CONTRIBUTIONS TO THE ASYMMETRIC CATALYSIS OF C-C COUPLINGS, AND TO THE CHEMICAL INDUCTION OF CARDIOMYOGENESIS FROM EMBRYONIC STEM CELLS

Inaugural Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

Diplom-Chemikerin Bianca Seelig

aus Hilden

Köln 2009

Gutachter:	Pror. Dr. A. Berkessel
	Prof. Dr. H.-G. Schmalz
Tag der mündlichen Prüfung:	06. Juli 2009

“Organic chemistry just now is enough to drive one mad.”

F. Wöhler, in a letter to his mentor J. J. Berzelius

dedicated to B.

Acknowledments

Research work reported in this thesis was carried out from March 2006 until Mai 2009 at the Institute for Organic Chemistry of the University of Cologne under the supervision of Prof. Dr. Albrecht Berkessel. Additionally, a part of the research was done at the Graduate School of Pharmaceutical Sciences of the University of Tokyo, Japan, during October 2008 - February 2009, in the research group of Prof. Dr. Masakatsu Shibasaki.

First of all, I would like to acknowledge Prof. Dr. Albrecht Berkessel for providing me with those challenging and rewarding thesis projects. His constant help, critical advices and active encouragement were very important for the good outcome of this work.

Shibasaki-sensei deserves my sincerest gratitude for giving me the opportunity to work under his guidance at the tôdai. どうもありがとうございます。

I would like to thank Prof. Dr. Hans Günther Schmalz for reviewing the thesis.

I would also like to mention the people who have been collaborating on the projects described in this thesis: Prof. Dr. J. Hescheler, Prof. Dr. A. Sachinidis and Dr. Silke Schwengberg as part of the stem cell project and the people at the Shibasaki group, especially Mastunaga-sensei and Morimoto-san, contributed in great extent to the success of this work.

I am very grateful to all my colleagues for providing a wonderful working atmosphere, for their help, patience and support. A standing ovation is to Ilona Jurkiewicz for being much more then the best lab mate one can imagine. I am thankful to my practical students, particulary for Jan Krämer, for sharing some of my experimental work. Thanks are also due to all of my Japanese colleagues, for the wonderful time in Japan.

For the critical reading of this work, I would like to thank Dr. Burkhard Koch, Dr. Nicolas Leconte, Angela Heinsch, Ilona Jurkiewicz, Eva Leitterstorf, David Müller, Mei Ching Ong and Dr. Silke Schwengberg.

My special thanks are to all the employees of the Institute for Organic Chemistry: Sarwar Aziz (HPLC), Dr. Nils Schlörer (NMR), Christoph Schmitz (GCMS), Dr. Johann Lex and Dr. Jörg Neudörfl (X-ray), Michael Neihs and Dr. Mathias Schäfer (MS). I also want to thank Susanne Geuer and Dr. Wolfgang Klug for their help in organisational

problems and Dietmar Rutsch and the members of the workshop, particulary Herbert Hartmann and Peter Küpper for their kind help with technical problems.

A big hug goes to all my dear friends for being there to remind me of the more important aspects of life.

I am gratefull for my parents for giving me the freedom to find my own interests and goals and for their support and tolerance.

B., thank you for your encouragement, your support, and for always being there when I needed you.

TABLE OF CONTENTS

1 Summary

This thesis deals with

- the investigation of chemically induced cardiomyogenesis of mouse ES cells,
- a simplified procedure for the synthesis of Takemoto's catalyst,
- the development of the first Lewis acid catalysed asymmetric aza-BH reaction.

1.1 Chemically Induced Cardiomyogenesis of mES Cells

Cardiomyocytes are used as donor cells in cell replacement therapies to treat serious heart damage. Despite the significant demand for heart cells, there exists up to now no efficient method for the production of these donor cells. The most promising approach so far is the *in vitro* cardiomyogenesis from embryonic stem (ES) cells induced by small molecules. However, with this method, only a small ratio of stem cells evolve into cardiomyocytes. The design of new and highly selective small molecules is therefore of great interest.[1]

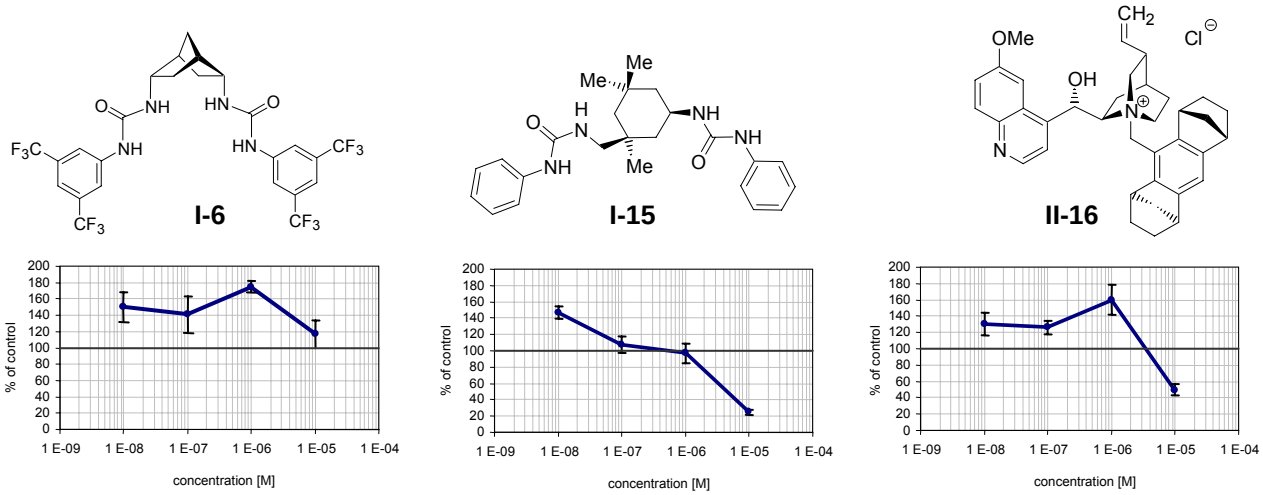
1.1.1 Substance Screening

For the identification of a cardiomyogenesis inducing substance, a rational drug design (RDD) was performed with a preliminary diversity orientated screening (DOS) focused on the synthesis and screening of (thio)urea and cinchona alkaloid based libraries. Derivatives of these (thio)ureas and cinchona alkaloids are known to exhibit bioactivities correlated to decreased proliferation rates of cancer cells[2] or cardiac action potential,[3] respectively.

A transgenic murine ES cell lineage, expressing enhanced green fluorescence protein (EGFP) under the control of the heart specific promoter α-myosine heavy chain (pα-MHC), was used to investigate the effects of the test substrates on cardiomyogenesis.

Diversity Orientated Screening (DOS). For a preliminary DOS, two substance libraries **I** and **II** containing (thio)urea (**I**, 19 compounds) or cinchona-alkaloid (**II**, 20 compounds) derivatives with extensively modified substitution patterns were synthesised and tested for their cardiomyogenesis inducing efficiency.

Among the substrates tested, especially compounds **I-6**, **I-15** and **II-16** induced an increase of 80 %, 50 % or 60 %, respectively, of the EGFP fluorescence which correlates with a positive cardiomoygenic effect (Scheme 1-1, p. 2).

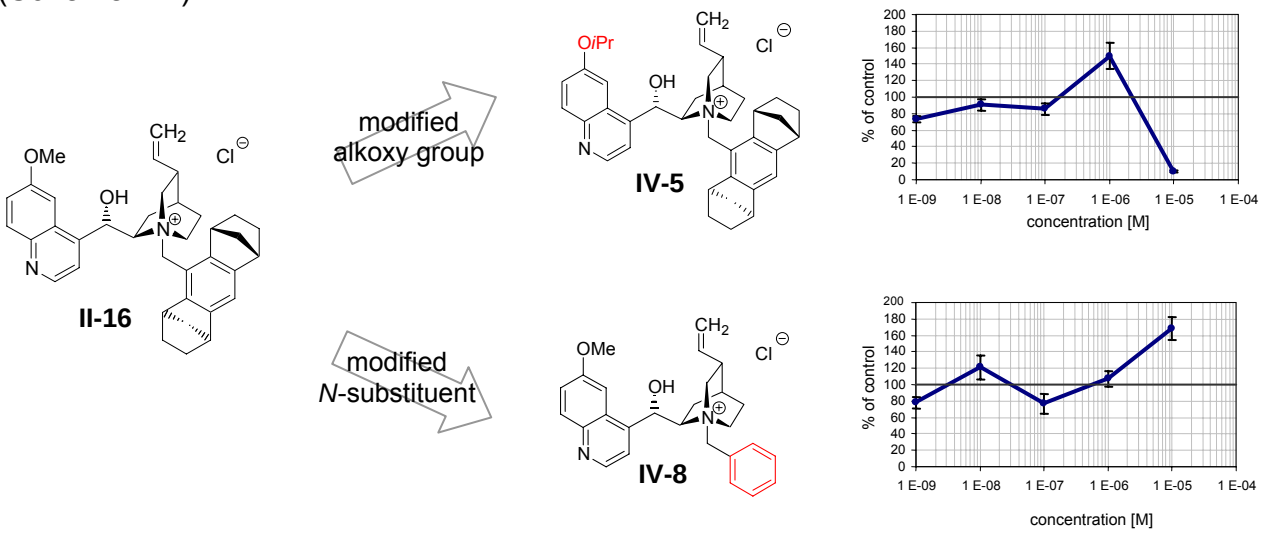


Scheme 1-1 Influence of the compounds **I-6**, **I-15** and **II-16** on the expression of EGFP. Both a significant increase and decrease of values, compared to controls, represent an effect on the generation of cardiac cells.

Rational Drug Design (RDD). On the basis of the three lead structures (**I-6**, **I-15**, **II-16**), a focused (thio)urea (**III**, 8 compounds) and a focused cinchona-alkaloid based library (**IV**, 9 compounds) were created, tested for their cardiomyogenic activity, and analysed in a RDD approach.

The results obtained with the (thio)urea based library did not provide a sufficiently solid and reliable basis for a meaningful structure-activity relationship (in this case of small molecule induced cardiomyogenesis).

In contrast, RDD analysis of the physiological screening of the cinchona alkaloid based library **IV** showed that especially variations on the alkoxy group (**IV-5**) and the quarternary ammonium moiety (**IV-8**) lead to an increased formation of cardiomyocytes (Scheme 1-2).



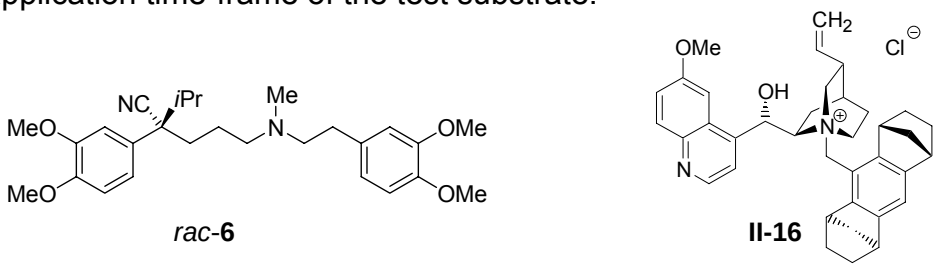
Scheme 1-2 Influence of the variations on the alkoxy and quarternary ammonium substituents (compounds **IV-5** and **IV-8**) on the expression of EGFP. Both a significant increase and decrease of values, compared to controls, represent an effect on generation of cardiac cells.

Based on these positively tested substances (compounds **IV-5** and **IV-8**), a new quinidine based substance library **V** (8, compounds) was developed and screened for its cardiomyogenesis inducing effectivity. This library contains substances which differ either in the alkoxy group attached to the quinidine moiety, or in the quinuclidine *N*-substituent compared to the lead structures **IV-5** and **IV-8**. However, these structural modifications did not improve the cardiomyogenesis inducing effect of the lead structures.

Conclusion. The substance screenings led to the identification of cardiomyogenesis inducing compounds with good (**IV-5**) to very good activities (**II-16**, **IV-8**) determined by a 50 to 80 % increase of the EGFP fluorescence compared to untreated cells.

1.1.2 Identification of Signalling Cascades Involved in Cardiomyogenesis

Verapamil *rac*-**6**, a cardiomyogensis inducing agent found in previous studies,[4] and the most active compound identified in the substance screening **II-16** were used for the identification of signalling cascades involved in the cardiomyogenesis by a time-dependent screening approach. In this method, an increased cardiomyogenesis indicates interaction of the compound tested with a target expressed at a certain point in time during the application time-frame of the test substrate.



rac-**6** **II-16**

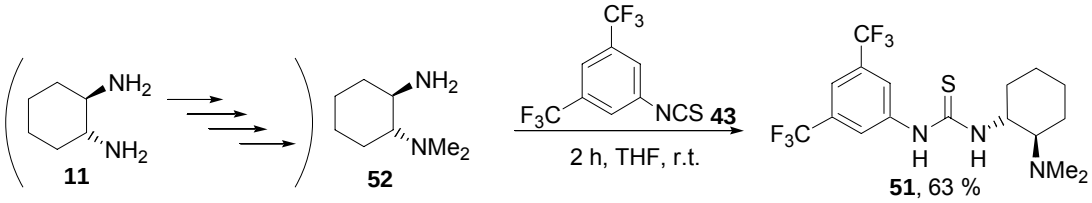
Scheme 1-3 Cinchona alkaloid **II-16** (left), verapamil *rac*-**6** (racemic mixture, right).

Since both substrates exhibited different cardiomyogenesis inducing profiles in each test series, the cardiomyogenesis inducing effect of the tested substrates appeared to rely on an interaction of the tested substances with several target proteins expressed at different points of time. In this case, time-dependent correlations between cardiomyogenesis and concentrations of the substrates are not suitable to limit the number of potential targets associated to the various developmental stages.

1.2 A Simplified Synthesis of Takemoto's Catalyst

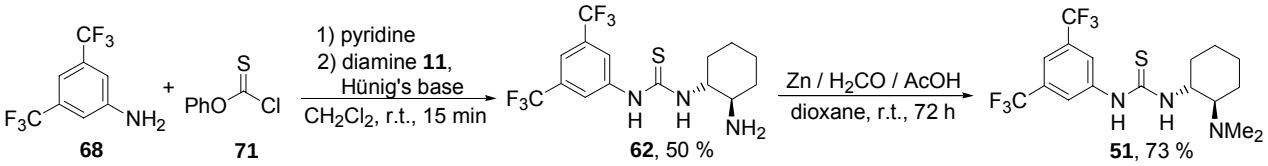
A significant number of C-C couplings can be catalysed enantioselectively by *Takemoto's* bifunctional amino thiourea derivative **51**.[5] The original synthesis of the amino

thiourea catalyst **51** consists in the facile addition of isothiocyanate **43** to *N*,*N*-dimethyl-*trans*-1,2-diaminocyclohexane **11**. The preparation of the *N*,*N*-dimethyl-*trans*-1,2-diaminocyclohexane building block **52** is a laborious four step procedure (Scheme 1-4).[6,7]



Scheme 1-4 Synthesis of the amino thiourea **51** developed by *Takemoto*.

An improved synthesis of *Takemoto's* catalyst **51** was realised consisting of only two steps: The thiourea moiety was obtained by condensation of 3,5-bistrifluoromethyl-aniline **68** with phenyl chlorothioformate **71** and subsequent reaction with *trans*-1,2-diaminocyclohexane **11**, according to a modified reaction protocol of *Nagasawa*.[8] Subsequent reductive dimethylation using formaldehyd / zinc afforded *Takemoto's* catalyst in an overall yield of 36 % (Scheme 1-5).



Scheme 1-5 Improved synthesis of *Takemoto's* catalyst **51**.

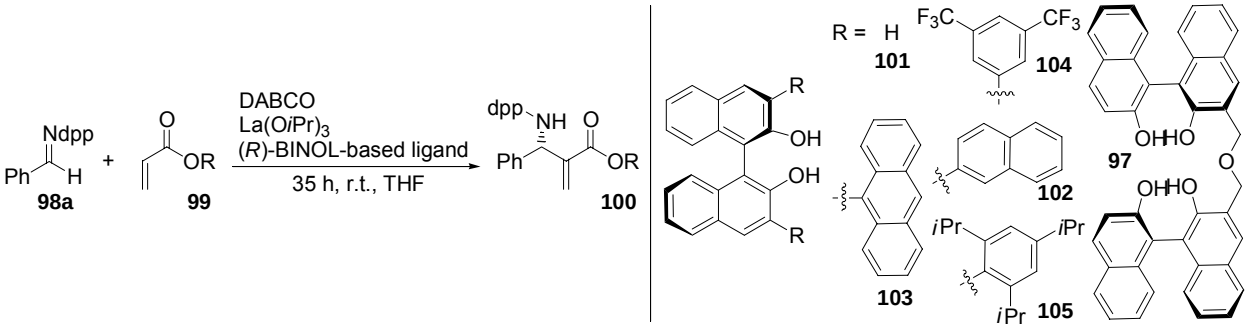
1.3 La-linked BINOL Catalysed Asymmetric aza-BH reaction

The asymmetric aza-Baylis-Hillman (aza-BH) reaction of achiral imines with acrylates provides a direct access to chiral β-amino acid esters, which have gained considerable interest due to their important biological properties, their occurrence in natural products, and as potential precursors for β-lactams.[9]

In this work, the first aza-Baylis-Hillman reaction catalysed by a chiral Lewis acidic complex was developed. Diphenylphosphinoyl- (dpp-) protected imines were employed as electrophiles, due to the ease of removal of this activating and protecting group.

In an initial ligand screening with La(O*i*Pr)$_3$ and various (*R*)-BINOL-based ligands (Scheme 1-6, p. 5), high enantioselectivity was achieved with linked-(*R*,*R*)-BINOL **97**. Since raising the ligand to metal ration for (*R*)-BINOL **101** from 1:1 to 2:1 showed no beneficial effect, the increase in enantioselectivity for linked-(*R*,*R*)-BINOL **97** complex was

discussed as effect of its higher stability compared to the La-(*R*)-BINOL complex. The introduction of sterically more demanding substituents in the (*R*)-BINOL 3,3' position lead to catalysts with low reactivity and enantioselectivity.

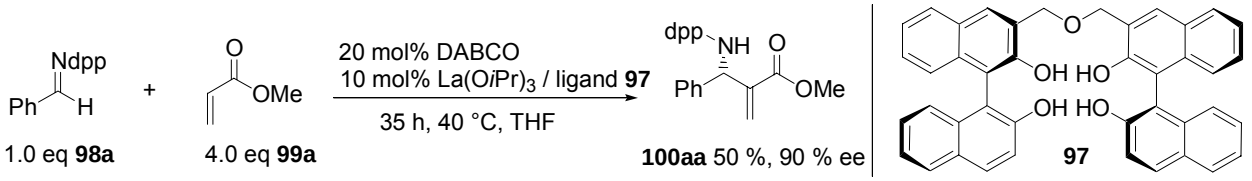


Scheme 1-6 Screening of (*R*)-BINOL-based ligands.

The La-linked-(*R*,*R*)-BINOL complex is well known for its dual reactivity as Lewis acid and Brønsted base. However, increasing the basicity of the catalyst by deprotonation with KHMDS, NaHMDS or *n*-BuLi did not result in higher yields or enantioselectivities relative to the non-metallated species.

A variation of the catalyst's Lewis-acidity by complexation of linked-(*R*,*R*)-BINOL **97** with various M(O*i*Pr)$_x$, identified Ln-complexes as the most efficient catalysts, whereas the more Lewis-acidic Ti-complex as well as the more Lewis basic Sr-complex led to decreased yields and enantioselectivities.

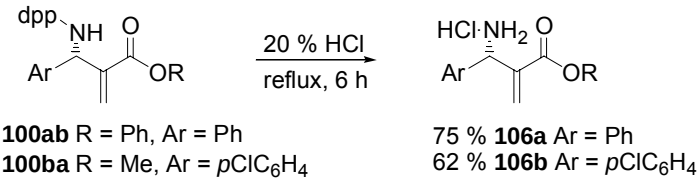
An extensive screening of the reaction parameters was performed. The optimised conditions are shown in Scheme 1-7.



Scheme 1-7 Optimised reaction conditions for the aza-BH reaction catalysed by La-linked-BINOL

In a substrate screening, among the acrylates tested, the highest yields were achieved with 2-naphthyl acrylate **99d** (up to 67 %), while methyl acrylate **99a** led to the highest enantioselectivities (up to 90 % ee). Comparison of dpp-protected benzaldimine **98a** with its *para*-Cl-substituted analogue **98b** revealed in most cases higher enantioselectivities at virtually unchanged yields for the *para*-Cl-substituted and dpp-protected benzaldimine **98b**. Aliphatic imines were also tested, but only traces of the corresponding aza-BH adducts were detected.

Hydrolytic deprotection of the aza-Baylis-Hillman products **100** provided single step access to the corresponding β-amino acids **106** in up to 75 % yield (Scheme 1-8).



100ab R = Ph, Ar = Ph
100ba R = Me, Ar = pClC$_6$H$_4$

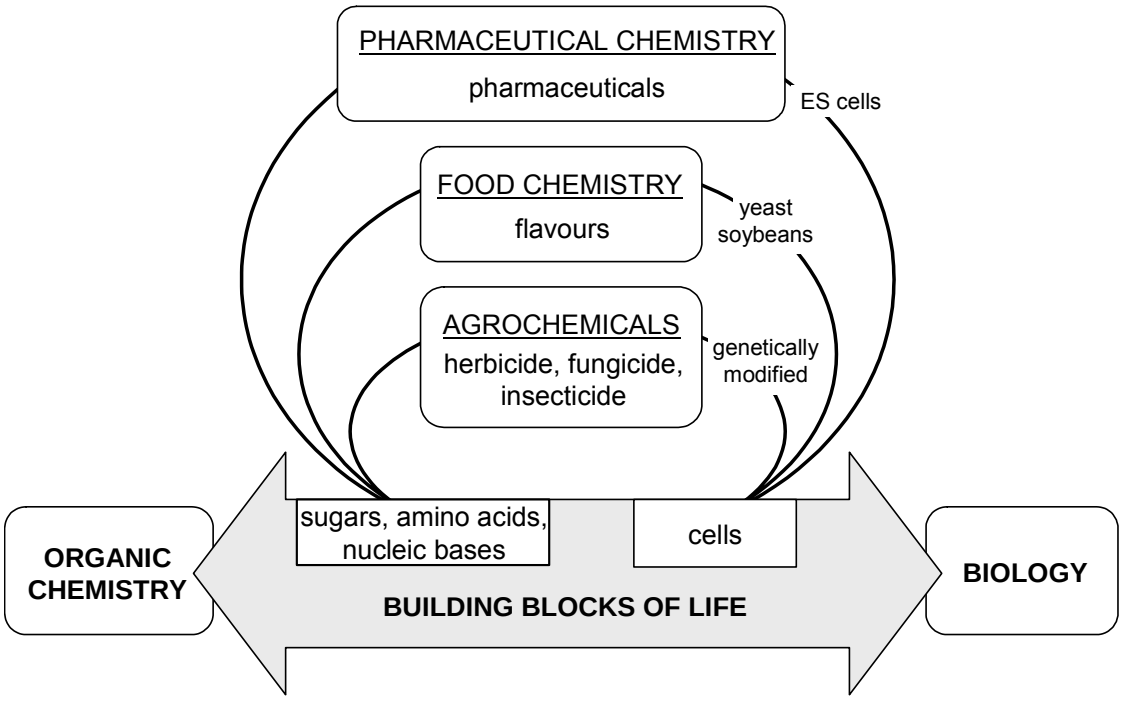
75 % **106a** Ar = Ph
62 % **106b** Ar = pClC$_6$H$_4$

Scheme 1-8 Single step conversion of aza-BH adducts **100** to β-amino acids **106**.

Conclusions. It was shown for the first time that the aza-Baylis-Hillman reaction can be catalyzed enantioselectively by a chiral Lewis acidic metal complex. Using the La-linked-(*R*,*R*)-BINOL complex as catalyst and dpp-protected imines as substrates the corresponding aza-BH adducts could be obtained in up to 68 % yield and up to 90 % ee. Hydrolytic deprotection of the highly enantio-enriched aza-Baylis-Hillman products thus obtained grants access to α-methylene-β-aminoacids in a simple one pot-procedure.

2 Introduction

In modern sciences, interdisciplinary fields become more and more important. For example, organic chemistry and biology are linked by the building blocks of life. Chemically, such building blocks of life are small organic molecules (i.e. amino acids, sugars and nucleic bases) or polymers (i.e. proteins, nucleic acids, carbonhydrates). Biologically, the building blocks of life are cells (Scheme 2-1).



Scheme 2-1: Building block of life as interdisciplinary field of organic chemistry and biology and their relations to applied chemistry.

Cellular biology, studied since the 1800s, is the basis of current stem cell research. The advent of the microscope provided scientists a first look at human cells. At this time cells were identified as the building blocks of life, capable of reproducing and differentiating into all the cell types that make up the living body.

Although the history of stem cell research started in the early 1900s with the discovery that the various types of blood cells all came from a particular 'stem cell', the potential of these stem cells has been discovered in the late 1960s: *McCulloch* and *Till* detected self-renewing cells in mouse bone marrow[10] and *Altman* as well as *Das* showed that adult neurogenesis was accompanied by stem cell activity in the brain.[11]

Stem cell research has basically focused on bone marrow transplants. Since 1950, theses transplants have been used in patients additionally treated with radiation and

chemotherapy.[12] However, first the pioneering discovery by *Dausset* in 1958, which identified the first human histocompatibility antigens, enabled the establishment of this stem cell theraphy.[13]

Developments in biotechnology in the 1980s and 1990s afforded techniques for targeting and altering genetic material as well as methods for growing human cells in the laboratory. These advances opened the doors for stem cell research.[14]

In 1998, *Thompson* isolated cells from the inner cell mass of early embryos, and developed the first embryonic stem cell lines.[15] The blastocysts used for human stem cell research typically originate from *in vitro* fertilization procedures.

These discoveries stimulated further research. The growing interest in stem cell research is evident from the exponential increase of publications in this research area. The possibilities for stem cell research are indeed endless, but yet unpredictable. If scientists can master the biochemistry behind stem cell development, stem cell technology could be used, e.g. to produce replaceable tissues or organs.

A contribution to the stem cell development is the subject of the present work. In the first part of this thesis, the *in vitro* development of cardiomyocytes from ES cells induced by small molecules is described.

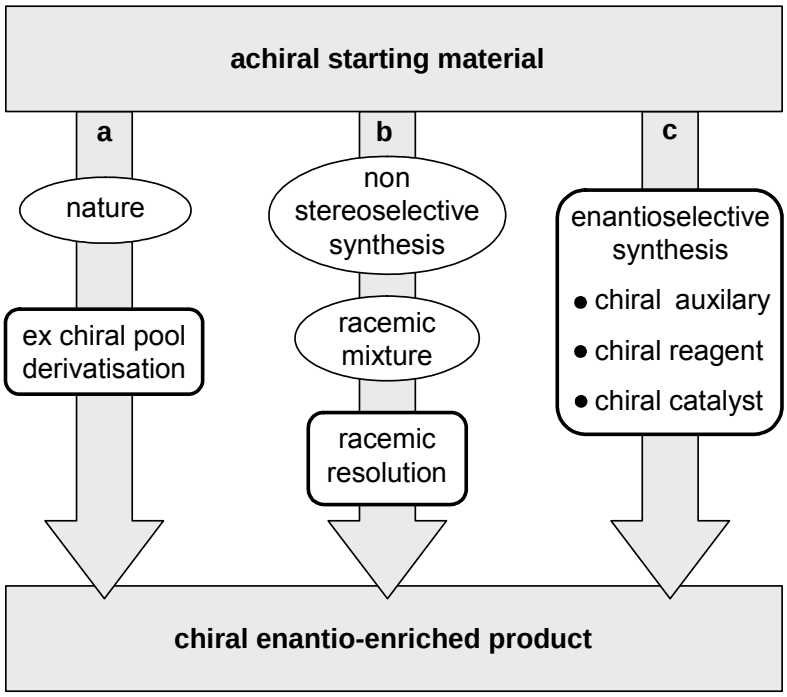
Organic compounds such as pharmaceuticals, agrochemicals, flavors and fragrances play an improtant role in modern life. These compounds possess biological activity which arises from the interaction of the organic compound with a biological target. These biomolecules are single enantiomers of chiral compounds since they are constructed from chiral building blocks such as amino acids or carbohydrates, which are present in nature as a single enantiomer.

Besides ex chiral pool syntheses (Scheme 2-2 (**a**)), there are two general methods for obtaining enantiomerically enriched compounds: (Scheme 2-2 (**b**)) they may be synthesised in racemic form and resolved into their optical antipodes, or (Scheme 2-2 (**c**)) the synthesis may be directly performed in an enantioselective manner.

(**b**) An example for the separation of enantiomers by chemical resolution based on the selective crystallisation of one enantiomer by diastereomeric salt formation. Although this method is not generally applicable, it is still quite popular for the large scale preparation of enantiomerically pure acids or amines. Other approaches of resolution involve the formation of covalent bonds between the racemic substrate and an enantiomerically pure compound. The resulting diastareomeric compounds can be separated by chromatographic technics and the desired enantiomer can be regenerated from the

appropriate diastereomer by chemical transformations. However, these methods yield only up to 50 % of the desired enantiomer.

(**c**) For asymmetric syntheses, i.e. the generation of enantio-enriched compounds from achiral precursors, chiral auxilaries, chiral reagents or chiral catalysts, all in enantio-pure form, can be employed. There are prominent auxiliar-based synthesis, e.g. *Ender's* SAMP/RAMP[16] or *Schöllkopf's* bislactim ether[17] syntheses. However, the covalent binding to and cleaving from the substrate makes these procedures less efficient. Chiral enantio-pure reagents, e.g. BINAL-H,[18] must be employed in stoichiometric quantities, this likewise decreases the efficiency of the synthesis. Hence, enantioselective catalyses with small amounts of "chiral information" are the most efficient procedures to obtain enantiopure chiral compounds. There are three different kinds of chiral catalysts employed in asymmetric synthesis, biocatalysts,[19] metal ligand complexes derived from chiral ligands,[20] and chiral organocatalysts.[21] Enzymes as biocatalysts are very selective and efficient, but often tolerate only small temperature ranges, few solvents and a narrow range of sustrates. Metal complex catalysts are widely applied in organic chemistry, although the ligands and metals are often quite expensive. Catalysis by small organic molecules, e.g. proline, complements enantioselective synthesis, as such catalysts are often cheap, easy to modify and less toxic.



Scheme 2-2 Three methods to obtain chiral, enantio-enriched compounds.

Altough impressive results were achieved in the field of asymmetric catalysis in the last decades, there is still the need for the development of highly selective and effective catalytic systems.

A contribution to this is the subject of the present work. In the second and third part of this thesis, an improved two step synthesis of a prominent organocatalyst as well as the devopment of a new catalytic system for an established C-C coupling is described.

3 Chemically Induced Cardiomyogenesis of mES Cells

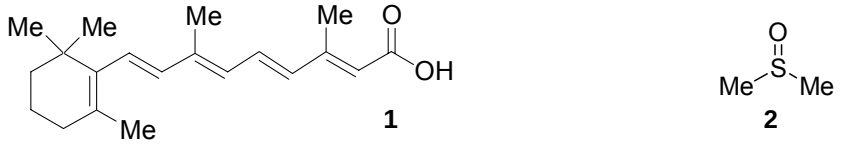
3.1 Background

Cardiomyopathy results from the loss of functional heart muscle cells, impairing the ability of the heart to maintain adequate blood circulation. As adult differentiated cardiomyocytes lack prominent regenerative capacity, heart transplantation remains the only effective causal treatment. An increasing number of patients suffer from severe heart failure, and the shortage of available donor organs emphasise the demand for alternative therapy methods, such as cellular cardiomyoplasty. In this context, several animal studies demonstrated a successful engraftment of cardiac myocytes into the adult heart.[22,23] However, the limiting factor for a general application of cell therapy for the treatment of cardiovascular diseases still is the insufficient number of donor cells.

Embryonic stem (ES) cells isolated from the inner cell mass of early blastocyst-stage embryos are pluripotent. They are capable of differentiating *in vitro* into any somatic cell type, including cardiomyocytes, haematopoietic progenitors, skeletal myocytes, smooth muscle cells, adipocytes, chondrocytes, endothelial cells, neurons and glia and pancreatic islet cells.[15] When cultured in the presence of leukaemia inhibitory factor (LIF), ES cells remain undifferentiated and can be propagated indefinitely. Upon withdrawal of LIF, ES cells spontaneously and irreversibly differentiate into multicellular aggregates, so-called embryoid bodies (EBs). These aggregates resemble early post-implantation embryos and contain derivatives of all three germ layers.[24]

So far, the *in vitro* differentiation of ES cells into cardiomyocytes offers a new approach for cellular therapy of degenerative heart diseases. Therefore, the development of new approaches allowing a direct differentiation of ES cells into cardiomyocytes is of growing interest.[25]

The differentiation programs of ES cells can be shifted toward cardiomyogenic or neuronal differentiation by treatment with small molecules, such as retinoic acid **1** or dimethyl sulfoxide (DMSO) **2** (Scheme 3-1), at specific stages of differentiation.[26,27]



Scheme 3-1: Retinoic acid **1** (left) and DMSO **2** (right).

However, this approach is not very efficient, and normaly requires selection to enrich specific cell lineages. Several small molecules have recently been found to control the differentiation of ES cell into a specific cell lineage as well as to affect the self renewal of ES cells:

Ascorbic acid. In 2003 *Takashi et al.*[28] screened 880 compounds of the *FDA2000 Drug Library*, which were approved for human use, for their potential to induce cardiac differentiation of mouse ES cells in a monolayer culture. They used CGR8 mouse ES cells stably transfected with the cardiac muscle-specific α-MHC promotor driven EGFP as a reporter. They found that upon treatment with ascorbic acid **3** (vitamin C, Scheme 3-2, p. 14) increased cardiac differentiation of ES cells in a dose-dependent manner. It can be assumed that this effect of ascorbic acid is independent of its antioxidative property[29], because antioxidants normally inhibit ES cell differentiation into cardiac myocytes, whereas ROS (reactive oxygen species) like H_2O_2 or radical-generating menadione enhance cardiogenesis[30]. Other antioxidative agents such as *N*-acetylcystein, Tiron (brenzcatechine-3,5-disulfonic acid disodiumsalt) or vitamin E also do not mimic the effect of ascorbic acid on the cardiac differentiation. In addition, there was no significant effect of vitamin C **3** on the cardiomyogenesis *via* embryoid body formation. This suggests that ascorbic acid induces permissive changes that occur during the formation of embryoid bodies, rather than induction of autonomous commitment of ES cells to cardiac myocytes.

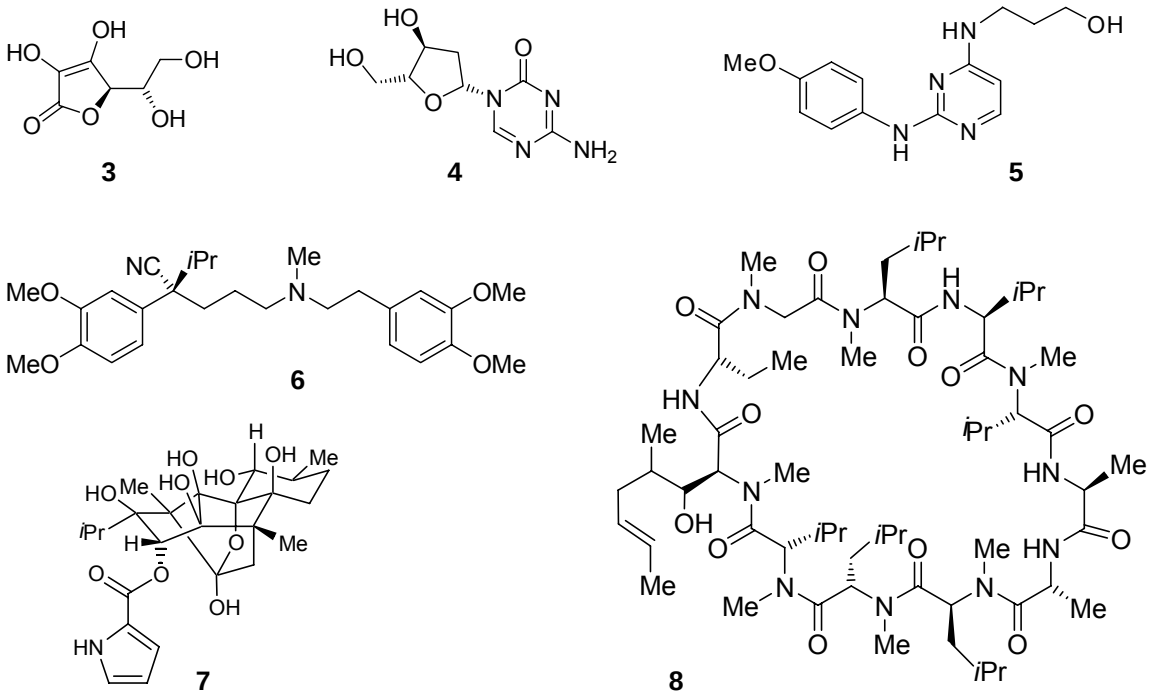
5-Aza-2'-deoxycytidine. Based on the results of *Fukuda et al.*[31], who ascertained an increased cardiomyogenesis of mesenchymal stem cells by adding the DNA-demethylating agent 5-aza-2'-deoxycytidine **4** (Scheme 3-2, p. 14), *Xu et al.*[32] tested the effect of this compound on the differentiation of human ES cells into cardiomyocytes *via* embryoid body formation. Using immunostaining and real-time PCR they observed an increase of cardiomyocyte formation by treating the human ES cells with 5-aza-2'-deoxycytidine **4**. Interestingly, DMSO and retinoic acid, which have been shown to induce cardiomyocyte differentiation in mouse ES cells (see above), did not enhance human ES cell cardiomyocyte differentiation.

Cardiogenol C. *Schultz et al.*[33] screened a 100.000 compound library of so called privileged heterocycles[34] using a stable engineered mouse embryonic carcinoma cell line P19 expressing luciferase under the control of the ANF promoter under monolayer

conditions. Like ES cells, P19 cells are pluripotent and are capable of differentiating into cardiac cells under specific culture conditions.[35] They identified 80 compounds that increased luciferase activity. In addition, 35 compounds induced a parallel expression of sarcomeric α-MHC, a cardiac specific protein. In particular, these compounds share significant structure similarities possessing a 2-hydroxylamino substitution at the C-4 position and large, hydrophobic groups at the C-2 position. To confirm that these compounds are general cardiomyogenesis inducing agents, they analysed their effects on undifferentiated R1 mouse ES cells also in a monolayer culture. They detected more than 90 % positive cardiomyocyte formation of ES cells treated with Cardiogenol C **5** (Scheme 3-2, p.14). Furthermore, they observed that compound treatment slowed down cellular proliferation with no significant cell death, indicating that this process is not simply a selection for cardiac precursor cells with the death of cells in other lineages.

Verapamil, Ryanodine, Cyclosporin. *Sachinidis et al.*[4] investigated the effects of 33 small molecules interfering with several signalling cascades on cardiomyognesis. They used a transgenic ES cell lines expressing enhanced green fluorescent protein (EGFP) under the conrol of the α-myosin heavy chain promoter (pαMHC). In this screening, especially the L-type Ca^{2+} channel blocker Verapamil **6** as well as Ryanodine **7**, an inhibitor of the protein phosphatase 2B, and Cyclosporin A **8**, an Calcineurin inhibitor (Scheme 3-2, p. 14), exerted the most striking pro-cardiomyogenic effect. Treatment of the EBs with Verapamil **6** caused a pronounced 94 % increase and treatment with Ryanodine **7** resulted in a significant 75 % increase of the EGFP fluorescence compared to untreated cells. Ryanodine **7** is a natural compound acting through binding to the Ryanodine receptor (RyR), the Ca^{2+} channel of the sarcoplasmatic reticulum (SR), resulting in the release of Ca^{2+} into the sarcoplasm. Depending on the cell type and concentration, Ryanodine **7** induces an inhibition or activation of the release of Ca^{2+} from SR.[36] Verapamil **6** is an antagonist of the plasma membrane L-type Ca^{2+} channel thereby inhibiting the influx of extracellular Ca^{2+} into the cytosol.[37] Furthermore, *Sachinidis et al.* observed that spontaneous contractions of ES cell derived cardiomyocytes were totally inhibited when Verapamil **6** was present at late stages of cardiac differentiation. From these results, *Sachinidis et al*. concluded that cyctosolic Ca^{2+} is involved in the differentiation of cardiomyocytes from ES cells and that $[Ca^{2+}]_i$ lowering agents promote cardiomyogenesis even if the physiological activity of beating is inhibited.

Exposure of EBs to Cyclosporin A **8** caused an increase of the EGFP fluorescence up to 140 %. Cyclosporin A **8** is an amino acid cyclic peptide used as immunosuppressant compound. Complexes of Cyclosporin A **8** with the immunophilin Cyclophilin A within the cell inhibit the Ca^{2+}- and calmodulin-dependent protein phosphatase 2B (Calcineurin). Futhermore there is an accumulating evidence that Calcineurin is also involved in the regulation of other cellular processes such as embryonic development and cancer, as well as cardiac valve formation and cardiac hypertrophy.[38]

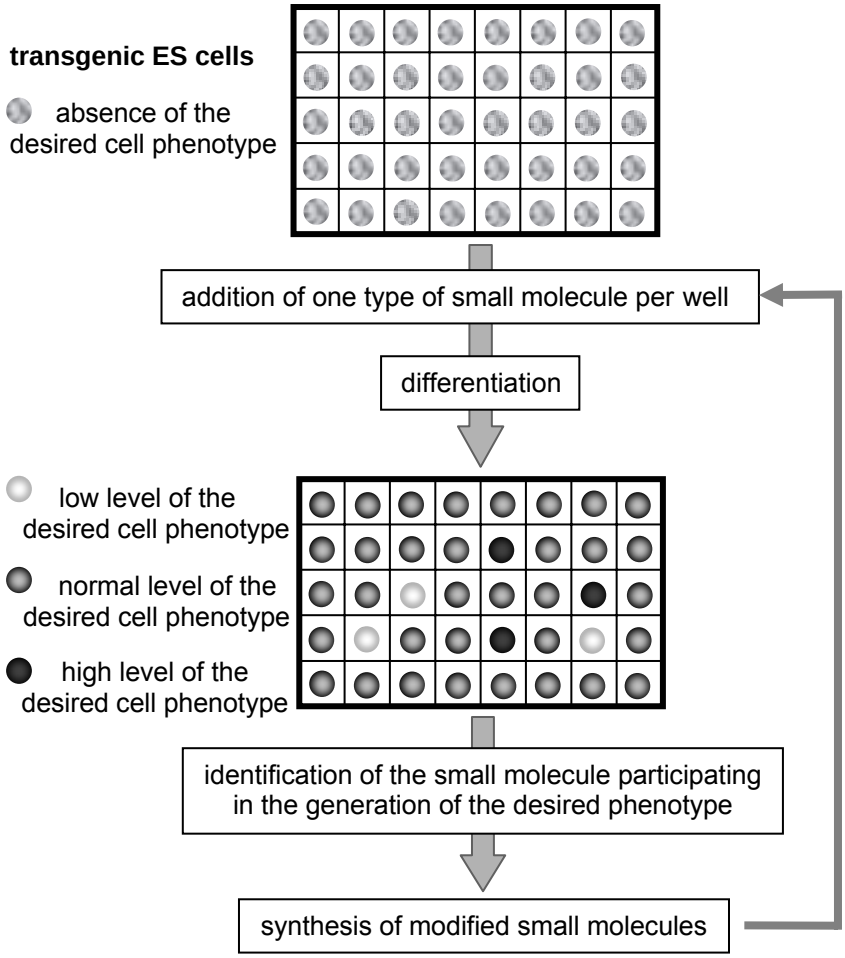


Scheme 3-2 Cardiomyogenesis inducing substances: ascorbic acid **3**, 5-aza-2'deoxycytidine **4**, Cardiogenol C **5**, Verapamil **6**, Ryanodine **7** and Cyclosporine A **8**.

3.2 Concept

3.2.1 Forward Chemical Genetics Approach

The *in vitro* differentiation of ES cells into cardiomyocytes offers a new approach for cellular therapy of degenerative heart diseases. For a direct differentiation of ES cells into cardiomyocytes experimental methods are required allowing rapid and "easy to handle" parallel testing of small molecules which may influence the differentiation of ES cells towards cardiomyocytes. For this aim, a transgenic ES cell lineage is used, expressing enhanced green fluorescent protein (EGFP) under the control of the heart specific promoter α–myosine heavy chain (pα-MHC) to test selected substances for their cardiomyogenesis inducing activity (Scheme 3-3, p. 15).[39,40]



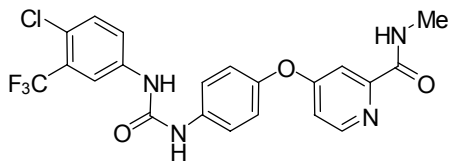
Scheme 3-3 Forward chemical genetics approach. After culturing of transgenic ES cells in multi-well plates, a library of small molecules can be screened by addition of one single compound per well. After differentiation, small molecules can be identified participating in the generation of the desired phenotype. Further modifications based on the structure of these active test substrates should lead to an enhanced differentiation potential of the tested compounds.

In general, two strategies exist for the identification of cardiomyogenesis stimulating substances: structure based drug design (SBDD), or rational drug design (RDD).[41,42,43]
The SBDD involves the search for a small molecule that perfectly fits in the binding pocket of a target protein, and thus influences the signalling pathway. An application of this method requires knowledge about the signalling pathways, the proteins involved and their structures.
The RDD describes the relationship between chemical structures and their biological effects. For a successful correlation all tested compounds must bind to the same target. To fulfil this requirement, the test substrates must have very similar chemical structures. To find a capable lead structure for the RDD, a foregoing diversity orientated screening (DOS) is performed. Within this screening, substances are tested which cover a broad structural spectrum with fixed core structures.

As the structures of the proteins involved in the signalling pathways - which are assumed to promote cardiomyogenesis of ES cells - are mostly unknown, the requirements for a SBDD approach are not fulfiled. Therefore a RDD with a preliminary DOS was performed focused on the synthesis and screening of (thio)urea and cinchona-alkaloid based compound libraries.

3.2.2 Selection of Substrates

Stem cells and cancer cells share the similarity that both cell types exhibit high proliferation rates. According to the cancer stem cell theory, drugs which induce differentiation of stem cells thereby also decrease their proliferation rates. If the same applies for cancer cells, drugs for cell differentiation could be used for anti-cancer therapies and vice versa. The prominent anti cancer drug Nexavar® (Sorafenib) harbours a thiourea moiety (Scheme 3-4).[2] Therefore, the selection of substances has been focused first on (thio)urea derivatives.



Scheme 3-4 Chemical structure of the prominet anti cancer drug Nexavar®.

A second selection of substances has concentrated on ammonium salts derived from cinchona alkaloids, as they are well known for their phase transfer abilities[44] which should promote membrane permeation by drugs. Furthermore, quinidine is a pharmaceutical agent that acts as a class I antiarrhythmic agent in the heart. The effect of quinidine on the ion channels is to prolong the cardiac action potential and thereby prolonging the QT interval on the surface electrocardiogram (ECG).[3]

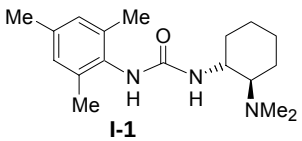
3.3 Results and Discussion

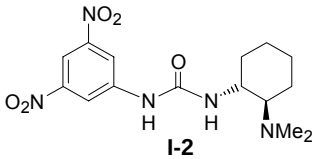
3.3.1 Substance Screening

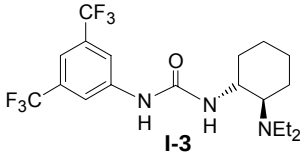
For the identification of a cardiomyogenesis stimulating substance, a rational drug design with a preliminary DOS focused on the synthesis and screening of (thio)urea and cinchona-alkaloid based compound libraries was performed.

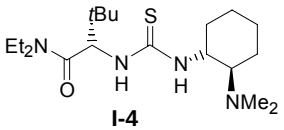
Differentiation Protocol – for DOS, RDD[45]. A transgenic murine ES cell lineage expressing enhanced green fluorescent protein (EGFP) under control of α-myosine heavy chain (α-MHC) promoter (pα-MHC- EGFP) was used to investigate the effects of (thio)urea and cinchona-alkaloid derivatives on cardiomyogenesis. To start differentiation, ES cells were cultured in suspension for 12 h to form EBs. About fifty EBs were transferred to each well of bacterial 6-well plates and the test compounds were added. On day 7, fresh medium and fresh test compounds were added. On day 14, EBs on each plate were counted, lysed and the EGFP fluorescence in the lysates was measured at an excitation wavelength of 476 nm and an emission wavelength of 508 nm.

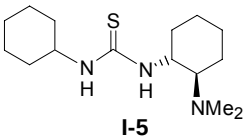
Diversity Orientated Screening (DOS). For a first DOS, two substance libraries **I** and **II** containing (thio)urea (**I**) or cinchona-alkaloid (**II**) derivatives with extensivly modified substitution patterns (Scheme 3-5 and Scheme 3-6, p. 18-19) were synthesised and tested for their cardiomyogenesis inducing efficiency.

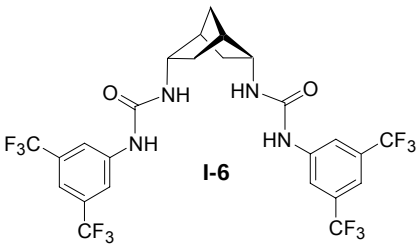

I-1

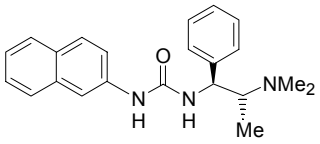

I-2

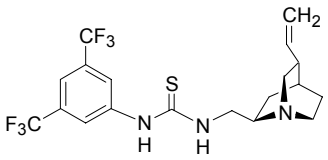

I-3

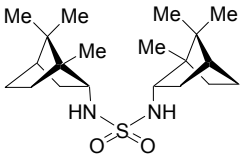

I-4

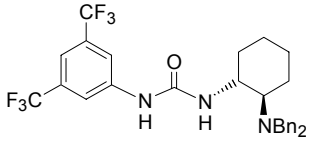

I-5

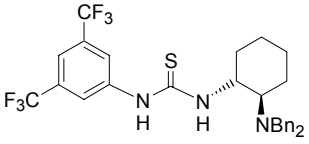

I-6

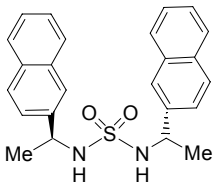

I-7

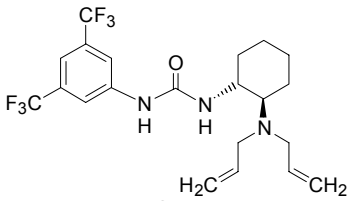

I-8

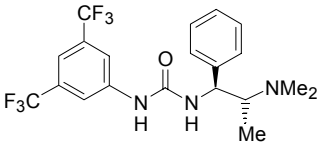

I-9

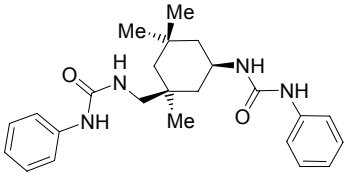

I-10

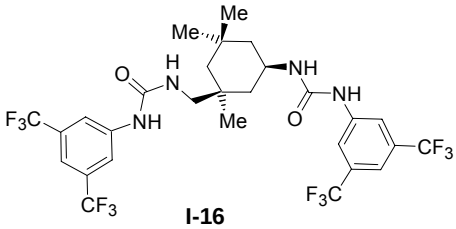

I-11

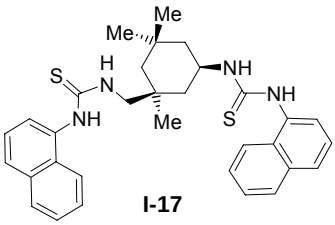

I-12

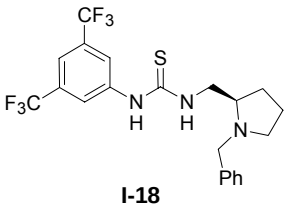

I-13

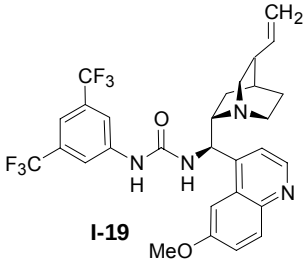

I-14


I-15

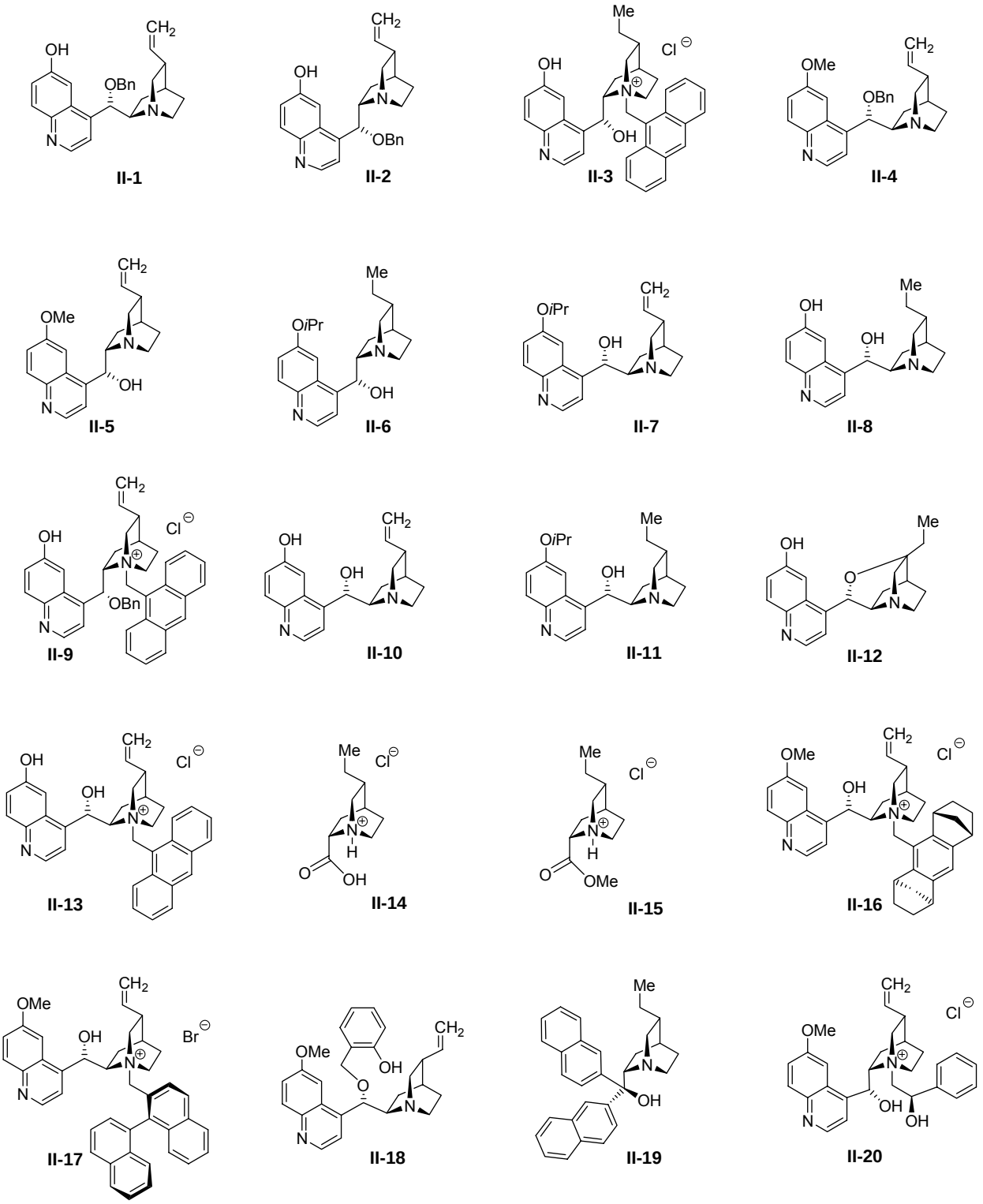

I-16


I-17


I-18


I-19

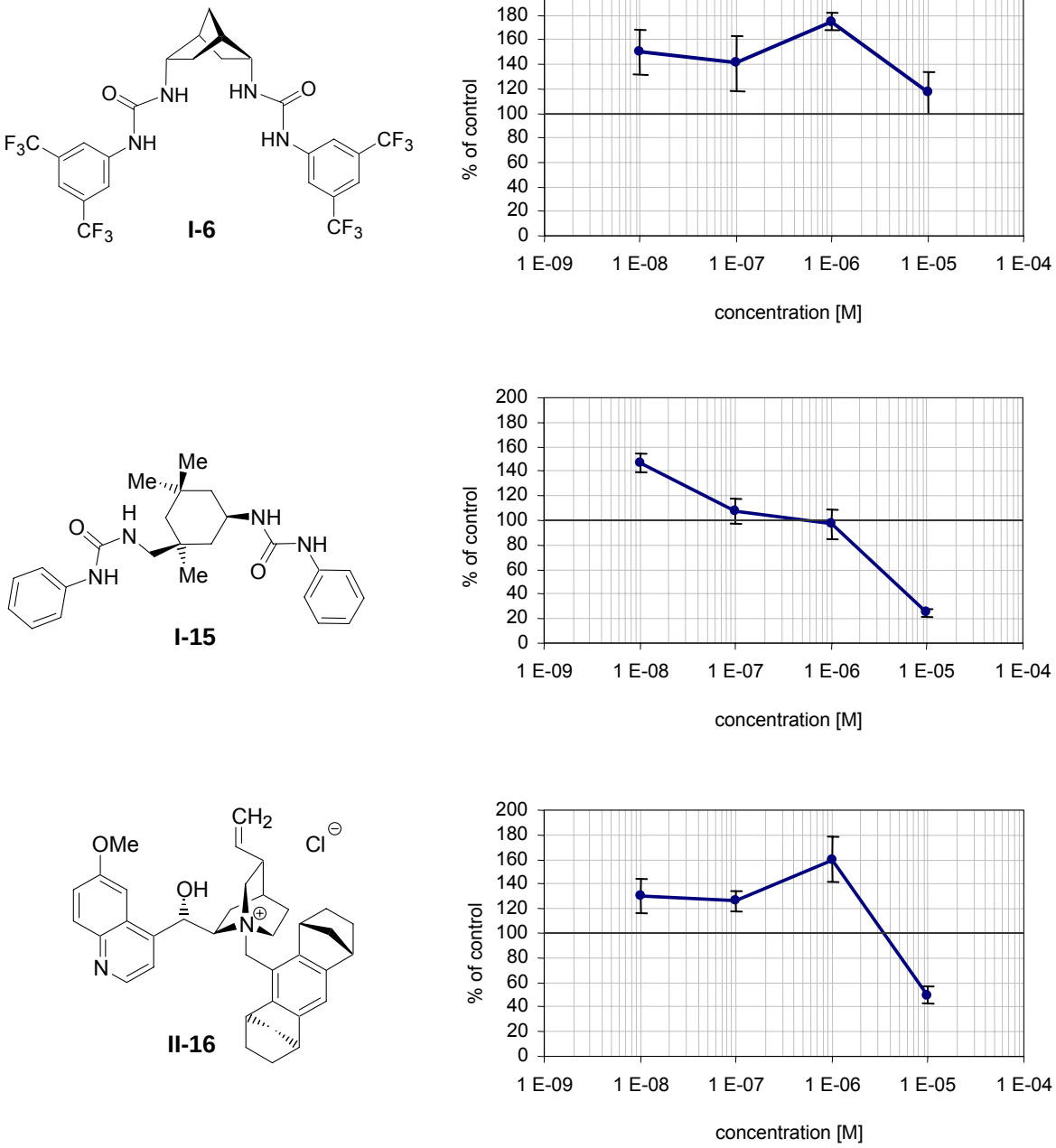
Scheme 3-5 Structures contained in the (thio)urea-based library **I**.



