Synthesis of some furanone derivatives: Putative quorum sensing or chitinase inhibitors

INAUGURALDISSERTATION

Zur

Erlangung der Würde eines Doktors der Philosophie

Vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Benoît JOLIVET

aus

Paris (Frankreich)

Basel 2005

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
auf Antrag der Herren

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Basel, den 25. Oktober 2005

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Dekan

The following work was carried out from July 2001 to August 2005 under the supervision of Prof. Dr. *U. Séquin* at the Departement Chemie, Universität Basel.

I would like to thank Prof. Dr. *U. Séquin* for the interesting research projects, his availability, his help and the friendly work atmosphere.

I thank Prof. Dr. *M. Rohmer* for agreeing to be a member of the dissertation committee.

I also thank *A. Schlatter*, *M. Graber*, *M. Enzler* and *A. Senti* for their help during their "Wahlpraktikum" work and diploma thesis.

I would especially like to thank Dr. *K. Kulicke* for his help and his availability for NMR analyses, *M. Neuburger* for X-ray structure analyses, and Dr. *S. Gunzenhauser* for his help and his advice in laboratory work.

I thank Dr. *H. Nadig* for mass spectra, *W. Kirsch* for the elemental analyses, Prof. Dr. *K.-D. Spindler* (Universität Ulm), Prof. Dr. *R. Bachofen* and Dr. *D. Martinelli* (Universität Zürich) for the biological tests.

Finally I would like to thank all my lab colleagues, *G. Grossmann*, *M. Senn*, *C. Mura* as well as all people from the institute for their help and the friendly work atmosphere.

I thank all people who helped me to achieve this PhD and the Swiss National Science Foundation for financial support.

A mes parents Françoise et Jean-Pierre

et à ma soeur Céline

"Sich allen Abend ernstlich zu befragen was man an dem Tage Neues gelernt hat"

Georg Christoph Lichtenberg (1742-1799).

Parts of this work have been published:

Bioactive butenolides from *Streptomyces antibioticus* TÜ 99: absolute configurations and synthesis of analogs, G. Grossmann, M. Poncioni, M. Bornand, B. Jolivet, M. Neuburger, U. Séquin, *Tetrahedron*, **2003**, *59*, 3237-3251.

***Streptomyces*-derived quorum sensing systems engineered for adjustable transgene expression in mammalian cells and mice**, W. Weber, R. Schoenmakers, M. Spielmann, M. Daoud El-Baba, M. Folcher, B. Keller, C. C. Weber, N. Link, P. van de Wetering, C. Heinzen, B. Jolivet, U. Séquin, D. Aubel, C. J. Thompson, M. Fussenegger, *Nucleic Acids Research*, **2003**, *31 (14)*, e71.

Synthesis and biological evaluation of some furanones as putative chitinase inhibitors, G. Grossmann, B. Jolivet, M. Bornand, U. Séquin, K.-D. Spindler, *Synthesis*, **2005** (9), 1543-1549.

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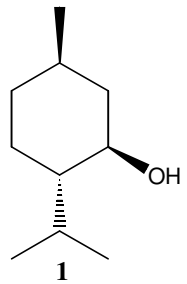
I Introduction

1. Primary and secondary metabolites

In the living world, metabolism is the ability to produce energy and all compounds useful for life through complex bioprocesses from suitable nutriments. In the case of plants, these nutriments can be extracted from the soil, or produced by photosynthesis. For animals, the nutriments come from the food.

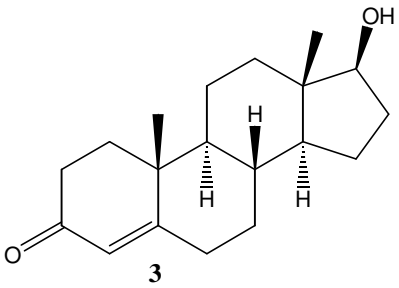
The first stage is the primary metabolism. It concerns the processes which occur on a large scale, such as cell respiration, growth, and energy storage. The products are called *primary metabolites*. These metabolites can be for instance polysaccharides like chitin, cellulose, starch, as well as amino acids and proteins.

Under particular conditions, regulated by enzymes or signalling compounds, some of these primary metabolites are involved in other bioprocesses whereby they can be transformed into very specific molecules. This is the secondary metabolism, and the products are the *secondary metabolites*. These metabolites are always produced in small amounts. They have very specific activities and can have very different purposes. Secondary metabolites can be pigments, flavour compounds, pheromones, hormones, antibiotics, and so on. They belong to various families such as terpenes, which can be flavour compounds like (–)-menthol (**1**), carotenoids like β-carotene (**2**), steroids like testosterone (**3**). Other well-known secondary metabolites are alkaloids like nicotine (**4**), morphine (**5**), or taxol (**6**), as well as antibiotics such as the penicillins (*e.g.* **7**, penicillin G), pristinamycin (**8**), spiramycin, which is a mixture mainly composed of the compounds **9a**, **9b**, and **9c**.



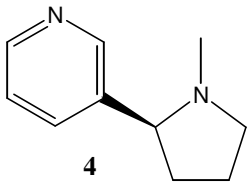
1

(−)-menthol



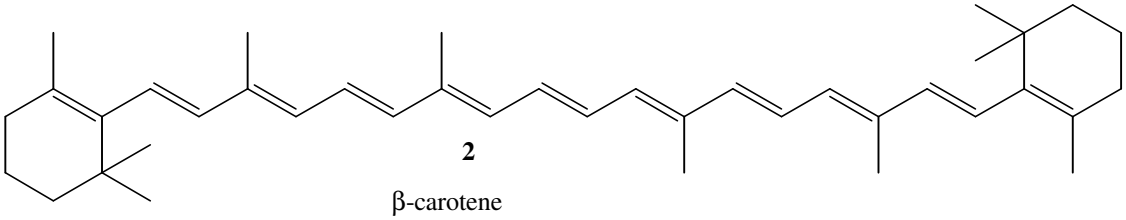
3

testosterone



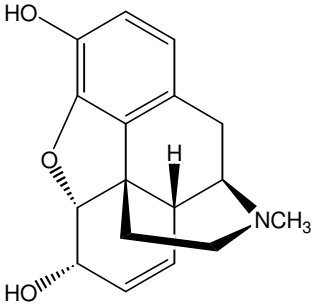
4

nicotine

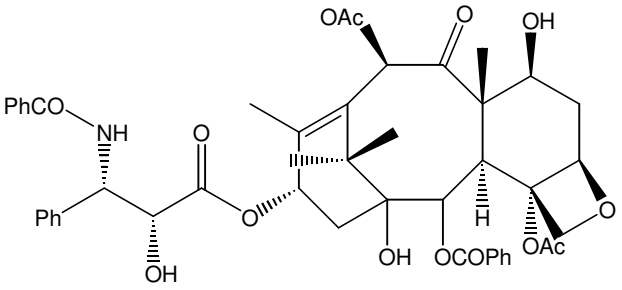


2

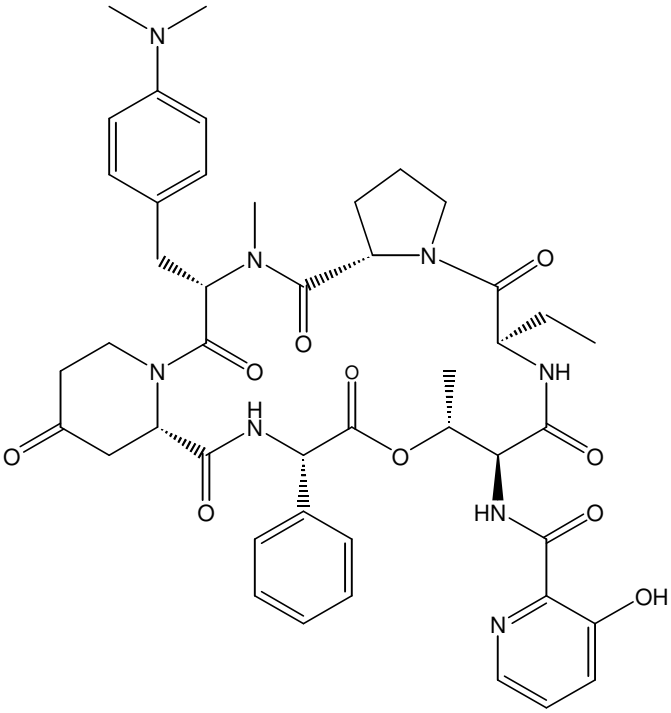
β-carotene



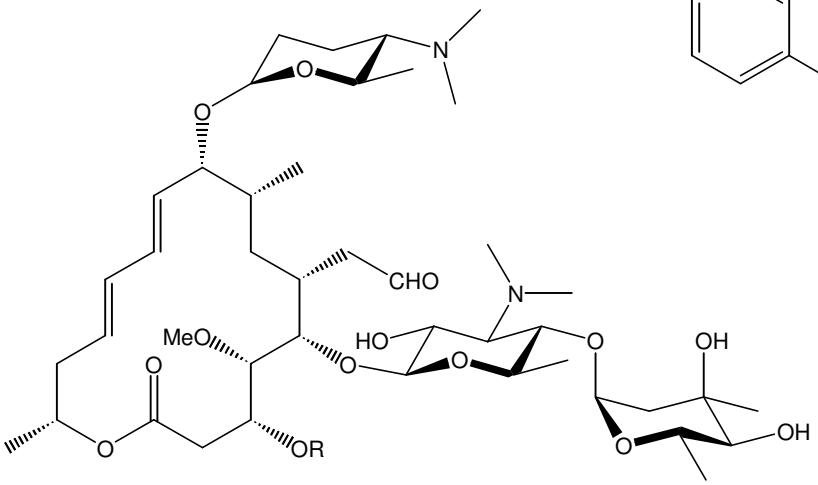
5 morphine



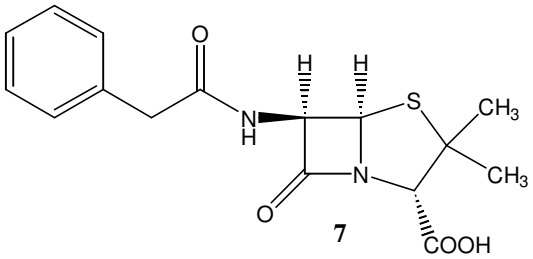
6 taxol



8 pristinamycin



spiramycin I R = H **9a**
spiramycin II R = COMe **9b**
spiramycin III R = COEt **9c**



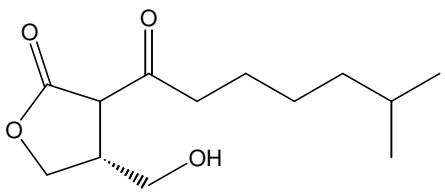
7

penicillin G

2. Secondary metabolites and cell-to-cell communication

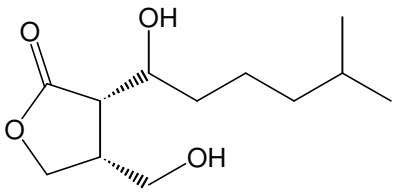
2.1 Autoregulatory factors

Very specific conditions are required for the production of secondary metabolites in bacteria. For instance, a γ-butyrolactone called A-factor (**10**) induces the production of the natural antibiotic streptomycin **16** by the mutant strain *Streptomyces griseus* [1,2]. Moreover, it is also essential for aerial mycelium formation, and it can activate sporulation and even the synthesis of a yellow pigment. In fact, A-factor is involved in a regulatory cascade. It binds to a specific protein, called A-factor binding protein, which regulates the expression of the genes responsible for streptomycin synthesis, sporulation and so on.

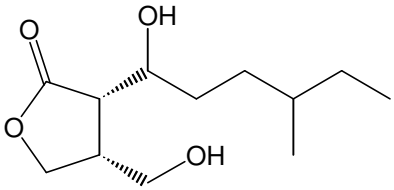


10 A-factor

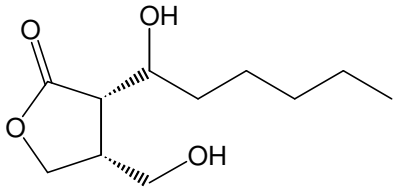
Other natural γ-butyrolactones have similar properties. Virginiae butanolides VB-A (**11**), VB-B (**12**), and VB-C (**13**) [2] extracted from *Streptomyces virginiae* are regulatory factors for the synthesis of virginiamycin, which is a mixture of two main components: virginiamycin M (**14**) and virginiamycin S (**15**). Although the structures of the three compounds **11-13** are very close to that of the A-factor (**10**), they only regulate the virginiamycin synthesis. They are not active on *Streptomyces griseus*, so they do not regulate the synthesis of streptomycin **16**. In the same way, A-factor (**10**) does not activate virginiamycin production.



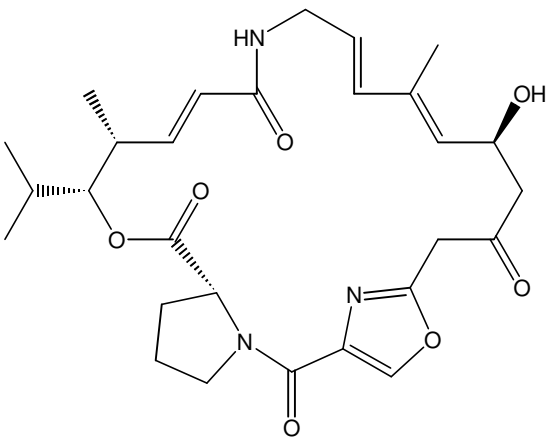
11 VB-A



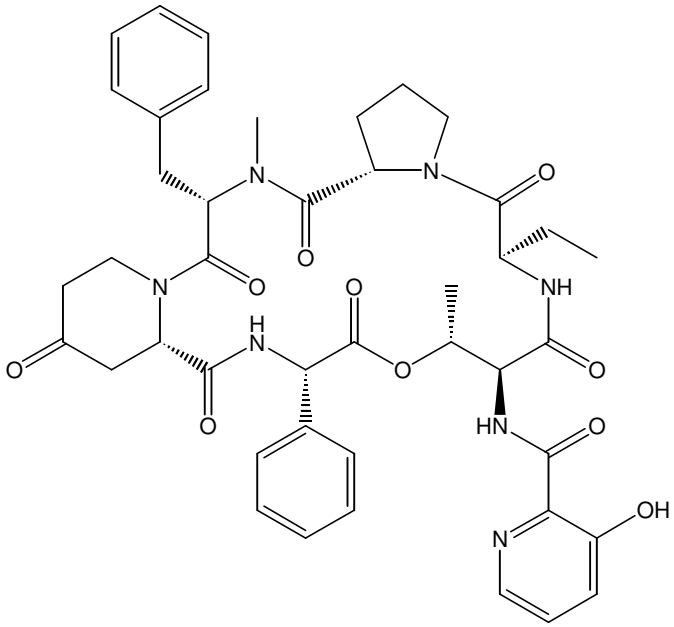
12 VB-B



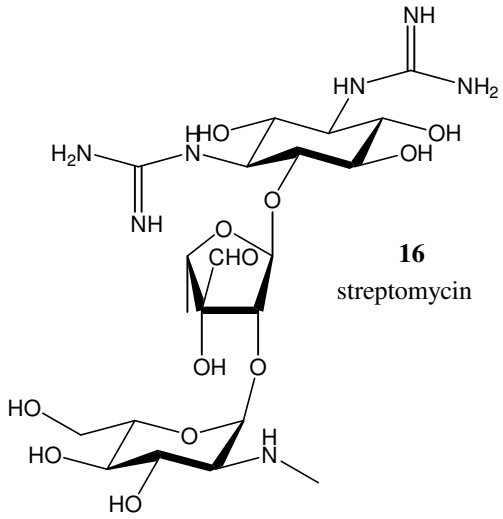
13 VB-C



14 virginiamycin M



15 virginiamycin S



16
streptomycin

2.2 Biofilms

Free-living bacteria cannot survive very long. They can be destroyed by antibiotics, other bacteria, or by an immune system. In order to survive, bacteria have to form colonies. These colonies must be protected from aggressive agents. Such colonies are called biofilms.

Nowadays, the mechanism of biofilm formation is well-known (Figure 1). In a first stage, bacteria find a favourable environment. They fix themselves onto a surface and begin to proliferate. When a critical concentration of bacteria, which is called *quorum*, is obtained, they begin to form a matrix. This matrix is generally made from polysaccharide derivatives. The colony grows inside the matrix, which contains channels in order to allow the circulation of nutriments. In addition, the matrix offers the bacteria a physical protection. At a later time, free-swimming bacteria leave the colony and try to find other places for new colonies, and so on [3].

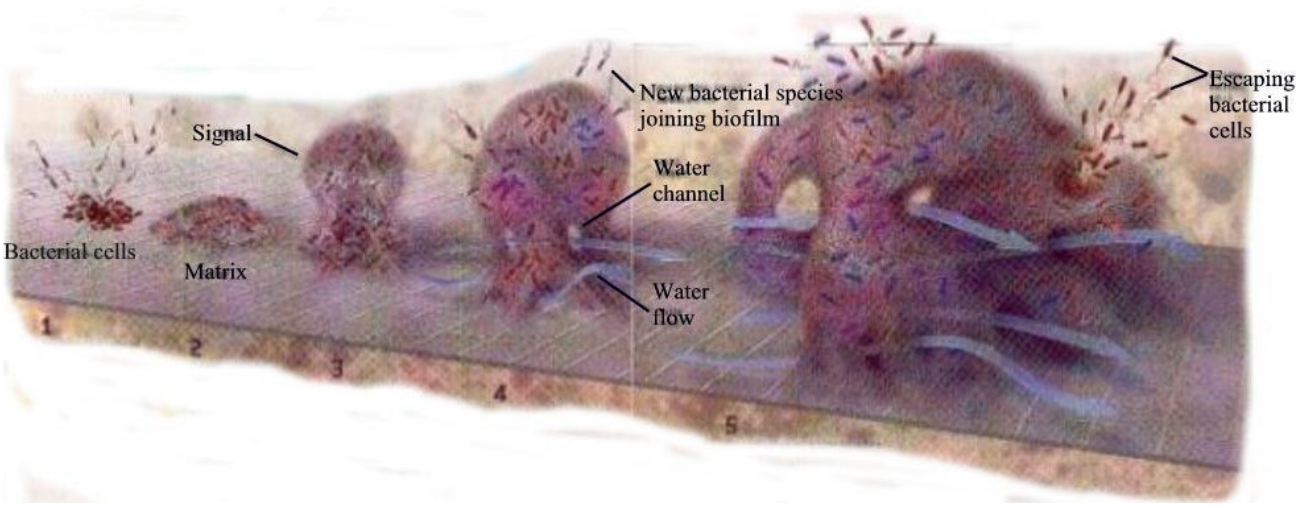


Figure 1: *Different steps of the formation of a biofilm [3].*

The main issue with biofilms is that it is almost impossible to remove them, chemically and physically. In fact, the matrix has very strong adherence forces onto the surface: even mechanical cleaning is difficult. Moreover, bacteria inside the matrix produce enzymes in order to prevent toxic compounds to penetrate into the colony. As a consequence, it is impossible to destroy biofilms with strong acids or bases or other corrosive chemicals. Even antibiotics are not efficient either, since they cannot diffuse into the matrix.

2.3 How to fight biofilms of pathogens?

People who suffer from cystic fibrosis are very sensitive to bacterial infections of their lungs. As they are treated essentially with antibiotics, they are more and more exposed to resistant pathogens. When resistant bacteria form biofilms, the only way to cure these patients, up to date, is lung transplantation. This extreme example shows the potential interest of finding new techniques to decrease the virulence of pathogens.

A first possibility against biofilm formation is to prevent bacteria from adhering to a surface. In fact, if bacteria cannot fix themselves onto a surface, they will never be able to form a biofilm. This is particularly interesting for drinking-water pipes, air conditioning systems and hot water networks. Recently, in France, several cases of contamination with *Legionella pneumophila* were reported. Some of these contaminations occurred in French

hospitals, where bacteria had colonized the hot water system network. Patients were contaminated by having a shower for instance.

We explained that biofilm formation involves a change in the behaviour of bacteria: starting from free-living bacteria, they differentiate and specialize their functions to perform specific tasks in the colony. Which factors contribute to this behaviour changes? When bacteria form an organized colony, how can they communicate to each other? If we could interfere with this system, would it be possible to block biofilm formation?

3. Quorum sensing

3.1 Definition

Free-living bacteria produce signalling compounds, which enable them to estimate their population. When the concentration of these signalling compounds is too low, bacteria simply proliferate in order to increase the population. The concentration of the signalling compounds increases parallel to the bacteria population. When a critical concentration is reached, the bacterial virulence increases significantly and infection or biofilm formation occurs. This phenomenon is called quorum sensing.

3.2 Quorum sensing in Vibrio fischeri

3.2.1 Symbiose between Vibrio fischeri *and the squid* Euprymna scolopes

Vibrio fischeri is a Gram-negative bacterium which becomes luminescent at high cell density. This bacterium can live in symbiosis with *Euprymna scolopes*, a small nocturnal squid which lives in the shallow sand flats associated with coral reefs in the Hawaiian archipelago [4.]

At night, predators can locate their preys through their shadows from the moonlight. The bioluminescence of *Vibrio fischeri* enables *Euprymna scolopes* to camouflage itself by

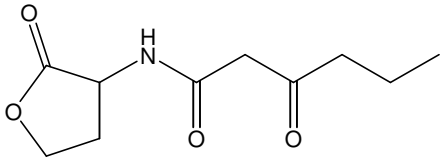
counter-illumination. It can control the intensity of the light it projects downward, so that it can eliminate the visible shadow created by the moonlight.

3.2.2 Mechanism of bioluminescence in Vibrio fischeri

Eight different genes (*luxA-E*, *luxG*, *luxI* and *luxR*) encode the proteins that are responsible for the bioluminescence system of *Vibrio fischeri*. In particular, the genes *luxI* and *luxR* are responsible for the regulation of the bioluminescence. Beside these two genes, *luxA* and *luxB* encode the synthesis of an heterodimeric luciferase, an enzyme which catalyses the oxidation of an aldehyde and of reduced flavin mononucleotide. The reaction gives a long-chain fatty acid, water and flavin mononucleotide. In addition the reaction produces an excess of energy, which is liberated as blue-green light: this gives the bioluminescence. The other genes *luxC-E* encode the compounds responsible for the synthesis of the aldehyde substrate, which is used by the luciferase, and *luxG* encodes a probable flavin reductase.

3.2.3 Quorum sensing in Vibrio fischeri

At low cell concentration, the different *lux* genes are not active, therefore no bioluminescence occurs. However, the bacteria produce an autoinducer, which is the *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL, **17**). This signalling compound can diffuse out of the cell, as it can go very easily through the membrane. As bacteria proliferate, the concentration of OHHL increases. When a critical OHHL concentration is reached, which means that a certain cell concentration, also called *quorum*, occurs, the *lux* genes are activated and the bacteria become luminescent (Figure 2).



17

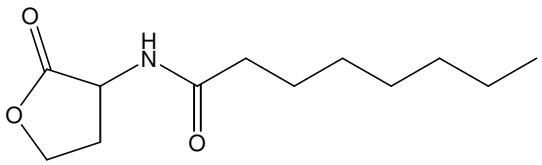
OHHL

At this critical concentration, the OHHL binds to the LuxR receptor, which probably changes its conformation. Then it is supposed that the activated LuxR binds to a part of the

DNA chain, called *lux box*, and induces the transcription of other *lux* genes. Moreover, the production of OHHL is amplified, which activates more and more the bioluminescence of the population (Figure 2).

On the other hand, at low concentration of OHHL, the LuxR receptor induces the transcription of *luxR*, but in higher concentration of OHHL, LuxR inhibits the transcription of *luxR*. This property might be a regulating process that self-limits the autoinduction of bioluminescence.

In addition to these processes, *Vibrio fischeri* produces *N*-octanoyl-L-homoserine lactone (OHL, **18**). This compound binds to LuxR competitively with OHHL, but contrary to this latter, OHL inhibits the LuxR activation. At low cell concentration the inhibition of the formation of the complex LuxR-OHHL prevents the premature induction of bioluminescence. The gene that regulates the synthesis of OHL is called *ainS* and is completely independent from *luxI*.



18
OHL

3.3 Quorum sensing and pathogens: the example of Pseudomonas aeruginosa

In the early 1990's other Gram-negative bacteria showed regulatory systems analogous to the LuxI-LuxR system of *Vibrio fischeri*. One of the most famous examples is the quorum sensing system in the virulence of the pathogenic bacterium *Pseudomonas aeruginosa* [5]. This bacterium is involved in tissues infections after wounds or at burn sites. It is also responsible of chronic lung infections in patients who suffer from cystic fibrosis. The strongest issue with this disease is that the chronic antibiotics treatment makes the bacterium resistant, and the only way to avoid death of the patient is a lung transplantation. That is why quorum sensing of *Pseudomonas aeruginosa* was widely studied during the last years.

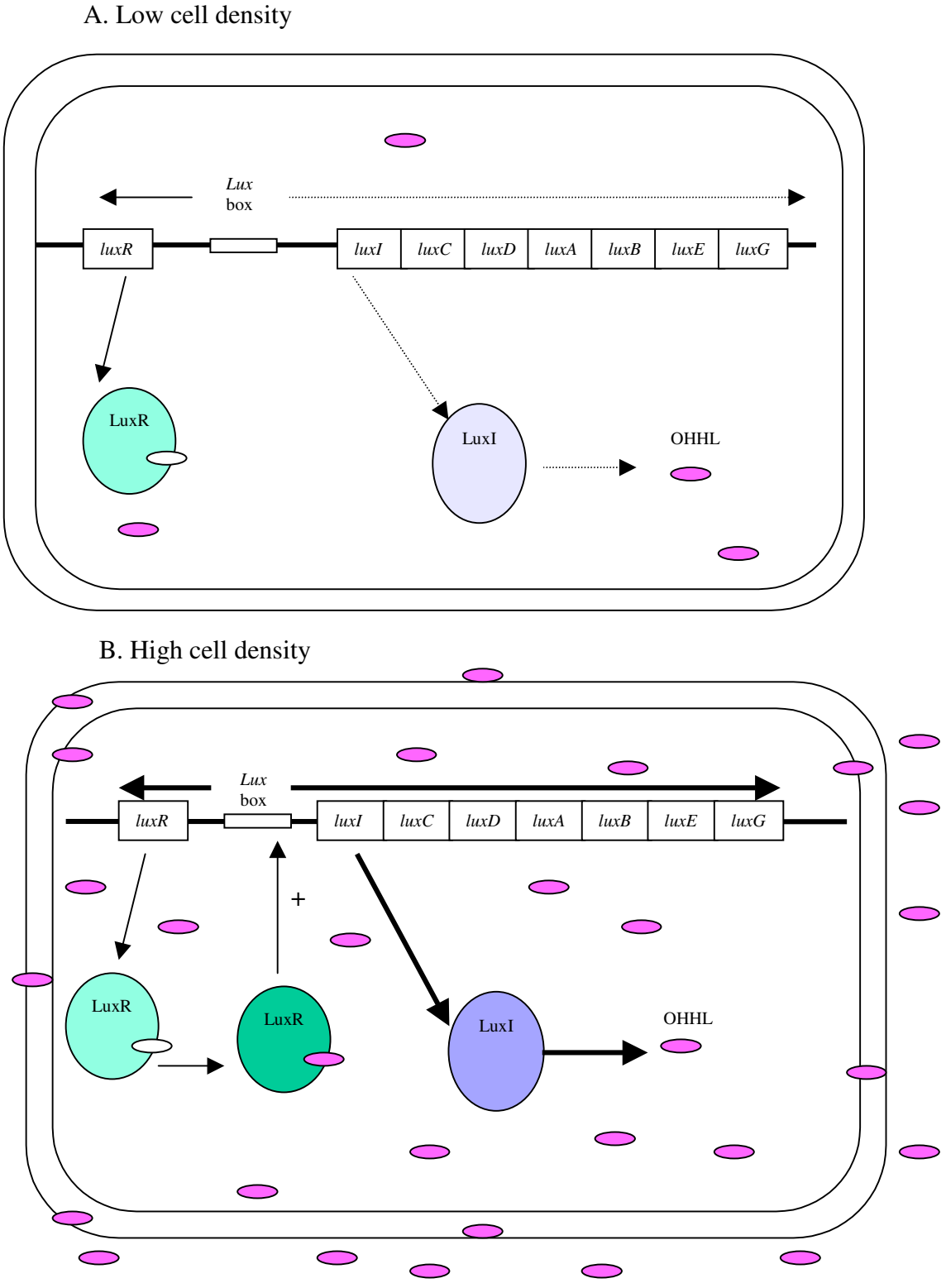
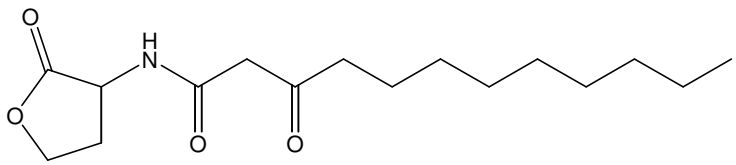


Figure 2: *The regulation of bioluminescence in* Vibrio fischeri*: the quorum sensing system.*

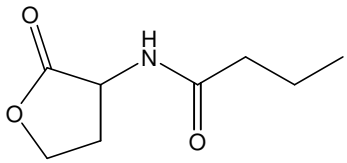
*A. At low cell density, transcription of the genes for bioluminescence (*luxICDABEG*) is weak and insufficient for the light emmission due to the low levels of OHHL.*
B. At high cell density, a critical concentration of OHHL is reached. OHHL binds to LuxR and stimulates transcription of luxICDABEG*, leading to rapid amplification of the OHHL signal and emission of light [4].*

The pathogenic activity of *Pseudomonas aeruginosa* consists in producing different extracellular virulence factors such as proteases, haemolysins, exotoxin A, exoenzyme S and pyocyanin. These compounds are responsible for the destruction of tissues in mammals, and particularly in humans.

Two quorum sensing systems coexist in *Pseudomonas aeruginosa* (Figure 3). The first system which had been described was the *las* system. The *lasR* gene encodes the receptor protein LasR, which is activated by the *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL, **19**). In addition, the gene *lasI* encodes a synthase that is necessary for the OdDHL synthesis. At a critical concentration, which occurs between the exponential growth and stationary stages of the bacterium, the OdDHL binds to two LasR proteins, which activates the transcription of the following genes: *lasA*, *lasB*, *aprA*, *toxA*, *xcpR*, *xcpP*, and *lasI*. The genes *lasA*, *lasB* and *aprA* encode two elastases and an alcaline protease, which are all responsible for tissue damages. The *toxA* gene encodes a toxin. The genes *xcpR* and *xcpP* encode the production of proteins which enable all the compounds listed before to be excreted out of the bacteria. Finally, the *lasI* gene enables a faster synthesis of the OdDHL, which amplifies the quorum sensing.



19 OdDHL



20 BHL

The second system, called *rhl*, functions in a similar way (Figure 3). The gene *rhlR* encodes the regulating protein RhlR, on which the *N*-butanoyl-L-homoserine lactone (BHL, **18**) binds. The gene *rhlI* encodes the protein RhlI which enables the synthesis of the BHL. Then the complex between RhlR and BHL induces the activation of several genes, such as *rhlAB*, *lasA*, *lasB*, *aprA*, *rhlI*. It is also interesting to note that the *las* system can activate the *rhl* system, because the OdDHL can either bind to LasR and RhlR proteins.

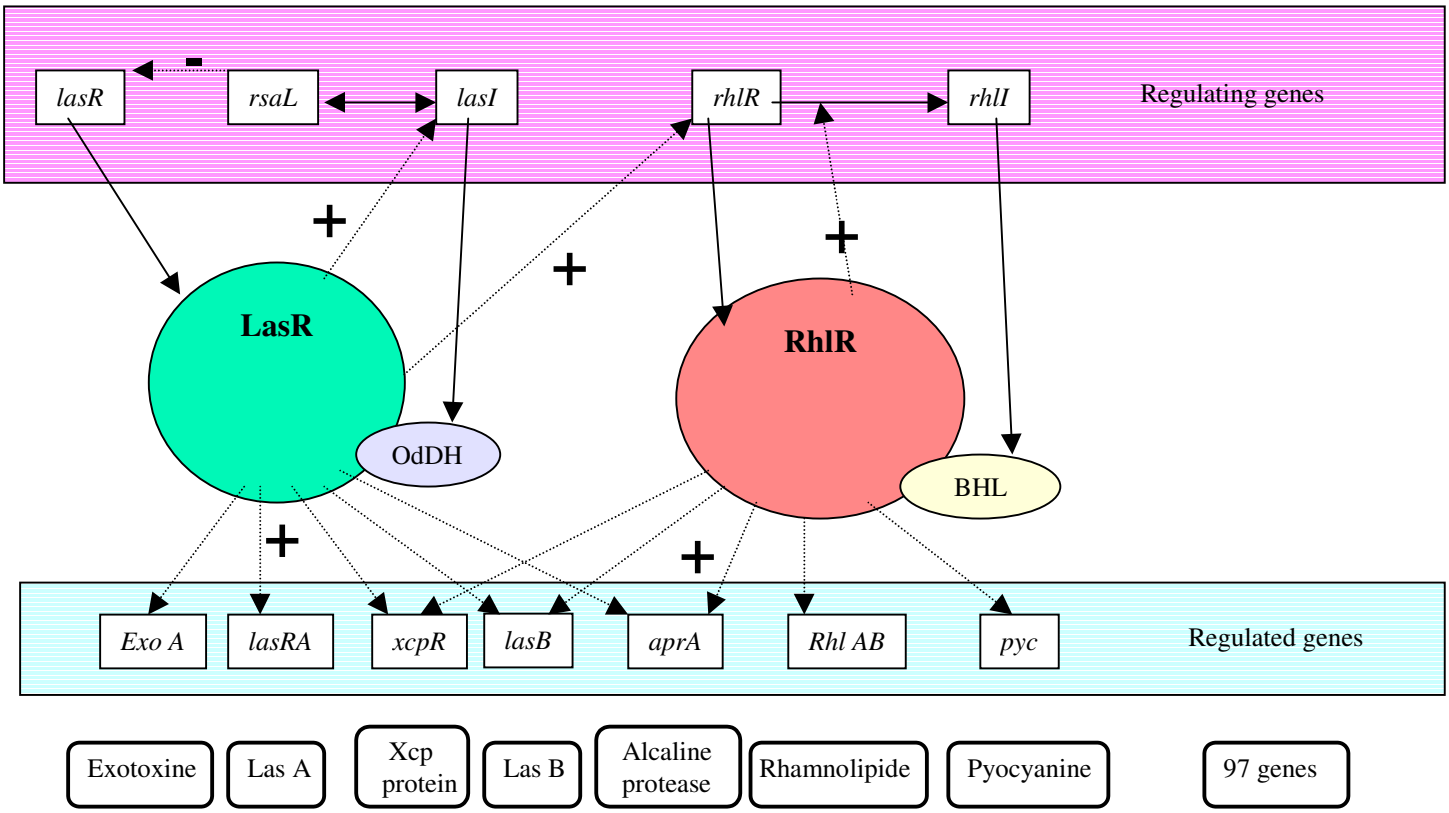
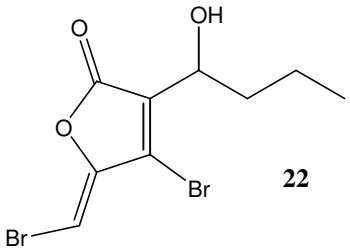


Figure 3: *Mechanism of quorum sensing in* Pseudomonas aeruginosa *[5].*

4. Is it possible to inhibit quorum sensing?

It was observed by *Kjelleberg* and *Steinberg* that the red alga *Delisea pulchra* was very rarely covered by biofilms [6,7], even when this alga grows in the presence of thousands of bacteria species. The study showed that this alga was able to produce halogenated furanones such as **22** and **23**. These furanones show a structure, which is very similar to the homoserine lactones seen above. Therefore, it could be assumed that the furanones produced by the alga were able to interfere with the quorum sensing systems of different bacteria. Moreover, it was seen for *Vibrio fischeri* that the bacterium was able to produce inhibitors of its own quorum sensing system at low cell concentration [4]. It could be imagined that the halogenated furanones have an analogous activity: the furanones could bind to the receptor proteins in competition with the homoserine lactones, and by this way inhibit the activation of the other genes responsible for the bacterial virulence.



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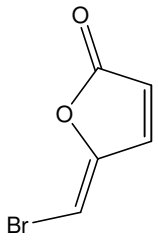


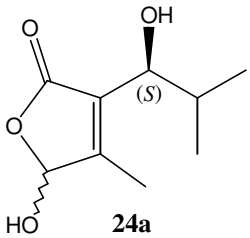
23

Following this idea, experiments were made by Hentzer et al. [8]. Their studies showed that the halogenated furanone **23** gave a significant inhibition effect on the quorum sensing systems of *Pseudomonas aeruginosa*. Although the furanone does not change the growth rate of the bacteria, it reduces their virulence through the inhibition of the expression of the genes responsible for it.

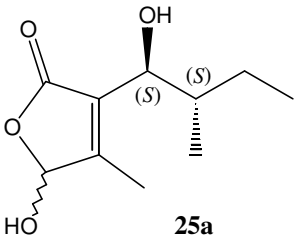
II Aims of the work

1. Natural furanones from *Streptomyces antibioticus*

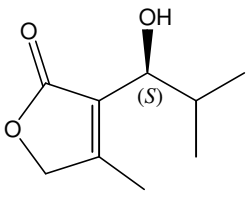
Four natural furanones **24a**, **25a**, **26a**, and **27a** extracted from *Streptomyces antibioticus* TÜ 99 showed a slight antibiotic activity as well as a slight inhibition effect on chitinases from *Serratia marcescens* [9]. The furanone **26a**, *e.g.*, had a slight antibiotic activity against *Bacillus brevis* and *Arthrobacter oxydans*. It was also active against *Pseudomonas fluorescens* [10]. Similarly, the four furanones **24a**, **25a**, **26a**, and **27a** also have antibiotic properties against the human pathogenic agent *Pseudomonas aeruginosa* [9].



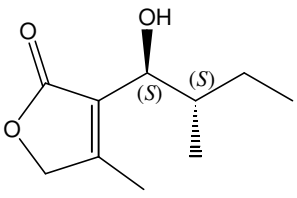
24a



25a



26a



27a

2. Natural furanones and quorum sensing

The natural furanones **24a**-**27a** shown above have structures similar to the halogenated compounds from *Delisea pulchra*, and also resemble somewhat the homoserine lactones from quorum sensing systems of bacteria. The main idea was to synthesize new compounds that could interfere with quorum sensing systems, and which are analogous to these natural furanones.

As they were extracted in too small amounts, the furanones **24a**-**27a** were chemically synthesized in order to determine not only their chemical and biological properties, but also their absolute configurations. A synthetic pathway was developed by Grossmann [11] in order to have a convenient synthesis for larger amounts. This method was then applied to the synthesis of new furanones [12].

3. The goals of the work

● The key step of the synthesis is a condensation of the menthylated furanone **38b** and an aldehyde to introduce a side chain at C(3) of the furanone. This step had to be optimized.

● The synthetic procedure developed by Grossmann should be used to obtain additional furanones with different side chains in position 3 of the lactone.

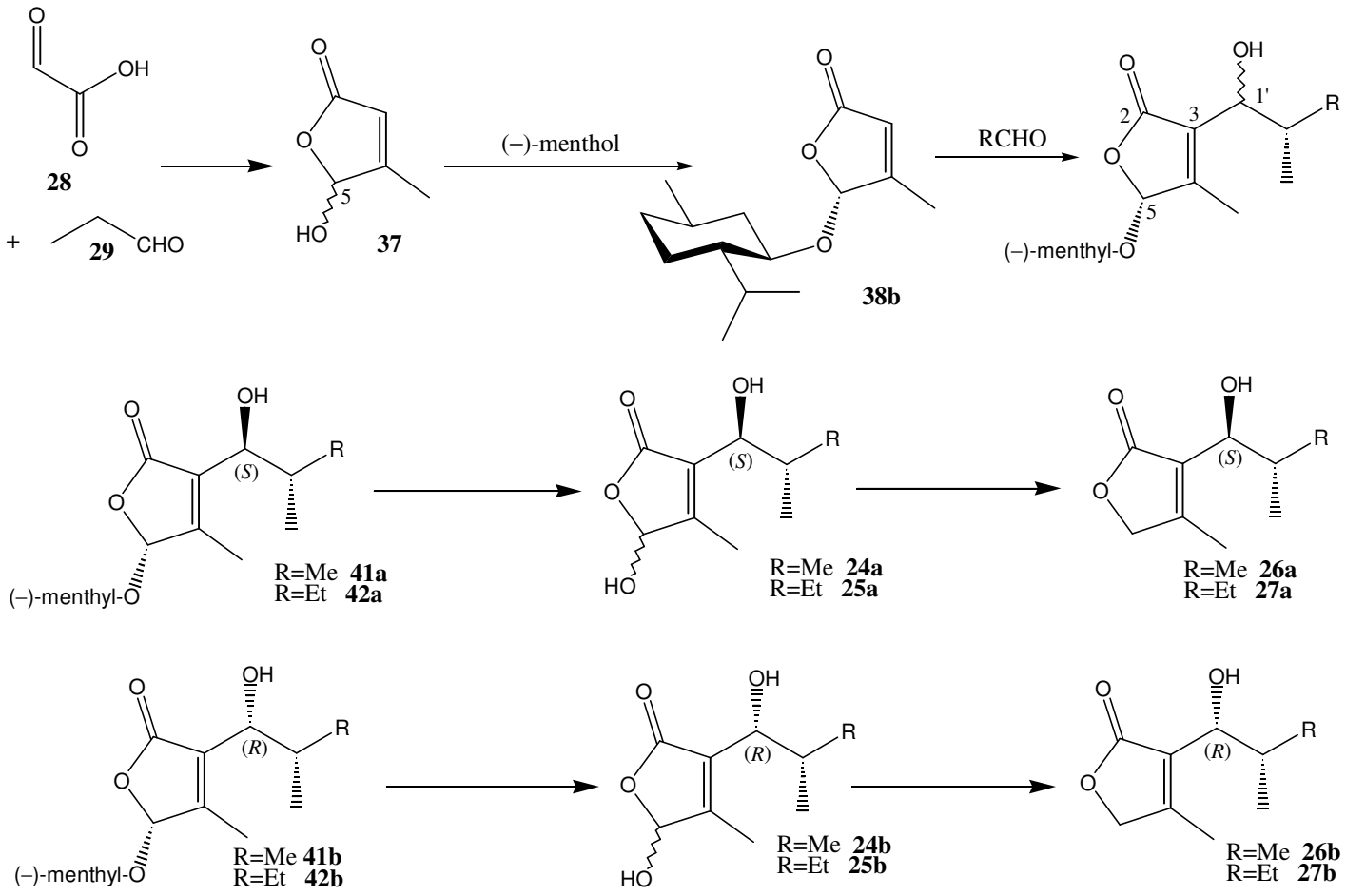
● The new synthetic furanones will have to be tested in order to investigate their ability to inhibit the quorum sensing system of bacteria.

III Synthesis of the furanones

1. Overview of the method

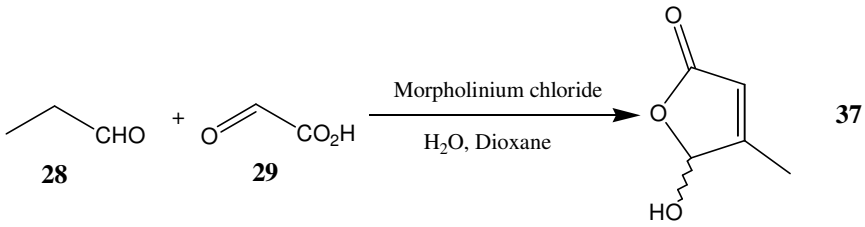
A five step synthetic pathway was designed by Grossmann for the preparation of the natural compounds **24a**-**27a** [11]. The two step preparation of the menthylated furanone **38b**

had been previously described in the literature [13,14]. A *Mannich*-type reaction between glyoxylic acid (**28**), propionaldehyde (**29**) and morpholinium chloride (**30**) affords the 5-hydroxyfuran-2-one (**37**), which is menthylated under acidic conditions. A fractional crystallization gives the pure 5(*R*) epimer **38b**. This furanone is then added to 2-methylpropanal or 2(*S*)-methylbutanal in the presence of LDA to give the respective epimer mixtures **41a/b** and **42a/b** (Scheme 1). The isomers were separated by flash-chromatography on silica gel. Then the menthyl ethers **41a**, **41b**, **42a**, **42b** were cleaved with BBr_3 and the obtained 5-hydroxy furanones **24a**, **24b**, **25a**, **25b** were reduced with $NaBH_4$ to give the compounds **26a**, **26b**, **27a**, **27b**. The absolute configurations of the natural products could then be deduced from a comparison of their NMR spectra with those of the synthetic compounds.

