthesis pathway 3*: methyl ester **50** was hydrogenated in *n*-pentane with a hydrogen pressure of 8-9 bar over Lindlar catalyst and an additional catalytic amount of quinoline. Lactone formation, epoxidation and amidation were performed in the same manner as described in chapter 3.3.3. However, oxidation from substances **52a-b** to **15a-b** was executed differently: a more convenient method from Griffith et al.[213] was chosen using tetrapropylammonium perruthenate (TPAP) as a mild oxidant that tolerates potentially reactive groups such as halides, amides and epoxides. TPAP was used

with the co-oxidant *N*-methylmorpholine *N*-oxide (NMO), so TPAP could be added only in catalytic amounts.[214] Therefore, TPAP with NMO displays a good alternative to the chromium trioxide-pyridinium complex and oxidation to the *Z*,*E*-cerulenin analogues **15a-b** proceeded successfully in 62 - 65 % yield.

3.4.3 Synthesis attempts to further cerulenin analogues

Further cerulenin analogues were tried to be synthesized with different side chains with two double bonds which should be integrated into synthesis pathway 4.

3.4.3.1 Synthesis attempt to (6E,9E)-cis-2,3-epoxy-4-hydroxyundeca-6,9-dienamides

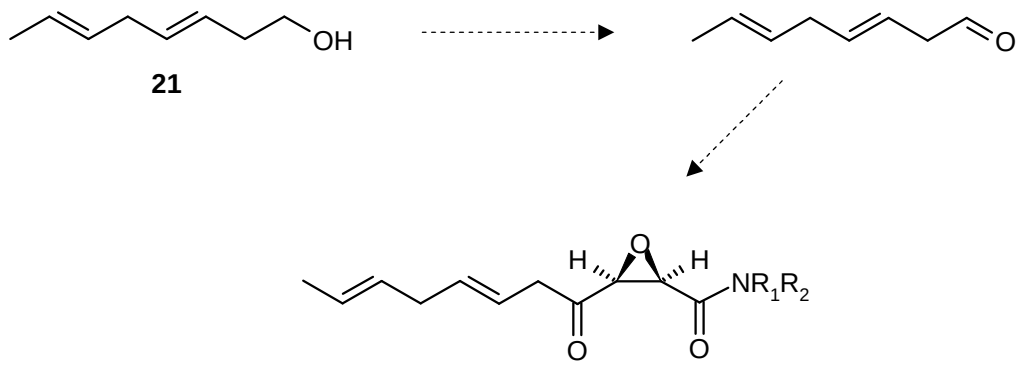


Fig. 3.23 Synthesis attempt to further E,E-cerulenin analogues 1

It was aimed to get products lacking one methylene group compared to the original cerulenin structure; thus, experiments were carried out to oxidize octadienol **21** (see synthesis pathway 1, chapter 3.1) to the corresponding aldehyde and to use the before chosen synthesis route (chapter 3.3) to gain (3*Z*,6*E*)-cis-2,3-epoxy-4-hydroxyundeca-3,6-dienamide cerulenin analogues. However, oxidation of **21** was not successful. Neither Swern nor oxidation with Jones reagent proved to be possible and NMR spectra showed after purification mostly starting material with small amounts of rearranged (2*E*,6*E*)-2,6-nonadien-1-al.

3.4.3.2 Synthesis attempt to (5E,7E)-cis-2,3-epoxy-4-hydroxyundeca-3,6-dienamides

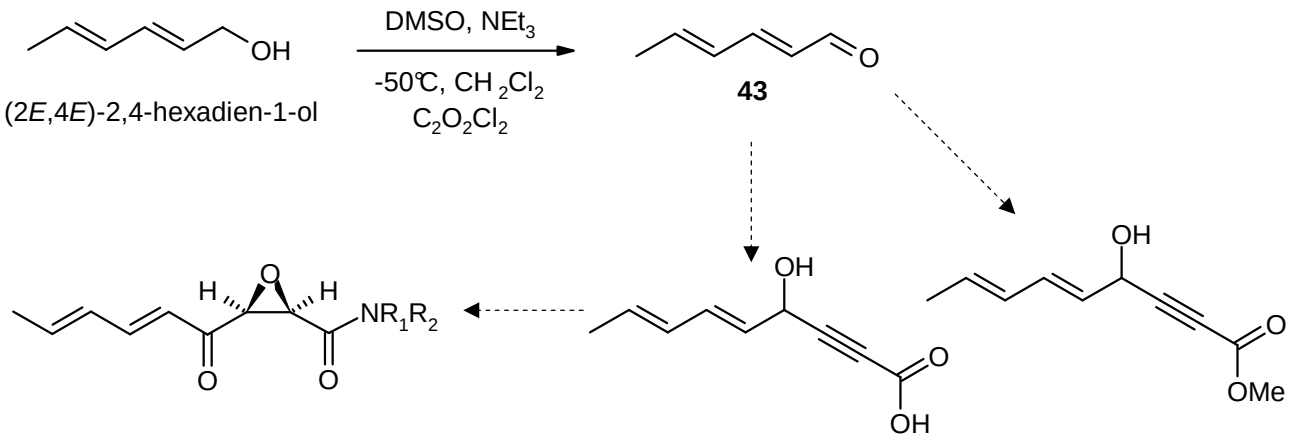


Fig. 3.24 Synthesis attempt to further E,E-cerulenin analogues 2

Experiments were executed to obtain (5*E*,7*E*)-cis-2,3-epoxy-4-hydroxyundeca-3,6-dienamides. Starting material was commercially available (2*E*,4*E*)-2,4-hexadien-1-ol, which was oxidized by a common Swern oxidation to give (2*E*,4*E*)-2,4-hexadienal in 40 % yield after distillation. However, coupling to either propiolic acid via a Grignard reagent or to methyl propiolate via the corresponding lithium reagent (details see chapter 3.3.1) failed to give any desired product but resulted according to the NMR spectra in a complicated mixture and only starting material could be isolated after purification.

3.5 Synthesis pathway 5: Synthesis of aromatic cerulenin analogues

In order to synthesise other cerulenin derivatives, a procedure to aromatic analogues was invented similar to that of Lawrence et al.[187]

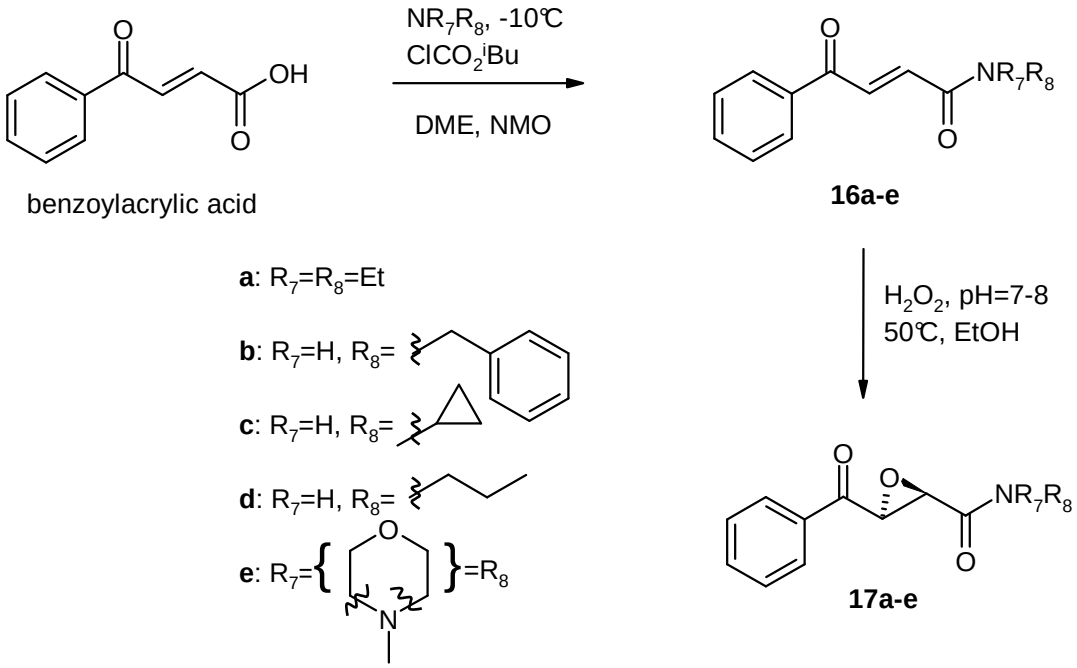


Fig. 3.25 Synthesis route to aromatic cerulenin analogues

In the first step to these aromatic cerulenin analogues the formation of the benzoyl acrylamides **16a-e** from benzoylacrylic acid was executed via a mixed anhydride which was prepared *in situ* with isobutylchloroformate followed by subsequent addition of a corresponding amine at -10 ℃ in DME under argon. The yields of this reaction ranged between 36 and 99 %. Subsequent epoxidation with H_2O_2 in basic EtOH gave the cerulenin analogues **17a-e** in 65 - 93 % yields.

3.6 Stereochemistry of cerulenin analogues

3.6.1 Stereochemistry of the epoxides

3.6.1.1 Stereochemistry of acyclic cerulenin analogues

All cerulenin analogues with an epoxy function synthesized in this study contain a *cis*-configuration for the epoxide protons with exception of the aromatic analogues where the epoxide protons are configured *trans* due to a different synthesis pathway. The latter will therefore be discussed separately (see 3.6.1.2).

The *cis* configuration at the epoxide of the alkyl cerulenins is generated due to the synthesis pathway (for details see 3.3.2) utilizing a lactone epoxydation with sodium hypochlorite.

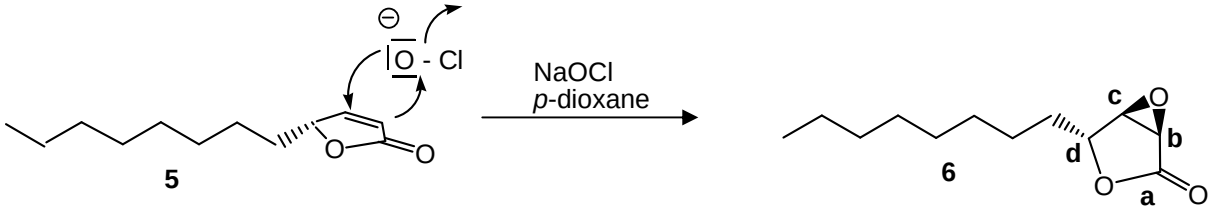


*Fig. 3.26 Synthesis of epoxylactone **6***

Only one isomeric epoxy lactone is formed as a racemate whose configuration can be determined via the coupling constants in the ^{1}H NMR spectrum. Jakubowski et al.[206] observed coupling constants for epoxide protons **b** and **c** of **6** (see Fig. 3.26) of 2.5 Hz for this *cis* geometry confirmed by the Karplus equation, which is in accordance with the constants observed in this study (J=2.6 Hz). In addition, the coupling constants for protons **c** and **d** of **6** leading to tetrahydrocerulenins had a coupling constant of J=1.2 Hz (see experimental section) which is consistent with a *trans* orientation for protons **c** and **d** of **6** confirmed by the Karplus equation. A *cis* configuration of protons **c** and **d** would require a coupling constant of J=2-3 Hz.[215] A *trans* stereochemistry of **c** and **d** is also consistent with the attack of the hypochlorite from the less hindered side of the molecule.

These observations hold also true for other epoxylactones of dihydrocerulenins or cerulenin analogues. (7*E*)-*cis*-2,3-epoxytridec-7-enoic acid γ-lactone (**39**) shows a coupling constant of J=2.5 Hz for the epoxide protons (**b**,**c**) and a constant of J=0.8 Hz for the corresponding protons **c** and **d** of **39**. (7*Z*,10*E*)-*cis*-2,3-epoxydodeca-7,10-

dienoic acid γ-lactone (**14**) shows a similar behaviour for the epoxide protons; a coupling constant of *J*=2.5 Hz for protons **b** and **c** and *J*=0.5 Hz for protons **c** and **d** was observed.

Due to these observations, also the stereochemistry for the subsequent cerulenin analogues (except for the aromatic derivatives) remains *cis* for the epoxide substituents and only a racemic mixture but no diastereomers were observed for the epoxylactones **6**, **14** and **39**.

3.6.1.2 Stereochemistry of aromatic cerulenin analogues

Due to a different synthesis route, the stereochemistry for the epoxide protons for the aromatic cerulenin analogues is observed to be *trans* (see Fig. 3.27). This is consistent with the results gained by Lawrence et al.[187] and Carr et al.[188] Furthermore, a *cis* configuration would require coupling constants of *J*=~5-6 Hz as observed by Jakubowski et al.[206] The constants observed in this work were *J*=~2Hz for the epoxide protons **b** and **c** of compounds **17a-e** (see experimental section).

A *trans* configuration of the epoxide protons is also consistent with the preferred arrangement of the bulky aromatic ring and the amide function during the epoxidation via hydrogen peroxide.

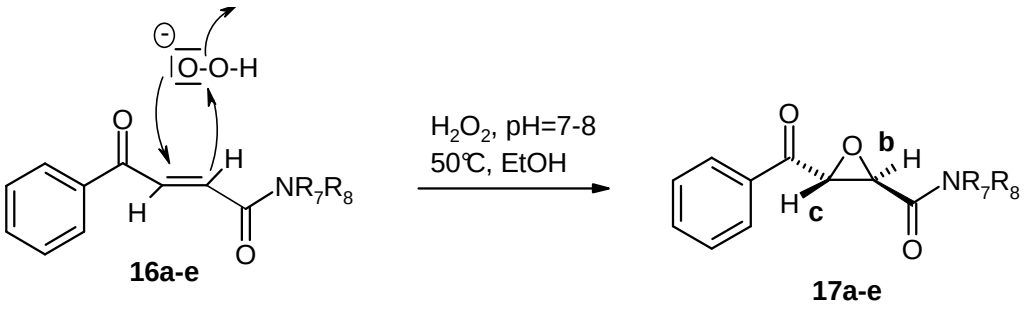


Fig. 3.27 Stereochemical arrangement of the aromatic cerulenin analogues

3.6.2 Hydroxylactam formation

3.6.2.1 Tetrahydrocerulenin analogues

Cerulenin shows special properties concerning its behaviour in solutions. Jakubowski et al.[206] realized that cerulenin shows a NMR spectrum of a single compound in $CDCl_3$. When the solvent is changed to MeOH-d_4, a second set of signals of the epoxy protons develops over time, diminishing at one position and arising at another until finally only the new set of signals is present. A similar observation can be made for the methylene group next to the keto group. The IR spectrum shows a reduction in the NH_2 bending frequency with concomitant appereance of a hydroxyl absorption (for details see Jakubowski et al.[206]). These observations suggest a rapid equilibrium between cerulenin (**I**) and its diastereomic hydroxylactam forms (**II**) in methanol (see Fig. 3.28), because the isomeric compounds **I** and **II** generate the same molecular ion in the mass spectrum as does cerulenin.[206]

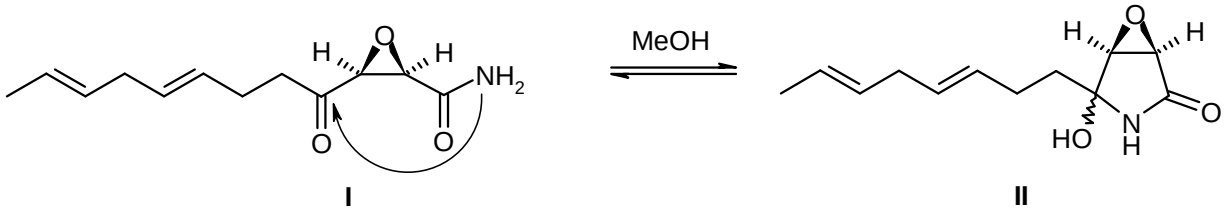


Fig. 3.28 Equilibration between cerulenin and its hydroxylactam forms

For the newly synthesized cerulenin analogues an additional phenomenon concerning the hydroxylactam forms could be observed. Although all NMR spectra were recorded in $CDCl_3$, lactam formation for analogues with substituents at a secondary amide function could be observed depending on the substituent on the nitrogen. This phenomenon of substituent-dependent ring-chain tautomerism could be observed for other 4-keto amides before.[216]

This is demonstrated exemplary for the tetrahydrocerulenin derivatives, which are the largest library of compounds synthesized here. Tetrahydrocerulenin **8a** consists of about 90 % (measured from the integrals of epoxy protons **Ic,d** and **IIc,d**, see Fig. 3.29) of the open isomer (analoguous to **I** in Fig. 3.28) and of only a small amount of the cyclized forms **II** according to the NMR spectra in $CDCl_3$.

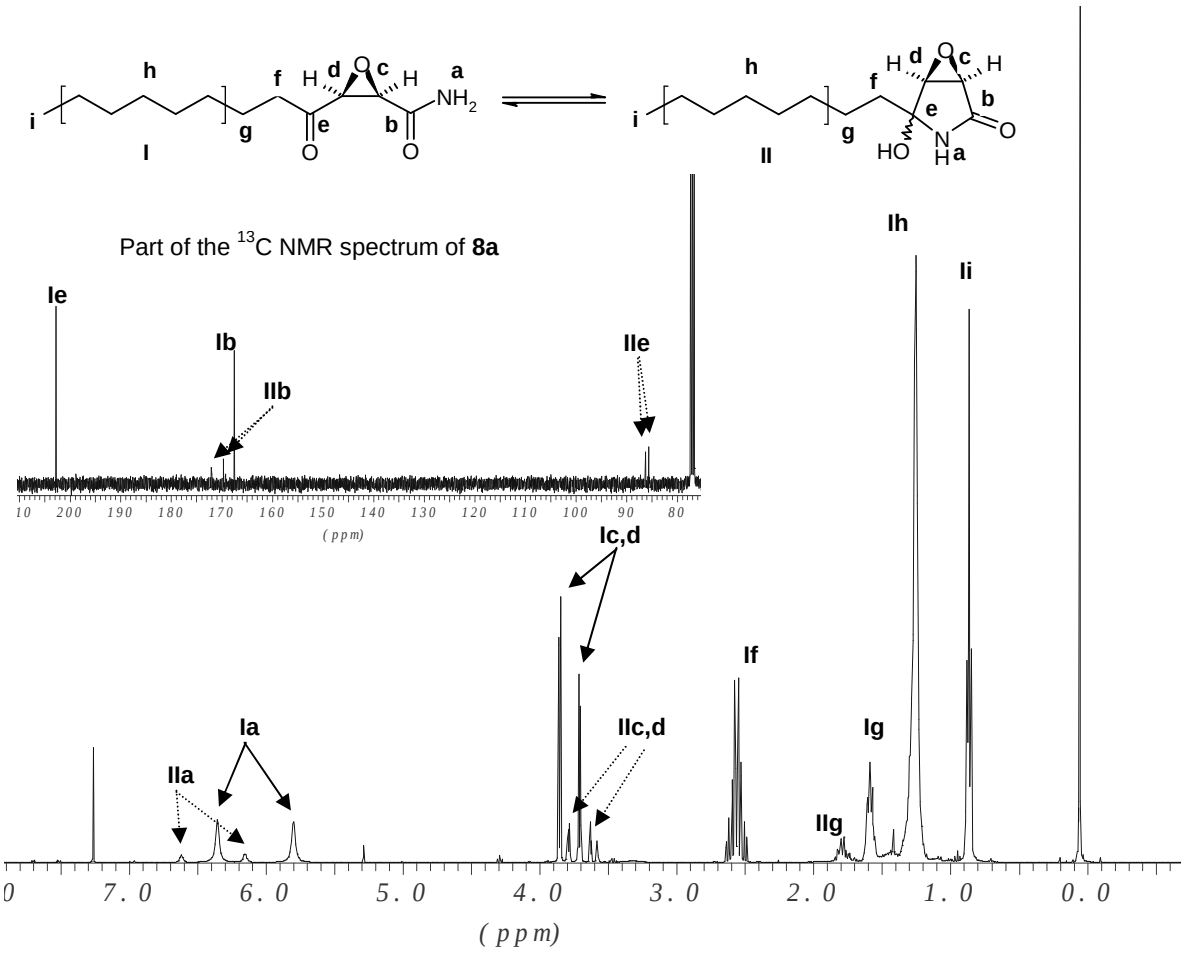


*Fig. 3.29 ^{1}H NMR spectra of tetrahydrocerulenin **8a** and its hydroxylactam forms; only the visible signals were picked for hydroxylactams **II***

This is confirmed especially by the signal of **Ie** in the ^{13}C spectrum, because only for the open form **I (8a)** a ketone signal at δ=202.76 ppm can be found. Hydroxylactams **II (8a)** show signals for the quaternary carbon atoms **IIe (8a)** at δ=85.61 and δ=86.26 ppm, which are no longer present in the ^{13}C DEPT spectrum (data not shown). All these findings confirm the open form **I (8a)** of tetrahydrocerulenin as the main isomer (ca. 90 %, measured from integrals of the epoxy protons in the ^{1}H NMR spectrum) in $CDCl_3$ and hydroxylactam forms **II (8a)** as the minor ones (ca. 10 %) and correspond to the observation of Jakubowski et al.[206] that cerulenin exists only in the open form **I** in aprotic solvents.

However, when attaching alkyl substituents to the nitrogen atom of the amide function like in *N*-cyclopropyl-tetrahydrocerulenin **8c** a change in the ratio of the open form **I (8c)** and the hydroxylactam forms **II (8c)** can be observed in the aprotic solvent $CDCl_3$ (see Fig. 3.30).

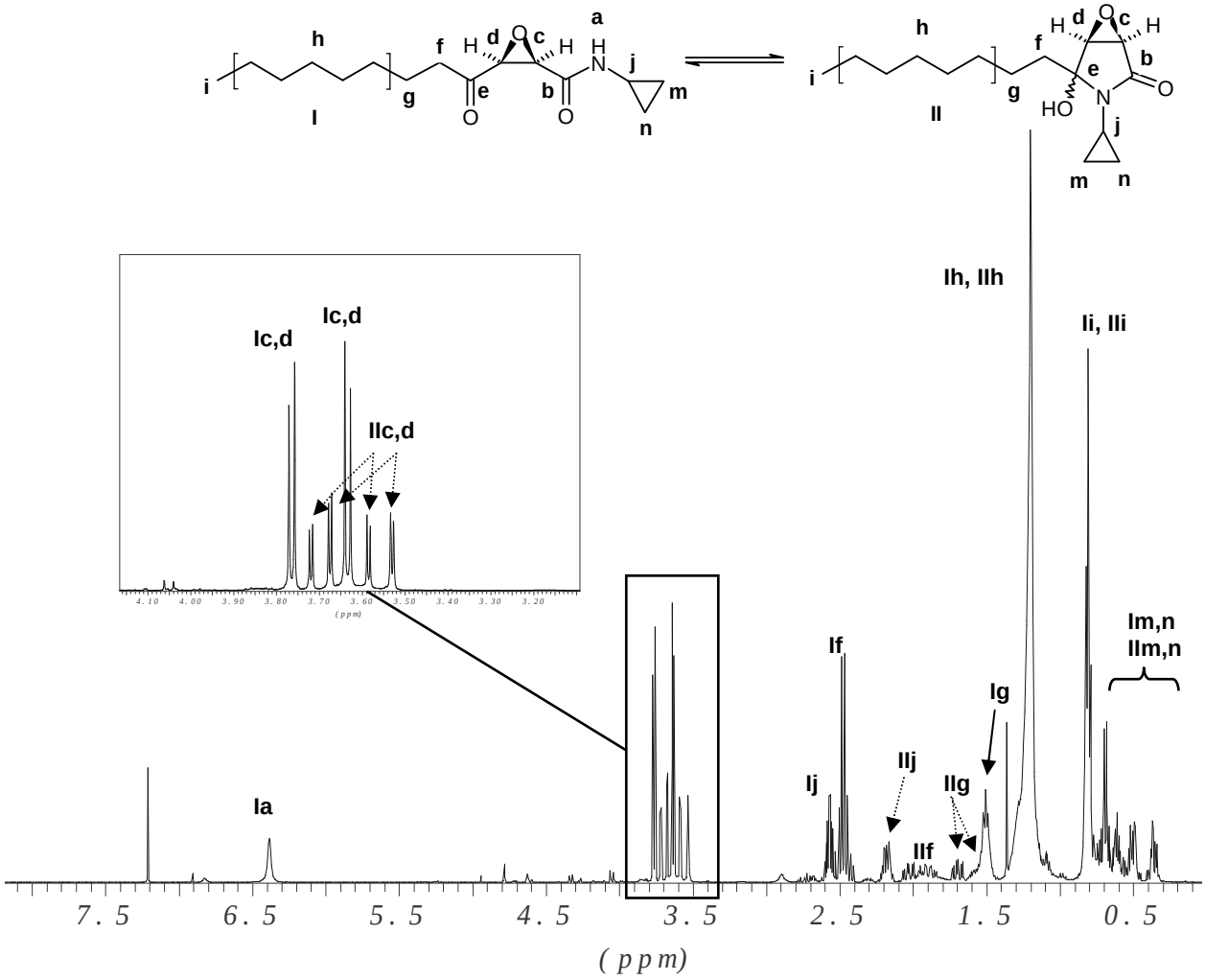


*Fig. 3.30 ^{1}H NMR spectrum of N-cyclopropyl tetrahydrocerulenin **8c** and its hydroxylactam forms*

In contrast to cerulenin and tetrahydrocerulenin, about 40 % of the hydroxylactam forms **II** of **8c** are existent (measured from the integrals of epoxy protons **Ic,d** and **IIc,d**, see Fig. 3.30) in the spectrum compared to ca. 60 % of the open form **I** of **8c**.

The ^{1}H spectrum of **8c** therefore looks more complicated. Some of the signals in Figure 3.30 are overlapping (e.g. Ig/IIg, Im,n/IIm,n), but the integrals correspond to the predicted number of protons of **8c**. Furthermore, in the ^{13}C NMR spectrum (not shown) 3 sets of signals are observed corresponding to the open form **I (8c)** and the two diastereomeric hydroxylactam isomers **II (8c)**.

Introduction of the cyclopropyl group on the nitrogen atom with a positive inductive effect obviously seems to facilitate the attack of the nitrogen **Ia (8c)** to the carbonyl carbon atom **Ie (8c)** and thus makes the formation of hydroxylactams **II (8c)** (ratio 57:43, see Table 3.1) easier.

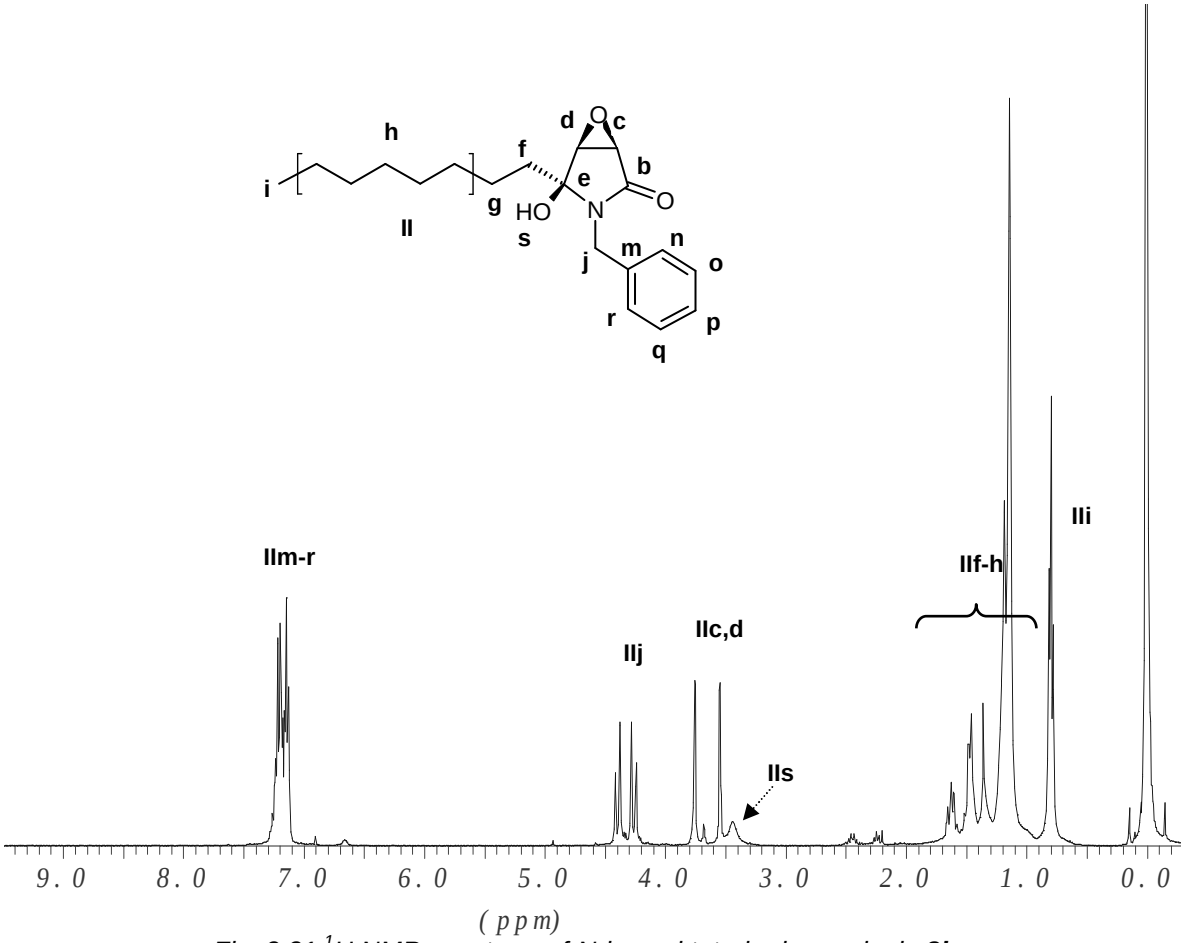


*Fig. 3.31 ^{1}H NMR spectrum of N-benzyl tetrahydrocerulenin **8b***

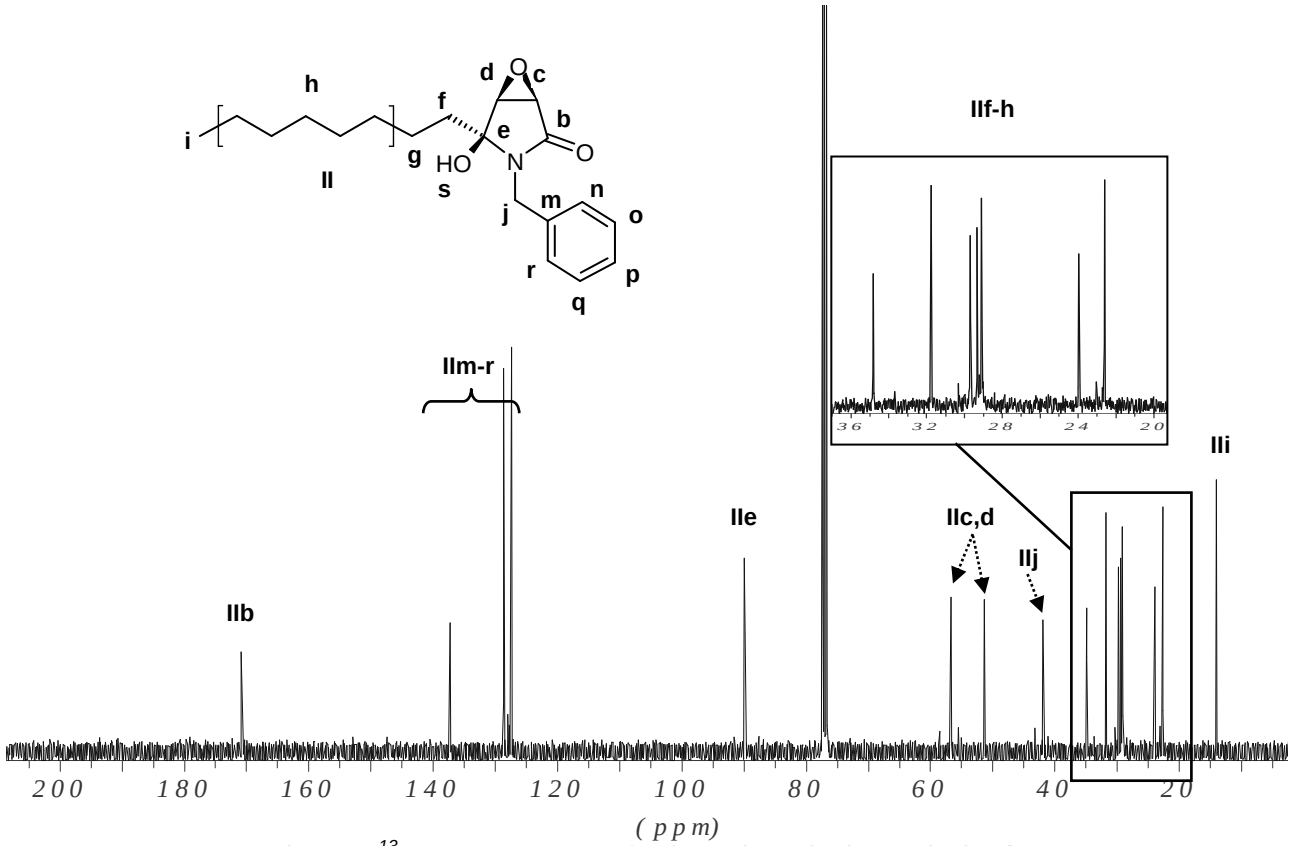


*Fig. 3.32 ^{13}C NMR spectrum of N-benzyl tetrahydrocerulenin **8b***

Interestingly, ^{1}H and ^{13}C NMR spectra show that the *N*-benzyl-tetrahydrocerulenin **8b** exists only in the hydroxylactam form **II**. In the ^{1}H NMR spectrum (see Fig. 3.31) the NH_2 protons cannot be observed and in the ^{13}C NMR spectrum no ketone is existent (Fig. 3.32). A further evidence for this fact reveals the carbon atom at δ=89.89 ppm; its chemical shift and the fact of being quarternary (^{13}C DEPT not shown) confirm the hydroxylactam form **II** of **8b**.
Considering the other spectra of tetrahydrocerulenins **8a** and **8c** two diastereomeric hydroxylactam forms are always present. In contrast, in case of compound **8b** the NMR spectra show only one set of signals. The steric bulk of the benzyl group probably leads to steric interference with the likewise bulky octyl group resulting in an attack only from one side of the nitrogen to the keto group at the carbon atom **e**. Thus, enantiomers of only one diastereomeric hydroxylactam form of **8b** are existent, probably the form where the epoxide and the alkyl chain are positioned *trans* due to steric reasons (see Fig. 3.31/3.32).

Regarding the remaining tetrahydrocerulenin analogues, the stereochemistry stays clear, because for these compounds (**8d**, **8e**) the nitrogen atom is substituted with three subsitutents and therefore has no possibility to form any of the hydroxylactam forms **II**. Thus, only the open form **I** can be observed in the ^{1}H and ^{13}C NMR spectra of **8d** and **8e** (exemplified on **8d** in Fig. 3.33). The signal of the keto carbon atom at δ=205.56 ppm (Fig. 3.34) and the absence of the signal of the quarternary carbon atom at about 90 ppm provide evidence for the open form.

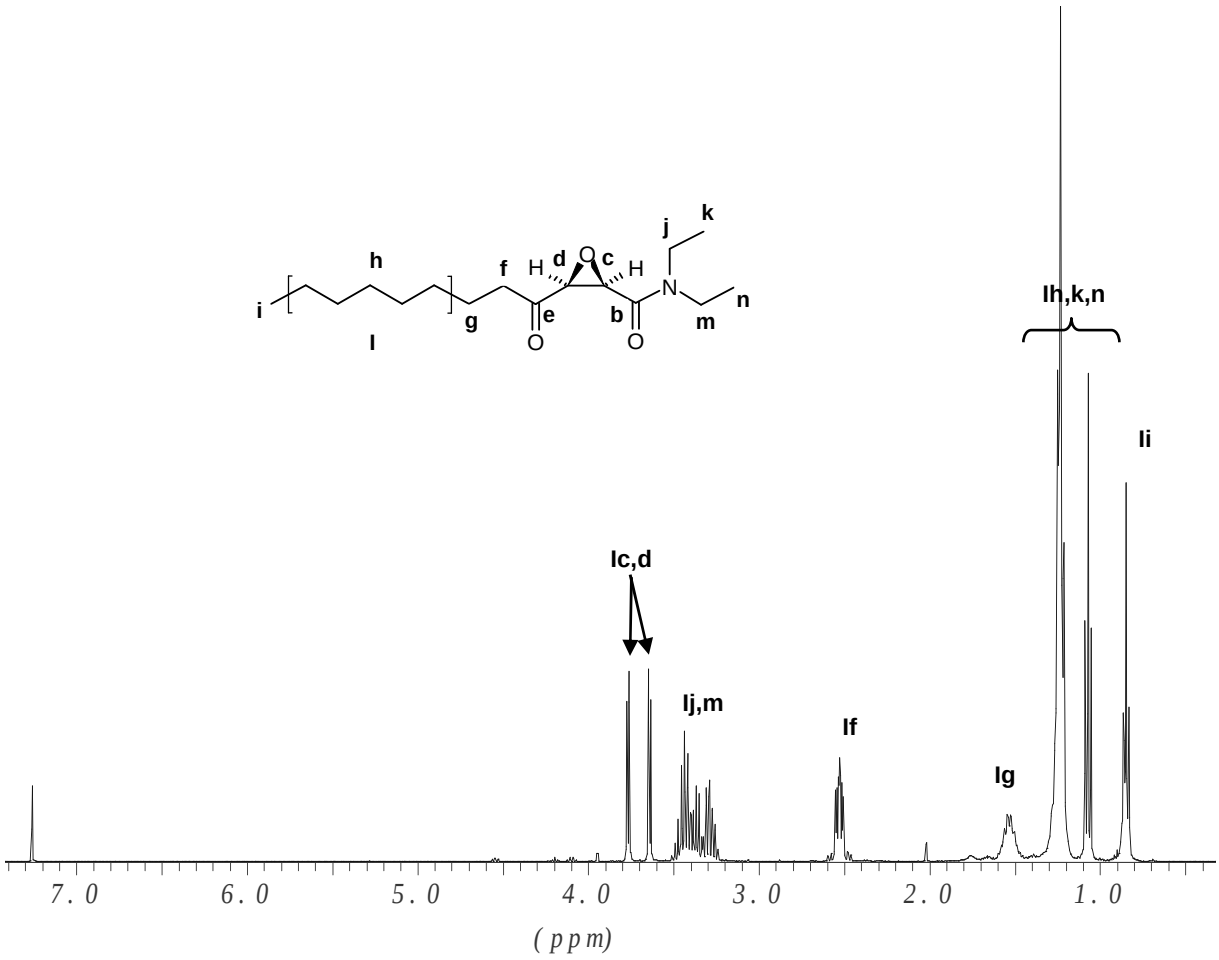


*Fig. 3.33 ^{1}H NMR spectrum of N,N-diethyl tetrahydrocerulenin **8d***

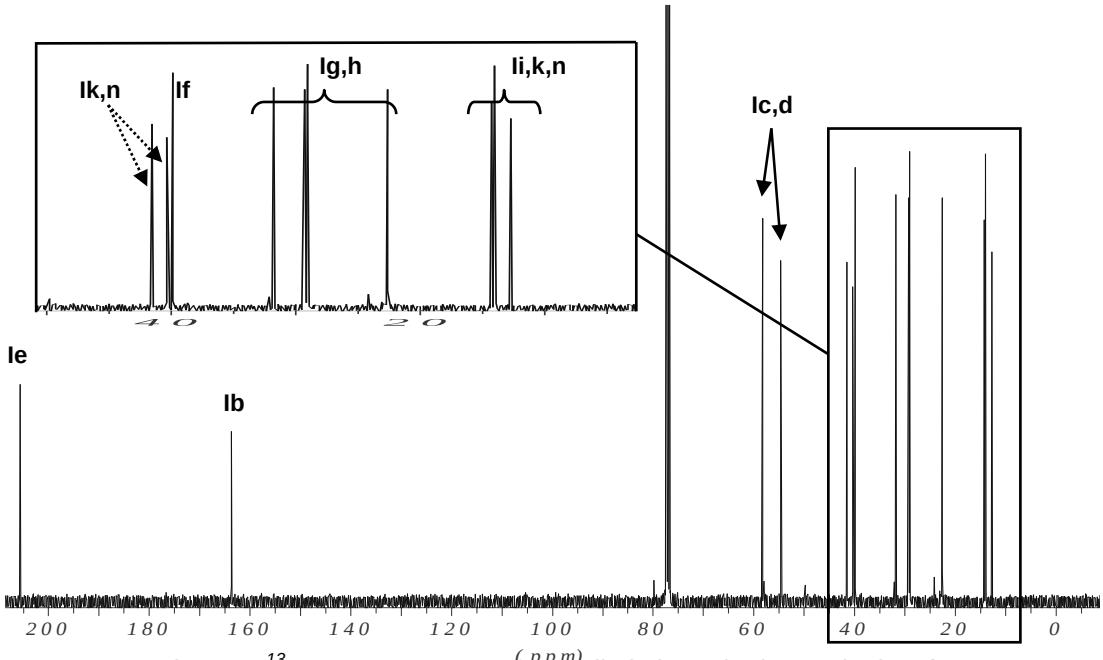


*Fig. 3.34 ^{13}C NMR spectrum of N,N-diethyl tetrahydrocerulenin **8d***

3.6.2.2 Dihydrocerulenin analogues

As expected, similar observations are made for dihydrocerulenin analogues **10b** and **10c** compared to the tetrahydrocerulenins of chapter 3.6.2.1.

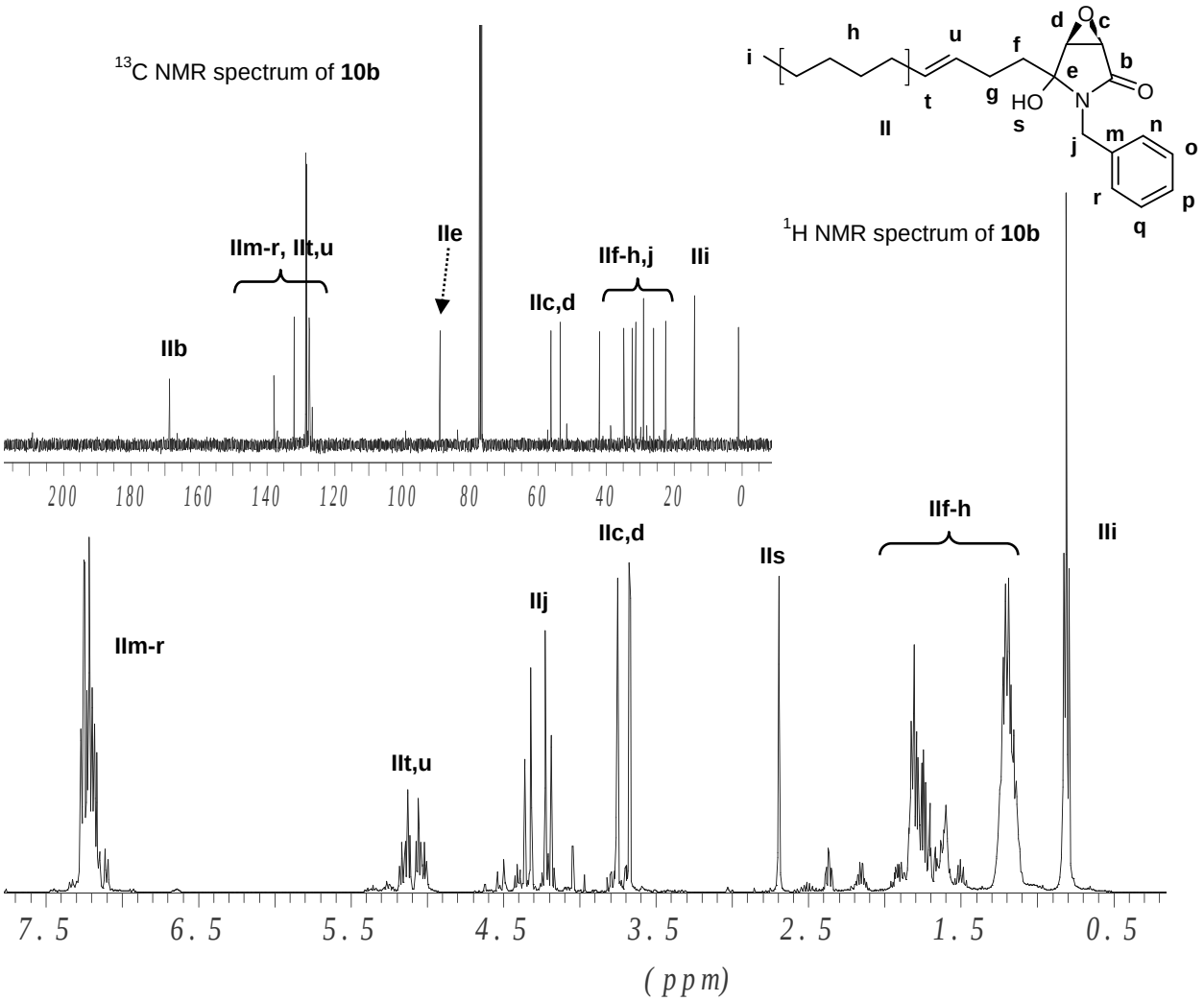


*Fig. 3.35 NMR spectra of dihydrocerulenin **10b**; only signals for the main isomer of the hydroxylactam forms were picked which is existent ~90 %*

^{1}H and ^{13}C NMR spectra of (7*E*)-*N*-benzyl-*cis*-2,3-epoxy-4-oxotridec-7-enamide **10b** reveal in correspondence to tetrahydrocerulenin **8b** the hydroxylactam form **II** of **10b** to be present only (see Fig. 3.35) and thus, in the ^{13}C NMR spectrum no ketone but a signal for the quaternary carbon atom at δ=88.95 ppm (^{13}C DEPT not shown) is existent.

