Peptide Catalyzed Conjugate Addition Reactions of Aldehydes to Nitroolefins

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Mechanistic Investigations and Challenging Substrates

Inauguraldissertation

zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

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aus Basel (BS)

Basel 2013

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Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von

Prof. Dr. Helma Wennemers

Prof. Dr. Andreas Pfaltz

Basel, den 21. 05. 2013

Prof. Dr. Jörg Schibler

Dekan

Für Melanie
Du und ich – Hand in Hand

The present thesis was carried out under the guidance of Prof. Dr. Helma Wennemers during a period from April 2009 to May 2013 at the Department of Chemistry of the Faculty of Science at the University of Basel (4/09 – 11/11) and the Laboratory of Organic Chemistry at the ETH Zürich (12/11 – 5/13).

Parts of this thesis have been published or are prepared for publication:

"Adapting to Substrate Challenges: Peptides as Catalysts for Conjugate Addition Reactions of Aldehydes to α,β-Disubstituted Nitroolefins"
J. Duschmalé, H. Wennemers, *Chem. Eur. J.* **2012**, *18*, 1111–1120.

"Effects of Internal and External Carboxylic Acids on the Reaction Pathway of Organocatalytic 1,4-Addition Reactions between Aldehydes and Nitroolefins"
J. Duschmalé, J. Wiest, M. Wiesner, H. Wennemers, *Chem. Sci.* **2013**, *4*, 1312-1318.

"Peptide-catalyzed 1,4-Addition Reactions of Aldehydes to Nitroolefins"
R. Kastl, Y. Arakawa, J. Duschmalé, M. Wiesner, H. Wennemers, *Chimia* **2013**, *67*, 279-282.

"Stoichiometric Reactions of Enamines Derived from Diphenylprolinol Silyl Ethers with Nitro Olefins and Lessons for the Corresponding Organocatalytic Conversions – a Survey"
D. Seebach, X. Sun, M.-O. Ebert, W. B. Schweizer, N. Purkayastha, A. Beck, J. Duschmalé, H. Wennemers, T. Mukaiyama, M. Benohoud, Y. Hayashi, M. Reiher, *Helv. Chim. Acta* **2013**, *96*, 799-852.

"Organocatalytic Asymmetric Conjugate Addition of Aldehydes to Nitroolefins: Identification of Catalytic Intermediates and the Stereoselectivity-Determining Step by ESI-MS"
F. Bächle, J. Duschmalé, C. Ebner, A. Pfaltz, H. Wennemers, *manuscript in preparation*.

"Peptide Catalyzed 1,4-Addition Reactions of Aldehydes to Nitroolefins in Aqueous Media"
J. Duschmalé, S. Kohrt, H. Wennemers, *manuscript in preparation*.

Book chapter:
J. Duschmalé, Y. Arakawa, H. Wennemers, "Peptide Catalysis" in Science of Synthesis: Asymmetric Organocatalysis" *Vol. 2* (Ed: K. Maruoka), Georg Thieme Verlag, **2012**, Chapter 2.3.7.

Parts of this thesis have been presented at the following events:

Oral presentations

Fallmeeting of the Swiss Chemical Society
Zürich, Switzerland. September 13, 2012.
"Peptide Catalyzed 1,4-Addition Reactions of Aldehydes to Nitroolefins –Challenging Nitroolefins and Mechanistic Investigations"

6th Annual Workshop of the International Research Training Group CCROS
Basel, Switzerland, June 21 – 22, 2012.
"Peptide Catalyzed 1,4-Addition Reactions of Aldehydes to Nitroolefins"

Meeting of the COST Action on Organocatalysis (ORCA)
Marseille, France. March 20 – 30, 2012.
"Peptide Catalyzed Conjugate Addition Reactions of Aldehydes to α,β-Disubstituted Nitroolefins"

Meeting of the European Marie Curie Research-Training Network: REVCAT
Cologne, Germany. November 4 – 7, 2009.
"Peptide Catalyzed Conjugate Addition Reactions of Aldehydes to α,β-Disubstituted Nitroolefins"

Poster Presentations

32nd European Peptide Symposium
Athens, Greece. September 2 – 7, 2012
"Adapting to Substrate Requirements: Peptide Catalyzed 1,4-Addition Reactions of Aldehydes to α,β-Disubstituted Nitroolefins"

47th Euchem Conference on Stereochemistry, "Bürgenstock Conference"
Brunnen, Switzerland. April 29 – May 4, 2012.
"Peptide Catalyzed Conjugate Addition Reactions"

Fallmeeting of the Swiss Chemical Society
Lausanne, Switzerland. September 9, 2011.
"Peptide Catalyzed 1,4-Addition Reactions of Aldehydes to α,β-Disubstituted Nitroolefins"

10th German Peptide Symposium
Berlin, Germany. March 7 – 10, 2011.
"Peptide Catalyzed 1,4-Addition Reactions of Aldehydes to α,β-Disubstituted Nitroolefins"

Acknowledgements

First of all, I want to thank Prof. Dr. Helma Wennemers for giving me the opportunity to carry out this thesis within her research group and for allowing me to work on such a fascinating topic. I am grateful for her trust in me and my research as well as for the freedom to follow up on my own ideas. She has clearly been very influential both scientifically as well as personally.

Many thanks go to Prof. Dr. Andreas Pfaltz for agreeing to be the second examiner of this thesis.

I am also grateful to Prof. Dr. Wolf-Dietrich Woggon for chairing the examination.

In the course of this thesis, I was fortunate to work with several people in the context of precious collaborations. My sincere gratitude in that respect goes to Prof. Dr. Andreas Pfaltz, Dr. Christian Ebner and Florian Bächle, to PD Dr. Daniel Häussinger as well as to Prof. Dr. Dieter Seebach and Dr. Xiaoyu Sun.

Two talented Master's students, Johannes Wiest and Sonja Kohrt contributed to this work during their research projects in our group. Their enthusiastic lab-work is gratefully acknowledged. I also want to thank all students I had the pleasure to teach as a laboratory assistant. I thoroughly enjoyed teaching them and they taught me so much about myself.

The services of the scientific and technical staff of both institutions, the Department of Chemistry of the University of Basel as well as the Laboratory for Organic Chemistry at the ETH Zürich were of invaluable importance for the successful outcome of this thesis. Many thanks to all the helping hands that are too numerous to be named individually.

I am deeply grateful to Dr. Markus Wiesner for providing me with the tools of the trade for the hunt for 99% ee.

I also want to thank Dr. Yukihiro Arakawa, Robert Kastl and Claudio Grünenfelder for being great colleagues in the peptide catalysis team. Many thanks go to Claudio Grünenfelder and Christiane Siebler for proof-reading this manuscript. I cordially want to thank my past and present lab-mates Dr. Michael Kümin, Dr. Markus Wiesner, Dr. Roman Erdmann, Dr. Gaetano Angelici, Robert Kastl, Alba Mascarin and Christiane Siebler for their friendship and constant support. Special thanks also go to all past and present members of the Wennemers group for the good working atmosphere and the good times we shared. Furthermore, I have to

thank a good friend, Dr. Paolo Clerici, for all his support and friendship not only in the laboratory but also during football games and train rides.

I extend my gratitude to the University of Basel, the ETH Zürich as well as the Swiss National Science Foundation and Bachem AG for financially supporting this work.

I want to thank my family, especially my parents, my parents-in-law and my brother, for being there whenever I needed their advice and support or simply a helping hand.

My most heartfelt gratitude goes to Melanie. Her love and support especially during the more difficult periods of the last years was invaluable and crucial for the successful outcome of this thesis.

Finally I also want to thank Cohiba and Wanda since *"le bichon semble dès l'origine n'avoir eu aucune autre raison d'être que d'apporter la joie et le bonheur aux hommes"* (M. Houellebecq in "La carte et le territoire").

Abstract

In this thesis mechanistic investigations into the peptide catalyzed conjugate addition reaction between aldehydes and nitroolefins are described. Additionally, the extension of this reaction to special reaction conditions as well as challenging substrate combinations is presented. The tripeptide H-D-Pro-Pro-Glu-NH_2 is an excellent catalyst for conjugate addition reactions between aldehydes and β-nitroolefins. Mechanistic investigations comparing the tripeptide H-D-Pro-Pro-Glu-NH_2 with its methyl ester analogue H-D-Pro-Pro-Glu(OCH_3)-NH_2 revealed that the reaction pathway and thus the rate determining steps of the reaction depend on the presence or absence of a suitably positioned carboxylic acid moiety within the catalyst. These findings have important implications for future catalyst design and optimization and offer an explanation why the peptide H-D-Pro-Pro-Glu-NH_2 bearing an intramolecular carboxylic acid moiety is such a successful catalyst for this reaction. Further mechanistic studies were directed at the elucidation whether an enamine or an enol is the active nucleophile in the peptide catalyzed conjugate addition reactions. ESI-MS back reaction screening using mass-labeled pseudo-enantiomeric substrate mixtures revealed that in the presence of several peptides of the type Pro-Pro-Xaa (Xaa = variable amino acid bearing a carboxylic acid group) the selectivity of the attack of the enamine onto the nitroolefin equals the selectivity of the preparative reaction. Thus, an enamine is involved in the selectivity determining step of reactions in the presence of such peptidic catalysts.

In the second part of this thesis, amphiphilic analogues of H-D-Pro-Pro-Glu-NH_2 were examined as catalysts for conjugate addition reactions between aldehydes and nitroolefins in aqueous reaction media. Introduction of a hydrophobic alkyl chain to the C-terminus gave the peptide H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ as an excellent catalyst. This amphiphilic peptide serves as both, catalyst for the reaction as well as a detergent stabilizing an emulsion of the substrates in the aqueous environment.

Finally, peptides of the type Pro-Pro-Xaa were examined as catalysts for conjugate addition reactions between aldehydes and α,β-disubstituted nitroolefins, a much more challenging and far less examined substrate class than their β-mono-substituted counterparts. The testing of a small collection of peptides led to the identification of H-Pro-Pro-D-Gln-OH and H-Pro-Pro-Asn-OH as effective catalysts allowing for addition reactions between different combinations of aldehydes and α,β-disubstituted nitroolefins. The resulting γ-nitroaldehydes bearing three consecutive stereogenic centres were obtained in good yields and excellent stereoselectivities.

Chiral pyrrolidines as well as fully substituted γ-butyrolactams and γ-amino acids were easily accessible from the γ-nitroaldehydes.

The results described within this thesis not only highlight the value of peptides of the type Pro-Pro-Xaa as successful catalysts in enamine catalysis but might also pave the way for future research into addition reactions of carbonyl compounds to other challenging electrophiles or the application of peptidic catalysts under physiological conditions in a cellular environment.

Zusammenfassung

In der vorliegenden Arbeit werden mechanistische Untersuchungen zu peptidkatalysierten 1,4-Additionen von Aldehyden an Nitroolefine beschrieben. Zusätzlich wird die Erweiterung der Reaktion auf spezielle Reaktionsbedingungen sowie schwierige Substratkombinationen dargestellt. Das Tripeptid H-D-Pro-Pro-Glu-NH_2 ist ein exzellenter Katalysator für 1,4-Additionsreaktionen zwischen Aldehyden und β-Nitroolefinen. Mechanistische Untersuchungen, in welchen das Peptid H-D-Pro-Pro-Glu-NH_2 mit seinem Methylester Analog H-D-Pro-Pro-Glu(OCH_3)-NH_2 verglichen wurde, zeigten dass der Reaktionsweg sowie die geschwindigkeitsbestimmenden Schritte von der Gegenwart einer günstig positionierten Carbonsäuregruppe innerhalb der Katalysatorstruktur abhängen. Diese Erkenntnisse haben wichtige Auswirkungen auf die zukünftige Katalysatorenentwicklung und erklären weshalb das Peptid H-D-Pro-Pro-Glu-NH_2, welches eine intramolekulare Säuregruppe trägt, ein derart erfolgreicher Katalysator ist. Weitere mechanistische Arbeiten dienten der Aufklärung ob ein Enamin oder ein Enol als aktives Nukleophil in 1,4-Additionsreaktionen zwischen Aldehyden und Nitroolefinen involviert ist. ESI-MS Studien der Rückreaktion mittels pseudo-enantiomerer Substrate ergaben dass in Gegenwart von Peptiden des Typs Pro-Pro-Xaa (Xaa = variable säurehaltige Aminosäure) die Selektivität der Reaktion zwischen Enamin und Nitroolefin der Selektivität der präparativen Reaktion entspricht. Dies zeigt dass ein Enamin im Selektivitätsbestimmenden Schritt involviert ist.

Im zweiten Teil dieser Arbeit wurden amphiphile Analoga des Peptids H-D-Pro-Pro-Glu-NH_2 als Katalysatoren für 1,4-Additionsreaktionen zwischen Aldehyden und Nitroolefinen in einem wässrigen Reaktionsmedium untersucht. Einführung einer hydrophoben Alkylkette ergab den erfolgreichen Katalysator H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$. Dabei dient dieses

amphiphile Peptid sowohl als Katalysator für die Reaktion als auch als Detergens, welches eine Emulsion der Substrate im wässrigen Medium stabilisiert.

Letztendlich wurden Peptide der Art Pro-Pro-Xaa als Katalysatoren untersucht für 1,4-Additionen zwischen Aldehyden und α,β-disubstituierten Nitroolefinen, eine bedeutend anspruchsvollere und viel seltener benutzte Substratklasse als β-mono-substituierte Analoga. Aus einer kleinen Katalysatorensammlung wurden die beiden Peptide H-Pro-Pro-D-Gln-OH sowie H-Pro-Pro-Asn-OH identifiziert, welche effektive Katalysatoren für Additionsreaktionen verschiedener Kombinationen von Aldehyden und α,β-disubstituierten Nitroolefinen darstellen. Die resultierenden γ-Nitroaldehyde mit drei aufeinanderfolgenden Stereozentren wurden in guten Ausbeuten und hervorragenden Stereoselektivitäten erhalten und konnten zu chiralen Pyrrolidinen sowie γ-Butyrolaktonen und γ-Aminosäuren umgesetzt werden.

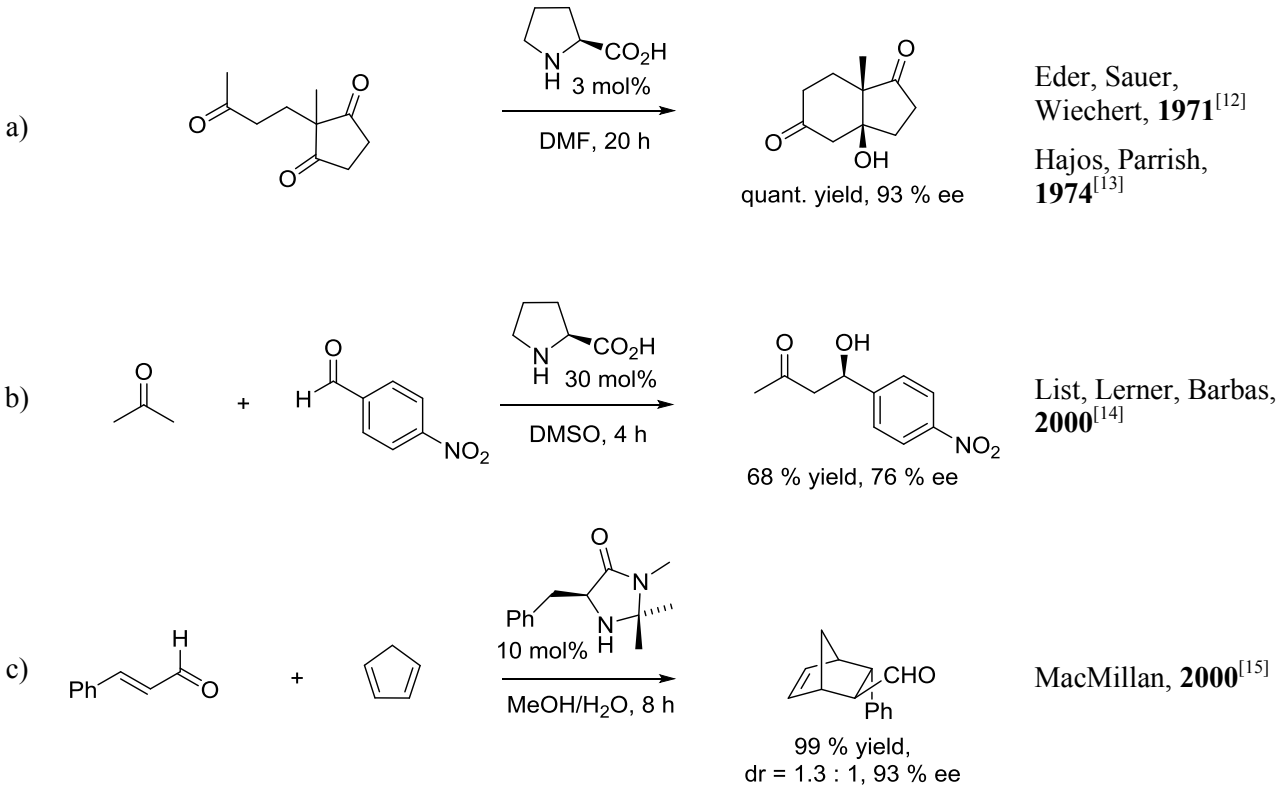
Die vorliegenden Resultate dieser Arbeit heben nicht nur hervor dass Peptide der Art Pro-Pro-Xaa wertvolle Enaminkatalysatoren darstellen sondern ebnen ebenfalls den Weg für die zukünftige Erforschung von Additionsreaktionen zwischen Carbonylverbindungen und anderen anspruchsvollen Elektrophilen oder die Anwendung von Peptidkatalysatoren unter physiologischen Bedingungen innerhalb einer Zelle.

Contents

1 Introduction

1.1 Organocatalysis

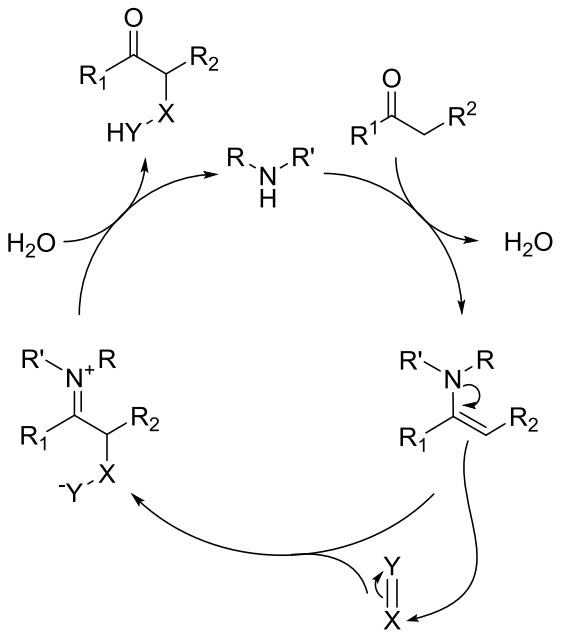
Organocatalysis, the catalytic application of small organic molecules in the absence of metal centres, has become a well recognized concept and for more than a decade now, a highly active field of research.[1-6] Additionally, in recent years, reactions catalyzed by purely organic molecules have found increasing application in the synthesis of complex molecules.[7-8] As a consequence, today, organocatalysis is undisputedly one of the pillars of asymmetric catalysis together with transition metal catalysis and biocatalysis.[4] While this "gold rush"[5] in organocatalysis is a relatively new phenomenon, the origins of the field date back about a century. In one of the earliest examples, Bredig reported in the early 20th century that in the presence of natural alkaloids a measurable enantiomeric excess could be observed in the products of reactions such as decarboxylation of camphorsulfonic acid[9] or the hydrocyanation of benzaldehyde.[10] Over the succeeding decades multiple examples of catalytic applications of small organic molecules were reported. Among these the enantioselective addition of methanol to methyl phenyl ketene was investigated by Pracejus in 1960 where in the presence of a quinine derivative the first synthetically useful selectivity (74% *ee*) was obtained.[11] A second famous example is the Hajos-Parrish-Eder-Sauer-Wiechert reaction (Scheme 1-1, a), where proline was used as a catalyst in an intramolecular aldol reaction providing an enantioselective route to the Wieland-Miescher ketone (93% *ee*).[12-13] Despite these early successes, where remarkable levels of enantioselectivity were achieved, research activity into catalysis by small organic molecules remained relatively limited until the year 2000, when two highly influential articles were published nearly simultaneously. In the first article, intrigued by class II aldolases and catalytic antibodies, List, Lerner and Barbas re-examined the proline catalyzed aldol reaction (Scheme 1-1, b).[14] The second article by MacMillan reported on chiral imidazolidinone catalysts for asymmetric Diels-Alder reactions (Scheme 1-1, c).[15] These two articles triggered an explosion of research activity leading in less than a decade to the diversity of concepts, catalysts and organocatalytic processes that are at the disposal of the synthetic organic chemist today.



Scheme 1-1 Early examples of organocatalytic transformations.

1.2 Asymmetric Enamine Catalysis

Organocatalysis can be classified into four distinct areas according to the mechanism by which the catalyst activates a substrate: (1) Lewis base catalysis, where the catalytic cycle is initiated by a nucleophilic addition of the catalyst to an electrophilic substrate. Examples include carbene as well as primary or secondary amine catalysts. (2) On the contrary, in Lewis acid catalysis, nucleophilic substrates are activated by reaction with an electrophilic catalyst (e.g. silyl or phosphonium cation-based catalysts). (3) Brønsted base catalysis includes catalysts that initiate a catalytic cycle by deprotonation. Tertiary amines such as cinchona alkaloids are the most prominent examples. (4) Finally, in Brønsted acid catalysis, the catalytic cycles are initiated by a (partial) protonation. Important catalyst classes are (thio)ureas and chiral phosphoric acids.[2-3] In addition, bi- and multifunctional catalysts have been developed that combine two or more of these properties within one molecule.[16] Within Lewis base catalysis one of the most prominent concepts is the enamine activation of carbonyl compounds, where a ketone or an aldehyde reacts with a secondary amine catalyst to form an enamine intermediate (Scheme 1-2). Such an enamine is a nucleophilic species which is



Scheme 1-2 General principle of enamine activation of carbonyl compounds.

activated towards reaction with electrophiles. The resulting adducts are then hydrolyzed to the desired reaction products and the free amine.[2,17] Enamines were already investigated by Storck as early as in the 1950ies as a base free way of achieving carbonyl enolate reactivity.[18-19] In these studies, preformed enamines were used as stoichiometric nucleophiles in α-functionalizations of carbonyl compounds. A first catalytic example was the proline catalyzed Robinson annulation in the Hajos-Parrish-Eder-Sauer-Wiechert reaction mentioned in chapter 1.1.[12-13] After the pioneering work by List, Lerner and Barbas on proline catalyzed intermolecular aldol reactions in the year 2000[14] and the successive explosion of research interest in organocatalysis, the general concept of enamine catalysis was extensively applied to a wide variety of α-functionalizations of carbonyl compounds such as aldol reactions, α-aminations, α-oxidations and α-halogenations, Mannich reactions as well as Michael and hetero Michael reactions (Figure 1-1).[17] Furthermore, the nucleophilic attack of enamines to electrophiles has often been used as the initializing step of various elegant cascade and domino processes.[20]

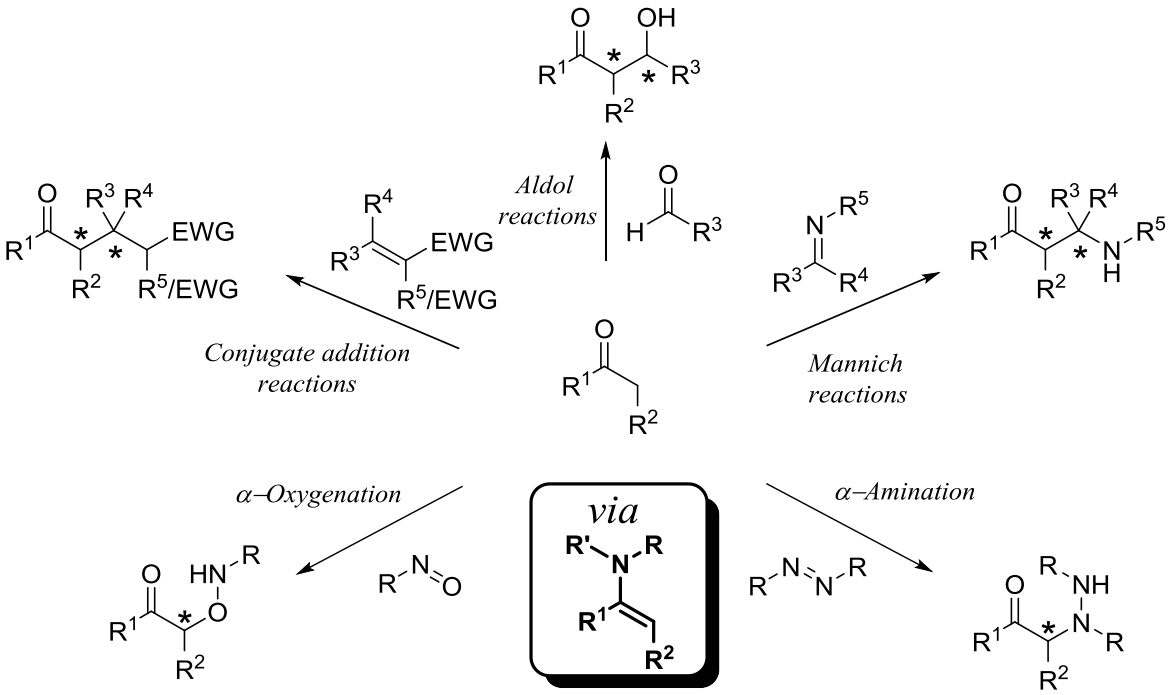
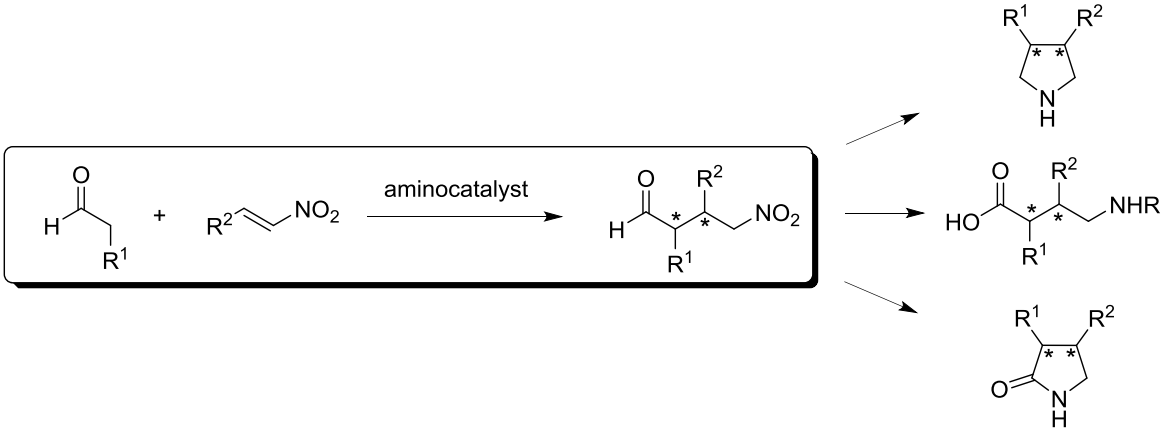


Figure 1-1 Some representative examples of the diverse chemistry accessible with enamine activation of carbonyl compounds by a secondary amine organocatalyst.

1.3 Conjugate Addition Reactions of Aldehydes to Nitroolefins

Conjugate addition reactions of carbon nucleophiles to electron deficient double bonds are widely used in organic synthesis in general[21] and in organocatalytic processes with enamine activation in particular.[22-24] In this context, many different Michael acceptors have been used such as nitroalkenes, enones, vinyl sulfones, maleimides or alkylidene malonates.[22-24] Nitroalkenes are particularly attractive Michael acceptors due to their high electrophilicity[25] and the synthetic utility of the nitro group.[26] The conjugate addition of aldehydes to nitroolefins provides γ-nitroaldehydes that are versatile intermediates for the synthesis of, for example, chiral γ-butyrolactones,[27] pyrrolidines,[28] or γ-amino acids[29] (Scheme 1-3). As a consequence, the conjugate addition of aldehydes to nitroolefins is one of the most studied transformations in which the concept of enamine activation has been applied. After initial studies focusing on organocatalytic conjugate additions of ketones to nitroolefins by List[30] and Enders,[31] it was Barbas in 2001 who reported the first example of the addition of aldehydes to nitroolefins.[32] In the presence of a morpholine functionalised pyrrolidine



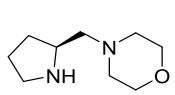
Scheme 1-3 Conjugate addition reaction of aldehydes to nitroolefins.

catalyst, his group was able to isolate the conjugate addition products of aldehydes with different aromatic β-substituted nitroolefins in selectivities of up to 78 % *ee*. Following these ground-breaking initial studies a wide variety of primary and secondary amine catalysts have been developed for this reaction (Figure 1-2).[27-29,32-89] However, most of these catalysts suffer from one or more general drawbacks: (1) high catalyst loadings (>5mol%) are often required to obtain good conversions, (2) a high excess of aldehyde has to be employed due to side reactions such as *homo*-aldol additions and (3) the substrate scope is often limited to aromatic β-nitroalkenes. The best catalysts that overcome these challenges are arguably a prolinol silyl ether catalyst first used in this reaction by Hayashi,[39] its ionic liquid functionalized analogue investigated by Lombardo[63] and the tripeptide H-D-Pro-Pro-Glu-NH_2 developed by Wennemers (Figure 1-2).[90-94]

1.4 Peptide Catalysis

Nature uses short peptides for a variety of functions, for example as hormones, neurotransmitters or toxins.[95] Despite these many crucial roles, peptides are not known as natural catalysts. Yet they consist of the same amino acid building blocks as enzymes, nature's catalysts. Thus, and also because of the large structural and functional diversity of their building blocks together with their modular nature, peptides are interesting candidates for the development of organocatalysts.[96-98] In the late 1970ies, Oku and Inoue investigated cyclic dipeptides (diketopiperazines) as catalysts in the hydrocyanation of benzaldehydes (Scheme 1-4, a).[99-101] At approximately the same time, Julià and Colonna reported the use of

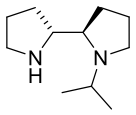
Barbas **2001**[32]



20 mol%
10 eq of aldehyde

42 – 96% yield
dr = 6:1 – 49:1
56 – 78 % *ee*

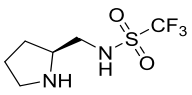
Alexakis **2002**[33]



15 mol%
10 eq of aldehyde

71 – 99% yield
dr = 3:1 – 24:1
61 – 88 % *ee*

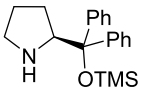
Wang **2005**[38]



20 mol%
10 eq of aldehyde

67 – 96% yield
dr = 22:1 – 50:1
89 – 99 % *ee*

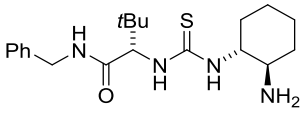
Hayashi **2005**[39]



10 mol%
10 eq of aldehyde

52 - 85% yield
dr = 13:1 – 24:1
99 % *ee*

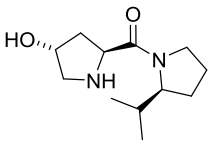
Jacobsen **2006**[41]



20 mol%
2 eq of aldehyde

81 - 94% yield
dr = 3:1 – >50:1
96 - 99 % *ee*

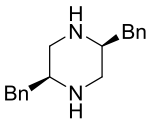
Palomo **2006**[27]



5 – 10 mol%
1.5 – 2 eq of aldehyde

70 – 90% yield
dr = 9:1 – >99:1
92 – >99 % *ee*

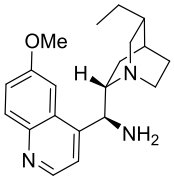
Barros **2007**[52]



10 mol%
10 eq of aldehyde

57 – 78% yield
dr = 4:1 – 32:1
68 – 85 % *ee*

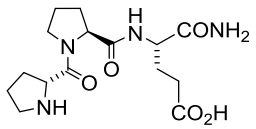
Connon **2007**[53]



10 - 20 mol%
5 eq of aldehyde

76 – 97% yield
dr = 2:1 – >60:1
65 – 95 % *ee*

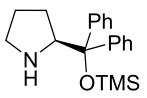
Wennemers **2008**[90-94]



0.1 - 1 mol%
1 eq of aldehyde

84 – 99% yield
dr = 6:1 – 99:1
90 – 98 % *ee*

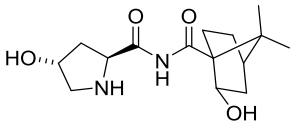
Ma **2008**[58]



0.5 - 2 mol%
2 eq of aldehyde

81 - 96% yield
dr = 12:1 – 49:1
> 99 % *ee*

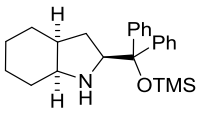
Chen **2009**[61]



5 mol%
3 eq of aldehyde

42 - 92% yield
dr = 9:1 – 99:1
79 - 99 % *ee*

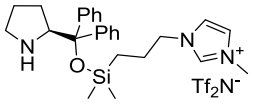
Lu **2009**[62]



10 mol%
10 eq of aldehyde

65 – 95% yield
dr = 3:1 – 99:1
99 % *ee*

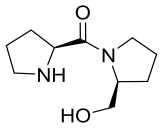
Lombardo **2009**[63]



0.25 – 5 mol%
1.2 – 2 eq of aldehyde

43 – 99% yield
dr = 4:1 – 49:1
95 – >99 % *ee*

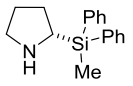
Gong **2010**[68]



1.5 - 10 mol%
3 eq of aldehyde

71 – 97% yield
dr = 9:1 – 99:1
77 - 97 % *ee*

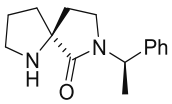
Bolm **2010**[71]



10 mol%
10 eq of aldehyde

87 – 99% yield
dr = 19:1 – 32:1
78 - 90 % *ee*

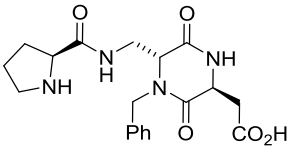
Kelleher **2010**[72]



5 mol%
1.5 eq of aldehyde

22 – 94% yield
dr = 2:1 – 9:1
4 – 82 % *ee*

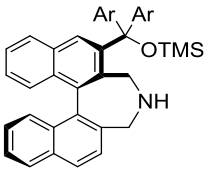
Piarulli **2011**[84]



5 mol%
3 eq of aldehyde

40 – 99% yield
dr = 7:1 – 99:1
92 – 98 % *ee*

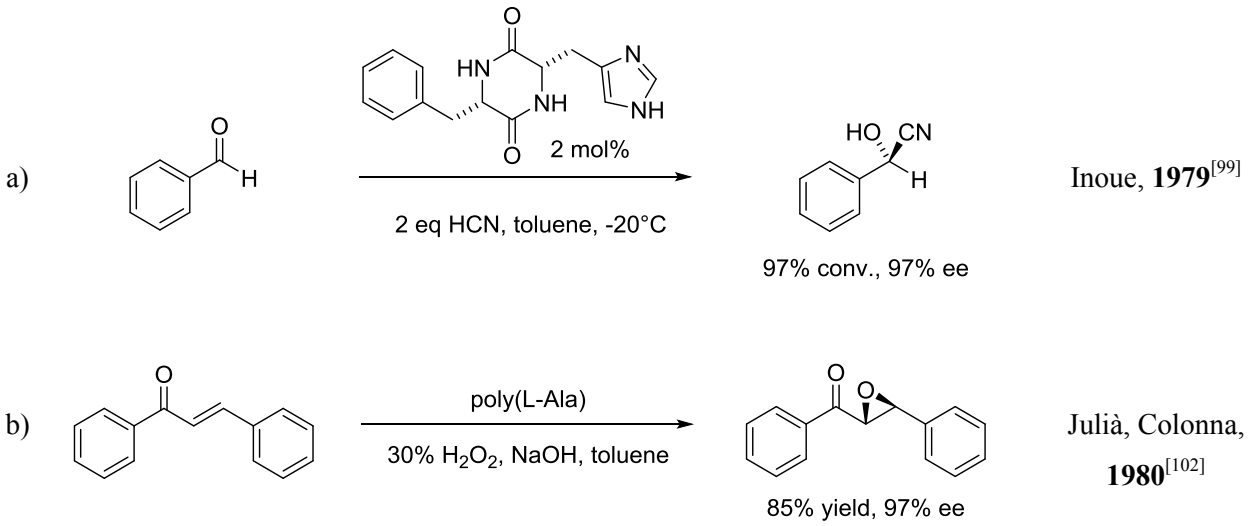
Lu **2012**[87]



10 mol%
10 eq of aldehyde

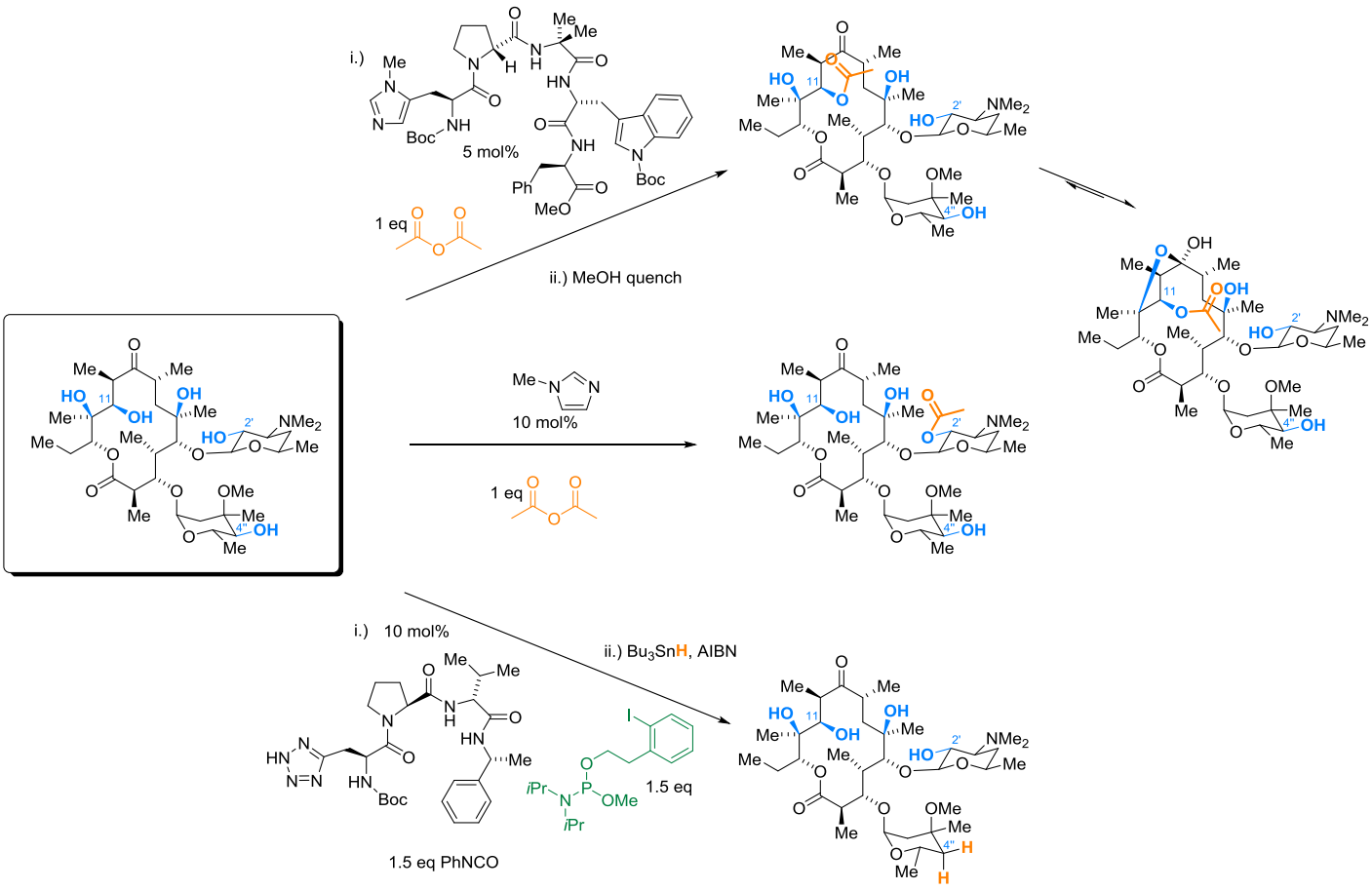
85 – 95% yield
dr = 3:1 – 99:1
86 – >99 % *ee*

Figure 1-2 Selected examples of organocatalysts for the conjugate addition reaction of aldehydes to nitroolefins.



