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Combined biocatalytic and transition metal-catalyzed approaches for the synthesis of biologically active peptides and proteins

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Abstract

Peptides are important drugs produced by the pharmaceutical industry. Although they cannot be applied orally, nowadays a large amount of peptide-based drugs have entered on the market.

Commonly solid-phase peptide synthesis (SPPS) is used to synthesize medium-sized or long peptides (10–50 amino acid residues). Often it is necessary to link several smaller peptide fragments together via fragment condensation reactions – a process which is frequently associated with partial epimerization.

In this thesis a novel chemoenzymatic peptide synthesis strategy was developed which operates free of epimerization. Peptide-enolesters were prepared by Ru-catalyzed addition of peptides across alkynes, which were subsequently used as starting materials for enzymatic peptide coupling reactions with Ser-hydrolase alcalase-CLEA. It was shown that the enolester formation is compatible with a series of unprotected amino acid side chains. Various tripeptides could be synthesized by this chemoenzymatic process.

In nature quite frequently various post-translational modifications (PTM) are used to modulate the biological activity of peptides and proteins. For example, one important PTM is prenylation (farnesylation) on the cysteine residue in oncogenic Ras proteins.

In the continuing effort to make transition metal catalysis available for the modification of biopolymers, we have started investigations to prenylate proteins by Pd-catalyzed allylation of cysteine derivatives. This endeavor faces several challenges which have been tackled in this thesis.

In an extensive screening effort we have identified suitable ligands and reaction conditions which allow the allylation of thiols and cysteines in peptides to proceed with remarkable catalytic turnovers and excellent *n/iso* ratio. We have already succeeded in the efficient synthesis of farnesylated cysteine containing peptide derivatives.

Kurzfassung

Peptide sind wichtige Wirkstoffe, die von der Pharmazeutischen Industrie hergestellt werden. Trotz ihrer mangelnden oralen Verfügbarkeit sind diese Medikamente mittlerweile auf dem Markt etabliert.

Um mittelgroße oder lange Peptide (10-50 Aminosäuren) zu synthetisieren, verwendet man für gewöhnlich die Festphasen-Peptid-Synthese (SPPS). Häufig ist es notwendig, kleinere Peptide-Fragmente über Fragmentkupplungen miteinander zu verbinden - ein Prozess der häufig mit teilweiser Epimerisierung verbunden ist.

In dieser vorliegenden Arbeit wurde eine neue chemoenzymatische Peptid-Synthesestrategie entwickelt, welche ohne Epimerisierung abläuft. Peptid-enolester wurden mit Hilfe von Ru-katalysierter Addition von Peptiden an Alkine hergestellt. Nachfolgend wurden diese als Startmaterial für die enzymatische Peptidkupplung unter zu Hilfenahme der Serin-Hydrolase Alcalase-CLEA verwendet. Die Enolester-Synthese wurde erfolgreich für eine ganz Reihe ungeschützter Aminosäure-Reste gezeigt. Verschiedene Tripeptide konnten auf diese Weise synthetisiert werden.

In der Natur werden verschiedene Formen der post-translationalen Modifikation (PTM) genutzt, um die biologische Aktivität von Peptiden und Proteinen zu steigern. Ein wichtiges Beispiel hierfür ist die Prenylierung (Farnesylierung) eines Cysteinrestes in onkogenen Ras-Proteinen.

Im Rahmen unserer anhaltenden Bemühungen Übergangsmetallkatalyse auf dem Gebiet der Modifizierung von Biomolekülen anzuwenden, haben wir begonnen die Prenylierung von Proteinen mit Hilfe von Pd-katalysierter Allylierung von Cystein-Derivaten zu untersuchen. Um unser Ziel zu erreichen, mussten viele Hindernisse aus dem Weg geräumt werden.

Im Rahmen umfangreicher Screenings konnten geeignete Liganden und Reaktions-bedingungen identifiziert werden, um die Allylierung an Thiolen und an Cystein-haltigen Peptiden mit bemerkenswerten katalytischen Umsätzen und exzellenten *n/iso*-Verhältnissen zu realisieren. Wir konnten bereits erfolgreich farnesylierte Cystein-haltige Peptid-Derivate synthetisieren.

1. Introduction

Peptides are important drugs produced by the pharmaceutical industry. Although they cannot be applied orally, nowadays a large amount of peptide-based drugs are on the market.[1-4]

There are several ways of synthesizing peptides commercially. Depending on the size of the peptide different methods are suitable. Commonly solid-phase peptide synthesis (SPPS), first established by Merrifield in 1962[5-6] is used for this purpose, allowing higher coupling yields due to its linkage to a solid support. A recognizable example for the ongoing improvements in this field is the commercially on a ton scale produced 36 amino acid containing peptide Enfuvirtide, an HIV fusion inhibitor. Due to limitations of SPPS, three peptide fragments are prepared by SPPS and linked via fragment condensation reactions in DMF.

However, fragment coupling often proceeds with concomitant partial epimerization.[7] To avoid racemization new techniques are necessary. In this regard especially enzymatic peptide synthesis appears attractive. It is mainly used for the synthesis of smaller peptides usually without or low amount of epimerization.[8] A very successful approach is the synthesis of different peptide-esters as starting material and the subsequent use of a hydrolase to perform an enzymatic peptide coupling reaction with amino acids or peptide fragments.[9] However, during the chemical formation of these esters certain amounts of epimerization can occur, so there is a strong need to find new ways for a *C*-terminal activation of peptides without epimerization.

In this regard enolester of amino acids are known for their excellent acyl-donor abilities and their stablility under moderately acidic conditions makes them outstanding candidates for enzymatic peptide synthesis.[10]

In nature not exclusively peptides and proteins are responsible for biological activity but often peptide and proteins become biologically active only after being processed by post-translational modification reactions, such as phosphorylations, glycosylations, prenylations etc.[11-12] Therefore, techniques which introduce such modification in peptides and proteins are desperately needed to study their function and location within the cell. The combination of cell biology and organic synthesis is a new perspective to study biological processes.[13]

Different methods are used and beside tyrosine, cysteine is the most widely used amino acid residue exploited for modifications.[14-15] The post-translational modification of cysteine via prenylation plays an important role in Ras proteins which are one of the most common oncoproteins present in ~30 % of human tumors. The Ras protein belongs to the group of small GTPases and is required for the regulation of cell differentiation and cell growth.[13],[16-20]

In this thesis transition-metal catalyzed reactions are explored to enable the chemoenzymatic synthesis of peptides and proteins, and the modification of these products to introduce lipid residues typical for post-translational modification of Ras proteins.

2. Theoretical background

2.1. Peptides

Peptides and proteins are composed of amino acids (Figure 1), which are linked via an amide (peptide) bond. These amino acids are the 22 proteinogenic amino acids (including selenocysteine and pyrrolysine), which are encoded by DNA. Typically, peptides contain less than 50 amino acids.

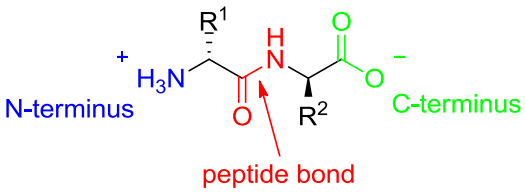


Figure 1: Peptide bond between two amino acids.

Many important physiological and biochemical functions of life are influenced by peptides. They act as neurotransmitters, hormones in the human body and are associated with a number of biochemical processes. For this reason, peptides are of increasing medical importance and represent a growing market for the pharmaceutical industry. They serve as active ingredients in various drugs to fight cardiovascular diseases, tumors, autoimmune diseases and infectious diseases.[21] An example for a therapeutical peptide is the hormone insulin which is of great medicinal importance due to its effect on blood glucose level.[22] Beside the use of peptides as drugs there are non-medical applications as well, such as the artificial sweetener aspartame.[23]

The need for synthetic peptides is continuously rising, so various synthetic methods for the production of peptides have been developed. An overview about several important methods for the synthesis of peptides is provided in the next chapter.

2.2. Methods for the synthesis of peptides

2.2.1. Solution-phase chemical peptide synthesis

In solution-phase chemical peptide synthesis a peptide bond is formed by connecting two amino acids. To form the peptide bond an activation of the carboxyl group is necessary. Without the right protection strategy, there are several peptide products possible. Therefore peptide synthesis consists of a three-step procedure. The first step is the preparation of

partially protected amino acids. In addition, all functional groups of the amino acid side chains also need to be protected to avoid the formation of byproducts. The second step is the formation of a peptide bond by activating the carboxyl function of *N*-protected amino acid and the subsequent reaction with another amino acid. Afterwards only a selective cleavage of the protecting group is conducted if the peptide synthesis is continued. After the final completion of the peptide synthesis a complete removal of the protecting groups is necessary to obtain the unprotected peptide.[21]

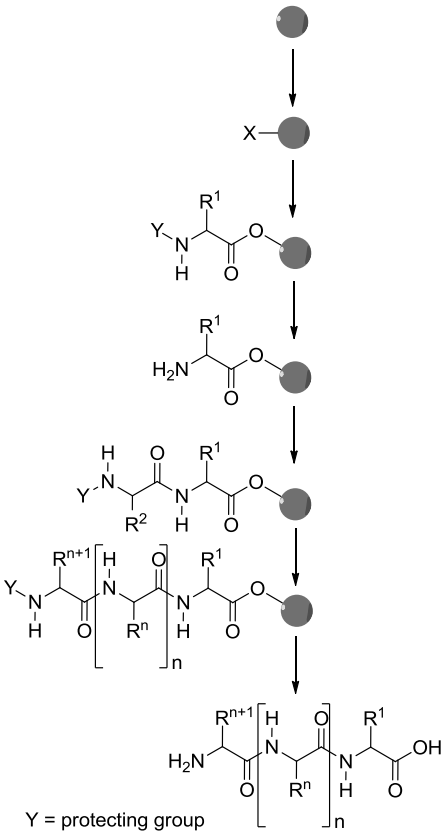
The earliest pioneers in the field of peptide bond formation were Curtius (1881)[24] and Fischer (1902).[25] Later improvements have been made by the introduction of mixed anhydrides,[26-27] dicyclohexylcarbodiimide[28] and active esters[29-30]. In addition, several important amino-protecting groups were developed for example the benzyloxycarbonyl group (Cbz or Z),[31] Boc-protecting group[32] and the Fmoc-protecting group.[33]

Most commonly only small peptides containing 2 - 10 amino acid residues are synthesized using solution phase chemical peptide synthesis. Unfortunately, expensive coupling reagents are required and uncontrolled *C*-terminal racemization during fragment assembly is a major problem of this synthesis method.[9] To overcome these problems an alternative method using solid supports was developed.

2.2.2. Solid-phase peptide synthesis (SPPS)

The first successful solid-phase synthesis of a peptide (H-Leu-Ala-Gly-Val-OH) was reported by Robert Bruce Merrifield in 1963.[5] The concept of peptide synthesis on a solid support represented a breakthrough.

In SPPS the solid support is typically polystyrene containing 1-2 % divinylbenzene as a crosslinking agent. As solvents mainly DMF and DCM are used. On this solid support an anchor group (linker) is attached. The *N*-protected amino acid is then covalently bound to the linker via an esterification. After deprotection of the linked amino acid the coupling with a second amino acid is accomplished using coupling reagents such e.g. DCC. This procedure is repeated, moving from the *C* to *N* direction, until the desired peptide size is reached. Then the linkage between linker and peptide is cleaved, the remaining protecting groups are removed and the desired peptide is obtained by filtration (Scheme 1).[21]



Scheme 1: Solid-phase peptide synthesis (SPPS).[21]

Depending on the peptide sequence SPPS requires the right protection strategy. For SPPS two different strategies are used. The Boc strategy uses the acid-labile tert-butyloxycarbonyl protecting group for the temporary protection of the amino group, while the Fmoc strategy uses the base-labile 9-fluorenyl methyloxycarbonyl-protecting group.[21]

The main advantage of SPPS is that the growing peptide chain is bound to the carrier between the couplings and so the excess of reagents and byproducts can be removed simply by filtration. Additionally, the simple technical procedure and the great potential for automation led to the triumph of the SPPS in the field of peptide synthesis. The synthesis has been steadily improved so that today high reproducibility and almost quantitative yields can be achieved.[21]

Nowadays, the SPPS method is the most commonly used if medium-sized or long peptides (10–50 amino acid residues) have to be synthesized. Nevertheless besides the tremendous success of SPPS it has disadvantages as well. It requires full protection of the functionalized side chains and it is necessary to use at least stoichiometric amounts of environmentally unfriendly coupling reagents. All these requirements, especially the use of reagent excess makes SPPS an expensive method.[9]

2.2.3. Recombinant DNA technologies

Mainly pharmacologically active peptides and proteins are produced in a large scale by recombination and expression of genetic material e.g. by a bacterium.[34]

A process for peptide synthesis based on this method involves the following steps:[21]

1. The isolation of the desired peptide coding DNA fragment.
2. Insertion of DNA into a vector (circular, double stranded DNA).
3. Transformation (contribution) of the vector into a suitable host (eg. micro-organism)
4. Cultivation of the host, leading to gene amplification, mRNA synthesis and protein synthesis.
5. Isolation of the recombinant protein

Such recombinant DNA technologies are especially useful for large peptides (>50 amino acid residues) and proteins, but having the disadvantage of high development time and costs.[9]

A famous example for this method is the industrial production of the hormone insulin by the bacteria *Escherichia coli*.[22]

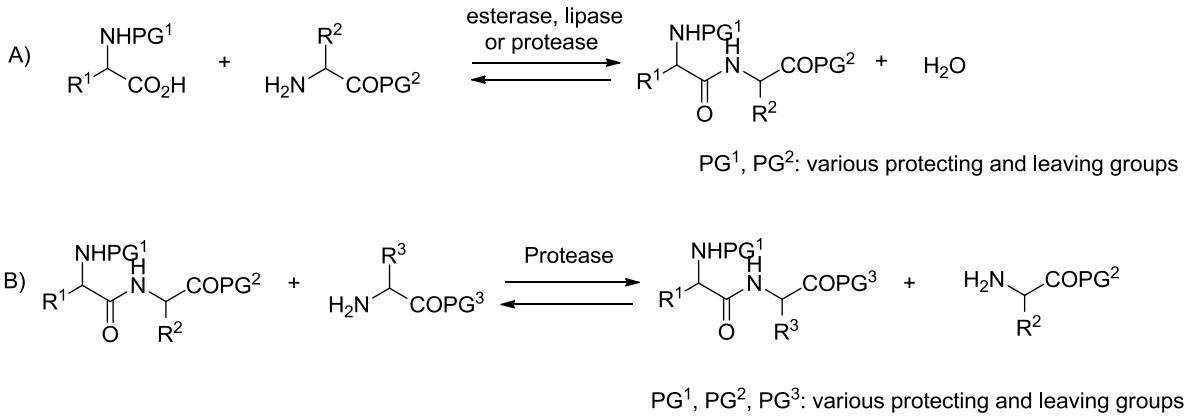
2.2.4. Enzymatic peptide synthesis

A promising way how to overcome the problems associated with conventional chemical approaches to synthesize peptides is the use of enzymes (hydrolases) to form peptides. The two major problems are racemization occurring during the chemical peptide synthesis and the separation of the desired peptide from side products and isomeric peptides having a similar sequence. To avoid these problems, the outstanding specificity of enzymes is used for peptide synthesis. Particularly proteases, which belong to the group of hydrolases, are used for this purpose.[35-41] The first enzymatic peptide synthesis was published already in 1936 by Bergmann.[8] Due to several advantages of enzymatic peptide synthesis contra conventional chemical approaches this method was intensively studied during the last years. The advantages of enzymatic peptide synthesis are the high stereoselectivity, high regioselectivity, low demand for protection groups, only moderate purity of the starting materials required and least but not last no risk of racemization. There are basically three different ways to synthesize peptides using enzyme catalysis, the reversal of hydrolysis, transpeptidation and aminolysis of esters (Scheme 2 and 3).[42]

2.2.4.1. Thermodynamic control of the reaction

The reversal of hydrolysis and transpeptidation reaction both have a reversible mechanism and are therefore thermodynamically controlled (Scheme 2). Under physiological conditions the equilibrium of the reaction is far on the side of the reactants. Therefore, various measures including the use of a reactant in excess, the removal of the reaction product from the reaction mixture[43-44] or the use of solvents with lower water content[45] by adding water-mixable organic cosolvents are necessary to shift the equilibrium in the desired direction.[42]

Reversal of hydrolysis A) and Transpeptidation B) (thermodynamic control):

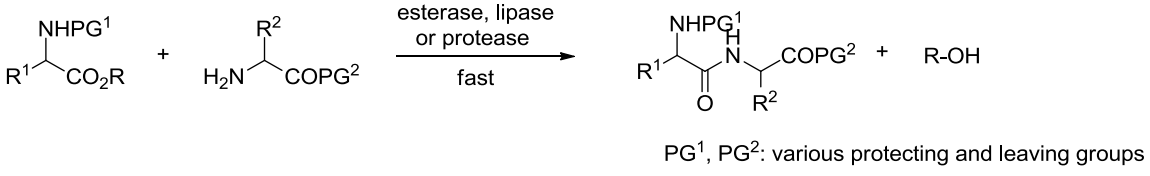


Scheme 2: Schematic illustration of thermodynamically controlled enzymatic peptide synthesis.[42]

2.2.4.2. Kinetic control of the reaction

The kinetically controlled third method is the aminolysis of esters catalyzed mainly by serine proteases (Scheme 3). In this type of reaction two nucleophiles (water and amine) compete for the acyl-enzyme intermediate.[46] In the presence of water it is necessary to terminate the reaction before the equilibrium is reached, because both the starting ester and the product can be hydrolytically cleaved by water.[47] Peptide hydrolysis can be avoided by using esterases and lipases.[42]

Aminolysis of esters (kinetic control):



Scheme 3: Schematic illustration of kinetically controlled enzymatic peptide synthesis.[42]

So far, substrates for this method mainly are L-amino acid esters of short chain alcohols such as methyl or ethyl esters. Other esters, such as 2,2,2-trifluorethyl- (Tfe)[48], p-nitrophenyl-[49], carboxamidomethyl- (Cam)[50] or guanidinophenyl-ester (Gp)[51] have been used for amino acids as well (Figure 2).[42]

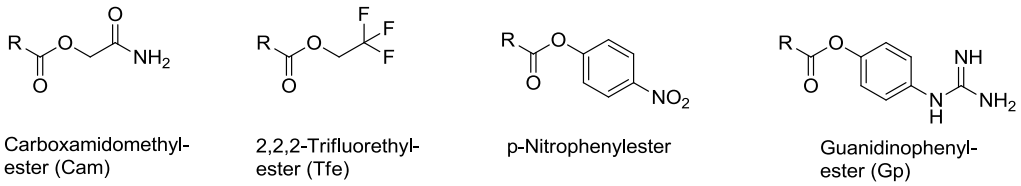


Figure 2: Various esters used for enzymatic peptide synthesis.

The main disadvantage of the enzymatic peptide synthesis so far is the substrate selectivity of the used enzymes. This substrate selectivity is typical for hydrolases. The search for enzymes (proteases) to cover the whole range of peptide bonds continues.[42]

The enzymatic peptide synthesis is particularly cost effective for the synthesis of short peptide sequences of up to 5 amino acid residues, even on a large scale. Mostly, this synthesis is performed in *N* → *C* terminal direction, so only an excess of comparably cheap *C*-terminally protected amino acids are required.[52]

If the *C*-terminal amino acid is protected as an amide it furthermore can be converted after the enzymatic peptide synthesis into a carboxylic acid by an enzyme-catalyzed hydrolysis[53] or by enzymatic reactions with alcohols to the corresponding esters. Additionally, terminal amides used as protecting group support the recognition of this coupling partner and they potentially increase the conversion of peptide coupling if the enzyme is a protease.[52]

2.3. Enzymes in organic media and enzyme immobilization

2.3.1. Enzyme catalyzed reactions in organic media

There are several disadvantages using water as a solvent, such as that many organic compounds are often insoluble in water. Side reactions such as hydrolysis, racemization and decomposition are often promoted in the presence of water.[54-57] Considering these facts the use of biocatalysts in non-aqueous media can help to significantly improve the performance of a reaction or even make the reaction possible.[58]

On the other hand, the majority of enzyme catalyzed reactions are carried out in aqueous solutions, because there enzyme activity is the highest, while it is assumed that most organic solvents affect the catalytic activity of enzymes or even destroying it.[59]

One important requirement to enable enzymes to catalyzed reactions in organic media efficiently, is that water molecules (“structural water”) remain on the surface of the enzyme. Subtilisin for example only needs 50 water molecules per enzyme molecule to remain its catalytic activity.[60] Water, present in biological transformations can be divided into two categories. While the majority of the water (> 98 %) only serves as the solvent ("bulk water"), a small part of it is bound tightly to the surface of the enzyme ("structural water" or "bound water"). This "structural water" is an important component of the enzyme having even a lower melting point than the “bulk water” and the “structural water” is essential for structure and activity of the enzyme. In principle it should be possible to remove "bulk water" without influencing the "structural water" of the active enzyme and therefore its structure and activity using apolar organic solvents.[61-63] Therefore performing enzyme catalyzed reactions in organic media can lead in the right context to a better solubility of nonpolar substrates, suppression of side reactions such as hydrolysis and improved yields due to a shift of the equilibrium in favor of the products.[54-57],[64]

2.3.2. Enzyme immobilization

When performing enzyme catalyzed reactions on industrial scale, enzymes suffer quite often under instability and a loss in catalytic activity due to auto-oxidation, self-digestion and denaturation. Additionally, the use for industrial processes is limited by the low tolerance of many enzymes to high substrate and product concentrations.[42]

A solution to this problem is immobilization of enzymes.[65-70] Immobilized biocatalysts can be regarded rather as heterogeneous catalysts then as homogeneous catalysts in the case of native enzymes. There are basically three different options for the immobilization of enzymes.[42]

A) Binding of the enzyme to a carrier:

Enzymes can be bound to carriers due to adsorption, ionic bonding or covalent bonding. Carriers are cellulose,[71] activated carbon,[72] Celite,[73] aluminium oxide[74] and glass.[75]

B) Enzyme inclusion, the insertion of the enzyme in a semi-permeable membrane:

Enzyme can be also enclosed in gel,[76-77] synthetic membranes,[78] vesicles and micelles.[79-81]

C) Polymerisation or „cross-linking“:

The above briefly mentioned methods to bind or include enzymes on or into a carrier unfortunately lead to a reduction in catalytic activity and thus to a lower productivity. Cross-linked enzyme aggregates (CLEA) offer many advantages because they can easily be obtained from unprepared enzyme extracts and do not require expensive substrates. They show improved storage and process stability towards denaturation by heat, organic solvents and autoproteolysis. Furthermore, they show high catalytic productivity and are easy to recover and can be recycled. It is even possible to "coimmobilize" several enzymes together in order to run several biotransformations in a catalytic cascade.[82]

The precipitation of the enzymes from an aqueous solution takes place using salts such as ammonium sulfate or organic solvents such as *tert*-butanol. The resulting enzyme aggregates are held together by noncovalent bonds without damaging their tertiary structure. Cross-linking of these aggregates by the addition of bifunctional reagents such as glutaraldehyde keeps the enzymes permanently insoluble while their structure and thus their catalytic activity is mainly maintained. The cross-linking is caused by the reaction of lysine residues on the enzyme surface with glutaraldehyde due to imine formation. By this method, purification of enzymes and cross-linking of the units can be combined.[82]

The majority of CLEA used are hydrolases, mainly due to their wide industrial application. Therefore, they are also readily available in large quantities and they are probably the easiest enzymes to work with.[82]

2.4. Serine proteases

Serine proteases are a class of enzymes which cleave peptide bonds of proteins, being digestive enzymes. Their common feature is their mechanism of cleaving peptide bonds having a serine residue involved in their active center. They all show a so called “catalytic triad”.[83] Two additional amino acids (Asp and His) are located closely to the serine residue which is the actual reactive amino acid reacting during the mechanism of cleaving peptide bonds. Due to this arrangement these three residues work together to decrease the pKa value of the serine hydroxyl group. This serine is able to perform a nucleophilic attack on the carbonyl group of the substrate which is then covalently attached to the enzyme. This acyl-enzyme intermediate is then attacked by a nucleophile, usually water and the substrate is released from the enzyme. The substrate contains now a carboxylic acid group, if water was used as nucleophile.[42]

Due to their structural characteristics serine proteases are divided into three classes, which are known as chymotrypsin-, subtilisin- and carboxypeptidase II–family.[84]

Alcalase-CLEA:

Being a cross-linked serine protease belonging to the subtilisin-family, especially alcalase-CLEA also called subtilisin Carlsberg[85] should be mentioned in this thesis, because alcalase-CLEA was used in the experimental work.

Subtilisins are extracellular serine endopeptidases with a broad substrate specificity. Endopeptidases are enzymes that hydrolyze peptide bonds within the peptide chain and not at the end, resulting in water-soluble peptides. Alcalase was first isolated in 1947 from *Bacillus licheniformis* and is among the first proteases that have ever been described. [86-87]

The enzyme alcalase consists of a 7-string β-sheet, which is located between two layers of α-helices (Figure 3). This structure consists of 274 amino acid residues with a weight of ca. 27 kDa.[85] The "catalytic triad" of this serine protease is composed of three amino acid residues Ser221, His64 and Asp32 (Figure 4). The optimum conditions for maximum enzymatic activity is a pH 8-9 and a temperature of about 60°C.[87] The main applications of this enzyme are predominantly in the detergent and food industries.[85]

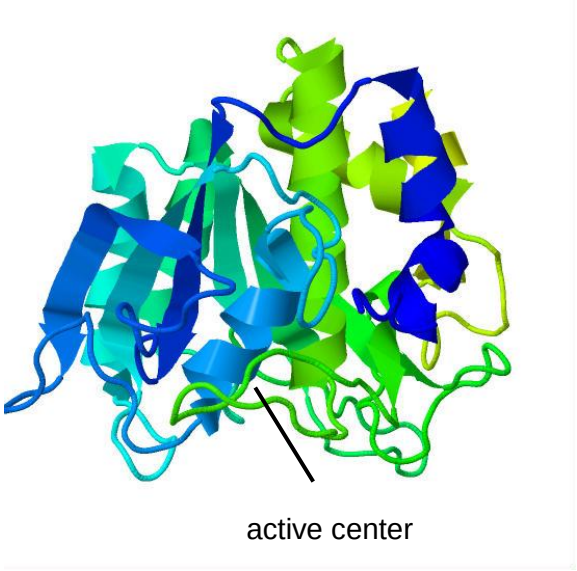


Figure 3: Enzyme crystal structure of alcalase (resolution 2Å) in anhydrous acetonitrile.[88]

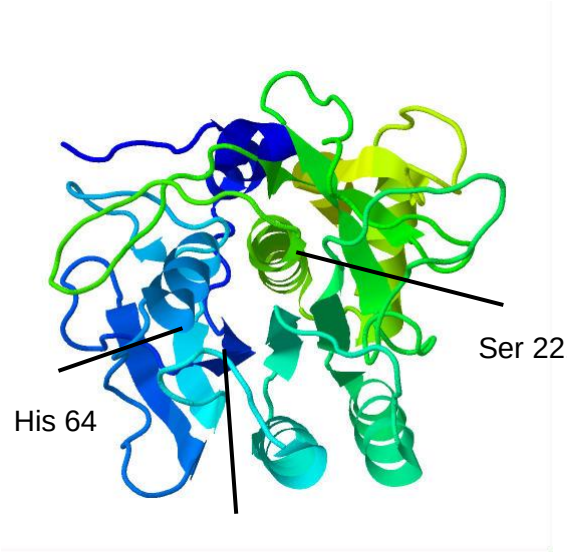
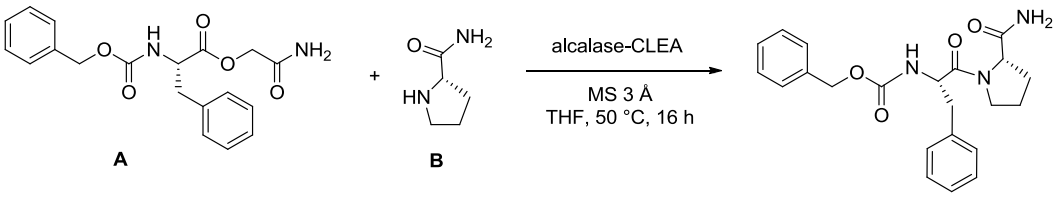


Figure 4: Active center of alcalase showing the “catalytic triade”. [84],[88-89]

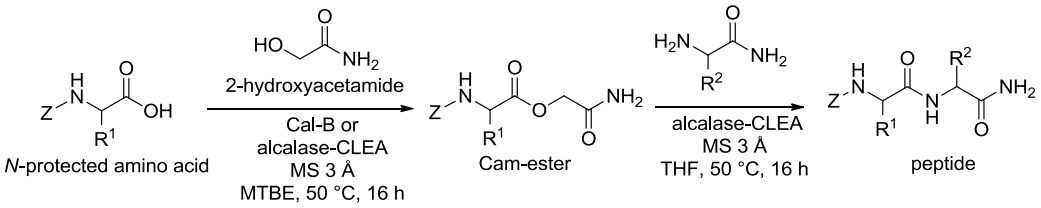
Alcalase or subtilisin Carlsberg used in the experimental part of this thesis has a specificity for the amino acid sequence Trp (Tyr, Phe, Leu, Met)-X, where X is any amino acid. Especially proline-containing peptides are considered as poor substrates not only for subtilisins but generally.[42] However Quaedflieg et al. reported the use of Alcalase-CLEA as enzymatic catalyst for coupling of *N*-protected amino acid esters, such as Z-Phe-OCam (**A**) with Pro-NH_2 (**B**) in THF (Scheme 4).[9]



Scheme 4: Synthesis of proline containing peptides using alcalase-CLEA.[9]

<u>General example for enzymatic peptide synthesis using alcalase-CLEA:</u>

Quaedflieg et al. circumvent the problems of the aminolysis-method by working with an immobilized Alcalase-CLEA in anhydrous THF. Working in anhydrous THF prevents hydrolysis of the products effectively and results in good conversions. As amino acid ester 2,2,2-trifluoroethyl (Tfe) and carboxamidomethyl esters (Cam-ester) were used which could be obtained by the use of Cal-B lipase or by alcalase-CLEA (Scheme 5). Both enzymes can be used in a "one-pot" system. Due to the use of enzymes during the synthesis no racemization occurred.[9]



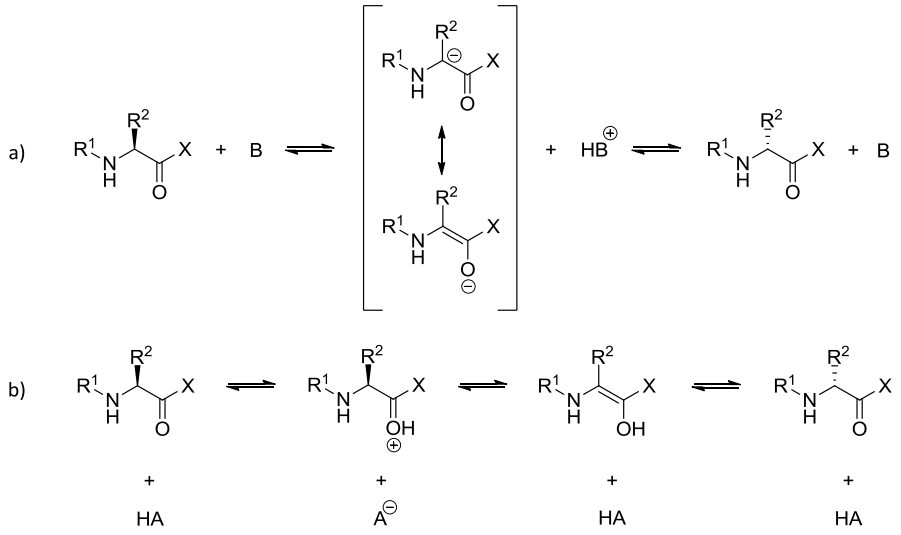
Scheme 5: General scheme for enzymatic peptide synthesis using alcalase-CLEA after Quaedflieg et al.[9]

2.5. Racemization

The minimization of racemization during the synthesis of peptides as well as during the synthesis of activated amino acids as starting material for peptide synthesis is very important, because the biological activity of peptides or proteins depends mainly on the configuration of the stereogenic centers. Although naturally occurring amino acids are usually configurationally stable, their behavior towards racemization is different if carboxyl-activated amino acid derivatives are present. This behavior is due to the effects of electron-withdrawing groups. Further influences on the amount of racemization are the reaction temperature and the properties of the solvent. Formally, this erosion of optical purity can be described by two different mechanisms: direct enolization and formation of 4*H*-5-oxazolones.[21]

2.5.1. Direct enolisation

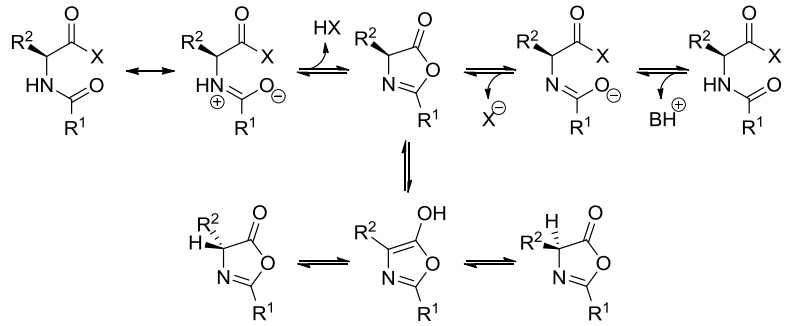
Racemization mainly occurs when peptide coupling reactions are performed under basic conditions. In a basic environment racemization is due to the abstraction of the acidic protons Hα and subsequent enolization observed as a side reaction during peptide formation. This configurational change takes place under different coupling conditions and is observed typically during ester saponification (Scheme 6a). Racemization because of acid-catalyzed enolization is due to the reaction of *N*-substituted amino acids with the acid mixtures, such as HBr / HOAc (Scheme 6b).[21]



Scheme 6: Mechanism of a) base catalyzed or b) acid catalyzed epimerization of activated amino acids.[21]

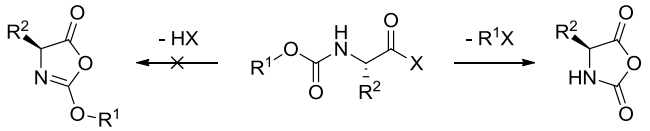
2.5.2. 4*H*-5-Oxazolone mechanism

Frequently, racemization happens upon activation of the amino acid or during peptide coupling reactions due to the formation of a stereochemically labile 4*H*-5-oxazolone. The tendency to form these heterocyclic compounds increases with the degree of activation on the carbonyl group attached to the leaving group. Also acyl groups on the nitrogen, such as acetyl or benzoyl, mostly lead to the formation of 4*H*-5-oxazolone due to the nucleophilic carbonyl oxygen atom of the amide bond (Scheme 7).[21]



Scheme 7: Racemization due to a 4*H*-5-Oxazolon-formation.[21]

The use of urethane-type protecting groups is supposed to reduce racemization and therefore regarded as the superior *N*-protecting groups. It is assumed that they lead to cyclization giving *N*-carboxy anhydrides and no 2-alkoxy-5(4H)-oxazolones (Scheme 8).[21]



Scheme 8: Cyclisation of urethane type *N*-protected amino acids to *N*-carboxy anhydrides.[21]

However, several groups showed that the formation of 2-alkoxy-5(4H)-oxazolones is possible.[90],[91] A plausible possible reason that urethane-type protected amino acids seem to be less prone to racemization is probably due to the lower acidity of the NH group in urethane-type protected amino acids compared to an amide.[21]

2.6. Enolester – starting material for chemoenzymatic peptide synthesis

2.6.1. Enolester properties and classical synthesis

Enolesters are important intermediates for the synthesis of organic compounds. Due to their good acyl-donor ability enolester enable carbon-carbon- and carbon-heteroatom bond formation under comparativly mild conditions. For this reason, enolesters can be regarded as a good starting point for enzymatic peptide synthesis. The three different types of enolesters are shown in Figure 5.

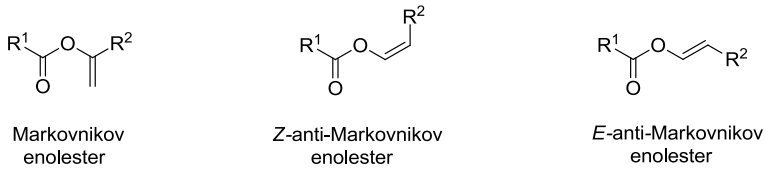
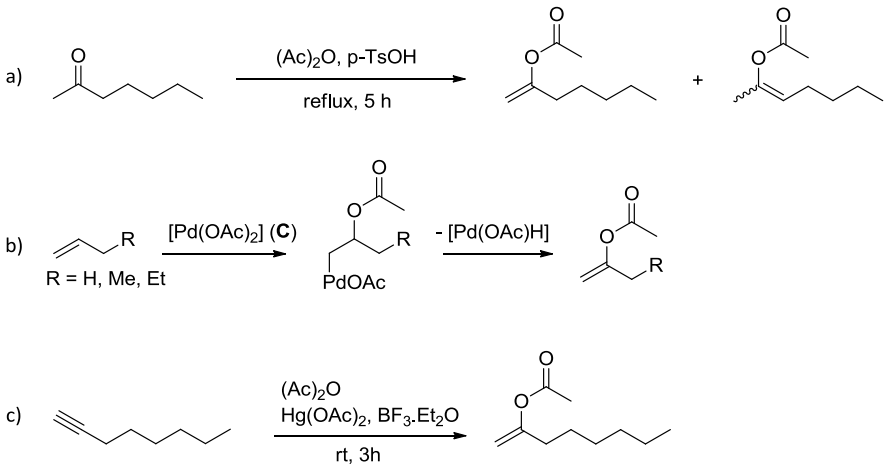


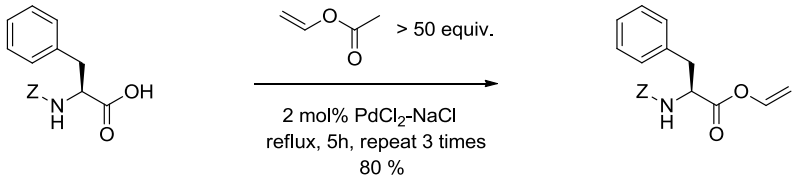
Figure 5: Schematic representation of the generally possible types of enolesters.

Historically, a variety of methods has been developed to produce these substances using different strategies. Several of these strategies are not efficient enough in terms of reaction conditions, yields, or toxicity. One attempt was made to obtain activated esters via acetylation of enolates,[92] however, with limited success. Other routes were acetoxylation reactions of olefins with palladium acetate (**C**),[93] transvinylation of carboxylic acids[94] using mercury salts as well as alkyne addition on carboxylic acids also using toxic mercury salts as catalyst (Scheme 9).[95]



Scheme 9: Formation of enolesters through a) acetylation of enolates,[92] b) acetoxylation of olefins[93] and c) addition of carboxylic acids to alkynes[95]

Another interesting synthesis is the Pd-catalyzed transesterification used exclusively for the formation of vinyl acetates from amino acids,[96-97] which was later modified by Loeffler et al. (Scheme 10).[98]



Scheme 10: Pd-catalyzed transesterification.[98]

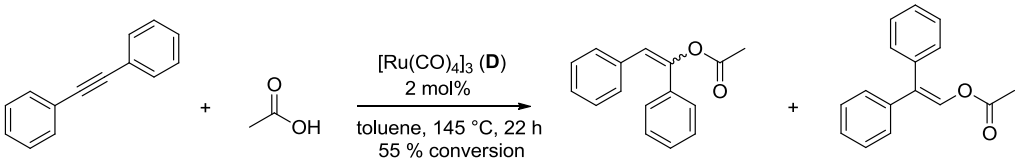
A further improvement of this method was provided by Kawamoto et al. and Schneider et al. using palladium(II) acetate and potassium hydroxide.[99-100]

Concerning biological applications of enolesters e.g. enzymatic peptide synthesis it was necessary to develop more effective and less toxic ways to form enolesters selectively.

2.6.2. Enolester synthesis via Ru-catalyzed alkyne addition – the beginning

The carboxylic acid addition on alkynes is a simple and efficient process that takes place with perfect atom economy. So far, one drawback of carboxylic acid addition on alkynes, was the use of toxic mercury salts as catalysts.[95]

In 1983, the first ruthenium-based synthesis of enolesters by addition of carboxylic acids to alkynes was reported by Rotem and Shvo.[101] They used $[Ru(CO)_4]_3$ (**D**) and $[Ru(OAc)(CO)_2]_n$ (**E**) as catalyst precursors to obtain guite good conversions and variable stereoselectivity (Scheme 11). With Ru-complex $[Ru(CO)_4]_3$ (**D**) it was possible to use both aliphatic and aromatic carboxylic acids and structurally different alkynes for this transformation. Their investigations concentrated on the use of internal symmetric alkynes. With these alkynes appropriate carboxylic acid-alkyne combinations were accessible in quantitative yields, but under the rather high temperatures of 145°C.

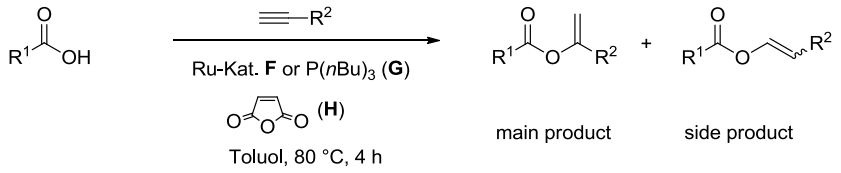


Scheme 11: First Ru-catalyzed method for the preparation of enolesters.[101]

This promising start inspired others to use ruthenium for the carboxylic acid addition on alkynes and efforts were made to further improve the catalytic system to obtain isomerically pure products.

2.6.3. Ru-catalyzed alkyne addition – the work of Mitsudo et al.

Soon after the report of Rotem and Shvo, Mitsudo et al. were able to come up with an improved systems for the formation of enolesters. Using catalytic amounts of [Ru(1,5-COD)$_2$] (**F**) and P*n*Bu$_3$ (**G**) milder conditions could be chosen, in which, after reaction times of only 4 h and average temperatures of 80°C quantitative yields could be achieved. However, this catalytic system was limited to unsaturated carboxylic acids, benzoic acid and terminal alkynes as starting materials. Additionally, this catalytic system showed a high regioselectivity for the mentioned substrates.[102] Later Mitsudo et al. published an improvement of the mentioned system by adding catalytic amounts of maleic anhydride (**H**), which allows the use of saturated carboxylic acids in the Ru-catalyzed alkyne addition (Scheme 12). Furthermore, it was shown that alkyl substituents at the α-carbon atom of the carboxylic acid have no appreciable effect on the yield and selectivity of the reaction.[103]



Scheme 12: Improved catalytic system for the addition of a carboxylic acid to a terminal alkyne.[103]

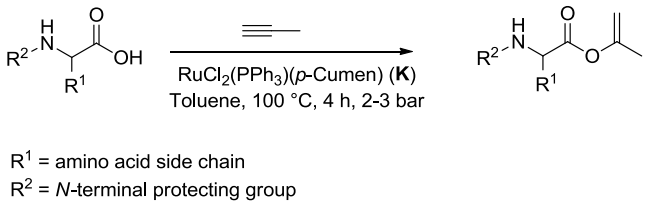
Further studies from Mitsudo et al. showed, that the choice of different phosphine ligands does influence the regioselectivity of the obtained enolesters. Electron-withdrawing carboxylic acids or unsaturated carboxylic acids, are responsible for an acceleration of reductive elimination which leads to a faster enolester formation.[104] In respect of using enolester as starting material for enzymatic peptide synthesis another important aspect needs attention. Regarding the degree of racemization it was described in the case of the present catalytic system that low amounts of racemization could be detected when *N*-acetyl-protected amino acid were used.

2.6.4. Ru-catalyzed alkyne addition – the work of Dixneuf et al.

After Mitsudo et al. also Dixneuf et al. published a synthesis of enolesters using the catalyst [RuCl$_2$(p-cumene)P(Me)$_3$] (**J**). This Ru-complex provided good results in producing

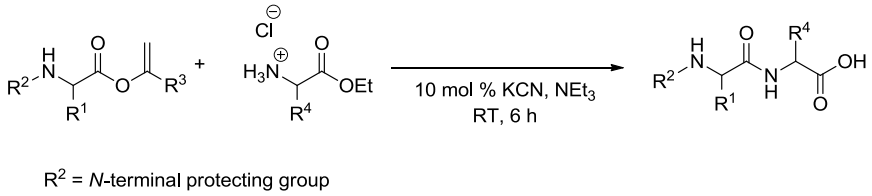
enolesters with regard to yield and selectivity.[105] Compared to the synthesis published by Mitsudo et al. the catalytic system decribed by Dixneuf et al. needed higher temperatures of at least 120°C and much longer reaction times to obtain satisfactory yields.

In 1988, Dixneuf et al. published a synthesis method to form isopropenyl esters of *N*-protected amino acid by the addition of propyne in toluene using ruthenium (II) complex, [$RuCl_2$(p-cumene)PPh_3] (**K**) as catalyst (Scheme 13). The reaction was carried out regioselectively and without racemization.[106]



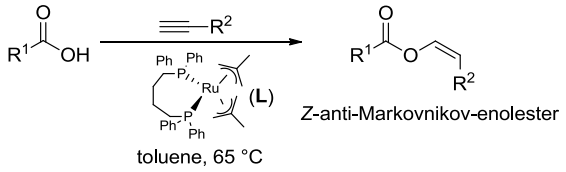
Scheme 13: Synthesis of amino acid isopropenyl esters via Ru-catalyzed propyne addition.[106]

In 1991 Dixneuf et al. reported, based on ruthenium (II) complex, [$RuCl_2$(p-cumene)PPh_3] (**K**), about the formation of enolesters from *N*-protected amino acids using 1-hexyne instead of propyne without racemization. These enolesters were used to form different dipeptides by reacting them with amino acid ethyl ester hydrochloride in triethylamine and 10 % KCN, which acts as the catalyst (Scheme 14).[107-108]



Scheme 14: Synthesis of dipeptides from enolesters using KCN as a catalyst.[107]

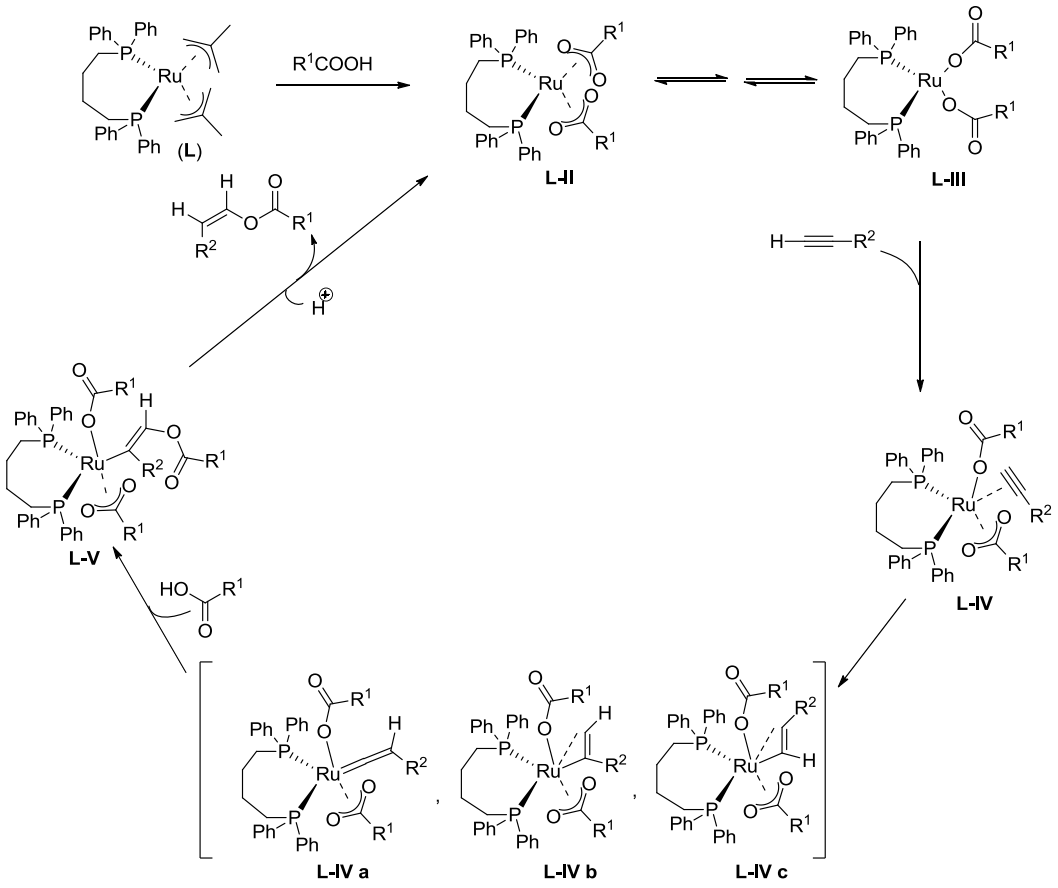
So far the protocols decribed by Dixneuf formed mainly Markovnikov-enolesters. In 1993 Dixneuf reported about a breakthrough observing a regioselective and stereoselective addition of carboxylic acids to terminal alkynes. With the developed pre-catalyst precursors of the general structure [$Ru(\eta^3\text{-}CH_2C(Me)=CH_2)_2(Ph_2P(CH_2)_nPPh_2)$] (n = 1, 2, 3, 4) Dixneuf obtained *Z*-anti-Markovnikov-enolesters in good yields. Pre-catalyst [$Ru(\eta^3\text{-}CH_2C(Me)=CH_2)_2(Ph_2P(CH_2)_4PPh_2)$] (**L**) was the best catalytic system which made it possible to synthesize optically pure *Z*-anti-Markovnikov-enolester at 65°C with reaction times below 20 h (Scheme 15). Responsible for the observed good regioselectivity and stereoselectivity are mainly steric factors of this diphosphine ligand.[109]



Scheme 15: Regioselective addition of a carboxylic acid with a terminal alkyne with Ru-catalyst **L** described by Dixneuf et al.[109]

In a subsequent publication a more detailed study of using catalyst precursors [Ru(η^3-$CH_2C(Me)=CH_2)_2(Ph_2P(CH_2)_nPPh_2)$] (n = 1, 2, 3, 4) providing more examples was disclosed.[110] Summarizing, it can be stated that bulky alkynes improve the regioselectivity in favor of the desired product. Small carboxylic acids with a low pKa value allow lower reaction temperatures and therefore lead to products having a higher isomeric purity and a higher stereoselectivity.

A possible mechanism for the formation of *Z*-anti-Markovnikov-enolester using alkyne addition to carboxylic acids with pre-catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2(Ph_2P(CH_2)_4PPh_2)$] (**L**) was provided by Dixneuf et al. (Scheme 16).[110]



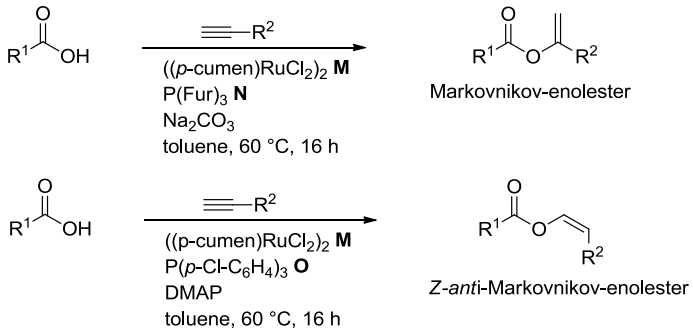
Scheme 16: Postulated reaction mechanism for the formation of *Z*-anti-Markovnikov-enolester.[110]

Dixneuf et al. postulated that the first step is a substitution of the allylic groups by the carboxylates with elimination of isobutene to produce complex **L-II**. After a rearrangement of the complex **L-II** into **L-III** the coordination of the alkyne is possible to give intermediate **L-VII.** After the coordination of the alkyne (oxidative addition) several resonance forms (**L-IV b** and **L-IV c**) and a vinylidene tautomer **L-IVa** can be postulated depending on the bidentate ligands used. The external addition of a free carboxylate to the electronically activated, unsubstituted carbon atom of the alkyne might then occur to form intermediate **L-V**. Probably due to subsequent protonation of the ruthenium center followed by reductive elimination, the desired *Z*-anti-Markovnikov-enolester is liberated. The resulting complex **L-II** is then used again for another catalytic cycle.[110]

2.6.5. Ru-catalyzed alkyne addition – the work of Goossen et al.

2003 Goossen et al. published a method for the production of Markovnikov-enolesters and *Z*-anti-Markovnikov-enolesters from terminal alkynes and carboxylic acids. The catalyst system was formed in situ using air- and water-stable and commercially available pre-catalyst ((*p*-cumen)$RuCl_2)_2$ (**M**) and $P(Fur)_3$ (**N**). By adding small amounts of additives to the reaction mixture mentioned below the selectivity of the reaction could be influenced. By adding an inorganic base such as Na_2CO_3, the Markovnikov-product formed in high yields, whereas through the addition of an organic base such as DMAP together with P(*p*-Cl-$C_6H_4)_3$ (**O**) to pre-catalyst ((*p*-cumen)$RuCl_2)_2$ (**M**) the *Z*-anti-Markovnikov-product was obtained. A variety of alkynes and carboxylic acids, as well as *N*-protected amino acids were used for this transformation at the moderate temperature of 60°C. The resulting products could be obtained in high yields (Scheme 17).[111]

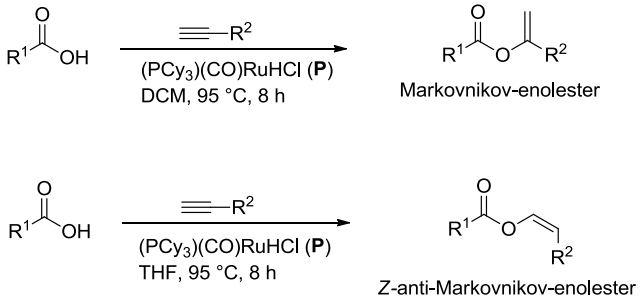
Through further development of this catalyst system Goossen et al. were also able to produce amides with terminal alkynes with high regioselectivity and good yields.[112]



Scheme 17: Preparation of Markovnikov-enolester and *Z*-anti-Markovnikov-enolester by Goossen et al.[111]

2.6.6. Ru-catalyzed alkyne addition – the work of Yi et al.

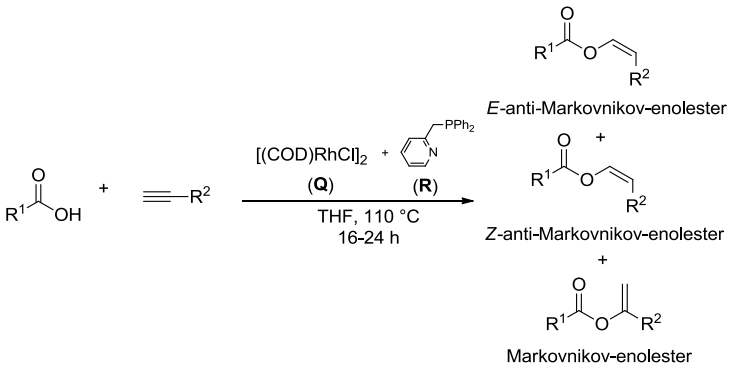
2009 Yi et al. reported a highly efficient enolester synthesis from terminal alkynes and carboxylic acids by the ruthenium hydride complex $(PCy_3)_2(CO)RuHCl$ (**P**). The regio- and stereoselectivity could be controlled by the solvent used. In the case of DCM used as solvent Markovnikov product was found and in the case of THF *Z*-anti-Markovnikov product was produced. However, high temperatures of 95°C are needed for this synthesis (Scheme 18).[113]



Scheme 18: Selective enolester synthesis after Yi et al.[113]

2.6.7. Rhodium catalyzed synthesis of *Z*-anti-Markovnikov-enolester

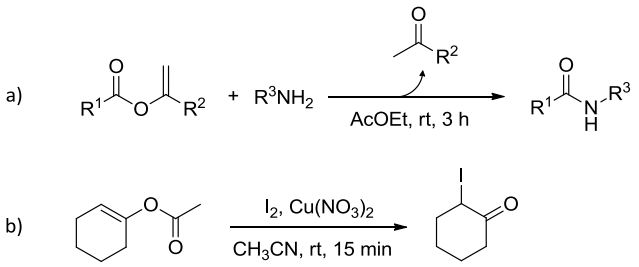
In a paper by Breit et al. the synthesis of *Z*-anti-Markovnikov-enolesters from terminal alkynes and carboxylic acids was described.[114] Here a rhodium complex $[(COD)RhCl]_2$ (**Q**) was used as pre-catalyst. The active catalyst species could be generated in situ by adding 2-((diphenylphosphino)methyl)pyridine ligand (**R**). With this catalytic system the desired product could be formed in high yields and with good regio-and stereoselectivity (Scheme 19). Also, an iridium complex $[(COD)IrCl]_2$ (**S**) was tested for this reaction, but led to unsatisfactory yields and selectivities.



Scheme 19: Rhodium catalyzed enolester synthesis after Breit et al.[114]

2.6.8. Applications of enolesters

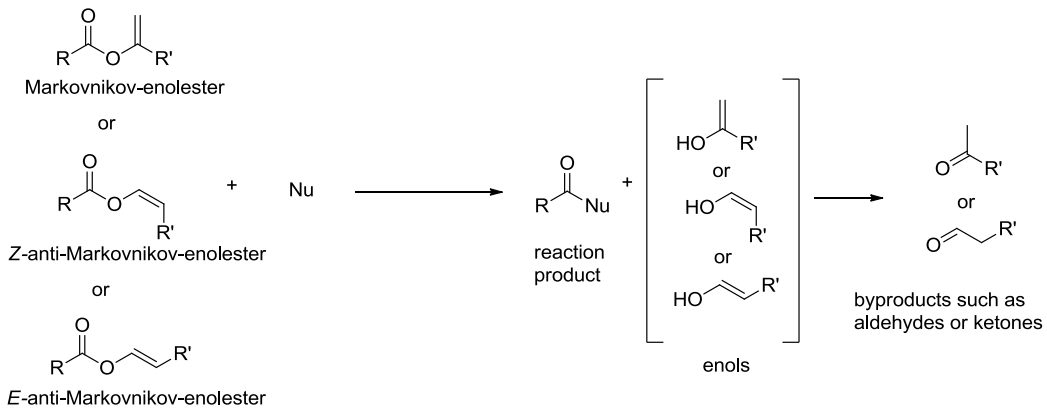
Starting from one of three possible enolester isomers shown in Figure 5 various reactions can be carried out using enolesters as starting material. The formation of amides[107] and esters is possible due to a nucleophilic attack of amines and alcohols on the enolester group. Also α-halogen ketones are accessible under mild conditions in quantitative yields (Scheme 20).[115]



Scheme 20: Preparation of a) amides[107] and b) α-halo-ketones[115] from enolesters.

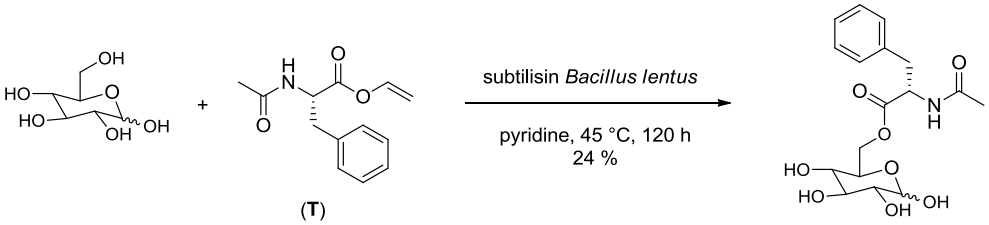
Further reactions were carried out starting from enolesters such as Diels-Alder reactions [116-117], [2+2]-,[118] [3+2],[119] 1,3-dipolar cycloaddition reactions,[120] as well as asymmetric hydrogenation,[121-123] hydroformylation[124] and Pd-catalyzed enantioselective allylations.[125-126]

Especially useful are enolesters such as vinyl- and isopropenyl-enolesters in enzyme-catalyzed esterification reactions. In the course of a reaction of the enolesters with a nucleophile, enols are released as byproducts. These enols are converted into the thermodynamically more stable aldehydes or ketones as a result of keto-enol tautomerisation, shifting the equilibrium in favor of the reaction products such as esters and amides (Scheme 21).[127]



Scheme 21: Reactions of various types of enolester with a nucleophile.

In 2001 Davis et al. described an enzyme-catalyzed esterification reaction, using *N*-protected amino acid vinyl esters, such as *N*-acetyl L–phenylalanine vinylester (**T**), which were prepared by Pd-catalyzed transesterfication reaction with vinyl acetate. These enolesters were subsequently esterified using different carbohydrates in pyridine at 45°C. The key to the success of this esterification was the use of immobilized protease subtilisin *Bacillus lentus* (Scheme 22).[10]



Scheme 22: Transesterification reactions of *N*-protected L–phenylalanine vinylester **T** with glucopyranose.[10]

2.7. Modification of biopolymers (protein modification)

2.7.1. Post-translational modifications - introduction

According to the central dogma of molecular biology the genetic information is transferred from the DNA to mRNA via transcription and afterwards from mRNA this information is translated into proteins (Figure 6).[128]

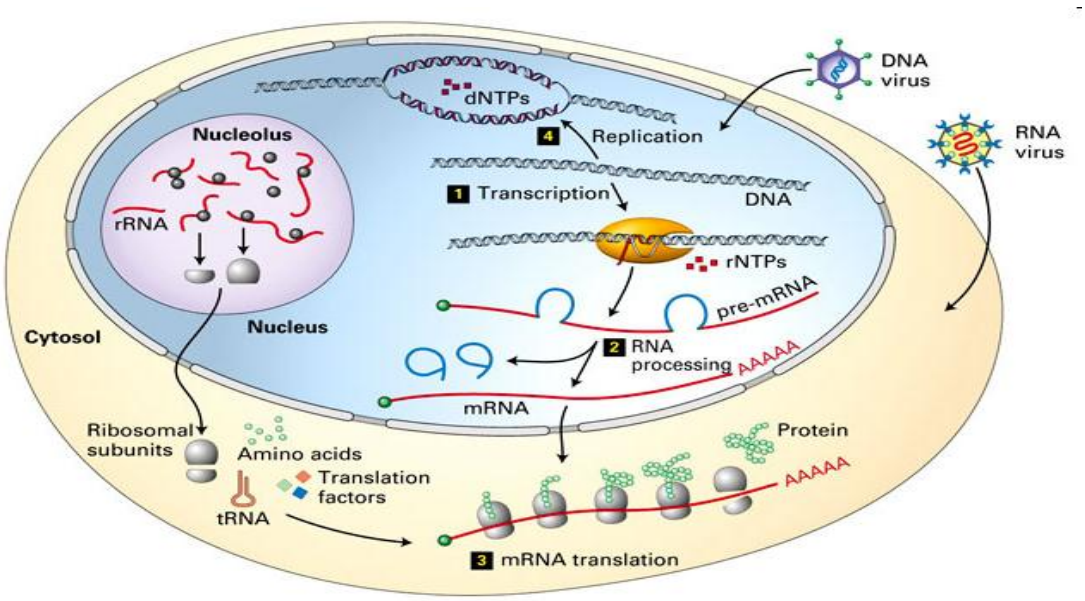


Figure 6: Central dogma of molecular biology.[128]

After the translation of mRNA into proteins, many of these proteins are still not biologically active. To obtain their biological activity they are further modified by different enzymes within the cell. In nature this additional reactions are accomplished by different post-translational modifications (PTM) that in turn mediate protein activity.[11-12],[129] One of the most important post-translational modification is the addition of functional groups. Probably the most important additions are phosphorylations, hydroxylations, glycosylations, ubiquitylations, acetylations, methylations and prenylations.

2.7.2. Prenylation – Ras protein

Prenylation is a post-translational modification process to covalently attach terpene residues to cysteine residues of proteins, discovered by Crain et al. in 1990.[130] In eukaryotic cells, cysteine residues of proteins are modified with farnesyl- and geranylgeranyl-groups, providing a hydrophobic anchor for fixing proteins to membranes or to allow an easier

binding to other proteins. Furthermore, the prenylation is crucial for the binding or the transport of proteins between different cellular membranes.[16-17],[131-132]

In recent times, prenylation of proteins gained significant interest, due to its appearance in many signal proteins, being part of the signal connection between cell surface receptors and the inside of the cell.[16]

One of the most important group of proteins which are prenylated via post-translational modification is the group of Ras proteins (H-Ras is shown as an example in Figure 7). Ras proteins are one of the most common oncoproteins present in ~30 % of human tumors.[16],[19-20]

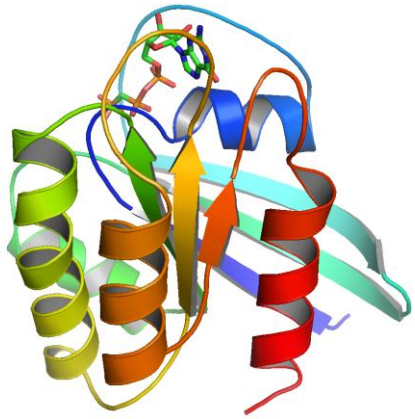


Figure 7: Tertiary structure of the human H-Ras-Protein with GTP.[133]

The Ras protein belongs to the group of small GTPases, which is activated by release of bound GDP against GTP and deactivated via an hydrolysis of GTP back to GDP, so function primarily as a molecular binary switch. Ras is required for the regulation of cell differentiation and cell growth. The Ras protein is only biologically active if prenylated, which shows the crucial role of prenylation in biological processes.[13],[16-19]

The natural occurring enzymatic pathway for the modification of prenylated proteins is believed to be the following:

First, the farnesyl group from farnesyl diphosphate is attached to the cysteine SH of the CaaX motif at the C terminus of proteins via protein farnesyltransferase (PFT). Afterwards in the endoplasmic reticulum the endoprotease Rce1p C-terminal aaX motif followed by S-adenosylmethionine (SAM)-dependent methylation of the COOH terminus by isoprenylcysteine carboxylmethyltransferase (Icmt). Some proteins, such as N-Ras and H-Ras, are further modified by palmitoylation on one or two cysteines near the prenylated

cysteine after transfer to Golgi membranes. Probably via vesicle transport completely lipidated N-Ras and H-Ras are transferred to the plasma membrane (Figure 8).[16]

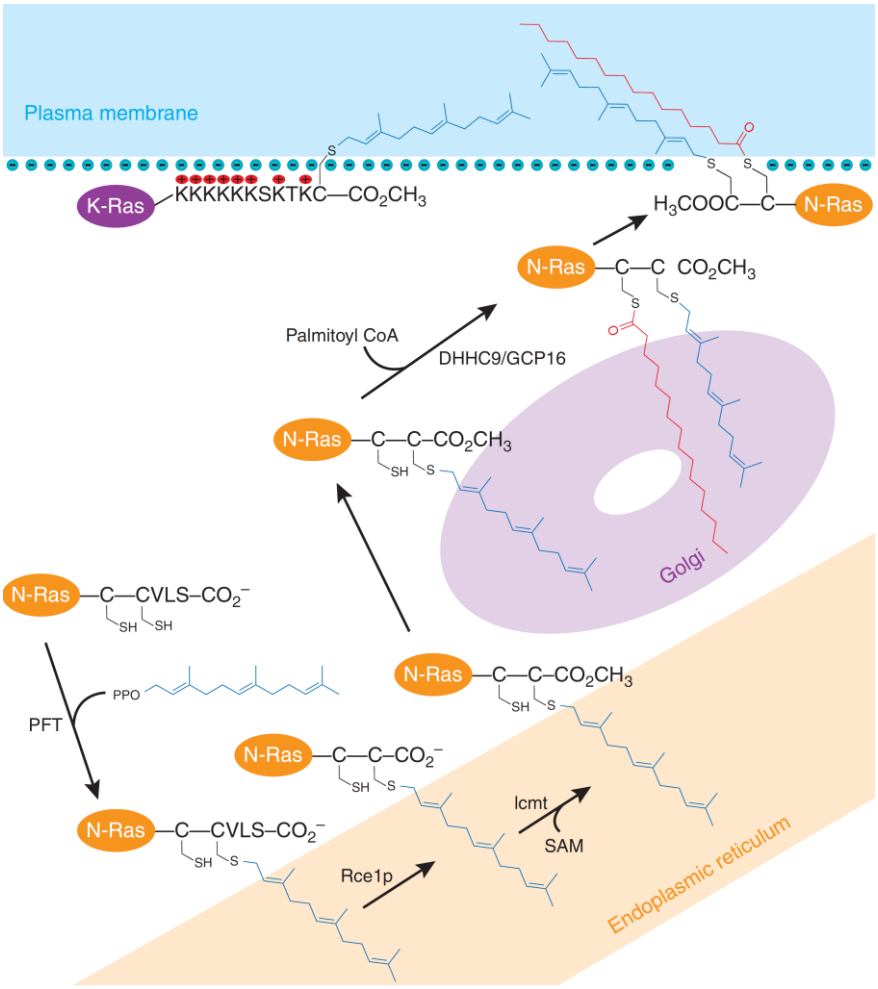


Figure 8: Enzymatic reaction pathway for the modification of Ras protein via prenylation and palmitoylation.[16]

With regard to their biological relevance, only a few chemical syntheses of S-prenylated peptides have been developed.[134] Solid phase synthesis, starting from the unmodified peptide is used for their synthesis, followed by a prenylation reaction on the SH of the cysteine using for example farnesylbromide. The last step of the synthesis is then deprotection, which should not be performed under acidic conditions due to the acid lability of the prenyl group.[16]

The prenyl group is usually introduced at the last steps of synthesis after synthesizing the peptide on solid support. The prenylation reaction can be performed both under basic and moderately acidic conditions. However, several problems might lead to incomplete conversion. Under basic conditions, the main problem is the oxidation of the thiol group to

the disulfide, which can be prevented by working under an inert atmosphere. Additionally solubility problems, alkylation of other functional groups beside thiols present in the peptide and hydrolysis of the prenylation reagent can occur.[16]

The ultimate aim of this thesis is to contribute to establish a new method to farnesylate peptides or even proteins via homogenous metal catalysis obtaining a thiol selective reaction.

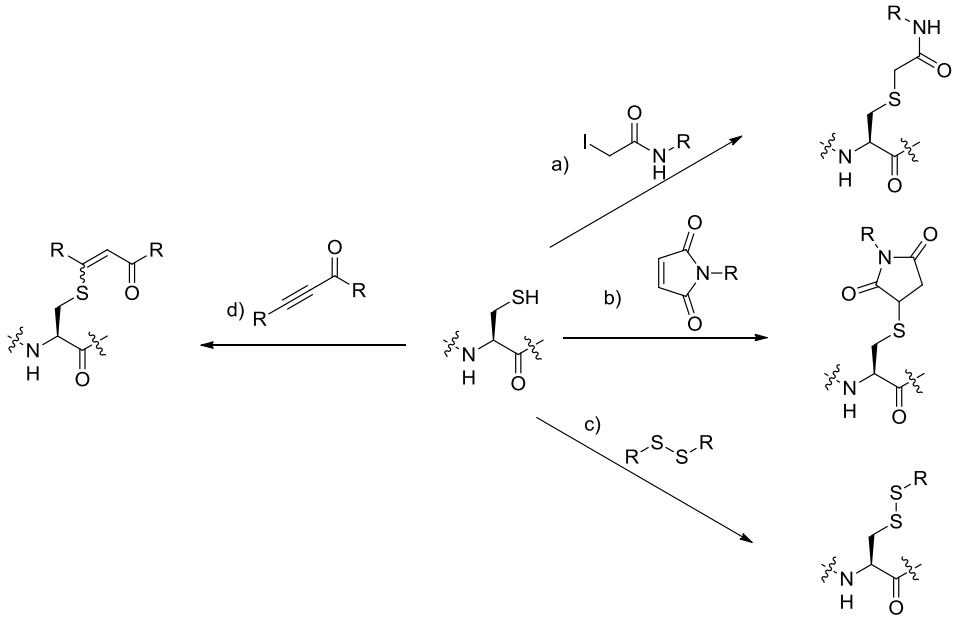
2.7.3. Methods for the modification of cysteine residues in biopolymers

The challenge of finding suitable reactions for protein modifications is that this reaction must be very selective. Among several competing side chains in a polypeptide only the desired residue should be modified. Additionally, there is a strong need to work under conditions which are required to prevent protein denaturation. These reactions must be performed in water near neutral pH and low temperature. Finally, this reaction must be able to tolerate salts and surfactants required for protein stability and these reactions must be fast to achieve full conversion due to low concentrations present.[11],[135]

Our aim was to find a selective modification on the protein residue cysteine. Cysteine is a good target for a modification due to its stongly nucleophilic side chain and its relatively low natural abundance in proteins.[11],[136]

A common way to modify cysteine in peptides or proteins is using alkylation reactions with electrophiles by taking great care of the pH-value not to alkylate histidine or lysine.[137]

The earliest and widely used examples are summarized in Scheme 23.



Scheme 23: Different methods for the modification of Cysteine within biopolymers a) iodoacetamides, b) maleimides, c) disulfides d) electron-deficient alkynes.[15],[138]

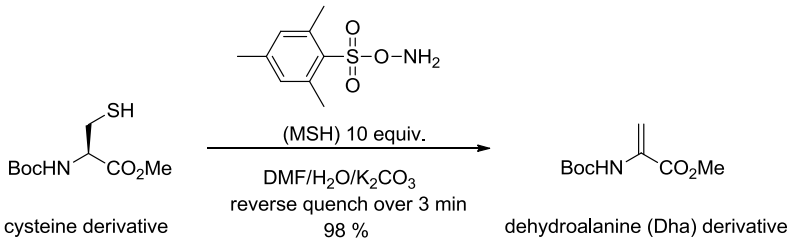
One of the first methods for the alkylation of cysteine was the use of iodoacetamides in 1935. They were used for the modification of cysteines in keratin.[139] The modification of lysine as a side-reaction is known but can be avoided by the use of chloroacetamide.[140] The protein papain could be modified by the use of α-bromoacetophenone derivatives (Scheme 23 a)).[11],[141-142]

The use of Michael acceptors such as maleimides[137],[143] and vinyl sulfones,[144] is a widely used method for the selective alkylation of the cysteine side chain. One of the first example for the use of maleimides for peptide modification was reported by Moore and Ward in 1956 (Scheme 23b).[145] Until now maleimides are still widely used to alkylated cysteine in peptides and proteins.[11],[143],[146]

Another method to modify cysteine is the formation of disulfide via simple air oxidation. The disulfide is generated by adding a thiol to the cysteine containing protein in a basic buffer open to air. This method is useful for the conjugation of small molecules to proteins.[137] Beside long reaction times the limited control of product distribution is a disadvantage (Scheme 23c).[11]

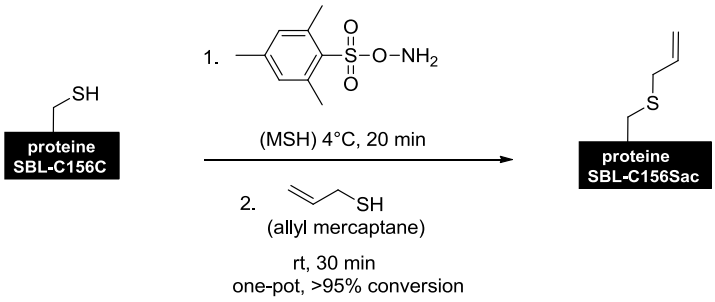
Che et al. reported about the formation of a vinyl sulfide linkage using different electron-deficient alkynes for chemoselective modifications on cysteine of unprotected peptides and proteins in water (Scheme 23d).[138]

A more sophisticated method is the oxidative elimination of cysteine followed by metal mediated modifications at cysteine and cysteine derivatives described by Davis et al.[148] First step is the desulfurization of cysteine towards dehydroalanine (Dha) respectively towards dehydroalanine derivatives via oxidative elimination upon treatment with *O*-mesitylenesulfonylhydroxylamine (MSH) (Scheme 24). Holmes and Lawton were one of the first describing the desulfurization of cysteine towards dehydroalanine (Dha).[147]



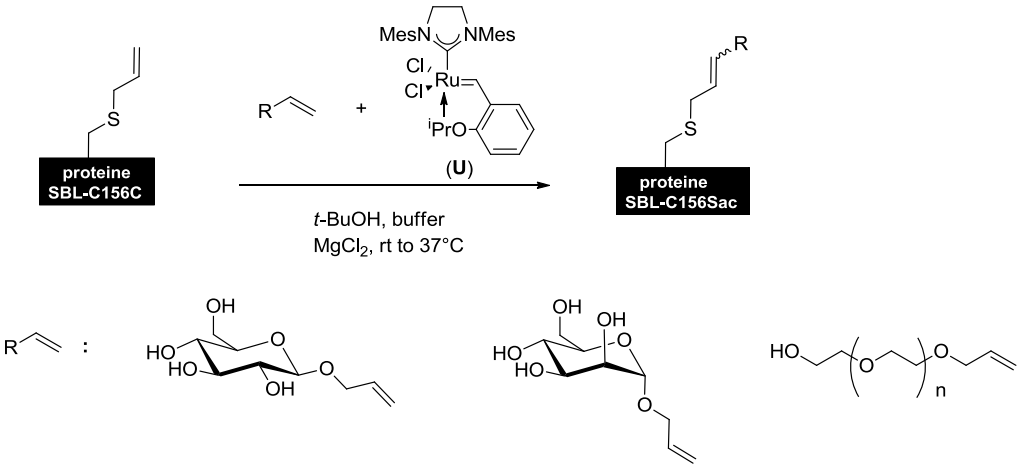
Scheme 24: Oxidative elimination of cysteine derivative to dehydroalanine (Dha) derivative.[148]

The formation of dehydroalanine is a veritable alternative to the direct alkylation of cysteine using acetamides and maleimides, because it serves as a good Michael acceptor for thiol nucleophiles. Dehydroalanines were formed by the oxidative elimination of alkylcysteine[149] and selenocysteine[150-151] and this method has been applied to biosynthetic peptides[152] and proteins.[153] In 2008 Davis et al. discovered that when cysteine is added to excess O-mesitylenesulfonylhydroxylamine (MSH) under basic conditions, Dha is formed rapidly.[148] After having obtained Dha from a serine protease mutant subtilisin *Bacillus lentus* (SBL) S156C, they were able to establish different modifications such as glycosylations, phosphorylations and farnesylations by the addition of thiols.[11],[148] In Scheme 25 this is illustrated by allyl thiol (allyl mercaptane). A disadvantage of this method is that it is associated with epimerization at the α-carbon of the amino acid, because diastereoselectivity of the thiol addition in simple Dha peptides is reported to be low.[154-155]



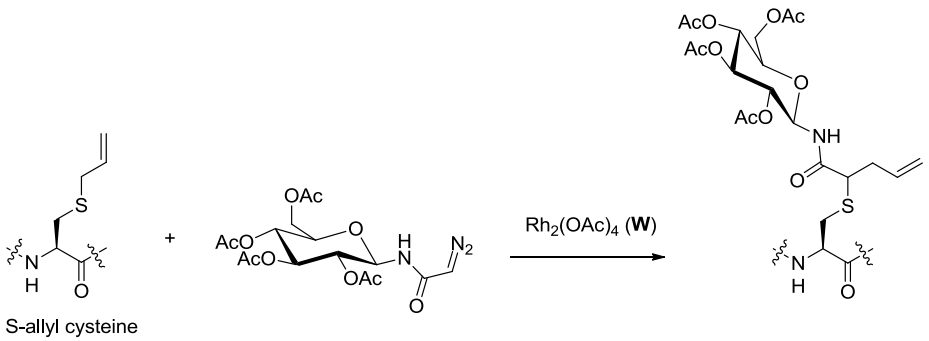
Scheme 25: Chemical formation of S-allyl cysteine (Sac).[156]

Using metal mediated chemistry on cysteine is not new. For example a nickel and palladium mediated selective reduction of cysteine to alanine is known in literature.[157] Due to ongoing efforts to improve metathesis reactions Davis et al. have observed that Hoveyda–Grubbs 2nd generation metathesis catalyst **U** is able to catalyze the crossmetathesis reaction in aqueous media using allylic sulfides.[156] Although allylic sulfides have been used in organic solvents so far their rate enhancement kept unnoticed.[158],[159] Together with the preliminarily obtained Dha they were able to glycosylate and PEGylate the protein surface so reporting the first examples of cross-metathesis on a protein (Scheme 26).[11],[156]



Scheme 26: Cross-methathesis used for site-selective protein modification in water.[156]

Beside this striking result allyl cysteines can be used for a Kirmse–Doyle reaction to functionalize peptides (Scheme 27).[160] Several peptide conjugates have been synthesized using a sulfur ylide formed due to an attack of a rhodium carbenoid **W**.[11],[161-162]



Scheme 27: Kirmse–Doyle strategy for S-allyl cysteine modification.[11],[160]

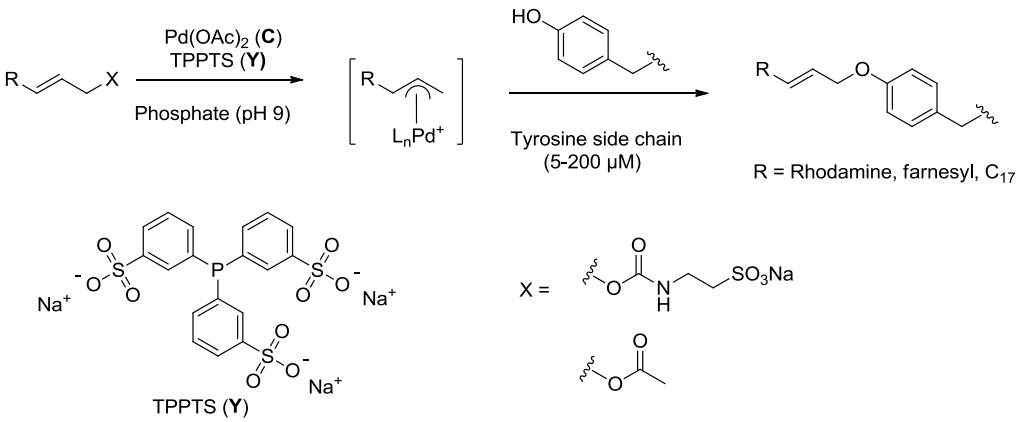
Similar to these methods described above Hackenberger et al. modify the same model protein (SBL-156) by simple alkylation of the cysteine residue by aryl iodide and subsequent

glycosylation with a vinyl boronic linker using Pd-catalyzed Suzuki-Miyaura reaction[163-164] in water.[165]

Also Chapman et al. reported that they obtained phenylalanine derivatives of di- and tripeptides through site-selective conversion of the cysteine residue. Also here they first obtained a common dehydroalanine intermediate by elimination. The subsequent use of Rh-catalyzed 1,4-addition leads to the phenylalanine derivative.[166]

The for our purpose most inspiring reaction was performed by Francis et al.[167] They site selectively modified a tyrosine residue. Modifying tyrosine can be considered as an alternative to cysteine targets in native proteins due to the low abundance of solvent-accessible tyrosine residues on most proteins.[168]

They used a π-allylpalladium complex arriving from a Pd precursor **C** and a water soluble phosphine ligand **Y**, to modify tyrosine residue within the protein α-chymotrypsinogen A and coat protein of bacteriophage MS2 at a concentration of 5 µM. They considered the use of a "disposable" polar allylic carbamate leaving group as beneficial, because it is cleaved during the bioconjugation reaction (Scheme 28).[15],[167]

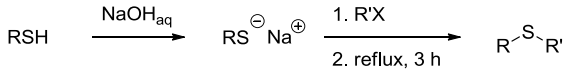


Scheme 28: Site-selective tyrosine residue modification with π-allylpalladium complexes.[15]

2.8. Thioether synthesis

2.8.1. Classical reaction

One of the first reports about the formation of dialkyl sulfides was provided by McAllan et al. First, the thiol gets deprotonated by an inorganic base in aqueous media. This thiolate undergoes nucleophilic substitution with organic halogen compounds forming a dialkyl sulfide (Scheme 29).[169] Also organic base can be used to deprotonate thiols in organic media.[131]

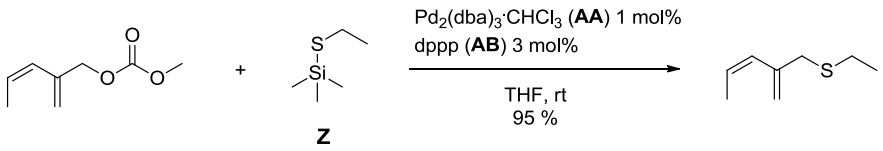


Scheme 29: Dialkyl sulfides formed by nucleophilic substitution.

Nevertheless, for our purpose to modify cysteine containing biomolecules, this way of making dialkyl sulfides cannot be considered as effective. The necessary alkyl bromides or iodides used in this transformation are strong electrophiles with a high reactivity and therefore they can be hardly used for our purpose. For this reason, it is necessary to search for more selective reactions, that only the thiol is converted to the desired dialkyl sulfide. One reaction which is known to be selective is the Pd-catalyzed allylation reaction.[170-171]

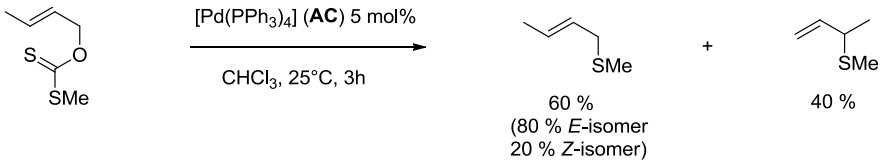
2.8.2. First Pd-catalyzed allylic alkylation of thiols – the work of Trost et al. and Bosnich et al.

The first Pd-catalyzed allylation in combination with sulfur containing compounds was reported by Trost et al. already in 1986 allowing milder reaction conditions. First he tried to substitute allyl halides by thiols. It turned out, however, that due to strong electron-withdrawing properties of the chloride leaving group large quantities of unwanted disulfides were formed. To avoid the formation of these byproducts, a weaker leaving group was necessary. Carbonates were used, since these are easily prepared and because they form excellent allylation reagents in the presence of palladium. Instead of a thiol the nucleophile used was a thiotrimethylsilan **Z** (Scheme 30). Therefore it was possible under mild reaction conditions to obtain a chemo-, regio- and diastereoselective substitution of allylic carbonates with sulfur nucleophiles using pre-catalyst $Pd_2(dba)_3 \times CHCl_3$ (**AA**) and ligand dppp (**AB**). The frequently occurring poisoning of the catalyst by sulfur in heterogeneous catalysis could not be observed here in this homogeneous catalysis reaction.[172]



Scheme 30: Early protocol of a Pd-catalyzed allylic thioether formation by Trost et al.[172]

Bosnich et al. published in 1986 the use of *O*-allyl *S*-alkyl dithiocarbonate substrates as starting material for allylation reactions using catalyst $[Pd(PPh_3)_4]$ (**AC**) (Scheme 31).[173]

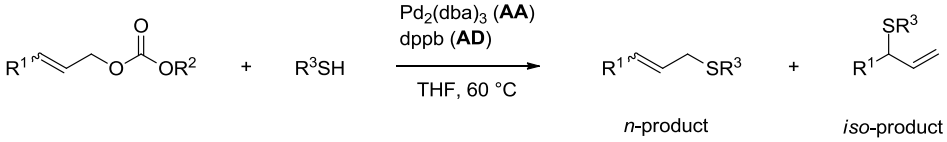


Scheme 31: Early protocol of a Pd-catalyzed allylic thioether formation by Bosnich et al.[173]

Very similar to the reported work by Trost et al. also this protocol leaves space for further improvements regarding selectivity of the Pd-catalyzed allylation.

2.8.3. Pd-catalyzed allylic alkylation of thiols – the work of Sinou et al.

In the following years, many improvements were achieved in the field of Pd-catalyzed allylic substitution. Inspired by the concept of Trost et al. using silylated thiols, Sinou et al. described the formation of various allylic thioethers starting from various allylic carbonates using the catalyst-ligand system tris(dibenzylideneacetone)dipalladium(0) $Pd_2(dba)_3$ (**AA**) and diphenylphosphinobutane (dppb) (**AD**) (Scheme 32). They described the formation of allylic thioether from aromatic thiol compounds such as thiophenol, 2-mercaptopyridine, 2-mercaptopyrimidine, 4-hydroxythiopenol and thiobenzoxazolone in good to moderate yields. Depending on the allylic carbonate used *n/iso* ratios of typically 95/5 were found. Also different *E/Z*-ratios were found, although the configuration was predominantly the one of the starting material. Furthermore, they could show that primary, secondary and tertiary allyl groups can be used for this transformation.[170],[174] It has to be pointed out that Tsuji et al. have employed allyl carbonates for Pd-catalyzed allylation using conventional *C*- and *O*-nucleophiles before.[175],[176]



Scheme 32: General reaction scheme for the Pd-catalyzed formation of allylic thioether by Sinou et al.[174]

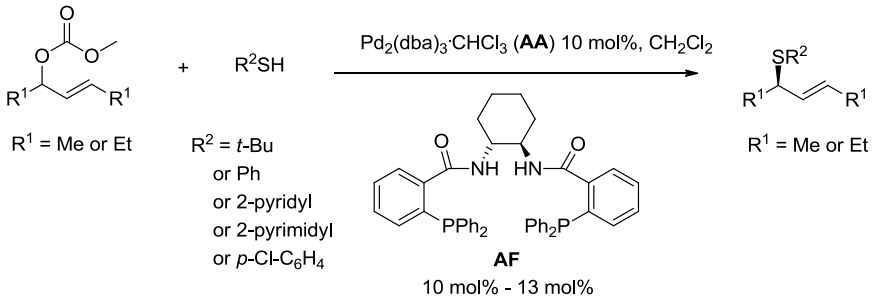
In addition, the influence of different temperatures on *n/iso* ratio of the obtained products was studied. They could show that temperatures between -90 °C to +60 °C have no significant impact on the *n/iso* ratio and no conversion of the *iso*-isomer to the linear *n*-isomer could be observed. Based on these results they postulated, that sulfur, being a large nucleophile, attacks on the less substituted end of the allylic complex. As a consequence this reaction is

regioselective, forming the less substituted alkylated sulfide and stereospecific by retention of configuration. Concerning our aim to modify cysteine containing biomolecules the main disadvantage of this reaction developed by Sinou et al., is its limitation to aromatic thiols. [170],[174],[177] Beside this limitation, there are hints in the literature, that such reactions are also possible in presence of water (at least in two-phase systems), which is important regarding the aim of modifying biomolecules.[178-179]

It has to be mentioned that nearly parallel to the work of Sinou also Manas et al. published similar results working with 2-mercaptopyridine, 2-mercaptopyrimidine and thiobenzoxazolone in combination with cinnamyl and allylic carbonate using a mixture of $Pd(acac)_2$ (**AE**) and triphenylphosphine as catalyst.[180-181]

2.8.4. Pd-catalyzed allylic alkylation of thiols – the work of Gais et al.

Gais et al. described the asymmetric synthesis of allylic thioether from the corresponding racemic carbonates with 2-mercaptopyridine, 2-mercaptopyrimidine and *p*-chlorothiophenol in good to moderate yields and ee-values (Scheme 33). Using quite high amounts of $Pd_2(dba)_3{\cdot}CHCl_3$ (**AA**) as pre-catalyst and Trost ligand **AF** they were not able to obtain any conversion in the case of aliphatic thiols like tert-butyl thiol or aromatic thiophenol. However, the use of again quite high amounts of DIOP (**AG**) (8 mol%) as ligand led to moderate conversion in the case of aliphatic *tert*-butyl thiol and good conversion when aromatic thiophenol was used. They obtained in both cases very low ee-values.[182]

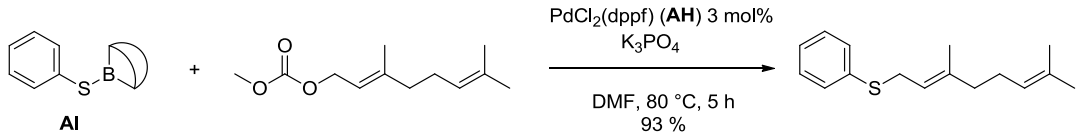


Scheme 33: Asymmetric synthesis of allylic thioether by Gais et al.[182]

They further described this Pd-catalyzed kinetic resolution of racemic allylic carbonates still using aromatic thiols in a full paper 2003.[183]

2.8.5. Pd-catalyzed allylic alkylation of thiols – using organo thioboranes

A regio-and stereoselective synthesis of dialkyl sulfides using the conversion of organo thioboranes with organic halides or carbonates under mild conditions was reported by Miyaura et al. The coupling occurred in the presence of a base and different Pd catalysts, tolerating a wide variety of functional groups. This reaction could be accelerated by increasing the polarity of the solvent used. An additional accelerating effect could be detected in the presence of K_3PO_4. The preferred catalyst-ligand system used was $PdCl_2$(dppf) (**AH**). Yields of 80 % to 98 % were achieved by the use of 3 mol% Pd catalyst and a three-fold excess of K_3PO_4 used as base (Scheme 34).[184]



Scheme 34: Pd-catalyzed formation of dialkyl sulfides using organo thioboranes as starting material.[184]

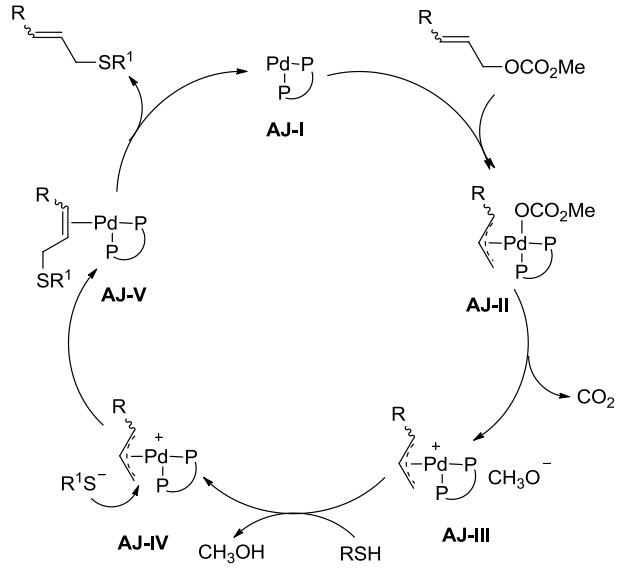
However this method is hardly usable for our aim to modify cysteine containing biomolecules, due to the necessary dehydrogenative condensation reaction of 9-BBN and thiols to obtain the starting material (**AI**).[185-186]

2.8.6. Mechanism of the Pd-catalyzed allylic substitution of thiols

In this thesis the Pd-catalyzed allylic alkylation reaction was chosen for our purpose to modify cysteine containing biomolecules, because classical alkylation of thiols need highly reactive allylic bromides or iodides and many other functional groups within the biopolymer (especially peptide) will react too.

A mechanism for the Pd-catalyzed allylic substitution on thiols was provided by Sinou et al. and for other carbonucleophiles by Tsuji et al. (Scheme 35). The postulated mechanism is initiated by coordination of the palladium complex **AJ-I** with the allylic substrate. By the subsequent oxidative addition an allylic η^3-coordinated species **AJ-II** is formed. In the case of phosphine ligand an equilibrium forms between a neutral and a cationic complex. The cationic complex is favored if bidentate phosphine ligands are present.[171] The allylpalladium carbonate complex **AJ-II** undergoes decarboxylation to form allylpalladium alkoxide complex **AJ-III**. Due to the removal of carbon dioxide from the equilibrium the cationic species **AJ-III** plus the corresponding alkoxide is formed predominantly. This leads to the deprotonation of

the thiol and after subsequent nucleophilic substitution and dissociation from the cationic complex **AJ-III** the desired allylic sulfide is obtained. Parallel the original reactive catalyst species is generated being able to proceed in the next catalytic cycle.[170],[187]



Scheme 35: Mechanism of the Pd-catalyzed allylic substitution of thiols.[170],[187]

Although this mechanism is described for the use of carbonates, in principle also other leaving groups such as acetates, halides, sulfones, carbamates, epoxides, and phosphates can be used. Carbonates are preferably used, since carbon dioxide is liberated during the catalytic cycle, which evaporates out of solution and therefore the equilibrium is shifted to the product side.[170-171]

One important aspect for the potential use of Pd-catalyzed substitution for the modification of biomolecules is the ability to tune the selectivity of the reaction by varying the ligands on the Pd. The sterical and electronic properties of different ligands used have a major effect on both the rate of reaction and the control of its regioselectivity, as Åkermark et al. could show.[188-189] A substantial acceleration of the reaction is mainly caused by π-acceptor ligands such as phosphines. This is not the case with ligands having only σ-donor properties It is regarded as a fact that phosphites are better π-acceptor ligands as phosphines.[190-191] Therefore, phosphites are better suited for the allylic substitution reaction as the corresponding phosphines. Due to the electron-withdrawing property electron density is removed from the metal center, resulting in a higher electropositive character on the coordinating allyl ligand. Therefore a subsequent nucleophilic attack on the coordinated allylic compound is more likely.[171]

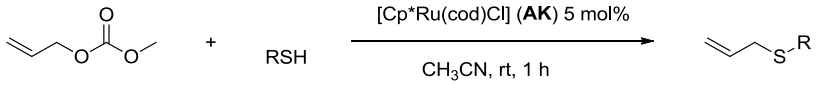
2.8.7. Alternative ways to synthesize carbon-sulfur bonds

A review on metal-catalyzed carbon-sulfur bond formation is provided by Kondo and Mitsudo.[192]

2.8.7.1. Ru-catalyzed allylic alkylation of thiols

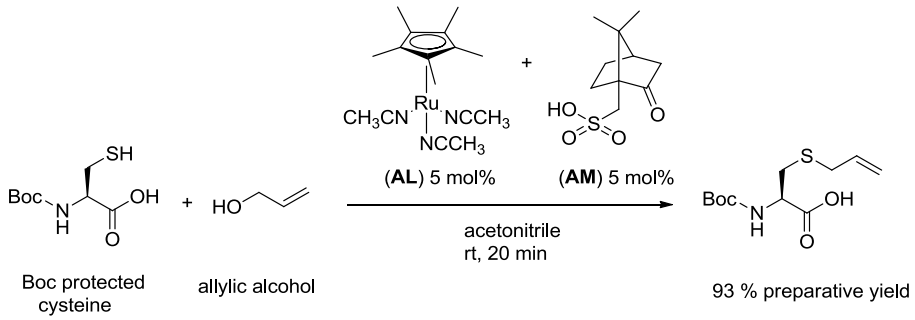
Beside an Ir-catalyzed allylation protocol using the for our purpose incompatible sodium metal salt of thiophenol[193], the transition metal ruthenium can be regarded as competitive for the allylic alkylation reaction of thiols.

Allylic sulfide products can be obtained both from aliphatic and aromatic thiols in good yields by the use of Ru catalyst [Cp*Ru(cod)Cl] (**AK**) (Scheme 36). However, the right choice of solvent is important. The best results could be achieved by using acetonitrile. In fact, acetonitrile is not only acting as the solvent but it is believed to also coordinate to the active ruthenium center, preventing poisoning of the catalyst in the presence of thiols.[194]



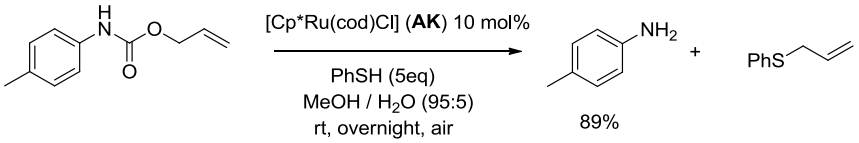
Scheme 36: Ru-catalyzed formation of dialkyl sulfides.[194]

Continuous improvements in this field published during this thesis made it possible to obtain even an allylic sulfide from Boc-protected cysteine via Ru-catalyzed allylic alkylation using allyl alcohol in combination with Ru catalyst (**AL**) and camphor sulfonic acid (**AM**) (Scheme 37). The results obtained by Pregosin and Veiros even indicate that there is no additional advantage using carbonates instead of allyl alcohol used in this publication. However, due to its good coordinating ability of acetonitrile to Ru exclusively acetonitrile was used for this transformation here, providing not enough solubility for various biomolecules.[195]



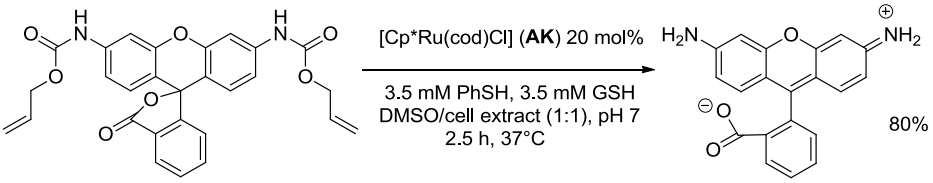
Scheme 37: Ru-catalyzed allylic alkylation of cysteine.[195]

Another important and inspiring method was developed by Streu and Meggers. They could show the cleavage of different allylcarbamates with thiophenol in the presence of Ru-catalyst [Cp*Ru(cod)Cl] (**AK**) in the solvent mixture of MeOH/H_2O (95:5) under air (Scheme 38).



Scheme 38: Ru-induced allylcarbamate cleavage.[196]

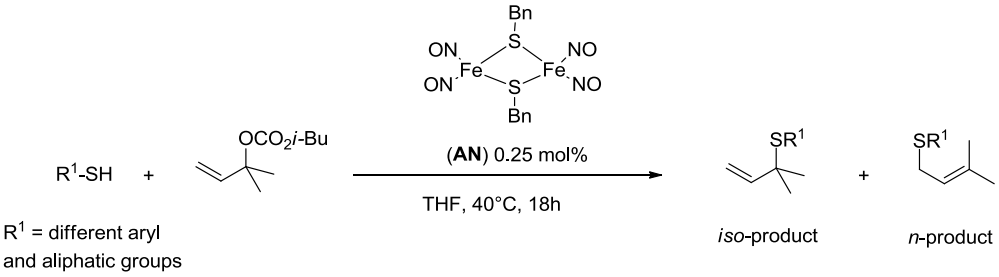
They were even able to perform this type of reaction under physiological conditions in living mammalian cells (HeLa cells) (Scheme 39).[196]



Scheme 39: Ru-induced allylcarbamate cleavage in a living cell.[196]

2.8.7.2. Fe-catalyzed ligand free allylic alkylation of thiols

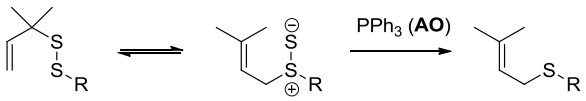
In 2011 Plietker et al. reported an allylic alkylation of thiols using different binuclear Fe-complexes. Among several Fe-complexes tested ‘‘reduced Roussin’s Red Ester (rRRE)’’ (**AN**) was found as the best catalyst allowing a ligand free allylic sulfenylation with a low catalyst loading. Many different aryl and also aliphatic thiols were allylated in good to excellent regioselectivities and yields. Depending on the structure of the carbonate used as starting material they could obtain *iso*-substituted allylic thioether or *n*-substituted allylic thioether (Scheme 40).[197]



Scheme 40: Fe-catalyzed ligand free allylic alkylation of thiols.[197]

2.8.7.3. Formation of allylic sulfides through allylic disulfide rearrangement

The phosphine based desulfurative allylic rearrangement of alkyl alkyl disulfides is a useful selective preparation of dialkyl sulfides. The first step is the selective formation of disulfides, which is still a widely used method for the ligation of peptides and proteins.[146],[198-199] The second step is the desulfurative allylic rearrangement of the disulfide towards dialkyl sulfides by the use of phosphine, more precisely PPh_3 (**AO**) (Scheme 41), having a high affinity towards sulfur. This desulfurative allylic rearrangement proceeds as a [2,3]-sigmatropic rearrangement via a diallyl thiosulfoxide intermediate.[200-201] The strength of this method is its chemoselectivity towards the thiol group of cysteine residues in the presence of all other functional groups present in natural occuring peptides. This desulfurative allylic rearrangement was performed using glutathione in the presence of a sulfenylating agent in a 2/1/1 mixture of tris-buffer/MeCN/THF yielding 70 % of the modified glutathione peptide.[202]

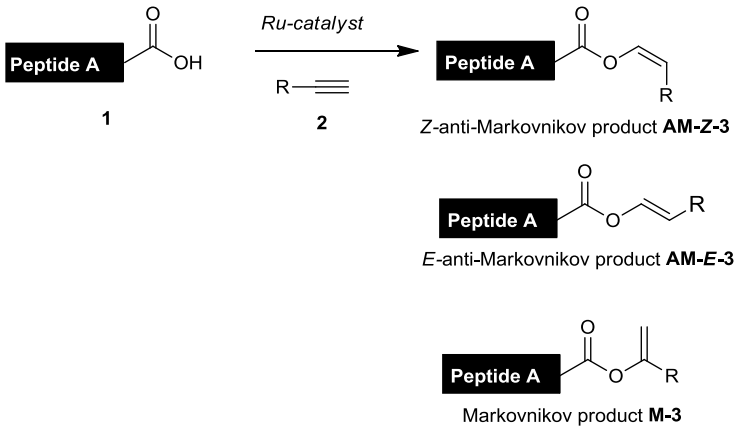


Scheme 41: Allylic alkyl disulfide rearrangement resulting in allylic thioether.[202]

3. Aims of the dissertation

The decreasing numbers of approved small molecules drugs developed by the pharmaceutical industry raised interest in the development of peptides as potential drug candidates. This interest resulted in the discovery of a large number of peptide-based drug candidates, for which synthetic access on a large scale has to be established.[1] One of the major challenge in synthesizing peptides is the elimination of racemization during the peptide formation. In this regard an attractive method is the chemoenzymatic synthesis of peptides using hydrolases, which allows the conversion of non-side chain protected peptides. A challenge associated with this strategy is the preparation of activated peptide esters without racemization, which are suitable substrates for this enzyme.

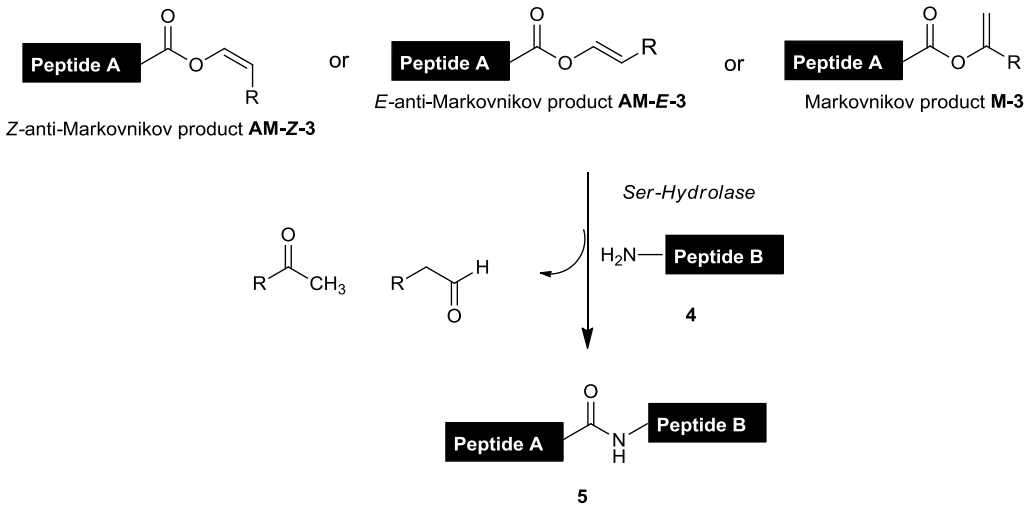
The development of a racemization free synthesis of amino acid and peptide enolesters was the major aim for this work. We envisaged that the Ru-catalyzed addition of the *C*-terminal carboxyl group across a terminal alkyne moiety would result in the atom-economical production of enolesters (Scheme 42).



Scheme 42: General scheme for the preparation of peptide enolesters.

Based on previous work by Dixneuf et al.[110] and Goossen et al.[111] amino protected amino-acids and peptide enolesters using different alkynes should be synthesized. All literature known synthetic routes of making enolesters using ruthenium catalysts have only been developed for protected amino acids but not for peptides, which would require considerable optimization. Therefore, the performance of the catalyst complexes should be increased by screening of different ligands and different solvents. The reduction of epimerization during the formation of peptide enolesters should always receive highest attention during the development of new catalyst-ligand species.

Once a synthetic route to peptide enolesters will be established, they should function as substrates for peptide couplings with a Ser-hydrolase (Scheme 43).

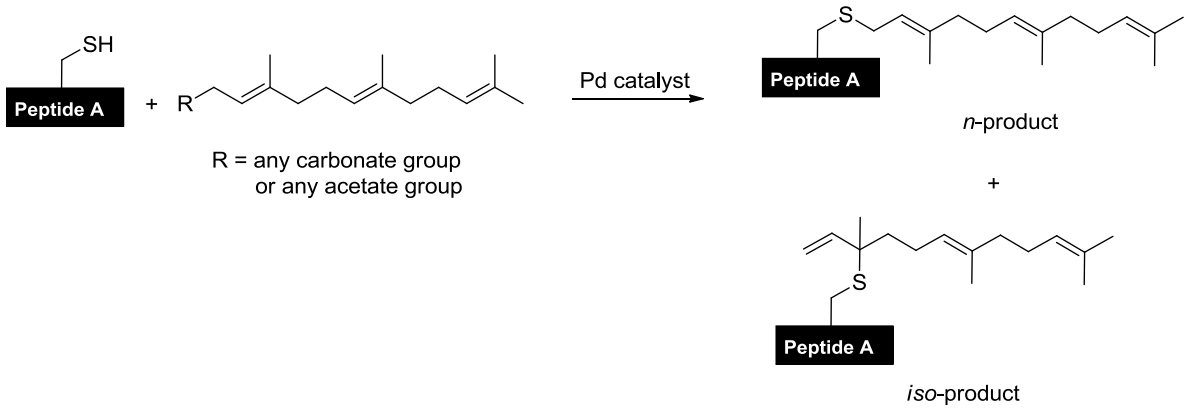


Scheme 43: General scheme for the use of peptide enolesters in enzymatic peptide couplings.

Alcalase-CLEA should be used as a Ser-hydrolase This enzyme, is a serine endoprotease which is cross-linked via glutaraldehyde. An alcalase-CLEA OM, especially designed for organic media, should be applied in the enzymatic peptide synthesis. Advantages of Alcalase –CLEA are its well proven ability to accept the ester moieties carboxamidomethyl (Cam)[50] and trifluoroethyl (Tfe)[48] for the synthesis of small peptides.

Peptides or even proteins are often not biologically active by itself, but they become biologically active by post-translational modifications. In this thesis the focus should be on lipidation (prenylation) as one possible post-translational modification of peptides and proteins.

A Pd-catalyzed allylic alkylation should be envisioned to be able to modify peptides and biopolymers (or even proteins) selectively. Several challenges will have to be addressed. First, the poisoning effect of sulfur atoms in low oxidation states e.g. thiols on transition metals needs to be overcome.[203],[204] Second, an allylic alkylation can lead to several isomeric products, but only the linear isomeric product is the desired one, as in nature. Therefore, a selective way of modifying peptides containing thiol groups has to be developed (Scheme 44).



Scheme 44: General scheme for Pd-catalyzed post-translational modification of peptides.

Besides the modification of peptides, another aim of this work was to be able to synthesize aliphatic thiol ethers by using Pd allyl-chemistry, obtaining them as a single isomer (*n*-product).

