Investigation on Structure-Bioactivity Relationship and Determination of the Absolute Configuration of Natural Products

Der Fakultät für Naturwissenschaften
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“The way to succeed is to keep one´s courage and patience,
and to work on energetically.”
(Vincent van Gogh, 1853-1890)

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1 Introduction

The unambiguous knowledge of the stereo structure of biologically active synthetic and natural products is an important prerequisite for structure-activity relationship (SAR) investigation. For this reason this thesis involves both the studies on structure-activity relationship and the determination of the absolute stereo structure.

Other applications, such as targeted drug design and delivery can also profit from the investigation on structure-activity relationship. SAR is a means by which the effect of a drug or toxic chemical on an animal, plant or the environment can be related to its molecular structure. This type of relationship may be assessed by considering a series of molecules and making gradual changes to them, noting the effect upon their biological activity of each change. In these investigations the first task is to discover which part of the molecule is responsible for the biological activity. Using this information, the next step is to modify the other part of the compound to reach the required activity.

1.1 Cancer

The most important investigation on structure-bioactivity relationship of this thesis is the synthesis of benzamide riboside derivatives. Benzamide riboside (**2-2**) exhibits potent antitumor activity against several human cancer cells.[1,2] Therefore **2-2** might be a promising antitumor agent in the cancer therapy.

Although there are many kinds of cancer, they all start because of out-of-control growth of abnormal cells. Cancer develops when cells in a part of the body begin to grow out of control because of damage to DNA. Most of the time when DNA gets injured, either the cell dies or it is able to repair the DNA. In cancer cells, the damaged DNA is not repaired. People can inherit damaged DNA, which accounts for inherited development of cancers.[3,4] Many times though, a person's DNA can be harmed by exposure to carcinogens in the environment, like smoking. Cancer usually forms as a tumor. Some cancers, like leukemia, do not form tumors. Instead, these cancer cells involve the blood and blood-forming organs, and circulate through other tissues where they grow. Cancer cells often spread to other parts of the body where they begin to grow and replace normal tissue. This process, called metastasis, occurs as the cancer cells get into the bloodstream or lymph vessels of our body. Tumors can be classified into two groups:

- **Benign tumors** are not cancerous. They can often be removed and, in most cases, they do not come back. Cells from benign tumors do not spread to other parts of the body. Most importantly, benign tumors are rarely a threat to life.
- **Malignant tumors** are cancerous. Cells in these tumors are abnormal and divide uncontrollably and disorderly. They can invade and damage nearby tissues and organs. Also, cancer cells can break away from a malignant tumor and enter the bloodstream or the lymphatic system. That is how cancer spreads from the original cancer site to form new tumors in other organs.

Leukemia and lymphoma are cancers that arise in blood-forming cells. The abnormal cells circulate in the bloodstream and lymphatic system.[5] They can also invade (infiltrate) body organs and form tumors.

1.1.1 Treatment

Treatment for cancer depends on the type of cancer; the size, location, and stage of the disease; the person's general health; and other factors. Treatment for cancer can be either local or systemic. Local treatments affect cancer cells in the tumor and the surrounding area. Systemic treatments travel through the bloodstream, reaching cancer cells all over the body. Surgery and radiation therapy are types of local treatment. Chemotherapy, hormone therapy, and biological therapy are examples of systemic treatment.[6,7]

It is hard to protect healthy cells from the harmful effects of cancer treatment. This is because treatment does damage healthy cells and tissues. Moreover, it often causes side effects. The side effects of cancer treatment depend mainly on the type and extent of the treatment. Also, the effects may not be the same for each person.

Chemotherapy is the use of drugs to kill cancer cells. It may be the only kind of treatment a patient needs, or it may be combined with other forms of treatment. Neoadjuvant chemotherapy refers to drugs given before surgery to shrink a tumor; adjuvant chemotherapy refers to drugs given after surgery to help prevent the cancer from recurring. Chemotherapy also may be used (alone or along with other forms of treatment) to relieve symptoms of the disease. Sometimes the anticancer drugs are given in other ways. For example, in an approach called *intraperitoneal chemotherapy*, anticancer drugs are placed directly into the *abdomen* through a catheter. To reach cancer cells in the *central nervous system* (CNS), the patient may receive *intrathecal chemotherapy*. In this type of treatment, the anticancer drugs enter the

cerebrospinal fluid through a needle placed in the spinal column or a device placed under the scalp.

The side effects of chemotherapy depend mainly on the drugs and the doses the patient receives. Generally, anticancer drugs affect cells that divide rapidly. In addition to cancer cells, blood cells, which fight infection, help the blood to clot, and carry oxygen to all parts of the body that are affected. When blood cells are affected, patients are more likely to get infections, may bruise or bleed easily, and may feel unusually weak and very tired. Rapidly dividing cells in hair roots and cells that line the digestive tract may also be affected. As a result, loss of hair, poor appetite, nausea and vomiting, diarrhea, or mouth and lip sores are possible side effects that may arise.

Inhibiting cancer involves more than just locating the right molecule. Most cancers engage multiple growth factor, angiogenic, cell cycle, and apoptosis pathways. Frequently, redundant pathways exist, so that as drugs shut one pathway down another pathway takes over. This is one way that cancer becomes resistant to targeted agents. Early stage tumors tend to secrete a small number of pro-angiogenic factors, whereas late stage tumors secrete a larger number of pro-angiogenic factors.

1.2 Apoptosis

Until several years ago, cell death has been regarded as an event that is in principle negative for the organism. The fact that cell death possesses an important regulating function during development of the organism and it represents a central part of the life, was a surprising discovery. Programmed cell death, the apoptosis, is a suicide program of the cell, which completely eliminates the cell within a few hours. This phenomenon has already been well-known for decades. However, in 1972, after the detailed description of the morphologic changes during apoptosis[8], the physiological cell elimination was recognized as an independent and genetically controlled form of cell death, and the term Apoptosis was given. The term apoptosis (apo = off, away; ptosis = lowering) originates from Greek and describes the drop of leaves in autumn. Five years later the first genes, which are responsible for the apoptosis, were discovered in the thread worm "*Caenorhabditis elegans*". The genes were found by mutations, which lead to the disturbance of the apoptosis during the growth.[9] In 1990 researchers discovered that a tumor production gene in case of overexpression prevents apoptosis.[10] Thus the apoptosis had become one of the most attractive new research fields

with constantly rising publication numbers. So far, this development has appeared in over 50000 scientific publications relating to this topic.

Apoptosis is an important possibility for multi-cell organism to organize themselves. It plays an important role in directing apoptotic death of certain cells during the development of the embryo. The shaping of body and organs of the embryo during the development also takes place via apoptosis; for example the skins between toes and fingers are removed by apoptosis during the embryonic growth. Nerve cells are produced in excess during the embryonic development and as soon as they do not have contact with the neighboring neurons its level is lowered through apoptosis to 40-85%. Without contact to the neighbors the nerve growth factors (NGF) or ChAT (choline acetyltransferase) development factors (CDF)[11] are missing. Later on the central nerve-system shows only a small cell-elimination-rate. Each diseased change in the genetic material of the cells would be passed on inevitably to the descendants.[12]

A cell begins the apoptosis program, if internal or external signals command the self destruction.

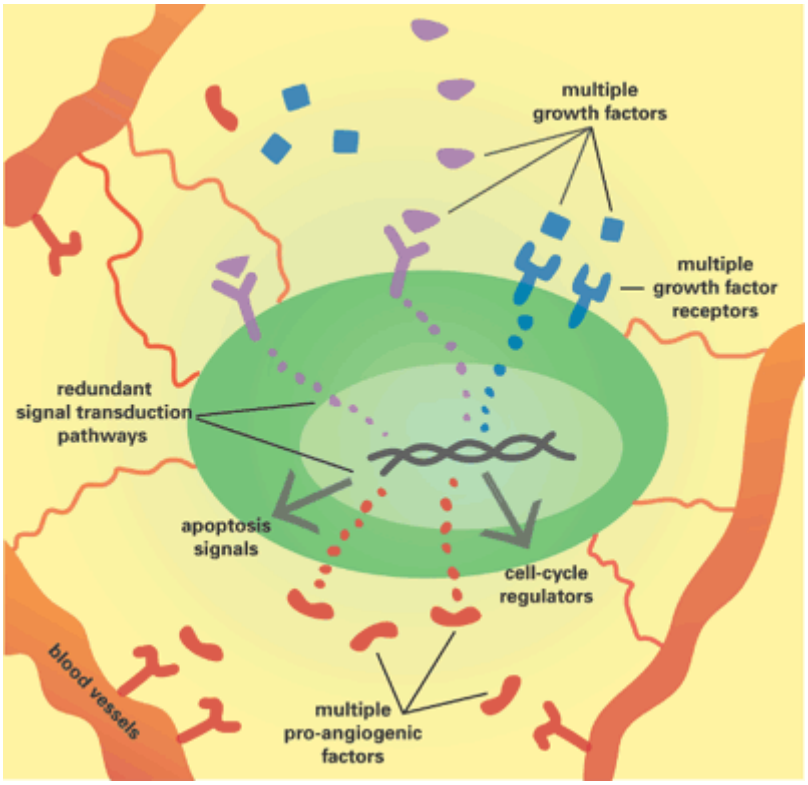


Figure 1.1: Pathway of apoptosis

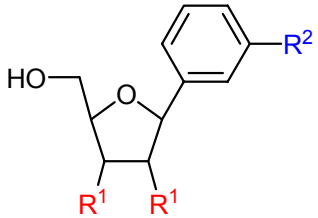
Each cell of our body is in constant contact to its neighboring cells by chemical messenger materials (e.g. growth factor NGF or Interleukin-2), which is bound to the receptors of the cell. This contact is extracted and usually leads to the release of apoptosis.
High doses of UV or X-ray radiation can also lead to damage of the genetic material in the cell. These cells then have the choice between repairs of smaller DNA damage, or if the damage can not be repaired any more then apoptosis. In case of doubt, the cell kills itself, in order not to degenerate.
Cancer cells differ from other cells in particular by the fact that they proliferate unresisted. A change in the genetic code made these cells immortal. The mutated genes are often dealing with apoptosis-regulation. In 50 % of the human tumors genes, which the Tumor-suppressor-protein P53 code, are inactive. In the case of some kinds of cancer, high concentrations of apoptosis inhibitors Bcl-2 were found. The aim of the medical research is to find a medicine against such apoptosis resistant cancer cells.

Depending on cell type and elicitor, the apoptosis can be introduced by three different signal paths. The common goal of the different apoptosis activating way is the initiation of *Caspase cascade*, which introduces irreversible cell death.[13]

1.3 Aims and Scopes

In order to improve the biological activity of some natural and synthetic compounds, several of their derivatives had to be synthesized and tested. Through systematic changing of the different moieties and/or substitution patterns of the molecules, and performing biological tests it can be established which part of the molecule is responsible for the bioactivity. This thesis consists of five topics, from which four are concentrating on structure-bioactivity relationship investigation. The fifth deals with the determination of the absolute configuration of natural products.

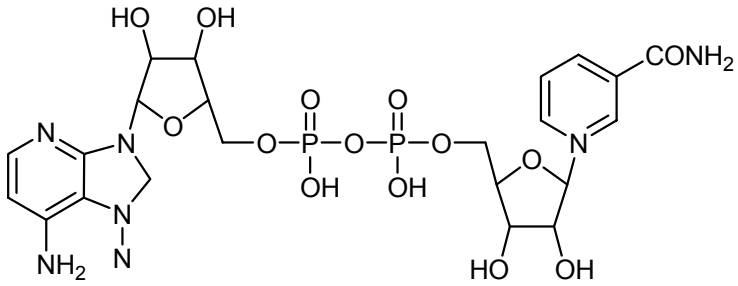
(1) The first project concentrates on the synthesis of antitumor benzamide riboside derivatives in hope of improving a higher biological selectivity against cancer cells.



R^2: $CONH_2$, OMe, or OH, F, H

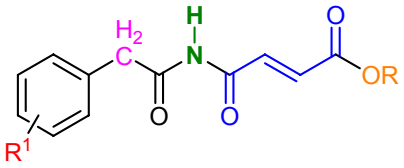
R^1: OH, or H

(2) The second part involves the preparation of NAD analogous BAD in order to investigate the enzyme-substrate complex with ubichinon. X-ray crystallographic study could provide information about the active site of reductases.



BAD

(3) The third topic describes the synthesis and biological study of antifungal coniothyriomycin analogues to find stable derivatives with high activity against plant fungi.



R: Changing the hydrophobicity of the alcohol component

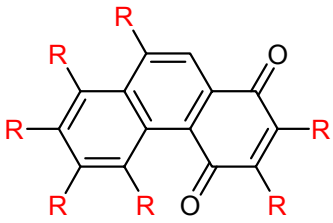
R^1: Variation of the substitution pattern of the aromatic ring

Replacement of the phenylacetic acid with benzoic acid

Variation of the degree of saturation in the side chain

Replacement of the imide function to hydrazide, thio, etc. derivatives

(4) The fourth project is to prove a so far unknown correlation between the molecule orbital coefficients (LUMO) and the biological activity on the example of several phenanthrenequinones.



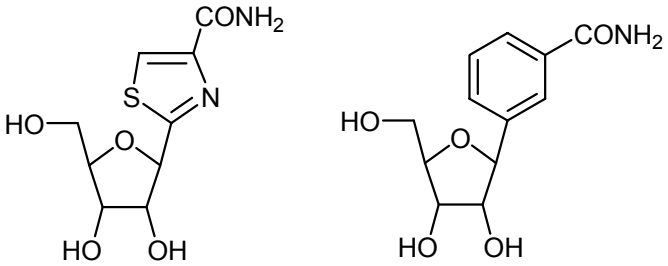
R: H or OMe

(5) The last topic focuses on the determination of the absolute structure of natural products using a quantum mechanical approach. By calculating the CD-spectra of compounds and comparing them with that of the experimental ones, the absolute configuration of the given molecule can be elucidated.

2 Synthesis of Benzamide Riboside Derivatives with Antitumor Activity

2.1 Introduction

Benzamide riboside, a recently discovered inhibitor of inosine 5′-monophosphate dehydrogenase (IMPDH) exhibits oncolytic activity.[2] IMPDH is the key enzyme of the *de novo* guanylate biosynthesis including GTP and dGTP, and was shown to be linked with proliferation. Therefore, IMPDH is a very good target for antitumor therapy. In order to be active, benzamide riboside has to be converted to BAD, an NAD analogue that binds to the NAD site on IMPDH. Inhibition of the enzyme by benzamide riboside selectively inhibits tumor cells growth and induces apoptosis in various human tumor cell lines.
The discovery of tiazofurin **(2-1)** as a specific inhibitor of IMPDH with antitumor properties led to a greater understanding of the IMPDH function and its importance in therapy.



Scheme 2.1: Structure of tiazofurin **(2-1)** (TR) and benzamide riboside **(2-2)** (BR)

Benzamide riboside **(2-2)** (BR) was developed to find a better inhibitor of IMPDH.[1] BR exhibited dual mechanisms of action; first by inhibiting IMPDH and second by inducing apoptosis in highly proliferating cells, such as cancer. The previous investigation ensures that benzamide riboside influences the adenosine-receptor-transport-activity. These unfavorable influences should be reduced by chemical modifications. For a high biological selectivity in the cells new derivatives must be synthesized, which have less structural similarity to NAD. To prepare these molecules, a general method for the synthesis of benzamide riboside derivatives must be developed.

2.2 Mechanism of Action of Benzamide Riboside

Benzamide riboside [(1-*β*-D-ribofuranosyl)benzene-3-carboxamide)] **(2-2)**, a *C*-glycoside analogue of nicotinamide riboside, has generated interest because of its excellent toxic activity.[1] Benzamide riboside (BR) was examined for its activity against different human tumor cell lines.[2] A detailed comparative study of the activity was conducted with selenazofurin (SR) and tiazofurin (TR).[14] The mechanisms of action of SR and TR were shown to be due to their conversion to an analogue of NAD that inhibits the NAD utilization by IMPDH. This causes an arrest of cancer cell proliferation, leading to cancer cell death.[15,16,17] The enzyme inosine 5´-monophosphate dehydrogenase (IMPDH) is responsible for the formation of xanthine-monophosphate from IMP (inosine-monophosphate) and was shown to be the rate limiting enzyme for the de novo formation of GTP (guanosine-triphosphate) and dGTP (deoxyguanosine-triphosphate).[18] Due to the requirement of high concentration of GTP and dGTP in rapidly proliferating cells, tumor cells have, in contrast to slowly proliferating cells, high activity of IMPDH. First, Jackson showed in a spectrum of hepatomas that the enzyme activity is increased linked with proliferation and malignant transformation.[18] Therefore the enzyme is considered to be an excellent target for antitumor therapy. Malignant cells can be killed without causing much harm to normal, slowly growing cells.

2.3 Synthesis of Benzamide Riboside

2.3.1 Synthesis of *C*-glycosides

The study and synthesis of *C*-nucleosides has been extensively investigated owing to their biological activity and potential as drug candidates for antiviral and anticancer therapy.[19,20] Nucleosides are generally considered to be compounds which contain a heterocyclic aglycon and a carbohydrate moiety that are joined together by a carbon–nitrogen bond. However, *C*-nucleosides differ from the more common nucleosides in the way that the sugar and heterocyclic aglycon are connected by a C–C, rather than a C–N bond. The C–C bond is responsible for the resistance of *C*-nucleosides to hydrolytic and enzymatic cleavage.

Numerous synthetic strategies have also been investigated in order to optimize yields and stereo selectivity in the glycosylation reaction. Methods for the synthesis of *C*-nucleosides

have been studied extensively;[21,22] however, synthetic obstacles in terms of low yield and/or low stereoselectivity have been frequently encountered. Two major synthetic approaches to *C*-nucleosides have been established:

(1) introduction of a functional group at the anomeric position of a sugar derivative, followed by the construction of a heterocyclic base; and

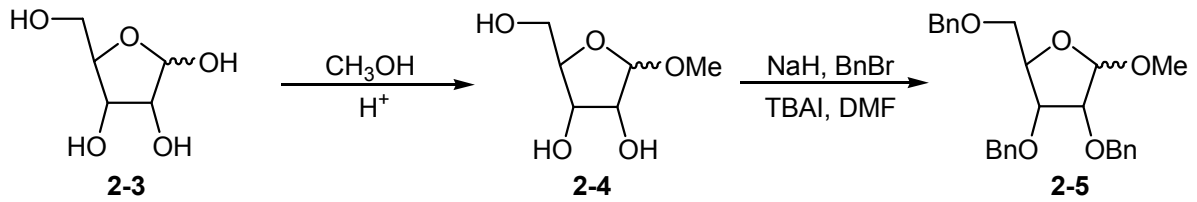
(2) direct attachment of a pre-formed aglycon unit to an appropriate carbohydrate moiety.

The first synthetic approach, which has been reviewed in detail,[19,21] is a versatile method for the preparation of *C*-nucleosides since numerous heterocyclic bases can be constructed starting from an appropriate functional group at the anomeric carbon atom of a sugar component. However, this approach involves a large number of steps, results in rather low yields, and often results in unsatisfactory stereoselectivity. The possible methods for preparation of *C*-glycosides are as follows:

- Coupling of ribofuranose derivatives with organometallic reagents
- Heck-type coupling reaction
- Coupling of protected ribofuranosyl chlorides with organometallic reagents
- Coupling of 1,4-ribonolactone derivatives with organometallic reagents
- Coupling reactions mediated by Lewis acids

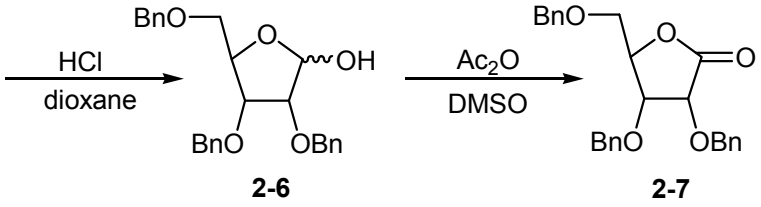
2.3.2 Preparation of 2,3,5-tri-*O*-benzyl-γ-ribonolactone (2-7)

The preparation of benzamide riboside was performed as described in literature.[23] Starting from D-ribose **(2-3)** lactone **2-7** could easily be prepared in a four step synthesis. In the first step, the glycosidic OH of ribose **2-3** was methylated in methanol using H_2SO_4 as catalyst.[24] Benzylation[25] of methyl glycoside **2-4** gave the protected ribose **2-5** in a yield of 89%. As solvent abs. DMF was employed, NaH was used for deprotonation, benzyl bromide as nucleophile and tetrabutyl-ammonium iodide as phase transfer catalyst (Scheme 2.2).



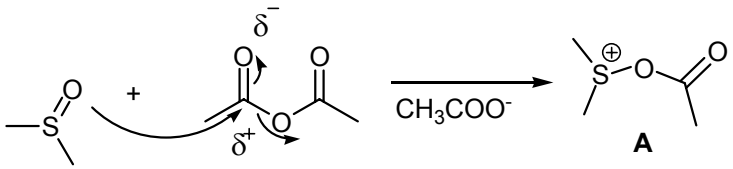
Scheme 2.2: Transformation of D-ribose **2-3** to methyl-benzyl ether **2-5**

Methyl glycoside protecting group of **2-5** was cleaved by HCl in dioxane to afford lactol **2-6**, which was used for the next step without purification. To prepare lactone **2-7**, oxidation[26] was performed in DMSO with acetic acid anhydride. After crystallization from ether/pentane, lactone **2-7** was isolated as white needles in a yield of 85 %.



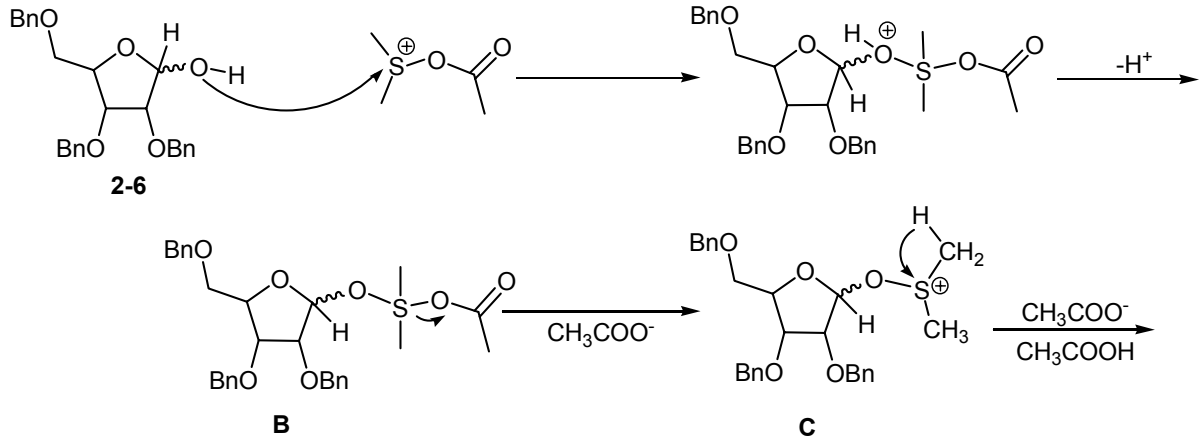
Scheme 2.3: Preparation of lactone **2-7**

The oxidation begins with the activation of DMSO through a nucleophilic attack of an oxygen atom of the DMSO on one of the carbonyl carbon atoms of the acetic acid anhydride. This step results in the formation of sulfoxonium ion **A**, which is the activated form of DMSO.



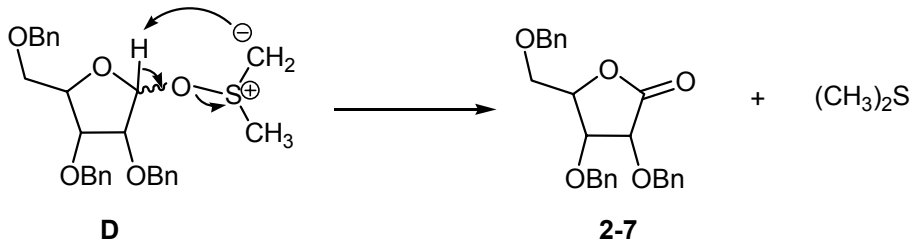
Scheme 2.4: Mechanism of the oxidation *Part I*.

In the next step, sulfoxonium ion **A** reacts with a hydroxyl group of lactol **2-6**, that generates a sulfurane **B** from which, after the cleavage of another acetate anion, sulfoxonium salt **C** is formed.



Scheme 2.5: Mechanism of the oxidation *Part II*.

Acetate ion, which is now present in high concentration, serves as a base and deprotonates one of the methyl groups of intermediate **C**. The obtained sulfonium-ylide **D** provides lactone **2-7** through β-elimination over a cyclic transition state.

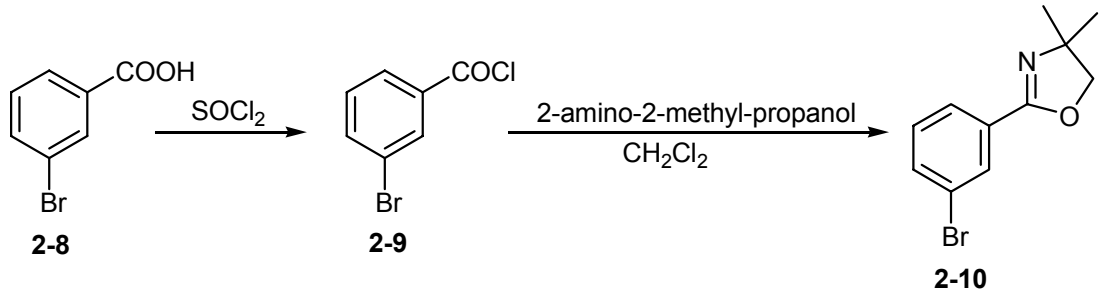


Scheme 2.6: Mechanism of the oxidation *Part III*.

2.3.3 Coupling of 1,4–Ribonolactone (2-7) with organometallic reagent 2-11

The key step of the synthesis is the coupling of the sugar moiety with the aromatic part and the reduction of the tertiary hydroxyl group to achieve satisfactory β/α ratio with the β-anomer **2-14** as the major product.

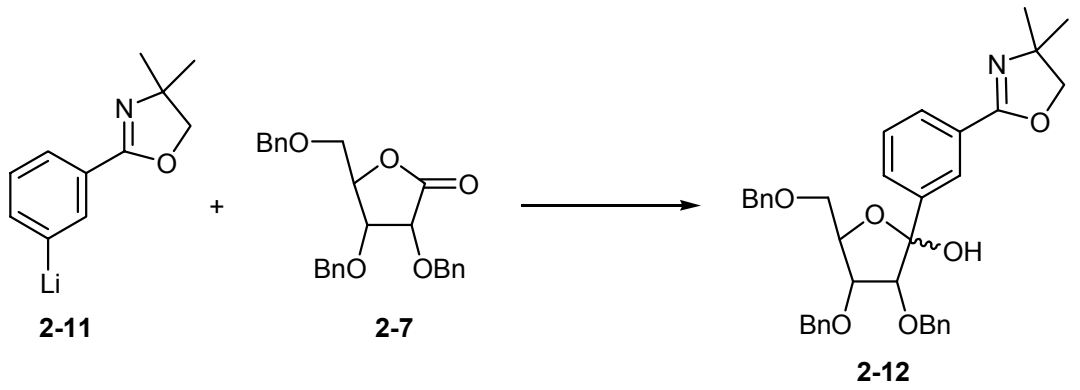
To protect the amide function of the target molecule **2-20**, the oxazoline group was applied for the coupling reaction with lactone **2-7,** since it is stable against both Grignard- and lithium-organic compounds. The preparation of oxazoline **2-10** was accomplished in a three step synthesis starting from 3-bromo benzoic acid (**2-8**), which was transformed into acid chloride **2-9** by treatment with $SOCl_2$. The addition of 2-amino-2-methyl-propanol and ring closure with $SOCl_2$ afforded oxazoline **2-10**.[27]



Scheme 2.7: Preparation of oxazoline **2-10**

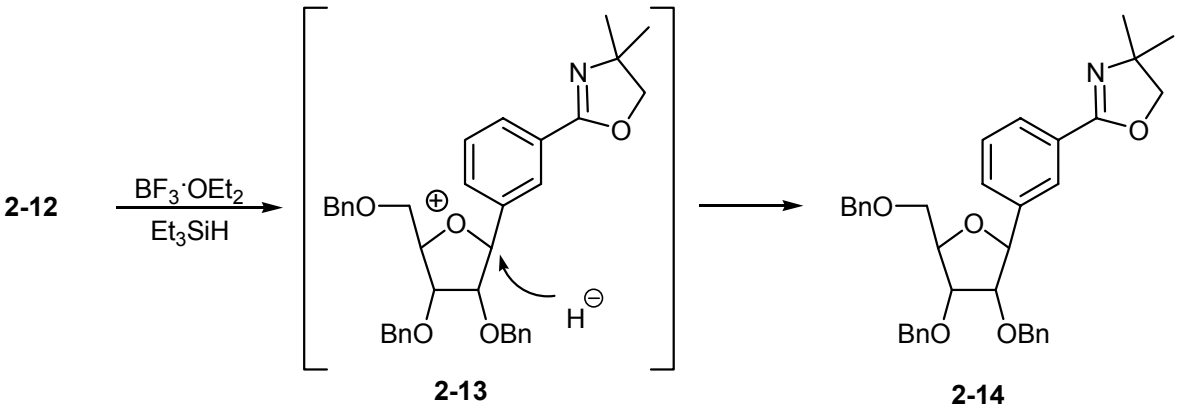
The lithiation of oxazoline **2-10** with butyllithium was carried out at −78 °C in abs. THF under argon atmosphere. TLC monitoring of the reaction showed that the starting material was completely consumed after 30 min. The following reaction at −78 °C between the lithiated oxazoline **2-11** and the lactone **2-7** proceeded surprisingly fast, so that the reaction mixture was worked up after 3 hours. This step gave cleanly the intermediate lactol **2-12**, which was not isolated, but directly subjected to the triethylsilane reduction. To assure the complete conversion of the reduction, the reaction was left to warm up from −78 °C to +10 °C

overnight. After column chromatography only the sterically uniform β-C-riboside **2-14** was isolated as the single product in 87 % yield over two steps.[28,29]



Scheme 2.8: Coupling reaction of the lithiated oxazoline **2-11** and lactone **2-7**

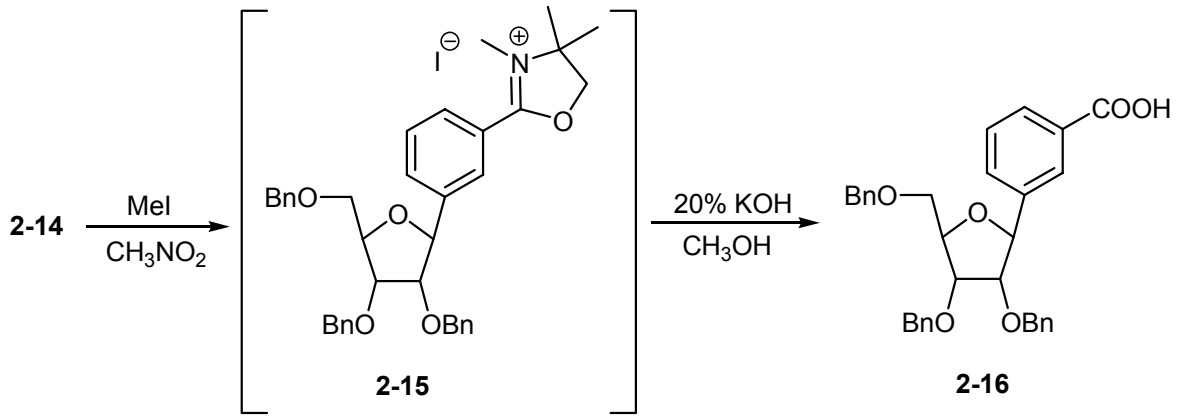
During reduction of the hemiketal **2-12** with triethylsilane in the presence of Lewis acid ($BF_3 \cdot OEt_2$), the nucleophile addition of the hydride ion occurred from the α- and accordingly from the equatorial side of the oxonium ion **2-13**. The stereochemical control can be explained by the anomeric effect of the ring-oxygen and by the higher stability of the β-anomer.



Scheme 2.9: Mechanism of the reduction by Et_3SiH

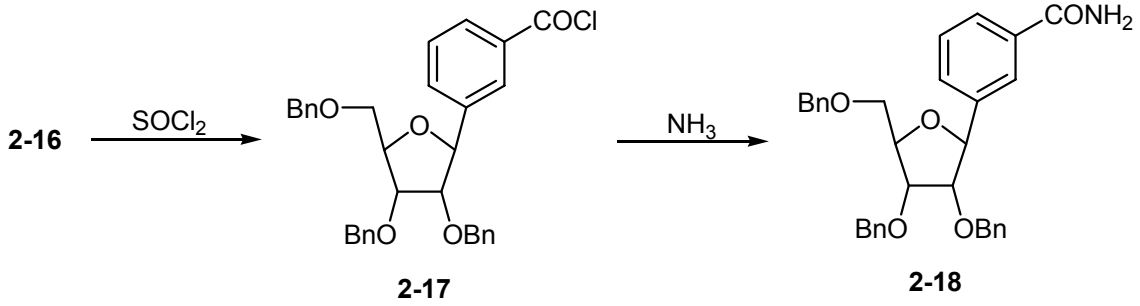
2.3.4 Cleavage of the oxazoline group and transformation to benzamide riboside (2-2)

To prepare the amide function of the target molecule **2-2** the cleavage of the oxazoline group was performed under basic conditions.[30] The desired *C*-ribofuranosylbenzoic acid **2-16** was obtained by first heating oxazoline **2-14** with methyl iodide in nitromethane and then refluxing the intermediate iodide salt **2-15** with 20 % methanolic KOH for 2 days.



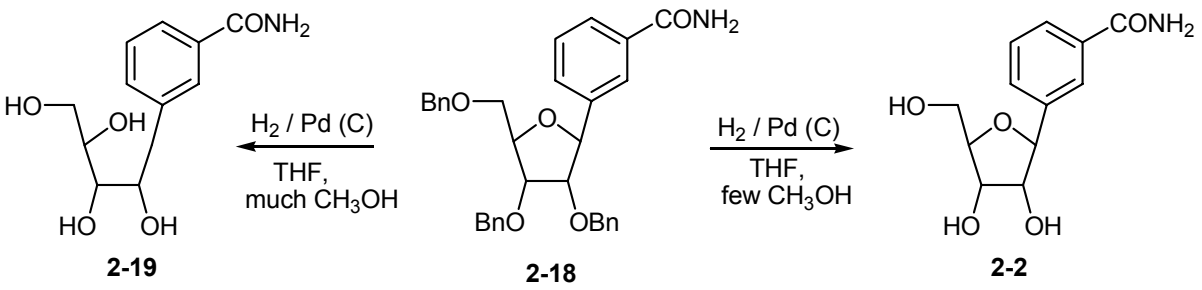
Scheme 2.10: Cleavage of the oxazoline protecting group

Acid **2-16** could easily be transformed to the acid chloride **2-17** by heating it with $SOCl_2$ without any solvent. According to the traditional Schotten-Baumann reaction,[31] treatment of acid chloride **2-17** with ammonia resulted in amide **2-18**, which readily crystallized from ether/pentane after column chromatography.



Scheme 2.11: Transformation of acid **2-16** to amide **2-18**

The cleavage of the benzyl ether was performed by hydrogenolysis with palladium/charcoal as catalyst at room temperature under normal pressure. Changing the polarity of the solvent had a very strong influence on the product composition. If hydrogenation was carried out in THF, without any additional methanol, then either no product was formed, or the reaction took place very slowly. By adding a large amount of methanol to the reaction mixture the benzyl amide **2-18** was converted into the open chain benzamide riboside **2-19** exclusively. If only a few drops of methanol were added to the THF solution, the reaction was complete in 3−4 hours. Just 5−10 % open chain side product was produced, and the desired target molecule **2-2** was obtained in a good yield.



Scheme 2.12: Hydrogenation of benzyl ether **2-18**

The separation of the open-chained **2-19** and main product **2-2** was performed by column chromatography on silica gel with gradually increasing the polarity of the eluting solvents. The products were identified by NMR analysis.

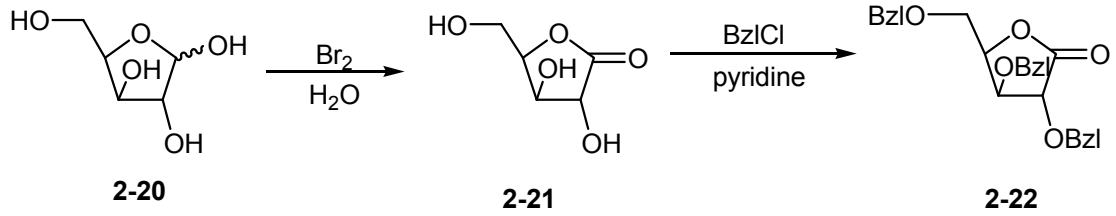
2.4 Synthesis of Benzamide Riboside Analogues

2.4.1 Preparation of 3-deoxy sugar derivative (2-32)

Since the key step of the synthesis is the coupling of a sugar-lactone with oxazoline **2-10**, the preparation of the 3-deoxy-ribonolactone (**2-26**) was necessary in the first step.
Commercially available xylose **(2-20)** was used as starting material, because it is much cheaper than the *C-3*-epimeric ribose. During the synthesis the *C-3* hydroxyl group of xylose was removed by the DeLederkremer method[32] in order to obtain the desired 3-deoxy sugar derivative.

2.4.1.1 Synthesis of 3-deoxy-ribonolactone (2-26)

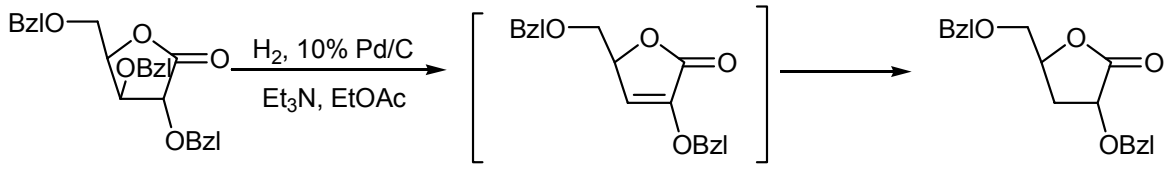
Selective oxidation of the anomeric hydroxyl group was carried out in aqueous solution with bromine[33] to afford the lactone **2-21**. After completion of the reaction (5 days), the excess of bromine was extracted with diethyl ether into the organic phase, and the water phase containing the lactone **2-21** was lyophilized. Treatment of lactone **2-21** with benzoyl chloride in pyridine/chloroform afforded benzoyl ester **2-22** in good yield.