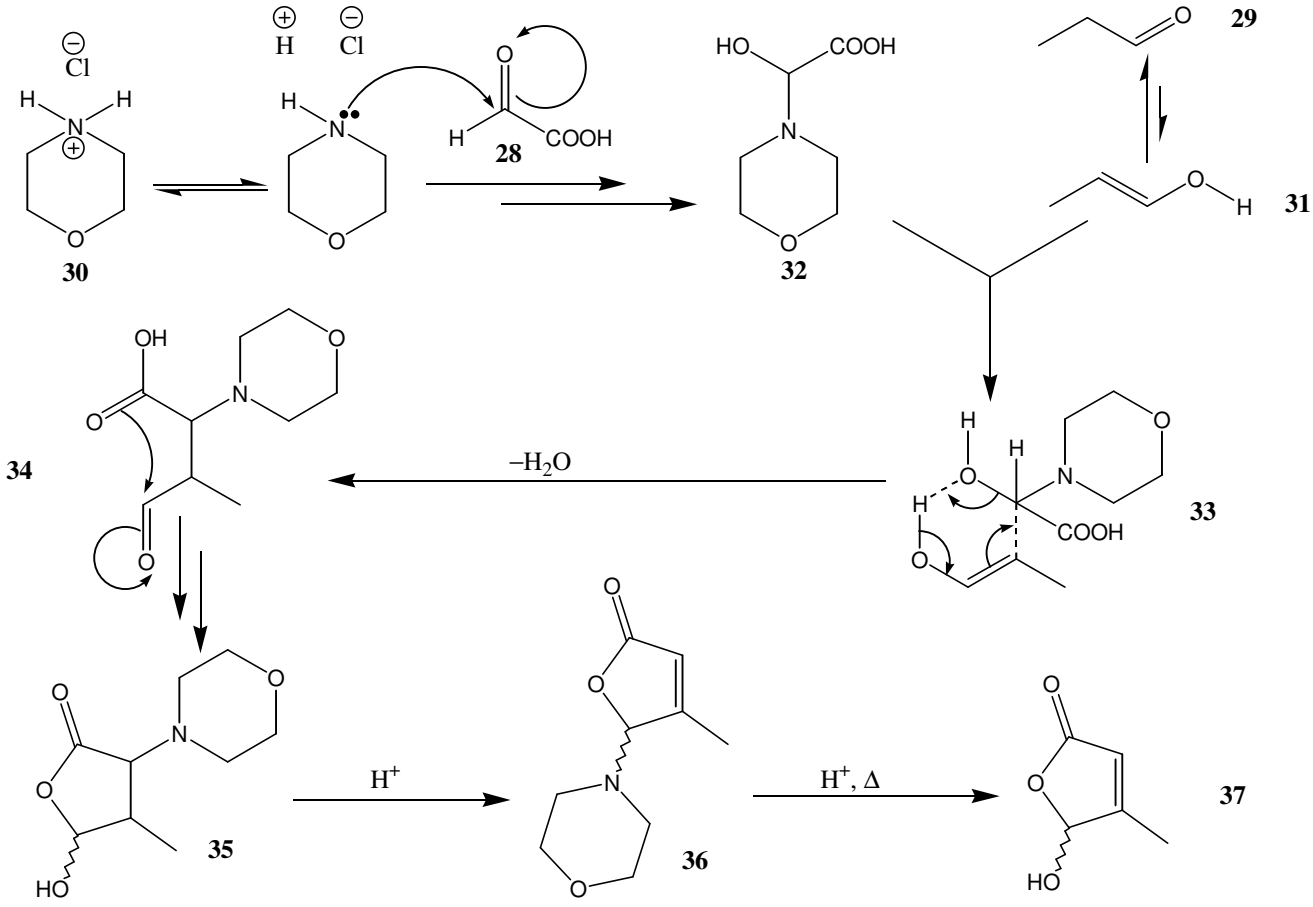


Scheme 1: *Synthesis of the natural furanones* ***24a-27a*** *and their enantiomers.*

2. First step: synthesis of the starting furanone (**37**)



Although this reaction was published in the early 80's [13], its mechanism – being particularly interesting – will be discussed in the following paragraph.

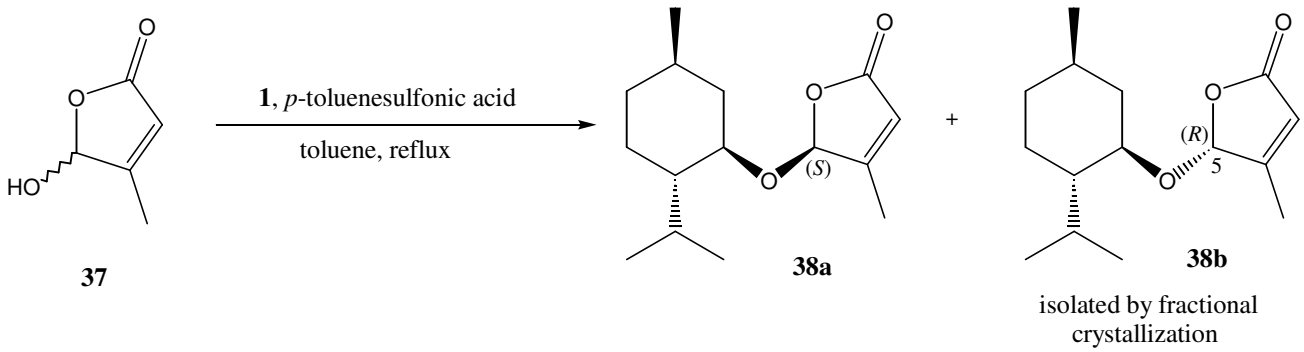


Scheme 2: *Mechanism of 5-hydroxy-4-methyl-5H-furan-2-one (**37**) synthesis.*

Morpholinium chloride (**30**) reacts with glyoxylic acid (**28**) to form the Mannich base **32** (Scheme 2), which reacts with the enol **31** corresponding to propionaldehyde (**29**). Formula **33** shows the 6-membered ring transition state of this Mannich-like reaction.

Condensation of both reagents with simultaneous departure of water gives the intermediate **34**. Then an intramolecular cyclization occurs to **35**, followed by an acid catalysed rearrangement and elimination of the morpholine to afford the furanone **37**.

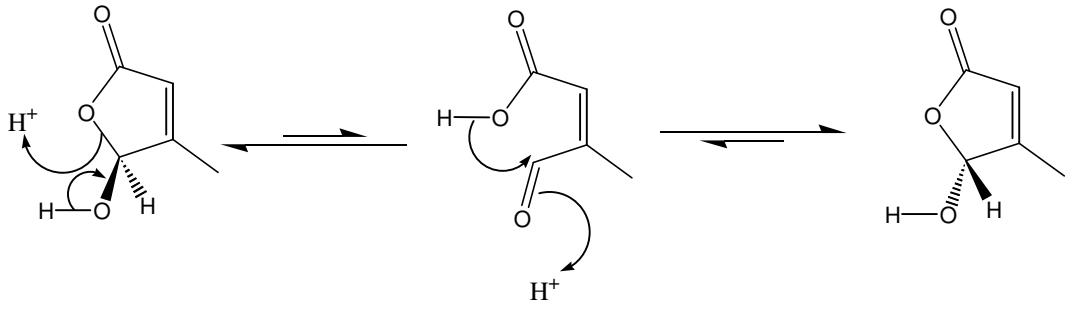
3. Second step: the menthylation



37 **38a** **38b**

isolated by fractional crystallization

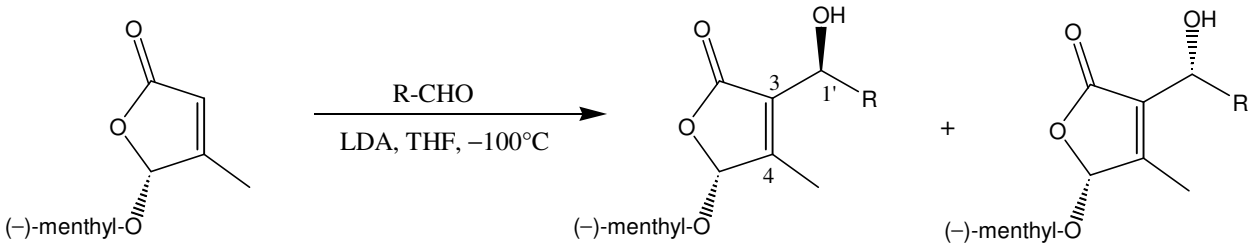
At room temperature, the furanone **37** racemizes very quickly (Scheme 3). This property makes the separation of isomers like **24a** and **24b** impossible, because one has to deal with an equilibrium mixture of two pairs of enantiomers. Blocking the epimerization at C(5) facilitates the separation of the epimers after the coupling with the aldehyde. The use of a chiral blocking group could lead to chiral induction in the condensation of furanone and aldehyde. (−)-Menthol is perfect for this purpose as it is a natural product, available in enantiomerically pure form at a modest price. The menthylation of the starting furanone **37** was published by Feringa et al. at the end of the 80's [14]. The reaction occurs in refluxing toluene with *p*-toluenesulfonic acid as a catalyst, and the water which is produced during the reaction can be collected in a Dean-Stark apparatus.



Scheme 3: *Racemization of the furanone at room temperature.*

The reaction itself yields both epimers **38a** and **38b**, which can be separated through fractional crystallization. After precipitation of both epimers at –18°C in petrol ether, the wanted epimer **38b** is isolated after successive recrystallizations from petrol ether.

4. Third step: nucleophilic addition of the furanone on the aldehyde

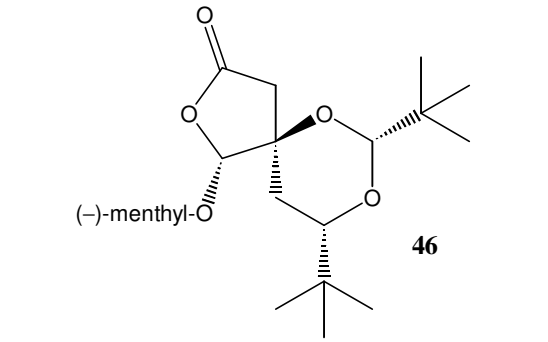


38b

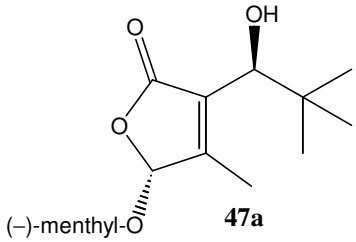
4.1 General remarks

The typical procedure was described by Grossmann. The condensation was first performed at –78°C in dry THF. The furanone **38b** was treated with LDA as a base to afford the anion at C(3), then the aldehyde was added and the reaction carried out at –78°C.

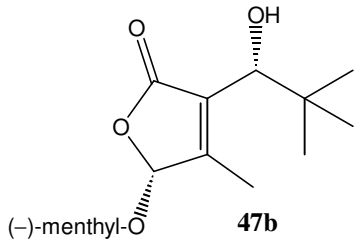
Following these conditions, the reaction was tried with pivalaldehyde, heptanal and decanal in order to obtain the respective compounds **47a**, **47b**, **50**, **51**. In fact, the *tert*-butylated furanones **47a** and **47b** were obtained in poor yields (about 20% yield for the mixture). Another product, **46**, was isolated in significantly larger amounts, lacking the C(3)-C(4) double bond according to the NMR spectra. Addition of heptanal or decanal to the furanone proved to be almost impossible under the above conditions (–78°C).



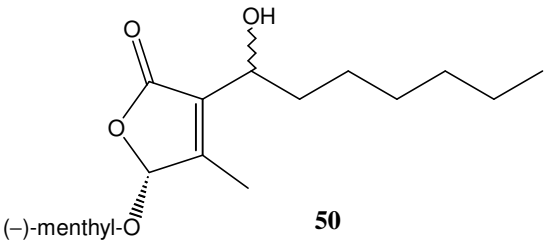
46



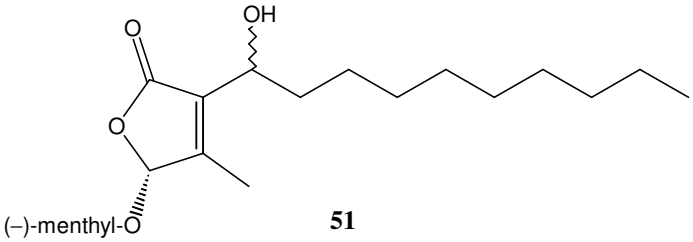
47a



47b



50



51

4.2 Enolate reactivity

The crystallization of the new spiro-compound **46** followed by an X-ray structure determination (Figure 4) revealed that the reaction conditions for the condensation were not optimal. The structure showed that two equivalents of pivalaldehyde were added to the furanone. How could this surprising result be explained?

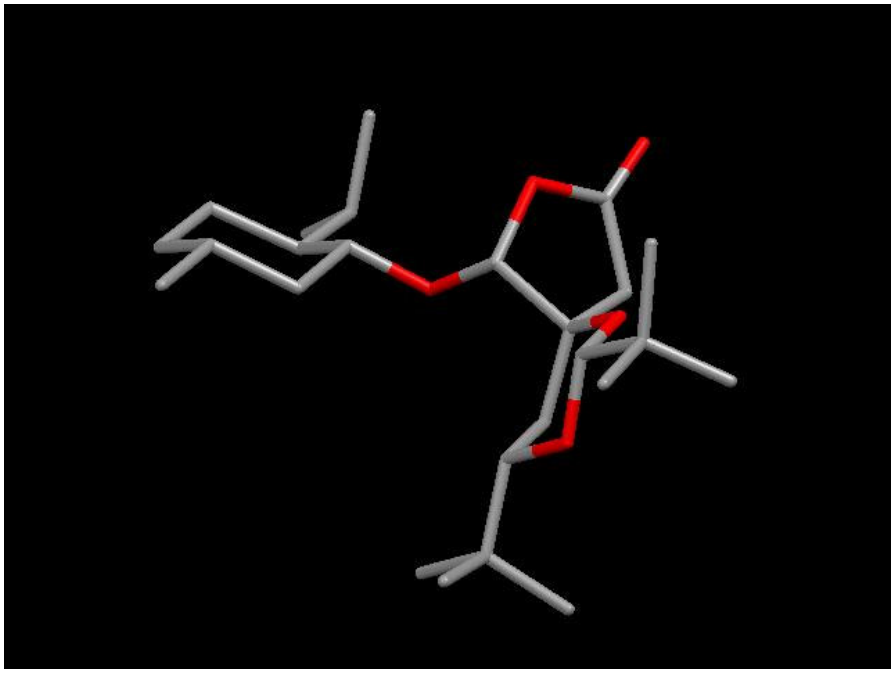
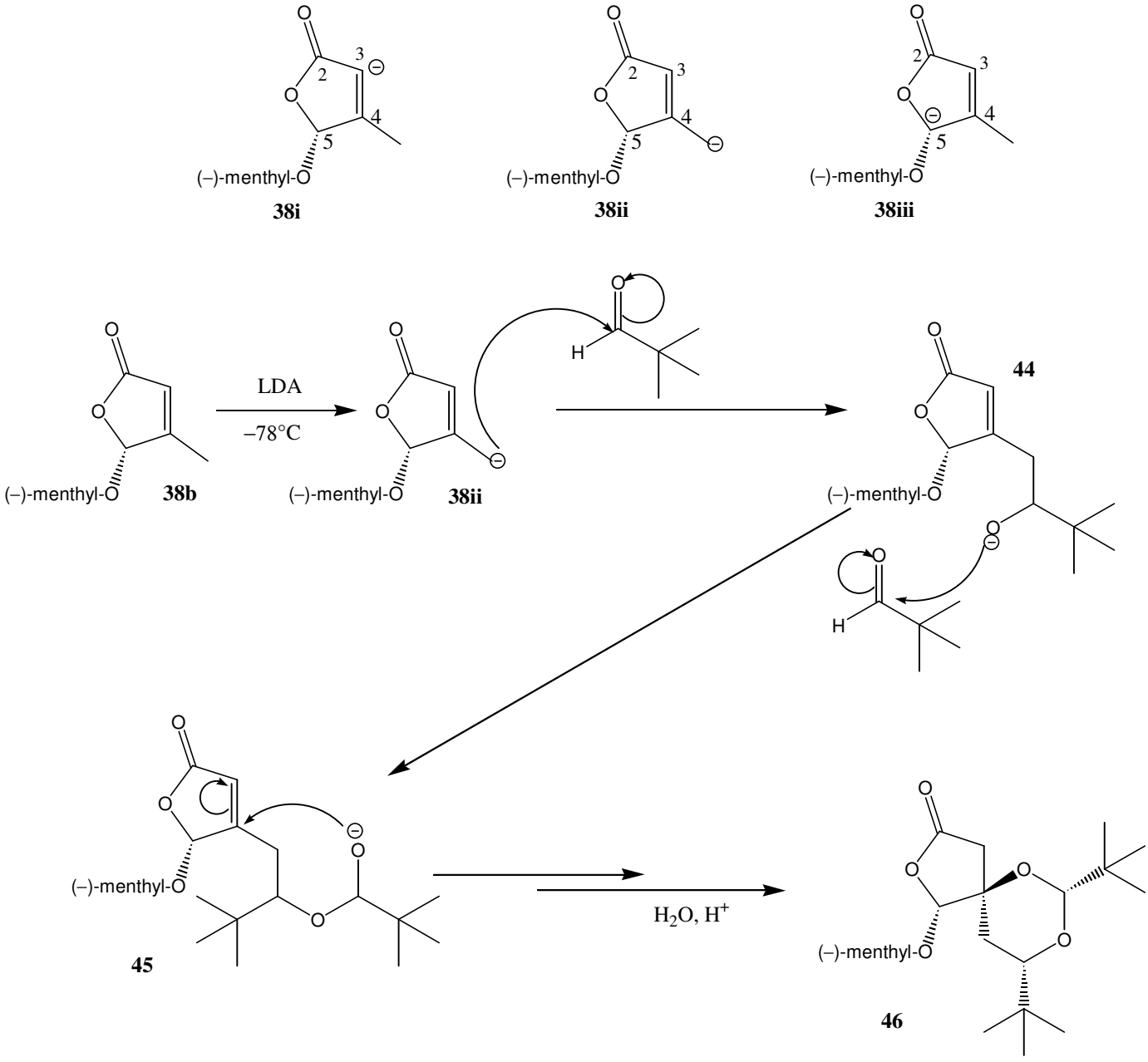


Figure 4: *X-ray structure of the spiro-compound* ***46****.*

By treating furanone **38b** with LDA, three different anions **38i**, **38ii**, and **38iii** are theoretically possible: with the negative charge on C(3) (**38i**), on C(5) (**38iii**), or on the methyl carbon (**38ii**), respectively. In fact, the proton on the C(5) might not be acidic because this position is a ketal function, and the corresponding anion should not be very stable. Normally, formation of the anion is expected at C(3). However, the appearance of compound **46** clearly shows that the charge can also be formed at the methyl position. We assume that the anion **38ii** adds first to pivalaldehyde (Scheme 4), then the new anion **44** adds to a second molecule of pivalaldehyde to form the intermediate **45**. Then, cyclization via intramolecular Michael addition occurs to give after protonation the observed spiro-compound **46**.

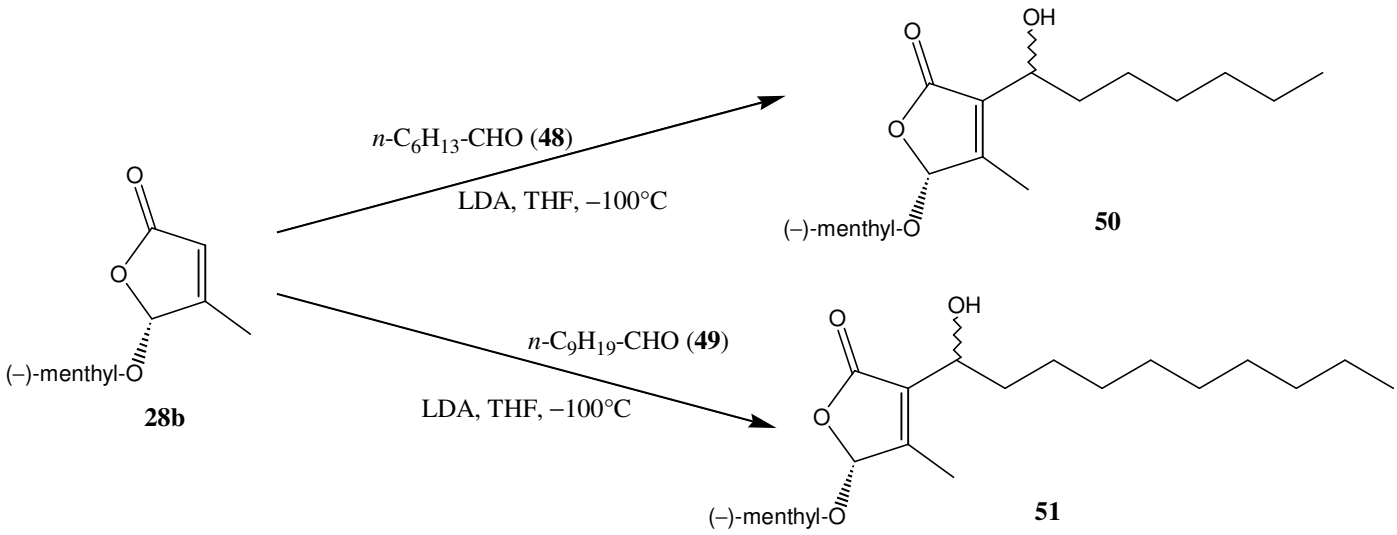


Scheme 4: *Hypothetical mechanism of the formation of* ***46.***

This mechanism explains reasonably well the observed result. The configuration of the spiro carbon can be explained by steric consideration. The intramolecular Michael addition will most likely take place from the side opposite to the menthyloxy group. In addition, the *tert*-butyl groups are observed in equatorial positions. The main issue now was to figure out how to promote anion formation at C(3).

In general with α,β-unsaturated ketones, the proton that is lost during the enolate formation is located at the γ-carbon (vinylogous effect). So, we can assume that the conjugated enolate **38ii** should be more stable than the C(3) anion **38i**. For this latter, the negative charge is located in an electron rich area and almost no resonance stabilization is possible. However, the C(3) anion **38i** does exist, because condensation does occur at this position. We conclude that the C(3) anion **38i** is kinetically favoured, whereas the conjugated enolate **38ii** is thermodynamically more stable. This assumption led us to work at a lower temperature. When the reaction is carried out at –100°C, no spiro-compound is obtained, and the two expected epimers **47a** and **47b** were isolated in approximately 20% yield each.

The same procedure was applied for the condensation of the menthylated furanone **38b** with heptanal **48** and decanal **49** to give the corresponding products **50** and **51** (Scheme 5) as mixtures of epimers in 30% (heptanal) and 37% yield (decanal), respectively. The lower reaction temperature enabled in all the cases a very significant improvement of the yields. The assumption about the kinetic behaviour of the C(3) anion was corroborated by these experiments.



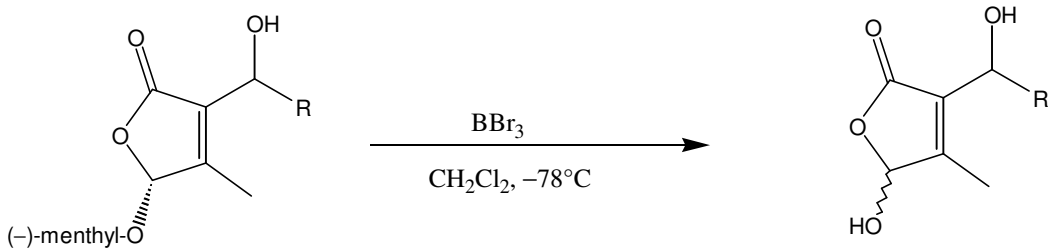
Scheme 5: *Condensation of **38b** with heptanal and decanal.*

4.3 Separation of the epimers

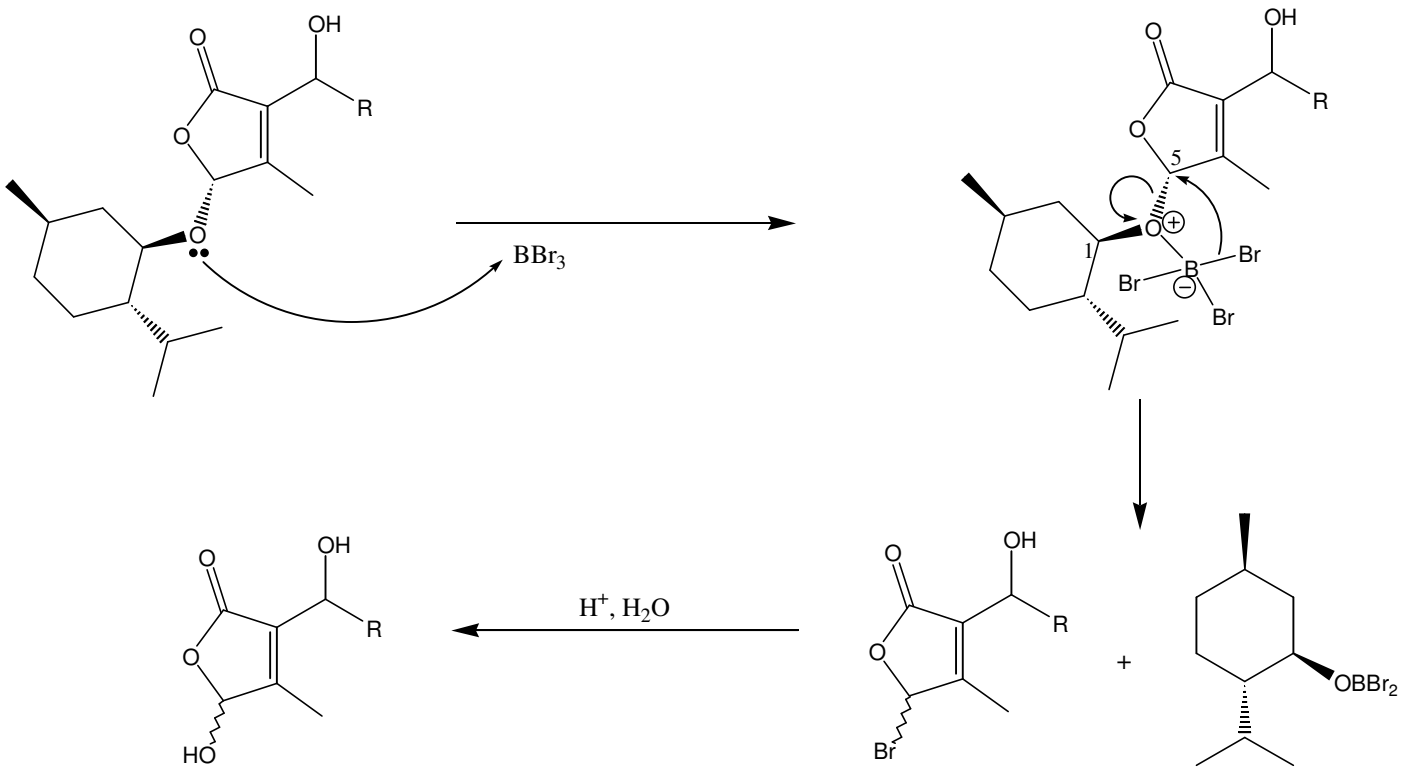
So far, the separation of the epimers formed in the condensation of the furanone with the aldehyde could always be achieved by column chromatography on silica gel [12]. In this way, pure diastereomers **47a** and **47b** could be obtained. Unfortunately, the epimers of the compounds **50** and **51** could not be separated under these conditions.

5. Fourth step: cleavage of the menthyloxy bond

The classical ether cleavage reaction with BBr_3 was used. In general, the Lewis acid BBr_3 is used for the cleavage of aryl methyl ethers, which allows very mild conditions. This is particularly useful when the substrate contains a lot of functional groups and limits the risk of a decomposition of the compounds.

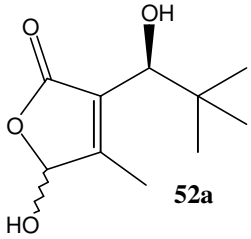


The mechanism of this cleavage is quite simple [15]. The oxygen of the ether adds to the empty *p*-orbital of BBr_3 (Scheme 6). Simultaneously, a bromide binds to C(5) of the furanone, which is much more electrophilic than C(1) of the menthyl moiety. After work-up, the respective alcohols are obtained. When the reaction was performed with **50**, brominated furanone **54** was isolated in addition to **53**, which confirms the mechanism outlined above.

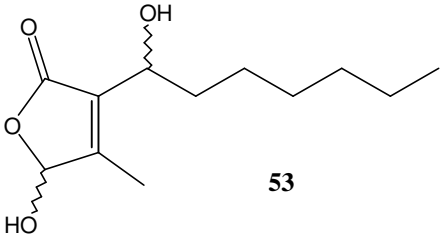


Scheme 6: *Mechanism of the ether cleavage with BBr_3.*

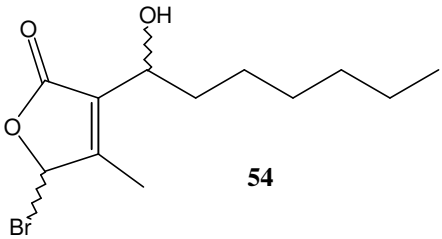
The yield of this cleavage reaction is generally about 40-60%; the method was used for the preparation of **52a**, **52b**, **53**, and **55** starting from the respective menthylated furanones.



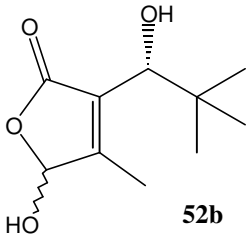
52a



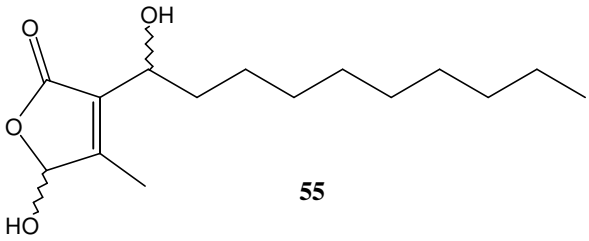
53



54



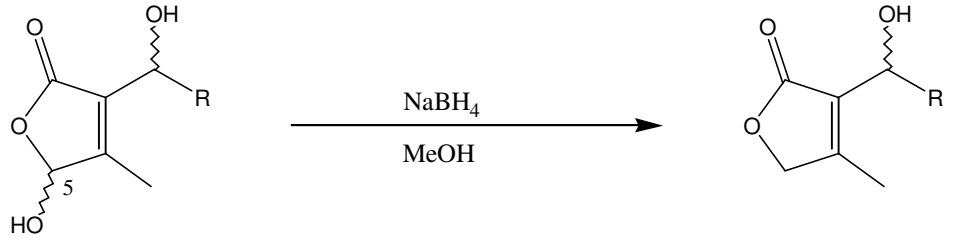
52b

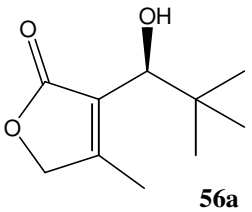


55

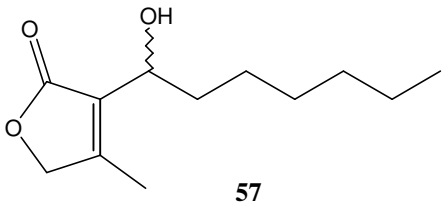
6. Fifth step: reduction of C(5)

The carbon C(5) can be reduced with $NaBH_4$. The use of this hydride enables a selective reduction, as it does not react with an ester, which is crucial for the synthesis of the reduced furanones. The reaction occurs in methanol at room temperature, and the yields generally range from 60% to 70%. Following these conditions, compounds **52a**, **52b**, **53**, and **55** yielded the furanones **56a**, **56b**, **57**, and **58**, respectively (Figure 5).





56a



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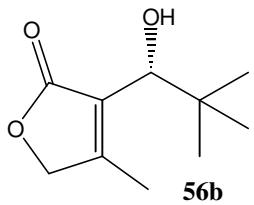
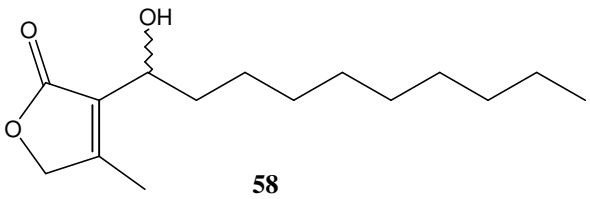


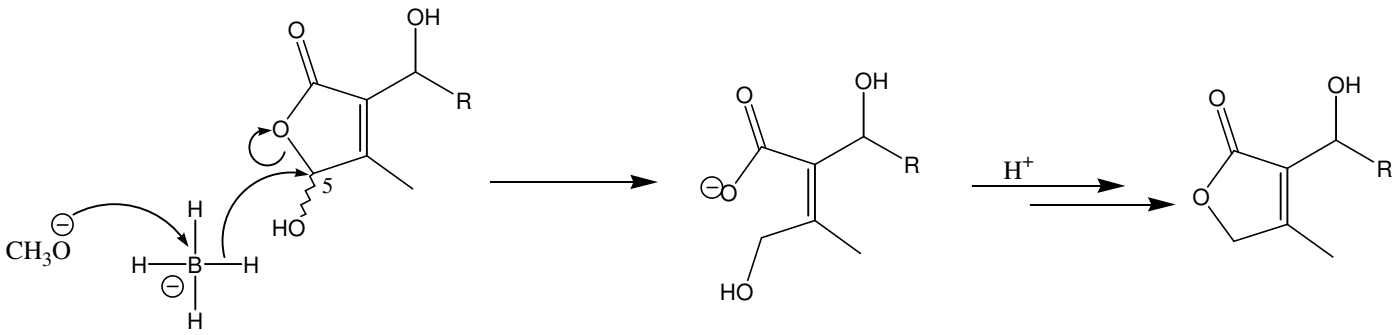
56b



58

Figure 5: *Reduction of furanone C(5).*

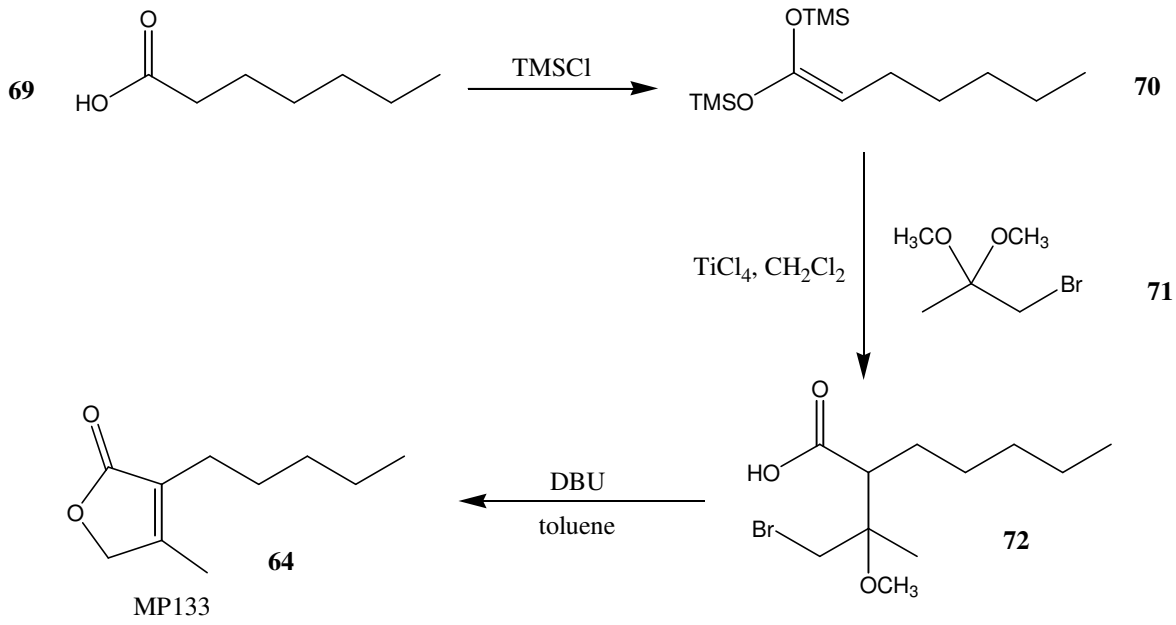
The mechanism of the reduction of an aldehyde by $NaBH_4$ involves a transition state including the aldehyde, the BH_4^- anion and a molecule of the solvent [16]. In the present case, C(5) is a hemiketal derivate. We can imagine a similar mechanism for its reduction. Since the reaction occurs under basic conditions, part of the added $NaBH_4$ reacts with methanol to give methanolate ions, with hydrogen formation. Then the methanolate adds to BH_4^-, with simultaneous hydride transfer to the furanone (Scheme 7). We suppose that the lactone ring opens to promote this hydride transfer, but it could also be imagined that the hydroxy group can leave C(5) without lactone opening. An opened lactone ring will reclose during the work-up.



Scheme 7: *Mechanism of the reduction with $NaBH_4$.*

7. Synthesis of MP133 (**64**)

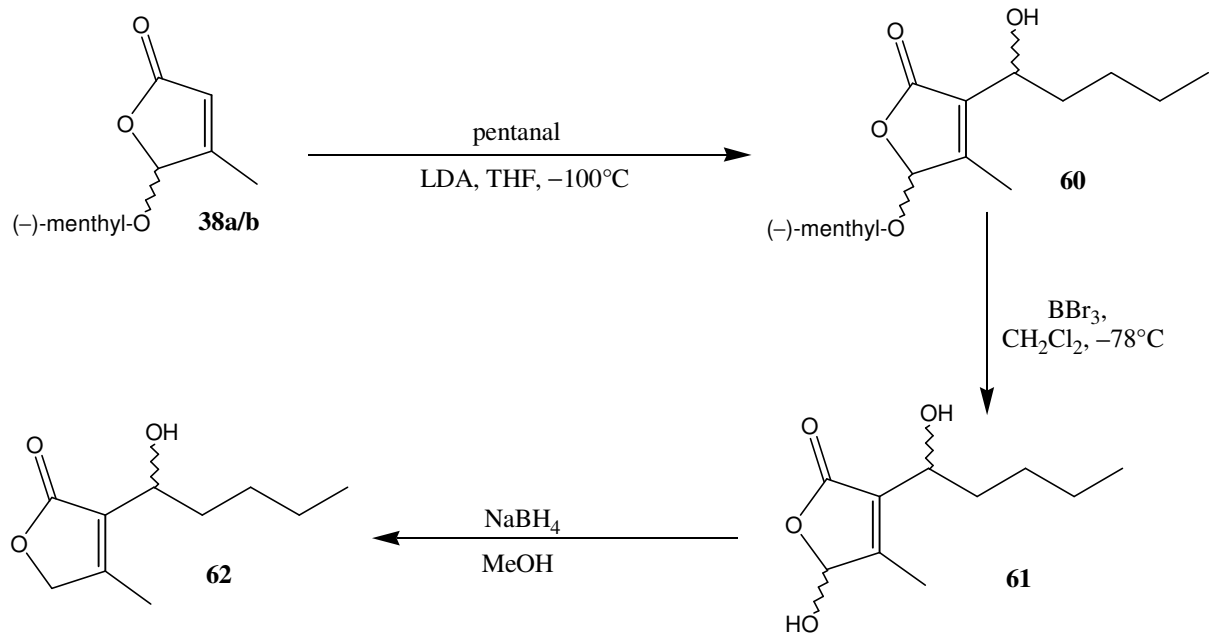
This compound was obtained by Poncioni during the search for synthetic pathways to furanones **26a** and **27a** [17]. The preparation of MP133 (**64**) was originally carried out according to Demnitz's method (Scheme 8) [18]. Heptanoic acid (**69**) was treated with TMSCl under basic conditions to afford the corresponding ketene bis(trimethylsilyl) ketal (**70**), which was condensed with 1-bromo-2,2-dimethoxypropane (**71**) under $TiCl_4$ catalysis to afford the β-methoxy-γ-bromo carboxylic acid (**72**). This latter was treated with DBU in toluene to give furanone **64**.



Scheme 8: *Preparation of MP133 (**64**) according to Demnitz's method [17].*

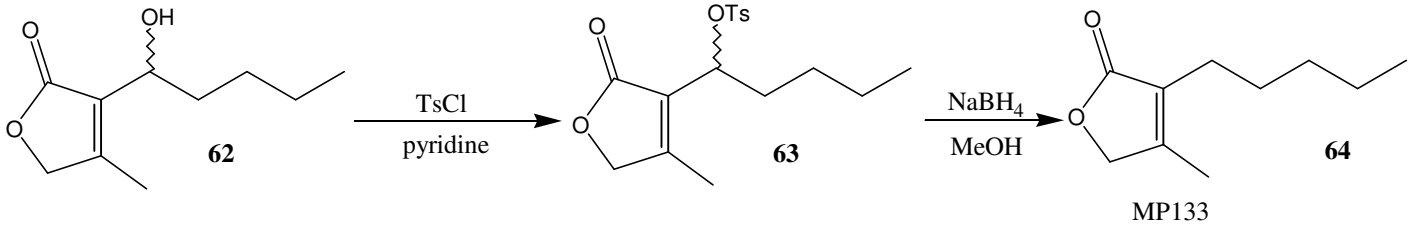
Interstingly, MP133 (**64**) proved to have a weak activity as A-factor analog in *Streptomyces pristinaespiralis* [19]. However, the Demnitz method did not afford MP133 (**64**) in sufficient yields, and more material was necessary for additional biological tests. As **64** is also a flavour compound, a completely different synthesis of this furanone had already been patented by Givaudan [20]: starting from acetylmethyl 2-bromoheptanoate, an intramolecular Reformatsky reaction followed by a dehydratation gives **64**.

Having elaborated in our group a versatile route to furanones (Grossmann's method, see above), we decided to try the latter for the synthesis of **64**. The starting furanone **37** was menthylated, but no fractional crystallization was performed. Both epimers **38a** and **38b** (Scheme 8) were treated with LDA at –100°C and condensed with pentanal to give **60** as a mixture of four diastereomers in 60% yield. Then, the menthyl group was removed with BBr_3 to afford a mixture of two pairs of enantiomers **61** in 45% yield. All isomers of **61** were reduced together with $NaBH_4$ to give racemic **62** in 60% yield.



Scheme 9: *Towards MP133 (**64**) according the Grossmann's method.*

At this stage, an additional reduction was necessary to remove the OH group in the side chain and give MP133 (**64**). A convenient general method to reduce secondary alcohols is the preparation of a sulfonic ester followed by a nucleophilic substitution with a hydride. In our case, a tosylation of **62** in pyridine (Scheme 10) to **63** and an immediate reduction of this latter with $NaBH_4$ after dilution in methanol gave the MP133 (**64**) in 43% yield [21].



Scheme 10: *Reduction of **62** into MP133 (**64**).*

The synthesis was performed with isomer mixtures as the final product MP133 (**64**) is an achiral molecule. This avoids the loss of material, especially in the menthylation step with the furanone **37**: instead having to isolate a pure diastereomer in 15-20% yield, the non-fractional recrystallization affords easily 50-60% yield of the mixture **38a/b**. However, this

menthylation step is necessary to avoid side reactions during the basic condensation with pentanal.

In addition, in order to save a step, a double tosylation of both hydroxy groups of **61**, followed by the reduction with $NaBH_4$ was attempted. However, these conditions did not allow the direct preparation of MP133 (**64**) from **61**.

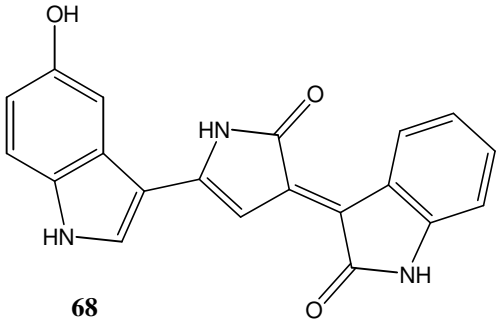
IV Biological Tests, results and discussion

1. General remarks

Our synthetic furanones were expected to be analogues to natural homoserine lactones, A-factor, and related compounds. Therefore, they might interact in two different ways with the quorum sensing system. They can either promote or inhibit the quorum sensing phenomena. This latter is more interesting, as it could prevent biofilm formation as well as significantly reduce the virulence of pathogens.

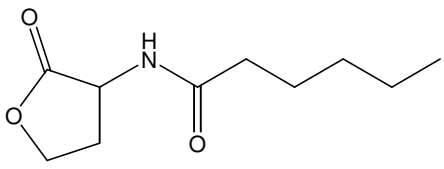
2. Biological tests with *Chromobacterium violaceum* CV026

The bacterium *Chromobacterium violaceum* is able to produce a purple pigment called violacein (**68**) which also has antibiotic properties. The biosynthesis of this pigment is induced when a critical concentration of HHL (**21**) is reached in a similar way to triggering of the bioluminescence of *Vibrio fischeri*.



68

Violacein



21

HHL

The wild-type bacterium is able to produce HHL, therefore a mutant strain of *Chromobacterium violaceum*, called CV026, was used. This mutant is not able to produce HHL, but it can synthesize violacein. This means that if HHL is added to the culture, then the purple pigmentation is observed.