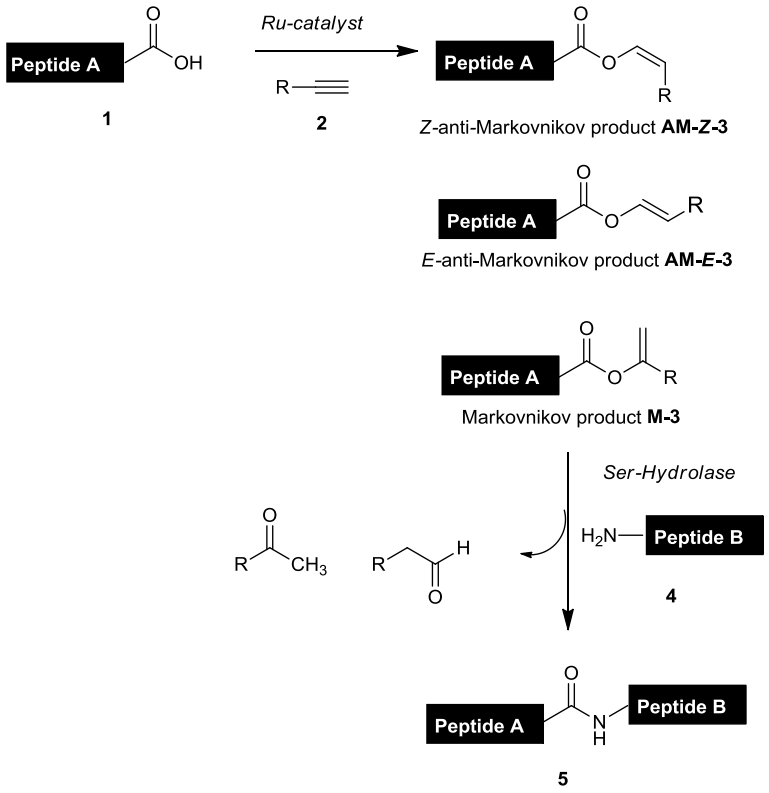
4. Chemoenzymatic synthesis of peptides and Pd-catalyzed alkylation of cysteine containing peptides – results and discussion

4.1. Synthesis of amino acid and peptide enolesters via ruthenium-catalyzed alkyne addition

4.1.1. Introduction

In recent years the scalable synthesis of peptides, ranging from simple dipeptides to oligopeptides, has gained significant importance, as such products have been increasingly investigated and marketed in the pharmaceutical, cosmetics and nutrition industry.[1-4] Although several excellent methods and reagents are available for the formation of peptides, the issue of racemisation free fragment coupling remains a problem without a general solution.[205-210] One attractive method is the chemoenzymatic synthesis of peptides using hydrolases,[211-214] where either suitable reaction conditions shift the equilibrium to the coupling product[215-216], or activated esters[97],[217] or thioesters[218] are used. In principle, chemoenzymatic peptide synthesis is distinguished by the advantage of enzymatic chemoselectivity, which allows the conversion of non-side chain protected peptides. Quaedflieg et al. reported in a series of publications that the serine endopeptidase Alcalase is a practical enzyme for the preparation of oligopeptides.[9],[52],[219-221] One challenge associated with this strategy is the preparation of activated peptide esters which are suitable substrates for this enzyme. Activation of peptide donors for chemoenzymatic synthesis has so far relied on the chemical or enzymatic synthesis of thioesters, or the chemical synthesis of activated esters. Most of these strategies involve the activation of the *C*-terminal carbonyl group followed by addition of a nucleophile – a process which is often associated with a certain amount of racemization. Quaedflieg[9],[52],[219-221] and others [222-223] could demonstrate that the activation of peptides as Cam-esters, in which the carboxyl-group serves as a nucleophile in the reaction with the strong electrophile iodoacetamide is a viable alternative method for activation. However *S*-containing amino acids or unprotected tyrosine cannot be used with this method. In order to provide a more general solution to this problem, we envisaged that the addition of the *C*-terminal carboxyl group across a terminal alkyne moiety would result in the atom-economical production of enolesters, which have been recognized as ideal reagents in enzymatic acylations, although so far only for simple and more or less unfunctionalized acids.[224-226] One exception is the protease-catalyzed synthesis of

carbohydrate-amino acid conjugates with amino acid vinyl esters.[10] Also vinylesters of protected amino acids have been prepared by a formal transesterification with vinyl acetate using $PdCl_2$/NaCl.[98] These peptide enolesters would then function as substrates for peptide couplings with a Ser-hydrolase (Scheme 45).



Scheme 45: General scheme for the preparation of peptide enolesters and their use in enzymatic peptide couplings.

The key challenge for our approach was therefore to develop a mild and racemization free method for the synthesis of enolesters of quite complex peptide fragments, which are significantly more prone to racemization than single amino acids. Among several methods considered, we identified the reports by Watanabe,[102-104] Dixneuf,[105],[108-110] and Goossen [111] on the Ru-catalyzed addition of carboxylic acids to alkynes as rewarding starting points for our investigations.[108],[227-229] It has to be noted that other metals like rhodium,[114],[230] rhenium,[231] iridium,[232] and gold[233] were applied as catalysts in the alkyne addition. Dixneuf and Goossen have reported about the synthesis of a single amino acid enolester without racemization.[105-111]

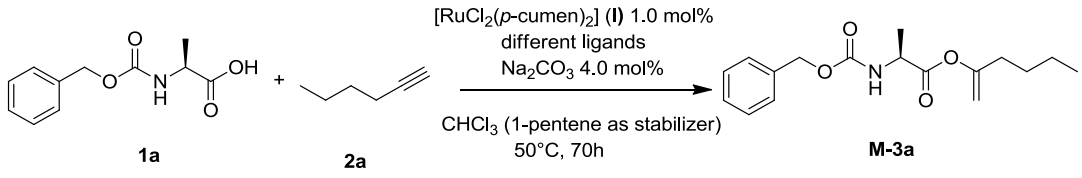
The Markovnikov product **M-3** shown in Scheme 45, which is accessible by Goossen's method, was the most desired isomer compared to the *E*- or *Z*-anti-Markovnikov-products **AM-*E*-3** resp. **AM-*Z*-3**. The advantage of applying the Markovnikov-enolesters **M-3** in

enzymatic peptide synthesis originates from the release of a less reactive ketone instead of a more reactive aldehyde as a side product. This is important, because the enzymatic peptide synthesis is best performed under anhydrous conditions using molecular sieves as a drying agent. Therefore, the first experiments focused on the synthesis of Markovnikov-enolesters. As a test substrate we used Cbz-Ala-OH (**1a**) and 1-hexyne (**2a**), as this reaction has already been reported in the literature.[111]

4.1.2. Synthesis of amino acid and peptide Markovnikov-enolester

4.1.2.1. Synthesis of Z-Ala-Markovnikov-enolester

For the synthesis of Markovnikov-enolesters **M-3** we applied the method of Goossen et al. Here, due to the addition of the base Na_2CO_3 to the catalytic system of Ru-catalyst [$RuCl_2$(p-cumen)$_2$] (**I**) and ligand P(Fur)$_3$ (**6**) different Markovnikov-enolesters of carboxylic acids, without a functional group in α-position could be obtained with good to excellent conversions and selectivities.[111] With Z-Ala-OH (**1a**) as a substrate both conversion and selectivity are reduced. Therefore, we started a small ligand screening effort in the hope to be able to improve conversions and selectivities (Scheme 46). We used Z-Ala-OH (**1a**), Na_2CO_3 as base, ruthenium complex [$RuCl_2$(p-cumen)$_2$] (**I**) and different ligands to yield (2*S*)-1'-hexen-2'-yl-2-(*N*-benzyloxycarbonylamino)-propanoate (**M-3a**) (Z-Ala-Markovnikov-enolester) (Figure 9, Table 1). The conversion was monitored via thin-layer chromatography and the isomers were quantified by GC-MS.



Scheme 46: Synthesis of Z-Ala-Markovnikov-enolester **M-3a**.

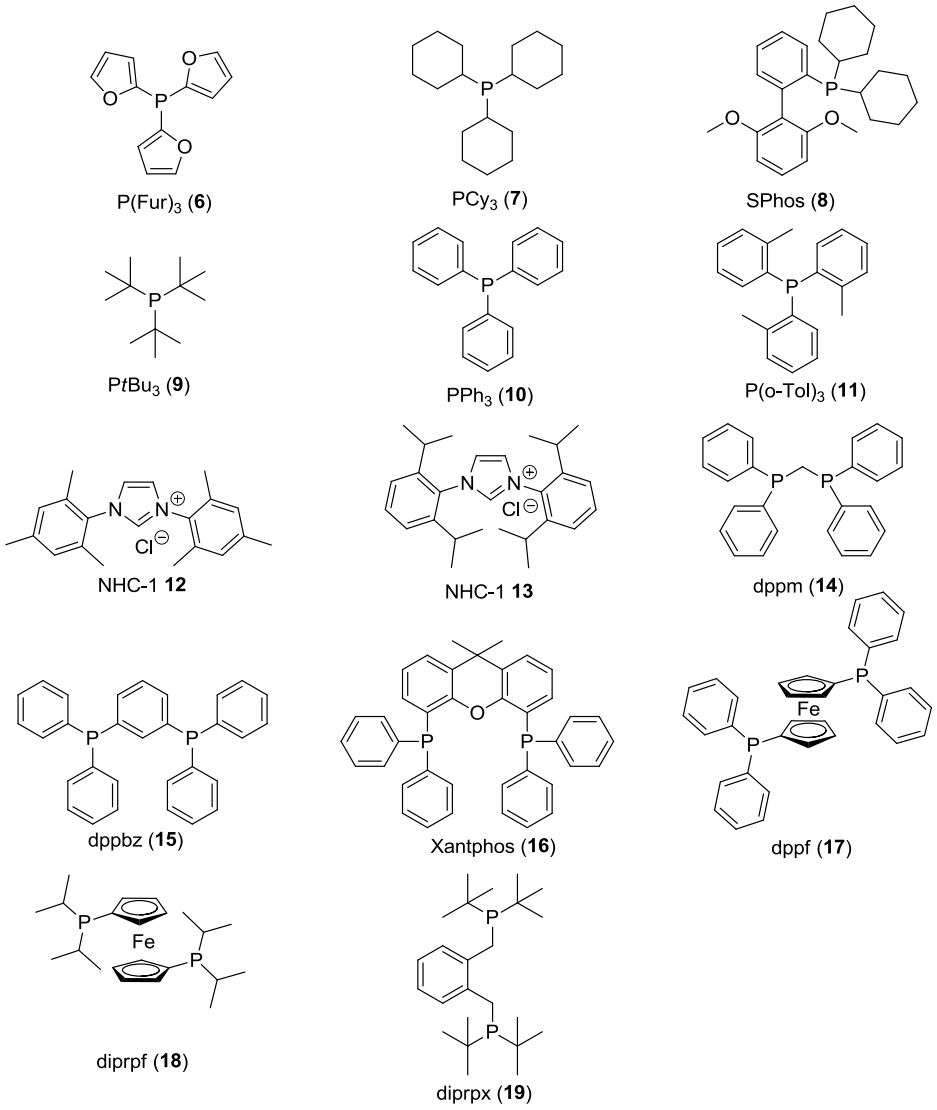


Figure 9: Ligands used in the ligand screening to optimize the formation of Z-Ala-Markovnikov-enolester **M-3a**.

Table 1: Results of the ligand screening; Catalysis-conditions: 135 mg (0.60 mmol, 1.0 eq) Cbz-L-alanine (**1a**), 90 µL (0.78 mmol, 1.3 eq) 1-hexyne (**2a**), 1.0 mol% catalyst-precursor **I**, 4.0 Mol% Na_2CO_3, $CHCl_3$ (1-pentene), 50 °C, 70 h; [a]Catalysis-conditions: 230 mg (1.03 mmol, 1.0 eq) Cbz-L-alanine (**1a**), 154 µL (1.33 mmol, 1.3 eq) 1-hexyne (**2a**), 1.0 mol% Catalyst-precursor **I**, 2.0 mol% P*t*Bu_3 (14), 4.0 mol% Na_2CO_3, $CHCl_3$ (1-Penten), 50 °C, 70 h; [b]Deprotonation with 1.4 mg (12.5 µmol) K*t*OBu in 1.0 mL THF.

Entry	Ligand	Mol [%]	Conversion after 70 h [%]	M-3a [%]	AM-*Z*-3a [%]	AM-*E*-3a [%]
1	$P(Fur)_3$ (**6**)	2.0	> 99	88	11	1
2	PCy_3 (**7**)	2.0	> 99	90	9	1
3	SPHOS (**8**)	2.0	> 99	80	19	1
4	PPh_3 (**10**)	2.0	> 99	88	11	1
5	P*t*Bu_3[a] (**9**)	2.0	low	14	60	26
6	P(*o*-Tol)$_3$ (**11**)	2.0	low	19	54	27
7	NHC-1[b] (**12**)	2.0	low	38	50	12
8	NHC-2[b] (**13**)	2.0	low	34	51	15
9	dppm (**14**)	2.0	low	16	68	16
10	dppbz (**15**)	2.0	low	16	53	31
11	Xantphos (**16**)	2.0	low	23	75	2
12	dppf (**17**)	2.0	low	3	95	2
13	dppf (**17**)	1.0	low	62	34	4
14	diprpf (**18**)	1.0	low	70	24	6
15	diprpx (**19**)	1.0	low	52	37	11

The best results were obtained using monodentate phosphine-ligands, which have an electron donating character. Comparison of entry 2 with entry 5 shows that to ensure sufficient coordination of Z-Ala-OH (**1a**) and 1-hexyne (**2a**) to the metal center ligands should not be too bulky. PCy_3 (**6**) and P*t*Bu_3 (**9**) have similar electronic properties, but ligand P*t*Bu_3 (**9**) is due to a ligand cone angle (Tolman cone angle) of 182° bulkier than ligand PCy_3 (**6**) with a ligand cone angle of 170°.[234-235] Other bulky ligands such as P*t*Bu_3 (**9**) and P(*o*-Tol)$_3$ (**11**) are not appropriate for this reaction.

Bidentate ligands (entries 9-15) are also not suitable ligands for this enolester formation, as only low conversions were found when using them. Interestingly, depending on the amount of equivalents used the formation of *Z*-anti-Markovnikov-product **AM-*Z*-3a** is favored. This is the case if 2.0 eq of bidentate ligands are used (entries 9-12), which was also shown by Dixneuf *et. al.*[110] when using 1.0 eq of bidentate ligands, Markovnikov-product **M-3a** is the major product (entries 13-15).

Regarding entries 7-8 carbenes were also applied as ligands in this reaction, but with only little success.

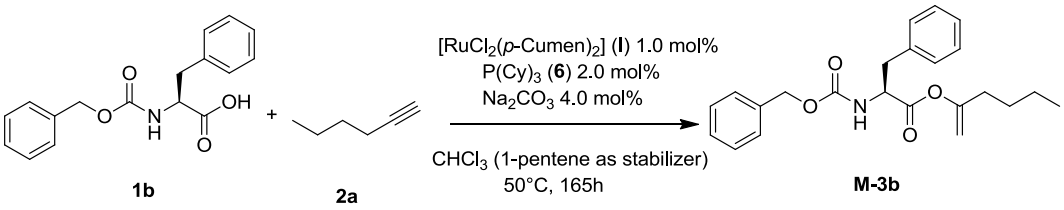
Among the tested set of ligands PCy_3 (**6**) was the best for this transformation (Table 1, entry 2). Performing the reaction described in Scheme 46 with PCy_3 (**6**) yielded a mixture of the isomers in 92 % isolated yield. After purification using flash column chromatography, Markovnikov-Product **M-3a** could be isolated in 33 % yield and with an isomeric purity of >99 %.

The ee-value of Z-Ala-Markovnikov-enolester **M-3a** was determined as 99.8 % ee by chiral HPLC. The HPLC-method had been validated by synthesizing first the racemic Z-DL-Ala-Markovnikov-enolester under the same conditions and then analyzing the racemate with chiral HPLC to ensure that peak separation is achieved with the chosen HPLC conditions which are further described in the experimental section.

To further improve the selectivity of this reaction we wanted to perform this transformation at different temperatures (50°C, 40°C, rt). In respect to a commonly increasing amount of racemization at higher temperatures, lower reaction temperatures are favored. Surprisingly, decreasing the temperature from 50°C to 40°C resulted in an approximately 3 times longer reaction time and no change in selectivity towards the Markovnikov-enolester **M-3a**. Performing this reaction at room temperature resulted in incomplete conversion even after 8 days and the selectivity of the reaction remained unchanged. It seems that changing the temperature has no effect on the selectivity of ruthenium-catalyzed reaction.

4.1.2.2. Synthesis of Z-Phe-Markovnikov-enolester

The successful reproduction of Goossen's work with Z-Ala-OH (**1a**) made us hopeful that we can use this method also for more challenging amino acid substrates. Phenylalanine is an amino acid which is known to be prone to racemization.[236] Therefore, it provides an excellent test case for validating our synthetic method of enolester formation regarding racemization.



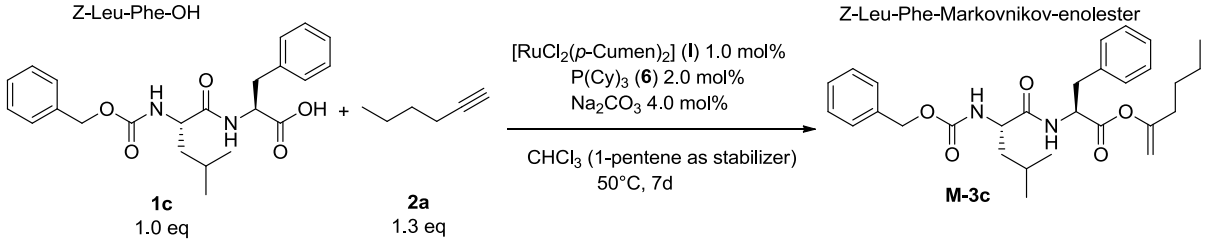
Scheme 47: Synthesis of Z-Phe-Markovnikov-enolester **M-3b**.

Using the conditions as above we could convert substrate Z-Phe-OH (**1b**) to Z-Phe-enolester **3b** in 86 % yield as a mixture of isomers (Scheme 47). After purification using flash column chromatography the Markovnikov-product **M-3b** could be isolated in 20 % yield and with an isomeric purity of >99 %.

Chiral HPLC analysis of the Z-Phe-Markovnikov-enolester **M-3b** revealed only the L-enantiomer demonstrating that again no epimerization has occurred under the reaction conditions.

4.1.2.3. Synthesis of Z-Leu-Phe-Markovnikov-enolester

As experiments in chapter 4.1.2 have confirmed the results of Goossen for amino acids, we were excited to investigate if this method is also applicable to peptides, as it is well known that epimerization becomes a greater problem when moving from amino acids to peptides. The first experiment for the synthesis of peptide enolester was conducted applying Goossen's conditions to dipeptide Z-Leu-Phe-OH (**1c**) as substrate (Scheme 48).



Scheme 48: Synthesis of Z-Leu-Phe-Markovnikov-enolester **M-3c**.

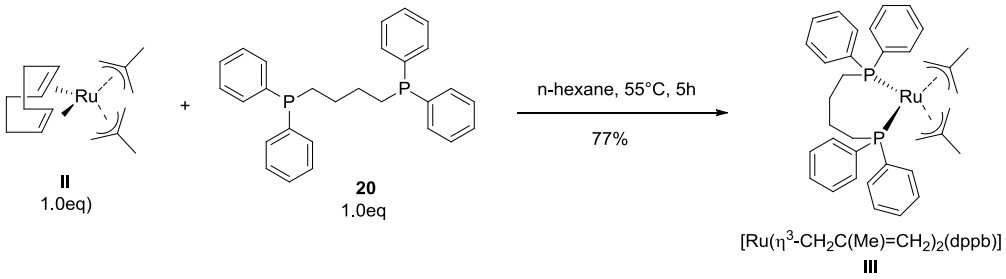
Unfortunately, even after a long reaction time of 7 days, we could find only 28 % conversion towards the product **M-3C** detected by HPLC-MS. In the HPLC analysis at 210 nm, we found two peaks with a ratio of almost 1:1 for the starting material Z-Leu-Phe-OH (**1c**). This finding indicates considerable racemization of the substrate during the reaction. In addition we observed the formation of side products, which we did not assign further. Because of the low conversion, we did not attempt the isolation of the product. It seems that the low reaction rate

and the necessity of a base are limiting factors for further optimizations. Unable to continue forward with the even optimized Goossen protocol, we surmised that switching to another protocol for making enolesters might alleviate these problems.

4.1.3. Synthesis of amino acid and peptide *Z*-anti-Markovnikov-enolester

4.1.3.1. Initial approaches

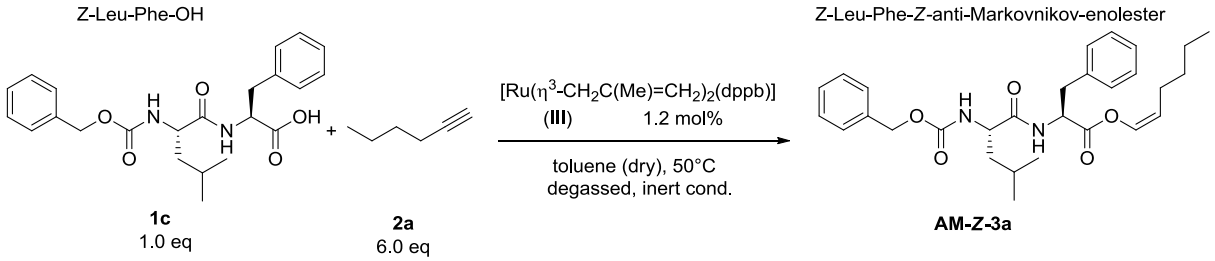
At this stage, the key question centered around providing fast conversion with a low amount of epimerization that would lead most efficaciously to enolesters. As a consequence of the disappointing results obtained with Goosen's method for dipeptides (chapter 4.1.2.3), we decided to investigate the alternative protocols from Dixneuf et al.[110] Ruthenium complex **III** was prepared by displacement of cyclooctadiene from bis(2-methylpropenyl)(cycloocta-1,5-diene)ruthenium (**II**) by diphenylphosphinobutane (dppb) (**20**) according to methods reported for phosphites (Scheme 49).[237]



Scheme 49: Synthesis of [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**).

According to literature, with the switch from Goossen´s method to the Dixneuf protocol, *Z*-anti-Markovnikov-enolesters are to be expected as the main products instead of the Markovnikov-enolesters. One consequence is that during peptide synthesis there will be a release of a more reactive aldehyde instead of a less reactive ketone. Problems associated with this fact of a highly electrophilic side product will be discussed in chapter 4.2.2.2.

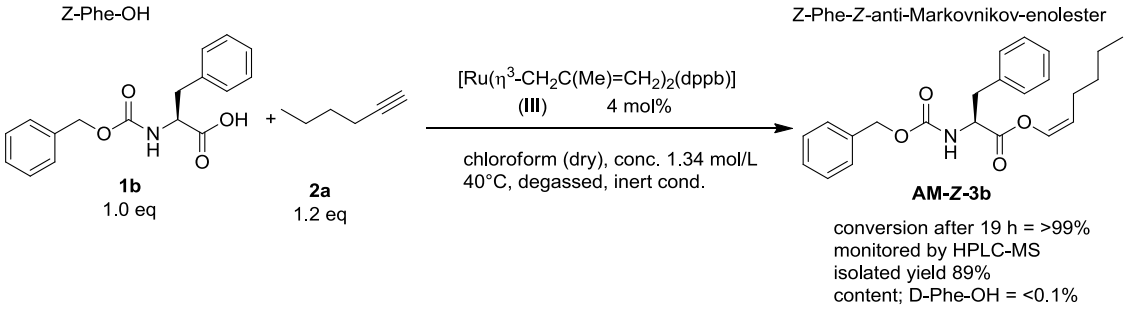
When we applied the original Dixneuf protocol[110] to the substrate Z-Leu-Phe-OH (**1c**) in toluene, we could observe not more than 1 % conversion after 36 h (Scheme 50). One reason for this disappointing result could be that because of solubility problems with substrate **1c** we had to use a concentration of 0.07 mol/L, compared to 0.5 mol/L in the original Dixneuf publication, in which rather small substrates such as acetic acid, trifluoroacetic acid, benzoic acid, valeric acid, Boc-phenylalanine, Z-alanine, etc. but no dipeptides have been studied so far.[110]



Scheme 50: Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-Z-3a** in toluene.

4.1.3.2. Synthesis of Z-Phe-*Z*-anti-Markovnikov-enolester

Since almost no conversion toward the desired dipeptide enolesters could be observed, we decided to use chloroform as solvent which provides us with a better solubility for the peptide substrate. Simultaneously, we increased the amount of catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) to 4 mol % to be able to detect conversion even if the catalyst turnover is low (Scheme 51). We first tested this approach with the Cbz-protected single amino acid phenylalanine, because a single amino acid is easier to convert into an enolester as peptides, which we learned from the previous reaction under Goossen's conditions.

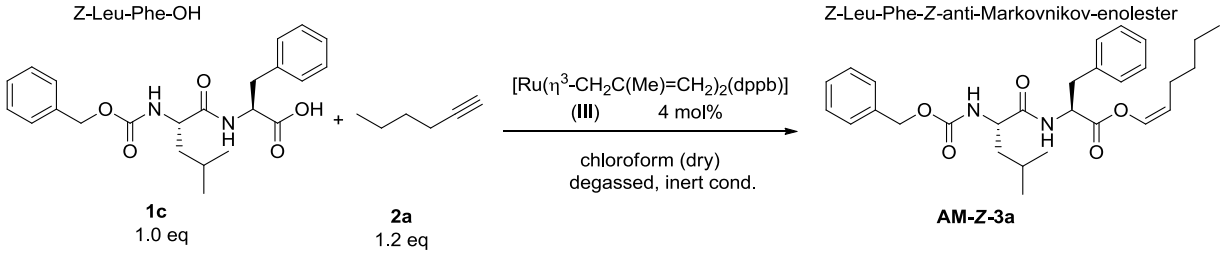


Scheme 51: Synthesis of Z-Phe-*Z*-anti-Markovnikov-enolester **AM-Z-3b**.

We were pleased to see that under these conditions after 19 h full conversion towards the *Z*-anti-Markovnikov-enolester resulted in 89 % isolated yield (Scheme 51). Analyzing the amount of racemization, the content of D-Phe-OH was <0.1 %. To analyze the amount of racemization we used a method which is further described in chapter 4.1.8.

4.1.3.3. Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester and Z-Leu-Ala-*Z*-anti-Markovnikov-enolester

After this encouraging result, we applied this modified Dixneuf method to our test substrate Z-Leu-Phe-OH (**1c**). As already mentioned in chapter 4.1.2.2, phenylalanine is known to be highly prone to racemization during peptide coupling reactions using chemical methods.[236] This is especially true if phenylalanine is at the *C*-terminal end of peptide. For our further studies we have selected Z-Leu-Phe-OH (**1c**) as a model substrate, because it is a rather large and bulky substrate, which is highly prone to epimerization. We hoped that reaction conditions which lead to high conversion and a low amount of epimerization for Z-Leu-Phe-OH (**1c**) should be transferable to less challenging amino acids at the *C*-terminus (Scheme 52).



Scheme 52: Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-Z-3a** in chloroform.

Table 2: Conversion towards Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-Z-3a** in chloroform.

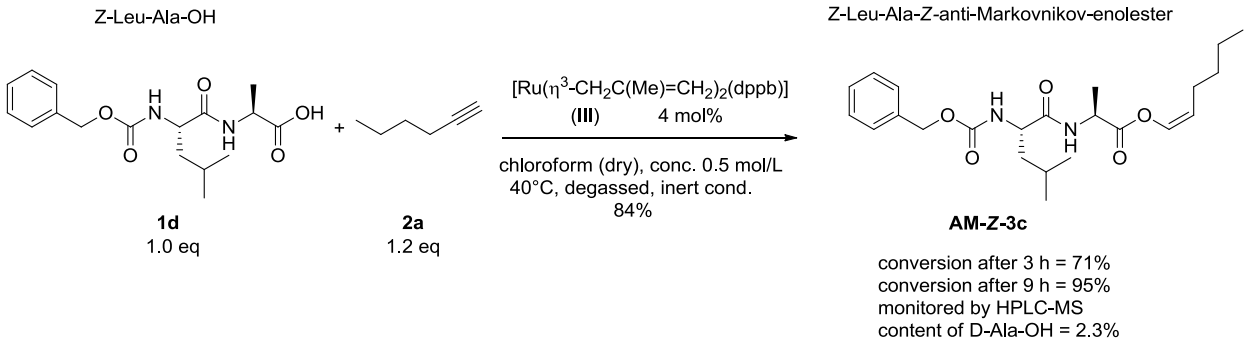
Entry	Starting material	Temp. [°C]	Conc. [mol/L]	Conversion[a] [%]			Isolated yield after column chrom. [%]	D-Phe-OH content[b] [%]
				3h	7h	20h		
1	**1c**	50	0.5	-	-	>99	90	2.9
2	**1c**	40	0.5	64	89	99	85	2.0
3	**1c**	rt	0.5	20	38	67	77[c] (95 % conv.)[c]	3.5[c]

[a] monitored by HPLC-MS (210 nm)
[b] Analysis of racemization: hydrolysis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester with 6N HCl and HPLC-analysis on a Chirobiotic-R column; D-Phe-OH content after hydrolysis of Z-Leu-Phe-OH (**1c**) under identical conditions was subtracted
[c] after 4d

The reaction was performed at different temperatures. We were able to achieve full conversion to the dipeptide enolester in chloroform at 50°C and 40°C, but not at rt (Table 2, entry 1-3). Unfortunately, in all experiments some epimerization occurred. At 40°C 2.0 % of D-Phe-OH (entry 2) could be monitored by the method described in chapter 4.1.8. This result

confirmed that Cbz-protected amino acids are less prone to racemization than dipeptides. Interestingly, performing the reaction at 40°C (entry 2) instead of 50°C (entry 1) lowered the amount of D-Phe-OH by almost 1 %. The reaction performed at room temperature was much slower than at 40°C. Also the amount of D-Phe-OH of 3.5 % (entry 3) is even higher than in entries 1 and 2, although the temperature was lower. These findings suggest that the amount of epimerization during ruthenium catalysis is not only depending on the temperature but also depending on the reaction time.

40°C was the temperature of choice for the conversion of Z-Leu-Ala-OH (**1d**) into enolester **AM-*Z*-3c**. The formation of enolester **AM-*Z*-3c** proceeded even a little bit faster compared to reaction entry 2 in Table 2. This confirmed the assumption that bulky amino acids at the *C*-terminal end of a peptide, like phenylalanine, are more difficult to convert into their corresponding enolester.



Scheme 53: Synthesis of Z-Leu-Ala-*Z*-anti-Markovnikov-enolester **AM-Z-3c** in chloroform.

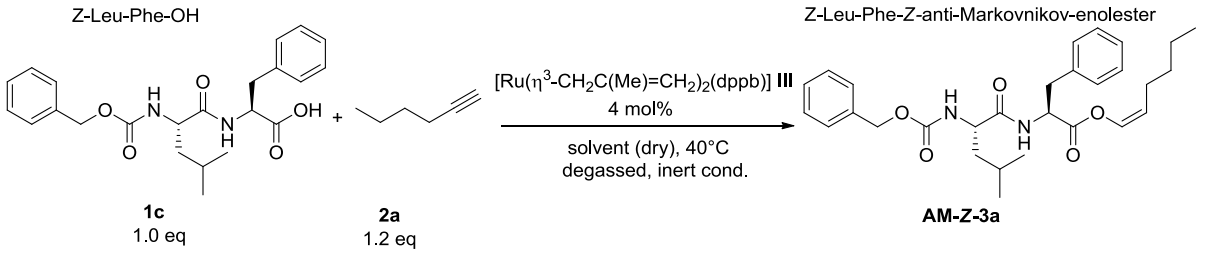
Similar to the Z-Leu-Phe-product **AM-*Z*-3a** described before, Z-Leu-Ala-*Z*-anti-Markovnikov-enolester **AM-*Z*-3c** also contained a few percent of *C*-terminal D-amino acid. To analyze the amount of racemization we used the method which is described in chapter 4.1.8 for the amino acid alanine.

With these experiments we could show that the Dixneuf-approach will be viable for the synthesis of dipeptide enolesters. But the problem of too much epimerization made a further optimization of the reaction condition necessary.

4.1.3.4. Solvent screening

Due to the toxicity of chloroform alternative solvents, which are easier to handle, would be preferred for this reaction. For many different homogeneous metal catalysis reactions THF is often the solvent of choice. Using THF as the solvent in the same reaction and under the

same conditions as described in chapter 4.1.3.3 (Table 2, entry 2) gave similar results in terms of conversion rates, but fortunately with a significantly reduced amount of D-amino acid formation. This motivated us to perform a broader solvent screening. The main goal of this solvent screening was to identify a solvent in which the reaction (Scheme 54) works fast and without racemization.



Scheme 54: Solvent screening for the synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-Z-3a**.

In Table 3 the results are summarized. Interestingly, especially ether-type solvents (entries 8, 10, 11) and alcohols (entries 13-15) gave the best conversions and generally low amounts of epimerization. 2-Propanol (entry 15) performed best among all tested solvents showing the by far fastest conversion and lowest amount of D-Phe-OH formation at the *C*-terminal end of the peptide. Fortunately and to our surprise, we could not observe any transesterification with the alcohols, which is probably due to the absence of a base. In order to avoid base introduced side reactions by base-bath contaminated Schlenk glassware, the Schlenk flasks were first washed with diluted HCl and secondly with distilled H_2O before drying them in the oven. During the solvent-screening in all cases we provided a homogeneous solution of Z-Leu-Phe-OH (**1c**), the catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) and the applied solvent. If necessary the suspension was heated to reflux for a few seconds until everything was dissolved and afterwards immediately cooled to 40°C.

Table 3: Solvents used for the synthesis of *Z*-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a**.

Entry	Solvent	Conc. [mol/L]	Conversion[a] [%]				Isolated Yield [%]	Amount of H-D-Phe-OH[b] [%]
			3h	18h	24h	48h		
1	NMP	0.5	15	-	29	33	-	n.d.
2	DMF	0.5	13	-	36	39	-	n.d.
3	DCM	0.5	54	-	90	98	-	13.2
4	aceton	0.5	52	-	82	87	-	14.2
5	sulfolane	0.5	65	-	86	89	-	3.5
6	DMSO	0.5	<1	-	<1	-	-	n.d.
7	acetonitrile	0.5	0.5	1	-	-	-	n.d.
8	THF	0.5	36	91	95	-	68	0.6
9	THF+1 % H_2O	0.5	40	57	57	-	-	1.8
10	methyl-THF	0.5	70	98	-	-	-	0.4
11	1,4-dioxane	0.5	55	-	97	-	-	0.4
12	DME	0.5	55	-	92	96	-	5.1
13	methanol	0.25	25	61	65	-	-	0.5
14	ethanol	0.5	85	98	-	-	-	0.7
15	2-propanol	0.5	98	-	-	-	91	0.3[c]
16	*tert*-butanol	0.25	65	90	92	-	-	1.9

[a] monitored by HPLC-MS (210 nm)

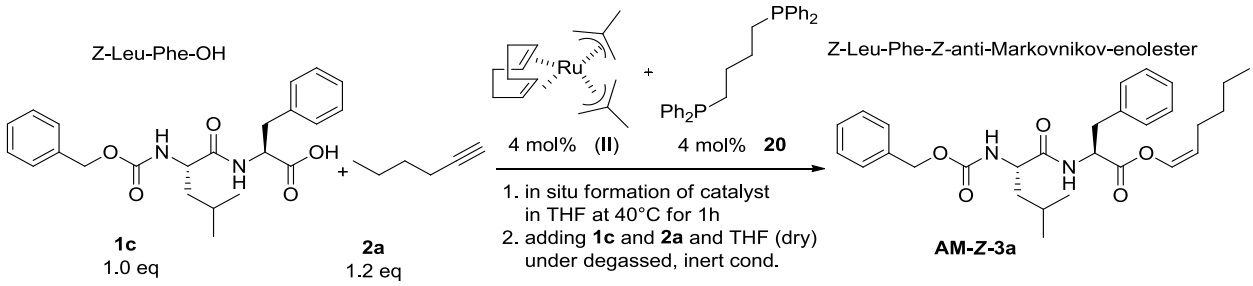
[b] HPLC: determination of racemization: hydrolysis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** with 6N HCl HPLC-analysis with Chirobiotic-R control-experiment: hydrolysis of Z-Leu-Phe-OH (**1c**) under identical conditions

[c] amount of racemization determined after flash column chromatography

Our data from the solvent screen also suggest that polar, aprotic solvents like DMF and NMP (entries 1 and 2) are less suitable for this reaction. They might interfere with the active species of the Ru-catalyzed enolester formation, as alkyne addition with amide nucleophiles is already known in literature.[238] Interestingly, DMSO and also acetonitrile (entries 6 and 7) gave no conversion at all. Acetonitrile may act in a similar fashion like 1-hexyne (**2a**) on the active ruthenium intermediate and therefore blocks the ruthenium catalysis via coordination.[194] In our reaction this ability of acetonitrile might hinder the conversion of the dipeptide **1c** to the enolester **AM-*Z*-3a**. Using DCM, acetone and sulfolane (entries 3-5) as solvents in this reaction we found quite satisfying conversions. In addition to the conversion we also determined the amount of D-Phe-OH created during the catalysis after the indicated reaction time (Table 3) in different solvents (see chapter 4.1.8). Much to our delight with the solvents THF, methyl-THF, 1,4-dioxane, methanol and ethanol the amount of D-Phe-OH was <1 %. Once again, the solvent 2-propanol stood out, for which we monitored only 0.3 % of D-Phe-OH (Table 3 entry 15). Unfortunately, good solvents regarding their ability to solubilize the dipeptide **1c**, like DCM and acetone (entries 3 and 4), formed D-Phe-OH in an inacceptable high amount during the reaction. Considering the amount of 2.0 % D-Phe-OH found when using chloroform (chapter 4.1.3.3, Table 2, entry 2), the amount of 13.2 % of epimerization using DCM as solvent is surprisingly high. From the solvent screening we got the impression that in general short reaction times are accompanied with only low amounts of epimerization with DCM as a notable exception to this rule. Therefore, with 2-propanol in hand, we envisioned a ligand screening to hopefully find ligands which would lead to even faster conversions and even lower amounts of epimerization (chapter 4.1.3.5).

4.1.3.5. Ligand screening

The solvent screening led already to a significant improvement in yield and reduced the problem of racemization. From the solvent screening we also got the impression that the faster the reaction proceeds the lower the amount of epimerization. Therefore, we wanted to screen a variety of phosphorus ligands to find a more effective catalyst-ligand system. Instead of using isolated ruthenium complexes which would be time consuming to prepare, we hoped that we could perform such a ligand screening with in situ generated Ru-ligand complexes. The feasibility of using an in situ formation of catalyst was tested in dry THF (Scheme 55 and Table 4, entry 1). Fortunately, the results were quite similar to the results obtained by using the isolated catalyst **III** (chapter 4.1.3.4, Table 3, entry 8).



Scheme 55: In situ formation of [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) in THF and 2-propanol and subsequent formation of **AM-*Z*-3a**.

Table 4: In situ formation of [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) in THF and 2-propanol and subsequent formation of **AM-*Z*-3a**.

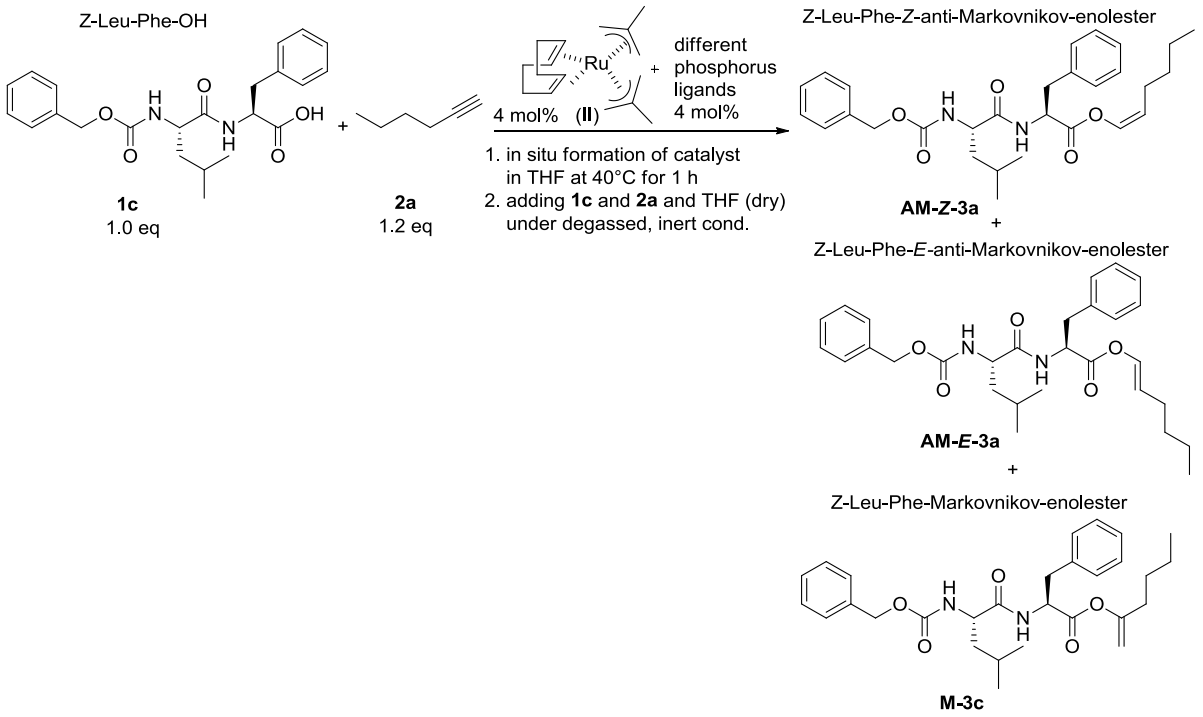
Entry	Solvent	Temp. [°C]	Conc. [mmol/mL]	Conversion[a] [%]			H-D-Phe-OH content[b] [%]
				3h	7h	20h	
1	THF	40	0.5	36	-	97	0.8
2	2-Propanol	40	0.5	85	97	-	1.2

[a] monitored by HPLC-MS (210 nm)
[b] HPLC: test for racemization: described in hydrolysis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester with 6N HCl HPLC-analysis with Chirobiotic-R control-experiment: hydrolysis of Z-Leu-Phe-OH (**1c**) under identical conditions

As a disclaimer it has to be noticed, that the in situ produced catalyst does not perfectly reproduce results, regarding conversion and racemization. In Table 4 (entry 2) the conversion towards **AM-*Z*-3a** with the in situ catalysis is slower and the amount of epimerization is higher compared to the reaction where the isolated catalyst-complex [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) is used in 2-propanol (chapter 4.1.3.4, Table 3, entry 15). The amount of racemization obtained with the in situ produced catalyst is usually higher than with the isolated catalyst-complex [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**). Nevertheless, the in situ method provided sufficiently precise data, to identify trends in the ligand screening.

For this ligand screening we used different phosphorus ligands, mainly phosphine ligands. We hoped or even expected an influence of the bite angle of the phosphorus ligand concerning the rate of the catalysis. Some indications for that were already given by Dixneuf, who had investigated dppm **21**, dppe **22**, dppp **23** and the already mentioned dppb **20** ligand.[109] He was studying the synthesis of hex-1-en-1-yl benzoates from 1-hexyne (**2a**) and benzoic acid. He found moderate to good conversion with dppe **22** (72 % after 24 h) and dppp **23** (69 % after 24 h), but dppb **20** gave the best conversion of 98 % after 2.5 h.[110]

In our ligand screening the ruthenium complex was formed in situ in THF using stoichiometric amounts of bis(2-methylpropenyl)(cycloocta-1,5-diene)ruthenium (**II**) and the different phosphorous ligands. Afterwards Z-Leu-Phe-OH (**1c**) and 1-hexyne (**2a**) were added and the conversion was monitored by HPLC-MS (Scheme 56, Figure 10). The objective of this ligand screening was to identify a more reactive ruthenium-ligand combination, as we assumed that shorter reaction times should decrease the amount of epimerisation. During the ligand-screening we observed in all cases a homogeneous solution through all steps of the reaction.



Scheme 56: Screening of different types of phosphorus ligands **20-42** in the Ru-catalyzed addition of 1-hexyne (**2a**) to Z-Leu-Phe-OH (**1c**) in THF at 40°C.

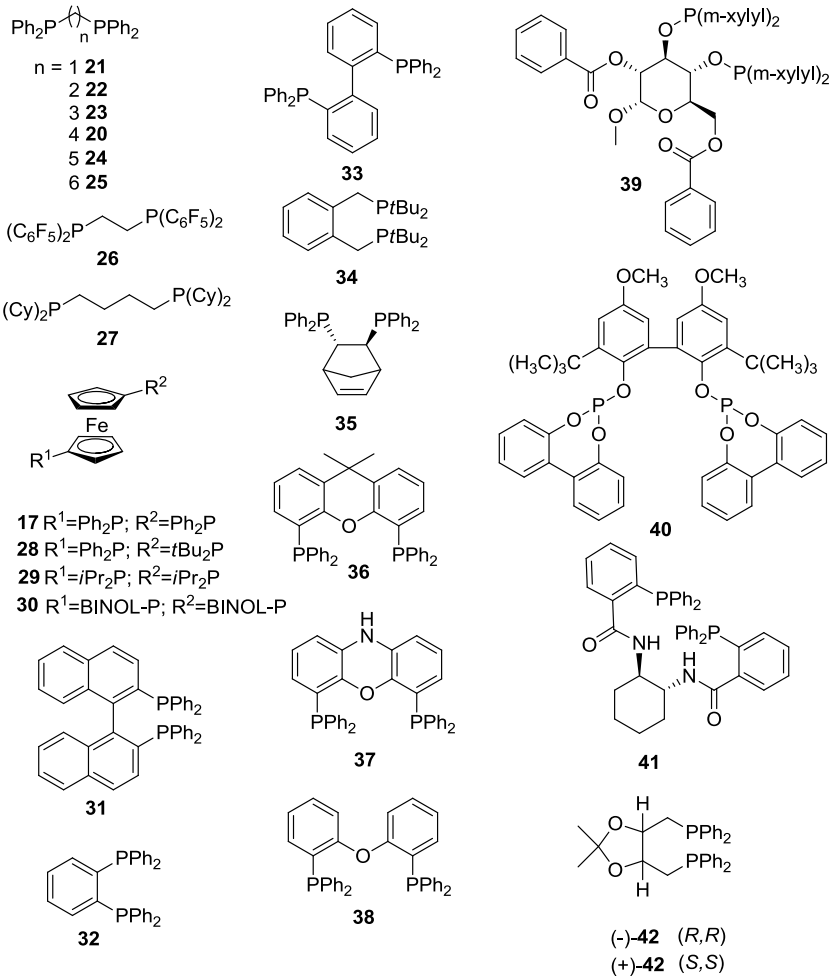


Figure 10 : Structures of phosphorus ligands **20-42** used for the Ru-catalyzed alkyne addition to Z-Leu-Phe-OH (**1c**).

Table 5: Screening of different types of phosphorus ligands **20**-**42** in the Ru-catalyzed addition of 1-hexyne (**2a**) to Z-Leu-Phe-OH (**1c**) in THF at 40°C.

Entry	Ligand[a)]	Conversion after 24h[b)] [%]	AM-Z-3a/AM-E-3a/M-3a[b)]
1	**21**	43	93/-/6
2	**22**	18	41/-/59
3	**23**	57	29/-/71
4	**20**	97	98/-/2
5	**24**	57	84/-/16
6	**25**	43	53/-/47
7	**26**	51	91/-/9
8	**27**	77	25/-/75
9	**17**	54	93/-/7
10	**28**	22	19/-/81
11	**29**	40	78/-/22
12	**30**	5	nd
13	**31**	46	97/-/3
14	**32**	15	83/-/17
15	**33**	78	99/-/1
16	**34**	18	64/-/36
17	**35**	57	98/-/2
18	**36**	10	nd
19	**37**	5	nd
20	**38**	56	99/-/1
21	**39**	17	78/-/22
22	**40**	21	75/-/25
23	**41**	8	nd
24	(-)-**42**	98	96/-/4
25	(+)-**42**	99	>99/-/<1

[a)] 4 mol% of ligand used; [b)] monitored by HPLC-MS (210 nm)

In an extensive screening of different types of ligands in which we measured the conversion after 24 h using in situ prepared Ru-complexes we recognized that indeed the bite angle of the phosphorous ligand imposes an important influence on the reaction. In the homologous series of bis(diphenylphosphino)alkane ligands **20-25** (Table 5, entries 1-3 and 5-6), dppb ligand **20** (Table 5, entry 4) showed the highest activity leading to complete conversion of substrate in 24 h, and with a high selectivity towards the *Z*-anti-Markovnikov-product **AM-*Z*-3a**. Testing ligands **26**-**41** (Table 5, entries 7-23) which possess a more rigid backbone, gave mixed results, but no improvement in comparison with dppb **20**. However, the DIOP ligand **42** (Table 5, entries 24 and 25), which can be regarded as a conformationally restricted version of dppb **20** gave superior results. Interestingly, (+)-**42** (Table 5, entry 25) showed higher activity than (-)-**42** (Table 5, entry 24) indicating a possible cooperative interaction between the chiral catalyst and the chiral substrate (89 % conversion after 3 h in THF with (+)-**42** vs. 40 % conversion after 3 h in THF with (-)-**42**). Our studies also allowed us to conclude that a strong electronic effect of the phosphorous ligands is exerted, leading to an improvement of conversion by shifting towards more electron poor phosphines (see entries 2 and 7 resp. 4 and 8, Table 5).

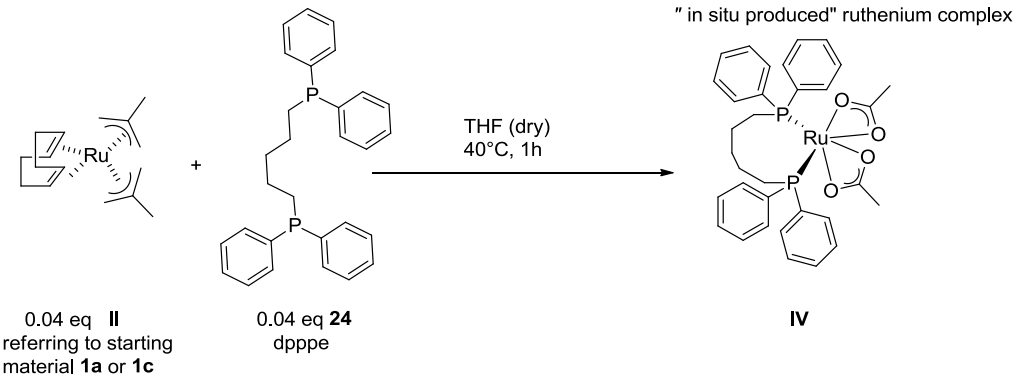
Monitoring the conversion of the reaction via HPLC-MS we observe in most cases a mixture of isomers **AM-*Z*-3a** and **M-3c**. It can be summarized that in the reactions with higher conversions also the amount of **AM-*Z*-3a** is usually higher. Especially the result obtained with ligand (+)-**42** was encouraging. Not only the conversion was nearly complete, but also the formation of **M-3c** was <1 % (Table 5, entry 25), which indicates that we have a straight-forward catalysis reaction with high regioselectivity.

4.1.3.6. Structural assignment of isomers

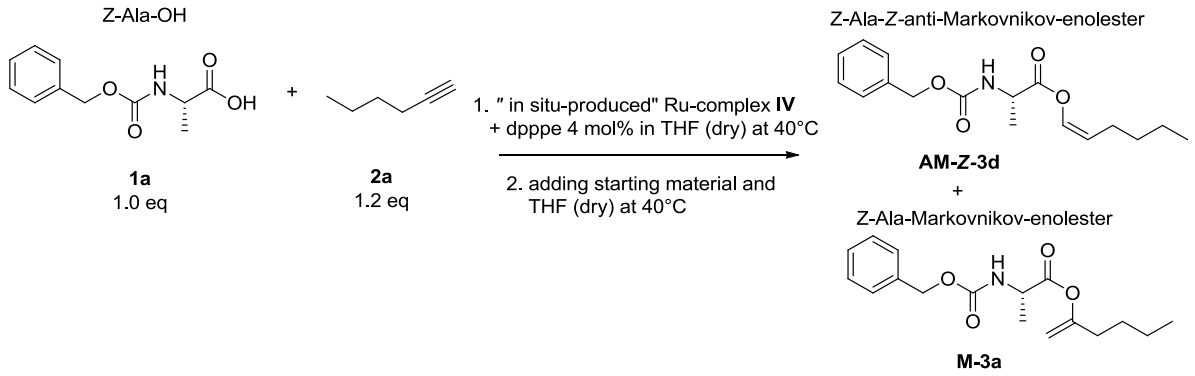
To assign the isomers occurring in the ligand screening we picked the reaction with 1,5-bis(diphenylphosphino)pentane dpppe **24** (Table 5, entry 5), as the conversion and the ratio of isomers on the HPLC-traces at 210 nm was acceptable for a good distinction of the isomers via ^{1}H- and ^{13}C-NMR.

The first step was an in situ preparation of ruthenium-complex **IV** (Scheme 57) by combining 1,5-bis(diphenylphosphino)pentane dpppe **24** and bis(2-methylpropenyl)(cycloocta-1,5-diene)ruthenium (**II**) as already reported in chapter 4.1.3.5. Ruthenium-complex **IV** converted the starting material Z-Ala-OH (**1a**) into the corresponding enolesters **AM-*Z*-3d** and **M-3a** (Scheme 58). After flash column chromatography we were able to compare our results with the literature using ^{1}H-NMR, building on a recent publication by Breit et al.[114] in which the

spectroscopic assignment of the isomers shown in Scheme 58 occurring from the formation of Z-Ala-OH (**1a**) and 1-hexyne (**2a**) has been reported.

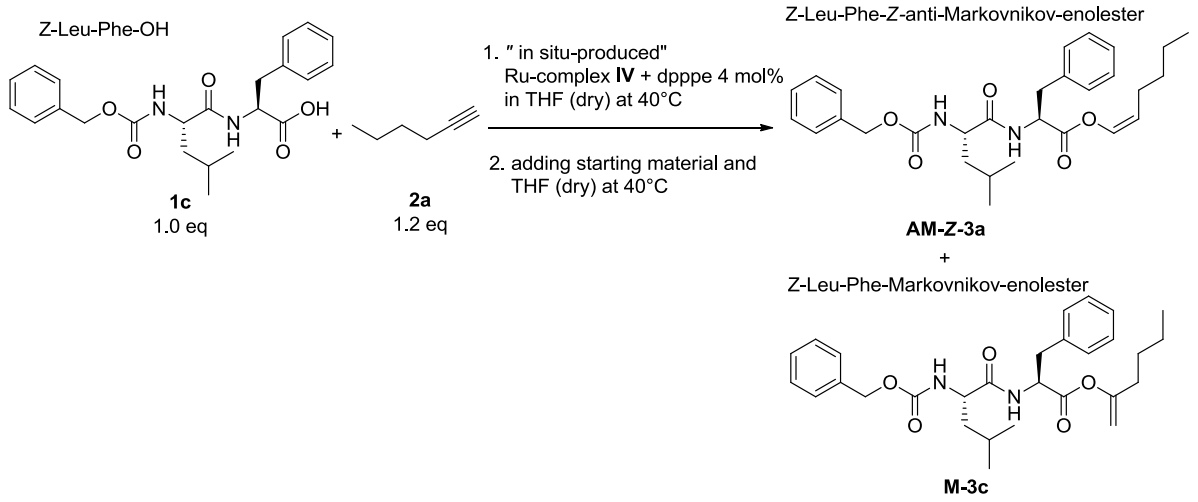


Scheme 57: In situ formation of [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dpppe)] **IV** in THF.



Scheme 58: Synthesis of Z-Ala-enolester as a mixture of isomers **AM-*Z*-3d** and **M-3a**.

Also the conversion of Z-Leu-Phe-OH (**1c**) into the corresponding enolesters by using dpppe **24** as a ligand was checked by HPLC-MS (Scheme 59). After flash column chromatography we could clearly identify the Markovnikov-product **AM-*Z*-3a** and the *Z*-anti-Markovnikov-product **M-3c** via ^{1}H-NMR and ^{13}C-NMR (Figure 11 and 12).



Scheme 59: Synthesis of Z-Leu-Phe-enolester as a mixture of isomers **AM-*Z*-3a** and **M-3c**.

Both results for Z-Ala-enolesters **AM-*Z*-3d**, **M-3a** and the Z-Leu-Phe-enolesters **AM-*Z*-3a**, **M-3c** are summarized in Table 6. We took great care not to lose any product during flash column chromatography, so checking the ratio of isomers before and afterwards by HPLC was essential.

Table 6: Results of synthesizing enolesters as a mixture of isomers using Z-Ala-OH **1a**, Z-Leu-Phe-OH (**1c**), 1-hexyne (**2a**) and the in situ formed [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dpppe)]-complex **IV** in THF.

Entry	Acids	Conc. [mol/l]	Conv. after 24h [a] [%]	Product-ratio[a] [%]		Isolated yield[%]	Amount of H-D-amino acid[b] [%]
				Z-anti-Markovnikov-Isomer	Markovnikov-Isomer		
1	**1a**	0.5	68	56 **AM-*Z*-3d**	44 **M-3a**	62	10.3
2	**1c**	0.5	57	84 **AM-*Z*-3a**	16 **M-3c**	44	9.3

[a] monitored with HPLC-MS (210 nm, Poroshell C-18)
[b] amount of H-D-amino acid: detected by GC-MS using Chirasil-L-Val column

The conversion of Z-Leu-Phe-OH (**1c**) into the corresponding enolesters **AM-*Z*-3a**, **M-3c** by using dpppe **24** as a ligand produced the enolester with a 16 % share of the Markovnikov-enolester **M-3c**. The remaining percentages were exclusively *Z*-anti-Markovnikov-enolester **AM-*Z*-3a**, whose identity was confirmed by NOESY-NMR. We could not find any evidence for the formation of *E*-anti-Markovnikov-enolester via HPLC-MS or ^{1}H NMR, neither with the reaction of Z-Leu-Phe-OH (**1c**) or the reaction of Z-Ala-OH (**1a**) into the corresponding enolesters by using dpppe **24** as a ligand.

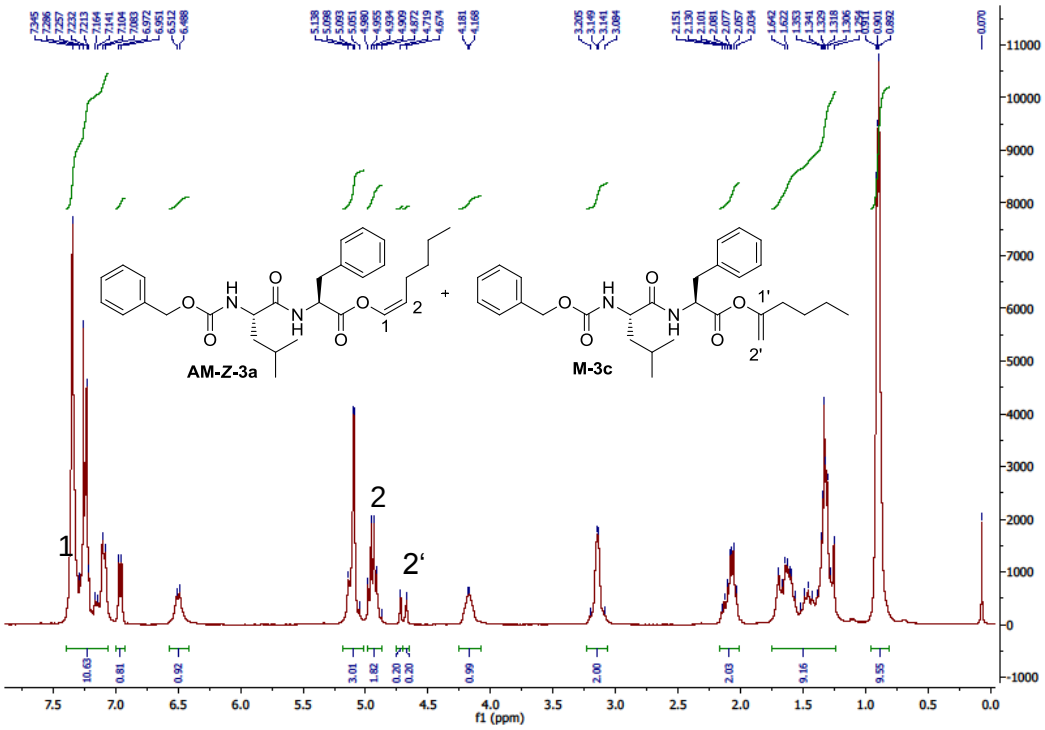


Figure 11 : ^{1}H-NMR of compound (**AM-*Z*-3a** + **M-3c**).

The 300 MHz-^{1}H-spektrum shows the two isomers **AM-*Z*-3a** and **M-3c** is the isolated mixture (Figure 11). The doublet at 6.96 ppm belongs to the proton of the *Z*-anti-Markovnikov-enolester **AM-*Z*-3a** (-CO_2-C<u>H</u>=CH-CH_2-) with a ^{3}J(H,H) = 6.3 Hz, which is characteristic for a (*Z*)-configuration of the double bond The second proton of the double bond (-CO_2-CH=C<u>H</u>-CH_2-) appears at 4.91-4.98 as a multiplet together with proton of the (-CO-NH-C<u>H</u>(CH_2-Ph)-CO_2-) stereocenter of phenylalanine. In contrast the two protons form the double bond of the Markovnikov-enolester **M-3a** appear at 4.67 and 4.72 as singlets.

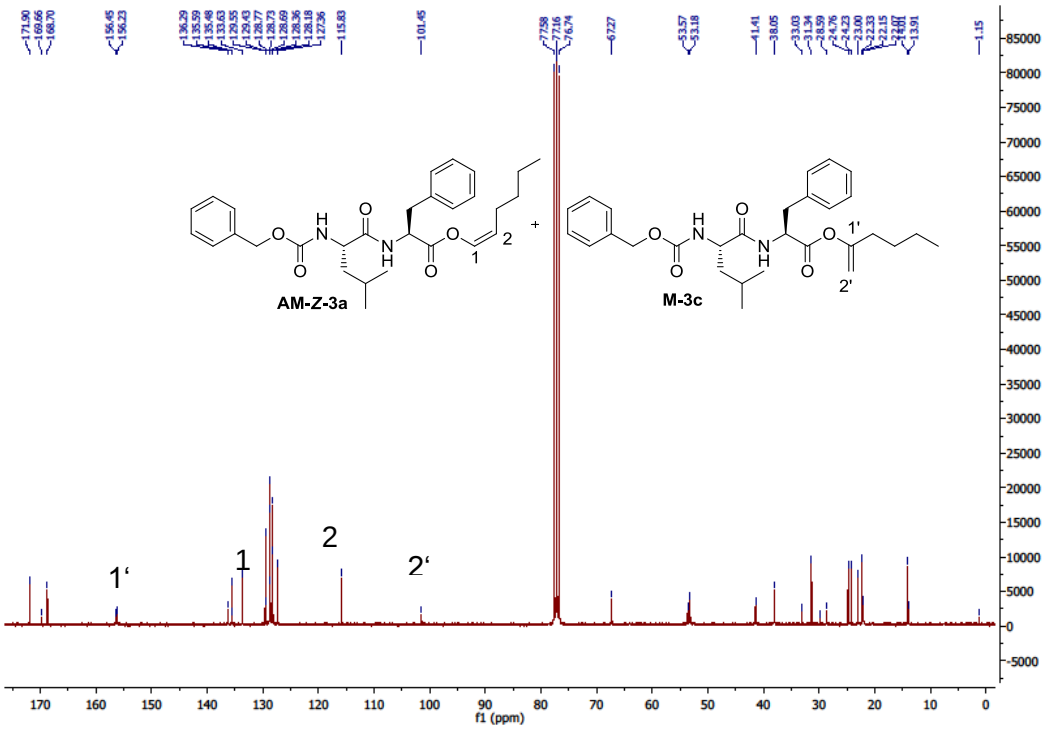


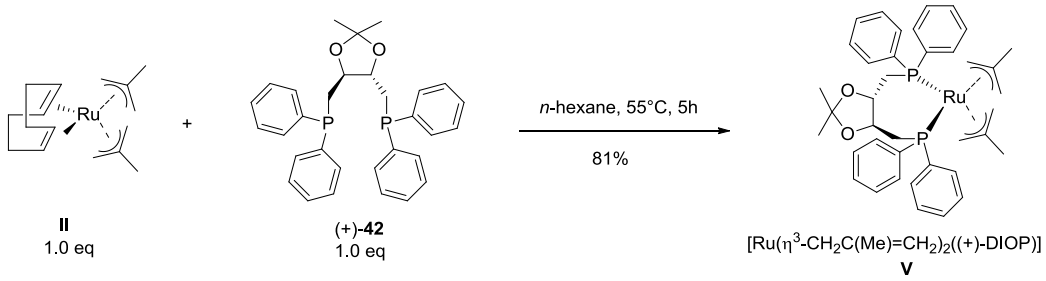
Figure 12: ^{13}C-NMR of compound (**AM-*Z*-3a** + **M-3c**).

Again the mixture of isomers **AM-*Z*-3a** and **M-3c** is depicted in the ^{13}C-NMR (Figure 12). For the *Z*-anti-Markovnikov-enolester **AM-*Z*-3a** the signals at 115.8 ppm (CO_2-CH=C<u>H</u>-CH_2-) and 133.6 ppm (CO_2-C<u>H</u>=CH-CH_2-) could be assigned to the enolester group. In comparison the characteristic signals for the double bond of the Markovnikov-enolester **M-3c** are at 101.5 ppm (CO_2-C($C_{alkylchain}$)=C<u>H</u>$_2$) and 156.5 ppm (CO_2-C<u>(</u>$C_{alkylchain}$)=CH_2).

4.1.3.7. [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)]-complex for enolester synthesis

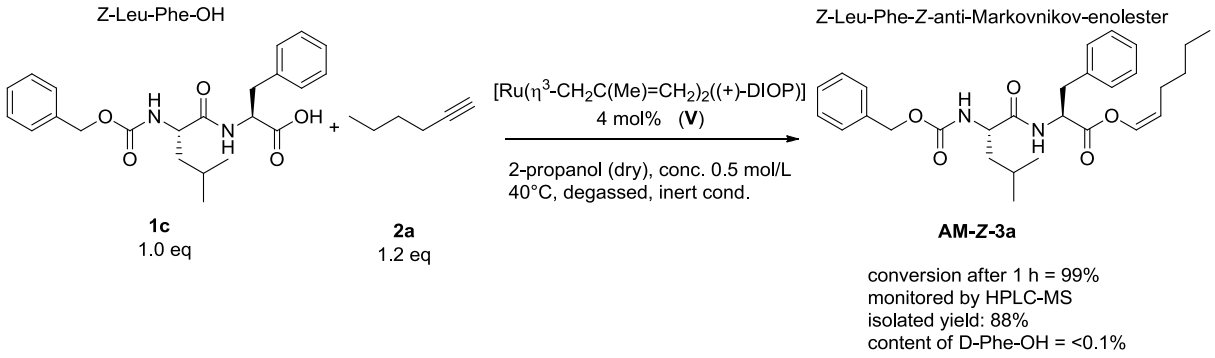
From the ligand screening in chapter 4.1.3.5 (+)-DIOP (+)-**42** emerged as the best ligand for our purposes (Table 5, entry 25).

As we wanted to have the isolated Ru-complex in hands to get reproducible conversions, we synthesised the [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) complex according to the method reported by Genet.[237] Ruthenium complex **V** was prepared by displacement of cyclooctadiene from bis(2-methylpropenyl)(cycloocta-1,5-diene)ruthenium (**II**) by (+)-2,3-O-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane ((+)-DIOP) ((+)-**42**) using *n*-hexane as solvent (Scheme 60).



Scheme 60: Synthesis of [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**).

With this catalyst in hand we managed to transform the Cbz-protected dipeptide *Z*-Leu-Phe-OH (**1c**) into the corresponding hexenyl ester **AM-*Z*-3a** with a conversion of 99 % in 1 h discovering only *Z*-anti-Markovnikov-isomer via HPLC-MS at 210 nm UV-trace (Scheme 61). No detectable epimerization could be detected within the experimental error applying the method described in chapter 4.1.8. After purification via flash column chromatography we obtained exclusively the *Z*-anti-Markovnikov-product **AM-*Z*-3a** in 88 % yield. The formation of *Z*-anti-Markovnikov-enolester was confirmed by NOESY NMR.

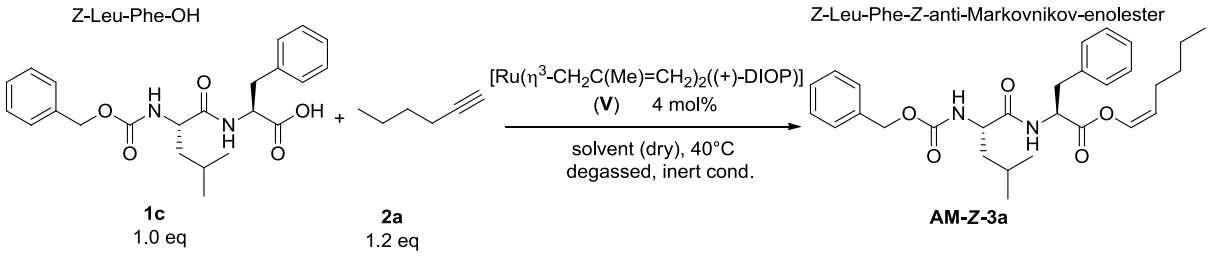


Scheme 61: Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** using ruthenium-complex **V**.

With the isolated catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) we pursued a second solvent screening.

4.1.3.8. Solvent screening using [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)]-complex

Due to the sometimes problematic solubility of peptides we tested several alternative solvents and solvent mixtures using Z-Leu-Phe-OH (**1c**) as substrate to identify alternatives for 2-propanol (Scheme 62). Basically, alcohols and chloroform are good solvents for the alkyne addition reaction.



Scheme 62: Solvent screening for the Ru-catalysed 1-hexyne addition using [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**).

In comparison with these mentioned solvents, especially reactions in dichloromethane (DCM) resulted in a high amount of 1.5 % of D-Phe-OH (Table 7, entry 6). The addition of 10 % of 2-propanol to DCM accelerated catalysis and also reduced the amount of D-Phe-OH formed (Table 7, entry 7). Comparing the results obtained with ruthenium-complex [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) with the result obtained using [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) it can be concluded that the conversion is in all cases faster and also the amount of epimerization is always lower if catalyst **V** is applied. For an example the use of [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) in chloroform leads to full conversion in 3 h and the amount of D-Phe-OH is only 0.2 % (Table 7, entry 5), compared to the previous used complex [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) with a conversion of 99 % after 20 h and 2.0 % D-Phe-OH (chapter 4.1.3.3, Table 2 , entry 2).

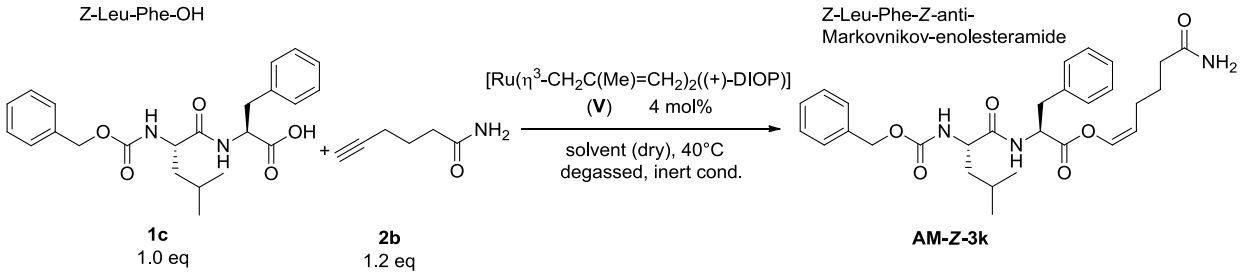
Table 7: Solvent screening for the Ru-catalysed 1-hexyne addition using [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**).

Entry	Solvent	Conc. [mol/l]	Conversion[a)] [%]				Isolated Yield[%]	Amount of H-D-Phe-OH[b)] [%]
			1h	3h	6h	24h		
1	Methanol	0.25	26	51	-	95[c)]	85	0
2	Ethanol	0.5	73	93	98	-	86	0
3	1-Propanol	0.5	70	91	98	-	87	0.1
4	MTBE+20 %DMF	0.5	9	42	-	99	90	0.3
5	Chloroform	0.5	66	98	-	-	87	0.2
6	DCM	0.5	39	73	99[d)]	-	88	1.5
7	DCM+10 % 2-propanol	0.5	72	98	-	-	89	0.7

[a)] monitored by HPLC-MS (210 nm)
[b)] analysis of racemization after hydrolysis with 6N HCl; HPLC-analysis with Chirobiotic-R; comparison with results obtained after hydrolysis of Z-Leu-Phe-OH (**1c**) under identical conditions
[c)] after 22h
[d)] contains 3 % Markovnikov-enolester **M-3c** monitored by HPLC-MS at 210 nm; 8 h reaction time

With MTBE containing 20 % of DMF in the Ru-catalyzed enolester formation we obtained nearly full conversion after 24 h (Table 7, entry 4). This a good result considering the quite low conversion of 39 % after 48 h found when only DMF was used with catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) (chapter 4.1.3.4, Table 3, entry 2). However, it needs to be mentioned that the reaction rate using MTBE containing 20 % of DMF is rather slow, because DMF might interfere with the active species of the Ru-catalyzed enolester formation as already illustrated in chapter 4.1.3.4.

To be able to screen even more polar solvents instead of 1-hexyne (**2a**) we had to use a more polar alkyne like hex-5-ynamide (**2b**). Due to its nonpolar nature 1-hexyne (**2a**) is not soluble in very polar solvents.



Scheme 63: Ruthenium-catalysed hex-5-ynamide (**2b**) addition to Z-Leu-Phe-OH (**1c**) using highly polar solvents.

In the case of propylene carbonate, diethylene glycol, 1,2-propandiol and ethylene glycol the procedure was the same as mentioned above, with the change that hex-5-yneamide (**2b**) was added as a solid (Scheme 63). The use of hex-5-yneamide (**2b**) for Ru-catalyzed alkyne addition will be further discussed in chapter 4.1.4.3.

Table 8: Solvent screening for the Ru-catalysed hex-5-ynamide (**2b**) addition using highly polar solvents.

Entry	Solvent	Temp. [°C]	Conc. [mol/l]	Conversion[a)] [%]			Isolated yield	Amount of H-D-Phe-OH
				1h	3h	24h		
1	propylene carbonate	40	0.5	85	98	-	not isolated	n.d.
2	diethylene glycol	40	0.5	47	76	94	not isolated	n.d.
3	1,2-propandiol	40	0.5[a),b)]	0.6	1.4	11	not isolated	n.d.
4	ethylene glycol	40	0.167[a),b)]	0.8	1.1	6	not isolated	n.d.

[a)] monitored by HPLC-MS (210 nm)
[b)] [Ru(η3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) not soluble!!!

In fact the catalysis does not work efficiently in highly polar solvents such as 1,2-propandiol and ethylene glycol (Table 8, entries 3 and 4), very likely because the catalyst **V** is not sufficiently soluble under these conditions. For propylene carbonate it can be said that the conversion of starting material **1a** towards product **AM-*Z*-3k** is with 98 % quite fast (Table 8, entry 1). Even in diethylene glycol the conversion is quite reasonable with 94 % after 24 h

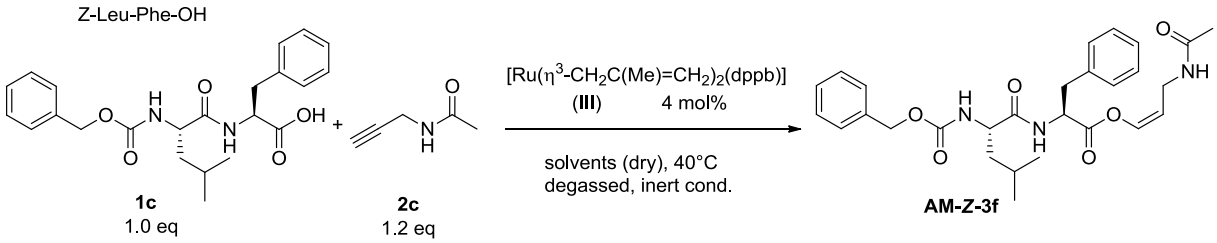
(Table 8, entry 2). In summary the described Ru-catalyzed enolester formation proceeds even in highly polar solvents as long as the catalyst **V** is still soluble. If one would add polyethylene glycol polymer chains on the isopropylidene moiety of the (+)-DIOP-ligand (+)-**42**, it would be possible to increase the solubility of the ligand. It is highly likely that this would provide the necessary solubility of this new ruthenium complex in solvents like 1,2-propandiol and ethylene glycol.

4.1.4. Amide bond containing leaving groups

4.1.4.1. Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester containing an amide bond in the leaving group

Our effort in enolester formation has focused so far on the synthesis of 1-hexenyl-enolesters such as **AM-*Z*-3a**. The application of enolester **AM-*Z*-3a** in the enzyme catalyzed peptide coupling described in chapter 4.2.2.1 showed good acceptance of the substrate by alcalase-CLEA, but full conversion could not be reached. Therefore, as a new objective we wanted to develop a new type of enolester that embodies an amide motif as an advantageous feature. We speculated that this amide motif will lead to better recognition of the substrate by alcalase-CLEA, our enzyme used in the peptide coupling reaction. This idea was based on the fact, that alcalase is a protease and therefore naturally cleaves peptide bonds in the presence of water. The ability of recognizing an amide bond containing enolester should in principle be better than the recognition of a C-6-alkyl chain containing enolester. Therefore, we synthesized *N*-(2-propynyl)acetamide (**2c**) by acylation of propargylamine with acetic anhydride in triethylamine and DMAP.[239]

At this stage of the work on the PhD thesis we had not yet discovered the superior DIOP-complex. Therefore, we used for the first test reaction [Ru(η^3-$CH_2C(Me)=CH_2)_2$(dppb)] (**III**) under the same conditions as already mentioned in chapter 4.1.3.4.



Scheme 64: Synthesis of enolester **AM-*Z*-3f** containing an amide motif.

THF, methyl-THF and 2-propanol were used as solvents in the Ru-catalyzed enolester formation. Reacting Z-Leu-Phe-OH (**1c**) with *N*-(2-propynyl)acetamide (**2c**), we could monitor an excellent conversion towards the product **AM-*Z*-3f** (Scheme 64). The conversion is a little bit slower than with 1-hexyne (**1a**). We found that after 15-30 min a precipitate formed in methyl-THF and 2-propanol, which is probably due to the low solubility of the desired product in these solvents (Table 9, entries 1 and 3). However, these encouraging results shown in Table 9 aroused our interest to synthesize more enolesters containing a variety of amide bond containing motifs.

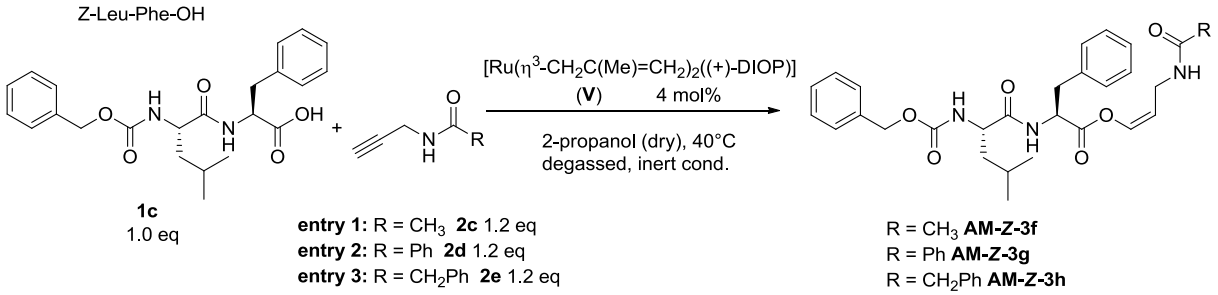
Table 9: Results of the synthesis of enolester **AM-*Z*-3f** containing an amide motif.

Entry	Solvent	Conc. [mol/l]	Conversion[a] [%]			Isolated yield	Amount of H-D-Phe-OH[b]
			3h	18h	24h		
1	methyl-THF	0.5	84	-	97.5 [c]	84	0.6
2	THF	0.5	78	96	-	-	-
3	2-propanol	0.5	90	97[c]	-	-	-

[a] monitored by HPLC-MS (210 nm)
[b] HPLC: test for racemisation: hydrolysis of Z-Leu-Phe-*Z*-anti-Markovnikov-Enolester with 6N HCl HPLC-analysis with Chirobiotic-R control-experiment: hydrolysis of Z-Leu-Phe-OH under identical conditions
[c] product not soluble in methyl-THF, 2-propanol at 40°C, but soluble at reflux -> samples for HPLC were taken after re-dissolving the material at higher temperatures

4.1.4.2. Synthesis of enolesters containing different internal amide motives as the leaving group

The application of **AM-*Z*-3f** in the enzyme catalyzed peptide coupling reaction with alcalase-CLEA described in chapter 4.2.3.1, showed that indeed leaving groups containing an amide bond are better recognized than those without an amide motif and converted faster to the envisaged products. In order to explore the ideal size of this amide motif in the side chain we synthesized besides *N*-(2-propynyl)acetamide (**2c**) two additional amide containing leaving groups (Table 10, entries 2 and 3).



Scheme 65: Ru-catalyzed addition of amide containing alkynes to Z-Leu-Phe-OH (**1c**).

This time we used [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) as catalyst in the enolester formation of Z-Leu-Phe-OH (**1c**) under the same conditions as already mentioned in chapter 4.1.3.4 (Scheme 65). 2-Propanol was used as the solvent because of its outstanding performance in the solvent screening (Chapter 4.1.3.4).

Table 10: Ru-catalyzed addition of amide containing alkynes to Z-Leu-Phe-OH (**1c**) in 2-propanol at 40°C.

Entry	Conc. [mol/l]	Conversion[a] [%]		Isolated yield[%]	Amount of H-D-Phe-OH[b] [%]
		1h[c]	**3h**		
1	0.25	96[d] (ratio:86 % **AM-*Z*-3f**[e] / 14 % **43**)	98[d] (ratio:86 % **AM-*Z*-3f**[e] / 14 % **43**)	75	0.1
2	0.25	90 %[d] (ratio:80 % **AM-*Z*-3g**[e] / 20 % **44**)	94 %[d] (ratio:81 % **AM-*Z*-3g**[e] / 19 % **44**)	42	0.2
3	0.25	90 %[d] (ratio:79 % **AM-*Z*-3h**[e] / 21 % **45**)	94 %[d] (ratio:80 % **AM-*Z*-3h**[e] / 20 % **45**)	44	<0.1

[a] monitored by HPLC-MS (210 nm)
[b] analysis of racemization after hydrolysis with 6N HCl; HPLC-analysis with Chirobiotic-R; comparison with results obtained after hydrolysis of Z-Leu-Phe-OH under identical conditions
[c] reaction run 30 min at a concentration of 0.5 mmol/mL but was then diluted to 0.25 mmol/mL to dissolve all material. Heating for a few seconds to reflux temperature dissolved all material but when reaching 40°C again some material became insoluble
[d] product not soluble in 2-propanol at 40°C but soluble at reflux temperature; samples for HPLC were taken after re-dissolving the material at higher temperatures and analyzed on a Purospher Star Rp-18e column
[e] up to 2 % Markovnikov-product monitored via HPLC-MS at 210 nm; not characterized

The conversion towards the enolester products **AM-*Z*-3f**, **AM-*Z*-3g**, **AM-*Z*-3h** is very good and the amount of D-Phe-OH is negligible. Besides that, in all reactions listed in Table 10 we observed a considerable amount of oligomers, which resulted from a subsequent alkyne addition with the amide bond in the side chain of the desired reaction product using catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**)[238] (see byproducts **43**, **44**, **45** determined only by MS in Figure 13). In entry 3 we found a ratio of 4:1 of the desired compound **AM-*Z*-3h** and probably isomeric bis-coupling products **45**. Although we have not used them in experiments, it is likely that these bis-coupling products might be still good substrates for the enzymatic peptide coupling reaction. Again during the reaction we found that after 15-30 min a precipitate formed in 2-propanol, which is probably due to the low solubility of the desired products **AM-*Z*-3f**, **AM-*Z*-3g**, **AM-*Z*-3h** in 2-propanol.

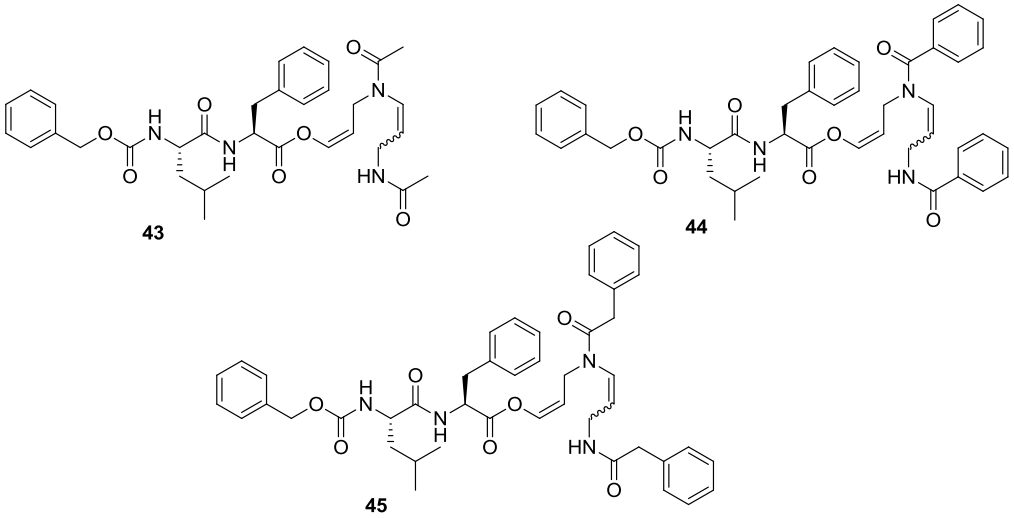
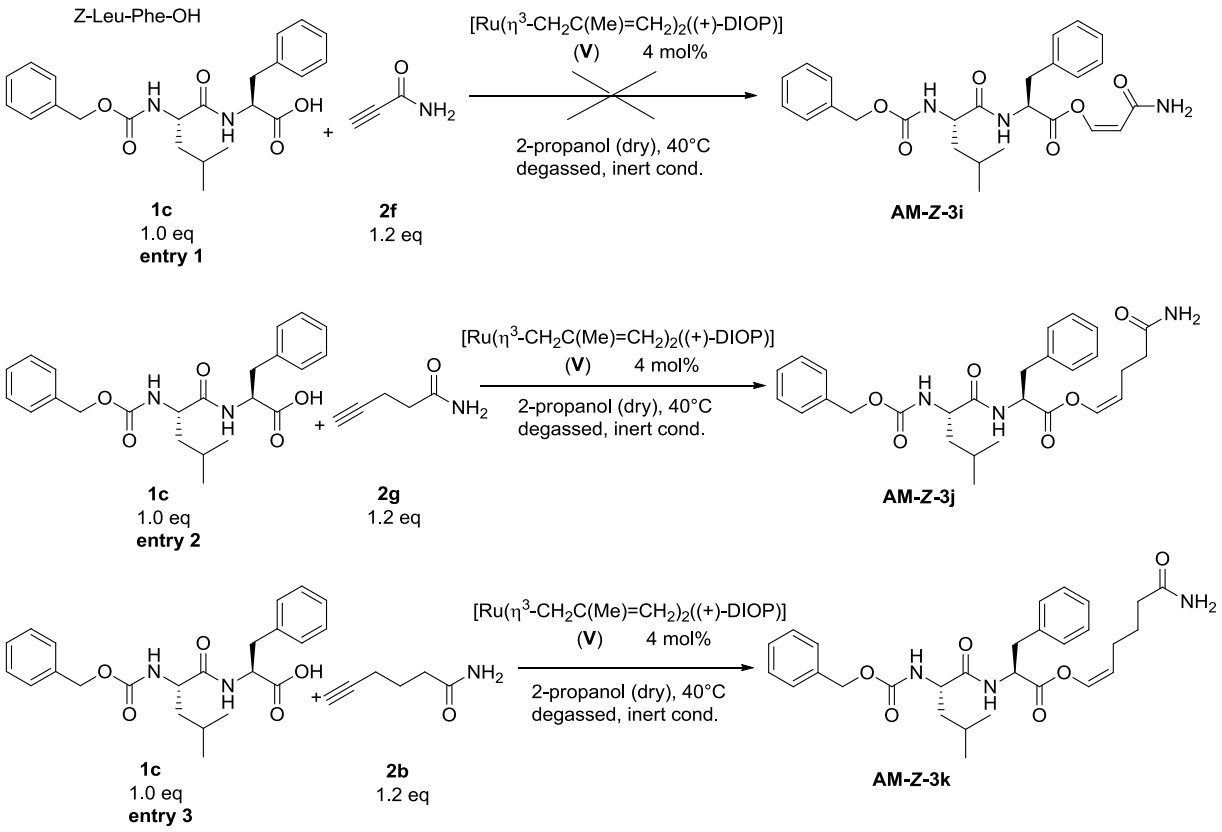


Figure 13: Putative side-products of the Ru-catalyzed addition of amide containing alkynes to Z-Leu-Phe-OH (**1c**).

4.1.4.3. Synthesis of enolesters containing different terminal amide motives as the leaving group

As the Z-Leu-Phe-OCam-ester (**52**) showed good conversion as shown in chapter 4.2.2.1, we planned to synthesize enolesters bearing terminal amide bonds. Therefore we synthesized hex-5-ynamide (**2b**), pent-4-yneamide (**2g**)[240] and propiolamide (**2f**)[241] according to literature procedures. Our hope was that enolesters containing a terminal amide bond in the leaving group, would be recognized by our chosen enzyme as well as the same peptide containing a Cam-ester moiety. Once again we used Z-Leu-Phe-OH (**1c**) as starting material for the ruthenium catalysed alkyne addition (Scheme 66).



Scheme 66: Ru-catalyzed addition of terminal amide containing alkynes to Z-Leu-Phe-OH (**1c**).

The enolester formation (Table 11, entry 1-3) was performed under the same conditions as already mentioned in chapter 4.1.3.4 with the exception that ruthenium complex [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) was used as catalyst.

Concerning the reaction of hex-5-ynamide (**2b**) and pent-4-yneamide (**2g**) with Z-Leu-Phe-OH (**1c**) into the corresponding Z-Leu-Phe-O(*Z*)-6-amino-6-oxohex-1-enyl **AM-*Z*-3k** and Z-Leu-Phe-O(*Z*)-5-amino-5-oxopent-1-enyl **AM-*Z*-3j** enolesters it is clear that the reaction is almost as efficient as with 1-hexyne (**2a**).

Table 11: Ru-catalyzed addition of terminal amide containing alkynes to Z-Leu-Phe-OH (**1c**) in 2-propanol at 40°C and a peptide concentration of 0.5 mol/l.

Entry	Conversion[a] [%]		Isolated yield[%]	Amount of H-D-Phe-OH[b] [%]
	1h[c]	**2h**		
1	0	0	not isolated	n.d.
2	97 (ratio: 98 % **AM-*Z*-3j** / 2 % **46**)	97 (ratio: 98 % **AM-*Z*-3j** / 2 % **46**)	82	<0.1
3	97 (ratio: 98 % **AM-*Z*-3k**[d] / 2 % **47**)	98 (ratio: 98 % **AM-*Z*-3k**[d] / 2 % **47**)	80	<0.1

[a] monitored by HPLC-MS (210 nm)
[b] analysis of racemization after hydrolysis with 6N HCl; HPLC-analysis with Chirobiotic-R; comparison with results obtained after hydrolysis of *Z*-Leu-Phe-OH under identical conditions
[c] after heating for 3 seconds to reflux all material was dissolved and remained soluble at 40°C
[d] up to 2 % Markovnikov-product monitored via HPLC-MS at 210nm; not characterized

As already mentioned in chapter 4.1.4.2 again the reaction with ruthenium complex [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) and terminal amide containing alkynes produced byproducts **46** and **47** but only in low amounts. These oligomers result from a second alkyne addition with the excess of alkyne amides **2b** and **2g** (see bis-coupling products **46**, **47** determined by MS in Figure 14) applied. Fortunately, with a ratio of just 2 % each compared to the desired *Z*-anti-Markovnikov-product **AM-*Z*-3k** and **AM-*Z*-3j** these byproducts are almost negligible. Concerning conversion and side-product formation the use of alkynes containing terminal amide motives in the Ru-catalyzed enolester formation is superior to the use of internal amide motives containing alkynes mentioned in chapter 4.1.4.2. A reasonable explanation would be that in the case of **AM-*Z*-3j** and **AM-*Z*-3k** during the formation of the postulated vinylidene species the amide bond is not so close to the catalytic center of the ruthenium. However, these bis-coupling products might be still good substrates for the enzymatic peptide coupling reaction, which would make its separation obsolete.

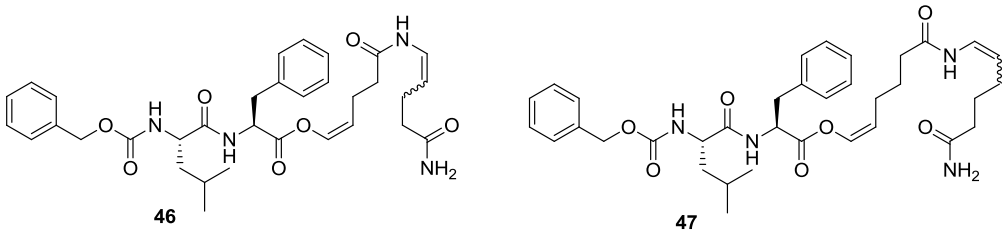


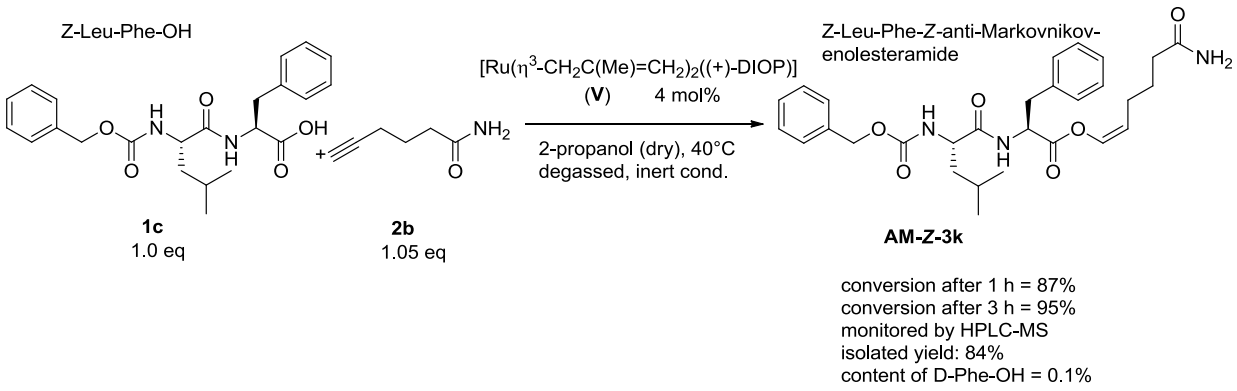
Figure 14: Putative by-products of the Ru-catalyzed addition of terminal amide containing alkynes to Z-Leu-Phe-OH (**1c**) as assigned by MS.

It is very important to mention that not only the conversions towards **AM-*Z*-3j** and **AM-*Z*-3k** are higher but also the isolated yields are higher compared to the enolesters **AM-*Z*-3f**, **AM-*Z*-3g**, **AM-*Z*-3h** formed from addition of alkynes containing an internal amide bond like **2c**, **2d**, **2e**. Another attractive feature of this formation is that no racemization, within the experimental error, occurred (analyzed as described in chapter 4.1.8).

Unfortunately, but not surprisingly we could not obtain any enolester formation towards the desired product **AM-*Z*-3i** using propiolamide (**2f**) as alkyne source, which is due to the different electronic and steric properties of propiolamide (**2f**).

Due to the similar R_f-values of hex-5-yneamide (**2b**) and product Z-Leu-Phe-O(*Z*)-6-amino-6-oxohex-1-enyl enolester **AM-*Z*-3k**, the compounds are difficult to separate by flash column chromatography. Therefore, the reaction was reproduced with only 1.05 equivalents of hex-5-ynamide (**2b**) to minimize this problem (Scheme 67).

After 3 h the reaction showed 95 % conversion (monitored by HPLC-MS), which is similar to the reaction with 1.2 equivalents of hex-5-ynamide (**2b**) as reported in Table 11 (entry 3). The reaction led to a content of only 0.1 % D-Phe-OH. Importantly, the amount of side-product **46** is reduced to only 0.5 % as monitored by HPLC-MS.



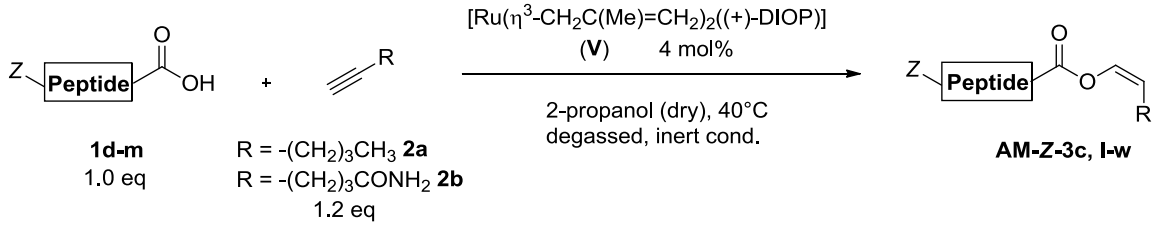
Scheme 67: Ru-catalyzed addition of 1.05 eq of terminal amide containing alkyne **2b** to Z-Leu-Phe-OH (**1c**) with a peptide concentration of 0.5 mol/L.

4.1.5. Expanding the scope of enolester formation to various peptides

The next step was to extend the Ru-catalyzed enolester formation developed for Z-Leu-Phe-OH (**1c**) to other peptides using [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) as catalyst. In all reactions we took great care, that all of the starting material was dissolved for an efficient

transformation towards the enolesters. The reactions were performed at a concentration of 0.5 mol/L of peptide in 2-propanol. For many peptides it was necessary to heat the substrate in the solvent to reflux for a few seconds to obtain a clear solution. As a consequence if the concentration of the reaction is reduced this will lead to a lower reaction rate in the catalysis reaction.

With (+)-DIOP (+)-**42** as the best ligand and 2-propanol as the best solvent we had conditions at hand with which we set out to explore the scope of this reaction for different peptide substrates **1d-k** and alkyne partners **2a** and **2b** (Scheme 68, Table 12). All reactions were performed as mentioned in chapter 4.1.3.7 using 1-hexyne and in chapter 4.1.4.3 using alkyne amides.



Scheme 68: *Z*-anti-Markovnikov Ru-catalyzed addition of alkynes **2a** and **2b** to peptide fragments **1d-k**.

We were pleased to see that our method allowed the quantitative conversion of a great variety of peptide substrates and also functionalized alkynes. Even the tripeptide Z-Phe-Leu-Ala-OH (**1f**) was converted excellently towards the enolesters **AM-*Z*-3o** and **AM-*Z*-3p** (entries 5-6). Notably, the method tolerates unprotected serine and tyrosine in substrates (entries 7-8). Interestingly, the conversion of Boc-Phe-Tyr-OH (**1g**) into enolester **AM-*Z*-3q** is much faster than the conversion of Z-Ile-Ser-OH (**1h**) into enolester **AM-*Z*-3r**. One simple explanation is, that in the case of Z-Ile-Ser-OH (**1h**), ethanol was used instead of 2-propanol due to better solubility in ethanol. As we know from the solvent screening (Chapter 4.1.3.4), catalysis in 2-propanol occurs 6 times faster than in ethanol. During the transformation of Z-Ile-Ser-OH (**1h**) to its corresponding enolester **AM-*Z*-3r**, we detected formation of 1.5 % of ethyl ester via HPLC-MS. This was the only time that we observed such an ethyl ester formation.

Fortunately, no or negligible amounts of racemization were observed in the Ru-catalyzed alkyne addition using [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) as catalyst.

The *S*-containing amino acid methionine in dipeptide Z-Phe-Met-OH (**1k**) was also tolerated, although for complete conversion towards enolester **AM-*Z*-3u** we had to add an additional amount of catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) (entry 11).

Without a second amount of catalyst **V** the catalysis stops after ~5 h at a conversion of ~ 65 %. It can be speculated, that during catalysis a "poisoning effect" of the ruthenium, due to the thioether motif in the amino acid methionine, might occur.

The amount of racemization in the case of methionine is with 0.7 % slightly higher, compared to the other amino acids. This apparent poisoning effect by sulfur could result in a modification of the ruthenium-complex, which may cause more epimerization.

Table 12: Racemization-free Ru-catalyzed synthesis of various peptide enolesters **AM-*Z*-3** using [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) at 40°C and 2-propanol as solvent.

Entry	Peptide	Alkyne	Time [h]	Conversion [%]	Yield [%]	D-AA [%][a)]
1	Z-Leu-Ala-OH **1d**	**2a**	1	99	92 **AM-*Z*-3c**	0.1
2	Z-Leu-Ala-OH **1d**	**2b**	1	99[b)]	89 **AM-*Z*-3l**	0.2
3	Z-Phe-Leu-OH **1e**	**2a**	1	99	93 **AM-*Z*-3m**	0.3
4	Z-Phe-Leu-OH **1e**	**2b**	1	98[b)]	92 **AM-*Z*-3n**	0.1
5	Z-Phe-Leu-Ala-OH **1f**	**2a**	1	99	87 **AM-*Z*-3o**	0.1
6	Z-Phe-Leu-Ala-OH **1f**	**2b**	1	97 [b)]	86 **AM-*Z*-3p**	0.1
7	Boc-Phe-Tyr-OH **1g**	**2a**	3	99	86 **AM-*Z*-3q**	0.2
8	Z-Ile-Ser-OH **1h**	**2a**	24	97[c)]	83 **AM-*Z*-3r**	0.1
9	Z-Phe-Pro-OH **1i**	**2b**	5	97 [b)]	78 **AM-*Z*-3s**	0.3
10	Z-Phe-Val-OH **1j**	**2b**	2	98 [b)]	83 **AM-*Z*-3t**	0.2
11	Z-Phe-Met-OH **1k**	**2a**	7	97[d)]	87 **AM-*Z*-3u**	0.7
12	Z-Ala-Pro-Leu-OH **1l**	**2a**	24	2	n.d. **AM-*Z*-3v**	n.d.
13	Z-Leu-Gly-Gly-Phe-OH**1m**	**2a**	24	64[d)]	n.d. **AM-*Z*-3w**	n.d.

[a)] Amount of *C*-terminal racemizsation measured after the indicated reaction time and hydrolysis with 6N HCl.
[b)] Bis-addition products up to 2 % relative to product and starting material were detected.[238]
[c)] Ethanol used as solvent, 1.5 % ethyl ester detected.
[d)] Addition of 4 mol% of catalyst after 3 h.

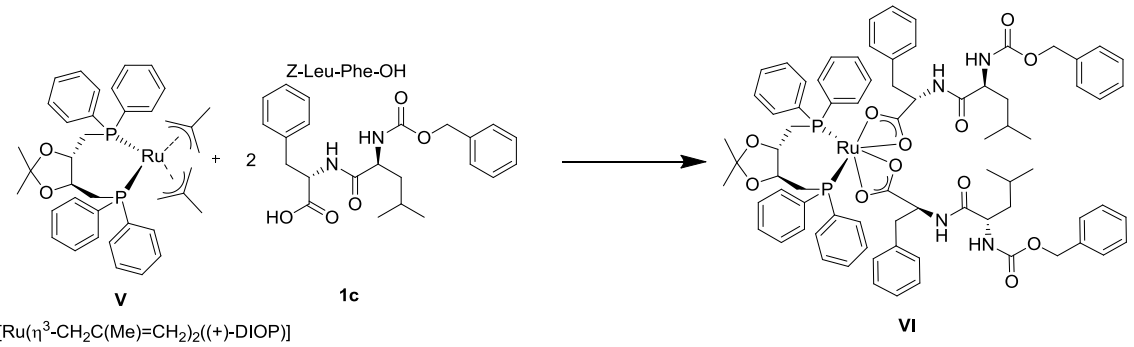
The reaction with Z-Phe-Pro-OH (**1i**) delivered the desired product **AM-*Z*-3s** in satisfactory yield and purity (entry 9). It needed a longer reaction time than the other enolesteramide synthesis. It is believed that this behavior is caused by the steric properties of the molecule. Proline contains a cyclic secondary amine which lowers the degree of freedom. Compared to other aliphatic amino acids, the conformation of proline is quite rigid and it leads to a bend in the peptide chain. Considering this it seems that the reaction rate is reduced because the active ruthenium-center is less accessible.

Having successfully established the enolester synthesis for dipeptides, we converted the tripeptide Z-Ala-Pro-Leu-OH (**1l**) into its enolester **AM-*Z*-3v** (entry 12). However, after 24 h the UV- trace at 210 nm showed only a conversion of 1.9 % enolester **AM-*Z*-3v**. One possible reason for this finding is the presence of proline in the tripeptide. If proline is in the middle of the tripeptide **1l** sequence the consequence for the rate of the catalysis seems to be more dramatic. Another reason may be the use of the solvent mixture 60 % TFE and 40 % 2-propanol to dissolve the starting material. The influence of TFE as a solvent in the Ru-catalyzed enolester formation is described in chapter 4.1.6.3.

Also the tetra-peptide Z-Leu-Gly-Gly-Phe-OH (**1m**) was successfully employed as a substrate in enolester formation using catalyst [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**), although for 64 % conversion to enolester **AM-*Z*-3w** we had to add a second amount of catalyst. It appears that the size of the peptide cannot be neglected. To obtain a more detailed view on this fact one should try to convert more and larger peptides into their enolesters.

4.1.6. Synthesis of [Ru(TFA)$_2$(dppb)] and [Ru(TFA)$_2$((+)-DIOP)]

Due to the fact that *Z*-Leu-Phe-OH (**1c**) acts not only as a substrate, but also as a ligand in the proposed ruthenium-complex **IV** during catalysis with 4 mol% of ruthenium complex **III** and **V** (a structure proposal with two peptide fragment coordinating to the ruthenium is shown in Scheme 69), apparently around 8 % of the substrate might possibly remain coordinated to the ruthenium and therefore remains not converted. This proposal is supported by work from Dixneuf et al.[110] Using ruthenium complex **III** as catalyst precursor they studied the addition of benzoic acid to 1-hexyne. After complete conversion of the starting materials and formation of hex-1-en-1-yl benzoate, they were able to recover a ruthenium complex which contained benzoic acid as ligand coordinated to the ruthenium center at the end of the reaction. It is assumed that this is the actual catalyst precursor or the catalyst itself. This finding is illustrated in the case of Z-Leu-Phe-OH (**1c**) which adds to the ruthenium complex **V** (Scheme 69).



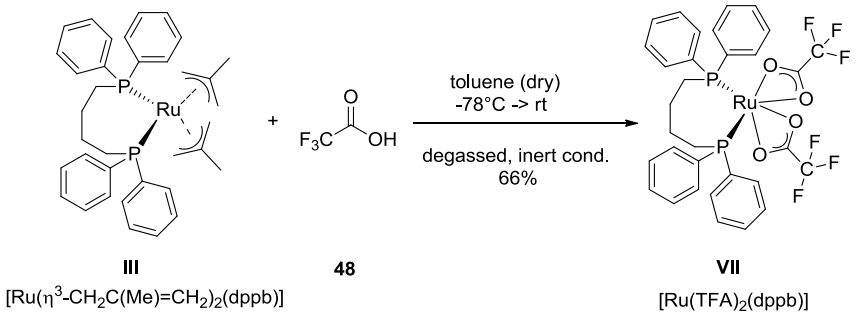
Scheme 69: Coordination of Z-Leu-Phe-OH **1c** to the ruthenium complex **V**.

To overcome the problem of losing starting material, other such as small, inexpensive acids are needed, which than would act as ligands for the ruthenium. We reasoned that no ligand exchange between trifluoroacetic acid and amino acid or Z-protected peptides will occur.[110] Dixneuf however performed a reaction with 1 equiv. of $[Ru(TFA)_2(dppb)]$ (**VII**) (Scheme 70) and 2 equiv. benzoic acid. They used phenylacetylene as alkyne and obtained exclusively (*Z*)-styryl benzoate without a trace of styryl trifluoroacetate. They even could recover the unchanged complex $[Ru(TFA)_2(dppb)]$ (**VII**), as no trifluoroacetate was exchanged by benzoic acid.

4.1.6.1. Synthesis of $[Ru(TFA)_2(dppb)]$

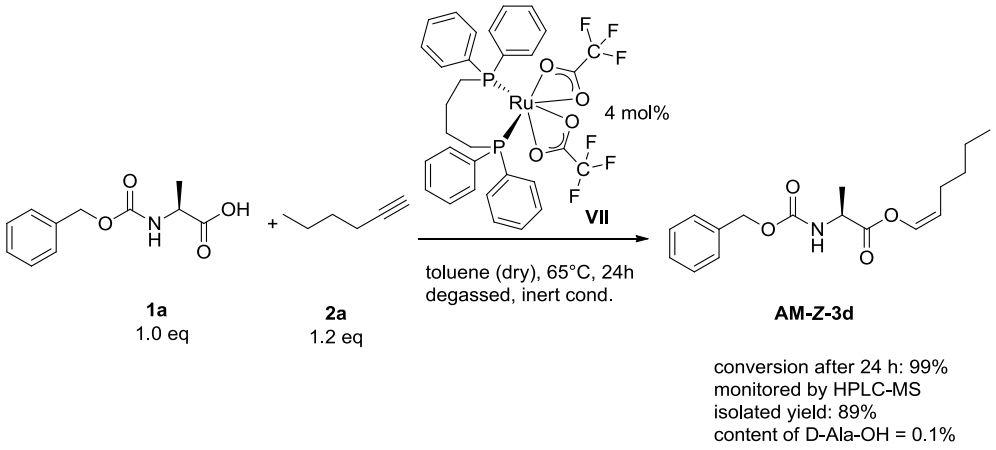
To test this approach we used the original catalyst **III** used by Dixneuf and converted it into catalyst $[Ru(TFA)_2(dppb)]$ (**VII**) by adding trifluoroacetic acid (**48**) to catalyst **III** in toluene. This reaction (Scheme 70) was performed similar to the literature procedure from Weberndörfer et al.[242]

As substrate we used Z-Ala-OH (**1a**) and 1-hexyne (**2a**) in toluene as this is already described using the original catalyst **III** by Dixneuf et al.[110] This made it easier for us to decide whether this approach is successful or not.



Scheme 70: Synthesis of $[Ru(TFA)_2(dppb)]$ (**VII**).