Scheme 3-6 Structures contained in the cinchona-alkaloid-based library **II**.

The cardiomyogenic effect of the substances is expressed as percent of the EGFP fluorescence of the vehicle treated cells (100%). An increased production of cardiomyocytes gives percent of control values higher then 100 %, a decreased production of cardiomyocytes lower than 100 %.

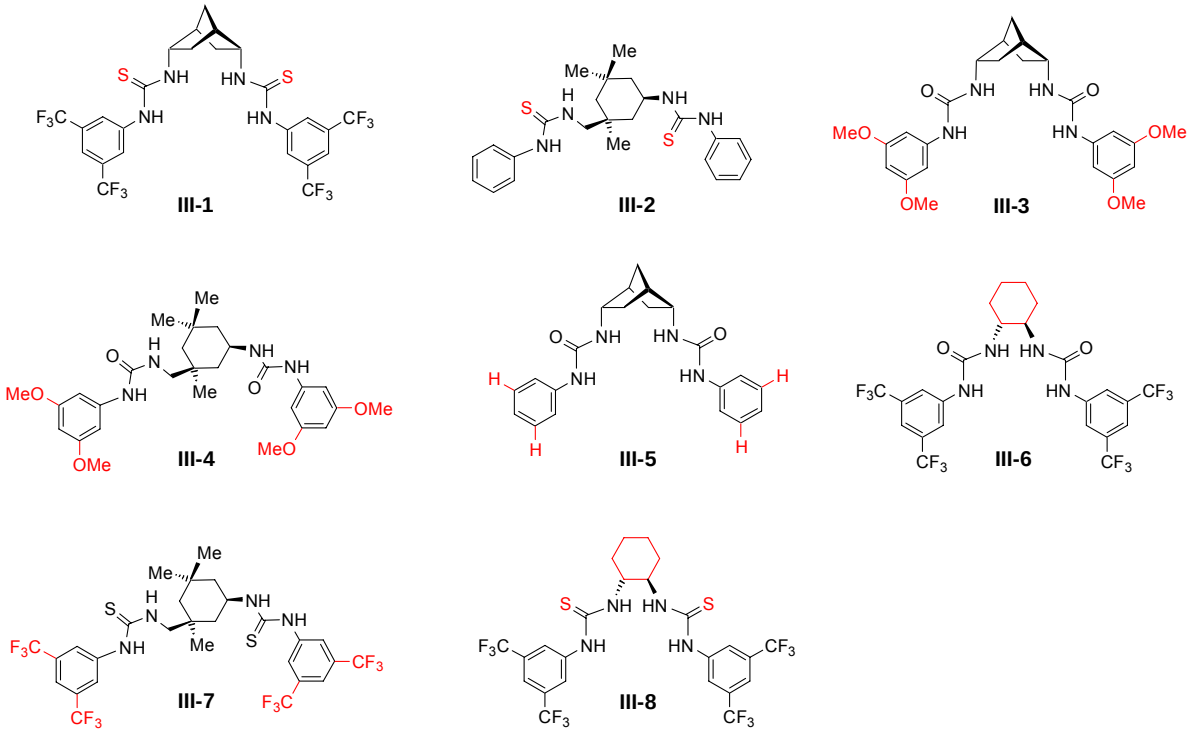
Among the (thio)urea derivatives (library **I**) tested, especially the bis-ureas **I-6** and **I-15** induced an increase of 80 % or 50 %, respectively, of the EGFP fluorescence which correlates with a positive cardiomoygenic effect. In the cinchona-alkaloid based library **II** the best result could be obtained with the quinidine based salt **II-16** which induced a 60 % increase (Scheme 3-7).



Scheme 3-7 Influence of the compounds **I-6**, **I-15** and **II-16** on the expression of EGFP, normalised to the number of EBs. Values from four independent experiments (mean ± SD, n=4), each performed in triplicate, are displayed as percent of control values (=100 %, only vehicle, without test substrate). Both a significant increase and decrease of values, compared to controls, represent an effect on the generation of cardiac cells.

Rational Drug Design (RDD). On the basis of the three lead structures (**I-6**, **I-15**, **II-16**) found in the foregoing diversity orientated screening, a focused (thio)urea (**III**, Scheme 3-8) and a focused cinchona-alkaloid based library (**IV**,Scheme 3-9) were created. These libraries were tested for their cardiomyogenic activity and analysed in a rational drug design. As mentioned before, a meaningful RDD requires binding to the same target protein. Therefore, analogues were synthesised which differ only slightly compared to the starting compounds, as smaller structural differences can be assumed to lead to a higher probability of binding to the same target protein.

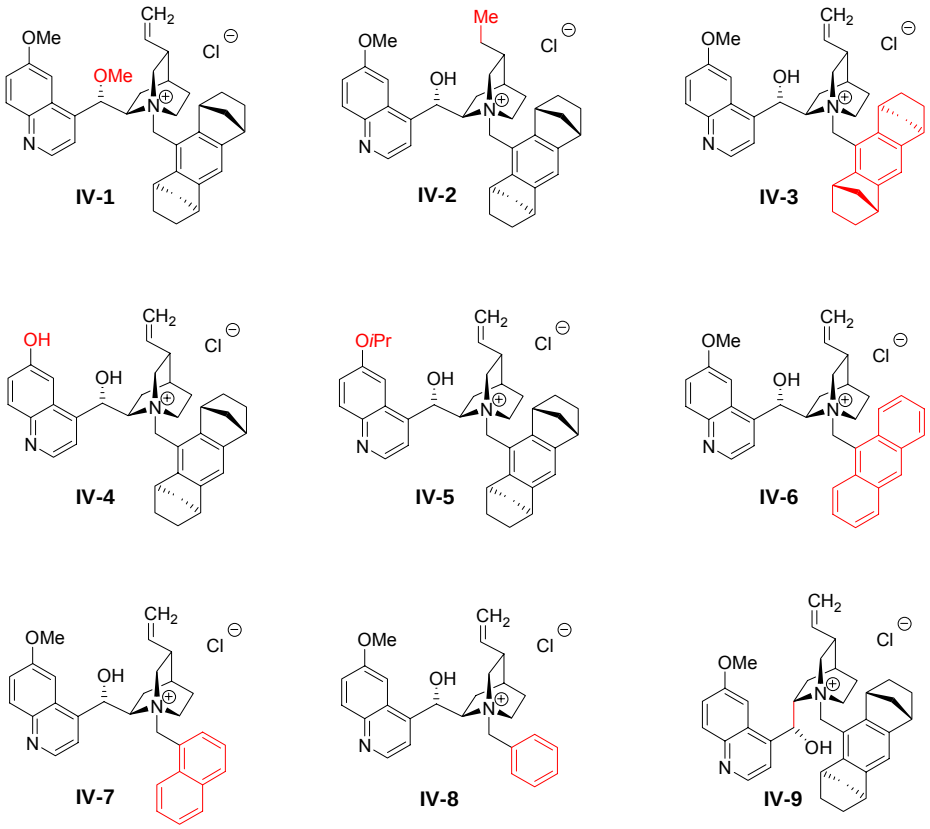
The substrates **III-1**, **III-3** and **III-5** (Scheme 3-8) are based on the lead structure **I-6**. They differ from this lead compound in the thiourea moiety (**III-1**) or in the aromatic 3,5-subtituents (**III-3**, **III-5**). Test compounds **III-2**, **III-4** and **III-7** were evolved from the lead structure **I-15**. Substrate **III-2** harbours a thiourea function instead of the urea moiety and the test compounds **III-4** and **III-7** exhibit additional aromatic 3,5-subtituents. Variation of the chiral diamino backbone led to the test compounds **III-6** and **III-8**.



Scheme 3-8 Structures contained in the focused (thio)urea-based library **III**. Differences to the lead structures **I-6** resp. **I-15** are coloured in red.

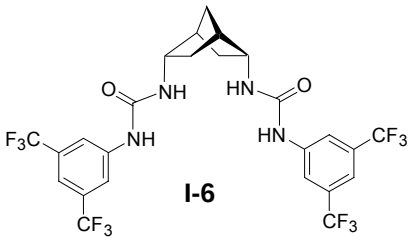
The substrates in library **IV** (Scheme 3-9) differ from the lead structure **II-16** in the methylated hydroxyfunction (**IV-1**), the hydrogenated double bond (**IV-2**), the inversion

of the stereocenters at C8 and C9 (**IV-9**), a variation in the alkoxy group (**IV-4** and **IV-5**) or in the quaternary ammonium moiety (**IV-3**, **IV-6**, **IV-7**, **IV-8**).

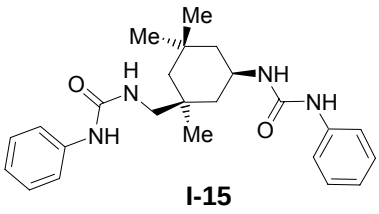


Scheme 3-9 Structures contained in the focused cinchona alkaloid-based library **IV**. Differences to the lead structure **II-16** are coloured in red.

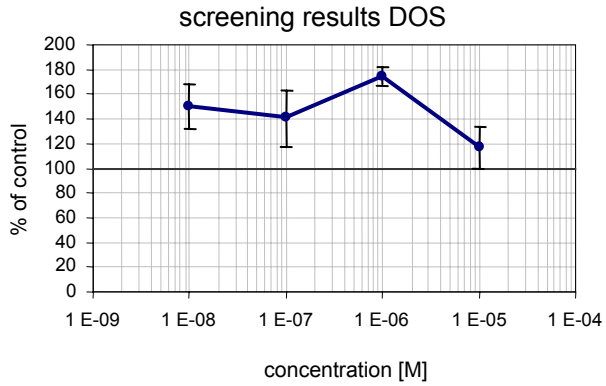
The cardiomyogenesis inducing effect of the lead structures **I-6**, **I-15**, identified in the DOS, could not be reproduced in the RDD screening, despite the fact that the same screening method (see differentiation protocol) was used (Scheme 3-10, p. 23). Consequently, (thio)ureas and their structural variations appear not to provide a sufficiently solid and reliable basis for a meaningful structure-activity relationship (in this case of small molecule-induced cardiomyogenesis).

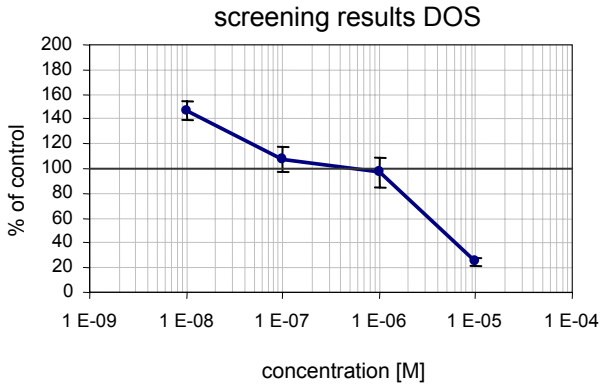


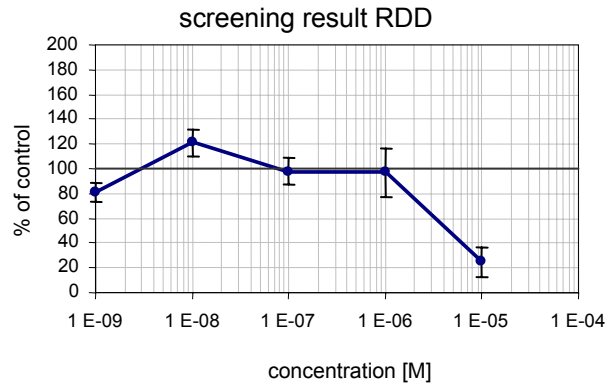
I-6

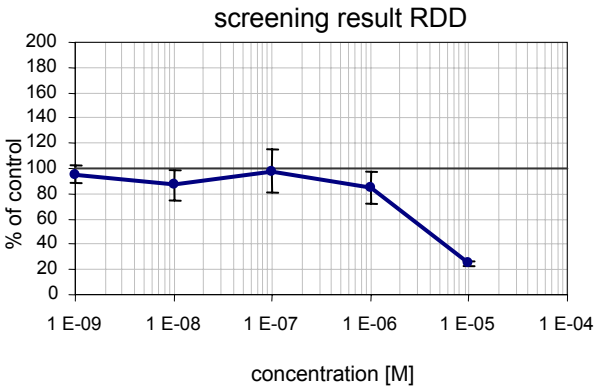


I-15







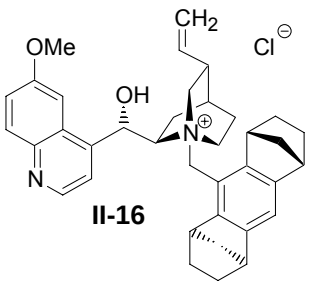


Scheme 3-10 Influence of the compounds **I-6** and **I-15** on the expression of EGFP, normalised to the number of EBs. Values from four independent experiments (mean ± SD, n=4), each performed in triplicate, are displayed as percent of control values (=100 %, only vehicle, without test substrate). Both a significant increase and decrease of values, compared to controls, represent an effect on the generation of cardiac cells (left – results of the physiological screening of compound **I-6**; right – results of the physiological screening of compound **I-15**).

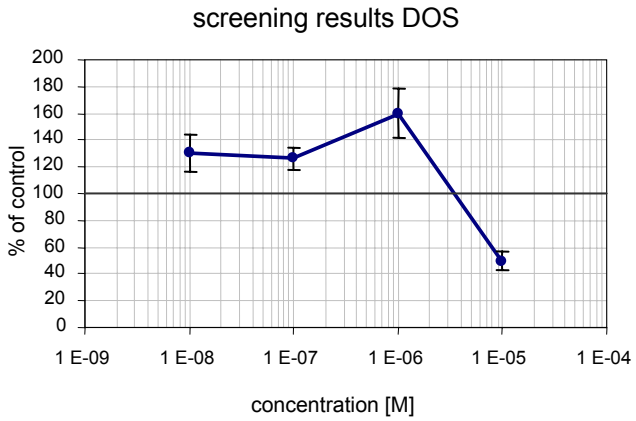
A possible explanation for these results might be that the thio(urea)-substrate binding is not specific, but rather depends on medium parameters, such as pH value or temperature, which are influenced by metabolism of the ES cells and therefore can hardly be controlled. Such small changes in the experimental environment would then lead to a significant influence of a possible (thio)urea induced cardiomyogenesis.

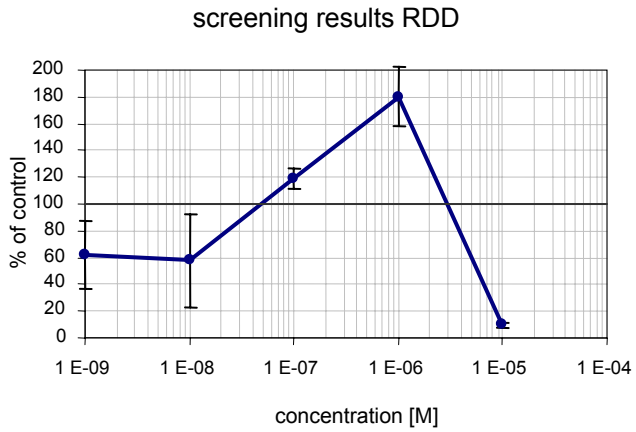
In contrast, in the quinidine based library **IV**, the positive effect of the lead structure **II-16** on the generation of cardiac cells identified in the DOS could be reproduced in the RDD,

i.e. the shapes of the curves are very similar, both show maximal effect (in between 60 to 80 % increase) at a substrate concentration of $1 \cdot 10^{-5}$ M (Scheme 3-11).



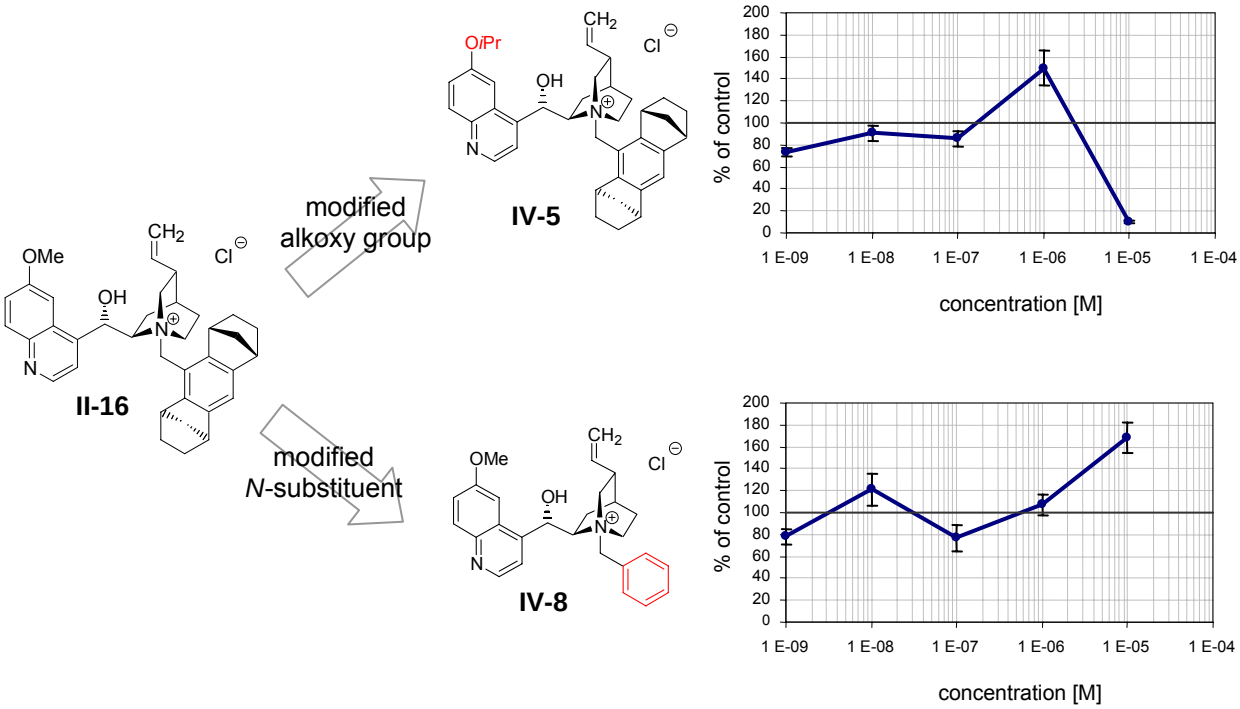
II-16





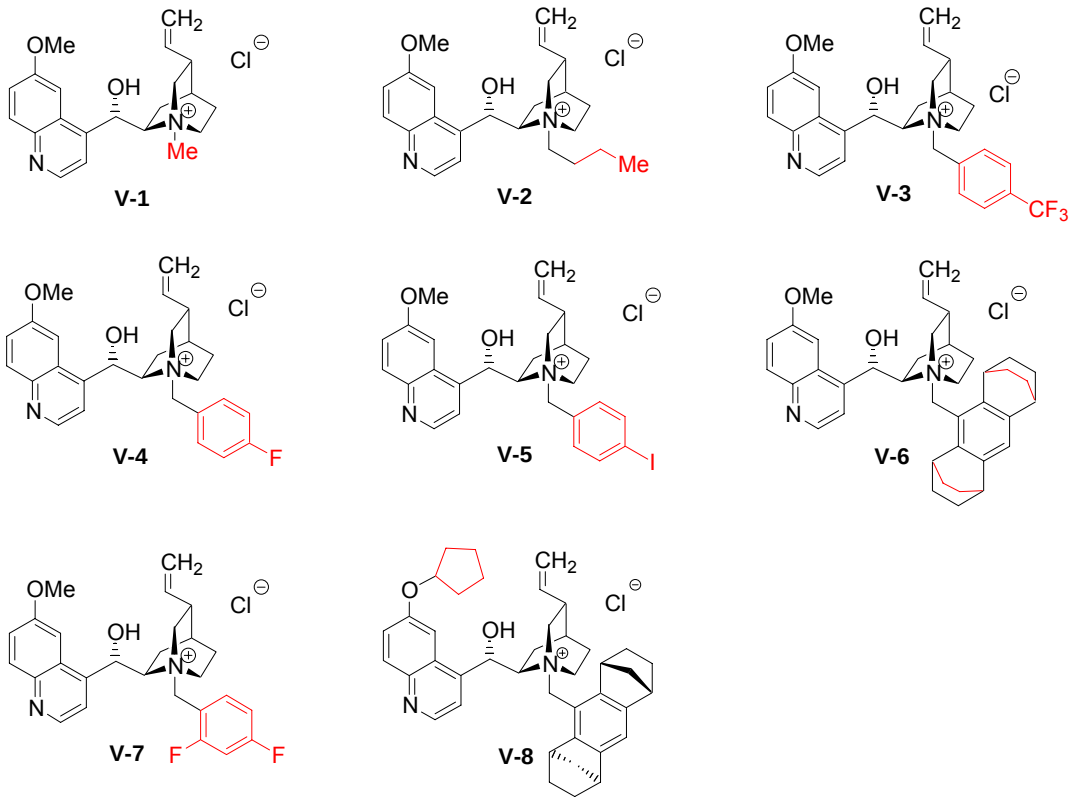
Scheme 3-11 Influence of the compound **II-16** on the expression of EGFP, normalised to the number of EBs. Values from four independent experiments (mean ± SD, n=4), each performed in triplicate, are displayed as percent of control values (=100 %, only vehicle, without test substrate). Both a significant increase and decrease of values, compared to controls, represent an effect on generation of cardiac cells (top – results of the physiological screening for the DOS; bottom – results of the physiological screening for the RDD).

RDD analysis of the physiological screening of the cinchona alkaloid based library **IV** shows that, compared to the lead structure **II-16**, the inversion of the C8 and C9 stereo-centers (**IV-9**), the hydrogenation of the double bond (**IV-2**) or the methylation of the hydroxyfunction (**IV-1**) result in the loss of cardiomyogenesis-inducing activity. In contrast, especially variations on the alkoxy group (**IV-5**) and the quarternary ammonium moiety (**IV-3**,**6**,**7**,**8**) lead to an increased formation of cardiomyocytes. The best hits could be obtained with the quinidine derivatives (**IV-5**) (up to 50 % increase) and (**IV-8**) (up to 70 % increase) (Scheme 3-12, p. 25).



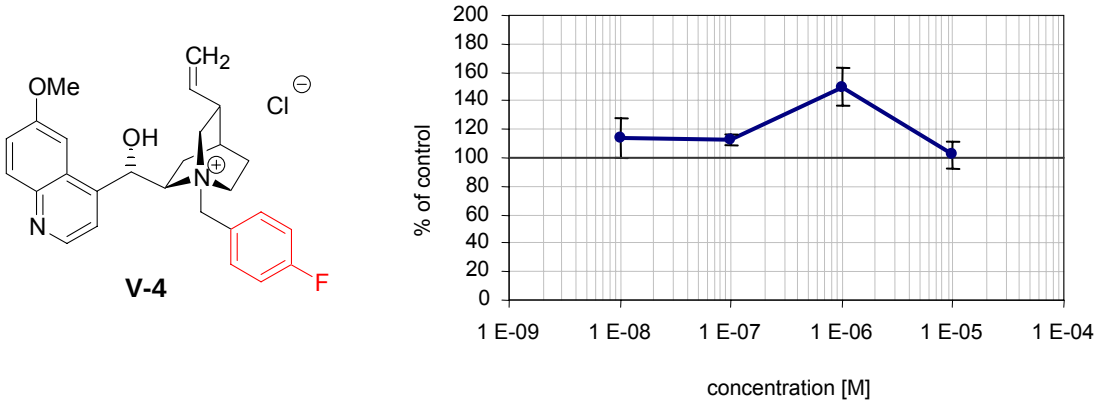
Scheme 3-12 Influence of the variations on the alkoxy and quarternary ammonium substituents (compounds **IV-5** and **IV-8**) on the expression of EGFP, normalised to the number of EBs. Values from four independent experiments (mean ± SD, n=4), each performed in triplicate, are displayed as percent of control values (=100 %, only vehicle, without test substrate). Both a significant increase and decrease of values, compared to controls, represent an effect on generation of cardiac cells.

Based on these positively tested substances (compounds **IV-5** and **IV-8**), a new quinidine based substance library **V** was developed and screened for its cardiomyogenesis inducing effectivity. This library contains substances which differ either in the alkoxy group attached to the quinidine moiety (**V-8**), or in the quinuclidine *N*-substituent (**V-1** - **V-7**) compared to the lead structures **IV-5** and **IV-8** (Scheme 3-13, p. 26)



Scheme 3-13 Structures contained in the quinidine based library **V**. Differences to the lead structures **IV-5** resp. **IV-8** are coloured in red.

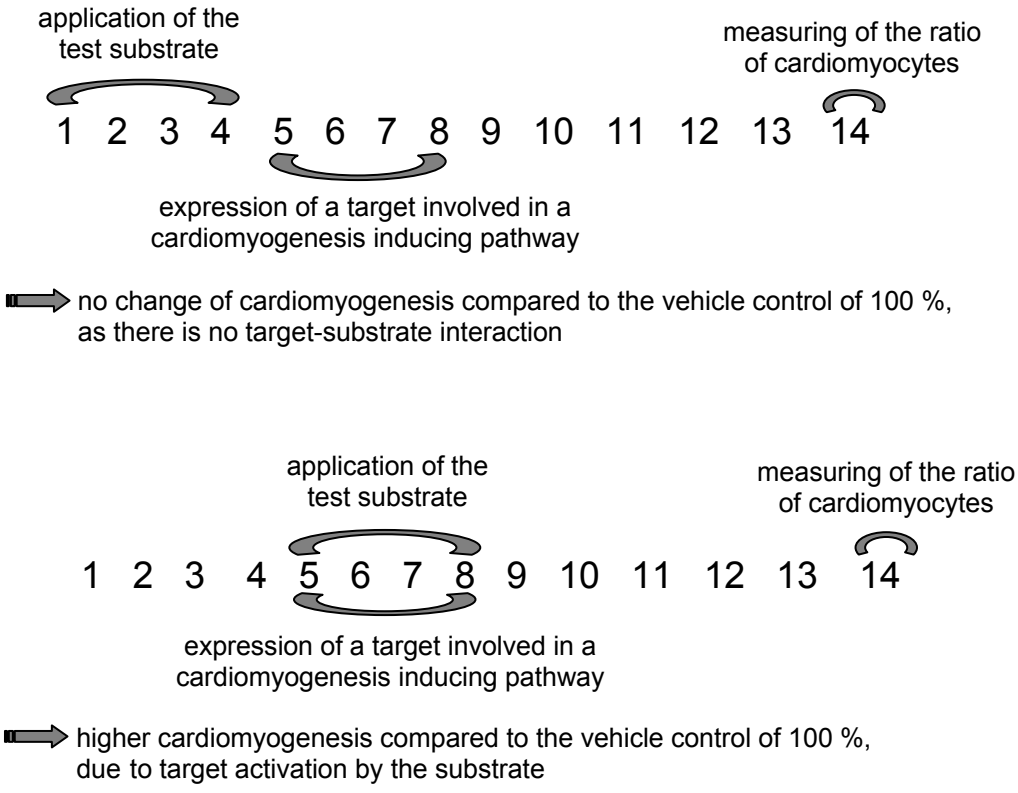
Physiological screenings of the quinidine based library **V** identified compound **V-4** as the most effective cardiomyogenesis inducing substance with a maximal 50 % increase of the EGFP fluorescence at a concentration of $1 \cdot 10^{-6}$ M (Scheme 3-14). In summary, the structural modifications introduced by compounds **V-1** – **V-8** did not improve the cardiomyogenesis inducing effect of the original hit, i.e. compound **II-16** (60-80 % increase in cardiomyogenesis).



Scheme 3-14 Influence of compound **V-4** on the expression of EGFP, normalised to the number of EBs. Values from four independent experiments (mean ± SD, n=4), each performed in triplicate, are displayed as percent of control values (=100 %, only vehicle, without test substrate). Both a significant increase and decrease of values, compared to controls, represent an effect on the generation of cardiac cells.

3.3.2 Identification of Signalling Cascades Involved in Cardiomyogenesis

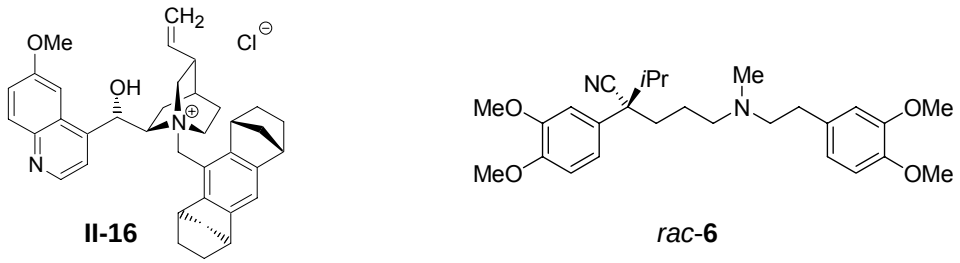
The best hit of the substance screening **II-16** and Verapamil **6**, a cardiomyogensis inducing agent found in previous studies,[4] were used for the identification of signalling cascades involved in the cardiomyogenesis by the time-dependent screening approach. In contrast to previous screenings, in this approach, the test substrates were only applied for 2 instead of 14 days. An increased cardiomyogenesis, detected after 14 days, indicates interaction of the compound tested with a target expressed at a certain point in time during the application time-frame of the test substrate. Thus, time-dependent correlations between cardiomyogenesis and concentrations of the substrates are expected to limit the number of potential targets associated to the various developmental stages (Scheme 3-14).



Scheme 3-15 Limitation of potential targets by a time-dependent screening.

Differentiation Protocol - Time Dependent Screening. In contrast to the differentiation protocol described above (p. 16), in time-dependent studies, the test substrates are applied at different intervals (day 1-3, 3-5, 5-7, 7-9).

Time Dependent Screening. For the time-depending screening, the cinchona-alkaloid **II-16** and (racemic) Verapamil *rac*-**6** were used as test substrates (Scheme 3-16). Among all substances tested in the physiological screenings, these two led to the highest ratio of cardiomyocytes and may best contribute to the identification of the signalling pathways involved in cardiomyogenesis.

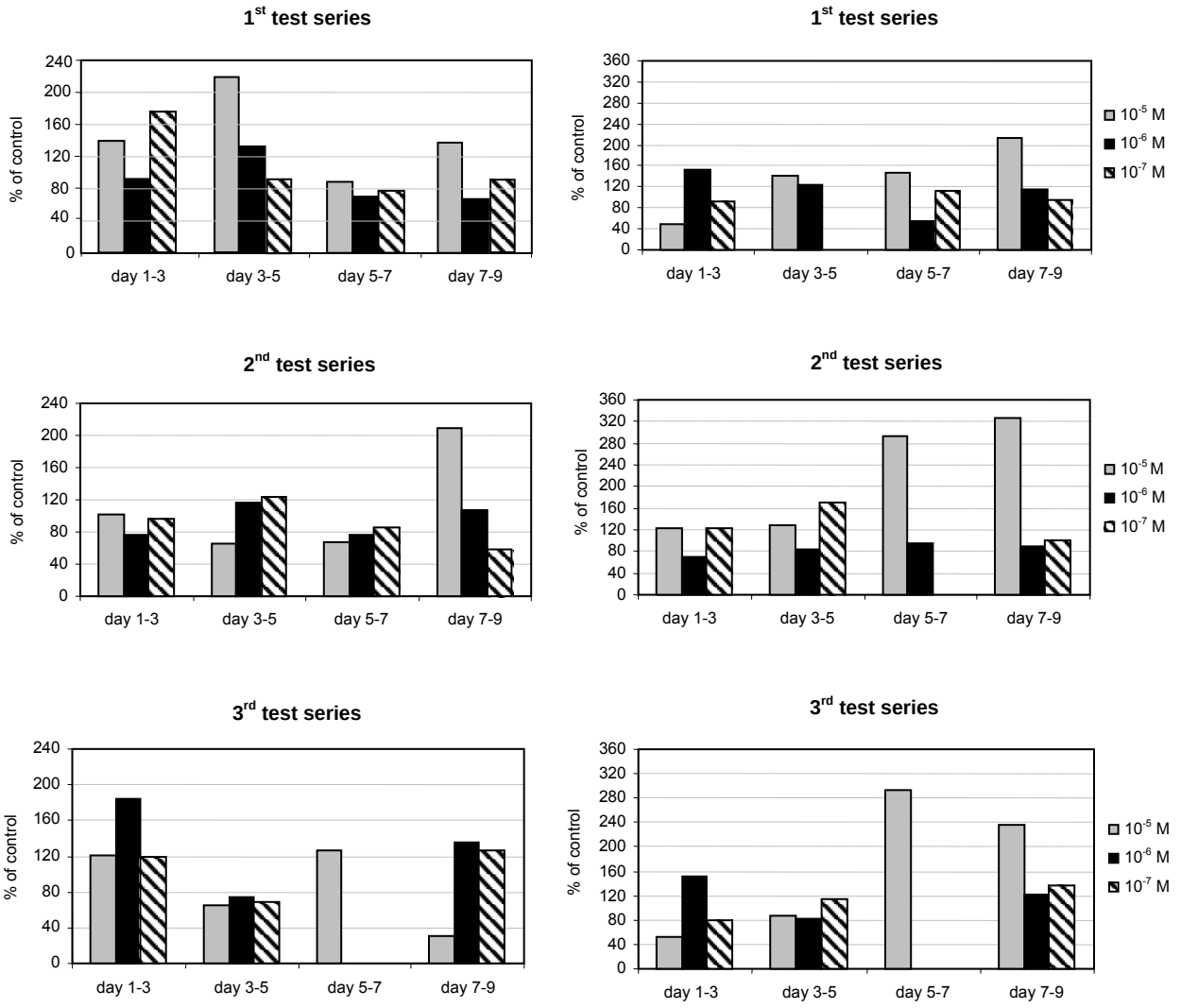


II-16 *rac*-**6**

Scheme 3-16 Cinchona alkaloid **II-16** (left), verapamil *rac*-**6** (racemic mixture, right).

For each application time-frame (days 1-3, 3-5, 5-7 and 7-9) three different concentrations (10^{-5} M, 10^{-6} M and 10^{-7} M) of the quinidine derivative **II-16** and Verapamil *rac*-**6** were tested in three identical test series. The cardiomyogenic effect of the substances is expressed as percent of the EGFP fluorescence of the vehicle treated cells (100%). An increased production of stem cells gives percent of control values higher then 100 %, a decreased production of stem cells lower than 100 %.

As shown in Scheme 3-17 (p. 29), both substrates exhibit different cardiomyogenesis inducing profiles in each test series. Based on these results, the cardiomyogenesis inducing effect of the test substrate **II-16** and verapamil *rac*-**6** appeared to rely on an interaction of the tested substances with several target proteins expressed at different points of time. In this case, time-dependent correlations between cardiomyogenesis and concentrations of the substrates are not suitable to limit the number of potential targets associated to the various developmental stages.



Scheme 3-17 Time-dependent effects of quinidine derivative **II-16** (left) and Verapamil *rac*-**6** (right) on the expression of EGFP, normalised to the number of EBs. Values of three independent test series for four application time frames, each performed in triplicate, are displayed as percent of control values (=100 %, only vehicle, without test substrate). Both a significant increase and decrease of values, compared to controls, represent an effect on generation of cardiac cells.

Conclusions. The substance screening led to the identification of cardiomyogenesis inducing compounds with good (**IV-5**, **V-4**) to very good activities (**II-16**, **IV-8**) determined by a 50 to 80 % increase of the EGFP fluorescence compared to untreated cells. Time-dependent screenings in which compounds were added at different developmental stages of the ES cell were of limited suitability for the identification of possible targets addressed by active reagents. Further insight on target binding might be achieved by the expression of isolated targets and their induction with test substances.

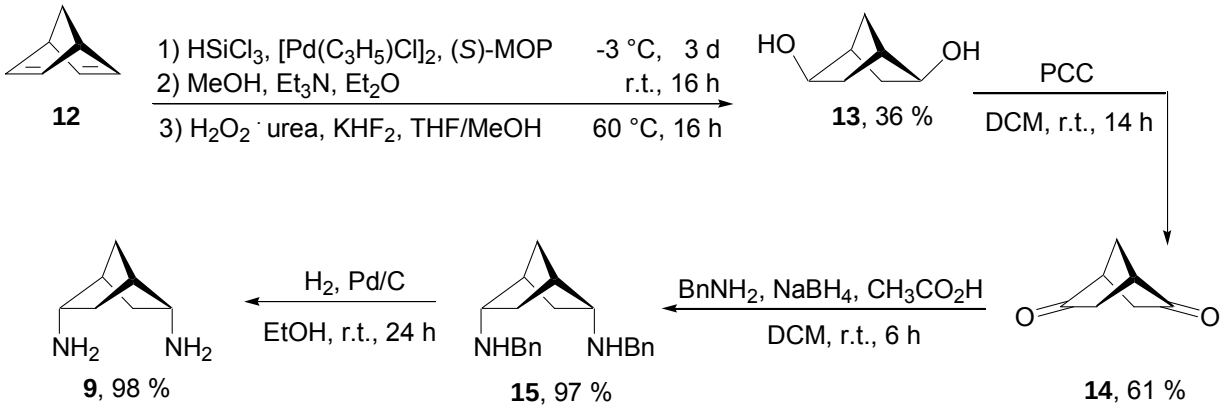
3.3.3 Synthesis of the Test Substrates

Substance Library I. The test substrates of the substance library **I** were synthesised by *Dr. M. Brandenburg,*[46] *Dr. F. Cleemann,*[47] *Dr. S. Mukherjee,*[48] *Dr. T. Müller,*[49] *Dr. S. Schnippering*[50] and *Dr. K. Roland.*[51]

Substance Library II. The test substrates of the substance library **II** were synthesised by *Dr. M. Guixà*[52] and *Dr. F. Schmidt*.[53]

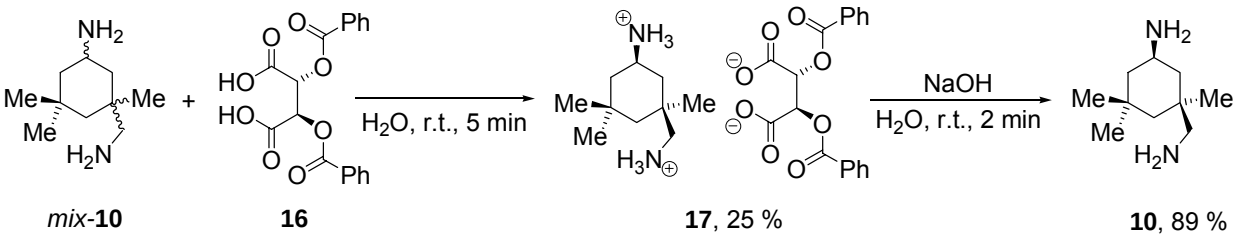
Substance Library III. To generate the (thio)urea based library **III** the chiral diamino-backbones, consisting of (1*S*,2*S*,4*S*,5*S*)-2,5-diamino-bicyclo[2.2.1]heptane (DIANANE) **9**, (1*R*,3*S*)-3-aminomethyl-3,5,5-trimethylcyclohexylamine (IPDA) **10** and (1*R*,2*R*)-1,2-diaminocyclohexane (DACH) **11**, were synthesised first.

For the synthesis of DIANANE **9**, norbornadiene **12** was first hydrated to form the corresponding diol **13** in 36 % yield. Oxidation of the hydroxy-functions led to the diketone **14** in 61 % yield. Reductive amination of the carbonyl-functions resulted in the benzyl protected diamine **15** in 97 % yield and deprotection of **15** afforded the desired DIANANE **9** in 98 % yield (Scheme 3-18).



Scheme 3-18 Synthesis of (1*S*,2*S*,4*S*,5*S*)-DIANANE **9**.

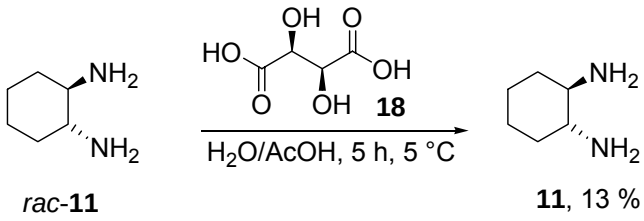
Commercially available IPDA *mix*-**10** is a mixture of the racemic *cis*- and *trans*-diastereomers in a 3 : 1 ratio. The optical resolution of IPDA *mix*-**10** was performed via a diastereoselective salification with (*R*,*R*)-dibenzoyltartaric acid **16** (Scheme 3-19, p. 31).



mix-**10** **16** **17**, 25 % **10**, 89 %

Scheme 3-19 Synthesis of (1*R*,3*S*)-IPDA **10** .

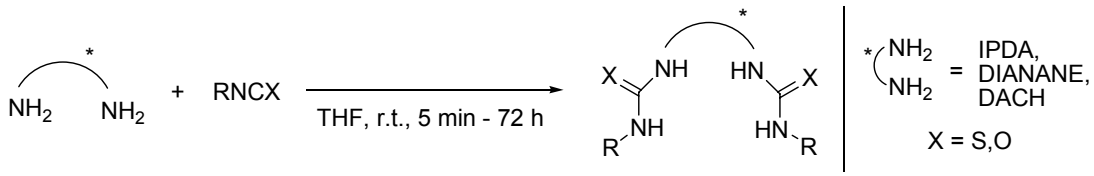
The optical resolution of the commercially available racemic 1,2-diaminocyclohexane *rac*-**11** was carried out *via* a diastereoselective salification using L-(+)-tartaric acid **18** (Scheme 3-20).



rac-**11** **11**, 13 %

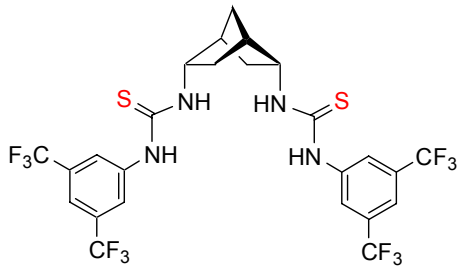
Scheme 3-20 Synthesis of (1*R*,2*R*)-DACH **11**.

Addition of the chiral diamines (1*S*,2*S*,4*S*,5*S*)-DIANANE **9**, (1*R*,3*S*)-IPDA **10** and (1*R*,2*R*)-DACH **11** to the iso(thio)cyanates, according to a general procedure (Scheme 3-21), yields the corresponding (thio)urea derivatives of the substance library **III**.

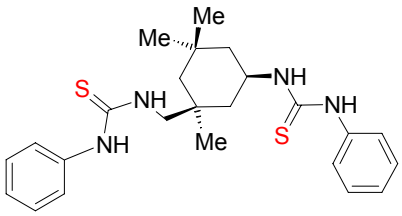


Scheme 3-21 General procedure for the addition of the chirale diamines **9**, **10** and **11** to the corresponding iso(thio)cyanates.

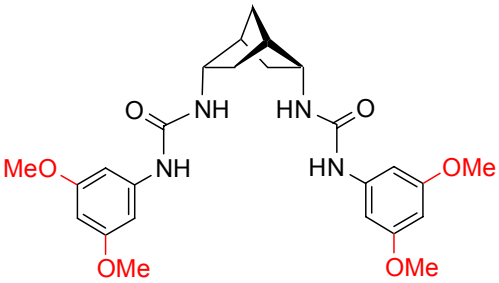
The yields of these syntheses are given in Scheme 3-22, p. 32.



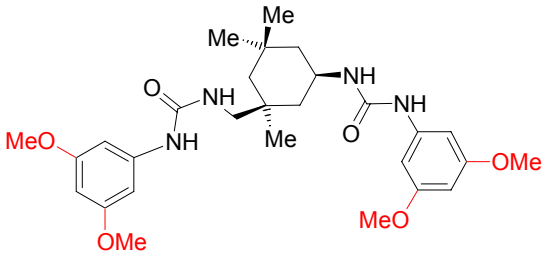
III-1, 66 %[i]



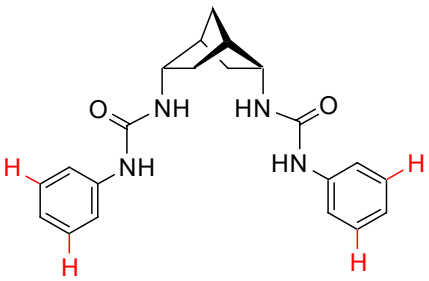
III-2, 67 %



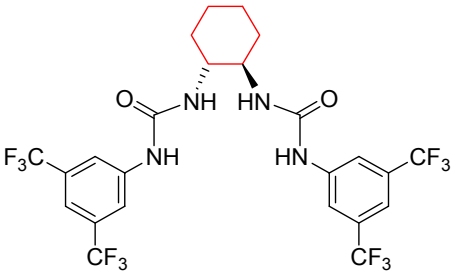
III-3, 97 %



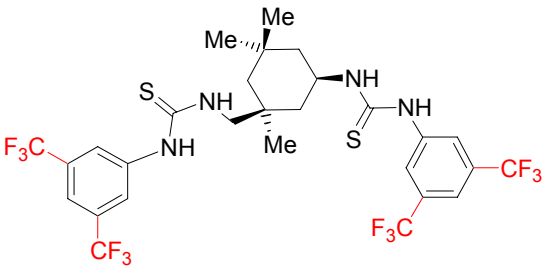
III-4, 90 %



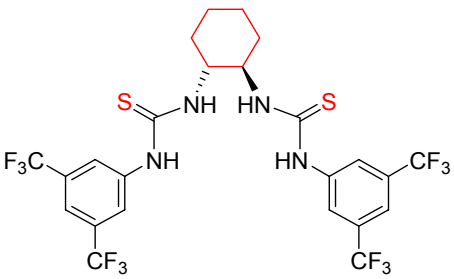
III-5, 95 %



III-6, 61 %



III-7, 72 %



III-8, 65 %

Scheme 3-22 Compounds contained in the (thio)urea based library **III**.

Substance Library IV. For the preparation of the cinchona-alkaloid based library **IV**, quinidine **19** was first transformed to the derivatives **20**, **21** and **22**.

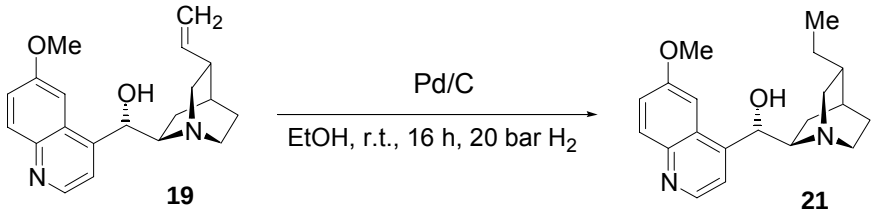
[i] Compound **III-1** was synthesised by *Dr. M. Brandenburg*.

Methylation of the hydroxy function of quinidine **19** with methyl iodide **23** resulted in the quinidine derivative **20** in 90 % yield (Scheme 3-23).

19 **20,** 90 %

Scheme 3-23 Synthesis of 9-*O*-methylquinidine **20**.

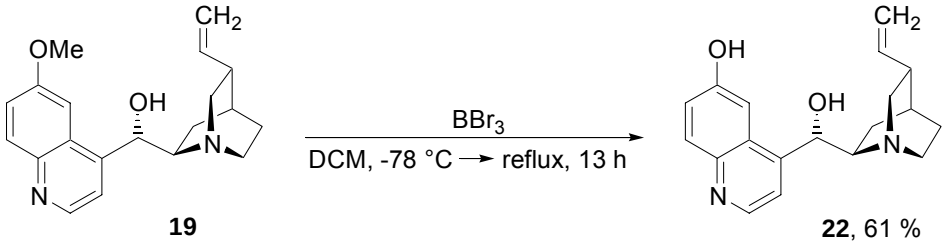
Hydrogenation of the aliphatic double bond of quinidine **19** with Pd/C led to the quinidine derivative **21** in 94 % yield (Scheme 3-24).



19 **21**

Scheme 3-24 Synthesis of 10,11-dihydroquinidine **21**.

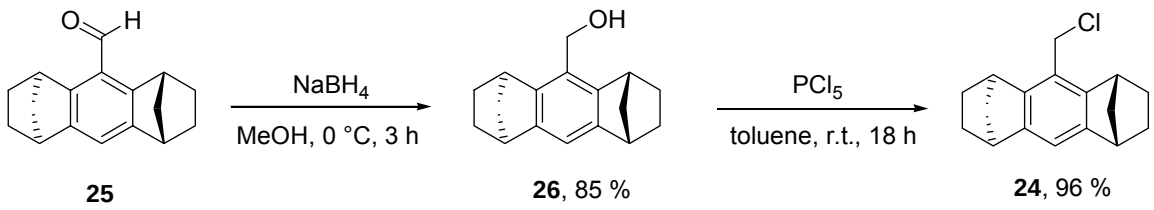
Quinidine derivative **22** was obtained in 61 % yield by cleavage of the aromatic ether function of quinidine **19** with BBr_3 (Scheme 3-25).



19 **22**, 61 %

Scheme 3-25 Synthesis of 6'-hydroxy-cinchonine **22**.

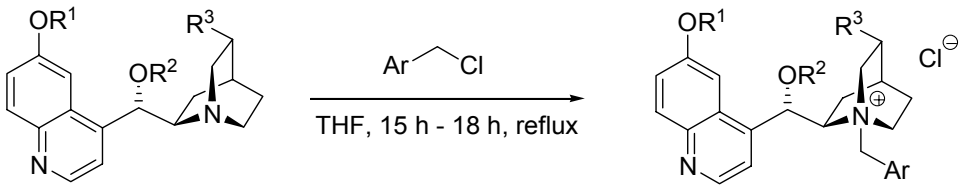
Furthermore, the benzyl chloride derivative 9-(chloromethyl)-(1,8-*R*;4,5-*S*)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene **24** was synthesised by reduction of the aldehyde **25** to the corresponding alcohol **26** in 85 % yield. Subsequent substitution of the hydroxy function led to the chloride **24** in 96 % yield (Scheme 3-26, p.34).



25 **26**, 85 % **24**, 96 %

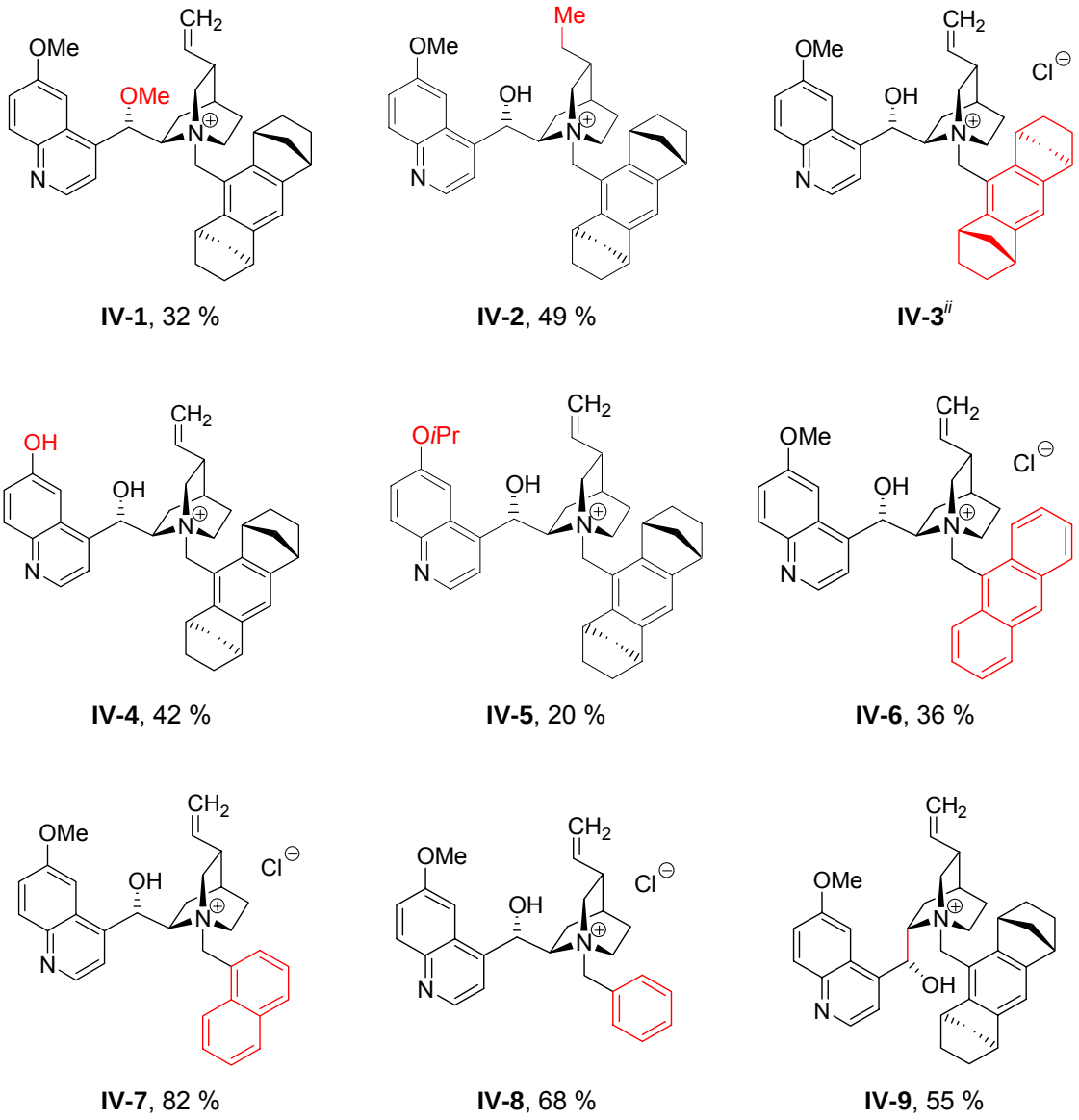
Scheme 3-26 Synthesis of the 9-(chloromethyl)-(1,8-*R*;4,5-*S*)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene **24**.

Accoding to the general procedure in Scheme 3-27, quaternisation of the quinidine derivatives with the corresponding benzyl chloride derivatives led to the quinidinium chlorides of substance library **IV** (Scheme 3-28).



Scheme 3-27 General synthetic procedure for the quaternisation of the quinidine derivatives.

The yields obtained in the synthesis of these quinidinium chlorides are summarised in Scheme 3-28, p.35.

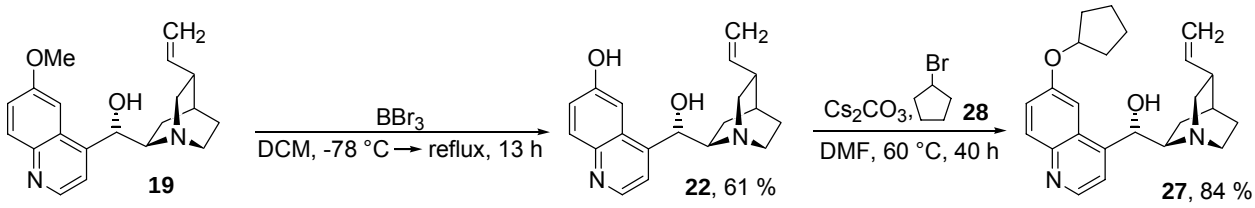


Scheme 3-28 Compounds contained in the cinchona alkaloid-based library **IV**.