2-20 **2-21** **2-22**

Scheme 2.13: Conversion of xylose **2-20** to benzoyl ester **2-22**

The reduction of the esterified butyrolactone **2-22** in basic medium with Raney nickel or palladium-charcoal[34] gave rise to β-elimination and the intermediate enol lactone **2-23** was subsequently reduced by hydrogenation of the endocyclic bond to deoxy lactone **2-24**.

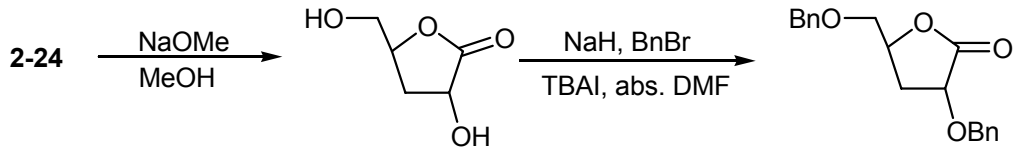


2-22 **2-23** **2-24**

Scheme 2.14: β-elimination followed by hydrogenation of ester **2-22**

Deoxygenation was accomplished in a yield of 68 % over two steps; and product **2-24** crystallized readily from ethanol.

However, the ester function reacts with metalloorganic reagents. Therefore, lactone **2-24** was converted to benzyl ether **2-26**. To cleave the benzoyl ester group, Zemplén saponification was applied using freshly prepared sodium methanolate in methanol to obtain unprotected 3-deoxy-ribonolactone **2-25**. TLC monitoring showed that the reaction was completed in 30 minutes. The reaction mixture was worked up by addition of acidic ion exchange resin to the solution, and after filtration the solvent was evaporated. Without further purification, benzylation of **2-25** was performed in abs. DMF using NaH for deprotonation of the alcohol function, benzyl bromide as nucleophile, and TBAI as phase transfer catalyst. Benzyl ether **2-26** could be isolated as an oil (yield 46 %).

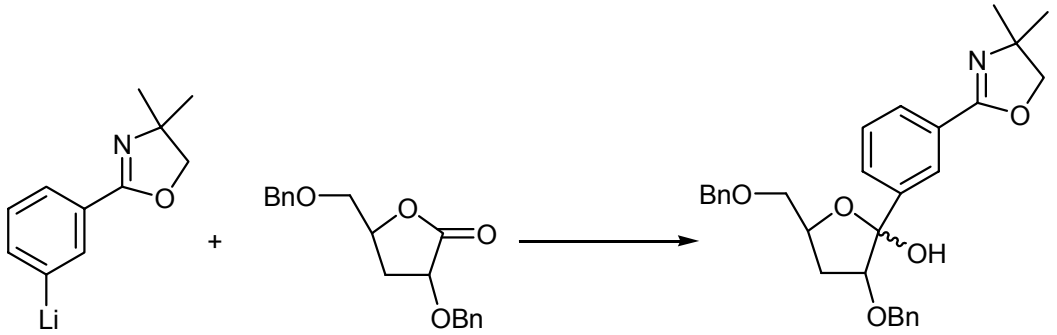


2-25 **2-26**

Scheme 2.15: Zemplén reaction of **2-24** and benzylation to ether **2-26**

2.4.1.2 Coupling of 1,4–Ribonolactone 2-26 with organometallic reagent (2-11)

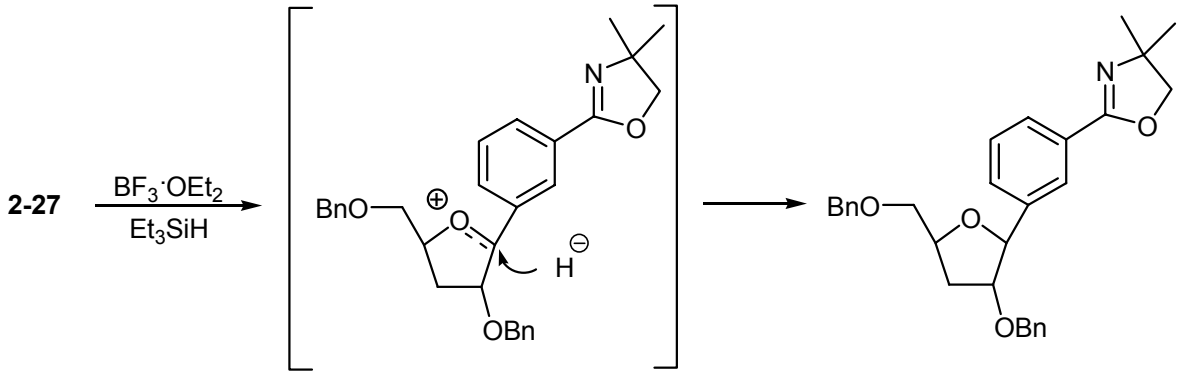
Using the conditions of the previously mentioned metalloorganic reaction,[29,30] the coupling between bromo oxazoline **2-10** and 3-deoxy lactone **2-26** was performed in abs. THF at −78 °C. Lithium oxazoline **2-11**, generated from oxazoline **2-10** by treatment with *n*-butyllithium, was reacted *in situ* with 3-deoxy lactone **2-26** to afford the tertiary alcohol **2-27** with a yield of 67 %.



2-11 **2-26** **2-27**

Scheme 2.15: Coupling reaction of lithiated oxazoline **2-11** and lactone **2-26**

Deprotonation and removal of the tertiary OH group took place under the same reaction conditions as described for BR. Due to thermodynamic control the reduction could be performed stereoselectively.

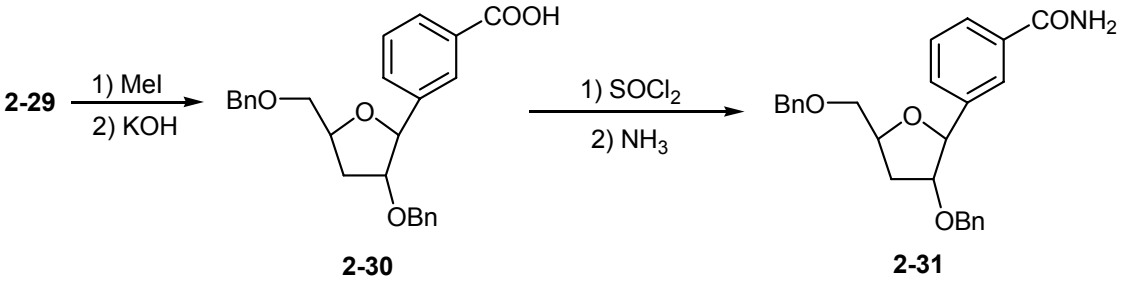


2-28 **2-29**

Scheme: 2.16: Mechanism of reduction by Et_3SiH

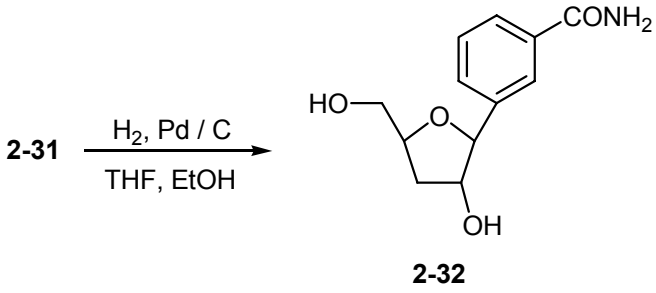
2.4.1.3 Transformation of *C*-glycoside 2-29 to 3-deoxy benzamide riboside (2-32)

Basic cleavage of the oxazoline protecting group resulted in acid **2-30**, which was purified by column chromatography on silica gel and then treated with thionyl chloride and subsequently with aqueous ammonia solution to obtain amide **2-31** in a yield of 88 % over 2 steps.[31]



Scheme 2.17: Preparation of benzyl protected 3-deoxy ribose phenyl amide **2-31**

Reductive cleavage of the benzyl ether by careful hydrogenation of **2-31** was carried out in abs. THF containing 5 % of ethanol. The reaction proceeded fast; TLC monitoring showed the complete consumption of the starting material **2-31** in 2 hours.



Scheme 2.18: Reductive cleavage of benzyl ether **2-31**

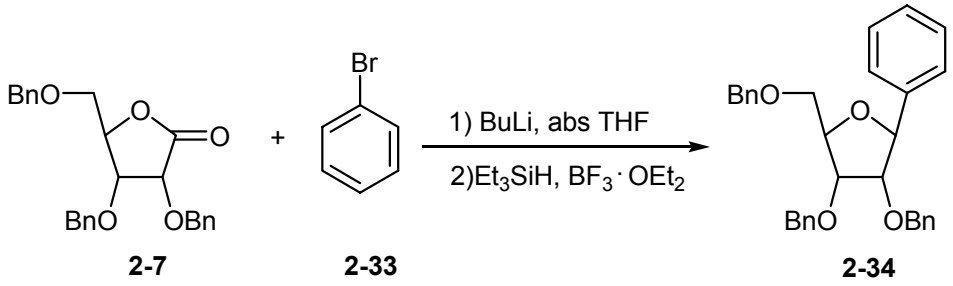
3-Deoxy-benzamide riboside **(2-32)** was isolated in a yield of 86 %. The formation of open-chain side product was not observed.

2.4.2 Preparation of derivatives with different substituents on the aromatic ring

New *C*-glycosides were prepared by coupling of lactone **2-7** with different *meta*-substituted bromo benzene derivatives in order to investigate if the amide function of the benzamide riboside (**2-2**) is necessary for the antitumor activity.

2.4.2.1 Synthesis of β-D-ribofuranosyl-benzene (2-35)

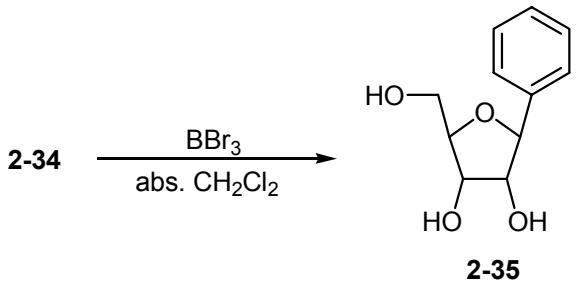
Primarily unsubstituted bromo benzene **(2-33)** was used in the same procedure[29,30] as mentioned before (see Chapter 2.2.3). The coupling reaction was performed in abs. THF at −78 °C and proceeded quickly. The reduction took place in dry dichloromethane by the addition of triethylsilane and boron trifluoride diethyl etherate giving an over-all yield of 67 % for the two steps.



2-7 **2-33** **2-34**

Scheme 2.19.: Preparation of C-glycoside **2-34**

Hydrogenation of benzyl ether **2-34** in abs. THF / 5 % methanol unfortunately did not lead to the desired ribofuranyl benzene (**2-35**). Only partially hydrogenated products were observed. Even increasing the polarity of the solvent did not result in the target molecule **2-35**. The cleavage of the benzyl ether protecting groups was therefore carried out by acidic cleavage using BBr_3 as a Lewis acid.



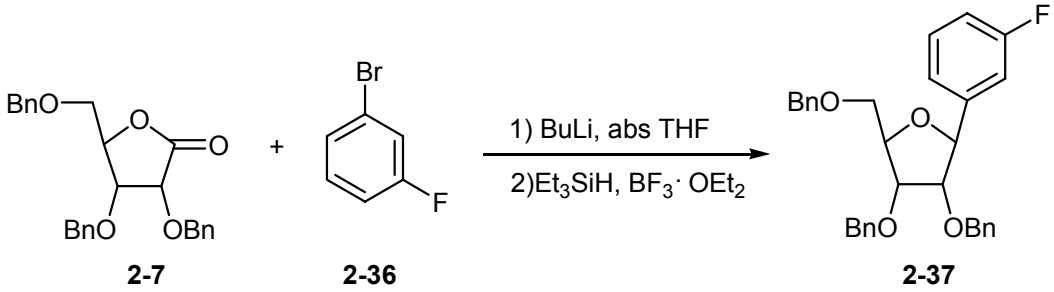
2-35

Scheme 2.20: Acidic cleavage of benzyl ether protecting group

Starting material **2-34** was entirely consumed after the reaction time of 10 min. at 0 °C. The reaction mixture was then neutralized by the addition of saturated aqueous $NaHCO_3$ solution and the water phase was evaporated under reduced pressure. The resulting white crystals (product **2-35** and inorganic salts) were dissolved in methanol and filtered. Ribofuranyl benzene (**2-35**) was isolated as the only product with a yield of 92 %.

2.4.2.2 Synthesis of β-D-ribofuranosyl-3´-fluorobenzene (2-38)

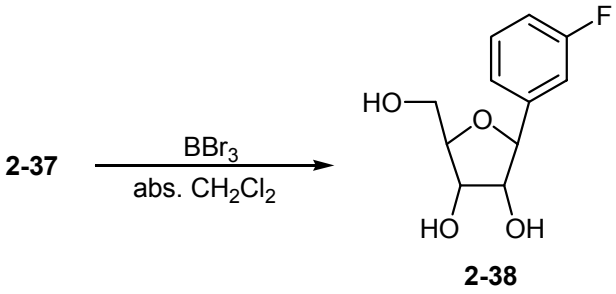
Since several fluoro sugar derivatives are potential anticancer and anti human immuno-deficiency virus agents (HIV),[35,36] the preparation of a fluoro derivative of benzamide riboside (**2-2**) was worth trying. Coupling reaction of commercially available 1-bromo-3-fluorobenzene (**2-36**) with ribonolactone **2-7** afforded the *C*-glycoside **2-37** with a good yield (83 %, after reduction of the tertiary hydroxyl group by $BF_3{\cdot}OEt_2$, Et_3SiH). Because aryl bromide reacts considerably faster with *n*-BuLi than aryl fluoride no other product was isolated from the reaction mixture.



2-7 **2-36** **2-37**

Scheme 2.21: Coupling reaction of 1-bromo-3-fluorobenzene (**2-36**) with ribonolactone **2-7**

As the last step, the cleavage of benzyl ether was performed. Also in this case, hydrogenation was not successful. However, using BBr_3 as Lewis acid in dry dichloromethane, the target compound **2-38** could be obtained after purification with a yield of 75 %.

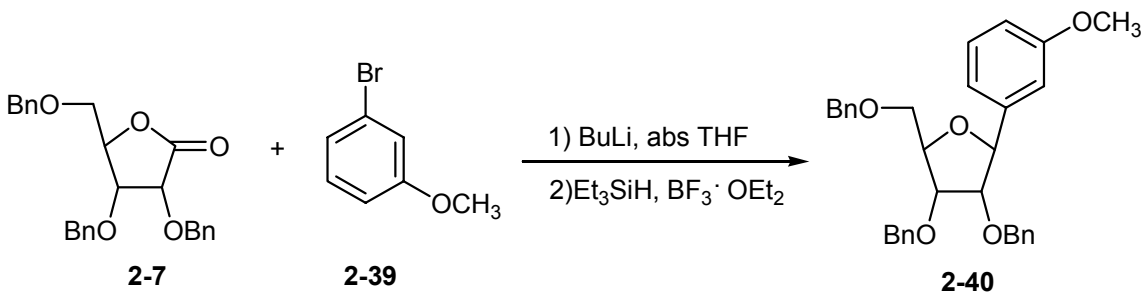


2-38

Scheme 2.22.: Preparation of β-D-ribofuranosyl-3´-fluorobenzene (**2-38**)

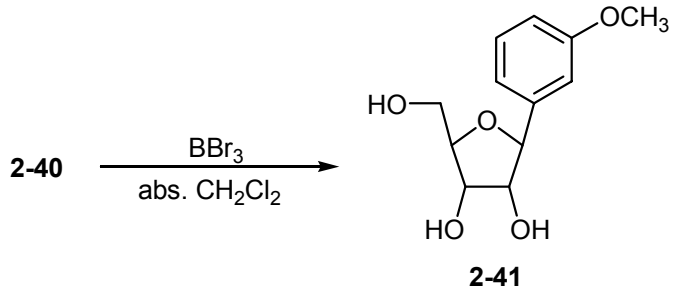
2.4.2.3 Synthesis of β-D-ribofuranosyl-*m*-anisol (2-41)

For use in the following investigation on structure activity relationship of benzamide riboside (**2-2**), a strong electron donating substituent in *meta* position was applied. 3-Bromoanisol (**2-39**) was coupled with ribonolactone **2-7** and the intermediate tertiary alcohol was reduced. (see Chapter 2.2.3).



Scheme 2.23.: Coupling of 3-bromoanisol (**2-39**) and ribonolactone **2-7**

Due to the fact that the activation energy of the cleavage of a benzyl group by a Lewis acid is lower then that of a methyl ether, the selective debenzylation could be performed at −78 °C using BBr_3. TLC monitoring showed that the reaction was completed after 1.5 hours at this temperature. After neutralization by the addition of aqueous $NaHCO_3$ solution, the target compound **2-41** could be isolated from the water phase. Using a small column of silica gel for purification, *β*-D-ribofuranosyl-*m*-anisol was obtained in 85 % yield.



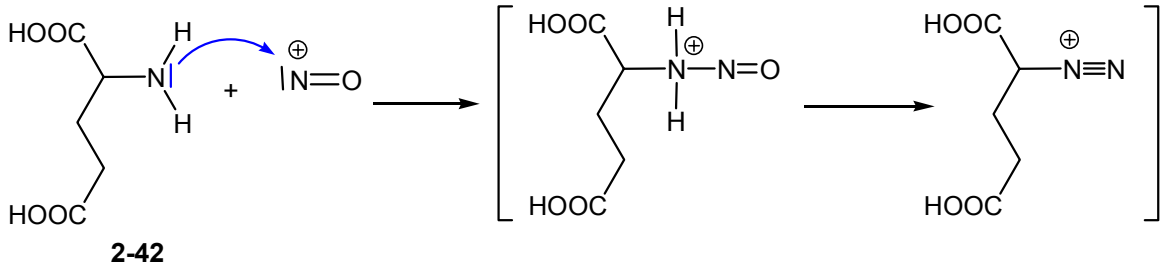
Scheme 2.24.: Debenzylation to **2-41**

2.5 Investigation Towards the Synthesis of 2-deoxy- and 2,3-dideoxy-Benzamide Riboside

2.5.1 Preparation of 2,3-dideoxy-5-benzyl-ribonolactone (2-46)

2,3-Dideoxy ribonolactone (**2-45**) was prepared from L-glutamic acid (**2-42**) as described in the literature.[37] In the presence of HCl, glutamic acid (**2-42**) was treated with aqueous $NaNO_2$ solution; HNO_2 was generated during this process. Protonation and cleavage of a water molecule resulted in a mesomerically stabilized nitrosyl cation. In the next step a nucleophile attack of the amine on the nitrosyl cation intermediate took place and a

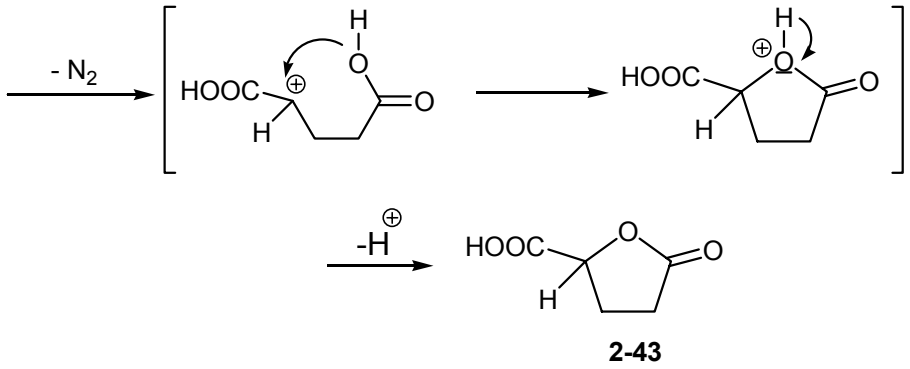
nitrosoamine salt was formed. After several deprotonation and protonation steps, H_2O was eliminated and a diazonium ion is formed.



2-42

Scheme 2.25: Formation of diazonium ion

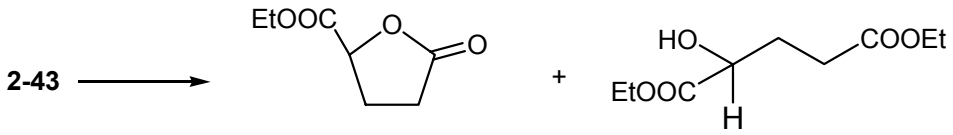
Nitrogen is eliminated to give a very reactive cation. Ring closure occured and subsequent deprotonation yielded acid **2-43**.



2-43

Scheme 2.26: Ring closure to acid **2-43**

The acid **2-43** was reduced to the corresponding alcohol in two steps. Esterification was performed in benzene/ethanol using *p*-toluene sulfonic acid as catalyst and resulted in the formation of **2-44a** and open chained diester **2-44b**.

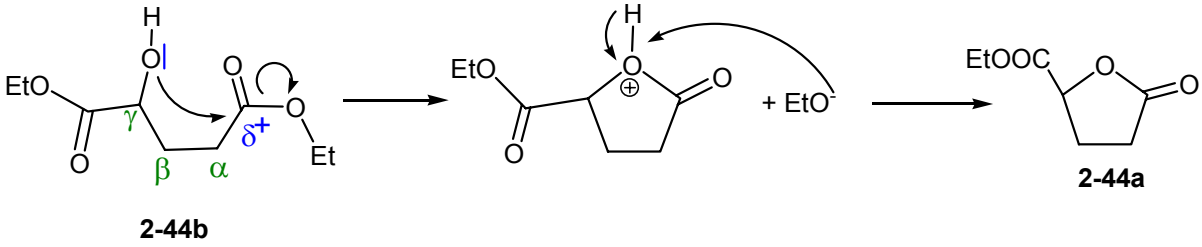


2-44a **2-44b**

Scheme: 2-27: Esterification to **2-44a** and **2-44b**

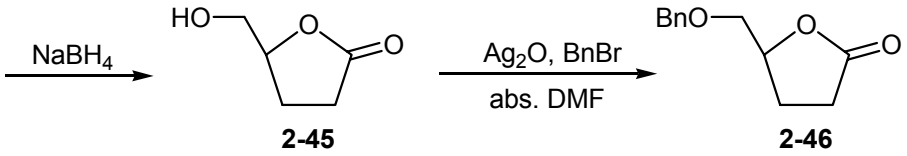
The separation of cyclic- **2-44a** and open-chained esters **2-44b** was not necessary because during the reduction with sodium borohydride in ethanol the open-chained product **2-44b**

cyclisized to **2-44a** and secondary alcohol **2-45** was isolated as a single product of the reduction.



Scheme: 2-28: Ring closure of ester **2-44b** to **2-44a**

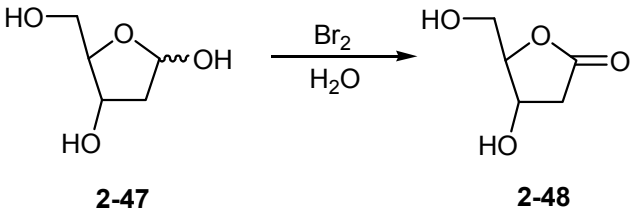
The benzylation of the secondary alcohol **2-45** was performed in abs. DMF using freshly prepared Ag_2O as a base instead of NaH to avoid the opening of the lactone ring.[38]



Scheme 2.29: Preparation of 2,3-didesoxy-5-benzyl-ribonolactone (**2-46**)

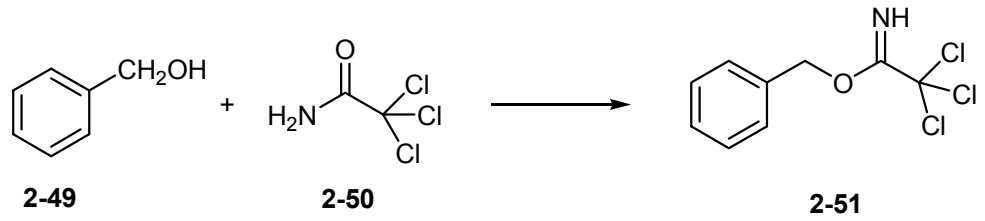
2.5.2 Preparation of 2-deoxy-3,5-dibenzyl-ribonolactone (2-52)

Commercially available 2-deoxy ribose (**2-47**) was employed as starting material. Using the previously mentioned oxidation procedure,[33] 2-deoxy (**2-47**) ribose was converted into 2-deoxy ribonolactone (**2-48**) (see Chapter 2.3.1.1).



Scheme 2.30: Oxidation to 2-deoxy-lactone **2-48**

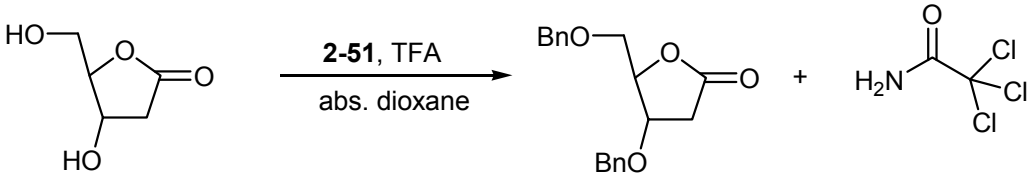
For benzylation, the trichloroacetimidate procedure of Schmidt was chosen.[39] The benzylation agent benzyl-2,2,2-trichloroacetimidate (**2-51**) was prepared from benzyl alcohol (**2-49**) and trichloroacetamide (**2-50**) in dry diethyl ether under acidic conditions. The imide formation proceeded smoothly within 10 minutes.[40]



2-49 **2-50** **2-51**

Scheme 2.31: Preparation of benzyl-2,2,2-trichloroacetimidate (**2-51**)

2-Deoxy-3,5-dibenzyl-ribonolactone (**2-52**) was obtained after purification in a very good yield by treating diol (**2-48**) with benzyl-2,2,2-trichloroacetimidate (**2-51**) as benzylation agent in the presence of trifluoromethanesulfonic acetic at room temperature. Trichloroacetamide (**2-53**) that formed during the reaction crystallized readily and could be separated by filtration.

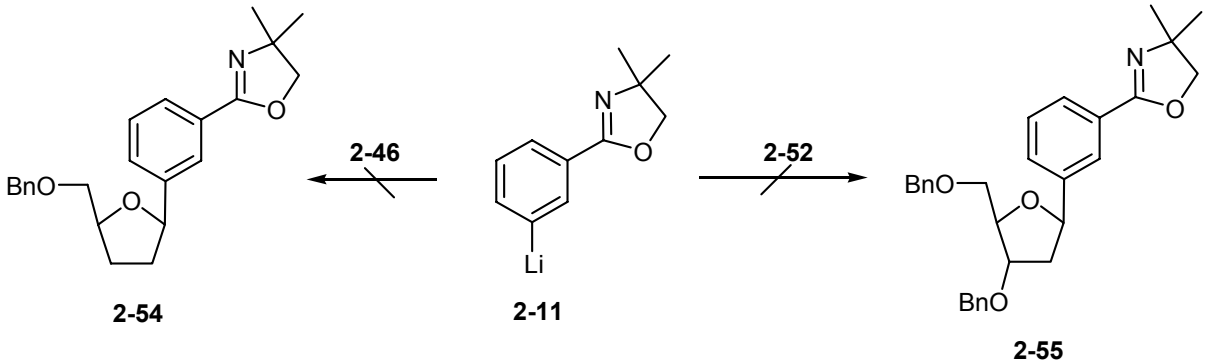


2-48 **2-52** **2-53**

Scheme 2.32: Benzylation of ribonolactone **2-48** by benzyl-2,2,2-trichloracetimidate (**2-51**)

2.5.3 Investigation on the coupling of deoxy-lactones 2-46 and 2-52 with lithiated oxazoline 2-11

Both ribonolactone derivatives **2-46** and **2-52** were subjected to metalloorganic coupling reaction with lithiated oxazoline **2-11** in order to prepare the *C*-glycosides **2-54** and **2-55** using the methods mentioned in Chapter 2.2.4.



2-54 **2-11** **2-55**

Scheme 2.33: The attempted coupling of metal-organic reagent **2-11** with the lactones **2-46** and **2-52**

Unfortunately no transformation was observed. In the literature the coupling reaction of various substituted lithiated aryl compounds with cyclic or open chained ketons was performed by the addition of TMEDA[41,42]. However, the addition of TMEDA to the reaction mixture in the case of both metalloorganic coupling reactions did not yield the desired products **2-54** and **2-55**.

This reaction of lithiated aryls with lactones was a convenient method for the preparation of benzamide riboside (**2-2**), 3-deoxy-benzamide riboside (**2-32**), and BR-derivatives **2-35, 2-38, 2-41**, but gave no product for **2-46** and **2-52**. The failure could be explained by the absence of a benzyloxy substituent in position *C*-2 of ribonolactone. Evidently, this group plays a significant role during the reaction. The existence of the neighboring group effect could provide an explanation. Probably the oxygen atom of the benzyloxy group participated in the coupling reaction in a way (chelat formation), that the presence of it is indispensable to perform the reaction.

2.6 Biological test results

The cytotoxicity of compounds **2-2**, **2-32**, **2-35**, **2-38**, **2-41** were examined against both human myelogenous leukemia K562 cells and human colon carcinoma HT-29 cells.

Benzamide riboside (**2-2**)**,** at a concentration which kills about 20 % of the lymphoma cells, only slightly increases the toxicity of dexamethasone on 549.1 lymphoma cells (in contrast to benzamide). Therefore, it appears that the poly(ADP-ribose) synthetase is not preferentially affected by benzamide riboside (**2-2**) or the benzamide containing NAD analogue compared to other NAD-dependent enzymes. Although benzamide riboside (**2-2**) does not potentiate the toxicity of glucocorticoids in S49.1 lymphoma cells, it could be an interesting agent in antitumor cell therapy. Since the concentration of pyridine dinucleotides in malignant cells has been shown to be low as compared to normal tissue, tumors could be more susceptible to compounds such as benzamide riboside (**2-2**)**.** The riboside (**2-2**) is presently being tested for antitumor activity *in vivo*. The compound did not show any stimulatory or suppressive behavior in immunomodulation tests with human lymphocytes.

It was shown that 5 μM BR (**2-2**) induces apoptosis of HL-60 cells. Increasing the concentration to 20 μM BR provokes necrosis of HL-60 cells, which is reflected by typical membrane and organelle disintegration. A rapid and sustained decrease in the intracellular concentration of GTP and dGTP was observed. 10μM BR (**2-2**) treatment of K562 cells for 2

hours significantly decreases GMP, GDP, GTP and dGTP levels without changing the ATP level.

3-Deoxy benzamide riboside (**2-32**), and derivatives **2-35, 2-38, 2-41** did not show inhibition both in human myelogenous leukemia K562 and human colon carcinoma HT-29 cells up to 100 μM concentration. That is, both the amide function of the benzene ring and the *C*-2 hydroxyl group are necessary for the antitumor activity.

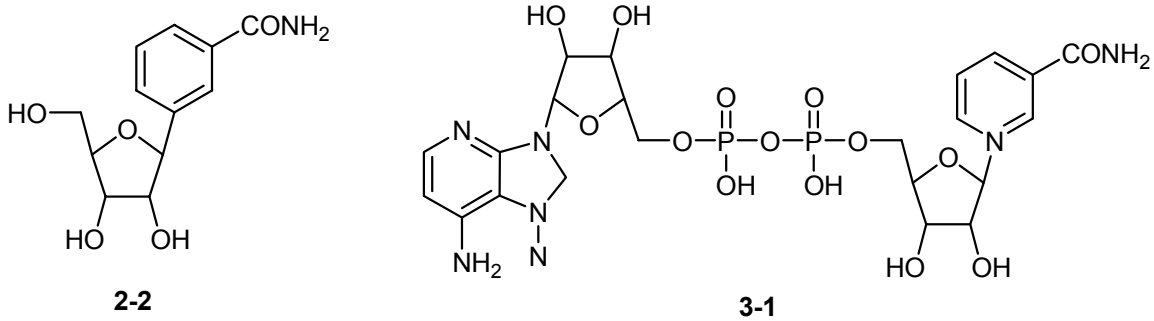
Benzamide riboside (**2-2**) exhibits potent antitumor activity against a diverse panel of human cancer cells. The cytotoxic potency is time related and dependent on duration of exposure to the drug. Benzamide riboside (**2-2**) might be a new addition as a promising antitumor agent. Further *in vivo* studies in animals should provide the basis for future clinical studies.

3 Synthesis of Benzamide Riboside Adenine Dinucleotide

This chapter focuses on the chemical synthesis of benzamide riboside adenine dinucleotide (BAD) (**3-5**). The enzymatic synthesis from benzamide riboside 5´-monophosphate (**3-3**) and ATP (adenosine triphosphate) has already been described in the literature,[43] utilizing a partially purified commercially available preparation of NAD pyrophosphorylase from hog liver. In cooperation with the Max Plank Institute for Biophysics in Frankfurt, Germany enzyme-substrate complex studies will be performed using BAD (**3-5**), to investigate the active site of various reductase enzymes.

3.1 Introduction

Benzamide riboside (**2-2**) is related to NAD (**3-1**) (nicotinamide adenine dinucleotide), in which the pyridine ring of NAD is replaced by a benzene ring. However, benzamide riboside (**2-2**) binds to the NAD bonding site of enzymes. NAD plays a very important role in many biochemical processes. NADH formed during the glycolysis is reoxidised under anaerobic conditions. In the citric acid cycle, it acts as a coenzym of the isocitrate-dehydrogenase, which catalyses the reduction to α-ketoglutarate. Since benzamide riboside is a *C*-glucoside, it is unable either to accept or to donate protons. This way the biological processes are stopped and the cell dies.



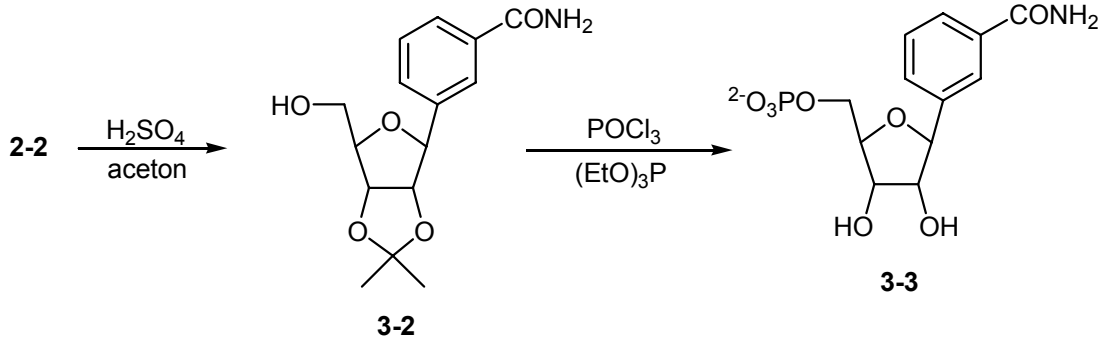
2-2 **3-1**

Scheme 3.1: Structure of benzamide riboside (**2-2**) and NAD (**3-1**)

In order to accomplish these studies, the NAD analogue BAD (benzamide riboside adenine dinucleotide) (**3-5**) was prepared *in vitro*.

3.2 Synthesis of benzamide riboside adenine dinucleotide (3-5)

The starting material of this synthesis was the target compound of the previous Chapter 2.3. To get monophosphate **3-3**, the tertiary OH-groups of benzamide riboside (**2-2**) were protected by an isopropylidene group, which was prepared in acetone and a few drops of H_2SO_4 was applied as a catalyst.

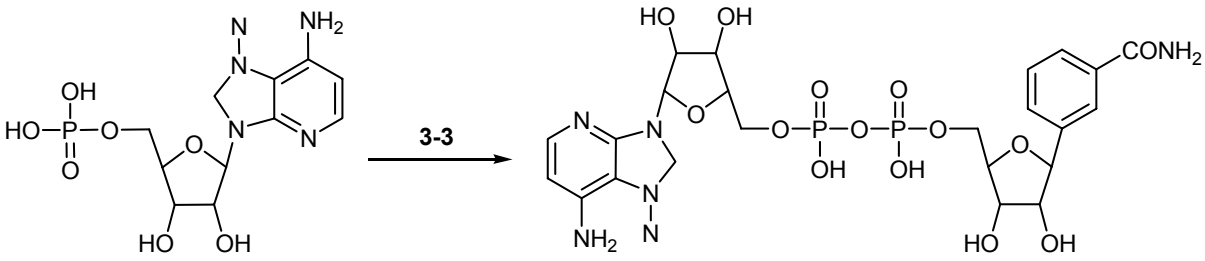


Scheme 3.2: Preparation of monophosphate **3-3**

Phosphorylation of **2-2** and cleavage of the acetonide group proceeded in a one-pot reaction after the standard method of Ludwig[44] to generate the monophosphate **3-3**. The coupling of the monophosphate **3-3** with adenosine monophosphate (**3-4**) was carried out by Michael Morr in Braunschweig at the GBF, Germany according to the method of Marquez et al.[45,46]. This procedure consisted of activating one of the nucleotides (as usual, the five-membered base nucleotide) with carbonyldiimidazole.

The formation of the imidazolidates was easily monitored by HPLC and required no isolation. When the reaction was completed, methanol was added to hydrolyze the excess of reagent, followed immediately by the addition of AMP (**3-4**) as a DMF solution containing tri-*n*-butylamine. Formation of the dinucleotide was complete after 48 h. The aqueous extract containing both the product **3-5** and its 2',3'-cyclic carbonate analogue, was treated by triethylamine to convert it to the deblocked product **3-5**.[47]

In those cases where this last step was omitted, significant amounts of the corresponding cyclic carbonate dinucleotide was obtained as verified by MS analysis. After the triethylamine treatment product **3-5** was purified by chromatography on Hamilton X4-H4 anion exchange column.