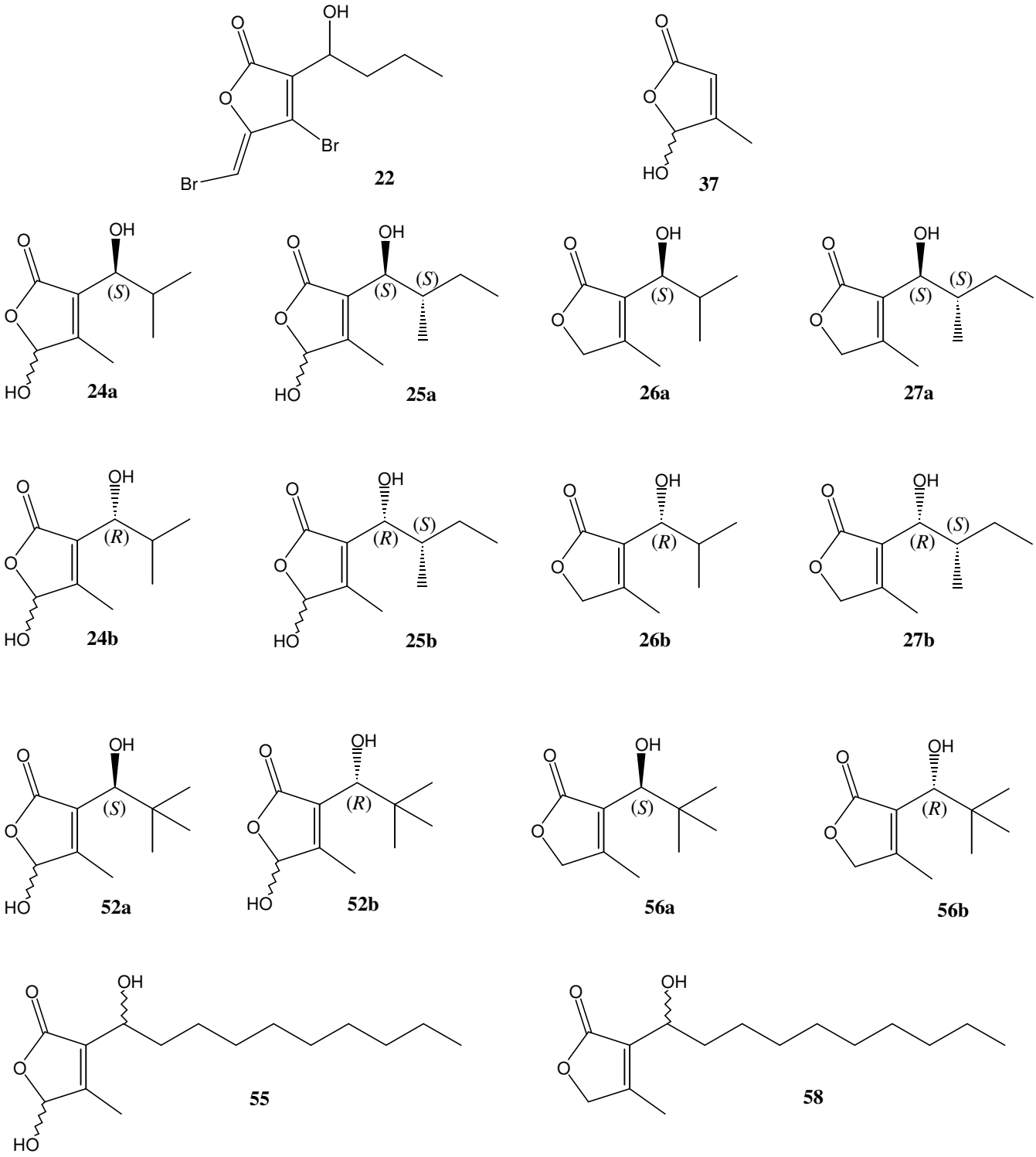


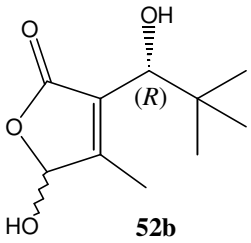
Figure 6: *Structures of the tested furanones.*

The biological tests for quorum sensing interference were carried out with *C. violaceum* CV026 and furanones from *Delisea pulchra*, flavouring furanones from Givaudan, and the synthetic furanones mentioned above. Only the most significantly active compounds, whose formula are given above (Figure 6), will be discussed further.

Two different experiments were made with the furanones. In the first experiment, the furanone and HHL were added simultaneously to the mutant bacterium. The absence of the purple coloration meant that the tested furanone was able to inhibit the quorum sensing system, whereas the apparition of the coloration proved the furanone was not active in this way. The second experiment consisted in adding only the furanone to the bacterium. The coloration meant that the furanone could work in a similar way to HHL, whereas no coloration meant that the furanone had no effect.

As an example, the complete activity tests for compound **52b** are given. Figure 7 shows the bioassay plate. The concentration of the furanone **52b** decreases horizontally from 1 to 12 and the concentration of HHL decreases from row A to F. For each concentration, three tests were made. *E.g.*, the samples A1, A2, and A3 contain the same mixture.

We explain here the conditions for the tests. The concentrations of the furanone were 10^{-2} M in columns 1 to 3, 10^{-3} M in columns 4 to 6, 10^{-4} M in columns 7 to 9, and 10^{-5} M in columns 10 to 12. The concentrations of HHL were 10^{-6} M in row A, $3.3*10^{-7}$ M in row B, $1.1*10^{-7}$ M in row C, $3.7*10^{-8}$ M in row D, $1.2*10^{-8}$ M in row E, and $4.1*10^{-9}$M in row F. The calibration with HHL alone was made with the following concentrations: 10^{-6} M in G1-G3, 10^{-7} M in G4-G6, 10^{-8} M in G7-G9, 10^{-9} M in G10-G12, 10^{-10} M in H1-H3, and 10^{-11} M in H4-H6. A growth control of the mutant CV026 lacking HHL was made in H7-H9, and the growth inhibition was tested with the bacteria in the presence of **52b** at 10^{-2} M in H10-H12 [22]. Quorum sensing inhibition was measured by absorbance at 590 nm after evaporation and resolubilization in DMSO of the samples, and growth inhibition (or activation) was measured by turbidity at 660nm.



52b

At maximal concentration of **52b** (A1-F1), no purple coloration was observed: this showed that the quorum sensing was completely inhibited. In addition, the purple coloration became stronger with decreasing furanone concentration indicating decreasing inhibition (*e.g.* A1-A12). Full development of the purple coloration was observed for the HHL calibration samples, where no furanone was added (G1-G12, H1-H6). The colourless samples H7-H12 corresponded to the growth inhibition test.

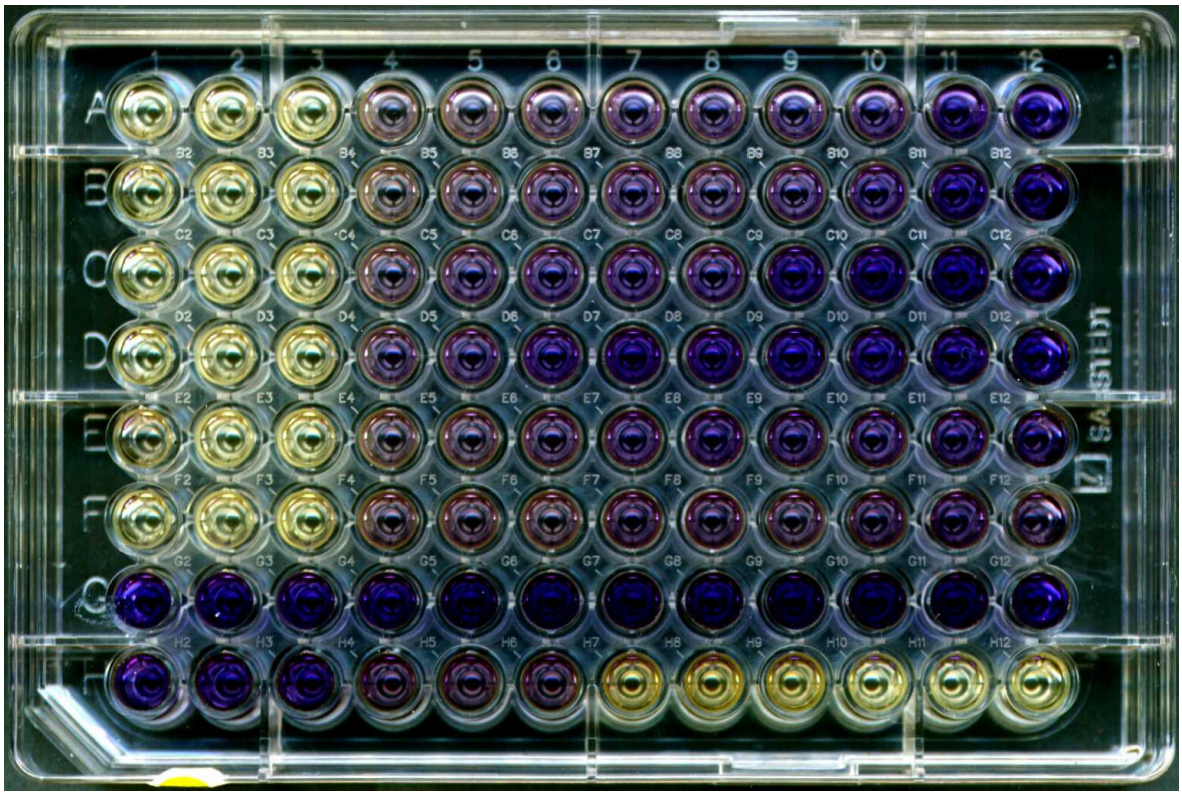


Figure 7: *Bioassay for quorum sensing demonstrating effects of* ***52b*** *on violacein formation [22].*

Similar series of tests were made with the other furanones. The results are summarized in Table 1, while all compounds tested are compiled in Figure 6. Activation of quorum sensing is shown in the second column; growth control = 0 and $3.7*10^{-8}$ M HHL = 100. In the

third column, the growth activation is given; growth control = 100, significant activation (>115) is emphasized in bold green and significant toxicity (<60) in bold red. In the fourth column, the inhibition of quorum sensing is given for HHL at $3.7*10^{-8}$ M and compound to be tested at 10^{-4} M: control = 0 and complete inhibition = 100. Significant inhibitions (>40) are emphasized in bold red. The fifth column also gives inhibition properties: HHL concentration is 10^{-6} M and compound concentration is 10^{-4} M. Significantly better inhibition effects compared to the fourth column are given in bold red, whereas significantly smaller effects are given in bold green. The change in activity is considered as significant when the difference is more than 15. The sixth column gives the synergistic effect of the tested compounds at 10^{-5} M with HHL at $4.6*10^{-9}$ M: control = 100, and significantly enhanced expressions (>115) are given in bold green.

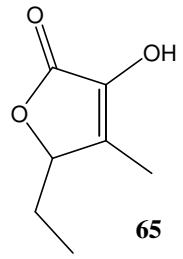
Compound	Activation	Growth activation	Inhibition 1	Inhibition 2	Synergistic effect
22	–4	**59**	**68**	61	96
24a	–1	96	31	**55**	**121**
24b	0	97	**69**	82	18
25a	–2	75	27	**46**	**127**
25b	–2	83	17	5	**241**
26a	–5	65	**64**	64	52
26b	–1	86	**42**	33	94
27a	–3	72	26	**48**	**164**
27b	–3	76	34	**49**	**137**
37	–4	65	22	23	**174**
52a	–8	**38**	**49**	**72**	105
52b	–5	**50**	**80**	**64**	82
55	–6	**52**	13	6	115
56a	–4	**53**	**53**	49	90
56b	–6	**49**	**56**	**68**	86
58	3	**117**	30	**2**	97

Table 1: *Effects of the furanones on quorum sensing and growth [22].*

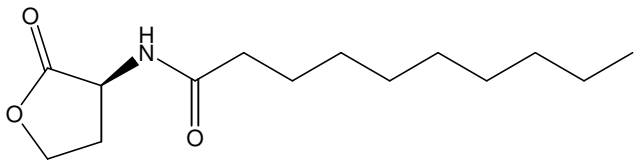
3. Discussion

Even if some of the tested compounds proved to be toxic at 10^{-2} M, such as **52a**, **52b**, **55**, **56a**, and **56b**, none of them inhibited cell growth at 10^{-4} M. As the tests were made at this concentration, or at a lower one, we can assume that the results are only due to the interaction between the compounds and the quorum sensing system.

Only the furanone **65** activated the quorum sensing without HHL. It is also not surprising to notice that the DHL (**66**) strongly activates the formation of violacein [22].



65



66

The halogenated furanone **22** extracted from *Delisea pulchra* significantly inhibits the violacein formation. In addition, this furanone is toxic as it also reduces the bacterial growth. This result is in accordance with the observation in nature: the red alga *Delisea pulchra* is rarely covered by biofilms. It confirms that halogenated furanones are able to inhibit the quorum sensing in nature.

Furanones **24b** and **26a** showed quite good inhibition. The furanone **24b** is not toxic, as the growth activation was not significantly different from the control, whereas the compound **26a** showed a slight toxicity at 10^{-2} M. The most active compounds were the furanones **52a**, **52b**, **56a**, and **56b**. For these compounds, the inhibition of violacein production ranged from 49% to 80%. All these compounds are derived from the condensation of the menthylated furanone **38b** with pivalaldehyde. Furthermore, these compounds proved to be toxic for bacteria, as they inhibited the growth from 48% to 62%. It shows that these derivatives not only inhibit the quorum sensing but also disable the bacterial proliferation at higher concentration. Compared with the halogenated furanones from *Delisea pulchra*, the compounds **52a**, **52b**, **56a**, and **56b** have similar bioactivities.

The natural furanones as well as the synthetic compounds from Grossmann have quite short side chains: this is an important difference to the structures of the homoserine lactones. A longer side chain at C(3) should resemble in a better way the homoserine lactone structure. The derivatives **55** and **58** were synthesized following this idea: it was supposed that the biological activity could have been improved by the introduction of a long aliphatic chain at C(3). Unfortunately, furanones **55** and **58** showed very poor inhibition effects.

Some of the furanones showed a synergistic effect with HHL. *E.g.* **25b** and **65** at 10^{-5} M concentration activated the violacein (**68**) production two times better than HHL alone at $4.6*10^{-9}$ M. Approximately half of the compounds tested led to an enhanced violacein production.

The results show, that it is not possible to class the compounds tested in agonists or antagonists of the quorum sensing receptors. In addition, there is a competition between these compounds and HHL to the binding sites of the receptors.

A comparison of compounds **24a** and **26b** with their respective epimers **24b** and **26a** shows significantly different activities. The furanones **24a** and **26b** are less active than their respective epimers. In contrast, the enantiomers **56a** and **56b** have similar activities, even if **56b** is a bit more active at higher HHL concentration.

Thus, it is very difficult to find a relationship between the structures of the synthetic furanones and their activities. Therefore it is not possible to predict which structure would have the best effect. There is for instance no correlation between the configuration of the carbon bearing the secondary alcohol in the side chain and activity in the quorum sensing system.

3. Biological activity of MP133 (**64**)

In a former study, MP133 (**64**) had been tested with a protein from *Streptomyces pristinaespiralis*. This protein is a receptor, which is involved in the pristinamycin biosynthesis. Natural butyrolactones bind to this receptor, which induces the antibiotic

production. MP133 (**64**) showed an ability to weakly inhibit this binding to the receptor protein [19].

According to these results, the biological activity of MP133 (**64**) was investigated further with mammalian cells. The aim was to test a possible activity on gene regulation in a similar way with the quorum sensing of *Streptomyces*. However, the compound proved to be toxic: it significantly inhibited the cell growth [21].

V Conclusion

The procedure developped by Grossmann [11] enables the synthesis of a large family of furanones in moderate yields through simple reactions, and it was successfully used for the preparation of furanones containing longer side chains. The reduction of the secondary alcohol in the side chain is also possible. However, the separation of the epimers after the condensation of the menthylated furanone and a long chain aliphatic aldehyde remains difficult. On the other hand, the yield of this condensation could be significantly improved by the use of new reaction conditions.

Some of the compounds we had prepared proved to be biologically active. They inhibited more or less the quorum sensing of *Chromobacterium violaceum* CV026. A few of these inhibitors also showed toxic properties since the growth of the bacteria was reduced in a significant manner. However, none of the synthetic furanones was significantly more active than the natural halogenated furanones from *Delisea pulchra*.

Part two:
Substituted furanones as putative chitinase inhibitors

I Introduction

1. Glycosidic biopolymers

In the living world, the most abundant polymers that we can find are carbohydrate derivatives. These glycopolymers can be found in a large variety of structures and for very different purposes. The best known glycopolymers are cellulose, starch, chitin and glycogen.

Cellulose is the most important material for the plant cell walls. It is a linear polymer of glucose. The monomers are linked through a beta 1-4 glycosidic bond. The linear chain could be very flexible if there were no hydrogen bond interactions between monomers. In the same way, inter-molecular hydrogen bond interactions make the structure rigid. Cellulose polymers linked together can arrange themselves in sheets, which are very resistant. Then, cohesion forces between the sheets gives hydrophobic properties, as well as more rigidity. So, the formation of micro-fibrils can occur, and this enables the production of a very resistant material.

Starch is also a polymer of glucose. The main difference to cellulose is that there are ramifications in the polymer structure. Starch is a convenient way for plants to store large amounts of energy. Therefore cereals and potatoes, which are rich in starch, are very energizing food.

Animals are also able to store large amounts of energy through the polymerization of glucose: they produce glycogen. This glycopolymer, like starch, is a ramified polymer of glucose. The difference to starch is the number of ramifications. In glycogen, this is much more important than in starch.

Chitin is different: the monomer is *N*-acetylglucosamine. Chitin is the most abundant polymer containing nitrogen in the living world. The structure is analogous to cellulose: chitin is a linear polymer in which the monomers are linked through beta 1-4 glycosidic bonds [23]. Like cellulose, chitin can form sheets in order to assemble itself in very rigid structures like insect exoskeletons or crustacean shells. In addition, chitin can be found in the cell wall of micro-organisms such as bacteria, mushrooms, fungi, and yeasts.

2. Glycosidases

The biosynthesis of the polymers involves very complicated processes. The enzymes used for the synthesis, as well as the degradation must have the suitable selectivity. We can imagine that there are as many different glycosidases as animal or plant species in nature. In fact, it is possible to classify the different enzymes in several families. Such a classification has been introduced by Henrissat [24-26]. All chitinases, the enzymes which are mainly responsible for chitin degradation, are classified into families 18 and 19.

3. Different roles of chitinases in nature

Chitinases are essential in chitin metabolism processes. They enable the regulation of the insect exoskeleton formation, as well as the degradation of chitin. As chitin is too rigid to enable the growth, insects have to change their exoskeleton regularly. This process is the moulting. The exoskeleton is degraded in certain parts so that the insect can get rid of it: it is precisely during this moment that chitinases are crucial. Then insects need several days to grow a new exoskeleton that fits their new size.

Chitin is also an essential constituent of the membranes of bacteria and mushrooms, including in particular pathogenic bacteria and different species of parasites. Unlike animals, plants have no immune system, therefore chitinases are useful for plants to fight pathogens through the destruction of their cell wall: thus chitinases are defence proteins. In the same way, animals also produce chitinases as well as lysozymes in addition to their immune system: these enzyme are complementary to the immune system in order to prevent infections.

Furthermore, chitinases are used by predators and carnivore plants. They enable the digestion of their preys. *E.g.*, the octopus *Eledone cirrhosa* generally eats crabs. It injects its saliva, which contains a potent mixture of chitinases and proteases into its preys. The saliva diffuses throughout the prey (the crab) and enables the predator to remove the meat from the carapace [27]. Some parasites can also produce chitinases to enable them to penetrate into an insect's body. In addition, chitinases also provide nutrients to the parasites, since the insect exoskeleton can be hydrolysed into glucosamine derivates. Moreover, the chitinases can participate to the release of progeny pathogens.

These examples of the uses of chitinases in nature are not exhaustive, but they show some of the most important functions of these enzymes.

4. General information on chitinases

Chitinases are proteins whose molecular weights range from 30 kDa to 120 kDa. Plant and alga chitinases have generally a molecular weight of 30 kDa. Chitinases from animals such as mollusks, arthropods, fishes, amphibians and mammals have molecular weights generally from 40 to 90 kDa, and some of them can be up 120 kDa. Bacterial and fungal chitinases have very different sizes, ranging from 30 kDA to 120 kDa [28].

The optimum activity occurs generally at an approximately neutral pH, or under very mild basic conditions as well as slightly acidic conditions. Plant and algal chitinases are active between pH 4 and pH 9. Chitinases from animals are best active at pH 4.8-7.5, whereas chitinases from microorganisms are active at pH 3.5-8.0.

It was possible to isolate chitinases from *Bacillus licheniformis* in hot springs. These chitinases were stable at 80°C. Other chitinases from plants also showed such thermal stability. On the other hand, chitinases from insects are much less stable at high temperature. For instance, chitinases from silkworm are unstable above 40°C. In fact, most of time, insects use chitinases for the production and the degradation of their exoskeleton. As they generally grow at moderate temperature, like 25°C, they do not need chitinases, which are stable at high temperature. In addition, chitinases from insect are generally larger than chitinases from

plants. It can be assumed that smaller chitinases are more stable at higher temperature than the bigger ones.

There are two different catalysis mechanisms for the hydrolysis of chitin: the catalysis with inversion of the configuration of the anomeric carbon of monomers and the catalysis with retention [29].

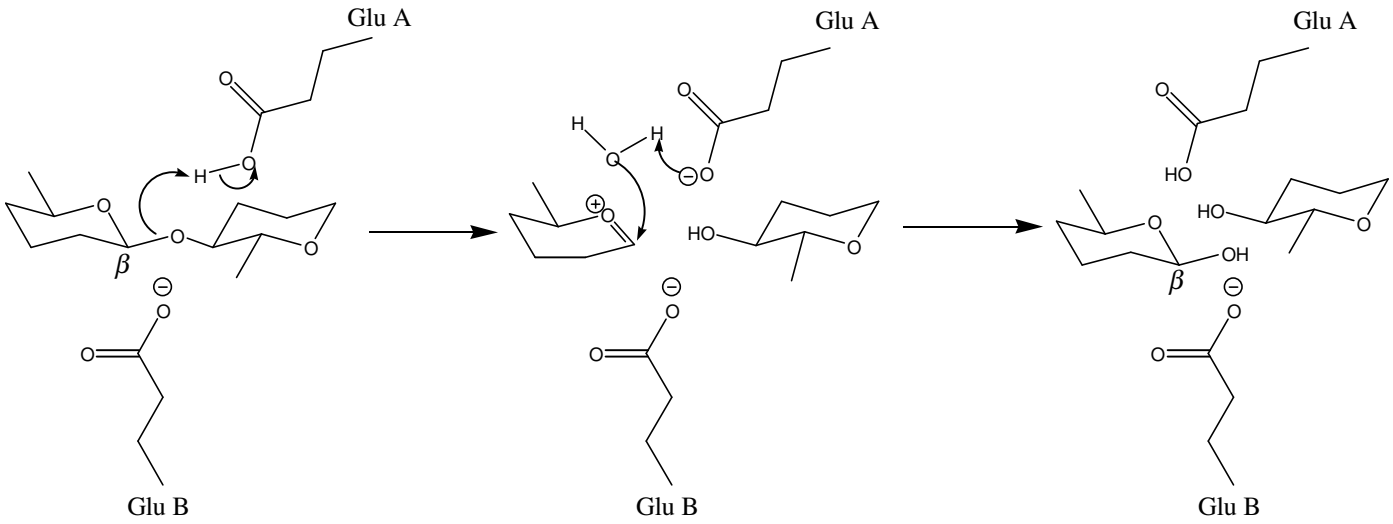


Figure 8: *Cleavage of a β-glycosidic bond with retention of the anomeric configuration [29].*

Figure 8 shows the mechanism of the degradation of chitin with retention of configuration of the anomeric carbon. The glycosidic bond breaks with a simultaneous protonation of the leaving alcohol. The oxocarbenium cation obtained is stabilized in the active site by carboxylate anions (*e.g.* from glutamate). Then, water adds to the intermediate to complete the cleavage.

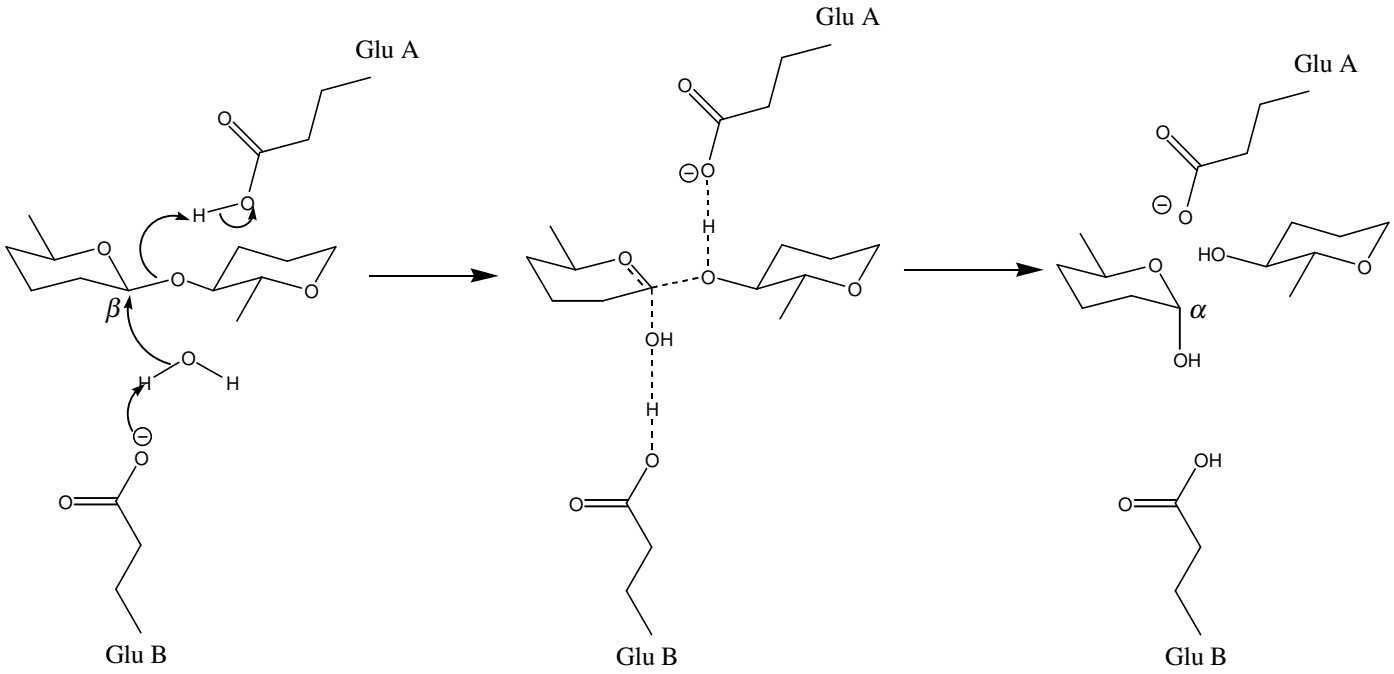


Figure 9: *Cleavage of a β-glycosidic bond with inversion of the anomeric configuration [29].*

The mechanism with inversion of the configuration gives the α-anomer (Figure 9). In this case, the mechanism resembles an S_N2 reaction. This mechanism is illustrated by the chitin degradation by a barley chitinase (Figure 13). The chitin fragment is maintained in the active site through hydrogen bonds (Figure 10). The glutamic acid (Glu 67) activates the oxygen of the leaving sugar. At the same time, the glutamate (Glu 89) polarizes water, which adds to the anomeric carbon.

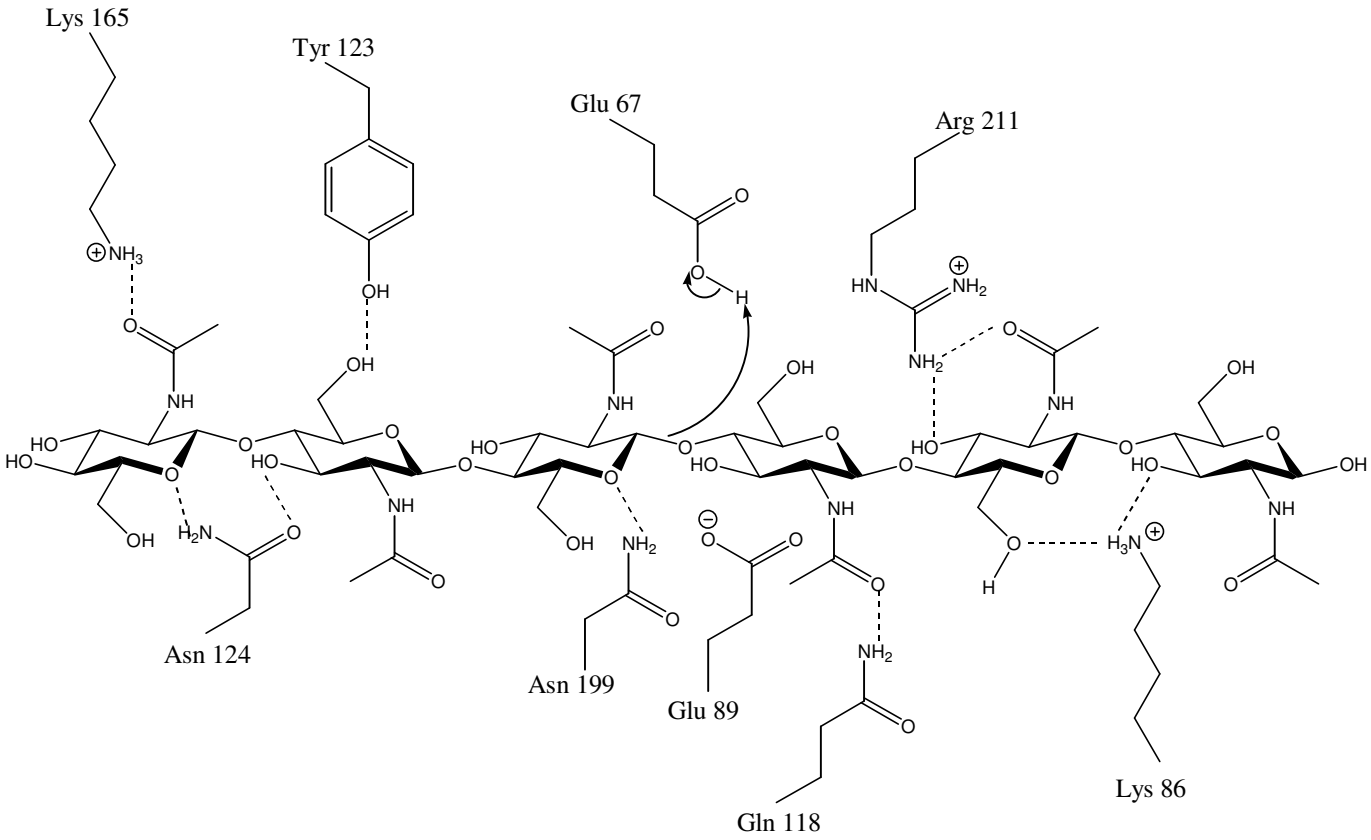


Figure 10: *Hypothetical binding and cleavage of a chitin polymer with barley chitinase [29].*

Both mechanisms presented above remain hypothetical. They are, however, in accordance with the configuration observed in the products. The inversion mechanism was in addition confirmed by NMR and kinetic studies [30].

5. Classification of chitinases

Henrissat and Bairoch developed a classification for all the glycosidases [24-26]. This classification is based on the amino-acids sequences of the proteins. It led to define more than 50 different families of glycoside hydrolases. As a consequence of this, two proteins from the same family have close structures and have similar hydrolysis mechanisms. Though chitinases are widely present in nature, they all belong to only two families: the family 18 and the family 19.

The family 18 is the largest family with approximately 180 members. It contains essentially chitinases from eukaryotes, prokaryotes and viruses. The family 18 chitinases have

an α/β barrel structure, in which the active site is located in the centre of the barrel. A structure analysis of the chitinase from *Serratia marcescens* (Figure 11) shows that the barrel is constituted of β-sheets, linked by return α-helices. Hevamine (Figure 12), a defence protein from the rubber tree *Hevea brasiliensis*, is also a family 18 chitinase, and has a structure close to that of the chitinase from *Serratia marcescens*. The catalysis mechanism of family 18 chitinases is with configuration retention with a double displacement mechanism, as discussed above [29].

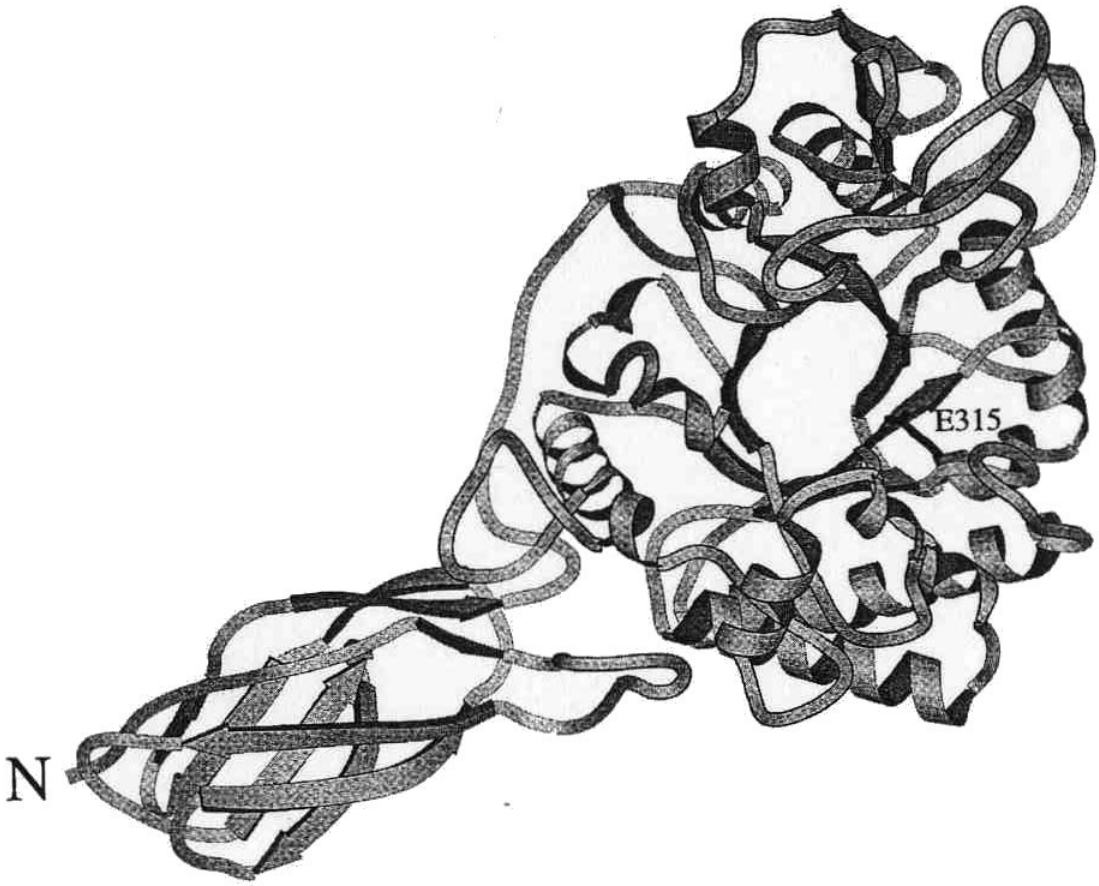


Figure 11: *Structure of a chitinase from* Serratia marcescens *[29].*

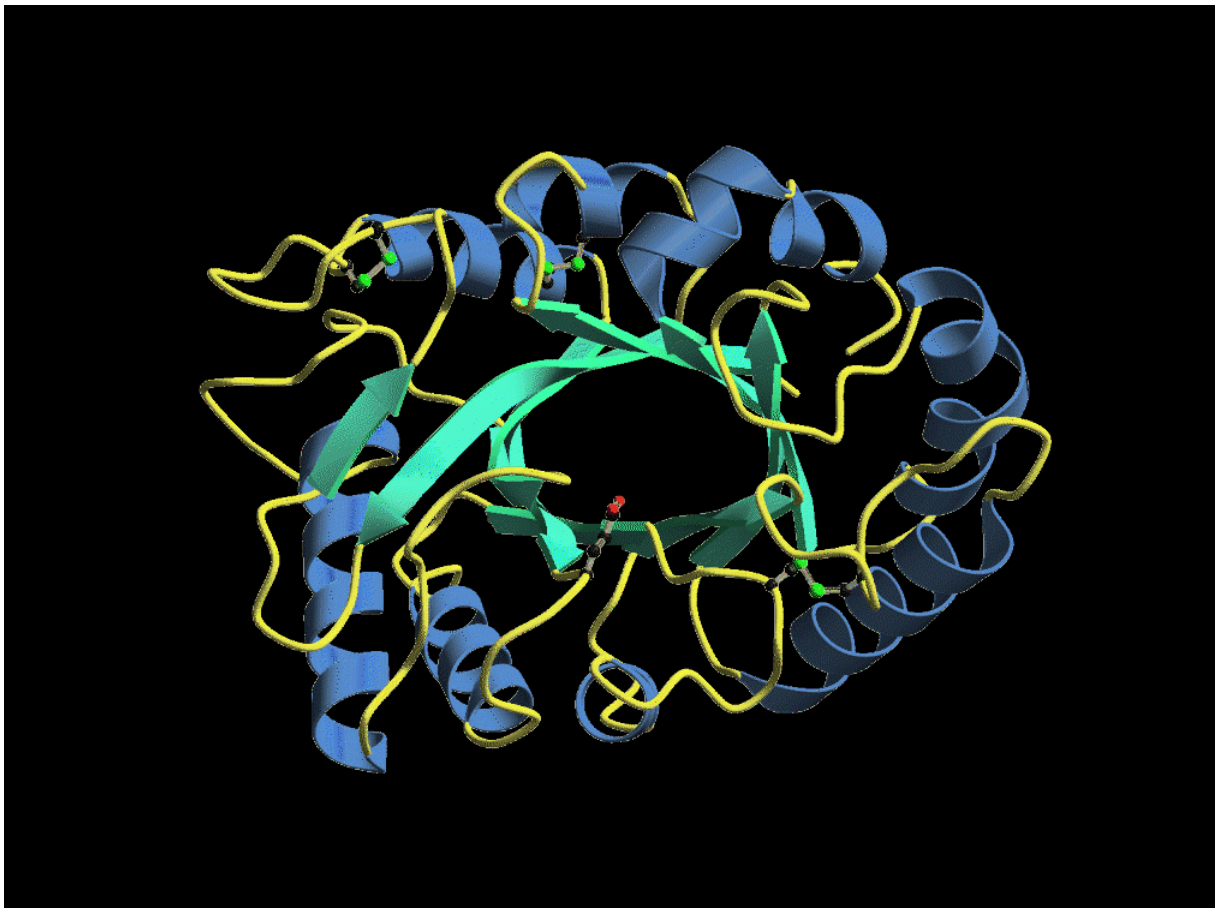


Figure 12: *Structure of hevamine [31].*

Family 19 is smaller: about 130 members are listed in this category. The family 19 chitinases were all extracted from plants, except a chitinase from *Streptomyces griseus*. The structure of these chitinases is essentially built from α-helices [29] like in the barley chitinase (Figure 13). The catalytic mechanism is with a configuration inversion. This explains the strong structural difference to the family 18 chitinases.

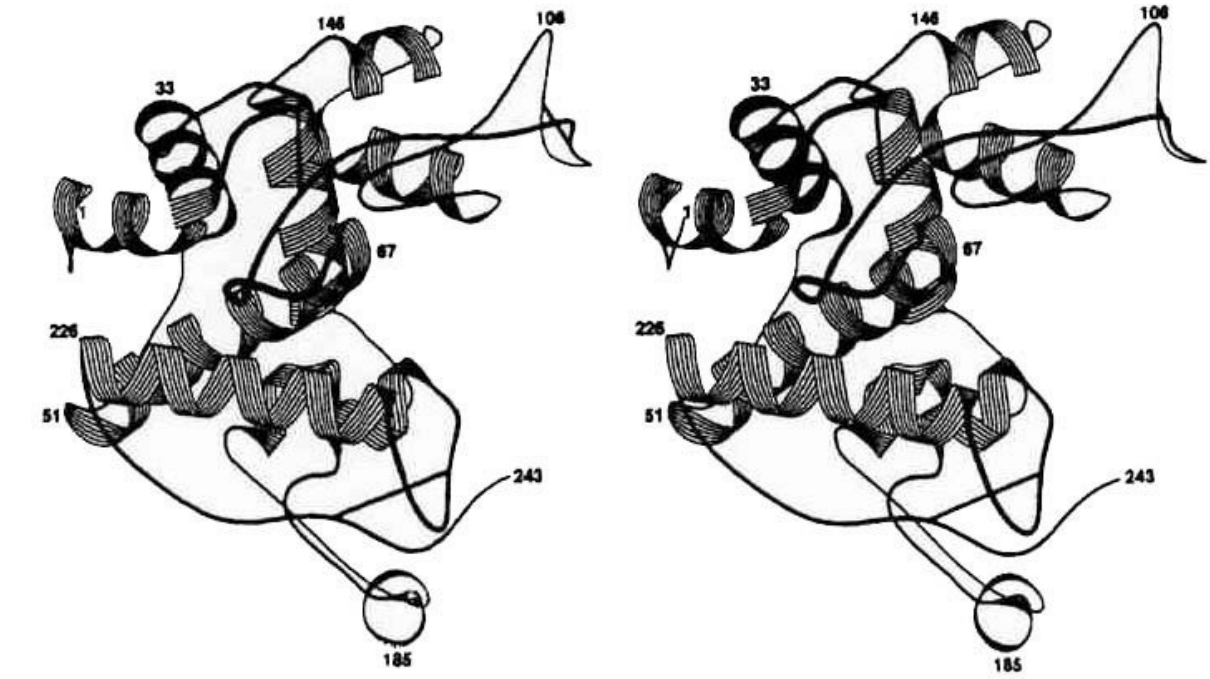
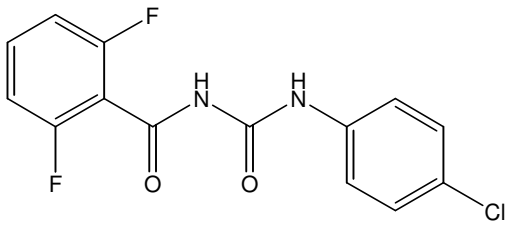


Figure 13: *Structure of barley seed endochitinase (stereo view) [32].*

6. Inhibitors of chitinases

Inhibitors are very useful to study the catalytic mechanism of enzymes. Furthermore, vertebrate do not produce chitin, so interfering with the chitin metabolism can be a suitable method to develop insecticides that are non-toxic for humans. Chitin synthesis inhibitors, *e.g.* diflubenzuron (**73**, Dimilin®), have been used in this way for more than 20 years [23, 33].



73

diflubenzuron (Dimilin®)

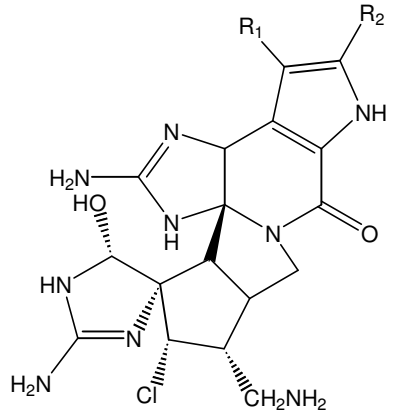
The main issue when developing chitinase inhibitors as insecticides is the selectivity. Plants as well as animals have pathogen defence chitinases. Therefore, the new chitinase

inhibitors have to be active against insect chitinases only: a non-selctive chitinase inhibitor would weaken plant and animal defences.

Spindler (University of Ulm) published several articles concerning chitinase inhibitors. It was shown that metal cations such as Cu^{2+}, Zn^{2+}, or Hg^{2+} could inhibit chitinases, particularly those of bacteria and of some insects [34]. Some plant chitinases were also inhibited by such divalent cations. However, other metal cations, such as Na^{+}, K^{+}, Ca^{2+}, or Mg^{2+} activated the chitinase from the marine bacterium *Alteromonas*.