Substance Library V. For the preparation of the cinchona-alkaloid based library **V**, quinidine **19** was first transformed to the derivative **27**.

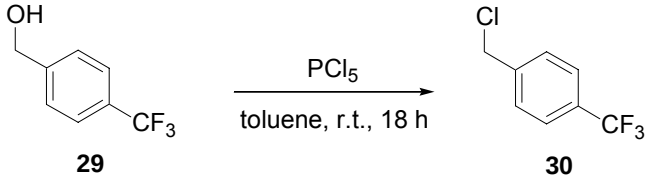
Cleavage of the aromatic ether function of quinidine **19** with BBr_3 led to the 6'-hydroxy-quinidine **22** in a yield of 61 %. Subsequent etherification with cyclopentylbromide **28** resulted in the desired quinidine derivative **27** in 84 % yield (Scheme 3-29, p.36).

[ii] Test substrate **IV-3** was synthesised by *Dr. M. Guixà.*



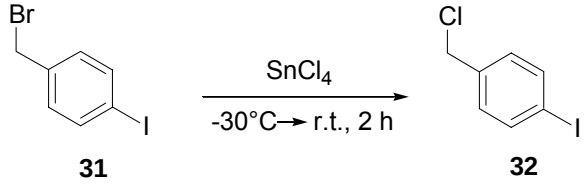
Scheme 3-29 Synthesis of cyclopentyloxy cinchonine **27**.

For the synthesis of the benzyl chloride derivatives, 4-(trifluoromethyl)benzyl alcohol **29** was converted to the corresponding chloride **30** with PCl_5. The crude product was used for the synthesis of **V-3** without further purification (Scheme 3-30).



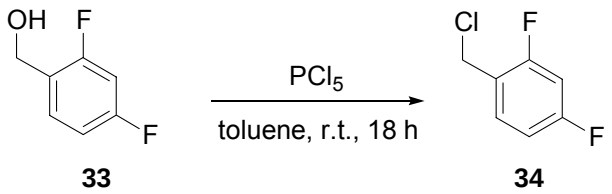
Scheme 3-30 Synthesis of 4-(trifluoromethyl)benzyl chloride **30**.

Reaction of 4-iodobenzyl bromide **31** with tin-(IV)-chloride resulted in the formation of the corresponding 4-iodobenzyl chloride **32** (Scheme 3-31). Again, the crude product could be used directly in the next step without further purification.



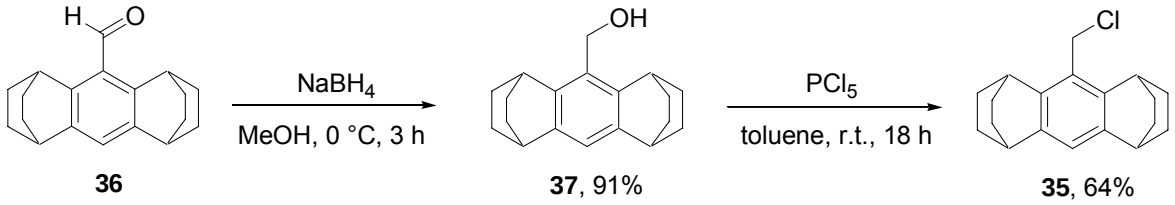
Scheme 3-31 Synthesis of 4-iodobenzyl chloride **32**.

Conversion of 2,4-difluorobenzylalcohol **33** with PCl_5 yielded the desired chloride **34**. This was directly used in the synthesis of test substrate **V-7**, without further purification (Scheme 3-32).



Scheme 3-32 Synthesis of 2,4-difluorobenzyl chloride **34**.

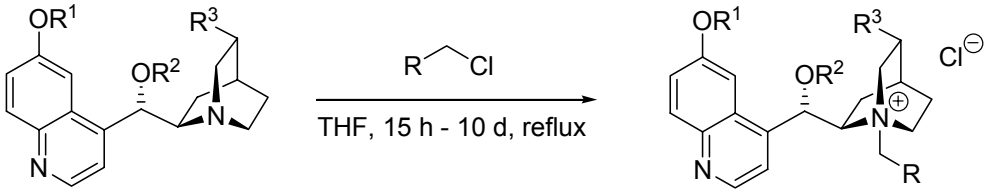
For the synthesis of the benzylchloride **35**, the corresponding aldehyde **36** was first reduced to the alcohol derivative **37**. Subsequent substitution of the hydroxy function with PCl_5 led to the benzylchloride **35** (Scheme 3-33).



36 **37**, 91% **35**, 64%

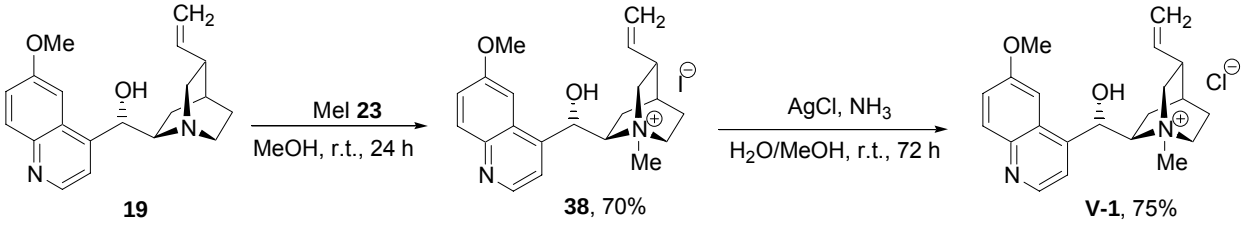
Scheme 3-33 Synthesis of 9-(chloromethyl)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-diethanoantracene **35**.

According to the general procedure in Scheme 3-34, quaternisation of the quinidine derivatives with the corresponding chlorides led to the quinidinium chlorides of substance library **V** (Scheme 3-36).



Scheme 3-34 General synthetic procedure for the quaternisation of the quinidine derivatives.

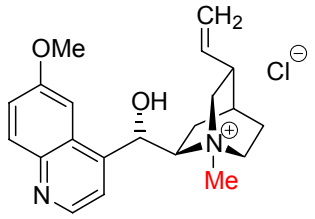
In contrast to the general procedure, for the synthesis of test substrate **V-1**, quinidine **19** was first converted to the quaternary ammonium iodide **38** with methyl iodide in 70 % yield. The desired chloride **V-1** was obtained by anion exchange with silver chloride in 75 % yield (Scheme 3-35).



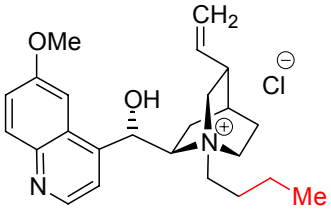
19 **38**, 70% **V-1**, 75%

Scheme 3-35 Synthesis of 1*N*-(methyl)quinidinium chloride **V-1**.

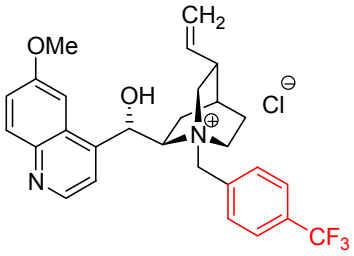
The synthetic yields of the substances of library **V** are shown in Scheme 3-36.



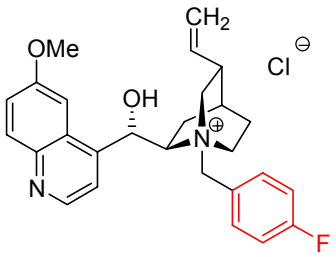
V-1, 75 %



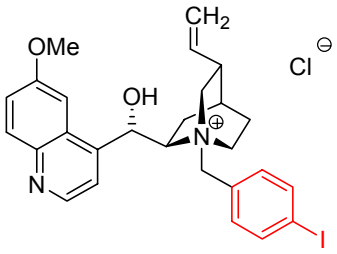
V-2, 32 %



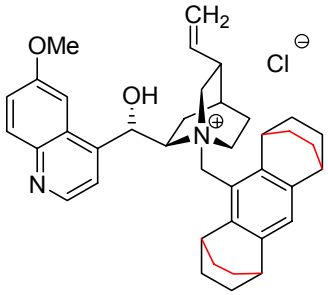
V-3, 49 %



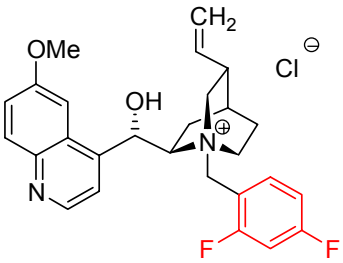
V-4, 38 %



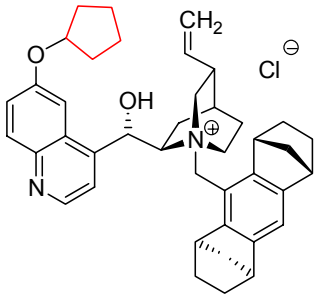
V-5, 34 %



V-6, 17 %



V-7, 12 %



V-8, 38 %

Scheme 3-36 Compounds contained in the cinchona alkaloid-based library **V**.

3.4 Experimental Part

3.4.1 Physiological ES cell screenings

The physiological tests were performed using a transgenic murine ES cell lineage expressing enhanced green fluorescent protein (EGFP) under the control of α-myosine heavy chain promoter (pα-MHC).

Culture of ES cells on mouse embryonic feeder cells. Transgenic mouse ES cells (αMHC Cl23) were cultured on 10 cm Petri dishes in Dulbecco's-modified Eagle's medium (DMEM) supplemented with 15 % fetal calf serum (FCS) and leukaemia inhibitory factor (LIF) on a layer of feeder cells (irradiated mouse embryonic fibroblasts). Cells were incubated at 37 °C, 7 % CO_2 and 95 % humidity. Cells were split every second day by trypsinising them to single cell suspension and seeding on a fresh dish coated with feeder cells.

ES cell aggregation. ES cells from one or more Petri dish were trypsinised, to obtain a single cell suspension, and collected by centrifugation. Cells were resuspended to a density of approximately $2 \cdot 10^6$ cells/ml in Iscove's modified Dulbecco's medium (IMDM) supplemented with 20 % FCS. 4 ml per 6 cm Petri dish of this suspension were incubated on a rocking table at 50 rpm, 37 °C, 5 % CO_2 and 95 % humidity for 6 h. After this time the suspension was diluted 1:20 in several T25 tissue culture flasks and incubated for additional 18 h. Under these conditions ES cell aggregates (embryoid bodies) formed, typically around 500 per ml of cell suspension.

ES cell differentiation. The embryoid bodies (EBs) were cultured on 6-well-plates in IMDM supplemented with 20 % FCS. The test substrates were dissolved in DMSO (10^{-2} M) and a dilution series was obtained by dilution with IMDM, 20 % FCS (10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M). The EBs were treated with the test substrates and the corresponding dilutions and afterwards they were incubated at 37 °C, 5 % CO_2 and 95 % humidity for 14 d. After 7 d fresh IMDM, 20 % FCS and the test substrate were added. After 14 d the expressed EGFP was quantified. Therefore the EBs per well were counted and subsequently lysed with Triton X. The lysate was transferred to 96-well plates and the emitted fluorescence per well was determined.

The physiological ES cell screenings were performed according to the *Cell Culture Quality Assurance Guide* and the *Standard Operating Protocols* (SOP 3-01: Culture of

mES cells; SOP 3-02: Production of EBs by mass culture, SOP 4-01: R.E.Tox Test for embryotoxicity) developed by Axiogenesis AG, Köln, Department for cell culture, toxicology and assay developments. These procedures cannot be explained in detail, as they are subject to privacy regulations.

3.4.2 Synthesis of the Test Substrates

General Methods. Flash chromatography was performed on silica gel (Macherey-Nagel MN-Kieselgel 60, 230-240 mesh). TLC was performed on aluminium backed silica plates (Macherey-Nagel, Polygram© SIL G/UV_{254}) which were developed by using UV fluorescence. Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded at 300 MHz on Bruker AC 300 and DPX 300 instruments, respectively; ^{13}C-NMR spectra were recorded at 75.5 MHz on a Bruker DPX 300 instrument. Chemical shifts (δ) are given in parts per million (ppm) referenced to TMS. For the fine-structure interpretation the abbreviations of the signals are the following: s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution mass spectra (HR ESI-MS) were recorded on a Finnigan MAT 900 ST spectrometer. Infrared spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR spectrometer using the ATR technique and on a Perkin Elmer 1600 Series FT-IR spectrometer. All commerically available chemicals were used without further purification. Compounds **25** and **36** were kindly provided by Sarwar Aziz. Anhydrous solvents were distilled from appropriate drying agents prior to use.

All experiments are characterised by a number within the bracket: [XX-SEE-XXX]. The roman number in front of the three-letter-code indicates the volume number of the laboratory notebook, whereas the number after the three-letter-code indicates the experiment number in the corresponding notebook volume.

3.4.3 Synthesis of (1S,2R,4S,5R)-2,5-Dihydroxybicyclo[2.2.1]heptane (13)

[II-SEE-123]

12

13

12	1. $HSiCl_3$, $[Pd(C_3H_5)Cl]_2$, (*S*)-MOP -3 °C, 3 d 2. MeOH, Et_3N, Et_2O, r.t, 16 h 3. $H_2O_2{\cdot}H_2NCONH_2$, KHF_2, THF/MeOH 60 °C, 16 h ⟶	**13**, 36 %

A solution of $[Pd(C_3H_5)Cl]_2$ (14.6 mg, 40 µmol, 0.05 mol%) and (*S*)-MOP (78.7 mg, 168 µmol, 0.20 mol%) in dry benzene (2 ml) was placed into a double-jacketed 50 ml Schlenk flask under argon. $HSiCl_3$ (19.9 ml, 197 mmol, 2.40 equiv), distilled from quinoline, and quinoline (236 µl, 2.00 mmol, 2.43 mol%) were added, and the solution was cooled to -3 °C. Norbornadiene **12** (8.34 ml, 82.0 mmol, 1.00 equiv), passed through neutral aluminia and subsequently distilled, was added slowly with magnetic stirring. The reaction mixture was stirred at -3 °C for 3 d, until it turned into a pale yellowish solid. The solvent and excess silane were removed in vacuo at r.t.. The residue was dissolved in dry Et_2O (50 ml) under argon and cooled to 0 °C. A mixture of dry MeOH (59.9 ml, 1.48 mol, 18.0 equiv), dry Et_3N (80.0 ml, 574 mmol, 7.00 equiv), and dry Et_2O (50 ml) was added dropwise. The solution was stirred at r.t. overnight. The precipitated salts were filtered off and washed with small quantities of Et_2O. The combined filtrates were concentrated in vacuo. To the residue was added KHF_2 (32.0 mg, 410 mmol, 5.00 equiv), THF (80 ml), MeOH (80 ml) and $H_2O_2{\cdot}$urea (57.8 g, 615 mmol, 7.50 equiv). The resulting white suspension was stirred overnight at 60 °C. After addition of a catalytic amount of MnO_2, stirring was continued at r.t. for 4 h. The solids were filtered off, and the filter cake was washed with MeOH. The combined filtrates were concentrated in vacuo. The residue was dissolved in H_2O (100 ml) and extracted with a $CHCl_3$/*i*PrOH mixture (5 x 100 ml, 3:1). The combined organic phases were dried over $MgSO_4$ and evaporated. The remaining solid was recrystallised from $CHCl_3$/*n*-hexane to give 3.82 g (30.0 mmol, 36 %) of the diol **13** as thin colourless crystals.

$C_7H_{12}O_2$ (128.17 g/mol)

m.p. 157 °C [m.p. ref.[54]: 158 °C]

^{1}H NMR (300 MHz, CH_3OH-d_4): δ [ppm] = 4.69 (s; 2H), 3.64-3.58 (m; 2H), 2.11-2.10 (m; 2H), 1.55 (s; 2H), 1.50-1.43 (m; 2H), 1.26- 1.19 (m; 2H).

^{13}C NMR (75.5 MHz, CH_3OH-d_4): δ [ppm] = 72.9, 42.4, 36.0, 29.6.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3245, 2958, 2886, 1666, 1439, 1346, 1286, 1184, 1089, 1039, 991, 930, 877, 813, 781.

GC-MS (HP-5; 100 °C (5 min), 20 °C/min, 200 °C (15 min); He; 1.00 ml/min) τ_R (min) = 6.64 (**13**): m/z = 128 [M^+], 110, 95, 81, 66, 55.

The spectroscopical data are in agreement with the literature.[54]

3.4.4 Synthesis of (1S,4S)-Bicyclo[2.2.1]heptane-2,5-dione (14)

[II-SEE-124]

13

14

13	PCC, MS 3 Å CH_2Cl_2, r.t., 14 h	**14**, 61 %

Diol **13** (2.05 g, 16.0 mmol, 1.00 equiv) and powdered 3 Å molecular sieves (10 g) were suspended in CH_2Cl_2 (250 ml). PCC (17.2 g, 80.0 mmol, 5.00 equiv) was added slowly and the resulting mixture was stirred at r.t. overnight. Et_2O (100 ml) was added with vigorous stirring, and stirring was continued for 30 min. The mixture was then allowed to stand for 2 h. The liquid was filtered through Florisil (20 g, 80-150 µm). The black residue was extracted with CH_2Cl_2/Et_2O (4 x 50 ml, 1:1) in an ultrasonic bath. The combined extracts were also filtered through the Florisil pad. Evaporation of the combined solutions yielded 1.21 g (9.75 mmol, 61 %) of the diketone **14** as a white solid.

$C_7H_8O_2$ (124.14 g/mol)

m.p. 115-126 °C [m.p. ref.[54]: 115-133 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 2.98 (s; 2H), 2.41-2.33 (m; 2H), 2.16-2.09 (m; 4H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 212.4, 48.7, 39.0, 36.5.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2954, 1732, 1404, 1230, 1186, 1123, 1089, 1053, 999, 960, 915, 893, 794, 731, 709.

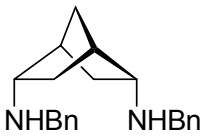
GC-MS (HP-5, 25 m; 100 °C (5 min), 20 °C/min, 200 °C (15 min); He; 1.00 ml/min) τ_R (min) = 5.55 (**14**): m/z = 124 [M^+], 95, 82, 67, 55.

The spectroscopical data are in agreement with the literature.[54]

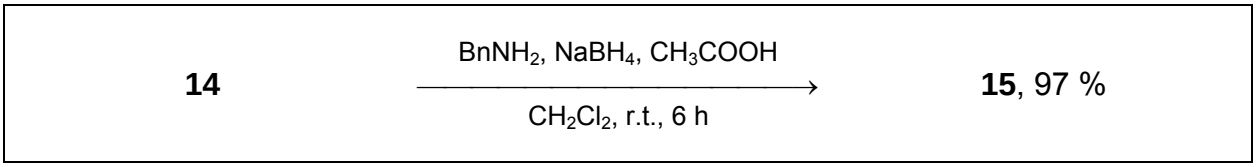
3.4.5 Synthesis of (1S,2S,4S,5S)-2,5-Dibenzylaminobicyclo[2.2.1]heptane (15)

[II-SEE-125]

14



15



Glacial acetic acid (4.38 ml, 76.0 mmol, 8.00 equiv) was added dropwise to a suspension of $NaBH_4$ (900 mg, 23.7 mmol, 2.50 equiv) in dry CH_2Cl_2 (80 ml) and the resulting mixture was heated to reflux for 30 min. A mixture of diketone **14** (1.18 g, 9.51 mmol, 1.00 equiv) in dry CH_2Cl_2 (20 ml) and freshly distilled $BnNH_2$ (2.59 ml, 23.7 mmol, 2.50 equiv), was added dropwise at r.t.. After being stirred for 6 h, the reaction mixture was quenched by the addition of 5 % aq. NaOH (18.9 ml, 23.7 mmol, 2.50 equiv). The mixture was extracted with 3 x 50 ml of 2 M HCl and the combined aqueous phases were basified by the addition of solid NaOH to pH 9. The resulting suspension was extracted with Et_2O (3 x 50 ml) and the combined organic phases were dried over $MgSO_4$. After concentration in vacuo a colourless oil remained, which soli-

dified slowly. This material was purified by Kugelrohr distillation at 150 °C, 3 x 10^{-3} mbar, to yield 2.82 g (9.22 mmol, 97 %) of (1*S*,2*S*,4*S*,5*S*)-2,5-dibenzylaminobicyclo-[2.2.1]heptane **15** as a white solid.

$C_{21}H_{26}N_2$ (306.44 g/mol)

m.p. 47 °C [m.p. ref.[54]: 48-49 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 7.36-7.27 (m; 10H), 3.71 (dd; *J* = 12.92 Hz; *J* = 9.0 Hz, 4H), 3.19-3.14 (m; 2H), 2.31 (s; 2H), 1.80-1.71 (m; 2H), 1.66 (s; 2H), 1.51 (s; 2H), 1.43 (dd; *J* = 12.7 Hz, *J* = 3.8 Hz, 2H).

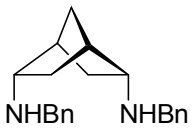
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 140.6, 128.2, 128.1, 126.7, 58.4, 52.5, 39.7, 38.1, 29.2.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3312, 3023, 2941, 2866, 2797, 1947, 1806, 1742, 1668, 1601, 1493, 1451, 1342, 1201, 1132, 1095, 1027, 977, 906, 730, 693.

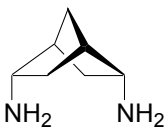
The spectroscopical data are in agreement with the literature.[54]

3.4.6 Synthesis of (1S,2S,4S,5S)-2,5-Diaminobicyclo[2.2.1]heptane (9)

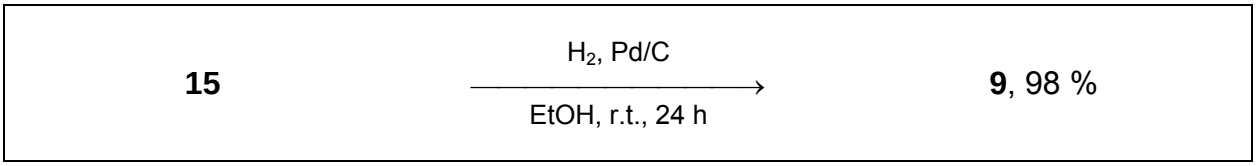
[II-SEE-126]



15



9



A suspension of the bis-benzylamine **15** (2.50 g, 8.15 mmol, 1.00 equiv) and $Pd(OH)_2$ (15-20 % on activated charcoal with 50 % H_2O, 2.50 g) in EtOH (70 ml) was stirred under H_2 -atmospheric pressure (balloon). After stirring for 24 h, the catalyst was filtered off and the solution was concentrated in vacuo to afford 1.01 g (8.00 mmol, 98 %) DIANANE **9** as colourless oil.

$C_7H_{14}N_2$ (126.20 g/mol)

^{1}H NMR (300 MHz, MeOH-d_4): δ [ppm] = 3.28-3.22 (m; 2H), 2.07 (t; J = 4.2 Hz, 2H), 1.75-1.64 (m; 2H), 1.50 (s; 2H), 1.26-1.20 (m; 2H).
The NH_2-protons could not be detected.

^{13}C NMR (75.5 MHz, MeOH-d_4): δ [ppm] = 54.1, 44.9, 39.6, 30.2.

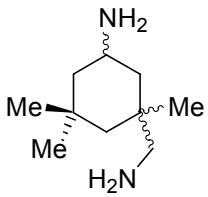
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3268, 2951, 2873, 1629, 1558, 1464, 1379, 1326, 820.

GC-MS (HP-5, 25 m; 100 °C (5 min), 20 °C/min, 280 °C (10 min); He; 1.00 ml/min)
τ_R (min) = 6.2 (**9**): m/z = 126 [M^+], 109, 94, 82, 68, 56.

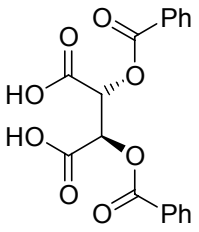
The spectroscopical data are in agreement with the literature.[54]

3.4.7 Synthesis of (2R,3R)-2,3-Bis(benzoyloxy)butanedioic acid (1S,5R)-(5-amino-1,3,3-trimethylcyclohexyl)-methaneamine salt (1:1) (17)

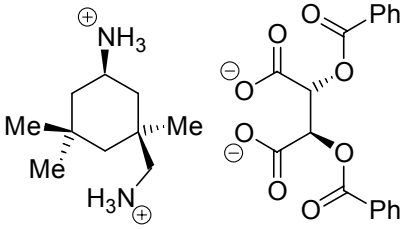
[II-SEE-127]



mix-**10** [iii]



16



17

mix-**10** [iii] + **16**	$\xrightarrow[H_2O,\ r.t.,\ 5\ min]{}$	**17**, 25 %

3-Aminomethyl-3,5,5-trimethylcyclohexylamine *mix*-**10** (135 ml, 124 g, 732 mmol, 2.50 equiv) was added at r.t. to (2*R*,3*R*)-dibenzoyl tartaric acid **16** (105 g, 293 mmol, 1.00 equiv) suspended in distilled water (1.35 L) with vigorous stirring. During the exothermic reaction the solution cleared, and the precipitation of the product started after

[iii] *rac*-**10** *cis* + *rac*-**10** *trans ca.* 3 : 1.

about 5 min. The reaction mixture was cooled to 0 °C and left at this temperature for 1.5 h. The solid was filtered off, washed with *i*PrOH (3 x 140 ml), and dried under reduced pressure over P_2O_5. Recrystallisation from *i*PrOH/water (650 ml, 2:1) yielded 39.0 g (73.7 mmol, 25 % based on the amount of *mix*-**10** used) of (2*R*,3*R*)-2,3-bis(benzoyloxy)butanedioic acid (1*S*,5*R*)-(5-amino-1,3,3-trimethylcyclohexyl)-methylamine salt (1:1) **17** as colourless crystals.

$C_{28}H_{36}N_2O_8$ (528.59 g/mol)

m.p. 205 °C [m.p. ref.[55]: 205 °C]

^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.99 (d; J = 7.2 Hz, 4H), 7.64-7.59 (m; 2H), 7.53-7.48 (m; 4H), 5.55 (s; 2H), 3.16-3.09 (m; 1H), 2.31 (s; 2H), 1.61-1.54 (m; 2H), 1.09-1.00 (m; 1H), 0.84 (s; 3H), 0.78 (s; 3H), 0.71 (s; 3H), 0.96-0.68 (m; 3H).
The NH_3^+-protons could not be detected.

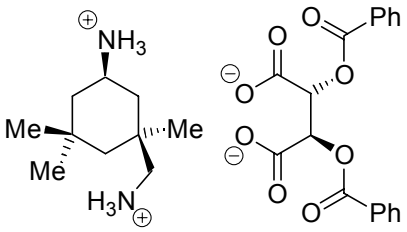
^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 169.6, 165.1, 132.7, 130.8, 129.2, 128.3, 75.5, 52.6, 45.5, 43.7, 42.1, 40.2, 34.2, 34.1, 30.8, 26.8, 22.1.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3428, 2954, 2713, 1723, 1607, 1512, 1407, 1333, 1280, 1122, 1025, 736, 716.

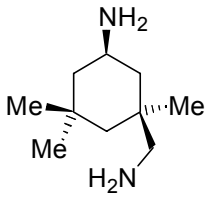
The spectroscopical data are in agreement with the literature.[55]

3.4.8 Synthesis of (1R,3S)-3-Aminomethyl-3,5,5-trimethylcyclohexylamine (10)

[II-SEE-128]



17



10

17	NaOH H_2O, r.t., 2 min	**10**, 89 %

(2*R*,3*R*)-2,3-Bis(benzoyloxy)butanedioic acid (1*S*,5*R*)-(5-amino-1,3,3-trimethylcyclohexyl)-methanamine salt (1:1) **17** (1.52 g, 2.88 mmol, 1.00 equiv) was dissolved in 5 M aq. NaOH (72 ml). The clear solution was extracted with CH_2Cl_2 (4 x 20 ml), the combined organic phases were dried over Na_2SO_4, and the main part of the solvent was evaporated. Vacuum destillation gave 437 mg (2.57 mmol, 89 %) of (1*R*,3*S*)-3-(aminomethyl)-3,5,5-trimethylcyclohexylamine **10** as a clear liquid (to be stored under argon).

$C_{10}H_{22}N_2$ (170.3 g/mol)

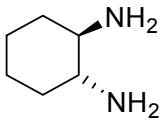
^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ = 2.90-2.80 (m; 1H), 2.22 (s; 2H), 1.50-1.44 (m; 1H), 1.40-1.33 (m; 1H), 1.07-1.03 (m; 1H), 0.97 (s; 4H), 0.89 (s; 3H), 0.86 (s; 3H), 0.79 (s; 3H), 0.83-0.59 (m; 3H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ = 57.5, 50.4, 47.1, 45.7, 43.9, 36.4, 35.2, 31.9, 27.9, 23.8.

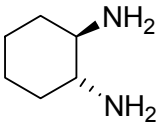
The spectroscopical data are in agreement with the literature.[55]

3.4.9 Synthesis of (1R,2R)-1,2-Diaminocyclohexane (11)

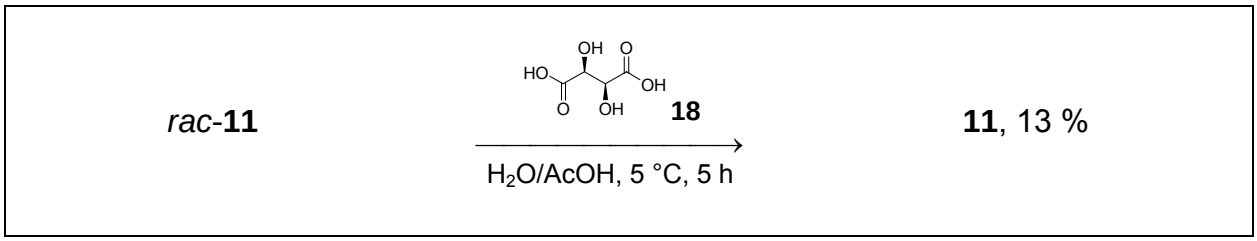
[III-SEE-233]



rac-**11**



11



A 2 L beaker is charged with 300 ml of distilled water. L-(+)-tartaric acid **18** (118 g, 786 mmol, 1.00 equiv) is added with stirring in one portion. The solution is stirred as 1,2-diaminocyclohexane *rac*-**10** (180 g, 1.57 mol, 2.00 equiv) and glacial acetic acid (82 ml) is added. An exothermic reaction was observed and the temperature rose up to 90 °C. The reaction mixture is allowed to cool to 5 °C, with stirring, over 4 h. The temperature is maintained at 5 °C for an additional hour and than the product is isolated by filtration. The crude product is washed with cold water (50 ml) followed by MeOH (4 x 50 ml). The filter cake is recrystallized from water (1.2 L). The solid is dissolved in CH_2Cl_2 (500 ml) and washed with 6 M aq. NaOH (3 x 100 ml) and brine (500 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure yielding 12.1 g (106 mmol, 13 %) (1*R*,2*R*)-1,2-diaminocyclohexane **10** as an off-white solid.

For the determination of the enantiomeric excess, a small amount of the product **10** was dissolved in 1 ml CH_2Cl_2, laced with one drop of trifluoroacetic anhydride, and analysed by chiral GC.

$C_6H_{14}N_2$ (114.19 g/mol)

m.p. 41 °C [m.p. ref.[56]: 42-43 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 2.09-2.07 (m; 2H), 1.70-1.66 (m; 2H), 1.52 (brs; 2H), 1.21 (s; 4H), 1.15-1.08 (m; 2H), 0.95-0.92 (m; 2H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 57.6, 35.4, 25.3.

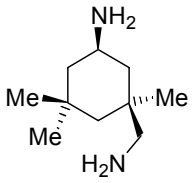
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3275, 2922, 2855, 1581, 1447, 1377, 1288, 1163, 1081, 959, 914, 843.

GC (CP-Chirasil-DexCB, 1.1 ml/min N_2; 60 °C, 15 °C/min 150 °C (30 min)) τ_R (min) = 14.95 (*ent*-**10**), 15.57 (**10**).

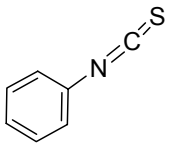
The spectroscopical data are in agreement with the literature.[7]

3.4.10 Synthesis of N-Phenyl-N'-[(1R,3S)-3-({[(phenyl)amino]-thioxomethyl}amino)-methyl-3,5,5-trimethylcyclohexyl]thiourea (III-2)

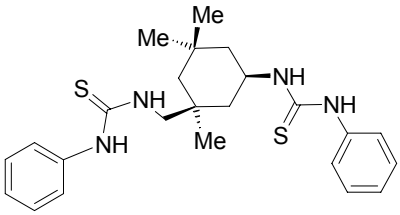
[II-SEE-158]



10



39



III-2

10 + **39**	THF, r.t., 72 h	**III-2**, 67 %

Phenyl isothiocyanate **39** (1.04 ml, 1.18 g, 8.75 mmol, 2.50 equiv) was added to a solution of (1*R*,3*S*)-3-(aminomethyl)-3,5,5-trimethylcyclohexylamine **10** (648 µl, 596 mg, 3.50 mmol, 1.00 equiv) in dry THF (5 ml). The resulting mixture was stirred at r.t. under argon for 72 h. The solvent was removed under reduced pressure. Purification of the residue by flash chromatography (*c*-hexane/EtOAc 2:1) afforded 1.04 g (2.36 mmol, 67 %) of the product **III-2** as colourless solid.

$C_{24}H_{32}N_4S_2$ (440.67 g/mol)

m.p. 182 °C [m.p. ref.[51]: 182 °C]

R_f 0.32 (*c*-hexane/EtOAc 2:1)

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.51 (s; 1H), 8.40 (s; 1H), 7.36-7.12 (m; 10 H), 6.11 (s; 1H), 5.78 (s; 1H), 4.56-4.53 (m; 1H), 3.44-3.30 (m; 2H), 1.87-1.74 (m; 2H), 1.14-0.77 (m; 4H), 1.01 (s; 6H), 0.83 (s; 3H).

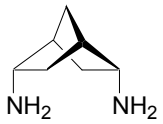
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 180.9, 179.1, 136.2, 136.1, 130.1, 127.3, 127.0, 125.2, 124.9, 58.9, 48.9, 47.7, 45.3, 41.2, 36.7, 34.9, 32.0, 27.6, 23.4.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3383, 3207, 2953, 2217, 1594, 1519, 1448, 1312, 1240, 1178, 1071, 1027, 1001, 905, 825, 724.

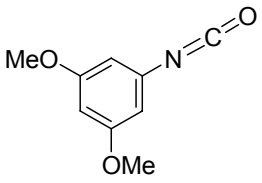
The spectroscopical data are in agreement with the literature.[51]

3.4.11 Synthesis of 1,1'-[(1S,2S,4S,5S)-Bicyclo[2.2.1]heptane-2,5-diyl]bis{3-[3,5-dimethoxyphenyl]urea} (III-3)

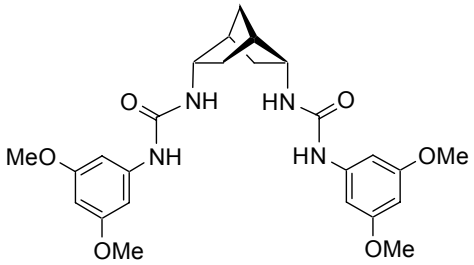
[II-SEE-140]



9



40



III-3

9 + **40**	THF, r.t., 72 h	**III-3**, 97 %

3,5-Dimethoxyphenyl isocyanate **40** (312 mg, 1.74 mmol, 2.20 equiv) was added to a solution of (1*S*,2*S*,4*S*,5*S*)-2,5-diamino-bicyclo[2.2.1]heptane **9** (100 mg, 792 µmol, 1.00 equiv) in dry THF (2 ml) and the resulting mixture was stirred at r.t. under argon for 40 h. The solvent was removed under reduced pressure. Purification of the residue by flash chromatography ($CHCl_3$/MeOH 5:1) afforded 372 mg (767 µmol, 97 %) of the product **III-3** as colourless solid.

$C_{25}H_{32}N_4O_6$ (484.54 g/mol)

R_f 0.83 ($CHCl_3$ /MeOH 5:1)

m.p. 123 °C

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 6.47 (s; 4H), 6.00 (s; 2H), 3.89-3.83 (m; 2H), 3.59 (s; 12H), 2.25 (s; 2H), 1.89-1.80 (m; 2H), 1.44 (s; 2H), 1.25-1.18 (m; 2H).
NH-protons could not be detected.

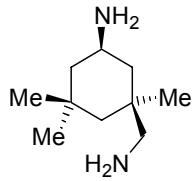
^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 161.1, 156.7, 141.1, 96.8, 94.1, 54.3, 51.2, 40.9, 36.9, 28.8.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3383, 2949, 2473, 1602, 1539, 1456, 1273, 1203, 1153, 1064, 961, 831, 758, 682.

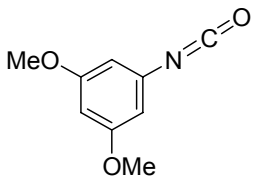
HR ESI-MS (m/z): exact mass $[M+Na]^+$: 507.2219 ; found $[M+Na]^+$: 507.222.

3.4.12 Synthesis of N-[3,5-Di(methoxy)phenyl]-N'-[(1R,3S)-3-{[({[3,5-bis(methoxy)-phenyl]amino}oxomethyl)amino]methyl}-3,5,5-trimethylcyclohexyl]urea (III-4)

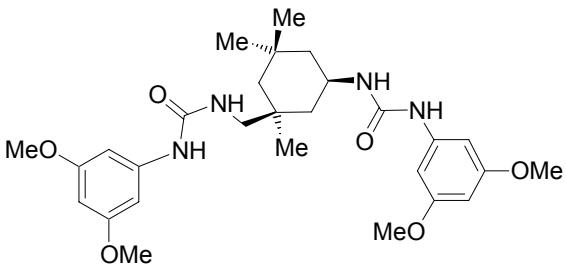
[II-SEE-119]



10



40



III-4

10 + **40**	THF, r.t., 1 h	**III-4**, 90 %

3,5-Dimethoxyphenyl isocyanate **40** (871 mg, 4.86 mmol, 2.00 equiv) was added to a solution of (1*R*,3*S*)-3-(aminomethyl)-3,5,5-trimethylcyclohexylamine **10** (450 µl, 414 mg, 2.43 mmol, 1.00 equiv) in dry THF (0.5 ml) and the resulting mixture was stirred at r.t. under argon for 1 h. The solvent was removed under reduced pressure. The residue was dried in vacuo and 1.15 g (2.17 mmol, 90 %) of the product **III-4** were obtained as colourless solid.

$C_{28}H_{40}N_4O_6$ (528.64 g/mol)

m.p. 110 °C

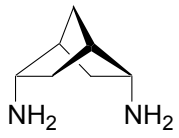
^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.41 (s; 1H), 8.30 (s; 1H), 6.62 (s; 4H), 6.22-6.20 (m; 1H), 6.06 (s; 2H), 5.97-5.94 (m; 1H), 3.81-3.76 (m; 1H), 3.69 (s; 12H), 2.95-2.78 (m; 2H), 1.63-1.60 (m; 2H), 1.19-1.15 (m; 1H), 1.05-0.87 (m; 3H), 1.04 (s; 3H), 1.01 (s; 3H), 0.92 (s; 3H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 160.4, 155.2, 154.2, 142.1, 95.6, 93.9, 54.8, 52.6, 46.8, 46.0, 42.3, 41.7, 36.0, 34.8, 31.5, 27.4, 23.1.

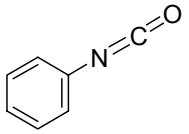
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3339, 2948, 1651, 1607, 1553, 1479, 1455, 1421, 1301, 1256, 1205, 1150, 1174, 1063, 833.

3.4.13 Synthesis of 1,1'-[(1S,2S,4S,5S)-Bicyclo[2.2.1]heptane-2,5-diyl]bisphenyl-urea (III-5)

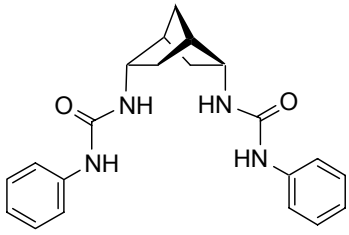
[II-SEE-112]



9



41



III-5

9 + **41**	THF, r.t., 72 h	**III-5**, 95 %

Phenyl isocyanate **41** (189 µl, 208 mg, 1.74 mmol, 2.20 equiv) was added to a solution of (1*S*,2*S*,4*S*,5*S*)-2,5-diamino-bicyclo[2.2.1]heptane **9** (100 mg, 792 µmol, 1.00 equiv) in dry THF (2 ml) and the resulting mixture was stirred at r.t. under argon for 72 h. The solvent was removed under reduced pressure. Purification of the residue by flash chromatography ($CHCl_3$/MeOH 7:1) afforded 274 mg (750 µmol, 95 %) of the product **III-5** as colourless solid.

$C_{21}H_{24}N_4O_2$ (364.44 g/mol)

R_f 0.24 ($CHCl_3$/MeOH 7:1)

m.p. 164 °C

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.30 (s; 2H), 7.22-7.11 (m; 8H), 6.93-6.89 (m; 2H), 6.17 (s; 2H), 3.98 (s; 2H), 2.32 (s; 2H), 1.86 (brs; 2H), 1.42 (s; 2H), 1.32-1.28 (m; 2H).

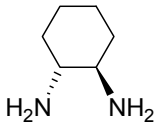
^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 156.7, 138.7, 128.9, 123.1, 119.8, 51.5, 41.0, 37.5, 29.4.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3323, 2951, 2875, 2242, 1642, 1594, 1538, 1495, 1438, 1310, 1276, 1226, 1173, 1119, 1076, 1028, 1002, 906, 861, 725, 689.

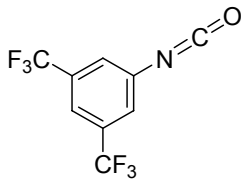
HR ESI-MS (m/z): exact mass $[M+Na]^+$: 387.1797 ; found $[M+Na]^+$: 387.180.

3.4.14 Synthesis of 1-[3,5-Bis(trifluoromethyl)phenyl]-3-{(1R,2R)-2-{3-[3,5-bis(trifluoromethyl)phenyl]ureido}cyclohexyl}urea (III-6)

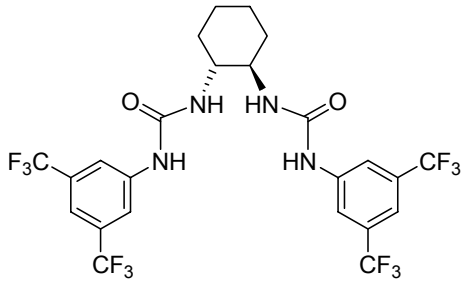
[II-SEE-114]



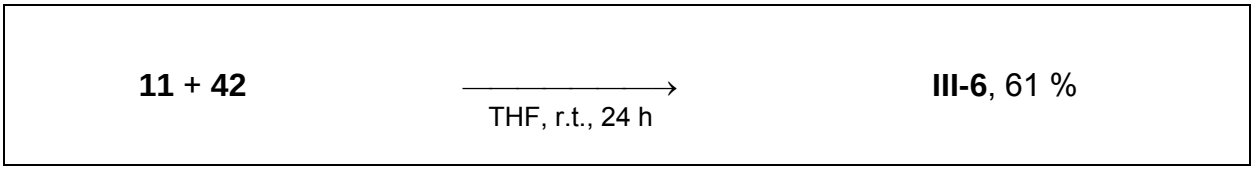
11



42



III-6



3,5-Bis(trifluoromethyl)phenyl isocyanate **42** (333 µl, 492 mg, 1.93 mmol, 2.20 equiv) was added to a solution of (1*R*,2*R*)-1,2-diaminocyclohexane **11** (100 mg, 876 µmol, 1.00 equiv) in dry THF (2 ml), and the resulting mixture was stirred at r.t. under argon for 24 h. The solvent was removed under reduced pressure. Purification of the residue by flash chromatography (*c*-hexane/EtOAc 2:1) afforded 334 mg (535 µmol, 61 %) of the product **III-6** as colourless solid.

$C_{24}H_{20}F_{12}N_4O_2$ (624.42 g/mol)

R_f 0.23 (*c*-hexane/EtOAc 2:1)

m.p. 248 °C [m.p. ref.[8]: 249-252 °C]

^{1}H NMR (300 MHz, MeOH-d_4): δ [ppm] = 7.84 (s; 4H), 7.32 (s; 2H), 4.62 (s; 2H), 2.04-2.02 (m; 2H), 1.82 (s; 2H), 1.41-1.39 (m; 4H).
NH-protons could not be detected.

^{13}C NMR (75.5 MHz, MeOH-d_4): δ [ppm] = 156.1, 141.6, 131.5 (q; $^2J_{C\text{-}F}$ = 33.2 Hz), 123.2 (d; $^1J_{C\text{-}F}$ = 271.9 Hz), 117.3, 114.0, 54.3, 32.2, 24.7.

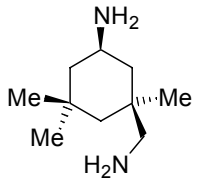
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3315, 2938, 2859, 2476, 1634, 1557, 1476, 1449, 1377, 1276, 1117, 1055, 1000, 973, 936, 881, 847, 728, 701, 681.

HR ESI-MS (m/z): exact mass $[M+Na]^+$: 647.1292; found $[M+Na]^+$: 647.129.

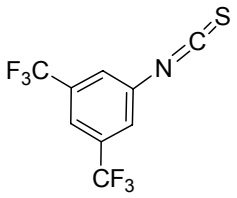
The spectroscopical data are in agreement with the literature.[8]

3.4.15 Synthesis of N-[3,5-Bis(trifluoromethyl)phenyl]-N'-[(1R,3S)-3-{[({[3,5-bis(trifluoromethyl)phenyl]amino}thioxomethyl)amino]methyl}-3,5,5-trimethylcyclohexyl]thiourea (III-7)

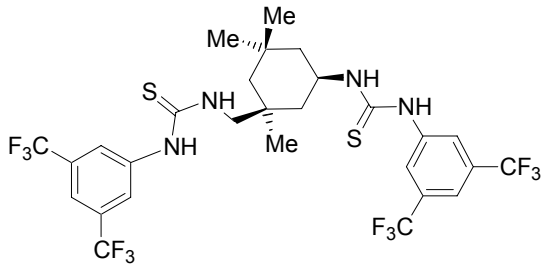
[II-SEE-157]



10



43



III-7

10 + **43**	THF, r.t., 4 d	**III-7**, 72 %

3,5-Bis(trifluoromethyl)phenyl isothiocyanate **43** (1.39 ml, 2.06 g, 7.60 mmol, 2.10 equiv) was added to a solution of (1*R*,3*S*)-3-(aminomethyl)-3,5,5-trimethylcyclohexylamine **10** (670 µl, 616 mg, 3.62 mmol, 1.00 equiv) in dry THF (5 ml) and the resulting mixture was stirred at r.t. under argon for 4 d. The solvent was removed under reduced pressure. Purification of the residue by flash chromatography (*c*-hexane/EtOAc 2:1) afforded 1.87 g (2.62 mmol, 72 %) of the product **III-7** as yellowish solid.

$C_{28}H_{28}F_{12}N_4S_2$ (712.66 g/mol)

R_f 0.35 (*c*-hexane/EtOAc 2:1)

m.p. 125 °C [m.p. ref.[51]: 120-122 °C]

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 10.09 (s; 1H), 9.86 (s; 1H), 8.31-8.11 (m; 6H), 7.68 (s; 2H), 4.60 (s; 1H), 3.45-3.40 (m; 2H), 1.83-1.71 (m; 2H), 1.33-1.29 (m; 1H), 1.18-1.06 (m; 3H), 1.14 (s; 3H), 1.09 (s; 3H), 0.96 (s; 3H).

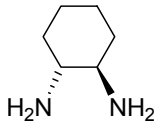
^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 181.7, 179.8, 142.4, 130.6 (q; $^2J_{C\text{-}F}$ = 32.6 Hz), 130.5 (q; $^2J_{C\text{-}F}$ = 32.6 Hz), 123.7 (q; $^2J_{C\text{-}F}$ = 272.5 Hz), 122.3, 121.9, 116.3, 116.2, 57.7, 47.8, 44.8, 44.3, 40.8, 36.9, 35.2, 32.1, 27.8, 23.6.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3234, 2964, 1621, 1539, 1466, 1380, 1303, 1274, 1176, 1134, 1107, 951, 889, 847, 702 681.

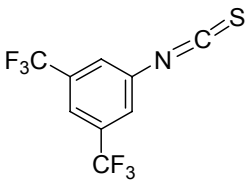
The spectroscopical data are in agreement with the literature.[8]

3.4.16 Synthesis of 1-[3,5-Bis(trifluoromethyl)phenyl]-3-{(1R,2R)-2-[3-[3,5-bis(trifluoromethyl)phenyl)thioureido]cyclohexyl}thiourea (III-8)

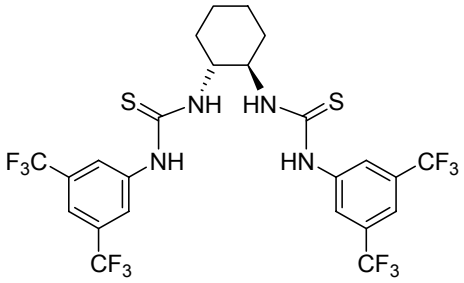
[II-SEE-115]



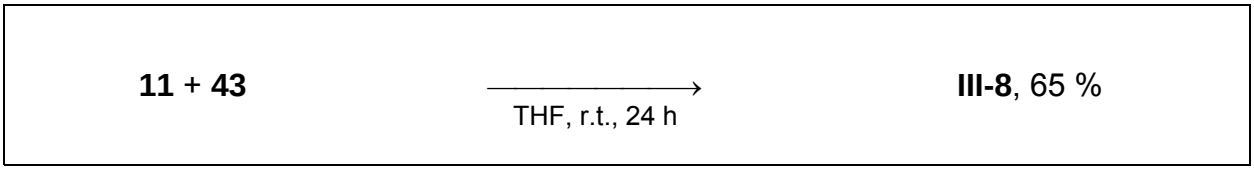
11



43



III-8



3,5-Bis-(trifluoromethyl)phenyl isocyanate **43** (352 µl, 523 mg, 1.93 mmol, 2.20 equiv) was added to a solution of (1*R*,2*R*)-1,2-diaminocyclohexane **11** (100 mg, 876 µmol, 1.00 equiv) in dry THF (2 ml), and the resulting mixture was stirred at r.t. under argon for 24 h. The solvent was removed under reduced pressure. Purification of the residue by flash chromatography (*c*-hexane/EtOAc 2:1) afforded 376 mg (573 µmol, 65 %) of the product **III-8** as colourless solid.

$C_{24}H_{20}F_{12}N_4S_2$ (656.55 g/mol)

R_f 0.40 (*c*-hexane/EtOAc 2:1)

m.p. 131-132 °C [m.p. ref.[57]: 132-133 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.41 (s; 2H), 7.84 (s; 4H), 7.68 (s; 2H), 7.19 (s; 2H), 4.38 (brs; 2H), 2.19 (brs; 2H), 1.80 (brs; 2H), 1.33 (brs; 4H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 180.4, 138.8, 132.6 (q; $^2J_{C\text{-}F}$ = 30.1 Hz), 123.9, 122.7 (d; $^1J_{C\text{-}F}$ = 274.1 Hz), 119.5, 59.3, 31.3, 24.4.

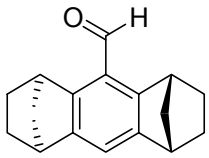
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3245, 3048, 2942, 2860, 1791, 1699, 1621, 1538, 1471, 1373, 1266, 1183, 999, 974, 956, 885, 847, 787, 731, 681.

HR ESI-MS (m/z): exact mass $[M+H]^+$: 657.1016; found $[M+H]^+$: 657.101.

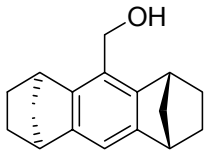
The spectroscopical data are in agreement with the literature.[57]

3.4.17 Synthesis of 9-(Hydroxymethyl)-(1,8-R;4,5-S)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene (26)

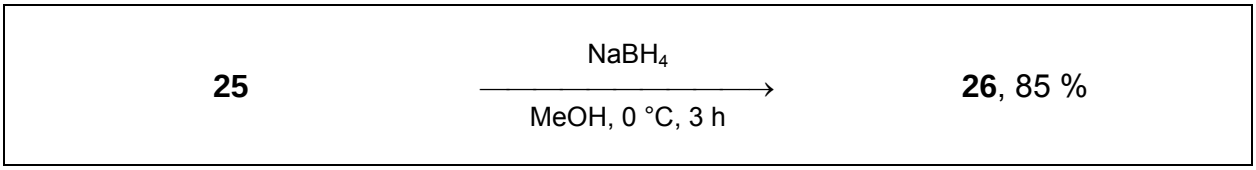
[II-SEE-163]



25



26



Under argon atmosphere, $NaBH_4$ (845 mg, 22.3 mmol, 5.20 equiv) was slowly added to a solution of aldehyde **25** (1.02 g, 4.30 mmol, 1.00 eq) in dry MeOH (40 ml) at 0 °C and the reaction mixture was stirred for 3 h. 4 M HCl (32 ml) was added and the precipitate was filtered off, washed with distilled water (3 x 10 ml) and dried under vacuo to give 877 mg (3.65 mmol, 85 %) of the alcohol **26** as colourless solid.

$C_{17}H_{20}O$ (240.34 g/mol)

m.p. 120-123 °C [m.p. ref.[58]: 120-122°C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 6.94 (s; 1H), 4.78-4.69 (m; 2H), 3.54 (s; 2H), 3.28 (s; 2H), 1.89-1.85 (m; 4H), 1.71-1.68 (m; 2H), 1.48-1.45 (m; 2H),

1.10-1.07 (m; 4H).
The OH-proton could not be detected.

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 145.7, 143.9, 125.1, 113.5, 60.4, 49.1, 43.9, 41.3, 27.2, 27.1.

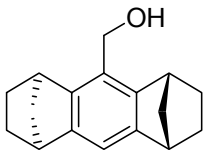
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3322, 2960, 2864, 1688, 1471, 1444, 1327, 1300, 1269, 1142, 1106, 1044, 1003, 972, 945, 867, 734.

GC MS (HP-5; 100 °C (5 min), 20 °C/min, 200 °C (15 min); He; 1.00 ml/min) τ_R (min) = 12.67 (**26**): m/z = 240 [M^+], 212, 194, 184, 166, 153, 141, 128, 115, 96, 89, 76, 63, 51.

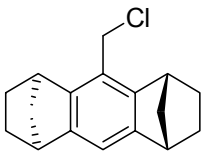
The spectroscopical data are in agreement with the literature.[58]

3.4.18 Synthesis of 9-(Chloromethyl)-(1,8-R;4,5-S)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene (24)

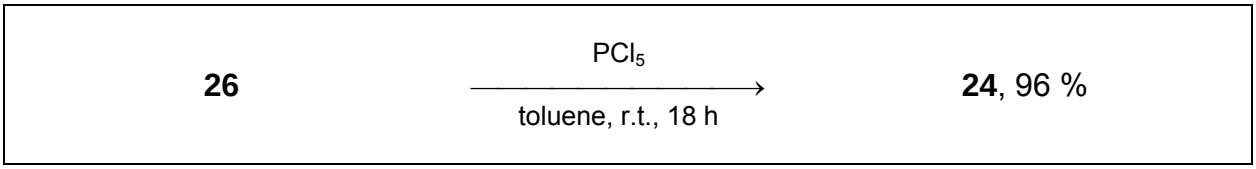
[II-SEE-165]



26



24



Under argon atmosphere, alcohol **26** (730 mg, 3.04 mmol, 1.00 equiv) was dissolved in dry toluene (30 ml). The mixture was cooled to 0 °C and PCl_5 (1.06 g, 5.16 mmol, 1.70 equiv) was added. After stirring at r.t. for 18 h, saturated aq. $NaHCO_3$ (30 ml) was added at 0 °C, and the mixture was stirred for 10 min. The phases were seperated and the aqueous layer was extracted with toluene (20 ml). The combined organic phases were washed with water (20 ml), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain 753 mg (2.91 mmol, 96 %) of the chloride **24** as colourless solid.

$C_{17}H_{19}Cl$ (258.79 g/mol)

m.p. 110-112 °C [m.p. ref.[58]: 111-112 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 6.96 (s; 1H), 4.77-4.65 (m; 2H), 3.54 (s; 2H), 3.30 (s; 2H), 1.95-1.83 (m; 4H), 1.74-1.72 (m; 2H), 1.51-1.48 (m; 2H), 1.20-1.08 (m; 4H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 145.8, 144.2, 122.4, 114.1, 49.1, 44.0, 41.3, 41.2, 27.2, 26.7.

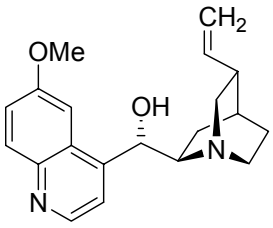
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2966, 2863, 1464, 1443, 1329, 1302, 1290, 1271, 1256, 1141, 1105, 1038, 941, 909, 883, 865, 815, 737, 711, 683.