About 90 % of one diastereomeric hydroxylactam (measured from the integrals of epoxy protons **IIc,d**) of **10b** are formed probably due to the bulky properties of the benzyl group (see chapter 3.6.1).

For dihydrocerulenin (7*E*)-*N,N*-diethyl-*cis*-2,3-epoxy-4-oxotridec-7-enamide **10a** and (7*E*)-*N*-morpholino-*cis*-2,3-epoxy-4-oxotridec-7-enamide **10c** the stereochemistry stays unambiguous as for tetrahydrocerulenins **8d** and **8e**, because for these compounds the nitrogen atom again is substituted with three subsitutents and therefore has no possibility to form any of the hydroxylactam forms **II**. Thus, only the open form **I** can be observed (exemplified on **10c** in Fig. 3.36).

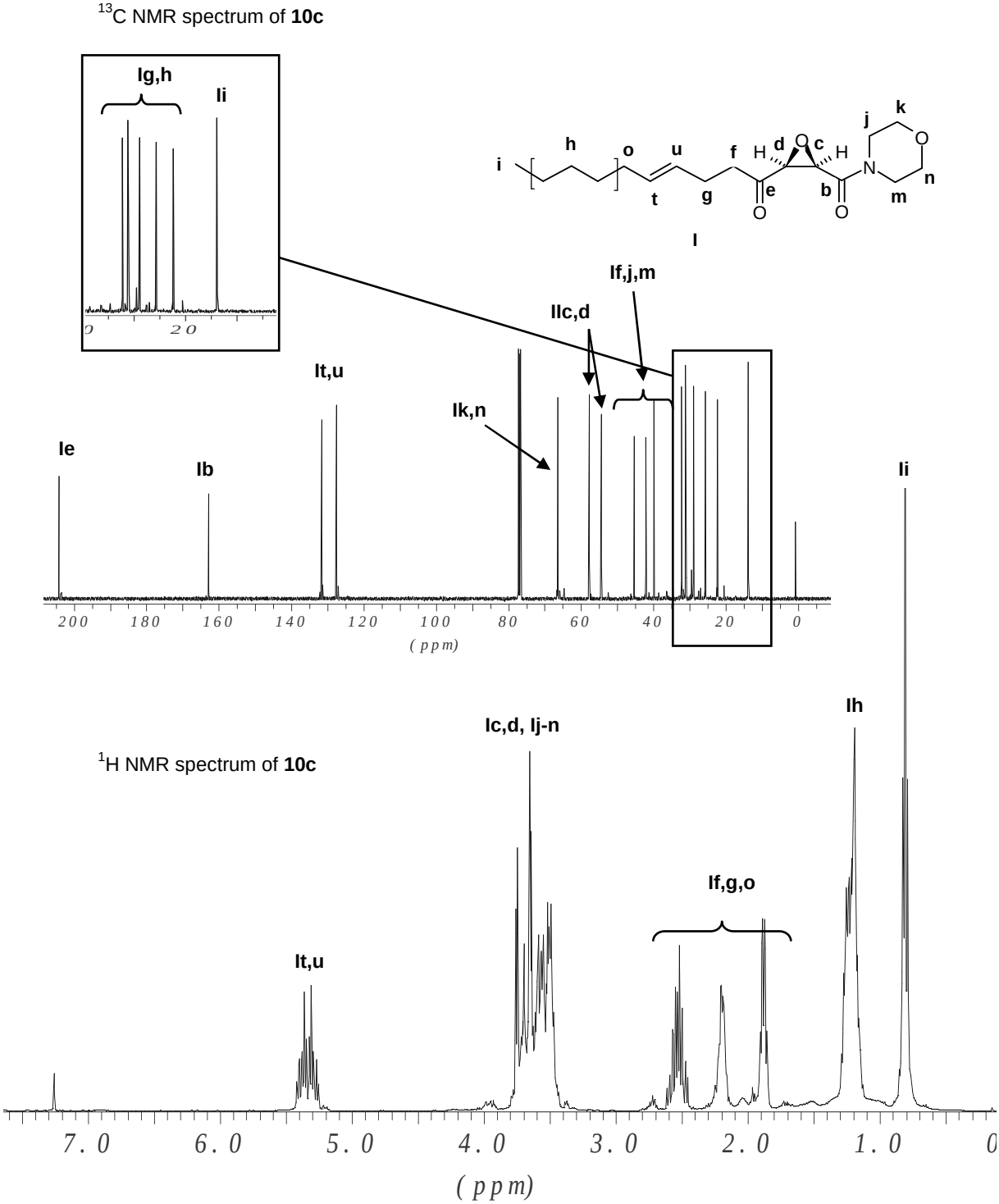


*Fig. 3.36 NMR spectra of dihydrocerulenin **10c***

3.6.2.3 (7Z,10E)-cerulenin analogues

The synthesized cerulenin analogues **15a** and **15b** contain only nitrogen atoms with three substituents and therefore only the open form **I** of both compounds **15a** (7*Z*,10*E*)-*N,N*-diethylcerulenin) and **15b** (7*Z*,10*E*)-*N,N*-morpholinocerulenin) is observed in the NMR spectra (exemplified on **15a** in Fig. 3.37).

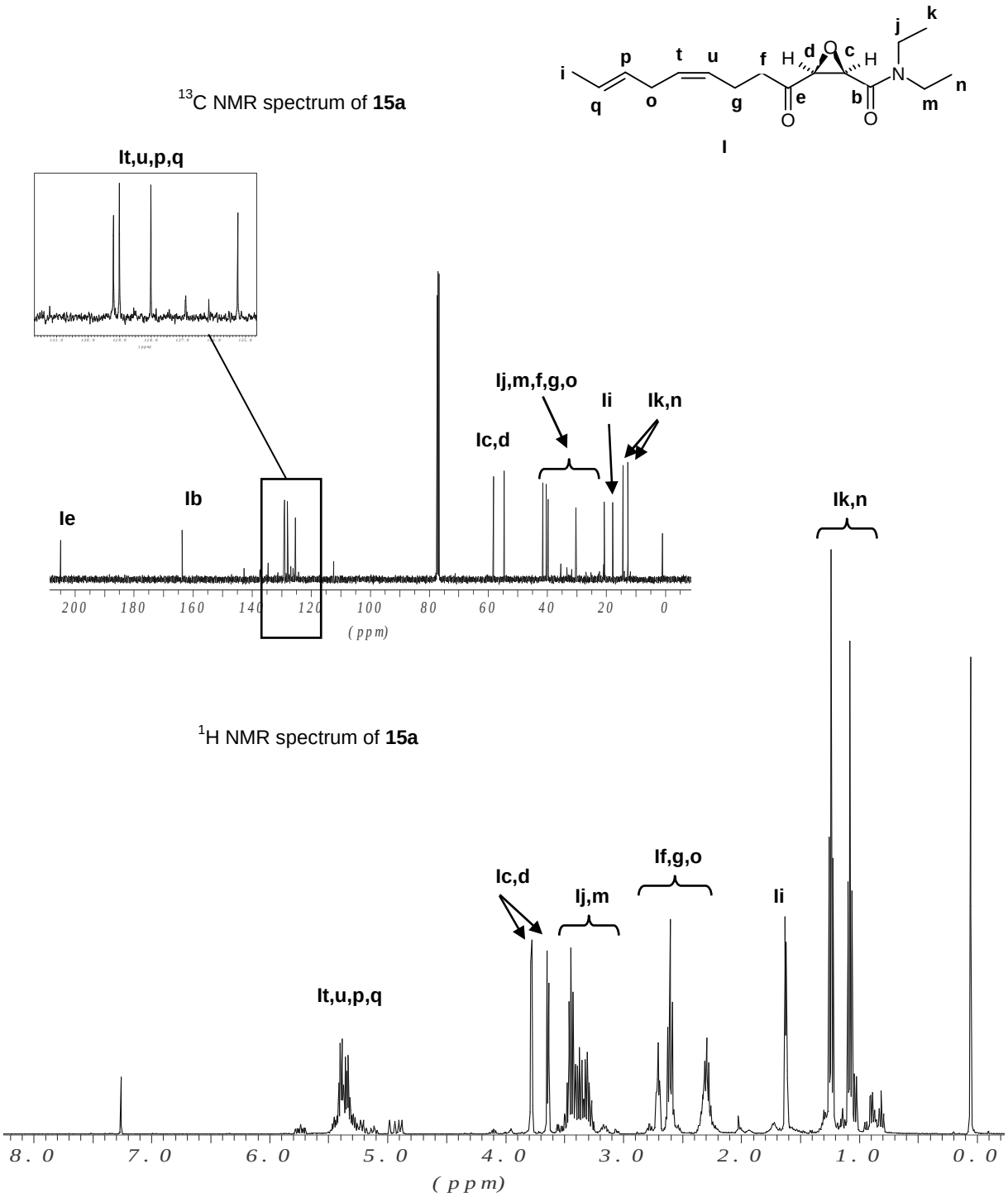


*Fig. 3.37 NMR spectra of cerulenin analogue **15a***

3.6.2.4 Aromatic cerulenin analogues

In case of the aromatic cerulenin analogues **17a-e** none of the synthesized compounds shows any indication of hydroxylactam formation independent from possessing a primary or secondary nitrogen atom. All of these compounds seem to exist in the open ketone form **I** when dissolved in the aprotic solvent $CDCl_3$. An explanation for this property can be found in the differences of the structure of these aromatic analogues in comparison to the alkyl cerulenins. First, the epoxide function shows a *trans* orientated configuration. This is confirmed by the ^{1}H NMR coupling constants of the epoxy protons **c,d** (see 3.6.1.2) and was also observed by Lawrence et al.[187] Therefore, the substituents (amide function and benzyl ring) are in *trans*-position too and cannot cyclize as easy to the five membered hydroxylactam ring as the other cerulenin analogues (see Fig. 3.27). Furthermore, the phenyl ring instead of the acyl chain represents a bulky residue and possibly detains the nitrogen from attacking the keto function. This probably results in the open form **I** for the aromatic cerulenin analogues as exemplified on **17b** in Fig. 3.38.

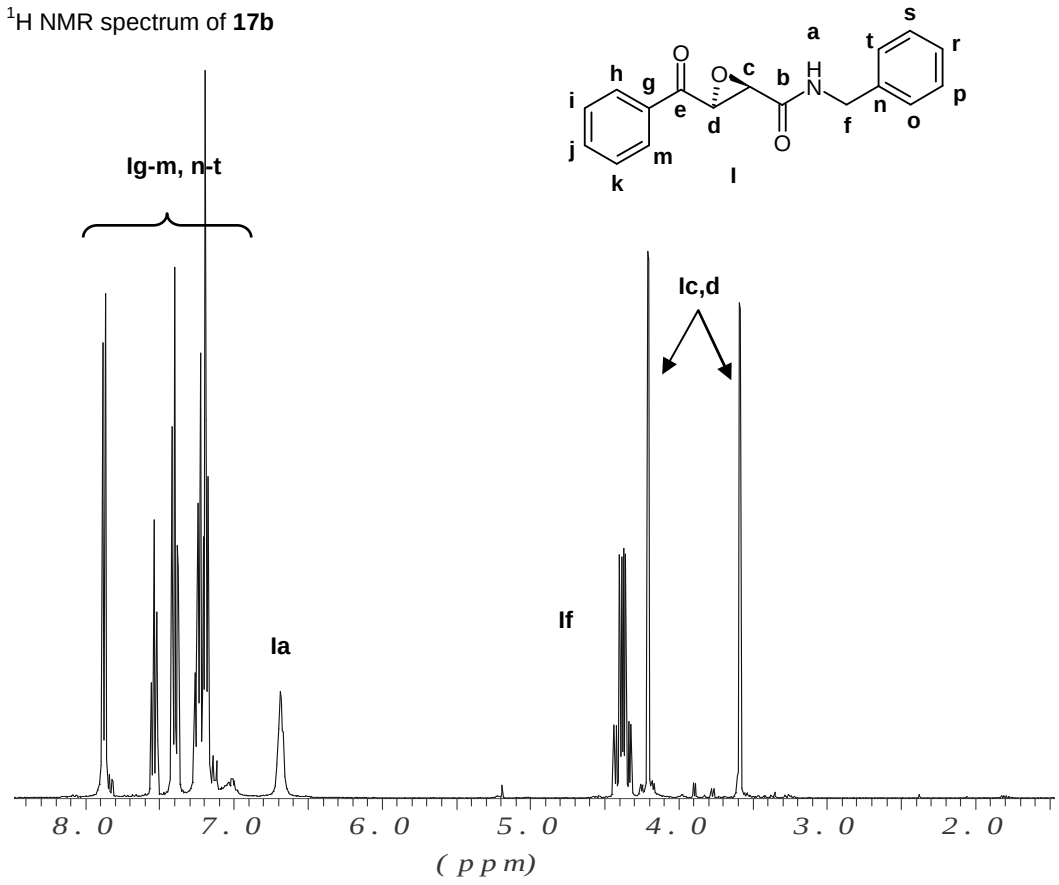


*Fig. 3.38 ^{1}H NMR spectrum of aromatic cerulenin analogue **17b***

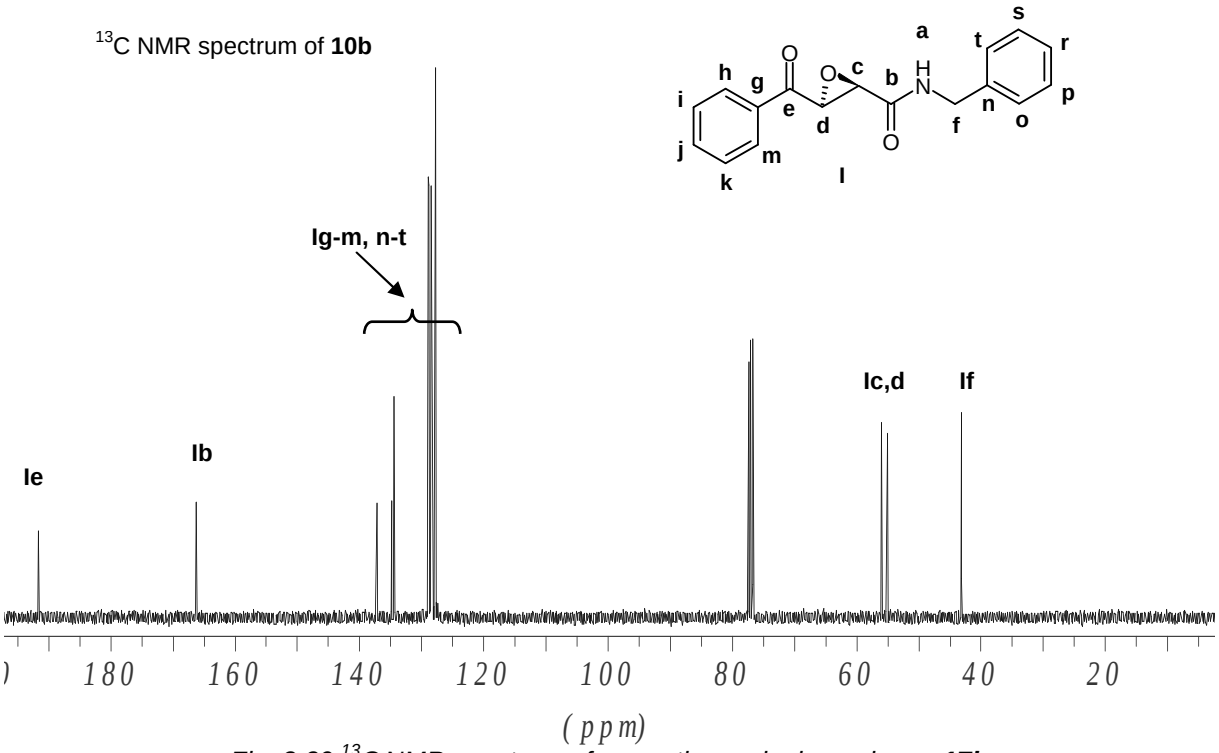


*Fig. 3.39 ^{13}C NMR spectrum of aromatic cerulenin analogue **17b***

3.6.2.5 Comparison of cerulenin analogues

Comparing the different cerulenin analogues in terms of the equilibrium of the open form and hydroxylactam formation (see Fig. 3.28) it can be observed that the hydroxylactam formation of the cerulenin-like substances does not only depend on the solvent as Jakubowski et al.[206] found out, but also on the substituent bound to the nitrogen atom as can be seen especially in the case of the tetrahydrocerulenins (see 3.6.1):

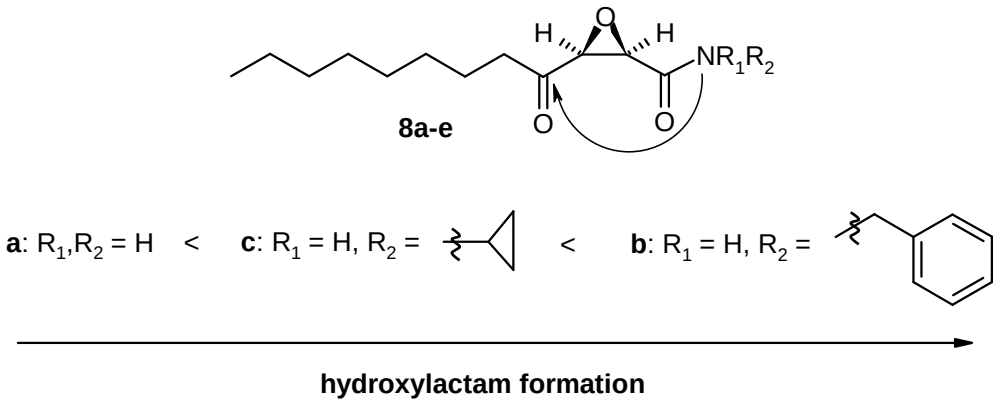
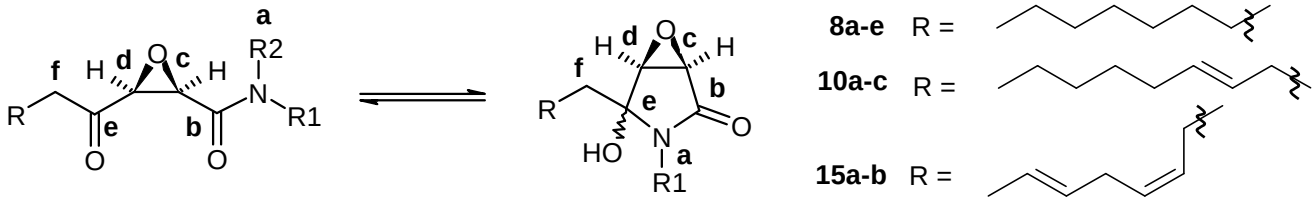


Fig. 3.40 Effect of the substituents on the nitrogen for tetrahydrocerulenins

The cerulenin analogues showing an open form **I** are only present as a racemic but not diastereomeric mixture due to the epoxidation process of the corresponding epoxylactone (see 3.6.1.1). Hydroxylactams **II** of the cerulenin analogues **8a-e**, **10a-c** and **15a-b** (when formed) show diastereomers although derivatives with a benzylamide function show only one (**8b**) or mostly one (**10b**) diastereomeric isomer. The most important signals of the synthesized alkyl cerulenins are summarized in Table 3.1, those of the aromatic analogues in Table 3.2. In addition, the ratio of the open isomer **I** and hydroxylactam forms **II** is reported. When hydroxylactam forms **II** are observed, also the ratio between the diastereomers of **II** is given.



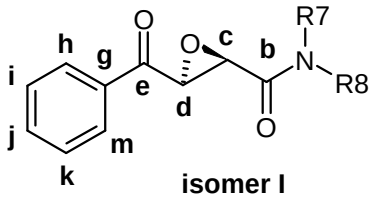
compound	open form I (%)	hydroxy-lactams II (%), (ratio/%)	δ epoxy protons **c,d** (ppm) (^{1}H NMR) isomer I	δ epoxy protons II **c,d** (ppm) (^{1}H NMR) isomers II	δ ketone signal **e** (ppm) (^{13}C NMR) isomer I	δ quarternary C atom **e** (ppm) (^{13}C NMR) isomers II
8a R1, R2=H	89	11	3.71, 3.85	3.63, 3.78 (OL)	202.76	85.61 86.26
8b R1=benzyl, R2 =H	0	100 (100:0)	-	3.55, 3.75	-	89.89
8c R1=cyclo-propyl, R2 =H	58	42 (57:43)	3.68, 3.81	3.58, 3.72 3.63, 3.77	202.60	89.55 90.58
8d R1, R2=Et	100	0	3.64, 3.77	-	205.56	-
8e R1, R2= morpholin	100	0	3.54-3.80	-	204.98	-
10a R1, R2=Et	100	0	3.63, 3.77	-	204.90	-
10b R1=benzyl, R2 =H	0	100 (90:10)	-	3.67, 3.75 3.80, 4.05	-	88.95 83.76
10c R1, R2= morpholin	100	0	3.46-3.76	-	204.22	-

15a R1, R2=Et	100	0	3.59, 3.73	-	204.87	-
15b R1, R2= morpholin	100	0	3.50-3.79	-	204.24	-

*Table 3.1 Most important NMR signals of alkyl cerulenins and ratios between open isomer **I** and hydroxylactam forms **II** and between the hydoxylactam forms **II**. The ratios were determined from the epoxy signals **c** and **d** of the ^{1}H NMR spectrum. OL = overlapping*

In case of the aromatic cerulenin analogues only the open form **I** can be observed.

compound	epoxy protons c,d (ppm) (^{1}H NMR) isomer I	ketone signal e (ppm) (^{13}C NMR) isomer I
17a R1, R2=Et	3.83, 4.58	192.92
17b R1=benzyl, R2 =H	3.59, 4.21	191.54
17c R1=cyclo-propyl, R2 =H	3.62, 4.29	191.78
17d R1=n-propyl, R2 =H	3.71, 4.46	191.99
17e R1, R2= morpholin	3.85, 4.55	192.48



isomer I

Table 3.2 Most important NMR signals of aromatic cerulenins

The observations made can be summarized as

- if the nitrogen atom is substituted with three substituents as is the case for **8d**, **8e**, **10c**, **15a** and **15b** hydroxylactam formation cannot take place and therefore only the open form **I** is present
- nitrogen substitution with bulky substituents as in *N*-benzyl-tetrahydrocerulenin **8b** and the dihydrocerulenin analogue **10b** show only hydroxylactam forms **II** and only (**8b**) or mostly (**10b**) one of the hydroxylactam diastereomers **II** due

to steric interference with the likewise bulky octyl group resulting in an attack only from one side of the nitrogen to the keto group

- nitrogen substitution with small alkyl substituents as for compound **8c** *N*-cyclopropyl tetrahydrocerulenin results in a mixture of the open form **I** and hydroxylactam forms **II**
- an unsubstituted amide function as in tetrahydrocerulenin **8a** shows mainly the open form **I** (~90 %).
- the aromatic cerulenin analogues show no hydroxylactam formation at all due to steric effects

4. Pharmacological results

The aim of this work was to synthesize compounds that target efflux pump resistant *Candida albicans* isolates and the elongation enzyme of the fatty acid synthesis pathway of *Mycobacterium tuberculosis*, KasA. Furthermore, the synthesized substances were tested on different other organisms within the Sonderforschungsbereich 630.

Testings on *Candida albicans* were executed by the research group of Prof. Morschhäuser, Department of Molecular Infection Biology, University of Würzburg. Testings on the enzyme KasA were carried out in the laboratory of Prof. Tonge, Institute for Chemical Biology & Drug Discovery, State University of New York at Stony Brook, New York, U.S.A. Testings on other organisms such as *Leishmania major*, *Trypanosoma brucei brucei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* were done in the Z1 project within the Sonderforschungsbereich 630.

4.1 Biological testings on Candida albicans

The testings were executed starting with the determination of the minimal inhibitory concentration (from now on referred to as MIC) of the synthesized cerulenin analogues. In the next step, a combination of the synthesized compounds and known inhibitors of *Candida albicans* (cerulenin, brefeldin A; also substrates of *Candida albicans* efflux pumps) were added to efflux pump-resistant and -susceptible isolates of *Candida albicans*. The known inhibitors were either appended at fixed subinhibitoric concentrations (see 4.1.2.1) or at various concentrations with DMSO as a control (see 4.1.2.2). In the first case inhibition could only occur if there was a synergistic effect between the known inhibitors and the newly synthesized cerulenin analogues. In the second case the extent of decrease in resistance was determined.

4.1.1 Inhibition studies of cerulenin analogues

To determine the MICs of the synthesized cerulenin analogues, different *Candida albicans* isolates were used. SC5314 was employed as a non-resistant control (see

Table 4.1), whereas isolate pairs Gr10/13 and Gu4/5 were isolated in each case from the same patient. Compared to Gr10 and Gu4 the isolates Gr13 and Gu5 showed an increased resistance towards fluconazole. Gr13 overexpressed the major facilitator efflux pump *MDR1p* whereas Gu5 overexpressed the ABC-transporters *CDR1p* and *CDR2p*.

Stock solutions of all synthesized cerulenin analogues were prepared in DMSO (5 mg/ml) and diluted in HR (high resolution) medium to a concentration of 200 µg/ml. Further dilutions of the latter solution were made (100 µg/ml, 50 µg/ml, 25 µg/ml etc.) and 100 µl of these were diluted with 100 µl cell suspension respectively. The highest concentration being tested therefore was 100 µg/ml. The suspensions were incubated at 37 ℃ for 24 hours.

compound	SC5314 control	Gr10 sensitive (MDR1)	Gr13 MDR1 resistance	Gu4 sensitive (CDR1/2)	Gu5 CDR 1/2 resistance
cerulenin	0.78	0.39	6.25	0.78	3.12
4a	>100	>100	>100	>100	>100
4b	>100	>100	>100	>100	>100
4c	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100
4e	>100	>100	>100	>100	>100
7a	>100	>100	>100	>100	>100
7b	>100	>100	>100	>100	>100
7c	>100	>100	>100	>100	>100
7e	>100	>100	>100	>100	>100
8a	12.5	6.25	25	12.5	50
8e	>100	>100	>100	>100	>100

Table 4.1 Extract from the tested compounds; MIC values are given in µg/ml.

From Table 4.1 can be observed that all compounds except for cerulenin and tetrahydrocerulenin (**8a**) do not show any inhibitory activity against either susceptible or resistant *Candida albicans* isolates.

These results were required to proceed with further testings, because a possible efflux pump inhibitor should be given to resistant isolates in addition to a known inhibitor. In order to block the efflux pump and to make the resistant *Candida*

albicans isolates again susceptible to an inhibitor the cerulenin derivatives therefore should not inhibit by themselves.

4.1.2 Inhibition studies of the compound combination

The created cerulenin analogues were tested in combination with known growth inhibitors of *Candida albicans,* cerulenin and brefeldin A, which are also substrates of the efflux pumps in *Candida albicans*. The first testings were executed with subinhibitoric inhibitor concentrations of cerulenin or brefeldin A in addition to the synthesized analogues (4.1.2.1). Second, testings with a constant concentration of the synthesized substances and various inhibitor concentrations of brefeldin A were carried out (4.1.2.2). Each compound combination was tested on susceptible and efflux pump resistant *Candida albicans* isolates.

4.1.2.1 Combination of fixed subinhibitoric inhibitor concentrations and cerulenin analogues

The synthesized cerulenin analogues **4a-e**, **7a-c,e**, **8a-c,e** and **17a-e** were tested in various concentrations (100 µg/ml, 50 µg/ml, 25 µg/ml etc.; preparations see 4.1.1) in combination with subinhibitoric cerulenin concentrations (1/2 MIC und 1/4 MIC) on different *Candida albicans* isolates. Isolates Gr13 and Gu5 showed increased resistance towards cerulenin due to the fact of overexpressing efflux pumps *MDR1p* (Gr13) and *CDR1p/CDR2p* (Gu5). The MIC of cerulenin for these resistant *Candida albicans* isolates was 6.25 µg/ml for Gr13 and 3.12 µg/ml for Gu5. The suspensions were incubated at 37 °C for 24 hours. The aim was to show if the synthesized substances could make the *Candida albicans* isolates sensitive to cerulenin again.

	Gr13			**Gu5**		
cerulenin	**without**	**3.12 µg/ml**	**1.56 µg/ml**	**without**	**1.56 µg/ml**	**0.78 µg/ml**
7c	>100	25	50	>100	>100	>100
7e	>100	25	100	>100	>100	>100
8a	25	12.5	25	12.5	25	25
8b	>200	50	50	>200	100	>100
8c	100	12.5	25	100	50	50
8e	>100	25	100	>100	>100	>100
17b	>200	-	12.5	>200	-	25

Table 4.2 Extract from the tested compounds only that showed effects; MIC values are given in µg/ml.

Table 4.2 shows that some of the synthesized compounds had an effect on the MIC values of cerulenin and could make the resistant *Candida albicans* isolates sensitive again to concentrations of cerulenin that were subinhibitoric before.

The synthesized cerulenin analogues **7c**, **8b**, **8c** and **17b** showing effects in the tests of table 4.2 even at lower concentrations (1.56 µg/ml for the *MDR1* overexpressing isolate Gr13 or 0.78 µg/ml for *CDR1/CDR2* overexpressing isolate Gu5) were used for further testings. Compounds **7c**, **8b**, **8c** and **17b** (**8a** was not taken due to its inhibitory properties in Table 4.1) were tested in various concentrations (100 µg/ml, 50 µg/ml, 25 µg/ml etc.; preparations see 4.1.1) in combination with subinhibitoric brefeldin A concentrations (1/16 - 1/8 MIC, see Fig. 4.3a) on different *Candida albicans* isolates (Table 4.3b). A synergistic effect between brefeldin A and the cerulenin analogues based on a blocking of the efflux pump by the synthesized analogues should only be observed for *MDR1* overexpressing isolates (F13, Gr13), but not for isolates that do not overexpress *MDR1* (F7, Gr10 from the same patients as F13 and Gr13) or do not possess the *MDR1* gene (mdr1 mutants F13M432 and Gr13M432, fabricated from F13 and Gr13). Therefore, effects should only be visible for isolates F13/Gr13 if the observed synergistic effect (table 4.3b) was due to a blocking of the efflux pump *MDR1p*.

isolates	**MIC brefeldin A (µg/ml)**	**used concentrations (µg/ml) of brefeldin A**
F7	6.25	0.8
F13	50	6.25
F13M432	6.25	0.8
Gr10	(3.1)	0.8
Gr13	(50)	6.25
Gr13M432	12.5	0.8
Gu4	6.25	0.8
Gu5	50	6.25

Table 4.3a Applied brefeldin A concentrations for the used Candida albicans isolates

	F7	F13	F13M432	Gr10		Gr13		Gr13M432
				without b.A	with b.A	without b.A	with b.A	
7c	12.5	>100	25	>100	50	>100	>100	>100
8b	50	100	50	>200	50	>200	100	100
8c	25	50	25	100	50	100	50	100
17b	100	>100	12.5	>200	100	>200	>100	100

Table 4.3b MIC (µg/ml) of the tested compounds on different isolates that did not overexpress (F7, Gr10), that overexpressed (F13, Gr13) and that did not possess (F13M432, Gr13M432) the MDR1 gene; b.A = brefeldin A

Table 4.3b shows that there are slight synergistic effects between some cerulenin derivatives and brefeldin A on *Candida albicans* isolates with *MDR1* overexpression (Gr13, F13). However, effects could also be observed for the isolates that either did not overexpress (Gr10, F7) or did not possess (Gr13M432, F13M432) the *MDR1* gene. Therefore, these synergistic effects examined are probably not due to a blocking of the *MDR1p* efflux pump but due to other synergistic effects.

4.1.2.2 Combination of various inhibitor concentrations and cerulenin analogues

The synthesized cerulenin analogues **8d**, **10a-c**, **14**, **15a-b** and the most potent compounds of 4.1.2.1 **7c**, **8b** and **17b** were tested with constant compound concentrations (50 µg/ml, 25 µg/ml; preparation see 4.1.1) in combination with various brefeldin A concentrations. Brefeldin displays a different mode of action than cerulenin and was used to exclude any synergistic inhibition effects between cerulenin and the synthesized analogues due to their similarity in structure. These testings were executed on different *Candida albicans* isolates to determine the MIC. SC5314 was employed as a non-resistant control (see Table 4.4), whereas isolate pairs Gr10/13, Gu4/5 and F7/F13 were isolated in each case from the same patient (for details see 4.1.1). DMSO was used as a compound control. The suspensions were incubated at 37 ℃ for 24 hours.

compound	SC5314 control	Gr10 sensitive (MDR1)	Gr13 MDR1 resistance	F7 sensitive (MDR1)	F13 MDR1 resistance	Gu4 sensitive (CDR1/2)	Gu5 CDR 1/2 resistance
DMSO	6,25	6,25	100	6,25	50	6,25	50
7c	6,25	3,125	12,5	6,25	12,5	-	-
8b	12,5	6,25	25	6,25	12,5	-	-
8d	12,5	3,125	12,5	6,25	25	6,25	50
10a	12,5	3,125	12,5	6,25	25	6,25	50
10b	12,5	6,25	50	-	-	6,25	100
10c	25	12,5	50	-	-	12,5	50
14	12,5	6,25	25	6,25	25	12,5	100
15b	6,25	6,25	50	6,25	25	-	-
17b	50	12,5	25	25	25	-	-

Table 4.4 MIC values (µg/ml) of brefeldin A with a constant compound concentration of the synthesised cerulenin analogues of 25 µg/ml; only compounds that showed effects are illustrated

As can be seen from Table 4.4, the synthesized cerulenin analogues showed higher MICs than the control DMSO for many of the sensitive isolates, which is demonstrated best on isolate SC5314: the control showed an MIC of 6,25 µg/ml and except for compound **7c** and **15b** all other compounds display MICs between 12,5 up to 50 µg/ml at a constant compound concentration. Most compounds therefore seemed to induce a resistance to the sensitive isolates against brefeldin A. However, some of the synthesized cerulenin analogues seemed to decrease the resistance of the MDR1 overexpressing isolates Gr13 and F13 compared to the control (DMSO) at a constant compound concentration of 25 µg/ml. The highest effect could be observed on the MDR1 overexpressing isolate Gr13 for substances **7c**, **8d** and **10a**. Examining isolate Gr13, the MDR1 mediated resistance decreased for these compounds 8fold (MIC of brefeldin A: 12,5 µg/ml with **7c**, **8d** and **10a**) while the control DMSO/brefeldin A displays the MIC value of 100 µg/ml. These substances were used for further investigations with lower constant compound concentrations (see Table 4.5).

Therefore, substances **7c**, **8d** and **10a** were tested at concentrations of 12.5 µg/ml and 6.25 µg/ml on their ability to still reduce the resistance of *MDR1* overexpressing *Candida albicans* isolate Gr13 as in Table 4.4 but with lower constant compound concentrations. The suspensions were again incubated at 37 ℃ for 24 hours.

	SC5314	Gr10	Gr13	F7	F13
DMSO	3,125	3,125	50	6,25	50
7c (12,5 µg/ml)	6,25	6,25	25	12,5	25
7c (6,25 µg/ml)	6,25	6,25	25	12,5	25
8d (12,5 µg/ml)	6,25	3,125	25	12,5	25
8d (6,25 µg/ml)	6,25	6,25	25	12,5	25
10a (12,5 µg/ml)	6,25	6,25	25	12,5	25
10a (6,25 µg/ml)	6,25	6,25	50	12,5	50

Table 4.5 MIC values (µg/ml) of brefeldin A with a constant compound concentration of the synthesised cerulenin analogues of 12.5 and 6.25 µg/ml of the most effective substances in preliminary tests (Table 4.4)

Again, the synthesized cerulenin analogues seemed to induce resistances towards the sensitive isolates SC5314, Gr10 and F7.

However, from Table 4.5 can also be figured out that at these lower constant compound concentrations of 12,5 or 6,25 µg/ml, substances **7c**, **8d** and **10a** can still slightly reduce the resistance of *MDR1* overexpressing isolates Gr13 and F13. Examining e.g. isolate Gr13, the MDR1 mediated resistance (control DMSO/brefeldin A MIC: 50 µg/ml) decreased 2fold for **7c**, **8d** at constant compound concentrations of 12,5 or 6,25 µg/ml. For substance **10a** the 2fold decrease of the MIC (compared to the control) can only be observed at a concentration of 12,5 µg/ml, but vanishes at 6,25 µg/ml.

4.1.3 Discussion

Because overexpression of efflux pumps is one of the most important reasons why *Candida albicans* becomes resistant to antimycotics (see introduction), potential inhibitors of these efflux pumps were aimed to examine. Cerulenin was chosen as the target structure due to the fact that it is a substrate of the known multidrug resistant efflux pumps (*MDR1p*, *CDR1/2p*) of *Candida albicans*.

The cerulenin analogues synthesized here were tested at first on the ability to inhibit the growth of *Candida albicans* (see 4.1.1) but did not show any inhibitory activity (except for **8a**). This was a requirement for proceeding with further testings, because the synthesized substances should not inhibit the growth but only the efflux pumps of *Candida albicans*. To find out if this was the case, *Candida albicans* isolates that overexpressed *MDR1* or *CDR1/2* were incubated with concentrations of cerulenin or brefeldin A, which were subinhibitory for these isolates, in combination with different concentrations of the synthesized analogues (see 4.1.2.1). The minimal inhibitory concentration was determined and it was shown that some of the combinations inhibited the growth of the *Candida albicans* isolates although the inhibitor (cerulenin or brefeldin A) was present in subinhibitoric concentrations (Table 4.3b). The highest effects in this case were observed for substances **7c**, **8b** and **17b**. However, this effect was also observed for *Candida albicans* isolates that did not overexpress or did not possess the *MDR1* efflux pump. Therefore, the synthesized cerulenin derivatives showed synergistic effects with the inhibitors on resistant *Candida albicans* isolates that were probably not due to a blocking of the efflux pumps.

Next, experiments were executed (see 4.1.2.2) of further synthesized cerulenin analogues and the most potent compounds of 4.1.2.1 with constant compound concentrations (50 µg/ml, 25 µg/ml) in combination with various brefeldin A concentrations. Brefeldin A is also a substrate of the efflux pumps Mdr1p or Cdr1p/Cdr2p, respectively, but displays a different mode of action than cerulenin. With a compound concentration of 25 µg/ml of the synthesized analogues and various brefeldin A concentrations, the synthesized cerulenin analogues **7c**, **8d** and **10a** showed a good decrease of the MIC (Table 4.4) in the resistant isolates up to 8fold compared to the control. However, some of the cerulenin analogues showed the tendency to induce resistance to sensitive isolates and at lower concentrations of the synthesized compounds the decrease of resistance shrinked (Table 4.5).

Although a blocking of the efflux pump in corresponding resistant *Candida albicans* isolates probably could not be achieved, a synergistic effect between known inhibitors and the synthesized cerulenin derivatives to decrease the resistance in resistant *Candida albicans* isolates is obviously present. The strongest effects were achieved by substances with either small residues at the nitrogen atom (**7c**, **8d**, **10a**; Fig. 4.1a) or interestingly compounds substituted with phenyl ring(s) (**8b, 17b**; Fig. 4.1b).

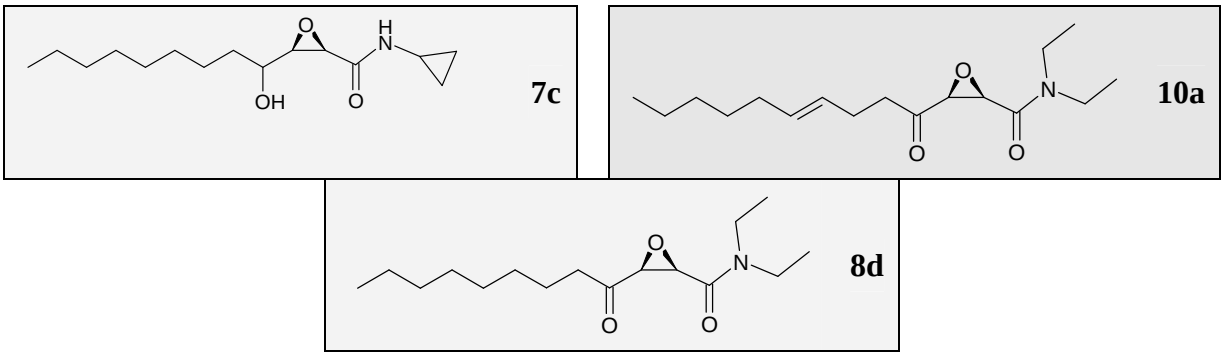


Fig. 4.1a Most effective cerulenin analogues with small residues at the nitrogen atom

Fig. 4.1b Most effective cerulenin analogues substituted with phenyl ring(s)

A lead compound to decrease efflux pump mediated resistance should therefore either possess a long (probably flexible) alkyl or aryl side chain and a small alkyl or aryl substituent at the amide function:

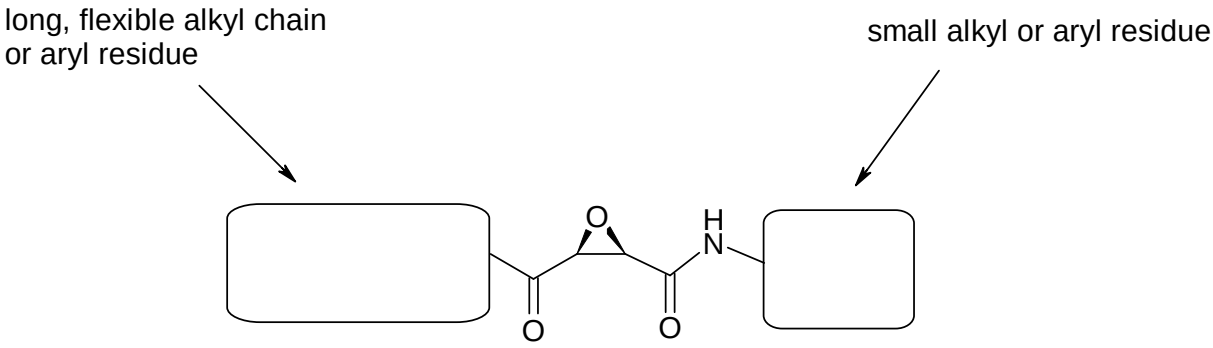


Fig. 4.2 Lead structure explored from the test results

4.2 Biological testings on KasA

Cerulenin is known to inhibit irreversibly the elongation enzymes of the fatty acid synthesis of *E. coli* FabB and FabF (see introduction). The corresponding enzyme in mycobacteria is KasA and therefore the synthesized substances should be tested on this enzyme, which was obtained from the group of Prof. Tonge, Insitute for Chemical Biology & Drug Discovery, State University of New York at Stony Brook, New York, U.S.A. KasA was used within 5 days after isolation.

Due to the fact that enzyme assays for KasA[69] are complex, another method was developed to screen the synthesized cerulenin analogues for possible inhibitors and to obtain the first test results. An inhibitor induced change of the auto fluorescence of KasA was developed based on the methods reported by Houtzager et al.[217] and Tsai and Johnson[218] in order to determine K_i values of the inhibitors to KasA.