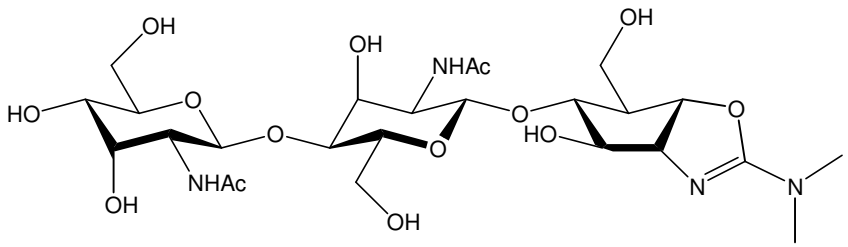
Some cyclic peptides showed interesting properties. Cyclo(L-Arg-D-Pro), isolated from marine *Pseudomonas* sp. showed an activity against chitinases from *Bacillus* sp., *Saccharomyces*, and *Candida*. [34]

Other compounds called styloguanidins (**74a**-**74c**) were extracted from the marine sponge *Stylotella aurantium* and showed an activity against a chitinase from *Shewanella* sp. Up to date, the inhibitory properties of the styloguanidins were not completely elucidated. [34]



styloguanidines **74a**: $R_1 = R_2 = H$
74b: $R_1 = Br$, $R_2 = H$
74c: $R_1 = R_2 = Br$

The most famous chitinase inhibitor is allosamidin (**75**). It was isolated from *Streptomyces* sp. by Sakuda et al. [35]. Allosamidin essentially inhibits family 18 chitinases, and particularly chitinases from insects. This good selectivity enables the development of a new generation of insecticides. However, allosamidin is not suitable for a commercial use, since the total synthesis is too expensive to allow an industrial production [36], and the compound is not stable under field conditions.



75

Allosamidin

II Aims of the work

1. Conditions for the development of allosamidin analogs

Allosamidin was first isolated, and the structure elucidated, by Sakuda et al. from *Streptomyces* sp. [35]. A mycelial extract of *Streptomyces* sp. inhibited significantly chitinases from the silkworm *Bombyx mori*.

Allosamidin is a pseudo-trisaccharide, whose structure contains two main parts: an allosamine disaccharide, and an aminocyclitol derivative called allosamizoline. The allosamine disaccharide has a structure which is very close to that of chitobiose, the dimer obtained after the degradation of chitin: this might be the recognition part. On the other hand, the allosamizoline moiety has a structure which differs from chitin much more than the allosamine part: this might be the inhibition part.

According to these assumptions, and after testing different inhibitors, Spindler concluded that good chitinases inhibitors must contain at least one glucosamine moiety, and the compound must mimic the spatial arrangement of the allosamizoline part [34].

2. Activity of menthylated furanones

Preliminary tests showed that the natural furanones **24a**-**27a**, in addition to their antibiotic properties, were also weak chitinase inhibitors [9]. These compounds were synthesized to get more material for further investigation of their biological properties. The synthetic furanones, as well as all intermediates, were tested as potential chitinase inhibitors

by Spindler (University of Ulm). Surprisingly, the natural compounds proved to be inactive, whereas some menthylated intermediates were active.

The furanones were tested with four different chitinases: chitinases A and B from *Serratia marcescens*, a chitinase from *Chironomus tentans*, and a chitinase from *Hevea brasiliensis*. We give here the most significant results for the chitinase B from *Serratia marcescens* with methylumbelliferyl-chitobiose (MUF-chitobiose) as substrate [37] (Figure 14, Table 2).

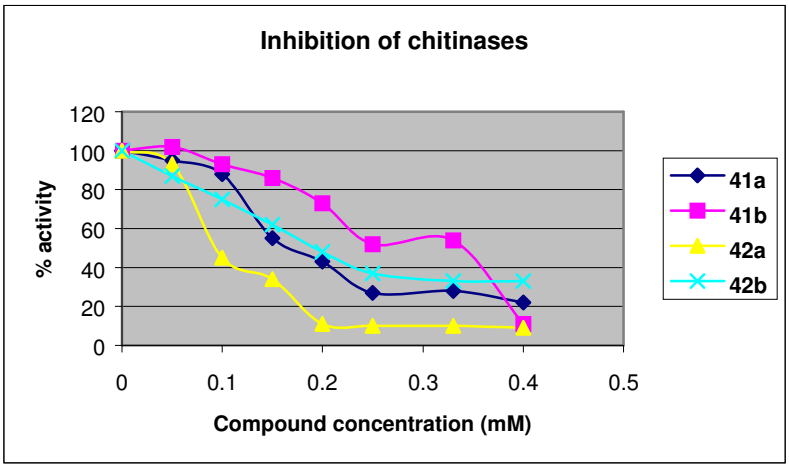
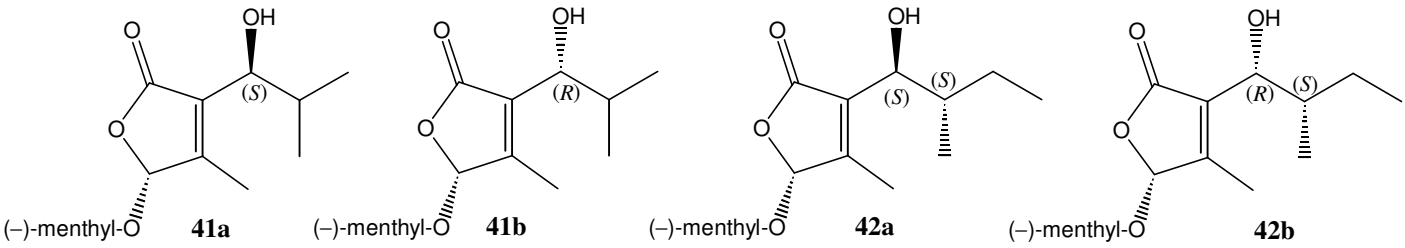


Figure 14: *Inhibition of chitinase B from* Serratia marcescens *by furanones* ***41a***, ***41b***, ***42a***, ***42b***, *with MUF-chitobiose as substrate.*



Compound	**41a**	**41b**	**42a**	**42b**
IC_{50} (µM)	170	280	100	190

Table 2: *IC_{50} values estimated from Figure 14. Inhibition of chitinase B from* Serratia marcescens *by furanones **41a**, **41b**, **42a**, **42b**, with MUF-chitobiose as substrate.*

It is noteworthy that the compounds **41a** and **42a** are significantly more active than their respective epimers **41b** and **42b**. In addition, compound **42a** is more active than **41a**.The same is true for **42b** and **41b** when the IC_{50}-values are compared. However, the latter compound is more active at higher concentration. The *S*-configuration of the secondary alcohols in the side chain of the furanones seems to give a better inhibitory effect to the compounds, in comparison to the respective epimers.

3. Glycosylated furanones

The activity of the furanones listed above could be explained as follow: the structure of the mentylated furanone could be analogous to the allosamidin. In fact, we could assume that the menthyl group, with its 6-membered-ring, should be analogous to a monosaccharide like glucosamine. The substituted furanone on the other hand could mimic the allosamizoline moiety of allosamidine.

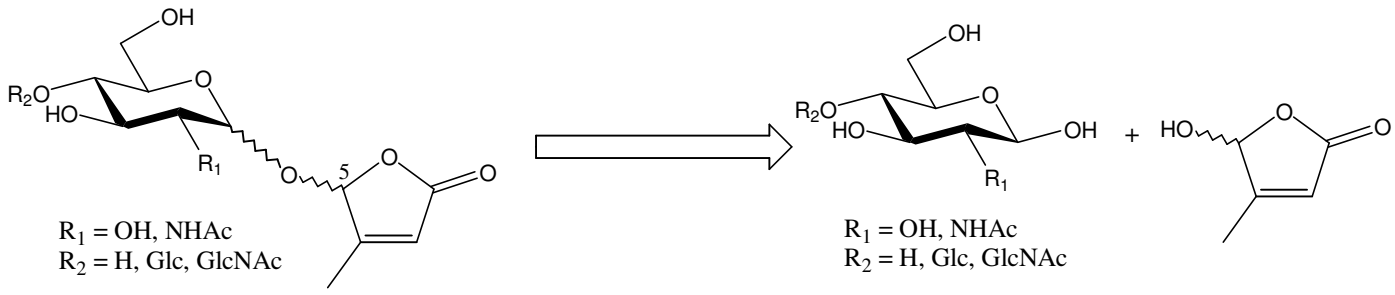
Therefore, it can be hoped that if the menthyl group is replaced by a saccharide, then the inhibitory activity of the compound should increase. The goal of the work therefore was to link to the furanone a carbohydrate through a suitable glycosylation method in order to obtain a new family of glycosylated furanones, which could be inhibitors of chitinases.

III Synthesis of glycosylated furanones

1. Ideas for the synthesis

The main purpose was to develop the simplest synthesis possible. The use of acetates as protecting groups for the glycosides should be a good idea, since the selective anomeric deprotection of acetylated sugars is very simple and gives generally good yields. The removal of acetyl groups should also be facile.

However, the C(5) configuration of the furanone after glycosylation cannot be predicted. In contrast, depending on the glycosylation method, it should be possible to obtain selectively the α- or β-anomer. In this way, two mixtures of C(5)-epimers would be obtained. If the stereoselective glycosylation is not possible, a mixture of four diastereomers will result:

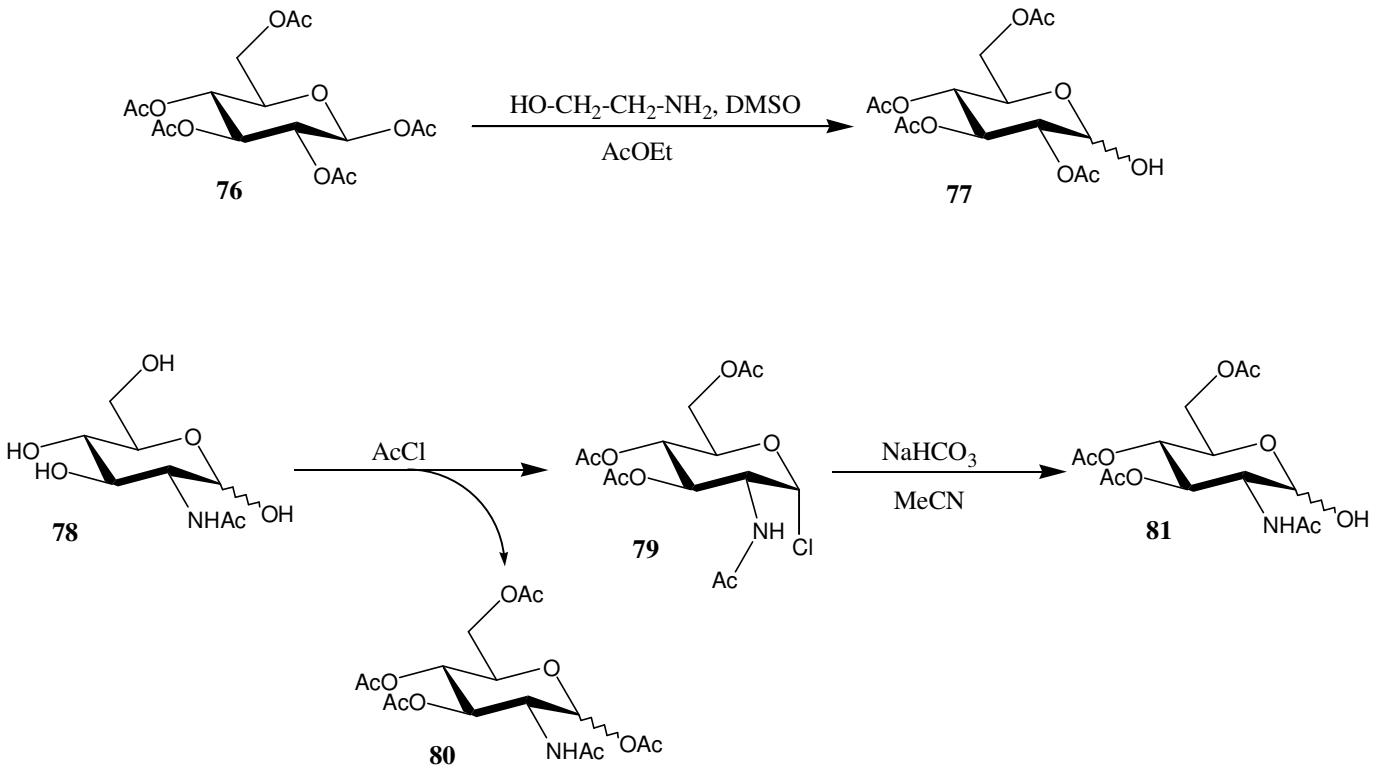


The biological activity of the isomer mixtures should be investigated. If an inhibitory effect is observed, the separation of the diastereomers should be carried out in order to identify the active isomer.

2. Preparation of the starting materials

The starting monosaccharides **77** and **81** are very simple to prepare in relatively good yields (Scheme 11). The tetraacetylated glucose **77** is obtained after selective cleavage of the ester at the anomeric center, with ethanolamine and DMSO in AcOEt, from the commercially available peracetylated glucose **76** [38]. This reaction gives compound **77** in 80% yield. The tetraacetylated glucosamine **81** is prepared from *N*-acetyl-D-glucosamine (**78**) in two steps. After acetylation with AcCl and chlorination of the anomeric position [39], the intermediate

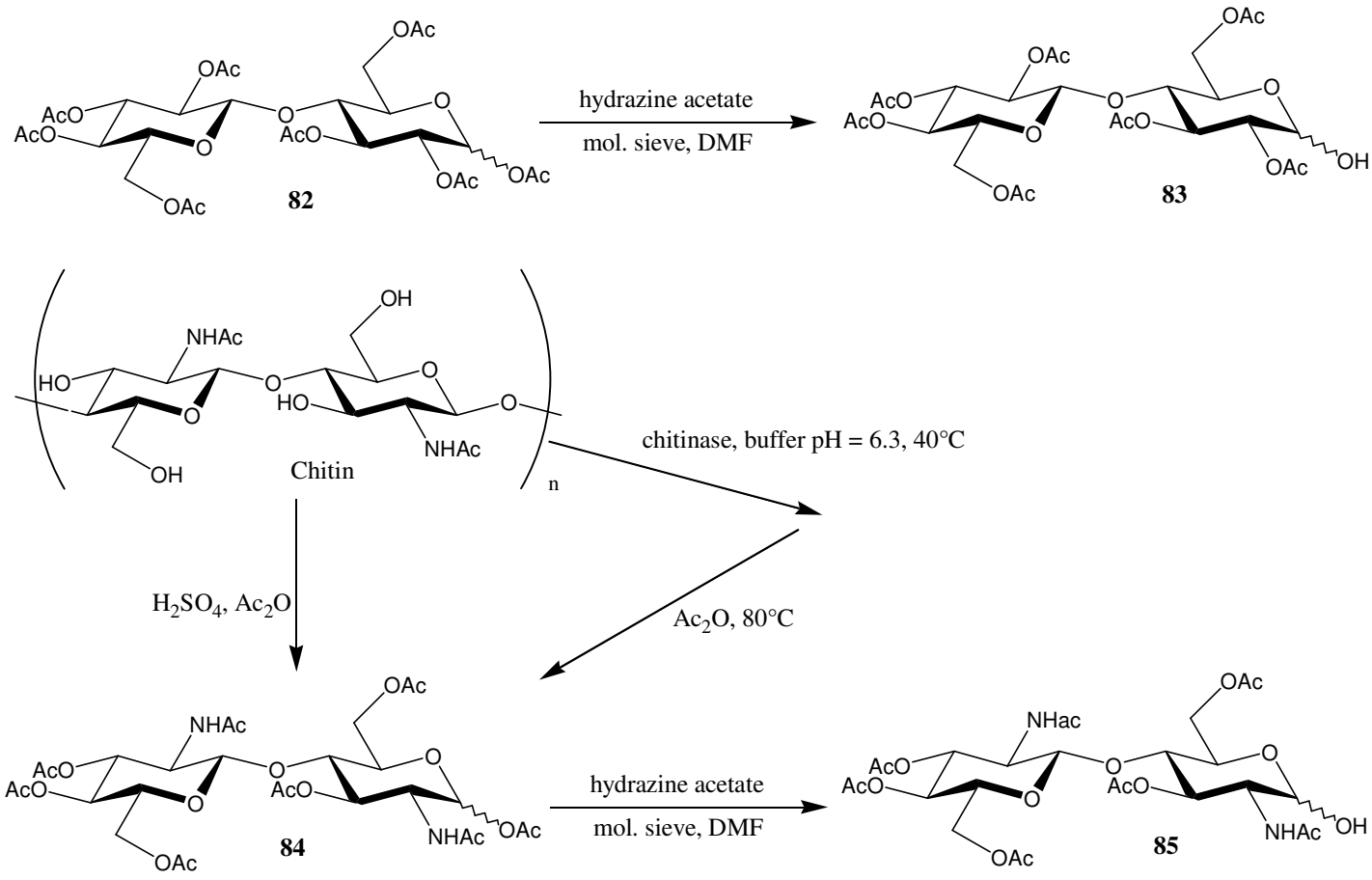
79 is hydrolysed with $NaHCO_3$ in MeCN. This hydrolysis can also be performed with Ag_2CO_3 [40] so that the chloride is removed as AgCl, but the use of $NaHCO_3$ is much cheaper and gives the same yield.



Scheme 11: *Preparation of the starting monosaccharides **77** and **81**.*

The acetylation of the glucosamine **78** is performed in a sealed flask, so that the hydrogene chloride, which is produced during the acetylation cannot escape and *in situ* replaces the anomeric OH to give **79**. If the hydrogen chloride is removed from the reaction, the exclusive formation of the peracetylated glucosamine **80** is observed. However, even in a sealed apparatus a small part of peracetylated glucosamine **80** was formed.

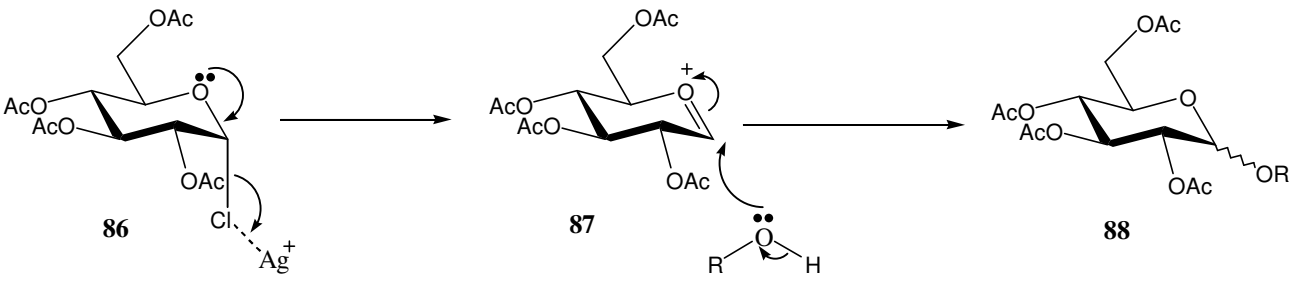
The acetylated disaccharides **83** and **85** were also prepared (Scheme 12). Commercial cellobiose octaacetate **82** was selectively deacetylated at the anomeric position with hydrazine acetate in DMF to give **83** in 89% yield [41,42]. Chitobiose octaacetate **84** was prepared by degradation of chitin with sulfuric acid and acetic anhydride [42], or by enzymatic degradation with chitinase from *Streptomyces griseus* [43] followed by acetylation with acetic anhydride. The octaacetate **84** was selectively deacetylated et the anomeric position to give **85** in 61% yield.



Scheme 12: *Preparation of the starting disaccharides* ***83*** *and* ***85****.*

3. Glycosylation

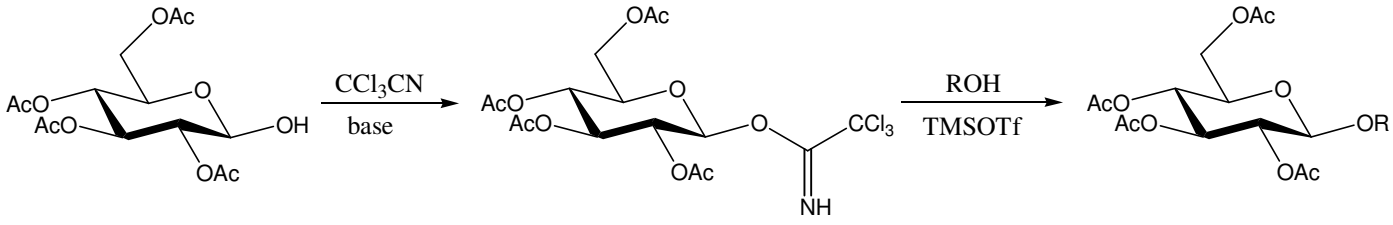
The most common methods for the synthesis of oligosaccharides are the use of halogenated carbohydrates, trichloroacetimidate derivatives, and thioglycosides. The use of halogenated carbohydrates as glycosyl donors was described by Koenigs and Knorr in 1901 [44,45]. The glycosylation is performed in the presence of a heavy metal salt in order to activate the halogenated carbohydrate (Scheme 13). For instance, a silver salt is often used as the silver chloride precipitates and the reaction is favoured by this way. In fact, the departure of the halide from the anomeric position of **86** gives an oxocarbenium cation **87**, which reacts with an alcohol to give the glycoside **88**. However, a Lewis acid can also be used to replace the heavy metal salt.



Scheme 13: *Koenigs-Knorr glycosylation.*

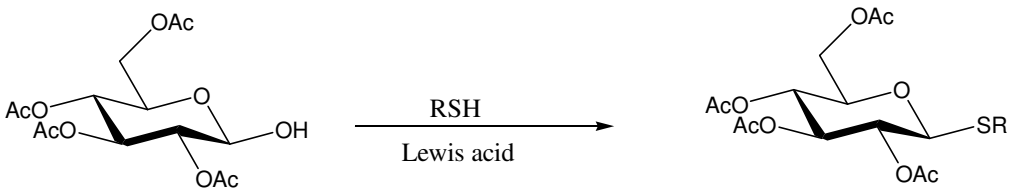
In addition, the reactivity of the glycosyl halide depends on the protecting groups of the glycosyl donor. For instance, ether protected carbohydrates are generally more reactive than the analogous ester protected ones. Furthermore, the protecting group at O-C(2) is of highest importance, as it gives a neighbouring group effect. The best example is the acetamido group, which can form an oxazoline after departure of the anomeric halide.

A more recent glycosylation method was designed by Schmidt [46]. The anomeric position is activated through the formation of a trichloroacetimidate. This trichloroacetimidate can be easily prepared by the treatment of the glycoside with a base, such as NaH or DBU, and trichloroacetonitrile (Scheme 14). The resulting trichloroacetimidate can be isolated and stored under anhydrous conditions. It is activated for glycosylation by a catalytic amount of a Lewis acid, such as trimethylsilyl triflate (TMSOTf) or boron trifluoride-diethyl etherate ($BF_3 \cdot Et_2O$) at low temperature.



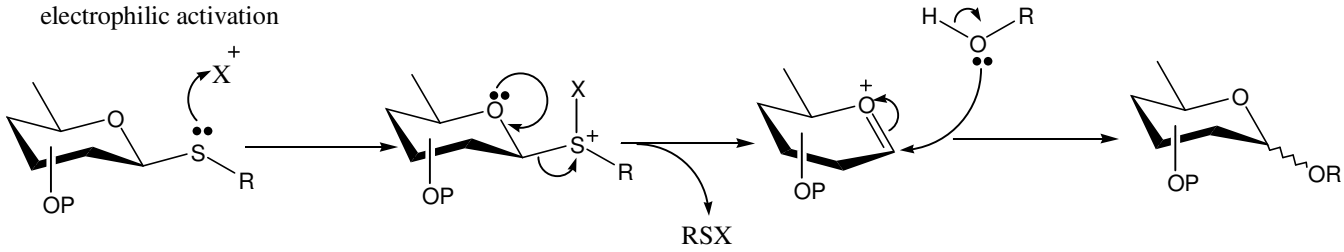
Scheme 14: *Schmidt glycosylation.*

Thioglycosides are another convenient way to synthesize new glycosidic bonds [47-49]. They can be prepared very easily by the treatment of the corresponding glycoside with a mercaptan, in the presence of a Lewis acid:



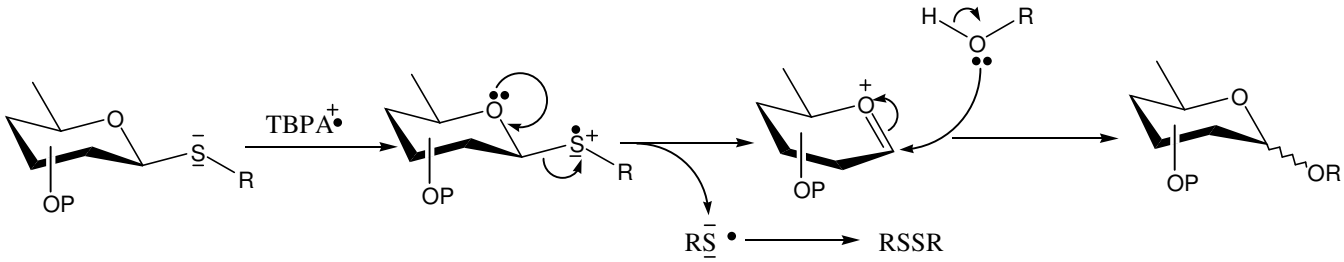
An alternative method is the treatment of the glycosyl halide with a mercaptide, which also gives good yields. The main interest of these thiocompounds is their stability. The anomeric thioether is compatible with various reaction conditions.

The activation of thioglycosides occurs with soft electrophiles (Scheme 15). The most commonly used reagents are methyl triflate (MeOTf) and dimethyl(methylthio) sulfonium triflate (DMTST), for instance. The electrophilic activator reacts with a lone pair of the sulfur. The resulting sulfonium intermediate is an excellent leaving group. Then, addition of an alcohol can lead to the glycosylation.



Scheme 15: *Activation of thioglycosides with soft electrophiles.*

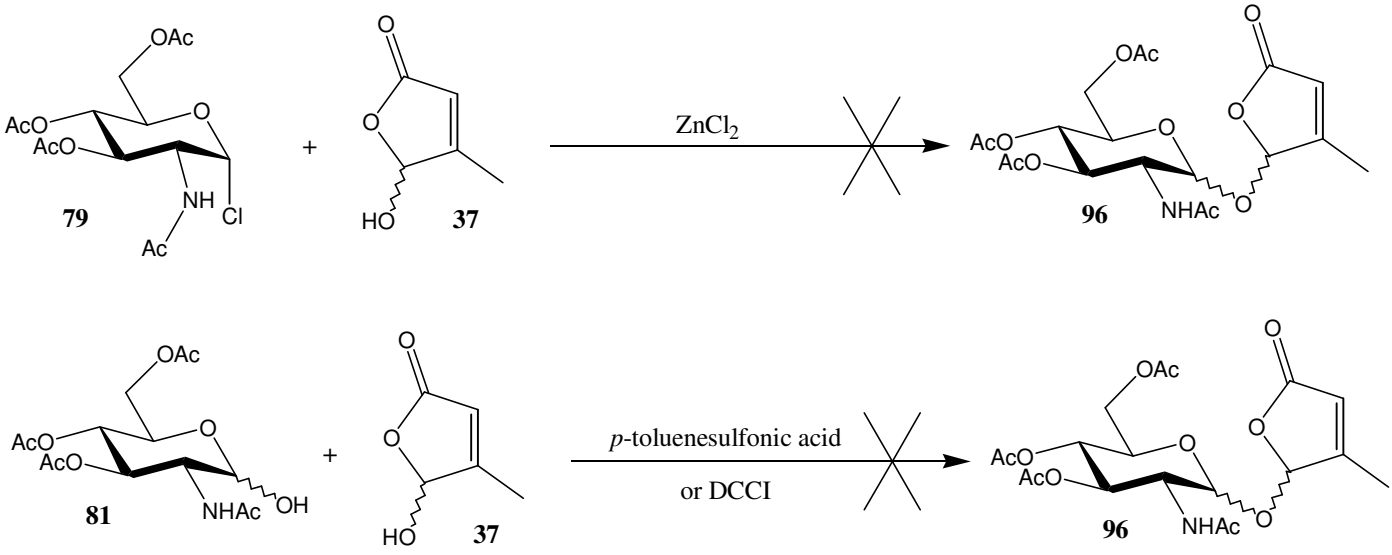
Activation of thioglycosides is also possible by treatment with tris-(4-bromophenyl)ammonium hexachloroantimonate ($TBPA^{+\bullet}$). In this case, a one-electron transfer occurs (Scheme 16), leading to a sulfonium radical cation. The thioglycoside is cleaved into an oxocarbenium ion and a sulfur radical, which dimerizes into a disulfide. Glycosylation is achieved by addition of an alcohol on the oxocarbenium ion.



Scheme 16: *Activation of thioglycosides by one-electron transfer.*

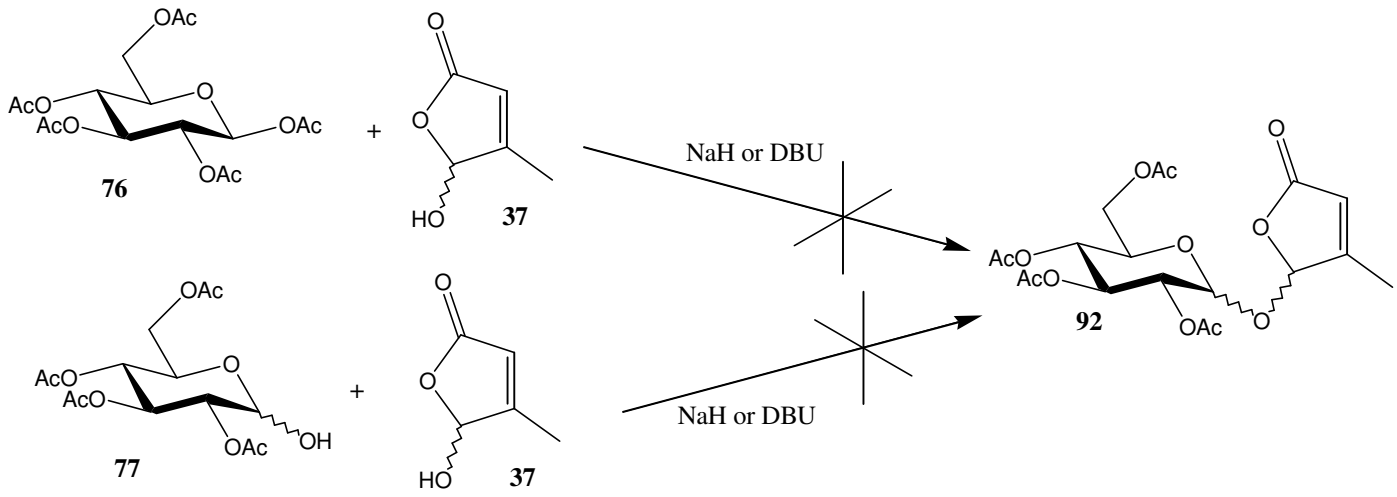
3.1 Searching for the best method

A first attempt to achieve the glycosylation was made with the halogenated glucosamine **79** and the furanone **37** in the presence of $ZnCl_2$ as Lewis acid (Scheme 17) [50,51]. No reaction occurred under these conditions. Then, the coupling between the acetylated glucosamine **81** and the furanone **37** was tried in refluxing toluene in the presence of *p*-toluenesulfonic acid, in analogy to the menthylation of the furanone. As there was degradation of the carbohydrate, the same reaction was also tried at room temperature, which remained again unsuccessful. The use of DCCI [52] as the coupling reagent did not give the expected compound **96**.



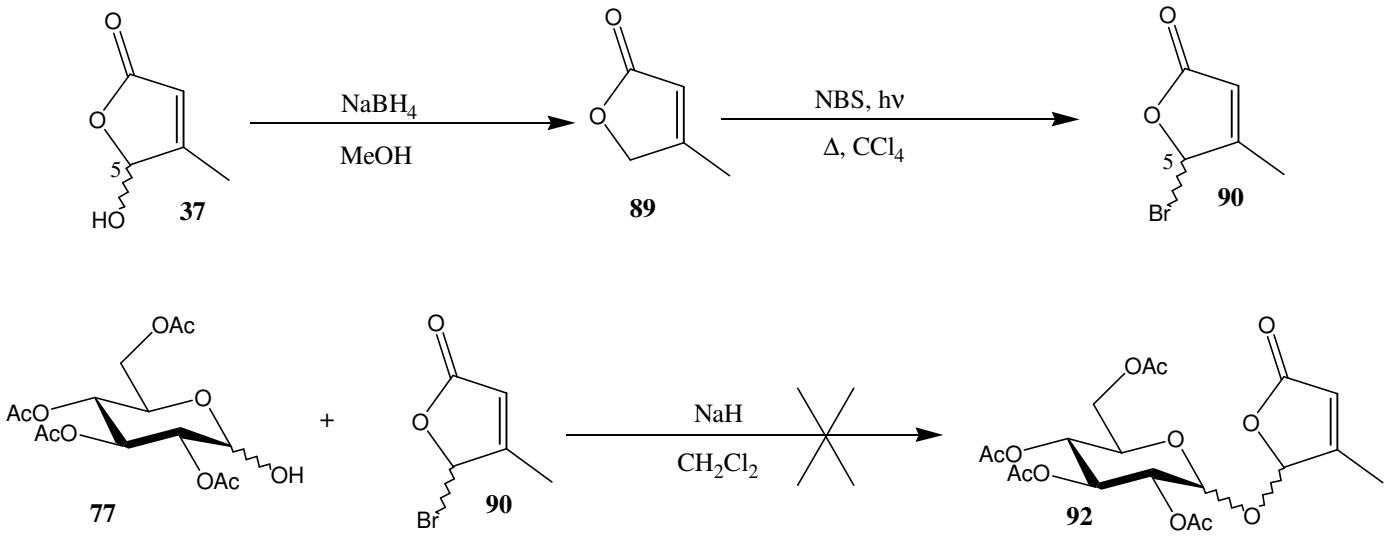
Scheme 17: *Unsuccessful glycosylations with glucosamine derivatives.*

As these methods gave no positive results, it was decided to first elaborate a synthetic method for **92** using the acetylated glucoses **76** or **77**, which are much cheaper than the acetylated glucosamine **81**. The glycosidation was tried under basic conditions. The furanone **37** was treated with the glucose pentaacetate **76** in the presence of DBU (Scheme 18). The same reaction was also carried out with the acetylated glucose **77**, the furanone and DBU, then NaH was used as the base. None of these reactions under basic conditions led to the desired products.



Scheme 18: *Unsuccessful glycosylations with acetylated glucose derivatives.*

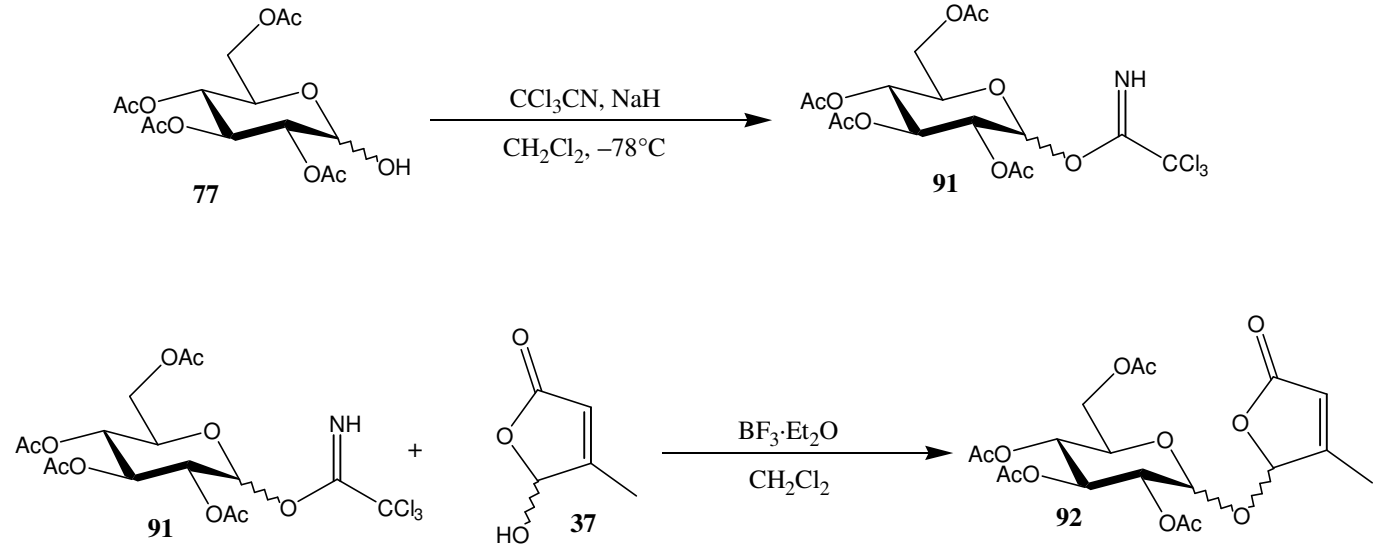
It was decided to prepare the brominated furanone **90**, in order to make C(5) more electrophilic. This halogenated compound was synthesized by reducing the starting furanone **37** with $NaBH_4$ in 81% yield to **89** (Scheme 19) [53], which was then brominated with *N*-bromosuccinimide and light in 85% yield to give **90** [54]. The glycosylation was tried with **90** and tetraacetyl glucose (**77**) with NaH as the base, but no glycosylated furanone **92** could be isolated.



Scheme 19: *glycosylation attempt with a brominated furanone.*

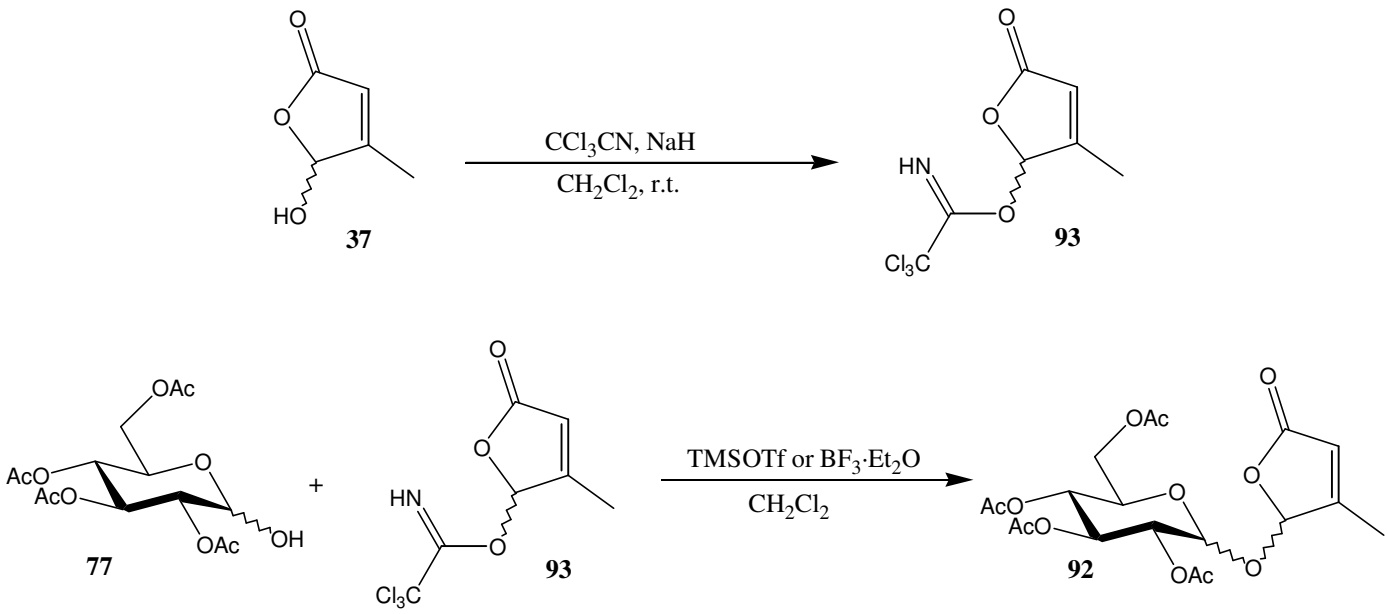
3.2 The Schimdt glycosylation

As direct glycosylation gave no result, the activation of the glucose through the preparation of the trichloroacetimidate **91** was performed (Scheme 20) [46]. This trichloroacetimidate was treated with the furanone **37** and $BF_3{\cdot}Et_2O$ [55] to afford the expected glycosylated furanone **92** in 38% yield.



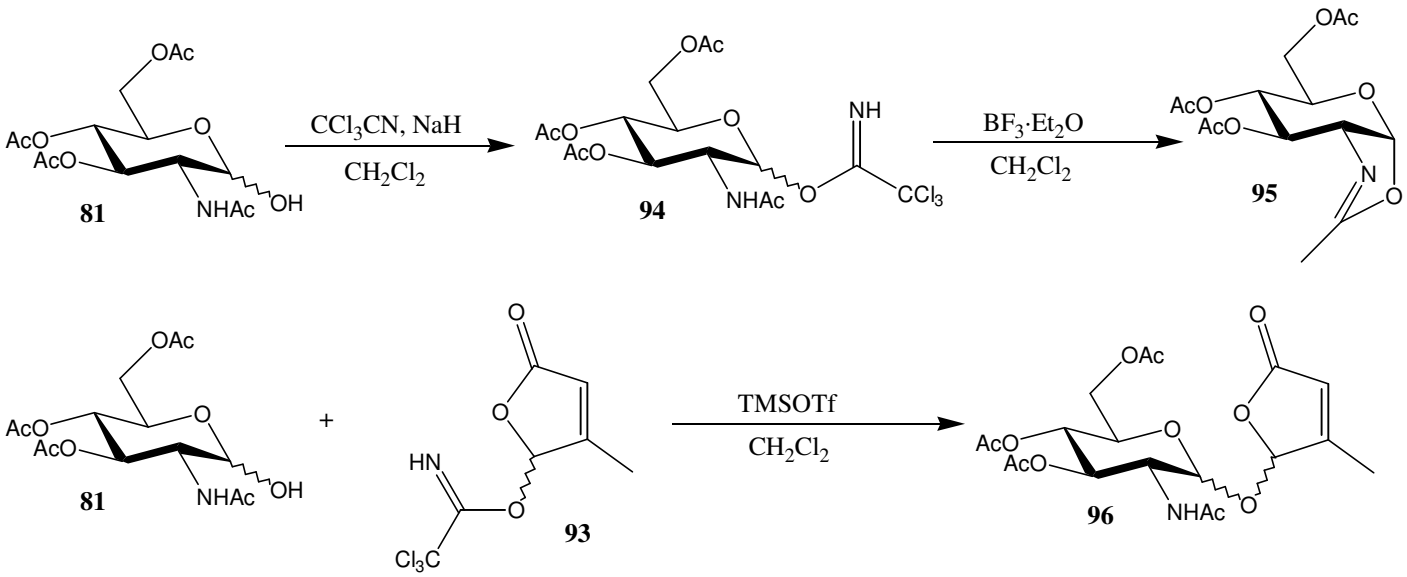
Scheme 20: *Preparation of **92** by Schmidt glycosylation.*

The same product **92** could be obtained in 70% yield in a "reversed type Schmidt reaction" [56,57] with the acetylated glucose **77** and the furanone **93** (Scheme 21), which was activated as the trichlororacetimidate. This latter was prepared by the treatment of the furanone **37** with trichloroacetonitrile in the presence of NaH [37]. The glycosylation was performed with either $BF_3 \cdot Et_2O$ or TMSOTf as the Lewis acid.



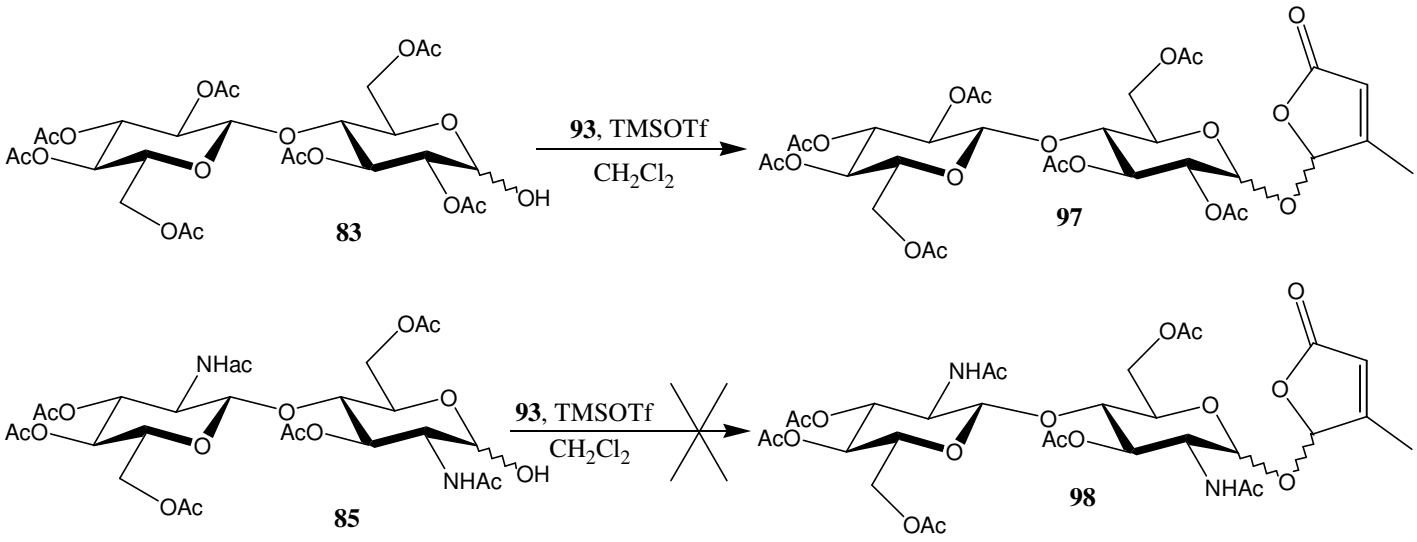
Scheme 21: *Synthesis of* ***92*** *by "reversed type Schmidt reaction".*

As the Schmidt glycosylation works with glucose derivatives, it was tried with glucosamine derivatives. The acetylated glucosamine **81** was transformed to the corresponding trichloroacetimidate **94**, which then was reacted with the furanone **37** using $BF_3 \cdot Et_2O$ as the Lewis acid. Unfortunately, the expected glycosylated product **96** was not obtained, but only the oxazoline **95** (Scheme 22) [58]. However, when the "reverse Schmidt glycosylation" was performed with the acetylated glucosamine **81** and the furanone trichloroacetimidate **93**, using TMSOTf as the Lewis acid, the expected product **96** was obtained in 42% yield.