Scheme 1-4 Early examples of asymmetric peptide catalysis: a) the hydrocyanation of benzaldehyde, b) the Julià-Colonna epoxidation.

poly(alanine) and poly(leucine) as catalysts for the enantioselective epoxidation of chalcones with basic hydrogen peroxide providing the desired products with excellent enantioselectivities (Scheme 1-4, b).[102-103] After the year 2000 the emerging concept of organocatalysis with the success of single amino acids as catalysts also led to a dramatically increased interest in the catalytic properties of peptides. Today, a wide variety of catalytically active short peptides have been developed for reactions as diverse as oxidation, acylation, phosphorylation and sulfonylation, the aldol and the conjugate addition reaction, the Stetter reaction or halogenation.[96-98] As a consequence, peptide catalysis is considered as an important area within organocatalysis. In these studies, it was often found that peptidic catalysts not only allow for the synthesis of important chiral molecules with high enantioselectivity but also provide solutions to challenges such as regio- or site selectivity that are often difficult to address with other catalysts. Among the most impressive examples in that respect are peptide catalyzed site selective manipulations of erythromycin A developed in the research group of Miller (Scheme 1-5).[104-105] While in enantioselective catalysis the challenge is to discriminate between two diastereomeric transition states, achieving site selectivity includes the inversion of the intrinsic reactivity of a substrate, an even more challenging task.[104] Erythromycin A is a complex natural product bearing three free secondary hydroxy groups with the intrinsic reactivity order towards acylation of C2'-OH > C4''-OH > C11-OH. Indeed, in the reaction of erythromycin A with acetic anhydride in the presence of the achiral catalyst *N*-methyl imidazole the C2' and C4'' positions are acetylated preferentially. However, screening a collection of peptides revealed a catalyst which in the



Scheme 1-5 Site selective manipulations of erythromycin A mediated by peptidic catalysts.[104-105]

same reaction allowed for acetylation to take place preferentially at C11.[104] Similarly, with the use of a peptidic tetrazole catalyst C4''-deoxy-erythromycin A can be prepared selectively in an elegant site selective phosphitylation radical deoxygenation sequence.[105]

Furthermore, given the success of single amino acids, the building blocks of peptides, in enamine catalysis, short peptides of 2-4 amino acids have been used regularly as enamine catalysts.[106-120] Among the most successful peptididic enamine catalysts are the peptides of the type Pro-Pro-Xaa, where the turn inducing Pro-Pro motive is combined with an arbitrary C-terminal amino acid bearing a carboxylic acid group (see section 1.5).

1.5 Peptides of the Type Pro-Pro-Xaa as Catalysts in Conjugate Addition Reactions between Aldehydes and Nitroolefins

Due to the high degree of rotational freedom even in short peptides, prediction of the conformational properties and thereby the spatial arrangement of functional groups of potential peptidic catalysts is a challenge complicating their rational design. Thus, smart

combinatorial methods are attractive for the discovery of peptide catalysts.[121-123] The modular nature of linearly linked amino acid building blocks combined with the established synthetic protocols in peptide synthesis (solid phase synthesis), allow for straightforward generation of diverse libraries by the split-and-mix method.[121-123] Such one-bead-one-compound libraries combined with a cleverly designed screening method and an elegant way of identifying active species allow for the discovery of very potent peptide catalysts.[122]

Using the concept of catalyst substrate co-immobilisation (Figure 1-3, a)[124] the two tripeptides H-Pro-D-Ala-D-Asp-NH_2 and H-Pro-Pro-Asp-NH_2 were identified as excellent catalysts for aldol reactions of acetone with aromatic aldehydes.[125-126] The corresponding aldol products were obtained in high enantioselectivities using as little as 1 mol% of the peptidic catalyst. Interestingly, in a lowest energy structure obtained by molecular modelling studies the distance between the secondary amine and the carboxylic acid of H-Pro-Pro-Asp-NH_2 is approximately 3 Å greater than in proline. Inspired by this fact and hypothesising that these 3 Å might provide enough space for two additional atoms (Figure 1-3, b), H-Pro-Pro-Asp-NH_2 and closely related peptides Pro-Pro-Xaa, combining the Pro-Pro motif with a C-terminal amino acid containing a carboxylic acid moiety (Xaa), were examined as catalysts for conjugate addition reactions. Indeed, H-Pro-Pro-Asp-NH_2 and in particular its diastereoisomer H-D-Pro-Pro-Asp-NH_2 proved to be very good catalysts for the conjugate addition reaction of various aldehydes to aromatic as well as aliphatic β-substituted nitroolefins providing the corresponding products in excellent yields, diastereo- and enantioselectivities.[90] Further studies revealed that the closely related analogue H-D-Pro-Pro-Glu-NH_2 is an even better catalyst for conjugate additions between aldehydes and nitroolefins.[92,94] Careful investigations revealed that within the catalyst structure the turn inducing D-Pro-Pro-motif as well as the C-terminal amide and the carboxylic acid moiety in the side chain are crucial for effective catalysis.[92] Kinetic studies showed, that the reaction rate depends more strongly on the concentration of the nitroolefin than on the concentration of the aldehyde. Furthermore, water was found to slow down the reaction.[93] Accordingly, highest reaction rates were observed when an excess of the nitroolefin rather than the aldehyde as well as dried solvents and reagents were used. Under these conditions the catalyst loading could be reduced to as little as 0.1 mol%, the lowest catalyst loading achieved thus far in enamine catalysis (Scheme 1-6, a).[93,127] In addition, in the presence of the peptide H-D-Pro-Pro-Glu-NH_2 nitroethylene, a challenging nitroolefin due to its high reactivity and disposition to polymerisation, is accepted as a substrate in the reaction with aldehydes

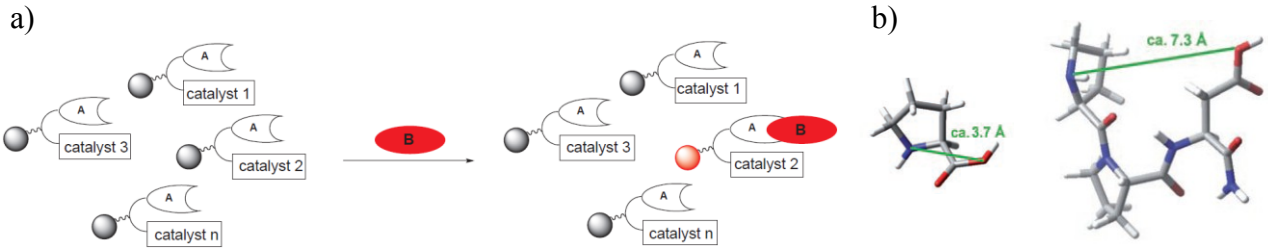
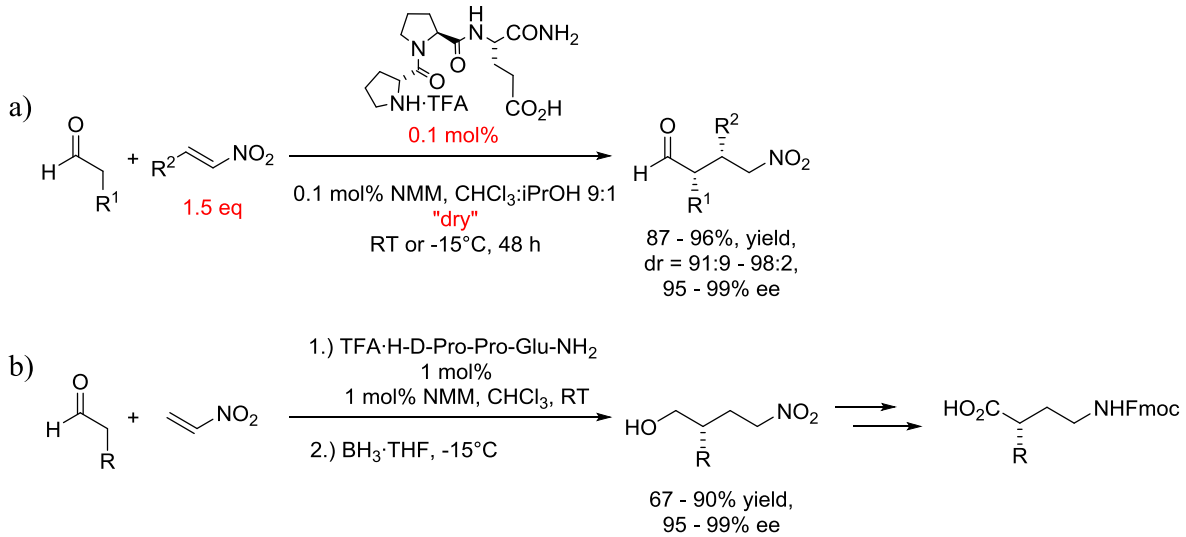


Figure 1-3 a.) The principle of catalyst-substrate co-immobilisation: the bead carrying an active catalyst (catalyst 2) becomes labelled with a red dye. b.) The additional distance between the carboxylic acid moiety and the secondary amine of H-Pro-Pro-Asp-NH_2 compared to proline.

offering in few synthetic steps a convenient entry into γ^2-amino acids in high enantiomeric purity that are difficult to obtain by other methods (Scheme 1-6, b).[91] Moreover, due to the high robustness of the peptide, the solid supported analogue H-D-Pro-Pro-Glu-NH-R (R=solid support) is a heterogeneous catalyst that does not show any irreversible deactivation.[128] Consequently, it can be reused numerous times and even be integrated into a continuous flow system.[129]

With their high catalytic activity and selectivity at very low catalyst loadings, the broad substrate scope while not requiring an excess of the aldehyde and the high stability, not showing any irreversible catalyst deactivation, peptides of the type Pro-Pro-Xaa (Xaa = variable acidic amino acid) and especially the tripeptide H-D-Pro-Pro-Glu-NH_2, solve the typical challenges (high catalyst loadings, limited substrate scope, need for a high excess of aldehyde and catalyst deactivation) often encountered with other amine-based catalysts in conjugate addition reactions between aldehydes and nitroolefins (compare Figure 1-2).



Scheme 1-6 a) Conjugate addition reactions of aldehydes to nitroolefins in the presence of H-D-Pro-Pro-Glu-NH_2 under optimized conditions. b) Conjugate addition of aldehydes to nitroethylene – an entry to γ^2-amino acids.

2 Objectives

The conjugate addition reaction between aldehydes and nitroolefins is one of the most intensely researched organocatalytic transformations.[23] The resulting substituted γ-nitro-aldehydes are highly versatile chiral building blocks for the synthesis of various useful molecules such as pyrrolidines, γ-butyrolactones or γ-amino acids. As a consequence over the first decade of research into this catalytic transformation the main focus was on the design of new catalysts and the optimisation of reaction conditions. Much less attention has been paid to mechanistic investigations aimed at the explanation why some catalysts are much more successful than others or why optimal conditions differ from catalyst to catalyst. Furthermore, the investigations of other nitroolefins with different substitution patterns than one substituent in the β-position have, with the exception of unsubstituted nitroethylene,[29,91] been mostly neglected.

Three general objectives were pursued within this thesis:

(1) With its high catalytic activity and excellent stereoselectivity the tripeptide H-D-Pro-Pro-Glu-NH_2 is a particularly successful organocatalyst for the conjugate addition reaction of various combinations of aldehydes and β-mono-substituted nitroolefins[92-94] as well as nitroethylene.[91] *Thus, the first aim of this thesis was to elucidate the mechanistic subtleties that render the peptide H-D-Pro-Pro-Glu-NH_2 such an excellent catalyst.*

(2) Despite the fact that peptides are not known as catalysts in nature, they consist of the same amino acid building blocks as the natural catalysts, enzymes. However, whereas enzymes work under physiological aqueous conditions, most peptidic catalysts perform well in organic solvents. Thus the use of peptidic catalysts in an aqueous environment is intriguing. *The second aim was the development of amphiphilic analogues of the peptide H-D-Pro-Pro-Glu-NH_2 as catalysts for the reaction to take place in water.*

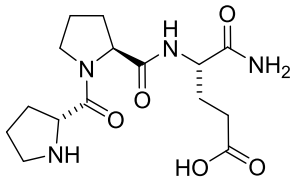
(3) Peptides of the general lead structure Pro-Pro-Xaa (Xaa = variable amino acid bearing a carboxylic acid group) offer the structural modularity to tune the catalytic properties in order to adapt to the requirements of a substrate. *The third aim of this thesis was therefore the investigation of peptides of the type Pro-Pro-Xaa as catalysts for conjugate addition reactions of aldehydes to α,β-disubstituted nitroolefin - more challenging substrates than their β-mono-substituted counterparts.*

3

Mechanistic Investigations into the Conjugate Addition Reaction of Aldehydes to β-Nitroolefins

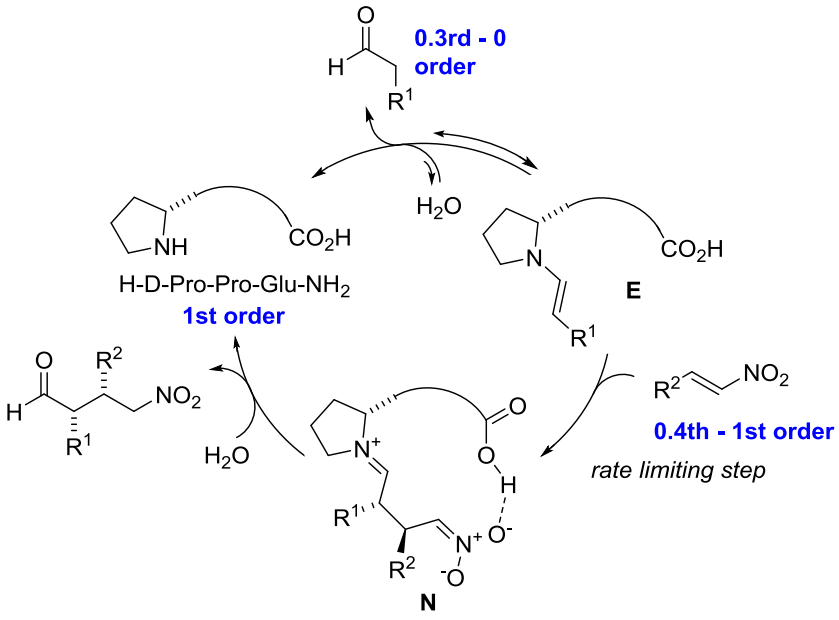
3.1 Background

In the development of the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) as a catalyst for conjugate addition reactions between aldehydes and β-nitroolefins kinetic investigations using *in situ* FT-IR spectroscopy proved to be a valuable tool for the optimization of the reaction



1a

conditions.[93,130] Using initial rate measurements the reaction orders with respect to the catalyst and the substrates were determined (Figure 3-1).[93,130] The reaction was found to be first order with respect to the catalyst. Depending on the nitroolefin used an order of 0.4^{th} – 1^{st} was obtained for this substrate. The dependence of the reaction rate on the aldehyde was determined to be around 0.3^{rd} order at low aldehyde concentrations and became 0 order at higher concentrations indicating saturation kinetics under these conditions. The concentration at which this 0 order regime is reached depended on the reactivity of the aldehyde, the catalyst loading and the amount of water in the system. In general, the concentration of water was

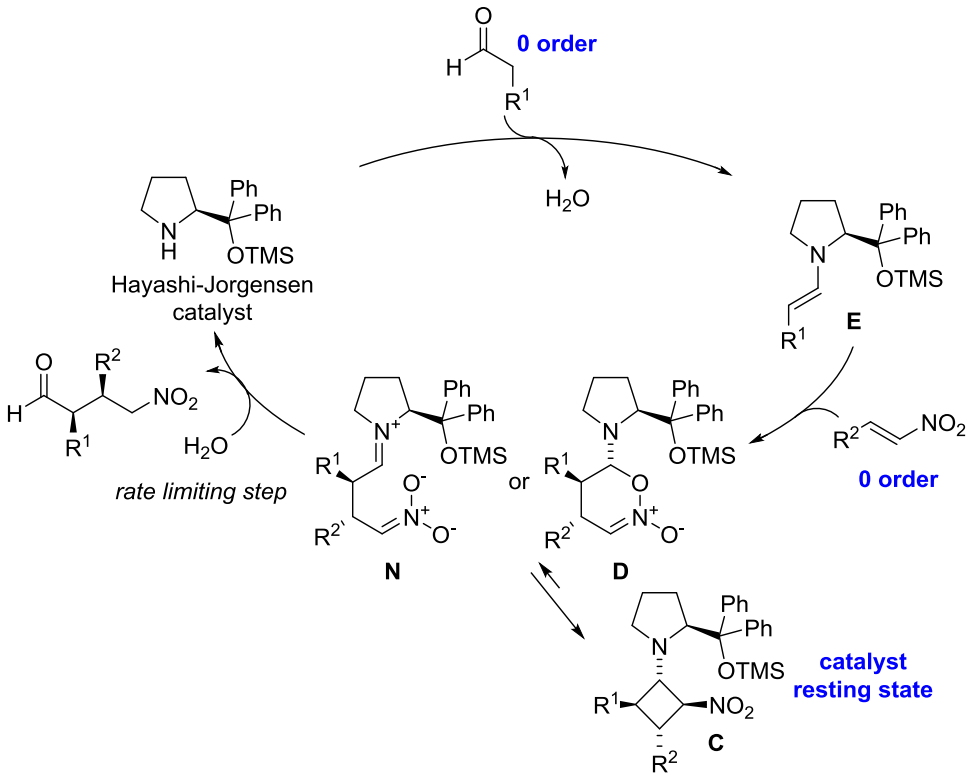


Scheme 3-1 Proposed catalytic cycle for the conjugate addition reaction of aldehydes to nitroolefins in the presence of the tripeptide **1a**.[93,130]

found to dramatically influence the reaction rate with fastest reactions observed by using carefully dried solvents and reagents. These experimental observations are in agreement with a mechanism consisting of enamine formation between the aldehyde and the catalyst in a rapid pre-equilibrium followed by a rate limiting conjugate addition between the enamine and the nitroolefin (Figure 3-1). Hydrolysis of the resulting intermediate (which is potentially also rate contributing) liberates the reaction product and frees the catalyst. These results clearly showed that optimal reaction conditions include the use of an excess of the nitroolefin rather than the aldehyde (because of the higher order in the nitroolefin) as well as dried solvents and reagents. Under these conditions the catalyst loading could be reduced to as little as 0.1 mol%, the lowest catalyst loading achieved so far for this reaction.[93]

Besides peptide **1a** arguably the most successful organocatalyst for the reaction between aldehydes and β-nitroolefins is α,α-diphenylprolinol trimethylsilyl ether, the Hayashi-Jørgensen catalyst (see Figure 1-2). Interestingly, while under optimized conditions in the presence of 1 mol% of this catalyst the product is formed in similar reaction times as with peptide **1a**, upon reduction of the catalyst loading to 0.1 mol% the reaction virtually comes to a halt (12% conversion after 7 days).[131]

In independent mechanistic investigations by the groups of Blackmond,[132-134] Hayashi and Seebach[131,135] as well as Pihko and Pàpai[136] cyclic intermediates such as cyclobutanes (**C**) or 1,6-dihydrooxazine-*N*-oxides (**D**) were observed in reactions in the presence of the Hayashi-Jørgensen catalyst. Such cyclic intermediates were NMR-spectroscopically characterized upon mixing a stoichiometric amount of the catalyst with an aldehyde and a nitroolefin.[131,134-136] They were also detected as the catalyst resting state under turnover conditions.[134] Additionally, kinetic studies revealed that the reaction rate neither depends on the concentration of the aldehyde nor on that of the nitroolefin (0 order).[134] However, a dramatic rate acceleration was observed in the presence of a carboxylic acid co-catalyst.[131,134,136] Based on these experimental observations the catalytic cycle depicted in Scheme 3-2 was proposed. Enamine formation between the aldehyde and the catalyst, followed by conjugate addition, gives rise to the short-lived zwitterion **N**.[131] Alternatively, based on computational calculations, it was suggested that the enamine reacts with the nitroolefin in an inverse-electron-demand *hetero*-Diels-Alder reaction directly to the 6-membered intermediate **D**.[136] Both, **N** and **D** would then collapse to the more stable cyclobutane **C**. Due to its high stability, **C** is the resting state of the catalyst and its opening to



Scheme 3-2 Proposed catalytic cycle for the conjugate addition reaction of aldehydes to nitroolefins in the presence of the Hayashi-Jørgensen catalyst (b.)[131-136]

the zwitterion **N** (or reaction back to **D**[136]) followed by protonation is the rate determining step of the overall reaction.[134] As none of the substrates is involved in the rate determining step, zero order dependence is observed. However, since protonation of intermediate **N** is rate limiting, an external acidic co-catalyst dramatically accelerates the reaction.[134]

Comparison of these observations with what has been described above for the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) reveals several striking differences: (1) reactions in the presence of the Hayashi-Jørgensen catalyst do not depend on the substrate concentration, whereas positive order dependence on both the aldehyde and the nitroolefin are observed in the presence of **1a**, (2) reactions in the presence of the peptide are fastest under "dry" conditions while no influence of water is observed in the presence of the Hayashi-Jørgensen catalyst and (3) the Hayashi-Jørgensen catalyst relies on an acidic co-catalyst for successful catalysis whereas no additive is necessary in the presence of H-D-Pro-Pro-Glu-NH_2. Furthermore, different rate limiting steps and catalyst resting states have been proposed for the two systems. We hypothesised that these differences could be the reason for the distinctly higher catalytic activity of **1a** at low catalyst loadings (0.1 mol%). We therefore decided to conduct further

mechanistic studies and investigate the factors in more detail that render the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) such an exceptionally successful catalyst.

3.2 Mechanistic Investigations Based on Kinetic and NMR-Spectroscopic Studies

3.2.1 Comparison of Structurally Related Catalysts Bearing and Lacking an Intramolecular Carboxylic Acid Moiety[137]

Major differences between the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) and the Hayashi-Jørgensen catalyst are their general structures but also the absence and presence, respectively, of an intramolecular carboxylic acid moiety. Both factors could be the reason for their distinctly different mechanistic features. To evaluate whether the presence or absence of the carboxylic acid group is mainly responsible for the different properties, we decided to compare **1a** with its analogue H-D-Pro-Pro-Glu(OMe)-NH_2 (**2a**) in which the carboxylic acid in the side chain is replaced by a methyl ester. Additionally, the two analogues H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (**1b**) and H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (**2b**) were also investigated. These derivatives bearing *n*-dodecyl chains at their C-termini are significantly more soluble in a range of organic solvents and allowed for the examination of the reaction in nonpolar solvents such as toluene. We chose the conjugate addition reaction between butanal and nitrostyrene as a model reaction and followed the conversion over time using *in situ* FT-IR as a non-invasive monitoring method, the same method we had used in our previous studies.[93,130]

Effect of the C-terminal alkyl chain: The obtained conversion-time curves in the presence of each of the different peptide catalysts in a 9:1 mixture of $CHCl_3$ and *i*PrOH, the best solvent system for this reaction, are shown in Figure 3-1. The data clearly shows that the C-terminal *n*-dodecyl chain has no influence on the catalytic activity of the peptidic catalysts. The stereoselectivities observed when the catalysts with an alkyl chain were used were slightly lower (see Figure 3-1; the lower diastereoselectivity determined in the presence of **2a** compared to **2b** is most likely due to post reaction epimerization rather than low diastereoselectivity of the reaction, see section 3.2.6). A small drop in enantioselectivity was expected based on previous studies that showed that the highest enantioselectivity was obtained when the catalyst bore a primary amide at the C-terminus.[92,130]

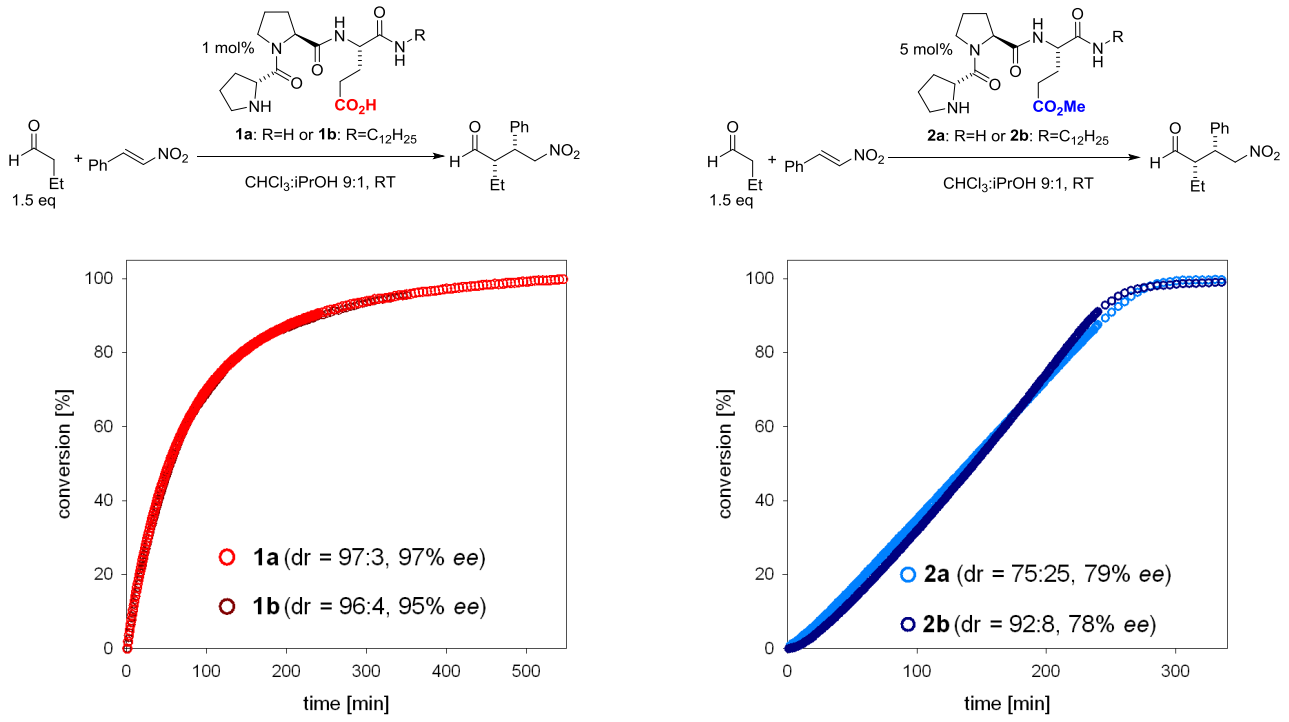


Figure 3-1 Conversion-time curves obtained in the presence of 1 mol% of peptidic catalysts **1a** and **1b** (left) as well as 5 mol% of **2a** and **2b** (right) in $CHCl_3$:*i*PrOH 9:1.

Shape of the conversion-time curves depending on the presence or absence of an intramolecular carboxylic acid group: Large differences were observed, however, depending on the presence or absence of the intramolecular carboxylic acid group within the catalyst. While 1 mol% of the carboxylic acid bearing peptides **1a** and **1b** was enough to achieve rapid product formation, 5 mol% of **2a** and **2b** were required for good conversions in similar reaction times. Additionally, the stereoselectivities obtained in the presence of the methyl ester catalysts (79% *ee* for **2a** and 78% *ee* for **2b**) were dramatically lower than the selectivities observed with the catalysts bearing an acid moiety (97% *ee* for **1a** and 95% *ee* for **1b**). The most striking differences were observed, though, in the shape of the conversion-time curves (Figure 3-1). The kinetic profiles obtained in the presence of **1a** and **1b** demonstrated that the reaction is fastest in the beginning and slows down as the starting materials are consumed (Figure 3-1, left). Such behaviour is in agreement with the previously determined positive order dependence on the substrate concentration[93,130] and expected for reactions where the substrates are involved in the rate determining step. In contrast, when the methyl ester catalysts **2a** and **2b** were used the corresponding kinetic profiles had a sigmoidal shape (Figure 3-1, right). This means that the reaction rate increases with increasing conversion. Furthermore, identical conversion-time curves were observed for reactions in the presence of

2b using different substrate concentrations demonstrating that the reaction rate does not depend on the concentration of the reactants.

Effect of the solvent: In order to investigate the effect of the protic and polar reaction medium used in the reactions described above ($CHCl_3$:*i*PrOH 9:1) the conjugate addition between butanal and nitrostyrene was performed and monitored in the aprotic non-polar solvent toluene comparing the more soluble peptidic catalysts **1b** and **2b** (Figure 3-2). When using peptide **1b** in toluene, an identical conversion-time curve was obtained as in the presence of **1a** in the chloroform *iso*-propanol mixture (Figure 3-2, red and dark red spheres). In the presence of the methyl ester peptide **2b**, however, the reaction in toluene (Figure 3-2, dark blue spheres) proceeded significantly slower than the corresponding reaction in the protic environment (in the presence of **2a**, Figure 3-2, blue spheres). Additionally, the sigmoidal shape of the conversion-time curve was even more pronounced in toluene.

Effect of acetic acid as an additive: To further investigate the effect of an external proton donor, reactions in the presence of the peptides bearing and lacking an intramolecular proton donor were also performed and monitored using 10 mol% of acetic acid as an acidic additive. Whereas the acetic acid additive did not affect the reaction in the presence of a peptidic catalyst bearing an intramolecular carboxylic acid group such as **1b** (Figure 3-2, dark red circles), such a co-catalyst accelerated the reaction with the methyl ester **2b** dramatically (Figure 3-2, dark blue circles). Furthermore, the corresponding kinetic profile was linear. These findings demonstrate that external proton sources from the solvent or an additive do not influence the reaction rate when the catalyst bears an intramolecular carboxylic acid group, whereas a protic environment dramatically accelerates reactions when the catalysts lack such an intramolecular proton donor.

NMR-spectroscopic investigation: In order to further probe the differences observed in our kinetic studies in the presence of peptidic catalysts with and without an intramolecular carboxylic acid group, butanal was exposed to an excess of the catalysts **1b** and **2b** in $CDCl_3$ or C_6D_6 and the mixtures were inspected by NMR-spectroscopy. In agreement with previous studies,[130] no signals corresponding to an enamine were observed in the presence of catalyst **1b** bearing an intramolecular carboxylic acid under these conditions. In the presence of the methyl ester **2b**, however, formation of the corresponding enamine proceeded readily and in high quantities (50% of the aldehyde in C_6D_6, 30% in $CDCl_3$). This shows that the enamine intermediate is formed in a higher concentration and is much more stable if the catalyst lacks

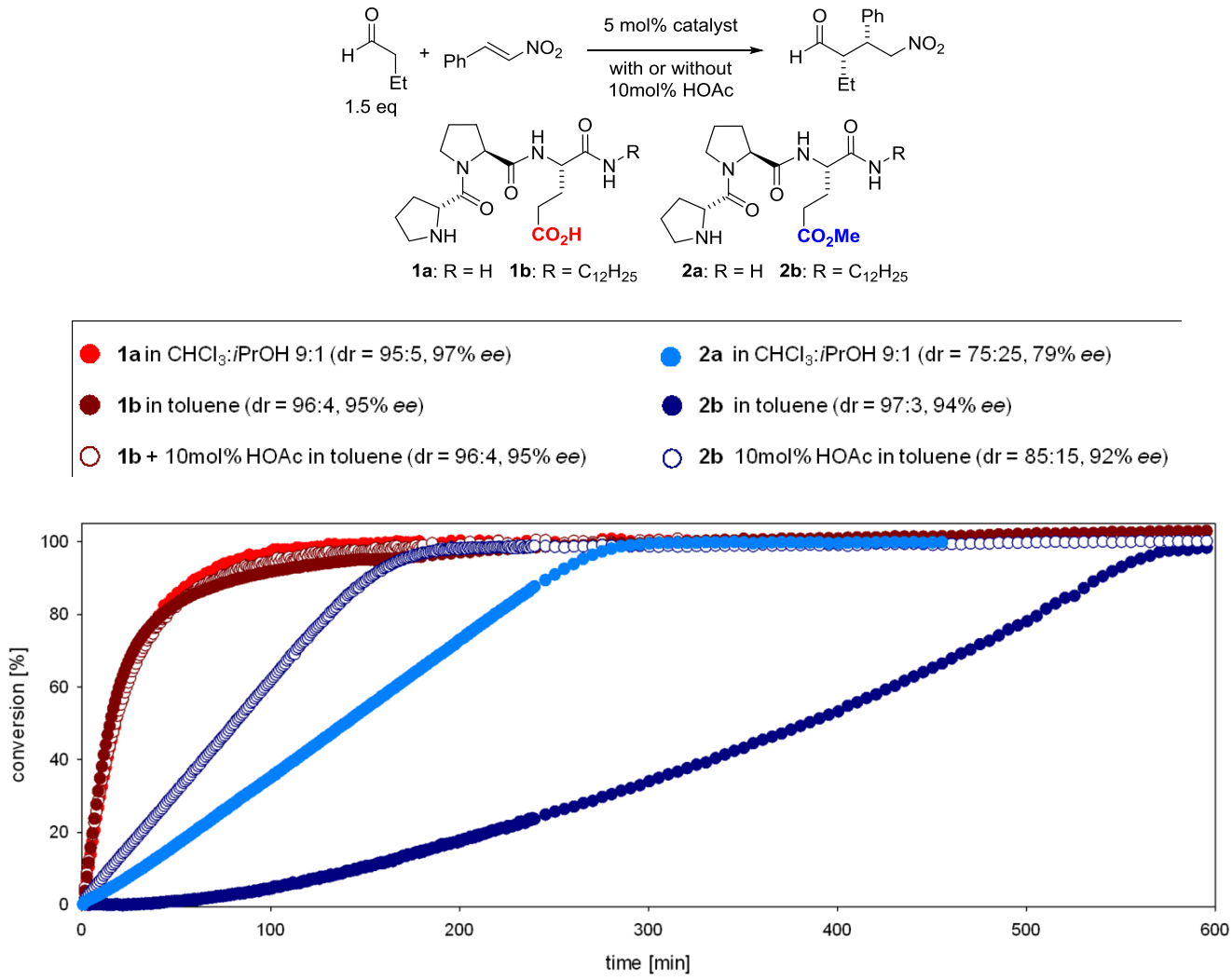
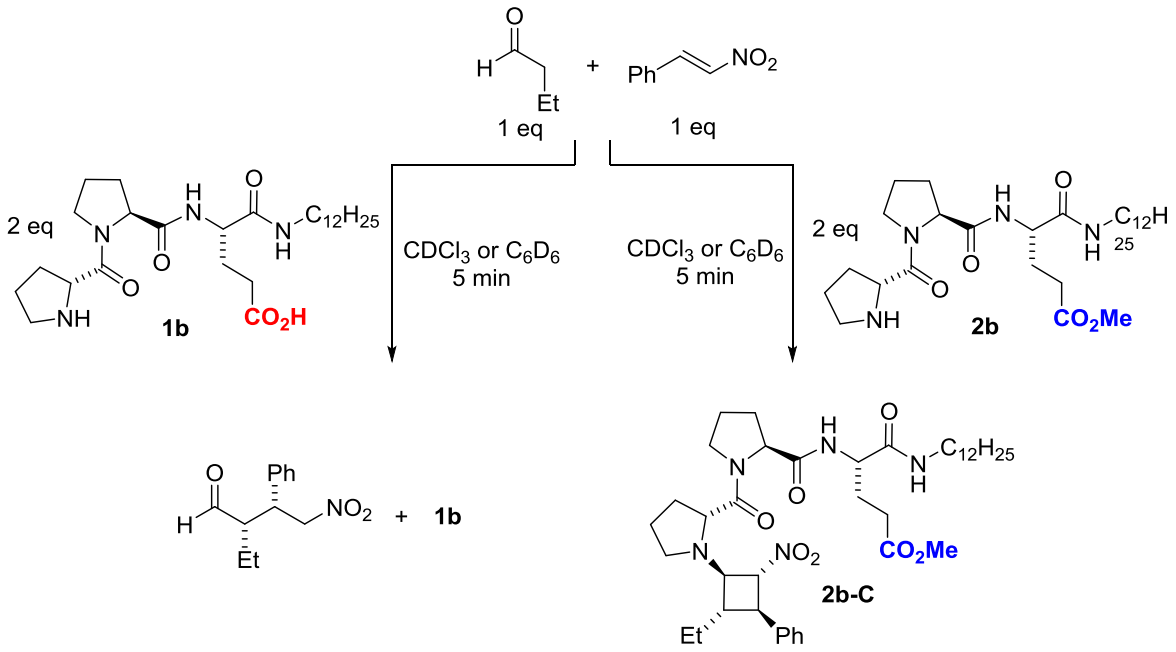


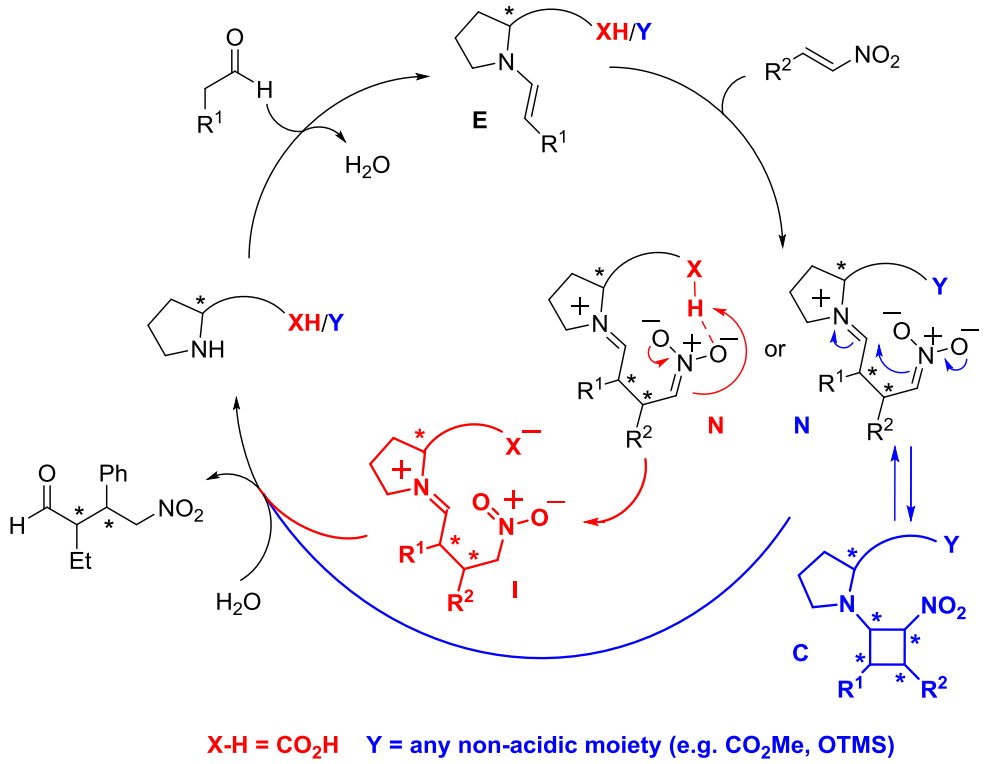
Figure 3-2 Conversion-time curves of the conjugate addition reaction of butanal to nitrostyrene in the presence of catalysts **1a**, **1b**, **2a** and **2b**.

an intramolecular carboxylic acid. An even more pronounced difference between the catalysts bearing and lacking an intramolecular carboxylic acid moiety was observed when an equivalent of nitrostyrene was added to these reactions (Scheme 3-3). In the presence of peptide **1a** bearing an intramolecular acid group the only observable new signals corresponded to the reaction product which started forming immediately after the addition of nitrostyrene (Scheme 3-3, left). In stark contrast, the methyl ester peptide **2b** reacted with the substrates to form cyclobutane **2b-C**, the only new species that was formed after a reaction time of 5 min (Scheme 3-3, right). This result suggests that while a cyclobutane intermediate is a likely intermediate and catalyst resting state for reactions in the presence of catalysts **2a** and **2b** lacking an intramolecular carboxylic acid moiety, it is not populated to a significant extent when the carboxylic acid bearing catalysts **1a** and **1b** are used.