The ruthenium complex **VII** could be obtained in 66 % yield as a powder. The experiment with Z-Ala-OH (**1a**) and catalyst **VII** revealed that this complex can be used to form enolesters, because catalyst **VII** is as active as the original catalyst **III** used by Dixneuf (Scheme 71). Z-Ala-*Z*-anti-Markovnikov-enolester **AM-*Z*-3d** could be obtained after 24 h with a conversion of 99 % and 89 % isolated yield after flash column chromatography.

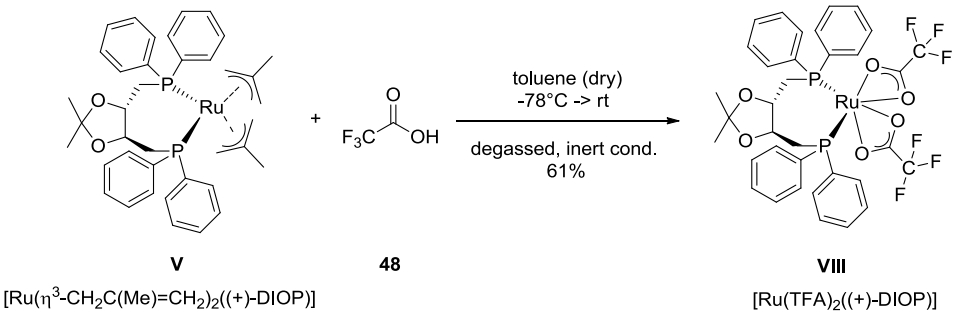


Scheme 71: Synthesis of Z-Ala-*Z*-anti-Markovnikov-enolester **AM-*Z*-3x**.

Having obtained this encouraging result and having showed that no ligand exchange between trifluoroacetic acid (**48**) and the dipeptide occurred, which was confirmed by HPLC-MS, we wanted to apply this method for the enolester formation of Z-Leu-Phe-OH (**1c**) mentioned in chapter 4.1.6.2.

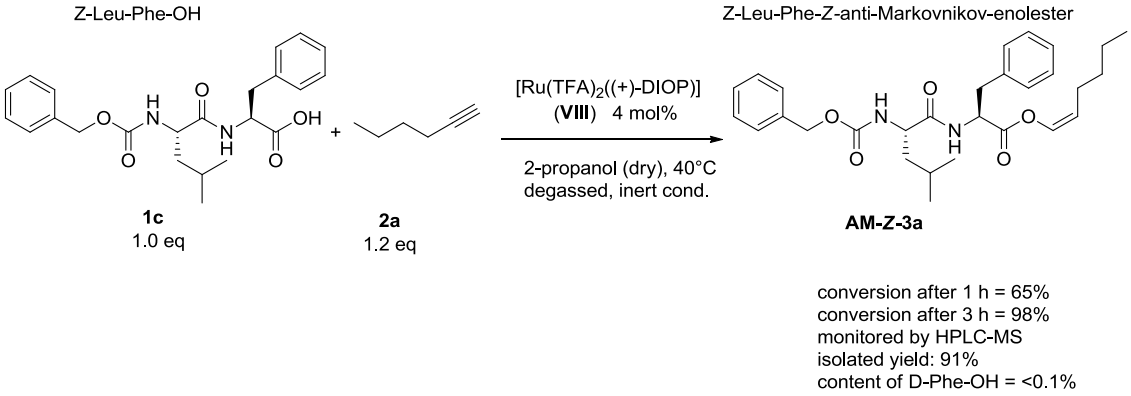
4.1.6.2. Synthesis and use of [Ru(TFA)$_2$((+)-DIOP)]

The use of (+)-DIOP (+)-**42** instead of dppb **20** as a ligand in the ruthenium-complex accelerates the formation of enolesters and additionally lowers the amount of racemization (Chapter 4.1.3.7). Hence we synthesized complex [Ru(TFA)$_2$((+)-DIOP)] (**VIII**) (Chapter 4.1.6.2). This reaction was performed similar to the literature procedure from Weberndörfer et al. (Scheme 72).[242]



Scheme 72: Synthesis of [Ru(TFA)$_2$((+)-DIOP)] (**VIII**).

With the use of [Ru(TFA)$_2$((+)-DIOP)] (**VIII**) in the alkyne addition reaction we obtained almost full conversion after 3 h. The reaction is only somewhat slower with 98 % conversion after 3 h compared with [Ru(η^3-CH$_2$C(Me)=CH$_2$)$_2$((+)-DIOP)] (**V**) where we could monitor via HPLC-MS 99 % conversion after already 1 h (Chapter 4.1.3.7). After flash column chromatography we obtained product **AM-*Z*-3a** in 91 % yield (Scheme 73). This encouraging result let us believe, that the use of trifluoroacetate ligands instead of methylallyl ligands can be of great value for enolester formations.



Scheme 73: Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** using ruthenium-complex **VIII**.

4.1.7. TFE in the ruthenium catalysed enolester formation

From our solvent screening we had identified 2-propanol as an optimal solvent for the Ru-catalyzed enolester formation (Chapter 4.1.3.4). However, because of solubility issues of various peptides due to their different size and polarity, 2-propanol will not be able to serve as a universal solvent for all cases. As already mentioned in chapter 4.1.3.4 it is advisable for the Ru-catalyzed enolester formation, that all starting materials are soluble in the chosen solvent because suspensions proved to be disadvantageous. Concerning the rate of

catalysis, higher concentrations of the starting materials are beneficial. Therefore, we considered 2,2,2-trifluoroethanol as an appropriate additive to 2-propanol. TFE is a particularly good solvent for many different peptides or even proteins due to its strong H-bond donor property, which dissolves secondary structures.

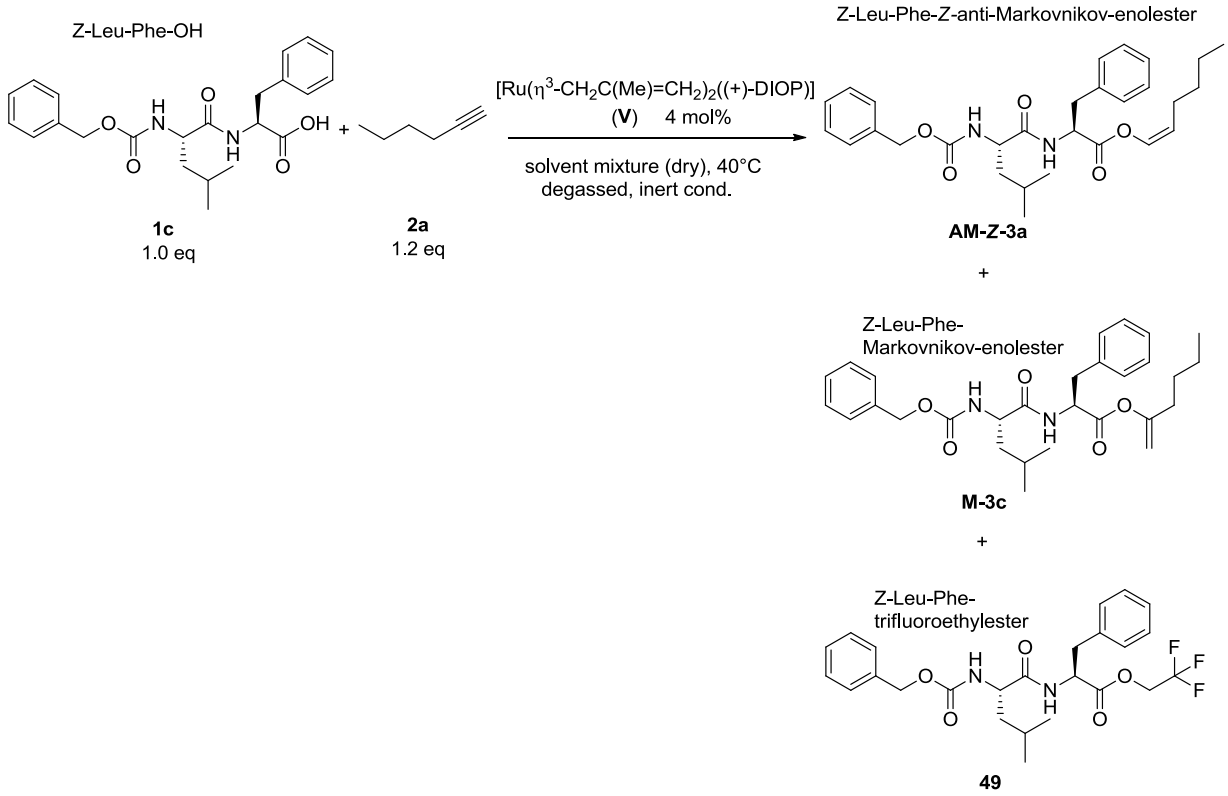
4.1.7.1. TFE as cosolvent

We tested three different solvent mixtures, which all contained 2-propanol and TFE. In the case of 2-propanol containing 10 % of TFE (Table 13, entry 1) the reaction with Z-Leu-Phe-OH (**1c**) and 1-hexyne (**2a**) worked smoothly (Scheme 74). 97 % conversion is just insignificantly slower than without the addition of TFE.

In experiments containing higher percentages of TFE, we observed the formation of Z-Leu-Phe-trifluoroethylester (**49**), which itself is a suitable substrate for alcalase catalyzed peptide formation. In the case of using the solvent mixture of TFE and just 10 % of 2-propanol (Table 13, entry 3), the TFE-ester **49** was even the major product, which was isolated by flash column chromatography and characterized by ^{1}H and ^{13}C-NMR (see experimental part).

Concerning the amount of racemization we found 1.1 % D-Phe-OH after 48 h (Table 13, entry 3). This higher amount of racemization might be caused due to the longer reaction time.

One possible explanation for the formation of Z-Leu-Phe-trifluoroethylester (**49**) and the higher amount of racemization is the pKa-value of 2,2,2-trifluoroethanol (12.4) compared to the pKa-value of 2-propanol (16.6). The higher acidity of trifluoroethanol appears to be responsible for the decomposition of the Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a**. This assumption is supported by the observation that the amount of Z-Leu-Phe-trifluoro-ethylester (**49**) increases slowly over time (Table 13, entry 3).



Scheme 74: Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** using TFE as a cosolvent.

Table 13: Synthesis of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** using TFE as a cosolvent.

Entry	Solvent	Conc. [mol/l]	Conversion [%][a)]				Isolated Yield [%]	Amount of H-D-Phe-OH[b)] [%]
			1h	3h	24h	48h		
1	2-Propanol + 10 % TFE	0.5	85	97 (99/<1)[c)]	n.d.	n.d.	88	0.1
2	2-Propanol + 50 % TFE	0.3	91 (99/<1)[c)]	96[d)] (96/4)[c)]	n.d.	n.d.	82	0.1
3	TFE + 10 % 2-Propanol	0.5	86[d)] (99/<1)[c)]	94[e)] (96/4)[c)]	92 (26/74)[c)]	91 (17/83)[c)]	73[e)]	1.1

[a)] Conversion measured with HPLC (210 nm, Poroshell C-18)
[b)] Analysis of racemisation after hydrolysis with 6N HCl; HPLC-analysis with Chirobiotic-R; comparison with results obtained after hydrolysis of *Z*-Leu-Phe-OH (**1c**) under identical conditions
[c)] ratio of **AM-*Z*-3a** / **49**
[d)] up to 2 % Markovnikov-product monitored via HPLC-MS at 210 nm; not characterized
[e)] yield of Z-Leu-Phe-trifluoroethylester (**49**)

Another factor favoring the formation of Z-Leu-Phe-trifluoroethylester (**49**) is the keto-enol tautomerization occurring during transesterification. During transesterification with TFE hex-1-en-1-ol is released. Hex-1-en-1-ol is immediately converted to the thermodynamically more stable aldehyde hexanal via keto-enol tautomerization. Therefore, the reaction equilibrium is shifted to the side of the Z-Leu-Phe-trifluoroethylester (**49**) (Figure 15).

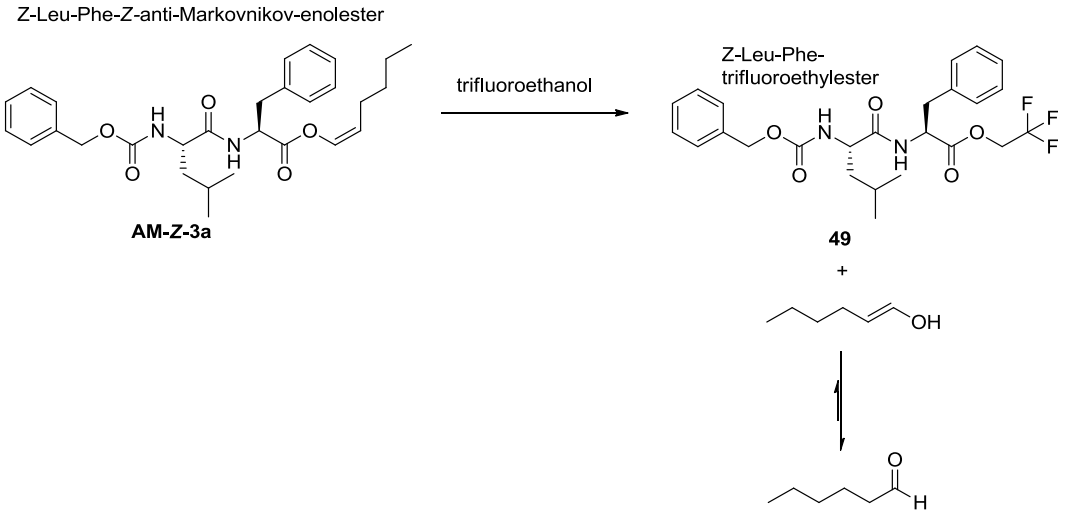


Figure 15 : Keto-enol tautomerization.

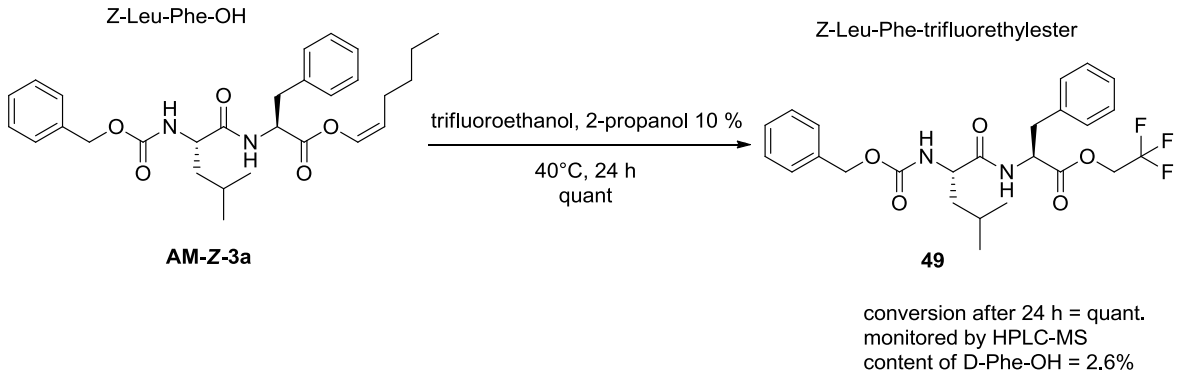
However, trifluoroethylesters[48] have already been applied for the enzymatic synthesis of peptides. Therefore, one has to emphasize that the formation of trifluoroethylester as a side-product arising from our desired enolesters when using TFE as cosolvent is not in vain. TFE as a cosolvent can be used to form activated esters from peptides without epimerization. Consequently, a mixture of trifluoroethylester **49** and enolester **AM-*Z*-3a** (Table 13, entry 2) can be applied to couple peptides enzymatically.

4.1.7.2. Synthesis of Z-Leu-Phe-trifluoroethylester

These results raised the question, if the synthesis of Z-Leu-Phe-trifluoroethylester (**49**) operated through ruthenium catalysis or whether TFE itself is able to initiate the transesterification process.

Therefore, the previously synthesised and isolated enolester **AM-*Z*-3a** was stirred in a solvent mixture of trifluoroethanol and 10 % 2-propanol for 24 h at 40°C in an open flask experiment. By using HPLC-MS for monitoring this reaction a quantitative conversion towards Z-Leu-Phe-trifluoroethylester (**49**) could be determined and confirmed by NMR. This proves that the formation of the Z-Leu-Phe-trifluoroethylester (**49**) during enolester synthesis

might not proceed over a mechanism involving ruthenium catalysis but can be explained by the reactivity of trifluoroethanol used as solvent (Scheme 75).



Scheme 75: Synthesis of Z-Leu-Phe-trifluoroethylester **49**.

4.1.8. Determination of racemization in various peptides

The precise determination of epimerization was an important part in addition to the experiments, which finally led to the development of a racemization free enolester synthesis.

In case of phenylalanine, we performed the hydrolysis of enolesters containing a *C*-terminal phenylalanine with 6N HCl and subjected the hydrolysis products to HPLC-analysis on a Chirobiotic-R column. The D-Phe-OH content after parallel hydrolysis of the starting material (**1**) under identical conditions was substracted to compensate for the background reaction.

To analyze the amount of racemization when alanine was the *C*-terminal amino acid, we performed the hydrolysis of enolesters with 6N HCl and analyzed the content of D-Ala-OH via HPLC-analysis after derivatization with the L-valinamide analogue of Marfey's reagent (FDAA, N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide) (**50**) on a Poroshell column (Figure 16).[243] The D-Ala-OH content after parallel hydrolysis of the starting material **1d** under identical conditions was subtracted to compensate for the background reaction.

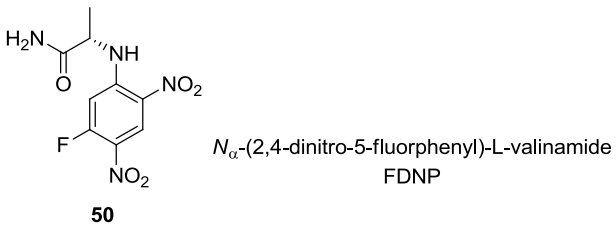


Figure 16: Analogue of Marfey's reagent (FDAA) used for ee-detection.

For all other amino acids we used a literature known protocol[244] also used by DSM. To analyze the amount of racemization when alanine was the *C*-terminal amino acid we performed the hydrolysis of enolesters with 6N DCl in D_2O followed by an esterification reaction with 2-3N DCl in 2-propanol and finally an acylation with pentafluoropropionic anhydride. The amount of epimerization was monitored via GC-MS using a Chirasil-L-Val column.

In certain cases, the amount of epimerization of alanine or phenylalanine as the *C*-terminal amino acid was also monitored via the GC-MS method as described above. We regarded this as necessary to validate the correctness of our methods used. In all cases the amount of epimerization was the same in the range of the experimental error for Chirobiotic-R column method and the GC-MS method used.

4.2. Chemoenzymatic peptide coupling

4.2.1. Introduction

An elegant alternative to the well-established methods solid phase peptide synthesis (SPPS) and solution-phase chemical peptide synthesis is the chemoenzymatic peptide synthesis for the formation of small and medium-sized peptides.[245] One excellent way to synthesize peptides enzymatically is the use of an *N*-terminally protected amino acid, which is *C*-terminally activated via an ester. Letting it react in the presence of a suitable enzyme together with a *C*-terminally protected amino acid, which acts as an acyl acceptor, forms the peptide. There are specially designed ester moieties known in literature that are particularly recognized by certain enzymes.

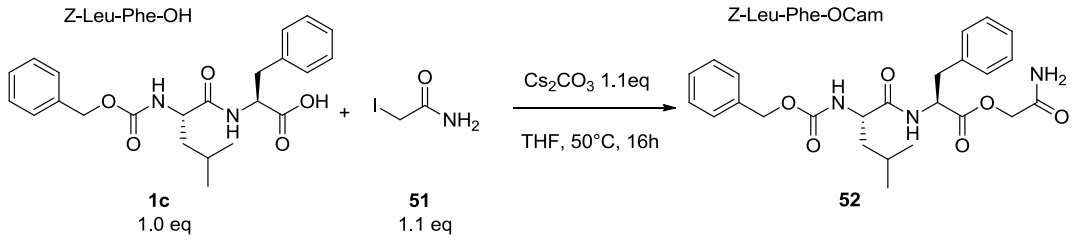
There are three outstanding ester moieties guanidinophenyl (Gp),[51],[246] carboxamidomethyl (Cam)[50] and trifluoroethyl (Tfe)[48] used for enzymatic peptide coupling. A disadvantage of these esters is the sometimes challenging synthesis. In this respect we wanted to establish the peptide enolester synthesized in 4.1. as substrates for enzymatic peptide synthesis. The enzyme chosen was alcalase-CLEA, because of its well proven ability to accept the ester moieties carboxamidomethyl (Cam) and trifluoroethyl (Tfe) for the synthesis of small peptides. Alcalase-CLEA is a serine endoprotease which has been cross-linked via glutaraldehyde. An especially for organic media designed alcalase-CLEA OM was applied for the enzymatic peptide synthesis, using two different batches named “alcalase-CLEA OM batch DSM1” and “alcalase-CLEA OM batch DSM2”. Both batches were slightly different in their optical appearance and ability to catalyze our envisioned biotransformations.

4.2.2. Synthesis of Z-Leu-Phe-Phe-NH_2 using Z-Leu-Phe-*Z*-anti-Markovnikov-enolester as a substrate

4.2.2.1. Initial attempts

After having obtained Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** and Z-Leu-Ala-*Z*-anti-Markovnikov-enolester **AM-*Z*-3c** via ruthenium catalysis without racemization, it was time to test their potential for enzymatic peptide synthesis. Central for the success of our approach using enolesters in chemoenzymatic peptide synthesis was the goal to obtain comparable results to literature known ester motives. In our case we decided to use the Cam-ester moiety as a reference for the feasibility of the applied enolesters. Therefore, Z-

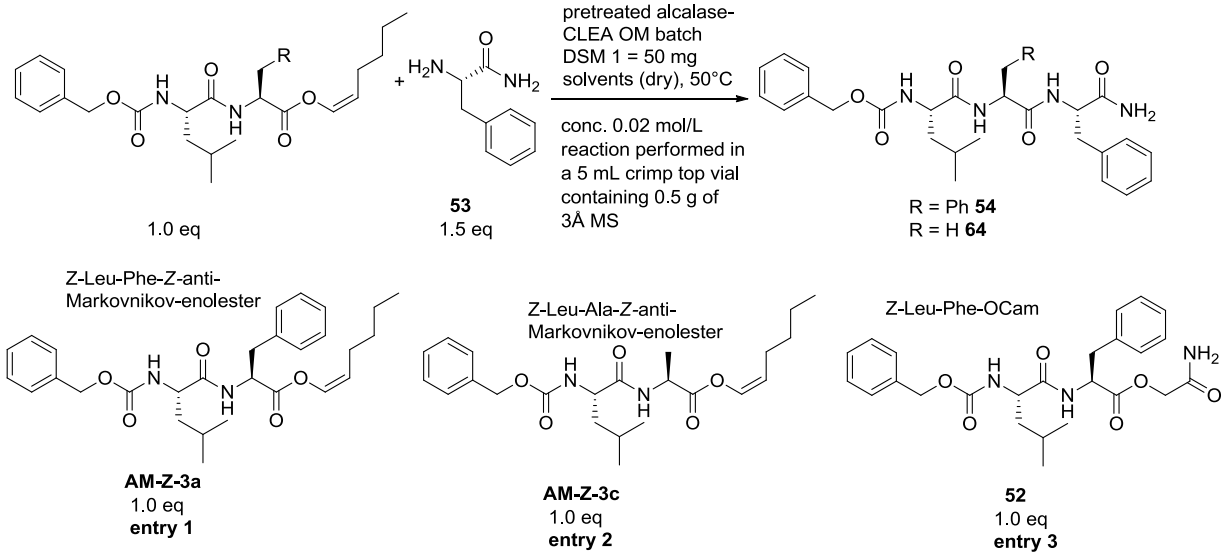
Leu-Phe-OCam (**52**) was synthesized using iodoacetamide (**51**) in the presence of Cs_2CO_3 (Scheme 76).[247]



Scheme 76: Synthesis of Z-Leu-Phe-OCam (**52**).

Before starting the enzymatic peptide coupling, the alcalase-CLEA OM batch DSM1 had to be pretreated. For that purpose alcalase-CLEA was washed with dry *tert*-butanol under argon to remove the bulk water of the enzyme. *Tert*-butanol was removed by filtration in a way so that it does not get too cold and becomes a solid. In the next step alcalase-CLEA was washed with MTBE (dry) to remove all *tert*-butanol and then the enzyme was dried under vacuum.

The enzymatic peptide coupling reactions were carried out in an oven dried crimp top vial. Dipeptide enolesters **AM-*Z*-3a**, **AM-*Z*-3c**, Z-Leu-Phe-OCam (**52**), H-Phe-NH_2 (**53**) and pretreated alcalase-CLEA were placed in this vial. Then dry THF and subsequently the drying agent MS 3Å was added. The necessity of molecular sieves is further explained in chapter 4.2.2.3. The crimp top vials were closed and the reactions were shaken (Scheme 77).



Scheme 77: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using alcalase-CLEA OM batch DSM1 and esters **AM-*Z*-3a**, **AM-*Z*-3c** and **52**.

Table 14: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using alcalase-CLEA OM batch DSM1 and esters **AM-*Z*-3a**, **AM-*Z*-3c** and **52**.

Entry	Starting material	Solvent	Time [h]	Amount of enzyme [mg/mL]	Product 54[a)] [%]	Hydro-lysis [%]	Imine formation[b)] [%]
1a	**AM-*Z*-3a**	toluene	18	50	79	<1	11
1b	**AM-*Z*-3a**	THF	18	50	46	0	20
2	**AM-*Z*-3c**	toluene	18	50	78 (product **64**)	0	28
3a	**52**	toluene	18	50	89	0	0
3b	**52**	THF	18	50	96	0	0

[a)] monitored by HPLC-MS (210 nm)
[b)] relative to product and starting material

As hoped the reaction showed, that Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** is well accepted by alcalase-CLEA. The conversion was monitored by HPLC-MS. The formation of Z-Leu-Phe-Phe-NH_2 (**54**) proceeded better in toluene (79 %) (Table 14, entry 1a) than in THF (46 %) (Table 14, entry 1b). Unfortunately, we observed some imine formation **56** as a side-reaction, which will be further discussed in chapter 4.2.2.2. Also Z-Leu-Ala-*Z*-anti-Markovnikov-enolester **AM-*Z*-3c** was well accepted by alcalase-CLEA and we could reach 78 % conversion to product **64** in toluene (Table 14, entry 2).

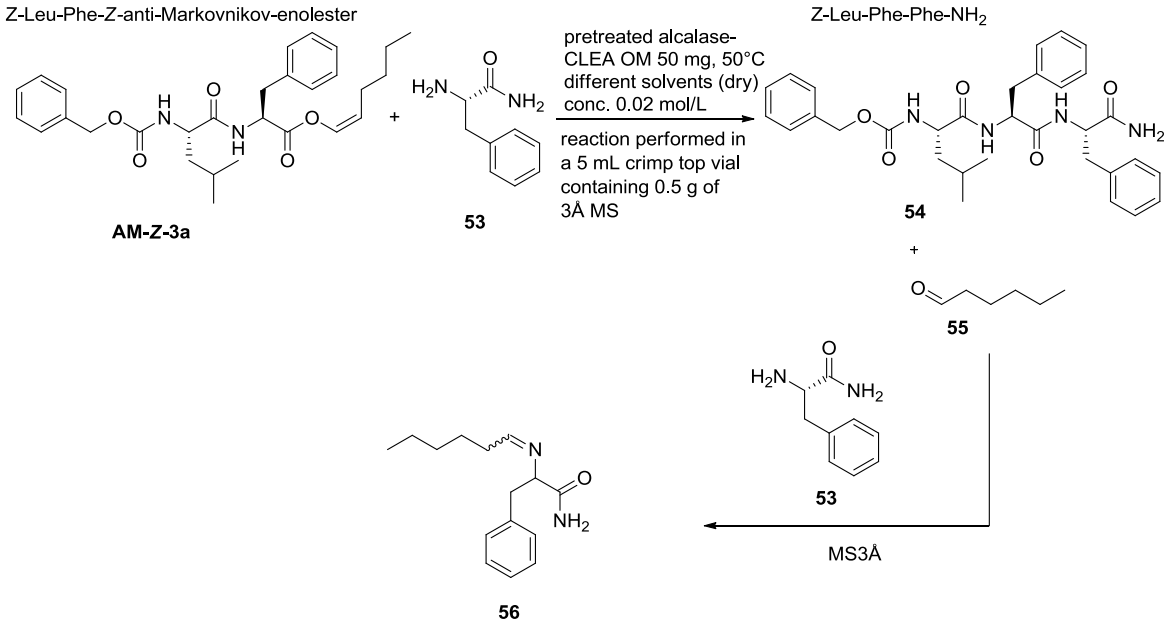
Table 14, entry 3 shows that Z-Leu-Phe-OCam (**52**) is preferably accepted by alcalase-CLEA than the *Z*-anti-Markovnikov-enolesters **AM-*Z*-3a** and **AM-*Z*-3c**. One reason is that alcalase-CLEA is a protease, so naturally cleaves amid-(peptide)-bonds. Cam esters, bear a terminal amide bond and therefore might function as a mimic of peptides. Our enolesters **AM-*Z*-3a** and **AM-*Z*-3c** instead contain a hexenyl-enolester chain at the *C*-terminal end of the peptide. These alkyl-chain motives might probably be more suitable, for reactions with esterases or lipases as enzyme catalysts.

In respect to solvent effects, for the conversion of the Cam-ester **52**, it can be seen, that THF is better than toluene. One reason for this result is the low solubility of Cam-ester **52** in toluene. Concerning future applications and high reaction rates it seems to be necessary to get the starting material in solution. Therefore, the decision was made to use THF as the

solvent for enzymatic peptide coupling. An advantage of using Cam-ester **52** for enzymatic peptide coupling is that there is no formation of imines possible.

4.2.2.2. Imine formation

In respect to the enzymatic peptide coupling reactions mentioned in chapter 4.2.2.1 the imine formation is due to a condensation reaction of released aldehyde 1-hexanal (**55**) and the excess of primary amine H-Phe-NH_2 (**53**). The first step of the formation of the imine is the formation of a hemiaminal. The imine forms after elimination of water. This reaction is an equilibrium which is mainly on the side of the aldehyde and the amine. But the presence of molecular sieves shifts the equilibrium in favour of the imine, and the acidic properties of the molecular sieves catalyze this reaction (Scheme 78).



Scheme 78: Undesired imine formation.

The amount of imine formation is in a range of 10 % to 30 % relative to the total peak area of peptide fragments at 210 nm in the UV-trace of the HPLC chromatogram. No attempt was made to exactly quantify the conversion, but one can conclude that the reaction is efficiently promoted by the elevated reaction temperature and presence of molecular sieves in the system, thus continuously reducing the available concentration of the amine fragment for the coupling.

In order to confirm the identity of the imine isomers **56** we synthesized them chemically (see experimental part). HPLC analysis of the reaction products confirmed the identity with those imines formed during the peptide coupling reactions.

In order to analyse if there is any racemization occurring during the formation of the imine we applied our racemization test protocol mentioned in chapter 4.1.8 using a Chirobiotic-R column for HPLC analysis. After hydrolysis of imine **56**, formed form H-Phe-NH_2 (**53**) and hexanal, we found 0.3 % racemization of phenylalanine.

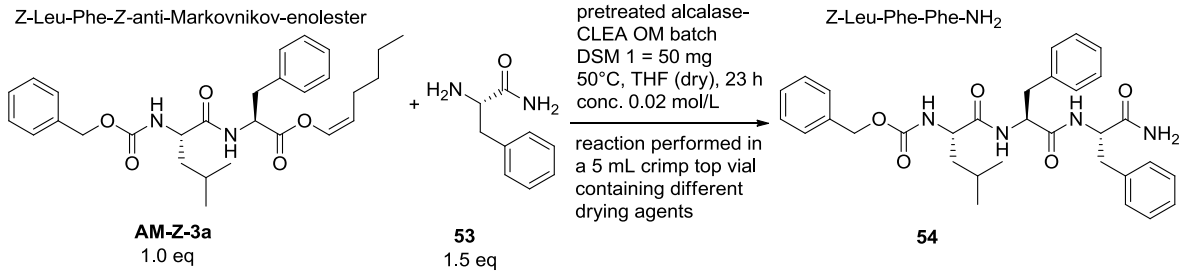
However, even small amounts of racemization and the loss of expensive starting material upon imine formation, as unwanted side reaction during the enzymatic peptide synthesis, showed the necessity to reduce or better avoid any imine formation.

4.2.2.3. Synthesis of *Z*-Leu-Phe-Phe-NH_2 with different drying agents

Central to the success of the proposal using enolesters for enzymatic peptide coupling, was the need to deal with undesired imine formation. We speculated that the presence of molecular sieves and its surface was responsible for the formation of imine **56**. Anyway the presence of a drying agent like molecular sieves is necessary to avoid hydrolysis. If water is not efficiently removed from the catalytic system the enzyme is in principle able to use the water molecules to saponify the precious enolesters **AM-*Z*-3a** and **AM-*Z*-3c**. Moreover this would be the naturally preferred way for alcalase, which belongs to the group of serine proteases, where a serine residue and water in the active site are responsible for the cleavage of amide bonds. If hydrolysis occurs a carboxylic acid is formed. This is undesired because the pKa-value of amino acids or peptides is very low. For instance the pKa-value of phenylalanine is 1.8. This pKa value is far away from the pH optimum of alcalase-CLEA. Regarding these facts it appeared important to us to know if other drying agents are also capable to prevent hydrolysis (Scheme 79).

In Table 15, entry 1 it is shown that activated 3 Å molecular sieves are responsible for the generation of imine **56** with 19 % relative to product and starting material monitored via HPLC-MS. Compared to molecular sieves, the use of Na_2SO_4 and $MgSO_4$ led to considerable hydrolysis in the enzymatic peptide coupling reactions (Table 15, entries 3-4) and consequently to a lower amount of Z-Leu-Phe-Phe-NH_2 (**54**). On the other hand, Na_2SO_4 and $MgSO_4$ showed almost no imine formation after 23 h at 50°C. Nevertheless, due to their inefficient water capture ability, sodium- or magnesium sulfate cannot replace molecular sieves as drying agent.

Another attempt was made to replace the molecular sieves by aluminium oxide. Unfortunately, this resulted in an even higher amount of imine formation (62 % relative to product and starting material).



Scheme 79: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using alcalase-CLEA OM batch DSM 1 and drying agents (MS 3Å, Alox, Na_2SO_4, $MgSO_4$).

Table 15: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using alcalase-CLEA OM batch DSM 1 and drying agents (MS 3Å, Alox, Na_2SO_4, $MgSO_4$).

Entry	Time [h]	THF[e] + drying agent	Conversion[a] [%]	Product-ratio[b] [%]			Imine formation[d] [%]
				54	57[c]	1c	
1	23	MS 3Å	69	99	<1	0	19
2	23	Alox	73	99	<1	0	62
3	23	Na_2SO_4	75	57	0	43	<1
4	23	$MgSO_4$	74	56	0	44	<1

[a] measured with HPLC (210 nm, Purospher Star Rp-18e)
[b] product-ratio: Z-Leu-Phe-Phe-NH_2 (**54**) / Z-Leu-Phe-Phe-Phe-NH_2[c] (**57**) / Z-Leu-Phe-OH (**1c**)
[c] identified as Z-Leu-Phe-Phe-Phe-NH_2 (**57**) by HPLC-MS
[d] relative to product and starting material
[e] THF dried over sodium

Taking the results from our peptide coupling reactions with different drying agents we assumed that the activated 3 Å molecular sieves catalyze the imine formation efficiently. This is probably due to their slightly acidic heterogeneous surface. As an attractive alternative we envisioned that it should be possible to avoid imine formation by using Soxhlet-conditions with molecular sieves separated from the reaction as further explained in chapter 4.2.2.4.

4.2.2.4. Synthesis of Z-Leu-Phe-Phe-NH_2 under Soxhlet-conditions

After having been unable to find an alternative to molecular sieves for the enzymatic peptide coupling using enolesters, we faced the tradeoff between hydrolysis and imine formation. We planned to separate the molecular sieves from the starting material and reaction product, but still get them into contact with the solvent. This proposition could be realized using Soxhlet-conditions (Figure 17). In our case we worked under reduced pressure (270 mbar) to obtain reflux conditions for THF at 50°C. Due to an azeotropic mixture of THF and water, water in the reaction system can be removed by an azeotropic distillation. After recondensation of the azeotropic water/THF mixture water can be removed by the molecular sieves in a Soxhlet set up before the solvent drips back to the reaction solution.

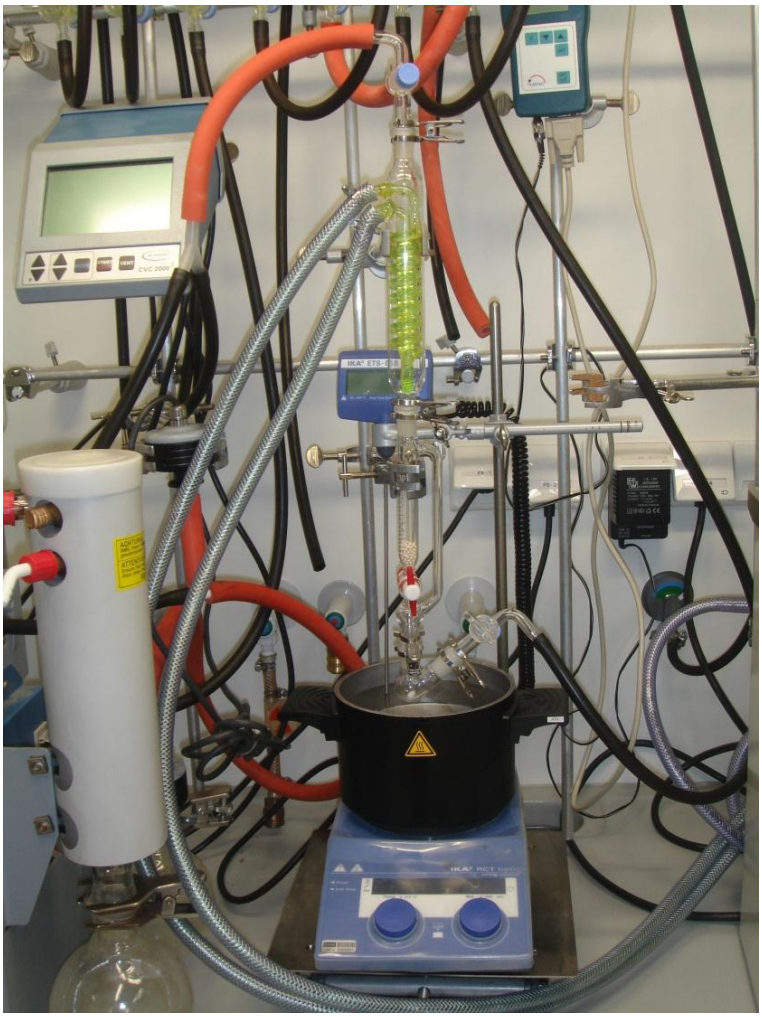
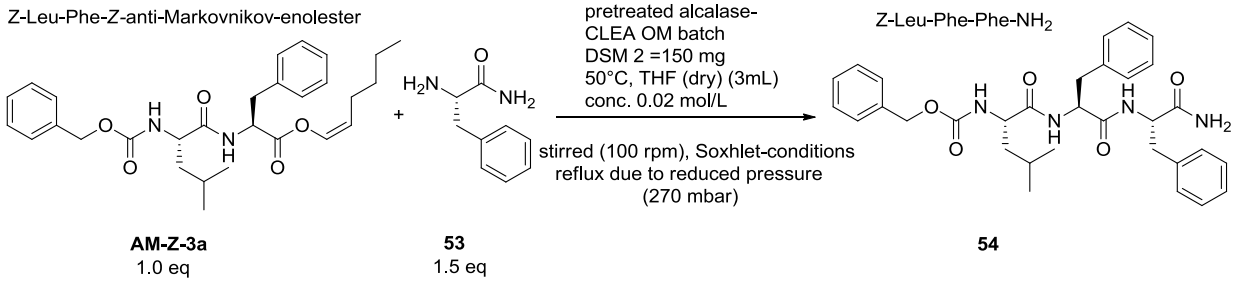


Figure 17: Soxhlet type reaction set up.

This approach indeed turned out to be successful. When performing the enzymatic peptide coupling using enolester **AM-*Z*-3a** and H-Phe-NH_2 (**53**) we could monitor 74 % conversion towards the desired product Z-Leu-Phe-Phe-NH_2 (**54**) via HPLC-MS (Scheme 80, Table 16, entry 1). With the formation of only 0.2 % of imine **56**, relative to product and starting material, we could prove that there is barely any imine-formation, if the starting material and the molecular sieves are separated. Much to our delight also the amount of hydrolysis is negligible. At this stage it was necessary to repeat this reaction to investigate its reproducibility (Scheme 80, Table 16, entry 2). Note, both reactions (Table 16, entry 1 and 2) were performed with alcalase-CLEA OM batch DSM 2. The only difference was that 0.06 mmol enolester **AM-*Z*-3a** (Table 16, entry 2) in 3 mL THF instead of 0.04 mmol enolester **AM-*Z*-3a** (Table 16, entry 1) in 2 mL THF were used.



Scheme 80: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using alcalase-CLEA OM batch DSM 2 performed under Soxhlet-conditions.

Table 16: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using alcalase-CLEA OM batch DSM 2 performed under Soxhlet-conditions.

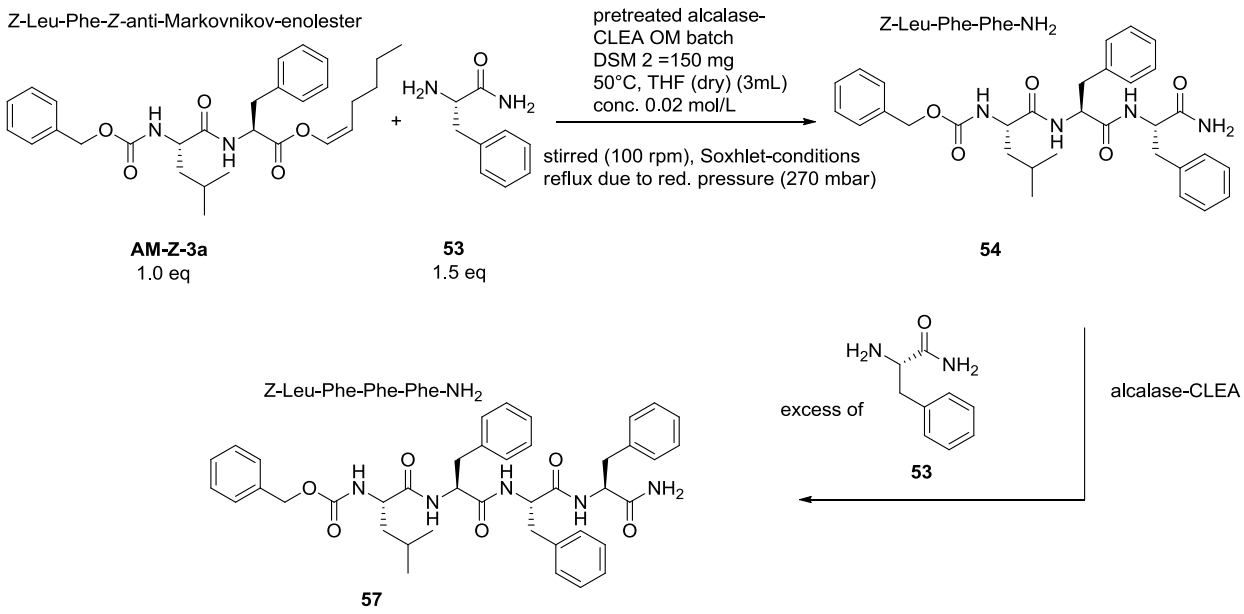
Entry	Time [h]	Solvent	Conversion[a] [%]	Product-ratio[b] [%]			Imine formation[d] [%]	Isolated yield [%]
				54	**57**[c]	**1c**		
1	17	THF[f]	75	98	1	<1	0.2	n.d.
2	23	THF[f]	65	92	7	<1	0	36 (60)[e]

[a] monitored with HPLC-MS (210 nm, Poroshell C-18)
[b] product-ratio: Z-Leu-Phe-Phe-NH_2 (**54**) / Z-Leu-Phe-Phe-Phe-NH_2[c] (**57**) / Z-Leu-Phe-OH (**1c**)
[c] identified as Z-Leu-Phe-Phe-Phe-NH_2 (**57**) by HPLC-MS
[d] relative to product and starting material
[e] 60 % yield after flash column chromatography; 36 % after recrystallization
[f] THF dried by distillation over sodium

This second experiment confirmed the results reported in Table 16, entry 1, although the conversion with 63 % was slightly lower. There was no imine formation **56** and also only a

negligible amount of hydrolysis was found. After 1-2 h the product started precipitating from the solution under Soxhlet-conditions. After 23 h DMSO was added to ensure that everything was dissolved before measuring the conversion via HPLC-MS. THF was removed in vacuo using a rotary evaporator. Afterwards DMSO was removed in vacuo (0.02 mbar) and the resulting solid was purified by flash column chromatography (DCM/MeOH 20:1 + traces of DMSO). 60 % of desired product **54** could be obtained. ^{13}C-NMR confirmed product **54** but ^{1}H showed small amounts of impurities. The crude product was recrystallized from ethylacetate (reflux) and cyclohexane yielding 36 % pure isolated product **54**.

Additionally, the formation of tetrapeptide Z-Leu-Phe-Phe-Phe-NH_2 (**57**) was detected via HPLC-MS (Table 16, entry 2). No attempt was made to isolate this side-product and to quantify the conversion exactly. Obviously, alcalase-CLEA is able to recognize the product Z-Leu-Phe-Phe-NH_2 (**54**) and convert it with excess of H-Phe-NH_2 (**53**) into tetrapeptide **57** (Scheme 81).



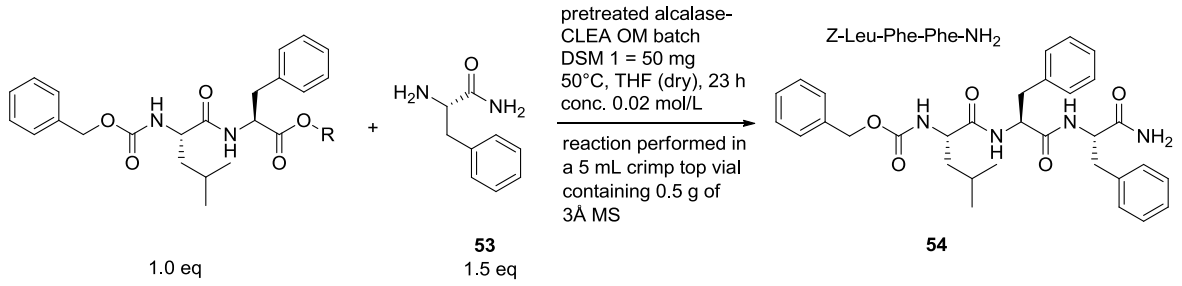
Scheme 81: Possible explanation for the formation of tetrapeptide Z-Leu-Phe-Phe-Phe-NH_2 (**57**).

4.2.3. Synthesis of Z-Leu-Phe-Phe-NH_2 using amide motif containing enolesters

4.2.3.1. Internal amide bonds in the leaving group

In order to identify a leaving group which is better recognized by alcalase-CLEA, ultimately leading to a faster conversion, we used *Z*-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3f** containing an amide motif in the leaving group (Scheme 82, Table 17, entry 1). At this stage of the PhD thesis alcalase-CLEA OM batch DSM 1 was used for this transformation. If not stated otherwise all reactions with an amide motif containing leaving group are performed under shaking in a 5 mL crimp top vial together with MS 3Å. Indeed the conversion toward the tripeptide Z-Leu-Phe-Phe-NH_2 (**54**) was faster compared to hexenyl-enolester **AM-*Z*-3a** and, fortunately, the amount of imine formation was considerably reduced.

We obtained 88 % conversion to product **54** after 23 h and just 7 % imine **58** formation, relative to product and starting material (Table 17, entry 1), compared to 69 % conversion and 19 % imine **56** formation, when using *Z*-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** (Chapter 4.2.2.3, Table 15, entry 1) containing a C-6 alkyl chain in the leaving group.



R = $CH=CHCH_2NHCOCH_3$ **AM-*Z*-3f**

R = $CH=CHCH_2NHCOPh$ **AM-*Z*-3g**

R = $CH=CHCH_2NHCOCH_2Ph$ **AM-*Z*-3h**

R = $CH=CH(CH_2)_3CONH_2$ **AM-*Z*-3k**

R = CH_2CONH_2 **52**

Scheme 82: Peptide coupling reactions with various esters of Z-Leu-Phe-OH (**1c**).

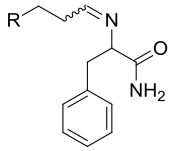
Table 17: Peptide coupling reactions with various esters of Z-Leu-Phe-OH (**1c**) in THF (dried over sodium) at 50°C using alcalase-CLEA OM batch DSM 1.

Entry	Substrate	Solvent[f]	Conversion[a] after 23 h [%]	Product-ratio[b] [%]			Imine formation[d] [%]	Impurity[d] $[MNa]^+$: 628
				54	57[c]	1c		
1	**AM-*Z*-3f**	THF	88	99	<1	0	7	0
2	**AM-*Z*-3g**	THF	69	99	<1	0	28	16
3	**AM-*Z*-3h**	THF	62	99	<1	0	23	16
4	**AM-*Z*-3k**	THF	91	99	<1	0	5	20
5	**52**	THF	90	98	<1	0	0	14

[a] monitored with HPLC-MS (210 nm)
[b] product-ratio: Z-Leu-Phe-Phe-NH_2 (**54**) / Z-Leu-Phe-Phe-Phe-NH_2[c] (**57**) / Z-Leu-Phe-OH (**1c**)
[c] identified as Z-Leu-Phe-Phe-Phe-NH_2 (**57**) by HPLC-MS
[d] relative to product and starting material
[f] THF dried over sodium

This encouraging result, shown in Table 17, entry 1 motivated us to test more amide motif containing enolesters. Their synthesis is described in chapter 4.1.4.2 and chapter 4.1.4.3. As a reference compound for the enzymatic peptide coupling reaction towards Z-Leu-Phe-Phe-NH_2 (**54**) we used again Z-Leu-Phe-OCam (**52**) to compare conversions with our enolesters **AM-*Z*-3f**, **AM-*Z*-3g**, **AM-*Z*-3h** and **AM-*Z*-3k**. In the reaction with Z-Leu-Phe-OCam (**52**) (Table 17, entry 5) 90 % conversion could be reached with alcalase-CLEA OM batch DSM 1.

Unfortunately, the high expectations concerning Z-Leu-Phe-*Z*-anti-Markovnikov-enolester containing an internal amide bond **AM-*Z*-3g** (Table 17, entry 2) and **AM-*Z*-3h** (Table 17, entry 3) were not fulfilled. The conversions of both enolesters **AM-*Z*-3g** and **AM-*Z*-3h** were just slightly higher compared to the hexenyl-enolester **AM-*Z*-3a** without an amide motif. Additionally, the amount of imine formation occurring during the formation of **AM-*Z*-3g** and **AM-*Z*-3h** was even slightly higher than the amount of imine formation using Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** containing no amide motif. Therefore, enolester **AM-*Z*-3g** (28 % of **59**, relative to product and starting material, Table 17, entry 2 and Figure 18) and **AM-*Z*-3h** (23 % of **60**, relative to product and starting material, Table 17, entry 3 and Figure 18) cannot be considered as a good option for enzymatic peptide coupling using alcalase-CLEA. An additional disadvantage of compounds **AM-*Z*-3g** and **AM-*Z*-3h** is the formation of a considerable amount of bis-coupling by-products **44** and **45** monitored by HPLC-MS during the ruthenium catalysis.(see chapter 4.1.4.2.)



R = $NHCOCH_3$ **58**
R = NHCOPh **59**
R = $NHCOCH_2Ph$ **60**

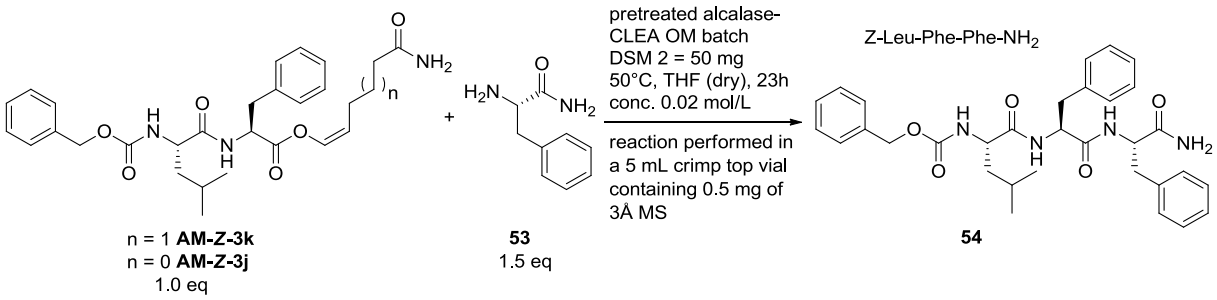
Figure 18: Putative imine side products **58, 59, 60** monitored via HPLC-MS at 210 nm.

In contrast enolester **AM-*Z*-3k** bearing a terminal amide bond in the leaving group gave a very promising performance with alcalase-CLEA OM batch 1. With Z-Leu-Phe-O(*Z*)-6-amino-6-oxohex-1-enyl (Z-Leu-Phe-*Z*-anti-Markovnikov-enolester) **AM-*Z*-3k** (Table 17, entry 4) a conversion of 91 % to product Z-Leu-Phe-Phe-NH_2 (**54**) could be detected by HPLC-MS. Fortunately, also the amount of imine **61** formed during the reaction (Table 17, entry 4) is with 5 %, relative to product and starting material, rather low compared to the previously mentioned amounts.

Summarising this chapter, enolester **AM-*Z*-3k**, which has a terminal amide motif is slightly better converted by alcalase-CLEA than compound **52** containing the already literature known Cam-ester motif. After having obtained these encouraging results further improvement of the conversion was necessary. These improvements are explained in the next chapter.

4.2.3.2. Terminal amide bonds in the leaving group

Starting from the best result from the previous chapter (Table 17, entry 4) the focus of enzymatic peptide coupling was now on the use of terminal amide bond containing enolesters **AM-*Z*-3k** and **AM-*Z*-3j**. Additionally, to obtain full or almost full conversion, the reaction conditions had to be improved. One important implementation was the change to another batch of alcalase-CLEA OM (alcalase-CLEA OM batch DSM 2) (Scheme 83). This resulted in 98 % conversion after 23 h using *Z*-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3k** containing a terminal amide bond in the leaving group (Table 18, entry 1). (With the previously used batch of alcalase-CLEA (alcalase-CLEA OM batch DSM 1) the conversion of the same starting material **AM-*Z*-3k** under identical conditions was only 91 % (chapter 4.2.3.1 Table 17 entry 4)). Gratifyingly, also the amount of imine formation was further reduced to 1.4 % of **62** (Figure 19). Applying the new batch of alcalase-CLEA we obtained the pleasant effect of a reduced amount of not characterized impurities compared to the alcalase-CLEA OM batch DSM 1.



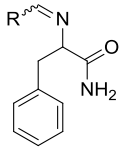
Scheme 83: Peptide coupling reactions with enolesters **AM-*Z*-3k** and **AM-*Z*-3j** in THF (dried over sodium) at 50°C using alcalase-CLEA OM batch DSM 2.

Table 18: Results of peptide coupling reactions with enolesters **AM-*Z*-3k** and **AM-*Z*-3j** in THF (dried over sodium) at 50°C using and alcalase-CLEA OM batch DSM 2 after 23h.

Entry	Starting material	Solvent[e)]	Conversion[a)] [%]	Product-ratio[b)] [%]			Imine formation[d)] [%]
				54	**57**[c)]	**1c**	
1	**AM-*Z*-3k**	THF	98	98	2	0	1.4
2	**AM-*Z*-3j**	THF	96	95	5	0	3.8

[a)] monitored with HPLC-MS (210 nm)
[b)] product-ratio: Z-Leu-Phe-Phe-NH_2 (**54**) / Z-Leu-Phe-Phe-Phe-NH_2[c)] (**57**) / Z-Leu-Phe-OH (**1c**)
[c)] identified as Z-Leu-Phe-Phe-Phe-NH_2 (**57**) by HPLC-MS
[d)] relative to product and starting material
[e)] THF dried over sodium

From Table 18, entry 2 also the employment of enolester Z-Leu-Phe-O(*Z*)-5-amino-5-oxopent-1-enyl **AM-*Z*-3j** can be regarded as success, although the conversion of 96 % after 23 h is a little bit lower compared to enolester **AM-*Z*-3k**. The amount of imine formation resulted in ~4 % **61** (Figure 19).

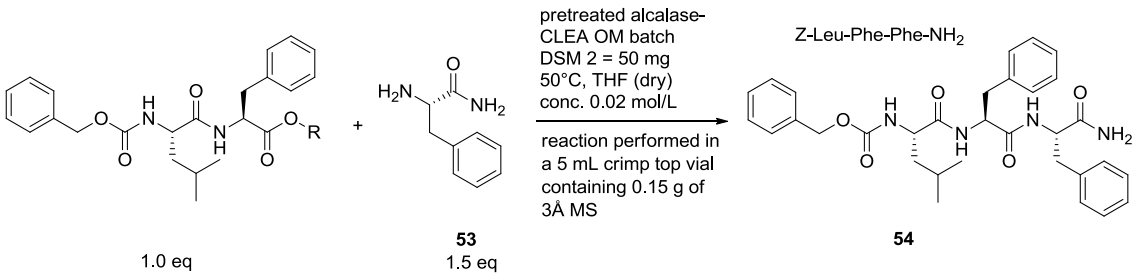


R = $(CH_2)_3CONH_2$ **61**
R = $(CH_2)_4CONH_2$ **62**

Figure 19: Putative imine side products **61, 62** monitored via HPLC-MS at 210 nm.

4.2.4. Investigation of peptide coupling kinetics

The investigation of the coupling kinetics of enolesters **AM-*Z*-3j** and **AM-*Z*-3k** appeared necessary to be able to better distinguish between the reaction rates of **AM-*Z*-3j** and **AM-*Z*-3k**. In parallel we also measured the conversions of hexenyl-enolester **AM-*Z*-3a** and Cam-ester **52** to benchmark these results with the results obtained from the conversion of enolesters **AM-*Z*-3j** and **AM-*Z*-3k** (Scheme 84). Additionally, as a further implementation we wanted to use BHT stabilized THF and which was dried with MS 3Å overnight instead of being dried over sodium and benzophenone. In preliminary experiments with Z-Leu-Phe-OCam (**52**) and alcalase-CLEA we had observed that the reaction with **52** reaches higher conversions when using THF which contains BHT and which was dried with MS 3Å overnight. It is known in the literature,[61] that enzymes need a certain amount of water to be still active. Performing a Karl-Fischer-Titration, it turned out that the amount of water in the THF which was dried with MS 3Å overnight was just 37 ppm. However, additional amounts of H_2O were subsequently introduced to the system by the oven dried 5 mL glass crimp top vials were not kept under an inert atmosphere when charging it with all starting materials. In case of too much water present in the reaction mixture, alcalase-CLEA is able to hydrolyze the designed enolesters. Nevertheless, at the beginning of the peptide coupling reaction, hydrolysis seems to be negligible because of an excess of the stronger amine nucleophile H-Phe-NH_2 (**53**).



R = $CH=CH(CH_2)_4$ **AM-*Z*-3a**

R = $CH=CH(CH_2)_3CONH_2$ **AM-*Z*-3k**

R = $CH=CH(CH_2)_2CONH_2$ **AM-*Z*-3j**

R = CH_2CONH_2 **52**

Scheme 84: Peptide coupling reactions with various esters of Z-Leu-Phe-OH (**1c**).

Table 19: Peptide coupling reactions with various esters of Z-Leu-Phe-OH (**1c**) in THF at 50°C.

Entry	Peptide ester	Time [h]	Conversion[a] [%]	Product-ratio[b,c] [%]		Schiff base formation relative to starting material + products [%]
				Z-Leu-Phe-Phe-NH_2 (54)	Z-Leu-Phe-Phe-Phe-NH_2 (57)	
1[d]	**AM-*Z*-3a**	1	56	92	8	<1
		3	70	90	10	2
		22	92	88	12	15
2[d]	**AM-*Z*-3k**	1	91	95	5	1
		3	99	92	8	3
		22	100	81	19	7
3[d]	**AM-*Z*-3j**	1	77	94	6	<1
		3	93	91	9	1
		22	100	85	15	1
4[d]	**52**	1	89	93	7	-
		3	98	89	11	-
		22	100	80	20	-

[a] monitored with HPLC-MS (210 nm)
[b] product-ratio: Z-Leu-Phe-Phe-NH_2 (**54**) / Z-Leu-Phe-Phe-Phe-NH_2 (**57**)
[c] no hydrolysis-product Z-Leu-Phe-OH (**1c**) detected
[d] THF (Fluka not dry) contains stabilizer BHT, dried with MS overnight; amount of H_2O in THF = 37ppm (Karl-Fischer Titration)

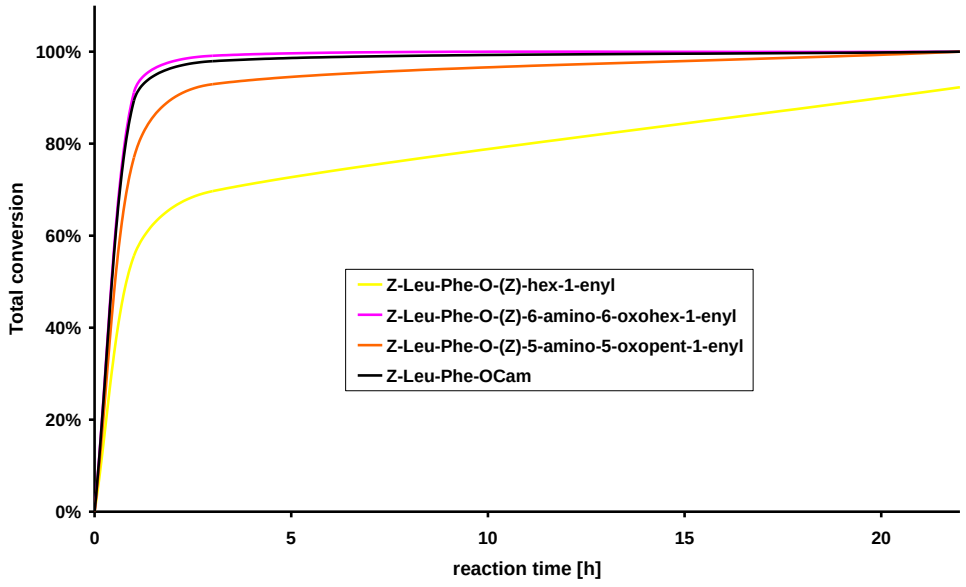


Figure 20: Alcalase-CLEA catalysed peptide coupling reactions of various esters of Z-Leu-Phe-OH (**1c**) and 1.5eq. H-Phe-NH_2 (**53**) in THF at 50°C.

Finally, our efforts were rewarded with full conversion of the starting materials **AM-*Z*-3j**, **AM-*Z*-3k** and **52** (Scheme 84, Figure 20) using alcalase-CLEA OM batch DSM 2 and THF which contains the stabilization agent BHT and which was dried over MS 3Å overnight. We found a huge difference in the reaction rate using hexenyl-enolester **AM-*Z*-3a** (Table 19, entry 1) compared to the amide motif containing enolesters **AM-*Z*-3j** and **AM-*Z*-3k** (Table 19, entries 2-3), concluding that enolesters containing a terminal amide motif are better substrates for alcalase-CLEA. The conversion of enolester **AM-*Z*-3j** (entry 3) occurs slightly slower than the conversion of enolester **AM-*Z*-3k** (entry 2), although **AM-*Z*-3j** reaches also full conversion after 23 h. Another very pleasant fact is that conversion of enolester **AM-*Z*-3k** is as fast as Z-Leu-Phe-OCam (**52**) (entry 4) or even slightly faster. With this result we have identified an enolester **AM-*Z*-3k** which is competitive in terms of conversion to the literature known Cam-ester motif. It should be mentioned, that the enolester **AM-*Z*-3k** can be synthesised from *C*-terminal amino acids like tyrosine and serine in contrast to their Cam-analoga which are difficult to prepare without a suitable protecting strategy using iodoacetamide (**51**) to form the corresponding Cam-ester. In all cases a significant amount of tetrapeptide Z-Leu-Phe-Phe-Phe-NH_2 (**57**) was formed. This is not due to the use of Cam-esters or enolesters but due to the good recognition of the tripeptide product Z-Leu-Phe-Phe-NH_2 (**54**) by alcalase-CLEA. The problem of imine formation still bothered us. Therefore, we applied Soxhlet-conditions already mentioned in chapter 4.2.2.4 for the enzymatic peptide coupling reaction using the enolester **AM-*Z*-3k**.

4.2.5. Synthesis of various tripeptides under Soxhlet-like-conditions

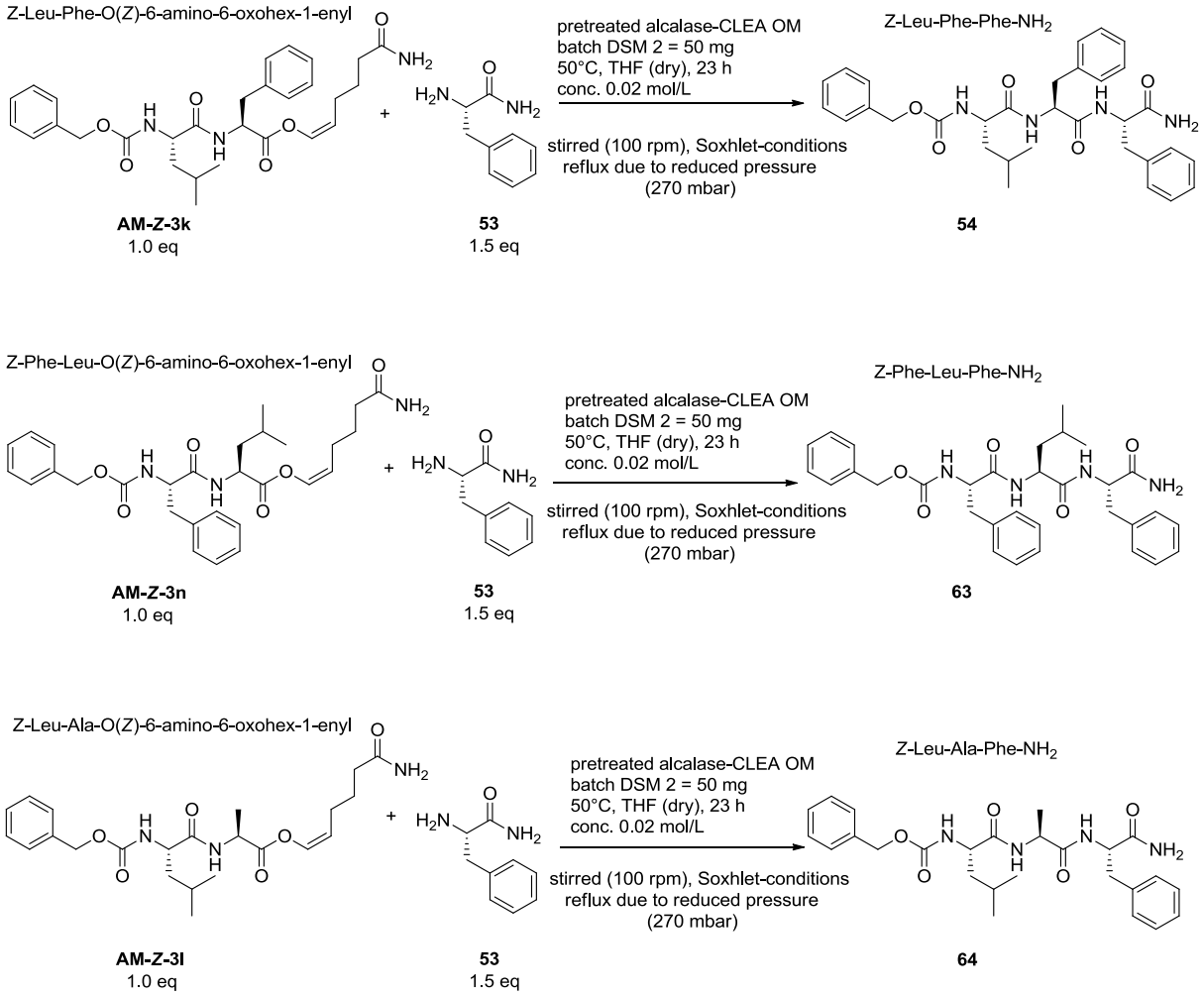
After having reached the goal of full conversion using enolester amide **AM-*Z*-3k**, it was time to address the last remaining challenge: the imine formation. Therefore, we combined Soxhlet-conditions with the superior enolester amide **AM-*Z*-3k**, the already in chapter 4.2.3.2 mentioned alcalase-CLEA OM batch DSM 2 and the BHT containing THF dried over molecular sieves overnight and containing BHT. The Soxhlet-conditions are explained and illustrated in chapter 4.2.2.4.

With Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3k** (Scheme 85) using Soxhlet-conditions we obtained 98 % conversion over all products with a ratio of 93 % of the desired product Z-Leu-Phe-Phe-NH_2 (**54**) (Table 20, entry 1) and only 4 % of the tetrapeptide Z-Leu-Phe-Phe-Phe-NH_2 (**57**) and 3 % formation of Z-Leu-Phe-OH (**1c**)**.** These 3 % of hydrolysis product Z-Leu-Phe-OH (**1c**) are a little bit higher than we had observed before using Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** containing a C-6 alkyl chain in the leaving group under the same conditions (chapter 4.2.2.4). Presumably, this can be explained if one takes into consideration that the terminal amide bond acts like a mimic for alcalase-CLEA.

Therefore, the hydrolysis should also be easier for alcalase-CLEA in the case of an enolester containing a terminal amide bond in the leaving group like **AM-*Z*-3k** compared to an enolester containing a C-6 alkyl chain in the leaving group like **AM-*Z*-3a**.

If THF is too dry (dried by distillation over sodium), the conversion is lower and the amount of hydrolysis is even higher than reported here. One possible reason for that is that the enzyme is less active due to the very low amount of water and therefore the enzyme is less able to perform this unnatural reaction. Although there is less water in the system the enzyme catalyzes more hydrolysis, which is the natural reaction pathway. Additionally, over time THF, due to its polarity, is able to interfere with the structural water directly on the surface of the enzyme. This putative release of bound water would enable the enzyme to hydrolyze the enolester containing a terminal amide bond. If the structural water is taken from the enzyme, unfolding and therefore a loss of the catalytic activity can occur. In summary it can be said that the better an enolester is accepted by alcalase-CLEA the better this enolester is also hydrolyzed even under Soxhlet-conditions. Depending on the scale and setup of the reaction it seems feasible to identify an optimal H_2O-content, which represents an ideal compromise between as little H_2O as possible to avoid hydrolysis, but sufficient amounts of H_2O to ensure optimal enzyme activity.

Having identified the critical parameters for a successful enzyme coupling reaction, we wanted to apply these insights for the synthesis of several peptides.



Scheme 85: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using dipeptide-enolester **AM-*Z*-3k**, **AM-*Z*-3n**, **AM-*Z*-3l** and alcalase-CLEA OM batch DSM 2 performed under Soxhlet-conditions.

Table 20: Results of synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using dipeptide-enolester **AM-*Z*-3k**, **AM-*Z*-3n**, **AM-*Z*-3l** and alcalase-CLEA OM batch DSM 2 performed under Soxhlet-conditions after 23h.

Entry	Starting material	Solvent	Conversion[a)][%]	Product-ratio[b)] [%]	Impurity[f)] [%]	Isolated yield [%]
1	**AM-*Z*-3k**	THF[g)]	98	(93/4/3)[c)]	3	91[h)]
2	**AM-*Z*-3n**	THF[g)]	75	(80/15/5)[d)]	2	n.d.
3	**AM-*Z*-3l**	THF[g)]	>99	(51/48/1)[e)]	8	n.d.

[a)] monitored with HPLC-MS (210 nm)
[b)] product-ratio: tripeptide / tetrapeptide / hydrolysis-product
[c)] product-ratio: Z-Leu-Phe-Phe-NH_2 (**54**) / Z-Leu-Phe-Phe-Phe-NH_2 (**57**) / Z-Leu-Phe-OH (**1c**)
[d)] product-ratio: Z-Phe-Leu-Phe-NH_2 (**63**) / Z-Phe-Leu-Phe-Phe-NH_2[c)] (**65**) / Z-Phe-Leu-OH (**1e**)
[e)] product-ratio: Z-Leu-Ala-Phe-NH_2 (**64**) / Z-Leu-Ala-Phe-Phe-NH_2[c)] (**66**) / Z-Leu-Ala-OH (**1d**)
[f)] relative to product and starting material; $[MH]^+$: 387, $[MNa]^+$: 409 detected by HPLC-MS
[g)] THF (Fluka not dry) contains stabilizer BHT, dried with MS overnight; amount of H_2O in THF = 37ppm (Karl-Fischer Titration)
[h)] 67 % isolated by precipitation + 24 % isolated by flash column chromatography

Using Soxhlet-conditions for the formation of Z-Leu-Phe-Phe-NH_2 (**54**) no imine formation was detected using terminal amide containing enolester **AM-*Z*-3k** as starting material. This is in good correlation with the results obtained in chapter 4.2.2.4. After reaching 98 % total conversion applying enolester amide **AM-*Z*-3k** for the enzymatic peptide coupling reaction performed under Soxhlet-conditions, the desired product Z-Leu-Phe-Phe-NH_2 (**54**) could be isolated in 91 % combined yield. Firstly, product **54** was precipitated from THF in a yield of 67 %. The remaining mother liquor was evaporated to dryness (0.02 mbar) and purified by flash column chromatography (same conditions as described in chapter 4.2.2.4) yielding an additional yield of 24 % of isolated product **54**.

The synthesis of Z-Phe-Leu-Phe-NH_2 (**63**) (Table 20, entry 2) was performed under identical conditions as mentioned above. Using Z-Phe-Leu-*Z*-anti-Markovnikov-enolester containing a terminal amide bond **AM-*Z*-3n** a total conversion of 75 % was monitored by HPLC-MS with a ratio of 80 % product Z-Phe-Leu-Phe-NH_2 (**63**), 15 % tetrapeptide Z-Phe-Leu-Phe-Phe-NH_2 (**65**) (Figure 21) and 5 % Z-Phe-Leu-OH (**1e**). This conversion is remarkably lower compared to the formation of tripeptide **54** mentioned above. One possible reason for the lower conversion is probably the reduced acceptance of alcalase-CLEA towards the *C*-terminal leucine in enolester **AM-*Z*-3n** compared to the *C*-terminal phenylalanine in enolester **AM-*Z*-3k** (Table 20, entry 1 and entry 2). However, the amount of hydrolysis is not much higher than in the previous reaction with enolester **AM-*Z*-3k**. Interestingly, the amount of the Z-Phe-Leu-Phe-Phe-NH_2 (**65**) occurring as a side-product is even higher than reported in Table 20, entry 1. This finding supports the idea that *C*-terminal phenylalanine can be regarded as a

good substrate for alcalase-CLEA. It is accepted even as an amide, when phenylalanine is at the end of the peptide chain. Otherwise it appears to us that *C*-terminal leucine is not an equally good coupling partner for alcalase-CLEA as phenylalanine.

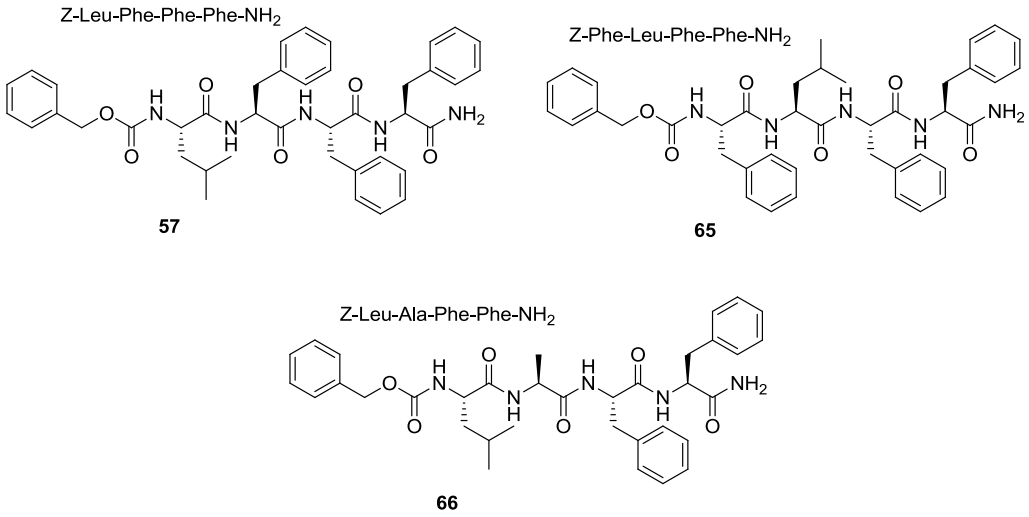


Figure 21: Side-products **57**, **65**, **66** of enzymatic peptide coupling reaction using alcalase-CLEA OM batch DSM 2 under Soxhlet-conditions.

The conversion of **AM-*Z*-3l** into the desired tripeptide Z-Leu-Ala-Phe-NH_2 (**64**) was performed under identical conditions as mentioned above. Despite the almost full conversion of the starting material Z-Leu-Ala-*Z*-anti-Markovnikov-enolester **AM-*Z*-3l** also containing a terminal amide bond, an almost equally large amount of undesired tetrapeptide Z-Leu-Ala-Phe-Phe-NH_2 (**66**) was formed. Due to the almost full conversion of starting material **AM-*Z*-3l** it appears that all 1.5 equiv. of H-Phe-NH_2 (**53**) were converted by alcalase-CLEA and therefore the ratio of tripeptide **64** and tetrapeptide **66** (Figure 21) is almost 1:1. Fortunately, hydrolysis towards product Z-Leu-Ala-OH (**1d**) is with 1 % very low.

We speculated that we could avoid or even significantly reduce the amount of side-product **66** by reducing the amount of Phe-NH_2 (**53**) and by applying shorter reaction times a reaction under identical conditions but with only 1.05 eq of H-Phe-NH_2 (**53**) was performed. Unfortunately, our hope kept unfulfilled, because a total conversion of 66 % after 6 h was monitored by HPLC-MS with a ratio of 74 % product **64**, 20 % tetrapeptide Z-Leu-Ala-Phe-Phe-NH_2 (**66**) and 6 % hydrolysis towards **1d**. Side-product Z-Leu-Ala-Phe-Phe-NH_2 (**66**) is formed although the starting enolester amide **AM-*Z*-3l** was still present in the reaction solution. Consequently, after 23 h the reaction does not reach full conversion of the starting

material **AM-*Z*-3l** (89 % total conversion), because H-Phe-NH_2 (**53**) is already completely consumed by alcalase-CLEA forming the undesired tetrapetide **66**.

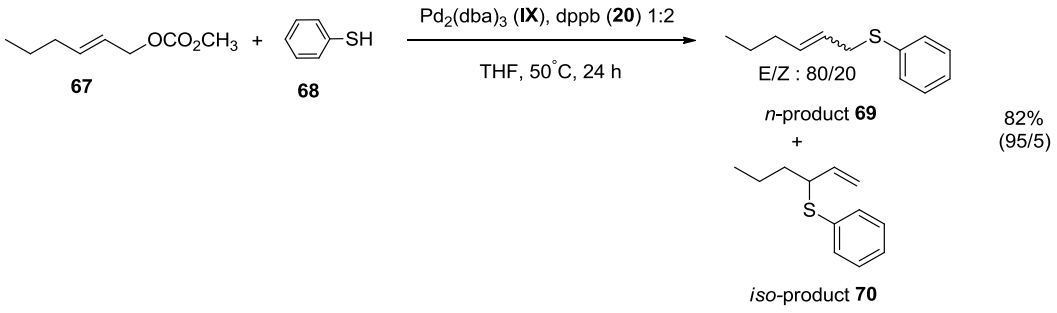
However, it can be said that enolester **AM-*Z*-3l** is an excellent substrate for alcalase-CLEA. In summary of all these results we are very optimistic that with a bit further optimization the combination of Soxhlet-condition and the use of enolesters like **AM-*Z*-3k** etc will be a powerful method for the synthesis of small to medium sized peptides.

4.3. Pd-catalyzed allylic alkylation reactions with sulfur-nucleophiles

4.3.1. Introduction

The synthesis of carbon sulfur bonds using metal-catalysis remains a challenge.[192],[248], [249] This is mainly due to the poisoning effect of sulfur atoms in low oxidation states e.g. thiols on transition metals.[203],[204] Sulfur may be able to lead to deactivation of the metal catalyst, because of the potential formation of relatively strong metal-sulfur bonds, preventing reactant access on the catalytic center.[197],[250] Additionally, organo sulfur compounds are prone to undergo oxidation and isomerization reactions.[251-253]

Sinou et al. described the formation of different allyl thioethers starting from allyl methyl carbonate using the catalyst-ligand system tris(dibenzylideneacetone)dipalladium(0) $Pd_2(dba)_3$ (**IX**) and diphenylphosphinobutane (dppb) (**20**). They only described the formation of allyl thioether from aromatic thiol compounds such as thiophenol, 2-mercaptopyridine, 2-mercaptopyrimidine, 4-hydroxythiopenol and thiobenzoxazolone in good to moderate yields. When (*E*)-hex-2-en-1-yl methyl carbonate (**67**) was reacted with thiophenol (**68**) an *n/iso*-mixture of 95/5 was obtained, where the *n*-product **69** consists of 80 % *E*- and 20 % *Z*-isomer (Scheme 86).[170]



Scheme 86: Pd-catalyzed allylation of thiophenol by Sinou *et al.*

Concerning the unwanted formation of different isomers efforts have to be made to improve the selectivity of this catalytic reaction to obtain only the desired *n*-isomer.

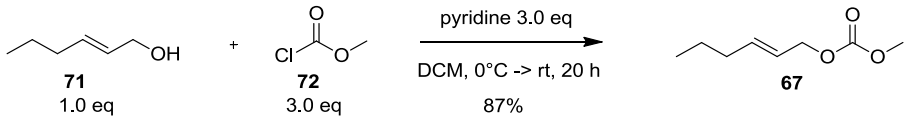
For our aim to develop a method using a Pd-catalyzed reaction for the farnesylation of a cysteine residue it was necessary to make aliphatic thiols accessible for this reaction. We envisioned that a screening of different bidentate phosphine ligands could possibly tune the catalyst to produce only the *n*-isomer. Concerning the above described poisoning effect of sulfur compounds on metal catalysis, we regarded bidentate phosphine ligands favourable as well accessible. Due to their relatively large bite angles[254-255]and their ability to cover

more of the palladium atom surface compared to mono phosphine ligands we hoped to prevent catalyst poisoning potentially provoked by the used thiols.

4.3.2. The Beginning

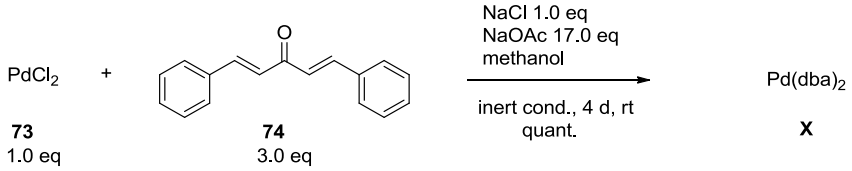
4.3.2.1. Synthesis of pre-catalyst and starting material

As a model substrate we used (*E*)-hex-2-en-1-yl methyl carbonate (**67**) which we synthesized in analogy to a procedure by Gilmore et al. (Scheme 87).[256-257] Using (*E*)-hex-2-en-1-ol (**71**) and methyl chloroformate (**72**) as starting materials, the reaction worked smoothly and after extraction and drying the product (*E*)-hex-2-en-1-yl methyl carbonate (**67**) could be isolated via vacuum distillation in 87 % yield.



Scheme 87: Synthesis of (*E*)-hex-2-en-1-yl methyl carbonate (**67**).

Additionally, we synthesized the pre-catalyst $Pd(dba)_2$ (**X**) (Scheme 88) following a protocol by Maitlis et al.[258] The reaction worked smoothly and we were able to obtain pre-catalyst $Pd(dba)_2$ (**X**) in quantitative yield.



Scheme 88: Synthesis of Bis(dibenzylideneacetone)palladium(0) $Pd(dba)_2$ (**X**).

4.3.2.2. Initial screening of a small amount of different ligands in different solvents

With pre-catalyst $Pd(dba)_2$ (**X**) and (*E*)-hex-2-en-1-yl methyl carbonate (**67**) in hand we screened a small set of ligands to explore, if our approach might be successful. It was regarded as necessary to work under inert conditions to avoid the formation of palladium black. It is a well known problem that precipitation of metal-catalysts can provoke the decomposition of the active catalyst species, going in hand with the loss of catalytic activity.

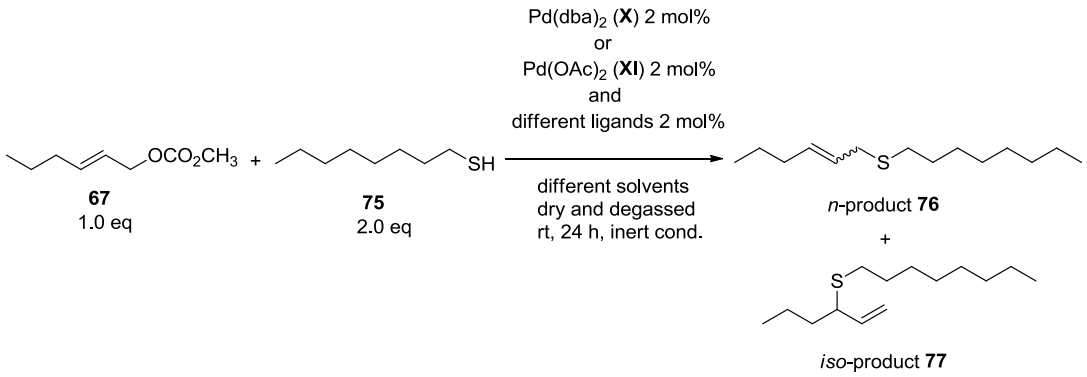
Especially, in homogeneous Pd-catalyzed reactions these aggregations, which form palladium black, are known.[259] In particular, this is true for our reaction system used, because in the catalytic cycle Pd(0) is also a putative intermediate necessary for the first step of oxidative addition in the catalytic cycle. Precipitation of the metal should not occur if the oxidative addition in the catalytic cycle is sufficiently fast.[260]

To be able to avoid the formation of palladium black we worked in all cases with degassed solvents, when this type of Pd reaction was used. Additionally, all magnetic stirring bars used were cleaned with *aqua regia*, neutralised and dried before use obtaining a perfectly clean surface to avoid any seeds for aggregations. All liquid starting materials like (*E*)-hex-2-en-1-yl methyl carbonate (**67**) and 1-octanethiol (**75**) were degassed freshly before usage. Working under inert conditions prevents oxidation reactions on thiols, which are sensitive towards oxidation forming disulfides.[251]

In our effort of using aliphatic thiol instead of aromatic thiols, which have been used before for this type of reaction, we chose 1-octanethiol (**75**) as test substrate due to its reduced unpleasant smell compared to other aliphatic thiols with lower boiling points.

The first step was the in situ formation of the putative catalytically active species. This was done by adding palladium pre-catalyst $Pd(dba)_2$ (**X**) and different phosphine ligands in a Schlenk-tube together with the solvent, degassed prior to use. Afterwards this homogeneous solution was stirred at 60°C for 30 min. In almost all cases one could observe a tiny amount of a black solid on the glass wall or on the magnetic stirring bar.

After the formation of the catalytically active species, the solution was cooled to rt and the starting materials (*E*)-hex-2-en-1-yl methyl carbonate (**67**) and 1-octanethiol (**75**) were added at rt using a Hamilton syringe under inert conditions (Scheme 89).



Scheme 89: Initial screening of ligands yielding a mixture of (*E*/*Z*)-hex-2-en-1-yl(octyl)sulfane (**76**) and hex-1-en-3-yl(octyl)sulfane (**77**).

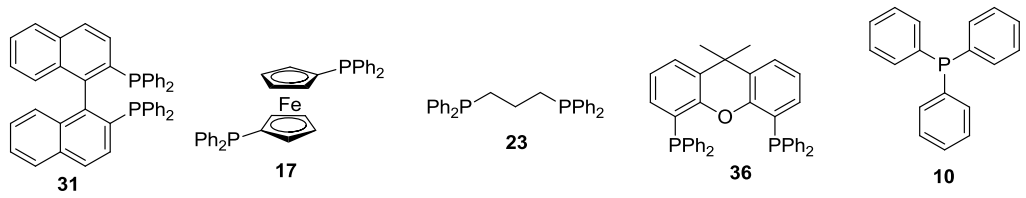


Figure 22: Phosphine ligands **10**, **17**, **23**, **31**, **36** used for initial screening.

Table 21: Results of the initial screening of ligands.

Entry	Pre-catalyst	Ligand	Conversion [%]					
			Toluene	DCM	THF	ACN	MeOH	H_2O/*tert*-butanol 1:3
1	$Pd(dba)_2$ (**X**)	BINAP (**31**)	0	0	0	0[b)]	0[b)]	0[b)]
2	$Pd(dba)_2$ (**X**)	dppf (**17**)	0	49	20	64[a)]	58	40
3	$Pd(dba)_2$ (**X**)	xantphos (**36**)	0	10	0	0[b)]	<1[b)]	0[b)]
4	$Pd(dba)_2$ (**X**)	dppp (**23**)	0	14	2	13	0	0[b)]
5	$Pd(dba)_2$ (**X**)	PPh_3 (**10**)[c)]	0	0	0	0	0[b)]	15
6	$Pd(OAc)_2$ (**XI**)	BINAP (**31**)	n.d.	0	n.d.	0	n.d.	n.d.
7	$Pd(OAc)_2$ (**XI**)	dppf (**17**)	n.d.	2	n.d.	0	n.d.	n.d.
8	$Pd(OAc)_2$ (**XI**)	xantphos (**36**)	n.d.	5	n.d.	0	n.d.	n.d.
9	$Pd(OAc)_2$ (**XI**)	dppp (**23**)	n.d.	0	n.d.	0	n.d.	n.d.
10	$Pd(OAc)_2$ (**XI**)	PPh_3 (**10**)[c)]	n.d.	0	n.d.	0	n.d.	n.d.

[a)] ratio: (*n*-product (**76**) / *iso*-product (**77**) = 95/5)
[b)] light orange-yellow to green suspensions occurred during formation of catalyst (active catalytic species)
[c)] 4 mol%

For the initial ligand screening we used BINAP (**31**), dppf (**17**), xantphos (**36**), dppp (**23**), PPh_3 (**10**) as ligands (Figure 22) and $Pd(dba)_2$ (**X**) and palladium(II) acetate $Pd(OAc)_2$ (**XI**) as palladium source (Table 21). The conversions after 24 h shown in Table 21 were monitored via GC-MS taking carbonate **67** as limiting component. This was considered as sufficient enough to obtain an impression whether our approach is successful or not. The reactions were performed using 2 equiv. thiol, to guarantee that during catalysis an excess of thiol is present to simulate the potential poisoning effect of thiols on palladium. In all cases using bidentate phosphine ligands BINAP (**31**), xantphos (**36**) and dppp (**23**) (Table 21, entry 1, 3,

4, 6, 8, 9) in the Pd-catalyzed allylic alkylation reaction on 1-octanethiol (**75**) no conversion or very low conversion was monitored via GC-MS. Also the use of monodentate phosphine ligand PPh_3 (**10**) did not lead to any conversion, with the exception of 15 % product formation using $Pd(dba)_2$ (**X**) in H_2O/*tert*-butanol 1:3 (Table 21, entry 5, 10). Fortunately, the utilization of 1,1'-bis(diphenylphosphino)ferrocene dppf (**17**) as bidentate phosphine ligand resoluted in acceptable conversions towards product hex-2-en-1-yl(octyl)sulfane (**76**) (Table 21, entry 2). It has to be mentioned that the pre-catalyst palladium(II) acetate $Pd(OAc)_2$ (**XI**) was not able to convert the starting material into the desired product **76**, even when using dppf (**17**) as ligand. However, the combination of $Pd(dba)_2$ (**X**) and dppf (**17**) seemed to be promising, especially regarding the reaction performed in acetonitrile. In acetonitrile the conversion was 64 % and an *n/iso* ratio of already 95/5 could be detected via GC-MS. Also the conversions in DCM and methanol or even in H_2O/*tert*-butanol 1:3 were quite encouraging (entry 2), indicating that this reaction might be compatible with several solvents with different polarities.

4.3.2.3. Initial ligand screening at higher temperature

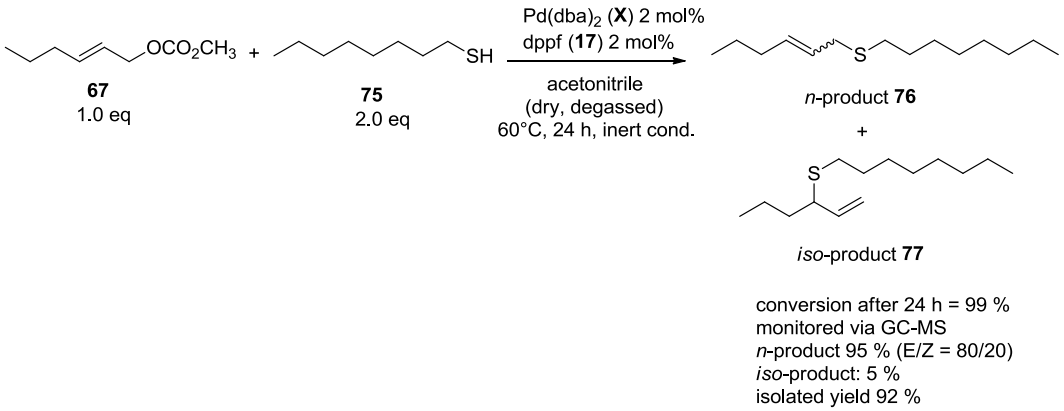
Encouraged by the good results obtained using $Pd(dba)_2$ (**X**) and dppf (**17**) in acetonitrile at rt, we were very optimistic that the conversion would increase with higher temperature. The reaction shown in Scheme 89 and in Table 21, entry 2 was performed in DCM under reflux conditions and in acetonitrile at 60°C. With the exception of BINAP ligand (**31**) and ligand PPh_3 (**10**) (Table 22, entry 1 and 5) the conversions could be improved. Regarding reactions performed with ligands xantphos (**36**) and dppp (**23**) it can be said that the conversion in DCM (reflux) and acetonitrile (60°C) is higher than at rt (Table 22, entry 3 and 4). Nevertheless, a conversion around 20 % in both cases was way too low for our ultimate goal to modify cysteines in proteins.

Table 22: Results of the ligand screening using higher temperature.

Entry	Pre-catalyst	Ligand	Conversion [%]	
			DCM under reflux conditions	ACN at 60°C
1	$Pd(dba)_2$ (**X**)	BINAP (**31**)	0	0[b]
2	$Pd(dba)_2$ (**X**)	dppf (**17**)	78 (95/5)[a]	99 (95/5)[a]
3	$Pd(dba)_2$ (**X**)	xantphos (**36**)	18 (96/4)[a]	15[b] (96/4)[a]
4	$Pd(dba)_2$ (**X**)	dppp (**23**)	22 (96/4)[a]	24 (96/4)[a]
5	$Pd(dba)_2$ (**X**)	PPh_3 (**10**)[c]	0	0[b]

[a] *n/iso* – ratio: (*n*-product (**76**) / *iso*-product (**77**))
[b] light orange-yellow suspensions occurred during formation of catalyst (active catalytic species)
[c] 4 mol%

Performing the Pd-catalyzed reaction together with $Pd(dba)_2$ (**X**) and dppf (**17**) under reflux condition, we could determine a conversion of ~80 % with a *n/iso* ratio of 95/5. Fortunately, the allylic sulfenylation reaction using dppf (**17**) as ligand in acetonitrile at 60°C led to almost full conversion measured via GC-MS after 24 h (Scheme 90). After purification via flash column chromatography a mixture of allylic thioether isomers **76** and **77** could be obtained in 92 % yield. GC-MS showed an *n/iso* ratio of 95/5, where the *n*-product **76** consist of 80 % *E*-isomer and 20 % *Z*-isomer analyzed via NOESY-NMR-spectroscopy.



Scheme 90: Results using dppf (**17**) as ligand at 60°C in acetonitrile.

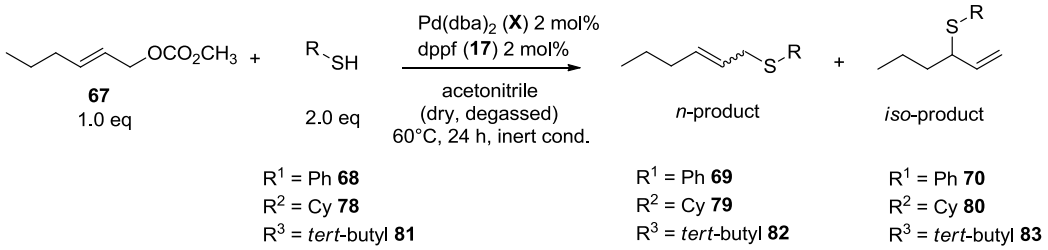
Interestingly, the *n/iso* ratio of 95/5 was the same whether dppf (**17**) was used in DCM or in acetonitrile. For the *n/iso* ratio also the temperature was not relevant, which was also observed by Sinou et al. using dppb (**20**) together with aromatic thiols (Scheme 86).[170],[174]

Our hope, that 1,2-dichloroethane instead of DCM at 60°C for the Pd-catalyzed reaction together with $Pd(dba)_2$ (**X**) and dppf (**17**) would result in full conversion was not fulfilled. Unfortunately, performing the above mentioned reaction in 1,2-dichloroethane (DCE), which has quite similar properties to DCM, only 3 % conversion could be monitored by GC-MS. Until now, no logic reason can be given for this dramatic solvent effect occurring during this Pd-catalyzed reaction.

4.3.3. Pd-catalyzed allylic alkylation reactions using dppf as ligand

4.3.3.1. Pd-catalyzed allylic alkylation reactions with different thiols

With reaction conditions in hand that lead to a conversion of 99 % using primary thiol 1-octanethiol (**75**) as starting material, it was of interest to know if also secondary or tertiary thiols will be appropriate substrates for Pd-catalyzed allylic sulfenylation.



Scheme 91: Pd-catalyzed allylic alkylation using dppf (**17**) as ligand and thiophenol (**68**), cyclohexanethiol (**78**), and 2-methyl-2-propanethiol (**81**) at 60°C in acetonitrile.

Therefore, thiophenol (**68**), cyclohexanethiol (**78**), and 2-methyl-2-propanethiol (**81**) were used for the reaction described in chapter 4.3.2.3 (Scheme 91).

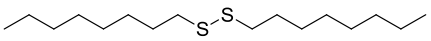
With thiophenol (**s10a**), we obtained >99 % conversion after 24 h and an isolated yield of 92 % of allylic thioether isomers **69** (*n*-product) and **70** (*iso*-product). The *n/iso* ratio was 95/5, where the *n*-product **69** consist of 80 % *E*-isomer and 20 % *Z*-isomer. This result cannot be considered as an improvement, but as comparable to the already described reaction from Sinou et al.[170]

However, more interestingly was the conversion of secondary and tertiary thiols. Using cyclohexanethiol (**78**) as starting material the catalysis worked smoothly with >99 % conversion after 24 h and an isolated yield of 90 % for cyclohexyl(hex-2-en-1-yl)sulfane (**79**). The *n/iso* ratio of the crude product (isomeric mixture of **79** and **80**) was 95/5, where the *n*-product **79** consists of 80 % *E*-isomer and 20 % *Z*-isomer.

After this great and encouraging result using the secondary thiol **78** to the Pd-catalyzed reaction, we were keen to know if also a tertiary thiol like **81** can be converted into thioether. Fortunately, we could obtain a conversion of 99 % after 24 h and an isolated yield of 84 % for *tert*-butyl(hex-2-en-1-yl)sulfane (**82**). The *n/iso* ratio of the crude product (isomeric mixture of **82** and **83**)was 78/22, where the *n*-product **82** consist of 80 % *E*-isomer and 20 % *Z*-isomer. Although we could observe 99 % conversion the *n/iso* ratio is not as good as we hoped. Nevertheless, due to the fact that a secondary thiol as well as a tertiary thiol can be converted under the conditions mentioned in chapter 4.2.3.2, we were optimistic, that also the conversion of molecules containing a cysteine moiety should be achievable.

4.3.3.2. Sensitivity of the Pd-catalyzed reaction towards air

As mentioned in chapter 4.3.2.2 we had difficulties performing a Pd-catalyzed alkylation under not inert conditions, we still were interested if these concerns are also true in this special case. Therefore, we performed the allylic alkylation reaction with $Pd(dba)_2$ (**X**) and dppf (**17**) under air. All reactions were performed in DCM and the first reaction was started as described in chapter 4.3.2.2 under argon. After 30 min a sample for GC-MS measurement was taken and at the same time the argon atmosphere was changed against air. GC-MS showed a conversion of ~20 % towards product thioether **76**. The conversion after 5 h (4.5 h under air) was 34 % and after 24 h the conversion was 34 %, indicating that working under air stops the catalysis. Interestingly, the formation of the disulfide 1,2-dioctyldisulfane (**84**) was only ~5 % as monitored with GC-MS (Figure 23). As a control experiment the same reaction was performed the whole time under argon atmosphere as already described in chapter 4.3.2.2 were the conversion was similar to the conversion mentioned in Table 21, entry 2. Performing the reaction under air right after the catalytic active species has formed under argon 12 % conversion towards product **76** were detected after 30 min and 14 % after 24 h. However, although the disulfide formation **84** is with 5 % not really high it is higher than under inert conditions where the disulfide formation is in a range < 1 % or in rare cases < 2 %. These low percentage of disulfide **84** detected might also be due to the GC-MS measurement which is done with not degassed solvents to dissolve the sample (usually in ethyl acetate or DCM). Nevertheless the experiments showed the necessity to work under inert conditions and with degassed solvents and degassed liquid starting materials to avoid disulfide formation **84**.[131] In rare cases even the use of nitrogen as inert gas atmosphere was not appropriate, because sometimes after taken a sample for GC-MS (under inert conditions see experimental part) the reaction stopped and the formation of disulfide **84** was higher than usual.



84

Figure 23: Side-product 1,2-dioctyldisulfane (**84**) formed from two molecules of 1-octanethiol (**75**).

As another attempt, we were interested, if the addition of butylhydroxy toluene (BHT) is beneficial to this reaction, because BHT is commonly used as an antioxidant to avoid or reduce product changes caused by oxidation.

Adding 2 equiv. BHT to the reaction mentioned in Scheme 88 (chapter 4.3.2.3), we observed only ~3 % product formation. Also the reaction performed with 2 equiv. of BHT in darkness led only to ~3 % product formation. Maybe, BHT interferes in the necessary oxidative addition step in the beginning of the palladium catalytic cycle, and prevents efficient catalysis.

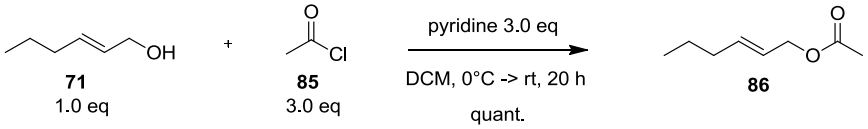
4.3.3.3. Varying equivalents of starting material and catalyst

For our purpose to prenylate cysteine containing molecules, it is clear, that the thiol moiety on cysteine will be the limiting component. Therefore, we decided to reduce the excess of 1-octanethiol (**75**) used to 1.1 equiv. Performing our model-reaction shown in Scheme 90 chapter 4.3.2.3 with 2 mol% of $Pd(dba)_2$ (**X**) and 2 mol% dppf (**17**) in acetonitrile we could show, that this reaction performs with 99 % conversion after 24 h as good using 2 equiv. of 1-octanethiol (**75**). Also the *n/iso* ratio was with 94/6 nearly the same.

Another interesting aspect regarding the Pd-catalyzed reaction with the catalyst ligand system $Pd(dba)_2$ (**X**) and dppf (**17**) was the reduction of the amount of catalyst used (Scheme 90). Reactions were performed to investigate how much catalyst is necessary to obtain still satisfying conversions. We could obtain 99 % conversion after 24 h, when instead of 2 mol% just 1.5 mol% $Pd(dba)_2$ (**X**) and 1.5 mol% dppf (**17**) were used. The *n/iso* ratio was 94/6 monitored by GC-MS. In contrast the use of 1 mol% $Pd(dba)_2$ (**X**) and 1 mol% dppf (**17**) to catalyze the formation of hex-2-en-1-yl(octyl)sulfane (**76**) and the corresponding *iso*-product **77** only led to 57 % conversion with an *n/iso* ratio of 94/6. Due to this result we decided to use 2 mol% of $Pd(dba)_2$ (**X**) and 2 mol% dppf (**17**) in our reactions, to have enough reserves left when it comes to more challenging substrates.

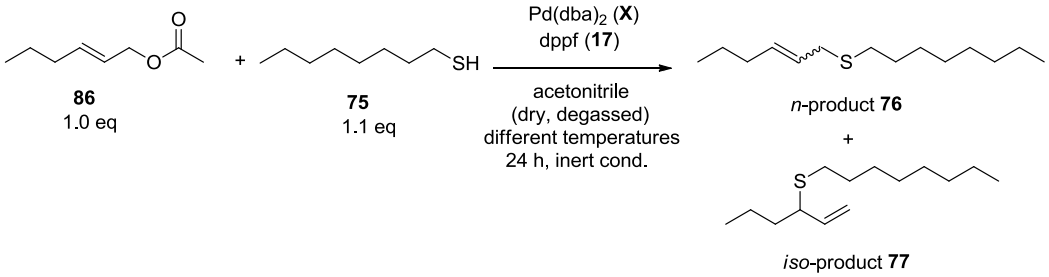
4.3.3.4. (*E*)-Hex-2-en-1-yl acetate as substrate for the Pd-catalyzed alkylation of 1-octanethiol

In chapter 4.3.2.3 we could show, that the use of (*E*)-hex-2-en-1-yl methyl carbonate (**67**) resulted in good conversions for the Pd-catalyzed reaction. The advantage of using carbonates is the loss of carbon dioxide during catalysis, which shifts the equilibrium in favor of the product side.[170-171] In principle also other leaving groups such as acetates, halides or carbamates are possible. We synthesized (*E*)-hex-2-en-1-yl acetate (**86**) using acetyl chloride (**85**) to test the suitability of allylacetates as substrates (Scheme 92).



Scheme 92: Synthesis of (*E*)-hex-2-en-1-yl acetate (**86**).

(*E*)-Hex-2-en-1-yl acetate (**86**) was used in the Pd-catalyzed reaction with catalyst-ligand system $Pd(dba)_2$ (**X**) and dppf (**17**) as illustrated in Scheme 93.



Scheme 93: Pd-catalyzed synthesis of *β*,*γ*-unsaturated thioether (**76**) using (*E*)-hex-2-en-1-yl acetate (**86**).

Table 23 summarizes the results we could obtain using different amounts of $Pd(dba)_2$ (**X**) and dppf (**17**) at different temperatures.

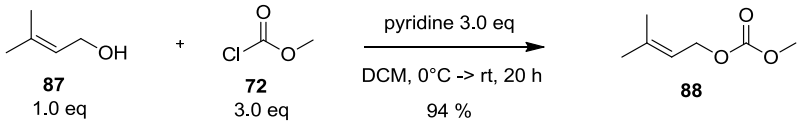
Table 23: Results of using (*E*)-hex-2-en-1-yl acetate (**86**) instead of the corresponding carbonate **67**.

Entry	$Pd(dba)_2$ (X)	dppf (17)	Temp [°C]	Conversion GC-MS [%] (*n*/*iso*-ratio)
1	2 mol%	2 mol%	60	60 (94/6)
2	2 mol%	2 mol%	reflux	72 (93/7)
3	4 mol%	4 mol%	60	85 (95/5)
4	6 mol%	6 mol%	60	99 (95/5)

With 2 mol% of $Pd(dba)_2$ (**X**) and dppf (**17**) we were able to obtain only 60 % conversion after 24 h (Table 23, entry 1) in comparison to the reaction where (*E*)-hex-2-en-1-yl methyl carbonate (**67**) with the same amount of catalyst, ligand and 1.1 equiv. of 1-octanethiol (**75**) was used (chapter 4.3.2.3), which resulted in full conversion after 24 h. Unfortunately, raising the temperature to reflux conditions of acetonitrile did not lead to full conversion, although the conversion is with 72 % higher than previously (entry 2). Even with 4 mol% $Pd(dba)_2$ (**X**) and 4 mol% dppf (**17**) we could only obtain 85 % conversion after 24 h (entry 3). Finally, the use of 6 mol% of $Pd(dba)_2$ (**X**) and 6 mol% of dppf (**17**) were enough to obtain 99 % conversion after 24 h (entry 4). From these experiments we learned that the use of carbonates is the best choice or that we had to face the necessity of searching for a better ligand than dppf (**17**). The decision was made to first investigate further acetates and carbonates.

4.3.3.5. The use of isoprenyl carbonate as starting material for the Pd-catalyzed alkylation of 1-octanethiol

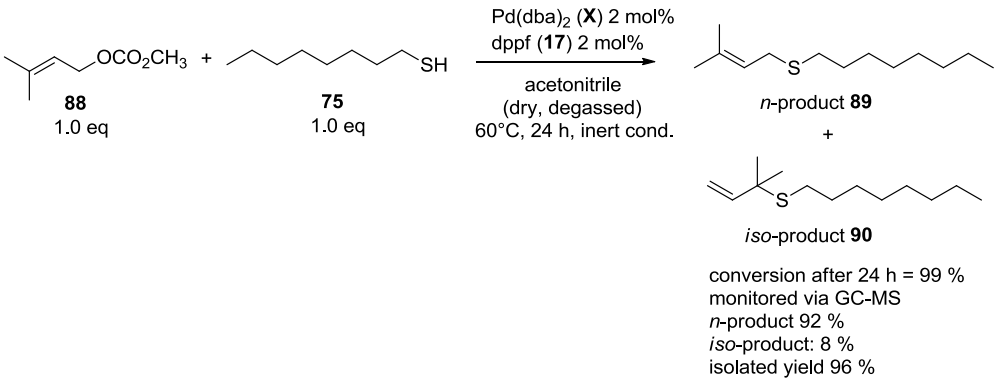
To pursue our goal to farnesylate cysteine containing biomolecules, it was necessary to have a cheap test substrate in hand, which contains already the main features of farnesol. We chose 3-methylbut-2-en-1-ol (isoprenyl alcohol) (**87**). It resembles farnesol, but is shorter in length simplifying the analytical characterization.



Scheme 94: Synthesis of methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**).

Following the protocol by Gilmore[256] we obtained a colorless liquid of methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) in 94 % yield after extraction and vacuum distillation (15 mbar) (Scheme 94).

We were very interested, how isoprenyl carbonate **88** would behave in the Pd-catalyzed allylic sulfenylation using $Pd(dba)_2$ (**X**) and dppf (**17**) (Scheme 95).



Scheme 95: Pd-catalyzed synthesis of *β*,*γ*-unsaturated thioether **89** starting from isoprenyl carbonate (**88**).