Scheme 22: *Preparation of* ***96****.*

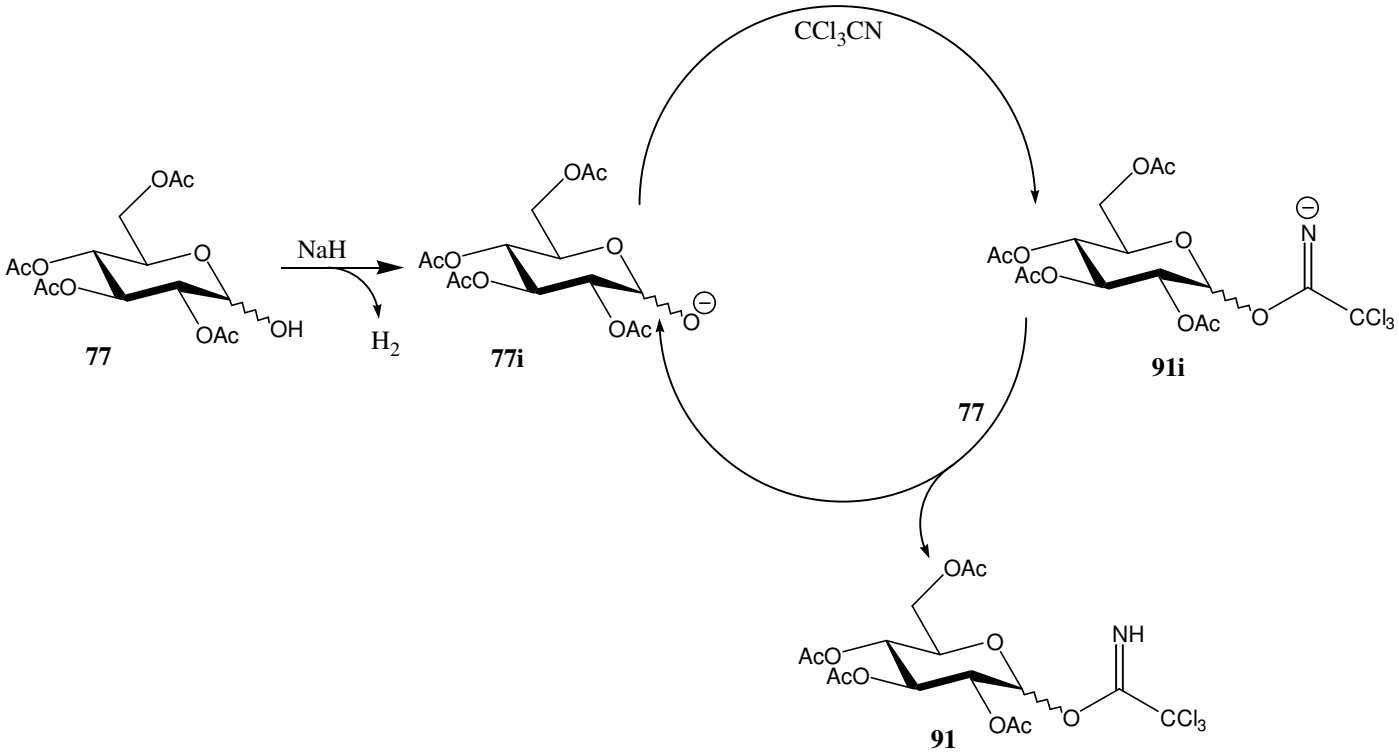
Similarly, the glycosylation of the acetylated cellobiose **83** yielded under analogous conditions the product **94** in 64% (scheme 23). In this preparation, the furanone **93** and the Lewis acid were used in larger excess than for monosaccharide glycosylation. Application of the same procedure to the acetylated chitobiose **85** did not afford the desired glycosylated furanone **98**.



Scheme 23: *"reverse Schmidt glycosylation" with acetylated cellobiose and chitobiose.*

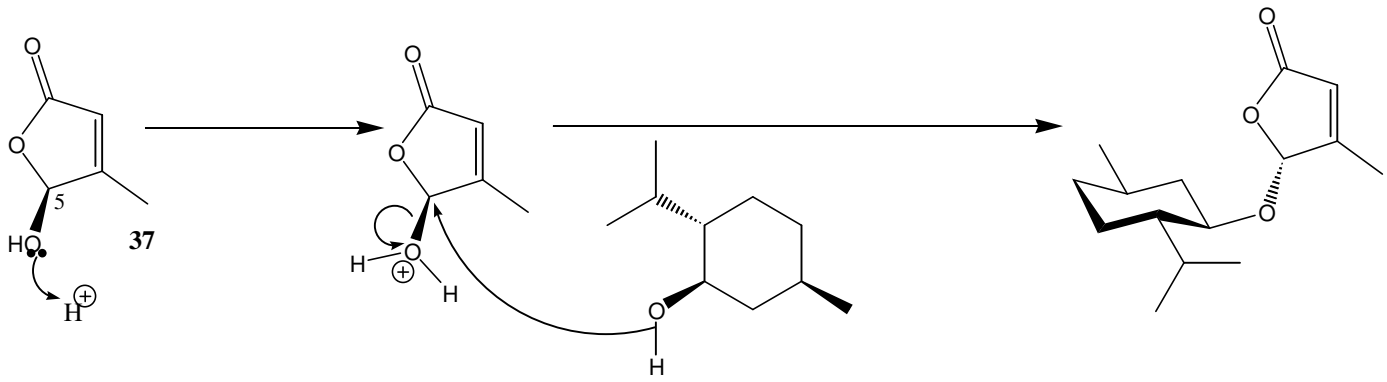
3.3 Discussion of the mechanism

The formation of the trichloroacetimidate is a base-catalyzed addition. Acetylated glucose **77** reacts with NaH (Scheme 24). The obtained alcoholate **77i** adds to trichloroacetonitrile to form the intermediate **91i**, which reacts with **77** in an acid-base reaction to give the trichloroacetimidate **91**.



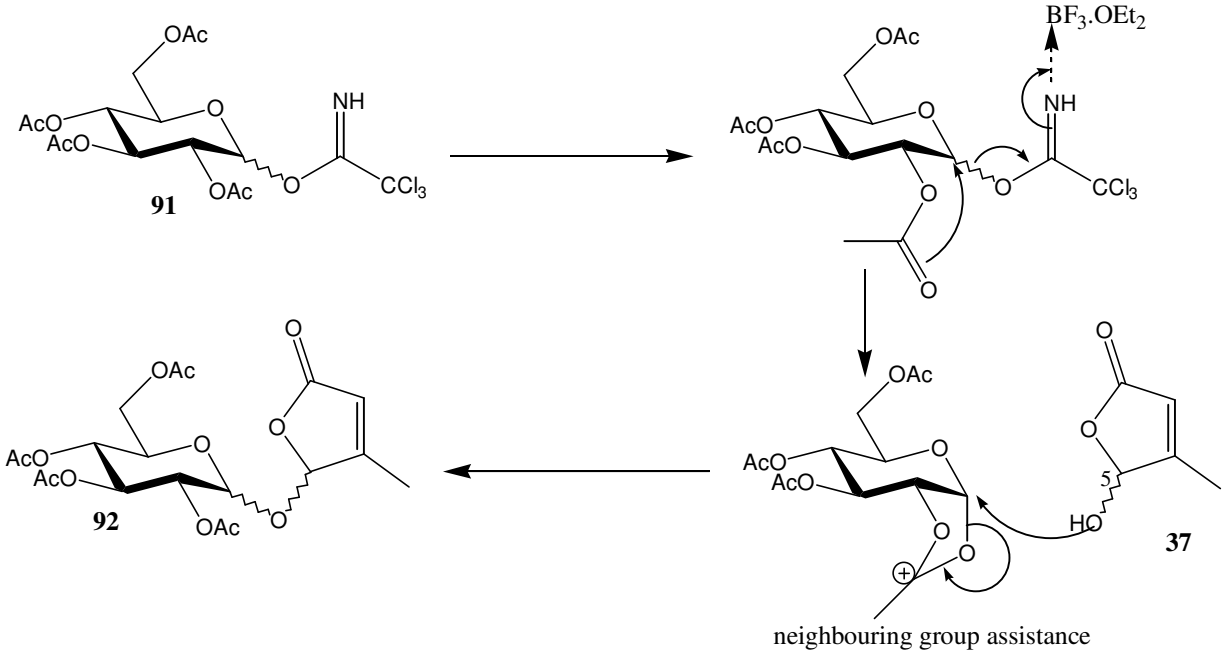
Scheme 24: *Mechanism of the formation of the trichloroacetimidate **91**.*

It was mentioned above that the menthylation of the starting furanone **37** gave two epimers differing in the configuration at C(5) of the furanone. There was no configuration change at C(1) of the (–)-menthol, which shows that the C(5) of the furanone is electrophilic: this is not surprising because this carbon is a ketal. The *p*-toluenesulfonic acid activates the OH group, which is normally a very bad leaving group (Scheme 25).



Scheme 25: *Acid catalyzed menthylation of* ***37****.*

On the other hand, the C(5) hydroxy group of furanone **37** has weak nucleophilic properties and thus can be added to the glucose trichloroacetimidate **91**, with the displacement of the trichloroacemidate in a "normal" Schmidt glycosylation. In addition, there is a neighbouring group assistance (Scheme 26), which promotes the formation of the β-glycoside. The same process does not occur with the stable oxazoline **95** (Scheme 22), showing that the furanone is not a strong enough nucleophile to open the oxazoline ring.



Scheme 26: *Mechanism of the Schmidt glycosylation with* ***91****.*

The "reverse Schmidt glycosylation" functions in a similar way. The trichloroacetimidate **93** is activated by the Lewis acid (*e.g.* $BF_3 \cdot Et_2O$) and the acetylated sugar (*e.g.* **77** or **81**) adds to the C(5). This confirms the electrophilic properties of the C(5) of the furanone.

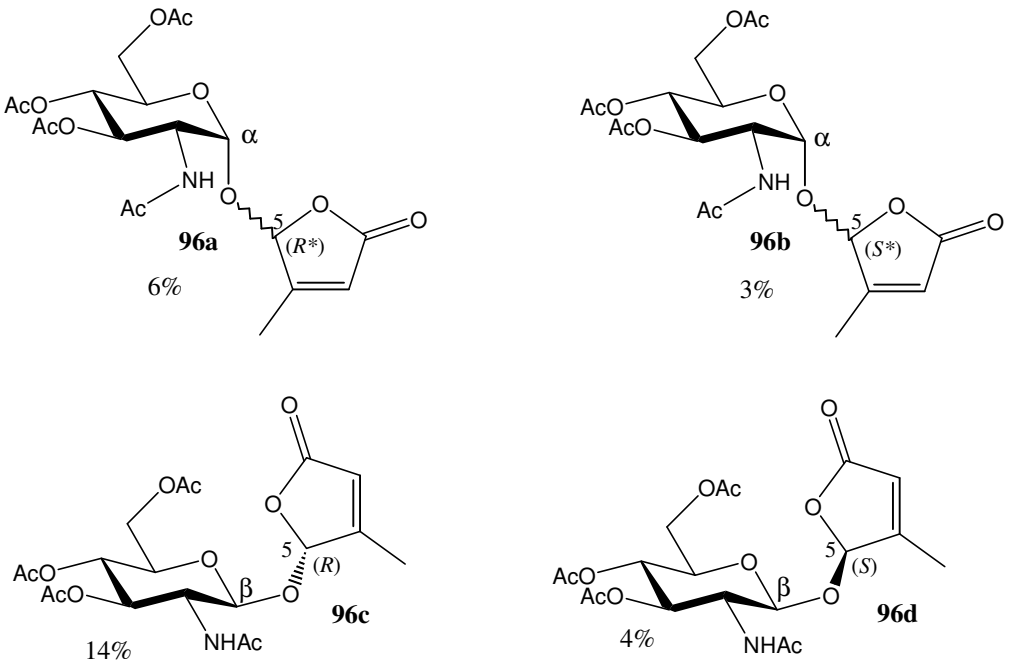
3.4 Stereochemistry

The "reversed Schmidt glycosylation" does not enable a selective formation of α or β anomers. As the addition of the sugar can occur on both sides of the furanone giving both epimers at C(5), all products can only be obtained as mixtures of four diastereomers..

The four stereoisomers of furanone **92** derived from acetylated glucose could not be fully separated by column-chromatography on silica gel. Nevertheless, one of the isomers (**92a**) could be isolated. In the proton NMR spectra, the signal observed for the anomeric proton was a doublet with a coupling constant equal to 8 Hz. Such a coupling constant is typical for the diaxial arrangement of the anomeric proton and H-C(2) in β-anomers. In α-anomers typically a coupling constant of about 4-5 Hz is observed.

The configuration of the furanone C(5) of **92** could not be determined by NMR analyses. The only way to elucidate it would be an X-ray structure determination, but unfortunately no suitable crystal could be obtained.

The four diastereomers **96a**, **96b**, **96c**, and **96d** of furanone **96** derived from acetylated glucosamine could be partially separated by column-chromatography on silica gel, with a slow gradient of MeOH in CH_2Cl_2. Some fractions contained two or more isomers. For each isomer, the anomeric configuration was determined by NMR. One of the two α-anomers could not be purified properly: the sample contained significant amounts of other isomers. The yields obtained of each isomer are give in Figure 15.



Remaining mixture **96a-d**: 16%

Total yield for the four isomers: 43%

Figure 15: *Separation of isomers of* ***96***.

Only for one of the four isomers (**96d**) a crystal suitable for X-ray structure determination was obtained. The structure derived from this measurement is shown in Figure 16. Since NMR spectra showed that **96c** was the epimer at furanone C(5) of **96d**, its structure could be deduced. However, the configuration of furanone C(5) of **96a** and **96b** could not be elucidated.

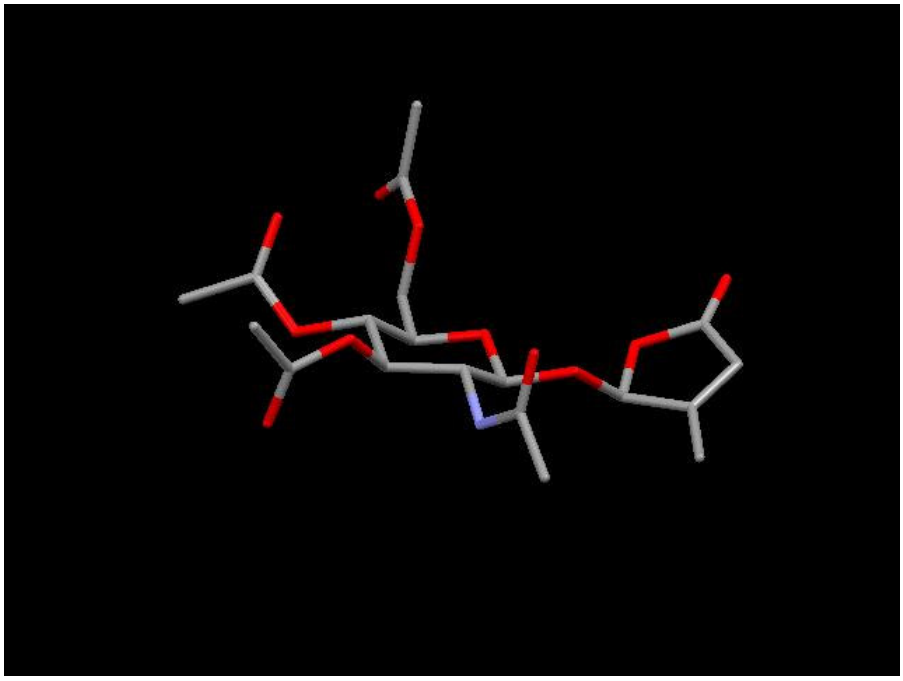


Figure 16: *Crystal structure of* ***96d****.*

The reaction of the acetylated cellobiose **83** with the trichloroacetimidate **93** gave also a mixture of four diastereomers **97** which could not be separated. Only one α-isomer could be concentrated enough by chromatography to allow for an NMR characterization, but since crystallization was not possible no X-ray structure determination could be made: the configuration at C(5) remained unknown.

3.5 Separation and purification

All glycosylated compounds were – as far as possible – separated and purified by column chromatography on silica gel. Due to the carbohydrate structure, these compounds had to be eluted with rather polar solvents, such as AcOEt in CH_2Cl_2 or 5% MeOH in CH_2Cl_2. With such polarities, it was very difficult not to elute impurities and the carbohydrate together.

Furthermore, carbohydrates can be very difficult to crystallize. So it is not surprising that we could not recrystallize the glycosylated compounds, even when amourphous solids

were easily obtained after evaporation of the solvent. Preparative HPLC was not suitable for our compounds, as they were not active enough in UV.

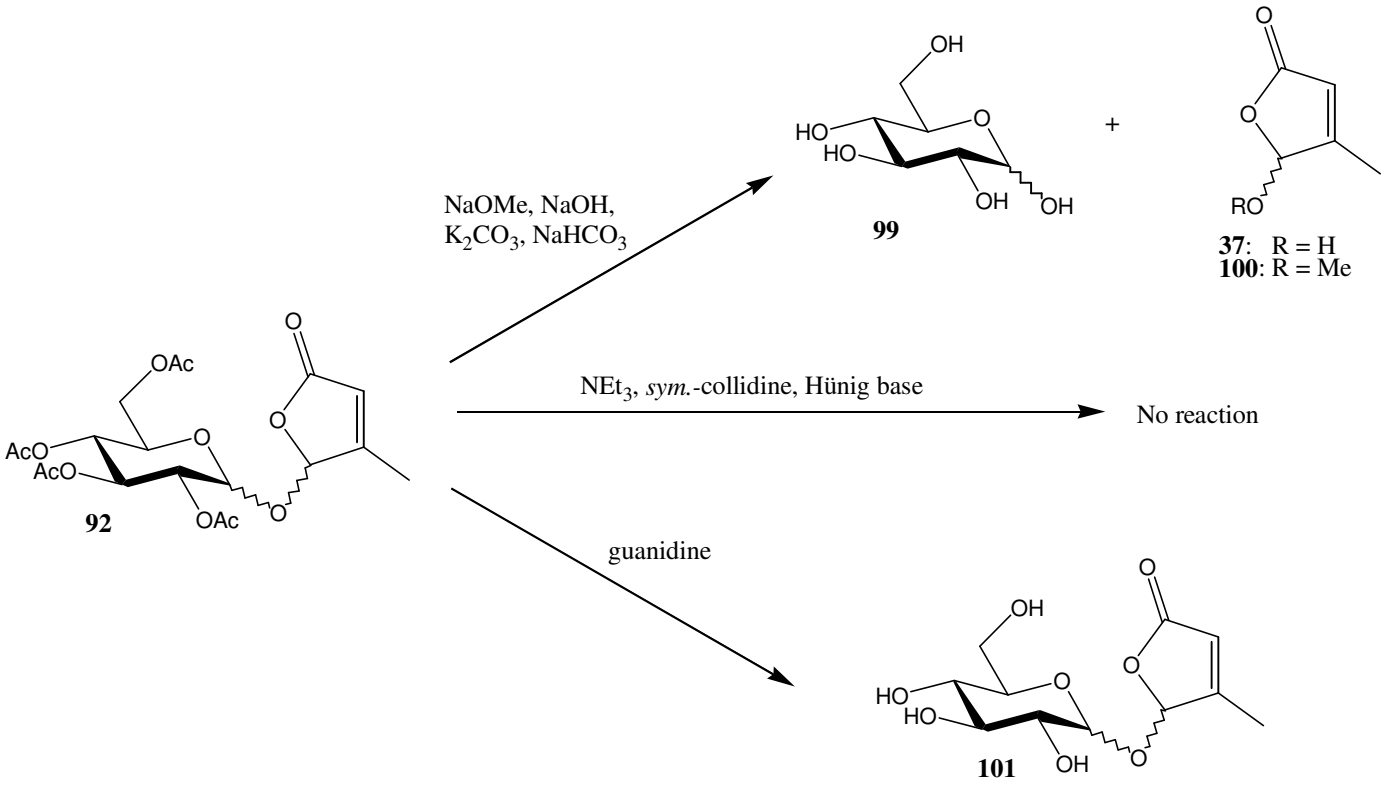
4. Deacetylation

In general, the cleavage of acetyl groups on carbohydrates is very simple. The reaction is usually carried out under basic conditions. The most common procedure is the use of MeONa in methanol, however any other strong base can be used. The cleavage can also be done in aqueous solution with NaOH, KOH, or K_2CO_3 for instance. Another possibility is the use of NH_3 in methanol. There are many other ways to achieve this cleavage: *e.g.* an enzymatic reaction with a lipase or an esterase [59, 60], or the use of Bu_2SnO as a catalyst [61] can be alternative routes. An acid catalysed deacetylation was described by Byramova et al. [62]; this process uses HCl gas, dissolved in anhydrous methanol and selectively cleaves acetates, whereas benzoates do not react.

4.1 Searching for the best method

The classical method to deacetylate carbohydrates is the basic reaction with NaOMe in MeOH. This method was applied to the compound **92**, and not only the deacetylation occurred, but also the cleavage of the new glycosidic bond: only glucose and a furanone derivative were isolated. All aqueous bases, like NaOH, K_2CO_3, and $NaHCO_3$, gave the same result (Scheme 27).

The use of nitrogenated organic bases, like triethylamine, *sym.*-collidine, *N*,*N*-diphenylguanidine, dicyanodiamide, diisopropylethylamine (Hünig base) gave no deacetylation.



Scheme 27: *results for different deacetylation methods.*

The only base, which gave complete deacetylation without cleaving the glycosidic bond was guanidine [63].

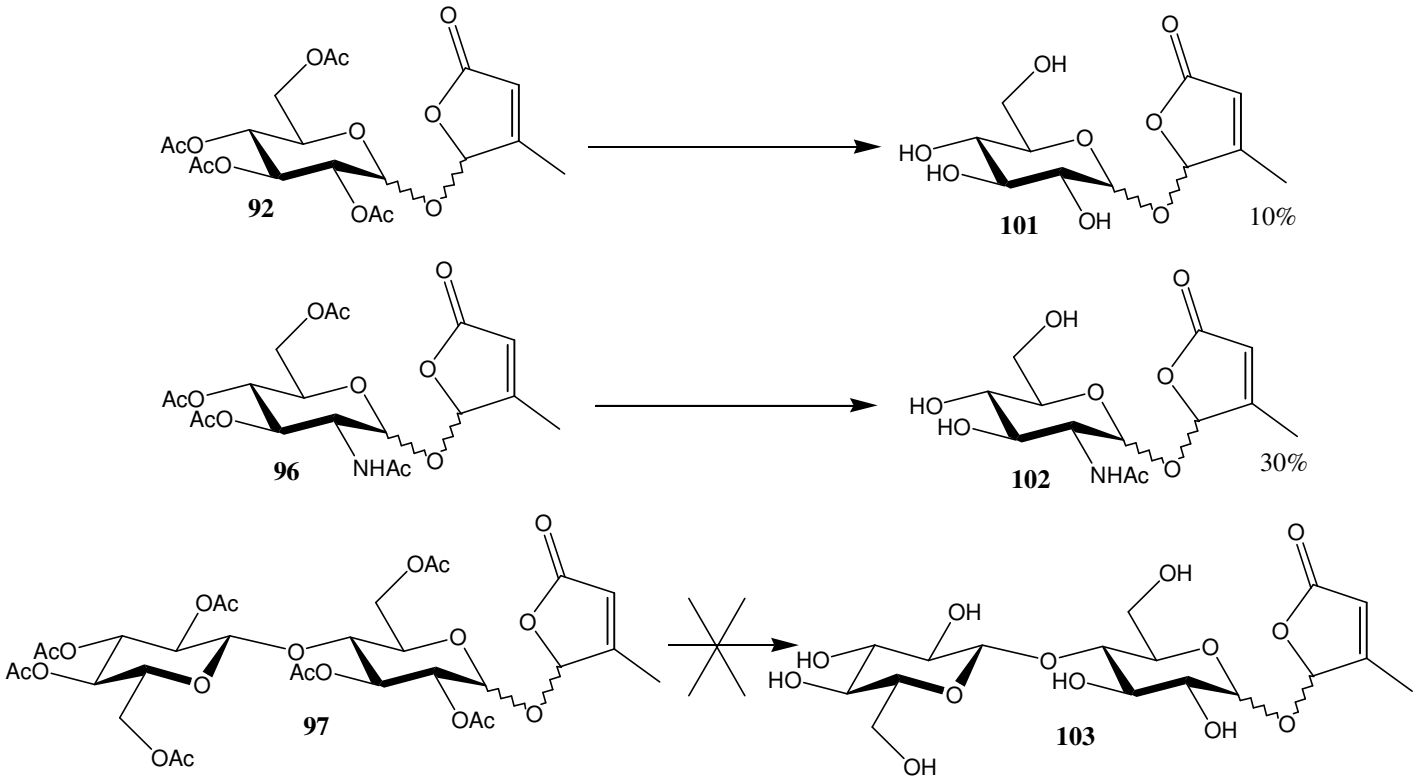
In the literature, a procedure using HCl in anhydrous methanol was published. Such a procedure could be interesting in our case, as the glycosidic bond in our compounds proved to be unstable under strong basic conditions. However, this deacetylation method requires the neutralization of the acid with a strong base. Therefore, such a process is not suitable for our compounds.

4.2 Deacetylation with guanidine

Guanidine base is prepared by running a solution of guanidine hydrochloride in MeOH through an anionic ion exchange resin, such as Amberlite IRA-900Cl. The deacetylation is carried out in CH_2Cl_2/EtOH 1/9 and is followed by TLC. The amount of guanidine used is very critical: too much base as well as too long reaction times lead to the cleavage of the

glycosidic bond. Therefore, guanidine is added in portions, depending on how the deacetylation is proceeding.

In fact, the reaction conditions are not anhydrous, since the guanidine was not dried. Moreover, each acetate requires one H_2O molecule for the cleavage. Therefore deacetylation cannot be carried out under strictly anhydrous conditions. Since in our case water is present in a large excess and the conditions are basic, the destruction of part of the deacetylated product cannot be avoided. A compromise between deacetylation and destruction must be found in order to optimize the yield.



Scheme 28: *Results of the deacetylations.*

Deacetylation of the compounds **92** and **96** gave the respective glycosylated furanones **101** and **102** (Scheme 28). Deprotection of **92** could only be achieved with a poor yield (10%). Deacetylation of **96** proceeded somewhat easier (30% yield) and afforded the acetamido-glucopyranosyl furanone **102**, thus only the *O*-acetyl groups had been removed whereas the amide function was left intact. On the other hand, deacetylation of the

peracetylated cellobiose derivative **97** afforded only partially deacetylated compounds, and it was not possible to isolate the wanted product **103**.

4.3 Purification

The reaction was stopped by pouring the reaction mixture directly onto a silica gel column. Evaporation of the reaction mixture before chromatography must be avoided, because the increasing concentration of the base would lead to cleavage of the glycosidic bond.

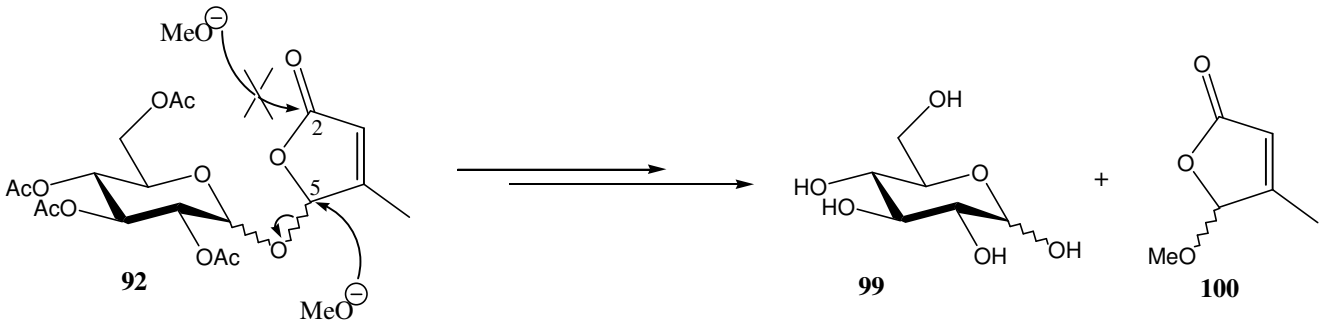
The deacetylated products needed a very polar solvent for the elution from the silica gel, such as MeOH in CH_2Cl_2 15%. Such a polar solvent did not enable a perfect removal of all impurities. Also, a separation of the diastereomer mixtures **101** and **102** into the single compounds was not possible.

Similarly to the acetylated derivatives (*e.g.* **96**), the deacetylated compounds **101** and **102** were obtained as amorphous solids after solvent evaporation.

4.4 Discussion

The deacetylated compounds **101** and **102** could not be purified by chromatography on aluminium oxide and on basic ion exchange resins either. These methods led to the destruction of the products. In addition, our experiments showed that the glycosidic bond sugar/furanone was not stable under basic conditions. This explains why the glycosylation did not work under basic conditions, for instance with DBU or NaH. However, the compounds are stable for more than several months when stored in an anhydrous argon atmosphere. What can explain the instability of the glycosides **101** and **102** in the presence of aqueous bases?

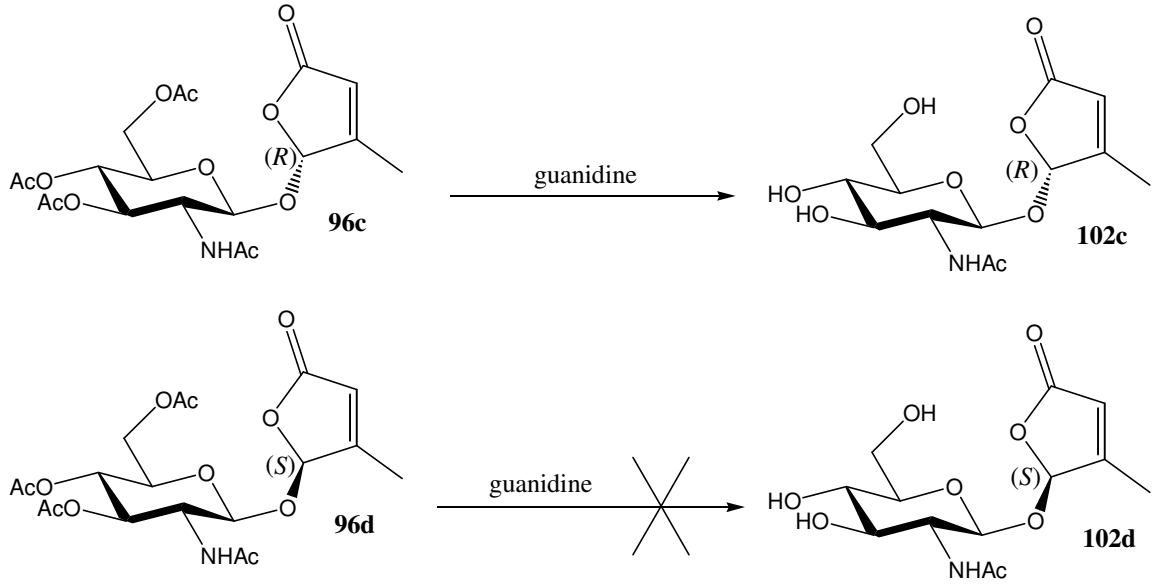
When the acetylated glycoside **92** is treated with MeONa in MeOH, the 4-methyl-5-methoxy-5*H*-furan-2-one (**100**) [64] is isolated. This means that the methoxide ion attacks the electrophilic C(5) (Scheme 29), followed by cleavage of the glycosidic bond; an attack at the carbonyl C(2) would lead to a methyl ester, which is not observed.



Scheme 29: *Cleavage of glycosidic bond with methoxide.*

We can assume that under basic aqueous conditions, not only opening of the lactone can occur, but also hydroxide can add to C(5) similarly to methoxide, leading to the cleavage of the glycosidic bond sugar/furanone. This could explain the instability of **101** and **102** under basic aqueous conditions.

Surprisingly, the deacetylation of the mixture **96** afforded the compound **102** as a mixture of only three diastereomers. According to the NMR analyses, both furanone C(5) epimers with an α-configuration of the sugar were obtained, but only one β-anomer. When the deacetylation was performed separately with each of the two β-epimers **96c** and **86d**, only the former gave the corresponding deacetylated carbohydrate **102c**. Thus, we suppose that the lacking isomer is **102d**. However, we cannot explain this different behaviour so far.



IV Biological tests

1. Test conditions

All biological tests were performed by the group of Prof. Spindler, University of Ulm, Germany.

The furanone derivatives were tested with four different enzymes: Chitinase A and Chitinase B from the bacterium *Serratia marcescens* and a chitinase, as well as a *N*-acetyl-β-D-glucosaminidase, from the insect *Chironomus tentans*. The activities were measured by fluorimetry and the substrates were methylumbelliferyl-*N*-acetylglucosamine (MUF-GlcNAc), methylumbelliferyl-chitobiose (MUF-chitobiose) and methylumbelliferyl-chitotriose (MUF-chitotriose) in a sodium citrate/phosphate buffer, at pH 5.5.

2. Results

The mentylated furanones **47a** and **47b**, as well as the compounds **104** and **105** were tested for chitinase inhibition. The latter two compounds were obtained from furanone **37** by derivatization with (+)-menthol and (+)-isomenthol, respectively (see Experimental Part). Compound **105** is the enantiomer of **38b**. Acetylated monosaccharides **92**, **96a**, **96b**, **96c**, **96d**, acetylated disaccharide **97**, and deacetylated glucosamine derivative **102** were also tested (Figure 17). The deacetylated glucose derivative **101** was not tested, since it was not possible to purify it completely.

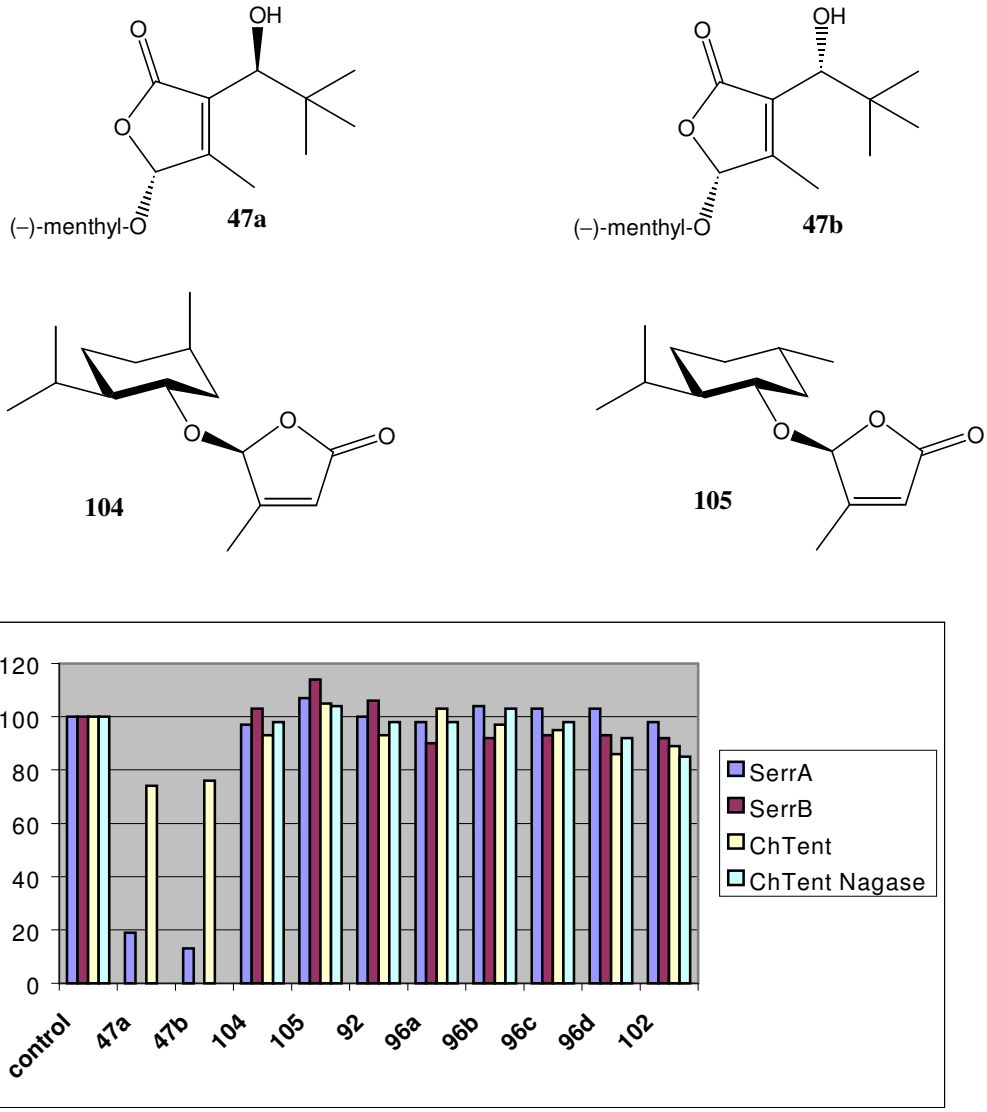


Figure 17: *Chitinase inhibition of furanone derivatives. Data of compound* ***97*** *(inactive) are not shown*

Only the compounds **47a** and **47b** showed a significant inhibition effect. This activity was strong with chitinase A from *Serratia marcescens*, and weak but still significant with the chitinase from *Chironomus tentans*. It can be concluded that **47a** and **47b** are specific inhibitors to chitinase A from *Serratia marcescens*. The derivatives **104** and **105** were not active.

The glycosylated furanones **92**, **96a**, **96b**, **96c** and **96d** did not have any significant activity. Only the deacetylated compound **102** showed a very slight inhibition, but this was not significant enough to be considered.

3. Discussion

Only compounds **47a** and **47b** were active. Other menthylated furanones were also active [37]. The derivatives without side chain at C(3) (**38b**, **104**, **105**) were not inhibitors: the presence of a menthyl group, or a similar derivative like isomenthyl, on the furanone is not sufficient to give an inhibiton effect.

The allosamizoline part of allosamidin can be easily protonated during the inhibition process [65] (Figure 18). With our furanones derivatives, such as compound **47a**, containing a secondary alcohol in the side chain, this is also possible. However, protonation would be much weaker than in allosamidin. Furanones **38b**, **104** and **105** contain no side chain, which means that protonation is not possible (Figure 19). This might explain why these compounds were not active.

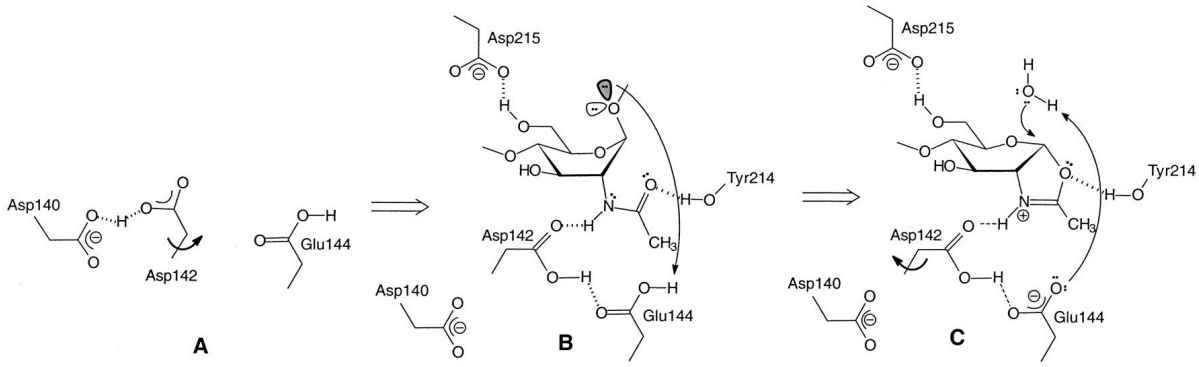


Figure 18: *"Proposed catalytic mechanism. Asp-140, Asp-142 and Glu-144, conserved in most family 18 chitinases, are shown during separate stages of catalysis. (*A*) Resting enzyme. Asp-142 is too far away to interact with Glu-144. (*B*) Binding of substrate causes distorsion of the pyranose ring to a boat or skewed boat conformation and rotation of Asp-142 toward Glu-144, enabling hydrogen bond interactions between the hydrogen of acetamido group, Asp-142, and Glu-144. (*C*) Hydrolysis of the oxazolinium ion intermediate leads to protonation of Glu-144 and rotation of Asp-142 to ist original position where it shares a proton with Asp-140." [65]*

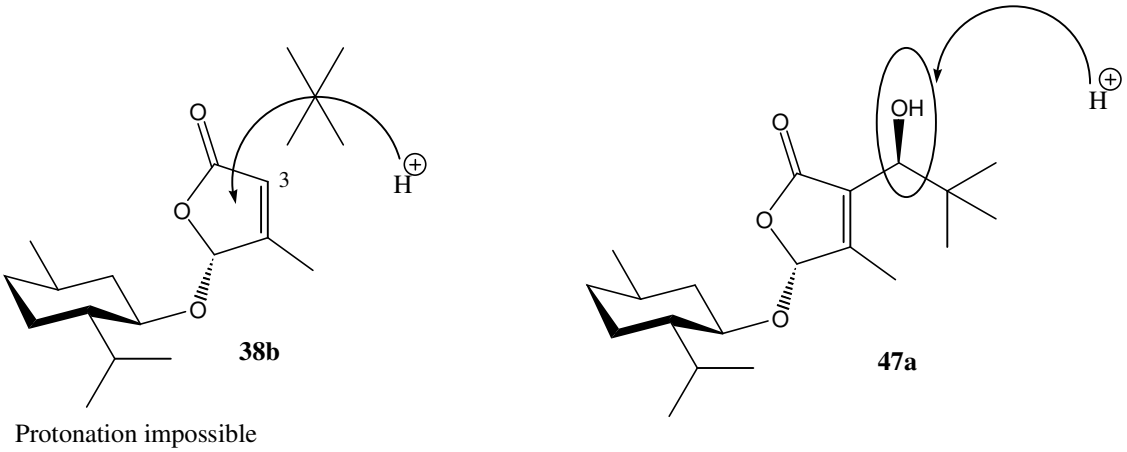


Figure 19: *Hypothetical reactivity of furanones in the active site of chitinases.*

The acetylated compounds **92**, **96a**, **96b**, **96c**, **96d** and **97** were not active. We had previously pointed out that the substrate chitin was held in the active site of the chitinase through hydrogen bonds. Acetylated sugar derivatives cannot have these interactions in the same way. This could explain why our acetylated furanone glycosides were found to be inactive. In this context it was interesting to note that the deacetylated compound **102** had a very slight inhibitory activity.

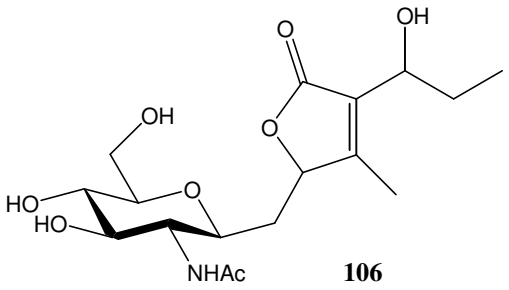
V Conclusion and perspective

The synthesis of glycosylated furanones could be achieved. However, the new glycosidic bond proved to be unstable under basic aqueous conditions. This led to low yields during the synthesis, particularly during the final deacetylation, during which it remained impossible to get a full deacetylation without degradation of a large part of the product.

Therefore, the use of an other protecting group was taken into account. The protection of glucose with phenoxyacetic acid was tried, but the preparation of the carbohydrate before the glycosylation was much less convenient than with acetic acid, and it was much more expensive. This synthetic route was not investigated further.

The biological tests also showed that the addition of a cyclohexane derivative or a sugar on the furanone was not sufficient to have a significant inhibition. The presence of a

side chain with a secondary alcohol is also required. Therefore, if an aldehyde is condensed with the glycosylated furanones, in the same way as the menthylated furanones, the bioactivity should increase. However, this condensation occurs under basic conditions, and it was seen that the glycosidic bond was not stable under basic conditions. The only way to get glycosylated furanones with a side-chain is to introduce the aldehyde first, and then to glycosylate the substituted furanone.