Scheme 3-3 Reactions of **1b** and **2b** with butanal and nitrostyrene monitored by NMR spectroscopy.

All of the observed features of peptides **2a** and **2b**, the sigmoidal or linear conversion-time curves, the independence of the reaction rate on the concentration of the substrates, the dramatic rate acceleration by an acidic additive and the straightforward formation of a cyclobutane when treated with an aldehyde and a nitroolefin, are comparable to the results reported for the Hayashi-Jørgensen catalyst.[134] The distinct differences observed depending on the presence or absence of an intramolecular carboxylic acid moiety within the catalyst structure demonstrate that the reactions proceed through alternative pathways and have different rate limiting steps (Scheme 3-4). The experimental evidence is in agreement with two pathways differing in the fate of the short-lived, zwitterionic iminium nitronate intermediate **N**. Irrespective of the presence or absence of an intramolecular acid in the catalyst structure **N** forms by reaction of the catalyst and the aldehyde to an enamine (**A**) followed by conjugate addition to the nitroolefin. In the presence of a suitably positioned intramolecular carboxylic acid moiety within the catalyst, zwitterion **N** is first intramolecularly stabilized by coordination of the acid to the nitronate[138-139] and then immediately trapped by protonation to form iminium ion **I** (Scheme 3-4, red pathway). **I** can then undergo hydrolysis thereby completing the catalytic cycle. In this pathway the protonation step proceeds intramolecularly and fast and is thus not the rate limiting step of the reaction. Instead, C-C bond formation between the enamine and the nitroolefin is decisive for the overall rate of the reaction. As a consequence the reaction rate depends on the



Scheme 3-4 Catalytic cycle proposed for conjugate addition reactions between aldehydes and nitroolefins in the presence of catalysts with (red) or without (blue) an intramolecular proton donor.

concentration of the nitroolefin but is not influenced by an external acidic additive. In the absence of such an intramolecular proton donor protonation of the reactive intermediate **N** has to proceed intermolecularly, mediated by the solvent, an acidic additive or the conjugate acid of the secondary amine organocatalyst. As a consequence, this protonation is slow and the short-lived zwitterionic intermediate **N** is intramolecularly stabilized by C-C bond formation to the much more stable cyclobutane **C**, which is the resting state of the catalyst (Scheme 3-4, blue pathway). As a consequence, the reopening of **C** to the zwitterion **N** and protonation is the rate determining step of the reaction. Reactions in the absence of an intramolecular proton donor are therefore accelerated by an external proton source facilitating this protonation but are unaffected by changes in the substrate concentration.

3.2.2 Effect of the Position of an Intramolecular Carboxylic Acid within the Catalyst Structure[137]

Based on this mechanistic rationale (Scheme 3-4), the reaction pathway and thus the rate determining steps are envisioned to be influenced by the position of an intramolecular carboxylic acid moiety within the catalyst structure: a transition from the pathway in which the intramolecular protonation of the iminium nitronate **N** is fast (Scheme 3-4, red pathway)

to that in which **N** cyclizes intramolecularly to a cyclobutane (Scheme 3-4, blue pathway) is expected for catalysts that bear a carboxylic acid moiety, but not in a position that allows for a favourable coordination of the acid group to the nitronate which is the prerequisite for the intramolecular proton transfer. If, for example, the carboxylic acid is positioned remotely from the backbone of the catalyst, the intramolecular protonation of **N** should become less favourable and, as in the case of catalysts lacking a proton donor, the protonation step is expected to become rate limiting. Thus, slower reaction rates and conversion-time profiles that are comparable to those of reactions with catalysts lacking an intramolecular carboxylic acid are expected with catalysts in which a carboxylic acid is present but not appropriately positioned to coordinate to the nitronate. Furthermore, since the coordination of the carboxylic acid to the nitronate is predicted to be involved in the transition state of the C-C bond formation step in which the stereogenic centres are formed, the stereoselectivity of the conjugate addition reaction is also expected to depend on the position of the carboxylic acid. In order to probe these mechanistic conclusions, we prepared peptides **1c** – **1f** with increasing length of the side chain as well as peptide H-D-Pro-Pro-Ada-OH (**1g**, Ada = 12-aminododecanoic acid), where a C-terminal carboxylic acid is connected to the D-Pro-Pro motif by a long and flexible alkyl chain. The conversion-time curves of the conjugate addition reaction of butanal to nitrostyrene in the presence of these different peptide catalysts are shown in Figure 3-3. While the observed reaction rates in the presence of peptides **1c** – **1e** with the short alkyl spacers were comparable, a significant drop in catalytic activity was observed for peptides **1f** and **1g** (Figure 3-3, olive and violet spheres). Moreover, the shapes of the curves differed significantly. Whereas in the presence of peptides **1c** – **1e** the kinetic profiles showed a distinct curvature, demonstrating a decrease in reaction rate with decreasing substrate concentration, this curvature was much less pronounced in the presence of catalyst **1f** and an essentially linear conversion-time curve was obtained, when **1g** with the longest and most flexible linker was used (Figure 3-3, violet spheres). In fact, the linear conversion-time curve obtained with peptide **1g** is almost identical to the curve observed in the presence of methyl ester H-D-Pro-Pro-Glu(OMe)-NH_2 (**2a**) in combination with an acetic acid co-catalyst (compare violet spheres with the inlet in Figure 3-3). These results suggest that the carboxylic acid moiety within peptide **1g** acts rather as an external acidic co-catalyst and not as the intramolecular carboxylic acid group of, for example, peptide **1a**. The long and flexible linker between the D-Pro-Pro motif and the C-terminal carboxylic acid prevents an effective coordination and protonation of the iminium nitronate **N** which is therefore stabilized by

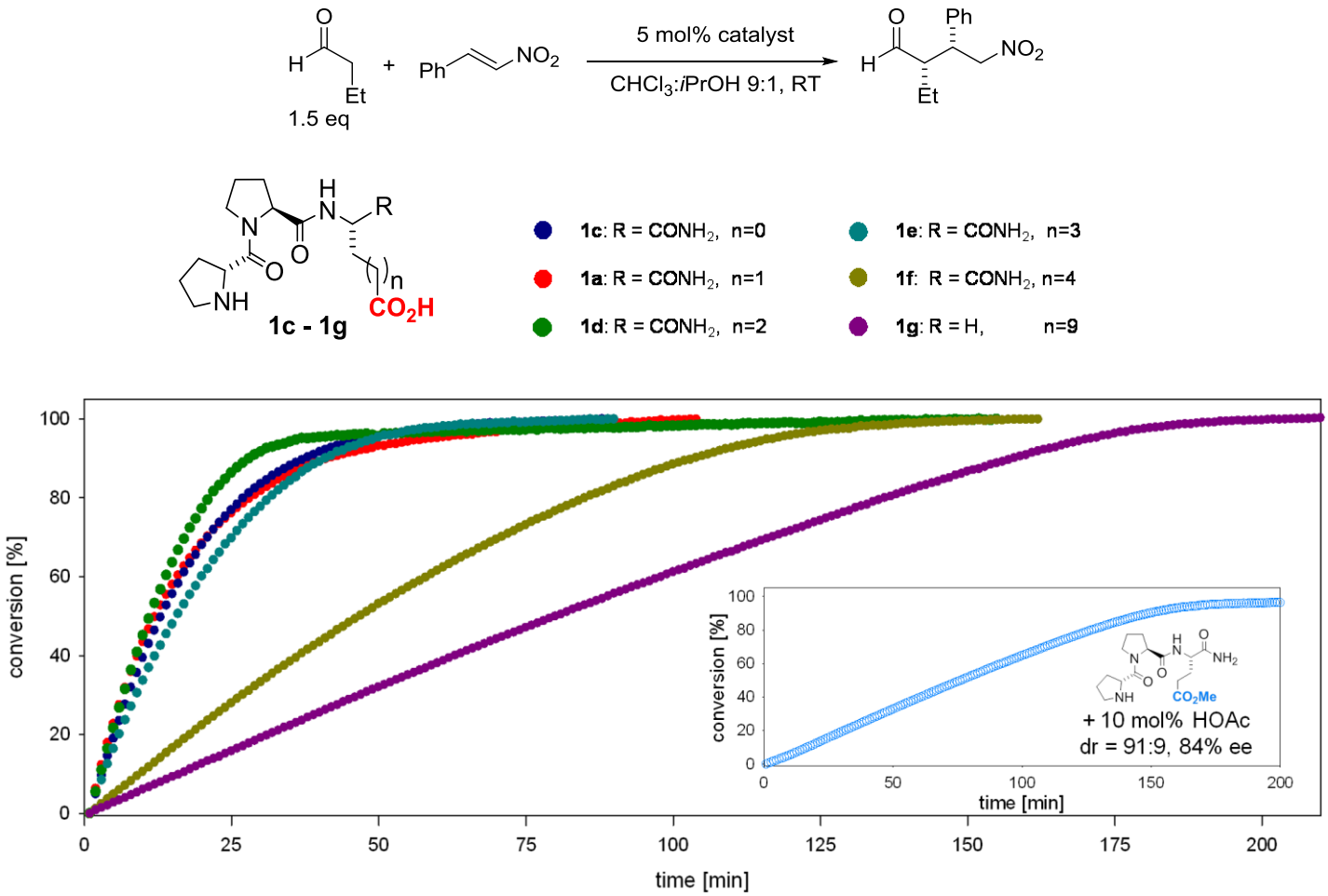
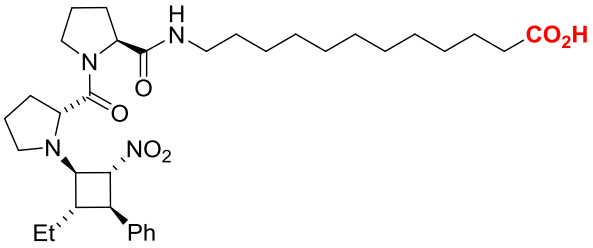


Figure 3-3 Conversion-time curves of the conjugate addition reaction of butanal to nitrostyrene in the presence of catalysts **1a** and **1c** – **1g**. Inlet: The same reaction in the presence of methyl ester **2a** and acetic acid as a co-catalyst under otherwise identical conditions.

cyclization to a cyclobutane **C**. As a consequence, the rate limiting step of the reaction is the protonation of this intermediate rather than C-C bond formation and, therefore, the conversion-time profile is linear. This mechanistic interpretation is further confirmed by the observation of cyclobutane **1g-C** as the resting state of the catalyst upon reaction of butanal with nitrostyrene under turnover conditions in the presence of **1g** in C_6D_6 and NMR-spectroscopic inspection of the reaction mixture.

Also the stereoselectivities of the peptides with the longer linkers proved to be significantly lower than those of the shorter analogues (Figure 3-4). The enantioselectivities of the catalysts with shorter chains **1c** – **1e** were all higher than 90% *ee* and had a maximum for the parent peptide H-D-Pro-Pro-Glu-NH_2 (**1a**, 97% *ee*). However, the enantiomeric excess dropped to 84% *ee* of catalyst **1f** and to 58% *ee* of the longest analogue **1g**. Thus, these results



1g-C

strongly suggest that coordination of the carboxylic acid group to the nitronate followed by intramolecular proton transfer not only controls the reaction rate and which step is rate determining but is also crucial for the enantioselectivity.

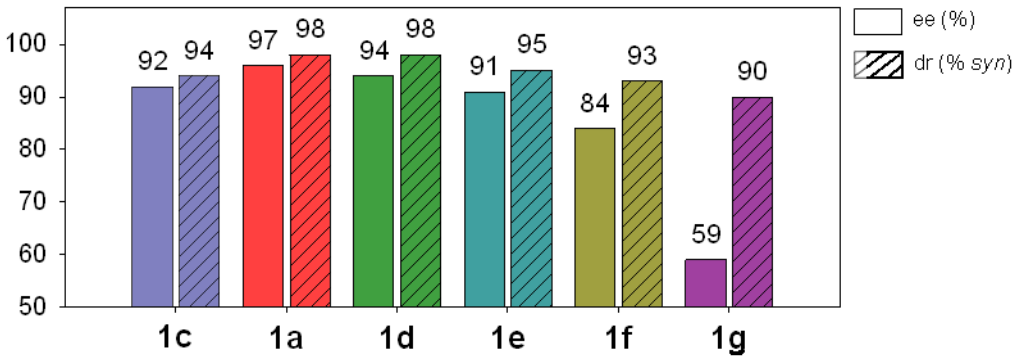


Figure 3-4 Stereoselectivities of the catalysts **1a** and **1c** – **1g**.

3.2.3 Effects of External Acidic Additives of Different Strengths[137]

To further probe the two different pathways in the presence of catalysts bearing or lacking a suitably positioned carboxylic acid moiety (red and blue pathways in Scheme 3-4), we next explored the effect of carboxylic acid additives with different acidities on the conjugate addition reaction. Based on the mechanistic conclusions distinctly different effects of weak and strong acidic co-catalysts on the two alternative pathways were expected.

Pathway via an intramolecular protonation of the iminium nitronate (Scheme 3-4, red pathway): The rate determining step of reactions with catalysts bearing a carboxylic acid moiety in an appropriate position for an intramolecular coordination to the nitronate is not the protonation of the iminium nitronate but the C-C bond formation between the enamine and the nitroolefin. Thus, an additive with an acidity that is comparable or lower compared to that of the intramolecular carboxylic acid group (pKa ~ 4 in water) is expected to be a mere spectator and not to affect the reaction rate or stereoselectivity of the catalyst. In contrast, a

stronger acid is expected to protonate the secondary amine of the catalyst and to destabilize the enamine. Thus, acids with pKa values lower than 4 should lead to a reduced amount of the enamine and, since the C-C bond formation between the enamine and the nitroolefin is determining the rate of the reaction, to a reduction of the overall reaction rate. Indeed, when 10 mol% of acetic acid (Figure 3-5, red circles) or 4-nitrophenol (Figure 3-5, green circles) were added to the reaction of butanal to nitrostyrene in the presence of H-D-Pro-Pro-Glu-NH_2 (**1a**) the conversion time curves observed were identical to the curve obtained in the absence of an additive (Figure 3-5, red spheres). Use of additives with higher acidities such as 4-nitrobenzoic acid (Figure 3-5, violet circles) and chloroacetic acid (dark red circles) led to dramatically slowed down reactions and the reactions did nearly not proceed at all in the presence of dichloroacetic acid (olive circles) or trifluoroacetic acid (cyan circles). These results further underline the mechanistic conclusions of Scheme 3-4 and also demonstrate that acidic additives cannot improve reactions in the presence of catalysts bearing a suitably positioned carboxylic acid moiety.

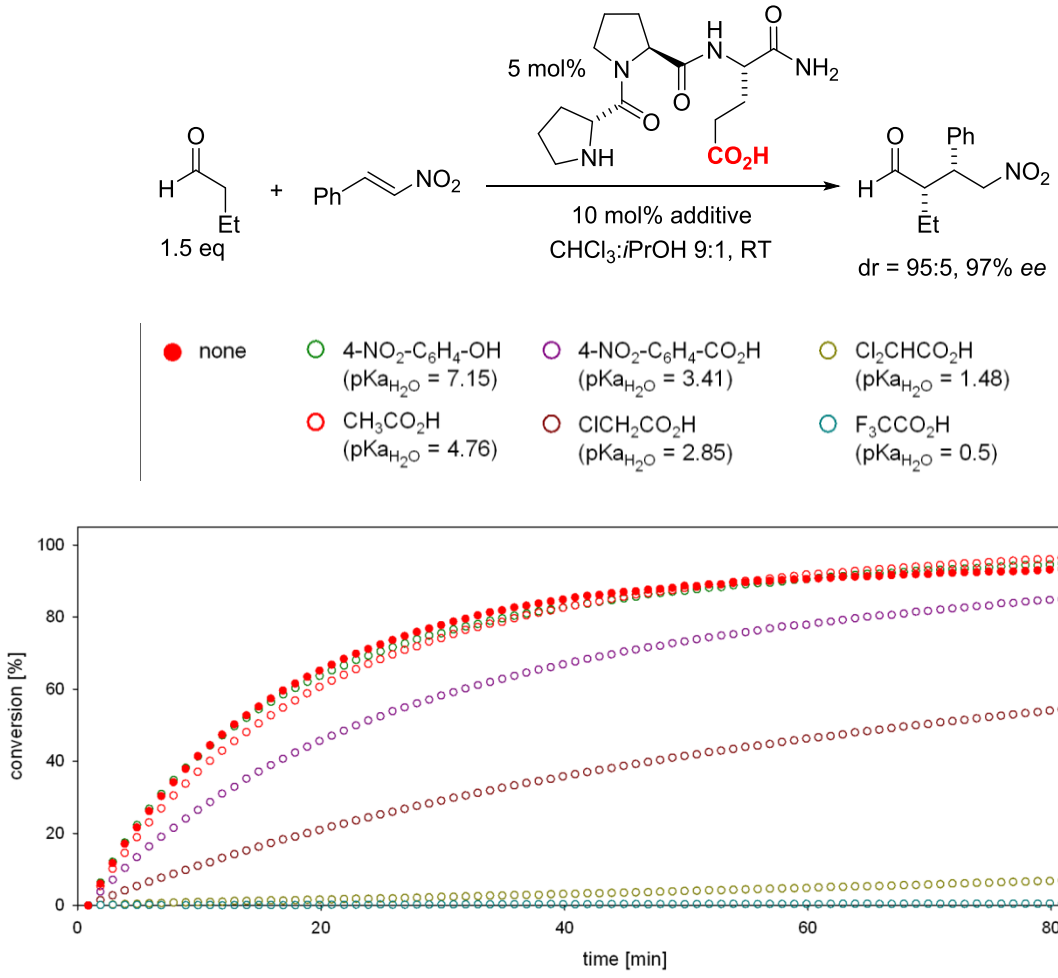


Figure 3-5 Conversion-time curves of the conjugate addition reaction of butanal to nitrostyrene in $CHCl_3$:*i*PrOH 9:1 in the presence of peptides **1a** and different acidic additives.

Pathway involving the formation of a cyclobutane intermediate (Scheme 3-4, blue pathway): For reactions in which the protonation of the iminium nitronate is the rate limiting step, an acidic additive is expected to accelerate catalyst turnover and therefore the reaction rate. As described in section 3.2.1, this is the case when protic solvents or acetic acid as a co-catalyst are used for reactions with catalysts lacking an intramolecular carboxylic acid moiety. Stronger acids can be expected to further facilitate the protonation of the iminium nitronate which would lead to an even faster reaction. However, as described above stronger acids will also protonate the secondary amino group within the catalyst and destabilize the enamine. Taking these two effects together, the use of strong acids as additives in reactions with catalysts lacking an intramolecular carboxylic acid can be expected to lead to a shift of the rate limiting step from the protonation of the iminium nitronate (Scheme 3-4, blue pathway) to the C-C bond formation between the enamine and the nitroolefin (Scheme 3-4, red pathway). As described in section 3.2.1, use of 10 mol% of acetic acid as a co-catalyst in the reaction between butanal and nitrostyrene using H-D-Pro-Pro-Glu(OMe)-$NHC_{12}H_{25}$ (**2b**) led indeed to dramatic rate acceleration (Figure 3-6, blue circles). In the presence of 4-nitrophenol

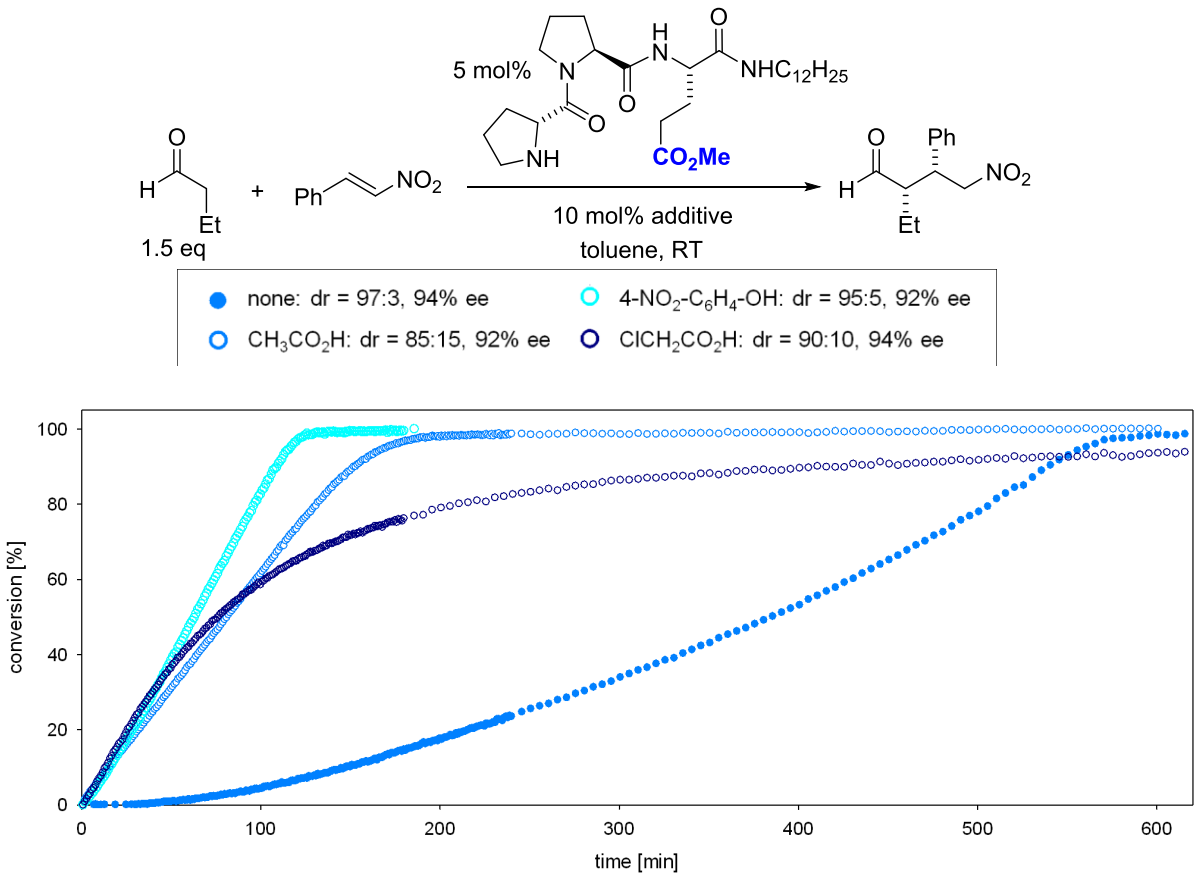
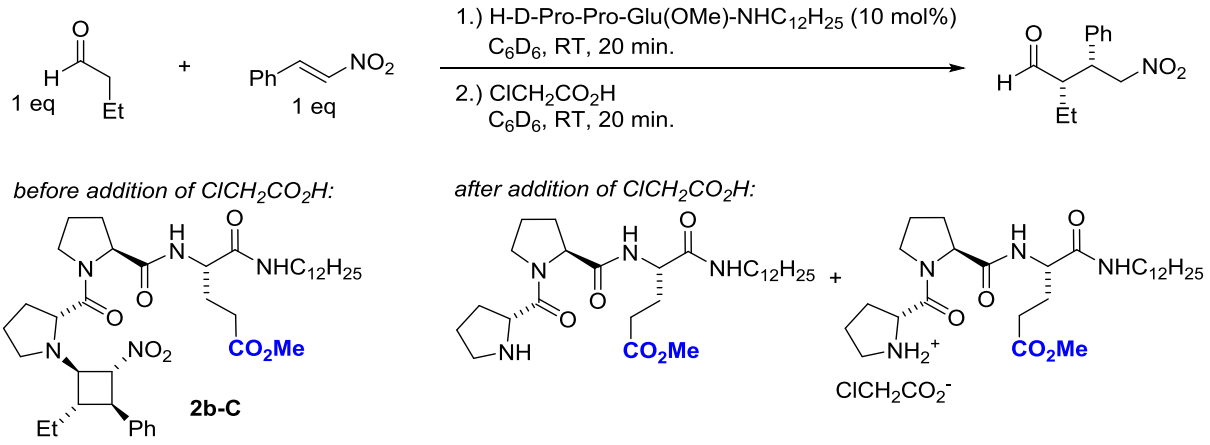


Figure 3-6 Conversion-time curves of the conjugate addition reaction of butanal to nitrostyrene in toluene in the presence of peptides **2b** and different acidic additives.

the reaction proceeded even faster (Figure 3-6, cyan circles). This is in agreement with studies on this reaction in the presence of the Hayashi-Jørgensen catalyst where 4-nitrophenol was identified as a particularly good co-catalyst.[131] When the stronger acid chloroacetic acid was examined (Figure 3-6, dark blue circles), the corresponding conversion-time curve showed a distinct curvature indicative of a decreasing reaction rate with increasing conversion and fully compatible with a concomitant change in the rate limiting step of the reaction from the protonation of the nitronate to the C-C bond formation. This change in the rate limiting step was also reflected by the observation of a change in the resting state of the catalyst by NMR-spectroscopic inspection of the reaction mixture (Scheme 3-5). When the reaction was conducted in the presence of 10 mol% of **2b** in C_6D_6 the reaction was sluggish (< 5% conversion to the γ-nitroaldehyde after 20 min) and the corresponding cyclobutane intermediate **2b-C** was the only peptide related species observed. Upon addition of 10 mol% of chloroacetic acid the reaction proceeded much faster (35% conversion within 20 min) and the cyclobutane intermediate disappeared. Instead the catalyst was present as a mixture of the protonated and non-protonated catalyst. This finding is in agreement with a change in the rate determining step from protonation of the nitronate to C-C bond formation as suggested by the kinetic experiments.



Scheme 3-5 Change of the catalyst resting state depending on the presence or absence of chloroacetic acid in the reaction of butanal to nitrostyrene in the presence of **2b** as observed by NMR-spectroscopy.

3.2.4 Comparison of Proline with Pyrrolidine

To probe the generality of the findings that the reaction pathways and thus the rate determining steps of conjugate addition reactions between aldehydes and nitroolefins depends on the presence or absence of an intramolecular carboxylic acid group within the catalyst structure, the reaction between butanal and nitrostyrene was also performed in the presence of proline and pyrrolidine, additional examples of organocatalysts bearing and lacking an intramolecular carboxylic acid moiety (Figure 3-7). Based on the rationale that a suitably positioned carboxylic acid group within the catalyst can trap the zwitterionic intermediate **N** and thereby causes C-C bond formation to be the rate limiting step, an exponential product formation curve was also expected for proline. In contrast, in the presence of pyrrolidine the reaction was expected to proceed through a cyclobutane intermediate and, thus, sigmoidal or linear conversion-time curves were expected. Indeed, in the presence of proline, an exponential product formation curve that was not influenced by the presence of an external acetic acid additive was obtained (Figure 3-7, left). This is again in agreement with C-C bond formation being the rate limiting step of the reaction (Scheme 3-4, red pathway). Interestingly, pyrrolidine turned out to be a much more reactive catalyst than proline for this reaction and full conversion was observed in reaction times of less than an hour (Figure 3-7, right). Furthermore, when pyrrolidine was used as catalyst, linear conversion-time curves

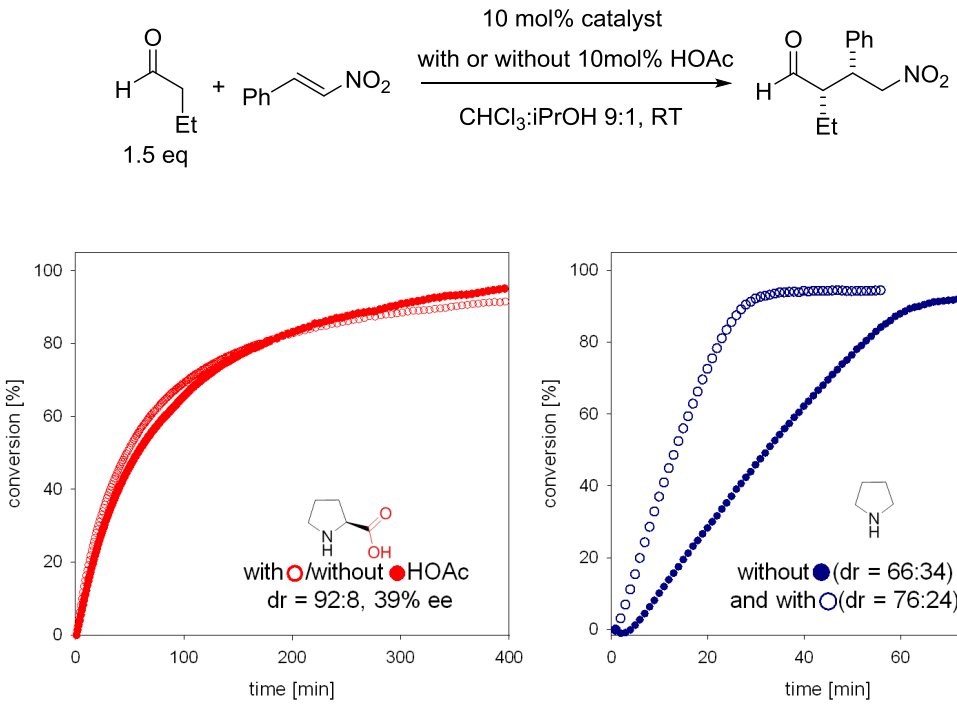
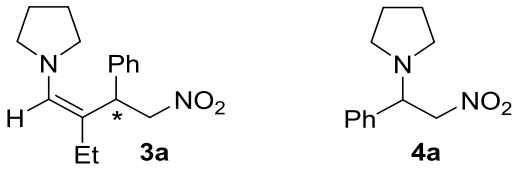


Figure 3-7 Conversion-time curves of the conjugate addition reaction of butanal to nitrostyrene in the presence of proline (left) and pyrrolidine (right).



Scheme 3-6 Additional intermediates involved in the conjugate addition reaction between butanal and nitrostyrene in the presence of pyrrolidine. Both are readily formed upon treatment of the reaction product or nitrostyrene with pyrrolidine.

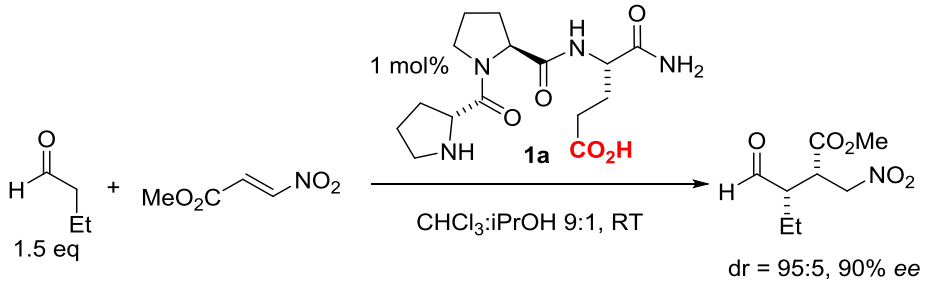
and significant rate acceleration by an acetic acid co-catalyst were observed, which is in agreement with formation of a cyclobutane intermediate and protonation as the rate determining step of the reaction (Scheme 3-4, blue pathway). However, great caution is appropriate when interpreting the curves obtained in the presence of pyrrolidine. First, when a diastereomerically pure sample of the γ-nitroaldehyde resulting from the reaction was treated with pyrrolidine in $CDCl_3$ a significant amount of the corresponding product enamine **3a** (Scheme 3-6, left) was observed together with a fast epimerization of the γ-nitroaldehyde. Second, upon mixing of nitrostyrene with pyrrolidine in $CDCl_3$ quantitative formation of the corresponding adduct **4a** resulting from conjugate addition of the secondary amine to the nitroolefin was observed (Scheme 3-6, right) No such adduct has been observed between nitrostyrene and the peptidic catalysts H-D-Pro-Pro-Glu-NH_2 (**1a**) even at high excesses of the nitroolefin demonstrating that the properties of **1a** cause the equilibrium of this aza-Michael addition to be on the side of the free catalyst. In the presence of pyrrolidine, both species **3a** and **4a** are likely to be involved in the catalytic reaction. While adduct **4a** probably plays a more prominent role at the beginning of the reaction (when nitroolefin concentration is high) the product enamine **3a** becomes more relevant towards its end (when product concentration is maximal). Both processes sequester the catalyst from the catalytic cycle and are potentially an alternative explanation for the linear conversion-time curves observed in the presence of pyrrolidine. Furthermore, in addition to the protonation of the nitronate intermediate, both the hydrolysis of the product enamine as well as the β-elimination of adduct **4a** to free the catalyst and the nitrostyrene are also acid catalyzed and thus in agreement with the positive influence of an acidic co-catalyst on the reaction rate.

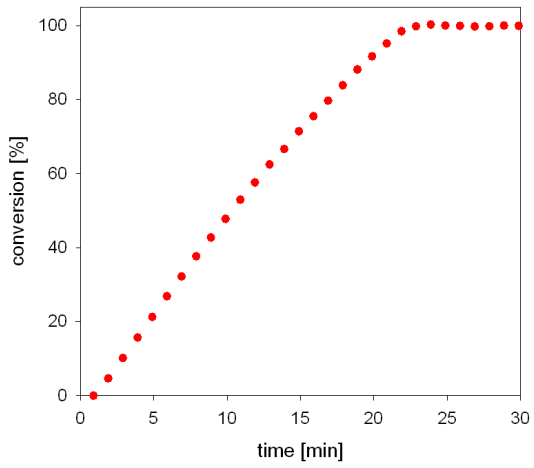
Despite this additional mechanistic complexity in the presence of pyrrolidine, at least when proline is used as a catalyst, the conclusions of Scheme 3-4 regarding the two different pathways and rate limiting steps depending on the presence and absence of a carboxylic acid in close proximity to the reaction centre that can protonate the iminium nitronate intermediate **N** appears to be general and not restricted to peptidic catalysts.

3.2.5 Reactions of Methyl 3-Nitroacrylate

3-Nitroacrylates are interesting substrates for conjugate addition reactions with aldehydes because the resulting γ-nitroaldehydes bearing an ester moiety are highly functionalized compounds that are useful intermediates in the synthesis of complex molecules.[140-141] In comparison to β-aryl or β-alkyl substituted nitroolefins, they are much more electrophilic due to the electron withdrawing ester group in the β-position. When butanal was allowed to react with methyl 3-nitroacrylate in the presence of 1 mol% of the peptide H-D-Pro-Pro-Glu-NH_2 (**1a**) the reaction proceeded to completion within only 20 min. Additionally the corresponding conversion-time curve was linear (Figure 3-8). This means that the reaction rate does not depend on the substrate concentration and thus excludes the C-C bond formation as the rate determining step of the reaction even though peptide **1a** bearing an intramolecular carboxylic acid moiety is used.

The reason for this linear conversion-time curve is most likely the conjugate addition of the secondary amine of the catalyst to the highly electrophilic nitroolefin. Indeed, when **1a** was





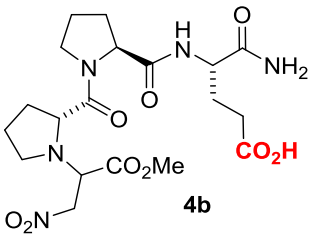


Figure 3-8 Conversion-time curve of the conjugate addition reaction of butanal to methyl 3-nitroacrylate in the presence of peptidic catalyst **1a**; structure of adduct **4b**.

mixed with a small excess of methyl 3-nitroacrylate (3 eq) in $CDCl_3$, the corresponding adduct **4b** was observed upon NMR-spectroscopic analysis of the mixture. Analogous adducts between 3-nitroacrylates and the catalyst have also been reported in studies with the Hayashi-Jørgensen catalyst.[142] Especially under catalytic conditions, where the nitroolefin is in large excess with respect to the catalyst, formation of this adduct as the catalyst resting state is highly likely. As a consequence, the catalyst is drawn off the catalytic cycle and the β-elimination of the adduct liberating the catalyst is the rate determining step of the reaction. Neither of the substrates is involved in this elimination step and, as a consequence, the reaction rate does not depend on their concentrations and the resulting conversion-time curve is linear.

This finding demonstrates that care needs to be taken when drawing general conclusions regarding the rate determining steps of reactions in the presence of **1a** when special substrates such as highly activated nitroolefins are used.

3.2.6 Catalyst Promoted Epimerization of the Reaction Product

Secondary amine catalyzed conjugate addition reactions between aldehydes and nitroolefins often have to be worked up directly after complete conversion is reached because the product γ-nitroaldehydes are prone to epimerization when they are left in contact with the catalyst for longer times until finally a thermodynamic ratio of approximately 60:40 in favour of the *syn* diastereoisomer is reached.[131-132,134] Such an epimerization is probably the result of the formation of a product enamine followed by unselective hydrolysis leading to products with an epimerized centre in the (2)-position. In this context it has been suggested[134] that in the presence of the Hayashi-Jørgensen catalyst the formation of a cyclobutane as the resting state of the catalyst helps to avoid product epimerization in the course of the reaction. Since no such cyclobutane intermediate is populated to a significant extent when the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) is used as a catalyst the catalyst promoted epimerization was investigated next.