3-4 **3-5**

Scheme 3.3: Preparation of BAD (**3-5**)

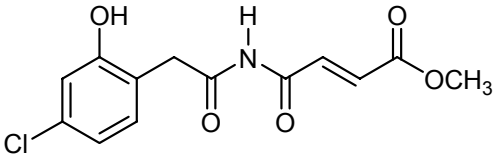
3.3 Enzym-substrate complex studies

Due to the high similarity between BAD (**3-5**) and NAD (**3-1**), BAD (**3-5**) also binds to the active site of NAD binding enzymes. However, BAD (**3-5**) probably binds irreversible, since it can neither accept nor donate protons. For this reason **3-5** is a suitable substrate for enzyme-substrate complex studies. X-ray crystallographic studies of the complex with reductase will be performed by Dr. Carola Hunte at the Max Plank Institute for Biophysics in Frankfurt, Germany. The combined approach of X-ray crystallography, biochemical analysis, site-directed mutagenesis and spectroscopy will provide valuable information about the active site of the enzyme. If the active site of the reductase is known, further benzamide riboside derivatives, with a better selectivity in tumor cells, can be developed. On the other hand the molecular mechanism of action of the reductase can be explored, since there are several possible pathways for proton uptake and release.

4 Synthesis and Structure-Activity Relationship of Antifungal Coniothyriomycin Analogues

4.1 Introduction

In the research group of Prof. Krohn, an open-chain imide named coniothyriomycin **(4-1)** with remarkable antifungal activity was isolated from an unidentified fungus *Coniothyrium sp.*[48,49]



coniotyriomycin **(4-1)**

Figure 4-1.: Structure of coniothyriomycin **(4-1)**

Unfortunately, these open-chain mixed amides of phenylacetic and fumaric acid, in spite of excellent short-term antifungal activity, did not show curative effects. One reason for this was the inherent instability of the imide functionality in the presence of nucleophiles such as water. Therefore, in the hope to increase the chemical stability and retain or even increase antifungal activity, different coniothyriomycin derivatives were prepared to examine the structure-activity relationship. This way, we extended our study to the preparation and biological testing of various analogues by systematically changing the structure of **4-1** by:

- replacement of the substituted phenylacetic acids with substituted benzoic acids,
- changing of hydrophobicity by variation of the alcohol component,
- variation in the degree of saturation of the fumaric acid moiety,
- replacement of carbon by nitrogen, oxygen or sulfur in the middle part of the molecule,
- incorporation of the open-chain part of the molecule into cyclic arrangements,
- changing the substitution pattern of the aromatic ring.

4.2 Developing a new method for the synthesis of mixed imides

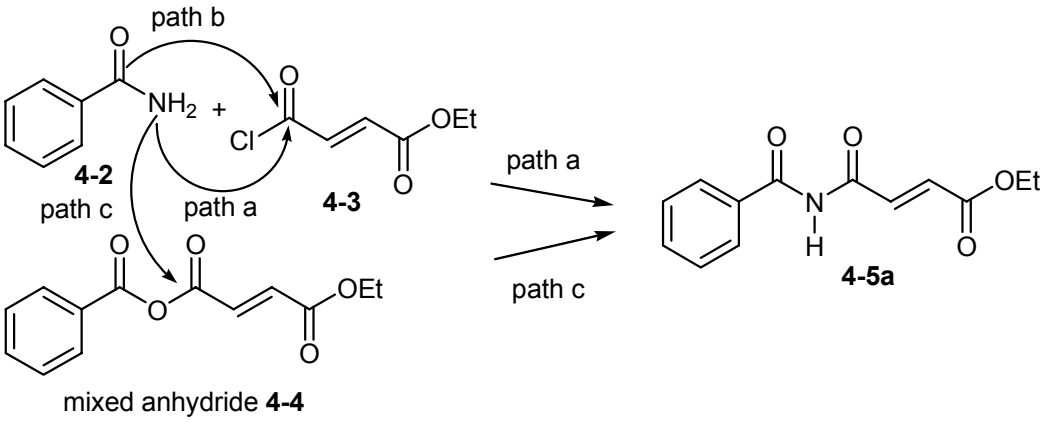
In the previous syntheses of coniothyriomycin analogues[48] the method of Suhara et al.[50] was used, starting from nitriles via the corresponding ethyl imidates. However, the yields using this method were poor (10–36 %) and the workup of the dark brown reaction mixture was tedious.

The efforts were therefore directed towards a simplification of the procedure. To that end, equivalent amounts of benzamide (**4-2**) were heated with the fumaric monoethyl ester chloride[51] (**4-3a**) in toluene (Scheme 1). However, starting with a 1:1 ratio, only modest yields of the desired mixed imide **4-5** were observed and the mixed anhydride **4-4** was identified as the major side product.

Evidently, both theoretically possible reaction pathways **a** and **b** were realized: attack by the amide nitrogen on the acyl chloride **(path a)** to form **4-5a**, and attack by the nucleophilic oxygen **(path b)** to form **4-4**. However, the acyl group transfer potential was still preserved in the mixed anhydride **4-4** and attack by **excess** benzoic acid amide {benzamide} **(path c)** on the mixed anhydride carbonyl might ultimately result in the formation of the mixed open chain imide **4-5a**.

In fact, using two equivalents of amide **4-2**, **pathway c** was followed consuming the mixed anhydride **4-4**, and resulted in the mixed imide **4-5a**. Yields of 50 % and more were achieved in a clean, easily worked-up reaction.

Interestingly, the formation of the symmetric benzoic acid imide, resulting by attack of the nitrogen on the benzoic acid part of anhydride **4-4**, was not observed.

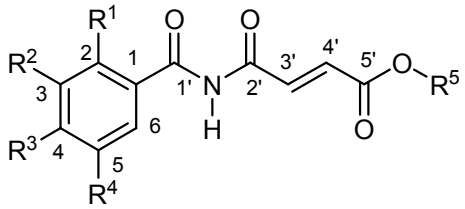


Scheme 4-1: Formation of mixed imide **4-5a** starting from amide **4-2** and acid chloride **4-3a** via the mixed anhydride **4-4**

The newly developed method proved to be general and most of the mixed imides **(4-5a to 4-5h, 4-10 to 4-14, 4-21),** were prepared using this simple procedure. However, in some cases it was advantageous to use the anion of amides such as **4-2**. Also, the yields were generally higher starting with benzamide (**4-2**) than with phenylacetic acid amide (**4-6a**) (Scheme 4-2).

4.3 Synthesis of coniothyriomycin analogues – Variation of the substitution pattern of the benzyl-ring

The first series of compounds were a number of mixed benzoic acid and fumaric ester open chain imides with variation in the substituents in the aromatic ring and the ester alcohol component. It was investigated whether omission of the methylene group in **4-5a** by replacement of phenylacetic acid in the coniothriomycin analogues[48] with benzoic acid would preserve their antifungal activity. In addition, variation of the substituents from methoxy to fluoride or nitro groups in **4-5b to 4-5g** (Figure 4-2) would show the influence of electron density on biological activity. Furthermore, the lipophilicity of the fungicide was increased in **4-5h** by linking the *n*-octanyl ester of fumaric acid chloride to benzamide. The data and substituents of the mixed benzoic acid-fumaric ester imides **4-5a to 4-5h** are listed in Table 4-1.



4-5a to 4-5h

Figure 4-2: Mixed benzoic acid-fumaric ester imides **4-5a** to **4-5h**

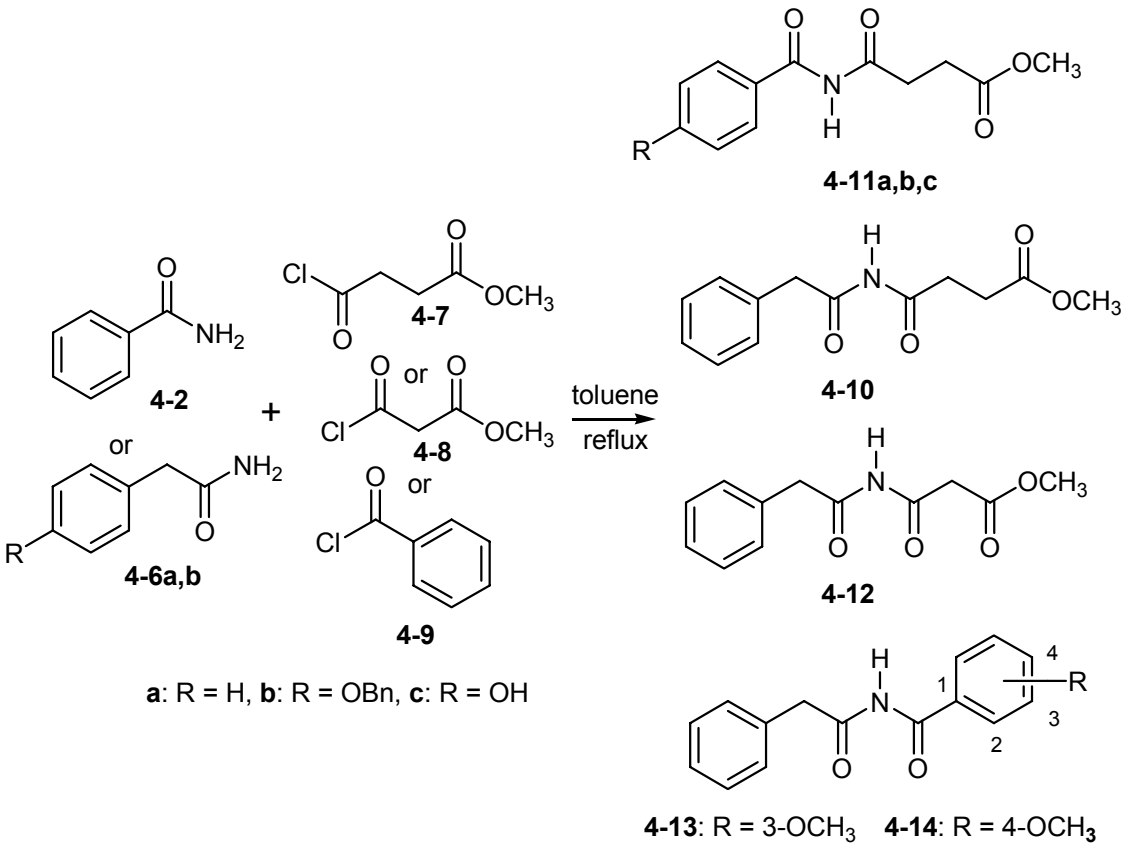
	R^1	R^2	R^3	R^4	R^5	mp (°C)	yield (%)
4–5a	H	H	H	H	C_2H_5	87–89 °C	51 %
4–5b	F	H	H	H	C_2H_5	54-55°C	42 %
4–5c	H	H	NO_2	H	C_2H_5	135-137°C	44 %

4–5d	OCH_3	H	H	H	C_2H_5	98-100°C	46 %
4–5e	H	H	OCH_3	H	C_2H_5	120-122°C	45%
4–5f	H	OCH_3	H	OCH_3	C_2H_5	162-164°C	43 %
4–5g	H	OCH_3	OCH_3	OCH_3	C_2H_5	114-116°C	65%
4–5h	H	H	H	H	*n*-C_8H_{15}	oil	33 %

Table 4-1: Data and substituents of mixed benzoic acid-fumaric ester imides **4-5a** to **4-5h**

4.4 Synthesis of coniothyriomycin analogues – Changing the degree of saturation, and hydrophopbicity in the fumaric ester side-chain

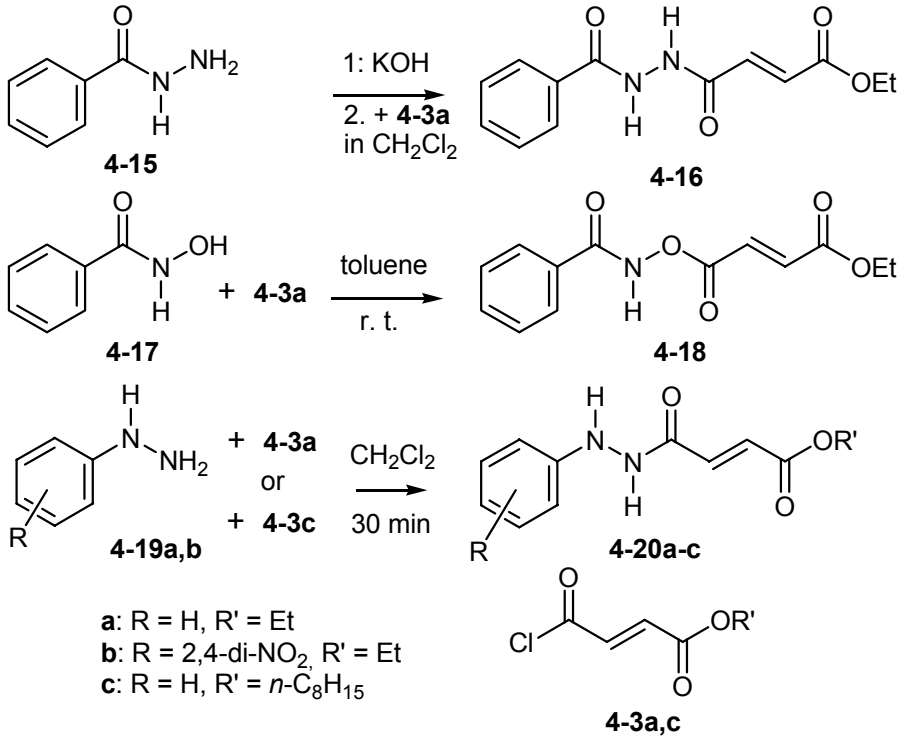
Next, the variation in the degree of saturation in the fumaric ester part was analyzed, starting from phenylacetic amide as well as from benzoic acid amide. Omission of a methine group, e.g. the shift from fumaric ester to malonic ester, or replacement with benzoic acid (incorporating the double bond of fumaric acid into a ring system) was also in line with this type of variation and worth testing for fungicidal activity. The construction of the mixed imides **4-10 to 4-14** by coupling of amides **4-2** and **4-6a,b** with the acid chlorides **4-7 to 4-9** is shown in Scheme 4.2, employing about two equivalents of the respective amides as outlined in Scheme 4.1. The phenolic imide **4-11c**, as present in the natural product coniothyriomycin (**4-1**), was prepared by reaction of benzyl ether **4-6b** with **4-7**, followed by hydrogenolysis of benzyl ether **4-11b** to **4-11c** in order to evaluate the influence of a phenolic hydroxy group on activity.



Scheme 4-2: Construction of mixed imides **4-10** to **4-13** by coupling of amides **4-2** and **4-6a,b** with the acid chlorides **4-7** to **4-9**.

4.5 Synthesis of coniothyriomycin analogues - Replacement of carbon by nitrogen, oxygen or sulfur in the middle part of the molecule

Next, we investigated the replacement of carbon by nitrogen, oxygen or sulfur in the middle part of the molecule to study the effect of these exchanges on bioactivity. Three different types of compounds resulted from these exchanges: The *bis*-acylated hydrazine **4-16**, the acylated hydroxamide **4-18**, and the acylated phenylhydrazines **4-20a to 4-20c**. The compounds were prepared by reaction of the hydrazide anion of **4**–**15**, the hydroxamic acid **4-4-17** or the phenylhydrazines **4-19a,b** with the acid chlorides **4-3** or **4-3c**, respectively, as outlined in Scheme 4-3.



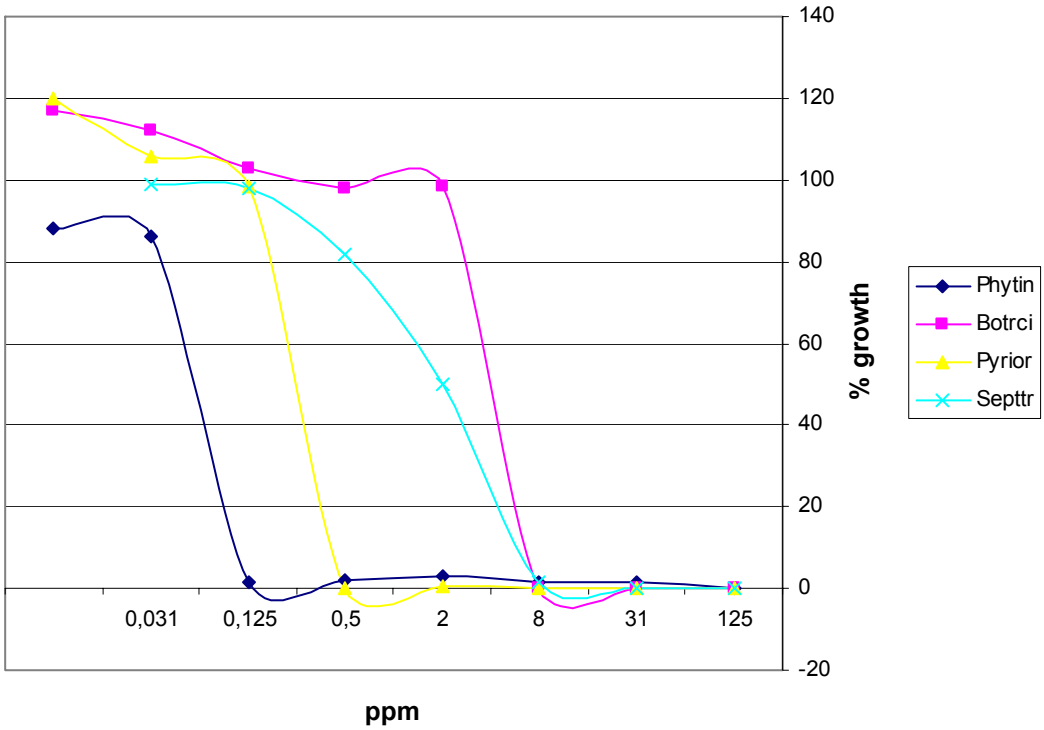
Scheme 4-3: Synthesis of *bis*-hydrazide **4-16**, acyl hydroxamic acid **4-18** and fumaric hydrazides **4-20a-c**

4.6 Biological Studies

The tested synthetic coniothyriomycin analogues showed primarily control of plant diseases caused by representative fungi belonging to the class of *Oomycetes*, for example late blight on tomatoes caused by *Phytophthora infestans* or downy mildew on grape vine caused by *Plasmopara viticola*. The fungicidal activity was tested *in vitro* in 96-well microtitre plates and on intact plants in a greenhouse.

The change of the molecular fragment phenylacetic amide to benzoic amide in **4-5a to 4-5h** retained the same good in vitro activity as the lead structure coniothyriomycin. **4-5a to 4-5d** controlled *P. infestans* with ED_{90}-values of less than 0.5 ppm, **4-5e** even at 0.125 ppm. Some additional control of the casual organism of rice blast, *Pyricularia oryzae*, and of the casual organism of leaf blotch on wheat, *Septoria tritici* was observed for **4-5a to 4-5e** with ED_{90}-values of less than 2 and 8 ppm respectively. The fungicidal *in vitro* activity could only partially be translated into *in vivo* activity in greenhouse tests on intact plants. Only **4-5f**

showed some initial protective control of *P. infestans* on tomatoes with additional initial good protective activity against *P. viticola* on grapes. The compounds **4-5d, 4-5g** and **4-5h** showed only some moderate control of *P. viticola*.



Scheme 4-4: Biological activity of **4-5a**.

The hydrogenation of the double bond in the fumaric acid fragment, which resulted in **4-10** and **4-11a**, led to loss of fungicidal activity both *in vitro* and *in vivo*. The other structural variations in the compounds **4-13** and **4-14** resulted in reduced *in vitro* fungicidal activity against *P. infestans*, with ED_{90}-values of 31 and 8 ppm respectively. All other structural variations in compounds **4-12, 4-16, 4-18, 4-20a to 4-20c** led to a loss of any significant *in vitro* or *in vivo* fungicidal activity.

5 Structure-Activity Relationships in Allergic Contact Dermatitis

5.1 Introduction

Non-terpenoid and diterpenoid phenanthrenequinones (PAC) have been found in the plant kingdom. Some of them occur in plants used in traditional Chinese medicine like Tan-Shen, while others are constituents of orchids that are popular as ornamental plants. Quinones are found mainly in plants, fungi, bacteria and the animal kingdom. By far the largest group in plants involves the anthraquinones, benzoquinones and naphthoquinones.

Phenanthrenequinones (PAC) of non-terpenoid origin are rarely detected, and some of them are simply oxidation products from their hydroxylated phenanthrene precursors. Currently known naturally occurring 1,4-phenanthrenequinones have been isolated from species of the orchid family, (denbinobin from *Dendrobium nobile* Lindl. and other orchid species,[52,53,54] cypripedin from *Cypripedium calceolus* L.),[55] Annonaceae (annoquinone-A from *Annona montana* MacFad.),[56] Labiatae (plectranthrones from *Plectranthus* species)[57,58] and Combretaceae (combretastatin C-1 from *Combretum sp*.).[59] Also tropical timbers of the Leguminosae-Papilionaceae family contain phenanthrenequinones such as latinone (in *Dalbergia latifolia* Roxb.)[60] and melatinone (in *D. melanoxylon* Guill. & Perr).[61]

A greater number of structurally related diterpenoid and abietanoid quinones, like the tanshinones, royleanones and miltirones, do occur in species of the *Dioscoraceae, Lamiaceae, Taxodiaceae and Compositae,*[62,63,64,65,66,67] while the seeds of *Taxodium distichum* (L.) Rich. contain taxodione and quinone methides.[68] (Both quinone methides are relatively stable).

Hazardous effects of phenanthrenequinones to the skin have been described as irritant or as causing allergic contact dermatitis as early as 1875. H. H. Babcock, a well-known botanist from Chicago, suffered from severe dermatitis of his hands and face after collecting field-grown lady's slippers (*Cypripedium calceolus*).[69] Similar skin lesions caused by related *Cypripedium* and *Paphiopedilum* species were observed by MacDougal several years later[70,71] Babcock's bad experiences motivated Nestler in Prague to rub leaves and extracts of different *Cypripedium* species on the skin of his arms. A month later strong itching developed, followed by redness and vesicles.[72] Retired individuals who spent all of their leisure time in breeding orchids developed allergic contact dermatitis as described between

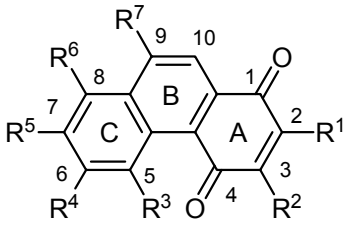
1957 and 1980[73,74,75] In the eighties several cases of occupational allergic contact dermatitis have been observed in female employees handling orchids of the genus *Cypripedium*, *Paphiopedilum*, *Cattleya* and *Cymbidium* in orchid nurseries in Lower Saxony (Germany). However, the sufferers refused any patch testing because they were deeply convinced that "such beautiful flowers never could be the sources of their skin lesions". Therefore, only chemical studies of wild and bred plant material from Germany and the USA were performed revealing the occurrence of quinonoid constituents in some of these species.[76] In two other nurseries two cultivators could be tested epicutaneously with leaves, petals and stems as well as with two isolated quinones giving strong responses. However, due to insufficient plant material (orchids were extremely expensive at that time) the reacting quinones could not be isolated in quantities sufficient for structure elucidation.[77] Nestler's assumption of 1907 that the 'toxic' constituent of one of the *Cypripedium* species might be a red quinone was corroborated more than 70 years later when the responsible allergen could be identified as 2,8-dimethoxy-7-hydroxy-1,4-phenanthrenequinone (cypripedin) (compound **5-6**, Tab. 5-1).[57]

The most valuable commercial timbers, East Indian rosewood (*Dalbergia latifolia*) and African blackwood (Grenadill) (*Dalbergia melanoxylon*), containing latinone and melatinone, respectively, have also been blamed for their sensitizing properties.[78]

Perianal contact dermatitis was observed in eight patients in Kenya using maigoya leaves from *Plectranthus barbatus* Andr. (family Lamiaceae) as toilet paper.[79] Black bryony (*Tamus communis* L.) has since been well known for its irritant properties.[80] Other plants and timbers of the Combretaceae, Dioscoraceae and Compositae family, containing phenanthrenequinones and related compounds, have not been described so far for causing skin reactions. However, Hopff & Schweizer[81] could already demonstrate in early calculations that some of these phenanthrenequinones are highly reactive in nucleophilic substitution. Furthermore, there is evidence that phenanthrenes, abundantly occurring as precursors in distinct plants and timbers, will be oxidized under proper conditions to either *ortho*- or *para*-phenanthrene-quinones.[82]

5.1.1 The investigated phenanthrene-quinones

Case reports suggest that some or even all of these PACs possess a distinct sensitizing potency. In order to study the relationship between chemical structure and sensitizing properties,[83] a number of known, as well as new PACs were synthesized chemically. In a first step, the Diels-Alder reaction of styrenes and benzoquinones was used, leading to a number of substituted PACs or the corresponding dihydro components. In an alternative approach, the well-known photocyclization of stilbenes was exploited to prepare the PACs. Details of these syntheses have been published recently in a chemical journal.[84] To obtain more details on structure-activity relationships, guinea pigs were sensitized to determine the sensitizing capacity of the 26 PACs (four naturally occurring and 22 synthetic PACs).



5-1 to **5-23**

Scheme 5-1: Substitutions pattern of phenanthrenequinones **5-1 to 5-23**

No	Phenanthrenequinone (PAC)	R^1	R^2	R^3	R^4	R^5	R^6	R^7
5-1	1,4-PAC	H	H	H	H	H	H	H
5-2	3-Methoxy-1,4-PAC (= Annoquinone-A)	H	OMe	H	H	H	H	H
5-3	6-Methoxy-1,4-PAC	H	H	H	OMe	H	H	H
5-4	7-Methoxy-1,4-PAC	H	H	H	H	OMe	H	H
5-5	8-Methoxy-1,4-PAC	H	H	H	H	H	OMe	H
5-6	2,8-Dimethoxy-7-hydroxy-1,4-PAC (= Cypripedin)	OMe	H	H	H	OH	OMe	H
5-7	3,6-Dimethoxy-1,4-PAC	H	OMe	H	OMe	H	H	H
5-8	3,7-Dimethoxy-5-hydroxy-1,4-PAC	H	OMe	OH	H	OMe	H	H

	(= Denbinobin)							
5-9	3,8-Dimethoxy-1,4-PAC	H	OMe	H	H	H	OMe	H
5-10	5,8-Dimethoxy-1,4-PAC	H	H	OMe	H	H	OMe	H
5-11	6,7-Dimethoxy-1,4-PAC	H	H	H	OMe	OMe	H	H
5-12	7,8-Dimethoxy-1,4-PAC	H	H	H	H	OMe	OMe	H
5-13	2,7,8-Trimethoxy-1,4-PAC (= Cypripedin methyl ether)	OMe	H	H	H	OMe	OMe	H
5-14	3,5,7-Trimethoxy-1,4-PAC	H	OMe	OMe	H	OMe	H	H
5-15	3,5,8-Trimethoxy-1,4-PAC	H	OMe	OMe	H	H	OMe	H
5-16	3,6,7-Trimethoxy-1,4-PAC	H	OMe	H	OMe	OMe	H	H
5-17	3,7,8-Trimethoxy-1,4-PAC	H	OMe	H	H	OMe	OMe	H
5-18	6,7,8-Trimethoxy-1,4-PAC	H	H	H	OMe	OMe	OMe	H
5-19	2,3,7,8-Tetramethoxy-1,4-PAC	OMe	OMe	H	H	OMe	OMe	H
5-20	3,5,6,8-Tetramethoxy-1,4-PAC	H	OMe	OMe	OMe	H	OMe	H
5-21	8-Benzyloxy-7-methoxy-1,4-PAC	H	H	H	H	OMe	Bn	H
5-22	3,6-Dimethoxy-7-hydroxy-9-phenyl-1,4-PAC (= Latinone)	H	OMe	H	OMe	OH	H	Ph
5-23	6,7-Dimethoxy-9-phenyl-1,4-PAC	H	H	H	OMe	OMe	H	Ph

Table 5-1: Substitutions pattern of phenanthrenequinones **5-1** to **5-23**

However, such experimental studies in guinea pigs have not been performed so far. Guinea pigs were sensitized by a modified Freund's complete adjuvant method (FCA, FCAT, GPMT)[85] in order to find out which and how many substituents at the carbons of the three rings of the PAC will influence the sensitizing power of the molecule.

5.2 Sensitization

A method developed from the Freund's complete adjuvant test (FCAT) and the guinea pig maximization test (GPMT); named *modified* FCAT has been used. Its advantage could be proven with a large number of compounds, especially in the determination of the sensitizing capacity of moderate and weak contact allergens. Ten guinea pigs of the Pirbright white strain were used for each substance. For induction, 15 mg of the PAC was dissolved in 4 mL FCA test solution and emulsified with 4 mL physiological saline. Then, six intradermal injections of 0.1 to 0.15 mL of the emulsion were administered in a semicircular arc on the clipped and shaved shoulder area (4×6 cm) from left to right. Starting on day 1, the procedure was repeated on the fifth and ninth day, leaving a gap of 2 to 3 cm between the rows of injections. Each guinea pig received a total of 4.5 mg of the PAC during the entire sensitization procedure. All sensitization experiments were performed by Prof. B. M. Hausen at the Department of Dermatology of the University Hospital, Hamburg, Germany.

Elicitation was performed 21 days after induction by open epicutaneous challenge with 0.05 mL of the compound dissolved in acetone in sub irritant molar concentrations and further dilutions on the left flank of the animals. In case the PAC did not dissolve sufficiently in acetone, 10 % ethanol, methanol, or ethyl acetate was added. Generally, molar concentrations (M) were used, starting with 0.3 mol/L and subsequent dilutions, in steps of 1:2 down to 0.0001 mol/L. Readings were performed after 24, 48, and 72 hours. The mean response (mr) was computed as the quotient of the sum of the reactions observed and the total number of guinea pigs treated. A mr of 0 to 1 was considered weak, 1 to 2 moderate, and a mr greater than 2 as strong.

Using the emulsion of FCA and physiological saline but without a PAC, ten guinea pigs were treated at the same intervals and under the same conditions as described above. One day before challenge, these animals were tested epicutaneously with 3 different concentrations of the PACs on the right flank (1.0 mol/L, 0.3 mol/L, and 0.1 mol/L). The results were read after 24 hours. A (+) reaction was considered the threshold of irritancy.

5.3 Sensitization test results

One quarter of the control animals developed a (+) reaction when challenged with the PACs in 1.0 mol/L concentration. Thus the safe sub irritant challenge concentration was chosen to be 0.1 mol/L. The results of the sensitization experiments are summarized in Table 3. The mr (mean response) given in the following table is the median of all 3 readings observed at a challenge concentration of 0.003 mol/L. Two PACs (**5-21**, **5-26**) showed a strong sensitizing capacity, eight a moderate one (**5-3**, **5-4**, **5-5**, **5-8**, **5-10**, **5-12**, **5-18**, **5-23**), and ten a weak sensitizing capacity (**5-1**, **5-2**, **5-6**, **5-7**, **5-9**, **5-11**, **5-15**, **5-17**, **5-22**, **5-25**). The five remaining PACs were nearly (**5-14**, **5-13**, **5-19**, **5-20**, **5-24**) or completely negative (**5-16**).

Phenanthrenequinone	**No**	**mr**
Strong		
8-Benzyloxy-7-methoxy-1,4-PAC	(**5-21**)	2.16
7,8-Dimethoxy-9,10-dihydro-1,4-PAC	(**5-26**)	2.10
Moderate		
8-Methoxy-1,4-PAC	(**5-5**)	1.93
7-Methoxy-1,4-PAC	(**5-4**)	1.82
7,8-Dimethoxy-1,4-PAC	(**5-12**)	1.72
5,8-Dimethoxy-1,4-PAC	(**5-10**)	1.63
6,7,8-Trimethoxy-1,4-PAC	(**5-18**)	1.60
6-Methoxy-1,4-PAC	(**5-3**)	1.42
6,7-Dimethoxy-9-phenyl-1,4-PAC	(**5-23**)	1.38
3,7-Dimethoxy-5-hydroxy-1,4-PAC (Denbinobin)	(**5-8**)	1.28
Weak		
1,4-Phenanthrenequinone	(**5-1**)	0.88
3-Methoxy-1,4-PAC (Annoquinone-A)	(**5-2**)	0.85
9,10-phenanthrenequinone	(**5-25**)	0.75

3,8-Dimethoxy-1,4-PAC	(**5-9**)	0.65
6,7-Dimethoxy-1,4-PAC	(**5-11**)	0.60
3,5,8-Trimethoxy-1,4-PAC	(**5-15**)	0.50
3,6-Dimethoxy-1,4-PAC	(**5-7**)	0.43
3,6-Dimethoxy-7-hydroxy-9-phenyl-1,4-PAC (Latinone)	(**5-22**)	0.30
3,7,8-Trimethoxy-1,4-PAC	(**5-17**)	0.25
2,8-Dimethoxy-7-hydroxy-1,4-PAC (Cypripedin)	(**5-6**)	0.18
Extremely weak and negative		
3,5,7-Trimethoxy-1,4-PAC	(**5-14**)	0.08
3,5,6,8-Tetramethoxy-1,4-PAC	(**5-20**)	0.03
2,7,8-Trimethoxy-1,4-PAC (Cypripedin-methylether)	(**5-13**)	0.02
2,3,7,8-Tetramethoxy-1,4-PAC	(**5-19**)	0.02
7,8-Dimethoxy-1,2-PAC	(**5-24**)	0.02
3,6,7-Trimethoxy-1,4-PAC	(**5-16**)	0.00

Table 5-2: Sensitization test results

The strongest reactions were obtained with PAC (**5-21)** with two substituents on the remote carbons of ring C (C-7 and C-8). The other PAC (**5-26)** with strong sensitizing capacity was the corresponding dihydrophenanthrenequinone, lacking the 9,10 double bond.

Those PACs with one (**5-4**, **5-5**), two (**5-10**, **5-12**) and in one case three methoxy groups (**5-18**) on ring C, ranged among the stronger sensitizers in the *moderate* group. However, 6-methoxy-1,4-PAC (**5-3**), denbinobin (**5-8**) and 6,7-dimethoxy-9-phenyl-1,4-PAC (**5-23**) remained below a mean response of mr =1.5 within the *moderate* group.

The *weak* group consisted of the two unsubstituted basic molecules of phenanthrene-quinones, namely 1,4-PAC (**5-1**) and 9,10-PAC (**5-25**). Six of the other seven weak sensitizers were substituted with a methoxy group at C-3 (**5-2**, **5-7**, **5-9**, **5-15**, **5-17, 5-22**) and one at C-2

(cypripedin (**5-6**)), of the quinonoid ring (ring A). 6,7-dimethoxy-1,4-PAC (**5-5** to **5-11**) revealed to be an exception.

The *extremely weak* group (mean response ≤ 0.08) comprised the rest of those PACs that were substituted at one or both carbon atoms (C-2, C-3) of the quinonoid ring A (**5-13**, **5-14**, **5-19**, **5-20**, **5-24**), including the naturally occurring latinone (**5-14**) as well as cypripedin methyl ether (**5-13**). Although possessing two substituents at C-7 and C-8 of ring C, the *ortho*-quinone **24** (1,2-phenanthrenequinone) was the weakest sensitizer. Only one of the 26 PACs remained completely negative (**5-16**).

The basic, unsubstituted *para*- and *ortho*-phenanthrenequinones 1,4-PAC (**5-1**) and 9,10-PAC (**5-25**) are weak sensitizers (mr = 0.88 and 0.75, respectively). 1,2-PAC, even when substituted with two methoxy groups at the remote positions of the ring C (**5-24**) is nearly negative in its sensitizing properties (mr = 0.02). The most important finding with the 1,4-PAC-derivatives certifies that substitution of the carbons C-7 and/or C-8 of the ring C, which are remote positions in the molecule, increases the sensitizing power to moderate (**5-4**, **5-5**, **5-10**, **5-12**, **5-18**) or even strong (**5-21**, **5-26**). Obviously, two substituents are necessary, but as expected no great differences are observed between them being either a methoxy or a benzyloxy group (**5-21**, **5-26**). However, one alkoxy substituent alone at either C-7 or C-8 is not sufficient enough for a strong sensitizing effect (**5-4**, **5-5**). Introduction of a second substituent at C-5, as in **5-10** or a third as in **5-18** of ring C**,** decreases the sensitizing capacity. With methoxy groups at the quinonoid ring itself (ring A), either in C-3 (e.g. **5-7, 5-14**, **5-15**, **5-17**, **5-20**, **5-22**) or C-2 (**5-6**, **5-13**) or in both (**5-19**) the sensitizing capacity weakens remarkably (mr ≤ 0.5), even if methoxy groups are present at C-7 or C-8 of ring C (**5-19**, **5-20**).

In contact allergy, side chains of amino acids in proteins function as nucleophiles. Nucleophilic substitution may occur by an electron-rich nucleophile on an electron-deficient electrophilic centre such as the double bonds in quinones (Michael-type addition).

5.4 Quantum mechanical calculations

In an attempt to rationalize the sensitizing capacity of the differently substituted PACs in terms of chemical reactivity with nucleophilic sites of the proteins, we calculated the LUMO coefficients at the reactive sites (C-2 and C-3) of a selected number of 1,4-phenanthrene-quinones using the Spartan Program, Version 1.07 (the AM1 and PM3 methods within the Spartan program gave nearly identical results). According to the frontier orbital theory,[86] reactivity is highest with greatest overlap of the corresponding LUMO of the electrophile and the HOMO of the nucleophile. Thus, with the HOMO of the protein nucleophilic centres to be assumed constant, chemical reactivity should depend on the size of the LUMO coefficient at the potential electrophilic centres at C-2 or/and C-3 of the 1,4-phenanthrenequinones. The LUMO energies and the coefficients at C-2 and C-3 are listed in Table 5-3. The LUMO calculations were extended to 2-Methoxy-1,4-PAC (**5-27**) and 9,10-Dihydro-PAC (**28**), which had not been included in the sensitizing experiments.

No	LUMO energy (eV)	LUMO coefficient at C-2	LUMO coefficient at C-3
5-1	1.743	0.0074	0.0075
5-3	2.021	0.0073	0.0053
5-4	2.021	0.0086	0.0069
5-5	2.002	0.0081	0.0070
5-7	1.518	0.0051	0.0038
5-12	1.657	0.0073	0.0073
5-13	1.551	0.0037	0.0086
5-26	1.514	0.0077	0.0085
5-27	-	0.0020	0.0045
5-28	-1.639	0.0107	0.0081

Table 5-3: Calculated LUMO coefficients of 10 phenanthrene-quinones

In fact, methoxy substitution at C-2 or C-3 (**5-2** or **5-27**) decreases the coefficients at the remaining electrophilic centres with respect to the unsubstituted 1,4-phenanthrene-quinone (**1**).