106

The instability of the linkage between the furanone and the sugar remains the strongest problem of these compounds: this glycosidic bond is very sensitive to hydrolysis. Therefore, even if the compound **102** would have been active *in vitro*, it might not be possible to use it *in vivo*. The synthesis of *C*-glycosides like **106** should be considered. In fact, *C*-glycosides are not sensitive to hydrolysis [66], and their structure analogy with *O*-glycosides can confer them interesting bioactivities. *E.g.* some *C*-disaccharides were found to be glycohydrolase inhibitors [67].

B. Experimental Part

General remarks

Chemicals were purchased from *Fluka AG*, *Merck GmbH*, *Acros Organics*, and *Sigma-Aldrich Chemical Company, Inc*.

Absolute solvents were bought in *puriss* quality. THF was distilled over sodium and benzophenone, under argon. Absolute methanol and dichloromethane were stored under argon over 4 Å molecular sieves.

Solvent evaporations were performed on a *Büchi* Rotavapor R-114 with a water bath *Büchi* B-480 at 40 °C; the final pressure was 15 mbar; all products were dried overnight under high vacuum at 0.06-0.1 mbar.

Flash chromatographies were eluted with distilled solvents from a technical quality and with air pressure at the top of the column. Silica gel was purchased from *Macherey-Nagel*, 60M 0.040-0.063 mm. Solvent ratios are given in volume percentage.

Meting points were measured on a *Büchi* 535.

Optical rotations were measured on a *Perkin Elmer* polarimeter 341 in a 10 cm cell.

Infrared spectra were measured on a spectrometer *Perkin Elmer* 1600 Series FT-IR, using potassium bromide pellets or a substance film between sodium chloride plates. Resonance frequencies are given in cm^{-1}.

NMR spectra were measured on a *Bruker* VRX 500 (^{1}H-NMR 500 MHz; ^{13}C-NMR 125 MHz) with the help of Dr. *K. Kulicke*. The chemical shifts δ are given in ppm. Reference for proton is TMS at 0.00 ppm. Reference for carbon is $CDCl_3$ at 77.0 ppm. Assignments were made from APT, COSY, HMQC, HMBC. $CDCl_3$ with 0.05% TMS was stored over a silver foil. CD_3OD was purchased in 1 g glass ampoules. DMSO-d6 containing 0.05 % TMS

was stored in a dessicator. All solvents were purchased from *Cambridge Isotope Laboratories*, Inc.

Mass spectra were measured by Dr. *H. Nadig* on the spectrometers *VG* 70SE, *Finnigan* MAT95Q (EI, 70 eV), and *Finnigan* MAT312 [FAB, matrix: 3-nitrobenzyl alcohol (NBA)]. We report *m/z* values with their respective intensities in parentheses

Elemental analysis were performed by *W. Kirsch* from the Microanalytical Laboratory.

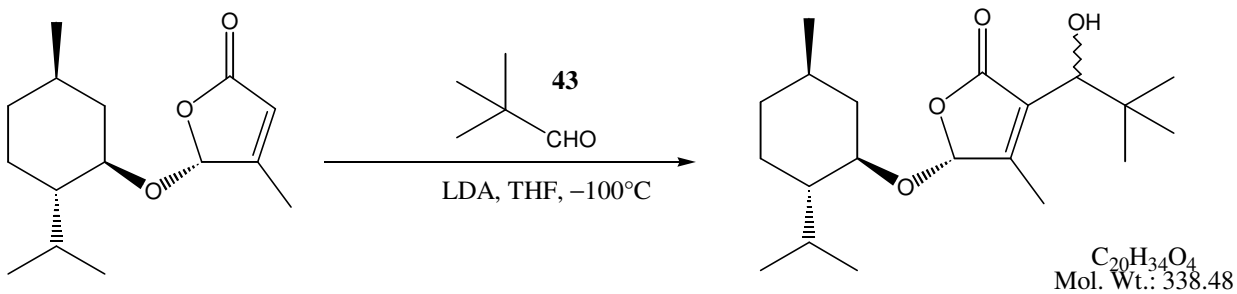
X-ray structures were measured by Dr. *M. Neuburger*, from the Institute of Inorganic Chemistry, University of Basel.

Part One

Furanones with an aliphatic side-chain

I Synthesis of both enantiomers of 3-[1-hydroxy-2,2-dimethylpropyl]-4-methyl-5*H*-furan-2-one 56a and 56b

1. Synthesis of the mixture of (5*R*)-3-[(1*S*)-hydroxy-2,2-dimethylpropyl]-5-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**47a**) and (5*R*)-3-[(1*R*)-hydroxy-2,2-dimethylpropyl]-5-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**47b**).

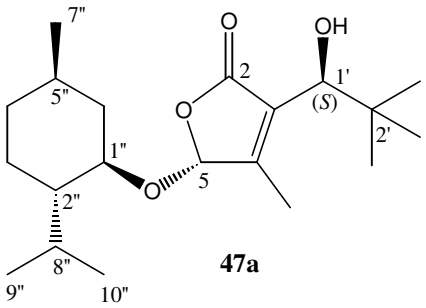


$C_{20}H_{34}O_4$
Mol. Wt.: 338.48

38b **47a-b**

A solution of freshly distilled diisopropylamine (11 ml, 77 mmol) in freshly distilled THF (450 ml) was cooled to 0°C under argon. *n*-BuLi in hexane (55 ml, 79 mmol) was added dropwise over 30 min and the resulting solution was stirred for 30 min at 0°C. Then, the mixture was cooled to −100°C (ether in hexane 20%, liquid N_2) and a solution of (5*R*)-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one [14] (**38b**, 15 g, 59 mmol) in THF (35 ml) was added dropwise during 30 min and the resulting solution was stirred for 30 min at −100°C. Pivalaldehyde (8.5 ml, 77 mmol) was added dropwise during 30 min and the mixture was stirred for further 30 min at −100°C. After a slow warm-up to −20°C, the reaction was quenched with saturated NH_4Cl solution (400 ml) and the mixture was extracted with CH_2Cl_2 (3 × 300 ml), washed with saturated $NaHCO_3$ solution (3 × 300 ml) and saturated NaCl solution (3 × 300 ml), dried ($MgSO_4$) and evaporated. The residue was chromatographed (SiO_2, CH_2Cl_2) to afford **47** as a mixture of 2 diastereomers. A second chromatography (SiO_2, EtOAc in hexane 10%) enabled the separation of **47a** and **47b**.

*1.1 Data of (5*R*)-3-[(1*S*)-hydroxy-2,2-dimethylpropyl]-5-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy-4-methyl-5*H*-furan-2-one (**47a**)*



47a

$C_{20}H_{34}O_4$
Mol. Wt.: 338.48

Yield: 3.85g, 19%

Colorless needles, Mp 112.7-113.6°C.

$[\alpha]_D^{20} = -153.5$ (c = 1.00, $CHCl_3$ stab. 1% EtOH).

IR (KBr): 3497, 2956, 1733, 1681, 1449, 1363, 1326, 1278, 1240, 1182, 1098, 1049, 1011, 937.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.75 (*s*, 1 H, H-C(5)); 4.18 (*d*, *J* = 8.7 Hz, 1 H, H-C(1')); 3.62 (*td*, *J* = 10.5, 4 Hz, 1 H, H-C(1'')); 3.36 (*d*, *J* = 8.6 Hz, 1 H, HO-C(1')); 2.15-2.06 (*m*, 2 H, H_{eq}-C(6'') and H-C(8'')); 1.99 (*s*, 3 H, H_3C-C(4)); 1.72-1.63 (*m*, 2 H, H_{eq}-C(4'') and H_{eq}-C(3'')); 1.48-1.35 (*m*, 1 H, H-C(5'')); 1.30-1.21 (*m*, 1 H, H-C(2'')); 1.12-0.85 (*m*, 3 H, H_{ax}-C(6''), H_{ax}-C(4'') and H_{ax}-C(3''));0.96 (*d*, *J* = 7 Hz, 3 H, H_3C(7'')); 0.95 (*s*, 9 H, $(H_3C)_3$-C(2')); 0.88 (*d*, *J* = 7 Hz, 3 H, H_3C(9'')); 0.80 (*d*, *J* = 7 Hz, 3 H, H_3C(10'')).

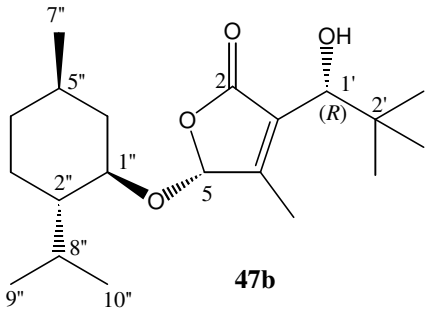
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 172.6 (C(2)); 158.0 (C(4)); 129.3 (C(3)); 100.9 (C(5)); 79.8 (C(1'')); 75.7 C(1')); 48.2 (C(2'')); 40.8 (C(6'')); 38.2 (C(2')); 34.6 (C(4'')); 31.9 (C(5'')); 26.4 ((CH_3)$_3$-C(2')); 25.7 (C(8'')); 23.6 (C(3'')); 22.6 (C(7'')); 21.2 (C(9'')); 16.2 (C(10'')); 13.0 (CH_3-C(4)).

EI-MS (70 eV, 200°C): *m/z* (%) = 282 (16), 281 (13), 166 (16), 144 (82), 143 (31), 139 (72), 127 (47), 126 (100), 97 (18), 95 (14), 83 (85), 81 (21), 69 (36), 57 (65), 55 (38), 43 (21), 41 (36).

FAB-MS (NBA): *m/z* (%) = 340 (21), 339 ([*M* + H]$^+$, 100), 201 (26), 183 (98), 165 (24), 139 (28), 127 (37), 125 (11), 97 (15), 83 (85), 81 (19),69 (32), 57 (49), 55 (32), 43 (14), 41 (20).

Anal. calc. for $C_{20}H_{34}O_4$ (338.48): C, 70.97; H, 10.12; O, 18.91. Found: C, 71.01; H, 10.04; O 18.77.

*1.2 Data of (5*R*)-3-[(1*R*)-hydroxy-2,2-dimethylpropyl]-5-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy-4-methyl-5*H*-furan-2-one (**47b**)*



47b

$C_{20}H_{34}O_4$
Mol. Wt.: 338.48

Yield: 3.50g, 17%.

Colorless needles, Mp 85.5-86.8°C.

$[\alpha]_D^{20} = -108.5$ (*c* = 1.00, MeOH).

IR (KBr): 3442, 2954, 1745, 1678, 1459, 1368, 1338, 1297, 1241, 1178, 1106, 1003, 952.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.68 (*s*, 1 H, H-C(5)); 4.17 (*d*, *J* = 9.5 Hz, 1 H, H-C(1')); 3.64 (*td*, *J* = 10.5, 4 Hz, 1 H, H-C(1'')); 3.53 (*d*, *J* = 9.5 Hz, 1 H, HO-C(1')); 2.15-2.03 (*m*, 2 H, H_{eq}-C(6'') and H-C(8'')); 1.97 (*s*, 3 H, H_3C-C(4)); 1.72-1.63 (*m*, 2 H, H_{eq}-C(4'') and H_{eq}-C(3'')); 1.47-1.37 (*m*, 1 H, H-C(5'')); 1.29-1.23 (*m*, 1 H, H-C(2'')); 1.09-0.85 (*m*, 3 H, H_{ax}-C(6''), H_{ax}-C(4'') and H_{ax}-C(3''));0.96 (*d*, *J* = 7 Hz, 3 H, H_3C(7'')); 0.95 (*s*, 9 H, $(H_3C)_3$-C(2')); 0.88 (*d*, *J* = 7 Hz, 3 H, H_3C(9'')); 0.81 (*d*, *J* = 7 Hz, 3 H, H_3C(10'')).

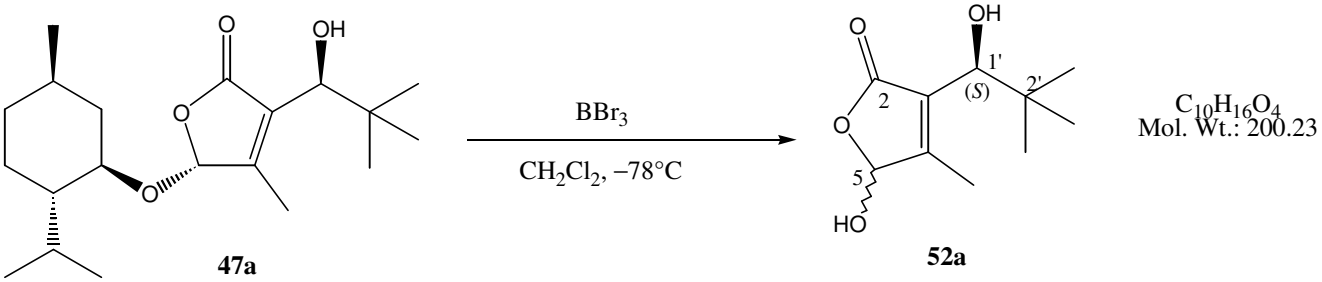
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 172.2 (C(2)); 157.2 (C(4)); 128.7 (C(3)); 100.6 (C(5)); 79.4 (C(1'')); 75.4 C(1')); 47.8 (C(2'')); 40.4 (C(6'')); 37.7 (C(2')); 34.4 (C(4'')); 31.5 (C(5'')); 26.0 ((CH_3)$_3$-C(2')); 25.8 (C(8'')); 23.7 (C(3'')); 22.2 (C(7'')); 20.7 (C(9'')); 16.2 (C(10'')); 12.7 (CH_3-C(4)).

EI-MS (70 eV, 200°C): *m/z* (%) = 282 (17), 281 (13), 166 (13), 144 (89), 143 (31), 139 (70), 127 (45), 126 (100), 97 (19), 95 (17), 83 (92), 81 (23), 69 (40), 57 (69), 55 (42), 43 (22), 41 (40).

FAB-MS (NBA): *m/z* (%) = 340 (22), 339 ([*M* + H]$^+$, 96), 201 (28),184 (12), 183 (100), 165 (23), 139 (29), 127 (35), 125 (10), 97 (14), 83 (88), 81 (20),69 (30), 57 (50), 55 (35), 43 (16), 41 (22).

Anal. calc. for $C_{20}H_{34}O_4$ (338.48): C, 70.97; H, 10.12; O, 18.91. Found: C, 70.93; H, 10.06; O 18.78.

2. Synthesis of 5-hydroxy-3-[(1*S*)-1-hydroxy-2,2-dimethylpropyl]-4-methyl-5*H*-furan-2-one (**52a**)



47a **52a**

$C_{10}H_{16}O_4$
Mol. Wt.: 200.23

(5*R*)-3-[(1*S*)-1-hydroxy-2,2-dimethylpropyl]-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**47a**, 1.0 g, 3.0 mmol) was dissolved in absolute CH_2Cl_2 (50 ml). The solution was cooled to −78°C and BBr_3 (0.86 ml, 8.9 mmol) was added. After a reaction time of 4 hours at −78°C, additional BBr_3 (0.86 ml, 8.9 mmol) was added at −78°C, the resulting mixture was allowed to warm up to −20°C and saturated

$NaHCO_3$ solution (25 ml) was added. After warming up to r.t., the mixture was extracted EtOAc (3 × 100 ml). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated. The residue was chromatographed (SiO_2, 33% EtOAc in hexane) to give **52a**.

Yield: 482 mg, 81%.

colorless cubes (EtOH), Mp 162.3-162.7°C.

$[\alpha]_D^{20} = -41.5$ (c = 1.00, MeOH).

IR (KBr): 3473, 3264, 2959, 2868, 1729, 1675, 1459, 1384, 1363, 1341, 1297, 1271, 1177, 1155, 1098, 1030, 1006, 950.

^{1}H-NMR (500 MHz, d_6-DMSO): δ = 7.75 (*d*, *J* = 7.5 Hz, 1 H, HO-C(5^A)); 7.66 (*d*, *J* = 8.5 Hz, 1 H, HO-C(5^B)); 5.93 (*d*, *J* = 7.5 Hz, 1 H, H-C($5^{A\text{ or }B}$)); 5.80 (*d*, *J* = 8.5 Hz, 1 H, H-C($5^{B\text{ or }A}$)); 5.12 (*d*, *J* = 4.9 Hz, 2×1 H, HO-C($1'^{A\text{ and }B}$)); 4.14 (*d*, *J* = 4.7 Hz, 1 H, H-C($1'^{A\text{ or }B}$)); 4.11 (*d*, *J* = 4.5 Hz, 1 H, H-C($1'^{2\text{ or }1}$)); 2.09 (*s*, 2×3 H, H_3C-C($4^{1\text{ and }2}$)); 0.90 (*s*, 9 H, $(H_3C)_3$-C($2'^{1\text{ or }2}$)); 0.89 (*s*, 9 H, $(H_3C)_3$-C($2'^{2\text{ or }1}$)).

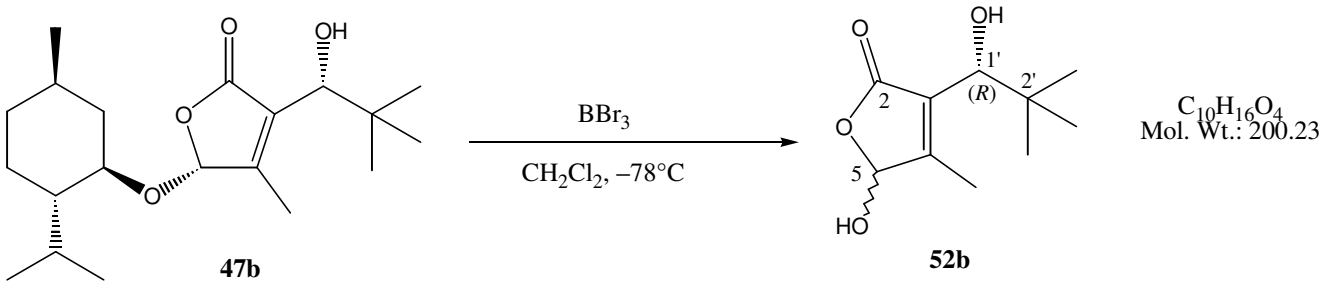
^{13}C-NMR (125 MHz, d_6-DMSO): δ = 172,72 and 172,36 (C(2)); 161.08 and 160.44 (C(4)); 130.2 (C(3)); 98.90 and 98.84 (C(5)); 73.82 and 73.73 (C(1')); 38.12 and 37.97 (C(2')); 26.9 ($(CH_3)_3$-C(2')); 13.88 and 13.67 (CH_3-C(4)).

EI-MS (70 eV, 300°C): *m/z* (%) = 167 (32), 149 (43), 144 (11), 127 (10), 126 (100), 98 (17), 97 (15), 70 (12), 69 (26), 57 (85), 55 (11), 44 (12), 43 (19), 41 (68), 39 (34).

FAB-MS (NBA): *m/z* (%) = 339 (9), 202 (10), 201 ($[M + H]^+$, 100), 183 (64), 165 (42), 139 (11), 137 (34), 136 (48), 127 (16), 120 (17), 107 (16), 91 (16), 90 (22), 83 (34), 81 (10), 77 (10), 69 (34), 67 (12), 57 (79), 55 (46), 53 (11), 43 (46), 41 (35).

Anal. calc. for $C_{10}H_{16}O_4$ (200.23): C, 59.98; H, 8.05; O, 31.96. Found: C, 60.08; H, 7.90; O, 32.04.

3. Synthesis of 5-hydroxy-3-[(1*R*)-1-hydroxy-2,2-dimethylpropyl]-4-methyl-5*H*-furan-2-one (**52b***)*



(5*R*)-3-[(1*R*)-1-hydroxy-2,2-dimethylpropyl]-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**47b**, 1.0 g, 3.0 mmol) was dissolved in absolute CH_2Cl_2 (50 ml). The solution was cooled to −78°C and BBr_3 (0.86 ml, 8.9 mmol) was added. After a reaction time of 4 hours at −78°C, additional BBr_3 (0.86 ml, 8.9 mmol) was added at −78°C, the resulting mixture was allowed to warm up to −20°C and saturated $NaHCO_3$ solution (25 ml) was added. After warming up to r.t., the mixture was extracted EtOAc (3 × 100 ml). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated. The residue was chromatographed (SiO_2, 33% EtOAc in hexane) to give **52b**.

Yield: 330 mg, 66%.

colorless cubes (EtOH), Mp 162.0-162.7°C.

$[\alpha]_D^{20}$ = +39.0 (*c* = 1.00, MeOH).

IR (KBr): 3473, 3342, 2959, 2868, 1729, 1714, 1675, 1460, 1384, 1363, 1340, 1278, 1177, 1155, 1098, 1030, 1006, 950

^{1}H-NMR (500 MHz, d_6-DMSO): δ = 7.71 (*d*, *J* = 7.5 Hz, 1 H, HO-C(5^1)); 7.62 (*d*, *J* = 8.5 Hz, 1 H, HO-C(5^2)); 5.90 (*dd*, *J* = 7.5, 0.6 Hz, 1 H, H-C($5^{1\text{ or }2}$)); 5.76 (*dd*, *J* = 8.5, 0.2 Hz, 1 H, H-C($5^{2\text{ or }1}$)); 5.08 (*d*, *J* = 5 Hz, 2×1 H, HO-C($1'^{1\text{ and }2}$)); 4.11 (*d*, *J* = 4.5 Hz, 1 H, H-C($1'^{1\text{ or }2}$)); 4.07 (*d*, *J* = 4.5 Hz, 1 H, H-C($1'^{2\text{ or }1}$)); 2.06 (*s*, 3 H, H_3C-C($4^{1\text{ or }2}$)); 2.05 (*s*, 3 H, H_3C-C($4^{2\text{ or }1}$)); 0.86 (*s*, 9 H, $(H_3C)_3$-C($2'^{1\text{ or }2}$)); 0.85 (*s*, 9 H, $(H_3C)_3$-C($2'^{2\text{ or }1}$)).

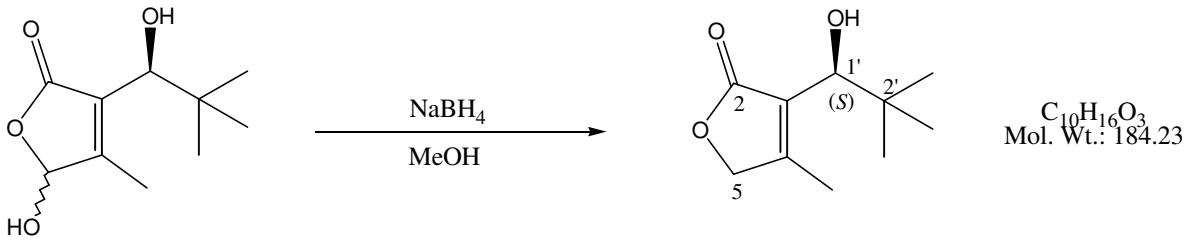
^{13}C-NMR (125 MHz, d_6-DMSO): δ = 172,71 and 172,36 (C(2)); 161.07 and 160.44 (C(4)); 130.18 (C(3)); 98.90 and 98.84 (C(5)); 73.81 and 73.73 (C(1')); 38.12 and 37.97 (C(2')); 26.9 ((*C*H$_3$)$_3$-C(2')); 13.89 and 13.67 (*C*H$_3$-C(4)).

EI-MS (70 eV, 300°C): *m/z* (%) = 144 (14), 126 (100), 97 (11), 69 (16), 57 (50), 41 (29), 39 (12).

FAB-MS (NBA): *m/z* (%) = 409 (18), 339 (9), 202 (11), 201 ([*M* + H]$^+$, 100), 183 (74), 165 (49), 139 (15), 137 (33), 136 (73), 127 (13), 125 (13), 121 (11), 120 (25), 107 (12), 97 (13), 91 (12), 90 (18), 83 (48), 81 (15), 69 (39), 57 (77), 55 (37), 43 (36), 41 (31).

Anal. calc. for $C_{10}H_{16}O_4$ (200.23): C, 59.98; H, 8.05; O, 31.96. Found: C, 60.20; H, 7.89; O, 31.71.

4. Synthesis of 3-[(1*S*)-1-hydroxy-2,2-dimethylpropyl]-4-methyl-5*H*-furan-2-one (**56a**)



52a **56a**

A solution of 5-hydroxy-3-[(1*S*)-1-hydroxy-2,2-dimethylpropyl]-4-methyl-5*H*-furan-2-one (**52a**, 200 mg, 1.0 mmol) in absolute MeOH (5 ml) was cooled at 0°C. $NaBH_4$ (200 mg, 5.3 mmol) was added in portions within 10 min and the solution was stirred 45 min at r.t. Then the reaction mixture was quenched with H_2O (5 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic layers were dried ($MgSO_4$), evaporated and the residue was chromatographed (SiO_2, EtOAc in hexane 33%) to give **56a**.

Yield: 134 mg, 73%.

colorless solid, Mp 77-78°C.

$[\alpha]_D^{20}$ = −28.4 (*c* = 1.00, CH_2Cl_2).

IR (KBr): 3454, 2975, 2861, 1734, 1662, 1483, 1469, 1440, 1389, 1363, 1350, 1293, 1243, 1219, 1179, 1121, 1088, 1039, 1016, 992, 943, 896.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.73 (*d*, *J* = 17.5 Hz, 1 H, H_A-C(5)); 4.65 (*d*, *J* = 17.5 Hz, 1 H, H_B-C(5)); 4.20 (*d*, *J* = 9.5 Hz, 1 H, H-C(1‘)); 3.66 (*d*, *J* = 9.5 Hz, 1 H, HO-C(1’)); 2.08 (*s*, 3 H, H_3C-C(4)); 0.96 (*s*, 9 H, $(CH_3)_3$-C(2’)).

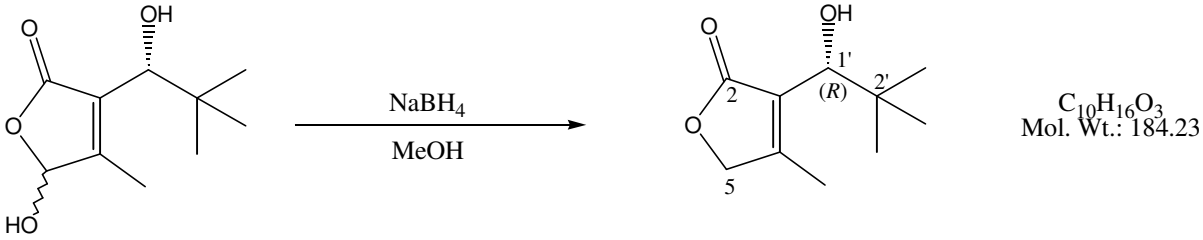
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 174.8 (C(2)); 159.3 (C(4)); 126.3 (C3)); 75.2 (C(1’)); 72.8 (C(5)); 37.8 (C(2’)); 25.9 ((*C*H_3)$_3$-C(2’)); 13.3 (*C*H_3-C(4));

EI-MS (70 eV, 100°C): *m/z* (%) = 151 (13), 128 (80), 127 (64), 110 (100), 99 (12), 82 (65), 57 (49), 53 (13), 43 (18), 41 (35), 39 (19).

FAB-MS (NBA): *m/z* (%) = 186 (10), 185 ([*M* + H]$^+$, 100), 167 (82), 137 (16), 136 (8), 127 (16), 77 (11), 57 (24), 41 (17), 39 (16).

Anal. calc. for $C_{10}H_{16}O_3$ (184.23): C, 65.19; H, 8.75; O, 26.05. Found: C, 64.97; H, 8.78; O, 26.12.

5. Synthesis of 3-[(1*R*)-1-hydroxy-2,2-dimethylpropyl]-4-methyl-5*H*-furan-2-one (**56b**)



52b **56b**

A solution of 5-hydroxy-3-[(1*R*)-1-hydroxy-2,2-dimethylpropyl]-4-methyl-5*H*-furan-2-one (**52b**, 200 mg, 1.0 mmol) in absolute MeOH (5 ml) was cooled at 0°C. $NaBH_4$ (200 mg, 5.3 mmol) was added in portions within 10 min and the solution was stirred 45 min at r.t. Then the reaction mixture was quenched with H_2O (5 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic layers were dried ($MgSO_4$), evaporated and the residue was chromatographed (SiO_2, EtOAc in hexane 33%) to give **56b**.

Yield: 118 mg, 64%.

colorless solid, Mp 77-78°C.

$[\alpha]_D^{20}$ = +27.0 (*c* = 1.00, CH_2Cl_2).

IR (KBr): 3454, 2975, 2861, 1734, 1662, 1483, 1469, 1440, 1382, 1363, 1350, 1293, 1243, 1219, 1179, 1121, 1088, 1039, 1016, 992, 943, 896.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.73 (*d*, *J* = 17.5 Hz, 1 H, H_A-C(5)); 4.65 (*d*, *J* = 17.5 Hz, 1 H, H_B-C(5)); 4.20 (*d*, *J* = 9.5 Hz, 1 H, H-C(1‘)); 3.66 (*d*, *J* = 9.5 Hz, 1 H, HO-C(1’)); 2.08 (*s*, 3 H, H_3C-C(4)); 0.96 (*s*, 9 H, $(CH_3)_3$-C(2’)).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 174.8 (C(2)); 159.3 (C(4)); 126.3 (C3)); 75.2 (C(1’)); 72.8 (C(5)); 37.8 (C(2’)); 25.9 ((CH_3)$_3$-C(2’)); 13.3 (CH_3-C(4));

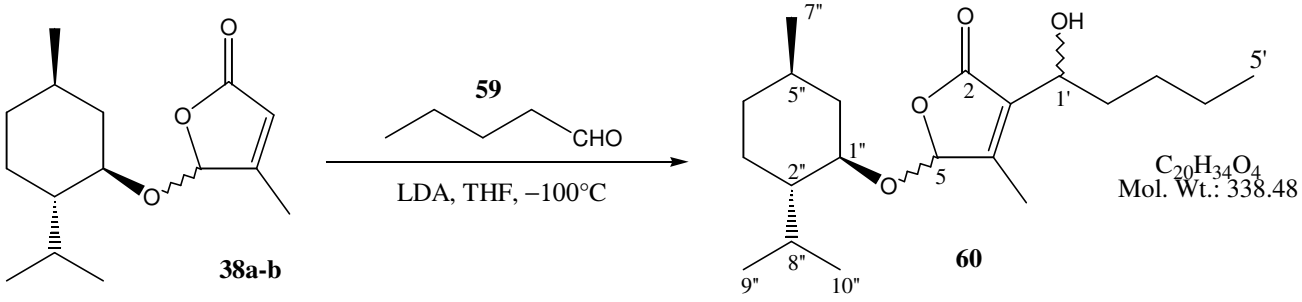
EI-MS (70 eV, 100°C): *m/z* (%) = 151 (13), 128 (80), 127 (64),123 (11), 110 (100), 99 (12), 82 (65), 57 (49), 53 (13), 43 (18), 41 (35), 39 (19).

FAB-MS (NBA): *m/z* (%) = 186 (10), 185 ($[M + H]^+$, 100), 167 (82), 137 (14), 136 (10), 127 (14), 77 (14), 57 (12), 41 (12), 39 (13).

Anal. calc. for $C_{10}H_{16}O_3$ (184.23): C, 65.19; H, 8.75; O, 26.05. Found: C, 65.24; H, 8.75; O, 26.34.

II Synthesis of 4-methyl-3-pentyl-5*H*-furan-2-one 52 (64, MP133)

1. Synthesis of 3-(1-hydroxypentyl)-5-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcycloheyl]-oxy}-4-methyl-5*H*-furan-2-one (**60**)



A solution of freshly distilled diisopropylamine (11 ml, 77 mmol) in freshly distilled THF (500 ml) was cooled to 0°C under argon. *n*-BuLi in hexane (53 ml, 80 mmol) was added dropwise over 30 min and the resulting solution was stirred for 30 min at 0°C. Then, the mixture was cooled to −100°C (hexane-ether 80:20, liquid N_2) and a solution of 5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one [14] (**38a-b**, 15 g, 59 mmol) in THF (30 ml) was added dropwise during 30 min and the resulting solution was stirred for 30 min at −100°C. Pentanal (**59**, 8 ml, 75 mmol) was added dropwise during 30 min and the mixture was stirred for further 30 min at −100°C. After a slow warm-up to –20°C, the reaction was quenched with saturated NH_4Cl solution (300 ml) and the mixture was extracted with CH_2Cl_2 (3 × 300 ml), washed with saturated $NaHCO_3$ solution (3 × 300 ml) and saturated NaCl solution (3 × 300 ml), dried ($MgSO_4$) and evaporated. The residue was chromatographed (SiO_2, EtOAc in CH_2Cl_2 0 to 10%) to afford **60** as a mixture of 4 diastereomers.

Light yellow oil, yield: 9.0g, 45%.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.72, 5.70, 5.61, 5.60 (4 *s*, 1 H, H-C(5)); 4.49-4.43 (*m*, 1 H, H-C(1')); 3.66-3.47 (2 *m*,1 H, H-C(1'')); 2.89 (*d*, *J* = 9.2 Hz), 2.88 (*d*, *J* = 9.0 Hz), 2.82 (*d*, *J* = 8.4 Hz), 2.75 (*d*, *J* = 8.4 Hz) (1 H, HO-C(1')); 2.33-2.07 (3 *m*, 2 H, H-C(8'') and H_{eq}-C(6'')); 2.04, 2.02, 1.99, 1.98 (4 *s*, 3 H, H_3C-C(4)); 1.88-1.78 (*m*, 1 H, H_A-C(2')); 1.71-1.62 (*m*, 3 H, H_{eq}-C(4''), H_{eq}-C(3'') and H_B-C(2')); 1.45-1.22 (*m*, 6 H, H_2C(3'), H_2C(4'),

H-C(2'') and H-C(5'')); 1.11-0.85 (*m*, 12 H, H_{ax}-C(6''), H_{ax}-C(4''), H_{ax}-C(3''), H_3C(5'), H_3C(7''), and H_3C(9'')), 0.83-0.77 (*m*, 3 H, H_3C(10'')).

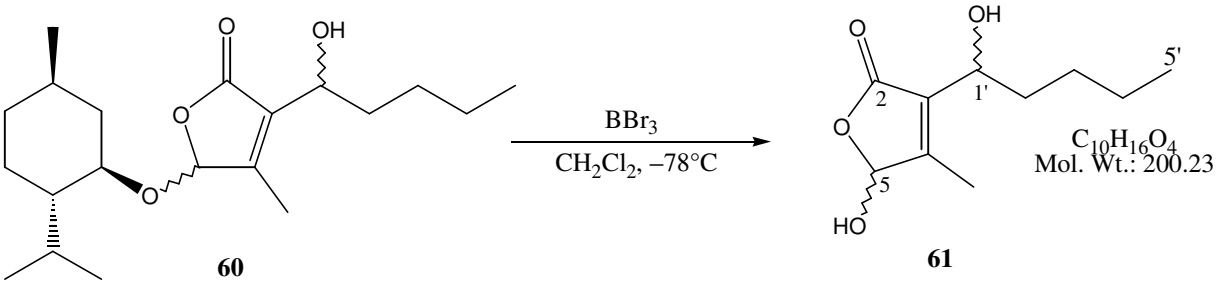
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.47, 171.42, 171.31, 171.28 (C(2)); 155.39, 155.35, 155.23, 155.06 (C(4)); 130.81, 130.73, 130.70, 130.58 (C(3)); 104.73, 104.50, 100.85, 100.69 (C(5)); 83.59, 83.43, 79.50, 79.45 (C(1'')); 66.96, 66.90, 66.87, 66.83 (C(1')); 48.12, 48.09, 47.70, 47.66 (C(2'')), 42.33, 40.42, 40.38 (C(6'')); 36.38, 36.34, 36.19, 36.13 (C(2')); 34.18, 34.05 (C(4'')); 31.63, 31.42 (C(5'')); 27.63, 27.56 (C(3')); 25.41, 25.39, 25.32, 25.13 (C(8'')); 23.19, 23.04, 22.84, 22.82 (C(3'')); 22.40, 22.38, 22.22, 22,21, 22.09 (C(4') and C(7'')); 20.93, 20.91, 20.84, 20.78 (C(9'')); 15.84, 15.70 (C(10'')); 13.96, 13.95 (C(5')), 11.62, 11.48 (CH$_3$-C(4)).

EI-MS (70 eV, 250 °C): *m/z* (%) = 281 (29), 183 (28), 182 (19), 167 (13), 166 (79), 155 (44), 143 (64), 140 (15), 139 (100), 138 (21), 137 (24), 127 (12), 126 (27), 123 (11), 111 (31), 110 (35), 109 (10), 97 (24), 95 (33), 85 (49), 83 (96), 82 (10), 81 (53), 71 (14), 69 (63), 67 (19), 57 (53), 55 (64), 43 (39), 41 (59), 39 (12).

FAB-MS (NBA): *m/z* (%) = 340 (14), 339 ([M + H]$^+$, 64), 201 (15), 184 (11), 183 (87), 97 (15), 85 (12), 83 (100), 81 (20), 77 (13), 69 (39), 67 (11), 57 (37), 55 (39), 43 (17), 41 (30), 39 (19).

Anal. calc. for $C_{20}H_{34}O_4$ (338.48): C, 70.97; H, 10.12. Found: C, 70.96; H, 10.21.

2. Synthesis of 5-hydroxy-3-(1-hydroxypentyl)-4-methyl-5*H*-furan-2-one (**61**)



60 **61**

3(1-Hydroxypentyl)-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**60**, 7.3 g, 21 mmol) was dissolved in absolute CH_2Cl_2 (300 ml). The solution was cooled to −78°C and BBr_3 (6 ml, 62 mmol) was added. After a reaction time of 4 hours at −78°C, additional BBr_3 (6 ml, 62 mmol) was added at −78°C, the resulting mixture was allowed to warm up to −20°C and saturated $NaHCO_3$ solution (800 ml) was added. After warming up to r.t., the mixture was extracted EtOAc (3 × 700 ml). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated. The residue was chromatographed (SiO_2, 10 to 50% EtOAc in hexane) to give **61** as a mixture of two pairs of enantiomers.

Yield: 2.6g (60%).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.97 (*br s*, 1 H, HO-C(5)); 5.88 and 5.85 (2 *s*, 1 H, H-C(5)); 4.50-4.45 (*m*, 1 H, H-C(1')); 3.43 (*br s*, 1 H, HO-C(1')); 2.08 and 2.06 (2 *s*, 3 H, H_3C-C(4)); 1.87-1.63 (2 *m*, 2 H, H_2C(2')); 1.41-1.19 (*m*, 2 H, H_2C(3') and H_2C(4')); 0.90 (*t*, 3 H, J = 7 Hz, H_3C(5')).

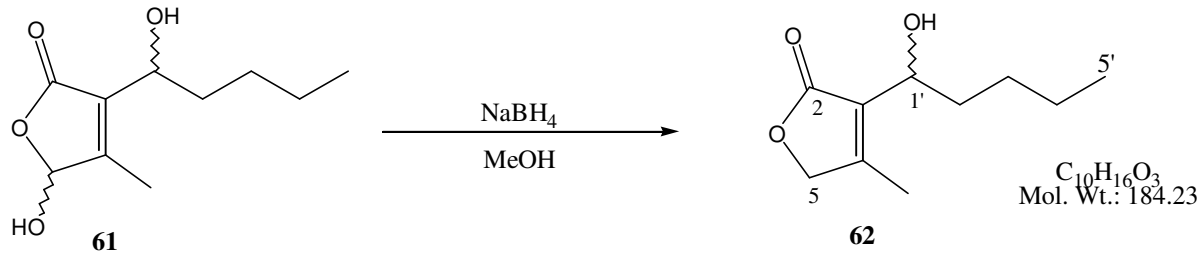
^{13}C-NMR (125 MHz,$CDCl_3$): δ = 172.2 and 171.8 (C(2)); 158.3 and 158.2 (C(4)); 130.2 and 129.8 (C(3)); 98.9 and 98.8 (C(5)); 66.6 and 66.4 (C(1')); 35.7 and 35.2 (C(2')); 27.6 and 27.5 (C(3')); 22.4 and 22.3 (C(4')); 13.9 (C(5')); 11.6 and 11.4 (CH$_3$-C(4)).

EI-MS (70 eV, 250 °C): *m/z* (%) = 182 (8) 144 (12), 143 (100), 139 (28),127 (13), 126 (38), 125 (66), 115 (19), 98 (16), 97 (72), 85 (17), 71 (11), 69 (45), 67 (11), 57 (27), 55 (11), 43 (20), 41 (55), 39 (31).

FAB-MS (NBA): *m/z* (%) = 202 (11), 201 ([M + H]$^+$, 100), 183 (81), 165 (16), 137 (23), 77 (11), 41 (14), 39 (13).

Anal. calc. for $C_{10}H_{16}O_4$ (200.23): C, 59.98; H, 8.05. Found: C, 60.05; H, 8.14.

3. Synthesis of 3-(1-hydroxypentyl)-4-methyl-5*H*-furan-2-one (**62**)



61 **62**

A solution of 5-hydroxy-3(1-hydroxypentyl)-4-methyl-5*H*-furan-2-one (**61**, 2.8 g, 14 mmol) in absolute MeOH (150 ml) was cooled at 0°C. $NaBH_4$ (2.8g, 74 mmol) was added in portions within 10 min and the solution was stirred overnight at room temperature. Then the reaction mixture was quenched with H_2O (150 ml) and extracted with CH_2Cl_2 (3 × 150 ml). The combined organic layers were dried ($MgSO_4$), and evaporated to give **62** without further purification.

Yield: 1.9g, 74%.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.66 (*s*, 2 H, $H_2C(5)$); 4.05 (*t*, 1 H, J = 7 Hz, H-C(1')); 3.23 (*br s*, 1 H, HO-C(1')); 2.10 (*s*, 3 H, H_3C-C(4)); 1.87-1.64 (*m*, 2 H, $H_2C(2')$); 1.43-1.22 (*m*, 4 H, $H_2C(3')$ and $H_2C(4')$); 0.90 (*t*, 3 H, J = 7 Hz, $H_3C(5')$).

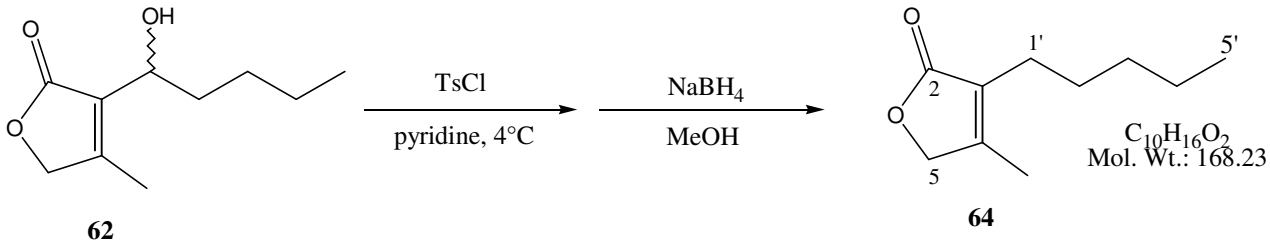
^{13}C-NMR (125 MHz,$CDCl_3$): δ = 174.0 (C(2)); 157.8 (C(4)); 128.0 (C(3)); 72.6 (C(5)); 66.6 (C(1')); 35.91 (C(2')); 27.5 (C(3')); 22.3 (C(4')); 13.8 (C(5')); 12.1 (CH_3-C(4)).

EI-MS (70 eV, 150 °C): *m/z* (%) =127 (100), 99 (22), 82 (5), 71 (5), 53 (7), 43 (10), 41 (10).

FAB-MS (NBA): *m/z* (%) = 186 (8), 185 ($[M + H]^+$, 81), 167 (100), 77 (11), 51 (11), 41 (14), 39 (16).

Anal. calc. for $C_{10}H_{16}O_3$ (184.24): C, 65.19; H, 8.75; O, 26.05. Found: C, 64.99; H, 8.63; O, 26.32.

4. Synthesis of 4-methyl-3-pentyl-5*H*-furan-2-one (**64**) (MP133)



62 **64**

3-(1-Hydroxypentyl)-4-methyl-5*H*-furan-2-one (**62**, 708 mg, 3.8 mmol) was added to a solution of tosyl chloride (817 mg, 4.3 mmol) in pyridine (4 ml) at 0°C. The reaction mixture was stirred overnight at 4°C. After dilution with MeOH (20 ml), $NaBH_4$ (700 mg, 19 mmol) was added and the reaction mixture was stirred for 24 hours at room temperature, after which it was poured into aqueous 1 M HCl at 0°C. The mixture was extracted with CH_2Cl_2 (3 × 50 ml), and the combined organic layers were washed with saturated $NaHCO_3$ (3 × 50 ml) and saturated NaCl solution (3 × 50 ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed (SiO_2, 5% to 30% EtOAc in hexane) to give **64**.

Yield: 280 mg, 43%.