4.2.1 Inhibitor induced fluorescence change of KasA

The fluorescence of KasA was measured in a time dependent manner; addition of an inhibitor resulted in a saturation-type fluorescence decrease (see Fig. 4.4a/b). A two step binding model is applied for the binding process: the initial formation of an enzyme-inhibitor complex (EI) is followed by the slower formation of a tightly bound enzyme-inhibitor complex with a conformational change (EI*):[217]

$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} EI \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EI^*$$

Fig. 4.3a Equation of the two step binding model

In the case of irreversibly bound inhibitors such as the cerulenin analogues synthesized in this work (due to the epoxide function of the molecule and the cysteine residue of the elongation enzyme, see introduction) the equation changes to:[217]

$$E + I \overset{K_i}{\rightleftharpoons} EI \xrightarrow{k_{on}} EI^*$$

Fig. 4.3b Equation of the two step binding model for irreversible inhibitors (E=enzyme kasA, I=inhibitor, K_i=dissociation constant, k_{on}=forward rate)

To find out the K_i and k_{on} values, the following procedure was applied:

At first, the fluorescence was measured time/concentration dependently. The fluorescence was dependent on the inhibitor concentration and decreased with an increasing amount of inhibitor. A typical fluorescence curve is represented in Figure 4.4a and 4.4b:

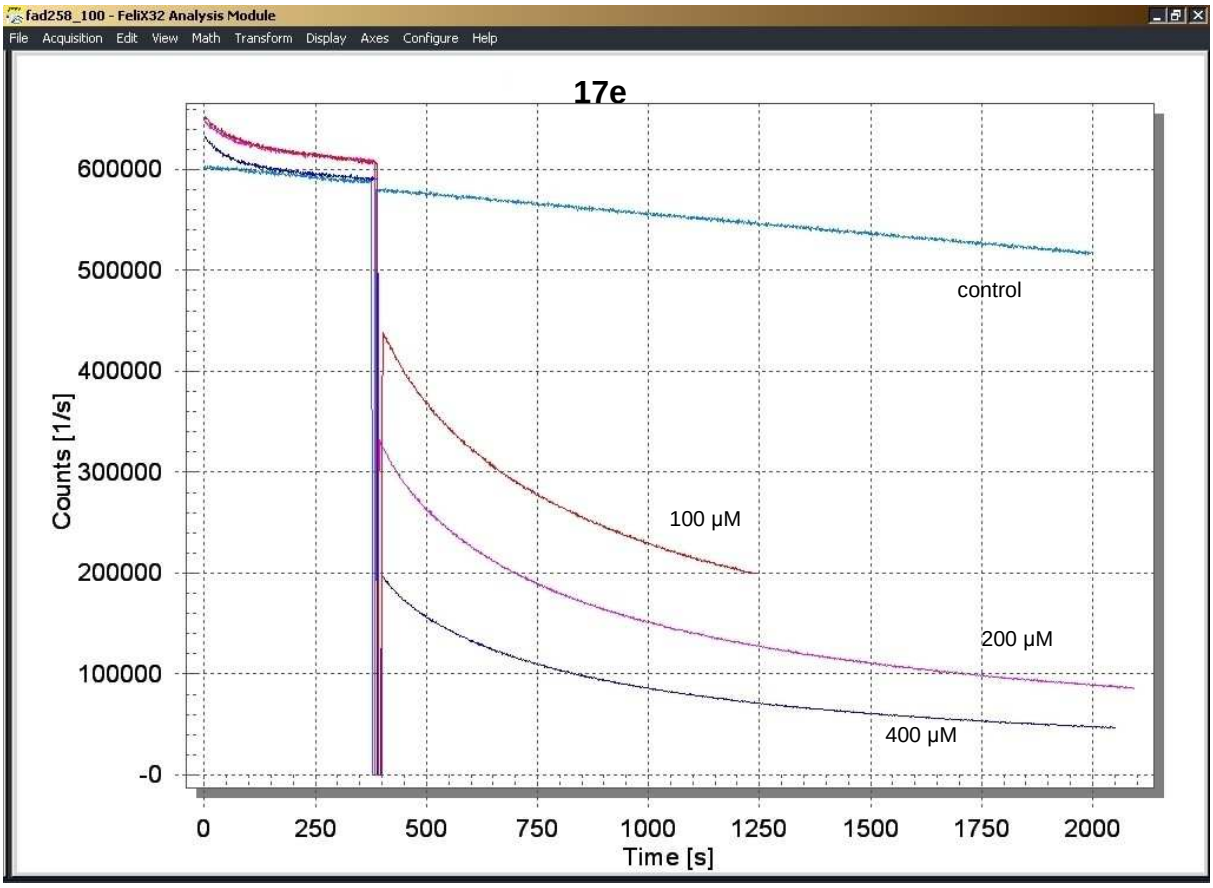


*Fig. 4.4a Fluorescence curve of the time and concentration dependent effects of **17e** on KasA; KasA was treated with the following concentrations: control (DMSO), 400µM, 200µM, 100µM (shown), 75µM, 50µM, 25µM, 5µM (not shown)*

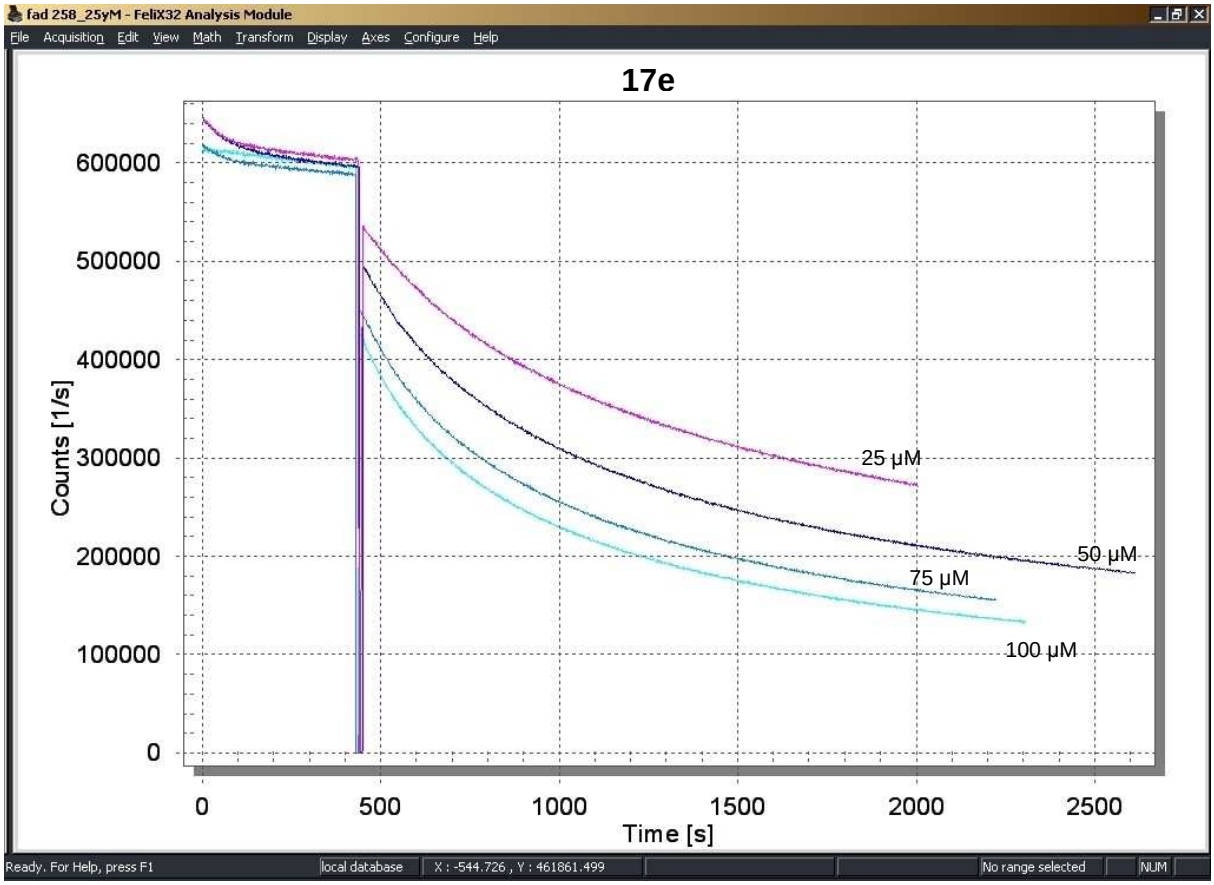


*Fig. 4.4b Flurescence curve of the time and concentration dependent effects of **17e** on KasA; KasA was treated with the following concentrations: control (DMSO), 400µM, 200µM, 100µM (not shown), 75µM, 50µM, 25µM (shown), 5µM (not shown)*

Each of the concentration curves was then fitted to the double exponential function $y=A*e^{-kobs*t}+B*e^{-k2*t}$ by nonlinear regression (exemplified for the concentration 100 µM on **17e** in Fig. 4.5):[218]

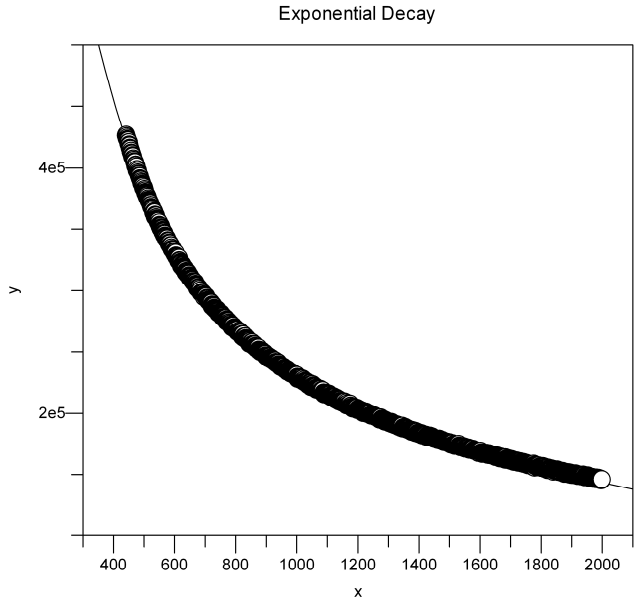
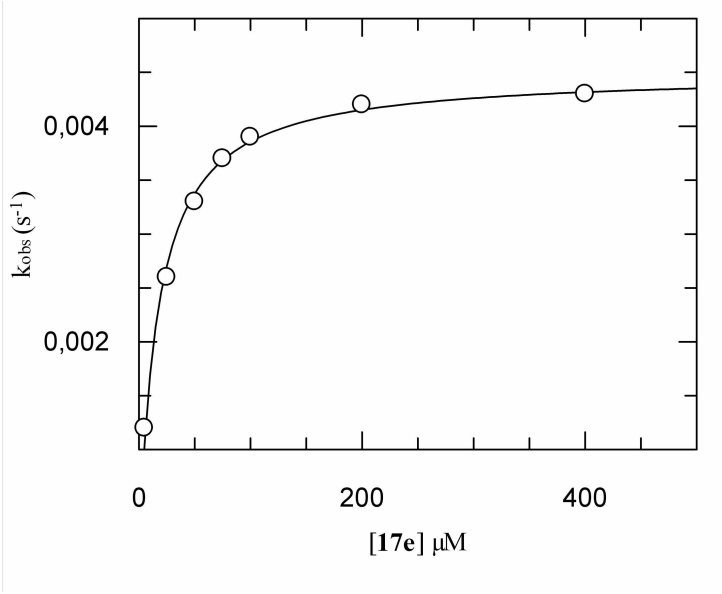


*Fig. 4.5 Fitting of the concentration 100µM of **17e** on KasA by non-linear regression (smooth line); x=time, y=fluorescence; the trace was fitted to a double-exponential function of the form $y=A*e^{-kobs*t}+B*e^{-k2*t}$; the fast (major) phase defined the rate of the reaction (k_{obs}), while the slow phase corrected for the drop of intensity of the enzyme (k_2, control line Fig.4.4a)*

The so obtained rate constants (k_{obs}) of the reaction were then fitted against the corresponding compound concentration to the equation $k_{obs} = k_{on} * [I] / (K_i + [I])$ to find out the dissociation constant K_i for the compound (exemplified on **17e**) and KasA (see Fig. 4.3b):[217]



17e (µM)	**k_{obs}** (s^{-1})
400	0,0043
200	0,0042
100	0,0039
75	0,0037
50	0,0033
25	0,0026
5	0,0012

Parameter	**Value**	**Std. Error**
forward	0,0045	$7{,}60 \cdot 10^{-5}$
dissociation constant	16,56	1,40

*Fig. 4.6 Fitting of the observed rate constants (k_{obs}) of **17e** on KasA to the different concentrations to the equation $k_{obs} = k_{on} * [I] / (K_i + [I])$ with k_{obs} = rate constant, [I] = compound concentration, k_{on} = forward rate, K_i = dissociation constant; K_i was obtained as 16.56 µM ± 1.40 µM*

As can be seen from Figure 4.6, the dissociation constant for substance **17e** was obtained as K_i = 16.56 µM ± 1.40 µM.

This procedure was also accomplished for compound **17c**. The K_i value acquired for this compound was K_i = 50.29 µM ± 10.02 µM.

4.2.2 Discussion

The results obtained from the inhibitor induced fluorescence change testings on KasA look encouraging. Interestingly, the K_i value for substance **17e** looks much better than for compound **17c**. The closed morpholine ring seems to have much better inhibition properties than the cyclopropyl residue with only a secondary amine (see Fig. 4.7).

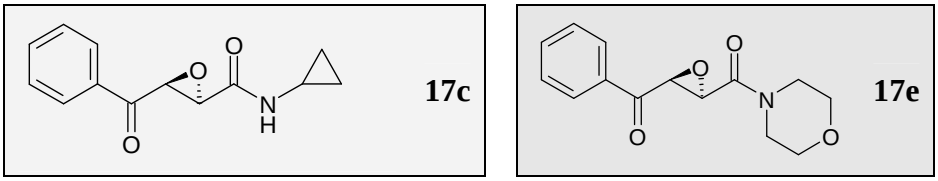


*Fig. 4.7 Structures of aromatic cerulenin analogues **17c** and **17e***

However, these results have to be treated carefully, because they are preliminary. The determined data has to be confirmed additionally; experiments with mass spectrometry similar to those of O´Farrell et al.[219] are currently investigated in the group of Prof. Tonge to confirm the data of the above described experiments. Furthermore, the data of compounds that show good results in the preliminary tests then should be confirmed via enzyme activity assays which are very complex.[69]

The remaining aromatic cerulenin analogues **17a**, **b** and **d** still have to be tested by the developed fluorescence assay (see 4.2.1). Cerulenin and some of the synthesized alkyl cerulenins analogues were also screened by this method but probably due to a missing chromophor showed no alteration of the KasA fluorescence signal. Corresponding derivatives with added fluorophores or pharmacological testings by either mass spectrometry or enzyme activity assays could be a possibility to evaluate the pharmacological properties for these cerulenin analogues.

4.3 Further biological testings

The synthesized cerulenin analogues were also tested on different other organisms within the SFB 630.

MIC values were determined for *Staphylococci*, *Trypanosoma* and *Leishmania* and the best results are represented below.

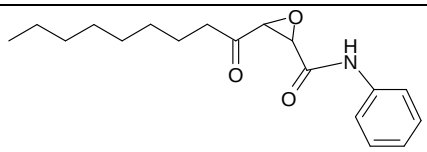
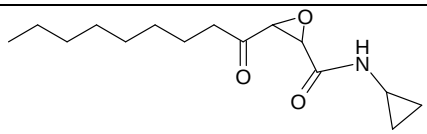
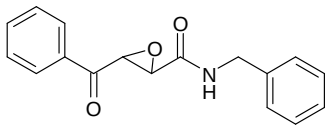
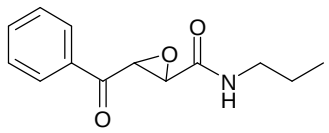
compound		MIC [μM]
8b		62,00
8c		34,00
17b		34,00
17d		31,00

Fig. 4.8 Structures of cerulenin analogues with inhibitory activity against Leishmania (L. major)

The substances listed in Fig. 4.8 show slight to moderate inhibitory activities against Leishmania.

Better testing results were obtained concerning the biofilm inhibition of *Staphylococcus epidermidis* (Fig. 4.9).

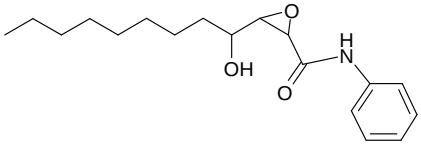
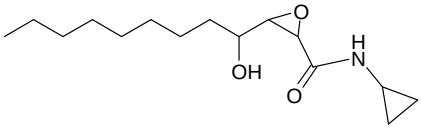
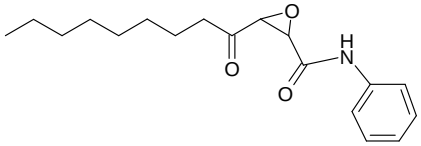
compound		inhibition of biofilm formation
7b		**~ 40 % (160μM)**
7c		**~ 30 % (160μM)**
8b		**~ 60 % (158μM)** **~ 40 % (78μM);** **~ 30 % (39 μM)** **~ 20 % (20 μM)**

Fig. 4.9 Structures of cerulenin analogues with biofilm inhibition of S. epidermidis

The best biofilm inhibition on *S. epidermidis* shows tetrahydrocerulenin analogue **8b**.

Further testings were executed on *Trypanosoma brucei brucei* (Fig. 4.10).

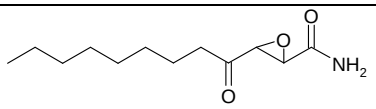
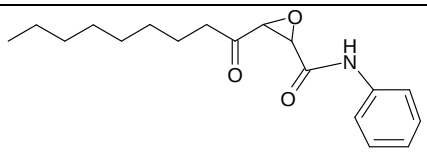
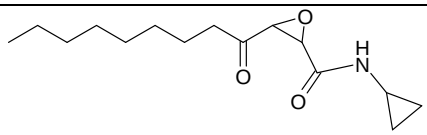
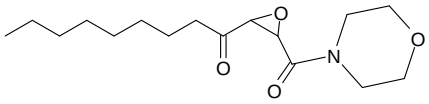
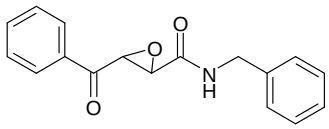
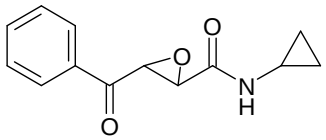
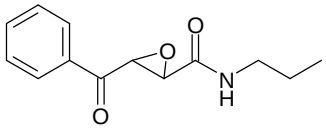
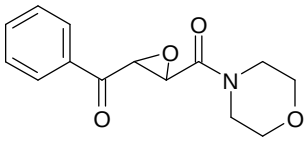
compound		MIC (μM)
8a		21,95
8b		36,37
8c		3,39
8e		32,84
17b		32,37
17c		33,25
17d		32,92
17e		30,47

Fig. 4.10 Structures of cerulenin analogues with inhibitory activity against T.b.brucei

Cerulenin analogue **8c** shows a good result with an inhibitory activity of 3.39 μM. Further synthesized substances still remain in the testing procedure and have to be evaluated.

5. Summary / Zusammenfassung

5.1 Summary

The present work deals with the synthesis and characterization of cerulenin analogues as inhibitors of efflux pump mediated resistance of *Candida albicans* isolates and as inhibitors of the fatty acid synthesis enzyme KasA of *Mycobacterium tuberculosis*.

Candida albicans represents one of the main pathogens causing fungal infections. *Candida* species (with *C. albicans* as the most common) are accountable for about 78 % of nosocomial fungal infections and represent the fourth most common microorganism in nosocomial sepsis cases in the U.S. Especially the latter bloodstream infections are associated with attributable mortality rates up to 49 % in the same institutions between 1997 and 2001. Due to a limited number but excessive use of antimycotics on increasing fungal infections an increasing amount of resistances can be observed not only of intrinsically resistant but also normally sensitive fungal isolates.

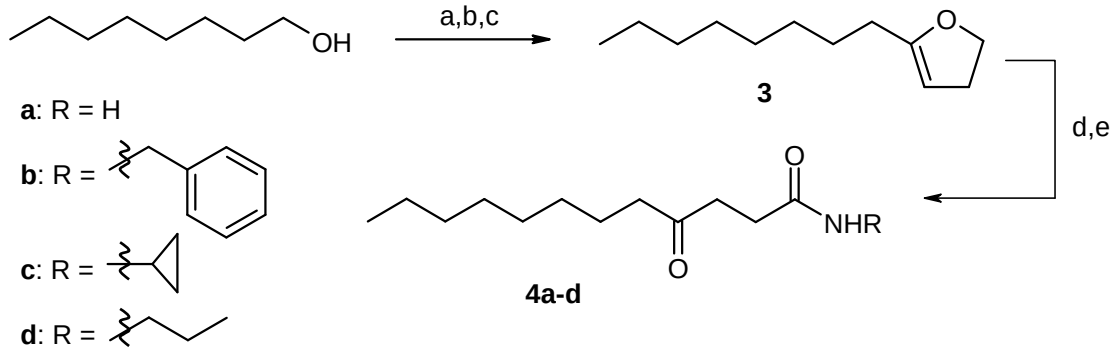
In case of Fluconazole as one of the mostly used drugs against *Candida* infections different mechanisms can be responsible for the development of a resistance. The overexpression of the efflux pumps MDR1p and CDR1p and CDR2p, where fluconazole is actively transported of out of the cell, is one of the most important mechanisms. The inhibition of this resistance mechanism via blocking of the efflux pumps could be a strategy to reduce the number of resistant *Candida* infections.

Mycobacterium tuberculosis is the main pathogen of tuberculosis, which represents one of the major health problems today with one third of the world population infected and 1.6 million people estimated to have died of tuberculosis in 2005 according to the WHO. Due to the fact that there are only few antituberculosis drugs at hand and that the number of multidrug resistant and extensively drug resistant tuberculosis cases is rising, there is an urgent need to develop new drugs against tuberculosis. The fatty acid synthase (FAS) pathway is an especially attractive target for future drugs against tuberculosis because its inhibition leads to the lack of the essential mycolic acids in the mycobacterial cell envelope. Furthermore, the organisation of FAS system components is different in mammals (type I FAS) and in bacteria (type II FAS) and its efficacy has been proven by the known antituberculosis drug isoniazid. Also

extant resistance mechanisms should be relatively ineffective against FAS targeting drugs because it is largely unexploited.

Cerulenin was chosen as the lead structure, being a substrate of the efflux pumps in *Candida albicans* on one hand and therefore variations on the structure could lead to a blocking of the efflux pumps as in the case of tetracycline and inhibitor 13-CPTC of the TetB efflux pump. On the other hand, cerulenin is a known inhibitor of the FAS system but inhibition is unselective in type I and II FAS. Therefore, analogues could result in increased selectivity towards the type II FAS system in *M. tuberculosis*.

The first cerulenin derivatives were prepared by coupling 2,3-dihydrofuran to the before synthesized 1-octaniodide, followed by ring opening and oxidation in one step by chromic acid and transfer of the resulting 4-keto acid to amides to give analogues **4a-d**, **4e** was prepared in analogy.

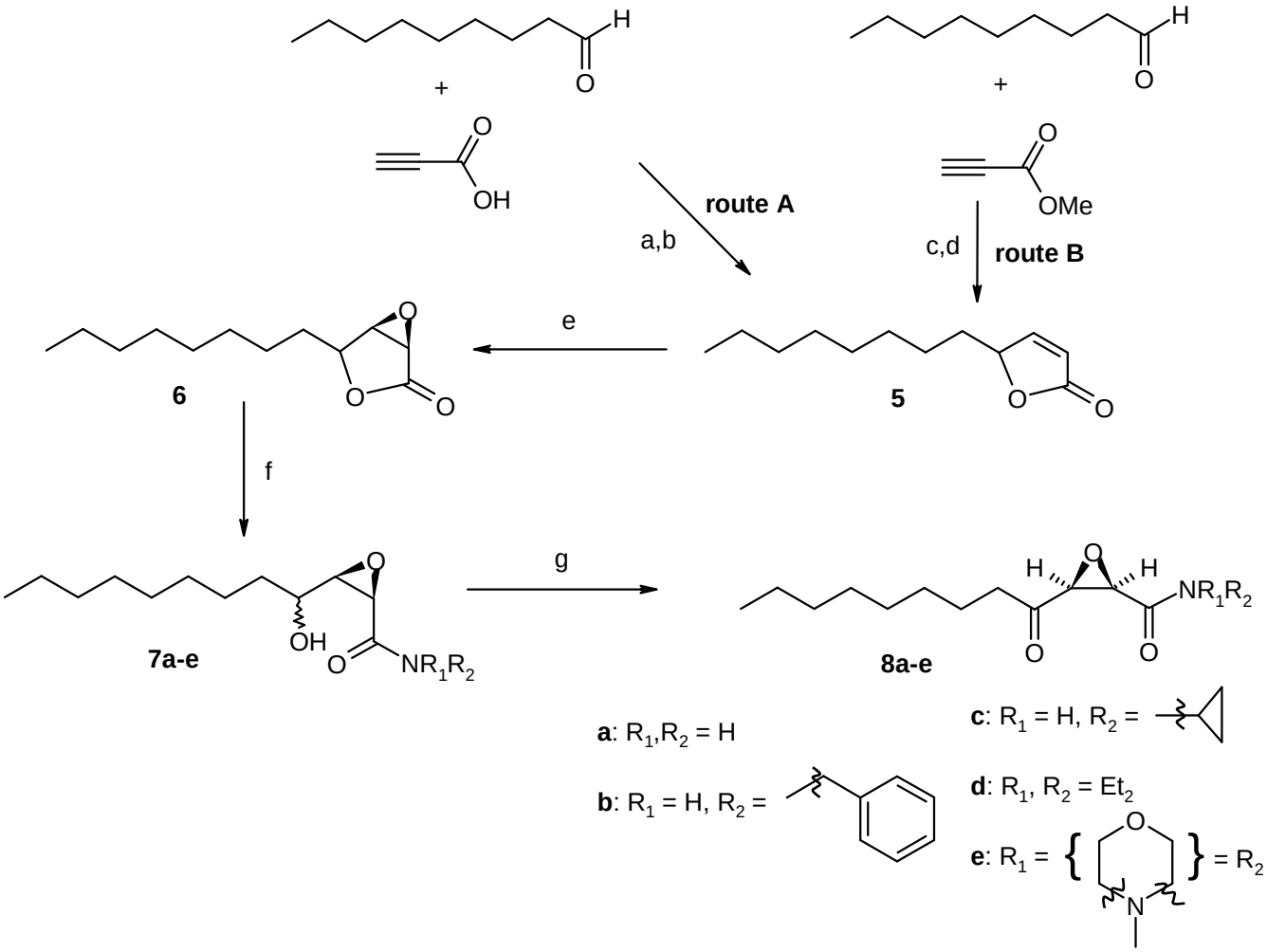


Reagents: (a) MsCl, NEt_3, CH_2Cl_2; (b) NaI, Aliquat 336; (c) ^{t}BuLi, -78°C, THF, 2,3-dihydrofuran; (d) H_2CrO_4, 0°C; (e) $ClCO_2{}^{i}Bu$, NEt_3, -10°C, NHR

Fig. 5.1 First cerulenin derivatives prepared

To include the epoxide function especially with regard to the mechanism of action of cerulenin in the FAS system (considering known crystal structures of cerulenin and the KasA analogue of *E. coli*) tetrahydro- and dihydrocerulenin analogues were synthesized. Starting from the corresponding aldehyde, lactone **5** (tetrahydrocerulenin analogues) was obtained via two different routes **A** and **B**. Route **A** included the coupling of the aldehyde 1-nonanal to propiolic acid via a Grignard reaction with subsequent hydrogenation with the Lindlar catalyst under hydrogen pressure to give **5**. Via Route **B** 1-nonanal was coupled to methyl propiolate by *n*-BuLi with subsequent hydrogenation under reflux with the catalytic system Lindlar cat./NH_4HCO_2 to yield **5**. These hydrogenations were also executed in a microwave oven resulting in better yields and/or reaction times. The lactone **5** was then epoxidized, the ring opened by amidation and the remaining alcohol was

oxidized via Collins oxidation to result in tetrahydrocerulenin analogues **8a-e**. The same procedure was used for dihydrocerulenin analogues **10a-c** except that to obtain the corresponding lactone **9a** only route **A** was used and a further step had to be executed for ring closure.

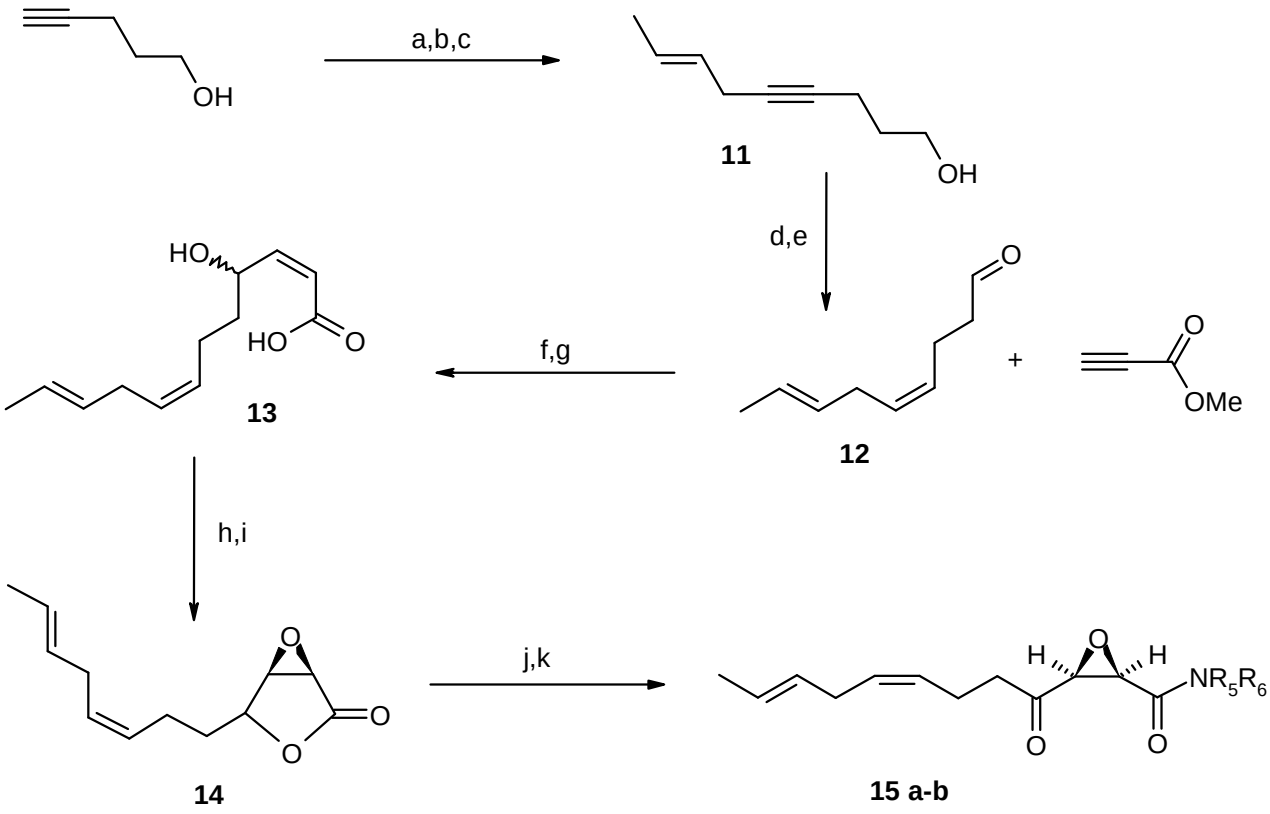


Reagents: (a) 2 eq. EtMgBr, Et_2O, 0℃, (b) Lindlar-cat., H_2, 5 bar, EtOAc; (c) $((H_3C)_2CH)_2NH$, *n*-BuLi, THF, -78℃ (d) Lindlar-cat., HCO_2NH_4, EtOH, reflux; (e) 5% NaOCl, *p*-dioxane, 0℃ →RT; (f) HNR_1R_2, MeOH, 0℃; (g) $CrO_3 \cdot pyr_2$, CH_2Cl_2, 0℃ →RT

Fig. 5.2 Synthesis route to tetrahydro- and dihydrocerulenin analogues

To obtain analogues with all structural features of cerulenin including two double bonds and the epoxide function, a third pathway was chosen. To obtain the future side chain, aldehyde **12** was synthesized by coupling protected 4-pentyn-1-ol to either crotyl bromide or crotyl chloride, which then was deprotected, hydrogenated with Lindlar catalyst under hydrogen pressure and oxidized via a Swern oxidation. The following synthesis sequence starting from **12** was executed similar to that of dihydrocerulenins via the corresponding lactone (**51**) with the major exception of the

oxidation procedure in the last step via TPAP/NMO to result in (4*Z*,7*E*)-cerulenin analogues **15a-b**.



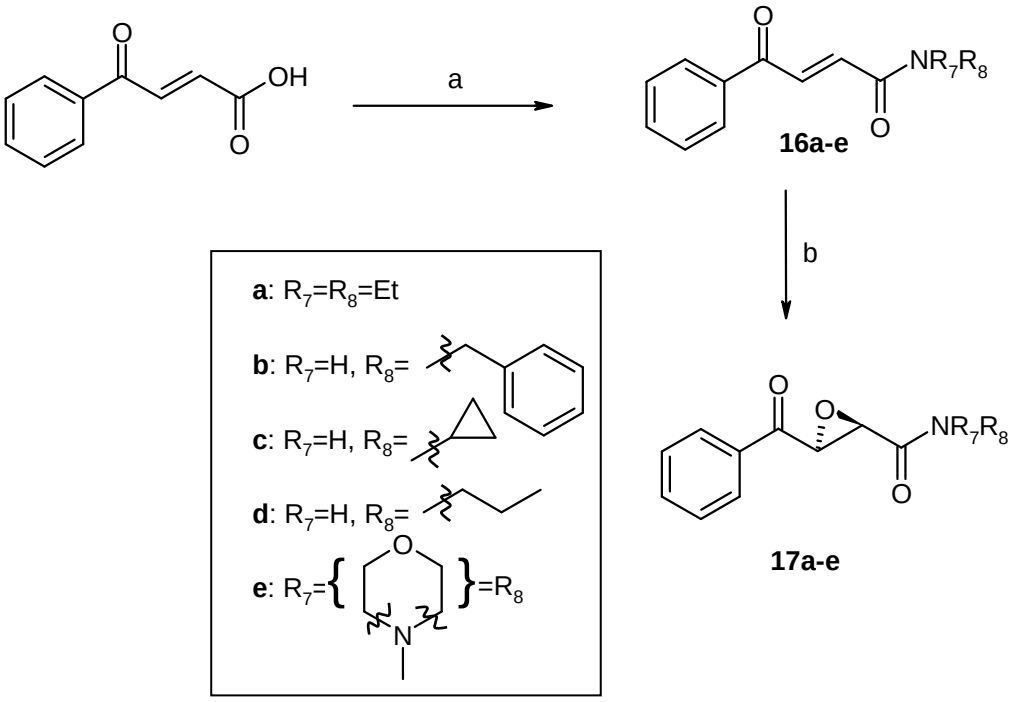
Reagents: (a) *p*-Tss, DHP; (b) EtMgBr, CuBr, CrotylBr, RT, THF (c) *p*-Tss, MeOH, reflux; (d) Lindlar-cat., H_2, 5 bar, quinoline, -10℃; (e) $C_2Cl_2O_2$, DMSO, NEt_3, -50℃; (f) $((H_3C)_2CH)_2NH$, *n*-BuLi, THF, -78℃; (g) Lindlar-cat., H_2, 7 bar, quinoline, -10℃; (h) *p*-Tss, Et_2O, RT; (i) 5% NaOCl, *p*-dioxane, 0℃→RT; (j) HNR_5R_6, MeOH, 0℃; (k) TPAP, NMO, 4 Å mol. siev., CH_2Cl_2, RT

a: R_5, R_6 = Et_2

b: R_5 = { O, N } = R_6

*Fig. 5.3 Synthesis route to cerulenin analogues **15a** and **15b***

A fourth class of cerulenin analogues was synthesized with the aromatic analogues **17a-e**. This synthesis pathway started with the formation of the benzoyl acrylamides **16a-e** from benzoylacrylic acid via a mixed anhydride which was prepared with isobutylchloroformate followed by the addition of the corresponding amine. Subsequent epoxidation with H_2O_2 in basic EtOH gave the aromatic cerulenin analogues **17a-e**.



Reagents: (a) $ClCO_2{}^{i}Bu$, DME, -10°C, HNR_7R_8; (b) H_2O_2, NaOH, pH=7-8

Fig. 5.4 Synthesis route to aromatic cerulenin analogues

Pharmacological testings for the synthesized substances were executed on efflux pump-resistant and -sensitive *Candida albicans* isolates, on the fatty acid synthesis enzyme KasA of *Mycobacterium tuberculosis* and on other organisms such as *Leishmania major*, *Trypanosoma brucei brucei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* within the Sonderforschungsbereich 630.

The testings on *Candida albicans* were executed starting with the determination of the minimal inhibitory concentration (MIC) of the synthesized cerulenin analogues to determine whether they showed inhibitory activity against *C. albicans* or not. In the next step, a combination of the synthesized compounds that did not show inhibitory activity and known inhibitors of *Candida albicans* (cerulenin, brefeldin A; also substrates of *Candida albicans* efflux pumps) were added to efflux pump-resistant and -susceptible isolates of *Candida albicans*. The known inhibitors were either appended at fixed subinhibitoric concentrations or at various concentrations with DMSO as a control. The synthesized cerulenin analogues **7c**, **8d** and **10a** seemed to decrease the resistance of the MDR1p efflux pump overexpressing isolates Gr13 and F13 in combination with brefeldin A at a constant compound concentration of 25 µg/ml 8fold (MIC of brefeldin A: 12,5 µg/ml in combination with **7c**, **8d** and **10a**)

compared to the control DMSO/brefeldin A (MIC of brefeldin A: 100 µg/ml). Even at lower concentrations of **7c** and **8d** (12,5 or 6,25 µg/ml) these compounds showed a 2fold decrease of the MDR1 mediated resistance. Also for the synthesized cerulenin analogues **8b** and **17b** a substantial decrease (4fold) of the MDR1 mediated resistance could be observed (MIC of brefeldin A: 25 µg/ml in combination with **7c**, **8d** and **10a**, each 25 µg/ml). A lead compound to decrease efflux pump mediated resistance should therefore either possess a long (probably flexible) alkyl or aryl side chain and a small alkyl or aryl substituent at the amide function:

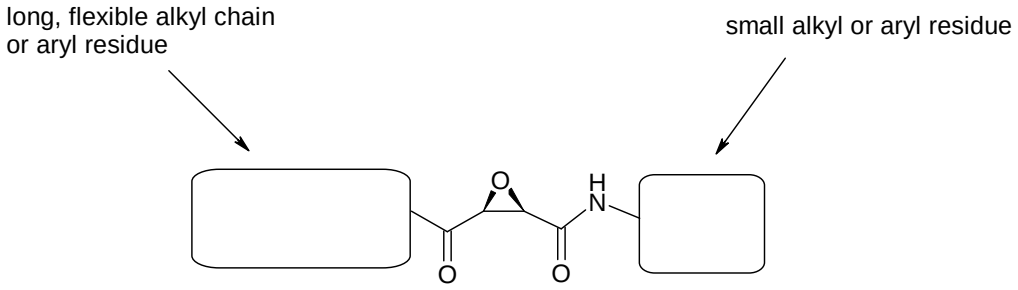


Fig. 5.5 Lead structure explored from the test results

Cerulenin is known to inhibit irreversibly the elongation enzymes of the fatty acid synthesis of *E. coli* FabB and FabF. The corresponding enzyme in *Mycobacterium tuberculosis* is KasA. Due to the fact that enzyme assays for KasA are complex, a system with an inhibitor induced change of the auto fluorescence of KasA was developed in order to determine K_i values of the inhibitors to KasA. The fluorescence of KasA and added cerulenin analogue was measured in a time dependent manner; the so obtained rate constants (k_{obs}) of the reaction were then fitted against the corresponding compound concentration to find out the dissociation constant K_i for the synthesized cerulenin analogues and KasA. The dissociation constant for substance **17e** was obtained as K_i = 16.56 µM $\pm$ 1.40 µM, for compound **17c** the acquired K_i value was K_i = 50.29 µM $\pm$ 10.02 µM. The closed morpholine ring and the tertiary amine of **17e** seem to have much better inhibition properties than the smaller cyclopropyl ring and the secondary amine of **17c**. The determination of the remaining aromatic cerulenin analogues is still under current investigation. The synthesized alkyl cerulenin analogues were also screened, but probably due to a missing chromophor showed no alteration of the KasA fluorescence signal. The synthesized cerulenin analogues were also tested on different other organisms such as *Leishmania major*, *Trypanosoma brucei brucei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* within

the SFB 630. The best results were obtained for inhibition of *L. major* by **8c** (MIC: 34,00 µM), **17b** (MIC: 34,00 µM) and **17c** (MIC: 31,00 µM), for biofilm inhibition of *S. epidermidis* by **7b** (~40 %/160 µM), **7c** (~30 %/160 µM) and **8b** (~20 %/20 µM) and for inhibition of *T.b.brucei* by **8a** (MIC: 21,95 µM) and **8c** (MIC: 3,39 µM).

5.2 Zusammenfassung

Die vorliegende Arbeit befasst sich mit der Synthese und Charakterisierung von Ceruleninanaloga als Inhibitoren effluxpumpenresistenter *Candida-albicans*-Isolate und als Inhibitoren des Fettsäuresyntheseenzyms KasA von *Mycobacterium tuberculosis*.

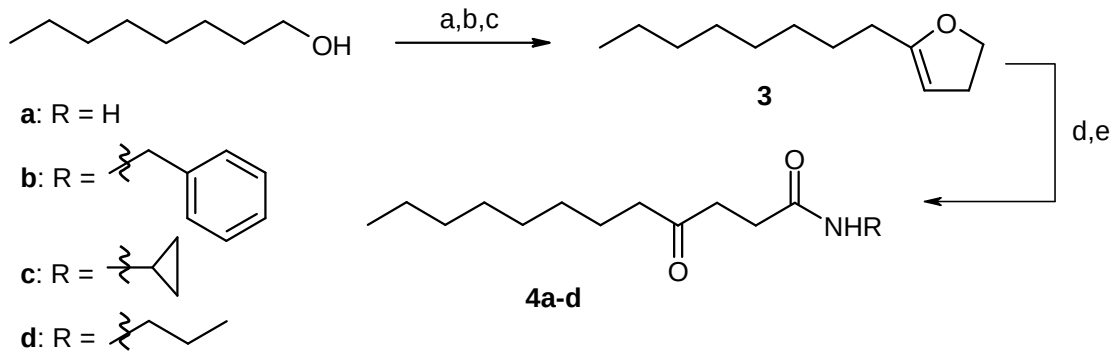
Candida albicans stellt einen der hauptverantwortlichen Pathogene mykotischer Infektionen dar. *Candida*-Arten (mit *C. albicans* als dem häufigsten Erreger) rufen ca. 78 % der nosokomialen Pilzinfektionen hervor und sind der vierthäufigste Mikroorganismus betreffend nosokomialer Sepsis in den U.S.A. Insbesondere die letztgenannten Infektionen der Blutbahn sind mit einer erheblichen Sterblichkeitsrate verbunden, die zwischen 1997 und 2001 bis zu 49 % betrug. Wegen der begrenzten Zahl an, aber exzessivem Gebrauch von Antimykotika bei einer steigenden Anzahl fungaler Infektionen kann ein Anstieg der Resistenzbildung nicht nur bei intrinsisch resistenten, sondern auch bei normalerweise sensitiven Isolaten beobachtet werden. Im Fall von Fluconazol als dem am häufigsten eingesetzten Antimykotikum gegen *Candida*-Infektionen können verschiedene Mechanismen für eine solche Resistenz verantwortlich sein. Die Überexpression der Effluxpumpen MDR1p, CDR1p und CDR2p, durch die Fluconazol aktiv aus der Zelle herausgepumpt wird, ist dabei einer der wichtigsten Mechanismen. Die Inhibition dieses Resistenzmechanismus mittels Blockierung der Effluxpumpen stellt daher eine Strategie zur Reduzierung resistenter *Candida*-Infektionen dar.

Mycobacterium tuberculosis ist einer der Haupterreger der Tuberkulose, die heute eines der größten Gesundheitsprobleme darstellt, da laut WHO im Jahr 2005 ein Drittel der Weltbevölkerung mit Tuberkulose infiziert war und die Zahl der tödlichen Verläufe auf 1,6 Millionen Fälle geschätzt wurde. Aufgrund der geringen Anzahl antimykobakterieller Wirkstoffe und einer steigenden Zahl multiresistenter Tuberkulosefälle gibt es einen hohen Bedarf zur Entwicklung neuer Wirkstoffe gegen Tuberkulose. Die Enzyme der Fettsäuresynthese stellen dabei ein attraktives Target für solche zukünftigen Wirkstoffe dar, da eine entsprechende Inhibition zum Fehlen

der essentiellen Mykolsäuren in der mykobakteriellen Zellwand führt. Weiterhin ist die Anordnung der Fettsäurezyklus-Komponenten in Säugern (Typ I FAS) und in Bakterien (Typ II FAS) verschieden und zudem sollten vohandene Resistenzmechanismen relativ uneffektiv gegen diesen Wirksmechanismus sein, da ihn nur wenige Wirkstoffe als Target besitzen. Die Effektivität der Inhibition der Fettsäuresynthese wurde für den gegen Tuberkulose eingesetzten Wirkstoff Isoniazid gezeigt.

