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Peptide-Catalyzed Stereoselective 1,4-Addition Reactions between Aldehydes and Nitroolefins Generating Quaternary Stereogenic Centers

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Abstract

This thesis describes the development and application of peptidic catalysts for 1,4-addition reactions between aldehydes and nitroolefins that provide synthetically valuable γ-nitroaldehydes bearing adjacent quaternary and tertiary stereocenters. Mechanistic investigations of these reactions utilizing β,β-disubstituted nitroolefins are also presented.

In the first part, peptides of the general type Pro-Pro-Xaa (Xaa = acidic amino acid) were evaluated as catalysts for 1,4-addition reactions between aldehydes and β,β-disubstituted nitroolefins, a much more challenging substrate class compared to their β-mono-substituted counterparts and to date not examined in such reactions. Modification of the peptide structures led to the development of H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4, a powerful catalyst allowing for reactions between different combinations of aldehydes and β,β-disubstituted nitroolefins under mild organocatalytic conditions. The desired γ-nitroaldehydes bearing a quaternary stereogenic center adjacent to a tertiary stereocenter were obtained in good yields and stereoselectivities as well as with high chemoselectivity. Chiral γ-butyrolactones and pyrrolidines as well as γ-amino acids with a quaternary stereogenic center that have so far not been prepared, were readily accessible from the γ-nitroaldehydes.

In the second part, mechanistic studies into the peptide-catalyzed reaction between aldehydes and β,β-disubstituted nitroolefins were performed in order to gain insight into the reaction pathway and to determine the rate-limiting step of the reaction. The investigations revealed a significant rate acceleration in the presence of weakly acidic co-catalysts and considerably slower reactions in the presence of strongly acidic co-catalysts. Furthermore, it was found that the rate-determining step of the reaction depends on the acidity of the co-catalyst. Initial mechanistic investigations using ESI-MS back-reaction screening with mass-labeled quasi-enantiomeric substrates suggested that the enantiomeric excess of the reaction product might be determined by the relative reactivity and stability of downstream intermediates in the catalytic cycle.

In the third part, short-chain peptides were examined as potential catalysts for the 1,4-addition reaction between α,α-disubstituted aldehydes and β-substituted nitroolefins. The use of aldehyde functionalized peptide libraries in a combinatorial screening led to

the identification of several peptides which, however, proved not to be particularly active catalysts when used in solution-phase conjugate addition reactions. More promising results were obtained with the previously developed tripeptide H-D-Pro-Pro-Glu-NH_2 and its thiourea-functionalized derivatives.

The results presented in this thesis not only show that peptides with the Pro-Pro motif offer the possibility to adapt to the structural requirements of challenging substrate combinations in 1,4-addition reactions but also are a good basis for future research to develop catalysts for other types of transformations.

Zusammenfassung

Die vorliegende Arbeit beschreibt die Entwicklung und Anwendung von peptidischen Katalysatoren für 1,4-Additionsreaktionen zwischen Aldehyden und Nitroolefinen welche synthetisch nützliche γ-Nitroaldehyde mit benachbarten quartären und tertiären Stereozentren liefern. Zusätzlich werden mechanistische Untersuchungen dieser Reaktionen beschrieben in welchen β,β-disubstituierte Nitroolefine verwendet werden.

Im ersten Teil dieser Arbeit wurden Peptide des Typs Pro-Pro-Xaa (Xaa = variable säurehaltige Aminosäure) als Katalysatoren untersucht für 1,4-Additionsreaktionen zwischen Aldehyden und β,β-disubstituierten Nitroolefinen, eine bedeutend anspruchsvollere Substratklasse als β-monosubstituierte Analoga und bisher nicht untersucht in diesen Reaktionen. Die Modifizierung der Peptidstrukturen führte zur Entwicklung des Peptids H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4, das einen effektiven Katalysator für Additionsreaktionen verschiedener Kombinationen von Aldehyden und β,β-disubstituierten Nitroolefinen unter milden Bedingungen darstellt. Die gewünschten γ-Nitroaldehyde mit einem quartären Stereozentrum benachbart zu einem tertiären Stereozentrum wurden in guten Ausbeuten und Stereoselektivitäten sowie mit hoher Chemoselektivität erhalten. Chirale γ-Butyrolactone und Pyrrolidine wie auch γ-Aminosäuren mit einem quartären Stereozentrum, welche bisher nicht hergestellt wurden, sind leicht zugänglich ausgehend von den γ-Nitroaldehyden.

Im zweiten Teil wurden mechanistische Untersuchungen zur peptidkatalysierten Reaktion zwischen Aldehyden und β,β-disubstituierten Nitroolefinen durchgeführt, um einen Einblick in den Reaktionsweg zu erhalten sowie zur Bestimmung des geschwindigkeitsbestimmenden Schrittes der Reaktion. Die Untersuchungen zeigten eine erhebliche Reaktionsbeschleunigung in Gegenwart von schwach sauren Cokatalysatoren und deutlich langsamere Reaktionen in Gegenwart von stark sauren Cokatalysatoren. Zusätzlich stellte sich heraus dass der geschwindigkeitsbestimmende Schritt der Reaktion von der Acidität des Cokatalysators abhängt. Erste ESI-MS Studien der Rückreaktion mittels quasi-enantiomerer Substrate deuteten darauf hin dass der Enantiomerenüberschuss des Reaktionsproduktes durch die relative Reaktivität und Stabilität von Intermediaten im Katalysezyklus bestimmt wird.

Im dritten Teil dieser Arbeit wurden kurzkettige Peptide als potentielle Katalysatoren für 1,4-Additionsreaktionen zwischen α,α-disubstituierten Aldehyden und β-substituierten Nitroolefinen untersucht. In einem kombinatorischen Screening mit aldehydfunktionalisierten Peptidbibliotheken wurden mehrere Peptide identifiziert, jedoch stellte sich heraus dass diese nicht besonders aktive Katalysatoren sind für 1,4-Additionsreaktionen in Lösung. Bessere Resultate wurden mit dem vorgängig entwickelten Tripeptid H-D-Pro-Pro-Glu-NH_2 sowie Thioharnstoffderivaten davon erhalten.

Die vorliegenden Resultate dieser Arbeit demonstrieren nicht nur dass Peptide mit dem Pro-Pro Motiv die Möglichkeit zur Anpassung an die strukturellen Erfordernisse von anspruchsvollen Substratkombinationen in 1,4-Additionsreaktionen bieten, sondern sind ebenfalls eine solide Basis für die zukünftige Entwicklung von peptidischen Katalysatoren für weitere Reaktionen.

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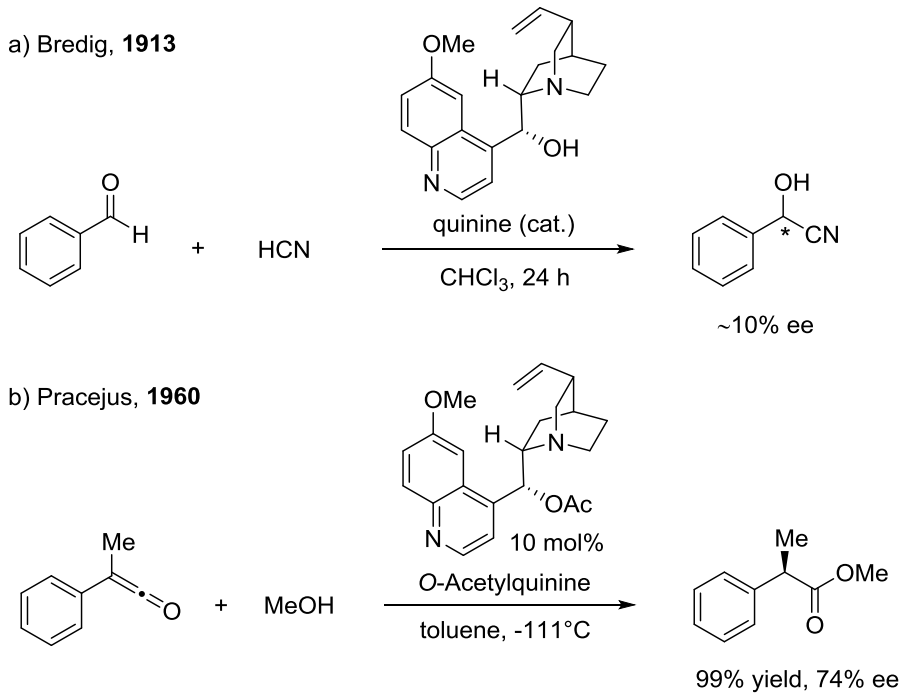
Introduction

1.1 Organocatalysis

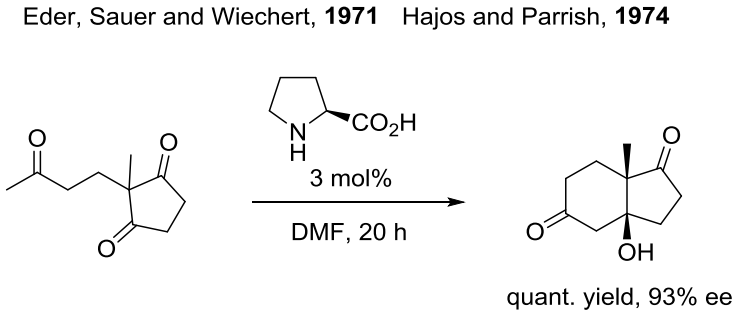
Organocatalysis describes chemical reactions that are accelerated by metal-free small organic molecules.[1] In the last decade, the stereoselective synthesis of chiral compounds catalyzed by such purely organic molecules has become a highly active field of research.[1-7] Many research groups have developed organocatalytic procedures for reactions that were typically carried out using traditional transition metal catalysis or enzymatic methods. Consequently, organocatalysis is nowadays, next to transition metal catalysis and biocatalysis, an established methodology in asymmetric synthesis. Compared to metal-catalyzed and biocatalyzed reactions, organocatalytic transformations offer certain advantages including the fact that organocatalysts are usually stable in air and water, often have low molecular weights, are generally non-toxic, and can either be readily prepared or are often commercially available.

While the interest in organocatalysis has greatly increased only since the beginning of the 21st century,[1] the origins of small organic molecules used as catalysts go back to the 19th century. Justus von Liebig's synthesis of oxamide from dicyan and water in the presence of acetaldehyde is nowadays considered as the first example of an organocatalytic reaction.[8] The first asymmetric organocatalytic transformations were reported by the German chemist Georg Bredig about 50 years later, in the early 20th century.[9-10] Bredig found that in the presence of natural alkaloids such as nicotine or quinine the decarboxylation of camphorcarboxylic acid[9] or the addition of hydrogen cyanide to benzaldehyde[10] proceeded in a stereoselective manner (Scheme 1-1, a). Despite the low enantiomeric excess observed in the products, these studies have inspired others and led to the development of further organocatalyzed asymmetric reactions, achieving higher stereoselectivities. For example, in 1960 Pracejus reported the addition of methanol to methyl phenyl ketene using a quinine derivative as catalyst, proceeding with good levels of enantioselectivity (Scheme 1-1, b).[11] One of the most famous examples in the history of organocatalysis was reported independently by two research groups in the early 1970s: the intramolecular aldol reaction catalyzed by L-proline, known as the



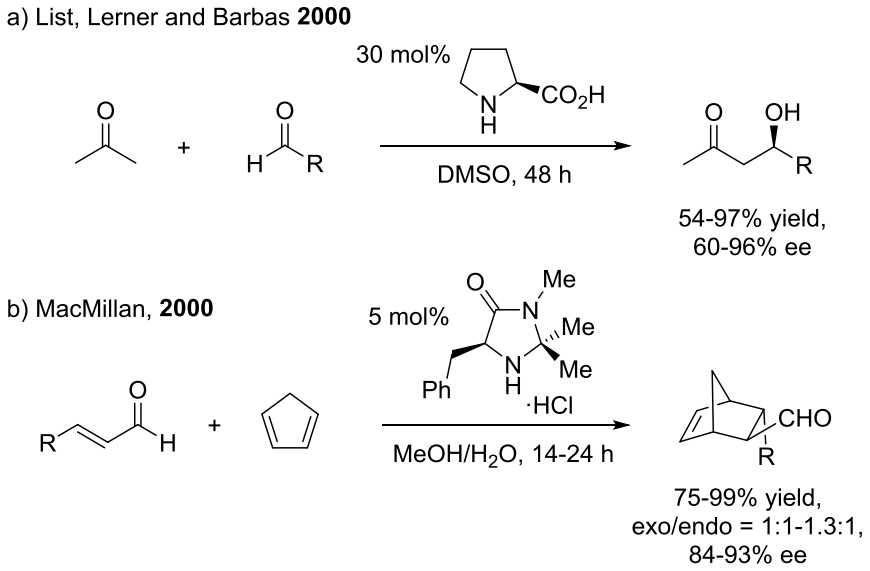
Scheme 1-1 Examples of the first asymmetric organocatalytic reactions: a) Hydrocyanation of benzaldehyde.[10] b) Conjugate addition of methanol to methyl phenyl ketene.[11]

Hajos-Parrish-Eder-Sauer-Wiechert reaction (Scheme 1-2).[12-13] In the following years, further organocatalyzed transformations were developed and although it was known by then that small organic molecules are able to catalyze different types of reactions, it was not until the beginning of the 21st century that the interest in organocatalysis started to grow rapidly.



Scheme 1-2 A milestone in the history of organocatalysis: the Hajos-Parrish-Eder-Sauer-Wiechert reaction.[12-13]

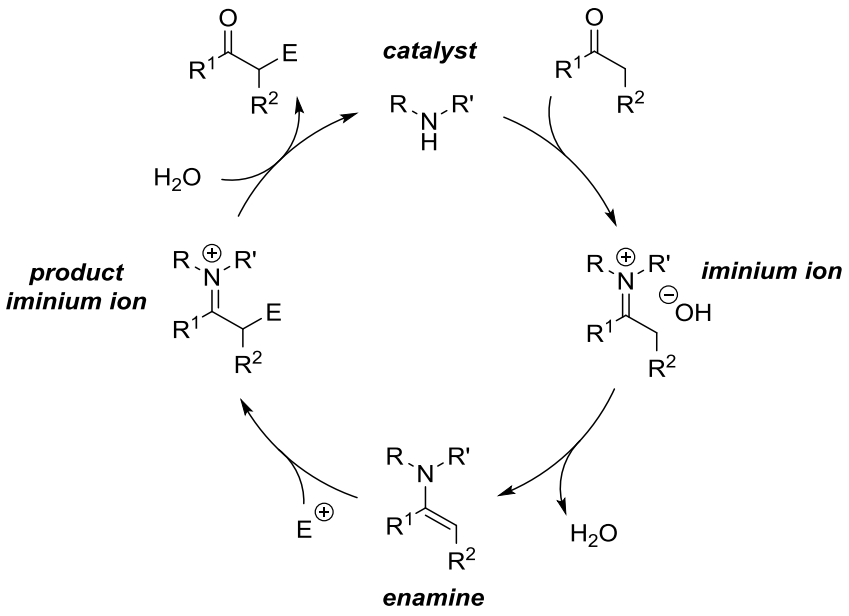
The publication of two articles in 2000 - one from List, Lerner and Barbas[14] on L-proline as catalyst for intermolecular aldol reactions and the other from MacMillan[15] on asymmetric Diels-Alder reactions catalyzed by a chiral imidazolidinone - marks the rebirth of organocatalysis as an active field of research (Scheme 1-3). Since then, a great number of organocatalyzed reactions have been reported using different activation concepts that provide the desired products in high yields and stereoselectivities.



Scheme 1-3 The two reports that initiated the launch of organocatalysis: a) L-Proline catalyzed intermolecular aldol reaction[14], and b) Imidazolidinone catalyzed Diels-Alder reaction.[15]

Based on a mechanistic classification, organocatalysts are typically divided into two groups according to the interactions between the catalyst and the substrate.[16] The first group, "non-covalent organocatalysis", describes the activation of the substrate by the catalyst *via* non-covalent interactions such as ionic interactions or hydrogen bonding. Examples include cinchona alkaloids, (thio)ureas or phosphoric acid catalysts. In the second group, "covalent organocatalysis", the activation of the substrate is achieved by formation of a covalent bond with the catalyst. *N*-heterocylic carbenes and chiral amine catalysts operate through this mode. Alternatively, organocatalysis can be divided into four areas according to the acid/base reactivity of the organocatalysts: Lewis base, Lewis acid, Brønsted base and Brønsted acid catalysis.[3] Many of the organocatalysts, however, function through both covalent and non-covalent activation modes and/or have a dual

acid/base character and can therefore be classified as multifunctional catalysts.[17] An important concept within covalent organocatalysis using amines as catalysts is the activation of carbonyl compounds *via* an enamine intermediate. It is also the basis of the catalytic reactions described in Chapters 3 – 5 in this thesis. In enamine activation, the catalyst (a primary or a secondary amine) reacts with the substrate (a ketone or an aldehyde) which results in the inital formation of an iminium ion. This species has an increased α-C-H acidity compared to the original carbonyl compound due to the LUMO lowering effect. As a result, α-deprotonation becomes more facile and leads to the formation of an enamine with a higher HOMO energy. This nucleophilic enamine intermediate allows for subsequent reaction with an electrophile to afford, after hydrolysis, α-functionalized carbonyl compounds and the regenerated amine catalyst that can participate in a subsequent catalytic cycle (Scheme 1-4).[2,18]



Scheme 1-4 General concept of enamine catalysis.[2,18]

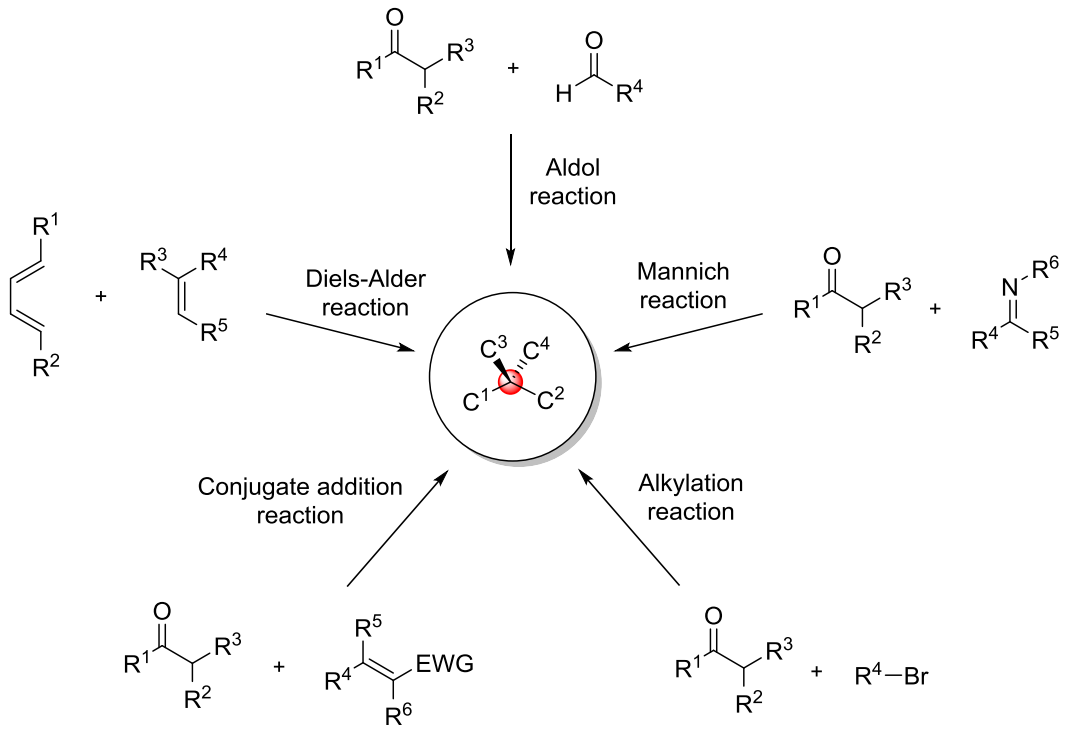
The synthetic utility of enamines as nucleophiles in stoichiometric reactions for α-functionalizations of carbonyl compounds was already reported by Stork and co-workers back in the 1950s.[19-20] The first catalytic application of enamines was demonstrated 20 years later in the Hajos-Parrish-Eder-Sauer-Wiechert reaction (Scheme 1-2).[12-13] Since the publication of the proline-catalyzed asymmetric aldol reaction by List, Lerner

and Barbas[14] at the beginning of this century, asymmetric enamine catalysis has become an efficient strategy for the stereoselective synthesis of α-functionalized carbonyl compounds. Many aminocatalysts have been developed and essentially all types of aldehydes and ketones have been shown to react with a broad range of electrophiles in various types of catalytic transformations including aldol, Mannich and Michael reactions as well as α-heteroatom functionalizations of carbonyl compounds.[18]

1.2 Organocatalyzed Formation of Quaternary Stereogenic Centers

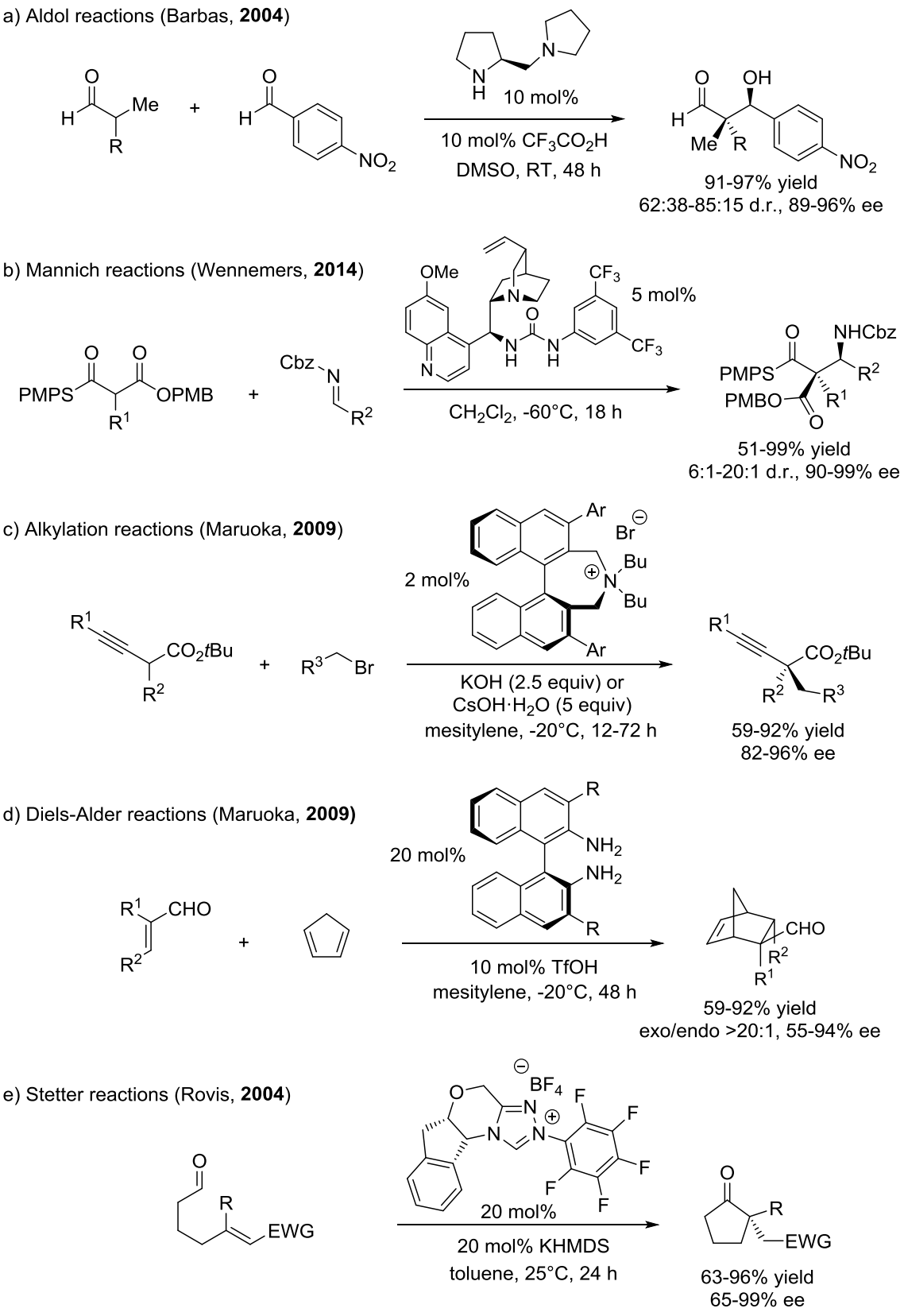
Carbon atoms connected to four different carbon substituents are found in many organic molecules, including natural products and biologically active compounds.[21] It should be mentioned that such quaternary stereogenic centers are commonly described as "*all-carbon* quaternary stereocenters" to stress that the central carbon atom is bonded to four distinct carbon substituents. However, this term is, in principle, redundant since "quaternary" in the context of carbon atoms/centers already stands for tetra-carbon-substituted. In this thesis, we therefore use the term quaternary stereogenic center for a carbon atom bearing four different carbon substituents.

The enantioselective formation of quaternary stereogenic centers is a demanding reaction due to steric repulsion between the carbon substituents and, particularly in a catalytic manner, represents a great challenge in organic synthesis.[22-30] The impressive progress in the field of asymmetric organocatalysis in recent years led to the development of new and sophisticated concepts for stereoselective C-C bond forming reactions.[1-7] These methodologies have also been applied in the formation of quaternary stereogenic centers and several reports on catalytic C-C bond forming reactions that afford compounds with one or more quaternary stereocenters under mild organocatalytic conditions have been reported.[24-25,27-29] Among these, the generation of quaternary stereocenters is particularly challenging in *acyclic* compounds due to their number of degrees of freedom.[27] Examples include aldol[31-34] and Mannich reactions,[35-40] α-alkylation of carbonyl compounds,[41-50] Diels-Alder reactions,[51-53] conjugate addition reactions[54-97] and rearrangement reactions[98] (Scheme 1-5). Selected examples of these reaction types providing the desired products in high yields and stereoselectivities are presented in



Scheme 1-5 Formation of quaternary stereogenic centers *via* different reaction types.

Scheme 1-6. For example, Barbas reported direct intermolecular aldol reactions using a chiral diamine catalyst in combination with trifluoroacetic acid as an additive. This bifunctional catalyst afforded the corresponding aldol products of α,α-dialkylaldehydes and aryl aldehydes in high yields and enantioselectivities (Scheme 1-6, a).[31] Another example of an organocatalyzed aldol reaction where the product contains a quaternary stereocenter is the famous Hajos-Parrish-Eder-Sauer-Wiechert reaction that was reported already 40 years ago (see above, Scheme 1-2).[12-13] Recently, our group developed a mild organocatalytic Mannich reaction of monothiomalonates providing highly stereoselective access to β-amino thioesters with an acyclic quaternary stereogenic center (Scheme 1-6, b).[40] Such β-amino thioesters, which are activated derivatives of β-amino acids, are attractive building blocks in organic synthesis and for foldamer research.[99-101] Highly enantioselective phase-transfer-catalyzed alkylations of α-alkyl-α-alkynyl esters with benzyl or allyl halides were recently reported by Maruoka (Scheme 1-6, c).[44] The chiral quaternary ammonium salt catalyst was also previously used in a slightly modified version for alkylation reactions of 1,3-dicarbonyl compounds.[41-42] Maruoka also



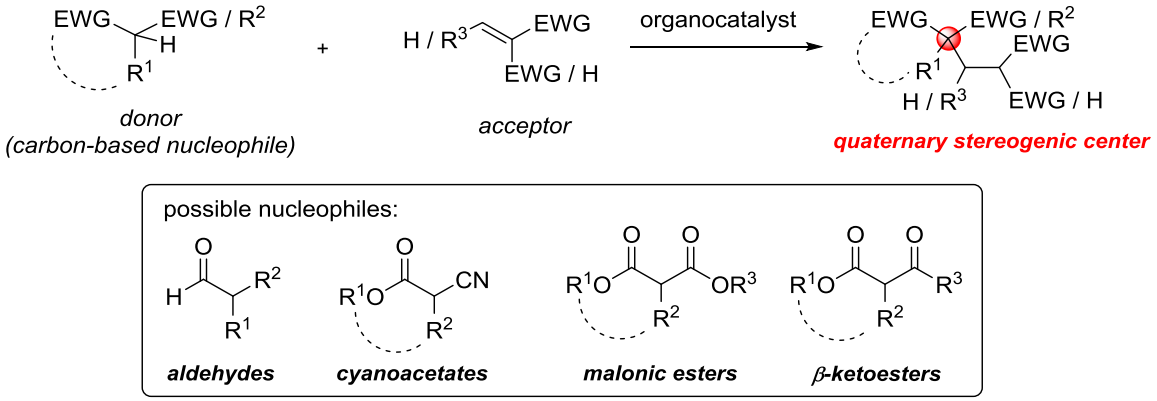
Scheme 1-6 Selected examples of organocatalyzed reactions generating quaternary stereogenic centers.[31,40,44,52,54]

developed a binaphthyl-based primary amine catalyst for asymmetric Diels-Alder reactions of acroleins bearing various α-alkyl substituents. The desired cycloaddition products having a quaternary stereocenter were obtained in good yields and enantioselectivities (Scheme 1-6, d).[52] The synthesis of highly enantioenriched quaternary stereogenic centers under mild organocatalytic conditions has also been demonstrated by Rovis *via* an intramolecular Stetter reaction of aromatic and aliphatic aldehyde substrates (Scheme 1-6, e).[54]

Despite the recent advances in organocatalytic methods for the generation of quaternary stereocenters, many difficulties still remain. For example, high catalyst loadings, limited reaction scope and inaccessibility of compounds with certain substitution patterns. Moreover, the incorporation of the new methodologies in the synthesis of more complex structures requires further development and/or optimization of the catalysts in order to allow for efficient reactions.

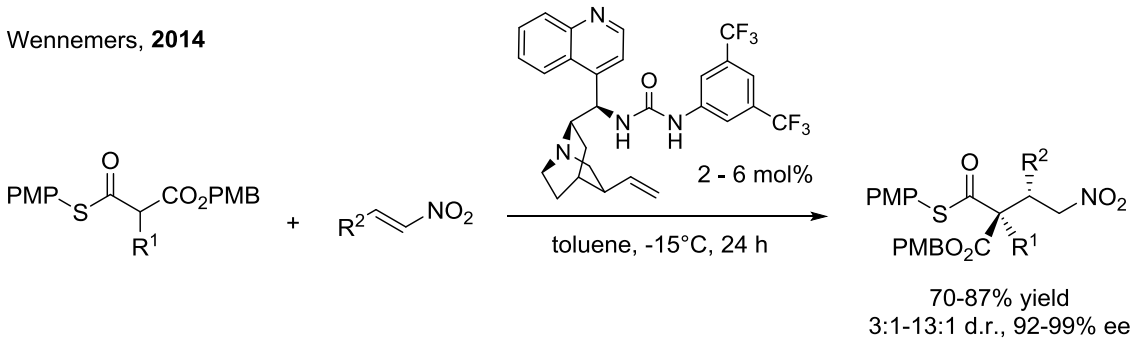
1.2.1 Conjugate Addition Reactions

The conjugate addition reaction of carbon-based nucleophiles to electron-deficient olefins is one of the most useful C-C bond forming reactions in the stereoselective synthesis of organic compounds and numerous reports on organocatalytic versions of this transformation have been reported.[16,102-103] Various carbon-based donors including aldehydes, ketones, esters and nitroalkanes that act as nucleophiles upon activation by a catalyst, have been added to a broad spectrum of different Michael acceptors such as enals, enones, alkylidene malonates, acrylonitriles, vinyl sulfones, vinyl phosphonates, maleimides and nitroalkenes providing a wide variety of functionalized addition products.[16,102-103] Despite these many variations, examples with sterically hindered carbon donors such as α-branched carbonyl compounds, that provide upon reaction with the above mentioned acceptors a direct method for the formation of quaternary stereogenic centers, are still quite limited. Only a few reports on conjugate addition reactions with substrates such as α-substituted β-ketoesters, malonic esters and cyanoacetates, α,α-disubstituted aldehydes or 3-substituted oxindoles as donors have appeared in the literature (Scheme 1-7).[55-75]



Scheme 1-7 Examples of donors and acceptors for organocatalytic conjugate addition reactions affording compounds with a quaternary stereogenic center.[55-75]

These sterically demanding and therefore less reactive nucleophiles require more reactive reaction partners to provide the desired adducts in good yields and acceptable time periods. Consequently, nitroalkenes are particularly interesting acceptors. The strong electron-withdrawing character of the nitro group makes nitroalkenes highly electrophilic[104] and in addition, the nitro group offers high synthetic versatility.[105] In this context, mostly 1,3-dicarbonyl compounds or α,α-disubstituted aldehydes were reacted as nucleophiles in reactions with β-monosubstituted nitroolefins.[76-89] For example, recently our group reported reactions between α-substituted monothiomalonates and nitroolefins that provide, in the presence of catalytic amounts of cinchona alkaloid derivatives, γ-nitrothioesters bearing a quaternary stereogenic center in excellent yields and stereoselectivities (Scheme 1-8).[80]



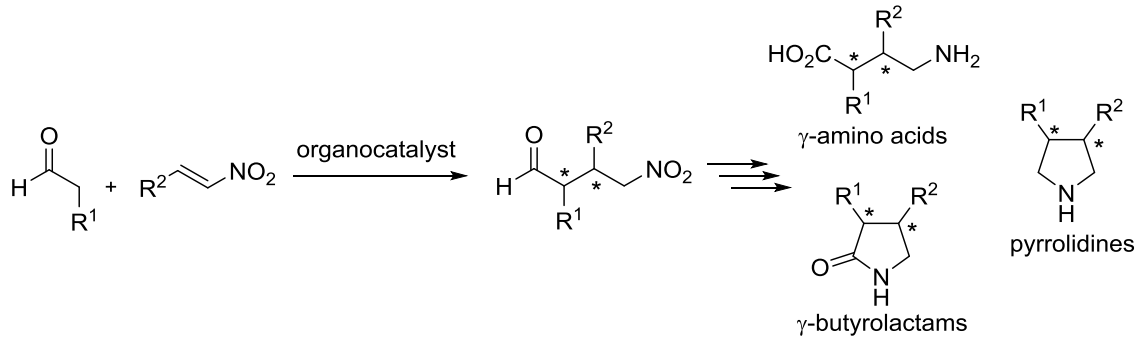
Scheme 1-8 Example for conjugate addition reactions with nitroolefins generating acyclic quaternary stereocenters.[80]

The addition products containing three orthogonal functional groups were readily converted into γ-nitro aldehydes and γ-butyrolactams or, as demonstrated more recently, into indolin-3-yl acetates with an exocyclic quaternary stereogenic center.[106]

In all of the mentioned examples, where an α-branched donor reacts with the nitroolefin, the quaternary stereogenic center is derived from the nucleophile. Even more challenging and therefore very rare are reactions in which the quaternary stereogenic center is derived from the electrophile.[90-97] For example, Ricci,[90] Melchiorre,[91] and more recently Akiyama[93] and Liu[92] reported conjugate additions reactions of carbon-based nucleophiles to β,β-disubstituted nitroolefins providing acyclic nitro compounds bearing a quaternary stereocenter. Kwiatkowski,[94] Kudo[95] (see Scheme 1-13, Chapter 1.3), Shibata[96] and Hayashi[97] all described the addition of nitromethane to α,β-unsaturated aldehydes or ketones. Although nitroolefins were not used as acceptors in these examples, also acyclic γ-nitrocarbonyl compounds with a quaternary stereocenter derived from the electrophile were formed as products.

1.2.2 Conjugate Addition Reactions between Aldehydes and Nitroolefins

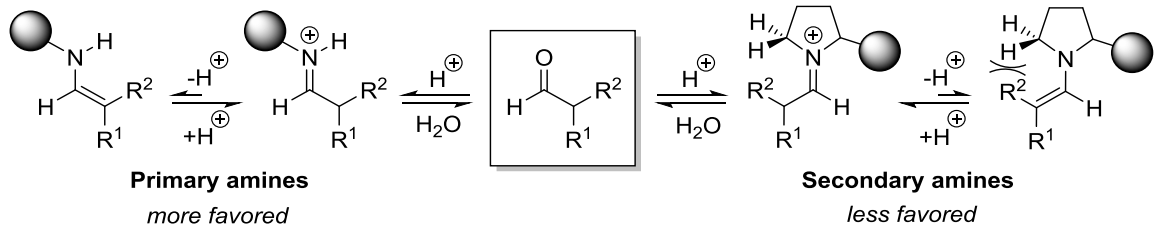
In the past decade, impressive progress has been made in the development of catalysts for stereoselective addition reactions of linear aldehydes to nitroolefins to provide synthetically valuable γ-nitroaldehydes.[107-134] These highly functionalized addition products can be straightforwardly transformed into useful compounds including chiral pyrrolidines, γ-butyrolactams and γ-amino acids (Scheme 1-9).



Scheme 1-9 Conjugate addition reaction between linear aldehydes and nitroolefins.

Although high yields and stereoselectivities were achieved with several catalysts, their often poor catalytic activity often required the use of 10 mol% or more of the catalyst and a high excess of the aldehyde since side-products from *homo*-aldol reactions are commonly observed.[107-108,111-118,126-127,134]

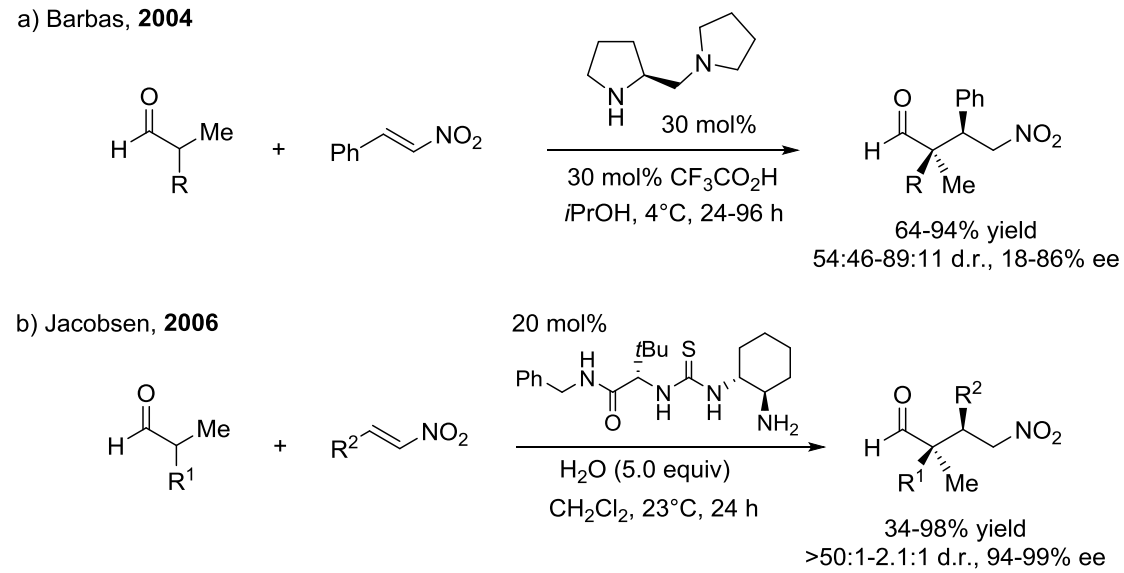
Compared to the many examples utilizing linear aldehydes, far fewer studies on α,α-disubstituted aldehydes are available which provide direct access to γ-nitroaldehydes bearing a quaternary stereocenter.[81-89] The difficulty of reactions with such sterically more demanding substrates lies not only in the lower reactivity of the hindered enamine but also in controlling the ratio between the *E*- and the *Z*-enamine intermediate by the catalyst to provide the product in high stereoselectivity. In that respect, primary amine catalysts generally provide better results than secondary amines, since secondary amine catalysts form more congested imine/enamine intermediates (Scheme 1-10).[135-136] In addition, primary amine catalysts may deliver higher equilibrium concentrations of the active enamine species and thus increase the reaction rate.



Scheme 1-10 Primary versus secondary amine catalysts in reactions with sterically hindered aldehydes.

The first example of the conjugate addition between various α-branched aldehydes and nitrostyrene was reported by Barbas in 2004.[81] In the presence of a chiral diamine/TFA catalyst system the corresponding γ-nitroaldehydes were produced in good yields but only in rather moderate stereoselectivities and long reaction times were necessary demonstrating the difficulties when using secondary amines as catalysts (Scheme 1-11, a). Not long after the report from Barbas, Jacobsen developed the first primary amine catalyzed addition of α-branched aldehydes to nitroolefins.[82] A wide range of α,α-disubstituted aldehyde and nitroolefin combinations reacted readily in the presence of the primary amine thiourea catalyst providing the products in high yields and excellent

enantioselectivities (Scheme 1-11, b). Although certain substrate combinations involving 2-phenylpropanal gave a high diastereomeric ratio (23:1 – >50:1), the d.r. with α,α-dialkyl aldehydes was rather moderate (2.1:1 – 7.1:1).



Scheme 1-11 The first examples of conjugate addition reactions between α,α-disubstituted aldehydes and nitroolefins catalyzed by a a) secondary amine catalyst[81] and b) primary amine-thiourea catalyst.[82]

In 2007, Connon introduced amino cinchona alkaloids as catalysts for addition reactions of aldehydes and ketones to nitroolefins.[85] Although the scope of chiral α-branched aldehydes was limited to only two examples, this publication demonstrated for the first time the potential of cinchona alkaloid based primary amine catalysts for the enamine activation of ketones and α,α-disubstituted aldehydes. Today, such cinchona alkaloid derived primary amines are among the most popular organocatalysts for sterically demanding reaction partners.[136]

Further important examples of conjugate addition reactions with α,α-disubstituted aldehydes and nitroolefins affording the products with quaternary stereocenters were reported by Nugent[88] and Yoshida[89] who used amino acids as catalysts. Nugent demonstrated that the desired γ-nitroaldehydes can be obtained in good yields (up to 84%) and excellent enantiomeric excess (91-99% ee) by using only 5 mol% of *tert*-butyl-protected L-threonine in combination with sulfamide as hydrogen bond donor and DMAP

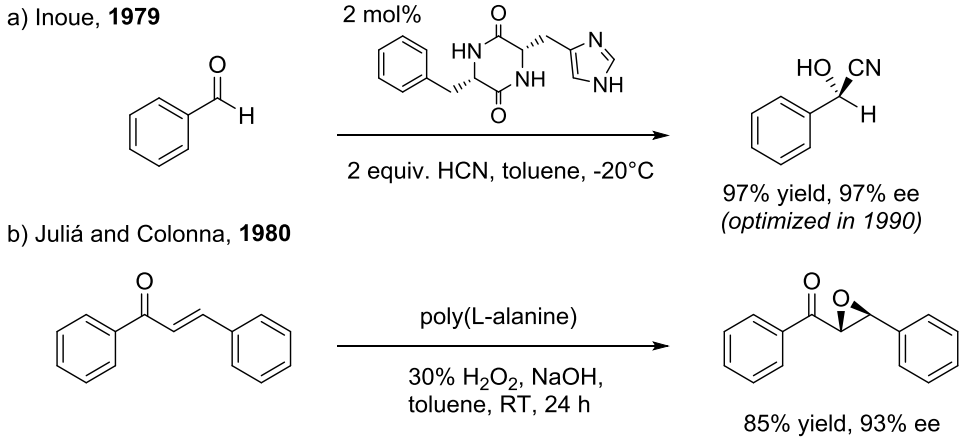
as base.[88] This catalyst loading is so far the lowest loading reported for organocatalyzed addition reactions between α-branched aldehydes and nitroolefins. Although high levels of enantioselectivity were achieved, the diastereoselectivity was again moderate (70:30 – 78:22) because of the possible *E*- or *Z*-enamine formation from the α-branched aldehydes. Yoshida disclosed the use of a 4:1 mixture of L-phenylalanine and its lithium salt as an effective catalyst for α-branched aldehydes to β-nitroacrylates to give the addition products having a quaternary carbon centre in good yields (up to 85%) and with high stereoselectivities (up to >20:1 d.r. and 98% ee).[89] Interestingly, Yoshida also presented in his work the reaction between cyclohexanecarboxaldehyde and the β,β-disubstituted nitroolefin methyl (*E*)-2-methyl-3-nitroprop-2-enoate. However, with these sterically congested substrates the desired addition product was not formed even with a higher catalyst loading. In fact, conjugate addition reactions of aldehydes to β,β-disubstituted nitroolefins have so far not been realized.

1.3 Asymmetric Catalysis with Peptides

In nature and our everyday life, peptides are widespread as, *e.g.*, hormones, neurotransmitters, toxins and therapeutics.[137] In spite of their structural and functional diversity, it is interesting that there have not been found any peptides in nature that serve as catalysts. Short peptides, consisting of only a few amino acid residues as building blocks, may already adopt defined secondary structures to serve as chiral scaffolds suitable to induce stereoselectivity into organic molecules. This feature, together with their modular nature, allowing for tuning reactivity and selectivity, and in addition their facile synthesis, renders short peptides attractive candidates as catalysts for asymmetric organocatalyzed reactions.[138-140]

The first examples of using peptides as catalysts for asymmetric induction were reported in the late 1970s and early 1980s by Oku and Inoue[141] as well as by Juliá and Colonna[142]. In their pioneering study on the addition of hydrogen cyanide to benzaldehyde, Oku and Inoue demonstrated in 1979 that cyclic dipeptides can afford the corresponding mandelonitrile with an excess of one enantiomer of the chiral product.[141] Further optimization of the reaction conditions including the use of diketopiperazine derived from phenylalanine and histidine provided (*R*)-mandelonitrile in excellent yield

and enantioselectivity (Scheme 1-12, a).[143] Shortly after the studies by Oku and Inoue, Juliá and Colonna presented their work on poly-L-alanine and poly-L-leucine catalyzed epoxidations of (*E*)-chalcone. Using hydrogen peroxide under basic conditions afforded the desired epoxide in high yield and enantioselectivity (Scheme 1-12, b).[142] In subsequent reports the scope of this reaction was evaluated.[144-145]

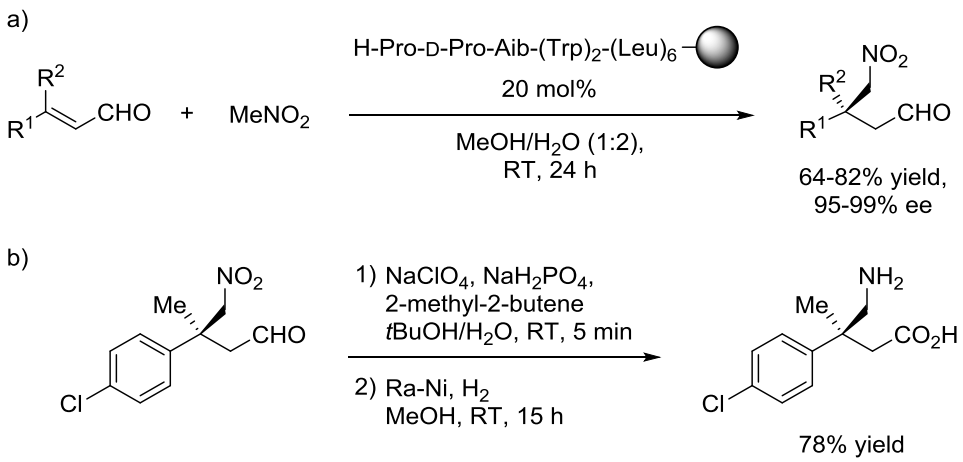


Scheme 1-12 Early examples of asymmetric peptide catalysis. a) Diketeopiperazine as catalyst for the hydrocyanation of benzaldehyde.[141,143] b) Juliá-Colonna epoxidation using poly-L-alanine.[142]

After these early examples, it was not until the turn of the century when the application and development of peptides as asymmetric catalysts became of great interest. With the development of smart combinatorial screening methods that allowed for identification of catalytically active candidates which otherwise may not have been discovered, the field started to flourish.[146-148] The "rediscovery" that even simple amino acids such as proline and derivatives thereof can catalyze important reactions has also added to the significantly increased interest in peptides and their catalytic properties. Nowadays, many short-chain peptides are known to be capable of promoting a wide variety of reactions including stereoselective acylations, phosphorylations, oxidations, halogenations and C-C bond forming reactions.[138-140] Considering the importance of C-C bond forming reactions and given the success of using proline derivatives as catalysts in aldol and conjugate addition reactions, short chain peptides have also been successfully applied in transformations proceeding through iminium ion and enamine activation modes.[149-160] Among the most reactive and selective peptidic catalysts in enamine catalysis are

tripeptides of the general type Pro-Pro-Xaa, with Xaa being a variable amino acid with a carboxylic acid group (see the following Chapter 1.4).

Recent advances in the field of asymmetric peptide catalysis also demonstrated that the unique features of peptides, compared to other less structurally diverse catalysts, can be used to address challenges such as chemoselectivity, regio- and site selectivity, as well as difficult enantioselective transformations.[138-139,161] As described earlier (see Chapter 1.2), the enantioselective generation of acyclic quaternary stereogenic centers represents a great challenge in organic synthesis. Whereas, a variety of organocatalysts has been developed for the asymmetric formation of tertiary stereocenters, protocols for the synthesis of such quaternary stereogenic centers are still limited.[27] Recently, in an important contribution to this field, Kudo reported peptide-catalyzed conjugate additions between nitromethane and β,β-disubstituted α,β-unsaturated aldehydes.[95] In the presence of a solid-supported helical 11-mer peptide the desired γ-nitroaldehydes bearing a quaternary stereocenter were obtained under mild aqueous conditions in good yields and very high enantioselectivites (Scheme 1-13, a). Furthermore, this transformation provided a simple route to highly congested β,β-disubstituted γ-amino acids which was illustrated by preparing a β-methylated analogue of baclofen, a drug for treating spasticity (Scheme 1-13, b).

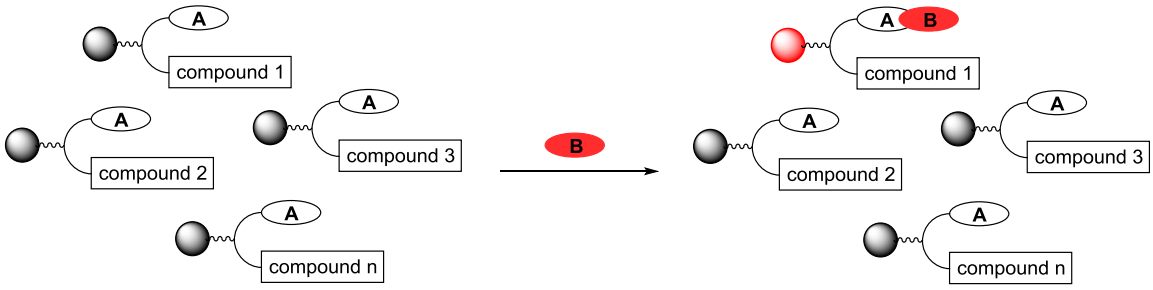


Scheme 1-13 a) Challenging enantioselective addition of nitromethane to β,β-disubstituted α,β-unsaturated aldehydes generating a quaternary stereocenter. b) Derivatization of the products to access β,β-disubstituted γ-amino acids.[95]

1.3.1 Conjugate Addition Reactions between Aldehydes and Nitroolefins Catalyzed by Peptides of the Type Pro-Pro-Xaa

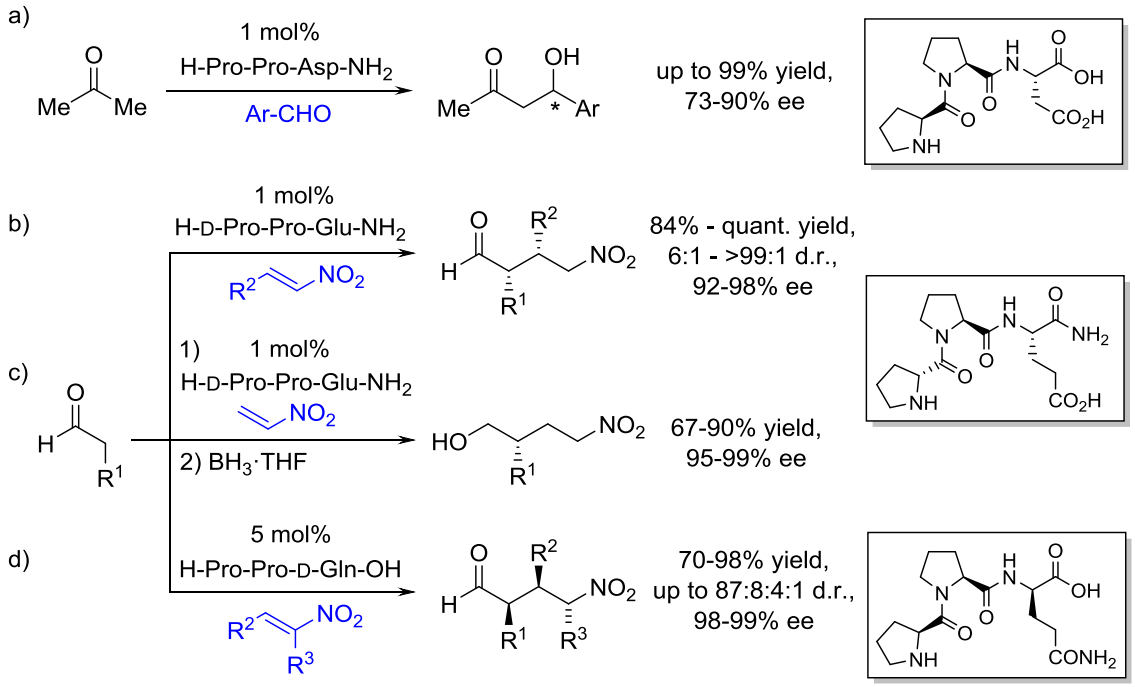
The large structural and functional diversity of peptides allowed the establishment of these molecules as efficient catalysts for a broad range of asymmetric reactions.[138-140] However, since short peptides are typically conformationally flexible due to many rotational degrees of freedom, the development of potential peptidic catalysts through rational design is extremely difficult. Consequently, the use of combinatorial chemistry which allows for the simultaneous generation and evaluation of a variety of diverse compounds is an attractive approach that offers an effective solution to this problem.[146-148] Among the strategies to create molecular diversity, split-and-mix synthesis is one of the most elegant methods, particularly suitable for building up diverse combinatorial peptide libraries due to the modularity of peptides (linearly connected amino acid residues) and the established synthetic protocols (solid-phase peptide synthesis). Such one-bead-one-compound peptide libraries allow, in combination with smart screening methods, for the identification of powerful peptidic catalysts.[146-148]

Our group identified catalytically active peptides in one-bead-one-compound peptide libraries by using the method of catalyst-substrate co-immobilization.[162-164] The concept of this method is that one substrate is immobilized together with one library member (potential catalyst) on the same bead. Subsequent reaction with the other substrate, which is labeled for example with a dye, results in colored beads if the library member catalyzed the reaction (Scheme 1-14). These colored beads can then be separated and analyzed to reveal the catalyst structure.[162]



Scheme 1-14 The principle of catalyst-substrate co-immobilization for the identification of potent catalysts.

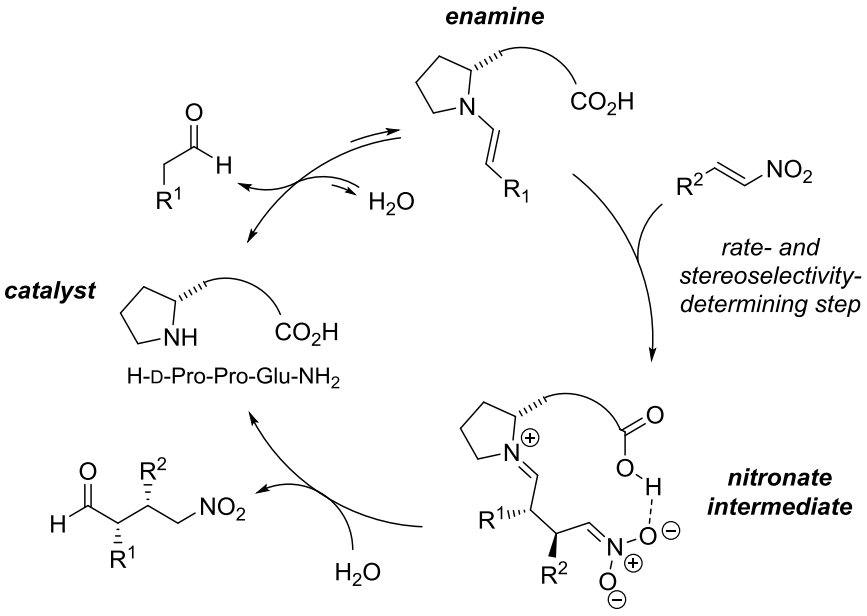
With this method, our group identified peptides of the general type Pro-Pro-Xaa (Xaa = variable amino acid with a carboxylic acid group) as excellent catalysts for aldol reactions between acetone and aromatic aldehydes. In particular, the tripeptide H-Pro-Pro-Asp-NH_2 proved to be a highly active and selective catalyst (Scheme 1-15, a).[163-164] Insights from molecular modeling studies showed that the distance between the secondary amine and the carboxylic acid within peptide H-Pro-Pro-Asp-NH_2 is greater than in proline.[165] This led to the hypothesis that H-Pro-Pro-Asp-NH_2 allows not only for catalysis of 1,2- but also of 1,4-addition reactions and revealed that H-Pro-Pro-Asp-NH_2, and especially the diastereoisomer H-D-Pro-Pro-Asp-NH_2, are very good catalysts for conjugate addition reactions between aldehydes and nitroolefins. Further structural and functional investigations showed that the closely related peptide H-D-Pro-Pro-Glu-NH_2 is an even more efficient catalyst for conjugate addition reactions of aldehydes to nitroolefins.[166] In the presence of only 1 mol% of H-D-Pro-Pro-Glu-NH_2 the desired γ-nitroaldehydes from various aldehydes and aromatic as well as aliphatic β-substituted nitroolefins were obtained in excellent yields and stereoselectivities (Scheme 1-15, b).[166-167]



Scheme 1-15 Addition reactions catalyzed by peptides of the type Pro-Pro-Xaa. a) Aldol reactions between acetone and aromatic aldehydes.[163-164] b) – d) Conjugate addition reactions of aldehydes to nitroolefins.[166-169]

In addition, even nitroethylene, known to be a challenging substrate due to its polymerization tendency, reacted readily with aldehydes in the presence of 1 mol% of H-D-Pro-Pro-Glu-NH_2 (Scheme 1-15, c). The corresponding γ-nitroaldehydes allowed facile conversion into monosubstituted γ^2-amino acids that are difficult to prepare by other methods.[168]

Kinetic studies showed that the rate-determining step of the reaction is the C-C bond formation between the enamine and the nitroolefin (Scheme 1-16).[170] Furthermore, the highest reaction rate was observed when anhydrous solvents and reagents as well as an excess of the nitroolefin with respect to the aldehyde were used. These optimized conditions allowed to reduce the catalyst loading to as little as 0.1 mol% and still afford the conjugate addition products in high yields and stereoselectivities.[170] This is thus far the lowest catalyst loading achieved in enamine catalysis with synthetic organocatalysts.



Scheme 1-16 Proposed catalytic cycle for conjugate addition reactions between aldehydes and nitroolefins catalyzed by peptide H-D-Pro-Pro-Glu-NH_2.[170-171]

More recently, further mechanistic studies by ESI-MS revealed that the C-C bond formation between the enamine and the nitroolefin is also the stereoselectivity-determining step[171] and that a suitably positioned carboxylic acid group within the catalyst controls the reaction pathway.[172]

The excellent features of the peptidic catalyst H-D-Pro-Pro-Glu-NH_2 such as high catalytic efficiency, high chemoselectivity and high robustness, made it possible to recover and reuse the resin-bound analogue H-D-Pro-Pro-Glu-NH-R (R = solid support, Tentagel or polystyrene) several times without loss in catalytic activity or stereoselectivity.[173] As a result, the immobilized catalyst was successfully applied in a continuous flow system to provide the addition products on a >100 gram scale.[174] Moreover, when H-D-Pro-Pro-Glu-NH_2 was equipped with a *n*-dodecyl chain at the C-terminus, the resulting peptide proved to be an excellent amphiphilic catalyst for conjugate addition reactions of aldehydes to β-substituted nitroolefins in aqueous medium.[175] The highly modular nature combined with the ease of solid-phase peptide synthesis is a clear benefit of peptidic catalyst of the type Pro-Pro-Xaa allowing for their straightforward derivatization and optimization of their properties. In this way, two new powerful catalysts for conjugate addition reactions of aldehydes and challenging α,β-disubstituted nitroolefins were developed. Using only 5 mol% of the peptides H-Pro-Pro-D-Gln-OH or H-Pro-Pro-Asn-OH provided synthetically useful γ-nitroaldehydes bearing three consecutive stereogenic centers in good yields, high diastereoselectivities and excellent enantioselectivities (Scheme 1-15, d).[169] The γ-nitroaldehydes were readily converted to chiral pyrrolidines, fully substituted γ-butyrolactams, and γ-amino acids.

Short-chain peptides of the type Pro-Pro-Xaa (Xaa = acidic amino acid) developed by our group represent a family of robust, modular and highly active, chemo- and stereoselective catalysts that allow to overcome typical problems of other amine-based organocatalysts such as high catalyst loadings, high excess of aldehyde and limited substrate scope in conjugate addition reactions of aldehydes to nitroolefins.

2

Objectives

The stereoselective generation of quaternary stereocenters is a difficult task in asymmetric catalysis. Particularly challenging is the synthesis of *acyclic* compounds with a quaternary stereogenic center under mild organocatalytic conditions.[22-30] Among C-C bond forming reactions, the conjugate addition of aldehydes to nitroolefins is a very attractive and intensively studied reaction providing access to highly functionalized γ-nitroaldehydes.[16] These valuable intermediates can be readily converted into useful compounds such as γ-amino acids, γ-butyrolactams or chiral pyrrolidines. Whereas a variety of examples of catalytic asymmetric reactions between linear aldehydes and β-monosubstituted nitroolefins in the presence of chiral amine-based catalysts have been reported, only a few studies with α,α-disubstituted aldehydes providing a direct route to γ-nitroaldehydes with a quaternary stereocenter are available.[29] Moreover, reactions of linear aldehydes with sterically demanding β,β-disubstituted nitroolefins to afford these γ-nitroaldehydes bearing an adjacent quaternary and tertiary stereogenic center have so far not been realized.

With the tripeptides of the type Pro-Pro-Xaa we have established highly active and stereoselective catalysts[163-166,168-170] that offer with their structural modularity the possibility to tune the catalytic properties in order to adapt to the requirements of a given substrate combination and thus suggest that these peptides might be potent catalyst for conjugate addition reactions with challenging substrates.

The objectives of this thesis were:

1) *The development and application of a peptidic catalyst for conjugate addition reactions of aldehydes to β,β-disubstituted nitroolefins providing acyclic γ-nitroaldehydes bearing a quaternary stereogenic center.*

2) *Once an appropriate peptidic catalyst was obtained to perform kinetic and NMR spectroscopic studies to gain insight into the mechanism of reactions between aldehydes and β,β-disubstituted nitroolefins.*

3) *To extend the substrate scope of peptide-catalyzed conjugate addition reactions to α,α-disubstituted aldehydes.*

3

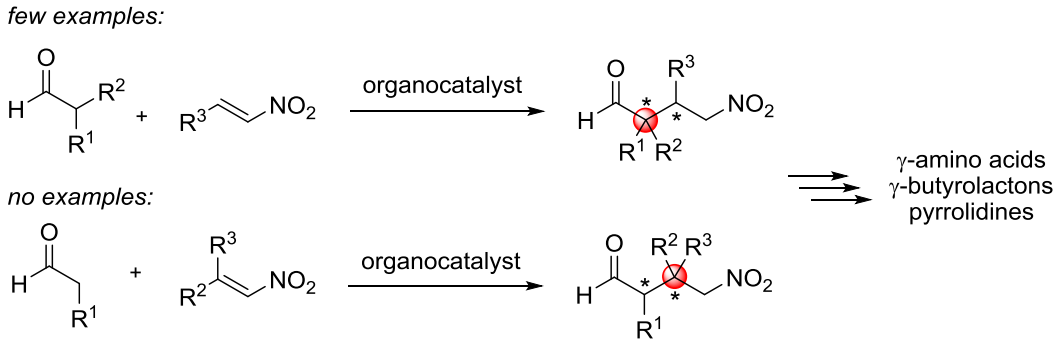
Peptide-Catalyzed Stereoselective 1,4-Addition Reactions between Aldehydes and β,β-Disubstituted Nitroolefins

Parts of this chapter were published:

R. Kastl, H. Wennemers, *Angew. Chem. Int. Ed.* **2013**, *52*, 7228-7232

3.1 Background

There are two possible ways to obtain synthetically valuable γ-nitroaldehydes with a quaternary stereocenter *via* conjugate addition reactions of aldehydes to nitroolefins. Either by reacting *α,α-disubstituted* aldehydes with *β-monosubstituted* nitroolefins, or by reacting *linear* aldehydes with *β,β-disubstituted* nitroolefins (Scheme 3-1). Whereas few examples have been reported with α,α-disubstituted aldehydes,[29] no such reactions have yet been achieved with β,β-disubstituted nitroolefins, although the asymmetric addition to β-monosubstituted nitroolefins is well-established.



Scheme 3-1 Challenging Conjugate addition reactions between aldehydes and nitroolefins generating adjacent quaternary and tertiary stereogenic centers.

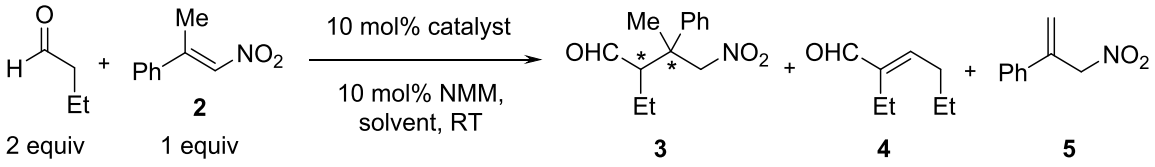
Previously, our group introduced tripeptides of the type Pro-Pro-Xaa (Xaa = acidic amino acid) as highly reactive and stereoselective catalysts for aldol reactions and conjugate addition reactions of aldehydes to nitroolefins.[163-170,173-175] For example, H-D-Pro-Pro-Glu-NH_2 (**1**) and H-Pro-Pro-D-Gln-OH catalyze conjugate addition reactions of aldehydes with β-monosubstituted nitroolefins[165-168,170,173-175] and α,β-disubstituted nitroolefins,[169] respectively. Products resulting from *homo*-aldol reactions that are typical side products in such conjugate addition reactions, form either not at all or only in minimal amounts. This high chemoselectivity of the peptidic catalysts is remarkable, particularly since the closely related peptide H-Pro-Pro-Asp-NH_2 is a very good catalyst for aldol reactions.[163-164] Thus, the modular nature of these peptidic catalysts allows for fine-tuning and optimizing their structural and functional properties to the desired reaction pathway and to accommodate the requirements of a given substrate combination.

These features suggest that members of the class of peptides Pro-Pro-Xaa might also fulfill the requirements for catalyzing stereoselective addition reactions between aldehydes and challenging β,β-disubstituted nitroolefins to provide acyclic γ-nitroaldehydes bearing a quaternary stereogenic center.

3.2 Initial Experiments

We started our investigations by testing the catalytic properties of peptide H-D-Pro-Pro-Glu-NH_2 (**1**) in the reaction of butanal with β,β-disubstituted nitroolefin *trans*-α-methyl-β-nitrostyrene (**2**). For the first experiment we chose to use the established conditions for β-monosubstituted nitroolefins.[166-167] Because we expected a slower reaction with the β,β-disubstituted nitroolefin **2**, 10 instead of 1 mol% of catalyst **1** and a slightly higher excess of butanal (2 instead of 1.5 equivalents) were used with 1 equivalent of the nitroolefin in a 9:1 mixture of chloroform and *i*PrOH (Table 3-1, entry 1). The peptidic catalyst was used as the trifluoroacetic acid (TFA) salt that is conveniently obtained by standard solid-phase peptide synthesis. Thus, the addition of a base was necessary to liberate the secondary amine and allow for catalysis.

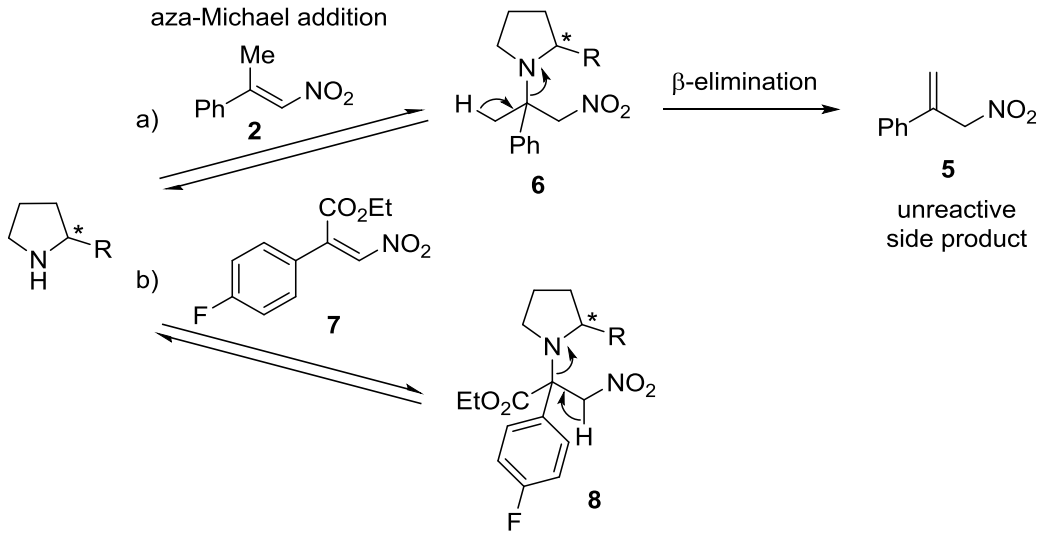
Table 3-1 Initial studies: conjugate addition reactions between butanal and α-methyl-β-nitrostyrene (**2**).



	catalyst	solvent	time [h]	conversion [%][a] **3**
1	TFA·H-D-Pro-Pro-Glu-NH_2	$CHCl_3$/*i*PrOH 9:1	196	< 1
2	TFA·H-D-Pro-Pro-Glu-NH_2	*i*PrOH	96	< 5
3	proline[b]	*i*PrOH	120	< 5
4	pyrrolidine[b]	*i*PrOH	120	~ 10

[a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Without addition of NMM.

In previous studies with β-monosubstituted nitroolefins, *N*-methylmorpholine (NMM) proved to be the base of choice to liberate the secondary amine within the catalyst.[166] Therefore, we also used NMM in the present reaction. Despite the increased catalyst loading of 10 mol%, the desired addition product **3** could not obtained in the chloroform/*i*PrOH mixture even after an extended reaction time of several days. The use of pure *i*PrOH, in which the reaction with β-monosubstituted nitroolefins proved to proceed faster,[165] still led only to trace amounts of addition product **3** (Table 3-1, entry 2). Even with proline or pyrrolidine as a catalyst, which are sterically less hindered compared to peptide **1**, the conversion to addition product **3** could not be significantly increased (Table 3-1, entries 3 and 4). Furthermore, in all of the reactions with *trans*-α-methyl-β-nitrostyrene (**2**) depicted in Table 3-1, a significant amount of the *homo*-aldol condensation product **4** formed and more than 25% of nitroolefin **2** isomerized into side product (3-nitroprop-1-en-2-yl)benzene (**5**) with a terminal double bond. The formation of **5** is expected to proceed *via* addition of the secondary amine catalyst to the nitroolefin, affording the corresponding aza-Michael adduct **6**, followed by β-elimination (Scheme 3-2, a).



Scheme 3-2 Aza-Michael addition followed by β-elimination as side reactions in amine catalyzed 1,4-addition reactions between aldehydes and β,β-disubstituted nitroolefins. a) Formation of the unreactive olefin **5** from nitroolefin **2** bearing an aliphatic substituent with β-hydrogens. b) Reversible reaction between amine catalyst and nitroolefin **7** bearing substituents at C(2) without β-hydrogens.

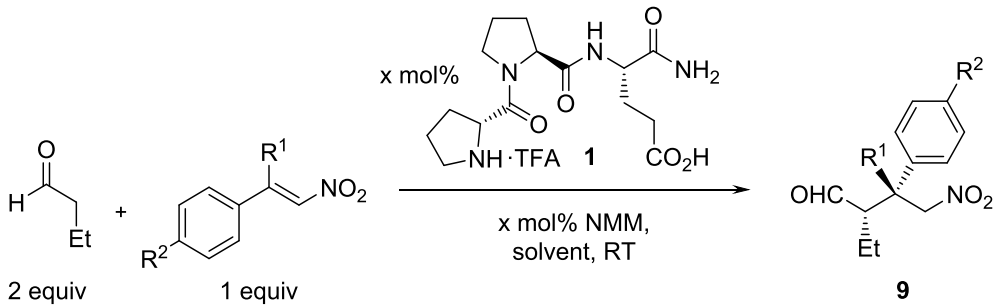
The elimination of a proton in the α position to the nitro group of adduct **6** results in the back-reaction to the catalyst and nitroolefin **2**, whereas irreversible β-elimination of a proton from the methyl group liberates the catalyst and affords side product **5** (Scheme 3-2, a). Such a mechanism was proposed by Shi and co-workers who used nitroolefins such as **2** in intermolecular cross-conjugate additions with enones and enals catalyzed by proline.[176]

In order to achieve higher conversion to the 1,4-addition product and to prevent the formation of side products such as **5**, we selected (*Z*)-ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**), bearing an ester group at C(2), as the substrate for further studies. First of all, the ester group is not only a sterically less demanding substituent than a methyl group (sp^2 hybridized carbonyl group vs. sp^3 hybridized carbon of the methyl group), but more importantly makes nitroacrylate **7** much more electrophilic due to its electron-withdrawing properties. Thus, more of the desired addition product should form. Another important advantage of **7** is that the aza-Michael adduct **8**, formed upon addition of the amine catalyst to nitroacrylate **7**, cannot undergo β-elimination as observed for adduct **6** because the ester group and the phenyl substituent at C(2) of **8** do not bear hydrogen atoms for β-elimination. Therefore, no unreactive side product such as **5** can form (Scheme 3-2, b). A further benefit of nitroacrylate **7** is that in 1,4-addition reactions with aldehydes highly functionalized γ-nitroaldehydes are obtained which might be used as valuable intermediates in the synthesis of more complex molecules.

Nitroacrylate **7** was therefore reacted with butanal in the presence of peptide **1** under the same conditions as for the previously described reaction (Table 3-1) providing the corresponding addition product **9** in 50% conversion within 3 days. In comparison, < 5% conversion to product **3** was achieved in the reaction of butanal to nitroolefin **2** after 4 days (see Table 3-1, entry 2 and Table 3-2, entry 3). This demonstrates that nitroacrylate **7** is indeed more reactive than **2**. Nevertheless, the β,β-disubstituted nitroolefin proved to be significantly less prone to form conjugate addition products with aldehydes than β-monosubstituted nitroolefins. Whereas β-nitroolefins react readily with aldehydes in the presence of as little as ≤ 1 mol% of H-D-Pro-Pro-Glu-NH_2 (**1**) in a mixture of $CHCl_3$ and *i*PrOH (*e.g.*, nitrostyrene with butanal; Table 3-2, entry 1),[165-167,170] only a small amount of the β,β-disubstituted nitroolefin **7** was

converted to the desired addition product **9** in the presence of 10 mol% of peptide **1** within three days under the same reaction conditions (Table 3-2, entry 2). Again, a significant amount of the *homo*-aldol reaction product **4** formed (as in the reaction with alkylsubstituted nitroolefin **2**). Reassuringly, the desired γ-nitroaldehyde **9** was obtained in good stereoselectivity (Table 3-2, entry 2).

Table 3-2 Initial studies: 1,4-addition reactions of butanal to (*Z*)-ethyl 2-(4-fluoro-phenyl)-3-nitroacrylate (**7**) compared to *trans*-β-nitrostyrene.



	R^1	R^2	x	solvent	time [h]	conv. [%][a]	d.r.[a]	ee [%][b]
1	H	H	1	$CHCl_3$/*i*PrOH 9:1	12	100	50:1	97
2	F	CO_2Et	10	$CHCl_3$/*i*PrOH 9:1	72	<10	6.4:1	86
3	F	CO_2Et	10	*i*PrOH	72	50	4.0:1	75

[a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis.

In agreement with previous studies with β-monosubstituted nitroolefins and as mentioned above, a higher conversion to the γ-nitroaldehyde **9** was observed when only *i*PrOH was used as a solvent although at the expense of stereoselectivity (Table 3-2, entry 3). These initial studies demonstrate that β,β-disubstituted nitroolefins are more challenging substrates than their monosubstituted counterparts and therefore catalysts are required that better accommodate the different structural as well as electronic properties within these substrates.

3.3 Screening for Peptidic Catalysts

The initial experiments showed that H-D-Pro-Pro-Glu-NH_2 (**1**) is able to catalyze the reaction between butanal and β,β-disubstituted nitroolefin **7**. However, long reaction times are necessary and the desired γ-nitroaldehyde **9** is formed only in moderate stereoselectivity. Based on these results, we hypothesized that a peptide structurally related to **1** might be a better catalyst for addition reactions of aldehydes to β,β-disubstituted nitroolefins. We therefore synthesized and tested peptidic catalysts bearing the Pro-Pro motif that differed in (1) the stereochemistry of the amino acids residues, (2) the functional groups at the C-terminus or in the side chain of the C-terminal amino acid, and (3) the spacer length to the functional group in the C-terminal amino acid (Figure 3-1).

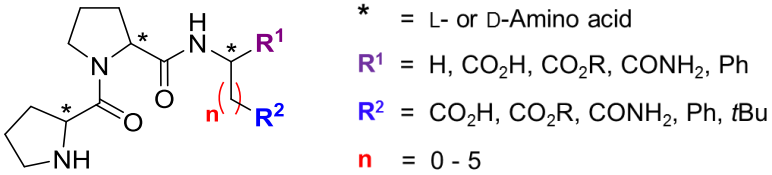
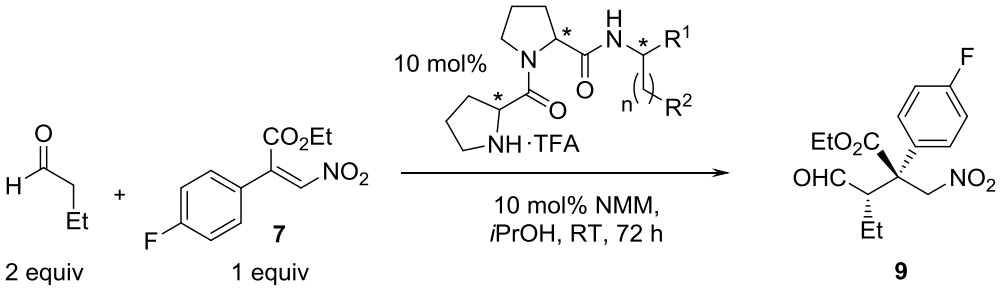


Figure 3-1 Variations in the structure of peptides with the Pro-Pro motif in order to identify an optimal catalyst for 1,4-addition reaction between aldehydes and β,β-disubstituted nitroolefins.

The modular nature of these peptides allowed for a straightforward preparation of a variety of different catalysts, either by solid-phase peptide synthesis or solution-phase synthesis. In total, more than 50 peptides were studied as catalysts in the conjugate addition reaction between butanal and nitroacrylate **7**. All reactions were carried out with the TFA-salts of the peptides, the equivalent amount of NMM, 2 equivalents of butanal and 1 equivalent of the nitroolefin in *i*PrOH. The concentration of the reactions was 0.4 M with respect to the nitroolefin. The following Table 3-3 and Scheme 3-3 illustrate examples in which the most significant differences in reactivity as well as stereoselectivity were observed in the development of the optimal catalyst. A more detailed table is given in the appendix.