A diastereomerically pure sample of the γ-nitroaldehyde derived from butanal and nitrostyrene was treated with **1a** (1 or 10 mol%) and the diastereomeric ratio was followed

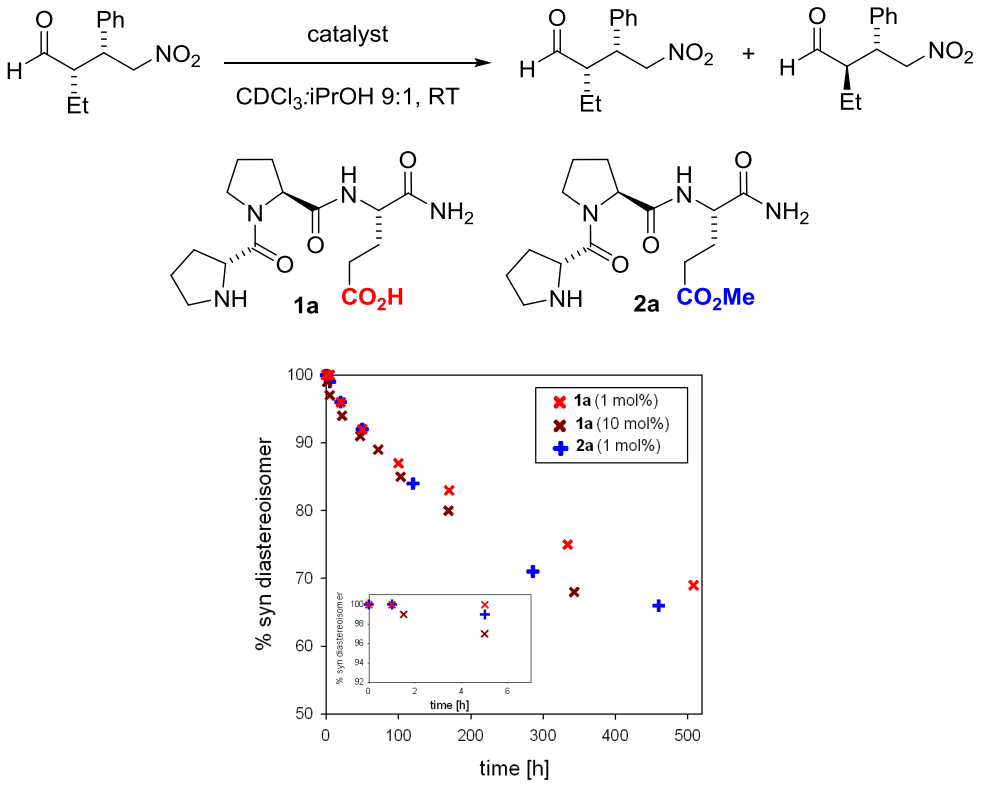


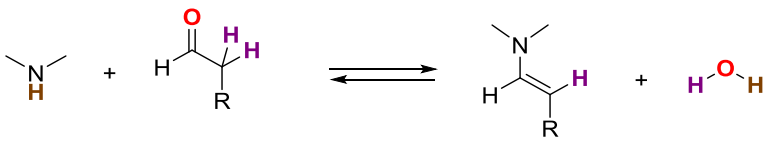
Figure 3-9 Catalyst promoted epimerization of the reaction product.

over time by ^{1}H-NMR spectroscopy (Figure 3-9). Additionally, to probe for an effect of the intramolecular carboxylic acid the methyl ester H-D-Pro-Pro-Glu(OMe)-NH_2 (**2a**) was also investigated (1 mol%, Figure 3-9). In all three reactions the diastereoselectivity gradually decreased over extended reaction times. Epimerization proceeded slightly faster at higher catalyst loadings or with the methyl ester peptide **2a**, with which formation of the product enamine is more favoured (Figure 3-9). However, the long reaction times necessary for high degrees of epimerization in the presence of **1a** together with the high configurational stability of the γ-nitroaldehyde during the first 20 hours of the reaction especially at low catalyst loading (Figure 3-9, inlet) suggest that catalyst promoted product epimerization during the conjugate addition reactions can be neglected for this system.

3.3 Investigations on the Enamine Formation Step by Isotope Labelling Experiments

The formation of an enamine intermediate upon reaction of the secondary amine of the peptide H-D-Pro-Pro-Glu-NH_2 (**1a**) with an aldehyde is the first step in the proposed catalytic cycle of conjugate addition reactions (Scheme 3-4). Unfortunately, the detection of this intermediate is difficult because the corresponding equilibrium is strongly on the side of the free catalyst and aldehyde when stoichiometric or nearly stoichiometric ratios of the reagents

are used.[130] However, as the formation of an enamine between a secondary amine and an aldehyde involves loss of a water molecule, enamine formation can be monitored indirectly by isotope labelling experiments (Scheme 3-7). For example, treatment of an aldehyde with the peptidic catalyst in protic deuterated solvents should allow for the indirect observation of enamine formation by a H/D exchange of the protons in the α-position of the aldehyde. Similarly, reactions conducted in the presence of ^{18}O-labelled water should result in the incorporation of the label into the product.



Scheme 3-7 Enamine formation between an aldehyde and a secondary amine.

3.3.1 H/D-Exchange

In order to investigate the H/D exchange of the α-protons of butanal in the presence of H-D-Pro-Pro-Glu-NH_2 (**1a**), butanal was treated with 20 mol% of the peptide in a 9:1 mixture of $CDCl_3$ and CD_3OD. In this solvent, the aldehyde was found to be present as a 4:1 mixture with the corresponding dimethyl acetal. In agreement with previous studies, no enamine was observed upon ^{1}H-NMR spectroscopic analysis of the reaction mixture. Strikingly, however, already after a reaction time of 10 min nearly quantitative exchange of the protons in the α-position were observed, both in the free aldehyde as well as the acetal (Figure 3-10).

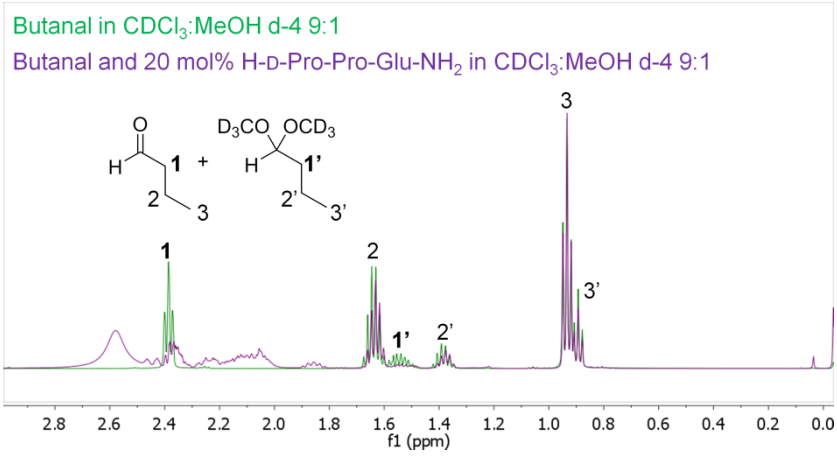


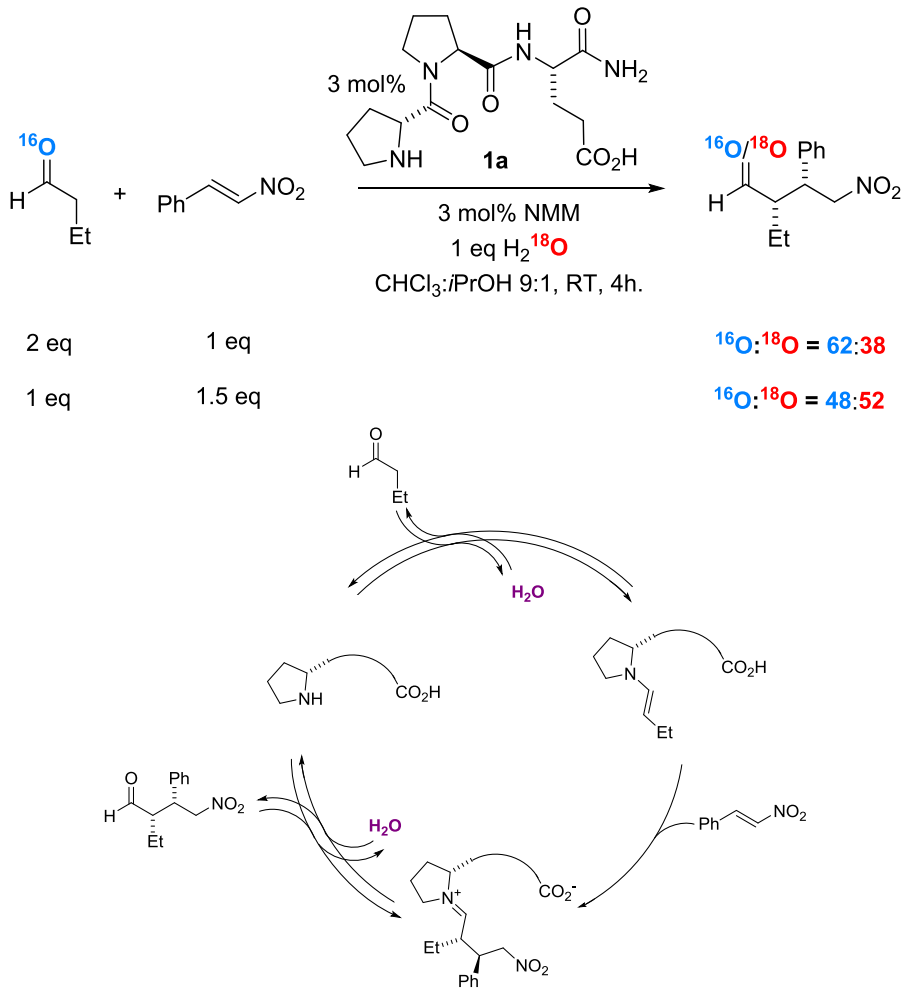
Figure 3-10 H/D-Exchange at the α-position of the aldehyde observed in the presence of peptide catalyst **1a**.

This result demonstrates that all the equilibria, the one between the aldehyde and the enamine as well as the one between the aldehyde and the corresponding acetal are fast and thus suggests that in catalytic conjugate addition reaction the enamine formation is not rate determining.

3.3.2 ^{18}O-Incorporation

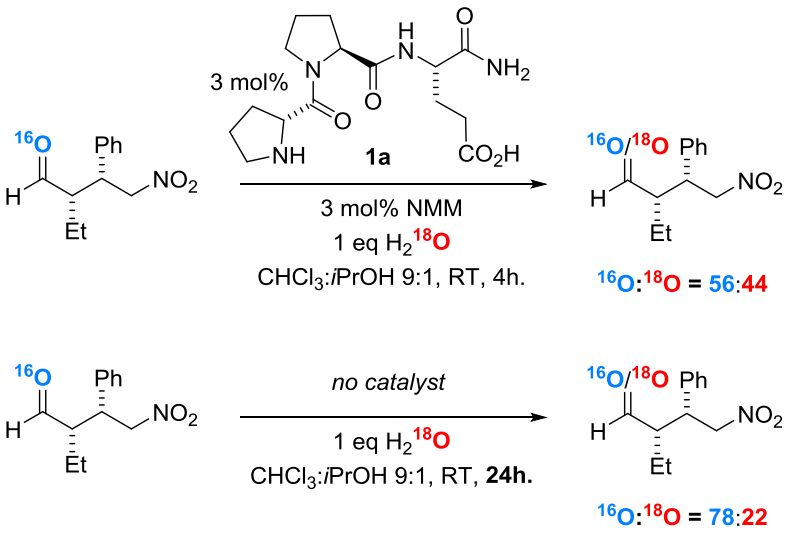
If the peptide catalyzed conjugate addition reaction proceeds through an enamine and when the reaction is performed in the presence of ^{18}O-labelled water, incorporation of the ^{18}O-label into the product γ-nitroaldehyde is expected. Similar studies have been reported by Alexakis for conjugate addition reactions between aldehydes and nitrodienes in the presence of the Hayashi-Jørgensen catalyst in an aqueous system.[143] They conducted the reaction in ^{18}O-labelled water and found a significantly lower incorporation of ^{18}O than would have been expected from the excess of the ^{18}O-label present in the reaction. Their conclusion was that in this case an enol-mechanism is operative (for further studies regarding a possible enol mechanism see 3.4). In order to address this question and to gain further insight into the enamine formation step, the peptide catalyzed conjugate addition reaction between butanal and nitrostyrene was investigated under rigorously dried conditions and in the presence of one equivalent of ^{18}O-labelled water. The ratio of ^{16}O and ^{18}O incorporated into the γ-nitroaldehyde was investigated by ESI-MS analysis (Scheme 3-8). Indeed, incorporation of the label into the reaction product was observed and in the presence of two equivalents of butanal, the γ-nitroaldehyde was obtained after 4 h with a ^{16}O:^{18}O ratio of 62:38. Use of one equivalent of butanal resulted in a ratio of 48:52 (Scheme 3-8, top). These ratios reflect the stoichiometry of the ^{16}O-aldehyde with respect to the ^{18}O-water and demonstrate that the entire label is scrambled between these two species in the course of the reaction presumably through fast equilibria between the aldehyde and the corresponding enamine both on the level of butanal as well as the γ-nitroaldehyde (Scheme 3-8, bottom). Indeed, control experiments studying the incorporation of the label into the γ-nitroaldehyde showed that while the background incorporation in the absence of the peptidic catalyst was slow (^{16}O:^{18}O ratio of 78:22 after 24 h), scrambling of the label was observed when the γ-nitroaldehyde was treated with the peptide in the presence of ^{18}O-labeled water (^{16}O:^{18}O ratio of 56:44 after 4 h, Scheme 3-9).

These results clearly demonstrate that there is a rapid enamine formation between the catalyst and both the substrate aldehyde as well as the product γ-nitroaldehyde. Thus the findings



Scheme 3-8 Conjugate addition reaction of butanal to nitrostyrene in the presence of **1a** and 1 equivalent of $H_2{}^{18}O$ (top). Catalytic cycle and the steps water is involved (bottom).

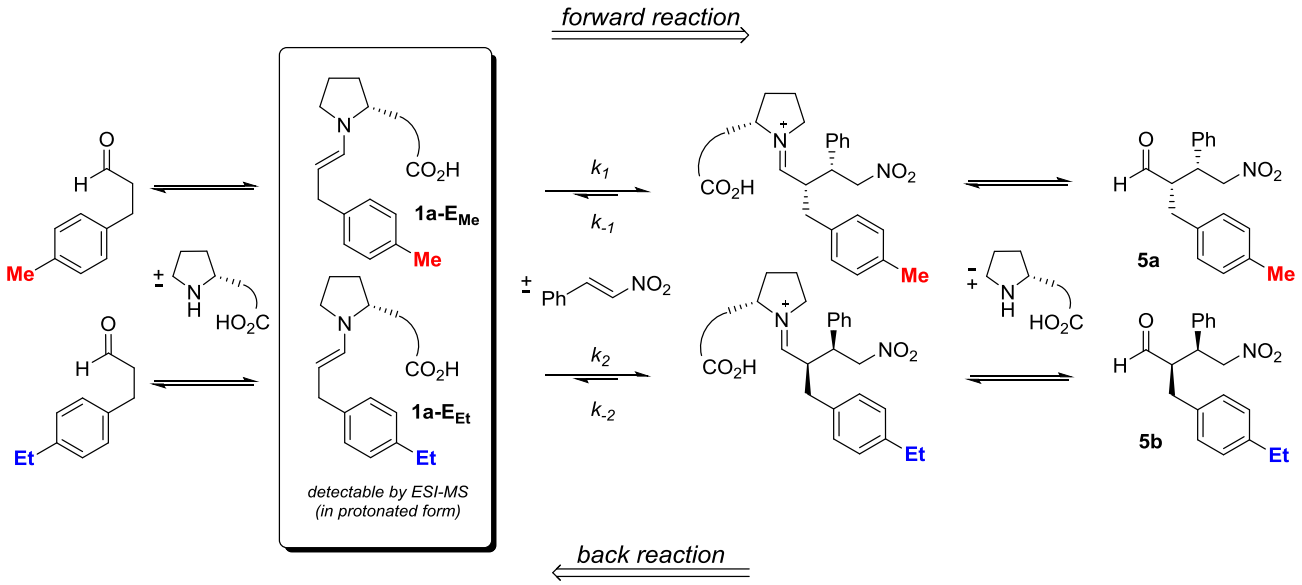
are in agreement with an enamine mechanism. The observation of complete scrambling of the label is indicative of a rapid enamine formation in a pre-equilibrium before the rate limiting C-C bond formation and further underlines the notion that enamine formation is not the rate determining step of the reaction. However, since in principle product enamine formation could be responsible for the observed incorporation of the ^{18}O-label into the γ-nitroaldehyde even if this product was initially formed in an enol mechanism, more sophisticated experiments are necessary to further clarify this issue (see Section 3.4).



Scheme 3-9 Control experiments demonstrating catalyst promoted incorporation of the ^{18}O label into the γ-nitroaldehyde.

3.4 Determination of the Selectivity Determining Step Using an ESI-MS Back Reaction Screening

ESI-MS back-reaction screening using mass-labelled pseudo-enantiomeric substrate mixtures is a powerful tool developed by Pfaltz and co-workers for the rapid determination of the intrinsic enantioselectivity of catalysts and has been successfully applied to the screening of catalysts and catalyst mixtures in a variety of metal-catalyzed as well as organocatalytic reactions.[144-150] Rather than studying the reaction of two prochiral substrates in the forward direction, in this elegant method the reverse reaction of a racemic mixture of pseudo-enantiomeric compounds differing in an appropriately positioned mass label is examined (Scheme 3-10). Based on the principle of microscopic reversibility, which states that the transition state of the forward reaction is identical to the one of the reverse process, the ratio of the intensities of two intermediates derived from the two pseudo-enantiomeric substrates reflects the intrinsic selectivity of the examined catalyst ($s_1 = s_{-1} = k_{-1}/k_{-2} = I(\mathbf{1a}\text{-}E_{Me})/I(\mathbf{1a}\text{-}E_{Et})$, Scheme 3-10). While this method has so far been successfully applied to the identification of highly enantioselective members within catalyst libraries, it should also be a valuable tool to answer mechanistic questions such as whether an enamine or an enol mechanism is active in the peptide catalyzed conjugate addition reaction between aldehydes and nitroolefins (see also chapter 3.3.2). In case of an enamine mechanism and if the attack of the enamine onto the nitroolefin is the selectivity determining step of the reaction, upon reaction of a 1:1 mixture of the two pseudo-enantiomeric γ-nitroaldehydes **5a** and **5b** in the



Scheme 3-10 Principle of the selectivity determination by ESI-MS back reaction screening in the conjugate addition reaction of pseudo-enantiomeric 3-arylpropanals and nitrostyrene in the presence of **1a**.

presence of a catalytic amount of the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) the selectivity of the preparative reaction in the forward direction is expected to be reflected in the ratio of signals corresponding to the two mass-spectrometrically distinguishable enamines **1a-E$_{Me}$** and **1a-E$_{Et}$** (Scheme 3-10).

In order to shed light on these mechanistic questions an ESI-MS back reaction screening was conducted for the peptide catalyzed conjugate addition reaction between aldehydes and nitroolefins in collaboration with Christian Ebner,[151] Florian Bächle and Prof. Andreas Pfaltz.

3.4.1 Investigations on the Forward Reaction

As a first step, the peptide catalyzed conjugate addition reaction between aldehydes and nitroolefins was studied in the forward direction in order to investigate whether the relevant intermediates can be observed using ESI-MS-techniques. Thus the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) was allowed to react with 10 equivalents of 3-phenylpropanal in a 9:1 mixture of chloroform and *iso*-propanol, the solvent mixture of choice for catalytic reactions in the forward direction. After a reaction time of 1 min the mixture was diluted with methanol and investigated with ESI-MS (Figure 3-11, a). In the resulting mass spectrum most signals corresponded to the peptide, which is observed in the protonated form, as the sodium adduct and as a protonated dimeric species. Additionally, the sought-after signal of the protonated

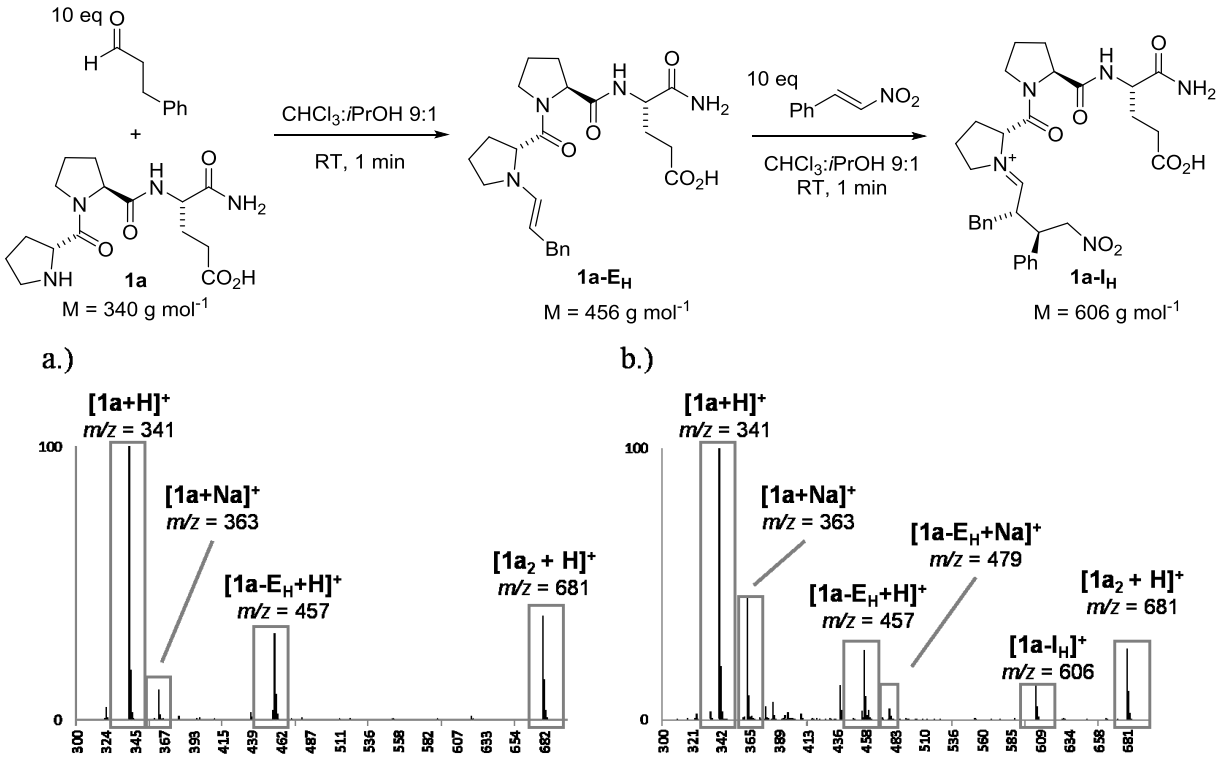
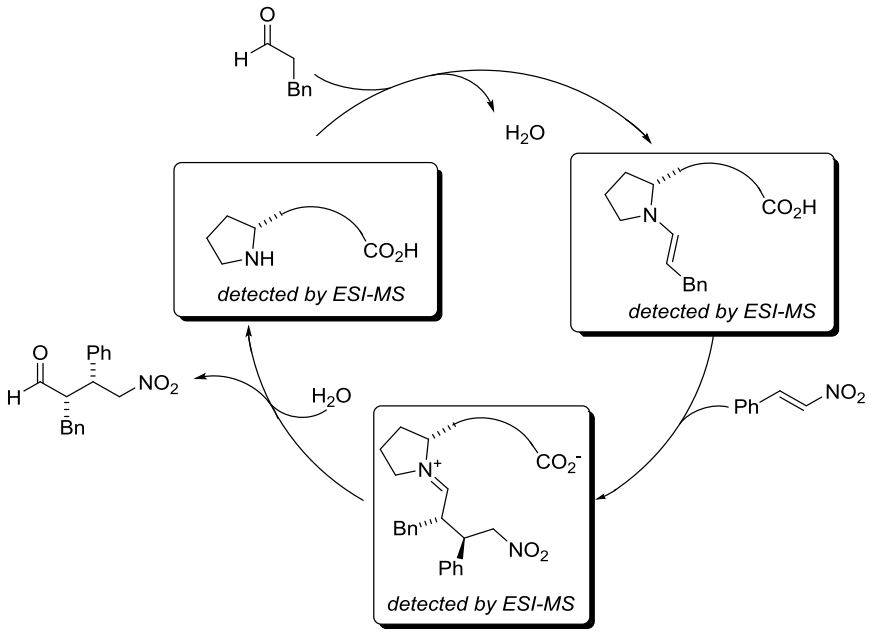


Figure 3-11 ESI-MS investigation of the forward reaction between 3-phenylpropanal and nitrostyrene in the presence of catalyst **1a**. (a) before addition of the nitroolefin, (b) after addition of the nitroolefin.

enamine **1a-E$_H$** was also observed. Next, additional 10 equivalents of nitrostyrene were added to the reaction under otherwise identical conditions. When the mixture was analyzed by ESI-MS a new signal corresponding to the iminium ion **1a-I$_H$** was observed in addition to the



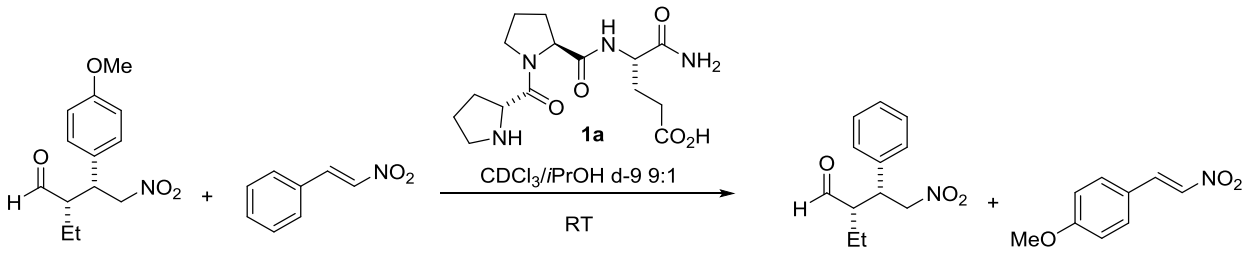
Scheme 3-11 Intermediates detected by ESI-MS analysis of the forward reaction.

catalyst and enamine signals (Figure 3-11, b). Thus by studying the reaction in the forward direction, all the intermediates of the catalyst proposed for an enamine mechanism were observed by ESI-MS (Scheme 3-11).

3.4.2 Reversibility of the Reaction

A prerequisite for a successful ESI-MS back reaction screening is that the reaction under study is reversible. Thus, the reversibility of the reaction of a γ-nitroaldehyde in the presence of the peptidic catalyst **1a** was next probed in a ^{1}H-NMR spectroscopic experiment. The γ-nitroaldehyde resulting from the conjugate addition reaction of butanal to *trans*-4-methoxy nitrostyrene was mixed with an equimolar amount of nitrostyrene in the presence of 2 mol% of the catalyst **1a** (Scheme 3-12). If the reverse reaction takes place, the enamine resulting after C-C bond cleavage is expected to preferentially react with nitrostyrene rather than the liberated 4-methoxy nitrostyrene, because the former is more electrophilic than the latter.[25] Thus, it should be possible to monitor the back reaction by the formation of free 4-methoxy nitrostyrene even if it only occurs to a small extent.

Indeed in this experiment the back reaction occurred and after an extended reaction time of two weeks approximately 20 % conversion to the cross-over product and the free 4-methoxy nitrostyrene was observed. However, the back reaction was found to be a slow process since on the time scale of the catalytic reaction (up to 48 h) the release of 4-methoxy nitrostyrene was determined to be less than 5 %. This finding demonstrates that whereas the reverse reaction is a too slow process to be considered relevant for preparative catalytic reactions, the conjugate addition is in principle a reversible process that can be followed by ESI-MS, a highly sensitive method that can also detect intermediates that are only present in low concentrations.



Scheme 3-12 Experimental setup for probing the reversibility of peptide catalyzed conjugate addition reactions between aldehydes and nitroolefins.

3.4.3 Investigations on the Reverse Reaction

After all the relevant intermediates of the peptide catalyzed conjugate addition reaction were observed by ESI-MS when studying the reaction in the forward direction and having shown that the reaction is in principle a reversible process, next the reverse reaction of a γ-nitroaldehyde with the catalyst was investigated. The γ-nitroaldehyde derived from the conjugate addition reaction of 3-phenylpropanal with nitrostyrene was treated with catalyst **1a** under the same reaction conditions used in the forward reaction. Besides signals corresponding to the peptide the iminium ion **1a-I$_H$** as well as enamine **1a-E$_H$** were observed in the mass spectrum (Figure 3-12). However, the intensity of the signal of the enamine was only very low. The detection of such a peptide derived enamine intermediate in the back reaction is challenging for several reasons: (1) the equilibrium between the iminium **I** and the enamine and nitroolefin strongly lies on the side of the iminium ion; (2) the equilibrium of enamine-hydrolysis also lies on the side of the aldehyde and the free catalyst; (3) the enamine formed in the process is a neutral species and thus needs to be protonated to be detectable by ESI-MS. If this protonation does not occur to a sufficiently high extent the enamine might still be formed but cannot be detected by ESI-MS. And (4) the stability of an enamine strongly depends on the solvent and is arguably lowered by the protic solvent mixture $CHCl_3$/*i*-PrOH 9:1. Several attempts were undertaken to increase the signal to noise ratio. When carboxylic acids or inorganic salts which should help to convert the enamine to a charged species

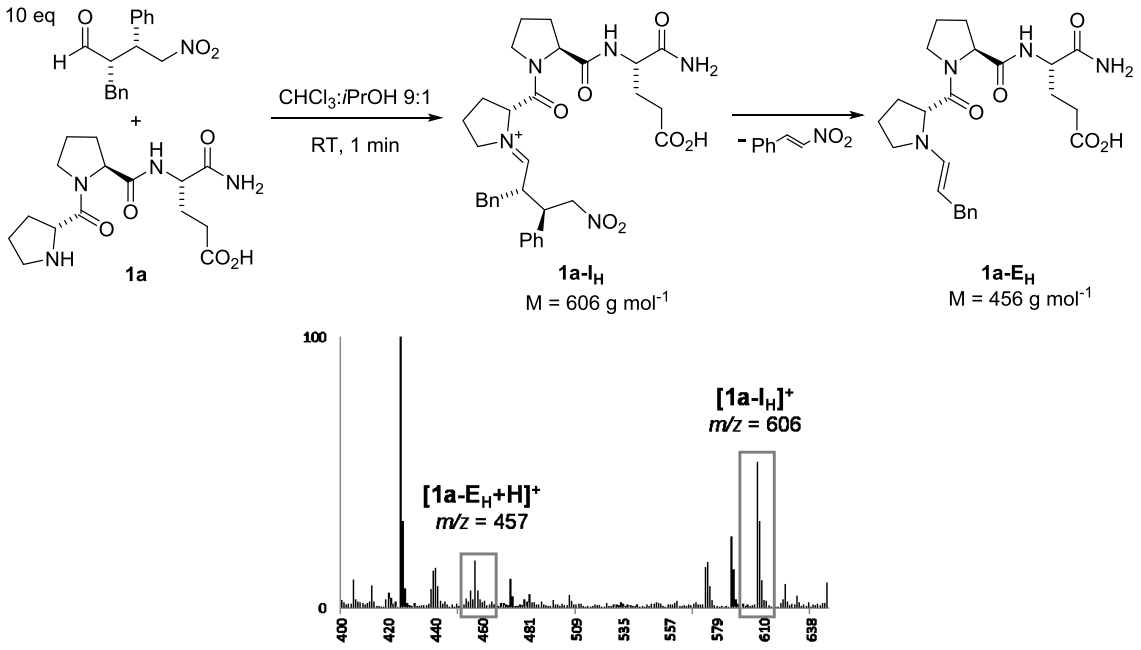


Figure 3-12 ESI-MS investigation of the back reaction.

observable by ESI-MS were added, the enamine signals disappeared completely. Furthermore, attempted trapping of the enamine with the highly reactive electrophile Dimethylmethylideneammonium iodide (Eschenmoser's salt)[152] was also unsuccessful and no mass signals corresponding to the expected adduct were observed in the ESI-MS-spectra.

3.4.4 Back Reaction Screening Using Pseudo-enantiomeric Substrates in the Presence of Different Catalysts of the Type Pro-Pro-Xaa

Even though the enamine was only observed in low quantities in our initial experiments studying the reverse reaction, the ESI-MS selectivity determination of the catalyst using pseudo-enantiomeric mass labelled substrates was attempted next. For this purpose, mass labelled pseudo-enantiomeric substrates were designed. The mass labels were placed on the aldehyde-derived part of the addition product, since the label needs to be incorporated into the enamine intermediate which ought to be detected by ESI-MS. Ethyl and methyl substituents were installed in the *para*-position of the phenyl ring since this position was envisioned to be far away from the reaction centre and not to influence the selectivity of the reaction. The mass-labelled pseudoenantiomeric γ-nitroaldehydes were synthesized by reacting the two aldehydes 3-(4-methylphenyl) propanal and 3-(4-ethylphenyl) propanal with nitrostyrene in the presence of H-D-Pro-Pro-Glu-NH_2 (**1a**) and H-Pro-D-Pro-D-Glu-NH_2 (*ent*-**1a**) respectively. Both substrates were obtained with the same enantiomeric excess of 97% *ee* demonstrating that the mass labels do not affect the reaction course.

When an equimolar solution of the pseudo-enantiomeric γ-nitroaldehydes **5a** and **5b** in $CHCl_3$:*i*PrOH 9:1 was exposed to the catalyst and the reaction mixture was analyzed by ESI-MS, the signals of the two iminiums **1a-I_{Me}** and **1a-I_{Et}** were observed together with the signals corresponding to the catalyst (Figure 3-13). Unfortunately, however, the detection of the enamine intermediate proved again to be difficult and in this experiment no signal corresponding to the two expected enamines was observed.

In order to increase the signal intensity of the enamine installation of a permanent charge tag within the catalyst structure was first examined. The tripeptidic catalyst **1h** bearing an imidazolium group connected to the 4-position of proline by a pentanoic acid linker was therefore prepared. Unsatisfactorily, even though **1h** provided the product in the preparative

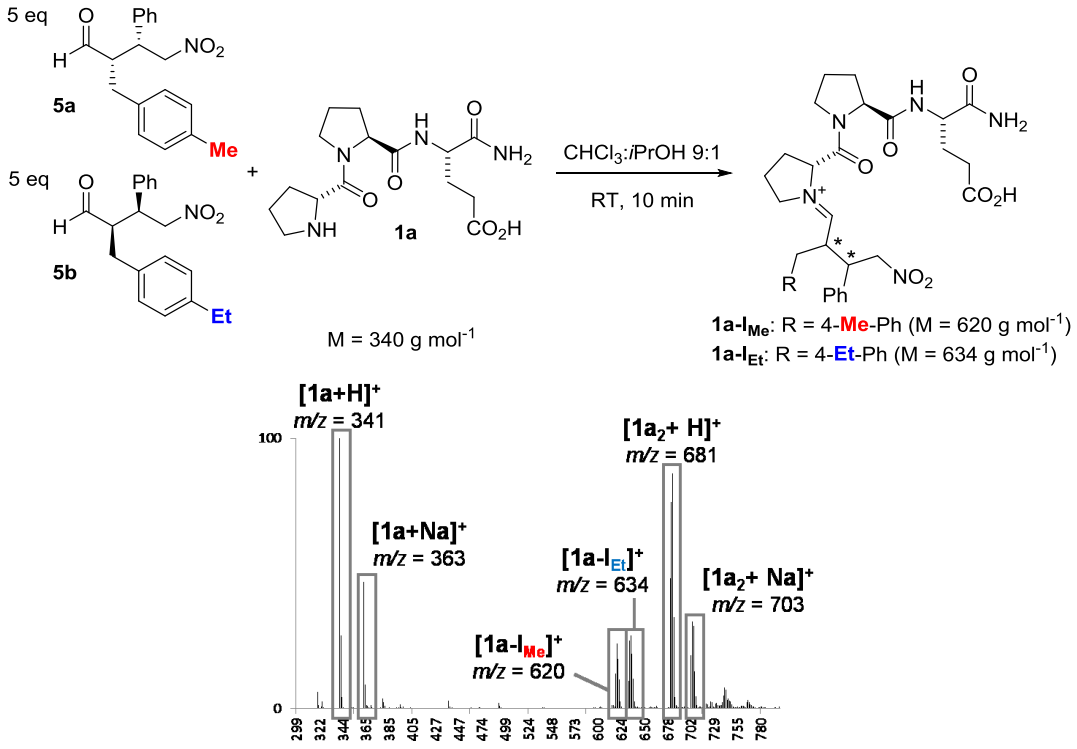
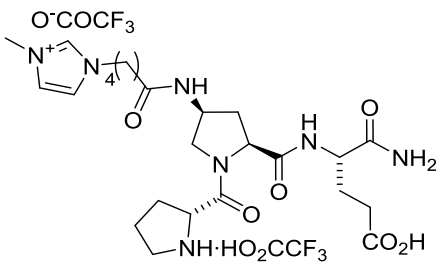


Figure 3-13 ESI-MS back reaction screening using mass labelled substrates in $CHCl_3$:*i*PrOH 9:1.

reaction with a good yield of 95 % after a reaction time of 22 h (dr = 92:8, 92% *ee*), none of the proposed catalytic intermediates, neither the product iminium nor the enamine, were observed with this catalyst. This is presumably due to the low solubility of this peptide in the reaction medium or a detrimental effect of the permanent charge on the stability of the intermediates.

Next we decided to study the more soluble peptidic catalyst H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (**1b**) in toluene with the hope that the enamine intermediate might be visible in a non-protic solvent in which enamines are known to be more stable. Indeed, when catalyst **1b** was treated with a solution containing a 1:1 mixture of the two mass labelled pseudo-enantiomeric substrates, the signal of the enamine **1b-E$_{Me}$** was observed in the mass spectrum



1h

(Figure 3-14). This enamine intermediate bearing the methyl label is derived from the γ-nitroaldehyde with the (2*S*,3*R*)-configuration, the enantiomer preferentially formed in the presence of catalyst **1b**. The intensity of the mass peak of enamine intermediate **1b-E$_{Et}$** derived from the opposite enantiomer of the γ-nitroaldehyde was again very low and the signal disappeared in the noise of the mass spectrum. While this makes it impossible to quantitatively determine the enantioselectivity of catalyst **1b**, the signal to noise ratio of 85:15 for the peak of **1b-E$_{Me}$** signifies that the corresponding enantiomeric excess must be higher than 70% *ee* (Figure 3-14). This was confirmed by the preparative reaction under the same reaction conditions, where the γ-nitroaldehyde was isolated with an enantiomeric excess of 97% *ee*.

DMSO is known to be the solvent of choice, when enamines derived from catalysts bearing a carboxylic acid group within their structure are desired. For example, the NMR-spectroscopic characterization of proline derived enamines has only been reported in this solvent.[153-154] We thus hypothesised that the use of DMSO as the reaction solvent in the ESI-MS back-reaction screening could improve the intensity of enamine derived signals in the mass spectrum.

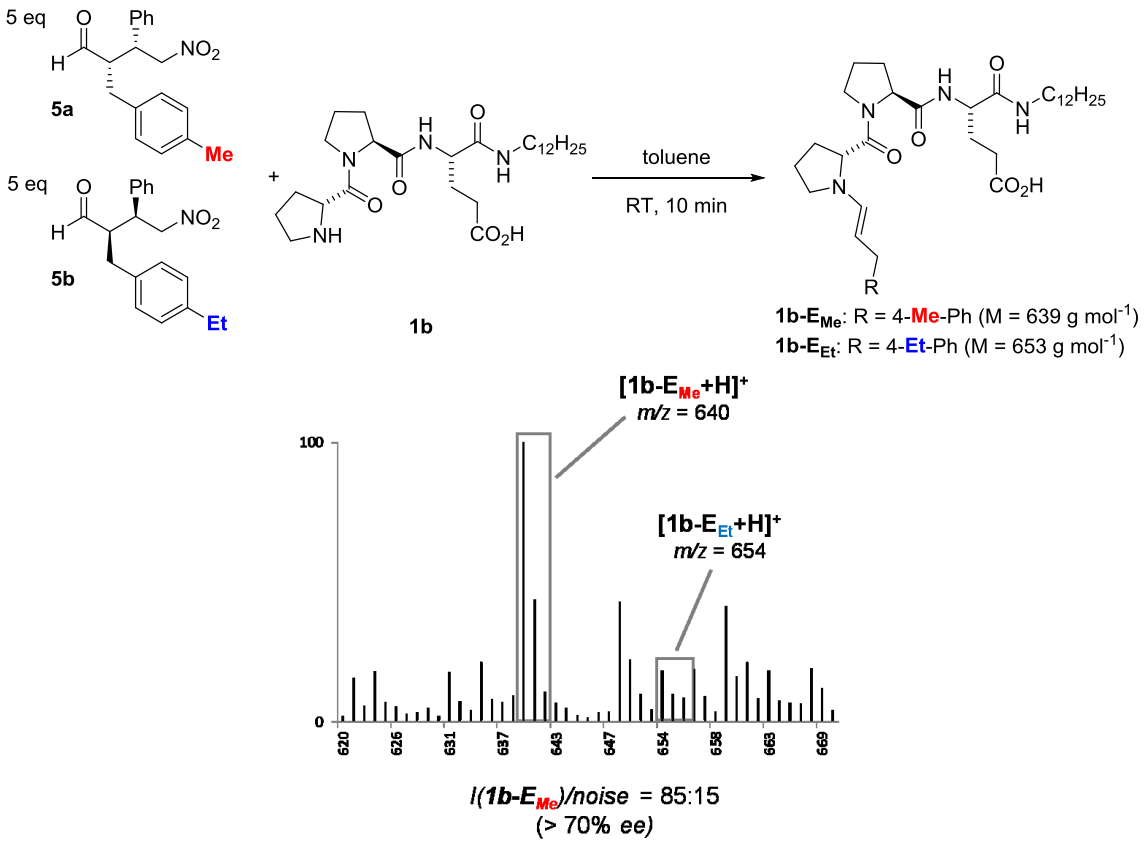


Figure 3-14 ESI-MS back reaction screening using peptide **1b** in toluene.