Der Naturstoff Cerulenin wurde als Leitstruktur gewählt, da er einerseits ein Substrat der bekannten Effluxpumpen von *Candida albicans* ist und deshalb Strukturvariationen zu einer Blockierung der Effluxpumpen, wie im Fall von Tetrazyklin und dem Inhibitor 13-CPTC der TetB Effluxpumpe, führen können. Andererseits ist Cerulenin ein bekannter Inhibitor der Fettsäuresynthese, allerdings unselektiv für Typ I und II. Ceruleninanaloga als Fettsäureinhibitoren könnten daher eine erhöhte Selektivität bezüglich des Typ II Fettsäuresystems in *M. tuberculosis* aufweisen.

Die in dieser Arbeit zuerst synthetisierten Ceruleninderivate wurden durch Kupplung von 2,3-Dihydrofuran und dem zuvor dargestellten 1-Octaniodid gefolgt von Ringöffnung und Oxidation mittels Chromsäure zur 4-Ketosäure, und Umsetzung zum entsprechenden Amid und somit den Ceruleninanaloga **4a-d** hergestellt. Substanz **4e** wurde entsprechend synthetisiert.

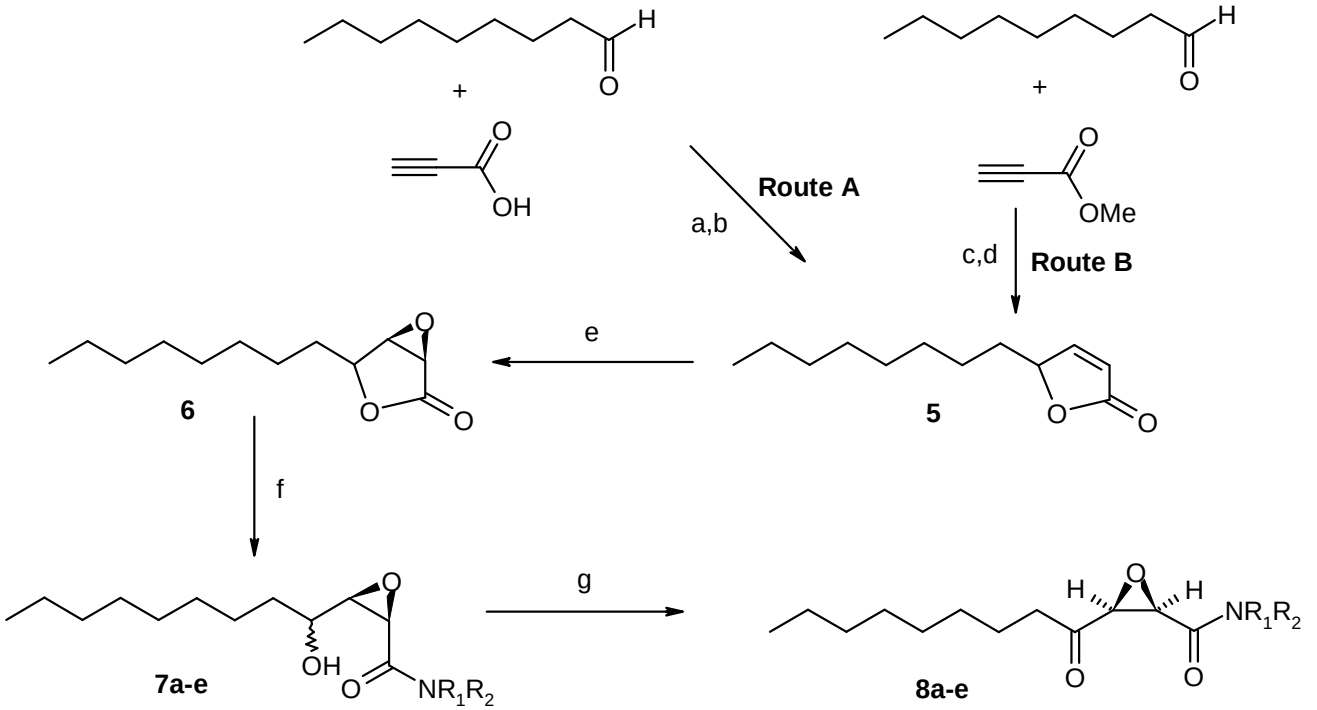


Reagenzien: (a) MsCl, NEt_3, CH_2Cl_2; (b) NaI, Aliquat 336; (c) ^{t}BuLi, -78°C, THF, 2,3-dihydrofuran; (d) H_2CrO_4, 0°C; (e) $ClCO_2{}^{i}Bu$, NEt_3, -10°C, H_2NR

Fig. 5.6 Syntheseweg der zuerst hergestellten Ceruleninderivate

Um die Epoxidfunktion der Leitstruktur zu integrieren, die besonders hinsichtlich des Wirkmechanismus von Cerulenin im FAS-System wichtig zu sein scheint (wenn man die bekannten Kristallstrukturen von Cerulenin und dem KasA-Analogon in *E. coli* berücksichtigt), wurden Tetrahydro- und Dihydroceruleninanaloga synthetisiert.

Ausgehend von dem entsprechendem Aldehyd wurde (im Fall der Tetrahydroceruleninе) Lacton **5** auf zwei verschiedene Arten dargestellt: mittels Route **A** und **B**. Route **A** beinhaltete die Kupplung des Aldehyds 1-Nonanal mit Propiolsäure durch eine entsprechende Grignardreaktion mit anschließender Hydrierung über Lindlar-Katalysator unter Wasserstoffdruck. Bei Route **B** wurden 1-Nonanal und Methylpropiolat mittels *n*-BuLi gekuppelt und anschließend hydriert durch Refluxieren mit Lindlar-Katalysator und NH_4HCO_2. Die Hydrierungen von Route **A** und **B** wurden auch in der Mikrowelle durchgeführt, wodurch bessere Ausbeuten und/oder Reaktionszeiten erzielt werden konnten. Das so dargestellte Lacton **5** wurde dann epoxidiert, der Lactonring durch den Angriff eines Amins geöffnet und der so entstandene Alkohol mittels Collins Oxidierung zu den Tetrahydroceruleninanaloga **8a-e** oxidiert. Dihydroceruleninanaloga **10a-c** wurden auf analogem Syntheseweg hergestellt mit dem Unterschied, dass die entsprechende Lactonzwischenstufe **9a** nur durch Route **A** synthetisiert und ein weiterer Zwischenschritt zum Ringschluss benötigt wurde.



a: R_1,R_2 = H

b: R_1 = H, R_2 =

c: R_1 = H, R_2 =

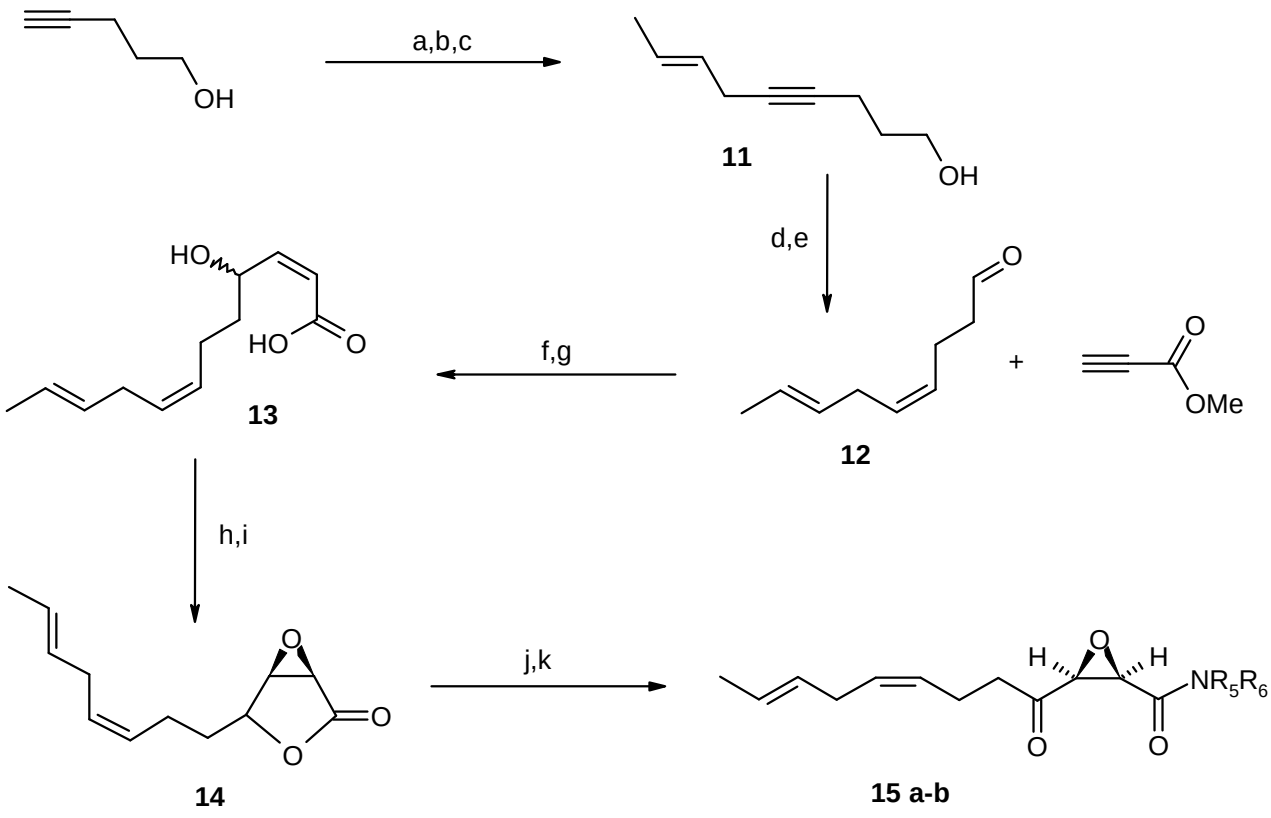
d: R_1, R_2 = Et_2

e: R_1 = { } = R_2

Reagenzien: (a) 2 eq. EtMgBr, Et_2O, 0℃, (b) Lindlar-cat., H_2, 5 bar, EtOAc; (c) $((H_3C)_2CH)_2NH$, *n*-BuLi, THF, -78℃ (d) Lindlar-cat., HCO_2NH_4, EtOH, reflux; (e) 5% NaOCl, *p*-dioxane, 0℃ →RT; (f) HNR_1R_2, MeOH, 0℃; (g) $CrO_3 \cdot pyr_2$, CH_2Cl_2, 0℃ →RT

Fig. 5.7 Syntheseroute der Tetrahydro- und Dihydroceruleninanaloga

Um Ceruleninanaloga mit allen strukturellen Komponenten des Cerulenins inklusive zweier Doppelbindungen und Epoxidfunktion zu erhalten, wurde ein dritter Syntheseweg gewählt. Zur Integration der späteren Seitenkette wurde zuerst Aldehyd **12** durch Kupplung von geschütztem 4-Pentyn-1-ol mit entweder Crotylbromid oder Crotylchlorid, anschließendem Entschützen und Hydrierung über Lindlar-Katalysator und unter Wasserstoffdruck und nachfolgender Swern Oxidation synthetisiert. Die anschließende Synthesesequenz startete von Substanz **12** und wurde in Anlehnung der Synthese an die Dihydroceruleninderivate über Lacton **51** durchgeführt. Die größte Abweichung stellte dabei die Oxidation im letzten Schritt dar, die mittels TPAP/NMO durchgeführt wurde und in den (4*Z*,7*E*)-Ceruleninanaloga **15a-b** resultierte.



Reagenzien: (a) *p*-Tss, DHP; (b) EtMgBr, CuBr, CrotylBr, RT, THF (c) *p*-Tss, MeOH, reflux; (d) Lindlar-cat., H_2, 5 bar, quinoline, -10℃; (e) $C_2Cl_2O_2$, DMSO, NEt_3, -50℃; (f) $((H_3C)_2CH)_2NH$, *n*-BuLi, THF, -78℃; (g) Lindlar-cat., H_2, 7 bar, quinoline, -10℃; (h) *p*-Tss, Et_2O, RT; (i) 5% NaOCl, *p*-dioxane, 0℃→RT; (j) HNR_5R_6, MeOH, 0℃; (k) TPAP, NMO, 4 Å mol. siev., CH_2Cl_2, RT

a: R_5, R_6 = Et_2

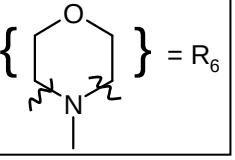
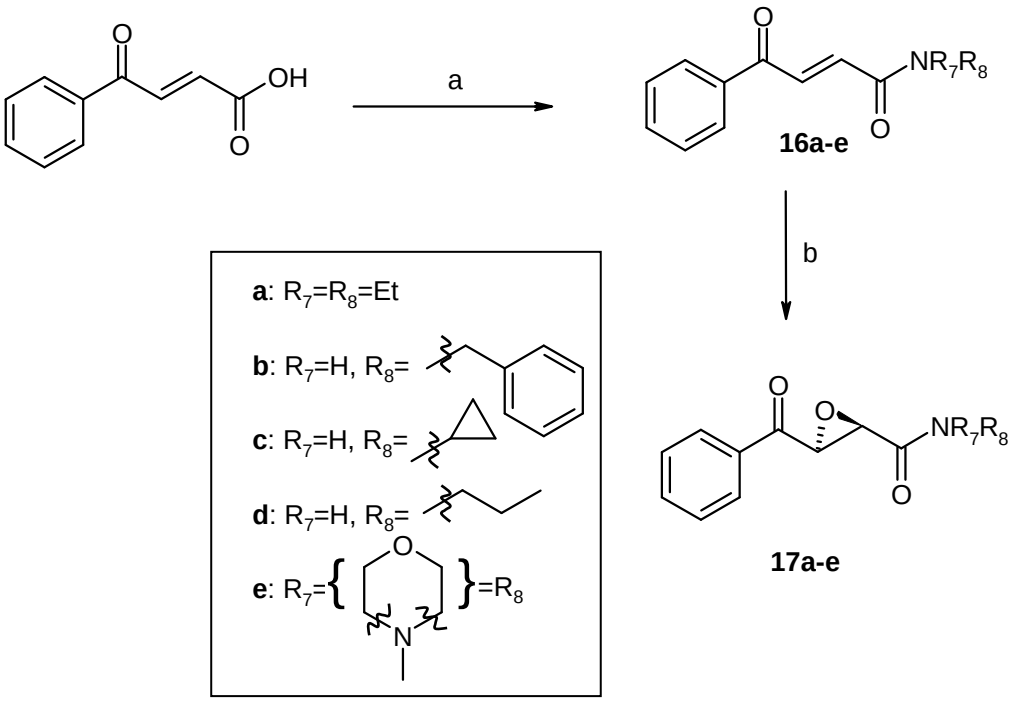
b: R_5 =  = R_6

*Fig. 5.8 Syntheseroute der Ceruleninanaloga **15a** and **15b***

Eine vierte Klasse von Ceruleninanaloga wurde mit den aromatischen Derivaten **17a-e** synthetisiert. Diese Route startete mit der Synthese der Benzoylacrylamide **16a-e** aus Benzoylacrylsäure über das gemischte Anhydrid, das mit Isobutylchloroformiat hergestellt wurde, gefolgt von der Zugabe des entsprechenden Amins. Die nachfolgende Epoxidierung mit H_2O_2 in basischem EtOH ergab die aromatischen Ceruleninanaloga **17a-e**.



Reagenzien: (a) $ClCO_2{}^{i}Bu$, DME, -10°C, HNR_7R_8; (b) H_2O_2, NaOH, pH=7-8

Fig. 5.9 Syntheseroute der aromatischen Ceruleninanaloga

Pharmakologische Testungen der synthetisierten Substanzen wurden an Efflux-pumpen-resistenten und -sensitiven *Candida albicans* Isolaten, am Fettsäuresyntheseenzym KasA von *Mycobacterium tuberculosis* und an anderen Mikroorganismen wie *Leishmania major*, *Trypanosoma brucei brucei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* und *Pseudomonas aeruginosa* im Sonderforschungsbereich 630 durchgeführt.

Bei den Testungen an *Candida albicans* wurde zuerst die minimale Hemmkonzentration (MHK) der synthetisierten Ceruleninanaloga bestimmt, um zu sehen ob sie inhibitorische Aktivität gegen *C. albicans* zeigten oder nicht. Im nächsten Schritt wurde eine Kombination der synthetisierten Substanzen, die keine

inhibitorische Aktivität zeigten, und bekannten Inhibitoren von *Candida albicans* (Cerulenin, Brefeldin A; Substrate der *Candida albicans* Effluxpumpen) an Efflux-pumpenresistenten und -sensitiven Isolaten von *Candida albicans* getestet. Die bekannten Inhibitoren wurden entweder in bestimmten festen oder variierenden Konzentrationen mit DMSO als Kontrolle zugesetzt. Die synthetisierten Ceruleninanaloga **7c**, **8d** und **10a** verringerten die Resistenz der MDR1p-Effluxpumpen-überexprimierenden Isolate Gr13 and F13 in Kombination mit einer konstanten Brefeldin A Konzentration von 25 µg/ml 8-fach (MHK von Brefeldin A: 12,5 µg/ml in Kombination mit **7c**, **8d** und **10a**) im Vergleich zur Kontrolle DMSO/Brefeldin A (MHK von Brefeldin A: 100 µg/ml). Sogar bei niedrigeren Konzentrationen von **7c** und **8d** (12,5 oder 6,25 µg/ml) zeigten diese Substanzen eine 2-fache Verringerung der durch MDR1p entstandenen Resistenz. Auch für die synthetisierten Ceruleninanaloga **8b** und **17b** konnte eine signifikante Verringerung (4-fach) der durch MDR1p entstandene Resistenz beobachtet werden (MHK von Brefeldin A: 25 µg/ml in Kombination mit **7c**, **8d** und **10a** (25 µg/ml)). Daraus geht hervor, dass eine Leitsubstanz zur Verringerung einer Effluxpumpen-Resistenz bei *Candida albicans* entweder eine lange (und flexible) Alkyl- oder Arylseitenkette und einen kleinen Alkyl- oder Arylsubstituent an der Amidfunktion besitzen sollte.

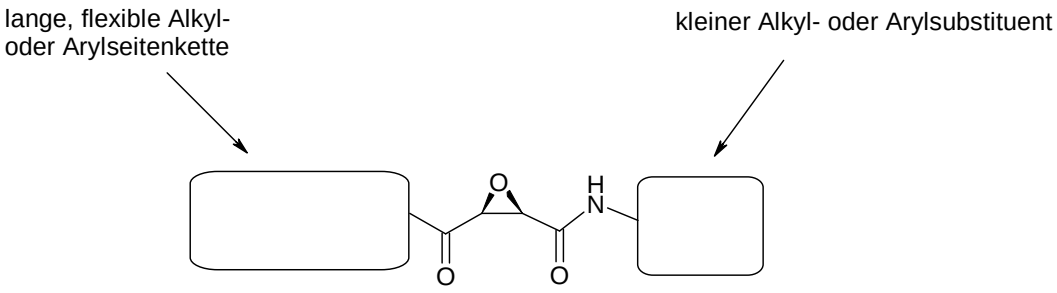


Fig. 5.10 Aus den Testergebnissen resultierende Leitstruktur

Cerulenin ist ein bekannter irreversibler Inhibitor der Elongationsenzyme des Fettsäuresynthesezyklus von *E. coli* FabB and FabF. Das entsprechende Enzym in Mykobakterien ist KasA. Da Enzymassays für KasA sehr komplex sind, wurde ein System mit einer inhibitor-induzierten Veränderung der Eigenfluoreszenz von KasA entwickelt, um die K_i-Werte der synthetisierten Ceruleninanaloga und KasA zu bestimmen. Die Fluoreszenz von KasA wurde zeitabhängig gemessen und die so erhaltenen Geschwindigkeitskonstanten (k_{obs}) wurden anschließend gegen die entsprechende Substanzkonzentration aufgetragen, um die K_i-Werte für die synthetisierten Ceruleninanaloga und KasA zu bestimmen. Die

Dissoziationskonstante für Substanz **17e** ergab K_i = 16.56 µM ± 1.40 µM und für Substanz **17c** K_i = 50.29 µM ± 10.02 µM. Der geschlossene Morpholinring und das tertiäre Amin von **17e** scheinen erheblich bessere Inhibitionseigenschaften als der kleinere Cyclopropylring und das sekundäre Amin von **17c** zu besitzen. Die Bestimmung der K_i-Werte der verbleibenden aromatischen Cerulenninanaloga wird zurzeit noch durchgeführt. Auch die synthetisierten Alkyl-cerulenninanaloga wurden mit dieser Methode gescreent, zeigten aber (wahrscheinlich wegen des fehlenden Chromophors) keine Veränderung des KasA-Fluoreszenz-Signals.

Die synthetisierten Cerulenninderivate wurden auch an anderen Mikroorganismen wie *Leishmania major*, *Trypanosoma brucei brucei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* und *Pseudomonas aeruginosa* innerhalb des Sonderforschungsbereichs 630 getestet. Die besten Resultate wurden für die Inhibition von *L. major* von **8c** (MHK: 34,00 µM), **17b** (MHK: 34,00 µM) und **17c** (MHK: 31,00 µM), für die Biofilminhibition von *S. epidermidis* von **7b** (~40 %/160 µM), **7c** (~30 %/160 µM) und **8b** (~20 %/20 µM) und für die Inhibition von *T.b.brucei* von **8a** (MHK: 21,95 µM) und **8c** (MHK: 3,39 µM) erzielt.

6. Experimental section

Melting points are uncorrected and taken on a Sanyo Gallenkamp (Sanyo Gallenkamp, Loughborough, UK) melting point apparatus.

^{1}H Nuclear magnetic resonance spectra (400.13 MHz) were recorded on a Bruker Avance 400 NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) and chemical shifts are reported in δ units with the centre of the deuterated solvent signal as internal reference ($CDCl_3$: 7.26 ppm; DMSO-d_6: 2.50 ppm, Acetone-d_6: 2.05 ppm).

^{13}C Nuclear magnetic resonance spectra (100.62 MHz) were recorded on a Bruker Avance 400 NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) and chemical shifts are reported in δ units with the centre of the deuterated solvent signal as internal reference ($CDCl_3$: 77.00 ppm; DMSO-d_6: 39.52 ppm, Acetone-d_6: 29.84 ppm). Concerning ^{1}H-NMR-spectra the following abbreviations were applied: s = singulet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet.

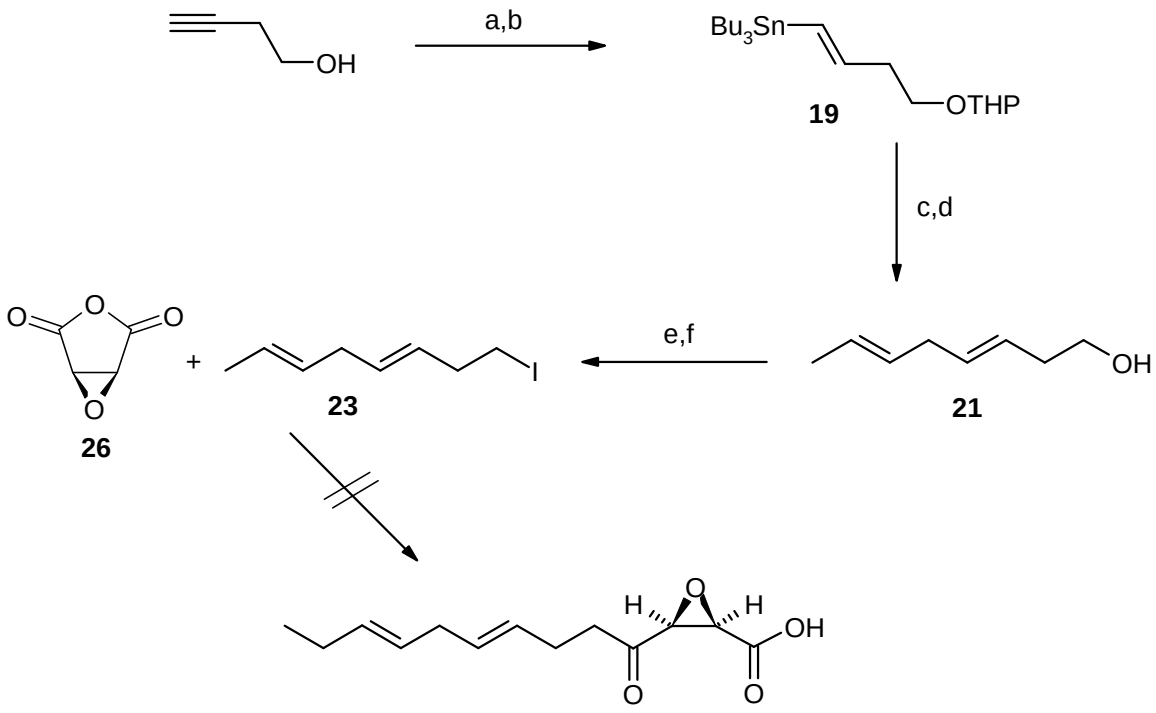
Infrared spectra were recorded on a Biorad-PharmalyzIR FT-IR spectrometer (Biorad, Cambridge, MA, USA). **Microwave reactions** were executed in a Milestone MLS-Ethos 1600 (MLS GmbH, Leutkirch, Germany). Reaction progress was monitored by **thin layer chromatography**; TLC aluminium sheets silica gel 60 F_{254} from Merck were used (Merck KGaA, Darmstadt, Germany). **Column chromatography** was carried out with silica gel 60, 0.063-0.2 mm (70-230 mesh) from Merck (Merck KGaA, Darmstadt, Germany).

Fluorescence spectra were recorded on a QM-4/2005-SE spectrometer from Photon Technology INT Inc. (London, Ontario, Canada).

Chemicals were obtained from either Acros Organics (Geel, Belgium) or Sigma-Aldrich (Taufkirchen, Germany). Reactions were performed in oven-dried glassware and anhydrous organic solvents were dried and distilled according to the usual methods.[220]

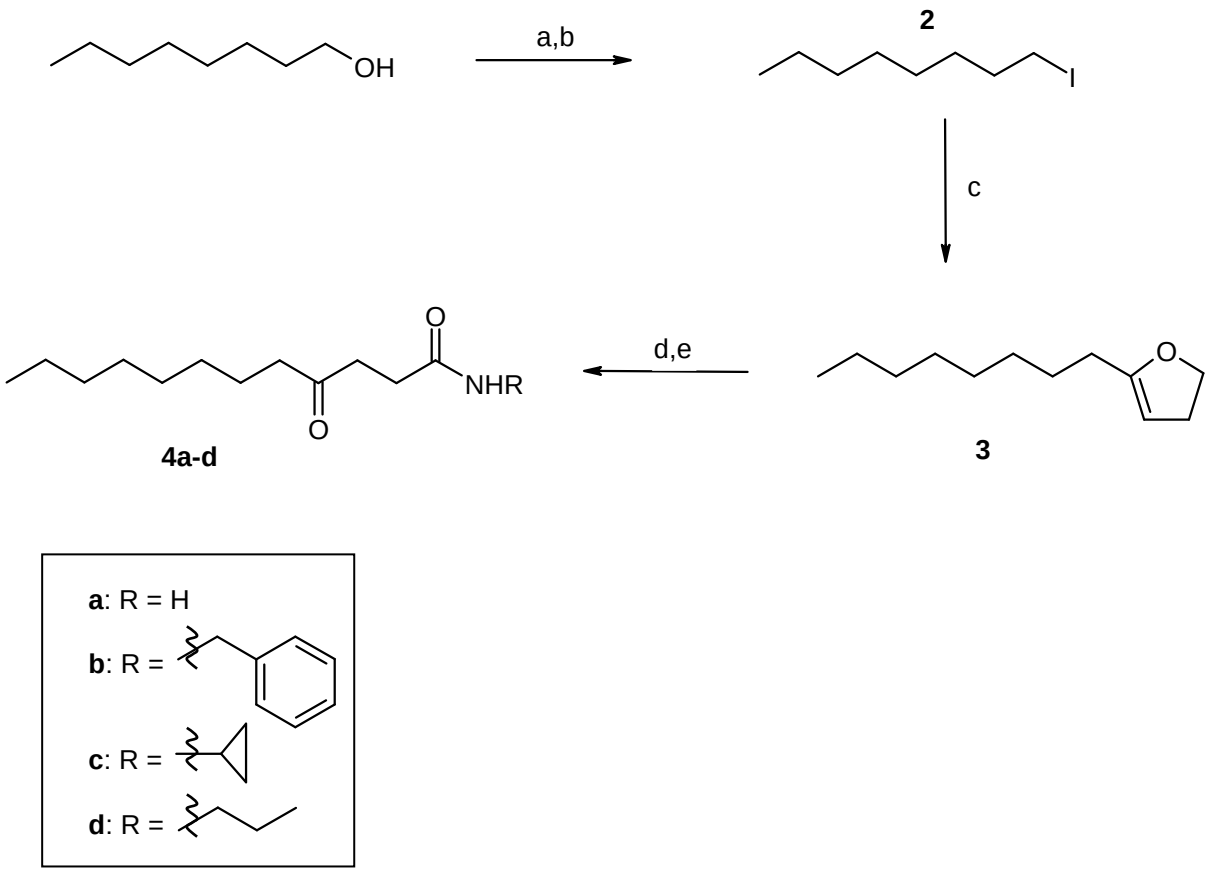
6.1 Synthesis procedures and analytical data

Scheme 1. First applied synthesis pathway to Cerulenin Analogues



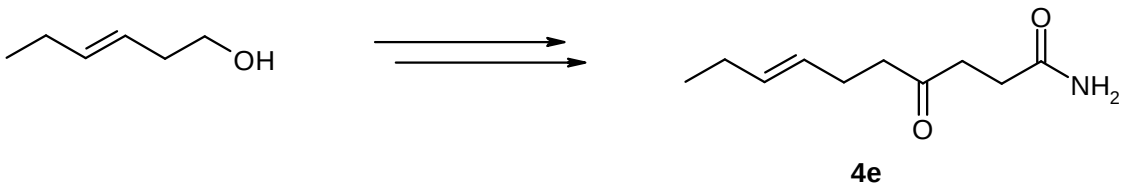
Reagents: (a) *p*-Tss, DHP; (b) AIBN, $HSnBu_3$, ΔT; (c) *n*-BuLi, CrotylBr, -78℃, THF; (d) Dowex50 WX8, MeOH, reflux (e) MsCl, CH_2Cl_2, NEt_3; (f) NaI, Aliquat 336

Scheme 2a. Synthesis of 4-Oxoalkanamide Cerulenin analogues

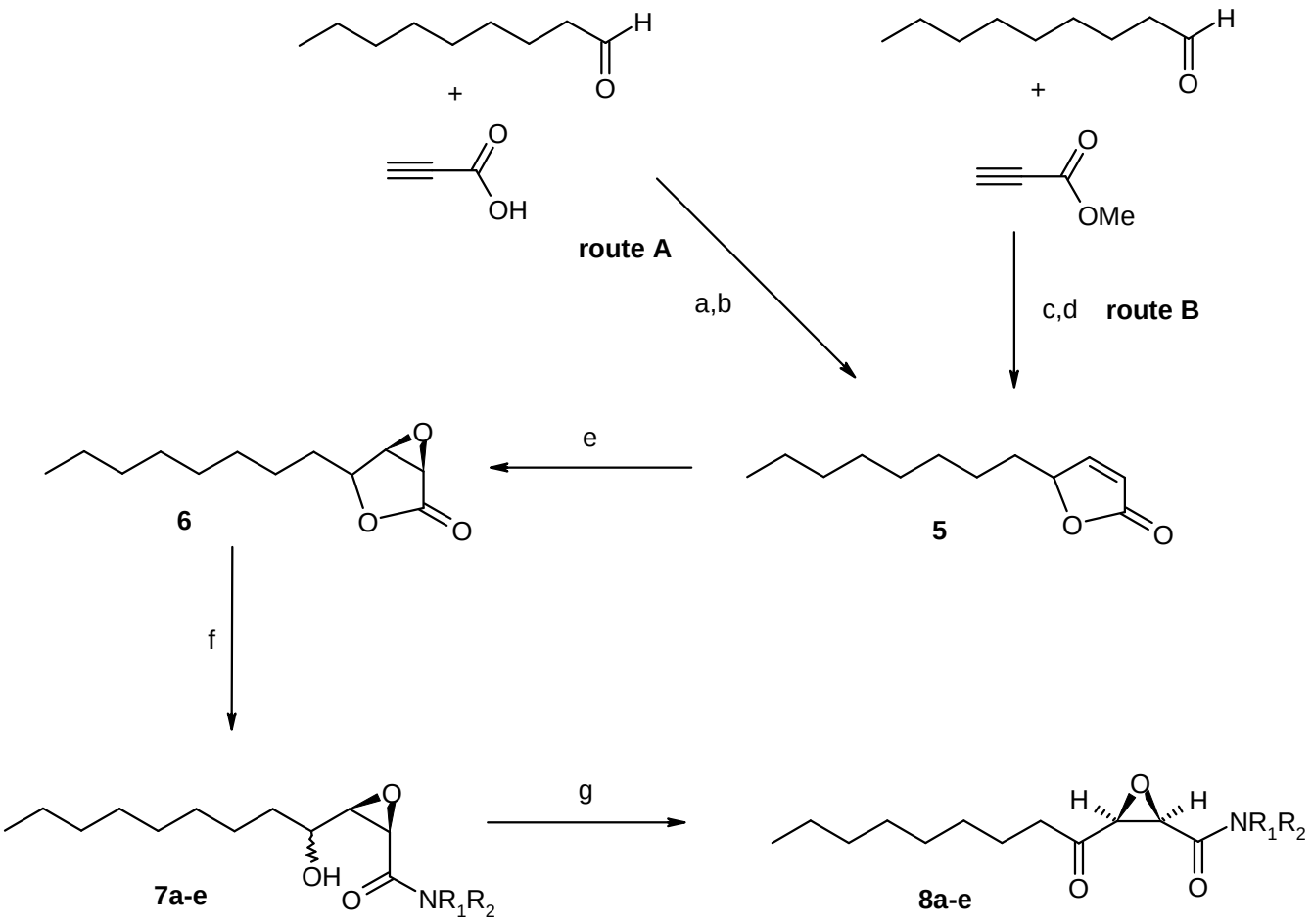


Reagents: (a) MsCl, NEt_3, CH_2Cl_2; (b) NaI, Aliquat 336; (c) ^{t}BuLi, -78°C, THF, 2,3-dihydrofuran; (d) H_2CrO_4, 0°C; (e) $ClCO_2{}^{i}Bu$, NEt_3, -10°C, H_2NR

Scheme 2b. Synthesis of a 4-Oxoalkenamide Cerulenin Analogue (pathway/reagents s. scheme 2a)



Scheme 3a. Synthesis of Tetrahydrocerulenin Analogues



a: R_1,R_2 = H

b: R_1 = H, R_2 =

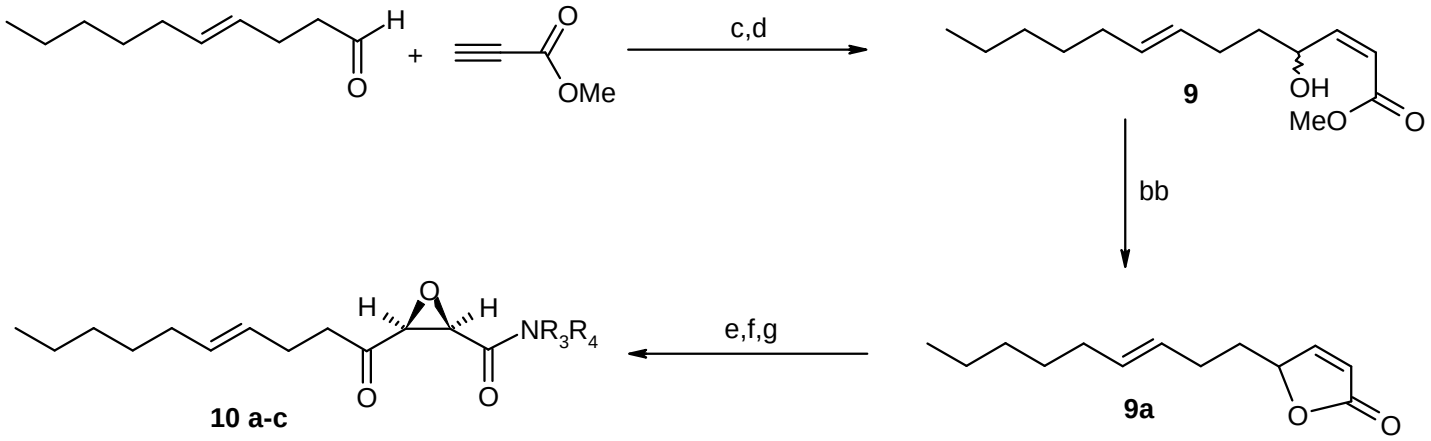
c: R_1 = H, R_2 =

d: R_1, R_2 = Et_2

e: R_1 = { } = R_2

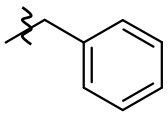
Reagents: (a) 2 eq. EtMgBr, Et_2O, 0℃, (b) Lindlar-cat., H_2, 5 bar, EtOAc; (c) $((H_3C)_2CH)_2NH$, BuLi, THF, -78℃ (d) Lindlar-cat., HCO_2NH_4, EtOH, reflux; (e) 5% NaOCl, *p*-dioxane, 0℃→RT; (f) HNR_1R_2, MeOH, 0℃; (g) $CrO_3 \cdot pyr_2$, CH_2Cl_2, 0℃→RT

Scheme 3b. Synthesis of Dihydrocerulenin Analogues

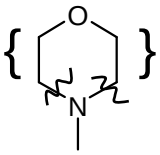


a: R_3, R_4 = Et_2

b: R_3 = H, R_4 =



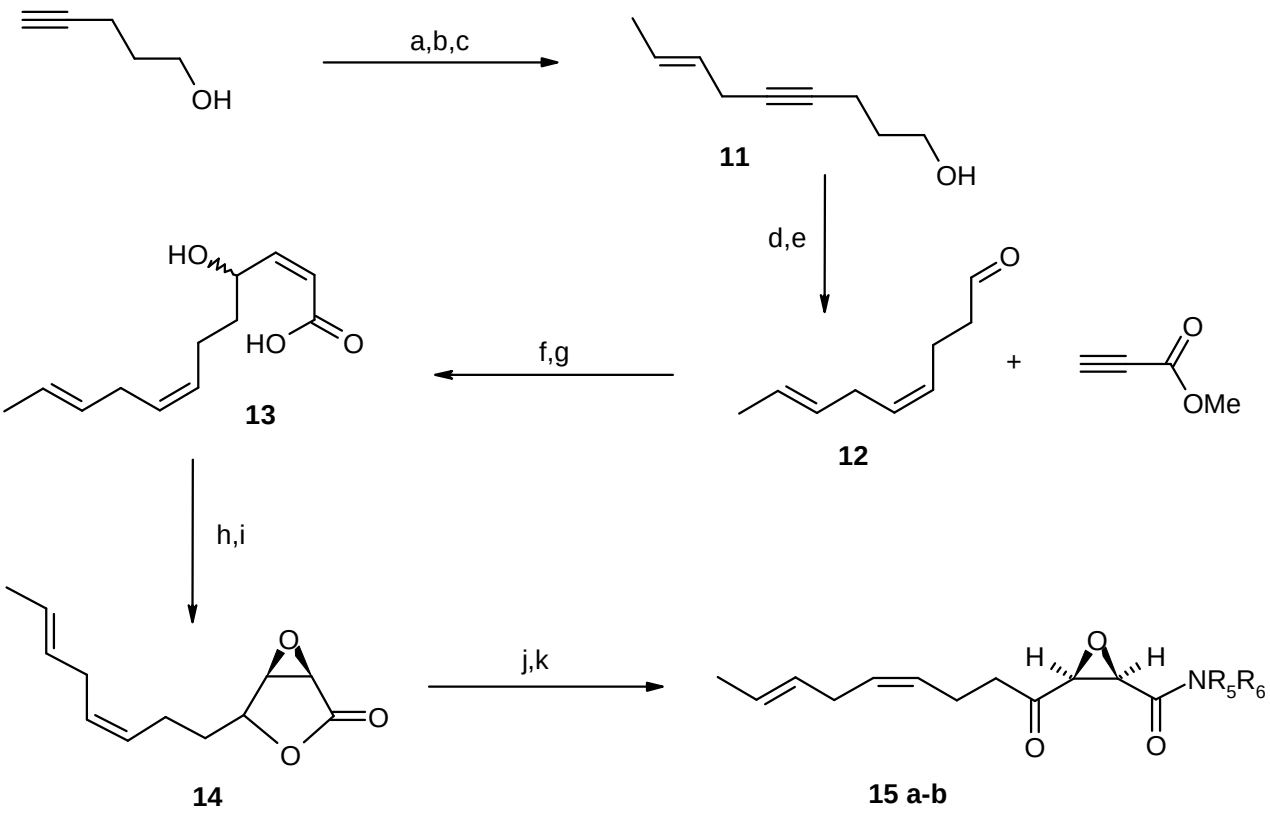
c: R_3 =



= R_4

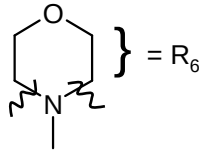
Reagents: see scheme 3a except (bb) *p*-Tss, Et_2O

Scheme 4. Synthesis of Cerulenin Analogues

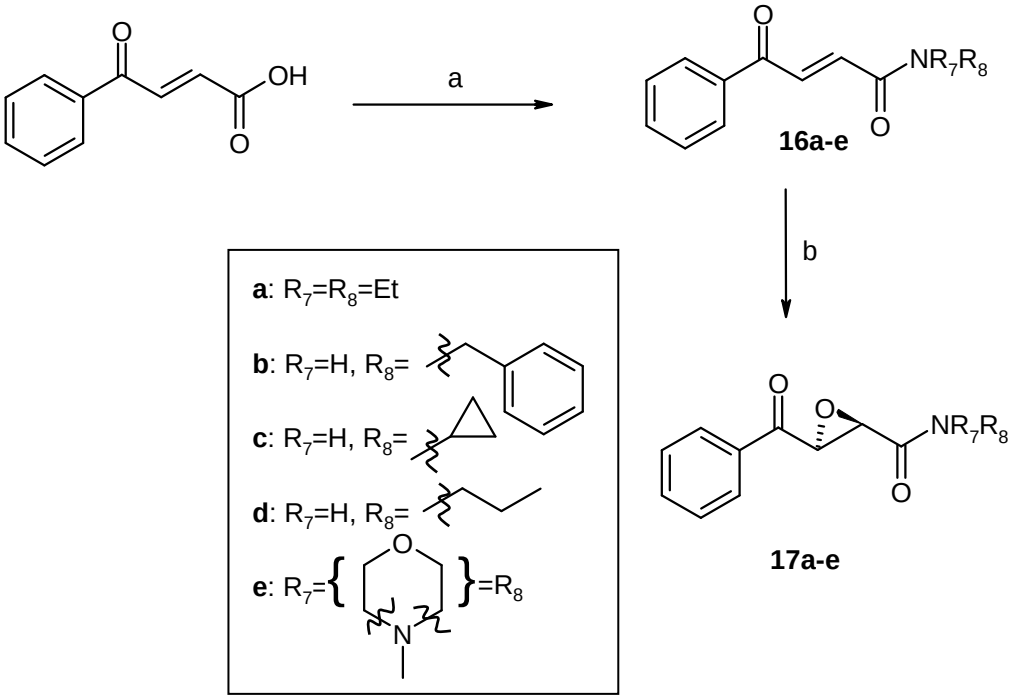


Reagents: (a) *p*-Tss, DHP; (b) EtMgBr, CuBr, CrotylBr, RT, THF (c) *p*-Tss, MeOH, reflux; (d) Lindlar-cat., H_2, 5 bar, quinoline, -10℃; (e) $C_2Cl_2O_2$, DMSO, NEt_3, -50℃; (f) $((H_3C)_2CH)_2NH$, BuLi, THF, -78℃; (g) Lindlar-cat., H_2, 7 bar, quinoline, -10℃; (h) *p*-Tss, Et_2O, RT; (i) 5% NaOCl, *p*-dioxane, 0℃ →RT; (j) HNR_5R_6, MeOH, 0℃; (k) TPAP, NMO, 4 Å mol. siev., CH_2Cl_2, RT

a: R_5, R_6 = Et_2

b: R_5 =  = R_6

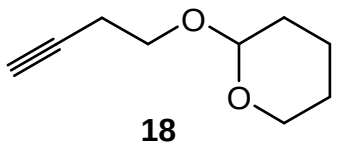
Scheme 5. Synthesis of aromatic Cerulenin Analogues



Reagents: (a) $ClCO_2{}^{i}Bu$, DME, -10℃, HNR_7R_8; (b) H_2O_2, NaOH, pH=7-8

6.1.1 Scheme 1. First applied synthetic pathway to cerulenin analogues

6.1.1.1 2-(3-Butynyloxy)-tetrahydro-2H-pyran (**18**)



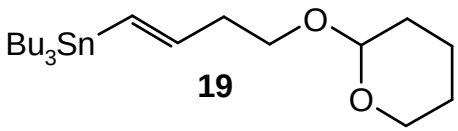
18

The synthesis of **18** was executed following mainly the procedure reported by Corey et al.[192] 2.60 g (37.1 mmol) 3-Butyn-1-ol was added to a solution of 3.74 g (44.6 mmol) 3,4-dihydro-2*H*-pyran in 30 ml of CH_2Cl_2 at 0 °C. A catalytic amount of *p*-toluene sulfonic acid was added (5 mg) and the mixture was stirred for 2 h at 0 °C. Afterwards the mixture was washed with 20 ml saturated aqueous Na_2CO_3 solution and 10 ml saturated NaOH solution, dried over anhydrous Na_2SO_4 and concentrated. The residue was distilled to give **18** as a colourless oil.