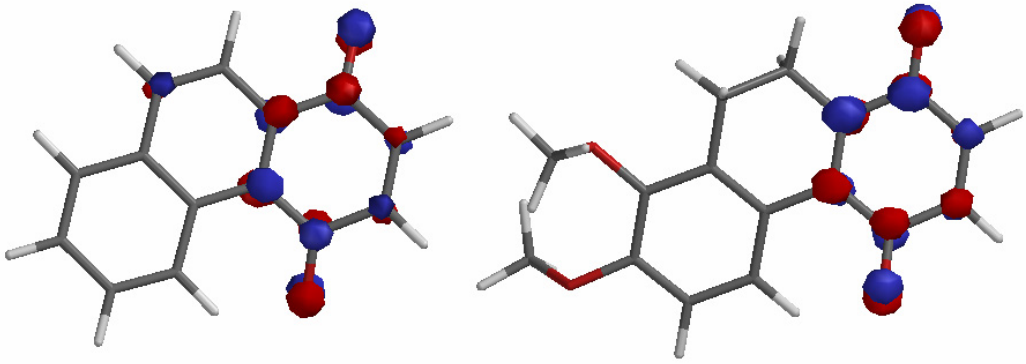


Figure 5-1: LUMO of **5-1** and **5-26**

In addition, the chemical reactivity is decreased considerably by sterical hindrance at the neighboring electrophilic site. On the other hand, the LUMO coefficients at C-2 considerably increased by substitution at C-7 or C-8 (**5-4** and **5-5**), whereas a methoxy group at C-6 (**5-3**) has practically no influence on the size of the LUMO coefficients at C-2, and decreases the coefficient at C-3. The increase of the coefficients are not influenced by hydrogenation of the 9,10 double bond (**5-26**) showing a high coefficient at C-3 (**5-28**).

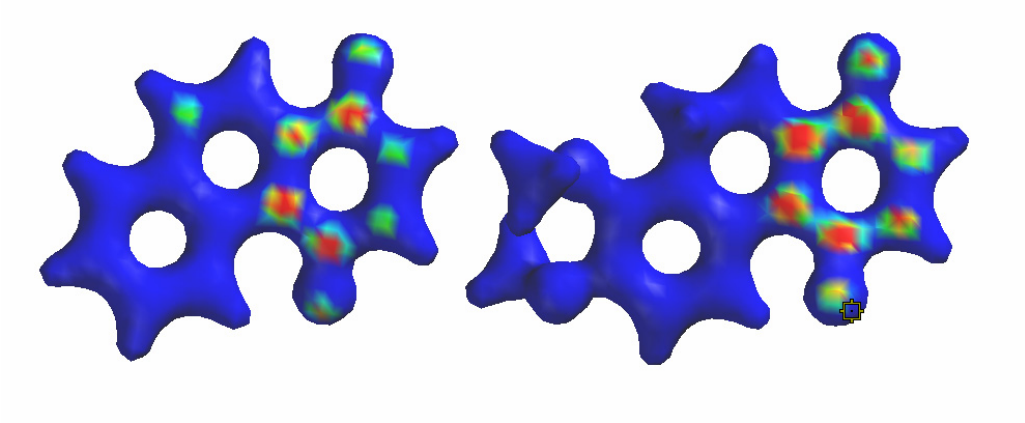


Figure 5-2: LUMO density of **5-1** and **5-26** (red low, blue high)

Thus, not surprisingly, phenanthrenequinone **5-26** is among the strongest sensitizers. The correlation and statistical evaluation of the LUMO coefficients at the reactive sites at C-2 and C-3 and the biologic parameter mr Δ, representing the strength of sensitizing potency, is shown in Figure 5.3. With the exception of three values, there is a good fit of the data for both electrophilic centers C-2 and C-3, with regression factors 0.903 and 0.943 respectively. This correlation is really remarkable considering a complicated biologic effect such as sensitizing

potency. One reason for this may be the relatively homogeneous series of PACs investigated, which had comparable polarities and pharmacologic properties.

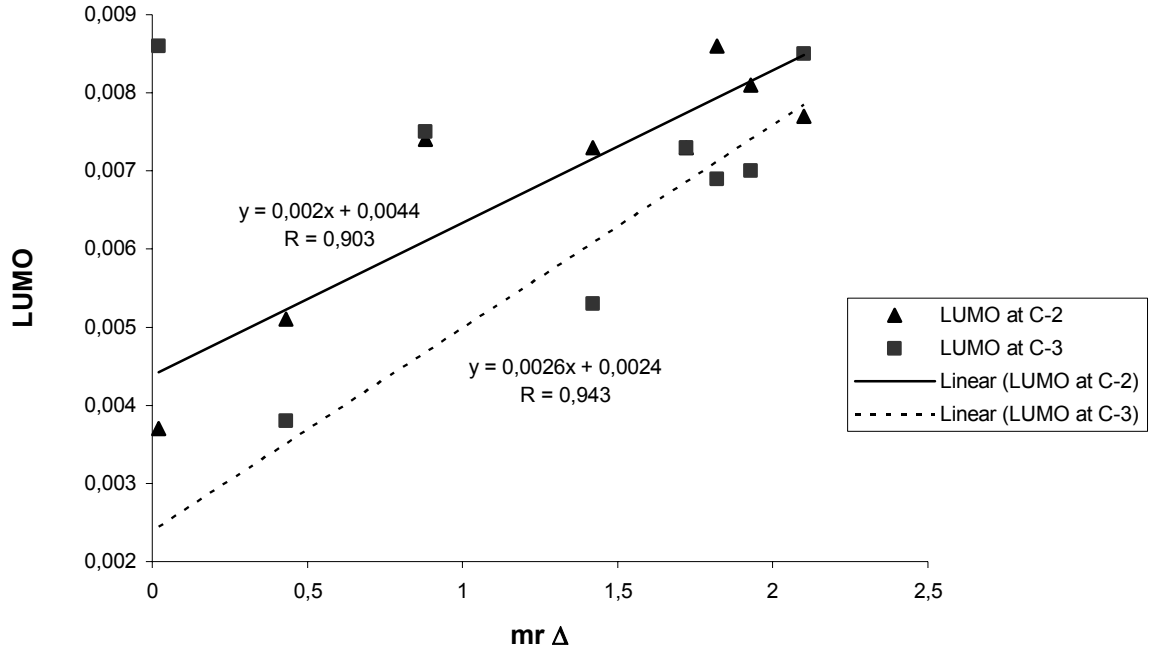


Figure 5-3: Statistical distribution of LUMO coefficients vs. sensitizing capacity

This is a very nice example that theoretically calculated chemical reactivity and the experimental data of sensitizing capacity are in a very good agreement in a large and homogeneous group of potential allergens. This approach is very meaningful in the case of the natural compounds latinone **(5-22**), cypripedin (**5-6**), annoquinone-A (**5-2**), denbinobine (**5-8**) and several selected synthetic phenanthrenequinones. It may also be useful in predicting the sensitizing capacity of potentially allergenic compounds of natural origin such as the royleanones and tanshinones, which have not been the subjects of sensitization studies so far. These results might contribute to a better understanding of contact allergy.

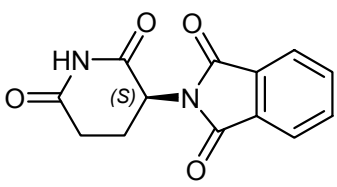
6 Determination of the Absolute Configuration by Quantum Chemical Calculation of CD Spectra

6.1 Introduction

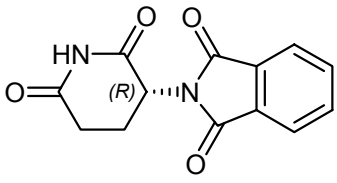
The knowledge of the stereo structure of biologically active natural products, in particular of their absolute configuration, is an important precondition, for example, for directed structure-activity relationship investigation. While the relative configuration can be established relatively easily spectroscopically (for example by NMR) or by X-ray diffraction, the assignment of the absolute stereo structure is sometimes a more difficult task, in particular for novel classes of compounds.

Enantiomers have the same scalar physical characteristics such as melting point, boiling point, density and chemical reactivity with achiral reaction partners. However, their reactivity with chiral reagents is different. The biological effect of chiral substances, which are generally used as herbicides or drugs, also depends on their absolute configurations. Two enantiomers can differ strongly in their strength and quality of effect depending considerably on the affinity and selectivity of the active substance to the active site.

The contergan scandal serves as an instructive example. The sedative and antinausea agent, contergan, with the active substance *rac*-thalidomide, led to the most serious of medicament scandals: the contergan disaster.



(*S*)-Thalidomide



(*R*)-Thalidomide

When taking contergan in the early stage of pregnancy, the children born had a high incidence of deformities in their limbs (phocomelia). From 1958 to 1961, approximately 10000 children world-wide were born with some kind of defects. The medicament contergan contained primarily the active substance *rac*-thalidomide (Phthalimidoglutarimid), whose *R*-(+)-

enantiomer carries the required pharmacological effect, while the (*S*)-enantiomer is a very potent teratogen.

Thalidomide was developed in 1956 by a chemist, Dr. Heinrich Mückler, and brought into trade in October of 1957 by Grünenthal in Stollberg at Aachen. Contergan was removed from the market in November 1961, when the drugs serious side effects became known.

6.2 Theoretical Principles - Calculation of CD Spectra

Conformation analysis by means of ^{1}H-NMR spectroscopy employs the so called Karplus rule, which describes the dependence of the vicinal-coupling constant on the torsional angle in a saturated system. Another method is the measurement of the NOE´s (Nuclear Overhauser Effect); hereby steric interactions are evaluated. The determination of the absolute configuration is not always that straightforward.[87]

One of the most efficient if not ideal methods for configurational assignment is the investigation of circular dichroism (CD). With low experimental demand, two enantiomers can be easily distinguished by their exactly opposite CD spectra. The assignment of the absolute configuration of the enantiomers, however, can be complicated, since it quite often can not be deduced solely from the measured CD spectrum exploiting empirical rules on the given chromophore (octant rule). Even the widely used exciton chirality method[88,89,90] is restricted in its application, since it requires the presence of certain functional groups that can be derivatized and this precondition is not fulfilled in each single case.

The quantum chemical calculation of CD spectra, by contrast, is not limited by any structural restrictions and thus constitutes a generally applicable procedure. When light passes through an absorbing optically active substance, not only do the left and the right circularly polarized rays travel at different speeds, $c_L \neq c_R$, which leads to $\lambda_L \neq \lambda_R$, but these two rays are also absorbed to a different extent; that is, $\varepsilon_L \neq \varepsilon_R$. The difference $\Delta\varepsilon \equiv \varepsilon_L - \varepsilon_R$ is called *circular dichroism*, and all commercially available "dichrographs" the difference in absorption for the two helical rays is recorded.[89] For a quantitative and standardized description of circular dichroism we refer to the Lambert-Beer-Bouguer law. If I_0 is the intensity of light impinging on the cell, and I that when leaving, the absorbancy $A = log_{10}(I_0/I) = \varepsilon cl$; that is, the recorded signal (A) is proportional to the concentration c and the path length l. If c is given in mol/L and l in cm, then ε is called the *molar (decadic) absorption coefficient* or *molar absorptivity*.

From the definition, it follows that CD can be observed only within absorption bands and hence it concerns electronic transitions in the VIS or UV range (electronic circular dichroism: ECD) and vibration bands in IR region (vibrational circular dichroism: VCD). In solution, a Cotton effect (CE) arises only if the examined substance is chiral. The CD spectra of enantiomers are mirror-image to the x axis. The rotatory power R_{0a} for a transition $0 \rightarrow a$ can be calculated from the scalar product of the magnetic and electrical transition moment as follows[91]:

$$R_{0a} = \mathrm{Im}\left\{\left\langle \psi_0 \left| \hat{\mu} \right| \psi_a \right\rangle \left\langle \psi_a \left| \hat{m} \right| \psi_0 \right\rangle\right\} = \mathrm{Im}\left\{\mu_{0a} - m_{a0}\right\} \qquad (1)$$

Whereas Im means that one should use only the imaginary part of the complex numbers, μ (electrical dipole transition moment) and m (magnetic dipole transition moment) are operators.

$$\mu_{0a} = \frac{e\hbar}{im(E_a - E_0)} p_{0a} \qquad (2)$$

Substituting term (2) into term (1) results in:

$$R_{0a} = \mathrm{Im}\left\{\frac{e\hbar}{im(E_a - E_0)} \left\langle \psi_0 \left| \hat{p} \right| \psi_a \right\rangle \left\langle \psi_a \left| \hat{m} \right| \psi_0 \right\rangle\right\} = \mathrm{Im}\left\{\frac{e\hbar}{im(E_a - E_0)} p_{0a} m_{0a}\right\} \qquad (3)$$

Term (3) has the advantage that it depends on origin of the advanced wave functions Ψ_0 and Ψ_a for the ground and excited states; and p is the operator of impulses. The wave functions are obtained by CNDO/S-CI calculations. The extension CI means that both the ground state and the excited state are considered. These calculations were carried out by the program package BDZDO/MCDSPD developed by Downing and modified by Fleischauer.[92]

For a better demonstration the rotatory power is calculated from the $\Delta\varepsilon$-curves as follows:

$$\Delta\varepsilon(\nu) = \frac{6.909 hc_0 \nu \sqrt{\varepsilon_0}}{8\pi^2 1000 N_A \sqrt{\mu_0}} \sigma_{0a}(\nu) R_{0a} \qquad (4)$$

Whereas N_A represents the Avogadro number and $\sigma_{0a}(\lambda)$ is the Gauss-function:

$$\sigma_{0a}(\lambda) = -\frac{1}{\Delta_a \sqrt{\pi}} e^{-\left(\frac{\lambda - \lambda_a}{\Delta_a}\right)^2} \qquad (5)$$

λ_a denotes the wavelength of the maximum of $\Delta\varepsilon$, and Δ_a is the full width at half maximum of the Gauss-curve in nm.

For the computation of the wave function, it is necessary to know the conformation of the molecule. At room temperature, the lowest energy conformer can equilibrate with some higher energy ones, some of which can not be neglected. The change of the relative orientation of the chromophore has an influence on the CD spectrum. For this reason,

conformational analysis of the arbitrarily chosen enantiomer is performed first accomplished by the program Spartan SGI Version 5.3. For this purpose, either a systematic or a Monte-Carlo search with force field MMFF94 is applied. Subsequently, the heat of formation $\Delta_f H$ in kJ/mol is computed semi-empirically by AM1-Model, which can be also gained from the Spartan program package.

The participation of each conformer is determined by the Boltzmann equation as:

$$\frac{N_i}{N_j} = e^{-\frac{E_i - E_j}{kT}} \qquad (6)$$

The CD data of all the conformational isomers of the chiral compound up to an energy cut-off (usually 10−12 kJ/mol) are taken into consideration, because these conformers have more than 1 % participation to the sum CD spectrum at room temperature (298 K). The CD-spectra of each conformer is computed and added up by the means of the Boltzmann statistics (6) to get the final CD-spectrum.

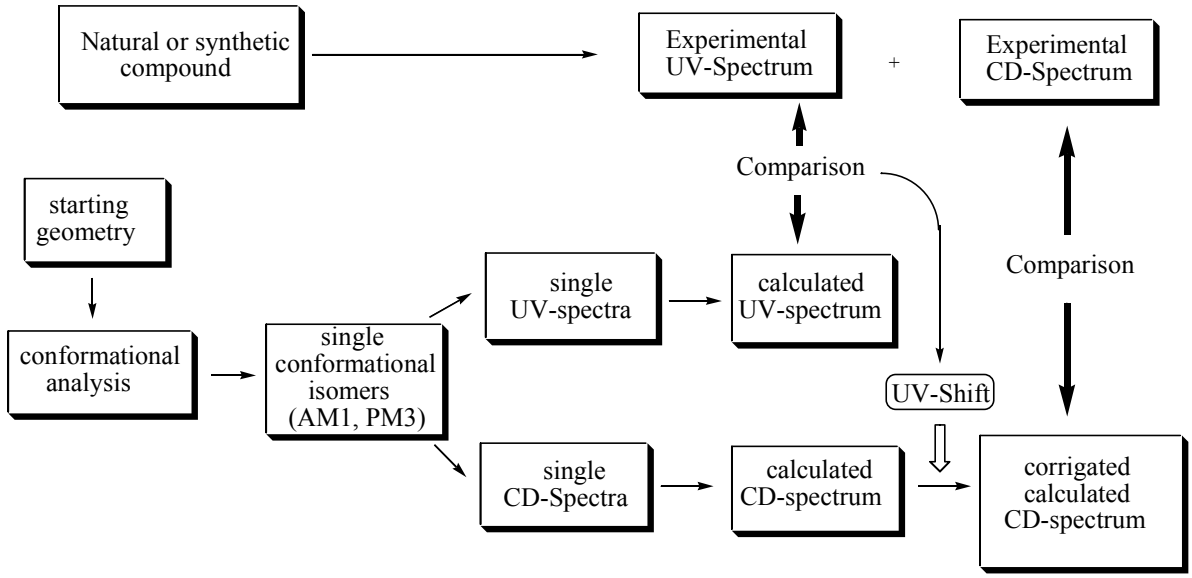


Figure 6.1: Diagram the calculation of CD spectrum

When comparing the calculated overall CD spectrum obtained in this manner with the experimental one, three possibilities arise:

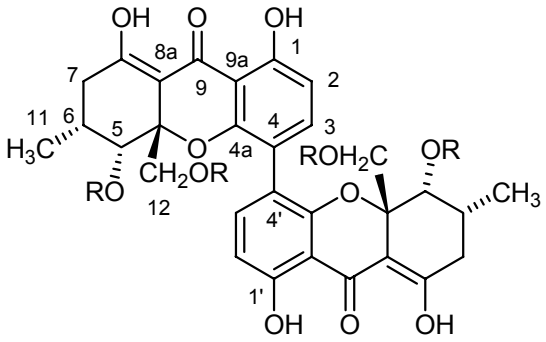
- the two spectra show essentially the same CD curve
- the experimental spectrum shows a virtually opposite CD behavior
- the two spectra have no similarity.

In the first case, the studied compound has the same absolute configuration as the one used for the calculation. In the second case, it must have the enantiomeric structure. If the two spectra differ from each other significantly (third case) no statement about the absolute configuration

can be made. Using this method, the absolute configuration of several natural products such as Ancistrocladin, Doncophyllin, Palmarumycine, Vismion H etc. have been elucidated.[87]

6.3 Determination of the absolute configuration of an ergochrome

Ergochromes are natural products, which belong to the class of anthranoids and are present in numerous microorganisms.[93] The first representatives were isolated from an ergot fungus *(Claviceps Purpurea)*. It has been discovered recently that ergochromes are toxins of food-fungi, and detailed investigation of their biological activities were initiated. The available toxicity studies on mice designate this class of compounds as fungus toxin of middle strength. Antibiotic 5049 (**6-1**), isolated by our research group from the strain *Costus sp*. (Costaceae) as yellow crystals, also belongs to the class of anthranoids, which derives from natural anthracen compounds.[94]



6-1

Figure 6.2: Antibiotic 5049, R=OAc

The yellow color of the compound can be explained by both the presence of the chromogenen tricycle ring system of the anthrone structure and the auxochromen hydroxy groups. The biosynthetic precursor of this natural product is the secalonic acid A (ergochrome A). All secalonic acids are dimers of two xanthon monomers connected at the 2,2´-position. The formation of secalonic acid A can be explained by the enzymatic oxidative ring-opening of Emodin (a tricyclic quinone derivative). Besides the different position of linkage of the two monomers, the methyl ester group of secalonic acid A is replaced by methylenacetoxy group. There are several secalonic acid derivatives known in the literature whose configurations at position 5,5´, 6,6´, or 10,10´ are different.[95]

The relative stereochemistry of the aliphatic moiety was elucidated by NOE-difference-spectrum. The strong correlation of the 12-methylene protons with 5-H (+50.3 %) suggested

the *trans*-orientation (most likely *trans*-diaxial) of the substituents at position C-5 and C-10a. In contrast, a similar strong enhancement of the C-16 protons was observed by irradiation implying the *cis* assignment of these positions. Finally, X-ray diffraction confirmed the former relative conformation.

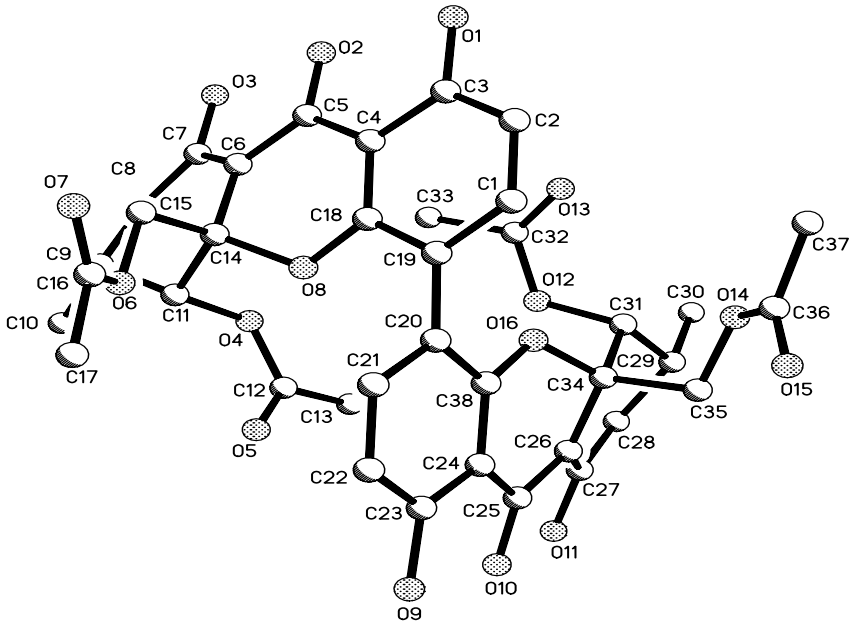


Figure 6.3: X-ray picture of Antibiotic 5049 (**6-1**)

The determination of the relative configuration was a prerequisite for the quantum chemical calculation of the CD-spectra.

The conformational analysis was performed by combining molecular mechanics and semi-empirical (AM1) methods using Monte-Carlo-Simulation in water-CT, setting 2 as degree of freedom of the aryl-aryl axes. 8 minimum-energy conformers were found, up to an energetic cut-off of 11 kJ/mol relatively to the lowest energy conformer whose energy was computed semi-empirically. The theoretical calculation of the CD-spectra was carried out by the BDZDO/MCDSPD program package.[92]

The calculated and experimental CD spectra are in a good agreement as shown in Figure 6.4.

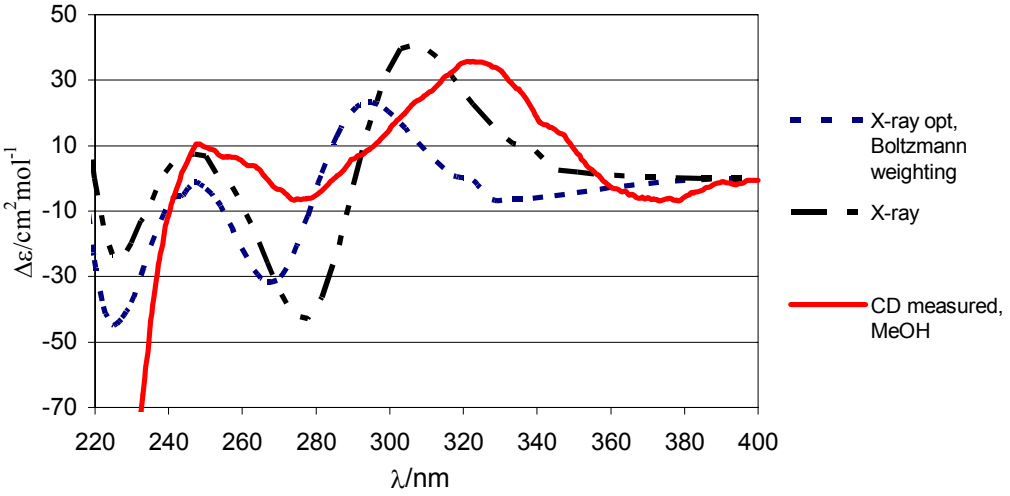


Figure 6.4: Comparison of the calculated and measured CD spectra of **6-1**

Since this molecule contains not only central but also axial chirality, it should be investigated which of these chirality elements determine the CD spectrum. In order to find the answer of this question, CD spectra of several conformers of Antibiotic 5049 (**6-1**) have been calculated, which showed that conformers with negative dihedral angle exhibit mirror-image CD-spectrum.

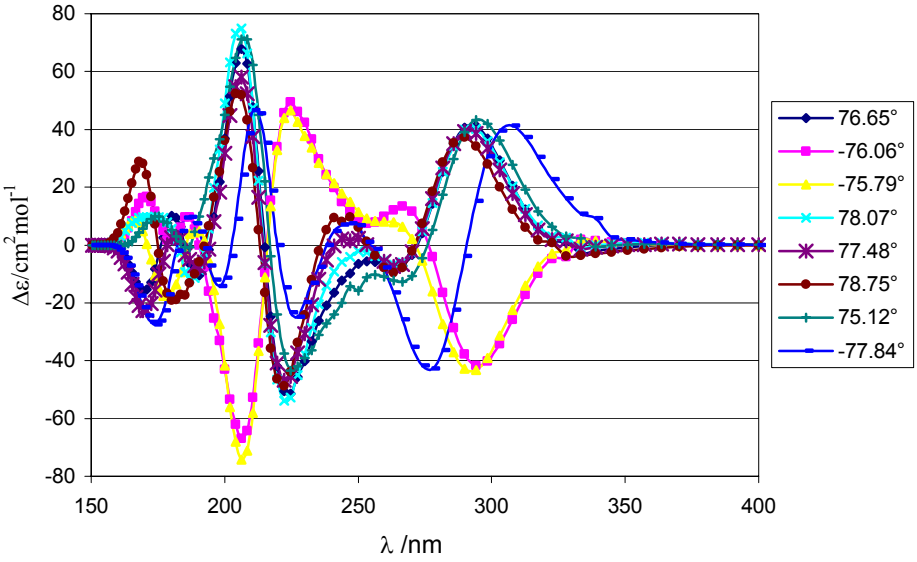
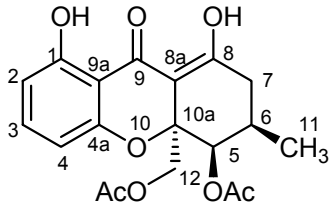


Figure 6.5: Calculated CD spectra of 8 conformers of **6-1**

This experiment points out that the axial chirality plays a decisive role in the CD-spectra. Since the Cotton-effect at 300 nm ($\Delta\varepsilon$= 41) derives from the carbonyl n-π^* transition, the CD spectrum of the monomer-moiety (**6-2**) was calculated in the next step. This monomer has the

C-10a chirality center, but has no axial chirality element. This way the axial chirality contribution, which has a dominating effect on the CD spectrum was ruled out.



6-2

Figure 6.6: Structure of monomer building block **(6-2)**

The calculated CD of the monomer **6-2** shows only a negative couplet between 192 nm and 238 nm with a negative peak at 209 nm ($\Delta\varepsilon = -33$) and a positive one at 290 nm ($\Delta\varepsilon = 7$), whereas this curve showed quite different characteristics.

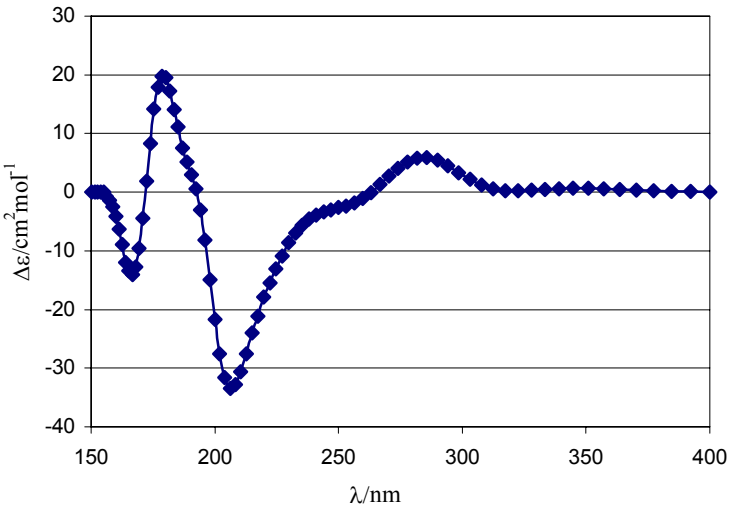


Figure 6.7: Calculated CD of monomer **6-2**

6.3.1 Atropismerism

Biphenyls with hindered rotation represent one of the most classical examples of atropisomerism. They form enantiomers because the two benzene rings are not coplanar and both rings are substituted unsymmetrically so that the plane passing through the pivot bond and one of the benzene rings can not be a σ plane. If we consider the conformations of

biphenyls in more detail, we recognize that there are two diastereomeric conformations possible.

In biphenyls, the restricted rotation is sustained by bulky atoms in the *ortho*-positions of the phenyl rings. Oki (1983)[96] arbitrarily predicted the existence of atropisomerism where isomers can be isolated for compounds that have a racemization half-life $t_{1/2} > 1000$ s (16.7 min). This value does not define the required free energy barrier, which evidently now depends on temperature; it is 22.3 kcal/mol (93.3 kJ/mol) at 300 K, 26.2 kcal/mol (109.6 kJ/mol) at 350 K and 14.7 kcal/mol (61.5 kJ/mol) at 200 K.[97]

6.3.2 Determination of the rotation barrier of ergochrome (6-1)

Quantum chemical calculations, in particular, *ab initio* molecular orbital calculations and density functional calculations, can be applied to furnish data for parameterizing empirical energy functions (molecular mechanics models). The data related to torsional motions are the most important and meanwhile these are the most difficult to obtain experimentally, since the empirical energy function needs to reflect the inherent periodicity. For example, the three-fold periodicity of rotation about the carbon-carbon bond in ethane may be described by the functional form:

$$E_i^{torsion}(\omega_i) = k_i^{torsion3}(1-\cos 3(\omega_i-\omega_i^{equilibrium}))$$

$\omega_i^{equilibrium}$ is the ideal dihedral angle and $k_i^{torsion3}$ is treated as a parameter. Proper description of bond torsion also requires, at the very least, a term which are one-fold and two fold periodic.

$$E_i^{torsion}(\omega_i) = k_i^{torsion1}(1-\cos 1(\omega_i-\omega_i^{eq})) + k_i^{torsion2}(1-\cos 2(\omega_i-\omega_i^{eq})) + k_i^{torsion3}(1-\cos 3(\omega_i-\omega_i^{eq}))$$

$k_i^{torsion1}$ and $k_i^{torsion2}$ are additional parameters. The one-fold term accounts for the difference in energy between *cis* and *trans* conformers, and the two-fold term for the difference in energy between planar and perpendicular conformers.

In the case of ergochrome (**6-1**), the so called “coordinate driving” was performed using AM1 semi-empirical model of the Spartan Program. First the dihedral angle was set to 0 ° and then it was driven from 0 ° to 180 ° in 19 steps. After the 19 geometry optimizations are completed the energy was calculated semi-empirically to get the following plot:

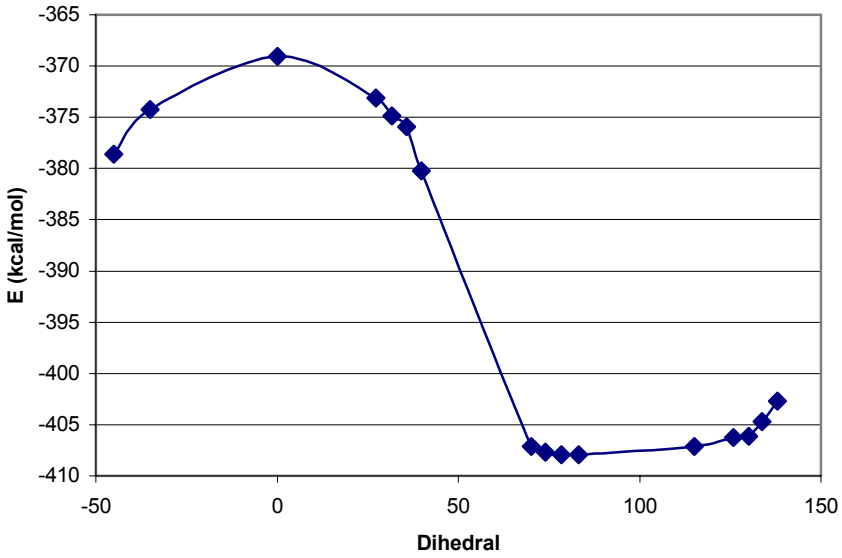
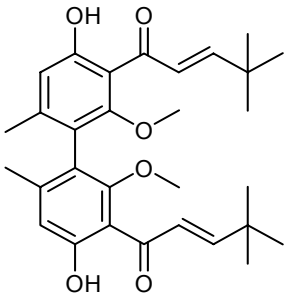


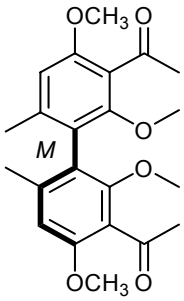
Figure 6.8: Coordinate Driving of Dihedral of Ergochrome **6-1**

Subtraction of the lowest value from the highest energy value resulted in 37 kcal/mol, which unambiguously showed the existence of a hindered rotation.

This indicates that in the case of ergochromes the axial chirality must dominate over the central chirality. In order to clarify this theory, the CD spectra of the biaryl-system **6-3a** was computed. This biaryl-system includes the same chromophore as Antibiotics 5049 (**6-1**) but has only the axial chirality element.



6-3a



(M)-**6-3b**

Figure 6.9: Structure of biaryl systems **6-3a** and **6-3b**

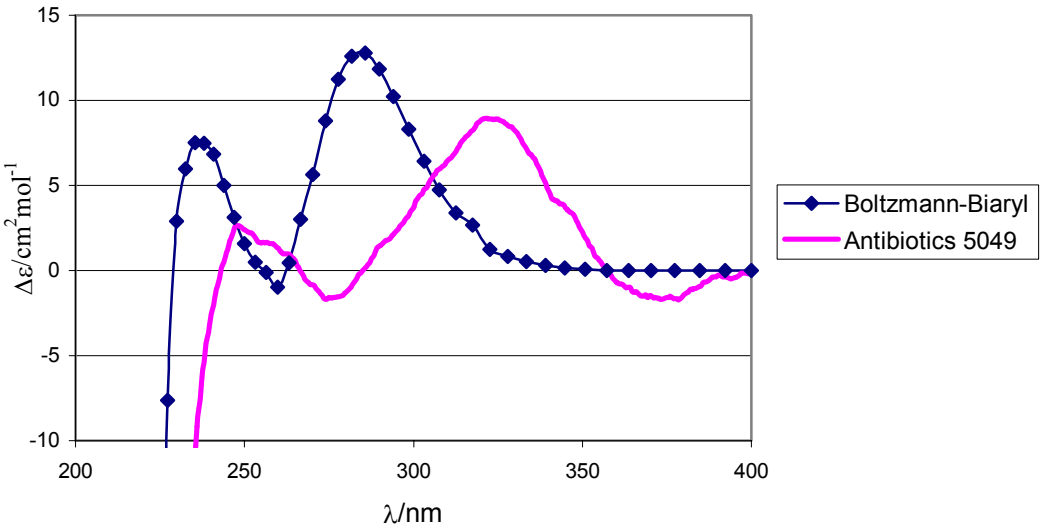


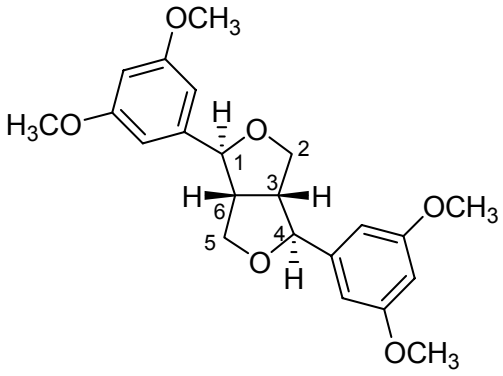
Figure 6.10: Calculated CD of biaryl system **6-3a** and measured CD of ergochrome **6-1**

This curve has a relatively good agreement with the shape of the CD spectrum of Antibiotic 5049 (**6-1**), having a positive couplet between 225 and 245 nm with a maximum at 232 nm ($\Delta\varepsilon$= 32), a minimum at 256 ($\Delta\varepsilon$= −1) nm and a positive peak at 317 nm ($\Delta\varepsilon$= 17). This proves that the axial chirality element is responsible for the chiroptical activity due to the hindered rotation around the aryl-aryl axes.

Enantiomeric pure biaryl compound (*M*)-**6-3b** was investigated by Bringmann and co-worker.[98] The chromophore system of **6-3a** and **6-3b** are very similar, the only difference is the aliphatic side chain in **6-3a**, and that the aryl-hydroxyl group is substituted by a methyl ether. These differences have only a small influence on the CD-spectra, since the main peaks belong to the interaction of the benzyl rings and to the n→π* carbonyl transition. The measured CD spectra of **6-3b** shows the same shape of curve as the calculated CD spectra of **6-3a** and the measured CD spectra of **6-1**, having a strong negative peak around 220 nm ($\Delta\varepsilon$ = −30), and two smaller positive band at 240 ($\Delta\varepsilon$= 15) and 290 nm ($\Delta\varepsilon$= 5). These results also confirm the previously established theory that the axial chirality dominates over the central chirality element in the case of these substance classes. These investigations permitted not only the first attribution of the absolute configuration of compound **6-1**, but more importantly, also showed a way to elucidate the axial configurations of related bisanthrones.[93,94,95]

6.4 Calculation of the absolute configuration of epieudesmin (6-4)

Lignan **6-4** (epieudesmin) was isolated by Krohn and Riaz from *Achillea holosericea*. Members of the genus Achillea are common in the Mediterranean area, Eurasia and North Africa.[99] Aerial parts of different species of this genus are widely used in folk medicine for the preparation of herbal teas with antiphlogostic and spasmolytic activity. The extract exhibits pharmacological activities including anti-bacterial, anti-inflammatory and antiallergic properties.



6-4

Figure 6.11: Structure of lignan **6-4**

A large and increasing number of lignans have been characterised and recent progress has been summarized in the *Natural Product Reports*.[100] The driving force for many of this work has been the diversity of biological activities shown by various lignans. Most notably, anti-tumour activity is displayed by podophyllotoxin. Some of its relatives are of clinical use against cancer, *e.g.* etoposide, and lately a new generation of derivatives active against etoposide resistant cells have been introduced. Anti-HIV, antagonism of viral reverse transcriptase and PAF, antifungal and immunosuppressive activities are among the other recorded properties.[100]

While the NMR data revealed the connectivities, the relative configuration of **6-4** was determined by X-ray analysis, which showed that the chirality centers have either 1*R*,3*S*,4*R*,6*S* or 1*S*,3*R*,4*S*,6*R* configuration.