The conversion appeared to be with 99 % after 24 h as good as hoped. Unfortunately, the *n/iso* ratio monitored by GC-MS was 92/8. This *n/iso* ratio was not as good as the *n/iso* ratio of 95/5 regarding the Pd-catalyzed allylic sulfenylation reaction with (*E*)-hex-2-en-1-yl methyl carbonate (**67**) as starting material as described in chapter 4.3.2.3. The product could be obtained in 96 % yield as a mixture of **89** and **90** after purification via flash column chromatography. Due to the fact that we wanted to obtain only a single isomer (*n*-product **89**) we performed an additional ligand screening to hopefully identify a ligand, which enables to fulfil this proposition. This screening is described in chapter 4.3.4.

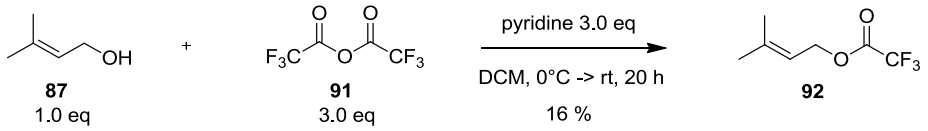
At this stage of the project we were interested to evaluate if our palladium reaction is able to isoprenylate cysteine. This is described in chapter 4.3.3.8.

To exclude that also the palladium pre-catalyst $Pd(dba)_2$ (**X**) by itself is able to catalyze this transformation, we performed the reaction in *tert*-butanol/H_2O 3:1 using exclusively $Pd(dba)_2$ (**X**) without any ligand. As expected we were not able to detect any formation of product **89**. However, it is important to mention that we did observe ~9 % disulfide **84** formation after 24 h, although during any time of the catalysis the work was performed under inert conditions. A repetition of the reaction under identical conditions showed almost the same results. Even after 30 min reaction time already 8 % of disulfide **84** was formed. It might be that oxidation

of 1-octanethiol (**75**) due to the not inert conditions during the GC measurement could have occured.

4.3.3.6. The use of methyl-3-methylbut-2-enyl 2,2,2-trifluoroacetate as starting material for the Pd-catalyzed alkylation of octanethiol

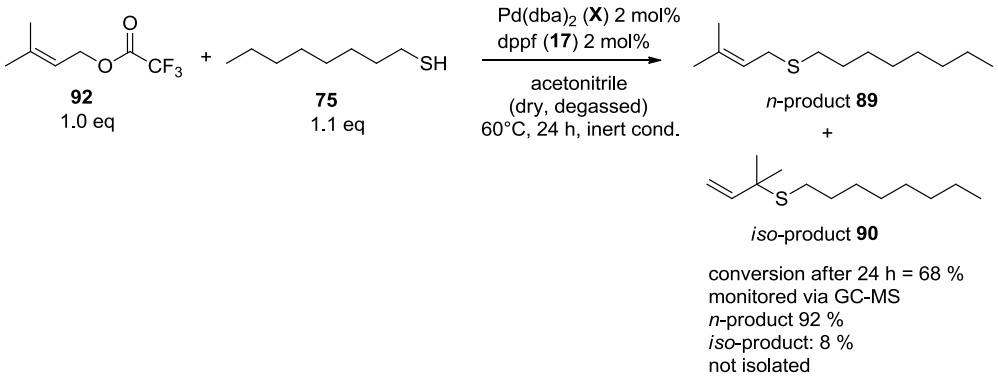
After having obtained allylic thioether **89** using (*E*)-hex-2-en-1-yl methyl carbonate (**67**) as starting material in very good yield, we wanted to know if 3-methylbut-2-en-1-yl 2,2,2-trifluoroacetate (**92**) would be also a good substrate for this catalysis reaction mentioned in chapter 4.3.3.5. Substrate **92** containing the isoprenyl moiety, would be the fluorinated analogue to (*E*)-hex-2-en-1-yl acetate (**86**), which did not show equal performance compared to carbonates used so far.



Scheme 96: Synthesis of methyl-3-methylbut-2-enyl 2,2,2-trifluoroacetate (**92**).

Adding 2,2,2-trifluoroacetic anhydride (**91**) to isoprenyl alcohol **87** under the same conditions mentioned in chapter 4.3.2.1 in Scheme 87, product 3-methylbut-2-en-1-yl 2,2,2-trifluoroacetate (**92**) was formed after 20 h. Unfortunately, due to the low boiling point of product **92** only 16 % yield were obtained after vacuum distillation (Scheme 96).

Product **92** was used for the Pd-catalyzed allylic sulfenylation using $Pd(dba)_2$ (**X**) and dppf (**17**) (Scheme 97).



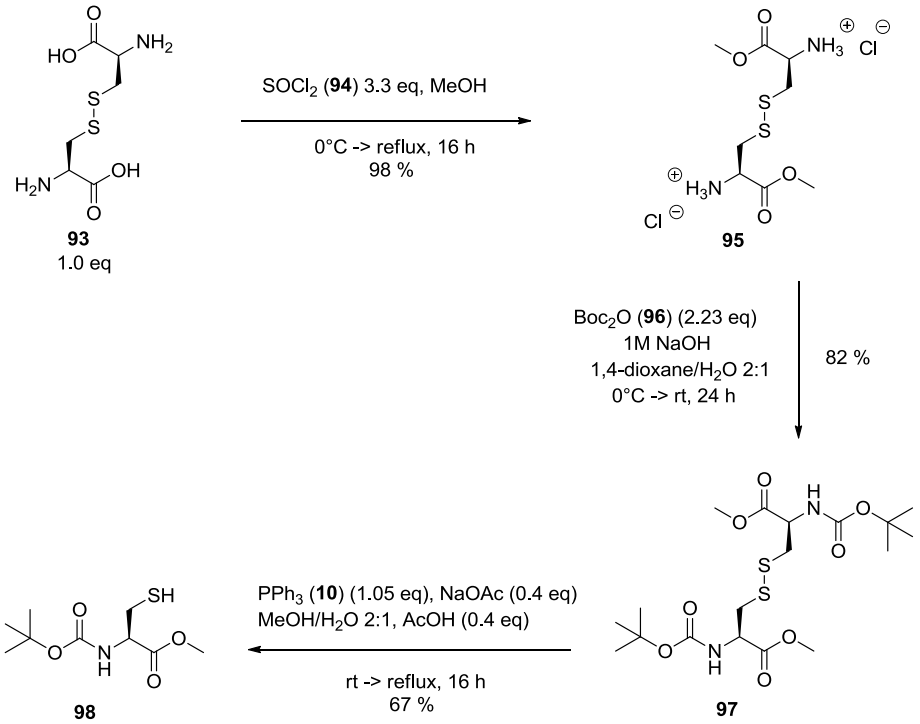
Scheme 97: Pd-catalyzed synthesis of *β*,*γ*-unsaturated thioether **89** starting from 3-methylbut-2-en-1-yl 2,2,2-trifluoroacetate **92**.

The reaction reached only 68 % product formation after 24 h with an *n/iso* ratio of the crude product (isomeric mixture of **89** and **90**) of 92/8, monitored by GC-MS. The conversion of 68 % towards **89** is in the same range as the use of (*E*)-hex-2-en-1-yl acetate (**86**) described in chapter 4.3.3.4, Table 23. The use of fluorinated acetate instead of acetate did not lead to a significant improvement of product formation. We did not take efforts to further investigate other leaving groups.

4.3.3.7. Synthesis of L-cysteine-derivatives as starting material for the Pd-catalyzed alkylation of 1-octanethiol

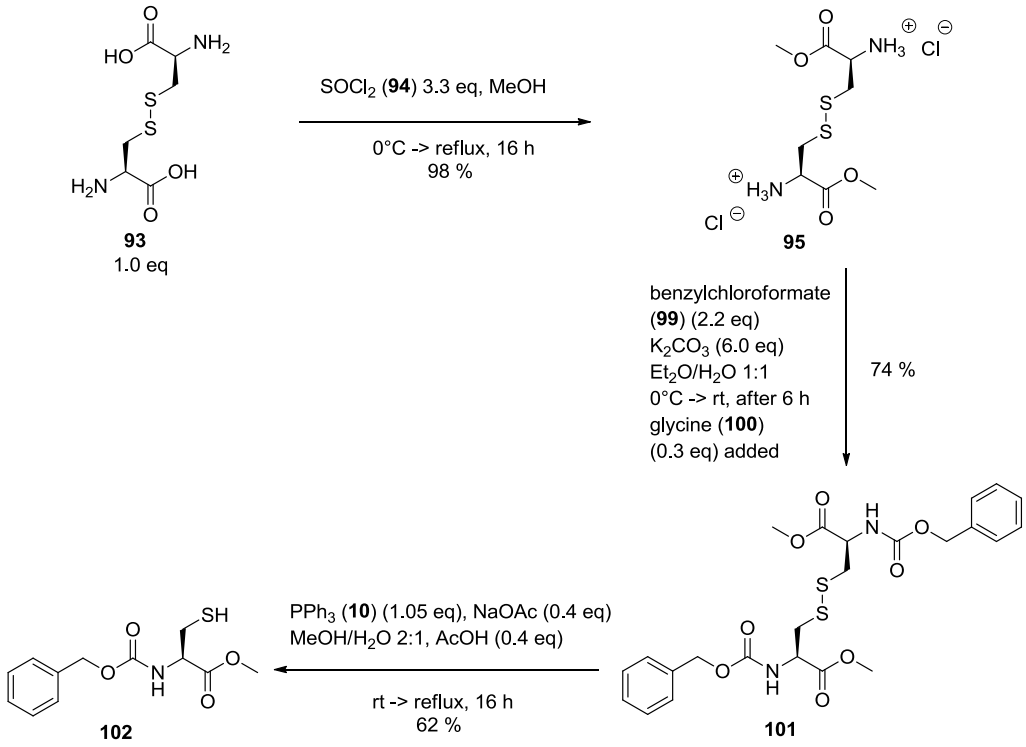
Our main goal was to farnesylate cysteine containing biomolecules. To be able to investigate if $Pd(dba)_2$ (**X**) and dppf (**17**) mediate the allylation of cysteine derivatives, we synthesized three different L-cysteine-derivatives: *N*-terminally Boc-, Cbz- and Bz- protected L-cysteines, which contain a methyl ester moiety at the *C*-terminus. There are two reasons to convert the carboxylic acid group of L-cysteine into a methyl ester group. First, substrates for naturally occurring prenylations e.g. farnesylation of Ras-protein contain a methyl ester group at the *C*-terminal end of the protein.[16] Second, better solubility of methyl ester group in organic solvents were beneficial for our synthetic efforts.

The formation of the methyl ester **95** was the same for all mentioned synthetic routes to carbamate-protected L-cysteine methyl esters. The synthesis of L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (**98**) was performed according to Boucher et al.[261] Starting from L-cysteine (**93**) it was possible to obtain L-cystine methyl ester hydrochloride **95** in an excellent yield of 98 % using thionylchloride (**94**) in methanol.



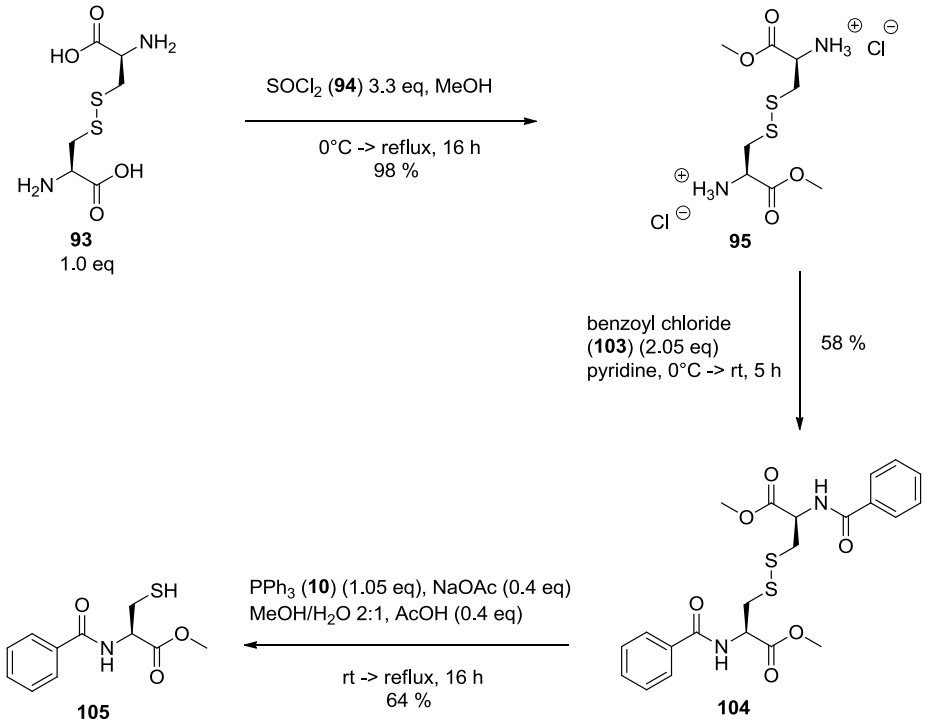
Scheme 98: Synthesis of L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (**98**).[261]

After recrystallization of L-cystine methyl ester hydrochloride **95**[262] di-*tert*-butyl-pyrocarbonate (**96**) was added together with 1M NaOH in 1,4-dioxane/H_2O 2:1. After extraction and purification using flash column chromatography product **97** could be obtained in 82 % yield. Boc-protected L-cystine methyl ester **97** was then reduced with triphenylphosphine (**10**) in MeOH/H_2O 2:1 together with sodium acetate and acetic acid. The addition of acetic acid helped to convert the formed suspension into a solution. After 16 h reaction time and after extraction and purification via flash column chromatography L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (**98**) containing the necessary free thiol-group was obtained in 67 % yield (Scheme 98).



Scheme 99: Synthesis of L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**).

As already described above L-cystine methyl ester hydrochloride **95** could be obtained in 98 % yield. With benzylchloroformate (**99**) and K_2CO_3 in diethylether/H_2O 1:1 L-cystine methyl ester hydrochloride **95** was Cbz-protected. After 6 h glycine (**100**) was added as a scavenger for the excess of benzylchloroformate (**99**) and the solution was stirred for an additional 18 h.[263] After Kugelrohr-distillation (50°C, 0.05 mbar, 5 h) product L-cystine *N,N'*-bis[(phenylmethoxy)carbonyl] 1,1' dimethyl ester (**101**) was obtained in 74 % yield as a clear oil that solidifies upon cooling. Product **101** was then reduced with triphenylphosphine (**10**) in MeOH/H_2O 2:1 together with sodium acetate and acetic acid. After 16 h reaction time, extraction and purification via flash column chromatography product L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) containing the necessary free thiol-group was obtained in 62 % yield (Scheme 99).



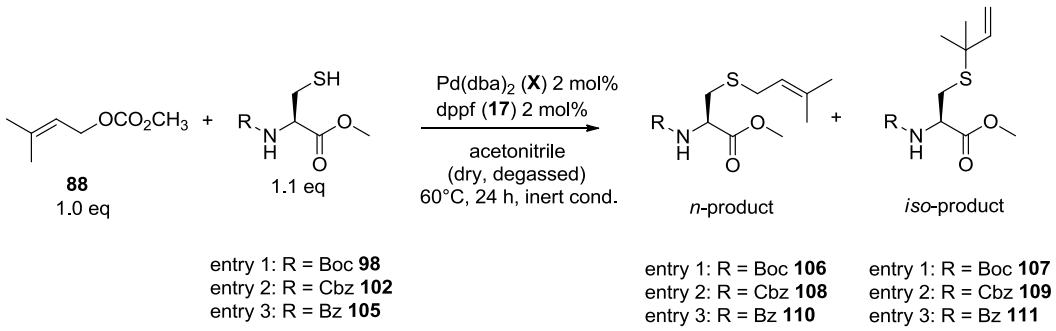
Scheme 100: Synthesis of L-cysteine *N*-benzoyl methyl ester (**105**).

Benzoylchloride (**103**) was added at 0°C to hydrochloride **95** in pyridine. After 5 h distilled water was added until a precipitate formed. The precipitate was collected by filtration and dried in vacuo (0.02 mbar) to yield product L-cystine *N,N'*-dibenzoyl-1,1' dimethyl ester (**104**).[262] The attempt to further purify the product **104** by flash column chromatography was not successful. However, the purity of product **104** was good enough to use it directly in the next reaction. Product **104** was reduced with triphenylphosphine (**10**) in MeOH/H_2O 2:1 together with sodium acetate and acetic acid. After 16 h, extraction and purification using via column chromatography L-cysteine *N*-benzoyl methyl ester (**105**) containing the necessary free thiol-group was obtained in 64 % yield (Scheme 100).

With these carbamate-protected L-cysteine methyl esters in hand we were now able to test them as substrates in the Pd-catalyzed allylic sulfenylation reaction described in the next chapter 4.3.3.8.

4.3.3.8. Pd-catalyzed alkylation of different L-cysteine-derivatives

After having synthesized starting materials **98**, **102** and **105** we were interested if it will be possible to convert these carbamate-protected L–cysteine methyl esters into their corresponding allylic thioethers using our developed Pd-catalyzed allylic sulfenylation reaction using $Pd(dba)_2$ (**X**) and dppf (**17**) as catalyst ligand system.



Scheme 101: Pd-catalyzed allylic alkylation using L-cysteine derivatives **98**, **102**, **105** as starting materials.

We were pleased to see that all three carbamate-protected L-cysteine methyl esters **98**, **102** and **105** gave full conversion after 24 h (Scheme 101, Table 24). For L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (**98**) as starting material the conversion was determined using TLC, because product thioether **106** was not detectable on GC-MS. One possible explanation is decomposition (especially of the Boc-group) due to heating (250-280°C) in the inlet of the GC-MS apparatus. Z-protected L-cysteine methyl esters **108** and Bz-protected L-cysteine methyl esters **110** could be monitored via GC-MS, but no separation of *n/iso*-product was achieved. The *n/iso* ratio was determined by 1H-NMR and TOCSY-NMR.

Table 24: Results for Pd-catalyzed allylic alkylation using L-cysteine derivatives **98**, **102**, **105** as starting materials.

Entry	Starting material	Products	Conversion GC/MS after 24 h [%]	*n*/*iso*-ratio (NMR)	Isolated yield [%]
1	Boc-L-cysteine methyl ester **98**	**106**/**107**	n.d.	8:1	83
2	Cbz-L-cysteine methyl esters **102**	**108**/**109**	>99	6:1	93
3	Bz-L-cysteine methyl esters **105**	**110**/**111**	>99	6:1	93

Unfortunately, the *n/iso* ratios obtained were not as good as expected from the experiments performed with 1-octanethiol (**75**) as starting material. The allylic sulfenylation isomeric product mixture **106**/**107** of starting material L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (**98**) showed a *n/iso* ratio of 8:1 (Table 24, entry 1). This mixture of *n*-product **106** and *iso*-product **107** contained a much higher amount of *iso*-product **107** than obtained before with starting material 1-octanethiol (**75**) with a *n/iso* ratio of 95:5. Using L-cysteine methyl ester **98** as substrate the amount of *iso*-product **107** detected is ~2 times higher than with 1-octanethiol (**75**) used as starting material for the allylic sulfenylation reaction. For L-

cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) and L-cysteine *N*-benzoyl methyl ester (**105**) used as substrates for the Pd-catalyzed allylic sulfenylation the *n/iso* ratios of 6:1 are even worse. Compared to the *n*-products **108** and **110** the amount of *iso*-products **109** and **111** detected is even ~3 times higher than with 1-octanethiol (**75**) used as starting material for the allylic sulfenylation reaction.

In summary our approach to prenylate cysteine containing biomolecules is in principle possible using the Pd-catalyzed allylic sulfenylation reaction. However, more work was necessary to improve the *n/iso* ratio. This work will be presented in the next chapter 4.3.4.

4.3.4. Screening of a large ligand set for the Pd-catalyzed allylic alkylation reaction

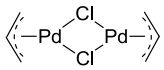
An isomeric mixture (*n/iso*) is undesirable for our ultimate purpose to farnesylate L-cysteine containing peptides, because in nature an isomerisation does not occur.[14],[132]

We initiated a new and larger ligand screening to achieve two aims. First we wanted to obtain full conversion of starting material as fast as possible, because a fast reaction would in principle also work under more difficult conditions as in the case where a cysteine residue would not be easily accessible. Second was the improvement of the *n/iso* ratio. Our hope was to find a new catalyst ligand system which is able to exclusively produce our desired *n*-product.

As a more or less extra feature we hoped to find a catalyst ligand system, which is less sensitive to the presence of air. For all reactions performed during the ligand screening the conversion was measured after 24 h. The main reason for that was the sensitivity of the Pd-catalyzed reaction against oxygen as already mentioned in chapter 4.3.3.2. Especially, when catalyst ligand system $Pd(dba)_2$ (**X**) and dppf (**17**) were used, it was of importance to work under strictly inert conditions. In rare cases the catalysis stopped after taking a sample for GC measurements, even when working under nitrogen. Therefore use of argon is recommended. The main structural element of dppf (**17**) is ferrocene, which is known to have an affinity towards oxygen, because it is used as an additive of petrol to ensure better oxygen binding and therefore a better burning of petrol.[264]

For a ligand screening we sticked to $Pd(dba)_2$ (**X**) as palladium pre-catalyst, due to previous experiments where we could show that use of $Pd(OAc)_2$ (**XI**) was less favourable. Also the use of allylpalladium(II) chloride dimer (**XII**) (Figure 24) and dppf (**17**) with methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) as substrates in

acetonitrile under the same conditions as described in chapter 4.3.3.5 and Scheme 95 led only to 28 % conversion after 24 h.



XII

Figure 24: Structure of allylpalladium(II) chloride dimer (**XII**).

The reaction conditions for the ligand screening were nearly identical to those mentioned in chapter 4.3.3.5 in Scheme 95. Again acetonitrile was the solvent of choice, due to its good solubility properties for a lot of different ligands. An important change was the use of 1.2 equiv. of methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1.0 equiv. of 1-octanethiol (**75**) (Scheme 102). The main reason was that in the case of L–cysteine containing biomolecules these molecules would be the expensive and limiting component and not the carbonate.

For accurate calculation of conversion of starting material, we used diethylene glycol dimethyl ether (**112**) as an internal standard. The same volume of diethylene glycol dimethyl ether (**112**) compared to 1-octanethiol (**75**) was used.

First the active catalytic species was formed in situ by stirring palladium pre-catalyst $Pd(dba)_2$ (**X**) and the ligand for 30 min at 60°C under inert conditions in acetonitrile, degassed prior to use. In some cases the solution contained almost negligible amounts of black solid. Afterwards 1-octanethiol (**75**) and diethylene glycol dimethyl ether (**112**), both degassed prior to use, were added under inert conditions to the 60°C hot solution. Right afterwards the first sample for the GC (FID) measurement was taken under inert conditions. Due to that first GC (FID) measurement, before the reaction has even started, it was possible to calculate the "initial ratio" defined below.

$$\text{initial ratio} = \frac{\text{area starting material}}{\text{area internal standard}}$$

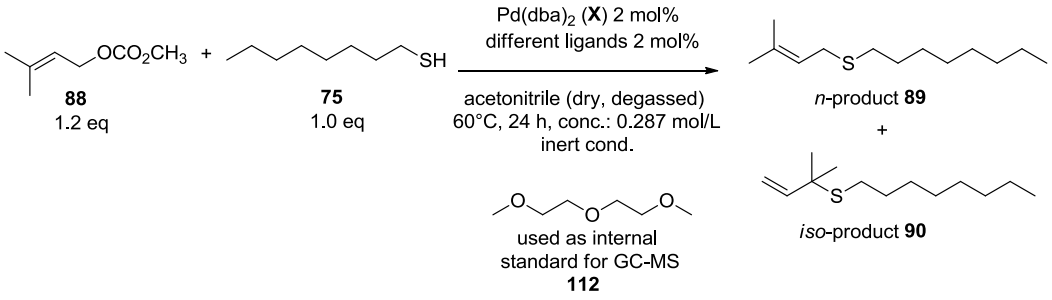
After the first sample for the GC to calculate the "initial ratio" was taken under inert conditions, methyl-3-methylbut-2-enyl carbonate (**88**), degassed prior to use, was added again under inert conditions. After 24 h a second sample for GC was taken under inert conditions. To calculate the conversion, the following formula was used.

$$\text{conversion}[\%] = 100 - (100 \cdot \frac{\text{area remaining starting material} / \text{area internal standard}}{\text{initial ratio}})$$

Our aim was to screen a diverse set of phosphine ligands featuring different steric and electronic properties (Figure 25). We expected that different amounts of conversion and different *n/iso* ratios of the product would teach us lessons about the influence of the steric and electronic property of the ligands on the reactivity and selectivity of the reaction.

The Tolman parameters[235] θ and χ are useful tools to quantify steric and electronic properties in monophosphine ligands.[265] An important parameter in the discussion of bidentate (chelating) phosphine ligands is the natural bite angle, which is widely used and determined by molecular mechanics calculations.[254] They are called bidentate (chelating) phosphine ligands, because these ligands contain two phosphorus atoms that bind through two sites to the palladium. Due to their bidentate character one can change steric interactions between ligand and pre-catalyst by the introduction of changes on the backbone of the ligand. For dppm (**21**) and dppe (**22**), to name just one example, this is possible without changing the substituents or the environment at the phosphorous atom. These changes of the natural bite angle due to the use of different bidentate (chelating) phosphine ligands alter the activity and selectivity of the catalytic active species, because the changes in steric interactions between catalyst and ligand can result in a change of energies of the transition state of the catalysis. [265] However, the natural bite angle cannot be regarded as fixed, there is a flexibility range for each ligand, for example xantphos ligand **36** has a natural bite angle of 111.7° with a flexibility range of 36°.[266]

Beside steric effects mentioned above we were also interested to investigate ligands with different electronic properties (electron rich or electron poor ligands), because obviously, this also changes energies of the transition state of the catalysis. Basicity of phosphorous ligands differ depending on electronic properties of the residue attached to the phosphorous atom. As an example, if in the case of phosphines PR_3, R represents an alkyl moiety. These electron donating substituents increase the basicity and therefore also the σ-donor ability of the phosphorous atom, which is important to form stable phosphorous-metal complexes. In contrast due to the electron withdrawing ability of the oxygen atoms connected to the phosphorous atom in phosphite ligands $P(OR)_3$ the basicity is lower compared to phosphine ligands. Nevertheless, phosphite ligands are strong π-acceptors and therefore at least form stable phosphorous-metal bonds with electron rich transition metals.[255]



Scheme 102: Screening conditions used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**).

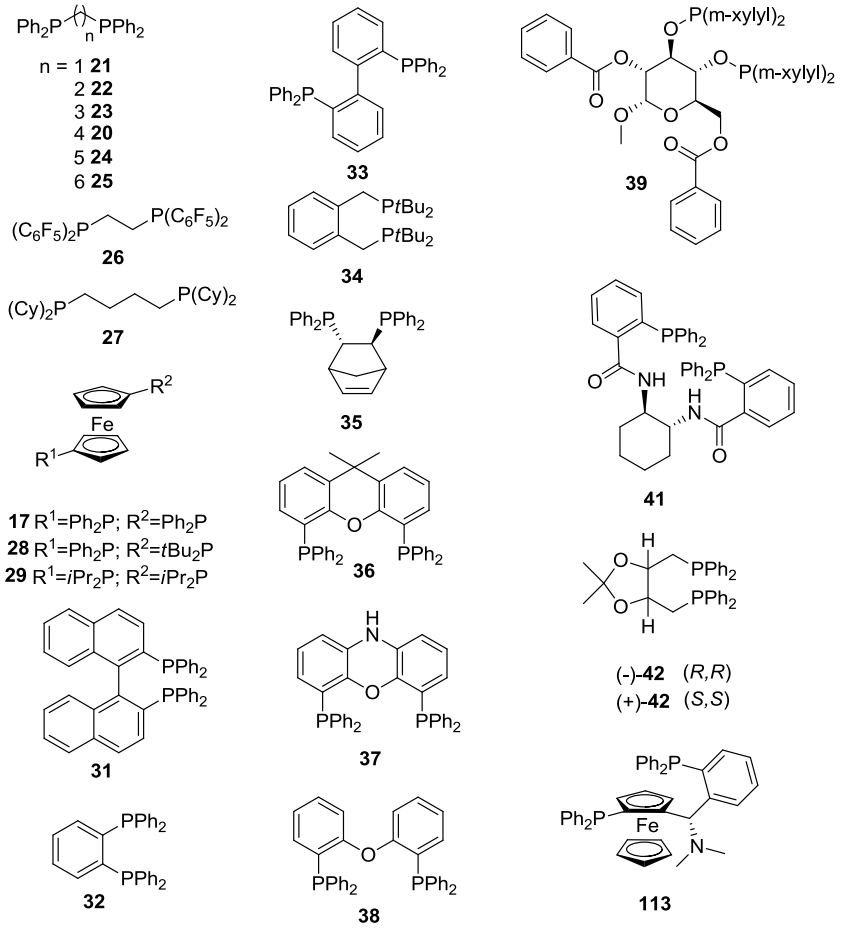


Figure 25: Structures of phosphorus ligands **20-42 and 113** used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**).

Figure 25 shows the ligands used for the Pd-catalyzed allylic sulfenylation employing methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) in acetonitrile (Scheme 102). With the exception of ligand **39** all used ligands were bidentate (chelating) phosphine ligands. Carbophos ligand **39** is due to its one single bond between the phosphorus atom and oxygen called a phosphinite ligand $R_2P(OR)$.

Table 25: Screening of different types of phosphorus ligands **20**-**42** and **113** in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) in acetonitrile at 60°C.

Entry	Ligand	Conversion after 24 h [%]	*n*/*iso*-ratio
1	**21**	0	n.d.
2	**22**	0	n.d.
3	**23**	5	>99/<1
4	**20**	7	92/8
5	**24**	0	n.d.
6	**25**	2	n.d.
7	**26**	6	89/11
8	**27**	42[b)]	62/38
9	**17**	99	92/8
10	**28**	89[a)]	77/23
11	**29**	96	41/59
12	**31**	0[b)]	n.d.
13	**32**	0	n.d.
14	**33**	0	n.d.
15	**34**	45	51/49
16	**35**	0	n.d.
17	**36**	2[c)]	n.d.
18	**37**	0[c)]	n.d.
19	**38**	0	n.d.
20	**39**	13	83/17
21	**41**	0	n.d.
22	(-)-**42**	33	88/12
23	(+)-**42**	92	92/8
24	**113**	72	63/37

a) disulfide formation detected; product composition: (**89**/**84** = 16/84)

[b] light orange suspensions occurred during formation of catalyst (active catalytic species)

[c] light green suspensions occurred during formation of catalyst (active catalytic species)

Table 25 shows that we were not able to identify a ligand, which performs better than the ligand 1,1'-bis(diphenylphosphino)ferrocene dppf (**17**) found already in a previous ligand screening (chapter 4.3.2.3). Again, with the catalyst ligand system $Pd(dba)_2$ (**X**) and dppf (**17**) we could obtain 99 % conversion after 24 h and an *n/iso* ratio of 92/8 showing that the chosen diethylene glycol dimethyl ether (**112**) as internal standard behaves inert and does not interfere with the Pd-catalyzed reaction (Table 25, entry 9). Concerning the natural bite angle of dppf (**17**) with 99.1° it is still somewhere in the middle of the range of natural bite angles of ligands used in this screening.[255] Ligands having low natural bite angles like dppm (**21**) with 72° and ligands having large natural bite angles like xantphos (**36**) with 111.7° or norphos (**35**) with 123.0° gave no or almost no conversion (entry 1, 16, 17).[255] Interestingly, other ligands, which also lead to good result (conversion) have similar natural bite angles as dppf (**17**). Using (+)-diop ligand (+)-**42** conversion reached 92 % with a product composition of *n/iso* 92/8 (entry 23). The natural bite angle of (+)-diop (+)-**42** is 102.2° which is quite similar to the natural bite angle of dppf (**17**).[255] Also, *iso*-propyl analog **29** of dppf (**17**), where the backbone between the two phosphorous atoms is identical, resulted in a good conversion of 96 % after 24 h (entry 11). Remarkably, the *n/iso* ratio of 41/59 using ligand **29** is by far not as good as in the case of dppf (**17**). This observation indicates that electron rich ligands like **29** are not the right ones concerning the necessity of obtaining only the *n*-isomer for our future plans. This concern is also supported by the results obtained from the use of electron rich ligands such as 1,4-bis(dicyclohexylphosphino)butane (**27**) and 1,2-bis(di-*tert*-butylphosphinomethyl)benzene (**34**) (entry 8, 15). Both ligands gave a conversion over 40 % and an *n/iso*-ratio of 62/38 in the case of **27** and an *n/iso*-ratio of 51/49 in the case of **34**. Although the more electron rich ligand **27** gave an unwanted *n/iso*-ratio it seems, that at least in this case the electron rich character of **27** has an accelerating effect on the conversion. Using its less electron rich analog 1,4-bis(diphenylphosphino)butane dppb (**20**) we only find 7 % product formation, but with a better *n/iso*-ratio of 92/8 (entry 4). Remarkable is the very high disulfide formation using ferrocene containing ligand **28**, beside a not favored *n/iso*-ratio of 77/23 (Table 25, entry 10). A repetition of this reaction showed almost identical results. With regard to the mechanism proposed by Tsuji[187] for allylic carbonate alkylation with carbonucleophiles and the mechanism shown by Sinou et al.[170] for allylic carbonate alkylation with *S*-nucleophiles one can speculate that after the oxidative addition step in the Pd catalytic cycle the active catalytic species is not able to proceed with the nucleophilic

substitution step. Maybe due to this 1-octanethiol (**75**) gets oxidized to disulfide **84** in the absence of oxygen.

Having learned lessons about the importance of natural bite angles and the electronic character of suitable ligands, we purchased further phosphine ligands (different commercially available ligand kits) and tested them for the Pd-catalyzed allylic sulfenylation reaction.

As we already experienced from the ligand screening shown in table 25, relatively small changes in the environment of the phosphorous atoms result in strong effects on the ability to catalyze our reaction with a good *n/iso*-ratio as this is the case comparing ligands **17** and **29** (Table 25, entry 9, 11) or ligands **20** and **27** (Table 25, entry 4, 8).

We decided to purchase a commercially available ligand kit from the company Solvias to test the concept of “privileged ligands families”. For the Josiphos ligand family (Figure 19) this concept was already employed.[267] The ligand class containing a ferrocene moiety seemed to be privileged in our reaction. The concept of “privileged ligand families” focuses on a small number of privileged ligand structures, which can be easily modulated regarding their sterical and electronical properties. For each ligand family the ligands are based on the same lead structure but having sufficient electronic and steric diversity.[268]

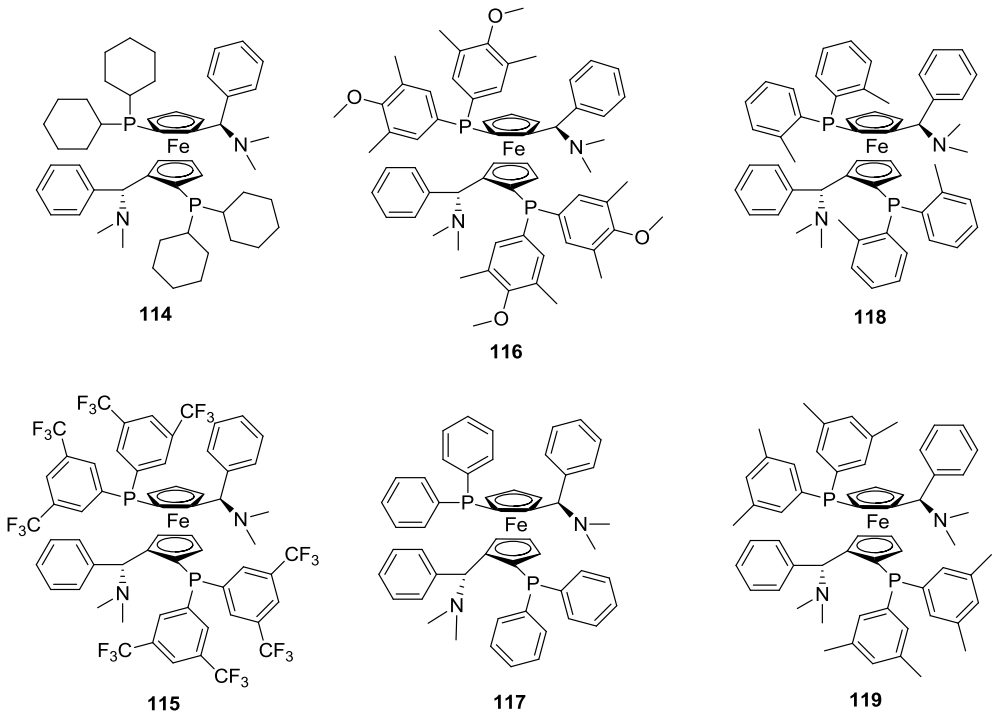


Figure 26: Structures of phosphine ligands **114-119** used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**).

Table 26: Screening of Mandyphos ligand kit, containing phosphine ligands **114**-**119**, in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) in acetonitrile at 60°C.

Entry	Ligand	Conversion after 24h [%]	*n/iso*-ratio
1	**114**	80 %	18/82
2	**115**	0	n.d.
3	**116**	13[a)]	24/76
4	**117**	0	n.d.
5	**118**	42[b)]	n.d.
6	**119**	53[b)]	n.d.

[a)] disulfide formation detected
[b)] conversion only due to disulfide **84** formation no product **89** formed

First we screened the so called “Mandyphos” ligand kit (Figure 26). The results are summarized in Table 26.

Unfortunately, our hope concerning the use of “Mandyphos” ligands were not fulfilled. Although, their basic structure is similar to ligands **17** and **29** also having a ferrocene attached to the phosphorous atoms, their performance was not as expected or hoped. Only ligand **114** showed a conversion of 80 %, but the *n/iso*-ratio of 18/82 favors the unwanted *iso*-isomer (Table 26, entry 1). Due to the cyclohexyl-groups on the phosphorous atoms the ligand **114** can be regarded as electron rich and probably due to this electronic effect the amount of unwanted *iso*-product is with 82 % very high.

All other tested ligands **115**-**119** of this “ligand family” showed no or only low conversion towards thioether product **89** (Table 26, entry 2-6). Due to the poor *n/iso*-ratio obtained using ligand **114** this unexpected behavior was not further investigated. Interestingly to mention is the formation of disulfide **84** using ligands **118** and **119**. Here no product formation could be monitored via GC, but exclusively disulfide formation. It has to be mentioned that, these reaction were performed strictly under an inert atmosphere. The formation of disulfide **84** was already discussed in chapter 4.3.3.5.

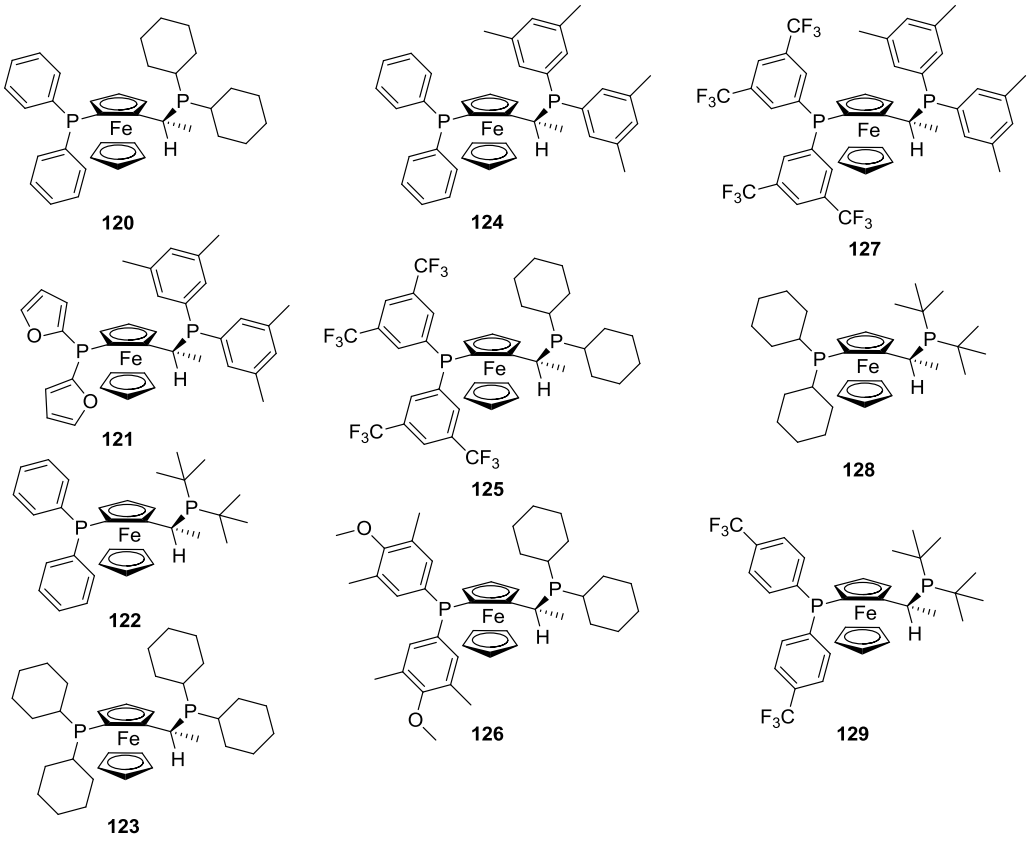


Figure 27: Structures of phosphine ligands **120-129** used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**).

Table 27: Screening of Josiphos ligand kit, containing phosphine ligands **120**-**129**, in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) in acetonitrile at 60°C.

Entry	Ligand	Conversion after 24h [%]	*n*/*iso*-ratio
1	**120**	0	n.d.
2	**121**	4	89/11
3	**122**	67[b)]	n.d.
4	**123**	61[b)]	n.d.
5	**124**	0	n.d.
6	**125**	5	80/20
7	**126**	0	n.d.
8	**127**	3	79/21
9	**128**	0	n.d.
10	**129**	4[a)]	82/18

[a)] disulfide formation detected
[b)] conversion only due to disulfide **84** formation no product **89** formed

The next commercially available ligand kit we screened belongs to the "Josiphos ligand family" (Figure 27), which showed for example a good performance regarding the Pd-catalyzed allylic substitution with *C*-nucleophiles.[269] Nevertheless, in our Pd-catalyzed reaction all ligands showed almost no performance (Table 27). The conversion was either zero or very low. One possible explanation can be the back bone between the two phosphorus atoms and therefore the natural bite angle. Comparing the best ligand dppf (**17**) so far with the "Josiphos ligand family" it is obvious, that in the case of the "Josiphos ligands" only one cyclopentadiene ring is disubstituted with phosphor giving it a totally different geometry than dppf (**17**) where the two cyclopentadiene ring are monosubstituted by phosphor.

Again, a formation of disulfide **84** using ligands **122** and **123** occurred. Here no product formation could be monitored via GC, but exclusively disulfide formation.

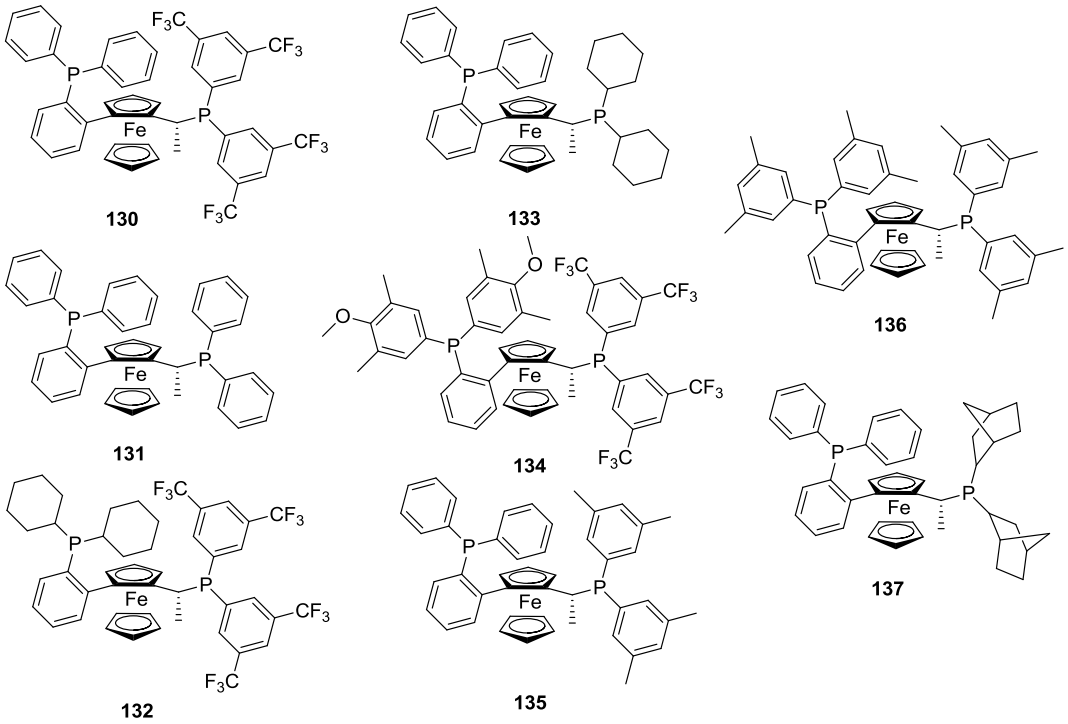


Figure 28: Structures of phosphine ligands **130-137** used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**).

Table 28: Screening of Walphos ligand kit, containing phosphine ligands **ra**-**ya**, in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) in acetonitrile at 60°C.

Entry	Ligand	Conversion after 24h [%]	*n*/*iso*-ratio
1	**130**	64	67/33
2	**131**	74[a)]	71/29
3	**132**	81	70/30
4	**133**	93	32/68
5	**134**	99	69/31
6	**135**	99	58/42
7	**136**	98	57/43
8	**137**	97	35/65

a) disulfide formation detected

The last commercially available ligand kit we screened belongs to the "Walphos ligand family" a ferrocenyl-aryl-based diphosphine ligand class which is also disubstituted on one cyclopentadiene ring system (Figure 28). The performance of the ligands from the "Walphos

ligand family" was much better than of the "Josiphos ligand family". Regarding conversion we obtained good to excellent conversions after 24 h (Table 28). Especially, ligands **134**-**137** gave very good conversion monitored via GC (Table 28, entry 5-8). Again one possible explanation is the back bone between the two phosphorus atoms and therefore the large natural bite angle, which is different from the "Josiphos ligands". Unfortunately, we obtained undesired *n/iso*-ratios. For ligand **130**, the *n/iso*-ratio is 67/33 (Table 28, entry 1). Ligand **130** can be considered as more electron poor compared to ligand **133**, due to the electron withdrawing ability of the CF_3-group on the aromatic ring attached to the one phosphorous atom. In contrast ligand **133** can be considered as more electron rich compared to ligand **130**, because of the electron donating effect of the cyclohexyl-groups attached to the one phosphorous atom (entry 4). For the more electron rich ligand **133** we obtained an *n/iso*-ratio of 32/68. Not as extreme, but still obvious this effect can also be seen for ligands **134**-**137** (Table 28, entry 5-8). In summary, without taking the conversion into consideration, we can say, that the more electron rich ligands form more unwanted *iso*-product **90**.

For the next steps we wanted to test ligands with a lower basicity, i.e. having less electron donating substituents attached to the phosphorous atoms. Therefore, we used the commercially available phosphonite-ligand **30**, where two oxygen atoms are attached to the phosphorous atoms of the ligand. We choose phosphonite-ligand **30** due to its structural similarity to dppf ligand (**17**), which showed the best performance in the Pd-catalyzed allylic sulfenylation reaction so far. Unfortunately, the use of phosphonite-ligand **30** resulted in no product formation (Figure 29 and Table 29, entry 1). This was indeed unexpected, but one possible explanation for this bad result could be the sterically demanding binaphthyl group on the phosphorus atoms.

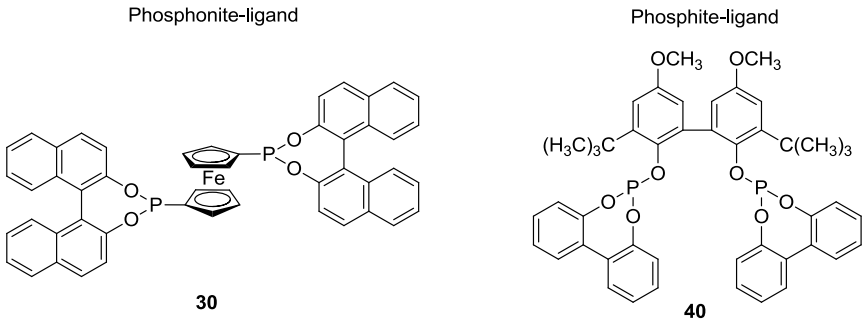


Figure 29: Structures of phosphonite ligand **30** and phosphite ligand biphephos (**40**) used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**).

Table 29: Use of phosphonite ligand **30** and phosphite ligand biphephos (**40**) in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) in acetonitrile at 60°C.

Entry	Ligand	Conversion after 24h [%]	*n/iso*-ratio
1	**30**	0	n.d.
2	**40**	>99	>99/<1

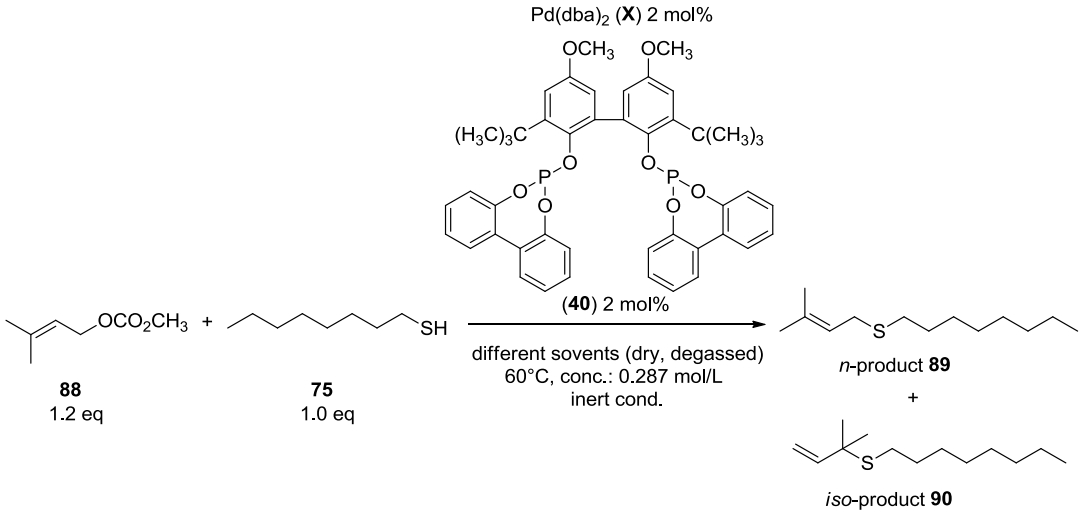
The next ligand used was the commercially available phosphite-ligand biphephos (**40**), where three oxygen atoms are attached to the phosphorous atoms of the ligand. We could not only achieve full conversion of the starting material after 24 h, but additionally for the product composition an *n/iso*-ratio of >99/<1 could be monitored via GC (Figure 29 and Table 29, entry 2). After purification via flash column chromatography we obtained pure *n*-product (3-methylbut-2-enyl)(octyl)sulfane (**89**) in 85 % yield. Due to an optimization in purification later this yield could be improved even further when no internal standard was present. This great result supports our idea that more electron-poor ligands (lower basicity) show a higher formation of *n*-product **89**.

4.3.5. Pd-catalyzed allylic alkylation reactions using biphephos as ligand

4.3.5.1. Time depending experiments

So far for all reaction performed during the ligand screening the conversion was measured after 24 h. The main reason for that was the sensitivity of the Pd-catalyzed reaction against oxygen as already mentioned in chapter 4.3.3.2. However it is of great importance to know how fast the Pd-catalyzed transformation towards allylic thioether **89** does occur. Additionally, it was necessary to investigate whether the ratio of *n/iso*-products formed was due to the catalyst-ligand system or a matter of time. Due to this need two reactions were performed (Scheme 103). The first reaction was the identical transformation as already described in the ligand screening chapter 4.3.4. The second reaction was identical to the first one but instead of acetonitrile a mixture of *tert*-butanol/H_2O 3:1 was used (Table 30). This mixture was used because of our goal to be able to perform the Pd-catalyzed allylic sulfenylation reaction in water. Both reactions were performed without the use of an internal standard. At this stage of the project testing this real case was not possible because so far used starting materials methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) are not soluble in water. Nevertheless, using a mixture of *tert*-butanol/H_2O

3:1 shows at least if this transformation is possible in the presence of such a polar and protic solvent as water. Samples were taken under inert conditions and measured via GC after 30 min, 2 h, 5 h, 18 h, 24 h and 48 h.



Scheme 103: Time depending experiments using biphephos (**40**) as ligand in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**).

Table 30: Time depending experiments using biphephos (**40**) as ligand in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) in different solvents at 60°C.

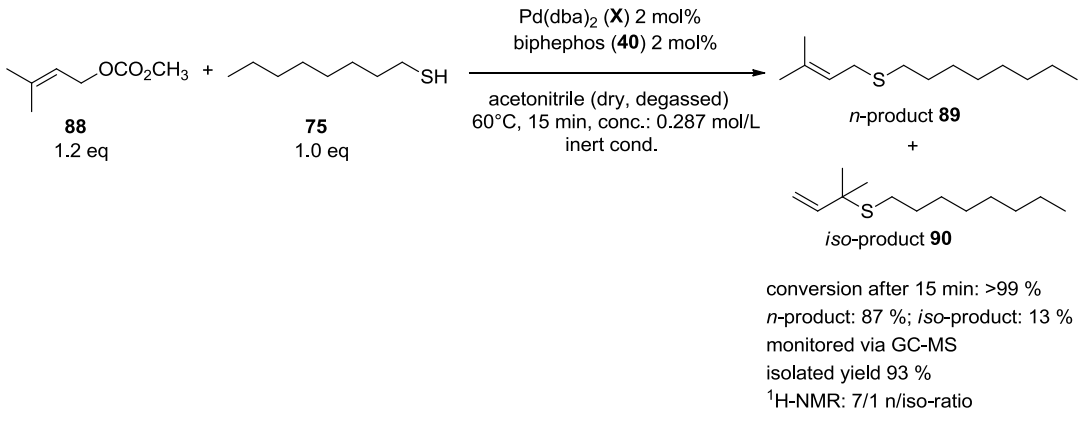
Entry	**Time [h]**	**Conversion and (*n/iso*-ratio)**	
		Acetonitrile	***t*-BuOH/$H_2$0 3:1**
1	0.5	>99 (89/11)	>99 (89/11)
2	2	>99 (94/6)	>99 (90/10)
3	5	>99 (99/1)	n.d.
4	18	n.d.	>99 (96/4)
5	24	>99 (>99/<1)	>99 (98/2)
6	48	n.d.	>99 (>99/<1)

Full conversion of 1-octanethiol (**75**) is already reached after 30 min in the case of acetonitrile and also in the case of *tert*-butanol/H_2O 3:1 used as solvents. Unfortunately, the amount of undesired *iso*-product **90** is with 11 % in both reactions quite high. However, after 24 h reaction time in acetonitrile almost no *iso*-product **90** was detected via GC. Even after 5

h only ~1 % of *iso*-product **90** was detected via GC. In the case of solvent mixture *tert*-butanol/H_2O 3:1 used as solvent after 48 h almost no *iso*-product **90** was detected via GC. After 18 h there are ~4 % and after 24 h there are ~2 % of undesired *iso*-product **90** still present (entries 4 and 5). This phenomenon of isomerization of *iso*-product **90** into *n*-product **89** can be due to the catalyst-ligand system used or simply a non-catalytic isomerization. This fact of isomerization is further investigated and discussed in chapter 4.3.5.3 and 4.3.5.4.

After having recognized the time dependence of the regioselectivity it was regarded as necessary to investigate the rate of the Pd-catalyzed reaction and to check whether isomerization occurs using the second best ligand dppf (**17**). This reaction was done under identical conditions as mentioned in Scheme 103 using acetonitrile as solvent. After 30 min a conversion of 90 % was monitored via GC, having an *n/iso*-ratio of the crude product (isomeric mixture of **79** and **80**) of 92/8. The sample taken after 2 h showed full conversion of starting material and a product composition of *n/iso*-ratio of 92/8. In all cases so far the *n/iso*-ratio after 24 h was also 92/8. Only in this reaction we could monitor after 24 h an *n/iso*-ratio of 95/5 still having full conversion. This clearly demonstrates the superiority of ligand biphephos (**40**) compared to ligand dppf (**17**).

Due to the fast formation of allylic thioether **89** using catalyst-ligand system $Pd(dba)_2$ (**X**) and biphephos (**40**) it was decided to stop the reaction after 15 min (Scheme 104). One important point to look at was if the amount of unwanted *iso*-product would be higher than after 30 min. The reaction was performed under identical conditions as described above in Scheme 103 using acetonitrile as solvent. Additionally, another aim was to compare the *n/iso*-ratio found in GC-MS with the *n/iso*-ratio obtained by ^{1}H-NMR.



Scheme 104: Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) using biphephos (**40**) as ligand after 15 min reaction time.

Fortunately, the reaction showed already full conversion after only 15 min and we obtained an *n/iso*-ratio of 87/13 of allylic thioethers **89** and **90** as monitored by GC-MS (Scheme 104). The reaction was stopped by evaporation of the solvent using a rotary evaporator leaving the crude product as a sticky oil, which was immediately purified by flash column chromatography. Great care was taken to isolate both spots on TLC together as a mixture of isomers. Directly after evaporation of the solvents used for purification by a rotary evaporator and drying for 30 min at 0.02 mbar (oilpump) the ^{1}H-NMR was measured. The ^{1}H-NMR showed a product composition of *n*-product/*iso*-product of 7:1. This is in good correlation with the *n/iso*-ratio of 87/13 monitored by GC-MS.

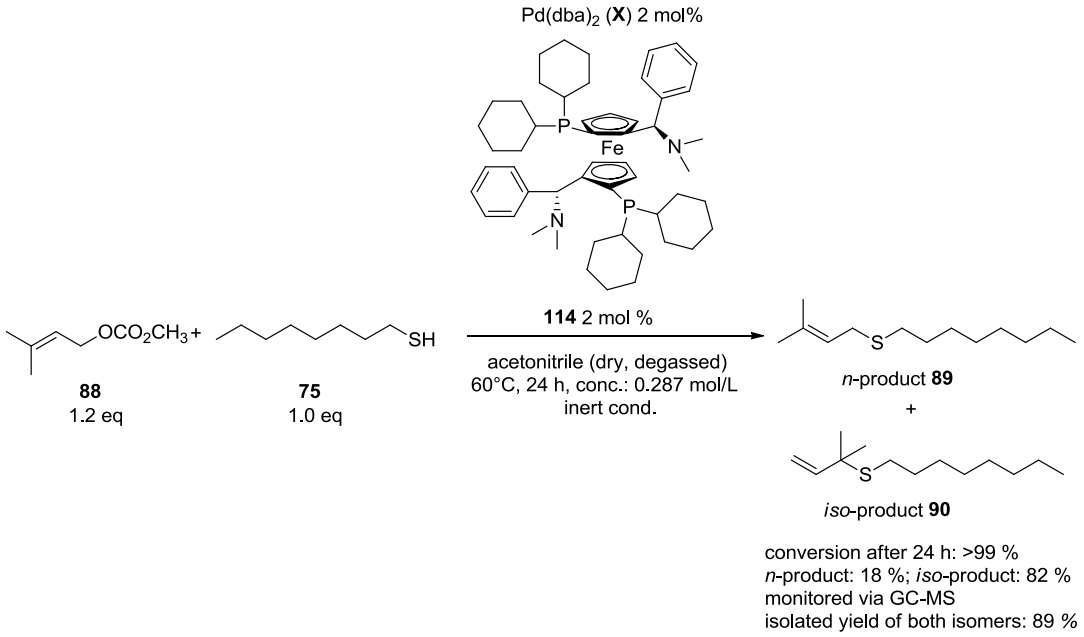
In summary, the use of ligand biphephos (**40**) in the Pd-catalyzed formation of allylic thioethers results in a very fast reaction and in a isomerization reaction towards the linear *n*-product **89**.

To prove that these described results are exclusively due to the use of ligand biphephos (**40**) and that there is no contribution of pre-catalyst $Pd(dba)_2$ (**X**) to this result a reaction without ligand was performed but under identical conditions mentioned above. This resulted in no product formation when acetonitrile was used as solvent. In the case of *tert*-butanol/H_2O 3:1 as solvent no product could be detected via GC-MS, but interestingly always 8 % of disulfide **84** was formed during the reaction after 30 min, after 2 h and after 24 h. It cannot be fully excluded, that there might occur an oxidation reaction due to the pre-catalyst. Nevertheless is seems more likely that the formation of disulfide **84** appears during the micro workup and during the GC measurement, because during this time 1-octanethiol (**75**) is exposed to air.

4.3.5.2. Structural characterization of *iso*-product via NMR

To support the results obtained by GC and GC-MS regarding the *n/iso*-ratio it was necessary to isolate and characterize the minor isomer, the so called *iso*-product **90**. Ligand **114**, belonging to the “Mandyphos” ligand family (Figure 26), was chosen because it showed the highest amount of *iso*-product formation **90** within the ligand screening mentioned in chapter 4.3.4.

In contrast to the reaction reported in chapter 4.3.4 no internal standard was used and for the measurement of conversion and *n/iso*-ratio GC-MS was used.



Scheme 105: The Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) using ligand **ba** belonging to "Mandyphos" ligand family.

During the reaction three samples were taken under inert conditions after 30 min, after 2 h and after 24 h. A conversion of 19 % and an *n/iso*-ratio of 25/75 was obtained after 30 min. After 2 h 79 % conversion and an *n/iso*-ratio of 20/80 was monitored via GC-MS. In contrast to the previously reported reaction (Table 26, entry 1) in chapter 4.3.4 here full conversion could be reached with an *n/iso*-ratio of 18/82 (Scheme 105). The *n/iso*-ratio of 18/82 is identical to the previously reported *n/iso*-ratio.

The 300 MHz-^{1}H-spectrum shows the *n*-isomer **89** (Figure 30). The triplett at 5.23 ppm belongs to the proton of the double bond of the linear (3-methylbut-2-enyl)(octyl)sulfane (**89**) (-C<u>H</u>=C$(CH_3)_2$) with ^{3}J(H,H) = 7.8 Hz. ^{13}C-NMR spectra shows two signals for the double bond at 121.0 ppm (-<u>C</u>H=C$(CH_3)_2$) and 135.0 ppm (-CH=<u>C</u>$(CH_3)_2$) (see experimental part).

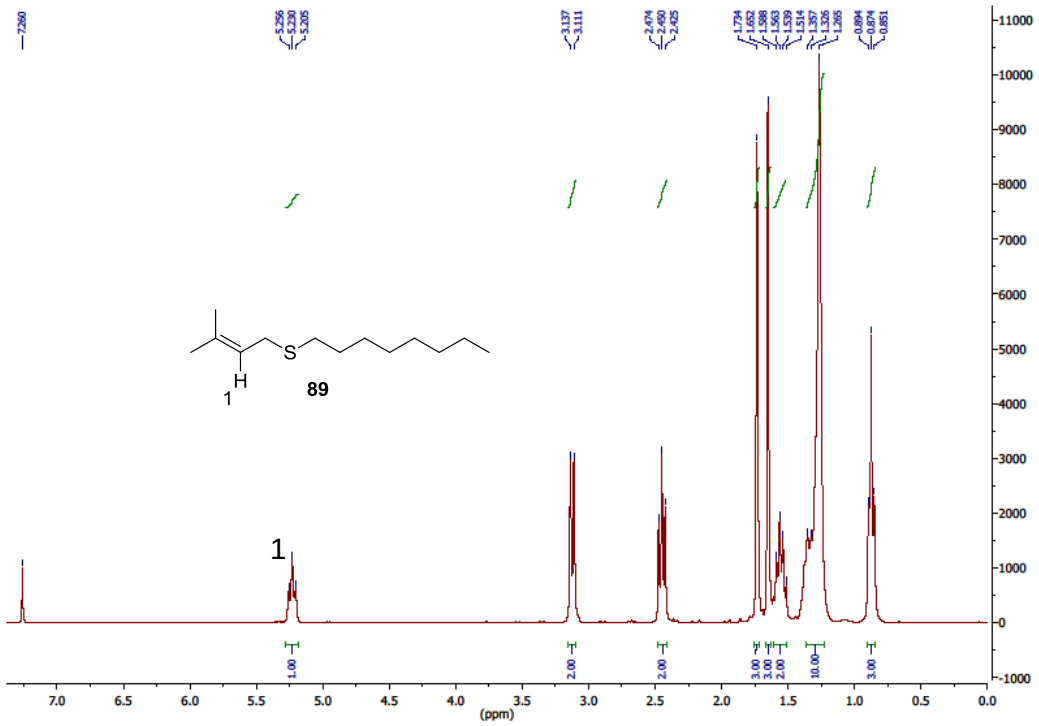


Figure 30: ^{1}H-NMR of *n*-isomer **89**.

In contrast the ^{1}H-NMR of the *iso*-substituted product (2-methylbut-3-en-2-yl)(octyl)sulfane (**90**) shows, as one would expect, two signals in the region of the double bond (Figure 31). A multiplet which integrates for two protons at 4.89-5.00 ppm (R-HC=C<u>H</u>$_2$) and doublet of doublet at 5.84 ppm (R-C<u>H</u>=CH$_2$) integrating for one proton with ^{3}J(H,H) = 17.1 Hz and ^{3}J(H,H) = 10.5 Hz. ^{13}C-NMR spectrum shows two signals for the double bond at 111.1 ppm (R-HC=<u>C</u>H$_2$) and 145.1 ppm (R-<u>C</u>H=CH$_2$) (see experimental part).

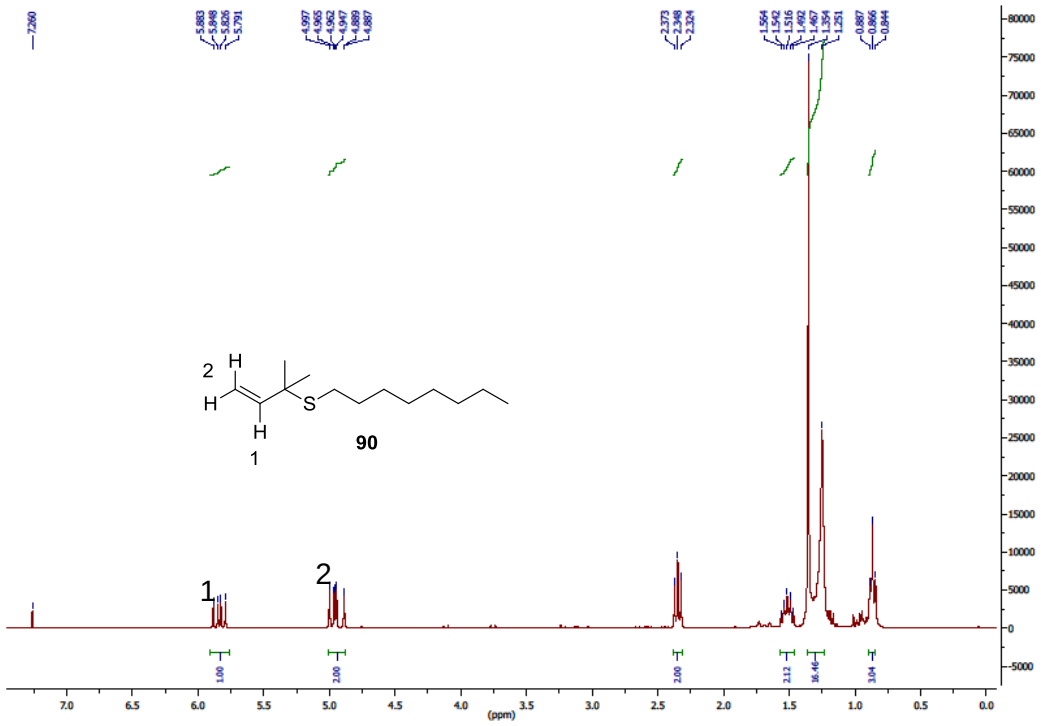


Figure 31: ^{1}H-NMR of *iso*-isomer **90**.

4.3.5.3. Studying the isomerization reaction of the iso-product into the linear *n*-product

To further investigate the isomerization reaction occurring over a long reaction time as already reported in chapter 4.3.5.1 a mixture of *n*-product **89** and *iso*-product **90** purified by flash column chromatography was used. This mixture did not contain anymore the catalyst $Pd(dba)_2$ (**X**) or ligand biphephos (**40**). In this mixture some disulfide **84** was present. The initial *n/iso*-ratio was 82/18 of isomers **89**/**90** monitored via GC. To this mixture degassed acetonitrile was added and heated to 60°C. Afterwards $Pd(dba)_2$ (**X**) 2 mol% and biphephos (**40**) 2 mol% were added under the identical inert conditions previously described.

After 2 h a GC sample was taken which showed an *n/iso*-ratio of 92/8. This *n/iso*-ratio of 92/8 remained the same after 24 h. Even after 72 h the detected *n/iso*-ratio was 93/7. This indicates a fast isomerization reaction in the beginning, which stops or is at least dramatically slower after long reaction times. One possible explanation would be that the used biphephos ligand (**40**) is responsible for this effect. This assumption is supported by the finding that the use of other ligands like dppf (**17**) or Mandyphos ligand **114** did not show such a remarkably isomerization effect. In the case of Mandyphos ligand **114** the amount of *iso*-product **90** is

even rising from 75 % after 30 min having 19 % conversion to 82 % *iso*-product after 24 h having full conversion (chapter 4.3.5.2).

In the case of an influence of the biphephos ligand (**40**) on the isomerization reaction between the *n*-product **89** and *iso*-product **90** an explanation could be the concept of microscopic reversibility. For allyl–palladium complexes this concept was already reported by Pfalz et al. The principle of microscopic reversibility postulates that the transition states of the forward and back reaction should be identical.[270] According to our catalytic system a back reaction is possible, because it is highly likely that after 15 min the actual catalytic species is still active. Maybe the catalytic reaction proceeds the back and forward step of the nucleophilic substitution at cationic allyl–palladium complex and therefore the amount of thermodynamically more stable linear *n*-product **89** in the reaction increases. However, a fast non-catalytic isomerization described by Johnson et al. and Warren et al. cannot be excluded, which is also the case for experiments decribed in chapter 4.3.5.4 and 4.3.5.5.[252-253]

4.3.5.4. Pd-catalyzed allylic sulfenylation reactions at different temperatures

Another important point of interest was to investigate the performance of the Pd-catalyzed allylic sulfenylation reactions at different temperatures using the best ligand biphephos (**40**). Additionally, from these reactions performed at different temperatures we would also obtain further data about the isomerization of the *iso*-product **90** into the linear *n*-product **89**. All reactions were performed under identical conditions described in chapter 4.3.5.1 in Scheme 104. The reactions were performed using degassed acetonitrile as solvent at rt, 37°C and reflux.

Table 31: Temperature dependent experiments using biphephos (**40**) as ligand in the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) in acetonitrile.

Entry	Temp. [°C]	Conversion after 30 min [%] (*n*/*iso*-ratio)	Conversion after 2 h [%] (*n*/*iso*-ratio)	Conversion after 24 h [%] (*n*/*iso*-ratio)
1	rt	>99 (87/13)	>99 (86/14)	>99 (90/10)
2	37	>99 (86/14)	>99 (87/13)	>99 (91/9)
3	60	>99 (89/11)	>99 (94/6)	>99 (>99/<1)
4	reflux	>99 (90/10)	>99 (91/9)	>99 (98/2)

The reactions at rt and 37°C showed also already full conversion after 30 min (Table 31, entry 1 and 2). Unfortunately, isomerization of the *iso*-product **90** into the linear *n*-product **89** does not proceed as fast as in the case of 60°C used as temperature (Table 31, entry 3). The *n*/*iso*-ratio at rt was 90/10 and using 37°C as reaction temperature the *n*/*iso*-ratio at rt was 91/9 after 24 h. According to these reactions it cannot be fully assigned if this isomerization reaction is exclusively caused by the use of biphephos (**40**) as ligand (see also chapter 4.3.5.3).

Due to the slow and not complete isomerization reaction at rt and 37°C of the *iso*-product **90** into the linear *n*-product **89**, we decided to use the reaction temperature of 60°C for further reactions. Expanding the scope of this reaction type to various other substrates seemed to be more important at this stage of the project.

However concerning reaction performed under reflux conditions it can be stated that the higher temperature lead to a faster isomerization of the *iso*-product **90** into the linear *n*-product **89** (Table 31, entry 4) compared to the reactions performed at rt and 37°C. Surprisingly, the rate of the isomerization reaction at reflux is slightly slower than at 60°C.

4.3.5.5. Studying the influence of lower amounts of catalyst $Pd(dba)_2$ and ligand biphephos used

At this stage of the project we wanted to know to which extent we are able to reduce the amount of $Pd(dba)_2$ (**X**) and biphephos (**40**) used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) at 60°C, because the lower the amount of catalyst and ligand, the lower the overall costs are for this transformation.

Table 32: Different amounts of $Pd(dba)_2$ (**X**) and biphephos (**40**) used for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) at 60°C.

Entry	Mol% of $Pd(dba)_2$ and biphephos	Conversion and (*n/iso*-ratio)		
		30 min	2 h	24 h
1	2.0	>99 (90/10)	>99 (94/6)	>99 (>99/<1)
2	1.0	>99 (82/18)	>99 (85/15)	>99 (93/7)
3	0.5	>99 (87/13)	>99 (88/12)	>99 (92/8)
4	0.1	38 (85/15)	37 (87/13)	45 (89/11)
5	0.05	3 (83/17)	5 (83/17)	6 (n.d.)

To test the influence of lower amounts of catalyst and ligand on conversion and on the *n/iso*-ratio 5 different amounts of $Pd(dba)_2$ (**X**) and biphephos (**40**) were used (Table 32). In the case of 0.1 and 0.05 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) used, stock solutions were prepared in a Schlenk tube in degassed acetonitrile. Afterwards the necessary amount was transferred under inert conditions into the reaction Schlenk tube, before the start of the reaction. Samples for GC-measurements were taken after 30 min, 2 h and 24 h. Using 2 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) full conversion was reached after 30 min and after 24 h just the desired *n*-product **89** was present (Table 32, entry 1). Using 1 and 0.5 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) full conversion was also already reached after 30 min (Table 32, entry 2 and 3). Unfortunately, the isomerization reaction of the *iso*-product **90** into the linear *n*-product **89** was much slower. In the case of 1 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) used we obtained an already higher *n/iso*-ratio of 82/18 after 30 min and after 24 h the isomerization reaction of the *iso*-product **90** into the linear *n*-product **89** was incomplete with an *n/iso*-ratio of 93/7 (entry 2). Similar results were found when 0.5 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) were used. When 0.1 or 0.05 mol% of $Pd(dba)_2$ (**X**) and

biphephos (**40**) were employed full conversion could not be reached after 24 h; 45 % after 24 h when 0.1 mol% were used and only 6 % after 24 h when 0.05 mol% were used (Table 32, entry 4 and 5). With 0.1 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) the isomerization reaction did not lead to a single *n*-isomer **89** after 24 h. The *n/iso*-ratio was 89/11.

These results indicate that there must be an influence of the catalyst ligand system $Pd(dba)_2$ (**X**) and biphephos (**40**) on the *n/iso*-ratio, which can be explained by the concept of microscopic reversibility mentioned in chapter 4.3.5.3. The main reason for this assumption is that the temperature is the same in all reactions and only the amount of $Pd(dba)_2$ (**X**) and biphephos (**40**) is different. It seems obvious that the ability to catalyze this isomerization reaction of the *iso*-product **90** into the linear *n*-product **89** is slower when lower amounts of the active catalytic specie are present in solution.

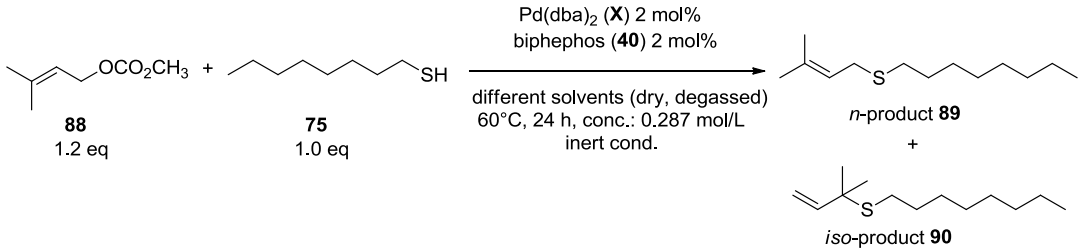
4.3.5.6. Solvent-screening

After finding an appropriate catalyst ligand system with $Pd(dba)_2$ (**X**) and biphephos (**40**) which is able to produce allylic thioether **89** in very short time it was of interest what classes of solvents are suitable for this kind of transformation. If this transformation would tolerate a large amount of solvents with different polarities it would be of greater use for application purposes, because of their different dissolving abilities for different starting materials.

Due to our aim to find in short time a lot of different solvents where this catalytic reaction works fast, we regarded measurements using GC-MS as sufficiently precise. No purification using flash column chromatography was done to obtain isolated yields.

For this solvent screening starting materials methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) were used, together with 2 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) 60°C. The solvents used were different, but without changing the concentration (Scheme 106). The temperature used was 60°C because previous experiments described in chapter 4.3.5.4 showed the lowest amount of undesired *iso*-product **90** at this reaction temperature. Samples for GC-MS-measurements were taken after 30 min and 24 h.

Two aspects received our special attention. The first point was the conversion especially after 30 min as the use of acetonitrile, which was the solvent of choice already before, shows full conversion after 30 min (Table 33 entry 1). The second point of interest was the *n/iso*-ratio at 30 min. Due to the isomerization of *iso*-product **90** into the linear *n*-product **89** the detection of the *n/iso*-ratio after 24 h was important as well.



Scheme 106: Conditions for solvent screening for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) using biphephos (**40**) as ligand.

Table 33: Solvent screening for the Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) using biphephos (**40**) as ligand at 60°C.

Entry	Solvent	Conversion (GC-MS) 30min [%]	*n/iso* - ratio (GC-MS) 30 min	Conversion (GC-MS) 24h [%]	*n/iso* - ratio (GC-MS) 24h
1	acetonitrile	>99	90/10	>99	>99/<1
2	propionitrile	>99	98/2	>99	>99/<1
3	methanol	>99	95/5	>99	>99/<1
4	ethanol	>99	95/5	>99	>99/<1
5	ethanol/H_2O (5:1)	>99	93/7	>99	97/3
6	1-propanol	>99	98/2	>99	99/1
7	2-propanol	>99	91/9	>99	94/6
8	1-butanol	>99	97/3	>99	99/1
9	*tert*-butanol	99[c)]	97/3	99[c)]	96/4
10	*tert*-butanol/H_2O (3:1)	>99	89/11	>99	97/3
11	benzyl alcohol	>99[a)]	87/13	>99[a)]	98/2
12	2,2,2-trifluorethanol	>99[a)]	93/7	>99[a)]	>99/<1
13	DCM[b)]	89	86/14	92	96/4
14	chloroform	99[a)]	94/6	>99[a)]	>99/<1
15	1,2-dichloroethane (DCE)	7	>99/<1	22	90/10
16	toluene	73[a)]	94/6	97[a)]	94/6

17	ethyl acetate	98	94/6	>99	97/3
18	acetone	>99	91/9	>99	96/4
19	1,4-dioxane	75[c)]	90/10	93[c)]	94/6
20	THF	82	93/7	86	93/7
21	2-methyl-THF	82	92/8	92	94/6
22	MTBE[b)]	89	95/5	90	91/9
23	NMP	>99	94/6	>99	>99/<1
24	DMF	>99	81/19	>99	83/17
25	*N,N*-dimethylacetamide	>99	95/5	>99	99/1
26	DMSO	>99	87/13	>99	98/2
27	sulfolane	>99	93/7	>99	97/3
28	propylene carbonate	>99	94/6	>99	>99/<1
29	diethylene glycol	99	92/8	99	92/8
30	DME	99	93/7	>99	98/2
31	acetic acid	87[a),c)]	78/22	>99[a),c)]	96/4

[a)] Unknown impurities relative to product and starting material monitored via GC-MS
[b)] reflux conditions due to a lower boiling point than 60°C
[c)] disulfide formation detected

At this stage of the project it was not possible to use H_2O as a solvent as the used starting materials methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) are not soluble in water.

Nevertheless, the use of water in combination with alcohols shows that in principle the Pd-catalyzed allylic sulfenylation in water should be possible. This assumption is supported by the use of water containing mixtures such as ethanol/H_2O (5:1) and *tert*-butanol/H_2O (3:1). In the case of the solvent mixture of ethanol/H_2O (5:1) we could monitor full conversion after 24 h and an acceptable *n/iso*-ratio of 97/3 after 24 h (Table 33, entry 5). Very similar to this result we could monitor using solvent mixture of *tert*-butanol /H_2O (3:1) full conversion after 24 h and an acceptable *n/iso*-ratio of 97/3 after 24 h via GC-MS (Table 33, entry 10).

Interestingly, especially primary alcohols appeared to be very good solvents for this kind of transformation. Methanol, ethanol, 1-propanol and 1-butanol showed full conversion after 30 min and almost no *iso*-product **90** could be detected via GC-MS after 24 h. In respect to the *n/iso*-ratio methanol and ethanol (Table 33, entries 3 and 4) seem to be even slightly better than 1-propanol and 1-butanol (Table 33, entries 6 and 8). The use of secondary alcohol 2-propanol (Table 33, entry 7) showed also full conversion after 30 min, but the *n/iso*-ratio is with 94/6 after 24 h was not as good as in the use of primary alcohols. A similar result was found when *tert*-butanol was used as solvent (Table 33, entry 9). Just 99 % conversion was reached after 30 min and 24 h. Also the *n/iso*-ratio of 96/4 after 24 h was slightly inferior to the *n/iso*-ratios obtained with primary alcohols. It is noteworthy to say that when *tert*-butanol was used as solvent also disulfide formation **84** was detected by GC-MS after 24 h having a ratio of sum of product isomers (**89** + **90**)/disulfide **84** of 76/24. A loss of solvent could also be observed, maybe due to a not airtight Schlenk tube. However, we focused now on screening other solvents, because we already could show that the mixture of *tert*-butanol /H_2O (3:1) is a good solvent mixture for this Pd-catalyzed allylic sulfenylation. Performing the reaction in benzyl alcohol led also to full conversion after 30 min and to a quite good *n/iso*-ratio of 98/2 after 24 h. Unfortunately, unknown impurities occurred during the reaction. In sum they are under 5 % relative to product and starting material. It cannot be fully excluded that benzyl alcohol also reacted with methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) in this Pd-catalyzed reaction.

2,2,2-Trifluorethanol was tested as solvent for the Pd-catalyzed allylic sulfenylation due to the fact that 2,2,2-trifluorethanol is known to be one of the best solvents for peptides. With 2,2,2-trifluorethanol as solvent we also could monitor full conversion after 30 min and after 24 h no *iso*-product **90** could be detected via GC-MS. Sadly, an unknown impurity occurred which was less than 3 %, relative to product and starting material, but having a mass spectra very similar to the mass spectra of linear *n*-product **89**. As we could not find this impurity on TLC, we were not able to further characterize this impurity. However, it is also possible that this side product does occur due to the conditions used of the GC-MS measurement. Maybe there is an isomerization present due to moving of the double bond caused by the heat in the GC inlet.

As already reported before in chapter 4.3.5.1 acetonitrile can be regarded as one of the outstanding solvents for the Pd-catalyzed allylic sulfenylation reaction using methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**). Therefore, we tried propionitrile as solvent for this kind of transformation (Table 33, entry 2). Indeed, propably due to its structural analogy to acetonitrile it is one of the best solvents used so far.

Full conversion and an almost perfect *n/iso*-ratio of 98/2 already after 30 min was found. After 24h no *iso*-product **90** could be monitored via GC-MS. Unfortunately, propionitrile is very toxic and therefore its application is limited.

With chlorinated solvents such as DCM, 1,2-dichloroethane (DCE) and chloroform the performance of the Pd-catalyzed allylic sulfenylation reaction using methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) was very different. The best chlorinated solvent was chloroform with 99 % conversion after 30 min (Table 33, entry 14). After 24 h the conversion was complete and no *iso*-product **90** was found in GC-MS spectra. Unfortunately, identical to 2,2,2-triuorethanol tested an unknown impurity <3 %, relative to product and starting material, was detected having a mass spectra very similar to the mass spectra of linear *n*-product **89**.

The use of DCM as solvent resulted in only 92 % conversion after 24 h (Table 33, entry 13). Even worse was the application of 1,2-dichloroethane yielding in only 22 % conversion after 24 h (Table 33, entry 15). This finding was already described in chapter 4.3.2.3 using dppf (**17**) as ligand.

The apolar solvent toluene was not a good solvent for this kind of transformation. Only 73 % conversion after 30 min and 97 % conversion after 24 h were detected via GC-MS (Table 33, entry 16). In this case the unknown impurity having a mass spectrum very similar to the mass spectrum of linear *n*-product **89** occurred again. This time its amount was ~7 % relative to product and starting material.

Interestingly, in ethyl acetate the performance of Pd-catalyzed allylic sulfenylation reaction was better. Although only 98 % conversion was reached after 30 min conversion is complete after 24 h obtaining an acceptable product composition with an *n/iso*-ratio of 97/3 (Table 33, entry 17). This effect cannot simply be explained by the polarity of the used solvent.

The use of more polar solvents such as acetone yielded in already full conversion after 30 min and an *n/iso*-ratio of 96/4 (Table 33, entry 18).

Interestingly, in the case of ether-type solvents such as 1,4-dioxane, THF, 2-methyl-THF and MTBE the Pd-catalyzed allylic sulfenylation reaction using methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) did not work as good as when using alcohols (Table 33, entries 19-22). However, conversion is in all cases around 90 % after 24 h, the *n/iso*-ratios are only moderate. The only ether-type solvent reaching full conversion was dimethoxyethane (DME), reaching full conversion after 24 h and having an acceptable *n/iso*-ratio of 98/2 (Table 33, entry 30).

Polar aprotic solvents such as NMP, *N,N*-dimethylacetamide, DMSO, sulfolane and propylene carbonate appeared as very good solvents for the Pd-catalyzed allylic sulfenylation reaction using methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) (Table 33, entry 23 and 25-28). Using these solvents full conversion could be monitored via GC-MS after already 30 min, additionally having very good *n/iso*-ratios after 24 h. Especially, NMP and propylene carbonate gave excellent *n/iso*-ratios after 24 h, with no *iso*-product **90** found in GC-MS spectra. The Pd-catalyzed allylic sulfenylation reaction using NMP as solvent was repeated three times under identical conditions. Everytime full conversion was monitored after 30 min. Two times no *iso*-product **90** could be found in GC-MS spectra. Only one time the *n/iso*-ratio was not that perfect with 98/2 after 24 h. This shows that the reactions performed are highly reproducible within the experimental error. In the group of polar aprotic solvents in Table 33 DMF is somehow an exception (Table 33, entry 24). Although the ability to reach full conversion in short time is as good as using the other polar aprotic solvents mentioned before, the *n/iso*-ratio of 83/17 obtained after 24 h is rather poor. Until now there is no explanation for this finding.

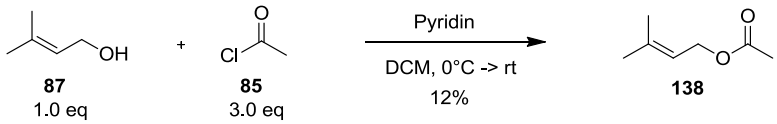
Due to the poor solubility of methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) in ethylene glycol, diethylene glycol was used as solvent to test the performance of glycols in the Pd-catalyzed allylic sulfenylation reaction. 99 % conversion was monitored via GC-MS, having a product composition of 92 % *n*-product **89** and 8 % *iso*-product **90** (Table 33, entry 29).

Last but not least, we tested pure acetic acid as solvent (Table 33, entry 31). Notably, the Pd-catalyzed allylic sulfenylation reached full conversion after 24 h having an *n/iso*-ratio of 96/4 (Table 33, entry 31). Regarding the proposed mechanism of this transformation, one would expect that the catalysis is hindered, because the formation of the π-allyl-palladium species and the loss of CO_2 generate an alkoxide (methanolate). Exchanging the proton with 1-octanethiol (**75**) should give the thiolate nucleophile attacking the π-allyl-palladium species yielding allyl alkyl sulfide **89**.[170] Having such a large excess of acid the formation of thiolate should be unlikely. However, maybe 1-octanethiol (**75**) is a sufficient nucleophile for this reaction itself. It should be noticed, that during the catalysis with acetic acid as solvent side reactions occurred. An unknown impurity already mentioned before in the case of 2,2,2-triuorethanol could be detected. The amount was higher, with ~8 % relative to product and starting material, than in all other reactions where this impurity (side-product) occurred so far. This not characterized impurity having a mass spectrum very similar to the mass spectrum of linear *n*-product **89**. Also ~1 % of disulfide **84** was observed.

To summarize we found alcohols, polar aprotic solvents and nitriles such as acetonitrile and propionitile as suitable solvents for the Pd-catalyzed allylic sulfenylation reaction using methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**). As a rule of thumb one can state that usually more polar solvents are superior to less polar solvents.

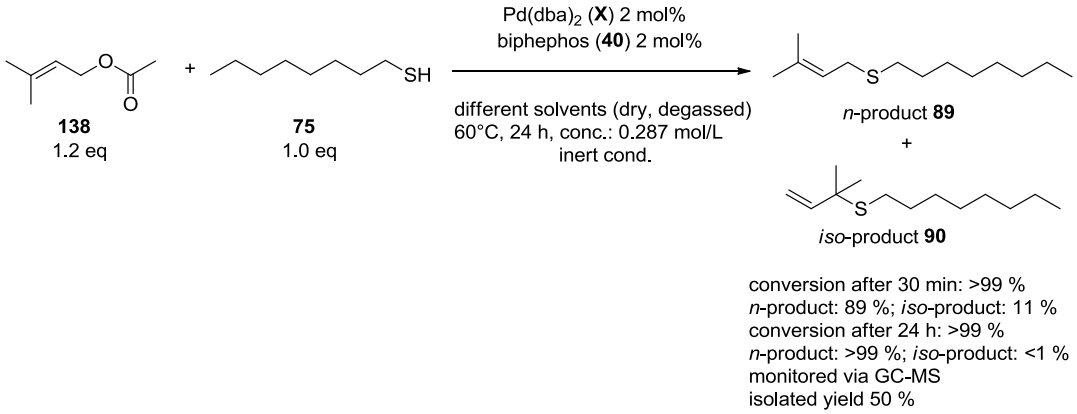
4.3.5.7. Using acetate leaving group containing starting material for the Pd-catalyzed alkylation of 1-octanethiol

As already mentioned in chapter 4.3.3.4 we were interested to investigate if also other leaving groups instead of a carbonate group are possible to use for the Pd-catalyzed allylic sulfenylation reaction using biphephos (**40**) as ligand. Therefore we synthesized 3-methylbut-2-enylacetate (isoprenyl acetate) (**138**) (Scheme 107). Probably because of the low boiling point of 3-methylbut-2-enylacetate (isoprenyl acetate) (**138**) we lost a lot of our desired product in the distillation workup yielding only 12 %. However, isoprenyl acetate (**138**) just served as a starting material, no efforts were made to improve the yield of this reaction.



Scheme 107: Synthesis of 3-methylbut-2-enylacetate (isoprenyl acetate) (**138**).

Isoprenyl acetate (**138**) was latter used for the Pd-catalyzed reaction to form (3-methylbut-2-en-1-yl)(octyl)sulfane (**89**) using catalyst ligand system $Pd(dba)_2$ (**X**) and biphephos (**40**) as illustrated in Scheme 108.



Scheme 108: Synthesis of (3-methylbut-2-en-1-yl)(octyl)sulfane (**89**) using 3-methylbut-2-enylacetate (**138**) as starting material.

Fortunately, GC-MS analysis showed full conversion after 30 min obtaining a product composition where the amount of *iso*-product **90** is <1 %. This illustrates that also other leaving groups than the thermodynamically favored carbonate group are possible for the Pd-catalyzed allylic sulfenylation reaction using biphephos (**40**) as ligand. However, after 24 h 5 % of disulfide **84** were detected via GC-MS giving a product composition of *n*-product **89**/disulfide **84** of 95/5. Unfortunately, after purification using flash column chromatography only 50 % yield was obtained. This is mainly due to loss of product during the purification process. Nevertheless, no efforts were made to further improve this yield or to repeat this reaction.

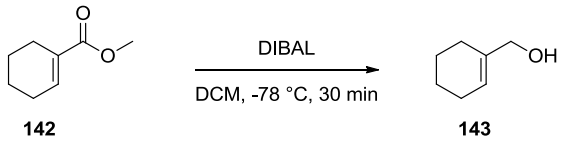
4.3.5.8. Synthesis of starting materials for the Pd-catalyzed allylic sulfenylation

In order to explore the scope and limitations of the Pd-catalyzed allylic sulfenylation reaction using $Pd(dba)_2$ (**X**) as pre-catalyst and biphephos (**40**) as ligand the synthesis of various carbonates as starting materials were necessary.

The syntheses were performed as already described in chapter 4.3.3.5 in Scheme 94. [256-257] The synthesized carbonates are listed in Table 34. (*E*)-Hex-2-en-1-yl methyl carbonate (**67**) (Table 34, entry 1) and methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) (Table 34, entry 3) have already been described in this thesis before, but are included in Table 34 again to present a complete list of carbonates used as starting materials.

In the case of cyclohex-1-en-1-ylmethyl methyl carbonate (**144**) (Table 34, entry 6) it was necessary to first reduce methyl cyclohex-1-enecarboxylate (**142**) to the corresponding alcohol **143** using DIBAL as reducing agent (Scheme 109). Reducing the ester without

reducing the double bond of the ring was only possible with DIBAL. Using the stronger reducing agent $LiAlH_4$ yielded in additional side product formation and the reduction of the double bond. Cyclohex-1-en-1-ylmethanol **143** was then used as starting material for the formation of carbonate **144**.

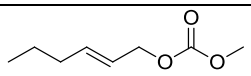
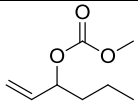
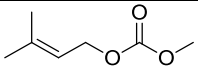
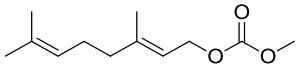
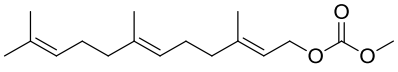
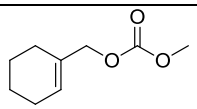
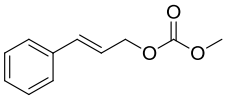
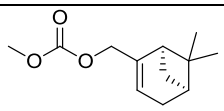


142 **143**

Scheme 109: Reduction of methylcyclohex-1-encarboxylate (**142**) using DIBAL to obtain starting material cyclohex-1-en-1-ylmethanol (**143**).

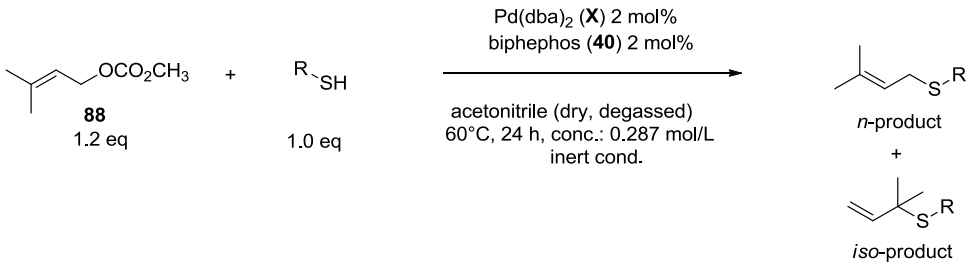
All carbonates shown in Table 34 could be obtained in excellent to good yields after extraction and purification using vacuum distillation (15 mbar). In case of problems separating the different layers, the organic phase was further diluted with diethyl ether to obtain a better separation of the layers.

Table 34: Different carbonates synthesized for the Pd-catalyzed allylic alkylation of different thiols.

entry	carbonate	number	Yield [%]
1		**67**	87
2		**139**	74
3		**88**	94
4		**140**	95
5		**141**	82
6		**144**	87
7		**145**	99
8		**146**	96

4.3.5.9. Pd-catalyzed allylic sulfenylation for the synthesis of different β,γ-unsaturated thioethers

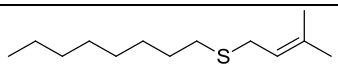
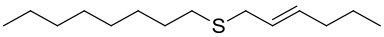
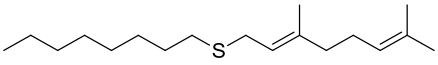
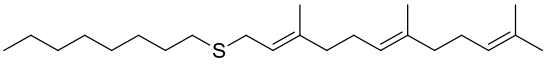
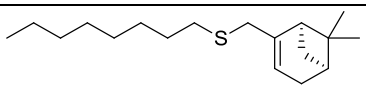
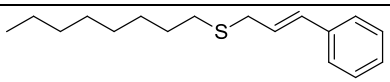
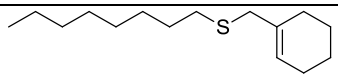
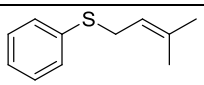
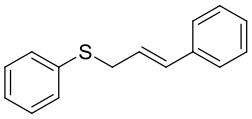
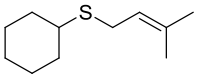
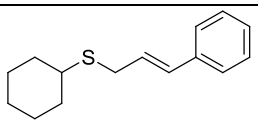
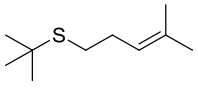
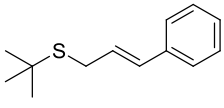
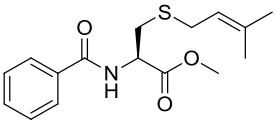
After having synthesized various carbonates (Table 34) to serve as starting material for the Pd-catalyzed formation of allylic thioethers we were ready to react them with different thiols. The aim was to cover different classes of substrates, meaning that branched and linear (non-branched) or aliphatic and aromatic compounds were used. Not only different carbonates, but also different thiols were used. These reactions should provide us with answers if this new Pd-catalyzed allylic sulfenylation reaction is of general use. The reaction conditions were kept the same, as in Scheme 110 showing isoprenyl carbonate **88** as an example. First the active catalytic species was formed in situ by stirring palladium pre-catalyst $Pd(dba)_2$ (**X**) and biphephos (**40**) as ligand for 30 min at 60°C under inert conditions in acetonitrile, degassed prior to use. In some cases the solution contained almost negligible amounts of black solid. Afterwards the desired thiol, degassed prior to use, was added under inert conditions to the 60°C hot solution. Directly afterwards the desired carbonate, degassed prior to use, was added under inert conditions to the 60°C hot solution. In case of having a solid starting material, it was added as a solid under inert conditions to the 60°C hot solution. All reactions shown in this chapter were worked up after 24 h, to ensure that isomerization reaction of the *iso*-product into the linear *n*-product occurred which is much slower than the actual bond forming reaction.

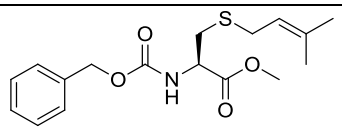
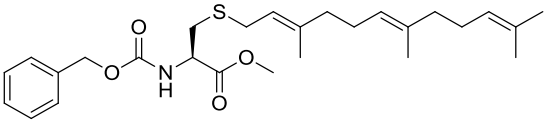


Scheme 110: General scheme for the Pd-catalyzed allylic sulfenylation reaction using $Pd(dba)_2$ (**X**) and biphephos (**40**). Isoprenyl carbonate (**88**) is shown as an example for all the different carbonates used as starting material (Table 34).

In Table 35 the obtained results are summarized. Pd-catalyzed allylic sulfenylation of peptides containing an cysteine moiety is described in chapters 4.3.6.1 and 4.3.6.2. Regarding table 35, entry 2 the synthesis of allylic thioether (*E*)-hex-2-en-1-yl(octyl)sulfane (**76**) was already mentioned in chapter 4.3.2.3 in Scheme 100. However, the Pd-catalyzed allylic sulfenylation reaction was performed with ligand dppf (**17**) instead of biphephos (**40**) in chapter 4.3.2.3.

Table 35: Different *β*,*γ*-unsaturated thioether obtained by Pd-catalyzed allylic alkylation of different thiols.

Entry	Product	No.	Conv. [%] after 24h	*n*/*iso*-ratio after 24h (GC/MS)	Isolated yield [%]
1		**89**	>99	99/1	91
2		**76**	>99	97/3	99
3		**147**	>99	99/1	95
4		**148**	>99	not assigned	99
5		**149**	>99	>99/<1	98
6		**150**	>99	98/2	94
7		**151**	>99	98/2	86
8		**152**	>99	>99/<1	95
9		**153**	>99	99:1	99
10		**154**	>99	98/2	90
11		**155**	>99	99/1	76
12		**156**	>99	>99/<1	15
13		**157**	>99	99/1	76
14		**110**	>99	>99/<1	82

15		**108**	>99	97/3	80
16		**158**	>99	>99/<1 [a)]	79

[a)] monitored by HPLC-MS (210 nm)

Fortunately, all reactions showed full conversion after 24 h, excellent to very good *n/iso*-ratios and good to excellent isolated yields. From reaction monitoring via GC-MS all reactions reached full conversion after only 30 min, but the reaction were worked up after 24 h to give the isomerization reaction of the *iso*-product into the linear *n*-product enough time to get into equilibration. Only once the conversion towards product (*R*)-methyl-2-(benzyloxycarbonylamino)-3-(3-methylbut-2-enylthio)propanoate **108** was with 96 % not complete after 24 h (Table 35, entry 15). After repeating this reaction we could already reach full conversion after 30 min.

The scope of this transformation turned out to be very broad. Independent of the carbonate used for this transformation all gave full conversions, whether an aliphatic one (Table 35, entries 1-8, 10, 12, 14-16) or cinnamylmethyl carbonate **145** containing an aromatic moiety, is used as starting material (Table 35, entry 9, 11, 13). Especially, the excellent results obtained using carbonates containing a terpene moiety like isoprenyl carbonate **88**, geranyl carbonate **140** and farnesyl carbonate **141** delighted us (Table 35, entries 1, 3, 4, 8, 10, 12, 14-16). With these results one big milestone of this project was fulfilled on our way to reach the ultimate goal to modify cysteine containing biomolecules using water soluble farnesyl carbonate.

The use of different thiols such as 1-octanethiol (**75**), thiophenol (**68**), cyclohexanethiol (**78**) and 2-methyl-2-propanethiol (**81**) resulted in mainly perfect conversions, *n/iso*-ratios and good to excellent isolated yields. Even the use of sterically more demanding thiols like cyclohexanethiol (**78**) and 2-methyl-2-propanethiol (**81**) gave full conversions, very good *n/iso*-ratios in good yields (Table 35, entry 10, 11, 13). Regarding the isolated yields there is one exception. The product *tert*-butyl(4-methylpent-3-enyl)sulfane **156** could only be obtained in 15 % yield although we saw full conversion on GC-MS (Table 35, entry 12). The reason for this poor isolated yield was the low boiling point of compound **156**. We lost most of it during evaporation of the solvent using a rotary evaporator. We are very optimistic that this poor yield of product **156** can be improved. However, no efforts were made to improve

the yield. One can assume that also other products listed in table 35 have a more or less high volatility. Slightly lower isolated yields after drying using vacuum, resulted from this fact.

In all cases in which cinnamylmethyl carbonate **145** (Table 34, entry 7) was used as starting material together with different thiols the resulting allylic thioether **150**, **153**, **155**, **157** consist only of the *trans*-isomers (*E*-configuration) shown in Table 35, entry 6, 9, 11, 13. This was confirmed by NOESY-NMR.

In GC-MS spectra of the reaction resulting in product (*E*)-(3,7-dimethylocta-2,6-dienyl)(octyl)sulfane (**147**) (entry 3) we found several peaks having very similar mass-spectra. This indicates isomerization due to the heat in the GC-inlet, because after purification using flash column chromatography only one product could be detected using ^{1}H and ^{13}C-NMR. Only the *trans*-isomer of **147** was found using NOESY-NMR Similar behavior was found when farnesyl carbonate **141** was analyzed via GC-MS, although NMR-spectra showed no other side products.

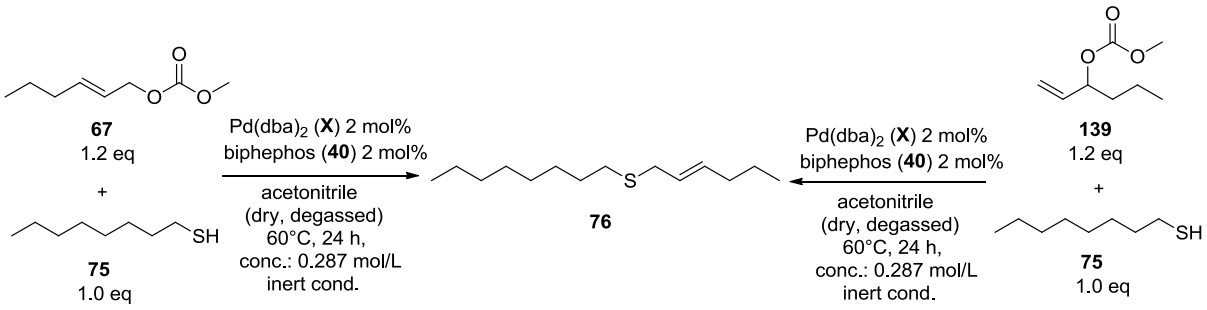
In the case of octyl((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)sulfane (**148**) (entry 4) one additional smaller peak could be found after 30 min in the GC-MS spectrum. The ratio between the two peaks was 89/11. The mass spectrum showed a high similarity to the mass spectra of product **148**. Unfortunately, no molecular peak could be found. However it was not possible to identify if the occurrence of this peak is due to the heat in the GC-inlet or if it is because of the formation of the corresponding *iso*-product. Nevertheless, the ^{1}H and ^{13}C-NMR after 24 h showed one single product **148**. The reaction was repeated and stopped after 30 min. TLC showed an additional tiny spot. Immediately, the product was purified using flash column chromatography taking care that both spots were collected. However, ^{1}H and ^{13}C-NMR only showed one single product **148**.

A very important milestone was fulfilled with the more challenging protected L-cysteines as substrates for the Pd-catalyzed allylic sulfenylation. L-Cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) and L-cysteine *N*-benzoyl methyl ester (**105**) were used as starting materials together with isoprenyl carbonate **88**. Due to the oily consistency of L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) it was dissolved first in acetonitrile, degassed prior to use and then added with a syringe to the 60°C hot solution of $Pd(dba)_2$ (**X**) and biphephos (**40**) under inert conditions. L-Cysteine *N*-benzoyl methyl ester (**105**) was added as a solid to the 60°C hot solution of $Pd(dba)_2$ (**X**) and biphephos (**40**) under inert conditions. Fortunately, in both cases we could find full conversion after 30 min and excellent to good *n/iso*-ratios (Table 35, entry 14, 15). Both products **110**, **108** could be isolated in good yields. Regarding the *n/iso*-ratio of *n*-product **108** and *iso*-product **109** with 97/3 monitored by GC-

MS we could not detect *iso*-product **109** in the ^{1}H-NMR-spectrum. This really made us enthusiastic that we will be able to farnesylate peptides or proteins. To come closer to this aim we used farnesyl carbonate **141** to farnesylate L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**). Full conversion was detected after 30 min via HPLC-MS (ESI, positive mode) using UV-trace at 210 nm to calculate conversion. After 24 h product **158** was isolated using flash column chromatography to obtain 79 % isolated yield. No *iso*-product could be found in HPLC-MS and in the NMR. It should be noticed that in the case of protected cysteines used as starting materials the isolated yields are lower than using simple thiols. Partly, this is due to the starting material itself. Using HPLC-MS to analyze the protected cysteines starting materials **102**, **105** before using them for the reactions, revealed ~5 % of disulfide present in the starting material. This is probably due to the long storage or also possible that the protected cysteines **102**, **105** get oxidized during dissolving them and measuring HPLC-MS.

Another important fact to mention is that the *n/iso*-ratios obtained via GC-MS are in good correlation with the *n/iso*-ratios found in ^{1}H-NMR after purification. In most cases no *iso*-product could be found in the corresponding ^{1}H-NMR. In the case of (*E*)-hex-2-en-1-yl(octyl)sulfane **76** the GC-MS spectrum shows an *n/iso*-ratio of 97/3 and ^{1}H-NMR spectrum shows 4 % *iso*-product **77**.

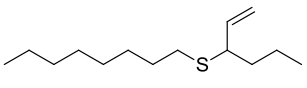
Using Pd-catalyzed allylic sulfenylation reaction with two constitutional isomers **67** and **139** it could be shown that only one single product (*E*)-hex-2-en-1-yl(octyl)sulfane (**76**) was formed (Scheme 111).



Scheme 111: Using constitutional isomers **67** and **139** and 1-octanethiol (**75**) as starting material for the Pd-catalyzed allylic sulfenylation reaction to form single product **76**.

This observation is in accordance with the mechanism of the Pd-catalyzed allylic sulfenylation reaction described in chapter 2. Right after the oxidative addition step, after the coordination of the allyl carbonate with the palladium, an allylic η^3-coordinated species is formed. It is obvious, that this allylic η^3-coordinated specie is the same for both carbonates

used. Therefore the thermodynamically more stable product **76** is formed. Although performing this reaction with hex-1-en-3-yl methyl carbonate **139** and 1-octanethiol (**75**) in principle also the formation of product **159** should be possible (Figure 32).



159

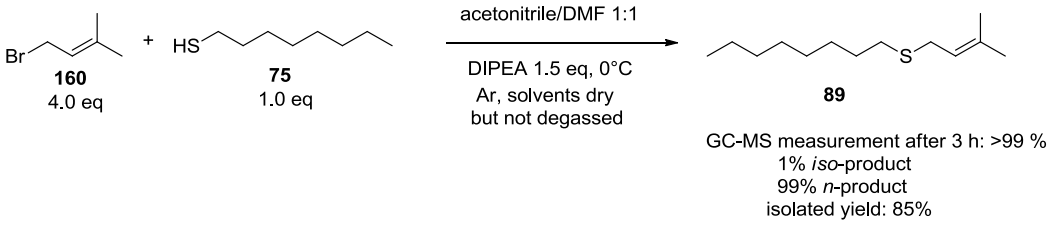
Figure 32: Possible product **159** for the Pd-catalyzed allylic sulfenylation reaction with hex-1-en-3-yl methyl carbonate **139** and 1-octanethiol (**75**).

According to the ^{1}H-NMR and ^{13}C-NMR spectra, product (*E*)-hex-2-en-1-yl(octyl)sulfane (**76**) formed using methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) only shows *E*-configuration. The product **76** obtained by using hex-1-en-3-yl methyl carbonate (**139**) and isoprenyl carbonate **88** showed a mixture of *E* and *Z*. The *E/Z*-ratio of 75/25 was confirmed by NOESY-NMR.

Summarizing this chapter it can be said that the Pd-catalyzed allylic sulfenylation reaction is a powerful tool to make allylic thioethers from a very diverse set of substrates.