A first screening included tripeptides of the type Pro-Pro-Xaa, with Xaa being a variable amino acid with a carboxylic acid group. Introduction of aspartic acid, glutamic acid,

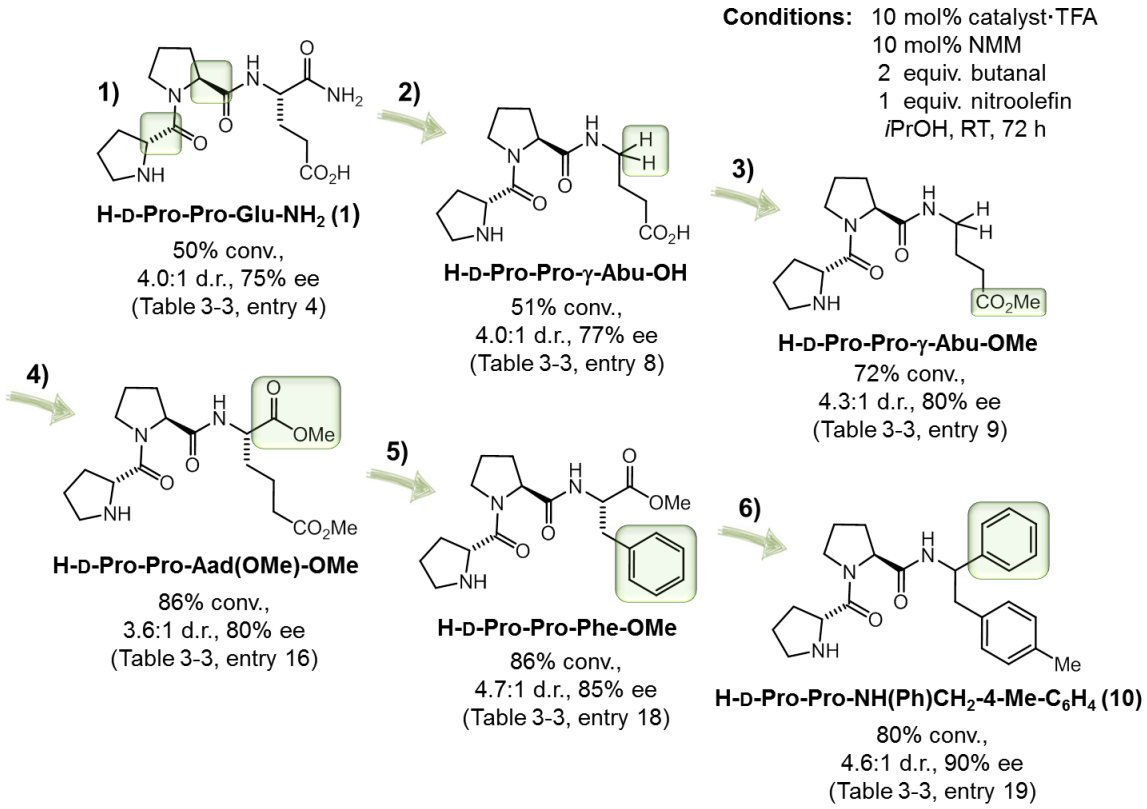
Table 3-3 1,4-Addition reactions of butanal to (*Z*)-ethyl 2-(4-fluoro-phenyl)-3-nitroacrylate (**7**) catalyzed by peptides with the D-Pro-Pro motif.



	catalyst[a]	R^1	R^2	conv. [%][b]	d.r.[b]	ee [%][c]
1	H-D-Pro-Pro-Asp-NH_2	$CONH_2$	CO_2H	30	2.2:1	70
2	H-D-Pro-Pro-Asn-OH	CO_2H	$CONH_2$	40	2.1:1	58
3	H-D-Pro-Pro-Gln-OH	CO_2H	CH_2CONH_2	47	2.3:1	74
4	H-D-Pro-Pro-Glu-NH_2 (**1**)	$CONH_2$	CH_2CO_2H	50	4.0:1	75
5	H-Pro-Pro-Glu-NH_2	$CONH_2$	CH_2CO_2H	33	3.5:1	59[d]
6	H-Pro-D-Pro-Glu-NH_2	$CONH_2$	CH_2CO_2H	40	3.5:1	68[d]
7	H-Pro-Pro-D-Glu-NH_2	$CONH_2$	CH_2CO_2H	43	3.6:1	60[d]
8	H-D-Pro-Pro-γ-Abu-OH	H	CH_2CO_2H	51	4.0:1	77
9	H-D-Pro-Pro-γ-Abu-OMe	H	CH_2CO_2Me	72	4.3:1	80
10	H-D-Pro-Pro-5-Ava-OMe	H	$(CH_2)_2CO_2Me$	80	4.2:1	80
11	H-D-Pro-Pro-6-Ahx-OMe	H	$(CH_2)_3CO_2Me$	76	3.7:1	80
12	H-D-Pro-Pro-7-Ahp-OMe	H	$(CH_2)_4CO_2Me$	75	3.6:1	81
13	H-D-Pro-Pro-5-Ava-O*i*Pr	H	$(CH_2)_2CO_2iPr$	80	4.0:1	79
14	H-D-Pro-Pro-5-Ava-OPh	H	$(CH_2)_2CO_2Ph$	78	4.0:1	81
15	H-D-Pro-Pro-5-Ava-OBn	H	$(CH_2)_2CO_2Bn$	59	3.0:1	80
16	H-D-Pro-Pro-Aad(OMe)-OMe	CO_2Me	$(CH_2)_2CO_2Me$	87	3.6:1	80
17	H-D-Pro-Pro-β-*tert*-butyl-Ala-OMe	CO_2Me	*tert*-butyl	67	4.7:1	79
18	H-D-Pro-Pro-Phe-OMe	CO_2Me	Ph	86	4.7:1	85
19	**H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (10)**	**Ph**	**4-Me-C_6H_4**	**80**	**4.6:1**	**90**

Abbreviations: Abu = aminobutyric acid, Ava = aminovaleric acid, Ahx = aminohexanoic acid, Ahp = aminoheptanoic acid, Aad = aminoadipic acid. [a] The peptidic catalysts were used as the TFA salts. [b] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [c] Determined by chiral-phase HPLC analysis. [d] The opposite enantiomer was formed.

asparagine or glutamine in the Xaa position showed that peptides with the D-Pro-L-Pro motif were better catalysts than their diastereomeric analogues (see Appendix, Table 9-1 for details). However, none of these catalysts performed better than H-D-Pro-Pro-Glu-NH_2 (**1**) (Table 3-3, entries 1-4. For a comparison between peptide **1** and its diastereoisomers see entries 4-7). Further variations at the C-terminal end revealed that the functional group affects the reactivity and chemoselectivity (aldol versus conjugate addition reaction) of the peptidic catalysts significantly. Whereas peptides bearing a carboxylic acid group led to the formation of larger quantities of *homo*-aldol reaction products, higher conversions to the conjugate addition product **9** were achieved with



Scheme 3-3 Summary scheme that shows variations in the structure of peptidic catalyst H-D-Pro-Pro-Glu-NH_2 (**1**) that resulted in the discovery of peptide **10** as optimal catalyst for 1,4-addition reactions of aldehydes to β,β-disubstituted nitroolefins. 1) Peptides with the D-Pro-L-Pro motif were better catalysts than their diastereomeric analogues. 2) and 3) The C-terminal amide had no significant influence on the reactivity and selectivity of the catalyst, whereas an ester group led to an increase in reactivity compared to a carboxylic acid. 4) Two ester groups were better than one. 5) and 6) C-terminal aromatic substituents provided better enantioselectivity.

peptides bearing an ester group at the C-terminus (for example, compare entries 8 and 9 in Table 3-3; step 2 and 3 in Scheme 3-3). This is remarkable, since the presence of a carboxylic acid moiety within the peptidic catalyst had been found to provide highest reactivity and stereoselectivity in the previously examined peptide-catalyzed conjugate addition reactions with β-mono- as well as α,β-disubstituted nitroolefins.[166,169,172] As can be seen from Table 3-3 (entry 16) and Scheme 3-3 (step 4), the highest conversion to γ-nitroaldehyde **9** was achieved with peptide H-D-Pro-Pro-Aad(OMe)-OMe (Aad = aminoadipic acid) bearing two methyl ester groups.

To investigate the effect of a sterically more demanding substituent in the catalyst on the reactivity and stereoselectivity, the addition reaction between butanal and nitroacrylate **7** was carried out next in the presence of the peptides H-D-Pro-Pro-β-*tert*-butyl-Ala-OMe and H-D-Pro-Pro-Phe-OMe bearing either a *tert*-butyl group or an aromatic substituent in the side chain of the C-terminal amino acid residue (Table 3-3, entries 17 and 18). Whereas the introduction of the *tert*-butyl group led to a less reactive catalyst (Table 3-3, entry 17), the aromatic residue of the C-terminal phenylalanine led to a higher enantioselectivity (Table 3-3, entry 18; Scheme 3-3, step 5). The best catalyst with respect to stereoselectivity and reactivity identified from the screening was found to be peptide H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) with two aromatic residues at the C-terminus (Table 3-3, entry 19; Scheme 3-3, step 6). In presence of **10**, 80% conversion to the desired γ-nitroaldehyde **9** was observed and good enantioselectivity (90%) was achieved. It needs to be noted that peptide **10** is a mixture of the two diastereo-isomers H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) and H-D-Pro-Pro-NH-(*S*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-S**; for further details see Chapter 3.4.2).

The question of why a peptidic catalyst lacking an intramolecular carboxylic acid moiety is better suited for the reaction with a β,β-disubstituted nitroolefin compared to peptides bearing a proton donor that had been found to be optimal for related reactions is not trivial. The findings suggest that steric shielding and/or interaction between the aromatic portion of catalyst **10** and the β,β-disubstituted nitroolefin are critical for favoring the desired conjugate addition over the competing aldol reaction.

The results achieved with peptide **10** were the starting point for further optimization of reaction parameters in order to find conditions, under which product **9** is formed in even higher yield and stereoselectivity.

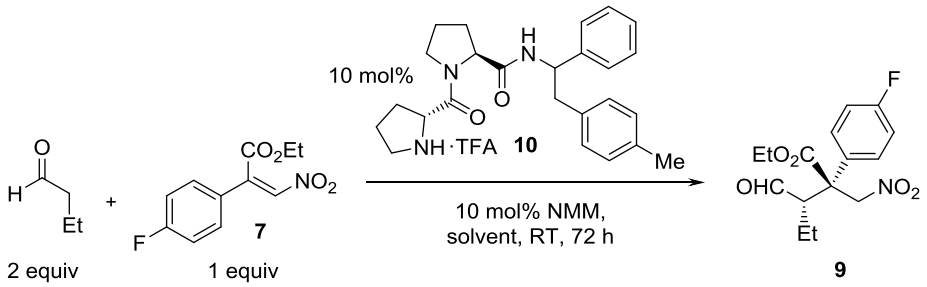
3.4 Optimization of Reaction Conditions

Having found peptide H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) as a promising catalyst for 1,4-addition reaction of aldehydes to β,β-disubstituted nitroolefins, we next studied different reaction conditions and their influence on the reaction outcome.

3.4.1 Solvent Screening

First, we investigated the effect of the solvent and performed the reaction between butanal and nitroacrylate **7** in the presence of H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) in different solvents with different polarities (Table 3-4).

Table 3-4 1,4-Addition reactions of butanal to (*Z*)-ethyl 2-(4-fluoro-phenyl)-3-nitroacrylate (**7**) catalyzed by H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) in different solvents.



	solvent	conv. [%][a]	d.r.[a]	ee [%][b]
1	MeOH	10	1.1:1	68
2	EtOH	33	3.0:1	81
3	*i*PrOH	80	4.6:1	90
4	***t*BuOH**	**96[c]**	**6.0:1**	**94**
5	CH_2Cl_2	12	4.1:1	85
6	$CHCl_3$	36	4.8:1	92
7	toluene	21	5.0:1	93
8	EtOAc	20	5.1:1	83
9	THF	27	6.1:1	89
10	CH_3CN	8	3.6:1	n.d.[d]
11	DMSO	14	3.7:1	40
12	DMF	11	2.9:1	55

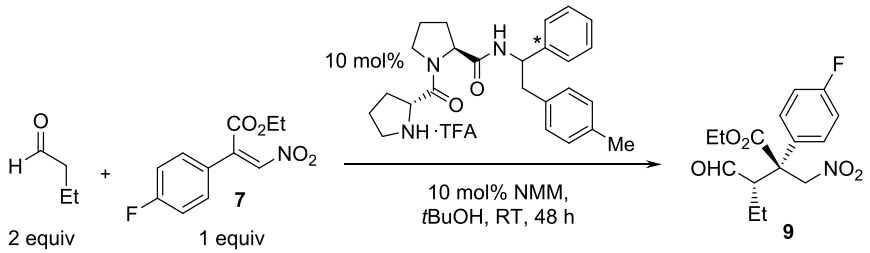
[a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis. [c] Reaction time was 48 h. [d] Not determined.

Whereas in primary alcohols such as MeOH and EtOH the reaction proceeded slowly and with poor stereoselectivity, better results were achieved when secondary and tertiary alcohols were used (Table 3-4, entries 1-4). *t*BuOH was found to be the best solvent in terms of both reactivity and stereoselectivity. Nitroacrylate **7** was converted nearly quantitatively to the 1,4-addition product **9**, which was obtained with a diastereoselectivity of 6:1 and an enantioselectivity of 94% ee within 48 h (Table 3-4, entry 4). In less polar solvents such as CH_2Cl_2, $CHCl_3$, toluene, ethyl acetate or THF, product **9** was formed with similar stereoselectivity, but the conversion was low (Table 3-4, entries 5-9). The use of polar aprotic solvents, for example acetonitrile, DMF or DMSO, resulted in poor reactivity and selectivity (Table 3-4, entries 10-12).

3.4.2 Effect of a C-terminal (*R*)- or (*S*)-Configured Stereocenter in the Catalyst H-D-Pro-Pro-NH-CH(Ph)CH_2-4-Me-C_6H_4

As mentioned above, catalyst **10** is a 1:1 mixture of the two diastereoisomers H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) and H-D-Pro-Pro-NH-(*S*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-S**), since it was synthesized from racemic 1-phenyl-2-(*p*-tolyl) ethylamine. Evaluation of the catalytic performance of the individual stereoisomers **10-R** and **10-S** in *t*BuOH showed that their reactivities and in particular stereoselectivities differ only slightly from those of the mixture (Table 3-5, entry 1-3).

Table 3-5 Comparison of the catalytic performance of H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) with its diastereoisomers **10-R** and **10-S** in the reaction of butanal to nitroacrylate **7**.



	catalyst[a]	conv. [%][b]	d.r.[b]	ee [%][c]
1	H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**)	96	6.0:1	94
2	H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**)	98	5.6:1	95
3	H-D-Pro-Pro-NH-(*S*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-S**)	86	6.0:1	93

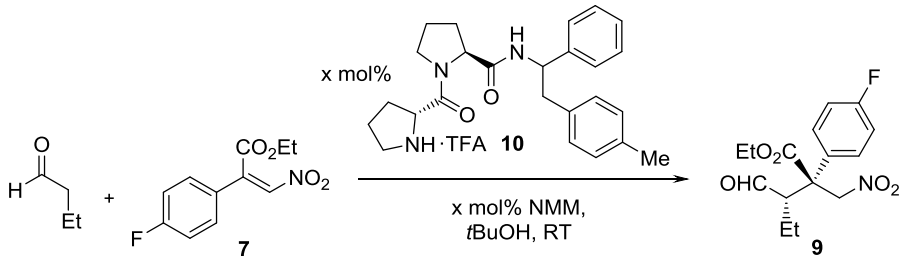
[a] The peptidic catalysts were used as the TFA salts. [b] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [c] Determined by chiral-phase HPLC analysis.

Since the enantiomerically pure amines needed for the synthesis of **10-R** and **10-S** are less readily available than the racemic mixture, all further studies described in Chapter 3 were performed with catalyst **10**.

3.4.3 Catalyst Loading, Substrate Ratio, Concentration and Temperature Influence

Next, we tested the influence of the catalyst loading, the ratio between the aldehyde and the nitroolefin, the concentration of the reaction mixture as well as the temperature on the outcome of the reaction (Table 3-6). When the reaction between butanal and nitroacrylate **7** was performed with less than 10 mol% of catalyst **10**, a significant drop in reactivity

Table 3-6 Variation of the reaction parameters and their influence on the 1,4-addition reaction of butanal to (*Z*)-ethyl 2-(4-fluoro-phenyl)-3-nitroacrylate (**7**).



	cat. [mol%][a]	aldehyde [equiv.]	nitroolefin [equiv.]	conc. [mol/L][b]	time [h]	conv. [%][c]	d.r.[c]	ee [%][d]
1	10	2	1	0.4	48	96	6.0:1	94
2	5	2	1	0.4	96	90	6.0:1	n.d.[e]
3	2	2	1	0.4	240	92	6.5:1	95
4	10	5	1	0.4	48	96	6.3:1	93
5	10	1	1	0.4	48	88	6.4:1	n.d.[e]
6	10	1	1.5	0.4	48	57	6.5:1	n.d.[e]
7	10	2	1	0.6	36	94	5.1:1	93
8	10	2	1	0.2	72	97	5.8:1	94
9	10	2	1	0.1	72	83	6.3:1	95

[a] The equivalent amount of NMM was used. [b] Concentration with respect to nitroacrylate (**7**). [c] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [d] Determined by chiral-phase HPLC analysis. [e] Not determined.

was observed (Table 3-6, entries 1-3). For example, reducing the catalyst loading to 2 mol%, the addition product **9** was still formed in the same stereoselectivity as with 10 mol% of **10** but more than 10 days were necessary to achieve high conversion to product **9** (Table 3-6, entry 3). An excess of butanal proved to be crucial for efficient catalytic performance. Using a twofold excess of the aldehyde with respect to the nitroolefin was found to be optimal (Table 3-6, entry 1). If more than two equivalents of butanal were used, the conversion to addition product **9** and the stereoselectivity were the same, but more *homo*-aldol reaction products were formed (Table 3-6, entry 4). With an equimolar quantity of butanal or even an excess of the nitroolefin, the reaction did not go to completion (Table 3-6, entries 5-6). Performing the reaction at a higher concentration than 0.4 M resulted in a faster reaction, whereas more diluted reactions were, as expected, slower but no significant change in stereoselectivity was noted (Table 3-6, entries 7-9). Finally, the influence of the temperature was also investigated. Since the temperature is an important parameter for both reactivity as well as stereoselectivity, we hoped to improve the latter at lower temperatures. Unfortunately, the optimal reaction solvent *t*BuOH has a melting point of 25°C and thus temperature effects on the reaction outcome could not be studied in this media. Therefore, mixtures of chloroform and *t*BuOH were chosen to perform the reactions at lower temperatures. However, already at 5°C, reaction times of several days were necessary to achieve full conversion to addition product **9** and the stereoselectivity was not significantly improved.

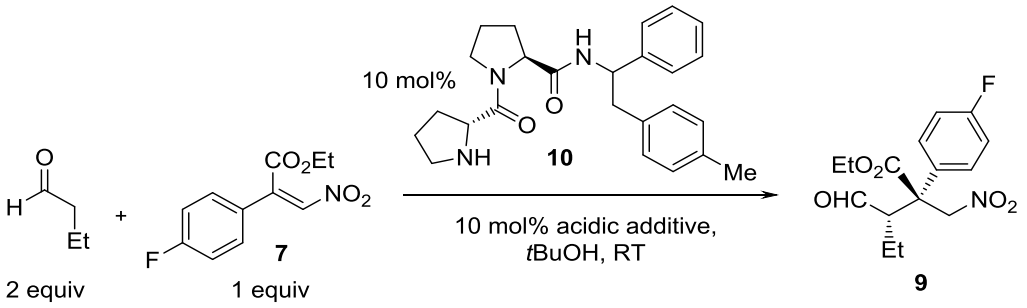
In conclusion, best results in terms of reactivity and stereoselectivity were achieved using 2 equivalents of butanal and 1 equivalent of nitroacrylate **7** in the presence of 10 mol% of **10** in *t*BuOH at room temperature and at a concentration of 0.4 M with respect to nitroacrylate **7** (Table 3-6, entry 1).

3.4.4 Effect of Additives

In the last step of the optimization of the reaction conditions we evaluated the effects of additives on the catalytic performance of the peptidic catalyst H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**). Previous studies on the mechanism of 1,4-addition reactions between aldehydes and β-monosubstituted nitroolefins had shown that catalysts bearing an appropriately positioned proton donor do not require any additives for high

reactivity and stereoselectivity, whereas an acidic co-catalyst is critical for catalysts lacking an intramolecular proton donor.[172,177-178] In fact, a direct correlation between the acidity of the additive and the reaction rate was observed in those experiments. Thus, for catalyst H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**), also lacking an intramolecular proton donor, the presence of an additional acid was expected to be important. In order to test the influence of acidic additives on the catalytic performance of catalyst **10**, first the TFA was removed from the peptide **10**·TFA salt by extraction with an aqueous sodium bicarbonate solution and dichloromethane. The resulting desalted peptide **10** was then studied for its catalytic efficiency in the reaction between butanal and nitroacrylate **7** in the presence of additives with different pK_a values (Table 3-7). A control experiment showed that when the desalted catalyst **10** was used in the presence of TFA and NMM, the same results were obtained as with the TFA salt of the catalyst and the equimolar amount of NMM (Table 3-7, entry 1). In contrast, performing the reaction with the desalted catalyst **10** in absence of any additive dramatically decreased the reaction rate (Table 3-7, entry 2). Additionally, the stereoselectivity of the reaction with the desalted catalyst (2.4:1 d.r. and 92% ee) was somewhat lower than the one obtained by using the catalyst in presence of TFA and NMM (6.2:1 d.r. and 94% ee, Table 3-7, entries 1-2). Performing the reaction in the presence of 10 mol% 4-nitrophenol (pK_a = 7.2), which was found to be a good co-catalyst in studies with β-monosubstituted nitroolefins,[172,178] did not result in a significant acceleration of the reaction with β,β-disubstituted nitroolefin **7** (Table 3-7, entry 3). With acetic acid (pK_a = 4.8) as additive, a faster reaction was observed, but still several days were required to achieve high conversion (Table 3-7, entry 4). The fastest reactions were observed with acids having pK_a values of ~ 3. In the presence of 4-nitrobenzoic acid (pK_a = 3.4), chloroacetic acid pK_a = 2.9) or 2,4-dichlorobenzoic acid (pK_a = 2.7), addition product **9** was formed with nearly full conversion reached within 24 h (Table 3-7, entries 5-7). However, the diastereoselectivity of these reactions was considerably lower than in the case of using TFA together with NMM as additives. Larger amounts of *homo*-aldol reaction products were also formed. The use of additives with higher acidities (pK_a values lower than 2) such as dichloroacetic acid (pK_a = 1.3) and TFA (pK_a = 0.2) led to dramatically slower reactions (Table 3-7, entries 9-10).

Table 3-7 Influence of acidic additives on the 1,4-addition reaction between butanal and (*Z*)-ethyl 2-(4-fluoro-phenyl)-3-nitroacrylate (**7**) in the presence of peptide **10**.



	acidic additive	pK_a in H_2O	time [h]	conversion [%][a]	d.r.[a]	ee [%][b]
1	TFA + NMM[c]	-	48	95	6.2:1	94
2	-	-	240	90	2.4:1	92
3	4-NO_2-C_6H_4-OH	7.2	96	24	2.0:1	90
4	AcOH	4.8	96	85	2.2:1	92
5	4-NO_2-C_6H_4-CO_2H	3.4	24	93	2.9:1	93
6	$ClCH_2CO_2H$	2.9	24	95	3.4:1	94
7	2,4-Cl_2-$C_6H_4CO_2H$	2.7	24	95	2.5:1	93
8	2-NO_2-C_6H_4-CO_2H	2.2	24	88	2.5:1	94
9	Cl_2CHCO_2H	1.3	48	70	5.3:1	94
10	TFA	0.2	120	22	8.8:1	93

[a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis. [c] 10 mol% TFA and 10 mol% NMM were used.

In summary, the results of the additive screening clearly showed that an acidic additive is important for an efficient reaction. The best results with respect to reactivity and stereoselectivity were obtained with TFA as an additive in combination with the equivalent amount of NMM. In comparison to reactions with β-monosubstituted nitroolefins, additives with lower pK_a values were better suited for the reaction with β,β-disubstituted nitroolefins under the optimal reaction conditions (see Chapter 4.2.1 for a more detailed discussion).[172,177-178] The outcome of the screening also suggests a similar reaction pathway for β,β-disubstituted nitroolefins as for their β-monosubstituted counterparts when using catalysts without a proton donor. In that reaction pathway, the rate-determining step is the protonation of the iminium nitronate intermediate, which

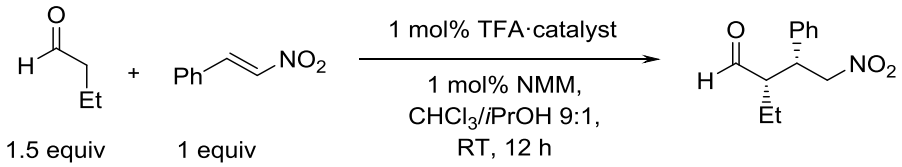
forms by addition of the enamine to the nitroolefin (see the blue pathway of the catalytic cycle in Scheme 4-1, Chapter 4.1). Weakly acidic additives facilitate the protonation of the iminium nitronate and therefore accelerate the reaction, whereas stronger acids also protonate the catalyst as well as destabilize the enamine, and thereby slowing down the reaction.[172,177-178] More detailed mechanistic studies to gain insight into the reaction pathway with β,β-disubstituted nitroolefins are described in Chapter 4.

Based on the results of the optimization, we defined 10 mol% of the TFA salt of peptidic catalyst **10** in combination with 10 mol% of NMM, 1 equivalent of nitroacrylate **7** and 2 equivalents of butanal in tBuOH at room temperature with a 0.4 M concentration of the reaction mixture with respect to nitroacrylate **7** as the optimal conditions to achieve highest conversions and stereoselectivities.

3.5 Substrate Specificity

With peptide H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) identified as an efficient catalyst for the 1,4-addition reaction of butanal to β,β-disubstituted nitroolefin **7**, we were curious to see how the peptidic catalyst **10** would perform in the conjugate addition reaction with the β-monosubstituted counterparts. We therefore reacted butanal and *trans*-β-nitrostyrene in the presence of **10** and compared its performance with that of H-D-Pro-Pro-Glu-NH_2 (**1**), which is the best catalyst for this reaction (Table 3-8). Under the conditions optimized for the addition of aldehydes to β-monosubstituted nitroolefins, catalyst **10** performed significantly poorer than **1** (Table 3-8, entries 1 and 2). Whereas quantitative conversion to the γ-nitroaldehyde and excellent stereoselectivity were achieved in presence of 1 mol% of peptide **1**, 2 mol% of peptidic catalyst **10** were required for high conversion and only moderate stereoselectivity was observed. In fact, poorer performance of **10** was expected, as careful studies revealed that a C-terminal primary amide and an appropriately positioned carboxylic acid within the peptidic catalyst structure are crucial for the catalysts efficiency in the addition of aldehydes to β-monosubstituted nitroolefins.[166]

Table 3-8 Comparison of H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) with H-D-Pro-Pro-Glu-NH_2 (**1**) in the 1,4-addition reaction between butanal and nitrostyrene.

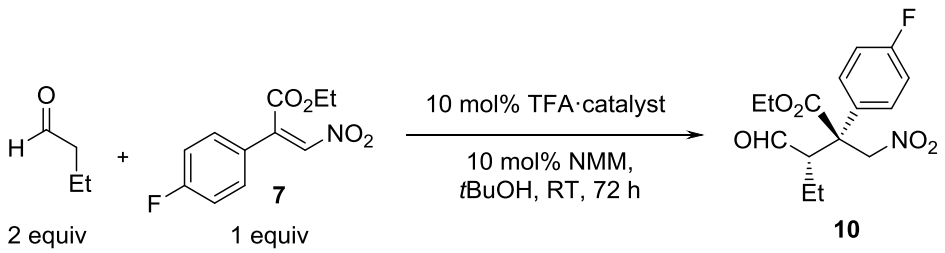


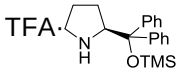
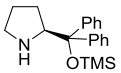
	catalyst	conversion [%][a]	d.r.[a]	ee [%][b]
1	TFA·H-D-Pro-Pro-Glu-NH_2 (**1**)	quant.	50:1	97
2	TFA·H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**)[c]	>95	10:1	73

[a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis. [c] 2 mol% TFA·H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) and 2 mol% NMM were used.

Next, we compared peptide H-D-Pro-Pro-Glu-NH_2 (**1**) and the Hayashi-Jørgensen catalyst with H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) in the reaction of butanal to β,β-disubstituted nitroolefin **7** using the previously optimized conditions (Table 3-9). Peptide **1** which is an excellent catalyst for conjugate additions with β-monosubstituted nitroolefins, led only to moderate stereoselectivity and conversion of β,β-disubstituted nitroolefin **7** (Table 3-9, entry 2). Interestingly, the Hayashi-Jørgensen catalyst did not perform better than peptide **1** in terms of conversion or diasteroselectivity. It did however allow to achieve a good level of enantioselectivity in the product (Table 3-9, entry 4). In summary, the substrate-catalyst compatibility studies showed that the different properties of β-monosubstituted and β,β-disubstituted nitroolefins can be optimally addressed by two similar yet sufficiently structurally different peptidic catalysts.

Table 3-9 Comparison of H-D-Pro-Pro-Glu-NH_2 (**1**) and α,α-diphenylprolinol trimethylsilyl ether with H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) in the 1,4-addition reaction between butanal and (*Z*)-ethyl 2-(4-fluoro-phenyl)-3-nitroacrylate (**7**).



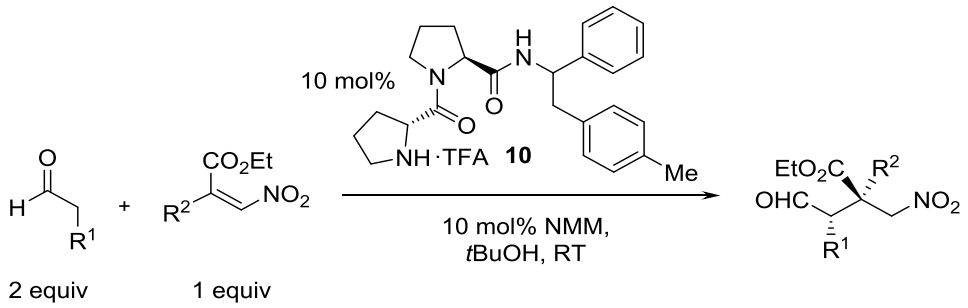
	catalyst	conversion [%][a]	d.r.[a]	ee [%][b]
1	TFA·H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**)	quant.	6.0:1	94
2	TFA·H-D-Pro-Pro-Glu-NH_2 (**1**)	71	3.1:1	84
3		50	1.7:1	84[c]
4[d]		17	3.8:1	92[c]

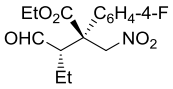
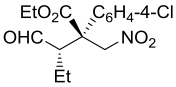
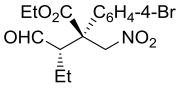
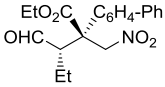
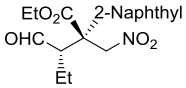
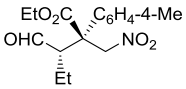
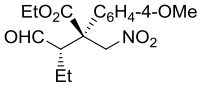
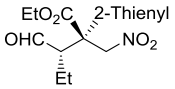
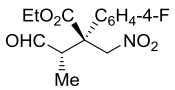
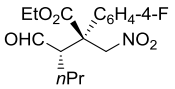
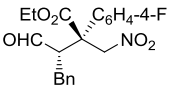
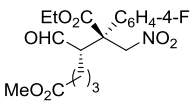
[a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis. [c] The opposite enantiomer was formed. [d] Reaction performed in toluene with 10 mol% α,α-diphenylprolinol trimethylsilyl ether and 10 mol% 4-nitrophenol - conditions optimized for β-monosubstituted nitroolefins.[178]

3.6 Substrate Scope

With the optimized reaction conditions defined, we next explored the scope of the peptide-catalyzed conjugate addition reaction and allowed a range of different aldehyde and β,β-disubstituted nitroolefin combinations to react with each other in the presence of peptidic catalyst **10** (Table 3-10). Variations in the aldehyde as well as in the nitroolefin were well tolerated. The desired γ-nitroaldehydes bearing a quaternary stereogenic center adjacent to a tertiary stereocenter were obtained in good yields and stereoselectivities. The best results with respect to the diastereoselectivity and enantioselectivity were achieved when electron-poor aromatic β,β-disubstituted nitroolefins were used (*e.g.*, Table 3-10, entry 11, 10:1 d.r. and 97% ee). However, good product yields and stereoselectivities were also obtained with nitroacrylates bearing electron-rich aromatic substituents (*e.g.*, Table 3-10, entries 7 and 8).

Table 3-10 Scope of the 1,4-addition reaction between aldehydes and β,β-disubstituted nitroolefins in the presence of peptide **10**.



	product	time [h]	yield [%][a]	d.r.[b]	ee [%][c]
1		48	82	6.0:1	94
2		48	85	5.0:1	94
3		48	84	5.5:1	94
4		72	85	5.5:1	96
5		96	83	6.5:1	91
6		72	90	6.5:1	94
7		96	72	6.0:1	94
8		48	88	5.5:1	89
9		48	83	3.0:1	95
10		60	85	6.5:1	95
11		60	87	10.0:1	97
12		72	85	4.5:1	91

[a] Yields correspond to γ-nitroaldehydes isolated as a mixture of stereoisomers. [b] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [c] Determined by chiral-phase HPLC analysis.

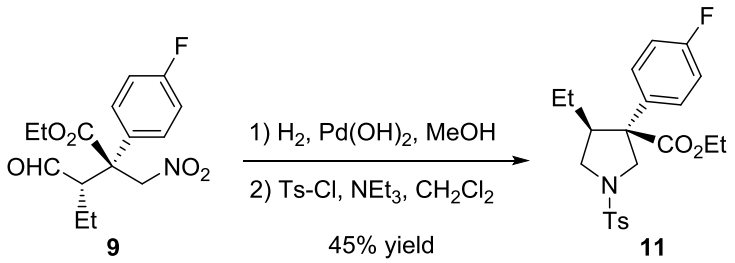
In addition to nitroacrylate **7**, also the analogue with the *tert*-butyl ester was reacted with butanal, but only 50% conversion was observed after 72 h and the corresponding addition product was obtained in 4:1 diastereoselectivity. Reactions with nitroacrylate **7** and homologues of butanal such as propanal and pentanal afforded the desired products in comparable results, except that the use of propanal resulted in a lower diastereoselectivity (Table 3-10, entries 9 and 10). Furthermore, aldehydes bearing functional groups such as esters also reacted readily with the β,β-disubstituted nitroolefins providing ester-functionalized γ-nitroaldehydes (*e.g.*, Table 3-10, entry 12).

Current limitations were found to be aliphatic nitroacrylates and β,β-disubstituted nitroolefins lacking an additional electron-withdrawing group. For example, no reaction to the desired 1,4-addition product was observed with (Z)-ethyl 2-cyclohexyl-3-nitroacrylate. Instead, this aliphatic nitroacrylate isomerized under the reaction conditions to ethyl 2-cyclohexylidene-3-nitropropanoate, as described earlier in Chapter 3.2 for *trans*-α-methyl-β-nitrostyrene (**2**). Also, the more sterically hindered α- or β-branched aldehydes did not react or only in trace amounts to the desired products.

3.7 Derivatization of the 1,4-Addition Products and Determination of the Relative and Absolute Configuration

The straightforward transformation of γ-nitroaldehydes into a variety of interesting compounds renders these molecules valuable synthetic intermediates. We therefore next explored the synthetic versatility of the 1,4-addition products for the preparation of γ-amino acids as well as heterocyclic compounds with quaternary stereocenters that might be valuable for the synthesis of therapeutically active compounds.[179] Furthermore, these studies also allowed for the unambiguous determination of the relative and absolute configuration of the major stereoisomer formed in the peptide-catalyzed addition reactions.

Chiral pyrrolidines are interesting structural units found in many biologically active substances as well as in organocatalysts.[180] We were therefore pleased to see that the addition products from the conjugate addition reaction between aldehydes and β,β-disubstituted nitroolefins were easily transformed into *N*-tosylated pyrrolidines bearing a quaternary stereocenter. For example, addition product **9** was converted to the corresponding *N*-tosylated pyrrolidine **11** following the same procedure as reported by Barbas for α,β-disubstituted γ-nitroaldehydes.[181] Using palladium hydroxide on carbon in a hydrogen atmosphere followed by tosylation, afforded the desired pyrrolidine **11** in 45% yield (Scheme 3-4).



Scheme 3-4 Conversion of 1,4-addition product **9** to the corresponding pyrrolidine **11** bearing a quaternary stereocenter.

NMR spectroscopic analyses including NOE spectroscopy of the major diastereoisomer of **11** supported, as expected from the related reactions with β- and α,β-substituted nitroolefins, the relative *syn* configuration of the ethyl group and the aromatic moiety within the major diastereoisomer of the addition product **9** (*anti* configuration within pyrrolidine **11**, Figure 3-2).

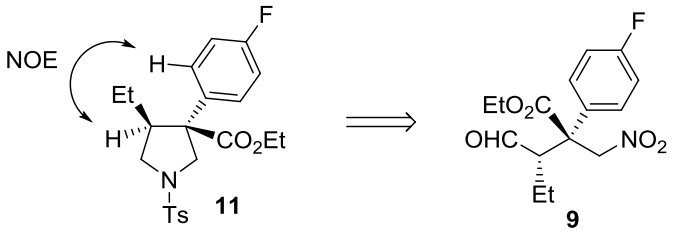
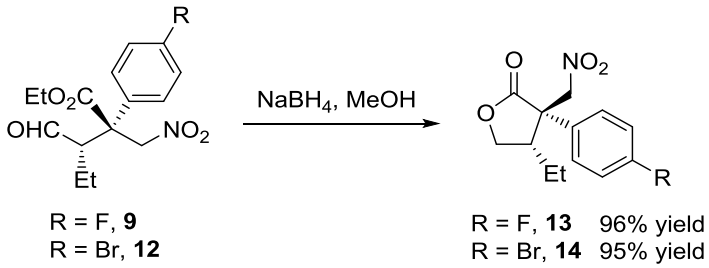


Figure 3-2 Determination of the relative configuration of 1,4-addition product **9** by NOE spectroscopy of pyrrolidine **11**.

In addition to pyrrolidines, γ-butyrolactones are other common structural motifs present in many biologically active compounds and natural products that proved to be easily accessible from the conjugate addition products with quaternary stereogenic centers. Reduction of the aldehyde moiety within γ-nitroaldehydes **9** and **12** using sodium borohydride yielded after spontaneous intramolecular cyclization of the initially formed hydroxyesters, the crystalline γ-butyrolactones **13** and **14** in ≥95% yields (Scheme 3-5).



Scheme 3-5 Conversion of the addition products **9** and **12** to the corresponding γ-butyrolactones **13** and **14** bearing a quaternary stereocenter.

Bromo-substituted lactone **14** allowed for determining the absolute configuration of the conjugate addition products formed from aldehydes and the β,β-disubstituted nitroolefins. Crystals suitable for X-ray crystal structure analysis of **14** were obtained from a saturated solution of diethyl ether and pentane. The crystal structure confirmed the relative configuration and allowed to determine unambiguously the absolute configuration of both stereogenic centers within the parent γ-nitroaldehyde **12** to be (2*S*,3*S*) (Figure 3-3).

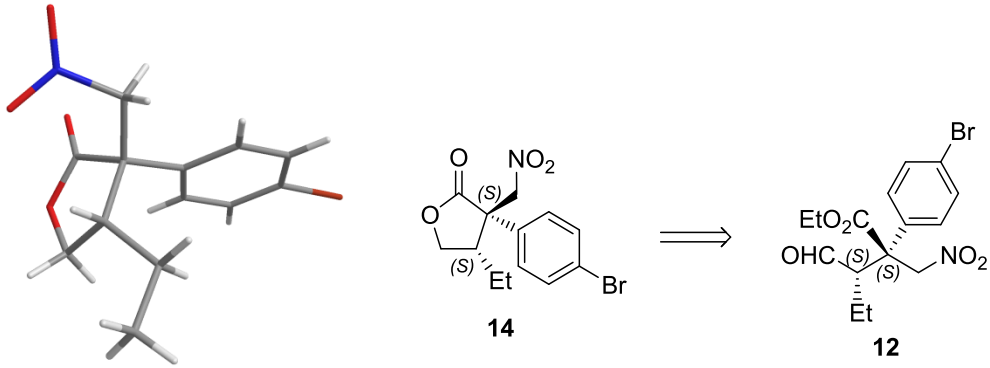
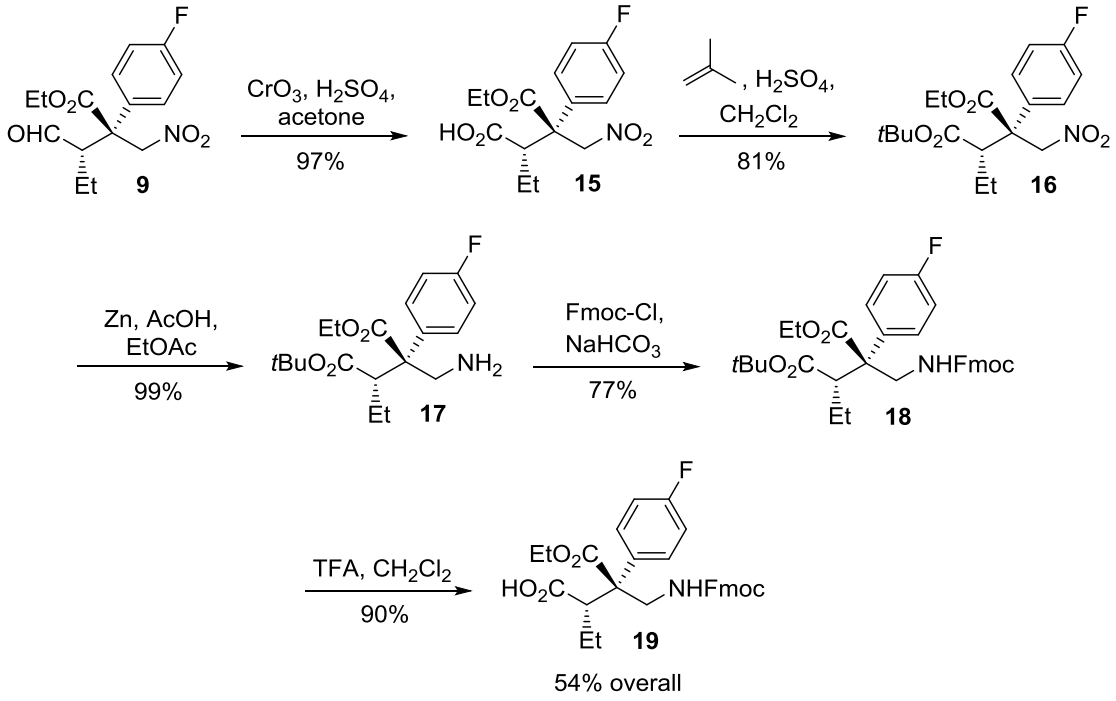


Figure 3-3 Determination of the absolute configuration of 1,4-addition product **12** by crystal structure analysis of γ-butyrolactone **14**.

Aside from the pyrrolidines and lactones, we also prepared a protected 2,3,3-trisubstituted γ-amino acid. Such amino acids with a quaternary stereogenic center next to a tertiary stereocenter have to our knowledge not been prepared to date and might not only be interesting for the development of therapeutics but also for foldamer research.[99,179,182-183] Towards this goal, γ-nitroaldehyde **9** was transformed to the corresponding Fmoc-protected $\gamma^{2,3,3}$-amino acid in five steps (Scheme 3-6).



Scheme 3-6 Synthesis of Fmoc-protected $\gamma^{2,3,3}$-amino acid **19**.

γ-Nitroaldehyde **9** was oxidized by Jones reagent to afford γ-nitrocarboxylic acid **15** in 97% yield. Reduction of the nitro group of carboxylic acid **15** in presence of palladium hydroxide or Raney nickel as catalysts provided the desired γ-amino acid but also a significant amount of the corresponding γ-butyrolactam. Therefore γ-nitrocarboxylic acid **15** was first transformed to the corresponding *tert*-butyl ester **16** which was then reduced to γ-amino ester **17**. A good yield of 81% was obtained for the esterification of carboxylic acid **15** with isobutylene and a catalytic amount of concentrated sulfuric acid. In contrast, Fischer esterification with *tert*-butyl acetate and aqueous perchloric acid or Steglich esterification with *tert*-butanol were less successful. When the reduction of the nitro

group of **16** was first attempted with palladium hydroxide in a hydrogen atmosphere, only partial conversion to γ-amino ester **17** was observed. Pleasingly, the use of zinc and acetic acid provided smoothly γ-amino ester **17** in quantitative yield. Finally, Fmoc-protection of γ-amino ester **17** followed by cleavage of the *tert*-butyl ester of fully protected amino acid **18** afforded the desired Fmoc-protected $\gamma^{2,3,3}$-amino acid **19** in an overall yield of 54% (Scheme 3-6).

3.8 Conclusions

In summary, we have developed the peptide H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) as a powerful catalyst for conjugate addition reactions of aldehydes to β,β-disubstituted nitroolefins to provide under mild organocatalytic conditions synthetically valuable γ-nitroaldehydes bearing a quaternary stereogenic center adjacent to a tertiary stereocenter. In the presence of 10 mol% of peptide **10** as its TFA salt in combination with 10 mol% NMM, a range of different aldehydes and electron-poor as well as electron-rich sterically demanding β,β-disubstituted nitroolefins reacted with high chemoselectivity to afford the desired γ-nitroaldehydes in high yields and stereoselectivities (up to 90% yield, 10:1 d.r. and 97% ee). Furthermore, the γ-nitroaldehydes were readily converted into γ-butyrolactones, chiral pyrrolidines and a γ-amino acid with a quaternary stereogenic center that have so far not been prepared.

Our results also demonstrate that the modularity of tripeptides of the class Pro-Pro-Xaa offers the possibility to adapt to the structural requirements of challenging substrates such as β,β-disubstituted nitroolefins. For example, while tripeptide H-D-Pro-Pro-Glu-NH_2 (**1**) is a highly active and selective catalyst for reactions with β-monosubstituted nitroolefins, a different catalyst had to be identified for the successful reaction with β,β-disubstituted nitroolefins that are significantly less inclined to form the corresponding conjugate addition products with aldehydes. H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) proved to be such a potent peptidic catalyst for these sterically congested substrates and shows high chemoselectivity for conjugate additions over competing *homo*-aldol reactions of the aldehyde. Interestingly, this optimal peptide is lacking a carboxylic acid

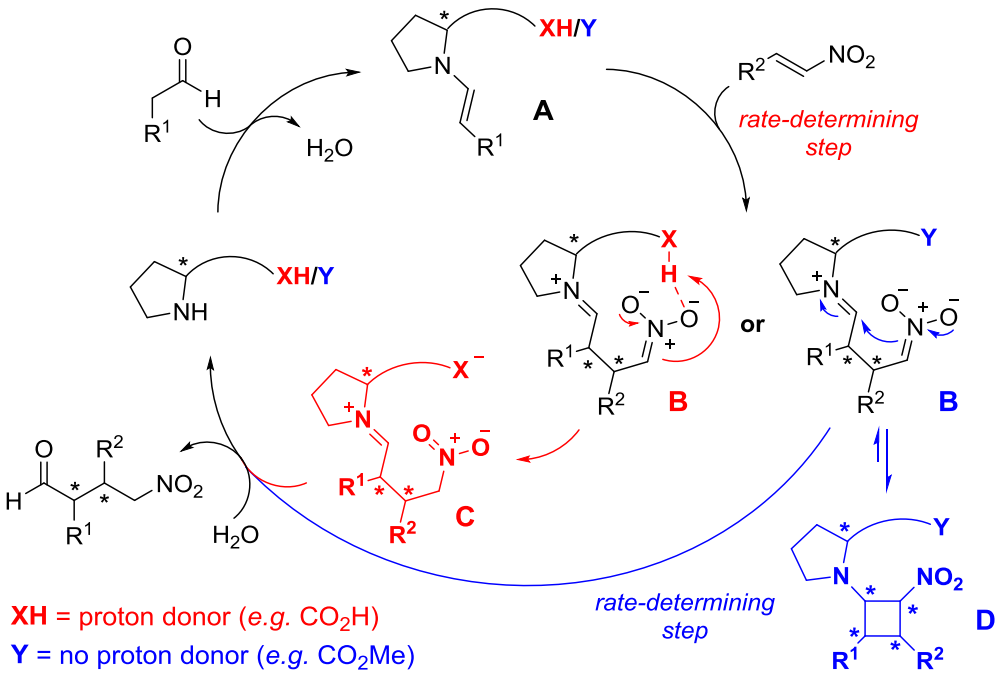
group at the C-terminal end. In our previous studies with β-mono- as well as α,β-disubstituted nitroolefins, peptides bearing a carboxylic acid have proven to be the best in terms of catalytic activity and stereoselectivtiy.[166,169] This suggests that for reactions with β,β-disubstituted nitroolefins, an interaction between the nitronate intermediate and the carboxylic acid group of the catalyst (see Scheme 1-16 for such an interaction of peptide **1** in reactions with β-monosubstituted nitroolefins) is obviously not as critical for the reaction rate and stereoselectivity and might be compensated by optimal steric dispositions within the catalyst. In addition, the lack of a proton donor within peptide **10** combined with the results of the acidic additives screening suggest a similar reaction pathway for conjugate addition reactions with β,β-disubstituted nitroolefins as previously reported for their monosubstituted analogues in the presence of catalysts without a proton donor. Mechanistic investigations into the conjugate addition reaction for β,β-disubstituted nitroolefins using *in situ* FT-IR and NMR spectroscopy are described in the following Chapter 4.

4

Mechanistic Investigations into 1,4-Addition Reactions between Aldehydes and β,β-Disubstituted Nitroolefins

4.1 Background

Our group has shown with kinetic and NMR spectroscopic studies that the pathway of peptide-catalyzed conjugate addition reactions of aldehydes to β-monosubstituted nitroolefins depends on the presence or absence of a suitably positioned carboxylic acid group within the catalyst structure.[172] The alternative pathways have different rate-limiting steps. Based on the experimental observations the catalytic cycle depicted in Scheme 4-1 was proposed.

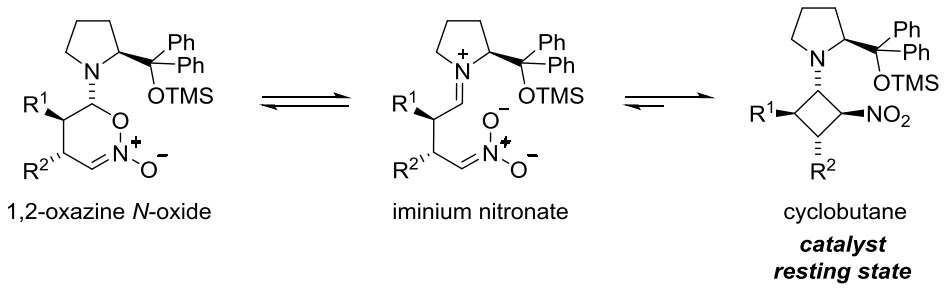


Scheme 4-1 Proposed catalytic cycle for conjugate addition reactions between aldehydes and β-substituted nitroolefins using catalysts with (red) or without (blue) an intramolecular proton donor.[172]

Enamine **A**, formed upon reaction of the catalyst with the aldehyde, reacts with the nitroolefin to form the intermediate nitronate **B**. In the presence of peptides bearing a suitably positioned intramolecular carboxylic acid group, the short-lived iminium nitronate intermediate **B** is first intramolecularly stabilized by the carboxylic acid and then immediately protonated to form iminium ion **C** (Scheme 4-1, red pathway). **C** can then be hydrolyzed with the release of the addition product and recovery of the catalyst to complete the catalytic cycle. In this reaction pathway, the protonation of **B** is fast and

therefore not rate-limiting. Instead, the C-C bond formation between the enamine and the nitroolefin is the rate-limiting step and thus the reaction rate depends on the concentration of the nitroolefin and the enamine. Furthermore, the reaction rate could not be increased by an acidic additive.[170,172] The optimal reaction conditions with catalysts bearing a suitably positioned carboxylic acid group were therefore achieved by using an excess of the nitroolefin rather than the aldehyde, dried solvents and reagents as well as no acidic additives.

On the contrary, with catalysts lacking an acidic moiety within their structure, the iminium nitronate **B** cannot be intramolecularly protonated and thus collapses to the much more stable cyclobutane species **D** which is the resting state of the catalyst (Scheme 4-1, blue pathway). The reopening of cyclobutane **D** and the subsequent intermolecular protonation of iminium nitronate **B** is slow and thus becomes the rate-determining step of the reaction. Therefore, reactions in presence of catalysts lacking an intramolecular proton donor can be accelerated by an external proton source such as the solvent or an acidic additive and they do not depend on the concentration of the substrates.[172] These observations for peptides lacking a proton donor are in agreement with the mechanistic studies on conjugate addition reactions between aldehydes and nitroolefins catalyzed by the Hayashi-Jørgensen catalyst, which were reported independently by the groups of Hayashi and Seebach,[178,184-185] Blackmond,[177,186-187] as well as Pápai and Pihko.[188] In addition to cyclobutane intermediates also 1,2-oxazine *N*-oxides were observed in the reactions with the Hayashi-Jørgensen catalyst. The six-membered species was found to be in equilibrium with the iminium nitronate (Scheme 4-2).



Scheme 4-2 1,2-oxazine *N*-oxides and cyclobutanes: cyclic intermediates observed in the conjugate addition reaction between aldehydes and nitroolefins in the presence of the Hayashi-Jørgensen catalyst.[177-178,184-188]

Since the catalyst H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) for conjugate addition reactions of aldehydes with β,β-disubstituted nitroolefins is lacking an intramolecular proton donor and a significant reaction rate acceleration was observed in combination with acidic additives (see Chapter 3.4.4), we became interested in whether these reactions proceed *via* a similar pathway as the analogous reactions with β-monosubstituted nitroolefins in the presence of catalysts without an intramolecular proton donor. Therefore, we decided to perform mechanistic studies using *in situ* FT-IR as well as NMR spectroscopy and then compare the results with those of the previous studies.[170,172] The results of the mechanistic investigations into the conjugate addition reactions between aldehydes and β,β-disubstituted nitroolefins are presented in the following.

4.2 Mechanistic Investigations with *in situ* IR and NMR Spectroscopy

4.2.1 Effects of Acidic Additives on the Reaction Profiles

We started our investigations by recording the profile of the reaction between butanal and (*Z*)-ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**) catalyzed by the peptide H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) which is a slightly more active catalyst than **10** (see Table 3-5, Chapter 3.4.2). As in our previous studies, we used *in situ* FT-IR spectroscopy as a non-invasive monitoring method.[189-190] The reaction progress was monitored over time by following the N-O-stretching absorbance of the forming product γ-nitroaldehyde **9** at 1563 cm^{-1} (Figure 4-1). At the same time, the consumption of substrate **7** was followed by the decreasing absorption band at 1530 cm^{-1} (N-O-stretching absorbance of **9**). These absorption bands are both undisturbed by other IR-absorbances within the reaction mixture (Figure 4-1). Spectra were collected every 5 or 10 minutes, each time by performing 256 scans, until nitroacrylate **7** was completely consumed (see experimental part, Chapter 7.2.5, Protocol R for further reaction details).

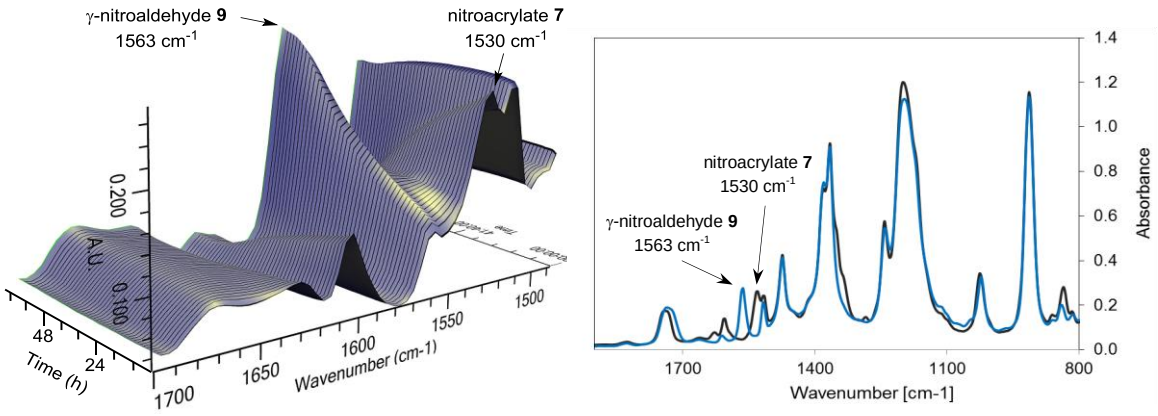


Figure 4-1 Three dimensional stack plot of IR spectra of the reaction between butanal and nitroacrylate **7** in presence of catalyst **10-R** (left). Superimposed IR spectra of nitroacrylate **7** and γ-nitroaldehyde **9** in *t*BuOH (right).

In order to reduce the reaction time, the catalyst loading was increased from 10 to 25 mol%. The reaction was evaluated in *t*BuOH, the optimal solvent for the reaction (see Chapter 3.4.1). In toluene or chloroform/*i*PrOH 9:1, which had been used in previous studies with β-monosubstituted nitroolefins, lower reactivity was observed (further investigations on the reaction in chloroform are described later in Chapter 4.2.3).

In the first experiment, we monitored the reaction only in the presence of desalted peptide **10-R** without an acidic additive. Even with the increased catalyst loading of 25 mol%, around 60 h were necessary for complete consumption of nitroacrylate **7**. Furthermore, it was observed that in the absence of an acidic additive the reaction did not proceed as cleanly as when an acidic co-catalyst was used (see later) and thus the conversion to γ-nitroaldehyde **9** was only around 75% (determined with 1,1,2,2-tetrachloroethane as internal standard by ^{1}H-NMR spectroscopy and by ^{19}F-NMR spectroscopy with respect to **7**). The obtained conversion-time curve had a sigmoidal shape meaning that the product formation is slowest in the beginning and becomes faster as the reaction progresses (Figure 4-2, left). This is in agreement with previous studies and due to the fact that γ-nitroaldehyde **9** is weakly acidic and thus promotes its formation by facilitating the protonation of the corresponding iminium nitronate (analogous to **B** in the catalytic cycle, Scheme 4-1), leading to the observed temporally increasing rate and the sigmoidal shape of the reaction profile.

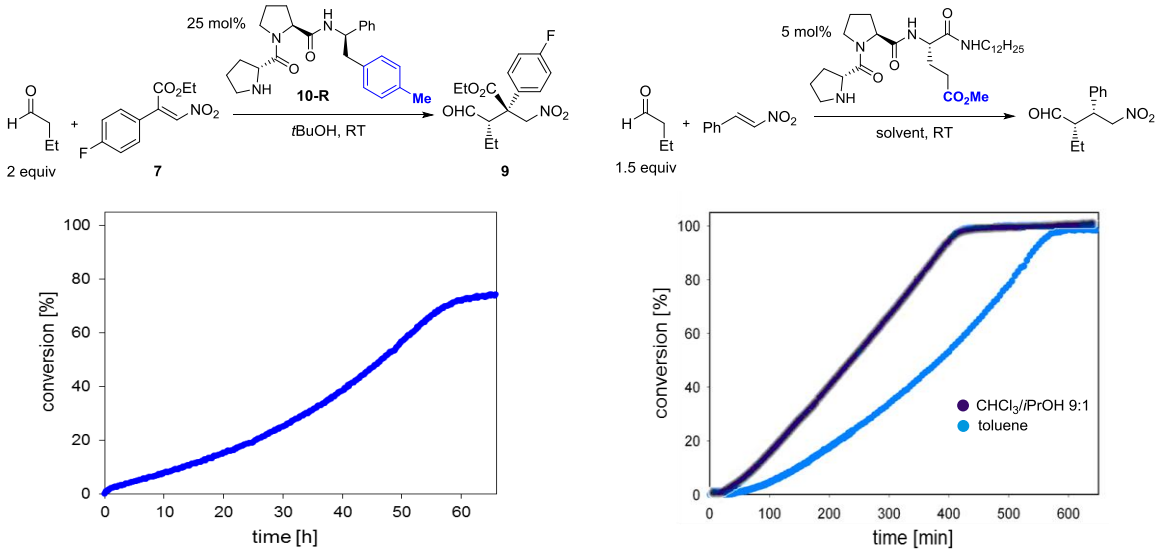


Figure 4-2 Comparison of the conversion-time curves of the conjugate addition reaction with β,β-disubstituted nitroacrylate **7** (left) and with nitrostyrene (right) in the presence of a peptidic catalyst that lacks an intramolecular proton donor.

Such a distinct kinetic profile was also observed previously in our studies of reactions between butanal and nitrostyrene both in the protic polar solvent mixture $CHCl_3$/*i*PrOH 9:1 as well as in the aprotic non-polar solvent toluene in the presence of a catalyst lacking an intramolecular proton donor (Figure 4-2, right).[172] The reaction proceeded significantly faster in the protic environment, however, the sigmoidal shape of the conversion-time curve was more pronounced in toluene. In addition, identical conversion-time curves were obtained for reactions performed at different substrate concentrations proving that the reaction rate for reactions with such a sigmoidal profile is independent of the concentration of the substrates.[172]

To further probe the similarities between the reactions of aldehydes with nitroacrylate **7** and nitrostyrene, we next monitored the reaction between butanal and nitroacrylate **7** using catalyst **10-R** in combination with additives of different acidities. If the protonation of the corresponding iminium nitronate is the rate-limiting step of the reaction, an acidic additive was expected to increase the reaction rate.[172,177-178]

Indeed, in the presence of 25 mol% of the weak acid 4-nitrophenol (pK_a in water = 7.2) as co-catalyst, the reaction was faster than in its absence (Figure 4-3, purple spheres). In addition, more γ-nitroaldehyde **9** was formed (ca. 85%) compared to the reaction without an additive. Interestingly, the conversion-time profile was still sigmoidal, which

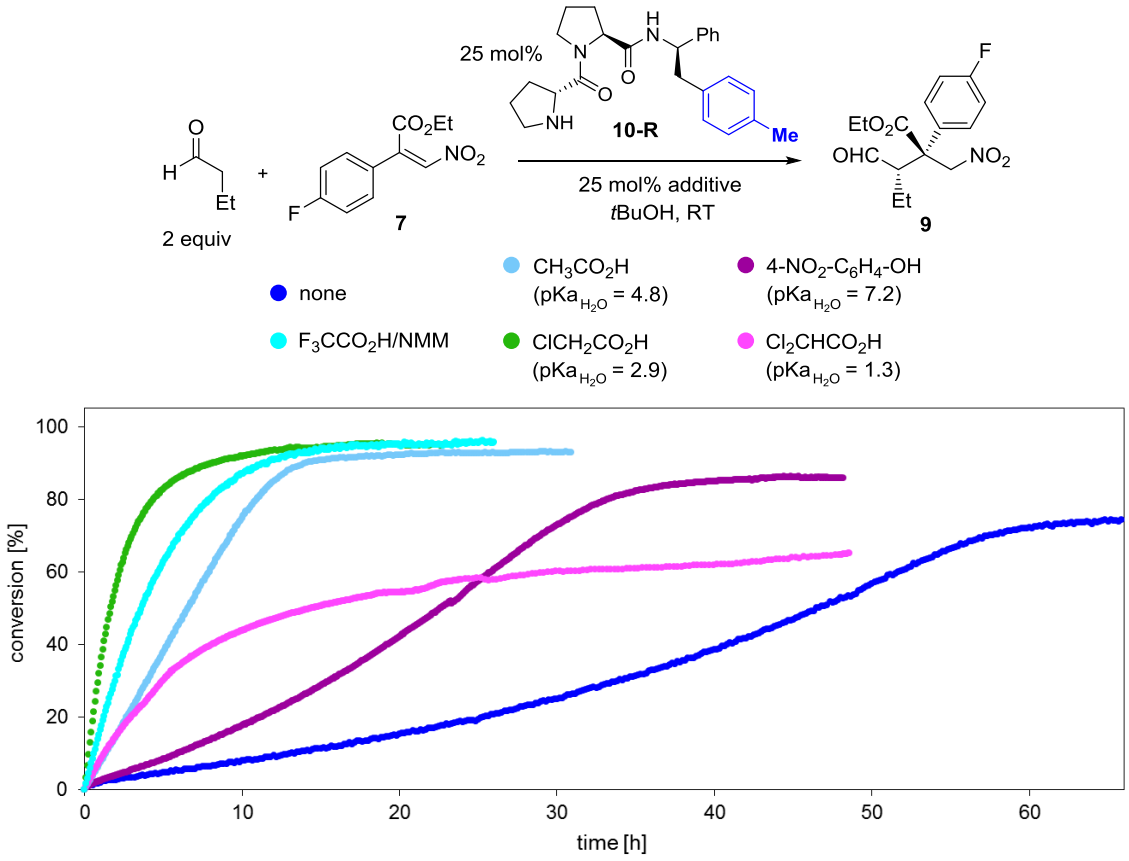
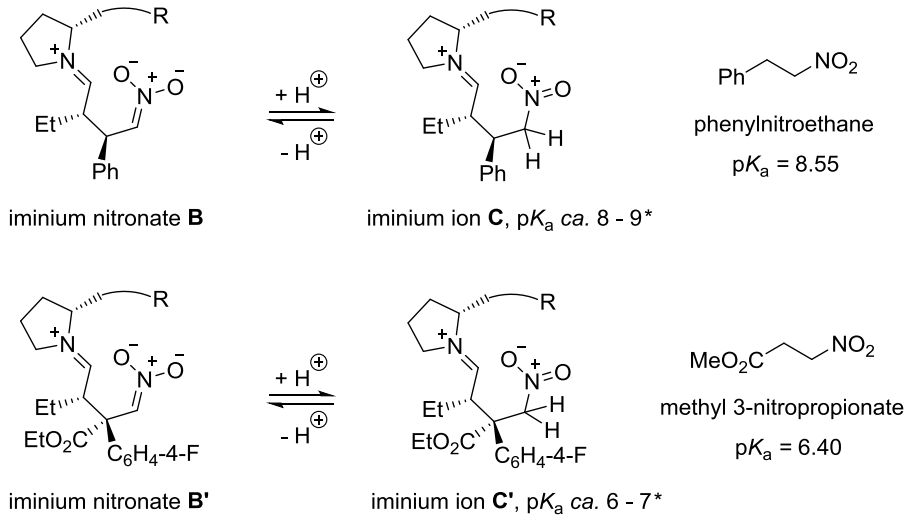


Figure 4-3 Conversion-time curves of the conjugate addition reaction between butanal and nitroacrylate **7** in the presence of peptidic catalyst **10-R** and different acidic additives.

is different to the reaction between butanal and nitrostyrene where a linear profile was observed using 4-nitrophenol as co-catalyst.[172] Furthermore, while the reaction with nitroacrylate **7** was only about 1.5 times faster in the presence of 4-nitrophenol than in its absence, the reaction with nitrostyrene was significantly faster (about 5 times) with this additive than without. This indicates that the iminium nitronates **B** and **B'** have different basicities, nitronate **B'** being a weaker base than **B** due to the additional electron-withdrawing ester substituent at C(3) (Scheme 4-3). Consequently, a stronger acid is better suited to protonate nitronate **B'**, while a weaker acid suffices to facilitate the protonation of nitronate **B**. In fact, due to the pK_a of about 6 – 7 (derived from methyl 3-nitropropionate; Scheme 4-3) of species **C'**, it can be expected that an additive with a pK_a < 6 is optimal for the reaction between butanal and nitroacrylate **7** in order to considerably facilitate the protonation of nitronate **B'** and thus significantly increase the reaction rate. Nevertheless, as can be seen from Figure 4-3 (purple spheres), a rate

acceleration occured also with 4-nitrophenol (pK_a = 7.2) having a pK_a > 6. However, this weakly acidic co-catalyst is expected to be only effective in the beginning of the reaction when its concentration is much higher than the one of the more acidic γ-nitroaldehyde product **9** (pK_a ~ 6 – 7) and therefore only a moderate rate acceleration was observed with 4-nitrophenol. Additionally, since γ-nitroaldehyde **9** is more likely to facilitate the protonation of the nitronate in the course of the reaction than the less acidic 4-nitrophenol, a sigmoidal conversion-time profile was obtained.



*The pK_a values of iminium **C** and **C'** were derived from phenylnitroethane and methyl 3-nitropropionate*

Scheme 4-3 Iminium nitronates **B** and **B'** derived from butanal, nitrostyrene or nitroacrylate **7** and a secondary amine-based catalyst lacking a proton donor and the corresponding protonated species **C** and **C'**. Reference of pK_a values: phenylnitroethane[191], methyl 3-nitropropionate[192]

As hypothesized, a more profound rate acceleration was observed when the reaction was performed in the presence of a co-catalyst with a pK_a < 6 such as acetic acid (pK_a = 4.8). Furthermore, the corresponding reaction profile was now linear (Figure 4-3 and Figure 4-4, light blue spheres; 92% conversion to **9**). This rate acceleration as well as the change in the shape of the conversion-time curve from sigmoidal to linear is in agreement with previous studies on reactions with β-monosubstituted nitroolefins and therefore strongly suggests an analogous pathway where the protonation of the iminium nitronate is the rate-determining step and the cyclobutane (analogous to **D** in the catalytic cycle, Scheme 4-1) is the resting state of the catalyst (blue pathway, Scheme 4-1).

The use of the stronger acid dichloroacetic acid (pK_a = 1.3) led to a significantly slower reaction (Figure 4-3, magenta spheres). The corresponding conversion-time curve was not linear anymore and showed that the reaction is fastest in the beginning and then becomes slower as the substrates are consumed. Such a kinetic profile was also observed for reactions of butanal with nitrostyrene in the presence of a stronger acid and a catalyst lacking a proton donor, and it corresponds to a change of the rate-determining step of the reaction from the protonation of the iminium nitronate to the C-C bond formation between the enamine and the nitroolefin.[172] This can be explained by the fact that stronger acids not only facilitate the protonation of the nitronate, but also protonate the amine catalyst and destabilize the enamine which results in a lower concentration of the enamine. As a consequence, protonation of the iminium nitronate becomes fast and is not rate-limiting anymore whereas, due to lower enamine concentration, the C-C bond formation with the nitroolefin is now slower and thus becomes the rate-limiting step of the reaction. The change of the rate-limiting step is reflected in the distinct curvature of the kinetic profile that is indicative of reactions in which at least one of the reactants is involved in the rate-determining step and demonstrates a decrease in reaction rate with decreasing substrate concentration.

When chloroacetic acid (pK_a = 2.9) with a pK_a value between acetic acid and dichloroacetic acid was used as an additive, the reaction proceeded even faster than in the presence of acetic acid (Figure 4-3 and Figure 4-4, green spheres; 95% conversion to **9**). Interestingly, however, the corresponding conversion-time profile was not linear anymore and showed a distinct curvature, similar to the kinetic profile of the reaction with dichloroacetic acid where the C-C bond formation is expected to be the rate-limiting step.

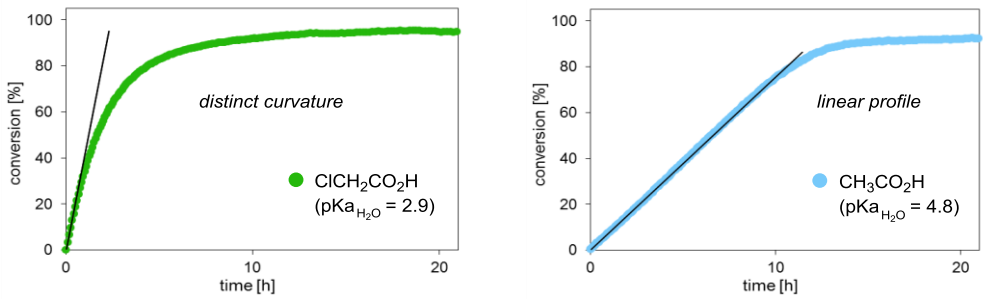


Figure 4-4 Comparison of the conversion-time curves of the conjugate addition between butanal and nitroacrylate **7** in the presence of catalyst **10-R** and chloroacetic acid (right) and acetic acid (left), respectively.

These results indicate that chloroacetic acid with a pK_a value of around 3 has an optimal acidity to further facilitate the protonation of the iminium nitronate. Apparently, the protonation becomes faster than the C-C bond formation between the enamine and the nitroacrylate **7** and thus a conversion-time curve with a distinct curvature is observed. Furthermore, chloroacetic acid obviously does not lower the enamine concentration to such an extent that the C-C bond formation becomes much slower as it is the case when stronger acids are employed as co-catalysts. Therefore, a faster overall reaction is observed using chloroacetic acid as an additive.

The use of chloroacetic acid as co-catalyst was also reported by Hayashi and co-workers for the synthesis of oseltamivir.[193-194] During the course of their synthetic studies they found that chloroacetic acid substantially enhanced the rate of the conjugate addition between 2-(pentan-3-yloxy)acetaldehyde and (*E*)-*tert*-butyl 3-nitroacrylate. This example further demonstrates that more acidic co-catalysts are better suited for conjugate addition reactions with nitroacrylates than for reactions with aromatic and aliphatic nitroolefins due to the lower basicity of the corresponding nitronate (Scheme 4-3).

As described earlier in Chapter 3.4.4, the slow reaction observed in presence of the strong acid TFA (pK_a = 0.2) could be dramatically accelerated when an equivalent amount of NMM was added, because the base is necessary to liberate the secondary amine of the catalyst and allow it to participate in catalysis (Figure 4-3, cyan spheres). In addition, γ-nitroaldehyde **9** was obtained in nearly quantitative conversion (≥ 95%) in these conditions. The shape of the conversion-time curve for the reaction with TFA in combination with NMM was not linear and thus suggests that the C-C bond formation is the rate-limiting step.

In order to further probe whether the rate-limiting step of the reactions in presence of acidic additives can be derived from the shape of their conversion-time curves, NMR experiments were performed to confirm the presence or absence of a cyclobutane intermediate. Moreover, the NMR studies were also used to verify if the enamine, the first crucial species in the catalytic cycle of conjugate addition reactions with nitroacrylate **7**, could be detected. The results of these studies are described in the following Chapter 4.2.2.

4.2.2 Investigations of Enamine and Cyclobutane Intermediates in Protic Solvents

4.2.2.1 Stoichiometric Reactions

As described above, the rate acceleration and the change in the kinetic profile from sigmoidal (in absence of an acidic additive) to linear (in presence of acetic acid) of the reaction between butanal and nitroacrylate **7** suggest that the reaction pathway involves the formation of a cyclobutane intermediate as the resting state of the catalyst.

To evaluate this hypothesis, we investigated the composition of the reaction mixture using NMR spectroscopy with the aim of identifying the resting state of the catalyst. In order to achieve a high concentration of the expected cyclobutane intermediate, in the first experiment stoichiometric amounts of butanal and the peptidic catalyst **10-R** were mixed in *i*PrOD-d_8, which is similar to *t*BuOH, the optimal solvent for the studied addition reactions (Figure 4-5). Analysis of the reaction mixture by ^{1}H-NMR spectroscopy revealed the presence of a new species which was expected to be the corresponding enamine. Unfortunately, the deuterated protic solvent used for the experiment led to a fast and quantitative H/D-exchange of exchangeable protons in the reagents which made unambiguous identification of the species very complicated.

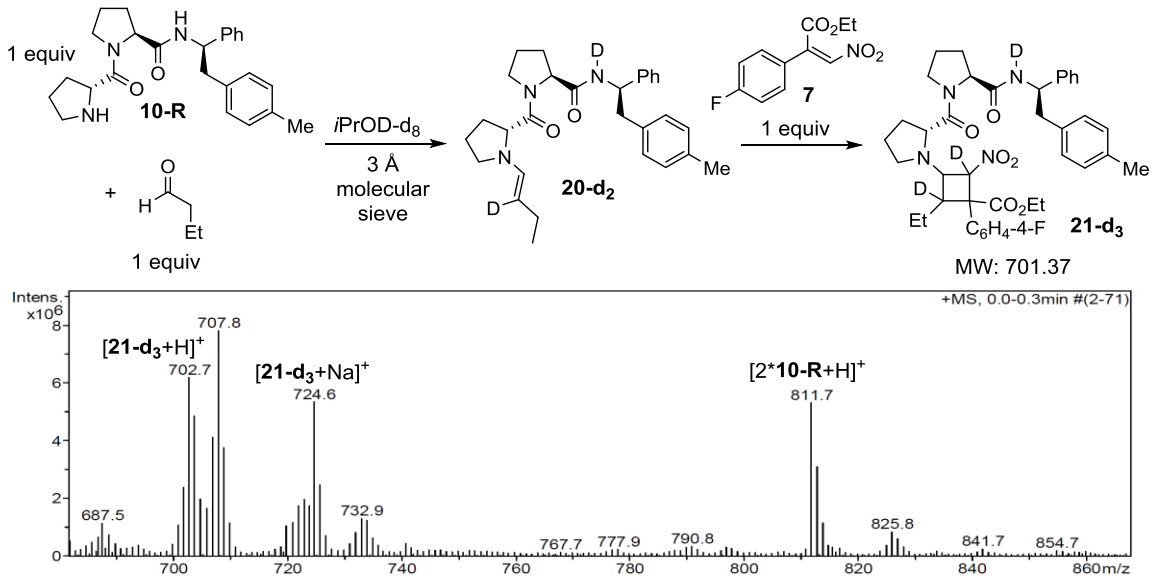


Figure 4-5 Formation of the expected deuterated cyclobutane species **21-d_3** in *i*PrOD-d_8 (top). ESI-MS spectrum of the reaction mixture with the peaks corresponding to the mass of the possible **21-d_3** and the sodium adduct (bottom).

When one equivalent of nitroacrylate **7** was added to the mixture the signals of the assumed deuterated enamine **20-d$_2$** disappeared within few minutes and a new species was formed (about 40% with respect to nitroacrylate **7**). It was however unclear whether this was the corresponding cyclobutane species and thus a small aliquot of the NMR sample was diluted with dry *i*PrOH and analyzed by ESI-MS. The major detected peaks corresponded to the catalyst **10-R**, but signals matching the mass of a cyclobutane species **21-d$_3$**, bearing three deuteriums within the structure, as well as its sodium adduct were also observed (Figure 4-5).

Due to the incorporation of deuterium into the observed species, their characterization by NMR spectroscopy proved to be difficult. Therefore, the above experiment was repeated using non-deuterated *i*PrOH instead of *i*PrOD-d$_8$ under otherwise identical conditions. Inspection of the reaction mixture by ^{1}H-NMR spectroscopy after dilution with *i*PrOD-d$_8$, revealed the presence of signals corresponding to the protons of the cyclobutane ring. However, due to superimposition by the intense signals of *i*PrOH present in the sample and rather low intensities of the cyclobutane signals, a detailed assignment was again not possible. A mass spectrum of the mixture of butanal and **10-R** in *i*PrOH showed that the main peak corresponded again to the peptidic catalyst **10-R** but the signal of the protonated enamine **20** was also observed. After addition of one equivalent of **7** to the mixture, another ESI-MS measurement was performed. The resulting spectrum showed characteristic signals most likely corresponding to the cyclobutane **21** and its sodium adduct, analogously to the experiment in *i*PrOD-d$_8$ as described above (Figure 4-6, left).

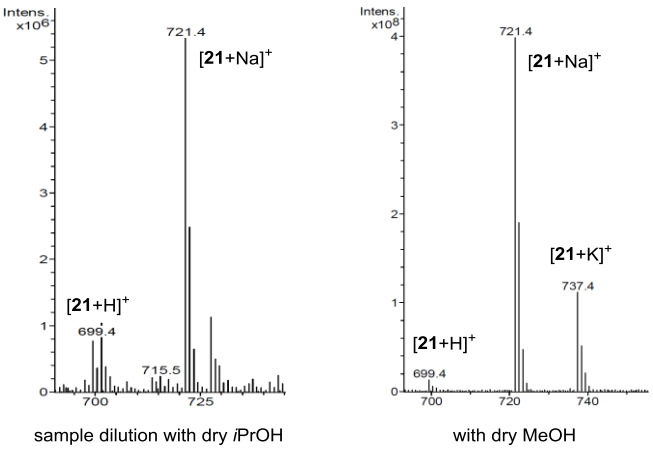
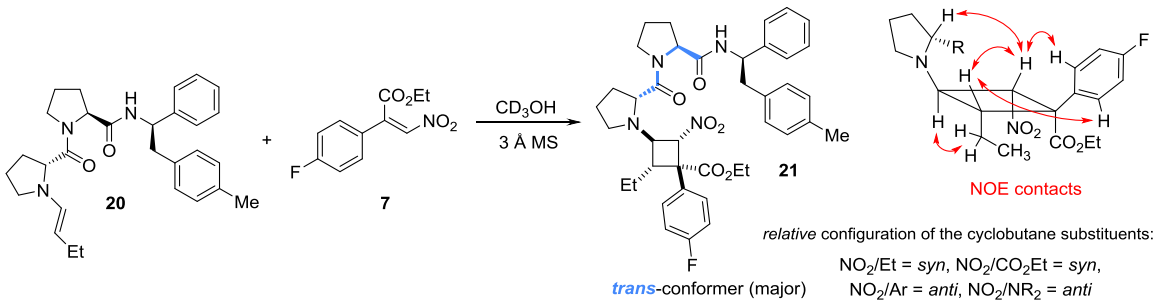


Figure 4-6 Excerpts of mass spectra resulting after addition of nitroacrylate **7** to preformed enamine **20**. The observed signals correspond to the mass of cyclobutane species **21**.

Higher signal intensities were observed due to better ionization, when the aliquot withdrawn from the mixture was diluted with dry methanol instead of *i*PrOH prior to injection (Figure 4-6, right).

In order to further investigate the formation of the enamine and cyclobutane intermediates in the reaction of butanal with β,β-disubstituted nitroolefin **7** in a protic solvent, we next mixed the substrates in equimolar amounts with catalyst **10-R** in CD_3OH-d_3 in the presence of molecular sieves and again analyzed the mixture by NMR spectroscopy. The enamine **20** was formed in less than 5 minutes in ca. 10% when catalyst **10-R** was mixed with the aldehyde and was observed as a mixture of *cis/trans*-conformers (amide bond in Pro-Pro) in a ratio of 1:1.7. In addition to the enamine, several other species were observed which, however, could not be assigned. Upon addition of nitroacrylate **7** to the preformed enamine **20**, immediate formation of the corresponding cyclobutane **21** was observed which was characterized by 1D- and 2D-NMR spectroscopy (see experimental part, Chapter 7.8.2 for details). The formation of **21** increased to about 50% (with respect to **7**) within 1 hour and after 24 hours **21** was still the major species present in the reaction mixture. The cyclobutane was also observed as a mixture of *cis/trans*-conformers (amide bond in Pro-Pro; *cis/trans* ratio = 1:1.5). The major conformer was expected to be the *trans*-conformer. A NOE spectroscopy experiment allowed for the assignment of the relative configuration of the cyclobutane substituents in **21** which is analogous to the related reactions with β-monosubstituted nitroolefins performed in the presence of catalysts lacking an intramolecular proton donor (Scheme 4-4).[172,177-178]



Scheme 4-4 Formation of cyclobutane **21** after addition of nitroacrylate **7** to the preformed enamine **20** in CD_3OH-d_3 (left) and the assignment of the relative configuration of **21** from NOE spectroscopy (right).

Compared to stoichiometric reactions between aldehydes and β-monosubstituted nitroolefins in the presence of the Hayashi-Jørgensen catalyst (performed in C_6D_6), where apart from the corresponding cyclobutane also the corresponding 1,2-oxazine *N*-oxide intermediate (Scheme 4-2) was detected (about 25%),[184-185] in the reaction between enamine **20** and nitroacrylate **7** such an 1,2-oxazine *N*-oxide species could not be clearly observed. The few tiny signals in the NMR spectra that were potentially indicative of that species corresponded to an amount of < 5%.