The conformational analysis of the 1*R*,3*S*,4*R*,6*S* enantiomer was carried out for the calculation of the CD spectra. The quantum chemical calculation gave 10 conformers whose heat of formation and CD spectra were computed.

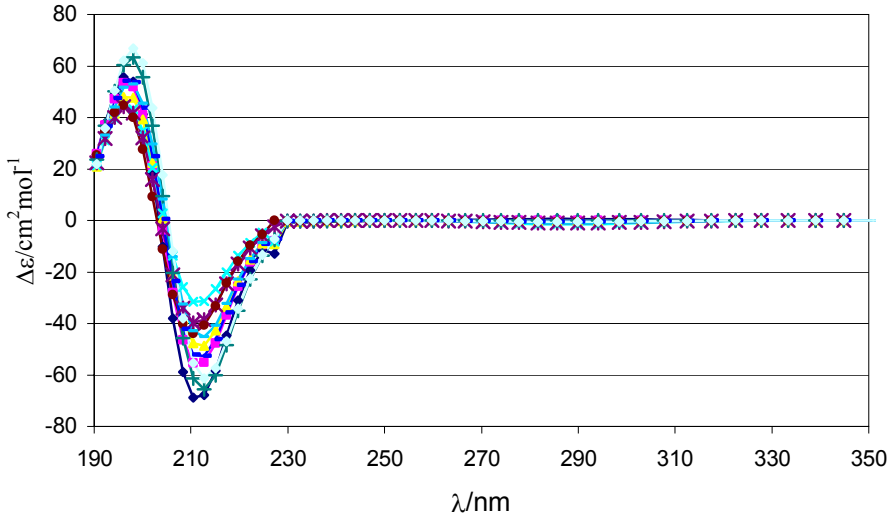


Figure 6.12: Calculated CD spectra of several conformers of lignan **6-4**

Using the Boltzmann-weighting, the CD-spectra were added according to their relative participation. Subsequently the calculated and experimental CD spectra were juxtaposed. Both curves show a negative peak around 210 nm ($\Delta\varepsilon = -69$) and a small Cotton-effect for the ^1L_a band around 237 and 240.5 nm ($\Delta\varepsilon = 1.5$).

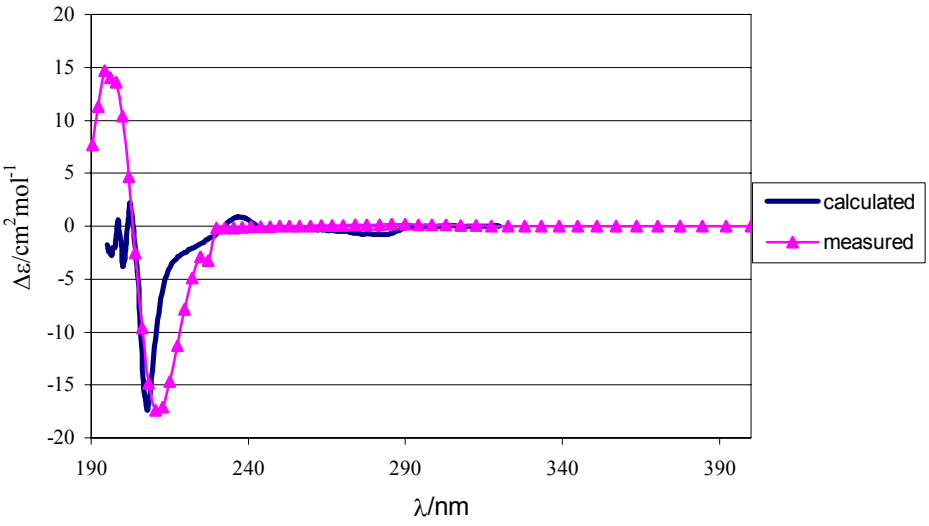


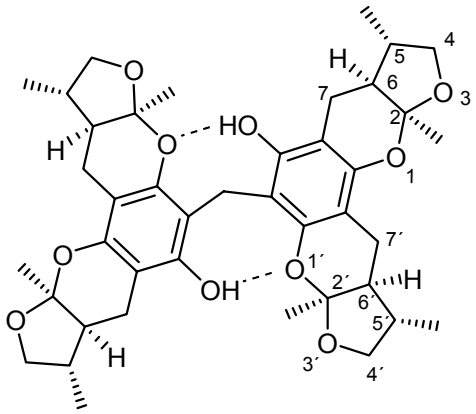
Figure 6.13: Comparison of calculated and experimental CD spectra of lignan **6-4**

Comparing these results with data from literature[101] of similar structures (sesamins) with known absolute configuration, it can be noticed that those substances also show a strong negative peak around 210 nm, and a positive Cotton-effect for the ^1L_a band near by 237 and 240.5 nm. Since the calculated and measured CD spectra are in good agreement, the structure

used for the calculation is identical with that of the isolated lignan and hence its absolute configuration is 1*R*,3*S*,4*R*,6*S*. Another related structure of lignan **6-4** is syringaresinol, whose absolute configuration has been established from the X-ray structure of its dibromo derivative.[102] However, the benzene rings of syringaresinol is substituted by OH-groups in *para* position. Those X-ray studies on absolute configuration are in a good agreement with our quantum chemical calculation results.

6.5 Determination of the absolute configuration of a Xyloketal (6-5)

Xyloketal (**6-5**) was isolated from a *Xylaria* species by Yongcheng Lin and coworkers. This fungus origins from the mangroves of the South China Sea. In the first biological test, xyloketal (**6-5**) inhibited acetyl choline esterase at the rate of 1.5×10^{-6} mol/L ($p < 0.01$). Xyloketals represent a novel class of natural products whose common structural element is a bicyclic ketal. Xyloketal (**6-5**) is a dimer of two bicyclic ketals, whose two moieties are connected by a CH_2 bridge. It has a rigid structure due to the hydrogen bonding between the two moieties as shown in Fig. 6.14.



6-5

Figure 6.14: Structure of Xyloketal **6-5**

The relative configuration of **6-5** was elucidated by X-ray crystallography and NMR-spectroscopy, which revealed that all the stereogenic centers have either *R* or *S* configuration. Since it has a rigid structure, the number of low energy conformers are limited, which allow the quantum mechanical calculations of its CD spectrum. Comparison with experimental data then leads to matching or mismatching curves, allowing the assignment of the absolute configuration.

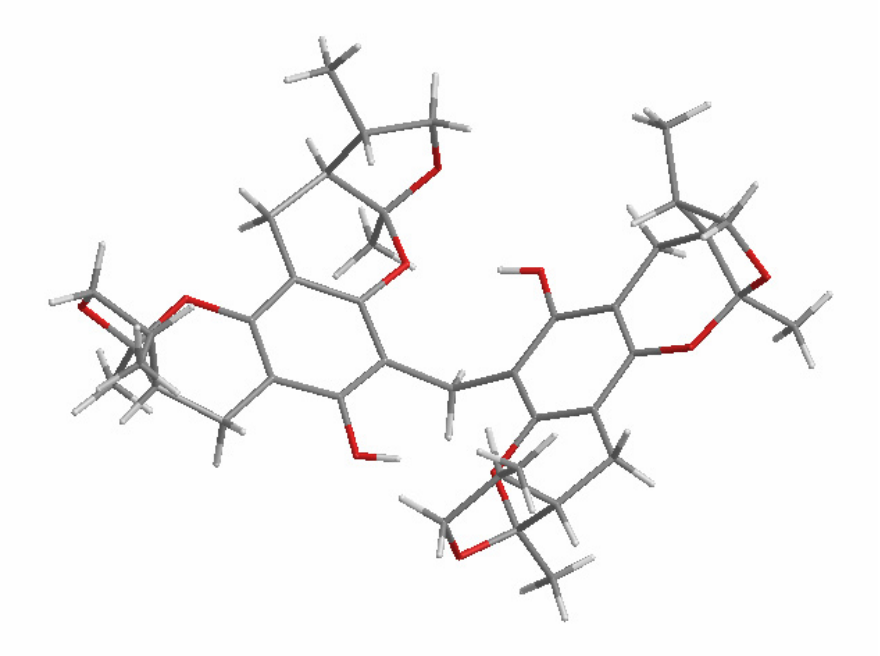


Figure 6.15: Most stabile conformer of Xyloketal **6-5**

For the CD calculation, the all-*R*-enantiomer was taken for conformational analysis. The calculation showed that the six-membered ring is relatively rigid but the five-membered ring exhibits some conformational flexibility (Figure 6.13). The calculation resulted in three conformers whose heat of formation and the CD spectra was computed. Boltzmann weighting was performed and this Boltzmann weighted curve was compared with the experimental one. The experimental curve shows a strong negative Cotton effect at 215 nm ($\Delta\varepsilon = -15.97$) and two weaker positive peaks at 245 and 270 nm ($\Delta\varepsilon = 8.71, 1.58$). Although the calculated CD spectrum has a strong negative band at 220 nm ($\Delta\varepsilon = -15.79$), it shows only a very weak positive peak at 245 nm ($\Delta\varepsilon = 0.71$). Since the most intense Cotton effect around 210−225 nm belongs to the main transition of the xyloketal (^1B_a and ^1B_b transition; 215 nm ($\Delta\varepsilon = -15.97$)[103] and its calculated and measured parameters showed good agreement, it could be used for the configurational assignment with a high degree of probability.

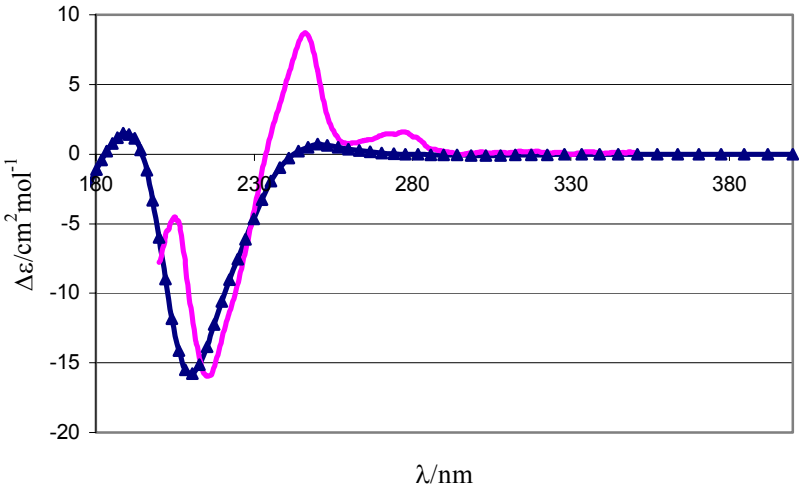
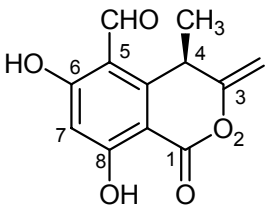


Figure 6.16: Boltzmann weighted (triangles) and experimental spectra (circles) of lignan **6-5**

Since the experimental CD spectrum had the same negative CD minimum for the ^1B_a and ^1B_b transition as the CD curve calculated for the all R-enantiomer, the absolute configurations of all the stereogenic centers are *all-R*.

6.6 Determination of the absolute configuration of Ascochin (6-6)

Isocumarine derivate **(6-6),** named Ascochin, derives from *Ascochyta* sp. has been isolated by Krohn and Kock from *Meliotus dentatus,* ingenious at the coast of the East Sea, Ahrenshoop. The strain showed fungicide activity in the agar-diffusion test and was cultivated for 28 days at room temperature on 12 L-Culture biomalt agar.



6-6

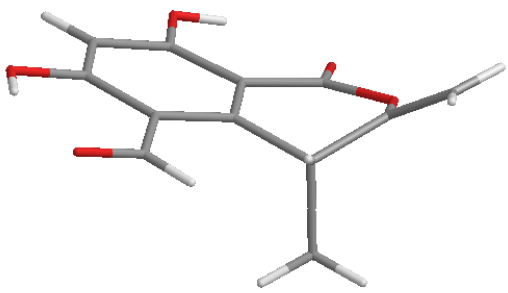


Figure 6.17: Structure and optimized conformation of ascochin (**6-6)**

Since X-ray diffraction does not give any information about the absolute configuration of ascochin **(6-6)**, it must be determined by calculation and measurement of its CD spectra. For this purpose, the *R* enantiomer was arbitrarily selected for quantum chemical conformational analysis, which resulted in only three optimized conformations, thanks to the rigid structure of the molecule. The structures of these conformers and the X-ray structure were compared and showed a meaningful correlation. In all cases, the *C*-4 methyl is axial thereby indicating the helicity of the hetero ring. The axial position of this methyl group can be explained by the position of the formyl group on the peri sp^2 carbon atom. To relieve the sterical strain, the *C*-4 methyl moves out the plane of the hetero ring to the axial position. All single CD spectra thus obtained were added up by Boltzmann statistics using appropriate heats of formation, to give the calculated overall CD spectrum for isocumarin **6-6**, which showed a strong positive Cotton-effect at 192 nm ($\Delta\varepsilon$= 20.75).

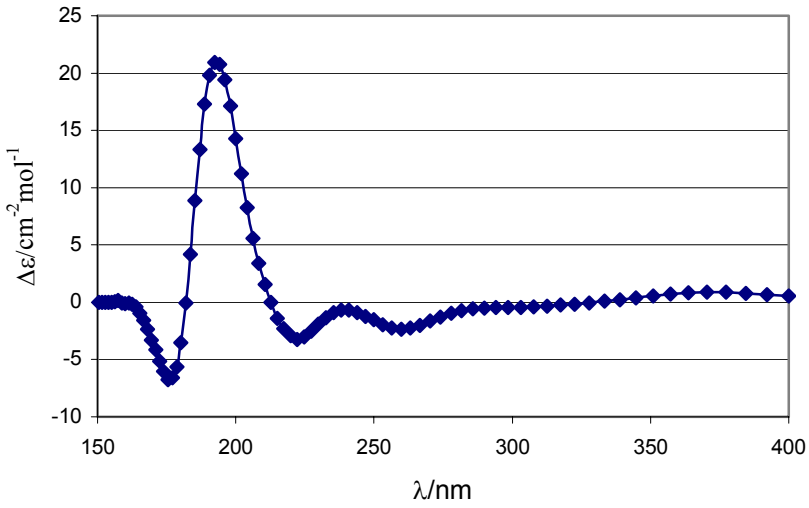


Figure 6.18: Calculated CD spectra of ascochin **(6-6)**

Subsequently, the CD spectrum of ascochin **(6-6)** was measured. The plot showed a strong positive Cotton-effect at 253 nm ($\Delta\varepsilon$= 52.44). Compared with the calculated value, a shift of approximately 60 nm was observed.

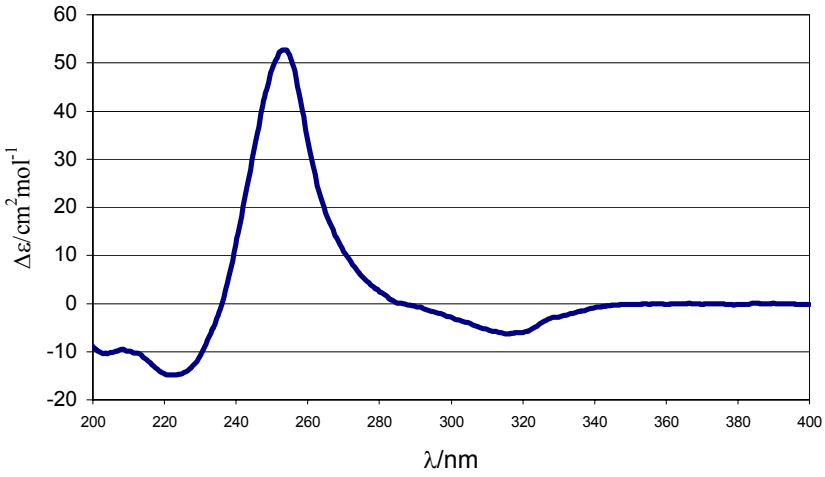
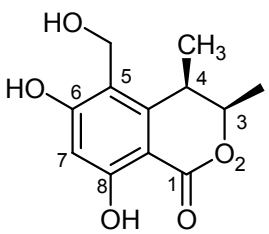


Figure 6.19: Measured CD of ascochin (**6-6)**

This shift can be explained by the structure of the molecule **6-6**. Since isocumarine **6-6** possesses a multichromophore system, it was assumed that the program was not able to consider it completely for the calculation. To avoid this problem, the chromophore of compound **6-6** was modified by catalytic hydrogenation to dihydro-isocumarin **6-7**. As expected, the reaction proceeded diastereoselectively. The hydrogenation gave only the *cis* diastereomer under the conditions used (MeOH, Pd/C, H_2). On the other hand, dihydro-isocumarins possess a benzoic ester chromophore, whose chiroptical properties have been systematically investigated.[104] It was found that the sign of the Cotton effect of n→π* origin could be safely used for establishing the absolute configuration of the hetero ring of this chromophore system, the sign of this type of Cotton effect is independent of the substitution pattern of the aromatic ring system.[105]

For conformation analysis the 3*R*,4*R*-dihydro-isocumarin derivate **6-7** was taken. The result provided three minimum energy conformers, whose CD-spectra were calculated quantum mechanically. Structure optimization and NMR studies show that *C*-4 methyl group remained in the axial position. Thus the helicity of the hetero ring has not changed.



6-7

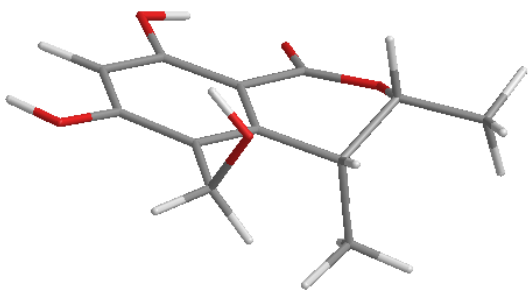


Figure 6.20: Structure and optimized conformation of dihydro-isocumarine **6-7**

The CD spectra of **6-7** showed a strong negative Cotton-effect at 200 nm ($\Delta\varepsilon = -21.40$) and two weak positive ones at 225 nm and 260 nm ($\Delta\varepsilon = 6.77, 4.06$)

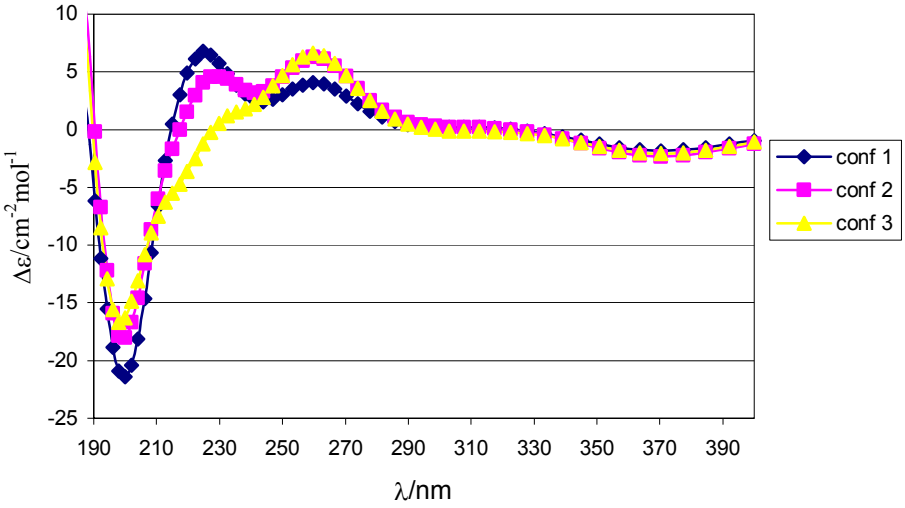


Figure 6.21: Calculated CD spectra of dihydro-isocumarine **(6-7)** conformers

According to the helicity rule of the chiral benzoic ester chromophore based on the sign of the n→π* band of conformationally fixed dihydo-isocumarin derivatives,[104] the heterocyclic ring of **6-7** must adopt a half-chair or a chair conformation, in which the methyl group at *C*-3 is oriented equatorially. The quantum chemically optimized structure of **6-7** (Figure 6.20) follows this rule, whereas the hetero ring is in distorted half-chair conformation and the *C*-3 methyl is equatorial, and the *C*-4 methyl group has axial position that also determines the helicity of the hetero ring. Therefore the 3*R* absolute configuration could also be deduced from its CD data.[105]

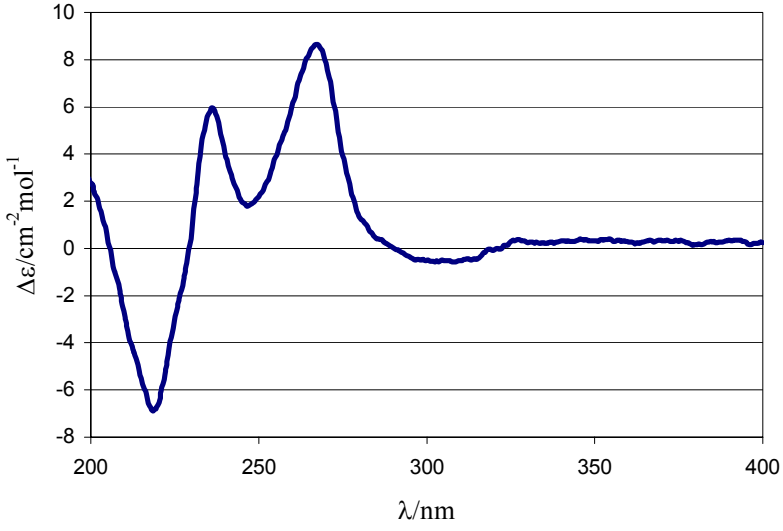


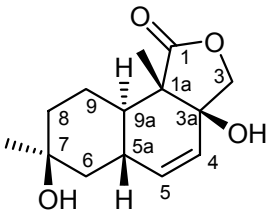
Figure 6.22: Experimental CD spectra of dihydro-isocumarine **6-7**

These results are in good agreement with other dihydro-isocumarin derivates described in literature.[105] Otherwise the calculated and measured CD spectra are also almost identical. Compound **6-7** has also a strong negative peak at 218 nm ($\Delta\varepsilon = -6.67$), and the two weak positive peaks at 236 nm ($\Delta\varepsilon = 5.96$) and 266 nm ($\Delta\varepsilon = 8.49$) were observed. A minimal shift of 10-20 nm was observed. The conformer, which was chosen for quantum chemical calculations, has 3*R*,4*R* configuration; therefore the absolute configuration of dihydro-isocumarine **(6-7)** is 3*R*,4*R* also. Since the pyranone-ring of both **6-6** and **6-7** have the same conformation, and the hydrogenation has not changed the absolute configuration of the stereo center of **6-6**, we can consider that the absolute configuration of isocumarine **6-6** is 4*R* also. This is a nice example that if the relative configuration is known, the absolute configuration could be established both by empirical (helicity) rules and by quantum chemical calculations.

6.7 Determination of the absolute configuration of a metabolic product of *Phomopsis oblonga* (6-8) using UV-correction

Phomopsis oblonga (Desm) Trav., a fungus frequently found in the outer bark of healthy *Ulmus* sp., particularly wych elm (*U. glabra*), can invade the phloem of stressed trees, principally those infected by *Ceratocystis ulmi*, the causative agent of Dutch elm disease. Bark beetles of *Scolytus* sp., the insect vector of the disease, reject *P. oblonga*-invaded phloem as being unsuitable for breeding and such trees do not become breed trees. Using a laboratory bioassay, it was discovered that *P. oblonga* produces *in vitro* a number of boring/feeding deterrents against *Scolytid* beetles.

Compound **6-8** belongs to a novel class of norsesquiterpene γ-lactones to which the name oblongolide was assigned.[106]



6-8

Figure 6.23 Relative structure of oblongolide **6-8**

After structure elucidation, the X-ray structure was used for conformational analysis, which resulted in a single low-energy conformer in the calculation.

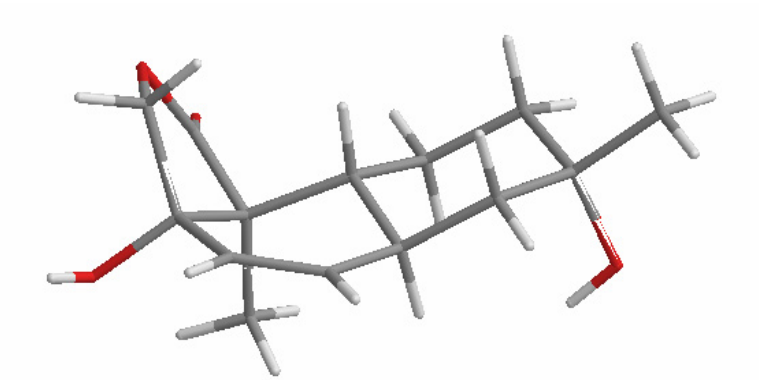


Figure 6.24: X-ray structure of lactone **6-8**

The CD spectrum of this conformer was calculated and it showed a strong negative couplet at 194 nm (Δε= –22.38), a positive one at 215 (12.85) with a shoulder at 235 nm (7.57).

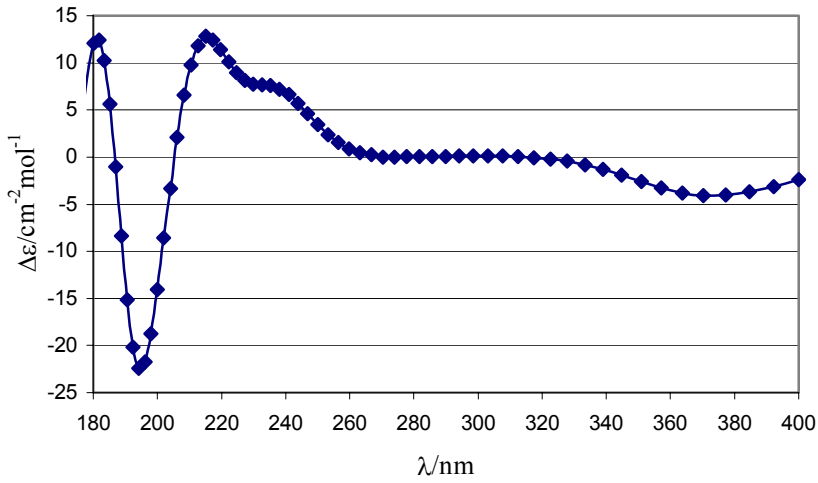


Figure 6.25: Calculated CD spectra of lactone **6-8**

Since the measured CD spectrum is a mirror image of the calculated one, having a strong positive transition at 236 nm (Δε= 3.48), a negative one at 259 (–4.68) with a shoulder at 292 (–2.49), the conformer, which was taken as starting geometry for quantum chemical calculations, is the mirror image of the natural product: 1a-*R*,3a-*R*,5a-*R*,7-*R*,9a-*S*.

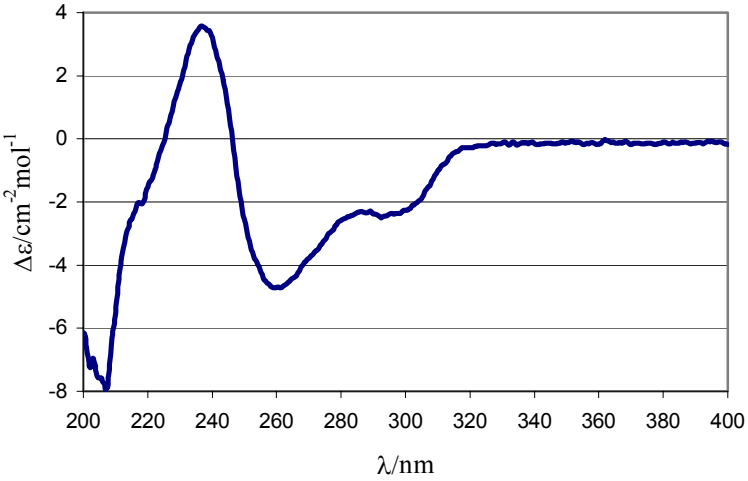


Figure 6.26: Measured CD spectra of lactone **6-8**

Jennings, Klyne and Scopes investigated the ORD and CD properties of lactones and established the "lactone sector rule", in which the molecules are viewed in the plane of the lactone group along the bisectix of the O-C-O angle, i.e., the line of the carboxy group and its attached carbon atom. Thus atoms lying in the back upper right and lower left sectors make positive contributions to the lactone Cotton effect, while atoms in the back upper left and lower right sectors make negative contributions.[107] They also studied the class of sesquiterpene lactones, and found that the compounds with *R,R,R,S* configuration showed a positive Cotton effect of the carbonyl n→π* transition at 236 nm. These results are in good agreement with the measured CD spectra of oblongolide **6-8**, since in this case the mirror image of CD curve is observed. (Fig. 6.26)

The shape of the experimental and the measured curves match also well. (Fig. 6.27) The only apparent difference is a 40 nm red shift of the measured CD spectrum compared with the calculated one –a systematic mistake of the quantum chemical calculations. According to the Brillouin theorem, the excited states and thus the transition energies may be calculated to be too low when compared to the ground state. This is due to the fact that these calculations can account for singly occupied excited configurations exclusively. This discrepancy can be eliminated by an efficient "UV correction".[87] When calculating the UV spectrum of **6-8** likewise (which should be subject to the same systematic mistake) by using the same program, the theoretical absorption spectrum was also found to be shifted by about 40 nm compared to the experimental one, i.e. by virtually the same amount as for the CD spectrum. A rational scaling of the CD spectrum, by shifting the theoretical CD spectrum by exactly the same amount, can be established.

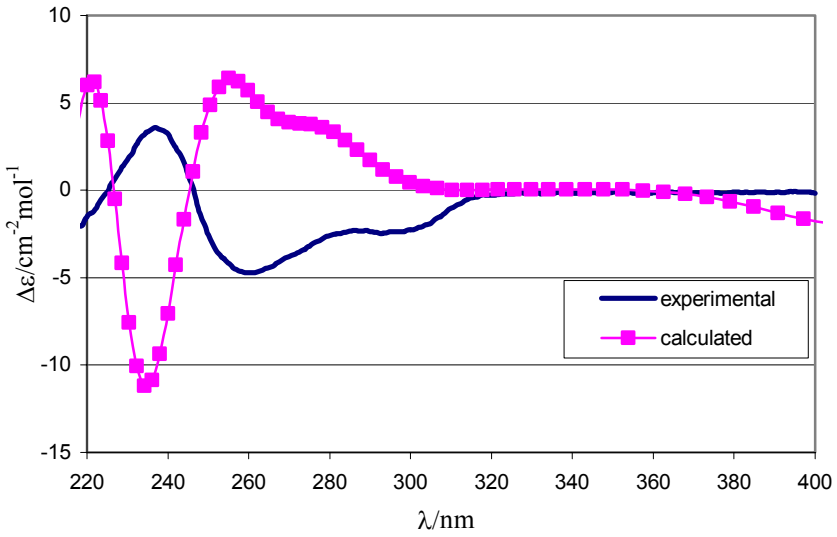
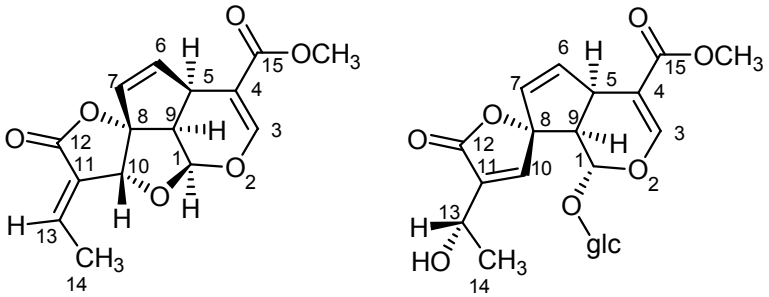


Figure 6.27 Comparison of experimental and calculated CD spectra of lactone **6-8** after UV correction

This UV-correction can be particularly helpful in those cases in which several CD bands of different signs follow each other within a smaller spectral range. Taking into consideration both theoretical[107] and computational results the absolute configuration can be deduced. In view of this UV-correction, the absolute configuration of **6-8** could be assigned as 1a-*S*,3a-*S*,5a-*S*,7a-*S*,9a-*R*.

6.8 Determination of absolute configuration of Plumericine (6-9) and Plumenoside (6-10)

Plumericin **(6-9)** was first isolated by Schönberg and Schmidt 1961[108] from the roots of *Plumeria rubra*. Plumenoside was first isolated by Abe from *Plumeria acutifolia*.[109] However, their absolute structure has not been elucidated yet. Both plumericin (**6-9**) and plumenoside (**6-10**) belong to *Apocynaceae* family to the class of iridoids, and show very good antibiotic, antifungal and antineoplastic activity.



6-9 **6-10**

Figure 6.28: Structure of plumericin (**6-9**) and plumenoside (**6-10**)

The monoterpenoid natural products are a large, structurally diverse class of compounds that occur in a wide variety of plant and animal species. A subgroup of this class, the cyclopentanoid monoterpenes, has been under extensive investigation during the past 30 years.[110,111] Most of the members of this subgroup contain a cyclopentano pyran ring system, and the name "iridoid" was suggested due to the structural similarity of these compounds to one of the simplest members, iridodial. Various plants containing iridoids have been used in a variety of folk medicines for centuries as a bitter tonic, an expectorant, a purgative, and as a treatment for certain skin diseases. Among the isolated iridoids, demonstrated biological activities include antibiotic (genepic acid, genipinic acid, plumericin, fulvoplumierin, udoteatrial), antifungal (plumericin, fulvoplumierin), hypotensive (oleuropein), analgesic (harpagoside), diuretic (catalposide), antipsychotic (gentianine).[112] All plumericin derivatives appear in the literature always in the same configuration but there is no reference about establishing the absolute configuration.

Both plumericin (**6-9**) and plumenoside (**6-10**) were isolated from the same plant by Krohn and Akthar. The relative configuration of **6-9** was established by NMR- and X-ray analysis. However, plumenoside (**6-10**) possesses a sugar moiety therefore X-ray diffraction of **6-10** could have provided even the absolute configuration. Unfortunately no single crystal could be grown. This way the absolute configuration could not be established.

In order to determine the absolute configuration, the X-ray structure of **6-9** was employed as a basis for quantum chemical calculations. Since plumericin **6-9** has a rigid conformation, it is a suitable structure for these studies. Comparison of the calculated and experimental CD spectra gave a very good agreement. There is only a slight shift of 5 nm.

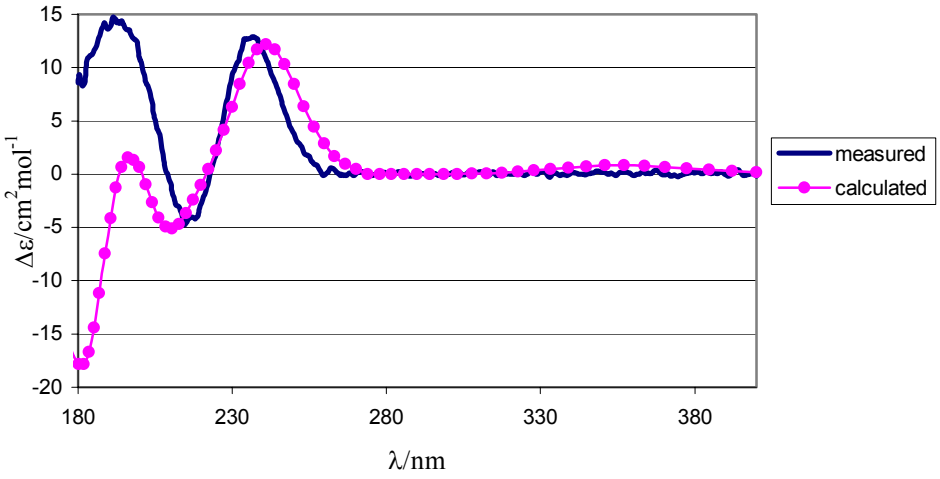


Figure 6.29: Calculated and measured CD spectra of plumericin **6-9**

The X-ray diffraction revealed that the enone chromophore has planar conformation and hence it is not inherently chiral. Thus the CD transitions derive from the perturbation effect of the neighboring stereogenic centers. Both curves show a positive Cotton effect around 191 nm due to the n→σ* transition of the enone, a negative one at 214.5 nm (Δε= −5.11) and a positive one at 237.5 nm (Δε= 12.16), which arises from the π→π* transition of the enone chromophore.[113]

For calculation the (1*S*,*5R*,9*R*,10*R)*-enantiomer was employed and the calculated and experimental curves are practically the same. Therefore, considering the good agreement of the calculated and measured CD-spectra the absolute configuration of plumericin (**6-9**) can be assigned as 1*S*,*5R*,9*R*,10*R*. Using this information, the absolute configuration of plumenoside (**6-10**) can be designated. On the one hand the optical rotation of **6-9** and **6-10** has the same sign, and both were found in the same strain. On the other hand, plumenoside (**6-10)** is a precursor for the synthesis of plumericin (**6-9)**. After cleavage of the glucose moiety, the ring closure can be performed by a Michael-addition.[112] This indicates that **6-9** and **6-10** must have the same absolute configuration.

In the literature several plumericin derivatives are described, whose sign of optical rotation is the same, but in all cases the mirror image of these substances are presented.[111,108,112,114,110]

The results of this quantum chemical calculation means that the old literature concerning the absolute structure of other substances of this class must be revised in the light of this recent finding.

7 Summary

Within the scope of this thesis, the synthesis of biologically active compounds was performed for a better understanding of the fundamentals of structure-bioactivity relationship, and in order to develop new agents for a more effective treatment of different plant or human diseases. These investigations could also open up new opportunities for other applications, such as targeted drug design and delivery.