However, it was time to compare our new method with the classical S_N2 reaction making allylic thioether. Therefore we used a protocol described by Kania et al.[131]

The reaction was performed with 1-bromo-3-methylbut-2-ene (isoprenyl bromide) (**160**) and 1-octanethiol (**75**) as our test substrate (Scheme 112). With the Pd-catalyzed allylic sulfenylation we found a competitive reaction, although the equilibration of isomers towards the desired *n*-product needs more time. Nevertheless, for our purpose to modify cysteine containing biomolecules, allylic bromide analogues as isoprenyl bromide (**160**) can be hardly used because of their high reactivity. Analogues of allylic bromide would also react with other functional groups present in biomolecules.



Scheme 112: Classical formation of allylic thioether (**89**) using isoprenyl bromide (**160**) and 1-octanethiol (**75**) as starting material.

4.3.5.10. Influence of cosubstrates on the Pd-catalyzed allylic alkylation

Having established a new method to alkylate or more importantly to farnesylate protected single amino acid L-cysteine it was time to test actually how tolerant is the Pd-catalyzed allylic sulfenylation using $Pd(dba)_2$ (**X**) and biphephos (**40**) compared to other functional groups.

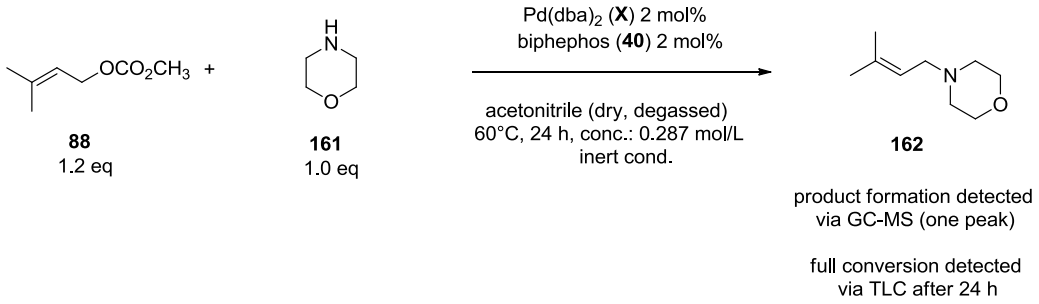
According to our aim to farnesylate peptides or proteins it was necessary to investigate the influence of molecules containing other functional groups than a thiol group. That is obvious, because peptides having also free amino and acid groups and also alcohol functionalities in the side chain. Reactions with acetic acid and alcohols as solvent were already performed successfully in chapter 4.3.5.6. However, we also wanted to use phenol as a cosubstrate to simulate tyrosine. Therefore, we used our model reaction with the starting materials methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) and 1-octanethiol (**75**) together with pre-catalyst $Pd(dba)_2$ (**X**) and and ligand biphephos (**40**) as shown in chapter 4.3.5.6 (Scheme 106). As solvents acetonitrile and ethanol, degassed prior to use, were used. Due to the fact that this Pd-catalyzed allylic sulfenylation reaction is a very fast reaction care was taken to add first 1-octanethiol (**75**) and directly afterwards 1 equiv. of phenol under inert conditions to the 60°C hot catalyst ligand solution. The last step was always addition of isoprenyl carbonate **88**. The results are shown in Table 36.

Table 36: Phenol used as a cosubstrate for the Pd-catalyzed formation of *β*,*γ*-unsaturated thioether (**89**).

Entry	Solvent	Additive	Conv. [%] after 24 h	(*n*/*iso*-ratio) after 24 h
1	acetonitrile	phenol 1.0 eq	>99	>99/<1
2	ethanol	phenol 1.0 eq	>99	>99/<1

With phenol as additive full conversion was reached after 30 min. Concerning, the *n*/*iso*-ratios the use of phenol as an additive was even advantageous. No *iso*-product **90** could be detected after 24 h via GC-MS.

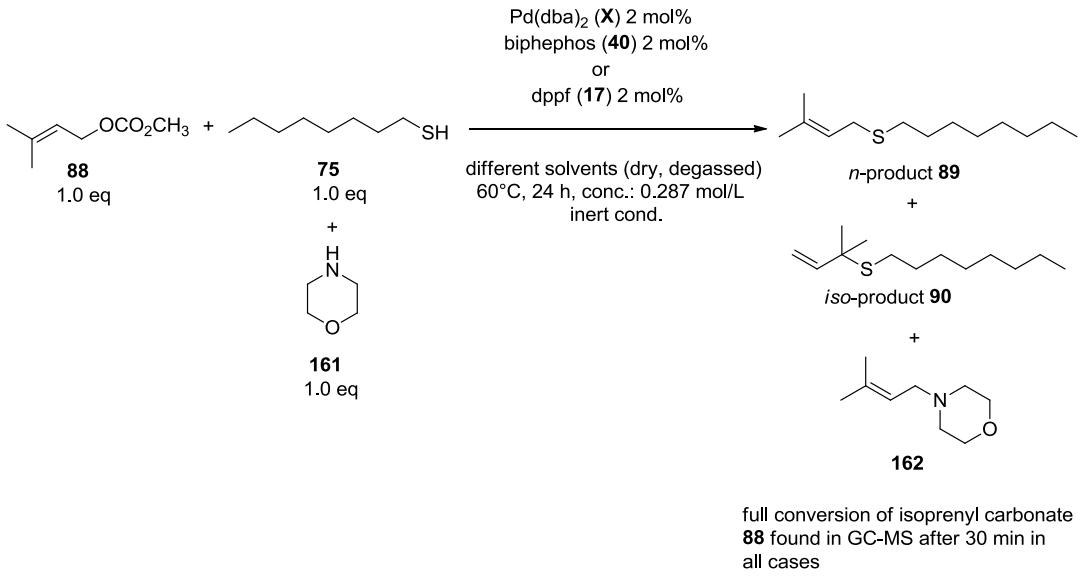
For simulation of basic amino functionalities we used morpholine (**161**). First, we decided to investigate the alkylation of this secondary amine without having a thiol group present (Scheme 113), because the Pd-catalyzed allylation of secondary amines with other ligands is well described in literature.



Scheme 113: Synthesis of 4-(3-methylbut-2-en-1-yl)morpholine (**162**).

Indeed, using biphephos (**40**) as ligand the tertiary amine product **162** formed. Due to the mini workup (silica gel) used to remove the palladium catalyst it was not possible to see starting material morpholine (**161**) on GC-MS. However, the formation of product **162** could be detected after already 30 min. We measured full conversion after 24 h using TLC.

Although in principle the amine functionality does react using our catalyst-ligand-system $Pd(dba)_2$ (**X**) and biphephos (**40**) it was still very interesting to see how morpholine does influence the allylation reaction on thiols. Therefore morpholine (**161**) was used as an additive to our model reaction shown in Scheme 114. For all three substrates (carbonate, thiol, amine) 1.0 equiv. was used. Concerning the proposed catalytic cycle, in principle, both functional groups can take part in the nucleophilic attack on the π-allyl group coordinated to the palladium. Although, our hope was that, due to potentially different kinetics, exclusively the thiol functionality will react and no reaction will occur with the amino functionality of the morpholine (**161**).



Scheme 114: Morpholine used as an additive for the Pd-catalyzed formation of *β*,*γ*-unsaturated thioether (**89**).

Three different reactions were performed. For all three reactions 2 mol% of $Pd(dba)_2$ (**X**) and biphephos (**40**) or dppf (**17**) and 1 equiv. each for morpholine (**161**), 1-octanethiol (**75**) and isoprenyl carbonate **88** were used. The reactions using biphephos (**40**) as ligand were performed in acetonitrile and in a second reaction in *tert*-butanol/H_2O 3:1. The reaction using dppf (**17**) as ligand was performed only in acetonitrile.

In all three reactions, we observed already full conversion of isoprenyl carbonate **88** after 30 min. Unfortunately but not surprisingly, we could monitor the formation of tertiary amine **162** beside the desired allylic thioether **89** even after 30 min. The use of biphephos (**40**) as ligand in acetonitrile revealed a product ratio of **162**:(**89** + **90**) 75:25 via GC-MS after 24 h. Concerning, the tertiary amine product **162** only one peak was found in GC-MS. The allylic thioether products were found as a mixture of isomers **89** and **90** having an unfavorable *n*/*iso*-ratio of 79/21. Regarding the reaction with biphephos (**40**) in *tert*-butanol/H_2O 3:1 we observed a product ratio of **162**: (**89** + **90**) 17:83 using GC-MS after 24 h. The *n*/*iso*-ratio of the isomeric thioethers **89** and **90** was 89/11. This results tells us that the use of water in contrast to acetonitrile is beneficial, which can be explained with a higher rate of protonated secondary amine present in *tert*-butanol/H_2O 3:1 than in acetonitrile. Due to the protonation of the amine functionality in morpholine (pka 8.36)[271] the nucleophilicity is reduced. However the *n*/*iso*-ratio even after 24 h is not as good as expected and no equilibration of *iso*-product **90** into desired *n*-product **89** could be observed.

The reaction performed using dppf (**17**) in acetonitrile also showed some formation of undesired teriary amine **162** monitored via GC-MS after already 30 min. We monitored a product ratio of **162**:(**89** + **90**) 8:92 after 24 h. The *n/iso*-ratio of the isomeric thioethers **89** and **90** was 94/6. In this special case ligand dppf (**17**) seems to be the better choice.

In the case of water used as solvent the amino groups in peptides and proteins are protonated. Therefore these protonated amino groups are much less nucleophilic and probably will not take part making a side reaction in the Pd-catalyzed allylic sulfenylation. Additionaly, working in water would enable us to chose different buffer-systems, adjusting the pH in a range that the amino functionalities present will probably not react.

4.3.6. Pd-catalyzed allylic alkylation reactions on protected peptides

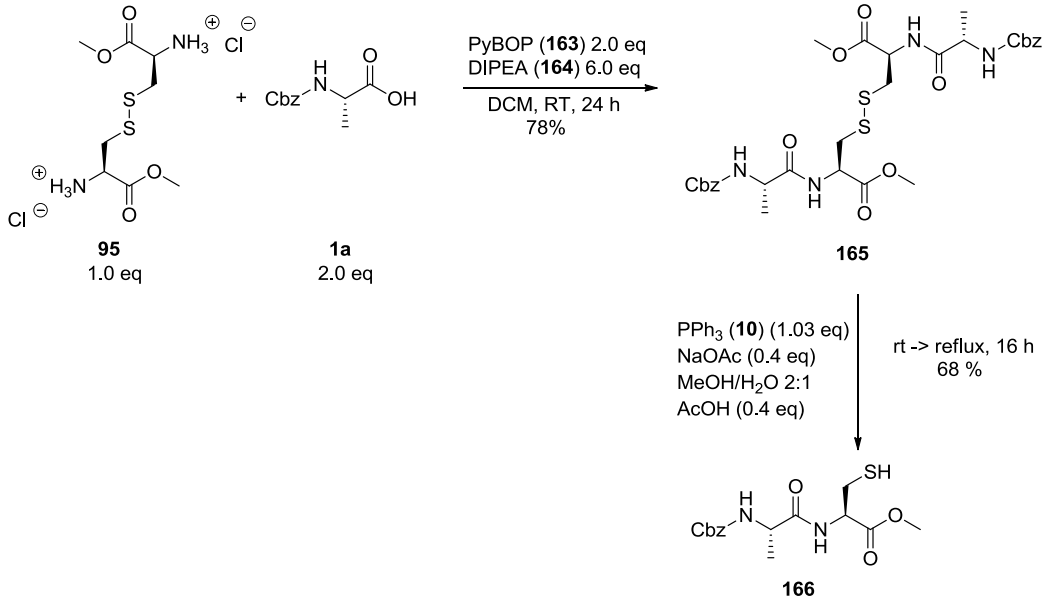
4.3.6.1. Synthesis of protected dipeptide Cbz-Ala-Cys-OMe and its use in the Pd-catalyzed allylic alkylation reaction

To achieve our main goal to modify biomolecules especially peptides and proteins in water it was necessary to synthesize first a dipeptide containing the amino acid L-cysteine. This L-cysteine should be later on alkylated using Pd-catalyzed allylic sulfenylation with the most efficient ligand biphephos (**40**). We first synthesized Cbz-Ala-Cys-OMe (**166**) because it is easy to prepare and it is soluble in acetonitrile or alcohols, which were used already as solvents for the Pd-catalyzed allylic sulfenylation. The use of unprotected peptides in water was not desired at this stage of the project due to the fact that the ligand biphephos (**40**) is not water soluble. Additionally, it was first necessary to test if the Pd-catalyzed allylic sulfenylation reaction proceeds using more complex and sterically demanding Cbz-protected peptides.

The synthesis of starting material Cbz-Ala-Cys-OMe (**166**) is shown in Scheme 115. In chapter 4.3.3.7 already described L-cystine methyl ester hydrochloride **95** together with Cbz-Ala-OH (**1a**) were used as starting materials. Together with 2 equiv. of coupling reagent PyBOP (**163**) and 6 equiv. DIPEA (**164**) the desired tetrapeptide $(Cbz\text{-}Ala\text{-}Cys\text{-}OMe)_2$ (**165**), containing a disulfide bridge, was synthesized in 78 % yield after purification via flash column chromatography. The coupling reagent PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) (**163**) was used as a substitute for the BOP reagent, to avoid the formation of carcinogenic side product HMPA (hexamethylphosphoramide).[272-273] The decision to use the Cbz protecting group instead of

Boc as protecting group for the amine functionality was due to the better visibility of the resulting Cbz-Ala-Cys-OMe in the UV at 210 nm using HPLC-MS.

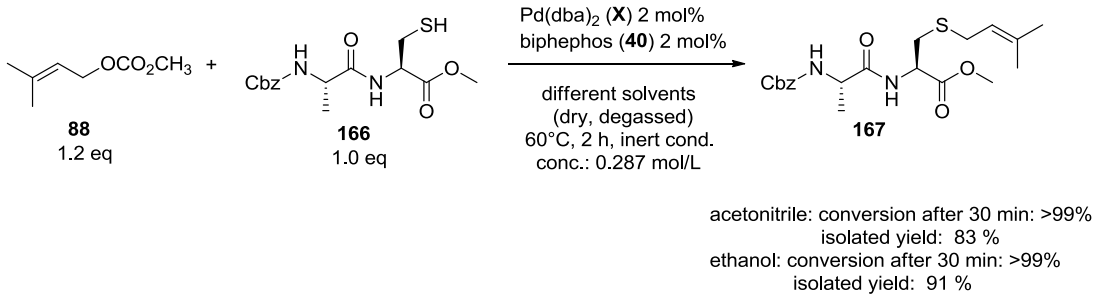
The second step was the reduction of the disulfide bond. Using the same protocol as already described in chapter 4.3.3.7 tetrapeptide $(Cbz\text{-}Ala\text{-}Cys\text{-}OMe)_2$ (**165**) was then reduced with triphenylphosphine (**10**) in $MeOH/H_2O$ 2:1 together with sodium acetate and acetic acid. After 16 h reaction time and after extraction and purification via flash column chromatography product Cbz-Ala-Cys-OMe (**166**) containing the necessary free thiol-group could be obtained in 68 % yield (Scheme 115).



Scheme 115: Synthesis of Cbz-protected dipeptide Cbz-Ala-Cys-OMe (**166**).

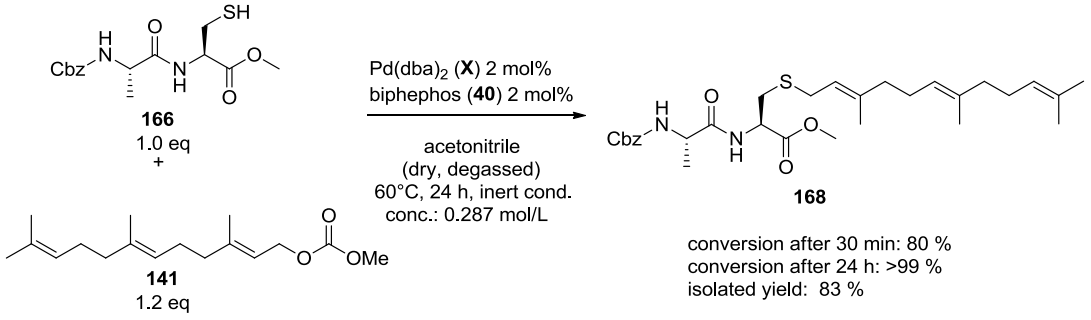
After having obtained Cbz-Ala-Cys-OMe (**166**) it was used as starting material together with isoprenyl carbonate **88** for the Pd-catalyzed allylic sulfenylation. Identical reaction conditions were used as already described in chapter 4.3.5.9. The conversion was monitored via HPLC-MS at 210 nm. This reaction was performed in acetonitrile and in ethanol. Fortunately, the Pd-catalyzed allylic sulfenylation showed already full conversion after 30 min. No differentiation between the two isomers (*n* or *iso*-product) was possible using Nucleosil C4 HPLC-column. After 2 h the reaction was controlled again still showing full conversion. Directly afterwards the product **167** was isolated using flash column chromatography obtaining allylic alkylation product **167**. In case of acetonitrile used as solvent 83 % yield could be reached containing only the *n*-product **167** shown in Scheme 116 as analyzed by ^{1}H-NMR. In case of ethanol as solvent we obtained 91 % overall yield having an *n/iso*-ratio of 95/5 as analyzed by ^{1}H-NMR. Probably the amount of putative *iso*-product, in the case of

ethanol used as solvent, would be much less after 24 h reaction time. Beside these fantastic results it should be mentioned that few percentages of unknown impurities could be found in the HPLC-trace at 210 nm, although a micro workup using silicagel was performed. Potentially, these can be degradation products of the active catalytic species present. No attempts were made to quantify and characterize these small impurities or side-products.



Scheme 116: Pd-catalyzed allylic alkylation using Cbz-Ala-Cys-OMe (**166**) and isoprenyl carbonate **88** as starting material.

Now it was time to test if a farnesylation of dipeptide Cbz-Ala-Cys-OMe (**165**) is also possible. Therefore an identical reaction to the above described one was performed. The only difference was the use of farnesyl carbonate **141** (Scheme 117). Acetonitrile was used as solvent. Again, the conversion was monitored via HPLC-MS at 210 nm after 30 min, 2 h and 24 h. The Pd-catalyzed allylic sulfenylation showed 80 % conversion towards product **168** (UV-trace: 210 nm) after 30 min. No differentiation between the two isomers (*n* or *iso*-product) was possible using Nucleosil C4 HPLC-column. After 2 h the reaction reached 97 % conversion. We obtained full conversion after 24 h. Afterwards the product was isolated using flash column chromatography obtaining allylic alkylation product **168** in 95 % yield. Again before purification we found some few percentages of unknown impurities in the HPLC-trace at 210 nm which was discussed already above.



Scheme 117: Pd-catalyzed allylic alkylation using Cbz-Ala-Cys-OMe (**166**) and farnesyl carbonate **141** as starting material.

This tremendously important result shows the power of this transformation (Scheme 117), so we were even more optimistic to be able to reach our goal to farnesylate peptides or proteins.

4.3.6.2. Synthesis of glutathione derivative and its use in the Pd-catalyzed allylic alkylation reaction

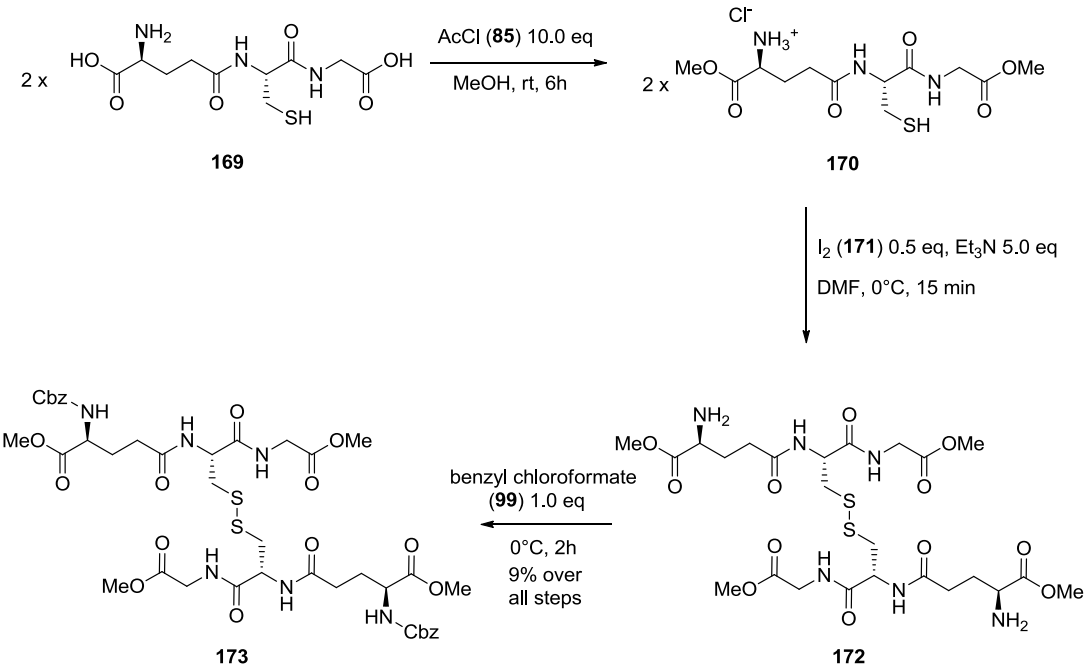
Taking into consideration, that we want to modify L-cysteine containing biomolecules such as the Ras-protein it appeared necessary to test an even more challenging substrate as starting material for the Pd-catalyzed allylic sulfenylation. In the posttranslational modification of Ras-protein in the endoplasmic reticulum a farnesyl-group is attached to the *C*-terminale cysteine and afterwards the *C*-terminal end is methylated.[16] However, at this stage of the project it would be too early to try to farnesylate the Ras-protein. Due to denaturation and often solubility issues of proteins in organic media the farnesylation of Ras-protein is not possible at this stage of the project. Nevertheless, we regarded glutathione as an interesting test substrate, due to its high natural abundance in cells and its cheap price. Glutathione is a pseudotripeptide (cysteine, glutamic acid and glycine) containing an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side-chain. Glutathione acts mainly as an antioxidant preventing free radicals and peroxides from harming the cell.[274] The synthesis of Cbz-protected and methylated glutathione with a free thiol group **176** would allow us to first test this substrate in organic solvents before testing the real case in water. Again Cbz protection of the amine functionality was favored, due to the better visibility of the resulting product in the UV-spectrum at 210 nm using HPLC-MS.

The strategy of using thionylchloride (**94**) to make the methylester of the carboxylic acid groups and subsequently protect the amino groups according to literature protocols using similar substrates failed.[263],[275-276] Due to the low solubility of the oxidized form of glutathione in methanol also the second strategy of making the corresponding methylester by using trimethylsilyldiazomethane (TMS-CHN_2) as reagent failed.[277],[278]

However, there were still at least two options left. One strategy was published by Hutton et al., starting the protection of the thiol group using trityl alcohol followed by Cbz-protection of the amino groups. Afterwards the carboxylic acid is esterified and in the end the trityl protecting group is cleaved.[202] We employed the protocol published by Davis et al., because it promised to be the most efficient synthetic strategy with the lowest expenditure of time due to less synthetic steps.[279] The synthetic steps are shown in Schemes 118 and 119. Although

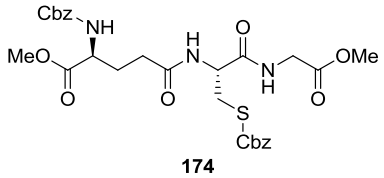
Davis et al. used the Boc protection group instead of the desired Cbz protection group as in our case we could obtain the desired product in sufficient yield.

The first step of the reaction scheme was the generation of HCl containing methanol solution by adding acetyl chloride (**85**) to ice cold methanol. After adding glutathione (**169**) and after 6 h reaction time the volatile compounds were evaporated using a rotary evaporator. Without further purification the esterified glutathione hydrochloride **170** was used directly for the oxidation step with iodine (**171**) and triethylamine in DMF at 0°C. Directly to this reaction solution containing crude product **172**, after 15 min benzyl chloroformate (**99**) was added for protection of the amino functionality and it was stirred for additional 2 h at 0°C.



Scheme 118: Synthesis of Cbz-protected glutathione oxidized (glutathione disulfide) **173**.

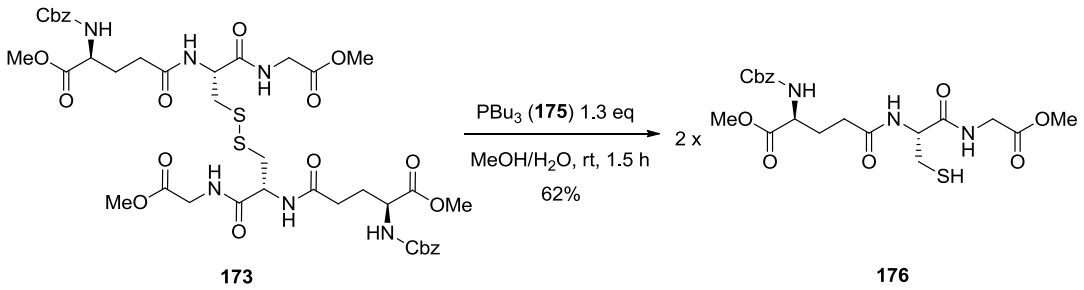
After extraction and evaporation of the volatile compounds using a rotary evaporator the crude product was purified via flash column chromatography. Unfortunately, this purification step did not lead to pure compound **173**. We recrystallized the product from DCM / petroleum ether. However, again we were not able to obtain pure product **173**. This was mainly due to side product formation during the reaction leading side product **174** having the thiol group protected by Cbz. This putative side product **174** was only assigned by its mass via HPLC-MS (Figure 33). Probably this formation of side product **174** is the main reason for the low yield of 9 % over all steps. Nevertheless, for our purpose of using Cbz-protected glutathione as starting material for Pd-catalyzed allylic sulfenylation this was good enough.



174

Figure 33: Putative side product **174** assigned by HPLC-MS.

The last step of this synthetic route was the reduction of disulfide product **173** containing small amounts of putative side product **174**. This time tributyl phosphine (**175**) in methanol/water 5/1 was used for the reduction of **173** instead of triphenyl phosphine (**10**) mentioned in chapter 4.3.6.1. The reason was that we wanted to stay as close as possible to the reported protocol form Davis et al.[279]

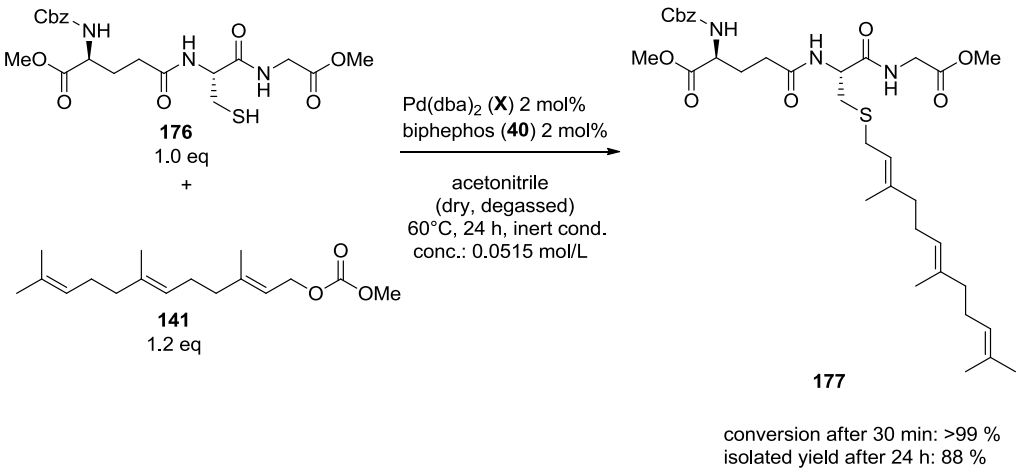


173 176

Scheme 119: Synthesis of Cbz-protected glutathione reduced **176**.

After 1.5 h the volatile compounds were evaporated using a rotary evaporator and the crude product was purified via flash column chromatography. Once again we could not obtain the desired pure product **176**. Therefore product **176** was recrystallized under inert conditions from DCM / petrolether twice to obtain Cbz-protected and esterified glutathione Cbz-GSH-OMe **176** in 62 % yield. NMR and HPLC confirmed product formation having a purity of >96 % at 210 nm UV-trace (HPLC-MS).

With starting material **176** in hand we were now able to test if the Pd-catalyzed allylic sulfenylation also works efficiently when using more challenging and sterically more demanding tripeptide Cbz-GSH-OMe **176**. As the starting carbonate farnesyl carbonate **141** was chosen to resemble the real case (Scheme 120).



Scheme 120: Pd-catalyzed allylic alkylation using glutathione derivative **176** and farnesyl carbonate **141** as starting materials.

Due to the low solubility of Cbz-GSH-OMe **176** we had to use a concentration (0.0515 mol/L), which was ~5 times lower than the concentration (0.287 mol/L) used so far for the Pd-catalyzed allylic sulfenylation. Our concerns of having lower conversions due to the lower concentration were falsified by the fact that we obtained full conversion after 30 min, which is an impressive result. The reaction was stopped after 24 h and the farnesylated product **177** was isolated using flash column chromatography to yield 88 % product with purity >97 % (HPLC-MS 210 nm). Only the corresponding *n*-product was found in NMR. As already described in chapter 4.3.6.1 a few percentages of unknown impurities could be found in the HPLC-trace at 210 nm. No differentiation between the two isomers (*n* or *iso*-product) was possible using Nucleosil C4 HPLC-column. However, we still wanted to kown whether there is a formation of *iso*-product or not. Therefore, this reaction was repeated and stopped after already 2 h ensuring full conversion. Fortunately, after flash column chromatography ensuring that all spots (3 in total; one big, 2 tiny ones) were collected the ^{1}H-NMR and ^{13}C-NMR did not show a formation of *iso*-product. Maybe in this case the use of sterically quite demanding glutathione derivative **176** led predominantly to the formation of desired *n*-product **177**.

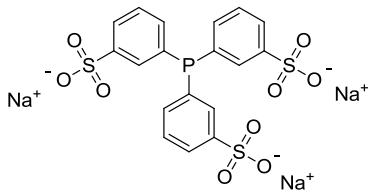
Due to the low solubility of Cbz-GSH-OMe **176** in acetonitrile we used 2,2,2-trifluorethanol as solvent for the Pd-catalyzed allylic sulfenylation. This reaction was performed using a concentration of 0.287 mol/L. Although we obtained full conversion already after 30 min we could only isolate one fraction of farnesylated product **177** with 32 % yield and a purity of >95 % (HPLC-MS 210 nm) after 24 h. Another fraction having a yield of 28 % did contain more unknown impurities. Perhaps the higher acidity of 2,2,2-trifluorethanol compared to

acetonitrile is responsible for the higher amount of unknown impurities found in HPLC-MS. However, no efforts were made to isolate or further characterize these unknown impurities.

As a summary it should be emphasized that we can report a very efficient method to farnesylate even complex and sterically demanding substrates like Cbz-GSH-OMe **176**.

4.3.6.3. Influence of Cbz-tyrosine as a cosubstrate on the Pd-catalyzed allylic alkylation reaction

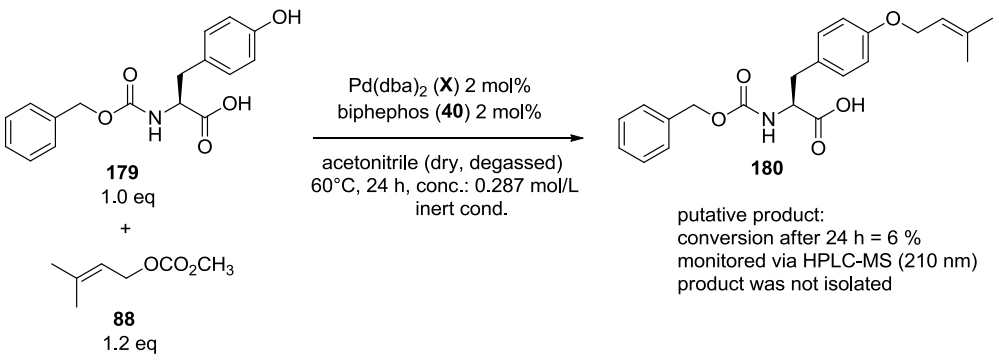
Concerning our ongoing interest in the modification of biomolecules, especially cysteine containing peptides or proteins, we have to ensure that the commonly occurring amino acid tyrosine is not influencing our Pd-catalyzed allylic sulfenylation. We worried about the behaviour of tyrosine using the protocol for the Pd-catalyzed allylic sulfenylation because first Francis et al. and later Ma et al. could report that allylpalladium complexes can selectively react with nucleophilic phenolic groups using $Pd(OAc)_2$ (**XI**) and TPPTS (**178**) (Figure 34) in 100 mM phosphate buffer pH 8.5 at rt.[167],[280]



178

Figure 34: TPPTS triphenylphosphine tris-(sulfonate) (**178**).

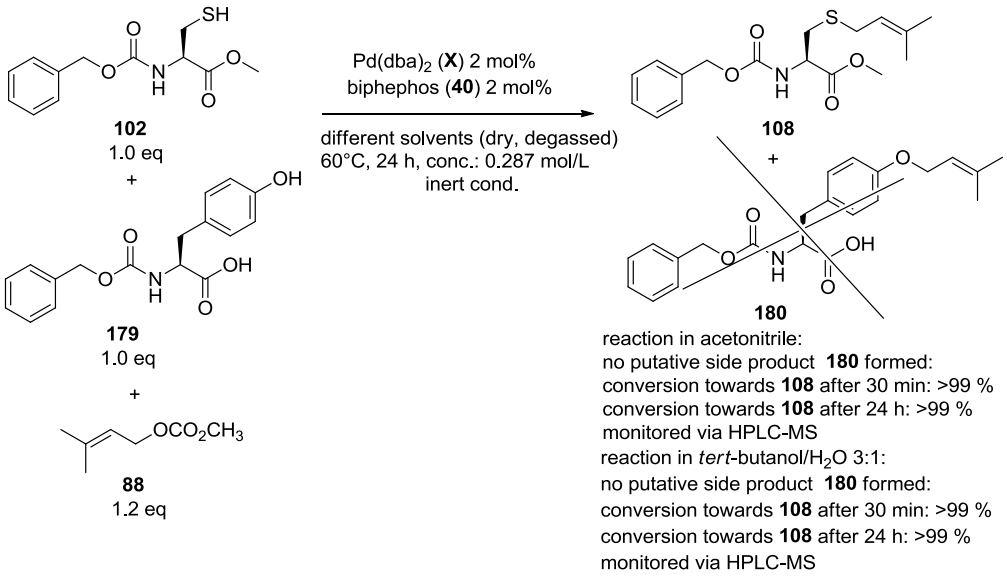
Although we had already successfully used phenol as an additive to the Pd-catalyzed allylic sulfenylation reaction of 1-octanethiol (**75**) with isoprenyl carbonate **88** in acetonitrile it appeared necessary to test also Cbz-Tyr-OH (**179**). The properties of this substrate are much closer to the properties of the amino acid tyrosine within a protein. As shown in Scheme 121 Cbz-Tyr-OH (**179**) and isoprenyl carbonate **88** are used as starting materials for the Pd-catalyzed allylic sulfenylation having ligand biphephos (**40**) and pre-catalyst $Pd(dba)_2$ (**X**) present.



Scheme 121: Pd-catalyzed allylic alkylation using Z-Tyr-OH (**179**) and isoprenyl carbonate **88** as starting materials.

We hoped to find no conversion of Cbz-Tyr-OH (**179**) but this was not the case. Using HPLC-MS (210 nm) we observed ~6 % putative product formation **180** after 24 h towards the allylated phenol group of tyrosine. This assignment was made only by using the mass-spectra of the corresponding peak obtained by HPLC-MS.

Although this can be considered as an unwanted result it already showed that thiols groups are the preferred substrates for this transformation. Without hesitation we tested Cbz-Tyr-OH (**179**) together with thiol group containing L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (Cbz-Cys-OMe) (**102**) in a 1:1 ratio in acetonitrile and in *tert*-butanol/H_2O 3/1 (Scheme 122). The same conditions were used as mentioned already in chapter 4.3.5.9 for the reactions shown in Schemes 121 and 122.



Scheme 122: Pd-catalyzed allylic alkylation using Z-Cys-OMe (**102**) together with Z-Tyr-OH (**179**) and isoprenyl carbonate **88** as starting materials.

Fortunatly, only product **108** was formed in both cases. No possible side reaction with Cbz-Tyr-OH (**179**) could be detected via HPLC-MS at 210 nm, although an excess of isoprenyl carbonate **88** was used. This result indicates that the desired product **108** is formed very fast and consumes most of the isoprenyl carbonate **88** and the rest left is probably too low to obtain detectable amounts of side product **180**. Due to the use of Nucleosil C4 HPLC-column no ***iso/n***-ratios are given. No efforts were made to isolate the obtained product **108**, because it was already characterized before (chapter 4.3.5.9). Although this result can be regarded as great, it cannot be fully excluded that side reactions due to a tyrosine residues within the peptide or protein can occur in a more complex setting.

4.3.7. Pd-catalyzed allylic alkylation reactions using TEGylated biphephos as ligand

4.3.7.1. Introduction

At this stage of the project at least one main challenge needed our attention before we would be able to modify cysteine containing biomolecules in water. It was necessary to further increase the water solubility of the starting material and the catalyst-ligand system used. Basically, in our case there are three different ways to achieve better water solubility for substrates not soluble yet in water. The first one is the use of a water soluble disposable group which is cleaved during the reaction.[167] In our case this strategy is just useful for the allyl containing starting material, but not for the ligand biphephos (**40**). On the other hand one can use surfactants like sodium dodecyl sulfate (SDS) as additives which help bringing apolar molecules into a solution of water, due to their amphiphilic character. This was already exploited for transition metal catalysis in water for example by Lipshutz et al.[281] However, also this strategy was abolished due to their well known denaturing effect on proteins. Last but not least we decided to PEGylate starting material **88** and the ligand biphephos (**40**) due to easier preparation concerning the ligand compared to sulfonated phosphite or tetraalkylammonium phosphite. Although sulfonated phosphines and tetraalkylammonium phosphines were already successfully used in water.[282-283] we preferred to use PEGylation. The preparation of PEGylated starting material and the ligand biphephos (**40**) are further described below in chapter 4.3.7.2.

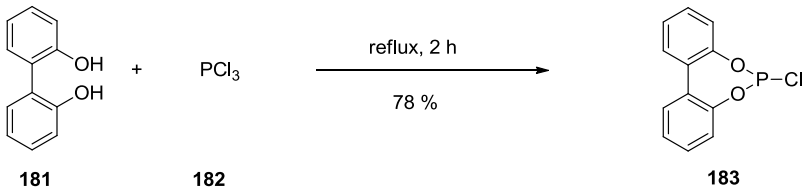
Beside the necessity of having water soluble starting materials and ligands, it is likely, that the use of water as solvent has beneficial effects on the catalytic reactions. Several studies revealed the phenomenon that transition metal catalyzed reactions are less sensitive to air

when performed in water compared to organic media. Although the exact reason for this phenomenon remains unclear, it is explained with the hard-soft and acid-base principle. The (hard) water molecules do not interfere so much with the (soft) late transition metals.[284-287]

4.3.7.2. Synthesis of TEGylated isoprenyl carbonate and TEGylated biphephos

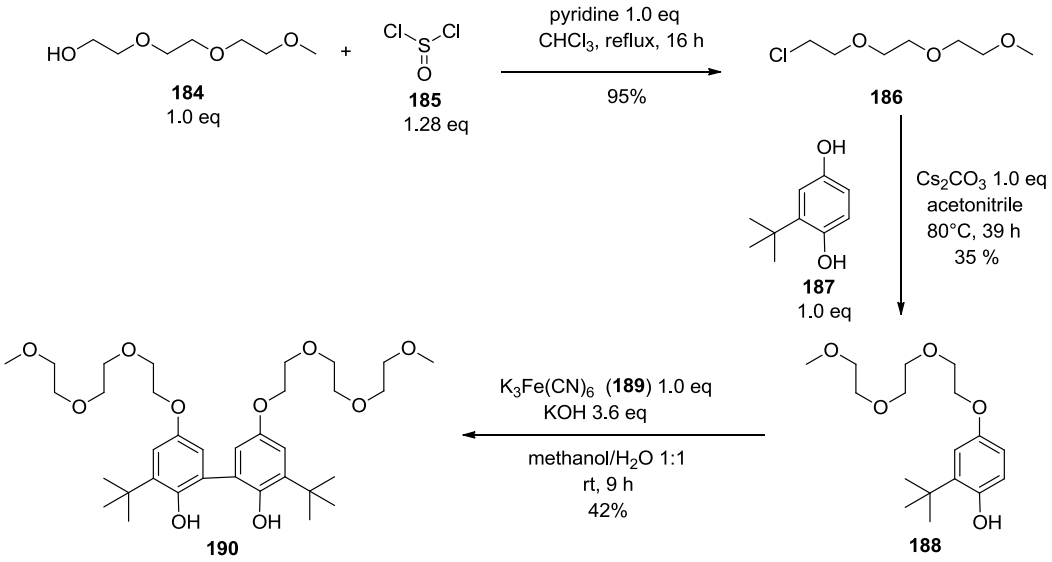
We dicided to increase the solubility of isoprenyl carbonate **88** and ligand biphephos (**40**) in water by using TEGylation (triethylene glycol-group). For this TEGylation of ligand biphephos (**40**) several reactions steps were necessary. These reaction steps were kindly performed by Nicole Mayer M.Sc.

The first step was the preparation of 6-chlorodibenzo[d,f][1,3,2]dioxaphosphepine (**183**) according to the literature (Scheme 123).[288] After distillation **183** could be obtained in 78 % yield.



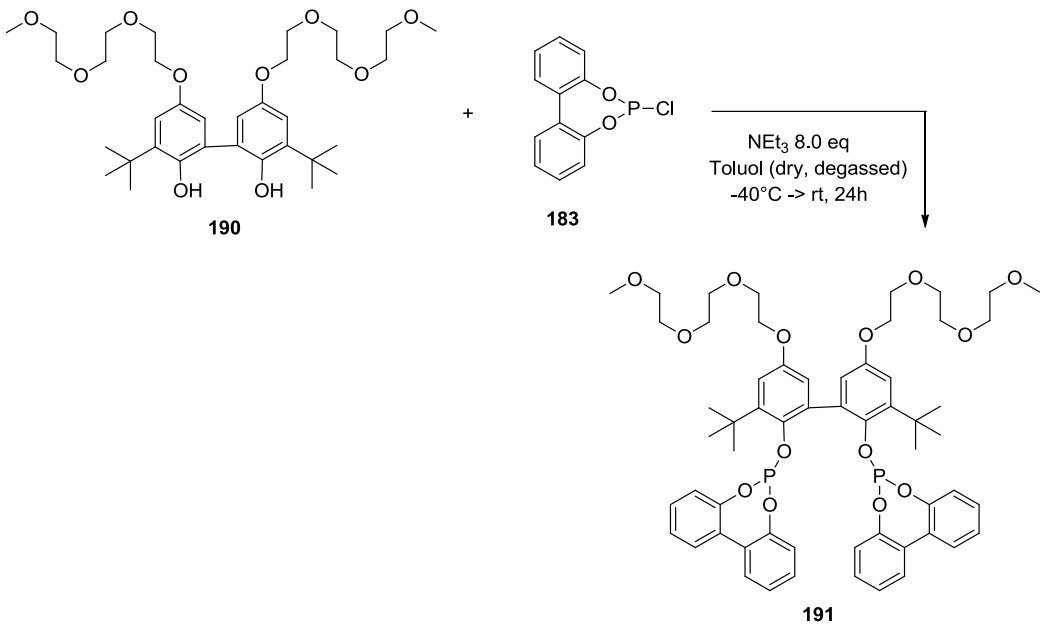
Scheme 123: Synthesis of 6-chlorodibenzo[d,f][1,3,2]dioxaphosphepine (**183**).

Parallel the synthesis of TEGylated precursor **190** for the TEGylated biphephos (Scheme 124) was performed. First part was the synthesis of 1-chloro-2-(2-(2-methoxyethoxy)ethoxy)ethane (**186**) according to Wilcox et al.[289] Afterwards 2-(tert-butyl)benzene-1,4-diol (**187**) was added to compound **186** to yield its TEGylated analog **188** in 35 % yield.[290-291] The last synthesis step in Scheme 124 was performed similar to a literature protocol published by Tunge et al.[292] Product **190** could be isolated with 42 % yield.



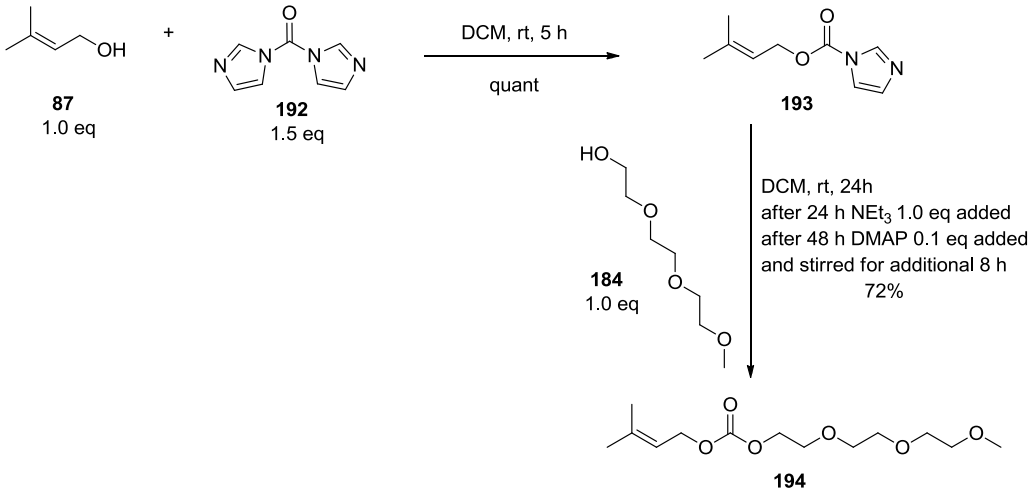
Scheme 124: Synthesis route to TEGylated compound **190**.

The last step was the formation of TEGylated biphephos (TEG-biphephos) **191** (Scheme 125) combining TEGylated product **190** and 6-chlorodibenzo[d,f][1,3,2]dioxaphosphepine (**183**) in toluene together with the base triethylamine (NEt_3).[288] TEG-biphephos **191** was purified using flash column chromatography under inert conditions with argon as inert gas. TEG-biphephos **191** was obtained in quantitative yield after purification. ^{1}H-NMR showed impurities probably due to the grease (Lithilen) used for the Schlenk-line. Triturating TEG-biphephos **191** in *n*-pentane, degassed prior to use reduced the amount of these peaks significantly.



Scheme 125: Synthesis of TEG-biphephos **191**.

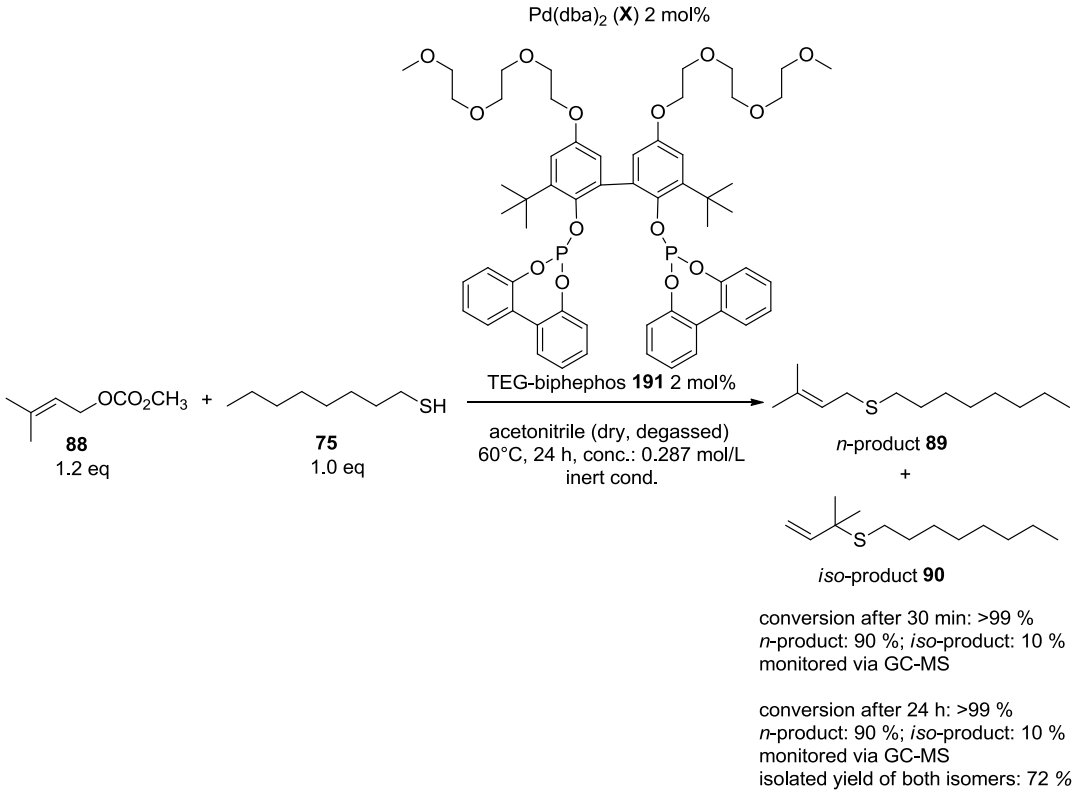
After having obtained TEG-biphephos **191** it was still necessary to make the starting material water soluble. The TEGylated analog to isoprenyl carbonate **88** was synthesized by Nicole Mayer M.Sc. Due to the use of isoprenyl carbonate **88** as test substrate in many reactions described before, we regarded also its anolog TEG-isoprenyl carbonate **194** as an ideal test substrate. For the synthesis of TEG-isoprenyl carbonate **194** we used the same TEG-group **184** as already employed for the formation of TEG-biphephos **191**. The first step was the activation of the alcohol group of isoprenyl alcohol **87** using 1,1'-carbonyldiimidazole (CDI) (**192**) in DCM to form product **193** in quantitative yield after trituration in *n*-hexane (Scheme 126). Afterwards TEG-group **184** was added to product **193** in DCM. Due to no conversion after 24 h the base triethylamine (NEt_3) was added. However only ~10 % conversion towards the desired product could be detected via GC-MS after 48 h. Therefore 10 mol% of the base DMAP were added yielding 97 % conversion after additional 8 h. After extraction and purification via flash column chromatography the product TEG-isoprenyl carbonate **194** could be obtained in 72 % yield.



Scheme 126: Synthesis of TEG-isoprenyl carbonate **194**.

4.3.7.3. Use of TEGylated biphephos as ligand in the Pd-catalyzed allylic alkylation reaction

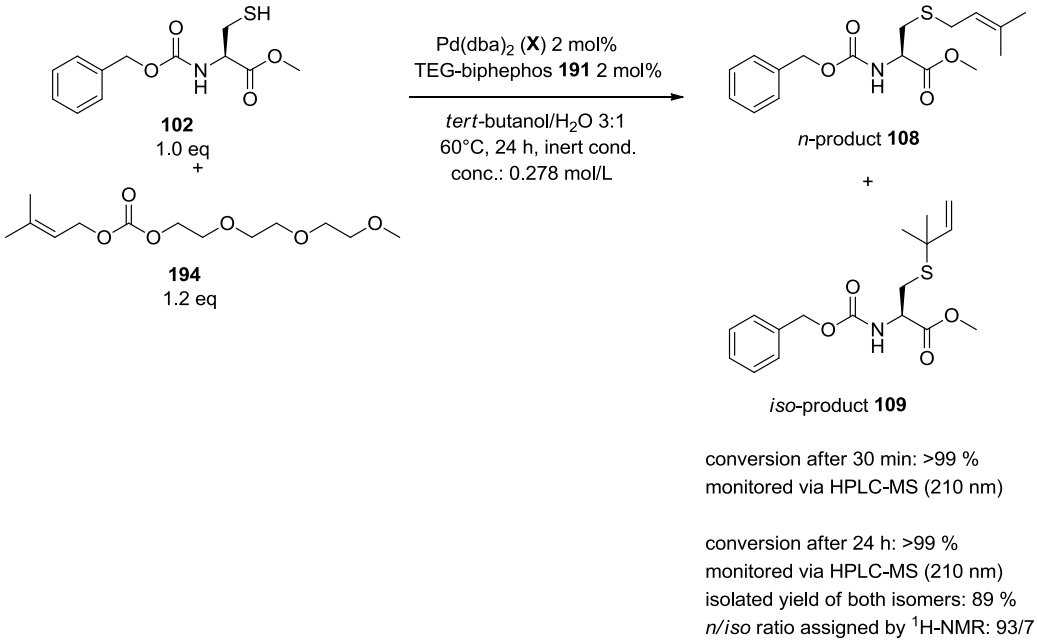
Having obtained TEG-biphephos **191** we were keen to first test if this PEGylated ligand is able to catalyze our model reaction as efficient as ligand biphephos (**40**) did. For this reaction the same conditions were used as already described in chapter 4.3.5.1. Acetonitrile, degassed prior to use, was used as solvent to obtain comparable results (Scheme 127).



Scheme 127: Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) using TEG-biphephos **191** as ligand.

The rate of the catalytic reaction of TEG-biphephos **191** compared to biphephos (**40**) was compatible. GC-MS showed full conversion after 30 min with an *n/iso* ratio 90/10. Unfortunately, we could not monitor any equilibration of the isomers after 24 h via GC-MS, meaning that the *n/iso* ratio remained with 90/10 still the same. Regarding the isomerization reaction TEG-biphephos **191** seems to be inferior compared to biphephos (**40**).

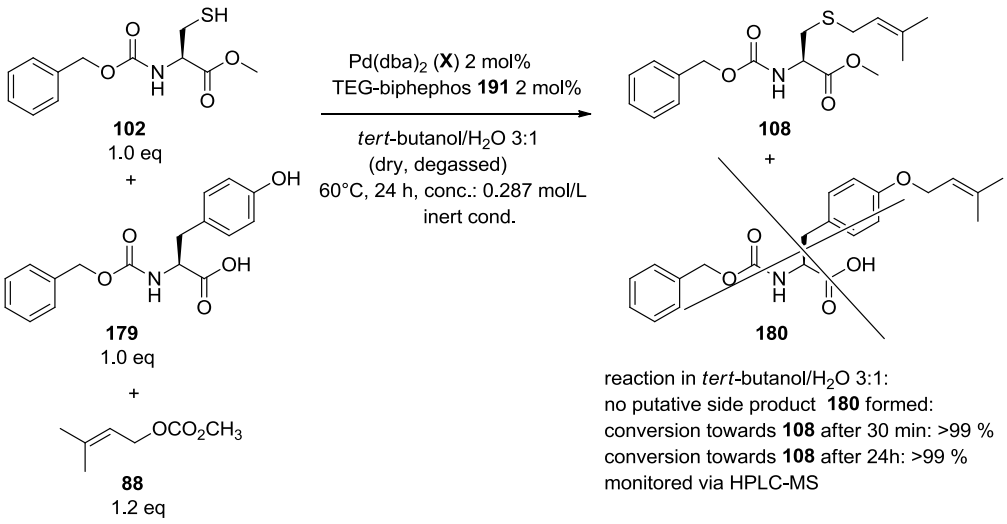
Nevertheless, it was of great importance for us to test TEG-biphephos **191** together with TEG-isoprenyl carbonate **194**. For this reaction the same conditions were used as already described in chapter 4.3.5.1. *Tert*-butanol/H_2O 3:1, degassed prior to use, was used as solvent (Scheme 128). We have chosen L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**), which is not water soluble, as substrate, because we already obtained product **108** using biphephos (**40**) as ligand. This fact helped us investigating if there will be also more *iso*-product **109** present in this case.



Scheme 128: Pd-catalyzed allylic alkylation of 1-octanethiol (**75**) using TEG-biphephos **191** as ligand and TEG-isoprenyl carbonate **194**.

The use of TEG-biphephos **191** instead of biphephos (**40**) showed no significant change in the conversion detected. Again full conversion is detected after 30 min via HPLC-MS (210 nm). Due to the use of Nucleosil C4 HPLC-column no *iso/n*-ratios are given. After 24 h the volatile compounds of the reaction mixture were evaporated using a rotary evaporator and purification was performed using flash column chromatography. The isolated yield of only 89 % (mixture of isomers) is more or less also due to ~ 5 % disulfide formation of starting material L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) monitored via HPLC-MS. With ^{1}H-NMR 4 % of *iso*-product **109** were found. The formation of *iso*-product observed in the ^{1}H-NMR-spectrum is with just 7 % a little bit higher than no *iso*-product found in ^{1}H-NMR reported in chapter 4.3.5.9 in Table 35, entry 15 using biphephos (**40**) as ligand in acetonitrile.

However we were interested to test if Cbz-Tyr-OH (**179**) is also not alkylated using TEG-biphephos **191** instead of biphephos (**40**) (Scheme 129). Therefore the same reaction with exception of the ligand used performed under identical conditions as described in chapter 4.3.6.3.



Scheme 129: Pd-catalyzed allylic alkylation using Z-Cys-OMe (**102**) together with Z-Tyr-OH (**179**) and isoprenyl carbonate **88** as starting material and TEG-biphephos **191** as ligand.

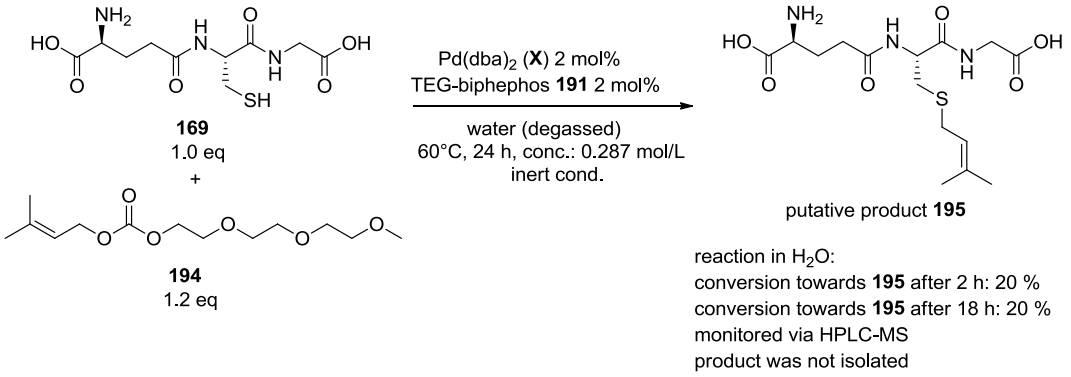
Not surprisingly, also in the case of TEG-biphephos **191** used as ligand no putative side product **180** could be monitored via HPLC-MS, but having full conversion towards the desired product **108**. Due to the use of Nucleosil C4 HPLC-column no *iso/n*-ratios are given.

Finally, it was time to test the Pd-catalyzed allylic sulfenylation reaction of glutathione reduced (**169**) with TEG-isoprenyl carbonate **194** in distilled water (Scheme 130). First we tested if TEG-isoprenyl carbonate **194** and TEG-biphephos **191** were water soluble under the conditions and concentrations used for the Pd-catalyzed allylic sulfenylation as shown in Scheme 86. TEG-isoprenyl carbonate **194** was not fully soluble at rt but it was fully dissolved at 60°C. Unfortunately, ligand TEG-biphephos **191** showed only low solubility in water at 60°C. Beside the not dissolved ligand, we only could observe that water was colored light green-brown. This color indicates that the ligand was at least slightly soluble in water.

This finding was indeed disappointing, but we still wanted to investigate if at least some product is formed. There was still hope that even low concentrations of the TEGylated catalyst-ligand system in water will catalyze the reaction shown in Scheme 130.

Due to the very poor solubility of TEG-biphephos **191** in water we decided to first form in situ the catalyst-ligand system in acetonitrile, degassed prior to use. Therefore $Pd(dba)_2$ (**X**) and TEG-biphephos **191** were dissolved in acetonitrile at 60°C and stirred for 30 min. Afterwards acetonitrile was evaporated under inert conditions using an oil-pump (0.02 mbar). After dryness was reached water, degassed prior to use, was added and heated to 60 °C. As expected the catalyst-ligand system was mainly not dissolved even when using an ultra-sonic bath for 1 min to support solution of the catalyst-ligand system. However, we could

observe some light green-brownish color in water. Afterwards both starting materials were added. Both were dissolved quickly.



Scheme 130: Pd-catalyzed allylic alkylation using glutathione reduced **169** and TEG-isoprenyl carbonate **194** as starting material together with $Pd(dba)_2$ (**X**) and TEG-biphephos **191** as catalyst-ligand system.

The reaction was monitored by HPLC-MS after 2 h and 18 h. Not surprisingly, we only could monitor ~20 % conversion towards putative product **195** using HPLC-MS (210 nm). Even after 18 h the conversion was still the same. Additionally, after 18 h we observed the formation of unknown impurities (~30 %, relative to product and starting material). No attempts were made to quantify and characterize these small impurities or side-products. Due to the low conversion we did not attempt to isolate the observe product. Using HPLC-MS we did not find another peak with the same mass. Therefore no differentiation between the putative *n*- and *iso*-product was possible. Additionally, this finding also means that probably no alkylation of the amino group did occur because this would definitely result in a different retention of this product on the HPLC-column used. However, it cannot be fully excluded that also the amino functionality did react.

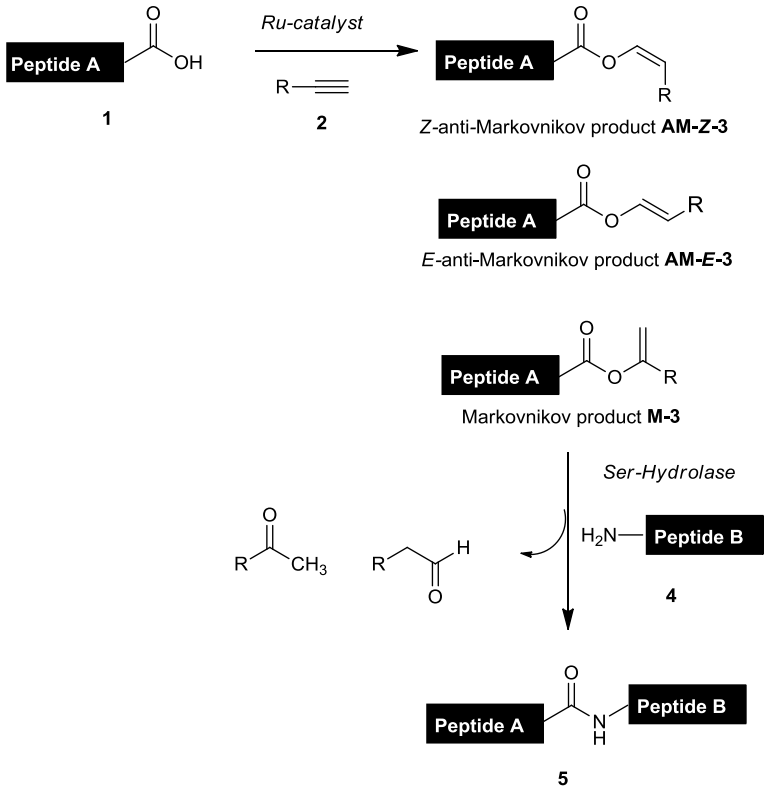
The results obtained using only $Pd(dba)_2$ (**X**) without any ligand in a mixture of *tert*-butanol/H_2O 3/1 mentioned in chapter 4.3.3.5, in which we did not observe any product formation, insinuate that the observed 20 % conversion are due to the catalyst-ligand system $Pd(dba)_2$ (**X**) and TEG-biphephos **191** used.

Although this result was not as good as hoped it already shows that we are moving into the right direction. This reaction shows that Pd-catalyzed allylic sulfenylation reaction of glutathione **169** is possible, but further efforts are necessary to reach our goal to modify L-cysteine containing biomolecules in water. The main focus should be on PEGylated biphephos ligands which contain longer PEG-chains providing better water solubility.

5. Summary and outlook

5.1. Ru-catalyzed formation of enolesters and their subsequent use in chemoenzymatic peptide synthesis

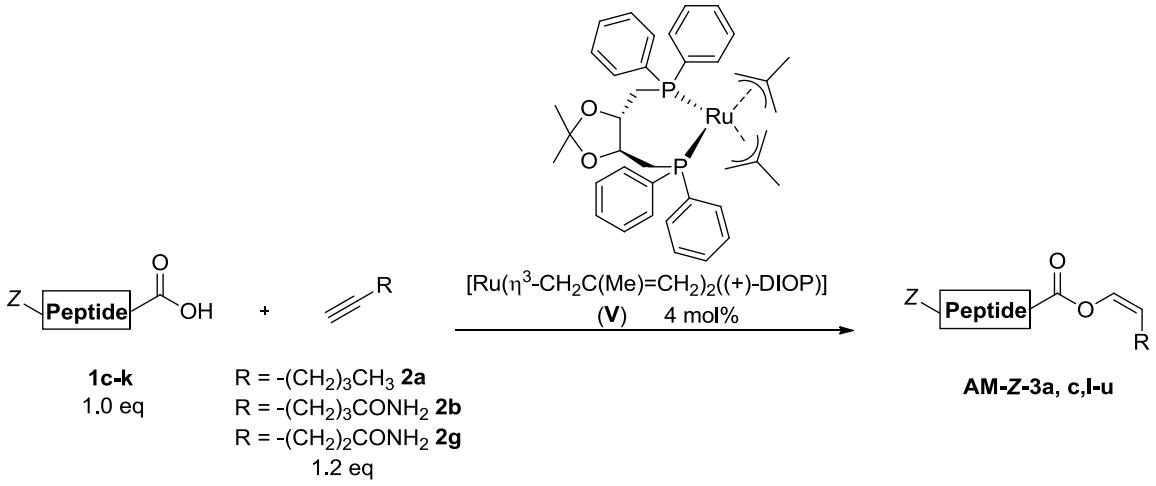
Oligopeptides have gained increasing interest in pharmaceutical, cosmetics and nutrition industry.[1-4] Several excellent methods and reagents are already available for the formation of peptides. However the issue of racemization free fragment coupling of peptides remains a challenge without a general solution.[205-210], One attractive method to overcome this problem is the chemoenzymatic synthesis of peptides using hydrolases.[9],[52],[97],[211-221] The main challenge associated with this strategy is the preparation of activated peptide esters which are suitable substrates for this enzyme (Scheme 131).



Scheme 131: General scheme for the preparation of peptide enolesters and their use in enzymatic peptide couplings.

The *C*-terminal activation of peptides is a process which is often associated with a certain amount of epimerization. In the thesis we have successfully developed a racemization free Ru-catalyzed enolester formation for the activation of the *C*-terminal carbonyl group of peptides. The ruthenium pre-catalyst which served our needs the best was bis(2-

methylpropenyl)(cycloocta-1,5-diene)ruthenium (**II**) In an extensive ligand (25 ligands) and solvent screening we could identify phosphine ligand (+)-2,3-O-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane ((+)-DIOP) ((+)-**42**) in combination with ruthenium pre-catalyst **II** in 2-propanol as the most efficient way to synthesize activated peptide-*Z*-anti-Markovnikov-enolesters. Especially, the use of the isolated Ru-complex [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) for this transformation led to no or negligible amounts of epimerization. Further we were able to also obtain peptide-*Z*-anti-Markovnikov-enolesters containing a terminal amide bond in the leaving group. The most important results are summarized in Scheme 132 and Table 37.

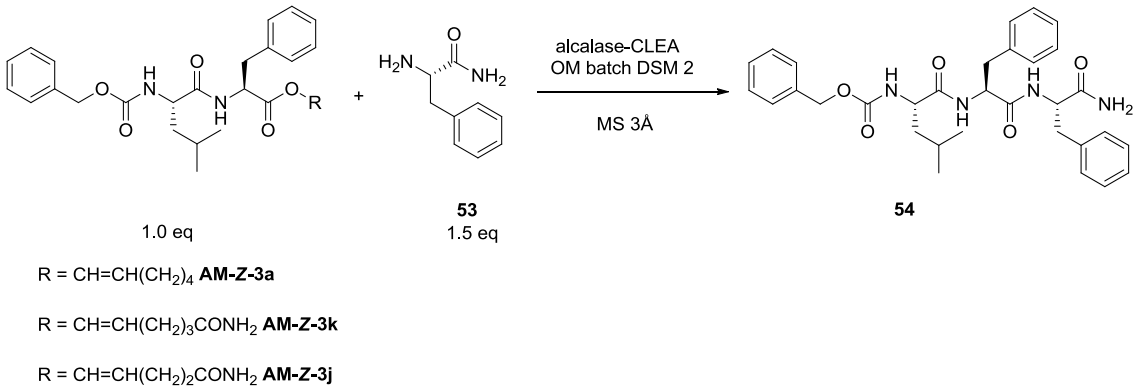


Scheme 132: *Z*-anti-Markovnikov Ru-catalyzed addition of alkynes **2a**, **2b** and **2g** to peptide fragments **1c-m**.

Table 37: Racemization-free Ru-catalyzed synthesis of various peptide enolesters **AM-*Z*-3** using [Ru(η^3-$CH_2C(Me)=CH_2)_2$((+)-DIOP)] (**V**) at 40°C and 2-propanol as solvent.

Entry	Peptide	Alkyne	Time [h]	Conversion [%]	Yield [%]	D-AA [%]
1	Z-Leu-Ala-OH **1d**	**2a**	1	99	92 **AM-*Z*-3c**	0.1
2	Z-Leu-Ala-OH **1d**	**2b**	1	99	89 **AM-*Z*-3l**	0.2
3	Z-Phe-Leu-OH **1e**	**2a**	1	99	93 **AM-*Z*-3m**	0.3
4	Z-Phe-Leu-OH **1e**	**2b**	1	98	92 **AM-*Z*-3n**	0.1
5	Z-Phe-Leu-Ala-OH **1f**	**2a**	1	99	87 **AM-*Z*-3o**	0.1
6	Z-Phe-Leu-Ala-OH **1f**	**2b**	1	97	86 **AM-*Z*-3p**	0.1
7	Boc-Phe-Tyr-OH **1g**	**2a**	3	99	86 **AM-*Z*-3q**	0.2
8	Z-Ile-Ser-OH **1h**	**2a**	24	97	83 **AM-*Z*-3r**	0.1
9	Z-Phe-Pro-OH **1i**	**2b**	5	97	78 **AM-*Z*-3s**	0.3
10	Z-Phe-Val-OH **1j**	**2b**	2	98	83 **AM-*Z*-3t**	0.2
11	Z-Phe-Met-OH **1k**	**2a**	7	97	87 **AM-*Z*-3u**	0.7
12	Z-Phe-Leu-OH **1c**	**2a**	1	99	88 **AM-*Z*-3a**	0.1
13	Z-Phe-Leu-OH **1c**	**2b**	1	97	80 **AM-*Z*-3k**	0.1
14	Z-Phe-Leu-OH **1c**	**2c**	1	97	82 **AM-*Z*-3j**	0.1

After the efficient and epimerization free synthesis of different peptide enolesters, the use of Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** in the enzymatic peptide coupling with alcalase-CLEA showed that the enolester is well accepted by the enzyme with 69 % conversion after 23 h. Terminal amide bond containing enolester **AM-*Z*-3k** and **AM-*Z*-3j** led to excellent conversions of 96-98 % showing that enolesters having these leaving groups are even better accepted by alcalase-CLEA than enolesters having only an alkyl chain in the leaving group (Scheme 133). Further optimization by using THF which was dried with MS 3Å overnight led to full conversion after 22 h using terminal amide bond containing enolesters **AM-*Z*-3k** and **AM-*Z*-3j**. With enolester **AM-*Z*-3k** 99 % conversion could be reached already after 3 h.



Scheme 133: Synthesis of Z-Leu-Phe-Phe-NH_2 (**54**) using alcalase-CLEA OM and drying agent MS 3Å.

In the course of the development of this reaction, we noticed that the aldehyde side product released upon the enzymatic reaction, led to unwanted imine formation with the amine nucleophile. To address this issue Soxhlet-conditions were developed. By avoiding contact of MS 3Å with the starting materials in solution it was possible to synthesize the desired tripeptide Z-Leu-Phe-Phe-NH_2 (**54**) without additional imine formation.

From analysis of the electronic and steric effects of our ligand screening we are optimistic that the use of more electron poor analogs such as fluorinated analogs of the (+)-DIOP ligand (+)-**42** together with Ru-catalyst bis(2-methylpropenyl)(cycloocta-1,5-diene)ruthenium (**II**) will lead to even higher reaction rates, allowing enolesters of longer peptide fragments to be formed more easily.

Additionally, a further solvent screening regarding the enzymatic peptide synthesis with alcalase-CLEA could provide us with solvents having better solubility properties for longer peptides.

5.2. Pd-catalyzed allylic alkylation with sulfur-nucleophiles to modify peptides

In nature quite frequently additional reactions are accomplished by different post-translational modifications (PTM) to obtain biologically active peptides and proteins that in turn mediate protein activity.[11-12],[129] For example, one important PTM is prenylation on the cysteine residue in Ras proteins.[130] Cysteine is the most widely used amino acid residue in proteins which is modified chemically.[14-15] We developed a method to form carbon sulfur bonds using a Pd-catalyzed reaction being able to overcome the poisoning effect of sulfur atoms in low oxidation states e.g. thiols on transition metals.[203-204] So far only a protocol using palladium for the formation of only simple thioethers arriving exclusively from aromatic thiols has been

known[170] being far away from our goal to farnesylate cysteine containing biomolecules. An extensive pre-catalyst and ligand screening (50 different phosphine, phosphonite and phosphite ligands) was performed in which we identified phosphine ligand dppf (**17**) and phosphite ligand biphephos (**40**) being the best ones for our purpose to synthesize allylic thioethers especially from aliphatic thiols (Figure 35).

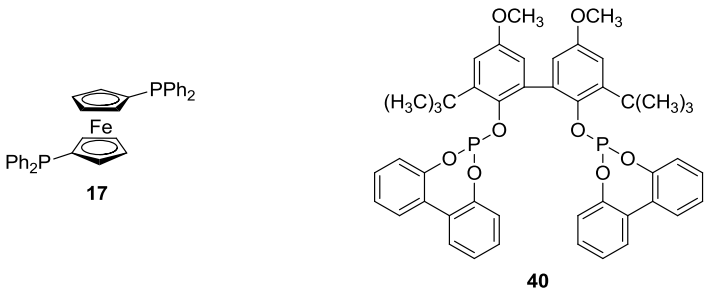
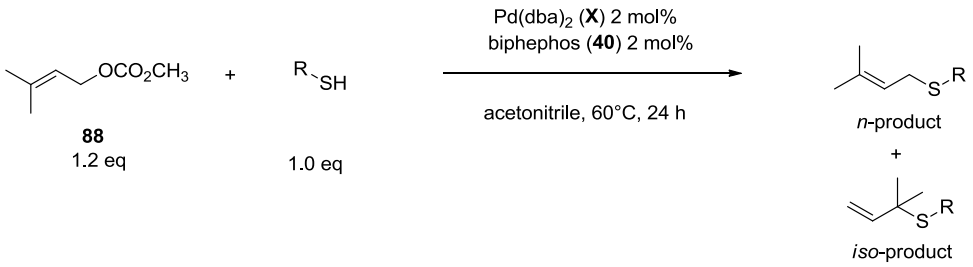


Figure 35: Ligands dppf (**17**) and biphephos (**40**).

The in situ formation of catalyst $Pd(dba)_2$ (**X**) and phosphite ligand biphephos (**40**) gave very fast and perfect conversions and in most cases also perfect *n*/*iso*-ratios in the Pd-catalyzed formation of allylic thioethers being even superior to ligand dppf (**17**). Using the reaction conditions shown in Scheme 134 the most important thioethers synthesized are summarized in Figure 36.



Scheme 134: General scheme for the Pd catalyzed allylic sulfenylation reaction using $Pd(dba)_2$ (**X**) and biphephos (**40**). Isoprenyl carbonate **88** is shown as an example for all the different carbonates used as starting material.

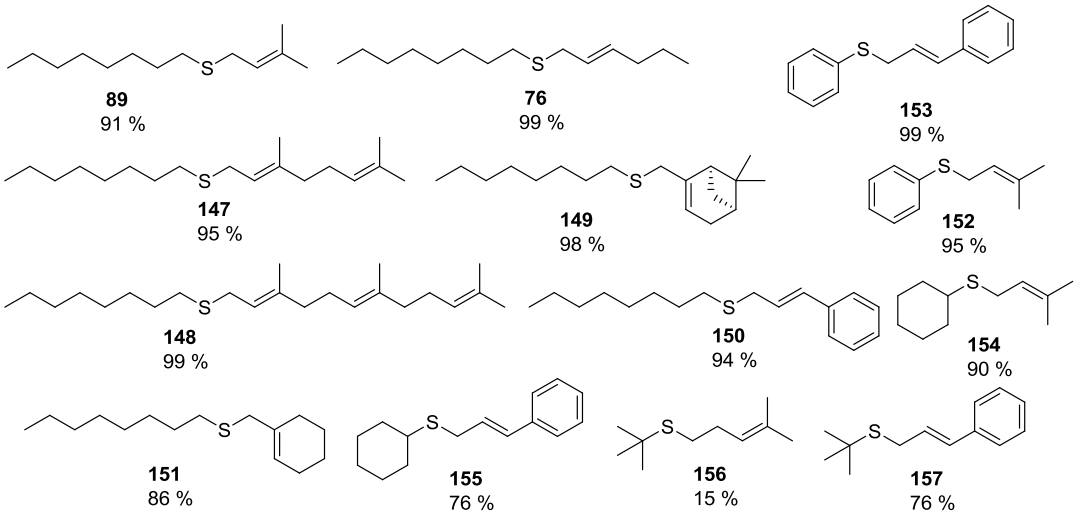


Figure 36: Summary of various thioethers synthesized using ligand biphephos (**40**) for the Pd-catalyzed allylic sulfenylation.

Fortunately, under identical conditions also cysteine containing amino acid and peptide derivatives could be alkylated and even farnesylated on the thiol functionality (Figure 37).

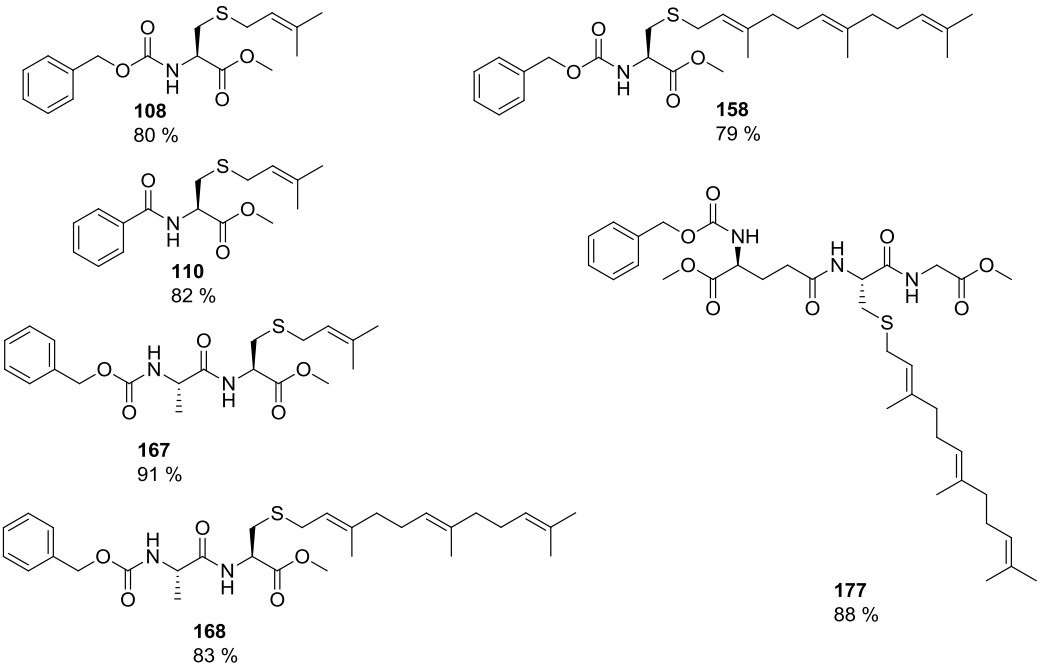
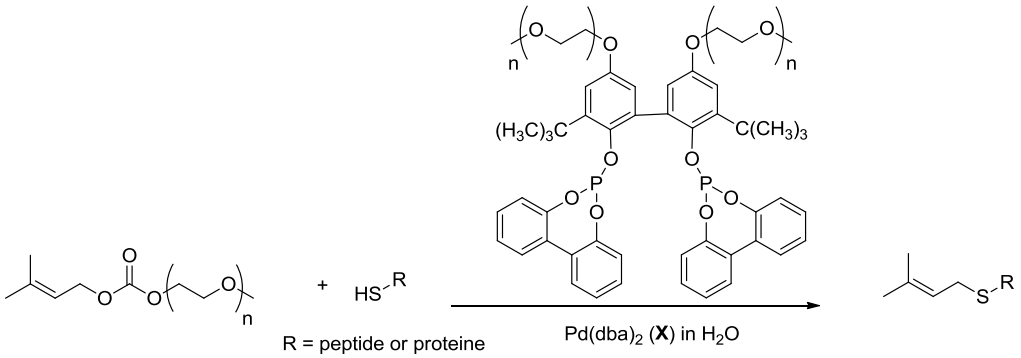


Figure 37: Summary of various thioethers from L–cysteine containing amino acid and peptide derivatives synthesized by Pd-catalyzed allylic sulfenylation in the presence of ligand biphephos (**40**).

In a solvent screening we found alcohols, polar aprotic solvents and nitriles such as acetonitrile and propionitrile as suitable solvents for the Pd-catalyzed allylic sulfenylation reaction. In general, more polar solvents are superior to less polar solvents.

We could show that additives such as phenol and Cbz-Tyr-OH (**179**) do not interfere in this reaction, but not surprisingly morpholine (**161**) as a secondary amine does.

To reach our ultimate goal to farnesylate proteins in water a water soluble biphephos (**40**) analog is needed. It is likely, that long PEG-chains will provide us with the necessary solubility of this phosphorous ligand in water (Scheme 135).



Scheme 135: Pd-catalyzed allylic alkylation of peptides in water using a PEGylated-biphephos ligand.

Another important challenge is to farnesylate peptides selectively on the thiol group of cysteine without alkylating other functionalities such as amino groups, present in peptides and proteins. We are very optimistic that with the right pH-value present in a water/buffer system the amino functionalities will not react due to their protonated state. Protonated amino groups are much less nucleophilic than amino groups.

If we master these challenges the way will be open to fulfill our ultimate goal to farnesylate a protein using the Pd catalyzed allylic sulfenylation reaction (Figure 38).

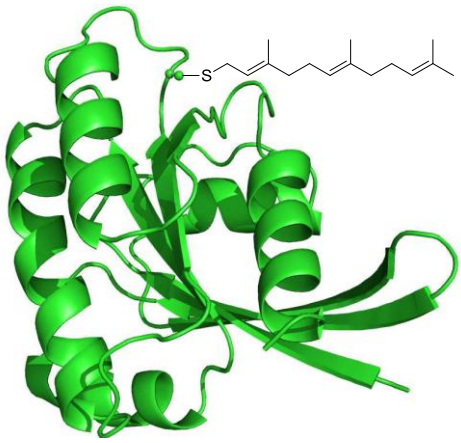


Figure 38: Lipidation of the cysteine in a native protein by Pd catalyzed allylic sulfenylation.

6. Experimental section

6.1. General

Reactions under inert atmosphere were carried out with standard Schlenk techniques. Syntheses which are sensitive against oxidation and humidity were accomplished under nitrogen or argon atmosphere using dry and degassed solvents. Two different protocols were used to degas the applied solvents: A) Degassing of solvents was performed by subjecting the frozen solvent (liquid nitrogen) in appropriate Schlenk tubes to vacuum (0.02 mbar). After melting of the solvent the Schlenk tube was refilled with argon. This procedure was repeated 3 times. B) Alternatively, degassing was carried out in a Schlenk tube with septum and cannula by passing a stream of argon through the solvent. The Schlenk tube was placed in an ultrasonic bath for 20 to 30 minutes, depending on the volume of solvent used.

6.2. Solvents

All solvents were used at commercial quality without further purification, unless otherwise noted. In certain cases activated molecular sieves were used to dry the solvents. For that purpose 3Å or 4Å molecular sieves were activated at oil pump vacuum (0.02 mbar) at 200°C for 2 days. Dry solvents, which were not commercially available, were stored in a Schlenk bottle under argon and light exclusion. The solvent was stored over activated molecular sieves for at least two days before usage. Other solvents were dried according to literature known procedures.

Acetic acid: Acetic acid was purchased from Aldrich with 99.85 % purity, refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. Acetic acid was stored in this Schlenk tube for at least two days before first usage.

Acetone: Acetone was purchased from ACROS Organics as extra dry solvent (99.8 %, over 3 Å molecular sieves and inert atmosphere, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

Acetonitrile: Acetonitrile was purchased from Sigma Aldrich (99.5 %) and first dried by passing through an aluminium oxide column (solvent purification system: Puresolv^{TM} from Innovative Technology Inc.) under inert conditions, and then dried over CaH_2 for 12 h. Afterwards it was distilled into a Schlenk tube over activated 3 Å molecular sieves for long-term storage under argon and light exclusion.

Benzyl alcohol: Benzyl alcohol was purchased from Fluka with 98.0 % purity, refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light

exclusion. Benzyl alcohol was stored in this Schlenk tube for at least two days before first usage.

1-Butanol: 1-Butanol was purchased from ACROS Organics as extra dry solvent (99.9 %, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

***tert*-Butanol:** *Tert*-butanol was purchased from Alfa Aesar as anhydrous solvent with ≥ 99.8 % purity packaged under argon in resealable ChemSeal™ bottle. It was refilled into a 1 L Schlenk bottle (brown glass) under argon, light exclusion and activated 4 Å molecular sieves for storage. *Tert*-butanol was stored in this bottle at 40°C for at least two days before first usage.

Chloroform: Chloroform was purchased from ACROS Organics as extra dry solvent (99.9 %, over 4 Å molecular sieves and inert atmosphere, H_2O <50 ppm, AcroSeal®, stabilizer agent: 1-pentene) and directly used in the reactions.

1,2-Dichloroethane (DCE): 1,2-Dichloroethane was purchased from ACROS Organics as extra dry solvent (99.8 %, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

Dichloromethane (DCM): Methode A: Dichloromethane was purchased from Fisher Scientific as analytical grade solvent (99.99 %) and first distilled over phosphorus pentoxide P_4O_{10} for 12 h and after distillation additionally refluxed over CaH_2 for 12 h and afterwards destilled under argon and stored in a Schlenk bottle (brown glass) over 4 Å molecular sieves, under argon atmosphere and light exclusion.

Method B: Dichloromethane was purchased from Fisher Scientific as analytical grade solvent (99.99 %) and first distilled over phosphorus pentoxide P_4O_{10} for 12 h and after distillation it was stored in a brown 1L bottle.

Diethylene glycol: Diethylene glycol was purchased from Sigma with 99 % purity and refilled into a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. Diethylene glycol was stored in a Schlenk tube for at least two days before first usage.

Diethylene glycol dimethyl ether: Diethylene glycol dimethyl ether was purchased from Sigma-Aldrich with 99.0 % purity, refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. Diethylene glycol dimethyl ether was stored in this Schlenk tube for at least two days before first usage.

Diethyl ether: Diethyl ether was distilled and stored over KOH pellets in a brown 1L bottle.

1,2-Dimethoxyethane (DME): 1,2-Dimethoxyethane was purchased from Sigma as anhydrous solvent (99.5 %, inert atmosphere, Sure/Seal™) and directly used in the reactions.

***N,N*-Dimethylacetamide:** *N,N*-Dimethylacetamide was purchased from ACROS Organics as extra dry solvent (99.5 %, over molecular sieves, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

***N,N*-Dimethylformamide (DMF):** *N,N*-Dimethylformamide was purchased from ACROS Organics as extra dry solvent (99.8 %, over 3 Å molecular sieves, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

Dimethylsulfoxide (DMSO): Dimethylsulfoxide was purchased from ACROS Organics as extra dry solvent (99.8 %, over molecular sieves, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

1,4-Dioxane: 1,4-Dioxane was purchased from ACROS Organics as dry solvent (99.5 %, over molecular sieves and inert atmosphere, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

Ethanol: Ethanol was purchased from Merck (99 %, 1 % ethylmethylketone as poisoning agent) was distilled over sodium and diethyl phthalate and finally stored in a Schlenk bottle (brown glass) over 3 Å molecular sieves, under argon atmosphere and light exclusion.

Ethyl acetate: Ethyl acetate was purchased from ACROS Organics as extra dry solvent (99.9 %, over molecular sieves, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

Ethylene glycol: Ethylene glycol was purchased from Sigma Aldrich with 99.5 % purity, refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. Ethylene glycol was stored in this Schlenk tube for at least two days before first usage.

***n*-Heptane**: *n*-Heptane was purchased from Sigma-Aldrich as CHROMASOLV®, for HPLC, ≥99 %.

***n*-Hexane:** a) used for part “chemoenzymatic peptide synthesis”: *n*-Hexane was purchased from Sigma Aldrich as anhydrous solvent with 95 % purity and refilled into a 1 L Schlenk bottle (brown glass) under argon, light exclusion and activated 4 Å molecular sieves were added.

b) used for part “Pd-catalyzed allylation of thiols”: *n*-Hexane was purchased from Sigma-Aldrich as puriss., absolute solvent (99.0 %, over molecular sieves, H_2O <100 ppm) and directly used in the reactions.

Methanol: Methanol was purchased from Fisher Scientific as analytical grade (99.99 %) and was dried over magnesium turnings and iodine, distilled under argon atmosphere and stored over 3 Å molecular sieves in a Schlenk bottle (brown glass) under argon atmosphere and light exclusion.

1-Methyl-2-pyrrolidinone (NMP): 1-Methyl-2-pyrrolidinone was purchased from ACROS Organics as extra dry solvent (99.5 %, over molecular sieves and inert atmosphere, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

Methyl *tert*-butyl ether (MTBE): Methyl *tert*-butyl ether was purchased from Aldrich as anhydrous solvent (99.8 %, inert atmosphere, Sure/Seal™) and directly used in the reactions.

2-Methyltetrahydrofuran: 2-Methyltetrahydrofuran was purchased from Sigma Aldrich as anhydrous solvent (99 %, inhibitor-free, inert atmosphere, Sure/Seal™) and directly used in the reactions.

***n*-Pentane:** *n*-pentane was purchased from Alfa Aesar (HPLC-Grade, >99 %) and stored in a brown 1L bottle.

Petroleum ether: Petroleum ether (boiling range 50-70°C) was purchased from Riedel de Haën (puriss p. a.) and stored in a brown 1L bottle.

1,2-Propandiol: 1,2-Propandiol was purchased from Sigma Aldrich with 99.5 % purity, refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. 1,2-Propandiol was stored in this tube for at least two days before first usage.

1-Propanol: 1-Propanol was purchased from ACROS Organics as extra dry solvent (99.5 %, over molecular sieves and inert atmosphere, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

2-Propanol: 2-Propanol was purchased from ACROS Organics as extra dry solvent (99.5 %, over molecular sieves and inert atmosphere, H_2O <50 ppm, AcroSeal®) and directly used in the reactions.

Propionitrile: Propionitile was purchased from Sigma-Aldrich with 99.0 % purity, refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. Propionitrile was stored in this Schlenk tube for at least two days before first usage.

Propylene carbonate (PC): Propylene carbonate was purchased from Sigma Aldrich as anhydrous solvent (99.7 %, inert atmosphere, Sure/Seal™) and directly used in the reactions.

Sulfolane: Sulfolane was purchased from Fluka with 99.5 % purity and refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. Sulfolane was stored in this tube at 40°C for at least two days before first usage.

Tetrahydrofuran (THF): Under inert conditions tetrahydrofuran was dried at reflux temperature under argon atmosphere over sodium and benzophenone until ketyl radical indicated dryness by the appearance of a deep blue color. THF was distilled and stored over 4 Å molecular sieves in a Schlenk bottle (brown glass) under argon atmosphere and light exclusion.

In the case of enzymatic peptide coupling reactions described in chapter 4.2.4. and 4.2.5. tetrahydrofuran from Fluka with 99.5 % purity was used. Tetrahydrofuran containing BHT as stabilization agent was stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. Tetrahydrofuran was stored in the Schlenk tube at least overnight before first usage.

2,2,2-Trifluoroethanol (TFE): 2,2,2-Trifluoroethanol was purchased from Sigma Aldrich with 99 % purity and refilled and stored in a Schlenk tube containing activated 3 Å molecular sieves under argon and light exclusion. 2,2,2-Trifluoroethanol was stored in the Schlenk tube for at least two days before first usage.

Toluene: Toluene was purchased from Sigma Aldrich (99.7 %) and dried by passing through an aluminium oxide column (solvent purification system: Puresolv^{TM} from Innovative Technology Inc.) under inert conditions and stored in a Schlenk bottle (brown glass) containing 4 Å molecular sieves under argon atmosphere and light exclusion.

6.3. Reagents

All used chemicals and reagents were purchased from the following companies: Aldrich, Sigma Aldrich, Fluka, Merck, ABCR, Alfa Aesar, Fisher Scientific, ACROS Organics and Strem. Reagents were used without further purification, unless stated otherwise.

Peptides used as starting material were obtained from Bachem and used as received.

3Å and **4Å molecular sieves:** (8 to 12 mesh) were obtained form Acros Organics and activated using an oil pump vacuum (0.02 mbar) at 200°C for 2 days before use.

Aluminium oxide: was obtained from Sigma Aldrich (activated, neutral, Brockmann I).

$Pd(dba)_2$: see Scheme 44 and chapter 6.7.5 note: selfmade catalyst is superior to the one commercially available from Acros
$Pd(OAc)_2$: $Pd(OAc)_2$ was purchased from Sigma Aldrich 98 %.

Pyridine (dry): Pyridine was purchased from ACROS Organics as extra dry solvent (99.5 %, over molecular sieves and inert atmosphere, H_2O <50 ppm, AcroSeal®) and directly used in the reactions**.**

Thionylchloride: Before use, thionylchloride was freshly distilled under inert conditions and stored in the fridge under argon atmosphere and light exclusion.

Preparation of stirring bars used for catalysis reactions: magnetic stirring bars were always pretreated with nitro-hydrochloric acid for 2 h. Afterwards the nitro-hydrochloric acid is decanted and the magnetic stirring bars were neutralized with diluted NaOH. Afterwards the liquid was decanted and the magnetic stirring bars were washed 3 times with distilled water and 2 times with acetone. Afterwards they were dried at 0.02 mbar and stored under inert atmosphere to exclude moisture.
For the reactions the magnetic stirring bars were transferred using tweezers to prevent contamination with dirt. This is important concerning Pd-catalyzed reactions, because impurities on the magnetic stirring bar can catalyze the formation of “palladium black”, which itself interferes in the Pd-catalyzed reaction by converting the active palladium species into palladium black which is inactive.

6.4. Analytical methods

6.4.1. Nuclear magnetic resonance spectroscopy

The described and attached nuclear magnetic resonance spectra (^{1}H-NMR, ^{13}C-NMR, COSY-NMR, APT-NMR, NOESY-NMR, TOCSY-NMR were recorded with the following equipment:
Varian GEMINI-200 BB: 200 MHz-^{1}H-NMR und 50 MHz-^{13}C-NMR

Bruker Avance DRX-400: 400 MHz-^{1}H-NMR und 100 MHz-^{13}C-NMR

Bruker AVANCE III: 300 MHz-^{1}H-NMR, 75 MHz-^{13}C-NMR

Varian Inova-500: 500 MHz-^{1}H-NMR, 125 MHz-^{13}C-NMR

Chemical shifts δ are referenced to residual protonated solvent signals as internal standard. Signal multiplicities *J* are abbreviated as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), dt (douplet of tripets), (triplet), td (triplet of doublets), q (quartet), p (pentet) and m (multiplet). If the word “pseudo” is used before the abbreviation, the multiplicity is reported by its appearance and not as the physically correct spin system. Additionally, quarternary carbon atoms are marked as C_q, aromatic carbon atoms are marked as C_{Ar}, and aromatic protons as H_{Ar}. For assignments of protons, COSY-NMR spectra were recorded. In the case of carbon atoms, APT spectra were recorded. For more detailed assignment of signals TOCSY and NOESY-spectra were recorded. Chemical shifts are stated in ppm (part per millions) and the coupling constants in Hz (Hertz).

6.4.2. Gas chromatography

Analytical gas chromatography was performed on two different instruments.

- *Hewlett Packard GC-system “HP 6890 series“* equipped with a HP-5MS column (30 m × 250 µm × 0.25 µm); injection occured by an autosampler “7683B injector“ used in split mode; carrier gas: helium 5.0; EI ionisation source with a potential of E = 70 eV and mass selective detector 5972 MSD.

  Temperature programs:

  HS-50-S2: 50 °C 1 min, ramp 40 °C/min linear to 300 °C, 5 min

  HS-100-L: 100 °C 1 min, ramp 40 °C/min linear to 300 °C, 15 min

- *Agilent Technologies 5975C inert MSD with Triple Axis Detector* with a HP-5MS column (30 m x 250 µm x 0.25 µm); injection occured by an autosampler “7683B injector“ used in split mode; carrier gas: helium 5.0; EI ionisation source with a potential of E = 70 eV and mass selective detector 5975C MSD.
  Temperature programs:

  HS-50-S28: 50 °C 1 min, ramp 40 °C/min linear to 300 °C, 5 min

  HS-100-L: 100 °C 1 min, ramp 40 °C/min linear to 300 °C, 15 min

6.4.3. High-performance liquid chromatography

Analytical high-performance liquid chromatography was performed on three different instruments.

HPLC analyses were conducted on an *HP 1100* instrument composed of vacuum degasser G1322A, quaternary pump G1313A, thermostated column compartment G1316A, refractive index detector G1362A and diode array detector (DAD) G1315A. Signals were detected at 210 nm.

HPLC analyses were also conducted on an *Agilent 1200* instrument composed of vacuum degasser G1379B, binary pump G1312B, autosampler G1367C with autosampler thermostat G1330B, thermostated column compartment G1316B and multi wavelength detector (MWD) G1365C connected to an Agilent quadrupol-MS 6120 with electrospray ionization (ESI) unit. Signals were detected at 210 nm.

HPLC analyses were also conducted on a *Shimadzu Nexera* instrument composed of vacuum degasser prominence DGU-20A, binary pump Nexera LC-30AD, autosampler Nexera SIL-30AC, diode array detector (DAD) Prominence SPD-M20A, column oven prominence CTO-20AC, communication bus module CBM-20A equipped with quadrupol liquid chromatograph mass spectrometer (*Shimadzu* LCMS - 2020). Signals were detected at 210 nm.

Columns:

The conversions of the reactions performed were analyzed on following columns:

Chromolith Performance Rp-18e (100×3.0 mm, *Merck*)

Chromolith Performance Rp-18e (100×2.0 mm, *Merck*)

Nucleodur Rp-18e (150×4.6 mm, 5 µm, *Macherey & Nagel*)

Purospher Star RP-18e (250×4.0 mm, 5 µm, *Merck*)

Poroshell 120 SB-C18 (100×3.0 mm, 2.7 µm, *Agilent*)

Chiralcel®-OD-H (250×4.6 mm, 5 µm *Daicel Chemical Industries*)

Astec Chirobiotic R (250×4.6 mm, 5 µm)

Nucleosil Rp-4e (120×4.6 mm, 5 µm, *Macherey & Nagel*)

General settings:

With the exception of the chiral column *Chiralcel®-OD-H* these general settings were applied: For the separation on these columns mixtures of water, containing formic acid (0.01 vol%), and acetonitrile were used. UV detection (210 nm) and ESI-MS detection, typically in positive mode, was used to identify the compounds.

Methods:

Method A: Agilent 1200; column: Nucleodur Rp18e (5 µm) 150×4 mm; 30°C, 1.0 mL/min; solvent A: H_2O+0.01 %HCOOH; B: MeCN; 0 min: A/B = 70/40; 13-15 min: A/B = 0:100; MS: ESI (pos. Mode)

Method B: Agilent 1200; column: Purospher STAR Rp18e (5 µm) 250×4 mm; 30°C, 0.7 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 50/50; 15-19 min: A/B = 0:100; MS: ESI (pos. Mode)

Method C: Shimadzu Nexera; column: Poroshell 120 SB-C18 (2.7 µm, Agilent) 100×3 mm; 40°C, 0.7mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 90/10; 9.0-9.5 min: A/B = 0:100; MS: ESI (pos.+ neg. Mode)

Method D: Shimadzu Nexera; column: Poroshell 120 SB-C18 (2.7 µm, Agilent) 100×3 mm; 40°C, 0.7 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 90/10; 4.5-5.0 min: A/B = 0:100; MS: ESI (pos.+ neg. Mode)

Method E: Agilent 1200; column: Nucleodur Rp18e (5 µm) 150×4 mm; 30°C, 1.0 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0min: A/B = 50/50; 13-15 min: A/B = 0:100; MS: ESI (pos. Mode)

Method F: Agilent 1200; column: Chromolith Performance Rp-18e 100×2 mm; 25°C, 0.65 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 60/40; 3.6-8.4 min: A/B = 35:65; 8.5-9.3 min: A/B = 0:100; MS: ESI (pos. Mode)

Method G: Agilent 1200; column: Chromolith Performance Rp-18e 100×3 mm; 25°C, 0.65 mL/min; solvent A: H_2O+0.05 % HCOOH; B: MeCN; 0 min: A/B = 65/35; 3.6-8.4 min: A/B = 40:60; 8.5-9.3 min: A/B = 0:100; MS: ESI (pos. Mode)

Method H: Agilent 1200; column: Purospher STAR Rp18e (5 µm) 250×4 mm; 30°C, 0.7 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 85/15; 4.5 min: A/B 50/50; 21-22 min: A/B = 0:100; MS: ESI (pos. Mode)

Method I: Agilent 1200; column: Nucleodur Rp18e (5µm) 150×4 mm; 30°C, 1.0 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 90/10; 13-14 min: A/B = 0:100; MS: ESI (pos. Mode)

Method J: Agilent 1200; column: Nucleodur Rp18e (5 µm) 150×4 mm; 30°C, 1.0 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 90/10; 6 min: A/B = 54/46, 15 min: A/B = 27/73; 19.5-20.5 min: A/B = 0:100; MS: ESI (pos. Mode)

Method K: Shimadzu Nexera; column: Poroshell 120 SB-C18 (2.7 µm, Agilent) 100×3 mm; 40°C, 0.7 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0-4.0 min: A/B = 65/35; 9.5 min: A/B = 60:40; 10.5-12.5 min: A/B = 0/100; MS: ESI (pos.+ neg. Mode)

Method L: Agilent 1200; column: Nucleosil Rp-4e (5 µm, Macherey & Nagel) 120×4.6 mm; 30°C, 1.0 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0-1 min: A/B = 90/10; 9-12 min: A/B = 0:100; MS: ESI (pos. Mode)

Method M: Shimadzu Nexera; column: Nucleosil Rp-4e (5 µm, Macherey & Nagel) 120×4.6 mm; 40°C, 1.0 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0 min: A/B = 90/10; 9-12 min: A/B = 0:100; MS: ESI (pos.+ neg. Mode)

Table 38: Chromatographic methods applied in ruthenium catalysed alkyne addition (chapter 4.1) t_R = retention time.

Substrate	**Method**	**Column**	**t_R starting material [min]**	**t_R product AM-*Z*-3 [min]**	**t_R of additional products [min]**
AM-*Z*-3a	A	Nucleodur Rp-18e	**1c** = 5.4	10.4	**-**
	D	Poroshell 120 SB-C18	**1c** = 3.7	5.0	**M-3c** = 4.8
	F	Chromolith Performance Rp-18e	**1c** =2.6	7.7	**M-3c** = 7.2
AM-*Z*-3b	F	Chromolith Performance Rp-18e	**1b** = 1.7	7.4	-
AM-*Z*-3c	G	Chromolith Performance Rp-18e	**1d** = 2.2	7.6	-
	B	Purospher STAR Rp18e	**1d** = 6.0	13.7	-

AM-*Z*-3d	D	Poroshell 120 SB-C18	**1a** = 2.6	4.5	**M-3a** = 4.3
AM-*Z*-3f	B	Purospher STAR Rp18e	**1c** = 8.8	8.4	**43** = 6.5 and 6.9
AM-*Z*-3g	B	Purospher STAR Rp18e	**1c** = 8.8	11.2	**44** = 11.4, 12.1
AM-*Z*-3h	B	Purospher STAR Rp18e	**1c** = 8.8	11.2	**45** = 11.4, 12.0
AM-*Z*-3j	C	Poroshell 120 SB-C18	**1c** = 5.7	5.8	**46** = 5.1 and 5.2
	A	Nucleodur Rp-18e	**1c** = 5.4	5.6	**46** = 4.3 and 4.6
AM-*Z*-3k	B	Purospher STAR Rp18e	**1c** = 8.9	8.5	**47** = 6.3 and 6.8
	C	Poroshell 120 SB-C18	**1c** = 5.8	6.1	**47** = 5.4 and 5.6
AM-*Z*-3l	C	Poroshell 120 SB-C18	**1d** = 4.8	5.2	**B3l** = 4.7 and 4.9
AM-*Z*-3m	B	Purospher STAR Rp18e	**1e** = 8.7	15.6	-
AM-*Z*-3n	C	Poroshell 120 SB-C18	**1e** = 5.7	6.0	**B3n** = 5.3 and 5.4
AM-*Z*-3o	A	Nucleodur Rp18e	**1f** = 4.7	9.4	-
AM-*Z*-3p	C	Poroshell 120 SB-C18	**1f** = 5.4	5.7	**B3p** = 5.1 and 5.2
AM-*Z*-3q	A	Nucleodur Rp18e	**1g** = 3.6	8.4	-
AM-*Z*-3r	D	Poroshell 120 SB-C18	**1h** = 2.9	4.3	-
AM-*Z*-3s	C	Poroshell 120 SB-C18	**1i** = 5.3	5.7	**B3s** = 5.1 and 5.2
AM-*Z*-3t	C	Poroshell 120 SB-C18	**1j** = 5.6	5.9	**B3t** = 5.2 and 5.4
AM-*Z*-3u	D	Poroshell 120 SB-C18	**1k** = 3.5	4.8	-
AM-*Z*-3v	D	Poroshell 120 SB-C18	**1l** = 3.1	3.9	-
AM-*Z*-3w	D	Poroshell 120 SB-C18	**1m** = 3.3	4.5	-
50	D	Poroshell 120 SB-C18	**1c** = 3.7	**50** = 4.4	-

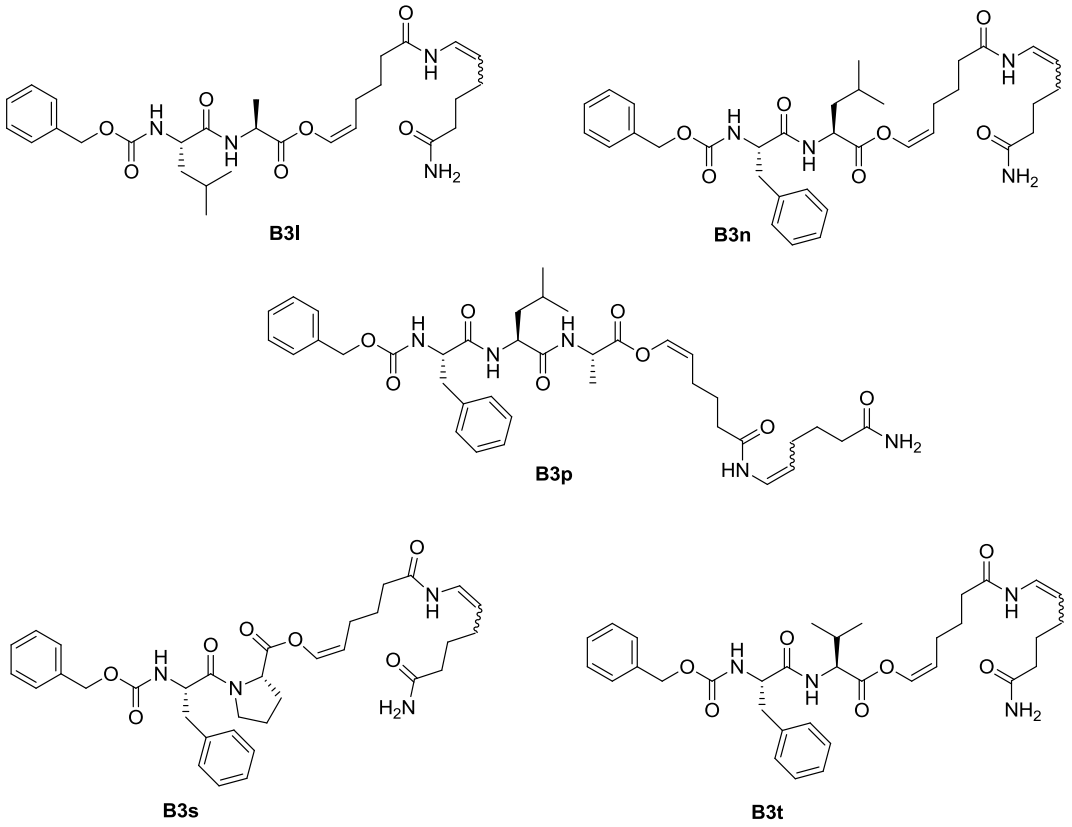


Figure 39: Putative byproducts of the Ru-catalyzed addition of terminal amide containing alkynes to Z-Leu-Ala-OH (**1d**), Z-Phe-Leu-OH (**1c**), Z-Phe-Leu-Ala-OH (**1f**), Z-Phe-Pro-OH (**1i**) and Z-Phe-Val-OH (**1j**) as assigned by MS.

Table 39: Chromatographic methods applied in enzymatic peptide coupling reactions (chapter 4.2) t_R = retention time.

Substrate	Method	Column	t_R starting material [min]	t_R product [min]	t_R of additional products [min]
54	D	Poroshell 120 SB-C18	**AM-*Z*-3a** = 4.9 **AM-*Z*-3j** = 3.6 **AM-*Z*-3k** = 3.7 **52** = 3.5	**54** = 3.8	**56** = 2.8 and 3.0 **57** = 4.1 **61** = 1.5 and 1.7 **62** = 1.5 and 1.6 **1c** = 3.7
	C	Poroshell 120 SB-C18	**AM-*Z*-3k** = 6.1	**54** = 6.3	**57** =6.9 **1c** = 5.9
	B	Purospher STAR Rp18e	**AM-*Z*-3a** = 16.0	**54** = 9.3	**56** = 5.4 and 5.9 **1c** = 9.0
	H	Purospher STAR Rp18e	**AM-*Z*-3f** = 14.0	**54** = 14.5	**58** =6.8 and 7.7
	I	Nucleodur Rp-18e	**AM-*Z*-3g** = 10.7 **AM-*Z*-3h** = 10.7	**54** = 9.6	**59** = 5.8 and 6.1 **60** = 6.0 and 6.2
	J	Nucleodur Rp-18e	**AM-*Z*-3j** = 11.0 **AM-*Z*-3k** = 11.4	**54** = 11.8	**61** = 3.6 and 4.0 **62** = 3.0 and 3.2
63	K	Poroshell 120 SB-C18	**AM-*Z*-3n** = 8.2	**63** = 8.4	**65** =10.7 **1e** = 7.3
64	C	Poroshell 120 SB-C18	**AM-*Z*-3l** = 5.2	**64** = 5.4	**66** = 6.3 **1d** = 4.8

Table 40: Chromatographic methods used in Pd-catalyzed allylic sulfenylation (chapter 4.3) t_R = retention time.