Furthermore, based on previous studies,[130] the selectivity of the peptide catalyzed conjugate addition reaction was expected to be lower using DMSO as solvent. A lower selectivity results in higher signal intensity of the enamine derived from the minor enantiomer and could thus further facilitate the ESI-MS based selectivity determination.

Indeed, when catalyst **1a** was allowed to react with the pseudo-enantiomeric substrates in DMSO and the back-reaction was monitored by ESI-MS, in addition to signals corresponding to the free catalyst and iminium ions **1a-I$_{Me}$** and **1a-I$_{Et}$**, the signals of the two enamines **1a-E$_{Me}$** and **1a-E$_{Et}$** were clearly visible in the mass spectrum (Figure 3-15, a). Their ratio of 73:27 (47% *ee*) matches well with the experimentally observed enantioselectivity of 46% *ee* in the forward reaction under otherwise identical conditions. The fact that the intrinsic selectivity of the attack of the enamine onto the nitroolefin determined by ESI-MS matches the stereochemical outcome of the preparative reaction shows that in the presence of peptide **1a** the reaction proceeds via an enamine rather than an enol intermediate and that indeed this C-C bond formation reaction is the selectivity determining step of the reaction.

To further probe the generality of these observations, the back reaction of the two pseudo-enantiomeric substrates was also followed by ESI-MS in the presence of the diastereoisomeric peptide catalyst **1i** (H-Pro-Pro-D-Glu-NH_2), the regioisomer **1j** (H-Pro-Pro-D-Gln-OH) as well as derivative **1b** bearing an aliphatic chain at the C-terminus (Figure 3-15). Peptides **1i** and **1j** were chosen because, based on previous studies,[130] they were expected to provide the γ-nitroaldehydes with the opposite absolute configuration and in a lower enantiomeric excess than peptide **1a**. In all three cases, the most intense mass-signals observed in the screening corresponded to the free catalysts and the iminium ions **I$_{Me}$** and **I$_{Et}$** (Figure 3-15, b - d). In addition, the protonated enamines **E$_{Me}$** and **E$_{Et}$** were detected in the presence of all three catalysts. Ratios of 34:66 and 36:64 in favour of the expected (2*R*,3*S*)-**5b** derived enamine **E$_{Et}$** were observed for peptides **1i** and **1j** respectively, whereas for catalyst **1b** a ratio of 76:24 favouring the enamine **1b-E$_{Me}$** was obtained. Based on these results peptidic catalysts **1i** and **1j** were expected to preferentially provide the (2*R*,3*S*)-enantiomer of the γ-nitroaldehyde with *ee*-values of 32% and 28% in the forward reaction under otherwise identical conditions. An enantiomeric excess of 52% for the (2*S*,3*R*)-enantiomer was expected in the presence of **1b**. Indeed, when the corresponding catalytic reactions were performed, the (2*R*,3*S*)-configured γ-nitroaldehyde was formed predominately and with an enantiomeric excess of 30% in cases of **1i** and **1j** (Table 3-1). In the presence of **1d** a preparative enantiomeric excess of 51% was

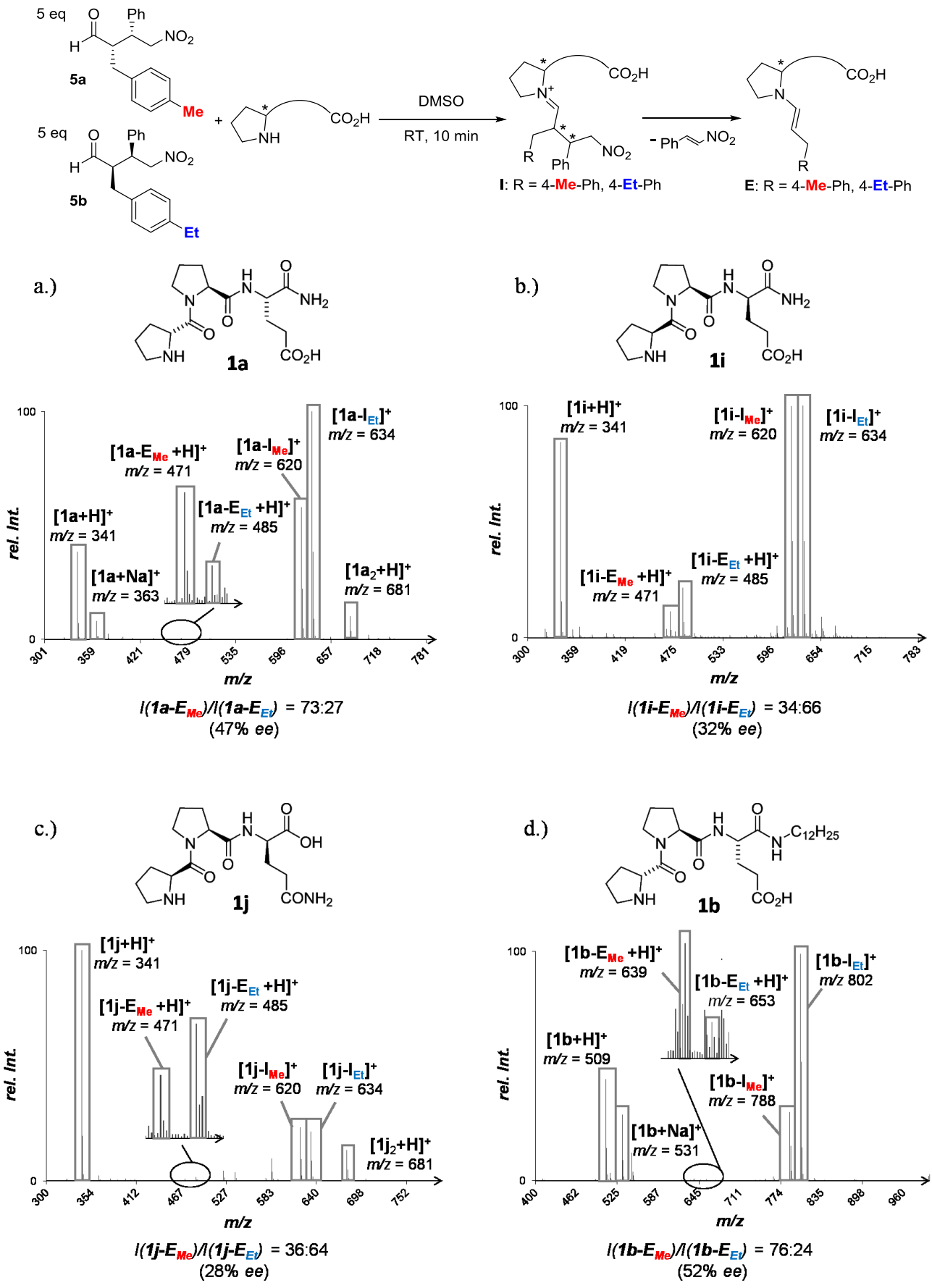


Figure 3-15 ESI-MS back reaction screening using mass-labelled substrates in DMSO in the presence of peptidic catalysts **1a**, **1i**, **1j** and **1b**.

Table 3-1 Comparison of the intrinsic selectivity of the enamine attack onto the nitroolefin determined by ESI-MS back reaction screening and the corresponding enantiomeric excess obtained in the preparative reaction in forward direction.

	catalyst	theoretical *ee* based on ESI-MS back reaction screening	*ee* obtained in preparative reaction in forward direction
1	**1a**	47% *ee*, (2*S*,3*R*)-enantiomer	46% *ee*, (2*S*,3*R*)-enantiomer
2	**1i**	32% *ee,* (2*R*,3*S*)-enantiomer	30% *ee,* (2*R*,3*S*)-enantiomer
3	**1j**	28% *ee,* (2*R*,3*S*)-enantiomer	30% *ee,* (2*R*,3*S*)-enantiomer
4	**1b**	52% *ee*, (2*S*,3*R*)-enantiomer	51% *ee* (2*S*,3*R*)-enantiomer

obtained ((2*S*,3*R*)-enantiomer). These values are again in excellent agreement with the selectivities determined by ESI-MS in terms of both, the absolute configuration of the major enantiomer as well as the enantiomeric excess of the formed product (Table 3-1). Interestingly, in the presence of peptides **1a** and **1b** a 1:2 ratio of iminium ions $\mathbf{I_{Me}}/\mathbf{I_{Et}}$ was determined (Figure 3-15, a and d). This demonstrates that the catalyst preferentially reacts with the (2*R*,3*S*)-configured γ-nitroaldehyde to form $\mathbf{I_{Et}}$. Nonetheless, on the level of the enamine a preference for the expected intermediate $\mathbf{E_{Me}}$ derived from the (2*S*,3*R*)-configured γ-nitroaldehyde was obtained. The ratios of the iminium ions $\mathbf{1i\text{-}I_{Me}}/\mathbf{1i\text{-}I_{Et}}$ as well as $\mathbf{1j\text{-}I_{Me}}/\mathbf{1j\text{-}I_{Et}}$ were found to be 1:1 (Figure 3-15, b and c). These findings demonstrate that the observed ratio of enamines reflects the selectivity of the C-C bond formation step and is not affected by a preference of the catalyst towards one of the pseudo-enantiomeric γ-nitroaldehydes in the formation of the iminium intermediates $\mathbf{I_{Me}}$ and $\mathbf{I_{Et}}$.

3.4.5 Comparison to Catalysts Lacking an Intramolecular Carboxylic Acid Group

For the Hayashi-Jørgensen catalyst Blackmond has suggested that the formation of a stable cyclobutane as the resting state of the catalyst in conjugate addition reactions between aldehydes and nitroolefins has not only an effect on catalyst turnover but also an important implication on the stereoselectivity of the reaction.[132] Since the rate determining step of the reaction is the protonation of the nitronate **N** (Scheme 3-2) and because the C-C bond formation is reversible, the enantioselectivity of the overall reaction was proposed to be correlated with the relative stability and reactivity of diastereoisomeric downstream intermediates such as cyclobutanes rather than determined by the C-C bond formation step where the stereogenic centres are originally created.[132] In order to probe for potential differences in the selectivity determining step between catalysts with and without an

intramolecular carboxylic acid group the ESI-MS back reaction screening was performed in the presence of the Hayashi-Jørgensen catalyst as well as the methyl ester peptide **2b** under otherwise identical conditions (Figure 3-16 and Figure 3-17).

In the resulting mass spectra, for both catalysts signals corresponding to the free catalysts, the enamines $\mathbf{E_{Me}}$ and $\mathbf{E_{Et}}$ as well as adducts between the catalysts and the pseudo-enantiomeric substrates were observed. Mass spectrometrical distinction between an iminium ion **I** and a protonated cyclobutane **C** is difficult because both species have the same mass. However, in the presence of the methyl ester peptide **2b** and the Hayashi-Jørgensen catalyst the signals corresponding to this adduct (**I** or **C**) between the catalyst and the pseudo-enantiomeric

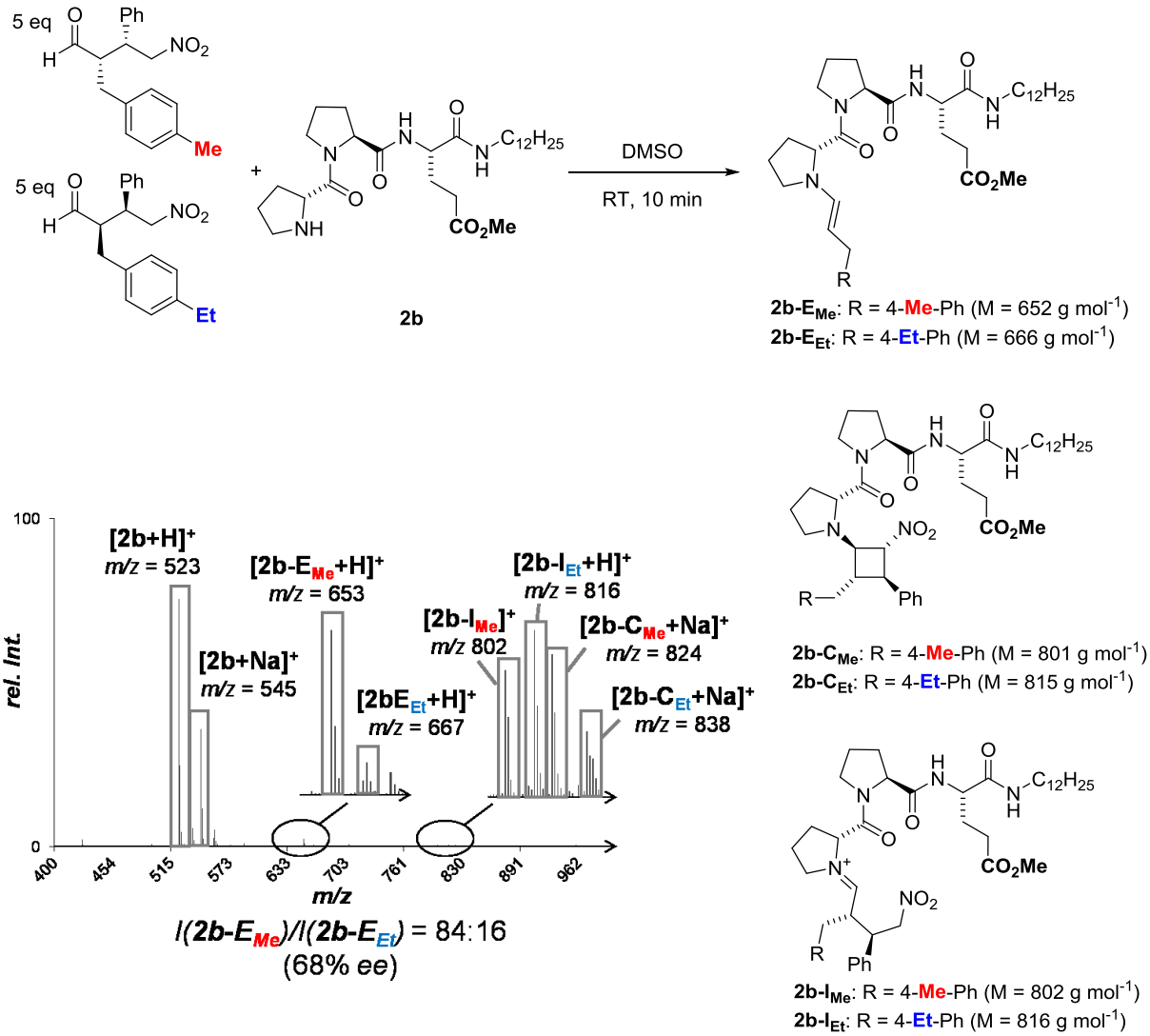


Figure 3-16 ESI-MS back reaction screening in DMSO in the presence of methyl ester peptide **2b**.

substrates were observed with a very low intensity. This intensity increased by a factor of 10 when the reaction mixture was diluted with methanol containing acetic acid (0.02 %) prior to injection into the mass spectrometer. Furthermore, in the case of peptide **2b** the signals of the protonated species as well as the corresponding sodium adduct were observed. Both, the low intensity as well as the observed sodium adducts suggest that in the presence of catalysts lacking an intramolecular carboxylic acid moiety, these primary products formed upon reaction of the catalyst with the pseudo-enantiomeric substrates are neutral cyclobutanes $\mathbf{C_{Me}}$ and $\mathbf{C_{Et}}$ rather than the positively charged iminium ions $\mathbf{I_{Me}}$ and $\mathbf{I_{Et}}$.

The enamine signals were readily observed with a good signal to noise ratio in the presence of both, peptide **2b** and the Hayashi-Jørgensen catalyst. Interestingly, in the presence of the methyl ester peptide **2b**, the two enamines were detected in a ratio of 84:16. From this selectivity of the C-C bond breaking step an enantiomeric excess of 68% favouring the formation of the γ-nitroaldehyde with (2*S*,3*R*)-configuration is expected for the preparative reaction if this step is selectivity determining. However, when the preparative reaction was carried out in DMSO the (2*S*,3*R*)-γ-nitroaldehyde was isolated with a significantly different

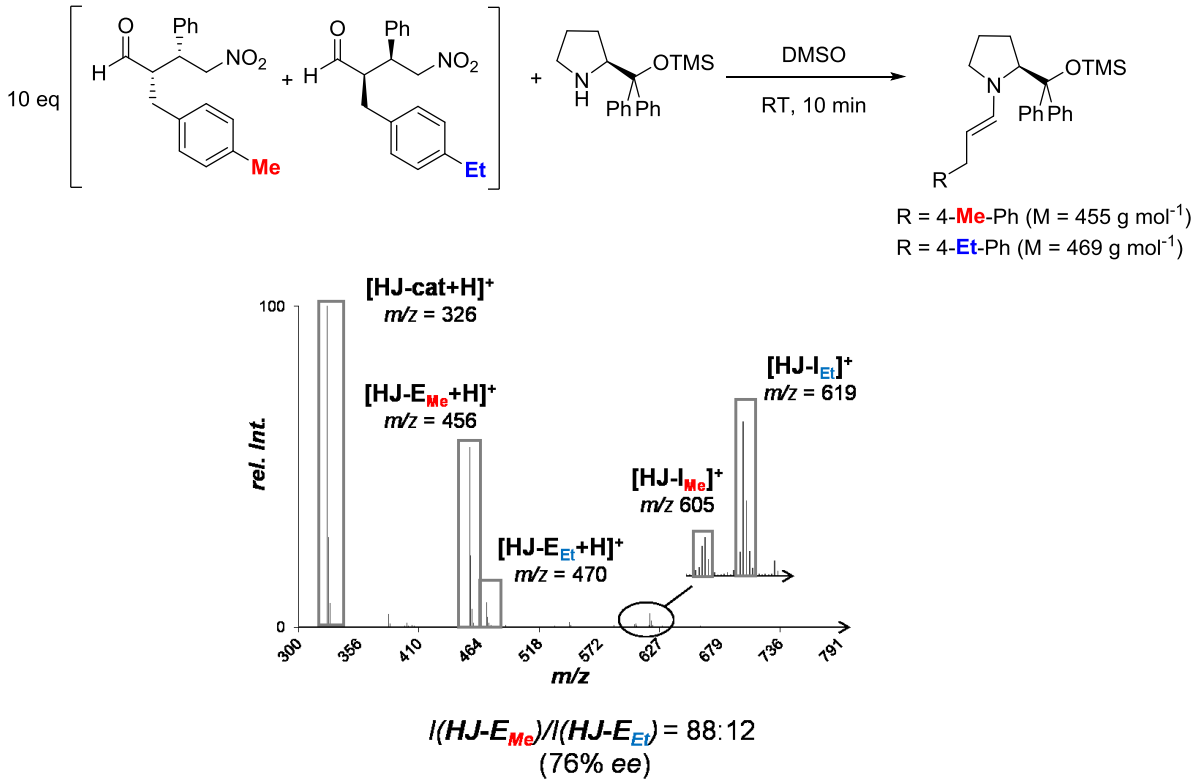


Figure 3-17 ESI-MS back reaction screening in DMSO in the presence of the Hayashi-Jørgensen catalyst.

enantiomeric excess of 49%. Even more strikingly, in the presence of the Hayashi-Jørgensen catalyst the enantioselectivity of the C-C bond formation determined from the ratio of the two enamine signals is 76% *ee* in favour of the (2*S*,3*R*)-configured γ-nitroaldehyde. However, the catalytic reaction in forward direction provided the corresponding γ-nitroaldehyde not only with a significantly different enantiomeric excess (63% *ee*) but also gave the opposite (2*R*,3*S*)-enantiomer as the major stereoisomer.

The formation of neutral cyclobutanes rather than a positively charged iminium species as the primary adduct between the catalyst and the substrates together with the difference in the selectivity of the conjugate addition step and the preparative reaction in forward direction, suggests that, in agreement with the suggestions made in case of the Hayashi-Jørgensen catalyst,[132] in the presence of catalysts lacking an intramolecular carboxylic acid moiety the enantiomeric excess of the reaction product might not just be determined by the selectivity of the C-C bond formation step but is also influenced by the relative stability of diastereomeric downstream intermediates such as cyclobutanes.

3.5 Conclusions

The tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) is a highly active and selective catalyst for organocatalytic conjugate addition reactions of aldehydes to nitroolefins. Its high catalytic activity, allowing for reactions to take place at catalyst loadings as low as 0.1 mol%, is particularly remarkable and has not been achieved with other amine based organocatalysts. In order to gain a better understanding of the reaction mechanism and the features that render **1a** such an exceptionally successful catalyst various mechanistic studies were performed.

Kinetic and NMR-spectroscopic investigations comparing catalysts H-D-Pro-Pro-Glu-NHR with their methyl ester analogues H-D-Pro-Pro-Glu(OCH_3)-NHR revealed that the presence or absence of a suitably positioned carboxylic acid moiety within the catalyst structure controls the reaction pathway and thereby the rate limiting steps of conjugate addition reactions between aldehydes and nitroolefins. If the catalyst bears a carboxylic acid group the zwitterionic iminium nitronate intermediate is protonated intramolecularly. As a result, this protonation is fast and C-C bond formation between the enamine and the nitroolefin is the rate determining step of the reaction. In contrast, in the presence of catalysts lacking an intramolecular acid moiety this intermediate collapses to a cyclobutane – a stable resting state of the catalyst. In consequence, ring opening and protonation of the resulting zwitterion is limiting the rate of the overall reaction. This knowledge allowed for influencing the reaction pathways and rate determining steps by manipulations in the system: If a catalyst bears a carboxylic acid group, but not in a position allowing for favourable coordination and protonation of the nitronate the pathway via a cyclobutane intermediate is favoured. Likewise, in the presence of strong carboxylic acid additives C-C bond formation is rate determining even when catalysts lacking an intramolecular acid group are used. Knowledge of the distinct differences in the rate determining steps and the factors that control them is crucial for future catalyst design and optimization because only parameters affecting the rate determining step will improve the overall reaction. For example, acidic additives or a protic environment can improve the reaction rate only when protonation of the nitronate is the rate limiting step but are either mere spectators or slow the reaction down if C-C bond formation is rate determining. In this case the use of conditions that favour enamine formation (e.g. use of dried reagents and solvents) leads to faster reactions. Furthermore, these insights also offer an explanation why H-D-Pro-Pro-Glu-NH_2 (**1a**) is such an exceptionally active catalyst even at very low catalyst loadings. When a catalyst lacking an intramolecular acid group is used in very low loadings it is trapped as a cyclobutane and limited catalyst turnover leads to low conversions. In contrast, the intramolecular carboxylic acid group of **1a** not only stabilizes the

transition state of the C-C bond formation by coordinating the developing nitronate but also ensures efficient protonation of the short-lived zwitterionic intermediate and thereby avoids formation and accumulation of cyclic intermediates leading to a significantly higher turnover frequency.

Direct spectroscopic observation of an enamine intermediate derived from the peptide **1a** is difficult as the equilibrium between aldehyde/**1a** and the enamine strongly lies on the side of the free species. Thus further mechanistic investigations were conducted to elucidate whether reactions in the presence of **1a** proceed through an enamine or an enol intermediate. Isotope labelling experiments revealed that the aldehyde oxygen as well as the α-protons are rapidly exchanged under the reaction conditions. Further evidence for the involvement of an enamine in the C-C bond formation with the nitroolefin was obtained by ESI-MS studies. Not only the enamine but also all other intermediates proposed for an enamine mechanism were readily observed in ESI-MS spectra. In addition, back reaction screening using equimolar mixtures of pseudo-enantiomeric substrates demonstrated that the intrinsic selectivity of the attack of the enamine onto the nitroolefin matches the enantiomeric excess observed for the γ-nitroaldehydes obtained in the preparative catalytic reactions, strong evidence for an enamine mechanism. An identical screening with catalysts lacking an intramolecular proton donor demonstrated that in this case the induction of enantioselectivity might be more complicated and possibly involves intermediates such as cyclobutanes.

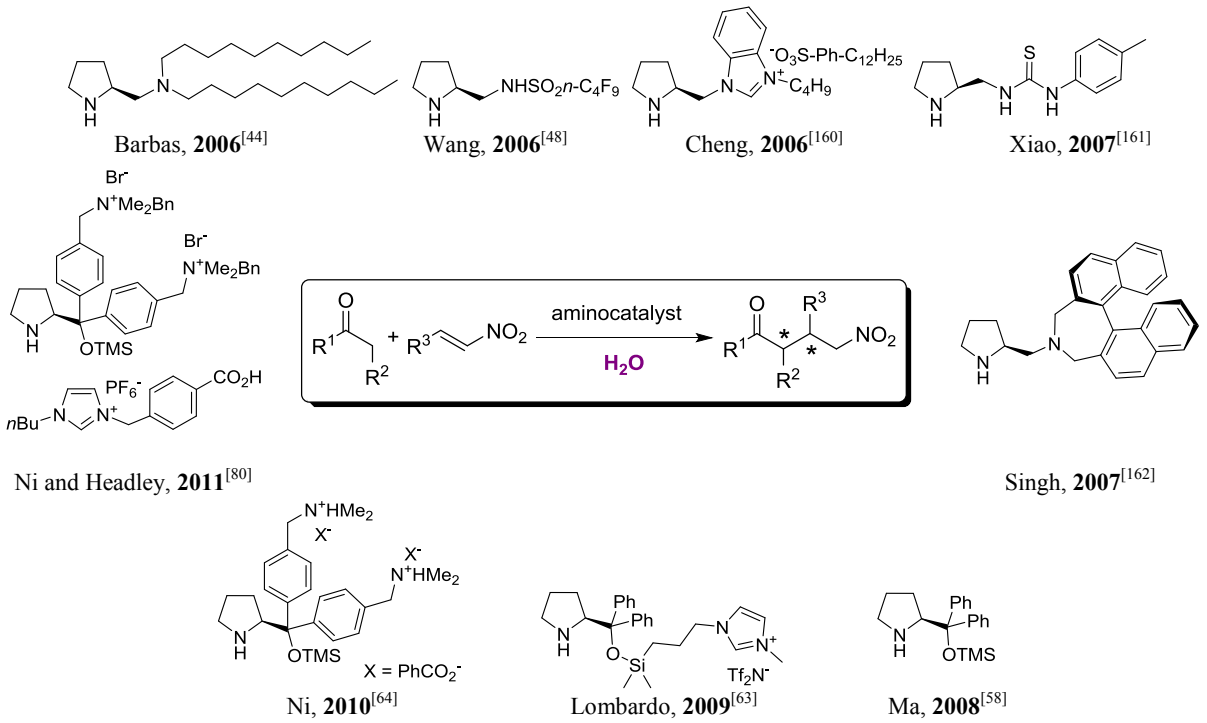
4

Peptide Catalysis in Aqueous Reaction Media

4.1 Background

Short peptides are of low molecular weight but, at the same time, consist of the same amino acid building blocks as enzymes. Thus, it is a highly interesting question if and in how far enzymes can be mimicked by their low molecular weight analogues. In addition to their high activity and selectivity, tripeptides of the type Pro-Pro-Xaa (Xaa = arbitrary amino acid bearing a carboxylic acid group) also display features like high chemoselectivity and substrate specificity that are generally attributed to enzymes.[92] Yet, while the peptide catalysts only perform well in organic solvents, enzymes operate under physiological aqueous conditions. However, the active sites of enzymes are often buried within hydrophobic pockets and, thus, the enzyme catalyzed reactions actually take place in a hydrophobic environment rather than in water.[155] This led us to hypothesise that conjugation of a suitable hydrophobic moiety to the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) might provide for an organic microenvironment that allows for peptide catalyzed conjugate addition reactions in an aqueous system. Furthermore, catalytic applications of peptides in C-C bond formation reactions in water are particularly interesting in light of their potential involvement in prebiotic chemistry, the development of *homo*-chirality and as intermediates in the evolution of enzymes.

The development of organocatalytic processes that use water as the reaction medium has attracted widespread attention over the last years because water is the most abundant solvent on earth, non-toxic, non-flammable and easy to handle and thus generally considered as a "green solvent".[155-159] Since the conjugate addition reaction of carbonyl compounds to nitroolefins is one of the most useful and widely researched organocatalytic transformations, the development and optimization of secondary amine catalysts that allow for this reaction to take place in an aqueous medium has received considerable research interest. [155,157-159] In this context, several catalysts have been reported that provide the corresponding γ-nitro carbonyl compounds in good yields and stereoselectivities (Scheme 4-1).[44,48,58,63-64,80,160-162] As the water-solubility of the substrates is generally low, the reaction mixtures are inhomogeneous and the reactions take place in or at the surface of a highly concentrated organic phase. Consequently, the secondary amine catalysts that perform best in an aqueous medium are amphiphilic, allowing for their solubility in the organic phase as well as stabilization of



Scheme 4-1 Selected examples of amphiphilic secondary amine organocatalysts for the conjugate addition reaction of aldehydes to nitroolefins under aqueous conditions.

an emulsion of the two phases. Being one of the most successful organocatalysts for the conjugate addition reaction between aldehydes and nitroolefins in organic solvents, it is highly desirable from a methodological point of view to investigate whether the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) or its analogues can be used as catalysts in conjugate addition reactions in water.

Based on both, this general interest in increasing the application range of the peptidic catalyst **1a** by making it water compatible as well as the special interest in peptide catalysis in water, we investigated peptide **1a** and some amphiphilic analogues thereof as catalysts in conjugate addition reactions between aldehydes and nitroolefins in an aqueous medium.

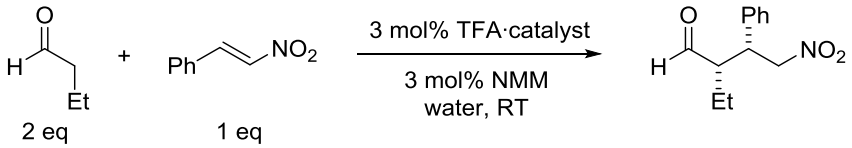
4.2 Reaction Development and Optimization

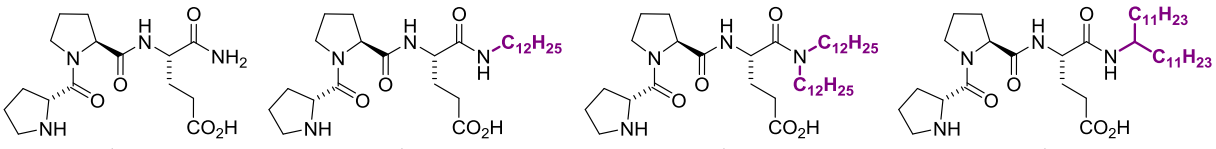
4.2.1 Screening of Amphiphilic Peptidic Catalysts

In order to evaluate the catalytic properties of the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) as well as amphiphilic analogues thereof as catalysts for conjugate addition reactions in an aqueous medium the 1,4-addition of butanal to nitrostyrene was chosen as a test reaction (Table 4-1). In a first experiment, the substrates were allowed to react in the presence of a catalytic amount of the peptide **1a** using water as the reaction medium. The resulting reaction mixture was heterogeneous, consisting of a slurry of the substrates in the aqueous solvent. Only a sluggish conversion was observed (28 % conversion after 96 h) but the product was isolated with an excellent diastereomeric ratio of 97:3 and a moderate enantiomeric excess of 73 % (Table 4-1, entry 1). Use of a catalytic amount of the detergent sodium dodecyl sulfate (SDS) together with the catalyst **1a** led to the emulsification of the insoluble starting materials in water. However, the reactivity and the selectivity were low (Table 4-1, entry 2). These initial results demonstrated that the conjugate addition reaction in an inhomogeneous aqueous reaction mixture is a challenging transformation and suggested that more sophisticated catalysts than the simple tripeptide were necessary to achieve water-compatibility. Based on the hypothesis that peptides combining catalytic activity with the capability to stabilize an emulsion of the substrates and the aqueous reaction medium might be particularly suitable catalyst candidates for these reactions in water, a variety of amphiphilic analogues of the peptide H-D-Pro-Pro-Glu-NH_2 (**1b**, **1g**, **1k-1p**) were synthesised and tested as catalysts in the conjugate addition reaction between butanal and nitrostyrene (Table 4-1).

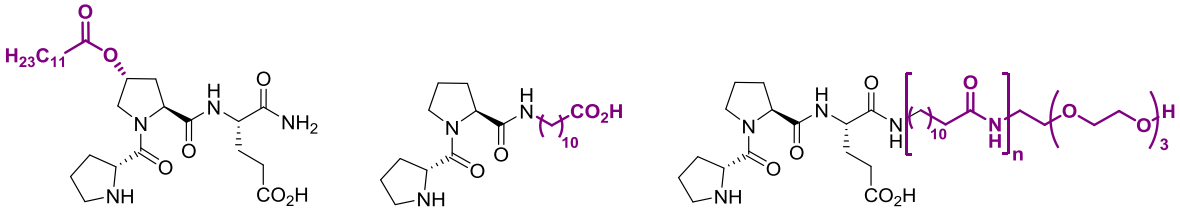
As the simplest amphiphilic analogue of **1a** the tripeptide H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (**1b**) bearing an *n*-dodecyl chain at the C-terminus of the peptide was investigated. In order to probe the catalytic performance of more hydrophobic analogues, peptides **1k**, with two *n*-dodecyl chains and a tertiary amide at the C-terminus, as well as **1l**, an example of a more hydrophobic peptide maintaining a secondary amide at the C-terminus, were examined. As the C-terminal amide hydrogen atoms of **1a** are known to be important for the conformational properties as well as the catalytic performance of this peptide,[92] analogue **1m**, where a hydrophobic alkyl chain is grafted to the 4-position of the second proline via an ester linkage was prepared and tested. Finally, peptides **1g** and **1n** – **1p**, where the hydrophobic part is embedded between two hydrophilic parts, were also synthesised and used as catalysts in conjugate addition reactions of butanal to nitrostyrene.

Table 4-1 Conjugate addition reaction between butanal and nitrostyrene in water in the presence of amphiphilic peptide catalysts.





1a **1b** **1k** **1l**



1m **1g**

1n (n=1)
1o (n=2)
1p (n=3)

	Catalyst	Time [h]	Conversion [%][a]	dr[a]	*ee* [%][b]
1	**1a**	96	28	97:3	73
2	**1a** + 3 mol% SDS [c]	96	20	97:3	50
3	**1b**	8	> 95	98:2	91
4	**1k**	18	> 95	96:4	85
5	**1m**	19	>95	97:3	80
6	**1l**	14	>95	97:3	87
7	**1g**	36	>95	92:8	83
8[f]	**1n**	18	20	95:5	86
9	**1o**	20	>95	97:3	87
10	**1p**	20	>95	97:3	71

a Determined by ^{1}H-NMR analysis of the crude reaction mixture. b Determined by chiral-phase HPLC analysis. c SDS = sodium dodecyl sulfate. f 20% conversion after 18 h.

Interestingly, the simple analogue **1b** bearing a single *n*-dodecyl chain attached *via* a secondary amide to the C-terminus, turned out to be the best catalyst providing the corresponding γ-nitroaldehyde in quantitative conversion within 8h and with excellent diastereo- (dr = 98:2) and enantioselectivity (91 % *ee*, Table 4-1, entry 3). This represents a dramatic increase in the enantioselectivity achieved compared to the parent catalyst **1a** (compare entries 1 and 2 with entry 3 in Table 4-1) and clearly demonstrates the positive effect of the introduction of a hydrophobic moiety on the enantioselectivity when the reaction is carried out in water. The more hydrophobic analogues **1k** and **1l**, catalyst **1m** with a

primary amide at the C-terminus, as well as analogues **1g** and **1n** – **1o** were all found to be suitable catalysts for the conjugate addition reaction in an aqueous medium and the resulting γ-nitroaldehyde was readily obtained with high stereoselectivity in all cases. Most of the amphiphilic catalysts provided the product with a higher enantiomeric excess than the parent peptide **1a** demonstrating that the introduction of hydrophobic elements into the catalyst is generally beneficial for reactions carried out in water. However, all of these more sophisticated peptides provided the product with a lower enantioselectivity than peptide **1b**.

As mentioned above, when the parent peptide **1a** is used as a catalyst in an aqueous system the substrates form an insoluble slurry (Figure 4-1, left). In contrast, when peptide **1b** is dissolved in water the formation of foam is observed (Figure 4-1, middle) and upon addition of the substrates, a microemulsion is formed, that remains stable throughout the reaction (Figure 4-1, right). Thus, unlike the parent peptide **1a** the amphiphilic nature of e.g. catalyst **1b** provides for the formation and stabilization of a highly concentrated organic microenvironment where the reaction takes place with high selectivity. The appearance of the reaction mixture therefore supports the conclusion that the peptide **1b** acts at the same time as both a catalyst and a detergent (Figure 4-1).

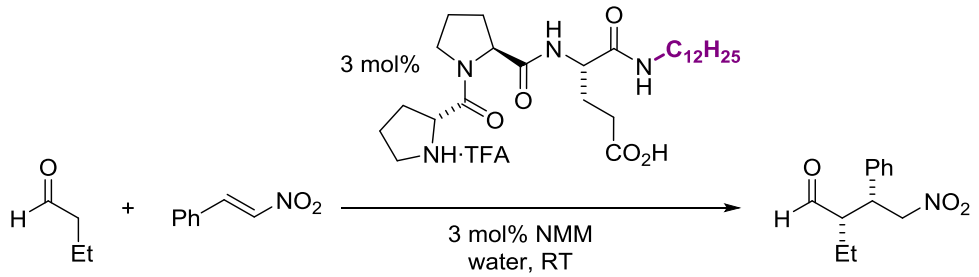
Figure 4-1 Appearance of the reaction mixture of the conjugate addition reaction of butanal to nitrostyrene in water in the presence of **1a** (left), of an aqueous solution of peptide **1b** before (middle) and after (right) the addition of butanal and nitrostyrene.

4.2.2 Optimization of Reaction Conditions

Having identified the amphiphilic tripeptide H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (**1b**) as a suitable catalyst for conjugate addition reactions of aldehydes to nitroolefins in an aqueous reaction medium, optimization of the reaction conditions was next pursued. Since the reaction takes place in an emulsion of the organic starting materials in the aqueous environment, concentration of the substrates as well as their equivalence were expected to influence the outcome of the reactions. As a first parameter the ratio of the reactants was varied (Table 4-2,

entries 1-5). While an excess of one of the reactants was required in order to push the reaction to completion (when the substrates were used in a 1:1 ratio only 80% conversion was observed after 18 h, Table 4-2, entry 2), only a small influence was observed on both, reaction rate as well as stereoselectivity (Table 4-2, entry 1-5). Using a twofold excess of the aldehyde was found to be optimal in order to achieve a high stability of the emulsion. Next the total concentration of both the catalyst as well as the substrates was varied applying a constant twofold excess of the aldehyde with respect to the nitroolefin (Table 4-2, entries 6-9). At concentrations lower than the initially used 0.44 M (Table 4-2, entries 6 and 7) the reactions proceeded significantly slower and with a slightly lower selectivity. When higher concentrations were used (Table 4-2, entries 8 and 9) the reaction mixture was not a stable emulsion anymore and phase separation of an organic and an aqueous phase was observed. Nonetheless, the product γ-nitroaldehydes formed with high conversions already after 2 h albeit with a significant drop in the diastereoselectivity. Thus all further experiments were conducted using a concentration of 0.44 M and 0.88 M of the nitroolefin and the aldehyde, respectively.