Chapter 2 describes the synthesis of benzamide riboside derivatives with potential anitumor activity. These *C*-glycosidic analogues of NAD (nicotinamide adenine dinucleotide) (**3-1**) are supposed to increase the effect of glucocorticoids, which are administered in the treatment of malignant lymphoma.[2]

The key step of these syntheses is a metalloorganic coupling-reaction that was carried out by reacting ribonolactones as electrophiles with organic lithium reagents. The formed tertiary alcohol was reduced by triethyl silane using borontrifluoride etherate to generate the oxonium ion. An oxazoline group (**2-10**) was used as a protecting group for the amide function. In this manner, the 3-deoxy derivative (**2-32**) could also be prepared. The coupling of ribonolactone **2-7** with different lithium aryl derivatives (**2-33**, **2-36**, **2-39**) proceeded in each case with yields greater than 75 % over two steps. Investigation on the synthesis of 2-deoxy- (**2-53**) and 2,3-dideoxy-derivatives (**2-54**) showed that the presence of a benzyloxy-group in position *C*-2 of the sugar moiety is indispensable for the coupling.

Clinical trials show that benzamide riboside (**2-2**) exhibits extreme toxicity against both human myelogenous leukemia K562 cells and human colon carcinoma HT-29 cells. Test with the derivatives also showed that both the amide function on the benzene ring and the *C*-2 hydroxyl group are necessary for the desired antitumor activity.

Chapter 3 The NAD analogue BAD was prepared chemically in order to perform enzyme-substrate investigations. In cooperation with the Max Plank Institute of Biophysics, in Frankfurt, X-ray crystallographic study of this complex with reductase will be performed to investigate the active site of the enzyme. Having information about the active site of the enzyme can help to decide which part of benzamide riboside (**2-2**) should be changed to achieve a better selectivity in tumor cells. Furthermore, this finding can also provide information about the mechanism of action of reductases. These results could open new ways for design of biologically more active benzamide riboside analogues.

Chapter 4 deals with the structure activity relationship investigation of a fungicide, named coniothyriomycin (**4-1**). Through systematic changing of the substitution pattern of **4-1** it was shown that fumaric side chain is necessary for the desired antifungal property. The replacement of the phenyl acetic acid moiety by benzoic acid does not decrease the biological activity. Electron donating substituents (e.g. OCH_3 groups) on the aromatic ring increase the fungicide effect. Changing the imide function to a hydrazide or thioimide decreases the activity.

Chapter 5 involves a very nice example that theoretically calculated chemical reactivity matches the experimental data of sensitizing capacity in a large and homogeneous group of potential allergens. LUMO coefficients of phenanthrenequinones were calculated semi-empirically and compared with sensitation tests performed on guinea pigs using Freund´s complete adjuvant. These results show a very good correlation between chemical reactivity and sensitizing capacity.

Chapter 6 comprises the determination of the absolute configuration of several natural compounds whose CD-spectra were calculated theoretically and compared with the experimental curve. If the two spectra show essentially the same shape of the curve, then the studied compound has the same absolute configuration as the one used for the calculation. If the two curves are the mirror image of each other, then the calculated structure is the other enantiomer. This way the absolute configuration of 6 natural products have been assigned. The knowledge of the absolute configuration is very important for further structure-activity relationship investigation.

8 Experimental Part

8.1 Instrumentation

Column Chromatography: Silica gel 60 (230-400 mesh, 0.04-0.063 nm) from Merck.

Thin Layer Chromatography (TLC): Macherey-Nagel, Silica gel 60/F_{254}. The compounds were visualized by irradiation with 254 or 366 nm light and/or using developing reagents:

- 8 % H_2SO_4 in ethanol
- Solution of 10 g Cer-(IV)-sulfate, 25 g Molybdatophosphoric acid and 60 mL cc. H_2SO_4 in 960 mL water.

IR Spectra: Nicolet FT-IR 510 p. The spectra were measured as KBr pellets or as thin films of neat compound. The absorptions are given in wave numbers (cm^{-1}).

Optical Rotation: Perkin-Elmer Polarimeter 241 using standard cuvette (d = 10 cm) and Na-lamp (D-line, λ = 589 nm).

CD-Spectroscopy: JASCO J-715/150S Spectrometer at 25 °C. Temperature control by Peltier Thermostate, solvent CH_3CN. The spectra were recorded by Dr. Tibor Kurtán, Department of Organic Chemistry, University of Debrecen, Hungary.

Mass Spectra: Fison MD 800. Relative intensity is related to basis peak.

NMR spectra: BRUKER ARX 200, AMX 300, ARX 500. All spectra were recorded at room temperature (RT). ^{1}H-NMR and ^{13}C-NMR spectra the chemical shift values are given in ppm relative to $SiMe_4$. The resonance multiplicity is described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Broad resonances are indicated broad (br).

Melting Point: Gallenkamp Melting point Apparatus, using one-side open capillary.

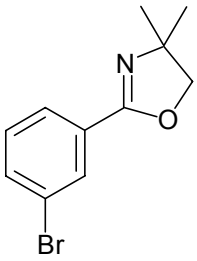
Purification of Solvents: Purification of solvents was performed by standard methods.[115,116]

Program for CD-Spectra calculation: BDZDO/MCDSPD Program packet Version Z-07A from J. W. Downing, Department of Chemistry and Biochemistry, University of Colorado, Boulder, USA; modified by J. Fleischauer, W. Schlecker und B. Kramer or DZDO Program Version 4.23, ZDO Program Version of 22.06.2001.

Program to calculate the conformers: Spartan SGI Version 5.1.3; Wavefunction Inc., Irvine, USA.

8.2 Experimental Part to Chapter 2

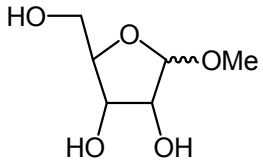
2-(3-Bromphenyl)-4,4-dimethyl-2-oxazoline (2-10)[27]



To a solution of 2-amino-2-methyl-1-propanol (14.4 mL, 0.15 mol) in abs. dichloromethane (30 mL) *m*-bromo benzoyl chlorid (10 mL, 0.075 mol) in dichloromethane (30 mL) was dropped slowly under argon atmosphere at 0 °C. The reaction mixture was stirred for 2 h at 25 °C, and white crystals precipitated. The crystals were filtered off and washed with water. The filtrate was evaporated and the precipitated crystals were filtered off, and washed with water.

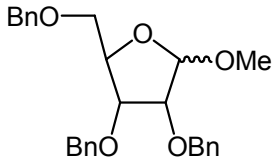
After drying this intermediate thionyl chloride (14.6 mL, 0.2 mol) was added slowly. Formation of a gas was observed. The reaction mixture was then poured into diethyl ether and the precipitated crystals were filtered off. The hydrochloride salt was neutralized by the addition of 20 % aqueous NaOH solution and extracted three times with ether. The organic phases were dried over Na_2SO_4. After evaporation of the solvent, the product could be isolated as a colorless oil.[27] (TLC: petrol ether/ethyl acetate: 7: 3). Yield: 11.8 g (75 %).–

Methyl-D-ribofuranoside (2-4)[24]



D-ribose (25.0 g, 0.17 mol) was dissolved in dry methanol (450 mL) at 0 °C and cc. sulfuric acid (2.5 mL) was added. After stirring for 16 h at 4 °C, the reaction mixture was neutralized by the addition of ion-exchange resin (Amberlite XAD-4 stored over saturated NaOH-methanol solution). After filtration, the solvent was evaporated under vacuum. The viscous oil was dried under high vacuum. Yield: 26.8 g (98 %).–

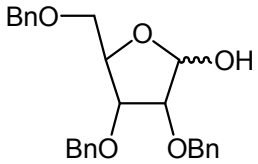
Methyl-2,3,5-tri-*O*-benzyl-D-ribofuranoside (2-5)[25]



Methyl riboside (**2-4**) (25.0 g, 0.162 mol) was dissolved in abs. DMF (300 mL), and 60 % sodium hydride (35.0 g, 0.875 mol) was added. Formation of hydrogen gas was observed; then tetrabutylammonium iodide (18.0 g, 0.016 mol) was added. After half an hour, benzyl bromide (60 mL, 0.875 mol) was added dropwise at 0 ° and the reaction mixture was kept at 50 °C overnight. (TLC monitoring: petrol ether: ethyl acetate = 3:1.) The reaction mixture was poured into 1 L of ice water and extracted once with diethyl ether and three times with dichloromethane. The combined organic phases were dried over Na_2SO_4 and evaporated at reduced pressure. After column chromatography (first petrol ether, then petrol ether/ethyl acetate 3:1) 59.9 g (84 %) benzyl ether (**2-5**) was obtained.–

^{1}H-NMR (200 MHz): δ = 3.31 (s, 3H, OCH_3), 3.49 (d, $J_{4,5a}$ = 3.3 Hz, J_{gem} = 10.6 Hz, 1H, 5a-H), 3.59 (dd, $J_{4,5b}$ = 3.3 Hz, J_{gem} = 10.6 Hz, 1H, 5b-H), 3.83 (d, J = 4.6 Hz, 1H, 3-H), 4.02 (dd, $J_{1,2}$ = 4.6, $J_{2,3}$ = 6.9 Hz, 1H, 2-H), 4.34 (m, 1H, 4-H), 5.21 (d, $J_{1,2}$ = 6.5 Hz, 1H, 1-H), 4.39-4.64 (m, 6H, 3 CH_2Ph), 7.26 (m, 15H, ArH).–

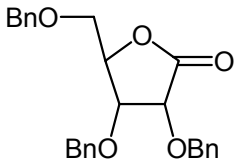
2,3,5-Tri-*O*-benzyl-D-ribofuranoside (2-6)[25]



Benzyl ether (**2-5**) (55.0 g, 0.13 mol) was dissolved in dioxane (300 mL) and aqueous 1N HCl (220 mL). The reaction mixture was heated under reflux for 3 hours. After cooling to room temperature, the reaction mixture was neutralized by the addition of aqueous saturated $NaHCO_3$ solution. The solvents were evaporated at reduced pressure, and the residue was dissolved in dichloromethane. The organic phase was washed with water, and the combined organic phases were dried over Na_2SO_4, and evaporated at reduced pressure. A yellow oil was obtained, which was purified by column chromatography (petrol ether/ethyl acetate: 2:1) to yield 42.5 g of **2-6** (84.4 %).–

^{1}H-NMR (200 MHz): δ = 3.41 (d, $J_{4,5a}$ = 3.5 Hz, J_{gem} = 10.4 Hz, 1H, 5a-H), 3.50 (m, 1H, 3-H), 3.60 (dd, $J_{4,5b}$ = 3.7 Hz, J_{gem} = 10.4 Hz, 1H, 5b-H), 3.63 (m, 1H, 2-H), 4.39 (m, 1H, 4-H), 5.23 (d, $J_{1,2}$ = 6.4 Hz, 1H, 1-H), 4.39-4.64 (m, 6H, 3 CH_2Ph), 7.26 (m, 15H, ArH).–

2,3,5-Tri-*O*-benzyl-D-1,4-ribonolactone (2-7)[26]

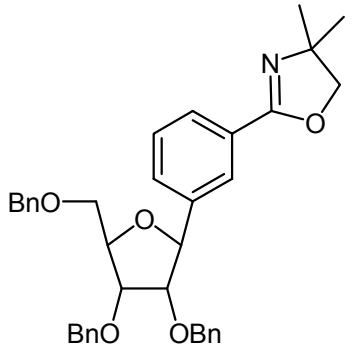


Acetic acid anhydride (90.0 mL) and dimethyl sulfoxide (400.0 mL) were stirred under argon atmosphere for 1 hour. 2,3,5-Tri-*O*-benzyl-D-ribofuranoside **2-6** (42.0 g, 0.103 mol) was then added and the reaction mixture was stirred at room temperature overnight, poured into ice water (250.0 mL) and stirred for another hour. The reaction mixture was extracted with dichloromethane and the organic phase was dried over Na_2SO_4. After filtration, the solvent was evaporated at reduced pressure to yield a yellowish-orange oil, which was purified by column chromatography (petrol ether/ethyl acetate: 4:1). The product **2-7** (40.0 g, 94 %) was dried under vacuum, and crystallized from ethanol. mp.: 34 °C.–

^{1}H-NMR (200 MHz): δ = 3.63 (dd, 1H, $J_{4,5a}$ = 2.7 Hz, J_{gem} = 11 Hz, 1H, 5a-H), 3.69 (dd, $J_{4,5b}$ = 2.8 Hz, J_{gem} = 11 Hz, 1H, 5b-H), 4.21 (m, 1H, 3-H), 4.49 (m, 1H, 2-H), 4.55 (m, 1H, 4-H), 4.61-4.85 (m, 6H, 3 CH_2Ph), 7.26 (m, 15H, ArH).–

^{13}C-NMR (200 MHz): δ = 69.3 (CH_2, C-5), 72.8 (OCH_2), 73.2 (OCH_2), 74.04 (OCH_2), 74.4 (CH, C-3), 75.9 (CH, C-4), 82.3 (CH, C-2), 128.1-129.01 (ArH), 137.5 (q, ArH), 137.7 (q, ArH), 137.8 (q, ArH), 174.4 (C=O).–

2-[3-(2,3,5-Tribenzyl-β-D-ribofuranosyl)-phenyl]-4,4-dimethyl-2-oxazoline (2-14)[64]



A solution of bromo oxazoline (**2-10**) (4.55 g, 18 mmol) in anhydrous THF (50 mL) was treated under nitrogen at −85 °C within 10 min with a solution of n-BuLi (12 mL, 1.5 M) in hexane. After 20 min at −85 °C, a solution of the lactone (**2-7**) (5.00 g, 12 mmol) in THF (30 mL) was added over 30 min and stirred for an additional 1 h and then warmed over 2 h to −30 °C (TLC control). The reaction was then quenched by the addition of water (30 mL) and extracted with diethyl ether (100 mL). The organic phase was dried with Na_2SO_4 and evaporated under reduced pressure to afford a slightly red oil.

The residue was dissolved in CH_2Cl_2 and treated at −78 °C with boron trifluoride diethyl etherate (4.5 mL, 35.43 mmol) and triethylsilane (5.7 mL, 35.78 mmol). The reaction mixture was stirred for 1 h at −78 °C, warmed overnight to 10 °C, neutralized by the addition of a saturated aqueous sodium hydrogencarbonate solution (ca. 15 mL), and extracted with CH_2Cl_2 (100 mL). The solution was dried over Na_2SO_4 and purified by column chromatography (hexane/5 % ethyl acetate) to afford **2-14** as a yellow oil (6.02 g, 87 %).–

IR (film): 3063 (aromatic), 2967, 2925 (CH_3, CH_2), 2892, 2867 (CH), 1779, 1651 (C=N), 1604 (aromatic), 1454, 1363, 1355, 1267, 1124 (ether), 1121, 1092, 1062, 1028, 994, 972, 911, 806.–

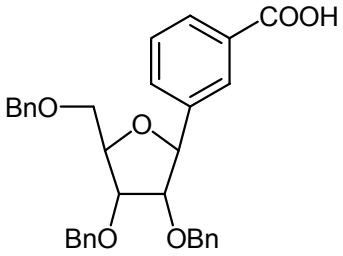
UV: 208 (4.64), 239 (sh, 4.06).–

^{1}H-NMR (300 MHz): δ = 1.37 (s, 6H, 2 CH_3), 3.66 (ddd, $J_{4,5a}$ = 3.9 Hz, $J_{4,5b}$ = 4.0 Hz, J_{gem} = 10.4 Hz, 2H, 5-H), 3.83 (dd, $J_{2,3}$ = 5.2 Hz, $J_{1,2}$ = 6.5 Hz, 1H, 2-H), 4.02 (dd, $J_{2,3}$ = 5.0 Hz, $J_{3,4}$ = 4.0 Hz, 1H, 3-H), 4.05 (2 s, 2H, CH_2), 4.34 (m, 1H, 4-H), 5.05 (d, $J_{1,2}$ = 6.5 Hz, 1H, 1-H), 7.31 (m, 16H, ArH), 7.52 (d, $J_{5,6}$ = 7.8 Hz, 1H, 6´-H), 7.88 (d, $J_{4,5}$ = 7.8 Hz, 1H, 4´-H), 7.99 (s, 1H, 2´-H).–

^{13}C-NMR (300 MHz): δ = 29.01 (CH_3), 29.02 (CH_3), 68.17 (CH), 70.91 (CH_2, 5-C), 72.55 (OCH_2), 72.88 (OCH_2), 74.04 (OCH_2), 78.03 (CH), 79.64 (CH_2, oxaz), 82.47 (CH), 82.87 (CH), 84.32 (CH), 126.64 (ArC), 128.19 (ArC), 128.26 (ArC), 128.30 (ArC), 128.35 (ArC), 128.45 (ArC), 128.54 (ArC), 128.62 (ArC), 128.74 (ArC), 128.87 (ArC), 128.91 (ArC), 128.96 (ArC), 129.10 (ArC), 129.16 (q, ArC), 129.28 (ArC), 129.82 (ArC), 138.28 (q, ArC), 138.53 (q, ArC), 138.78 (q, ArC), 141.33 (q, ArC), 162.63 (q, oxaz-C).–

Anal. Calcd. for $C_{37}H_{39}NO_5$: (577.28): C: 76.92, H: 6.80, N: 2.42;
Found.: C: 76.86, H: 6.77, N: 2.40.–

3-(2,3,5-Tribenzyl-β-D-ribofuranosyl)benzoic acid (2-16)[64]



A solution of oxazoline (**2-14**) (25.0 g, 47.7 mmol) in nitromethane (71 mL) was treated with methyl iodide (35 mL) and refluxed for 22 h. The solvents were evaporated under reduced pressure, and the residue was dissolved in methanol (200 mL) and 20 % aqueous KOH (250 mL) and refluxed for 44 h. Half of the solvent was removed under reduced pressure, and the mixture was then neutralized by the addition of HCl and extracted three times with ethyl acetate. The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to afford the crude acid **2-16** (35.0 g, 76 %) that could be used for the next reaction without purification. $[\alpha]_d^{23}$ = 12.41 ° (c = 0.6 in methanol).–

IR (film): 3040, 2880, 2920, 1690, 1610, 1580, 1500, 1460, 1410, 1360, 1200, 1100, 820, 750, 700.–

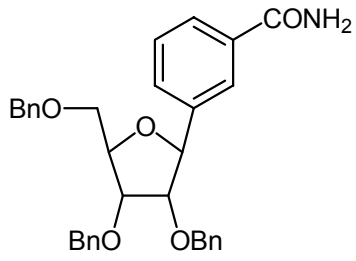
UV: 206 (4.18), 226 (sh, 3.76), 275 (2.79).–

^{1}H-NMR (300 MHz): δ = 3.64 (ddd, $J_{4,5a}$ = 3.7 Hz, $J_{4,5b}$ = 3.8 Hz, J_{gem} = 10.4 Hz, 2H, 5-H), 3.81 (dd, $J_{2,3}$ = 4.0 Hz, $J_{1,2}$ = 6.4 Hz, 1H, 2-H), 4.02 (dd, $J_{2,3}$ = 5.0 Hz, $J_{3,4}$ = 4.0 Hz, 1H, 3-H), 4.37 (m, 1H, 4-H), 4.41-4.67 (3 AB-systeme, 2H, 3 CH_2Ph), 5.07 (d, $J_{1,2}$ = 6.8 Hz, 1H, 1-H), 7.16-7.38 (m, 16H, ArH), 7.64 (d, $J_{5,6}$ = 7.5 Hz, 1H, 6′-H), 8.01 (d, $J_{4,5}$ = 7.5 Hz, 1H, 4′-H), 8.19 (s, 1H, 2′-H).–

^{13}C-NMR (300 MHz): δ = 69.85 (CH_2, 5-C), 73.52 (OCH_2), 73.95 (OCH_2), 75.14 (OCH_2), 78.03 (CH), 82.35 (CH), 82.76 (CH), 84.23 (CH), 126.62 (ArC), 128.21 (ArC), 128.27 (ArC), 128.31 (ArC), 128.37 (ArC), 128.44 (ArC), 128.53 (ArC), 128.64 (ArC), 128.75 (ArC), 128.88 (ArC), 128.89 (ArC), 128.95 (ArC), 129.11 (ArC), 129.26 (ArC), 129.80 (ArC), 130.08 (q, ArC), 138.27 (q, ArC), 138.55 (q, ArC), 138.80 (q, ArC), 141.35 (q, ArC), 169.33 (q, C=O).−

Anal. Calcd. for $C_{33}H_{32}NO_6$: (524.6): C: 75.55, H: 6.15;
Found.: C: 75.38, H: 6.21.–

3-(2,3,5-Tribenzyl-*β*-D-ribofuranosyl)benzamide (2-18)



A solution of the acid (**2-16**) (35.0 g, 66.8 mmol) in thionyl chloride (100.0 mL) was treated with ten drops of DMF and refluxed for 2-3 h. The residue of thionyl chloride was distilled off. The brown oil was dissolved in CH_2Cl_2 and the solution was added dropwise to a cold concentrated aqueous solution of ammonia (300 mL) and then extracted with water and dichloromethane**.** The organic phase was dried over Na_2SO_4, evaporated under reduced

pressure, and purified by column chromatography (first ethyl acetate then CH_2C1_2/5 % MeOH) to afford the amide **2-18** (4.61 g, 97 %): mp 98 °C (diethyl ether/pentane); $[\alpha]_d^{23}$ = –48 ° (c = 1.2 in CCl_4) .–

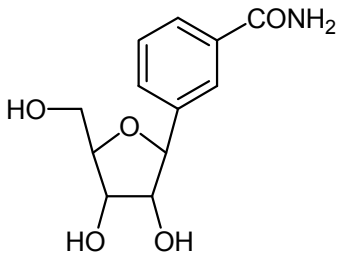
IR (film): 3360, 3210 (NH_2), 2940, 2880, 1670 (C=O), 1640 (amid II), 1610, 1590, 1500, 1460, 1390, 1140, 1100 (ether), 820 (aromatic), 750, 700.–

^{1}H-NMR (300 MHz): δ = 3.64 (ddd, $J_{4,5a}$ = 3.5 Hz, $J_{4,5b}$ = 3.8 Hz, J_{gem} = 10.4 Hz, 2H, 5-H), 3.70 (q, 1H, 4-H), 3.82 (dd, $J_{2,3}$ = 5.1 Hz, $J_{1,2}$ = 6.4 Hz, 1H, 2-H), 4.05 (t, 1H, 3-H),), 4.43-4.63 (3 AB-systems, each 2H, 3 C*H*$_2$Ph), 5.06 (d, $J_{1,2}$ = 6.4 Hz, 1H, 1-H), 6.01 (s, 2H, NH_2), 7.15-7.36 (m, 16H, ArH), 7.51 (d, $J_{5,6}$ = 7.5 Hz, 1H, 6´-H), 7.76 (d, $J_{4,5}$ = 7.5 Hz, 1H, 4´-H), 7.78 (s, 1H, 2´-H).–

^{13}C-NMR (300 MHz): δ = 70.29 (CH_2, 5´-C), 71.99 (OCH_2, CH_2Ph), 72.30 (OCH_2, CH_2Ph), 73.48 (OCH_2, CH_2Ph), 77.19 (CH, 3´-C), 81.81 (CH, 4´-C), 82.09 (CH, 1´-C), 83.66 (CH, 2´-C), 124.57-133.39 (19 ArC), 137.53 (q, ArC), 137.98 (q, ArC), 140.94 (q, ArC), 169.31 (q, C=O).–

Anal. Calcd. for $C_{33}H_{33}NO_5$: (523.6): C: 75.70, H: 6.35, N: 2.67;
Found: C: 75.42, H: 6.32, N: 2.55.–

3-(*β*-D-Ribofuranosyl)-benzamide (2-2)[64]



A solution of the benzyl ether (**2-18**) (2.00 g, 3.82 mol) in THF (50 mL) and methanol (2 mL) was hydrogenated at atmospheric pressure (200 mg 10 % palladium/charcoal, 18 h stirring). The catalyst was filtered off over Celite, the solvent was evaporated under reduced pressure. The product was purified by column chromatography (15 % CH_3OH/CH_2Cl_2) to afford **2-18**

as a colorless oil (895 mg, 93 %): $[\alpha]_d^{23} = -28$ ° (c = 0.7 in methanol) and open chained side product **2-19** as white powder.–

IR (film): 3360 (OH), 2925, 1663 (C=O), 1606, 1581, 1397, 1115, 1072, 1051.–

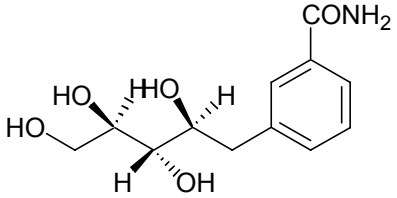
UV: 207 (3.25), 223 (sh, 2.93), 274 (2.17).–

^{1}H-NMR (D_6-DMSO, 300 MHz): δ = 3.55, 3.59 (2 dd, $J_{4,5}$ = 4.4 Hz, J_{gem} = 11.7 Hz, 2H, 5-H), 3.90 (dd, $J_{2,3}$ = 5.4 Hz, $J_{1,2}$ = 7.1 Hz, 1H, 2-H), 3.83 (q, 1H, 4-H), 3.90 (t, 1H, 3-H), 4.60 (d, $J_{1,2}$ = 7.1 Hz, 1H, 1-H), 7.36 (s, 2H, NH_2), 7.41 (t, J = 7.7 Hz, 1H, 5´-H), 7.56 (d, $J_{5,6}$ = 7.7 Hz, 1H, 4´-H), 7.77 (d, $J_{4,5}$ = 7.7 Hz, 1H, 6´-H), 7.96 (s, 1H, 2´-H).–

^{13}C-NMR (D_6-DMSO, 300 MHz): δ = 62.10 (CH_2, 5-C), 71.47 (CH), 77.60 (CH), 82.89 (CH), 85.31 (CH), 125.52 (ArC), 126.46 (ArC), 127.99 (ArC), 129.21 (ArC), 134.17 (q, ArC), 141.55 (q, ArC), 168.09 (q, C=O).–

Anal. Calcd. for $C_{12}H_{15}NO_5$: (253.25): C: 56.91, H: 5.97, N: 5.53;
Found: C: 55.87, H: 6.44, N: 5.45.–

(2*S*, 3*S*, 4*R*)-3-(2,3,4-Tetrahydroxypentyl)benzamide (2-19)



mp.: 134 °C, white powder.–

IR (KBr): 3303 (OH), 1643, 1607, 1577, 1387, 1070, 1016, 767.–

UV: 206 (3.22), 229 (2.72), 275 (1.90).–

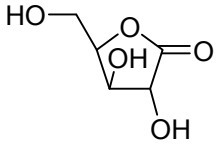
^{1}H-NMR (D_6-DMSO, 300 MHz): δ = 2.59 (dd, J_{gem} = 13.8 Hz, $J_{1b,2}$ = 9.4 Hz, 1H, 1b-H), 2.94 (dd, $J_{1a,2}$ = 2.3 Hz, J_{gem} = 13.8 Hz, 1H, 1a-H), 3.42-3.90 (m, 9H, 2, 3, 4, 5-H, 4 OH), 7.36 (s,

2H, NH_2), 7.38 (t, J = 7.5 Hz, 1H, 5′-H), 7.75 (d, $J_{5,6}$ = 7.6 Hz, 1H, 4′-H), 7.84 (d, $J_{4,5}$ = 7.6 Hz, 1H, 6′-H), 7.99 (s, 1H, 2′-H).–

^{13}C-NMR (D_6-DMSO, 300 MHz): δ = 13.16 (CH), 37.30 (CH_2), 62.46 (CH_2), 72.09 (CH), 73.66 (CH), 123.87 (ArC), 126.76 (ArC), 127.97 (ArC), 131.72 (ArC), 133.01 (q, ArC), 139.73 (q, ArC), 167.68 (q, C=O).–

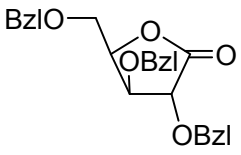
Anal. Calcd. for $C_{12}H_{17}NO_5$: (255.27): C: 56.46, H: 6.71, N: 5.49;
Found: C: 55.16, H: 6.80, N: 5.36.–

D-Xylono-1,4-lactone (2-21)[33]



A solution of D-xylose **2-20** (30 g, 0.2 mol) and water (100 mL) was cooled to 0 °C and K_2CO_3 (34 g, 0.232 mol) was added in small portions. Bromine (12 mL, 0.216 mol) was added dropwise. The solution was kept at 0 °C for a half an hour and warmed up to room temperature overnight.
The reaction mixture was worked up by the addition of formic acid. Evaporation of the solvent resulted in white crystals (a mixture of D-xylono-1,4-lactone and inorganic salts, which was used for the next step without further purification).–

2,3,5-Tri-*O*-benzoyl-D-1,4-xylonolactone (2-22)[34]



A reaction mixture of benzoyl chloride (32 mL, 0.13 mol), pyridine (40 mL), and chloroform (32 mL) was cooled with ice to 0 °C, and xylonolactone (**2-21**) (5.0 g, 0.043 mol) was added slowly. After 1 hour the reaction mixture was diluted with chloroform and extracted with saturated aqueous $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$ and the

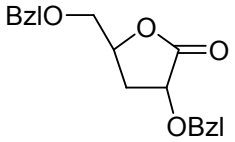
solvents were evaporated. The product was recrystallized from diethyl ether to yield **2-22** (9.0 g, 60 %), mp.: 156-158 °C, $[\alpha]_d^{23}$= 24 ° (in aceton).–

IR (KBr): 3062, 2958, 1797 (C=O), 1724 (C=O), 1452 (ν C=C), 1272 (C-O(C), 1114 (C-O(C), 707 (γ =CH).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 4.72 (m, 2H, 5a,b-H), 5.40 (m, 1H, 3-H), 6.15 (m, 1H, 4-H), 6.19 (m, 1H, 2-H), 7.36-7.67 (m, 9H, ArH), 7.98-8.14 (m, 6H, ArH) (m, 9H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 62.0 (CH_2, C-5), 71.8 (CH, C-3), 73.4 (CH, C-4), 75.9 (CH, C-2), 128.3-130.6 (ArC), 134.1 (ArC), 134.4, (ArC), 134.6 (ArC), 165.6 (C=O), 169.0 (C=O, C-1).–

3-Deoxy-2,5-di-*O*-benzoyl-D-1,4-xylonolactone (2-24)



2,3,5-Tri-*O*-benzyl-D-1,4-xylonolactone (**2-22**) (10.0 g, 0.0217 mol) was dissolved in ethyl acetate (75 mL) and triethylamine (2 mL). The solution was hydrogenated overnight using 10 % palladium/charcoal. The reaction mixture was filtered over Celite and washed first with aqueous sat. $NaHCO_3$ solution, and then with 4M HCl. The organic phase was dried over $MgSO_4$ and the solvent was evaporated to afford **2-24** (5.0 g), which was recrystallized from ethanol. Yield 67 %, mp.: 135-137 °C, $[\alpha]_d^{23}$ = –149 ° (in CH_2Cl_2).–

IR (KBr): 3059, 2977, 1784 (C=O), 1727 (C=O), 1452 (ν C=C), 1272 (C-O(C), 1114 (C-O(C), 707 (γ =CH).–

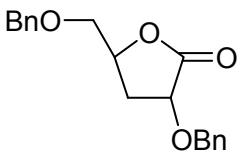
^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.25-2.32 (o, 1H 3a-H), 2.35-2.42 (o, 1H, 3b-H), 4.55 (dd, J_1= 3 Hz, J_2= 10 Hz, 1H 5a-H), 4.71 (dd, J_1= 5.5 Hz, J_2= 7 Hz, 1H, 5b-H), 4.94 (m, 1H, 4-H), 5.80 (t, J= 9 Hz, 1H, 2-H), 7.45-7.53 (m, 4H, ArH), 7.59-7.68 (m, 2H, ArH), 8.08-8.13 (m, 4H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 31.4 (CH_2, C-3), 65.3 (CH_2, C-5), 68.9 (CH, C-4), 74.8 (CH, C-2), 128.9-134.2 (ArC), 165.3 (C=O), 166.0 (C=O), 171.6 (C=O, C-1).–

Mass calcd.: 340.09;
MS (EI-CI) m/z %: 341.2 [M^+] (63%), 218 [M^+–OBzl], 105, 77, 51.–

Anal. Calcd. for $C_{19}H_{20}O_4$ (340.09) C: 67.05, H: 4.74;
Found: C: 66.53, H: 4.69.–

3-Deoxy-2,5-dibenzyl-D-1,4-xylonolactone (2-26)



3-Deoxy-2,5-di-*O*-benzoyl-D-1,4-xylonolactone (**2-24**) (4.3 g 12.6 mmol) was suspended in methanol (120 mL) and NaOMe (3 mL) solution was added. After 1 hour the solution was neutralized by the addition of Amberlite IR-120 on-exchange resin, evaporated, and dried at reduced pressure. The residue was dissolved in abs. DMF and NaH (0.912 g, 38.0 mmol) was added. After ½ h, tetrabutylammonium iodide (1.22 g, 3.8 mmol) and benzyl bromide (4.5 mL, 38.0 mmol) was added slowly and stirred at room temperature overnight.
The reaction mixture was poured into ice-water and extracted with dichloromethane. The organic phase was dried over $MgSO_4$ and the solvent evaporated. The residue was purified by column chromatography (PE: EE: 3:1) to yield **2-26** (1.81 g, 46 %) as a yellow oil. $[\alpha]_d^{23}$= –5.61 ° (in CH_2Cl_2).–

IR (film): 3064, 2925, 1789 (C=O), 1454 (ν C=C), 1272 (C-O(C)), 1122 (C-O(C), 698 (γ =CH).–

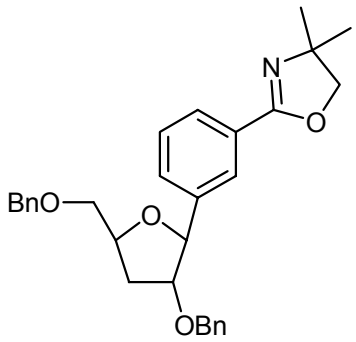
^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.08-2.27 (o, 1H 3a-H), 2.47-2.60 (o, 1H, 3b-H), 3.68 (d, 2H, 5a,b-H), 4.31 (t, *J*= 9 Hz, 1H, 2-H), 4.54 (m, 1H, 4-H), 6.41 (s, 2H, $ArCH_2$), 4.90 (dd, J_1= 12 Hz, J_2= 32 Hz, 2H, $ArCH_2$), 7.39 (m, 10H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 32.0 (CH_2, C-3), 71.4 (CH_2, C-5), 72.7 (OCH_2), 73.6 (CH, C-4), 73.9 (OCH_2), 76.1 (CH, C-2), 128.1-128.9 (ArC), 137.4 (ArC), 138.8 (ArC), 175.0 (C=O, C-1).–

Mass calcd.: 312.14;
MS (EI-CI): 313 [M+1] (24 %), 223.2, 181.2, 133.1, 57.1.–

Anal. Calcd. for $C_{19}H_{20}O_4$: (312.14): C: 67.05, H: 4.74;
Found: C: 67.01, H: 4.78.–

2-[3-(3-Deoxy-2,5-dibenzyl-*β*-D-ribofuranosyl)-phenyl]-4,4-dimethyl-2-oxazolin (2-29)



A solution of bromo oxazoline (**2-10**) (834 mg, 3.36 mmol) in anhydrous THF (20 mL) was treated under nitrogen atmosphere at −85 °C within 10 min with a solution of *n*-BuLi (2.2 mL, 1.6 M) in hexane. After 20 min at −85 °C a solution of the lactone (**2-26**) (700 mg, 2.24 mmol) in THF (30 mL) was added over 30 min and stirred for an additional 1 h and then let to warm up within 2 h to −30 °C (TLC control). The reaction was then quenched by the addition of water (10 mL) and extracted with diethyl ether (30 mL). The organic phase was dried with Na_2SO_4 and evaporated under reduced pressure to afford a slightly red oil.
The residue was dissolved in CH_2Cl_2 and treated at −78 °C with boron trifluoride diethyl etherate (0.84 mL, 6.67 mmol) and triethylsilane (1.06 mL, 6.67 mmol). The reaction mixture was stirred for 1 h at −78 °C, warmed overnight to 10 °C, neutralized by the addition of a saturated aqueous sodium hydrogencarbonate solution (ca. 15 mL), and extracted with CH_2Cl_2 (100 mL). The solution was dried over Na_2SO_4 and purified by column chromatography (hexane/ethyl acetate: 3: 1) to afford **2-29** as a colorless oil (700 mg, 67 %), $[\alpha]_d^{20}$ = –93 ° (in CH_2Cl_2).–

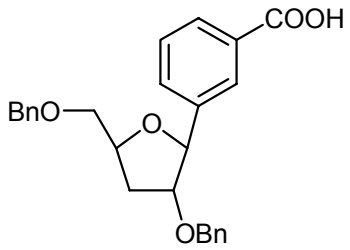
IR (film): 3031, 2927, 1722 (ν C=N), 1454 (ν C=C), 1272 (C-O(C)), 1106 (ν_{as} C-O-C), 698 (γ =CH).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 1.44 (s, 6H, 2xCH_3), 1.96-2.08 (o, 1H, 3a-H), 2.26-2.40 (o, 1H, 3b-H), 3.62 (dd, 1H, 5a-H), 3.77 (dd, 1H, 5a-H), 4.08 (t, *J*= 6 Hz, 1H, 2-H), 4.15 (s, 2H, OCH_2), 4.60 (m, 1H, 4-H), 4.56 (s, 2H, OCH_2), 4.67 (m, 2H, 2´-H), 5.12 (d, *J* = 4 Hz, 1H, 1-H), 7.23-7.53 (m, 12H, ArH), 7.78-7.99 (m, 2H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 28.8 (2xCH_3), 34.3 (CH_2, C-3), 68.0 (C-3´), 72.2 (CH_2, C-5), 73.4 (OCH_2), 73.8 (OCH_2), 78.3 (CH, C-2), 79.5 (CH_2, C-2´), 84.6 (CH, C-4), 85.9 (CH, C-1), 125.9 (ArC), 127.8-129.1 (ArC), 138.4 (ArC), 138.7 (ArC), 142.0 (ArC), 162.5 (C-1´).–

Anal. Calcd. for $C_{30}H_{33}NO_4$: (471.59): C: 76.41, H: 7.05;
Found: C: 76.34, H: 7.08.–

3-(3-Deoxy-2,5-dibenzyl-*β*-D-ribofuranosyl)benzoic acid (2-30)



A solution of oxazoline **2-29** (700 mg, 1.48 mmol) in nitromethane (20 mL) was treated with methyl iodide (1 mL) and refluxed for 22 h. The solvents were evaporated under reduced pressure, and the residue was dissolved in methanol (15 mL) and 20 % aqueous KOH (25 mL) and refluxed for 44 h. Half of the solvent was removed under reduced pressure, and the mixture was neutralized by the addition of aqueous 1N HCl solution and extracted three times with ethyl acetate. The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to afford the crude acid **2-30** (450 mg, 72 %) that could be used for the next reaction without purification. $[\alpha]_d^{23}$ = –32.26 ° (in CH_2Cl_2) .–