Substrate	Method	Column	t_R starting material [min]	t_R product [min]
108	D	Poroshell 120 SB-C18	**102** = 3.5	4.3
177	D	Poroshell 120 SB-C18	**176** = 2.8	4.4
	L	Nucleosil Rp-4e	**176** = 6.2	8.5
176	D	Poroshell 120 SB-C18	**173** = 3.4	2.8
	L	Nucleosil Rp-4e	**173** = 7.2	6.2
180	D	Poroshell 120 SB-C18	**179** = 2.9	4.0
195	D	Poroshell 120 SB-C18	**169** = 0.8	1.9
168	L	Nucleosil Rp-4e	**166** = 6.7	9.1
167	L	Nucleosil Rp-4e	**166** = 6.7	7.6
166	L	Nucleosil Rp-4e	**165** = 8.0	6.7
158	M	Nucleosil Rp-4e	**102** = 4.4	7.7

Semi-preparative HPLC:

For semi-preparative HPLC a *Knauer Smartline* instrument with Autosampler 3800, Manager 5000 Low Pressure Gradient, Pump 1000, UV Diode Array Detector 2600 and Fraction Collector Teledyne Isco Foxy Junior FC100 modules was used. Semi-preparative HPLC was carried out utilizing a *Macherey Nagel* VP 125/21 Nucleodur 100-5 C18 ec column with VP 50/21 Nucleodur 100-5 C18 ec pre-column at a flow rate of 15 mL/min.

6.4.4. Analysis of amino acid composition

AM 1:

Peptide samples were hydrolyzed in 6M hydrochloric acid at 95°C over a time span of 16h. Subsequently the reaction mixtures were evaporated in vacuo using a rotary evaporator to remove most of the acid. The residue was then dissolved in water and the pH adjusted to

approximately 9 using 0.4M potassium tetraborate buffer pH 10.5. Analysis of UV-active amino acids (phenylalanine) was directly done without derivatization on an *Astec Chirobiotic* column (250×4.6 mm) using a water/methanol mixture (40/60, v/v) at a flow rate of 0.7 mL/min, a temperature of 25°C and a detection wavelength of 210 nm. The retention times were: 8.2 min (L-Phe) and 12.1 min (D-Phe) (method L).

AM 2:

For all other amino acids we used a literature known protocol[244] also used by DSM. To analyze the amount of racemization with a *C*-terminal amino acid other than phenylalanine we performed first the hydrolysis of enolesters with 6N DCl in D_2O followed by an esterification reaction with 2-3N HCl in 2-propanol and finally an acylation with pentafluoropropionic anhydride. The amount of epimerization was monitored via GC-MS (*Agilent Technologies 5975C inert MSD with Triple Axis Detector)* using a Chirasil-L-Val column. With this method it is possible to measure the amount of epimerization occurring during ruthenium catalysis while excluding the amount of racemization occurring during hydrolysis under acidic conditions (DCl). This is the case because during hydrolysis with DCl minor amounts of protons of the α-carbon of the amino acid are exchanged by deuterium and therefore the mass is increased by 1. With this protocol it is possible to distinguish between the non- and the deuterated amino acid using GC-MS (sim-mode).

Sample pretreatment:

Hydrolysis:

In a GC-vial the polypeptide samples (5 mg peptide) were hydrolyzed with 6N DCl in D_2O (500 µL) during 16 hrs at 85°C. Afterwards the hydrolyzed material was dried using a rotary evaporator.

Derivatization-procedures:

Esterification:

To the dried hydrolyzed material 1 mL 2-3 N HCl in 2-propanol was added and heated for 1 h at 110 °C. After reaction the solution was concentrated to dryness with a gentle stream of nitrogen.

Acylation:

250 µL ethyl acetate and 50 µL of pentafluoropropionic acid anhydride were added to the residue of the esterification reaction and heated at 150 °C for 10 min in the case of serine

and tyrosine present in the peptide. In case of all other amino acids it was heated to 55°C for 16 h. Afterwards this solution was concentrated to dryness with a gentle stream of nitrogen.

The residue was dissolved in 750 µL acetonitrile and 1 µL of this solution was injected into GC-MS.

GC and MS parameters

Oven:

Initial temp: 50 °C Maximum temp: 200 °C

Initial time: 5.00 min Equilibration time: 0.50 min

Ramps:

Rate (°C/min)	Final temp	Final time
4.00	200	5.00

Table 41: Overview of all applied amino acids in the Ru-catalyzed enolester formation and listing of fragmentation masses and retention times t_R.

Name	**Ab.**	**Structure**	**Derivatization with pentafluoro-propionic acid anhydride**	**MW (sim-mode)**		**t_R [min]**	
				derivatized product	**-87**	**D-amino acid**	**L-amino acid**
alanine	Ala	$C_3H_7NO_2$	1[a)]	277	190	11.51	13.22
isoleucine	Ile	$C_6H_{13}NO_2$	1[a)]	319	232	n.d.	17.96
leucine	Leu	$C_6H_{13}NO_2$	1[a)]	319	232	18.66	20.43
methionine	Met	$C_5H_{11}NO_2S$	1[a)]	337	250	26.43	27.60
phenylalanine	Phe	$C_9H_{11}NO_2$	1[a)]	353	266	28.43	29.39
proline	Pro	$C_5H_9NO_2$	1[a)]	303	216	18.01	18.28
serine	Ser	$C_3H_7NO_3$	2[b)]	439	352	19.49	20.25
tyrosine	Tyr	$C_9H_{11}NO_3$	2[b)]	515	428	33.71	34.47
valine	Val	$C_5H_{11}NO_2$	1[a)]	305	218	14.24	15.27

a) acylation reaction of pentafluoropropionic acid anhydride with the amino functionality of the corresponding amino acid isopropylesters

b) acylation reaction of pentafluoropropionic acid anhydride with the amino and alcohol functionalities of the corresponding amino acid isopropylesters

AM 3:

To analyze the amount of racemization when alanine was the *C*-terminal amino acid, we performed the hydrolysis of enolesters with 6N HCl and analyzed the content of D-Ala-OH via HPLC-analysis after derivatization with the L-valinamide analogue of Marfey's reagent (FDAA, N_α-(2,4-dinitro-5-fluorophenyl)-L-alaninamide) on a Poroshell column (Figure 16).[243] The D-Ala-OH content after parallel hydrolysis of the starting material **1d** under identical conditions was substracted to compensate for the background reaction.

Method N: Shimadzu Nexera; column: Poroshell 120 SB-C18 (2.7 µm, Agilent) 100×3 mm; 40°C, 0.7 mL/min; solvent A: H_2O+0.01 % HCOOH; B: MeCN; 0-3 min: A/B = 70/30; 3.01-3.50 min: A/B = 0:100; UV (340 nm) and ESI-MS detection in positive mode, was used to identify the compounds

The retention times were: 1.9 min (L-Ala) and 2.7 min (D-Ala).

AM 4:

To analyze the ee-value of the Cbz-protected single amino acid Markovnikov-enolesters (**M-3a** and **M-3b**) chiral HPLC column Chiralcel®-OD-H (method N) was used. As a reference the appropriate racemic Cbz-protected single amino acid Markovnikov-enolesters were synthesized and analysed with chiral HPLC under the same conditions.

Method N: Agilent 1100; column: Chiralcel®-OD-H (5 µm) 250×4.6 mm, 15°C, 0.65 mL/min;

solvent A: *n*-heptane; B: 2-propanol; 0 min: A/B=80/20; isocratic

6.4.5. Determination of conversion (HPLC)

$$\text{conversion}\,[\%] = 100 \cdot \frac{\sum \text{products}}{\text{starting material} + \sum \text{products}}$$

6.4.6. High resolution mass spectroscopy

Electron impact (EI, 70 eV) HRMS spectra were recorded on a *Waters GCT Premier* equipped with direct insertion (DI) and GC (HP GC7890A).

HRMS spectra recorded with MALDI-TOF mass spectrometry were performed on a *Micromass TofSpec 2E* Time-of-Flight Mass Spectrometer.

6.4.7. Thin layer chromatography

Analytical thin layer chromatography was performed using TLC-plates from Merck (TLC aluminium foil, silica gel 60 F_{254}). Generally, the spots were visualized using a UV lamp (λ = 254, 366 nm) or by treatment with different staining reagents (listed below) followed by heating.

CAM-solution: 2.0 g cer(IV)-sulfate, 50.0 g ammonium molybdate and 50 mL conc. H_2SO_4 in 400 mL water

Potassium permanganate: 3.0 g potassium permanganate, 20.0 g K_2CO_3, 300 mL of a 5 % aqueous NaOH solution

The used solvent mixtures and R_f-values are stated in the individual experimental procedures.

6.4.8. Column chromatography

Preparative flash column chromatography was performed using silica gel 60 from ACROS Organics (35-70 µm particle size). The mass of silica gel used was 100 times (w/w) the amount of dry crude product. The length of columns used differ from 10 to 40 cm. The Rf-values are listed in the experimental section, the following abbreviations for the eluents were used: EE (ethyl acetate), CH (cyclohexane), DCM (methylene chloride) and MeOH (methanol).

All solvents used for flash column chromatography were purchased from Fisher Scientific as analytical grade solvents (99.99 %).

6.4.9. Determination of the melting point

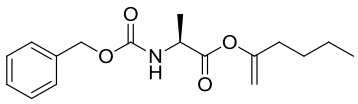
Melting points are uncorrected and were determined with the apparatus "*Mel-Temp*®" from *Electrothermal* with an integrated microscopical support. The temperature was measured with a mercury-in-glass thermometer.

6.4.10. Specific rotation

The determination of specific rotations was performed on a polarimeter *341 from Perkin Elmer*. A sodium vapor lamp (589 nm) was used as source of monochromatic light.

6.5. Experimental procedures and analytical data for the synthesis of amino acid and peptide Markovnikov-enolesters

6.5.1. Synthesis of Z-Ala-Markovnikov-enolester M-3a



5.56 g Z-alanine (**1a**) (24.9 mmol, 1.00 eq) together with 102 mg (996 µmol, 0.04 eq) sodium carbonate were added to an oven-dried Schlenk tube under argon and were then suspended in 17 mL degassed $CHCl_3$. In the meantime in another oven-dried Schlenk tube 153 mg (249 µmol, 0.01 eq) $((p\text{-cumene})RuCl_2)_2$ (**I**) together with 140 mg (498 µmol, 0.02 eq) tricyclohexylphosphine (**6**) were dissolved in 17 mL degassed $CHCl_3$ under argon and stirred for 30 min to form the catalyst of this reaction. The red catalyst solution was transferred to the colourless suspension, the Schlenk tube was rinsed with degassed $CHCl_3$ (4 x 17 mL) and this solution was also added to the reaction mixture. 3.72 mL (32.6 µmol, 1.30 eq) degassed 1-hexyne (**2a**) were added to the red suspension and the reaction mixture was stirred for 3 d in a 50 °C preheated oil bath under argon, while a yellow solution was formed. The solvent was removed in vacuo using a rotary evaporator. The brownish crude product was purified via flash column chromatography (650 g silica gel, 27 × 8.0 cm, cyclohexane/ethyl acetate = 7:1 (v/v), R_f = 0.29).

Yield: 2.52 g (8.24 mmol, 33 %) of Markovnikov-product **M-3a**, yellow oil

Yield of sum of isomers after flash chromatography: 7.02 g (23.0 mmol, 92 %)

Content of Z-D-Ala-Markovnikov-enolester: 0.2 % (chiral HPLC Method N)

$C_{17}H_{23}NO_4$ [305.38 $gmol^{-1}$]

$[\alpha]_D^{20}$ = -33.5 (c = 1 in ethanol)

GC-MS (HS_50_S2): t_R = 7.59 min; m/z = 91 (BP)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, 3H, ^3J(H,H) = 7.2 Hz, -CH_2-C$\underline{H}_3$), 1.24-1.47 (m, 7H, -CH_2-C$\underline{H}_2$-CH_3, -CH_2-C$\underline{H}_2$-CH_2-CH_3, -CH-C$\underline{H}_3$), 2.18 (t, 2H, ^3J(H,H) = 7.4 Hz, -(C=CH_2)-C$\underline{H}_2$-CH_2-CH_2-CH_3), 4.44 (p, 1H, ^3J(H,H) = 7.4 Hz, -NH-C$\underline{H}$(CH_3)-CO-), 4.73 (bs, 2H, -C=C$\underline{H}_2$), 5.10 (m, 2H, -O-C$\underline{H}_2$-Ph), 5.31 (d, 1H, ^3J(H,H) = 6.9 Hz, -CO-N$\underline{H}$-CH-), 7.29-7.35 (m, 5H, H_{Ar}).

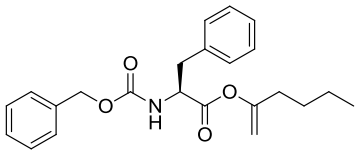
^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.8 (-CH_2-$\underline{C}H_3$), 18.7 (-CH-$\underline{C}H_3$), 22.0 (-CH_2-$\underline{C}H_2$-CH_3), 28.5 (-CH_2-$\underline{C}H_2$-CH_2-CH_3), 32.8 (-(C=CH_2)-$\underline{C}H_2$-CH_2-CH_2-CH_3), 49.8 (-NH-$\underline{C}$H(CH_3)-CO-), 67.0 (-

O-$\underline{C}H_2$-Ph), 101.4 (-C=$\underline{C}H_2$), 128.1 (2 × C_{Ar}), 128.2 (C_{Ar}), 128.6 (2 × C_{Ar}), 136.2 ($C_{q,Ar}$), 155.6 (-O-$\underline{C}$(=CH_2)-$C_{alkylchain}$), 156.3 (NH-$\underline{C}O_2$-CH_2-C_6H_5), 171.3 (C_q, -CH-$\underline{C}$O-O-).

6.5.2. General procedure for ligand screening to optimize the synthesis of Z-Ala-Markovnikov-enolester M-3a

135 mg Z-alanine (**1a**) (600 µmol, 1.00 eq) together with 2.6 mg (24.1 µmol, 0.04 eq) sodium carbonate were added to an oven-dried Schlenk tube under argon and were then suspended in 400 µL degassed $CHCl_3$. In the meantime in another oven-dried Schlenk tube 3.7 mg (6.0 µmol, 0.01 eq) ((p-cumene)$RuCl_2)_2$ (**I**) together with 0.02 eq of various phosphine ligands were dissolved in 400 µL degassed $CHCl_3$ under argon and stirred for 30 min to form the catalyst of this reaction. The catalyst solution was transferred to the colourless suspension of Z-alanine and sodium carbonate, the Schlenk tube was washed with degassed $CHCl_3$ (4 x 400 µL) and this solution was also added to the reaction mixture. 90 µL (780 µmol, 1.30 eq) degassed 1-hexyne (**2a**) were added to the coloured suspension and the reaction mixture was stirred at 50 °C in a preheated oil bath under argon.

6.5.3. Synthesis of Z-Phe-Markovnikov-enolester M-3b



2.00 g Z-phenylalanine (**1b**) (6.70 mmol, 1.00 eq) together with 28.4 mg (268 µmol, 0.04 eq) sodium carbonate were added to an oven-dried Schlenk tube with a magnetic stirring bar under argon and were then suspended in 4.5 mL degassed $CHCl_3$. In the meantime in another oven-dried Schlenk tube 41.0 mg (67.0 µmol, 0.01 eq) ((p-cumene)$RuCl_2)_2$ (**I**) together with 37.6 mg (134 µmol, 0.02 eq) tricyclohexylphosphine (**6**) were dissolved in 4.5 mL degassed $CHCl_3$ under argon and stirred for 30 min to form the catalyst complex. The red catalyst solution was transferred to the colourless suspension, the Schlenk tube was rinsed with degassed $CHCl_3$ (4 x 4.5 mL) and this solution was also added to the reaction mixture. 1.00 mL (715 µmol, 1.30 eq) degassed 1-hexyne (**2a**) were added to the red suspension and the reaction mixture was stirred for 7 d in a 50 °C preheated oil bath under argon, while a yellow solution was formed. After cooling down to room temperature, the solution was filtered through 40 g silica gel, eluted with 500 mL ethyl acetate. Then the solvent was removed in vacuo using a rotary evaporator and the brownish crude product was

purified via flash column chromatography (265 g silica gel, 22 x 5.7 cm, cyclohexane/ethyl acetate = 9:1 (v/v), R_f = 0.25).

Yield: 0.51 g (1.33 mmol, 20 %), of the Markovnikov-product **M-3b**, light brown crystals.

Yield of sum of isomers after flash chromatography: 2.20 g (5.77 mmol, 86 %)

Content of Z-D-Phe-Markovnikov-enolester: <0.1 % (chiral HPLC Method N)

$C_{28}H_{27}NO_4$ [381.46 $gmol^{-1}$]

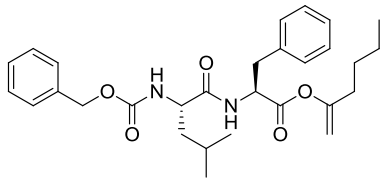
mp = 55-56 °C

$[\alpha]_D^{20}$ = -21.5 (c = 1.0 in EtOH)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.93 (t, 3H, ^3J(H,H) = 7.1 Hz, -CH_2-C$\underline{H}_3$), 1.27-1.45 (m, 4H, -CH_2-C$\underline{H}_2$-CH_3, -CH_2-C$\underline{H}_2$-CH_2-CH_3), 2.14-2.20 (m, 2H, -(C=CH_2)-C$\underline{H}_2$-CH_2-CH_2-CH_3), 3.13-3.26 (m, 2H, -CH-C$\underline{H}_2$-Ph), 4.71-4.80 (m, 3H, -C=C$\underline{H}_2$, -NH-C$\underline{H}$(CH_2-Ph)-CO-), 5.14 (s, 2H, -O-C$\underline{H}_2$-Ph), 5.28 (d, 1H, ^3J(H,H) = 7.8 Hz, -CO-N$\underline{H}$-CH-), 7.18-7.21 (m, 2H, H_{Ar}), 7.28-7.39 (m, 8H, H_{Ar}).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 (-$\underline{C}H_2$-CH_3), 22.2 (-CH_2-$\underline{C}H_2$-CH_3), 28.6 (-CH_2-$\underline{C}H_2$-CH_2-CH_3), 33.1 (-(C=CH_2)-$\underline{C}H_2$-CH_2-CH_2-CH_3), 38.4 (-CH-$\underline{C}H_2$-Ph), 55.0 (-NH-$\underline{C}$H(CH_2-Ph)-CO-), 67.2 (-O-$\underline{C}H_2$-Ph), 101.5 (-C=$\underline{C}H_2$), 127.4 (C_{Ar}), 128.3 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.9 (2 × C_{Ar}), 129.6 (2 × C_{Ar}), 135.7, 136.4 (2 × $C_{q,Ar}$), 155.8 (-O-$\underline{C}$(=CH_2)-$C_{alkylchain}$), 156.5 (NH-$\underline{C}O_2$-CH_2-C_6H_5), 170.0 (C_q, -CH-$\underline{C}$O-O-).

6.5.4. Approach to synthesize Z-Phe-Leu-Markovnikov-enolester M-3c



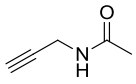
The approach to synthesize Z-Phe-Leu-Markovnikov-enolester **M-3c** was performed as described for single amino acid enolesters **M-3a** and **M-3b**.

Z-Leu-Phe-OH (**1c**) (150 mg, 0.36 mmol, 1.0 eq) and Na_2CO_3 (1.54 mg, 0.015 mmol, 0.04 eq) were suspended in chloroform (500 µL). Subsequently, a solution of ((*p*-cumene)$RuCl_2$)$_2$ (**I**) (2.2 mg, 3.6 µmol) and tricyclohexylphosphine (2.0 mg, 7.27 µmol) in chloroform (700 µL), and 1-hexyne (54 µL, 0.47 mmol) were added. The reaction mixture was heated to 50 °C. The conversions were determinated by HPLC Method G. Due to the low conversion towards **M-3c** of 28 % after 7 d and putative racemization, **M-3c** was not isolated and further characterized.

6.6. Experimental procedures and analytical data for the synthesis of amino acid and peptide *Z*-anti-Markovnikov-enolesters

6.6.1. Preparation of starting materials

6.6.1.1. General procedure for the synthesis of *N*-(2-propynyl)acetamide (2c), 2d and 2e



This reaction was conducted according to the literature.[239]

Propargylamine (1.28 mL, 20.0 mmol), triethylamine (4.19 mL, 30.0 mmol), 4-(dimethylamino)pyridine (48.8 mg, 0.40 mmol, 2 mol%) and dichloromethane (30 mL) were placed in a 100 mL round-bottomed flask and the mixture was cooled under argon atmosphere to 0°C in an ice bath. Then acetic anhydride (2.63 mL, 25.0 mmol) (or the appropriate acid chlorides for **2d** and **2e**) in dichloromethane (10 mL) was added dropwise under stirring over a time span of 15 min. The reaction was allowed to reach rt and further stirred overnight. Excess of reagent was hydrolyzed by the addition of water (10 mL) and prolonged stirring for 2 h. Then 0.2M hydrochloric acid (50 mL) and ethyl acetate (120 mL) were added and the aqueous phase was separated. The organic phase was washed with saturated sodium bicarbonate solution (1× 50 mL) and brine (1× 50 mL) and then dried over Na_2SO_4. Finally, the volatiles were evaporated and the residue was purified by flash column chromatography (85 g silica gel, 23 × 3.5 cm, cyclohexane/ethyl acetate = 1:2 (v/v) R_f = 0.31).

Yield: 0.802 g (41 %), colourless powder

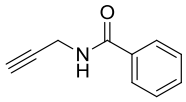
C_5H_7NO [97.12 $gmol^{-1}$]

mp = 86°C

^{1}H- NMR (300 MHz, $CDCl_3$): δ = 1.99 (s, 3H, -CO-C<u>H</u>$_3$), 2.21 (t, 1H, ^4J(H,H) = 2.5 Hz, <u>H</u>C≡C-C<u>H</u>$_2$-NH-), 4.02 (dd, 2H, ^3J(H,H) = 5.3 Hz, ^4J(H,H) = 2.5 Hz, HC≡C-C<u>H</u>$_2$-NH-), 6.07 5.75 (bs, 1H, HC≡C-CH$_2$-N<u>H</u>-).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 23.1 (-CO-<u>C</u>H$_3$), 29.3 (HC≡C-<u>C</u>H$_2$-NH-), 71.6 (H<u>C</u>≡C-CH$_2$-NH-), 79.7 (HC≡<u>C</u>-CH$_2$-NH-), 170.0 (-<u>C</u>O-CH$_3$).

6.6.1.2. Synthesis of *N*-(2-propynyl)benzamide (2d)



Procedure according to chapter 6.6.1.1. and literature.[239] The same quantity of reagent, solvents and starting materials were applied (propargylamine (1.28 mL, 20.0 mmol) and benzoyl chloride (2.90 mL, 25.0 mmol)). Purification: flash column chromatography (85 g silica gel, 23 × 3.5 cm, cyclohexane/ethyl acetate = 2:1 (v/v), R_f = 0.35)

Yield: 2.50 g (79 %), colourless powder

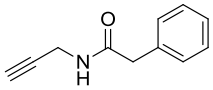
$C_{10}H_9NO$ [159.18 $gmol^{-1}$]

mp = 111°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.27 (t, 1H, ^4J(H,H) = 2.5 Hz, HC≡C-CH_2-NH-), 4.24 (dd, 2H, ^3J(H,H) = 5.2 Hz, ^4J(H,H) = 2.5 Hz, HC≡C-CH_2-NH-), 6.50 (bs, 1H, HC≡C-CH_2-NH-), 7.38-7.55 (m, 3H, H_{Ar}), 7.76-7.83 (m, 2H, H_{Ar}).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 29.9 (HC≡C-CH_2-NH-), 72.0 (HC≡C-CH_2-), 79.6 (HC≡C-CH_2-), 127.2 (2 × C_{Ar}), 128.7 (2 × C_{Ar}), 131.9 (C_{Ar}), 133.9 ($C_{q,Ar}$), 167.3 (-NH-CO-Ph).

6.6.1.3. Synthesis of 2-phenyl-*N*-(prop-2-ynyl)acetamide (2e)



Procedure according to chapter 6.6.1.1. and literature.[293]

The same quantity of reagent, solvents and starting materials were applied (propargylamine (1.28 mL, 20.0 mmol) and phenylacetyl chloride (3.31 mL, 25.0 mmol)). Purification: flash column chromatography (85 g silica gel, 23 × 3.5 cm, cyclohexane/ethyl acetate = 2:1 (v/v), R_f = 0.26)

Yield: 2.77 g (80 %), colourless powder

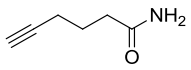
$C_{11}H_{11}NO$ [173.21 $gmol^{-1}$]

mp = 77°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.21 (t, 1H, ^{4}J(H,H) = 2.5 Hz, HC≡C-CH_2-NH-), 3.62 (s, 2H, -NH-CO-CH_2-Ph), 4.03 (dd, 2H, ^{3}J(H,H) = 5.3 Hz, ^{4}J(H,H) = 2.5 Hz, HC≡C-CH_2-NH-), 5.75 (bs, 1H, HC≡C-CH_2-NH-), 7.26-7.44 (m, 5H, H_{Ar}).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 29.4 (HC≡C-CH_2-NH-), 43.5 (-OC-CH_2-Ph), 71.6 (HC≡C-CH_2-), 79.4 (HC≡C-CH_2-), 127.5 (C_{Ar}), 129.1 (2 × C_{Ar}), 129.5 (2 × C_{Ar}), 134.4 ($C_{q,Ar}$), 170.6 (-NH-CO-CH_2-Ph).

6.6.1.4. Synthesis of hex-5-ynamide (2b)



5-Hexynoic acid (1.65 mL, 15.0 mmol, 1.0 eq) was placed in a 25 mL round-bottom flask and then thionyl chloride (3.27 mL, 45.0 mmol, 3.0 eq) was added. The mixture was heated at 50°C for 10 min and another 2.5 h under reflux. After all thionyl chloride had been distilled off, the residual oil was dissolved in dry methyl *tert*-butyl ether (10 mL) and added dropwise under stirring to NH_3 in dioxane (0.5M, 30 mL) in an 100 mL round-bottom flask. After stirring overnight, the volatiles were evaporated and the residue was purified by flash column chromatography (180 g silica gel, 27 × 6 cm, cyclohexane/ethyl acetate = 1:5 (v/v), R_f = 0.17).

Yield: 711 mg (43 %), colourless to pale yellow powder

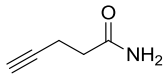
C_6H_9NO [111.14 $gmol^{-1}$]

mp = 79-80°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.82 (p, 2H, ^{3}J(H,H) = 7.0 Hz, -CH_2-CH_2-CH_2-), 1.96 (t, 1H, ^{4}J(H,H) = 2.6 Hz, HC≡C-), 2.25 (td, 2H, ^{3}J(H,H) = 6.8 Hz, ^{4}J(H,H) = 2.6 Hz, HC≡C-CH_2-), 2.33 (t, 2H, ^{3}J(H,H) = 7.4 Hz, -CH_2-CH_2-CO-NH_2-), 5.76 (brs, 1H, -$CONH_2$), 6.15 (brs, 1H, -$CONH_2$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.5 (HC≡C-CH_2-), 23.6 (HC≡C-CH_2-CH_2-), 33.9 (-CH_2-CH_2-$CONH_2$), 68.9 (HC≡C-CH_2-), 83.1 (HC≡C-CH_2-), 174.9 (-CH_2-$CONH_2$).

6.6.1.5. Synthesis of pent-4-ynamide (2g)



This reaction was conducted according to the literature.[240]

4-Pentynoic acid (1.29 g, 13.15 mmol, 1.0 eq) was dissolved in dry dichloromethane (30 mL) in a 100 ml round-bottom flask. Then DMF (51 µL, 0.66 mmol, 0.05 eq) and oxalyl chloride (1.67 mL, 19.73 mmol, 1.5 eq) were consecutively added while stirring under nitrogen at rt. After 4 h the excess of oxalyl chloride and dichloromethane were removed in vacuo and the residue was treated with NH_3 in dioxane (0.5M, 52.6 mL, 2 eq) and triethylamine (2.75 mL, 19.7 mmol, 1.5 eq) and left at room temperature overnight. Finally, the volatiles were evaporated and the residue was purified by flash column chromatography (180 g silica gel, 27 × 6 cm, ethyl acetate = 100 %, R_f = 0.30).

Yield: 558 mg (44 %), colourless powder

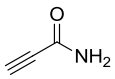
C_6H_9NO [97.11 $gmol^{-1}$]

mp = 112°C

^{1}H-NMR (300 MHz, CD_3OD): δ = 2.29 (t, 1H, ^4J(H,H) = 2.4 Hz, HC≡C-), 2.40-2.53 (m, 4H, HC≡C-CH_2-CH_2-CONH$_2$), 6.87 (brs, 1H, -CONH$_2$), 7.65 (brs, 1H, -CONH$_2$).

^{13}C-NMR (75 MHz, CD_3OD): δ = 15.5 (HC≡C-CH$_2$-CH$_2$-CONH$_2$), 35.5 (HC≡C-CH$_2$-CH$_2$-CONH$_2$), 70.2 (HC≡C-CH$_2$-CH$_2$-CONH$_2$), 83.6 (HC≡C-CH$_2$-CH$_2$-CONH$_2$), 176.9 (HC≡C-CH$_2$-CH$_2$-CONH$_2$).

6.6.1.6. Synthesis of propiolamide (2f)



Propiolamide (**2f**) was prepared according to the literature.[241]

Methyl propiolate (2.52 g, 30 mmol, 1.0 eq) was dissolved in 25 % NH_3/H_2O (120 mmol, 4.0 eq) and stirred for 1 h at -78°C (dry ice in acetone) and finally warmed to rt. Then, all volatile compounds were removed in vacuo using a rotary evaporator and crude product was purified

via flash column chromatography (85 g silica gel, 23 × 3.5 cm, cyclohexane/ethyl acetate = 1:2 (v/v), R_f = 0.48).

Yield: 1.93 g (93 %), colourless powder

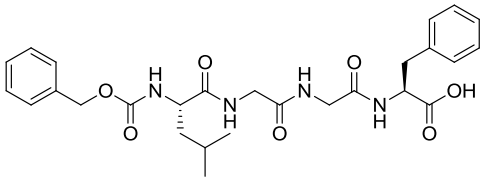
C_3H_3NO [97.11 $gmol^{-1}$]

mp = 59°C

^{1}H-NMR (300 MHz, CD_3OD): δ = 3.58 (s, 1H, H<u>C</u>≡C-$CONH_2$), 7.54 (br s, 1H, -CO-N<u>H</u>$_2$), 8.18 (br s, 1H, -CO-N<u>H</u>$_2$).

^{13}C-NMR (75 MHz, CD_3OD): δ = 76.4 (H<u>C</u>≡C-$CONH_2$), 78.1 (HC≡<u>C</u>-$CONH_2$), 156.8 (HC≡C-<u>C</u>ONH_2).

6.6.1.7. Z-Leu-Gly-Gly-Phe-OH (1m)



Acetyl chloride (**85**) (853 µL, 12.0 mmol, 2 eq) was dropwise added to methanol (12 mL) at 0°C under argon and stirring. After 1h H-Gly-Phe-OH (1.345 g, 6.0 mmol) was added and stirring was continued at rt for 20 h. Then all volatiles were evaporated leaving H-Gly-Phe-OMe·HCl (1.619 g, 99 %) as product. Without further purification the hydrochloride was redissolved in dichloromethane (5.0 mL) and then DIPEA (4.10 mL, 24.0 mmol), Z-Leu-Gly-OH (1.934 g, 6.0 mmol) and TBTU (2.31 g, 7.2 mmol, 1.2 eq) were added and the mixture was stirred for 18 h. The reaction mixture was diluted with dichloromethane (50 mL), washed consecutively with 1M HCl (1× 50 mL) and saturated $NaHCO_3$ (1× 50 mL) and dried over Na_2SO_4. After the removal of volatiles by evaporation, the crude product was purified by flash column chromatography on silica gel using mixtures of methanol (1-5 vol%) in dichloromethane. All fractions containing the product were combined and concentrated to dryness using a rotary evaporator yielding off-white Z-Leu-Gly-Gly-Phe-OMe (1.20 g, 2.22 mmol, 37 %). For the final step, Z-Leu-Gly-Gly-Phe-OMe was dissolved in a mixture of THF (16 mL) and water (4 mL). After cooling to 0°C, $LiOH·H_2O$ (112 mg, 2.66 mmol, 1.2 eq) was added and the reaction was stirred at the same temperature until HPLC analysis confirmed full conversion of the ester (after ca. 5 h). Then formic acid (113 µL, 3.0 mmol) was added and the solvent evaporated. The product was purified by flash column chromatography (150 g silica gel, 21 × 4.5 cm, dichloromethane/methanol = 4:1 (v/v) + 1 % acetic acid, R_f = 0.41).

Yield: 338 mg (33 %), almost colourless powder

$C_{27}H_{34}N_4O_7$ [526.58 $gmol^{-1}$]

mp = 117°C

^{1}H-NMR (300 MHz, CD_3OD): δ = 0.94 (t, 6H, ^3J(HH) = 7.0 Hz, -CH-($C\underline{H}_3)_2$), 1.57-1.74 (m, 3H, -CH_2-$C\underline{H}$-$(CH_3)_2$, -$C\underline{H}_2$-CH-$(CH_3)_2$), 2.97-3.22 (m, 2H, C_6H_5-$C\underline{H}_2$-CH-NH), 3.76-3.95 (m, 4H, 2 × NH-$C\underline{H}_2$-CO-NH-), 4.13 (t, 1H, ^3J(HH) = 7.3 Hz, -NH-$C\underline{H}$-), 4.58-4.62 (m, 1H, NH-$C\underline{H}$-), 5.02-5.13 (m, 2H, HN-CO_2-$C\underline{H}_2$-C_6H_5), 7.16-7.44 (m, 10 H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, CD_3OD): δ = 21.8, 23.1 (-CH-($\underline{C}H_3)_2$), 24.8 (-$\underline{C}$H-$(CH_3)_2$), 37.3 (C_6H_5-$\underline{C}H_2$-CH-NH-), 41.2, 42.9, 42.9 (2 x NH-$\underline{C}H_2$-CO-NH, -$\underline{C}H_2$-CH-$(CH_3)_2$), 54.1, 54.8 (2 x NH-$\underline{C}$H-), 67.4 (HN-CO_2-$\underline{C}H_2$-C_6H_5), 127.0 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.6 (2 × C_{Ar}), 128.7 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 138.0, 138.7 (2 × $C_{q,Ar}$), 158.9 (NH-$\underline{C}O_2$-CH_2-C_6H_5), 171.0 (C_q), 172.0 (C_q), 175.7 (C_q), 176.2 (C_q).

6.6.2. Preparation of ruthenium catalysts

6.6.2.1. Bis-(2-methylallyl)(dppb)ruthenium(II) (III)

An oven-dried Schlenk-tube with a magnetic stirring bar was charged with bis-(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (**II**) (500 mg, 1.57 mmol, 1.0 eq) and dppb (**20**) (669 mg, 1.57 mmol, 1.0 eq) under argon atmosphere. After evacuation and refilling with argon, dry *n*-hexane (8.5 mL) was added. After heating to 55 °C in a preheated oil bath, a brownish solution was formed. Stirring was continued for another 5 h at this temperature while the yellow product precipitated. Using an inert Schlenk filter funnel, the precipitate was collected and washed with cold and dry *n*-hexane (3 × 5 mL). The solid was dried under vacuum (0.02 mbar) for several hours yielding the product as a yellowish to greenish powder (765 mg, 1.20 mmol, 77 %).

6.6.2.2. Bis-(2-methylallyl)((+)-DIOP)ruthenium(II) (V)

An oven-dried Schlenk-tube with a magnetic stirrer was charged with bis-(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (**II**) (450 mg, 1.41 mmol, 1.0 eq) and (+)-DIOP ((+)-**42**) (717 mg, 1.41 mmol, 1.0 eq) under argon atmosphere. After evacuation and refilling with argon, dry *n*-hexane (8.5 mL) was added. After heating to 55 °C in a preheated oil bath, a brownish solution was formed. Stirring was continued for another 5 h at this temperature while the yellow product precipitated. Using an inert Schlenk filter funnel, the precipitate was collected and washed with cold and dry *n*-hexane (3 × 5 mL). The solid was dried under vacuum (0.02 mbar) for several hours yielding the product as a yellowish to greenish powder (877 mg, 1.24 mmol, 88 %).

The ^{1}H and ^{31}P NMR data were in complete agreement with those reported.[237]

6.6.2.3. [Ru(TFA)$_2$(dppb)] (VII)

The reaction (Scheme 26) was performed similar to the literature procedure from Weberndörfer et al.[242]

A solution of **III** (200 mg, 0.31 mmol, 1.00 eq) in 6 mL toluene in an oven-dried Schlenk-tube with a magnetic stirrer was treated dropwise at -78°C with trifluoroacetic acid (**48**) (48.8 µL, 0.63 mmol, 2.02 eq). The reaction was stirred for 1 h at -78°C and afterwards the reaction mixture was warmed to rt. During warming up a rapid change of the color from orange to brown occurred and a precipitate was formed. After stirring at rt for 15 min, the solvent was

evaporated in vacuo and the product **VII** was washed with *n*-hexane (1×2 mL). The solid was dried under vacuum (0.02 mbar) for several hours yielding the product as a yellowish to greenish powder (156 mg, 0.21 mmol, 66 %).

6.6.2.4. [Ru(TFA)$_2$((+)-DIOP)] (VIII)

The reaction (Scheme 28) was performed similar to the literature procedure from Weberndörfer et al.[242]

A solution of **V** (71 mg, 0.10 mmol, 1.00 eq) in 6 mL toluene in an oven-dried Schlenk-tube with a magnetic stirrer was treated dropwise at -78°C with trifluoroacetic acid (**48**) (15.4 µL, 0.20 mmol, 2.00 eq). The reaction was stirred for 1 h at -78°C and afterwards the reaction mixture was warmed to rt. During warming up a rapid change of the color from orange to brown occurred and a precipitate was formed. After stirring at rt for 15 min the solvent was evaporated in vacuo and the product **VIII** washed with *n*-hexane (1×2 mL). The solid was dried under vacuum (0.02 mbar) for several hours yielding the product as a green powder (50 mg, 0.06 mmol, 61 %).

6.6.3. Screening of phosphines: General procedure for Ru-catalyzed enolester formation of Z-Leu-Phe-OH (1c) using different phosphorous-ligands

All experiments were carried out under argon in previously oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis-(2-methylallyl)(cycloocta-1,5-diene)ruthenium(II) (**II**) (3.1 mg, 9.7 µmol, 0.04 eq) and different bidentate phosphine ligands (9.7 µmol, 0.04 eq) and dry THF (0.25 mL) degassed prior to use. The solution was stirred at 40°C for 1 h under argon. Afterwards Z-Leu-Phe-OH (**1c**) (100 mg, 0.24 mmol, 1.00 eq) was added and the Schlenk vessel was rinsed with dry THF (0.25 mL) degassed prior to use, under inert conditions at 40°C. Degassed 1-hexyne (**2a**) (34 µL, 0.29 mmol, 1.20 eq) was finally added with a Hamilton syringe to the solution at 40°C and the mixture was stirred at this temperature for the indicated time.

6.6.4. Screening of solvents: General procedure for the Ru-catalyzed enolester formation of Z-Leu-Phe-OH (1c) in different solvents

All experiments were carried out under argon in oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with Z-Leu-Phe-OH (**1c**) (100 mg, 0.24 mmol, 1.0 eq), Ru-catalyst **V** (6.9 mg, 9.7 µmol, 0.04 eq) or Ru-catalyst **III** (6.2 mg, 9.7 µmol, 0.04 eq) and dry solvent (0.5 mL) degassed prior to use. If necessary the materials were dissolved by heating to reflux temperature for a few seconds and then the vessel was immediately cooled and immersed in an oil bath preheated to 40°C. Then degassed 1-hexyne (**2a**) (34 µL, 0.29 mmol, 1.2 eq) was added to the reaction solution with a Hamilton syringe and the mixture was stirred at 40°C for the indicated time.

6.6.5. General procedure for the Ru-catalyzed enolester formation of dipeptides using 1-heyxne (2a)

All experiments were carried out under argon in oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with Z-Peptide-OH **1** (0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq) and dry solvent (0.5 mL) degassed prior to use. If necessary the materials were dissolved by heating to reflux temperature for a few seconds and then the vessel was immediately cooled and immersed in an oil bath preheated to 40°C. Then degassed 1-hexyne (**2a**) (0.3 mmol, 1.2 eq) was added to the reaction solution with a Hamilton syringe and the mixture was stirred at 40°C. After the

appropriate conversion was reached, the solution was concentrated to dryness. The tawny solid residue was then redissolved in dichloromethane, co-evaporated in the presence of the same quantity of silica gel and finally subjected to chromatographic purification. All fractions containing the product were combined and the solvents removed in vacuo using a rotary evaporator. The product was finally dried at 0.02 mbar.

6.6.6. General procedure for the Ru-catalyzed enolester formation of dipeptides using hex-5-ynamide (2b), *N*-(2-propynyl)acetamide (2c), *N*-(prop-2-yn-1-yl)benzamide (2d), 2-phenyl-*N*-(prop-2-yn-1-yl)acetamide (2e), propiolamide (2f), pent-4-ynamide (2g)

All experiments were carried out under argon in oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with Z-Peptide-OH **1** (0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq) and dry solvent (0.5 mL) degassed prior to use. The materials were dissolved by heating to reflux temperature for a few seconds and then the vessel was immediately cooled and immersed in an oil bath preheated to 40°C. The solid alkyne **2** (0.3 mmol, 1.2 eq) was added to the reaction solution and the mixture stirred at 40°C. After the appropriate conversion was reached the solution was concentrated to dryness. The tawny solid residue was then redissolved in dichloromethane, co-evaporated in the presence of the same quantity of silica gel and finally subjected to chromatographic purification. All fractions containing the product were combined and the solvents removed in vacuo using a rotary evaporator. The product was finally dried at 0.02 mbar.

6.6.7. Enolester formation of amino acids or dipeptides using 1-heyxne (2a) and [Ru(TFA)$_2$((+)-DIOP)] (VIII) or [Ru(TFA)2(dppb)] (VII)

All experiments were carried out under argon in oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with Z-Amino acid-OH or Z-Peptide-OH **1** (0.24 mmol, 1.0 eq), Ru-catalyst **VIII** (8.0 mg, 9.7 µmol, 0.04 eq) or Ru-catalyst **VII** (7.3 mg, 9.7 µmol, 0.04 eq) and dry solvent (0.5 mL) degassed prior to use. If necessary the materials were dissolved by heating to reflux temperature for a few seconds and then the vessel was immediately cooled and immersed in an oil bath preheated to 40°C. Then degassed 1-hexyne (**2a**) (0.29 mmol, 1.2 eq) was added to the reaction solution with a Hamilton syringe and the mixture was further on stirred at 40°C. After the appropriate conversion was reached the solution was concentrated to dryness. The tawny solid residue

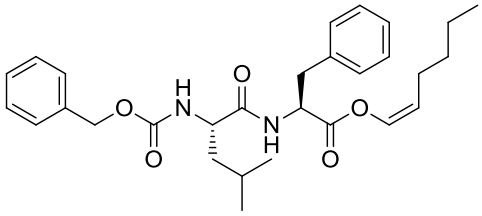
was then redissolved in dichloromethane, co-evaporated in the presence of the same quantity of silica gel and finally subjected to chromatographic purification. All fractions containing the product were combined and the solvents removed in vacuo using a rotary evaporator. The product was finally dried at 0.02 mbar.

6.6.8. Procedure for the structural assignment of *Z*-anti-Markovnikov- and Markovnikov-isomers AM-*Z*-3d, M-3a and AM-*Z*-3a, M-3c

The experiments were carried out under argon in previously oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis-(2-methylallyl)(cycloocta-1,5-diene)ruthenium(II) (**II**) (3.2 mg, 0.01 mmol, 0.04 eq) and the phosphine ligand dpppe (1,5-bis(diphenylphosphino)pentane) (**24**) (4.4 mg 0.01 mmol, 0.04 eq) and dry THF (0.25 mL) degassed prior to use. The solution was stirred at 40°C for 1 h under argon. Afterwards Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.00 eq) or Z-Ala-OH (**1a**) (55.8 mg, 0.25 mmol, 1.00 eq) were added and the Schlenk vessel was rinsed with dry THF (0.25 mL) degassed prior to use, under inert conditions at 40°C. Degassed 1-hexyne (**2a**) (34 µL, 0.30 mmol, 1.20 eq) was finally added with a Hamilton syringe to the solution at 40°C and the mixture was stirred at this temperature for 24 h.

6.6.9. Experimental information, according to Ru-catalyzed alkyne addition

6.6.9.1. Z-Leu-Phe-*Z*-anti-Markovnikov-enolester AM-*Z*-3a



Procedure according to chapter 6.6.5.

Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), dry 2-propanol (0.5 mL), 1-hexyne (**2a**) (34 µL, 23.9 mg, 1.2 eq)

Flash column chromatography: (16 g silica gel, 22 × 1.5 cm, cyclohexane/ethyl acetate = 5:1 (v/v), R_f = 0.30)

Yield: 0.105 g (88 %), colourless oil, which became a grey solid after 1 d

Content of D-Phe-OH: <0.1 % HPLC-MS method AM 1 and GC-MS method AM 2 (chapter 6.4.4)

$C_{29}H_{38}N_2O_5$ [494.62 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 517.2678

found: 517.2716

$[\alpha]_D^{25}$ = -13.1 (c = 0.5 in chloroform)

mp = 72-73°C

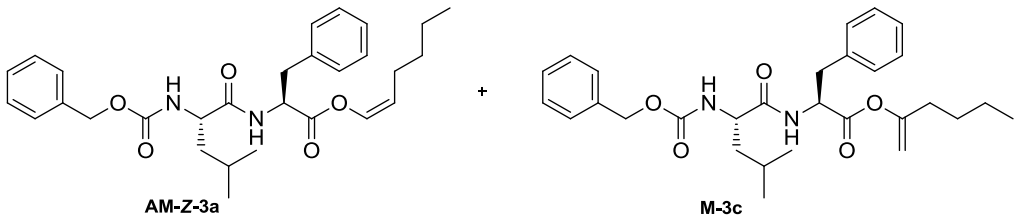
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.90-0.92 (m, 9H, -CH_2-C<u>H</u>$_3$, -CH-(C<u>H</u>$_3$)$_2$), 1.26-1.72 (m, 7H, -NH-CH-C<u>H</u>$_2$-CH-$(CH_3)_2$, -CH_2-C<u>H</u>-$(CH_3)_2$, -CO_2-CH=CH-CH_2-(C<u>H</u>$_2$)$_2$-CH_3), 2.04-2.11 (m, 2H, -CH=CH-C<u>H</u>$_2$-CH_2-), 3.15-3.17 (m, 2H, C_6H_5-C<u>H</u>$_2$-CH-NH), 4.17-4.22 (m, 1H, NH-C<u>H</u>(CH_2-$CH(CH_3)_2$)-CO-NH), 4.92-4.99 (m, 2H, CO-NH-C<u>H</u>(CH_2-Ph)-CO_2-, -CO_2-CH=C<u>H</u>-CH_2-), 5.06-5.15 (m, 3H, HN-CO_2-C<u>H</u>$_2$-C_6H_5, -N<u>H</u>-), 6.51 (d, 1H, ^3J(H,H) = 7.2 Hz, -N<u>H</u>-), 6.97 (d, 1H, ^3J(H,H) = 6.3 Hz, CO_2-C<u>H</u>=CH-CH_2), 7.09-7.36 (m, 10H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 (<u>C</u>H$_3$-$(CH_2)_3$-CH=CH-O_2C), 22.1, 22.3, 23.0, 24.2, 24.8, 31.3 ($C_{aliphatic}$), 38.1 (C_6H_5-<u>C</u>H$_2$-CH-NH-), 41.4 (NH-CH-<u>C</u>H$_2$-CH-$(CH_3)_2$), 53.2, 53.6 (2 × NH-<u>C</u>H), 67.3 (HN-CO_2-<u>C</u>H$_2$-C_6H_5), 115.8 (CO_2-CH=<u>C</u>H-CH_2-), 127.4 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.4

(C_{Ar}), 128.7 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 133.6 (CO_2-CH=CH-CH_2-), 135.5 ($C_{q,Ar}$), 136.3 ($C_{q,Ar}$), 156.2 (NH-CO_2-CH_2-C_6H_5), 168.7 (C_q), 171.9 (C_q).

NOESY: only Z-Leu-Phe-*Z*-anti-Markovnikov-enolester (**AM-*Z*-3a**) detected

6.6.9.2. *Z*-anti-Markovnikov- and Markovnikov-product isomers of AM-*Z*-3a and M-3c



Procedure according to chapter 6.6.8.

Flash column chromatography: (15 g silica gel, 22 × 1.5 cm, cyclohexane/ethyl acetate = 5:1 (v/v), R_f = 0.30 for both isomers)

Yield: 0.54 g (44 %), colourless oil, which became a grey solid after 1 d (mixture of isomers)

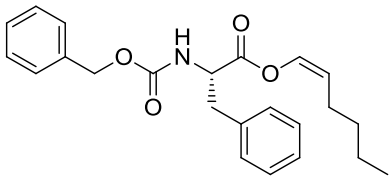
Content of D-Phe-OH: 9.3 % for both isomers GC-MS method AM 2 (chapter 6.4.4)

$C_{29}H_{38}N_2O_5$ [494.62 gmol^{-1}]

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.89-0.91 (m, 9H, -CH_2-CH_3, -CH-(CH_3)$_2$), 1.28-1.69 (m, 7H, -NH-CH-CH_2-CH-(CH_3)$_2$, -CH_2-CH-(CH_3)$_2$, -CO_2-CH=CH-CH_2-(CH_2)$_2$-CH_3), 2.03-2.15 (m, 2H, -CH=CH-CH_2-CH_2-), 3.18-3.21 (m, 2H, C_6H_5-CH_2-CH-NH), 4.17-4.18 (m, 1H, NH-CH(CH_2-CH(CH_3)$_2$)-CO-NH), 4.67 (s, 1H, CO_2-C($C_{alkyl\ chain}$)=CH_2, **M-3a**), 4.72 (s, 1H, CO_2-C($C_{alkyl\ chain}$)=CH_2, **M-3a**), 4.91-4.98 (m, 2H, CO-NH-CH(CH_2-Ph)-CO_2- + -CO_2-CH=CH-CH_2-), 5.05-5.14 (m, 3H, HN-CO_2-CH_2-C_6H_5, CO_2-NH-CH-CH_2-), 6.50 (d, 1H, ^3J(H,H) = 7.0 Hz, -CH-CO-NH-CH-), 6.96 (d, 1H, ^3J(H,H) = 6.3 Hz, CO_2-CH=CH-CH_2), 7.08-7.35 (m, 10H, H_{Ar}).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.9 ($C_{aliphatic}$, **M-3a**), 14.0 (CH_3-(CH_2)$_3$-CH=CH-O_2C), 22.1, 22.3, 23.0, 24.2, 24.8, 31.3 ($C_{aliphatic}$), 22.1, 28.6, 33.0 ($C_{aliphatic}$, **M-3a**), 38.1 (C_6H_5-CH_2-CH-NH-), 41.4 (NH-CH-CH_2-CH-(CH_3)$_2$), 53.2, 53.6 (2 × NH-CH), 67.3 (HN-CO_2-CH_2-C_6H_5), 101.5 (CO_2-C($C_{alkyl\ chain}$)=CH_2, **M-3a**), 115.8 (CO_2-CH=CH-CH_2-), 127.4 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 128.7, 129.6 (C_{Ar}, **M-3a**), 133.6 (CO_2-CH=CH-CH_2-), 135.5 ($C_{q,Ar}$), 136.3 ($C_{q,Ar}$), 156.2 (NH-CO_2-CH_2-C_6H_5), 156.5 (CO_2-C($C_{alkyl\ chain}$)=CH_2, **M-3a**), 168.7 (C_q), 169.7 (C_q , **M-3a**), 171.9 (C_q).

6.6.9.3. Z-Phe-*Z*-anti-Markovnikov-enolester AM-*Z*-3b



Procedure according to chapter 6.6.4.

Z-Phe-OH (**1b**) (100.0 mg, 0.33 mmol, 1.0 eq), Ru-catalyst **III** (8.5 mg, 13.4 µmol, 0.04 eq), dry Chloroform (0.25 mL), 1-hexyne (**2a**) (46 µL, 0.40 mmol, 1.2 eq)

Flash column chromatography: (15 g silica gel, 22 × 1.5 cm, cyclohexane/ethyl acetate = 6:1 (v/v), R_f = 0.40)

Yield: 0.114 g (89 %), yellow oil

Content of D-Phe-OH: <0.1 % HPLC-MS method AM 1 (chapter 6.4.4)

$C_{23}H_{27}NO_4$ [381.46 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 404.1838

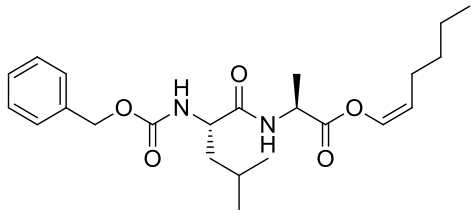
found: 404.1819

$[\alpha]_D^{25}$ $[\alpha]_D^{20}$ = +4.8 (c = 0.5 in chloroform)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, 3H, -CH_2-C<u>H</u>$_3$), 1.25-1.32 (m, 4H, -$CH_{2\ alkyl\ chain}$), 2.03-2.07 (m, 2H, -CH=CH-C<u>H</u>$_2$-CH_2-), 3.16 (d, 2H, ^3J(H,H) = 5.8 Hz, C_6H_5-C<u>H</u>$_2$-CH-NH), 4.73-4.80 (m, 1H, -NH-C<u>H</u>-), 4.94 (pseudo q, ^3J(H,H) = 6.6, 7.5 Hz, CO_2-CH=C<u>H</u>-CH_2-), 5.06-5.15 (m, 2H, HN-CO_2-C<u>H</u>$_2$-C_6H_5), 5.22 (d, 1H, ^3J(H,H) = 8.1 Hz, -N<u>H</u>-CH-), 6.97 (d, 1H, ^3J(H,H) = 6.2 Hz, CO_2-C<u>H</u>=CH-CH_2-), 7.10 (dd, 2H, ^3J(H,H) = 7.5 Hz, ^4J(H,H) = 1.8 Hz, H_{Ar}), 7.24-7.34 (m, 8 H, <u>H</u>$_{Ar}$).

^{13}C-NMR(75 MHz, $CDCl_3$): δ = 14.0 (<u>C</u>H_3-$C_{alkyl\ chain}$), 22.3, 24.2, 31.2 ($C_{aliphatic}$), 38.4 (C_6H_5-<u>C</u>H_2-CH-NH-), 54.8 (NH-<u>C</u>H), 67.2 (HN-CO_2-<u>C</u>H_2-C_6H_5), 115.8 (CO_2-CH=<u>C</u>H-CH_2-), 127.4 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 133.7 (CO_2-<u>C</u>H=CH-CH_2-), 135.5 ($C_{q,Ar}$), 136.3 ($C_{q,Ar}$), 155.8 (NH-<u>C</u>O_2-CH_2-C_6H_5), 169.0 (C_q).

6.6.9.4. Z-Leu-Ala-*Z*-anti-Markovnikov-enolester AM-*Z*-3c



Procedure according to chapter 6.6.5.

Z-Leu-Ala-OH (**1d**) (81.5 mg, 0.24 mmol, 1.0 eq), Ru-catalyst **V** (6.9 mg, 9.7 µmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), 1-hexyne (**1a**) (34 µL, 0.29 mmol, 1.2 eq)

Flash column chromatography: (12 g silica gel, 15 × 1.5 cm, cyclohexane/ethyl acetate = 4:1 (v/v), R_f = 0.26)

Yield: 93.4 mg (92 %), highly viscous grey oil

Content of D-Ala-OH: 0.1 % GC-MS method AM 2 (chapter 6.4.4)

$C_{23}H_{34}N_2O_5$ [418.53 $gmol^{-1}$]

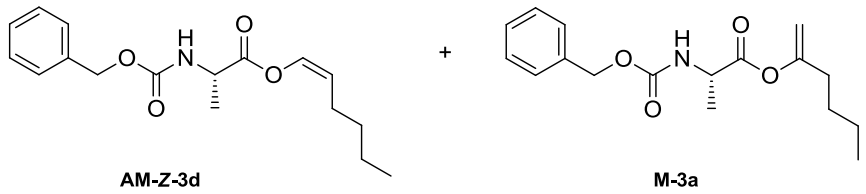
HRMS: $[MH]^+$:	calculated:	419.2546
	found:	419.2543
HRMS: $[MNa]^+$:	calculated:	441.2365
	found:	441.2366

$[\alpha]_D^{25}$ = -17.4 (c = 0.5 in chloroform)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.88-0.95 (m, 9H, -CH_2-C$\underline{H}_3$, -CH-($C\underline{H}_3$)$_2$), 1.28-1.74 (m, 7H, -CH-C$\underline{H}_2$-CH-(CH_3)$_2$, -CH_2-C$\underline{H}$-(CH_3)$_2$, -CH_2 $_{alkyl\ chain}$), 1.44 (d, 3H, ^3J(H,H) = 6.9 Hz, CONH-CH-C$\underline{H}_3$), 2.10-2.17 (m, 2H, -CH=CH-C$\underline{H}_2$-CH_2-), 4.22-4.26 (m, 1H, NH-C$\underline{H}$-CO-NH), 4.65 (pseudo p, 1H, ^3J(H,H) = 6.9, 7.2, 7.5 Hz, CO-NH-C$\underline{H}$-CH_3), 4.95 (pseudo q, 1H, ^3J(H,H) = 6.6, 7.5 Hz, CO_2-CH=C$\underline{H}$-CH_2-), 5.11 (s, 2H, HN-CO_2-C$\underline{H}_2$-C_6H_5), 5.23 (br d, 1H, ^3J(H,H) = 8.1 Hz, CO_2-N$\underline{H}$-CH-CH_2-), 6.57 (br d, 1H, ^3J(H,H) = 6.9 Hz, -CH-CO-N$\underline{H}$-CH-), 6.97 (d, 1H, ^3J(H,H) = 6.3 Hz, CO_2-C$\underline{H}$=CH-CH_2), 7.30-7.34 (m, 5H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 ($\underline{C}H_3$-$C_{alkyl\ chain}$), 18.3 ($\underline{C}H_3$-CH-NH-CO-), 22.1, 22.3, 23.1 24.2, 24.8, 31.3 ($C_{aliphatic}$), 41.7 (NH-CH-$\underline{C}H_2$-CH-(CH_3)$_2$), 48.1 (CH_3-$\underline{C}$H-NH-CO-), 53.5 (NH-$\underline{C}$H), 67.3 (HN-CO_2-$\underline{C}H_2$-C_6H_5), 115.7 (CO_2-CH=$\underline{C}$H-CH_2-), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 133.9 (CO_2-$\underline{C}$H=CH-CH_2-), 136.3 ($C_{q,Ar}$), 156.3 (NH-$\underline{C}O_2$-CH_2-C_6H_5), 170.1 (C_q), 171.9 (C_q).

6.6.9.5. *Z*-anti-Markovnikov- and Markovnikov-product isomers of AM-*Z*-3d and M-3a



AM-*Z*-3d + **M-3a**

Procedure according to chapter 6.6.8.

Flash column chromatography: (10 g silica gel, 14 × 1.5 cm, cyclohexane/ethyl acetate = 7:1 (v/v), R_f = 0.29 and 0.34 for both isomers)

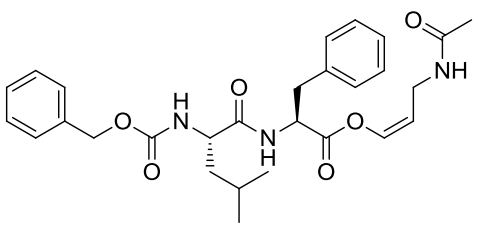
Yield: 0.47 g (62 %), yellow oil, (mixture of isomers)

Content of D-Ala-OH: 10.3 % for both isomers GC-MS method AM 2 (chapter 6.4.4)

$C_{17}H_{23}NO_4$ [305.38 $gmol^{-1}$]
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, 3H, ^3J(H,H) = 7.0 Hz, -CH_2-C$\underline{H}_3$), 1.26-1.49 (m, 7H, -CO_2-CH=CH-CH_2-(C$\underline{H}_2$)$_2$-CH_3, NH-CH-C$\underline{H}_3$), 2.10 -2.23 (m, 2H, -CH=CH-C$\underline{H}_2$-CH_2), 4.41-4.51 (m, 1H, NH-C$\underline{H}$-CH_3), 4.75 (s, 2H, CO_2-C($C_{alkylchain}$)=C$\underline{H}_2$, **M-3a**), 4.92-4.99 (pseudo q, 1H, ^3J(H,H) = 6.6 Hz, 7.5 Hz, -CO_2-CH=C$\underline{H}$-CH_2-), 5.12 (s, 2H, -C_6H_5, CO_2-N$\underline{H}$-CH-CH_2-), 5.33 (br. d, 1H, ^3J(H,H) = 6.5 Hz, N$\underline{H}$-CH-CH_3), 6.98 (d, 1H, ^3J(H,H) = 6.2 Hz, CO_2-C$\underline{H}$=CH-CH_2), 7.35 (s, 5H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.9, 14.0 (C$\underline{H}_3$-$(CH_2)_3$-CH=CH-O_2C + [C$\underline{H}_3$-$(CH_2)_3$-C(=CH_2)-O_2C **M-3a**]), 18.7, 18.8 (NH-CH-C$\underline{H}_3$ + [NH-CH-C$\underline{H}_3$ **M-3a**]), 22.3, 24.2, 31.3 ($C_{aliphatic}$), 22.1, 28.6, 32.9 ([$C_{aliphatic}$ **M-3a**]), 49.7 (NH-C$\underline{H}$-CH_3), 49.9 ([NH-C$\underline{H}$-CH_3 **M-3a**]), 67.1 (HN-CO_2-C$\underline{H}_2$-C_6H_5), 101.5 ([CO_2-C($C_{alkylchain}$)=C$\underline{H}_2$ **M-3a**]), 115.7 (CO_2-CH=C$\underline{H}$-CH_2-), 128.2 (2 × C_{Ar}), 128.3 (C_{Ar}), 128.7 (2 × C_{Ar}), 134.0 (CO_2-C$\underline{H}$=CH-CH_2-), 136.4 ($C_{q,Ar}$), 155.7 (C_q), 156.4 ([CO_2-C$\underline{(C_{alkylchain})}$=$CH_2$ **M-3a**]), 170.4 (C_q), 171.4 ([C_q **M-3a**])

6.6.9.6. Z-Leu-Phe-*Z*-anti-Markovnikov-enolester AM-*Z*-3f



Procedure according to chapter 6.6.6.

Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), dry 2-propanol (1.0 mL), *N*-(prop-2-yn-1-yl)acetamide (**2c**) (29.1 mg, 0.3 mmol, 1.2 eq)

Flash column chromatography: (16 g silica gel, 15 × 2 cm, cyclohexane/ethyl acetate = 1:5 (v/v), R_f = 0.26)

Yield: 92 mg (75 %), grey oil, which became a grey solid after 1d

Content of D-Phe-OH: 0.1 % HPLC-MS method AM 1 (chapter 6.4.4)

$C_{28}H_{35}N_3O_6$ [509.59 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 532.2424

found: 532.2449

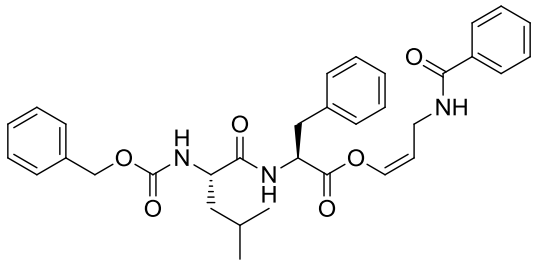
$[\alpha]_D^{25}$ = -19.5 (c = 0.5 in chloroform)

mp = 149°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.90-0.92 (m, 6H, -CH-($C\underline{H}_3$)$_2$), 1.43-1.52 (m, 1H, -CH_2-C$\underline{H}$-(CH_3)$_2$), 1.57-1.66 (m, 2H, -NH-CH-C$\underline{H}_2$-CH-(CH_3)$_2$), 1.94 (s, 3H, C$\underline{H}_3$-CO-NH-CH_2-CH=CH-CO_2-), 3.13 (d, 2H, ^3J(H,H) = 6.0 Hz, C_6H_5-C$\underline{H}_2$-CH-NH), 3.71-3.81 (m, 2H, CH_3-CO-NH-C$\underline{H}_2$-CH=CH-CO_2-), 4.19-4.21 (m, 1H, NH-C$\underline{H}$(CH_2-CH(CH_3)$_2$)-CO-NH), 4.87 (pseudo q, 1H, ^3J(H,H) = 6.6 Hz, 7.2 Hz, CO-NH-C$\underline{H}$(CH_2-Ph)-CO_2-), 5.03-5.08 (m, 3H, -CO_2-CH=C$\underline{H}$-CH_2 + HN-CO_2-C$\underline{H}_2$-C_6H_5), 5.37 (d, 1H, ^3J(H,H) = 8.1 Hz, -N$\underline{H}$-), 5.92 (pseudo s, 1H, -N$\underline{H}$-), 6.86 (d, 1H, ^3J(H,H) = 7.6 Hz, -N$\underline{H}$-), 7.04 (d, 1H, ^3J(H,H) = 6.0 Hz, CO_2-C$\underline{H}$=CH-CH_2), 7.13 (d, 2H, ^3J(H,H) = 6.6 Hz, $\underline{H}_{Ar}$), 7.23-7.33 (m, 8H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.0, 23.0, 23.2, 24.8 ($C_{aliphatic}$), 33.8 (CH_3-CO-NH-C$\underline{H}_2$-CH=CH-CO_2-), 37.9 (C_6H_5-C$\underline{H}_2$-CH-NH-), 41.1 (NH-CH-C$\underline{H}_2$-CH-(CH_3)$_2$), 53.6, 53.8 (2 × NH-C$\underline{H}$), 67.3 (HN-CO_2-C$\underline{H}_2$-C_6H_5), 111.2 (CO_2-CH=C$\underline{H}$-CH_2-), 127.4 (C_{Ar}), 128.1 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 135.6 (CO_2-C$\underline{H}$=CH-CH_2-), 136.1 ($C_{q,Ar}$), 136.2 ($C_{q,Ar}$), 156.4 (NH-C$\underline{O}_2$-CH_2-C_6H_5), 168.0 (C_q), 171.4 (C_q), 172.3 (C_q).

6.6.9.7. Z-Leu-Phe-*Z*-anti-Markovnikov-enolester AM-*Z*-3g



Procedure according to chapter 6.6.6.

Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst (**V**) (7.1 mg, 0.01 mmol, 0.04 eq), dry 2-propanol (1.0 mL), *N*-(prop-2-yn-1-yl)benzamide (**2d**) (47.8 mg, 0.3 mmol, 1.2 eq)

Flash column chromatography: (16 g silica gel, 15 × 2 cm, cyclohexane/ethyl acetate = 2:1 (v/v), R_f = 0.11)

Yield: 60.1 mg (42 %), grey oil, which became a green-grey solid after 1 d

Content of D-Phe-OH: 0.2 % HPLC-MS method AM 1 (chapter 6.4.4)

$C_{33}H_{37}N_3O_6$ [571.66 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 594.2580

found: 594.2621

$[\alpha]_D^{25}$ = -30.0 (c = 0.5 in chloroform)

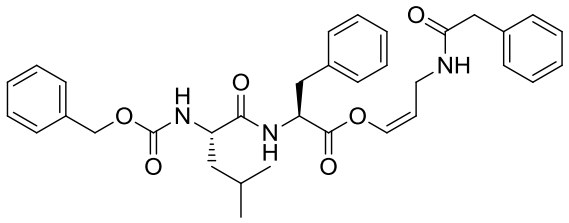
mp = 148°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.88-0.91 (m, 6H, -CH-(C<u>H</u>$_3$)$_2$), 1.43-1.52 (m, 1H, -CH$_2$-C<u>H</u>-(CH$_3$)$_2$), 1.62-1.66 (m, 2H, -NH-CH-C<u>H</u>$_2$-CH-(CH$_3$)$_2$), 3.13 (d, 2H, ^3J(H,H) = 5.7 Hz, C$_6$H$_5$-C<u>H</u>$_2$-CH-NH), 3.83-4.13 (m, 2H, C$_6$H$_5$-CO-NH-C<u>H</u>$_2$-CH=CH-CO$_2$-), 4.17-4.21 (m, 1H, NH-C<u>H</u>(CH$_2$-CH(CH$_3$)$_2$)-CO-NH), 4.88 (pseudo q, 1H, ^3J(H,H) = 6.9 Hz, 7.5 Hz, CO-NH-C<u>H</u>(CH$_2$-Ph)-CO$_2$-), 5.07 (s, 2H, HN-CO$_2$-C<u>H</u>$_2$-C$_6$H$_5$), 5.18 (pseudo q, 1H, ^3J(H,H) = 6.6 Hz, 6.9 Hz, -CO$_2$-CH=C<u>H</u>-CH$_2$), 5.29 (d, 1H, ^3J(H,H) = 7.2 Hz, -N<u>H</u>-), 6.48 (t, 1H, ^3J(H,H) = 4.9 Hz, -N<u>H</u>-), 6.83 (d, 1H, ^3J(H,H) = 7.7 Hz, -N<u>H</u>-), 7.08-7.16 (m, 3H, CO$_2$-C<u>H</u>=CH-CH$_2$ + <u>H</u>$_{Ar}$), 7.19-7.52 (m, 11H, <u>H</u>$_{Ar}$), 7.75-7.77 (m, 2H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 21.9, 23.0, 24.8 (C$_{aliphatic}$), 34.2 (C$_6$H$_5$-CO-NH-<u>C</u>H$_2$-CH=CH-CO$_2$-), 38.0 (C$_6$H$_5$-<u>C</u>H$_2$-CH-NH-), 41.1 (NH-CH-<u>C</u>H$_2$-CH-(CH$_3$)$_2$), 53.5, 53.8 (2 × NH-<u>C</u>H), 67.3 (HN-CO$_2$-<u>C</u>H$_2$-C$_6$H$_5$), 111.3 (CO$_2$-CH=<u>C</u>H-CH$_2$-), 127.1 (2 × C$_{Ar}$), 127.4 (C$_{Ar}$), 128.1 (2 × C$_{Ar}$), 128.4 (C$_{Ar}$), 128.7 (4 × C$_{Ar}$), 128.8 (2 × C$_{Ar}$), 129.4 (2 × C$_{Ar}$), 131.7 (C$_{Ar}$), 134.4 (C$_{q,Ar}$), 135.6

($C_{q,Ar}$), 136.1 (CO_2-<u>C</u>H=CH-CH_2-), 136.2 ($C_{q,Ar}$), 156.4 (NH-<u>C</u>O_2-CH_2-C_6H_5), 167.5 (C_q), 168.1 (C_q), 172.3 (C_q).

6.6.9.8. Z-Leu-Phe-*Z*-anti-Markovnikov-enolester AM-*Z*-3h



Procedure according to chapter 6.6.6.

Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst (**V**) (7.1 mg, 0.01 mmol, 0.04 eq), dry 2-propanol (1.0 mL), 2-phenyl-*N*-(prop-2-yn-1-yl)acetamide (**2e**) (52.0 mg, 0.3 mmol, 1.2 eq)

Flash column chromatography: (16 g silica gel, 15 × 2 cm, cyclohexane/ethyl acetate = 1:1 (v/v), R_f = 0.26)

Yield: 64.4 mg (44 %), grey oil, which became a grey solid after 1 d

Content of D-Phe-OH: <0.1 % HPLC-MS method AM 1 (chapter 6.4.4)

$C_{34}H_{39}N_3O_6$ [585.69 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 608.2737

found: 608.2726

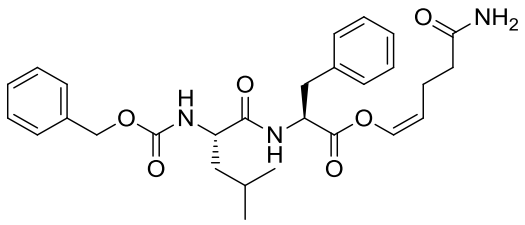
$[\alpha]_D^{25}$ = -17.3 (c = 0.5 in chloroform)

mp = 134°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.91 (d, 6H, ^3J(H,H) = 6.1 Hz, -CH-(C<u>H</u>$_3$)$_2$), 1.43-1.52 (m, 1H, -CH_2-C<u>H</u>-$(CH_3)_2$), 1.57-1.66 (m, 2H, -NH-CH-C<u>H</u>$_2$-CH-$(CH_3)_2$), 3.09 (d, 2H, ^3J(H,H) = 6.3 Hz, C_6H_5-C<u>H</u>$_2$-CH-NH), 3.53 (s, 2H, C_6H_5-C<u>H</u>$_2$-CO-NH-CH_2-CH=CH-CO_2-), 3.62-3.83 (m, 2H, C_6H_5-CH_2-CO-NH-C<u>H</u>$_2$-CH=CH-CO_2-), 4.18-4.20 (m, 1H, NH-C<u>H</u>$(CH_2$-$CH(CH_3)_2)$-CO-NH), 4.83 (pseudo q, 1H, ^3J(H,H) = 6.9 Hz, 7.5 Hz, CO-NH-C<u>H</u>$(CH_2$-Ph)-CO_2-), 4.97 (pseudo q, 1H, ^3J(H,H) = 6.3 Hz, 7.2 Hz, -CO_2-CH=C<u>H</u>-CH_2), 5.07 (s, 2H, HN-CO_2-C<u>H</u>$_2$-C_6H_5), 5.32 (d, 1H, ^3J(H,H) = 8.1 Hz, -N<u>H</u>-), 5.59 (t, 1H, ^3J(H,H) = 4.9 Hz, -N<u>H</u>-), 6.80 (d, 1H, ^3J(H,H) = 7.7 Hz, -N<u>H</u>-), 7.01 (d, 1H, ^3J(H,H) = 6.2 Hz, CO_2-C<u>H</u>=CH-CH_2), 7.08 (d, 2H, ^3J(H,H) = 6.9 Hz, <u>H</u>$_{Ar}$), 7.16-7.38 (m, 13H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.0, 23.0, 24.8 ($C_{aliphatic}$), 33.8 (C_6H_5-CH_2-CO-NH-$\underline{C}H_2$-CH=CH-CO_2-), 38.1 (C_6H_5-$\underline{C}H_2$-CH-NH-), 41.2 (NH-CH-$\underline{C}H_2$-CH-$(CH_3)_2$), 43.8 (C_6H_5-$\underline{C}H_2$-CO-NH-CH_2-CH=CH-CO_2-), 53.5, 53.7 (2 × NH-$\underline{C}$H), 67.3 (HN-CO_2-$\underline{C}H_2$-C_6H_5), 111.1 (CO_2-CH=$\underline{C}$H-CH_2-), 127.3 (C_{Ar}), 127.6 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.2 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 129.6 (2 × C_{Ar}), 134.9 ($C_{q,Ar}$), 135.6 ($C_{q,Ar}$), 135.8 (CO_2-$\underline{C}$H=CH-CH_2-), 136.3 ($C_{q,Ar}$), 156.3 (NH-$\underline{C}O_2$-CH_2-C_6H_5), 168.0 (C_q), 171.0 (C_q), 172.2 (C_q).

6.6.9.9. Z-Leu-Phe-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3j



Procedure according to chapter 6.6.6.

Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst (**V**) (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), pent-4-ynamide (**2g**) (29.1 mg, 0.3 mmol, 1.2 eq)

Flash column chromatography: (15 g silica gel, 22 × 1.5 cm, cyclohexane/ethyl acetate = 1:5 (v/v), R_f = 0.12)

Yield: 103.8 mg (82 %), grey gum-like solid

Content of D-Phe-OH: <0.1 % HPLC-MS method AM 1 (chapter 6.4.4)

$C_{29}H_{37}N_3O_6$ [509.61 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 532.2424

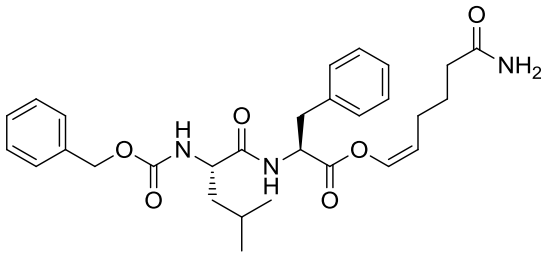
found: 532.2463

$[\alpha]_D^{25}$ = +11.2 (c = 0.5 in chloroform)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.92-0.94 (m, 6H, -CH(-C$\underline{H}_3$)$_2$), 1.45-1.65 (m, 3H, HN-CH-C$\underline{H}_2$-CH(-CH_3)$_2$, -CH_2-C$\underline{H}$(-CH_3)$_2$), 2.15-2.39 (m, 4H, H_2N-CO-C$\underline{H}_2$-CH_2-, H_2N-CO-CH_2-C$\underline{H}_2$-), 3.15 (d, 2H, ^3J(H,H) = 5.8 Hz, C_6H_5-C$\underline{H}_2$-CH-NH), 4.24- 4.29 (m, 1H, NH-C$\underline{H}$-CO-NH), 4.88-4.99 (m, 2H, CO-NH-C$\underline{H}$-CO_2-, CO_2-CH=C$\underline{H}$-CH_2-), 5.09 (s, 2H, HN-CO_2-C$\underline{H}_2$-C_6H_5), 5.40-5.68 (m, 3H, -CO_2-N$\underline{H}$-CH-, -CH_2-CO-N$\underline{H}_2$), 6.99-7.01 (m, 2H, CO_2-C$\underline{H}$=CH-CH_2- + -CH-CO-N$\underline{H}$-CH-CO_2-), 7.12-7.34 (m, 10H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.7 (CO_2-CH=<u>C</u>H-CH_2-), 22.0, 23.0 (2 × C, CH(-<u>C</u>H_3)$_2$), 24.8 (-CH_2-<u>C</u>H(-CH_3)$_2$), 35.2, 37.9 (2 × C, C_6H_5-<u>C</u>H_2-CH-NH-, NH_2-CO-C<u>H</u>$_2$-CH_2-), 41.4 (NH-CH-<u>C</u>H_2-CH-(CH_3)$_2$), 53.6, 53.9 (2 × NH-<u>C</u>H), 67.2 (HN-CO_2-<u>C</u>H_2-C_6H_5), 113.3 (CO_2-CH=<u>C</u>H-CH_2-), 127.2 (2 × C_{Ar}), 128.2 (C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.7 (2 × C_{Ar}), 129.7 (2 × C_{Ar}), 135.1 (CO_2-<u>C</u>H=CH-CH_2-), 135.8 ($C_{q,Ar}$), 136.3 ($C_{q,Ar}$), 156.4 (NH-<u>C</u>O_2-CH_2-C_6H_5), 168.1 (C_q), 172.3 (C_q), 174.9 (C_q).

6.6.9.10. Z-Leu-Phe-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3k



Procedure according to chapter 6.6.6.

Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), hex-5-ynamide (**2b**) (33.3 mg, 0.3 mmol, 1.2 eq)

Flash column chromatography: (16 g silica gel, 22 × 1.5 cm, cyclohexane/ethyl acetate = 1:5 (v/v), R_f = 0.31)

Yield: 0.110 g (0.210 mmol, 84 %), off-white gum-like solid

Content of D-Phe-OH: <0.1 % HPLC-MS method AM 1 (chapter 6.4.4)

$C_{29}H_{37}N_3O_6$ [523.62 gmol^{-1}]

HRMS: $[MH]^+$: calculated: 524.2761

found: 524.2740

HRMS: $[MNa]^+$: calculated: 546.2580

found: 546.2576

$[\alpha]_D^{25}$ = -1.5 (c = 0.5 in chloroform)

$[\alpha]_D^{25}$ = -35.2 (c = 0.5 in methanol)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.82-0.84 (m, 6H, -CH(-C<u>H</u>$_3$)$_2$), 1.36-1.70 (m, 5H, H_2N-CO-CH_2-C<u>H</u>$_2$- CH_2-CH=CH-O_2C-, HN-CH-C<u>H</u>$_2$-CH(-CH_3)$_2$, -CH_2-C<u>H</u>(-CH_3)$_2$), 1.87-1.96 (m, 2H,

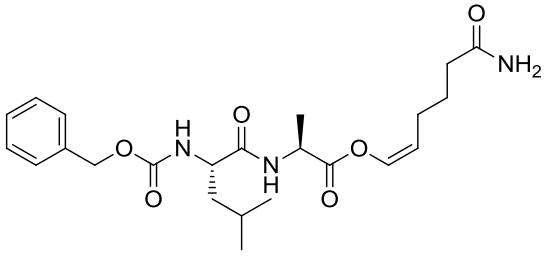
H_2N-CO-C$\underline{H}_2$-CH_2-), 2.01-2.12 (m, 2H, CO_2-HC=CH-C$\underline{H}_2$-CH_2-), 3.00-3.13 (m, 2H, C_6H_5-C$\underline{H}_2$-CH-NH), 4.17-4.27 (m, 1H, NH-C$\underline{H}$(CH_2-CH(CH_3)$_2$)-CO-NH), 4.79-4.91 (m, 2H, CO-NH-C$\underline{H}$(CH_2-Ph)-CO_2-, CO_2-CH=C$\underline{H}$-CH_2-), 5.02 (s, 2H, HN-CO_2-C$\underline{H}_2$-C_6H_5), 5.33 (d, 1H, ^3J(H,H) = 7.9 Hz, -CH-CO-N$\underline{H}$-CH-), 5.52 (br. s, 1H, -CH_2-CO-N$\underline{H}_2$), 5.71 (br. s, 1H, -CH_2-CO-N$\underline{H}_2$), 6.94 (d, 1H, ^3J(H,H) = 6.1 Hz, CO_2-C$\underline{H}$=CH-CH_2-), 7.04-7.27 (m, 11H, $\underline{H}_{Ar}$ + -CH-CO-N$\underline{H}$-CH(CH_2-Ph)-CO_2-).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.1, 23.0 (2 × C, CH(-C$\underline{H}_3$)$_2$), 23.7, 24.4 (CO_2-CH=CH-C$\underline{H}_2$-CH_2, CO_2-CH=CH-CH_2-C$\underline{H}_2$), 24.8 (-CH_2-C$\underline{H}$(-CH_3)$_2$), 34.4, 38.1 (C_6H_5-C$\underline{H}_2$-CH-NH-, NH_2-CO-C$\underline{H}_2$-CH_2-), 41.5 (NH-CH-C$\underline{H}_2$-CH-(CH_3)$_2$), 53.5, 53.7 (2 × NH-C$\underline{H}$), 67.2 (HN-CO_2-C$\underline{H}_2$-C_6H_5), 114.4 (CO_2-CH=C$\underline{H}$-CH_2-), 127.2 (C_{Ar}), 128.1 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.7 (2 × C_{Ar}), 129.5 (2 × C_{Ar}), 134.8 (CO_2-C$\underline{H}$=CH-CH_2-), 135.8 ($C_{q,Ar}$), 136.3 ($C_{q,Ar}$), 156.3 (NH-C$\underline{O}_2$-CH_2-C_6H_5), 168.2 (C_q), 172.3 (C_q), 175.4 (C_q).

NOESY: only *Z*-Leu-Phe-*Z*-anti-Markovnikov-enolester (**AM-*Z*-3k**) detected

TOCSY: Position of CO_2-HC=CH-C$\underline{H}_2$-CH_2- assigned by TOCSY-NMR

6.6.9.11. Z-Leu-Ala-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3l



Procedure according to chapter 6.6.6.

Z-Leu-Ala-OH (**1d**) (252.3 mg, 0.75 mmol, 1.0 eq), Ru-catalyst **V** (21.3 mg, 0.03 mmol, 0.04 eq) and degassed dry 2-propanol (1.5 mL), hex-5-ynamide (**2b**) (100.0 mg, 0.90 mmol, 1.2 eq)

Flash column chromatography: (43 g silica gel, 40 × 1.8 cm, cyclohexane/ethyl acetate = 1:7 (v/v), R_f = 0.25)

Yield: 297.0 mg (89 %), colourless oil, which became a grey solid after 1 d

Content of D-Ala-OH: 0.2 % GC-MS method AM 1 (chapter 6.4.4)

$C_{23}H_{33}N_3O_6$ [447.52 gmol^{-1}]

HRMS: $[MNa]^+$: calculated: 470.2267

found: 470.2254

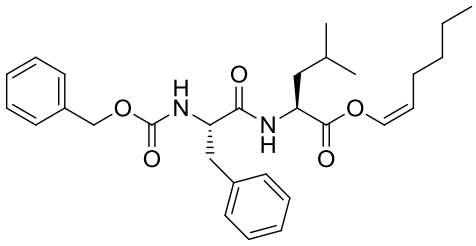
$[\alpha]_D^{25}$ = -13.4 (c = 0.5 in chloroform)

mp = 128-139°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.92 (d, 6H, ^3J(H,H) = 6.2 Hz, -CH_2-CH-(C$\underline{H}_3$)$_2$), 1.43 (d, 3H, ^3J(H,H) = 7.1 Hz, NH-CH-C$\underline{H}_3$), 1.48-2.47 (m, 9H, -CH_2-C$\underline{H}$-(CH_3)$_2$, -C$\underline{H}_2$-CH-(CH_3)$_2$, HC≡C-C$\underline{H}_2$-CH_2-, -CH_2-C$\underline{H}_2$-CH_2-CO-NH_2, -CH_2-CH_2-C$\underline{H}_2$-CO-NH_2), 4.20-4.40 (m, 1H, -CO_2-NH-C$\underline{H}$-CO), 4.60 (pseudo p, 1H, ^3J(H,H) = 7.4 Hz, NH-C$\underline{H}$-CH_3), 4.96 (pseudo q, 1H, ^3J(H,H) = 6.9, 7.5 Hz, CO_2-CH=C$\underline{H}$-CH_2), 5.09 (s, 2H, HN-CO_2-C$\underline{H}_2$-C_6H_5), 5.50 (d, 1H, ^3J(H,H) = 8.3 Hz, -CO_2-N$\underline{H}$-CH-CO), 5.83 (br s, 1H, -CH_2-CO-N$\underline{H}_2$), 5.90 (br s, 1H, -CH_2-CO-N$\underline{H}_2$), 7.02 (d, 1H, ^3J(H,H) = 6.2 Hz, CO_2-C$\underline{H}$=CH-CH_2), 7.17 (d, 1H, ^3J(H,H) = 6.8 Hz, N$\underline{H}$-CH-CH_3), 7.33 (s, 5H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0 (NH-CH-C$\underline{H}_3$), 22.1, 23.1 (-CH-(C$\underline{H}_3$)$_2$), 23.8, 24.5 (2 × C, CO_2-CH=CH-C$\underline{H}_2$-CH_2-, CO_2-CH=CH-CH_2-C$\underline{H}_2$-), 24.8 (CH_2-C$\underline{H}$-(CH_3)$_2$), 34.5 (-CH_2-C$\underline{H}_2$-CO-NH_2), 41.7 (C$\underline{H}_2$-CH-(CH_3)$_2$), 48.4 (NH-C$\underline{H}$), 53.5 (NH-C$\underline{H}$), 67.2 (HN-CO_2-C$\underline{H}_2$-C_6H_5), 114.4 (CO_2-CH=C$\underline{H}$-CH_2), 128.1 (2 × C_{Ar}), 128.3 (C_{Ar}), 128.7 (2 × C_{Ar}), 135.1 (CO_2-C$\underline{H}$=CH-CH_2), 136.3 ($C_{q,Ar}$), 156.4 (HN-C$\underline{O}_2$-CH_2-C_6H_5), 169.8 (C_q), 172.4 (C_q), 175.6 (C_q).

6.6.9.12. Z-Phe-Leu-*Z*-anti-Markovnikov-enolester AM-*Z*-3m



Procedure according to chapter 6.6.5.

Z-Phe-Leu-OH (**1e**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), 1-hexyne (**2a**) (34.5 µL, 0.3 mmol, 1.2 eq)

Flash column chromatography: (16 g silica gel, 15 × 2 cm, cyclohexane/ethyl acetate = 6:1 (v/v), R_f = 0.20)

Yield: 115.0 mg (93 %), highly viscous grey oil

Content of D-Leu-OH: 0.3 % GC-MS method AM 2 (chapter 6.4.4)

$C_{29}H_{37}N_3O_6$ [494.62 gmol^{-1}]

HRMS: $[MNa]^+$: calculated: 517.2678

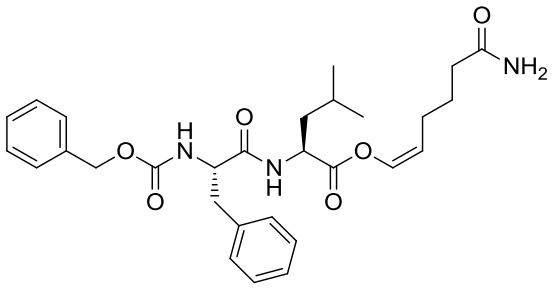
found: 517.2681

$[\alpha]_D^{25}$ = -1.5 (c = 0.5 in chloroform)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.90-0.91 (m, 9H, -CH_2-C$\underline{H}_3$, -CH-($C\underline{H}_3$)$_2$), 1.25-1.64 (m, 7H; found: 9H, H-N-CH-C$\underline{H}_2$-CH-(CH_3)$_2$, -CH_2-C$\underline{H}$-(CH_3)$_2$, -CO_2-CH=CH-CH_2-(C$\underline{H}_2$)$_2$-CH_3), 2.13 (pseudo q, 2H, ^3J(H,H) = 7.0 Hz, -CH=CH-C$\underline{H}_2$-CH_2-), 3.02-3.16 (m, 2H, C_6H_5-C$\underline{H}_2$-CH-NH), 4.42-4.46 (m, 1H, NH-C$\underline{H}$(CH_2-CH(CH_3)$_2$)-CO-NH), 4.60-4.67 (m, 1H, CO-NH-C$\underline{H}$(CH_2-Ph)-CO_2-), 4.95 (pseudo q, ^3J(H,H) = 7.4, 6.6 Hz, CO_2-CH=C$\underline{H}$-CH_2-), 5.09 (s, 2H, HN-CO_2-C$\underline{H}_2$-C_6H_5), 5.31 (d, 1H, ^3J(H,H) = 6.5 Hz, CO_2-N$\underline{H}$-CH-CH_2-), 6.22 (d, 1H, ^3J(H,H) = 7.4 Hz, -CH-CO-N$\underline{H}$-CH-), 6.95 (d, 1H, ^3J(H,H) = 6.3 Hz, CO_2-C$\underline{H}$=CH-CH_2), 7.17-7.34 (m, 10 H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 ($\underline{C}H_3$-(CH_2)$_3$-CH=CH-O_2C), 22.1, 22.2, 22.6, 24.1, 24.8, 31.2 ($C_{aliphatic}$), 38.3 (C_6H_5-$\underline{C}H_2$-CH-NH-), 41.5 (NH-CH-$\underline{C}H_2$-CH-(CH_3)$_2$), 50.8, 56.1 (2 × NH-$\underline{C}$H), 67.2 (HN-CO_2-$\underline{C}H_2$-C_6H_5), 115.5 (CO_2-CH=$\underline{C}$H-CH_2-), 127.2 (C_{Ar}), 128.1 (2 × C_{Ar}), 128.3 (C_{Ar}), 128.6 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 133.7 (CO_2-$\underline{C}$H=CH-CH_2-), 136.1 ($C_{q,Ar}$), 136.2 ($C_{q,Ar}$), 155.9 (NH-$\underline{C}O_2$-CH_2-C_6H_5), 169.7 (C_q), 170.6 (C_q).

6.6.9.13. Z-Phe-Leu-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3n



Procedure according to chapter 6.6.6.

Z-Phe-Leu-OH (**1e**) (206.3 mg, 0.5 mmol, 1.0 eq), Ru-catalyst **V** (14.2 mg, 0.02 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), hex-5-ynamide (**2b**) (66.7 mg, 0.6 mmol, 1.2 eq)

Flash column chromatography: (30 g silica gel, 25 × 2.5 cm, cyclohexane/ethyl acetate = 1:3 (v/v), R_f = 0.22)

Yield: 240.5 mg (92 %), highly viscous grey oil

Content of D-Leu-OH: 0.1 % GC-MS method AM 2 (chapter 6.4.4)

$C_{29}H_{37}N_3O_6$ [523.62 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 546.2580

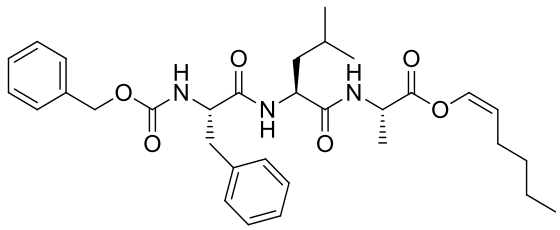
found: 546.2588

$[\alpha]_D^{25}$ = -5.3 (c = 0.5 in chloroform)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.91 (d, 6H, ^3J(H,H) = 5.6 Hz, -CH(-C<u>H</u>$_3$)$_2$), 1.51-1.84 (m, 5H, H_2N-CO-CH_2-C<u>H</u>$_2$-, H-N-CH-C<u>H</u>$_2$-CH(-CH_3)$_2$, -CH_2-C<u>H</u>(-CH_3)$_2$), 2.04-2.26 (m, 4H, H_2N-CO-C<u>H</u>$_2$-CH_2- and HC≡C-C<u>H</u>$_2$-CH_2-), 3.07 (d, 2H, ^3J(H,H) = 6.5 Hz, C_6H_5-C<u>H</u>$_2$-CH-NH), 4.56-4.65 (m, 2H, NH-C<u>H</u>-CO-NH + CO-NH-C<u>H</u>-CO_2), 4.96 (pseudo q, 1H,-^3J(H,H) = 6.6, 7.5 Hz, CO_2-CH=C<u>H</u>-CH_2-), 5.07 (s, 2H, NH-CO_2-C<u>H</u>$_2$-C_6H_5), 5.50 (d, 1H, ^3J(H,H) = 8.1 Hz, -CH-CO-N<u>H</u>-CH- or -CO_2-N<u>H</u>-CH-), 5.75 and 5.80 (br. s, 2H, -CH_2-CO-N<u>H</u>$_2$), 6.89 (d, 1H, ^3J(H,H) = 7.2 Hz, -CH-CO-N<u>H</u>-CH- or -CO_2-N<u>H</u>-CH-), 7.01 (d, 1H, ^3J(H,H) = 6.0 Hz, CO_2-C<u>H</u>=CH-CH_2-), 7.16-7.35 (m, 10H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.1, 22.8 (2 × C, CH(-<u>C</u>H_3)$_2$), 23.9, 24.5 (2 × C, CO_2-CH=CH-<u>C</u>H_2-CH_2, CO_2-CH=CH-CH_2-<u>C</u>H_2), 24.9 (-CH_2-<u>C</u>H(-CH_3)$_2$), 34.5, 38.6 (2 × C, C_6H_5-<u>C</u>H_2-CH-NH-, NH_2-CO-<u>C</u>H_2-CH_2-), 41.4 (NH-CH-<u>C</u>H_2-CH-(CH_3)$_2$), 51.1, 56.1 (2 × NH-<u>C</u>H), 67.2 (HN-CO_2-<u>C</u>H_2-C_6H_5), 114.2 (CO_2-CH=<u>C</u>H-CH_2-), 127.2 (C_{Ar}), 128.1 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.5 (2 × C_{Ar}), 135.0 (CO_2-<u>C</u>H=CH-CH_2-), 136.2 ($C_{q,Ar}$), 136.4 ($C_{q,Ar}$), 156.2 (NH-<u>C</u>O_2-CH_2-C_6H_5), 169.2 (C_q), 171.1 (C_q), 175.5 (C_q).

6.6.9.14. Z-Phe-Leu-Ala-*Z*-anti-Markovnikov-enolester AM-*Z*-3o



Procedure according to chapter 6.6.5.

Z-Phe-Leu-Ala-OH (**1f**) (48.4 mg, 0.1 mmol, 1.0 eq), Ru-catalyst **V** (2.8 mg, 4.0 µmol, 0.04 eq), degassed dry 2-propanol (0.2 mL), 1-hexyne (**2a**) (23 µL, 0.2 mmol, 1.2 eq)

Flash column chromatography: (10 g silica gel, 14 × 1.5 cm, cyclohexane/ethyl acetate = 3:1 (v/v), R_f = 0.13)

Yield: 49.0 mg (87 %), pale yellow oil, which became a yellow gum-like solid after 1 d

Content of D-Ala-OH: 0.1 % GC-MS method AM 2 (chapter 6.4.4)

$C_{32}H_{43}N_3O_6$ [565.70 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 588.3049

found: 588.3037

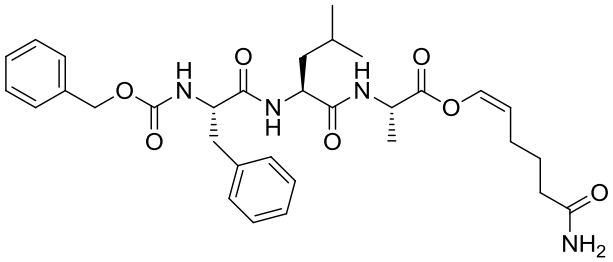
$[\alpha]_D^{25}$ = -23.5 (c = 0.5 in chloroform)

mp = 98°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.87-0.93 (m, 9H, $-CH_2-C\underline{H}_3$, $-CH(-C\underline{H}_3)_2$), 1.29-1.71 (m, 10H, H-N-CH-$C\underline{H}_2$-CH-$(CH_3)_2$, $-CH_2-C\underline{H}-(CH_3)_2$, $-CH_{2\ alkyl\ chain}$, CONH-CH-$\underline{C}H_3$), 2.11-2.18 (m, 2H, HC=CH-$C\underline{H}_2-CH_2$-), 3.08 (d, 2H, ^3J(H,H) = 6.6 Hz, $C_6H_5-C\underline{H}_2$-CH-NH), 4.41-4.48 (m, 2H, NH-C$\underline{H}$), 4.61 (p, 1H, ^3J(H,H) = 7.2 Hz, NH-C$\underline{H}$-CH_3), 4.95 (pseudo q, 1H, ^3J(H,H) = 6.3, 7.5 Hz, CO_2-CH=C$\underline{H}$-CH_2-), 5.07 (s, 2H, HN-$CO_2-C\underline{H}_2-C_6H_5$), 5.33 (d, 1H, ^3J(H,H) = 6.3 Hz, -CO-N$\underline{H}$-), 6.41 (d, 1H, ^3J(H,H) = 6.5 Hz, -CO-N$\underline{H}$-), 6.71 (d, 1H, ^3J(H,H) = 5.4 Hz, -CO-N$\underline{H}$-), 6.98 (d, 1H, ^3J(H,H) = 6.3 Hz, $CO_2-C\underline{H}$=CH-CH_2-), 7.15-7.37 (m, 10H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 ($\underline{C}H_3-C_{alkyl\ chain}$), 18.2 ($\underline{C}H_3$-CH-NH-CO-), 22.1, 22.3, 23.0 24.2, 24.7, 31.3 ($C_{aliphatic}$), 38.2 ($C_6H_5-\underline{C}H_2$-CH-NH-), 41.0 (NH-CH-$\underline{C}H_2$-CH-$(CH_3)_2$), 48.1, 51.8, 56.3 (3 × NH-$\underline{C}$H), 67.4 (HN-$CO_2-\underline{C}H_2-C_6H_5$), 115.7 ($CO_2$-CH=$\underline{C}$H-$CH_2$-), 127.3 ($C_{Ar}$), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.9 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 134.0 (CO_2-$\underline{C}$H=CH-CH_2-), 136.1 ($C_{q,Ar}$), 136.2 ($C_{q,Ar}$), 156.2 (NH-$\underline{C}O_2-CH_2-C_6H_5$), 170.0 ($C_q$), 171.1 ($C_q$), 171.2 ($C_q$).

6.6.9.15. Z-Phe-Leu-Ala-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3p



Procedure according to chapter 6.6.6.

Z-Phe-Leu-Ala-OH (**1f**) (96.7 mg, 0.2 mmol, 1.0 eq), Ru-catalyst **V** (5.7 mg, 8.0 µmol, 0.04 eq), degassed dry 2-propanol (0.4 mL), hex-5-ynamide (**2b**) (26.7 mg, 0.24 mmol, 1.2 eq)

Flash column chromatography: (12 g silica gel, 15 × 1.5 cm, cyclohexane/ethyl acetate = 1:15 (v/v), R_f = 0.31)

Yield: 102.2 mg (86 %, after semi-preparative HPLC) white solid

Semi-preparative HPLC method: crude product dissolved in 1.5 mL DMSO (HPLC grade) column: 125/21 Nucleodur 100-5 C18 ec column with VP 50/21 Nucleodur 100-5 C18 ec pre-column at a flow rate of 15 mL/min; solvent A: H_2O; B: MeCN; 0-24 min: A/B = 60/40; t_R = 15-18 min.

Content of D-Ala-OH: 0.1 % GC-MS method AM 2 and HPLC-method AM 3 (chapter 6.4.4)

$C_{32}H_{42}N_4O_7$ [594.70 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 617.2951

found: 617.2950

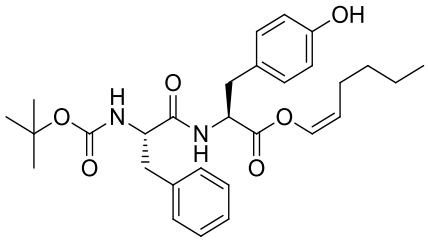
$[\alpha]_D^{25}$ = -13.3 (c = 0.5 in chloroform)

mp = 186-187°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.85-0.86 (m, 6H, -CH(-C<u>H</u>$_3$)$_2$), 1.40-1.53 (m, 4H, -CH_2-C<u>H</u>(-CH_3)$_2$, CONH-CH-<u>C</u>H_3), 1.62-1.85 (m, 4H, H_2N-CO-CH_2-C<u>H</u>$_2$-, HN-CH-C<u>H</u>$_2$-CH(-CH_3)$_2$, 2.06-2.29 (m, 4H, H_2N-CO-C<u>H</u>$_2$-CH_2- and HC=CH-C<u>H</u>$_2$-CH_2-), 3.06 (d, 2H, ^3J(H,H) = 6.1 Hz, C_6H_5-C<u>H</u>$_2$-CH-NH), 4.40-4.55 (m, 3H, NH-C<u>H</u>), 4.95 (pseudo q, 1H, ^3J(H,H) = 6.6, 7.5 Hz, CO_2-CH=C<u>H</u>-CH_2-), 5.05 (s, 2H, HN-CO_2-C<u>H</u>$_2$-C_6H_5), 5.40 (d, 1H, ^3J(H,H) = 7.1 Hz, -CO-N<u>H</u>-), 5.89 (bs, 1H, -CH_2-CO-N<u>H</u>$_2$), 6.02 (bs, 1H, -CH_2-CO-N<u>H</u>$_2$), 6.82 (d, 1H, ^3J(H,H) = 7.1 Hz, -CO-N<u>H</u>-), 7.01-7.08 (m, 2H, CO_2-C<u>H</u>=CH-CH_2-, -CO-N<u>H</u>-), 7.15-7.37 (m, 10H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.7 (<u>C</u>H_3-CH-NH-CO-), 22.9, 23.1 (2 × C, CH(-<u>C</u>H_3)$_2$), 23.8, 24.5 (2 × C, CO_2-CH=CH-<u>C</u>H_2-CH_2, CO_2-CH=CH-CH_2-<u>C</u>H_2), 24.8 (-CH_2-<u>C</u>H(-CH_3)$_2$), 34.6, 38.2 (2 × C, C_6H_5-<u>C</u>H_2-CH-NH-, NH_2-CO-<u>C</u>H_2-CH_2-), 41.8 (NH-CH-<u>C</u>H_2-CH-(CH_3)$_2$), 48.6, 51.1, 56.1 (3 × NH-<u>C</u>H), 67.4 (HN-CO_2-<u>C</u>H_2-C_6H_5), 114.2 (CO_2-CH=<u>C</u>H-CH_2-), 127.3 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.5 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.9 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 135.2 (CO_2-<u>C</u>H=CH-CH_2-), 136.1 ($C_{q,Ar}$), 136.1 ($C_{q,Ar}$), 156.4 (NH-<u>C</u>O_2-CH_2-C_6H_5), 169.5 (C_q), 171.3 (C_q), 171.6 (C_q), 175.7 (C_q).

6.6.9.16. Boc-Phe-Tyr-*Z*-anti-Markovnikov-enolester AM-*Z*-3q



Procedure according to chapter 6.6.5.

Boc-Phe-Tyr-OH (**1g**) (107.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), 1-hexyne (**2a**) (34 µL, 0.30 mmol, 1.2 eq)

Flash column chromatography: (16 g silica gel, 22 × 1.5 cm, cyclohexane/ethyl acetate = 3:1 (v/v), R_f = 0.29)

Yield: 110.0 mg (86 %), grey oil, which became a grey solid after 1 d

Content of D-Tyr-OH: 0.2 % GC-MS method AM 2 (chapter 6.4.4)

$C_{29}H_{38}N_2O_6$ [510.62 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 533.2628

found: 533.2591

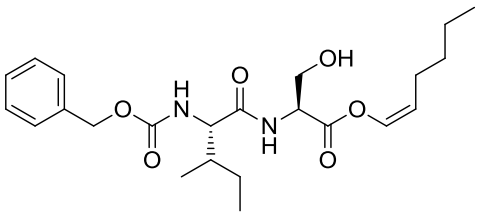
$[\alpha]_D^{25}$ = -2.5 (c = 0.5 in chloroform)

mp = 126°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.91 (t, 3H, ^3J(H,H) = 8.7 Hz, -CH_2-C$\underline{H}_3$), 1.25-1.40 (m, 13H, -C(C$\underline{H}_3$)$_3$, -CH_2-(C$\underline{H}_2$)$_2$-CH_3), 2.12 (pseudo q, 2H, ^3J(H,H) = 6.3, 6.9 Hz, -CH=CH-C$\underline{H}_2$-CH_2-), 3.02 (m, 4H, C_6H_5-C$\underline{H}_2$-CH-NH, HO-C_6H_4-C$\underline{H}_2$-CH-NH), 4.35 (m, 1H, NH-C$\underline{H}$-CO-NH or CO-NH-C$\underline{H}$-CO_2-), 4.86 (pseudo q, 1H, ^3J(H,H) = 5.4, 7.5 Hz, NH-C$\underline{H}$-CO-NH or CO-NH-C$\underline{H}$-CO_2-), 4.95 (pseudo q, 1H, ^3J(H,H) = 6.3, 7.5 Hz, CO_2-CH=C$\underline{H}$-CH_2-), 5.06 (d, 1H, ^3J(H,H) = 6.2 Hz, CO_2-N$\underline{H}$-CH-CH_2- or -CH-CO-N$\underline{H}$-CH-), 6.40 (s, 1H, C_6H_5-O$\underline{H}$), 6.53 (d, 1H, ^3J(H,H) = 7.1 Hz, -CH-CO-N$\underline{H}$-CH- or CO_2-N$\underline{H}$-CH-CH_2-), 6.66 (d, 2H, ^3J(H,H) = 8.3 Hz, H_{Ar}), 6.83 (d, 2H, ^3J(H,H) = 8.4 Hz, H_{Ar}), 6.94 (d, 1H, ^3J(H,H) = 6.3 Hz, CO_2-C$\underline{H}$=CH-CH_2), 7.15-7.30 (m, 5 H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 ($\underline{C}H_3$-$C_{alkyl\ chain}$), 22.3, 24.3 ($C_{aliphatic}$), 28.4 (-C($\underline{C}H_3$)$_3$), 31.4 ($C_{aliphatic}$), 37.3, 38.4 (2 × C, C_6H_5-$\underline{C}H_2$-CH-NH-, HO-C_6H_4-$\underline{C}H_2$-CH-NH-), 53.4, 55.8 (2 × C, NH-$\underline{C}$H), 80.7 ((-$\underline{C}$(CH_3)$_3$), 115.7, 115.9 (3 × C, CO_2-CH=$\underline{C}$H-CH_2-, $C_{Ar,Tyr}$), 126.9 ($C_{q,Ar}$), 127.2 (C_{Ar}), 128.8 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 130.5 (2 × C_{Ar}), 133.6 (CO_2-$\underline{C}$H=CH-CH_2-), 136.4 ($C_{q,Ar}$), 155.4, 155.7 (2 × C, NH-$\underline{C}O_2$-CH_2-C_6H_5, $C_{q,Ar,Tyr}$), 168.6 (C_q), 171.2 (C_q).

6.6.9.17. Z-Ile-Ser-*Z*-anti-Markovnikov-enolester AM-*Z*-3r



Procedure according to chapter 6.6.5.

Z-Ile-Ser-OH (**1h**) (88.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), 1-hexyne (**2a**) (34 µL, 0.30 mmol, 1.2 eq)

Flash column chromatography: (10 g silica gel, 14 × 1.5 cm, cyclohexane/ethyl acetate = 2:1 (v/v), R_f = 0.26)

Yield: 83 mg (76 %), white powder

Content of D-Ser-OH: 0.1 % GC-MS method AM 2 (chapter 6.4.4)

$C_{23}H_{34}N_2O_6$ [434.53 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 457.2314

found: 457.2359

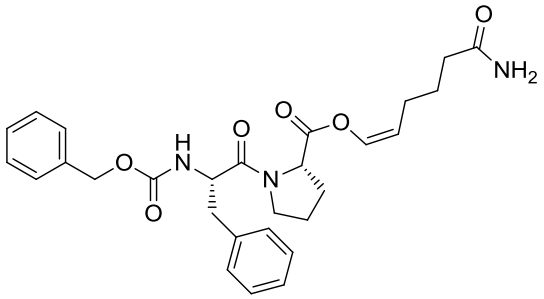
$[\alpha]_D^{25}$ = + 5.4 (c = 0.5 in chloroform)

mp = 148-149°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.88-0.93 (m, 6H, CH_2-CH_2-CH_2-C<u>H</u>$_3$, -CH(-CH_3)-CH_2-C<u>H</u>$_3$), 0.97 (d, 3H, ^3J(H,H) = 6.7 Hz, -CH(-C<u>H</u>$_3$)-CH_2-CH_3), 1.10-1.43 (m, 6H, CH_2-CH_2-C<u>H</u>$_2$-CH_3, CH_2-C<u>H</u>$_2$-CH_2-CH_3, -CH(-CH_3)-C<u>H</u>$_2$-CH_3), 1.56 (br s, 1H), 1.81-1.92 (m, 1H, -C<u>H</u>(-CH_3)-CH_2-CH_3), 2.08-2.19 (m, 2H, CO_2-CH=CH-C<u>H</u>$_2$-CH_2-), 3.92-4.02 (m, 2H, NH-CH-C<u>H</u>$_2$-OH), 4.07 (pseudo t, 1H, ^3J(H,H) = 7.8, 7.5 Hz, NH-C<u>H</u>-CH-(-CH_3)-CH_2-CH_3), 4.76-4.79 (m, 1H, NH-C<u>H</u>-CH_2-OH), 4.93-5.14 (m, 3H, CO_2-CH=C<u>H</u>-CH_2, HN-CO_2-C<u>H</u>$_2$-C_6H_5), 5.53 (d, 1H, ^3J(H,H) = 8.2 Hz, <u>H</u>N-CH-CH(-CH_3)-CH_2-CH_3), 7.00 (d, 1H, ^3J(H,H) = 6.3 Hz, CO_2-C<u>H</u>=CH-CH_2), 7.05 (d, 1H, ^3J(H,H) = 7.8 Hz, N<u>H</u>-CH-CH_2-OH), 7.28-7.37 (m, 5H, H_{Ar}).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 11.3 14.0, 15.6 (CH_2-CH_2-CH_2-<u>C</u>H_3, -CH(-CH_3)-CH_2-<u>C</u>H_3, -CH(-<u>C</u>H_3)-CH_2-CH_3), 22.3, 24.3, 25.0 (3 × C, CH_2-CH_2-<u>C</u>H_2-CH_3, <u>C</u>H_2-CH_2-CH_2-CH_3, -CH(-CH_3)-<u>C</u>H_2-CH_3), 31.3 (CH_2-<u>C</u>H_2-CH_2-CH_3), 37.4 (-<u>C</u>H(-CH_3)-CH_2-CH_3), 54.7, 60.1 (2 × C, HN-<u>C</u>H-), 62.9 (NH-CH-<u>C</u>H_2-OH), 67.4 (HN-CO_2-<u>C</u>H_2-C_6H_5), 116.0 (CO_2-CH=<u>C</u>H-CH_2), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 133.9 (CO_2-<u>C</u>H=CH-CH_2), 136.2 ($C_{q,Ar}$), 156.9 (HN-<u>C</u>O_2-CH_2-C_6H_5), 167.7 (C_q), 171.9 (C_q).