4.2.2.2 Catalytic Reactions

With the NMR spectra of the stoichiometrically prepared cyclobutane **21** in hand we returned to the reactions under catalytic conditions both in the absence as well as in the presence of an acidic co-catalyst and searched for the corresponding signals of the cyclobutane species in the reaction mixtures. For that purpose, butanal and nitroacrylate **7** were reacted in the presence of 25 mol% of catalyst **10-R** and the equimolar amount of an acidic additive under the same conditions as described for the reactions of the IR spectroscopic studies (see experimental part, Chapter 7.2.5, protocol R for reaction details). After a reaction time of 30 min, aliquots were withdrawn from the reaction mixtures, diluted with CD_3OD and immediately analyzed by ^{1}H- and ^{19}F-NMR spectroscopy. The spectra of the reactions carried out in the absence of an additive (Figure 4-7, a) as well as in presence of acetic acid (Figure 4-7, b) showed both small signals corresponding to cyclobutane **21**, which means that the proposed rate-limiting step in these two cases is indeed the protonation of the iminium nitronate. The NMR experiments also confirmed that no such cyclobutane intermediate was present in the reaction when a strong acid such as dichloroacidic acid or TFA was used as an additive (Figure 4-7, d and e). These findings strongly indicate that the rate-limiting step in the reactions with strong acidic additives is the C-C bond formation between the enamine and the nitroalkene, as reported previously for reactions with β-monosubstituted nitroolefins.[172]

When the reaction was performed with chloroacetic acid as additive, the C-C bond formation was expected to be the rate-determining step, based on the distinct shape of the kinetic reaction profile (see Figure 4.4, green curve). This would mean that a cyclobutane intermediate should not be present. However, ^{1}H- and ^{19}F-NMR spectroscopic analysis of

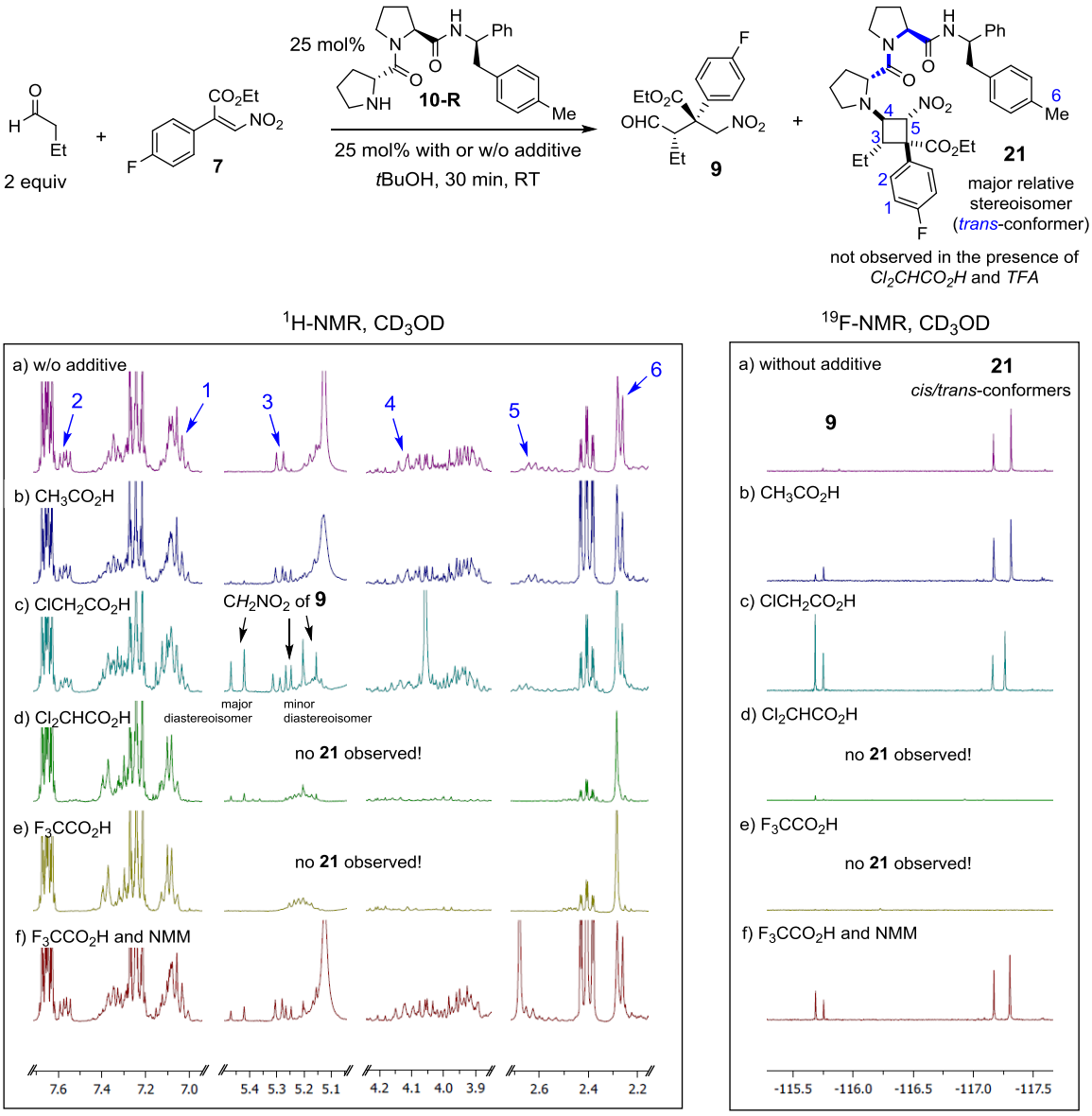


Figure 4-7 Comparison of ^{1}H-NMR (left) and ^{19}F-NMR (right) spectra of the conjugate addition reaction between butanal and nitroacrylate **7** in the presence of catalyst **10-R** and different acidic additives showing signals of the corresponding cyclobutane **21**.

this reaction also revealed the presence of cyclobutane **21** (Figure 4-7, c), suggesting that the protonation of the iminium nitronate should be the rate-limiting step. A plausible explanation for this apparent contradiction could be that the C-C bond formation and the iminium nitronate protonation have relatively similar rates, whereby the rate of the protonation must be slightly higher than that of the C-C bond formation. This would

explain both, the distinct curve shape (C-C bond formation is the rate-limiting step) and the observation of a cyclobutane (protonation of the nitronate is not extremely fast).
A similar conversion-time profile as for the reaction in the presence of chloroacetic acid was observed when TFA in combination with the equivalent amount of NMM was used (see Figure 4.3, cyan curve). This suggests that the rates of the C-C bond formation and the protonation are again similar, and the C-C bond formation is rate-limiting. This was further supported by ^{1}H- and ^{19}F-NMR spectroscopic inspection of the reaction mixture, revealing again the presence of cyclobutane **21** (Figure 4-7, f). The role of NMM in this reaction might be explained as follows: On the one hand, NMM partially liberates the protonated catalyst, which increases the concentration of free catalyst, therefore allowing for a faster C-C bond formation than in the absence of base. On the other hand, protonated NMM is a weaker acidic additive than TFA, slowing down the protonation of the nitronate. However, since the rate of the C-C bond formation is limiting the overall rate, the decrease of the rate of the nitronate protonation does not affect the reaction outcome. Clearly, these findings demonstrate that the combination of an acid and a base as additives creates a more complex situation in the catalytic pathway.

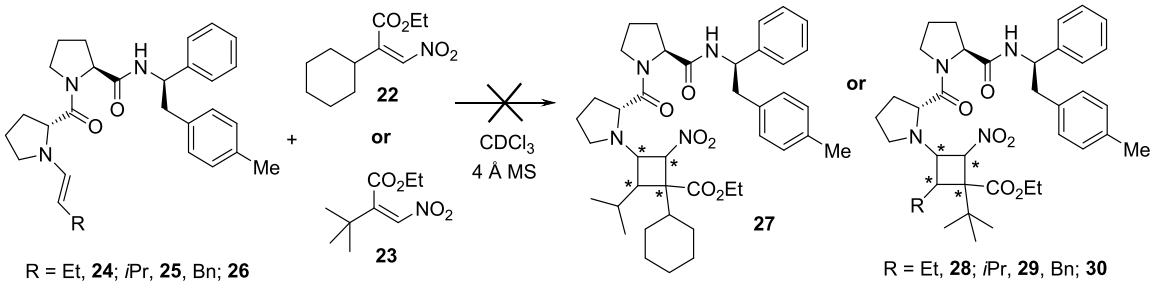
4.2.3 Investigations of Enamine and Cyclobutane Intermediates in Aprotic Solvents

4.2.3.1 Stoichiometric Reactions

In addition to the data obtained for the protic solvent MeOH, we also wanted to evaluate the pathway of the conjugate addition reaction between aldehydes and β,β-disubstituted nitroolefins in aprotic solvents. For this purpose we investigated the formation of enamine **20** and cyclobutane **21** in benzene and chloroform as these two solvents were also used in previous mechanistic studies of reactions between aldehydes and nitrostyrene.[172,177-178] Stoichiometric reactions in the presence of molecular sieves were performed as described in Chapter 4.2.2.1. Higher quantities of enamine **20** were formed in both solvents (ca. 30% of the aldehyde in C_6D_6, up to 90% in $CDCl_3$). Furthermore, the enamine was formed as a single conformer, presumably the *trans*-conformer. Addition of nitroacrylate **7** to the preformed enamine in $CDCl_3$ resulted in 30% conversion to cyclobutane **21** (with respect to **7**) within 5 min, formed in a *cis/trans*-conformer ratio of about 1:10 (see experimental part, Chapter 7.8.2 for details). After 24 h **7** was nearly quantitatively

converted into cyclobutane **21**. A complicated spectrum was obtained in C_6D_6 and was therefore not investigated further. As observed for the reaction in CD_3OH-d_3 (see Chapter 4.2.2.1), again only a very small amount (< 5%) of the potential 1,2-oxazine *N*-oxide intermediate was detected in $CDCl_3$. ESI-MS analysis of an aliquot of the NMR sample after dilution with dry acetonitrile or MeOH revealed the presence of mass peaks corresponding to the cyclobutane as well as its sodium and potassium adducts (see experimental part, Chapter 7.8.2 for details).

Attempts to isolate the cyclobutane **21** with the aim of determining its absolute configuration were unfortunately not successful due to decomposition of the compound. As shown by Seebach and co-workers, in their mechanistic studies on addition reactions between aldehydes and β-monosubstituted nitroolefins catalyzed by the Hayashi-Jørgensen catalyst, cyclobutanes formed from substrates with more bulky substituents were stable enough for isolation and characterization.[178,185] We therefore mixed nitroacrylates **22** or **23**, bearing a cyclohexyl or *tert*-butyl group, respectively, with the preformed enamines **24 - 26** in $CDCl_3$ in presence of molecular sieves (Scheme 4-5). Disappointingly, the desired cyclobutanes **26 - 30** were either not formed at all or only in trace amounts and even after several hours the reaction mixture contained mostly the starting materials. Further efforts to synthesize and isolate other cyclobutane intermediates were not successful and these studies were therefore not continued.



Scheme 4-5 Unsuccessful attempt to form cyclobutanes **26 - 30** bearing bulky substituents *via* the addition of nitroacrylates **22** or **23** to enamines **24 - 26**.

4.2.3.2 Catalytic Reactions in Chloroform

In order to examine the comparability of the reaction between butanal and nitroacrylate **7** in the presence of catalyst **10-R** in protic and aprotic solvents, we next performed the catalytic experiments in chloroform and followed the formation of γ-nitroaldehyde **9** over time. Due to the high volatility of chloroform which resulted in inaccurate results when *in situ* FT-IR spectroscopy was used, the reaction was monitored by ^{1}H-NMR spectroscopy and carried out in a NMR tube. Identical conditions were used as for the catalytic reactions in *t*BuOH monitored by IR spectroscopy. The concentration of the reaction was 0.42 M with respect to the nitroacrylate **7**. The conversion to γ-nitroaldehyde **9** was determined by the use of 1,1,2,2-tetrachloroethane as internal standard.

The reaction was first performed in the absence of an acidic additive and therefore a sigmoidal conversion-time curve was expected as for the reaction in *t*BuOH (Figure 4-8, left). However, the obtained kinetic profile was not sigmoidal but showed a distinct

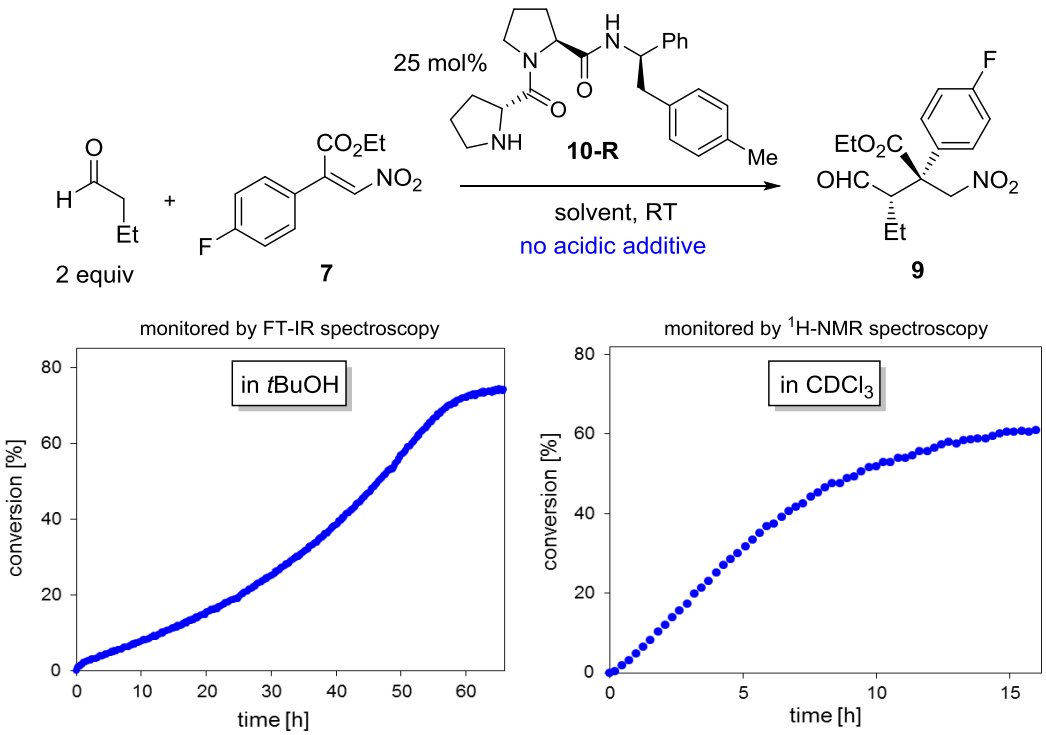
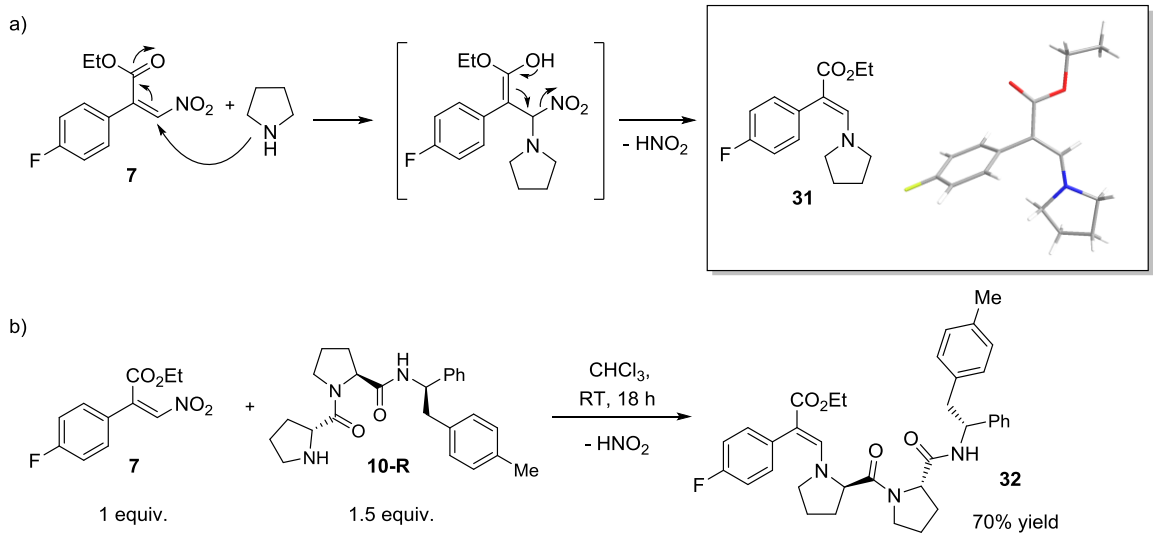


Figure 4-8 Comparison of the conversion-time curves of the conjugate addition reaction between butanal and nitroacrylate **7** in the presence of catalyst **10-R** and absence of an acidic additive in *t*BuOH (left) and in chloroform (right).

curvature (Figure 4-8, right). Furthermore, the reaction proceeded significantly faster than in *t*BuOH but interestingly only 60% conversion to product **9** was observed. Such behaviour would be expected for a reaction carried out in the presence of an acidic additive which is somewhat mysterious since no additive was used and the chloroform was filtered over basic aluminium oxide to remove traces of HCl. Careful inspection of the individual NMR spectra taken over the course of the reaction revealed the presence of the corresponding cyclobutane **21** which indicates that the rate-limiting step of this reaction is the protonation of the iminium nitronate. Furthermore, despite the fact that butanal and nitroacrylate **7** were still present after 15 h, further progress of the reaction was not observed. This is a strong indication of the presence of a background reaction involving catalyst deactivation. Indeed, apart from small amounts of the *homo*-aldol product **4** (< 5% of butanal) and the α-ketoester (~ 5% of **7**), which can form *via* Retro-Henry reaction from nitroacrylate **7**, the presence of another unknown species (~ 15% of **7**) that formed over the course of the reaction was observed. Isolation by preparative TLC (on neutral or basic aluminium oxide; decomposition was observed on silica gel) and subsequent analysis showed that it was an adduct between the peptidic catalyst **10-R** and nitroacrylate **7** but the exact structure could initially not be assigned. Consequently, for reasons of simplification, pyrrolidine instead of catalyst **10-R** was mixed with **7** in chloroform and the reaction mixture was allowed to stand overnight. Column chromatography of the mixture afforded a colorless oil which crystallized in the fridge providing crystals suitable for X-ray crystallographic analysis. The obtained crystal structure confirmed, as proposed by NMR spectroscopy, that it was the *trans*-isomer of pyrrolidinyl acrylate **31**. This compound probably forms upon addition of pyrrolidine to nitroacrylate **7** followed by elimination of nitrous acid (Scheme 4-6, a).

Subsequently, when peptidic catalyst **10-R** was reacted with nitroacrylate **7** the peptide-derived acrylate **32** was obtained and proved upon isolation and characterization to be the observed side product formed in the initial catalytic reaction in chloroform (Scheme 4-6, b). As a comparison, when nitroacrylate **7** was treated with catalyst **10-R** in *t*BuOH, only about half of the amount of adduct **32** formed as in chloroform under otherwise identical conditions. However, in the catalytic reaction between butanal and nitroacrylate **9** in presence of catalyst **10-R** in *t*BuOH, **32** was not observed, neither in the absence nor in the presence of an acidic additive.



Scheme 4-6 a) Proposed mechanism of the formation of pyrrolidinyl acrylate **31** and its crystal structure. b) Acrylate **32** analogously prepared as **31** from nitroacrylate **7** and peptidic catalyst **10-R**.

Having identified **32** as a side product and knowing that its formation is accompanied by the generation of an acid (HNO_2), a plausible explanation for the observed shape of the kinetic profile of the catalytic reaction in chloroform can be deduced: 1) during the first few hours of the reaction the kinetic profile can be interpreted as rather linear, meaning that the forming acid accelerates the reaction. Note: it is expected that the instable nitrous acid (pK_a in water = 3.4) rapidly decomposes under the reaction conditions into nitric acid (pK_a in water = -1.4), water and nitric oxide and therefore actually, it is nitric acid that should be considered as co-catalyst. 2) as the reaction progresses (from about 4 h on and 35% conversion) an increasing curvature is observed, indicating that the reaction rate drops due to a decreasing catalyst concentration, since **10-R** is being continuously trapped as the side product **32** and thus deactivated. Additionally, the increased amounts of acid present in the reaction mixture might protonate the remaining free catalyst and therefore also slow down the reaction. As a consequence of both factors, the reaction stops at a certain point and incomplete conversion to γ-nitroaldehyde **9** is observed.

Due to the fact that side product **32** forms when the reaction is performed in chloroform, it is expected that the addition of an acidic additive will not have a positive effect on the reaction's outcome in this solvent. A strong acidic additive will immediately protonate the catalyst which will result in a slow reaction, whereas a weak acid might only be effective

in the beginning but will become a mere "spectator" as soon as the stronger acid HNO_3 forms in the course of the reaction. An attempt of using acetic acid as the co-catalyst in the reaction performed in chloroform confirmed that, indeed, the reaction proceeded slightly faster in the beginning, however, the kinetic profile was, as suggested, similar to that of the reaction in the absence of acidic additive (Figure 4-8). In addition, comparable quantities of side product **32** were formed, resulting again in a moderate conversion to γ-nitroaldehyde **9** of about 60%.

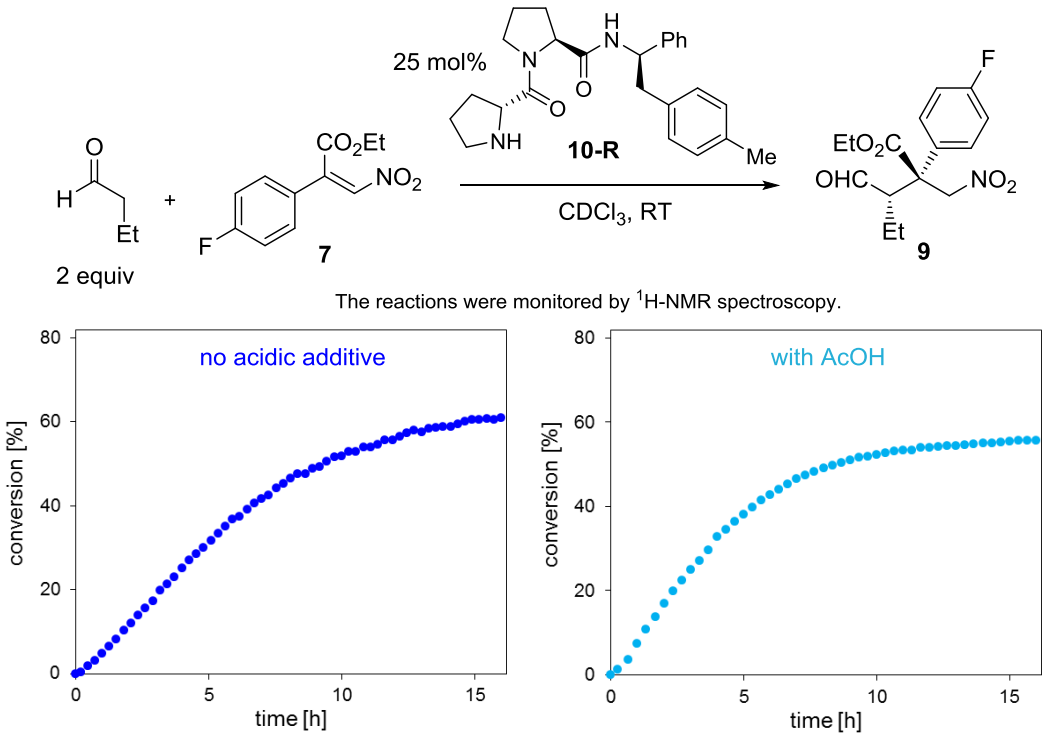


Figure 4-8 Comparison of the conversion-time curves of the conjugate addition reaction between butanal and nitroacrylate **7** with catalyst **10-R** in the absence (left) and presence (right) of acetic acid in chloroform.

4.2.4 Investigations on the Formation of an Aza-Michael Adduct

During the NMR spectroscopic investigation of the reaction between butanal and nitroacrylate **7** in CD_3OH-d_3 and $CDCl_3$ (see Chapters 4.2.2 and 4.2.3) we detected, apart from the expected cyclobutane intermediate, another unknown species in the reaction mixture. Under simplified reaction conditions using pyrrolidine as catalyst in equimolar amount with respect to butanal and nitroacrylate **7**, not only the corresponding

cyclobutane **33** but also aza-Michael adduct **34** was observed (Figure 4-9; see experimental part, Chapter 7.8.4 for details).

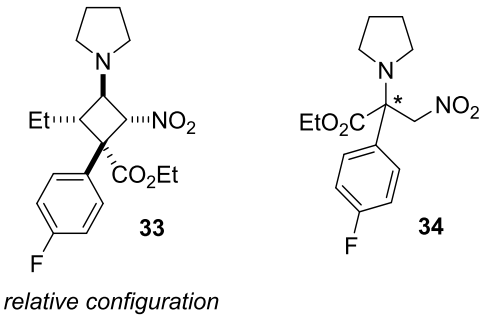


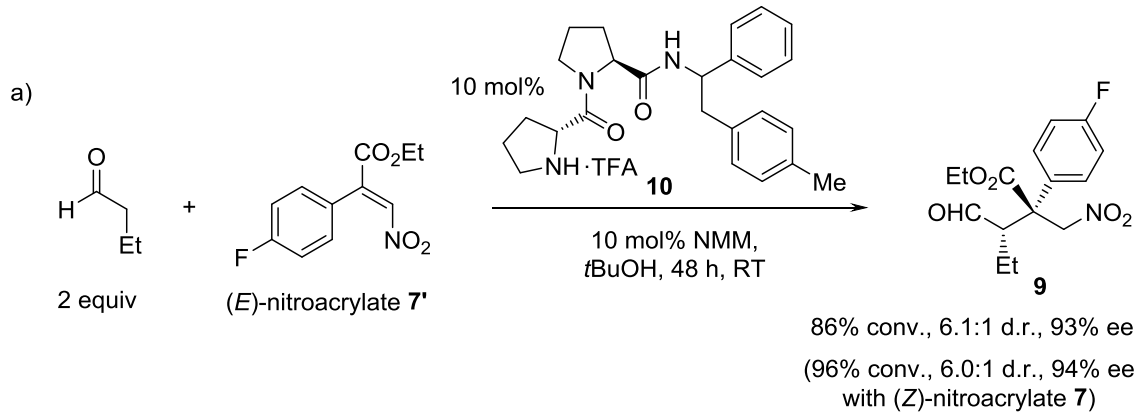
Figure 4-9 Cyclobutane intermediate **33** and aza-Michael adduct **34** which are readily formed in the stoichiometric reaction between pyrrolidine, butanal and nitroacrylate **7**.

The NMR experiments also showed that when pyrrolidine was mixed with nitroacrylate **7** exclusively, adduct **34** formed immediately and in high quantities, however, disappeared again within 2 h when the reaction was performed in $CDCl_3$ and within 30 min when carried out in CD_3OH-d_3. Further experiments revealed that the analogous reaction between peptidic catalyst **10-R** and nitroacrylate **7**, produced significantly smaller amounts of corresponding aza-Michael adduct **35** in both solvents, $CDCl_3$ as well as CD_3OH-d_3 under otherwise identical conditions. The peptide-derived adduct **35** was present in the reaction mixture also only for about 2 h ($CDCl_3$) or 30 min (CD_3OH-d_3).

Recently, Seebach reported in studies with unsubstituted 3-nitroacrylates and the Hayashi-Jørgensen catalyst that such aza-Michael adducts are expected to be the resting state of the catalyst.[185] Particularly when unsubstituted, highly reactive nitroacrylates are used and the nitroacrylate is in large excess to the catalyst, formation of the aza-Michael adduct as the catalyst resting state is plausible.

In our catalytic reaction between butanal and nitroacrylate **7** under standard conditions (10 mol% catalyst **10** and TFA/NMM as additives) the aza-Michael adduct could not be detected. An interesting observation, however, was made when the reaction was performed with the (*E*)-nitroacrylate **7'** instead of the standard (*Z*)-nitroacrylate **7** which was obtained when a diluted solution of **7** in CH_2Cl_2 was irradiated with UV light (366 nm) over several days (see experimental part, Chapter 7.5.3 for details). The reaction

with butanal proved to be somewhat slower but the γ-nitroaldehyde **9** was obtained with the same stereoselectivity as observed for the (*Z*)-nitroacrylate **7** (Figure 4-10, a). ^{1}H-NMR spectroscopic analysis of the reaction mixture revealed rapid isomerization of (*E*)-nitroacrylate **7'** back to (*Z*)-nitroacrylate **7** in the beginning of the reaction (Figure 4-10, b). As this isomerization is most likely to occur through the reversible formation of the aza-Michael adduct **35**, our observation suggests that such an adduct between the catalyst and the nitroacrylate seems to be present also under catalytic conditions.



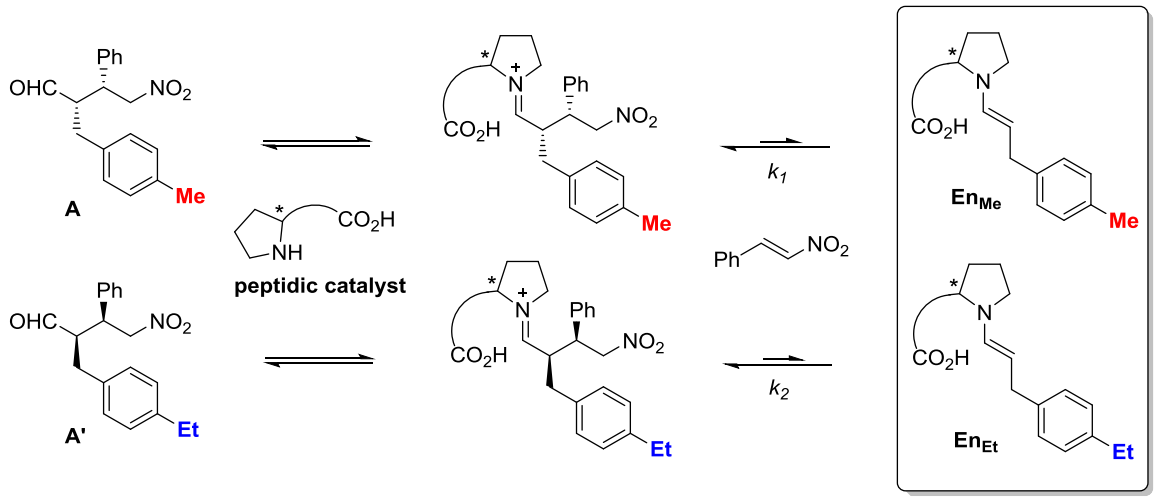
reaction time	ratio nitroacrylate (E) : (Z)
0 min	>20 : 1
15 min	1.6 : 1
60 min	1 : 1.7
3 h	1 : 5.1
20 h	1 : 23

Figure 4-10 a) Conjugate addition reaction with (*E*)-nitroacrylate **7'**. b) Isomerization of (*E*)-nitroacrylate **7'** back to the (*Z*)-nitroacrylate **7** in the reaction mixture *via* adduct **35**.

However, as observed in the stoichiometric experiments between catalyst **10-R** and nitroacrylate **7**, we expect that **35** forms also in rather small amounts with the less reactive 2-substituted 3-nitroacrylate **7** (compared to unsubstituted 3-nitroacrylates) and rapidly undergoes β-elimination liberating the catalyst and **7**. Therefore, it can be assumed that the catalyst is not really sequestered from the catalytic cycle and trapped within adduct **35**.

4.3 Investigations on the Stereoselectivity-Determining Step by ESI-MS

The ESI-MS back-reaction screening using mass-labeled quasi-enantiomeric substrates is an elegant method developed by Pfaltz and co-workers that allows for the fast determination of the intrinsic enantioselectivity of catalysts and has been successfully used for screening of a variety of metal- and organocatalyzed reactions.[195-201] Based on the principle of microscopic reversibility, which states that the transition states of the forward and back reaction are identical, the enantioselectivity of the catalyst can be determined by screening the intermediates derived from the two quasi-enantiomeric substrates in the reverse reaction. The ratio of the signal intensities (*I*) of two quasi-enantiomeric and thus mass-spectrometrically distinguishable intermediates (*e.g.*, enamines $\mathbf{En_{Me}}$ and $\mathbf{En_{Et}}$) reflects the enantioselectivity of the catalyst ($k_1/k_2 = I(\boldsymbol{En_{Me}})/I(\boldsymbol{En_{Et}})$, Scheme 4-7).



Scheme 4-7 The concept of the ESI-MS back-reaction screening illustrated by the *retro*-conjugate addition between mass-labeled quasi-enantiomeric substrates (**A** and **A'**) and a peptidic catalyst.[171]

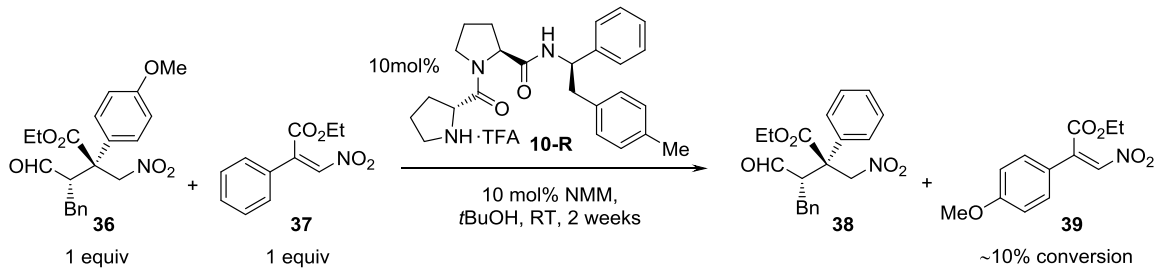
In collaboration with the Pfaltz group, we have successfully applied the concept of the ESI-MS screening in the peptide-catalyzed conjugate addition reaction between aldehydes and β-monosubstituted nitroolefins (Scheme 4-7).[171] The studies with 1:1 mixtures of the two quasi-enantiomeric γ-nitroaldehydes **A** and **A'** in the presence of peptides of the type Pro-Pro-Xaa (Xaa = acidic amino acid) bearing an intramolecular proton donor showed

that the ratio of signals corresponding to the two distinguishable enamines $\mathbf{En_{Me}}$ and $\mathbf{En_{Et}}$ was the same as the enantiomeric ratio of **A** and **A'** formed in the catalytic forward reaction. This unambiguously proved that these reactions proceed *via* an enamine mechanism and that the C-C bond formation between the enamine and the nitroolefin is the stereoselectivity-determining step.[171] In contrast, screening of the back reaction in presence of catalysts lacking an intramolecular proton donor showed that the ratio of the enamines was different from the enantiomeric ratio of the corresponding γ-nitroaldehyes **A** and **A'** and thus not the C-C bond formation but a different step determines the stereoselectivity. This is in agreement with recent mechanistic studies by Blackmond and co-workers where they proposed that for reactions of aldehydes with β-nitroolefins catalyzed by the Hayashi-Jørgensen catalyst (also lacking an intramolecular proton donor), the stereoselectivity is correlated with the relative stability and reactivity of diastereomeric downstream intermediates such as cyclobutanes.[186-187]

Having shown that the ESI-MS back-reaction screening is a valuable tool for probing the mechanism of the above described conjugate addition reaction, we became interested in performing analogous experiments for the reaction of aldehydes with β,β-disubstituted nitroolefins. Since we proposed that it progresses *via* a similar pathway as the reaction with β-monosubstituted nitroolefins, we expected that with catalysts lacking a proton donor within their structure, such as **10-R**, the ratio of the enamines observed in the back reaction will also be significantly different from the enantiomeric ratio of the product γ-nitroaldehydes formed in the forward reaction.

4.3.1 Initial Experiments

In order to evaluate whether the conjugate addition reaction between aldehydes and β,β-disubstituted nitroolefins can be investigated by the ESI-MS back-reaction screening, we first examined the reversibility of the reaction in a cross-over experiment analogous to the previously reported reactions with β-monosubstituted nitroolefins.[171] Therefore, γ-nitroaldehyde **36** was exposed to an equimolar amount of nitroacrylate **37** in the presence of 10 mol% of catalyst **10-R** (Scheme 4-8).

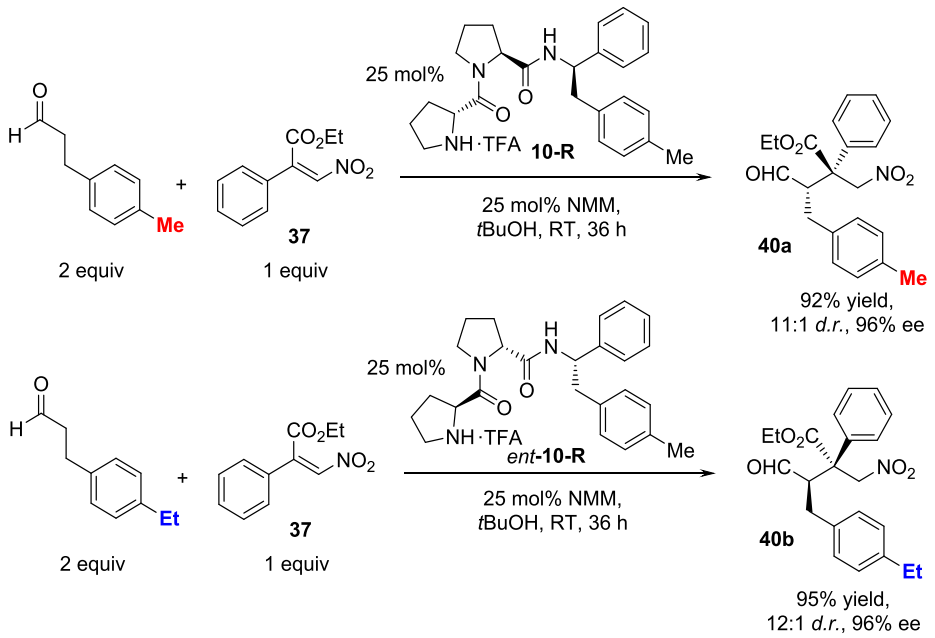


Scheme 4-8 Cross-over experiment to examine the reversibility of the peptide-catalyzed conjugate addition reaction between aldehydes and β,β-disubstituted nitroolefins.

If the reaction was reversible, we expected to observe the formation of the cross-over product **38** and the free 4-methoxy phenyl nitroacrylate **39** since the enamine, resulting from the adduct between **36** and catalyst **10-R** after C-C bond cleavage, should rather react with the more electrophilic nitroacrylate **37** than with the liberated **39**. Indeed, analysis of the reaction mixture by ^{1}H-NMR spectroscopy revealed that the reverse reaction occurred and after 2 weeks about 10% conversion to cross-over product **38** and the free 4-methoxy phenyl nitroacrylate **39** was observed (Scheme 4-8). Even though the back reaction was very slow, the experiment demonstrated that the conjugate addition with β,β-disubstituted nitroolefins is reversible. Thus, it should also be possible to perform the ESI-MS back-reaction screening of the process and to detect intermediates formed in the back reaction that are present in only low quantities by highly sensitive mass spectrometry.

4.3.2 Investigations on the Back Reaction Using Pseudo-Enantiomeric Substrates

In order to perform the ESI-MS back-reaction screening we required the pair of mass-labeled quasi-enantiomeric substrates. Analogously to our previous studies, we prepared the substrates **40a** with a methyl group and **40b** with an ethyl group in the *para*-position of the phenyl ring (Scheme 4-9). 3-(4-methylphenyl)propanal was reacted with nitroacrylate **37** using catalyst H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) to obtain **40a**, whereas 3-(4-ethylphenyl)propanal and **37** provided **40b** in the presence of enantiomeric catalyst *ent*-**10-R**.



Scheme 4-9 Synthesis of mass-labeled quasi-enantiomeric substrates **40a** and **40b** for the ESI-MS back-reaction screening.

The enantiomeric excess of both substrates was 96% demonstrating that the mass labels do not influence the stereoselectivity of the reaction. Since the diastereoselectivity was 11:1 for **40a** and 12:1 for **40b**, which could lead to a distortion of the screening, both substrates were purified by HPLC over a semi-preparative chiral column to afford the optically pure substrates (>60:1 d.r. and >99% ee, see experimental part for details).

We started our investigations by reacting a 1:1 mixture of the two quasi-enantiomeric substrates **40a** and **40b** with 10 mol% of desalted catalyst **10-R** in DMSO which was found to be an optimal solvent for the back-reaction screening in the previous studies (Figure 4-11).[171] After 10 min, the reaction mixture was diluted with acetonitrile and analyzed by ESI-MS. As the reaction is reversible, a short reaction time is important to prevent racemization of the substrates. In the resulting mass spectrum the major signals corresponded to the catalyst in the protonated form, the sodium adduct and the protonated dimeric species. The mass peaks of the iminium ions $\mathbf{Im_{Me}}$ and $\mathbf{Im_{Et}}$ (or cyclobutane species; both have the same mass) and the sought-after signals of the enamines $\mathbf{En_{Me}}$ and $\mathbf{En_{Et}}$ were observed only in very low intensities and disappeared in the noise of the mass spectrum (Figure 4-11, left).

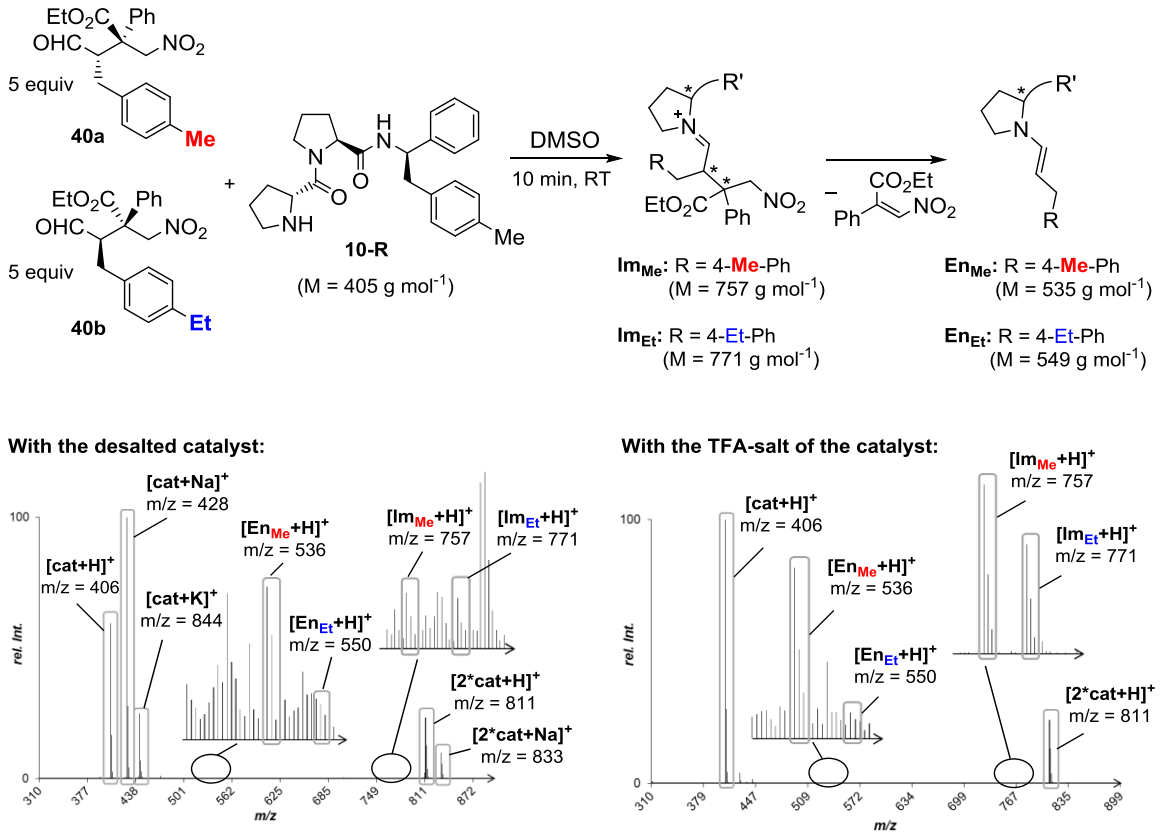


Figure 4-11 ESI-MS back-reaction screening using mass-labeled substrates in DMSO in the presence of catalyst **10-R**.

When the TFA-salt of the catalyst **10-R** was reacted with the two quasi-enantiomeric substrates **40a** and **40b** under otherwise identical conditions, a better signal to noise ratio and somewhat higher intensities of the two iminium ions ($\mathbf{Im_{Me}}$ and $\mathbf{Im_{Et}}$) and the enamine intermediate $\mathbf{En_{Me}}$ were observed (Figure 4-11, right). Even though the intensity of the enamine $\mathbf{En_{Me}}$ signal was still rather low, it was distinguishable from the noise of the spectrum. This enamine is derived from the γ-nitroaldehyde with the (2*S*,3*S*)-configuration and is the enantiomer that forms preferentially in the forward reaction in the presence of catalyst **10-R**. Unfortunately, the signal of the other enamine $\mathbf{En_{Et}}$, derived from the opposite enantiomer of the γ-nitroaldehyde could still not be clearly detected. Due to the low signal intensities it was impossible to quantitatively determine the ratio between the enamines and to compare it with the enantiomeric excess of the product γ-nitroaldehyde obtained in the forward reaction under the same conditions, which was 85:15 (70% ee).

In order to increase the signal intensities of the enamines, we next examined the back reaction between the TFA-salt of **10-R** and the 1:1 mixture of the substrates **40a** and **40b** in chloroform as in this solvent enamines derived from an aldehyde and catalyst **10-R** were readily formed (see Chapter 4.2.3.1). Additionally, we tested also the protic polar solvent *i*PrOH and found that the relative signal intensity of enamine $\mathbf{En_{Me}}$ was somewhat higher than in the back reaction in DMSO. However, the intensity of the mass peak of enamine $\mathbf{En_{Et}}$ was again very low and the signal disappeared in the noise of the spectrum. In chloroform the signal corresponding to enamine $\mathbf{En_{Me}}$ was more visible than in *i*PrOH (Figure 4-12).

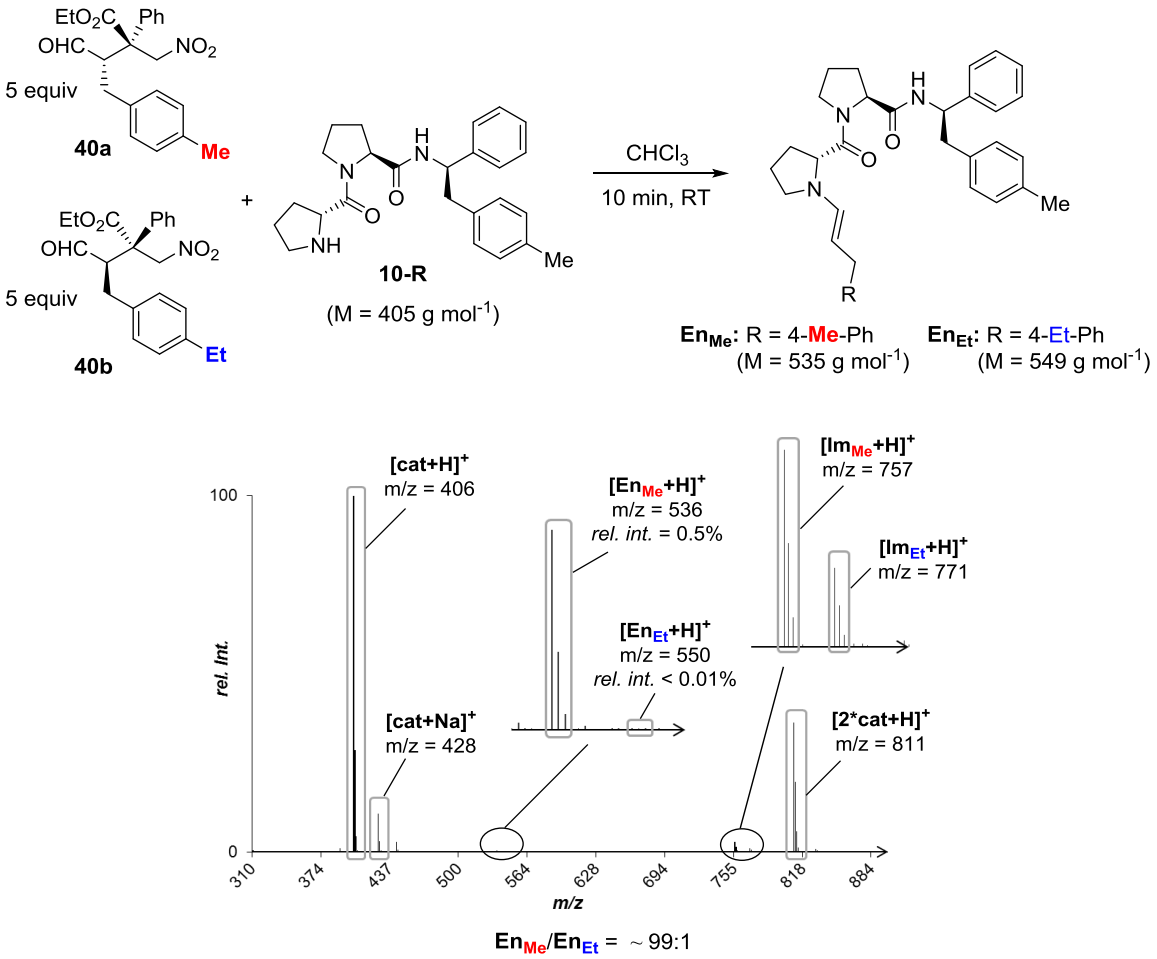
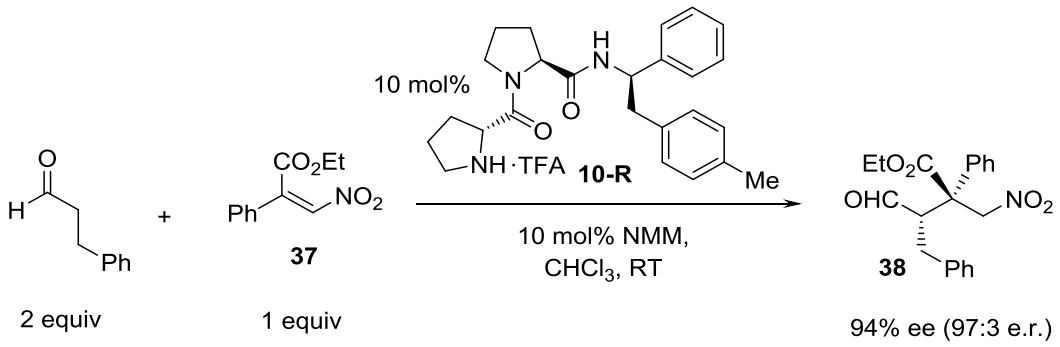


Figure 4-12 ESI-MS back-reaction screening using mass-labeled substrates in $CHCl_3$ in the presence of catalyst **10-R**.

Its relative intensity was around 0.5% of the major signal corresponding to the protonated catalyst **10-R**. The mass peak corresponding to the minor enamine $\mathbf{En_{Et}}$ was still of very low intensity (< 0.01%) in the noise of the mass spectrum. From the obtained data the ratio of $\mathbf{En_{Me}}$/$\mathbf{En_{Et}}$ was determined to be ~ 99:1, however, this value is more an estimate as the signal of $\mathbf{En_{Et}}$ was of extremely low intensity (Figure 4-12).

Attempts to further increase the intensities of the enamine signals such as addition of additives (base or acid) to the reaction mixture, performing the reaction with a higher catalyst loading (up to 30 mol%) or increasing the reaction concentration (from 0.2 M up to 0.5 M) were unfortunately not successful. Also using few other catalysts lacking an intramolecular proton donor (including the Hayashi-Jørgensen catalyst) did not result in an improvement of signal intensity.

Nevertheless, to compare the outcome of the back and forward reactions, we next performed the catalytic reaction between 3-phenylpropanal and nitroacrylate **37** in chloroform in presence of peptide **10-R** under otherwise identical conditions as the back reaction. The expected (2*S*,3*S*)-γ-nitroaldehyde **38** was formed with 94% ee (97:3 e.r., Scheme 4-10).



Scheme 4-10 Catalytic conjugate addition reaction in the forward direction in order to determine the enantiomerice excess of the addition product.

The similar stereochemical outcome observed in the back reaction (enamine ratio $\mathbf{En_{Me}}$/$\mathbf{En_{Et}}$ of ~ 99:1 in favor of the enamine $\mathbf{En_{Me}}$, derived from the γ-nitroaldehyde with the (2*S*,3*S*)-configuration) suggests that the selectivity of the forward reaction matches the intrinsic selectivity of the attack of the enamine onto the nitroolefin. This would mean that the C-C bond formation between the enamine and the nitroolefin is the

stereoselectivity-determining step of the reaction which would clearly be contrary to our expectations since the previous studies with β-monosubstituted nitroolefins demonstrated that the C-C bond formation is the stereoselectivity-determining step only in reactions with catalysts bearing an intramolecular proton donor.[171] To verify our observations, we then performed the ESI-MS back-reaction screening using the enantiomer of the catalyst **10-R** (Figure 4-13).

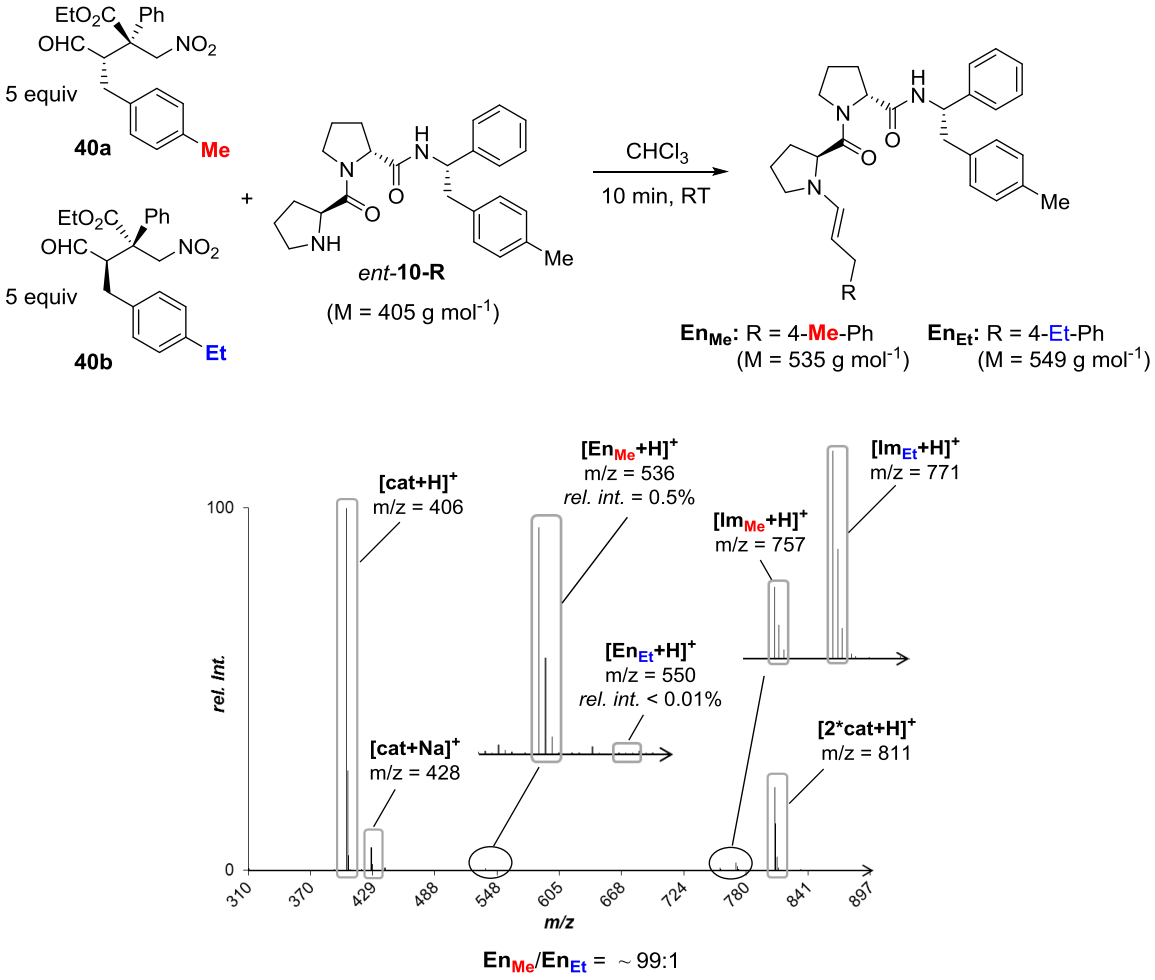


Figure 4-13 ESI-MS back-reaction screening using mass-labeled substrates in $CHCl_3$ in the presence of enantiomeric catalyst *ent*-**10-R**.

If the C-C bond formation between the enamine and the nitroolefin really were determining the enantioselectivity of the studied reactions, we expected to observe the mass peaks of the enamines $\mathbf{En_{Me}}$ and $\mathbf{En_{Et}}$ in opposite intensities providing an enamine ratio $\mathbf{En_{Me}/En_{Et}}$ of ~ 1:99 in favor of $\mathbf{En_{Et}}$ when using the enantiomer of catalyst **10-R**.

Following the back reaction between the two substrates **40a** and **40b** in the presence of *ent*-**10-R** we noticed that the signals corresponding to the iminium ions $\mathbf{Im_{Me}}$ and $\mathbf{Im_{Et}}$ were present in the expected opposite ratio, however, the signals corresponding to enamines $\mathbf{En_{Me}}$ and $\mathbf{En_{Et}}$ were observed in the same ratio as for the reaction performed with **10-R**, again ~ 99:1 in favor of enamine $\mathbf{En_{Me}}$ (Figure 4-13; the signal intensity of $\mathbf{En_{Et}}$ was again very low, < 0.01% of the major signal corresponding to the protonated catalyst **10-R**).

This result shows that there is a clear mismatch between the enantiomeric ratio of the forward reaction and the enamine ratio observed in the back reaction which indicates that the C-C bond formation between the enamine and the nitroolefin is, as initially expected, not the enantioselectivitiy-determining step of the reaction. Consequently, it can be speculated that the enantioselectivity is possibly determined by the relative stability and reactivity of downstream intermediates such as diastereomeric cyclobutanes as it was suggested by Blackmond and co-workers in their mechanistic studies on conjugate addition reaction between aldehydes and β-monosubstituted nitroolefins in presence of the Hayashi-Jørgensen catalyst.[186-187]

Nevertheless, given the fact that very low enamine signal intensities were observed in the ESI-MS screening with the substrates **40a** and **40b** in the presence of catalyst **10-R** and particularly that the minor enamine mass peak $\mathbf{En_{Et}}$ remained always around the noise limit, only careful conclusions should be drawn at this point from these preliminary findings.

Regardless of the above uncertainties, we were curious to find out whether the enantiomeric excess of the γ-nitroaldehyde obtained in the catalytic forward reaction will match the enamine ratio $\mathbf{En_{Me}/En_{Et}}$ of the back reaction when catalysts bearing an intramolecular carboxylic acid are used as it was the case for the analogous reactions using β-monosubstituted nitroolefins and the corresponding γ-nitroaldehydes.[171]

In order to test this, initial ESI-MS back-reactions were performed with the two peptides H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ and H-Pro-Pro-D-Glu-NH_2. When the back reaction was conducted with the two quasi-enantiomeric substrates **40a** and **40b** in presence of the TFA-salt of peptide H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$, a significantly more soluble analogue of H-D-Pro-Pro-Glu-NH_2 (**1**) in chloroform, the signals corresponding to the enamines $\mathbf{En_{Me}}$ and $\mathbf{En_{Et}}$ were observed with only very low intensities in the noise of the spectrum.

Therefore, the peptide H-Pro-Pro-D-Glu-NH_2, which showed the best results in terms of enamine signal intensities in the previous studies,[171] was used (Figure 4-14). The reaction was carried out with the TFA-salt of the peptide in DMSO as the peptide was not soluble in chloroform. Since H-Pro-Pro-D-Glu-NH_2 bears L-Pro instead of D-Pro residue at its N-terminus, the peptide was expected to provide the γ-nitroaldehyde in the forward reaction with the opposite absolute configuration (see also Table 3-3, entry 7) and thus also an enamine ratio $\mathbf{En_{Me}/En_{Et}}$ in favor of enamine $\mathbf{En_{Et}}$ in the back-reaction screening. Indeed, in the catalytic forward reaction the 1,4-addition product was obtained as the (2*R*,3*R*)-enantiomer and in 20% ee (60:40 e.r.). In the ESI-MS back reaction a $\mathbf{En_{Me}/En_{Et}}$ ratio of 51:49 was observed (Figure 4-14).

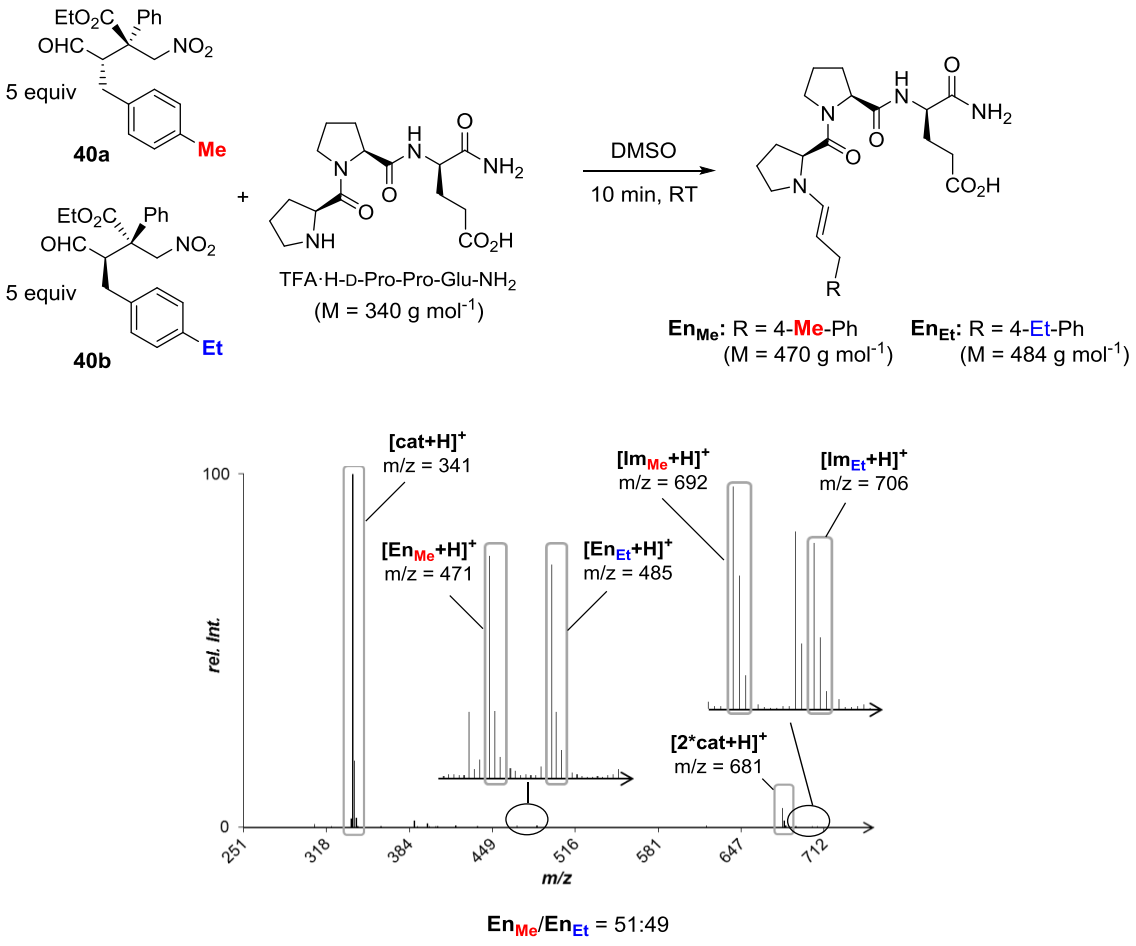


Figure 4-14 ESI-MS back-reaction screening using mass-labeled substrates in DMSO in the presence of catalyst H-Pro-Pro-D-Glu-NH_2.

Although these ratios are somewhat similar, this single result does not allow for a conclusion whether it is really a match case and the C-C bond formation between the enamine and the β,β-disubstituted nitroolefin is the stereoselectivity-determining step or if it is rather a mismatch case where not the C-C bond formation but another step determines the stereoselectivity of the reaction. Therefore, clearly additional experiments are required not only with catalysts bearing a carboxylic acid but also with non-acidic catalysts to gain insight into the stereoselectivty-determining step of the conjugate addition reaction of aldehydes to β,β-disubstituted nitroolefins.

4.4 Conclusions

Peptide H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) is an effective catalyst for the conjugate addition reaction between aldehydes and β,β-disubstituted nitroolefins. Since **10-R** is lacking an intramolecular proton donor, we assumed that this reaction proceeds *via* a similar pathway as the analogous reaction with β-monosubstituted nitroolefins in the presence of a catalyst without a proton donor. In order to gain further insight into the reaction mechanism, we performed mechanistic studies and compared the results with those obtained previously for rections with β-monosubstituted nitroolefins.

In situ FT-IR spectroscopic investigations on the reaction between butanal and ethyl (*Z*)-2-(4-fluorophenyl)-3-nitroacrylate (**7**) in the presence of **10-R** and weakly acidic additives (pK_a ~ 4 to 7) showed a significant rate acceleration compared to the reaction without an acidic additive. NMR spectroscopic experiments of these reactions revealed the presence of a cyclobutane intermediate. These results are in agreement with the analogous studies performed for β-monosubstituted nitroolefins and thus signify that the presently investigated reaction with β,β-disubstituted nitroolefins proceeds through a similar pathway. According to this pathway protonation of the zwitterionic iminium nitronate is the rate-determining step of the reaction and a cyclobutane is the resting state of the catalyst. When stronger acids (pK_a < 2) were used as co-catalysts, decreased reaction rates were observed and no cyclobutane was detected. This is also in agreement with the previous studies, and suggests that in this case C-C bond formation between the enamine and the nitroolefin is rate-limiting. Using an acid of a medium strength (pK_a ~ 3) or a stronger acid (*e.g.* TFA) in combination with a base as additives led to a special

situation. With these additives even faster reactions were observed than with the weakly acidic co-catalysts. Furthermore, the IR spectroscopic studies of these reactions indicated that the C-C bond formation is the rate-limiting step, while the NMR spectroscopic studies suggested that the protonation of the iminium nitronate is determing the reaction rate. These apparently contradictive observations led to the conclusion that the C-C bond formation and the protonation of the iminium nitronate must in this case have similar rates, with the protonation being slightly faster than the C-C bond formation. However, the protonation is expected to be not extremely fast and thus a cyclobutane intermediate can still form. The mechanistic studies also showed that due to the higher acidity of the iminium nitronate, co-catalysts with lower pK_a values were required for the reaction with β,β-disubstituted nitroolefin **7** than for the analogous reactions with β-monosubstituted nitroolefins in order to achieve a significant rate acceleration.

Investigations on the comparability of the reaction between butanal and nitroacrylate **7** catalyzed by **10**-**R** in protic and aprotic solvents revealed that the reaction proceeded significantly faster in chloroform than in *tert*-butanol, the optimal solvent for this reaction. However, due to a background reaction between **7** and the catalyst in chloroform, only moderate conversion to the desired 1,4-addition product was obtained. Additionally, the stereoselectivity was also somewhat lower than in *tert*-butanol.

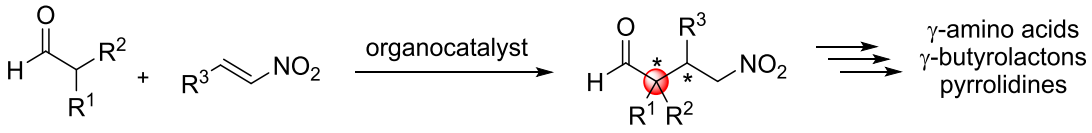
Finally, an ESI-MS back-reaction screening using mass-labeled quasi-enantiomeric substrates was performed in order to gain insight into the stereoselectivity-determining step of the conjugate addition reaction of aldehydes to β,β-disubstituted nitroolefins. Preliminary experiments suggested that the enantioselectivity might be determined by the relative stability and reactivity of downstream intermediates such as diastereomeric cyclobutanes as it was also proposed previously for the analogous reaction with β-monosubstituted nitroolefins in presence of a catalyst lacking an intramolecular proton donor. Nevertheless, since very low signal intensities were observed in the mass spectra, these results should be interpreted with caution and further experiments are clearly necessary in order to shed more light on the stereoselectivtiy-determining step of this reaction.

5

Preliminary Studies on Peptide-Catalyzed Stereoselective 1,4-Addition Reactions between α-Branched Aldehydes and β-Monosubstituted Nitroolefins

5.1 Background

γ-Nitroaldehydes with adjacent quaternary and tertiary stereogenic centers are valuable building blocks for organic synthesis.[29] Apart from reactions of aldehydes with β,β-disubstituted nitroolefins described in Chapter 3,[202] the addition of α,α-disubstituted aldehydes to β-nitroolefins is the other direct approach to obtain these types of compounds (Scheme 5-1).



Scheme 5-1 Generation of a quaternary stereogenic center: conjugate addition reactions of α,α-disubstituted aldehydes to β-monosubstituted nitroolefins.

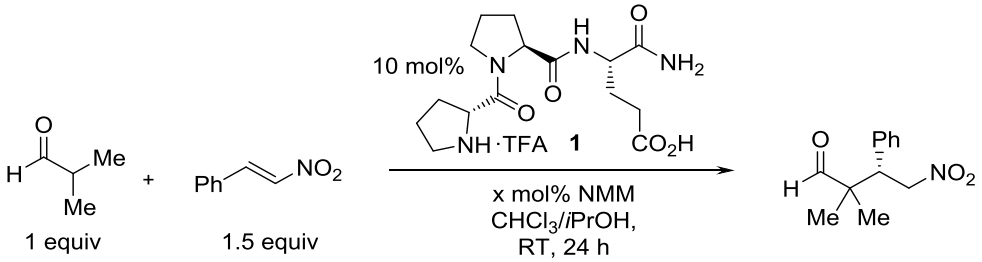
To the best of our knowledge, there are only two examples of that reaction in which amino acids have been used as catalysts.[88-89] However, studies on that reaction in presence of short-chain peptides have not been reported even though peptidic catalysts might be superior to simple amino acids and other organocatalysts due to their conformational flexibility and thus better adaptability to challenging substrates.

Having established peptidic catalysts with the Pro-Pro motif for conjugate addition reactions between linear aldehydes and β-monosubstituted,[165-167,170,173-175] α,β-disubstituted,[169] and β,β-disubstituted nitroolefins (see Chapter 3),[202] we next decided to aim at the development of peptides capable of catalyzing reactions between α,α-disubstituted aldehydes and β-monosubstituted nitroolefins providing the corresponding γ-nitroaldehydes bearing adjacent quaternary and tertiary stereogenic centers. In this chapter we present preliminary results in the search of an optimal peptidic catalyst for this transformation.

5.2 Initial Experiments

Since the peptide H-D-Pro-Pro-Glu-NH_2 (**1**) is an excellent catalyst for conjugate addition reactions between linear aldehydes and β-monosubstituted nitroolefins, it was evaluated first as a potential catalyst in the analogous reaction using α,α-disubstituted aldehydes. Initial studies, performed previously in our group with isobutyraldehyde and nitrostyrene as model substrates, showed that in presence of 10 mol% of catalyst **1** as the TFA-salt the corresponding γ-nitroaldehyde was obtained in only low conversion (20%) and moderate enantioselectivity (80%) after 24 h (Table 5-1, entry 1).[203] In agreement with the studies on reactions of linear aldehydes and β-monosubstituted nitroolefins,

Table 5-1 Conjugate addition reactions between isobutyraldehyde and nitrostyrene in the presence of H-D-Pro-Pro-Glu-NH_2 (**1**).[203]



	solvent	NMM [mol%]	conversion [%][a]	ee [%][b]
1[c]	$CHCl_3$/*i*PrOH 9:1	10	20	80
2	$CHCl_3$/*i*PrOH 7:3	10	70	75
3	$CHCl_3$/*i*PrOH 1:1	10	90	70
4	$CHCl_3$/*i*PrOH 7:3	50	98	83
5	$CHCl_3$/*i*PrOH 9:1	50	85	84

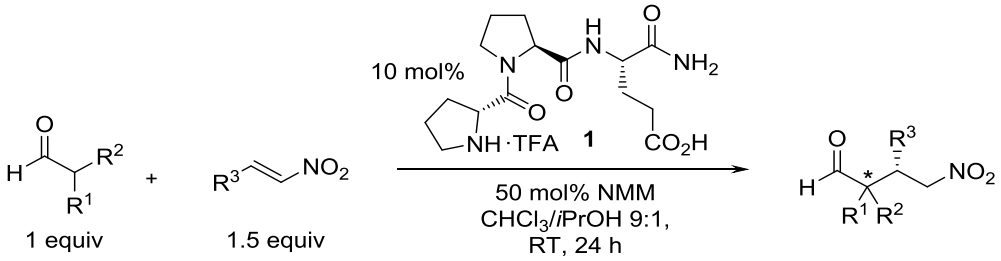
The concentration of the reactions was 0.88 M with respect to butanal. [a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis (DAICEL Chiralpak OD-H column, 250 x 4.6 mm, hexan/*i*PrOH 80:20, 25°C, 0.8 mL/min, UV254 nm, t_R (minor) = 15.9 min, t_R (major) = 22.4 min). [c] Catalyst was not fully dissolved in the reaction medium.

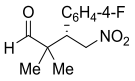
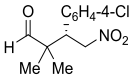
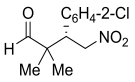
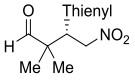
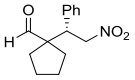
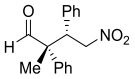
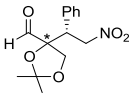
faster reactions but somewhat lower stereoselectivities were observed when more *i*PrOH was used as the solvent (Table 5-1, entries 2 and 3).[165] Interestingly, when the reaction was performed with more than equimolar amounts of NMM with respect to the peptide,

not only a faster reaction but also a higher enantioselectivity was observed (Table 5-1, entry 4). No such effect was observed in analogous reactions with linear aldehydes.[189] When a small substrate scope study was performed it was observed that, due to the rather high concentration of the reaction (0.88 M), a larger amount of chloroform in the solvent mixture was necessary in order to fully dissolve the different nitroolefins in the reaction medium. Therefore, a mixture of $CHCl_3$/*i*PrOH 9:1 was chosen as a solvent which again resulted in a somewhat slower reaction (Table 5-1, entry 5).

The substrate scope showed that variations in the nitroolefin did not have a significant influence on the conversion and the enantioselectivity (Table 5-2, entries 1-4).

Table 5-2 Initial substrate scope of the conjugate addition reaction between α,α-disubstituted aldehydes and β-monosubstituted nitroolefins in the presence of peptide **1**.[203]



	product	conversion [%][a]	d.r.[a]	ee [%][b]
1		75	-	86
2		89	-	82
3		80	-	82
4		87	-	80
5		82	-	79
6		20	18:1	rac.
7		>95	18:1	73

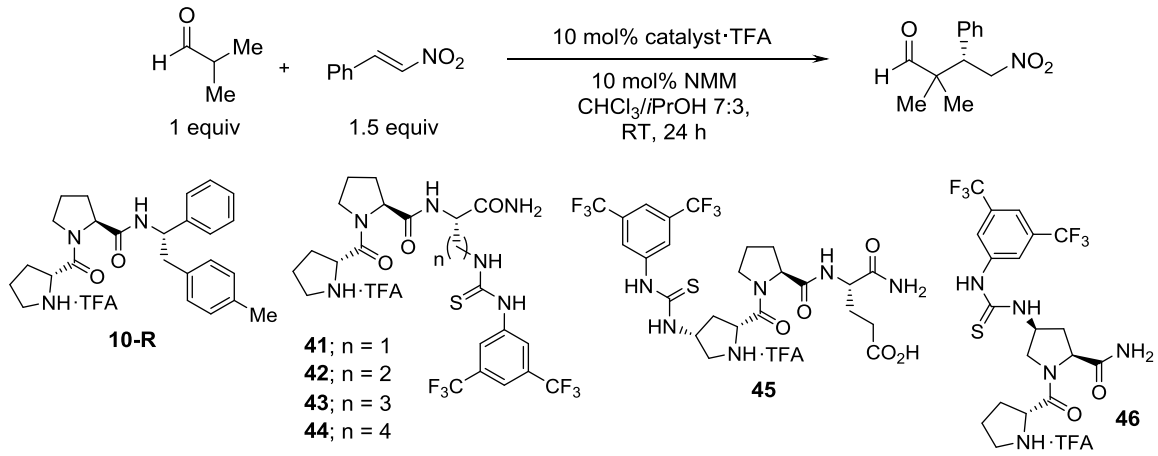
The concentration of the reactions was 0.88 M with respect to butanal. [a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis.

On the other hand, a dramatic drop in reactivity was observed when 2-phenylpropanal was reacted with nitrostyrene (Table 5-2, entry 6). Although the corresponding γ-nitroaldehyde was obtained with good diastereoselectivity (18:1), no enantiomeric excess was achieved. The highest conversion to the corresponding γ-nitroaldehyde was obtained in the reaction between 2,2-dimethyl-1,3-dioxolane-4-carboxaldehyde and nitrostyrene (Table 5-2, entry 7).

Following up on these initial studies, we tested H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) as catalyst in the reaction between isobutyraldehyde and nitrostyrene as this peptide proved before to be a powerful catalyst for the conjugate addition reaction with sterically challenging substrates (see Chapter 3). Since Jacobsen and co-workers showed that chiral primary amine thioureas were excellent catalysts for the conjugate addition reaction of α-branched aldehydes and nitroolefins (see Scheme 1-11),[82] we were also curious to find out whether the introduction of a thiourea moiety as H-bond donor in a peptide with the Pro-Pro motif would result in a better catalyst than H-D-Pro-Pro-Glu-NH_2 (**1**). Thus, the structurally related peptides **41 – 46** bearing a thiourea functional group were also evaluated (Table 5-3).[204]

We performed the experiments under the initially established conditions used (10 mol% of catalyst and 10 mol% NMM in $CHCl_3$/*i*PrOH 7:3; see Table 5-1, entry 2) in order to avoid the effect of increased catalyst activity and enantioselectivity by the excess of NMM as co-catalyst (see Table 5-1, entries 4 and 5). Furthermore, the concentration of the reaction mixture was lowered from 0.88 M to 0.44 M (with respect to the aldehyde) to ensure full solubility of the catalysts. Under these conditions and in the presence of 10 mol% of H-D-Pro-Pro-Glu-NH_2 (**1**), 50% conversion to the γ-nitroaldehyde and 73% ee were observed after 24 h (Table 5-3, entry 1). Unfortunately, peptide **10-R** turned out to be a poor catalyst for this reaction and provided the γ-nitroaldehyde in only 15% conversion and 25% ee (Table 5-3, entry 2). Better catalytic activity and enantioselectivity were observed with the peptidic catalysts **41 – 44** bearing a thiourea functional group in the side chain of the C-terminal amino acid (Table 5-3, entries 3 - 6). Peptide **41** with one methylene group as spacer between the backbone and the thiourea group provided the corresponding γ-nitroaldehyde in highest conversion (80% after 24 h; Table 5-3, entry 3). Nearly quantitative conversion was achieved after 48 h.

Table 5-3 Conjugate addition reactions between isobutyraldehyde and nitrostyrene in the presence of catalysts **10-R** and **41 – 46**.



	catalyst	conversion [%][a]	ee [%][b]
1	TFA · H-D-Pro-Pro-Glu-NH_2 (**1**)	50	73
2	**10-R**	15	25
3	**41**	80 (>95)[c]	79
4	**42**	67	77
5	**43**	70	78
6	**44**	70 (>95)[c]	82
7	**45**	60	73[d]
8	**46**	75	50

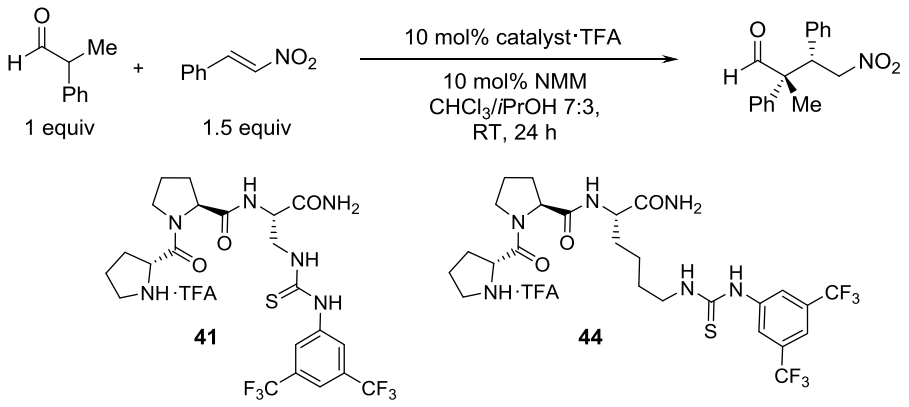
The concentration of the reactions was 0.44 M with respect to butanal. [a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis (DAICEL Chiralpak OD-H column, 250 x 4.6 mm, hexane/*i*PrOH 80:20, 25°C, 1 mL/min, UV254 nm, t_R (minor) = 13.3 min, t_R (major) = 18.5 min). [c] Conversion after 48 h in brackets. [d] The opposite enantiomer was formed.

The enantioselectivity (79% ee) was comparable to that of catalysts **42 – 44** bearing up to four methylene groups between the peptidic backbone and the thiourea group (Table 5-3, entries 4 - 6) which, however, led to lower conversions. H-D-Pro-Pro-Glu-NH_2 derivative **45** which bears the thiourea functionality at the N-terminal proline residue, afforded the conjugate addition product in slightly higher conversion (60%) than parent peptide **1**, but same enantioselectivity (73% ee; Table 5-3, entry 7). Interestingly, with this catalyst the opposite enantiomer of the γ-nitroaldehyde was formed. When the thiourea group was installed on the second proline residue of dipeptide H-D-Pro-Pro-NH_2 **46**, the resulting

catalyst displayed similar reactivity as peptides **41 – 45** but lower enantioselectivity (50% ee) was observed (Table 5-3, entry 8).

Catalysts **41** and **44**, which performed best in terms of reactivity and enantioselectivity, were also evaluated in the reaction between the more challenging nucleophile 2-phenylpropanal and nitrostyrene (Table 5-4). Unfortunately, as in the case of H-D-Pro-Pro-Glu-NH_2 (**1**), only a low conversion (32%) to the corresponding γ-nitroaldehyde was observed (compare Table 5-2, entry 6 with Table 5-4 entries 1 and 2). Moreover, the diastereoselectivity (7:1) was rather moderate and the enantioselectivity (5-15%) was poor. Higher conversion could be achieved when the reaction was carried out with 5 equivalents of water with respect to the aldehyde (Table 5-2, entry 3), which is consistent with the observations of Jacobsen and co-workers.[82]

Table 5-4 Conjugate addition reactions between 2-phenylpropanal and nitrostyrene in the presence of peptidic thiourea catalysts **41** and **44**.



	catalyst	conversion [%][a,b]	d.r.[a]	ee [%][c]
1	**41**	21 (32)	7:1	5
2	**44**	20 (30)	7:1	15
3[d]	**44**	57 (72)	9:1	10

The concentration of the reactions was 0.44 M with respect to butanal. [a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Conversion after 48 h in brackets. [c] Determined by chiral-phase HPLC analysis (DAICEL Chiralpak OD-H column, 250 x 4.6 mm, hexane/*i*PrOH 95:5, 25°C, 1 mL/min, UV254 nm, t_R (syn, major) = 22.2 min, t_R (syn, minor) = 30.6 min). [d] Reaction was performed with 5 equiv. of H_2O with respect to 2-phenylpropanal.