Colorless oil.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.62 (*q*, 2 H, J = 1 Hz, $H_2C(5)$); 2.25 (*t*, 2 H, J = 7.5 Hz, $H_2C(1')$); 2.03 (*s*, 3 H, H_3C-C(4)); 1.49 (*quint*, 2 H, J = 8 Hz, $H_2C(2')$); 1.36-1.25 (*m*, 4 H, $H_2C(3')$ and $H_2C(4')$); 0.89 (*t*, 3 H, J = 7 Hz, $H_3C(5')$).

^{13}C-NMR (125 MHz,$CDCl_3$): δ = 175.0 (C(2)); 156.4 (C(4)); 127.2 (C(3)); 72.3 (C(5)); 31.4 (C(3')); 27.5 (C(2')); 23.2 (C(1')); 22.3 (C(4')); 13.9 (C(5')); 12.1 (CH$_3$-C(4)).

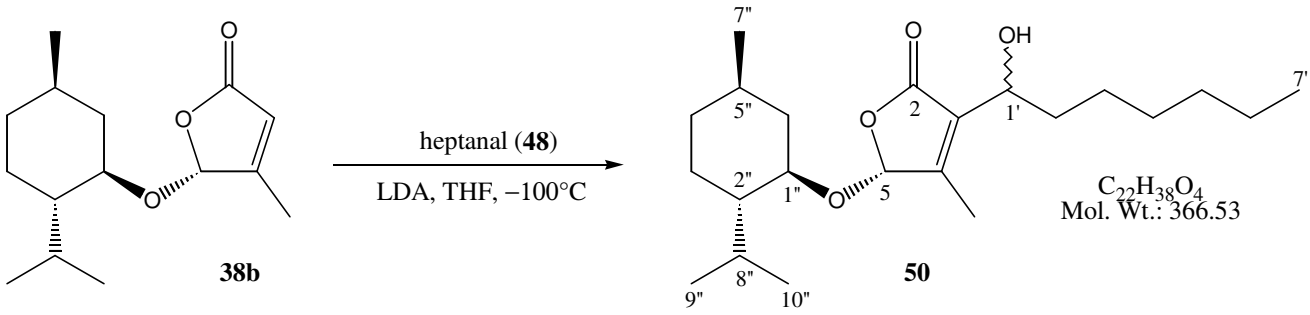
EI-MS (70 eV, 100 °C): *m/z* = 168 (10), 153 (19), 139 (19), 113 (12), 112 (100), 111 (11), 93 (10), 69 (12), 67 (11), 55 (24), 43 (15), 41 (24), 39 (12).

FAB-MS (NBA): *m/z* = 170 (8), 169 ($[M + H]^+$, 100), 167 (9), 57 (6).

Anal. calc. for $C_{10}H_{16}O_2$ (168.23): C, 71.39; H, 9.59; O, 19.02. Found: C, 71.09; H, 9.77; O, 19.14.

III Synthesis of 3-[1-hydroxyheptyl]-4-methyl-5*H*-furan-2-one (57)

1. Synthesis of (5*R*)-3-(1-hydroxyheptyl)-5-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**50**)



A solution of freshly distilled diisopropylamine (3.7 ml, 26 mmol) in freshly distilled THF (250 ml) was cooled to 0°C under argon. *n*-BuLi in hexane (16 ml, 26 mmol) was added dropwise over 30 min and the resulting solution was stirred for 30 min at 0°C. Then, the mixture was cooled to −100°C (ether in hexane 20%, liquid N_2) and a solution of (5*R*)-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one [14] (**38b**, 5 g, 20 mmol) in THF (16 ml) was added dropwise during 30 min and the resulting solution was stirred for 30 min at −100°C. Heptanal (3.6 ml, 26 mmol) was added dropwise during 30 min and the mixture was stirred for further 30 min at −100°C. After a slow warm-up to –20°C, the reaction was quenched with saturated NH_4Cl solution (100 ml) and the mixture was extracted with CH_2Cl_2 (3 × 300 ml), washed with saturated $NaHCO_3$ solution (3 × 100 ml) and saturated NaCl solution (3 × 100 ml), dried ($MgSO_4$) and evaporated. The residue was chromatographed (SiO_2, 0 to 10% EtOAc in hexane) to afford **50** as a mixture of 2 diastereomers.

Yield: 1.01g, 14%.

Colorless oil, R_f 0.7 (EtOAc in hexane 50%).

IR (NaCl): 3471, 2922, 1760, 1688, 1454, 1386, 1330, 1240, 1178, 1094, 943, 779, 730.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 5.71 (*s*, 1 H, H-C(5)); 4.47 (*m*, 1 H, H-C(1')); 3.62 (*td*, *J* = 8, 4 Hz, 1 H, H-C(1'')); 2.85 (*d*, *J* = 8 Hz, 1 H, HO-C(1'^{1}); 2.79 (*d*, *J* = 8 Hz, 1 H, HO-C(1'^{2}); 2.17-2.08 (*m*, 2 H, H-C(8'') and H_{eq}-C(6''); 1.99 (*s*, 3 H, H_3C-C(4)); 1.86-1.77 (*m*, 1 H, H_A-C(2')); 1.72-1.63 (*m*, 3 H, H_B-C(2'), H_{eq}-(3'') and H_{eq}(4'')); 1.47-1.37 (*m*, 2 H, H-C(5'') and H_A-C(3')); 1.34-1.21 (*m*, 8 H, H-C(2''), H_B-C(3'), H_2C(4'), H_2C(5'), H_2C(6')); 1.05-0.98 (*m*, 2 H, H_{ax}-C(3'') and H_{ax}-C(6'')); 0.95 (*d*, *J* = 6 Hz, 3 H, H_3C(7'')); 0.90-0.85 (*m*, 7 H, H_{ax}-C(4''), H_3C(9'') and H_3C(7')); 0.80 (*d*, *J* = 7.5 Hz, 3 H, H_3C(10'')).

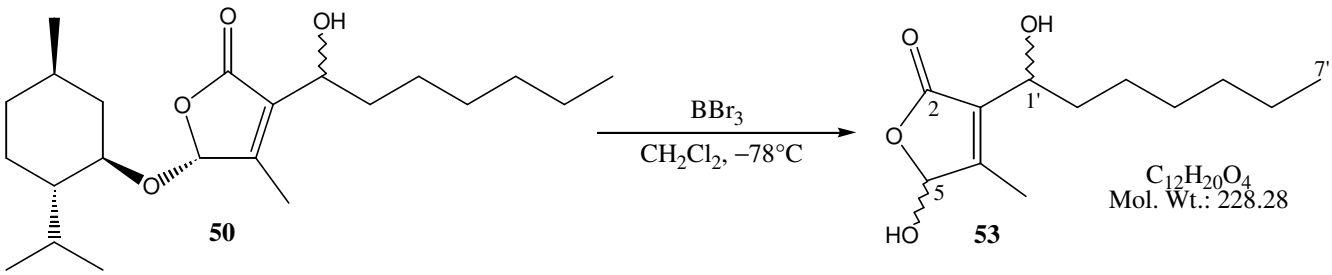
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 171.5 and 171.4 (C(2)); 155.4 (C(4)); 130.7 and 130.6 (C(3)); 101.9 and 101.0 (C(5)); 79.5 (C(1'')); 67.0 and 66.9 C(1')); 47.8 and 47.7 (C(2'')); 40.4 (C(6'')); 36.7 and 36.5 (C(2')); 34.2 (C(4'')); 31.7 (C(5')); 31.5 (C(5'')); 29.0 (C(4')); 25.5 (C(3')); 25.4 and 25.2 (C(8'')); 23.2 and 23.1 (C(3'')); 22.5 (C(6')); 22.3 (C(7'')); 20.9 and 20.8 (C(9'')); 15.9 and 15.7 (C(10'')); 14.1 (C(7')); 11.5 (CH$_3$-C(4)).

EI-MS (70 eV, 250°C): *m/z* (%) = 281 (11), 211 (14), 210 (12), 194 (33), 183 (33), 143 (45), 139 (80), 127 (10), 113 (28), 110 (25), 97 (24), 95 (34), 83 (100), 81 (50), 57 (38), 43 (63), 41 (56).

FAB-MS (NBA): *m/z* (%) = 368 (11), 367 ($[M + H]^+$, 46), 229 (9), 212 (11), 211 (81),139 (12), 137 (15), 97 (16), 95 (12), 83 (100), 81 (22), 69 (39), 57 (29), 55 (38), 43 (23), 41 (24).

Anal. calc. for $C_{22}H_{38}O_4$ (366.53): C, 72.09; H, 10.45; O, 17.46. Found: C, 72.00; H, 10.51; O, 17.28.

2. Synthesis of 5-hydroxy-3-(1-hydroxyheptyl)-4-methyl-5*H*-furan-2-one (**53**)



(5*R*)-3(1-Hydroxyheptyl)-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**50**, 4.0 g, 10.1 mmol) was dissolved in absolute CH_2Cl_2 (100 ml). The solution was cooled to −78°C and BBr_3 (3.1 ml, 32.7 mmol) was added. After a reaction time of 4 hours at −78°C, additional BBr_3 (3.1 ml, 32.7 mmol) was added at −78°C, the resulting mixture was allowed to warm up to −20°C and saturated $NaHCO_3$ solution (350 ml) was added. After warming up to r.t., the mixture was extracted EtOAc (3 × 500 ml). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated. The residue was chromatographed (SiO_2, 10 to 40% EtOAc in hexane) to give **53** as a mixture of two pairs of enantiomers.

Yield: 0.94g, 37%.

yellow oil, R_f 0.3 (EtOAc in hexane 50%).

IR (NaCl): 3370, 2929, 2857, 1747, 1683, 1456, 1386, 1336, 1181, 1123, 1091, 952, 774, 725.

^{1}H-NMR (400 MHz, $CDCl_3$, [1]): δ =5.87 (*d*, *J* = 6 Hz, 1 H, HO-C(5^A)); 5.84 (*d*, *J* = 6 Hz,1 H, 1 HO-C(5^B)); 5.67 (*d*, *J* = 6 Hz, 1 H, H-C(5^A)); 5.63 (*d*, *J* = 6 Hz, 1 H, H-C(5^B)); 4.52-4.41 (*m*, 2×1 H, H-C(1')); 3.47-3.38 (*m*, 1 H, HO-C($1'^A$)); 3.32-3.24 (*m*, 1 H, HO-C($1'^B$)); 2.07 (*d*, *J* = 11 Hz, 2×3 H, H_3C-C(4)); 1.88-1.75 (*m*, 2×1 H, H_A-C(2')); 1.72-1.60 (*m*, 2×1 H,

[1] where separate resonances for the two isomers could be observed, they are labelled with superscript A and B

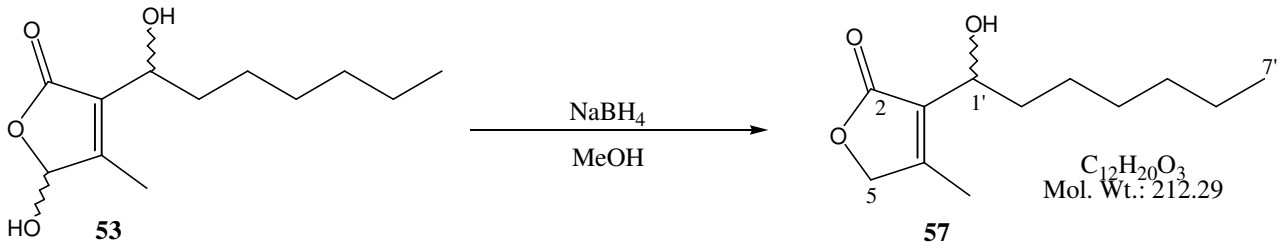
H_B-C(2')); 1.44-1.19 (*m*, 2×8 H, $H_2C(3')$, $H_2C(4')$, $H_2C(5')$ and $H_2C(6')$); 0.88 (*t*, *J* = 7 Hz, 2×3 H, $H_3C(7')$).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 172.1 and 171.7 (C(2)); 157.9 (C(4)); 130.4 and 129.9 (C(3)); 98.9 and 98.8 (C(5)); 66.7 and 66.6 (C(1')); 36.2 and 35.7 (C(2')); 31.7 (C(5')); 28.9 (C(4')); 25.4 (C(3')); 22.5 (C(6')); 14.0 (C(7')); 11.6 and 11.4 (CH_3-C(4)).

EI-MS (70 eV, 250°C): *m/z* (%) = 210 (9), 143 (100), 139 (43), 126 (49), 125 (53), 97 (74), 69 (45), 55 (28), 43 (80), 41 (86), 39 (38).

FAB-MS (NBA): *m/z* (%) = 229 ($[M + H]^+$, 100), 211 (90), 193 (17), 137 (36), 83 (20), 55 (19), 43 (29), 41 (20).

3. Synthesis of 3-(1-hydroxyheptyl)-4-methyl-5*H*-furan-2-one (**57**)



A solution of 5-hydroxy-3(1-hydroxyheptyl)-4-methyl-5*H*-furan-2-one (**53**, 1.0 g, 4.38 mmol) in absolute MeOH (40 ml) was cooled at 0°C. $NaBH_4$ (1.1g, 28.9 mmol) was added in portions within 10 min and the solution was stirred for 4 hours at r.t. Then the reaction mixture was quenched with H_2O (40 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The combined organic layers were dried ($MgSO_4$), and evaporated. The residue was chromatographed (SiO_2, 33% EtOAc in hexane) to give **57** as a mixture of enantiomers.

Yield: 707 mg, 76%.

colorless oil, R_f 0.3 (EtOAc in hexane 50%).

IR (NaCl): 3453, 2928, 2857, 1737, 1674, 1446, 1389, 1335, 1179, 1043, 940, 786.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.65 (*s*, 2 H, $H_2C(5)$); 4.49 (*dt*, *J* = 10, 8 Hz, 1 H, H-C(1')); 2.89 (*d*, *J* = 10 Hz, 1H, HO-C(1')); 2.08 (*s*, 3 H, H_3C-C(4)); 1.89-1.79 (*m*, 1 H, H_A-C(2')); 1.73-1.62 (*m*, 1 H, H_B-C(2')); 1.45-1.21 (*m*, 8 H, $H_2C(3')$, $H_2C(4')$, $H_2C(5')$ and $H_2C(6')$); 0.88 (*t*, *J* = 7 Hz, 3 H, H-C(7')).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 174 (C(2)); 157.2 (C(4)); 128.4 (C(3)); 72.7 (C(5)); 66.9 (C(1')); 36.6 (C(2')); 31.8 (C(5')); 29.0 (C(4')); 25.5 (C(3')); 22.5 (C(6')); 14.0 (C(7')); 12.2 (CH_3-C(4)).

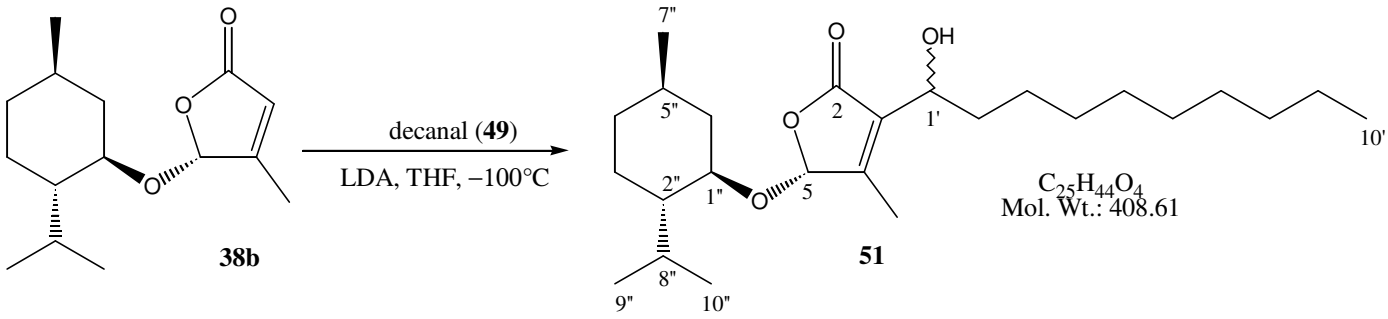
EI-MS (70 eV, 250°C): *m/z* (%) = 194 (7), 137 (11), 127 (100), 111 (31), 110 (37), 99 (30), 43 (25), 41 (44), 39 (22).

FAB-MS (NBA): *m/z* (%) = 214 (12), 213 ($[M + H]^+$, 95), 196 (13), 195 (100), 137 (22).

Anal. calc. for $C_{12}H_{20}O_3$ (212.29): C, 67.89; H, 9.50; O, 22.61. Found: C, 67.53; H, 9.47; O, 23.10.

IV Synthesis of 3-[1-hydroxydecyl]-4-methyl-5*H*-furan-2-one (58)

1. Synthesis of (5*R*)-3-(1-hydroxydecyl)-5-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methylfuran-2(5*H*)-one (**51**)



A solution of freshly distilled diisopropylamine (2.2 ml, 15 mmol) in freshly distilled THF (100 ml) was cooled to 0°C under argon. *n*-BuLi in hexane (10 ml, 15 mmol) was added dropwise over 30 min and the resulting solution was stirred for 30 min at 0°C. Then, the mixture was cooled to −100°C (ether in hexane 20%, liquid N_2) and a solution of (5*R*)-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one [14] (**38b**, 3 g, 12 mmol) in THF (10 ml) was added dropwise during 30 min and the resulting solution was stirred for 30 min at −100°C. Decanal (2.5 ml, 13 mmol) was added dropwise during 30 min and the mixture was stirred for further 30 min at −100°C. After a slow warm-up to −20°C, the reaction was quenched with saturated NH_4Cl solution (100 ml) and the mixture was extracted with CH_2Cl_2 (3 × 100 ml), washed with saturated $NaHCO_3$ solution (3 × 100 ml) and saturated NaCl solution (3 × 100 ml), dried ($MgSO_4$) and evaporated. The residue was chromatographed (SiO_2, CH_2Cl_2 in hexane 25%, with EtOAc from 0% to 10%) to afford **51** as a mixture of 2 diastereomers, which can be partially separated.

Yield: 1.88g, 37%.

Light yellow oil, R_f 0.36 (EtOAc in hexane 25%).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.70 (*s*, 1 H, H-C(5)); 4.47 (*q*, 1H, *J* = 7.5 Hz, 1 H, H-C(1')); 3.62 (*td*, *J* = 10, 3.2 Hz, 1 H, H-C(1'')); 3.13 (*br s*, 1 H, HO-C(1')); 2.15-2.09 (*m*, 2H, H-C(8'') and H_{eq}(6'')); 2.01 and 1.98 (2 *s*, 3 H, H_3C-C(4)); 1.84-1.76 (*m*, 1 H, H_A-C(2')); 1.71-1.63 (*m*, 3 H, H_B-C(2'), H_{eq}-C(3'') and H_{eq}-C(4'')); 1.46-1.37 (*m*, 2 H, H_A-C(3') and H-C(5'')); 1.33-1.22 (*m*, 14 H, H_B-C(3'), H-C(2''), H_2C(4'), H_2C(5'), H_2C(6'), H_2C(7'), H_2C(8') and H_2C(9')); 1.07-0.85 (*m*,12 H, H_{ax}-C(3''), H_{ax}-C(4''), H_{ax}-C(6''), H_3C(5'), H_3C(7'') and H_3C(9''); 0.808 (*d*, *J* = 7 Hz, 3 H, $H_3C(10''^A)$) and 0.802 (*d*, *J* = 7 Hz, 3 H, $H_3C(10''^B)$).

^{13}C-NMR (125 MHz, $CDCl_3$):
Resonances of isomer A:
δ = 171.38 (C(2)); 155.40 (C(4));130.71 (C(3)); 100.65 (C(5)); 79.40 (C(1'')); 66.82 (C(1')); 47.67 (C(2''); 40.35 (C(6'')); 36.43 (C(2')); 34.15 (C(4'')); 31.79 (C(8')); 31.39 (C(5'')); 29.48, 29.45, 29.29, 29.23 (C(4'), C(5'), C(6'), C(7')); 25.47 (C(3')); 25.10 (C(8'')); 23.02 (C(3''); 22.60 (C(9')); 22.19 C(7'')); 20.80 (C(9'')); 15.67 (C(10'')); 14.04 (C(10')); 11.44 (CH_3-C(4)).

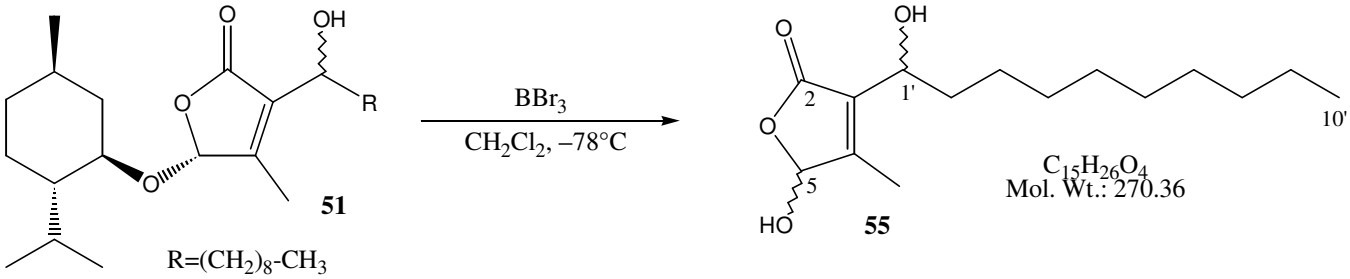
Resonances of isomer B:

δ = 171.43 (C(2)); 155.37 (C(4));130.56 (C(3)); 100.81 (C(5)); 79.45 (C(1'')); 66.91 (C(1')); 47.63 (C(2''); 40.38 (C(6'')); 36.57 (C(2')); 34.15 (C(4'')); 31.79 (C(8')); 31.39 (C(5'')); 29.45, 29.43, 29.26, 29.23 (C(4'), C(5'), C(6'), C(7')); 25.40 (C(3')); 25.28 (C(8'')); 23.15 (C(3'')); 22.60 (C(9')); 22.17 C(7'')); 20.75 (C(9'')); 15.81 (C(10'')); 14.04 (C(10')); 11.44 (*C*H_3-C(4)).

EI-MS (70 eV, 200°C): *m/z* (%) = 281 (43), 253 (11), 252 (10), 236 (20), 225 (20), 155 (15), 143 (80), 139 (100), 126 (13), 111 (14), 110 (13), 97 (15), 95 (22), 83 (84), 81 (37), 69 (41), 67 (12), 57 (33), 55 (44), 43 (33), 41 (29).

FAB-MS (NBA): *m/z* (%) = 409 ([*M* + H]$^+$, 29), 253 (54), 139 (11), 97 (22), 95 (18), 83 (100), 81 (28), 71 (13), 69 (48), 67 (17), 57 (43), 55 (53), 43 (33), 41 (35).

2. Synthesis of 5-hydroxy-3-(1-hydroxydecyl)-4-methyl-5*H*-furan-2-one (**55**)



(5*R*)-3(1-Hydroxydecyl)-5-{[(1*R*, 2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**51**, 440 mg, 1.08 mmol) was dissolved in absolute CH_2Cl_2 (20 ml). The solution was cooled to –78°C and BBr_3 (0.35 ml, 3.64 mmol) was added. After a reaction time of 4 hours at –78°C, additional BBr_3 (0.35 ml, 3.64 mmol) was added at –78°C, the resulting mixture was allowed to warm up to –20°C and saturated $NaHCO_3$ solution (50 ml) was added. After warming up to r.t., the mixture was extracted EtOAc (3 × 50 ml). The combined organic phases were dried ($MgSO_4$) and the solvent was evaporated. The residue was chromatographed (SiO_2, 5 to 45% EtOAc in hexane) to give **55** as a mixture of two pairs of enantiomers.

Yield: 180 mg, 62%.

Light yellow oil.

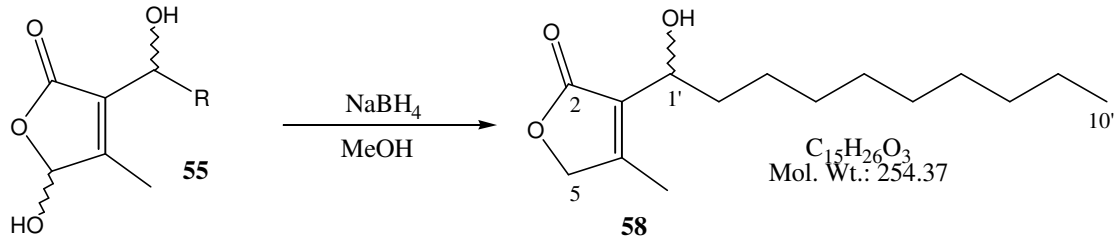
^{1}H-NMR (500 MHz, $CDCl_3$, [2]): δ = 6.17-6.12 (*m*, 1 H, HO-C(5)); 5.90-5.83 (*m*, 1 H, H-C(5)); 4.49 (*t*, *J* = 7 Hz, 1 H, H-C(1'^{A})) and 4.46 (*t*, *J* = 7 Hz, 1 H, H-C(1'^{B})); 3.63 (*br s*, 1 H, HO-C(1')); 2.08 and 2.06 (2 *s*, 3 H, H_3C-C(4')); 1.86-1.75 (*m*, 1 H, H_A-C(2')); 1.71-1.61 (*m*, 1 H, H_B-C(2')); 1.42-1.34 (*m*, 1 H, H_A-C(3')); 1.33-1.23 (*m*, 13 H, H_B-C(3'), H_2C(4'), H_2C(5'), H_2C(6'), H_2C(7'), H_2C(8') and H_2C(9')); 0.88 (*t*, *J* = 7 Hz, 3 H, H_3C(10')).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.82 and 171.39 (C(2)); 158.17 (C(4)); 129.76 and 129.41 (C(3)); 98.69 (C(5)); 66.10 and 65.89 (C(1')); 35.51 and 34.95 (C(2')); 31.42 (C(8')); 29.11 (2C), 28.93 and 28.84 (C(4'), C(5'), C(6'), and C(7')); 25.11 C(3')); 22.22 (C(9')); 13.66 (C(10')); 11.30 and 11.13 (CH$_3$C(4)).

EI-MS (70 eV, 100°C): *m/z* (%) = 155 (14), 153 (16), 143 (100), 139 (15), 126 (27), 125 (27), 115 (13), 97 (31), 86 (12), 71 (16), 69 (15), 57 (18), 55 (16), 43 (38), 41 (37).

FAB-MS (NBA): *m/z* (%) = 541 (5), 272 (16), 271 ([*M* + H]$^+$, 97), 253 (100), 235 (14), 139 (11), 137 (29), 123 (11), 115 (15), 111 (13), 97 (12), 95 (14), 83 (25), 81 (16), 77 (11), 69 (31), 67 (14), 57 (18), 55 (31), 43 (28), 41 (33), 39 (14).

3. Synthesis of 3-(1-hydroxydecyl)-4-methyl-5*H*-furan-2-one (**58**)



R=$(CH_2)_8$-CH_3

[2] where separate resonances for the two isomers could be observed, they are labelled with superscript A and B

A solution of 5-hydroxy-3(1-hydroxydecyl)-4-methyl-5*H*-furan-2-one (**55**, 290 mg, 1.07 mmol) in absolute MeOH (10 ml) was cooled at 0°C. $NaBH_4$ (300 mg, 7.9 mmol) was added in portions within 10 min and the solution was stirred for 3 hours at r.t. Then the reaction mixture was quenched with H_2O (10 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The combined organic layers were dried ($MgSO_4$), and evaporated to give **58** without further purification.

Yield: 202 mg, 74%.

white amorphous solid

IR (KBr): 3452, 2919, 2851, 2364, 1720, 1669, 1467, 1441, 1385, 1345, 1182, 1083, 1043, 996, 939, 874, 780, 701, 634, 546.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 4.66 (*br s*, 2 H, $H_2C(5)$); 4.49 (*q*, *J* = 7.5 Hz, 1 H, H-C(1')); 2.97 (*d*, *J* = 9 Hz, 1 H, HO-C(1')); 2.08 (*s*, 3 H, $H_3C(4)$); 1.88-1.80 (*m*, 1 H, H_A-C(2')); 1.71-1.67 (*m*, 1 H, H_B-C(2')); 1.44-1.35 (*m*, 1 H, H_A-C(3')); 1.35-1.23 (*m*, 13 H, H_B-C(3'), $H_2C(4')$, $H_2C(5')$, $H_2C(6')$, $H_2C(7')$, $H_2C(8')$, and $H_2C(9')$); 0.88 (*t*, *J* = 7 Hz, 3 H, $H_3C(10')$).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 174.0 (C(2)); 157.3 (C(4)); 128.2 (C(3)); 72.7 (C(5)); 66.8 (C(1')); 36.5 (C(2')); 31.8 (C(8')); 29.50, 29.47, 29.31, and 29.23 (C(4'), C(5'), C(6'), and C(7')); 25.5 (C(3')); 22.6 (C(9')); 14.1 (C(10')); 12.2 (CH$_3$-C(4)).

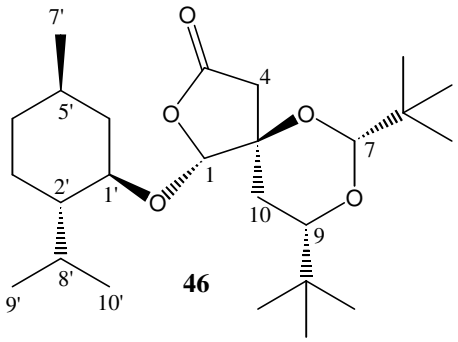
EI-MS (70 eV, 100°C): *m/z* (%) = 128 (9), 127 (100), 110 (8), 99 (14), 41 (12).

FAB-MS (NBA): *m/z* (%) = 256 (14), 255 ([*M* + H]$^+$, 84), 238 (17), 237 (100),167 (12), 153 (11), 137 (12), 125 (13), 95 (12), 83 (16), 81 (15), 69 (18), 67 (12), 57 (20), 55 (28), 43 (26), 41 (25).

Anal. calc. for $C_{15}H_{26}O_3$ (254.37): C, 70.83; H, 10.30; O, 18.87. Found: C, 70.75; H, 10.05; O, 18.87.

V Miscellaneous

1. (1*R*, 5*R*, 7*R*, 9*R*)-7,9-di-*tert*-butyl-1-{[(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-2,6,8-trioxa-spiro[4.5]decan-3-one (**46**)



46

$C_{25}H_{44}O_5$
Mol. Wt.: 424.61

colorless plates, Mp 162.3-162.7°C.

IR (KBr): 2959, 1787, 1459, 1369, 1291, 1181, 1115, 1041, 996, 948, 895.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.74 (*s*, 1 H, H-C(1)); 4.36 (*s*, 1 H, H-C(7)); 3.58 (*td*, *J* = 11, 4.5 Hz, 1 H, H-C(1')); 3.08 (*dd*, *J* = 12, 2 Hz, 1 H, H-C(9)); 2.58 (*d*, *J* = 17.5 Hz, 1 H, H_A-C(4)); 2.44 (*d*, *J* = 17.5 Hz, 1 H, H_B-C(4)); 2.17-2.12 (*m*, 1 H, H_{eq}-C(6')); 1.98 (*dd*, *J* = 13, 2 Hz, 1 H, H_A-C(10)); 2.00-1.92 (*m*, 1 H, H-C(7)); 1.72-1.62 (*m*, 2 H, H_{eq}-C(3') and H_{eq}-C(4')); 1.50 (*dd*, *J* = 13, 12 Hz, 1 H, H_B-C(10)); 1.46-1.38 (*m*, 1 H, H-C(5')); 1.22-1.16 (*m*, 1 H, H-C(2')); 1.06-0.99 (*m*, 1 H, H_{ax}-C(3')); 0.97 (*d*, *J* = 7 Hz, 3 H, H_3C(7')); 0.92-0.83 (*m*, 2 H, H_{ax}-C(4') and H_{ax}-C(6')); 0.89 (*s*, 9 H, $(CH_3)_3$C(7)); 0.88 (*s*, 9 H, $(CH_3)_3$C(9)); 0.87 (*d*, *J* = 7 Hz, 3 H, H_3C(9')); 0.77 (*d*, *J* = 7 Hz, 3 H, H_3C(10')).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 175.0 (C(3)); 102.5 (C(7)); 99.8 (C(1)); 80.4 (C(9)); 79.4 (C(5)); 77.04 (C(1')); 47.9 (C(2')); 41.5 (C(4)); 40.1 (C(6')); 35.1 ($(CH_3)_3$*C*-C(9)); 34.2 (C(4')); 33.9 ($(CH_3)_3$*C*-C(7)); 31.3 (C(5')); 27.3 (C(10)); 25.65 (C(8')); 25.39 (3 C, (CH_3)$_3$C-C(7)); 24.4 (3 C, (CH_3)$_3$C-C(9)); 23.0 C(3')); 22.2 (C(7')); 20.9 (C(9')); 15.6 (C(10')).

EI-MS (70 eV, 200°C): *m/z* (%) = 229 (22), 183 (12), 165 (13),156 (13), 155 (>100), 141 (13), 139 (18), 127 (14), 126 (17), 111 (38), 109 (10), 97 (14), 95 (14), 84 (13), 83 (71), 81 (16), 70 (10), 69 (34), 57 (39), 55 (28), 43 (22), 41 (34).

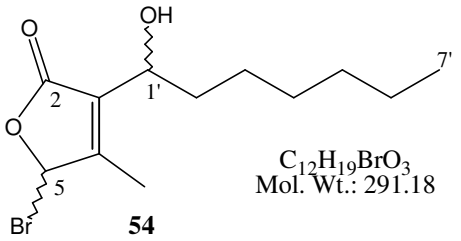
FAB-MS (NBA): *m/z* (%) = 426 (4), 425 ([*M* + H]$^+$, 15), 287 (12), 201 (22), 183 (28), 165 (12), 141 (30), 139 (26), 137 (24), 99 (12), 97 (14), 95 (18), 83 (100), 81 (25), 70 (10), 69 (47), 67 (11), 57 (55), 55 (34), 43 (21), 41 (35), 39 (12).

Anal. calc. for $C_{25}H_{44}O_5$ (424.61): C, 70.72; H, 10.44; O, 18.84. Found: C, 70.83; H, 10.20; O, 18.86.

X-ray crystal data

Formula:	$C_{25}H_{44}NO_5$
Mol. Weight:	424.62
Crystal system:	tetragonal
Space group:	P 4_3 2_1 2
a [Å]	11.1355(2)
b [Å]	11.1355(2)
c [Å]	43.9984(7)
α [°]	90
β [°]	90
γ [°]	90
Z:	8
V[Å^3]:	5455.8
F(000):	1872.519
Density [Mg m^{-3}]:	1.034
μ [mm^{-1}]:	0.070
Crystal size [mm]:	0.15 × 0.27 × 0.30
Temperature [K]:	293
Radiation:	MoK$_\alpha$ (λ = 0.71073 Å)
Θ_{max} [°]	23.30
No of measured reflections:	19582
No of independant reflections:	3938
No of observed reflections:	2540
No of refined parameters:	272
Final *R*:	0.0335
Final *Rw*:	0.0395

2. Data of 5-bromo-3-(1-hydroxyheptyl)-4-methyl-5*H*-furan-2-one (**54**)



$C_{12}H_{19}BrO_3$
Mol. Wt.: 291.18

54

Yellow oil, sensitive to air moisture, R_f 0.6 (EtOAc in hexane 50%).

IR (NaCl): 3396, 2955, 2928, 2857, 1764, 1674, 1457, 1386, 1335, 1193, 1092, 1006, 951, 779.

^{1}H-NMR (400 MHz, $CDCl_3$, [3]): δ = 5.88 (*s*, 2×1 H, H-C(5)); 4.72 (*d*, *J* = 7 Hz, 1 H, H-C(1'^{A})); 4.70 (*d*, *J* = 7 Hz, 1 H, HO-C(1'^{B}); 4.56 (*m*, 2×1 H, H-C(1')); 2.13 (*s*, 3 H, H_3C-C(4^A)); 2.12 (*s*, 3 H, H_3C-C(4^B)); 1.91-1.80 (*m*, 2×1 H, H_A-C(2)), 1.77-1.63 (*m*, 2×1 H, H_B-C(2)); 1.50-1.21 (*m*, 2×8 H, H_2C(3'), H_2C(4'), H_2C(5') and H_2C(6')); 0.88 (*t*, *J* = 7 Hz, 2×3 H, H_3C(7')).

^{13}C-NMR (100 MHz, $CDCl_3$): δ =169.9 (C(2); 159.0 (C4)); 129.5 (C(3)); 98.0 (C(5)); 66.9 (C(1')); 35.9 (C(2')); 31.6 (C(5')); 28.4 (C(4')); 25.4 (C(3')); 22.5 (C(6')); 14.0 (C(7')); 12.0 (*C*H_3-C(4)).

EI-MS (70 eV, 250°C): *m/z* (%) = 211 (16), 210 (18), 194 (20), 153 (25), 140 (26), 139 (74), 111 (100), 110 (99), 67 (46), 55 (43), 43 (53), 41 (94), 39 (47).

FAB-MS (NBA): *m/z* (%) = 293 (38), 291 ([*M* + H]$^+$, 38), 275 (21), 273 (21), 211 (78), 193 (100), 137 (96), 136 (36), 107 (35), 83 (40), 77 (40), 55 (58), 43 (59), 41 (63), 39 (43).

[3] where separate resonances for the two isomers could be observed, they are labelled with superscript A and B

Part Two

Synthesis of the putative chitinase inhibitors

I Preparation of the starting compounds 83 and 93

1. Synthesis of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-glucopyranose (**83**)

$C_{26}H_{36}O_{18}$
Mol. Wt.: 636.55

The preparation was carried out as described in the literature [42].

Amorphous white solid

IR (KBr): 3485, 2963, 2365, 1751, 1639, 1436, 1374, 1232, 1164, 1042, 906, 601.

EI-MS (70 eV, 200°C): *m/z* (%) = 331 (9), 169 (27), 157 (8), 115 (8), 109 (14), 97 (17), 43 (100).

FAB-MS (NBA): *m/z* (%) = 331 (32), 169 (63), 109 (35), 81 (11), 43 (100).

NMR data of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranose (**83α**)[4]

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.50 (*dd*, *J* = 10, 9.5 Hz, 1 H, H-C(3)); 5.37 (*pseudo-t*, *J* = 3.5 Hz, 1 H, H-C(1)); 5.15 (*pseudo-t, J* = 9.5 Hz, 1 H, H-C(3')); 5.08 (*pseudo-t*, *J* = 9.5 Hz, 1 H, H-C(4')); 4.94 (*dd*, *J* = 9.5, 8 Hz, 1 H, H-C(2')); 4.85-4.78 (*m*, 1 H, H-C(2)); 4.56-4.49 (*m*, 3 H, H-C(1') and $H_2C(6)$); 4.17 (*ddd*, *J* = 10, 4, 2 Hz, 1 H, H-C(5)); 4.14-4.09 (*m*, 2 H, $H_2C(6')$); 3.90-3.73 (*m*, 1 H, H-C(4)); 3.70-3.63 (*m*, 1 H, H-C(5')); 3.52 (*d*, *J* = 3 Hz, 1 H, HO-C(1)); 2.14, 2.094, 2.079, 2.036, 2.032, 2.013, 1.99 (7*s*, 21 H, CH_3CO).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.56, 170.44, 170.32, 170.27, 169.67, 169.30, 169.02 (7 C, $CH_3\mathit{C}O$); 100.63 (C(1')); 90.01 (C(1)); 76.47 (C(4)); 72.93 (C(3')); 71.85 (C(5')); 71.59 (C(2')); 71.18 (C(2)); 69.24 (C(3)); 68.18 (C(5)); 67.75 (C(4')); 61.70, 61.53 (C(6) and C(6')); 20.9-20.4 (7 C, $\mathit{C}H_3CO$).

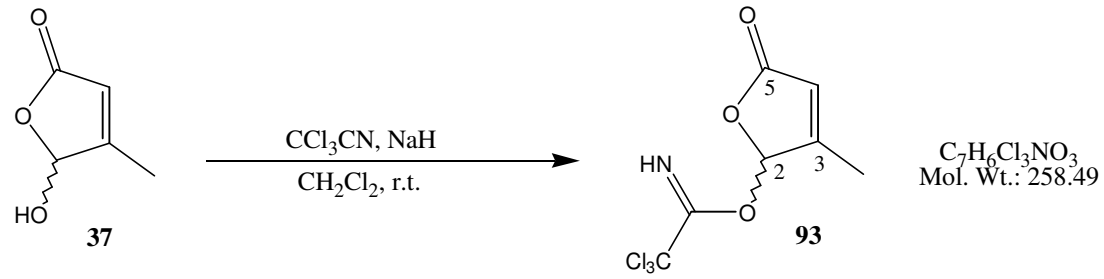
NMR data of 2,3,6-tri-O-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranose (**83β**)[4]

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.22 (*pseudo-t*, *J* = 9.5 Hz, 1 H, H-C(3)); 5.15 (*pseudo-t*, *J* = 9.5 Hz, 1 H, H-C(3')); 5.07 (*pseudo-t*, *J* = 9.5 Hz, 1 H, H-C(4')); 4.93 (*dd*, *J* = 9.5, 8 Hz, 1 H, H-C(2')); 4.85-4.78 (*m*, 1 H, H-C(2)); 4.73 (*pseudo-t*, *J* = 8 Hz, 1 H, H-C(1)); 4.56-4.49 (*m*, 1 H, H-C(1')); 4.40-4.35 (*m*, 2 H, $H_2C(6)$); 4.08-4.02 (*m*, 2 H, $H_2C(6')$); 3.84 (*d*, *J* = 8 Hz, 1 H, HO-C(1)); 3.80-3.72 (*m*, 1 H, H-C(4)); 3.70-3.63 (*m*, 2 H, H-C(5) and H-C(5')); 2.15-1.98 (7 *s*, 21 H, CH_3CO).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.9-169.0 (7 C, $CH_3\mathit{C}O$); 100.75 (C(1')); 95.2 (C(1)); 76.43 (C(4)); 73.28 (C(2)); 72.93 (C(5)); 72.86 (C(3')); 71.90, 71.78 (C(3) and C(5')); 71.53 (C(2')); 67.69 (C(4')); 61.86, 61.49 (C(6) and C(6')); 20.9-20.4 (7 C, $\mathit{C}H_3CO$).

[4] Data derived from spectra of the anomer mixture

2. Synthesis of 3-methyl-5-oxo-2,5-dihydrofuran-2-yl 2,2,2-trichloroacetimidate (**93**)



37 **93** $C_7H_6Cl_3NO_3$ Mol. Wt.: 258.49

To a suspension of NaH (498 mg of 55% dispersion in oil, 11 mmol) in CH_2Cl_2 (40 ml),were added slowly **37** [13] (9.9 g, 87 mmol) and trichloroacetonitrile (10 ml, 100 mmol). The mixture was stirred for 4 h at r.t. and then filtered. The filtrate was evaporated, and the residue was chromatographed on silica gel (EtOAc-hexane, 25:75) to give **93**.

Yield: 14.1 g (63%)

Colorless amorphous solid, R_f 0.55 (AcOEt/hexane 1/1), sensitive to moisture.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.90 (*br s*, 1 H, HN=C); 6.96 (*br s*, 1 H, H-C(2)); 6.04-6.02 (*qd*, *J* = 1.6,1 Hz, 1 H, H-C(4)); 2.18 (*dd*, *J* = 1.6, 0.7 Hz, 3 H, H_3C-C(3)).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 169.7 (C=O); 162.4 (C(3)); 160.8 (C=NH), 119.7 (C(4)); 97.7 (C(2)); 90.0 (CCl_3); 13.3 (CH_3-C(3)).

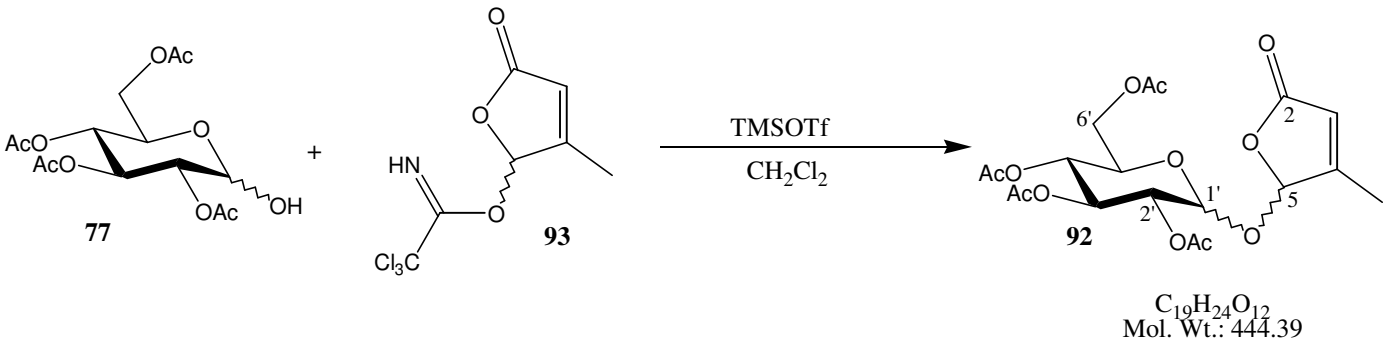
EI-MS (70 eV, 100°C): *m/z* (%) = 232 (1.5), 230 (4), 228 (4), 110 (12), 108 (18), 97 (100), 69 (16), 68 (13), 41 (28), 40 (11), 39 (17).