Formula	$C_9H_{14}O_2$
Molecular weight	154.09
Yield	4.69 g (82 %) [Lit.: 96 %][192]
Boiling point	75 °C (10mbar) [Lit.: 86 - 90 °C/20mm] [192]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.42-1.55 (m, 4H, $CH_2O[CHCH_2C\mathit{H}_2C\mathit{H}_2CH_2O]_{THP}$), 1.61-1.79 (m, 2H, $CH_2O[CHC\mathit{H}_2CH_2CH_2CH_2O]_{THP}$), 1.91 (t, *J* 2.52, 1H, $C\mathit{H}CCH_2CH_2OTHP$), 2.43 (td, *J* 7.08, *J* 2.52, 2H, $CHCC\mathit{H}_2CH_2OTHP$), 3.40-3.82 (m, 4H, $C\mathit{H}_2O[CHCH_2CH_2CH_2C\mathit{H}_2O]_{THP}$), 4.58 (t, *J* 3.28, 1H, $OC\mathit{H}O$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 19.35, 25.37, 30.49 ($CH_2O[CH\mathit{C}H_2\mathit{C}H_2\mathit{C}H_2CH_2O]_{THP}$), 19.90 ($CHC\mathit{C}H_2CH_2OTHP$), 62.18, 65.48 ($\mathit{C}H_2O[CHCH_2CH_2CH_2\mathit{C}H_2O]_{THP}$), 69.13 ($\mathit{C}HCCH_2CH_2OTHP$), 81.34 ($CH\mathit{C}CH_2CH_2OTHP$), 98.73 ($O\mathit{C}HO$); **IR** data were consistent with literature.[221]

6.1.1.2 Tributyl((E)-4-(tetrahydro-2H-2-pyranyloxy)1-butenyl)stannane (**19**)



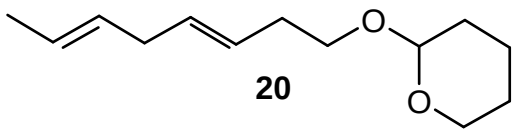
19

3.50 g (22.7 mmol) of alkyne **18** and 7.27 g (25.0 mmol, 1.1 eq.) tributyltin hydride were stirred for 3 h at 95 °C in the presence of a catalytic amount of azobisisobutyronitrile (150 mg) under argon. The mixture was cooled to RT and distilled under high vacuum to result in **19** and its *Z*-isomer as a colourless oil. [193]

Formula	$C_{21}H_{42}O_2Sn$
Molecular weight	444.92
Yield	8.38 g (83 %) [Lit.: 89 %][195]
Boiling point	140 - 144 °C (10^{-3} mbar) [Lit.: ~200 °C/0.1mbar] [195]

Composition of the *Z*/*E* ratio was determined from the intensity of the ^{1}H NMR signal of *δ* 2.44 and 2.34 (allylic-CH_2) of each isomer (*Z*:*E*=23:77).[222]
^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.85-1.90 (m, 33H, $Sn(CH_2CH_2CH_2CH_3)_3$, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 2.41-2.47 (m, 2H, SnCHCHC$H_2$$CH_2$OTHP), 3.40-3.90 (m, 4H, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 4.61 (t, *J* 3.28, 1H, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$); 5.91-5.94 (m, 2H, SnC*H*C*H*); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 9.38 ($Sn(CH_2CH_2CH_2CH_3)_3$), 13.67 ($Sn(CH_2CH_2CH_2CH_3)_3$), 19.49, 25.53, 30.68 ($CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 27.29, 29.17 ($Sn(CH_2CH_2CH_2CH_3)_3$), 38.13 (SnCHCHC$H_2$$CH_2$OTHP), 62.08, 66.86 ($CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 98.60 (OCHO), 129.92, 145.52 (SnCHCH); **IR** (cm^{-1}): 667, 869, 984, 1032, 1071, 1119, 1136, 1376, 1456, 1598, 2871, 2921, 2954.

6.1.1.3 2-((3E,6E)-3,6-Octadienyloxy)-tetrahydro-2H-pyran (**20**)



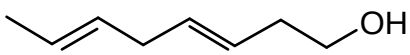
20

19.80 g (44.5 mmol) of the protected stannylbutenol **19** were diluted in 20 ml anhydrous THF and the solution was treated with 19.6 ml *n*-BuLi (2.5 M in hexane, 48.4 mmol) at -78 °C under argon. After 2 h of stir ring at -78 °C, 5.0 ml (6.60 g, 48.9 mmol) commercially available crotyl bromide was added and the mixture was stirred for 1 h at -78 °C and afterwards at room temperatur e for another 15 h. The solution was diluted with 50 ml Et_2O and washed with 50 ml saturated Na_2CO_3 solution. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. 80 ml MeOH was added to the obtained oil and $(n\text{-}Bu)_4Sn$ as the lower layer was removed and the remaining solution was evaporated. Crude **20** was used in the next step to **21**.[195]

Formula	$C_{13}H_{22}O_2$
Molecular weight	210.32
Yield	4.21 g (45 %) [Lit.: 72][192]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.54-1.87 (m, 9H, $CH_2O[CHC\mathit{H}_2C\mathit{H}_2C\mathit{H}_2CH_2O]_{THP}$, $C\mathit{H}_3$), 2.21-2.40 (m, 2H, $CHCHC\mathit{H}_2CH_2OTHP$), 2.60-2.64 (m, 2H, $CH_3CHCHC\mathit{H}_2CH$), 3.36-3.85 (m, 4H, $C\mathit{H}_2O[CHCH_2CH_2CH_2C\mathit{H}_2O]_{THP}$), 4.45-4.61 (m, 1H, $OC\mathit{H}O$), 5.28-5.57 (m, 4H, $4xC\mathit{H}_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 19.30 ($C\mathit{H}_3$), 19.90, 20.17, 25.59, 30.68, 34.88, 37.31 ($CH_2O[CH\mathit{C}H_2\mathit{C}H_2\mathit{C}H_2CH_2O]_{THP}$, $CH_3CHCH\mathit{C}H_2CH$), 63.24, 65.97 ($\mathit{C}H_2O[CHCH_2CH_2CH_2\mathit{C}H_2O]_{THP}$), 107.57 ($OC\mathit{H}O$), 126.71, 128.03, 128.81, 128.96 ($4xC\mathit{H}_{db}$); **IR** (cm^{-1}): 661, 684, 810, 869, 966, 1033, 1073, 1120, 1259, 1376, 1463, 2851, 2870, 2922, 2954.

6.1.1.4 (3E,6E)-3,6-Octadien-1-ol (**21**)



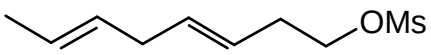
21

4.10 g (19.5 mmol) of **20** were dissolved in 70 ml MeOH, a catalytic amount of Dowex50 WX8 was added and the mixture was refluxed for 5 h at 80 °C. After cooling to RT the cationic ion exchanger was separated by filtering. Removal of the solvent gave crude **21** and its 3-*Z*-isomer (ratio see **19**) which were carefully distilled (vigreux column) to give a colourless oil.[195]

Formula	$C_8H_{14}O$
Molecular weight	126.20
Yield	1.01 g (41 %) [Lit.: 64 %][192]
Boiling point	88 - 92 °C (18 mbar) [Lit.: 92 - 94 °C/21mm] [192]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.65 (d, *J* 4.04, 3H, C*H*$_3$), 2.17 (br s, 1H, O*H*), 2.25-2.30 (m, 2H, C*H*$_2$CH$_2$OH), 2.67-2.76 (m, 2H, CH$_3$CHCHC*H*$_2$CH), 3.63 (t, *J* 6.30, 2H, C*H*$_2$OH), 5.40-5.54 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 17.85 (*C*H$_3$), 35.63, 35.90 (CH$_3$CHCH*C*H$_2$CH, *C*H$_2$CH$_2$OH), 61.99 (*C*H$_2$OH), 125.79, 126.38, 129.26, 132.38 (4x*C*H$_{db}$); **IR** (cm^{-1}): 696, 791, 863, 1012, 1257, 1412, 1717, 2962, 3329.

6.1.1.5 (3E,6E)-3,6-Octadienyl methanesulfonate (**22**)[196]



22

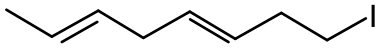
1.08 g (8.6 mmol) of **21** and 1.81 ml (10.3 mmol, 1.31 g) of NEt_3 were dissolved in 20 ml CH_2Cl_2 and cooled to 0 °C. To this solution 1.08 g (9.5 m mol, 0.73 ml) MsCl were added dropwise in 5-10 min and the mixture was stirred for further 15 min. Then, the reaction mixture was washed with 40 ml ice-cold water, 40 ml 10 % aqueous HCl-

solution, 20 ml saturated Na_2CO_3 solution and 40 ml saturated brine. After that the organic solution was dried over Na_2SO_4 and the solvent was removed to give **22** and 3-*Z*-isomers as a yellow oil. **22** was used as such in the next step to **23**.[196]

Formula	$C_9H_{16}O_3S$
Molecular weight	204.29
Yield	1.46 g (83 %) [Lit.: 94 %][195]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.58-1.66 (m, 3H, CH_3CHCH), 2.42-2.74 (m, 4H, CH_2CH$_2$OMs, CH$_3$CHCHCH_2CH), 2.99 (s, 3H, SCH$_3$), 4.22 (t, *J* 6.82, 2H, CH_2OMs), 5.38-5.60 (m, 4H, 4xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 17.85 (*C*H$_3$CHCH), 30.46, 32.34 (CH$_3$CHCH*C*H$_2$CH, *C*H$_2$CH$_2$OMs), 37.47 (S*C*H$_3$), 69.44 (*C*H$_2$OMs), 124.05, 126.07, 128.86, 133.16 (4x*C*H$_{db}$); **IR** (cm^{-1}): 734, 796, 912, 966, 1171, 1257, 1348, 1724, 2941, 2962, 3022.

6.1.1.6 (3E,6E)-3,6-Octadien-1-iodide (**23**)



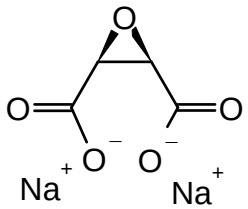
23

1.45 g (7.1 mmol) of **22**, 0.20 g (0.50 mmol, 0.23 ml) of Aliquat 336 and 9.31 g (62.1 mmol) of NaI were dissolved in 30 ml H_2O. The suspension was heated to 80 °C and stirred at that temperature over night. After cooling, the mixture was extracted twice with 40 ml *n*-pentane, water and dark impurities were separated and the organic layer was dried over anhydrous Na_2SO_4. After removal of the solvent **23** and 3-*Z*-isomers (ratio as determined for **19**) were obtained fairly pure.[195]

Formula	$C_8H_{13}I$
Molecular weight	236.09
Yield	1.17 g (70 %) [Lit.: 78][195]
Thin layer chromatography	CH_2Cl_2 (100 %); R_f (**21**)=0.32, R_f (**23**)=0.68

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.62-1.67 (m, 3H, C*H*$_3$), 2.54-2.69 (m, 4H, C*H*$_2$CH$_2$I, CH$_3$CHCHC*H*$_2$CH), 3.15 (t, *J* 7.32, 2H, C*H*$_2$I), 5.32-5.58 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 5.73 (*C*H$_2$I), 17.89 (*C*H$_3$), 35.47, 36.74 (CH$_3$CHCH*C*H$_2$CH, *C*H$_2$CH$_2$I), 125.94, 128.91, 129.03, 131.71 (4x*C*H$_{db}$); **IR** data were consistent with literature.[195]

6.1.1.7 Disodium cis-epoxysuccinate (**24**)



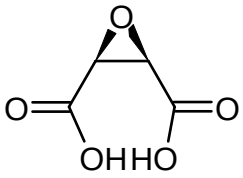
24

10.0 g (86.2 mmol) of maleic acid were dissolved in 30 ml H_2O. A solution of 5.17 g (129.2 mmol) NaOH in 15 ml H_2O was added carefully dropwise. To the warm mixture 0.57 g (1.73 mmol) $Na_2WO_4 \cdot 2H_2O$ and after that 3.52 g (1.04 mol) of 30 % H_2O_2 were added slowly. The temperature rose to 45 - 50 °C. The mixture was then heated to 65 - 68 °C and stirred at that temperature for 1.5 h. During that time the pH-value dropped; to maintain it at a minimum of 4, an aqueous solution of 5N NaOH was added dropwise as needed throughout that period. The mixture was then cooled down to 40 °C, concentrated to a volume of ca. 40 ml and then poured onto 50 ml acetone. The precipitate formed was filtered and dried over P_2O_5 to give **24** as a white solid which was used crude in the next step.[197]

Formula	$C_4H_2Na_2O_5$
Molecular weight	176.03
Yield	15.02 g (99 %) [Lit.: 100 %][197]
Melting point	259 - 261 °C (decomp.)

^{1}H NMR (400 MHz, D_2O, δ (ppm)): 4.82 (s, 2H, C*H*); **^{13}C NMR** (100 MHz, D_2O, δ (ppm)): 55.25 (*C*H), 173.67 (*C*O).

6.1.1.8 cis-Epoxysuccinic acid (**25**)



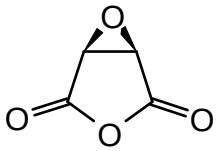
25

4.00 g (26.1 mmol) of **24** in 15 ml H_2O were added to a solution of 6.38 g (26.2 mmol) barium chloride in 20 ml H_2O. The barium salt of **24** precipitated immediately. The mixture was cooled and filtered. The precipitate was added to 1.00 g (8.31 mmol) anhydrous $MgSO_4$ in 25 ml anhydrous Et_2O and the suspension was stirred vigorously at 0 - 5 °C. Then, a solution of 1 ml conc. H_2SO_4 in 10 ml anhydrous Et_2O was added dropwise. The suspension was stirred overnight at RT, filtered, the filtrate dried over anhydrous Na_2SO_4 and concentrated *in vacuo* and dried again over P_2O_5. **25** was obtained as a white solid.[197]

Formula	$C_4H_4O_5$
Molecular weight	132.07
Yield	2.17 g (63 %) [Lit.: 77 %][197]
Melting point	147 °C [Lit.: 149 °C] [197]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm)): 4.31 (s, 2H, C*H*), acid protons could not be observed; **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 50.95 (*C*H), 162.18 (*C*O).

6.1.1.9 cis-Epoxysuccinic anhydride (**26**)



26

1.50 g (11.4 mmol) of **25** was dissolved in 10 ml trifluoroacetic anhydride, stirred at RT for 2d under argon, filtered and evaporated to afford **26** as a white solid.[198]

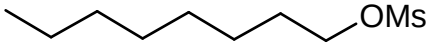
Formula	$C_4H_2O_4$
Molecular weight	114.06

Yield	0.75 g (58 %) [Lit.: 85 %][198]
Melting point	63 °C [Lit.: 63 - 64 °C] [198]

^{1}H NMR (400 MHz, DMSO, *δ* (ppm)): 3.77 (s, 2H, C*H*); **^{13}C NMR** (100 MHz, DMSO, *δ* (ppm)): 52.17 (*C*H), 167.38 (*C*O); **IR** data were consistent with literature.[199]

6.1.2 Scheme 2a. Synthesis of 4-oxoalkanamide cerulenin analogues

6.1.2.1 Octyl methanesulfonate (**27**)



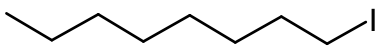
27

5.00 g (38.4 mmol) of commercially available 1-octanol and 8.08 ml (57.6 mmol) of NEt_3 were dissolved in 100 ml CH_2Cl_2 and stirred at 0 °C. To this solution 4.84 g (42.2 mmol, 3.27 ml) MsCl were added dropwise throughout 5 - 10 min and the mixture was stirred for further 15 min. Then, the reaction mixture was washed with 50 ml ice-cold water, 50 ml 10 % aqueous HCl-solution, 50 ml saturated Na_2CO_3 solution and 50 ml saturated brine. Afterwards the organic layer was dried over Na_2SO_4 and evaporated to give **27** as a yellow oil. Crude **27** was used in the next step to give **2**.

Formula	$C_9H_{20}O_3S$
Molecular weight	208.32
Yield	7.28 g (91 %)

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.87 (t, *J* 6.82, 3H, C*H*$_3$CH$_2$), 1.25-1.40 (m, 10H, CH$_3$C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$C*H*$_2$), 1.70-1.76 (m, 2H, C*H*$_2$CH$_2$OMs), 2.99 (s, 3H, SC*H*$_3$), 4.21 (t, *J* 6.60, 2H, C*H*$_2$OMs); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 14.00 (*C*H$_3$CH$_2$), 22.56, 25.38, 28.94, 29.03, 29.09, 31.66 (CH$_3$*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$*C*H$_2$), 37.32 (S*C*H$_3$), 70.18 (*C*H$_2$OMs); **IR** data were consistent with literature.[223]

6.1.2.2-Iodooctane (**2**)



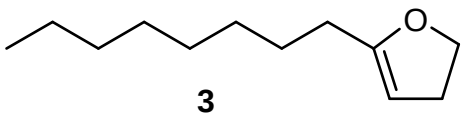
2

7.00 g (34.1 mmol) of **27**, 1.00 g (2.50 mmol, 1.20 ml) Aliquat 336 and 20.0 g (133.3 mmol) NaI were dissolved in 100 ml distilled H_2O. The suspension was heated to 80 ℃ and stirred at that temperature overnight. After cooling, the mixture was extracted twice with 50 ml *n*-pentane, water and dark impurities were separated and the organic layer was dried over anhydrous Na_2SO_4. After removal of the solvent the iodide **2** was obtained fairly pure and used as such in the next step.

Formula	$C_8H_{17}I$
Molecular weight	240.13
Yield	7.04 g (86 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.88 (t, *J* 7.04, 3H, C*H*$_3$), 1.26-1.39 (m, 10H, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.78-1.84 (m, 2H, CH_2CH_2I), 3.18 (t, *J* 7.08, 2H, CH_2I); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 7.27 (CH_2I), 14.05 (CH_3), 22.60, 28.50, 29.07, 30.51, 31.74, 33.57 ($CH_3CH_2CH_2CH_2CH_2CH_2CH_2$); **IR** data were consistent with literature.[224]

6.1.2.3 2,3-Dihydro-5-octylfuran (**3**)



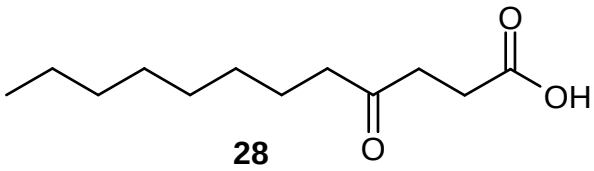
3

1.57 ml (20.80 mmol) of **2** were diluted in 120 ml anhydrous THF under argon and stirred for 30 min at -78 ℃. 15 ml (25.56 mmol, 1. 63 g) of 1.7M ^{t}BuLi solution in *n*-pentane was added throughout a period of 1h. The mixture was warmed to 0 ℃ for 1h, then again cooled down to -78 ℃. At that tempe rature a solution of 5.00 g (20.80 mmol) of 1-iodooctane in 10 ml anhydrous THF was added. The reaction mixture was warmed to RT and stirred for 1 h at that temperature. Afterwards, the solution was

cooled to 0 °C and 20 ml 50 % aqueous NH_4Cl solution were added carefully. The organic layer was separated and the aqueous layer was extracted three times with pentane/diethyl ether 1:1 (20 ml each). The organic solutions were combined, dried over anhydrous Na_2SO_4 and evaporated to obtain **3**, which was used without isolation in the next step to **28**.[201]

Formula	$C_{12}H_{22}O$
Molecular weight	182.30
Yield	3.72 g (98 %)

6.1.2.4 4-Oxododecanoic acid (**28**)



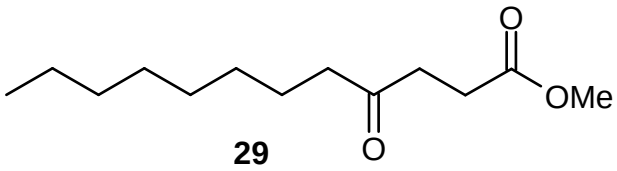
28

3.70 g (20.30 mmol) of **3** were dissolved in 90 ml THF, cooled to 0 °C and 27 ml of a 2.7M solution of Jones´ reagent[202] (chromic acid: 26.99 g CrO_3 were dissolved in 23 ml conc. H_2SO_4 and diluted with H_2O to 100 ml) were added very carefully over a period of 90 min. Then the solution was warmed to RT overnight and 70 ml H_2O and 70 ml Et_2O were added. After stirring for 30 min the layers were separated and the aqueous layer was extracted 4 times with 50 ml Et_2O each. The organic solutions were combined und washed three times with 30 ml H_2O and 40 ml 10 % NaOH solution each. The basic aqueous extracts were adjusted to pH=1 by 6N aqueous HCl solution and extracted 4 times with 40 ml CH_2Cl_2 each. The combined organic layers were dried over Na_2SO_4, the solvent was removed and the residue was recrystallised from *n*-hexane to give **28** as white crystals.[201]

Formula	$C_{12}H_{22}O_3$
Molecular weight	214.30
Yield	1.78 g (41 %)
Melting point	81 °C [Lit.: 80 - 81 °C] [225]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.88 (t, *J* 6.82, 3H, C*H*$_3$), 1.25-1.28 (m, 10H, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.55-1.61 (m, 2H, $CH_2CH_2COCH_2$), 2.44 (t, *J* 7.32, 2H, $CH_2CH_2COCH_2$), 2.60-2.74 (m, 4H, $COCH_2CH_2COOH$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.06 (*C*H_3), 22.62, 23.80, 27.65, 29.10, 29.18, 29.33, 31.79, 36.75 ($CH_3CH_2CH_2CH_2CH_2CH_2CH_2$, $COCH_2CH_2COOH$), 42.75 ($CH_2COCH_2CH_2COOH$), 177.90 (*C*OOH), 209.02 (*C*O); **IR** (cm^{-1}): 931, 1091, 1242, 1412, 1694, 1696, 2851, 2916, 2957, 3032.

6.1.2.5 Methyl-4-Oxododecanoate (**29**)



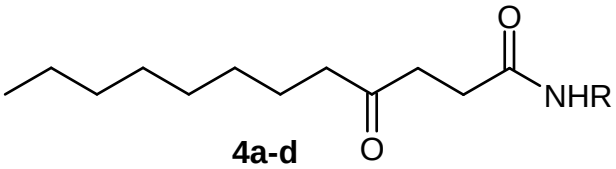
29

0.92 g (4.30 mmol) of oxododecanoic acid **28** were dissolved in a mixture of 20 ml anhydrous MeOH and anhydrous $CHCl_3$. 2.00 g (11.60 mmol) *p*-toluene sulfonic acid were added and the mixture was refluxed for 16 h under argon. After cooling down, the solution was washed with H_2O, saturated aqueous $NaHCO_3$ solution and H_2O again (20 ml each), dried over Na_2SO_4 and evaporated to yield pure **29** as a colourless oil.

Formula	$C_{13}H_{24}O_3$
Molecular weight	228.33
Yield	0.85 g (87 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.86 (t, *J* 6.82, 3H, C*H*$_3$CH$_2$), 1.24-1.27 (m, 10H, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.53-1.59 (m, 2H, $CH_2CH_2COCH_2$), 2.42 (t, *J* 7.46, 2H, $CH_2CH_2COCH_2$), 2.57, 2.70 (2x (t, *J* 6.44, 2H), $COCH_2CH_2COOMe$), 3.66 (OC*H*$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.03 (*C*H_3), 22.59, 23.78, 27.69, 29.07, 29.16, 29.31, 31.78, 36.97 ($CH_3CH_2CH_2CH_2CH_2CH_2CH_2$, $COCH_2CH_2COOMe$), 42.78 ($CH_2COCH_2CH_2COOMe$), 51.70 (O*C*H$_3$), 173.28 (*C*OOCH$_3$), 209.10 (*C*O); **IR** data were consistent with literature.[225]

6.1.2.6 4-Oxododecanamides (**4a-d**)



4a-d

a: R = H

b: R =

c: R =

d: R =

The following procedure was applied to obtain **4a-d** and is exemplified on **4a**:
0.50 g (2.34 mmol) of oxododecanoic acid **28** and 0.24 g (2.37 mmol, 0.33 ml) of NEt_3 were dissolved in 10 ml THF and cooled down to -10 °C. 0.32 g (2.34 mol, 0.31 ml) isobutyl chloroformate were added and stirring was continued for 30 min at -10 °C. Then, 0.33 g (9.43 mmol, 0.36 ml) conc. NH_4OH were added dropwise over a period of 30 min. Afterwards the solution was stirred for 2 h at RT. The precipitates were separated and the filtrate was concentrated *in vacuo*. The residue was dissolved in 100 ml Et_2O and washed with aqueous 5 % Na_2CO_3 solution and water (50 ml each). The organic layer was dried over Na_2SO_4 and evaporated. The residue was washed with acetone and isolated to yield pure **4a** as a white solid.[204]

6.1.2.6a **4a** (4-Oxodecanamide):

Formula	$C_{12}H_{23}NO_2$
Molecular weight	213.32
Yield	0.21 g (41 %)
Melting point	133 - 135 °C

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.87 (t, *J* 7.08, 3H, C*H*$_3$), 1.26 (br s, 10H, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.53-1.57 (m, 2H, $CH_2CH_2COCH_2CH_2CONH$), 2.44 (t, *J* 7.44, 2H, $CH_2COCH_2CH_2CONH$), 2.51, 2.78 (2x(t, *J* 6.20, 2H) $COCH_2CH_2CONH$), 3.05 (br s, NH), 5.96 (br s, NH); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.05 (CH_3), 22.60, 23.80, 28.90, 29.08, 29.17, 29.31, 31.78, 37.39, 42.77 (9xCH_2), 175.28 ($CONH_2$), 210.16 (*C*O); **IR** (cm^{-1}): 794, 1015, 1084, 1259, 1642, 1707, 2851, 2918, 2961, 3182, 3362; **MS** (m/z): 214 $(M+H)^+$.

6.1.2.6b **4b** (N-Benzyl-4-oxodecanamide):

Formula	$C_{19}H_{29}O_2N$
Molecular weight	303.19
Yield	0.62 g (44 %)
Melting point	98 °C

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.81 (t, *J* 6.82, 3H, C*H*$_3$), 1.20 (br s, 10H, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.46-1.52 (m, 2H, C*H*$_2$CH$_2$COCH$_2$CH$_2$CONH), 2.33-2.75 (m, 6H, C*H*$_2$COCH$_2$CH$_2$CONH, COC*H*$_2$C*H*$_2$CONH), 4.35 (d, *J* 5.56, 2H, NHC*H*$_2$), 5.95 (br s, NH), 7.16-7.25 (m, 5H, C*H*$_{ar}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.06 (*C*H$_3$), 22.61, 23.83, 29.10, 29.19, 29.32, 29.92, 31.79, 37.66, 42.85 (9x*C*H$_{2\ alkyl}$), 43.66 (NH*C*H$_{2\ benz}$), 127.44, 127.69, 128.67, 138.20 (*C*H$_{ar}$), 171.89 (*C*ONH), 210.31 (*C*O); **IR** (cm^{-1}): 695, 721, 1026, 1258, 1549, 1635, 1701, 2849, 2917, 2956, 3309.

6.1.2.6c **4c** (N-Cyclopropyl-4-oxodecanamide):

Formula	$C_{15}H_{27}O_2N$
Molecular weight	253.16
Yield	0.15 g (37 %)
Melting point	93 °C

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.45-0.48 (m, 2H, C*H*$_{2\ cyc}$), 0.71-0.75 (m, 2H, C*H*$_{2\ cyc}$), 0.87 (t, *J* 6.82, 3H, C*H*$_3$), 1.26 (br s, 10H, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.53-1.58 (m, 2H, C*H*$_2$CH$_2$COCH$_2$CH$_2$CONH), 2.37, 2.75 (2x (t, *J* 6.44, 2H), COC*H*$_2$C*H*$_2$CONH), 2.43 (t, *J* 7.46, 2H, C*H*$_2$COCH$_2$CH$_2$CONH), 2.64-2.67 (m, 1H, C*H*$_{cyc}$), 5.89 (br s, 1H, N*H*); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 6.52 (2xC*H*$_{2\ cyc}$ overlapping), 14.05 (*C*H$_3$), 22.61 (2 peaks overlapping), 23.81, 29.10, 29.18, 29.32, 29.76, 31.79, 37.64, 42.85 (9x*C*H$_{2\ alkyl}$, CH$_{cyc}$), 173.52 (*C*ONH$_2$), 210.15 (*C*O); **IR** (cm^{-1}): 798, 1017, 1089, 1260, 1641, 1700, 2848, 2916, 2955, 3300.

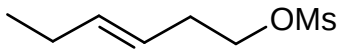
6.1.2.6d **4d** (N-Propyl-4-oxodecanamide):

Formula	$C_{15}H_{29}O_2N$
Molecular weight	255.15
Yield	0.40 g (42 %)
Melting point	78 °C

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.83-0.92 (m, 6H, 2xCH_3), 1.26 (br s, 10H, $CH_3CH_2CH_2CH_2CH_2CH_2$), 1.46-1.58 (m, 4H, $CH_2CH_2COCH_2CH_2CONH$, $NHCH_2CH_2$), 2.39-2.77 (m, 6H, $CH_2COCH_2CH_2CONH$, $COCH_2CH_2CONH$), 3.18 (dd, *J* 12.12, *J* 6.84, 2H, $NHCH_2$), 5.77 (br s, 1H, NH); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 11.30 ($NHCH_2CH_2CH_3$) 14.05 (CH_3), 22.61, 22.79, 23.83, 29.10, 29.18, 29.32, 30.01, 31.79, 37.83, 41.33, 42.86 (11xCH_2), 172.05 ($CONH_2$), 210.48 (CO); **IR** (cm^{-1}): 797, 1018, 1092, 1259, 1558, 1637, 1698, 2848, 2915, 1959, 3289.

6.1.3 Scheme 2b. Synthesis of a 4-oxoalkenamide cerulenin analogue

6.1.3.1 (E)-3-Hexenyl methanesulfonate (**30**)



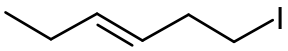
30

Compound **30** was synthesised starting from commercially available (*E*)-3-Hexen-1-ol and following the same procedure as for substance **27**.

Formula	$C_7H_{14}O_3S$
Molecular weight	178.25
Yield	3.40 g (96 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.97 (t, *J* 7.46, 3H, CH_3CH_2), 1.99-2.04 (m, 2H, CH_3CH_2), 2.43 (dd, *J* 6.82, *J* 1.26, 2H, CH_2CH_2OMs), 2.99 (s, 3H, SCH_3), 4.20 (t, *J* 6.82, 2H, CH_2OMs), 5.32-5.38, 5.58-5.64 2x(m, 1H, CH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 13.53 (CH_3CH_2), 25.53, 32.33 ($CH_3CH_2CHCHCH_2CH_2OMs$), 37.43 (SCH_3), 69.59 (CH_2OMs), 122.43, 136.39 (2xCH_{db}); **IR** (cm^{-1}): 728, 794, 816, 918, 955, 1170, 1348, 1462, 2938, 2964.

6.1.3.2 (E)-1-Iodo-3-hexene (**31**)



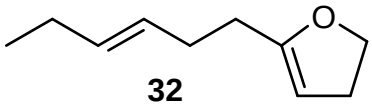
31

Compound **31** was synthesised starting from **30** and following the same procedure as for substance **2**.

Formula	$C_6H_{11}I$
Molecular weight	210.06
Yield	2.75 g (77 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.97 (t, *J* 7.46, 3H, C*H*$_3$), 1.96-2.02 (m, 2H, CH_3CH_2), 2.50-2.54 (m, 2H, CH_2CH_2I), 3.13 (t, *J* 7.32, 2H, CH_2I), 5.30-5.38, 5.51-5.58 2x (m, 1H, C*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 6.04 (CH_2I), 13.56 (CH_3), 25.47, 36.70 ($CH_3CH_2CHCHCH_2CH_2I$), 127.26, 134.99 (2xCH_{db}); **IR** (cm^{-1}): 895, 920, 1026, 1241, 1460, 1650, 2881, 2930, 2960, 3014.

6.1.3.3 (E)-5-(3-Hexenyl)-2,3-dihydrofuran (**32**)

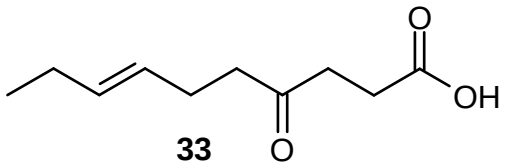


32

Compound **32** was synthesised starting from **31** and following the same procedure as for substance **3**. Product **32** was used without isolation in the next step to **33**.

Substance	**32**
Formula	$C_{10}H_{16}O$
Molecular weight	152.23
Yield	0.84 g (42 %)

6.1.3.4 (E)-4-Oxo-7-decenoic acid (**33**)



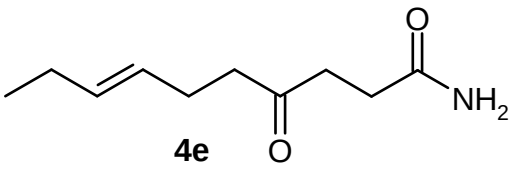
33

Compound **33** was synthesised starting from **32** and following the same procedure as for substance **28**.

Formula	$C_{10}H_{16}O_3$
Molecular weight	184.23
Yield	0.32 g (31 %)
Melting point	59 - 60 ℃

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.93 (t, *J* 7.46, 3H, C*H*$_3$), 1.93-1.99 (m, 2H, CH_3CH_2), 2.23-2.33 (m, 2H, $CH_2CH_2COCH_2CH_2COOH$), 2.50 (t, *J* 7.44, 2H, $CH_2COCH_2CH_2COOH$), 2.58-2.71 (m, 4H, $COCH_2CH_2COOH$), 5.31-5.49 (m, 2H, 2xC*H*$_{db}$), 9.22 (COO*H*); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 13.70 (*C*H_3), 25.44, 26.42, 27.72, 36.83, 42.52, (5x*C*H_2), 127.01, 133.20 (2x*C*H_{db}), 178.65 (*C*OOH), 208.39 (*C*O); **IR** (cm^{-1}): 933, 962, 1088, 1225, 1250, 1376, 1394, 1408, 1703, 2921, 2963.

6.1.3.5 (E)-4-Oxo-7-decenamide (**4e**)



4e

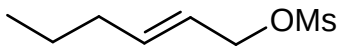
Compound **4e** was synthesised starting from **33** and following the same procedure as for substances **4a-d**.

Formula	$C_{10}H_{17}NO_2$
Molecular weight	183.25
Yield	0.10 g (42 %)

Melting point	104 °C

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.90-0.97 (m, 3H, C*H*$_3$), 1.94-2.00 (m, 2H, CH_3CH_2), 2.23-2.30 (m, 2H, $CH_3CH_2CHCHCH_2$), 2.45-2.77 (m, 6H, $CH_2COCH_2CH_2CONH$), 5.32-5.50 (m, 3H, 2xC*H*$_{db}$, NH), 5.68 (br s, NH); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 13.75 (*C*H$_3$), 25.47, 26.71, 29.09, 37.57, 42.61 (5x*C*H$_2$), 127.06, 133.22 (2x*C*H$_{db}$), 174.40 (*C*ONH$_2$), 209.46 (*C*O); **IR** (cm^{-1}): 964, 1089, 1239, 1305, 1375, 1406, 1658, 1707, 2931, 2961, 3177, 3338.

6.1.3.6 (E)-2-Hexenyl methanesulfonate (**34**)



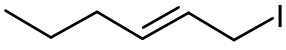
34

Compound **34** was synthesised starting from commercially available (*E*)-2-Hexen-1-ol and following the same procedure as for substance **27**.

Formula	$C_7H_{14}O_3S$
Molecular weight	178.25
Yield	8.80 g (99 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.98 (t, *J* 6.20, 3H, C*H*$_3$), 1.23-1.48 (m, 2H, CH_3CH_2), 1.89-2.19 (m, 2H, $CH_3CH_2CH_2$), 2.94 (s, 3H, SCH_3), 4.08 (d, *J* 6.82, 2H, C*H*$_2$OMs), 5.46-5.67 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.20 (*C*H$_3$), 23.36, 35.94 ($CH_3CH_2CH_2$), 38.07 (S*C*H$_3$), 65.44 (*C*H$_2$OMs), 128.85, 129.27 (2x*C*H$_{db}$); **IR** data were consistent with literature.[226]

6.1.3.7 (E)-1-Iodo-2-hexene (**35**)



35

To a solution of 3.00 g (20.0 mmol) NaI in 30 ml acetonitrile 2.17 g (20.0 mmol, 2.56 ml) trimethylsilyl chloride, 0.20 ml H_2O and 2.00 g (20.0 mmol, 2.37 ml) commercially

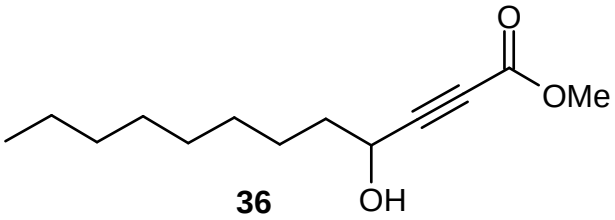
available (*E*)-2-hexen-1-ol were added slowly. The mixture was stirred at RT for 30 min and then diluted with 20 ml H_2O. The solution was extracted three times with Et_2O (30 ml each) and the organic layers were combined and washed with 10 % $Na_2S_2O_3$ solution, dried over Na_2SO_4 and evaporated to yield pure **35**. [227]

Formula	$C_6H_{11}I$
Molecular weight	210.06
Yield	2.14 g (51 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.88-0.90 (m, 3H, C*H*$_3$), 1.35-1.43 (m, 2H, CH_3CH_2), 1.98-2.02 (m, 2H, $CH_3CH_2CH_2$), 3.85-3.89 (m, 2H, C*H*$_2$I), 5.64-5.76, (m, 2H, C*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 6.90 (*C*H$_2$I), 13.54 (*C*H$_3$), 21.98, 34.02 ($CH_3CH_2CH_2$), 128.00, 135.07 (2x*C*H$_{db}$); **IR** data were consistent with literature.[227]

6.1.4 Scheme 3a. Synthesis of tetrahydrocerulenin analogues

6.1.4.1 Methyl 4-hydroxy-2-dodecynoate (**36**)



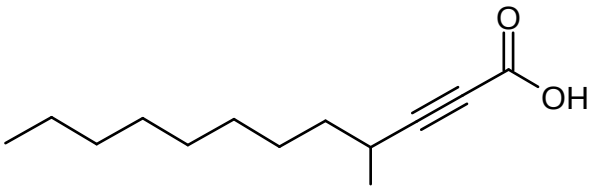
36

To a solution of 3.60 ml diisopropylamine (2.59 g, 25.6 mmol) in 50 ml dry THF at 0 ℃ 10.1 ml *n*-BuLi (25.3 mmol, 2.5M in hexane) were added dropwise. The solution was then cooled to -78 ℃ and 2.26 ml (2.12 g, 25.3 mmol) commercially available methyl propiolate were added dropwise. After 30 min, a solution of 3.27 g (3.95 ml, 23.0 mmol) commercially available 1-nonanal in 5 ml dry THF was added at that temperature. The mixture was stirred at -78 ℃ for 1.5 h, then a solution of 6 ml acetic acid in 30 ml dry THF was added, and the mixture was allowed to come to RT over 2h. The mixture was diluted with 50 ml Et_2O, washed successively with 30 ml saturated $NaHCO_3$ solution, water, brine and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was distilled to give pure **36**.

Formula	$C_{13}H_{22}O_3$
Molecular weight	226.31
Yield	3.09 g (54 %)
Boiling point	110 - 114 °C (10^{-3} mbar)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.81 (t, *J* 6.82, 3H, C*H*$_3$), 1.16-1.23 (m, 10H, $CH_3(CH_2)_5$), 1.37-1.41 (m, 2H, C*H*$_2$CH$_2$CHOHCCCOOMe), 1.65-1.72 (m, 2H, C*H*$_2$CHOHCCCOOMe), 2.40 (br s, 1H, CHO*H*), 3.71 (OC*H*$_3$), 4.42 (t, *J* 6.70, 1H, C*H*OHCCCOOMe); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.02 (*C*H$_3$), 22.59, 24.88, 29.11, 29.14, 29.34, 31.77, 36.82 (7x*C*H$_2$), 52.74 (O*C*H$_3$), 62.02 (*C*HOHCCCOOMe), 76.08, 88.48 (*CC*COOMe), 153.86 (*C*O).

6.1.4.2 4-Hydroxy-2-dodecynoic acid (**37**)



37

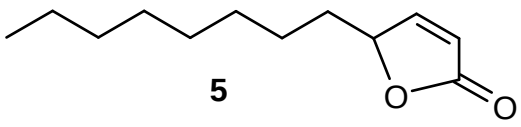
66 ml (0.2 mol, 3M in dry Et_2O) EtMgBr were added slowly at 0 °C to a vigorously stirred solution of 7.00 g (0.1 mol) commercially available propiolic acid in 200 ml anhydrous THF under argon. After the mixture was stirred for 2 h at 0 °C, 14.1 g (0.1 mol) commercially available 1-nonanal were added in one portion and the mixture was stirred at 0 °C for 1 h and allowed to warm to RT over 1h. The mixture was diluted with 50 ml Et_2O and acidified under cooling to pH 1 with 2M H_2SO_4. The organic phase was extracted three times with 50 ml saturated $NaHCO_3$ solution, and the combined $NaHCO_3$ solutions were acidified to pH 1 with 2M HCl and extracted three times with CH_2Cl_2 (50 ml each). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The residue was recrystallised from *n*-hexane and **37** was obtained as colourless crystals.[206]

Formula	$C_{12}H_{20}O_3$
Molecular weight	212.29

Yield	14.43 g (68 %) [53 %][187]
Melting point	70 - 71 ℃ [Lit.: 68 - 69 ℃] [206]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.87 (t, *J* 6.82, 3H, C*H*$_3$), 1.25-1.45 (m, 12H, $CH_3(CH_2)_6$), 1.72-1.78 (m, 2H, C*H*$_2$CHOHCCCOOH), 4.49 (t, *J* 6.70, 1H, C*H*OHCCCOOH), 7.09 (br s, COO*H*); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.03 (*C*H_3), 22.60, 24.88, 29.11, 29.15, 29.35, 31.79 ($CH_3(CH_2)_6$), 36.70 (*C*H_2CHOHCCCOOH), 62.11 (*C*HOHCOOH), 76.40, 89.26 (*CC*COOH), 155.67 (*C*O); **IR** data were consistent with literature.[206]

6.1.4.3 Dodec-2-enoic acid γ-lactone (**5**)



5

Route A (starting from **37**):[206]

Classical: 8.90 g (41.97 mmol) of acid **37** were dissolved in 160 ml EtOAc. A catalytic amount of Lindlar catalyst (500 mg) was added and the mixture was hydrogenated at 5 bar for 2 h in a sealed hydrogen tube at RT. Product formation was monitored by TLC. The mixture was filtered and the solvent was evaporated at 40 ℃ water bath temperature. After column chromatography, **5** was obtained pure in 57 % (4.70 g) [Lit.: 86 %][206] yield.