The initial results with H-D-Pro-Pro-Glu-NH_2 (**1**) and its thiourea-functionalized derivatives demonstrated the potential of peptidic catalysts in the conjugate addition reaction between α,α-disubstituted aldehydes and β-monosubstituted nitroolefins. Even though so far low to moderate reactivity and stereoselectivity was achieved in reactions with a challenging substrate, 2-phenylpropanal, the evaluated peptides represent a good basis for further modifications and development of potent catalysts of the type H-Pro-Pro-Xaa (Xaa = acidic amino acid) for this reaction. As was shown by Connon and Jacobsen, primary amine-based catalysts can be superior to secondary amine-based ones in reactions involving α,α-disubstituted aldehydes.[82,85] Therefore, apart from secondary amine-based peptidic catalysts with N-terminal proline residues, peptides bearing a primary amine within their structure should also be considered.

Since predicting the catalytic activity and selectivity of potential peptidic catalyst is very challenging due to their high degree of rotational freedom, rational catalyst design is very difficult. Consequently, we decided to use a combinatorial approach in order to increase the chance of identifying suitable catalysts. We aimed to prepare a functionalized peptide library and use it for a combinatorial screening applying our previously developed screening method of "catalyst-substrate co-immobilization".[162]

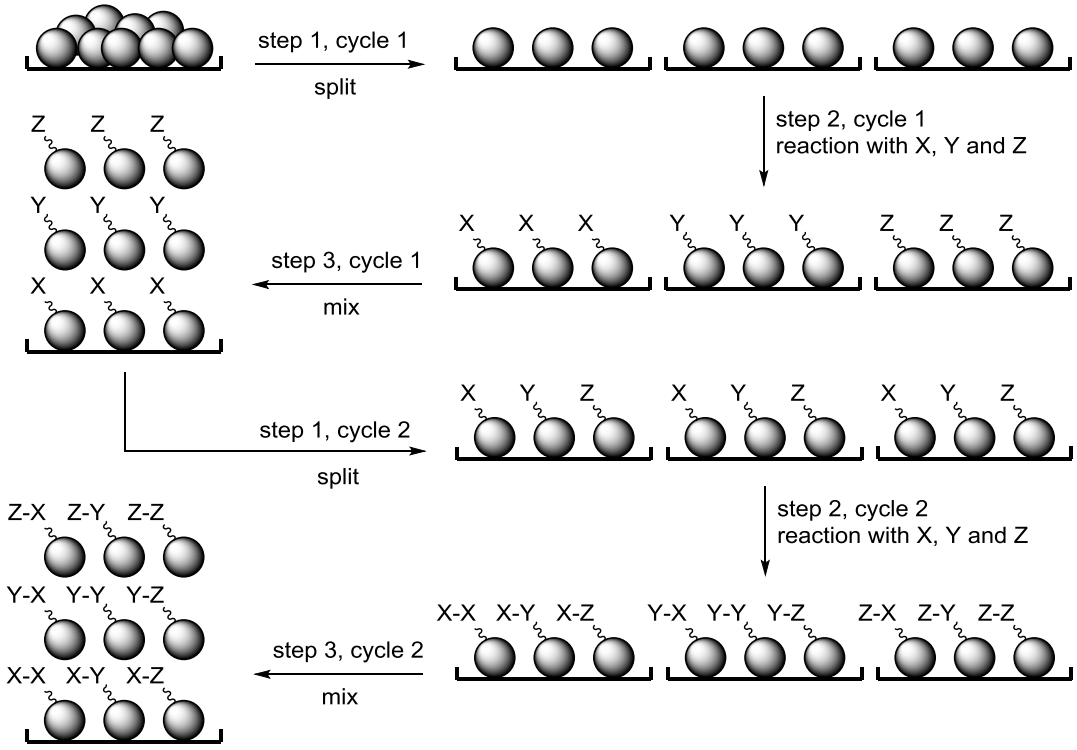
5.3 Combinatorial Screening of Peptidic Catalysts Using Catalyst-Substrate Co-Immobilized Libraries

5.3.1 Combinatorial Chemistry

Combinatorial chemistry is an empirical approach to the natural evolutionary principles of random mutation and selection of the fittest and allows for the simultaneous generation and evaluation of a large number of diverse catalytically active compounds.[146-148,205] Chemical approaches to create such high molecular diversity include the synthesis of parallel libraries, one-bead-one-compound libraries,[206] or dynamic combinatorial libraries.[207]

5.3.1.1 Split-and-Mix Synthesis

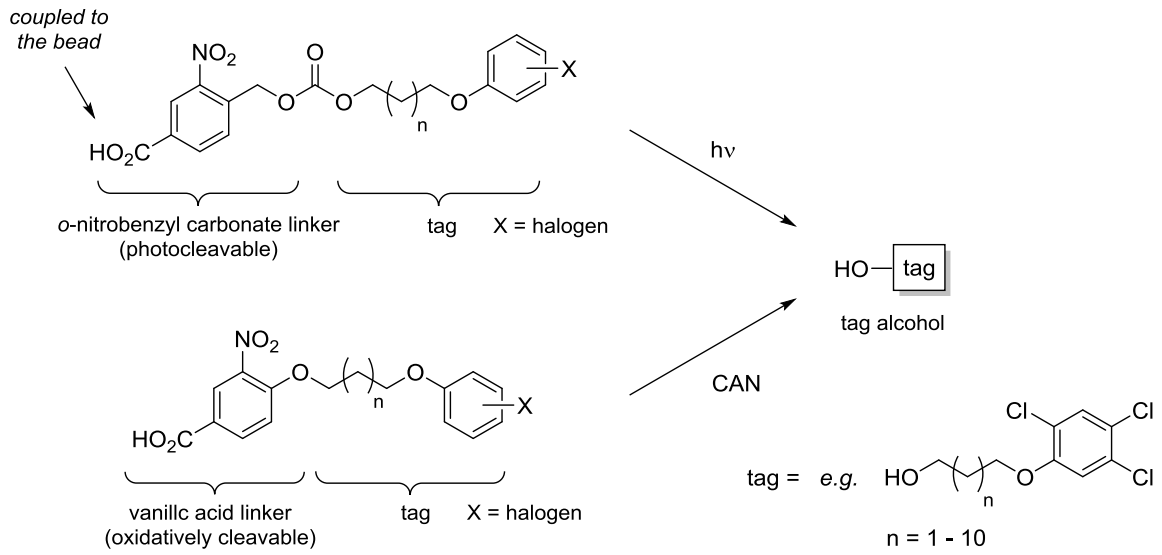
Among the strategies for the generation of combinatorial libraries, the split-and-mix technique is one of the most elegant methods and allows to achieve large molecular diversity without the need for automated synthesis.[208-210] The library components are prepared on solid support and therefore this strategy is particularly suited for building up diverse peptide libraries since peptides consist of linearly connected building blocks that are typically synthesized on solid phase according to established protocols. The protocol for the synthesis of such "one-bead-one-compound libraries" consists of successive cycles of 1) splitting the resin (beads) into equal portions, 2) reacting each portion with a different building block and 3) mixing of the beads (Scheme 5-2). This approach leads to an exponential increase in the compound diversity with respect to the number of reactions performed. For example, if 10 distinct amino acids were used and 4 split-and-mix cycles were performed, a library containing approximately 10000 (10^4) different members would be obtained.



Scheme 5-2 The principle of split-and-mix synthesis.

5.3.1.2 Chemical Encoding

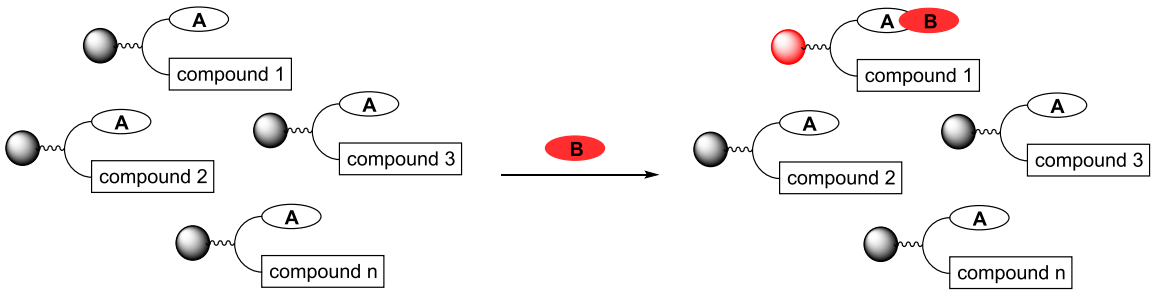
The identification of the compound attached to a particular bead is as essential as the screening of the library itself. The small amount of material per bead, typically around 100 pmol, makes its direct analysis quite difficult. Although NMR spectroscopy or mass spectrometry can in principle be used, the distinction between structurally similar molecules, for example stereoisomers, is very often impossible. A more elegant method to identify the compound on the bead is chemical encoding. The concept of chemical encoding has been developed by Still in 1993 and includes the attachment of inert tags which can be easily analyzed by gas chromatography using a highly sensitive electron capture detector (ECD-GC).[211-212] The tags are long alkyl chained alcohols bearing a polyhalogenated aromatic moiety and are coupled to 1 – 5% of the free functionalities of the bead *via* oxidatively cleavable (using ceric ammonium nitrate) or photocleavable linkers prior to each coupling of a building block (Scheme 5-3). The tags are used as a binary code and record the synthetic history of each bead. For each different compound and each different reaction step a unique combination of tags is needed. For example, using a set of four different tags allows for encoding of 2^4-1 = 15 different building blocks. Consequently, with 12 tags (3 x 4) a library of 15^3 = 3375 different compounds can be encoded.



Scheme 5-3 Examples of oxidatively cleavable and photocleavable linkers.[211-212]

5.3.1.3 Catalyst-Substrate Co-Immobilization

For the identification of catalytically active library members smart screening methods that are general, fast and reliable are required.[147] In reactions where the substrates and products are able to freely diffuse in solution, the identification of active compounds is very challenging or even impossible. To address this issue, techniques such as IR-thermography,[213] generation of insoluble colored reaction products[214] or reactions in gels where the diffusion is slower[215-216] have been developed. Among them, the catalyst-substrate co-immobilization is the most general method which is suitable for virtually any type of bimolecular reaction.[162] The concept of this method is based on the immobilization of one of the reaction partners (A) together with one of library members on the same bead (Scheme 5-4). The other reaction partner (B), which is marked for example with a dye, fluorophore or a radioactive label, is free in solution and will react with the substrate on the bead if the library member on that bead is able to mediate the reaction. This results in the covalent attachment of the marked substrate to the bead, which can then be identified easily. Subsequent isolation and analysis of the bead allows to reveal the structure of the catalytically active compound.[162]

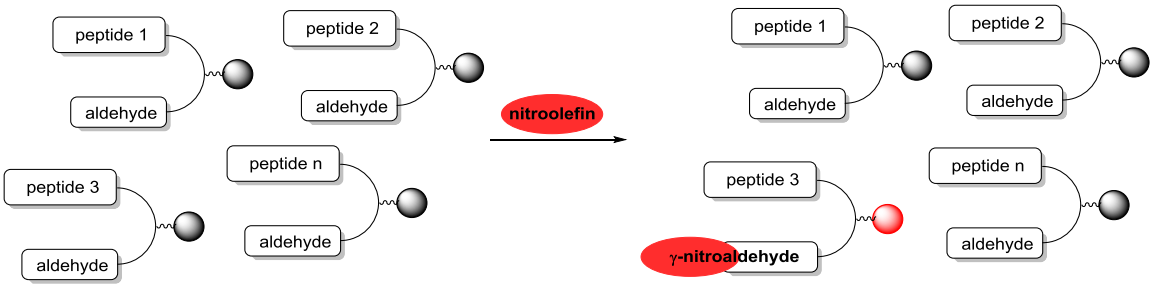


Scheme 5-4 The concept of catalyst-substrate co-immobilization for the identification of catalytically active compounds.

Catalyst-substrate co-immobilization is particularly suited for the identification of catalytically active peptides. For example, an encoded peptide library can be prepared (by the split-and-mix method, see above), with the member attached to one amino group of a lysine based linker. The other amine of the linker can be functionalized with the desired substrate for the reaction. The second substrate which is free in solution is then typically

labeled with a dye such as Disperse Red. After the reaction the resin is washed thoroughly and the beads which remain colored after the wash are then isolated and analyzed.
In previous studies, our group used the method of catalyst-substrate co-immobilization to identify peptides of the general type Pro-Pro-Xaa (Xaa = variable amino acid with a carboxylic acid group) as excellent catalysts for aldol reactions between acetone and aromatic aldehydes. Particularly, tripeptide H-Pro-Pro-Asp-NH_2 proved to be a highly active and selective catalyst.[163-164]

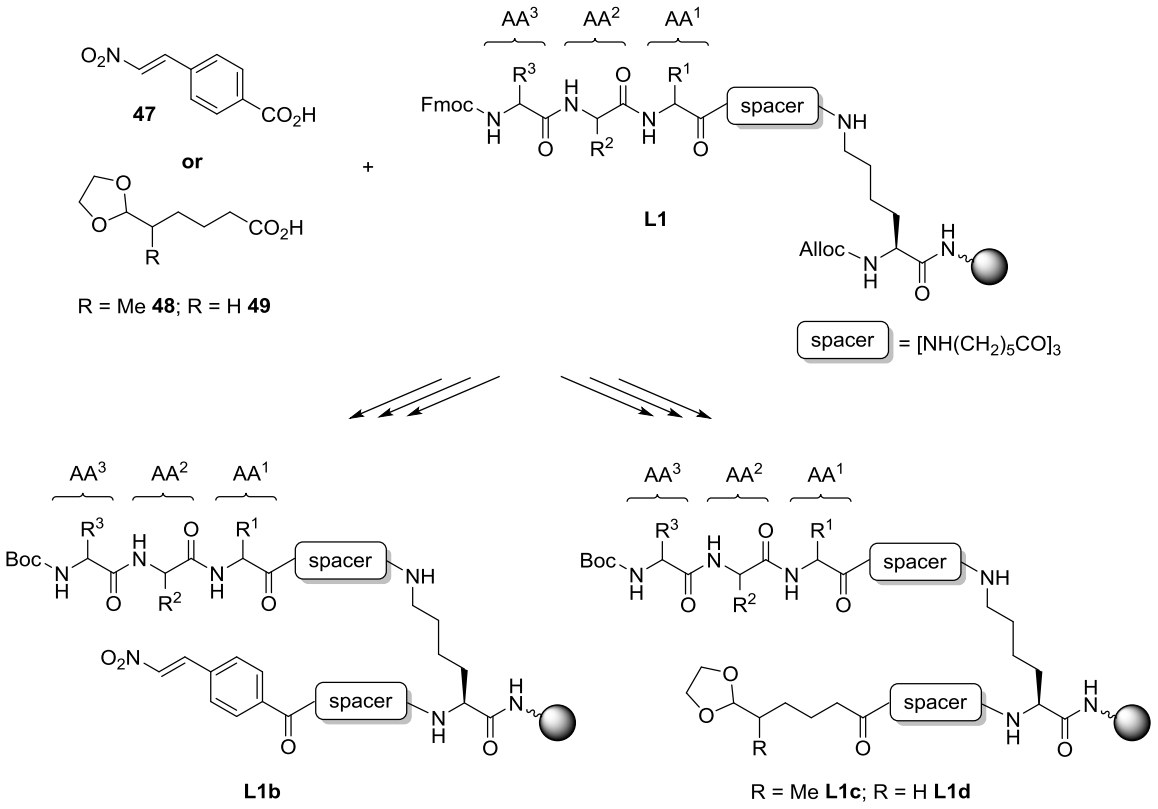
In order to find potential catalytically active peptides for the conjugate addition reaction between α,α-disubstituted aldehydes and β-monosubstituted nitroolefins we envisioned to use a structurally diverse peptide library functionalized with either an aldehyde (Scheme 5-5) or a nitroolefin.



Scheme 5-5 Example of a combinatorial screening approach using an aldehyde co-immobilized peptide library to identify peptidic catalysts for the conjugate addition reaction between α,α-disubstituted aldehydes and β-substituted nitroolefins.

5.3.2 Design and Functionalization of Peptide Library L1

We started the synthesis of a functionalized peptide library from library **L1** which had been synthesized previously in our group.[217] Library **L1** was immobilized on Tentagel *via* a bifunctional lysine linker and it consisted of 15 different D- and L-amino acids in positions AA1 and AA3. Position AA2 comprised of 15 different amino acids which were either flexible or turn-inducing rigid motifs to allow for large structural peptide diversities (Scheme 5-6). This combination of variable components provided a library consisting of a maximum of 3375 (15^3) potential catalysts.

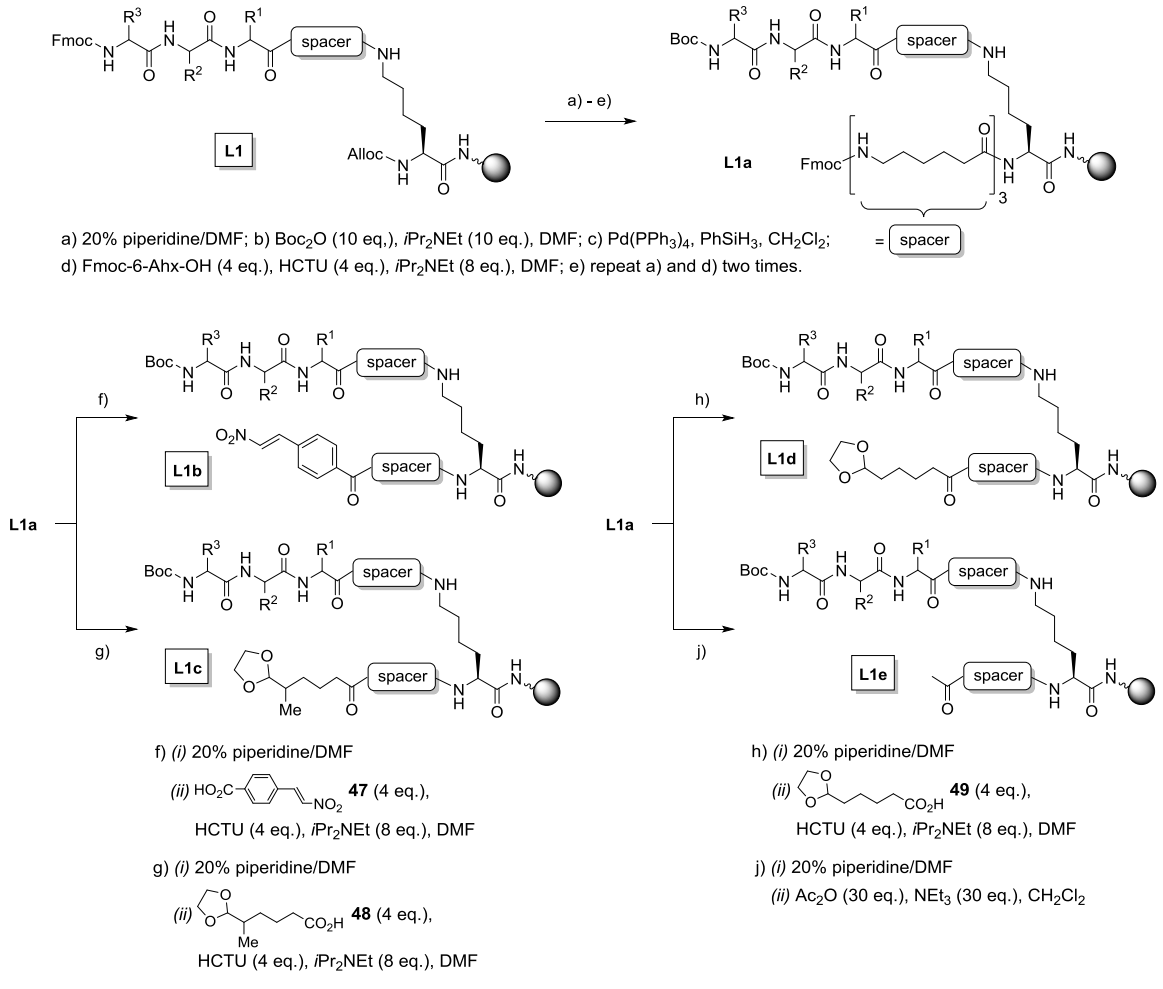


AA^1 = L-Pro, D-Arg, L-Lys, D-His, L-Trp, D-Asp, L-Glu, D-Asn, L-Gln, D-Ser, L-Thr, D-Tyr, L-Phe, D-Val, L-Ala.
AA^2 = L-Pro, L-Pro-Aib, L-Pro-Gly, (1*R*,2*R*)-Achc, (1*S*,2*S*)-Achc, Gly, L-/D-Leu, L-/D-Phe, L-/D-Val, L-Ala, Ahx, no motif
AA^3 = D-Pro, L-Arg, D-Lys, L-His, D-Trp, L-Asp, D-Glu, L-Asn, D-Gln, L-Ser, D-Thr, L-Tyr, D-Phe, L-Val, D-Ala.

Scheme 5-6 Nitroolefin or aldehyde functionalized peptide libraries immobilized on TentaGel.

We planned to couple one of the reaction partners to the other end of the bifunctional linker in order to obtain the catalyst-substrate co-immobilized library. We decided to prepare both versions, nitroolefin co-immobilized library **L1b** and aldehyde (protected as acetal) co-immobilized library **L1c** (Scheme 5-6). Additionally, we functionalized library **L1** also with linear aldehyde **49** (protected as acetal) in order to test whether with the corresponding library **L1d** peptides of the type Pro-Pro-Xaa (Xaa = acidic amino acid) such as H-D-Pro-Pro-Glu-NH_2 (**1**) will be identified which are excellent catalysts for reactions between linear aldehydes and β-monosubstituted nitroolefins.[165-166,173-174]

In order to obtain libraries **L1b**, **L1c** and **L1d**, functionalization of library **L1** was performed according to the strategy depicted in Scheme 5-7. First, the N-terminal Fmoc-protecting groups were removed and the resulting amines were reprotected with Boc groups. Subsequently, the Alloc group was removed using $Pd(PPh_3)_4$ and phenylsilane followed by coupling with Fmoc-protected 6-aminohexanoic acid as spacer using 2-(6-Chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) as coupling reagent (Scheme 5-7, top). The resulting library **L1a** was split into four equal portions (Scheme 5-7, bottom). Three of them were coupled with the substrates **47 – 49** to provide libraries **L1b**, **L1c** and **L1d**. The fourth portion was used to prepare the acetyl-protected library **L1e**.



Scheme 5-7 Preparation of catalyst-substrate co-immobilized libraries **L1b**, **L1c**, **L1d** and acetylated library **L1e**.

5.3.3 Screening of Functionalized Peptide Library L1

Screenings of libraries **L1b**, **L1c** and **L1d** were performed with the Disperse Red dye-labeled aldehyde **50** and nitroolefin **51**, respectively, as substrates in the conjugate addition reactions (Figure 5-1).

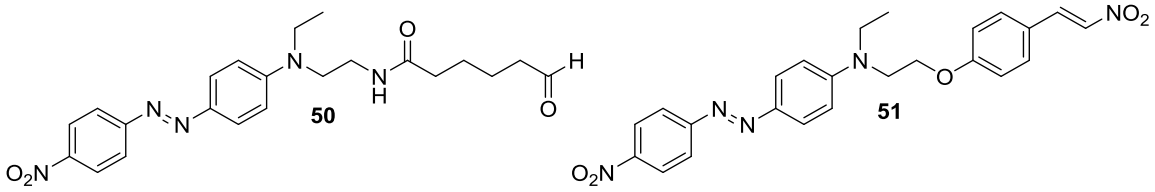


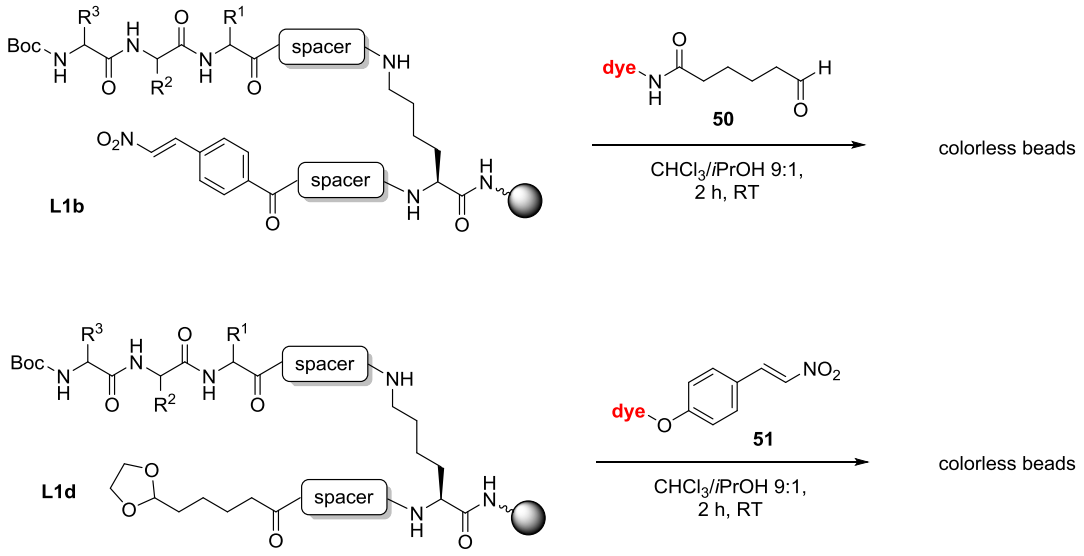
Figure 5-1 Disperse Red labeled reaction partners for the functionalized peptide libraries.

The screening assays were performed with 10 mg of a given library which corresponded to at least three copies of each library member. The library was globally deprotected (the acetal and N-terminal Boc group as well as the acid-labile side chain protecting groups) immediately prior to screening. The dye-labeled substrate **50** or **51** was reacted with the library in a 9:1 mixture of $CHCl_3$ and *i*PrOH in a concentration of 2.5 mM at room temperature for 2 h. Afterwards, the resin was washed thoroughly to remove any unbound dye-labeled substrate and subsequently examined by visual inspection under a low power microscope. The most intensely colored beads were isolated and transferred individually into capillary tubes. The tags, which were coupled to the resin during the synthesis of the peptide library, thus allowing for identification of every individual library member (see Chapter 5.3.1.2), were then cleaved by UV light and analyzed by GC-ECD in order to reveal the sequence of the tripeptide (see experimental part, Chapter 7.2.3, Protocol M for details).

5.3.3.1 Negative Controls

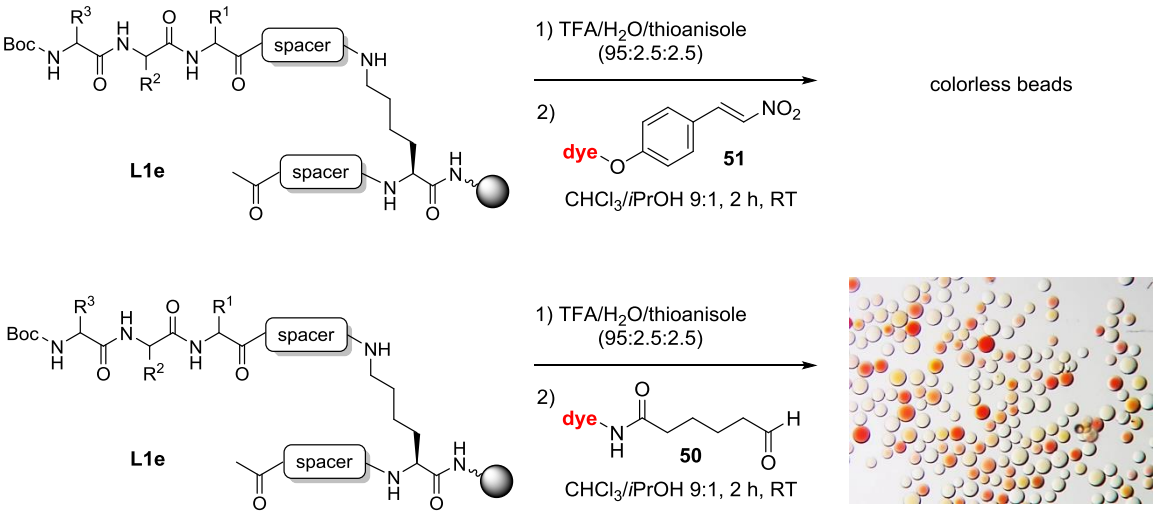
Before we started to screen libraries **L1b**, **L1c** and **L1d**, a series of negative control assays were conducted in order to test for false positive results. First, we exposed library **L1b** to dye-labeled aldehyde **50** and library **L1d** to dye-labeled nitroolefin **51** under the screening conditions described above with the exception that global deprotection of the libraries was omitted (Scheme 5-8). Since no free functionalities that could potentially

catalyze the reaction between the co-immobilized substrate and the dye-labeled substrate were present, and furthermore, the aldehyde within **L1d** was still protected as the acetal, we expected to observe no colored beads. Indeed, after 2 h reaction time and upon washing the resin briefly with CH_2Cl_2 only colorless beads were obtained (Scheme 5-8).



Scheme 5-8 Negative control assays with fully protected libraries **L1b** and **L1d**.

Next, two negative control assays were performed with the unfunctionalized library **L1e** (Scheme 5-9). The library was again subjected to the screening conditions but first globally deprotected. These negative controls would show whether reactions can occur between the peptides of the library and the dye-labeled substrates **50** or **51** leading to stable intermediates and thus to colored beads. Evaluation of library **L1e** in the presence of dye-labeled nitroolefin **51** resulted in colorless beads, meaning that there was no covalently bound dye-labeled substrate **51** (Scheme 5-9, top). In contrast, performing the control assay with dye-labeled aldehyde **50** revealed that some of the beads (about one out of 20) had picked up the red color indicating that the peptides on those beads had reacted with the dye-marked aldehyde **50** (Scheme 5-9, bottom). Even after intensive washing with different solvents, acid and base (see experimental part, Chapter 7.2.3, Protocol M for details), the beads remained red colored indicating that stable intermediates or side products had been formed.



Scheme 5-9 Negative control assays with unfunctionalized library **L1e**.

Several of the red beads were isolated and the peptide sequences on them were analyzed (Table 5-5). The results showed a high selectivity for three amino acids threonine, tryptophan and histidine. Threonine and histidine were found almost exclusively at the N-terminus and tryptophan was predominantly present at the C-terminus of the peptides.

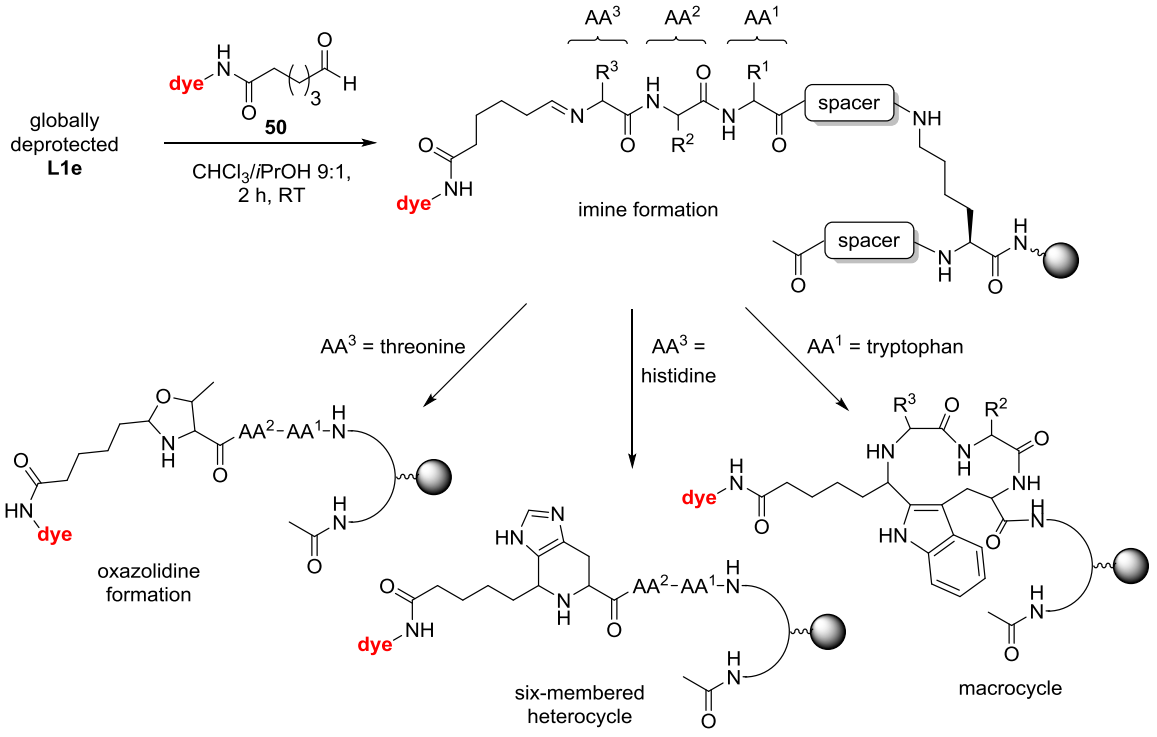
Table 5-5 Identified peptide sequences in the negative control assay of library **L1e** with dye-labled aldehyde **50**.

N-terminus		C-terminus	N-terminus		C-terminus
AA3	**AA2**	**AA1**	**AA3**	**AA2**	**AA1**
D-Thr	(1S,2S)-Achc	L-Ala	L-His	L-Pro-Aib	D-Tyr
D-Thr	(1S,2S)-Achc	L-Pro	L-His	Ahx	L-Pro
D-Thr	L-Pro-Gly	L-Trp	L-His	Ahx	L-Pro
D-Thr	L-Pro-Gly	L-Trp	L-His	L-Pro	L-Pro
D-Thr	L-Pro-Gly	L-Trp	L-His	L-Pro	L-Pro
D-Thr	L-Pro-Gly	L-Trp	L-His	L-Pro-Gly	L-Trp
D-Thr	Ahx	L-Trp	L-His	L-Pro-Gly	L-Trp
D-Thr	Ahx	L-Trp	L-His	Ahx	L-Trp
D-Thr	L-Pro-Aib	L-Trp	L-His	Ahx	L-Trp
D-Thr	L-Val	L-Trp	L-His	D-Leu	L-Trp
D-Thr	L-Pro	L-Trp	L-His	L-Pro	L-Trp
D-Thr	(1S,2S)-Achc	L-Trp	L-His	L-Pro	L-Trp
D-Thr	(1S,2S)-Achc	L-Trp	L-His	L-Pro	L-Trp
D-Thr	(1R,2R)-Achc	L-Trp	L-His	L-Pro	L-Trp
D-Thr	(1R,2R)-Achc	L-Trp	L-His	D-Val	L-Trp
D-Trp	(1R,2R)-Achc	L-Trp	L-His	(1R,2R)-Achc	L-Trp
D-Trp	(1R,2R)-Achc	L-Phe	L-Ser	L-Pro	L-Trp

Achc = 2-Aminocyclohexanecarboxylic acid, Ahx = 6-Aminohexanoic acid.

In the second position (AA2), amino acids, which were turn-inducing rigid motifs (*e.g.*, proline and 2-aminocyclohexanecarboxylic acid (Achc)), were found more often than flexible ones (*e.g.*, valine, leucine and 6-aminohexanoic acid (Ahx)). The two most frequent sequences found in this negative control assay were D-Thr-Taa-L-Trp and L-His-Taa-L-Trp (Taa = turn-inducing amino acid). It needs to be mentioned that only sequences with a D-Thr and L-His in position AA3 could be found since only D-Thr and L-His but not L-Thr and D-His were introduced in this position during the library synthesis (see Scheme 5-6).[217] The same goes for L-Trp in position AA1.

We assumed that in the negative control assay of library **L1e** with dye-labeled aldehyde **50**, first the free N-terminal amino groups of the peptides reacted with **50** to form the corresponding imines (Scheme 5-10).



Scheme 5-10 Suggested reactions of library members containing N-terminal threonine, histidine or C-terminal tryptophan in the presence of dye-marked aldehyde **50**.

The observed high selectivity for threonine, tryptophan and histidine indicated subsequent reactions between the side-chains of these residues and the formed imine. For example, peptides bearing threonine at the N-terminus (AA3 position) could have formed an oxazolidine ring through reaction of the imine and the hydroxyl group of the threonine

side chain. Furthermore, a Pictet-Spengler reaction could have occurred with peptides that contained histidine at the N-terminus leading to the formation of six-membered heterocycles. A similar reaction between the tryptophan residue at the C-terminus (AA1 position) and the imine resulting in formation of a macrocycle might also be envisioned (Scheme 5-10).[218-220]

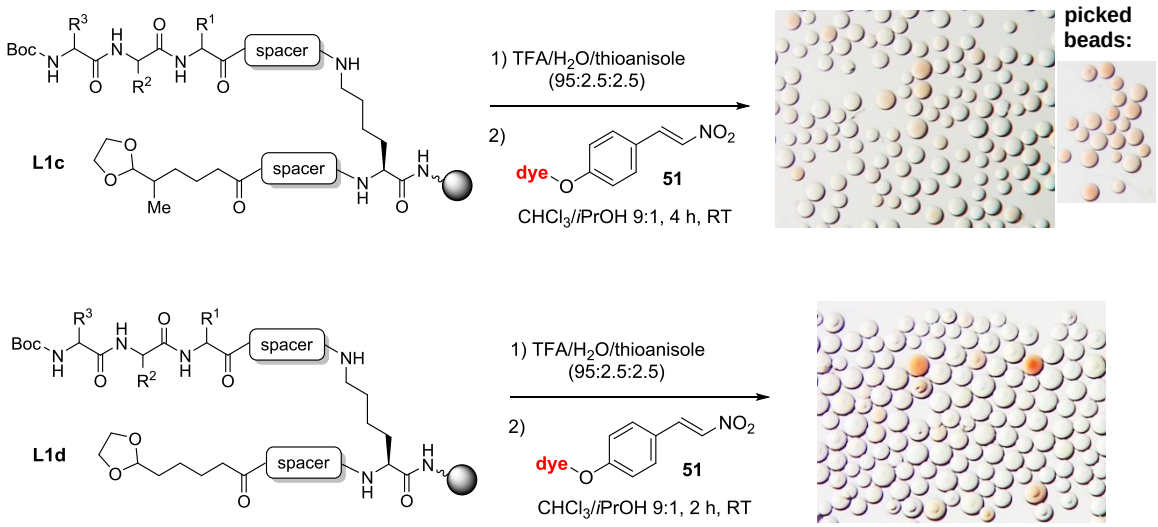
5.3.3.2 Screening Assays

The false positive results obtained in the negative control assay of library **L1e** using dye-labeled aldehyde **50** as reaction partner, led to the decision to perform the screenings for the identification of potential catalysts for the conjugate addition reaction with library **L1c** rather than **L1b**, thus avoiding the problem of potential side reactions of the peptides with the aldehyde in solution as mentioned above. Nevertheless, one has to keep in mind that, when the aldehyde is co-immobilized with the solid-supported peptide library and the nitroolefin is the dye-labeled reaction partner in solution, peptides containing threonine, tryptophan and histidine could still potentially react in an intramolecular fashion with the co-immobilized aldehyde leading to the formation of heterocycles similar to the ones depicted in Scheme 5-10. This would unfortunately eliminate those peptide sequences similar to those which led to false positive results in the negative control assay from potential catalysts of the conjugate addition reaction. We were however hoping that we should still be able to identify potent peptidic catalysts among the remaining library members.

After global deprotection of library **L1c** and screening with Disperse Red labeled nitroolefin **51** according to the conditions described above (Chapter 5.3.3; a reaction time of 4 h was necessary in the assay of **L1c** in order to achieve visible coloration of the beads), we observed that about one in 30 beads were of pale red color (Scheme 5-11, top). The colored beads indicated that a reaction between the bead-bound aldehyde and the nitroolefin **51**, mediated by the peptides on those beads, had occured.

Parallel to library **L1c** we also screened library **L1d** which was co-immobilized with a linear aldehyde in order to test whether the assay will reveal peptide sequences such as H-D-Pro-Pro-Glu-NH_2 (**1**) and H-D-Pro-Pro-D-Asp-NH_2 that are among the library members and powerful catalysts for the conjutate addition reaction between linear

aldehydes and β-monosubstituted nitroolefins.[165-166,173-174] After 2 h reaction time followed by an intensive washing of the resin about one out of 500 beads were orange colored (Scheme 5-11, bottom).



Scheme 5-11 Screening assay with libraries **L1c** and **L1d**.

Analysis of the most intense colored beads isolated from the two screenings revealed the corresponding peptide sequences (Table 5-6). Again, as in the negative control experiment of library **L1e** (Table 5-5), a high selectivity for tryptophan was observed in both assays. However, this time, tryptophan was predominantly found at the N-terminus and not at the C-terminus. Furthermore, sequences containing N-terminal threonine and histidine were not observed suggesting that these peptides might have reacted intramolecularly with the co-immobilized aldehyde leading to the proposed oxazolidine formation (Scheme 5-10). Besides tryptophan, glutamine was also found a few times at the N-terminus in the assay of library **L1c**. In the screening assay of library **L1d** arginine and phenylalanine were the most common residues at the C-terminus. In both assays, the sequence D-Trp-Pro-Phe was found and position AA2 contained only turn-inducing proline-derived motifs.

Notably, peptide sequences such as D-Pro-Pro-Glu or D-Pro-Pro-D-Asp were not found in the assay of library **L1d** with the co-immobilized linear aldehyde, suggesting that peptides with tryptophan were either more selective catalysts for the conjugate addition

reaction between the aldehyde and nitroolefin **51** or that they formed selectively a side product where the dye-marked nitroolefin was bound to the resin.

Table 5-6 Identified peptide sequences in the screenings of library **L1c** and **L1d** with dye-labled nitroolefin **51**.

Library **L1c**

(functionalized with branched aldehyde)

N-terminus		C-terminus
AA3	**AA2**	**AA1**
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro-Gly	L-Phe
D-Trp	L-Pro-Gly	L-Lys
D-Trp	L-Pro-Aib	L-Lys
D-Trp	L-Pro-Gly	L-Pro
D-Trp	L-Pro-Gly	L-Pro
D-Trp	L-Pro	D-Ser
D-Trp	L-Pro	L-Thr
D-Trp	L-Pro	D-Tyr
D-Trp	L-Pro	L-Trp
D-Phe	L-Pro	L-Trp
D-Gln	L-Pro-Aib	L-Trp
D-Gln	L-Pro-Aib	L-Trp
D-Gln	L-Pro	L-Lys
D-Gln	L-Pro-Aib	L-Thr
D-Gln	L-Pro	L-Phe
D-Lys	L-Pro-Gly	L-Trp
L-Tyr	L-Pro-Gly	L-Pro

Library **L1d**

(functionalized with linear aldehyde)

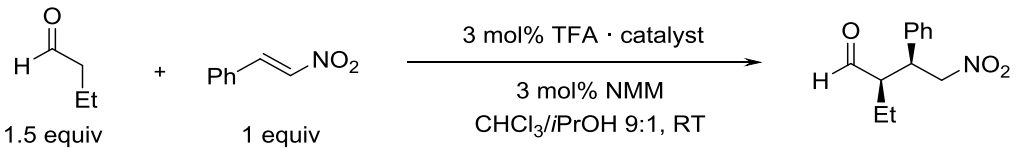
N-terminus		C-terminus
AA3	**AA2**	**AA1**
D-Trp	L-Pro-Gly	D-Arg
D-Trp	L-Pro-Gly	D-Arg
D-Trp	L-Pro-Gly	D-Arg
D-Trp	L-Pro-Gly	D-Arg
D-Trp	L-Pro-Gly	D-Arg
D-Trp	L-Pro-Gly	D-Val
D-Trp	L-Pro-Gly	D-Val
D-Trp	L-Pro-Gly	D-Tyr
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Phe
D-Trp	L-Pro	L-Ala
D-Trp	L-Pro	L-Ala

5.3.4 Investigations on the Identified Catalysts from Functionalized Library L1

In order to probe the catalytic properties of the tryptophan-containing peptide sequences found in the screening, we next synthesized peptides H-D-Trp-Pro-Gly-D-Arg-NH_2 (**52**) and H-D-Trp-Pro-Phe-NH_2 (**53**) and evaluated their activity in solution-phase conjugate addition reactions between butanal and nitrostyrene (Table 5-7). The two peptides were prepared by solid-phase peptide synthesis using the Fmoc/*t*Bu protocol and were obtained as their TFA-salts (see experimental part, Chapter 7.3.2 for details). Unfortunately compared to H-D-Pro-Pro-Glu-NH_2 (**1**), which is an excellent catalyst for the conjugate addition reaction, both peptides, **52** and **53**, provided the γ-nitroaldehyde

product in poor conversion and with low stereoselectivity. Peptide **52** was not fully soluble in the $CHCl_3$/*i*PrOH 9:1 solvent mixture and thus only about 20% conversion to the γ-nitroaldehyde was observed even after an extended reaction time of 72 h (Table 5-7, entry 1). Furthermore, a low diastereoselectivity (8:1) and enantioselectivity (38%) was observed. In the presence of 3 mol% of peptide **53**, the γ-nitroaldehyde was formed also in significantly smaller amounts (23% conversion) and lower stereoselectivity (3:1 d.r., 84% ee) than with catalyst **1** (90% conv., 43:1 d.r., 96% ee; Table 5-7, entries 2 and 3).

Table 5-7 Comparison of the catalytic properties of peptides H-D-Trp-Pro-Gly-D-Arg-NH_2 (**52**) and H-D-Trp-Pro-Phe-NH_2 (**53**) with H-D-Pro-Pro-Glu-NH_2 (**1**) in the 1,4-addition reaction between butanal and nitrostyrene.



	catalyst	time [h]	conversion [%][a]	d.r.[a]	ee [%][b]
1[c]	TFA·H-D-Trp-Pro-Gly-D-Arg-NH_2 (**52**)	72	20	8:1	38
2	TFA·H-D-Trp-Pro-Phe-NH_2 (**53**)	3	19 (25)[d]	3:1	84
3	TFA·H-D-Pro-Pro-Glu-NH_2 (**1**)	3	90	43:1	96[e]

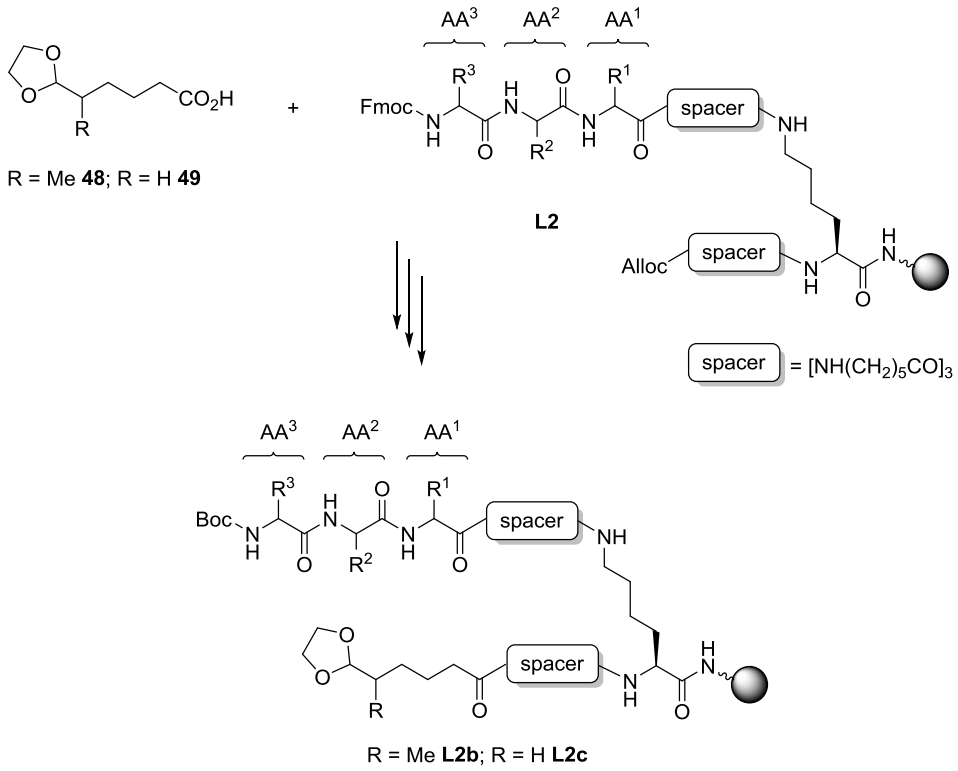
[a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis. [c] 6 mol% NMM was used. [d] Conversion after 24 h in brackets. [e] The opposite enantiomer was formed.

The observed low catalytic activity of peptides containing N-terminal tryptophan indicated that the coloration of the beads in the library screening assays presumably resulted from the covalent binding of dye-labeled nitroolefin **51** to the resin by formation of a side product derived from nitroolefin **51**, the immobilized aldehyde and the tryptophan residue rather than from the expected peptide-mediated conjugate addition of the immobilized aldehyde to dye-labeled nitroolefin **51**.

In order to avoid false positive results caused by tryptophan residues, we next performed the screening assays with another peptide library that did not contain tryptophan.

5.3.5 Design and Functionalization of Peptide Library L2

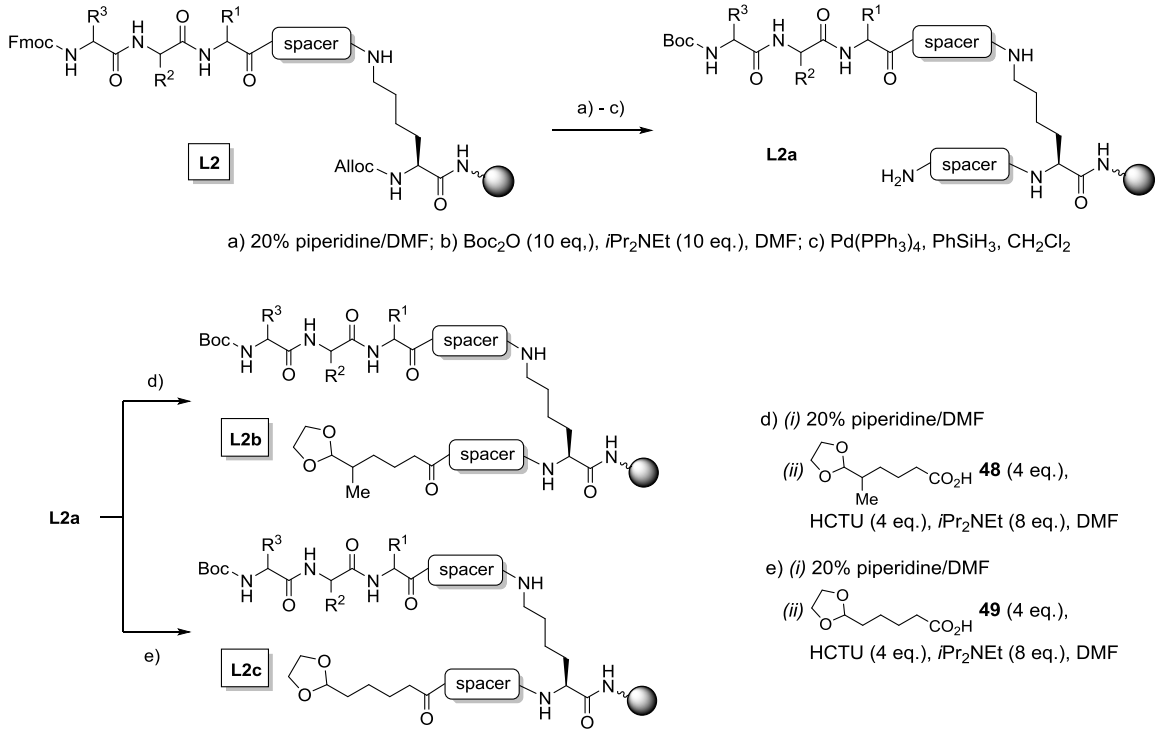
We used tripeptide library **L2** which had also been prepared previously in our group.[163-164] Similarly to library **L1**, it was immobilized on Tentagel *via* a bifunctional lysine linker and consisted of 15 different D- and L-amino acids in each of the three positions AA1 to AA3, again resulting in a maximum of 3375 (15^3) different tripeptides (Scheme 5-12). Instead of tryptophan and lysine, which were present in library **L1**, proline and glycine were introduced. We also functionalized library **L2** with an aldehyde (protected as the acetal) through attachment to the other amino group of the lysine linker. As before, not only library **L2b**, co-immobilized with a branched aldehyde, but also library **L2c** with a linear one was prepared to test if H-D-Pro-Pro-Glu-NH_2 (**1**) will be present among the identified hit sequences (Scheme 5-12).



AA^1 and AA3 = L-Pro, D-Pro, L-Arg, L-His, Gly, L-Asp, L-Glu, L-Asn, L-Gln, L-Ser, L-Thr, L-Tyr, L-Phe, L-Val, L-Ala.
AA^2 = L-Pro, D-Pro, D-Arg, D-His, Gly, D-Asp, D-Glu, D-Asn, D-Gln, D-Ser, D-Thr, D-Tyr, D-Phe, D-Val, D-Ala.

Scheme 5-12 Design of aldehyde functionalized peptide libraries **L2b** and **L2c** immobilized on TentaGel.

Libraries **L2b** and **L2c** were prepared from library **L2** according to Scheme 5-13. First, the N-terminal Fmoc-protecting groups were removed and the resulting amines were reprotected with Boc groups. Subsequently, the Alloc group was removed using $Pd(PPh_3)_4$ and phenylsilane (Scheme 5-13, top). The resulting library **L2a** was then split into two equal portions which were coupled with the substrates **48** and **49**, respectively, using 2-(6-Chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) as the coupling agent (Scheme 5-13, bottom).

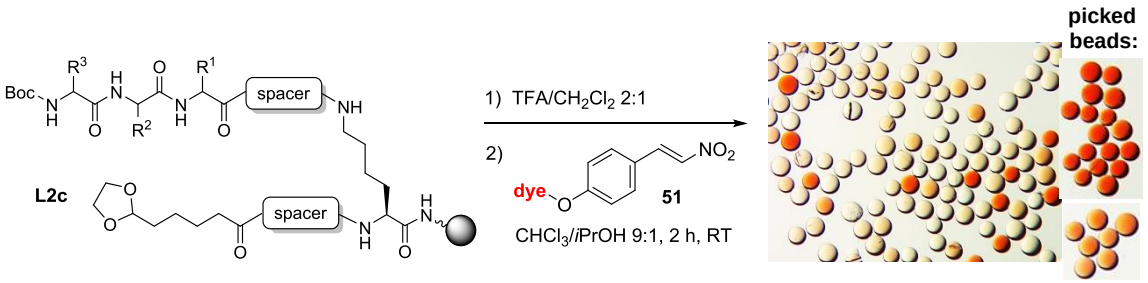


Scheme 5-13 Preparation of catalyst-substrate co-immobilized libraries **L2b** and **L2c**.

5.3.6 Screening of Functionalized Peptide Library L2

Screenings of libraries **L2b** and **L2c** were performed under the conditions described above for functionalized library **L1**, using the Disperse Red dye labeled nitroolefin **51** as the substrate in solution (see Chapter 5.3.3).

First, we screened library **L2c** functionalized with the linear aldehyde. After global deprotection of the library with TFA/CH_2Cl_2 2:1 the resin was exposed to the general screening conditions (Scheme 5-14).



Scheme 5-14 Screening assay of library **L2c**.

Approximately one out of 20 beads had turned red but orange colored beads were also observed (about one out of 20). Apart from the red beads a few orange ones were also isolated to compare whether different peptide sequences are found on those beads. The results are summarized in Table 5-8.

Almost all sequences found on both, the orange and the red colored beads, contained proline at the N-terminus and in position AA2. Interestingly, different types of amino acids were found at the C-terminus. In the case of orange colored beads acidic residues, aspartic acid and glutamic acid (highlighted in red), were predominantly found, whereas the sequences from red colored beads contained either amino acids with hydrophobic (highlighted in orange) or polar uncharged side chains (highlighted in green). These findings were somewhat unexpected since we previously showed that peptides of the type Pro-Pro-Xaa (Xaa = acidic amino acid) such as H-D/L-Pro-Pro-Asp-NH_2 and H-D-Pro-Pro-Glu-NH_2 (**1**) are catalytically more active than those lacking an intramolecular carboxylic acid group.[166,172] Thus, we expected to find sequences containing aspartic acid and glutamic acid in positon AA3, rather on darker colored beads than on the orange ones. Nevertheless, the results showed that the combinatorial screening assay of the aldehyde functionalized peptide library **L2c** with dye-labeled nitroolefin **51** allowed for the identification of effective catalysts for the conjugate addition reaction between linear aldehydes and β-monosubstituted nitroolefins.

Table 5-8 Identified peptide sequences in the screenings of library **L2c** with dye-labled nitroolefin **51**.

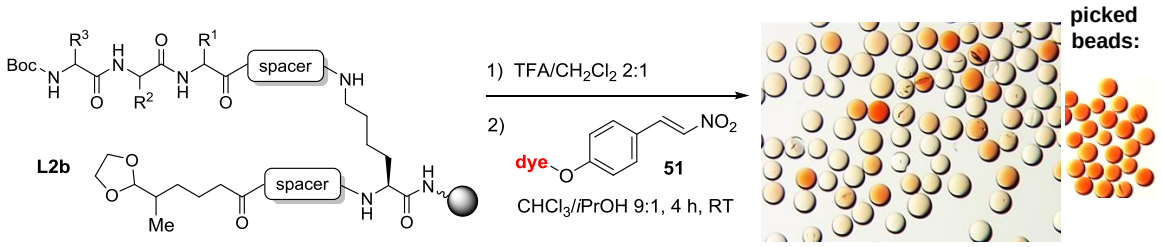
sequences on orange colored beads

N-terminus		C-terminus
AA3	**AA2**	**AA1**
L-Pro	L-Pro	L-Asp
L-Pro	L-Pro	L-Asp
D-Pro	L-Pro	L-Asp
L-Pro	D-Ala	L-Asp
L-Pro	D-Gln	L-Glu
L-Pro	D-Tyr	L-Glu
D-Pro	L-Pro	L-Arg

sequences on red colored beads

N-terminus		C-terminus
AA3	**AA2**	**AA1**
D-Pro	D-Pro	L-Ala
L-Pro	D-Pro	L-Ala
L-Pro	D-Pro	L-Val
L-Pro	D-Pro	L-Val
L-Pro	D-Pro	L-Val
L-Pro	D-Pro	D-Pro
L-Pro	L-Pro	D-Pro
D-Pro	D-Pro	L-Phe
L-Pro	L-Pro	Gly
D-Pro	L-Pro	L-Asn
L-Pro	L-Pro	L-Asn
L-Pro	D-Pro	L-Asn
L-Pro	D-Pro	L-Gln
L-Pro	L-Pro	L-Gln
L-Pro	L-Pro	L-Tyr
D-Pro	D-Pro	L-Tyr
D-Pro	L-Pro	L-Ser

Screening of library **L2b** under the previously described conditions was performed next. It resulted in orange coloration of every one out of 15 beads (Scheme 5-15).



Scheme 5-15 Screening assay of library **L2b**.

Analysis of the peptide sequences showed a high selectivity for glycine at the N-terminus in this assay (Table 5-9). In the second position (AA2) proline was the most abundant amino acid as in the screening of library **L2c**. Hydrophobic amino acids such as phenylalanine and valine (highlighted in orange) but also glycine were predominantly found at the C-terminus. Interestingly, no sequences with acidic amino acids were observed.

Table 5-9 Identified peptide sequences in the screenings of library **L2b** with dye-labled nitroolefin **51**.

N-terminus		C-terminus
AA3	**AA2**	**AA1**
Gly	D-Ala	L-Asn
Gly	D-Arg	L-Gln
Gly	L-Pro	L-Arg
Gly	D-Pro	L-Arg
Gly	D-Pro	L-Phe
Gly	L-Pro	L-Phe
Gly	L-Pro	L-Phe
Gly	L-Pro	L-Phe
Gly	L-Pro	L-Phe
Gly	L-Pro	Gly

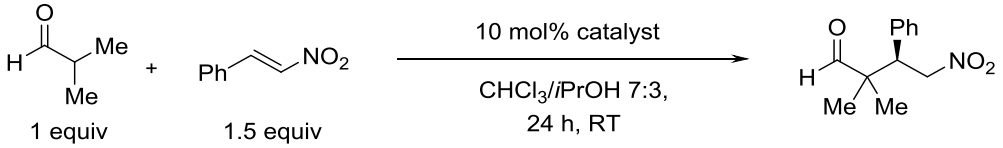
N-terminus		C-terminus
AA3	**AA2**	**AA1**
Gly	L-Pro	Gly
Gly	L-Pro	Gly
L-Ala	D-Pro	Gly
L-Ala	D-Pro	L-Val
L-Phe	D-Pro	L-Phe
L-Tyr	L-Pro	L-Val
L-Tyr	L-Pro	L-Phe
L-Tyr	D-Pro	Gly
L-Gln	D-Pro	Gly
L-Glu	D-Pro	L-Tyr

5.3.7 Investigations on the Identified Catalysts from Functionalized Library L2

The peptide sequence H-Gly-Pro-Phe-NH_2 (**54**), which occurred most frequently (four times) on beads collected in the assay of library **L2b**, was next resynthesized and tested as a catalyst in the solution phase addition reaction of isobutyraldehyde to nitrostyrene (Table 5-10). Additionally, a rationally designed peptide H-Gly-Pro-Glu-NH_2 (**55**) bearing glutamic acid, an intramolecular proton donor, at the C-terminus was also prepared.

In the presence of 10 mol% of peptide **54** the corresponding γ-nitroaldehyde was obtained in only low conversion of 20% and with 43% ee even after 48 h reaction time (Table 5-10, entry 1). No improvement was observed when TFA and NMM were used as additives in equimolar amounts to the catalyst (Table 5-10, entry 2). In the presence of 20 mol% acetic acid as additive hardly any conversion to the γ-nitroaldehyde was observed (Table 5-10, entry 3). Carrying out the reaction with an excess of aldehyde (5 eq) with respect to nitrostyrene proved to be worse than using the nitroolefin in excess (Table 5-10, compare entries 1 and 4). Peptide **55** with a C-terminal glutamic acid was an even poorer catalyst than **54**. Since **55** could not be fully dissolved in the $CHCl_3$/*i*PrOH 7:3 solvent mixture, a higher amount of *i*PrOH was used. Unfortunately, however, no improvement in reactivity was achieved (Table 5-10, entries 5 and 6).

Table 5-10 Catalytic properties of peptides H-Gly-Pro-Phe-NH_2 (**54**) and H-Gly-Pro-Glu-NH_2 (**55**) in the 1,4-addition reaction between isobutyraldehyde and nitrostyrene.



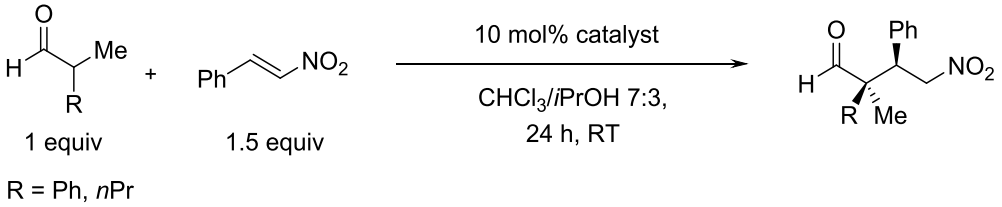
	catalyst	conversion [%][a]	ee [%][b]
1	H-Gly-Pro-Phe-NH_2 (**54**)	18 (20)[c]	43
2	H-Gly-Pro-Phe-NH_2 (**54**) + TFA/NMM[d]	15 (16)[c]	45
3	H-Gly-Pro-Phe-NH_2 (**54**) + AcOH[e]	4	n.d.[f]
4[g]	H-Gly-Pro-Phe-NH_2 (**54**)	8	n.d.[f]
5[h]	H-Gly-Pro-Glu-NH_2 (**55**)	3 (4)[c]	29
6[i]	H-Gly-Pro-Glu-NH_2 (**55**)	5	n.d.[f]

The concentration of the reactions was 0.44 M with respect to butanal. [a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis (DAICEL Chiralpak OD-H column, 250 x 4.6 mm, hexane/*i*PrOH 80:20, 25°C, 1 mL/min, UV254 nm, t_R (minor) = 13.3 min, t_R (major) = 18.5 min). [c] Conversion after 48 h in brackets. [d] 10 mol% TFA and 10 mol% NMM were used. [e] 20 mol% AcOH was used. [f] Not determined. [g] Reaction was carried out with 5 equiv. of isovaleraldehyde and 1 equiv. of nitrostyrene. [h] Catalyst was not fully dissolved in the reaction medium. [i] Reaction was carried out in $CHCl_3$/*i*PrOH 1:9.

Similarly disappointing results were obtained for peptides **54** and **55** in reactions with other aldehydes, 2-phenylpropanal or 2-methylvaleraldehyde, and nitrostyrene under the same reaction conditions (Table 5-11, entries 1-3).

Interstingly, using the same aldehyde substrates, 2-phenylpropanal or 2-methyl-valeraldehyde, and the thiourea peptide **44**, which proved to be a good catalyst for the reaction between isobutyraldehyde and nitrostyrene (see Table 5-3, entry 6), low conversions to the corresponding γ-nitroaldehydes were also observed (Table 5-11, entries 4 and 5). The γ-nitroaldehyde with the propyl substituent at C(2) was however formed with a relatively good 75% ee (Table 5-11, entry 5).

Table 5-11 Catalytic properties of peptides H-Gly-Pro-Phe-NH_2 (**54**) and H-Gly-Pro-Glu-NH_2 (**55**) in the 1,4-addition reaction of 2-phenylpropanal or 2-methylvaleraldehyde with nitrostyrene.



R = Ph, *n*Pr

	catalyst	R	conversion [%][a]	d.r.[a]	ee [%][b]
1	H-Gly-Pro-Phe-NH_2 (**54**)	Ph	5 (6)[c]	15:1	n.d.[d]
2	H-Gly-Pro-Phe-NH_2 (**54**)	*n*Pr	10 (10)[c]	3:1	33
3	H-Gly-Pro-Glu-NH_2 (**55**)	Ph	3 (4)[c]	-[e]	n.d.[d]
4	thiourea peptide **44**	Ph	20 (30)	7:1	14
5	thiourea peptide **44**	*n*Pr	10 (15)[c]	3:1	75[f]

The concentration of the reactions was 0.44 M with respect to butanal. [a] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [b] Determined by chiral-phase HPLC analysis (DAICEL Chiralpak OJ-H column, 250 x 4.6 mm, hexane/*i*PrOH 90:10, 25°C, 1 mL/min, UV254 nm, t_R (syn, major) = 21.7 min, t_R (syn, minor) = 34.0 min). [c] Conversion after 48 h in brackets. [d] Not determined. [e] The minor diastereoisomer was not visible in the ^{1}H-NMR spectrum. [f] The opposite enantiomer was formed.

5.4 Conclusions

The conjugate addition reaction between α,α-disubstituted aldehydes and β-substituted nitroolefins provides direct access to synthetically valuable γ-nitroaldehydes bearing adjacent quaternary and tertiary stereogenic centers. Today, there are only a few examples for this challenging transformation and to the best of our knowledge peptides have not been employed as catalysts.

Having developed peptidic catalyst H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) for the conjugate addition of linear aldehydes to challenging β,β-disubstituted nitroolefins, affording γ-nitroaldehydes with a quaternary stereocenter in β position to the carbonyl group (see Chapter 3), we then decided to aim at the development of short-chain peptides that can promote the conjugate addition of α,α-disubstituted aldehydes to β-monosubstituted nitroolefins. Preliminary experiments using H-D-Pro-Pro-NH-(*R*)-

$CH(Ph)CH_2$-4-Me-C_6H_4 (**10-R**) as catalyst for the model reaction of isovaleraldehyde to nitrostyrene revealed a poor catalytic activity and stereoselectivity of **10-R** for this reaction (15% conversion, 25% ee). Better results were obtained with H-D-Pro-Pro-Glu-NH_2 (**1**), which is an excellent catalyst for the analogous reaction with linear aldehydes,[165-167,170,173-175] and derivatives thereof bearing a thiourea functional group. In the presence of 10 mol% of these peptides, up to quantitative conversion and 85% ee to the corresponding γ-nitroaldehydes from isobutyraldehyde and different aromatic β-monosubstituted nitroolefins bearing a quaternary carbon were observed within 24 h. Performing the reaction with other α-branched aldehydes than isobutyraldehyde such as 2-phenylpropanal proved to be more challenging and the desired conjugate addition product was obtained in considerably lower conversions (20 – 57%) and stereoselectivities (up to 9:1 d.r. and 15% ee).

In order to find more suitable peptidic catalysts for the reaction between α,α-disubstituted aldehydes and β-substituted nitroolefins, a combinatorial screening was performed using the concept of catalyst-substrate co-immobilization. For that purpose, nitroolefin and aldehyde functionalized peptide libraries were prepared and screened against dye-labeled aldehyde and nitroolefin, respectively. Negative control experiments revealed false-positive results when the dye-labeled aldehyde was used as the substrate in solution, thus the library where the aldehyde is immobilized along with the peptides on the solid support was used for further investigations. The assay showed a high selectivity for peptides containing N-terminal tryptophan, however, these peptides proved not to be particularly active catalysts when examined in solution-phase conjugate addition reactions. This led to the conclusion that the selectivity observed in the combinatorial screening for tryptophan-containing peptides was presumably a result of side product formation involving the tryptophan residue. Consequently, the combinatorial screening was then repeated with another aldehyde functionalized tripeptide library lacking tryptophan. In that assay H-Gly-Pro-Phe-NH_2 (**54**) was the most commonly found peptide but also this peptide did not perform well as a catalyst in solution phase.

At this point, it remains unclear why H-Gly-Pro-Phe-NH_2 (**54**) was found selectively in the combinatorial screening but proved to be a rather poor catalyst for solution-phase conjugate addition reactions between α-branched aldehydes and nitrostyrene. Further experiments with peptide **54** are necessary to investigate whether, for example, the

Disperse Red label might have an influence on the observed selectivity of the combinatorial screening. In addition, di- and tetrapeptide libraries which are available in our laboratory should also be tested to search for a suitable catalyst for the reaction of α,α-disubstituted aldehydes to β-substituted nitroolefins. Furthermore, since peptides with the Pro-Pro motif bearing a thiourea group gave promising preliminary results, variations in their structure such as installation of a primary amine at the N-terminus or in the side chain of one of the two proline residues should also be studied.

6

Summary and Outlook

The direct asymmetric generation of quaternary stereogenic centers in acyclic compounds under mild organocatalytic conditions is a challenging task. While many organocatalysts have been developed for the stereoselective formation of tertiary stereocenters, methods for the synthesis of quaternary stereocenters are still limited. Taking advantage of the unique features of short-chain peptides as organocatalysts, peptide-catalyzed conjugate addition reactions between aldehydes and nitroolefins providing access to γ-nitroaldehydes with adjacent quaternary and tertiary stereogenic centers were investigated in this thesis. The general objectives were: 1) to develop a peptidic catalyst for conjugate additions of aldehydes to β,β-disubstituted nitroolefins, 2) to perform mechanistic studies in order to gain insight into the mechanism of these reactions, and 3) to develop a peptide that promotes conjugate addition reactions between α,α-disubstituted aldehydes and β-substituted nitroolefins.

Based on our previous studies of peptides with the Pro-Pro motif as excellent catalysts for conjugate additions of aldehydes to β-mono- and α,β-disubstituted nitroolefins, we have developed the peptide H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) as a potent catalyst for conjugate addition reactions of aldehydes to β,β-disubstituted nitroolefins to afford under mild organocatalytic conditions synthetically useful γ-nitroaldehydes bearing a quaternary stereogenic center adjacent to a tertiary stereocenter. Such reactions with sterically demanding β,β-disubstituted nitroolefins, which are significantly less inclined to form the corresponding addition products than their monosubstituted counterparts, have not been developed before. In the presence of 10 mol% of peptide **10** as its trifluoroacetic acid salt in combination with 10 mol% of the base *N*-methylmorpholine, a variety of aldehydes and both electron-poor as well as electron-rich β,β-disubstituted nitroolefins reacted with high chemoselectivity providing the desired γ-nitroaldehydes in high yields and stereoselectivities. Moreover, we have shown that the obtained γ-nitroaldehydes can be readily converted into chiral pyrrolidines, γ-butyrolactones, and γ-amino acids bearing a quaternary stereogenic center that have so far not been accessed. The results also demonstrated that peptides with the Pro-Pro motif offer with their modularity the possibility to adapt to the structural requirements of challenging substrates such as β,β-disubstituted nitroolefins.

In the second part of this thesis, mechanistic studies of the peptide-catalyzed conjugate addition reaction between aldehydes and β,β-disubstituted nitroolefins are described. FT-IR and NMR spectroscopic investigations revealed that in the presence of weakly acidic co-catalysts (pK_a ~ 4 to 7), the reactions were significantly faster than in the absence of such acidic additive and that a cyclobutane intermediate was formed. As a result, the protonation of the iminium nitronate was suggested as the rate-determining step of these reactions. The use of stronger acids (pK_a < 2) as co-catalysts led to significantly slower reactions and a shift of the rate-limiting step from the nitronate protonation to the C-C bond formation between the enamine and the β,β-disubstituted nitroolefin. These findings are in agreement with previous studies for the analogous reactions with β-monosubstituted nitroolefins and thus strongly indicate the same reaction pathway for both types of β-substituted nitroolefins. Initial mechanistic investigations using ESI-MS back-reaction screening with mass-labeled quasi-enantiomeric substrates suggested that the enantiomeric excess of the reaction product might be correlated with the relative stability and reactivity of downstream intermediates such as diastereomeric cyclobutanes.

Finally, we also aimed at the development of short-chain peptides capable of catalyzing conjugate addition reactions between α,α-disubstituted aldehydes and β-substituted nitroolefins. Preliminary experiments with peptide H-D-Pro-Pro-Glu-NH_2 (**1**) and thiourea-functionalized derivatives thereof gave promising results and demonstrated the potential of peptidic catalysts of the general type Pro-Pro-Xaa (Xaa = acidic amino acid) for these reactions. In order to evaluate other short-chain peptides bearing a N-terminal primary amine as catalysts, a combinatorial screening was performed using the concept of catalyst-substrate co-immobilization. A first attempt using an aldehyde functionalized tripeptide library showed high selectivity for peptides containing N-terminal tryptophan, however, these peptides proved not to be particularly active catalysts when examined in solution-phase. Repeating the combinatorial screening with another aldehyde functionalized tripeptide library lacking tryptophan revealed the sequence H-Gly-Pro-Phe-NH_2 (**54**), but this catalyst was also not a suitable catalyst for this reaction when used in solution phase.

This suggests that further experiments using peptide **54** are necessary to investigate the reason for the observed high selectivity of the combinatorial screening but the poor catalytic activity in solution. Additionally, di- and tetrapeptide libraries available in our laboratory should also be tested in the search of suitable catalysts for reactions of α,α-disubstituted aldehydes and β-substituted nitroolefins. Moreover, due to the promising results with thiourea-functionalized peptides, further structural variations within those peptides should also be examined.

In addition, peptides of the class Pro-Pro-Xaa offer the flexibility to tune their catalytic properties in order to adapt to the requirements of a given substrate combination and thus might also contain members capable of catalyzing other challenging conjugate addition reactions between carbonyl compounds and, for example, disubstituted acrylonitriles or disubstituted vinyl sulfones providing valuable molecules with quaternary stereogenic centers.

7

Experimental Part

7.1 General Aspects and Materials

Materials and reagents were of the highest commercially available grade and used without further purification. Reactions were monitored by thin layer chromatography using Merck silica gel 60 F254 plates. Compounds were visualized by UV, $KMnO_4$ or ninhydrin. Flash chromatography was performed using Merck or Simga Aldrich silica gel 60, particle size 40-63 μm. NMR spectra were recorded on Varian Mercury 300 MHz or Bruker AV300, AV400, DRX500 or DRX600 spectrometers. Chemical shifts (δ) are reported in ppm using TMS or the residual solvent peak as a reference. The assignment of the signals of complex compounds was carried out by COSY, HSQC and HMBC analysis. Ion exchange was performed using StratoSpheres^{TM} SPE cartridges (PL-HCO_3 MP Resin) from Agilent Technologies. Electrospray ionization (ESI) mass spectra were recorded on a Bruker Esquire 3000 Plus or Bruker Amazon Speed spectrometer. The ESI-MS back-reaction screening was performed on a Varian 1200L Quadrupol MS/MS spectrometer using mild desolvation conditions (39 psi nebulizing gas, 4.9 kV spray voltage, 19 psi drying gas at 200°C, 1300 V detector voltage, 110 V capillary voltage. High resolution mass spectra (HRMS) were performed on a Bruker Daltonics maXis spectrometer by the MS service at the Laboratory for Organic Chemistry, ETH Zürich. Elemental analysis was performed on a Perkin-Elmer 240 Analyser (Dr. W. Kirsch, University of Basel). Normal Phase HPLC analysis was performed on an analytical HPLC with a diode array detector SPD-M10A from Shimadzu or on a Dionex UltiMate 3000 HPLC system (Thermo-Fisher) using Chiracel columns (250 mm x 4.6 mm) from Daicel. GC-ECD measurements were performed using an Agilent 7890A Series GC system with electron capture detection from Agilent Technologies with a GC capillary of the type 19091A-102E HP_Ultra 1 (25 m x 0.200 mm x 0.33 μm) from J&W Scientific. *In situ* FT-IR spectroscopy was carried out with a ReactIR 15 spectrometer using a SiComb probe. Optical rotations were measured on a Perkin Elmer Polarimeter 341. Automated solid-phase peptide synthesis was performed on a Syro I Peptide Synthesizer (MultiSynTech).

7.2 General Protocols

7.2.1 General Protocols for Peptide Synthesis in Solution

Most of the peptides were prepared in solution according to the following general protocols.

Protocol A: *General protocol for peptide couplings in solution*

To a suspension of Boc-D-Pro-Pro-OH (1.0 mmol), EDC·HCl (1.2 mmol) and HOBt·H_2O (1.2 mmol) in dry CH_2Cl_2 (10 mL) was added *i*PrNEt_2 (1.2 mmol). The resulting solution was stirred for 5 min before the respective amino acid or amine (1.0 mmol) was added. The reaction mixture was stirred at room temperature for 5-12 h and then diluted with 0.1 M HCl (10 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (10 mL) and water (10 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (EtOAc then EtOAc/MeOH 10:1, TLC visualized with ninhydrin or $KMnO_4$ solution) to afford the Boc-protected peptide.

Protocol B: *General protocol for the hydrolysis of peptide methyl esters*

To a solution of the peptide methyl ester (1.0 mmol) in MeOH (5 mL) was added 1 M NaOH (1.5 mL) and the resulting solution was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and the resulting aqueous phase was diluted with water (5 mL) and washed with CH_2Cl_2 (2 x 5 mL). The aqueous phase was then acidified with concentrated HCl (to pH 2) and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases were washed with brine (10 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford the peptide with the free carboxylic acid group.

Protocol C: *General protocol for Boc-deprotection*

The Boc-protected peptide (1.0 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and TFA (1 mL) was added. The solution was stirred at room temperature for 2 h and then concentrated under reduced pressure. The remaining TFA was co-evaporated with toluene (3x). The crude peptide was precipitated from Et_2O/pentane 1:2, centrifuged and the solvent was decanted. The white solid was again suspended in Et_2O/pentane 1:2,

sonicated, centrifuged and the solvent was decanted. This procedure was repeated 3 times to afford the peptide TFA-salt as a white solid which was dried under high vacuum. If the crude peptide could not be precipitated, it was washed/sonicated 3 times with Et_2O/pentane 1:2 to afford the peptide TFA-salt as a colorless oil which was dried under high vacuum.

7.2.2 General Protocols for Solid-Phase Peptide Synthesis

Some of the peptides were prepared on solid-phase polymeric support (Rink Amide AM or Rink Amide ChemMatrix) according to the following general protocols. All values given as equivalents (eq) are relative to the initial commercial loading of the resin.

Protocol D: *General protocol for peptide couplings (automated peptide synthesis)*
iPr_2NEt (12 eq as a 3 M solution in *N*-methylpyrrolidone) was added to a solution of Fmoc-protected amino acid (4 eq) and HCTU (4 eq) in DMF. The coupling cocktail was added to the amino-functionalized resin, swollen in DMF (≈100 mM concentration) and the mixture was agitated for 1.5 h before washing with DMF (5x). The Fmoc deprotection was performed by the addition of 40% (v/v) piperidine in DMF to the resin (preswollen in DMF). The reaction mixture was agitated for 3 min, drained and the piperidine treatment repeated for 10 min. Finally the resin was washed with DMF (7x). The entire protocol was then repeated for the next cycle.

Protocol E: *General protocol for cleavage of peptides from the solid-support*
The solid-supported peptide was cleaved from the Rink amide resin by agitation in a mixture of TFA/CH_2Cl_2 2:1 for 1 h and then repeated a second time for 30 min. The filtrates were combined and all volatiles were removed under reduced pressure. The crude peptide was precipitated with cold Et_2O, centrifuged and the ether phase was decanted. The white solid was again suspended in Et_2O, sonicated, centrifuged and the ether phase was decanted. This procedure was repeated 3 times to afford the peptide TFA-salt as a white solid which was dried under high vacuum.

Protocol F: *General protocol for the ion exchange of peptides*
The TFA salt of the peptide (50 - 100 mg) was dissolved in water (2 mL) and loaded on a StratoSpheres^{TM} SPE cartridge (PL-HCO_3 MP Resin, Agilent Technologies) which was

previously rinsed with MeOH (2 mL). The cartridge was washed with water or acetonitrile/water mixtures until the peptide was fully eluted (TLC spots visualised with ninhydrin). Peptide-containing fractions were pooled and lyophilized. The absence of TFA was confirmed by ^{19}F-NMR analysis.