GC MS (HP-5; 100 °C (5 min), 20 °C/min, 200 °C (15 min); He, 1.00 ml/min) τ_R (min) = 12.60 (**24**): m/z = 258 [M^+], 230, 202, 178, 167, 152, 139, 128, 115, 96, 89, 76, 63, 51.

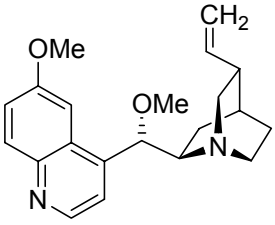
The spectroscopical data are in agreement with the literature.[58]

3.4.19 Synthesis of 9-O-Methylquinidine (20)

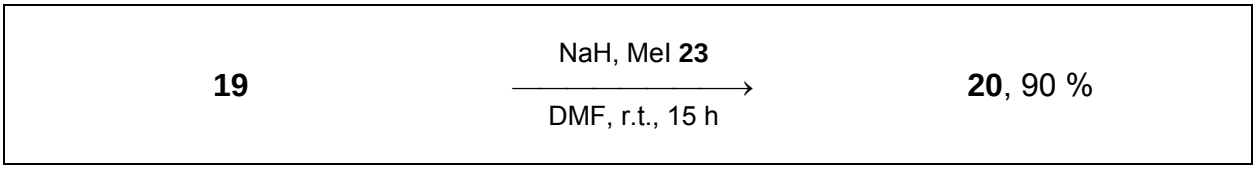
[II-SEE-122]



19



20



Under argon atmosphere, NaH (55 % dispersion in mineral oil) (336 mg, 7.70 mmol, 2.50 equiv) was added to a solution of quinidine **19** (1.00 g, 3.08 mmol, 1.00 equiv) in DMF (10 ml) and the suspension was stirred at r.t. for 2 h. Methyliodide **23** (211 µl,

481 mg, 3.39 mmol, 1.10 eq) was added dropwise within 10 min and the mixture was stirred for 13 h at r.t. The reaction was quenched with brine (10 ml) and the phases were separated. The aqueous layer was extracted with EtOAc (3 x 7 ml) and the organic layer was washed with brine (20 ml). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography ($CHCl_3$/MeOH 5:1) to yield 940 mg (2.78 mmol, 90 %) of the product **29** as yellow oil.

$C_{21}H_{26}N_2O_2$ (338.44 g/mol)

R_f 0.42 ($CHCl_3$/MeOH = 5:1)

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.71 (d; *J* = 4.5 Hz, 1H), 8.00 (d; *J* = 8.8 Hz, 1H), 7.40 (d; *J* = 4.5 Hz, 1H), 7.35-7.32 (m; 2H), 6.11-5.99 (m; 1H), 5.18 (s; 1H), 5.09-5.04 (m; 2H), 3.92 (s; 3H), 3.39-3.35 (m; 1H), 3.31 (s; 3H), 3.02-2.95 (m; 2H), 2.89-2.81 (m; 1H), 2.79-2.71 (m; 1H), 2.29-2.20 (m; 1H), 2.13-2.05 (m; 1H), 1.73 (s; 1H), 1.57-1.39 (m; 2H), 1.15-1.05 (m; 1H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 162.3, 147.2, 144.3, 143.9, 140.1, 131.5, 127.1, 121.6, 118.3, 114.5, 100.8, 82.4, 59.4, 57.0, 55.6, 49.8, 49.2, 39.7, 28.0, 26.0, 20.8.

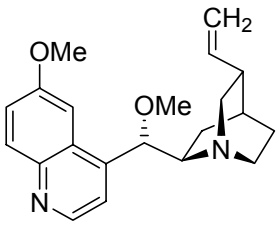
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2930, 2866, 1673, 1618, 1589, 1505, 1470, 1430, 1359, 1302, 1238, 1226, 1117, 1065, 1027, 911, 882, 827, 759, 728, 715.

HR ESI-MS (m/z): exact mass $[M+H]^+$: 339.2072; found $[M+H]^+$: 339.207.

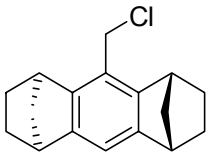
The spectroscopical data are in agreement with the literature.[59]

3.4.20 Synthesis of 1-N-[9-((1,8-R;4,5-S)-1,2,3,4,5,6,7,8-Octahydro-1,4:5,8-dimethanoanthracenyl)methyl]-9-O-methylquinidinium chloride (IV-1)

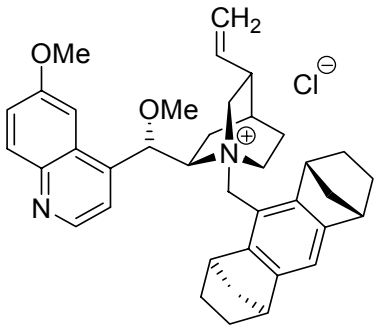
[II-SEE-148]



20



24



IV-1

20 + **24**	DMF/EtOH/$CHCl_3$, 100°C, 5 h	**IV-1**, 32 %

Under argon atmosphere, 9-(chloromethyl)-(1,8-*R*;4,5-*S*)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene **24** (250 mg, 966 µmmol, 1.10 equiv) and 9-*O*-methyl-quinidine **20** (297 mg, 878 µmol, 1.00 equiv) were dissolved in a mixture of DMF/ EtOH/$CHCl_3$ (8 ml, 9:4:1). The solution was stirred at 100 °C for 5 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 9:1→ 5:1). 170 mg (284 µmol, 32 %) of the product **IV-1** were obtained as off-white solid.

$C_{38}H_{45}N_2O_2Cl$ (597.23 g/mol)

R_f 0.25 ($CHCl_3$/MeOH 9:1)

m.p. 150 °C (decomposition)

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 8.85 (d; *J* = 4.3 Hz, 1H), 8.03 (d; *J* = 9.2 Hz, 1H), 7.64 (d; *J* = 4.3 Hz, 1H), 7.50-7.47 (s; 2H), 7.25 (s; 1H), 6.43 (s; 1H), 6.12-6.00 (m; 1H), 5.31-5.20 (m; 2H), 5.01 (d; *J* = 12.7 Hz, 1H), 4.60 (d; *J* = 12.7 Hz, 1H), 4.30-4.19 (m; 2H), 4.11-4.05 (m; 4H), 3.91 (brs; 1H), 3.54 (s; 3H), 3.52-3.31 (m; 4H), 3.27-3.17 (m; 1H), 2.80-2.77 (m; 1H), 2.51-2.41 (m; 1H), 1.97-1.58 (m; 11H), 1.24-0.99 (m; 5H).

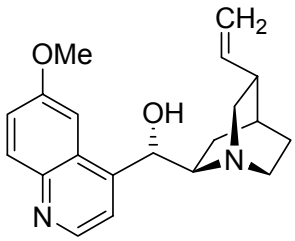
^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 157.2, 147.6, 147.4, 147.3, 144.0, 138.6, 137.3, 131.1, 126.1, 122.2, 119.6, 116.8, 116.5, 113.8, 101.8, 74.5, 66.6, 59.6, 56.2, 55.1, 55.0, 54.9, 43.8, 43.3, 42.2, 36.5, 26.3, 26.0, 25.8, 23.3, 20.9.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2956, 2865, 1620, 1506, 1473, 1428, 1353, 1328, 1294, 1240, 1205, 1109, 1073, 1023, 999, 949, 923, 867 828, 772, 717.

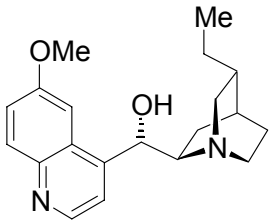
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 561.3481; found $[M\text{-}Cl]^+$: 561.348.

3.4.21 Synthesis of 10,11-Dihydroquinidine (21)

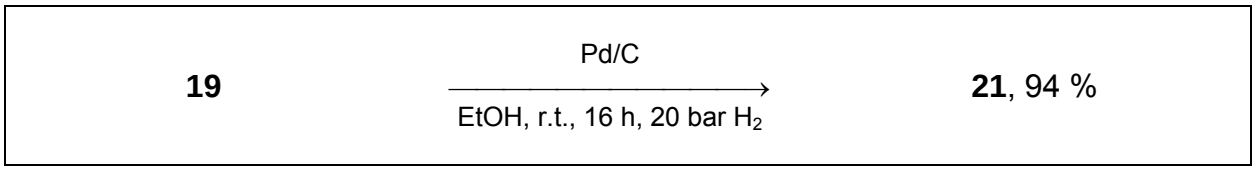
[II-SEE-116]



19



21



Palladium on charcoal (300 mg, 5 % Pd/C, 55 % H_2O) was added to a solution of quinidine **19** (400 mg, 1.23 mmol, 1.00 equiv) in ethanol (40 ml), and the resulting suspension was stirred for 16 h at 20 bar H_2-pressure. The reaction mixture was filtered through a pad of celite, the filtrate was concentrated under reduced pressure and the residue was dried in vacuo to yield 378 mg (1.16 mmol, 94 %) of the product **21** as colourless solid

$C_{20}H_{26}N_2O_2$ (326.43 g/mol)

m.p. 167-168 °C [m.p. ref.[53]: 166-167 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.51 (d; *J* = 4.5 Hz, 1H), 7.86 (d; *J* = 9.2 Hz, 1H), 7.52 (d; *J* = 4.5 Hz, 1H), 7.20 (d; *J* = 9.2 Hz, 1H), 7.05 (s;

1H), 5.57 (s; 1H), 3.71 (s; 3H), 3.22-3.16 (m; 1H), 2.94-2.66 (m; 4H), 2.05-1.98 (m; 1H), 1.65 (s; 1H), 1.65-1.36 (m; 5H), 1.00-0.93 (m; 1H), 0.87 (t; *J* = 7.2 Hz, 3H).

The OH-proton could not be detected.

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 157.5, 148.3, 147.2, 143.7, 131.1, 126.3, 121.4, 118.3, 101.0, 71.4, 59.6, 55.4, 51.1, 50.2, 37.3, 27.1, 26.2 25.1, 20.1, 12.0.

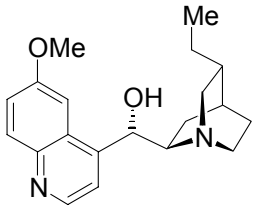
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3134, 2933, 2866, 2216, 1919, 1620, 1589, 1505, 1469, 1430, 1363, 1329, 1239, 1226, 1201, 1113, 1082, 1030, 998, 977, 909, 884, 859, 828, 729, 640.

HR ESI-MS (m/z): exact mass $[M+H]^+$: 327.2072; found $[M+H]^+$: 327.207.

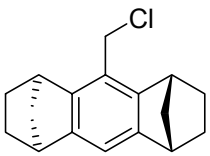
The spectroscopical data are in agreement with the literature.[53]

3.4.22 Synthesis of 1-N-[9-((1,8-R;4,5-S)-1,2,3,4,5,6,7,8-Octahydro-1,4:5,8-dimethanoanthracenyl)methyl]10,11-dihydroquinidinium chloride (IV-2)

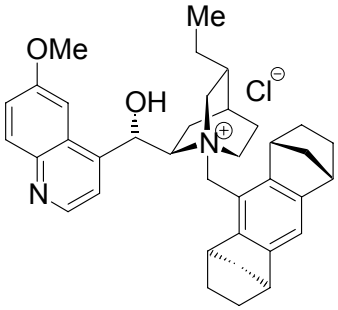
[II-SEE-149]



21



24



IV-2

21 + **24**	THF, reflux, 18 h	**IV-2**, 49 %

Under argon atmosphere, 9-(chloromethyl)-(1,8-*R*;4,5-*S*)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene **24** (250 mg, 966 µmmol, 1.10 equiv) and 10,11-dihydroquinidine **21** (287 mg, 878 µmol, 1.00 equiv) were dissolved in dry THF (7 ml), and the solution was refluxed for 20 h. The solvent was removed under reduced pressure. The

residue was dissolved in MeOH (5 ml) and added dropwise to Et_2O (200 ml). The suspension was stirred for 15 min, the precipitate was filtered off and dried in vacuo to yield 252 mg (431 µmol, 49 %) of the product **IV-2** as off-white solid.

$C_{37}H_{45}ClN_2O_2$ (585.22 g/mol)

m.p. 210 °C

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.66 (d; *J* = 4.5 Hz, 1H), 7.95 (d; *J* = 9.2 Hz, 1H), 7.76 (d; *J* = 4.5 Hz, 1H), 7.59 (d; *J* = 5.5 Hz, 1H), 7.29 (d; *J* = 9.2 Hz, 1H), 7.12 (s; 1H), 7.06 (s; 1H), 6.59 (d; *J* = 5.5 Hz, 1H), 5.88 (d; *J* = 12.7 Hz, 1H), 4.39 (d; *J* = 12.7 Hz, 1H), 3.95 (s; 3H), 3.78-3.64 (m; 2H), 3.55-3.48 (m; 1H), 3.29-3.26 (m; 4H), 3.05-2.95 (m; 1H), 2.81 (brs; 1H), 2.47-2.40 (m; 1H), 1.88-1.47 (m; 14H), 1.25 (brs; 1H), 1.03-0.95 (m; 4H), 0.86 (t; *J* = 6.8 Hz, 3H).

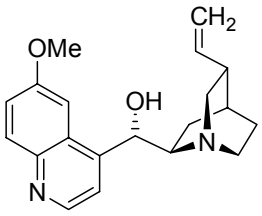
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 157.9, 148.6, 148.5, 147.6, 144.1, 143.6, 131.9, 125.6, 121.2, 120.5, 117.3, 113.3, 100.7, 69.3, 64.4, 60.1, 56.9, 56.0, 55.5, 48.4, 44.4, 43.3, 36.4, 27.2, 26.3, 25.0, 24.9, 24.8, 21.2, 11.6.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2958, 2866, 2197, 1619, 1590, 1506, 1457, 1429, 1329, 1254, 1238, 1225, 1110, 1027, 996, 949, 906, 864, 825, 718.

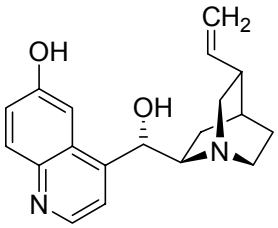
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 549.3481; found $[M\text{-}Cl]^+$: 549.348.

3.4.23 Synthesis of 6'-Hydroxy-cinchonine (22)

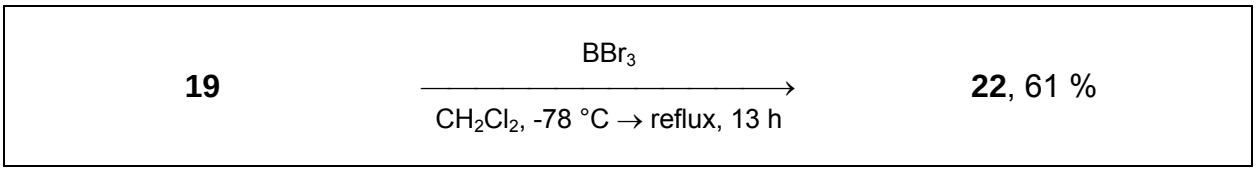
[II-SEE-100]



19



22



In a Schlenk flask, boron tribromide (3.09 g, 12.3 mmol, 4.00 equiv) in dry CH_2Cl_2 (12 ml) was slowly added under vigorous stirring to a solution of quinidine **19** (1.00 g, 3.08 mmol, 1.00 equiv) in dry CH_2Cl_2 (100 ml) at -78 °C. The reaction mixture was allowed to warm up to r. t.. It was refluxed at 40 °C for 1 h and then cooled to 5 °C. While stirring and maintaining the temperature aq. NaOH (10 %, 30 ml) was added. The aqueous solution was separated from the organic phase and was washed with CH_2Cl_2 (30 ml). HCl (2 M) was added dropwise until a colourless solid precipitated (approx. pH 8). Extraction with $CHCl_3$, drying of the organic phase over $MgSO_4$ and evaporating to dryness yielded 580 mg (1.87 mmol, 61 %) of the desired product **22** as yellowish solid.

$C_{19}H_{22}N_2O_2$ (310.39 g/mol)

m.p. >168 °C (decomposition) [m.p. ref.[53]: >165 °C (decomposition)]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.57 (brs; 1H), 7.91 (d; *J* = 8.5 Hz, 1H), 7.60 (s; 1H), 7.41 (s; 1H), 7.29 (d; *J* = 8.5 Hz, 1H), 6.04 (brs; 2H), 4.99-4.87 (m; 2H), 3.91-3.72 (m; 1H), 3.06-2.90 (m; 1H), 2.88-2.69 (m; 1H), 2.61-2.43 (m; 1H), 2.35-2.14 (m; 2H), 2.11-1.99 (m; 1H), 1.64 (s; 1H), 1.40-1.12 (m; 2H), 0.90-0.74 (m; 1H).
The OH-protons could not be detected.

^{13}C NMR (75 MHz, $CHCl_3$-d_1): δ [ppm] = 158.0, 147.2, 146.1, 142.7, 139.7, 131.3, 126.6, 123.5, 117.8, 115.2, 104.0, 70.4, 59.3, 49.2, 49.0, 39.5, 28.0, 25.5,

18.4.

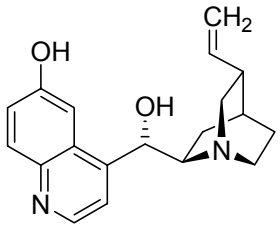
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3000, 2868, 1708, 1652, 1614, 1590, 1557, 1506, 1455, 1405, 1325, 1226, 1129, 1103, 996, 907, 881, 854, 829, 795, 761, 725.

HR ESI-MS (m/z): exact mass $[M+H]^+$: 311.1760; found $[M+H]^+$: 311.176.

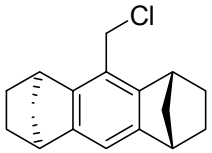
The spectroscopical data are in agreement with the literature.[53]

3.4.24 Synthesis of 1-N-[9-((1,8-R;4,5-S)-1,2,3,4,5,6,7,8-Octahydro-1,4:5,8-dimethanoanthracenyl)methyl]- 6'-hydroxycinchoninium chloride (IV-4)

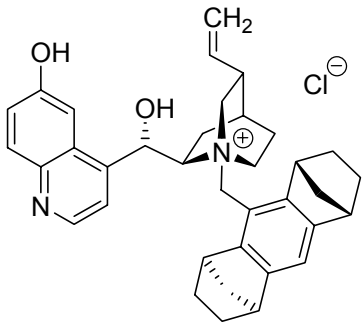
[II-SEE-150]



22



24



IV-4

22 + **24** ⟶ (THF, reflux, 18 h) **IV-4**, 42 %

Under argon atmosphere, 9-(chloromethyl)-(1,8-*S*;4,5-*R*)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene **24** (250 mg, 966 µmmol, 1.10 equiv) and 6'-hydroxy-cinchonine **22** (273 mg, 878 µmol, 1.00 equiv) were dissolved in dry THF (7 ml) and the solution was refluxed for 18 h. The precipitate was filtered off and washed with THF to yield 210 mg (369 µmol, 42 %) of the product as light red solid.

$C_{36}H_{41}ClN_2O_2$ (569.18 g/mol)

m.p. 210 °C

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 10.53 (s; 1H), 8.73 (d; *J* = 4.3 Hz, 1H), 7.93 (d; *J* = 9.2 Hz, 1H), 7.85 (s; 1H), 7.69 (d; *J* = 4.3 Hz, 1H), 7.38 (d; *J* = 9.2 Hz, 1H), 7.22 (s; 1H), 7.10 (s; 1H), 6.47 (s; 1H), 6.12-6.01 (m; 1H),

5.29-5.13 (m; 3H), 4.94 (d; *J* = 12.8 Hz, 1H), 4.25-4.18 (m; 3H), 4.00-3.74 (m; 2H), 3.61-3.22 (m; 4H), 2.71-2.64 (m; 1H), 2.30-2.22 (m; 1H), 1.92-1.50 (m; 11H), 1.14-1.04 (m; 5H).

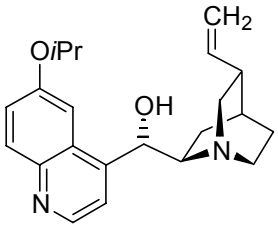
^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 156.2, 148.1, 148.0, 146.3, 142.5, 142.3, 135.7, 130.5, 124.9, 121.6, 119.2, 117.7, 116.9, 114.1, 104.6, 66.3, 66.1, 58.2, 56.3, 54.2, 48.4, 44.2, 44.1, 38.0, 26.9, 26.8, 26.6, 24.0, 21.5.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2963, 2868, 1619, 1531, 1464, 1403, 1328, 1225, 1110, 1000, 924, 864, 832.

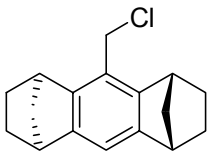
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 533.3161; found $[M\text{-}Cl]^+$: 533.316.

3.4.25 Synthesis of 1-N-[9-((1,8-R;4,5-S)-1,2,3,4,5,6,7,8-Octahydro-1,4:5,8-dimethanoanthracenyl)methyl]-6'-isopropoxy-cinchoninium chloride (IV-5)

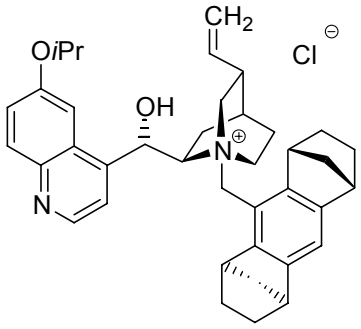
[II-SEE-164]



44



24



IV-5

44 + **24**	THF, reflux, 18 h	**IV-5**, 20 %

Under argon atmosphere, 9-(chloromethyl)-(1,8-*S*;4,5-*R*)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene **24** (250 mg, 966 µmmol, 1.10 equiv) and 6'-isopropoxy-cinchonine **44** (309 mg, 878 µmol, 1.00 equiv) were dissolved in dry THF (7 ml) and the solution was refluxed for 18 h. The solvent was removed under reduced pressure. The residue was dissolved in MeOH (5 ml) and added dropwise to Et_2O (200 ml). The suspension was stirred for 15 min, the precipitate was filtered off and dried in vacuo to yield 106 mg (173 mmol, 20 %) of the product as off-white solid.

$C_{39}H_{47}ClN_2O_2$ (611.26 g/mol)

m.p. 170 °C (decomposition)

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.64 (d; *J* = 4.3 Hz, 1H), 7.99 (d; *J* = 9.2 Hz, 1H), 7.77 (d; *J* = 4.3 Hz, 1H), 7.70 (s; 1H), 7.30 (d; *J* = 9.2 Hz, 1H), 7.20 (s; 1H), 7.04 (s; 1H), 6.60 (s; 1H), 6.03-5.91 (m; 2H), 5.19-5.11 (m; 2H), 4.73-4.60 (m; 2H), 4.41 (d; *J* = 12.7 Hz, 1H), 4.07 (brs; 1H), 3.71-3.65 (m; 1H), 3.57-3.47 (m; 3H), 3.28 (brs; 2H), 3.09-2.99 (m; 1H), 2.52-2.33 (m; 2H), 1.96-1.72 (m; 6H), 1.67 (d; *J* = 8.6 Hz, 2H), 1.46 (d; *J* = 8.7 Hz, 2H), 1.35-0.98 (m; 12H).

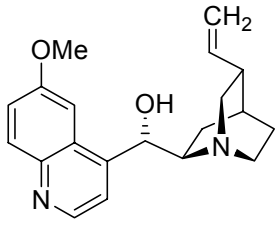
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 156.0, 148.8, 147.7, 145.6, 143.7, 143.1, 135.5, 132.0, 125.7, 120.8, 120.5, 118.3, 117.0, 113.2, 104.9, 70.5, 69.4, 64.6, 60.1, 56.1, 54.9, 48.4, 44.2, 43.8, 38.4, 26.8, 26.8, 26.7, 24.4, 22.2, 21.3.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2962, 2866, 1616, 1504, 1457, 1383, 1326, 1238, 1110, 1003, 968, 926, 863, 826.

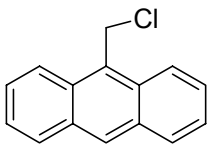
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 575.3638; found $[M\text{-}Cl]^+$: 575.364.

3.4.26 Synthesis of 1-N-(9-Anthrylmethyl)quinidinium chloride (IV-6)

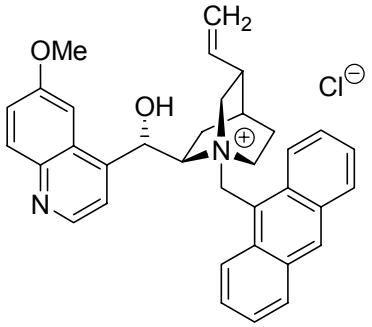
[II-SEE-117]



19



45



IV-6

19 + **45** ⟶ (THF, reflux, 16 h) **IV-6**, 36 %

Under argon atmosphere, quinidine **19** (1.00 g, 3.08 mmol, 1.00 equiv) and 9-chloromethylanthracene **45** (768 mg, 3.08 mmol, 1.00 equiv) were dissolved in dry THF (25 ml), and the solution was refluxed for 16 h. The precipitate was filtered off and was washed with THF. The solid was redissolved in CH_2Cl_2 (10 ml) and was added dropwise to Et_2O (150 ml). The suspension was stirred for 15 min, the precipitate was filtered off and was dried in vacuo. The crude product was purified by flash chromatography ($CHCl_3$/MeOH 9:1→ 6:1) to yield 617 mg (1.12 mmol, 36 %) of the product **IV-6** as a yellow solid.

$C_{35}H_{35}ClN_2O_2$ (551.12 g/mol)

R_f 0.53 ($CHCl_3$/MeOH = 6:1)

m.p. 160 °C (decomposition) [m.p. ref.[52]: >155 °C (decomposition)]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 9.04 (d; *J* = 8.9 Hz, 1H), 8.43 (d; *J* = 8.9 Hz, 1H), 8.29-8.21 (m; 3H), 8.06 (d; *J* = 4.5 Hz, 1H), 8.07 (s; 1H), 7.87 (d; *J* = 9.2 Hz, 1H), 7.63 (d; *J* = 7.5 Hz, 1H), 7.54 (d; *J* = 8.5 Hz, 1H), 7.36-23 (m; 3H), 7.14-7.10 (m; 2H), 7.01 (s; 1H), 6.87 (d, *J* = 13.5 Hz, 1H), 6.45 (d; *J* = 13.5 Hz, 1H), 5.74-5.60 (m; 1H), 5.07-4.90 (m; 2H), 4.77-4.72 (m; 1H), 4.52-4.45 (m; 1H), 4.22-4.14 (m; 1H), 3.83 (s; 3H), 2.71-2.64 (m; 1H), 2.28-2.18 (m; 2H), 1.85-1.69 (m; 2H), 1.59 (s; 1H), 1.41-1.31 (m; 1H), 0.95-0.86 (m; 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 157.6, 147.0, 144.3, 143.1, 135.7, 132.8, 131.1, 130.3, 128.6, 127.6, 127.4, 127.3, 126.1, 124.8, 121.3, 120.5, 118.1, 117.2, 104.2, 70.2, 68.0, 56.8, 56.2, 54.3, 54.2, 38.0, 26.2, 24.2, 22.5.

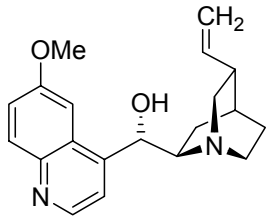
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3078, 2200, 1620, 1584, 1539, 1506, 1471, 1447, 1430, 1353, 1239, 1225, 1124, 1081, 1027, 997, 908, 867, 825, 792, 724.

HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 515.2701; found $[M\text{-}Cl]^+$: 515.270.

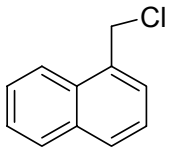
The spectroscopical data are in agreement with the literature.[52]

3.4.27 Synthesis of 1-N-(1-Naphthylmethyl)quinidinium chloride (IV-7)

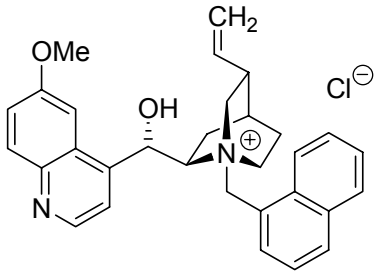
[II-SEE-103]



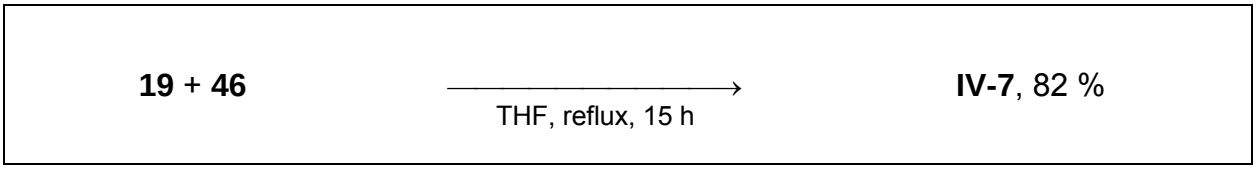
19



46



IV-7



Under argon atmosphere, 1-chloromethylnaphthaline **46** (465 µl, 544 mg, 3.08 mmol, 1.00 equiv) was added to a solution of quinidine **19** (1.00 g, 3.08 mmol, 1.00 equiv) in dry THF (20 ml), and the solution was refluxed for 15 h. The precipitate was filtered off and was washed with THF. The solid was redissolved in MeOH (10 ml) and added dropwise to Et_2O (200 ml). The suspension was stirred for 15 min, the precipitate was filtered off and dried in vacuo to yield 1.27 g (2.53 mmol, 82 %) of the product **IV-7** as colourless solid.

$C_{31}H_{33}ClN_2O_2$ (501.06 g/mol)

m.p. 187 °C (decomposition) [m.p. ref.[52]: >186 °C (decomposition)]

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 8.83 (d; *J* = 4.1 Hz, 1H), 8.58 (d; *J* = 8.2 Hz, 1H), 8.18 (d; *J* = 8.1 Hz, 1H), 8.12-8.08 (m; 2H), 8.03 (d; *J* = 9.2 Hz, 1H), 7.84 (d; *J* = 4.1 Hz, 1H), 7.73-7.62 (m; 5H) 7.50 (d; *J* = 9.13 Hz, 1H), 6.76 (s; 1H), 6.13-6.00 (m; 1H), 5.95 (d; *J* = 12.8 Hz, 1H), 5.30 (d; *J* = 12.8 Hz, 1H), 5.28-5.14 (m; 2H), 4.49-4.43 (m; 1H), 4.21-4.16 (m; 5H), 3.45-3.40 (m; 1H), 2.89-2.85 (m; 1H), 2.57-2.54 (m; 1H), 2.44-2.41 (m; 1H), 1.85 (s; 1H), 1.71 (brs; 2H), 1.11 (s; 1H).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 157.8, 147.8, 144.3, 144.2, 137.8, 134.9, 134.2, 133.5, 131.7, 131.7, 129.6, 127.8, 126.7, 126.0, 125.8, 124.5,

124.4, 122.3, 120.9, 117.4, 102.8, 68.0, 65.0, 59.1, 56.8, 56.0, 54.7, 37.5, 26.5, 23.9, 21.4.

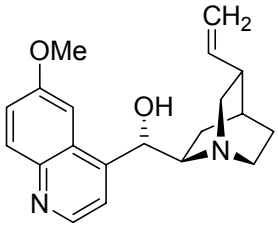
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3376, 3124, 1623, 1539, 1515, 1474, 1433, 1365, 1336, 1260, 1241, 1227, 1173, 1122, 1080, 1026, 994, 967, 930, 866, 850, 834, 807.

HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 465.254; found $[M\text{-}Cl]^+$: 465.254.

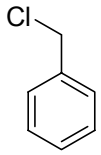
The spectroscopical data are in agreement with the literature.[52]

3.4.28 Synthesis of 1-N-(Benzyl)quinidinium chloride (IV-8)

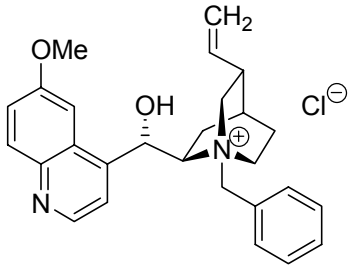
[II-SEE-108]



19



47



IV-8

19 + **47**	THF, reflux, 16 h	**IV-8**, 68 %

Under argon atmosphere, benzyl chloride **47** (496 µl, 546 mg, 4.31 mmol, 1.40 equiv) was added to a solution of quinidine **19** (1.00 g, 3.08 mmol, 1.00 equiv) in dry THF (15 ml), and the solution was refluxed for 16 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 9:1→1:1) to yield 944 mg (2.09 mmol, 68 %) of the product **IV-8** as colourless solid.

$C_{27}H_{31}ClN_2O_2$ (451.00 g/mol)

R_f 0.28 ($CHCl_3$/MeOH 9 :1)

m.p. 178 °C [m.p. ref.[60]: 180 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.51 (d; *J* = 4.5 Hz, 1H), 7.83 (d; *J* = 9.2 Hz, 1H), 7.68-7.65 (m; 3H), 7.49 (d; *J* = 5.7 Hz, 1H), 7.63-7.14 (m; 5H),

6.38 (brs; 1H), 5.94-5.83 (m; 1H), 5.66 (d, *J* = 11.9 Hz, 1H), 5.17-5.11 (m, 3H), 4.53-4.46 (m, 1H), 4.31 (brs; 1H), 3.99-3.90 (m; 4H), 3.33-3.26 (m; 1H), 2.80-2.70 (m; 1H), 2.37-2.26 (m; 2H), 1.71 (s; 1H), 1.56 (brs; 2H), 0.82 (brs, 1H).

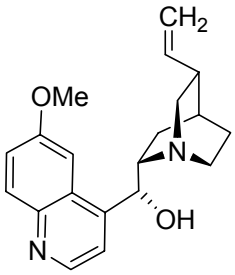
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 157.9, 147.1, 144.0, 143.3, 135.8, 133.9, 131.4, 130.1, 128.9, 127.3, 126.1, 121.4, 120.7, 117.9, 102.1, 67.8, 65.9, 62.2, 56.3, 56.1, 53.7, 38.1, 27.2, 23.9, 21.5.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3135, 2834, 2201, 1921, 1828, 1616, 1587, 1505, 1472, 1430, 1356, 1255, 1239, 1173, 1117, 1088, 1024, 1000, 904, 867, 827, 717, 643.

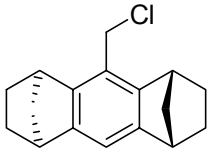
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 415.2385; found $[M\text{-}Cl]^+$: 415.239.

3.4.29 Synthesis of 1-N-[9-((1,8-R;4,5-S)-1,2,3,4,5,6,7,8-Octahydro-1,4:5,8-dimethanoanthracenyl)methyl]quininium chloride (IV-9)

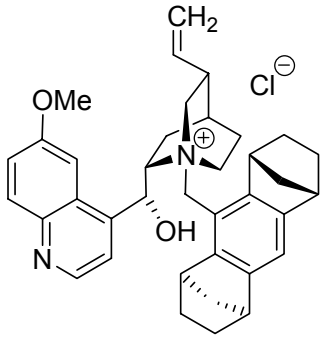
[II-SEE-151]



48



24



IV-9

48 + **24** —(toluene, reflux, 15 h)→ **IV-9**, 55 %

Under argon atmosphere, a solution of quinine **48** (285 mg, 878 µmol, 1.00 equiv) and 9-(chloromethyl)-(1,8-*S*;4,5-*R*)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-dimethanoanthracene **24** (250 mg, 966 µmmol, 1.10 equiv) in toluene (10 ml) was refluxed for 15 h. The precipitate was filtered off, washed with toluene, and dried in vacuo to yield 281 mg (483 µmol, 55 %) of the product **IV-9** as colourless solid.

$C_{37}H_{43}ClN_2O_2$ (583.20 g/mol)

m.p. >207 °C (decomposition) [m.p. ref.[52]: >205 °C (decomposition)]

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 8.82 (d; *J* = 4.4 Hz, 1H), 8.02 (d; *J* = 9.1 Hz, 1H), 7.83 (d; *J* = 4.4 Hz, 1H), 7.55-7.50 (m; 3H), 7.27-7.14 (m; 1H), 6.68 (s; 1H), 5.87-5.76 (m; 1H), 5.48 (d; *J* = 12.7 Hz, 1H), 5.12-5.00 (m; 2H), 4.74-4.63 (m; 2H), 4.31 (brs; 1H), 4.04 (s; 3H), 3.91 (s; 2H), 3.64 (brs; 1H), 3.41-3.30 (m; 5H), 2.75 (brs; 1H), 2.23 (brs; 2H), 2.00 (s; 1H), 1.87 (brs; 5H), 1.52-1.50 (m; 4H), 1.10 (brs; 4H).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 156.9, 147.4, 147.3, 144.3, 143.6, 138.0, 131.1, 125.3, 121.4,120.3, 116.3, 116.2, 114.3, 102.6, 68.3, 63.5, 59.6, 59.3, 55.1, 50.4, 45.4, 43.6, 41.9, 37.0, 26.5, 26.4, 25.7, 24.4, 20.5.

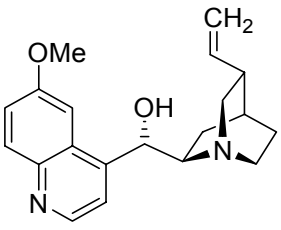
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2961, 2866, 1619, 1507, 1471, 1329, 1239, 1110, 1060, 1027, 946, 910, 860, 826, 713.

HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 547.3325; found $[M\text{-}Cl]^+$: 547.332.

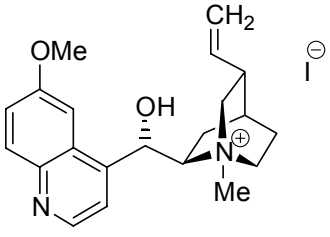
The spectroscopical data are in agreement with the literature.[52]

3.4.30 Synthesis of N-Methyl-quinidinium iodide (38)

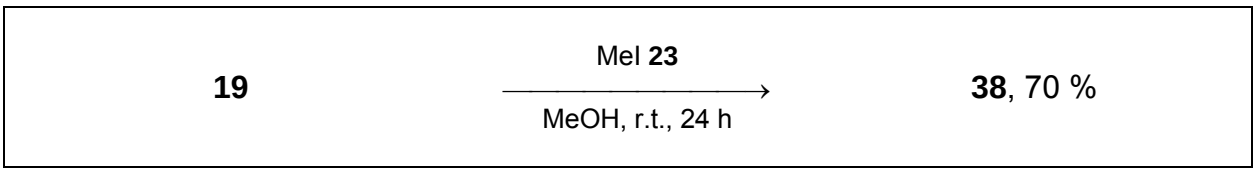
[III-SEE-208]



19



38



Under argon atmosphere, methyl iodide **23** (437 µl, 1.00 g, 7.09 mmol, 1.15 equiv) was added to a solution of quinidine **19** (2.00 g, 6.18 mmol, 1.00 equiv) in MeOH (60 ml), and the reaction mixture was stirred for 24 h at r.t.. The solvent was removed under reduced pressure and the residue was washed with THF. The crude product was dissolved at reflux in a mixture of MeOH/H_2O (30 ml, 1:1). Activated charcoal was added and the suspension was stirred for 15 min before it was filtered hot. The filtrate was concentrated under reduced pressure, and the residue was dried in vacuo to yield 2.02 g (4.32 mmol, 70 %) of the product **38** as off-white solid.

$C_{21}H_{27}IN_2O_2$ (466.36 g/mol)

m.p. 235 °C (decomposition) [m.p. ref.[61]: 236-237 °C (decomposition)]

^{1}H NMR (300 MHz, MeOH-d_4): δ [ppm] = 8.76 (d; *J* = 4.7 Hz, 1H), 7.99 (d; *J* = 9.3 Hz, 1H), 7.51 (d; *J* = 4.7 Hz, 1H), 7.53-7.47 (m; 1H), 7.34-7.29 (m; 1H), 6.37 (brs; 1H), 6.16-6.04 (m; 1H), 5.36-5.28 (m; 2H), 4.53-4.45 (m; 1H), 4.11 (s; 3H), 3.85-3.61 (m; 4H), 3.52 (s; 3H), 2.95-2.86 (m; 1H), 2.52-2.45 (m; 1H), 2.04-1.93 (m; 3H), 1.16-1.08 (m; 1H).
The OH-proton could not be detected.

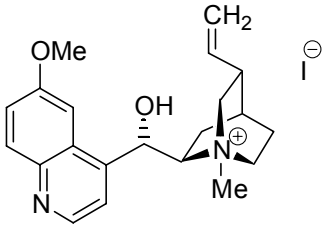
^{13}C NMR (75.5 MHz, MeOH-d_4): δ [ppm] = 158.6, 146.8, 144.24, 143.3, 136.3, 130.3, 125.9, 122.0, 119.9, 116.5, 100.9, 66.5, 65.6, 60.8, 59.2, 48.5, 38.0, 26.9, 23.7, 19.9.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3509, 2992, 2947, 2830, 1621, 1589, 1511, 1472, 1451, 1432, 1359, 1243, 1227, 1205, 1133, 1115, 1023, 921, 902, 876, 826, 718.

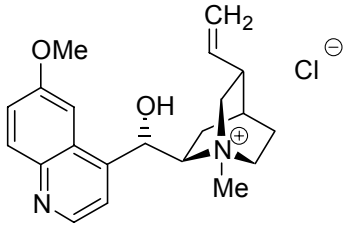
HR ESI-MS (m/z): exact mass $[M-I]^+$: 339.2072; found $[M-I]^+$: 339.208.

3.4.31 Synthesis of N-Methyl-quinidinium chloride (V-1)

[III-SEE-238]



38



V-1

38	$\xrightarrow[H_2O/MeOH,\ r.t.,\ 72\ h]{AgCl,\ NH_3}$	**V-1**, 75 %

Aqueous ammonia was added dropwise to a suspension of silver(I)chloride (138 mg, 965 µmol, 1.50 equiv) in dist. H_2O (5 ml) until the silver(I)chloride was dissolved completely. This solution was added slowly to a solution of *N*-methyl-quinidinium iodide **38** (300 mg, 643 µmol, 1.00 equiv) in MeOH (5 ml), and the reaction mixture was stirred for 72 h at r.t. The solvents were removed under reduced pressure and the residue was dissolved in $CHCl_3$ (5 ml). Insoluble components were filtered off and the filtrate was concentrated under reduced pressure. The residue was dried in vacuo to yield 180 mg (481 µmol, 75 %) of the product **V-1** as off-white solid.

$C_{21}H_{27}ClN_2O_2$ (374.91 g/mol)

m.p. 255 °C (decomposition) [m.p. ref.[60]: 250-251 °C (decomposition)]

^{1}H NMR (300 MHz, MeOH-d_4): δ [ppm] = 8.76 (d; *J* = 4.7 Hz, 1H), 8.01 (d; *J* = 9.3 Hz, 1H), 7.85 (d; *J* = 4.7 Hz, 1H), 7.52-7.48 (m; 1H), 7.35-7.29 (m; 1H), 6.37 (brs; 1H), 6.16-6.04 (m; 1H), 5.36-5.28 (m; 2H), 4.55-4.47 (m; 1H), 4.09 (s; 3H), 3.83-3.56 (m; 4H), 3.46 (s; 3H), 2.93-2.85 (m; 1H), 2.53-2.45 (m; 1H), 2.04-1.92 (m; 3H), 1.16-1.08 (m; 1H).
The OH-proton could not be detected.

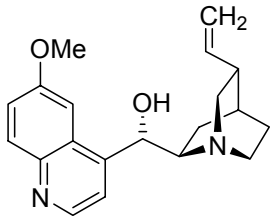
^{13}C NMR (75.5 MHz, MeOH-d_4): δ [ppm] = 158.6, 146.8, 144.32, 143.3, 136.3, 130.3, 125.9, 122.0, 119.9, 116.5, 100.9, 66.5, 65.5, 60.7, 59.2, 48.2, 38.0, 27.0, 23.7, 19.9.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3356, 2953, 2510, 1619, 1589, 1507, 1472, 1451, 1430, 1353, 1290, 1226, 1178, 1134, 1100, 1019, 918, 861, 828, 717.

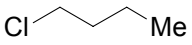
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 339.2072; found $[M\text{-}Cl]^+$: 339.208.

3.4.32 Synthesis of N-1-Butyl-quinidinium chloride (V-2)

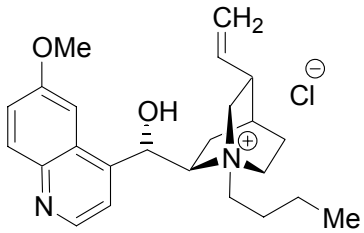
[III-SEE-218]



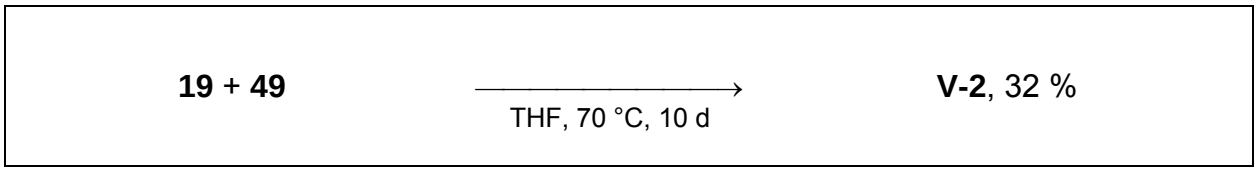
19



49



V-2



Under argon atmosphere, 1-butyl chloride **49** (10.0 ml, 8.00 g, 95.1 mmol, 15.4 equiv) was added to a solution of quinidine **19** (2.00 g, 6.18 mmol, 1.00 equiv) in dry THF (30 ml), and the solution was stirred for 10 d at 70 °C. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 5:1) to yield 834 mg (2.00 mmol, 32 %) of the product **V-2** as off-white solid.

$C_{24}H_{33}ClN_2O_2$ (416.98 g/mol)

R_f 0.55 ($CHCl_3$/MeOH 5:1)

m.p. 140 °C

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.76 (d; *J* = 4.7 Hz, 1H), 8.02 (d; *J* = 9.4 Hz, 1H), 7.86 (d; *J* = 4.7 Hz, 1H), 7.52-7.48 (m; 1H), 7.30-7.27 (m; 1H), 6.29 (brs; 1H), 6.14-6.02 (m; 1H), 5.36-5.28 (m; 2H), 4.87 (s; 1H), 4.47-4.39 (m; 1H), 4.08 (s; 3H), 3.86-3.58 (m; 5H), 3.58-3.48 (m; 1H), 2.91-2.82 (m; 1H), 2.49-2.37 (m; 1H), 2.15-1.88 (m; 5H), 1.68-1.61 (m; 2H), 1.14-1.08 (m; 4H).

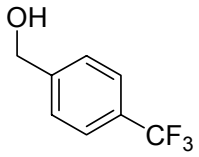
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 158.5, 146.8, 144.4, 143.3, 136.3, 130.3, 126.0, 122.0, 120.2, 116.5, 101.0, 65.7, 65.5, 60.2, 57.1, 56.0, 55.1, 37.9, 26.8 , 24.8, 23.5, 20.7, 20.1, 12.8.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3140, 2960, 2928, 2869, 2830, 2197, 1619, 1588, 1506, 1470, 1430, 1362, 1323, 1254, 1238, 1225, 1173, 1132, 1095, 1079, 1026, 997, 917, 865, 827, 728, 639.

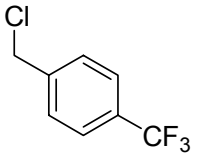
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 381.2542; found $[M\text{-}Cl]^+$: 381.254.

3.4.33 Synthesis of 4-(Trifluoromethyl)benzyl chloride (30)

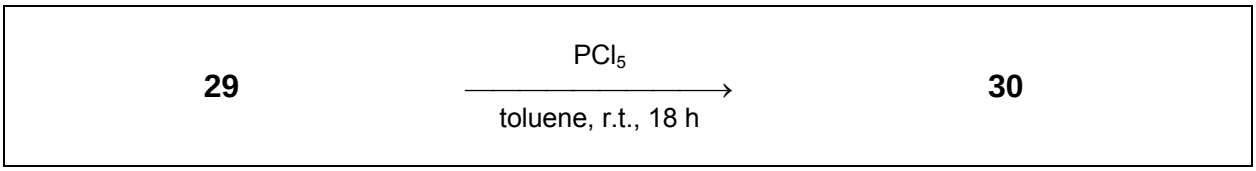
[III-SEE-244]



29



30



Under argon atmosphere, PCl_5 (4.02 g, 19.3 mmol, 1.70 equiv) was added to 4-(trifluoromethyl)benzyl alcohol **29** (1.56 ml, 2.00 g, 11.4 mmol, 1.00 equiv) in dry toluene (100 ml) and the reaction mixture was stirred for 18 h at r.t.. Saturated aqueous $NaHCO_3$ solution (100 ml) was added and the mixture was stirred for 15 min. The organic layer was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was used for the synthesis of 1-*N*-(4-trifluoromethyl-benzyl)quinidinium chloride **V-3** without further purification.

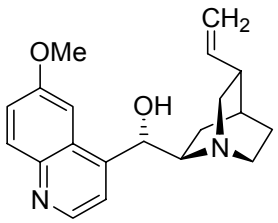
$C_8H_6ClF_3$ (194.58 g/mol)

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 7.68 (d; *J* = 8.1 Hz, 2H), 7.56 (d; *J* = 8.1 Hz, 2H), 4.66 (s; 2H).

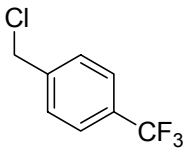
^{13}C NMR (75 MHz, $CHCl_3$-d_1): δ [ppm] = 141.3, 130.6 (q; $^2J_{C\text{-}F}$ = 32.1 Hz), 128.8, 125.7, 124.0 (q; $^1J_{C\text{-}F}$ = 273.1 Hz), 45.1.

3.4.34 Synthesis of 1-N-(4-Trifluoromethylbenzyl)quinidinium chloride (V-3)

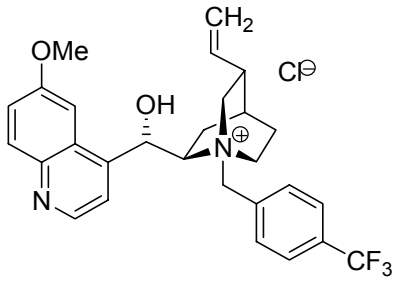
[III-SEE-245]



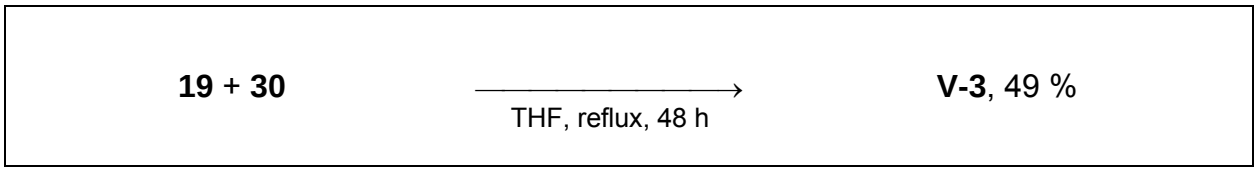
19



30



V-3



Under argon atmosphere, quinidine **19** (2.23 g, 6.87 mmol, 1.00 equiv) and 4-(trifluoromethyl)benzyl chloride **30** (2.00 g, 10.3 mmol, 1.50 equiv) were dissolved in dry THF (60 ml). The reaction mixture was refluxed for 48 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1) to yield 1.75 g (3.37 mmol, 49 %) of the product **V-3** as off-white solid.

$C_{28}H_{30}ClF_3N_2O_2$ (519.00 g/mol)

R_f 0.47 ($CHCl_3$/MeOH 7:1)

m.p. 195 °C

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.47 (d; *J* = 4.2 Hz, 1H), 7.88 (d; *J* = 7.9 Hz, 2H), 7.76 (d; *J* = 9.2 Hz, 1H), 7.60 (d; *J* = 4.2 Hz, 1H), 7.41-7.35 (m; 4H), 6.97 (d; *J* = 9.2 Hz, 1H), 6.32 (brs; 1H), 5.86-5.74 (m; 2H), 5.58 (d; *J* = 12.1 Hz, 1H), 5.15-5.08 (m; 2H), 4.52-4.45 (m; 1H), 4.31 (brs; 1H), 4.07-4.01 (m; 1H), 3.77 (s; 3H), 3.19-3.12 (m; 1H), 2.66-2.56 (m; 1H), 2.30-2.16 (m; 2H), 1.69 (s; 1H), 1.51 (brs; 1H), 0.78-0.73 (m; 1H).
The OH-proton could not be detected.

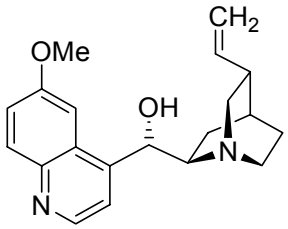
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 157.9, 146.9, 143.9, 142.8, 135.3, 134.4, 132.1 (q; $^2J_{C\text{-}F}$ = 32.7 Hz), 131.6, 131.4, 125.9, 125.5, 123.4 (q; $^1J_{C\text{-}F}$ = 272.2 Hz), 120.4, 120.4, 118.1, 102.4, 67.6, 66.4, 60.9, 56.0, 56.1, 54.0, 38.0, 27.0, 23.7, 21.7.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3131, 2962, 1619, 1587, 1506, 1471, 1427, 1322, 1238, 1225, 1168, 1126, 1067, 1020, 1003, 927, 864, 829, 750, 661.

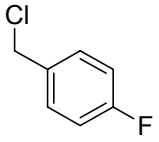
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 483.2259; found $[M\text{-}Cl]^+$: 483.225.

3.4.35 Synthesis of 1-N-(4-Fluorobenzyl)quinidinium chloride (V-4)

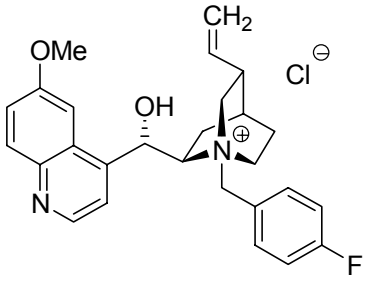
[III-SEE-205]



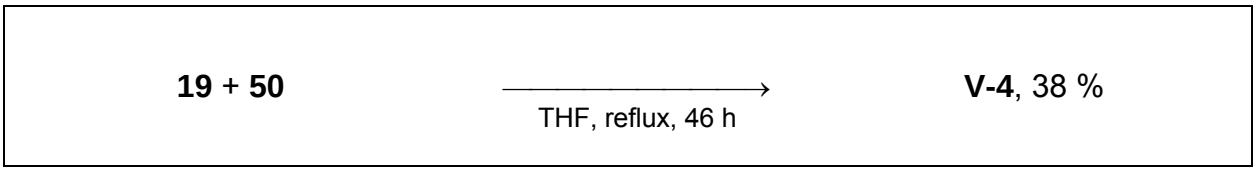
19



50



V-4



Under argon atmosphere, 4-fluorobenzylchloride **50** (1.04 ml, 1.25 g, 8.65 mmol, 1.40 equiv) was added to a solution of quinidine **19** (2.00 g, 6.18 mmol, 1.00 equiv) in dry THF (30 ml) and the reaction mixture was refluxed for 46 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1). 1.10 g (2.35 mmol, 38 %) of the product **V-4** were isolated as violet solid.

$C_{27}H_{30}ClFN_2O_2$ (468.99 g/mol)

R_f 0.29 ($CHCl_3$/MeOH 7:1)

m.p. 166-168 °C (decomposition)

^{1}H NMR (300 MHz, MeOH-d_4): δ [ppm] = 8.77 (d; *J* = 4.6 Hz, 1H), 8.02 (d; *J* = 10.1 Hz, 1H), 7.92 (d; *J* = 4.3 Hz, 1H), 7.83-7.79 (m; 2H), 7.53-7.50 (m; 2H), 7.36-7.30 (m; 2H), 6.64 (s; 1H) 6.17-6.05 (m; 1H), 5.35-5.18 (m; 3H) 4.92 (s; 1H), 4.49-4.42 (m; 1H), 4.10 (s; 3H), 4.02-3.97 (m; 2H), 3.66-3.58 (m; 1H), 3.18-3.08 (m; 1H), 2.72-2.53 (m; 2H), 1.98-1.88 (m; 3H), 1.20-1.11 (m; 1H).

The OH-proton could not be detected.

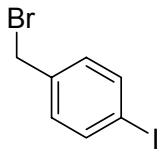
^{13}C NMR (75.5 MHz, MeOH-d_4): δ [ppm] = 164.1 (d; $^1J_{C\text{-}F}$ = 250.0 Hz), 158.6, 146.8, 144.3, 143.4, 136.4, 135.8 (d; $^2J_{C\text{-}F}$ = 22.0 Hz), 130.3, 126.1, 123.5, 121.7, 120.3, 116.5, 115.9 (d; $^3J_{C\text{-}F}$ = 8.7 Hz), 101.6, 67.8, 65.6, 62.9, 56.7, 55.2, 54.3, 37.5, 27.1, 23.4, 21.0.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3371, 3148, 2942, 2830, 1616, 1601, 1557, 1538, 1506, 1471, 1453, 1430, 1354, 1256, 1239, 1225, 1163, 1117, 1084, 1023, 1001, 933, 917, 867, 828, 784, 759, 718, 638, 620.