Table 4-2 Conjugate addition reaction of butanal to nitrostyrene in the presence of peptide **1b** using different substrate concentration and equivalence.

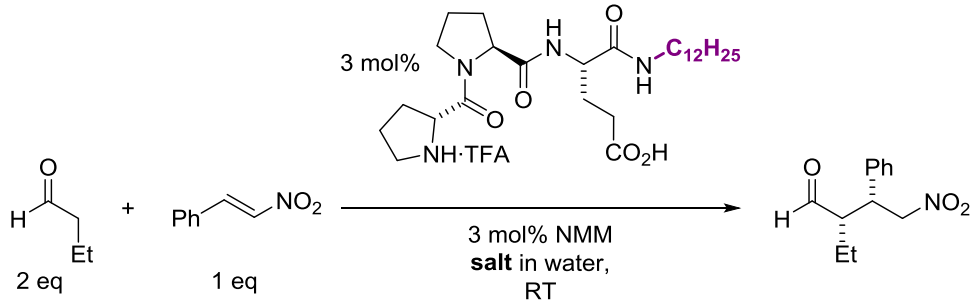


	Butanal conc. [M]	Nitrostyrene conc. [M]	Ratio	Time [h]	Conversion [%][a]	dr[a]	*ee* [%][b]
1	0.88	0.44	2:1	8	>95	98:2	91
2	0.44	0.44	1:1	18	80	96:4	89
3	0.44	0.88	1:2	8	>95	97:3	90
4	1.32	0.44	3:1	8	>95	97:3	91
5	2.20	0.44	5:1	8	>95	97:3	91
6	0.30	0.15	2:1	24	>95	95:5	88
7	0.44	0.22	2:1	24	>95	97:3	90
8[c]	1.76	0.88	2:1	2	80	91:9	91
9[c]	4.40	2.20	2:1	2	90	89:11	90

a Determined by ^{1}H-NMR analysis of the crude reaction mixture. b Determined by chiral-phase HPLC analysis. c The reaction mixture consisted of large droplets in water rather than an emulsion.

One of the appealing features of conducting reactions in an aqueous environment is the possibility of tuning the reaction-medium by the addition of inorganic salts. In order to investigate this parameter, the reaction of butanal to nitrostyrene in the presence of the amphiphilic peptide **1b** was performed in 1 M solutions of NaCl, NH_4Cl, $NaHCO_3$, KOAc, $NaNO_3$ and $NaHSO_4$ (Table 4-3). Additionally, a saturated solution of NaCl was also used as the reaction medium. Neutral and slightly acidic salts had no significant influence on the outcome of the reaction, neither in terms of the reaction rate nor the stereoselectivity (Table 4-3, entries 2-4). In the presence of a strongly acidic salt such as $NaHSO_4$ no conversion was observed at all (Table 4-3, entry 5). Interestingly, when the reactions were performed in the presence of basic salts ($NaHCO_3$ and NaOAc) complete conversion to the γ-nitroaldehyde was observed already after 2 h. However this fast reaction was accompanied by a significant drop in the enantioselectivity (Table 4-3, entries 6 and 7). This finding is indicative of an unselective base promoted background reaction competing with the enantioselective enamine catalysis thereby reducing the observed enantioselectivity.

Table 4-3 Conjugate addition reaction of butanal to nitrostyrene in the presence of catalyst **1b** in aqueous solutions of inorganic salts



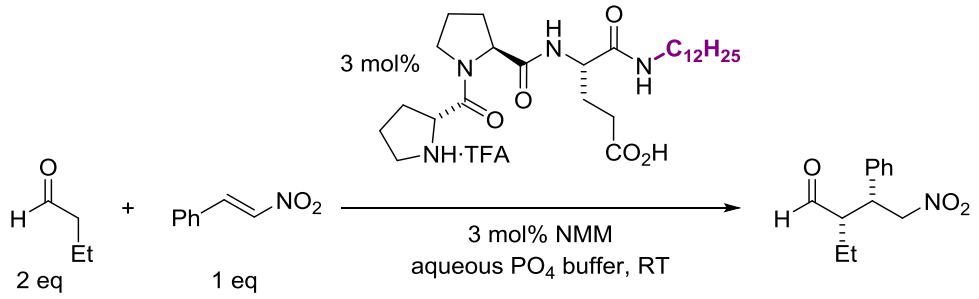
	salt	Time [h]	Conversion [%][a]	dr[a]	*ee* [%][b]
1	*none*	8	>95	98:2	91
2	NaCl	8	>95	97:3	91
3	NaCl (sat.)	8	>95	92:8	91
4	NH_4Cl	8	>95	96:4	90
5[c]	$NaHSO_4$	--	--	--	--
6	$NaHCO_3$	4	>95[d]	94:6	83
7	KOAc	2	>95	90:10	83
8	$NaNO_3$	2	>95	97:3	84

a Determined by ^{1}H-NMR analysis of the crude reaction mixture. b Determined by chiral-phase HPLC analysis. c No reaction. d Large amounts of a precipitate formed in the course of the reaction.

In order to exclude that the enantioselectivity of reactions in the presence of peptide **1b** is corrupted by such an unselective base promoted background reaction, butanal and nitrostyrene were allowed to react in the presence of the peptide using phosphate buffers of different pH and concentrations as the reaction medium (Table 4-4). In general, when neutral or slightly acidic buffer systems were used the resulting reactivities and selectivities were comparable to the results from the non-buffered system (Table 4-4, entries 1 - 5). Also the concentration of the buffer had no influence on the reaction outcome (Table 4-4, entry 3). Only when basic buffer solutions were used, the stereoselectivity dropped to some extent (Table 4-4, entries 6 and 7). This result excludes an unselective background reaction affecting the enantioselectivity of peptide **1b** under standard catalytic conditions in the absence of basic additives.

Next the influence of the temperature on the reaction between butanal and nitrostyrene in the presence of **1b** was investigated (Table 4-5). Upon reduction of the reaction temperature from room temperature to 12°C, 5°C and 0°C a dramatic drop in the reactivity was observed

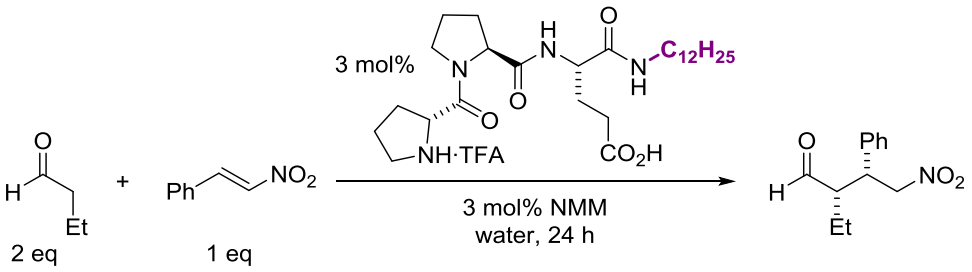
Table 4-4 Conjugate addition reaction between butanal and nitrostyrene in aqueous phosphate buffer



	pH	buffer conc. [mM]	Time[h]	Conversion [%][a]	dr[a]	*ee* [%][b]
1	4.8	10	<36	>95	97:3	90
2	5.0	10	<36	>95	96:4	91
3	5.0	1	8	73	95:5	90
4	5.5	10	8	>95	97:3	90
5	6.5	10	8	>95	96:4	91
6	8.0	10	8	>95	95:5	87
7	8.5	1000	8	>95	95:5	86

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

Table 4-5 Temperature effects on the conjugate addition reaction of butanal to nitrostyrene in the presence of peptide **1b** in water



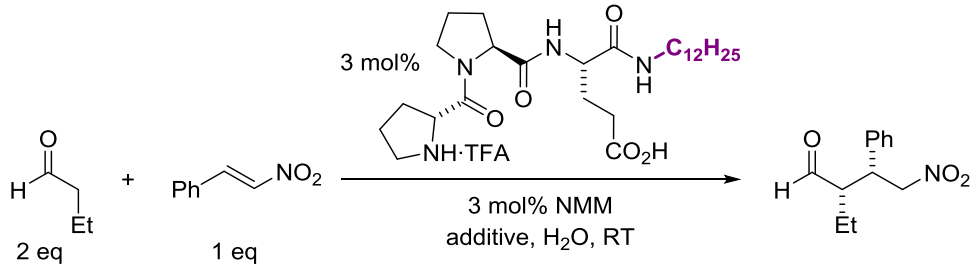
	temp [°C]	conversion [%]	dr[a]	*ee* [%][b]
1	RT	quant.	98:2	91
2	12	57	96:4	90
3	5	12	99:1	91
4	0	5	99:1	90
5	-10	no reaction	--	--

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

(Table 4-5, entries 2-4). Together with this drop in reactivity a concomitant loss of the stability of the emulsion was observed and at lower temperatures (5° C) the appearance of the reaction mixture was not a stable emulsion but rather a slurry of the substrates finely dispersed in the aqueous medium. When the reaction was conducted at -10°C the reaction mixture froze and no reaction took place (Table 4-5, entry 5). Whereas lowering the temperature had a positive effect on the diastereoselectivity of the reaction, the enantiomeric excess remained constant and no improvement was observed (Table 4-5, entries 2-4).

Neither of the parameters examined above in the conjugate addition reaction of butanal to nitrostyrene in the presence of **1b** led to an increase of the enantioselectivity of the reaction and an enantiomeric excess of 91% appeared to be an upper barrier in these aqueous systems. However the peptidic catalyst **1b** provides the γ-nitroaldehyde with an enantioselectivity of 95% *ee* when the organic solvent mixture $CHCl_3$:*i*PrOH 9:1 or toluene is used. Furthermore, a control experiment where butanal and nitrostyrene were allowed to react in the presence of peptide H-D-Pro-Pro-Glu-NH_2 (**1a**) in a highly concentrated hexane solution, imitating potential conditions within a microdroplet, provided the γ-nitroaldehyde with an enantiomeric excess of only 92% (97% *ee* are obtained with this catalyst in $CHCl_3$:*i*PrOH 9:1). We therefore hypothesised that the dilution of the organic phase of the emulsion might be beneficial for the enantioselectivity of the reaction when **1b** is used as a catalyst in aqueous conditions (Table 4-6).

Table 4-6 Conjugate addition reaction of butanal to nitrostyrene in the presence of peptide **1b** in water using organic additives



	additive	Time [h]	Conversion [%][a]	dr[a]	*ee* [%][b]
1	*none*	8	>95	98:2	91
2	lauric acid (10 mol%)	14	>95	93:7	91
3	lauric acid (50 mol%)	14	>95	95:5	90
4	EtOH (10% v/v)	8	>95	99:1	92
5	*i*PrOH (10% v/v)	8	79	99:1	92
6	*t*BuOH (10% v/v)	8	85	99:1	92
7	DMSO (10% v/v)	8	>95	97:3	91
8	acetone (10% v/v)	6	77	97:3	90
9	Et_2O (10% v/v)	<36	>95	95:5	91
10	dioxane (10% v/v)	<36	>95	95:5	91
11	DME (10% v/v)	6	>95	99:1	92
12	THF (10% v/v)	6	68	97:3	92
13	EtOAc (10% v/v)	<36	>95	96:4	91
14	MeCN (10% v/v)	<36	>95	96:4	90
15	$CHCl_3$ (10% v/v)	14	>95	96:4	93
16	toluene (10% v/v)	14	>95	97:3	93
17	PEG (700 g/mol, 100 mg)	5	32	91:9	91
18	PEG (6000 g/mol, 100 mg)	5	17	96:4	94
19	$CHCl_3$ (15% v/v)	5	>95	97:3	95
20	toluene (15% v/v)	14	53	97:3	94

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

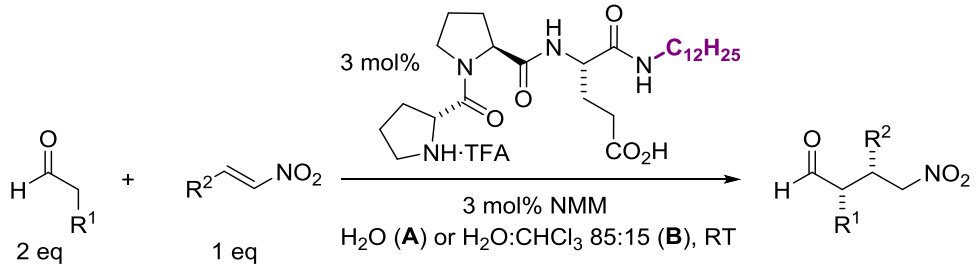
While initial experiments using lauric acid as an additive were not successful (Table 4-6, entries 1 and 2), the addition of 10% v/v of organic solvents to the reaction mixtures led in many cases to an increase in enantiomeric excess (Table 4-6, entries 3-18). In agreement with their success when used as solvents of this reaction, chloroform and toluene were particularly beneficial as additives (Table 4-6, entries 15 and 16). Additionally, high molecular weight polyethylene glycol was also found to be an excellent organic additive increasing the enantiomeric excess of the resulting γ-nitroaldehyde to a value of 94% *ee* (Table 4-6, entry 20). Finally, the same enantiomeric excess of 95% originally obtained with the peptide **1b** in toluene or the chloroform/*iso*-propanol mixture was achieved under inhomogeneous aqueous conditions when the reaction was performed using 15 % v/v of chloroform as an organic additive (Table 4-6, entry 20).

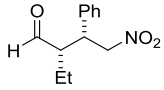
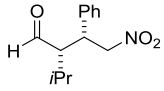
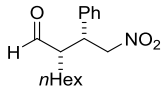
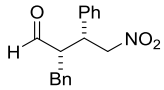
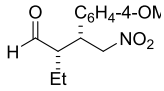
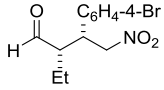
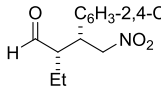
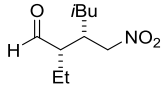
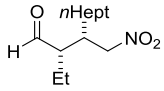
4.3 Substrate Scope

In order to investigate the substrate scope of the peptide catalyzed conjugate addition reaction between aldehydes and nitroolefins in an aqueous medium, structurally different aldehydes were allowed to react with both aromatic nitroolefins with different substitution patterns on the aromatic ring as well as branched and linear aliphatic nitroolefins in the presence of the amphiphilic peptide catalyst **1b** (Table 4-7). To probe the generality of **1b** as a water-compatible peptidic catalyst, reactions were performed under purely aqueous conditions (Table 4-7, conditions **A**). With the expectation that the enantioselectivity is higher when the microemulsion is diluted with an organic solvent, the same set of reactions was also conducted using 15% v/v of chloroform as an organic additive (Table 4-7, conditions **B**). In general, high yields and excellent levels of stereoselectivity were observed in all the substrate combinations examined. As expected, the enantioselectivities were higher in the presence of 15% v/v of chloroform (Table 4-7, conditions **B**, 91 – 95 % *ee*) than in its absence (Table 4-7, conditions **A**, 84 – 94 % *ee*).

Most interestingly, and in contrast to the analogous reactions in organic solvents, the observed rate of product formation appeared not only to depend on the reactivity of the nitroolefin but to a large extent also on its disposition to form a stable emulsion with the aldehyde and peptide **1b**. For example 4-bromo nitrostyrene is a much more reactive substrate than 4-methoxy nitrostyrene in **1a** catalyzed conjugate addition reactions with aldehydes in organic solvents. This is due to its higher electrophilicity and thus higher reactivity. However, under

Table 4-7 Scope of the conjugate addition reaction between aldehydes and nitroolefins in the presence of peptide **1b** both in aqueous medium as well as in water containing 15% v/v of $CHCl_3$.



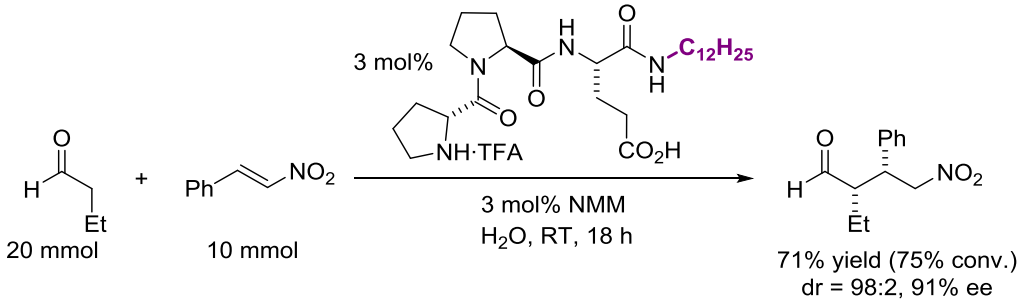
	product	conditions	Time [h]	yield [%]	dr[a]	*ee* [%][b]
1		A	8	82	98:2	91
		B	5	95	97:3	95
2		A	14	92	99:1	92
		B	15	88	99:1	94
3		A	14	97	95:5	93
		B	18	86	98:2	95
4		A	14	87	97:3	94
		B	16	90	97:3	95
5		A	22	81	99:1	88
		B	14	95	95:5	93
6		A	24	66[c]	93:7	89
		B	13	95	92:8	95
7		A	18	89	93:7	88
		B	18	81	97:3	92
8		A	18	99	99:1	84
		B	14	99	98:2	94
9		A	16	76[d]	99:1	89
		B	19	61[d]	97:3	91

a Determined by ^{1}H-NMR spectroscopy of the crude reaction mixture. b Determined by chiral phase HPLC analysis. c 70% conversion d Yield affected by the volatility of the γ-nitroaldehyde.

aqueous conditions in the presence of **1b** 4-bromo nitrostyrene does not form a stable emulsion and is present as a solid under the reaction conditions. As a consequence, only 70% of this nitroolefin were converted to the γ-nitroaldehyde after a reaction time of 24 h (Table 4-7, entry 6, conditions **A**) whereas full conversion of the less reactive 4-methoxy nitrostyrene was observed after the same reaction time under otherwise identical reaction conditions (Table 4-7, entry 5, conditions **A**). Similarly, aliphatic nitroolefins, generally considered challenging β-substituted nitroolefins in reactions in organic solvents, formed stable emulsions and reacted readily under the aqueous inhomogeneous conditions providing the corresponding adducts in high yields (Table 4-7, entries 8 and 9). These results demonstrate that the amphiphilic peptide catalyst **1b** is an excellent catalyst for various combinations of different aldehydes and nitroolefins in aqueous reaction media. Good reactivity and stereoselectivities are obtained even when electron-poor or aliphatic nitroolefins are employed, substrates traditionally considered challenging.

4.4 Gram-Scale Synthesis of 4-Nitro-3-Phenyl-2-Ethylbutanal

In order to probe the scalability of the peptide catalyzed conjugate addition reaction in an inhomogeneous aqueous reaction medium the reaction was also performed on a 10 mmol scale. In the presence of 3 mol% of the amphiphilic catalyst **1b**, 1.50 g of nitrostyrene were reacted with a twofold excess of butanal under optimized reaction conditions. The crude conjugate addition product was obtained without the need for extraction with an organic solvent by saturation of the reaction mixture with sodium chloride followed by centrifugation and phase separation. Filtration through a short plug of silica gel eluting with cyclohexane and ethyl acetate provided 1.57 g (71%) of the desired γ-nitroaldehyde together with 361 mg (24%) of unreacted nitrostyrene.



Scheme 4-2 Conjugate addition reaction between butanal and nitrostyrene in water on a 10 mmol scale.

This experiment demonstrates that the conjugate addition in water is highly efficient since the crude product can be separated from the aqueous reaction medium without the need for extraction with organic solvents.

4.5 Conclusions

The development of a peptidic catalyst that allows for performing conjugate addition reactions between aldehydes and nitroolefins in an aqueous reaction medium is highly desirable both from a methodological point of view and in order to analyze if and in how far enzymes, natures catalysts, can be imitated by peptides, their short analogues.

Testing of multiple amphiphilic analogues of the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) demonstrated that the simple introduction of an alkyl chain at the C-terminus of **1a** provides a highly active and selective catalyst for the conjugate addition reaction in water (H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$, **1b**). The reaction takes place in an emulsion and the peptidic catalyst serves as both catalyst for the reaction and detergent stabilizing the emulsion.

Several combinations of aldehydes and nitroolefins reacted readily with each other in the presence of **1b**, providing the corresponding γ-nitroaldehydes in good yields and stereoselectivities. Interestingly, electron rich aromatic or aliphatic nitroolefins, generally considered challenging substrates for conjugate addition reactions, reacted readily under inhomogeneous conditions whereas with 4-bromonitrostyrene, an activated nitroolefin, no stable emulsion was formed and the reaction was only sluggish. Generally, it was possible to improve the enantioselectivity of the reactions when chloroform or toluene (15 % v/v) were added to the solvent. The reaction was performed on a gram scale and the resulting γ-nitroaldehyde was separated from the water by simple centrifugation without the need of extraction with an organic solvent.

In a similar manner to the active site of enzymes, which often contain hydrophobic pockets, the peptidic catalyst **1b** provides an organic micro-environment within the aqueous medium in which the reaction takes place with high efficiency. Thus, these findings demonstrate that peptidic catalysts can act as intriguing intermediates between small molecule organocatalysts and enzymes, their natural high molecular weight analogues.

The obtained water compatibility of peptide **1b** is remarkable in light of the fact that the parent catalyst **1a**, when employed in water, is not only much less reactive but also

dramatically less stereoselective and opens the way for future applications of catalytic peptides of the type Pro-Pro-Xaa in a more biologically oriented setting.

5

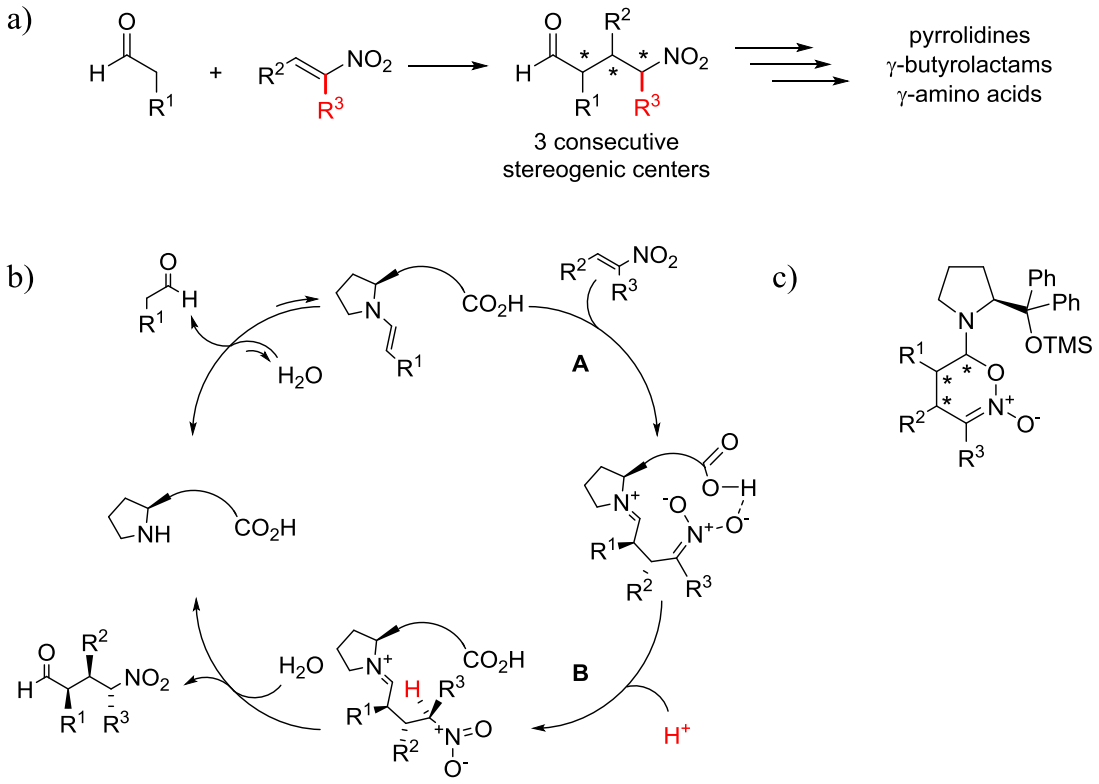
Peptide Catalyzed Conjugate Addition Reactions of Aldehydes to α,β-Disubstituted Nitroolefins

5.1 Background

Whereas an immense amount of research activity has been directed towards the organocatalytic conjugate addition reaction of aldehydes to β-monosubstituted nitroolefins,[23] examples of additions of carbonyl compounds to nitroolefins which not only bear a substituent in the β- but also in the α-position are scarce.[136,163-173] These substrates are highly interesting, because upon reaction with aldehydes or ketones, γ-nitro carbonyl compounds bearing three consecutive stereogenic centres are formed providing access to, for example, trisubstituted chiral pyrrolidines or fully substituted γ-butyrolactams and γ-amino acids (Scheme 5-1, a).

Considering a simplistic catalytic cycle, such α,β-disubstituted substrates are even more interesting: while two of the three stereogenic centres formed in the reaction are set during the conjugate addition step (Scheme 5-1, b., **A**), the configuration of the third chiral centre at the carbon bearing the nitro group is only defined in a subsequent protonation of a nitronate intermediate (Scheme 5-1, b., **B**). Thus, it is highly interesting whether such a protonation can proceed in a diastereocontrolled manner allowing for stereoselective formation of the desired trisubstituted γ-nitroaldehyde. Indeed, early examples of conjugate additions of ketones[174-175] or aldehydes[163] to α,β-disubstituted nitroolefins provided the corresponding products in low diastereo- and enantioselectivities mostly due to poor control over the stereogenic centre at this carbon bearing the nitro group.

α,β-Disubstituted nitroolefins are much more challenging substrates than their β-monosubstituted counterparts and reactions with these substrates often proceed only sluggishly. Most α,β-disubstituted nitroolefins that have been used successfully as electrophiles in enamine catalysis are either cyclic[164,168,172] or bear a proton donor in the α-position which intramolecularly activates the nitro group.[165,167,169] Apart from our studies[176] (see below), only very recently conditions have been developed that allow for the conjugate addition of aldehydes to acyclic, unfunctionalised α,β-disubstituted nitroolefins in the presence of a biphenylprolinol silyl ether organocatalyst (the Hayashi-Jørgensen catalyst).[136,173]



Scheme 5-1 a) Conjugate addition reactions of aldehydes to α,β-disubstituted nitroolefins. b) Simplistic catalytic cycle of conjugate addition reactions of aldehydes to α,β-disubstituted nitroolefins. c) 1,6-dihydrooxazine-*N*-oxides observed as intermediates in the presence of the Hayashi-Jørgensen catalyst.

Challenges of α,β-disubstituted nitroolefins include their lower reactivity compared to their β-mono-substituted counterparts[169] as well as the formation of stable cyclic intermediates, hampering catalyst turnover.[135-136] Whereas with β-mono-substituted nitroolefins and in the absence of a suitably positioned carboxylic acid group within the catalyst, cyclobutanes are formed as cyclic intermediates (see Chapter 3.2),[131-132,134,137] the conjugate addition of prolinol silyl ether derived enamines to α,β-disubstituted nitroolefins results in the formation of 1,6-dihydrooxazine-*N*-oxide intermediates (Scheme 5-1, c.).[135-136] The hydrolytic conversion of these intermediates to the reaction products is acid catalysed and unsurprisingly optimized conditions for catalytic reactions include the use of an acidic co-catalyst (up to 40 mol%!).[136,173]

We hypothesised that peptides of the general type Pro-Pro-Xaa (Xaa = variable amino acid bearing a carboxylic acid moiety) might contain members capable of catalyzing conjugate addition reactions between aldehydes and α,β-disubstituted nitroolefins. This hypothesis was based on: (1) The modular nature of the Pro-Pro-Xaa motif, which combines a powerful lead

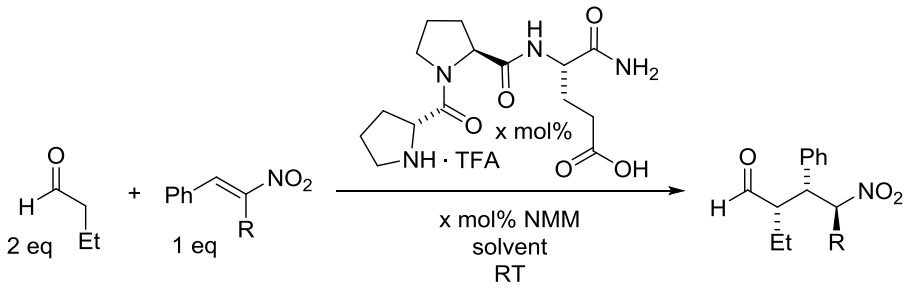
structure with the ease of accessing structurally diverse catalysts by solid phase synthesis, offers the possibility to adapt to substrate requirements. (2) whereas the peptide H-Pro-Pro-Asp-NH_2 is an excellent catalyst for aldol reactions, the closely related analogue H-D-Pro-Pro-Glu-NH_2 strongly favours conjugate addition over homo-aldol reactions of the aldehyde. This demonstrates that slight variations in the structure of peptides of the type Pro-Pro-Xaa allow for a fine tuning of their chemoselectivity. A high chemoselectivity for conjugate addition reactions over homo-aldol reactions becomes even more important when less reactive substrates, such as α,β-disubstituted nitroolefins, are employed. (3) our mechanistic studies (see chapter 3.2) show that in the presence of peptides of the type Pro-Pro-Xaa bearing a suitably positioned carboxylic acid moiety, the formation of stable cyclic intermediates is avoided by the coordination of the zwitterionic intermediate followed by intramolecular protonation. We therefore decided to study such peptides as catalysts for conjugate addition reactions between aldehydes and α,β-disubstituted nitroolefins. The results of these studies are presented in the following.

5.2 Reaction Development and Optimization[176]

5.2.1 Initial Experiments

We started our investigation by evaluating the catalyst H-D-Pro-Pro-Glu-NH_2 (**1a**) in the reaction of butanal to β-methyl-β-nitrostyrene in a 9:1 mixture of chloroform and *iso*-propanol (Table 5-1, entry 2). β-Methyl-β-nitrostyrene was chosen as the model substrate because it is an acyclic, unactivated, disubstituted nitroolefin. The reaction was much slower than with nitrostyrene demonstrating the challenging nature of this α,β-disubstituted substrate (Table 5-1, compare entries 1 and 2). Nonetheless, in the presence of 5 mol% of **1a** 30 % conversion to the desired conjugate addition product was observed within a week and the product was isolated with a high diastereoselectivity and excellent enantiomeric excess (Table 5-1, entry 2). This finding demonstrates that in the presence of the peptidic catalyst all three stereogenic centres including the one at the carbon bearing the nitro group are formed in a controlled manner. In agreement with previous studies with β-mono-substituted nitroolefins, a somewhat faster reaction at the expense of stereoselectivity was observed when more of an aliphatic alcohol was used as the solvent (Table 5-1, entry 3).[90] These initial studies clearly demonstrate that catalysts were required that are better adapted to the challenges set by these α,β-disubstituted nitroolefins.

Table 5-1 Initial studies: conjugate addition reactions of aldehydes to β-mono- and α,β-disubstituted nitroolefins in the presence of H-D-Pro-Pro-Glu-NH_2 (**1a**).



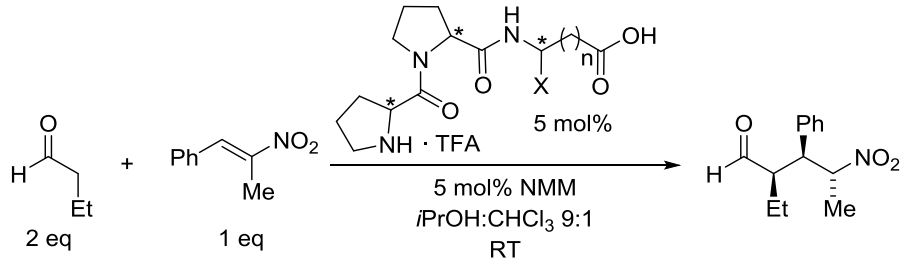
	R	x	solvent	time [h]	conv. [%][a]	dr[a]	*ee* [%][b]
1	H	1	$CHCl_3$:*i*PrOH 9:1	12	100	97:3	97
2	Me	5	$CHCl_3$:*i*PrOH 9:1	150	30	86:8:3:3	99
3	Me	5	*i*PrOH:$CHCl_3$ 9:1	150	40	67:15:10:8	98

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

5.2.2 Screening of peptidic catalysts of the type Pro-Pro-Xaa

Our initial experiments show that while in the presence of the tripeptide H-D-Pro-Pro-Glu-NH_2 (**1a**) the γ-nitroaldehyde is formed with good control over all three stereogenic centres, the reactions are slow and even after extended reaction times of several days only low conversions were achieved. We therefore hypothesised that a structurally related peptide of the type Pro-Pro-Xaa with geometrically different properties than **1a** might be better suited to accommodate α,β-disubstituted nitroolefins. Thus we synthesised and tested a small collection of 15 tripeptides all sharing a Pro-Pro motif and a carboxylic acid moiety in the C-terminal amino acid (Table 5-2). The individual members of this collection differed with respect to (1) the stereochemistry of all three amino acid residues, (2) the position of the carboxylic acid group either at the C-terminus or in the side chain of the C-terminal amino acid and (3) the length of the spacer between the backbone of the peptide and the carboxylic acid or primary amide moieties in the side chain. Owing to the ease of solid phase peptide synthesis a peptide collection of this size can easily be prepared within a few days. Pleasingly, all of the synthesised peptides were catalytically active (Table 5-2) demonstrating the value of the general lead structure Pro-Pro-Xaa. Significant differences in reactivity as well as stereoselectivity were observed depending on the stereochemistry of all three amino acids as well as the position of the carboxylic acid. Even seemingly small differences in the catalyst structures (such as inversion of a single stereogenic center; compare e.g. entries 13 and 14 in

Table 5-2 1,4-Addition reactions of butanal to β-methyl-β-nitrostyrene in the presence of different tripeptides of the type Pro-Pro-Xaa.



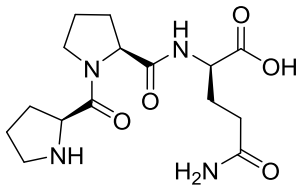
	Catalyst	time [h]	conv. [%][a]	dr[a]	*ee* [%][b] (enantiomer)
1	TFA·H-D-Pro-Pro-Glu-NH_2	150	40	67:15:10:8	98 (2*S*,3*R*,4*S*)
2	TFA·H-Pro-Pro-Glu-NH_2	150	100	67:16:10:7	96 (2*R*,3*S*,4*R*)
3	TFA·H-Pro-D-Pro-Glu-NH_2	150	40	48:23:19:10	91 (2*R*,3*S*,4*R*)
4	TFA·H-Pro-Pro-D-Glu-NH_2	150	85	55:21:15:9	96 (2*R*,3*S*,4*R*)
5	TFA·H-Pro-Pro-Asp-NH_2	72	100	71:18:6:5	97 (2*R*,3*S*,4*R*)
6	TFA·H-Pro-Pro-D-Asp-NH_2	150	100	65:17:10:8	96 (2*R*,3*S*,4*R*)
7	TFA·H-Pro-Pro-β-*homo*-Asp-NH_2	84	100	75:15:6:4	97 (2*R*,3*S*,4*R*)
8	TFA·H-D-Pro-D-Pro-β-*homo*-Asp-NH_2	150	90	70:13:9:8	98 (2*S*,3*R*,4*S*)
9	TFA·H-Pro-Pro-Gln-OH	150	100	66:21:8:5	97 (2*R*,3*S*,4*R*)
10	TFA·H-D-Pro-Pro-Gln-OH	150	30	42:27:19:12	84 (2*S*,3*R*,4*S*)
11	**TFA·H-Pro-Pro-D-Gln-OH (1j)**	**54**	**100**	**84:10:5:1**	**99 (2*R*,3*S*,4*R*)**
12	TFA·H-Pro-D-Pro-Gln-OH	150	40	48:20:17:15	86 (2*R*,3*S*,4*R*)
13	**TFA·H-Pro-Pro-Asn-OH (1q)**	**54**	**100**	**82:12:4:2**	**99 (2*R*,3*S*,4*R*)**
14	TFA·H-D-Pro-Pro-Asn-OH	150	60	42:27:18:13	90 (2*S*,3*R*,4*S*)
15	TFA·H-Pro-Pro-D-Asn-OH	54	100	75:16:6:3	97 (2*R*,3*S*,4*R*)
16	TFA·H-Pro-Pro-Cysteic acid-NH_2	150	50	53:17:17:13	89 (2*R*,3*S*,4*R*)

a Determined by ^{1}H-NMR analysis of the crude reaction mixture. b Of the major diastereoisomer; determined by chiral-phase HPLC analysis.

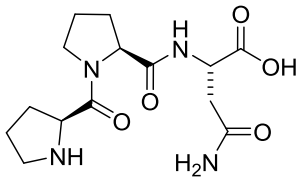
Table 5-2) had profound effects on conversion as well as stereoselectivity. In general, the most reactive catalysts were at the same time most stereoselective. In agreement with previous studies[90,92] the absolute configuration of the N-terminal proline moiety was decisive for which of the two enantiomers of the major diastereoisomer was formed preferentially (Table 5-2, compare e.g. entries 1 and 2).

The best catalysts identified from these studies were the two peptides H-Pro-Pro-D-Gln-OH (**1j**, Table 5-2, entry 11) and H-Pro-Pro-Asn-OH (**1q**, Table 5-2, entry 13). In the presence of 5 mol% of either of the two peptides full conversion of the substrates was observed within approximately 2 days and the resulting conjugate addition product was obtained in good diastereoselectivities (84:10:5:1 for **1j** and 82:12:4:2 for **1q**) as well as excellent

enantioselectivity for the major diastereoisomer (99% *ee*). Interestingly, both optimal catalysts differ from the parent peptide **1a** with respect to the configuration of the Pro-Pro-motif (L-Pro-L-Pro is preferred to D-Pro-L-Pro which was found to be best for conjugate addition reactions of aldehydes to β-mono-substituted nitroolefins) and the position of the C-terminal carboxylic acid which is within **1j** and **1q** at the C-terminus and not in the side chain.



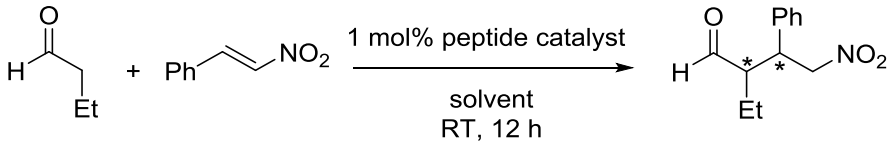
1j



1q

Next we became interested in the question whether the two new peptides were better catalysts for conjugate addition reactions of aldehydes to nitroolefins in general, or whether they are specifically successful catalysts for the addition reaction to α,β-disubstituted nitroolefins. We therefore allowed butanal and β-mono-substituted nitrostyrene to react in the presence of the two new peptidic catalysts **1j** and **1q** and compared their performance with that of the parent peptide **1a** (Table 5-3). Both, under conditions optimized for the addition of aldehydes to mono-substituted nitroolefins as well as under the conditions of Table 5-2 the new peptides **1j** and **1q** performed significantly poorer than **1a**. This remarkable "substrate specificity" demonstrates that the different properties of β-mono- versus α,β-disubstituted nitroolefins are optimally addressed by similar yet different peptidic catalysts. Since with peptides of the type

Table 5-3 Comparison of H-Pro-Pro-D-Gln-OH and H-Pro-Pro-Asn-OH with H-D-Pro-Pro-Glu-NH_2 in the conjugate addition reaction of butanal to nitrostyrene.