7.2.3 General Protocols for the Synthesis and Screening of Catalyst-Substrate Co-immobilized Libraries

The catalyst-substrate co-immobilized libraries were prepared according to the following general protocols in plastic syringes equipped with a PTFE filter or in a Merrifield vessel. The completeness of each coupling, protection/deprotection or acetylation was evaluated by using Chloranil and/or a Kaiser test. The entire protocol was repeated in the case of incomplete functionalization.

Protocol G: *General protocol for Fmoc-deprotection*

A solution of 20% piperidine in DMF was added to the pre-swollen resin (15 min in DMF) and the reaction mixture was agitated for 5 min, drained, and the piperidine treatment was repeated a second time for 10 min. Finally the resin was washed with DMF (3x) and CH_2Cl_2 (3x).

Protocol H: *General protocol for Boc-protection*

A solution of di-*tert*-butyl dicarbonate (10 eq) and *i*PrNEt$_2$ (10 eq) in DMF was added to the resin and the reaction mixture was agitated for 1.5 h before washing with DMF (3x) and CH_2Cl_2 (3x).

Protocol I: *General protocol for Alloc-deprotection*

To a suspension of the Alloc-protected resin in anhydrous CH_2Cl_2 (10 mL/g) was added phenylsilane (5 eq) and the mixture was gently sparged with argon gas over 5 minutes. Maintaining the reaction mixture under inert conditions, $Pd(PPh_3)_4$ (20 mol%) was added in one shot and the reaction mixture was vigorously shaken for 2 h. The resin was filtered and then washed with a solution of sodium diethyldithiocarbamate and *i*PrNEt$_2$ in DMF (3x, 30 mM), DMF (3x) and CH_2Cl_2 (3x).

Protocol J: *General protocol for substrate couplings*

*i*PrNEt$_2$ (8 eq) was added to a solution of the carboxylic acid compound (4 eq) and HCTU (3.6 eq) in the minimum amount of DMF necessary to solubilize. The coupling cocktail was aged for 2 minutes and then added to the amino-functionalized resin (pre-swollen in DMF and drained). The reaction mixture was agitated for 1 h and then thoroughly washed with DMF (3x) and CH_2Cl_2 (3x).

Protocol K: *General protocol for N-terminal acetylation*

A solution of NEt_3 (30 eq) and Ac_2O (30 eq) in CH_2Cl_2 (300 eq) was added to the amine-functionalized resin (pre-swollen in CH_2Cl_2) and the mixture was agitated for 15 min before washing with CH_2Cl_2 (5x).

Protocol L1: *General protocol for global deprotection of the library*

To a portion of the library (pre-swollen in CH_2Cl_2) was added a mixture of TFA/H_2O/thioanisole 95:2.5:2.5 (0.5 mL/10 mg resin). After agitation of the reaction mixture for 1 min the resin was drained and then again treated with fresh TFA/H_2O/thioanisole for a further 5 min and lastly for a further 75 min period. Then the resin was washed with H_2O (3 x 5 min, 2 x 2 min), DMF (5 x 2 min), CH_2Cl_2/10% NEt_3 (5 x 2 min) and lastly CH_2Cl_2 (5 x 2 min).

Protocol L2: *General protocol for global deprotection of the library*

To a portion of the library (pre-swollen in CH_2Cl_2) was added a mixture of TFA/CH_2Cl_2 2:1 (0.5 mL/10 mg resin). After agitation of the reaction mixture for 1 min the resin was drained and then again treated with fresh TFA/CH_2Cl_2 2:1 for a further 5 min and lastly for a further 75 min period. Then the resin was washed with H_2O (3 x 5 min, 2 x 2 min), DMF (5 x 2 min), CH_2Cl_2/10% NEt_3 (5 x 2 min) and lastly CH_2Cl_2 (5 x 2 min).

Protocol M: *General protocol for screening of the peptide library*

To a portion (1.5 μmol) of the globally deprotected library in a 2 mL syringe with a PTFE filter was added a solution of dye-labeled nitroolefin **51** (2.5 mM, 0.9 mL) in $CHCl_3$/*i*PrOH 9:1. The syringe was closed with the plunger and after 2 or 4 h agitation at RT, the resin was filtered and washed with CH_2Cl_2 (5x), DMF (5x), CH_2Cl_2 (5x), CH_2Cl_2/TFA 3:1 (3x), CH_2Cl_2 (3x), 1 M HCl (3x), DMF (3x), *i*PrOH/NEt_3 3:1 (3x) and finally *i*PrOH (3x). The most intensively colored resin beads were isolated under a

microscope and transferred by pipette (10 μL micro tips) individually into 20 μL capillaries (closed at one end and cut to a length of 4.5 cm). The beads were centrifuged to the closed ends of the capillaries and were subsequently washed with GC quality DMF (3 x 2 μL) with an analytical syringe (10 μL). To each capillary was added GC quality DMF (1 μL) in preparation for tag cleavage. The capillaries were closed at the open end and irradiated under UV light (254 nm) for 2 h liberating the tag alcohols contained on each bead into the supernatant DMF. The tag alcohol solutions were silylated *in situ* by addition of BSA (0.1 μL) and analyzed by GC-ECD (temperature gradient: 200-320 ºC in 11 min).

Protocol N: *General protocol for quantitative Fmoc test of the peptide library*

The Fmoc-protected library was dried under high vacuum for 24 h. To a portion of the dried library (20 mg) was added a solution of 20% piperidine in DMF and the reaction mixture was agitated for 30 min. Then 400 μL of the supernatant piperidine/DMF solution was diluted with 5 mL DMF in a volumetric flask. The resulting solution was placed into a quartz cuvette (d = 0.5 cm) and measured in a UV spectrometer at 280 nm in order to determine the absorption. The loading of the resin was determined using the following equation:

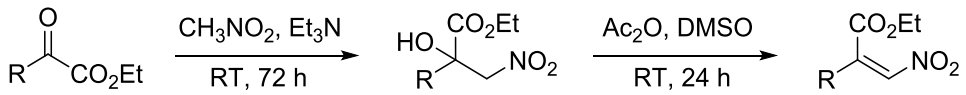
$$\text{Loading (mmol/g)} = \frac{Absorption}{\varepsilon_{280} * d_{cuvette}} * \frac{V_{sample}+V_{dilution}}{V_{sample}} * \frac{V_{cleavage\ solution}}{m_{sample}}$$

ε_{280} = 7800 M^{-1} cm^{-1}; V_{sample} = 0.400 mL; $V_{dilution}$ = 5.0 mL; $V_{cleavage\ sol.}$ = 2.0 mL; m_{sample} = 0.020 g.

7.2.4 General Protocols for the Synthesis of β,β-Disubstituted Nitroolefins

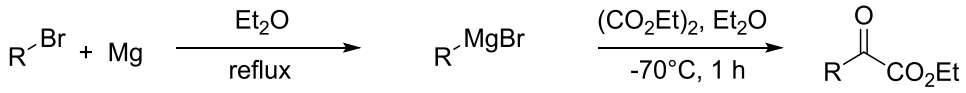
The nitroolefins were synthesized from the corresponding α-keto esters according to Protocol O. Some of the α-keto esters were synthesized according to Protocol P, the others were purchased from commercial suppliers and used without further purification.

Protocol O: *General protocol for the synthesis of β,β-disubstituted nitroolefins*[221]



Reagents and solvents were dried over 4 Å molecular sieves prior to use. In a dried round-bottom flask, triethylamine (693 μL, 5.00 mmol) was added to a solution of the α-keto ester (25.0 mmol) in nitromethane (100 mL). The yellow solution was stirred at room temperature for 72 h under an atmosphere of nitrogen. The solvent was removed under reduced pressure and the crude β-nitro-α-hydroxyester was treated without further purification with DMSO (90 mL) and Ac_2O (7.09 mL, 75.0 mmol). The reaction mixture was stirred at room temperature for 16–24 h (monitored by ^1H NMR) under an atmosphere of nitrogen, then poured into water (600 mL) and extracted with CH_2Cl_2 (1 x 250 mL, 2 x 150 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ (150 mL) and brine (150 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography to afford the β,β-disubstituted nitroolefin.

Protocol P: *General protocol for the synthesis of α-keto esters*[221]



In a dried two-neck round-bottom flask with condenser, about 2 mL of a solution of arylbromide (40.0 mmol) in Et_2O (20 mL) was added to magnesium turnings (972 mg, 40.0 mmol) and Et_2O (10 mL). Once the reaction started the remaining arylbromide solution was added slowly so that the reaction mixture was refluxing gently. The mixture was refluxed for another 1 h after the addition.

The freshly prepared Grignard reagent was added dropwise to a solution of diethyloxalate (5.14 mL, 38.0 mmol) in Et_2O (100 mL) at -70°C. The mixture was stirred for 1 h at -70°C and then warmed to 10°C before being hydrolyzed with 3 M HCl (20 mL) and H_2O (20 mL). The aqueous layer was extracted with Et_2O (2 x 100 mL) and the combined organic layers were washed with brine (100 mL) and dried over $MgSO_4$. The solvent was

removed under reduced pressure and the crude product was purified by flash chromatography to afford the α-keto ester.

7.2.5 General Protocols for 1,4-Addition Reactions

Protocol Q: *General protocol for the 1,4-addition reaction between aldehydes and β,β-disubstituted nitroolefins*

The nitroolefin (0.42 mmol) was added to a solution of the peptide (as the TFA-salt in combination with NMM or as the desalted peptide in combination with an acidic additive, 42 μmol, 10 mol%) and the aldehyde (0.84 mmol) in *t*BuOH (1 mL). The reaction mixture was agitated at room temperature. After consumption of the nitroolefin all volatile components were removed at reduced pressure and the resulting crude product was purified by flash column chromatography on silica gel by using a mixture of pentanes and EtOAc as eluent to afford the γ-nitroaldehyde.

Protocol R: *General protocol for 1,4-addition reactions between butanal and nitroacrylate **7** monitored by* in situ *FT-IR spectroscopy or NMR spectroscopy*

The catalyst (105 μmol, 25 mol%) and the respective acidic additive (105 μmol, 25 mol%) were placed into a volumetric flask (1 mL) and solvent was added. The resulting mixture was sonicated until a homogeneous solution was obtained. Then 1,1,2,2-tetrachloroethane (as internal standard, 10.0 μL, 94.5 μmol) and butanal (75.4 μL, 0.836 mmol) were added (the mixture was briefly vortexed) followed by the addition of nitroacrylate **7** (100 mg, 0.418 mmol). Further solvent was added until the total volume was exactly 1 mL. The mixture was again sonicated for 2 min to completely dissolve the nitroacrylate. The resulting clear solution was immediately transferred to a 5 mL round bottom flask containing the IR probe and a magnetic stirrer. The reaction mixture was gently stirred during the reaction. Reaction progress was monitored by following the N-O-stretching absorbance of the forming γ-nitroaldehyde **9** at 1563 cm^{-1}. Spectra were collected every 5 or 10 minutes, each time by performing 256 scans, until nitroacrylate **7** was completely consumed. Then an aliquot (50 μL) was withdrawn from the reaction mixture, diluted with DMSO-d_6 or $CDCl_3$ and analyzed by ^{1}H- and ^{19}F-NMR spectroscopy to determine the conversion to γ-nitroaldehyde **9**.

Protocol S: *General protocol for the 1,4-addition reaction between butanal and trans-β-nitrostyrene*

To a solution of the peptide (as the TFA-salt in combination with NMM, 2.2 μmol, 1 mol% or 6.6 μmol, 3 mol%) and butanal (0.33 mmol) in $CHCl_3$/*i*PrOH 9:1 (0.5 mL) was added *trans*-β-nitrostyrene (0.22 mmol). The reaction mixture was agitated at room temperature and the conversion and the diastereomeric ratio were determined by ^{1}H-NMR spectroscopy analysis ($CDCl_3$) of the crude reaction mixture. In order to determine the enantiomeric excess, a small amount of the γ-nitroaldehyde was isolated from the reaction mixture by preparative TLC (EtOAc/pentane 1:10) and subjected to HPLC using a Chiracel AD-H column (*n*-hexane/*i*PrOH 99.5:0.5, 25°C) at 0.9 mL/min, UV detection at 254 nm, t_R (*syn*, minor) = 36.8 min, t_R (*syn*, major) = 47.9 min.

Protocol T: *General protocol for the 1,4-addition reaction between α,α-disubstituted aldehydes and trans-β-nitrostyrene*

To a solution of the peptide (as the TFA-salt in combination with NMM or as the desalted peptide, 22 μmol, 10 mol%) and the aldehyde (0.22 mmol) in $CHCl_3$/*i*PrOH 7:3 (0.5 mL) was added *trans*-β-nitrostyrene (0.33 mmol). The reaction mixture was agitated at room temperature and the conversion and the diastereomeric ratio were determined by ^{1}H-NMR spectroscopy analysis ($CDCl_3$) of the crude reaction mixture. In order to determine the enantiomeric excess, a small amount of the γ-nitroaldehyde was isolated from the mixture by preparative TLC (EtOAc/pentane 1:10) and subjected to HPLC using a chiral column.

7.2.6 General Protocol for the ESI-MS Back-Reaction Screening

Protocol U: *General protocol for the ESI-MS back-reaction screening*

To an equimolar mixture of (2*S*,3*S*)-Ethyl 3-formyl-2-(nitromethyl)-2-phenyl-4-(*p*-tolyl)butanoate (**40a**, 5 μmol) and (2*S*,3*S*)-Ethyl 3-(4-ethylbenzyl)-2-(nitromethyl)-4-oxo-2-phenylbutanoate (**40b**, 5 μmol) was added the catalyst (1 μmol, 10 mol%) and 50 μL of solvent. The mixture was briefly sonicated to obtain a clear solution, then stirred for 10 min and then diluted with 1 mL of MeCN. This mixture was immediately analyzed by ESI-MS under mild desolvation conditions (see Chapter 7.1, General Aspects and Materials).

7.3 Peptide Synthesis

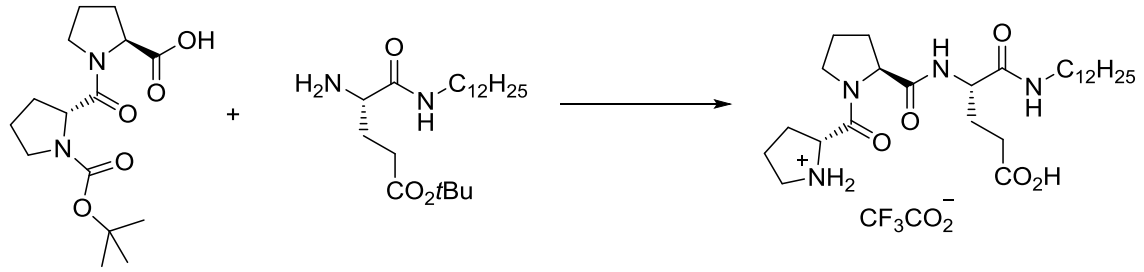
7.3.1 Synthesis of Peptidic Catalysts for Reactions between Butanal and β,β-Disubstituted Nitroolefins

The peptides in the following table had been synthesized by members of our group on solid-phase according to general protocols for solid-phase peptide synthesis (Fmoc/*t*Bu protocol). The corresponding reference for their synthesis and analytical data is given.

Peptide	Ref.	Peptide	Ref.
TFA·H-D-Pro-Pro-Glu-NH_2 (**1**)	[166]	TFA·H-Pro-Pro-Asp-NH_2	[165]
TFA·H-Pro-D-Pro-Glu-NH_2	[222]	TFA·H-D-Pro-Pro-Asn-OH	[222]
TFA·H-Pro-Pro-D-Glu-NH_2	[222]	TFA·H-Pro-Pro-D-Asn-OH	[222]
TFA·H-Pro-Pro-Glu-NH_2	[222]	TFA·H-Pro-Pro-Asn-OH	[222]
TFA·H-D-Pro-Pro-Glu-OH	[189]	TFA·H-D-Pro-Pro-Gly-OH	[189]
TFA·H-D-Pro-Pro-Gln-NH_2	[168]	TFA·H-D-Pro-Pro-β-Ala-OH	[166]
TFA·H-D-Pro-Pro-Gln-OH	[169]	TFA·H-D-Pro-Pro-Aad-NH_2	[166]
TFA·H-Pro-D-Pro-Gln-OH	[169]	TFA·H-D-Pro-Pro-Api-NH_2	[166]
TFA·H-Pro-Pro-D-Gln-OH	[169]	TFA·H-D-Pro-Pro-Asu-NH_2	[166]
TFA·H-Pro-Pro-Gln-OH	[169]	TFA·H-D-Pro-Pro-Ser-OH	[189]
TFA·H-D-Pro-Pro-Asp-NH_2	[165]	TFA·H-D-Pro-Pro-His-OH	[189]
TFA·H-Pro-Pro-D-Asp-NH_2	[165]		

The following peptides were prepared by solution-phase peptide synthesis. Most of the building blocks were synthesized according to procedures described in Chapter 7.5.1.

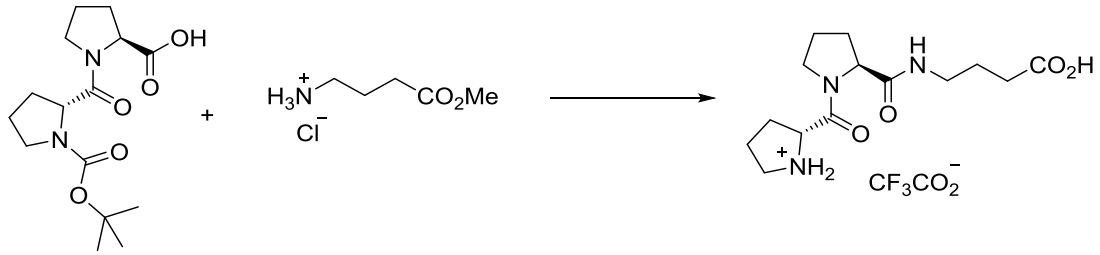
TFA·H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$:



Prepared from Boc-D-Pro-Pro-OH (1.00 g, 640 μmol) and H-Glu(O*t*Bu)NH-$C_{12}H_{25}$ (1.19 g, 3.2 mmol) according to the general protocols A and C. The peptide was obtained as a white powder (1.50 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C) δ = 8.85 (d, *J* = 7.4 Hz, 1H), 6.60 (t, *J* = 5.8 Hz, 1H), 4.78 (dd, *J* = 9.0, 6.4 Hz, 1H), 4.56 (dd, *J* = 8.7, 2.2 Hz, 1H), 4.44 (td, *J* = 7.2, 2.1 Hz, 1H), 3.92 – 3.72 (m, 2H), 3.52 (td, *J* = 9.6, 6.8 Hz, 1H), 3.40 (dt, *J* = 10.9, 7.0 Hz, 1H), 3.25 (d, *J* = 6.7 Hz, 1H), 3.21 (d, *J* = 6.7 Hz, 1H), 2.65 – 2.46 (m, 3H), 2.45 – 1.92 (m, 8H), 1.88 – 1.70 (m, 1H), 1.49 (t, *J* = 6.8 Hz, 2H), 1.26 (d, *J* = 8.2 Hz, 18H), 0.88 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C) δ = 181.6, 170.7, 170.1, 169.4, 62.0, 59.3, 54.7, 47.5, 45.3, 39.7, 32.4, 32.1, 29.8, 29.8, 29.8, 29.5, 29.5, 29.5, 28.0, 27.1, 25.8, 25.0, 24.7, 22.8, 14.3; HRMS (ESI): *m/z* calcd $C_{27}H_{49}N_4O_5$+H^+: 509.3697 [*M*+H^+]; found: 509.3709.

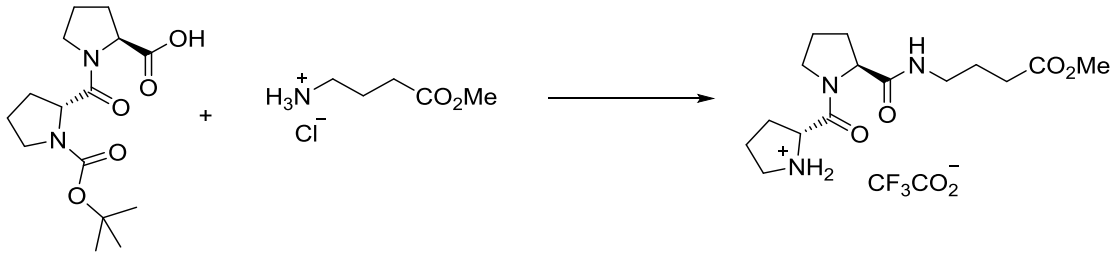
TFA·H-D-Pro-Pro-γ-Abu-OH:



Prepared from Boc-D-Pro-Pro-OH (200 mg, 640 μmol) and γ-aminobutyric acid methyl ester hydrochloride (100 mg, 640 μmol) according to the general protocols A – C. The peptide was obtained as a colorless oil (152 mg, 58%).

^{1}H NMR (400 MHz, D_2O, 25°C): δ = 4.68 (dd, J = 9.0, 6.8 Hz, 1H), 4.42 (dd, J = 8.9, 3.9 Hz, 1H), 3.82 – 3.72 (m, 1H), 3.69 – 3.59 (m, 1H), 3.54 – 3.39 (m, 2H), 3.28 (td, J = 6.7, 3.8 Hz, 2H), 2.65 – 2.54 (m, 1H), 2.44 (t, J = 7.4 Hz, 2H), 2.38 – 2.27 (m, 1H), 2.20 – 1.95 (m, 6H), 1.84 (p, J = 7.0 Hz, 2H); ^{13}C NMR (101 MHz, D_2O, 25°C): δ = 180.9, 176.7, 170.9, 64.1, 62.2, 51.6, 49.6, 41.5, 33.9, 32.7, 31.1, 27.2, 26.9, 26.8; HRMS (ESI): *m/z* calcd for $C_{14}H_{23}N_3O_4$+H^+: 298.1761 [M+H^+]; found: 298.1766.

TFA·H-D-Pro-Pro-γ-Abu-OMe:

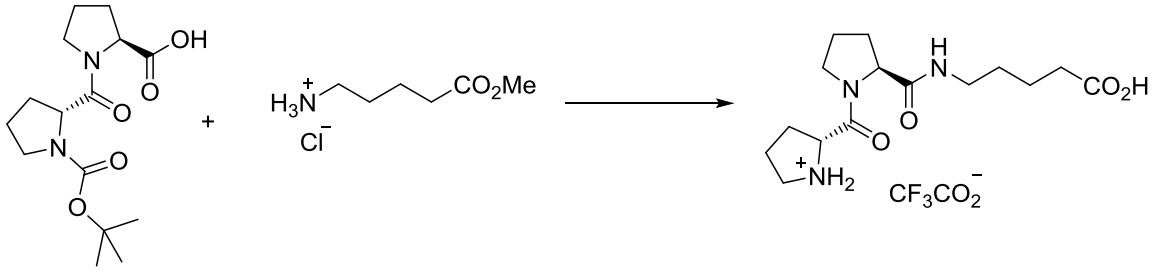


Prepared from Boc-D-Pro-Pro-OH (200 mg, 640 μmol) and γ-aminobutyric acid methyl ester hydrochloride (100 mg, 640 μmol) according to the general protocols A – C. The peptide was obtained as a colorless oil (212 mg, 78%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): *Cis/trans* conformers were observed in a ratio of 10:1. Major conformer: δ = 4.54 (dd, J = 8.8, 6.9 Hz, 1H), 4.36 (dd, J = 8.6, 3.7 Hz, 1H),

3.73 (ddd, *J* = 9.8, 7.3, 4.7 Hz, 1H), 3.66 (s, 3H), 3.55 (dt, *J* = 9.9, 7.2 Hz, 1H), 3.45 – 3.32 (m, 2H), 3.29 – 3.15 (m, 2H), 2.52 (ddt, *J* = 13.0, 8.8, 6.4 Hz, 1H), 2.42 – 2.33 (m, 2H), 2.24 (ddt, *J* = 12.1, 8.8, 4.6 Hz, 1H), 2.14 – 1.92 (m, 6H), 1.80 (p, *J* = 7.2 Hz, 2H); minor conformer: δ = 4.59 – 4.51 (m, 1H)*, 4.42 (t, *J* = 8.0 Hz, 1H), 3.66 (s, 3H)*, 3.69 – 3.60 (m, 1H)*, 3.60 – 3.50 (m, 1H)*, 3.47 – 3.29 (m, 2H)*, 3.29 – 3.15 (m, 2H)*, 2.58 – 2.47 (m, 1H)*, 2.42 – 2.33 (m, 2H)*, 2.28 – 2.19 (m, 1H)*, 2.15 – 1.89 (m, 6H)*, 1.86 – 1.74 (m, 2H)*. *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformer: δ = 174.0, 172.8, 167.0, 60.8, 59.2, 50.7, 47.0, 46.2, 38.3, 30.6, 29.6, 28.0, 24.3, 24.2, 23.9; visible signals of the minor conformer: δ = 172.5, 167.4, 60.3, 58.9, 50.7, 46.0, 38.5, 32.0, 30.6, 28.8, 24.2, 23.9; HRMS (ESI): *m/z* calcd for $C_{15}H_{25}N_3O_4$+Na^+: 334.1737 [*M*+Na^+]; found: 334.1739.

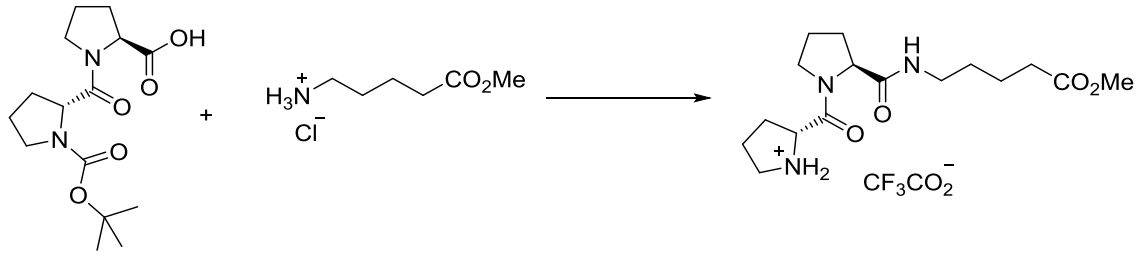
TFA·H-D-Pro-Pro-5-Ava-OH:



Prepared from Boc-D-Pro-Pro-OH (175 mg, 560 μmol) and 6-aminohexanoic acid methyl ester hydrochloride (94 mg, 560 μmol) according to the general protocols A – C. The peptide was obtained as a colorless oil (176 mg, 75%).

^{1}H NMR (400 MHz, D_2O, 25°C): δ = 4.50 (dd, *J* = 7.0, 8.8 Hz, 1H), 4.26 (dd, *J* = 4.2, 8.9 Hz, 1H), 3.60 (td, *J* = 6.3, 6.3 Hz, 10.2 Hz, 1H), 3.48 (ddd, *J* = 3.4, 7.3, 14.2 Hz, 1H), 3.30 (m, 2H), 3.90 (m, 2H), 2.43 (ddd, *J* = 6.8, 8.9, 13.0 Hz, 1H), 2.27 (t, *J* = 7.1 Hz, 2H), 2.16 (ddd, *J* = 5.8, 8.8, 12.4 Hz, 1H), 1.70-2.00 (m, 6H), 1.36-1.52 (m, 4H); ^{13}C NMR (101 MHz, D_2O, 25°C): δ = 178.3, 171.4, 168.8, 62.0, 59.9, 47.5, 47.1, 39.3, 33.0, 29.8, 28.9, 28.1, 25.4, 24.6, 21.0; MS (ESI): *m/z* calcd $C_{15}H_{25}N_3O_4$+H^+: 312.4 [*M*+H^+]; found: 312.2.

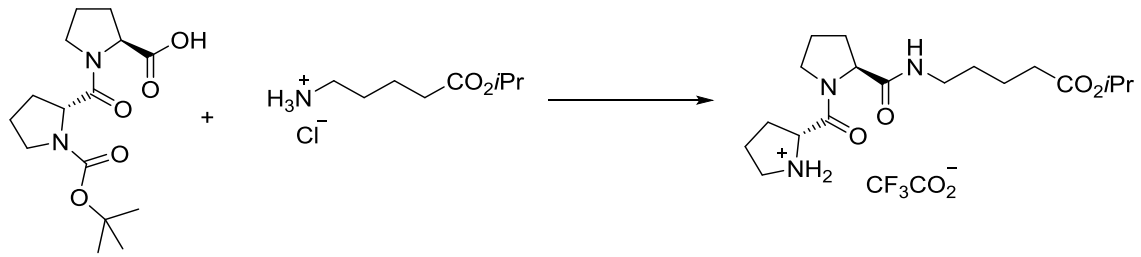
TFA·H-D-Pro-Pro-5-Ava-OMe:



Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and 5-aminovaleric acid methyl ester hydrochloride (54 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a colorless oil (116 mg, 83%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): The ratio of *cis/trans* conformers could not be determined due to overlapping signals. Major conformer: δ = 4.57 – 4.49 (m, 1H), 4.37 (dd, *J* = 8.6, 3.8 Hz, 1H), 3.72 (ddd, *J* = 10.2, 7.5, 4.8 Hz, 1H), 3.65 (s, 3H), 3.59 – 3.51 (m, 1H), 3.46 – 3.27 (m, 2H), 3.26 – 3.13 (m, 2H), 2.58 – 2.46 (m, 1H), 2.40 – 2.31 (m, 2H), 2.30 – 2.18 (m, 1H), 2.15 – 1.91 (m, 6H), 1.69 – 1.59 (m, 2H), 1.58 – 1.47 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformer: δ = 175.8, 174.1, 168.3, 62.1, 60.6, 52.0, 48.4, 47.6, 39.9, 34.3, 31.1, 29.7, 29.5, 25.6, 25.3, 23.1; HRMS (ESI): *m/z* calcd for $C_{16}H_{27}N_3O_4+H^+$: 326.2074 [$M+H^+$]; found: 326.2080.

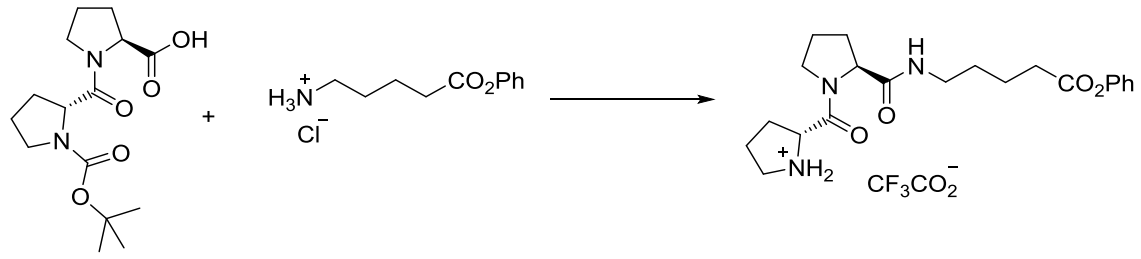
TFA·H-D-Pro-Pro-5-Ava-O*i*Pr:



Prepared from Boc-D-Pro-Pro-OH (300 mg, 960 μmol) and 5-aminovaleric acid isopropyl ester hydrochloride (190 mg, 960 μmol) according to the general protocols A and C. The peptide was obtained as a colorless oil (303 mg, 68%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 7.54 (t, *J* = 5.7 Hz, 1H), 4.95 (hept, *J* = 6.3 Hz, 1H), 4.68 – 4.59 (m, 1H), 4.54 (dd, *J* = 7.4, 4.3 Hz, 1H), 3.87 (ddd, *J* = 9.7, 7.7, 4.1 Hz, 1H), 3.56 – 3.39 (m, 3H), 3.39 – 3.29 (m, 1H), 3.16 – 3.03 (m, 1H), 2.48 – 2.36 (m, 1H), 2.31 – 1.90 (m, 9H), 1.61 – 1.37 (m, 4H), 1.21 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 174.2, 171.3, 168.1, 68.0, 61.6, 59.5, 47.3, 46.2, 39.1, 34.2, 29.8, 28.5, 28.3, 25.3, 24.4, 21.9, 21.9, 21.8; HRMS (ESI): *m/z* calcd for $C_{18}H_{31}N_3O_4$+H^+: 354.2 [*M*+H^+]; found: 354.3.

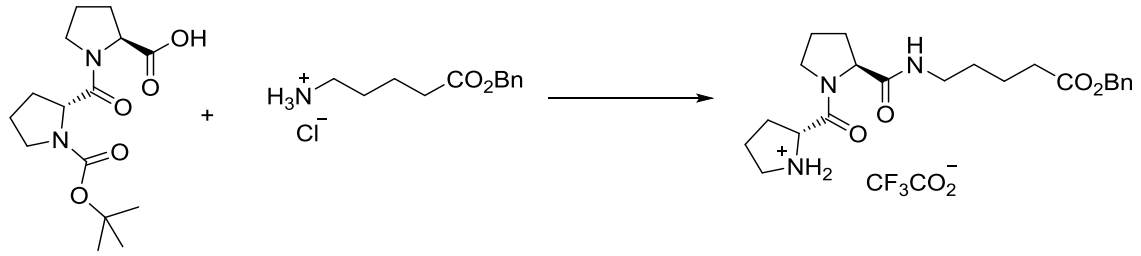
TFA·H-D-Pro-Pro-5-Ava-OPh:



Prepared from Boc-D-Pro-Pro-OH (115 mg, 370 μmol) and 5-aminovaleric acid phenyl ester hydrochloride (85 mg, 370 μmol) according to the general protocols A and C. The peptide was obtained as a colorless oil (113 mg, 61%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): *Cis/trans* conformers were observed in a ratio of ~7:1. Major conformer: δ = 7.42 – 7.34 (m, 2H), 7.27 – 7.19 (m, 1H), 7.11 – 7.03 (m, 2H), 4.54 (dd, *J* = 8.8, 6.9 Hz, 1H), 4.38 (dd, *J* = 8.8, 3.5 Hz, 1H), 3.72 (ddd, *J* = 9.5, 7.5, 4.5 Hz, 1H), 3.53 (dt, *J* = 9.9, 7.1 Hz, 1H), 3.42 – 3.27 (m, 2H), 3.25 (t, *J* = 6.7 Hz, 2H), 2.61 (t, *J* = 7.3 Hz, 2H), 2.55 – 2.43 (m, 1H), 2.28 – 2.18 (m, 1H), 2.14 – 1.90 (m, 6H), 1.82 – 1.70 (m, 2H), 1.68 – 1.58 (m, 2H); visible signals of the minor conformer: δ = 4.60 (dd, *J* = 8.5, 2.3 Hz, 1H), 4.46 – 4.41 (m, 1H), 3.66 – 3.59 (m, 1H), 2.37 – 2.25 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformer: δ = 174.1, 173.8, 168.5, 152.3, 130.5, 126.8, 122.8, 62.3, 60.6, 48.4, 47.6, 39.9, 34.5, 31.1, 29.7, 29.4, 25.6, 25.3, 23.1; visible signals of the minor conformer: δ = 173.6, 168.8, 162.7, 162.5, 119.5, 116.6, 61.7, 60.2, 47.4, 40.1, 34.5, 33.5, 30.2, 29.8, 23.3, 23.1; MS (ESI): *m/z* calcd for $C_{21}H_{29}N_3O_4$+H^+: 388.2 [*M*+H^+]; found: 388.2.

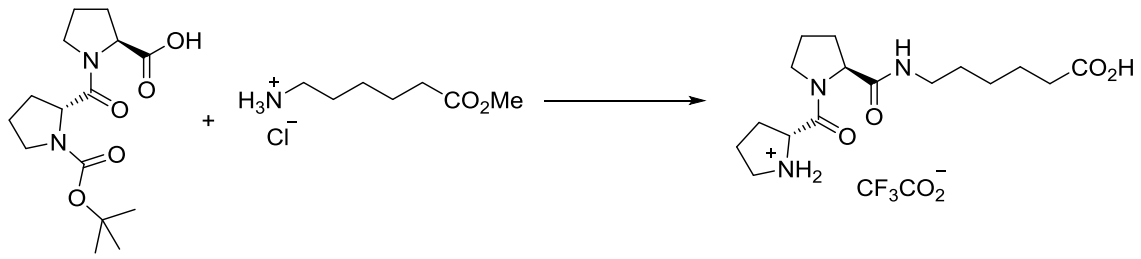
TFA·H-D-Pro-Pro-5-Ava-OBn:



Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and 5-aminovaleric acid benzyl ester hydrochloride (78 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a colorless oil (138 mg, 84%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 7.37 – 7.20 (m, 5H), 5.11 (s, 2H), 4.54 (dd, *J* = 8.8, 6.8 Hz, 1H), 4.37 (dd, *J* = 8.5, 3.6 Hz, 1H), 3.72 (ddt, *J* = 9.9, 7.5, 4.9 Hz, 1H), 3.55 (dt, *J* = 10.0, 7.1 Hz, 1H), 3.45 – 3.31 (m, 2H), 3.20 (t, *J* = 6.7 Hz, 2H), 2.52 (ddt, *J* = 13.0, 8.9, 6.6 Hz, 1H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.28 – 2.17 (m, 1H), 2.13 – 1.90 (m, 6H), 1.69 – 1.59 (m, 2H), 1.59 – 1.48 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 177.4, 174.1, 168.4, 137.7, 129.5, 129.2, 129.2, 67.2, 62.2, 60.6, 48.4, 47.6, 39.9, 34.4, 31.0, 29.7, 29.4, 25.6, 25.3, 23.2; MS (ESI): *m/z* calcd for $C_{22}H_{31}N_3O_4$+H^+: 402.2 [*M*+H^+]; found: 402.3.

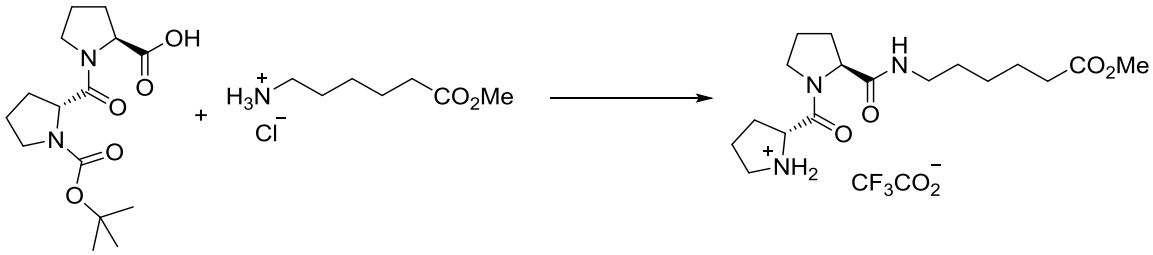
TFA·H-D-Pro-Pro-6-Ahx-OH:



Prepared from Boc-D-Pro-Pro-OH (106 mg, 340 μmol) and 6-aminohexanoic acid methyl ester hydrochloride (62 mg, 340 μmol) according to the general protocols A – C. The peptide was obtained as a colorless oil (90 mg, 46%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 11.48 (br s, 1H), 4.48 (dd, *J* = 7.0, 8.4 Hz, 1H), 4.33 (dd, *J* = 6.0, 8.6 Hz, 1H), 3.59 – 3.54 (td, *J* = 7.0 Hz, 10.1 Hz, 1H), 3.45 – 3.35 (m, 1H), 3.16 – 3.28 (m, 3H), 3.04 – 2.97 (m, 1H), 2.36 – 2.27 (m, 1H), 2.23 – 2.18 (m, 1H), 2.18 – 2.13 (m, 2H), 2.04 – 1.71 (m, 6H), 1.46 – 1.28 (m, 4H), 1.17 – 1.10 (m, 2H); MS (ESI): *m/z* calcd $C_{16}H_{27}N_3O_4$+H^+: 326.2 [*M*+H^+]; found: 326.3.

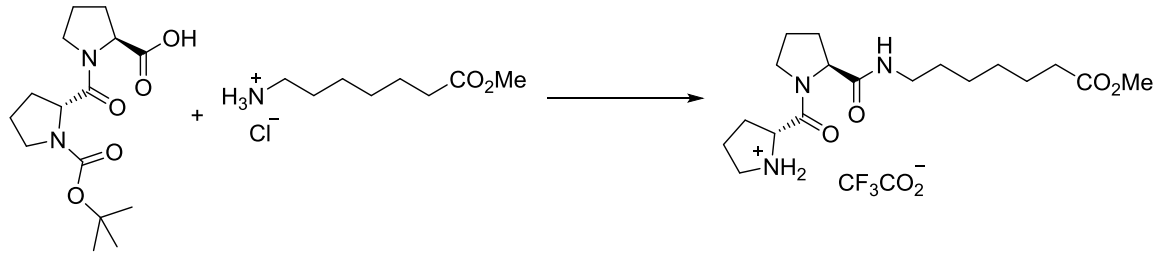
TFA·H-D-Pro-Pro-6-Ahx-OMe:



Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and 6-aminohexanoic acid methyl ester hydrochloride (58 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a pale yellow oil (121 mg, 83%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.49 (t, *J* = 5.9 Hz, 1H), 4.67 – 4.52 (m, 1H), 4.51 (dd, *J* = 8.3, 2.8 Hz, 1H), 3.94 – 3.79 (m, 1H), 3.66 (s, 3H), 3.61 – 3.46 (m, 1H), 3.46 – 3.35 (m, 2H), 3.24 (dq, *J* = 13.1, 6.5 Hz, 1H), 3.13 (dq, *J* = 12.9, 6.6 Hz, 1H), 2.46 – 2.35 (m, 1H), 2.34 – 1.99 (m, 9H), 1.92 (dq, *J* = 13.0, 8.1 Hz, 1H), 1.64 – 1.40 (m, 4H), 1.32 – 1.19 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 174.9, 170.7, 167.9, 61.6, 59.5, 51.6, 47.1, 45.8, 39.6, 34.1, 29.9, 28.8, 28.3, 26.3, 25.3, 24.7, 24.2; MS (ESI): *m/z* calcd for $C_{17}H_{29}N_3O_4$+H^+: 340.2 [*M*+H^+]; found: 340.3.

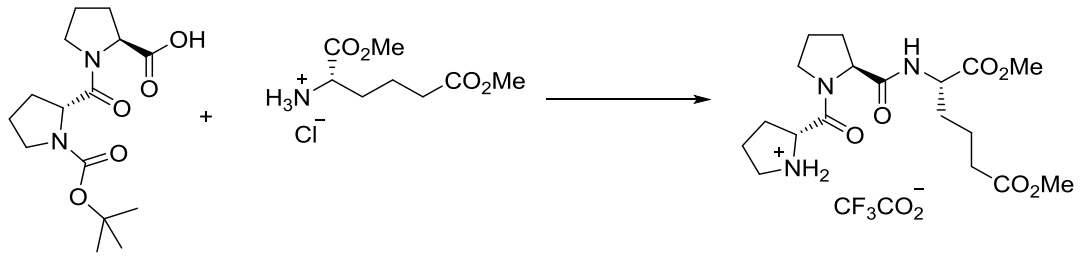
TFA·H-D-Pro-Pro-7-Ahp-OMe:



Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and 7-aminoheptanoic acid methyl ester hydrochloride (63 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a pale yellow oil (129 mg, 86%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.47 (t, *J* = 5.9 Hz, 1H), 4.65 – 4.55 (m, 1H), 4.53 (dd, *J* = 8.3, 2.8 Hz, 1H), 3.85 (ddd, *J* = 9.6, 7.2, 3.9 Hz, 1H), 3.66 (s, 3H), 3.60 – 3.49 (m, 1H), 3.48 – 3.37 (m, 2H), 3.27 – 3.08 (m, 2H), 2.47 – 2.37 (m, 1H), 2.36 – 1.98 (m, 8H), 1.92 (dq, *J* = 12.8, 8.1 Hz, 1H), 1.65 – 1.54 (m, 2H), 1.49 – 1.40 (m, 2H), 1.36 – 1.19 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 174.9, 170.8, 168.0, 61.9, 59.6, 51.6, 47.1, 45.8, 39.6, 33.9, 30.0, 29.0, 28.6, 28.2, 26.2, 25.2, 24.8, 24.1; MS (ESI): *m/z* calcd for $C_{18}H_{31}N_3O_4$+H^+: 354.2 [*M*+H^+]; found: 354.3.

TFA·H-D-Pro-Pro-Aad(OMe)-OMe:

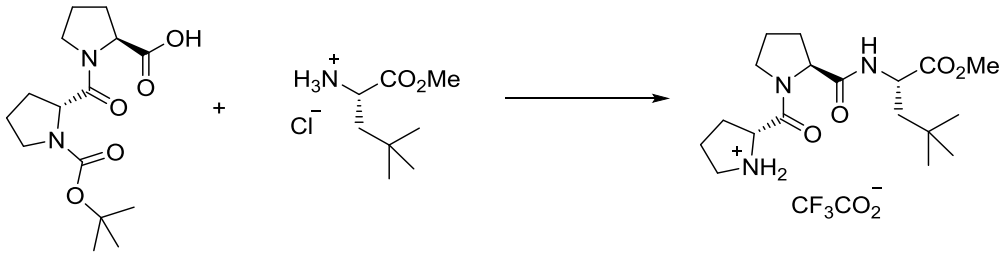


Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and (*S*)-dimethyl 2-aminohexanedioate hydrochloride (72 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a hygroscopic white foam (70 mg, 44%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): *Cis/trans* conformers were observed in a ratio of 10:1. Major conformer: δ = 7.56 (d, *J* = 8.2 Hz, 1H), 4.71 (t, *J* = 8.0 Hz, 1H), 4.53 (dd,

J = 8.0, 2.9 Hz, 1H), 4.43 (td, *J* = 8.8, 4.7 Hz, 1H), 3.90 (ddd, *J* = 9.8, 7.8, 3.6 Hz, 1H), 3.69 (s, 3H), 3.66 (s, 3H), 3.55 – 3.40 (m, 3H), 3.24 (br s, 1H), 2.51 – 2.39 (m, 1H), 2.39 – 2.25 (m, 3H), 2.23 – 1.91 (m, 6H), 1.91 – 1.69 (m, 2H), 1.67 – 1.57 (m, 2H); minor conformer: δ = 7.48 (d, *J* = 7.6 Hz, 1H), 4.79 (dd, *J* = 8.3, 2.5 Hz, 1H), 4.50 – 4.39 (m, 2H)*, 3.74 (s, 3H), 3.67 – 3.62 (m, 1H)*, 3.66 (s, 3H)*, 3.55 – 3.40 (m, 3H)*, 2.51 – 2.39 (m, 1H)*, 2.39 – 2.25 (m, 3H)*, 2.23 – 1.91 (m, 6H)*, 1.91 – 1.69 (m, 2H)*, 1.67 – 1.57 (m, 2H)*. *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Only signals of the major conformer were visible: δ = 174.4, 172.3, 170.5, 168.3, 61.3, 58.9, 52.4, 52.0, 51.7, 47.2, 46.1, 33.0, 30.4, 29.0, 28.6, 25.1, 24.5, 21.0; HRMS (ESI): *m/z* calcd for $C_{18}H_{29}N_3O_6$+H^+: 384.2129 [*M*+H^+]; found: 384.2129.

TFA·H-D-Pro-Pro-β-*tert*-butyl-Ala-OMe:

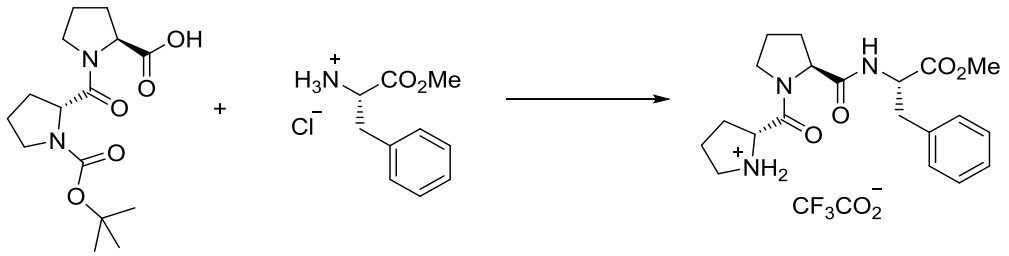


Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and β-*tert*-butyl-L-alanine methyl ester hydrochloride (63 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a white foam (130 mg, 87%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): *Cis/trans* conformers were observed in a ratio of 6:1. Major conformer: δ = 7.46 (d, *J* = 8.6 Hz, 1H), 4.71 – 4.60 (m, 1H), 4.57 (td, *J* = 8.2, 4.0 Hz, 1H), 4.51 (t, *J* = 5.9 Hz, 1H), 3.91 (ddd, *J* = 9.7, 7.5, 4.0 Hz, 1H), 3.64 (s, 3H), 3.62 – 3.47 (m, 1H), 3.49 – 3.32 (m, 2H), 2.41 (dtd, *J* = 13.1, 7.6, 5.3 Hz, 1H), 2.27 – 1.88 (m, 7H), 1.74 – 1.62 (m, 2H), 0.91 (s, 9H); visible signals of the minor conformer: δ = 6.99 (d, *J* = 7.9 Hz, 1H), 4.79 (q, *J* = 6.5, 5.9 Hz, 1H), 4.77 – 4.67 (m, 1H), 4.45 (ddd, *J* = 10.9, 7.2, 3.1 Hz, 1H), 3.71 (s, 3H), 3.68 – 3.60 (m, 1H), 1.81 (dd, *J* = 14.5, 3.5 Hz, 1H), 1.53 (dd, *J* = 14.5, 8.2 Hz, 1H), 0.94 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Only signals of the major conformer were visible: δ = 173.8, 170.9, 168.2, 61.7, 59.4,

52.5, 50.0, 47.3, 46.2, 44.5, 30.7, 29.5, 29.3, 28.4, 25.1, 24.5; MS (ESI): *m/z* calcd for $C_{18}H_{31}N_3O_4$+H^+: 354.2 [*M*+H^+]; found: 354.2.

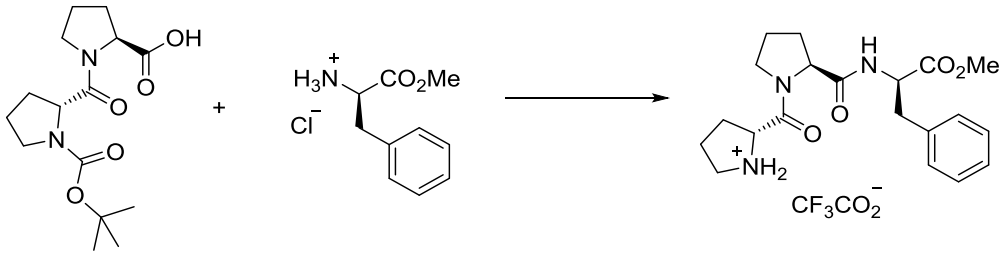
TFA·H-D-Pro-Pro-Phe-OMe:



Prepared from Boc-D-Pro-Pro-OH (200 mg, 640 μmol) and L-Phenylalanine methylester hydrochloride (138 mg, 640 μmol) according to the general protocols A and C. The peptide was obtained as a white powder (275 mg, 88%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): *Cis/trans* conformers were observed in a ratio of 5:1. Major conformer: δ = 7.51 (d, *J* = 9.0 Hz, 1H), 7.30 – 7.14 (m, 5H), 4.89 (ddd, *J* = 10.6, 8.8, 5.1 Hz, 1H), 4.58 (t, *J* = 8.5 Hz, 1H), 4.39 (dd, *J* = 8.4, 2.7 Hz, 1H), 3.75 – 3.66 (m, 1H), 3.68 (s, 3H), 3.52 (dt, *J* = 11.7, 6.9 Hz, 1H), 3.40 – 3.27 (m, 2H), 3.25 (dd, *J* = 14.1, 5.1 Hz, 1H), 3.02 (dd, *J* = 14.1, 10.6 Hz, 1H), 2.37 (dtd, *J* = 13.1, 7.7, 5.4 Hz, 1H), 2.17 – 2.05 (m, 2H), 1.97 – 1.70 (m, 4H), 1.57 – 1.44 (m, 1H); Minor conformer: δ = 7.37 (d, *J* = 8.3 Hz, 1H), 7.30 – 7.14 (m, 5H)*, 4.77 (ddd, *J* = 10.0, 8.1, 4.6 Hz, 1H), 4.71 (dd, *J* = 8.3, 2.3 Hz, 1H), 4.54 – 4.64 (m, 1H)*, 4.33 (dd, *J* = 8.8, 3.0 Hz, 1H), 3.73 (s, 3H), 3.75 – 3.66 (m, 1H)*, 3.60 – 3.48 (m, 1H)*, 3.40 – 3.27 (m, 2H)*, 3.16 (dd, *J* = 11.3, 7.2 Hz, 1H), 2.94 (dd, *J* = 14.2, 10.1 Hz, 1H), 2.17 – 2.05 (m, 2H)*, 1.97 – 1.59 (m, 4H)*, 1.58 – 1.43 (m, 1H)*. *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Major conformer: δ = 172.0, 170.4, 168.0, 137.3, 129.2, 128.3, 126.6, 61.2, 59.2, 52.8, 52.4, 46.9, 45.7, 36.7, 29.4, 28.2, 24.9, 23.7; minor conformer: δ = 171.8, 167.9, 136.6, 129.1, 128.6, 128.2, 127.0, 59.8, 58.2, 53.7, 52.5, 46.7, 45.9, 37.3, 31.78, 28.7, 22.1; HRMS (ESI): *m/z* calcd for $C_{20}H_{27}N_3O_4$+H^+: 374.2074 [*M*+H^+]; found: 374.2079.

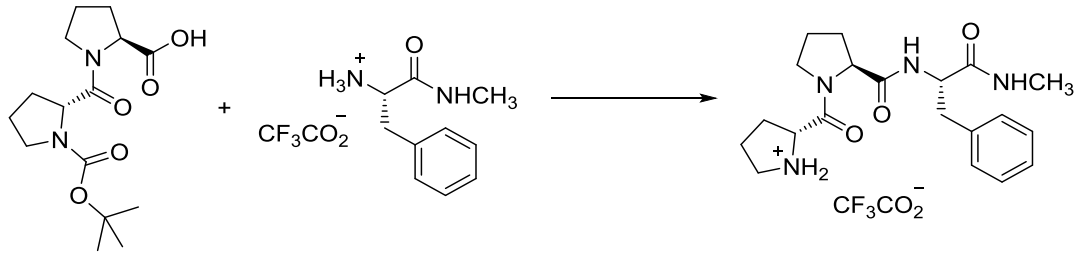
TFA·H-D-Pro-Pro-D-Phe-OMe:



Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and D-Phenylalanine methylester hydrochloride (69 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a white hygroscopic foam (60 mg, 38%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): *Cis/trans* conformers were observed in a ratio of 3:1. Major conformer: δ = 7.68 (d, *J* = 8.1 Hz, 1H), 7.32 – 7.11 (m, 5H), 4.73 (td, *J* = 8.6, 5.4 Hz, 1H), 4.62 (t, *J* = 8.2 Hz, 1H), 4.49 – 4.44 (m, 1H), 3.75 (td, *J* = 8.9, 8.0, 2.8 Hz, 1H), 3.68 (s, 3H), 3.46 – 3.32 (m, 3H), 3.18 (dd, *J* = 14.1, 5.5 Hz, 1H), 3.00 (dd, *J* = 14.1, 9.0 Hz, 1H), 2.49 – 2.36 (m, 1H), 2.15 – 1.84 (m, 6H), 1.83 – 1.73 (m, 1H); visible signals of the minor conformer: δ = 7.83 (d, *J* = 8.2 Hz, 1H), 4.80 – 4.75 (m, 1H)*, 4.46 – 4.40 (m, 1H)*, 3.75 (td, *J* = 8.9, 8.0, 2.8 Hz, 1H)*, 3.71 (s, 3H), 3.54 (dd, *J* = 8.6, 5.8 Hz, 1H), 3.24 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.93 (dd, *J* = 14.2, 9.8 Hz, 1H), 2.29 – 2.19 (m, 1H), 2.17 – 2.10 (m, 1H)*, 1.64 – 1.55 (m, 1H)*. *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Major conformer: δ = 172.3, 170.8, 168.0, 136.9, 129.2, 128.3, 126.7, 60.8, 59.1, 53.6, 52.4, 47.1, 46.0, 36.9, 29.3, 28.5, 24.9, 24.1; visible signals of the minor conformer: δ = 136.2, 129.1, 128.5, 127.0, 58.6, 53.3, 47.7, 46.8, 37.6, 29.4, 24.9; MS (ESI): *m/z* calcd for $C_{20}H_{27}N_3O_4$+H^+: 374.2 [*M*+H^+]; found: 374.2.

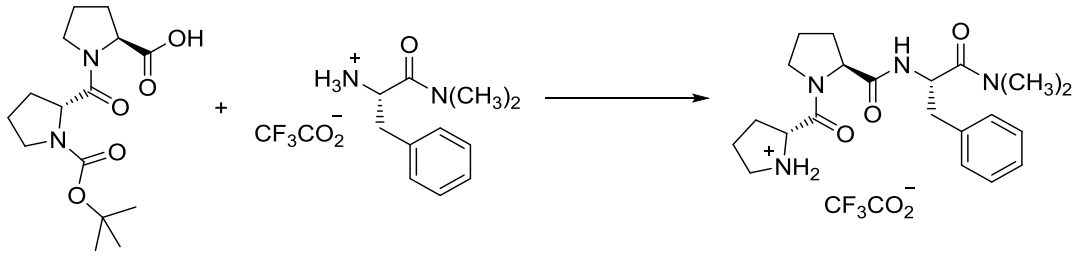
TFA·H-D-Pro-Pro-Phe-NHCH$_3$:



Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and the TFA-salt of (*S*)-2-amino-*N*-methyl-3-phenylpropanamide (TFA·H-Phe-NHCH$_3$, 94 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a white powder (96 mg, 62%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): The *cis/trans* conformers were observed in a ratio of 7:1. Major conformer: δ = 7.34 – 7.16 (m, 5H), 4.60 – 4.50 (m, 2H), 4.37 (dd, *J* = 8.8, 3.3 Hz, 1H), 3.69 (ddd, *J* = 9.6, 7.3, 4.2 Hz, 1H), 3.59 – 3.47 (m, 1H), 3.42 (dt, *J* = 11.3, 7.0 Hz, 1H), 3.38 – 3.31 (m, 1H), 3.14 (dd, *J* = 13.7, 6.4 Hz, 1H), 2.96 (dd, *J* = 13.8, 8.6 Hz, 1H), 2.67 (s, 3H), 2.52 (ddt, *J* = 13.0, 8.9, 6.8 Hz, 1H), 2.20 – 2.10 (m, 1H), 2.07 (ddd, *J* = 13.4, 6.9, 2.0 Hz, 2H), 2.03 – 1.75 (m, 4H); visible signals of the minor conformer: δ = 4.63 – 4.57 (m, 1H)*, 4.55 – 4.49 (m, 1H)*, 4.13 (dd, *J* = 8.9, 7.2 Hz, 1H), 3.18 – 3.08 (m, 1H)*, 2.88 (dd, *J* = 13.9, 10.7 Hz, 1H), 2.72 (s, 3H), 2.31 – 2.18 (m, 1H)*, 1.79 – 1.67 (m, 1H)*, 1.66 – 1.52 (m, 1H), 1.46 – 1.35 (m, 1H). *Superimposed by signals of the ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformer δ = 173.8, 173.7, 168.9, 138.6, 130.3, 129.5, 127.8, 62.3, 60.7, 56.3, 48.5, 47.5, 38.6, 30.7, 29.3, 26.3, 25.5, 25.4; visible signals of the minor conformer: δ = 173.9, 173.6, 168.7, 138.8, 130.4, 129.6, 128.0, 61.4, 60.1, 56.4, 47.4, 39.1, 33.2, 29.5, 26.4, 25.6; MS (ESI): *m/z* calcd $C_{20}H_{28}N_4O_3$+H^+: 372.2 [*M*+H^+]; found: 372.2.

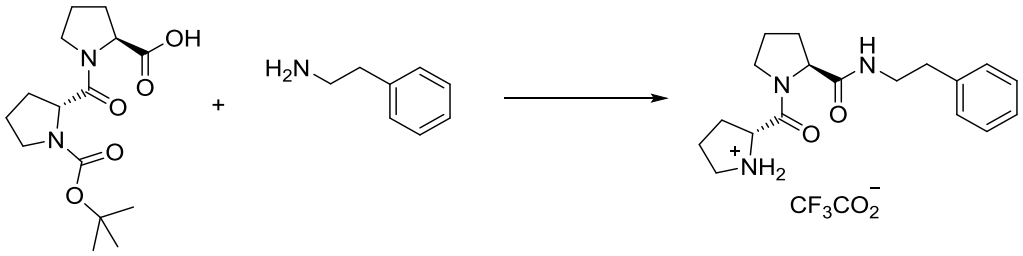
TFA·H-D-Pro-Pro-Phe-N(CH_3)$_2$:



Prepared from Boc-D-Pro-Pro-OH (100 mg, 320 μmol) and the TFA salt of (*S*)-2-amino-*N*,*N*-dimethyl-3-phenylpropanamide (TFA·H-Phe-N(CH_3)$_2$, 98 mg, 320 μmol) according to the general protocols A and C. The peptide was obtained as a white powder (80 mg, 50%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): The *cis/trans* conformers were observed in a ratio of 7:1. Major conformer: δ = 7.36 – 7.17 (m, 5H), 5.01 (dd, *J* = 8.4, 6.5 Hz, 1H), 4.55 (dd, *J* = 8.8, 6.8 Hz, 1H), 4.48 – 4.41 (m, 2H), 3.76 – 3.66 (m, 1H), 3.59 – 3.47 (m, 1H), 3.46 – 3.32 (m, 2H), 3.07 – 2.94 (m, 2H), 2.83 (s, 3H), 2.76 (s, 3H), 2.52 (ddt, *J* = 12.9, 9.0, 6.6 Hz, 1H), 2.20 (ddt, *J* = 10.5, 8.1, 5.4 Hz, 1H), 2.13 – 2.03 (m, 2H), 2.03 – 1.90 (m, 3H); visible signals of the minor conformer: δ = 5.06 (dd, *J* = 9.4, 6.0 Hz, 1H), 4.18 (t, *J* = 8.1 Hz, 1H), 3.26 – 3.15 (m, 1H), 2.60 – 2.53 (m, 1H)*, 2.30 – 2.20 (m, 1H)*, 1.93 – 1.85 (m, 1H)*, 1.85 – 1.75 (m, 1H), 1.73 – 1.53 (m, 2H). *Superimposed by signals of the ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformer δ = 173.5, 173.1, 168.5 137.9, 130.4, 129.6, 128.1, 61.9, 60.6, 52.2, 48.4, 47.7, 39.2, 37.5, 36.1, 30.7, 29.5, 25.5, 25.3; visible signals of the minor conformer: δ = 129.7, 128.2, 60.2, 52.5, 47.4, 37.6, 36.2, 33.1; MS (ESI): *m/z* calcd $C_{21}H_{30}N_4O_3$+H^+: 387.2 [*M*+H^+]; found: 387.2.

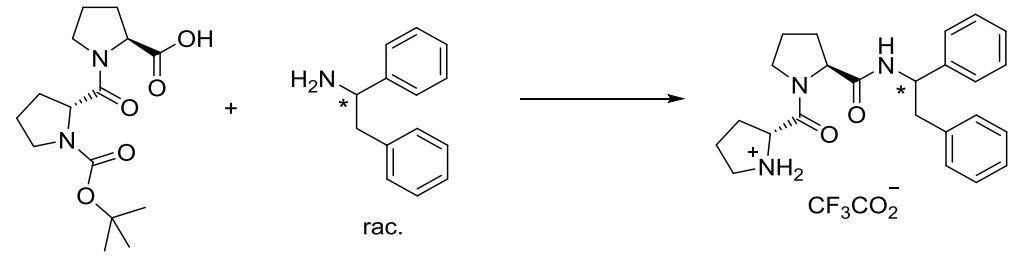
TFA·H-D-Pro-Pro-CH_2CH_2Ph:



Prepared from Boc-D-Pro-Pro-OH (312 mg, 1.00 mmol) and 2-phenethylamine (126 µL, 1.00 mmol) according to the general protocols A and C. The peptide was obtained as a white powder (285 mg, 77%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 7.33 – 7.11 (m, 5H), 4.58 – 4.49 (m, 1H), 4.34 (dd, *J* = 8.6, 3.6 Hz, 1H), 3.74 – 3.64 (m, 1H), 3.60 – 3.47 (m, 1H), 3.45 – 3.32 (m, 4H), 2.91 – 2.72 (m, 2H), 2.51 (ddt, *J* = 13.1, 9.1, 6.7 Hz, 1H), 2.23 – 2.12 (m, 1H), 2.11 – 2.02 (m, 2H), 2.02 – 1.91 (m, 3H), 1.89 – 1.80 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 174.1, 168.4, 140.5, 130.0, 129.5, 127.4, 62.1, 60.6, 47.6, 42.0, 36.5, 31.1, 29.5, 25.5, 25.3; MS (ESI): *m/z* calcd $C_{18}H_{25}N_3O_2+H^+$: 316.2 [$M+H^+$]; found: 316.2.

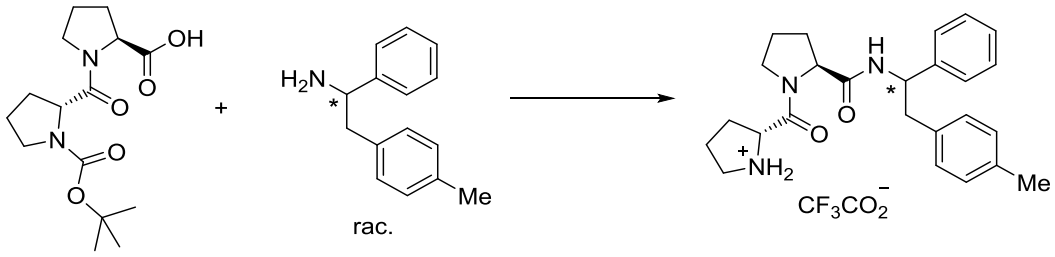
TFA·H-D-Pro-Pro-NHCH(Ph)-CH_2-Ph:



Prepared from Boc-D-Pro-Pro-OH (150 mg, 480 µmol) and racemic 1-phenyl-(2-*p*-tolyl)ethylamine (95 mg, 480 µmol) according to the general protocols A and C. The peptide was obtained as a white powder (195 mg, 80%). The peptide is a 1:1 mixture of the two diastereoisomers H-D-Pro-Pro-NH-(*R*)-CH(Ph)-CH_2-Ph and H-D-Pro-Pro-NH-(*S*)-CH(Ph)-CH_2-Ph.

^{1}H NMR (400 MHz, CD_3OD, 25°C): The *cis/trans* conformers were observed in a ratio of ~5:1. Only the major conformers are reported: δ = 7.53 – 7.00 (m, 2 x 10H), 5.24 (dd, *J* = 10.6, 5.2 Hz, 1H), 5.11 (t, *J* = 7.6 Hz, 1H), 4.53 (dd, *J* = 8.8, 7.1 Hz, 1H), 4.47 – 4.41 (m, 2x 1H), 4.37 (dd, *J* = 8.6, 3.6 Hz, 1H), 3.71 – 3.57 (m, 2 x 1H), 3.56 – 3.29 (m, 5H), 3.28 – 3.11 (m, 3H), 3.10 – 3.02 (m, 1H), 2.93 (dd, *J* = 13.9, 10.5 Hz, 1H), 2.49 (ddt, *J* = 13.1, 8.9, 6.6 Hz, 2 x 1H), 2.21 – 1.66 (m, 13H), 1.46 – 1.35 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformers: δ = 173.4, 173.3, 168.5, 168.1, 143.5, 143.1, 139.8, 139.5, 130.4, 130.3, 129.5, 129.4, 129.3, 129.3, 128.2, 128.2, 127.9, 127.6, 127.5, 127.4, 62.1, 61.9, 60.6, 60.5, 56.6, 55.7, 48.3, 48.3, 47.5, 47.5, 43.8, 43.3, 31.0, 30.8, 29.5, 29.4, 25.4, 25.3, 25.3, 25.3; MS (ESI): *m/z* calcd for $C_{24}H_{29}N_3O_2$+H^+: 392.2 [*M*+H^+]; found: 392.3.

TFA·H-D-Pro-Pro-NHCH(Ph)-CH_2-4-Me-C_6H_4 (10):

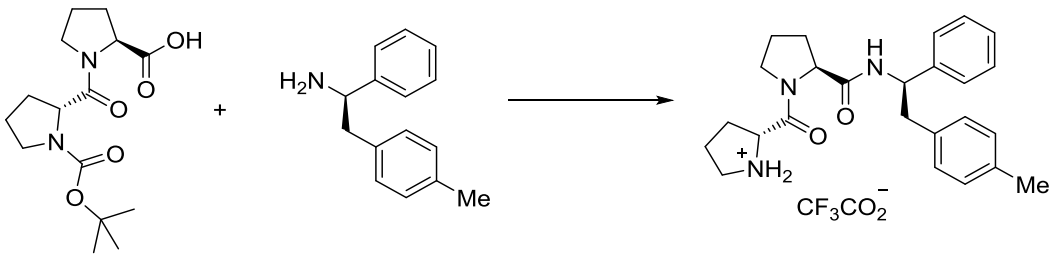


Prepared from Boc-D-Pro-Pro-OH (2.00 g, 6.40 mmol) and racemic 1-phenyl-(2-*p*-tolyl)ethylamine (1.35 g, 6.40 mmol, TCI chemicals, cas 30275-30-0) according to the general protocols A and C. The peptide was obtained as a white powder (3.10 g, 93%). The peptide is a 1:1 mixture of the two diastereoisomers H-D-Pro-Pro-NH-(*R*)-CH(Ph)-CH_2-4-Me-C_6H_4 (**10-R**) and H-D-Pro-Pro-NH-(*S*)-CH(Ph)-CH_2-4-Me-C_6H_4 (**10-S**).

^{1}H NMR (400 MHz, CD_3OD, 25°C): The ratio of *cis/trans* conformers could not be determined due to overlapping signals. Major conformers: δ = 8.58 (d, *J* = 8.9 Hz, 1H), 8.54 (d, *J* = 8.2 Hz, 1H), 7.42 – 7.17 (m, 10H), 7.15 – 7.05 (m, 4H), 7.03 – 6.95 (m, 4H), 5.26 – 5.16 (m, 1H), 5.06 (t, *J* = 7.6 Hz, 1H), 4.53 (dd, *J* = 8.8, 7.1 Hz, 1H), 4.47 – 4.42 (m, 2H), 4.37 (dd, *J* = 8.6, 3.5 Hz, 1H), 3.63 (2 x ddd, *J* = 9.8, 7.6, 4.6 Hz, 2H), 3.57 – 3.28 (m, 5H), 3.25 (dd, *J* = 11.4, 7.1 Hz, 1H), 3.19 – 3.07 (m, 2H), 3.01 (dd, *J* = 13.7,

7.7 Hz, 1H), 2.89 (dd, *J* = 13.9, 10.4 Hz, 1H), 2.49 (2 x ddt, *J* = 13.1, 8.8, 6.6 Hz, 2H), 2.29 (s, 3H), 2.25 (s, 3H), 2.22 – 1.70 (m, 13H), 1.49 – 1.40 (m, 1H); minor conformers: δ = 8.73 – 8.66 (m, 2H), 7.41 – 7.18 (m, 12H)*, 7.14 – 7.10 (m, 6H)*, 5.25 – 5.12 (m, 2H)*, 4.49 – 4.41 (m, 2H)*, 4.15 – 4.07 (m, 2H)*, 3.57 – 3.38 (m, 4H)*, 3.38 – 3.20 (m, 3H)*, 3.19 – 2.95 (m, 5H)*, 2.30 (s, 3H), 2.29 (s, 3H)*, 2.22 – 1.39 (m, 15H)*, 1.32 – 1.21 (m, 1H). *Superimposed by signals of the major conformers; ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformers: δ = 173.4, 173.2, 168.5, 168.0, 143.5, 143.1, 137.1, 137.0, 136.6, 136.3, 130.3, 130.2, 129.9, 129.9, 129.5, 129.4, 128.2, 128.2, 127.9, 127.7, 62.1, 61.9, 60.6, 60.5, 56.8, 55.8, 48.4, 48.3, 47.6, 47.5, 43.4, 42.9, 31.0, 30.8, 29.5 (two signals), 25.4, 25.3 (two signals), 25.3, 21.1 (two signals); visible signals of the minor conformers: δ = 173.0, 130.3, 130.2, 130.2, 130.0, 129.7, 127.5, 61.4, 61.3, 59.9, 56.3, 47.4, 47.2, 42.8, 29.4; HRMS (ESI): *m/z* calcd for $C_{25}H_{31}N_3O_2$+H^+: 406.2489 [*M*+H^+]; found: 406.2482.

TFA·H-D-Pro-Pro-NH-(*R*)-CH(Ph)-CH_2-4-Me-C_6H_4 (10-R):



Prepared from Boc-D-Pro-Pro-OH (718 mg, 2.30 mmol) and (*R*)-1-phenyl-(2-*p*-tolyl)ethylamine (485 mg, 2.30 mmol) according to the general protocols A and C. The peptide was obtained as a white powder (1.10 g, 92%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): The *cis/trans* conformers were observed in a ratio of 5:1. Major conformer: δ = 8.58 (d, *J* = 8.9 Hz, 1H), 7.41 – 7.36 (m, 2H), 7.35 – 7.20 (m, 3H), 7.15 – 7.05 (m, 4H), 5.20 (dd, *J* = 10.4, 5.4 Hz, 1H), 4.44 (dd, *J* = 8.7, 6.9 Hz, 1H), 4.37 (dd, *J* = 8.6, 3.5 Hz, 1H), 3.61 (ddd, *J* = 9.7, 7.5, 4.7 Hz, 1H), 3.51 – 3.42 (m, 1H), 3.35 (dd, *J* = 11.4, 7.0 Hz, 1H), 3.25 (dd, *J* = 11.3, 7.0 Hz, 1H), 3.15 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.89 (dd, *J* = 13.9, 10.4 Hz, 1H), 2.46 (ddt, *J* = 13.0, 8.8, 6.6 Hz, 1H), 2.29 (s, 3H),

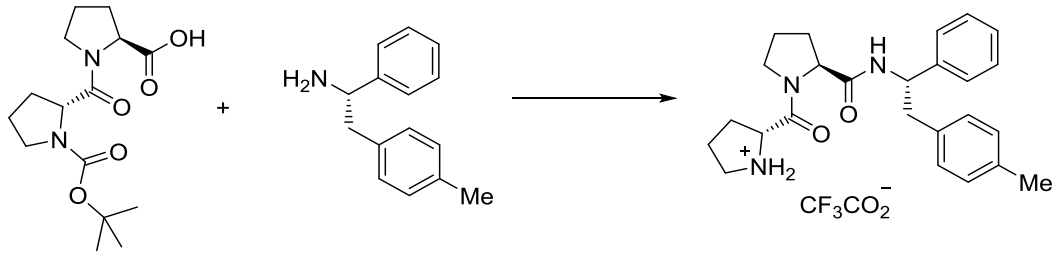
2.08 – 1.98 (m, 3H), 1.98 – 1.77 (m, 3H), 1.48 – 1.40 (m, 1H); minor conformer: δ = 8.70 (d, J = 8.8 Hz, 1H), 7.41 – 7.36 (m, 2H)*, 7.35 – 7.20 (m, 3H)*, 7.15 – 7.05 (m, 4H)*, 5.25 – 5.16 (m, 1H)*, 4.47 – 4.41 (m, 1H)*, 4.12 (t, J = 8.1 Hz, 1H), 3.55 – 3.45 (m, 2H)*, 3.38 – 3.21 (m, 2H)*, 3.11 (dd, J = 13.8, 5.8 Hz, 1H),* 3.01 (dd, J = 14.0, 10.1 Hz, 1H), 2.29 (s, 3H),* 2.21 – 2.08 (m, 1H), 2.07 – 1.61 (m, 5H)*, 1.60 – 1.51 (m, 1H), 1.51 – 1.39 (m, 1H)*. *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformer: δ = 173.4, 168.1, 143.5, 137.1, 136.6, 130.3, 129.9, 129.5, 128.2, 127.7, 61.9, 60.5, 55.8, 48.3, 47.5, 43.4, 31.0, 29.5, 25.4, 25.3, 21.1; visible signals of the minor conformer: δ = 172.9, 168.4, 143.7, 137.3, 136.4, 130.2, 130.0, 129.7, 128.6, 127.9, 61.4, 60.2, 56.3, 47.2, 42.9, 33.3, 30.0, 25.2, 23.1; HRMS (ESI): *m/z* calcd for $C_{25}H_{31}N_3O_2+H^+$: 406.2489 [M+H^+]; found: 406.2489; $[\alpha]_D^{23}$ = +20.7° (c = 1.0, MeOH).

Desalted H-D-Pro-Pro-NH-(*R*)-CH(Ph)-CH_2-4-Me-C_6H_4 (10-R):

The TFA was removed by extraction with $NaHCO_3$ solution. The TFA·**10-R** (1.0 g, 1.92 mmol) was suspended in a saturated aqueous solution of $NaHCO_3$ (40 mL) and sonicated until a clear solution was obtained. The solution was then extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phases were washed with water (40 mL), dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The resulting white foam was dissolved in water/MeCN 1:1 (20 mL) and lyophilized to afford the desalted peptide as a fluffy white solid (685 mg, 88%). The absence of TFA was confirmed by ^{19}F-NMR analysis.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.63 (q, J = 8.3 Hz, 1H), 7.30 – 7.23 (m, 2H), 7.23 – 7.17 (m, 1H), 7.17 – 7.11 (m, 2H), 7.03 (d, J = 8.0 Hz, 2H), 6.96 (d, J = 7.9 Hz, 2H), 5.11 (q, J = 7.5 Hz, 1H), 4.56 (dd, J = 8.1, 1.8 Hz, 1H), 3.78 (dd, J = 8.5, 6.1 Hz, 1H), 3.54 (ddd, J = 11.2, 8.3, 2.9 Hz, 1H), 3.38 (td, J = 9.6, 7.1 Hz, 1H), 3.21 – 3.11 (m, 1H), 3.04 – 2.96 (m, 2H), 2.84 (dt, J = 10.8, 6.8 Hz, 1H), 2.33 (ddt, J = 9.1, 6.7, 2.4 Hz, 1H), 2.28 (s, 3H), 2.15 – 2.04 (m, 1H), 2.04 – 1.94 (m, 1H), 1.94 – 1.63 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 174.9, 170.0, 142.1, 135.9, 134.6, 129.4, 129.0, 128.5, 127.2, 126.5, 60.2, 59.6, 55.1, 47.9, 46.9, 43.0, 30.1, 27.0, 26.6, 24.8, 21.2.

TFA·H-D-Pro-Pro-NH-(*S*)-CH(Ph)-CH_2-4-Me-C_6H_4 (10-S):

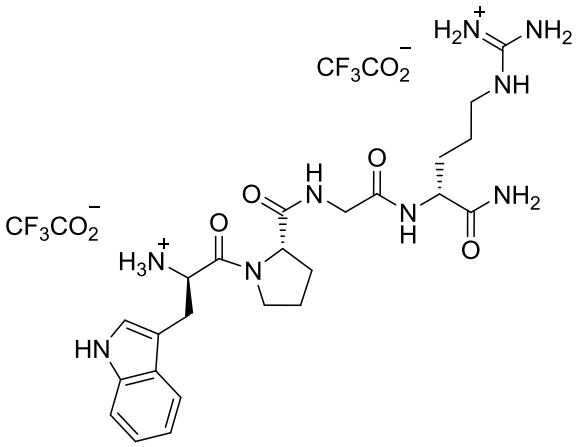


Prepared from Boc-D-Pro-Pro-OH (300 mg, 960 μmol) and (*S*)-1-phenyl-(2-*p*-tolyl)ethylamine (203 mg, 960 μmol, TCI chemicals, cas 30339-30-1) according to the general protocols A and C. The peptide was obtained as a white powder (420 mg, 84%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): The *cis/trans* conformers were observed in a ratio of 4:1. Major conformer: δ = 7.30 – 7.17 (m, 5H), 7.03 – 6.94 (m, 4H), 5.06 (t, *J* = 7.5 Hz, 1H), 4.52 (dd, *J* = 8.7, 7.0 Hz, 1H), 4.45 (dd, *J* = 8.7, 3.7 Hz, 1H), 3.65 (ddd, *J* = 9.8, 7.4, 4.8 Hz, 1H), 3.57 – 3.49 (m, 1H), 3.42 (dt, *J* = 11.3, 7.0 Hz, 1H), 3.36 – 3.29 (m, 1H), 3.11 (dd, *J* = 13.7, 7.2 Hz, 1H), 3.00 (dd, *J* = 13.6, 7.9 Hz, 1H), 2.52 (ddt, *J* = 13.0, 8.9, 6.6 Hz, 1H), 2.25 (s, 3H), 2.17 (dtd, *J* = 12.6, 8.9, 6.9 Hz, 1H), 2.12 – 2.04 (m, 2H), 2.03 – 1.95 (m, 1H), 1.95 – 1.83 (m, 2H), 1.83 – 1.74 (m, 1H); minor conformer: δ = 7.41 – 7.33 (m, 4H), 7.29 – 7.18 (m, 3H)*, 7.12 (d, *J* = 8.0 Hz, 2H), 5.16 (dd, *J* = 10.8, 4.8 Hz, 1H), 4.48 – 4.43 (m, 1H)*, 4.08 (dd, *J* = 9.0, 7.3 Hz, 1H), 3.57 – 3.47 (m, 2H)*, 3.33 – 3.26 (m, 1H)*, 3.16 – 2.95 (m, 3H)*, 2.30 (s, 3H), 2.23 – 1.71 (m, 5H)*, 1.71 – 1.58 (m, 1H), 1.55 – 1.41 (m, 1H), 1.32 – 1.21 (m, 1H). *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, CD_3OD, 25°C): Major conformer: δ = 173.2, 168.4, 143.1, 137.0, 136.3, 130.2, 129.9, 129.4, 128.2, 127.9, 62.0, 60.6, 56.8, 48.4, 47.6, 42.9, 30.8, 29.5, 25.3 (two signals), 21.1; visible signals of the minor conformer: δ = 173.0, 144.0, 130.3, 130.2, 129.7, 128.5, 127.5, 61.3, 59.9, 56.9, 47.4, 33.1, 29.4, 25.5, 25.2, 21.2; HRMS (ESI): *m/z* calcd $C_{25}H_{31}N_3O_2$+H^+: 406.2489 [*M*+H^+]; found: 406.2483.