FAB-MS (NBA): *m/z* (%) = 262 (1.6), 260 (5), 258 ($[M + H]^+$, 5), 250 (14), 137 (14), 136 (12), 107 (7), 98 (14), 97 (100), 89 (7), 77 (8), 41 (8), 39 (7).

Anal. calc. for $C_7H_6Cl_3NO_3$ (258.49): C 32.53, H 2.34, N 5.42; found: C 32.56, H 2.48, N 5.47.

II Synthesis of the glycosylated furanones 92, 96, 97, 101 and 102

1. Synthesis of 4-methyl-5-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyloxy)-5*H*-furan-2-one (**92**)



2,3,4,6-Tetra-*O*-acetyl-D-glucopyranose [38] (**77**; 8.16 g, 23 mmol) and **93** (8.5 g, 33 mmol) were dissolved at r.t. in anhydrous CH_2Cl_2 (150 ml). TMSOTf (5.0 ml, 28 mmol) was added and the mixture was stirred for 5 hours at r.t. Then it was washed with H_2O (30 ml), dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was chromatographed (SiO_2, 0 to 5% MeOH in CH_2Cl_2) to give **92** as a mixture of 4 diastereomers.

Yield: 7.41 g (70%)

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.6-168.7 (C=O); 163.65, 163.36, 162.35, 161.88 (C(4)); 119.9-118.9 (C(3)); 103.5-88.9 (C(5) and C(1')); 72.5-67.7 (C(2'), C(3'), C(4'), and C(5')); 61.7-61.3 (C(6')); 20.7-20.3 (CH$_3$-CO); 13.32, 13.29, 13.21, 12.99 (CH$_3$-C(4)).

Isolation of one of the β-isomers: from some fractions of the chromatography of **92**.

4-methyl-5-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-5*H*-furan-2-one.

White amorphous solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.97 (*br s*, 1 H, H-C(5)); 5.89 (*qd J* = 1.5, 1 Hz, 1 H, H-C(3)); 5.24 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(3')); 5.12 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(4')); 5.05 (*dd*, *J* = 10, 8 Hz, 1 H, H-C(2')); 4.94 (*d*, *J* = 8 Hz, 1 H, H-C(1')); 4.27 (*dd*, *J* = 12.5, 5 Hz, 1 H, H_A-C(6')); 4.19 (*dd*, *J* = 12.5, 2.5 Hz, 1 H, H_B-C(6')); 3.79 (*ddd*, *J* = 12.5, 5, 2.5 Hz, 1 H, H-C(5')); 2.10 (*s*, 3 H, H_3C-CO); 2.08 (*dd*, *J* = 1.5, 0.5 Hz, 3 H, H_3C-C(4)); 2.05, 2.03, 2.00 (3 *s*, 9 H, H_3C-CO).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.5, 170.2, 170.0, 169.44, 169.41 (C=O); 163.4 (C(4)), 119.0 (C(3)); 99.5 (C(5)); 98.1 (C(1')); 72.49 (C(3')); 72.35 (C(5')); 70.6 (C(2')); 68.1 (C(4')); 61.7 (C(6')); 20.70, 20.59, 20.55, 20.54 (CH$_3$-CO); 13.3 (CH$_3$-C(4)).

EI-MS (70 eV, 350 °C): *m/z* (%) = 347 (4), 331 (4), 259 (13), 245 (20), 169(7), 157(21), 139 (28), 115 (8), 98 (26), 97 (73), 69 (7), 43 (100), 41 (10).

FAB-MS (NBA): *m/z* (%) = 445 ($[M + H]^+$, 8), 331 (48), 169 (76), 137 (27), 136 (15), 127 (12), 109 (41), 107 (10), 97 (38), 89 (12), 77 (14), 43 (100), 41 (12), 39 (11).

2. Synthesis of 5-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one (**96a-d**)

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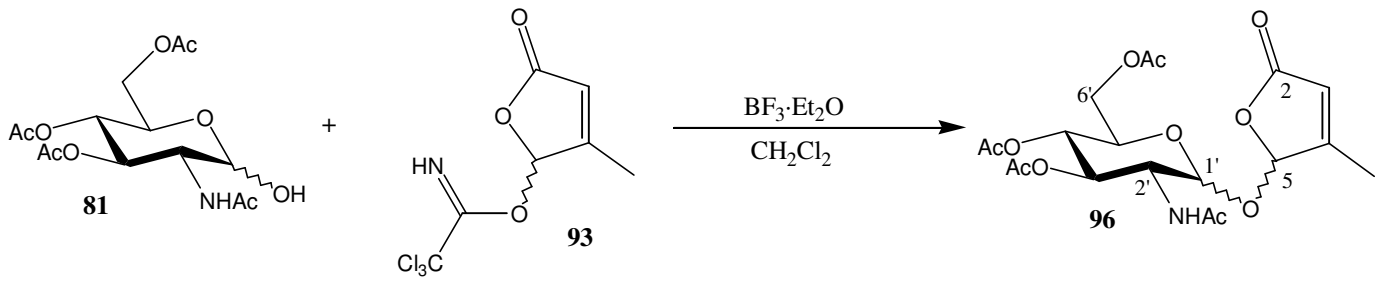
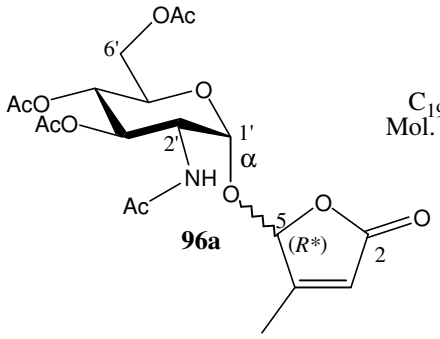
2.1 Synthesis and separation of the isomers



2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose [40][5] (**81**, 3.41 g, 9.8 mmol) and 5-trichloroacetimidate-4-methyl-5*H*-furan-2-one (**93**; 5.25 g, 20 mmol) were dissolved at r.t. in anhydrous CH_2Cl_2 (300 ml). TMSOTf (3.4 ml, 19 mmol) was added and the mixture was stirred overnight at r.t. Then it was washed with H_2O (50 ml), dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was chromatographed (200 mm × 55 mm i.d., SiO_2, 0-3% MeOH in CH_2Cl_2). Fractions containing the same isomer according to TLC were pooled to give **96a** (265 mg, 6%), **96b** (123 mg, 3%), **96c** (630 mg, 14%), and **96d** (160 mg, 4%); the remaining fractions contained mixtures of isomers (678 mg, 16%).

[5] The hydrolysis of the chloride was carried out using $NaHCO_3$ instead of Ag_2CO_3 giving the same yield.

*2.2 Data of (5*R**)-5-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one (**96a**)*



96a

$C_{19}H_{25}NO_{11}$
Mol. Wt.: 443.40

light brown amorphous solid, R_f 0.39 (5% MeOH in CH_2Cl_2)

$[\alpha]_D^{20}$: +92.2 (589 nm), +96.3 (578 nm), 108.8 (546 nm), +181.7 (436 nm) (*c* = 1.09 CH_2Cl_2).

IR (KBr): 3363, 2958, 1750, 1674, 1538, 1441, 1375, 1232, 1128, 1047, 964, 892.

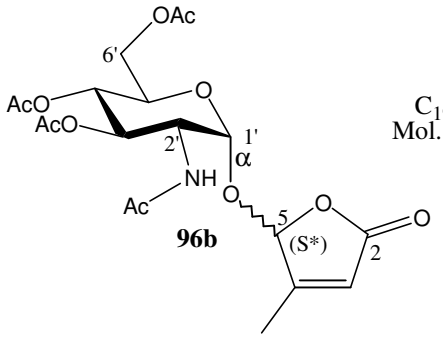
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.00-5.99 (*m*, 1H, H-C(3)); 5.89 (*s*, 1 H, H-C(5)); 5.66 (*d*, *J* = 9.5 Hz, 1 H, H-N); 5.29 (*d*, *J* = 3.5 Hz, 1 H, H-C(1')); 5.20-5.14 (*m*, 2 H, H-C(3') and H-C(4')); 4.46 (*m*, 1 H, H-C(2')); 4.24 (*dd*, *J* = 12.5, 5 Hz, 1 H, H_A-C(6')); 4.15 (*dd*, *J* = 12.5, 2.5 Hz, 1 H, H_B-C(6')); 4.06 (*ddd*, *J* = 9.5, 5, 2.5 Hz, 1 H, H-C(5')); 2.14-2.13 (*br s*, 3 H, H_3C-C(4)); 2.107, 2.051, 2.037, 1.972 (4 *s*, 12 H, H_3C-CO).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.28, 170.55, 170.29, 169.67, 169.14 (C=O); 162.0 (C(4)); 120.0 (C(3)); 100.3 (C(5)); 97.0 (C(1')); 70.3 (C(3')); 69.0 (C(5')); 67.9 (C(4')); 61.9 (C(6')); 51.2 (C(2')); 23.09, 20.66, 20.64, 20.54 (*C*H_3-CO); 13.4 (*C*H_3-C(4)).

EI-MS (70 eV, 200°C): *m/z* (%) = 346 (8), 330 (5), 318 (22), 304 (18), 286 (26), 259 (43), 244 (36), 226 (14), 198 (14), 184 (27), 168 (16), 166 (40), 157 (18), 156 (88), 150 (16), 142 (17), 140 (10), 139 (98), 138 (44), 126 (12), 114 (56), 101 (12), 98 (16), 97 (100), 96 (50), 84 (10), 69 (14), 60 (10), 43 (79), 41 (12).

FAB-MS (NBA): *m/z* (%) = 444 ($[M + H]^+$, 36), 330 (63), 270 (14), 210 (38), 168 (43), 150 (72), 126 (22), 108 (30), 97 (55), 43 (100).

*2.3 Data of (5*S**)-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one (**96b**)*



96b

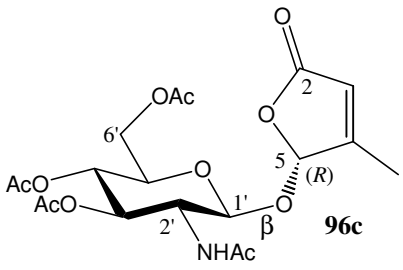
$C_{19}H_{25}NO_{11}$
Mol. Wt.: 443.40

Amorphous solid, containing some **96a** and **96c**; R_f 0.35 (5% MeOH in CH_2Cl_2).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.96 (*d*, *J* = 9.5 Hz, 1 H, H-N); 5.93 (*dd*, *J* = 1.6, 1.0 Hz, 1 H, H-C(3)); 5.77 (*s*, 1 H, H-C(5)); 5.26 (*d*, *J* = 3.5 Hz, 1 H, H-C(1')); 5.18-5.06 (*m*, 2 H, H-C(3') and H-C(5')); 4.29 (*ddd*, *J* = 10.6, 8.5, 3.7 Hz, 1 H, H-C(2')); 4.24-4.19 (*m*, 1 H, H-C(4')); 4.18-4.09 (*m*, 2 H, H_2C(6')); 2.093, 2.065, 2.014, 2.011, 1.937 (5 *s*, 15 H, CH_3).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.61, 170.70, 170.12, 169.71, 169.12 (C=O); 162.3 (C(4)); 119.6 (C(3)); 103.1 (C(5)); 97.9 (C(1')); 70.4 (C(3')); 69.0 (C(4')); 67.4 (C(5')); 61.5 (C(6')); 52.3 (C(2')); 22.87, 20.6 (2 C), 20.5 (CH$_3$CO); 13.2 (CH$_3$-C(4)).

*2.4 Data of (5*R*)-5-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one (**96c**)*



96c

$C_{19}H_{25}NO_{11}$
Mol. Wt.: 443.40

Colorless needles; Mp 162°C; R_f 0.30 (5% MeOH in CH_2Cl_2).

$[\alpha]_D^{20}$: –43.1 (589 nm), –44.9 (578 nm), –51.1 (546 nm), –88.3 (436 nm) (*c*=1.07 CH_2Cl_2).

IR (KBr): 3271, 3108, 2958, 2364, 2345, 1797, 1747, 1676, 1654, 1560, 1437, 1369, 1295, 1224, 1127, 1082, 1034, 967, 888 cm^{-1}.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.99 (*br s*, 1 H, H-C(5)); 5.90-5.85 (*br s*, 1 H, H-N); 5.88 (*qd*, *J* = 1.5, 1 Hz, 1 H, H-C(3)); 5.20 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(3')); 5.12 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(4')); 4.96 (*d*, *J* = 8.5 Hz, 1 H, H-C(1')); 4.26 (*dd*, *J* = 12.5, 4.5 Hz, 1 H, H_A-C(6')); 4.21 (*dd, J* = 12.5, 2.5 Hz, 1 H, H_B-C(6')); 4.15 (*ddd*, *J* = 10.5, 9.4, 8.6 Hz, 1 H, H-C(2')); 3.80 (*ddd*, *J* = 10, 4.5, 2.5 Hz, 1 H, H-C(5')); 2.11 (*s*, 3 H, H_3C-CO-O-C(6')); 2.09 (*dd*, *J* = 1.6, 0.6 Hz, 3 H, H_3C-C(4)); 2.04 (2 *s*, 6 H, H_3C-CO); 1.94 (*s*, 3 H, *H_3*C-CO-NH).

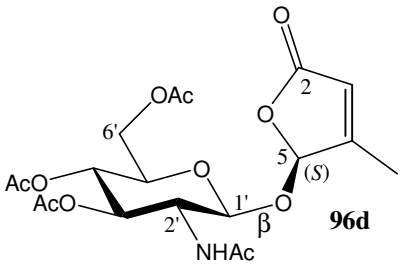
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.95, 170.62, 170.47, 170.43, 169.3 (C=O); 163.9 (C(4)); 118.8 (C(3)); 99.9 (C(5)); 98.7 (C(1')); 72.42 (C(3')); 72.36 (C(5')); 68.2 (C(4')); 61.8 (C(6')); 53.6 (C(2')); 23.2 (*C*H_3-CO-NH); 20.72, 20.61, 20.58 (*C*H_3-CO); 13,4 (*C*H_3-C(4)).

EI-MS (70 eV, 400°C): *m/z* (%) = 346 (3), 318 (5), 304 (6), 286 (7), 259 (10), 244 (11), 184 (7),166 (17), 156 (31), 139 (31),138 (17), 114 (22), 97 (54), 96 (25), 84 (9), 69 (9), 43 (100), 41 (14).

FAB-MS (NBA): *m/z* (%) = 444 ($[M + H]^+$, 21), 330 (64), 210 (19), 168 (32), 150 (45), 136 (17), 126 (17), 108 (25), 97 (48), 90 (12), 89 (15), 77 (17), 73 (37), 63 (9), 51 (12), 43 (100), 41 (11), 39 (15).

Anal. calc. for $C_{19}H_{25}NO_{11}$ (443.40): C 51.47, H 5.68, N 3.16, O 39.69; found: C 51.27, H 5.56, N 3.00, O 39.66.

*2.5 Data of (5*S*)-5-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one (**96d**)*



96d

$C_{19}H_{25}NO_{11}$
Mol. Wt.: 443.40

Colorless blocks; Mp 168-169°C; R_f 0.23 (5% MeOH in CH_2Cl_2)

$[\alpha]_D^{20}$: +15.1 (589 nm), +15.9 (578 nm), +17.9 (546 nm), +30.4 (436 nm) (*c* = 1.01 CH_2Cl_2).

IR (KBr): 3356, 3116, 2958, 1748, 1698, 1660, 1534, 1436, 1375, 1245, 1078, 1042, 967, 889 cm^{-1}.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.69 (*d*, *J* = 9 Hz, 1 H, H-N); 5.97 (*s*, 1 H, H-C(5)); 5.91 (*qd*, *J* = 1.5, 1 Hz, 1 H, H-C(3)); 5.35 (*dd*, *J* = 10.5, 9.5 Hz, 1 H, H-C(3')); 5.03 (*dd*, *J* = 10, 9.5 Hz, 1 H, H-C(4')); 4.98 (*d*, *J* = 10 Hz, 1 H, H-C(1')); 4.17-4.15 (*m*, 2 H, H-C(6')); 4.01 (*pseudo-dt*, *J* = 10.5, 8.5 Hz, 1 H, H-C(2')); 3.81 (*ddd*, *J* = 10, 4.5, 3 Hz, 1 H, H-C(5')); 2.14 (*dd*, *J* = 1.6, 0.6 Hz, 3 H, H_3C-C(4)); 2.09, 2.03, 2.02, 2.01 (4 *s*, 12 H, H_3C-CO).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.9, 170.52, 170.44, 170.10, 169.3 (C=O); 164.5 (C(4)); 119.2 (C(3)); 101.7 (C(5)); 95.8 (C(1')); 71.94, 71.86 (C(3') and *C*(5')); 68.4 (C(4')); 61.9 (C(6')); 54.0 (C(2')); 23.1, 20.59, 20.53, 20.46 (*C*H_3-CO); 13.5 (*C*H_3-C(4)).

EI-MS (70 eV, 200°C): *m/z* (%) = 346 (4), 318 (11),304 (17), 286 (16), 259 (31), 244 (28), 184 (22), 166 (41), 156 (72), 139 (80), 114 (49), 97 (100), 43 (74).

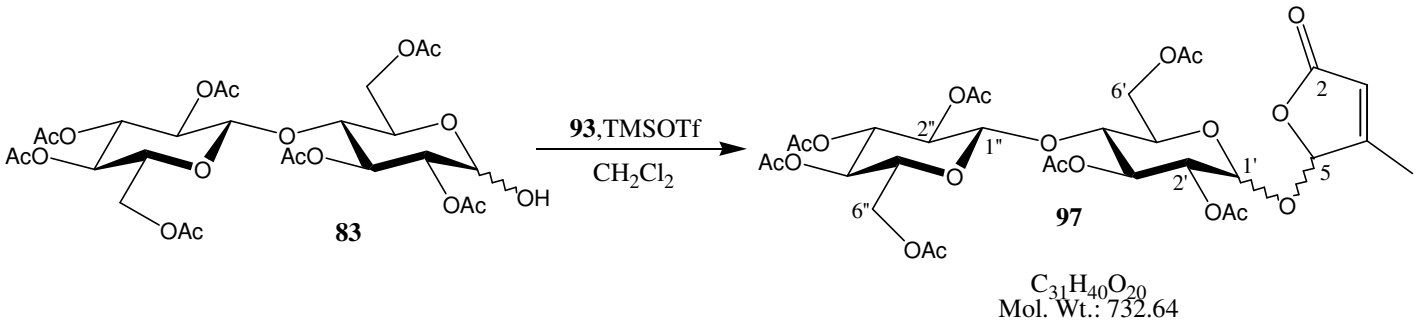
FAB-MS (NBA): *m/z* (%) = 445 (7.2), 444 ([*M* + H]$^+$, 30), 330 (79), 270 (7), 210 (25), 168 (36), 150 (51), 126 (19), 108 (23), 97 (48), 43 (100).

Anal. calc. for $C_{19}H_{25}NO_{11}$ (443.40): C 51.47, H 5.68, N 3.16, O 39.69; found: C 51.20, H 5.62, N 3.24, O 39.67.

X-ray crystal data

Formula:	$C_{19}H_{25}NO_{11}$
Mol. Weight:	443.42
Crystal system:	monoclinic
Space group:	P 2_1
a [Å]	9.5269(4)
b [Å]	9.7034(9)
c [Å]	11.8179(7)
α [°]	90
β [°]	98.059(5)
γ [°]	90
Z:	2
V[Å^3]:	108170(13)
F(000):	468
Density [Mg m^{-3}]:	1.361
μ [mm^{-1}]:	0.113
Crystal size [mm]:	$0.18 \times 0.20 \times 22$
Temperature [K]:	173
Radiation:	MoK_α (λ = 0.71073 Å)
Θ_{max} [°]	31.996
No of measured reflections:	66999
No of independant reflections:	3954
No of reflections in refinement:	3153
No of variables:	281
Final *R*:	0.0407
Final *Rw*:	0.0598

3. Synthesis of 4-methyl-5-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-glucopyranosyloxy]-5*H*-furan-2-one(**97**)



$C_{31}H_{40}O_{20}$
Mol. Wt.: 732.64

3.1 Preparation

To a solution of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6–tetra-*O*-acetyl-β-D-glucopyranosyl)-D-glucopyranose (1.774 g, 2.8 mmol) and 3-methyl-5-oxo-2,5-dihydrofuran-2-yl 2,2,2-trichloroacetimidate **93** (2.496 g, 9.7 mmol) in CH_2Cl_2 (100 ml), was added TMSOTf (0.8 ml, 4.4 mmol). The mixture was stirred overnight at r.t., then H_2O (30 ml) was added. The organic phase was dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was flash-chromatographed (SiO_2, gradient of EtOAc-hexane, 25:75 to EtOAc) to give **97** (1.30 g, 64%) as a mixture of 4 diastereomers.

3.2 Data of the mixture of all isomers

Colorless amorphous solid; R_f 0.5-0.6 (EtOAc).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.3-168.8 (C=O); 163.44, 163.16, 162.29, 162.01 (C(4)); 119.79, 119.46, 119.29, 118.92 (C(3)); 103.64, 101.99, 100.76, 100.65, 100.53, 100,49, 100.36, 99.70, 97.98, 97.19, 96.77, 94.6 (C(5), C(1') and C(1'')); 76.34, 75.98, 75.93, 75.87 (C(4')); 73.1-68.8 (C(2'), C(3'), C(5'), C(2''), C(3''), C(5'')); 67.63, 67.61, 67.58, 67.53 (C(4'')); 61.5-61.2 (C(6') and C(6'')); 20.9-20.3 (CH$_3$-CO); 13.35, 13.22, 13.10, 12.96 (CH$_3$-C(4)).

EI-MS (70 eV, 300°C): *m/z* (%) = 385 (29), 331 (33), 317 (13), 271 (6), 229 (6), 169 (100), 109 (36), 97 (52), 43 (52).

FAB-MS (NBA): *m/z* (%) = 733 ($[M + H]^+$, 0.9), 619 (5), 331 (17), 169 (49), 127 (9), 109 (31), 97 (15), 43 (100).

*3.3 Data of 4-methyl-5-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6–tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranosyloxy]-4-methyl-5*H*-furan-2-one.*

White amorphous solid; R_f 0.54 (EtOAc).

$[\alpha]_D^{20}$: +43.4 (589 nm), +45.4 (578 nm), +51.0 (546 nm), +83.1 (436 nm), +120.8 (365 nm) (*c* = 1.06 CH_2Cl_2).

IR (KBr): 3482, 2962, 1753, 1658, 1439, 1374, 1230, 1154, 1045, 966, 894.

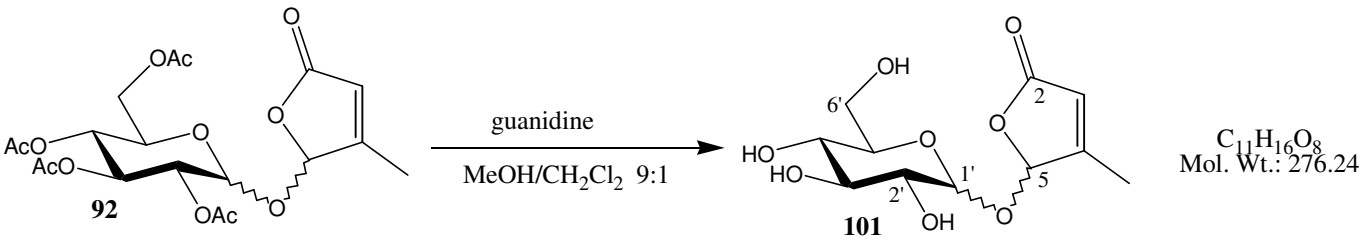
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.96-5.95 (*br s*, 1 H, H-C(2)); 5.74 (*s*, 1 H, H-C(5)); 5.41 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(3')); 5.36 (*d*, *J* = 4 Hz, 1 H, H-C(1')); 5.15 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(3'')); 5.07 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(4'')); 4.92 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(2'')); 4.84 (*dd*, *J* = 10, 4 Hz, 1 H, H-C(2')); 4.57 (*d*, *J* = 11 Hz, 1 H, H_A-C(6')); 4.40 (*dd*, *J* = 12.5, 4.5 Hz, 1 H, H_A-C(6'')); 4.15-4.08 (*m*, 2 H, H_B-C(6') and H-C(5')); 4.04 (*d*, *J* = 12.5 Hz, 1 H, H_B-C(6'')); 3.76 (*pseudo-t*, *J* = 10 Hz, 1 H, H-C(4')); 3.68 (*ddd*, *J* = 10, 4, 2Hz, 1 H, H-C(5'')); 2.170, 2.088, 2.076, 2.056, 2.045, 2.039, 2.011, 1.982 (8 *s*, 24 H, CH_3-C(4) and CH_3-CO).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 170.31, 170.19, 170.05, 170.00, 169.70, 169.47, 169.15, 169.08 (C=O); 162.3 (C(4)); 119.4 (C(3)); 103.6 (C(5)); 100.6 (C(1'')); 96.8 (C(1')); 76.0 (C(4')); 72.8 (C(3'')); 71.7 (C(5'')); 71.3 (C(2'')); 70.5 (C(2')); 69.5 (C(5')); 69.0 (C(3')); 67.6 (C(4'')); 61.4 (C(6'')); 61.2 (C(6')); 20.7-20.3 (CH$_3$-CO); 12.9 (CH$_3$-C(4)).

EI-MS (70 eV, ca. 300°C): *m/z* (%) = 431 (4), 385 (37), 331 (29), 317 (17), 271 (5), 243 (7), 242 (6), 169 (100), 109 (35), 97 (54), 43 (43).

FAB-MS (NBA): *m/z* (%) = 734 (2.3), 733 ([*M* + H]$^+$, 4.7), 619 (5), 331 (31), 169 (54), 109 (30), 97 (36), 43 (100).

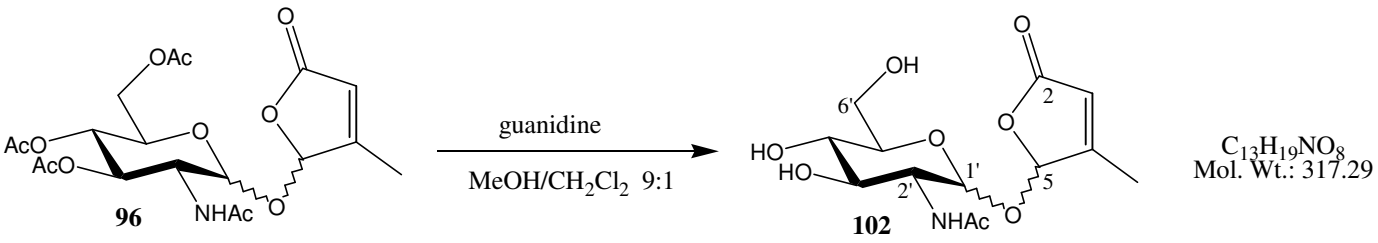
4. Synthesis of 5-(D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one (**101**)



To a solution of **92** (1097 mg, 2.5 mmol) in EtOH-CH_2Cl_2 (9:1, 30 ml), was added guanidine (114 mg, 1.9 mmol) in EtOH-CH_2Cl_2 (9:1, 2 ml). After a reaction time of 1.5 h at r.t., additional guanidine (284 mg, 4.8 mmol) in EtOH-CH_2Cl_2 (9:1, 5 ml) was added. Each hour, additional guanidine (57 mg, 0.95 mmol) in EtOH-CH_2Cl_2 (9:1, 1 ml) was added. The reaction was followed by TLC until the presence of glucose was detected. Then, the mixture was poured directly onto a silica gel column (0 to 10% MeOH in CH_2Cl_2). A mixture of isomeric **101** (219 mg, 32%) containing partially deacetylated compounds was isolated.

^{13}C-NMR (125 MHz, CD_3OD): δ = 173.14, 173.04, 172.99, 172.92, 172.84 (C=O); 167.19, 167.13, 166.63, 166.31 (C(4)); 119.72, 119.41, 118.98, 118.93 (C(3)); 105.6, 105.1, 103.4, 102.1, 101.84, 101.80, 100.7, 98.6, 98.0, 93.8 (C(5) and C(1')); 78.35, 78.32, 77.71, 77.67, 76.02, 75.09, 74.98, 74.68, 74.60, 74.53, 74.47, 74.37, 73.58, 73.07, 72.56, 71.8, 71.5, 71.3, 70.87, 70.85, 70.46 (C(2'), C(3'),C(4'), and C(5')); 62.62, 62.50, 62.24, 61.74 (C(6')); 13.62, 13.55, 13.46, 13.37 (*C*H_3-C(4)).

5. Synthesis of 5-(2-acetamido-2-deoxy-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one (**102**)



To a solution of 5-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one **96** (199 mg, 0.45 mmol) in EtOH-CH_2Cl_2 (90:10, 5 ml), was added guanidine (56 mg, 0.95 mmol) in EtOH-CH_2Cl_2 (90:10, 1 ml). After a reaction time of 1.5h at r.t., guanidine (56 mg, 0.95 mmol) in EtOH-CH_2Cl_2 (90:10, 1 ml) was added. The mixture was stirred for 2 h at r.t., and then poured directly onto a silica gel column. Elution with 10% MeOH in CH_2Cl_2 gave a mixture of isomeric 5-(2-acetamido-2-deoxy-D-glucopyranosyloxy)-4-methyl-5*H*-furan-2-one **102** (43 mg, 30%), consisting of three stereoisomers.

White amorphous solid; R_f 0.3 (20% MeOH in CH_2Cl_2).

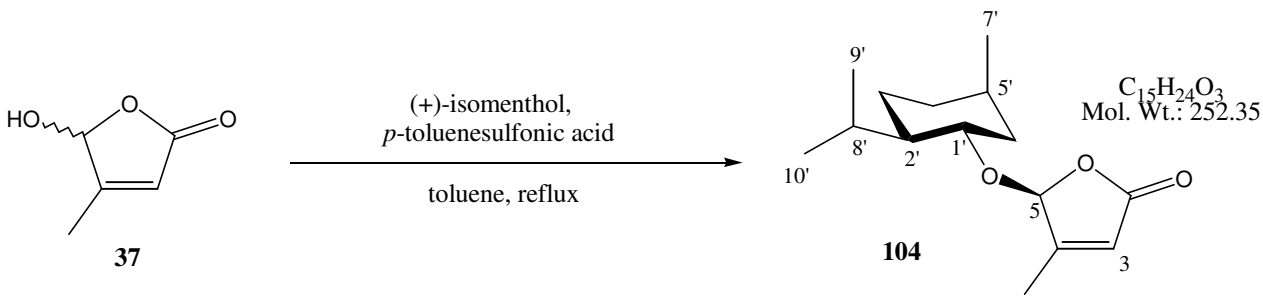
^{13}C-NMR (125 MHz, CD_3OD): δ = 173.88, 173.82, 173.64 (CH_3-*C*O-NH); 172.91, 172.85, 172.77 (C(2)); 166.68 (2 C), 166.14 (C(4)); 119.77, 119.27, 119.25 (C(3)); 105.29, 102.34, 101.54 (C(5)); 100.97, 99.84, 97.69 (C(1')); 78.53, 75.64, 75.28, 74.62, 71.98, 71.84, 71.52 (C(3'), C(4') and C(5')); 62.69, 62.58, 61.85 (C(6')); 56.92, 55.27, 54.80 (C(2')); 22.94, 22.50 (2 C) (*C*H_3-CO); 13.55, 13.36, 13.33 (*C*H_3-C(4)).

EI-MS (70 eV, 250°C): *m/z* (%) = 244 (4), 220 (3), 202 (16), 156 (13), 142 (19), 130 (76), 114 (47), 102 (35), 97 (92), 88 (26), 60 (100), 43 (75).

FAB-MS (NBA): *m/z* (%) = 319 (7), 318 ([M + H]$^+$], 40), 264 (6), 204 (49), 102 (100), 97 (26), 60 (35), 43 (27).

III Miscellaneous

1. Synthesis of (5*S*)-5-{[(1*S*,2*R*,5*R*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**104**)



37 **104**

To a solution of 5-hydroxy-4-methyl-5*H*-furan-2-one **37** [13] (6.63 g, 58 mmol) in toluene (20 ml), (1*S*, 2*R*, 5*R*)-2-isopropyl-5-methylcyclohexanol ((+)-isomenthol, 10g, 64 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. The solution was refluxed overnight (a dropping funnel filled with molecular sieve 4Å was put between the reaction flask and the condenser). After cooling at r.t., the mixture was washed with saturated $NaHCO_3$ solution (3 × 20 ml) and saturated NaCl solution (3 × 20 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated. The product was purified by successive recrystallizations in pentane.

Yield: 729 mg, 11%.

Colorless needles, Mp 63°C.

IR (KBr): 3099, 2924, 1756, 1656, 1442, 1352, 1295, 1127, 912, 729, 569, 507.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.85 (*br s*, 1 H, H-C(2)); 5.75 (*s*, 1 H, H-C(5)); 4.00 (*td*, *J* = 6, 3 Hz, 1 H, H-C(1')); 2.05 (*dd*, *J* = 1.5 0.5 Hz, 3 H, H_3C-C(4)); 1.94-1.79 (*m*, 2 H, H-C(8') and H-C(5')); 1.69-1.57 (*m*, 2 H, H_{eq}-C(3') and H_{eq}-C(6')); 1.55-1.41 (*m*, 3 H, H_{ax}-C(3'), H_{eq}-C(4'), and H_{ax}-C(6')); 1.40-1.34 (*m*, 1 H, H-C(2')); 1.26-1.17 (*m*, 1 H, H_{ax}-C(4')); 0.92 and 0.91 (2 *d*, *J* = 7 Hz, 6 H, H_3C(7') and H_3C(9')); 0.88 (*d*, *J* = 7 Hz, 3 H, H_3C(10')).

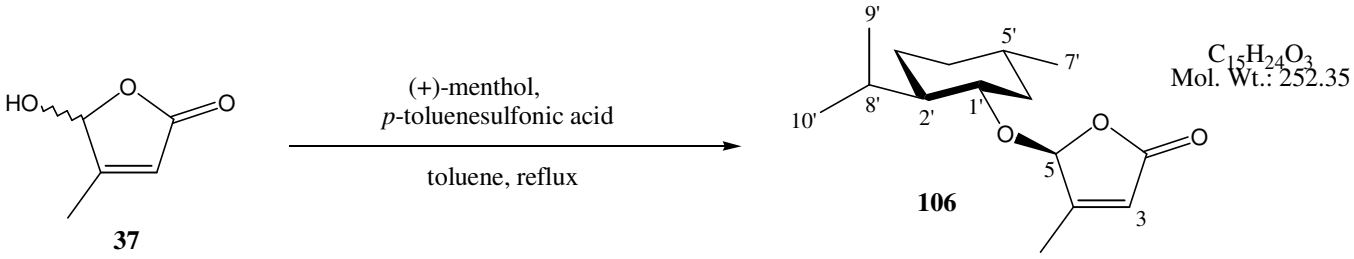
^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.2 (C(2)); 163.8 (C(4)); 118.8 (C(3));102.5 (C(5)); 77.4 (C(1')); 46.3 (C(2')); 35.2 (C(6')); 29.8 (C(4')); 27.2 (C(5')); 25.8 (C(8')); 20.93 and 20.80 (C(7') and C(9')); 20.42 (C(3')); 19.0 (C(10')); 13.3 (*C*H_3-C(4)).

EI-MS (70 eV, 50°C): *m/z* (%) = 167 (7), 138 (39), 137 (22), 98 (55), 97 (100), 95 (30), 93 (13), 81 (47), 69 (29), 55 (18), 41 (22).

FAB-MS (NBA): *m/z* (%) = 505 (7), 254 (6), 253 ([*M* + H]$^+$, 37), 139 (63), 137 (17), 115 (100), 97 (43), 95 (11), 83 (96), 81 (21), 69 (34), 57 (33), 55 (32), 43 (15), 41 (28).

Anal. calc. for $C_{15}H_{24}O_3$ (252.35): C, 71.39; H, 9.59; O, 19.02. Found: C, 71.40; H, 9.65; O, 19.16.

2. Synthesis of (5*S*)-5-{[(1*S*,2*R*,5*S*)-2-isopropyl-5-methylcyclohexyl]oxy}-4-methyl-5*H*-furan-2-one (**106**)



Preparation

To a solution of (1*S*,2*R*,5*S*)-2-isopropyl-5-methylcyclohexanol ((+)-menthol, 2.023 g, 13 mmol) and a catalytic amount of *p*-toluenesulfonic acid in toluene (10 ml), a solution of 5-hydroxy-4-methyl-5*H*-furan-2-one **37** [13] (1.016 g, 8.9 mmol) in toluene (2 ml) was added dropwise. The mixture was refluxed overnight (a dropping funnel filled with molecular sieve 4Å was put between the reaction flask and the condenser). After cooling at r.t., the mixture was washed with saturated $NaHCO_3$ solution (3 × 20 ml) and saturated NaCl solution (3 × 20 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated. Petrol ether was added to the residue and the mixture was left in the freezer (–18°C) overnight. The obtained

crystals were filtrated and the product was purified after two successive recrystallization in petrol ether.

Yield: 179 mg, 8%.

colorless needles, Mp 98.8-99.2°C.

$[\alpha]_D^{20}$ = +161.2 (589 nm), +167.7 (578 nm), +190.5 (546 nm); +325.9 (436 nm), +526.9 (365 nm) (*c* = 1.07, CH_2Cl_2).

IR (KBr): 2957, 2925, 2866, 2372, 1747, 1655, 1457, 1370, 1346, 1296, 1241, 1156, 1121, 952, 901, 861, 733, 628, 575.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 5.84 (*br s*, 1 H, H-C(3)); 5.78 (*s*, 1 H, H-C(5)); 3.64 (*td*, *J* = 10.5, 4.5 Hz, 1 H, H-C(1')); 2.16-2.09 (*m*, 2 H, H-C(8') and H_{eq}-C(6')); 2.04 (*dd*, *J* = 1.6, 0.6 Hz, 3 H, H_3C-C(4)); 1.72-1.63 (*m*, 2 H, H_{eq}-C(4') and H_{eq}-C(3')); 1.47-1.37 (*m*, 1 H, H-C(5')); 1.29-1.22 (*m*, 1 H, H-C(2')); 1.08-0.85 (*m*, 3 H, H_{ax}-C(3'), H_{ax}-C(4'), and H_{ax}-C(6')); 0.95 (*d*, *J* = 6.5 Hz, 3 H, H_3C(7')); 0.87 (*d*, *J* = 7 Hz, 3 H, H_3C(9')); 0.81 (*d*, *J* = 7 Hz, 3 H, H_3C(10')).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 171.2 (C(2)); 163.8 (C(4)); 118.8 (C(3)); 101.6 (C(5)); 79.4 (C(1')); 47.7 (C(2')); 40.4 (C(6')); 34.2 (C(4')); 31.4 (C(5')); 25.2 (C(8')); 23.1 (C(3')); 22.2 (C(7')); 20.9 (C(9')); 15.7 (C(10')); 13.3 (CH$_3$-C(4)).

EI-MS (70 eV, 50°C): *m/z* (%) = 167 (7), 138 (48), 137 (27), 123 (15), 115 (10), 98 (52), 97 (100), 95 (36), 83 (17), 82 (11), 81 (62), 69 (31), 55 (23), 43 (12), 41 (30).

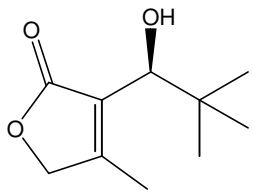
FAB-MS (NBA): *m/z* (%) = 505 (7), 254 (7), 253 ([*M* + H]$^+$, 42), 139 (47), 137 (14), 115 (100), 97 (39), 95 (10), 83 (85), 81 (20), 69 (31), 57 (31), 55 (32), 43 (16), 41 (27).

Anal. calc. for $C_{15}H_{24}O_3$ (252.35): C, 71.39; H, 9.59; O, 19.02. Found: C, 71.42; H, 9.29; O, 18.92.

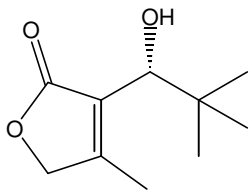
C. Summary

Bacteria are able to communicate through chemical signals. They can for instance estimate their population by the use of signalling compounds: this phenomenon is called *quorum sensing*. Some of these signalling compounds are derivatives of homoserine lactones. When their concentration reaches a certain level, bacterial genes are triggered, which leads to virulence, bacterial film formation, and so on. Natural halogenated furanones **22** and **23** extracted from the marine alga *Delisea pulchra* proved to inhibit the quorum sensing. Other natural furanones **24a**-**27a** extracted from *Streptomyces antibioticus* TÜ 99 were also quorum sensing inhibitors. As the structures of these natural compounds were similar to the homoserine lactones, additional furanones were synthesized in order to investigate their properties towards the quorum sensing.

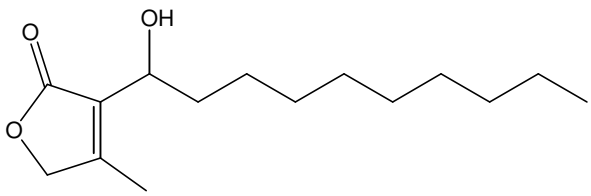
Following the synthetic route published by Grossmann [11], the compounds **56a**, **56b**, and **58** were prepared. The key step of the synthesis, which is a condensation of the menthylated furanone **38b** and an aldehyde with LDA under kinetic conditions, was optimized. The biological activity of these three compounds, as well as of all intermediates, was investigated. The tests were carried out with a mutant strain of *Chromobacterium violaceum*, called CV026.



56a



56b



58

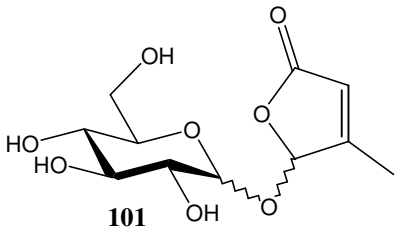
Some of the synthetic furanones proved indeed to be weak quorum sensing inhibitors, however none of them was more active than the natural compound **22** [22]. In addition, some of these compounds were toxic for *Chromobacterium violaceum*.

The Grossmann method, followed by a reduction of the side chain, was also used for the preparation of the flavour furanone **64**, which had previously shown a weak inhibition of

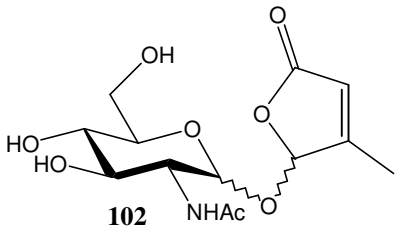
the pristinamycin production by *Streptomyces pristinaespiralis*. Further tests were carried out with mammalian cells, but the compound **64** proved to be toxic [21].

Other biological tests showed that some menthylated furanones were weak chitinase inhibitors. Starting from the assumption that menthylated furanones could have structure analogy with the most potent chitinase inhibitor allosamidin (**75**), the synthesis of glycosylated furanones was attempted. The main purpose was to replace the menthyl group by a monosaccharide or a disaccharide in a very short and very cheap process.

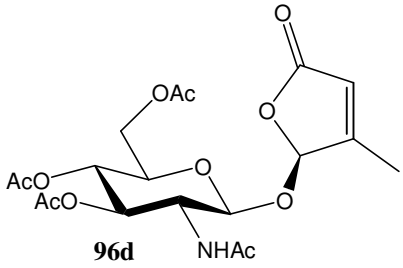
The starting furanone **37** was treated with trichloroacetonitrile to give the trichloroacetimidate **93**, which was submitted to glycosylation following a "reversed type Schmidt glycosylation" procedure using TMSOTf as Lewis acid. The obtained acetylated compounds **92**, **96**, and **97** were treated with guanidine. The deacetylated monosaccharides **101** and **102** were obtained in low yields whereas **97** was not completely deacetylated.



101



102



96d

The glycosylation afforded for each compounds **92**, **96**, and **97** a mixture of four diastereomers. Only the diastereomers of **96** could be partially separated by chromatography on silica gel. The structures of the α-anomers could not be completely elucidated, whereas the structures of the β-anomers could be obtained from an X-ray structure determination of **96d**.

The starting furanone **37** was also treated with (+)-isomenthol and (+)-menthol to give the compounds **104** and **105** respectively. These compounds, as well as the glycosylated furanones **92**, **96a**, **96b**, **96c**, **96d**, **102**, **104**, and **105**, were tested but none of these synthetic furanones were active as chitinase inhibitors.

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I thank the "*Nationalfonds zur Förderung der wissenschaftlichen Forschung*" for the financial support during my thesis. I would also like to thank the following Professors for the lectures I attended during the time I spent at the University of Basel:

H. Gampp, *M. Oehme*, *A. Pfaltz*, *E. Reich*, *U. Séquin*, *W. Woggon*.