Microwave: 9.00 g (42.44 mmol) of **37** were dissolved in 160 ml EtOAc. A catalytic amount of Lindlar catalyst (500 mg) was added and the mixture was heated in the microwave oven for 2 h at 100 ℃ (500W max.) at 6 b ar of hydrogen pressure with a heating rate of 5 min and cooling down for 10 min. Product formation was monitored by TLC. After cooling to RT, the catalyst was filtered and the filtrate evaporated. After column chromatography, **5** was obtained pure in 66 % (5.50 g) yield.

Column chromatography	EtOAc/petroleum ether (20:80);

R_f (**37**)=0.14, R_f (**5**)=0.46

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.86 (t, *J* 6.82, 3H, CH_3), 1.20-1.45 (m, 12H, $CH_3(CH_2)_6$), 1.62-1.76 (m, 2H, CH_2[CHCHCHCOO]$_{lactone}$), 5.02 (td, *J* 6.44, *J* 1.76, 1H, CH_2[C*H*CHCHCOO]$_{lactone}$), 6.08, 7.44 (2x(d, *J* 5.56, 1H) CH_2[CHC*H*C*H*COO]$_{lactone}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.00 (*C*H_3), 22.56, 24.91, 29.07, 29.23, 29.26, 31.74, 33.13 (7x*C*H_2), 83.39 (CH_2[*C*HCHCHCOO]$_{lactone}$), 121.43, 156.29 (CH_2[CHC*H*C*H*COO]$_{lactone}$), 173.11 (*C*O); **IR** data were consistent with literature.[206]

Route B (starting from **36**):

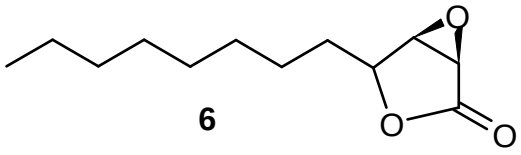
Classical: 0.25 g (1.11 mmol) of **36** were dissolved in 30 ml ethanol. A catalytic amount of Lindlar catalyst (100 mg) and 0.35 g (5.55 mmol) ammonium formate were added and the mixture was heated to reflux for 5 h. Product formation was monitored by TLC. After cooling to RT, the catalyst was filtered and the filtrate evaporated. The residue was diluted with Et_2O and the mixture was filtered again. The solvent was removed *in vacuo* and the remaining oil was purified by column chromatography to gain pure **5** in 40 % (88 mg) yield.

Microwave: 9.00 g (39.80 mmol) of **36** were dissolved in 250 ml EtOH. A catalytic amount of Lindlar catalyst (1 g) and 12.55 g (199.00 mmol) ammonium formate were added and the mixture was heated in the microwave oven for 3 h at 78 °C (500W max.) with a heating rate of 5 min and cooling down for 10 min. Product formation was followed by TLC. After cooling to RT, the catalyst was filtered and the filtrate evaporated at 40 °C water bath temperature. The residue was diluted with Et_2O and the mixture was filtered again. The solvent was removed and the remaining oil was purified by column chromatography to gain pure **5** in 51 % (3.98 g) yield.

Formula	$C_{12}H_{20}O_2$
Molecular weight	196.29
Column chromatography	EtOAc/petroleum ether (20:80); R_f (**36**)=0.80, R_f (**5**)=0.46

Further analytical data see above.

6.1.4.4 cis-2,3-Epoxydodecanoic acid γ-lactone (**6**)



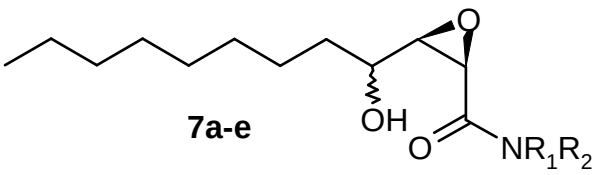
6

2.15 g (10.96 mmol) of the γ-lactone **5** were dissolved in 95 ml *p*-dioxane, cooled to 0 ℃ and treated with 30.14 ml (18.69 mmol) aqueous 5 % NaOCl solution added slowly. The mixture was stirred at 0 ℃ for 1 h and then allowed to come to RT over 1h. Afterwards, 100 ml Et_2O and 100 ml 5 % aqueous Na_2SO_3 solution were added, layers were separated and the aqueous phase was extracted twice with 50 ml Et_2O. The combined organic layers were washed with 50 ml 5 % aqueous NaOH solution, dried over Na_2SO_4 and the solvent was removed. The residue was purified by column chromatography to obtain pure **6**.[187]

Formula	$C_{12}H_{20}O_3$
Molecular weight	212.29
Yield	1.21 g (52 %) [Lit.: 47 %][187]
Column chromatography	*n*-hexane/EtOAc (90:10); R_f (**5**)=0.30, R_f (**6**)=0.49

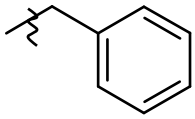
^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.87 (t, *J* 6.56, 3H, C*H*$_3$), 1.19-1.46 (m, 12H, CH_3(C*H*$_2$)$_6$), 1.62-1.69 (m, 2H, C*H*$_2$[CHCHOCHCOO]$_{lactone}$), 3.76 (dd, *J* 2.56, *J* 1.24, 1H, CH_2[CHC*H*OCHCOO]$_{lactone}$), 3.95 (d, *J* 2.56, 1H, CH_2[CHCHOC*H*COO]$_{lactone}$), 4.55 (td, *J* 6.70, *J* 1.24, 1H, CH_2[C*H*CHOCHCOO]$_{lactone}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 14.02 (*C*H_3), 22.56, 24.16, 29.07, 29.18, 29.24, 31.73, 32.04 (7x*C*H_2), 49.75 (CH_2[CH*C*HOCHCOO]$_{lactone}$), 57.98 (CH_2[CHCHO*C*HCOO]$_{lactone}$), 79.81 (CH_2[*C*HCHOCHCOO]$_{lactone}$), 170.32 (*C*OO); **IR** data were consistent with literature.[206]

6.1.4.5 cis-2,3-Epoxy-4-hydroxyalkanamides (**7a-e**)



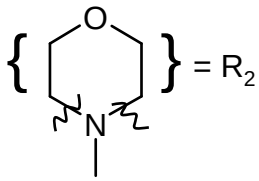
7a-e

a: R_1,R_2 = H

b: R_1 = H, R_2 = 

c: R_1 = H, R_2 = 

d: R_1, R_2 = Et_2

e: R_1 =  = R_2

6.1.4.5a. **7a** (cis-2,3-Epoxy-4-hydroxydodecanamide):

1.00 g (4.70 mmol) of epoxylactone **6** were dissolved in 10 ml MeOH and cooled to 0 °C for 15 min. 6.60 ml (88.2 mmol) concentrated NH_4OH (25 %) were added and after precipitation the solvent was evaporated, diluted with CH_2Cl_2, filtered, dried over Na_2SO_4 and either purified by recrystallisation from *n*-hexane or column chromatography.[187]

Formula	$C_{12}H_{23}NO_3$
Molecular weight	229.32
Yield	0.70 g (65 %)
Melting point	109 °C [Lit.: 108 - 109 °C] [187]
Column chromatography	CH_2Cl_2/MeOH (95:5) R_f (**6**)=0.84, R_f (**7a**)=0.52

^{1}H NMR (400 MHz, acetone-D_6, *δ* (ppm), *J* (Hz)): 0.88 (t, *J* 6.82, 3H, C*H*$_3$), 1.22-1.68 (m, 14H, CH_3(C*H*$_2$)$_7$, 3.00 (dd, *J* 8.08, *J* 4.56, 1H, C*H*OCHCONH), 3.41 (d, *J* 4.56, 1H, C*H*CONH), 3.48-3.54 (m, 1H, $CH_3(CH_2)_7$C*H*OH), 4.01 (d, *J* 5.04, 1H, O*H*), 6.70, 6.84 2x(br s, NH); **^{13}C NMR** (100 MHz, acetone-D_6, *δ* (ppm)): 14.35 (*C*H_3), 23.34, 25.70, 32.64, 36.12, 36.17 (5x*C*H_2) (3 *C*H_2 signals overlaid by the solvent), 55.07 (*C*HCONH), 60.74 (*C*HOCHCONH), 68.59 ($CH_3(CH_2)_7$*C*HOH), 169.85 (*C*ONH_2); **IR** (cm^{-1}): 720, 797, 906, 920, 1016, 1073, 1105, 1323, 1338, 1466, 1683, 2845, 2913, 3142.

6.1.4.5b-e **7b-e**:

The following procedure was applied to obtain **7b-e** and is exemplified on **7b**:

0.25 g (1.18 mmol) of **6** were dissolved in 5 ml MeOH and cooled to 0 °C. 1. 21 ml (2.33 mmol) of 2M benzylamine solution in THF was added and the mixture was stirred at 0 °C for 2 h. Then, the mixture was allo wed to warm to RT overnight. The solvent was removed and the residue was purified by column chromatography.

6.1.4.5b **7b** (N-Benzyl-cis-2,3-epoxy-4-hydroxydodecanamide):

7b was obtained as a white solid.

Formula	$C_{19}H_{29}NO_3$
Molecular weight	319.44
Yield	0.35 g (93 %)
Melting point	112 °C
Column chromatography	CH_2Cl_2/MeOH (95:5); R_f (**6**)=0.84, R_f (**7b**)=0.60

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.80 (t, *J* 6.82, 3H, C*H*$_3$), 1.16-1.37 (m, 12H, $CH_3(CH_2)_6$), 1.50-1.55 (m, 2H, $CH_3(CH_2)_6CH_2$), 2.33 (br s, 1H, O*H*), 3.01 (dd, *J* 8.08, *J* 4.56, 1H, C*H*OCHCON), 3.17-3.23 (m, 1H, $CH_3(CH_2)_7CH$OH), 3.50 (d, *J* 4.56, 1H, C*H*CON), 4.29-4.46 (m, 2H, NHC*H*$_2$), 6.40 (br s, 1H, NH), 7.16-7.23 (5xC*H*$_{arom}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.08 (*C*H$_3$), 22.64, 24.85, 29.20, 29.47 (2 peaks overlapping), 31.85, 34.74 (7x*C*H$_2$), 43.18 (NH*C*H$_2$), 54.59 (*C*HCON), 60.19 (*C*HOCHCON), 69.28 ($CH_3(CH_2)_7$*C*HOH), 127.90, 128.86, (*C*$_{arom.}$ overlapping), 137.31 (*C*$_{arom.\ q}$), 167.20 (*C*ONH); **IR** (cm^{-1}): 697, 735, 1027, 1063, 1238, 1276, 1295, 1448, 1546, 1632, 2846, 2918, 2957, 3081, 3231, 3532.

6.1.4.5c **7c** (N-Cyclopropyl-cis-2,3-epoxy-4-hydroxydodecanamide):

7c was obtained fairly pure without column chromatography as a yellow solid.

Formula	$C_{15}H_{27}NO_3$
Molecular weight	269.36
Yield	0.85 g (96 %)

Melting point	89 - 90 ℃

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.52-0.82 (m, 4H, C$H_{2\ cyc}$), 0.87 (t, *J* 6.82, 3H, CH_3), 1.21-1.66 (m, 14H, $CH_3(CH_2)_7$), 2.49-2.76 (m, br s overlapping, 2H, CH_{cyc}, O*H*), 3.08 (dd, *J* 6.30, *J* 4.80, 1H, C*H*OCHCON), 3.26-3.33 (m, 1H, $CH_3(CH_2)_7$C*H*OH), 3.51 (d, *J* 4.80, 1H, C*H*CON), 6.28 (br s, N*H*); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 6.49, 6.50 (*C*$H_{2\ cyc.}$), 14.07 (*C*H_3), 22.08 (*C*$H_{cyc.}$) 22.63, 24.84, 29.23, 29.46, 29.53, 31.83, 34.72 (7x*C*H_2), 54.53 (*C*HCON), 60.21 (*C*HOCHCON), 68.98 ($CH_3(CH_2)_7$*C*HOH), 168.77 (*C*ONH); **IR** (cm^{-1}): 698, 829, 873, 1025, 1071, 1283, 1463, 1537, 1644, 2846, 2918, 2955, 3303, 3522.

6.1.4.5d **7d** (N,N-Diethyl-cis-2,3-epoxy-4-hydroxydodecanamide):
7d was obtained fairly pure without column chromatography as a colourless oil.

Formula	$C_{16}H_{31}NO_3$
Molecular weight	285.42
Yield	0.67 g (98 %)

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.85 (t, *J* 6.12, 3H, C$H_3(CH_2)_6$), 1.09-1.42 (m, 18H, CH_3(C$H_2)_6$, 2xNCH_2CH_3), 1.61-1.67 (m, 2H, CH_2CHOH), 3.03 (dd, *J* 8.34, *J* 4.30, 1H, C*H*OCHCON), 3.13-3.17 (m, 1H, $CH_3(CH_2)_7$C*H*OH), 3.32-3.56 (m, 5H, C*H*CON, 2xNC$H_2$$CH_3$), 3.96 (br s, O*H*); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 12.53, 14.21 (2xNCH_2*C*H_3), 14.01 (*C*$H_3(CH_2)_6$)) 22.58, 24.91, 29.17, 29.42, 29.56, 31.79, 34.72 (7x*C*H_2), 39.96, 41.84 (2xN*C*H_2), 53.74 (*C*HCON), 59.46 (*C*HOCHCON), 71.60 ($CH_3(CH_2)_7$*C*HOH), 166.64 (*C*ON); **IR** (cm^{-1}): 666, 693, 783, 901, 1009, 1229, 1449, 1622, 1781, 2854, 2924.

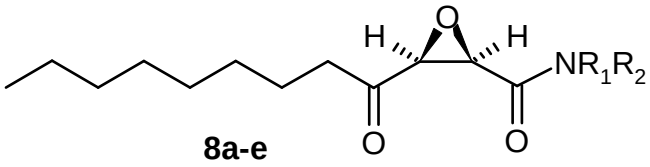
6.1.4.5d **7e** (N,N-Morpholino-cis-2,3-epoxy-4-hydroxydodecanamide):
7e was obtained fairly pure without column chromatography as a white solid.

Formula	$C_{16}H_{29}NO_4$
Molecular weight	299.41
Yield	0.50 g (89 %)

Melting point 81 °C

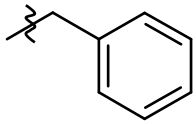
^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.86 (t, *J* 6.56, 3H, C*H*$_3$), 1.18-1.51 (m, 12H, $CH_3(CH_2)_6$), 1.63-1.69 (m, 2H, C*H*$_2$CHOH), 2.91 (br s, 1H, O*H*), 3.05-3.19 (m, 2H, $CH_3(CH_2)_7$C*H*OHC*H*OCH), 3.54-3.80 (m, 9H, C*H*CON, 2xNC*H*$_2$C*H*$_2$O); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.05 (*C*H_3), 22.61, 24.96, 29.19, 29.44, 29.60, 31.82 (6x*C*H_2), 34.74 (*C*H_2CHOH), 42.21, 45.84 (2x N*C*H_2CH_2O), 53.43 (*C*HCON), 59.65 (*C*HOCHCON), 66.63, 66.80 (2x NCH_2*C*H_2O), 71.64 ($CH_3(CH_2)_7$*C*HOH), 165.81 (*C*ON); **IR** (cm^{-1}): 803, 852, 998, 1015, 1100, 1244, 1444, 1477, 1642, 2854, 2926, 3479.

6.1.4.6 Tetrahydrocerulenin analogues (**8a-e**)



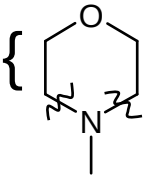
8a-e

a: R_1,R_2 = H

b: R_1 = H, R_2 = 

c: R_1 = H, R_2 = 

d: R_1, R_2 = Et_2

e: R_1 =  = R_2

6.1.4.6a **8a** (Tetrahydrocerulenin):

8a-e:

The following procedure was applied to obtain **8a-e** and is exemplified on **8a**:

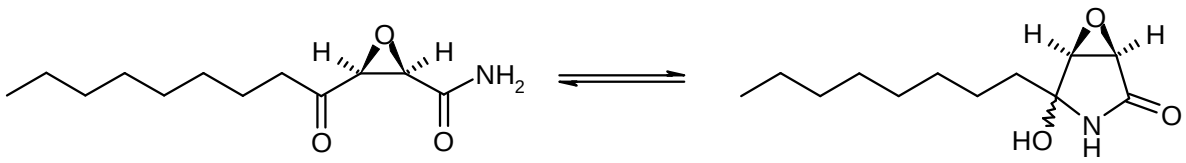
1.24 g (15.68 mmol) dry pyridine were dissolved in 20 ml dry CH_2Cl_2 and cooled to 0 °C. 0.79 g (7.90 mmol) dried CrO_3 were added at once and the mixture was stirred at 0 °C for another 5 min and was allowed to warm to R T for 1h. Then, a solution of 0.30 g (1.31 mmol) of **7a** in 5 ml dry CH_2Cl_2 was added and the mixture was stirred for 30 min and decanted afterwards over Celite®. The residue was washed three times with 10 ml CH_2Cl_2 and filtered. The combined organic extracts were washed with H_2O until the aqueous layer became colourless. The organic layer was dried over Na_2SO_4, evaporated and the residue was purified by column chromatography

first over a short column charged with Florisil® to remove chromic salts and second over a silica gel column. [228]

8a was obtained as a white solid.

Formula	$C_{12}H_{21}NO_3$
Molecular weight	227.32
Yield	119 mg (40 %) [Lit.: 37 %][187]
Melting point	78 °C [Lit.: 79 - 80 °C] [206]
Column chromatography	CH_2Cl_2/Et_2O (5:1); R_f (**7a**)=0.20, R_f (**8a**)=0.42

The NMR spectra also contained small peaks (ca. 10 %) of the cyclized isomers (**II**) (mostly overlapping with peaks of isomer **I**):



I **II**

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.87 (t, *J* 7.76, 3H, C*H*$_3$), 1.13-1.34 (m, 10H, $CH_3(CH_2)_5$), 1.76-1.81 (m, 2H, $CH_3(CH_2)_5CH_2CH_2COCH$), 2.52-2.60 (m, 2H, $CH_3(CH_2)_5CH_2CH_2COCH$), 3.71, 3.85 (2x (d, *J* 5.32, 1H), COC*H*OC*H*CONH), 5.80, 6.36 2x(br s, NH); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.03 (*C*H$_3$), 22.52, 22.57, 23.06, 29.02, 29.39, 31.80 ($CH_3(CH_2)_6$), 41.08 ($CH_3(CH_2)_6CH_2CO$), 55.30, 58.32 (CO*C*HO*C*HCONH), 167.52 (*C*ONH$_2$), 202.76 (*C*O); **IR** (cm^{-1}): 795, 1029, 1087, 1259, 1457, 1664, 1707, 2847, 2915, 2953, 3154, 3246, 3383.

6.1.4.6b **8b** (N-Benzyl-tetrahydrocerulenin):

8b was obtained as a white solid.

Formula	$C_{19}H_{27}NO_3$
Molecular weight	317.42

Yield	(140 mg) 21 %
Melting point	97 - 98 °C
Column chromatography	CH_2Cl_2/Et_2O (1:1); R_f (**7b**)=0.14, R_f (**8b**)=0.46

The NMR spectra showed only the cyclized isomers (see **8a**)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.79 (t, *J* 6.70, 3H, C*H*$_3$), 1.18-1.66 (m, 14H, $CH_3(CH_2)_7$), 3.55, 3.75 (2x (s, 1H), COC*H*OC*H*CONH), 4.23-4.42 (m, 2H, NC*H*$_2$), 7.11-7.22 (m, 5H, C*H*$_{aromat.}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.05 (*C*H$_3$), 22.59, 23.94, 29.09, 29.32, 29.68, 31.76, 34.81 ($CH_3(CH_2)_7$), 41.88 (N*C*H$_2$), 51.29, 58.72 (CO*C*HO*C*HCON), 89.89 (*C*$_q$OH), 127.39, 127.42, 128.62 (*C*$_{aromat.}$ overlapping), 137.21 (*C*$_{aromat.\ q}$), 170.77 (*C*ON); **IR** (cm^{-1}): 791, 1012, 1082, 1259, 1684, 2854, 2926, 2962, 3321.

6.1.4.6c **8c** (N-Cyclopropyl-tetrahydrocerulenin):
8c was obtained as a colourless oil.

Formula	$C_{15}H_{25}NO_3$
Molecular weight	267.36
Yield	(195 mg) 25 %
Column chromatography	CH_2Cl_2/Et_2O (1:1); R_f (**7c**)=0.14, R_f (**8c**)=0.42

The NMR spectra showed mostly the open form, but also peaks of the cyclized isomers (ca. 43 %) (see **8a**).

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)) **isomer I:** 0.52-0.75 (m, 4H, C*H*$_{2\ cyc}$), 0.86 (t, *J* 6.82, 3H, C*H*$_3$), 1.12-1.58 (m, 12H, $CH_3(CH_2)_6$), 2.48-2.64 (m, 3H, C*H*$_{cyc}$, $CH_3(CH_2)_6CH_2CO$), 3.68, 3.81 (2x (d, *J* 5.28, 1H), COC*H*OC*H*CONH), 6.43 (br s, NH); **isomers II** (ratio corresponding to 43 %): 0.52-0.75 (m, 14H, C*H*$_{2\ cyc}$, C*H*$_3$, overlapping with isomerI), 1.12-1.82 (m, 24H, $CH_3(CH_2)_6$, overlapping with isomerI), 1.88-2.25 (m, 6H, C*H*$_{cyc}$, $CH_3(CH_2)_6CH_2CO$), 3.58, 3.63, 3.72, 3,77 (2x (d, *J* 2.76, 1H), 2x (d, *J* 3.0, 1H), COC*H*OC*H*CONH); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm))

isomer I: 6.33, 6.50 (*C*$H_{2\ cyc.}$), 14.01 (*C*H_3), 21.92 (*C*$H_{cyc.}$) 22.56, 23.07, 28.98, 28.99, 29.16, 31.70, 41.01 (CH_3(*C*H_2)$_7$), 55.14, 58.32 (CO*C*HO*C*HCONH), 166.12 (*C*ONH), 202.60 (*C*O); **isomers II:** 0.96, 3.29, 3.42, 5.02 (*C*$H_{2\ cyc.}$), 14.01 (*C*H_3, overlapping with isomerI), 21.39, 21.65 (*C*$H_{cyc.}$) 22.60, 22.62, 22.86, 23.97, 29.09, 29.28, 29.44, 29.55, 29.86, 30.27, 31.73, 31.79, 34.26, 34.62 (CH_3(*C*H_2)$_7$), 51.61, 53.29, 55.96, 56.84 (CO*C*HO*C*HCONH), 89.55, 90.58 (*C*$_q$), 169.64, 171.07 (*C*ONH);**IR** (cm^{-1}): 666, 722, 765, 829, 862, 1025, 1365, 1427, 1667, 1703, 2851, 2920, 3300.

6.1.4.6d **8d** (N,N-Diethylamin-tetrahydrocerulenin):

8d was obtained as a colourless oil and purified by column chromatography successively from CH_2Cl_2 to EtOAc (100 % CH_2Cl_2 - 1:1 - 100 % EtOAc).

Formula	$C_{16}H_{29}NO_3$
Molecular weight	283.42
Yield	90 mg (36 %)

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.85 (t, *J* 6.96, 3H, C*H*$_3$(CH$_2$)$_7$), 1.04-1.27 (m, 16H, CH$_3$(C*H*$_2$)$_5$, 2xNCH$_2$C*H*$_3$), 1.51-1.55 (m, 2H, CH$_3$(CH$_2$)$_5$C*H*$_2$CH$_2$CO), 2.53 (dd, *J* 7.32, *J* 2.78, 2H, CH$_3$(CH$_2$)$_6$C*H*$_2$CO), 3.24-3.48 (m, 4H, 2xNC*H*$_2$), 3.64, 3.77 (2x (d, *J* 5.04, 1H), COC*H*OC*H*CON); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 12.66, 14.26 (2xNCH$_2$*C*H$_3$), 14.01 (*C*H$_3$(CH$_2$)$_7$) 22.56, 22.72, 29.01, 29.04, 29.28, 31.74 (CH$_3$(*C*H$_2$)$_6$), 39.88 (CH$_3$(CH$_2$)$_6$*C*H$_2$CO), 40.25, 41.45 (2xN*C*H$_2$), 54.57, 58.21 (CO*C*HO*C*HCON), 163.66 (*C*ON), 205.56 (*C*O); **IR** (cm^{-1}): 723, 786, 848, 1144, 1218, 1267, 1364, 1379, 1462, 1645, 1711, 2855, 2925.

6.1.4.6e **8e** (N,N-Morpholino-tetrahydrocerulenin):

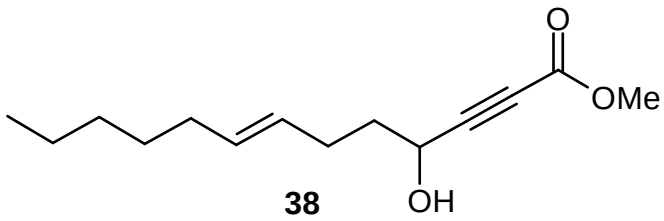
8e was obtained as a yellow oil and purified by column chromatography successively from CH_2Cl_2 to EtOAc (100 % CH_2Cl_2 - 1:1 - 100 % EtOAc).

Formula	$C_{16}H_{27}NO_4$
Molecular weight	297.39
Yield	360 mg (66 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.87 (t, *J* 6.68, 3H, C*H*$_3$), 1.19-1.27 (m, 10H, $CH_3(CH_2)_5$), 1.55-1.58 (m, 2H, $CH_3(CH_2)_5CH_2CH_2CO$), 2.54 (dd, *J* 14.02, *J* 7.56, 2H, $CH_3(CH_2)_6CH_2CO$), 3.54-3.80 (m, 10H, COC*H*OC*H*CON, 2xNC*H*$_2$C*H*$_2$O); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.05 (*C*H_3), 22.61, 22.77, 29.05, 29.08, 29.29, 31.78 ($CH_3(CH_2)_6$), 40.18 ($CH_3(CH_2)_6CH_2CO$), 42.28, 45.53 (2xNCH_2CH_2O), 54.55 57.84 (CO*C*HO*C*HCON$_{morph}$), 66.66, 66.67 (2xNCH$_2$*C*H$_2$O), 163.02 (*C*ON), 204.98 (*C*O); **IR** (cm^{-1}): 798, 1021, 1069, 1113, 1232, 1464, 1644, 1712, 2854, 2921.

6.1.5 Scheme 3b. Synthesis of dihydrocerulenin analogues

6.1.5.1 (E)-Methyl 4-hydroxytridec-7-en-2-ynoate (**38**)



38

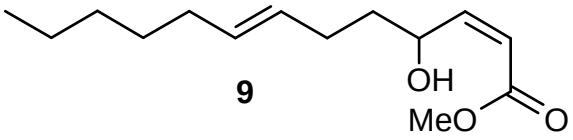
Compound **38** was synthesised starting from commercially available *trans*-4-decenal and following the same procedure as for substance **36**.

Formula	$C_{14}H_{22}O_3$
Molecular weight	238.16
Yield	9.70 g (75 %)
Column chromatography	EtOAc/petroleum ether (15:85); R_f (**start. material**)=0.92, R_f (**38**)=0.46

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.87 (t, *J* 6.94, 3H, C*H*$_3$), 1.20-1.33 (m, 6H, $CH_3(CH_2)_3$), 1.78-1.84 (m, 2H, CH_2CHOHCCCOOMe), 1.92-1.99 (m, 2H, $CH_3(CH_2)_3CH_2$CHCH), 2.12-2.18 (m, 2H, CH_2CH_2CHOHCCCOOMe), 2.50 (br s, 1H O*H*), 3.76 (OC*H*$_3$), 4.48 (dd, *J* 12.62, *J* 6.58, 1H, C*H*OH), 5.36-5.46 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 13.98 (*C*H_3), 22.45, 27.93, 29.08, 31.32, 32.45, 36.51 ($CH_3(CH_2)_4$CHCHCH_2CH_2CHOH), 52.74 (OCH_3), 61.46 (*C*HOHCCCOOMe),

76.27, 88.25 (*CC*CO), 128.05, 132.23 (*2xC*H_{db}), 153.83 (*C*O); **IR** (cm^{-1}): 937, 1095, 1246, 1442, 1662, 1752, 2232, 2916, 3320.

6.1.5.2 (2Z,7E)-Methyl 4-hydroxytrideca-2,7-dienoate (**9**)



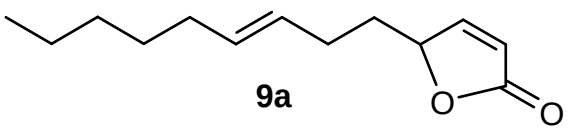
9

Compound **9** was synthesised starting from **38** and following the same procedure as for substance **37** / route B classical. The product **9** was used as such in the next step.

Formula	$C_{14}H_{24}O_3$
Molecular weight	240.34
Yield	7.35 g (87 %)

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.87 (t, *J* 6.94, 3H, C*H*$_3$), 1.23-1.33 (m, 6H, CH_3(C*H*$_2$)$_3$), 1.65-1.71 (m, 2H, C*H*$_2$CHOHCHCHCOOMe), 1.94-1.97 (m, 2H, $CH_3(CH_2)_3$C*H*$_2$CHCH), 2.08-2.14 (m, 2H, C*H*$_2$CH$_2$CHOHCHCHCOOMe), 3.45 (br s, 1H, O*H*), 3.73 (OC*H*$_3$), 4.86-4.90 (m, 1H, C*H*OHCHCHCOOMe), 5.39-5.44 (m, 2H, $CH_3(CH_2)_4$C*H*C*H*), 5.83 (d, *J* 11.86, 1H, C*H*COOMe), 6.28 (dd, *J* 11.86, *J* 7.06, 1H, C*H*CHCOOMe); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 14.01 (*C*H$_3$), 22.50, 28.41, 29.19, 31.35, 32.51, 36.31 (CH_3(*C*H$_2$)$_4$CHCH*C*H$_2$*C*H$_2$CHOH), 51.62 (O*C*H$_3$), 67.78 (*C*HOH), 119.70, 152.66 (*C*H*C*HCOOMe), 129.16, 131.32 ($CH_3(CH_2)_4$*C*H*C*H), 167.12 (*C*OOMe); **IR** (cm^{-1}): 942, 1090, 1242, 1455, 1608,1665, 1729, 2920, 3323.

6.1.5.3 (2Z,7E)-Trideca-2,7-dienoic acid γ-lactone (**9a**)



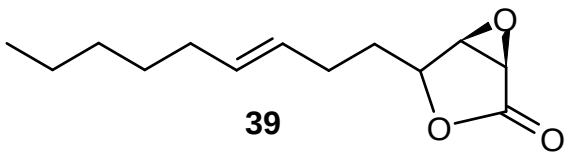
9a

7.38 g (35.44 mmol) of the ester **9** were dissolved in 100 ml Et_2O, a catalytic amount of *p*-toluene sulfonic acid (200 mg) was added and the mixture was stirred at RT overnight. The suspension was filtered, dried over Na_2SO_4 and evaporated. To obtain pure **9a** column chromatography was applied.

Formula	$C_{13}H_{20}O_2$
Molecular weight	208.29
Yield	5.32 g (72 %)
Column chromatography	CH_2Cl_2; R_f (**9**)=0.95, R_f (**9a**)=0.53

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.87 (t, *J* 6.94, 3H, C*H*$_3$), 1.24-1.33 (m, 6H, CH_3(C*H*$_2$)$_3$), 1.62-1.81 (m, 2H, C*H*$_2$[CHCHCHCOO]$_{lactone}$), 1.92-2.19 (m, 4H, $CH_3(CH_2)_3CH_2CHCHCH_2$), 5.04 (td, *J* 6.44, *J* 2.04, 1H, CH_2[C*H*CHCHCOO]$_{lactone}$), 5.32-5.51 (m, 2H, $CH_3(CH_2)_4CHCH$), 6.08, 7.44 (2x (d, *J* 5.80, 1H CH_2[CHC*H*C*H*COO]$_{lactone}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.00 (*C*H_3), 22.45, 28.06, 29.07, 31.33, 32.45, 33.09 ($CH_3(CH_2)_4CHCHCH_2CH_2$), 82.71 (CH_2[*C*HCHCHCOO]$_{lactone}$), 121.45, 156.25 (CH_2[CH*C*HCHCOO]$_{lactone}$), 127.74, 132.65 ($CH_3(CH_2)_4CHCH$), 173.03 (*C*O); **IR** (cm^{-1}): 818, 918, 1101, 1162, 1738, 2859, 2929, 2955, 3457.

6.1.5.4 (7E)-cis-2,3-Epoxytridec-7-enoic acid γ-lactone (**39**)



39

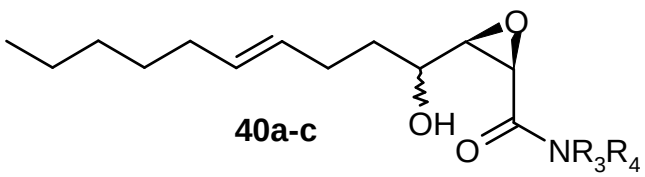
Compound **39** was synthesised starting from **9a** and following the same procedure as for substance **6**.

Formula	$C_{13}H_{20}O_3$
Molecular weight	224.29
Yield	0.91 g (34 %)
Column chromatography	CH_2Cl_2;

R_f (**9a**)=0.53, R_f (**39**)=0.67

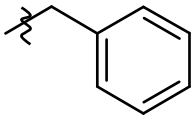
^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.87 (t, *J* 6.94, 3H, C*H*$_3$), 1.25-1.32 (m, 6H, $CH_3(CH_2)_3$), 1.69-1.77 (m, 2H, C*H*$_2$[CHCHOCHCOO]$_{lactone}$), 1.93-2.19 (m, 4H, $CH_3(CH_2)_3CH_2CHCHCH_2$), 3.75 (dd, *J* 2.52, *J* 0.76, 1H, CH_2[CHC*H*OCHCOO]$_{lactone}$), 3.96 (d, *J* 2.52, 1H, CH_2[CHCHOC*H*COO]$_{lactone}$), 4.58 (t, *J* 6.56, 1H, CH_2[C*H*CHOCHCOO]$_{lactone}$), 5.24-5.58 (m, 2H, 2xC*H*C*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 14.00 (*C*H_3), 22.45, 27.22, 29.02, 31.33, 31.86, 32.42 ($CH_3(CH_2)_4CHCHCH_2CH_2$), 49.78 (CH_2[CH*C*HOCHCOO]$_{lactone}$), 57.97 (CH_2[CHCHO*C*HCOO]$_{lactone}$), 79.19 (CH_2[*C*HCHOCHCOO]$_{lactone}$), 127.33, 132.87 (2x*C*H$_{db}$), 170.26 (*C*O); **IR** (cm^{-1}): 817, 916, 970, 1103, 1160, 1335, 1457, 1747, 2858, 2926, 2954.

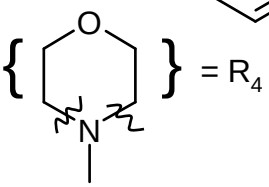
6.1.5.5 cis-2,3-Epoxy-4-hydroxyalkenamides (**40a-c**)



40a-c

a: R_3, R_4 = Et_2

b: R_3 = H, R_4 = 

c: R_3 =  = R_4

Compounds **40a-c** were synthesised starting from **39** and following the same procedure as for substances **7b-e**.

6.1.5.5a **40a** ((7E)-N,N-Diethyl-cis-2,3-epoxy-4-hydroxytridec-7-enamide):
40a was isolated as a yellow oil.

Formula	$C_{17}H_{31}NO_3$
Molecular weight	297.43
Yield	180 mg (34 %)
Column chromatography	CH_2Cl_2; R_f (**39**)=0.67, R_f (**40a**)=0.38

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.86 (t, *J* 6.94, 3H, C*H*$_3$(CH$_2$)$_4$, 1.18-1.32 (m, 12H, CH$_3$(C*H*$_2$)$_3$, 2xNCH$_2$C*H*$_3$), 1.70-1.76 (m, 2H, C*H*$_2$CHOHCHOCHCON), 1.91-2.18 (m, 4H, CH$_3$(CH$_2$)$_3$C*H*$_2$CHCHC*H*$_2$), 3.06 (dd, *J* 8.08, *J* 4.32, 1H, CHOHC*H*OCHCON), 3.16-3.22 (m, 1H, C*H*OHCHOCHCON), 3.33-3.58 (m, 5H, CHCHOC*H*CON, 2xNC*H*$_2$CH$_3$), 3.95 (br s, O*H*), 5.39-5.43 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 12.55, 14.24 (2xNCH$_2$*C*H$_3$), 14.01 (*C*H$_3$(CH$_2$)$_3$), 22.48, 27.98, 29.20, 31.34, 32.49, 34.57 (6x*C*H$_2$), 40.05, 41.90 (2xN*C*H$_2$), 53.83 (CHOHCHO*C*HCON), 59.39 (CHOH*C*HOCHCON), 71.05 (C*H*OHCHOCHCON), 129.13, 131.19 (2x*C*H$_{db}$), 166.64 (*C*ONH); **IR** (cm^{-1}): 967, 1072, 1271, 1363, 1380, 1436, 1489, 1621, 1787, 2855, 2923, 3395.

6.1.5.5b **40b** ((7E)-N-Benzyl-cis-2,3-epoxy-4-hydroxytridec-7-enamide):
40b was obtained as a white solid.

Formula	$C_{20}H_{29}NO_3$
Molecular weight	331.45
Yield	250 mg (56 %)
Melting point	82 - 83 ℃
Column chromatography	CH_2Cl_2/EtOAc (80:20); R_f (**39**)=0.84, R_f (**40b**)=0.67

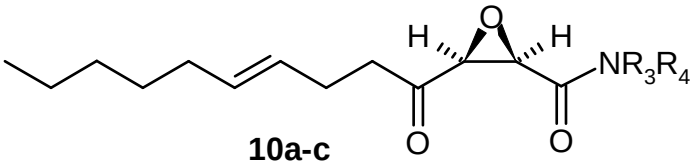
^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.81 (t, *J* 7.08, 3H, C*H*$_3$), 1.17-1.25 (m, 6H, CH$_3$(C*H*$_2$)$_3$), 1.60-1.64 (m, 2H, C*H*$_2$CHOHCHOCHCON), 1.83-2.10 (m, 4H, CH$_3$(CH$_2$)$_3$C*H*$_2$CHCHC*H*$_2$), 2.62 (br s, 1H, O*H*), 3.04 (dd, *J* 8.10, *J* 4.52, 1H, CHOHC*H*OCHCON), 3.24-3.30 (m, 1H, C*H*OHCHOCHCON), 3.51 (d, *J* 4.52, 1H, CHOHCHOC*H*CON), 4.39 (d, *J* 5.80, 2H, NHC*H*$_2$), 5.28-5.37 (m, 2H, 2xC*H*$_{db}$), 6.45 (br s, 1H, N*H*), 7.18-7.28 (5xC*H*$_{arom}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.03 (*C*H$_3$), 22.50, 27.97, 29.22, 31.37, 32.50, 34.59 (6x*C*H$_2$), 43.16 (NH*C*H$_2$), 54.65 (CHOHCHO*C*HCON), 60.18 (CHOH*C*HOCHCON), 68.61 (*C*HOHCHOCHCON), 127.85 (2 signals overlapping), 128.83, 128.93, 131.47 (*C*$_{arom.}$), 137.29 (*C*$_{arom.\ q}$), 167.21 (*C*ONH); **IR** (cm^{-1}): 698, 737, 968, 1027, 1060, 1261, 1441, 1541, 1631, 1682, 2853, 2922, 3239, 3535.

6.1.5.5c **40c** ((7E)-N-Morpholino-cis-2,3-epoxy-4-hydroxytridec-7-enamide):
40c was obtained as a yellow solid.

Formula	$C_{17}H_{29}NO_4$
Molecular weight	311.42
Yield	500 mg (72 %)
Melting point	63 - 64 ℃
Column chromatography	EtOAc; R_f (**39**)=0.95, R_f (**40c**)=0.36

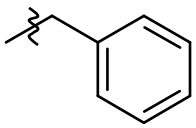
^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.88 (t, *J* 6.96, 3H, C*H*$_3$), 1.24-1.33 (m, 6H, CH_3(C*H*$_2$)$_3$), 1.72-1.78 (m, 2H, C*H*$_2$CHOHCHOCHCON), 1.96-2.22 (m, 2H, $CH_3(CH_2)_3$C*H*$_2$CHCHC*H*$_2$), 3.10 (dd, *J* 8.08, *J* 4.28, 1H, CHOHC*H*OCHCON), 3.19-3.22 (m, 1H, C*H*OHCHOCHCON), 3.55-3.77 (m, 10H, CHOHCHOC*H*CON, 2xNC*H*$_2$C*H*$_2$O, O*H*), 5.39-5.47 (m, 2H, 2xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 14.03 (*C*H_3), 22.50, 28.02, 29.23, 31.35, 32.51, 34.57 (6x*C*H_2), 42.25, 45.88 (2x N*C*H_2CH_2O), 53.49 (CHOHCHO*C*HCON), 59.52 (CHOH*C*HOCHCON), 66.65, 66.83 (2xNCH_2*C*H_2O), 71.22 (*C*HOHCHOCHCON), 129.10, 131.31 (2x*C*H_{db}), 165.78 (*C*ON); **IR** (cm^{-1}): 853, 966, 1012, 1102, 1114, 1240, 1445, 1477, 1635, 2852, 2922, 2956, 3471.

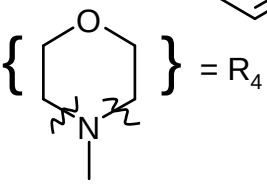
6.1.5.6 Dihydrocerulenin analogues (**10a-c**)



10a-c

a: R_3, R_4 = Et_2

b: R_3 = H, R_4 = 

c: R_3 =  = R_4

Compounds **10a-c** was synthesised starting from **40a-c** and following the same procedure as for substances **8a-e**.

6.1.5.6a **10a** ((7E)-N,N-Diethyl-cis-2,3-epoxy-4-oxotridec-7-enamide)):
10a was obtained as a yellow oil.

Formula	$C_{17}H_{29}NO_3$
Molecular weight	295.42
Yield	90 mg (50 %)
Column chromatography	CH_2Cl_2; R_f (**40a**)=0.38, R_f (**10a**)=0.81

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.85 (t, *J* 6.84, 3H, C*H*$_3$(CH$_2$)$_3$), 1.03-1.27 (m, 12H, CH$_3$(C*H*$_2$)$_3$, 2xNCH$_2$C*H*$_3$), 1.89-1.94 (m, 2H, C*H*$_2$COCHOCHCON), 2.19-2.62 (m, 4H, CH$_3$(C*H*$_2$)$_3$C*H*$_2$CHCHC*H*$_2$), 3.28-3.47 (m, 4H, 2xNC*H*$_2$), 3.63, 3.77 (2x (d, *J* 5.04, 2H), COC*H*OC*H*CON), 5.31-5.41 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 12.64, 14.26 (2xNCH$_2$*C*H$_3$), 13.96 (*C*H$_3$(CH$_2$)$_4$) 22.43, 25.81, 29.04, 31.28, 32.38 (5x*C*H$_2$), 39.77, 40.29 (2xN*C*H$_2$), 41.49 (*C*H$_2$COCHOCHCON), 54.56, 58.21 (CO*C*HO*C*HCON), 127.88, 131.57 (2x*C*H$_{db}$), 163.67 (*C*ON), 204.90 (*C*O); **IR** (cm^{-1}): 968, 1145, 1218, 1265, 1363, 1379, 1462, 1644, 1713, 2856, 2925.

6.1.5.6b **10b** ((7E)-N-Benzyl-cis-2,3-epoxy-4-oxotridec-7-enamide):
10b was obtained as a colourless oil.

Formula	$C_{20}H_{27}NO_3$
Molecular weight	329.43
Yield	180 mg (35 %)
Melting point	94 - 96 ℃
Column chromatography	*n*-hexane/EtOAc (60:40); R_f (**40b**)=0.22, R_f (**10b**)=0.30

The NMR spectra showed only the cyclized isomers.