7.3.2 Synthesis of Peptidic Catalysts for Reactions between α,α-Disubstituted Aldehydes and Nitrostyrene

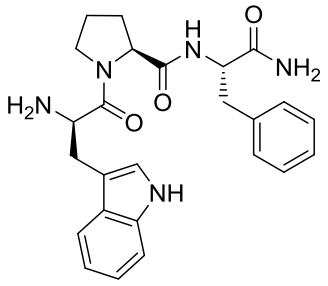
TFA·H-D-Trp-Pro-Gly-D-Arg-NH$_2$ (52):



The peptide was prepared on Rink Amide ChemMatrix resin (0.48 mmol/g) on a 72 μmol scale according the general protocols D and E. In protocol E TFA/H_2O/TIS 95:2.5:2.5 was used as cleavage solution. The peptide was obtained as its TFA-salt and was a white solid (22 mg, 42%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 7.58 – 7.48 (m, 2H), 7.44 – 7.33 (m, 1H), 7.29 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.20 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 4.57 (dd, *J* = 9.3, 5.6 Hz, 1H), 4.31 (dd, *J* = 9.1, 5.0 Hz, 1H), 4.15 – 4.03 (m, 1H), 4.04 – 3.83 (m, 2H), 3.52 – 3.30 (m, 3H), 3.18 (q, *J* = 6.9 Hz, 2H), 1.97 – 1.53 (m, 8H), 1.31 – 1.15 (m, 1H); MS (ESI): *m/z* calcd $C_{24}H_{35}N_9O_4$+H^+: 514.3 [*M*+H^+]; found: 514.3.

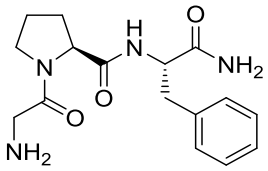
H-D-Trp-Pro-Phe-NH_2 (53):



The peptide was prepared on Rink Amide ChemMatrix resin (0.48 mmol/g) on a 144 μmol scale according the general protocols D and E. In protocol E TFA/H_2O/TIS 95:2.5:2.5 was used as cleavage solution. The TFA was removed according to general protocol F. The peptide was obtained as a white solid (26 mg, 45%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 7.47 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.32 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.27 – 7.12 (m, 5H), 7.11 – 7.06 (m, 2H), 7.01 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 4.60 (dd, *J* = 10.4, 4.9 Hz, 1H), 3.95 (dd, *J* = 6.9, 5.8 Hz, 1H), 3.85 (dd, *J* = 10.0, 5.2 Hz, 1H), 3.31 – 3.25 (m, 1H), 3.22 (ddd, *J* = 9.9, 7.2, 5.5 Hz, 1H), 3.11 (ddd, *J* = 13.6, 5.1, 0.7 Hz, 1H), 3.03 (ddd, *J* = 13.6, 10.0, 0.7 Hz, 1H), 2.86 (dd, *J* = 14.1, 10.5 Hz, 1H), 2.35 (dt, *J* = 9.8, 7.2 Hz, 1H), 1.32 – 1.18 (m, 3H), 0.94 – 0.83 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 177.2, 176.2, 174.2, 139.0, 138.0, 130.1, 129.4, 128.6, 127.7, 124.6, 122.7, 119.9, 119.3, 112.4, 111.2, 71.6, 62.3, 55.6, 55.4, 48.1, 37.8, 33.0, 30.0, 24.9; HRMS (ESI): *m/z* calcd $C_{25}H_{29}N_5O_3$+H^+: 448.2343 [*M*+H^+]; found: 448.2343.

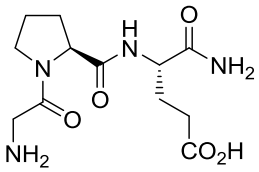
H-Gly-Pro-Phe-NH_2 (54):



The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 216 μmol scale according the general protocols D and E. The TFA was removed according to general protocol F. The peptide was obtained as a white solid (46 mg, 67%).

^{1}H NMR (400 MHz, D_2O, 25°C): The *cis/trans* conformers were observed in a ratio of 4:1. Major conformer: δ = 7.44 – 7.36 (m, 2H), 7.36 – 7.28 (m, 3H), 4.65 (dd, *J* = 9.0, 6.3 Hz, 1H), 4.36 (dd, *J* = 8.8, 4.4 Hz, 1H), 3.55 – 3.44 (m, 4H), 3.23 (dd, *J* = 13.9, 6.2 Hz, 1H), 3.05 (dd, *J* = 14.0, 9.1 Hz, 1H), 2.14 (dtd, *J* = 12.7, 8.5, 6.8 Hz, 1H), 1.92 (dtt, *J* = 12.0, 6.6, 5.4 Hz, 1H), 1.84 – 1.74 (m, 1H), 1.74 – 1.63 (m, 1H); visible signals of the minor conformer: 4.79 – 4.75 (m, 1H), 4.35 – 4.31 (m, 1H), 3.28 (dd, *J* = 14.2, 5.3 Hz, 1H), 2.98 (dd, *J* = 14.0, 10.6 Hz, 1H), 2.28 (dddd, *J* = 13.0, 10.8, 8.8, 7.0 Hz, 1H) ^{13}C NMR (101 MHz, D_2O, 25°C): Major conformer: δ = 175.7, 174.3, 173.5, 136.5, 129.1, 128.7, 127.1, 60.5, 54.3, 46.7, 42.6, 36.5, 29.1, 24.1; minor conformer δ = 175.4, 173.7, 173.1, 136.6, 129.1, 128.8, 127.1, 59.5, 54.2, 47.3, 42.0, 37.1, 31.7, 21.8; HRMS (ESI): *m/z* calcd $C_{16}H_{22}N_4O_3$+Na^+: 341.1584 [*M*+Na^+]; found: 341.1588.

H-Gly-Pro-Glu-NH_2 (55):

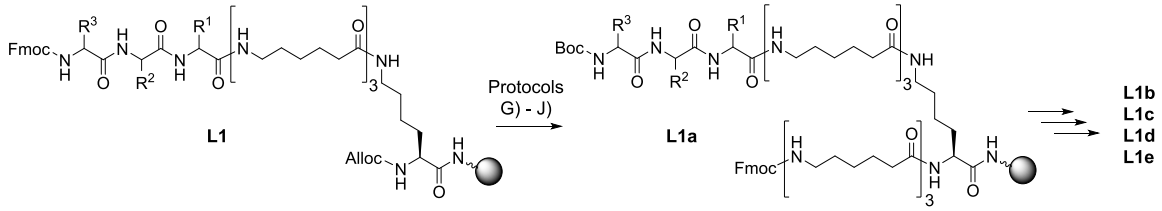


The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 216 μmol scale according the general protocols D and E. The TFA was removed according to general protocol F. The peptide was obtained as a white solid (41 mg, 75%).

^{1}H NMR (400 MHz, D_2O, 25°C): The *cis/trans* conformers were observed in a ratio of 7:1. Major conformer: δ = 4.47 (dd, *J* = 8.7, 4.3 Hz, 1H), 4.25 (dd, *J* = 8.6, 4.8 Hz, 1H), 4.06 (d, *J* = 16.5 Hz, 1H), 3.97 (d, *J* = 16.4 Hz, 1H), 3.69 – 3.52 (m, 2H), 2.40 – 2.23 (m, 3H), 2.13 – 1.92 (m, 5H); visible signals of the minor conformer: δ = 4.54 (dd, *J* = 8.7, 2.9 Hz, 1H), 4.32 (dd, *J* = 9.4, 5.0 Hz, 1H), 3.93 (d, *J* = 16.5 Hz, 3H), 2.44 – 2.38 (m, 1H), 2.18 (ddt, *J* = 13.1, 6.6, 3.4 Hz, 1H), 1.90 – 1.82 (m, 1H); ^{13}C NMR (101 MHz, D_2O, 25°C): Major conformer: δ = 181.7, 176.3, 174.1, 166.3, 60.7, 53.9, 47.0, 40.56 33.3, 29.4, 27.0, 24.3; minor conformer δ = 181.3, 176.1, 173.5, 166.2, 59.7, 53.7, 47.6, 40.3, 33.6, 31.8, 27.4, 22.0; HRMS (ESI): *m/z* calcd $C_{12}H_{20}N_4O_5$+Na^+: 323.1326 [*M*+Na^+]; found: 323.1327.

7.4 Synthesis of Catalyst-Substrate Co-Immobilized Libraries

7.4.1 Synthesis of Libraries L1a – L1e



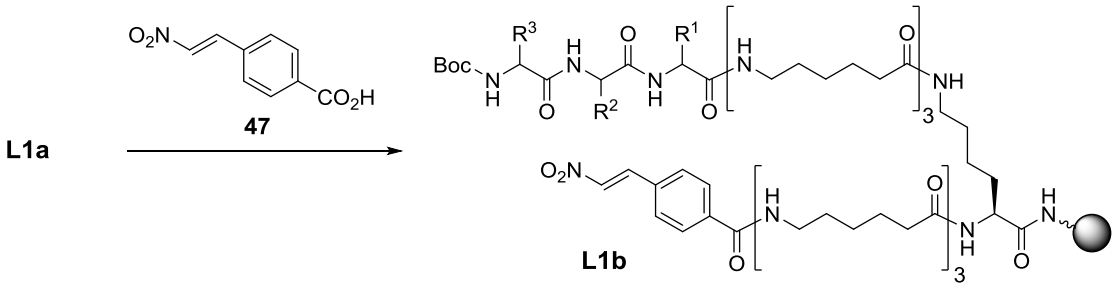
G) 20% piperidine/DMF; H) Boc_2O (10 eq,), iPr_2NEt (10 eq.), DMF; I) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2;
J) Fmoc-6-Ahx-OH (4 eq.), HCTU (4 eq.), iPr_2NEt (8 eq.), DMF then repeat G) and J) two times.

Boc/Fmoc-protected unfunctionalized library **L1a** was prepared according to Protocols G – J from Fmoc/Alloc-protected unfunctionalized library **L1** (600 mg, 90 μmol) which had previously been prepared in our group by C. Pfumbidzai.[217] Fmoc-6-aminohexanoic acid was used in Protocol J and was coupled 3 times.

Catalyst-substrate co-immobilized libraries **L1b** – **L1d**, and acetylated library **L1e** were prepared from library **L1a** according to Protocols G, J and K.

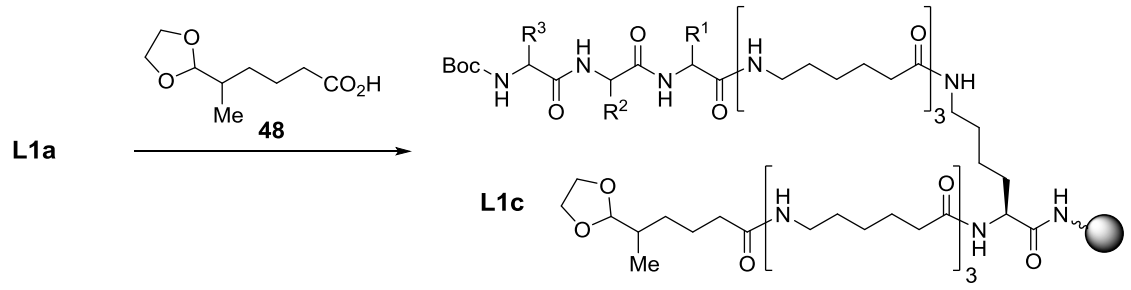
Quantitative Fmoc-analysis (Protocol N) of library **L1** indicated a loading of ~ 0.15 mmol g^{-1}. This loading was also assumed for libraries **L1a** – **L1e**.

Nitroolefin Functionalized Library L1b:



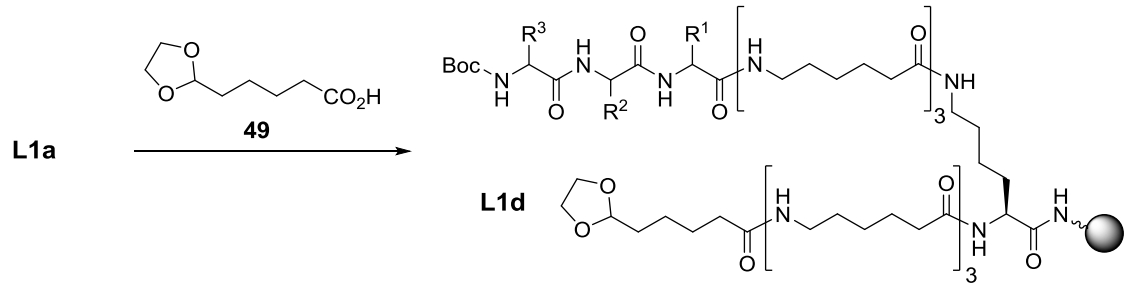
Library **L1b** was prepared from library **L1a** (150 mg, 22.5 μmol) according to Protocols G and J using nitroolefin **47** in Protocol J.

Branched Aldehyde Functionalized Library L1c:



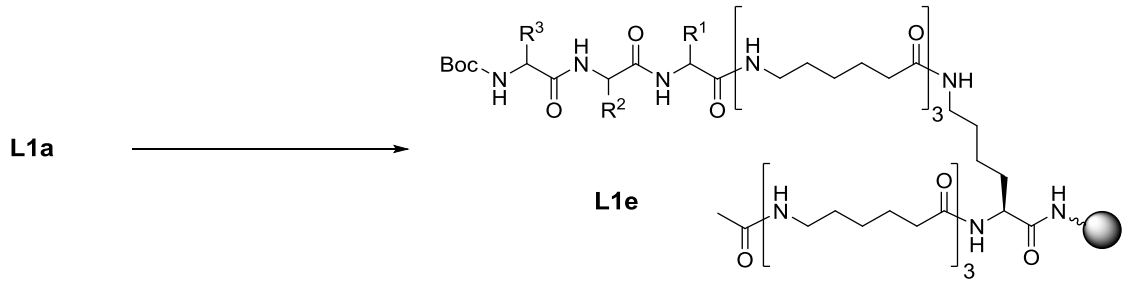
Library **L1c** was prepared from library **L1a** (150 mg, 22.5 μmol) according to Protocols G and J using carboxylic acid **48** in Protocol J. A 10 mg portion of library **L1c** was globally deprotected according to Protocol L1 immediately prior to the screening with Disperse-Red labeled nitroolefin **51** (Protocol M).

Linear Aldehyde Functionalized Library L1d:



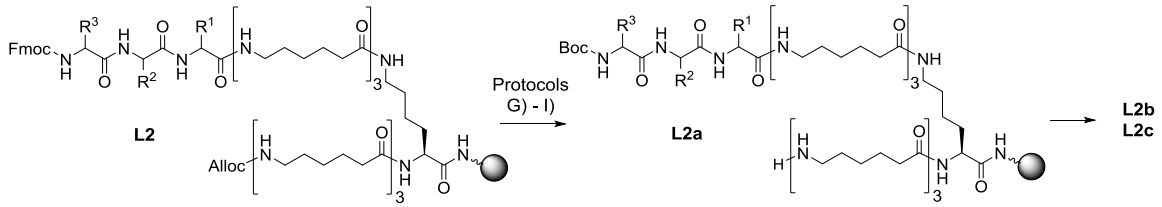
Library **L1d** was prepared from library **L1a** (150 mg, 22.5 μmol) according to Protocols G and J using carboxylic acid **49** in Protocol J. A 10 mg portion of library **L1d** was globally deprotected according to Protocol L1 immediately prior to the screening with Disperse-Red labeled nitroolefin **51** (Protocol M).

Acetylated Library L1e:



Library **L1e** was prepared from library **L1a** (150 mg, 22.5 μmol) according to Protocols G and K. 10 mg portions of library **L1e** were globally deprotected according to Protocol L1 immediately prior to the screening with Disperse-Red labeled aldehyde **50** or nitroolefin **51** (Protocol M).

7.4.2 Synthesis of Libraries L2a – L2c



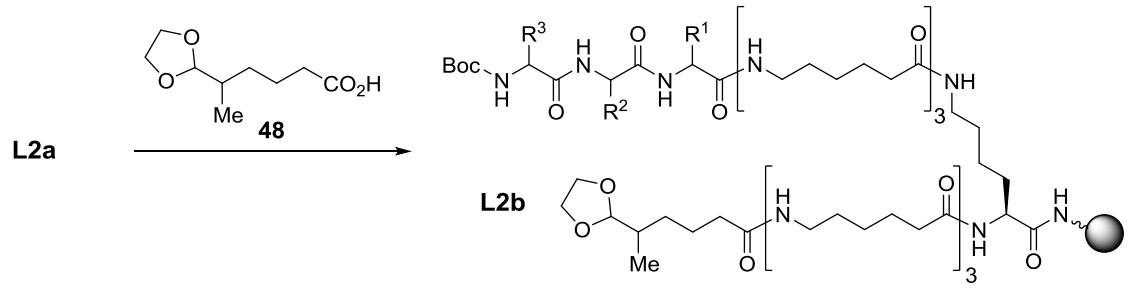
G) 20% piperidine/DMF; H) Boc_2O (10 eq,), iPr_2NEt (10 eq.), DMF; I) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2

Boc/Fmoc-protected unfunctionalized library **L2a** was prepared according to Protocols G – I from Fmoc/Alloc-protected unfunctionalized library **L2** (600 mg, 60 μmol) which had previously been prepared in our group.[163-164]

Catalyst-substrate co-immobilized libraries **L2b** and **L2d** were prepared from library **L2a** according to Protocol J.

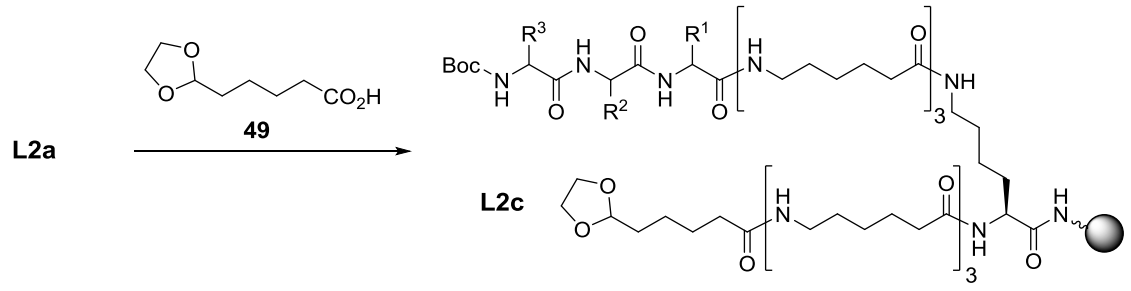
Quantitative Fmoc-analysis (Protocol N) of library **L2** indicated a loading of ~ 0.10 mmol g^{-1}. This loading was also assumed for libraries **L2a** – **L2c**.

Branched Aldehyde Functionalized Library L2b:



Library **L2b** was prepared from library **L2a** (300 mg, 30 μmol) according to Protocol J using carboxylic acid **48** in Protocol J. A 15 mg portion of library **L2b** was globally deprotected according to Protocol L2 immediately prior to the screening with Disperse-Red labeled nitroolefin **51** (Protocol M).

Linear Aldehyde Functionalized Library L2c:

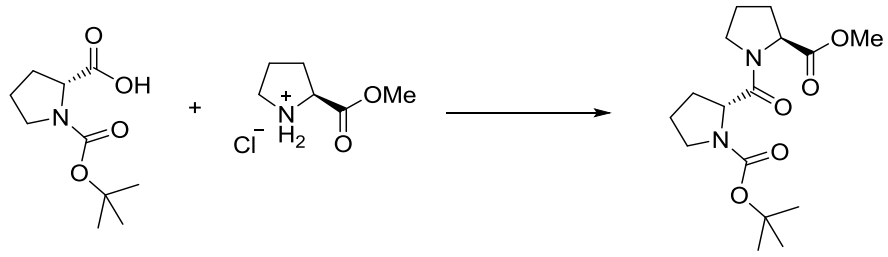


Library **L2c** was prepared from library **L2a** (300 mg, 30 μmol) according to Protocols J using carboxylic acid **49** in Protocol J. A 15 mg portion of library **L2c** was globally deprotected according to Protocol L2 immediately prior to the screening with Disperse-Red labeled nitroolefin **51** (Protocol M).

7.5 Synthesis of Substrates and Building Blocks

7.5.1 Building Blocks for Peptidic Catalysts

Boc-D-Pro-Pro-OMe:

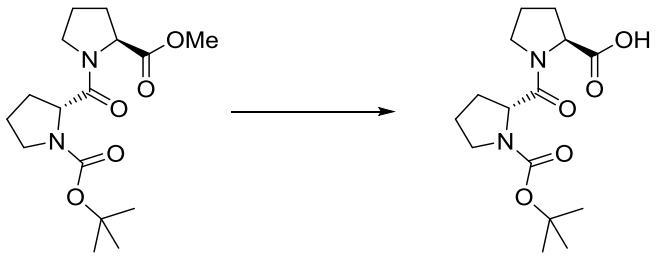


Boc-D-Pro-OH (10.0 g, 46.5 mmol), EDC·HCl (10.7 g, 55.8 mmol) and HOBt·H_2O (8.6 g, 55.8 mmol) were suspended in dry CH_2Cl_2 (200 mL) and cooled to 0°C under a nitrogen atmosphere. *i*PrNEt$_2$ (17.4 mL, 102 mmol) was added dropwise over 10 minutes, and the resulting yellow solution was stirred for an additional 10 minutes before HCl·H-Pro-OMe (7.3 g, 44.3 mmol) was added as a solid in 3 equal portions. The resulting homogeneous yellow reaction mixture was stirred at room temperature for 4 hours and diluted with 200 mL of 0.1 M HCl. The layers were separated and the aqueous phase extracted with CH_2Cl_2 (3 x 30 mL). The combined organic phases were washed with 1 M $NaHCO_3$ solution (100 mL), water (100 mL) and brine (100 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (EtOAc, TLC visualised with ninhydrin) to afford the title compound as a colorless oil (10.4 g, 72%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): *Cis/trans* conformers were observed in a ratio of 2:1:1:1. Major conformer: δ = 4.48 – 4.39 (m, 2H), 3.81 – 3.34 (m, 4H), 3.72 (s, 3H), 2.41 – 1.74 (m, 8H), 1.47 (s, 9H); Signals of the three minor conformers: δ = 5.00 (dd, *J* = 8.8, 2.6 Hz, 1H), 4.63 (dd, *J* = 8.2, 3.2 Hz, 1H), 4.55 (dd, *J* = 7.8, 2.9 Hz, 1H), 4.48 – 4.39 (m, 1H)*, 4.24 (dd, *J* = 7.8, 4.2 Hz, 1H), 4.15 (dd, *J* = 7.3, 5.6 Hz, 1H), 4.02 – 3.94 (m, 1H), 3.81 – 3.34 (m, 12H)*, 3.77 (s, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 2.41 – 1.74 (m, 24H)*, 1.45 (s, 9H), 1.43 (s, 9H), 1.40 (s, 9H);. *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Mixture of all conformers δ = 172.8, 172.6, 172.5, 172.4, 171.8, 154.5, 153.9, 79.8, 79.6, 79.4, 79.4, 59.6, 59.2, 59.2 58.0, 57.7, 57.4, 57.3, 52.6, 52.4, 52.1, 52.0, 47.2, 47.0, 46.9, 46.8, 46.7, 46.6, 46.5, 31.7, 31.5,

31.0, 30.4, 29.9, 29.4, 29.0, 28.9, 28.5, 28.4, 28.1, 25.0, 24.5, 23.7, 23.3, 22.6, 22.5; MS (ESI): *m/z* calcd for $C_{16}H_{26}N_2O_5+H^+$: 326.2 [$M+H^+$]; found: 326.4; $[\alpha]_D^{23}$ = -42.3° (c = 1.0, MeOH).

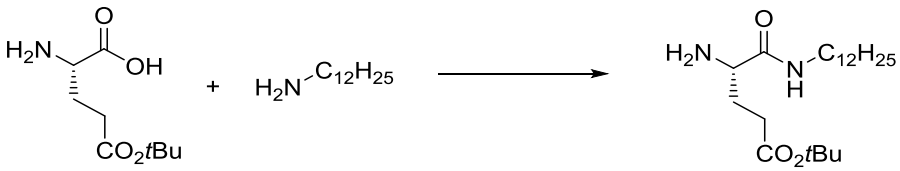
Boc-D-Pro-Pro-OH:



To a solution of Boc-D-Pro-Pro-OMe (8.3 g, 26.6 mmol) in MeOH (100 mL) was added 1M NaOH (40 mL) and the resulting yellow solution was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the resulting aqueous phase was washed with CH_2Cl_2 (3 x 50 mL) and was then acidified to pH 1 with HCl (32% aq.). The resulting colorless suspension was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford the title compound as a white solid (7.9 g, 95%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): *Cis/trans* conformers were observed in a ratio of 6:3:1. Major conformer: δ = 10.27 (br s, 1H), 4.61 (dd, *J* = 8.1, 2.1 Hz, 1H), 4.45 (dd, *J* = 8.5, 4.8 Hz, 1H), 3.77 (dt, *J* = 10.2, 5.3 Hz, 1H), 3.62 (ddd, *J* = 10.3, 7.4, 5.8 Hz, 1H), 3.57 – 3.34 (m, 2H), 2.59 – 1.83 (m, 8H), 1.40 (s, 9H); Mixture of the two minor conformers: δ = 4.99 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.67 – 4.58 (m, 1H)*, 4.51 – 4.39 (m, 1H)*, 4.26 (dd, *J* = 7.1, 5.2 Hz, 1H), 4.19 (dd, *J* = 7.5, 5.1 Hz, 1H), 4.05 – 3.96 (m, 1H), 3.69 – 3.34 (m, 6H)*, 2.62 – 1.71 (m, 16H)*, 1.43 (s, 9H), 1.44 (s, 9H). *Superimposed by signals of the major conformer; ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Mixture of all conformers δ = 176.0, 174.5, 171.7, 171.1, 155.0, 153.4, 80.7, 80.5, 60.7, 60.6, 57.9, 57.7, 47.6, 47.6, 46.9, 46.7, 30.3, 29.2, 28.5, 28.5, 28.4, 28.1, 28.1, 26.9, 24.9, 24.8, 24.7, 23.8; MS (ESI): *m/z* calcd for $C_{15}H_{24}N_2O_5+H^+$: 312.4 [$M+H^+$]; found: 312.4.

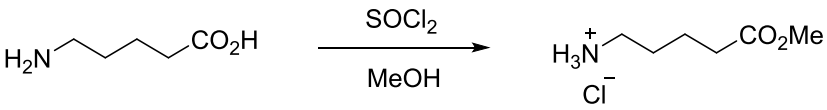
***tert*-Butyl (*S*)-4-amino-5-(dodecylamino)-5-oxopentanoate (H-Glu(O*t*Bu)NH-$C_{12}H_{25}$):**



Z-Glu(O*t*Bu)-OH (1.00 g, 2.97 mmol), *n*-dodecylamine (550 mg, 2.97 mmol) and EDC·HCl (680 mg, 3.55 mmol) were suspended in dry CH_2Cl_2 (20 mL) and *i*PrNEt$_2$ (0.60 mL, 3.55 mmol) was added. The resulting solution was stirred at room temperature for 5 h and then washed with 0.1 M HCl (2 x 10 mL), an aqueous solution of 1 M Na_2CO_3 (2 x 10 mL) and brine (10 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The resulting colorless solid was dissolved in MeOH (15 mL). Pd/C (10 % w/w, 100 mg) was added and the mixture was stirred under a hydrogen atmosphere at room temperature for 4 h. The reaction mixture was filtered over a pad of Celite. The Celite was washed with MeOH (3 x 5 mL). The solvent was removed under reduced pressure to give a colorless solid (1.08 g, 98 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.19 (t, *J* = 5.9 Hz, 1H), 3.30 (dd, *J* = 7.5, 5.0 Hz, 1H), 3.16 (td, *J* = 7.2, 5.9 Hz, 2H), 2.28 (t, *J* = 7.7 Hz, 1H), 2.27 (t, *J* = 7.2 Hz, 1H), 2.01 (ddt, *J* = 14.0, 7.2, 5.0 Hz, 1H), 1.73 (dtd, J = 14.0, 7.7, 7.5 Hz, 1H), 1.37 (s, 9H), 1.29 – 1.10 (m, 20H), 0.81 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 174.3, 172.8, 80.5, 54.7, 39.1, 32.0, 31.9, 30.4, 29.7, 29.6, 29.6, 29.6, 29.4, 29.3, 28.1, 27.0, 22.7, 14.1; MS (ESI): *m/z* calcd $C_{21}H_{43}N_2O_3$+H^+: 371.3 [*M*+H]$^+$; found: 371.3.

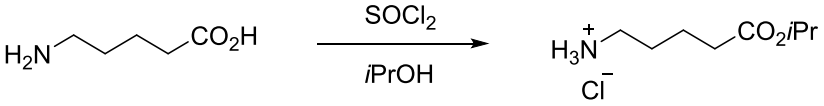
5-Aminovaleric acid methyl ester hydrochloride (HCl·H-5-Ava-OMe):



The building block was used for the synthesis of peptide H-D-Pro-Pro-5-Ava-OMe.

Thionyl chloride (7.7 mL, 53.4 mmol) was added dropwise to a cold suspension (0°C) of 5-aminovaleric acid (5.0 g, 42.7 mmol) in 30 mL of MeOH. The resulting solution was stirred at room temperature overnight. The solvent and the excess of thionyl chloride were removed under reduced pressure. Trituration with EtOAc of the resulting yellow sticky mass yielded the title compound as a white powder (6.30 g, 80%). ^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 3.67 (s, 3H), 2.94 (t, J = 6.8 Hz, 2H), 2.41 (t, J = 6.9 Hz, 2H), 1.73 – 1.67 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 175.2, 52.1, 40.4, 34.0, 28.0, 22.7; MS (ESI): *m/z* calcd $C_6H_{13}NO_2$+H^+: 132.1 [M+H^+]; found: 132.1.

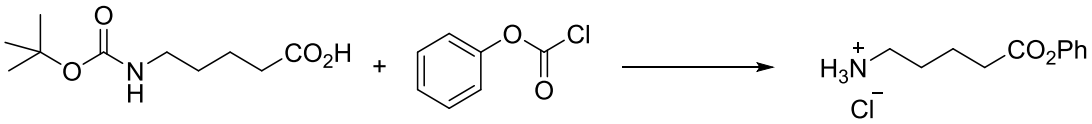
5-Aminovaleric acid isopropyl ester hydrochloride (HCl·H-5-Ava-O*i*Pr):



The building block was used for the synthesis of peptide H-D-Pro-Pro-5-Ava-O*i*Pr.

Thionyl chloride (1.6 mL, 21.4 mmol) was added dropwise to a cold suspension (0°C) of 5-aminovaleric acid (1.0 g, 8.54 mmol) in 10 mL of *i*PrOH. The resulting solution was refluxed overnight. The solvent and the excess of thionyl chloride were removed under reduced pressure. Trituration with EtOAc of the resulting yellow sticky mass yielded the title compound as a white powder (0.71 g, 42%). ^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 4.98 (hept, J = 6.3 Hz, 1H), 4.86 (br s, 3H), 2.99 – 2.89 (m, 2H), 2.40 – 2.32 (m, 2H), 1.77 – 1.64 (m, 4H), 1.23 (d, J = 6.3 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 174.3, 69.1, 40.4, 34.6, 27.9, 22.8, 22.1; MS (ESI): *m/z* calcd $C_8H_{17}NO_2$+H^+: 160.1 [M+H^+]; found: 160.1.

5-Aminovaleric acid phenyl ester hydrochloride (HCl·H-5-Ava-OPh):

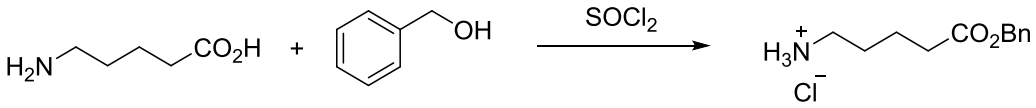


The building block was used for the synthesis of peptide H-D-Pro-Pro-5-Ava-OPh.

To a solution of Boc-5-aminovaleric acid (0.50 g, 2.30 mmol) in acetonitrile (10 mL) was added phenyl chloroformate (330 µL, 2.53 mmol), Et_3N (350 µL, 2.53 mmol) and DMAP (30 mg, 0.23 mmol). The reaction mixture was stirred at room temperature for 24 h, then filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and extracted with a saturated solution of $NaHCO_3$ (20 mL). The organic phase was washed with water (3 x 10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc/MeOH 20:1) and the obtained oil was dissolved in CH_2Cl_2 (30 mL) and TFA (3 mL). The solution was stirred at room temperature for 2 h and then the solvent and TFA were evaporated under reduced pressure. The resulting yellow mass was suspended in Et_2O/EtOAc 2:1 (20 mL) and sonicated until a fine suspension was obtained. The suspension was filtered and the solid was dried under high vacuum to afford the title compound as a white powder (202 mg, 40%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 7.43 – 7.35 (m, 2H), 7.27 – 7.20 (m, 1H), 7.12 – 7.06 (m, 5H), 3.04 – 2.95 (m, 2H), 2.72 – 2.64 (m, 2H), 1.84 – 1.77 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 173.3, 152.2, 130.4, 126.9, 122.7, 40.4, 34.2, 27.9, 22.7; MS (ESI): *m/z* calcd $C_{11}H_{15}NO_2$+H^+: 194.1 [*M*+H^+]; found: 194.1.

5-Aminovaleric acid benzyl ester hydrochloride (HCl·H-5-Ava-OBn):

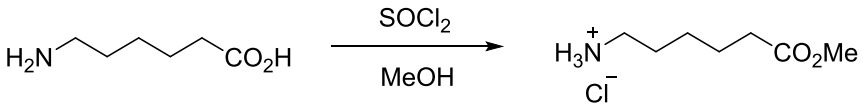


The building block was used for the synthesis of peptide H-D-Pro-Pro-5-Ava-OBn.

Thionyl chloride (3.9 mL, 53.4 mmol) was added dropwise to a cold suspension (0°C) of 5-aminovaleric acid (1.0 g, 8.54 mmol) in 5 mL of benzyl alcohol and the resulting solution was stirred at 60°C overnight. The mixture was cooled to 5°C and Et_2O (40 mL) was added. The resulting white suspension was sonicated for 5 min, the solvent was decanted and the white solid was once more suspended in Et_2O, sonicated and the solvent was decanted. The white solid was dried under high vacuum to afford the title compound as a white powder (1.69 g, 81%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 7.37 – 7.27 (m, 5H), 5.12 (s, 2H), 2.93 (t, J = 6.7 Hz, 2H), 2.48 – 2.41 (m, 2H), 1.80 – 1.60 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 174.5, 137.6, 129.5, 129.2 (2 signals), 67.3, 40.4, 34.2, 27.9, 22.7; MS (ESI): *m/z* calcd $C_{12}H_{17}NO_2$+H^+: 208.1 [M+H^+]; found: 208.1.

6-Aminohexanoic acid methyl ester hydrochloride (HCl·H-6-Ahx-OMe):

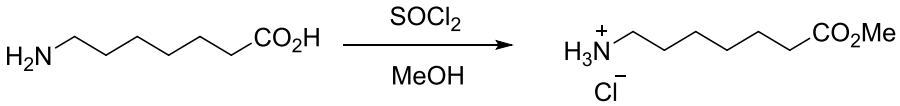


The building block was used for the synthesis of peptide H-D-Pro-Pro-6-Ahx-OMe.

Thionyl chloride (6.9 mL, 95.3 mmol) was added dropwise to a cold suspension (0°C) of 6-aminohexanoid acid (5.0 g, 38.1 mmol) in 30 mL of MeOH. The resulting solution was stirred at room temperature overnight. The solvent and the excess of thionyl chloride were removed under reduced pressure. Trituration with EtOAc of the resulting yellow sticky mass yielded the title compound as a white powder (5.65 g, 82%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 3.66 (s, 3H), 3.02 – 2.84 (m, 2H), 2.37 (t, J = 7.3 Hz, 2H), 1.77 – 1.58 (m, 4H), 1.50 – 1.36 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 175.6, 52.0, 40.6, 34.4, 28.2, 26.9, 25.4; MS (ESI): *m/z* calcd $C_7H_{15}NO_2$+H^+: 146.1 [M+H^+]; found: 146.1.

7-Aminoheptanoic acid methyl ester hydrochloride (HCl·H-7-Ahp-OMe):

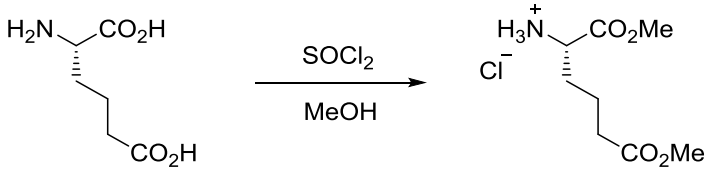


The building block was used for the synthesis of peptide H-D-Pro-Pro-7-Ahp-OMe.

Thionyl chloride (200 µL, 2.76 mmol) was added dropwise to a cold suspension (0°C) of 6-aminohexanoid acid (200 mg, 1.38 mmol) in 2 mL of MeOH. The resulting solution was stirred at room temperature overnight. The solvent and the excess of thionyl chloride were removed under reduced pressure. Trituration with EtOAc/pentane 4:1 of the residue yielded the title compound as an off-white powder (240 mg, 89%).

^{1}H NMR (400 MHz, D_2O, 25°C): δ = 3.68 (s, 3H), 2.97 (t, J = 7.5 Hz, 2H), 2.39 (t, J = 7.4 Hz, 2H), 1.71 – 1.53 (m, 4H), 1.44 – 1.25 (m, 4H); ^{13}C NMR (101 MHz, D_2O, 25°C): δ = 178.3, 52.5, 39.8, 33.9, 28.0, 26.9, 25.6, 24.4; MS (ESI): *m/z* calcd $C_8H_{18}NO_2$+H^+: 160.1 [M+H^+]; found: 160.1.

Dimethyl (*S*)-2-aminohexanedioate hydrochloride (HCl·H-Aad(OMe)-OMe):

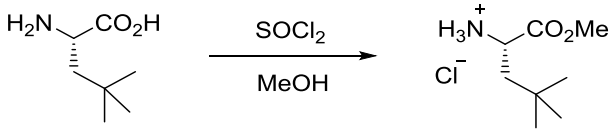


The building block was used for the synthesis of peptide H-D-Pro-Pro-Aad(OMe)-OMe.

Thionyl chloride (270 µL, 3.72 mmol) was added dropwise to a cold suspension (0°C) of L-2-aminoadipic acid (200 mg, 1.24 mmol) in 2 mL of MeOH. The resulting solution was stirred at room temperature overnight. The solvent and the excess of thionyl chloride were removed under reduced pressure. The crude product was purified by flash chromatography (EtOAc/MeOH 1:1, TLC visualised with ninhydrin) to yield the title compound as a colorless oil (5.65 g, 82%).

^{1}H NMR (400 MHz, CD_3OD, 25°C): δ = 4.01 (t, J = 6.3 Hz, 1H), 3.83 (s, 3H), 3.67 (s, 3H), 2.41 (t, J = 7.1 Hz, 2H), 2.02 – 1.60 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD, 25°C): δ = 174.9, 171.3, 53.8, 53.6, 52.1, 33.8, 31.1, 21.4; MS (ESI): *m/z* calcd $C_8H_{15}NO_4$+H^+: 190.1 [M+H^+]; found: 190.1.

β-*tert*-Butyl-L-alanine methyl ester hydrochloride (HCl·H-β-*tert*-butyl-Ala-OMe):

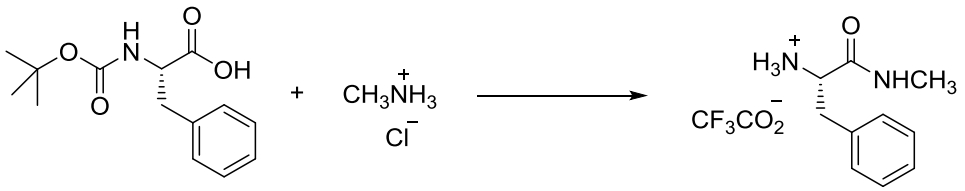


The building block was used for the synthesis of peptide H-D-Pro-Pro-β-*tert*-butyl-Ala-OMe.

Thionyl chloride (200 µL, 2.76 mmol) was added dropwise to a cold suspension (0°C) of 6-aminohexanoid acid (200 mg, 1.38 mmol) in 2 mL of MeOH. The resulting solution was stirred at room temperature overnight. The solvent and the excess of thionyl chloride were removed under reduced pressure. Trituration with EtOAc/pentane 4:1 of the residue yielded the title compound as a hygroscopic white powder (175 mg, 65%)

^{1}H NMR (400 MHz, D_2O, 25°C): δ = 4.13 (t, J = 5.9 Hz, 1H), 3.83 (s, 3H), 2.02 (dd, J = 14.9, 6.0 Hz, 1H), 1.68 (dd, J = 14.9, 5.8 Hz, 1H), 0.97 (s, 9H); ^{13}C NMR (101 MHz, D_2O, 25°C): δ = 172.4, 54.0, 50.9, 44.1, 30.0, 28.7; MS (ESI): *m/z* calcd $C_8H_{18}NO_2$+H^+: 160.1 [M+H^+]; found: 160.1.

(*S*)-2-Amino-*N*-methyl-3-phenylpropanamide TFA-salt (TFA·H-Phe-NHCH$_3$):



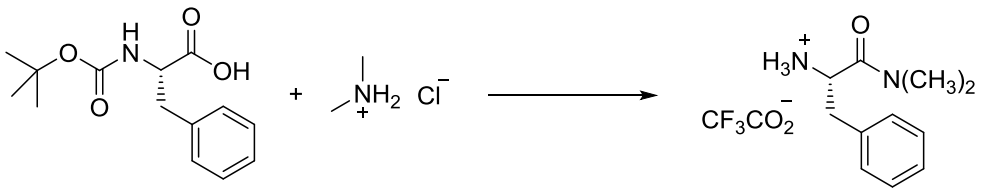
The building block was used for the synthesis of peptide H-D-Pro-Pro-Phe-NHCH$_3$.

To a suspension of Boc-L-phenylalanine (500 mg, 1.89 mmol), EDC·HCl (434 mg, 2.26 mmol) and HOBt·H_2O (346 mg, 2.26 mmol) in dry CH_2Cl_2 (10 mL) was added *i*PrNEt_2 (705 µL, 4.15 mmol) and methylamine hydrochloride (128 mg, 1.89 mmol). The reaction mixture was stirred at room temperature overnight and then diluted with 0.1 M HCl (20 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (20 mL) and water (20 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 50:1 – 20:1) to provide the Boc-protected intermediate which was dissolved in CH_2Cl_2/TFA 9:1 (10 mL). After stirring the solution at room temperature for 2 h, the solvent and the TFA were evaporated under reduced pressure. The remaining TFA in the resulting residue was co-evaporated with toluene (3x). The crude product was precipitated from Et_2O and dried under high vacuum to afford the title compound as a white powder (255 mg, 46%).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): δ = 8.34 (q, J = 4.6 Hz, 1H), 7.41 – 7.30 (m, 2H), 7.30 – 7.24 (m, 1H), 7.24 – 7.17 (m, 2H), 3.91 (t, J = 7.0 Hz, 1H), 3.00 (qd, J = 13.7, 7.0 Hz, 2H), 2.59 (d, J = 4.6 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): δ = 168.2,

135.0, 129.4, 127.1, 53.7, 39.5, 37.1, 25.5; MS (ESI): *m/z* calcd $C_{10}H_{15}N_2O+H^+$: 179.1 [$M+H^+$]; found: 179.1.

(*S*)-2-Amino-*N*,*N*-dimethyl-3-phenylpropanamide hydrochloride (HCl·H-Phe-N(CH$_3$)$_2$):

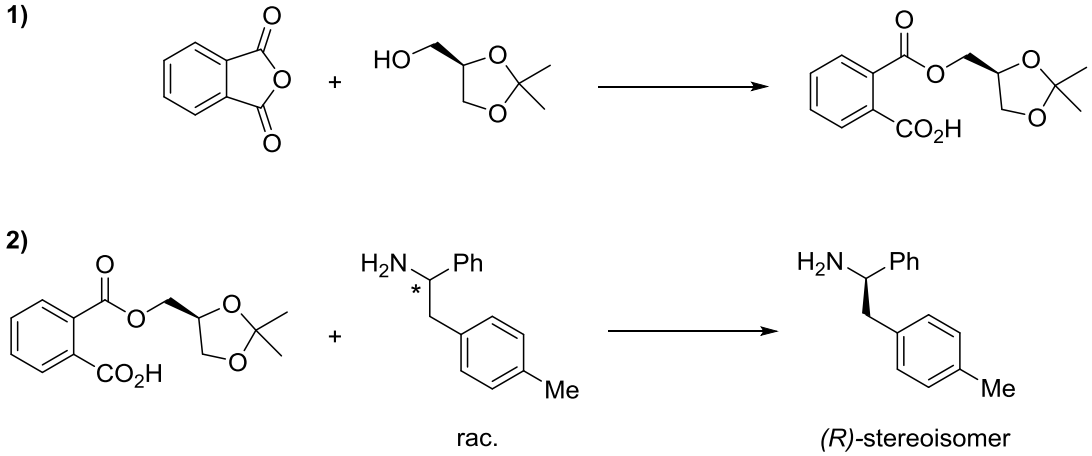


The building block was used for the synthesis of peptide H-D-Pro-Pro-Phe-N(CH$_3$)$_2$.

To a suspension of Boc-L-phenylalanine (500 mg, 1.89 mmol), EDC·HCl (434 mg, 2.26 mmol) and HOBt·H_2O (346 mg, 2.26 mmol) in dry CH_2Cl_2 (10 mL) was added *i*PrNEt$_2$ (705 µL, 4.15 mmol) and dimethylamine hydrochloride (154 mg, 1.89 mmol). The reaction mixture was stirred at room temperature overnight and then diluted with 0.1 M HCl (20 mL). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (20 mL) and water (20 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 50:1 – 20:1) to provide the Boc-protected intermediate which was dissolved in CH_2Cl_2/TFA 9:1 (10 mL). After stirring the solution at room temperature for 2 h, the solvent and the TFA were evaporated under reduced pressure. The remaining TFA in the resulting residue was co-evaporated with toluene (3x). The crude product was precipitated from Et_2O and dried under high vacuum to afford the title compound as a white powder (345 mg, 60%).

^{1}H NMR (400 MHz, DMSO-d$_6$, 25°C): δ = 8= 7.37 – 7.27 (m, 3H), 7.24 – 7.19 (m, 2H), 4.57 (dd, *J* = 7.9, 6.3 Hz, 1H), 3.04 (dd, *J* = 13.5, 6.3 Hz, 1H), 2.94 (dd, *J* = 13.5, 7.9 Hz, 1H), 2.79 (s, 3H), 2.61 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d$_6$, 25°C): δ = 167.9, 134.6, 129.5, 128.5, 127.3, 50.2, 39.5, 36.8, 36.3, 35.1; MS (ESI): *m/z* calcd $C_{11}H_{16}N_2O+H^+$: 193.1 [$M+H^+$]; found: 193.1.

(*R*)-1-Phenyl-(2-*p*-tolyl)ethylamine:



The building block was used for the synthesis of peptide H-D-Pro-Pro-NH-(*R*)-CH(Ph)-CH_2-4-Me-C_6H_4 (**10-R**). It was prepared by resolution from racemic 1-phenyl-(2-*p*-tolyl)ethylamine with the hemiphthalate of (*R*)-isopropylidene glycerol according to known procedures.[223-224]

1) A mixture of (*R*)-isopropylidene glycerol (8.40 g, 61.7 mmol, TCI chemicals, cas 14347-78-5), phthalic anhydride (9.14 g, 61.7 mmol) and pyridine (5.5 mL, 67.9 mmol) was stirred at 90°C for 1 h. The reaction mixture was allowed to cool to room temperature and was acidified to pH 3 with 1 M H_2SO_4. The aqueous phase was extracted with EtOAc (2 x 100 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford the hemiphthalate of (*R*)-isopropylidene glycerol as a colorless oil (17.1 g, 99%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.95 – 7.87 (m, 1H), 7.72 – 7.68 (m, 1H), 7.58 (m, 2H), 4.48 – 4.40 (m, 1H), 4.38 (dd, *J* = 5.4, 2.5 Hz, 2H), 4.13 (dd, *J* = 8.5, 6.4 Hz, 1H), 3.85 (dd, *J* = 8.6, 5.7 Hz, 1H), 1.43 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 171.5, 168.0, 133.1, 132.4, 131.1, 130.1, 130.0, 129.0, 110.1, 73.5, 66.6, 66.1, 26.8, 25.4; MS (ESI): *m/z* calcd $C_{14}H_{16}O_6$+Na^+: 303.1 [*M*+Na^+]; found: 303.1. The analytical data is in agreement with published data.[223]

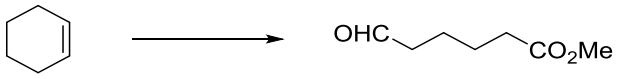
2) To a solution of the hemiphthalate of (*R*)-isopropylidene glycerol (17.0 g, 60.7 mmol) in *i*PrOH/H_2O 93:7 (v/v, 200 mL) was added racemic 1-phenyl-(2-*p*-tolyl)ethylamine (13.1 g, 60.7 mmol, TCI chemicals, cas 30275-30-0). The resulting clear solution was

stirred at room temperature overnight. The resulting white suspension was filtered and the filter cake was washed with EtOAc (2 x 50 mL) to isolate the salt of the hemiphthalate of (*R*)-isopropylidene glycerol and the (*R*)-1-phenyl-(2-*p*-tolyl)ethylamine as a white solid. The salt was suspended in EtOAc (150 mL) and extracted with 1 M H_2SO_4 (150 mL). The aqueous phase was washed with another portion of EtOAc (100 mL), then adjusted to pH >10 with 4 M NaOH and extracted with CH_2Cl_2 (3 x 150 mL). The CH_2Cl_2 phases were combined, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure to afford the title (*R*)-1-phenyl-(2-*p*-tolyl)ethylamine as a colorless oil (3.55 mg, 54%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.38 – 7.30 (m, 4H), 7.28 – 7.22 (m, 4H), 7.13 – 7.04 (m, 4H), 4.16 (dd, *J* = 8.9, 4.8 Hz, 1H), 2.98 (dd, *J* = 13.4, 4.8 Hz, 1H), 2.77 (dd, *J* = 13.4, 9.0 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 145.9, 136.1, 136.0, 129.4, 129.2, 128.5, 127.2, 126.6, 57.7, 46.2, 21.2; MS (ESI): *m/z* calcd $C_{15}H_{17}N+H^+$: 212.1 [$M+H^+$]; found: 212.1.

7.5.2 Aldehydes and Acetals

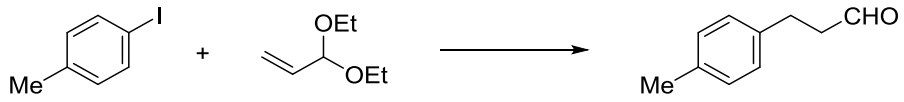
Methyl 6-oxohexanoate:



To a solution of cyclohexene (3.70 mL, 36.5 mmol) in CH_2Cl_2 (100 mL) and MeOH (25 mL) was added $NaHCO_3$ (1.00 g, 11.9 mmol). The resulting mixture was cooled to -78°C (acetone/dry ice) and then ozone enriched oxygen was bubbled through the reaction mixture until a blue coloration was observed (35 min). Bubbling with oxygen was continued until the coloration disappeared. The reaction mixture was filtered, diluted with toluene (40 mL) and concentrated to a volume of 25 mL. CH_2Cl_2 (100 mL) was added followed by triethylamine (7.26 mL, 54.7 mmol) and acetic anhydride (10.4 mL, 110 mmol) at 0°C. The solution was stirred at 0°C for 20 min and then at room temperature for 20 h. The reaction mixture was washed with 1 M HCl (100 mL) and 2 M NaOH (100 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified by distillation to obtain the title compound as a colorless liquid (3.66 g, 70 %).

b.p. 110°C (25 mbar); ^{1}H NMR (250 MHz, $CDCl_3$, 25°C): δ = 9.76 (t, J = 1.3 Hz, 1H), 3.66 (s, 3H), 2.51 – 2.38 (m, 2H), 2.38 – 2.25 (m, 2H), 1.71 – 1.58 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 202.0, 173.7, 51.5, 43.5, 33.7, 24.4, 21.5.
The analytical data is in agreement with published data.[225]

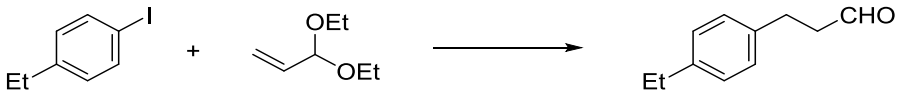
3-(4-Methylphenyl)propanal:



1-Iodo-4-methylbenzene (4.81 g, 22.0 mmol, 1.0 eq) was dissolved in 85 mL DMF. Acrolein diethyl acetal (9.44 mL, 61.8 mmol, 2.8 eq), tetrabutylammonium acetate (14.3 g, 47.4 mmol, 2.2 eq), potassium chloride (1.64 g, 22.0 mmol, 1.0 eq), potassium carbonate (4.27 g, 30.9 mmol, 1.4 eq) and palladium(II) acetate (111 mg, 494 µmol, 2.0 mol%) were added and the mixture was stirred at 95°C for 3 h. The black reaction mixture was allowed to cool to room temperature and then 100 mL 2 M HCl solution was added dropwise. After stirring for 10 min 300 mL of Et_2O were added. The phases were separated and the organic phase was washed with H_2O (3 x 100 mL). After drying over Na_2SO_4 the solvent was removed under reduced pressure. Purification by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (10:1) afforded 2.65 g of a light yellow oil. The cinnamaldehyde derivative was dissolved in 20 mL MeOH and 271 mg of Pd/C (10% w/w) was added. The resulting mixture was stirred under a H_2 atmosphere for 1.5 h. Filtration over Celite and evaporation of the solvent under reduced pressure afforded a colorless oil that was purified by column chromatography on silica gel (pentane/EtOAc 10:1) to provide 1.84 g (69 %) of a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.83 (t, J = 1.5 Hz, 1H), 7.18 – 7.08 (m, 4H), 2.94 (t, J = 7.6 Hz, 2H), 2.77 (t, J = 8.0 Hz, 1H), 2.34 (s, 3H); ^{13}C NMR (101 MHz, CDCl3): δ = 201.6, 137.1, 135.7, 129.2, 128.1, 45.3, 27.6, 20. 9.

3-(4-Ethylphenyl)propanal:

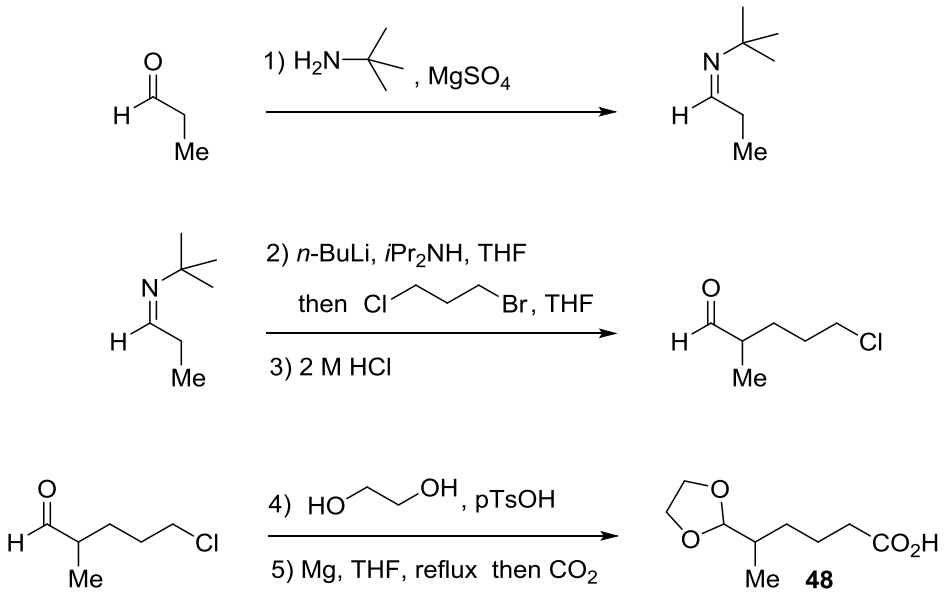


1-Iodo-4-ethylbenzene (5.11 g, 22.0 mmol, 1.0 eq) was dissolved in 85 mL DMF. Acrolein diethyl acetal (9.44 mL, 61.8 mmol, 2.8 eq), tetrabutylammonium acetate (14.3 g, 47.4 mmol, 2.2 eq), potassium chloride (1.64 g, 22.1 mmol, 1.0 eq), potassium carbonate (4.27 g, 30.9 mmol, 1.4 eq) and palladium(II) acetate (111 mg, 494 µmol, 2.0 mol%) were added and the mixture was stirred at 95°C for 3 h. The black reaction mixture was allowed to cool to room temperature and then 100 mL 2 M HCl solution was added dropwise. After stirring for 10 min 300 mL of Et_2O were added. The phases were separated and the organic phase was washed with H_2O (3 x 100 mL). After drying over Na_2SO_4 the solvent was removed under reduced pressure. Purification by column chromatography on silica gel eluting with a mixture of pentane/EtOAc (10:1) afforded 3.20 g of a light yellow oil. The cinnamaldehyde derivative was dissolved in 20 mL MeOH and 240 mg of Pd/C (10% w/w) was added. The resulting mixture was stirred under a H_2 atmosphere for 3 h. Filtration over Celite and evaporation of the solvent under reduced pressure afforded a colourless oil that was dissolved in 3 mL DMF and treated with 3 mL 4 M HCl overnight. To the mixture was added EtOAc (20 mL) and the organic phase was washed with water (3 x 10 mL). The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography on silica gel (pentane/EtOAc 10:1) to provide the title compound as a colourless liquid (2.57 g, 72 %).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.83 (t, J = 1.5 Hz, 1H), 7.22 – 7.10 (m, 4H), 2.96 (t, J = 7.6 Hz, 2H), 2.84 – 2.74 (m, 2H), 2.65 (q, J = 7.6 Hz, 2H), 1.26 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 201.6, 142.1, 137.4, 128.1, 128.0, 45.3, 28.3, 27.6, 15.5.

5-(1,3-Dioxolan-2-yl)hexanoic acid (48)

The title compound was prepared in five steps according to the following strategy:



1) *tert*-Butylamine (5.85 g, 80.0 mmol) was added dropwise to propanal (4.65 g, 80.0 mmol) at 0°C. Following addition, $MgSO_4$ (10 g) was added and the mixture was stirred at 0°C for 30 min. The mixture was filtered and residual $MgSO_4$ was rinsed with dry Et_2O (3 x 5 mL). The combined filtrate and Et_2O washings were concentrated under reduced pressure to afford the corresponding *tert*-butyl aldimine as a colorless liquid (5.80 g, 64%) which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.59 (t, J = 5.0 Hz, 1H), 2.24 (qd, J = 7.6, 5.0 Hz, 2H), 1.17 (s, 9H), 1.07 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 160.2, 56.5, 32.7, 29.8, 10.8.
The analytical data is in agreement with published data.[226]

2) and 3) To a stirred solution of *i*Pr_2NH (3.52 g, 34.8 mmol) in dry THF (30 mL) was added dropwise *n*-BuLi (1.6 M in hexane, 20.7 mL, 33.1 mmol) under N_2 at -10 °C. Subsequently, *tert*-butyl aldimine (2.50 g, 22.1 mmol) in dry THF (3 mL) was added slowly. After the addition, the mixture was stirred at -5°C for 2 h followed by the addition of 1-bromo-3-chloropropane (6.87g, 44.2 mmol) in THF (3 mL). The reaction mixture was stirred for 4 h before being poured into 0.5 M aqueous NaOH (50 mL) and extracted

with Et_2O (3 x 30 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude alkylated imine was taken up in 2 M HCl (10 mL) and stirred at room temperature overnight. The orange emulsion was extracted with CH_2Cl_2 (3 x 20 mL). The organic extracts were combined, washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the residue by flash column chromatography (Et_2O/pentane 1:20 – 1:10) afforded 5-chloro-2-methylpentanal as a colorless liquid (1.52 g, 50%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.64 (d, *J* = 1.8 Hz, 1H), 3.56 (t, *J* = 6.3 Hz, 2H), 2.45 – 2.31 (m, 1H), 1.93 – 1.74 (m, 4H), 1.59 – 1.49 (m, 1H), 1.14 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 204.5, 45.8, 44.8, 30.0, 27.8, 13.6.

4) A 100 mL round-bottomed flask containing a mixture of 5-chloro-2-methylpentanal (1.40 g, 10.4 mmol), ethylene glycol (0.64 mL, 11.4 mmol) and 4-toluenesulfonic acid monohydrate (90 mg, 5 mol%) in toluene (50 mL) was fitted with a Dean-Stark apparatus and the mixture was refluxed for 2 h. The cooled reaction mixture was diluted with 50 mL toluene and washed with a saturated aqueous solution of $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting yellow oil was purified by flash chromatography (Et_2O/pentane 1:20 – 1:10) to afford 2-(5-chloropentan-2-yl)-1,3-dioxolane as a colorless oil (1.34 g, 72%).

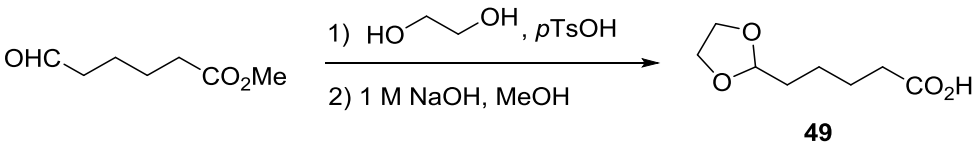
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 4.68 (d, *J* = 4.3 Hz, 1H), 4.01 – 3.79 (m, 4H), 3.60 – 3.46 (m, 2H), 1.96 – 1.61 (m, 4H), 1.39 – 1.26 (m, 1H), 0.96 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 107.6, 65.1, 45.4, 36.6, 30.5, 29.0, 14.1.

5) To magnesium turnings (195 mg, 8.00 mmol) in dry THF (2 mL) under an atmosphere of argon was added dropwise a solution of 2-(5-chloropentan-2-yl)-1,3-dioxolane (1.30 g, 7.28 mmol) in dry THF (1 mL). Following addition, the reaction mixture was refluxed for 5 h. Then the reaction mixture was cooled down to -5°C, diluted with dry THF (2 mL) and CO_2 was passed into the solution for 20 min allowing the reaction to warm to room temperature. A saturated aqueous solution of NH_4Cl (10 mL) was added and the mixture was extracted with Et_2O (2 x 20 mL). These two organic extracts were discarded. The

aqueous phase was acidified with 1 M HCl to pH 4 and extracted with Et_2O (5 x 20 mL). The organic extracts were combined, dried ($MgSO_4$) and the solvent was evaporated under reduced pressure to yield the title compound **48** as a colorless oil (361 mg, 26%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 4.68 (d, J = 4.3 Hz, 1H), 4.00 – 3.90 (m, 2H), 3.90 – 3.80 (m, 2H), 2.43 – 2.29 (m, 2H), 1.85 – 1.50 (m, 4H), 1.30 – 1.18 (m, 1H), 0.96 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 178.7, 107.4, 65.0, 36.7, 34.1, 30.9, 22.3, 13.8; MS (ESI): *m/z* calcd for $C_9H_{16}O_4$+Na^+: 211.1 [M+Na^+]; found: 211.1.

5-(1,3-dioxolan-2-yl)pentanoic acid (49):



49

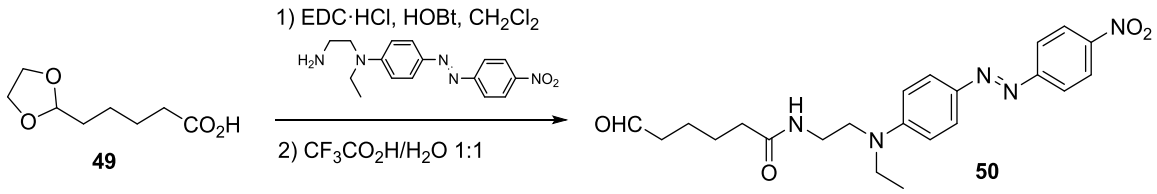
1) A 100 mL round-bottomed flask containing a mixture of methyl 6-oxohexanoate (2.00 g, 13.9 mmol), ethylene glycol (0.85 mL, 15.3 mmol) and 4-toluenesulfonic acid monohydrate (130 mg, 5 mol%) in toluene (50 mL) was fitted with a Dean-Stark apparatus and the mixture was refluxed for 2 h. The cooled reaction mixture was diluted with 50 mL toluene and washed with a saturated aqueous solution of $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting yellow oil was purified by flash chromatography (Et_2O/pentane 1:4 – 1:2) to afford methyl 5-(1,3-dioxolan-2-yl)pentanoate as a colorless oil (1.84 g, 70%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 4.85 (t, J = 4.7 Hz, 1H), 4.01 – 3.91 (m, 2H), 3.89 – 3.80 (m, 2H), 3.67 (s, 3H), 2.33 (t, J = 7.5 Hz, 3H), 1.73 – 1.63 (m, 4H), 1.51 – 1.40 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 174.1, 104.4, 65.0, 51.6, 34.1, 33.6, 24.9, 23.7.

2) The acetal ester (1.80 g, 9.56 mmol) was dissolved in MeOH (40 mL) and 1 M NaOH (14.3 mL, 14.3 mmol) was added. The mixture was stirred at room temperature for 2 h and then the MeOH was evaporated under reduced pressure. The resulting aqueous phase was first washed with CH_2Cl_2 (2 x 20 mL), then acidified with 3 M HCl (to pH 3) and finally extracted with CH_2Cl_2 (3 x 20 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The resulting colorless solid was washed/triturated with pentane (3 x 10 mL) to yield the title compound as a fine white powder (1.25 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 11.33 (br s, 1H), 4.87 (t, J = 4.7 Hz, 1H), 4.03 – 3.92 (m, 2H), 3.92 – 3.81 (m, 2H), 2.38 (t, J = 7.5 Hz, 2H), 1.70 (ddt, J = 9.0 Hz, 7.5, 3.0, 4H), 1.58 – 1.42 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 179.8, 104.4, 64.9, 34.1, 33.6, 24.7, 23.6.

(*E*)-*N*-(2-(Ethyl(4-((4-nitrophenyl)diazenyl)phenyl)amino)ethyl)-6-oxohexanamide (50):



1) To a mixture of pentanoic acid **49** (35 mg, 0.20 mmol), EDC·HCl (44 mg, 0.23 mmol) and HOBt·H_2O (35 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) was added *i*Pr_2NEt (39 µL, 0.23 mmol). After 5 min the amine[227] of Disperse Red 1 (60 mg, 0.19 mmol) was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 0.1 M HCl (10 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 20 mL). All organic phases were combined, washed with a saturated aqueous solution of $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The resulting dark red solid was purified by flash chromatography (1 – 5% v/v MeOH/CH_2Cl_2) to afford the acetal-protected aza-compound as a dark red powder (85 mg, 95%).

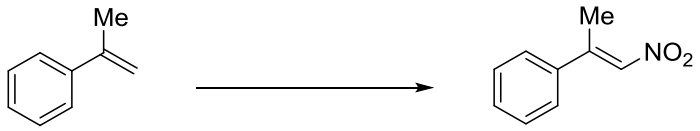
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 8.32 (d, *J* = 8.9 Hz, 2H), 7.95 – 7.87 (m, 4H), 6.86 – 6.80 (m, 2H), 5.68 (t, *J* = 5.9 Hz, 1H), 4.83 (t, *J* = 4.7 Hz, 1H), 3.99 – 3.89 (m, 2H), 3.88 – 3.79 (m, 2H), 3.58 (t, *J* = 6.4 Hz, 2H), 3.51 (q, *J* = 7.0 Hz, 4H), 2.23 – 2.14 (m, 2H), 1.74 – 1.64 (m, 4H), 1.51 – 1.42 (m, 2H), 1.25 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 173.3, 156.8, 151.4, 147.4, 143.8, 126.3, 124.7, 122.6, 111.4, 104.3, 64.9, 49.3, 45.5, 37.5, 36.9, 33.5, 25.4, 23.7, 12.3; MS (ESI): *m/z* calcd for $C_{24}H_{31}N_5O_5+H^+$: 470.2 [*M*+H^+]; found: 470.4.

2) The acetal-protected aza-compound (80 mg, 0.17 mmol) was stirred in a 1:1 mixture of trifluoroacetic acid and water for 30 min. The mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2 (3 x 20 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated under reduced pressure. Flash column chromatography (2 – 5% v/v MeOH/CH_2Cl_2) of the residue yielded the title compound **50** as a dark red powder (60 mg, 83%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.77 (t, *J* = 1.4 Hz, 1H), 8.35 – 8.25 (m, 2H), 7.94 – 7.82 (m, 4H), 6.87 – 6.77 (m, 2H), 5.89 (t, *J* = 6.0 Hz, 1H), 3.62 – 3.56 (m, 2H), 3.55 – 3.47 (m, 4H), 2.52 – 2.44 (m, 2H), 2.24 – 2.18 (m, 2H), 1.72 – 1.58 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 202.3, 173.1, 156.9, 151.6, 147.5, 143.8, 126.5, 124.8, 122.8, 111.6, 49.4, 45.6, 43.7, 37.6, 36.3, 24.9, 21.6, 12.4; MS (ESI): *m/z* calcd for $C_{22}H_{27}N_5O_4+H^+$: 426.2 [*M*+H^+]; found: 426.5.

7.5.3 Nitroolefins

***trans*-α-Methyl-β-nitrostyrene (2):**

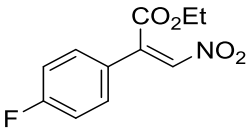


Nitric acid (65%, 4.0 mL, 57.8 mmol) was added to acetic anhydride at -15°C. After 10 min α-methylstyrene was added dropwise to the mixture. Following addition, the mixture was stirred at -15°C for 2h and then quenched with 60 mL iced water. After 1 h stirring at 0-5°C, the solution was extracted with Et_2O (3 x 60 mL). The combined organic phases were washed with a saturated aqueous solution of $NaHCO_3$ (400 mL) and brine (100 mL) before being dried over $MgSO_4$ and concentrated under reduced pressure. To the resulting orange residue was added chloroform (40 mL) and aqueous NaOH (15%, 20 mL) and the mixture was vigorously stirred at room temperature overnight. The phases were separated and the basic aqueous phase was extracted with chloroform (2 x 20 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. Column chromatography over silica gel (Et_2O/pentan 1:40) of the resulting brown oil afforded the title compound as a yellow solid (680 mg, 27%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.49-7.41 (m, 5H), 7.32 (q, J = 1.4 Hz, 1H), 2.66 (d, J = 1.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 150.0, 138.2, 136.2, 130.3, 129.0, 126.8, 18.6.

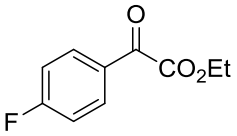
The analytical data is in agreement with published data.[228]

(*Z*)-Ethyl 2-(4-fluorophenyl)-3-nitroacrylate (7):



Prepared from ethyl 2-(4-fluorophenyl)-2-oxoacetate (5.00 g, 25.5 mmol) according to general protocol O and purified twice by flash chromatography (first Et_2O/pentane 1:20 – 1:10, second CH_2Cl_2/pentane 1:1 to pure CH_2Cl_2). The nitroacrylate was obtained as a yellow crystalline solid (3.79 g, 62%).

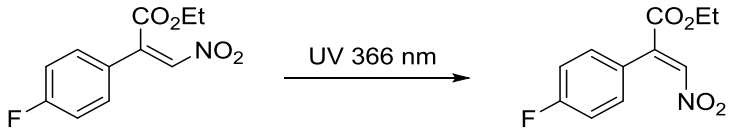
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.55 – 7.49 (m, 2H), 7.31 (s, 1H), 7.20 – 7.13 (m, 2H), 4.48 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 165.1 (d, $^1J_{C,F}$ = 255.0 Hz), 164.8, 142.4, 134.5, 129.9 (d, $^3J_{C,F}$ = 8.9 Hz), 125.8 (d, $^4J_{C,F}$ = 3.4 Hz), 117.1 (d, $^2J_{C,F}$ = 22.3 Hz), 63.1, 14.0; ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -106.1; elemental analysis calcd (%) for $C_{11}H_{10}FNO_4$: C 55.23, H 4.21, N 5.86; found: C 55.34, H 4.42, N 5.86.



Ethyl 2-(4-fluorophenyl)-2-oxoacetate was prepared from 1-bromo-4-fluorobenzene (7.00 g, 40.0 mmol) according to general protocol P and was purified by flash chromatography (EtOAc/pentane 1:40 – 1:20). The compound was obtained as a colorless oil (6.14 g, 82%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 8.13 – 8.04 (m, 2H), 7.24 – 7.14 (m, 2H), 4.45 (q, J = 7.2 Hz, 2H), 1.44 (t, J = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 184.5, 166.8 (d, $^1J_{C,F}$ = 258.3 Hz), 163.4, 133.0 (d, $^3J_{C,F}$ = 9.8 Hz), 129.0 (d, $^4J_{C,F}$ = 2.9 Hz), 116.3 (d, $^2J_{C,F}$ = 22.2 Hz), 62.5, 14.1; ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -101.3.
The analytical data is in agreement with published data.[229]

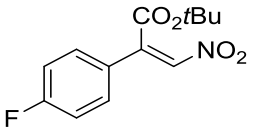
(*E*)-Ethyl 2-(4-fluorophenyl)-3-nitroacrylate (7'):



A solution of (*Z*)-Ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**, 500 mg, 2.09 mmol) in CH_2Cl_2 (500 mL) was irradiated with UV light (366 nm) for 72 h. The resulting yellow solution was concentrated under reduced pressure and the crude brown oil was purified by flash chromatography (CH_2Cl_2/pentane 1:4 – 1:1). The title compound was obtained as a yellow oil (424 mg, 85%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.79 (s, 1H), 7.29 – 7.22 (m, 2H), 7.14 – 7.06 (m, 2H), 4.33 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 164.3, 163.5 (d, $^1J_{C,F}$ = 250.4 Hz), 143.5, 135.9, 130.7 (d, $^3J_{C,F}$ = 8.6 Hz), 125.7 (d, $^4J_{C,F}$ = 3.6 Hz), 115.7 (d, $^2J_{C,F}$ = 22.1 Hz), 63.0, 14.0; ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -110.5; HRMS (EI): *m/z* calcd for $[C_{11}H_{10}FNO_4]^+$: 239.0588; found: 239.0588.

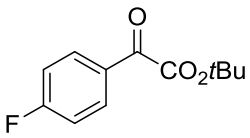
(*Z*)-*tert*-Butyl 2-(4-fluorophenyl)-3-nitroacrylate:



Prepared from *tert*-butyl 2-(4-fluorophenyl)-2-oxoacetate (2.00 g, 8.92 mmol) according to general protocol O and purified twice by flash chromatography (first Et_2O/pentane 1:20 – 1:10, second Et_2O/CH_2Cl_2/pentane 2:5:50 – 2:5:50). The nitroacrylate was obtained as a yellow crystalline solid (1.88 g, 79%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.57 – 7.49 (m, 2H), 7.26 (s, 1H), 7.20 – 7.11 (m, 2H), 1.62 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 164.8 (d, $^1J_{C,F}$ = 254.7 Hz), 163.4, 142.6, 133.5, 129.7 (d, $^3J_{C,F}$ = 8.9 Hz), 126.2 (d, $^4J_{C,F}$ = 3.4 Hz), 116.9 (d, $^2J_{C,F}$ = 22.2 Hz), 85.1, 27.9; elemental analysis calcd (%) for $C_{13}H_{14}FNO_4$: C 58.42, H 5.28, N 5.24; found: C 58.77, H 5.31, N 5.13.

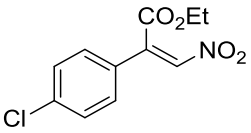
The analytical data is in agreement with published data.[221]



*t*BuOH (3.6 mL, 38 mmol) was added dropwise to a stirred solution of oxalyl chloride (3.3 mL, 38 mmol) in dry CH_2Cl_2 (70 mL) at 0°C under nitrogen. The mixture was stirred at 0°C for 1 h and then a solution of imidazole (7.76 g, 114 mmol) in dry CH_2Cl_2 (30 mL) was added within 30 min. The resulting yellow suspension was filtered and the filtrate was concentrated under reduced pressure to afford *tert*-butyl 2-(1*H*-imidazol-1-yl)-2-oxoacetate (6.20 g, 83%) as a yellow oil which was used immediately and without further purification. The *tert*-butyl 2-(4-fluorophenyl)-2-oxoacetate (5.50 g, 28.0 mmol) was reacted with 1-bromo-4-fluorobenzene (4.66 g, 26.6 mmol) according to general protocol P to yield *tert*-butyl 2-(4-fluorophenyl)-2-oxoacetate which was purified by flash chromatography (EtOAc/pentane 1:40 – 1:20) and obtained as a colorless oil (3.77 g, 63%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 8.06 – 8.00 (m, 2H), 7.22 – 7.15 (m, 2H), 1.63 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 185.2, 166.8 (d, $^1J_{C,F}$ = 257.7 Hz), 163.5, 132.9 (d, $^3J_{C,F}$ = 9.8 Hz), 129.2 (d, $^4J_{C,F}$ 3.0 Hz), 116.4 (d, $^2J_{C,F}$ 22.2 Hz), 85.1, 28.2.
The analytical data is in agreement with published data.[221]

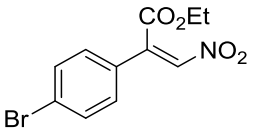
(*Z*)-Ethyl 2-(4-chlorophenyl)-3-nitroacrylate:



Prepared from commercially available ethyl 2-(4-chlorophenyl)-2-oxoacetate (1.00 g, 4.70 mmol, Fluorochem, cas 34966-48-8) according to general protocol O and purified twice by flash chromatography (first Et_2O/pentane 1:20–1:10, second Et_2O/CH_2Cl_2/pentane 2:5:50 – 2:5:40). The nitroacrylate was obtained as a yellow crystalline solid (575 mg, 48%).

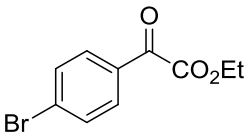
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.45 (s, 4H), 7.32 (s, 1H), 4.48 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 164.4, 142.1, 138.6, 134.7, 129.9, 128.7, 128.0, 63.0, 13.9; elemental analysis calcd (%) for $C_{11}H_{10}ClNO_4$: C 51.68, H 3.94, N 5.48; found: C 51.76, H 4.00, N 5.36.

(*Z*)-Ethyl 2-(4-bromophenyl)-3-nitroacrylate:



Prepared from ethyl 2-(4-bromophenyl)-2-oxoacetate (1.00 g, 3.89 mmol) according to general protocol O and purified twice by flash chromatography (first Et_2O/pentane 1:20 – 1:10, second Et_2O/CH_2Cl_2/pentane 2:5:50 – 2:5:40). The nitroacrylate was obtained as a yellow crystalline solid (610 mg, 52%).

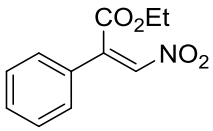
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.63 – 7.60 (m, 2H), 7.39-7.36 (m, 2H), 7.33 (s, 1H), 4.48 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 164.4, 142.2, 134.7, 132.9, 128.9, 128.4, 127.0, 63.0, 13.9; elemental analysis calcd (%) for $C_{11}H_{10}BrNO_4$: C 44.02, H 3.36, N 4.67; found: C 44.22, H 3.35, N 4.63.



Ethyl 2-(4-bromophenyl)-2-oxoacetate was prepared from 1,4-dibromobenzene (5.00 g, 21.2 mmol) according to general protocol P and was purified by flash chromatography (EtOAc/pentane 1:20). The compound was obtained as a colorless oil (3.51 g, 68%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.92 – 7.88 (m, 2H), 7.69 – 7.64 (m, 2H), 4.45 (q, *J* = 7.2 Hz, 2H), 1.43 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 185.2, 163.3, 132.4, 131.6, 131.5, 130.6, 62.7, 14.2.
The analytical data is in agreement with published data.[229]

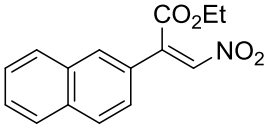
(*Z*)-Ethyl 3-nitro-2-phenylacrylate (37):



Prepared from commercially available ethyl 2-oxo-2-phenylacetate (1.00 g, 5.61 mmol, ABCR, cas 1603-79-8) according to general protocol O and purified twice by flash chromatography (first acetone/pentane 1:20 – 1:10, second Et_2O/CH_2Cl_2/pentane 2:5:40). The nitroacrylate was obtained as a yellow crystalline solid (660 mg, 53%).

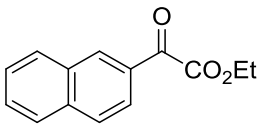
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.57 – 7.43 (m, 5H), 7.35 (s, 1H), 4.48 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 164.9, 143.5, 134.6, 132.3, 129.7, 129.7, 127.6, 63.0, 14.0; elemental analysis calcd (%) for $C_{11}H_{11}NO_4$: C 59.73, H 5.01, N 6.33; found: C 59.76, H 5.05, N 6.41.
The analytical data is in agreement with published data.[221]

(*Z*)-Ethyl 2-(naphthalen-2-yl)-3-nitroacrylate:



Prepared from ethyl 2-(naphthalen-2-yl)-2-oxoacetate (0.95 g, 4.16 mmol) according to general protocol O and purified by flash chromatography (Et_2O/pentane 1:20 – 1:10). The nitroacrylate was obtained as a yellow powder (470 mg, 42%).

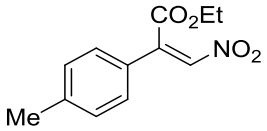
^{1}H NMR (400 MHz, DMSO-d_6, 25°C): δ = 8.32 (s, 1H), 8.20 (d, *J* = 1.6 Hz, 1H), 8.11 – 8.04 (m, 2H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.82 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.71 – 7.60 (m, 2H), 4.50 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): δ = 164.7, 142.0, 135.6, 134.1, 132.4, 129.4, 129.2, 129.0, 128.6, 127.6, 127.3, 126.4, 123.4, 62.4, 13.6; elemental analysis calcd (%) for $C_{15}H_{13}NO_4$: C 66.41, H 4.83, N 5.16; found: C 66.48, H 4.85, N 5.20.