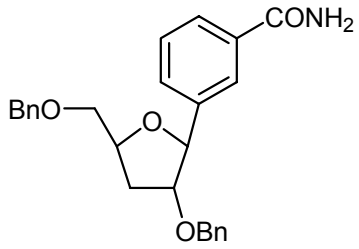
IR (film): 3040 (acid), 2880, 2920, 1690 (C=O), 1454 (ν C=C), 1272 (C-O(C)), 1106 (ν_{as} C-O-C), 698 (γ =CH).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 1.66-1.86 (o, 1H, 3a-H), 2.09-2.22 (o, 1H, 3b-H), 3.51 (dd, 1H, 5a-H), 3.66 (dd, 1H, 5a-H), 3.91 (m, 1H, 4-H), 4.41 (s, 2H, OCH_2), 4.60 (s, 2H, OCH_2), 4.67 (t, *J*= 6 Hz, 1H, 2-H), 5.03 (d, *J* = 4 Hz, 1H, 1-H), 7.26-7.34 (m, 12H, ArH), 7.96-8.10 (m, 2H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 34.0 (CH_2, C-3), 72.0 (CH_2, C-5), 73.3 (OCH_2), 73.7 (OCH_2), 78.2 (CH, C-2), 84.6 (CH, C-4), 85.9 (CH, C-1), 117.8 (ArC), 127.6-129.8 (ArC), 138.3 (ArC), 138.6 (ArC).–

Anal. Calcd. for $C_{26}H_{26}O_5$: (418.48) C: 74.62, H: 6.26;
Found: C: 74.56, H: 6.24.–

3-(3-Deoxy-2,5-dibenzyl-β-D-ribofuranosyl)benzamide (2-31)



A mixture of acid **2-30** (450 mg, 1.12 mmol) thionyl chloride (5 mL) and 2 drops of DMF were heated to 100 °C for 2 h. The reaction mixture was cooled down, toluene was added and the solvent was evaporated. The residue was dissolved in dry dichloromethane, cooled to 0 °C and poured into aqueous ice cold ammonia solution (30 mL). The organic phase was evaporated, dissolved in ethyl acetate and extracted with water. The ethyl acetate phase was dried over $MgSO_4$, evaporated and purified by column chromatography (first using CH_2Cl_2, then CH_2Cl_2: MeOH = 18:1) to yield amide **2-31** (400 mg, 88 %), $[\alpha]_d^{23}$ = – 42.73 ° (in CH_2Cl_2).–

IR (film): 3355, 3216 (NH_2), 2923, 2856, 1720 (C=O), 1697 (amid II), 1452 (ν C=C), 1272 (C-O(C)), 1108 (ν_{as} C-O-C), 698 (γ =CH).–

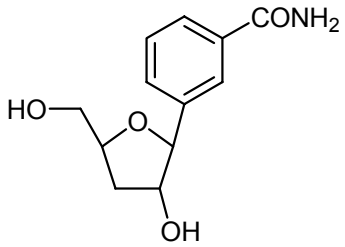
^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 1.92-2.11 (o, 1H, 3a-H), 2.25-2.45 (o, 1H, 3b-H), 3.62 (dd, 1H, 5a-H), 3.76 (dd, 1H, 5a-H), 4.07 (m, 1H, 4-H), 4.55 (s, 2H, OCH_2), 4.60 (t, *J*= 6 Hz, 1H, 2-H), 4.66 (s, 2H, OCH_2), 5.12 (d, *J* = 5 Hz, 1H, 1-H), 6.47 (s, 2H, NH_2), 7.23-7.55 (m, 12H, ArH), 7.45-7.91 (m, 2H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 34.3 (CH_2, C-3), 72.3 (CH_2, C-5), 73.5 (OCH_2), 73.8 (OCH_2), 78.4 (CH, C-2), 84.4 (CH, C-4), 85.9 (CH, C-1), 124.8 (ArC), 127.2-129.8 (ArC), 134.0 (ArC), 138.2 (ArC), 138.6 (ArC), 142.2 (ArC), 170.2 ($CONH_2$).–

Mass calcd.: 417.19;
MS (CI, HR 7500): 417.3 [M^+], 392.3, 340.3, 312.3, 221.1, 185.2, 129.1, 91.0, 55.0, 29.0.–

Anal. Calcd. for $C_{26}H_{27}NO_4$: (417.19): C: 74.80, H: 6.52;
Found: C: 74.23, H: 6.48.–

3-(3-Deoxy-β-D-Ribofuranosyl)-benzamide (2-32)



Amid **2-31** (100 mg, 3.82 mmol) was dissolved in abs. tetrahydrofuran (10 mL) and ethanol (1 mL) under argon atmosphere. 20 mg palladium/charcoal was added, and stirred under H_2-atmosphere for 10 h. The catalyst was filtered off over Celite and the filtrate evaporated to afford the product (48 mg, 86 %) as a colorless oil. $[\alpha]_d^{23}$ = –12 ° (in methanol).–

IR (film): 3337 (ν NH_2), 2921, 1662 (C=O), 1394, 1272, 1103 (ν C-O(H)), 624 (γ OH).–

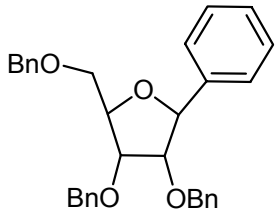
^{1}H-NMR (MeOD, 200 MHz): δ = 1.83-2.02 (o, 1H, 3a-H), 2.19-2.41 (o, 1H, 3b-H), 3.72 (m, 2H, 5a,b-H), 4.24 (m, 1H, 4-H), 4.38 (t, *J*= 6 Hz, 1H, 2-H), 4.87 (d, *J* = 5 Hz, 1H, 1-H), 7.41-7.61 (m, 2H, ArH), 7.82 (d, 1H, ArH), 7.93 (s, 1H, ArH).–

^{13}C-NMR (MeOD, 200 MHz): δ = 35.6 (CH_2, C-3), 64.7 (CH_2, C-5), 78.4 (CH, C-4), 79.5 (CH, C-4), 86.6 (CH, C-1), 125.0 (ArC), 126.8 (ArC), 128.6 (ArC), 129.3 (ArC), 134.0 (ArC), 142.3 (ArC), 162.0 ($CONH_2$).–

Mass calcd.: 237.1;
MS (EI, HR 7500): 237.1 [M^+], 219.1 [M–H_2O], 150.1, 134.1, 105.0, 91.0, 57.0.–

Anal. Calcd. for $C_{12}H_{15}NO_4$: (210.09): C: 60.75, H: 6.37;
Found: C: 60.46, H: 6.11.–

(2,3,5-Tribenzyl-β-D-ribofuranosyl)-benzene (2-34)



A solution of bromo benzene (**2-33**) (0.194 mL, 1.845 mmol) in anhydrous THF (20 mL) was treated under nitrogen at −85 °C within 10 min with a solution of *n*-BuLi (1.23 mL, 1.5 M in hexane). After 30 min at −85 °C a solution of lactone (**2-7**) (500 mg,1.23 mmol) in THF (10 mL) was added and stirred for an additional 1 h and then warmed over 2 h to −30 °C (TLC control). The reaction was then quenched by the addition of water (10 mL) and extracted with diethyl ether (30 mL). The organic phase was dried with Na_2SO_4 and evaporated under reduced pressure to afford a slightly red oil.
The residue was dissolved in CH_2Cl_2 and treated at −78 °C with boron trifluoride diethyl etherate (0.63 mL, 2.5 mmol) and triethylsilane (0.375 mL, 2.5 mmol). The reaction mixture was stirred for 1 h at −78 °C, warmed overnight to 10 °C, neutralized by the addition of a saturated aqueous sodium hydrogencarbonate solution (ca. 15 mL), and extracted with CH_2Cl_2 (30 mL). The solution was dried over Na_2SO_4 and purified by column chromatography (hexane/ethyl acetate: 6: 1) to afford white crystals (400 mg, 69.5 %), mp.: 55–57 °C, $[\alpha]_d^{23}$ = −36 ° (in CCl_4).–

IR (KBr): 3087, 3064, 3031, 2865, 1454 (ν C=C), 1272 (C-O(C), 1106 (ν_{as} C-O-C), 698 (γ =CH).–

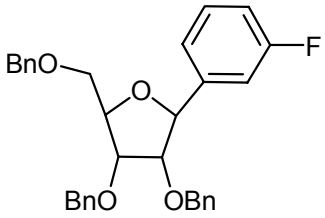
1H-NMR ($CDCl_3$, 200MHz): δ = 3.65 (ddd, J_{gem}= 10.4 Hz, $J_{4,5a}$= 4.2 Hz, $J_{4,5b}$=4.0 Hz; 2H, 5a,b-H), 3.81 (d, $J_{1,2}$= 6.5 Hz, $J_{2,3}$= 5.3 Hz, 1H, 2-H), 4.01 (dd, 1H, 3-H), 4.35 (q, 1H, 4-H), 4,47 (dd, AB-system, *J*= 12 Hz, 2H, OCH_2), 4,56 (dd, AB-system, *J*= 12 Hz, 2H, OCH_2), 4,58 (dd, AB-system, *J*= 12 Hz, 2H, OCH_2), 5.02 (d, $J_{1,2}$= 6.5 Hz, 1H, 1-H), 7.5 (m, 20H, ArH).–

13-NMR ($CDCl_3$, 200MHz): δ = 70.95 (OCH_2), 72.44 (OCH_2), 72.69 (OCH_2), 73.94 (CH_2, C-5), 82.21 (CH, C-2), 83.141 (CH, C-3), 84.27 (CH, C-1), 126.77 (ArC), 128.06−128.82 (ArC), 138.34 (ArC), 138.49 (ArC), 138.68 (ArC), 140.94 (ArC).–

Mass calcd.: 480.23;
MS (EI / 200 °C): m/z (%) = 480.22 (100 %), 389.2 [M^+– 91], 181, 105, 57, 23.–

Anal. Calcd. for $C_{32}H_{32}O_4$: (480.59): C: 79.97, H: 6.71;
Found: C: 78.96, H: 6.57.–

3-(2,3,5-Tribenzyl-β-D-ribofuranosyl)-fluoro-benzene (2-37)



A solution of 1-fluoro-3-bromo benzene **2-36** (0.202 mL, 1.845 mmol) in anhydrous THF (20 mL) was treated under nitrogen at −85 °C within 10 min with a solution of *n*-BuLi (1.23 mL, 1.5 M in hexane). After 30 min at −85 °C a solution of the lactone (**2-7**) (500 mg, 1.23 mmol) in THF (10 mL) was added and stirred for an additional 1 h and then warmed over 2 h to −30 °C (TLC control). The reaction was then quenched by the addition of water (10 mL) and extracted with diethyl ether (30 mL). The organic phase was dried with Na_2SO_4 and evaporated under reduced pressure to afford a slightly red oil.
The residue was dissolved in CH_2Cl_2 and treated at −78 °C with boron trifluoride diethyl etherate (0.63 mL, 2.5 mmol) and triethylsilane (0.375 mL, 2.5 mmol). The reaction mixture was stirred for 1 h at −78 °C, let it warm up overnight to 10 °C, neutralized by the addition of a saturated aqueous sodium hydrogencarbonate solution (ca. 15 mL), and extracted with

CH_2Cl_2 (30 mL). The solution was dried over Na_2SO_4 and purified by column chromatography (hexane/ethyl acetate: 6: 1) to afford **2-37** as a yellow oil (500 mg, 83 %), $[\alpha]_d^{23} = -30.52$ ° (in CH_2Cl_2).–

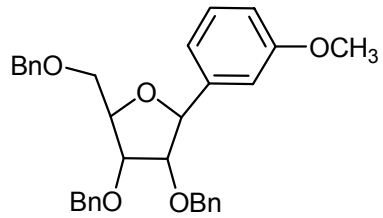
IR (KBr): 3336, 3031, 2865, 1727, 1614, 1590, 1496, 1454 (ν C=C), 1268 (ν C-F), 1106 (ν_{as} C-O-C), 698 (γ =CH).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.76 (t, $J_{4,5a}$= 4.5 Hz, $J_{4,5b}$=4.2 Hz, 2H, 5a,b-H), 3.92 (dd, J_1= 5.2 Hz, J_2= 0.5 Hz, 1H, 3-H), 4.14 (dd, $J_{1,2}$= 4.9 Hz, $J_{2,3}$= 3.9 Hz, 1H, 2-H), 4.48 (q, 1H, 4-H), 4,60 (dd, AB-system, J= 6 Hz, 2H, OCH_2), 4,68 (dd, AB-system, J= 3 Hz, 2H, OCH_2), 4,72 (s, 2H, OCH_2), 5.14 (d, $J_{1,2}$= 6.8 Hz, 1H, 1-H), 7.43 (m, 20H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 70.87 (CH_2, C-5), 72.49 (OCH_2), 72.88 (OCH_2), 74.04 (OCH_2), 77.97 (CH, C-4), 82.34 (CH, C-2), 82.45 (CH, C-3), 84.35 (CH, C-1), 113.35 ($J_{C\text{-}F}$= 22.05 Hz, ArC, C-4´), 114.799 ($J_{C\text{-}F}$= 21.05 Hz, ArC, C-2´), 122.43 ($J_{C\text{-}F}$= 2.8 Hz, ArC, C-6´), 128.17-128.91 (ArC, benzyl), 130.17 ($J_{C\text{-}F}$= 8.15 Hz, ArC, C-5´), 138.17, 138.40, 138.55 (q, 3 ArC, benzyl), 143.75 ($J_{C\text{-}F}$= 7.05 Hz, ArC, C-1´), 161.02 ($J_{C\text{-}F}$= 241 Hz, ArC, C-3´).–

Mass calcd.: 498.58;
MS (EI / 200 °C, HR): m/z (%) = 498.22 [M^+] (61 %), 407.2 [M^+– 91, CH_2Ph], 241, 181, 91, 65, 39.–

3-(2,3,5-Tribenzyl-β-D-ribofuranosyl)-anisol (2-40)



A solution of 3-bromo anisole **2-39** (0.231 mL, 1.845 mmol) in anhydrous THF (20 mL) was treated under nitrogen at −85 °C within 10 min with a solution of *n*-BuLi (1.23 mL, 1.5 M in hexane). After 30 min at −85 °C a solution of the lactone **2-7** (500 mg, 1.23 mmol) in THF (10 mL) was added and stirred for an additional 1 h and then warmed over 2 h to −30 °C (TLC control). The reaction was then quenched by the addition of water (10 mL) and

extracted with diethyl ether (30 mL). The organic phase was dried with Na_2SO_4 and evaporated under reduced pressure to afford a slightly red oil.
The residue was dissolved in CH_2Cl_2 and treated at −78 °C with boron trifluoride diethyl etherate (0.63 mL, 2.5 mmol) and triethylsilane (0.375 mL, 2.5 mmol). The reaction mixture was stirred for 1 h at −78 °C, let it warm up overnight to 10 °C, neutralized by the addition of a saturated aqueous sodium hydrogencarbonate solution (ca. 15 mL), and extracted with CH_2Cl_2 (30 mL). The solution was dried over Na_2SO_4 and purified by column chromatography (hexane/ethyl acetate: 6: 1) to afford **2-40** as a yellow oil (480 mg, 78.2 %), $[\alpha]_d^{23}$ = –34.4 ° (in CH_2Cl_2).–

IR (KBr):3345, 3087, 3029, 2911, 2867, 1602, 1454 (ν C=C), 1261 (C-O(C)), 1047 (ν_{as} C-O-C), 696 (γ =CH).–

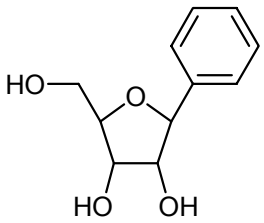
^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.72 (m, 2H, 5a,b-H), 3.78 (s, 3H, OCH_3), 3.94 (t, *J*= 4 Hz, 1H, 3-H), 4.14 (t, *J* = 4.4 Hz, 1H, 2-H), 4.48 (q, 1H, 4-H), 4,60 (s, 2H, OCH_2), 4,67 (d, *J*= 3 Hz, 2H, OCH_2), 4,70 (d, *J*= 2 Hz, 2H, OCH_2), 5.12 (d, $J_{1,2}$= 6.3 Hz, 1H, 1-H), 6.89 (m, 1H, ArH), 7.08 (m, 2H, ArH), 7.40 (m, 17H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 55.55 (OCH_3), 70.88 (CH_2, C-5), 72.47 (OCH_2), 72.72 (OCH_2), 73.97 (OCH_2), 78.02 (CH, C-4), 82.14 (CH, C-2), 83.10 (CH, C-3), 84.20 (CH, C-1), 111.84 (ArC, C-4´), 114.12 (ArC, C-2´), 119.11 (ArC, C-6´), 128.07-128.86 (ArC, benzyl), 129.80 (ArC, C-5´), 138.34, 138.47, 138.65 (q, ArC, benzyl), 142.65 (ArC, C-1´), 160.19 (ArC, C-3´).–

Mass calcd.: 510.24;
MS (EI / 200 °C): m/z (%) = 510.24 (61 %), 419.2 [M^+–91, CH_2Ph], 253.1, 135.1, 91.0, 65.0.–

Anal. Calcd. for $C_{33}H_{34}O_5$: (510.24): C: 77.62, H: 6.71;
Found: C: 77.53, H: 6.98.–

β-D-Ribofuranosyl-benzene (2-35)



A solution of benzyl ether **2-34** (100 mg, 0.21 mol) in abs. dichloromethane (10 mL) was cooled to 0 °C under N_2 atmosphere and 1N BBr_3 solution (0.65 mL, 0.65 mol) was added slowly. The reaction was completed in 30 min. (TLC monitoring). The reaction mixture was neutralized by the addition of sat. aqueous $NaHCO_3$ solution and the water phase was evaporated under reduced pressure. The residue was dissolved in methanol, filtered, and purified by column chromatography (CH_2Cl_2: methanol: 9: 1) to yield **2-35** (39 mg, 88.4 %). mp.: 120−121 °C, white crystals. $[\alpha]_d^{23}$ = –9.7 ° (in methanol).–

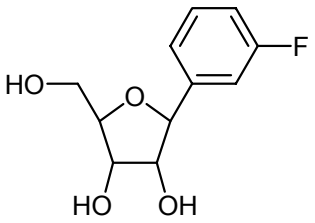
IR (KBr): 3306 (ν OH), 1454 (ν C=C), 1272 (C-O(C)), 1106 (ν_{as} C-O-C).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.74 (m, 1H, 5a-H), 3.80 (m, 1H, 3-H), 3.83 (m, 1H, 5b-H), 3.90 (m, 1H, 4-H), 4.05 (m, 1H, 3-H), 5.03 (d, *J*= 6.5 Hz, 1H, 1-H), 7.27−7.54 (m, 5H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 63.2 (CH_2, C-5), 79.0 (CH, C-3), 79.2 (CH, C-2), 84.8 (CH, C-4), 87.9 (CH, C-1), 126.4 (ArC), 127.7 (ArC), 127.9 (ArC), 128.5 (ArC), 141.2 (q, ArC).–

Mass calcd: 210.09;
MS (HR, EI / 200 °C): m/z (%) = 210 (2 %) [M^+], 192 (93 %) [M^+–H_2O], 179, 174, 131, 107, 91, 73.–

β-D-Ribofuranosyl-3´-fluoro-benzene (2-38)



A solution of benzyl ether **2-37** (110 mg, 0.225 mol) in abs. dichloromethane (10 mL) was cooled to 0 °C under N_2 atmosphere and 1N BBr_3 solution (0.7 mL, 0.7 mol) was added slowly. The reaction was completed in 30 min. (TLC monitoring). The reaction mixture was neutralized by the addition of sat. aqueous $NaHCO_3$ solution and the water phase was evaporated under reduced pressure. The residue was dissolved in methanol, filtered, and purified by column chromatography (CH_2Cl_2: methanol: 9: 1) to afford **2-38** as white crystals (38 mg, 74.5 %). mp.: 68 °C, $[\alpha]_d^{23}$ = –6.2 ° (in methanol).–

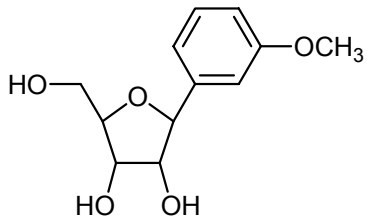
IR (KBr): 3473, 3413 (ν OH), 1454 (ν C=C), 1272 (C-O(C)), 1256 (ν C-F), 1106 (ν_{as} C-O-C).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.68 (m, 1H, 5a-H), 3.79 (m, 1H, 3-H), 3.86 (m, 1H, 5b-H), 4.02 (m, 1H, 4-H), 4.05 (m, 1H, 3-H), 5.11 (d, *J*= 6.5 Hz, 1H, 1-H), 7.23−7.43 (m, 4H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 62.5 (CH_2, C-5), 71.9 (CH, C-3), 78.2 (CH, C-2), 83.6 (CH, C-4), 85.5 (CH, C-1), 112.6 (J_{C-F}= 22.3 Hz, C-4´), 114.0 (J_{C-F}= 21.3 Hz, C-2´), 122.0 (J_{C-F}= 2.8 Hz, C-6´), 129.9 (J_{C-F}= 3.25 Hz, C-5´), 144.0 (J_{C-F}= 7.15 Hz, C-1´), 160.9 (J_{C-F}= 242.65 Hz, C-3´).–

Mass calcd.: 228.08;
MS (EI / 200 °C, HR 7500): m/z (%) = 228.08 (3 %) [M^+], 210.1 [M^+–F], 192.1 [–H_2O], 167.1, 149.0, 125.1, 109.1, 57.0.–

3-β-D-Ribofuranosyl-anisol (2-41)



A solution of benzyl ether **2-40** (250 mg, 0.55 mol) in abs. dichloromethane (10 mL) was cooled to −78 °C under N_2 atmosphere and 1N BBr_3 solution (0.7 mL, 0.7 mol) was added slowly. The reaction was completed in 90 min. (TLC monitoring). The reaction mixture was neutralized by the addition of sat. aqueous $NaHCO_3$ solution and the water phase was evaporated under reduced pressure. The residue was dissolved in methanol, filtered, and purified by column chromatography (CH_2Cl_2: methanol: 9: 1) to yield **2-41** (101 mg, 85 %). mp.: 52.5 °C, white crystals. $[\alpha]_d^{23}$ = –7.5 ° (in methanol).–

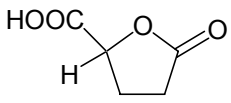
IR (KBr): 3473, 3413 (ν OH), 1617, 1454 (ν C=C), 1263 (C-O(C)), 1108 (ν_{as} C-O-C).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 3.68 (m, 1H, 5a-H), 3.79 (m, 1H, 3-H), 3.86 (m, 1H, 5b-H), 4.02 (m, 1H, 4-H), 4.05 (m, 1H, 3-H), 5.11 (d, *J*= 6.5 Hz, 1H, 1-H), 7.23−7.43 (m, 4H, ArH).–

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 54.9 (OCH_3), 63.2 (5-C, CH_2), 78.8 (3-C, CH), 84.8 (4-C, CH) 86.5 (2-C, CH), 87.7 (1-C, CH), 111.75 (4′-C), 113.1 (2′-C), 118.6 (6′-C), 129.5 (5′-C), 143.0 (1′-C), 160.2 (3′-C).–

Mass calc.: 240.1;
MS (EI / 200 °C, HR 7500): m/z (%) = 240.1 (18 %) [M^+], 222.2 [M^+–H_2O], 183.2, 152.1, 137.1, 109.1, 97.1, 57.1.–

(*S*)-(+)-γ-Carbonyl-butyrolactone (2-43)[37]



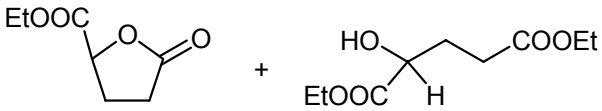
A solution of $NaNO_2$ (12,6 g, 0.183 mol) in H_2O (27 mL) was added dropwise to the solution of L-glutamic acid (**2-42**) (18 g, 0.122 mol) in water (48 mL)and cc. HCl (25.2 mL)at 0 °C and stirred for 6 h. The colorless reaction mixture was warmed up to room temperature overnight. Evaporation of water under reduced pressure resulted in a mixture of white crystals and a colorless oil. This residue was dissolved in ethyl acetate and filtered. The organic phase was dried over $NaSO_4$ and the solvent evaporated. The product was isolated as a viscous oil 14.64 g (92%). Recrystallisation from ethyl acetate/hexane yielded white powder. mp.: 71-73 °C; $[\alpha]_d^{20} = +15.6$ ° (c = 2.0 in EtOH).–

IR (KBr): 1775 (lacton C=O), 1723 (acid C=O), 1175 (C–O).–

^{1}H-NMR (CD_3OD, 200 MHz): δ = 1.8-2.3 (m, 4H, CH_2–CH_2), 4.2 (m, 1H, CH-O).–

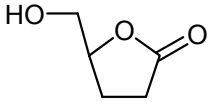
^{13}C-NMR (DMSO-d_6 200 MHz): δ = 26.0 (CH_2), 27.1 (CH_2), 76.0 (CH), 172.5 (C=O), 177.5 (C=O).–

(*S*)-(+)-γ-Ethoxycarbonyl-γ-butyrolactone (2-44a), **diethyl-α-hydroxyglutarate (2-44b)**[37]



A solution of (*S*)-(+)-γ-carbonyl-butyrolactone **2-43** (14.0 g, 0.107 mol) and *p*-TsOH (0.6 g, 3.8 mmol) in ethanol (30 mL) and benzene (70 mL) was heated under reflux for 7.5 h. The solvents were distilled off. The residue was dissolved in benzene and extracted with H_2O, aqueous 10 % Na_2CO_3, and H_2O. The organic phase was dried over $NaSO_4$ and evaporated. A colorless oil was obtained, which was used in the next step without purification.–

2,3-Didesoxy-γ-ribonolacton (2-45)[37]



To a suspension of $NaBH_4$ (2.0 g, 0.052 mol) in ethanol (30 mL) the mixture of (*S*)-(+)-γ-ethoxycarbonyl-γ-butyrolacton (**2-44a**) and diethyl-α-hydroxyglutarat (**2-44b**) (12.64 g) in

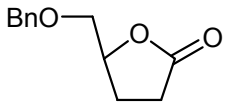
ethanol (40 mL) was added slowly at room temperature. After 90 min. the pH value of the solution was set to pH= 3 with aqueous 10 % HCl solution at 0 °C. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (dichloromethane: methanol : 9:1) to afford **2-45** as a colorless oil (8.2 g, 71 %). $[\alpha]_d^{20}$ = +29.6 ° (c = 0.4 in EtOH).–

IR (KBr): 3400 (O-H), 1765 (lacton C=O), 1180 (C–O).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.0-2.8 (m, 4H, CH_2-CH_2), 3.55-4.06 (m, 2H, 5a,b-H), 4.66 (m, 1H, CH).–

Anal. Calcd. for $C_5H_8O_3$: (116.05): C 51.72, H 6.94;
Found: C 51.67, H 6.95.–

5-Benzyl-2,3-didesoxy-γ-ribonolacton (2-46)[38]



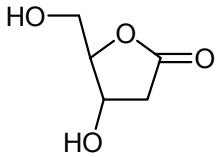
Freshly prepared Ag_2O (4.05 g, 0.017 mol) was suspended to a solution of 2,3-didesoxy-γ-ribonolactone (**2-45**) (1.35 g, 0.011 mol) in abs. DMF (20 mL). Benzyl bromide (2.07 mL) was added. The reaction mixture was protected from light and stirred at room temperature for 2 days. The unsolved material was filtered off and washed with $CHCl_3$ (50 mL). The filtrated was kept in the refrigerator overnight and pyridine (5 mL) was added. The solution was washed with H_2O, aqueous 10 % HCl, H_2O, aqueous 10 % Na_2CO_3 solution, and H_2O consecutively. The organic phase was dried over Na_2SO_4. After evaporation of the solvents the residue was purified by column chromatography (dichloromethane: methanol: 9:1) to afford **2-46** (1.3 g, 60 %) as a yellow oil. F.p.$_{Lit}$ 160-164°/0.02 mm[37], $[\alpha]_d^{20}$ = +18.1 ° (c = 2.70, EtOH).–

IR (KBr): 3064, 2925, 1789 (C=O), 1454 (ν C=C), 1272 (C-O(C)), 1122 (C-O(C)), 698 (γ =CH).–

^{1}H-NMR ($CDCl_3$, 200 MHz): δ = 2.38 (m, 2H, 3-H), 2.48 (m, 2H, 2-H), 3.41-3.66 (m, 2H, $5_{a,b}$-H), 4.43 (s, 2H, OCH_2), 4.70 (m, 1H, 4-H), 7.29 (m, 5H, ArH).−

^{13}C-NMR ($CDCl_3$, 200 MHz): δ = 26.01 (CH_2, C-3), 27.48 (CH_2, C-2), 71.68 (CH_2, C-5), 73.19 (OCH_2), 73.81 (CH, 4-C), 127.83 (ArC), 128.04 (ArC), 128.26 (ArC), 128.36 (ArC), 137.42 (q, ArC), 176.87 (C=O). −

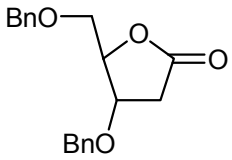
2-Deoxy-ribonolactone (2-48)[117]



A solution of 2-deoxy-D-ribose **2-47** (3.0 g, 11.36 mmol) and water (100 mL) was cooled to 0 °C. Bromine (6 mL, 0.108 mol) was added dropwise. The solution was kept at 0 °C for 30 minutes and stirred at room temperature for 5 days.

The reaction mixture was extracted with diethyl ether. Evaporation of the water phase resulted in a pale yellow viscous oil (3 g), which was used for the next step without further purification.

3,5-Dibenzyl-2-deoxy-ribonolactone (2-52)[118]



The mixture of 2-deoxy-ribonolactone (**2-48**) (1.5 g, 11.36 mmol) and benzyl trichloro-acetimidate (**2-51**) (12.57 g, 47.77 mmol) was dissolved in dry dioxan (25 mL) and trifluoro-methansulfonic acid (0.3 mL) was added. The reaction mixture was stirred at room temperature overnight. The solvent was distilled off at reduced pressure. The residue was dissolved in dichloromethane and extracted with water. The organic phase was dried over $MgSO_4$, and evaporated to obtain a yellow oil, and white crystals. Byproduct trichloro-acetamide was crystallized from pentane: dichloromethane 1:1, and the residue was purified by column chromatography (PE: EE:3: 1) to afford pure **2-52** (3.1g, 87.4 %) as a yellow oil. $[\alpha]_d^{20} = +31°$ (c = 2.0, $CHCl_3$)[118].–

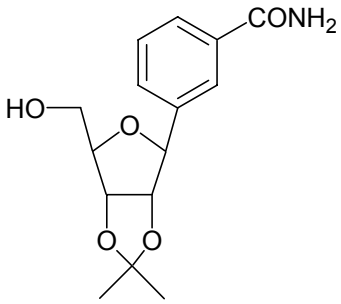
IR: 3064, 2925, 1789 (C=O), 1454 (ν C=C), 1272 (C-O(C)), 1122 (C-O(C)), 698 (γ =CH).–

^{1}H-NMR (200 MHz): δ = 2.64 (dd, $J_{3,2a}$ = 2.4 Hz, J_{gem} = 18.1 Hz, 1H, 2a-H), 2.87 (dd, $J_{3,2b}$ = 4.2 Hz, J_{gem} = 18.1 Hz, 1H, 2b-H), 3.68 (m, 2H, 5a,b-H), 4.30 (dt, $J_{3,2a}$ = 2.1 Hz, $J_{3,2b}$ = 6 Hz, 1H, 3-H), 4,56 (2s, 4H, OCH_2), 4.65 (m, 1H, 4-H), 7.32–7.40 (m, 10H, ArH).–

^{13}C-NMR (200 MHz): δ = 36.1 (CH_2, C-2), 70.0 (CH_2, C-5), 71.6 (OCH_2), 74.1 (OCH_2), 76.5 (CH, C-4), 84.5 (CH, C-3), 128.0-129.3 (ArC), 137.4 (q, ArC), 137.7 (q, ArC), 176.1 (C=O).–

8.3 Experimental Part to Chapter 3

3-(2,3-Isopropyliden-β-D-ribofuranosyl)benzamide (3-2)



A solution of the amide (**2-2**) (300 mg, 1.2 mmol) in anhydrous acetone (40 mL) was treated with concentrated sulfuric acid (1 mL) at 0 °C and stirred for 18 h at 20 °C. The solution was then neutralized by the addition of a saturated aqueous solution of sodium hydrogen carbonate and evaporated under reduced pressure. The residue was dissolved in ethyl acetate, filtered, and evaporated under reduced pressure. The product was purified by column chromatography (CH_2Cl_2 / 5 % MeOH) to afford the acetonide (320 mg**,** 93 %) as a white foam: $[\alpha]_d^{23}$ = –32 ° (c = 0.1, MeOH).–

IR (CCl_4): 3531, 3415, 3010, 2996, 2938, 1677, 1587, 1384, 1375.–

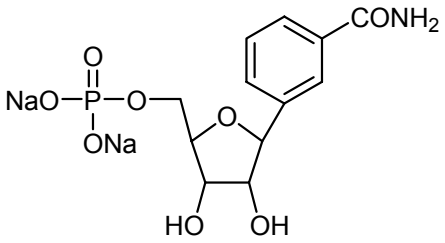
UV: 207 (3.24), 226 (sh, 2.96), 272 (1.87), 280 (1.78).–

^{1}H-NMR (300 MHz): δ = 1.31 (s, 1H, CH_3), 1.59 (s, 1H, CH_3), 3.73 (dd, $J_{4,5a}$ = 4.0 Hz, J_{gem} = 12.3 Hz, 1H, 5a-H), 3.89 (dd, $J_{4,5b}$ = 2.6 Hz, J_{gem} = 12.3 Hz, 1H, 5b-H), 4.14 (m, 1H, 4-H), 4.26 (s, 1H, OH), 4.45 (dd, $J_{2,3}$ = 6.8 Hz, $J_{1,2}$ = 5.5 Hz, 1H, 2-H), 4.72 (dd, $J_{2,3}$ = 6.8 Hz, $J_{3,4}$ = 4.2 Hz, 1H, 3-H), 4.84 (d, $J_{1,2}$ = 5.5 Hz, 1H, 1-H), 6.64-7.15 (s, 2H, NH_2), 7.33 (t, J = 7.7 Hz, 1H, 5´-H), 7.45 (d, $J_{5,6}$ = 7.7 Hz, 1H, 4´-H), 7.70 (d, $J_{4,5}$ = 7.7 Hz, 1H, 6´-H), 7.92 (s, 1H, 2´-H).–

^{13}C-NMR (300 MHz): δ = 24.98 (CH_3), 27.02 (CH_3), 61.74 (CH_2, C-5), 80.78 (CH), 84.85 (CH), 86.26 (CH), 114.59 (q, $(CH_3)_2C$), 124.06 (ArC), 126.60 (ArC), 128.21 (ArC), 129.21 (ArC), 133.02 (q, ArC), 139.68 (q, ArC), 169.72 (q, C=O).–

Anal. Calcd. for $C_{15}H_{19}O_5$: (293.13): C 61.42, H 6.53, N 4.78;
Found: C 61.40, H 6.57, N 4.70.–

3-(β-D-Ribofuranosyl)benzamide-5´-phosphate bisodium salt (3-3)



A solution of the acetonide (**3-2**) (1.0 g, 3.4 mmol) and freshly distilled phosphoryl chloride (0.75 mL**,** 8.1 mmol) in trimethyl phosphate (7.0 mL, 60.0 mmol) was stirred for 4 h at 0 °C and 6 h at 5 °C. The reaction was hydrolyzed with ice-water (16.0 g) and extracted with diethyl ether (30 mL). (TLC: on Cellulose F, isopropanol/water/ammonia = 7:2:1).
The aqueous phase was adjusted to pH 1.5 by the addition of 1 N NaOH and heated for 45 min at 70 °C. The solution was then neutralized with 1 N NaOH and evaporated under reduced pressure. The residue was purified by column chromatography (DEAE-sephadex; gradient: 1 L of water/l L of 0.18 M TEAB-buffer, pH 7.5) to afford the pseudonucleotide (778 mg, 53 %) in form of the TEAE-salt. The conversion to the disodium salt was effected with ion exchange resin; $[\alpha]^{23}$ –24.4 ° (c = 0.1, MeOH).–

IR (KBr): 3340 (OH), 2940 (C-H), 1680 (C=O), 1617, 1450, 1230 (phosphate), 1190, 1100 (ether), 1050 (phosphate), 918, 856.–

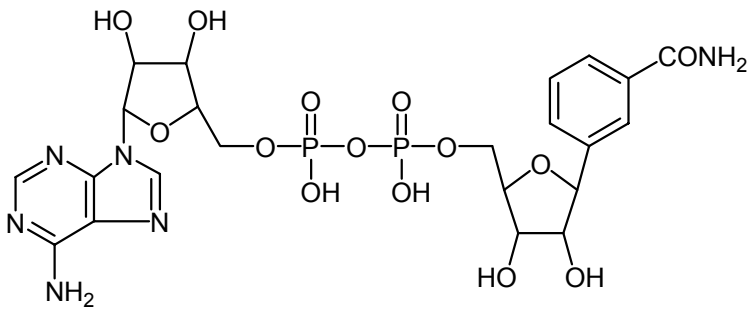
^{1}H-NMR (D_2O, 300 MHz): δ = 3.65 (t, 1H, 3-H), 3.91 (q, 1H, 4-H), 4.01 (dd, $J_{2,3}$ = 5.4 Hz, $J_{1,2}$ = 7.1 Hz, 1H, 2-H), 4.03, 4.28 (2 dd, $J_{4,5}$ = 4.4 Hz, J_{gem} = 11.7 Hz, 2H, 5-H) , 5.11 (d, $J_{1,2}$ = 7.1 Hz, 1H, 1-H), 7.41 (t, J = 7.7 Hz, 1H, 5´-H), 7.56 (d, $J_{5,6}$ = 7.7 Hz, 1H, 4´-H), 7.77 (d, $J_{4,5}$ = 7.7 Hz, 1H, 6´-H), 7.96 (s, 1H, 2´-H).–

^{13}C-NMR (D_2O, 300 MHz): δ = 65.13 (C-5), 72.33 (CH), 77.64 (CH), 83.83 (CH), 84.41 (CH), 126.01 (ArC), 128.25 (ArC), 129.80 (ArC), 131.36 (ArC), 133.46 (ArC), 140.11 (q, ArC), 173.01 (q, C=O).–

^{31}P-NMR (D_2O, 300 MHz): δ = 3.71.−

Anal. Calcd. for $C_{12}H_{14}NNa_2O_8P$: (377.03): C: 38.21, H: 3.74, N: 3.71;
Found: C: 34.17, H: 4.64, N: 3.67.–

3-(*β*-D-Ribofuranosyl)benzamide-adenine-dinucleotide (3-5)



The corresponding free acid monophosphate **3-3** (0.1 mmol) was rigorously dried and suspended in DMF (0.5 mL). Carbonyldiimidazole (0.5 mmol) was added and the reaction mixture became homogeneous in a few minutes. After 3 h, HPLC analysis showed the disappearance of starting material t_R= 13−15 min) and the appearance of a peak at t_R= 4−5 min. Methanol (33 pL) was added to hydrolyze excess carbonyldiimidazole and after 30 min, AMP (adenosine monophosphate) (0.15 mmol), dissolved in of anhydrous DMF (2 mL) containing tri-*n*-butylamine (0.15 mmol), was added. The reaction mixture was stirred for 48 h at room temperature and monitored by HPLC. When the reaction was completed, 5 mL of water was added and the solution was reduced to dryness. The gummy residue was dissolved again in 20 mL of water containing sodium acetate (30 mg) and extracted twice with 20 mL

each of chloroform and diethyl ether. The aqueous layer was treated with triethylamine (200 pL) until pH 10 was reached and stirred for 8−24 h. After such time, the entire mixture was lyophilized and the residue chromatographied on a Hamilton HA-X4 anion-exchange column (HCO_2-form, 0.8 x 28 cm). During a 7 h gradient from water to 2 M $(H_4N)HCO_2$, with a flow rate of 2 mL/min, samples of 12 mL were collected and monitored by W at 290 nm. The desired fractions were collected and passed through a strong cation-exchange resin (AG50W-X8, H^+ form, 1.5 x 15 cm) and eluted with water. After collection of 15 mL fractions from this column, the product was always present in fractions 2-4. These fractions were lyophilized to give the dinucleotide as white fluffy solids. Yield 71 %.