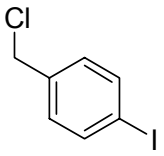
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 433.2291; found $[M\text{-}Cl]^+$: 433.229.

3.4.36 Synthesis of 4-Iodobenzyl chloride (32)

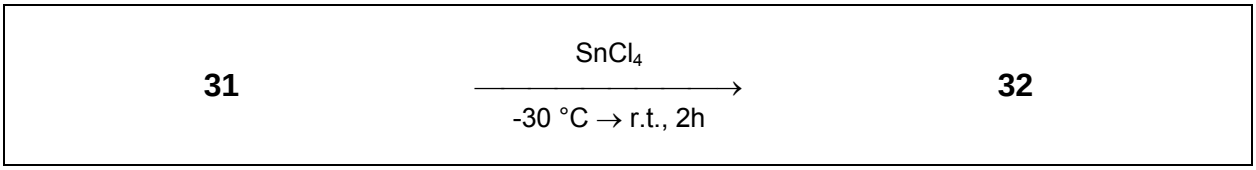
[III-SEE-222]



31



32



4-Iodobenzyl bromide **31** (445 mg, 1.50 mmol, 1.00 equiv) was added portionwise at -30 °C to tin(IV)chloride (1.58 ml, 3.51 g, 13.5 mmol, 9.00 eq). The reaction mixture was allowed to warm up to r.t. within 2 h. It was then added to ice water (5 ml). The mixture was extracted with Et_2O (3 x 3 ml) and the combined organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was used

for the synthesis of 1-*N*-(4-iodobenzyl)quinidinium chloride **V-5** without further purification.

C_7H_6ClI (252.48 g/mol)

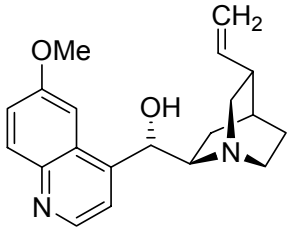
^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 7.73 (d; *J* = 8.2 Hz, 2H), 7.23 (d; *J* = 8.2 Hz, 2H), 4.69 (s; 2H).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 137.6, 137.6, 131.2, 94.7, 45.5.

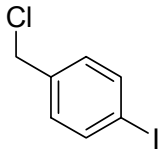
GC MS (HP-5; 100 °C, 20 °C/min, 200 °C (15 min); He; 1.00 ml/min), τ_R (min) = 8.49 (**32**): m/z = 252 [M^+], 217, 127, 90, 63.

3.4.37 Synthesis of 1-N-(4-Iodobenzyl)quinidinium chloride (V-5)

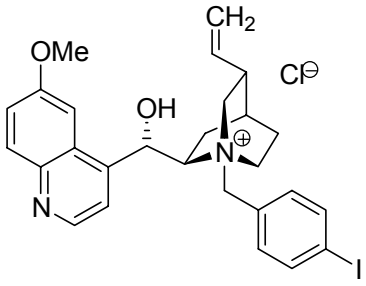
[III-SEE-235]



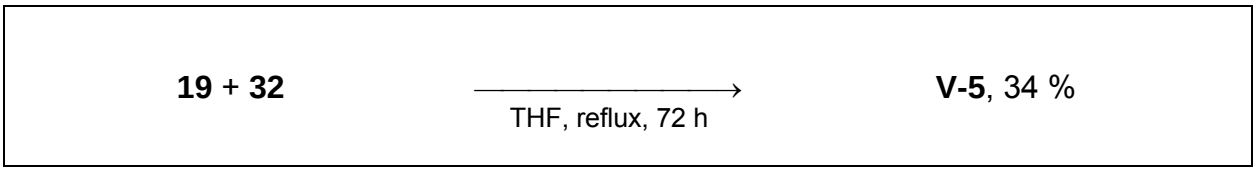
19



32



V-5



Under argon atmosphere, 4-iodobenzylchloride **32** (200 mg, 792 µmol, 1.10 equiv) was added to a solution of quinidine **19** (234 mg, 720 µmol, 1.00 equiv) in dry THF (5 ml), and the reaction mixture was refluxed for 72 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 5:1) to yield 140 mg (243 µmol, 34 %) of the product as yellow solid.

$C_{27}H_{30}ClIN_2O_2$ (576.99 g/mol)

R_f 0.47 ($CHCl_3$/MeOH 5:1)

m.p. >210 °C (decomposition)

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.40 (d; *J* = 4.5 Hz, 1H), 7.73 (d; *J* = 9.2 Hz, 1H), 7.67-7.63 (m; 1H), 7.50 (d; *J* = 7.8 Hz, 2H), 7.43-7.33 (m; 4H), 7.00 (d; *J* = 7.2 Hz, 1H), 6.29 (s; 1H), 5.85-5.74 (m; 1H), 5.61 (brs; 1H), 5.37 (brs; 1H), 5.15-5.09 (m; 2H), 4.42-4.38 (m; 1H), 3.99 (brs; 1H), 3.80 (s; 3H), 3.64-3.59 (m; 1H), 3.22-3.14 (m; 1H), 2.70-2.67 (m; 1H), 2.31-2.13 (m; 2H), 1.72-1.61 (m; 3H), 0.81-0.77 (m; 1H).

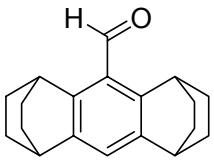
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 157.8, 146.9, 143.7, 143.0, 138.0, 135.5, 135.3, 131.3, 126.9, 126.0, 120.5, 120.4, 118.0, 102.8, 97.3, 67.5, 65.6, 61.2, 56.2, 56.1, 53.8, 38.0, 27.1, 23.8, 21.8.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3123, 2956, 2206, 1721, 1619, 1588, 1506, 1460, 1429, 1598, 1239, 1225, 1122, 1078, 1024, 1008, 904, 863, 821, 725, 642, 620.

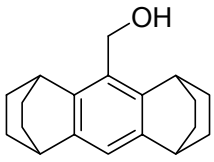
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 541.1351; found $[M\text{-}Cl]^+$: 541.135.

3.4.38 Synthesis of 1,2,3,4,5,6,7,8-Octahydro-1,4:5,8-diethano-9-anthracenemethanol (37)

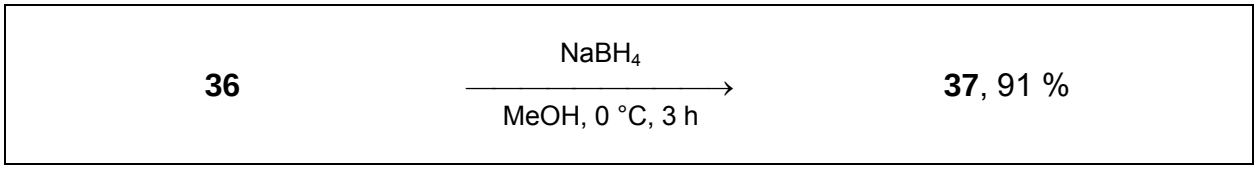
[III-SEE-227]



36



37



Under argon atmosphere, $NaBH_4$ (96.0 mg, 2.54 mmol, 5.20 equiv) was slowly added to a solution of 1,2,3,4,5,6,7,8-octahydro-1,4:5,8-diethano-9-anthracenecarboxaldehyde **36** (130 mg, 488 µmol, 1.00 eq) in dry MeOH (40 ml) at 0 °C and the reaction mixture was stirred for 3 h. 4 M HCl (4 ml) was added and the precipitate was filtered

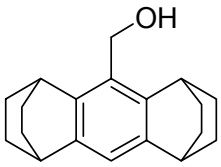
off, washed with distilled water (3 x 1 ml) and dried under vacuo to give 119 mg (443 µmol, 91 %) of the alcohol **37** as colourless solid. This was used for the synthesis of the chloride **35** without further purification

$C_{19}H_{24}O$ (268.39 g/mol)

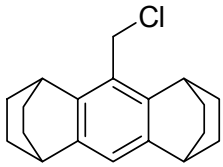
GC-MS (HP-5; 100 °C, 20 °C/min, 200 °C (15 min); He; 1.00 ml/min), τ_R (min) = 14.00 (**37**): m/z = 268 [M^+], 237, 221, 193, 165, 141, 115, 89, 76.

3.4.39 Synthesis of 9-(Chloromethyl)-1,2,3,4,5,6,7,8-octahydro-1,4:5,8-diethanoanthracene (35)

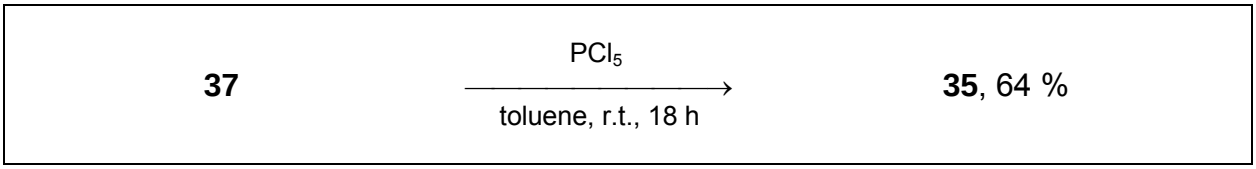
[III-SEE-228]



37



35



Under argon atmosphere, alcohol **37** (119 mg, 443 µmol, 1.00 equiv) was dissolved in dry toluene (5 ml). The mixture was cooled to 0 °C and PCl_5 (156 mg, 753 µmol, 1.70 equiv) was added. After stirring at r.t. for 18 h, saturated aq. $NaHCO_3$ (5 ml) was added at 0 °C, and the mixture was stirred for 10 min. The phases were separated and the aqueous layer was extracted with toluene (4 ml). The combined organic phases were washed with water (4 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain 80.6 mg (282 µmol, 64 %) of the chloride **35** as colourless solid.

$C_{19}H_{23}Cl$ (286.84 g/mol)

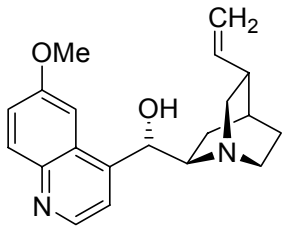
^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 6.94 (s; 1H), 4.82 (s; 2H), 3.37 (s; 2H), 2.98 (s; 2H), 1.83-1.78 (m; 8H), 1.42-1.40 (m; 8H).

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2938, 2858, 2352, 1606, 1450, 1358, 1325, 1281, 1252, 1220, 1182, 1135, 1110, 1026, 910, 859, 810, 733, 695.

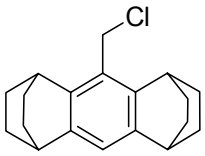
GC MS (HP-5; 100 °C, 20 °C/min, 200 °C (15 min); He; 1.00 ml/min) τ_R (min) = 13.58 (**35**): m/z = 286 [M^+], 258, 221, 179, 155, 89, 63.

3.4.40 Synthesis of (9S)-9-Hydroxy-6'-methoxy-1-[(1,2,3,4,5,6,7,8-octahydro-1,4:5,8-diethano-9-anthraceny)methyl]quinidinium chloride (V-6)

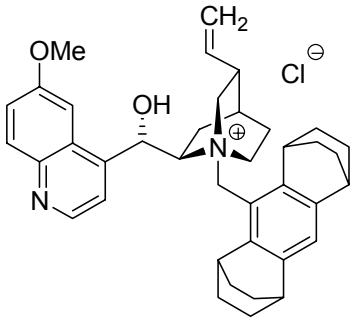
[III-SEE-224]



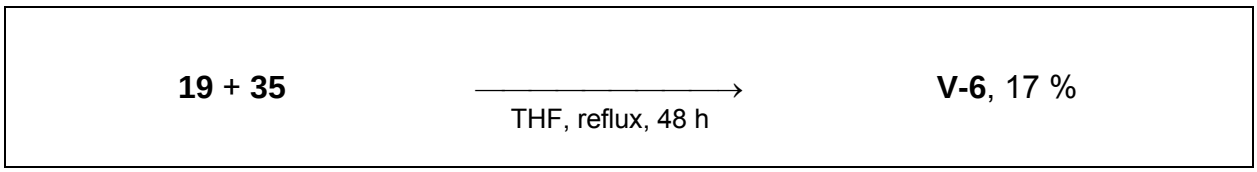
19



35



V-6



Under argon atmosphere, quinidine **19** (79.0 mg, 244 µmol, 1.00 equiv) and the corresponding chloride **35** (70.0 mg, 244 µmol, 1.00 equiv) were dissolved in dry THF (6 ml) and the reaction mixture was refluxed for 48 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 10:1) yielding 25.0 mg (40.7 µmol, 17 %) of the product **V-6** as off white-solid.

$C_{39}H_{47}ClN_2O_2$ (613.27 g/mol)

R_f 0.28 ($CHCl_3$/MeOH 10:1)

m.p. >210 °C

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.59 (s; 1H), 8.01-7.98 (m; 2H), 7.87 (d; *J* = 4.5 Hz; 1H), 7.35-7.32 (m; 2H), 7.09 (s; 1H), 6.80 (s; 1H), 6.13 (d; *J* = 12.5 Hz; 1H), 6.07-5.95 (m, 1H), 5.23-5.14 (m; 2H), 4.94 (d; *J* = 12.5 Hz,

1H), 4.73-4.66 (m; 1H), 3.97 (s; 3H), 3.80-3.70 (m; 2H), 3.52-3.41 (m; 3H, 3.05-2.99 (m; 2H), 2.58-2.50 (m; 1H), 2.45-2.37 (m; 1H), 1.95-1.80 (m; 9H), 1.68-1.62 (m; 2H), 1.45-1.20 (m; 9H), 1.05-0.97 (m; 1H).

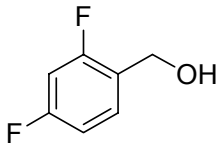
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 157.2, 147.8, 144.4, 144.2, 143.9, 143.4, 142.5, 141.9, 135.7, 132.2, 125.9, 123.3, 121.1, 120.7, 118.2, 116.3, 101.2, 69.5, 57.2, 55.8, 55.5, 54.4, 38.7, 35.0, 34.6, 31.1, 25.9, 25.7, 25.5, 25.4, 24.4, 21.6.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2954, 2858, 1721, 1619, 1585, 1505, 1469, 1432, 1357, 1321, 1288, 1239, 1176, 1133, 1090, 1074, 1025, 999, 928, 864, 825, 746, 715, 657.

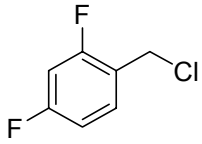
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 575.3638; found $[M\text{-}Cl]^+$: 575.364.

3.4.41 Synthesis of 2,4-Difluorobenzyl chloride (34)

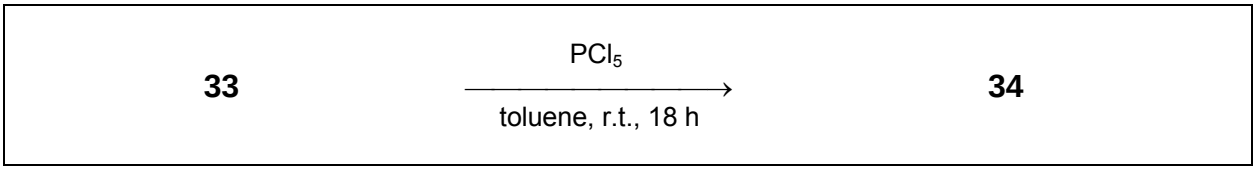
[III-SEE-243]



33



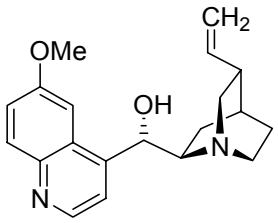
34



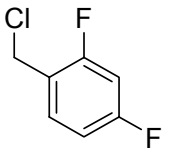
Under argon atmosphere, PCl_5 (2.95 g, 14.1 mmol, 1.70 equiv) was added to a solution of 2,4-difluorobenzyl alcohol **33** (930 µl, 1.20 g, 8.33 mmol, 1.00 equiv) in toluene (70 ml), and the reaction mixture was stirred at r.t. for 18 h. Saturated aqueous $NaHCO_3$ (70 ml) was added and the mixture was stirred for additional 15 min. The organic layer was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was used for the synthesis of 1-*N*-(2,4-difluorobenzyl)quinidinium chloride **V-7** without further purification or characterisation.

3.4.42 Synthesis of 1-N-(2,4-Difluorobenzyl)quinidinium chloride (V-7)

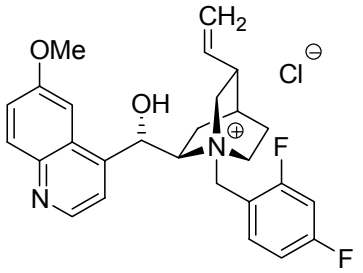
[III-SEE-246]



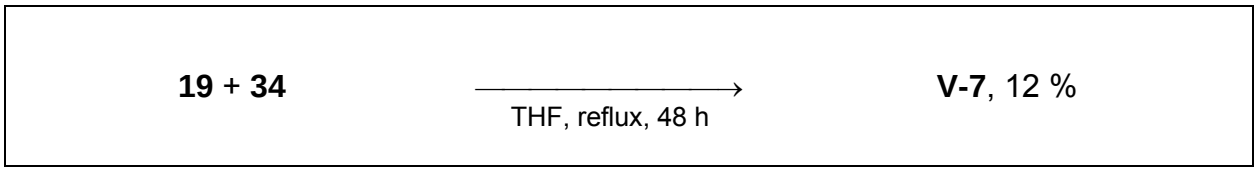
19



34



V-7



Under argon atmosphere, quinidine **19** (665 mg 2.05 mmol, 1.00 equiv) and 2,4-difluorobenzyl chloride **34** (500 mg 3.08 mmol, 1.50 equiv) were dissolved in dry THF (10 ml). The reaction mixture was refluxed for 48 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1) yielding 117 mg (240 µmol, 12 %) of the product **V-7** as off-white solid.

$C_{27}H_{29}ClF_2N_2O_2$ (486.98 g/mol)

R_f 0.61 ($CHCl_3$/MeOH 7:1)

m.p. 175 °C

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.51 (d; *J* = 4.4 Hz, 1H), 8.08-8.00 (m; 1H), 7.81 (d; *J* = 9.2 Hz, 1H), 7.68 (d; *J* = 4.4 Hz, 1H), 7.44 (s; 1H), 7.19 (s; 1H), 7.12 (d; *J* = 9.3 Hz, 1H), 6.87-6.83 (m; 1H), 6.76-6.73 (m; 1H), 6.40 (s; 1H), 5.91-5.76 (m; 2H), 5.17-5.11 (m; 3H), 4.56-4.50 (m; 1H), 4.02-3.89 (m; 2H), 3.78 (s; 3H), 3.20-3.12 (m; 1H), 2.83-2.73 (m; 1H), 2.42-2.26 (m; 2H), 1.78 - 1.68 (m; 3H), 0.84-0.76 (m; 1H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 164.4 (dd; $^3J_{C\text{-}F}$ = 12.3 Hz, $^1J_{C\text{-}F}$ = 255.1 Hz), 162.1 (dd; $^3J_{C\text{-}F}$ = 12.3 Hz, $^1J_{C\text{-}F}$ = 252.7 Hz), 158.0, 147.1, 143.9, 143.0, 137.5 (d; $^3J_{C\text{-}F}$ = 7.4 Hz), 135.4, 131.5, 125.8, 121.0, 120.4,

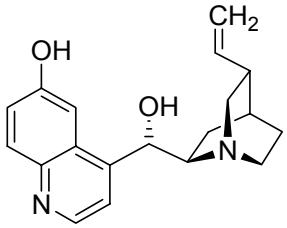
118.1, 112.7 (d; $^2J_{C\text{-}F}$ = 21.7 Hz), 111.1 (d; $^2J_{C\text{-}F}$ = 13.6 Hz), 104.5 (t; $^2J_{C\text{-}F}$ = 25.8 Hz), 101.6, 68.4, 65.8, 56.1, 55.9, 55.5, 54.3, 38.1, 26.9, 23.9, 21.5

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3107, 2204, 1617, 1505, 1471, 1430, 1324, 1287, 1239, 1225, 1144, 1101, 1024, 1002, 966, 912, 853, 826, 729, 661, 639.

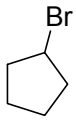
HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 451.2197; found $[M\text{-}Cl]^+$: 451.219.

3.4.43 Synthesis of 6'-Cyclopentyloxy-cinchonine (27)

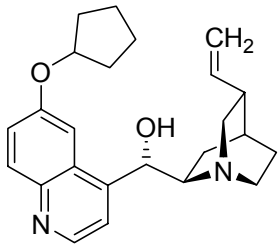
[III-SEE-203]



22



28



27

22 + **28**	$\xrightarrow[\text{DMF, 60 °C, 40 h}]{Cs_2CO_3}$	**27**, 84 %

Under argon atmosphere, cesium carbonate (1.31 g, 4.03 mmol, 2.50 equiv) and cyclopentyl bromide **28** (345 µl, 450 mg, 3.22 mmol, 2.00 eq) were added to a solution of 6'-hydroxycinchonine **22** (500 mg, 1.61 mmol, 1.00 equiv) in dry DMF (60 ml). The reaction mixture was stirred for 40 h at 60 °C. After cooling to r.t., the solids were filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (20 ml) and was washed with dist. H_2O (20 ml). The solvent was removed under reduced pressure and the residue was dried in vacuo to yield 510 mg (1.35 mmol, 84 %) of the product **27** as light orange solid.

$C_{24}H_{30}N_2O_2$ (378.23 g/mol)

m.p. 173 °C

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.43 (d; *J* = 4.3 Hz, 1H), 7.80 (d; *J* = 9.2 Hz, 1H), 7.42 (d; *J* = 4.3 Hz, 1H), 7.20-7.06 (m; 2H), 6.04-5.92 (m; 1H),

5.53 (s; 1H), 5.30 (brs; 1H), 4.99-4.94 (m; 2H), 4.62 (s; 1H), 3.36-3.29 (m; 1H), 2.94-2.92 (m; 1H), 2.84-2.61 (m; 3H), 2.17-2.09 (m; 1H), 1.99-1.92 (m; 1H), 1.73-1.40 (m; 11H), 1.03-0.97 (m; 1H).

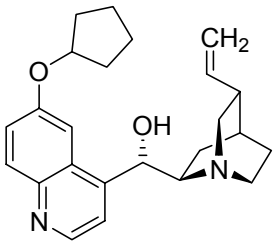
^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 155.9, 147.8, 147.1, 143.7, 140.6, 131.2, 126.4, 122.5, 118.5, 114.4, 103.0, 79.5, 71.9, 59.7, 50.3, 49.7, 40.1, 32.9, 32.5, 28.3, 26.4, 24.1, 21.0.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3064, 2936, 2868, 1723, 1616, 1588, 1505, 1456, 1353, 1238, 1218, 1196, 1168, 1107, 1078, 1047, 986, 906, 858, 828, 729, 641.

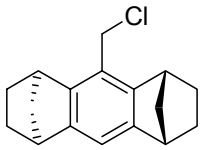
HR ESI-MS (m/z): exact mass $[M+H]^+$: 379.2385; found $[M+H]^+$: 379.239.

3.4.44 Synthesis of 1-N-[9-((1,8-R;4,5-S)-1,2,3,4,5,6,7,8-Octahydro-1,4:5,8-dimethanoanthracenyl)methyl]-6'-(cyclopentyloxy)cinchonine (V-8)

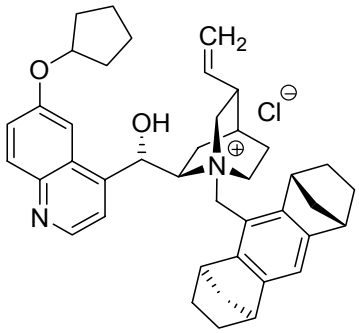
[III-SEE-204]



27



24



V-8

27 + **24** → (THF, reflux, 18 h) **V-8**, 38 %

Under argon atmosphere, 6'-cyclopentyloxy-cinchonine **27** (200 mg, 536 µmol, 1.00 equiv) and 9-chloromethyl-[(1,8-*S*;4,5-*R*)-1,2,3,4,5,6,7,8-octahydro-1,4;5,8-dimethanoanthracene **24** (153 mg 590 µmol, 1.10 equiv) were dissolved in dry THF (5 ml) and refluxed for 18 h. The solvent was removed under reduced pressure. The residue was dissolved in MeOH (1 ml) and was added dropwise to Et_2O (100 ml). The suspension was stirred for 15 min. The precipitate was filtered off and was further

purified by flash chromatography ($CHCl_3$/MeOH 8:1) to yield 129 mg (203 µmol, 38 %) of the product **V-8** as off-white solid.

$C_{41}H_{49}ClN_2O_2$ (637.29 g/mol)

R_f 0.40 ($CHCl_3$/MeOH 8:1)

m.p. 181 °C

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.62 (d; *J* = 4.2 Hz, 1H), 7.96 (d; *J* = 9.2 Hz, 1H), 7.76 (d; *J* = 4.2 Hz, 1H), 7.26 (brs; 1H), 7.28 (d; *J* = 9.2 Hz, 1H), 7.17 (s; 1H), 7.04 (s; 1H), 6.61 (s; 1H), 6.00-5.81 (m; 2H), 5.18-5.11 (m; 2H), 4.91 (s; 1H), 4.61-4.55 (m; 1H), 4.40 (d; *J* = 12.8 Hz, 1H), 3.96 (brs; 1H), 3.73-3.67 (m; 1H), 3.57-3.50 (m; 1H), 3.31 (brs; 4H), 3.10-3.04 (m; 1H), 2.47-37 (m; 2H), 1.88-59 (m; 17H), 1.48-1.45 (m; 2H), 1.18-0.90 (m; 5H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 156.4, 148.8, 147.4, 145.7, 143.6, 143.3, 135.6, 131.8, 125.8, 121.4, 120.5, 118.1, 117.1, 113.3, 103.9, 79.8, 69.1, 65.0, 60.0, 56.4, 54.0, 50.5, 48.4, 44.1, 43.0, 38.3, 33.0, 26.9, 26.7, 26.5, 24.5, 24.2, 21.7.

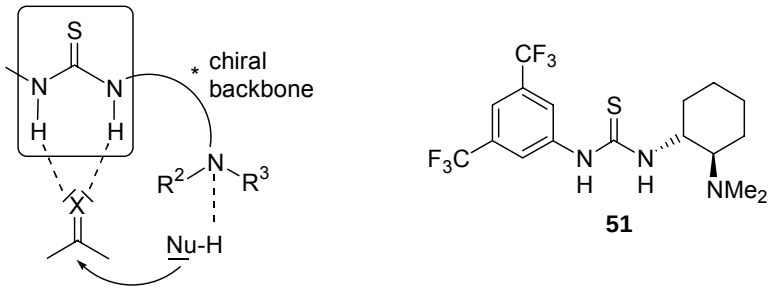
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2957, 2868, 2200, 1706, 1617, 1586, 1504, 1459, 1350, 1328, 1256, 1238, 1220, 1169, 1110, 1090, 1045, 988, 907, 864, 826, 723.

HR ESI-MS (m/z): exact mass $[M\text{-}Cl]^+$: 601.3794; found $[M\text{-}Cl]^+$: 601.380.

4 A Simplified Synthesis of Takemoto's Catalyst

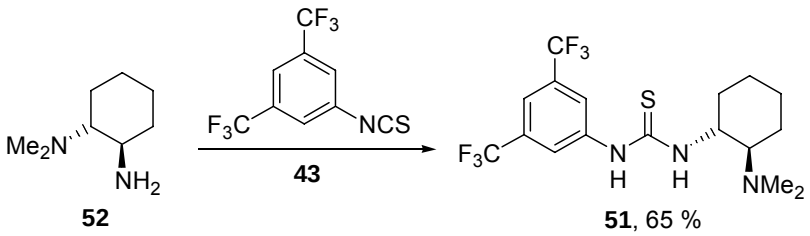
4.1 Background

Urea and thiourea derivatives have been subject to extensive investigations in the area of molecular recognition due to their strong hydrogen-bonding ability. *Kelly*[62] and *Etter*[63] reported that (thio)urea not only recognise organic compounds but also activate substrates by acting as general acidic catalysts. Since then, novel urea and thiourea derivatives have been developed for a variety of diastereo- and enantioselective reactions. Their versatility as general acids has been successfully demonstrated by several groups.[64] However, the use of these catalysts for enantioselective reactions has been rather limited as ureas are less acidic than metallic Lewis acids. To address this problem, bifunctional thiourea catalysts have been developed, which activate the nucleophile by deprotonation and the electrophile by hydrogen bonding (Scheme 4-1). Among the bifunctional systems developed, *Takemoto's* catalyst is the most prominent.[5] A significant number of C-C couplings, for example, Michael-,[65,66] Mannich-,[67] aza-Henry-[68] reactions, or the alcoholytic dynamic kinetic resolution of azlactones[69] are catalysed enantioselectively by *Takemoto's* bifunctional aminothiourea derivative **51**.



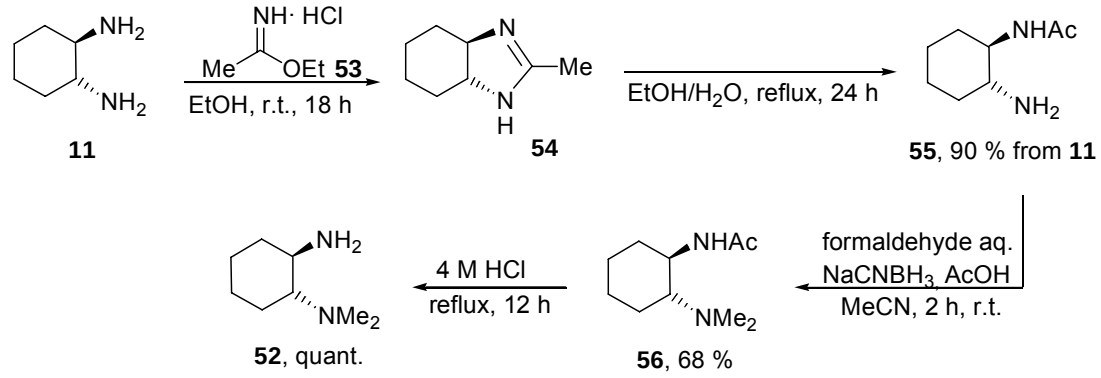
Scheme 4-1 Principle of the dual activation by bifunctional (thio)ureas (left), amino thiourea **51** (*Takemoto's* catalyst, right).

The original synthesis of *Takemoto's* catalyst **51** involves the addition of isothiocyanate **43** to *N*,*N*-dimethyl-*trans*-1,2-diaminocyclohexane **52** (Scheme 4-2).[65]



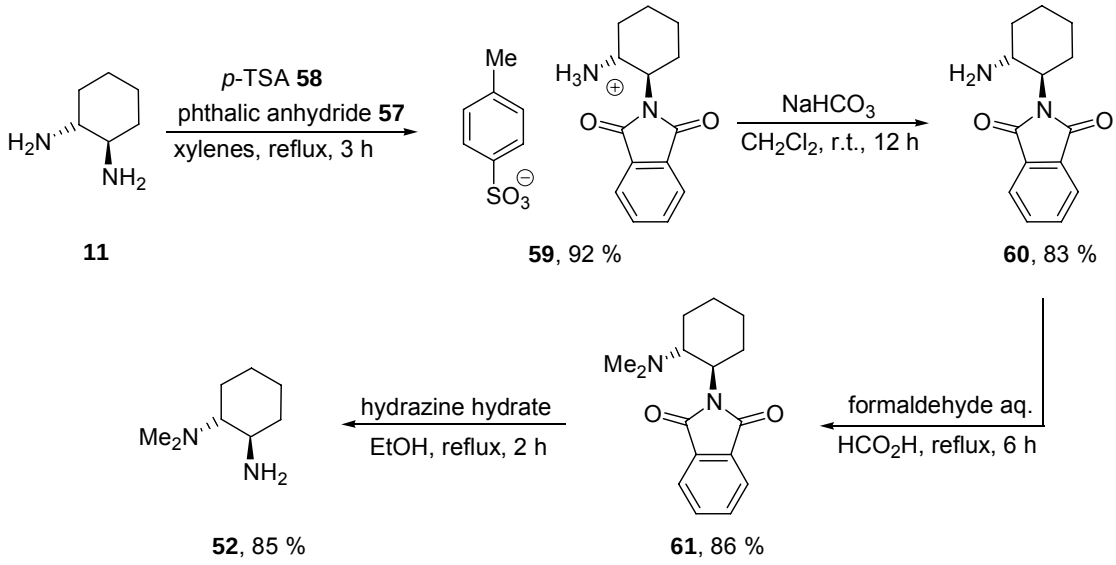
Scheme 4-2 Synthesis of the amino thiourea **51** developed by *Takemoto*.[65]

The main drawback of this approach is the laborious synthesis of the *N*,*N*-dimethyl-*trans*-1,2-diaminocyclohexane building block **52**, which was first described by *Finney* and coworkers in 2000.[6] As shown in Scheme 4-3, condensation of (1*R*,2*R*)-1,2-diaminocyclohexane **11** with the Pinner salt **53** derived from acetonitrile provides the corresponding imidazoline **54**. Refluxing a solution of **54** in ethanol-water mixture leads to the corresponding mono-acetyl diamine **55**. The diamine **55** is converted to the *N*,*N*-dimethyl derivative **56** by reductive amination, followed by acidic cleavage of the acetamide **56**.



Scheme 4-3 Synthesis of *N*,*N*-dimethylamine **52** according to *Finney.*[6]

An alternative approach for the synthesis of the *N*,*N*-dimethyl-*trans*-1,2-diaminocyclohexane building block **52** was described in 2003 by *Kaik* and *Gawronski.*[7] The desired compound **52** was synthesised *via* a monoprotection of the (1*R*,2*R*)-1,2-diaminocyclohexane **11** with phthalic anhydride **57**. However, this approach also involves a tedious four-step procedure (Scheme 4-4).



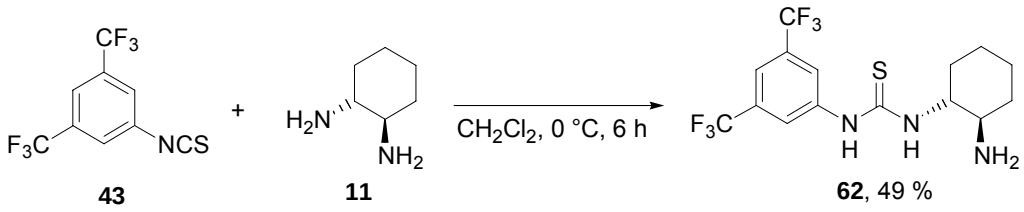
Scheme 4-4 Synthesis of *N*,*N*-dimethylamine **52** reported by *Kaik* and *Gawronski.*[7]

In the first step, mono-protection of the (1*R*,2*R*)-1,2-diaminocyclohexane **11** is achieved by reaction with phthalic anhydride **57** in the presence of *p*-toluenesulfonic acid **58**. Deprotonation of the corresponding salt **59** yields the mono-protected diamine **60**. Reductive amination affords the *N'*-phthaloyl-protected *N*,*N*-dimethylamine **61**. Finally, deprotection yields the desymmetrized *N,N*-dimethyl diamine **52**.

4.2 Concept

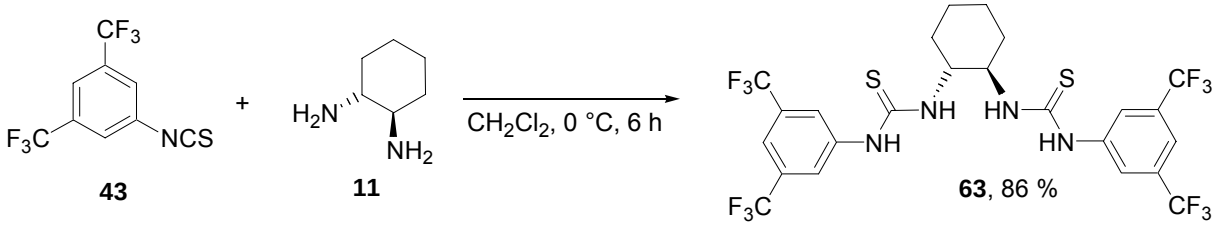
An improved synthesis of *Takemoto's* catalyst **51** could be realised in two steps if the readily available and cheap (1*R*,2*R*)-1,2-diaminocyclohexane **11** can be transformed to the mono-thiourea **62**. A subsequent dimethylation would lead to the fully assembled catalyst **51**. A literature survey afforded valuable information regarding the synthesis of mono-thiourea derivatives of diamines:

Connon and coworkers reported the direct addition of the (1*R*,2*R*)-1,2-diamino-cyclohexane **11** to isothiocyanate **43** in 49 % yield (Scheme 4-5).[70]



Scheme 4-5 Synthesis of the mono-thiourea **62** as reported by *Connon*.[70]

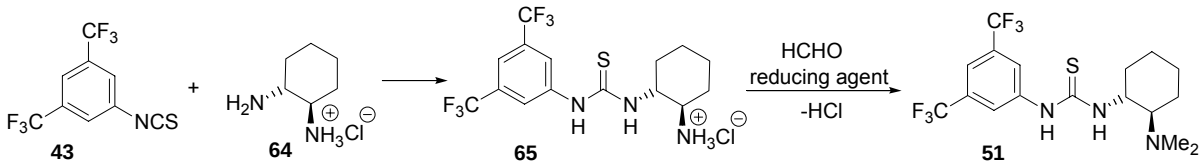
However, in our hands, following *Connon's* procedure led to the bis-thiourea **63** in 86 % yield from equimolar amounts of **43** and **11** (Scheme 4-6). This observation has been confirmed by several other working groups.[71,72]



Scheme 4-6 Formation of the bis-thiourea derivative **63**, following *Connon's* protocol.[71,72]

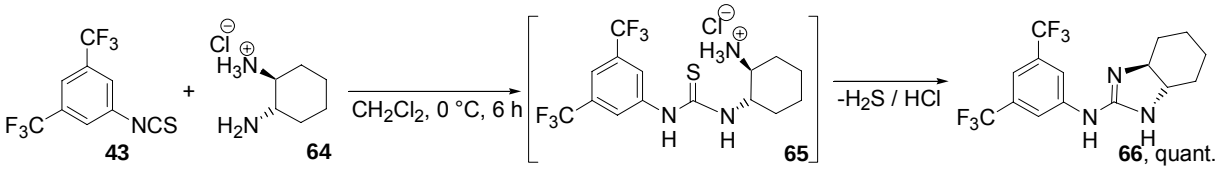
The formation of the corresponding bis-thiourea **63** could be avoided either (1) by mono-protection of the diamine **11** or (2) by decreasing the reactivity of the isothiocyanate **43**.

(1) Since covalent mono-protection of the diamines is normally accompanied by two additional protection/deprotection steps, this approach seems to be less favorable. In principle, the diamine **11** could be also protected as an ammonium chloride **64**, since the HCl "protecting group" can be cleaved *in situ* with $NaBH_3CN$[73] in the following methylation step (Scheme 4-7).



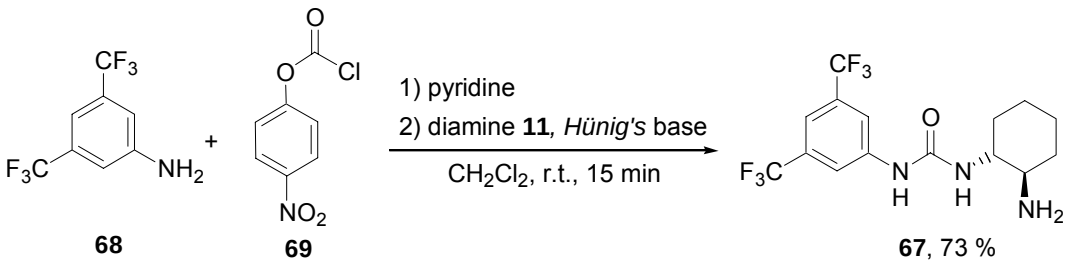
Scheme 4-7 Proposed addition of isothiocyanate **43** to the HCl mono-protected diamine **64** and *in situ* cleavage of the protecting group in the following methylation step.

However, as described by *Moreau*, the addition of aromatic isothiocyanate **43** to HCl mono-protected (1*R*,2*R*)-1,2-diaminocyclohexane **64** does not result in the corresponding mono-thiourea **65**. Instead, the guanidine derivative **66** is formed by cyclisation of the thiourea **65** and elimination of H_2S (Scheme 4-8).[71,72]



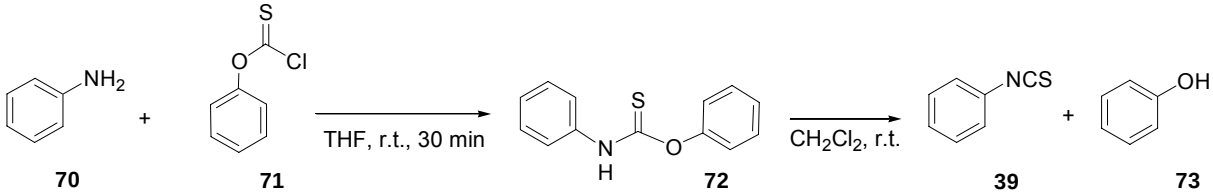
Scheme 4-8 Formation of guanidine derivative **66** by addition of isothiocyanate **43** to HCl mono-protected diamine **64**.[72]

(2) The group of *Nagasawa* observed that the urea analogue **67** can be prepared by condensation of 3,5-bis(trifluoromethyl)aniline **68** with 4-nitrophenylchloroformate **69** followed by addition of (1*R*,2*R*)-1,2-diaminocyclohexane **11** (Scheme 4-9, p. 94).[8]



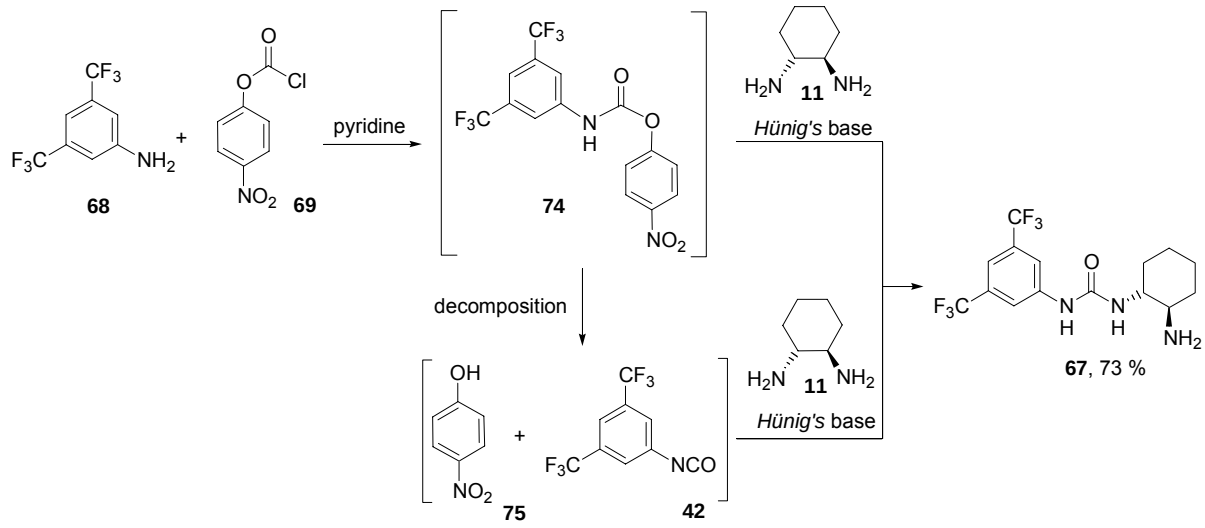
Scheme 4-9 Synthesis of the amino urea **67** according to *Nagasawa*.[8]

Rivier and coworkers[74] reported the reaction of aniline **70** with phenyl chlorothiocarbonate **71** to form phenyl thiophenylcarbamate **72** (Scheme 4-10). Furthermore, when stirring the phenyl thiophenylcarbamate **72** in CH_2Cl_2 at r.t. *Schneider*[75] observed its slow decomposition to the corresponding isothiocyanate **39** and phenol **73** (Scheme 4-10).



Scheme 4-10 Formation of phenyl thiophenylcarbamat **72** and its decomposition to isothiocyanate **39** and phenol **73**.[74,75]

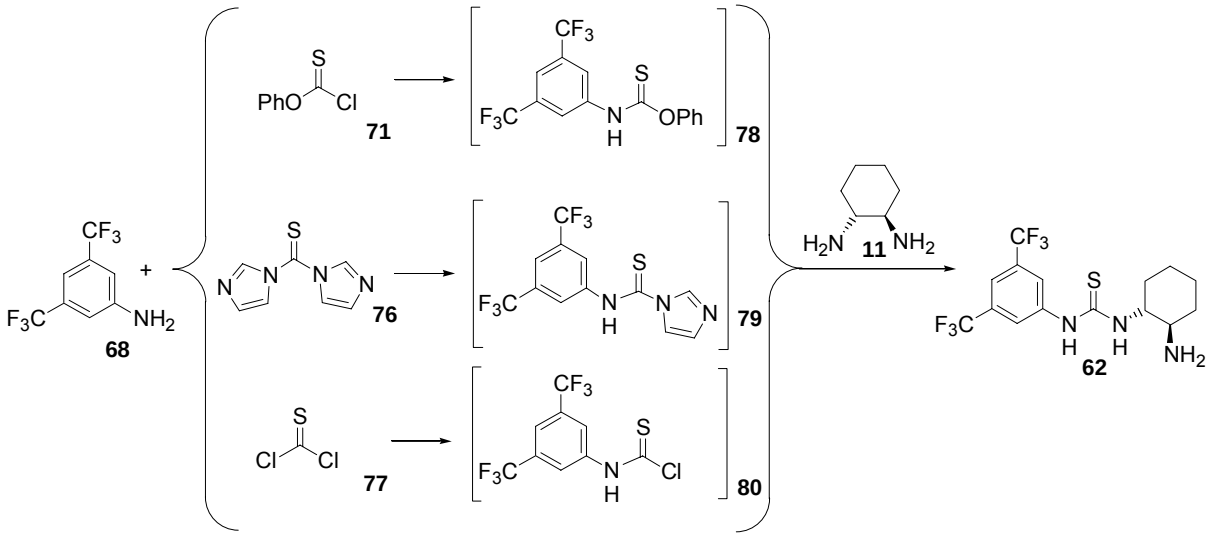
Based on the work of *Rivier*[74] and *Schneider*[75] the reaction described by *Nagasawa*[8] (Scheme 4-9) can be interpreted as follows:



Scheme 4-11 Proposed formation of intermediate **74**.

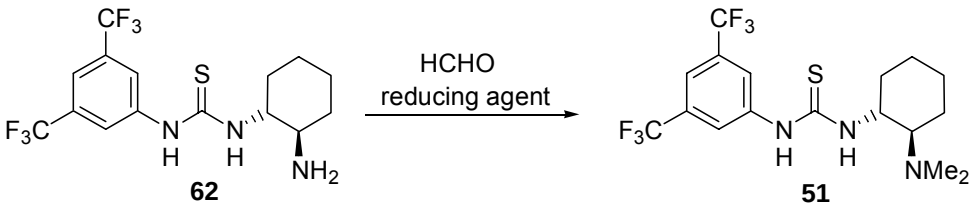
The aniline derivative **68** first reacts with 4-nitrophenylchloroformate **69** to form the corresponding intermediate **74**. Then it reacts either directly with the (1*R*,2*R*)-1,2-diaminocyclohexane **11** with elimination of the phenolate moiety or first decomposes to the isocyanate **42** which adds to (1*R*,2*R*)-1,2-diaminocyclohexane **11** (Scheme 4-11, p. 94).

Since the reaction of intermediate **74** with the diamine **11** yields only the monothiourea, it can be assumed that intermediate **74** represents a less reactive synthetic equivalent for isocyanate **43**.
Hence, to obtain the desired thiourea equivalent **62**, 4-nitrophenylchloroformate **69** could be replaced by a suitable, commercially available thio-analogue, e.g. **71**, **76**, and **77** (Scheme 4-12).



Scheme 4-12 Thiocarbonyl derivatives for the synthesis of thiourea **62**.

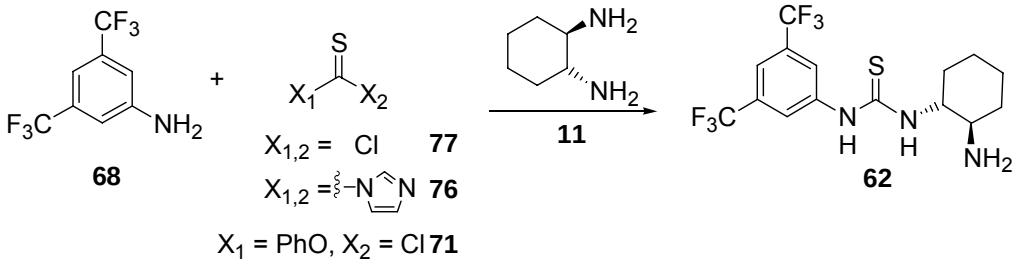
Finally, methylation of the primary amino function should yield the desired *Takemoto*'s catalyst **51** (Scheme 4-13).



Scheme 4-13 Methylation with formaldehyde.

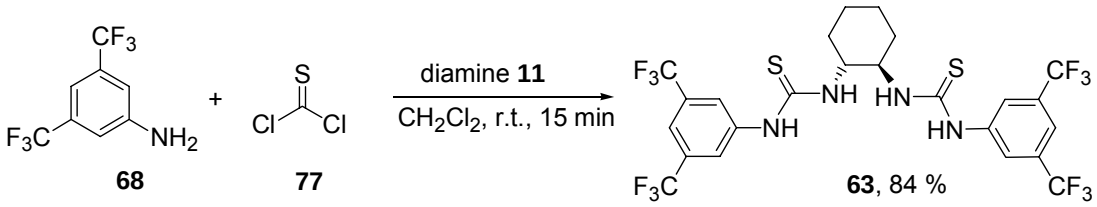
4.3 Results and Discussion

The approach for the synthesis of the thiourea **62** proceeds *via* the reaction of aniline derivative **68** with the *Staab*-reagent **76**, thiophosgene **77** or phenyl chlorothioformate **71** according to the synthetic procedure described by *Nagasawa*[8] (Scheme 4-14).



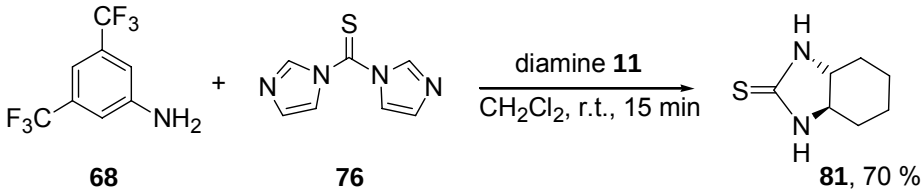
Scheme 4-14 Screening of different thiocarbonyl compounds, i.e. **71**, **76** and **77**, for the synthesis of mono-thiourea **62**.

Unfortunately, neither employing thiophosgene **77** nor the *Staab*-reagent **76** led to the desired product **62**. Using thiophosgene **77** resulted in the formation of bis-thiourea **63** in 84 % yield (Scheme 4-15).



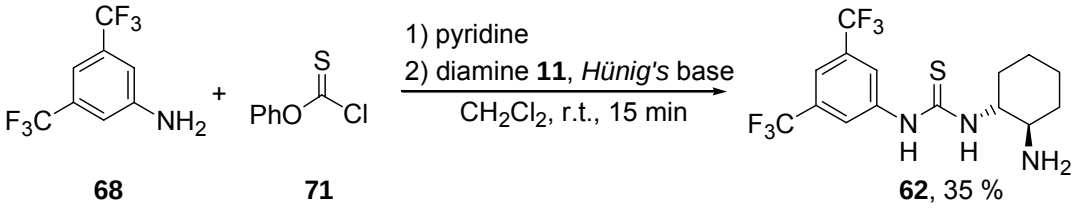
Scheme 4-15 Formation of bis-thiourea §§ from thiophosgene §§.

Application of the *Staab*-reagent **76** led predominantly to the formation of *trans*-4,5-tetramethyleneimidazolidine-2-thione **81** (Scheme 4-16).



Scheme 4-16 Formation of *trans*-4,5-tetramethyleneimidazolidine-2-thione **81** using *Staab*-reagent **76**.

Using phenyl chlorothioformate **71**, the desired mono-thiourea **62** was obtained in 35 % yield (Scheme 4-17, p. 97).

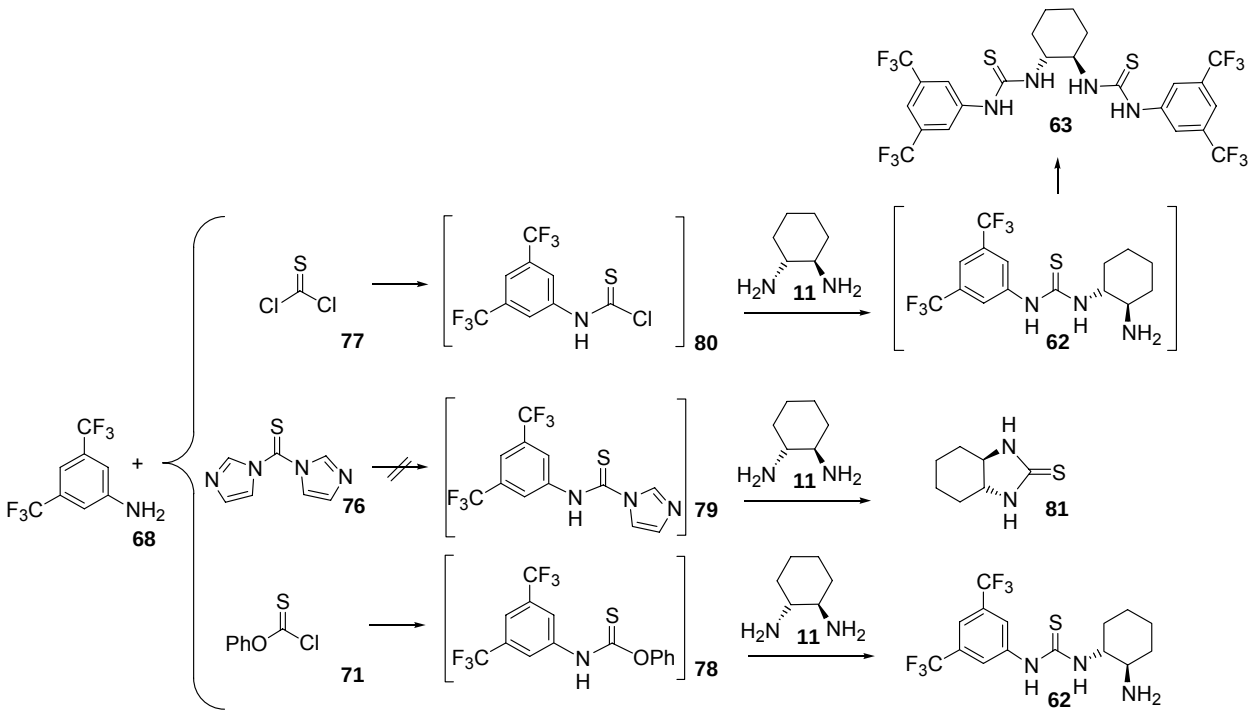


Scheme 4-17 Formation of thiourea **62** using phenylchlorothioformate **71**.

Based on the different reactivities of the thiocarbonyl compounds **71**, **76** and **77** an explanation for the observed differences may be as follows:

The very electrophilic thiophosgene **77** couples with the aniline derivative **68** to the highly reactive intermediate **80**, which then reacts with the diamine **11** to form the mono-thiourea **62**. After mono-thioacylation, the diaminocyclohexane derivative **62** should exhibit a lower NH_2-nucleophilicity than the free diaminocyclohexane **11**. Despite of that, the high electrophilicity of the intermediate **80** leads to fast bis-thioacylation and therefore results in the formation of the bis-thiourea **63** (Scheme 4-18).

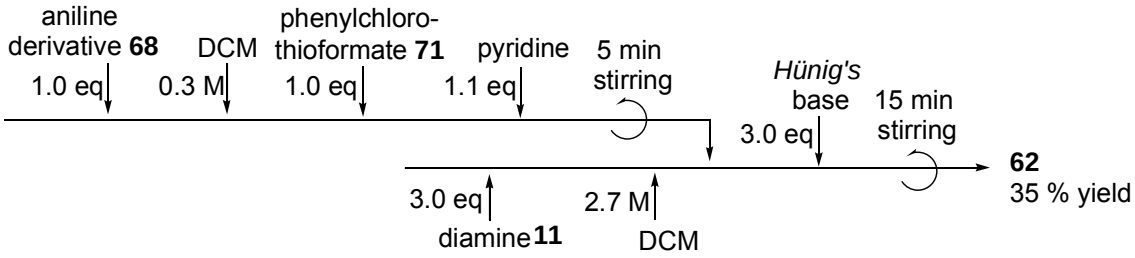
The less electrophilic *Staab*-reagent **76** does not react with the weakly nucleophilic NH_2-function of the aniline derivative **68**, but with the more nucleophilic NH_2-function of the diamine **11**, forming the cyclic thiourea **81** (Scheme 4-18).