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.81 (t, *J* 7.08, 3H, C*H*$_3$(CH$_2$)$_3$), 1.11-1.24 (m, 6H, CH$_3$(C*H*$_2$)$_3$), 1.69-1.83 (m, 6H, CH$_3$(CH$_2$)$_3$C*H*$_2$CHCHC*H*$_2$C*H*$_2$), 2.69 (s, 1H, O*H*), 3.67, 3.75 (2x (d, *J* 2.80, 1H), COC*H*OC*H*CON), 4.17-4.36 (m, 2H, NC*H*$_2$),

5.29-5.36 (m, 2H, 2xC*H*$_{db}$), 6.45 (br s, 1H, N*H*), 7.18-7.26 (m, 5H, C*H*$_{aromat.}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 14.01 (*C*H_3), 22.46, 25.97, 28.95, 31.28, 32.32, 24.84 (6x*C*H_2), 42.04 (N*C*H_2), 53.48, 56.28 (CO*C*HO*C*HCON), 88.95 (*C*$_q$OH), 127.42, 128.23 (2 signals overlapping), 128.52 (2 signals overlapping) (*C*$_{aromat.}$), 127.55, 131.96 (2x*C*H$_{db}$), 137.86 (*C*$_{aromat.\ q}$), 168.61 (*C*ON); **IR** (cm^{-1}): 656, 671, 696, 724, 778, 875, 965, 1439, 1681, 2852, 2924, 2959, 3315.

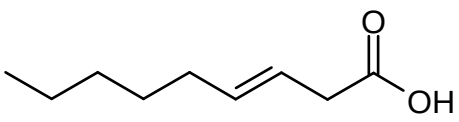
10b was also obtained via the procedure to **15a-b** and obtained in 79 % yield.

6.1.5.6c **10c** ((7E)-N-Morpholino-cis-2,3-epoxy-4-oxotridec-7-enamide):
10c was obtained as a yellow oil and purified by column chromatography successively from CH_2Cl_2 to EtOAc (100 % CH_2Cl_2 - 1:1 - 100 % EtOAc).

Substance	**10c**
Formula	$C_{17}H_{27}NO_4$
Molecular weight	309.40
Yield	210 mg (42 %)

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.81 (t, *J* 6.96, 3H, C*H*$_3$), 1.15-1.28 (m, 6H, CH_3(C*H*$_2$)$_3$), 1.84-2.22 (m, 4H, $CH_3(CH_2)_3CH_2CHCHCH_2$), 2.48-2.57 (m, 2H, C*H*$_2$COCHOCHCON), 3.46-3.76 (m, 10H, COC*H*OC*H*CON, 2xNC*H*$_2$C*H*$_2$O), 5.26-5.41 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 13.84 (*C*H_3), 22.30, 25.66, 28.89, 31.15, 32.25 (5x*C*H_2), 39.85 (*C*H_2COCHOCHCON), 42.09, 45.37 (2x NCH_2CH_2O), 54.37, 57.73 (CO*C*HO*C*HCON), 66.43, 66.46 (2x NCH$_2$*C*H$_2$O), 127.58, 131.66 (2x*C*H$_{db}$), 162.88 (*C*ON), 204.22 (*C*O); **IR** (cm^{-1}): 966, 1114, 1232, 1272, 1443, 1650, 1712, 2855, 2922, 2956.

6.1.5.7 (E)-Non-3-enoic acid (**41**)



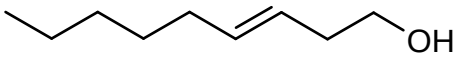
41

10.0 g (87.6 mmol) of commercially available *n*-heptanal and 10.0 g (96.1 mmol) malonic acid were dissolved in 20 ml triethanolamine and stirred at RT overnight. In addition, the mixture was refluxed for 8h. After cooling, 40 ml H_2O were added and the organic layer was separated, washed with 40 ml 20 % HCl and diluted with 50 ml ether. The solution was dried over Na_2SO_4, evaporated and distilled to afford pure **41**.[209]

Formula	$C_9H_{16}O_2$
Molecular weight	156.12
Boiling point	144 °C (20 mbar) [Lit.: 108 °C (0.8mm][209]
Yield	5.50 g (40 %) [Lit.: 20 %][209]

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.88 (t, *J* 6.82, 3H, C*H*$_3$), 1.24-1.37 (m, 6H, $CH_3(CH_2)_3$), 1.98-2.05 (m, 2H, $CH_3(CH_2)_3CH_2$), 3.06 (dd, *J* 6.56, *J* 1.04, 2H, C*H*$_2$COOH), 5.46-5.62 (m, 2H, 2xC*H*$_{db}$), 8.51 (br s, 1H, COO*H*); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 14.00 (*C*H_3), 22.47, 28.76, 31.33, 32.41 ($CH_3(CH_2)_4$), 37.78 (*C*H_2COOH), 120.65, 135.55 (2x*C*H$_{db}$), 178.35 (*C*OOH); **IR** data were consistent with literature.[229]

6.1.5.8 (E)-Non-3-en-1-ol (**42**)



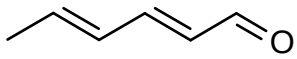
42

1.50 g (39.52 mmol) $LiAlH_4$ were diluted in anhydrous Et_2O under argon and stirred. 4.90 g (31.39 mmol) of the acid **41** in 100 ml anhydrous Et_2O were added slowly at RT, refluxed for 3 h afterwards and stirred overnight at RT. 40 ml ice cold water were added and the remaining solid was dissolved with 10 % H_2SO_4. The formed layers were separated and the aqueous phase was washed three times with 50 ml Et_2O each. The organic layers were combined, washed with 50 ml sat. aqueous NaCl solution and dried over Na_2SO_4. After removal of the solvent, the residue was distilled to give pure **42**. [230]

Formula	$C_9H_{18}O$
Molecular weight	142.14
Boiling point	116 °C (17 mbar) [Lit.: 60 - 61 °C (0.1 mm)][209]
Yield	2.70 g (61 %) [Lit.: 25 %][209]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.88 (t, *J* 6.86, 3H, C*H*$_3$), 1.24-1.36 (m, 6H, $CH_3(CH_2)_3$), 1.98-2.27 (m, 4H, $CH_3(CH_2)_3CH_2CHCHCH_2$), 3.61 (t, *J* 6.32, 2H, C*H*$_2$OH), 4.02 (s, 1H, O*H*), 5.31-5.58 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 14.01 (*C*H$_3$), 22.49, 29.74, 31.38, 32.61, 35.97 (5xC*H*$_2$), 62.02 (*C*H$_2$OH), 125.65, 134.34 (2x*C*H$_{db}$); **IR** data were consistent with literature.[231]

6.1.5.9 (2E,4E)-Hexa-2,4-dienal (**43**)



43

5.10 ml (56.4 mmol) oxalyl chloride in 100 ml dry CH_2Cl_2 were cooled to -50 °C under argon. 8.75 ml (112.8 mmol) dry DMSO were added and the mixture was stirred at -50 °C for 15 min. Afterwards 5.00 g (51.0 mmol) of commercially available (2*E*,4*E*)-hexa-2,4-dien-1-ol in 20 ml dry CH_2Cl_2 were added and the solution was stirred at -50 °C for another 30 min. 70 ml NEt_3 were added and the mixture was warmed to RT overnight. 100 ml H_2O was added and the aqueous layer was extracted 3 times with 100 ml CH_2Cl_2. The organic layers were combined, washed with 100 ml sat. NaOH solution, dried over anhydrous Na_2SO_4 and evaporated. The residue was distilled to afford pure **43**.[232]

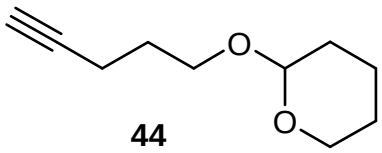
Formula	C_6H_8O
Molecular weight	96.13
Boiling point	61 °C (10mbar) [Lit.: 43 °C (5 mm)][233]
Yield	1.95 g (40 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.82 (d, *J* 5.60, 3H, C*H*$_3$), 5.82-6.01 (m, 1H, CHC*H*CHO), 6.22-6.34 (m, 2H, $CH_3CHCHCHCHCHO$), 6.89-7.05 (m, 1H,

$CH_3CHCHCH$), 9.38 (d, 1H, $CHCHCHO$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 18.81 (CH_3), 129.81, 130.05, 141.85, 152.57 ($CH_3CHCHCHCH$), 193.86 (CHO); **IR** data were consistent with literature.[234]

6.1.6 Scheme 4. Synthesis of cerulenin analogues

6.1.6.1 2-(4-Pentynyloxy)-tetrahydro-2H-pyran (**44**)



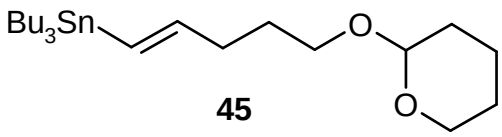
44

Compound **44** was synthesised starting from commercially available 4-pentyn-1-ol and following the same procedure as for substance **18**. The product was obtained as a colourless oil.[192]

Formula	$C_{10}H_{16}O_2$
Molecular weight	168.23
Yield	17.95 g (89 %)
Boiling point	98 - 100 ℃ (10 mbar)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), J (Hz)): 1.42-2.30 (m, 11H, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$, $CHCCH_2CH_2$), 3.40-3.89 (m, 4H, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 4.58 (t, J 4.28, 1H, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 15.30, 19.46, 25.43, 28.66, 30.62 ($CH_2CH_2CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 62.15, 65.73 ($CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 68.38, 83.93 ($CHCCH_2$), 98.76 ($CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$); **IR** ($cm^{-1}$): 812, 869, 905, 980, 1031, 1069, 1120, 1134, 1732, 2362, 2872, 2942, 3290.

6.1.6.2 Tributyl((E)-5-(tetrahydro-2H-2-pyranyloxy)1-pentenyl)stannane (**45**)



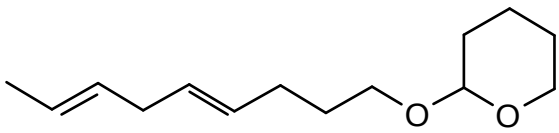
45

Compound **45** was synthesised starting from **44** and following the same procedure as for substance **19**. The product was obtained as a colourless oil.

Formula	$C_{22}H_{44}O_2Sn$
Molecular weight	459.29
Yield	33.71 g (69 %)
Boiling point	153 ℃ (10^{-3} mbar)

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.85-1.73, 2.19-2.23 (m, 39H, $Sn(CH_2CH_2CH_2CH_3)_3$, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$, $Bu_3SnCHCHCH_2CH_2CH_2$), 3.35-3.89 (m, 4H, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 4.57 (t, *J* 3.42, 1H, $CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 5.90-5.97 (m, 2H, $Bu_3SnCHCH$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 9.38 ($Sn(CH_2CH_2CH_2CH_3)_3$), 13.67 ($Sn(CH_2CH_2CH_2CH_3)_3$), 19.62, 25.51, 29.19, 30.75, 34.35 ($CH_2CH_2CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 27.29, 29.09 ($Sn(CH_2CH_2CH_2CH_3)_3$), 62.21, 66.99 ($CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 98.81 ($CH_2O[CHCH_2CH_2CH_2CH_2O]_{THP}$), 127.67, 148.76 ($Bu_3SnCHCH$); **IR** ($cm^{-1}$): 661, 869, 969, 988, 1020, 1034, 1075, 1119, 1136, 1200, 1455, 1599, 2871, 2922, 2953.

6.1.6.3 2-((4E,7E)-4,7-Nonadienyloxy)-tetrahydro-2H-pyran (**46**)

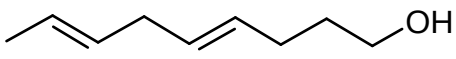


46

Compound **46** was synthesised starting from **45** and following the same procedure as for substance **20**. The product was obtained as a colourless oil and used without isolation in the next step to **47**.

Formula	$C_{14}H_{24}O_2$
Molecular weight	224.34
Yield	15.07 g (62 %)

6.1.6.4 (4E,7E)-4,7-Nonadien-1-ol (**47**)



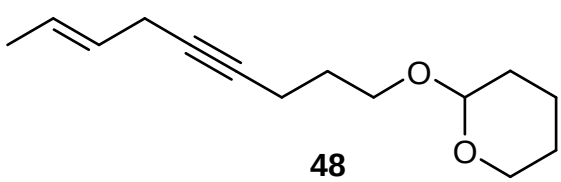
47

Compound **47** was synthesised starting from **46** and following the same procedure as for substance **21**. Product **47** and its *Z*-isomer were obtained as a colourless oil. Before separation, the composition of the *Z*/*E* ratio was determined from the intensity of the ^{1}H NMR signal of *δ* 2.72 and 2.65 (allylic-CH_2 between the double bonds) of each isomer (*Z*:*E*=27:73). The *Z*-isomer was then separated by column chromatography with 20 % $AgNO_3$ silica gel.[210]

Formula	$C_9H_{16}O$
Molecular weight	140.22
Yield	1.80 g (18 %)
Column chromatography	CH_2Cl_2; R_f (**46**)=0.80, R_f (**47**)=0.32

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 1.59-1.66 (m, 5H, C*H*$_3$, C*H*$_2$CH$_2$OH), 2.05-2.08 (m, 2H, C*H*$_2$CH$_2$CH$_2$OH), 2.64-2.67 (m, 2H, CH$_3$CHCHC*H*$_2$CHCH), 3.59-3.65 (m, 2H, C*H*$_2$OH), 5.39-5.44 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 17.82 (*C*H$_3$), 28.80, 32.30, 35.52 (3x*C*H$_2$), 62.41 (*C*H$_2$OH), 125.45, 129.31, 129.59, 130.03 (4x*C*H$_{db}$); **IR** (cm^{-1}): 970, 1034, 1177, 1371, 1445, 1717, 2929, 3391.

6.1.6.5 2-((E)-7-Nonen-4-ynyloxy)-tetrahydro-2H-pyran (**48**)



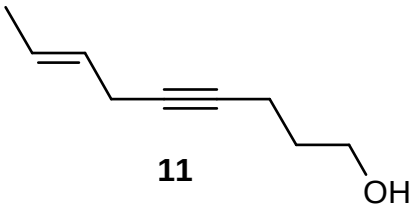
48

10.0 g (60.5 mmol) of compound **44** dissolved in anhydrous THF were added to 23.6 ml (66.6 mmol) of a 3M solution EtMgBr in Et_2O. After the addition was completed, the mixture was refluxed for 1h. After cooling to RT, 0.6 g (3.8 mmol) CuBr were added, followed by dropwise addition of 7.53 ml (62.1 mmol) commercially available crotyl bromide or 6.1 g (62.1 mmol) synthesized crotyl chloride **54** in 20 ml dry THF over 1h. Afterwards, the mixture was refluxed for 1h, stirred at RT for 24 h and quenched with 10 % aqueous NH_4Cl solution. The organic layer was washed again with 40 ml 10 % NH_4Cl solution, dried over anhydrous Na_2SO_4 and evaporated. The compound obtained was fairly pure **48**.[212]

Formula	$C_{14}H_{22}O_2$
Molecular weight	222.32
Yield	12.64 g (94 %) [Lit.: 67 %][212]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.51-1.80 (m, 11H, $CH_2O[CHC\mathit{H}_2C\mathit{H}_2C\mathit{H}_2CH_2O]_{THP}$, $C\mathit{H}_3$, $C\mathit{H}_2CH_2OTHP$), 2.26-2.31 (m, 2H, $C\mathit{H}_2CH_2CH_2OTHP$), 2.82-2.86 (m, 2H, $CH_3CHCHC\mathit{H}_2CC$), 3.44-3.50, 3.76-3.87 (m, 4H, $C\mathit{H}_2O[CHCH_2CH_2CH_2C\mathit{H}_2O]_{THP}$), 4.56-4.61 (m, 1H, $CH_2O[C\mathit{H}CH_2CH_2CH_2CH_2O]_{THP}$), 5.37-5.69 (m, 2H, 2x$C\mathit{H}_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 15.70 19.47, 21.98, 25.47, 29.19, 30.65 (6x$C\mathit{H}_2$), 17.59 ($C\mathit{H}_3$), 62.09, 66.04 ($C\mathit{H}_2O[CHCH_2CH_2CH_2C\mathit{H}_2O]_{THP}$), 77.95, 81.26 ($CH_3CHCHCH_2\mathit{CC}$), 98.71 ($CH_2O[\mathit{C}HCH_2CH_2CH_2CH_2O]_{THP}$), 125.86, 126.26 (2x$C\mathit{H}_{db}$); **IR** data were consistent with literature.[212]

6.1.6.6 (E)-7-Nonen-4-yn-1-ol (**11**)



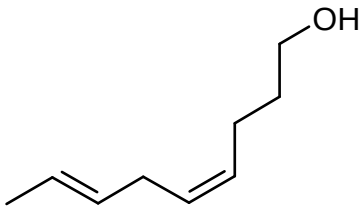
11

12.4 g (55.8 mmol) of the protected alcohol **48** were dissolved in 75 ml MeOH and 7 ml H_2O containing a catalytic amount of *p*-toluene sulfonic acid (0.89 g). The resulting mixture was refluxed for 2h, concentrated *in vacuo* and diluted with 40 ml H_2O and 50 ml *n*-pentane. Layers were separated and the aqueous phase was extracted twice with 40 ml *n*-pentane. Organic layers were combined, dried over anhydrous Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography to yield pure **11**.

Formula	$C_9H_{14}O$
Molecular weight	138.21
Yield	4.70 g (77 %) [Lit.: 86 %][212]
Column chromatography	CH_2Cl_2; R_f (**48**)=0.72, R_f (**11**)=0.28

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.64-1.76 (m, 5H, C*H*$_2$CH$_2$OH, C*H*$_3$), 2.27-2.32 (m, 2H, C*H*$_2$CH$_2$CH$_2$OH), 2.83-2.86 (m, 2H, CH$_3$CHCHC*H*$_2$CC), 3.74 (t, *J* 6.18, 2H, C*H*$_2$OH), 5.37-5.69 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 15.41 (CC*C*H$_2$CH$_2$CH$_2$OH), 17.58 (*C*H$_3$), 21.92 (CH$_3$CHCH*C*H$_2$CC), 31.53 (*C*H$_2$CH$_2$OH), 61.95 (*C*H$_2$OH), 78.42, 80.99 (CH$_2$*CC*CH$_2$), 125.70, 126.42 (2x*C*H$_{db}$); **IR** data were consistent with literature.[212]

6.1.6.7 (4Z,7E)-4,7-Nonadien-1-ol (**49**)



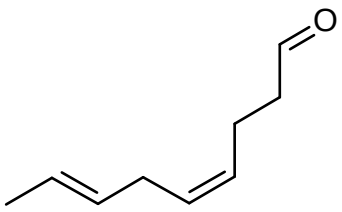
49

1.00 g (7.25 mmol) of alkynol **11** was hydrogenated at -10 °C and 7 bar over Lindlar catalyst (200 mg) dissolved in 50 ml *n*-pentane containing a catalytic amount of quinoline (0.05 ml). Hydrogen absorption ceased after 1 h and the catalyst was filtered off, the filtrate was evaporated and the product obtained was pure **49**.

Formula	$C_9H_{16}O$
Molecular weight	140.22
Yield	3.83 g (92 %) [Lit.: 95 %][212]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.59-1.68 (m, 5H, C*H*$_3$, C*H*$_2$CH$_2$OH), 2.09-2.15 (m, 2H, C*H*$_2$CH$_2$CH$_2$OH), 2.69-2.74 (m, 2H, CHCHC*H*$_2$CHCH), 3.63 (t, *J* 6.44, 2H, C*H*$_2$OH), 5.39-5.42 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 17.81 (*C*H$_3$), 23.39, 30.31, 32.45 (3x*C*H$_2$), 62.37 (*C*H$_2$OH), 125.17, 128.44, 129.38 (2 peaks overlapping) (4x*C*H$_{db}$); **IR** data were consistent with literature.[212]

6.1.6.8 (4Z,7E)-4,7-Nonadien-1-al (**12**)



12

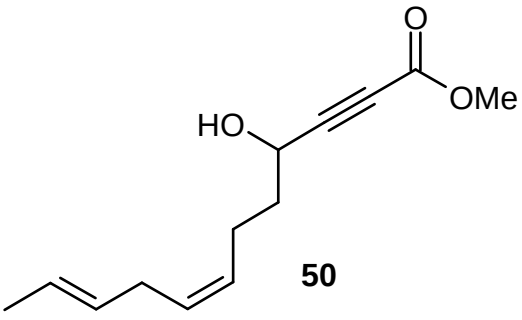
2.74 ml (30.27 mmol) oxalyl chloride in 90 ml dry CH_2Cl_2 were cooled to -50 °C under argon. 4.70 ml (60.53 mmol) dry DMSO were added and the solution was stirred for 10 min at that temperature. 3.83 g (27.34 mmol) of **49** in 20 ml dry CH_2Cl_2 were added dropwise over 5 min and the mixture was stirred at -50 °C for another 15 min. 37.30 ml NEt_3 were added and the solution was warmed to RT over 2 h (controlled by

TLC). 90 ml H_2O were added, layers were separated, the organic phase was washed with 90 ml H_2O and the combined aqueous layers were washed twice with 50 ml CH_2Cl_2. Combined organic layers were washed with 50 ml sat. aqueous NaOH solution, dried over anhydrous Na_2SO_4 and the solvent was removed. The residue was purified by column chromatography to afford pure **12**.

Formula	$C_9H_{14}O$
Molecular weight	138.21
Yield	2.80 g (74 %)
Column chromatography	CH_2Cl_2; R_f (**49**)=0.60, R_f (**12**)=0.41

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)):1.62-1.66 (m, 3H, C*H*$_3$), 2.35-2.40 (m, 2H, C*H*$_2$CH$_2$CHO), 2.45-2.51 (m, 2H, C*H*$_2$CHO), 2.71-2.74 (m, 2H, CH$_3$CHCHC*H*$_2$CHCH), 5.36-5.45 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 17.84 (*C*H$_3$), 19.96, 30.34, 43.71 (3x*C*H$_2$), 125.49, 127.63, 128.96, 129.44 (4x*C*H$_{db}$), 202.06 (*C*HO); **IR** (cm^{-1}): 971, 1032, 1175, 1373, 1453, 1717, 2930, 3383.

6.1.6.9 (7Z,10E)-Methyl 4-hydroxydodeca-7,10-dien-2-ynoate (**50**)



50

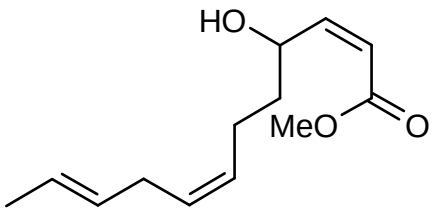
Compound **50** was synthesised starting from **12** and following the same procedure as for substance **36**. The product was purified by column chromatography.

Formula	$C_{13}H_{18}O_3$
Molecular weight	222.28
Yield	3.75 g (83 %)

Column chromatography	EtOAc/*n*-hexane (15:85); R_f (**12**)=0.93, R_f (**50**)=0.34

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.63 (d, *J* 5.56, 3H, C*H*$_3$CHCH), 1.78-1.83 (m, 2H, C*H*$_2$CHOHCCCOOMe), 2.18-2.26 (m, 2H, C*H*$_2$CH$_2$CHOHCCCOOMe), 2.59 (br s, 1H, O*H*), 2.68-2.75 (m, 2H, CH$_3$CHCHC*H*$_2$CHCH), 3.76 (OC*H*$_3$), 4.44-4.50 (m, 1H, C*H*OHCCCOOMe), 5.36-5.43 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 17.79 (*C*H$_3$CHCH), 22.61, 30.31, 36.49 (3x*C*H$_2$), 52.74 (O*C*H$_3$), 61.40 (*C*HOHCCCOOMe), 76.24, 88.19 (*CC*COOMe), 125.30, 128.05, 129.18, 129.47 (4x*C*H$_{db}$), 153.80 (*C*OOMe); **IR** (cm^{-1}): 902, 966, 1088, 1241, 1333, 1445, 1662, 1749, 2236, 2922, 3414.

6.1.6.10 (2Z,7Z,10E)-Methyl 4-hydroxydodeca-2,7,10-trienoate (**13**)



13

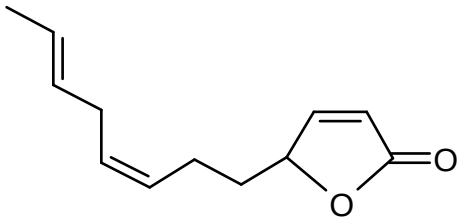
1.61 g (7.25 mmol) of the ester **50** was hydrogenated at -10 ℃ and 8-9 bar over Lindlar catalyst (200 mg) dissolved in 50 ml *n*-pentane containing a catalytic amount of quinoline (0.1 ml). Hydrogen absorption ceased after 2 h and the catalyst was filtered off, the filtrate was evaporated and the product obtained as pure **13**.

Formula	$C_{13}H_{20}O_3$
Molecular weight	224.30
Yield	2.05 g (94 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.60-1.66 (m, 5H, C*H*$_3$CHCH, C*H*$_2$CHOHCHCHCOOMe), 2.16-2.21, 2.70-2.74 (2x (m, 2H), CH$_3$CHCHC*H*$_2$CHCHC*H*$_2$), 3.41 (br s, 1H, O*H*), 3.73 (OC*H*$_3$), 4.87-4.91 (m, 1H, C*H*OHCHCHCOOMe), 5.39-5.44 (m, 4H, CH$_3$C*H*C*H*CH$_2$C*H*C*H*), 5.82 (d, *J* 11.86, 1H, C*H*COOMe), 6.29 (dd, *J* 11.86, *J* 7.08, 1H, C*H*CHCOOMe); **^{13}C NMR** (100 MHz,

$CDCl_3$, *δ* (ppm)): 17.84 (*C*H_3CHCH), 23.02, 30.36, 36.22 (3x*C*H_2), 51.62 (O*C*H_3), 67.65, 119.76 (*C*H*C*HCOOMe), 125.18, 128.62, 129.20, 129.41 (CH_3*C*H*C*HCH_2*C*H*C*H), 167.07 (*C*OOMe); **IR** (cm^{-1}): 903, 968, 1088, 1166, 1334, 1450, 1663, 1743, 2928, 3423.

6.1.6.11 (2Z,7Z,10E)-Dodeca-2,7,10-trienoic acid γ-lactone (**51**)



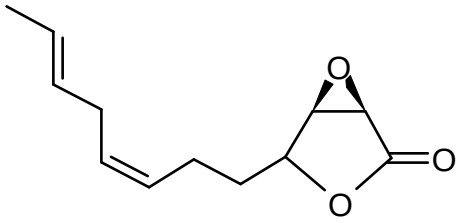
51

Compound **51** was synthesised starting from **13** and following the same procedure as for substance **9a**. The product was purified by column chromatography.

Formula	$C_{12}H_{16}O_2$
Molecular weight	192.25
Yield	1.27 g (87 %)
Column chromatography	CH_2Cl_2
R_f (educt)	0.95
R_f (product)	0.55

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 1.60-1.81 (m, 5H, C*H*$_3$, C*H*$_2$[CHCHCHCOO]$_{lactone}$), 2.18-2.26 (m, 2H, C*H*$_2$CH$_2$[CHCHCHCOO]$_{lactone}$), 2.72 (t, *J* 6.32, 2H, CH_3CHCHC*H*$_2$CHCH), 4.99-5.06 (m, 1H, CH_2[C*H*CHCHCOO]$_{lactone}$), 5.36-5.47 (m, 4H, CH_3C*H*C*H*CH_2C*H*C*H*), 6.10, 7.45 (2x (d, *J* 5.68, 1H), CH_2[CHC*H*C*H*COO]$_{lactone}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 17.84 (*C*H_3), 22.75, 30.35, 33.09 (3x*C*H_2), 82.71 (CH_2[*C*HCHCHCOO]$_{lactone}$), 121.55, 156.14 (CH_2[CH*C*H*C*HCOO]$_{lactone}$), 125.47, 127.79, 128.94, 129.79 (CH_3*C*H*C*HCH$_2$*C*H*C*H), 172.99 (*C*O); **IR** (cm^{-1}): 818, 901, 970, 1103, 1161, 1334, 1445, 1742, 2929, 3413.

6.1.6.12 (7Z,10E)-cis-2,3-Epoxydodeca-7,10-dienoic acid γ-lactone (**14**)



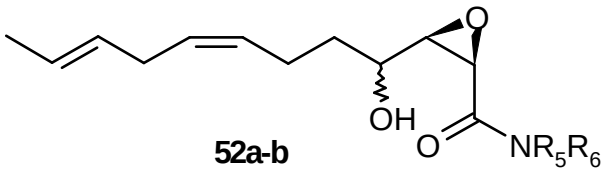
14

1.20 g (6.25 mmol) γ-lactone **51** were dissolved in 65 ml *p*-dioxane, chilled to 0 °C and treated with 17.16 ml (10.64 mmol) aqueous 5 % NaOCl solution added slowly. The mixture was stirred at 0 °C for 1 h and was then allowed to come to RT overnight. Afterwards, 100 ml Et_2O were added, layers were separated and the aqueous phase was extracted twice with 50 ml Et_2O. The combined organic layers were dried over Na_2SO_4 and the solvent was removed. The residue was purified by column chromatography to obtain pure **14**.

Formula	$C_{12}H_{16}O_3$
Molecular weight	208.25
Yield	0.47 g (36 %)
Column chromatography	CH_2Cl_2; R_f (**51**)=0.33, R_f (**14**)=0.52

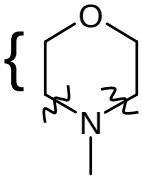
^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 1.61-1.75 (m, 5H, C*H*$_3$, C*H*$_2$[CHCHOCHCOO]$_{lactone}$), 2.20-2.26 (m, 2H, C*H*$_2$CH$_2$[CHCHOCHCOO]$_{lactone}$), 2.68-2.75 (m, 2H, CH$_3$CHCHC*H*$_2$CHCH), 3.76 (dd, *J* 2.52, *J* 0.52, 1H, CH$_2$[CHCHOC*H*COO]$_{lactone}$), 3.97 (d, *J* 2.52, 1H, CH$_2$[CHC*H*OCHCOO]$_{lactone}$), 4.56 (t, *J* 6.56, 1H, CH$_2$[C*H*CHOCHCOO]$_{lactone}$), 5.35-5.49 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 17.82 (*C*H$_3$), 21.96, 30.32, 31.89 (3x*C*H$_2$), 49.73 (CH$_2$[CH*C*HOCHCOO]$_{lactone}$), 57.95 (CH$_2$[CHCHO*C*HCOO]$_{lactone}$), 79.18 (CH$_2$[*C*HCHOCHCOO]$_{lactone}$), 125.57, 127.34, 128.80, 130.07 (4x*C*H$_{db}$), 170.21 (*C*O); **IR** (cm^{-1}): 852, 968, 1025, 1178, 1283, 1350, 1450, 1650, 1780, 2855, 2929, 3450.

6.1.6.13 cis-2,3-Epoxy-4-hydroxyalkadienamides (**52a-b**)



52a-b

a: R_5, R_6 = Et_2

b: R_5 =  = R_6

Compounds **52a-b** were synthesised starting from **14** and following the same procedure as for substances **7b-e**.

6.1.6.13a **52a** ((7Z,10E)-N,N-Diethyl-cis-2,3-epoxy-4-hydroxydodeca-7,10-dienamide):

52a was purified by gradient column chromatography (CH_2Cl_2/EtOAc (70:30) to EtOAc) and isolated as a pale yellow oil.

Formula	$C_{16}H_{27}NO_3$
Molecular weight	281.93
Yield	130 mg (71 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.13 (t, *J* 7.08, 3H, NCH_2CH_3), 1.22 (t, *J* 7.08, 3H, NCH_2CH_3), 1.61 (d, *J* 4.52, 3H, CH_3CHCH), 1.69-1.73 (m, 2H, $CH_2CHOHCHOCHCON$), 2.16-2.22 (m, 2H, $CH_2CH_2OHCHOCHCON$), 2.68-2.71 (m, 2H, $CH_3CHCHCH_2CHCH$), 3.01-3.06 (m, 1H, $CHOHCHOCHCON$), 3.13-3.18 (m, 1H, $CHOHCHOCHCON$), 3.33-3.96 (m, 5H, $CHOHCHOCHCON$, 2xNCH_2CH_3), 5.35-5.41 (m, 4H, 4xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$ δ (ppm)): 12.51, 14.21 (2xNCH_2CH_3), 17.79 (CH_3), 22.68, 30.30, 34.50 (3xCH_2), 39.97, 41.83 (2xNCH_2CH_3), 53.79, 59.30 ($CHOHCHOCHCON$), 71.06 ($CHOHCHOCHCON$), 125.07, 128.48, 129.20, 129.39 (4xCH_{db}), 166.56 (CON); **IR** (cm^{-1}): 964, 1140, 1218, 1266, 1451, 1643, 2935, 2970, 3362.

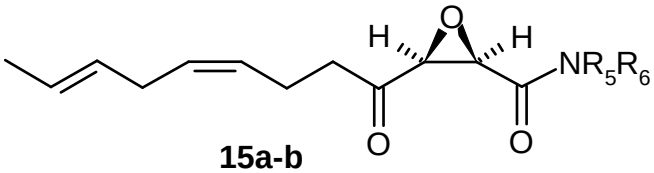
6.1.6.13b **52b** ((7Z,10E)-N,N-Morpholino-cis-2,3-epoxy-4-hydroxydodeca-7,10-dienamide):

52b was purified by gradient column chromatography (CH_2Cl_2/EtOAc (70:30) to EtOAc) and isolated as a pale yellow oil.

Formula	$C_{16}H_{25}NO_4$
Molecular weight	295.37
Yield	280 mg (89 %)

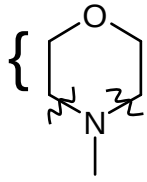
^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 1.19-1.23 (m, 2H, C*H*$_2$CHOHCHOCHCON), 1.60 (d, *J* 4.80, 3H, C*H*$_3$), 1.67-1.72 (m, 2H, C*H*$_2$CH$_2$CHOHCHOCHCON), 1.99 (s, 1H, O*H*), 2.16-2.21 (m, 2H, CH$_3$CHCHC*H*$_2$CHCH), 2.69, 3.16 (2x (d, *J* 6.32, 1H), CHOHC*H*OC*H*CON), 3.03-3.09 (m, 1H, C*H*OHCHOCHCON), 3.52-3.72 (m, 8H, 2xNC*H*$_2$C*H*$_2$O), 5.33-5.39 (m, 4H, 4xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 17.76 (*C*H$_3$), 22.60, 30.25, 34.43 (3x*C*H$_2$), 42.12, 45.72 (2xN*C*H$_2$CH$_2$O), 53.42, 59.35 (CHOH*C*HO*C*HCON), 66.50, 66.67 (2xNCH$_2$*C*H$_2$O), 70.85 (*C*HOHCHOCHCON), 125.07, 128.56, 129.04, 129.28 (4x*C*H$_{db}$), 165.62 (*C*ON); **IR** (cm^{-1}): 967, 1112, 1446, 1634, 1772, 2854, 2916, 3368.

6.1.6.14 Cerulenin analogues (**15a-b**)



15a-b

a: R_5, R_6 = Et_2

b: R_5 =  = R_6

The following procedure was applied to obtain **15a-b** and is exemplified on **15b**:[180]

To a mixture of 0.28 g (0.95 mmol) the amide **52b**, 4Å molecular sieves and 0.39 g (2.81 mmol) *N*-methylmorpholine *N*-oxide in 20 ml dry CH_2Cl_2 were added 0.03 g (0.09 mmol) tetrapropylammonium perruthenate under argon. The mixture was stirred at RT for 2h, diluted with 30 ml Et_2O and filtered through a short column filled with Celite®. The filtrate was evaporated and the residue was purified by column chromatography to afford pure **15b**.

6.1.6.14a **15a** ((7Z,10E)-N,N-Diethylcerulenin):

15a was obtained as a pale yellow oil.

Formula	$C_{16}H_{25}NO_3$
Molecular weight	279.37
Yield	90 mg (62 %)
Column chromatography	EtOAc/*n*-hexane (90:10); R_f (**52a**)=0.21, R_f (**15a**)=0.46

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.03 (t, *J* 7.08, 3H, NCH_2CH_3), 1.19 (t, *J* 7.08, 3H, NCH_2CH_3), 1.57 (d, *J* 4.80, 3H, CH_3), 2.20-2.27 (m, 2H, $CH_2CH_2COCHOCHCON$), 2.52-2.57 (m, 2H, $CH_2COCHOCHCON$), 2.62-2.67 (m, 2H, $CH_3CHCHCH_2CHCH$), 3.22-3.42 (m, 4H, 2xNCH_2CH_3), 3.59, 3.73 (2x (d, *J* 4.80, 1H), $COCHOCHCON$), 5.24-5.37 (m, 4H, 4xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 12.69, 14.32 (2xNCH_2CH_3), 17.83 (CH_3), 20.63, 30.30, 39.74 (3xCH_2), 40.32, 41.51 (2x NCH_2CH_3), 54.60, 58.23 ($COCHOCHCON$), 125.25, 128.01, 129.01, 129.20 (4xCH_{db}), 163.65 (CON), 204.87 (CO); **IR** (cm^{-1}): 965, 1144, 1218, 1264, 1450, 1645, 1712, 2935, 2970.

6.1.6.14b **15b** ((7Z,10E)-N,N-Morpholinocerulenin):

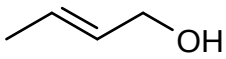
15b was obtained as a pale yellow oil.

Formula	$C_{16}H_{23}NO_4$
Molecular weight	293.36
Yield	180 mg (65 %)
Column chromatography	EtOAc/*n*-hexane (90:10); R_f (**52b**)=0.18, R_f (**15b**)=0.42

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.60 (d, *J* 4.80, 3H, CH_3), 2.26-2.31 (m, 2H, $CH_2CH_2COCHOCHCON$), 2.53-2.58 (m, 2H, $CH_2COCHOCHCON$), 2.66-2.71 (m, 2H, $CH_3CHCHCH_2CHCH$), 3.50-3.79 (m, 10H, $COCHOCHCON$, 2xNCH_2CH_2O), 5.26-5.41 (m, 4H, 4xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 17.76 (CH_3), 20.50, 30.23, 39.84 (3xCH_2), 42.16, 45.43 (2x NCH_2CH_2O), 54.42, 57.82 ($COCHOCHCON$), 66.52, 66.54 (2xNCH_2CH_2O), 125.26, 127.72, 129.03, 129.18 (4xCH_{db}), 162.89

(*C*ON), 204.24 (*C*O); **IR** (cm^{-1}): 966, 1031, 1068, 1092, 1232, 1271, 1443, 1681, 1712, 2857, 2919, 2962.

6.1.6.15 Crotyl alcohol (**53**)



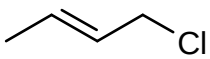
53

170 ml dry Et_2O were added to 5.42 g (0.14 mol) $LiAlH_4$ under argon. 35 g (0.5 mol) commercially available croton aldehyde were added slowly while stirring vigorously so that the mixture was refluxing gently. 20 min after the last addition, ice-cold H_2O was carefully added while the suspension was cooled with ice. The mixture was poured carefully onto 60 ml ice cold H_2O and 250 ml 10 % H_2SO_4 were added. After the remaining solids were dissolved, layers were separated and the aqueous phase was extracted twice with 50 ml Et_2O each. The organic phases were combined, dried over anhydrous Na_2SO_4 and evaporated. Distillation gave **53** as a colourless liquid.

Formula	C_4H_8O
Molecular weight	72.11
Yield	22.71 g (63 %)
Boiling point	119 - 120 °C [Lit.:118 - 122 °C] [235]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.68 (d, *J* 4.80, 3H, C*H*$_3$), 1.86 (br s, 1H, O*H*), 4.03 (s, 2H, C*H*$_2$OH), 5.60-5.69 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 17.57 (*C*H$_3$), 63.54 (*C*H$_2$), 127.95, 130.16 (2x*C*H$_{db}$); **IR** data were consistent with literature.[236]

6.1.6.16 Crotyl chloride (**54**)



54

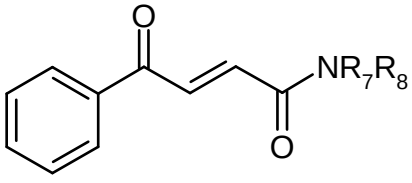
20.90 g (0.29 mol) crotyl alcohol were dissolved in 40 ml *s*-collidine under argon, cooled to 0 °C and 12.29 g (0.30 mol) LiCl in 190 m l dry DMF were added. 36.53 g (0.32 mol) of MsCl were added dropwise and the mixture was stirred for another 1.5 h at 0 °C. Afterwards, the reaction mixture was pou red onto 100 ml ice water and extracted twice with 100 ml *n*-pentane/diethyl ether (1:1). Organic layers were combined and washed twice with 50 ml sat. $CuNO_3$ solution, dried over anhydrous Na_2SO_4 and evaporated (300 mbar). The residue was distilled to yield pure **54**.[211]

Formula	C_4H_7Cl
Molecular weight	90.55
Yield	21.50 g (81 %) [Lit.: 50 %][211]
Boiling point	82 - 84 °C [Lit.: 84 °C] [237]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.72 (d, *J* 6.82, 3H, C*H*$_3$), 4.01 (d, *J* 7.08, 2H, C*H*$_2$Cl), 5.58-5.82 (m, 2H, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 17.52 (*C*H$_3$), 45.34 (*C*H$_2$), 127.14, 130.87 (2x*C*H$_{db}$); **IR** data were consistent with literature.[238]

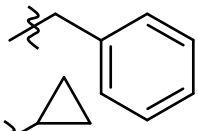
6.1.7 Scheme 5. Synthesis of aromatic cerulenin analogues

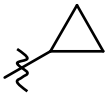
6.1.7.1 3-Benzoylacrylamides (**16a-e**)

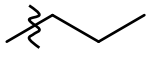


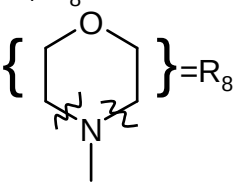
16a-e

a: R_7=R_8=Et

b: R_7=H, R_8= 

c: R_7=H, R_8= 

d: R_7=H, R_8= 

e: R_7= 

=R_8

The following procedure was applied to obtain **16a-e** and is exemplified on **16a**:
2.25 g (12.8 mmol) of commercially available 3-benzoylacrylic acid were dissolved in 25 ml dimethoxyethane and cooled to -10 °C under argon. 2.36 ml (15.4 mmol) isobutylchloroformate were added, followed by 1.70 ml (15.4 mmol) *N*-methylmorpholine. Afterwards, 18.86 ml (31.6 mmol) of a 2M solution of *N*,*N*-diethylamine in dry THF was added and stirred for 15 min at -10 °C and 1 h at RT. 20 ml H_2O were added and the mixture was extracted three times with 30 ml CH_2Cl_2 each. Organic layers were combined, washed with 20 ml sat. aqueous NaOH solution and H_2O, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified to yield pure **16a**.[187]

6.1.7.1a N,N-Diethyl-3-benzoyl-acrylamide (**16a**):
16a was purified by column chromatography and obtained as yellow oil.

Formula	$C_{14}H_{17}NO_2$
Molecular weight	231.29
Yield	2.96 g (99 %)
Column chromatography	EtOAc/Et_2O (50:50); R_f (**start. material**)=0.11, R_f (**16a**)=0.58

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.13-1.23 (m, 6H, 2xCH_3), 3.39-3.50 (m, 4H, 2xCH_2CH_3), 7.38-7.97 (m, 7H, 5x$CH_{aromat.}$, 2xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 12.89, 15.00 (2xCH_3), 41.00, 42.39 (2xCH_2), 128.67 (4 peaks overlapping), 132.86, 133.50, 133.72, 136.89 (6x$C_{aromat.}$, 2xCH_{db}), 164.12 (*C*ON), 189.55 (*C*O); **IR** (cm^{-1}): 688, 723, 789, 968, 1008, 1139, 1205, 1290, 1314, 1429, 1446, 1612, 1633, 2933, 2973.

6.1.7.1b N-Benzyl-3-benzoyl-acrylamide (**16b**):
16b was recrystallized from CH_2Cl_2/EtOAc (90:10) and obtained as a white solid.

Formula	$C_{17}H_{15}NO_2$
Molecular weight	265.31
Yield	1.20 g (36 %)
Melting point	152 - 153 °C [Lit.:153 - 154 °C] [239]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 4.59 (d, *J* 5.80, 2H, NHCH_2), 6.63 (br s, 1H, N*H*), 7.08-8.03 (m, 12H, 10xC$H_{aromat.}$, 2xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 44.12 (CH_2), 127.76, 127.93 (2 peaks overlapping), 128.81 (2 peaks overlapping), 128.83 (4 peaks overlapping), 133.41, 133.79, 135.21, 136.76, 137.47 (12x$C_{aromat.}$, 2xCH_{db}), 163.92 (*C*ON), 189.78 (*C*O); **IR** (cm^{-1}): 685, 731, 747, 985, 1193, 1309, 1445, 1540, 1634, 3026, 3285.