6.6.9.18. Z-Phe-Pro-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3s



Procedure according to chapter 6.6.6.

Z-Phe-Pro-OH (**1i**) (99.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), hex-5-ynamide (**2b**) (33.3 mg, 0.30 mmol, 1.2 eq)

Flash column chromatography: (15 g silica gel, 20 × 1.5 cm, cyclohexane/ethyl acetate = 1:10 (v/v), R_f = 0.22)

Yield: 98.9 mg (78 %), off-white gum-like solid

Content of D-Pro-OH: 0.3 % GC-MS method AM 2 (chapter 6.4.4)

$C_{28}H_{33}N_3O_6$ [507.58 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 530.2267

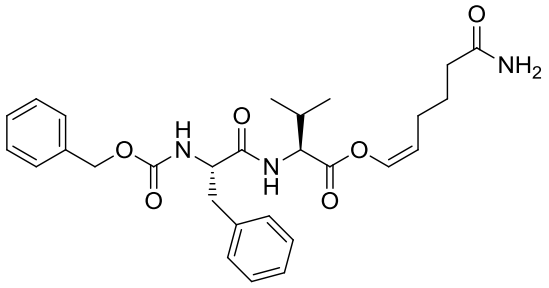
found: 530.2245

$[\alpha]_D^{25}$ = +6.6 (c = 0.5 in chloroform)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.59-2.39 (m, 10H, C<u>H</u>$_2$-CH_2-CH_2-CO-NH_2, -CH_2-C<u>H</u>$_2$-CH_2-CO-NH_2, -CH_2-CH_2-C<u>H</u>$_2$-CO-NH_2, -C<u>H</u>$_2$-CH_2-CH_2-N-CH-CO_2, -CH_2-C<u>H</u>$_2$-CH_2-N-CH-CO_2), 2.89-3.13 (m, 2H, C_6H_5-C<u>H</u>$_2$-CH-NH-), 3.23-3.71 (m, 2H, -CH_2-CH_2-C<u>H</u>$_2$-N-CH-CO_2), 4.49-4.53 (m, 1H, -CH_2-CH_2-CH_2-N-C<u>H</u>-CO_2), 4.70 (pseudo q, 1H, ^3J(H,H) = 8.1, 6.9 Hz, C_6H_5-CH_2-C<u>H</u>-NH-), 4.93-5.10 (m, 3H, CO_2-C<u>H</u>=CH-CH_2, HN-CO_2-C<u>H</u>$_2$-C_6H_5), 5.41 (br s, 1H, -CH_2-CO-N<u>H</u>$_2$), 5.50 (d, 1H, ^3J(H,H) = 8.1 Hz, C_6H_5-CH_2-CH-N<u>H</u>-), 6.08 (br. s, 1H, -CH_2-CO-N<u>H</u>$_2$), 7.06 (d, 2H, ^3J(H,H) = 6.2 Hz, CO_2-C<u>H</u>=CH-CH_2), 7.22-7.37 (m, 10H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 23.8, 24.1, 25.2 (3 × C, <u>C</u>H_2-CH_2-CH_2-CO-NH_2, -CH_2-<u>C</u>H_2-CH_2-CO-NH_2, -CH_2-<u>C</u>H_2-CH_2-N-CH-CO_2), 29.1 (-<u>C</u>H_2-CH_2-CH_2-N-CH-CO_2), 34.4, 38.8 (2 × C, C_6H_5-<u>C</u>H_2-CH-NH, -CH_2-<u>C</u>H_2-CO-NH_2), 47.2 (-CH_2-CH_2-<u>C</u>H_2-N-CH-CO_2), 59.1 (-CH_2-CH_2-CH_2-N-<u>C</u>H-CO_2), 67.0 (C_6H_5-CH_2-<u>C</u>H-NH-), 114.4 (CO_2-CH=<u>C</u>H-CH_2), 127.2 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.3 (C_{Ar}), 128.7 (4 × C_{Ar}), 129.8 (2 × C_{Ar}), 135.3 (CO_2-<u>C</u>H=CH-CH_2), 135.9, 136.4 (2 × $C_{q,Ar}$), 155.9 (HN-<u>C</u>O_2-CH_2-C_6H_5), 169.3 (C_q), 170.8 (C_q), 175.6 (C_q).

6.6.9.19. Z-Phe-Val-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3t



Procedure according to chapter 6.6.6.

Z-Phe-Val-OH (**1j**) (99.6 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), hex-5-ynamide (**2b**) (33.3 mg, 0.30 mmol, 1.2 eq)

Flash column chromatography: (15 g silica gel, 20 × 1.5 cm, cyclohexane/ethyl acetate = 1:3 (v/v), R_f = 0.26)

Yield: 106.0 mg (83 %), off-white gum-like solid

Content of D-Val-OH: 0.2 % GC-MS method AM 2 (chapter 6.4.4)

$C_{28}H_{35}N_3O_6$ [509.59 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 532.2424

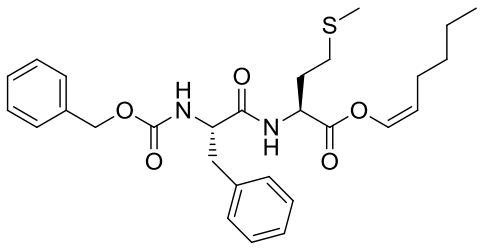
found: 532.2412

$[\alpha]_D^{25}$ = -4.1 (c = 0.5 in chloroform)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.89 (pseudo t, 6H, ^3J(H,H) = 6.9, 7.2 Hz, -CH(-C<u>H</u>$_3$)$_2$), 1.62-1.75 (m, 2H, H_2N-CO-CH_2-C<u>H</u>$_2$-), 1.99-2.26 (m, 5H, H_2N-CO-C<u>H</u>$_2$-CH_2-, HC=C-C<u>H</u>$_2$-CH_2-, -C<u>H</u>(-CH_3)$_2$), 2.99-3.13 (m, 2H, C_6H_5-C<u>H</u>$_2$-CH-NH), 4.53 (dd, 1H, ^3J(H,H) = 8.8, 5.4 Hz, H-N-C<u>H</u>-CH(-CH_3)$_2$) 4.58-4.65 (m, 1H, NH-C<u>H</u>-CO-NH), 4.97 (pseudo q, ^3J(H,H) = 7.8, 6.3 Hz, 1H, CO_2-CH=C<u>H</u>-CH_2-), 5.07 (s, 2H, C_6H_5-C<u>H</u>$_2$-CO_2-NH), 5.57 (d, 1H, ^3J(H,H) = 7.7 Hz, -N<u>H</u>-CH-), 5.69 (br s, 1H, -CH2-CO-N<u>H</u>$_2$), 5.93 (br s, 1H, -CH_2-CO-N<u>H</u>$_2$), 6.95 (d, 1H, ^3J(H,H) = 6.2 Hz, CO_2-C<u>H</u>=CH-CH_2-), 7.08 (d, 1H, ^3J(H,H) = 8.0 Hz, -N<u>H</u>-CH-), 7.16-7.38 (m, 10H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0, 18.9 (-CH-(<u>C</u>H$_3$)$_2$), 24.0, 24.5 (2 × C, CO_2-CH=CH-<u>C</u>H_2-CH_2-, CO_2-CH=CH-CH_2-<u>C</u>H_2-), 31.5 (-<u>C</u>H-(CH_3)$_2$), 34.5 (C_6H_5-<u>C</u>H_2-CH-NH), 38.7 (-CH_2-<u>C</u>H_2-CO-NH_2), 56.2, 57.7 (NH-<u>C</u>H-), 67.2 (HN-CO_2-<u>C</u>H_2-C_6H_5), 114.1 (CO_2-CH=<u>C</u>H-CH_2), 127.1 (C_{Ar}), 128.1 (2 × C_{Ar}), 128.3 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 129.4 (2 × C_{Ar}), 134.8 (CO_2-<u>C</u>H=CH-CH_2), 136.3 ($C_{q,Ar}$), 136.5 ($C_{q,Ar}$), 156.2 (HN-<u>C</u>O_2-CH_2-C_6H_5), 168.1 (C_q), 171.3 (C_q), 175.5 (C_q).

6.6.9.20. Z-Phe-Met-*Z*-anti-Markovnikov-enolester AM-*Z*-3u



Procedure according to chapter 6.6.5.

Z-Phe-Met-OH (**1k**) (107.6 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.5 mL), 1-hexyne (**2a**) (34 µL, 0.30 mmol, 1.2 eq)

Flash column chromatography: (15 g silica gel, 20 × 1.5 cm, cyclohexane/ethyl acetate = 4:1 (v/v), R_f = 0.26)

Yield: 111.0 mg (87 %), dark yellow solid

Content of D-Met-OH: 0.7 % GC-MS method AM 2 (chapter 6.4.4)

$C_{27}H_{34}N_2O_5S$ [512.66 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 535.2242

found: 535.2215

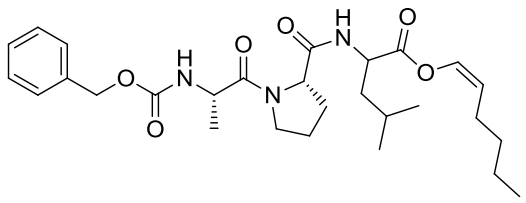
$[\alpha]_D^{25}$ = +1.0 (c = 0.5 in chloroform)

mp = 108-109°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, 3H, ^3J(H,H) = 7.1 Hz, -CH_2-C$\underline{H}_3$), 1.28-1.41 (m, 5H, -CH_2-CH_2-C$\underline{H}_2$-CH_3, -CH_2-C$\underline{H}_2$-CH_2-CH_3), 1.91-2.20 (m, 7H, -C$\underline{H}_2$-CH_2-S-CH_3, -CH_2-CH_2-S-C$\underline{H}_3$, -CH=CH-C$\underline{H}_2$-CH_2-), 2.41 (t, 2H, ^3J(H,H) = 7.3 Hz, -CH_2-C$\underline{H}_2$-S-CH_3), 2.97-3.26 (m, 2H, C_6H_5-C$\underline{H}_2$-CH-NH), 4.45 (pseudo q, 1H, ^3J(H,H) = 7.2, 6.9 Hz, C_6H_5-CH_2-C$\underline{H}$-NH-), 4.71-4.77 (m, 1H, -CO-NH-C$\underline{H}$-CO_2-), 4.96 (pseudo q, 1H, ^3J(H,H) = 7.5, 6.3 Hz, -CH=C$\underline{H}$-CH_2-CH_2-), 5.09 (s, 2H, -HN-CO_2-C$\underline{H}_2$-C_6H_5), 5.29 (d, 1H, ^3J(H,H) = 4.8 Hz, C_6H_5-CH_2-CH-N$\underline{H}$-), 6.54 (d, 1H, ^3J(H,H) = 7.6 Hz, -CO-N$\underline{H}$-CH-CO_2-), 6.96 (d, 2H, ^3J(H,H) = 6.3 Hz, CO_2-C$\underline{H}$=CH-CH_2-CH_2-), 7.17-7.38 (m, 10H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.0 ($\underline{C}H_3$-$C_{alkyl\ chain}$), 15.5 (CH_2-S-$\underline{C}H_3$), 22.3, 24.3, 29.8, 31.3, 31.5 (5 x C, -CH_2-$C_{alkyl\ chain}$), 38.4 (C_6H_5-CH_2-$\underline{C}$H-NH), 51.6, 56.3 (2 × C, NH-$\underline{C}$H), 67.4 (HN-CO_2-$\underline{C}H_2$-C_6H_5), 115.9 (-CH=$\underline{C}$H-CH_2-CH_2-), 127.3 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.9 (2 × C_{Ar}), 129.5 (2 × C_{Ar}), 133.8 (-C$\underline{H}$=CH-CH_2-CH_2-), 136.2 (2 × $C_{q,Ar}$), 156.0 (HN-$\underline{C}O_2$-CH_2-C_6H_5), 168.8 (C_q), 170.9 (C_q).

6.6.9.21. Z-Ala-Pro-Leu-*Z*-anti-Markovnikov-enolester AM-*Z*-3v



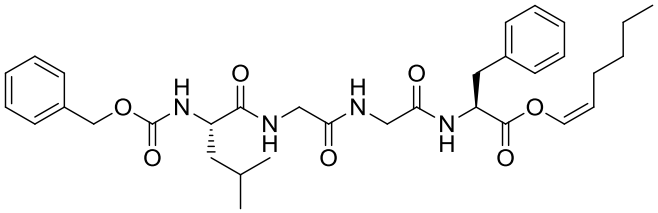
Procedure according to chapter 6.6.5.

Z-Ala-Pro-Leu-OH (**1l**) (43.4 mg, 0.1 mmol, 1.0 eq), Ru-catalyst **V** (2.8 mg, 0.04 mmol, 0.04 eq), degassed dry 2-propanol (0.2 mL), degassed dry trifluoroethanol (0.3 mL), 1-hexyne (**2a**) (23 µL, 0.20 mmol, 2.0 eq)

$C_{28}H_{41}N_3O_6$ [515.64 $gmol^{-1}$]

The conversions were checked by HPLC Method D. Due to the low conversion towards **AM-*Z*-3v** of 2 % after 24h, **AM-*Z*-3v** was not isolated and further characterized.

6.6.9.22. Z-Leu-Gly-Gly-Phe-*Z*-anti-Markovnikov-enolester AM-*Z*-3w



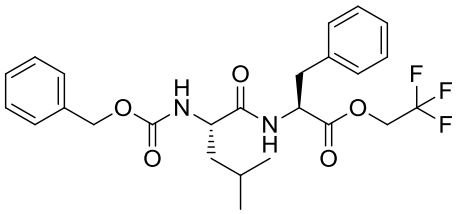
Procedure according to chapter 6.6.5.

Z-Leu-Gly-Gly-Phe-OH (**1m**) (52.7 mg, 0.1 mmol, 1.0 eq), Ru-catalyst **V** (2.8 mg, 0.04 mmol, 0.04 eq), degassed dry 2-propanol (0.2 mL), 1-hexyne (**2a**) (14 µL, 0.12 mmol, 1.2 eq). A second amount of Ru-catalyst **V** (2.8 mg, 0.04 mmol, 0.04 eq) was added after 3 h.

$C_{33}H_{44}N_4O_7$ [608.73 $gmol^{-1}$]

The conversions were checked by HPLC Method D. Due to the not satisfying conversion towards **AM-*Z*-3w** of 64 % after 24h, **AM-*Z*-3w** was not isolated and further characterized.

6.6.9.23. Z-Leu-Phe-trifluoroethylester (49)



Procedure according to chapter 6.6.5. Z-Leu-Phe-trifluoroethylester was formed as a side-product.

Z-Leu-Phe-OH (**1c**) (103.1 mg, 0.25 mmol, 1.0 eq), Ru-catalyst **V** (7.1 mg, 0.01 mmol, 0.04 eq), degassed dry 2-propanol (0.05 mL), degassed dry trifluoroethanol (0.5 mL), 1-hexyne (**2a**) (34 µL, 0.30 mmol, 1.2 eq)

Flash column chromatography: (16 g silica gel, 22 × 1.5 cm, cyclohexane/ethyl acetate = 5:1 (v/v), R_f = 0.14)

Yield: 90.7 mg (73 %), colourless solid

Content of D-Phe-OH: 1.1 % GC-MS method AM 2 (chapter 6.4.4)

Procedure according to chapter 4.1.7.2.

Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** (37.1 mg, 75 µmol) was stirred together with dry trifluorethanol (150 µL) and dry 2-propanol (15 µL) in a GC-vial with cap. During this reaction no inert atmosphere was present. After 24 h full conversion was monitored via HPLC-MS (method D). The work up was the same as mentioned in chapter 6.6.5.

Yield: quantitative, colourless solid

Content of D-Phe-OH: 2.6 % GC-MS method AM 2 (chapter 6.4.4)

$C_{25}H_{29}F_3N_2O_3$ [494.50 $gmol^{-1}$]

HRMS: $[MK]^+$: calculated: 533.1666

found: 533.1703

$[\alpha]_D^{25}$ = -9.4 (c = 0.5 in DMSO)

mp = 149-150°C

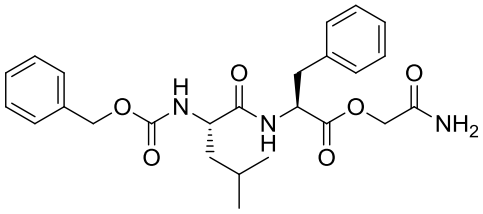
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.90 (d, 6H, ^3J(H,H) = 6.1 Hz, -CH-(C<u>H</u>$_3$)$_2$), 1.41-1.67 (m, 3H, -C<u>H</u>-(CH$_3$)$_2$, -C<u>H</u>$_2$-CH-(CH$_3$)$_2$), 3.04-3.21 (m, 2H, C_6H_5-C<u>H</u>$_2$-CH-NH), 4.14-4.19 (m, 1H, H-N-C<u>H</u>-CH$_2$-CH-(CH$_3$)$_2$), 4.35-4.58 (m, 2H, CF_3-C<u>H</u>$_2$-), 4.88-4.95 (pseudo q, 1 H, ^3J(H,H) = 6.3 Hz, 7.5 Hz, CO-NH-C<u>H</u>(CH$_2$-Ph)-CO$_2$-), 5.04-5.13 (HN-CO$_2$-C<u>H</u>$_2$-C$_6$H$_5$, CO$_2$-N<u>H</u>-CH-CH$_2$-),

6.49 (d, 1H, ^{3}J = 6.4 Hz, C_6H_5-CH_2-CH-N<u>H</u>-), 7.11 (d, 2H, ^{3}J(H,H) = 6.3 Hz, <u>H_{Ar}</u>), 7.23-7.35 (m, 8H, <u>H_{Ar}</u>).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.0, 22.9 (2 × C, -CH-(<u>C</u>H_3)$_2$), 24.7 (-CH_2-<u>C</u>H(-CH_3)$_2$), 37.7 (HN-CO_2-C<u>H_2</u>-C_6H_5), 41.1 (-C<u>H_2</u>-CH-(CH_3)$_2$), 53.1, 53.5 (2 × NH-<u>C</u>H), 60.4, 60.9, 61.4, 61.9 (q, ^{2}J(C,F) = 37.0 Hz, -CO_2-<u>C</u>H_2-CF_3), 67.3 (HN-CO_2-<u>C</u>H_2-C_6H_5), 117.3, 120.9, 124.6, 128.3 (q, ^{1}J(C,F) = 275.3 Hz, -CO_2-CH_2-<u>C</u>F_3), 127.5 (C_{Ar}), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 128.9 (2 × C_{Ar}), 129.3 (2 × C_{Ar}), 135.2 ($C_{q,Ar}$), 136.2 ($C_{q,Ar}$), 156.3 (NH-<u>C</u>O_2-CH_2-C_6H_5), 170.0 (C_q), 172.0 (C_q).

6.6.10. Enzymatic peptide coupling: general procedure

6.6.10.1. Synthesis of starting material *Z*-Leu-PheOCam-ester (52)



Under argon the reaction vessel was charged with *Z*-Leu-Phe-OH (**1c**) (2.062 g, 5.0 mmol, 1.0 eq), dry THF (20 mL) and cesium carbonate (1.80 g, 5.5 mmol, 1.1 eq). Then iodoacetamide (**51**) (1.02 g, 5.5 mmol, 1.1 eq) was added and the mixture was stirred overnight at 50°C. Finally, the volatiles were evaporated and the residue was purified by flash column chromatography (260 g silica gel, 22 × 5.7 cm, EtOAc, R_f = 0.35).

Yield: 2.256 g (96 %) colourless solid

Content of D-Phe-OH: 1.1 % GC-MS method AM 2 (chapter 6.4.4)

$C_{25}H_{31}N_3O_6$ [469.53 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 492.2111

found: 492.2095

$[\alpha]_D^{25}$ = -28.0 (c = 0.5 in chloroform)

mp = 134°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.92 (t, 6H, ^3J(H,H) = 6.2 Hz, -CH-(C<u>H</u>$_3$)$_2$), 1.43-1.57 (m, 1H, -CH$_2$-C<u>H</u>-(CH$_3$)$_2$), 1.57-1.74 (m, 2H, -C<u>H</u>$_2$-CH-(CH$_3$)$_2$), 3.13 (m, 2H, C$_6$H$_5$-C<u>H</u>$_2$-CH-NH), 4.17-4.27 (m, 1H, NH-C<u>H</u>(CH$_2$-CH(CH$_3$)$_2$)-CO-NH), 4.39-4.46 (pseudo d, 1H), 4.57-4.70 (m, 2H), 5.09 (s, 3H, HN-CO$_2$-C<u>H</u>$_2$-C$_6$H$_5$), 5.53 (d, 1H, ^3J(H,H) = 7.5 Hz, -CO-N<u>H</u>-), 5.96 (br. s, 1H, -CO-N<u>H</u>$_2$), 6.70 (br. s, 1H, -CO-N<u>H</u>$_2$), 6.93 (d, 1H, ^3J(H,H) = 4.2 Hz , -CO-N<u>H</u>-), 7.18 (d, 2H, ^3J(H,H) = 6.6 Hz, H$_{Ar}$), 7.24-7.44 (m, 8H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 21.8, 22.9, 24.6 (C$_{aliphatic}$), 37.1(C$_6$H$_5$-<u>C</u>H$_2$-CH-NH-), 40.6 (NH-CH-<u>C</u>H$_2$-CH-(CH$_3$)$_2$), 53.3, 54.4 (2 × NH-<u>C</u>H), 62.8 (-CO$_2$-CH$_2$-CO-NH$_2$), 67.3 (HN-CO$_2$-<u>C</u>H$_2$-C$_6$H$_5$), 127.5 (C$_{Ar}$), 128.0 (2 × C$_{Ar}$), 128.3 (C$_{Ar}$), 128.6 (2 × C$_{Ar}$), 128.96 (2 × C$_{Ar}$), 129.03 (2 ×

C_{Ar}), 135.3 ($C_{q,Ar}$), 136.0 ($C_{q,Ar}$), 156.6 (NH-$\underline{C}O_2$-CH_2-C_6H_5), 169.6 (C_q), 170.3 (C_q), 173.3 (C_q).

6.6.10.2. Procedure for the synthesis of Z-Leu-Phe-Phe-NH_2 (54) and Z-Leu-Ala-Phe-NH_2 (64) in chapter 4.2.2.1

For the pretreatment of the enzyme alcalase-CLEA OM (batch DSM 1) 0.8 g alcalase-CLEA was washed under inert conditions with dry *tert*-butanol (3 × 5 mL; 50°C) under a gentle stream of argon in a funnel with a sintered glass disc. *Tert*-butanol was removed by suction filtration taking care that *tert*-butanol never became too cold to avoid solidification. The enzyme was washed with dry MTBE (3 × 5 mL) under a gentle stream of argon. The solvent was removed by suction filtration. The enzyme was dried under vacuum (20 mbar) for 30 min at rt.

For the enzymatic peptide coupling reaction using pretreated alcalase-CLEA OM (batch DSM 1) all manipulations were conducted under a gentle stream of argon.

Dipeptide-enolester **AM-*Z*-3a** and **AM-*Z*-3d** (0.02 mmol, 1.0 eq), Z-Leu-Phe-OCam (**52**) (9.4 mg, 0.02 mmol, 1.0 eq), H-Phe-NH_2 (**53**) (4.9 mg, 0.03 mmol, 1.5 eq) and pretreated alcalase-CLEA OM batch DSM 1 (50 mg) were placed in a 5 mL crimp top vial. Then dry THF (1 mL) was added with a syringe and subsequently the drying agent 3Å molecular sieves (beads, 0.5 g) was added. The 5 mL crimp top vials were sealed and the reactions were carried out at 50°C and 150 rpm using incubation shaker Infors Multitron AJ 112.

6.6.10.3. Confirmation of imine formation (56)

To investigate the formation of imine **56** (chapter 4.2.2.2) under the reaction conditions applied for the coupling of peptide fragments the following reaction was performed:

H-Phe-NH_2 (**53**) (6.0 mg, 36.5 µmol) and hexanal (**55**) (4.5 µL, 36.5 µmol) were placed in an HPLC vial. After dissolving the starting materials in THF (0.5 mL) and adding 5 beads of molecular sieves 3Å, the mixture was warmed to 50°C for 20 h. 100 µL of reaction mixture were withdrawn, diluted with 500 µL acetonitrile and analyzed by HPLC. The remaining approximately 400 µL of each reaction were evaporated to dryness, hydrolyzed by the action of 6M HCl at 95°C for 16 h and then used for the amino acid determination as described in AM 1 in chapter 6.4.4.

Following amounts of D-Phe-OH were found after hydrolysis of the crude imine **56**: 0.6 %

An identical hydrolysis treatment with H-Phe-NH_2 leads to 0.3 % of D-Phe-OH.

By substraction a value of 0.3 % of D-Phe-OH formation during the formation of imine **56** is calculated.

6.6.10.4. Synthesis of Z-Leu-Phe-Phe-NH_2 (54) with different drying agents

For the pretreatment of the enzyme alcalase-CLEA OM (batch DSM 1) 0.8 g alcalase-CLEA was washed under inert conditions with dry *tert*-butanol (3 × 5 mL; 50°C) under a gentle stream of argon in a funnel with a sintered glass disc. *Tert*-butanol was removed by suction filtration under argon, taking care that *tert*-butanol never became too cold to avoid solidification. The enzyme was washed with dry MTBE (3 × 5 mL) under a gentle stream of argon. The solvent was removed by suction filtration under argon. The enzyme was dried under vacuum (20 mbar) for 30 min at rt.
For the enzymatic peptide coupling reaction using pretreated alcalase-CLEA OM (batch DSM 1) all manipulations were conducted under a gentle stream of argon.
Dipeptide-enolester **AM-*Z*-3a** (9.9 mg, 0.02 mmol, 1.0 eq), H-Phe-NH_2 (**53**) (4.9 mg, 0.03 mmol, 1.5 eq) and pretreated alcalase-CLEA OM (batch DSM 1) (50 mg) were placed in a 5 mL crimp top vial. Then dry THF (1 mL) was added with a syringe and subsequently the drying agents (3Å molecular sieves, aluminium oxide, Na_2SO_4 and $MgSO_4$) (each of 0.5 g) were added. The 5 mL crimp top vials were sealed and the reactions were carried out at 50°C and 150 rpm using incubation shaker Infors Multitron AJ 112.

6.6.10.5. Synthesis of Z-Leu-Phe-Phe-NH_2 (54) using different enolesters as starting material

The enzymatic peptide coupling reaction was performed as described in chapter 6.6.10.2.
All enolesters used as starting material are shown in the table below.

Table 42: Enolesters used in enzymatic peptide coupling.

Enolester (mg, mmol, eq)	alcalase-CLEA OM batch
AM-*Z*-3f (10.2 mg, 0.02 mmol, 1.0 eq)	alcalase-CLEA OM batch DSM 1
AM-*Z*-3g (11.4 mg, 0.02 mmol, 1.0 eq)	alcalase-CLEA OM batch DSM 1
AM-*Z*-3h (11.7 mg, 0.02 mmol, 1.0 eq)	alcalase-CLEA OM batch DSM 1
AM-*Z*-3j (10.2 mg, 0.02 mmol, 1.0 eq)	alcalase-CLEA OM batch DSM 2
AM-*Z*-3k (10.5 mg, 0.02 mmol, 1.0 eq)	alcalase-CLEA OM batch DSM 1 and batch DSM 2

6.6.10.6. Kinetic investigations of the synthesis of Z-Leu-Phe-Phe-NH_2 (54) using different enolesters as starting material

For the pretreatment of the enzyme alcalase-CLEA OM (batch DSM 2) all manipulations were conducted under air. First 0.8 g alcalase-CLEA was washed with distilled water (20 mL) in a funnel with a sintered glass disc. Alcalase-CLEA was washed with dry *tert*-butanol (4 × 5 mL; 30°C). *Tert*-butanol was removed by suction filtration taking care that *tert*-butanol never became too cold to avoid solidification. The enzyme was washed finally with dry MTBE (4 × 5 mL). The solvent was removed by suction filtration. The enzyme was dried under vacuum (20 mbar) for 30 min at rt.

The 3Å molecular sieves used was activated at approximately 200°C and 20 mbar over a time span of 24 h.

For the enzymatic peptide coupling reaction using pretreated alcalase-CLEA OM batch 2 all manipulations were conducted under air.

Dipeptide-esters (0.02 mmol, 1.0 eq), H-Phe-NH_2 (**53**) (4.9 mg, 0.03 mmol, 1.5 eq) and pretreated alcalase-CLEA OM batch DSM 2 (50 mg) were placed in a 5 mL crimp top vial. After addition of THF (1 mL, containing BHT and dried over 3Å molecular sieves (chapter 6.2)) and 3Å molecular sieves (beads, 150 mg) the vials were sealed and then heated under shaking (150 rpm) at 50°C using incubation shaker Infors Multitron AJ 112. Samples (100 µL) were taken from the reaction at appropriate times, diluted with acetonitrile (500 µL), filtered (0.2 µm, Pall) and then subjected to rp-HPLC analysis (method D).

Table 43: Esters used in kinetic investigations of the synthesis of Z-Leu-Phe-Phe-NH_2 (**54**).

Starting material	amount
AM-*Z*-3a	(9.9 mg, 0.02 mmol, 1.0 eq)
AM-*Z*-3j	(10.2 mg, 0.02 mmol, 1.0 eq)
AM-*Z*-3k	(10.5 mg, 0.02 mmol, 1.0 eq)
52	(9.4 mg, 0.02 mmol, 1.0 eq)

6.6.11. Enzymatic peptide coupling under Soxhlet-conditions

6.6.11.1. Synthesis of Z-Leu-Phe-Phe-NH_2 (54) using Z-Leu-Phe-*Z*-anti-Markovnikov-enolester AM-*Z*-3a as starting material

For the pretreatment of the enzyme alcalase-CLEA OM (batch DSM 2) all manipulations were conducted under a gentle stream of argon. First 0.3 g alcalase-CLEA was washed with

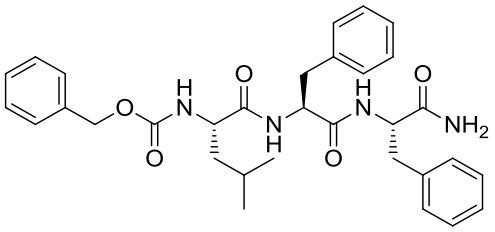
distilled water (10 mL) under inert conditions in a funnel with a sintered glass disc and with dry *tert*-butanol (3 × 4 mL; 50°C). *Tert*-butanol was removed by suction filtration under argon, taking care that *tert*-butanol never became too cold to avoid solidification. Finally, the enzyme was washed with dry MTBE (3 × 4 mL). The solvent was removed by suction filtration under argon and then the enzyme was dried under vacuum (20 mbar) for 30 min at rt.

For the enzymatic peptide coupling reaction using pretreated alcalase-CLEA OM batch DSM 2 all manipulations were conducted under a gentle stream of argon.

A reflux-condenser, a 10 mL dropping funnel with pressure release, a 10 mL two-necked flask and a magnetic stirring bar were assembled, dried under vacuum with a heat gun, and flushed with argon. The two-necked flask was charged with pretreated alcalase-CLEA OM batch DSM 2 (100 mg) and dry THF (2 mL, dried over sodium). The 3Å molecular sieves (beads, 1.5 g) were placed into the dropping funnel together with a plug of cotton to prevent that the beads of molecular sieves fall into the reaction solution. The reflux-condenser was connected to a cryostat containing a cooling agent at -15°C (according to Figure 17). The magnetic stirrer was adjusted to 50 rpm and the oil bath was heated to 50°C. After adjusting the reduced pressure to enable reflux conditions (270 mbar), the reaction mixture was refluxed for 1 h in the presence of alcalase-CLEA OM batch DSM 2, allowing the recondensing solvent to flow through the molecular sieves back to the reaction flask. Afterwards pressure was adjusted by using argon to atmospheric pressure. Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** (19.8 mg, 0.04 mmol, 1.0 eq) and H-Phe-NH_2 (**53**) (9.9 mg, 0.06 mmol, 1.5 eq) were added under a gentle stream of argon and the reaction was continued under reduced pressure at 50°C and magnetic stirring (50 rpm).

After the indicated reaction time the reaction mixture was diluted with DMSO (HPLC-grade) until all precipitated compounds were in solution. The enzyme was removed by filtration (0.2 µm, Pall) and the resulting solution was subjected to rp-HPLC analysis (method D).

The second reaction was performed under identical conditions but on a slightly larger scale, using Z-Leu-Phe-*Z*-anti-Markovnikov-enolester **AM-*Z*-3a** (29.7 mg, 0.06 mmol, 1.0 eq) and H-Phe-NH_2 (**53**) (14.8 mg, 0.09 mmol, 1.5 eq), pretreated alcalase-CLEA OM batch DSM 2 (150 mg) and dry THF (3 mL, dried over sodium). To the second reaction 0.9 mL of DMSO were added after 23 h before measuring the conversion via HPLC-MS (method D). THF was removed in vacuo using a rotary evaporator. Afterwards DMSO was removed in vacuo (0.02 mbar) and the resulting solid was purified by flash column chromatography (DCM/MeOH 20:1 + traces of DMSO). Afterwards **54** was recrystallized from ethylacetate (reflux) and cyclohexane yielding 36 % of pure isolated product **54**.



Yield: 20.0 mg (60 %, after flash column chromatography)

12.1 mg (36 % after recrystallization), white solid

R_f = 0.15 (dichloromethane/methanol = 20:1 (v/v))

Content of D-Phe-OH: 0.1 % GC-MS method AM 1 (chapter 6.4.4)

$C_{32}H_{38}N_4O_5$ [581.67 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 581.2740

found: 581.2767

$[\alpha]_D^{25}$ = -17.3 (c = 0.5 in DMSO)

mp = 182-183°C

^{1}H-NMR (300 MHz, DMSO-*d6*): δ = 0.78-0.83 (m, 6H, -CH(-C<u>H</u>$_3$)$_2$), 1.24-1.49 (m, 3H, HN-CH-C<u>H</u>$_2$-CH(-CH$_3$)$_2$, -CH$_2$-C<u>H</u>(-CH$_3$)$_2$), 2.73-3.02 (m, 4H, 2 × C_6H_5-C<u>H</u>$_2$-CH-NH), 3.97 (m, 1H, CO_2-NH-C<u>H</u>-CO-NH), 4.44-4.49 (m, 2H, CO-NH-C<u>H</u>-CO-NH-, CO-NH-C<u>H</u>-CO-NH$_2$), 5.01 (s, 2H, HN-CO_2-C<u>H</u>$_2$-C_6H_5), 7.09-7.34 (m, 18H, <u>H</u>$_{Ar}$, 3 × NH), 7.92 (d, 1H, ^3J(H,H) = 7.0 Hz, N<u>H</u>), 8.09 (d, 1H, ^3J(H,H) = 7.2 Hz, N<u>H</u>).

^{13}C-NMR (75 MHz, DMSO-*d6*): δ = 22.4, 22.9, 24.1 (3 × C, CH(-<u>C</u>H$_3$)$_2$, -CH$_2$-<u>C</u>H(-CH$_3$)$_2$), 37.4, 37.6 (2 × C, C_6H_5-<u>C</u>H$_2$-CH-NH-), 40.7 (NH-CH-<u>C</u>H$_2$-CH-(CH$_3$)$_2$), 53.2, 53.6, 53.7 (3 × NH-<u>C</u>H), 65.4 (HN-CO_2-<u>C</u>H$_2$-C_6H_5), 126.1 (C_{Ar}), 126.2 (C_{Ar}), 127.6 (2 × C_{Ar}), 127.7 (C_{Ar}), 127.9 (2 × C_{Ar}), 128.0 (2 × C_{Ar}), 128.3 (2 × C_{Ar}), 129.1 (2 × C_{Ar}), 129.2 (2 × C_{Ar}), 137.0 ($C_{q,Ar}$), 137.5 ($C_{q,Ar}$), 137.7 ($C_{q,Ar}$), 155.8 (NH-<u>C</u>O$_2$-CH$_2$-C_6H_5), 170.6 (C_q), 171.9 (C_q), 172.5 (C_q).

6.6.11.2. Synthesis of Z-Leu-Phe-Phe-NH_2 (54) using Z-Leu-Phe-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3k as starting material

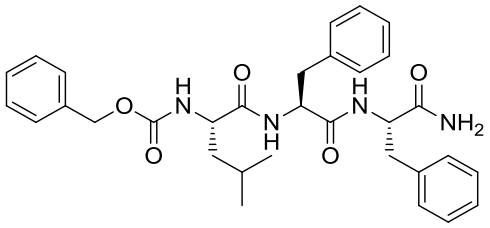
For the pretreatment of the enzyme alcalase-CLEA OM (batch DSM 2) all manipulations were conducted under air. First 0.8 g alcalase-CLEA was washed with distilled water (20 mL)

in a funnel with a sintered glass disc. Alcalase-CLEA was washed with dry *tert*-butanol (4 × 5 mL; 30°C). *Tert*-butanol was removed by suction filtration, taking care that *tert*-butanol never became too cold to avoid solidification. Finally, the enzyme was washed with dry MTBE (4 × 5mL). The solvent was removed by suction filtration. The enzyme was dried under vacuum (20 mbar) for 30 min at rt.

The 3Å molecular sieves used were activated at approximately 200°C and 20 mbar over a time span of 24 h.

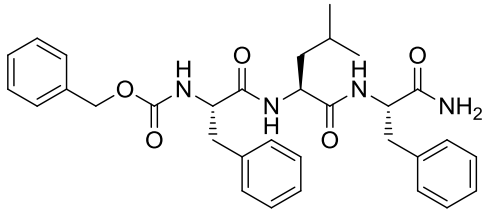
For the accomplishment of the enzymatic peptide coupling reaction pretreated alcalase-CLEA OM batch 2 was used.

A reflux-condenser, a 10 mL dropping funnel with pressure release, a 25 mL two-necked flask and a magnetic stirring bar were assembled, dried under vacuum with a heat gun, and flushed with argon. Under argon the 3Å molecular sieves (1.5 g) were placed into the dropping funnel together with a cotton plug to prevent that the beads of molecular sieves fall into the reaction solution. The reflux-condenser was connected to a cryostat containing a cooling agent at -15°C (according to Figure 17). Afterwards only the two-necked flask was unconnected form the rest of the apparatus. Now the two necked flask was exposed to air. The rest of the system (reflux-condenser and dropping funnel containing 3Å molecular sieves) was kept under inert conditions. Under non-inert conditions the two-necked flask was charged with alcalase-CLEA (150 mg) and H-Phe-NH_2 (**53**) (14.8 mg, 0.09 mmol, 1.5 eq). Afterwards the two-necked flask was connected again to the dropping funnel with a pressure release under a gentle stream of argon. Due to the gum-like solid form of Z-Leu-Phe-*Z*-anti-Markovnikov-enolesteramide **AM-*Z*-3k** (31.4 mg, 0.06 mmol, 1.0 eq) the compound was dissolved in 3 mL of THF (containing BHT, dried over 3Å molecular sieves (chapter 6.2), 37 ppm H_2O content) in an oven dried Schlenk-flask under argon before the two-necked flask was charged with this stock solution using a syringe under a gentle stream of argon. The magnetic stirrer was adjusted to 100 rpm and the oil bath was heated to 50°C. Immediately the reduced pressure was adjusted to 270 mbar to enable reflux conditions, allowing the recondensing solvent to flow through the molecular sieves back to the reaction flask. 0.9 mL DMSO was added after 23 h before measuring the conversion by HPLC-MS (method D). THF was removed in vacuo using a rotary evaporator. Afterwards DMSO was removed in vacuo (0.02 mbar). Product **54** partially precipitated from THF. The product **54** remaining dissolved in THF was purified by flash column chromatography (DCM/MeOH 20:1).



Yield: 22.4 mg 67 % precipitated, 8.0 mg 24 % isolated by flash column chromatography (combined yield: 30.4 mg, 91 %), white solid
Analytical data are identical to the data shown in chapter 6.6.11.1.

6.6.11.3. Synthesis of Z-Phe-Leu-Phe-NH_2 (63) using Z-Phe-Leu-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3n as starting material

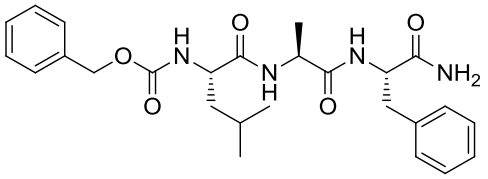


The procedure was the same as described in chapter 6.6.11.2.

Z-Phe-Leu-Z-anti-Markovnikov-enolesteramide **AM-*Z*-3n** (31.4 mg, 0.06 mmol, 1.0 eq), H-Phe-NH_2 (**53**) (14.8 mg, 0.09 mmol, 1.5 eq), THF (3 mL) (containing BHT, dried over 3Å molecular sieves (chapter 6.2), 37 ppm H_2O content), 3Å molecular sieves (1.5 g) and pretreated alcalase-CLEA OM batch 2 (150 mg)

0.9 mL DMSO was added after 23 h before measuring the conversion by HPLC-MS (method K). Due to the not satisfying conversion of starting material **AM-*Z*-3n** of 75 % after 23 h and the presence of a product mixture of (80/15/5): Z-Phe-Leu-Phe-NH_2 (**63**) / Z-Phe-Leu-Phe-Phe-NH_2 (**65**) / Z-Phe-Leu-OH (**1e**), **63** was not isolated and further characterized.

6.6.11.4. Synthesis of Z-Leu-Ala-Phe-NH_2 (64) using Z-Leu-Ala-*Z*-anti-Markovnikov-enolesteramide AM-*Z*-3l as starting material



The procedure was the same as described in chapter 6.6.11.2.

Z-Leu-Ala-Z-anti-Markovnikov-enolesteramide **AM-*Z*-3l** (26.9 mg, 0.06 mmol, 1.0 eq), H-Phe-NH_2 (**53**) (14.8 mg, 0.09 mmol, 1.5 eq), THF (3 mL) (containing BHT, dried over 3Å molecular sieves (Chapter 6.2), 37 ppm H_2O content), 3Å molecular sieves (1.5 g) and pretreated alcalase-CLEA OM batch 2 (150 mg)

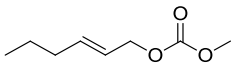
0.9 mL DMSO was added after 23 h before measuring the conversion by HPLC-MS (method C). Although there was an excellent conversion of starting material **AM-*Z*-3l** of >99 % after 23 h but due to the formation of a product mixture of (80/15/5): Z-Leu-Ala-Phe-NH_2 (**64**) / Z-Leu-Ala-Phe-Phe-NH_2 (**66**) / Z-Leu-Ala-OH (**1d**), **64** was not isolated and further characterized.

6.7. Experimental procedures and analytical data for Pd-catalyzed allylic thioether formation

6.7.1. General procedure for the synthesis of allylic carbonates as starting material for Pd-catalyzed allylic thioether formation

A three-necked flask with a magnetic stirring bar, a dropping funnel with pressure release and a pressure compensator were assembled, dried under vacuum with a heat gun, and flushed with argon. The three-necked flask was charged with dry DCM (method B), alcohol (1.0 eq) and dry pyridine (3.0 eq) and cooled to 0°C in an ice-bath. Methyl chloroformate (3.0 eq) was added dropwise to the heavily stirred and ice cold solution. After adding ca. 1/3 of methyl chloroformate a white precipitate formed. After all methyl chloroformate had been added the reaction mixture was warmed to rt and stirred for additional 20 h. The reaction mixture was diluted with diethyl ether (free of stabilisation agent, 1 to 1.5 times the amount of DCM used in the reaction), washed three times consecutively with distilled H_2O (the same amount as diethyl ether used) and one time with brine (the same amount as diethyl ether used). The combined water phases were washed again one time with diethyl ether (1 to 1.5 times the amount of DCM used in the reaction) and dried over Na_2SO_4. After filtration the volatiles were removed in vacuo (15 mbar) using a rotary evaporator. If possible the final purification was accomplished by vacuum distillation yielding the pure product.

6.7.1.1. Synthesis of (*E*)-hex-2-en-1-yl methyl carbonate (67)



Procedure according to chapter 6.7.1.

(*E*)-Hex-2-en-1-ol (**71**) (21.0 mL, 177 mmol, 1.0 eq), dry pyridine (43 mL, 530 mmol, 3.0 eq), methyl chloroformate (**72**) (40 mL, 530 mmol, 3.0 eq), dry DCM (method B) (400 mL).

Purification was accomplished by vacuum distillation (15 mbar) yielding the pure product.

Yield: 24.4 g (87 %), colorless liquid

Bp: 65°C (15 mbar)

$C_8H_{14}O_3$ [158.20 $gmol^{-1}$]

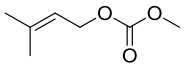
GC-MS (HS_50_S2): t_R = 4.26 min; *m/z* = 55 (58 %), 59 (39 %), 67 (100 %, BP), 71 (62 %), 77 (12 %), 82 (50 %), 83 (35 %), 85 (9 %), 99 (4 %), 115 (2 %), 158 (2 %, MP).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, 3H, ^3J(H,H) = 7.4 Hz, CH_2-C<u>H</u>$_3$), 1.40 (pseudo sext, 2H, ^3J(H,H) = 7.5 Hz, C<u>H</u>$_2$-CH_3), 2.02 (pseudo q, 2H, ^3J(H,H) = 7.1 Hz, CH_3-CH_2-C<u>H</u>$_2$-CH=CH), 3.75 (s, 3H, -OCO_2-C<u>H</u>$_3$), 4.48 (dd, 2H, ^3J(H,H) = 6.4 Hz, ^4J(H,H) = 0.9 Hz, CH=CH-C<u>H</u>$_2$-OCO_2-CH_3), 5.57 (dtt, 1H, ^3J(H,H) = 15.4 Hz, 6.5 Hz, ^4J(H,H) = 1.3 Hz, CH=C<u>H</u>-CH_2-OCO_2-CH_3), 5.80 (dt, 1H, ^3J(H,H) = 14.4 Hz, 6.7 Hz, C<u>H</u>=CH-CH_2-OCO_2-CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.7 (CH_2-<u>C</u>H$_3$), 22.1 (<u>C</u>H$_2$-CH_3), 34.4 (CH_3-CH_2-<u>C</u>H$_2$-CH=CH-), 54.8 (OCO_2-<u>C</u>H$_3$), 68.8 (<u>C</u>H$_2$-OCO_2-CH_3), 123.5 (CH=<u>C</u>H-CH_2-OCO_2-CH_3), 137.4 (<u>C</u>H=CH-CH_2-OCO_2-CH_3), 155.8 (O<u>C</u>O_2-CH_3).

Characterized by Marsden *et.al*.[294]

6.7.1.2. Synthesis of methyl (3-methylbut-2-en-1-yl) carbonate (88)



Procedure according to chapter 6.7.1.

3-Methylbut-2-en-1-ol (**87**) (8.30 mL, 81.3 mmol, 1.0 eq), dry pyridine (19.7 mL, 244 mmol, 3.0 eq), methyl chloroformate (**72**) (18.7 mL, 244 mmol, 3.0 eq), dry DCM (method B) (180 mL).

Purification was accomplished by vacuum distillation (15 mbar) yielding the pure product.

Yield: 10.94 g (76.2 mmol, 94 %), colorless liquid

Bp: 64°C (15 mbar)

$C_7H_{12}O_3$ [144.08 gmol^{-1}]

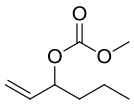
GC-MS (HS_50_S2): t_R = 3.87 min; *m/z* = 85 (21 %), 69 (65 %), 68 (100 %, BP), 67 (83 %), 59 (24 %), 53 (43 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.64 (s, 3H, (C<u>H</u>$_3$)$_2$-C_q=CH), 1.69 (s, 3H, (C<u>H</u>$_3$)$_2$-C_q=CH), 3.70 (s, 3H, C<u>H</u>$_3$-O), 4.55 (d, 2H, ^3J(H,H) = 7.5 Hz, C<u>H</u>$_2$-O), 5.30 (m, 1H, (CH_3)$_2$-C_q=C<u>H</u>).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0 (($\underline{C}H_3)_2$-C_q=CH), 25.7 (($\underline{C}H_3)_2$-C_q=CH), 54.6 ($\underline{C}H_3$-O), 64.7 ($\underline{C}H_2$-O), 118.0 ($(CH_3)_2$-C_q=$\underline{C}$H), 140.0 ($(CH_3)_2$-$\underline{C}_q$=CH), 155.9 ($\underline{C}O_3$).

Characterized by Watanabe *et al.*[295]

6.7.1.3. Synthesis of hex-1-en-3-yl methyl carbonate (139)



Procedure according to chapter 6.7.1.

Hex-1-en-3-ol (**e1**) (3.6 mL, 30.0 mmol, 1.0 eq), dry pyridine (7.30 mL, 89.9 mmol, 3.0 eq), methyl chloroformate (**72**) (7.00 mL, 89.9 mmol, 3.0 eq), dry DCM (method B) (65 mL).

Purification was accomplished by vacuum distillation (15 mbar) yielding the pure product.

Yield: 3.5 g (22.1 mmol, 74 %), colorless liquid

Bp: 56°C (15 mbar)

$C_8H_{14}O_3$ [158.09 $gmol^{-1}$]

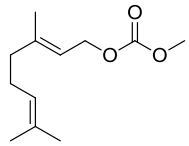
GC-MS (HS_50_S2): t_R = 3.83 min; m/z = 83 (30 %), 82 (52 %), 71 (100 %, BP), 67 (94 %), 59 (56 %), 55 (83 %), 54 (24 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.91 (t, 3H, ^3J(H,H) = 7.4 Hz, $C\underline{H}_3$-CH_2), 1.36 (m, 2H, $C\underline{H}_2$), 1.62 (m, 2H, $C\underline{H}_2$), 3.75 (s, 3H, $C\underline{H}_3$-O), 5.04 (q, 1H, ^3J(H,H) = 6.6 Hz, C$\underline{H}$-O), 5.18 (d, 1H, ^3J(H,H) = 10.5 Hz, CH=C$\underline{H}_2$), 5.27 (d, 1H, ^3J(H,H) = 18.3 Hz, CH=C$\underline{H}_2$), 5.78 (m, 1H, C$\underline{H}$=CH_2).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.7 ($C\underline{H}_3$-CH_2), 18.2 ($C\underline{H}_2$), 36.2 ($C\underline{H}_2$), 54.5 ($C\underline{H}_3$-O), 78.9 (C$\underline{H}$-O), 117.2 (CH=$C\underline{H}_2$), 136.0 (C$\underline{H}$=CH_2), 155.3 (CO_3).

Characterized by Sandri *et al.*[296]

6.7.1.4. Synthesis of (*E*)-3,7-dimethylocta-2,6-dien-1-yl methyl carbonate (140)



Procedure according to chapter 6.7.1.

Geraniol (**e2**) (2.80 mL, 16.2 mmol, 1.0 eq), dry pyridine (3.90 mL, 48.4 mmol, 3.0 eq), methyl chloroformate (**72**) (3.70 mL, 48.4 mmol, 3.0 eq), dry DCM (method B) (55 mL).

The reaction temperature was -40°C, maintained by a cryostat.

Purification was accomplished by vacuum distillation (0.2 mbar) yielding the pure product.

Yield: 3.30 g (15.5 mmol, 95 %), colorless liquid

Bp: 57°C (0.2 mbar)

R_f = 0.67 (cyclohexane/ethyl acetate = 5:1 (v/v))

$C_{12}H_{20}O_3$ [212.14 g.mol^{-1}]

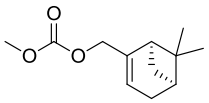
GC-MS (HS_50_S2): t_R = 5.69 min; *m/z* = 121 (15 %), 93 (22 %), 81 (16 %), 69 (100 %, BP), 68 (38 %), 67 (38 %), 59 (21 %), 53 (30 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.59 (s, 3H, C$\underline{H}_3$-C_q=CH), 1.67 (s, 3H, C$\underline{H}_3$-C_q=CH), 1.71 (s, 3H, C$\underline{H}_3$-C_q=CH), 2.02-2.11 (m, 4H, C$\underline{H}_2$), 3.77 (s, 3H, C$\underline{H}_3$-O), 4.65 (d, 2H, ^3J(H,H) = 7.2 Hz, C$\underline{H}_2$-O), 5.03-5.10 (m, 1H, CH_3-C_q=C$\underline{H}$), 5.37 (dt, 1H, ^3J(H,H) = 7.2 Hz, ^4J(H,H) = 1.2 Hz, CH_3-C_q=C$\underline{H}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.5 ($\underline{C}H_3$-C_q=CH), 17.6 ($\underline{C}H_3$-C_q=CH), 25.6 ($\underline{C}H_3$-C_q=CH), 26.2 ($\underline{C}H_2$), 39.5 ($\underline{C}H_2$), 54.6 ($\underline{C}H_3$-O), 64.7 ($\underline{C}H_2$-O), 117.7 (O-CH_2-$\underline{C}$H=C_q), 123.7 (-$\underline{C}$H=C_q), 131.9 (-CH=$\underline{C}_q$), 143.2 (-CH=$\underline{C}_q$), 155.9 ($\underline{C}O_3$).

Characterized by Ohashi *et al.*[297]

6.7.1.5. Synthesis of ((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl-methylcarbonate (146)



Procedure according to chapter 6.7.1.

((1*R*,5*S*)-6,6-Dimethylbicyclo-[3.1.1]hept-2-en-2-yl)methanol (**e3**) (5.10 mL, 32.9 mmol, 1.0 eq), dry pyridine (8.00 mL, 98.7 mmol, 3.0 eq), methyl chloroformate (**72**) (7.60 mL, 98.7 mmol, 3.0 eq), dry DCM (method B) (70 mL).

After extraction and drying the obtained pale yellow oil was not further purified. All volatile compounds were removed in vacuo (0.02 mbar)

Yield: 6.56 g (31.2 mmol, 96 %), pale yellow oil

$C_{12}H_{18}O_3$ [210.13 g.mol^{-1}]

$[\alpha]_D^{20}$ = -48.4 (c = 1 in Acetonitril)

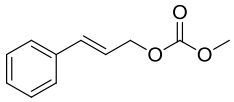
GC-MS (HS_50_S2): t_R = 5.53 min; *m/z* = 134 (11 %), 119 (44 %), 105 (14 %), 93 (18 %), 92 (29 %), 91 (100 %, BP), 79 (28 %), 77 (26 %), 65 (12 %), 53 (12 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.81 (s, 3H, C$\underline{H}_3$), 1.18 (d, 1H, ^3J(H,H) = 8.7 Hz, $\underline{H}$-alkyl), 1.28 (s, 3H, C$\underline{H}_3$), 2.09 – 2.43 (m, 5H, $\underline{H}$-alkyl), 3.76 (s, 3H, C$\underline{H}_3$-O), 4.49 (m, 2H, C$\underline{H}_2$-O), 5.60 (m, 1H, C$\underline{H}$=C$_q$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 20.9 (C$\underline{H}_3$), 26.0 (C$\underline{H}_3$), 31.3 (C-alkyl), 31.5 (C-alkyl), 38.0 (C-alkyl), 40.6 (C-alkyl), 43.4 (C-alkyl), 54.6 (C$\underline{H}_3$-O), 70.5 (C$\underline{H}_2$-O), 122.4 (C$_q$=C$\underline{H}$), 142.4 (C$_q$=CH), 155.8 (C$\underline{O}_3$).

Characterized by Watanabe *et al.*[295]

6.7.1.6. Synthesis of cinnamyl methyl carbonate (145)



Procedure according to chapter 6.7.1.

(*E*)-3-Phenylprop-2-en-1-ol (**e4**) (5.00 g, 37.3 mmol, 1.0 eq), dry pyridine (8.6 mL, 112 mmol, 3.0 eq), methyl chloroformate (**72**) (9.0 mL, 112 mmol, 3.0 eq), dry DCM (method B) (80 mL).

After extraction and drying the obtained pale yellow oil was not further purified. All volatile compounds were removed in vacuo (0.02 mbar)

Yield: 7.16 g (37.3 mmol, 99 %), pale yellow oil, which solidifies upon cooling to give white crystals

$C_{11}H_{12}O_3$ [192.08 g.mol^{-1}]

mp = 24 - 25 °C

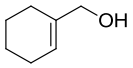
GC-MS (HS_50_S2): t_R = 5.13 min; *m/z* = 192 (16 %, MP), 133 (15 %), 117 (76 %), 116 (59 %), 115 (100 %, BP), 105 (35 %), 91 (29 %), 77 (31 %), 51 (21 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.84 (s, 3H, C$\underline{H}_3$-O), 4.83 (dd, 2H, ^3J(H,H) = 12.6 Hz, ^4J(H,H) = 1.2 Hz, C$\underline{H}_2$-O), 6.34 (td, 1H, ^3J(H,H) = 15.9 Hz, ^3J(H,H) = 6.3 Hz, C$\underline{H}$), 6.73 (d, 1H, ^3J(H,H) = 15.6 Hz, C$\underline{H}$), 7.28-7.45 (m, 5H, $\underline{H}$-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 54.4 ($\underline{C}H_3$-O), 67.9 ($\underline{C}H_2$-O), 122.0 (CH_2-$\underline{C}$H=CH-Ph), 126.2 (2 × $\underline{C}$-Ar), 127.7 ($\underline{C}$-Ar), 128.1 (2 × $\underline{C}$-Ar), 134.3 (CH_2-CH=$\underline{C}$H-Ph), 135.6 ($C_{q,Ar}$), 155.2 ($\underline{C}O_3$).

Characterized by Ohashi *et al.*[297]

6.7.1.7. Synthesis of cyclohex-1-en-1-ylmethanol (143)



A 250 mL two-necked flask with a magnetic stirring bar and a pressure compensator were assembled, dried under vacuum with a heat gun, and flushed with argon. The two-necked flask was charged with DIBAL 1M solution in pentane (33.0 mL, 32.8 mmol, 2.3 eq) and 40 mL of dry DCM (method B). This solution was cooled to -78°C using acetone and dry ice for the cooling bath. Under inert conditions methylcyclohex-1-encarboxylate (**142**) (1.95 mL, 14.3 mmol, 1.0 eq) was added drop wise using a syringe. Afterwards this solution was stirred at -78°C for additional 25 min. The excess of DIBAL was destroyed carefully by adding 20 mL of methanol and 40 mL of a 15 % NaOH to the -78°C solution. The reaction mixture became slightly hot and a formation of white precipitate could be observed. The two layers were separated using a separating funnel and the water phase was washed again with 10 ml of DCM. The combined organic phases were dried using $MgSO_4$ and volatile compounds were removed using a rotary evaporator. The greenish crude product was purified via flash column chromatography (180 g silica gel, 15 x 7 cm, cyclohexane/ethyl acetate = 5:1 (v/v), Rf = 0.32).

Yield: 1.40 g (12.5 mmol, 87 %), pale yellow oil

$C_7H_{12}O$ [112.09 $g.mol^{-1}$]

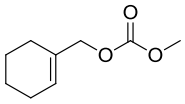
GC-MS (HS_50_S2): t_R = 3.99 min; *m/z* = 112 (46 %, MP), 94 (24 %), 83 (25 %), 81 (66 %), 79 (100 %, BP), 77 (29 %), 67 (22 %), 55 (32 %), 53 (30 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.56-1.64 (m, 5H, H-alkyl), 2.00-2.02 (m, 4H, H-alkyl), 3.96 (s, 2H, CH_2-OH), 5.67 (m, 1H, CH=C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.0 (C-alkyl), 22.1 (C-alkyl), 24.5 (C-alkyl), 25.1 (C-alkyl), 67.2 (CH_2-OH), 122.5 (CH=C_q), 137.1 (CH=C_q).

Characterized by Hartman *et al.*[298]

6.7.1.8. Synthesis of cyclohex-1-en-1-ylmethyl methyl carbonate (144)



Procedure according to chapter 6.7.1.

Cyclohex-1-en-1-ylmethanol (**143**) (1.43 mL, 12.3 mmol, 1.0 eq), dry pyridine (2.98 mL, 37.0 mmol, 3.0 eq), methyl chloroformate (**72**) (2.86 mL, 37.0 mmol, 3.0 eq), dry DCM (method B) (25 mL).

Purification was accomplished by vacuum distillation (15 mbar) yielding the pure product.

Yield: 1.83 g (10.8 mmol, 97 %), colorless liquid

$C_9H_{14}O_3$ [170.09 g.mol^{-1}]

b_p = 90 °C / 15 mbar

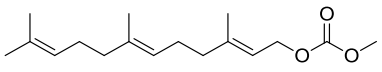
HRMS (TOF MSEI+):	calculated:	170.0943
	found:	170.0938

GC-MS (HS_50_S2): t_R = 4.98 min; *m/z* = 95 (29 %), 94 (38 %), 79 (100 %, BP), 77 (20 %), 67 (15 %), 55 (12 %), 53 (13 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.54-1.68 (m, 4H, H-alkyl), 2.00-2.02 (m, 4H, H-alkyl), 3.78 (s, 3H, CH_3-O), 4.48 (s, 2H, CH_2-O), 5.76 (m, 1H, CH=C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.2 (C-alkyl), 22.5 (C-alkyl), 25.2 (C-alkyl), 26.0 (C-alkyl), 54.9 (CH_3-O), 72.7 (CH_2-O), 127.5 (CH=C_q), 132.6 (CH=C_q), 156.1 (CO_3).

6.7.1.9. Synthesis of methyl ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) carbonate (farnesyl carbonate) (141)



Procedure according to chapter 6.7.1.

(*E*,*E*)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol,*trans*,*trans*-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol (*trans,trans*-farnesol) (**e5**) (1.02 mL, 4.05 mmol, 1.0 eq), dry pyridine (0.99 mL, 12.1 mmol, 3.0 eq), methyl chloroformate (**72**) (0.94 mL, 12.1 mmol, 3.0 eq), dry DCM (method B) (25 mL).

The reaction temperature was -40°C, maintained by a cryostat.

Purification was accomplished by vacuum distillation (0.04 mbar) yielding the pure product.

Yield: 842 mg (74 %), colorless liquid

$C_{17}H_{28}O_3$ [280.40 g.mol^{-1}]

b_p = 110°C / 0.04 mbar

GC-MS (HS_50_S28): t_R = 6.997 min; *m/z* (EI) = 280 (1 %, MP), 136 (16 %), 121 (7 %), 107 (22 %), 93 (48 %), 21 (36 %), 69 (100 %, BP), 53 (20 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.59 (s, 6H, 2 × C$\underline{H}_3$), 1.67 (s, 3H, C$\underline{H}_3$), 1.72 (s, 3H, C$\underline{H}_3$), 1.91-2.18 (m, 8H, 4 × C$\underline{H}_2$), 3.77 (s, 3H, OC$\underline{H}_3$), 4.65 (d, 2H, ^3J(H,H) = 7.2 Hz, OC$\underline{H}_2$), 5.08 (m, 2H, 2 × C$\underline{H}$), 5.37 (m, 1H, OCH$_2$C$\underline{H}$).

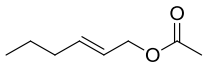
^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.0 (C-alkyl), 16.5 (C-alkyl), 17.6 (C-alkyl), 25.7 (C-alkyl), 26.1 (C-alkyl), 26.7 (C-alkyl), 39.5 (C-alkyl), 39.7 (C-alkyl), 54.6 (O$\underline{C}$H$_3$), 64.7(O-$\underline{C}$H$_2$-CH=C(CH$_3$)-$\underline{C}$H$_2$-), 117.7 (O-CH$_2$-$\underline{C}$H=C$_q$), 123.5 (-$\underline{C}$H=C$_q$), 124.3 (-$\underline{C}$H=C$_q$), 131.3 (-CH=$\underline{C}_q$), 135.5 (-CH=$\underline{C}_q$), 143.2 (-CH=$\underline{C}_q$), 155.9 (CO$_3$).

Characterized by Ahmad *et al.*[299]

6.7.2. General procedure for the synthesis of allylic acetates as starting material forPd-catalyzed thioether formation

A dropping funnel with pressure release, a three-necked flask with a magnetic stirring bar and a pressure compensator were assembled, dried under vacuum with a heat gun, and flushed with argon. The three-necked flask was charged with dry DCM (method B), alcohol (1.0 eq) and dry pyridine (3.0 eq) and cooled to 0°C in an ice-bath. Acetyl chloride (**85**) (3.0 eq) was added dropwise to the heavily stirred and ice cold solution. Immediately, after adding acetyl chloride (**85**) a white precipitate formed. After all acetyl chloride (**85**) had been added the reaction mixture was warmed to rt and stirred for additional 20 h. The reaction mixture was diluted with diethyl ether (free of stabilisation agent, 1 to 1.5 times the amount of DCM used in the reaction), washed three times consecutively with distilled H_2O (the same amount as diethyl ether used) and one time with brine (the same amount as diethyl ether used) and dried over Na_2SO_4. After filtration the volatiles were removed in vacuo (15 mbar) using a rotary evaporator. If possible the final purification was accomplished by vacuum distillation yielding the pure product.

6.7.2.1. Synthesis of (*E*)-hex-2-en-1-yl acetate (86)



Procedure according to chapter 6.7.2.

(*E*)-Hex-2-en-1-ol (**71**) (7.1 mL, 60 mmol, 1.0 eq), dry pyridine (14.5 mL, 180 mmol, 3.0 eq), acetyl chloride (**85**) (12.8 mL, 180 mmol, 3.0 eq), dry DCM (method B) (150 mL).

Purification was accomplished by vacuum distillation (15 mbar) yielding the pure product.

Yield: 8.5 g (quantitative), colorless liquid

Bp: 50°C (15 mbar)

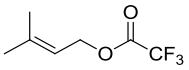
$C_8H_{14}O_2$ [142.10 $gmol^{-1}$]

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, 3H, ^3J(H,H) = 7.4 Hz, CH_2-C<u>H</u>$_3$), 1.40 (pseudo sext, 2H, ^3J(H,H) = 7.5 Hz, C<u>H</u>$_2$-CH_3), 1.96-2.05 (m, 5H, CH_3-CH_2-C<u>H</u>$_2$-CH=CH, -OCOC<u>H</u>$_3$), 3.76 (s, 3H, OCO_2C<u>H</u>$_3$), 4.56 (dd, 2H, ^3J(H,H) = 6.5 Hz, ^4J(H,H) = 0.8 Hz, CH=CH-C<u>H</u>$_2$-OCO_2-CH_3), 5.54 (dtt, 1H, ^3J(H,H) = 15.4 Hz, 6.5 Hz, ^4J(H,H) = 1.4 Hz, CH=C<u>H</u>-CH_2-OCO_2-CH_3), 5.75 (dtt, 1H, ^3J(H,H) = 12.3 Hz, 6.6 Hz, ^4J(H,H) = 1.1 Hz , C<u>H</u>=CH-CH_2-OCO_2-CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.7 (CH_2-$\underline{C}H_3$), 21.1 ($C\underline{H}_2$-OCO-$\underline{C}H_3$), 22.2 ($\underline{C}H_2$-CH_3), 34.4 (CH_3-CH_2-$\underline{C}H_2$-CH=CH-), 65.4 ($C\underline{H}_2$-OCO-$\underline{C}H_3$), 124.0 (CH=$\underline{C}$H-CH_2-OCO_2-CH_3), 136.5 ($\underline{C}$H=CH-CH_2-OCO_2-CH_3), 170.9 (O$\underline{C}O_2$-CH_3).

Characterized by Sjögren *et al.*[300]

6.7.2.2. Synthesis of methyl-3-methylbut-2-enyl 2,2,2-trifluoroacetate (92)



Procedure according to chapter 6.7.2.

3-Methylbut-2-en-1-ol (**87**) (1.2 mL, 11.6 mmol, 1.0 eq), dry pyridine (1.4 mL, 17.4 mmol, 1.5 eq), trifluoroacetic anhydride (**91**) (2.4 mL, 17.4 mmol, 1.5 eq), dry DCM (method B) (24 mL).

Purification was accomplished by vacuum distillation (10 mbar) yielding the pure product.

Yield: 342.6 mg (16 %), colorless liquid

Bp: 39°C (10 mbar)

$C_7H_9F_3O_2$ [182.14 $gmol^{-1}$]

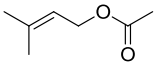
GC-MS (HS_50_S2): t_R =2.48 min; *m/z* = 53 (35 %), 67 (60 %), 68 (70 %), 69 (100 %, BP), 167 (3 %), 182 (2 %, MP).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.75 (s, 3H, -C$\underline{H}_3$), 1.79 (s, 3H,-C$\underline{H}_3$), 4.83 (d, 2H, ^3J(H,H) = 7.5 Hz, -C$\underline{H}_2$-CH=C$(CH_3)_2$), 5.39 (t, 1H, ^3J(H,H) = 7.5 Hz, -CH_2-C$\underline{H}$=C$(CH_3)_2$).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 18.2 (-$\underline{C}H_3$), 25.9 (-C$\underline{H}_3$), 65.0 (CF_3-CO_2-$\underline{C}H_2$-), 111.3, 113.6, 115.6, 118.2 (q, ^1J(C,F) = 248.0 Hz, -CO_2-$\underline{C}F_3$), 116.4 (-$\underline{C}$H=C$(CH_3)_2$), 142.7 (-CH=$\underline{C}$$(CH_3)_2$), 157.2, 157.5, 157.9, 158.2 (q, ^2J(C,F) = 42.0 Hz, -$\underline{C}O_2$-CF_3).

Characterized by Mellor *et al.*[301]

6.7.2.3. Synthesis of 3-methylbut-2-en-1-yl acetate (138)



Procedure according to chapter 6.7.2.

3-Methylbut-2-en-1-ol (**87**) (3.50 mL, 34.8 mmol, 1.0 eq), dry pyridine (8.40 mL, 105.0 mmol, 3.0 eq), acetyl chloride (**85**) (7.5 mL, 105.0 mmol, 3.0 eq), dry DCM (method B) (65 mL).

Purification was accomplished by vacuum distillation (15 mbar) yielding the pure product.

Yield: 530 mg (4.14 mmol, 12 %), colorless liquid

Bp: 30°C (15 mbar)

$C_7H_{13}O_2$ [128.08 g.mol^{-1}]

GC-MS (HS_50_S2): t_R = 3.39 min; *m/z* = 86 (15 %), 71 (31 %), 69 (41 %), 68 (100 %, BP), 67 (82 %), 57 (11 %), 53 (42 %).

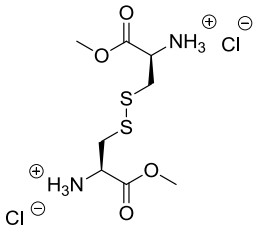
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.69 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 1.75 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 2.03 (s, 3H, CH_3-O), 4.55 (d, 2H, ^3J(H,H) = 7.2 Hz, CH_2-O), 5.33 (m, 1H, $(CH_3)_2$-C_q=C$\underline{H}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.9 ($(\underline{C}H_3)_2$-C_q=CH), 21.0 ($\underline{C}H_3$), 25.7 ($\underline{C}H_3$), 61.3 ($\underline{C}H_2$-O), 118.5 ($(CH_3)_2$-C_q=$\underline{C}$H), 139.0 ($(CH_3)_2$-$\underline{C}_q$=CH), 171.0 (CH_3-$\underline{C}O_2$).

Characterized by Webb et al.[302]

6.7.3. Further syntheses of differently protected L–cysteine derivatives as starting materials for Pd-catalyzed thioether formation

6.7.3.1. Synthesis of L-cystine 1,1'-dimethyl ester hydrochloride (95)



Procedure according to Boucher *et al.*[261]

A 500 mL three-necked flask with a magnetic stirring bar, a dropping funnel with pressure release, a reflux-condenser and a pressure compensator were assembled. The three-necked flask was charged with methanol (240 ml) and cooled to 0°C in an ice-bath. Thionyl chloride (**94**) (15 mL, 206 mmol, 3.3 eq) was added dropwise to the heavily stirred and ice cold methanol. Afterwards L-cystine (**93**) was added at once and the mixture was refluxed for 20 h.

The volatile compounds were evaporated in vacuo using a rotary evaporator yielding a white solid, which was recrystallized from MeOH (100 mL) by dissolving it at reflux temperature, cooling it to 0°C and then adding Et_2O (100 mL) according to literature procedure from Photaki *et al.*[262]

Yield: 21.0 g (61.5 mmol, 98 %), colorless solid

$C_8H_{18}Cl_2N_2O_4S_2$ [341.28 $gmol^{-1}$]

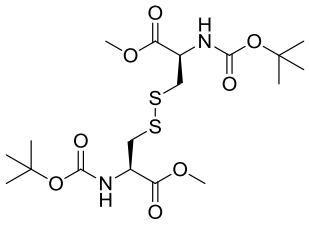
mp: 166-170°C

^{1}H-NMR (300 MHz, D_2O): δ = 3.33-3.47 (m, 4H, -C<u>H</u>$_2$-S-S-C<u>H</u>$_2$-), 3.88 (s, 6H, 2 × CO_2-C<u>H</u>$_3$), 4.58-4.62 (m, 2H, 2 × -S-CH_2-C<u>H</u>(NH_3Cl)-CO_2Me).

^{13}C-NMR (75 MHz, D_2O): δ = 35.7 (2 × C, -<u>C</u>H$_2$-S-S-<u>C</u>H$_2$-), 51.6 (2 × C, <u>C</u>H or <u>C</u>H$_3$), 54.0 (2 × C, <u>C</u>H or <u>C</u>H$_3$), 169.1 (2 × C, -CH-<u>C</u>O$_2$-CH$_3$).

Characterized by Sastry *et al.*[303]

6.7.3.2. Synthesis of L-cystine *N*,*N*'-bis[(1,1-dimethylethoxy)carbonyl]-, 1,1'-dimethyl ester (97)



Procedure according to Boucher *et al.*[261]

To a stirred solution of L-cystine dimethyl ester dihydrochloride (**95**) (6.9 g, 20.2 mmol, 1.0 eq) in 1M NaOH (40.4 mL) and dioxane/water (2:1, 60 mL), di-*tert*-butyl pyrocarbonate (**96**) (10.1 g, 46.3 mmol, 2.23 eq) was added in one portion at 0°C. The ice bath was removed, and the solution was stirred for 24 h at room temperature. After concentrating the mixture to ~50 mL using a rotary evaporator, ethyl acetate (60 mL) was added. The pH of the mixture was adjusted to 3.0 with aqueous 2M $KHSO_4$ (5 mL). The aqueous layer was extracted with ethyl acetate (2 × 40 mL) and the combined organic layers were washed with brine (40 mL) and water (40 mL), and dried over $MgSO_4$. Evaporation of ethyl acetate in vacuo using a rotary evaporator yielding a white solid, which was recrystallized from ethyl acetate (5 mL) by dissolving it at reflux temperature, cooling it to rt and then adding pentane (10 mL).

Yield: 7.75 g (16.5 mmol, 82 %), colorless solid

$C_{18}H_{32}N_2O_8S_2$ [468.59 $gmol^{-1}$]

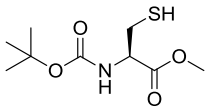
mp: 95-97°C

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.43 (s, 18H, 2 × -C($C\underline{H}_3$)$_3$), 3.15 (d, 4H, -$C\underline{H}_2$-S-S-$C\underline{H}_2$-), 3.75 (s, 6H, 2 × CO_2-$C\underline{H}_3$), 4.58-4.59 (m, 2H, 2 × -C<u>H</u>-NH-), 5.39 (d, 2H, ^3J(H,H) = 6.9 Hz, -CH-N<u>H</u>-).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.4 (6 × C, -C($\underline{C}H_3$)$_3$), 41.4 (2 × C, -$\underline{C}H_2$-S-S-$\underline{C}H_2$-), 52.8 (2 × C, <u>C</u>H or $\underline{C}H_3$), 52.9 (2 × C, <u>C</u>H or $\underline{C}H_3$), 80.4 (2 × C, -$\underline{C}(CH_3)_3$), 155.2 (2 × C, -NH-$\underline{C}O_2$-$C(CH_3)_3$), 171.3 (2 × C, -CH-$\underline{C}O_2$-CH_3).

Characterized by Boucher *et al.*[261]

6.7.3.3. Synthesis of L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (98)



Procedure according to Boucher *et al.*[261]

L-Cystine *N,N'*-bis[(1,1-dimethylethoxy)carbonyl]-, 1,1'-dimethyl ester (**97**) (2.5 g, 5.34 mmol, 1.0 eq), triphenylphosphine (**10**) (1.48 g, 5.49 mmol, 1.05 eq), and sodium acetate (0.17 g, 2.11 mmol, 0.4 eq) were suspended in a mixture of methanol (20 mL), water (10 mL). A white precipitate was formed. After adding glacial acetic acid (0.17 mL) the precipitate was dissolved again. Afterwards it was heated under reflux for 24 h. The mixture was diluted with 260 mL CH_2Cl_2, washed with 100 mL of water, 50 mL of saturated sodium chloride solution, and dried over $MgSO_4$. Ater filtration the solvent was removed in vacuo using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (270 g silica gel, 22 × 5.7 cm, cyclohexane/ethyl acetate = 4:1 (v/v), R_f = 0.4).

Yield: 1.67 g (7.1 mmol, 67 %), yellow oil

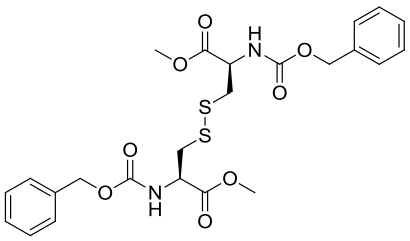
$C_9H_{17}NO_4S$ [235.30 $gmol^{-1}$]

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.38 (t, 1H, ^3J(H,H) = 8.9 Hz, -CH-S<u>H</u>), 1.43 (s, 9H, -C(C<u>H</u>$_3$)$_3$), 2.93-2.97 (m, 2H, -C<u>H</u>$_2$-SH), 3.76 (s, 3H, CO_2-C<u>H</u>$_3$), 4.58 (m, 1H, -C<u>H</u>-NH-), 5.42 (d, 1H, ^3J(H,H) = 4.5 Hz, -CH-N<u>H</u>-).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 27.4 (-<u>C</u>H$_2$-SH), 28.4 (-C(<u>C</u>H$_3$)$_3$), 52.8 (<u>C</u>H or <u>C</u>H$_3$), 55.0 (<u>C</u>H or <u>C</u>H$_3$), 80.4 (-<u>C</u>(CH$_3$)$_3$), 155.2 (-NH-<u>C</u>O$_2$-C(CH$_3$)$_3$), 170.9 (-CH-<u>C</u>O$_2$-CH$_3$).

Characterized by Boucher *et al.*[261]

6.7.3.4. Synthesis of L-cystine *N*,*N*'-bis[(phenylmethoxy)carbonyl] 1,1' dimethyl ester (101)



Procedure according to Truong *et al.*[263]

A 100 mL three-necked flask with a magnetic stirring bar, dropping funnel with pressure release and a thermometer were assembled. The three-necked flask was charged with L-cystine 1,1'-dimethyl ester hydrochloride (**95**) (7.0 g, 20.5 mol. 1.0 eq), potassium bicarbonate (17.0 g, 123.1 mmol, 6 eq), distilled water (40 mL), and diethyl ether (35 mL), and the solution is cooled to 0°C (ice-bath). Benzyl chloroformate (**99**) (6.41 mL, 45.1 mmol, 2.2 eq) was added dropwise over 30 min, the cooling bath was removed and the solution was stirred at rt for 5 h. Glycine (**100**) (0.46 g, 6.15 mmol, 0.3 eq) was added (to scavenge excess chloroformate) and the solution was stirred for an additional 18 h. The organic layer was separated and the aqueous layer was extracted with diethyl ether (2 × 15 mL). The combined organic layers were washed with 0.01 M hydrochloric acid (2 × 25 mL) water (2 × 25 mL), and saturated brine (25 mL) and then dried (Na_2SO_4), filtered and concentrated using a rotary evaporator. The resulting oil was further dried in a Kugelrohr oven (50°C, 0.05 mbar, 5 h) to leave product **101** as a clear oil that solidifies upon cooling (gum-like solid).

Yield: 7.90 g (14.7 mmol, 72 %), off-white, gum-like solid

$C_{24}H_{28}N_2O_8S_2$ [536.62 $gmol^{-1}$]

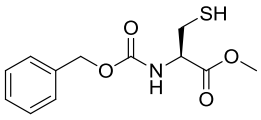
R_f = 0.24, cyclohexane/ethyl acetate = 2:1 (v/v)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.16 (d, 2H, ^3J(H,H) = 5.0 Hz, -C$\underline{H}_2$-S-S-C$\underline{H}_2$-), 3.74 (s, 3H, CO_2-C$\underline{H}_3$), 4.66 (pseudo q, 2H, ^3J(H,H) = 7.8 Hz, 5.1 Hz, -C$\underline{H}$-NH-), 5.11 (s, 4H, -NH-CO_2-C$\underline{H}_2$-C_6H_5), 5.72 (d, 1H, ^3J(H,H) = 7.7 Hz, -CH-N$\underline{H}$-), 7.30-7.35 (m, 10H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 41.2 (2 × C, -$\underline{C}H_2$-S-S-$\underline{C}H_2$-), 52.9 (2 × C, $\underline{C}$H or $\underline{C}H_3$), 53.4 (2 × C, $\underline{C}$H or $\underline{C}H_3$), 67.3 (2 × C, -NH-CO_2-$\underline{C}H_2$-C_6H_5), 128.3 (4 × C_{Ar}), 128.4 (2 × C_{Ar}), 128.6 (4 × C_{Ar}), 136.2 (2 × $C_{q,Ar}$), 155.8 (2 × C, -NH-$\underline{C}O_2$-CH_2-C_6H_5), 170.9 (2 × C, -CH-$\underline{C}O_2$-CH_3).

Characterized by Clive *et al.*[304]

6.7.3.5. Synthesis of L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (102)



Procedure according to Boucher *et al.*[261]

L-Cystine *N,N'*-bis[(phenylmethoxy)carbonyl]-, 1,1'-dimethyl ester (**101**) (4.65 g, 8.67 mmol, 1.0 eq), triphenylphosphine (**10**) (2.39 g, 8.76 mmol, 1.05 eq), and sodium acetate (0.28 g, 3.46 mmol, 0.4 eq) were suspended in a mixture of methanol (32.5 mL) and water (16.25 mL). A white precipitate formed. After adding glacial acetic acid (0.28 mL) the precipitate dissolved again. Afterwards it was heated under reflux for 24 h. The mixture was diluted with 475 mL CH_2Cl_2, washed with water (2 × 200 mL), saturated sodium chloride solution (2 × 200 mL) and dried over $MgSO_4$. The solvent was removed in vacuo using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (625 g silica gel, 25 × 8.0 cm, cyclohexane/ethyl acetate = 4:1 (v/v), R_f = 0.21).

Yield: 3.88 g (14.4 mmol, 83 %), white solid

$C_{12}H_{15}NO_4S$ [269.32 $gmol^{-1}$]

$[\alpha]_D^{20}$ = -26.7 (c = 0.5 in DMSO)

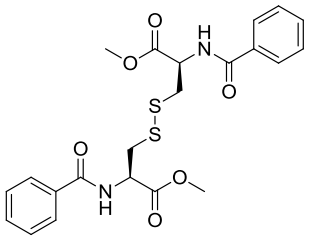
mp.: 20-25°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.39 (t, 1H, ^3J(H,H) = 9.0 Hz, -CH-S<u>H</u>), 2.98-3.02 (m, 2H, -C<u>H</u>$_2$-SH), 3.78 (s, 3H, CO_2-C<u>H</u>$_3$), 4.68 (dt, 1H, ^3J(H,H) = 7.8 Hz, 4.0 Hz, -C<u>H</u>-NH-), 5.13 (s, 2H, -NH-CO_2-C<u>H</u>$_2$-C_6H_5), 5.71 (d, 1H, ^3J(H,H) = 6.9 Hz, -CH-N<u>H</u>-), 7.32-7.36 (m, 5H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 27.3 (-<u>C</u>H$_2$-SH), 52.9 (<u>C</u>H or <u>C</u>H$_3$), 55.4 (<u>C</u>H or <u>C</u>H$_3$), 67.3 (-NH-CO_2-<u>C</u>H$_2$-C_6H_5), 128.3 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 136.1 ($C_{q,Ar}$), 155.8 (-NH-<u>C</u>O$_2$-CH_2-C_6H_5), 170.6 (-CH-<u>C</u>O$_2$-CH_3).

Characterized by Sang-Gyeong *et al.*[305]

6.7.3.6. Synthesis of L-cystine *N*,*N*'-dibenzoyl-, 1,1' dimethyl ester (104)



Procedure according to Photaki *et al.*[262]

L-Cystine 1,1'-dimethyl ester hydrochloride (**95**) (5.0 g, 14.7 mmol, 1.0 eq) was suspended in pyridine (300 mL) in a 4 L round-bottom flask and cooled to 0 °C (ice-bath). Benzoyl chloride (**103**) (3.46 mL, 30.0 mmol, 2.05 eq) was added dropwise and the reaction mixture was stirred for 1.5 h at 0°C and afterwards stirring was continued for 3.5 h at rt. After addition of water (2 L) the precipitate was filtered off and dried in vacuo (0.02 mbar). The attempt to further purify the product **104** by flash column chromatography on silica gel (180 g silica gel, 27 × 6 cm, DCM/MeOH = 80:1 (v/v), R_f = 0.21) was not successful.

Yield: 4.05 g (8.5 mmol, 58 %), white solid

$C_{22}H_{24}N_2O_6S_2$ [476.57 $gmol^{-1}$]

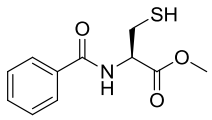
mp.: 179-180°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.34 (d, 4H, ^3J(H,H) = 5.0 Hz, -C$\underline{H}_2$-S-S-C$\underline{H}_2$-), 3.77 (s, 6H, CO_2-C$\underline{H}_3$), 5.07 (pseudo q, 2H, ^3J(H,H) =6.9 Hz, 5.1 Hz, -C$\underline{H}$-NH-), 7.15 (d, 2H, ^3J(H,H) = 7.0 Hz, -CH-N$\underline{H}$-), 7.38-7.53 (m, 6H, $\underline{H}_{Ar}$), 7.81 (d, 4H, ^3J(H,H) = 7.3 Hz, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 41.0 (2 × C, -$\underline{C}H_2$-S-S-$\underline{C}H_2$-), 52.4 (2 × C, $\underline{C}$H or $\underline{C}H_3$), 53.0 (2 × C, $\underline{C}$H or $\underline{C}H_3$), 127.3 (4 × C_{Ar}), 128.8 (4 × C_{Ar}), 132.1 (2 × C_{Ar}), 133.6 (2 × $C_{q,Ar}$), 167.2 (2 × C, -NH-$\underline{C}$O-C_6H_5), 171.1 (2 × C, -CH-$\underline{C}O_2$-CH_3).

Characterized by Plietker *et al.*[197]

6.7.3.7. Synthesis of L-cysteine *N*-benzoyl methyl ester (105)



L-Cystine *N,N'*-dibenzoyl-, 1,1' dimethyl ester (**104**) (250 mg, 0.53 mmol, 1.0 eq), triphenylphosphine (**10**) (145 mg, 0.55 mmol, 1.05 eq), and sodium acetate (17.2 mg, 0.21 mmol, 0.4 eq) were dissolved in acetone (6 mL, hplc-grade), water (1 mL) and glacial acetic acid (17.2 µL) were added. The clear solution was heated under reflux for 22 h. The mixture was diluted with 20 mL CH_2Cl_2, washed with water (2 × 10 mL), saturated sodium chloride solution (10 mL) and dried over $MgSO_4$. The solvent was removed in vacuo using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (35 g silica gel, 26 × 2 cm, cyclohexane/ethyl acetate = 4:1 (v/v), R_f = 0.18).

Yield: 160 mg (0.67 mmol, 64 %), white solid

$C_{11}H_{13}NO_3S$ [239.29 $gmol^{-1}$]

$[\alpha]_D^{20}$ = -40.3 (c = 0.5 in DMSO)

mp.: 59-60°C

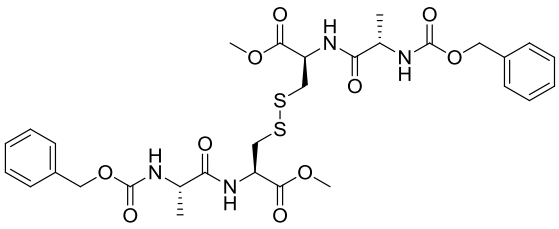
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.40 (t, 1H, ^3J(H,H) = 9.0 Hz, -CH-S<u>H</u>), 3.15 (ddd, 2H, ^3J(H,H) = 8.8 Hz, 3.7 Hz, ^4J(H,H) = 1.7 Hz, -C<u>H</u>$_2$-SH), 3.84 (s, 3H, CO_2-C<u>H</u>$_3$), 5.07-5.12 (m, 1H, -C<u>H</u>-NH-), 7.06 (d, 1H, ^3J(H,H) = 5.8 Hz, -CH-N<u>H</u>-), 7.44-7.57 (m, 3H, <u>H</u>$_{Ar}$), 7.84 (d, 2H, ^3J(H,H) = 7.4 Hz, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 27.1 (-<u>C</u>H$_2$-SH), 53.1 (<u>C</u>H or <u>C</u>H$_3$), 54.1 (<u>C</u>H or <u>C</u>H$_3$), 127.3 (2 × C_{Ar}), 128.8 (2 × C_{Ar}), 132.2 (C_{Ar}), 133.7 ($C_{q,Ar}$), 167.1 (-NH-<u>C</u>O-C_6H_5), 170.8 (-CH-<u>C</u>O$_2$-CH_3).

Characterized by Plietker *et al.*[197]

6.7.4. Syntheses of Cbz-protected L–cysteine containing peptide derivatives as starting materials for Pd-catalyzed thioether formation

6.7.4.1. Synthesis of (5*S*,8*R*,13*R*)-methyl 13-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-8-(methoxycarbonyl)-5-methyl-3,6-dioxo-1-phenyl-2-oxa-10,11-dithia-4,7-diazatetradecan-14-oate (165)



L-Cystine 1,1'-dimethyl ester hydrochloride (**95**) (1.0 g, 2.94 mmol, 1.0 eq) and Cbz-Ala-OH (**1a**) (1.31 g, 5.88 mmol, 2.0 eq) were added into a 100 mL round-bottom flask and dissolved in 60 mL of DMF. DIPEA (**164**) (3 mL, 17.6 mmol, 6.0 eq) and PyBOP (**163**) (3.06 g, 5.88 mmol, 2.0 eq) were added slowly under stirring obtaining a pale yellow solution. After stirring at rt for 24 h all volatile compounds were removed using a rotary evaporator and the resulting residue was purified by flash column chromatography on silica gel (140 g silica gel, 28 × 3.5 cm, cyclohexane/ethyl acetate = 1:5 (v/v)), R_f = 0.68).

Yield: 1.71 g (2.52 mmol, 86 %); colorless powder

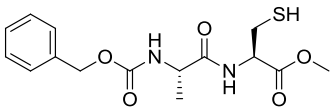
$C_{30}H_{38}N_4O_{10}S_2$ [678.77 gmol^{-1}]

mp.: 145-147°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.39 (d, 6H, ^3J(H,H) = 7.2 Hz, 2 x C$\underline{H}_3$), 2.97-3.10 (m, 4H, 2 x S-C$\underline{H}_2$), 3.73 (s, 6H, 2 x O-C$\underline{H}_3$), 4.42 (pseudo t, 2H, ^3J(H,H) = 6.3 Hz, 2 x CH_3-CO_2-C$\underline{H}$), 4.85 (pseudo q, 2H, ^3J(H,H) = 6.6 Hz, 2 x CH_3-C$\underline{H}$), 5.04-5.13 (m, 4H, 2 x Ph-C$\underline{H}_2$), 5.86 (d, 2H, ^3J(H,H) = 7.8 Hz, 2 x CO-N$\underline{H}$), 7.28-7.32 (m, 10H, $\underline{H}_{Ar}$), 7.38 (d, 2H, ^3J(H,H) = 7.5 Hz, 2 x CO_2-N$\underline{H}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.4 (2 × C, $\underline{C}H_3$), 40.8 (2 × C, S-$\underline{C}H_2$), 50.4 (2 × C, O-$\underline{C}H_3$), 52.3 (2 × C, CH_3-$\underline{C}$H or CH_3-CO_2-$\underline{C}$H), 52.9 (2 × C, CH_3-$\underline{C}$H or CH_3-CO_2-$\underline{C}$H), 67.2 (2 × C, Ph-$\underline{C}H_2$), 128.2 (4 × C_{Ar}), 128.3 (2 × C_{Ar}), 128.7 (4 × C_{Ar}), 136.4 (2 × $C_{q,Ar}$), 56.4 (2 × C, $\underline{C}O_2$-NH), 170.6 (2 × C_q), 172.6 (2 × C_q).

6.7.4.2. Synthesis of (*R*)-methyl 2-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-3-mercaptopropanoate (166)



In a 50 mL round-bottom flask (5*S*,8*R*,13*R*)-methyl-13-((*S*)-2-(((benzyloxy)carbonyl)amino)propanamido)-8-(methoxycarbonyl)-5-methyl-3,6-dioxo-1-phenyl-2-oxa-10,11-dithia-4,7-diazatetradecan-14-oate (**165**), (1.50 g, 2.21 mol, 1.0 eq) triphenylphosphine (**10**) (0.60 g, 2.29 mmol, 1.03 eq), and sodium acetate (80.0 mg, 0.98 mmol, 0.44 eq) were dissolved in methanol (8.3 mL), water (4.2 mL) and glacial acetic acid (81.0 µL) was added. The obtained suspension was heated under reflux for 24 h producing a pale yellow solution after 30 min. The mixture was diluted with 116 mL CH_2Cl_2, washed with water (2 × 46 mL), saturated sodium chloride solution (46 mL) and dried over $MgSO_4$. The solvent was removed in vacuo using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (180 g silica gel, 27 × 6 cm, cyclohexane/ethyl acetate = 1:1 (v/v), R_f = 0.40).

Yield: 526 mg (mmol, 70 %); colorless crystalls

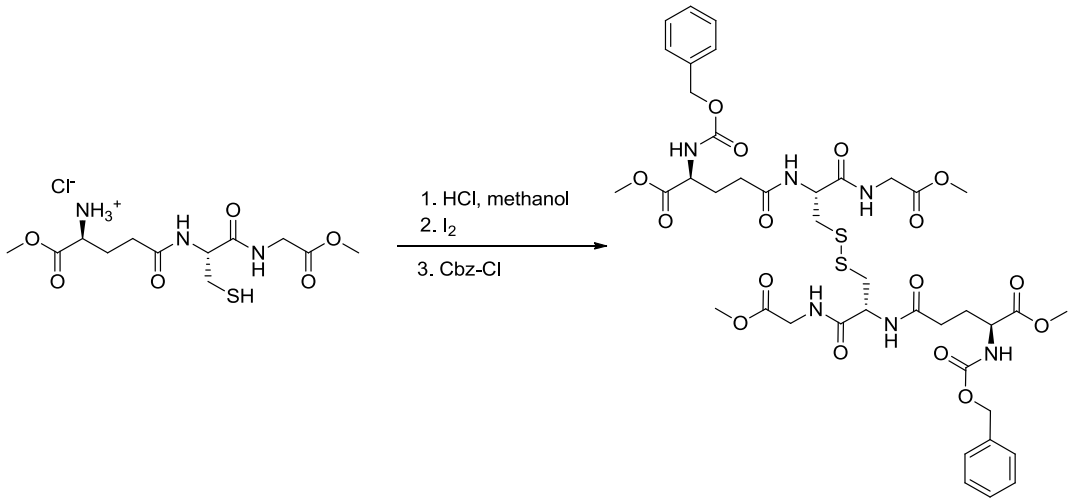
$C_{16}H_{21}N_2O_5S$ [340.39 $gmol^{-1}$]

mp.: 112-114°C

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.39-1.45 (m, 4H, CH-C<u>H</u>$_3$ and S<u>H</u>), 2.98 (dd, 2H, ^3J(H,H) = 8.8 Hz, 4.0 Hz, SH-C<u>H</u>$_2$), 3.78 (s, 3H, O-C<u>H</u>$_3$), 4.31 (pseudo t, 1H, ^3J(H,H) = 6.8 Hz, CH_3-CO_2-<u>C</u>H), 4.83-4.85 (m, 1H, CH_3-C<u>H</u>), 5.11 (s, 2H, Ph-C<u>H</u>$_2$), 5.41 (d, 1H, ^3J(H,H) = 7.2 Hz, CO-N<u>H</u>), 6.96 (d, 1H, ^3J(H,H) = 5.4 Hz, CO-N<u>H</u>), 7.30-7.34 (m, 5H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.4 (<u>C</u>H$_3$), 26.8 (SH-<u>C</u>H$_2$), 50.7 (O-<u>C</u>H$_3$), 53.0 (CH_3-<u>C</u>H or CH_3-CO_2-<u>C</u>H), 53.8 (CH_3-<u>C</u>H or CH_3-CO_2-<u>C</u>H), 67.3 (Ph-<u>C</u>H$_2$), 128.2 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 136.2 ($C_{q,Ar}$), 156.1 (<u>C</u>O$_2$-NH), 170.4 (C_q), 172.3 (C_q).

6.7.4.3. Synthesis of product 173



Procedure according to Davis et al.[279]

A 250 mL two-necked flask with a magnetic stirring bar, a dropping funnel with pressure release and a pressure compensator were assembled, dried under vacuum with a heat gun, and flushed with argon. The two-necked flask was charged with dry methanol (40 mL) and cooled to 0°C in an ice-bath. Acetyl chloride (**85**) (9.29 mL, 130 mmol, 10 eq) was added slowly using the dropping funnel and the solution was stirred for 15 min at 0°C. Afterwards L-glutathione (**169**) (4.0 g, 13.0 mmol, 1.0 eq) was added in one portion and the ice-bath was removed. The reaction was stirred for 6 h at rt. All volatiles were removed using a rotary evaporator to yield crude product **170**.

This crude residue was dissolved in DMF (66.8 mL) and this solution was cooled to 0°C before adding slowly triethylamine (9.1 mL, 65.1 mmol, 5.0 eq) under air. Under strong stirring iodine beads (**171**) (1.65 g, 6.51 mmol, 0.5 eq) were added. After 15 min benzyl chloro formate (**99**) (1.85 mL, 13.0 mmol, 1 eq) was added and the reaction flask was rinsed with 13.2 mL DMF. The reaction was stirred for additional 2 h. The reaction solution was diluted with ethyl acetate (320 mL). This solution was successively washed with 1M HCl (320 mL), H_2O (130 mL), and brine (130 mL). The organic layer was then dried over $MgSO_4$ and filtered. All volatiles were removed using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (650 g silica gel, 27 × 8.0 cm, 5 % methanol in DCM, R_f = 0.21). Afterwards the isolated product was recrystallized using DCM (100 mL at 40°C) and petroleum ether (40 mL). After cooling to -18°C over night, product **173** could be obtained by filtration. Unfortunately, product **173** contained side-product **174** (chapter

4.3.6.2) monitored via HPLC-MS. However, product **173** was used like this for the next reaction without further filtration.

Yield: 567.7 mg (mmol, 9 %); white to pale yellow powder

$C_{40}H_{52}N_6O_{16}S_2$ [937.00 $gmol^{-1}$]

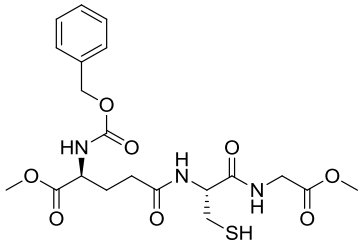
mp.: no melting point was measured due to the obtained mixture of **173** and **174** (see chapter 4.3.6.2.

HRMS: $[MNa]^+$: calculated: 959.2779

found: 959.2823

NMR: no NMR is provided due to the obtained mixture of **173** and **174** (see chapter 4.3.6.2.

6.7.4.4. Synthesis of Cbz-GSH-OMe 176



Crude product **173** (542.4 mg, 0.58 mmol, 1.0 eq) was added to a 25 mL two-necked flask with a magnetic stirring bar and a pressure compensator, which were dried under vacuum before with a heat gun, and flushed with argon. Methanol (6.76 mL), degassed prior to use, and distilled H_2O (1.35 mL), degassed prior to use, were added. PBu_3 (**175**) (281 µL, 0.75 mmol, 1.3 eq) was added to this solution and the reaction mixture was stirred vigorously for 1.5 h. All volatiles were removed using a rotary evaporator and the resulting residue was purified by flash column chromatography on silica gel (85 g silica gel, 23 × 3.5 cm, 5 % methanol in DCM, R_f = 0.23). Afterwards the isolated product was recrystallized twice using DCM (68 mL at 40°C) and petroleum ether (45 mL). After cooling to -18°C overnight product **176** could be collected by filtration as white power.

Yield: 335.7 mg (mmol, 62 %); white powder

$C_{20}H_{27}N_3O_8S$ [469.51 $gmol^{-1}$]

mp.: 176°C

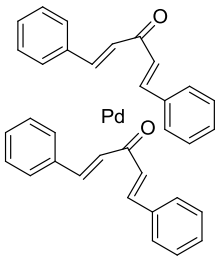
$[\alpha]_D^{20}$ = -35.0 (c = 0.25 in MeOH)

No HRMS could be measured due to disulfide formation using Maldi-TOF MS.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.77 (t, 1H, ^3J(H,H) = 8.5 Hz, S<u>H</u>), 1.85–2.09 (m, 1H, $H_{\beta\ Glu}$), 2.11–2.29 (m, 1H, $H'_{\beta\ Glu}$), 2.35 (t, 2H, ^3J(H,H) = 6.6 Hz, $H_{\gamma\ Glu}$), 2.66–2.86 (m, 1H, C<u>H</u>HSH), 2.96–3.12 (m, 1H, CH<u>H</u>SH), 3.70 (s, 3H, $CO_2C\underline{H}_3$), 3.72 (s, 3H, $CO_2C\underline{H}_3$), 3.99 (m, 2H, $H_{\alpha\ Gly}$), 4.39 (m, 1H, $H_{\alpha\ Glu}$), 4.66 (m, 1H, $H_{\alpha\ Cys}$), 5.07 (s, 2H, $C\underline{H}_{2\ Cbz}$), 5.71 (d, 1H, ^3J(H,H) = 7.8 Hz, N<u>H</u>$_{Glu}$), 6.79 (d, 1H, ^3J(H,H) = 7.7 Hz, N<u>H</u>$_{Cys}$), 7.05 (m, 1H, N<u>H</u>$_{Gly}$), 7.33 (m, 5H, H_{Ar}).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 26.5 ($C_{\beta\ Cys}$), 28.5 ($C_{\beta\ Glu}$), 32.2 ($C_{\gamma\ Glu}$), 41.4 ($C_{\alpha\ Gly}$), 52.6, 52.9, 53.4 ($C_{\alpha\ Glu}$ and 2 × $CO_2\underline{C}H_3$), 54.4 ($C_{\alpha\ Cys}$), 67.4 ($\underline{C}H_{2\ Cbz}$), 128.4 (2 × C_{Ar}), 128.5 (C_{Ar}), 128.8 (2 × C_{Ar}), 136.3 ($C_{q\ Cbz}$), 156.4 ($C{=}O_{Cbz}$), 170.3, 170.3, 172.4, 172.7 (4 × C=O).

6.7.5. Synthesis of pre-catalyst $Pd(dba)_2$ (X)



A Schlenk tube (250 ml) containing a stirring bar was charged with $PdCl_2$ (**73**) (4.03g, 33.6 mmol, 1.0 eq), NaCl (1.37 g, 22.6 mmol, 1.0eq) and methanol (125 ml) under a gentle stream of argon. The brown suspension was degassed and stirred for 20 h at rt. The dark red solution was filtered through a plug of cotton under not inert conditions, rinsed with methanol (600 mL) and transferred into a two-necked flask (1L), containing an argon atmoshere. The dark red solution was degassed and heated to 60 °C (oil-bath temperature) and dibenzylideneacetone (dba) (**74**) (15.82 g, 67.5 mmol, 3.0 eq) was added under a gentle stream of argon and stirred for 15 min. Afterwards sodium acetate (31.45 g, 383.5 mmol, 17 eq) was added under a gentle stream of argon and the temperature of the oil-bath was lowered to 40°C. After 45 min the oil-bath was removed and without stirring the reaction was kept at rt for 3 d. The purple-black precipitate was collected by filtration, washed with

methanol (5 × 30 mL), distilled H_2O (3 × 30 mL), acetone (2 × 10 mL) and dried under vacuum at 0.02 mbar.

Yield: 13.7 g (quant), purple-black solid

$C_{34}H_{28}O_2Pd$ [575.01 $gmol^{-1}$]

6.7.6. Ligand screenings

6.7.6.1. Initial Screening of phosphines: General procedure for Pd-catalyzed thioether formation using (*E*)-hex-2-en-1-yl methyl carbonate (67) and different phosphorous-ligands

All experiments were carried out under argon in previously oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (7.0 mg, 12.1 µmol, 0.02 eq) and different bidentate phosphine ligands (12.1 µmol, 0.02 eq) and dry solvent (2.0 mL) degassed prior to use. The solution was stirred at 60°C for 30 min under argon (exception: DCM 1.5 h at rt). Afterwards degassed (*E*)-hex-2-en-1-yl methyl carbonate (**67**) (100 µL, 0.60 mmol, 1.00 eq) and 1-octanethiol (**75**) (210µL, 1.21 mmol, 2.0 eq) were added with a Hamilton syringe at rt to the solution under inert conditions. The mixture was stirred at this temperature for 24 h. The conversion was monitored via GC-MS by taking a sample of the reaction after 24 h.

6.7.6.2. Screening of phosphines: General procedure for Pd-catalyzed thioether formation using methyl-3-methylbut-2-enylcarbonate (88) and different phosphorous-ligands

All experiments were carried out under argon in previously oven-dried Schlenk vessels containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (3.3 mg, 5.7 µmol, 0.02 eq) and different bidentate phosphorous-ligands (5.7 µmol, 0.02 eq) and dry acetonitrile (1.0 mL) degassed prior to use. The solution was stirred at 60°C for 30 min under argon. Afterwards 1-octanethiol (**75**) (50 µL, 0.29 mmol, 1.0 eq) degassed prior to use was added with a Hamilton syringe to the 60°C hot catalyst solution under inert conditions.

Directly after this, dry diethylene glycol dimethyl ether (**112**) (50 µL), as internal standard for GC-MS, degassed prior to use, was added with a Hamilton syringe to the 60°C hot catalyst solution under inert conditions. Now, a sample for GC analysis was taken under inert conditions with a continuously argon flushed Pasteur-pipette (reference sample at time zero). Afterwards degassed methyl-3-methylbut-2-enylcarbonat (**88**) (50 µL, 0.34 mmol, 1.2 eq) was added with a Hamilton syringe to the 60°C hot solution under inert conditions. The mixture was stirred at this temperature for 24 h and samples for GC (FID) analysis were taken under inert conditions with a continuously argon flushed Pasteur-pipette. For all GC

samples the palladium catalyst was removed by silica gel filtration of the aliquot taken from the reaction mixture using ethyl acetate 100 % as eluent.

Determination of conversion:

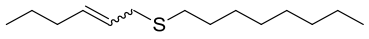
$$\text{initial ratio} = \frac{\text{area starting material}}{\text{area internal standard}}$$

$$\text{conversion}[\%] = 100 - (100 \cdot \frac{\text{area remaining starting material / area internal standard}}{\text{initial ratio}})$$

6.7.7. General method Pd-catalyzed allylic alkylation reaction using dppf (17) as ligand (Method A)

Reactions were carried out under argon in previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (7.0 mg, 12.1 µmol, 0.02 eq) and dppf (**17**) (6.7 mg, 12.1 µmol, 0.02 eq) and acetonitrile (2.0 mL) degassed prior to use. The solutions, containing almost negligible amounts of black solid, were stirred at 60°C for 30 min under argon. Afterwards first different thiols (1.21 mmol, 2.0 eq), degassed prior to use, and secondly degassed (*E*)-hex-2-en-1-yl methyl carbonate (**67**) (100 µL, 0.60 mmol, 1.00 eq) were added with a Hamilton syringe. The solution was stirred at this temperature for 24 h. The conversion was monitored via GC-MS by taking a sample of the reaction after 24 h. After cooling to room temperature the solvent was removed in vacuo using a rotary evaporator and the resulting crude products were purified via flash column chromatography.

6.7.7.1. Synthesis of (*E*)-hex-2-enyl(octyl)sulfane (76)



Procedure according to chapter 6.7.7.

The reaction was carried out under argon in a previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (7.0 mg, 12.1 µmol, 0.02 eq), dppf (**17**) (6.7 mg, 12.1 µmol, 0.02 eq) and acetonitrile (2.0 mL) degassed prior to use. The orange solution, containing almost negligible amounts of black solid, was stirred at 60°C for 30 min under argon. Afterwards 1-octanethiol (**75**) (210µL, 1.21 mmol, 2.0 eq) degassed prior to

use, and secondly degassed (*E*)-hex-2-en-1-yl methyl carbonate (**67**) (100 µL, 0.60 mmol, 1.00 eq) were added with a Hamilton syringe to the 60°C hot solution under inert conditions. The solution was stirred at this temperature for 24 h. After cooling to room temperature the solvent was removed in vacuo using a rotary evaporator and the orange crude product was purified via flash column chromatography (18 g silica gel, 20 x 1.5 cm, petroleum ether (boiling point 35-65°C) = 100 %, R_f = 0.33).