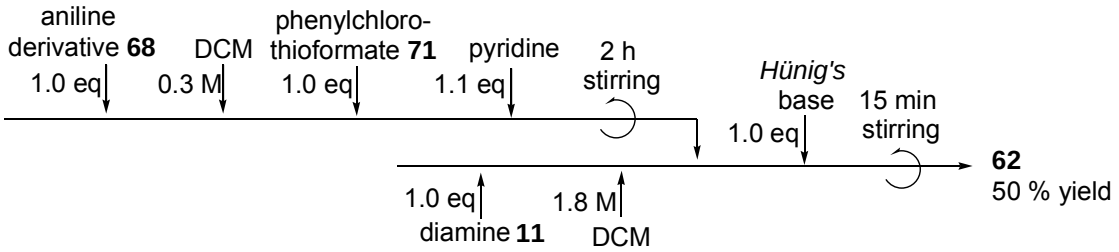
Scheme 4-18 Different reaction outcomes employing **71**, **76** or **77**.

In contrast, the phenyl chlorothioformate **71** has nucleofuges (Cl, OPh) with rather different reactivities. The more reactive Cl-leaving group is substituted by the weakly nucleophilic NH_2-group of the aniline derivative **68**. The intermediate formed **78** is less reactive than intermediate **80**. Therefore, reaction with diaminocyclohexane **11** leads predominantly to the desired mono substituted amino thiourea **62** (Scheme 4-18, p. 97).

To improve the yield of the mono-thiourea **62**, different reaction conditions were screened, based on the original reaction protocol[8] [values in parentheses] (Scheme 4-19). Stirring the solution of the aniline derivative **68** and phenyl chlorothioformate **71** for 2 h [5 min] and using a 1.8 M [2.7 M] solution of the diamine **11** led to an optimised yield of 50 % by using only 1.0 eq of the diamine **11** [3.0 eq] and 1.0 eq of *Hünig's* base [3.0 eq] (Scheme 4-20).



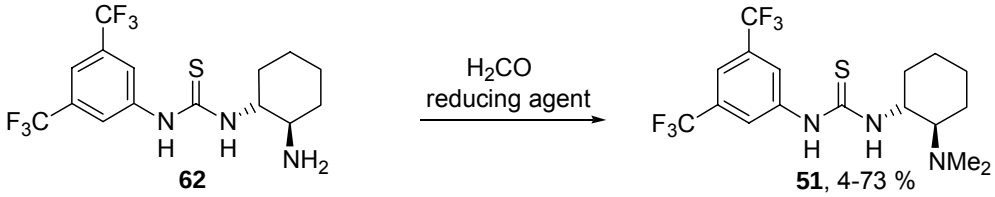
Scheme 4-19 Reaction protocol according to *Nagasawa*.[8]

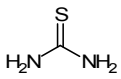
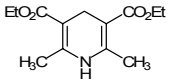


Scheme 4-20 Reaction protocol after optimisation.

The final step in the synthesis of *Takemoto's* catalyst **51** is the reductive methylation of the free primary amino function with formaldehyde. In a screening for the most suitable reducing agent (Table 4-1, p. 99), zinc powder proved best (Table 4-1, entry 2, 73 %).

Table 4-1 Screening of reducing agents for the reductive methylation of amino thiourea **62**.



Entry	Additive	Reducing Agent	Time (h)	Solvent	Temp (°C)	Yield (%)
1[76]			72	toluene	60	27
2[77]	CH_3CO_2H	Zn	72	dioxane	r.t.	73
3[78]	-	NaH_2PO_3	1	dioxane	60	68
4[7]	-	HCO_2H	6	HCO_2H	reflux	4
5[6]	CH_3CO_2H	$NaCNBH_3$	2	MeCN	r.t.	32

In conclusion, an efficient two-step synthesis of *Takemoto's* catalyst **51**, using commercially available starting materials has been developed. As the key step, the amino thiourea **62** was prepared by condensation of 3,5-bis(trifluoromethyl)aniline **68** with phenyl chlorothioformate **71**, and substitution of phenol by *trans*-1,2-diaminocyclohexane **11**. Reductive dimethylation with formaldehyde / zinc yielded *Takemoto's* catalyst **51** in an overall yield of 37 %.

4.4 Experimental Part

4.4.1 General Experimental Conditions

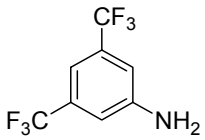
Flash chromatography was performed on silica gel (Macherey-Nagel, MN-Kieselgel 60, 230-240 mesh). TLC was performed on aluminium backed silica plates (Macherey-Nagel, Polygram© SIL G/UV_{254}), detection by UV fluorescence. Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker DPX 300 instrument; ^{13}C-NMR spectra at 75.5 MHz. Chemical shifts (δ) are given in parts per million (ppm) referenced to TMS. For the fine-structure interpretation the abbreviations of the signals are the following: s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution mass spectra (HR ESI-MS) were recorded on a Finnigan MAT 900 ST spectrometer. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer using the ATR

technique and on a Perkin Elmer 1600 Series FT-IR spectrometer. All commercially available chemicals were used without further purification.

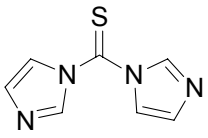
All experiments are characterised by a number within the bracket: [XX-SEE-XXX]. The roman number in front of the three-letter-code indicates the volume number of the laboratory notebook, whereas the number after the three-letter-code indicates the experiment number in the corresponding notebook volume.

4.4.2 Preparation of trans-4,5-Tetramethyleneimidazolidine-2-thione (81)

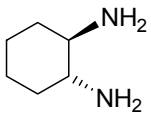
[III-SEE-250]



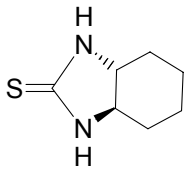
68



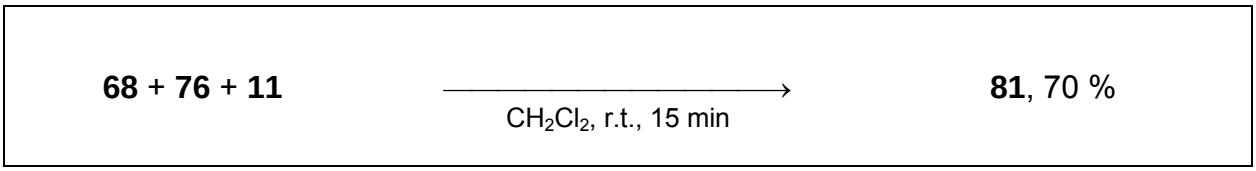
76



11



81



1,1'-thiocarbonyl diimidazole **76** (806 mg, 4.52 mmol, 1.00 equiv) was added to a solution of 3,5-bis(trifluoromethyl)aniline **68** (700 µl, 1.03 mg, 4.52 mmol, 1.00 equiv) in CH_2Cl_2 (15 ml) at r.t.. The mixture was stirred for 5 min, then added dropwise to a solution of (1*R*,2*R*)-1,2-diaminocyclohexane **11** (1.54 g, 13.5 mmol, 3.00 equiv) in CH_2Cl_2 (5 ml) and the resulting mixture was stirred for 15 min. Saturated aq. $NaHCO_3$ (20 ml) was added and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 15 ml). The combined organic layers were washed with brine (40 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 8:1) to give the product **81** as light yellow crystals (491 mg, 3.14 µmol, 70 %).

$C_7H_{12}N_2S$ (156.25 g/mol)

m.p. 151 °C [m.p. ref.[79]:148-150 °C]

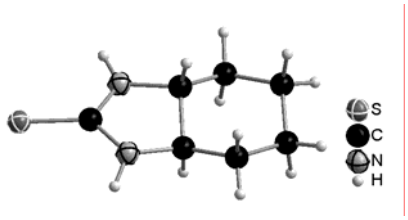
R_f 0.68 ($CHCl_3$/MeOH 8:1)

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 6.91 (brs; 2H), 3.28-3.24 (m; 2H), 2.04-2.01 (m; 2H), 1.82-1.79 (m; 2H), 1.47-1.26 (m; 4H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 187.2, 64.9, 28.9, 23.7.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3430, 2940, 1580, 1350.

X-RAY yellow crystals from chloroform



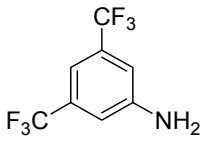
Empirical formula:	$C_7H_{12}N_2S$
Formula weight (M):	156.25
Temperature (T):	100(2) K
Wavelength (λ):	0.71073 A
Crystal system:	monoclinic, P21
Unit cell dimensions:	a = 5.9624(13) Å α = 90 °
	b = 8.6670(9) Å β = 101.184(6) °
	c = 8.2615(16) Å γ = 90 °
Unit cell volume:	418.82(13) $Å^3$
Z:	2
Calculated density:	1.239 mg/m^3
Absorption coefficient:	0.315 mm^{-1}
F(000):	168
Crystal size:	0.3 x 0.3 x 0.07 mm
Theta range for data collection:	2.51 ° to 26.98 °
Limiting indices	$-7 \le h \le 7$, $-8 \le k \le 10$, $-7 \le l \le 10$
Reflections collected:	2131
unique reflections:	1647 [R_{int} = 0.0223]
Reflection observed [$I > 2\sigma(I)$]:	1457
Completeness to Θ (= 26.98 °):	99.7 %
Refinement method:	Full-matrix least-squares on F^2
Data / restraints / parameters:	1647 / 1 / 139
Goodness-of-fit on F^2:	0.933
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0312, ωR2 = 0.0651
R indices (all data):	R1 = 0.0391, ωR2 = 0.0677
Absolute structure parameter:	0.03(8)

Largest diff. peak and hole: 0.263 and -0.225 e·Å^{-3}

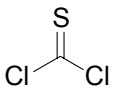
The spectroscopical data are in agreement with the literature.[79]

4.4.3 Preparation of 1-(3,5-Bis-trifluoromethyl-phenyl)-3-{(1R,2R)-2-[3-(3,5-bis-trifluoromethyl-phenyl)-thioureido]-cyclohexyl}-thiourea (63)

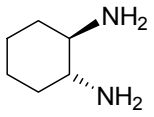
[IV-SEE-253]



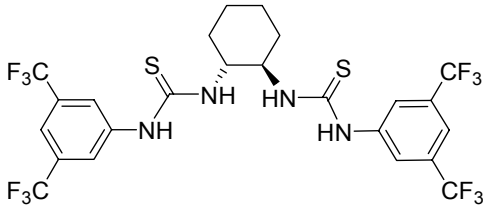
68



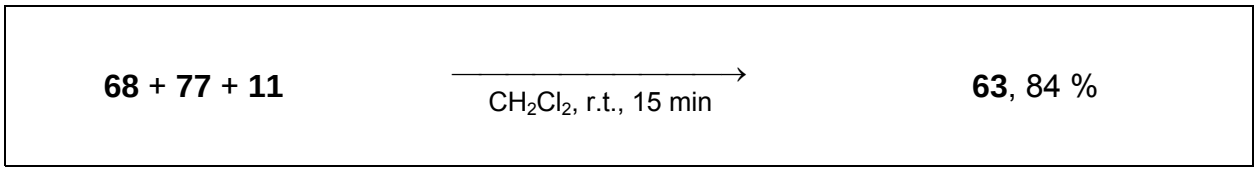
77



11



63



Thiophosgene **77** (344 µl, 520 mg, 4.52 mmol, 1.00 equiv) was added to a solution of 3,5-bis(trifluoromethyl)aniline **68** (700 µl, 1.03 mg, 4.52 mmol, 1.00 equiv) in CH_2Cl_2 (15 ml) at r.t.. The mixture was stirred for 5 min, then added dropwise to a solution of (1*R*,2*R*)-1,2-diaminocyclohexane **11** (1.54 g, 13.5 mmol, 3.00 equiv) in CH_2Cl_2 (5 ml). The resulting mixture was stirred for 15 min. Saturated aq. $NaHCO_3$ (20 ml) was added, the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 15 ml). The combined organic layers were washed with brine (40 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (*c*-hexane/EtOAc 2:1) to give the product **63** as light yellow crystals (1.24 g, 1.89 mmol, 84 %).

$C_{24}H_{20}F_{12}N_4S_2$ (656.55 g/mol)

m.p. 128-129 °C [m.p. ref.[57]: 131-132 °C]

R_f 0.40 (*c*-hexane/EtOAc 2:1)

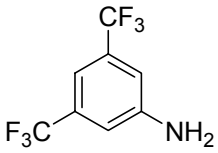
^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 8.41 (s; 2H), 7.84 (s; 4H), 7.68 (s; 2H), 7.19 (s; 2H), 4.38 (s; 2H), 2.19 (s; 2H), 1.80 (s; 2H), 1.33 (s; 4H).

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 180.4, 138.8, 132.6 (q; $^{2}J_{C\text{-}F}$ = 30.1 Hz), 123.9, 122.7 (d; $^{1}J_{C\text{-}F}$ = 274.1 Hz), 119.5, 59.3, 312.7, 24.4.

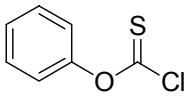
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3245, 3048, 2942, 2860, 1791, 1699, 1621, 1538, 1471, 1373, 1266, 1183, 999, 974, 956, 885, 847, 787, 731, 681.

The spectroscopical data are in agreement with the literature.[57]

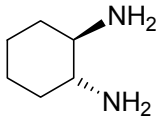
4.4.4 Preparation of 1-[(1R,2R)-2-Aminocyclohexyl]-3-[3,5-bis(trifluoromethyl)-phenyl]thiourea (62)



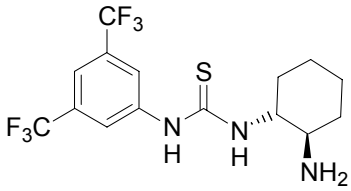
68



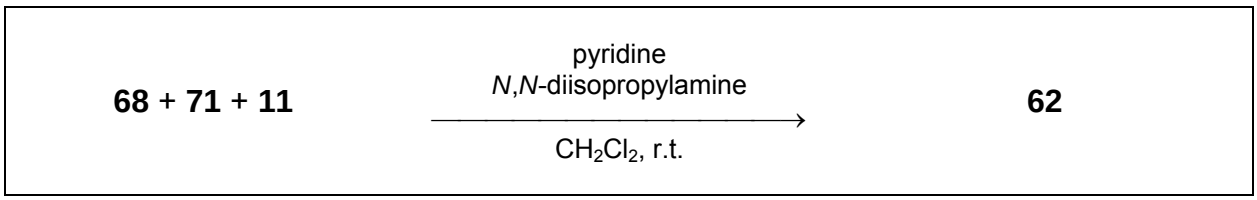
71



11



62



[III-SEE-247] (Scheme 4-19, p. 98)[8]

Phenyl chlorothioformate **71** (250 µl, 312 mg, 1.81 mmol, 1.00 equiv) was added to a solution of 3,5-bis(trifluoromethyl)aniline **68** (280 µl, 410 mg, 1.82 mmol, 1.00 equiv) and pyridine (160 µl, 157 mg, 1.99 mmol, 1.10 equiv) in CH_2Cl_2 (6 ml) at r.t.. The mixture was stirred for 5 min, then added dropwise to a solution of (1*R*,2*R*)-1,2-diaminocyclohexane **11** (620 mg, 5.43 mmol, 3.00 equiv) in CH_2Cl_2 (2 ml). *N*,*N*-Diisopropylethylamine (896 µl, 699 mg, 5.43 mmol, 3.00 eq) was added and the mixture was stirred for 15 min. Saturated aq. $NaHCO_3$ (7 ml) was added, the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 ml). The combined organic layers were washed with brine (10 ml) and dried over $MgSO_4$. The solvent was removed under

reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1) to give the product **62** as light yellow crystals (245 mg, 638 µmol, 35 %).

[IV-SEE-288] (Scheme 4-20, p. 98)

Phenyl chlorothioformate **78** (250 µl, 312 mg, 1.81 mmol, 1.00 equiv) was added to a solution of 3,5-bis(trifluoromethyl)aniline **68** (280 µl, 410 mg, 1.82 mmol, 1.00 equiv) and pyridine (160 µl, 157 mg, 1.99 mmol, 1.10 equiv) in CH_2Cl_2 (6 ml) at r.t.. The mixture was stirred for 2 h, then added dropwise to a solution of (1*R*, 2*R*)-1,2-diamino-cyclohexane **11** (207 mg, 1.81 mmol, 1.00 equiv) in CH_2Cl_2 (1 ml). *N*,*N*-Diisopropyl-ethylamine (300 µl, 234 mg, 1.81 mmol, 1.00 eq) was added and the mixture was stirred for 15 min. Saturated aq. $NaHCO_3$ (7 ml) was added, the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 ml). The combined organic layers were washed with brine (10 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1) to give the product **62** as light yellow crystals (351 mg, 911 µmol, 50 %).

$C_{15}H_{17}F_6N_3S$ (385.37 g/mol)

m.p. 69 °C [m.p. ref.[70]: 70-72 °C]

R_f 0.26 ($CHCl_3$/MeOH 7:1)

^{1}H NMR (300 MHz, DMSO-d_6): δ [ppm] = 8.30 (s; 2H), 7.68 (s; 1H), 4.06 (brs; 1H), 2.76 (s; 1H), 2.04-1.92 (m; 2H), 1.75-1.58 (m; 2H), 1.34-1.20 (m; 4H).
The NH-protons could not be detected.

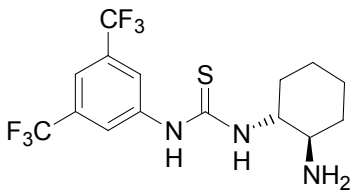
^{13}C NMR (75.5 MHz, DMSO-d_6): δ [ppm] = 180.9, 142.6, 130.50 (q; $^2J_{C\text{-}F}$ = 31.9 Hz), 124.4 (q; $^1J_{C\text{-}F}$ = 271.3 Hz), 122.1, 116.2, 58.5, 54.0, 33.2, 31.2, 24.7, 24.5.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 2936, 1539, 1472, 1380, 1332, 1269, 1177, 1129, 1100, 964, 881, 846, 754, 700, 675.

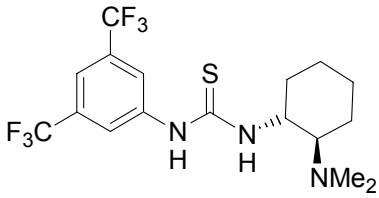
HR ESI-MS (m/z): exact mass $[M+H]^+$: 386.1125; found $[M+H]^+$: 386.113.

The spectroscopical data are in agreement with the literature.[70]

4.4.5 1-[3,5-Bis(trifluoromethyl)phenyl]-3-[(1R,2R)-2-(dimethylamino)cyclohexyl]-thiourea (51, Takemoto's Catalyst)



62



51

[IV-SEE-292] (Table 4-1, Entry 1, p. 99)[76]

Under argon atmosphere, a mixture of the aminothiourea **62** (110 mg, 285 µmol, 1.00 equiv) in toluene (1.5 ml) and aqueous formaldehyde 37 % (55.0 µl, 684 µmol, 2.40 equiv) was treated with Hantzsch ester (216 mg, 8.55 µmol, 3.00 equiv) and thiourea (2.10 mg, 28.5 µmol, 10.0 mol%). The mixture was stirred at 60 °C for 72 h. After filtration through celite, the solvent was evaporated and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1). The product **51** was obtained as an off-white crystalline solid (32.0 mg, 77.0 µmol, 27 %).

[IV-SEE-296] (Table 4-1, Entry 2, p. 99)[77]

Zinc powder (102 mg, 1.56 mmol, 4.00 equiv), AcOH (180 µl, 187 mg, 3.12 mmol, 8.00 equiv) and aqueous formaldehyde 37 % (95.0 µl, 1.17 mmol, 3.00 equiv) were added to a solution the of aminothiourea **62** (150 mg, 389 µmol, 1.00 equiv) in dioxane (0.5 ml), and the resulting mixture was stirred for 72 h at r.t.. Aqueous NH_3 (500 µl) was added, the aqueous phase was extracted with CH_2Cl_2 (2 x 1 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1). The product **51** was obtained as an off-white crystalline solid (117 mg, 283 µmol, 73 %).

[IV-SEE-294] (Table 4-1, Entry 3, p. 99)[78]

Aqueous NaH_2PO_3 solution (2 M, 1.4 ml, 2.85 mmol, 10.0 equiv) and aqueous formaldehyde 37 % (231 µl, 2.85 mmol, 10.0 equiv) were added to a solution of the amino thiourea **62** (110 mg, 285 µmol, 1.00 equiv) in dioxane (1.4 ml). The resulting mixture was stirred for 1 h at 60 °C. The pH was adjusted to 8 by addition of aqueous NaOH (2 M) and the reaction mixture was extracted with CH_2Cl_2 (3 x 3 ml). The organic layer was dried over $MgSO_4$, the solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1). The product **51** was obtained as an off-white crystalline solid (80.0 mg, 193 µmol, 68 %).

[IV-SEE-269] (Table 4-1, Entry 4, p. 99)[7]

A mixture of the amino thiourea **62** (313 mg, 812 µmol, 1.00 equiv), formic acid (337 µl, 8.94 mmol, 11.0 eq) and aqueous formaldehyde 37 % (149 µl, 1.79 mmol, 2.20 equiv) was stirred under reflux for 6 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (500 µl) and washed with saturated $NaHCO_3$ (500 µl) solution. The organic layer was dried over $MgSO_4$, the solvent was evaporated and the residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1). The product **51** was obtained as an off white crystalline solid (15.0 mg, 36.0 µmol, 4 %).

[III-SEE-234] (Table 4-1, Entry 5, p. 99)[6]

Aqueous formaldehyde 37 % (252 µl, 3.38 mmol, 5.00 equiv) was added to a solution of the amino thiourea **62** (260 mg, 675 µmol, 1.00 equiv) in acetonitrile (5 ml), and the resulting mixture was stirred for 15 min at r.t.. $NaBH_3CN$ (85.0 mg, 1.35 mmol, 2.00 equiv) was added, followed by acetic acid (85.0 mg, 1.35 mmol, 2.00 equiv) after 15 min. After stirring at r.t. for 2 h, the reaction mixture was diluted with 2 % MeOH/$CHCl_3$ mixture (10 ml) and washed with 1 N NaOH solution (12 ml). The aqueous layer was extracted with $CHCl_3$ (3 x 6 ml), the combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography ($CHCl_3$/MeOH 7:1). The product **51** was obtained as an off-white crystalline solid (90.0 mg, 218 µmol, 32 %).

$C_{17}H_{21}F_6N_3S$ (413.42 g/mol)

R_f 0.24 ($CHCl_3$/MeOH 7:1)

m.p. 110 °C [m.p. ref.[48]: 111-113 °C]

^{1}H NMR (300 MHz, $CHCl_3$-d_1): δ [ppm] = 7.97 (s; 2H), 7.52 (s; 1H), 4.28 (brs; 1H), 2.90 (brs; 1H), 2.48 (s; 6H), 2.40 (s; 1H), 1.96-1.71 (m; 3H), 1.38-1.19 (m; 4H).
The NH-protons could not be detected.

^{13}C NMR (75.5 MHz, $CHCl_3$-d_1): δ [ppm] = 180.5, 140.6, 131.8 (q; $^{2}J_{C\text{-}F}$ = 33.6 Hz), 123.1 (q, $^{1}J_{C\text{-}F}$ = 272.8 Hz), 122.2, 117.6, 67.2, 53.9, 39.8, 32.4, 24.4, 24.2, 22.2.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3302, 2939, 2860, 2215, 1617, 1538, 1471, 1381, 1272, 1170, 1127, 1061, 1040, 993, 963, 907, 883, 847, 730, 696, 679.

HR ESI-MS (m/z): exact mass $[M+H]^+$: 414.1438; found $[M+H]^+$: 414.145

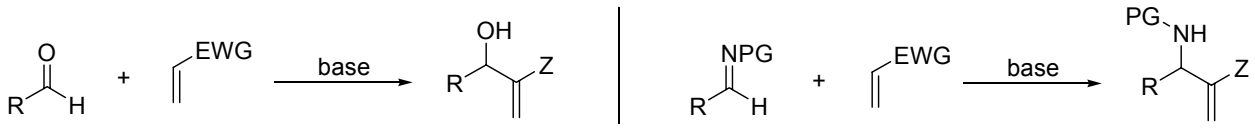
The spectroscopical data are in agreement with the literature.[48]

5 La-linked BINOL Catalysed Asymmetric Aza-BH Reaction

5.1 Background

The Morita-Baylis-Hillman reaction has developed to a highly efficient C-C bond forming process since it combines two important requirements: atom economy and functional group generation. Although it has been discovered as early as 1968 by *Morita*[80] (phosphine-catalysis) and was successively further improved in 1972 by *Baylis* and *Hillman*[81] (amine-catalysis), the reaction and its applications have received a growing interest only since the mid 1990s.

The Morita-Baylis-Hillman reaction consists of a C-C coupling between the α-position of an EWG-substituted olefin and a carbonyl group in the presence of an appropriate nucleophilic catalyst, e.g. a tertiary amine or a phosphine. The transformation leads to the formation of a multifunctionalised molecule (Scheme 5-1, left).

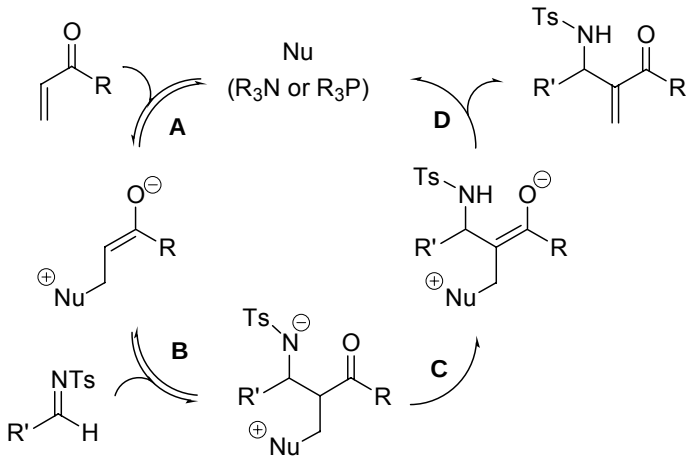


Scheme 5-1 Original Morita-Baylis-Hillman reaction (left), aza-Baylis-Hillman variation (right).

Replacing the carbonyl by an imine leads to the aza-Baylis Hillman reaction (Scheme 5-1, right). This reaction yields α-methylene-β-amino derivatives and, in particular, β-amino esters if acrylates are used as Michael acceptors.

5.1.1 Mechanistic Studies

Both the Baylis-Hillman- (BH) and aza-Baylis-Hillman (aza-BH) reactions are based on an addition-elimination sequence. The generally accepted mechanism of the aza-Baylis-Hillman reaction is shown in Scheme 5-2, p.109.[82,83] After addition of a nucleophile to the olefin (**A**), the resulting enolate adds to the imine (**B**). A proton transfer (**C**), followed by the elimination of the catalyst (**D**) and the release of the aza-Baylis-Hillman adduct.



Scheme 5-2 Proposed mechanism of the aza-BH reaction.

Jacobsen et al. reported aza-BH reactions of methyl acrylate and nosylimines in $CHCl_3$ catalysed by DABCO.[83] The initial rates were found to follow Equation (5-1).

$$\text{rate} = \frac{a[\text{DABCO}][\text{Acrylate}][\text{Imine}]}{1 + b[\text{Imine}]} \qquad \textbf{(5-1)}$$

A prominent primary kinetic isotope effect (kH/kD = 3.81) was observed after comparison of the initial reaction rates of methyl acrylate with the corresponding α-deutero-methyl acrylate, suggesting that deprotonation of the α-H(D) (step **C**) was rate limiting. *Leitner et al.*[82] analysed the PPh_3 catalysed aza-Baylis-Hillman reaction between methyl vinyl ketone and a fluorinated *N*-tosylated imine in THF. Initial rates are shown as a function of concentration for the individual components (Equation 5-2). The order of 0.5 for the imine indicates that the rate-determing step (RDS) is influenced by the proton transfer.

$$\text{rate} = k_{obs}[PPh_3]^1[MVK]^1[\text{Imine}]^{0.5} \qquad \textbf{(5-2)}$$

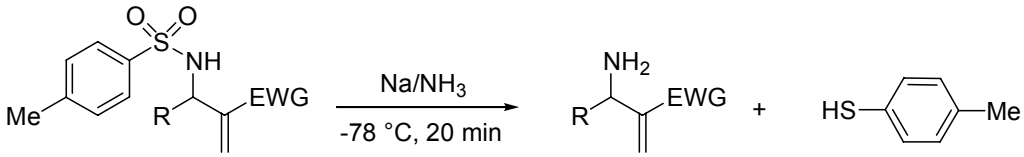
$$\text{rate} = k_{obs}[PPh_3]^1[MVK]^1[\text{Imine}]^{0.5} \qquad \textbf{(5-3)}$$

Examination of the kinetics in the presence of phenol as a prototypical additive revealed that the rate law of the reaction changes in the presence of a Brønsted acid, showing first order dependence for the imine (Equation (5-3)). Hence, the elimination step is no longer involved in the RDS and the proton transfer is accelerated by the protic additive.

5.1.2 Substrate Diversity

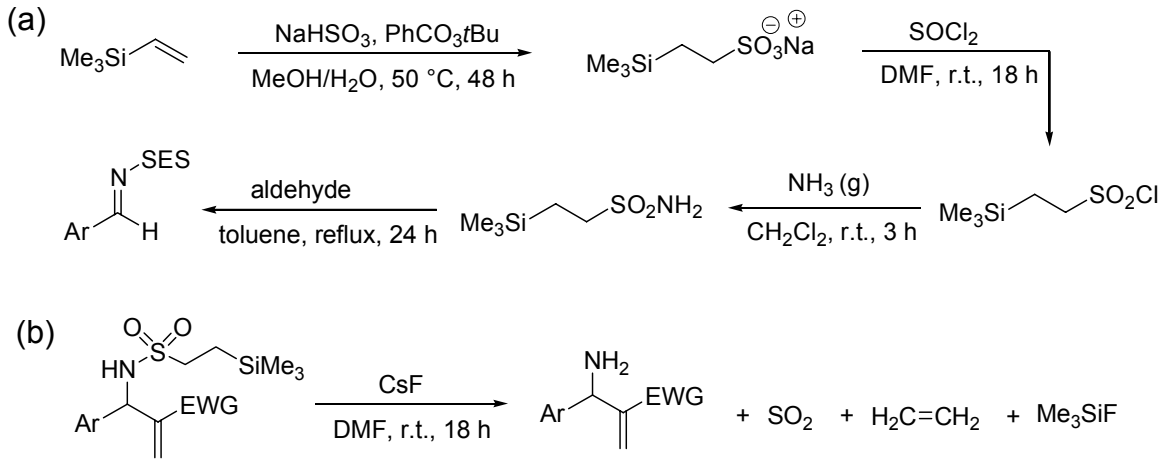
Imines.[84] Various activated aromatic aldimines like tosyl- (Ts-) imines, nosyl (Ns-) imines, 2-trimethylsilylethanesulfonyl- (SES-) imines, chiral sulfinyl- and thiophosphorylimines[iv], diphenylphosphinoyl- (dpp-) imines, and very recently, even *in situ* generated *tert*-butyloxycarbonyl- (Boc-) and carboxybenzyl- (Cbz-) imines can be used as electrophiles in aza-BH reactions. However, the reaction outcome strongly depends on the appropriate protecting group, as highly activated imines are required for most aza-BH reactions.

Tosylimines are the most often employed imines in the aza-BH-reaction. Since the Ts-protecting group exhibits a strong electron withdrawing effect, the resulting imines are very electrophilic. However, harsh conditions are required to cleave the Ts-protecting group. This limits the synthetic utility of the amino adducts as many functional groups do not tolerate these conditions (Scheme 5-3).[85]



Scheme 5-3 Cleavage of the Ts-protecting group.

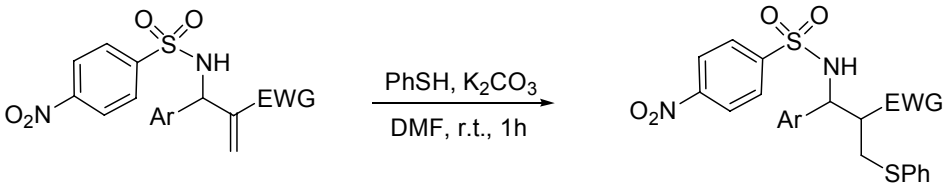
Although the Ns- and SES-protecting groups also lead to highly electrophilic imines, these groups are rarely used in the aza-BH reaction. Even though the SES-protecting group can be easily removed under relatively mild conditions (Scheme 5-4, (b)), the laborious four step synthesis of SES-imines is the main drawback (Scheme 5-4, (a))[85]



Scheme 5-4 Synthesis (a) and cleavage (b) of the SES-protecting group.

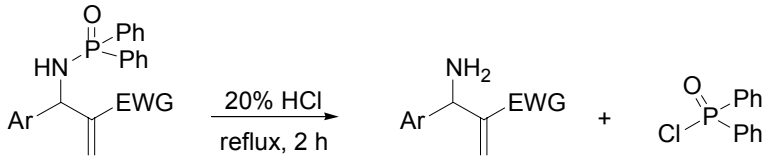
[iv] For the application of chiral imines see: *5.1.3 Development of Asymmetric Aza-Baylis-Hillman Reactions*

The use of Ns-imines is rather unusual as the Ns-protecting group cannot be cleaved off from the aza-BH adduct. The cleaving thiol reagents add to the double bond of the products instead of deprotecting the amino function (Scheme 5-5).



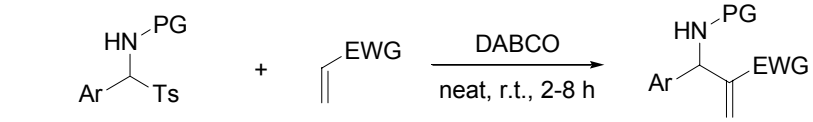
Scheme 5-5 Addition of thiophenol on the double bond of the aza-BH adduct.

Phosphinoylimines can be easily synthesised and their dpp-protecting group can be removed under mild conditions. However, dpp-protected imines are rarely used in aza-BH reactions, since they are weaker electrophiles compared to their sulfonylated analogues (Scheme 5-6).[85]



Scheme 5-6 Cleavage of the dpp-protecting group.

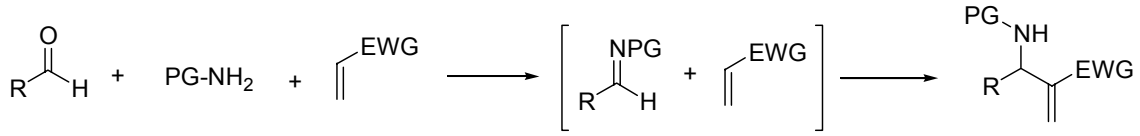
Recently, several working groups explored the use of *in situ* generated imines in aza-BH reactions.[86] For example, *Gajda et al*. described the DABCO catalysed reaction between electron deficient alkenes and Boc- or Cbz-protected imines, generated *in situ* from stable *N*-carbamate protected amidoalkyl-*p*-tolylsulfones (Scheme 5-7).[86a]



Scheme 5-7 Application of *in situ* generated Boc- or Cbz-imines in the aza-BH reaction.

In contrast to aromatic imines the use of aliphatic imines in aza-BH reactions is poorly illustrated in literature.[87] Indeed, activated aliphatic imines are enolisable, are more tedious to synthesise, are less stable, and their application in aza-BH reactions gave poorer results compared to their aromatic counterparts.

It is possible to synthesise the imine electrophile in the aza-BH reaction *in situ* from the corresponding aldehyde and the diphenylphosphinamid,[88] SES-NH_2,[89] tosylamide,[87b,90] or Boc-amide[87b] as it is shown in Scheme 5-8.

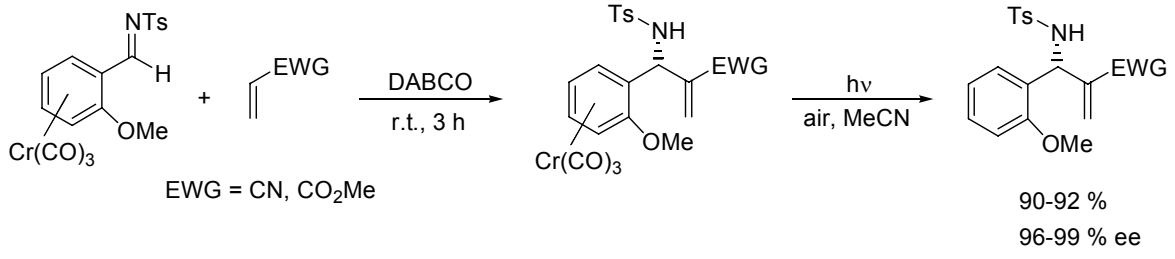


Scheme 5-8 One-pot procedure of the aza-BH reaction.

Enone components.[84] Vinyl ketones and acrylates are the most frequently employed enone components. Vinyl ketones are particulary interesting substrates due to their versatile applicability in aza-BH reactions while the use of acrylates in the aza-BH reaction gives synthetically valuable β-amino acid esters. The use of cyclic enones,[91] activated allenes,[92] alkynes[93] and conjugated dienes,[94] acrylonitrile,[87a] nitroalkenes,[95] acrylamide,[96] and acroleine[97] as enone components in the aza-BH reaction is less common.

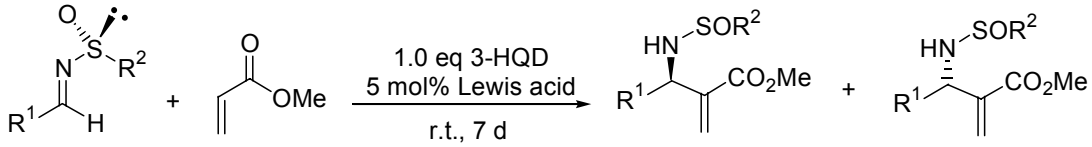
5.1.3 Development of Asymmetric Aza-Baylis-Hillman Reactions

Chiral Imines. The first example of an asymmetric aza-BH reaction has been developed by *Kündig et al*.[98] in 1994. Chiral planar Cr-complexes of sulfonylimines derived from *ortho*-substituted aromatic aldehydes were utilised. The chiral aldehydes were first transformed into tosylimines and then reacted with Michael acceptors in the presence of DABCO to give α-methylene-β-amino esters in good yields and good enantiomeric excesses. At the end of the reaction, the $Cr(CO)_3$ group is removed oxidatively. The main disadvantage of this method is the applicability only to imines derived from *ortho*-substituted aromatic aldehydes (Scheme 5-9).



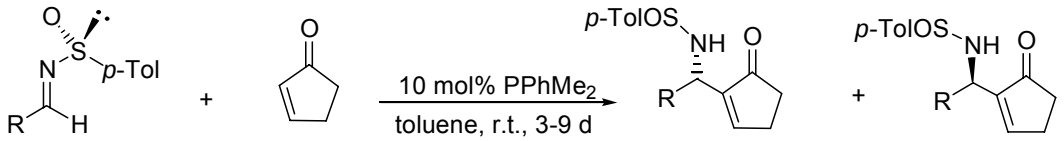
Scheme 5-9 Diastereoselective aza-BH reaction with enantiopure planar chiral *o*-substituted $Cr(CO)_3$.[98]

Later on, *Aggarwal et al*.[87c] employed enantiopure *N*-sulfinimines in aza-BH reactions with methyl acrylate in the presence of 3-hydroxyquinuclidine (3-HQD) and a Lewis acid. The desired products were obtained in good to moderate diastereoselectivities (Scheme 5-10).



Scheme 5-10 Use of enantiopure *N*-sulfinimines in aza-BH reactions with methyl acrylates.[87c]

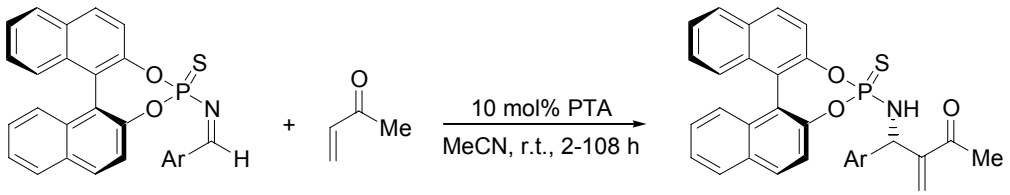
Subsequently, enantiopure sulfinimines were also used by *Shi et al*.[87d] for reactions with cyclopentenone. Good yields and diastereoselectivities were achieved (Scheme 5-11).



Scheme 5-11 Diastereoselective aza-BH reation with enantiopure *N*-sulfinimines according to *Shi*.[87d]

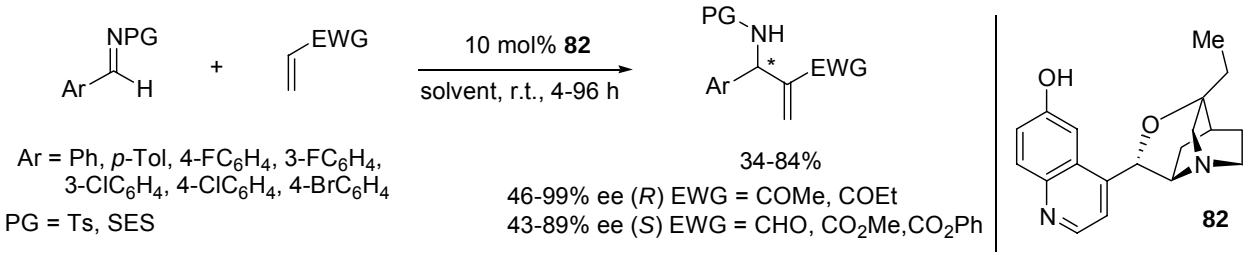
The *N*-sulfinyl groups not only induce high selectivity but also exhibit the advantage of being easily removed under relatively mild conditions. Their synthesis usually requires several steps[99] but a fast one-pot synthesis of chiral *N*-*p*-toluenesulfinylimines starting from a commercial chiral sulfinate has also been developed.[100]

Recently, *Zhou et al*.[101] used BINOL-based *N*-thiophosphoryl imines as chiral substrates in the 1,3,5-triaza-phosphaadamantane (PTA) catalysed aza-BH reaction and obtained excellent diastereomeric excesses and good yields (Scheme 5-12).



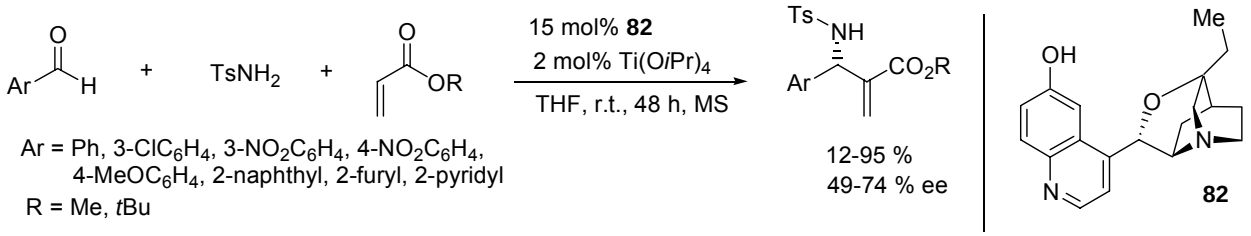
Scheme 5-12 BINOL-based *N*-thiophosphoryl imines in the aza-BH reaction with methyl vinyl ketone.[101]

Chiral Nucleophiles - Amines. The first asymmetric aza-Baylis Hillman reaction with chiral amines as catalysts was published by *Shi et al*.[102] in 2002. Based on the work of *Hatakeyama*[103] who first employed β-isocupreidine **82** as a chiral catalyst in the MBH-reaction, *Shi* and co-workers used this chiral amine quinidine derivative in the reaction between *N*-sulfonyl imines and various activated alkenes. The corresponding adducts were obtained in moderate yields with excellent enantiomeric excesses (Scheme 5-13).



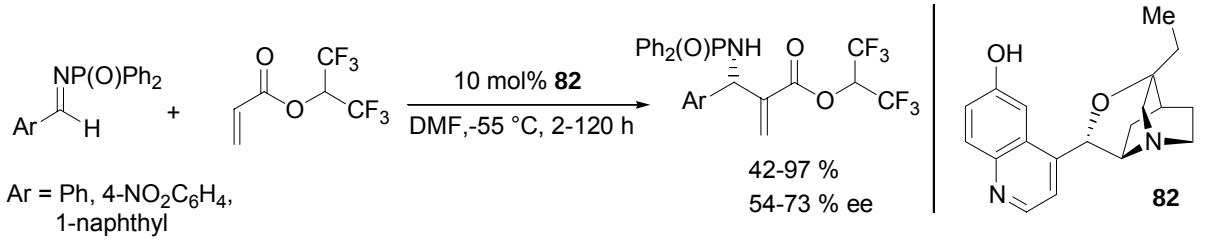
Scheme 5-13 First enantioselective aza-BH reaction catalysed by β-isocupreidine **82**.

Adolffson and *Balan*[90c] adopted the same catalyst (**82**) in the three component reaction between acrylates, aldehydes and tosylamines in the presence of Ti(O*i*Pr)$_4$ and molecular sieves as additive. Good yields and enantiomeric excesses were achieved (Scheme 5-14).



Scheme 5-14 Enantioselective *in situ* aza-BH reaction catalysed by β-isocupreidine **82**.[90]

This catalyst (**82**) was also employed by *Hatakeyama*[104] in reactions between dpp-protected imines and hexafluoroisopropyl acrylate, giving the corresponding aza-BH products in moderate yields with good enantiomeric excesses (Scheme 5-15).

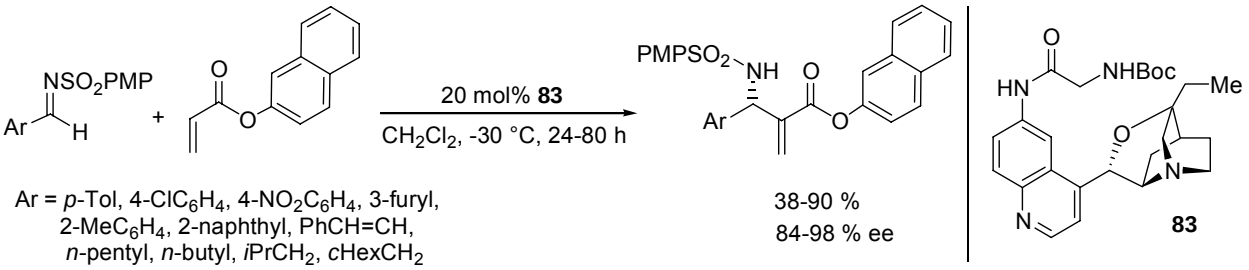


Scheme 5-15 β-Isocupreidine **82** catalysed aza-BH reaction of dpp-imines with HFIPA.[104]

From the work of *Shi,*[103] *Adolffson* and *Balan,*[90c] and *Hatakeyama*[104] it follows that both the ridgid structure of the β-isocupreidine **82** and the phenolic OH group are essential for a high degree of asymmetric induction and sufficient reactivity. Overall, the nucleophilic nitrogen atom in the quinuclidine moiety of **82** acts as a Lewis base to initiate the asymmetric aza-BH reaction, whereas the phenolic hydroxyl group acts as a Lewis acid to stabilise and organise the enolate intermediate and also to promote the subsequent aldol addition.

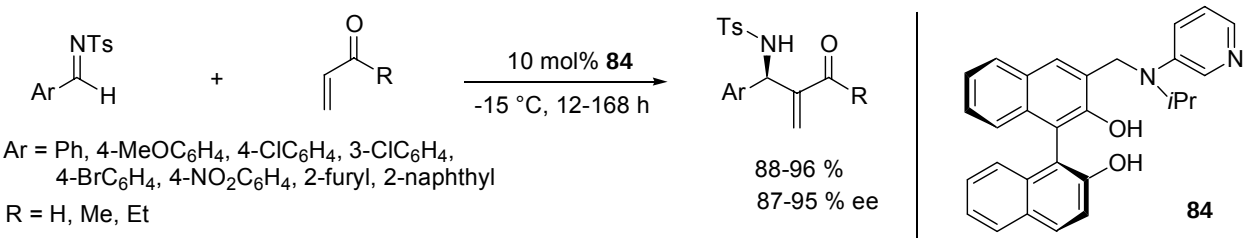
For the protected imines employed, it turned out that only those imines that have a nitrogen atom attached to electron withdrawing groups were electrophilic enough to participate in the aza-BH reaction.

Recently, modified β-isocupreidine catalysts were reported by *Zhu et al.*.[105] In these catalysts the 6'-OH moiety was replaced by various amide or thiourea functions. Excellent yields and enantioselectivities were obtained with the modified catalyst **83** in the aza-BH reaction of $PMPSO_2$-protected imines with 2-naphthyl acrylate (Scheme 5-16).



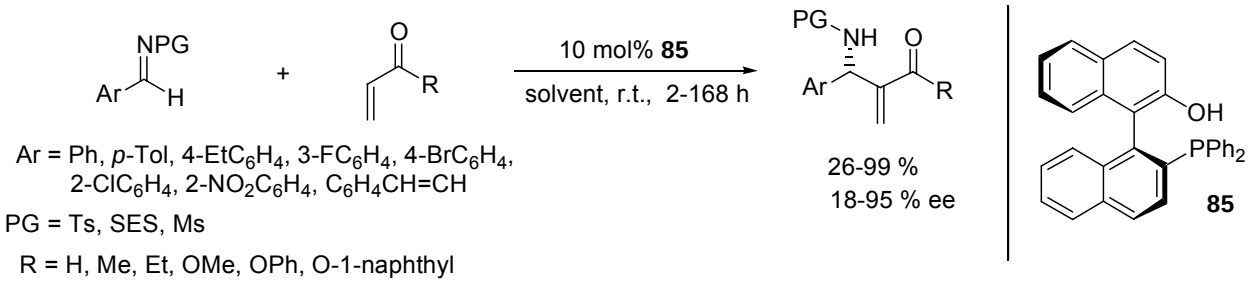
Scheme 5-16 Use of the β-isocupreidine derived bifunctional catalyst **83** in the aza-BH reaction.[105]

The group of *Sasai*[106] developed a chiral tertiary amine derived from BINOL (**84**). Like β-isocupreidine **82**, this base contains both a subunit possessing a Lewis basic character and two hydroxyl groups exhibiting Lewis acid properties. This ensures the stabilisation of the zwitterionic intermediates. The reaction yields and enantioselectivities were good, albeit with rather long reaction times (Scheme 5-17).



Scheme 5-17 Enantioselective aza-BH reaction catalysed by the BINOL-based tertiary amine **84**.[106]

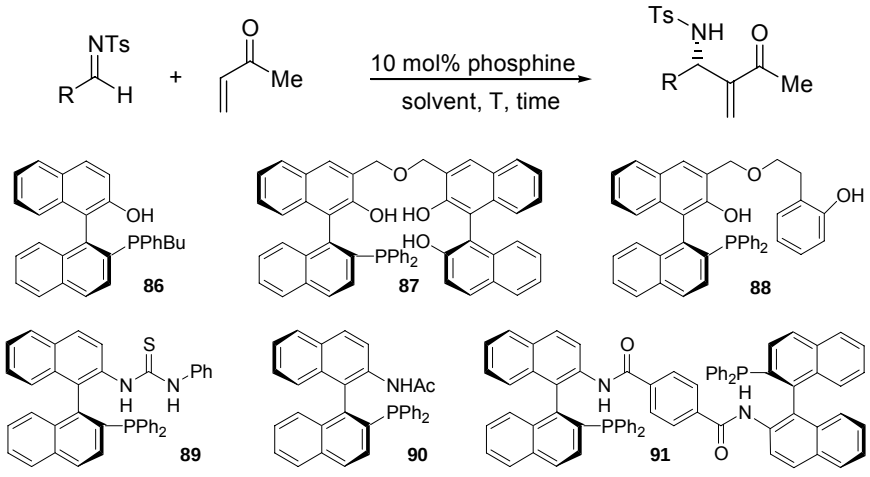
Chiral Nucleophiles - Phosphines. Starting in 2003, *Shi et al*.[107] explored the use of chiral phosphines to induce enantioselectivity in the aza-BH reaction. A chiral phosphine derived from BINAP **85** was investigated in the reaction of Ts-imines with methyl vinyl ketone. Good yields and moderate enantioselectivites were achieved, although long reaction times were required. However, poor yields and enantiomeric excesses were obtained with acrylates as substrates (Scheme 5-18).



Scheme 5-18 First asymmetric aza-BH reaction catalysed by a chiral phosphine **85** according to *Shi*.[107]

Since then, numerous new chiral phosphines have been designed and screened to further improve these pioneering results. Some representative chiral phosphine catalysts evolved from **85** are shown in Table 5-1. These catalysts were employed in the aza-BH reaction of Ts-protected imines with methyl vinyl ketones as Michael acceptors.

Table 5-1 Enantioselective aza-BH reaction of Ts-imines with methyl vinyl ketone catalysed by chiral phosphines.[108-111]



entry	phosphine	solvent	T (°C)	time (h)	yield (%)	ee (%)
1	**86**	THF	r.t.	1-5	80-99	44-89
2	**87**	THF	-20	24-48	70-97	90-96
3	**88**	THF	0	10-164	71-quant.	87-96
4	**89**	CH_2Cl_2	r.t.	5-80	61-98	67-97
5	**90**	DCE	r.t.	12-60	75-99	46-93
6	**91**	CH_2Cl_2	0	24-48	72-98	68-87

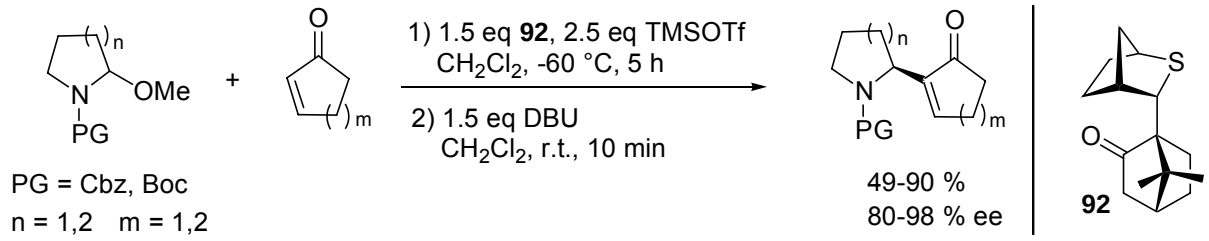
A slight modification of the phosphine **85** by replacement of one phenyl group with an alkyl chain led to a dramatic reduction of the reaction time. The best results were obtained with phosphine **86**, bearing a *n*-butyl group. However, in most cases, lower enantioselectivities were observed (Table 5-1, Entry 1).[108]

The group of *Shi*[109] has developed a new chiral phosphine **87** comprising several hydroxy groups. These types of catalysts ensure a better stabilization of the zwitterionic intermediate by creating more hydrogen bonding interactions. Employing phosphine **87** as chiral catalyst in the aza-BH reaction resulted in improved enantiomeric excesses and good yields (Table 5-1, Entry 2). Further improvements in this family of catalysts have been made by *Ito et al..*[110] Replacing the binaphthol unit of **87** by a phenol led to the organocatalyst **88** with improved catalytic properties, both in terms of yields and enantiomeric excesses (Table 5-1, Entry 3).

Because of the hydrogen bond donating properties of amide groups or thioureas, the group of *Shi*[111] incorporated these functionalities into chiral phosphine ligands. However, the phosphine-thiourea catalyst **89** as well as the phosphine-amide catalyst **90** led only to moderate enantioselectivities but good yields were obtained (Table 5-1, Entry 4,5). The sterically more demanding phosphine-amide bifunctional catalyst **91** gave similar results as organocatalyst **90** (Table 5-1, Entry 6).

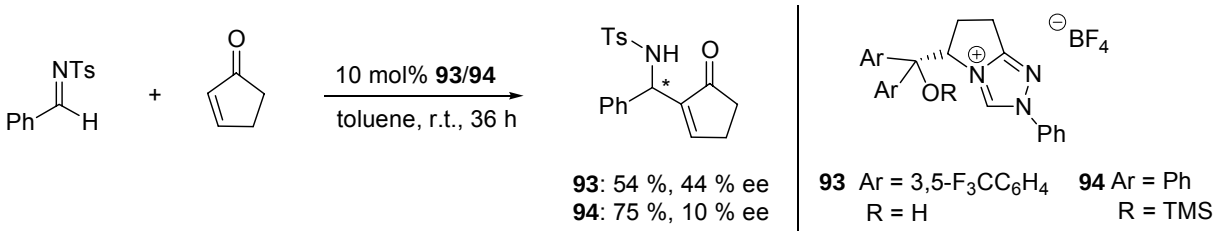
In summary, the chiral phosphine catalyst **87** showed the best results when used in the aza-BH reaction of Ts-protected imines with ketones. However, up to now acrylates remain challenging substrates for chiral phosphine catalysts.

Chiral Nucleophiles - Sulfides. *Aggarwal et al.*[86c] have applied the chiral sulfide **92** in the aza-BH-reaction between *in situ* generated iminium ions and various α,β-unsaturated ketones. While cyclic enones provided the adducts with good yields and enantioselectivities, the latter were very poor when acylic enones were used (Scheme 5-19).