2-(Naphthalen-2-yl)-2-oxoacetate was prepared from 2-bromo-naphthalene (4.50 g, 21.7 mmol) according to general protocol P and was purified by flash chromatography (Et_2O/CH_2Cl_2/pentane 2:5:40). The compound was obtained as a pale yellow oil (1.60 g, 34%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 8.56 (s, 1H), 8.05 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.98 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.65 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H), 7.58 (ddd, *J* = 8.1, 6.9, 1.3 Hz, 1H), 4.51 (q, *J* = 7.2 Hz, 2H), 1.46 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 186.4, 164.1, 136.5, 133.6, 132.4, 130.1, 130.0, 129.7, 129.1, 128.1, 127.3, 124.1, 62.5, 14.3.
The analytical data is in agreement with published data.[230]

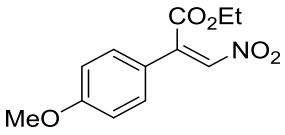
(*Z*)-Ethyl 3-nitro-2-*p*-tolylacrylate



Prepared from commercially available ethyl 2-oxo-2-*p*-tolylacetate (2.00 g, 10.4 mmol, Fluorochem, cas 5524-56-1) according to general protocol O and purified twice by flash chromatography (first Et_2O/pentane 1:20 – 1:15, second Et_2O/CH_2Cl_2/pentane 2:5:50). The nitroacrylate was obtained as a yellow crystalline solid (1.17 g, 48%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.42 – 7.38 (m, 2H), 7.35 (s, 1H), 7.29 – 7.25 (m, 2H), 4.48 (q, *J* = 7.2 Hz, 2H), 2.41 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 165.0, 143.4, 143.2, 133.6, 130.3, 127.5, 126.6, 62.8, 21.6, 13.9; elemental analysis calcd (%) for $C_{12}H_{13}NO_4$: C 61.27, H 5.57, N 5.95; found: C 61.41, H 5.60, N 6.00.
The analytical data is in agreement with published data.[221]

(*Z*)-Ethyl 2-(4-methoxyphenyl)-3-nitroacrylate (39):

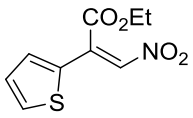


Prepared from commercially available ethyl 2-(4-methoxyphenyl)-2-oxoacetate (1.00 g, 4.80 mmol, Fluorochem, cas 40140-16-7) according to general protocol O and purified by flash chromatography (Et_2O/pentane 1:10 – 1:5). The nitroacrylate was obtained as a yellow oil (625 mg, 52%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°): δ = 7.48 – 7.43 (m, 2H), 7.34 (s, 1H), 6.99 – 6.94 (m, 2H), 4.49 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 3H), 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 165.2, 163.0, 143.2, 132.4, 129.5, 121.5, 115.1, 62.8, 55.6, 13.9; elemental analysis calcd (%) for $C_{12}H_{13}NO_5$: C 57.37, H 5.22, N 5.58; found: C 57.42, H 5.32, N 5.43.

The analytical data is in agreement with published data.[221]

(*E*)-Ethyl 3-nitro-2-(thiophen-2-yl)acrylate:

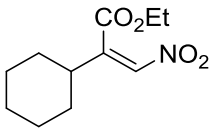


Prepared from commercially available ethyl 2-oxo-2-(thiophen-2-yl)acetate (3.0 g, 16.3 mmol, Alfa Aesar, cas 4075-58-5) according to general protocol O and purified twice by flash chromatography (first Et_2O/pentane 1:15 – 1:8, second acetone/CH_2Cl_2/pentane 2:5:50). The nitroacrylate was obtained as a yellow powder (1.31 g, 35%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.58 (dd, *J* = 5.1, 1.1 Hz, 1H), 7.37 (s, 1H), 7.37 (dd, *J* = 5.1, 1.1 Hz, 1H), 7.15 (dd, *J* = 5.1, 3.8 Hz, 1H), 4.50 (q, *J* = 7.2 Hz, 2H), 1.42 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 164.0, 137.8, 133.1, 132.7, 131.6, 131.6, 129.1, 63.2, 13.9; elemental analysis calcd (%) for $C_9H_9NO_4S$: C 47.57, H 3.99, N 6.16; found: C 47.55, H 3.88, N 6.09.

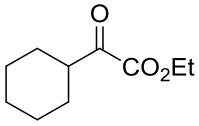
The analytical data is in agreement with published data.[221]

(*Z*)-Ethyl 2-cyclohexyl-3-nitroacrylate (22):



Prepared from ethyl 2-cyclohexyl-2-oxoacetate (1.73 g, 9.52 mmol) according to general protocol O and purified twice by flash chromatography (first Et_2O/pentane 1:20 – 1:10, second Et_2O/CH_2Cl_2/pentane 2:5:50 – 2:5:40). The nitroacrylate was obtained as a yellow powder (1.25 g, 58%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 6.81 (d, J = 1.3 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 2.44 – 2.33 (m, 1H), 1.92 – 1.79 (m, 4H), 1.77 – 1.68 (m, 1H), 1.35 (t, J = 7.2 Hz, 3H), 1.38 – 1.12 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 165.8, 150.7, 134.9, 62.2, 40.8, 30.8, 25.8, 25.5, 13.9.

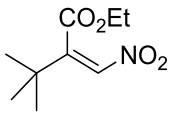


Ethyl 2-cyclohexyl-2-oxoacetate was prepared from bromocyclohexane (5.00 g, 30.7 mmol) according to general protocol P and was purified by flash chromatography (Et_2O/pentane 1:40 – 1:20). The compound was obtained as a colorless oil (1.75 g, 33%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 4.32 (q, J = 7.1 Hz, 2H), 3.10 – 2.96 (m, 1H), 1.98 – 1.86 (m, 2H), 1.85 – 1.75 (m, 2H), 1.73 – 1.64 (m, 1H), 1.37 (t, J = 7.16 Hz, 3H), 1.40 – 1.16 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 197.3, 161.8, 62.1, 46.2, 27.3, 25.6, 25.1, 13.8.

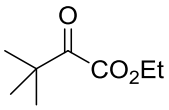
The analytical data is in agreement with published data.[231]

(*Z*)-Ethyl 3,3-dimethyl-2-(nitromethylene)butanoate (23):



Prepared from ethyl 3,3-dimethyl-2-oxobutanoate (2.60 g, 16.4 mmol) according to general protocol O and purified twice by flash chromatography (first Et_2O/CH_2Cl_2/pentane 2:5:50 – 2:5:40, second CH_2Cl_2/pentane 2:1 to pure CH_2Cl_2). The nitroacrylate was obtained as a yellow oil (1.62 g, 49%).

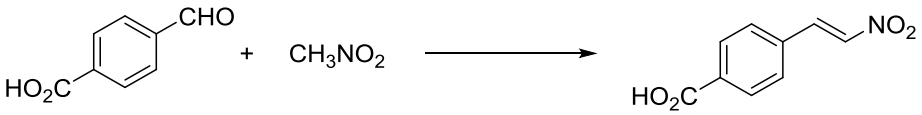
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 6.90 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 1.35 (t, J = 7.2 Hz, 3H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 165.3, 154.0, 135.0, 62.1, 35.3, 28.7, 13.9; HRMS (EI): *m/z* calcd for $[C_9H_{15}NO_4\text{-}NO_2]^+$: 155.1067; found: 155.1067.



Ethyl 3,3-dimethyl-2-oxobutanoate was prepared from *tert*-butylmagnesium chloride (1 M in THF, 30 mL, 30 mmol) according to general protocol P and was purified by flash chromatography (EtOAc/pentane 1:60 – 1:20). The compound was obtained as a yellow oil (2.65 g, 61%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 4.32 (q, J = 7.2 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H), 1.26 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 202.1, 163.9, 61.7, 42.6, 25.7, 14.1. The analytical data is in agreement with published data.[232]

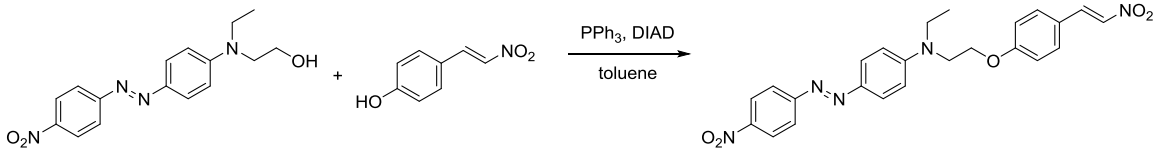
(*E*)-4-(2-Nitrovinyl)benzoic acid) (47):



4-Formylbenzoic acid (150 mg, 1.00 mmol), nitromethane (3 mL, dried over molecular sieves) and ammonium acetate (25 mg, 0.33 mmol) were charged into a microwave vial (10 mL). The reaction mixture (white suspension) was irradiated in the microwave at 90°C for 1 h. Then, the reaction mixture was diluted with EtOAc (40 mL) and extracted with 0.5 M HCl (10 mL). The organic phase was washed with water (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The resulting yellow powder was recrystallized from water/*i*PrOH 2:1 (30 mL) and dried under high vacuum to yield the title compound as a fine light brown powder (126 mg, 65%).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): δ = 13.24 (s, 1H), 8.30 (d, J = 13.7 Hz, 1H), 8.18 (d, J = 13.6 Hz, 1H), 8.02 – 7.94 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 166.5, 139.6, 137.7, 134.3, 133.2, 129.7, 129.7.

Disperse Red Labeled Nitroolefin 51:



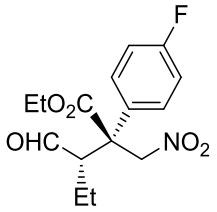
The title compound was prepared previously in our group from Disperse Red 1 dye and *trans*-4-hydroxy-β-nitrostyrene *via* Mitsunobu reaction.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 8.35 – 8.30 (m, 2H), 7.96 (d, J = 13.6 Hz, 1H), 7.94 – 7.89 (m, 4H), 7.52 (d, J = 8.3 Hz, 1H), 7.54 – 7.45 (m, 2H), 6.97 – 6.91 (m, 2H), 6.85 – 6.79 (m, 2H), 4.25 (t, J = 5.8 Hz, 2H), 3.89 (t, J = 5.8 Hz, 2H), 3.62 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 161.8, 156.9, 151.2, 147.7, 144.1, 138.8, 135.5, 131.3, 126.4, 124.8, 123.3, 122.8, 115.5, 111.6, 65.8, 49.8, 46.4, 12.5; MS (ESI): *m/z* calcd $C_{24}H_{23}N_5O_5$+H^+: 462.2 [M+H^+]; found: 462.3.

7.6 Synthesis and Analytical Data of 1,4-Addition Products

The diastereoselectivity was determined by ^{1}H-NMR analysis of the crude reaction mixture. The assignment of the stereoisomers was performed by comparison with chromatographic data of racemic samples obtained by using a 1:1 mixture of peptide H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**) and its enantiomer H-Pro-D-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 for reactions performed under otherwise identical conditions. The diastereoisomers of the products were not separated from each other for the analysis but can be separated by preparative HPLC. *Note:* Purification by column chromatography or analysis by HPLC leads to a decrease of the diastereomeric ratio due to epimerization at the α-position of the aldehyde moiety.

(2*S*,3*S*)-Ethyl 2-(4-fluorophenyl)-3-formyl-2-(nitromethyl)pentanoate (9):



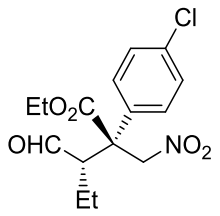
Prepared from butanal and (*Z*)-ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**) according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:10). The title compound was obtained as a colorless oil (82%, 6.0:1 d.r., 94% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.59 (d, *J* = 3.2 Hz, 1H), 7.41 – 7.38 (m, 2H), 7.26 – 7.21 (m, 2H), 5.62 (d, *J* = 15.1 Hz, 1H), 5.33 (d, *J* = 15.1 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 2.90 (dt, *J* = 11.0, 2.8 Hz, 1H), 1.49 – 1.41 (m, 1H), 1.38 – 1.28 (m, 1H), 1.18 (t, *J* = 7.1 Hz, 3H), 0.75 (t, *J* = 7.3 Hz, 3H); minor diastereomer δ = 9.48 (d, *J* = 2.4 Hz, 1H), 7.48 – 7.45 (m, 2H), 7.26 – 7.21 (m, 2H)*, 5.41 (s, 2H), 4.23 (q, *J* = 7.1 Hz, 2H)*, 3.08 (dt, *J* = 9.7, 2.7 Hz, 1H), 1.49 – 1.41 (m, 1H)*, 1.38 – 1.28 (m, 1H)*, 1.19 (t, *J* = 7.1 Hz, 3H)*, 0.81 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.3, 170.8, 161.4 (d, $^{1}J_{C,F}$ = 245.5 Hz), 131.7 (d, $^{4}J_{C,F}$ = 3.3 Hz), 129.6 (d, $^{3}J_{C,F}$ =

8.2 Hz), 115.3 (d, $^{2}J_{C,F}$ = 21.5 Hz), 78.7, 61.9, 56.2, 55.5, 18.3, 13.6, 12.3; visible signals of the minor diastereomer δ = 201.5, 170.4, 161.3 (d, $^{1}J_{C,F}$ = 245.3 Hz), 132.7 (d, $^{4}J_{C,F}$ = 3.5 Hz), 78.4, 62.0, 56.8, 55.3, 17.9, 13.6, 12.4; ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): Major diastereomer δ = -112.72; minor diastereomer δ = -112.67; elemental analysis calcd (%) for $C_{15}H_{18}FNO_5$: C 57.87, H 5.83, N 4.50; found: C 57.86, H 5.98, N 4.65.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 99:1, 25°C) at 1 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 26.3 min, t_R (*syn*, major) = 31.8 min.

(2*S*,3*S*)-Ethyl 2-(4-chlorophenyl)-3-formyl-2-(nitromethyl)pentanoate:

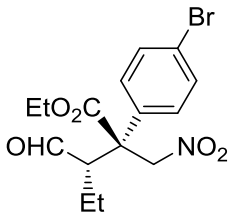


Prepared from butanal and (*Z*)-ethyl 2-(4-chlorophenyl)-3-nitroacrylate according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:20 – 1:10). The title compound was obtained as a colorless oil (85%, 5.0:1 d.r., 94% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.60 (d, *J* = 3.1 Hz, 1H), 7.49 – 7.45 (m, 2H), 7.41 – 7.37 (m, 2H), 5.61 (d, *J* = 15.1 Hz, 1H), 5.34 (d, *J* = 15.1 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 2.90 (dt, *J* = 10.8, 2.9 Hz, 1H), 1.51 – 1.29 (m, 2H), 1.18 (t, *J* = 7.1 Hz, 3H), 0.76 (t, *J* = 7.3 Hz, 3H); minor diastereomer δ = 9.48 (d, *J* = 2.5 Hz, 1H), 7.49 – 7.45 (m, 4H)*, 5.43 (s, 2H), 4.23 (q, *J* = 7.1 Hz, 2H)*, 3.08 (dt, *J* = 9.6, 2.9 Hz, 1H), 1.51 – 1.29 (m, 2H)*, 1.19 (t, *J* = 7.1 Hz, 3H), 0.82 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.3, 170.6, 134.6, 132.7, 129.4, 128.5, 78.5, 61.9, 56.2, 55.6, 18.4, 13.6, 12.3; minor diastereomer δ = 201.5, 170.2, 135.6, 132.6, 129.1, 128.1, 78.2, 62.1, 56.8, 55.4, 17.9, 13.6, 12.4; elemental analysis calcd (%) for $C_{15}H_{18}ClNO_5$: C 54.97, H 5.54, N 4.27; found: C 55.45, H 5.64, N 4.30.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 99:1, 25°C) at 1 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 26.6 min, t_R (*syn*, major) = 35.5 min.

(2*S*,3*S*)-Ethyl 2-(4-bromophenyl)-3-formyl-2-(nitromethyl)pentanoate:

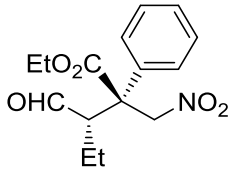


Prepared from butanal and (*Z*)-ethyl 2-(4-bromophenyl)-3-nitroacrylate according to the general protocol Q. Purified by flash chromatography (CH_2Cl_2/Et_2O/pentane 1:2:10). The title compound was obtained as a colorless oil (84%, 5.5:1 d.r., 94% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.59 (d, *J* = 3.1 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.34 – 7.29 (m, 2H), 5.60 (d, *J* = 15.1 Hz, 2H), 5.33 (d, *J* = 15.1 Hz, 2H), 4.22 (q, *J* = 7.1 Hz), 2.89 (dt, *J* = 10.8, 2.9 Hz, 1H), 1.53 – 1.27 (m, 2H), 1.17 (t, *J* = 7.1 Hz, 3H), , 0.75 (t, *J* = 7.4 Hz, 3H); minor diastereomer δ = 9.47 (d, *J* = 2.5 Hz, 1H), 7.62 – 7.57 (m, 2H)*, 7.41 – 7.37 (m, 2H), 5.42 (s, 2H), 4.24 (q, *J* = 7.1 Hz, 2H)*, 3.07 (dt, *J* = 9.7, 2.9 Hz, 2H), 1.54 – 1.28 (m, 2H)*, 1.19 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.3, 170.6, 135.0, 131.4, 129.7, 121.4, 78.5, 61.9, 56.1, 55.6, 18.4, 13.6, 12.3; elemental analysis calcd (%) for $C_{15}H_{18}BrNO_5$: C 48.40, H 4.87, N 3.76; found: C 48.63, H 4.86, N 3.78.

The enantiomeric excess was determined by HPLC using a Chiracel OD-H column (*n*-hexane/EtOH 97.5:2.5, 25°C) at 0.5 mL/min, UV detection at 254 nm: t_R (*syn*, major) = 35.2 min, t_R (*syn*, minor) = 40.0 min.

(2*S*,3*S*)-Ethyl 3-formyl-2-(nitromethyl)-2-phenylpentanoate:

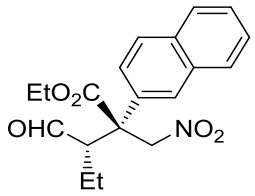


Prepared from butanal and (*Z*)-ethyl 3-nitro-2-phenylacrylate (**37**) according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:20 – 1:10). The title compound was obtained as a colorless oil (85%, 5.5:1 d.r., 96% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.57 (d, *J* = 3.4 Hz, 1H), 7.44 – 7.29 (m, 4H), 5.65 (d, *J* = 15.1 Hz, 1H), 5.30 (d, *J* = 15.1 Hz, 1H), 4.23 (qd, *J* = 7.1, 1.9 Hz), 2.85 (dt, *J* = 11.0, 3.5 Hz, 1H), 1.55 – 1.43 (m, 1H), 1.37 – 1.26 (m, 1H), 1.17 (t, *J* = 7.1 Hz, 3H), 0.74 (t, *J* = 7.4 Hz, 3H); minor diastereomer δ = 9.51 (d, *J* = 2.4 Hz, 1H), 7.44 – 7.29 (m, 4H)*, 5.43 (d, *J* = 14.9 Hz 1H), 5.38 (d, *J* = 14.9 Hz 1H), 4.23 (qd, *J* = 7.1, 1.9 Hz)*, 3.11 (dt, *J* = 9.8, 2.8 Hz, 1H), 1.55 – 1.43 (m, 1H)*, 1.37 – 1.26 (m, 1H)*, 1.18 (t, *J* = 7.1 Hz, 3H)*, 0.81 (t, *J* = 7.4 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.1, 171.1, 135.4, 128.6, 128.0, 127.3, 78.7, 61.8, 56.1, 55.9, 18.3, 13.6, 12.3; minor diastereomer δ = 201.6, 170.6, 136.6, 128.2, 127.9, 127.1, 78.4, 61.9, 56.7, 55.8, 17.9, 13.7, 12.5; elemental analysis calcd (%) for $C_{15}H_{19}NO_5$: C 61.42, H 6.53, N 4.78; found: C 61.45, H 6.53, N 4.78.

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/EtOH 98.75:1.25, 25°C) at 0.9 mL/min, UV detection at 254 nm: t_R (*syn*, major) = 42.8 min, t_R (*syn*, minor) = 64.7 min.

(2*S*,3*S*)-Ethyl 3-formyl-2-(naphthalen-2-yl)-2-(nitromethyl)pentanoate:

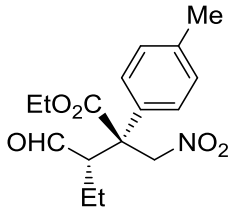


Prepared from butanal and (*Z*)-ethyl 2-(naphthalen-2-yl)-3-nitroacrylate according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:10). The title compound was obtained as a colorless oil (83%, 6.5:1 d.r., 91% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.65 (d, *J* = 3.3 Hz, 1H), 7.97 – 7.87 (m, 4H), 7.59 – 7.53 (m, 2H), 7.47 (dd, *J* = 8.8, 2.1 Hz, 1H), 5.75 (d, *J* = 15.0 Hz, 1H), 5.45 (d, *J* = 15.0 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 2.98 (dt, *J* = 11.0, 2.8 Hz, 1H), 1.60 – 1.48 (m, 1H), 1.44 – 1.31 (m, 1H), 1.20 (t, *J* = 7.1 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H); minor diastereomer δ = 9.58 (d, *J* = 2.3 Hz, 1H), 7.97 – 7.87 (m, 4H)*, 7.59 – 7.53 (m, 3H)*, 5.57 (d, *J* = 14.9 Hz, 1H), 5.49 (d, *J* = 15.0 Hz, 1H), 4.29 (q, *J* = 7.0 Hz, 2H)*, 3.25 (dt, *J* = 10.0 Hz, 2.5 Hz, 1H), 1.60 – 1.48 (m, 1H)*, 1.44 – 1.31 (m, 1H)*, 1.20 (t, *J* = 7.1 Hz, 3H)*, 0.83 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.2, 171.0, 132.8, 132.4, 132.0, 128.1, 128.0, 127.2, 126.7, 126.5, 126.5, 124.8, 78.6, 61.9, 56.2, 56.0, 18.4, 13.6, 12.2; visible signals of the minor diastereomer δ = 201.5, 170.5, 134.0, 131.9, 128.1, 124.7, 78.3, 61.8, 56.5, 55.9, 17.8, 13.6, 12.5; HRMS (ESI): *m/z* calcd for $C_{19}H_{21}NO_5$+Na^+: 366.1312 [*M*+Na^+]; found: 366.1307.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 97.5:2.5, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 18.1 min, t_R (*syn*, major) = 24.3 min.

(2*S*,3*S*)-Ethyl 3-formyl-2-(nitromethyl)-2-*p*-tolylpentanoate:

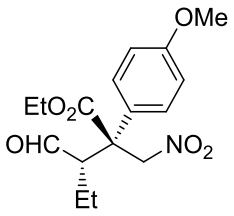


Prepared from butanal and (*Z*)-ethyl 3-nitro-2-*p*-tolylacrylate according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:20 – 1:10). The title compound was obtained as a colorless oil (90%, 6.5:1 d.r., 94% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.55 (d, *J* = 3.4 Hz, 1H), 7.22 – 7.18 (m, 4H), 5.62 (d, *J* = 15.0 Hz, 1H), 5.26 (d, *J* = 15.0 Hz, 1H), 4.21 (qd, *J* = 7.1, 1.6 Hz, 2H), 2.82 (dt, *J* = 11.3, 2.9 Hz, 1H), 2.29 (s, 3H), 1.55 – 1.42 (m, 1H), 1.36 – 1.22 (m, 1H), 1.16 (t, *J* = 7.1 Hz, 3H), 0.73 (t, *J* = 7.4 Hz, 3H); minor diastereomer δ = 9.51 (d, *J* = 2.3 Hz, 1H), 7.28 – 7.24 (m, 2H), 7.22 – 7.18 (m, 2H)*, 5.38 (d, *J* = 14.9 Hz, 1H), 5.33 (d, *J* = 14.9 Hz, 1H), 4.21 (qd, *J* = 7.1, 1.6 Hz, 2H)*, 3.09 (dt, *J* = 9.8, 2.6 Hz, 1H), 2.29 (s, 3H)*, 1.55 – 1.42 (m, 1H)*, 1.36 – 1.22 (m, 1H)*, 1.17 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.0, 171.1, 137.3, 132.2, 129.1, 127.0, 78.6, 61.6, 56.0, 55.5, 20.4, 18.2, 13.5, 12.2; visible signals of the minor diastereomer δ = 201.6, 170.6, 133.5, 127.0, 78.4, 61.7, 56.6, 55.4, 17.8, 13.6, 12.4; elemental analysis calcd (%) for $C_{16}H_{21}NO_5$: C 62.53, H 6.89, N 4.56; found: C 62.60, H 6.83, N 4.60.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 99.25:0.75, 25°C) at 0.8 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 26.3 min, t_R (*syn*, major) = 35.5 min.

(2*S*,3*S*)-Ethyl 3-formyl-2-(4-methoxyphenyl)-2-(nitromethyl)-pentanoate:

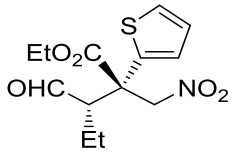


Prepared from butanal and (*Z*)-ethyl 2-(4-methoxyphenyl)-3-nitroacrylate (**39**) according to the general protocol Q. Purified by flash chromatography (CH_2Cl_2/Et_2O/pentane 1:2:10). The title compound was obtained as a yellow oil (72%, 6.0:1 d.r., 94% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.55 (d, *J* = 3.4 Hz, 1H), 7.27 – 7.20 (m, 2H), 6.98 – 6.90 (m, 2H), 5.61 (d, *J* = 15.0 Hz, 1H), 5.25 (d, *J* = 15.1 Hz, 1H), 4.22 (qd, *J* = 7.1, 1.9 Hz, 2H), 3.76 (s, 3H), 2.81 (dt, *J* = 11.1, 3.0 Hz, 1H), 1.56 – 1.43 (m, 1H), 1.37 – 1.24 (m, 1H), 1.17 (t, *J* = 7.1 Hz, 3H), 0.74 (t, *J* = 7.4 Hz, 3H); minor diastereomer δ = 9.52 (d, *J* = 2.4 Hz, 1H), 7.32 – 7.28 (m, 2H), 6.98 – 6.90 (m, 2H)*, 5.37 (d, *J* = 14.8 Hz, 1H), 5.32 (d, *J* = 14.8 Hz, 1H), 4.30 – 4.13 (m, 2H)*, 3.76 (s, 3H)*, 3.08 (dt, *J* = 9.6, 2.8 Hz, 1H), 1.56 – 1.43 (m, 1H)*, 1.37 – 1.24 (m, 1H)*, 1.18 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.1, 171.2, 158.5, 128.4, 126.9, 113.8, 78.7, 61.6, 56.0, 55.2, 55.0, 18.2, 13.5, 12.2; visible signals of the minor diastereomer δ = 201.7, 170.6, 158.4, 128.4, 78.5, 61.7, 56.6, 17.8, 13.6, 12.4; elemental analysis calcd (%) for $C_{16}H_{21}NO_6$: C 59.43, H 6.55, N 4.33; found: C 59.42, H 6.57, N 4.38.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 97.5:2.5, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 25.3 min, t_R (*syn*, major) = 27.0 min.

(2*S*,3*S*)-Ethyl 3-formyl-2-(nitromethyl)-2-(thiophen-2-yl)pentanoate:

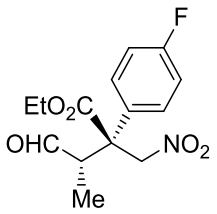


Prepared from butanal and (*E*)-ethyl 3-nitro-2-(thiophen-2-yl)acrylate according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:10). The title compound was obtained as a yellow oil (88%, 5.5:1 d.r., 89% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.58 (d, *J* = 3.3 Hz, 1H), 7.53 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.29 (dd, *J* = 3.8, 1.2 Hz, 1H), 7.03 (dd, *J* = 5.2, 3.7 Hz, 1H), 5.60 (d, *J* = 15.2 Hz, 1H), 5.55 (d, *J* = 15.2 Hz, 1H), 4.26 (qd, *J* = 7.1, 1.8 Hz, 2H), 2.74 (ddd, *J* = 7.5, 6.2, 3.3 Hz, 1H), 1.48 – 1.37 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H), 0.72 (t, *J* = 7.4 Hz, 3H); minor diastereomer δ = 9.47 (d, *J* = 2.9 Hz, 1H), 7.53 (dd, *J* = 5.2 Hz, 1.2 Hz, 1H)*, 7.23 (dd, *J* = 3.8, 1.2 Hz, 1H), 7.01 (dd, *J* = 5.2, 3.7 Hz, 1H)*, 5.60 (d, *J* = 15.2 Hz, 1H)*, 5.55 (d, *J* = 15.2 Hz, 1H)*, 4.26 (qd, *J* = 7.1, 1.8 Hz, 2H)*, 2.84 (dt, *J* = 10.7, 2.9 Hz, 1H), 1.50 – 1.60 (m, 1H), 1.48 – 1.37 (m, 1H)*, 1.22 (t, *J* = 7.1 Hz, 3H)*, 0.78 (t, *J* = 7.4 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.3, 169.8, 137.9, 127.2, 126.6, 126.3, 79.7, 62.2, 58.5, 53.7, 17.9, 13.7, 12.1; visible signals of the minor diastereomer δ = 201.1, 169.7, 138.5, 127.1, 126.4, 126.4, 78.5, 62.2, 59.0, 53.3, 13.7, 12.2; elemental analysis calcd (%) for $C_{13}H_{17}NO_5S$: C 52.16, H 5.72, N 4.68; found: C 52.40, H 5.74, N 4.61.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 98.5:1.5, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 19.5 min, t_R (*syn*, major) = 22.6 min.

(2*S*,3*S*)-Ethyl 2-(4-fluorophenyl)-3-methyl-2-(nitromethyl)-4-oxo-butanoate:

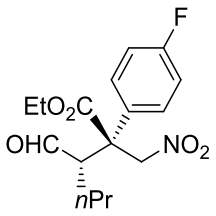


Prepared from propanal and (*Z*)-ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**) according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:20 – 1:10). The title compound was obtained as a colorless oil (83%, 3.0:1 d.r., 95% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.65 (d, *J* = 1.6 Hz, 1H), 7.41 – 7.36 (m, 2H), 7.29 – 7.20 (m, 2H), 5.61 (d, *J* = 14.9 Hz, 1H), 5.34 (d, *J* = 14.9 Hz, 1H), 4.24 (qd, *J* = 7.1, 1.1 Hz, 2H), 3.28 (dq, *J* = 7.3, 1.5 Hz, 1H), 1.19 (t, *J* = 7.1 Hz, 3H), 0.94 (d, *J* = 7.2 Hz, 3H); minor diastereomer δ = 9.43 (s, 1H), 7.53 – 7.47 (m, 2H), 7.29 – 7.20 (m, 2H)*, 5.39 (d, *J* = 14.6 Hz 1H)*, 5.32 (d, *J* = 14.6 Hz 1H)*, 4.25 (q, *J* = 7.1 Hz, 2H)*, 3.46 (q, *J* = 6.9 Hz, 1H), 1.17 (t, *J* = 7.1 Hz, 3H)*, 1.01 (d, *J* = 6.9 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.8, 171.1, 161.4 (d, $^{1}J_{C,F}$ = 245.5 Hz), 132.9 (d, $^{4}J_{C,F}$ = 3.3 Hz), 129.8 (d, $^{3}J_{C,F}$ = 8.2 Hz), 115.6 (d, $^{2}J_{C,F}$ = 21.6 Hz), 78.6, 62.1, 55.8, 49.6, 13.6, 10.2; visible signals of the minor diastereomer δ = 200.8, 170.4, 131.5 (d, $^{4}J_{C,F}$ = 3.3 Hz), 129.6 (d, $^{3}J_{C,F}$ = 8.3 Hz), 115.3 (d, $^{2}J_{C,F}$ = 21.5 Hz), 78.6, 61.9, 55.0, 49.1, 13.6, 9.4; elemental analysis calcd (%) for $C_{14}H_{16}FNO_5$: C 56.56, H 5.42, N 4.71; found: C 56.57, H 5.29, N 4.71.

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*PrOH 98.25:1.75, 25°C) at 1 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 31.3 min, t_R (*syn*, major) = 34.4 min.

(2*S*,3*S*)-Ethyl 2-(4-fluorophenyl)-3-formyl-2-(nitromethyl)hexanoate:

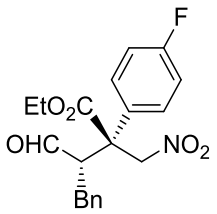


Prepared from pentanal and (*Z*)-ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**) according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:20:1 – 1:10). The title compound was obtained as a colorless oil (85%, 6.5:1 d.r., 95% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.58 (d, *J* = 3.2 Hz, 1H), 7.44 – 7.38 (m, 2H), 7.27 – 7.20 (m, 2H), 5.63 (d, *J* = 15.1 Hz, 1H), 5.33 (d, *J* = 15.1 Hz, 1H), 4.24 (q, *J* = 7.0 Hz, 2H), 3.03 – 2.93 (m, 1H), 1.38 – 1.30 (m, 2H), 1.28 – 1.17 (m, 1H), 1.18 (t, *J* = 7.1 Hz, 3H), 1.11 – 1.00 (m, 1H), 0.75 (t, *J* = 7.3 Hz, 3H); minor diastereomer δ = 9.50 (d, *J* = 2.5 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.27 – 7.20 (m, 2H)*, 5.44 (d, *J* = 15.0 Hz, 1H), 5.39 (d, *J* = 15.0 Hz, 1H), 4.24 (q, *J* = 7.0 Hz, 2H)*, 3.14 (dt, *J* = 10.3 Hz, 2.1 Hz, 1H), 1.54 – 1.43 (m, 1H), 1.42 – 1.29 (m, 2H)*, 1.25 – 1.13 (m, 1H)*, 1.19 (t, *J* = 7.1 Hz, 3H)*, 0.81 (t, *J* = 7.2 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.4, 170.8, 161.4 (d, $^{1}J_{C,F}$ = 245.6 Hz), 131.7 (d, $^{4}J_{C,F}$ = 3.4 Hz), 129.7 (d, $^{3}J_{C,F}$ = 8.2 Hz), 115.3 (d, $^{2}J_{C,F}$ = 21.4 Hz), 78.7, 61.9, 55.4, 54.2, 27.0, 20.4, 13.6, 13.6; visible signals of the minor diastereomer δ = 201.6, 170.4, 78.3, 62.0, 55.3, 54.9, 26.6, 20.7, 13.7, 13.6; elemental analysis calcd (%) for $C_{16}H_{20}FNO_5$: C 59.07, H 6.20, N 4.31; found: C 59.20, H 6.32, N 4.35.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 99:1, 25°C) at 0.7 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 28.6 min, t_R (*syn*, major) = 44.4 min.

(2*S*,3*S*)-Ethyl 3-benzyl-2-(4-fluorophenyl)-2-(nitromethyl)-4-oxo-butanoate:

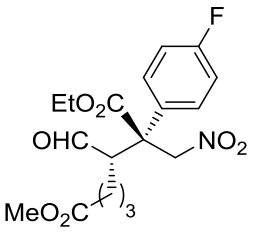


Prepared from 3-phenylpropanal and (*Z*)-ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**) according to the general protocol Q. Purified by flash chromatography (EtOAc/pentane 1:20 – 1:10). The title compound was obtained as a colorless oil (87%, 10.0:1 d.r., 97% ee).

^{1}H NMR (400 MHz, DMSO-d$_6$, 25°C): Major diastereomer δ = 9.61 (d, *J* = 2.8 Hz, 1H), 7.52 – 7.48 (m, 2H,), 7.32 – 7.12 (m, 5H), 7.05 – 7.00 (m, 2H), 5.75 (d, *J* = 15.2 Hz, 1H), 5.43 (d, *J* = 15.2 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 3.41 (ddd, *J* = 9.0, 4.7, 2.8 Hz), 2.69 – 2.80 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H); minor diastereomer δ = 9.52 (d, *J* = 2.5 Hz, 1H), 7.57 – 7.48 (m, 2H)*, 7.35 – 7.10 (m, 7H)*, 5.59 (d, *J* = 15.2 Hz, 1H), 5.54 (d, *J* = 15.2 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H)*, 3.54 (ddd, *J* = 9.5, 3.7, 2.4 Hz, 1H), 2.69 – 2.80 (m, 2H)*, 1.23 (t, *J* = 7.1 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d$_6$, 25°C): Major diastereomer δ = 201.1, 170.5, 161.5 (d, $^{1}J_{C,F}$ = 245.7 Hz), 138.5, 131.8 (d, $^{4}J_{C,F}$ = 3.3 Hz), 129.8 (d, $^{3}J_{C,F}$ = 8.2 Hz), 128.8, 128.4, 126.4, 115.5 (d, $^{2}J_{C,F}$ = 21.5 Hz), 78.8, 62.0, 56.9, 55.7, 31.2, 13.6; visible signals of the minor diastereomer δ = 201.0, 170.3, 161.4 (d, $^{1}J_{C,F}$ = 245.3 Hz), 138.6, 128.9, 128.3, 126.3, 115.4 (d, $^{2}J_{C,F}$ = 21.4 Hz), 62.2, 57.0, 55.5, 13.7; elemental analysis calcd (%) for $C_{20}H_{20}FNO_5$: C 64.34, H 5.40, N 3.75; found: C 64.40, H 5.53, N 3.39.

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*PrOH 98.25:1.75, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 32.1 min, t_R (*syn*, major) = 36.4 min.

(2*S*,3*S*)-1-Ethyl 7-methyl 2-(4-fluorophenyl)-3-formyl-2-(nitro-methyl)-heptane-dioate:

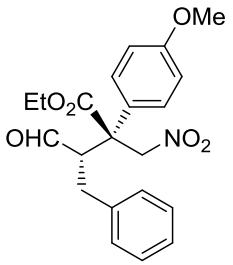


Prepared from methyl 6-oxohexanoate and (*Z*)-ethyl 2-(4-fluorophenyl)-3-nitroacrylate (**7**) according to the general protocol Q. Purified by flash chromatography (CH_2Cl_2/EtOH 400:1). The title compound was obtained as a colorless oil (85%, 4.5:1 d.r., 91% ee).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 9.60 (d, *J* = 3.0 Hz, 1H), 7.43 – 7.38 (m, 2H), 7.27 – 7.21 (m, 2H), 5.60 (d, *J* = 15.1 Hz, 1H), 5.32 (d, *J* = 15.1 Hz, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.52 (s, 3H), 3.01 (dt, *J* = 9.7, 2.8 Hz, 1H), 2.28 – 2.13 (m, 2H), 1.57 – 1.26 (m, 4H), 1.18 (t, *J* = 7.1 Hz, 3H); minor diastereomer δ = 9.48 (d, *J* = 2.0 Hz, 1H), 7.50 – 7.46 (m, 2H), 7.27 – 7.21 (m, 2H)*, 5.42 (d, *J* = 15.0 Hz 1H), 5.37 (d, *J* = 15.0 Hz 1H), 4.23 (q, *J* = 7.1 Hz, 2H)*, 3.55 (s, 3H), 3.22 – 3.16 (m, 1H), 2.28 – 2.13 (m, 2H)*, 1.57 – 1.26 (m, 4H)*, 1.19 (t, *J* = 7.1 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 201.3, 172.8, 170.7, 161.4 (d, $^1J_{C,F}$ = 245.6 Hz), 131.7 (d, $^4J_{C,F}$ = 3.4 Hz), 129.7 (d, $^3J_{C,F}$ = 8.3 Hz), 115.4 (d, $^2J_{C,F}$ = 21.4 Hz), 78.7, 61.9, 55.4, 54.3, 51.2, 32.7, 24.2, 22.7, 13.6; visible signals of the minor diastereomer δ = 201.3, 172.9, 170.3, 132.6 (d, $^4J_{C,F}$ = 3.2 Hz), 78.3, 62.1, 54.7, 32.7, 23.7, 22.9, 13.6; elemental analysis calcd (%) for $C_{18}H_{22}FNO_7$: C 56.39, H 5.78, N 3.65; found: C 56.43, H 5.75, N 3.82.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/EtOH 96:4, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R (*syn*, minor) = 30.6 min, t_R (*syn*, major) = 47.2 min.

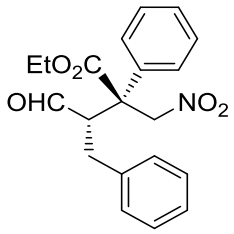
(2*S*,3*S*)-Ethyl 3-benzyl-2-(4-methoxyphenyl)-2-(nitromethyl)-4-oxobutanoate (36):



Prepared from butanal and (*Z*)-ethyl 2-(4-methoxyphenyl)-3-nitroacrylate (**39**) according to the general protocol Q. Purified by flash chromatography (CH_2Cl_2/Et_2O/pentane 1:2:10). The title compound was obtained as a yellow oil (83%, 10:1 d.r.).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 9.67 (d, *J* = 2.2 Hz, 1H), 7.30 – 7.20 (m, 2H), 7.22 – 7.16 (m, 1H), 7.16 – 7.06 (m, 4H), 6.95 – 6.90 (m, 2H), 5.19 (d, *J* = 14.0 Hz, 1H), 5.10 (d, *J* = 14.0 Hz, 1H), 4.41 – 4.28 (m, 2H), 3.82 (s, 3H), 3.61 (ddd, *J* = 10.8, 3.3, 2.2 Hz, 1H), 2.99 (dd, *J* = 14.1, 10.8 Hz, 1H), 2.87 (dd, *J* = 14.1, 3.3 Hz, 1H), 1.31 (t, *J* = 7.1 Hz, 3H); minor diastereomer δ = 9.70 (d, *J* = 1.5 Hz, 1H), 7.31 – 7.07 (m, 7H)*, 6.96 – 6.89 (m, 2H)*, 5.18 (s, 2H)*, 4.37 – 4.26 (m, 2H)*, 3.82 (s, 3H), 3.73 (ddd, *J* = 7.8, 5.6, 1.5 Hz, 1H), 2.83 – 2.77 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 201.8, 171.0, 159.5, 138.3, 128.9, 128.77, 128.0, 126.9, 126.8, 114.5, 79.2, 62.5, 56.8, 55.5, 55.3, 32.7, 13.9; visible signals of the minor diastereomer δ = 171.1, 159.5, 138.6, 129.0, 128.6, 127.5, 126.7, 114.5, 77.8, 62.6, 56.4, 55.5, 55.3, 32.0.

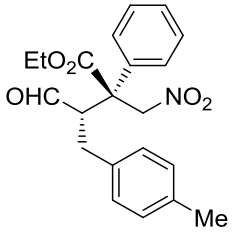
(2*S*,3*S*)-Ethyl 3-benzyl-2-(nitromethyl)-4-oxo-2-phenylbutanoate (38):



Prepared from 3-phenylpropanal and (*Z*)-Ethyl 3-nitro-2-phenylacrylate (**37**) according to the general protocol Q in $CHCl_3$ and in DMSO in order to compare the enantiomeric excess with the enamine ratio $\mathbf{En_{Me}/En_{Et}}$ observed in the back reaction with the quasi-enantiomeric substrates **40a** and **40b** (see Chapter 4.3.2).

In order to determine the enantiomeric excess, a small amount of the title compound was isolated from the reaction mixture by preparative TLC (EtOAc/pentane 1:10) and subjected to HPLC using a Chiracel AD-H column (*n*-hexane/EtOH 96:4, 40°C) at 1.0 mL/min and UV detection at 210 nm. t_R (*syn*, major) = 18.1 min, t_R (*syn*, minor) = 26.3 min. The enantiomeric excess was 94% (97:3 e.r.) for the reaction in $CHCl_3$ with catalyst **10-R**, 70% (85:15 e.r.) in DMSO with catalyst **10-R** and 20% (40:60 e.r., the (2*R*,3*R*)-enantiomer was the major one) in DMSO with catalyst H-Pro-Pro-D-Glu-NH_2.

(2*S*,3*S*)-Ethyl 3-formyl-2-(nitromethyl)-2-phenyl-4-(*p*-tolyl)butanoate (40a):

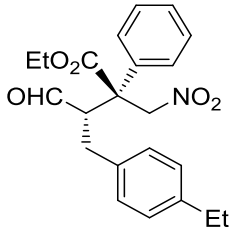


Prepared from 3-(4-methylphenyl)propanal and (*Z*)-ethyl 3-nitro-2-phenylacrylate (**37**) according to the general protocol Q. The title compound was first purified by flash chromatography (EtOAc/pentane 1:15) to yield a colorless oil which was a mixture of the four stereoisomers (88%, 11:1 d.r., 96% ee). The major stereoisomer was isolated by semi-preparative HPLC using a DAICEL Chiralpak IA column (250 mm x 20 mm, *n*-hexane/*i*PrOH 95:5, 25°C, 18 mL/min, UV 210 nm) in >60:1 d.r. and >99% ee.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.66 (d, *J* = 2.2 Hz, 1H), 7.47 – 7.33 (m, 3H), 7.25 – 7.18 (m, 2H), 7.06 (d, *J* = 7.8 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 5.22 (d, *J* = 14.1 Hz, 1H), 5.13 (d, *J* = 14.1 Hz, 1H), 4.44 – 4.29 (m, 2H), 3.61 (ddd, *J* = 10.9, 3.3, 2.3 Hz, 1H), 2.97 (dd, *J* = 14.2, 10.9 Hz, 1H), 2.83 (dd, *J* = 14.2, 3.3 Hz, 1H), 2.29 (s, 3H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 202.0, 171.0, 136.6, 135.5, 135.3, 129.6, 129.3, 128.9, 128.8, 126.9, 79.3, 62.7, 57.0, 56.1, 32.5, 21.1, 14.0; HRMS (EI): *m/z* calcd for $[C_{19}H_{21}NO_5\text{-}NO_2]^+$: 323.1642; found: 323.1636.

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*PrOH 95:5, 40°C) at 1.0 mL/min, UV detection at 210 nm: t_R (*syn*, major) = 14.8 min, t_R (*syn*, minor) = 33.0 min.

(2*S*,3*S*)-Ethyl 3-(4-ethylbenzyl)-2-(nitromethyl)-4-oxo-2-phenylbutanoate (40b):



Prepared from 3-(4-ethylphenyl)propanal and (*Z*)-ethyl 3-nitro-2-phenylacrylate (**37**) according to the general protocol Q. The title compound was first purified by flash chromatography (EtOAc/pentane 1:15) to yield a colorless oil which was a mixture of the four stereoisomers (92%, 12:1 d.r., 96% ee). The major stereoisomer was isolated by semi-preparative HPLC using a DAICEL Chiralpak IA column (250 mm x 20 mm, *n*-hexane/*i*PrOH 80:20, 25°C, 18 mL/min, UV 210 nm) in >99:1 d.r. and >99% ee.

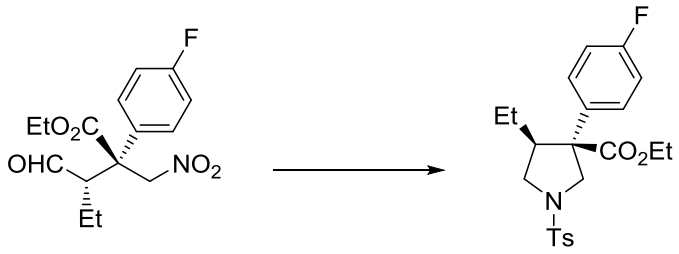
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.66 (d, *J* = 2.2 Hz, 1H), 7.46 – 7.34 (m, 3H), 7.24 – 7.19 (m, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 2H), 5.22 (d, *J* = 14.1 Hz, 1H), 5.13 (d, *J* = 14.0 Hz, 1H), 4.43 – 4.29 (m, 2H), 3.63 (ddd, *J* = 10.8, 3.3, 2.2 Hz, 1H), 2.98 (dd, *J* = 14.2, 10.9 Hz, 1H), 2.84 (dd, *J* = 14.2, 3.3 Hz, 1H), 2.59 (q, *J* = 7.6 Hz, 2H), 1.31 (t, *J* = 7.1 Hz, 3H), 1.20 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 202.0, 171.0, 142.9, 135.5, 135.5, 129.3, 128.9, 128.8, 128.4, 126.9, 79.3, 62.6, 56.9, 56.1, 32.5, 28.5, 15.7, 14.0; HRMS (EI): *m/z* calcd for $[C_{19}H_{21}NO_5\text{-}NO_2]^+$: 337.1798; found: 377.1794.

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*PrOH 95:5, 40°C) at 1.0 mL/min, UV detection at 210 nm: t_R (*syn*, minor) = 14.6 min, t_R (*syn*, major) = 32.1 min.

7.7 Derivatization of 1,4-Addition Products

7.7.1 Synthesis of Pyrrolidine 11

(3*S*,4*S*)-Ethyl 4-ethyl-3-(4-fluorophenyl)-1-tosylpyrrolidine-3-carboxylate (11):



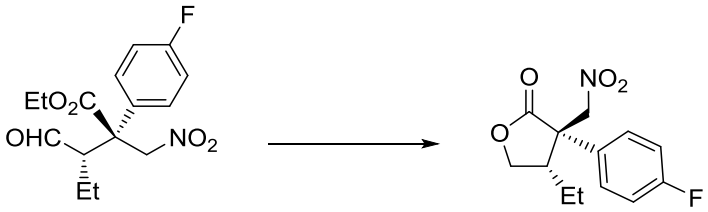
To a solution of γ-nitroaldehyde **9** (50 mg, 0.16 mmol, 5.5:1 d.r.) in MeOH (3 mL) was added $Pd(OH)_2/C$ (8 mg, 15 – 20 % Pd). The reaction mixture was stirred under an atmosphere of H_2 (20 bar) at room temperature for 24 h, then filtered over Celite and the solvent was evaporated under reduced pressure. The residue was dissolved in dry CH_2Cl_2 (1.5 mL) and cooled to 0°C. Tosyl chloride (34 mg, 0.18 mmol) and NEt_3 (67 µL, 0.48 mmol) were added and the mixture was stirred at room temperature for 18 h. The solution was diluted with CH_2Cl_2 (5 mL) and washed successively with water, saturated aqueous solution of $NaHCO_3$ and brine (3 mL each). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (EtOAc/pentane 1:10) to afford pyrrolidine **11** as a colorless oil (30 mg, 45%, 7:1 d.r.).

^{1}H NMR (600 MHz, CD_3OD, 25°C): Major diastereomer δ = 7.67 – 7.63 (m, 2H), 7.35 – 7.31 (m, 2H), 7.27 – 7.23 (m, 2H), 7.00 – 6.95 (m, 2H), 3.99 (d, *J* = 7.1 Hz, 2H), 3.96 (d, *J* = 10.8 Hz, 1H), 3.66 (d, *J* = 10.7 Hz, 1H), 3.46 (dd, *J* = 10.7, 6.3 Hz, 1H), 3.22 (dd, *J* = 10.0, 5.1 Hz, 1H), 2.59 (dddd, *J* = 11.1, 6.2, 5.1, 2.8 Hz, 1H), 2.42 (s, 3H), 1.57 – 1.49 (m, 1H), 1.06 (t, *J* = 7.1 Hz, 3H), 1.00 – 0.93 (m, 1H), 0.91 (t, *J* = 7.1 Hz, 3H); minor diastereomer δ = 7.80 – 7.77 (m, 2H), 7.45 – 7.42 (m, 2H), 7.16 – 7.13 (m, 2H), 7.05 – 7.01 (m, 2H), 4.12 (d, *J* = 9.9 Hz, 1H), 3.93 (q, *J* = 7.1 Hz, 2H), 3.63 (d, *J* = 9.9 Hz, 1H), 3.46 (dd, 1H)*, 3.22 (dd, 1H)*, 2.73 (ddt, *J* = 11.6, 6.5, 3.4 Hz, 1H), 2.44 (s, 3H), 1.06 (t, *J* = 7.1 Hz, 3H), 0.97 – 0.92 (m, 1H)*, 0.72 (t, *J* = 7.4 Hz, 3H), 0.28 (ddq, *J* = 13.5, 11.6, 7.2 Hz, 1H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (150 MHz, CD_3OD, 25°C): Major diastereomer δ = 173.2, 163.4 (d, $^1J_{C,F}$ = 245.6 Hz), 145.2, 136.6

(d, $^{4}J_{C,F}$ = 3.2 Hz), 135.2, 130.8, 129.4 (d, $^{3}J_{C,F}$ = 8.2 Hz), 128.5, 116.4 (d, $^{2}J_{C,F}$ = 21.7 Hz), 62.4, 61.1, 56.4, 51.7, 47.9, 23.3, 21.5, 14.3, 12.9; minor diastereomer δ = 174.2, 163.5 (d, $^{1}J_{C,F}$ = 245.9 Hz), 145.5, 135.1, 134.3 (d, $^{4}J_{C,F}$ = 3.3 Hz), 131.0, 130.2 (d, $^{3}J_{C,F}$ = 8.1 Hz), 128.7, 116.4 (d, $^{2}J_{C,F}$ = 21.7 Hz), 62.8, 61.5, 54.1, 51.7, 47.0, 22.5, 21.5, 14.1, 12.4; HRMS (ESI): *m/z* calcd for $C_{22}H_{26}FNO_4S+H^+$: 420.1639 [M+H^+]; found: 420.1636.

7.7.2 Synthesis of Lactones 13 and 14

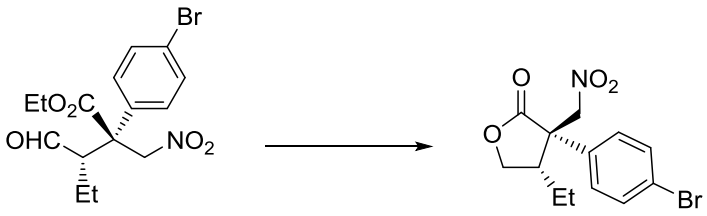
(3*S*,4*S*)-4-Ethyl-3-(4-fluorophenyl)-3-(nitromethyl)dihydrofuran-2(3*H*)-one (13):



To a cold solution (0°C) of γ-nitroaldehyde **9** (80 mg, 257 μmol, 6.0:1 d.r.) in 1 mL of MeOH was added $NaBH_4$ (10 mg, 257 μmol). The reaction mixture was stirred at room temperature for 2 h and then quenched with acetic acid (50 μL). The volatiles were removed under reduced pressure and the residue was suspended in EtOAc. The white suspension was filtered over a plug of silica gel and the filtrate was concentrated under reduced pressure to afford lactone **13** as a colorless oil which crystallized slowly upon standing at room temperature to yield a white crystalline solid (66 mg, 96%, 6.1:1 d.r.).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 7.16 – 7.06 (m, 4H), 5.08 (d, *J* = 14.5 Hz, 1H), 5.05 (d, *J* = 14.5 Hz, 1H), 4.60 (dd, *J* = 9.1, 8.0 Hz, 1H), 3.78 (dd, *J* = 10.6, 9.1 Hz, 1H), 2.85 – 2.74 (m, 1H), 1.49 – 1.37 (m, 1H), 0.87 (t, *J* = 7.2 Hz, 3H), 0.83 – 0.74 (m, 1H); minor diastereomer: δ = 7.51 – 7.45 (m, 2H), 7.15 – 7.07 (m, 2H)*, 4.90 (d, *J* = 14.2 Hz, 1H), 4.83 (d, *J* = 14.3 Hz, 1H), 4.26 (dd, *J* = 9.4, 1.8 Hz, 1H), 4.16 (dd, *J* = 9.4, 5.1 Hz, 1H), 3.16 (dddd, *J* = 11.5, 5.1, 3.4, 1.8, 1H), 2.04 – 1.92 (m, 1H), 1.54 – 1.45 (m, 1H)*, 1.14 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 176.9, 162.6 (d, $^{1}J_{C,F}$ = 250.0 Hz), 128.8 (d, $^{3}J_{C,F}$ = 8.2 Hz), 128.0 (d, $^{4}J_{C,F}$ = 3.7 Hz), 116.5 (d, $^{2}J_{C,F}$ = 21.7 Hz), 76.9, 70.2, 54.2, 43.3, 22.0, 11.7; visible signals of the minor diastereomer δ = 128.6 (d, $^{3}J_{C,F}$ = 8.3 Hz), 116.6 (d, $^{2}J_{C,F}$ = 21.6 Hz), 69.1, 52.5, 44.4, 21.2.

(3*S*,4*S*)-3-(4-Bromophenyl)-4-ethyl-3-(nitromethyl)dihydrofuran-2-(3*H*)-one (14):



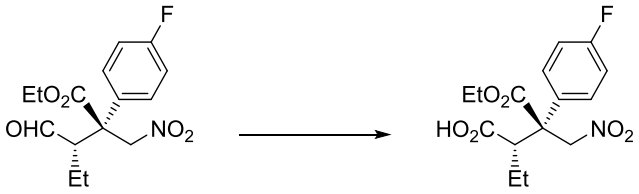
To a cold solution (0°C) of γ-nitroaldehyde **12** (120 mg, 322 μmol, 5.5:1 d.r.) in 1.5 mL of MeOH was added $NaBH_4$ (12 mg, 322 μmol). The reaction mixture was stirred at room temperature for 2 h and then quenched with acetic acid (50 μL). The volatiles were removed under reduced pressure and the residue was suspended in EtOAc. The white suspension was filtered over a plug of silica gel and the filtrate was concentrated under reduced pressure to afford lactone **14** as a colorless oil which crystallized slowly upon standing at room temperature to yield a white crystalline solid (100 mg, 95%, 5.5:1 d.r.).

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 7.67 – 7.60 (m, 2H), 7.19 – 7.13 (m, 2H), 5.67 (d, *J* = 15.4 Hz, 1H), 5.28 (d, *J* = 15.5 Hz, 1H), 4.66 (dd, *J* = 9.3, 8.0 Hz, 1H), 3.82 (dd, *J* = 10.3, 9.3 Hz, 1H), 2.79 (tdd, *J* = 10.3, 8.0, 4.3 Hz, 1H), 1.42 (ddq, *J* = 15.0, 7.6, 4.1 Hz, 1H), 0.75 (t, *J* = 7.4 Hz, 3H), 0.50 (ddq, *J* = 14.5, 10.4, 7.2, Hz, 1H); minor diastereomer: δ = 7.67 – 7.60 (m, 2H)*, 7.50 – 7.44 (m, 2H), 5.43 (d, *J* = 14.4 Hz, 1H), 5.16 (d, *J* = 14.4 Hz, 1H), 4.30 (dd, *J* = 9.4, 6.3 Hz, 1H), 4.25 (dd, *J* = 9.4, 5.0 Hz, 1H), 2.96 (dtd, *J* = 11.1, 5.6, 3.8 Hz, 1H), 1.73 (ddd, *J* = 13.4, 7.5, 3.7 Hz, 1H), 1.39 – 1.28 (m, 1H)*, 0.93 (t, *J* = 7.4 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 176.9, 132.6, 131.7, 129.3, 121.5, 76.2, 70.0, 53.7, 43.1, 21.2, 11.2; minor diastereomer: δ = 175.1, 135.6, 131.7, 129.1, 121.6, 76.2, 69.5, 53.5, 46.1, 19.9, 11.3; elemental analysis calcd (%) for $C_{13}H_{14}BrNO_4$: C 47.58, H 4.30, N 4.27; found: C 47.87, H 4.43, N 4.28.

7.7.3 Synthesis of γ-Amino Acid 19

γ-Amino acid **19** was prepared from γ-nitroaldehyde **9** in five steps in an overall yield of 54% following previously established procedures.[169]

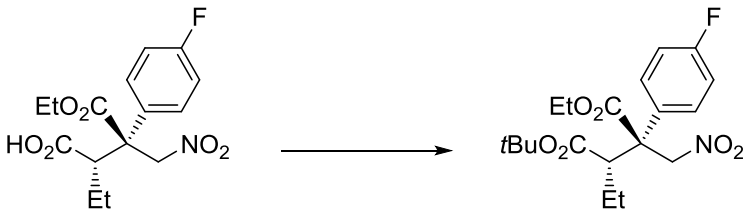
(2*S*,3*S*)-4-Ethoxy-2-ethyl-3-(4-fluorophenyl)-3-(nitromethyl)-4-oxo-butanoic acid (15):



To a cold solution (0°C) of γ-nitroaldehyde **9** (200 mg, 642 μmol) in acetone (2 mL) was added slowly Jones-Reagent[233] (1 mL, prepared as a standard reagent, 8 N). After 10 min the reaction was quenched with *i*PrOH (400 μL). The resulting green suspension was filtered over Celite and the filter cake was washed with acetone (2 x 5 mL). The filtrate was concentrated under reduced pressure and then diluted with 1 M HCl (5 mL) and extracted with CH_2Cl_2 (5 x 5 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 2% to CH_2Cl_2/MeOH 10%) to afford the γ-nitrocarboxylic acid **15** as a colorless oil (204 mg, 97%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 7.24 – 7.18 (m, 2H), 7.08 – 7.01 (m, 2H), 5.44 (d, *J* = 14.6 Hz, 1H), 5.15 (d, *J* = 14.5 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 3.22 (dd, *J* = 11.7, 2.7 Hz, 1H), 1.78 (ddd, *J* = 15.2, 13.4, 7.6 Hz, 1H), 1.59 (ddq, *J* = 14.0, 11.8, 7.1 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H); minor diastereomer δ = 7.28 – 7.24 (m, 2H), 7.08 – 7.01 (m, 2H)*, 5.38 (d, *J* = 15.5 Hz, 1H), 5.19 (d, *J* = 15.5 Hz, 1H), 4.26 (q, *J* = 7.1 Hz, 2H), 3.22 (dd, *J* = 11.7, 2.7 Hz, 1H)*, 1.42 – 1.13 (m, 2H)*, 1.24 (t, *J* = 7.1 Hz, 3H)*, 0.86 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, DMSO-d_6, 25°C): Major diastereomer δ = 178.3, 170.5, 162.2 (d, $^1J_{C,F}$ = 248.9 Hz), 132.3 (d, $^4J_{C,F}$ = 3.6 Hz), 128.4 (d, $^3J_{C,F}$ = 8.2 Hz), 115.8 (d, $^2J_{C,F}$ = 21.6 Hz), 77.9, 62.4, 54.5, 54.1, 22.0, 13.8, 12.9; visible signals of the minor diastereomer δ = 129.0 (d, $^3J_{C,F}$ = 8.7 Hz), 115.6 (d, $^2J_{C,F}$ = 21.5 Hz), 62.7, 54.7, 22.0, 13.7, 12.4; HRMS (ESI): *m/z* calcd for $C_{15}H_{18}FNO_6$+Na^+: 350.1010 [*M*+Na^+]; found: 350.1010.

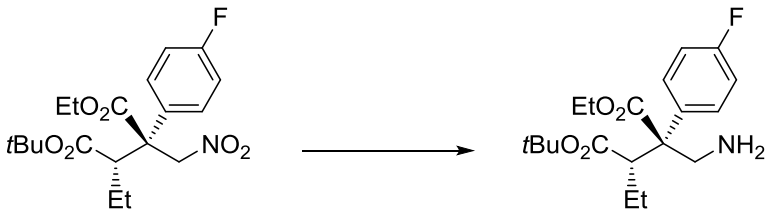
(2*S*,3*S*)-4-*t*-Butyl 1-ethyl 3-ethyl-2-(4-fluorophenyl)-2-(nitro-methyl)succinate (16):



A solution of the γ-nitrocarboxylic acid **15** (195 mg, 596 μmol) and H_2SO_4 (50 μL) in 2 mL of CH_2Cl_2 was stirred under an atmosphere of isobutylene (balloon) at room temperature and the reaction progress was monitored by TLC (CH_2Cl_2/MeOH 5%). After 3 h full conversion to the desired product was observed. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and extracted with a saturated solution of $NaHCO_3$ (3 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (short column, EtOAc/pentane 1:20) to afford the corresponding nitro-*tert*-butyl ester **16** as a colorless oil (186 mg, 81%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 7.29 – 7.23 (m, 2H), 7.07 – 7.01 (m, 2H), 5.51 (d, *J* = 14.8 Hz, 1H), 5.23 (d, *J* = 14.8 Hz, 1H), 4.25 (q, *J* = 7.1 Hz, 2H), 3.09 (dd, *J* = 12.0, 2.8 Hz, 1H), 1.80 – 1.69 (m, 1H), 1.63 – 1.51 (m, 1H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.22 (s, 9H), 0.91 (t, *J* = 7.3 Hz, 3H); minor diastereomer δ = 7.29 – 7.23 (m, 2H)*, 7.07 – 7.01 (m, 2H)*, 5.30 (d, *J* = 15.7 Hz, 1H), 5.16 (d, *J* = 15.7 Hz, 1H), 4.29 (qd, *J* = 7.2, 2.2 Hz, 2H)*, 3.13 (dd, *J* = 12.0, 2.8 Hz, 1H)*, 1.49 – 1.41 (m, 1H)*, 1.34 – 1.22 (m, 1H)*, 1.44 (s, 9H)*, 1.26 (td, *J* = 7.1, 1.5 Hz, 3H)*, 0.83 (t, *J* = 7.3 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 172.1, 170.6, 162.1 (d, $^1J_{C,F}$ = 248.3 Hz), 133.1 (d, $^4J_{C,F}$ = 3.5 Hz), 128.5 (d, $^3J_{C,F}$ = 8.1 Hz), 115.4 (d, $^2J_{C,F}$ = 21.5 Hz), 81.6, 77.2, 62.1, 55.6, 54.2, 27.7, 22.2, 13.8, 12.8; visible signals of the minor diastereomer δ = 172.2, 171.3, 129.4 (d, $^3J_{C,F}$ = 8.1 Hz), 115.2 (d, $^2J_{C,F}$ = 21.5 Hz), 82.2, 62.3, 52.8, 27.9, 23.0, 14.2, 12.4; HRMS (ESI): *m/z* calcd for $C_{19}H_{26}FNO_6$+Na^+: 406.1636 [*M*+Na^+]; found: 406.1635.

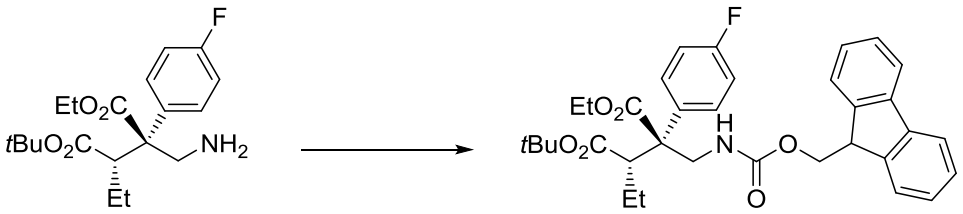
(2*S*,3*S*)-4-*t*-Butyl 1-ethyl 2-(aminomethyl)-3-ethyl-2-(4-fluoro-phenyl)succinate (17):



To a solution of the nitro-*tert*-butyl ester **16** (175 mg, 456 µmol) in 15 mL of EtOAc was added acetic acid (2.5 mL) and freshly activated zinc powder (washed with 2 M HCl, H_2O, EtOH and Et_2O, dried under high vacuum). The reaction mixture was stirred at room temperature for 18 h and then filtered over Celite. The filter cake was washed with EtOAc (3 x 10 mL) and the filtrate was then extracted with concentrated aqueous ammonia (50 mL). The aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure to afford the amino ester **17** as a colorless hygroscopic solid (160 mg, 99%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 7.19 – 7.12 (m, 2H), 7.08 – 7.01 (m, 2H), 5.62 (br s, 2H), 4.28 (qd, *J* = 7.2, 2.1 Hz, 2H), 3.31 (d, *J* = 13.8 Hz, 1H), 3.05 (d, *J* = 13.8 Hz, 1H), 2.87 (dd, *J* = 11.6, 2.5 Hz, 1H), 1.90 – 1.72 (m, 1H), 1.60 – 1.47 (m, 1H), 1.41 (s, 9H), 1.30 (t, *J* = 7.1 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H); minor diastereomer δ = 7.19 – 7.12 (m, 2H)*, 7.08 – 7.01 (m, 2H)*, 5.62 (br s, 2H)*, 4.19 (qd, *J* = 7.1, 2.4 Hz, 2H), 3.44 (d, *J* = 13.7 Hz, 1H), 3.33 (d, *J* = 13.6 Hz, 1H)*, 3.12 (dd, *J* = 8.6, 5.3 Hz, 1H), 2.02 (s, 9H), 1.90 – 1.72 (m, 1H)*, 1.60 – 1.47 (m, 1H)*, 1.21 (t, *J* = 7.1 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 173.4, 173.1, 161.7 (d, $^1J_{C,F}$ = 246.4 Hz), 135.0 (d, $^4J_{C,F}$ = 3.5 Hz), 129.3 (d, $^3J_{C,F}$ = 7.9 Hz), 115.4 (d, $^2J_{C,F}$ = 21.2 Hz), 81.3, 61.1, 58.9, 54.8, 48.9, 28.1, 22.8, 14.2, 13.1; HRMS (ESI): *m/z* calcd for $C_{19}H_{28}FNO_4$+H^+: 354.2075 [*M*+H^+]; found: 354.2073.

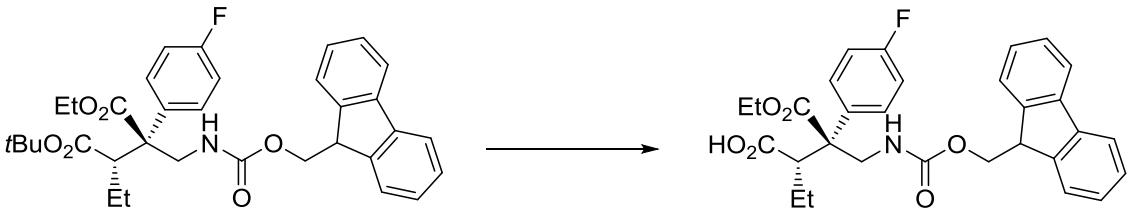
(2*S*,3*S*)-4-*t*-*B*utyl 1-ethyl 2-((((9H-fluoren-9-yl)methoxy)-carbonylamino)methyl)-3-ethyl-2-(4-fluorophenyl)succinate (18):



To a suspension of the amino ester **17** (150 mg, 424 μmol) in 3 mL of 1,4-dioxane/water 1:1 was added $NaHCO_3$ (107 mg, 1.27 mmol) and Fmoc-Cl (165 mg, 636 μmol). The reaction mixture was stirred at room temperature for 30 min, then diluted with water (30 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (EtOAc/toluene 1:20) to afford the Fmoc-protected amino ester **18** as a colorless oil (189 mg, 77%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 7.74 (d, J = 7.6 Hz, 2H), 7.50 (d, J = 7.4 Hz, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.38 (t, J = 7.4 Hz, 2H), 7.30 – 7.22 (m, 4H), 7.01 – 6.94 (m, 2H), 5.98 (t, J = 6.0 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 4.24 (dd, J = 7.6, 3.0 Hz, 1H), 4.20 (dd, J = 10.4, 7.2 Hz, 1H), 4.12 (t, J = 7.3 Hz, 1H), 3.83 – 3.97 (m, 2H), 3.08 (dd, J = 11.4, 2.7 Hz, 1H), 1.90 – 1.75 (m, 1H), 1.62 – 1.49 (m, 1H), 1.36 (s, 9H), 1.30 (t, J = 7.1 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H); minor diastereomer δ = 7.77 – 7.71 (m, 2H)*, 7.56 (d, J = 7.8 Hz, 1H), 7.53 – 7.48 (d, J = 7.6 Hz, 1H)*, 7.38 (t, J = 7.4 Hz, 2H)*, 7.30 – 7.22 (m, 4H)*, 7.09 – 7.01 (m, 2H), 5.37 (br s, 1H), 4.33 – 4.09 (m, 5H)*, 3.64 – 3.74 (m, 2H), 2.89 (d, J = 11.2 Hz, 1H), 1.90 – 1.75 (m, 1H)*, 1.62 – 1.49 (m, 1H)*, 1.56 (s, 9H)*, 1.30 (t, J = 7.1 Hz, 3H)*, 0.88 (t, J = 7.1 Hz, 3H). *Superimposed by signals of the major diastereomer. ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): Major diastereomer δ = 173.6, 173.3, 162.0 (d, $^1J_{C,F}$ = 247.0 Hz), 156.4, 144.2, 144.1, 141.4, 141.4, 133.7 (d, $^4J_{C,F}$ = 3.3 Hz), 129.4 (d, $^3J_{C,F}$ = 8.0 Hz), 127.7, 127.7, 127.1 (two signals), 125.3, 125.2, 120.1 (two signals), 115.3 (d, $^2J_{C,F}$ = 21.3 Hz), 81.8, 66.9, 61.8, 57.0, 55.3, 47.3, 45.0, 28.0, 23.2, 14.2, 13.2; HRMS (ESI): *m/z* calcd for $C_{34}H_{38}FNO_6$+Na^+: 598.2575 [M+Na^+]; found: 598.2578.

(3*S*,4*S*)-3-((((9*H*-Fluoren-9-yl)methoxy)carbonylamino)methyl)-4-ethoxy-2-ethyl-3-(4-fluorophenyl)-4-oxobutanoic acid (19):

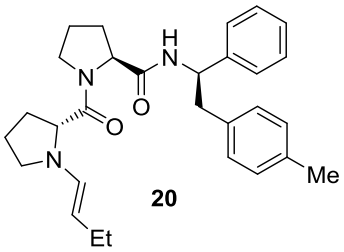


To a solution of the fully protected amino acid **18** (150 mg, 261 μmol) in CH_2Cl_2 (4 mL) was added TFA (1 mL). The reaction mixture was stirred at room temperature for 2 h and then concentrated under reduced pressure. The remaining TFA was co-evaporated with toluene (3 x 5 mL). The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 2% to CH_2Cl_2/MeOH 8%) to afford a colorless oil which was precipitated from pentane to yield the desired Fmoc-protected $\gamma^{2,3,3}$-amino acid **19** as a white powder (122 mg, 90%).

^{1}H NMR (600 MHz, CD_3OD, 25°C): Major diastereomer δ = 7.80 (d, J = 7.8 Hz, 2H), 7.55 (d, J = 7.6 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.40 (t, J = 7.5 Hz, 2H), 7.32 – 7.25 (m, 4H), 6.99 (t, J = 8.6 Hz, 2H), 4.27 (qd, J = 10.8, 7.1 Hz, 2H), 4.20 (dd, J = 10.2, 6.9 Hz, 1H), 4.13 (dd, J = 10.2, 7.1 Hz, 1H), 4.09 (t, J = 7.0 Hz, 1H), 3.90 (d, J = 14.1 Hz, 1H), 3.79 (d, J = 14.1 Hz, 1H), 3.11 (d, J = 11.0 Hz, 1H), 1.76 – 1.86 (m, 1H), 1.63 – 1.54 (m, 1H), 1.29 (t, J = 7.1 Hz, 3H), 0.99 (t, J = 7.3 Hz, 3H); minor diastereomer δ = 7.87 – 7.83 (m, 2H), 7.59 (t, J = 7.3 Hz, 2H), 7.46 – 7.33 (m, 4H)*, 7.04 – 7.00 (m, 2H), 7.01 – 6.96 (m, 2H)*, 4.33 – 4.17 (m, 4H)*, 4.16 – 4.07 (m, 1H)*, 3.62 (d, J = 14.2 Hz, 1H), 3.40 (d, J = 14.2 Hz, 1H), 2.94 (br s, 1H), 1.80 – 1.71 (m, 1H)*, 1.50 – 1.42 (m, 1H), 1.25 (t, J = 7.1 Hz, 3H)*, 0.99 (t, J = 7.3 Hz, 3H)*. *Superimposed by signals of the major diastereomer. ^{13}C NMR (150 MHz, CD_3OD, 25°C): Major diastereomer δ = 179.4, 175.1, 163.2 (d, $^1J_{C,F}$ = 245.2 Hz), 158.5, 145.3, 145.2, 142.6, 142.5, 135.8 (d, $^4J_{C,F}$ = 2.9 Hz), 130.8 (d, $^3J_{C,F}$ = 8.0 Hz), 128.8 (two signals), 128.2 (two signals), 126.2, 126.2, 120.9 (two signals), 115.9 (d, $^2J_{C,F}$ = 21.5 Hz), 68.0, 62.6, 58.6, 55.7, 48.3, 47.0, 24.2, 14.3, 13.6; visible signals of the minor diastereomer δ = 174.8, 163.1 (d, $^1J_{C,F}$ = 245.4 Hz), 158.0, 145.4, 145.4, 142.8, 135.9, 130.7, 128.9, 128.3, 128.3, 126.0, 121.1, 68.0, 58.4, 48.0; HRMS (ESI): *m/z* calcd for $C_{30}H_{30}FNO_6$+Na^+: 542.1949 [M+Na^+]; found: 542.1952.

7.8 Synthesis, Identification and Characterization of Reaction Intermediates and Side Products

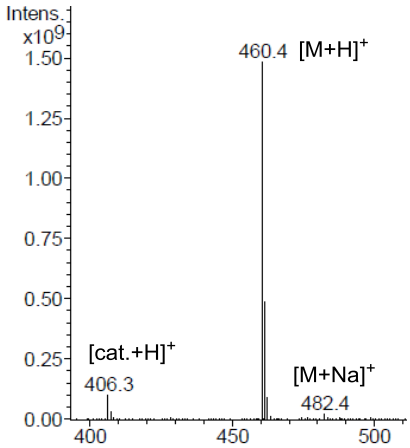
7.8.1 Enamine 20



20

To a solution of H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) (21 mg, 0.05 mmol) in CD_3OH-d_3 or $CDCl_3$ (600 µL) with molecular sieves (3 Å in CD_3OH-d_3, 4 Å in $CDCl_3$) in a NMR tube was added freshly distilled butanal (4.5 µL, 0.05 mmol). After 5 min a ^{1}H-NMR spectrum was recorded.

^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ = 7.91 (d, *J* = 8.3 Hz, 1H), 7.30 – 7.13 (m, 5H), 7.06 – 6.92 (m, 4H), 5.97 (d, *J* = 13.7 Hz, 1H), 5.09 (dd, *J* = 7.5 Hz, 1H), 4.62 (dd, *J* = 8.0, 1.6 Hz, 1H), 4.18 (dt, *J* = 13.6, 6.7 Hz, 1H), 3.91 (dd, *J* = 8.6, 4.8 Hz, 1H), 3.52 (ddd, *J* = 9.7, 8.3, 2.9 Hz, 1H), 3.39 (tt, *J* = 7.3, 5.6 Hz, 2H), 3.06 – 2.91 (m, 2H), 2.46 – 2.34 (m, 1H), 2.28 (s, 3H), 2.26 – 2.13 (m, 1H), 2.10 – 1.97 (m, 2H), 1.97 – 1.76 (m, 5H), 1.76 – 1.57 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H).

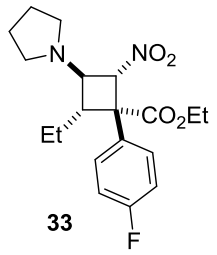


ESI-MS analysis of NMR sample of enamine **20**:

A small aliquot of the NMR sample in $CDCl_3$ was diluted with dry acetonitrile an injected into an ESI-MS spectrometer. In the resulting spectrum the mass of the protonated enamine as well as its sodium adduct were observed. The mass peak of the protonated catalyst was also observed.

7.8.2 Cyclobutanes

Cyclobutane 33:



33

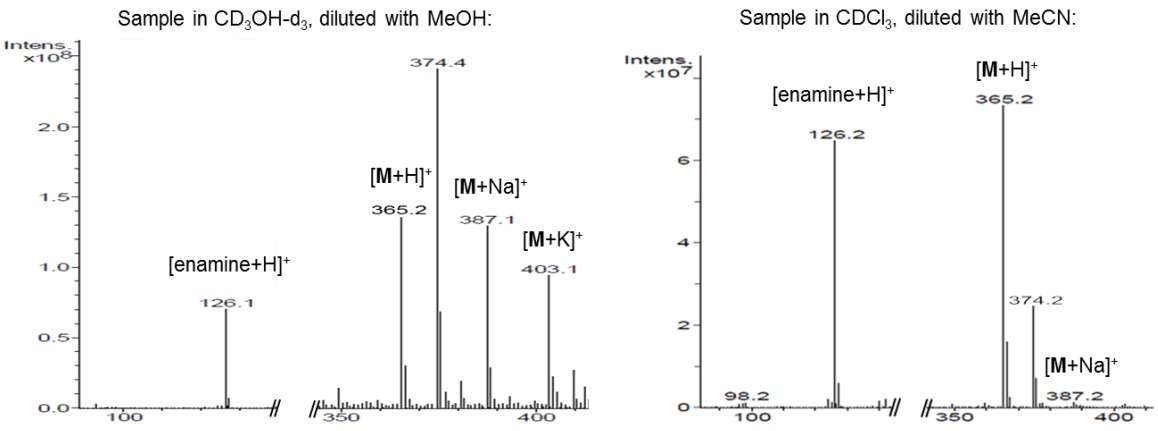
relative configuration

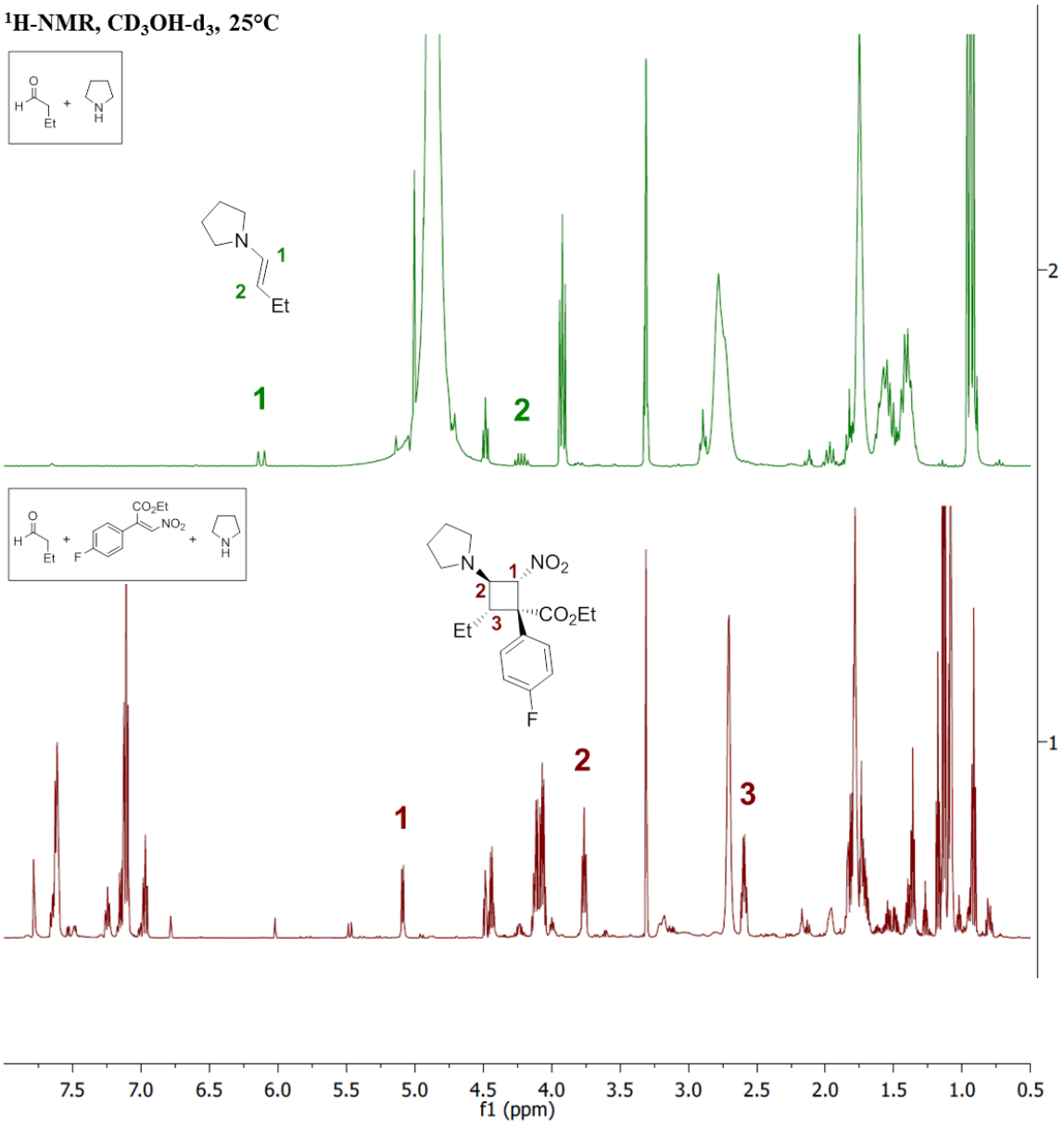
To a solution of pyrrolidine (4.1 µL, 0.05 mmol) in CD_3OH-d_3 or $CDCl_3$ (600 µL) with molecular sieves (3 Å in CD_3OH-d_3, 4 Å in $CDCl_3$) in a NMR tube was added freshly distilled butanal (4.5 µL, 0.05 mmol). After 5 min a ^{1}H-NMR spectrum was recorded that showed the corresponding enamine (see below). Then nitroacrylate **7** (12 mg, 0.05 mmol) was added to the mixture and ^{1}H-, ^{13}C- and ^{19}F-NMR spectra were recorded immediately.