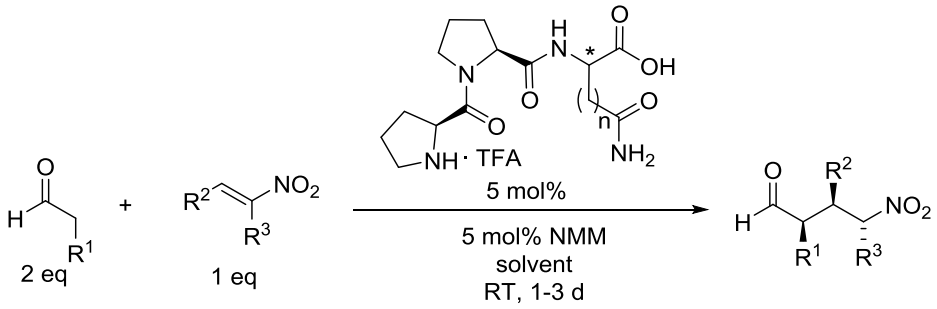
solvent	H-D-Pro-Pro-Glu-NH2 (**1a**)	H-Pro-Pro-D-Gln-OH (**1j**)	H-Pro-Pro-Asn-OH (**1q**)
$CHCl_3$:*i*PrOH 9:1	dr = 50:1, 97% *ee*	dr = 31:1, 83% *ee*	dr = 16:1, 85% *ee*
*i*PrOH:$CHCl_3$ 9:1	dr = 9:1, 82% *ee*	dr = 18:1, 63 % *ee*	dr = 12:1, 77% *ee*

Pro-Pro-Xaa parameters such as catalytic activity, stereo- and chemoselectivity can be fine-tuned by small changes in the catalyst structure, a small collection of 15 tripeptides was enough to provide two very good catalysts for the conjugate addition reaction of aldehydes to challenging α,β-disubstituted nitroolefins.

5.2.3 Solvent Screening

Having identified two very good catalysts for conjugate addition reactions between aldehydes and α,β-disubstituted nitroolefins, we next investigated effects of the solvent. Thus the conjugate addition reaction of butanal to β-methyl-β-nitrostyrene in the presence of H-Pro-Pro-D-Gln-OH (**1j**) as well as H-Pro-Pro-Asn-OH (**1q**) was examined in different solvents (Table 5-4). While the reaction proceeded well in other aliphatic alcohols such as EtOH or *t*BuOH, *i*PrOH turned out to be the best choice in terms of reactivity and selectivity

Table 5-4 Conjugate addition reaction between butanal and β-methyl-β-nitrostyrene using different solvents.



	catalyst	solvent	time [h]	conv. [%][a]	dr[a]	*ee* [%][b]
1	**1q**	10% $CHCl_3$ in *i*PrOH	54	100	82:12:4:2	99 %
2	**1j**	10% $CHCl_3$ in *i*PrOH	54	100	84:10:5:1	99 %
3	**1q**	10% $CHCl_3$ in EtOH	60	100	70:15:12:3	97 %
4	**1j**	10% $CHCl_3$ in EtOH	60	90	78:11:9:2	94 %
5	**1q**	10% $CHCl_3$ in *t*BuOH	70	100	82:13:3:2	99 %
6	**1j**	10% $CHCl_3$ in *t*BuOH	70	90	89:7:3:1	99 %
7	**1q**	THF	150	55	79:12:8:1	97 %
8	**1j**	THF	150	30	81:9:8:2	98 %
9	**1q**	CHCl3	150	35	88:8:3:1	99 %
10	**1j**	CHCl3	150	20	90:6:3:1	99 %
11	**1q**	DMSO	60	100	67:15:14:4	96 %
12	**1j**	DMSO	60	100	67:15:14:4	96 %

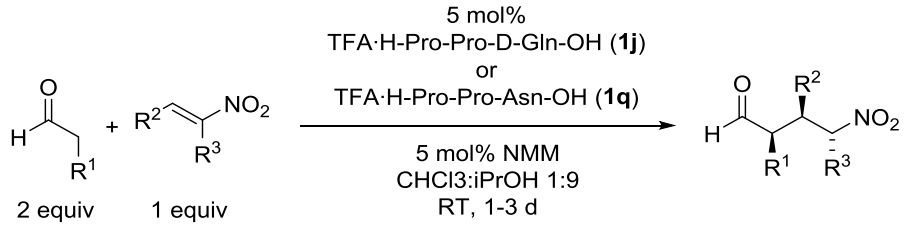
a Determined by ^{1}H-NMR spectroscopic analysis of the crude reaction mixture. b Determined by chiral-phase HPLC analysis.

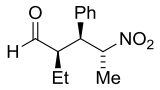
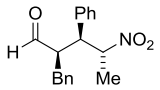
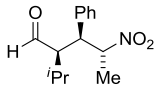
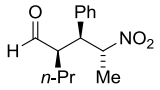
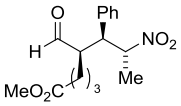
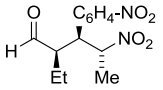
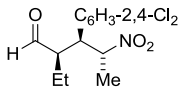
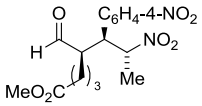
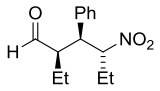
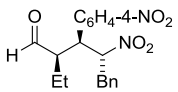
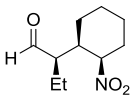
(Table 5-4, entries 1-6). When using aliphatic alcohols, 10% v/v of $CHCl_3$ were necessary in order to fully dissolve the nitroolefin in the reaction medium. In less polar solvents such as THF or $CHCl_3$ the products were obtained with similar selectivities but required longer reaction times, presumably due to low solubility of the peptide in the reaction medium (Table 5-4, entries 7-10). The use of DMSO resulted in a lower stereoselectivity albeit with good reactivity (Table 5-4, entries 11-12). This demonstrates that the original solvent system consisting of *i*PrOH and $CHCl_3$ is a good choice combining selectivity and high reactivity.

5.3 Substrate Scope[176]

To probe the substrate scope of the peptide-catalyzed conjugate addition reaction the two catalysts identified from the catalyst screening were applied to different combinations of aldehydes and α,β-disubstituted nitroolefins (Table 5-5). In order to avoid epimerisation at C(2) and to allow for easier isolation of diastereomerically pure samples for chiral stationary phase HPLC-analysis , the resulting γ-nitroaldehydes isolated from the catalysis reaction were subsequently reduced to the corresponding δ-nitroalcohols using $NaBH_4$. In all cases diastereomerically pure samples of major diastereomers were obtained by column chromatography or semipreparative HPLC allowing for the determination of the enantiomeric excess. In general, both catalysts performed similarly well in all the reactions examined. Variations in the aldehyde were well tolerated, though in agreement with conjugate addition reactions of aldehydes to mono-substituted nitroolefins, increasing steric demand in the β-position of the aldehyde required longer reaction times (Table 5-5, entries 2 - 4). The corresponding γ-nitroaldehydes were isolated in diastereomeric ratios between 77:23 and 87:13 (main diastereoisomer : all other diastereoisomers) and with *ee*'s of 99% for all but one reaction. Additionally, an ester functionalised aldehyde also reacted smoothly to provide an ester-functionalised γ-nitroaldehyde (Table 5-5, entry 5) in high yields (> 90%) and stereoselectivities (dr of 74:18:6:2 and 98% *ee* for peptide **1j** as well as a dr of 72:19:6:3 and 99% *ee* for peptide **1q**). Expectedly, β-methyl-nitrostyrenes bearing electron withdrawing groups on the aromatic ring showed higher reactivity compared to unsubstituted β-methyl-β-nitrostyrene while maintaining excellent levels of stereoselectivity (dr's between 71:29 and 86:14 and *ee*'s of more than 98%; Table 5-5, entries 6 and 7). Additionally, variations in the α-position of the nitroolefin were also well tolerated (Table 5-5, entries 9 and 10), providing the desired products in enantioselectivities of more than 97%, though in these cases somewhat lower diastereoselectivity was observed (beween 58:42 and 71:29). Only an α,β-disubstituted

Table 5-5 Scope of the conjugate addition reaction between aldehydes and α,β-disubstituted nitroolefins in the presence of peptides **1j** and **1q**.



	product	catalyst	yield [%][a]	dr[b]	*ee* [%][c]
1[d]		**1j**	81	84:10:5:1	99
		1q	88	82:12:4:2	99
2		**1j**	65	87:8:4:1	99
		1q	74	87:7:3:3	99
3[e]		**1j**	73	83:10:6:1	99
		1q	72	83:9:7:1	99
4[e]		**1j**	87	77:15:6:2	97
		1q	89	85:11:3:1	99
5		**1j**	98	74:18:6:2	98
		1q	90	72:19:6:3	99
6		**1j**	77	75:13:9:3	99
		1q	75	71:17:8:4	99
7		**1j**	81	86:7:6:1	99
		1q	73	86:7:5:2	98
8		**1j**	59	65:20:10:5	94
		1q	61	54:29:10:7	92
9[e]		**1j**	71	71:26(overlap):3	98
		1q	80	66:30(overlap):4	97
10		**1j**	66	61:29:7:3	98
		1q	66	58:31:7:4	98
11		**1j**	74	79:9:8:4	99
		1q	72	72:14:8:6	99

a Yields correspond to γ-nitroaldehydes isolated as a mixture of diastereoisomers. b.Determined by ^{1}H-NMR spectroscopy of the crude reaction mixture. c Determined by chiral phase HPLC analysis of the isolated major diastereomers of the corresponding γ-nitro-alcohols obtained after $NaBH_4$ reduction. d The enantiomeric excess was determined at the level of the γ-nitroaldehyde. d Reaction times of 4-5 days were necessary.

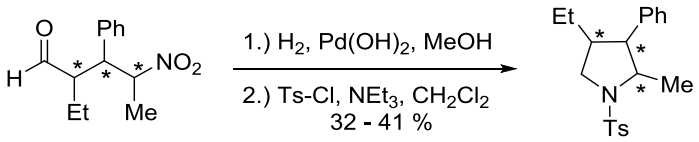
nitroolefin bearing aromatic residues in both positions did not provide the desired products and the starting materials were reisolated. While reactions of acyclic purely aliphatic nitroolefins were too slow to be practical, nitrocyclohexene proved to be an excellent substrate for the conjugate addition reaction (Table 5-5, entry 11) yielding a cyclic γ-nitroaldehyde with high diastereoselectivities (79:9:8:4 for peptide **1j** and 72:14:8:6 for peptide **1q**) and an enantioselectivity of 99 % *ee*. In general, it is noteworthy that in all substrate combinations good diastereo- and very high enantioselectivities of typically more than 97 % were observed. These results demonstrate that the peptides **1j** and **1q** are excellent catalysts for conjugate addition reactions of various aldehydes to different α,β-disubstituted nitroolefins.

5.4 Product Derivatization and Determination of the Relative and Absolute Configurations of γ-Nitroaldehydes[176]

One of the reasons why the conjugate addition reaction between aldehydes and nitroolefins is such a valuable transformation is the straightforward derivatization of the resulting γ-nitro-aldehydes to various interesting chiral molecules. The trisubstituted γ-nitroaldehydes from the conjugate addition reaction of aldehydes and α,β-disubstituted nitroolefins were derivatized to pyrrolidines, γ-butyrolactams and γ-amino acids. Furthermore, this derivatization was used for the determination of the relative and absolute configuration of the products from the catalytic reaction.

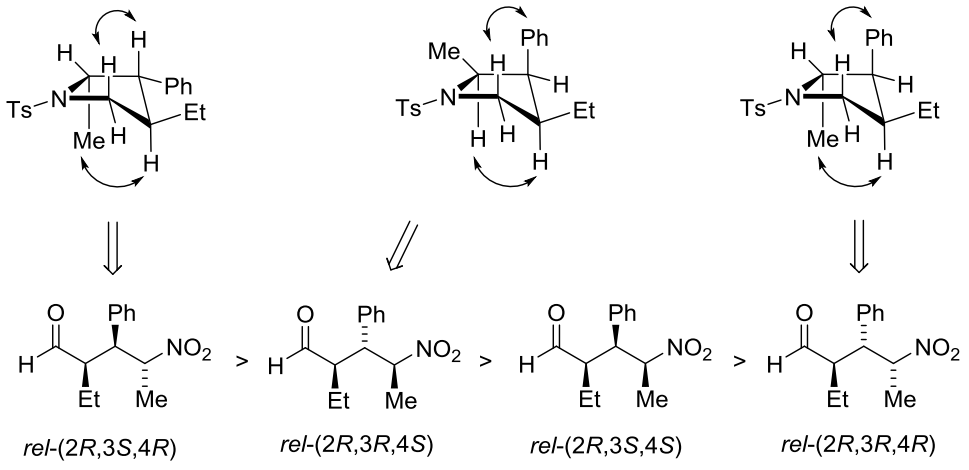
5.4.1 Synthesis of Pyrrolidines and Determination of the Relative Configuration

The γ-nitroaldehydes from the catalytic reaction were transformed to the corresponding trisubstituted pyrrolidines by a method that was introduced by Barbas for the conversion of disubstituted γ-nitroolefins to pyrrolidines.[177] In the presence of a catalytic amount of palladium hydroxide in a hydrogen atmosphere (4.5 bar) the desired pyrrolidine was formed through reduction of the nitro group followed by intramolecular reductive amination of the resulting γ-aminoaldehyde. The resulting pyrrolidine was then *N*-tosylated in order to facilitate isolation. Following this procedure three of the four diastereoisomers formed in the catalytic reaction between butanal and β-methyl-β-nitrostyrene were transformed to the corresponding pyrrolidines in yields of 32 – 41% (Scheme 5-2).



Scheme 5-2 Conversion of γ-nitroaldehydes to the corresponding pyrrolidines.

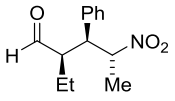
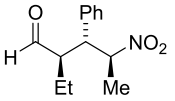
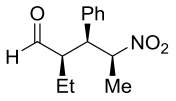
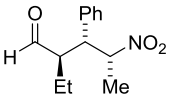
These derivatives allowed for the determination of their relative configuration by one and two dimensional ^{1}H-NMR spectroscopy and thereby for the assignment of all four diastereoisomers of γ-nitroaldehydes formed in the catalytic reaction (Scheme 5-3). As expected from analogy to conjugate additions of aldehydes to β-monosubstituted nitroolefins, the two stereogenic centres at C(2) and C(3) of the major diastereoisomer that are formed in the conjugate addition step are in a relative *syn* orientation. The configuration at C(4), the centre that is formed in the protonation step of the nitronate, is *anti* relative to the other two centres. Interestingly, the same *rel*-(2*R*,3*S*,4*R*) major diastereoisomer is also obtained in the presence of the Hayashi-Jørgensen catalyst[136,173] as well as in stoichiometric addition reactions to disubstituted nitroolefins.[178] Somewhat surprisingly, the second most abundant diastereoisomer has the *rel*-(2*R*,3*R*,4*S*)-configuration, where the substituents at C(2) and C(3) are in an *anti* arrangement. Only the third most abundant diastereoisomer is the epimer with respect to the centre at position C(4) next to the nitro group (*rel*-2*R*,3*S*,4*S*). Taken together, this means that in reactions catalyzed by the peptides there is a higher selectivity for the relative configurations at centres C(3) and C(4) (*anti*) than at centres C(2) and C(3) (*syn*) and implies that the protonation (*anti:syn*) of the iminium nitronate intermediate (or alternatively,



Scheme 5-3 Observed strong long range NOE contacts within the three analyzed diastereoisomeric pyrrolidines (top) and assignment of the corresponding diastereoisomers formed in the catalytic reaction (bottom).

as recently suggested by Pihko[136] for the Hayashi-Jørgensen catalyst, the protonation of a 1,6-dihydrooxazine-*N*-oxide intermediate) proceeds with an even higher diastereoselectivity than the conjugate addition step of the enamine to the nitroolefin (*syn*:*anti*). In principle, the diastereoselectivity of this protonation step could be either induced by the peptidic catalyst (catalyst control) or by the two stereogenic centres already formed in the conjugate addition step (substrate control). In order to probe whether the configuration at C(4) is controlled by the catalyst or the substrate we performed the conjugate addition between butanal and β-methyl-β-nitrostyrene in the presence of pyrrolidine and proline and compared the resulting distribution of diastereoisomers with the one obtained in the presence of the peptidic catalysts. While in the presence of the peptidic catalysts high diastereoselectivity for the *rel*-(2*R*,3*S*,4*R*) diastereoisomer was observed, both in the presence of pyrrolidine and proline the epimer with respect to the stereogenic centre at C(4) which is formed in the protonation step was obtained preferentially but with very low selectivity. Additionally, especially in the presence of proline the observed 1:1 ratios with respect to the epimers at C(4) suggest that this catalyst fails completely to control the protonation of the nitronate. These findings thus demonstrate that the high diastereoselectivity of the protonation observed in the presence of **1j** and **1q** is indeed controlled by the peptidic catalysts and not by the stereogenic centres already formed in the conjugate addition step.

Table 5-6 Ratio of the diastereoisomers of the γ-nitroaldehyde derived from butanal and β-methyl-β-nitrostyrene formed in the presence of different secondary amines.

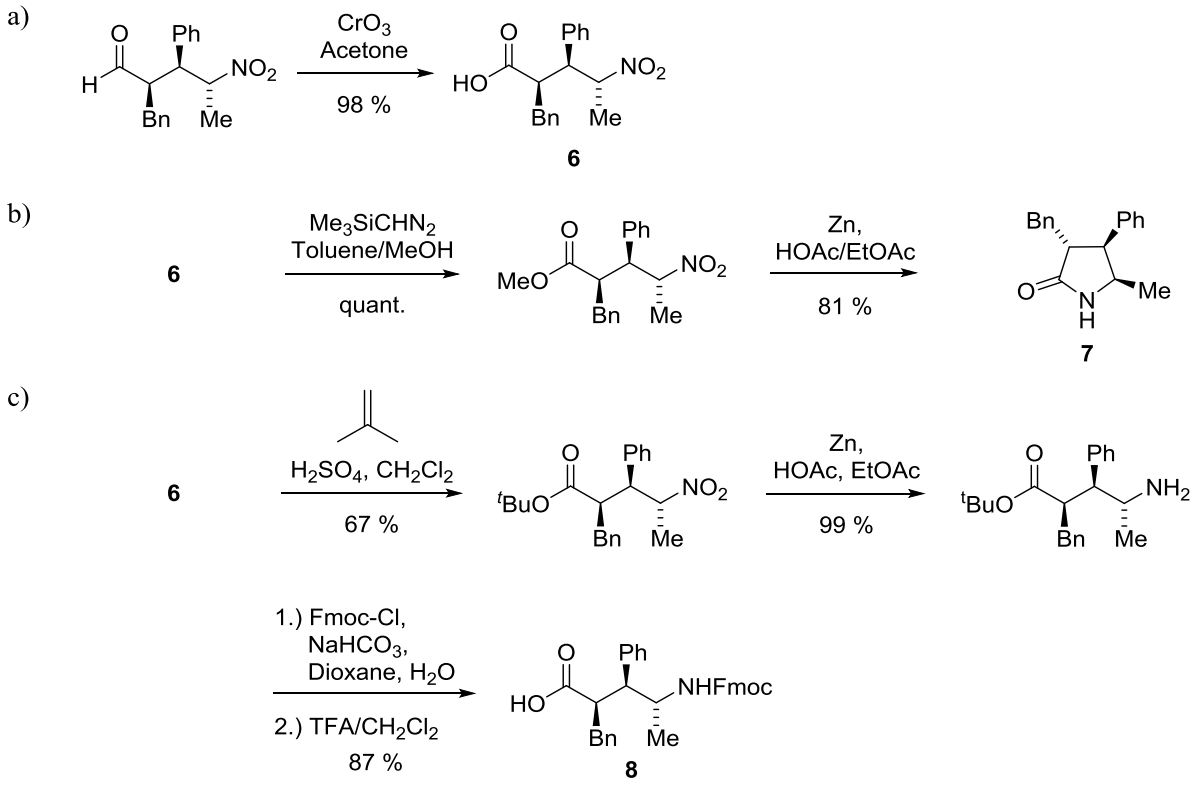
	catalyst	 *rel*-(2*R*,3*S*,4*R*)	 *rel*-(2*R*,3*R*,4*S*)	 *rel*-(2*R*,3*S*,4*S*)	 *rel*-(2*R*,3*R*,4*R*)
1	pyrrolidine	22	18	40	20
2	proline	32 (31% *ee*)	16	36	16
3	H-Pro-Pro-D-Gln-OH	84 (99% *ee*)	10	5	1
4	H-Pro-Pro-Asn-OH	82 (99% *ee*)	12	4	2

5.4.2 Synthesis of γ-Butyrolactams and γ-Amino Acids – Determination of the Absolute Configuration

In addition to chiral pyrrolidines, fully substituted γ-amino acids and γ-butyrolactams are other useful chiral building blocks that proved to be easily accessible from the γ-nitroaldehydes resulting from the conjugate addition of aldehydes to α,β-disubstituted nitroolefins. γ-Amino acids are interesting compounds for medicinal chemistry

applications[179] as well as for the incorporation into foldamers.[180-181] The syntheses of enantioenriched chiral γ-amino acids mostly rely on either chiral resolution or on the use of chiral auxiliaries.[182] The γ-nitro-aldehyde derived from 3-phenylpropanal and β-methyl-β-nitrostyrene was chosen as a starting material for the synthesis of the corresponding γ-butyrolactam and γ-amino acid. This γ-nitroaldehyde is an ideal candidate for further transformations because it is a crystalline solid that was obtained in diastereomerically and enantiomerically pure form by a simple precipitation from pentane.

Jones oxidation of the γ-nitroaldehyde provided γ-nitrocarboxylic acid **6** in a yield of 98 % (Scheme 5-4, a). Carboxylic acid **6** is a common intermediate *en route* to both, fully substituted γ-butyrolactam **7** as well as trisubstituted γ-amino acid **8**. Lactam **7** was easily prepared *via* the corresponding methyl ester (Scheme 5-4, b). Treatment of the carboxylic acid with trimethylsilyl diazomethane followed by reduction of the nitro group using zinc in an acidic environment gave rise to a γ-amino ester which cyclised spontaneously to lactam **7** under these conditions. γ-Amino acid **8** was obtained when carboxylic acid **6** was first transformed to the corresponding *tert.*-butyl ester (Scheme 5-4, c). The bulky *tert.*-butyl ester



Scheme 5-4 a) Oxidation of the γ-nitroaldehyde to γ-nitrocarboxylic acid **6**. b) Synthesis of γ-butyrolactam **7**. c) Synthesis of γ-amino acid **8**.

moiety prevented, upon reduction of the nitro group, the spontaneous cyclization and the open chain γ-amino ester was isolated in nearly quantitative yield. The corresponding Fmoc-protected γ-amino acid building block **8** was then obtained straightforwardly *via* Fmoc protection and deprotection of the *tert*.-butyl ester.

γ-Nitrocarboxylic acid **6** also proved to be a valuable intermediate towards the aim of determining the absolute configuration of the major diastereoisomer obtained in the addition reaction of aldehydes to α,β-disubstituted nitroolefins. Intermediate **6** was coupled to the methyl ester of (L)-phenylalanine using EDC as coupling reagent (Figure 5-1). The resulting protected dipeptide is a crystalline solid and crystals suitable for X-ray crystallographic analysis were obtained by the slow evaporation of a solution in hexane. Due to the known absolute configuration of the phenylalanine, the absolute configuration of the three stereogenic centres of the major stereoisomer formed in the catalytic reaction were unambiguously determined to be (2*R*,3*S*,4*R*).

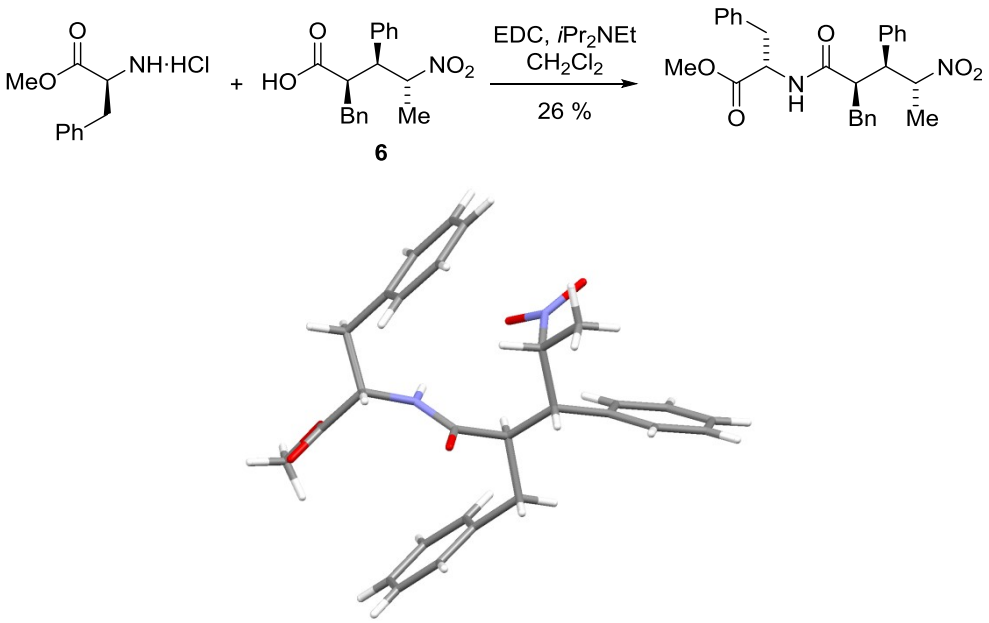


Figure 5-1 Determination of the absolute configuration of the major isomer of the γ-nitroaldehyde by synthesis (top) and crystal structure analysis of a (L)-phenylalanine derived dipeptide.

5.5 Mechanistic Considerations

5.5.1 Comparison of the Reactivities of β-Mono- and α,β-Disubstituted Nitroolefins

Enamine catalyzed conjugate addition reactions of carbonyl compounds to α,β-disubstituted nitroolefins proceed significantly slower than analogous reactions with β-monosubstituted nitroolefins (see above). *A priori* there are two possible explanations for these dramatically longer reaction times: (1) α,β-disubstituted nitroolefins are less electrophilic and therefore the conjugate addition of an enamine intermediate is slower or (2) stable intermediates are formed in the course of the reaction hampering catalyst turnover. Indeed 1,6-dihydrooxazine-*N*-oxides have been observed as stable catalyst resting states in the presence of the Hayashi-Jørgensen catalyst.[135-136]

To probe the first explanation we directly compared the reactivity of β-nitrostyrene and β-methyl-β-nitrostyrene in reactions with the potassium salt of Meldrum's acid. We chose the potassium salt of Meldrum's acid because it is a well characterized carbon based nucleophile with a relatively low nucleophilicity.[183] Furthermore the conjugate addition of the potassium salt of Meldrum's acid with β-nitrostyrene has also been used for the determination of the electrophilicity of nitroolefins.[25] Preliminary studies demonstrated the suitability of this nucleophile since upon mixing with the nitroolefins in DMSO d-6 both the reactions with β-nitrostyrene as well as β-methyl-β-nitrostyrene proceeded to completion within a few minutes without the formation of any detectable side products (^{1}H-NMR spectroscopy). The corresponding protonated products were isolated after aqueous workup as pure compounds in yields of >90%.

In order to directly compare the reactivity of the two nitroolefins, their reaction with the potassium salt of Meldrum's acid was followed by *in situ* IR spectroscopy monitoring the N-O stretching mode of the addition product. The corresponding conversion-time curves are depicted in Figure 5-2. Whereas in the reaction with β-nitrostyrene (Figure 5-2, blue dots) complete conversion was observed after 3 minutes, the corresponding reaction with β-methyl-β-nitrostyrene (Figure 5-2, green dots) took approximately one hour to go to completion. This finding demonstrates that α,β-disubstituted nitroolefins are clearly less reactive than their monosubstituted counterparts.

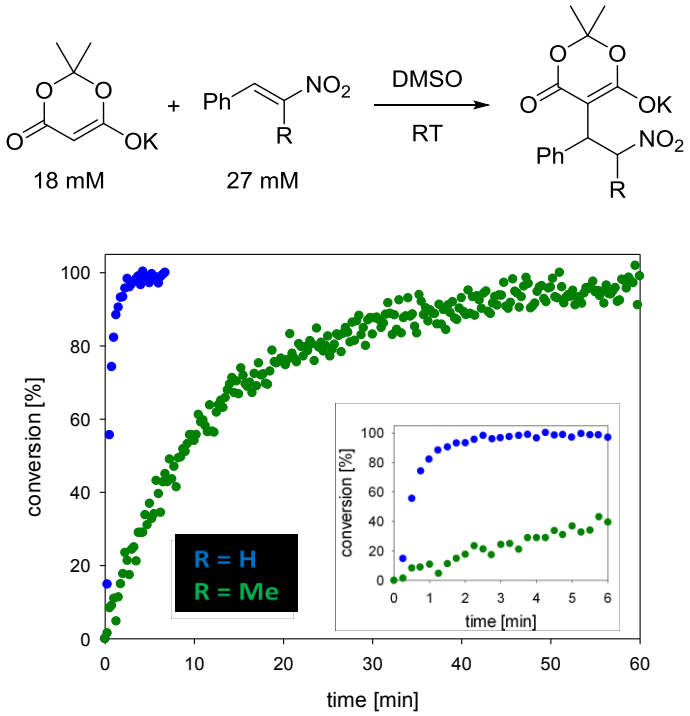


Figure 5-2 Conversion-time curves comparing the reactivity of β-nitrostyrene with β-methyl-β-nitrostyrene in their reactions with the potassium salt of Meldrum's acid. Inlet: magnification of the first 6 min.

Such a significantly reduced reactivity can be rationalized by comparing the structures of these substrates. Comparison of the X-ray crystal structures of β-methyl-β-nitrostyrene with β-nitrostyrene reveals a distinct difference (Figure 5-3). Whereas the monosubstituted nitroolefin is a planar molecule, the phenyl ring in the disubstituted analogue is twisted with respect to the plane of the nitroalkene due to allyl strain. Such a twist is not only responsible for an increased steric demand of this nitroolefin, but also hampers conjugation of the double bond and thereby renders the substrate less electrophilic. This distinct structural difference between mono- and α,β-disubstituted nitroolefins was also observed in *ab initio* calculations (R1-MP2/cc-pVTZ) of β-methyl-β-nitrostyrene (Figure 5-3).

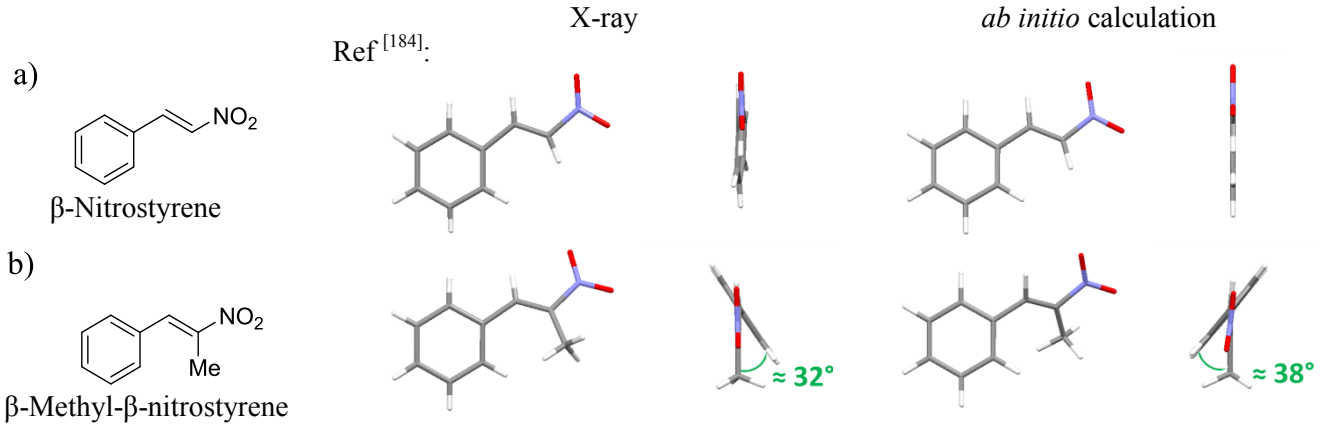


Figure 5-3 Structures of β-nitrostyrene (a) and β-methyl-β-nitrostyrene (b) obtained from X-ray crystallography and *ab initio* calculations.

5.5.2 Product Epimerization

In chapter 3.2 it was demonstrated that the γ-nitroaldehydes obtained in the reaction between aldehydes and β-monosubstituted nitroolefins slowly epimerize when exposed to the catalysts H-D-Pro-Pro-Glu-NH_2 (**1a**) and H-D-Pro-Pro-Glu(OCH_3)-NH_2 (**2a**) for extended reaction times. This catalyst promoted epimerization is presumably due to a non-stereospecific formation of an enamine between the reaction product and the catalyst followed by a non-stereospecific re-protonation of this enamine which results in the formation of the other diastereoisomer. The trisubstituted γ-nitroaldehydes resulting from the conjugate addition of aldehydes to α,β-disubstituted nitroolefins bear acidic protons both at C(2) and C(4) that are therefore epimeriszble stereocenters. In analogy to the disubstituted γ-nitroaldehydes the stereogenic centre at C(2) can undergo catalyst promoted epimerization. Additionally, due to the high acidity of the proton at C(4) a base mediated epimerization at this centre is also possible. In order to test for product epimerization under the reaction conditions the reaction between butanal and β-methyl-β-nitrostyrene was set up under standard conditions and the diastereomeric ratio was followed over the course of the reaction and for further 13 days (Figure 5-4). Additionally, after 13 days an equivalent amount of triethylamine was added in order to determine the thermodynamic distribution of the four diastereoisomers in the presence of a base that can readily epimerize the stereogenic centres as C(2) and C(4). During the course of the reaction (50 h) the diastereomeric ratio was almost constant and only decreases slightly from 87:7:3:3 to 84:10:5:1. Once full conversion to the γ-nitroaldehyde was reached, catalyst promoted epimerization of the stereogenic centre at C(2) was observed. The *syn-anti*- diastereoisomer (Figure 5-4, red crosses) was converted to the *anti-anti*-epimer (Figure 5-4, blue crosses) presumably again by formation and unselective hydrolysis of an enamine between the γ-nitroaldehyde and the catalyst. Over the course of 5 days the diastereomeric ratio dropped to 61:26:9:4 and remained stable for a further week. This demonstrates that the stereogenic centre at C(4), the carbon bearing the nitro group, is stable under these conditions. Only when an equivalent of triethylamine was added significant formation of the epimers with respect to this centre was observed (Figure 5-4, green and turquoise crosses). Within three days the diastereomeric ratio dropped to 28:20:28:24 suggesting that neither of the diastereoisomers is thermodynamically preferred to a significant extent.

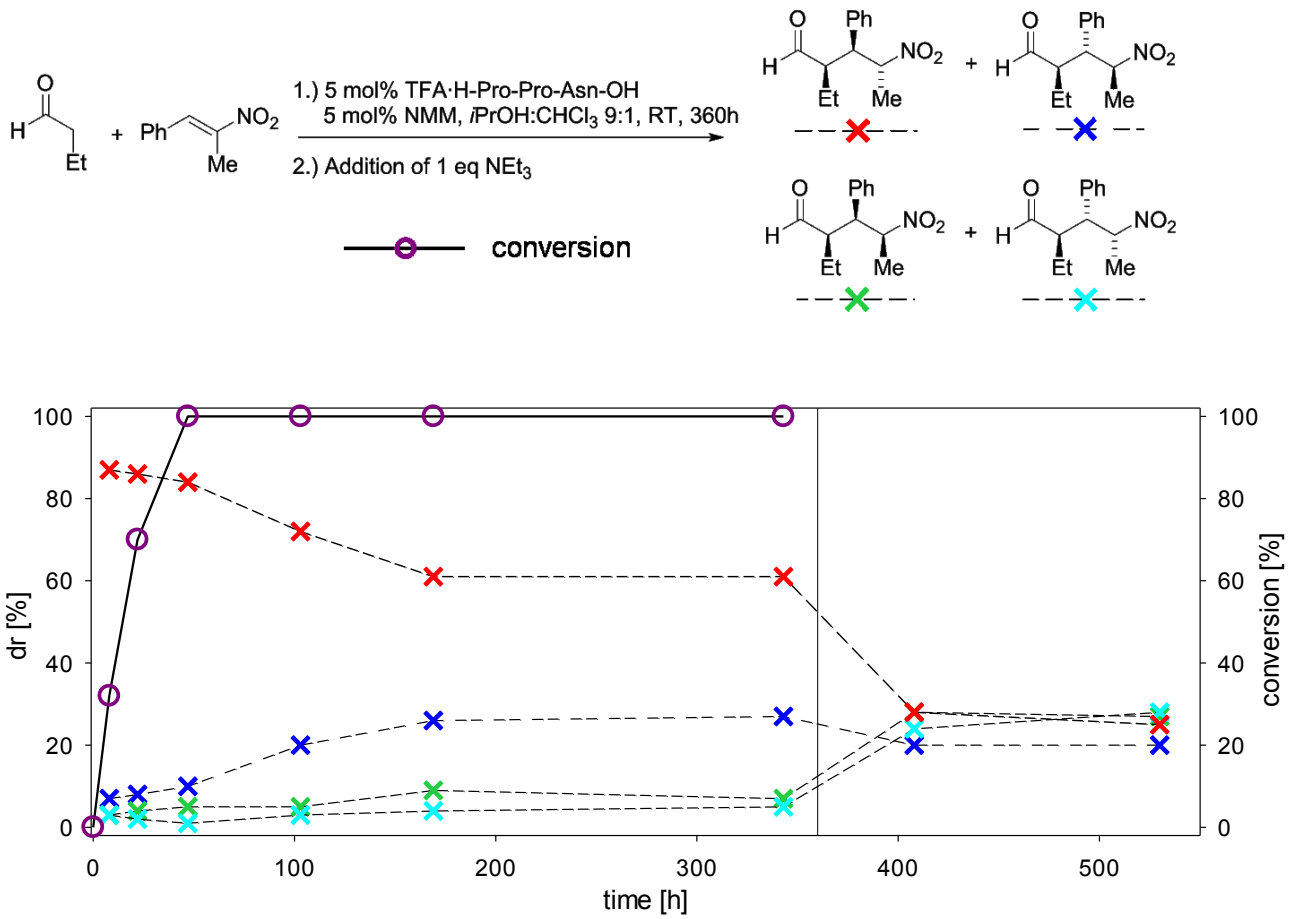


Figure 5-4 Distribution of diastereoisomers in the conjugate addition of butanal to β-methyl-β-nitrostyrene during the reaction (0 – 50 h) after the reaction (50 – 360 h) and after the addition of NEt_3 (360 – 530 h).

These results not only demonstrate that it is important to work up the reaction immediately after full conversion is reached but also that a secondary amine catalyst for this reaction should not be a strong base, since base promoted epimerization can lead to a complete erosion of the diastereoselectivity.