Yield: 127 mg (0.557 mmol, 92 %, mixture of isomers), yellow oil

A second purification of the product via flash column chromatography (18 g silica gel, 20 x 1.5 cm, petroleum ether (boiling point 35-65°C) = 100 %, R_f = 0.33) to separate the *n*-product **76** from the *iso*-product **77** yielded 55 mg (0.241 mmol, 40 %, mixture of *E/Z* isomers of *n*-product **76**

$C_{14}H_{28}S$ [228.19 $gmol^{-1}$]

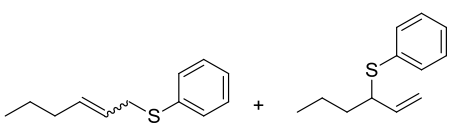
GC-MS (HS_50_S2): t_R = 6.32 min; *m/z* = 228 (7 %, MP), 145 (6 %), 87 (11 %), 82 (100 %, BP), 67 (49 %), 55 (75 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.86-0.93 (m, 6H, 2 × C$\underline{H}_3$), 1.27-1.43 (m, 12H, 6 × C$\underline{H}_2$), 1.51-1.60 (m, 2H, C$\underline{H}_2$), 2.01 (pseudo q, 2H, ^3J(H,H) = 7.2 Hz, C$\underline{H}_2$-CH=CH-C$\underline{H}_2$-S-), 2.44 (m, 2H, CH_2-C$\underline{H}_2$-S-), 3.08 (d, 2H, ^3J(H,H) = 5.9 Hz, CH=CH-C$\underline{H}_2$-S-), 3.15 (d, 2H, ^3J(H,H) = 6.8 Hz, CH=CH-C$\underline{H}_2$-S- (***Z*-configuration of 76**)), 5.35 – 5.53 (m, 2H, C$\underline{H}$=C$\underline{H}$).

^{13}C-NMR (APT) (100 MHz, $CDCl_3$): δ = 13.8 (CH_3), 13.9 (CH_3 (***Z*-configuration of 76**)), 14.2 (C$\underline{H}_3$), 22.7 (CH_2), 22.8 (CH_2), 23.0 (CH_2 (***Z*-configuration of 76**)), 28.5 (CH_2 (***Z*-configuration of 76**)), 29.1 (CH_2), 29.1 (CH_2 (***Z*-configuration of 76**)), 29.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2 (***Z*-configuration of 76**)), 29.8 (CH_2), 30.8 (CH_2), 31.3 (CH_2 (***Z*-configuration of 76**)), 32.0 (CH_2), 34.1 (CH_2), 34.5 (CH_2), 126.1 (C$\underline{H}$=CH (***Z*-configuration of 76**)), 126.4 (C$\underline{H}$=CH), 132.5 (C$\underline{H}$=CH (***Z*-configuration of 76**)), 133.4 (C$\underline{H}$=CH).

E/*Z* = 80/20 NOESY-NMR

6.7.7.2. Synthesis of hex-2-en-1-yl(phenyl)sulfane (69) obtained as isomeric mixture (69 and 70)



n-product **69** *iso*-product **70**

Procedure according to chapter 6.7.7.

$Pd(dba)_2$ (**X**) (7.0 mg, 12.1 µmol, 0.02 eq), dppf (**17**) (6.7 mg, 12.1 µmol, 0.02 eq), degassed (*E*)-hex-2-en-1-yl methyl carbonate (**67**) (100 µL, 0.60 mmol, 1.00 eq), degassed thiolphenol (**68**) (123 µL, 1.21 mmol, 2.0 eq), degassed acetonitrile (2.0 mL)

Flash column chromatography: (18 g silica gel, 20 x 1.5 cm, petroleum ether (boiling point 35-65°C) = 100 %, R_f = 0.36)

Yield: 107 mg (92 % mixture of isomers), colorless oil

$C_{12}H_{16}S$ [192.32 $gmol^{-1}$]

GC-MS (HS_50_S2): *n*-product: t_R = 5.97 min

iso-product: t_R = 5.64 min

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.82-0.96 (m, 3H, C$\underline{H}_3$), 1.30-1.39 (m, 2H, C$\underline{H}_2$), 2.01 (m, 2H, C$\underline{H}_2$-CH=CH-CH$_2$-S-), 3.52-3.54 (m, 2H, CH=CH-C$\underline{H}_2$-S-), 3.59-3.60 (m, 2H, CH=CH-C$\underline{H}_2$-S-(***Z*-configuration of 69**)), 4.86-4.97 (m, 2H, CH=C$\underline{H}_2$ (**70**)), 5.47-5.59 (m, 2H, C$\underline{H}$=C$\underline{H}$), 5.65-5.74 (m, 2H, C$\underline{H}$=CH$_2$ (**70**)), 7.17-7.39 (m, 5H, $\underline{H}_{Ar}$).

^{13}C-NMR (APT) (100 MHz, $CDCl_3$) (*n*-product): δ = 13.7 (C$\underline{H}_3$), 22.5 (CH$_3$-C$\underline{H}_2$), 34.5 (C$\underline{H}_2$), 36.7 (C$\underline{H}_2$), 125.2 (C_{Ar}), 126.2 (C$\underline{H}$=CH), 128.8 (2 × C_{Ar}), 130.0 (2 × C_{Ar}), 134.5 (C$\underline{H}$=CH), 136.4 ($C_{q,Ar}$).

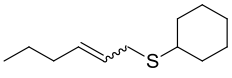
Additional peaks appearing in ^{13}C-MNR: additional peaks were not assigned. No differentiation made between both possible isomers. Peaks for *iso*-product **70** and the *Z*-configuration of **69** are shown together.

δ = 13.9, 20.5, 22.7, 29.3, 31.4, 36.4, 52.2, 115.6, 124.7, 126.3, 127.1, 127.3, 127.6, 128.5, 128.9, 129.2, 130.0, 132.8, 133.6

E/*Z* = 80/20 assigned by ^{1}H-NMR

Characterized by Cohen *et al.*[306]

6.7.7.3. Synthesis of cyclohexyl(hex-2-en-1-yl)sulfane (79)



Procedure according to chapter 6.7.7.

$Pd(dba)_2$ (**X**) (7.0 mg, 12.1 µmol, 0.02 eq), dppf (**17**) (6.7 mg, 12.1 µmol, 0.02 eq), degassed (*E*)-hex-2-en-1-yl methyl carbonate (**67**) (100 µL, 0.60 mmol, 1.00 eq), degassed cyclohexanethiol (**78**) (148 µL, 1.21 mmol, 2.0 eq), degassed acetonitrile (2.0 mL)

Flash column chromatography: (18 g silica gel, 20 x 1.5 cm, petroleum ether (boiling point 35-65°C) = 100 %, R_f = 0.16)

Using flash column chromatography it was possible to separate the *iso*-product from the *E/Z*-mixture of the *n*-product. (*iso*-product, R_f = 0.22) Only the the *E/Z*-mixture of the *n*-product was characterized via NMR.

Yield: 108 mg (90 % only mixture of *E/Z*-isomers of *n*-product), colorless oil

$C_{12}H_{22}S$ [198.37 $gmol^{-1}$]

GC-MS (HS_50_S2): *n*-product: t_R = 5.94 min

iso-product: t_R = 5.59 min

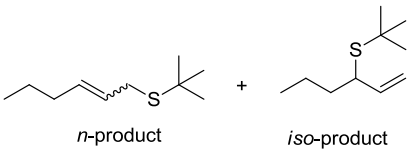
^{1}H-NMR (200 MHz, $CDCl_3$): δ = 0.89 (t, 3H, ^3J(H,H) = 7.4 Hz, C$\underline{H}_3$), 1.24-1.43 (m, 8H, C$\underline{H}_2$), 1.57-1.61 (m, 1H), 1.75 (m, 2H, C$\underline{H}_2$), 1.92-2.06 (m, 4H, C$\underline{H}_2$-CH=CH-CH$_2$-S- and C$\underline{H}_2$), 2.57-2.63 (m, 1H, S-C$\underline{H}$), 3.12 (d, 2H, ^3J(H,H) = 6.7 Hz, CH=CH-C$\underline{H}_2$-S-), 3.18 (d, 2H, ^3J(H,H) = 6.4 Hz CH=CH-C$\underline{H}_2$-S- (***Z*-configuration of 79**)), 5.39-5.54 (m, 2H, C$\underline{H}$=C$\underline{H}$).

^{13}C-NMR (50 MHz, $CDCl_3$) (*n*-product): δ = 13.8 (C$\underline{H}_3$), 13.9 (C$\underline{H}_3$ (***Z*-configuration of 79**)), 22.7 (CH$_3$-C$\underline{H}_2$), 23.0 (CH$_2$ (***Z*-configuration of 79**)), 26.0 (C$\underline{H}_2$), 26.2 (2 × C$\underline{H}_2$), 26.3 (CH$_2$ (***Z*-configuration of 79**)), 27.0 (CH$_2$ (***Z*-configuration of 79**)), 29.3 (CH$_2$ (***Z*-configuration of 79**)), 32.5 (C$\underline{H}_2$), 33.6 (2 × C$\underline{H}_2$), 33.8 (CH$_2$ (***Z*-configuration of 79**)), 34.5 (C$\underline{H}_2$), 42.3 (S-C$\underline{H}$), 42.9 (S-C$\underline{H}$ (***Z*-configuration of 79**)), 126.4 (C$\underline{H}$=CH (***Z*-configuration of 79**)), 126.7 (C$\underline{H}$=CH), 132.2 (C$\underline{H}$=CH (***Z*-configuration of 79**)), 133.1 (C$\underline{H}$=CH).

E/*Z* = 80/20 assigned by ^{1}H-NMR

Characterized according to allyl cyclohexyl sulfide by Mitsudo *et al.*[194]

6.7.7.4. Synthesis of tert-butyl(hex-2-en-1-yl)sulfane (82) obtained as isomeric mixture (82 and 83)



Procedure according to chapter 6.7.7.

$Pd(dba)_2$ (**X**) (7.0 mg, 12.1 µmol, 0.02 eq), dppf (**17**) (6.7 mg, 12.1 µmol, 0.02 eq), degassed (*E*)-hex-2-en-1-yl methyl carbonate (**67**) (100 µL, 0.60 mmol, 1.00 eq), degassed 2-methyl-2-propanethiol (**81**) (136 µL, 1.21 mmol, 2.0 eq), degassed acetonitrile (2.0 mL)

Flash column chromatography: (18 g silica gel, 20 x 1.5 cm, petroleum ether (boiling point 35-65°C) = 100 %, R_f = 0.36)

Yield: 88 mg (84 % mixture of isomers), colorless oil

$C_{10}H_{20}S$ [172.33 $gmol^{-1}$]

GC-MS (HS_50_S2): *n*-product: t_R = 4.76 min

iso-product: t_R = 4.70 min

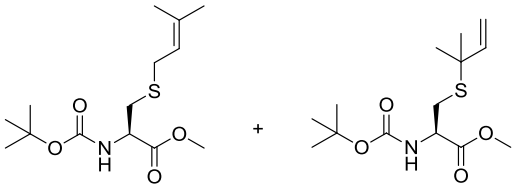
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.89 (t, 3H, ^3J(H,H) = 7.4 Hz, C$\underline{H}_3$), 0.92 (t, 3H, ^3J(H,H) = 7.3 Hz, C$\underline{H}_3$ ***Z*-configuration of 82**), 1.33 (s, 9H, -C(C$\underline{H}_3$)$_3$), 1.34 (s, 9H, -C(C$\underline{H}_3$)$_3$ ***Z*-configuration of 82**), 1.35-1.43 (m, 2H, CH_3-C$\underline{H}_2$-), 1.99 (td, 2H, ^3J(H,H) = 7.4 Hz, 0.7 Hz, C$\underline{H}_2$-CH=CH-CH_2-S-), 2.07 (pseudo q, 2H, ^3J(H,H) = 6.8 Hz, C$\underline{H}_2$-CH=CH-CH_2-S- ***Z*-configuration of 82**), 3.18 (dd, 2H, ^3J(H,H) = 6.9 Hz, ^4J(H,H) = 0.8 Hz, CH=CH-C$\underline{H}_2$-S-), 3.21 (d, 2H, ^3J(H,H) = 6.6 Hz, CH=CH-C$\underline{H}_2$-S- ***Z*-configuration of 82**), 5.47 (m, 1H, C$\underline{H}$=CH), 5.60 (m, 1H, C$\underline{H}$=CH).

No *iso*-product **83** detected in ^{1}H-NMR.

^{13}C-NMR (APT) (100 MHz, $CDCl_3$): δ = 13.8 (C$\underline{H}_3$), 13.9 (C$\underline{H}_3$ (***Z*-configuration of 82**)), 22.5 (CH_3-C$\underline{H}_2$), 22.8 (CH_2 (***Z*-configuration of 82**)), 25.7 (CH_2 (***Z*-configuration of 82**)), 29.3 (CH_2 (***Z*-configuration of 82**)), 31.0 (-C(C$\underline{H}_3$)$_3$ ***Z*-configuration of 82**), 31.1 (-C(C$\underline{H}_3$)$_3$), 31.3 (C$\underline{H}_2$), 34.6 (C$\underline{H}_2$), 42.4 (-C(C$\underline{H}_3$)$_3$ ***Z*-configuration of 82**), 42.5 (-C(C$\underline{H}_3$)$_3$), 126.0 (C$\underline{H}$=CH (***Z*-configuration of 82**)), 126.7 (C$\underline{H}$=CH), 132.4 (C$\underline{H}$=CH (***Z*-configuration of 82**)), 133.3 (C$\underline{H}$=CH).

E/*Z* = 80/20 assigned by ^{1}H-NMR

6.7.7.5. Isoprenylation of L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (98) to yield a mixture of isomers (*n*-product 106/*iso*-product 107)



n-product **106** + *iso*-product **107**

The experiment was carried out under argon in a previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (7.8 mg, 13.6 µmol, 0.02 eq) and dppf (**17**) (7.5 mg, 13.6 µmol, 0.02 eq) and dry acetonitrile (0.5 mL) degassed prior to use. The solution was stirred at 60°C for 30 min under argon. A stock solution of L-cysteine *N*-[(1,1-dimethylethoxy)carbonyl] methyl ester (**98**) (175.5 mg, 0.75 mmol, 1.1 eq) in 0.5 mL of degassed acetonitrile was prepared in an additional Schlenk vessel. The stock solution was added to the catalyst solution and the Schlenk vessel was rinsed with degassed acetonitrile (1.2 mL) and this solution was also added to the reaction mixture. Afterwards degassed methyl-3-methylbut-2-enylcarbonat (**88**) (100 µL, 0.68 mmol, 1.00 eq) was added with a Hamilton syringe to the 60°C hot solution under inert conditions. The mixture was stirred at this temperature for 24 h. The solvent was removed in vacuo using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (20 g silica gel, 15 × 2 cm, cyclohexane/ethyl acetate = 8:1 (v/v), R_f = 0.24).

Yield: 195 mg (0.64 mmol, 95 %), pale yellow oil

$C_{14}H_{25}NO_4S$ [303.15 $gmol^{-1}$]

HRMS (TOF MSEI+): calculated: 303.1504

found: 303.1484

$[\alpha]_D^{20}$ = -11.8 (c = 0.5 in MeOH)

mp.: 22-23°C

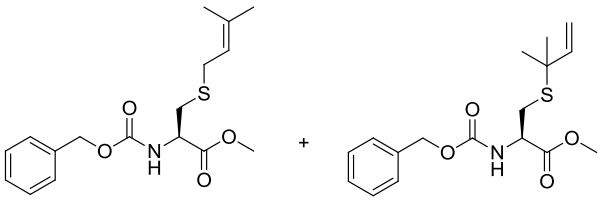
n/iso-ratio (8:1) TOCSY-NMR

NMR of *n*-product **106** together with the minor *iso*-product **107**:

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.34 (s, 6H, -S-C(C$\underline{H}_3$)$_2$-CH=CH_2 (**107**)), 1.43 (s, 9H, -C(C$\underline{H}_3$)$_3$), 1.65 (s, 3H, -C$\underline{H}_3$), 1.73 (s, 3H, -C$\underline{H}_3$), 2.78-2.92 (m, 2H, -CH-C$\underline{H}_2$-S-), 3.10-3.19 (m, 2H, -S-C$\underline{H}_2$-CH=C(CH_3)$_2$), 3.73 (s, 3H, CO_2-C$\underline{H}_3$ (**107**)), 3.75 (s, 3H, CO_2-C$\underline{H}_3$), 4.50 (m, 1H, -C$\underline{H}$-NH-), 4.98 (m, 2H, -S-C(CH_3)$_2$-CH=C$\underline{H}_2$ (**107**)), 5.19 (t, 1H, ^{3}J(H,H) = 7.8 Hz, -S-CH_2-C$\underline{H}$=C(CH_3)$_2$), 5.30 (d, 1H, ^{3}J(H,H) = 7.2 Hz, -CH-N$\underline{H}$-), 5.78 (dd, 1H, ^{3}J(H,H) = 17.3 Hz, 10.5 Hz, -S-C(CH_3)$_2$-C$\underline{H}$=CH_2 (**107**)).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 17.9 (CH_3), 25.8 (CH_3), 27.4 (C$\underline{H}_3$, (**107**)), 27.5 (C$\underline{H}_3$, (**107**)), 28.4 (-C(C$\underline{H}_3$)$_3$), 30.3 (-S-C$\underline{H}_2$-CH=C(CH_3)-), 31.8 (C, (**107**)), 33.9 (NH-CH(CO_2-CH_3)-C$\underline{H}_2$-S-), 47.0 (C_q, (**107**)), 52.6 (C$\underline{H}$ or C$\underline{H}_3$), 53.1 (C, (**107**)), 53.4 (C$\underline{H}$ or C$\underline{H}_3$), 80.2 (-C$\underline{C}$(CH_3)$_3$), 112.2 (-S-C(CH_3)$_2$-CH=C$\underline{H}_2$, (**107**)), 120.0 (-S-CH_2-C$\underline{H}$=C$_q$), 136.4 (-CH=C$\underline{C}_q$), 144.2 (-S-C(CH_3)$_2$-C$\underline{H}$=CH_2, (**107**)), 155.3 (-NH-C$\underline{C}O_2$-C(CH_3)$_3$), 171.6 (C_q, (**107**)), 171.8 (-CH-C$\underline{C}O_2$-CH_3).

6.7.7.6. Isoprenylation of L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (102) to yield a mixture of isomers (*n*-product 108/*iso*-product 109)



n-product **108** + *iso*-product **109**

The experiment was carried out under argon in a previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) (Pd(dba)$_2$) (**X**) (7.8 mg, 13.6 µmol, 0.02 eq) and dppf (**17**) (7.5 mg, 13.6 µmol, 0.02 eq) and dry acetonitrile (0.5 mL) degassed prior to use. The solution was stirred at 60°C for 30 min under argon. A stock solution of L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) (200.9 mg, 0.75 mmol, 1.1 eq) in 0.5 mL of degassed acetonitrile was prepared in an additional Schlenk vessel. The stock solution was added to the catalyst solution and the Schlenk vessel was rinsed with degassed acetonitrile (1.2 mL) and this solution was also added to the reaction mixture. Afterwards degassed methyl-3-methylbut-2-enylcarbonat (**88**) (100 µL, 0.68 mmol, 1.00 eq) was added with a

Hamilton syringe to the 60°C hot solution under inert conditions. The mixture was stirred at this temperature for 24 h. The solvent was removed in vacuo using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (25 g silica gel, 17 × 2 cm, cyclohexane/ethyl acetate = 8:1 (v/v), R_f = 0.20).

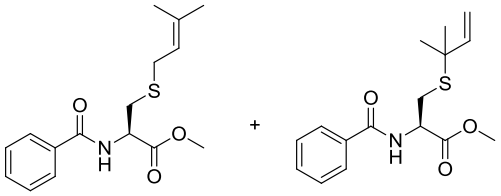
Yield: 212 mg (0.63 mmol, 93 %), colorless oil

$C_{17}H_{23}NO_4S$ [337.43 $gmol^{-1}$]

n/iso-ratio (6:1) TOCSY-NMR

Further analytical data are given in chapter 6.7.8.15 where this product was synthesized using biphephos (**40**) as ligand for the Pd-catalyzed allylic alkylation.

6.7.7.7. Isoprenylation of L-cysteine *N*-benzoyl methyl ester (105) to yield a mixture of isomers (*n*-product 110/*iso*-product 111)



n-product **110** + *iso*-product **111**

The experiment was carried out under argon in a previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (2.4 mg, 4.2 µmol, 0.02 eq) and dppf (**17**) (2.3 mg, 4.2 µmol, 0.02 eq) and dry acetonitrile (0.5 mL) degassed prior to use. The solution was stirred at 60°C for 30 min under argon. Under inert conditions L-cysteine *N*-benzoyl methyl ester (**105**) (50 mg, 0.21 mmol, 1.0 eq) was added as solid to the catalyst solution and this Schlenk vessel was rinsed with degassed acetonitrile (0.5 mL). Due to this procedure no solid remained on the glaswall of the Schlenk vessel after adding L-cysteine *N*-benzoyl methyl ester (**105**). Afterwards degassed methyl-3-methylbut-2-enylcarbonat (**88**) (35 µL, 0.24 mmol, 1.15 eq) was added with a Hamilton syringe to the 60°C hot solution under inert conditions. The mixture was stirred at this temperature for 24 h. The solvent was removed in vacuo using a rotary evaporator and the residue was purified by flash column chromatography on silica gel (10 g silica gel, 9.5 × 2 cm, cyclohexane/ethyl acetate = 4:1 (v/v), R_f = 0.26).

Yield: 60 mg (0.20 mmol, 93 %), colorless oil

$C_{16}H_{21}NO_3S$ [337.43 $gmol^{-1}$]

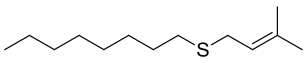
n/iso-ratio (6:1) TOCSY-NMR

Further analytical data are given in chapter 6.7.8.14 where this product was synthesized using biphephos (**40**) as ligand for the Pd-catalyzed allylic alkylation.

6.7.8. General method for products obtained by Pd-catalyzed allylic alkylation reaction using biphephos (40) as ligand (Method B)

Reactions were carried out under argon in a previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (0.02 eq) and biphephos (**40**) (0.02 eq) and acetonitrile degassed prior to use. The solutions containing almost negligible amounts of black solid, was stirred at 60°C for 30 min under argon. Afterwards first different thiols (1.0 eq), degassed prior to use and secondly different degassed carbonates (1.2 eq) were added with a Hamilton syringe. In the case of solid carbonates or thiols the procedure is further explained in the corresponding chapters. The solution was stirred at this temperature for 24 h. The conversion was monitored via GC-MS by taking a sample of the reaction after 24 h. After cooling down to room temperature the solvent was removed in vacuo using a rotary evaporator and the resulting crude products were purified via flash column chromatography.

6.7.8.1. Synthesis of (3-methylbut-2-enyl)(octyl)sulfane (89)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (6.6 mg, 11.5 µmol, 0.02 eq), biphephos (**40**) (9.0 mg, 11.5 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (100 µL, 0.689 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (100 µL, 0.574 mmol, 1.0 eq), degassed acetonitrile (2.0 mL)

Flash column chromatography: (18 g silica gel, 20 x 1.5 cm, cyclohexane = 100 %, R_f = 0.32)

Yield: 112 mg (0.522 mmol, 91 %), yellow oil

$C_{13}H_{26}S$ [214.41 $gmol^{-1}$]

GC-MS (HS_50_S2): t_R = 6.05 min; *m/z* = 214 (63 %, MP), 144 (21 %), 101 (10 %), 68 (100 %, BP).

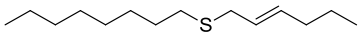
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, 3H, ^3J(H,H) = 6.6 Hz, C$\underline{H}_3$-CH_2), 1.27-1.42 (m, 10H, 5 x C$\underline{H}_2$), 1.50-1.61 (m, 2H, C$\underline{H}_2$), 1.65 (s, 3H, C$\underline{H}_3$-C_q), 1.73 (s, 3H, C$\underline{H}_3$-C_q), 2.45 (t, 2H,

^{3}J(H,H) = 7.5 Hz, C$\underline{H}_2$-S), 3.12 (d, 2H, ^{3}J(H,H) = 7.8 Hz, C$\underline{H}_2$-S), 5.23 (t, 1H, ^{3}J(H,H) = 7.5 Hz, C$\underline{H}$-C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.1 ($\underline{C}H_3$-CH_2), 17.7 ($\underline{C}H_3$-C_q), 22.7 (<u>C</u>-alkyl), 25.7 ($\underline{C}H_3$-C_q), 27.0 (<u>C</u>-alkyl), 29.1 (<u>C</u>-alkyl), 29.2 (<u>C</u>-alkyl), 29.5 (<u>C</u>-alkyl), 29.9 (<u>C</u>-alkyl), 31.3 (<u>C</u>-alkyl), 31.9 (<u>C</u>-alkyl), 120.9 ($\underline{C}H$-C_q), 134.81 ($\underline{C}_q$).

Characterized by Alper *et al.*[307]

6.7.8.2. Synthesis of (*E*)-hex-2-enyl(octyl)sulfane (76) using (*E*)-hex-2-en-1-yl methyl carbonate (67) as starting material



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (9.9 mg, 17.2 µmol, 0.02 eq), biphephos (**40**) (13.5 mg, 17.2 µmol, 0.02 eq), degassed (*E*)-hex-2-en-1-yl methyl carbonate (**67**) (175 µL, 1.03 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (150 µL, 0.861 mmol, 1.0 eq), degassed acetonitrile (3.0 mL)

Flash column chromatography: (25 g silica gel, 18 x 2.0 cm, cyclohexane = 100 %, R_f = 0.46)

Yield: 195 mg (0.853 mmol, 99 %), yellow oil

$C_{14}H_{28}S$ [228.43 $gmol^{-1}$]

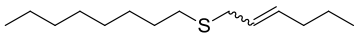
HRMS (TOF MSEI+):	calculated:	228.1912
	found:	228.1920

GC-MS (HS_50_S2): t_R = 6.32 min; *m/z* = 228 (7 %, MP), 145 (6 %), 87 (11 %), 82 (100 %, BP), 67 (49 %), 55 (75 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.85-0.93 (m, 6H, 2 x C$\underline{H}_3$), 1.27-1.62 (m, 14H, 7 x C$\underline{H}_2$), 2.01 (m, 2H, CH_2-C$\underline{H}_2$-CH=CH), 2.43 (t, 2H, ^{3}J(H,H) = 7.4 Hz, CH_2-C$\underline{H}_2$-S), 3.08 (d, 2H, ^{3}J(H,H) = 6.6 Hz, CH=CH-C$\underline{H}_2$-S), 5.37-5.53 (m, 2H, C$\underline{H}$=C$\underline{H}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.6 (C$\underline{H}_3$), 14.0 (C$\underline{H}_3$), 22.5 (<u>C</u>-alkyl), 22.6 (<u>C</u>-alkyl), 28.9 (<u>C</u>-alkyl), 29.2 (<u>C</u>-alkyl), 29.2 (<u>C</u>-alkyl), 29.4 (<u>C</u>-alkyl), 30.6 (<u>C</u>-alkyl), 31.8 (<u>C</u>-alkyl), 33.9 (<u>C</u>-alkyl), 34.3 (<u>C</u>-alkyl), 126.3 (<u>C</u>H=CH), 133.3 (<u>C</u>H=CH).

6.7.8.3. Synthesis of (*E*)-hex-2-enyl(octyl)sulfane (76) using hex-1-en-3-yl methyl carbonate (139) as starting material



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 5.74 µmol, 0.02 eq), degassed hex-1-en-3-yl methyl carbonate (**139**) (60 µL, 0.344 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (50 µL, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

Flash column chromatography: (6.5 g silica gel, 17 x 1.0 cm, cyclohexane = 100 %, R_f = 0.46)

Yield: 58 mg (0.254 mmol, 89 %), yellow oil

$C_{14}H_{28}S$ [228.43 $gmol^{-1}$]

HRMS (TOF MSEI+):	calculated:	228.1912
	found:	228.1920

GC-MS (HS_50_S2): t_R = 6.32 min; *m/z* = 228 (7 %, MP), 145 (6 %), 87 (11 %), 82 (100 %, BP), 67 (49 %), 55 (75 %).

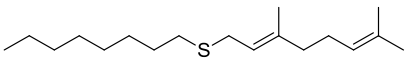
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.85-0.93 (m, 6H, 2 x C$\underline{H}_3$), 1.27-1.62 (m, 14H, 7 x C$\underline{H}_2$), 2.01 (m, 2H, CH_2-C$\underline{H}_2$-CH=CH), 2.43 (t, 2H, ^3J(H,H) = 7.4 Hz, CH_2-C$\underline{H}_2$-S), 3.08 (d, 2H, ^3J(H,H) = 6.6 Hz, CH=CH-C$\underline{H}_2$-S), 3.16 (d, 2H, ^3J(H,H) = 6.9 Hz, CH=CH-C$\underline{H}_2$-S (***Z*-configuration of 76**)), 5.37-5.53 (m, 2H, C$\underline{H}$=C$\underline{H}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 13.8 (CH_3), 13.9 (CH_3 (***Z*-configuration of 76**)), 14.2 (C$\underline{H}_3$), 22.7 (CH_2), 22.8 (CH_2), 23.0 (CH_2 (***Z*-configuration of 76**)), 28.6 (CH_2 (***Z*-configuration of 76**)), 29.1 (CH_2), 29.2 (CH_2 (***Z*-configuration of 76**)), 29.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2 (***Z*-configuration of 76**)), 29.8 (CH_2), 30.8 (CH_2), 31.3 (CH_2 (***Z*-configuration of 76**)), 32.0 (CH_2), 34.1 (CH_2), 34.5 (CH_2), 126.1 (C$\underline{H}$=CH (***Z*-configuration of 76**)), 126.4 (C$\underline{H}$=CH), 132.5 (C$\underline{H}$=CH (***Z*-configuration of 76**)), 133.4 (C$\underline{H}$=CH).

4 % of *iso*-product detected via ^{1}H-NMR

E/*Z* = 75/25 assigned by ^{1}H-NMR and NOESY-NMR

6.7.8.4. Synthesis of (*E*)-(3,7-dimethylocta-2,6-dien-1-yl)(octyl)sulfane (147)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (6.6 mg, 11.5 µmol, 0.02 eq), biphephos (**40**) (9.0 mg, 11.5 µmol, 0.02 eq), degassed (*E*)-3,7-dimethylocta-2,6-dien-1-yl methyl carbonate (**140**) (155 µL, 0.689 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (100 µL, 0.574 mmol, 1.0 eq), degassed acetonitrile (2.0 mL)

Flash column chromatography: (20 g silica gel, 14 x 2.0 cm, cyclohexane = 100 %, R_f = 0.17)

Yield: 154 mg (0.545 mmol, 95 %), yellow oil

$C_{18}H_{34}S$ [284.52 $gmol^{-1}$]

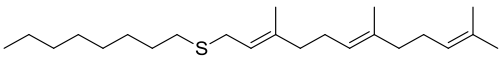
HRMS (TOF MSEI+):	calculated:	282.2381
	found:	282.2390

GC-MS (HS_50_S2): t_R = 7.33 min; *m/z* = 282 (1 %, MP), 213 (3 %), 169 (6 %), 145 (7 %), 136 (8 %), 121 (11 %), 93 (41 %), 81 (20 %), 69 (100 %, BP), 55 (21 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, 3H, ^3J(H,H) = 6.6 Hz, $C\underline{H}_3$-CH_2), 1.26 – 1.37 (m, 10H, <u>H</u>-alkyl), 1.51 – 1.67 (m, 11H, <u>H</u>-alkyl), 2.04 – 2.10 (m, 4H, -$C\underline{H}_2$-$C\underline{H}_2$-CH=$C(CH_3)_2$), 2.44 (t, 2H, ^3J(H,H) = 7.2 Hz, CH_2-$C\underline{H}_2$-S), 3.13 (d, 2H, ^3J(H,H) = 7.7 Hz, -S-$C\underline{H}_2$-CH=$C(CH_3)$-CH_2-), 5.06 – 5.10 (m, 1H, -CH_2-$C\underline{H}$=$C(CH_3)_2$), 5.23 (pseudo dt, 1H, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 0.9 Hz, -S-CH_2-$C\underline{H}$=C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.2 ($C\underline{H}_3$-CH_2), 16.1 ($\underline{C}H_3$), 17.8 ($\underline{C}H_3$), 22.8 ($\underline{C}H_2$), 25.8 ($\underline{C}H_3$), 26.6 ($\underline{C}_{Alkyl}$), 29.2 ($\underline{C}_{Alkyl}$), 29.3 ($\underline{C}_{Alkyl}$), 29.4 ($\underline{C}_{Alkyl}$), 29.4 ($\underline{C}_{Alkyl}$), 29.9 ($\underline{C}_{Alkyl}$), 31.2 ($\underline{C}_{Alkyl}$), 32.0 ($\underline{C}_{Alkyl}$), 39.8 (-S-CH_2-CH=$C(CH_3)$-$\underline{C}H_2$-), 120.8 (-S-CH_2-$\underline{C}$H=$C(CH_3)$-CH_2-), 124.0 (-CH_2-$\underline{C}$H=$C(CH_3)_2$), 131.6 (CH=$\underline{C}_q$), 138.4 (CH=$\underline{C}_q$).

6.7.8.5. Synthesis of octyl((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)sulfane (148)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (1.7 mg, 2.9 µmol, 0.02 eq), biphephos (**40**) (2.3 mg, 2.9 µmol, 0.02 eq), degassed (*E*)-3,7-dimethylocta-2,6-dien-1-yl methyl carbonate (**140**) (51 µL, 0.172 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (25 µL, 0.144 mmol, 1.0 eq), degassed acetonitrile (0.5 mL)

Flash column chromatography: (6.5 g silica gel, 35 x 0.5 cm, cyclohexane = 100 %, R_f = 0.20)

Yield: 49.9 mg (0.142 mmol, 99 %), yellow oil

$C_{23}H_{42}S$ [350.64 $gmol^{-1}$]

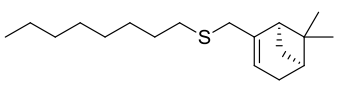
HRMS (TOF MSEI+):	calculated:	350.3007
	found:	350.3057

GC-MS (HS_50_S2): t_R = 8.44 min; *m/z* = 350 (2 %, MP), 255 (3 %), 237 (4 %), 204 (15 %), 189 (8 %), 161 (16 %), 136 (17 %), 133 (17 %), 119 (29 %), 107 (28 %), 93 (72 %), 81 (31 %), 69 (100 %, BP), 55 (29 %).

^{1}H-NMR (300 MHz; $CDCl_3$): δ = 0.88 (t, 3H, ^3J(H,H) = 6.7 Hz, C<u>H</u>$_3$-C<u>H</u>$_2$-), 1.27-1.38 (m, 10 H, 5 × CH_2), 1.52-1.74 (m, 14 H, 4 × CH_3, 1 × CH_2), 1.95-2.11 (m, 8 H, -C<u>H</u>$_2$-C<u>H</u>$_2$-CH=C(CH_3)-C<u>H</u>$_2$-C<u>H</u>$_2$-), 2.45 (t, 2H, ^3J(H,H) = 7.2 Hz, CH_2-C<u>H</u>$_2$-S-), 3.14 (d, 2H, ^3J(H,H) = 7.7 Hz, -S-C<u>H</u>$_2$-CH=C(CH_3)-), 5.07-5.11 (m, 2H, 2 × -C<u>H</u>=C_q), 5.24 (pseudo dt, 1 H, ^3J(H,H) = 7.7 Hz, ^4J(H,H) = 1.0 Hz, -S-CH_2-C<u>H</u>=C_q).

^{13}C-NMR (75 MHz; $CDCl_3$): δ = 14.2 (<u>C</u>H_3-CH_2), 16.2 (CH_3), 16.2 (CH_3), 17.8 (CH_3), 22.8 (CH_3-<u>C</u>H_2-), 25.8 (CH_3), 26.6 (CH_2), 26.9 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.9 (CH_2), 31.3 (CH_2), 32.0 (CH_2-<u>C</u>H_2-S-), 39.8 (-CH=C(CH_3)-<u>C</u>H_2-), 39.9 (-CH=C(CH_3)-<u>C</u>H_2-), 120.9 (-S-CH_2-<u>C</u>H=C_q), 124.0 (-<u>C</u>H=C_q), 124.5 (-<u>C</u>H=C_q), 131.5 (-CH=<u>C</u>$_q$), 135.4 (-CH=<u>C</u>$_q$), 138.6 (-CH=<u>C</u>$_q$).

6.7.8.6. Synthesis of (((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl)(octyl)sulfane (149)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (6.6 mg, 11.5 µmol, 0.02 eq), biphephos (**40**) (9.0 mg, 11.5 µmol, 0.02 eq), degassed ((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl-methylcarbonate (**146**) (140 µL, 0.689 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (100 µL, 0.574 mmol, 1.0 eq), degassed acetonitrile (2.0 mL)

Flash column chromatography: (20 g silica gel, 14 x 2.0 cm, cyclohexane = 100 %, R_f = 0.50)

Yield: 158 mg (0.563 mmol, 98 %), yellow oil

$C_{18}H_{32}S$ [280.51 $gmol^{-1}$]

HRMS (TOF MSEI+):	calculated:	280.2225
	found:	280.2222

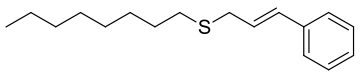
$[\alpha]_D^{20}$ = -6.3 (c = 1 in *n*-heptane)

GC-MS (HS_50_S2): t_R = 7.32 min; *m/z* = 280 (10 %, MP), 134 (53 %), 119 (100 %, BP), 91 (79 %), 79 (24 %), 55 (16 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.83-0.90 (m, 6H, 2 x C<u>H</u>$_3$), 1.14 (d, 1H, ^3J(H,H) = 8.6 Hz, <u>H</u>-alkyl), 1.27-1.37 (m, 13H, <u>H</u>-alkyl), 1.55 (p, 2H, ^3J(H,H) = 7.5 Hz, <u>H</u>-alkyl), 2.09-2.11 (m, 1H, <u>H</u>-alkyl), 2.19-2.27 (m, 3H, <u>H</u>-alkyl), 2.37-2.44 (m, 3H, <u>H</u>-alkyl), 3.05 (m, 2H, -S-C<u>H</u>$_2$-C_q), 5.34 (pseudo bs, 1H, CH_2-C<u>H</u>-C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.1 (<u>C</u>H$_3$), 21.1 (<u>C</u>-alkyl), 22.7 (<u>C</u>H$_3$), 26.2 (<u>C</u>H$_3$), 29.0 (<u>C</u>-alkyl), 29.2 (<u>C</u>-alkyl), 29.2 (<u>C</u>-alkyl), 29.3 (<u>C</u>-alkyl), 31.1 (<u>C</u>-alkyl), 31.3 (<u>C</u>-alkyl), 31.8 (<u>C</u>-alkyl), 31.8 (<u>C</u>-alkyl), 38.1 (<u>C</u>-alkyl), 38.2 (<u>C</u>-alkyl), 40.6 (<u>C</u>-alkyl), 45.1 (<u>C</u>-alkyl), 119.5 (<u>C</u>=C), 143.8 (<u>C</u>=C).

6.7.8.7. Synthesis of cinnamyl(octyl)sulfane (150)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (6.6 mg, 11.5 µmol, 0.02 eq), biphephos (**40**) (9.0 mg, 11.5 µmol, 0.02 eq), degassed cinnamyl methyl carbonate (**145**) (120 µL, 0.689 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (100 µL, 0.574 mmol, 1.0 eq), degassed acetonitrile (2.0 mL)

Due to the solid form of cinnamyl methyl carbonate (**145**) it was heated to 40°C, degassed at 40°C and added using a Hamilton syringe at this temperature to the 60°C hot $Pd(dba)_2$ (**X**) and biphephos (**40**) solution in acetonitrile.

Flash column chromatography: (20 g silica gel, 14 x 2.0 cm, cyclohexane = 100 %, R_f = 0.13)

Yield: 141 mg (0.537 mmol, 94 %), yellow oil

$C_{17}H_{26}S$ [262.45 $gmol^{-1}$]

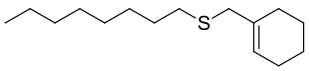
HRMS (TOF MSEI+):	calculated:	262.1755
	found:	262.1755

GC-MS (HS_50_S2): t_R = 7.61 min; *m/z* = 262 (7 %, MP), 117 (100 %, BP), 105 (35 %), 91 (17 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 3H, ^3J(H,H) = 6.8 Hz, C<u>H</u>$_3$-CH$_2$), 1.25-1.35 (m, 10H, 5 x C<u>H</u>$_2$), 1.52-1.64 (m, 2H, C<u>H</u>$_2$), 2.48 (t, 2H, ^3J(H,H) = 7.2 Hz, CH$_2$-C<u>H</u>$_2$-S), 3.29 (d, 2H, ^3J(H,H) = 7.2 Hz, S-C<u>H</u>$_2$-CH=CH), 6.13-6.23 (m, 1H, S-CH$_2$-C<u>H</u>=CH), 6.42 (d, 1H, ^3J(H,H) = 15.9 Hz, S-CH$_2$-CH=C<u>H</u>), 7.19-7.38 (m, 5H, <u>H</u>-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.1 (C<u>H</u>$_3$), 22.6 (<u>C</u>-alkyl), 28.9 (<u>C</u>-alkyl), 29.2 (<u>C</u>-alkyl), 29.2 (<u>C</u>-alkyl), 29.4 (<u>C</u>-alkyl), 30.8 (<u>C</u>-alkyl), 31.8 (<u>C</u>-alkyl), 34.3 (<u>C</u>-alkyl), 126.3 (<u>C</u>H=CH and 2 × <u>C</u>-Ar), 127.4 (<u>C</u>-Ar), 128.5 (2 × <u>C</u>-Ar), 131.9 (<u>C</u>H=CH), 136.8 ($C_{q,Ar}$).

6.7.8.8. Synthesis of (cyclohex-1-en-1-ylmethyl)(octyl)sulfane (151)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 5.74 µmol, 0.02 eq), degassed cyclohex-1-en-1-ylmethyl methyl carbonate (**144**) (55 µL, 0.344 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (50 µL, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

Flash column chromatography: (6.5 g silica gel, 15.5 x 1.0 cm, cyclohexane = 100 %, R_f = 0.43)

Yield: 59.2 mg (0.246 mmol, 86 %), yellow oil

$C_{15}H_{28}S$ [240.45 gmol^{-1}]

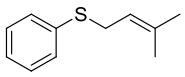
HRMS (TOF MSEI+): calculated: 240.1912
found: 240.1913

GC-MS (HS_50_S2): t_R = 6.86 min; *m/z* = 240 (23 %, MP), 145 (21 %), 127 (13 %), 95 (100 %, BP), 79 (63 %), 67 (38 %), 55 (25 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.81 (t, 3H, ^3J(H,H) = 6.6 Hz, C$\underline{H}_3$-CH_2), 1.20-1.30 (m, 11H, $\underline{H}$-alkyl), 1.45-1.59 (m, 6H, $\underline{H}$-alkyl), 1.95-2.00 (m, 4H, $\underline{H}$-alkyl), 2.32 (t, 2H, ^3J(H,H) = 7.2 Hz, CH_2-C$\underline{H}_2$-S), 2.98 (s, 2H, S-C$\underline{H}_2$-C_q=CH), 5.47 (pseudo bs, 1H, C$\underline{H}$-C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.1 ($\underline{C}H_3$), 22.3 ($\underline{C}$-alkyl), 22.7 ($\underline{C}$-alkyl), 22.8 ($\underline{C}$-alkyl), 25.3 ($\underline{C}$-alkyl), 26.8 ($\underline{C}$-alkyl), 29.0 ($\underline{C}$-alkyl), 29.2 ($\underline{C}$-alkyl), 29.2 ($\underline{C}$-alkyl), 29.4 ($\underline{C}$-alkyl), 31.0 ($\underline{C}$-alkyl), 31.8 ($\underline{C}$-alkyl), 39.7 ($\underline{C}$-alkyl), 124.6 (C_q=$\underline{C}$H), 133.9 ($\underline{C}_q$=CH).

6.7.8.9. Synthesis of (3-methylbut-2-en-1-yl)(phenyl)sulfane (152)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (16.8 mg, 29.2 µmol, 0.02 eq), biphephos (**40**) (23.0 mg, 29.2 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (260 µL, 1.75 mmol, 1.2 eq), degassed thiophenol (**68**) (150 µL, 1.46 mmol, 1.0 eq), degassed acetonitrile (5.0 mL)

Flash column chromatography: (35 g silica gel, 23.0 x 2.5 cm, *n*-pentane = 100 %, R_f = 0.41)

Yield: 247 mg (1.39 mmol, 95 %), colorless oil

$C_{11}H_{14}S$ [178.29 gmol^{-1}]

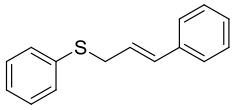
GC-MS (HS_50_S2): t_R = 5.58 min; *m/z* = 178 (25 %, MP), 110 (100 %, BP), 69 (93 %), 65 (27 %), 51 (13 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51 (s, 3H, C$\underline{H}_3$), 1.63 (s, 3H, C$\underline{H}_3$), 3.46 (d, 2H, ^3J(H,H) = 7.8 Hz, S-C$\underline{H}_2$), 5.22 (m, 1H, S-CH_2-C$\underline{H}$), 7.09-7.27 (m, 5H, $\underline{H}$-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.7 ($\underline{C}H_3$), 25.6 ($\underline{C}H_3$), 32.2 (S-$\underline{C}H_2$), 119.3 (S-CH_2-$\underline{C}H$-C_q), 125.9 ($\underline{C}$-Ar), 128.7 (2 × $\underline{C}$-Ar), 129.7 (2 × $\underline{C}$-Ar), 136.4 ($C_{q,Ar}$ or CH=$\underline{C}_q$), 136.8 ($C_{q,Ar}$ or CH=$\underline{C}_q$).

Characterized by Martin *et al.*[308]

6.7.8.10. Synthesis of cinnamyl(phenyl)sulfane (153)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (16.8 mg, 29.2 µmol, 0.02 eq), biphephos (**40**) (23.0 mg, 29.2 µmol, 0.02 eq), degassed cinnamyl methyl carbonate (**145**) (330 µL, 1.75 mmol, 1.2 eq), degassed thiophenol (**68**) (150 µL, 1.46 mmol, 1.0 eq), degassed acetonitrile (5.0 mL)

Due to the solid form of cinnamyl methyl carbonate (**145**) it was heated to 40°C, degassed at 40°C and added using a Hamilton syringe at this temperature to the 60°C hot $Pd(dba)_2$ (**X**) and biphephos (**40**) solution in acetonitrile.

Flash column chromatography: (90 g silica gel, 25.0 x 3.0 cm, cyclohexane/ethyl acetate = 40:1 (v/v), R_f = 0.41)

Yield: 326 mg (1.44 mmol, 99 %), pale yellow crystals

$C_{15}H_{14}S$ [226.34 $gmol^{-1}$]

m_p = 75 - 76 °C

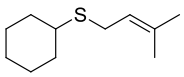
GC-MS (HS_50_S2): t_R = 7.29 min; *m/z* = 226 (7 %, MP), 117 (100 %, BP), 109 (10 %), 91 (23 %), 65 (14 %), 51 (8 %).

^{1}H-NMR (300.36 MHz, $CDCl_3$): δ = 3.63 (dd, 2H, ^3J(H,H) = 6.9 Hz, ^4J(H,H) = 0.6 Hz, S-C$\underline{H}_2$-CH=CH), 6.12 – 6.22 (m, 1H, S-CH_2-C$\underline{H}$=CH-Ph), 6.35 (d, 1H, ^3J(H,H) = 15.6 Hz, S-CH_2-CH=C$\underline{H}$-Ph), 7.09 – 7.32 (m, 10H, $\underline{H}$-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 37.1 (S-<u>C</u>H_2-CH=CH), 125.0 (<u>C</u>H=CH or <u>C</u>-Ar), 126.3 (2 × <u>C</u>-Ar), 126.4 (<u>C</u>H=CH or <u>C</u>-Ar), 127.5 (<u>C</u>H=CH or <u>C</u>-Ar), 128.5 (2 × <u>C</u>-Ar), 128.8 (2 × <u>C</u>-Ar), 130.2 (2 × <u>C</u>-Ar), 132.8 (S-CH_2-C<u>H</u>=CH-Ph), 135.8 ($C_{q,Ar}$), 136.7 ($C_{q,Ar}$).

Characterized by Jafarpour *et al.*[309]

6.7.8.11. Synthesis of cyclohexyl(3-methylbut-2-en-1-yl)sulfane (154)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (9.4 mg, 16.3 µmol, 0.02 eq), biphephos (**40**) (13.0 mg, 16.3 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (145 µL, 0.98 mmol, 1.2 eq), degassed cyclohexanethiol (**78**) (100 µL, 0.817 mmol, 1.0 eq), degassed acetonitrile (2.8 mL)

Flash column chromatography: (20 g silica gel, 14.5 x 2.0 cm, *n*-pentane/DCM = 1000:1 (v/v), R_f = 0.17)

Yield: 136 mg (0.739 mmol, 90 %), colorless oil

$C_{11}H_{20}S$ [184.34 $gmol^{-1}$]

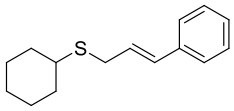
HRMS (TOF MSEI+):	calculated:	184.1286
	found:	184.1281

GC-MS (HS_50_S2): t_R = 5.55 min; *m/z* = 184 (35 %, MP), 114 (17 %), 101 (7 %), 81 (20 %), 69 (100 %, BP), 55 (12 %), 55 (55 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.25-1.37 (m, 6H, <u>H</u>-alkyl), 1.57-1.77 (m, 8H, <u>H</u>-alkyl), 1.94-1.97 (m, 2H, <u>H</u>-alkyl), 2.56-2.63 (m, 1H, S-C<u>H</u>-), 3.16 (d, 2H, ^3J(H,H) = 7.5 Hz, S-C<u>H</u>$_2$-CH=C_q), 5.24 (t, 1H, ^3J(H,H) = 7.7 Hz, C<u>H</u>=C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.9 (<u>C</u>H_3), 25.8 (C<u>H</u>$_3$ or C<u>H</u>$_2$), 26.0 (C<u>H</u>$_3$ or C<u>H</u>$_2$), 26.3 (2 ×<u>C</u>-alkyl), 28.0 (<u>C</u>-alkyl), 33.8 (2 × <u>C</u>-alkyl), 43.1 (S-<u>C</u>H-), 121.2 (<u>C</u>H=C_q), 134.7 (CH=<u>C</u>$_q$).

6.7.8.12. Synthesis of cinnamyl(cyclohexyl)sulfane (155)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (4.7 mg, 8.17 µmol, 0.02 eq), biphephos (**40**) (6.5 mg, 8.17 µmol, 0.02 eq), degassed cinnamyl methyl carbonate (**145**) (85 µL, 0.588 mmol, 1.2 eq), degassed cyclohexanethiol (**78**) (50 µL, 0.409 mmol, 1.0 eq), degassed acetonitrile (1.4 mL)

Due to the solid form of cinnamyl methyl carbonate (**145**) it was heated to 40°C, degassed at 40°C and added using a Hamilton syringe at this temperature to the 60°C hot $Pd(dba)_2$ (**X**) and biphephos (**40**) solution in acetonitrile.

Flash column chromatography: (6.0 g silica gel, 16.0 x 1.0 cm, cyclohexane = 100 %, R_f = 0.18)

Yield: 86.4 mg (0.372 mmol, 76 %), yellow oil

$C_{15}H_{20}S$ [232.38 $gmol^{-1}$]

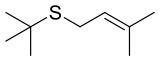
GC-MS (HS_50_S2): t_R = 7.30 min; *m/z* = 232 (24 %), 149 (4 %), 117 (100 %, BP), 91 (11 %), 55 (7 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.16-1.35 (m, 5H, H-alkyl), 1.48-1.57 (m, 1H, H-alkyl), 1.68-1.74 (m, 2H, H-alkyl), 1.87-1.91 (m, 2H, H-alkyl), 2.54-2.61 (m, 1H, S-CH-), 3.26 (dd, 2H, ^3J(H,H) = 6.3 Hz, ^4J(H,H) = 0.9 Hz, S-CH_2-CH=CH), 6.08–6.18 (m, 1H, S-CH_2-CH=CH-Ph), 6.36 (d, 1H, ^3J(H,H) = 15.6 Hz, S-CH_2-CH=CH-Ph), 7.12-7.31 (m, 5H, H-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 25.6 (C-alkyl), 25.7 (2 × C-alkyl), 32.5 (S-CH_2-CH=CH), 33.2 (2 × C-alkyl), 42.1 (S-CH-), 126.0 (2 × C-Ar), 126.5 (CH=CH or C-Ar), 127.1 (CH=CH or C-Ar), 128.3 (2 × C-Ar), 131.3 (S-CH_2-CH=CH-Ph), 136.7 ($C_{q,Ar}$).

Characterized by Jafarpour *et al.*[309]

6.7.8.13. Synthesis of *tert*-butyl(3-methylbut-2-en-1-yl)sulfane (156)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (5.1 mg, 8.90 µmol, 0.02 eq), biphephos (**40**) (7.0 mg, 8.90 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (80 µL, 0.533 mmol, 1.2 eq), degassed 2-methyl-2-propanethiol (**81**) (50 µL, 0.444 mmol, 1.0 eq), degassed acetonitrile (1.5 mL)

Flash column chromatography: (6.1 g silica gel, 16.0 x 1.0 cm, *n*-pentane = 100 %, R_f = 0.39)

Yield: 10.4 mg (65.8 µmol, 15 %), colorless oil

$C_9H_{18}S$ [158.30 $gmol^{-1}$]

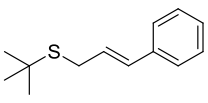
GC-MS (HS_50_S2): t_R = 4.21 min; *m/z* = 158 (24 %, MP), 102 (30 %), 69 (100 %, BP), 57 (50 %), 53 (23 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.33 (s, 9H, $(C\underline{H}_3)_3$-C_q-S), 1.68 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 1.71 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 3.17 (d, 2H, ^3J(H,H) = 7.8 Hz, S-$C\underline{H}_2$), 5.25 (m, 1H, $(CH_3)_2$-C_q=C$\underline{H}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 17.9 ($\underline{C}$-alkyl), 25.9 ($\underline{C}$-alkyl), 26.7 ($\underline{C}$-alkyl), 31.2 (($\underline{C}H_3)_3$-C_q-S), 42.4 ($(CH_3)_3$-$\underline{C}_q$-S), 120.5 ($\underline{C}$H=C_q), 135.2 (CH=$\underline{C}_q$).

Characterized by Vonwiller *et al.*[310]

6.7.8.14. Synthesis of *tert*-butyl(cinnamyl)sulfane (157)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (5.1 mg, 8.90 µmol, 0.02 eq), biphephos (**40**) (7.0 mg, 8.90 µmol, 0.02 eq), degassed cinnamyl methyl carbonate (**145**) (90 µL, 0.533 mmol, 1.2 eq), degassed 2-methyl-2-propanethiol (**81**) (50 µL, 0.444 mmol, 1.0 eq), degassed acetonitrile (1.5 mL)

Flash column chromatography: (6.0 g silica gel, 16.0 x 1.0 cm, cyclohexane/ethyl acetate = 30:1 (v/v), R_f = 0.49)

Yield: 69.6 mg (0.337 mmol, 76 %), yellow oil

$C_{13}H_{18}S$ [206.35 $gmol^{-1}$]

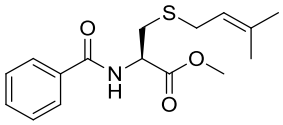
GC-MS (HS_50_S2): t_R = 6.27 min; *m/z* = 206 (14 %, MP), 117 (100 %, BP), 91 (15 %), 77 (7 %), 65 (6 %), 57 (21 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.29 (s, 9H, $(C\underline{H}_3)_3$-C_q-S), 3.31 (dd, 2H, ^3J(H,H) = 6.3 Hz, ^4J(H,H) = 0.9 Hz, S-$C\underline{H}_2$-CH=CH-Ph), 6.11-6.21 (m, 1H, S-CH_2-$C\underline{H}$=CH-Ph), 6.44 (d, 1H, ^3J(H,H) = 15.6 Hz, S-CH_2-CH=$C\underline{H}$-Ph), 7.10-7.29 (m, 5H, $\underline{H}$-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 31.3 ($(\underline{C}H_3)_3$-C_q-S), 31.9 (S-$\underline{C}H_2$-CH=CH-Ph), 43.0 ($(CH_3)_3$-$\underline{C}_q$-S), 126.5 (2 × $\underline{C}$-Ar), 127.1 ($\underline{C}$H=CH or $\underline{C}$-Ar), 127.6 ($\underline{C}$H=CH or $\underline{C}$-Ar), 128.8 (2 × $\underline{C}$-Ar), 132.0 (S-CH_2-$\underline{C}$H=CH-Ph), 137.3 ($C_{q,Ar}$).

Characterized by Veiros *et al.*[311]

6.7.8.15. Synthesis of (*R*)-methyl 2-benzamido-3-((3-methylbut-2-en-1-yl)thio)propanoate (110)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (7.2 mg, 12.5 µmol, 0.02 eq), biphephos (**40**) (9.9 mg, 12.5 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (110 µL, 0.752 mmol, 1.2 eq), L-cysteine *N*-benzoyl methyl ester (**105**) (150 mg, 0.627 mmol, 1.0 eq), degassed acetonitrile (2.2 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred for 30 min at 60°C in 1.0 mL of acetonitrile, degassed prior to use. Meanwhile L-cysteine *N*-benzoyl methyl ester (**105**) was dissolved in another dry Schlenk tube containing 1.2 mL of acetonitrile, degassed prior

to use. This solution was then transferred completely into the Schlenk tube containing $Pd(dba)_2$ (**X**) and biphephos (**40**) after 30 min stirring at 60°C. The transfer was performed under gentle stream of argon using a Hamilton syringe. Afterwards methyl (3-methylbut-2-en-1-yl) carbonate (**88**) was added as described in chapter 6.7.8.

After 24 h the reaction was stopped and purified using flash column chromatography: (20.0 g silica gel, 14.0 x 2.0 cm, cyclohexane/ethyl acetate = 4:1 (v/v), R_f = 0.35)

Yield: 165 mg (0.537 mmol, 82 %), white powder

$C_{16}H_{21}NO_3S$ [307.41 $gmol^{-1}$]

m_p = 83 - 84 °C

$[\alpha]_D^{20}$ = -72.4 (c = 1 in acetonitrile)

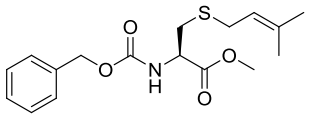
GC-MS (HS_50_S2): t_R = 8.24 min; *m/z* = 307 (1 %, MP), 238 (30 %), 206 (4 %), 148 (8 %), 122 (10 %), 105 (100 %, BP), 77 (45 %), 69 (24 %), 51 (10 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.63 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 1.70 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 2.98-3.19 (m, 4H, 2 x $C\underline{H}_2$-S), 3.80 (s, 3H, $C\underline{H}_3$-O), 5.00 (dt, 1H, ^3J(H,H) = 7.5 Hz, 5.2 Hz, CO-NH-C<u>H</u>), 5.15-5.21 (m, 1H, $(CH_3)_2$-C_q=C<u>H</u>), 6.97 (d, 1H, ^3J(H,H) = 6.9 Hz, N<u>H</u>), 7.42-7.55 (m, 3H, <u>H</u>-Ar), 7.81-7.84 (m, 2H, <u>H</u>-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0 ($(\underline{C}H_3)_2$-C_q=CH), 25.9 ($(\underline{C}H_3)_2$-C_q=CH), 30.5 ($\underline{C}H_2$-S), 33.8 ($\underline{C}H_2$-S), 52.5 ($C\underline{H}_3$-O or CO-NH-<u>C</u>H), 53.0 ($C\underline{H}_3$-O or CO-NH-<u>C</u>H), 120.0 ($(CH_3)_2$-C_q=<u>C</u>H), 127.4 (2 × <u>C</u>-Ar), 128.9 (2 × <u>C</u>-Ar), 132.1 (<u>C</u>=C or <u>C</u>-Ar), 134.0 (<u>C</u>=C or <u>C</u>-Ar), 136.7 ($C_{q,Ar}$), 167.2 (<u>C</u>O-NH), 171.7 ($\underline{C}O_2$-CH_3).

Characterized by Casadio *et al.*[312]

6.7.8.16. Synthesis of (*R*)-methyl 2-(((benzyloxy)carbonyl)amino)-3-((3-methylbut-2-en-1-yl)thio)propanoate (108)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (6.4 mg, 11.1 µmol, 0.02 eq), biphephos (**40**) (8.8 mg, 11.1 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (85 µL, 0.668 mmol, 1.2 eq), L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) (150 mg, 0.557 mmol, 1.0 eq), degassed acetonitrile (2.0 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred for 30 min at 60°C in 1.0 mL of acetonitrile, degassed prior to use. Meanwhile L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) was dissolved in another dry Schlenk tube containing 1.0 mL of acetonitrile, degassed prior to use. This solution was then transferred completely into the Schlenk tube containing $Pd(dba)_2$ (**X**) and biphephos (**40**) after 30 min stirring at 60°C. The transfer was performed under a gentle stream of argon using a Hamilton syringe. Afterwards methyl (3-methylbut-2-en-1-yl) carbonate (**88**) was added as described in chapter 6.7.8.

After 24 h the reaction was stopped and purified using flash column chromatography: (20.0 g silica gel, 14.0 x 2.0 cm, cyclohexane/ethyl acetate = 6:1 (v/v), R_f = 0.30)

Yield: 151 mg (0.448 mmol, 80 %), yellow oil

$C_{17}H_{23}NO_4S$ [337.43 $gmol^{-1}$]

HRMS (TOF MSEI+):	calculated:	337.1348
	found:	337.1368

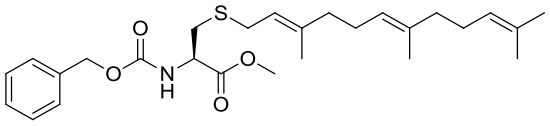
$[\alpha]_D^{20}$ = -38.3 (c = 1 in acetonitrile)

GC-MS (HS_50_S2): t_R = 8.51 min; *m/z* = 246 (3 %), 202 (34 %), 146 (14 %), 91 (100 %, BP), 69 (32 %).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.64 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 1.72 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 2.91 (t, 2H, ^3J(H,H) = 5.4 Hz, $C\underline{H}_2$-S), 3.14 (t, 2H, ^3J(H,H) = 7.5 Hz, $C\underline{H}_2$-S), 3.76 (s, 3H, $C\underline{H}_3$-O), 4.59 (dt, 1H, ^3J(H,H) = 7.8 Hz, 5.3 Hz, CO-NH-C$\underline{H}$), 5.12 (s, 2H, -NH-CO_2-$C\underline{H}_2$-C_6H_5), 5.18 (t, 1H, ^3J(H,H) = 7.8 Hz, $(CH_3)_2$-C_q=C$\underline{H}$), 5.58 (d, 1H, ^3J(H,H) = 7.8 Hz, N$\underline{H}$), 7.33-7.35 (m, 5H, $\underline{H}$-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0 ($(\underline{C}H_3)_2$-C_q=CH), 25.9 ($(\underline{C}H_3)_2$-C_q=CH), 30.4 ($\underline{C}H_2$-S), 33.9 ($\underline{C}H_2$-S), 52.8 ($\underline{C}H_3$-O or CO-NH-$\underline{C}$H), 53.9 ($\underline{C}H_3$-O or CO-NH-$\underline{C}$H), 67.3 (-NH-CO_2-$\underline{C}H_2$-C_6H_5), 120.0 ($(CH_3)_2$-C_q=$\underline{C}$H), 128.3 (2 × $\underline{C}$-Ar), 128.4 ($\underline{C}$-Ar), 128.8 (2 × $\underline{C}$-Ar), 136.4 ($(CH_3)_2$-$\underline{C}_q$=CH or $C_{q,Ar}$), 136.6 ($(CH_3)_2$-$\underline{C}_q$=CH or $C_{q,Ar}$), 156.0 ($\underline{C}O_2$-NH), 171.6 ($\underline{C}O_2$-CH_3).

6.7.8.17. Synthesis of (*R*)-methyl 2-(((benzyloxy)carbonyl)amino)-3-(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)thio)propanoate (159158)



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (2.1 mg, 3.71 µmol, 0.02 eq), biphephos (**40**) (2.9 mg, 3.71 µmol, 0.02 eq), degassed methyl ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) carbonate (farnesyl carbonate) (**141**) (76 µL, 0.222 mmol, 1.2 eq), L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) (50 mg, 0.186 mmol, 1.0 eq), degassed acetonitrile (0.75 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred for 30 min at 60°C in 0.75 mL of acetonitrile, degassed prior to use. Afterwards first L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) was added as a solid under a gentle stream of argon. Afterwards methyl ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) carbonate (farnesyl carbonate) (**141**) was added as described in chapter 6.7.8.

After 24 h the reaction was stopped and purified using flash column chromatography: (10.0 g silica gel, 20.0 x 1.5 cm, cyclohexane/ethyl acetate = 10:1 (v/v), R_f = 0.20)

Yield: 69.7 mg (0.147 mmol, 79 %), colorless oil

$C_{27}H_{39}NO_4S$ [473.68 $gmol^{-1}$]

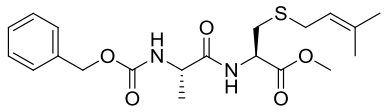
HRMS: $[MNa]^+$:	calculated:	496.2498
	found:	496.2472
HRMS: $[MK]^+$:	calculated:	512.2237
	found:	512.2264

$[\alpha]_D^{20}$ = -39.4 (c = 0.5 in methanol)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.60 (s, 6H, 2 × C<u>H</u>$_3$-), 1.64 (s, 3H, C<u>H</u>$_3$-), 1.68 (s, 3H, C<u>H</u>$_3$-), 1.96-2.10 (m, 8 H, -C<u>H</u>$_2$-C<u>H</u>$_2$-CH=C(CH_3)-C<u>H</u>$_2$-C<u>H</u>$_2$-), 2.84-2.98 (m, 2H, CH_2-C<u>H</u>$_2$-S-), 3.09-3.23 (m, 2H, -S-C<u>H</u>$_2$-CH=C(CH_3)-), 3.76 (s, 3H, C<u>H</u>$_3$-O), 4.59 (pseudo q, 1H, ^3J(H,H) = 7.8 Hz, 5.4 Hz, -NH-C<u>H</u>-), 5.07-5.12 (m, 4H, C_6H_5-C<u>H</u>$_2$-CO_2-NH-, 2 × -C<u>H</u>=C_q), 5.19 (t, 1H, ^3J(H,H) = 7.6 Hz, -S-CH_2-C<u>H</u>=C_q), 5.55 (d, 1H, ^3J(H,H) = 7.7 Hz, N<u>H</u>), 7.33-7.37 (m, 5H, <u>H</u>$_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.2 ($\underline{C}H_3$), 16.3 ($\underline{C}H_3$), 17.8 ($\underline{C}H_3$), 25.8 ($\underline{C}H_3$), 26.6 (CH_2), 26.9 (CH_2), 30.2 (-S-$\underline{C}H_2$-CH=C(CH_3)-), 33.7 (NH-CH(CO_2-CH_3)-$\underline{C}H_2$-S-), 39.8 (-CH=C(CH_3)-$\underline{C}H_2$-), 39.9 (-CH=C(CH_3)-$\underline{C}H_2$-), 52.7 (CH_3-O or -NH-$\underline{C}H$), 53.8 (CH_3-O or -NH-$\underline{C}H$), 67.3 (C_6H_5-$\underline{C}H_2$-CO_2-NH-), 119.8 (-S-CH_2-$\underline{C}H$=C_q), 123.9 (-$\underline{C}H$=C_q), 124.5 (-$\underline{C}H$=C_q), 128.3 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 131.5 (-CH=$\underline{C}_q$), 135.5 (-CH=$\underline{C}_q$), 136.3 ($C_{q,Ar}$), 140.2 (-CH=$\underline{C}_q$), 156.0 ($\underline{C}O_2$-NH), 171.5 ($\underline{C}O_2$-CH_3).

6.7.8.18. Isoprenylation of Cbz-Ala-Cys-OMe (166) to yield product 167



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 5.74 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (50 µL, 0.344 mmol, 1.2 eq), Cbz-Ala-Cys-OMe (**166**) (97.5 mg, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred for 30 min at 60°C in 1.0 mL of acetonitrile, degassed prior to use. Afterwards first Cbz-Ala-Cys-OMe (**166**) was added as a solid under a gentle stream of argon. Afterwards methyl (3-methylbut-2-en-1-yl) carbonate (**88**) was added as described in chapter 6.7.8.

After 24 h the reaction was stopped and purified via flash column chromatography: (15.0 g silica gel, 17.0 x 2.0 cm, cyclohexane/ethyl acetate = 2:1 (v/v), R_f = 0.27)

Yield: 97.0 mg (0.238 mmol, 83 %), colorless powder

$C_{20}H_{28}N_2O_5S_2$ [408.51 gmol^{-1}]

HRMS: $[MNa]^+$: calculated: 431.1617
found: 431.1602

mp.: 79-80°C

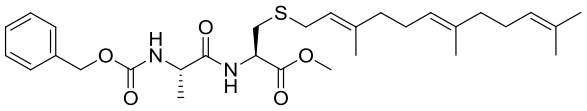
$[\alpha]_D^{22}$ = -28.7 (c = 0.5 in acetonitrile)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.39 (d, 3H, ^3J(H,H) = 6.9 Hz, C$\underline{H}_3$-CH), 1.62 (s, 3H, C$\underline{H}_3$-C_q), 1.71 (s, 3H, C$\underline{H}_3$-C_q), 2.80-2.93 (m, 2H, S-C$\underline{H}_2$), 3.08-3.17 (m, 2H, S-C$\underline{H}_2$), 3.73 (s, 3H,

$C\underline{H}_3$-O), 4.30 (pseudo t, 1H, ^3J(H,H) = 6.6 Hz, CO-C$\underline{H}$), 4.74 (pseudo q, 1H, ^3J(H,H) = 7.0, CO-C$\underline{H}$), 5.12-5.18 (m, 3H, Ph-C$\underline{H}_2$ and $(CH_3)_2$-C_q=C$\underline{H}$), 5.36 (d, 1H, ^3J(H,H) = 6.6 Hz, CO-N$\underline{H}$), 6.74 (d, 1H, ^3J(H,H) = 6.6 Hz, CO-N$\underline{H}$), 7.28-7.32 (m, 5H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0 ($\underline{C}H_3$-CH or $\underline{C}H_3$-C_q), 18.8 ($\underline{C}H_3$-CH or $\underline{C}H_3$-C_q), 25.9 ($\underline{C}H_3$-C_q), 30.3 (S-$\underline{C}H_2$), 33.5 (S-$\underline{C}H_2$), 50.6 ($\underline{C}H_3$-O or CH_3-$\underline{C}$H or CH_3-CO_2-$\underline{C}$H); 52.0 ($\underline{C}H_3$-O or CH_3-$\underline{C}$H or CH_3-CO_2-$\underline{C}$H), 52.9 ($\underline{C}H_3$-O or CH_3-$\underline{C}$H or CH_3-CO_2-$\underline{C}$H), 67.3 (Ph-$\underline{C}H_2$), 119.9 ($(CH_3)_2$-C_q=$\underline{C}$H), 128.3 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 136.4 ($(CH_3)_2$-$\underline{C}_q$=CH or $C_{q,Ar}$), 136.6 ($(CH_3)_2$-$\underline{C}_q$=CH or $C_{q,Ar}$), 156.1 ($\underline{C}O_2$-NH), 171.3 (C_q), 172.3 (C_q).

6.7.8.19. Farnesylation of Cbz-Ala-Cys-OMe (166) to yield product 168



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 5.74 µmol, 0.02 eq), degassed methyl ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) carbonate (farnesyl carbonate) (**141**) (104 µL, 0.344 mmol, 1.2 eq), Cbz-Ala-Cys-OMe (**166**) (97.5 mg, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred at 60°C for 30 min in 1.0 mL of acetonitrile, degassed prior to use. First Cbz-Ala-Cys-OMe (**166**) was added as a solid under a gentle stream of argon. Secondly methyl ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) carbonate (farnesyl carbonate) (**141**) was added as described in chapter 6.7.8.

After 24 h the reaction was stopped and purified via flash column chromatography: (20.0 g silica gel, 16.0 x 2.5 cm, cyclohexane/ethyl acetate = 3:1 (v/v), R_f = 0.18)

Yield: 148 mg (0.272 mmol, 95 %), pale yellow oil, which became a solid after 24 h storage at 4°C

$C_{30}H_{44}N_2O_5S$ [544.75 gmol^{-1}]

HRMS: $[MNa]^+$:	calculated:	567.2869
	found:	567.2872

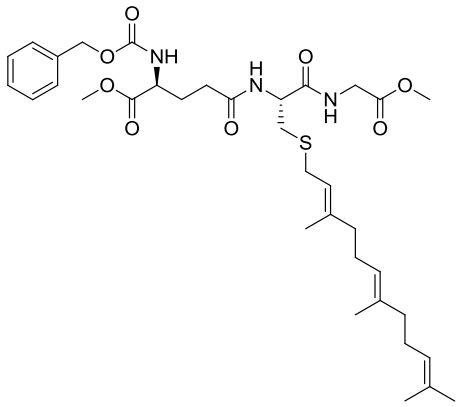
mp.: 43-45°C

$[\alpha]_D^{22}$ = -27.2 (c = 0.6 in acetonitrile)

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.39 (d, 3H, ^3J(H,H) = 6.9 Hz, C$\underline{H}_3$-CH), 1.57 (s, 6H, 2 × C$\underline{H}_3$-C_q), 1.63 (s, 3H, C$\underline{H}_3$-C_q), 1.65 (s, 3H, C$\underline{H}_3$-C_q), 1.94-2.08 (m, 8H, -C$\underline{H}_2$-C$\underline{H}_2$-CH=C(CH_3)-C$\underline{H}_2$-C$\underline{H}_2$-), 2.80-2.96 (m, 2H, S-C$\underline{H}_2$), 3.04-3.15 (m, 2H, S-C$\underline{H}_2$), 3.73 (s, 3H, C$\underline{H}_3$-O), 4.29 (pseudo t, 1H, ^3J(H,H) = 6.6 Hz, CO-C$\underline{H}$), 4.74 (pseudo q, 1H, ^3J(H,H) = 6.0 Hz, 5.7 Hz, CO-C$\underline{H}$), 5.05-5.12 (m, 4H, C_6H_5-C$\underline{H}_2$-CO_2-NH-, 2 × -C$\underline{H}$=C_q), 5.18 (t, 1H, ^3J(H,H) = 7.8 Hz, -S-CH_2-C$\underline{H}$=C_q), 5.33 (d, 1H, ^3J(H,H) = 6.6 Hz, CO-N$\underline{H}$), 6.71 (d, 1H, ^3J(H,H) = 7.2 Hz, CO-N$\underline{H}$), 7.30-7.33 (m, 5H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.2 ($\underline{C}H_3$-C_q), 16.3 ($\underline{C}H_3$-C_q), 17.9 ($\underline{C}H_3$-CH or $\underline{C}H_3$-C_q), 18.8 ($\underline{C}H_3$-CH or $\underline{C}H_3$-C_q), 25.9 ($\underline{C}H_3$-C_q), 26.6 ($\underline{C}H_2$), 26.9 ($\underline{C}H_2$), 30.1 (-S-$\underline{C}H_2$-CH=C(CH_3)-), 33.4 (NH-CH(CO_2-CH_3)-$\underline{C}H_2$-S-), 39.8 (-CH=C(CH_3)-$\underline{C}H_2$-), 39.9 (-CH=C(CH_3)-$\underline{C}H_2$-), 50.7 ($\underline{C}H_3$-O or CH_3-$\underline{C}H$ or CH_3-CO_2-$\underline{C}H$), 52.0 ($\underline{C}H_3$-O or CH_3-$\underline{C}H$ or CH_3-CO_2-$\underline{C}H$), 52.9 ($\underline{C}H_3$-O or CH_3-$\underline{C}H$ or CH_3-CO_2-$\underline{C}H$), 67.3 (C_6H_5-$\underline{C}H_2$-CO_2-NH-), 119.7 (-S-CH_2-$\underline{C}H$=C_q), 123.9 (-$\underline{C}H$=C_q), 124.5 (-$\underline{C}H$=C_q), 128.3 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.8 (2 × C_{Ar}), 131.5 (-CH=$\underline{C}_q$), 135.6 (-CH=$\underline{C}_q$ $\underline{C}_q$), 136.4 ($C_{q,Ar}$), 140.3 (-CH=$\underline{C}_q$), 156.0 ($\underline{C}O_2$-NH), 171.2 (C_q), 172.2 (C_q).

6.7.8.20. Farnesylation of Cbz-GSH-OMe 176 to yield product 177



Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (1.3 mg, 2.23 µmol, 0.02 eq), biphephos (**40**) (1.8 mg, 2.23 µmol, 0.02 eq), degassed methyl ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) carbonate (farnesyl carbonate) (**141**) (40 µL, 0.134 mmol, 1.2 eq), Cbz-GSH-OMe **176** (52.3 mg, 0.111 mmol, 1.0 eq), degassed acetonitrile (5.4 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred for 30 min at 60°C in 1.0 mL of acetonitrile, degassed prior to use. Afterwards first Cbz-GSH-OMe **176** was added as

a solid under a gentle stream of argon. Afterwards under inert conditions the wall of the Schlenk tube was rinsed with 4.4 mL of acetonitrile, degassed prior to use. This was necessary to dissolve all Cbz-GSH-OMe **176** at 60°C. Directly after having obtained a green solution methyl ((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) carbonate (farnesyl carbonate) (**141**) was added as described in chapter 6.7.8.

After 24 h the reaction was stopped and purified using flash column chromatography: (10.0 g silica gel, 20.0 x 1.5 cm, 3.3 % methanol in DCM, R_f = 0.19)

Yield: 66.0 mg (97.9 µmol, 88 %), off-white gum-like solid, which further solidified after storage at -18°C for 24 h.

$C_{35}H_{51}N_3O_8S$ [673.86 $gmol^{-1}$]

HRMS: $[MNa]^+$: calculated: 696.2395
found: 696.2359

mp.: 96°C

$[\alpha]_D^{20}$ = -29.0 (c = 0.25 in methanol)

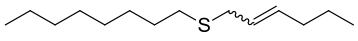
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.59 (s, 6H, 2 × C$\underline{H}_3$-C_q), 1.65 (s, 3H, 2 × C$\underline{H}_3$-C_q), 1.94-2.12 (m, 9H, -C$\underline{H}_2$-C$\underline{H}_2$-CH=C(CH_3)-C$\underline{H}_2$-C$\underline{H}_2$- and $H_{\beta\ Glu}$), 2.20-2.38 (m, 3H, H'$_{\beta\ Glu}$ and C$\underline{H}_{2\ \gamma\ Glu}$), 2.80-2.93 (m, 2H, S-C$\underline{H}_2$), 3.15-3.28 (m, 2H, S-C$\underline{H}_2$), 3.73 (s, 3H, C$\underline{H}_3$-O), 3.74 (s, 3H, C$\underline{H}_3$-O), 3.93-4.09 (m, 2H, C$\underline{H}_{2\ \alpha\ Gly}$), 4.45 (pseudo q, 1H, ^3J(H,H) = 7.8 Hz, CO-C$\underline{H}_{\ \alpha\ Glu}$ or CO-C$\underline{H}_{\ \alpha\ Cys}$), 4.57 (pseudo q, 1H, ^3J(H,H) = 6.7 Hz, CO-C$\underline{H}_{\ \alpha\ Glu}$ or CO-C$\underline{H}_{\ \alpha\ Cys}$), 5.06-5.14 (m, 4H, C_6H_5-C$\underline{H}_2$-CO_2-NH-, 2 × -C$\underline{H}$=C_q), 5.24 (t, 1H, ^3J(H,H) = 7.4 Hz, -S-CH_2-C$\underline{H}$=C_q), 5.71 (d, 1H, ^3J(H,H) = 7.6 Hz, CO-N$\underline{H}$), 6.66 (d, 1H, ^3J(H,H) = 7.0 Hz, CO-N$\underline{H}$), 7.02 (t, 1H, ^3J(H,H) = 5.1 Hz, CO-N$\underline{H}_{\ Gly}$), 7.31-7.36 (m, 5H, $\underline{H}_{Ar}$).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 16.2 (C$\underline{H}_3$-C_q), 16.3 (C$\underline{H}_3$-C_q), 17.8 (C$\underline{H}_3$-C_q), 25.8 (C$\underline{H}_3$-C_q), 26.6 (C$\underline{H}_2$), 26.9 (C$\underline{H}_2$), 28.4 ($CH_{2\ \beta\ Glu}$), 30.1 (-S-C$\underline{H}_2$-CH=C(CH_3)-), 32.2 (C$\underline{H}_{2\ \gamma\ glu}$ or NH-CH(CO_2-CH_3)-C$\underline{H}_2$-S-), 33.1 (C$\underline{H}_{2\ \gamma\ glu}$ or NH-CH(CO_2-CH_3)-C$\underline{H}_2$-S-), 39.8 (-CH=C(CH_3)-C$\underline{H}_2$-), 39.9 (-CH=C(CH_3)-C$\underline{H}_2$-), 41.4 ($CH_{2\ \alpha\ Gly}$), 52.5 (C$\underline{H}_3$-O or CO-C$\underline{H}$-), 52.6 (C$\underline{H}_3$-O or CO-C$\underline{H}$-), 52.7 (C$\underline{H}_3$-O or CO-C$\underline{H}$-), 53.4 (C$\underline{H}_3$-O or CO-C$\underline{H}$-), 67.3 (C_6H_5-C$\underline{H}_2$-CO_2-NH-), 119.7 (-S-CH_2-C$\underline{H}$=C_q), 123.9 (-C$\underline{H}$=C_q), 124.5 (-C$\underline{H}$=C_q), 128.3 (2 × C_{Ar}), 128.4 (C_{Ar}), 128.7 (2 × C_{Ar}), 131.5 (-CH=C$\underline{_q}$), 135.5 (-CH=C$\underline{_q}$), 136.3 ($C_{q,Ar}$), 140.2 (-CH=C$\underline{_q}$), 156.4 (C$\underline{O}_2$-NH), 170.1 (C_q), 170.7 (C_q), 172.2 (C_q), 172.6 (C_q).

6.7.9. General method for Pd-catalyzed allylic alkylation reaction using acetates as starting material (Method C)

Reactions were carried out under argon in a previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (0.02 eq) and biphephos (**40**) (0.02 eq) or dppf (**17**) (0.02 eq) and acetonitrile degassed prior to use. The solution containing almost negligible amounts of black solid, was stirred at 60°C for 30 min under argon. Afterwards first 1-octanethiol (**75**), degassed prior to use and secondly different degassed acetates were added with a Hamilton syringe. The solution was stirred at this temperature for 24 h. The conversion was monitored via GC-MS by taking a sample of the reaction after 24 h. After cooling to rt the solvent was removed in vacuo using a rotary evaporator and the resulting crude products were purified via flash column chromatography.

6.7.9.1. Synthesis of (*E*)-hex-2-enyl(octyl)sulfane (76) using (*E*)-hex-2-en-1-yl acetate (86) as starting material

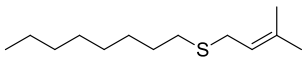


Procedure according to chapter 6.7.9.

$Pd(dba)_2$ (**X**) (7.3 mg, 12.6 µmol, 0.02 eq), dppf (**28**) (7.0 mg, 12.6 µmol, 0.02 eq), degassed (*E*)-hex-2-en-1-yl acetate (**86**) (100 µL, 0.63 mmol, 1.0 eq), degassed 1-octanethiol (**75**) (220 µL, 1.26 mmol, 2.0 eq), degassed acetonitrile (2.0 mL)

After 24 h the reaction was stopped and the conversion was measured via GC-MS. Results are shown in chapter 4.3.3.6. No isolation was attempted because conversion was incomplete and this product was already characterized in chapter 6.7.7.1.

6.7.9.2. Synthesis of (3-methylbut-2-en-1-yl)(octyl)sulfane (89) using 3-methylbut-2-en-1-yl 2,2,2-trifluoroacetate (92) as starting material

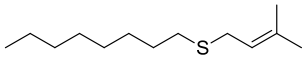


Procedure according to chapter 6.7.9.

$Pd(dba)_2$ (**X**) (7.0 mg, 12.2 µmol, 0.02 eq), dppf (**17**) (6.8 mg, 12.2 µmol, 0.02 eq), degassed 3-methylbut-2-en-1-yl 2,2,2-trifluoroacetate (**92**) (100 µL, 0.61 mmol, 1.0 eq), degassed 1-octanethiol (**75**) (117 µL, 0.67 mmol, 1.1 eq), degassed acetonitrile (2.0 mL)

After 24 h the reaction was stopped and the conversion was measured via GC-MS. Results are shown in chapter 4.3.3.4. No isolation was attempted because conversion was incomplete and this product was already characterized in chapter 6.7.7.1.

6.7.9.3. Synthesis of (3-methylbut-2-en-1-yl)(octyl)sulfane (89) using 3-methylbut-2-enylacetate (isoprenyl acetate) (138) as starting material



Procedure according to chapter 6.7.9.

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 5.74 µmol, 0.02 eq), degassed 3-methylbut-2-enylacetate (isoprenyl acetate) (**138**) (50 µL, 0.344 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (50 µL, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

After 24 h the reaction was stopped and purified via flash column chromatography: (6.0 g silica gel, 16.0 x 1.0 cm, cyclohexane 100 %, R_f = 0.46)

Yield: 30.9 mg (0.144 mmol, 50 %), yellow oil

$C_{13}H_{26}S$ [214.41 $gmol^{-1}$]

GC-MS (HS_50_S2): t_R = 6.05 min; *m/z* = 214 (63 %, MP), 144 (21 %), 101 (10 %), 68 (100 %, BP).

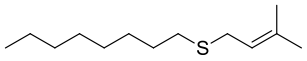
^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, 3H, ^{3}J(H,H) = 6.6 Hz, C$\underline{H}_3$-CH_2), 1.27-1.42 (m, 10H, 5 x C$\underline{H}_2$), 1.50-1.61 (m, 2H, C$\underline{H}_2$), 1.65 (s, 3H, C$\underline{H}_3$-C_q), 1.73 (s, 3H, C$\underline{H}_3$-C_q), 2.45 (t, 2H, ^{3}J(H,H) = 7.5 Hz, C$\underline{H}_2$-S), 3.12 (d, 2H, ^{3}J(H,H) = 7.8 Hz, C$\underline{H}_2$-S), 5.23 (t, 1H, ^{3}J(H,H) = 7.5 Hz, C$\underline{H}$-C_q).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.1 (C$\underline{H}_3$-CH_2), 17.7 (C$\underline{H}_3$-C_q), 22.7 (C$\underline{H}_3$-C_q), 25.7 (C-alkyl), 27.0 (C-alkyl), 29.1 (C-alkyl), 29.2 (C-alkyl), 29.5 (C-alkyl), 29.9 (C-alkyl), 31.3 (C-alkyl), 31.9 (C-alkyl), 120.9 (C$\underline{H}$-C_q), 134.81 ($\underline{C}_q$).

Characterized by Alper *et al.*[307]

6.7.10. Pd-catalyzed allylic alkylation in the presence of cosubstrates

6.7.10.1. Synthesis of (3-methylbut-2-en-1-yl)(octyl)sulfane (89) using cosubstrates



Cosubstrate: Phenol

Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 5.74 µmol, 0.02 eq), degassed methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) (50 µL, 0.344 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (50 µL, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

Phenol (26.2 mg, 0.278 mmol, 1.0 eq) was added under inert conditions as a solid directly after the addition of degassed 1-octanethiol (**75**) and before the addition of degassed methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**).

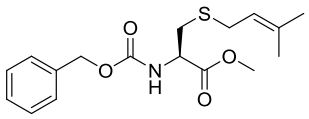
Cosubstrate: Morpholine

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 57.4 µmol, 0.02 eq), degassed methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) (42 µL, 0.287 mmol, 1.0 eq), degassed 1-octanethiol (**75**) (50 µL, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

Degassed morpholine (25 µL, 0.278 mmol, 1.0 eq) was added under inert conditions using a Hamilton syringe directly after the addition of degassed 1-octanethiol (**75**) and before the addition of methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**).

In both cases the conversion was analyzed using GC-MS after 24 h. The corresponding results are provided in chapter 4.3.5.10. No attemps were made to isolate the corresponding products, because product (3-methylbut-2-en-1-yl)(octyl)sulfane (**89**) was already characterized in chapter 6.7.8.1.

6.7.10.2. Synthesis of (*R*)-methyl 2-(((benzyloxy)carbonyl)amino)-3-((3-methylbut-2-en-1-yl)thio)propanoate (108) having cosubstrate Cbz-Tyr-OH (179) present



Procedure according to chapter 6.7.8.

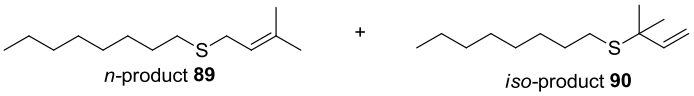
$Pd(dba)_2$ (**X**) (1.6 mg, 2.78 µmol, 0.02 eq), biphephos (**40**) (2.2 mg, 2.78 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (25 µL, 0.167 mmol, 1.2 eq), L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) (37.4 mg, 0.139 mmol, 1.0 eq), Cbz-Tyr-OH (**179**) (43.8 mg, 0.139 mmol, 1.0eq), degassed acetonitrile (0.5 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred for 30 min at 60°C in 0.5 mL of acetonitrile, degassed prior to use. L-Cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) and Cbz-Tyr-OH (**179**) were added as solids under inert conditions into the Schlenk tube containing $Pd(dba)_2$ (**X**) and biphephos (**40**) at 60°C. Afterwards methyl (3-methylbut-2-en-1-yl) carbonate (**88**) was added as described in chapter 6.7.8.

The conversion was analyzed using HPLC-MS at 210 nm after 24 h. The corresponding results are provided in chapter 4.3.6.3. No attempts were made to isolate the corresponding product, because (*R*)-methyl 2-(((benzyloxy)carbonyl)amino)-3-((3-methylbut-2-en-1-yl)thio)propanoate (**108**) was already characterized in chapter 6.7.8.16.

6.7.11. Pd-catalyzed allylic alkylation using TEG-biphephos 191 as ligand

6.7.11.1. Synthesis of (3-methylbut-2-en-1-yl)(octyl)sulfane (89) using TEG-biphephos 191 as ligand



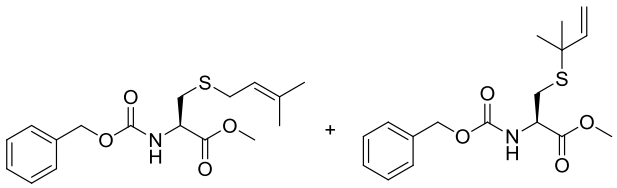
n-product **89** *iso*-product **90**

Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.2 mg, 5.6 µmol, 0.02 eq), TEG-biphephos **191** (5.9 mg, 5.6 µmol, 0.02 eq), degassed methyl-3-methylbut-2-enyl carbonate (isoprenyl carbonate) (**88**) (50 µL, 0.344 mmol, 1.2 eq), degassed 1-octanethiol (**75**) (50 µL, 0.287 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

Conversion was analyzed using GC-MS after 24 h. The corresponding result is provided in chapter 4.3.7.3. No attemps were made to isolate the corresponding products, because (3-methylbut-2-en-1-yl)(octyl)sulfane (**89**) was already characterized in chapter 6.7.8.1 and *iso*-product (2-methylbut-3-en-2-yl)(octyl)sulfane (**90**) is characterized in chapter 6.7.12.

6.7.11.2. Synthesis of (*R*)-methyl 2-(((benzyloxy)carbonyl)amino)-3-((3-methylbut-2-en-1-yl)thio)propanoate (108) using TEG-biphephos 191 as ligand and TEG-isoprenyl carbonate 194



n-product **108** *iso*-product **109**

Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.2 mg, 5.6 µmol, 0.02 eq), TEG-biphephos **191** (5.8 mg, 5.6 µmol, 0.02 eq) (synthesized by Nicole Mayer M.Sc.), degassed TEG-isoprenyl carbonate **194** (88 µL, 0.344 mmol, 1.2 eq) (synthesized by Nicole Mayer M.Sc.), L-cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) (74.9 mg, 0.278 mmol, 1.0 eq), degassed *tert*-butanol/H_2O 3:1 (1 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and TEG-biphephos **191** were stirred for 30 min at 60°C in 1 mL *tert*-butanol/H_2O 3:1, degassed separately prior to use and combined afterwards. L-Cysteine *N*-[(phenylmethoxy)carbonyl] methyl ester (**102**) was added as solid under inert conditions into the Schlenk tube containing $Pd(dba)_2$ (**X**) and TEG-biphephos **191** at 60°C. Afterwards degassed TEG-isoprenyl carbonate **194** was added as described in chapter 6.7.8.

The conversion was analyzed using HPLC-MS at 210 nm after 24 h. The corresponding results are provided in chapter 4.3.7.3.

After 24 h the reaction was stopped and purified via flash column chromatography: (14.0 g silica gel, 20.0 x 1.5 cm, cyclohexane/ethyl acetate = 6:1 (v/v), R_f = 0.30)

Yield: 83.3 mg (0.247 mmol, 89 %), pale yellow oil

$C_{17}H_{23}NO_4S$ [337.43 $gmol^{-1}$]

t_R = 4.29 min (Nucleosil 120-5-C4)

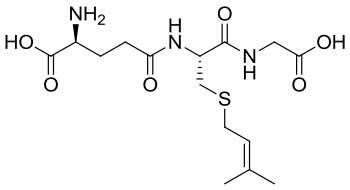
NMR of *n*-product **108** together with the minor *iso*-product **109**:

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.64 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 1.72 (s, 3H, $(C\underline{H}_3)_2$-C_q=CH), 2.91 (t, 2H, ^3J(H,H) = 5.4 Hz, $C\underline{H}_2$-S), 3.14 (t, 2H, ^3J(H,H) = 7.5 Hz, $C\underline{H}_2$-S), 3.76 (s, 3H, $C\underline{H}_3$-O), 4.59 (dt, 1H, ^3J(H,H) = 7.8 Hz, 5.3 Hz, CO-NH-C<u>H</u>), 4.91-5.01 (m, 2H, -S-$C(CH_3)_2$-CH=$C\underline{H}_2$ (**109**)), 5.12 (s, 2H, -NH-CO_2-$C\underline{H}_2$-C_6H_5), 5.18 (t, 1H, ^3J(H,H) = 7.8 Hz, $(CH_3)_2$-C_q=C<u>H</u>), 5.58 (d, 1H, ^3J(H,H) = 7.8 Hz, N<u>H</u>), 5.77 (dd, 1H, ^3J(H,H) = 17.3 Hz, 10.5 Hz, -S-$C(CH_3)_2$-C<u>H</u>=CH_2 (**109**)), 7.33-7.35 (m, 5H, <u>H</u>-Ar).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 18.0 ($(\underline{C}H_3)_2$-C_q=CH), 25.9 ($\underline{C}H_3)_2$-C_q=CH), 27.4 ($\underline{C}H_3$, (**109**)), 27.5 ($\underline{C}H_3$, (**109**)), 30.4 ($\underline{C}H_2$-S), 33.9 ($\underline{C}H_2$-S), 52.8 ($\underline{C}H_3$-O or CO-NH-<u>C</u>H), 53.9 ($\underline{C}H_3$-O or CO-NH-<u>C</u>H), 67.3 (-NH-CO_2-$\underline{C}H_2$-C_6H_5), 112.3 (-S-$C(CH_3)_2$-CH=$\underline{C}H_2$, (**109**)), 120.0 ($(CH_3)_2$-C_q=<u>C</u>H), 128.3 (2 × <u>C</u>-Ar), 128.4 (<u>C</u>-Ar), 128.8 (2 × <u>C</u>-Ar), 136.4 ($(CH_3)_2$-$\underline{C}_q$=CH or $C_{q,Ar}$), 136.6 ($(CH_3)_2$-$\underline{C}_q$=CH or $C_{q,Ar}$), 144.2 (-S-$C(CH_3)_2$-<u>C</u>H=CH_2, (**109**)), 156.0 ($\underline{C}O_2$-NH), 171.6 ($\underline{C}O_2$-CH_3).

n/iso-ratio (93:7) assigned by ^{1}H-NMR

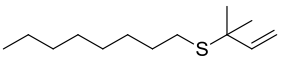
6.7.11.3. Synthesis of putative product 195 using TEG-biphephos 191 as ligand and TEG-isoprenyl carbonate 194



Due to the very poor solubility of TEG-biphephos **191** in water the decision was made to first form in situ the catalyst-ligand system in acetonitrile, degassed prior to use. Therefore $Pd(dba)_2$ (**X**) (3.2 mg, 5.6 µmol, 0.02 eq) and TEG-biphephos **191** (5.8 mg, 5.6 µmol, 0.02 eq) (synthesized by Nicole Mayer M.Sc.) were dissolved in acetonitrile (1.0 mL) at 60°C and stirred for 30 min. Afterwards acetonitrile was evaporated under inert conditions using an oil-pump (0.02 mbar). After dryness was reached distilled H_2O (1 mL), degassed prior to use, was added and heated to 60 °C. The catalyst-ligand system was mainly not dissolved even when using an ultra-sonic bath for 1 min to assist solubilization of the catalyst-ligand system. A light green-brownish color was observed in H_2O. Glutathione (**169**) (85.4 mg, 0.287 mmol, 1.0 eq) was added under inert conditions as a solid and dissolved quickly at 60°C. Degassed TEG-isoprenyl carbonate **194** (88 µL, 0.344 mmol, 1.2 eq) (synthesized by Nicole Mayer M.Sc.) was added via a Hamilton syringe which was also dissolved at 60°C. Still the catalyst-ligand system was mainly not dissolved. This mixture was stirred at 60°C for 18 h. The conversion was monitored via GC-MS.

The corresponding result is provided in chapter 4.3.7.3. No attemps were made to isolate the corresponding product.

6.7.12. Synthesis of (2-methylbut-3-en-2-yl)(octyl)sulfane (90)



The reaction was carried out under argon in a previously oven-dried Schlenk vessel containing a magnetic stirring bar. Under a flow of argon the vessel was charged with bis(dibenzylideneacetone)palladium(0) ($Pd(dba)_2$) (**X**) (6.6 mg, 11.5 µmol, 0.02 eq) and Mandyphos ligand (**114**) (9.7 mg, 11.5 µmol, 0.02 eq) and acetonitrile (2.0 mL) degassed prior to use. The orange solution containing almost negligible amounts of black solid, was stirred at 60°C for 30 min under argon. Afterwards first 1-octanethiol (**75**) (100 µL, 0.574 mmol, 1.0 eq), degassed prior to use and secondly degassed methyl-3-methylbut-2-enyl

carbonate (isoprenyl carbonate) (**88**) (100 µL, 0.688 mmol, 1.2 eq) were added with a Hamilton syringe. The solution was stirred at this temperature for 24 h. The conversion was monitored via GC-MS. After cooling to room temperature the solvent was removed in vacuo using a rotary evaporator and the resulting crude product were purified via flash column chromatography. (13.0 g SiO_2, 15.0 × 2.0, Cyclohexan 100 %, R_f = 0.38)

Yield: 61.1 mg *iso*-product **90** (299 µmol, 52 %), yellow oil

(46.3 mg mixture of *n*-product **89** and *iso*-product **90** (216 µmol, 38 %), yellow oil)

$C_{13}H_{26}S_2$ [214.41 g.mol^{-1}]

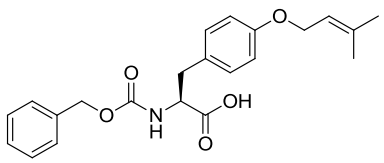
HRMS (TOF MSEI+):	calculated:	214.1755
	found:	214.1765

GC-MS (HS_50_S2): t_R = 5.58 min; *m/z* = 214 (7 %, MP), 145 (7 %), 101 (4 %), 69 (100 %, BP).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.87 (t, 3H, ^3J(H,H) = 6.5 Hz, C<u>H</u>$_3$-CH_2-CH_2-), 1.25-1.35 (m, 16H, 2 x CH_3, 5 x CH_2), 1.52 (p, 2H, ^3J(H,H) = 7.3 Hz, -CH_2-C<u>H</u>$_2$-CH_2-S-), 2.35 (t, 2H, ^3J(H,H) = 7.4 Hz, -CH_2-C<u>H</u>$_2$-S-), 4.89-5.00 (m, 2H, C_q-CH=C<u>H</u>$_2$), 5.84 (dd, 1H, ^3J(H,H) = 17.1 Hz, 10.5 Hz, C_q-C<u>H</u>=CH_2).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.2 (<u>C</u>H$_3$-CH_2-CH_2-), 22.8 (CH_2), 27.6 (2 × (<u>C</u>H$_3$)$_2$-C_q), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.8 (CH_2), 32.0 (CH_2), 46.3 ((CH$_3$)$_2$-<u>C</u>$_q$), 111.1 (C_q-CH=<u>C</u>H$_2$), 145.1 (C_q-<u>C</u>H=CH_2).

6.7.13. Synthesis of product 180



Procedure according to chapter 6.7.8.

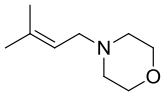
$Pd(dba)_2$ (**X**) (3.2 mg, 5.6 µmol, 0.02 eq), biphephos (**40**) (4.4 mg, 5.6 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (50 µL, 0.334 mmol, 1.2 eq), Cbz-Tyr-OH (**179**) (87.7, 0.278 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

In a dry Schlenk tube $Pd(dba)_2$ (**X**) and biphephos (**40**) were stirred for 30 min at 60°C in 1.0 mL of acetonitrile, degassed prior to use. After 30 min Cbz-Tyr-OH (**179**) is added as a solid under inert conditions to the solution of $Pd(dba)_2$ (**X**) and biphephos (**40**) at 60°C. Afterwards methyl (3-methylbut-2-en-1-yl) carbonate (**88**) was added as described in chapter 6.7.8.

After 24 h the reaction was stopped and analyzed via HPLC-MS (210 nm). The corresponding result is provided in chapter 4.3.6.3. No attemps were made to isolate the corresponding product.

t_R = 4.008 min (Nucleosil 120-5-C4)

6.7.14. Synthesis of 4-(3-methylbut-2-en-1-yl)morpholine (162)



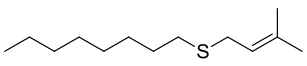
Procedure according to chapter 6.7.8.

$Pd(dba)_2$ (**X**) (3.3 mg, 5.74 µmol, 0.02 eq), biphephos (**40**) (4.5 mg, 5.74 µmol, 0.02 eq), degassed methyl (3-methylbut-2-en-1-yl) carbonate (**88**) (50 µL, 0.334 mmol, 1.2 eq), morpholine (**161**) (25 µL, 0.278 mmol, 1.0 eq), degassed acetonitrile (1.0 mL)

After 24 h the reaction was stopped and analyzed via GC-MS (210 nm). The corresponding result is provided in chapter 4.3.5.10. No attemps were made to isolate the corresponding product.

GC-MS (HS_50_S2): t_R = 4.51 min; *m/z* = 155 (20 %, MP), 140 (39 %), 100 (20 %), 87 (100 %, BP), 69 (77 %).

6.7.15. Synthesis of (3-methylbut-2-en-1-yl)(octyl)sulfane (89) using isoprenyl bromide (160) (classical S_N2 reaction)



A Schlenk tube with a magnetic stirring bar was dried under vacuum with a heat gun, and flushed with argon. The Schlenk tube was charged with dry DMF (1 mL), dry acetonitrile (1

mL), DIPEA (**164**) (180 µL, 1.03 mmol, 1.5 eq) and 1-bromo-3-methylbut-2-ene (isoprenyl bromide) (**160**) (0.32 mL, 2.74 mmol, 4.0 eq) and cooled to 0°C in an ice-bath. 1-Octanethiol (**75**) (119 µL, 0.684 mmol, 1.0 eq) was added dropwise to the heavily stirred and ice cold solution. After all 1-octanethiol (**75**) was added the reaction mixture was stirred for 3 h. Afterwards the reaction mixture was diluted with DCM (5 mL), washed with distilled H_2O (10 mL) and brine (10 mL). The combined organic layers were dried over Na_2SO_4. After filtration the volatiles were removed by evaporation (15 mbar) using a rotary evaporator and the resulting crude product was purified via flash column chromatography (20.0 g SiO_2, 15.0 × 2.0, cyclohexane 100 %, R_f = 0.31).

Yield: 123 mg (0.574 mmol, 85 %), yellow oil

For further characterization see chapter 6.7.8.1.

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Abbreviations

general

Å	Ångström
$[\alpha]_D^{20}$	angle of optical rotation
APT	attached proton test
bp	boiling point
BP	basis peak
bs	broad singlet
°C	grad celsius
C_{Ar}	aromatic carbon atoms
cm	centimeter
^{13}C-NMR	nuclear magnetic resonance spectroscopy for carbon
chrom.	chromatography
conc.	concentration
COSY	Correlation Spectroscopy
COSY-NMR	Correlation Spectroscopy - nuclear magnetic resonance
conv.	conversion
C_q	quarternary carbon atom
d	doublet
d	day
DAD	diode array detector
dd	doublet of doublet
DI	direct insertion
DNA	deoxyribonucleic acid
dt	dublet of triplet
EI	Elektron ionisation
eq	equivalents
equiv.	equivalents
ESI	Elektro spray ionisation
ESI-MS	Elektro spray ionisation – mass spectrometry
eV	elektron volt
g	gramm
GC	Gaschromatography
GC-MS	Gaschromatography-Mass spektrometry
h	hour

H_{Ar}	aromatic hydrogen atoms
^{1}H-NMR	proton nuclear magnetic resonance
HPLC	High Performance Liquid Chromatography
HRMS	high-resolution mass spectrometry
HS	Hilmar Schröder
Hz	Hertz
kDa	kilo dalton
J	coupling constant
L	liter
M	molar
m	multiplet
MALDI	matrix-assisted laser desorption/ionization
MHz	megahertz
µL	mikroliter
µm	mikrometer
µmol	mikromol
min	minute
mbar	millibar
mg	milligramm
mL	milliliter
mmol	millimol
mol%	mol percent
mol/L	mol per liter
mp	melting point
MP	molecule peak
mRNA	messenger RNA
MS	mass spectrometry
MSD	mass selective detector
MWD	multi wavelength detector
m/z	mass-load-relation
nm	nano meter
NMR	nuclear magnetic resonance
NOESY - NMR	Nuclear Overhauser Effect Spektroskopy
NOESY-1D	Nuclear Overhauser Effect Spektroskopy
o	ortho
p	pentet

p	para
%	percent
PG	protecting group
pKa	logarithmic acid dissociation constant
ppm	parts per million
PTM	post-translational modifications
q	quartet
quant.	quantitative
R_f	retention factor (retardation factor or ratio of fronts)
RNA	ribonucleic acid
rpm	rotations per minute
rt	room temperature
s	singlet
SPPS	solid-phase peptide synthesis
t	triplet
Temp.	temperature
tert	tertiary
td	triplet of doublets
TLC	Thin layer chromatography
TOCSY	Total Correlation Spectrosopy
TOCSY-1D	Total Correlation Spectrosopy
TOF	Time of Flight
t_R	retention time
UV	ultra violett
Vol.	volume
vol%	volume percent
v/v	relation of volume to volume
δ	chemical shift in ppm (parts per million)
λ	wave length
π	pi

Solvents and reagents

ACN	acetonitrile
Ala	alanine
9-BBN	9-borabicyclo(3.3.1)nonane

BHT	*tert.*-butylhydroxytoluene
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
BINOL	1,1′-bi-2-naphthol
Bn	benzyl
BiPhePhos	6,6'[(3,3'-di-tert-butyl-5,5'-dimethoxy-1,1'-biphenyl-2,2'-diyl)bis(oxy)]bis (dibenzo[d,f][1,3,2]dioxaphosphepin)
Boc	*tert*-butyloxycarbonyl-
BOP	Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
t-BuOH	*tert*-butanol
Cam	carboxamindomethyl ester
Cal-B	*Candida Antarctica Lipase B*
Cbz	benzyloxycarbonyl
CDI	*N,N´*-carbonyldiimidazole
CLEA OM	cross-linked enzyme aggregate organic media
Cys	cysteine
dba	dibenzylideneacetone
DCC	*N,N*'-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DCM	methylene chloride
DIBAL	diisobutyl aluminium hydride
DIOP	*O*-isopropyliden-2,3-dihydroxy-1,4-bis(diphenylphosphino)butan
DIPEA	*N,N*-ethyldiisopropylamine
diprpf	1,1'-bis(diisopropylphosphino)ferrocene
diprpx	1,1'-bis(di-tert-butylphosphino)-o-xylene
DMAP	4-dimethylamino pyridine
DME	1,2-dimethoxyethane
DMF	*N,N'*-dimethylformamide
DMSO	dimethyl sulfoxide
D-Phe-OH	D-phenylalanine
dppb	1,4-bis(diphenylphosphino)butane
dppbz	1,2-bis(diphenylphosphino)benzene
dppe	1,2-bis(diphenylphosphino)ethane
dppf	1,1'-bis(diphenylphosphino)ferrocene
dpppe	1,5-bis(diphenylphosphino)pentane
dppm	bis(diphenylphosphino)methane

dppp	1,3-bis(diphenylphosphino)propane
ee	enantiomeric excess
Et	ethyl
Et_2O	diethylether
EtOAc	ethyl acetate
EtOH	ethanol
Fmoc	Fluorenylmethyloxycarbonyl
Glu	glutamic acid
Gly	glycine
GDP	guanosine diphosphate
Gp	guanidinophenyl-ester
GSH	glutathione
GTP	guanosine triphosphate
HMPA	Hexamethylphosphoramide
H_2O	water
HOAc	acetic acid
Ile	isoleucine
Leu	leucine
Me	methyl
MeCN	acetonitrile
MeOH	methanol
Met	methionine
MS	molecular sieves
MSH	O-mesitylenesulfonylhydroxylamine
MTBE	methyl-*tert* -butylether
NEt_3	triethylamine
NHC	*N*-heterocyclic carbene
NMP	1-*N*-methyl-2-pyrrolidone
PCy_3	tricylclohexylphosphine
$Pd(acac)_2$	palladium(II) acetylacetonate
$Pd(dba)_2$	bis(dibenzylidenaceton)palladium(0)
$Pd_2(dba)_3$	tris(dibenzylideneacetone)dipalladium(0)
$Pd(OAc)_2$	palladium(II) acetate
PEG	polyethylene glycol
PFT	protein farnesyltransferase
$P(Fur)_3$	trifurfurylphosphine

Phe	phenylalanine
PPh_3	triphenylphosphine
Pro	proline
P*t*Bu_3	tri-*tert*-butylphosphine
P(*o*-Tol)$_3$	tri(ortho-tolyl)phosphine
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
SAM	S-adenosylmethionine
SBL	subtilisin *Bacillus lentus*
Ser	serine
SPHOS	dicyclohexyl-(2‘,6‘-dimethoxybiphenyl-2-yl)phosphine
*t*Bu	*tert*-butyl-
TFA	2,2,2-trifluoroacetic acid
TFE	2,2,2-trifluoroethylester
THF	tetrahydrofuran
TMS	trimethylsilyl-
TMS-CHN_2	trimethylsilyldiazomethane
TPPTS	3,3′,3″-phosphinidynetris(benzenesulfonic acid) trisodium salt
Trp	tryptophan
Tyr	tyrosine
Val	valine
Xantphos	4,5-bis(diphenylphosphano)-9,9-dimethylxanthene
Z	benzyloxycarbonyl

Others

e.g.	for example
et al.	et al.
etc.	et cetera
Inc.	incorporation
n.d.	not determined
no.	number
resp.	respective
Tab.	table

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