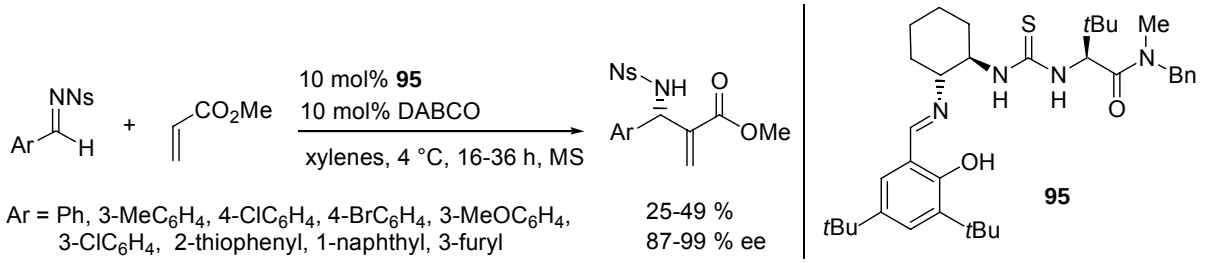
Scheme 5-19 First enantioselective aza-BH reaction with *in situ* generated iminium ions.[86c]

Chiral Nucleophiles - Carbenes. Recently, *Ye et al.*[112] demonstrated that chiral *N*-heterocyclic carbene precursors with a proximal hydroxy group can act as catalysts in aza-BH reactions of cyclopentenone with a Ts-protected imine. The corresponding aza-BH adduct was obtained in good yields but only moderate enantioselectivity (Scheme 5-20).



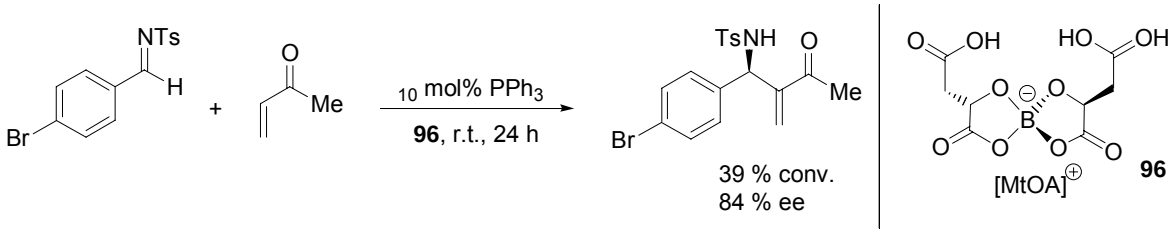
Scheme 5-20 First asymmetric aza-BH reaction with chiral *N*-heterocyclic carbene precursors.[112]

Chiral Additives - Thioureas. *Jacobsen et al.*[83] have developed highly enantioselective catalytic aza-BH reactions between nosylimines and methyl acrylate in the presence of the chiral thiourea derivative **95** and DABCO as nucleophile (Scheme 5-21). Good enantioselectivities but only moderate yields were obtained.



Scheme 5-21 Enantioselective aza-BH reaction catalysed by chiral thiourea derivative **95**.

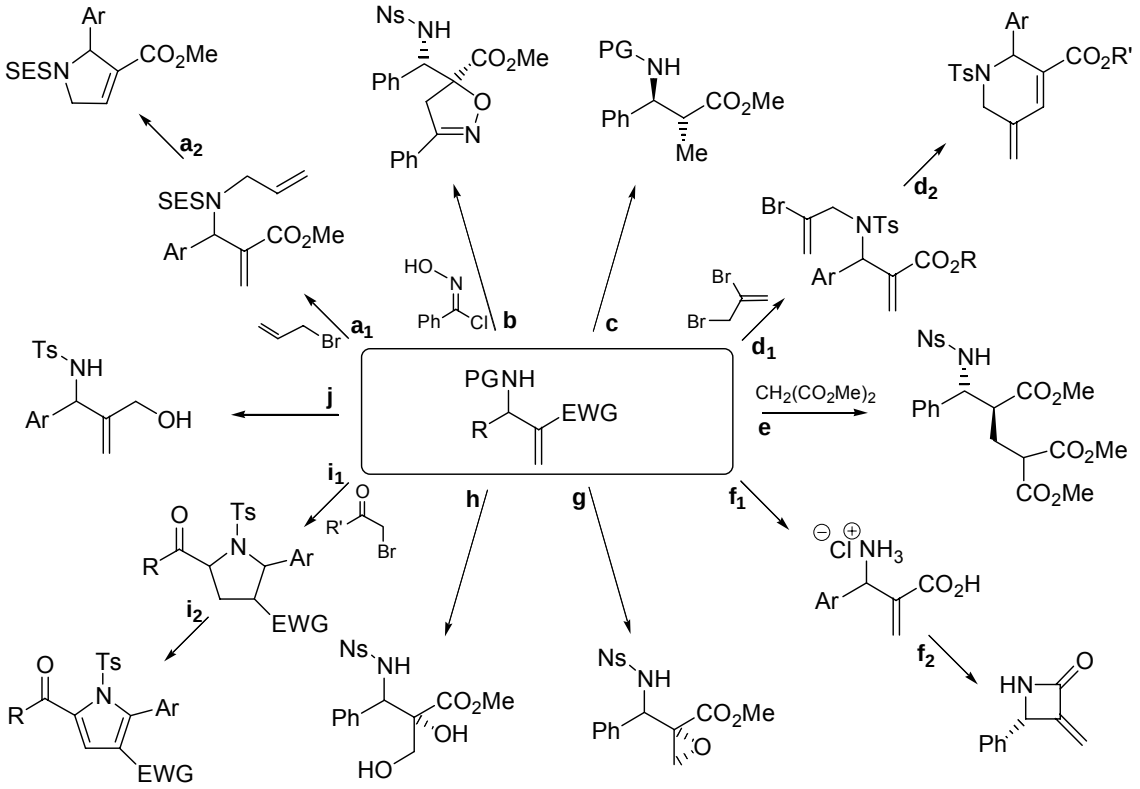
Chiral Solvent - Ionic Liquids. Besides chiral catalysts, chiral reaction media can also induce high asymmetric induction. *Leitner et al.*[113] performed reactions in the chiral ionic liquid methyltrioctylammonium dimalatoborate **96**. In the aza-BH reaction between methyl vinyl ketone and tosylimines, good enantioselectivities but only moderate conversions were observed (Scheme 5-22).



Scheme 5-22 Chiral ionic liquid **96** as reaction medium in the aza-BH reaction.

5.1.4 Use of Aza-Baylis-Hillman Adducts in Synthesis

The aza-Baylis-Hillman reaction has gained considerable interest because it leads to highly functionalised products.[114] The synthetic utility of the aza-BH adducts is illustrated in the variety of transformations shown in Scheme 5-23.



Scheme 5-23 Some representative synthetic tranformations of aza-BH aduccts. *Conditions:* (**a$_1$**) K_2CO_3, DMF; (**a$_2$**) Grubbs II, CH_2Cl_2; (**b**) Et_3N, CH_2Cl_2; (**c**) H_2 (1 atm), MeOH; (**d$_1$**) K_2CO_3, DMF; (**d$_2$**) $Pd(OAc)_2$, K_2CO_3, PEG-OH 3400, DMF; (**e**) DBU, THF; (**f$_1$**) 20 % HCl; (**f$_2$**) BOPCl, Et_3N, THF; (**g**) *t*-BuOOH, Triton B, THF; (**h**) OsO_4, NMO, H_2O/acetone; (**i$_1$**) K_2CO_3, MeCN; (**i$_2$**) DBU, MeCN.

Various transformations of Ns-protected α-methylene-β-amino esters to new β-amino esters have been described by *Jacobsen et al*..[83] These transformations include [3+2] cycloadditions of aldoximines (Scheme 5-23, (**b**)), conjugate additions of 1,3-dicarbonyl compounds (Scheme 5-23, (**e**)), epoxidations (Scheme 5-23, (**g**)), and dihydroxylations (Scheme 5-23, (**h**)).

In particular, the synthesis of new β-amino ester analogues by hydrogenation of type-B β-amino esters have been intensively investigated by several research groups.[115] For example, *Brown et al*.[115a] have studied the hydrogenation of *N*-Boc-α-methylene-β-amino esters. The reaction was performed in the presence of a chiral phosphine and

resulted in the kinetic resolution of the starting material, which was recovered with high enantiomeric excess (Scheme 5-23, (**c**)).

Another transformation of aza-BH adducts is the conversion to pyrrolidines, pyrrolines and pyrroles.[89,116] For example, *Lamaty et al*.[89] concentrated on the synthesis of SES-protected pyrrolidines using ring closing metathesis as a key step (Scheme 5-23, (**a**)). *Kim et al*.[116d] have recently synthesised polysubstituted pyrroles from β-amino-carbonyl compounds (Scheme 5-23, (**i**)).

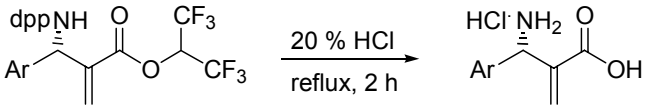
The syntheses of piperidines, tetrahydropiperidines and pyridines starting from aza-BH adducts were described by several groups.[94,117] For example, *Kim et al*.[117c] transformed suitably substituted β-amino esters to tetrahydropyridines by an intramolecular Heck reaction using a solvent mixture of PEG and DMF (Scheme 5-23, (**d**)).

The syntheses of β-amino alcohols by reduction of the corresponding aza-BH adducts with $LiAlH_4$ was published by *Shi et al.*[118] (Scheme 5-23, (**j**)).

β-Lactams are accessible from aza-BH adducts as illustrated by *Hatakeyama et al*..[119] The synthesis of β-lactams was carried out from dpp-protected β-amino esters following a deprotonation-cyclisation two step procedure (Scheme 5-23, (**f**)).

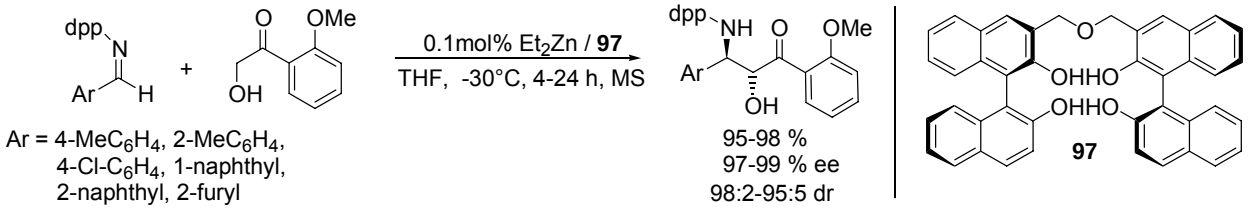
5.2 Concept

The application of acrylates as olefinic component in the aza-BH reaction yields enantio-enriched unsaturated β-amino acid esters, which have gained considerable interest due to their important biological properties, their occurrence in natural products and as potential precursors for β-lactams.[9] The most common approach for the synthesis of β-amino acid esters *via* aza-BH reactions is the use of Ts-protected imines and chiral Lewis bases as catalysts.[84] However, harsh conditions are required to cleave the Ts-protecting group (see 5.1.2 Substrate Diversity, p. 110). Therefore, the dpp-protecting group should be employed as it activates the imine and can be removed under mild conditions. Furthermore, using dpp-protected imines in combination with various acrylates provide a direct access to β-amino acids by simultaneous removal of the dpp-protecting group and hydrolysis of the ester function (Scheme 5-24, p. 121).[104]



Scheme 5-24 According to *Hatakeyama*,[104] hydrolytic *C*,*N*-terminal deprotection provides direct access to enantio-enriched α-methylene-β-amino acids.

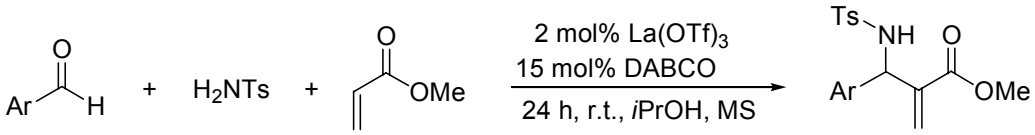
For the aza-BH reaction between dpp-protected imines and less activated acrylates a literature survey revealed that the use of chiral Lewis bases only provides moderate enantioselectivities. Alternatively, dpp-protected imines can be activated by Zn-(*R*,*R*)-linked-BINOL complexes as shown by *Shibasaki* in asymmetric Mannich-type reactions (Scheme 5-25).[120]



Scheme 5-25 Asymmetric Mannich–type reactions catalysed by a Zn-BINOL complex.

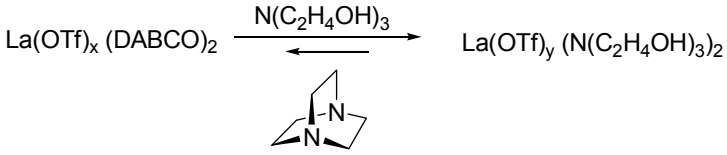
However, so far no example of an enantioselective protocol with chiral metal Lewis acid catalysts is known for the aza-BH reaction.[121] The following section describes the first applications of non-chiral metal Lewis acids in (aza)-BH reactions:

Adolfsson observed that the *in situ* aza-BH reaction can be accelerated by $La(OTf)_3$ (Scheme 5-26).[90a]

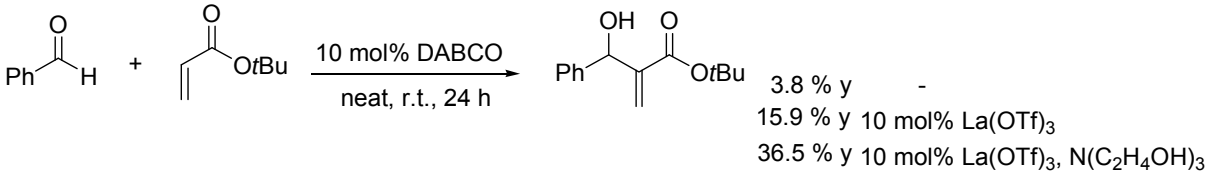


Scheme 5-26 *In situ* aza-BH reaction accelerated by $La(OTf)_3$ according to *Adolfsson*.[90a]

Unfortunately, the combination of Lewis acids and tertiary amines in MBH reactions causes the formation of amine-Lewis acid complexes. In these adducts, the *N*-nucleophile is not catalytically active. Therefore, in the classic MBH reaction, *Aggarwal* made use of triethanolamine to liberate the lanthanum-coordinated DABCO, which can then act as a nucleophile (Scheme 5-28, p. 122). In addition, the use of oxygen-rich ligands leads to more Lewis acidic metal complexes and therefore to a rate acceleration of the MBH reaction.[122]

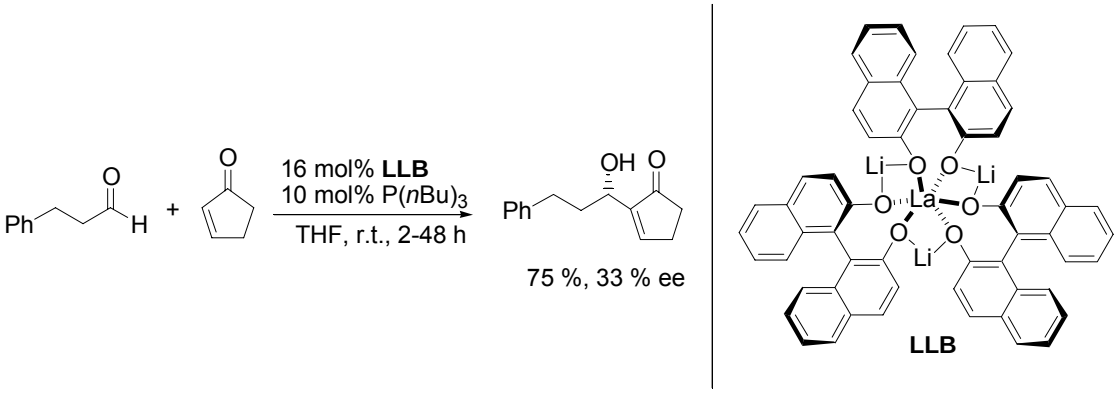


Scheme 5-27 DABCO-lanthanum, triethanolamine-lanthanum equilibrium.[122]



Scheme 5-28 Rate acceleration of the MBH reaction by Lewis acidic metal complexes.[122]

Furthermore, *Sasai et al.* successfully employed La-BINOL-derived heterobimetallic complexes in the MBH reaction (Scheme 5-29).[121b]

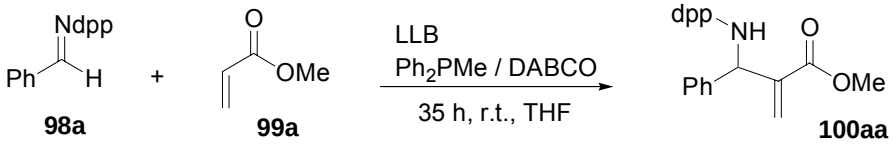


Scheme 5-29 Enantioselective MBH reaction promoted by heterobimetallic complexes.

Based on these results, BINOL-based lanthanum complexes should be tested as potential chiral catalysts for aza-Baylis-Hillman reactions of dpp-protected imines.

Shi et al. performed a screening of different nucleophiles for the aza-BH reaction of dpp-protected imine **98a** with methyl acrylate **99a** and obtained the highest yields when Ph_2PMe is employed (Table 5-2, entry 2 and 4, p. 123).[123] However, using Ph_2PMe in a preliminary screening in combination with (*R*)-LLB (La-Li-(*R*)-BINOL) resulted in poor enantioselectivities, while the less reactive DABCO provided much higher enantioselectivities (Table 5-2, entries 1 and 3, p. 123).

Table 5-2 Preliminary screening for the best nucleophile in the aza-BH reaction of dpp-imine **98a**.[a]



98a **99a** **100aa**

entry	nucleophile	(*R*)-LLB	yield (%)[b]	ee (%)[c]
1	DABCO	10 mol%	30	75
2	DABCO	-	7[d]	-
3	Ph_2PMe	10 mol%	33	5
4	Ph_2PMe	-	29[d]	-

[a] Reactions were carried out at r.t. in THF (1.0 M) using imine **98a** (1.0 eq), acrylate **99a** (2.0 eq), DABCO/Ph_2PMe (20 mol%), (*R*)-LLB (10 mol%). [b] Isolated yield. [c] Determined by HPLC. [d] Yields are in agreement with literature values.[123]

In conclusion, dpp-protected imines combined with various acrylates and DABCO as nucleophile should be employed as test system for the screening of Lewis acidic BINOL-based lanthanum complexes.

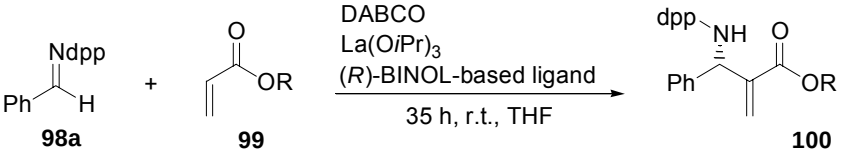
5.3 Results & Discussion

For the application of chiral Lewis acid complexes in the aza-Baylis-Hillman reaction, an initial ligand screening was performed using La(O*i*Pr)$_3$ and various (*R*)-BINOL-based ligands in the reaction of dpp-protected benzaldimine **98a** and methyl acrylate **99a** as test system (Table 5-3, p. 124).[v] Using ligand **101** ((*R*)-BINOL), the corresponding aza-Baylis-Hillman adduct was obtained in 25 % yield and 70 % ee (Table 5-3, entry 1). Increasing the ligand to metal ratio from 1:1 to 2:1 yielded the same result (Table 5-3, entry 2). Sterically more demanding groups in the BINOL 3,3'-positions, i.e. 2-naphthyl **102**, 8-anthracenyl **103**, as well as 3,5-bis-CF_3- and 2,4,6-tri-*i*Pr-substituted phenyl groups **104** and **105** decreased the yields and enantioselectivities (Table 5-3, entries 3-6 and 9-12). In contrast, a high enantioselectivity of 89 % ee was achieved with linked-(*R*,*R*)-BINOL **97** (Table 5-3, entry 7). Since increasing the ligand to metal ratio for (*R*)-BINOL **101** gave no improvement (Table 5-3, entry 2), it is postulated that the increase in enantioselectivity for linked-(*R*,*R*)-BINOL **97** complex was due to its higher stability as compared to the La-(*R*)-BINOL complex.[124] Similar reactivity and selectivity patterns

[v] Absolute product configuration was rigorously established for the aza-BH adducts **100aa** and **100ae** by comparison of the HPLC elution profiles with those reported by *Hatakeyama*.[104] For all other aza-BH products, absolute configuration is drawn in analogy.

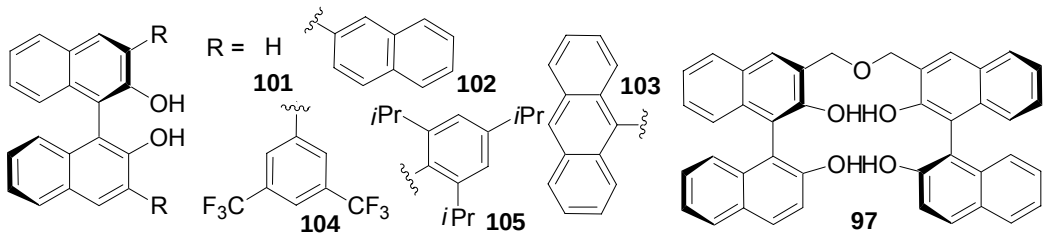
were observed by replacing methyl acrylate with phenyl acrylate **100ab** (Table 5-3, entry 9-14).

Table 5-3 Screening of (*R*)-BINOL-based ligands.[a]



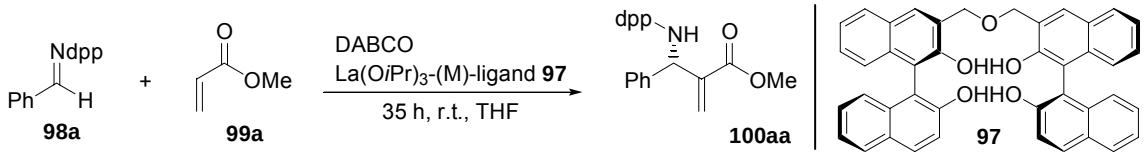
entry	R	ligand	yield (%)[b]	ee (%)[c]
1	Me	**101**	25	70
2[d]	Me	**101**	27	71
3	Me	**102**	19	36
4	Me	**103**	17	22
5	Me	**104**	22	46
6	Me	**105**	13	5
7	Me	**97**	36	89
8[e]	Me	**97**	32	87
9	Ph	**101**	51	19
10	Ph	**102**	48	12
11	Ph	**103**	55	22
12	Ph	**104**	46	rac
13	Ph	**105**	61	70
14[e]	Ph	**97**	60	74

[a] Reactions were carried out at r.t. in THF (1.0 M) using imine **98a** (1.0 eq), acrylate **99a,b** (2.0 eq), DABCO (20 mol%), $La(OTf)_3$ (10 mol%), and (*R*)-BINOL based ligand **97**, **101**-**105** (10 mol%). [b] Isolated yield. [c] Determined by HPLC. [d] 20 mol% ligand. [e] Catalyst loading: 20 mol% La(O*i*Pr)$_3$-ligand.



The La-linked-(*R*,*R*)-BINOL complex is well known for its dual reactivity as Lewis acid and Brønsted base.[125] The basicity of the catalyst was increased by deprotonation with KHMDS, NaHMDS or *n*-BuLi. However, applying these heterobimetallic complexes in the aza-Baylis-Hillman reaction did not result in higher yields or enantioselectivities (Table 5-4, entries 2-4, p. 125) relative to the non-metallated species.

Table 5-4 Screening of heterobimetallic La-M-linked-(*R*,*R*)-BINOL complexes.[a]

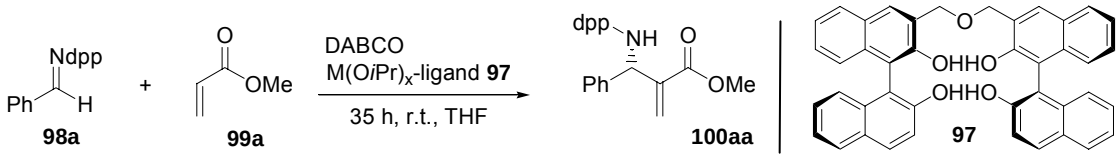


entry	M source	yield (%)[b]	ee (%)[c]
1	-	35	89
2	KHMDS	28	82
3	NaHMDS	29	86
4	*n*-BuLi	30	75

[a] Reactions were carried out at r.t. in THF (1.0 M) using imine **98a** (1.0 eq), methyl acrylate **99a** (2.0 eq), DABCO (20 mol%), $La(OTf)_3$ (10 mol%) and ligand **97** (10 mol%). [b] Isolated yield. [c] Determined by HPLC.

To vary the catalyst's Lewis acidity, linked-(*R*,*R*)-BINOL **97** was coordinated to several $M(OiPr)_3$. The activity and selectivity of the resulting complexes were compared to the corresponding La-linked-(*R*,*R*)-BINOL complex. The Sm- and Y-complexes showed similar reactivity and selectivity patterns (Table 5-5, entries 2,3). In contrast, the more Lewis-acidic $Ti(iPrO)_4$ did not lead to a catalytically active complex (Table 5-5, entry 4). The increased basicity of the Sr-complex led to poor yields and enantioselectivities (Table 5-5, entries 5,6). In the absence of $M(OiPr)_x$, ligand **97** did not show any catalytic activity (Table 5-5, entry 7).

Table 5-5 Screening of $M(OiPr)_x$–ligand **97** complexes.[a]

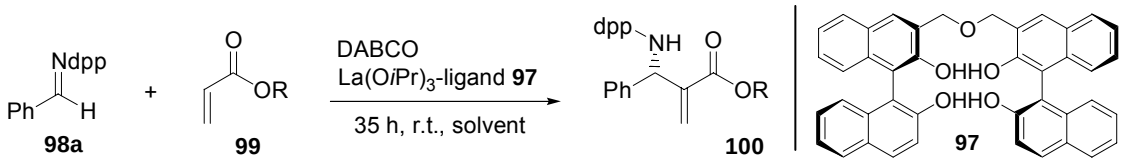


entry	$M(OiPr)_3$	yield (%)[b]	ee (%)[c]
1	$La(OiPr)_3$	35	89
2	$Sm(OiPr)_3$	35	89
3	$Y(OiPr)_3$	24	81
4	$Ti(OiPr)_4$	traces	n.d.
5	$Sr(OiPr)_2$	25	25[e]
6[d]	$Sr(OiPr)_2$	42	12[e]
7	-	traces	rac

[a] Reactions were carried out at r.t. in THF (1.0 M) using imine **98a** (1.0 eq), methyl acrylate **99a** (2.0 eq), DABCO (20 mol%), $La(OTf)_3$ (10 mol%), and ligand **97** (10 mol%). [b] Isolated yield. [c] Determined by HPLC. [d] 20 mol% $Sr(OiPr)_2$, [e] The opposite enantiomer was formed in excess.

To further optimise the reaction conditions, a test system consisting of the La-linked-(*R*,*R*)-BINOL complex as catalyst in combination with dpp-protected benzaldimine **98a** and methyl acrylate **99a** as substrates was employed. Among the three solvents tested, the best yield was achieved in MeCN (Table 5-6, entry 2), while the highest enantioselectivity was observed in THF (Table 5-6, entry 3). Higher yields were obtained in THF upon increasing the substrate concentration, without affecting the ee values (Table 5-6, entry 4). No improvements were observed with higher DABCO loadings or addition of molecular sieves (Table 5-6, entries 5 and 6). Increased yields resulted from higher amounts of methyl acrylate **99a** (Table 5-6, entry 7). Raising the temperature from r.t. to 40 °C only led to a slight increase in yield due to the increased formation of byproducts as well, while the enantioselectivity was not adversely affected (Table 5-6, entry 8). These conditions could not be applied to phenyl acrylate **99b**, due to its limited solubility in THF. Instead, the best reaction conditions were found to be a 1 M solution of the imine **98a** in THF, using 2 eq of the acrylate **99b** (Table 5-6, entry 9).

Table 5-6 Optimised reaction conditions of the aza-Baylis-Hillman reaction.[a]



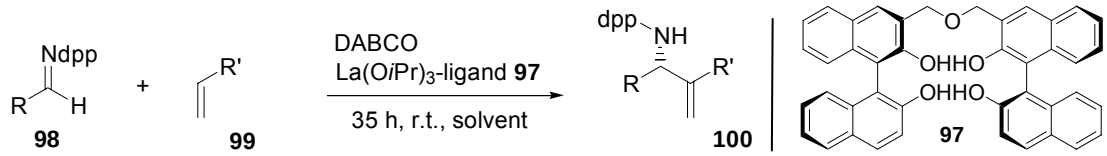
entry	acrylate	R	solvent	yield (%)[b]	ee (%)[c]
1	**99a**	(2 eq) Me	(1 M) EtOAc	38	86
2	**99a**	(2 eq) Me	(1 M) MeCN	40	83
3	**99a**	(2 eq) Me	(1 M) THF	35	89
4	**99a**	(2 eq) Me	(2 M) THF	41	89
5[d]	**99a**	(2 eq) Me	(2 M) THF	40	88
6[e]	**99a**	(2 eq) Me	(2 M) THF	38	89
7	**99a**	(4 eq) Me	(2 M) THF	46	88
8[f]	**99a**	(4 eq) Me	(2 M) THF	50	90
9[f]	**99b**	(2 eq) Ph	(1 M) THF	68	70

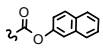
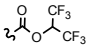
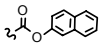
[a] Reactions were carried out at r.t. in THF using imine **98a** (1.0 eq), acrylate **99a,b**, DABCO (20 mol%), $La(OTf)_3$ (10 mol%) and ligand **97** (10 mol%). [b] Isolated yield. [c] Determined by HPLC. [d] Molecular sieves added (200mg/mmol). [e] 40 mol% DABCO, [f] Reaction performed at 40 °C.

Among the acrylates tested in a substrate screening, the highest yields were achieved using the very reactive phenyl acrylate **99b** and 2-naphthyl acrylate **99d** (Table 5-7, entries 2,8 and 4,10, p. 127). Employing the even more reactive 1,1,1,3,3,3-hexafluoro-isopropyl acrylate **99e** led to reduced yields, due to the increased formation of bypro-

ducts.[vi] Employing methyl acrylate **99a** led to the highest enantioselectivities (Table 5-7, entries 1 and 7). Acrylonitrile **99f** gave only racemic products, due to its fast background reaction (Table 5-7, entry 6). Applying the dpp-protected *para*-Cl-substituted benzaldimine derivative **99b** instead of the dpp-protected benzaldimine **98a** led in most cases to higher enantioselectivities at virtually unchanged yields (Table 5-7, entries 7-11 vs. 1-6). Aliphatic imines were also tested, but only provided traces of the corresponding aza-BH adducts.

Table 5-7 Substrates screening.[a]

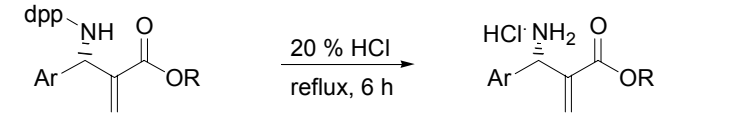


entry	imine	R	acrylate	R'	yield (%)[b]	ee (%)[c]
1	**98a**		**99a**		34	89
2	**98a**		**99b**		59	71
3	**98a**		**99c**		31	70
4	**98a**		**99d**		67	63
5	**98a**		**99e**		41	80
6	**98a**		**99f**		48	*rac*
7	**98b**		**99a**		39	90
8	**98b**		**99b**		56	69
9	**98b**		**99c**		27	83
10	**98b**		**99d**		59	79
11	**98b**		**99f**		52	11[e]

[a] Reactions were carried out at r.t. in THF (1.0 M) using imine **98a,b** (1.0 eq), olefin component **99a-f** (2.0 eq), DABCO (20 mol%), $La(OTf)_3$ (10 mol%) and ligand **97** (10 mol%). [b] isolated yield, [c] determined by HPLC.[e] The opposite enantiomer was formed in excess.

Acidic hydrolysis of the aza-Baylis-Hillman products **100** provides a single step access to the β-aminoacids (Scheme 5-30, p. 128).[104] The viability of this approach to α-methylene-β-aminoacids **106** was proven by the hydrolysis of the aza-Baylis-Hillman adducts **100**: After refluxing in 20 % aqueous HCl for 6 h, the corresponding β-amino acids **106** were isolated in up to 75 % yield.

[vi] Therefore, *Hatakeyama* performed aza-BH reactions with 1,1,1,3,3,3-hexafluoroisopropyl acrylate **99e** in DMF at -55 °C.[104] Unfortunately, these reaction conditions could not be applied for the La-linked-BINOL catalysed aza-BH reaction due to solubility problems.



100ab R = Ph, Ar = Ph
100ba R = Me, Ar = *p*ClC$_6$H$_4$

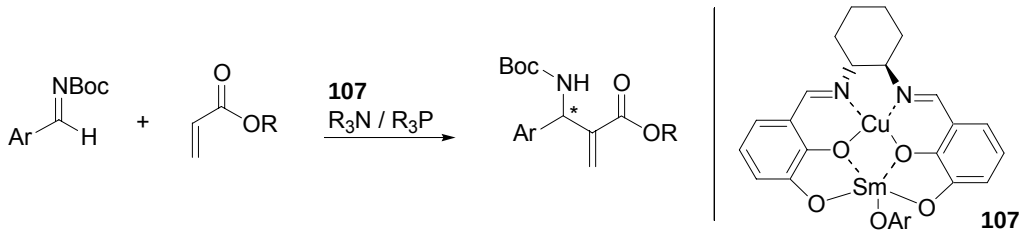
75 % **106a** Ar = Ph
62 % **106b** Ar = *p*ClC$_6$H$_4$

Scheme 5-30 Conversion of **100** to β-amino acids **106**.

In conclusion, it has been shown for the first time that the aza-Baylis-Hillman reaction can be catalysed enantioselectively by a chiral Lewis acidic metal complex. Using the La-linked-(*R*,*R*)-BINOL complex as catalyst and dpp-protected imines as substrates the corresponding aza-BH adducts could be obtained in up to 68 % yield and up to 90 % ee. Hydrolytic deprotection of the highly enantio-enriched aza-Baylis-Hillman products thus obtained grants access to α-methylene-β-aminoacids.

5.4 Outlook

In this work, it was shown for the first time that the aza-Baylis-Hillman reaction can be catalysed with chiral Lewis acidic complexes. Based on these studies, many different Lewis acidic metal complexes, which are known to activate protected imines, should be also tested in a future work. For example, the chiral heterobimetallic Cu/Sm/Schiff base/OAr complex **107**, which has been successfully employed in the asymmetric nitro-Mannich reaction for the activation of Boc-protected imines might also be used in the aza-BH reaction (Scheme 5-31).[126]

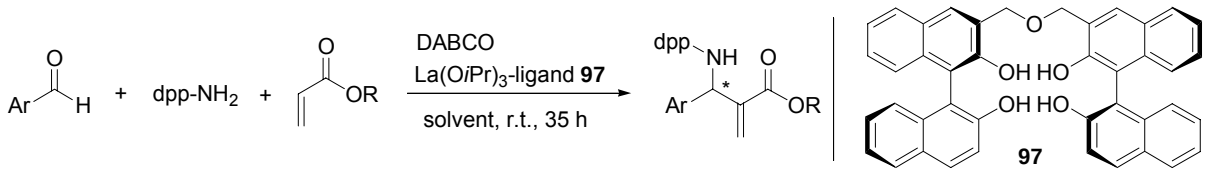


Scheme 5-31 Proposed asymmetric aza-BH reaction with *N*-Boc-protected imines catalysed by the chiral heterobimetallic complex **107**.

To further optimise the results of the present work, two main goals should drive future researches: (a) simplifying the procedure and (b) improving the yields.

(a) According to *Shi*, it is possible to synthesise the imine electrophile *in situ* in the aza-BH reaction from the corresponding aldehyde and diphenylphosphine amide.[88] This

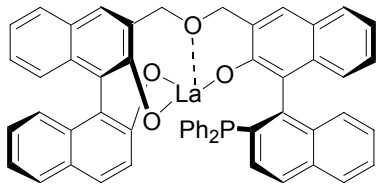
in situ protocol should be applied to the system developed in the present thesis (Scheme 5-32).



Scheme 5-32 Proposed asymmetric *in situ* aza-BH reaction catalysed by the La-linked-(*R*,*R*)-BINOL complex.

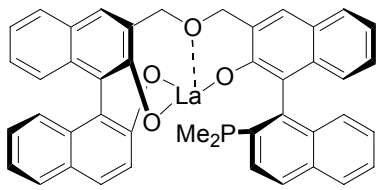
(b) According to previous studies, the low yields observed when employing DABCO as catalyst could be improved by replacement with a more reactive phosphine. However, in combination with the La-linked-(*R*,*R*)-BINOL complex, Ph_2PMe led to lower enantioselectivities than DABCO. This low selectivity might be due to a fast background reaction (see Table 5-2, p. 123).

An intramolecular combination of Ph_2PMe and the La-linked-(*R*,*R*)-BINOL complex might solve this problem and might increase the yield while maintaining high enantioselectivities (Scheme 5-33).



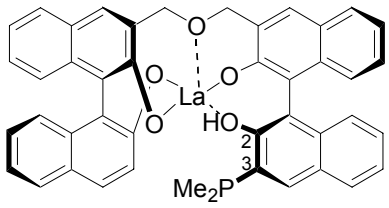
Scheme 5-33 Complex derived from La(O*i*Pr)$_3$ and a linked-(*R*,*R*)-BINOL-diphenylphosphine derivative.

A further improvement could be the substitution of the diphenylphosphino group by the dimethylphosphino group (Scheme 5-34). As shown by *Shi*, the chiral dimethylphosphino-derived catalyst exhibits much higher reactivity in the aza-BH reaction than its corresponding diphenyl analogue.[127]



Scheme 5-34 Complex derived from La(O*i*Pr)$_3$ and a linked-(*R*,*R*)-BINOL-dimethylphosphine derivative.

However, La-coordination by the phosphine might decrease its nucleophilicity. Therefore, designing a chiral ligand with the dimethylphosphine moiety in the 3-position and a La-coordinating hydroxy group in the 2-position might lead to a highly reactive and highly selective bifunctional catalyst (Scheme 5-35).



Scheme 5-35 La –linked-(*R*,*R*)-BINOL complex with non La-coordinated dimethylphosphine group.

5.5 Experimental Part

5.5.1 General Experimental Conditions

Preparative thin layer chromatography was performed with Merck F-254 TLC plates. Flash chromatography was performed on silica gel (Macherey-Nagel MN-Kieselgel 60, 230-240 mesh). Analytical TLC was performed on aluminium backed silica plates (Macherey-Nagel, Polygram© SIL G/UV_{254}), detection by using UV fluorescence. Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded at 500 MHz on a JEOL JNM-LA500 spectrometer and at 300 MHz on Bruker AC 300 and DPX 300 instruments, respectively; ^{13}C NMR spectra at 75.5 MHz on a Bruker DPX 300. Chemical shifts (δ) are given in parts per million (ppm) referenced to TMS. For the fine-structure interpretation the abbreviations of the signals are the following: s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet. HPLC analyses were performed using an Agilent 1100 Series or on a JASCO HPLC system. High resolution mass spectra (HR ESI-MS) were recorded on a Finnigan MAT 900 ST spectrometer. Infrared spectra were recorded on a *Perkin-Elmer* Paragon 1000 FT-IR spectrometer using ATR technique. All commercially available chemicals were used without further purification. Anhydrous solvents were distilled from appropriate drying agents prior to use.

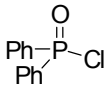
All experiments are characterised by a number within the bracket: [XX-SEE-XXX]. The roman number in front of the three-letter-code indicates the volume number of the

laboratory notebook, whereas the number after the three-letter-code indicates the experiment number in the corresponding notebook volume.

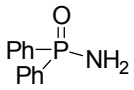
5.5.2 Syntheses of the Substrates for the Aza-BH Reaction

*5.5.2.1 Synthesis of P,P-Diphenylphosphinic amide (**109**)[128]*

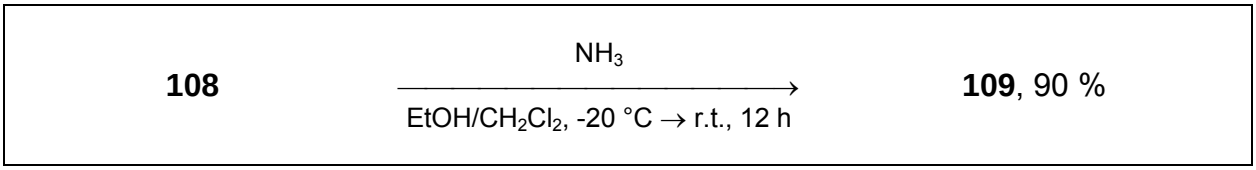
[IV-SEE-300]



108



109



A solution of diphenylphosphinic chloride **108** (20.0 g, 84.5 mmol, 1.00 eq) in dry CH_2Cl_2 (40 ml) was added dropwise with stirring to a saturated ethanolic ammonia solution (170 ml) and CH_2Cl_2 (70 ml) at -20 °C. The reaction mixture was stirred for 12 h at r.t.. The precipitated solid was filtered off and the solvents were removed under reduced pressure. The residue was dissolved in $CHCl_3$ (240 ml) and washed with 5 % aqueous K_2CO_3 (3 x 50 ml) and distilled water (70 ml). The organic layer was separated, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was recrystallised from toluene to yield 16.6 g (76.0 mmol, 90 %) of *P*,*P*-diphenylphosphinic amide **109** as an off-white solid.

$C_{12}H_{12}NOP$ (217.2 g/mol)

m.p. 163-164 °C [m.p. ref.[128]: 164-166 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.93-7.86 (m; 4H), 7.50-7.37 (m; 6H), 3.53 (s; 2H).

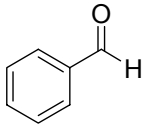
^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 134.5, 132.7, 131.9, 131.8, 128.5, 128.4.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3288, 3235, 3115, 1557, 1435, 1180, 1123, 909, 750.

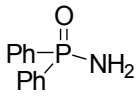
The spectroscopical data are in agreement with the literature.[128]

*5.5.2.2 Synthesis of P,P-Diphenyl-N-(phenylmethylene)phosphinic amide (**98a**)[128]*

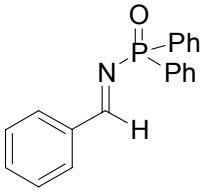
[IV-SEE-299]



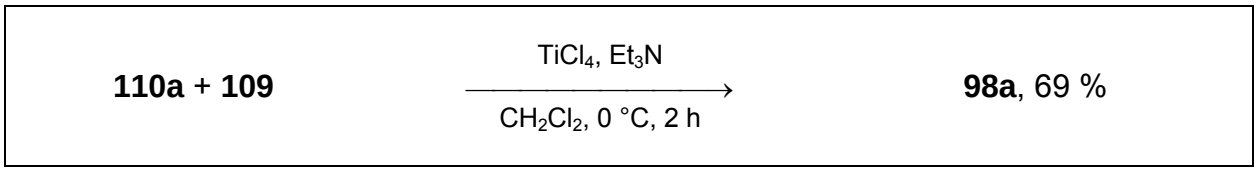
110a



109



98a



P,*P*-Diphenylphosphinic amide **109** (10.8 g, 50.0 mmol, 1.00 eq) was dissolved in CH_2Cl_2 (190 ml), then benzaldehyde **110a** (5.09 ml, 5.31 g, 50.0 mmol, 1.00 eq) and Et_3N (20.9 ml, 15.2 mg, 150 mmol, 3.00 eq) were added. The reaction mixture was cooled to 0 °C and $TiCl_4$ (3.01 ml, 5.21 g, 27.5 mmol, 55.0 mol%) was added dropwise. The mixture was stirred for 2 h at r.t. and the solvent was evaporated under reduced pressure. The residue was redissolved in toluene (100 ml), filtrated through a pad of celite and the solvent was removed under reduced pressure. The residue was redissolved in EtOAc (70 ml), filtrated, and the solvent was evaporated under reduced pressure. The crude product was recrystallised from EtOAc to yield 10.5 g (34.5 mmol, 69 %) of *P*,*P*-diphenyl-*N*-(phenylmethylene)phosphinic amide **98a** as light yellow crystals.

$C_{19}H_{16}NOP$ (305.3 g/mol)

m.p. 141-143 °C [m.p. ref.[128]: 139-141 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 9.37 (d; *J* = 32.0 Hz, 1H), 8.06-7.95 (m; 6H), 7.63-7.47 (m; 9H).

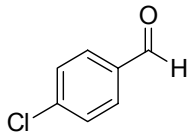
^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 173.8, 173.7, 136.1, 135.7, 133.9, 133.7, 132.2, 131.8, 131.7, 131.5, 130.2, 128.9, 128.5, 128.4.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 1613, 1576, 1451, 1436, 1204, 1124, 1106, 912, 850, 828, 748, 726.

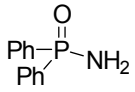
The spectroscopical data are in agreement with the literature.[128]

*5.5.2.3 Synthesis of P,P-Diphenyl-N-(4-chlorophenylmethylene)phosphinic amide (**98b**)*[128]

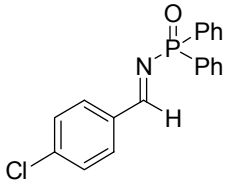
[IV-SEE-301]



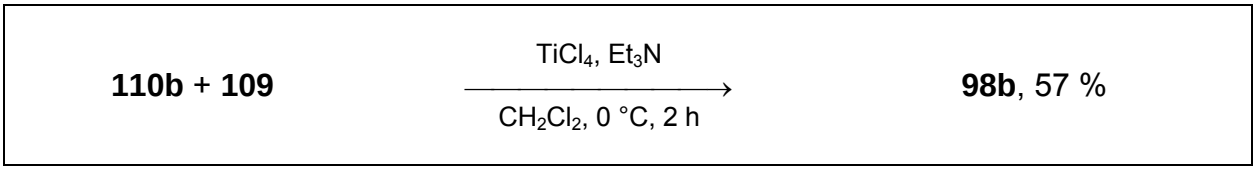
110b



109



98b



P,*P*-Diphenylphosphinic amide **109** (3.70 g, 17.0 mmol, 1.00 eq) was dissolved in CH_2Cl_2 (65 ml), then 4-chlorobenzaldehyde **110b** (2.00 ml, 2.38 g, 17.0 mmol, 1.00 eq) and Et_3N (7.11 ml, 5.16 mg, 51.0 mmol, 3.00 eq) were added. The reaction mixture was cooled to 0 °C and $TiCl_4$ (1.02 ml, 1.77 g, 9.35 mmol, 55.0 mol%) was added dropwise. The mixture was stirred for 2 h at r.t. and the solvent was evaporated under reduced pressure. The residue was redissolved in toluene (30 ml), filtrated through a pad of celite and the solvent was removed under reduced pressure. The residue was redissolved in EtOAc (20 ml), filtrated, and the solvent was evaporated under reduced pressure. The crude product was recrystallised from EtOAc to yield 3.32 g (9.77 mmol, 57 %) of *P*,*P*-diphenyl-*N*-(4-chlorophenylmethylene)phosphinic amide **98b** as light yellow crystals.

$C_{19}H_{15}NOPCl$ (339.8 g/mol)

m.p. 128-130 °C [m.p. ref.[128]:127-130 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 9.21 (d; *J* = 31.7 Hz, 1H), 7.88-7.82 (m; 6H), 7.43-7.34 (m; 8H).

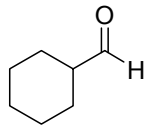
^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 172.3, 172.2, 134.1, 133.6, 131.9, 131.6, 131.5, 131.3, 129.4, 128.4, 128.6, 128.5.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3055, 2225, 1701, 1614, 1591, 1566, 1487, 1436, 1405, 1198, 1123, 1107, 1087, 1012, 849, 819, 751.

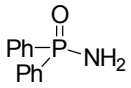
The spectroscopical data are in agreement with the literature.[128]

*5.5.2.4 Synthesis of N-[Cyclohexyl[(4-methylphenyl)sulfonyl]methyl]-P,P-diphenylphosphinic amide (rac-**112**)*[129]

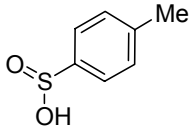
[IV-SEE-306]



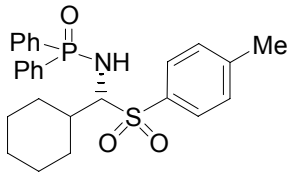
110c



109



111



rac-**112**

110c + **109** + **111** $\xrightarrow[Et_2O,\ r.t.,\ 12\ h]{}$ *rac*-**112**, 85 %

Under argon atmosphere, cyclohexane carbaldehyde **110c** (1.11 g, 9.90 mmol, 1.50 eq) was added to a suspension of *P*,*P*-diphenylphosphinic amide **109** (1.43 g, 6.60 mmol, 1.00 eq) and *p*-toluene sulfinic acid **111**[vii] (1.76 g, 9.90 mmol, 1.50 eq) in dry Et_2O. The mixture was stirred for 15 h at r.t.. The precipitate was filtered, washed with Et_2O and dried in vacuo to yield 2.63 g (5.62 mmol, 85 %) of *N*-[cyclohexyl[(4-methylphenyl)sulfonyl]methyl]-*P*,*P*-diphenylphosphinic amide *rac*-**112** as colourless solid.

$C_{26}H_{30}NO_3PS$ (467.6 g/mol)

m.p. 112-114 °C [m.p. ref.[129]: 113-115 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.80 (dd; *J* = 7.2 Hz, *J* = 12.1 Hz, 2H), 7.68

[vii] *p*-Toluene sulfinic acid was prepared by dissolving its hydrated sodium salt in 10 % HCl (pH < 3) and crystallisation at 4 °C. Filtration and drying under vacuo led to the crude product, which was directly used for the synthesis of of *N*-[cyclohexyl[(4-methylphenyl)sulfonyl]methyl]-*P*,*P*-diphenylphosphinic amide **111**.

(d; *J* = 8.1 Hz, 2H), 7.55-7.36 (m; 6H), 7.31-7.24 (m; 4H), 4.17 (t; *J* = 11.9 Hz, 1H), 3.73-3.66 (m; 1H), 2.47 (s; 3H), 2.39-2.31 (m; 1H), 2.03 (s; 1H), 1.77-1.73 (m; 2H), 1.66-1.53 (m; 2H), 1.43-1.12 (m; 5H).

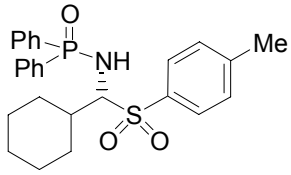
^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 144.8, 134.9, 132.4, 132.1, 131.9, 129.7, 129.1, 128.7, 128.5, 128.4, 128.3, 76.6, 37.8, 30.7, 26.9, 26.4, 25.8, 21.7.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3177, 3055, 2926, 2849, 1700, 1592, 1437, 1299, 1184, 1124, 1081, 1033, 1007, 905, 813, 750, 724.

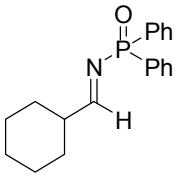
The spectroscopical data are in agreement with the literature.[129]

*5.5.2.5 Synthesis of N-(Cyclohexylmethylene)-P,P-diphenylphosphinic amide (**98c**)*[130]

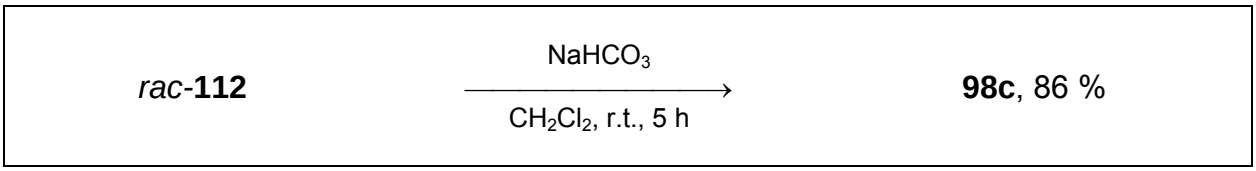
[IV-SEE-303]



rac-**112**



98c



Saturated aqueous $NaHCO_3$ (43 ml) was added to a mixture of *N*-[cyclohexyl[(4-methylphenyl)sulfonyl]methyl]-*P*,*P*-diphenylphosphinic amide *rac*-**112** in CH_2Cl_2 (43 ml). The mixture was stirred for 5 h at r.t.. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 30 ml). The combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was dried in vacuo to obtain 570 mg (1.83 mmol, 86 %) of *N*-(cyclohexyl-methylene)-*P*,*P*-diphenylphosphinic amide **98c** as colourless oil. The crude product was used in the aza-BH reaction without further purification.

$C_{19}H_{22}NOP$ (311.4 g/mol)

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.81 (dd, *J* = 3.7 Hz, *J* = 33.3 Hz, 1H), 7.91-7.85 (m; 4H), 7.51-7.41 (m; 6H), 2.48-2.42 (m; 1H), 1.98-1.95 (m; 2H), 1.80-1.68 (m; 3H), 1.42-1.22 (m; 5H).

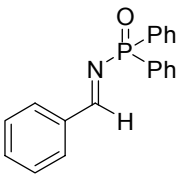
^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 185.6, 185.5, 131.6, 131.5, 131.4, 128.5, 128.3, 46.6, 46.3, 28.6, 25.9, 25.3.

The spectroscopical data are in agreement with the literature.[130]

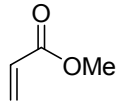
5.5.3 Syntheses of Racemic Reference Substances[131]

*5.5.3.1 Synthesis of Methyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (rac-**100aa**)*

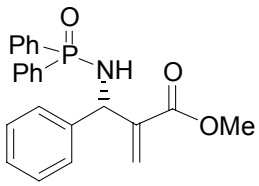
[IV-SEE-336]



98a



99a



rac-**100aa**

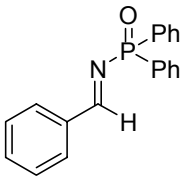
98a + **99a**	Ph_2PMe, THF, r.t., 72 h	*rac*-**100aa**, 35 %

Under argon atmosphere, methyl acrylate **99a** (70.8 µl, 67.6 mg, 786 µmol, 1.20 eq) was added to a mixture of imine **98a** (200 mg, 655 µmol, 1.00 eq) and diphenylmethylphosphine (24.3 µl, 26.2 mg, 131 µmol, 20.0 mol%) in dry THF (1 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by preparative-TLC (*c*-hexane/EtOAc 3:1) to obtain 90.0 mg (229 µmol, 35 %) of methyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate *rac*-**100aa** as colourless solid.

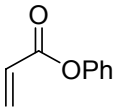
The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

*5.5.3.2 Synthesis of Phenyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (rac-**100ab**)*

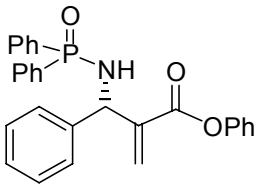
[IV-SEE-332]



98a



99b



rac-**100ab**

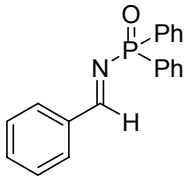
98a + **99b**	$\xrightarrow[\text{THF, r.t., 72 h}]{Ph_3P}$	*rac*-**100ab**, 50 %

Under argon atmosphere, phenyl acrylate **99b** (544 µl, 582 mg, 3.93 mmol, 1.20 eq) was added to a mixture of imine **98a** (1.00 g, 3.26 mmol, 1.00 eq) and triphenylphosphine (206 mg, 786 µmol, 20.0 mol%) in dry THF (3 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by column chromatography (*c*-hexane/EtOAc 3:1) to obtain 740 mg (1.63 mmol, 50 %) of phenyl (β*S*)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate *rac*-**100ab** as colourless solid.

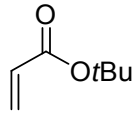
The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

*5.5.3.3 Synthesis of 1,1-Dimethylethyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (rac-**100ac**)*

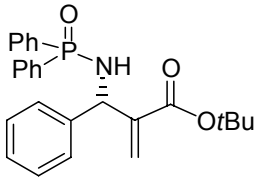
[IV-SEE-311]



98a



99c



rac-**100ac**

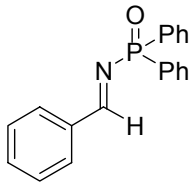
98a + **99c**	$\xrightarrow[\text{THF, 72 h, r.t.}]{Ph_2PMe}$	*rac*-**100ac**, 30 %

Under argon atmosphere*, tert*-butyl acrylate **99c** (580 µl, 512 mg, 4.00 mmol, 4.00 eq) was added to a mixture of imine **98a** (305 mg, 1.00 mmol, 1.00 eq) and diphenylmethylphosphine (37.0 µl, 40.0 mg, 200 µmol, 20.0 mol%) in dry THF (1 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by preparative-TLC (*c*-hexane/EtOAc 3:1) to obtain 130 mg (300 µmol, 30 %) of 1,1-dimethylethyl (β*S*)-β-[(diphenylphosphinoyl)-amino]-α-methylenebenzenepropanoate *rac*-**100ac** as colourless solid.

The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

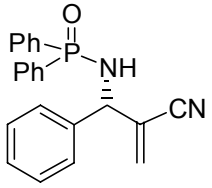
*5.5.3.4 Synthesis of (βS)-β-[(Diphenylphosphinoyl)amino]-α-methylenebenzenepropanenitrile (rac-**100af**)*

[IV-SEE-323]



98a

99f



rac-**100af**

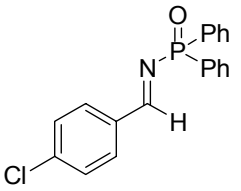
98a + **99f**	DABCO ⟶ MeCN, r.t., 72 h	*rac*-**100af**, 64 %

Under argon atmosphere, acrylonitrile **99af** (99.5 µl, 79.6 mg, 1.50 mmol, 1.50 eq) was added to a mixture of imine **98a** (305 mg, 1.00 mmol, 1.00 eq) and 1,4-diazabicyclo-[2.2.2]octane (16.8 mg, 150 µmol, 15.0 mol%) in dry MeCN (1 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by preparative-TLC (*c*-hexane/EtOAc 3:1) to obtain 230 mg (642 µmol, 64 %) of (β*S*)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzene-propanenitrile *rac*-**100af** as colourless solid.