^{1}H NMR (600 MHz, CD_3OH-d_3, 25°C): δ = 7.63 – 7.60 (m, 2H), 7.13 – 7.09 (m, 2H), 5.09 (d, *J* = 7.3 Hz, 1H), 4.12 (dq, *J* = 10.8, 7.1 Hz, 1H), 4.07 (dq, *J* = 10.8, 7.1 Hz, 1H), 3.76 (dd, *J* = 8.6, 7.3 Hz, 1H), 2.75 – 2.66 (m, 4H), 2.59 (td, *J* = 8.5, 6.2 Hz, 1H), 1.87 – 1.78 (m, 1H), 1.81 – 1.75 (m, 4H), 1.77 – 1.66 (m, 1H), 1.13 (t, *J* = 7.1 Hz, 3H), 1.09 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (151 MHz, CD_3OH-d_3, 25°C): δ = 171.5, 163.3 (d, $^1J_{C,F}$ = 245.2 Hz), 138.7 (d, $^4J_{C,F}$ = 3.2 Hz), 130.5 (d, $^3J_{C,F}$ = 8.1 Hz), 115.8 (d, $^2J_{C,F}$ = 21.6 Hz), 84.4, 66.1, 62.7, 59.3, 51.6, 49.0, 45.7, 26.0, 24.0, 14.1, 12.9. ^{19}F NMR (282 MHz, CD_3OH-d_3, 25°C): δ = -117.2.

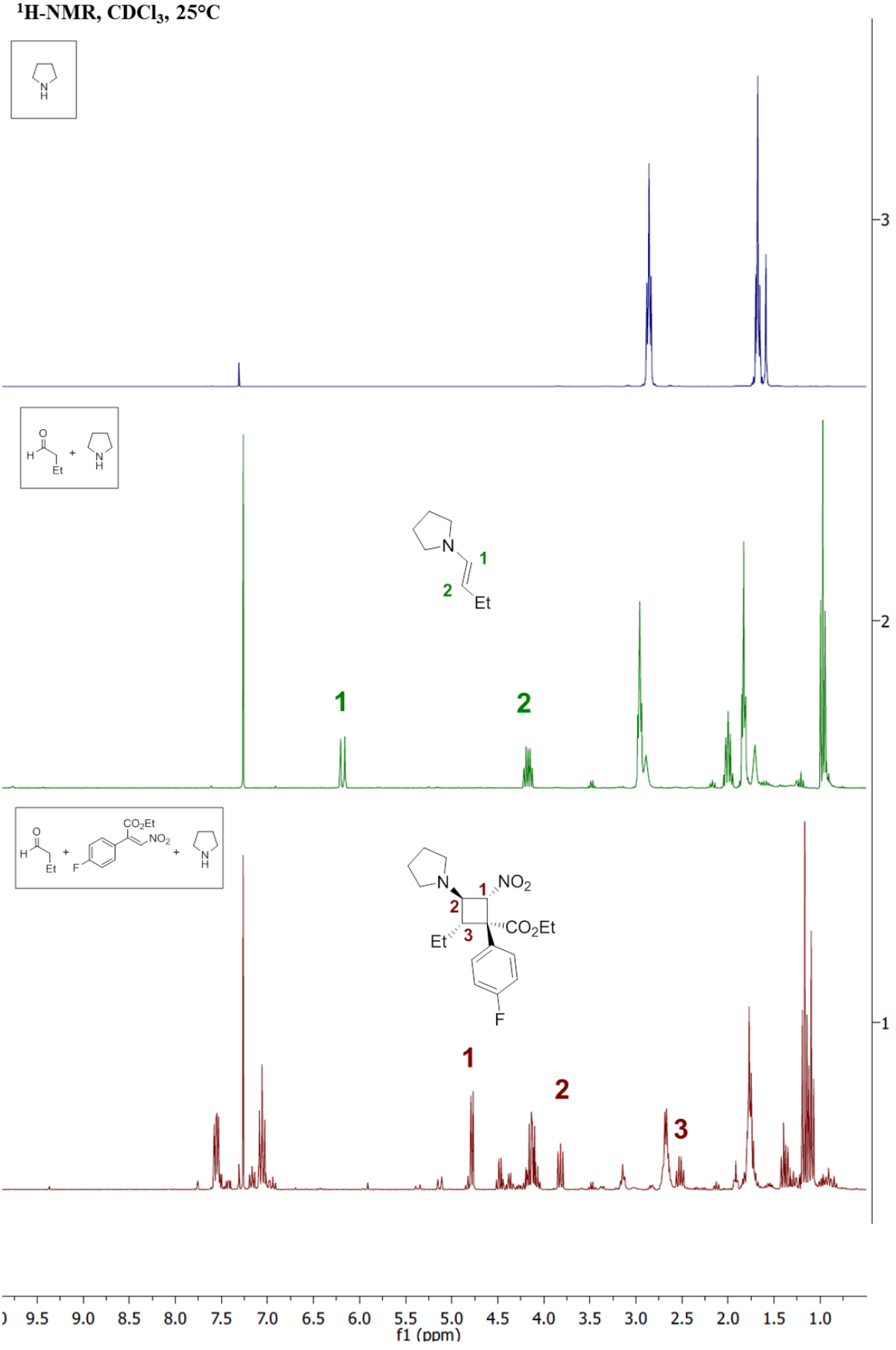
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.61 – 7.51 (m, 2H), 7.10 – 7.03 (m, 2H), 4.79 (d, *J* = 7.3 Hz, 1H), 4.21 – 4.05 (m, 2H), 3.82 (dd, *J* = 8.6, 7.3 Hz, 1H), 2.75 – 2.62 (m, 4H), 2.53 (td, *J* = 8.3, 6.5 Hz, 1H), 1.83 – 1.69 (m, 6H), 1.17 (t, *J* = 7.1 Hz, 3H), 1.10 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 170.0, 162.0 (d, $^1J_{C,F}$ = 246.7 Hz), 137.3 (d, $^4J_{C,F}$ = 3.4 Hz), 129.1 (d, $^3J_{C,F}$ = 8.1 Hz), 115.2 (d, $^2J_{C,F}$ = 21.4 Hz), 83.8, 64.8, 61.9, 58.2, 50.8, 45.0, 25.0, 23.4, 13.9, 12.8. ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -114.9.

ESI-MS analysis of NMR samples of cyclobutane **33**:
Small aliquots of the NMR samples in CD_3OH-d_3 and $CDCl_3$, respectively, were diluted with dry MeOH or dry acetonitrile an injected into an ESI-MS spectrometer. The spectra showed mass peaks corresponding to cyclobutane **33** as well as its sodium and potassium adducts. The mass peak of the protonated enamine was also observed.

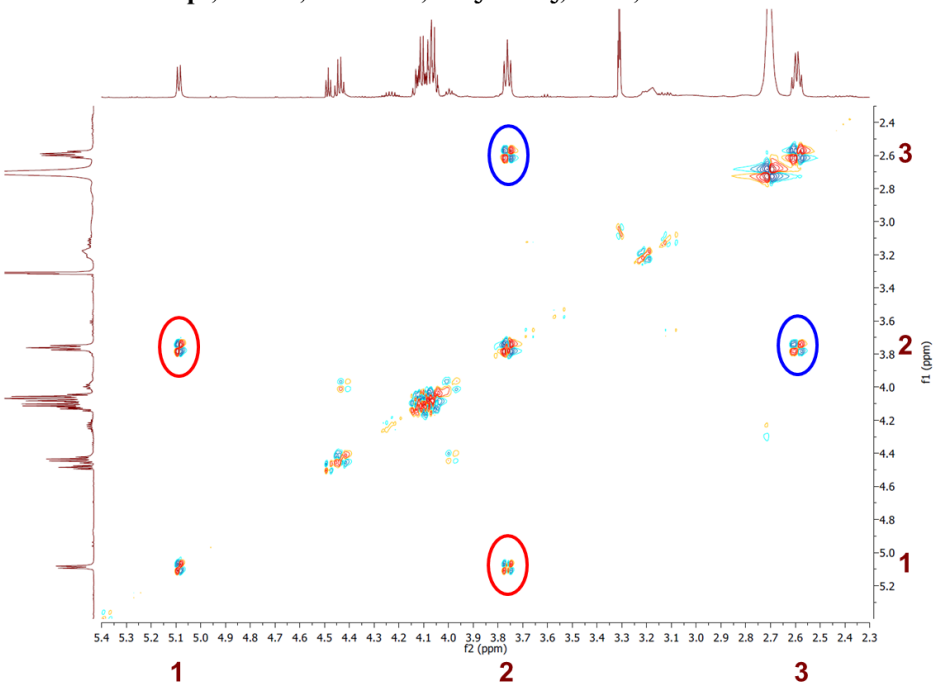

Sample in CD_3OH-d_3, diluted with MeOH:
Intens.
x10⁸
2.0
1.5
1.0
0.5
0.0
[enamine+H]⁺
126.1
374.4
[M+H]⁺
365.2
[M+Na]⁺
387.1
[M+K]⁺
403.1
100
350
400
Sample in $CDCl_3$, diluted with MeCN:
Intens.
x10⁷
6
4
2
0
[enamine+H]⁺
126.2
98.2
[M+H]⁺
365.2
374.2
[M+Na]⁺
387.2
100
350
400

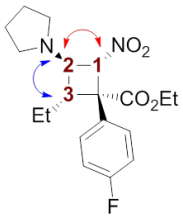

^{1}H-NMR, CD_3OH-d_3, 25°C
Et
N
H
1
2
Et
1
2
CO_2Et
NO_2
F
1
2
3
2
1
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

^{1}H-NMR, $CDCl_3$, 25°C



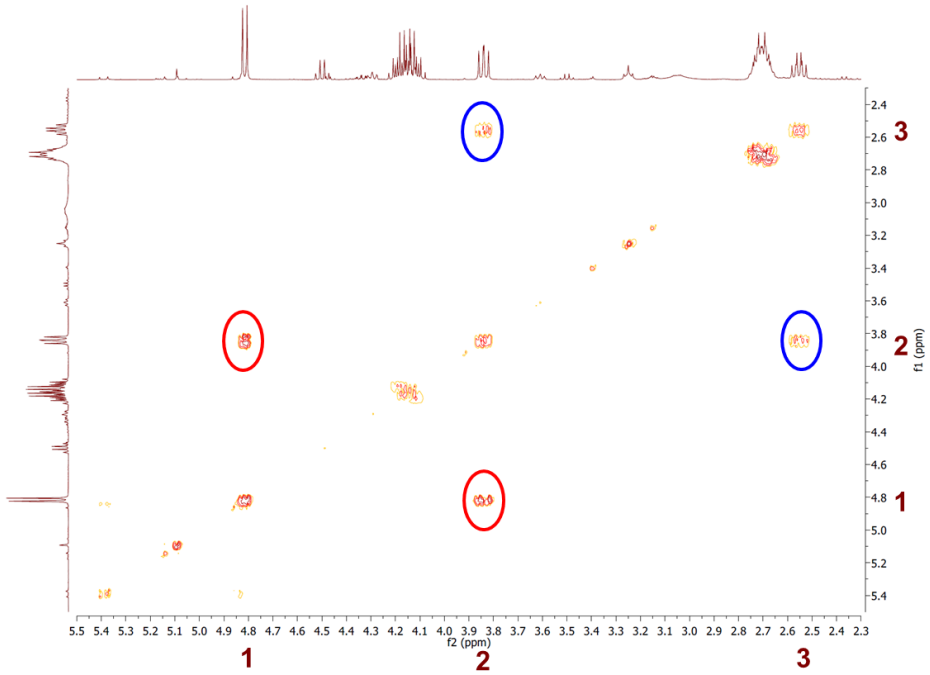
Relevant excerpt, COSY, 600 MHz, CD_3OH-d_3, 25°C, after 2 h

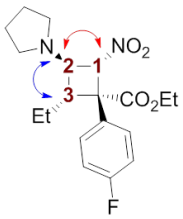




H1: 5.09 ppm
H2: 3.76 ppm
H3: 2.59 ppm

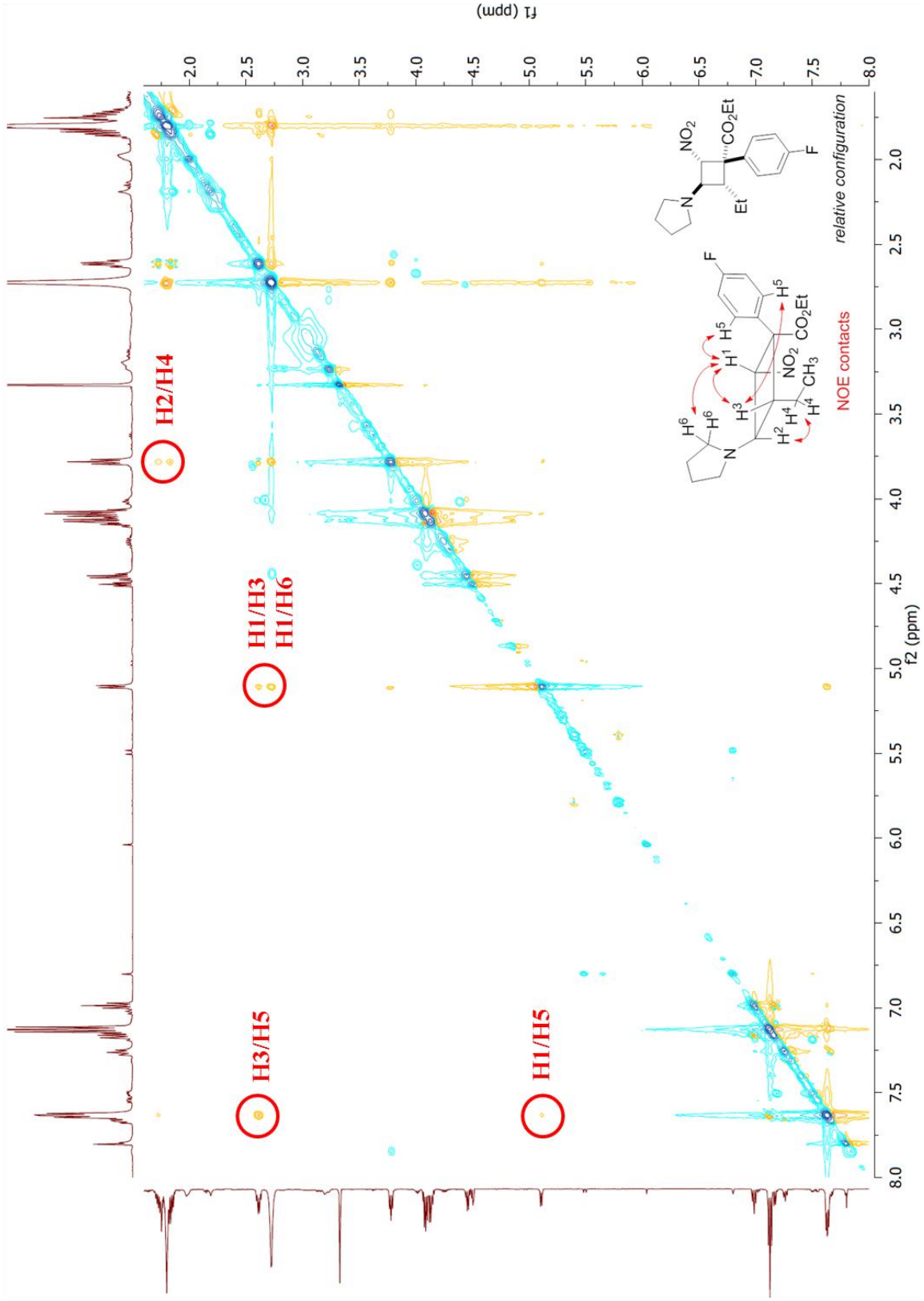
Relevant excerpt, COSY, 400 MHz, $CDCl_3$, 25°C, after 4 h



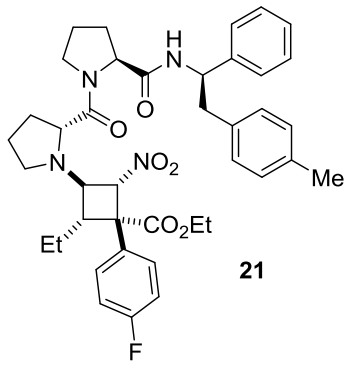


H1: 4.79 ppm
H2: 3.82 ppm
H3: 2.53 ppm

Relevant excerpt, NOESY, 600 MHz, CD_3OH-d_3, 25°C, after 2 h



Cyclobutane 21:



relative configuration of cyclobutane

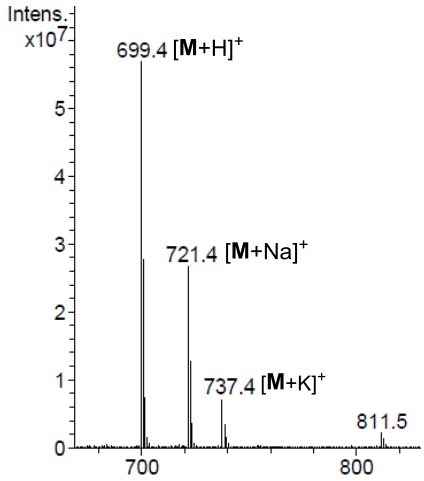
To a solution of peptide **10-R** (21 mg, 0.05 mmol) in CD_3OH-d_3 or $CDCl_3$ (600 μL) with molecular sieves (3 Å in CD_3OH-d_3, 4 Å in $CDCl_3$) in a NMR tube was added freshly distilled butanal (4.5 μL, 0.05 mmol). After 5 min a ^{1}H-NMR spectrum was recorded that showed the corresponding enamine (see below). Then nitroacrylate **7** (12 mg, 0.05 mmol) was added to mixture and ^{1}H-, ^{13}C- and ^{19}F-NMR spectra were recorded immediately.

^{1}H NMR (600 MHz, CD_3OH-d_3, 25°C): The *cis/trans* conformers were observed in a ratio of 1:1.5. Major conformer: δ = 7.88 (d, *J* = 8.8 Hz, 1H), 7.59 – 7.54 (m, 2H), 7.41 – 7.17 (m, 5H), 3.09 – 3.03 (m, 1H), 7.13 – 7.00 (m, 6H), 5.29 (d, *J* = 7.6 Hz, 1H), 5.21 – 5.12 (m, 1H), 4.42 (dd, *J* = 8.6, 3.5 Hz, 1H), 4.10 (dd, *J* = 9.2, 7.6 Hz, 1H), 4.10 – 3.91 (m, 2H), 3.89 (dd, *J* = 8.3, 7.2 Hz, 1H), 3.73 (ddd, *J* = 9.7, 7.7, 4.4 Hz, 1H), 3.54 – 3.47 (m, 1H), 3.15 – 2.92 (m, 2H), 2.63 (dt, *J* = 9.2, 7.2 Hz, 1H), 2.63 – 2.56 (m, 1H), 2.25 (s, 3H), 2.25 – 2.14 (m, 1H), 2.03 – 1.88 (m, 1H), 1.88 – 1.74 (m, 4H), 1.76 – 1.54 (m, 2H), 1.74 – 1.63 (m, 2H), 1.02 (t, *J* = 7.5 Hz, 3H), 0.99 (t, *J* = 7.1 Hz, 3H); minor conformer: δ = 8.62 (d, *J* = 8.9 Hz, 1H), 7.59 – 7.54 (m, 2H)*, 7.41 – 7.17 (m, 5H)*, 7.15 – 7.00 (m, 6H)*, 2.96 – 2.91 (m, 1H), 5.24 – 5.17 (m, 1H), 5.16 (d, *J* = 7.6 Hz, 1H)*, 4.63 (dd, *J* = 8.6, 2.8 Hz, 1H), 4.11 – 3.91 (m, 2H)*, 3.93 (dd, *J* = 9.5, 7.7 Hz, 1H), 3.56 – 3.45 (m, 2H)*, 3.15 – 2.92 (m, 2H)*, 2.96 – 2.91 (m, 1H)*, 2.55 (dt, *J* = 9.4, 7.3 Hz, 1H), 2.45 – 2.39 (m, 1H), 2.25 (s, 3H), 2.19 – 2.06 (m, 1H)*, 1.76 – 1.65 (m, 2H)*, 1.77 – 1.54 (m, 2H)*, 1.62 – 1.52 (m, 2H)*, 1.62 – 1.52 (m, 1H)*, 1.44 – 1.20 (m, 2H), 1.30 – 1.20 (m, 1H), 1.09 (t, *J* = 7.1 Hz, 3H), 1.06 (t, *J* = 7.5 Hz, 3H). *Superimposed by signals of the major conformer. ^{13}C NMR (151 MHz, CD_3OH-d_3, 25°C): Major conformer: δ = 175.1, 173.2, 171.6, 163.2 (d, $^1J_{C,F}$ = 245.1 Hz), 143.3, 138.6 (d, $^4J_{C,F}$ = 3.3 Hz), 136.6, 136.5, 130.5 (d, $^3J_{C,F}$ = 8.2 Hz), 130.2, 129.6, 129.2, 127.9, 127.8, 115.8 (d, $^2J_{C,F}$ = 21.7 Hz), 83.7, 64.0, 63.4, 62.7, 62.1, 59.0, 56.2, 48.7, 47.9, 45.3, 43.0, 30.3, 29.9, 25.8, 25.4, 23.7, 21.0, 14.1, 12.5; minor conformer: δ = 174.8, 173.3, 171.5, 163.3 (d, $^1J_{C,F}$ = 245.2 Hz), 143.6, 138.6 (d, $^4J_{C,F}$ = 3.1 Hz), 137.0, 136.3, 130.4 (d, $^3J_{C,F}$ = 8.5 Hz), 130.0, 129.8, 129.4, 128.2, 127.6, 115.8 (d, $^2J_{C,F}$ = 21.6 Hz), 83.4, 63.6, 63.1, 62.7, 61.2, 58.9, 55.9,

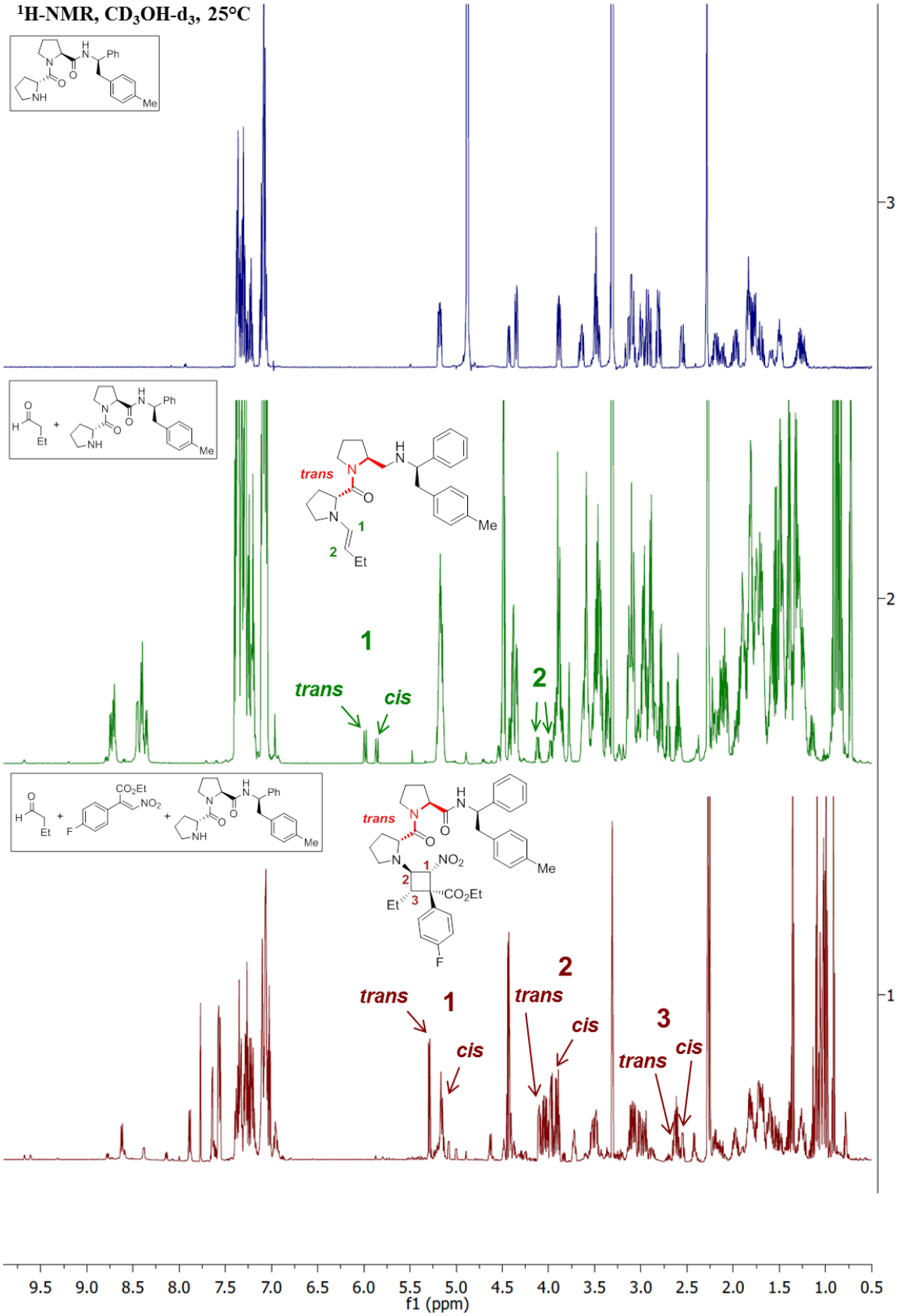
48.5, 47.9, 45.5, 42.9, 33.3, 30.4, 25.7, 23.4, 23.0, 21.0, 14.1, 12.5. ^{19}F NMR (565 MHz, CD_3OH-d_3, 25°C): Major conformer: δ = -117.3; minor conformer: δ = -117.2.

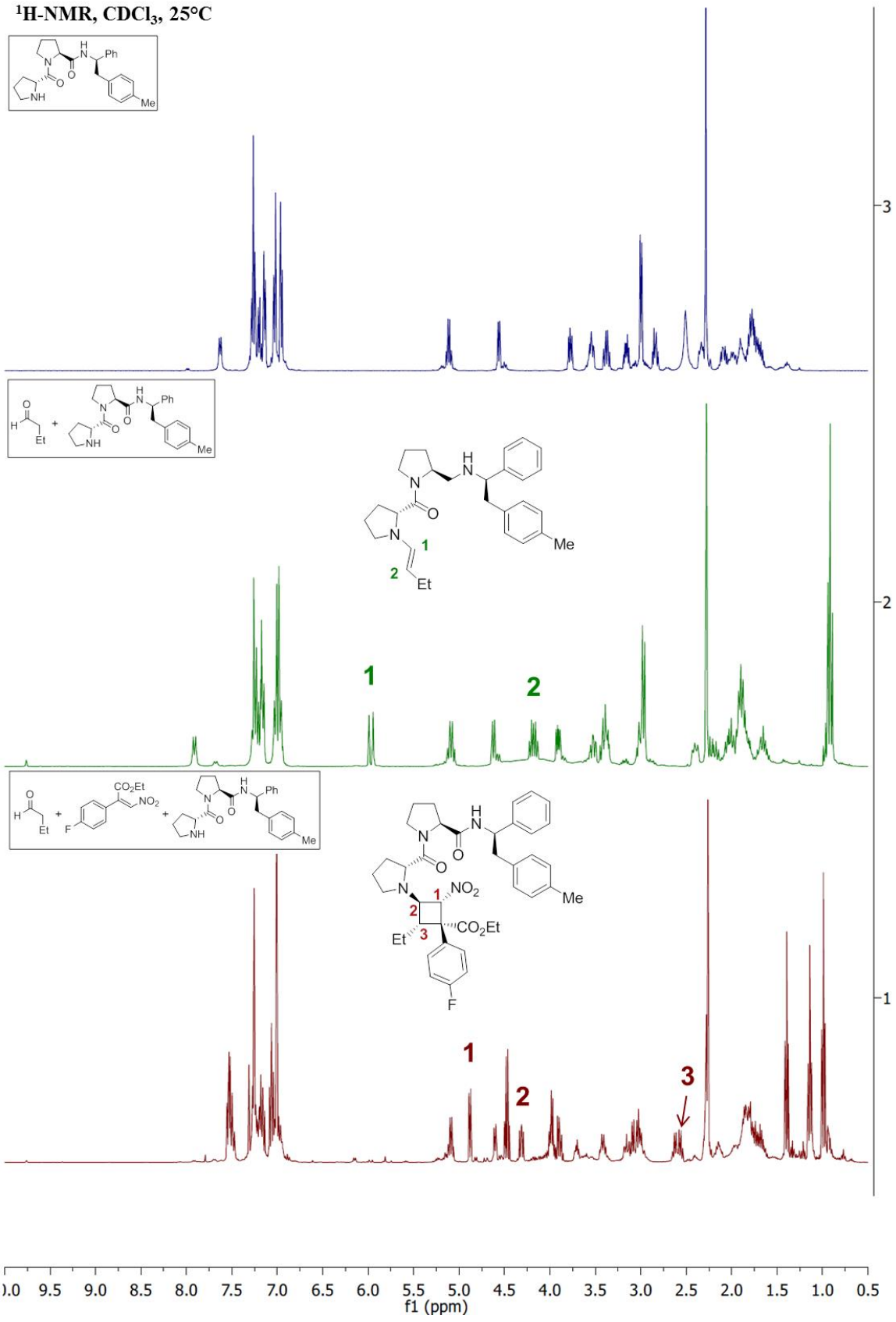
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.56 – 7.51 (m, 2H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.29 – 7.19 (m, 5H), 7.09 – 7.04 (m, 2H), 7.03 – 6.94 (m, 4H), 5.09 (dd, *J* = 7.6 Hz, 1H), 4.88 (d, *J* = 7.6 Hz, 1H), 4.60 (dd, *J* = 7.8, 2.2 Hz, 1H), 4.31 (dd, *J* = 9.4, 7.6 Hz, 1H), 3.99 (dq, 1H), 3.89 (dq, *J* = 10.8, 7.1 Hz, 1H), 3.75 – 3.67 (m, 1H), 3.46 – 3.38 (m, 1H), 3.16 (td, *J* = 8.1, 3.6 Hz, 1H), 3.10 (dd, *J* = 13.6, 7.5 Hz, 1H), 3.02 (dd, *J* = 13.6, 7.6 Hz, 1H), 3.03 – 2.92 (m, 1H), 2.68 – 2.59 (m, 1H), 2.57 (dt, *J* = 9.4, 7.4 Hz, 1H), 2.27 (d, *J* = 10.1 Hz, 3H), 2.20 – 2.08 (m, 1H), 2.02 – 1.60 (m, 9H), 1.14 (t, *J* = 7.5 Hz, 3H), 0.99 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 173.4, 170.3, 169.8, 162.1 (d, $^{1}J_{C,F}$ = 246.8 Hz), 142.2, 137.1 (d, $^{4}J_{C,F}$ = 3.3 Hz), 135.5, 135.1, 129.4, 129.0 (d, $^{3}J_{C,F}$ = 8.1 Hz), 128.8, 128.3, 126.9, 126.8, 115.4 (d, $^{2}J_{C,F}$ = 21.4 Hz), 82.6, 62.5, 62.3, 62.0, 60.9, 57.8, 55.8, 46.8, 46.7, 44.7, 42.5, 29.0, 28.0, 24.9, 24.7, 22.8, 21.1, 13.9, 12.2. ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -114.6.

ESI-MS analysis of NMR sample of cyclobutane **21**:

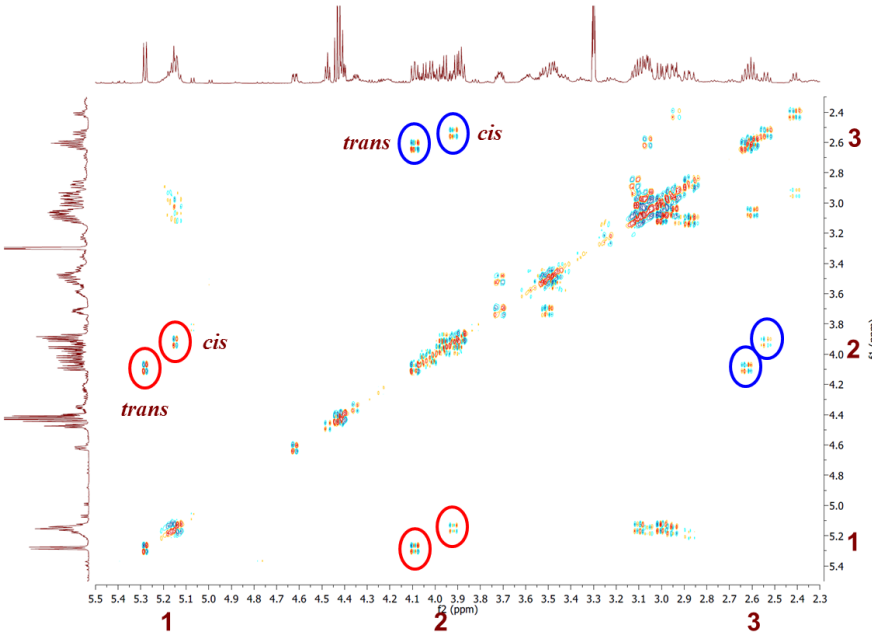


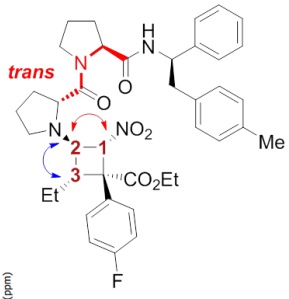
A small aliquot of the NMR sample in $CDCl_3$ was diluted with dry MeOH an injected into an ESI-MS spectrometer. In the resulting spectrum the mass peak corresponding to cyclobutane **21** as well as its sodium and potassium adducts were observed.


1H-NMR, CD3OH-d3, 25°C
trans
1
2
Et
Me
Ph
NH
trans
cis
1
2
trans
cis
2
trans
cis
3
trans
cis
NO2
CO2Et
F
3
2
1
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


1H-NMR, CDCl3, 25°C
Ph
Me
NH
Et
1
2
3
NO2
CO2Et
F
f1 (ppm)

Relevant excerpt, COSY, 600 MHz, CD_3OH-d_3, 25°C, after 1 h

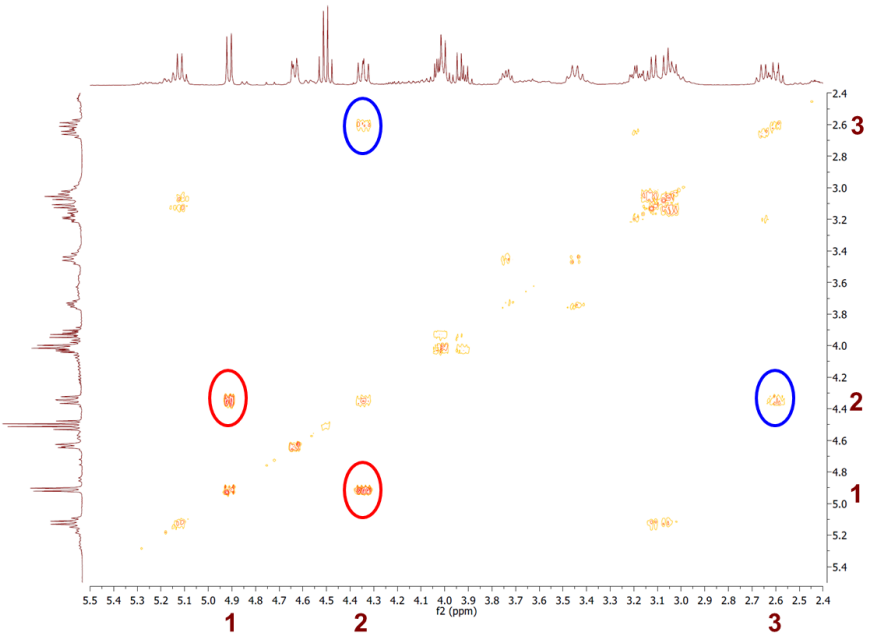


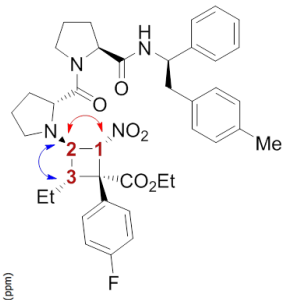


trans-conformer:
H1: 5.29 ppm
H2: 4.10 ppm
H3: 2.63 ppm

cis-conformer:
H1: 5.16 ppm
H2: 3.93 ppm
H3: 2.55 ppm

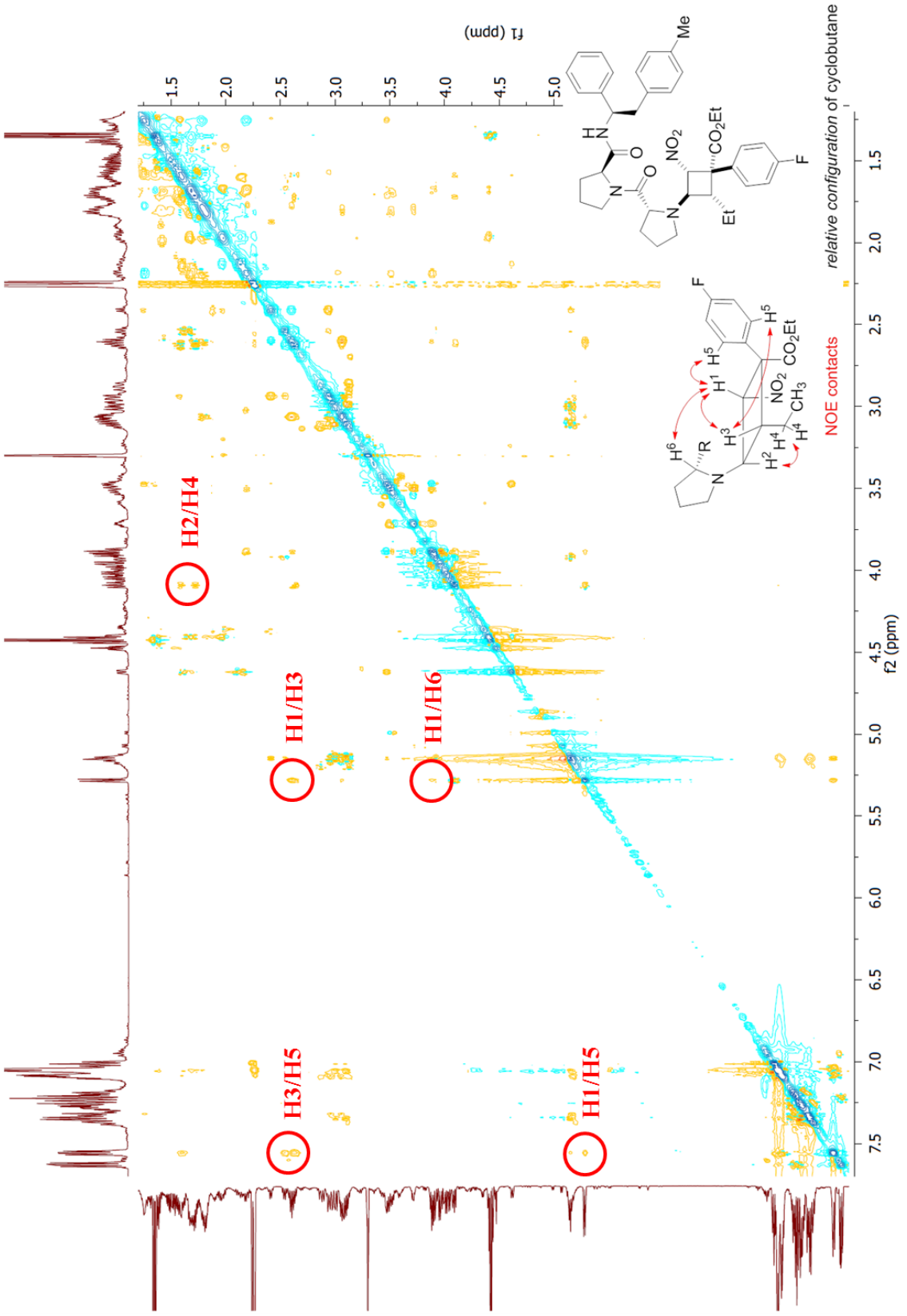
Relevant excerpt, COSY, 400 MHz, $CDCl_3$, 25°C, after 1 h





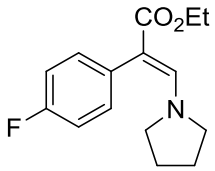
H1: 4.88 ppm
H2: 4.31 ppm
H3: 2.57 ppm

Relevant excerpt, NOESY, 600 MHz, CD_3OH-d_3, 25°C, after 1 h



7.8.3 Pyrrolidinyl Acrylates

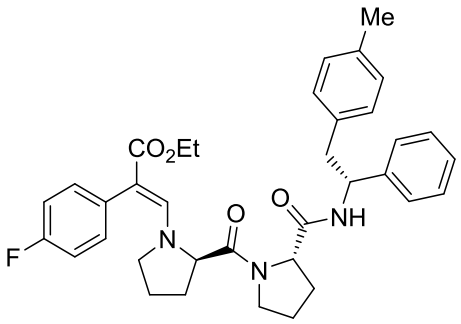
Ethyl (*E*)-2-(4-fluorophenyl)-3-(pyrrolidin-1-yl)acrylate 31:



To a solution of nitroacrylate **7** (96 mg, 0.40 mmol) in $CHCl_3$ (1 mL) was added pyrrolidine (33 μL, 0.40 mmol) and the reaction mixture was allowed to stand overnight at room temperature. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography over basic aluminium oxide (EtOAc/pentane 1:10) to yield the title compound as a colorless oil (30 mg, 28%) which crystallized in the fridge providing crystals suitable for X-ray crystallographic analysis (see Chapter 7.9 for details).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.76 (s, 1H), 7.19 – 7.13 (m, 2H), 6.98 – 6.90 (m, 2H), 4.12 (q, J = 7.1 Hz, 2H), 3.03 (broad s, 4H), 1.79 – 1.68 (m, 4H), 1.20 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 169.9, 161.4 (d, $^{1}J_{C,F}$ = 244.4 Hz), 146.0, 133.6 (d, $^{3}J_{C,F}$ = 7.8 Hz), 132.8 (d, $^{4}J_{C,F}$ = 3.4 Hz), 113.8 (d, $^{2}J_{C,F}$ = 21.1 Hz), 98.6, 59.5, 51.5, 25.3, 14.6. ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -117.0. HRMS (ESI): *m/z* calcd for $C_{15}H_{18}FNO_2$+H^+: 264.1394 [M+H^+]; found: 264.1394.

Pyrrolidinyl acrylate 32:

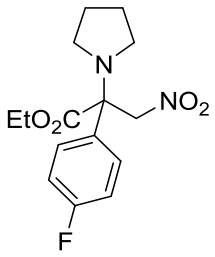


To a solution of H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10**-**R**) (76.0 mg, 0.188 mmol) in $CHCl_3$ (2 mL) was added nitroacrylate **7** (29.9 mg, 0.125 mmol) and the reaction mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography over basic aluminium oxide (EtOAc/pentane 1:1 – 2:1) to yield the title compound as a white foam (52 mg, 70%).

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.85 (broad s, 1H), 7.57 (broad s, 1H), 7.36 – 7.09 (m, 9H), 7.05 – 6.94 (m, 4H), 5.18 (d, *J* = 7.7 Hz, 1H), 5.14 (d, *J* = 7.9 Hz, 1H), 4.49 – 4.39 (m, 1H), 4.15 – 3.98 (m, 2H), 3.50 (s, 1H), 3.14 – 2.86 (m, 4H), 2.38 (ddt, *J* = 12.5, 6.3, 2.0 Hz, 1H), 2.28 (s, 3H), 2.13 (ddd, *J* = 16.0, 10.1, 5.1 Hz, 1H), 2.06 – 1.56 (m, 6H), 1.47 – 1.34 (m, 1H), 1.13 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 172.6, 169.5, 169.2, 161.6 (d, $^1J_{C,F}$ = 245.1 Hz), 144.7, 141.5, 135.9, 134.7, 133.6 (d, $^3J_{C,F}$ = 7.8 Hz), 132.3 (d, $^4J_{C,F}$ = 3.6 Hz), 129.4, 129.0, 128.7, 127.3, 126.7, 126.4, 114.2 (d, $^2J_{C,F}$ = 20.3 Hz), 100.7, 60.5, 59.7, 55.5, 47.4, 46.9, 43.4, 29.7, 26.7, 25.1, 22.2, 21.2, 14.6. ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -116.4. HRMS (ESI): *m/z* calcd for $C_{36}H_{40}FN_3O_4$+H^+: 598.3076 [*M*+H^+]; found: 598.3070.

7.8.4 Aza-Michael Adducts

Aza-Michael adduct 34:

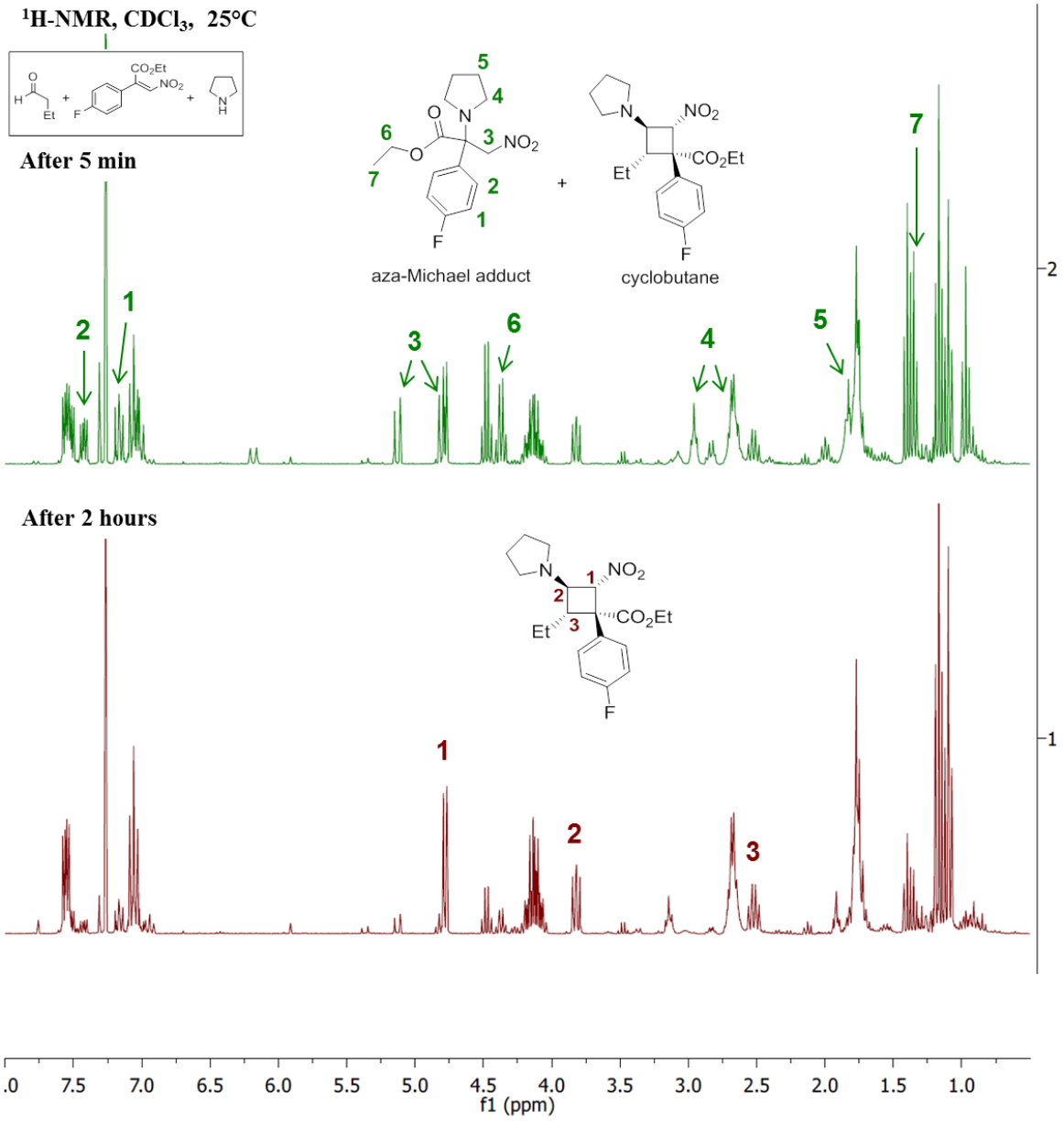


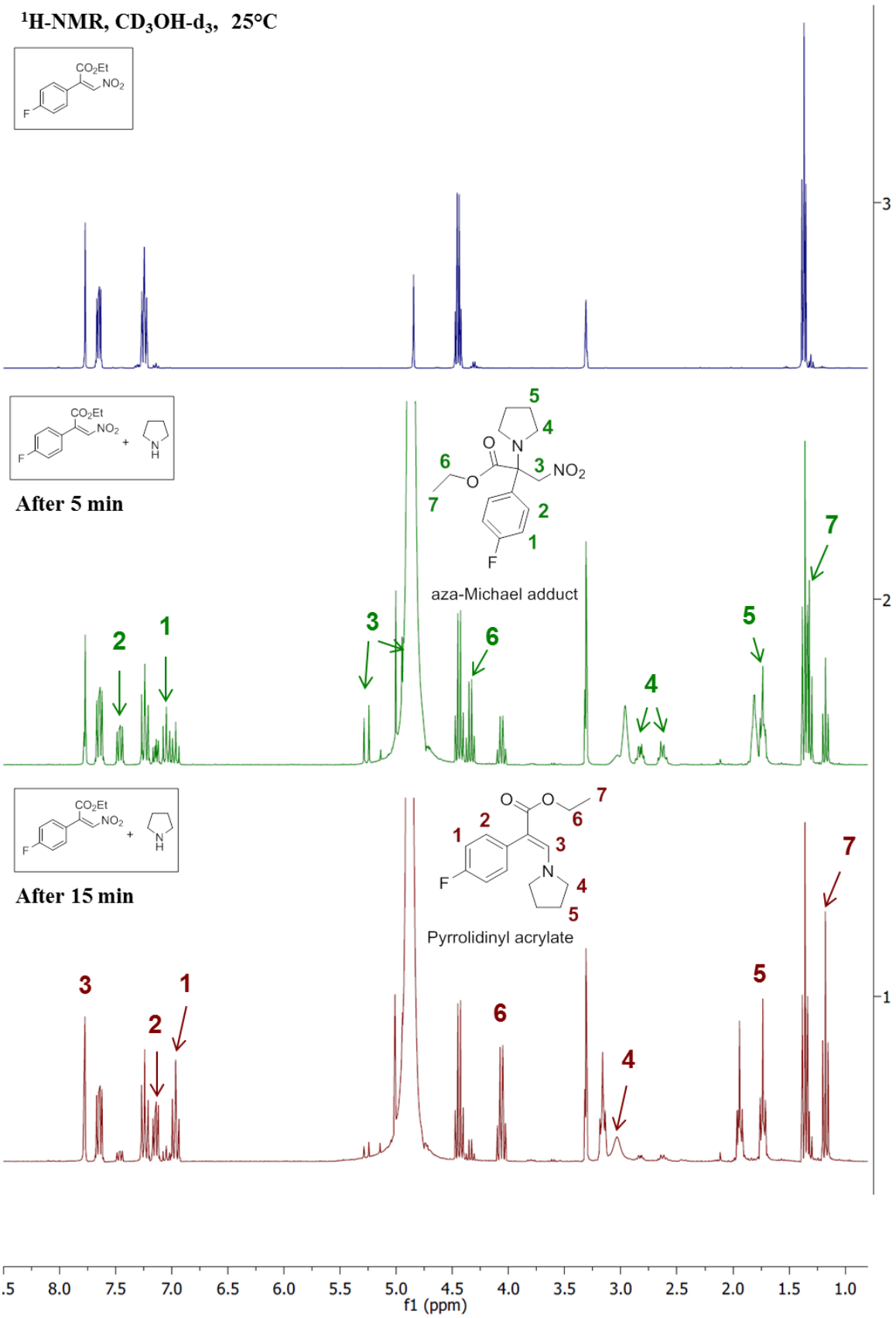
To a solution of nitroacrylate **7** (57 mg, 0.24 mmol) in $CDCl_3$ or CD_3OH-d_3 (600 μL) in a NMR tube was added pyrrolidine (33 μL, 0.40 mmol) and the reaction mixture was analyzed by NMR spectroscopy.

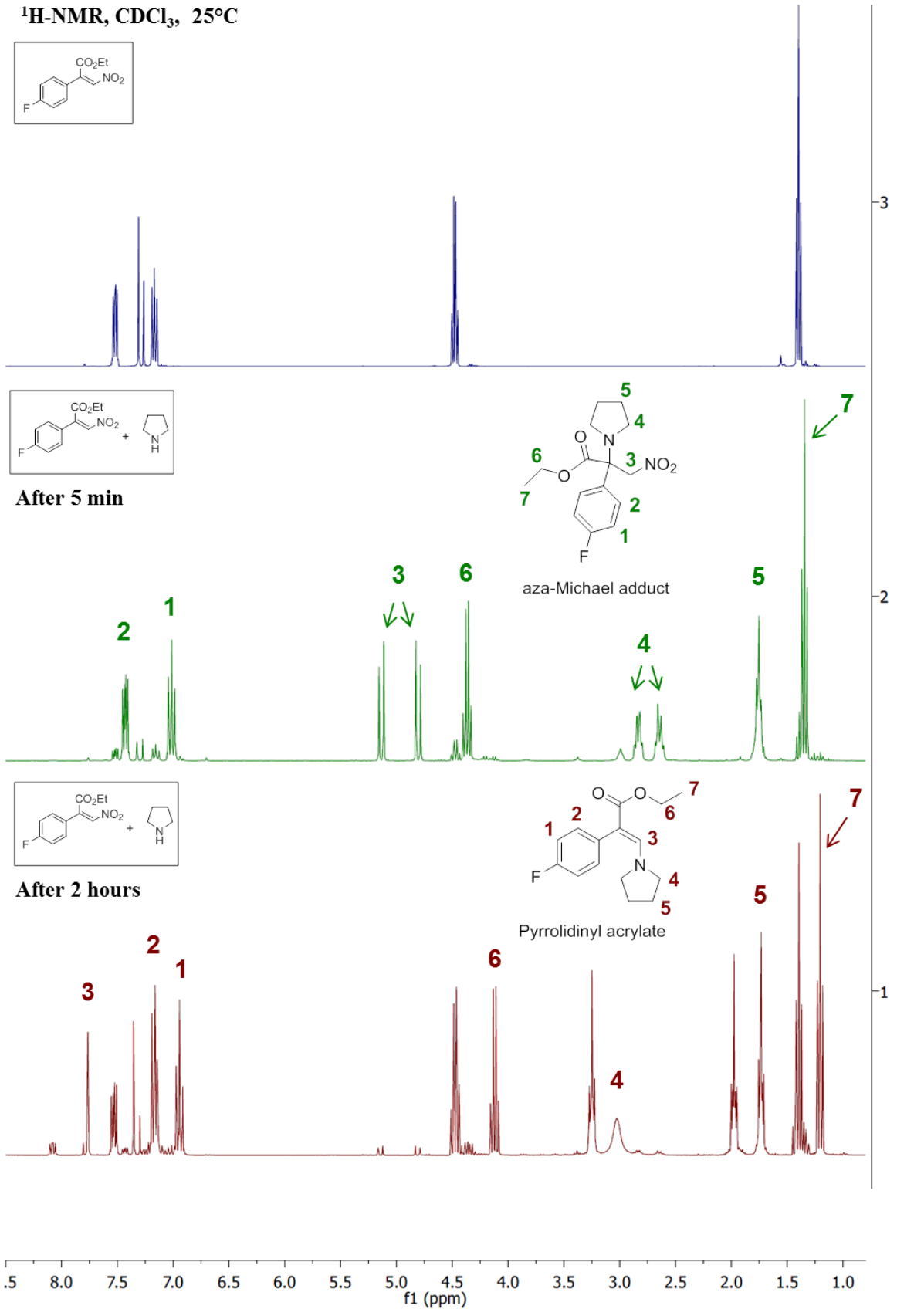
^{1}H NMR (300 MHz, CD_3OH-d_3, 25°C): δ = 7.51 – 7.42 (m, 2H), 7.18 – 7.10 (m, 2H), 5.27 (d, *J* = 12.9 Hz, 1H), 4.93 (d, *J* = 12.9 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 2.88 – 2.77 (m, 2H), 2.68 – 2.57 (m, 2H), 1.78 – 1.68 (m, 4H), 1.32 (t, *J* = 7.1 Hz, 3H). ^{19}F NMR (282 MHz, CD_3OH-d_3, 25°C): δ = -116.0.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.46 – 7.39 (m, 2H), 7.05 – 6.98 (m, 2H), 5.13 (d, *J* = 12.6 Hz, 1H), 4.81 (d, *J* = 12.6 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 2.90 – 2.79 (m, 2H), 2.70 – 2.60 (m, 2H), 1.83 – 1.68 (m, 4H), 1.35 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, 25°C): δ = 168.3, 162.4 (d, $^1J_{C,F}$ = 247.9 Hz), 132.7 (d, $^4J_{C,F}$ = 3.3 Hz), 129.9 (d, $^3J_{C,F}$ = 8.2 Hz), 115.0 (d, $^2J_{C,F}$ = 21.5 Hz), 81.9, 70.7, 61.5, 47.8, 24.2, 14.4. ^{19}F NMR (377 MHz, $CDCl_3$, 25°C): δ = -113.5.

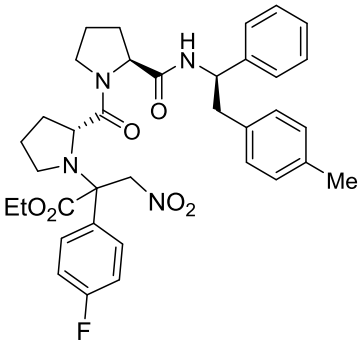
Stoichiometric reaction between butanal, nitroacrylate **7** and pyrrolidine (analogously to the procedure of cyclobutane **33**, Chapter 7.8.2):



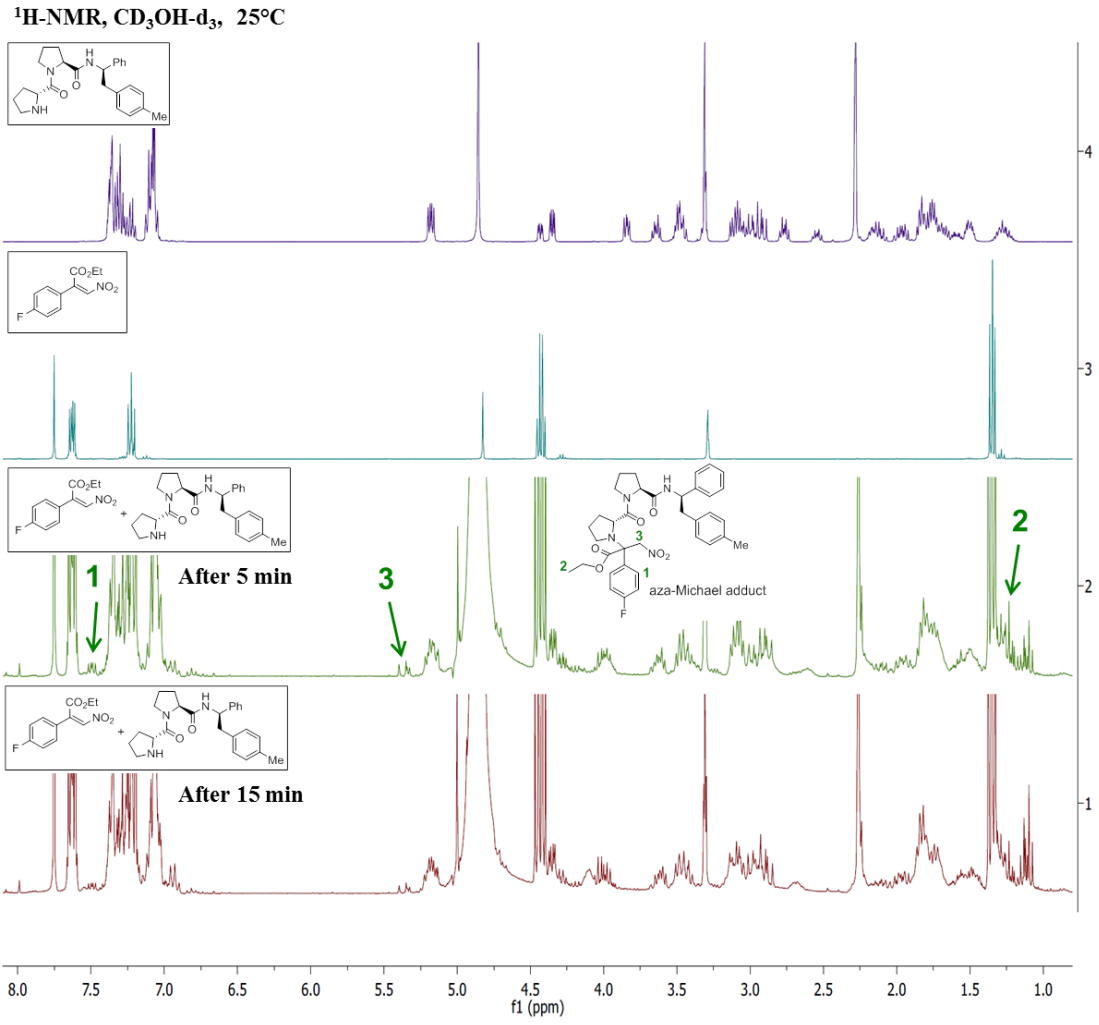

^{1}H-NMR, CD_3OH-d_3, 25°C
After 5 min
aza-Michael adduct
After 15 min
Pyrrolidinyl acrylate
f1 (ppm)

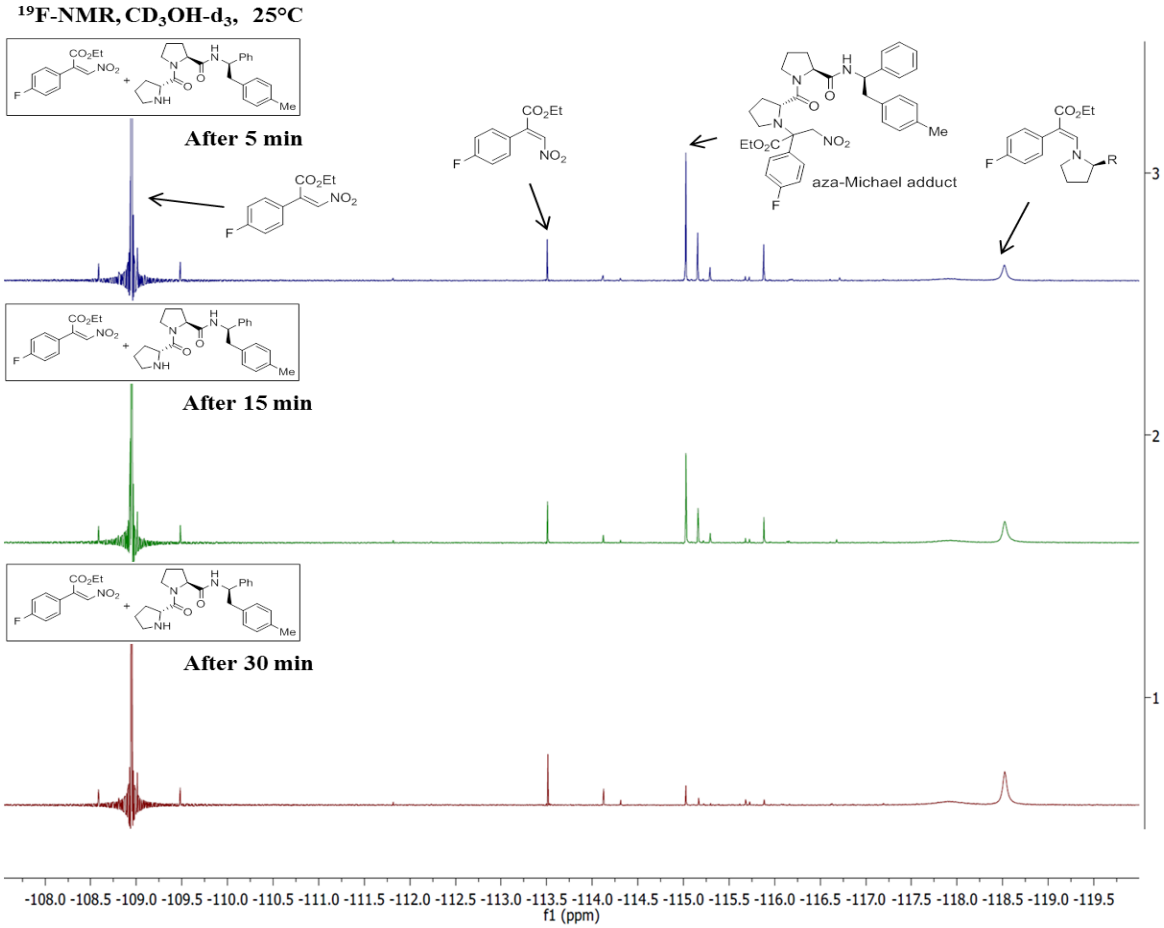

1H-NMR, CDCl3, 25°C
After 5 min
aza-Michael adduct
After 2 hours
Pyrrolidinyl acrylate
f1 (ppm)

Aza-Michael adduct 35:



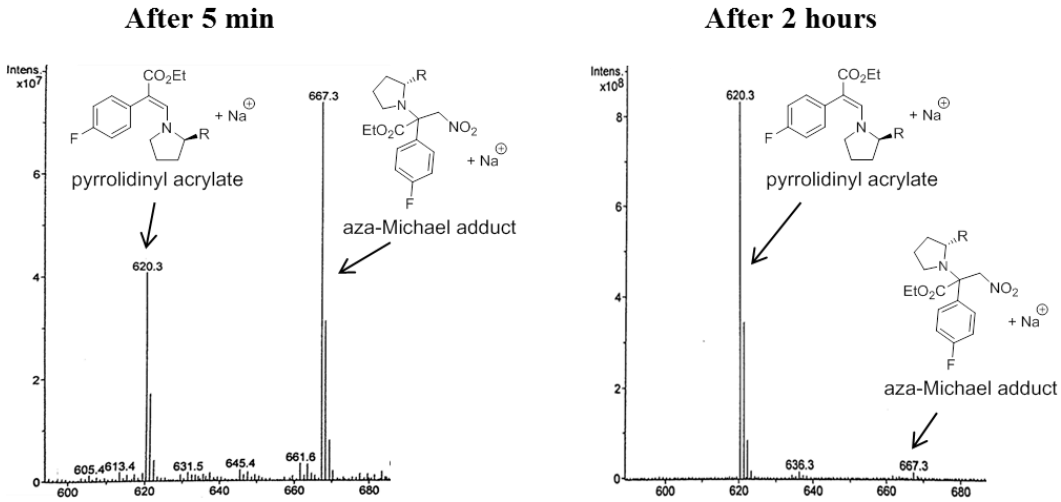
To a solution of nitroacrylate **7** (24 mg, 0.10 mmol) in CD_3OH-d_3 (600 μL) in a NMR tube was added H-D-Pro-Pro-NH-(*R*)-CH(Ph)CH_2-4-Me-C_6H_4 (**10-R**) (42 mg, 0.10 mmol) and the reaction mixture was analyzed by NMR spectroscopy.





ESI-MS analysis of aza-Michael adduct **35**:

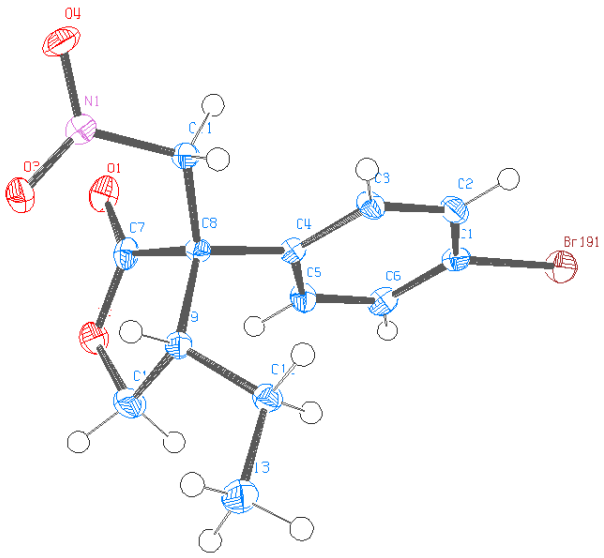
A small aliquot of the NMR sample was diluted with dry MeOH an injected into an ESI-MS spectrometer. In the resulting spectra the mass peaks corresponding to aza-Michael adduct **35** and pyrrolidinyl acrylate **32** were observed.



7.9 X-Ray Crystallographic Data

(3*S*,4*S*)-3-(4-Bromophenyl)-4-ethyl-3-(nitromethyl)dihydrofuran-2-(3*H*)-one (14):

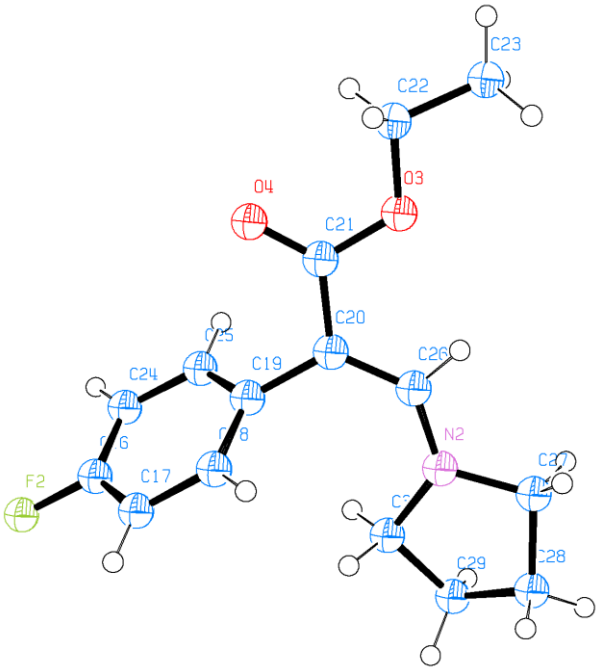
Formula $C_{13}H_{14}Br_1N_1O_4$, M = 328.16, F(000) = 332, colourless needle, size 0.030 · 0.090 · 0.230 mm^3, monoclinic, space group P 2_1, Z = 2, a = 6.0556(6) Å, b = 9.3123(9) Å, c = 11.7244(12) Å, α = 90°, β = 96.837(7)°, γ = 90°, V = 656.46(11) $Å^3$, $D_{calc.}$ = 1.660 Mg · m^{-3}. The crystal was measured on a Bruker Kappa Apex2 diffractometer at 123K using graphite-monochromated Mo K_α-radiation with λ = 0.71073 Å, Θ_{max} = 36.318°. Minimal/maximal transmission 0.75/0.91, μ = 3.140 mm^{-1}. The Apex2 suite has been used for data collection and integration. From a total of 23733 reflections, 6337 were independent (merging r = 0.051). From these, 4257 were considered as observed (I>2.0σ(I)) and were used to refine 173 parameters. The structure was solved by direct methods using the program SIR92. Least-squares refinement against F was carried out on all non-hydrogen atoms using the program CRYSTALS. R = 0.0363 (observed data), wR = 0.0522 (all data), GOF = 0.9730. Minimal/maximal residual electron density = -1.04/0.83 e $Å^{-3}$. Sheldrick weights were used to complete the refinement. Plots were produced using ORTEP3 for Windows. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center, the deposition number is CCDC 897771. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].



Ethyl (*E*)-2-(4-fluorophenyl)-3-(pyrrolidin-1-yl)acrylate (31):

Crystal Data for $C_{15}H_{18}FNO_2$ (*M* = 263.30 g/mol): F(000) = 560.0, crystal size 0.2 × 0.18 × 0.03 mm^3, triclinic, space group P-1 (no. 2), *a* = 10.6583(11) Å, *b* = 10.6600(11) Å, *c* = 13.0518(14) Å, α = 70.572(3)°, β = 77.754(4)°, γ = 74.717(3)°, *V* = 1336.3(2) $Å^3$, *Z* = 4, μ(MoKα) = 0.096 mm^{-1}, *Dcalc* = 1.309 g/cm^3, 19746 reflections measured (4° ≤ 2Θ ≤ 55.052°), 5906 unique (R_{int} = 0.0429, R_{sigma} = 0.0760) which were used in all calculations. The final R_1 was 0.0540 (I > 2σ(I)) and wR_2 was 0.1340 (all data).

Experimental: The crystal was measured on a ETH_LOC_ApexII Nonius_Mo diffractometer. The crystal was kept at 100.0(2) K during data collection. Radiation MoKα (λ = 0.71073). Using Olex2[234], the structure was solved with the XS[235] structure solution program using Direct Methods and refined with the XL[235] refinement package using Least Squares minimization.



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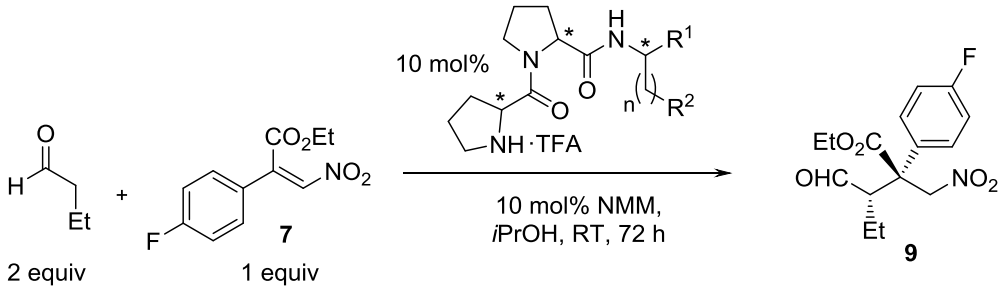
9

Appendix

Extended Table with Tested Peptidic Catalysts

Table 9-1 1,4-Addition reactions of butanal to (*Z*)-ethyl 2-(4-fluoro-phenyl)-3-nitroacrylate (**7**) catalyzed by peptides with the Pro-Pro motif.



	catalyst[a]	R^1	R^2	conv. [%][b]	d.r.[b]	ee [%][c]
1[d]	Proline	-	-	14	4.2:1	n.d.
2	H-D-Pro-Pro-His-NH_2	$CONH_2$	imidazole	25	3.0:1	n.d.
3	H-D-Pro-Pro-Ser-NH_2	$CONH_2$	OH	48	2.7:1	n.d.
4	H-Pro-Pro-Asp-NH_2	$CONH_2$	CO_2H	20	3.0:1	45[e]
5	H-D-Pro-Pro-Asp-NH_2	$CONH_2$	CO_2H	30	2.2:1	70
6	H-Pro-Pro-D-Asp-NH_2	$CONH_2$	CO_2H	37	3.0:1	n.d.
7	H-Pro-Pro-Asn-OH	CO_2H	$CONH_2$	24	2.5:1	42[e]
8	H-D-Pro-Pro-Asn-OH	CO_2H	$CONH_2$	40	2.1:1	58
9	H-Pro-Pro-D-Asn-OH	CO_2H	$CONH_2$	21	3.0:1	n.d.
10	H-Pro-Pro-Gln-OH	CO_2H	CH_2CONH_2	25	3.1:1	n.d.
11	H-D-Pro-Pro-Gln-OH	CO_2H	CH_2CONH_2	47	2.3:1	74
12	H-Pro-D-Pro-Gln-OH	CO_2H	CH_2CONH_2	33	1.8:1	n.d.
13	H-Pro-Pro-D-Gln-OH	CO_2H	CH_2CONH_2	14	3.1:1	n.d.
14	H-D-Pro-Pro-Gln-NH_2	$CONH_2$	CH_2CONH_2	50	4.1:1	82
15	H-Pro-Pro-Glu-NH_2	$CONH_2$	CH_2CO_2H	33	3.5:1	59[e]
16	H-D-Pro-Pro-Glu-NH_2 (**1**)	$CONH_2$	CH_2CO_2H	50	4.0:1	75
17	H-D-Pro-Pro-Glu-OH	CO_2H	CH_2CO_2H	44	2.9:1	74
18	H-Pro-D-Pro-Glu-NH_2	$CONH_2$	CH_2CO_2H	40	3.5:1	68[e]
19	H-Pro-Pro-D-Glu-NH_2	$CONH_2$	CH_2CO_2H	43	3.6:1	60[e]
20	H-D-Pro-Pro-Aad-NH_2	$CONH_2$	$(CH_2)_2CO_2H$	48	5.0:1	79

21	H-D-Pro-Pro-Api-NH_2	$CONH_2$	$(CH_2)_3CO_2H$	42	4.4:1	77
22	H-D-Pro-Pro-Asu-NH_2	$CONH_2$	$(CH_2)_4CO_2H$	51	4.4:1	76
23	H-D-Pro-Pro-Gly-OH	H	CO_2H	22	3.5:1	48[e]
24	H-D-Pro-Pro-β-Ala-OH	H	CO_2H	40	3.3:1	74
25	H-D-Pro-Pro-γ-Abu-OH	H	CH_2CO_2H	51	4.0:1	77
26	H-D-Pro-Pro-5-Ava-OH	H	$(CH_2)_2CO_2H$	72	4.1:1	77
27	H-D-Pro-Pro-6-Ahx-OH	H	$(CH_2)_3CO_2H$	68	3.7:1	77
28	H-D-Pro-Pro-γ-Abu-OMe	H	CH_2CO_2Me	72	4.3:1	80
29	H-D-Pro-Pro-5-Ava-OMe	H	$(CH_2)_2CO_2Me$	80	4.2:1	80
30	H-D-Pro-Pro-6-Ahx-OMe	H	$(CH_2)_3CO_2Me$	76	3.7:1	80
31	H-D-Pro-Pro-7-Ahp-OMe	H	$(CH_2)_4CO_2Me$	75	3.6:1	81
32	H-D-Pro-Pro-5-Ava-O*i*Pr	H	$(CH_2)_2CO_2iPr$	80	4.0:1	79
33	H-D-Pro-Pro-5-Ava-OPh	H	$(CH_2)_2CO_2Ph$	78	4.0:1	81
34	H-D-Pro-Pro-5-Ava-OBn	H	$(CH_2)_2CO_2Bn$	59	3.0:1	80
35	H-D-Pro-Pro-Aad(OMe)-OMe	CO_2Me	$(CH_2)_2CO_2Me$	87	3.6:1	80
36	H-D-Pro-Pro-β-*tert*-butyl-Ala-OMe	CO_2Me	*tert*-butyl	67	4.7:1	79
37	H-D-Pro-Pro-Phe-OMe	CO_2Me	Ph	86	4.7:1	85
38	H-D-Pro-Pro-D-Phe-OMe	CO_2Me	Ph	75	3.0:1	82
39	H-D-Pro-Pro-Phe-NHMe	CONHMe	Ph	42	3.3:1	74
40	H-D-Pro-Pro-Phe-$N(Me)_2$	$CON(Me)_2$	Ph	81	3.8:1	74
41	H-D-Pro-Pro-$NHCH_2CH_2Ph$	H	Ph	80	4.0:1	78
42	H-D-Pro-Pro-NHCH(Ph)CH_2Ph	Ph	Ph	82	4.2:1	90
43	H-D-Pro-Pro-NHCH(Ph)CH_2-4-Me-C_6H_4 (**10**)	Ph	4-Me-C_6H_4	80	4.6:1	90

Abbreviations: Aad = aminoadipic acid, Abu = aminobutyric acid, Ahp = aminoheptanoic acid, Ahx = aminohexanoic acid, Api = aminopimelic acid, Asu = aminosuberic acid, Ava = aminovaleric acid. [a] The peptidic catalysts were used as the TFA salts. [b] Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. [c] Determined by chiral-phase HPLC analysis. [d] The Reaction was performed without TFA and NMM. [e] The opposite enantiomer was formed.

Curriculum Vitae

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7/2009 – 1/2010	Master Thesis with Prof. Dr. H. Wennemers at the University of Basel, Switzerland. Title: *"Peptide als Katalysatoren für asymmetrische 1,4-Additionsreaktionen von Aldehyden an β,β-disubstituierte Nitroolefine".*
9/2008 – 5/2009	Master of Science in Chemistry at the University of Basel, Switzerland.
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Publications

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R. Kastl, Y. Arakawa, J. Duschmale, M. Wiesner, H. Wennemers, *"Peptide-catalyzed 1,4-Addition Reactions of Aldehydes to Nitroolefins" Chimia,* **2013**, *67,* 279-282.