6.1.7.1c N-Cyclopropyl-3-benzoyl-acrylamide (**16c**):

16c was purified by column chromatography, recrystallized from MeOH and obtained as a pale yellow solid.

Formula	$C_{13}H_{13}NO_2$
Molecular weight	215.25
Yield	1.20 g (44 %)
Melting point	94 - 95 °C
Column chromatography	CH_2Cl_2/acetone (98:2); R_f (**start. material**)=0.11, R_f (**16c**)=0.52

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.59-0.84 (m, 4H, NHCHCH_2CH_2), 2.86-2.92 (m, 1H, NHC*H*CH_2CH_2), 7.10 (d, *J* 15.16, 1H, 1xCH_{db}), 7.32 (br s, 1H, N*H*), 7.43-7.99 (m, 6H, 5xC$H_{aromat.}$, 1xCH_{db}); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 6.55 (2 peaks overlapping) (NHCH*C*H_2*C*H_2), 23.11 (NH*C*HCH_2CH_2), 128.72, 128.75 (3 peaks overlapping), 132.49 (5x$C_{aromat.}$), 133.71, 135.66 (2x*C*H*C*H_{db}), 136.78 ($C_{aromat.\ q}$), 165.56 (*C*ON), 189.98 (*C*O); **IR** (cm^{-1}): 680, 758, 966, 1018, 1194, 1291, 1326, 1446, 1535, 1641, 1690, 3291.

6.1.7.1d N-Propyl-3-benzoyl-acrylamide (**16d**):

16d was recrystallized from EtOAc and obtained as a pale yellow solid.

Formula	$C_{13}H_{15}NO_2$
Molecular weight	217.26
Yield	1.70 g (61 %)
Melting point	88 - 89 °C

^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 0.92 (t, *J* 7.44, 3H, C*H*$_3$), 1.54-1.62 (m, 2H, NHCH$_2$C*H*$_2$CH$_3$), 3.30-3.37 (m, 2H, NHC*H*$_2$CH$_2$CH$_3$), 7.20 (d, *J* 15.12, 1H, 1xC*H*$_{db}$), 7.30 (br s, 1H, N*H*), 7.39-7.97 (m, 6H, 5xC*H*$_{aromat.}$, 1xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 11.30 (*C*H$_3$), 22.51, 41.65 (NH*C*H$_2$*C*H$_2$CH$_3$), 128.63 (2 peaks overlapping), 128.66 (2 peaks overlapping), 132.39 (5x*C*$_{aromat.}$), 133.61, 136.09 (2x*C*H$_{db}$), 136.74 (*C*$_{aromat.\ q}$), 164.18 (*C*ON), 190.03 (*C*O); **IR** (cm^{-1}): 682, 703, 966, 1003, 1193, 1287, 1325, 1443, 1556, 1634, 2871, 2931, 2958, 3286.

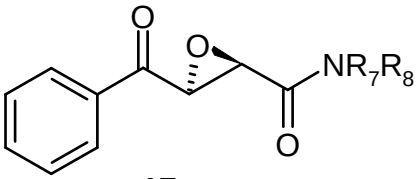
6.1.7.1e N,N-Morpholino-benzoyl-acrylamide (**16e**):

16e was purified by column chromatography and obtained as a white solid.

Substance	**16e**
Formula	$C_{14}H_{15}NO_3$
Molecular weight	245.27
Yield	2.40 g (77 %)
Melting point	130 - 131 °C [Lit.: 126.5 - 127.5 °C] [240]
Column chromatography	CH_2Cl_2/acetone (95:5); R_f (**start. material**)=0.14, R_f (**16c**)=0.58

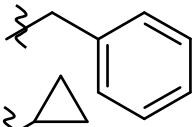
^{1}H NMR (400 MHz, $CDCl_3$, *δ* (ppm), *J* (Hz)): 3.60-3.73 (m, 8H, 2xNC*H*$_2$C*H*$_2$O), 7.43-8.03 (m, 7H, 5xC*H*$_{aromat.}$, 2xC*H*$_{db}$); **^{13}C NMR** (100 MHz, $CDCl_3$, *δ* (ppm)): 42.52, 46.35 (2xN*C*H$_2$CH$_2$O), 66.69 (2 peaks overlapping) (2xNCH$_2$*C*H$_2$O), 128.75 (2 peaks overlapping), 128.79 (2 peaks overlapping), 131.71, 136.77 (6x*C*$_{aromat.}$), 133.71, 134.48 (2x*C*H$_{db}$), 163.87 (*C*ON), 189.33 (*C*O); **IR** (cm^{-1}): 685, 718, 889, 962, 1114, 1263, 1283, 1444, 1598, 1632, 1672, 2860, 2967, 3292.

6.1.7.2 3-Benzoyloxiranecarboxamides (**17a-e**)

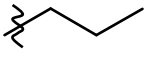


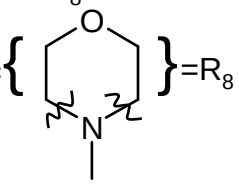
17a-e

a: R_7=R_8=Et

b: R_7=H, R_8= 

c: R_7=H, R_8=

d: R_7=H, R_8= 

e: R_7= 

=R_8

The following procedure was applied to obtain **17a-e** and is exemplified on **17a**:
1.32 g (5.70 mmol) of compound **16a** were dissolved in 50 ml 80 % EtOH, 1ml (9.79 mmol) of a 30 % aqueous H_2O_2 solution were added and adjusted to pH 7-8 with an aqueous 0.1M NaOH solution. The mixture was heated to 50 ℃ and during the following 3h 3 ml 30 % aqueous H_2O_2 solution was added and the pH was kept at 7-8 with aqueous 0.1M NaOH solution at that temperature. Then, the solution was stirred at RT overnight, evaporated, the residue dissolved in CH_2Cl_2, dried over anhydrous Na_2SO_4 and again evaporated to afford pure **17a**.

6.1.7.2a N,N-Diethyl-3-benzoyloxiranecarboxamide (**17a**):
17a was obtained fairly pure as a yellow oil.

Formula	$C_{14}H_{17}NO_3$
Molecular weight	247.29
Yield	0.92 g (65 %)

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 1.13-1.25 (m, 6H, 2xCH_3), 3.39-3.53 (m, 4H, 2xCH_2CH_3), 3.83, 4.58 (2x (d, *J* 2.04, 1H), COC*H*OC*H*CON), 7.47-8.09 (m, 5H, 5x$CH_{aromat.}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 12.69, 14.72 (2xCH_3), 41.11, 41.80 (2xCH_2), 53.79, 55.52 (CO*C*HO*C*HCON), 128.57 (2 peaks overlapping), 128.89 (2 peaks overlapping), 134.34 (5x$C_{aromat.}$), 134.94 ($C_{aromat.\ q}$), 164.87 (*C*ON), 192.92 (*C*O); **IR** (cm^{-1}): 712, 784, 1070, 1253, 1449, 1598, 1708, 2936, 2975.

6.1.7.2b N-Benzyl-3-benzoyloxiranecarboxamide (**17b**):

17b was purified by column chromatography and obtained as a white solid.

Formula	$C_{17}H_{15}NO_3$
Molecular weight	281.31
Yield	0.94 g (89 %)
Melting point	136 °C
Column chromatography	CH_2Cl_2/EtOAc (90:10); R_f (**16b**)=0.71, R_f (**17b**)=0.50

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 3.59, 4.21 (2x (d, *J* 2.28, 1H, COC*H*OC*H*CON), 4.30-4.44 (m, 2H, NCH_2), 6.68 (br s, 1H, N*H*), 7.16-7.89 (m, 10H, 10xC$H_{aromat.}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 43.12 (*C*H_2), 55.03, 55.97 (CO*C*HO*C*HCON), 127.76 (3 peaks overlapping), 128.40 (2 peaks overlapping), 128.75 (2 peaks overlapping), 128.90 (2 peaks overlapping), 134.35, 134.77, 137.13 (12x$C_{aromat.}$), 166.16 (*C*ON), 191.54 (*C*O); **IR** (cm^{-1}): 659, 670, 686, 725, 741, 787, 889, 995, 1029, 1227, 1566, 1662, 1681, 3074, 3228.

6.1.7.2c N-Cyclopropyl-3-benzoyloxiranecarboxamide (**17c**):

17c was purified by column chromatography and obtained as a pale yellow solid.

Formula	$C_{13}H_{13}NO_3$
Molecular weight	231.25
Yield	0.85 g (66 %)
Melting point	123 - 124 °C
Column chromatography	CH_2Cl_2/MeOH (95:5); R_f (**16c**)=0.79, R_f (**17c**)=0.64

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 0.52-0.80 (m, 4H, NHCHCH_2CH_2), 2.70-2.76 (m, 1H, NHC*H*CH_2CH_2), 3.62, 4.29 (2x (d, *J* 2.04, 1H), COC*H*OC*H*CON), 6.67 (br s, 1H, N*H*), 7.42-7.96 (m, 5H, 5xC$H_{aromat.}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 6.18, 6.28 (NHCH*C*H_2*C*H_2), 22.27 (NH*C*HCH_2CH_2), 54.96, 55.81 (CO*C*HO*C*HCON), 128.40 (2 peaks overlapping), 128.88 (2 peaks overlapping), 134.38 (5x$C_{aromat.}$), 134.73 ($C_{aromat.\ q}$), 167.81 (*C*ON), 191.78 (*C*O); **IR** (cm^{-1}): 670, 690, 741, 789, 899, 993, 1001, 1226, 1270, 1343, 1561, 1656, 1692, 3088, 3267.

6.1.7.2d N-Propyl-3-benzoyl-acrylamide (**17d**):

17d was purified by column chromatography and obtained as a yellow solid.

Formula	$C_{13}H_{15}NO_3$
Molecular weight	233.26
Yield	1.22 g (92 %)
Melting point	98 - 99 °C
Column chromatography	CH_2Cl_2/MeOH (90:10); R_f (**16d**)=0.95, R_f (**17d**)=0.76

^{1}H NMR (400 MHz, $CDCl_3$ δ (ppm), *J* (Hz)): 0.88 (t, *J* 7.46, 3H, C*H*$_3$), 1.49-1.57 (m, 2H, NHCH$_2$C*H*$_2$CH$_3$), 3.18-3.27 (m, 2H, NHC*H*$_2$CH$_2$CH$_3$), 3.71, 4.46 (2x (d, *J* 1.76, 1H), COC*H*OC*H*CON), 7.26 (br s, 1H, N*H*), 7.41-7.96 (m, 5H, 5xC*H*$_{aromat.}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 10.76 (*C*H$_3$), 21.92 (NHCH$_2$*C*H$_2$CH$_3$), 40.69 (NH*C*H$_2$CH$_2$CH$_3$), 54.68, 55.23 (CO*C*HO*C*HCON), 127.96 (2 peaks overlapping), 128.43 (2 peaks overlapping), 133.94 (5x*C*$_{aromat.}$), 134.32 (*C*$_{aromat.\ q}$), 166.25 (*C*ON), 191.99 (*C*O); **IR** (cm^{-1}): 663, 684, 698, 760, 902, 1009, 1228, 1448, 1545, 1654, 1685, 2875, 2936, 2965, 3310.

6.1.7.2e N,N-Morpholino-benzoyl-acrylamide (**17e**):

17e was obtained fairly pure as a white solid.

Formula	$C_{14}H_{15}NO_4$
Molecular weight	261.27
Yield	1.38 g (93 %)
Melting point	139 - 140 °C [Lit.: 137 - 139 °C] [188]

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm), *J* (Hz)): 3.59-3.72 (m, 8H, 2xNC*H*$_2$C*H*$_2$O), 3.85, 4.55 (2x (d, *J* 2.28, 1H), COC*H*OC*H*CON), 7.46-8.08 (m, 5H, 5xC*H*$_{aromat.}$); **^{13}C NMR** (100 MHz, $CDCl_3$, δ (ppm)): 42.54, 45.60 (2xN*C*H$_2$CH$_2$O), 53.90, 55.68 (CO*C*HO*C*HCON), 66.49, 66.59 (2xNCH$_2$*C*H$_2$O), 128.54 (2 peaks overlapping), 128.92 (2 peaks overlapping), 134.39 (5x*C*$_{aromat.}$), 134.88 (*C*$_{aromat.\ q}$), 164.14 (*C*ON), 192.48 (*C*O); **IR** (cm^{-1}): 664, 694, 780, 849, 889, 1001, 1037, 1111, 1228, 1398, 1447, 1635, 1656, 1691, 2858, 2979.

6.2 Inhibitor induced fluorescence change of KasA

6.2.1 Sample preparation

6.2.1.1 Preparation of the Tris buffer (pH 8,5)

7.88 g (0.05 mol) tris(hydroxymethyl)aminomethane hydrochloride and 17.53 g (0.30 mol) NaCl were dissolved in approximately 500 ml of distilled water. 0.1M aqueous NaOH solution was added in order to adopt a pH value of 8.5 and distilled water was added until the solution reached a volume of nearly 1000ml. The pH was checked again, corrected with 0.1M aqueous NaOH solution, and the solution was diluted to 1000ml with distilled water.

6.2.1.2 Preparation of the sample solutions
(exemplified for a compound concentration of 100 µM for **17e**)

21.3 µl of a 47 µM solution of KasA Tris buffer pH 8,5 were diluted with 968.7 µl of the Tris buffer in a 1ml quartz cuvette. The fluorescence was recorded (see below) and after approximately 400s (with stirring) 10.0 µl of a 10 mM solution in DMSO of the compound to be tested or (in case of the control) 10.0 µl DMSO were added.

6.2.1.3 Fluorimeter conditions

Fluorescence measurements were executed on a QM-4/2005-SE spectrometer from Photon Technology INT Inc. (London, Ontario, Canada). The excitation wavelength was adjusted to 280 nm and the emission wavelength was adjusted to 337 nm. Monochromator slit widths were 4.0 and 2.0 nm for the excitation and 2.0 and 2.0 nm for the emission. Measurements were carried out at 25 ℃ in a 1ml quartz cuvette and the solutions were mixed continuously by magnetic stirring. The data points were collected every 1 second. Experiments were executed three times.

6.2.2 Data analysis

The recorded fluorescence spectra were fitted by nonlinear regressing with GraFit 6 (Erithacus Software Limited) for each determined concentration (400 µM, 200 µM, 100 µM, 75 µM, 50 µM, 25 µM, 5 µM). The data were fitted to a double exponential function of the form $Y=A_0*e^{-kobs*t} + B_0*e^{-k2*t}$ (Y = fluorescence, t = time, A_0, B_0 = amplitude, k = rate constant).[218] The obtained rate constants k_{obs} were then fitted to a hyperbola of the form $k_{obs} = k_{on} * [I] / (K_i + [I])$ with k_{obs} = rate constant, [I] = compound concentration, k_{on} = forward rate, K_i = dissociation constant.[217] The spectra and the fittings are discussed in chapter 4.2.1.

6.2.2.1 Recorded fluorescence spectra

Compound **17c**:

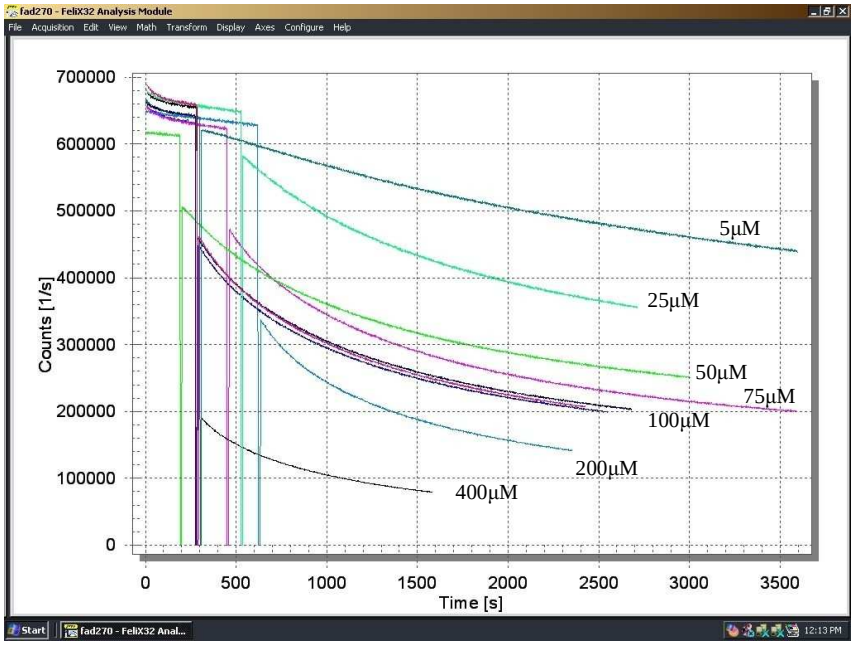


*Fig. 6.2.1 Fluorescence curve of the time and concentration dependent effects of **17c** on KasA; KasA was treated with the following concentrations: control (DMSO) (not shown), 400µM, 200µM, 100µM, 75µM, 50µM, 25µM, 5µM*

Compound **17e**:

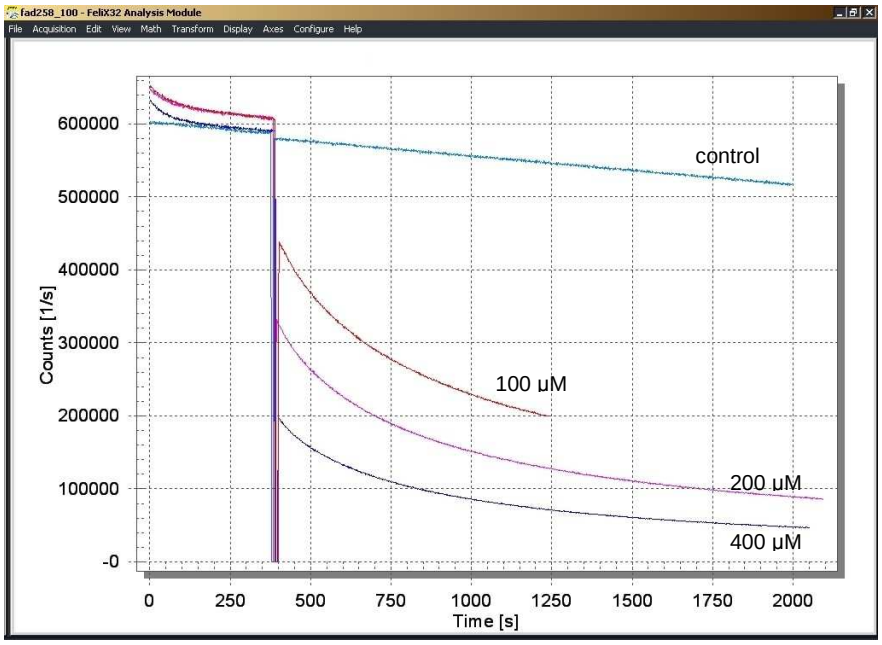


*Fig. 6.2.2a Fluorescence curve of the time and concentration dependent effects of **17e** on KasA; KasA was treated with the following concentrations: control (DMSO), 400µM, 200µM, 100µM (shown), 75µM, 50µM, 25µM, 5µM (not shown)*

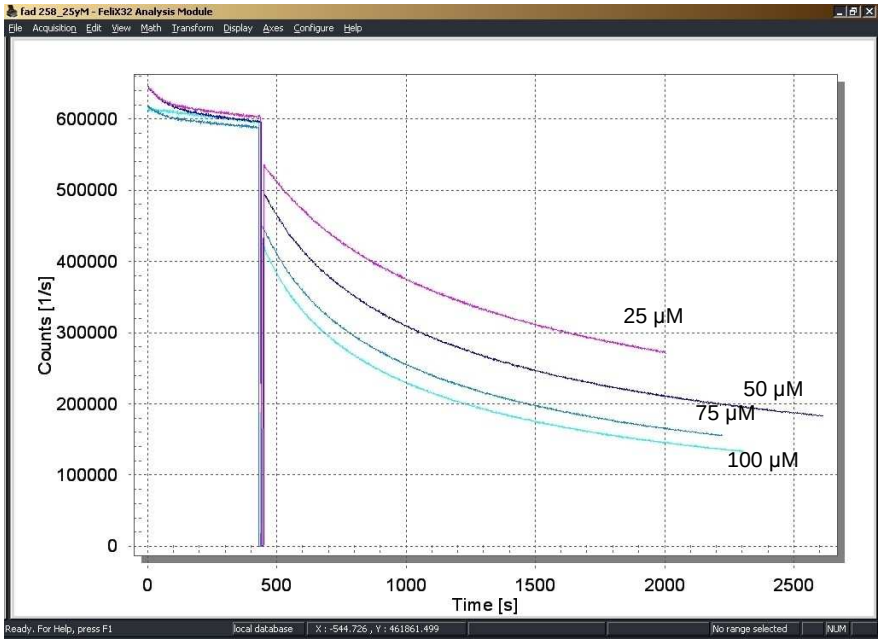
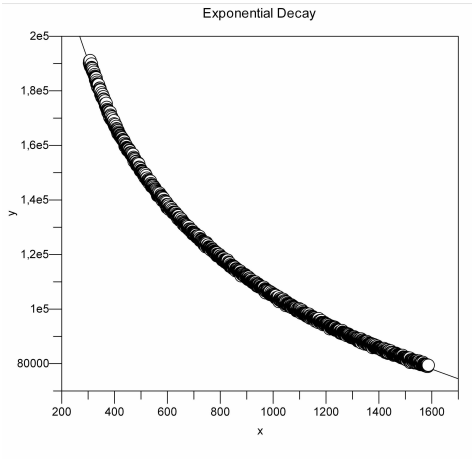


*Fig. 6.2.2b Fluorescence curve of the time and concentration dependent effects of **17e** on KasA; KasA was treated with the following concentrations: control (DMSO), 400µM, 200µM, 100µM (not shown), 75µM, 50µM, 25µM, 5µM (shown)*

6.2.2.2 Fitting of the fluorescence spectra

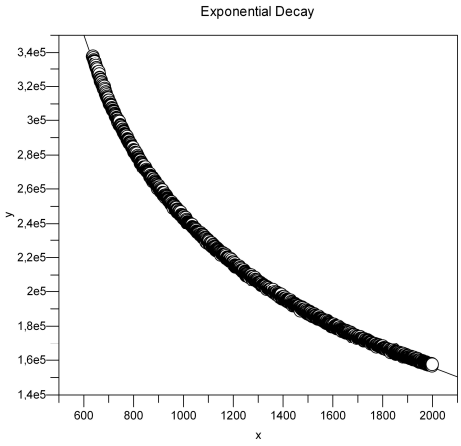
Each determined concentration (400 µM, 200 µM, 100 µM, 75 µM, 50 µM, 25 µM, 5 µM) was fitted to a double exponential function[218] of the form $Y=A_0*e^{-kobs*t} + B_0*e^{-k2*t}$:

Compound **17c**:



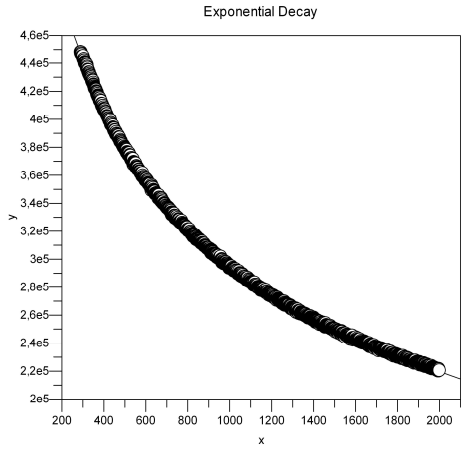
Parameter	Value	Std. Error
Initial value	165011,3280	722,2909
Rate constant	0,0038	3,05143e-005
B	159218,5918	516,1606
K2	0,0004	2,15464e-006

400 µM



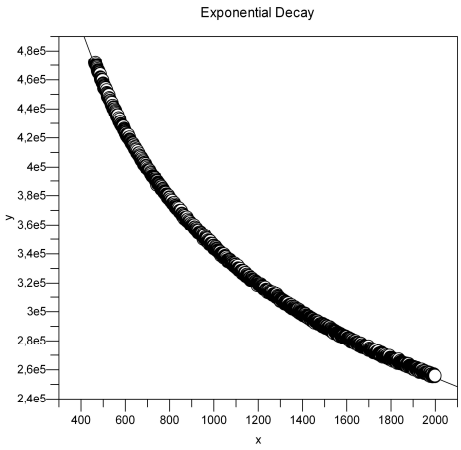
Parameter	Value	Std. Error
Initial value	790735,3682	8208,1740
Rate constant	0,0034	2,37523e-005
B	306545,9545	974,6758
K2	0,0003	1,64144e-006

200 µM



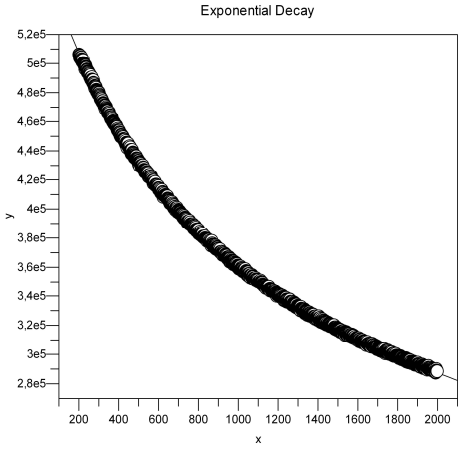
Parameter	Value	Std. Error
Initial value	262138,3855	416,1132
Rate constant	0,0025	1,31075e-005
B	343592,9769	721,8466
K2	0,0002	1,05809e-006

100 µM



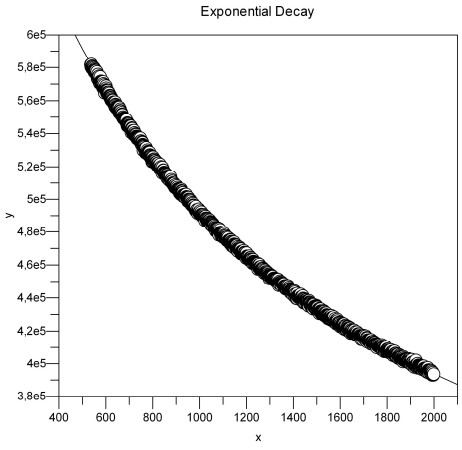
Parameter	Value	Std. Error
Initial value	379078,3788	1184,1767
Rate constant	0,0026	1,66931e-005
B	396755,7576	964,4485
K2	0,0002	1,18631e-006

75 µM



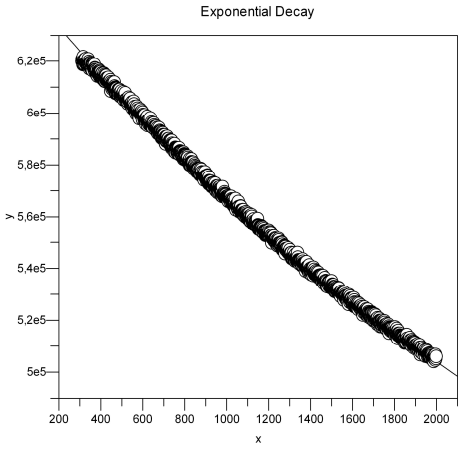
Parameter	Value	Std. Error
Initial value	191848,3853	702,8385
Rate constant	0,0019	1,13570e-005
B	388561,5507	913,6918
K2	0,0002	1,05365e-006

50 µM



Parameter	Value	Std. Error
Initial value	288185,6903	1964,1568
Rate constant	0,0016	2,41413e-005
B	489713,2701	3300,3209
K2	0,0001	2,54742e-006

25 µM

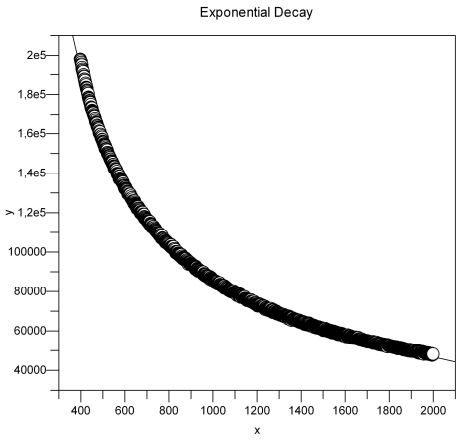


Parameter	Value	Std. Error
Initial value	86803,1271	2149,1244
Rate constant	0,0006	1,14376e-005
B	565684,6318	2169,6297
K2	8,44725e-005	8,32656e-007

5 µM

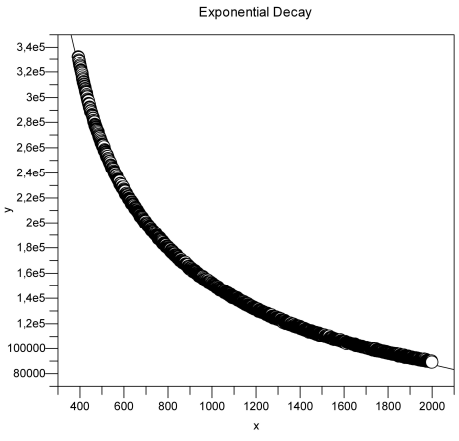
Fig. 6.2.3 Fitted Fluorescence curves: data points were taken 1s after injection of the compound ***17c****; each concentration* was *fitted to a double exponential function*[218] *of the form* $Y=A_0*e^{-kobs*t} + B_0*e^{-k2*t}$ *with Grafit6 (Erithacus Software Limited); y=fluorescence (units), x=time (s), rate constant=*k_{obs}*, initial value=*A_0

Compound **17e**:



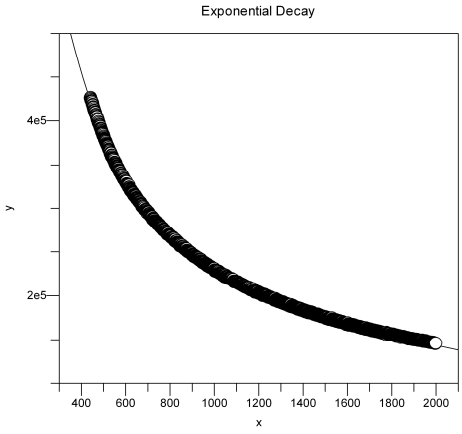
Parameter	Value	Std. Error
Initial value	468334,6535	3157,9163
Rate constant	0,0043	2,25745e-005
B	133560,4649	515,7185
K2	0,0005	2,31513e-006

400 µM



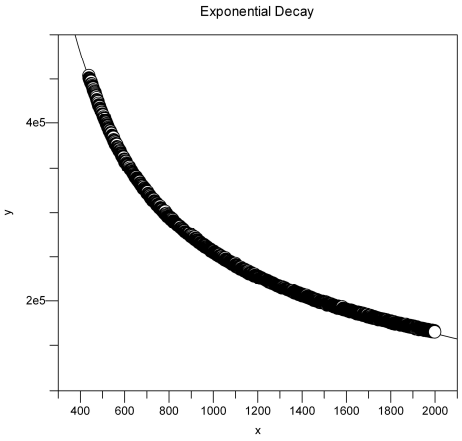
Parameter	Value	Std. Error
Initial value	732428,5882	4560,8522
Rate constant	0,0042	2,09711e-005
B	224920,2717	744,4089
K2	0,0005	1,98109e-006

200 µM



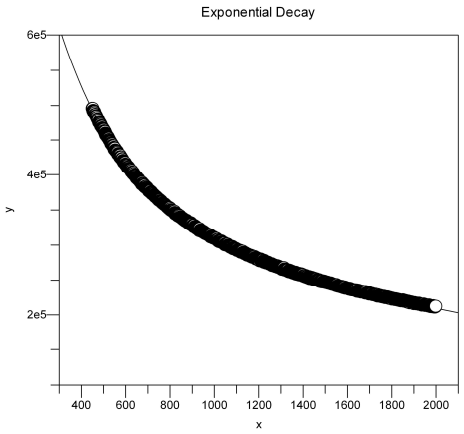
Parameter	Value	Std. Error
Initial value	904909,1076	5188,3989
Rate constant	0,0039	1,76561e-005
B	310229,7112	820,5751
K2	0,0004	1,51403e-006

100 µM



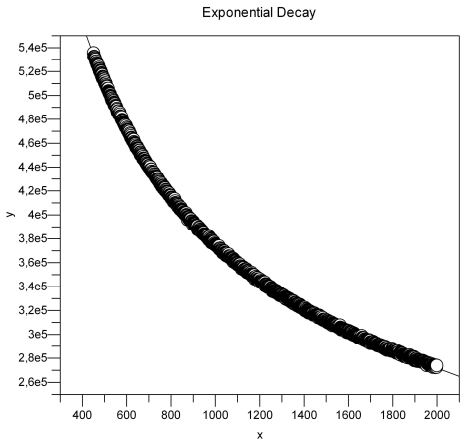
Parameter	Value	Std. Error
Initial value	828520,6248	3941,8363
Rate constant	0,0037	1,56003e-005
B	333188,7204	805,2569
K2	0,0004	1,35292e-006

75 µM



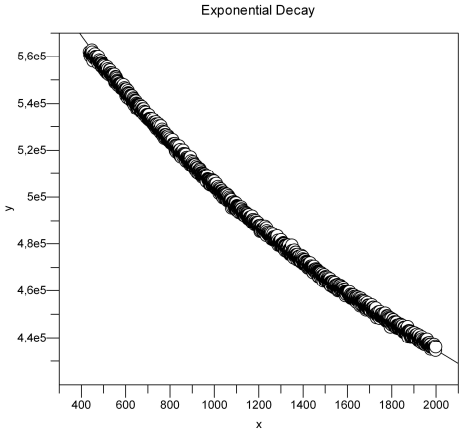
Parameter	Value	Std. Error
Initial value	697296,3868	2713,8518
Rate constant	0,0033	1,42254e-005
B	381126,2138	849,6039
K2	0,0003	1,19009e-006

50 µM



Parameter	Value	Std. Error
Initial value	481544,2339	1062,6919
Rate constant	0,0026	1,25518e-005
B	426904,1669	957,8689
K2	0,0002	1,09463e-006

25 µM



Parameter	Value	Std. Error
Initial value	98775,1199	695,3334
Rate constant	0,0012	1,13492e-005
B	531474,7087	708,1177
K2	0,0001	4,70609e-007

5 µM

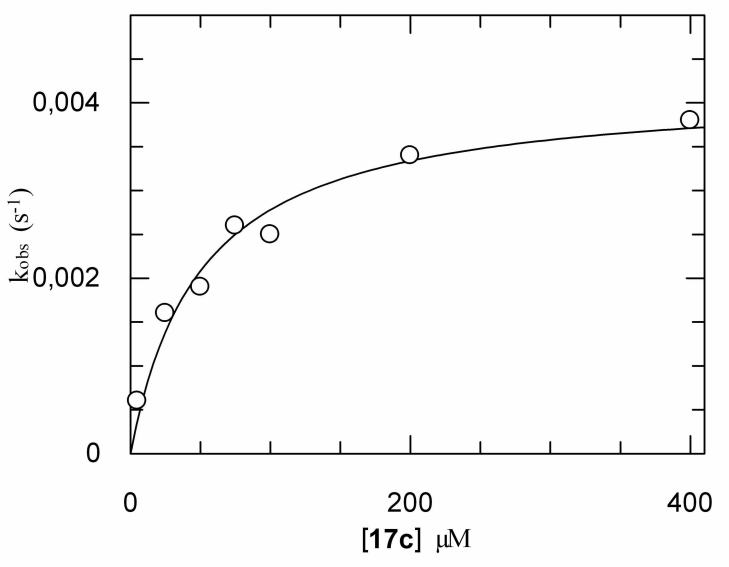
Fig. 6.2.4 Fitted Fluorescence curves: data points were taken 1s after injection of the compound ***17e****; each concentration* was *fitted to a double exponential function*[218] *of the form* $Y=A_0*e^{-kobs*t} + B_0*e^{-k2*t}$ *with Grafit6 (Erithacus Software Limited); y=fluorescence (units), x=time (s), rate constant=*k_{obs}*, initial value=*A_0

6.2.2.3 Fitting of the rate constants to the compound concentrations

The observed rate constants (k_{obs}) obtained from the fitting of the fluorescence spectra (Fig. 6.2.1 and 6.2.2) were then fitted to the compound (**17c**/**17e**) concentration to the equation $k_{obs} = k_{on} * [I] / (K_i + [I])$ with k_{obs} = rate constant, [I] = compound concentration, k_{on} = forward rate, K_i = dissociation constant[217] to determine the dissociation constant K_i of the compound and KasA (Fig. 6.2.5). For compound **17c** a K_i of 50.29 µM ± 10.02 µM was obtained (see Fig. 6.2.6) and for compound **17e** a K_i value of 16.56 µM ± 1.40 µM was determined (Fig. 6.2.7).

Fig. 6.2.5 Equation of the two step binding model for irreversible inhibitors (E=enzyme kasA, I=inhibitor, K_i=dissociation constant, k_{on}=forward rate)

Compound **17c**:

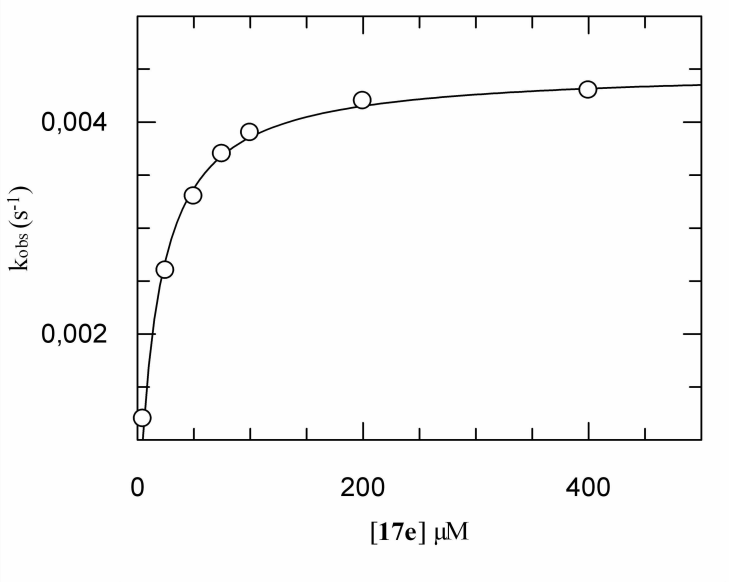


17c (µM)	**k_{obs}** (s^{-1})
400	0,0038
200	0,0034
100	0,0025
75	0,0026
50	0,0019
25	0,0016
5	0,0006

Parameter	**Value**	**Std. Error**
forward	0,0042	$3{,}00 \cdot 10^{-4}$
dissociation constant	50,29	10,02

*Fig. 6.2.6 Fitting of the rate constants (obtained from Fig. 8.3) to the compound (**17c**) concentration to the equation $k_{obs} = k_{on} * [I] / (K_i + [I])$ with k_{obs} = rate constant, [I] = compound concentration, k_{on} = forward rate, K_i = dissociation constant. Grafit6 (Erithacus Software Limited) was used; the dissociation constant was determined as 50.29 µM ± 10.02 µM for **17c**.*

Compound **17e**:



17e (µM)	**k_{obs}** (s^{-1})
400	0,0043
200	0,0042
100	0,0039
75	0,0037
50	0,0033
25	0,0026
5	0,0012

Parameter	Value	Std. Error
forward	0,0045	$7{,}60 \cdot 10^{-5}$
dissociation constant	16,56	1,40

*Fig. 6.2.7 Fitting of the rate constants (obtained from Fig. 8.4) to the compound (**17e**) concentration to the equation $k_{obs} = k_{on} * [I] / (K_i + [I])$ with k_{obs} = rate constant, [I] = compound concentration, k_{on} = forward rate, K_i = dissociation constant. Grafit6 (Erithacus Software Limited) was used; the dissociation constant was determined as 16.56 µM ± 1.40 µM for **17e**.*

7. Appendix

7.1 Publications

7.1.1 Articles

- Diwischek F, Arnone M, Engels B, Holzgrabe U
  Studies on the stereochemistry of 1,2,6-trimethyl-4-piperidone
  TETRAHEDRON 61 (29): 6993-7001 **2005**
- Diwischek F, Heller E, Holzgrabe U
  Comparison of conventional and microwave assisted reactions giving aromatic oxazolidines
  MONATSHEFTE FUR CHEMIE 134 (8): 1105-1111 **2003**

7.1.2 Oral presentations

- F. Diwischek, J. Morschhäuser, U. Holzgrabe, Efflux pump inhibitors of azole resistant Candida albicans, *New Trends in Infectious Disease Research, Second Joint Ph.D. Students Meeting of the Collaborative Research Centers SFB 630 & SFB 544*, Heidelberg, **2006**

7.1.3 Poster presentations

- F. Diwischek, , U. Holzgrabe, Elucidation of the stereochemistry of 1,2,6-trimethyl-4-piperidone, *DPhG-Jahrestagung Joint Meeting 2004*, Regensburg, **2004**
- F. Diwischek, J. Morschhäuser, U. Holzgrabe, Cerulenin and derivatives as possible efflux pump inhibitors of Candida albicans, *New Trends in Infectious Disease Research, Joint Ph.D. Students Meeting of the Collaborative Research Centers SFB 630 & SFB 544*, Würzburg, **2004**
- F. Diwischek, J. Morschhäuser, U. Holzgrabe, Cerulenin analogues as possible inhibitors of the Candida albicans efflux pumps, *Novel agents against Infectious Diseases – an Interdisciplinary Approach, Research, International Symposium SFB 630*, Würzburg, **2006**
- F. Diwischek, J. Morschhäuser, U. Holzgrabe, Cerulenin derivatives as efflux pump inhibitors of azole resistant Candida albicans, *DPhG-Doktorandentagung*, Nürnberg, **2006**

- F. Diwischek, J. Morschhäuser, U. Holzgrabe, Analogues of cerulenin as efflux pump inhibitors of azole resistant Candida albicans, *DPhG-Jahrestagung Joint Meeting 2006*, Marburg, **2006**
- F. Diwischek, J. Morschhäuser, U. Holzgrabe, Efflux pump inhibitors of resistant Candida albicans based on Cerulenin as a lead structure, *Pre-Satellite Meeting of the 3rd Pharmaceutical Sciences World Congress 2007*, Amsterdam, **2007**
- F. Diwischek, J. Morschhäuser, U. Holzgrabe, Cerulenin analogues as possible efflux pump inhibitors of resistant Candida albicans and inhibitors of the fatty acid synthesis of *M. Tuberculosis*, *3rd Joint Ph.D. Students Meeting of the Collaborative Research Centers SFB 630 & SFB 544*, Retzbach, **2007**
- F. Diwischek, C. Kisker, C. Machutta, P. Tonge, U. Holzgrabe, Development of synthesis pathways to cerulenin-type inhibitors of the fatty acid biosynthesis, *DPhG-Jahrestagung Joint Meeting 2007*, Erlangen, **2007**

8. Abbreviations

Abbreviation	Meaning
Å	Angström
$AgNO_3$	Silver nitrate
AIBN	Azobisisobutyronitrile
aq.	Aqueous
n-BuLi	*n*-Butyl lithium
$(n\text{-Bu})_4Sn$	Tetrabutylstannane
$CDCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
$ClCO_2{}^{i}Bu$	Isobutylchloroformate
$C_2Cl_2O_2$	Oxalyl chloride
$CrO_3 \cdot pyr_2$	Chromium trioxide-pyridine complex
CrotylBr	Crotyl bromide
CuBr	Copper(I)bromide
$CuNO_3$	Copper(II)nitrate
DHP	Dihydropyran
DME	Dimethoxyethane
DMSO	Dimethyl sulfoxide
EtMgBr	Ethyl magnesium bromide
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
MeOH	Methanol
h	Hour(s)
HCO_2NH_4	Ammonium formate
H_2CrO_4	Chromic acid
H_2O_2	Hydrogen peroxide
$HSnBu_3$	Tribuyltin hydride
H_2SO_4	Sulfuric acid
Jones´ reagent	Chromium trioxide/sulfuric acid
$LiAlH_4$	Lithium aluminium hydride
LiCl	Lithium chloride
Lindlar-cat.	5% Pd/$CaCO_3$/3.5%Pb
mol. siev.	Molecular sieves
MsCl	Mesyl chloride
Na_2CO_3	Sodium carbonate
$NaHCO_3$	Sodium bicarbonate
NaI	Sodium iodide
NaOCl	Sodium hypochlorite
Na_2SO_3	Sodium sulfite
Na_2SO_4	Sodium sulfate
$Na_2S_2O_3$	Sodium thiosulphate
$Na_2WO_4 \cdot 2H_2O$	Sodium wolframate
NEt_3	Triethyl amine
NH_4Cl	Ammonium chloride
NMO	*N*-methylmorpholine *N*-oxide
P_2O_5	Phosphorus pentoxide
reflux	Refluxing

RT	Room temperature
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TPAP	Tetrapropylammonium perruthenate
p-Tss	*p*-Toluene sulfonic acid

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