^{1}H-NMR (D_2O, 300 MHz): δ = 4.07 (dd, 1H, 2′(B)-H, $J_{1',2'}$= 7.2 Hz, $J_{2',3'}$= 5.3 Hz), 4.15-4.26 (m, 6H, 3′(B)-H, 4′(B)-H, 5′,5′′(A)-H, 5′,5′′(B)-H), 4.35 (m, 1H, 4′(A)-H), 4.44 (pseudo t, 1H, 3′(A)-H), 4.59 (pseudo t, 1H, 2′(A)-H), 4.75 (d, 1H, 1′-(B)-H, $J_{1',2'}$= 7.2 Hz), 6.01 (d, 1H, 1′(A)-H, $J_{1',2'}$= 5.3 Hz), 7.41 (t, 1H, 5-H, *J*= 7.7 Hz), 7.57 (d, 1H, 4-H, *J*= 7.7 Hz), 7.66 (d, 1H, 6-H), 7.73 (s, 1H 2(B)-H], 8.31 (s, 1H, 2-H), 8.55 (s, 1H, H8-(A)-H).−

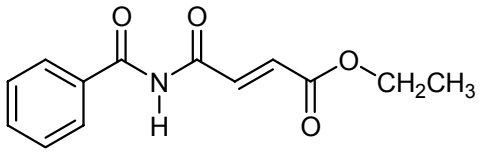
^{31}P-NMR (D_2O), AB-system: δ = 9.36 P_1, 9.59 P_2, $J_{P,P}$= 21.2 Hz .−

8.4 Experimental part to Chapter 4.

Condensation of amides with acid chlorides, General Procedure A and B.

Procedure A: To a boiling solution of the amide (10 mmol) in dry toluene (20 mL) a solution of the acid chloride (5 mmol) in toluene (5 mL) was added dropwise. The mixture was refluxed overnight (TLC monitoring), the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (dichloromethane) and recrystallized from diethyl ether. (For yields and mp of **4-5a** to **4-5h** see *Table 4-1*.)
Procedure B: Alternatively, the sodium salt of the amide (prepared by reaction of the amide solution with sodium hydride) was employed, with fumaric acid monoethyl ester anhydride.

4-Benzoylamino-4-oxobut-2-enoic acid ethyl ester (4-5a)



Benzamide (**4-2**) (1.23 g, 10 mmol) in dry toluene (20 mL) was reacted according to procedure A with fumaric acid monoethyl ester chloride (**4-3a**) (1.78 g, 7.5 mmol) in toluene (5 mL) to yield **4-5a** (1.18 g, 51 %), mp: 87–89 °C.−

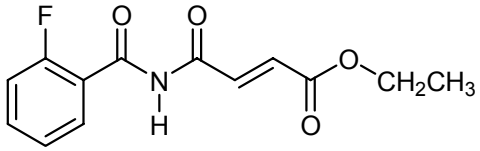
IR (KBr) $\tilde{\nu}$ = 3291 cm^{-1} (N-H), 1724 (C=O), 1703 (C=O), 1672 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.36 (t, *J* = 7 Hz, 3H, CH_3), 4.32 (q, *J* = 7 Hz, 2H, CH_2), 6.90 (d, *J* = 2 Hz, 2H, CH), 7.02 (d, *J* = 2 Hz, 2H, CH), 7.61 (m, 3H, ArH), 7.96 (m, 2H, 5ArH), 9.43 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.6 (CH_3), 61.8 (CH_2), 128.5 (CH), 129.4 (CH), 133.3 (ArC), 134.1 (ArC), 134.3 (ArC), 135.2 (ArC), 165.4 (C=O), 166.3 (C=O), 167.3 (C=O).−

Anal. Calcd for $C_{12}H_{11}NO_4$: (233.22): C 63.09, H 5.26;
Found: C 61.66, H: 5.29.−

4-(2-Fluorobenzoylamino)-4-oxobut-2-enoic acid ethyl ester (4-5b)



2-Fluorobenzoic acid amide (2.0 g, 14.4 mmol) in dry toluene (15 mL) was reacted with fumaric acid chloride (**4-3a**) (1.3 g, 9.6 mmol) as described in procedure A to afford imide **4-5b** (890 mg, 42 %), mp: 54–55 °C.−

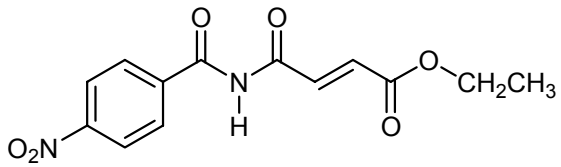
IR (KBr) $\tilde{\nu}$ = 3388 cm^{-1} (N-H), 1720 (C=O), 1705 (C=O), 1678 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.35 (t, *J* = 7 Hz, 3H, CH_3), 4.30 (q, *J* = 7 Hz, 2H, CH_2), 6.94 (d, *J* = 14 Hz, 1H, CH), 7.38 (m, 2H, ArH), 7.61 (m, 1H, ArH), 7.88 (d, *J* = 14 Hz, 1H, CH), 8.08 (m, 1H, ArH), 9.19 (d, *J* = 13, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.5 (CH_3), 61.8 (CH_2), 116.9 (CH), 120.4 (ArC), 125.8 (CH), 132.7 (ArC), 134.7 (ArC), 135.4 (ArC), 135.8 (ArC), 158.5 (C=O), 162.5 (C=O), 163.5 (C=O), 165.6 (ArC).−

Anal. Calcd for $C_{13}H_{12}FNO_4$: (265.24): C 58.87, H 4.56, N 5.28;
Found: C 58.39, H 4.46, N 5.24.−

4-(4-Nitrobenzoylamino)-4-oxobut-2-enoic acid ethyl ester (4-5c)



4-Nitrobenzoic acid amide (550 mg, 3.3 mmol) was reacted with fumaric acid chloride (**4-3a**) (200 mg, 1.5 mmol) as described in procedure A to afford imide **4-5c** (158 mg, 44 %) , mp: 135–137 °C.−

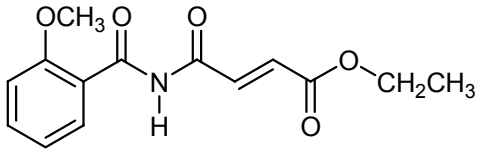
IR (KBr) $\tilde{\nu}$ = 3244 cm^{-1} (N-H), 1720 (C=O), 1713 (C=O), 1678 (C=O).−

^{1}H-NMR (200 MHz, DMSO): δ = 1.28 (t, *J* = 7 Hz, 3H, CH_3), 4.24 (q, *J* = 7 Hz, 2H, CH_2), 6.75 (d, *J* = 16 Hz, 1H, CH), 7.54 (d, *J* = 16 Hz, 1H, CH), 8.15 (d, *J* = 9 Hz, 2H, ArH), 8.37 (d, *J* = 9 Hz, 2H, ArH), 11.73 (s, 1H, NH).−

^{13}C-NMR (200 MHz, DMSO): δ = 14.8 (CH_3), 59.6 (CH_2), 134.4 (CH), 137.8 (CH), 123.7 (ArC), 123.8 (ArC), 128.2 (ArC), 128.3 (ArC), 139.4 (ArC), 150.6 (ArC), 165.1 (C=O), 165.5 (C=O), 166.4 (C=O).–

Anal. Calcd for $C_{13}H_{12}N_2O_6$ (292.24): C 53.43, H 4.14, N 9.59;
Found: C: 55.29, H 4.53, N 8.56.−

4-(2-Methoxybenzoylamino)-4-oxobut-2-enoic acid ethyl ester (4-5d)



2-Methoxybenzoic acid amide (2.1 g, 13.9 mmol) was reacted with fumaric acid chloride (**4-3a**) (1.0 g, 7.4 mmol) as described in procedure A to afford imide **4-5d** (785 mg, 46 %), mp: 98–100 °C.−

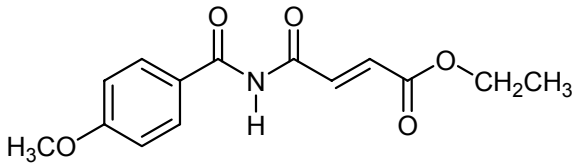
IR (KBr) $\tilde{\nu}$ = 3303 cm^{-1} (N-H), 1720 (C=O), 1705 (C=O), 1684 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.38 (t, *J* = 7 Hz, 3H, CH_3), 4.09 (s, 3H, OCH_3), 4.32 (q, *J* = 7 Hz, 2H, CH_2), 6.94 (d, *J* = 14 Hz, 1H, CH), 7.18 (m, 2H, ArH), 7.62 (m, 1H, ArH), 8.00 (d, *J* = 14 Hz, 1H, CH), 8.24 (m, 1H, ArH), 10.44 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.5 (CH_3), 56.7 (OCH_3), 61.7 (CH_2), 112.2 (CH), 120.1 (ArC), 122.2 (CH), 133.3 (ArC), 135.6 (ArC), 136.3 (ArC), 136.9 (ArC), 158.2 (ArC), 164.2 (C=O), 165.6 (C=O), 166.4 (C=O).−

Anal. Calcd for $C_{14}H_{15}NO_5$ (277.27): C 60.64, H 5.45, N 5.05;
Found: C 60.38, H 5.47, N 5.19.−

4-(4-Methoxybenzoylamino)-4-oxobut-2-enoic acid ethyl ester (4-5e)



4-Methoxybenzoic acid amide (500 mg, 3.3 mmol) in dry toluene (8 mL) was reacted with fumaric acid chloride (**4-3**) (240 mg, 1.8 mmol) as described in A to afford **4-5e** (185 mg, 45 %), mp: 120–122 °C.−

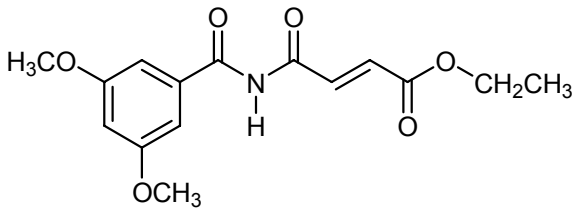
IR (KBr) $\tilde{\nu}$ = 3271 cm^{-1} (N-H), 1726 (C=O), 1709 (C=O), 1668 (νC=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.38 (t, *J* = 7 Hz, 3H, CH_3), 3.93 (s, 3H, OCH_3), 4.32 (q, *J* = 7 Hz, 2H, CH_2), 6.98 (d, *J* = 14 Hz, 1H, CH), 7.03 (d, *J* = 8 Hz, 2H, ArH), 7.89 (d, *J* = 8 Hz, 2H, ArH), 8.04 (d, *J* = 14 Hz, 1H, CH), 8.70 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.5 (CH_3), 55.9 (OCH_3), 61.7 (CH_2), 114.6 (CH), 124.6 (ArC), 130.8 (CH), 133.8 (ArC), 135.4 (ArC), 136.7 (ArC), 137.5 (ArC), 164.4 (ArC), 165.5 (C=O), 165.7 (C=O), 167.5 (C=O).−

Anal. Calcd for $C_{14}H_{15}NO_5$ (277.27): C 60.64, H 5.45, N 5.05;
Found: C: 59.97, H: 5.62, N 5.22.−

4-(3,5-Dimethoxybenzoylamino)-4-oxobut-2-enoic acid ethyl ester (4-5f)



3,4-Dimethoxy-benzoic acid amide (500 mg, 2.8 mmol) in dry toluene (8 mL) was reacted with fumaric acid chloride (**4-3a**) (200 mg, 1.5 mmol) as described in procedure A to afford **4-5f** (362 mg, 43 %), mp: 162–164 °C.−

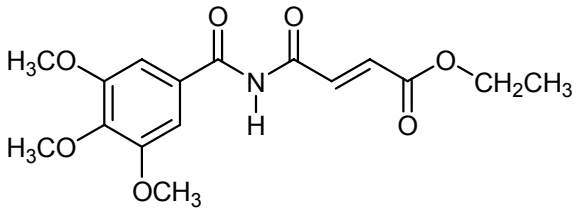
IR (KBr) $\tilde{\nu}$ = 3271 cm^{-1} (N-H), 1722 (C=O), 1705 (C=O), 1675 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.38 (t, *J* = 7 Hz, 3H, CH_3), 3.88 (s, 6H, 2 x OCH_3), 4.33 (q, *J* = 7 Hz, 2H, CH_2), 6.72 (d, *J* = 14 Hz, 1H, CH), 7.01 (s, 1H, ArH), 7.30 (d, *J* = 14 Hz, 1H, CH), 8.02 (d, *J* = 8 Hz, 2H, ArH), 8.78 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.5 (CH_3), 56.1 (OCH_3), 61.6 (OCH_2), 112.6 (CH), 124.8 (ArC), 130.4 (CH), 133.2 (ArC), 136.1 (ArC), 136.8 (ArC), 137.9 (ArC), 164.4 (ArC), 165.8 (C=O), 166.1 (C=O), 167.3 (C=O).−

Anal. Calcd for $C_{15}H_{17}NO_6$ (307.30): C 58.63, H 5.58, N 4.56;
Found: C 59.77, H 5.52.−

4-(3,4,5-Trimethoxybenzoylamino)-4-oxobut-2-enoic acid ethyl ester (4-5g)



The sodium salt of the amide was prepared by stirring a mixture 3,4,5-trimethoxy-benzoic acid amide (4 g, 18.9 mmol) and NaH (1 g, 20.8 mmol) in THF (100 mL) for 30 min, and reacted with fumaric acid monoethyl ester anhydride (5.1 g, 18.9 mmol) in 30 mL THF (procedure B). After column chromatography, **4-5g** was isolated (4.1 g, 65 %). mp: 114–116 °C.−

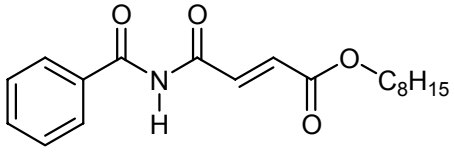
IR (KBr) $\tilde{\nu}$ = 3255 cm^{-1} (N-H), 1729 (C=O), 1707 (C=O), 1672 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.38 (t, *J* = 7 Hz, 3H, CH_3), 3.97 (s, 9H, 3 x OCH_3), 4.32 (q, *J* = 7 Hz, 2H, CH_2), 6.94 (d, *J* = 14 Hz, 1H, CH), 7.21 (s, 2H, ArH), 8.05 (d, *J* = 14 Hz, 1H, CH), 9.42 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.5 (CH_3), 56.6 (OCH_3), 56.7 (OCH_3), 61.3 (OCH_3), 61.9 (OCH_2), 106.2 (CH), 124.7 (ArC), 127.1 (CH), 133.6 (ArC), 135.3 (ArC), 143.2 (ArC), 153.3 (ArC), 165.2 (ArC), 166.0 (C=O), 168.5 (C=O), 169.2 (C=O).−

Anal. Calcd for $C_{16}H_{19}NO_7$ (337.32): C 56.97, H 5.68.;
Found: C 56.91, H 6.02.−

4-Benzoylamino-4-oxobut-2-enoic acid octyl ester (4-5h)



The sodium salt of benzamide was prepared by stirring a mixture of benzamide (500 mg, 4.1 mmol) and NaH (172 mg, 4.1 mmol) in THF (35 mL) for 30 min, and reacted with fumaric acid monooctyl ester anhydride (1.82 g, 4.1 mmol) in THF (15 mL) (procedure B). After column chromatography, **4-13** was isolated as an oil (190 mg, 13 %).−

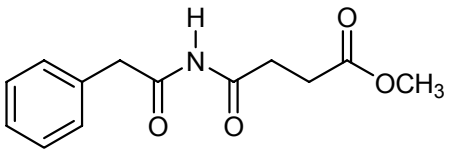
IR (KBr) $\tilde{\nu}$ = 3292 cm^{-1} (N-H), 1724 (C=O), 1709 (C=O), 1687 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.91 (t, *J* = 6 Hz, 3H, CH_3), 1.31 (m, 10H, CH_2), 4.25 (q, *J* = 3 Hz, 2H, CH_2), 6.95 (d, *J* = 14 Hz, 1H, CH), 7.55 (m, 2H, ArH), 7.68 (m, 1H, ArH), 7.98 (m, 2H, ArH), 8.06 (d, *J* = 14 Hz, 1H, CH), 9.02 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.5 (CH_3), 23.1 (CH_2), 26.3 (CH_2), 28.9 (CH_2), 29.6 (CH_2), 32.2 (CH_2), 66.1 (OCH_2), 112.6 (CH), 128.4 (ArC), 129.4 (CH), 132.6 (ArC), 134.0 (ArC), 134.3 (ArC), 135.2 (ArC), 165.6 (ArC), 166.3 (C=O), 166.7 (C=O), 167.0 (C=O).−

Anal. Calcd for $C_{19}H_{25}NO_4$ (331.41): C 68.86, H 7.60;
Found: C 69.67, H 7.51.−

4-Oxo-4-phenylacetylaminobutyric acid methyl ester (4-10)



Phenylacetic acid amide (**4-6a**) (1.35 g, 10 mmol) in dry toluene (20 mL) was reacted with succinic acid monomethyl ester chloride (**4-7**)[119] (1.65 g, 12.26 mmol) as described in general procedure **A** to afford 692 mg (28 %) of **4-10**.−

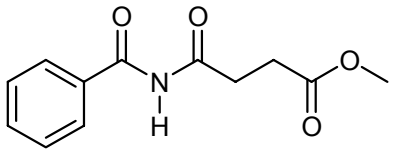
IR (KBr): $\tilde{\nu}$ = 3260 cm^{-1}, 1735 (C=O), 1705 (C=O), 1657 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.69 (t, *J* = 2 Hz, 4H, CH_2), 3.69 (s, 2H, $PhCH_2$), 3.70 (s, 3H, OCH_3), 7.37 (m, 5H, ArH), 9.05 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 28.7 (CH_2), 28.9 (CH_2), 39.3 ($PhCH_2$), 50.4 (CH_3), 127.4 (ArC), 129.0 (ArC), 129.8 (ArC), 135.9 (ArC), 170.7 (C=O), 172.0 (C=O), 175.2 (C=O).−

Anal. Calcd for $C_{13}H_{15}NO_4$: (249.26): C 62.58, H 6.01;
Found: C: 61.33, H: 5.33.−

4-Benzoylamino-4-oxobutyric acid methyl ester (4-11a)



Benzamide (**4-2**) (1.23 g, 10 mmol) was reacted with succinic acid monomethyl ester chloride (**4-7**) (1.65 g, 12.26 mmol) in toluene (5 mL) according to general procedure A to afford **4-11a** (1.06 g, 43 %).−

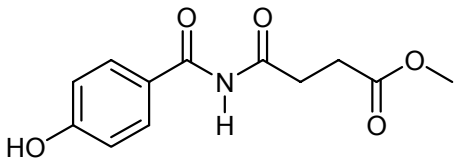
IR (KBr) $\tilde{\nu}$ = 3291 cm^{-1} (N-H), 1740 (C=O), 1709 (νC=O), 1677 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.72 (t, *J* = 4 Hz, 2H, CH_2), 3.34 (t, *J* = 4 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 7.61 (m, 3H, ArH), 7.93 (m, 2H, ArH), 9.35 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 28.6 (CH_2), 33.3 (CH_2), 52.3 (OCH_3), 128.3 (ArC), 129.3 (ArC), 132.9 (ArC), 133.6 (ArC), 166.2 (C=O), 173.4 (C=O), 175.7 (C=O).−

Anal. Calcd for $C_{12}H_{13}NO_4$: (235.24): C 61.22, H 5.53;
Found: C 61.23, H 5.45.−

4-(4-Hydroxybenzoylamino)-4-oxobutyric acid ethyl ester (4-11c)



A solution of 4-benzyloxybenzamide (500 mg, 2.2 mmol) in dry THF (10 mL) was treated with NaH (88 mg, 2.2 mmol) and stirred for 1 h at 20 °C. Fumaric acid monoethyl ester anhydride (600 mg, 2.2 mmol) was added and the mixture was heated overnight (procedure B). The product was purified by column chromatography on silica gel (CH_2Cl_2) and crystallized from diethyl ether. A sample of the resulting benzyl ether (150 mg) was dissolved in dry THF (10 mL) and hydrogenated over palladium/charcoal (10 %) for 3 h. The reaction mixture was filtered and the solvent removed at reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2) to yield **4-11c** (76 mg, 69 %), mp: 146–148 °C.

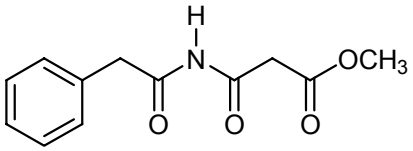
IR (KBr) $\tilde{\nu}$ = 3091 cm^{-1} (N-H), 1715 (C=O), 1675 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.31 (t, 3H, CH_3), 2.76 (t, *J* = 6 Hz, 2H, CH_2), 3.78 (t, *J* = 6 Hz, 2H, CH_2), 4.22 (q, 2H, CH_2), 7.76 (d, *J* = 7 Hz, 2H, ArH), 8.11 (d, *J* = 7 Hz, 2H, ArH), 8.62 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.1 (CH_3), 29.2 (CH_2), 29.6 (CH_2), 61.7 (CH_2), 116.5 (ArC), 117.0 (ArC), 125.9 (ArC), 128.8 (ArC), 129.7 (ArC), 161.1 (ArC), 168.2 (C=O), 172.3 (C=O), 172.4 (C=O).−

Anal. Calcd for $C_{13}H_{13}NO_5$ (263.25): C 59.31, H 4.98;
Found: C 59.38, H 4.95.−

3-Oxo-3-phenylacetylaminopropionic acid methyl ester (4-12)



Phenylacetic acid amide (**4-6a**) (1.0 g, 7.4 mmol) was reacted with malonic acid monomethyl ester chloride (**4-8**)[120] (1.22 g, 8.1 mmol) in toluene (5 mL) to afford **4-12** (520 mg, 30 %) after column chromatography (petroleum ether/ethyl acetate 3/1), mp 93–96 °C.−

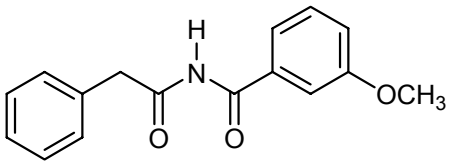
IR (KBr) $\tilde{\nu}$ = 3266 cm^{-1} (N-H), 1750 (C=O), 1735 (C=O), 1698.2 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 3.76 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.85 (s, 2H, $ArCH_2$), 7.31 (m, 3H, ArH), 7.40 (m, 2H, ArH), 8.62 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 44.2 (CH_2), 44.8 (CH_2), 53.1 (OCH_3), 128.3 (ArC), 129.6 (ArC), 129.9 (ArC), 135.9 (ArC), 170.7 (C=O), 171.8 (C=O).−

Anal. Calcd for $C_{12}H_{13}NO_4$: (235.24): C 61.22, H 5.53;
Found: C 60.85, H 5.28.−

3-Methoxy-*N*-phenylacetylbenzamide (4-13)



Phenylacetic acid amide (**4-6a**) (1.35 g, 10 mmol) in dry toluene (20 mL) was reacted with 3-methoxybenzoic acid chloride (1.26 g, 9.4 mmol) as described in procedure A to afford imide **4-13** (926 mg, 35 %), mp: 112–114 °C.−

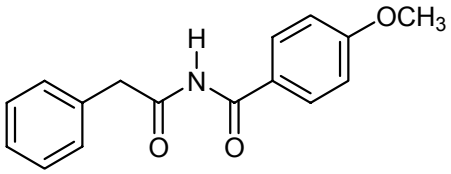
IR (KBr) $\tilde{\nu}$ = 3312 (N-H) cm^{-1}, 1709 (C=O), 1683 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 3.86 (s, 3H, OCH_3), 4.36 (s, 2H, $PhCH_2$), 7.17 (t, *J* = 2 Hz, 1H, ArH), 7.37 (m, 8H, ArH), 9.05 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 44.3 (CH_2), 55.9 (OCH_3), 113.0 (ArC), 119.9 (ArC), 120.2 (ArC), 127.6 (ArC), 129.0 (ArC), 130.2 (ArC), 130.4 (ArC), 134.1 (ArC), 134.4 (ArC), 160.4 (ArC), 167.7 (C=O), 175.2 (C=O).−

Anal. Calcd for $C_{16}H_{15}NO_3$ (269.30) C 71.29, H 5.57;
Found: C 71.35, H: 5.54.−

4-Methoxy-*N*-phenylacetylbenzamide (4-14)



Phenylacetic acid amide (**4-6a**) (1.35 g, 10 mmol) was reacted with 4-methoxybenzoic acid chloride (1.26 g, 9.4 mmol) as described in procedure A to afford imide **4-14** (807 mg, 30 %), mp: 162–164 °C.−

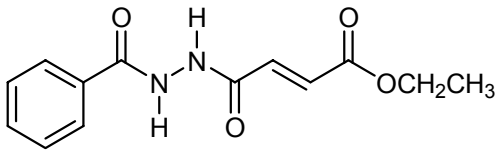
IR (KBr) $\tilde{\nu}$ = 3462 cm^{-1} (N-H), 1786 (C=O), 1601 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 3.88 (s, 3H, OCH_3), 4.37 (s, 2H, $PhCH_2$), 6.95 (t, *J* = 5 Hz, 2H, ArH), 7.37 (m, 5H, ArH), 7.60 (d, *J* = 4 Hz, 2H, ArH), 7.86 (d, *J* = 4 Hz, 2H, ArH), 8.12 (m, 5H, ArH), 9.45 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 39.3 (CH_2), 56.0 (OCH_3), 114.2 (ArC), 125.8 (ArC), 127.4 (ArC), 128.3 (ArC), 129.0 (ArC), 129.8 (ArC), 135.9 (ArC), 165.4 (ArC), 167.7 (C=O), 170.7 (C=O).−

Anal. Calcd for $C_{16}H_{15}NO_3$ (269.30): C 71.29, H 5.57;
Found: C 71.34, H: 5.54.−

4-(*N′*-Benzoylhydrazino)-4-oxobut-2-enoic acid ethyl ester (4-16)



The potassium salt of benzoylhydrazine was prepared by reaction of benzoylhydrazine hydrochloride (300 mg, 2.2 mmol) in a hot methanolic solution (5 mL) of KOH (98 mg) and evaporation of the methanol at reduced pressure. The residue was dissolved in CH_2Cl_2 (10 mL) and fumaric acid chloride ethyl ester (360 mg, 2.7 mmol) was added. The solution was stirred at room temperature for 1 hour, the precipitate was filtered off and washed with cold CH_2Cl_2 to yield **4-16** (256 mg, 50 %), mp: 236–238 °C.−

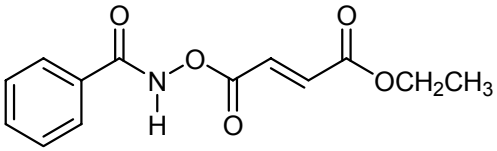
IR (KBr) $\tilde{\nu}$ = 3498 cm^{-1} (N-H), 3390 (N-H), 1654 (C=O), 1650 (C=O).−

^{1}H-NMR (200 MHz, DMSO): δ = 1.26 (t, 3H, CH_3), 4.20 (q, 2H, CH_2), 6.72 (d, *J* = 15 Hz, 1H, CH), 7.09 (d, *J* = 15 Hz, 1H, CH), 7.58 (m, 3H, ArH), 7.92 (m, 2H, ArH), 10.53 (s, 1H, NH), 11.15 (s, 1H, NH).−

^{13}C-NMR (200 MHz, DMSO): δ = 14.9 (CH_3), 61.7 (CH_2), 128.3 (ArC), 129.4 (ArC), 130.8 (ArC), 131.5 (ArC), 132.7 (ArC), 133.4 (ArC), 135.4 (CH), 137.5 (CH), 161.4 (C=O), 165.6 (C=O), 166.7 (C=O).−

Anal. Calcd for $C_{13}H_{14}N_2O_4$: (262.26) C: 59.54, H: 5.38;
Found: C 59.48, H 5.34.−

4-Benzoylaminoxy-4-oxobut-2-enoic acid ethyl ester (4-18)



A mixture of benzoic acid (6.10 g, 50 mmol), hydroxylamine (65 mmol) and dicyclohexyl carbodiimide (10.30 g, 50 mmol) in methanol (30 mL) was stirred for 1 h. The methanol was evaporated at reduced pressure and the residue extracted with 10 % aqueous NaOH (10 mL). The basic phase was acidified with aqueous 10 % HCl (10.3 mL) and extracted with CH_2Cl_2 (30 mL). The organic phase was dried (Na_2SO_4) and the solvent removed at reduced pressure. A sample of this crude hydroxamic acid **4-17** (250 mg), fumaric acid ethyl ester (**4-3a**) (300 mg, 1.8 mmol), and triethylamine (184 mg, 1.8 mmol) was dissolved in toluene (20 mL) and stirred for 1 hour at room temperature to yield **4-18** (290 mg, 61 %) after column chromatography on silica gel, mp: 76–78 °C.−

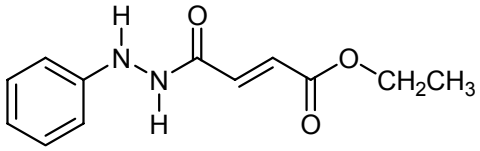
IR (KBr): $\tilde{\nu}$ = 3076 cm^{-1} (N-H), 1796 (C=O), 1720 (C=O), 1634 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.28 (t, 3H, CH_3), 4.24 (q, 2H, CH_2), 6.84 (d, *J* = 13 Hz, 1H, CH), 6.98 (d, *J* = 13 Hz, 1H, CH), 7.48 (m, 2H, ArH), 7.59 (m, 1H, ArH), 8.09 (m, 2H, ArH), 9.13 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.4 (CH_3), 61.9 (CH_2), 128.8 (ArC), 129.3 (ArC), 130.5 (ArC), 131.7 (ArC), 132.4 (ArC), 133.5 (ArC), 135.5 (CH), 137.5 (CH), 162.7 (C=O), 169.8 (C=O), 172.3 (C=O).−

Anal. Calcd for $C_{13}H_{13}NO_5$: (263.25): C 59.31, H 4.98;
Found: C 60.03, H 5.03.−

3-(*N´*-Phenylhydrazinocarbonyl)-acrylic acid ethyl ester (4-20a)



A mixture of phenylhydrazine (1.08 g, 10 mmol) and fumaric acid chloride ethyl ester (1.34 g, 10 mmol) in dichloromethane (25 mL) and 2–3 drops of triethyl amine was stirred for 30 minutes. The precipitate was filtered off and washed with CH_2Cl_2 to yield **4-20a** (976 mg, 48 %), mp: 140–142 °C.−

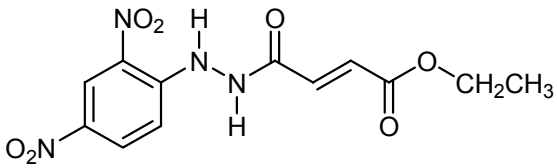
IR (KBr): $\tilde{\nu}$ = 3368 cm^{-1} (N-H), 3096 (N-H), 1644 (C=O), 1614 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.27 δ (t, *J* = 7 Hz, 3H, CH_3), 4.22 (q, *J* = 7 Hz, 2H, CH_2), 6.77 (d, *J* = 14 Hz, 1H, CH), 6.96 (m, 2H, ArH), 7.27 (d, *J* = 14 Hz, 1H, CH), 7.49 (m, *J* = 8 Hz, 3H, ArH), 9.98 (s, 1H, NH), 10.15 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 13.8 (CH_3), 61.4 (OCH_2), 113.2 (ArC), 113.3 (ArC), 119.1 (ArC), 130.0 (ArC), 131.2 (ArC), 135.1 (CH), 137.8 (CH), 144.6 (ArC), 165.2 (C=O), 166.4 (C=O).−

Anal. Calcd for $C_{12}H_{14}N_2O_3$: (234.25): C 61.53, H 6.02;
Found: C 61.60, 6.08.−

3-[*N´*-(2,4-dinitrophenyl)-hydrazinocarbonyl]-acrylic acid ethyl ester (4-20b)



A mixture of 2,4-dinitrophenylhydrazine (1.5 g, 7.6 mmol) and fumaric acid chloride ethyl ester (1.13 g, 8.4 mmol) in dichloromethane (30 mL) and 2–3 drops of triethyl amine was stirred for 30 minutes. The precipitate was filtered off, and washed with CH_2Cl_2 to yield **4-20b** (604 mg, 78 %), mp: 161–163 °C.−

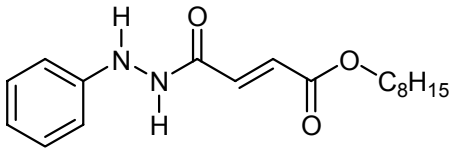
IR (KBr) $\tilde{\nu}$ = 3278 cm^{-1} (N-H), 3024 (N-H), 1695 (C=O), 1670 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.28 δ (t, *J* = 7 Hz, 3H, CH_3), 4.24 (q, *J* = 7 Hz, 2H, CH_2), 6.75 (d, *J* = 14 Hz, 1H, CH), 7.16 (d, *J* = 14 Hz, 1H, CH), 7.56 (s, 1H, ArH), 8.37 (m, 2H, ArH), 10.08 (s, 1H, NH), 10.28 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 14.8 (CH_3), 61.8 (OCH_2), 110.0 (ArC), 113.5 (ArC), 134.4 (ArC), 135.2 (CH), 137.6 (CH), 143.5 (ArC), 148.9 (ArC), 150.1 (ArC), 165.0 (C=O), 166.1 (C=O).−

Anal. Calcd for $C_{12}H_{12}N_4O_7$: (324.25): C 44.45, H 3.73;
Found: C 44.36, 3.69.−

3-(*N′*-phenyl)-hydrazinocarbonyl-acrylic acid octyl ester (4-20c)



A solution of phenylhydrazine (504 mg, 4.6 mmol) and fumaric acid monooctyl ester anhydride (1.15 g, 5.0 mmol) in CH_2Cl_2 (10 mL) and 2-3 drops of triethyl amine was stirred at room temperature for 1 hour. The solution was cooled to 0 °C, the precipitate was filtered off and washed with cold CH_2Cl_2 to yield hydrazide **4-20c** (800 mg, 57 %), mp: 130−132 °C.

IR (KBr): $\tilde{\nu}$ = 3383 cm^{-1} (N-H), 3283 (N-H), 1675 (C=O), 1594 (C=O).−

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.91 (t, 3H, CH_3), 1.32 (m, 10H, 5 x CH_2), 4.23 (t, *J* = 4 Hz, 2H, CH_2), 6.73 (d, *J* = 14 Hz, 1H, CH), 7.01 (d, 2H, ArH), 7.21 (d, *J* = 14 Hz, 1H, CH), 7.51 (m, 3H, ArH), 9.88 (s, 1H, NH), 10.11 (s, 1H, NH).−

^{13}C-NMR (200 MHz, $CDCl_3$): δ = 13.9 (CH_3), 22.8 (CH_2), 26.1 (CH_2), 29.8 (CH_2), 30.2 (CH_2), 30.5 (CH_2), 32.1 (CH_2), 65.4 (OCH_2), 112.9 (ArC), 118.7 (ArC), 129.1 (ArC), 133.8 (CH), 134.0 (CH), 134.2 (ArC), 165.0 (C=O), 165.7 (C=O).−

Anal. Calcd for $C_{12}H_{12}N_4O_7$: (324.25): C 68.65, H 8.49;

Found: C 68.05, 8.43.−

9 Abbreviations

abs.	absolute
Bn	benzyl
Bzl	benzoyl
cat.	catalyst, catalytic
cc.	concentrated
CD	circular dichroism
d	doublett
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
h.	hour(s)
HOMO	highest occupied molecule orbital
IR	infrared spectra
LUMO	lowest unoccupied molecule orbital
m.	multiplet
Me	methyl
MeOH	methanol
min.	minute
mp.	melting point
MS	mass spectrometry
NMR	nuclear magnetic resonance spectroscopy
PAC	phenanthrenequinone
PE	petrolether
Ph	phenyl
q	quartet
s	singlet
t	triplet
temp.	temperature
THF	tetrahydro furan
TLC	thin layer chromatography

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