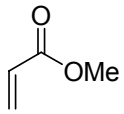
The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

*5.5.3.5 Synthesis of Methyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (rac-**100ba**)*

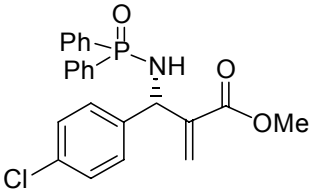
[IV-SEE-314]



98b



99a



rac-**100ba**

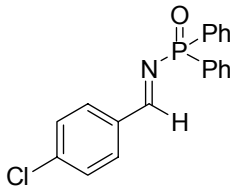
98b + **99a**	$\xrightarrow[CH_2Cl_2,\ r.t.,\ 72\ h]{Ph_2PMe}$	*rac*-**100ba**, 47 %

Under argon atmosphere, methyl acrylate **99a** (360 µl, 344 mg, 4.00 mmol, 4.00 eq) was added to a mixture of imine **98b** (339 mg, 1.00 mmol, 1.00 eq) and diphenylmethylphosphine (37.0 µl, 40.0 mg, 200 µmol, 20.0 mol%) in dry CH_2Cl_2 (1 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by preparative-TLC (*c*-hexane/EtOAc 3:1) to obtain 200 mg (470 µmol, 47 %) of methyl (β*S*)-4-chloro-β-[(diphenylphosphinoyl)-amino]-α-methylenebenzenepropanoate *rac*-**100ba** as colourless solid.

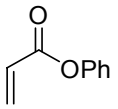
The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

*5.5.3.6 Synthesis of Phenyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylene-benzenepropanoate (rac-**100bb**)*

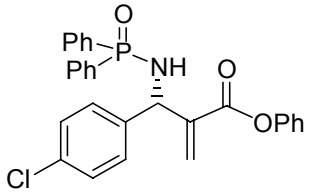
[IV-SEE-328]



98b



99b



rac-**100bb**

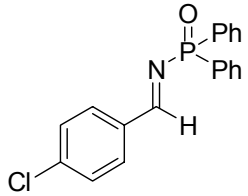
98b + **99b**	Ph_3P ⟶ MeCN, r.t., 72 h	*rac*-**100bb**, 51 %

Under argon atmosphere, phenyl acrylate **99b** (345 µl, 370 mg, 2.50 mmol, 2.50 eq) was added to a mixture of imine **98b** (339 mg, 1.00 mmol, 1.00 eq) and triphenylphosphine (39.3 mg, 150 µmol, 15.0 mol%) in dry THF (1 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by preparative-TLC (*c*-hexane/EtOAc 3:1) to obtain 241 mg (510 µmol, 51 %) of phenyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylene-benzenepropanoate *rac*-**100bb** as colourless solid.

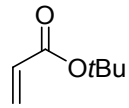
The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

*5.5.3.7 Synthesis of 1,1-Dimethylethyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (rac-**100bc**)*

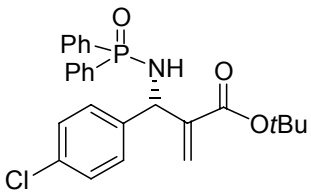
[IV-SEE-316]



98b



99c



rac-**100bc**

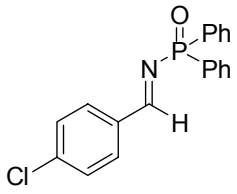
98b + **99c**	$\xrightarrow[CH_2Cl_2,\ r.t.,\ 72\ h]{Ph_2PMe}$	*rac*-**100bc**, 34 %

Under argon atmosphere*, tert*-butyl acrylate **99c** (580 µl, 512 mg, 4.00 mmol, 4.00 eq) was added to a mixture of imine **98b** (339 mg, 1.00 mmol, 1.00 eq) and diphenylmehtylphosphine (37.0 µl, 40.0 mg, 200 µmol, 20.0 mol%) in dry CH_2Cl_2 (1 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by preparative-TLC (*c*-hexane/EtOAc 3:1) to obtain 160 mg (342 µmol, 34 %) of 1,1-dimethylethyl (β*S*)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate *rac*-**100bc** as colourless solid.

The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

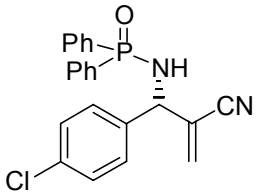
*5.5.3.8 Synthesis of (βS)-4-Chloro-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanenitrile (**100bf**)*

[IV-SEE-317]



98b

99f



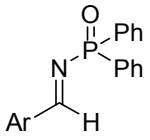
rac-**100bf**

98b + **99f**	DABCO MeCN, r.t., 72 h	*rac*-**100bf**, 51 %

Under argon atmosphere, acrylonitrile **99f** (99.5 µl, 79.6 mg, 1.50 mmol, 1.50 eq) was added to a mixture of imine **98b** (339 mg, 1.00 mmol, 1.00 eq) and 1,4-diazabicyclo-[2.2.2]octane (22.4 mg, 200 µmol, 20.0 mol%) in dry MeCN (1 ml). The suspension was stirred for 72 h at r.t.. The solvent and excess acrylate were removed under reduced pressure. The residue was purified by preparative-TLC (*c*-hexane/EtOAc 3:1) to obtain 200 mg (509 µmol, 51 %) of (β*S*)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylene-benzenepropanenitrile *rac*-**100bf** as colourless solid.

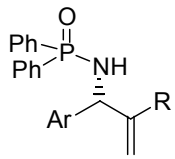
The spectroscopical data are in agreement with the data in chapter 5.5.4, p.144 et sqq..

5.5.4 General Procedure for the (R,R)-La-Linked BINOL Catalysed Aza-Baylis-Hillman Reaction of N-Diphenylphosphinoyl-Protected Imines with Olefins



98

99



100

98 + **99**	DABCO BINOL-based ligand / M(O*i*Pr)$_x$ ⟶ solvent, r.t., 35 h	**100**

A 0.2 M solution of La(O*i*Pr)$_3$ in THF (150 µl, 30.0 µmol, 10.0 mol%) was added to linked-(*R*,*R*)-BINOL **97** (18.4 mg, 30.0 µmol, 10.0 mol%) at 0 °C. After stirring for 15 min at 0 °C and 2 h at r.t., the *i*PrOH and THF were removed under reduced pressure. DABCO (6.73 mg, 60.0 µmol, 20.0 mol%) and the imine **98** (300 µmol, 1.0 eq) were added. The solids were dissolved in THF (300 µl), and the olefin component **99** (600 µmol, 2.00 eq) was added. After 35 h, the solvent and excess acrylate were removed under reduced pressure and the crude mixture was purified by preparative thin layer chromatography. The enantiomeric excess was determined by HPLC analysis using a chiral column.

*5.5.4.1 Methyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (**100aa**)*

Compound **100aa** was obtained as a colourless solid from **98a** and **99a** in 34 % yield. The enantiomeric purity was determined to be 89 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 38.7 min (*S*), 42.8 min (*R*)).

$C_{23}H_{22}NO_3P$ (391.2 g/mol)

m.p. 159-160 °C [m.p. ref.[131]: 157-160°C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.84-7.76 (m; 4H), 7.41-7.13 (m; 11H), 6.26 (s; 1H), 5.80 (s; 1H), 5.02 (t; *J* = 10.9 Hz, 1H), 4.24 (dd; *J* = 10.9 Hz, *J* = 8.6 Hz, 1H), 3.53 (s; 3H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 166.2, 141.2, 141.1, 133.0, 132.2, 132.1, 132.0, 128.7, 128.6, 128.5, 128.4, 127.3, 127.1, 126.5, 57.1, 51.9.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3174, 1717, 1436, 1273, 1190, 1151, 1122, 1108, 1068, 907, 696.

HR ESI-MS calcd for $C_{23}H_{22}NO_3P$ $[M+Na]^+$: 414.1235, found: 414.124.

The spectroscopical data are in agreement with the literature.[131]

*5.5.4.2 Phenyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (**100ab**)*

Compound **100ab** was obtained as a colourless solid from **98a** and **99b** in 59 % yield. The enantiomeric purity was determined to be 71 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 35.5 min (*S*), 45.5 min (*R*)).

$C_{28}H_{24}NO_3P$ (453.5 g/mol)

m.p. 157-159 °C [m.p. ref.[131]: 156-157 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.85-7.77 (m; 4H), 7.41-7.17 (m; 13H), 7.09-7.04 (m; 1H), 6.80-6.77 (m; 2H), 6.50 (s; 1H), 6.03 (s; 1H), 5.14 (t; *J* = 10.9 Hz, 1H), 4.18 (dd; *J* = 10.9 Hz, *J* = 8.6 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 164.4, 150.3, 141.1, 141.0, 141.0, 140.9, 132.8, 132.4, 132.3, 132.1, 132.1, 132.0, 129.4, 128.7, 128.6, 128.5, 127.5, 126.6, 126.0, 121.5, 57.1.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3061, 2916, 2220, 1730, 1590, 1437, 1250, 1187, 1121, 1063, 906, 847, 688.

HR ESI-MS calcd for $C_{28}H_{24}NO_3P$ $[M+Na]^+$: 476.1392, found: 476.139.

The spectroscopical data are in agreement with the literature.[131]

*5.5.4.3 1,1-Dimethylethyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzene-propanoate (**100ac**)*

Compound **100ac** was obtained as a colourless solid from **98a** and **99c** in 31 % yield. The enantiomeric purity was determined to be 70 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 0.12 ml/min; τ_R = 138.1 min (*S*), 144.6 min (*R*)).

$C_{26}H_{28}NO_3P$ (433.5 g/mol)

m.p. 121-123 °C

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.93-7.87 (m; 4H), 7.50-7.38 (m; 8H), 7.34-7.29 (m; 3H), 6.27 (s; 1H), 5.79 (s; 1H), 5.08 (t; *J* = 10.9 Hz, 1H), 4.18 (dd; *J* = 10.9 Hz, *J* = 8.7 Hz, 1H), 1.28 (s; 9H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 165.1, 142.7, 141.7, 133.3, 132.3, 132.2, 132.1, 132.1, 131.9, 128.6, 128.5, 128.4, 128.3, 127.1, 126.5, 125.9, 81.5, 57.1, 27.8.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3175, 2974, 1708, 1437, 1366, 1275, 1187, 1147, 1122, 1066, 748, 695.

HR ESI-MS calcd for $C_{26}H_{28}NO_3P$ $[M+Na]^+$: 456.1705, found: 456.170.

*5.5.4.4 2-Naphthalenyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzene-propanoate (**100ad**)*

Compound **100ad** was obtained as a colourless solid from **98a** and **99d** in 67 % yield. The enantiomeric purity was determined to be 63 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 56.6 min (*S*), 65.0 min (*R*)).

$C_{32}H_{26}NO_3P$ (503.5 g/mol)

m.p. 171-172 °C

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.99-7.91 (m; 4H), 7.83-7.73 (m; 3H), 7.54-7.28 (m; 14H), 7.04 (dd; *J* = 8.9 Hz, *J* = 2.2 Hz, 1H), 6.68 (s; 1H), 6.18 (s; 1H), 5.38 (t; *J* = 10.7 Hz, 1H), 4.29 (dd; *J* = 10.7 Hz, *J* = 8.6 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 164.5, 147.9, 141.1, 141.0, 133.6, 133.5, 132.9, 132.4, 132.3, 132.1, 132.1, 132.0, 131.8, 131.5, 131.2, 129.3, 128.7, 128.6, 128.6, 128.5, 128.5, 127.7, 127.6, 127.5, 126.6, 126.5, 125.8, 120.9, 118.5, 57.1.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3165, 3065, 1730, 1628, 1599, 1510, 1436, 1238, 1188, 1154, 1122, 1060, 960, 906, 808, 724, 696.

HR ESI-MS calcd for $C_{32}H_{26}NO_3P$ $[M+Na]^+$: 526.1548, found: 526.155.

*5.5.4.5 2,2,2-Trifluoro-1-(trifluoromethyl)ethyl (βS)-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (**100ae**)*

Compound **100ae** was obtained as a colourless solid from **98a** and **99e** in 41 % yield. For the determination of the enantiomeric purity, the 1,1,1,3,3,3-hexafluoroisopropyl acrylate was transformed to the corresponding methyl acrylate. To this end, Et_3N (500 µl, 360 µmol) was added to a stirred solution of the 2,2,2-Trifluoro-1-(trifluoromethyl)ethyl (*βS*)-*β*-[(diphenylphosphinyl)amino]-*α*-methylenebenzenepropanate **100ae** (50 µmol) in MeOH (500 µl) at r.t.. After stirring for 1 h, the mixture was acidified with DOWEX 50WX4-200, additionally stirred for 15 min, filtered, and evaporated. The enantiomeric purity was determined to be 80 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 38.7 min (*S*), 42.8 min (*R*)).

$C_{25}H_{20}F_6NO_3P$ (527.4 g/mol)

m.p. 190-192 °C [m.p. ref.[104]:192-193 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.83-7.74 (m; 4H), 7.47-7.35 (m; 6H), 7.25-7.19 (m; 5H), 6.49 (s; 1H), 6.13 (s; 1H), 5.57 (hept; *J* = 6.0 Hz, 1H), 5.11 (t; *J* = 10.5 Hz, 1H), 3.77 (dd; *J* = 10.5 Hz, *J* = 8.8 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 162.3, 140.2, 140.0, 139.0, 138.9, 132.9, 132.5, 132.3, 132.0, 130.7, 128.8, 128.7, 128.6, 128.5, 128.4, 127.9, 126.4, 121.1 (q; *J* = 283.4 Hz), 66.6 (hept; *J* = 35.0 Hz), 56.4, 30.8.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3168, 1748, 1436, 1384, 1355, 1287, 1230, 1194, 1107, 904, 724.

HR ESI-MS calcd for $C_{25}H_{20}F_6NO_3P$ $[M+Na]^+$: 550.0983, found: 550.098.

The spectroscopical data are in agreement with the literature.[104]

5.5.4.6 *(βS)-β-[(Diphenylphosphinoyl)amino]-α-methylenebenzenepropanenitrile (**100af**)*

Compound **100af** was obtained as a colourless solid from **98a** and **99f** in 48 % yield. The material was determined to be racemic by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 39.4 min (*S*), 47.5 min (*R*)).

$C_{22}H_{19}N_2OP$ (358.4 g/mol)

m.p. 179-180 °C [m.p. ref.[131]: 176-178 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.87-7.77 (m; 4H), 7.49-7.33 (m; 6H), 7.30-7.25 (m; 5H), 5.90 (d; *J* = 6.8 Hz, 2H), 4.92, (t; *J* = 9.8 Hz, 1H) 3.52 (dd; *J* = 9.8 Hz, *J* = 7.5 Hz, 1H)

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 138.9, 138.8, 132.3, 132.2, 132.1, 131.2, 129.2, 128.8, 128.7, 128.6, 128.6, 128.5, 126.8, 117.2, 57.5.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3053, 2867, 2221, 1437, 1185, 1123, 1106, 928, 696.

HR ESI-MS calcd for $C_{22}H_{19}N_2OP$ $[M+Na]^+$: 381.1133, found: 381.113.

The spectroscopical data are in agreement with the literature.[131]

5.5.4.7 *Methyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanoate (**100ba**).*

Compound **100ba** was obtained as a colourless solid from **98b** and **99a** in 39 % yield. The enantiomeric purity was determined to be 80 % by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 35.6 min (*S*), 42.4 min (*R*)).

$C_{23}H_{21}ClNO_3P$ (425.8 g/mol)

m.p. 173-174 °C [m.p. ref.[131]: 173-175 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.83-7.74 (m; 4H), 7.43-7.33 (m; 6H), 7.28-7.18 (m; 4H), 6.25 (s; 1H), 5.78 (s; 1H), 4.97 (t; *J* = 10.9 Hz, 1H), 4.26 (dd; *J* = 10.9 Hz, *J* = 8.6 Hz, 1H), 3.56 (s; 3H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 166.1, 140.6, 140.6, 139.8, 139.7, 133.2, 132.3, 132.2, 132.1, 132.1, 128.7, 128.6, 128.6, 128.4, 127.9, 127.4, 56.8, 52.0.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3171, 3054, 2954, 1719, 1489, 1436, 1268, 1187, 1122, 1073, 1014, 751, 696.

HR ESI-MS calcd for $C_{23}H_{21}ClNO_3P$ $[M+Na]^+$: 448.0846, found: 448.085.

The spectroscopical data are in agreement with the literature.[131]

*5.5.4.8 Phenyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylenebenzene-propanoate (**100bb**).*

Compound **100bb** was obtained as a colourless solid from **98b** and **99b** in 56 % yield. The enantiomeric purity was determined to be 69 % by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 36.3 min (*S*), 44.9 min (*R*)).

$C_{28}H_{23}ClNO_3P$ (487.9 g/mol)

m.p. 178-179 °C [m.p. ref.[131]: 180-182 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.83-7.74 (m; 4H), 7.43-7.17 (m; 12H), 7.12-7.07 (m; 1H), 6.83 (d; *J* = 7.6 Hz, 2H), 6.50 (s; 1H), 6.00 (s; 1H), 5.09 (t; *J* = 11.0 Hz, 1H), 4.24 (dd; *J* = 11.0 Hz, *J* = 8.7 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 164.2, 150.2, 140.5, 140.5, 139.7, 139.6, 133.4, 133.2, 132.8, 132.3, 132.2, 132.0, 131.5, 131.1, 129.4, 128.7, 128.6, 128.6, 128.5, 128.1, 126.1, 121.4, 56.7.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3161, 1731, 1592, 1484, 1435, 1248, 1186, 1159, 1122, 1066, 1012, 905, 809, 722, 684, 641.

HR ESI-MS calcd for $C_{28}H_{23}ClNO_3P$ $[M+Na]^+$: 510.1002, found: 510.100.

The spectroscopical data are in agreement with the literature.[131]

*5.5.4.9 1,1-Dimethylethyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methylene-benzenepropanoate (**100bc**).*

Compound **100bc** was obtained as a colourless solid from **98b** and **99c** in 27 % yield. The enantiomeric purity was determined to be 83 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 0.12 ml/min; τ_R = 151.3 min (*S*), 172.8 min (*R*)).

$C_{26}H_{27}ClNO_3P$ (467.9 g/mol)

m.p. 136-138 °C

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.83-7.75 (m; 4H), 7.42-7.33 (m; 6H), 7.27-7.17 (m; 4H), 6.17 (s; 1H), 5.66 (s; 1H), 4.94 (t; *J* = 11.0 Hz, 1H), 4.16 (dd; *J* = 11.0 Hz, *J* = 8.7 Hz, 1H), 1.23 (s; 9H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 164.9, 142.2, 142.1, 140.3, 140.3, 133.2, 132.9, 132.3, 132.2, 132.0, 128.7, 128.6, 128.5, 128.4, 128.0, 126.4, 81.8, 56.7, 27.9.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3172, 2975, 1707, 1489, 1436, 1366, 1274, 1186, 1122, 1085, 1014, 848, 751, 695.

HR ESI-MS calcd for $C_{26}H_{27}ClNO_3P$ $[M+Na]^+$: 490.1315, found: 490.132.

The spectroscopical data are in agreement with the literature.[131]

*5.5.4.10 2-Naphthalenyl (βS)-4-chloro-β-[(diphenylphosphinoyl)amino]-α-methyleneben-zenepropanoate (**100bd**)*

Compound **100bd** was obtained as a colourless solid from **98b** and **99d** in 59 % yield. The enantiomeric purity was determined to be 79 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 58.9 min (*S*),73.4 min (*R*)).

$C_{32}H_{25}ClNO_3P$ (537.9 g/mol)

m.p. 190-191 °C

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.97-7.75 (m; 7H), 7.55-7.33 (m; 13H), 7.09-7.05 (m; 1H), 6.67 (s; 1H), 6.15 (s; 1H), 5.24 (t; *J* = 10.8 Hz, 1H), 4.32 (dd; *J* = 10.8 Hz, *J* = 8.6 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 164.4, 147.8, 140.6, 140.6, 139.7, 139.6, 133.6, 132.3, 132.2, 132.0, 131.5, 129.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.1, 127.8, 127.7, 126.7, 125.9, 120.8, 118.5, 56.8.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3163, 3051, 2914, 1730, 1489, 1437, 1238, 1186, 1155, 1123, 1065, 907, 725, 697.

HR ESI-MS calcd for $C_{32}H_{25}ClNO_3P$ $[M+Na]^+$: 560.1159, found: 560.115.

*5.5.4.11 (βS)-4-Chloro-β-[(diphenylphosphinoyl)amino]-α-methylenebenzenepropanenitrile (**100bf**)*

Compound **100bf** was obtained as a colourless solid from **98b** and **99f** in 52 % yield. The enantiomeric purity was determined to be 11 % ee by HPLC (Chiralpak AD-H, 90:10 *i*PrOH/*n*-hexane, 1.0 ml/min; τ_R = 46.8 min (*S*), 51.3 min (*R*)).

$C_{22}H_{18}ClN_2OP$ (392.8 g/mol)

m.p. 142-144 °C [m.p. ref.[131]:142-143 °C]

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.94-7.84 (m; 4H), 7.58-7.44 (m; 6H), 7.37-7.30 (m; 4H), 5.98 (d; *J* = 17.8 Hz, 2H), 4.98 (t; *J* = 10.2 Hz, 1H), 3.66 (dd; *J* = 10.2 Hz, *J* = 7.4 Hz, 1H).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ [ppm] = 137.4, 137.4, 134.6, 132.5, 132.3, 132.2, 132.1, 131.9, 131.5, 130.7, 130.5, 129.3, 128.9, 128.8, 128.7, 128.6, 128.3, 125.4, 125.1, 116.9.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3144, 2866, 2218, 1490, 1436, 1183, 1106, 1090, 751, 725.

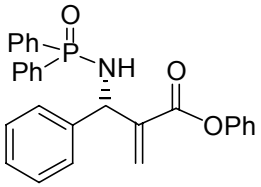
HR ESI-MS calcd for $C_{22}H_{18}ClN_2OP$ $[M+Na]^+$: 415.0743, found: 415.074.

The spectroscopical data are in agreement with the literature.[131]

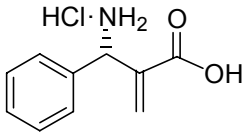
5.5.5 Hydrolytic C,N Terminal Deprotection[104]

*5.5.5.1 Synthesis of (βS)-β-Amino-α-methylenebenzenepropionic acid hydrochloride (**106a**)*

[IV-SEE-334]



100ab



106a

100ab	20 % HCl, reflux, 6 h	**106a**, 75 %

A mixture of **100ab** (300 mg, 661 µmol, 1.00 eq) in 20 % HCl (7 ml) was refluxed for 6 h. The reaction mixture as washed with Et_2O (3 x 4 ml) and EtOAc (2 x 4 ml) and the solvent was evaporated. The residue was dried under vacuo to give amino acid hydrochloride **106a** (105 mg, 496 µmol, 75 %) as a colourless solid.

$C_{10}H_{12}ClNO_2$ (213.6 g/mol)

m.p. 217-219 °C (decomposition)

^{1}H NMR (300 MHz, D_2O): δ [ppm] = 7.43-7.34 (m; 5H), 6.59 (s; 1H), 5.97 (s; 1H), 5.36 (s; 1H).
The OH- as well as the NH_3^+- protons could not be detected.

^{13}C NMR (75.5 MHz, D_2O): δ [ppm] = 167.9, 136.1, 134.1, 129.9, 129.5, 129.3, 127.3.

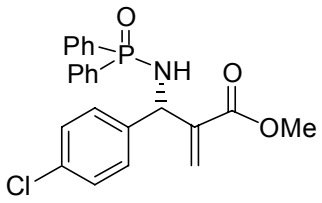
IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3385, 2870, 2600, 1962, 1697, 1626, 1577, 1493, 1435, 1395, 1252, 1164, 1116, 1004, 970, 919, 827, 759.

HR ESI-MS calcd for $C_{10}H_{12}ClNO_2$ $[M-Cl]^+$: 178.087 , found: 178.0868

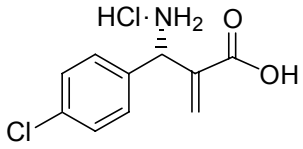
The spectroscopical data are in agreement with the literature.[104]

*5.5.5.2 (βS)-4-Chloro-β-amino-α-methylenebenzenepropionic acid hydrochloride (**106b**)*

[IV-SEE-335]



100ba



106b

100ba	20 % HCl reflux, 6 h	**106b**, 62 %

A mixture of **100ba** (200 mg, 470 µmol, 1.00 eq) in 20 % HCl (5 ml) was refluxed for 6 h. The reaction mixture as washed with Et_2O (3 x 3 ml) and EtOAc (2 x 3 ml) and the solvent was evaporated. The residue was dried under vacuo to give amino acid hydrochloride **106b** (72.0 mg, 291 µmol, 62 %) as a colourless solid.

$C_{10}H_{11}Cl_2NO_2$ (248.1 g/mol)

m.p. 209-211 °C (decomposition)

^{1}H NMR (300 MHz, D_2O) δ [ppm] = 7.60-7.52 (m; 4H), 6.80 (s; 1H), 6.17 (s; 1H), 5.56 (s; 1H).
The OH- as well as the NH_3^+- protons could not be detected.

^{13}C NMR (75.5 MHz, D_2O) δ [ppm] = 167.8, 135.9, 134.8, 132.8, 130.1, 129.3, 129.0, 54.3.

IR (ATR) $\tilde{\nu}$ [cm^{-1}] = 3387, 2886, 1962, 1700, 1629, 1595, 1494, 1412, 1198, 1157, 1092, 1014, 973, 834, 722.

The spectroscopical data are in agreement with the literature.[131]

6 Appendix

6.1 Abbrevations

For SI units the generally accepted abbreviations were used.

abs.	absolute (distilled and dried)
Ac	Acetyl
ANF	Atrial natriuretic factor
aq.	Aqueous
Ar	Aryl
ATR	Attenuated total reflection
BH	Baylis Hillman
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
BINOL	1,1'-Binaphthalene-2,2'-diol
Boc	*tert*-butyloxycarbonyl
BOP	(Benzotriazol-1-yloxy)-tris(dimethylamino)-phosphonium
Bu	Butyl
Cbz	Carboxybenzyl
d	Day(s)
DABCO	1,4-Diazabicyclo[2.2.2]octane
DACH	1,2-Diaminocyclohexane
DBU	1,8-Diazabicyclo[5.4.0]undec-7-en
DCE	Dichloroethane
DCM	Dichloromethane
DIANANE	2,5-Diamino-bicyclo[2.2.1]heptane
DMEM	Dulbecco's-modified eagle's medium
DMF	*N*,*N*-Dimethyl formamide
DMSO	Dimethylsulfoxide
DNA	Desoxyribonucleic acid
DOS	Diversity orientated sceening
dpp	Diphenylphosphinoyl
EB	Embryoid bodies
ECG	Electrocardiogram
ee	Enantiomeric excess
EGFP	Enhanced green fluorescence protein
ent-	Enantiomer
eq(uiv)	Equivalent(s)
(m)ES	(murine) Embryonic stem
Et	Ethyl
et sqq.	And the following (et sequentia)
EWG	Electron withdrawing group

FCS	Fetal calf serum
FDA	Food and Drug Administration
FT	Fourier transformation
GC	Gas chromatography
GC-MS	Gas chromatography connected with mass spectra
h	Hour(s)
HPLC	High performance liquid chromatography
3-HQD	3-Hydroxyquinuclidine
HR ESI-MS	High resolution electrospray ionisation mass spectra
Hz	Hertz
IMDM	Iscove's modified dulbecco's medium
IPDA	3-Aminomethyl-3,5,5-trimethylcyclohexylamine
*i*Pr	iso-Propyl
IR	Infrared Spectroscopy
J	Spin Coupling
LLB	La-Li-BINOL
LIF	Leukaemia inhibitor factor
m	Meta
α-MHC	α-Myosine heavy chain
m.p.	Melting point
m/z	Mass-to-charge ratio
Me	Methyl
min	Minute
MOP	2'-(Methoxy-[1,1']-binaphthalin-2-yl)-diphenyl-phosphin
MS	Molecular sieves
MtOA	Methyltrioctylammonium
MVK	Methyl vinyl ketone
n.d.	Not detected
NMR	Nuclear magnetic resonance
Ns	Nosyl
Nu	Nucleophile
o	Ortho
p	Para
pα-MHC	α-Myosine heavy chain promoter
PCC	Pyridinium chlorochromate
PCR	Polymerase chain reaction
PEG	Polyethyleneglycol
PG	Protecting group
Ph	Phenyl
PMP	*p*-Methoxyphenyl
PTA	1,3,5-Triaza-phosphaadamantane

p-TLC	Preparative thin layer chromatography
quant.	quantitative
r.t.	Room temperature
rac	Racemic
RDD	Rational drug design
RDS	Rate determing step
ref.	Reference
R_f	Retention factor
ROS	Reactive oxygen species
RyR	Ryanidine receptor
SBDD	Structur based drug design
SES	2-Trimethylsilylethanesulfonyl
SR	Sarcoplasmic reticulum
*t*Bu	*tert*-Butyl
Tf	Triflate
THF	Tetrahydrofuran
Tol	Toluene
Ts	Tosyl
TSA	Toluene sulfonic acid
®	Trade mark
τ_R	Retention time

6.2 References

[1] A. Sachinidis, B. K. Fleischmann, E. Kolossov, M. Wartenberg, H. Sauer, J. Hescheler, *Cardiovasc. Res*. **2003**, *58*, 278-291.

[2] Committee for orphan medicinal products, Public Summary of the positive opinion for orphan designation of sorafenib-tosylate **2007**, Doc. Ref: EMEA/COMP/294.

[3] J. R. Milne, K. J. Hellestrand, R. S. Bexton, P. J. Burnett, N. M. Debbas, A. J. Camm, *Eur. Heart J.* **1984**, *5*, 99–107.

[4] A. Sachinidis, S. Schwengberg, R. Hippler-Altenburg, D. Mariappan, N. Kamisetti, B. Seelig, A. Berkessel, J. Hescheler, *Cell. Physiol. Biochem.* **2006**, *18*, 303-314.

[5] For a review see: Y. Takemoto, H. Miyabe, *Chimia* **2007**, *61*, 269-275.

[6] J. M. Mitchell, N. S. Finney, *Tetrahedron Lett.* **2000**, *41*, 8431-8434.

[7] M. Kaik, J. Gawronski, *Tetrahedron: Asymmetry* **2003**, *14*, 1559-1563.

[8] Y. Sohtome, A. Tanatani, Y. Hashimoto, K. Nagasawa, *Chem. Pharm. Bull*. **2004**, *52*, 477-480.

[9] (a) E. Juaristi (Ed.), *Enantioselective Synthesis of β-Amino Acids*, Wiley, New York, **1997**; (b) D. Seebach, A. K. Beck, S. Capone, G. Deniau, U. Grošelj, E. Zass *Synthesis* **2009**, *1*, 1-39.

[10] E. J. Till, E. A. McCulloch, L. Siminovitch, A. M. Wu, *Proc. Natl. Acad. Sci. USA* **1968**, *59*, 1209-1215.

[11] J. Altman, G. D. Das, *Nature* **1967**, *214*, 1098-101.

[12] P. L. Davis, M. H. Shumway, *Medical Times* **1958**, *86*, 141-145.

[13] J. Dausset, *Revue Francaise d'Etudes Cliniques et Biologiques* **1958**, *3*, 825-828.

[14] R. G. Edwards, *Nature* **2001**, *413*, 349-351.

[15] J. A. Thomson, A. James, J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, S. V. Marshall, J. M. Jones, *Science* **1998**, *282*, 1145-1147.

[16] D. Enders, "Alkylation of chiral Hydrazones" in J. D. Morrison (Ed.), *Asymm. Synthesis 3*, Academic Press, New York, **1984**.

[17] U. Schöllkopf, *Topics in Current Chemistry*, **1983**, *109*, 65-68.

[18] (a) R. Noyori, I. Tomino, Y. Tanimoto, *J. Am. Chem. Soc*. **1979**, *101*, 3129-3131; (b) R. Noyori, *Pure Appl. Chem*. **1981**, *53*, 2316-2319.

[19] K. Drauz, H. Waldmann, *Enzyme Catalysis in Organic Synthesis*, 2nd Ed. Wiley-VCH, Weinheim, **2002**.

[20] E. N. Jacobsen, A. Pfaltz, H. Yamamoto, *Comprehensive Asymmetric Catalysis*, Springer, Berlin, **1999**.

[21] A. Berkessel, H. Gröger, *Asymmetric Organocatalysis*, Wiley-VCH, Weinheim, **2005**.

[22] J. Müller-Ehmsen, K. L. Peterson, L. Kedes, O. Whittaker, J. S. Dow, T. I. Long, P.W. Laird, R. A. Kloner, *Circulation* **2002**, *105*, 1720-1726.

[23] W. Roell, Z. J. Lu, W. Bloch, S. Siedner, K. Tiemann, Y. Xia, E. Stoecker, M. Fleischmann, H. Bohlen, R. Stehle, E. Kolossov, G. Brehm, K. Addicks, G. Pfitzer, A. Welz, J. Hescheler, B. K. Fleischmann, *Circulation* **2002**,*105*, 2435-2441.

[24] J. Itzkovitz-Eldor, M. Schuldiner, D. Karsenti, A. Eden, O. Yanuka, M. Amit, H. Soreq, N. Benvenisty, *Mol. Med.* **2000**, *6*, 88-95.

[25] for a review see: (a) S. Ding, P. G. Schultz, *Nature Biotechnol*. **2004**, *22*, 833-840, (b) A. Sachinidis, I. Sotiriadou, B. Seelig, A. Berkessel, J. Hescheler, *Comb. Chem. High Throughput Screening* **2008**, *11*, 70-82.

[26] C. Ventura, M. Maioli, *Circ. Res.* **2000**, *87*, 189-194.

[27] (a) A. M. Wobus, G. Kaomei, J. Shan, M. C. Weller, J. Rohwedel, G. Ji, B. Fleischmann, H. A. Katus, J. Hescheler, W. M. Franz, *J. Mol. Cell. Cardiol*. **1997**, *29*, 1525-1539. (b) K. Hidaka, J.-K. Lee, H. S. Kim, C. H. Ihm, A. Iio, M. Ogawa, S.-I. Nishikawa, I. Kodama, T. Morisaki, *FASEB J.* **2003**, *17*, 740-742.
[28] T. Takahashi, B. Lord, C. Schulze, R. M. Fryer, S. S. Sarang, S. R. Gullans, R.T. Lee, *Circulation* **2003**, *107*, 1912-1916.
[29] O. Arrigoni, M. C. de Tullio, *Biochim. Biophys. Acta* **2002**, *69*, 167-174.
[30] H. Sauer, M. Wartenberg, J. Hescheler, *Cell. Physiol. Biochem.* **2001**, *11*, 173-186.
[31] K. Fukada, *Artif. Organs* **2001**, *25*, 187-193.
[32] C. Xu, S. Police, N. Rao, M. K. Carpenter, *Circ. Res.* **2002**, *91*, 501-508.
[33] X. Wu, S. Ding, Q. Ding, N. S. Gray, P. G. Schultz*, J. Am. Chem. Soc.* **2004**, *126*, 1590-1591.
[34] S. Ding, N. S. Gray, X. Wu, Q. Ding, P. G. Schultz, *J. Am. Chem. Soc*. **2002**, *124*, 1594-1596.
[35] I. S. Skerjanc, *Trends Cardiovasc. Med*. **1999**, *9*, 139-143.
[36] J. J. Feher, G. B. Lipford, *Biochim. Biophys. Acta* **1985**, *813*, 77-86.
[37] J. Striessnig, M. Grabner, H. Glossmann, *Trends of Pharmacol. Sci*. **1998**, *19*, 108-115.
[38] J. D. Molkentin, *Cardiovasc. Res*. **2004**, *63*, 467-475.
[39] K. R. Boheler, J. Czyz, D. Tweedie, H.-T. Yang, S. V. Anisimov, A. M. Wobus, *Circ. Res*. **2002**, *91*, 189-201.
[40] E. Kolossov, Z. Lu, I. Drobinskaya, N. Gassanov, Y. Duan, H. Sauer, O. Manzke, W. Bloch, H. Bohlen, J. Hescheler, B. K. Fleischmann, *FASEB J.* **2005**, *19*, 577-579.
[41] H.-J. Böhm, G. Klebe, H. Kubini, *Wirkstoffdesign*, Spektrum, Heidelberg, **1996**.
[42] T. Wunberg, M. Hendrix, A. Hillisch, M. Lobell, H. Meier, C. Schmeck, H. Wild, B. Hinzen, *Drug Discovery Today* **2006**, *11*, 175-180.
[43] S. L. Schreiber, *Chem. Eng. News* **2003**, *3*, 51-61.
[44] A. Berkessel, M. Guixà, F. Schmidt, J. M. Neudörfl, J. Lex, *Chem. Eur. J.* **2007**, *13*, 4483-4498.
[45] For a detailed test protocoll of the physiological screenings see supporting information.
[46] M. Brandenburg, *Dissertation*, Köln, **2008**.
[47] F. Cleemann, *Dissertation*, Köln, **2006**.
[48] S. Mukherjee, *Dissertation*, Köln, **2005**.
[49] T. Müller, *Dissertation*, Köln, **2005**.
[50] S. Schnippering, *Dissertation*, Köln, **2007**.
[51] K. Roland, *Dissertation*, Köln, **2006**.
[52] M. Guixà, *Dissertation*, Köln, **2005**.
[53] F. Schmidt, *Dissertation*, Köln, **2005**.
[54] A. Berkessel, M. Schröder, C. A. Sklorz, S. Tabanella, N. Vogl, J. Lex. J. M. Neudörfl, *J. Org. Chem*. **2004**, *69*, 3050-3056.
[55] A. Berkessel, K. Roland, M. Schröder, J. M. Neudörfl, J. Lex, *J. Org. Chem.* **2006**, *71*, 9212-9318.
[56] D. Conlon, N. Yasuda, *Adv. Synth. Cata*. **2001**, *343*, 137-138.
[57] Y. Sohtome, N. Takemura, R. Takagi, Y. Hashimoto, K. Nagasawa, *Tetrahedron* **2008**, *64*, 9423-9429.
[58] A. Berkessel, M. Guixà, F. Schmidt, J. M. Neudörfl, J. Lex, *Chem. Eur. J*. **2007**, *13*, 4483-4498.

[59] C. Exner, A. Pfaltz, M. Studer, H.-U. Blaser, *Adv. Synth. Catal.* **2003**, *345*, 1253-1260.
[60] W. A. Jacobs, M. Heidelberger, *J. Am. Chem. Soc.* **1919**, *41*, 2090-2120.
[61] R. T. Major, J. Finkelstein, *J. Am. Chem. Soc*. **1941**, *63*, 1368-1371.
[62] T. R. Kelly, P. Meghani, V. S. Ekkundi, *Tetrahedron Lett*. **1990**, *31*, 3381-3384.
[63] M. C. Etter, *Acc. Chem. Res*. **1990**, *23*, 120-126.
[64] Y. Takemoto, *Org. Biomol. Chem*. **2005**, *3*, 4299-4306 and references herein.
[65] (a) T. Okino, Y. Hoashi, Y. Takemoto, *J. Am. Chem. Soc*. **2003**, *125*, 12672-12673; (b) T. Okino, Y. Hoashi, T. Furukawa, X. Yu, Y. Takemoto, *J. Am. Chem. Soc*. **2005**, *127*, 119-125.
[66] (a) T. Okino, Y. Hoashi, Y. Takemoto, *Angew. Chem. Int. Ed.* **2005**, *44*, 4032-4037; (b) T. Inokuma, Y. Hoashi, Y. Takemoto, *J. Am. Chem. Soc.* **2006**, *128*, 9413-9418.
[67] Y. Takemoto, H. Miyabe, *Chimia* **2007**, *61*, 269-275.
[68] T. Okino, S. Nakamura, T. Furukawa, Y. Takemoto, *Org. Lett*. **2004**, *6*, 625-631.
[69] (a) A. Berkessel, F. Cleemann, S. Mukherjee, T. N. Müller, J. Lex, *Angew. Chemie Int. Ed.* **2005**, *44*, 807-811; (b) A. Berkessel, S. Mukherjee, F. Cleemann, T. N. Müller, J. Lex, *Chem. Commun.* **2005**, 1898-1900; (c) A. Berkessel, S. Mukherjee, T. N. Müller, F. Cleemann, K. Roland, M. Brandenburg, J. M. Neudörfl, *J. Org. Biomol. Chem.* **2006**, *4*, 4319-4330.
[70] B. Procuranti, S. J. Connon, *Chem. Comm.* **2007**, 1421-1451.
[71] F. Touchard, F. Fache, M. Lemaire, *Tetrahedron: Asymmetry* **1997**, *8*, 3319-3326.
[72] C. Bied, J. J. E. Moreau, M. W. Chi Man, *Tetrahedron: Asymmetry* **2001**, *12*, 329-336.
[73] A. Berkessel, M. Brandenburg, E. Leitterstorf, J. Frey, J. Lex, M. Schäfer, *Adv. Synth. Catal.* **2007**, *349*, 2385-2391.
[74] H. Rivier, *Bull. Soc. Chim. France* **1906**, *35*, 837-841.
[75] F. Wrede, W. Schneider, *Chem. Ber*. **1914**, *47*, 2038-2043.
[76] D. Menche, S. Böhm, J. Li, S. Rudolph, W. Zander, *Tetrahedron Lett.* **2007**, *48*, 365-369.
[77] R. A. da Silva, I. H. S. Estevam, L. W. Bieber, *Tetrahedron Lett.* **2007**, *48*, 7680-7682.
[78] H. Loibner, A. Pruckner, A. Stütz, *Tetrahedron Lett.* **1984**, *25*, 2535-2536.
[79] S. G. Davies, A. A. Mortlock, *Tetrahedron* **1991**, *49*, 4419-4438.
[80] K.-i. Morita, Z. Suzuki, H. Hirose, *Bull. Chem. Soc. Jpn*. **1968**, *41*, 2815.
[81] A. B. Baylis, M.E.D. Hillman, *Ger. Offen*. 2155113, **1971**.
[82] P. Buskens, J. Klankermayer, W. Leitner, *J. Am. Chem. Soc*. **2005**, *127*, 16762-16763.
[83] I. T. Raheem, E. N. Jacobsen, *Adv. Synth. Catal*. **2005**, *347*, 1701-1708.
[84] For a review see: (a) G. Masson, C. Housseman, J. Zhu, *Angew. Chem*. **2007**, *119*, 4698-4712; (b) Y.-L. Shi, M. Shi, *Eur. J. Org. Chem*. **2007**, 2905-2916; (c) V. Dekleck, J. Matinez, F. Lamaty, *Chem. Rev*. **2009**, *109*, 1-48.
[85] T. Greene, P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 4th Ed., John Wiley & Sons, Inc., New York, **2006**.
[86] (a) A. Gajda, T. Gajda, *J. Org. Chem*. **2008**, *73*, 8643-8646; (b) B. Das, K. Damodar, N. Chowdhury, D. Saritha, B. Ravikanth, M. Krishnaiah, *Tetrahedron* **2008**, *64*, 9396-9400, (c) E. L. Myers, J. G. de Vries, V. K. Aggarwal, *Angew. Chem. Int. Ed*. **2007**, *46*, 1893-1896.

[87] For example: (a) J. Cyrener, K. Burger, *Monatsh. Chem*. **1995**, *126*, 319-331; (b) S. Bertenshaw, M. Kahn, *Tetrahedron Lett*. **1989**, *30*, 2731-2732; (c) V. K. Aggarwal, A. M. M. Castro, A. Mereu, H. Adams, *Tetrahedron Lett.* **2002**, *43*, 1577-1580; (d) M. Shi, Y.-M. Xu, *Tetrahedron: Asymmetry* **2002**, *13*, 1195-1199.
[88] M. Shi, G.-L. Zhao, *Tetrahedron Lett*. **2002**, *43*, 9171-9172.
[89] V. Declerck, P. Ribière, J. Martinez, F. Lamaty, *J. Org. Chem*. **2004**, *69*, 8372-8375.
[90] (a) D. Balan, H. Adolffson, *J. Org. Chem.* **2001**, *66*, 6498-6502; (b) D. Balan, H. Adolfsson, *J. Org. Chem.* **2002**, *67*, 2329-2332; (c) D. Balan, H. Adolfsson, *Tetrahedron Lett*. **2003**, *44*, 2521-2524.
[91] For example: M. Shi, Y.-M. Xu, *Chem. Commun*. **2001**, 1876-1878.
[92] For example: G.-L. Zhao, J.-W. Huang, M. Shi, *Org. Lett*. **2003**, *5*, 4737-4739.
[93] For example: G.-L. Zhao, M. Shi, *J. Org. Chem*. **2005**, *70*, 9975-9978.
[94] For example: T. G. Back, D. A. Rankic, J. M. Sorbetti, J. E. Wulff, *Org. Lett.* **2005**, *7*, 2377-2379.
[95] For example: N. Rastogi, R. Mohan, D. Panda, S. M. Mobin, I. N. N. Namboothiri, *Org. Biomol. Chem.* **2006**, *4*, 3211-3216.
[96] For example : Z. Wu, G. Zhou, J. Zhou, W. Gou, *Synth. Commun*. **2006**, *36*, 2491-2493.
[97] For example: X. Meng, Y. Huang, R. Cheng, *Chem.-Eur. J.* **2008**, *14*, 6852-6854.
[98] E. P. Kündig, L. Xu, B. Schnell, *Synlett* **1994**, 413-414
[99] D. A. Cogan, G. Liu, K. Kim, B. J. Backes, J. A. Ellman, *J. Am. Chem. Soc*. **1998**, *120*, 8011-8012.
[100] F. A. Davis, Y. Zhang, T. Fang, D. L. Fanelli, H. Zhang, *J. Org. Chem*. **1999**, *64*, 1403- 1407.
[101] A. Lu, X. Xu, P. Gao, Z. Zhou, H. Song, C. Tang, *Tetrahedron: Asymmetry* **2008**, *19*, 1886-1890.
[102] M. Shi, Y.-M. Xu, *Angew. Chem. Int. Ed*. **2002**, *41*, 4507-4510.
[103] Y. Iwabuchi, M. Nakatani, N. Yokoyama, S. Hatakeyama, *J. Am. Chem. Soc*. **1999**, *121*, 10219-10220.
[104] S. Kawahara, A. Nakano, T. Esumi, Y. Iwabuchi, S. Hatakeyama, *Org. Lett*. **2003**, *5*, 3103-3105.
[105] N. Abermil, G. Masson, J. Zhu, *J. Am. Chem. Soc.* **2008**, *130*, 12596-1597.
[106] K. Matsui, S. Takizawa, H. Sasai, *J. Am. Chem. Soc*. **2005**, *127*, 3680-3682.
[107] M. Shi, L.-H. Chen, *Chem. Comm*. **2003**, 1310-1311.
[108] Z.-Y. Lei, G.-N. Ma, M. Shi, *Eur. J. Org. Chem*. **2008**, 3817-3823.
[109] Y.-H. Liu, L.-H. Chen, M. Shi, *Adv. Synth. Catal*. **2006**, *348*, 973-977.
[110] K. Ito, K. Nishida, T. Gotanda, *Tetrahedron Lett*. **2007**, *48*, 6147-6149.
[111] (a) M.-J. Qi, T. Ai, M. Shi, G. Li, *Tetrahedron* **2008**, *64*, 1181-1183; (b) Y.-L. Shi; M. Shi, *Adv. Synth. Catal*. **2007**, *349*, 2129-2132; (c) Y. Guan, Y.-Q. Jiang, M. Shi, *Eur. J. Org. Chem*. **2008**, 2150-2155; (d) Y.-H. Liu, M. Shi, *Adv. Synth. Catal*. **2008**, *350*, 122-126.
[112] L. He, Y.-R. Zhang, X.-L. Huang, S. Ye, *Synthesis* **2008**, *17*, 2825-2829.
[113] R. Gausepohl, P. Buskens, J. Kleinen, A. Bruckmann, C. W. Lehmann, J. Klankermayer, W. Leitner, *Angew. Chem. Int. Ed.* **2006**, *45*, 3689-3692.
[114] V. Declerck, J. Martinez, F. Lamaty, *Chem. Rev.* **2009**, *109*, 1-48.
[115] (a) J. M. Brown, A. P. James, L. M. Prior, *Tetrahedron Lett.* **1987**, *28*, 2179-2183; (b) M. Tagaki, K. Yamamoto, *Tetrahedron* **1991**, *47*, 8869-8875; (c) H. Huang, X. Liu, J. Deng, M. Zqui, Z. Zheng, *Org. Lett*. **2006**, *8*, 3359-3362; (d) J. Deng, X.-P.

Hu, J.-D. Huang, S.-B. Yu, D.-Y. Wang Z.-C. Duan, Z. Zheng, *Org. Chem.* **2008**, *73*, 2015-2019; (e) P. Ribière, C. Enjalbal, J.-L. Aubagnac, N. Yadav-Bhatnagar, J. Martinez, F. Lamaty, *J. Comb. Chem*. **2004**, *6*, 464-468.
[116] (a) D. Balan, H. Adolfsson, *Tetrahedron Lett*. **2004**, *45*, 3089-3091; (b) S. Gowrisankar, K. Y. Lee, J. N. Kim, *Tetrahedron Lett*. **2005**, *46*, 4859-4862; (c) H. S. Lee, J. M. Kim, J. N. Kim, *Tetrahedron Lett*. **2007**, *48*, 4119-4121; (d) S. J. Kim, H. S. Kim, T. H. Kim, J. N. Kim *Bull. Korean Chem. Soc*. **2007**, *28*, 1605-1611.
[117] (a) D. Y. Park, M. J. Lee, T. H. Kim, J. N. Kim, *Tetrahedron Lett.* **2005**, *46*, 8799-8802; (b) S. Gowrisankar, H. S. Lee, J. M. Kim, J. N. Kim *Tetrahedron Lett.* **2006**, *47*, 5785-5788; (c) S. Gowrisankar, H. S. Lee, J. M. Kim, J. N. Kim *Tetrahedron Lett.* **2008**, *49*, 1670-1673.
[118] Y.-M. Xu, M. Shi, *J. Org. Chem.* **2004**, *69*, 417-422.
[119] S. Kawahara, A. Nakano, T. Esumi, Y. Iwabuchi, S. Hatakeyama, *Org. Lett*. **2003**, *5*, 3103-3105.
[120] S. Matsunaga, N. Kumagai, S. Harada, M. Shibasaki, *J. Am. Chem. Soc.* **2003**, *125*, 4712-4714.
[121] For enantioselective Morita-Baylis-Hillman reactions catalysed by chiral Lewis acid complexes see: (a) K.-S. Yang, W.-D. Lee, J.-F. Pan, K. Chen, *J. Org. Chem*. **2003**, *68*, 915. (b) K. Matsui, S. Takizawa, H. Sasai, *Tetrahedron Lett.* **2005**, *46*, 1943.
[122] V. K. Aggarwal, A. Mereu, G. J. Tarver, R. Mc Cague, *J. Org. Chem*. **1998**, *63*, 7183-7189.
[123] M. Shi, G.-L. Zhao, *Tetrahedron Lett*. **2002**, *43*, 4499-4502.
[124] S. Y. Kim, S. Matsunaga, J. Das, A. Sekine, T. Oshima, M. Shibasaki, *J. Am. Chem. Soc.* **2000**, *122*, 6505-6507.
[125] M. Shibasaki, S. Matsunaga, *Chem. Soc. Rev*. **2006**, 269-279.
[126] S. Handa, V. Gnanadesikan, S. Matsunaga, M. Shibasaki, *J. Am. Chem. Soc*. **2007**, *129*, 4900-4901.
[127] M. Shi, C.-Q. Li, *Tetrahedron: Asymmetry* **2005**, *16*, 1385-1391.
[128] W. B. Jennings, C. J. Lovely, *Tetrahedron* **1991**, *47*, 5561-5568.
[129] A. Côté, A. A. Boezio, A. Charrette, *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 5405-5410.
[130] B. M. Trost, J. Jaratjaroonphong, V. Reutrakul, *J. Am. Chem. Soc*. **2006**, *128*, 2778-2779.
[131] M. Shi, G.-L. Zhao, *Adv. Synth. Catal.* **2004**, *346*, 1205-1219.

6.3 Abstract - Kurzzusammenfassung

Abstract

In this work, new substances for the chemical induction of cardiomyogenesis from embryonic stem (ES) cells have been developed and contributions to asymmetric C-C couplings were made.

A transgenic murine ES cell lines was used to investigate the effects of (thio)urea and cinchona-alkaloid derivatives on cardiomyogenesis. Various compound screenings yielded substances which led to a 50 % to 80 % increased cardiomyogenesis compared to untreated cells. In the test system investigated, time dependent screening approaches appeared to be of limited suitability for the identification of potential cellular targets.

A facile two-step procedure for the preparation of *Takemoto's* catalyst was developed. The thiourea moiety was obtained by condensation of 3,5-bis(trifluoromethyl)aniline with phenylchlorothioformate and direct reaction with *trans*-1,2-diaminocyclohexane. Subsequent reductive dimethylation using formaldehyde / zinc afforded *Takemoto's* catalyst in an overall yield of 36 %.

For the first time, an enantioselective aza-Baylis-Hillman reaction was catalysed by a chiral Lewis acidic metal complex. *N*-dpp-α-methylene-β-amino acid esters were obtained in up to 68 % yield and up to 90 % ee, using a La-linked-(*R*,*R*)-BINOL complex as catalyst and *N*-dpp-protected imines as substrates. The subsequent hydrolytic *C*,*N*-terminal deprotection provided direct access to enantio-enriched α-methylene-β-amino acids in up to 75 % yield.

Kurzzusammenfassung

In Rahmen dieser Arbeit wurden neue Substanzen für die chemisch induzierte Kardiomyogenese von embryonalen Stammzellen entwickelt und Beiträge zu asymmetrischen C-C Kupplungen geleistet.
Die Einfüsse von (Thio)Harnstoffderivaten und Cinchona-Alkaloiden auf die Bildung von Kardiomyocyten wurden an einer transgenen mausartigen embyronalen Stammzelllinie untersucht. In Screenings dieser Testsubstrate wurden Verbindungen identifiziert, welche eine 50 - 80 %ige Zunahme der Kardiomyogenese, verglichen zu unbehandelten Stammzellen, bewirken. In den untersuchten Testsystemen konnten durch zeitabhängige Screeningverfahren keine Eingrenzung potentieller Targetproteine vorgenommen werden.

Eine vereinfachte zwei-Stufen Synthese zur Darstellung des *Takemoto* Katalysators wurde entwickelt. Die Thioharnstoffeinheit wurde dabei durch Kondensation von 3,5-(Bistrifluormethyl)anilin mit Phenylchlorothioformiat und Reaktion mit *trans*-1,2-Diaminocyclohexan erhalten. Anschließende reduktive Dimethylierung mit Formaldehyd / Zink ergab den *Takemoto* Katalysator in einer Gesamtausbeute von 36 %.

Weiterhin wurde eine enantioselektive aza-Baylis-Hillman Reaktion entwickelt, die erstmals durch einen chiralen Lewis sauren Metallkomplex katalysiert wurde. Einsatz eines (*R*,*R*)-BINOL-basierten La-Komplexes als Katalysator und dpp - geschützter Imine als Substrate ergab *N*-dpp-α-Methylen-β-aminosäureester in bis zu 68 % Ausbeute und bis zu 90 % ee. Hydrolytische *C*,*N*-terminale Entschützung führte in einem Schritt zu der entsprechenden freien α-Methylen-β-aminosäure in bis zu 75 % Ausbeute.

6.4 Erklärung

“Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist, sowie, dass ich eine solche Veröffentlichung vor Abschluß des Promotionsverfahren nicht vornehmen werde.
Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. A. Berkessel betreut worden.“

Köln, 2009-04-28

Bianca Seelig

Bisher sind folgende Teilpublikationen veröffentlicht worden:

„*Identification of Small Signalling Molecules Promoting Cardiac Specific Differentiation of Mouse Embryonic Stem Cells*”,
A. Sachinidis, S. Schwengberg, R. Hippler-Altenburg, D. Mariappan, N. Kamisetti, J. Hescheler, A. Berkessel, B. Seelig, *Cell. Physiol. Biochem*. **2006**, *18*, 303-314.

„*A Chemical Genetics Approach for Specific Differentiation of Stem Cells to Somatic Cells: A Promising Therapeutical Approach*”
A. Sachinidis, I. Sotiriadou, B. Seelig, J. Hescheler, A. Berkessel, *Comp. Chem. High Throughput Screening* **2008**, *11*, 70-82.

„*A Simplified Synthesis of Takemoto’s Catalyst*“,
A. Berkessel, B. Seelig, *Synthesis* **2009**, *in press*.

„*Chemically Induced Cardiomyogenesis of Embryonic Stem Cells*”,
A. Berkessel, B. Seelig, S. Schwengberg, J. Hescheler, A. Sachinidis, *submitted*.

„*Enantioselective Aza-Baylis-Hillman Reaction Catalysed by a La-Linked-BINOL Complex*“,
H. Morimoto, B. Seelig, S. Matsunaga. M. Shibasaki, A. Berkessel, *submitted.*