5.5.3 Kinetic and NMR-Spectroscopic Investigations

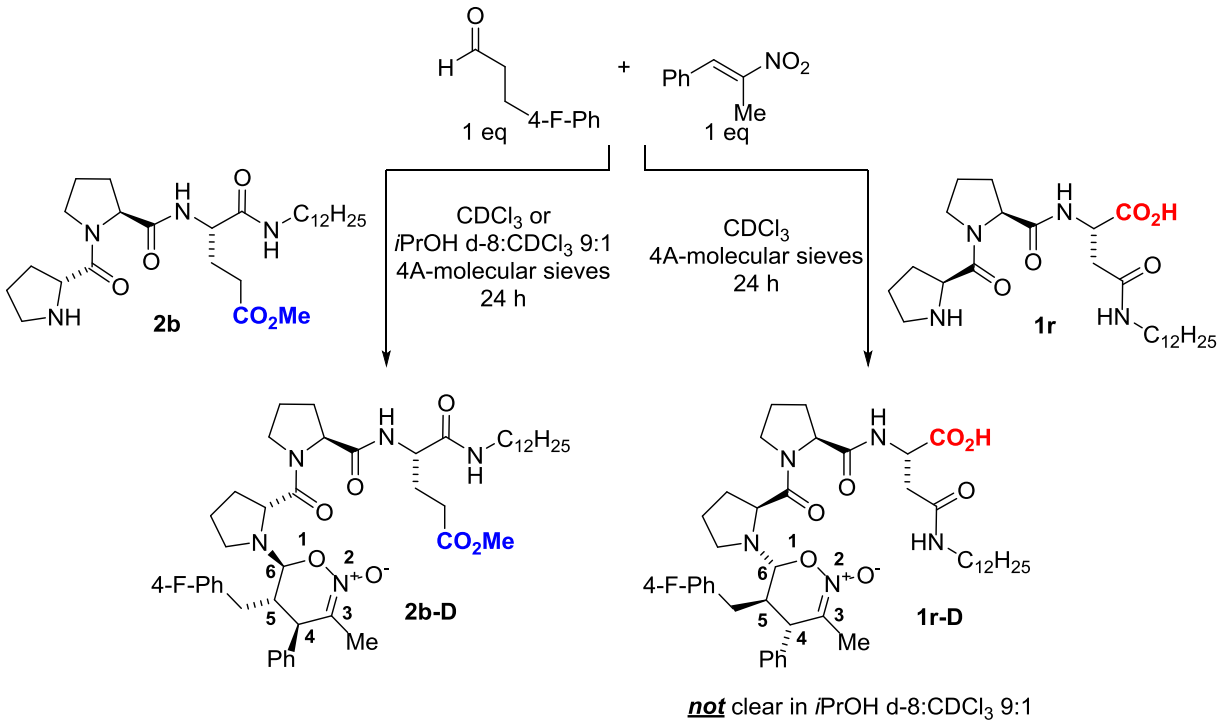
As mentioned in section 5.1 one of the possible challenges encountered in conjugate addition reactions between aldehydes and α,β-disubstituted nitroolefins is, besides the lower reactivity of these electrophiles, the formation of 1,2-dihydrooxazine-*N*-oxides as cyclic catalyst resting states. In the presence of the Hayashi-Jørgensen catalyst 1,2-dihydrooxazine-*N*-oxides are readily observed by NMR spectroscopic inspection of the reaction mixture.[135-136] The conversion of these cyclic intermediates to the γ-nitroaldehyde and the free catalyst is acid

promoted and indeed in the presence of the Hayashi-Jørgensen catalyst large amounts of a carboxylic acid co-catalyst is necessary in order to achieve catalyst turnover.[135-136]

In conjugate addition reactions of aldehydes to β-mono-substituted nitroolefins our mechanistic studies demonstrated that the presence of a suitably positioned carboxylic acid within the catalyst structure renders a catalyst particularly successful by trapping a short-lived zwitterionic intermediate and thus avoiding the formation of a stable cyclobutane intermediate (see chapter 3.2).[137] In peptide catalyzed conjugate addition reactions of aldehydes to α,β-disubstituted nitroolefins, again catalysts bearing a carboxylic acid within their C-terminal amino acid were found to be particularly successful. Interestingly, the position of the carboxylic acid within the catalyst was also found to strongly influence the catalytic performance of a catalyst (with optimal catalysts bearing a carboxylic acid at the C-terminus rather than in the side chain). Thus coordination to and trapping of a short lived zwitterionic or a 1,2-dihydrooxazine-*N*-oxide intermediate by intramolecular protonation again appears to be a plausible role for the carboxylic acid group within the catalyst structure.

In order to probe for the formation of peptide derived 1,2-dihydrooxazine-*N*-oxides the methyl ester peptide H-D-Pro-Pro-Glu(OMe)-NH-$C_{12}H_{25}$ (**2b**) was allowed to react with 3-(4-fluorophenyl)-propanal and β-methyl-β-nitrostyrene in $CDCl_3$ as well as in a 9:1 mixture of *i*PrOH d-8 and $CDCl_3$ and the resulting mixtures were analyzed by NMR-spectroscopy (Scheme 5-5, left). In both solvent systems the immediate formation of the 6-membered oxazine **2b-D** was observed. This intermediate was found to be surprisingly stable even towards the addition of acidic compounds such as 4-nitrophenol, 4-nitrobenzoic acid or chloroacetic acid.

As a next step, identical NMR-experiments were performed in the presence of the peptide H-Pro-Pro-Asn($C_{12}H_{25}$)-OH (**1r**) a more soluble analogue of **1q**. Interestingly, when $CDCl_3$ was used as the solvent the formation of a 1,6-dihydrooxazine-*N*-oxide **1r-D** was observed after 24 h (Scheme 5-5, right). The most characteristic signal of **1r-D** is a cross-peak between the carbon atom C(6) (Scheme 5-5) of the oxazine ring (97.9 ppm) and the corresponding proton (5.12 ppm) in HSQC-spectra. Use of a 9:1 mixture of *i*PrOH d-8 and $CDCl_3$ led to a complicated mixture of peptide derived compounds the assignment of which proved to be difficult. HSQC-spectra showed several cross-peaks that are potentially indicative of 1,6-dihydrooxazine-*N*-oxides (possibly diastereoisomers or conformers). At this point, it is not clear in how far these observations made after long reaction times (24 h) in stoichiometric



Scheme 5-5 NMR-spectroscopic investigations into the conjugate addition reaction of aldehydes to α,β-disubstituted nitroolefins in the presence of catalysts **2b** and **1r**.

reactions and in the presence of molecular sieves are relevant for reactions under catalytic conditions and further mechanistic studies comparing different catalysts and conditions are clearly necessary.

In addition to NMR-spectroscopic experiments initial kinetic studies were conducted in order to investigate the mechanism of these conjugate addition reactions to α,β-disubstituted nitroolefins. Thus, butanal and β-methyl-β-nitrostyrene were allowed to react in the presence of 10 mol% of the peptide H-Pro-Pro-Asn-OH (**1q**) and the formation of the corresponding γ-nitroaldehyde was followed by *in situ* FTIR-spectroscopy. As discussed in detail in chapter 3 involvement of the substrates in the rate determining step of the reaction results in a decrease of the reaction rate with increasing conversion and consequently a distinct curvature is observed in the product formation curve. If, however, protonation and hydrolysis of a cyclic intermediate is the rate determining step, the reaction rate does not depend on the substrate concentration and thus a linear conversion-time curve is expected. The resulting conversion-time curve is depicted in Figure 5-5. Interestingly, during the first 10 h of the reaction the curve is rather linear. This is indicative of the formation and the rate limiting hydrolysis of a stable intermediate. However, towards the end of the reaction a distinct curvature is observed

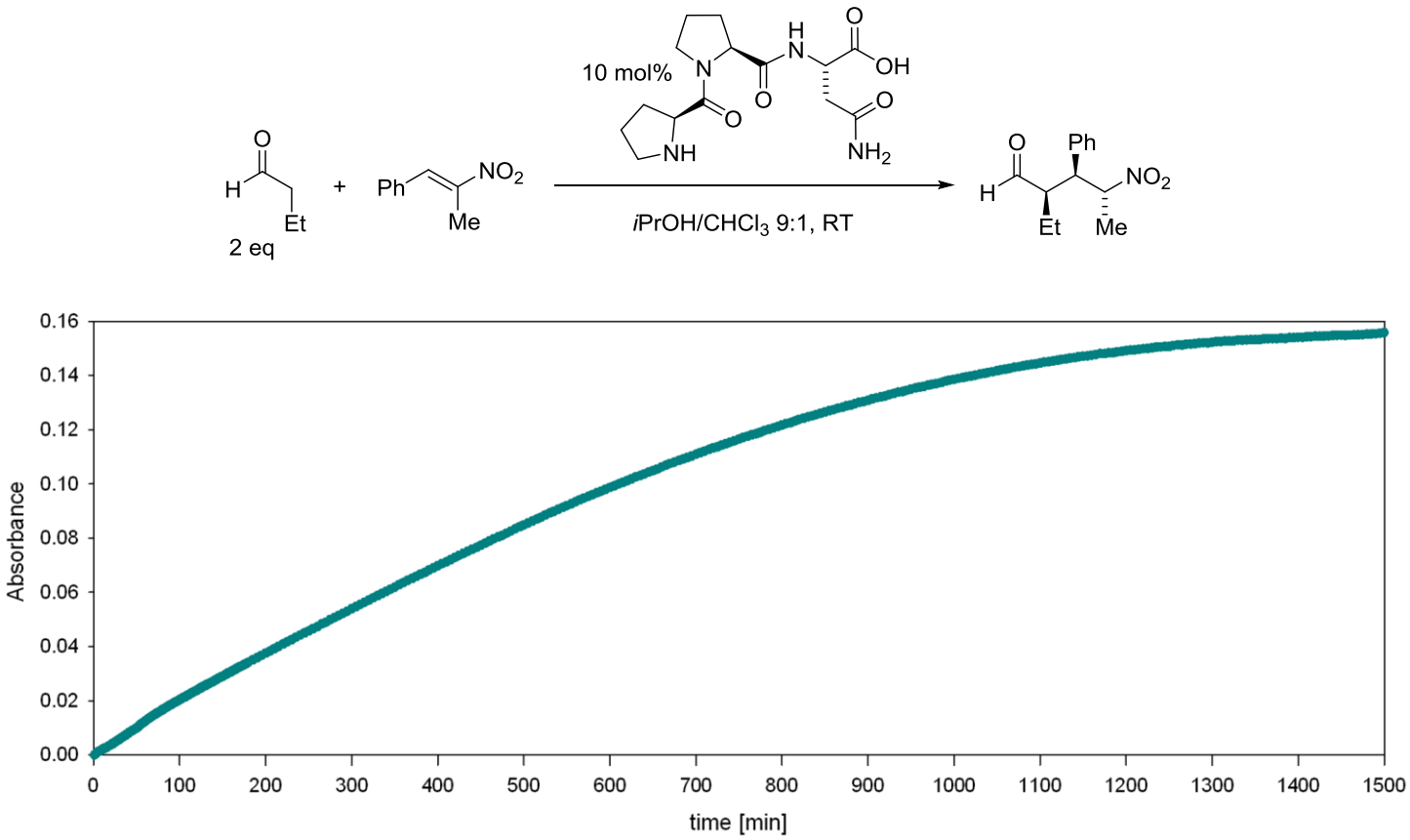


Figure 5-5 Conversion-time curve of the reaction of butanal with β-methyl-β-nitrostyrene in the presence of catalyst **1q**.

suggesting the involvement of the substrates in the rate determining step. Based on these results it can be speculated that over the course of the reaction different steps within the catalytic cycle are rate determining: when at the beginning of the reaction the substrate concentration is still high, C-C bond formation is fast. Consequently, the rate of the (slower) protonation is determining the overall rate and a linear curve is observed. In contrast, with increasing conversion the substrate concentration decreases, the C-C bond formation is slowed down and increasingly contributes to the over-all rate resulting in an increasing curvature of the conversion-time curve.

Even though the findings of both the NMR-spectroscopic as well as the kinetic studies are still preliminary at this point and somewhat inconclusive, they allow for cautious mechanistic speculations. The prompt formation of a methyl ester **2b** derived 1,6-dihydrooxazine-*N*-oxide of very high stability offers an explanation why in the presence of peptide **2b** no product formation was observed in reactions with α,β-disubstituted nitroolefins. Furthermore, the straightforward observation of intermediate **1q-D** in $CDCl_3$ together with a more complex situation in the protic solvent *i*PrOH d-8 clearly reflects the different reaction rate of catalytic

reactions in these solvents (see Table 5-4). Additionally, the potential involvement of 1,6-dihydrooxazine-*N*-oxides in the catalytic cycle of these reactions that need to be protonated for catalyst turnover offers an intriguing explanation why in these studies catalysts **1q** and **1j** with an intramolecular carboxylic acid at the C-terminus ($pK_a \approx 2$ in water) rather than in the side chain (pKa ≈ 4 in water) were found to give fastest reactions. The shape of the conversion-time curve together with the NMR-spectroscopic investigations clearly show that the mechanism and the rate limiting steps are more complicated when α,β-disubstituted nitroolefins are used rather than their β-mono-substituted analogues. Most certainly, future mechanistic investigations into this reaction will reveal an additional level of mechanistic complexity.

5.6 Conclusions

In summary it was shown that simple tripeptides are powerful catalysts for 1,4-addition reactions of aldehydes to α,β-disubstituted nitroolefins. In the presence of only 5 mol% of the peptides H-Pro-Pro-D-Gln-OH (**1j**) or H-Pro-Pro-Asn-OH (**1q**) synthetically useful γ-nitroaldehydes bearing three consecutive stereogenic centres were obtained in good yields, high diastereo- and excellent enantioselectivities. Furthermore, the resulting γ-nitroaldehydes were easily transformed to chiral pyrrolidines bearing three consecutive stereogenic centres as well as fully substituted γ-butyrolactams and γ-amino acids.

α,β-Disubstituted nitroolefins are much more challenging substrates than their monosubstituted counterparts. Interestingly peptides differing from the previously reported optimal catalysts had to be identified in order to overcome the difficulties posed by these nitroalkenes. While maintaining a powerful lead structure peptides of the type Pro-Pro-Xaa offer the necessary modularity of the C-terminal amino acid building block in order to adapt to the challenges set by α,β-disubstituted nitroolefins. This modularity combined with the ease of solid phase peptide synthesis is a clear benefit of peptidic catalysts and allowed for the identification of two highly successful catalysts from only a small peptide collection. Both peptides exhibit a high chemoselectivity for conjugate addition reactions over competing *homo*-aldol reactions of the aldehyde as well as a remarkable substrate specificity for α,β-disubstituted nitroolefins. Furthermore, the peptidic catalysts control all three stereogenic centres formed in the reaction including the one at the carbon atom bearing the nitro group. Thereby the major diastereoisomer obtained in the reaction is not the thermodynamically most stable γ-nitroaldehyde.

Lower reactivity[169] as well as the formation of stable cyclic intermediates[135-136] have been postulated as possible reasons for the dramatically lower reaction rates observed with α,β-disubstituted nitroolefins compared to reactions with β-mono-substituted analogues. Mechanistic investigations revealed that both reasons have to be considered. Whether the significantly lower reactivity of disubstituted nitroolefins or the formation of cyclic intermediates are more significant depends on the structure of the catalyst (presence or absence of an intramolecular carboxylic acid) and the nitroolefin as well as potentially on the degree of conversion of the reaction.

Clearly further investigations into the mechanism of these reactions are necessary. While such investigations most certainly will unearth a high complexity of mechanistic subtleties, they might also provide enough insight in order to further optimize the peptide design and potentially find even better catalysts for the conjugate addition reaction of aldehydes to α,β-disubstituted nitroolefins.

6 Summary and Outlook

Within this thesis, peptide catalyzed conjugate addition reactions of aldehydes to nitroolefins were examined with the aim to (1) gain a better understanding of the mechanistic subtleties that render the tripeptidic catalyst H-D-Pro-Pro-Glu-NH_2 such an excellent catalyst for 1,4-addition reactions between aldehydes and β-substituted nitroolefins, (2) develop amphiphilic analogues of H-D-Pro-Pro-Glu-NH_2 that allow for performing these conjugate addition reactions in an aqueous medium, and (3) to identify peptides of the type Pro-Pro-Xaa (Xaa = variable amino acid bearing a carboxylic acid group) that catalyze 1,4-addition reactions between aldehydes and α,β-disubstituted nitroolefins, a much more challenging and much less researched substrate class than their β-mono-substituted counterparts.

In kinetic and NMR-spectroscopic investigations we have shown that the presence or absence of a suitably positioned carboxylic acid moiety within the catalyst controls the reaction pathway of conjugate addition reactions between aldehydes and nitroolefins. The iminium nitronate intermediate is trapped intramolecularly either by protonation or cyclization to a cyclobutane intermediate. As a result, C-C bond formation between the enamine and the nitroolefin limits the rate in case of catalysts bearing an acid in a position that allows for coordination to the nitronate, whereas the protonation step is rate determining in case of catalysts lacking an acid. This knowledge of the rate limiting steps and the factors that control them has not only important implications for catalyst design and optimisation but also offers an intriguing explanation why the tripeptide H-D-Pro-Pro-Glu-NH_2 bearing an intramolecular carboxylic acid group is such an exceptionally successful catalyst for this reaction. Further mechanistic studies were directed at the elucidation whether an enamine or an enol is the active nucleophilic species in the peptide catalyzed conjugate addition reactions. Isotope exchange experiments allowed for the indirect observation of enamine formation. In addition, ESI-MS back reaction screening using mass-labelled pseudo-enantiomeric substrate mixtures revealed that in the presence of several peptides of the type Pro-Pro-Xaa the selectivity of the attack of the enamine onto the nitroolefin equals the overall selectivity of the preparative reaction. This clearly shows that C-C bond formation between an enamine and the nitroolefin is the selectivity determining step of the reaction. Thus, an enamine- rather than an enol-mechanism is taking place with this type of catalyst.

In search of a peptidic catalyst for conjugate addition reactions between aldehydes and nitroolefins in aqueous media, simple attachment of a hydrophobic alkyl chain to the C-terminus of the peptide H-D-Pro-Pro-Glu-NH_2 gave the analogue H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ as an excellent catalyst that allows the reaction to take place in an emulsion where the peptide serves as both, catalyst for the reaction as well as a detergent stabilising the emulsion. Thus, providing an organic microenvironment within an aqueous system where the reaction can take place efficiently the peptide uses the same mode of action as many enzymes do within their hydrophobic pockets.

Finally, we have introduced the tripeptides H-Pro-Pro-D-Gln-OH and H-Pro-Pro-Asn-OH as effective catalysts for conjugate addition reactions of aldehydes to α,β-disubstituted nitroolefins. These substrates are much more challenging than their β-mono-substituted counterparts because of their significantly lower reactivity as well as their disposition to form stable cyclic intermediates upon reactions with enamines. In the presence of 5 mol% of either of the two catalysts synthetically useful γ-nitroaldehydes bearing three consecutive stereogenic centres were obtained in good to excellent yields, diastereoselectivities, and enantiomeric excesses. Chiral pyrrolidines, γ-butyrolactams bearing three consecutive stereogenic centers, and fully substituted γ-amino acids are easily accessible in good yields from the γ-nitroaldehydes. The research also highlights the versatility of peptidic catalysts of the general type Pro-Pro-Xaa to accommodate different structural as well as electronic properties within the substrates. The distinctly different properties of α,β-disubstituted nitroolefins compared to β-monosubstituted nitroolefins require a distinctly different catalyst to allow for efficient reactions of these significantly more challenging electrophiles. The modular nature of peptides of the type Pro-Pro-Xaa allowed to solve this challenge by the testing of a small collection of different yet closely related peptides that are synthetically easily accessible. The versatility of the peptidic catalysts is further highlighted by the high chemoselectivity of the two peptides for conjugate addition reactions over homo-aldol reactions. Thus peptides of the type Pro-Pro-Xaa were demonstrated to be powerful tools for the fine-tuning of the catalyst structure to adapt to different substrate combinations and favour desired reaction pathways.

Future research directed at a better understanding of the mechanistic features that render α,β-disubstituted nitroolefins so much more challenging than their *mono*-substituted counterparts and investigating the underlying reasons why the two optimal peptides perform so much

better than others might provide the necessary information in order to design even better catalysts for this challenging transformation.

Furthermore, the successful lead structure Pro-Pro-Xaa with its flexibility to tune the structural and functional properties of the resulting peptides might allow for the development of catalysts for other challenging transformations such as the addition of carbonyl compounds to electrophiles as diverse as maleimides, acrylonitriles, vinyl sulfones and carbonates or azodicarboxylates.

Additionally, these investigations together with further studies on peptide catalysis under aqueous and, ultimately, physiological conditions might allow for catalysis in a cellular environment and will potentially help to answer fundamental questions such as whether there are catalytically active peptides in nature or whether peptides played a role in the evolution of enzymes.

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, $KMnO_4$ and/or ninhydrin solutions. Flash chromatography was performed using Merck or Sigma Aldrich silica gel 60, particle size 40-63 μm. Solvents for extractions and for column chromatography were of technical grade and distilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DMX600, DPX500, DPX400, av250 or Varian Mercury 300 MHz spectrometers. Chemical shifts 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 VariPureTM IPE tubes from Varian. Electrospray (ESI) mass spectra were recorded on a Bruker amaZon speed spectrometer. High resolution mass spectroscopy (HRMS) was carried out on an Applied Biosystems Sciex QStar Pular spectrometer (MS Service University of Bern) or a Bruker Daltonics maXis spectrometer (MS Service ETH-Zürich). Elemental analysis was performed on a Perkin-Elmer 240 Analyser (Dr. W. Kirsch, University of Basel). Normal Phase HPLC analysis was carried out on an analytical HPLC with a diode array detector SPD-M10A from Shimadzu or a on an Dionex UltiMate 3000 HPLC system (Thermo-Fisher) using Chiracel columns (AD-H, AS-H, OD-H) (250 mm x 4.6 mm) from Daicel. *In-situ* FT-IR spectroscopy was carried out on ReactIR R4000 or a ReactIR 15 spectrometers using SiComb probes.

7.2 General Protocols

7.2.1 General Protocols for Solid Phase Peptide Synthesis

Most peptides were prepared on solid phase. Rink Amid resin or Wang resin was used as the solid support depending on whether C-terminal primary amides or carboxylic acids were desired. The general protocol for Fmoc/tBu peptide synthesis was followed according to the general procedures below.

a) *Functionalization of Wang resin:* To a pre-swollen suspension of Wang OH resin in CH_2Cl_2, was added a solution of the Fmoc amino acid (3 equiv.), *N*-methylimidazole (2.5 equiv.) and MSNT (3 equiv.) in CH_2Cl_2. The reaction mixture was agitated at room temperature for 1 h, then washed with DMF (3x) and CH_2Cl_2 (5x). Quantitative Fmoc tests were performed as spot checks.

b) *Functionalization of Rink Amid resin:* The first amino acid was coupled to a pre-swollen suspension of Rink Amid resin according to the "*General procedure for peptide couplings"* described below.

c) *General procedure for peptide couplings:* $^{i}PrNEt_2$ (9 eq as a 3M solution in N-methylpyrrolidone) was added to a solution of Fmoc-Xxx-OH (3 eq) and HCTU (3 eq) in DMF. The activated amino acid was added as a solution in DMF (≈100 mM concentration) to the amino-functionalized resin, swollen in DMF and the mixture was agitated for 1.5 h before washing with DMF (5x).

d) *General procedure for Fmoc-deprotections*: 40% piperidine in DMF was added to the resin (preswollen in DMF) and 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).

e) *General procedure for cleavage of peptides from the solid support*: The solid supported peptides were cleaved from the Rink Amide resin by stirring in a mixture of TFA:CH_2Cl_2 2:1 for 1 h and a second time for 20 min. Pooling of filtrates and removal of all volatiles under reduced pressure followed by precipitation with Et_2O afforded the peptides as their TFA salts.

f) *General Protocol for the Ion-Exchange of Peptides:* The TFA salt of the peptide (50-100 mg) was dissolved in water (1.5 mL) and loaded on a VariPureTM IPE tube (Varian, Inc.) which was previously rinsed with MeOH (2 mL). The tube 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 lyophilised. The desalted peptide was obtained as a white solid (~80%). The absence of TFA was confirmed by ^{19}F-NMR analysis.

7.2.2 General Protocols for Conjugate Addition Reactions

Catalytic reactions were performed according to the general procedures below.

a) *General procedure for the conjugate addition reaction between aldehydes and nitroolefins followed by* in situ-*FTIR spectroscopy:* A volumetric flask (1 mL) was charged with the catalyst (22 mol, 5 mol%) and the nitroolefin (440 μmol, 1.0 eq.). Solvent was added and the resulting mixture was sonicated until a homogeneous

solution was obtained. Then the aldehyde (660 - 880 μmol, 1.5 – 2.0 eq.) was added followed by the addition of the solvent until the total volume of 1 mL was reached. The clear solution was immediately transferred to a 3 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 mode of the product γ-nitroaldehyde at 1554 cm^{-1}. Spectra were collected every minute (154 scans) for the first three hours and thereafter every 5 minutes (256 scans) until completion of the reaction. Upon completion of the reaction an aliquot (100 μL) was withdrawn from the reaction mixture, diluted with $CDCl_3$ and subjected to ^1H-NMR spectroscopic analysis to determine the diastereoselectivity. The remaining reaction mixture was used to isolate the γ-nitroaldehyde and determine the enantioselectivity of the reaction.

b) *General procedure for the conjugate addition reaction between aldehydes and nitroolefins in organic medium:*To a solution of the peptide (as the TFA salt in combination with NMM or as the desalted peptide, 4.40 μmol, 1 mol%) in the respective solvent was added the aldehyde (660 μmol, 1.5 eq) followed by the nitroolefin (440 μmol, 1.0 eq). The reaction mixture was agitated at the indicated temperature. After consumption of the nitroolefin the reaction mixture was directly applied to a chromatography column packed with silica gel and the products were eluted using mixtures of pentane and EtOAc.

c) *General procedure for the conjugate addition reaction between aldehydes and nitroolefins in aqueous medium:* To a solution of the peptide (as the TFA salt, 13.2 μmol, 3 mol %) and NMM (13.6 μmol, 3 mol %) in water (1 mL) or water and $CHCl_3$ (85:15, 1.2 mL), was added aldehyde (880 μmol, 2.0 eq.) and the nitroolefin (440 μmol, 1.0 eq.). The reaction mixture was sonicated until no more solid nitroolefin was observed and a stable white emulsion was obtained (approximately 2 min). The resulting emulsion was agitated at room temperature. After consumption of the nitroolefin, sodium chloride (330 mg) was added. The aqueous layer was extracted with $CHCl_3$ (5 x 1 mL). The combined organic layers were concentrated and directly purified by flash column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc.

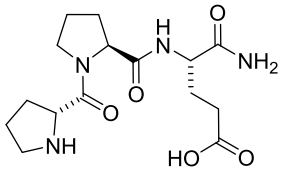
d) *General procedure for the conjugate addition reaction of aldehydes to α,β-disubstituted nitroolefins:* To a solution of the peptide (as the TFA salt, 22.0 mmol, 5

mol%), NMM (22 mmol,5 mol%), and the aldehyde (880 mmol, 2 eq.) in *i*PrOH and $CHCl_3$ (9:1, 1 mL) the nitroolefin (440 mmol, 1 eq.) was added. The resulting solution 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 eluting with a mixture of pentanes and EtOAc yielding 2,3,4-trisubstituted γ-nitroaldehydes.

e) *General procedure for the reduction of 2,3,4-trisubstituted γ-nitroaldehydes to 2,3,4-trisubstituted δ-nitroalcohols:* The isolated γ-nitroaldehyde (220 mmol, 1 eq.) was dissolved in MeOH (1 mL). The resulting solution was cooled to 0°C and $NaBH_4$ (220 mmol, 1 equiv) was added in one portion. The reaction mixture was stirred at 0°C. After consumption of the γ-nitroaldehyde the reaction was quenched by the addition of HOAc (220 mmol, 1 equiv). All volatiles were removed at reduced pressure and the resulting residue was purified by flash column chromatography on silica gel by eluting with a mixture of pentane and EtOAc. All δ-nitroalcohols were obtained in yields above 85%. Diastereomerically pure samples were either obtained by flash column chromatography on silica gel or by semi preparative HPLC by using a LichroCart 250-4 HPLC-Cartridge (LiChrospher Si 60 5 μm).

7.3 Peptide Synthesis

7.3.1 Synthesis of H-D-Pro-Pro-Glu-NH_2 (1a)

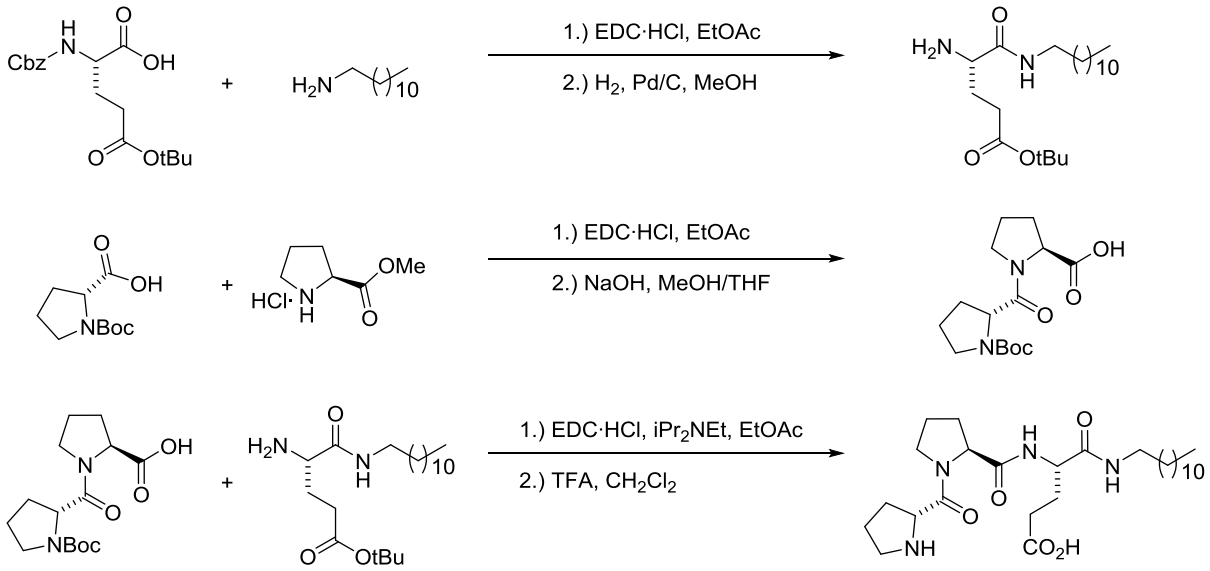


Peptide **1a** was prepared by solid phase peptide synthesis on Rink Amide AM resin (0.62 mmol/g) on up to a 3.1 mmol scale using general procedures 7.2.1 b – f. H-D-Pro-Pro-Glu-NH_2 was obtained as a white solid.

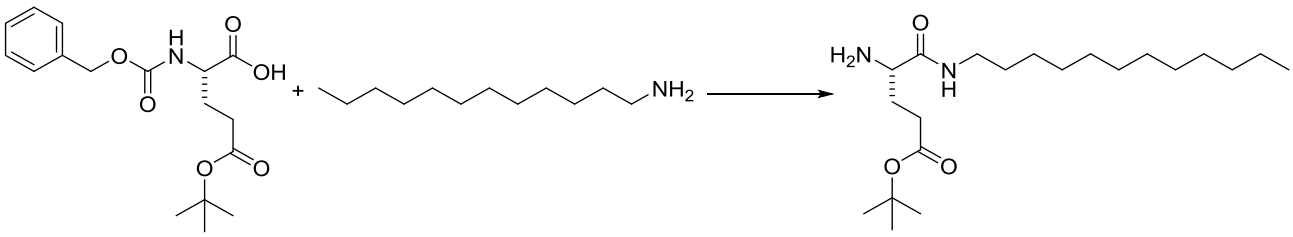
^{1}H NMR (400 MHz, DMSO-d6) δ 9.52 (d, *J* = 5.9 Hz, 1H), 7.17 (d, *J* = 2.4 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 4.42 (t, *J* = 8.4 Hz, 1H), 4.20 (dd, *J* = 8.6, 3.0 Hz, 1H), 3.89 (td, *J* = 5.9, 3.0 Hz, 1H), 3.83 – 3.73 (m, 1H), 3.60 – 3.48 (m, 1H), 3.26 (ddd, *J* = 10.9, 7.8, 5.8 Hz, 1H), 3.00 (dt, *J* = 11.1, 7.9 Hz, 1H), 2.31 – 1.52 (m, 12H). ^{13}C NMR (101 MHz, DMSO) δ 177.8, 173.1, 170.0, 169.1, 60.9, 59.2, 54.0, 46.8, 44.0, 32.5, 29.0, 26.8, 25.5, 24.0, 23.7. HRMS (ESI, $[M+H]^+$) Calcd for $C_{15}H_{25}N_4O_5$: 341.1824. Found: 341.1821.

7.3.2 Synthesis of H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (1b)

Peptide **1b** was prepared by solution phase peptide synthesis according to the strategy outlined below:



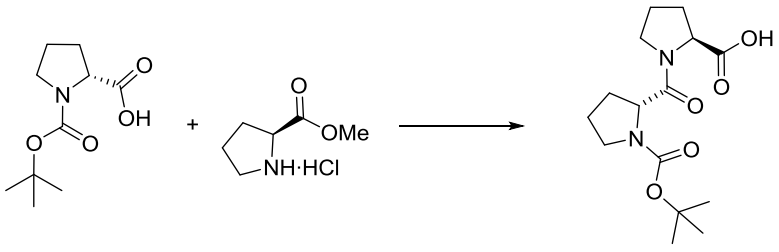
H-Glu(O*t*Bu)NH-$C_{12}H_{25}$:



Z-Glu(OtBu)-OH (1.00 g, 2.97 mmol, 1.0 eq), dodecylamine (550 mg, 2.97 mmol, 1.0 eq) and EDC·HCl (680 mg, 3.55 mmol, 1.2 eq) were suspended in EtOAc (15 mL) and stirred at room temperature for 3 h. The mixture was diluted with EtOAc (70 mL) and washed with 0.1 M HCl (2 × 10 mL), 5 % Na_2CO_3 (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_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 × 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, $[M+H]^+$) Calcd for $C_{21}H_{43}N_2O_3$: 371.3. Found: 371.3.

Boc-D-Pro-Pro-OH:

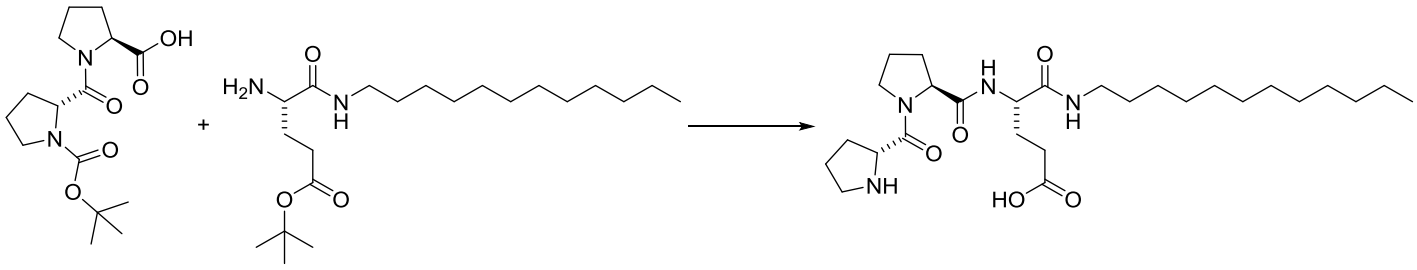


Boc-D-Pro-OH (1.94 g, 9.00 mmol, 1.05 eq), HOBt·H_2O (1.65 g, 10.8 mmol, 1.2 eq) and EDC·HCl (2.07 g, 10.8 mmol, 1.2 eq) were dissolved in CH_2Cl_2 (24 mL) and cooled to 0°C. Then *i*Pr_2NEt (1.92 mL, 11.3 mmol, 1.3 eq) was added and the mixture was stirred for 10 min

before H-Pro-OMe·HCl (1.42 g, 8.57 mmol, 1.0 eq) was added. The mixture was stirred at room temperature for 4 h. 0.1 M HCl (100 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with 10 % $NaHCO_3$ (30 mL), H_2O (20 mL) and brine (30 mL), dried over Na_2SO_4 and filtered through a short plug of silica gel. The solvent was removed under reduced pressure. The resulting colorless solid was dissolved in THF/MeOH 1:1 (15 mL), 4 M NaOH (8 mL) was added slowly and the reaction mixture stirred at room temperature for 1 h. The aqueous layer was washed with CH_2Cl_2 (3 × 10 mL), acidified (pH ≈ 2) with concentrated HCl and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure yielded Boc-D-Pro-Pro-OH as a colorless solid (2.33 g, 87 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.21 (s br, 1 H), 4.54 (m, 1H), 4.39 (m, 1H), 3.95 – 3.28 (m, 4H), 2.45 – 1.68 (m, 8H), 1.37 and 1.33 (2 s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 175.6, 174.3, 172.0, 171.5, 154.9, 153.4, 143.7, 80.6, 80.4, 60.5, 57.9, 57.7, 47.5, 46.9, 46.6, 30.2, 29.1, 28.5, 28.4, 28.3, 28.1, 28.0, 27.0, 24.8, 24.7, 24.7, 23.7 (Mixture of two conformers in a ratio of approximately 2:1). MS (ESI, $[2M+Na]^+$) Calcd for $C_{30}H_{48}N_4NaO_{10}$: 647.3. Found: 647.3.

H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (1b):

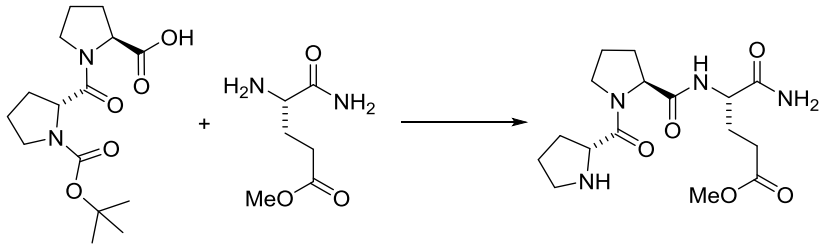


Boc-D-Pro-Pro-OH (1.00 g, 3.20 mmol, 1.0 eq) and EDC·HCl (736 mg, 3.84 mmol, 1.2 eq) were suspended in 20 mL EtOAc and *i*Pr_2NEt (660 mL, 1.2 eq) was added. After stirring for 10 min at room temperature H-Glu(OtBu)NH-$C_{12}H_{25}$ (1.19 g, 3.2 mmol, 1.0 eq) was added and the suspension was stirred at room temperature for 3h. The reaction mixture was diluted with 40 mL of EtOAc, washed with 0.1 M HCl (2 x 10 mL), 5 % Na_2CO_3 (10 mL) and brine (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give a

colourless oil which was purified by flash column chromatography on silica gel eluting with 7 % MeOH in EtOAc. The protected peptide was dissolved in 5 mL of TFA/CH_2Cl_2 2:1 and stirred at room temperature for 30 min. All volatile components were removed under reduced pressure to afford peptide H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ as the TFA salt (1.50 g, 75 %). The TFA was removed according to the general protocol 7.2.1 f).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (d, *J* = 6.0 Hz, 1H), 6.85 (t, *J* = 5.5 Hz, 1H), 4.54 (t, *J* = 7.7 Hz, 1H), 4.44 (dd, *J* = 6.9, 5.3 Hz, 1H), 4.30 (td, *J* = 6.0, 3.0 Hz, 1H), 3.95 (dt, *J* = 9.9, 5.9 Hz, 1H), 3.51 (dt, *J* = 9.8, 7.8 Hz, 1H), 3.43 – 3.31 (m, 1H), 3.31 – 3.13 (m, 3H), 2.45 – 1.84 (m, 9H), 1.58 – 1.43 (m, 1H), 1.38 – 1.13 (m, 22H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) 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, $[M+H]^+$) Calcd for $C_{27}H_{49}N_4O_5$: 509.3697. Found: 509.3709.

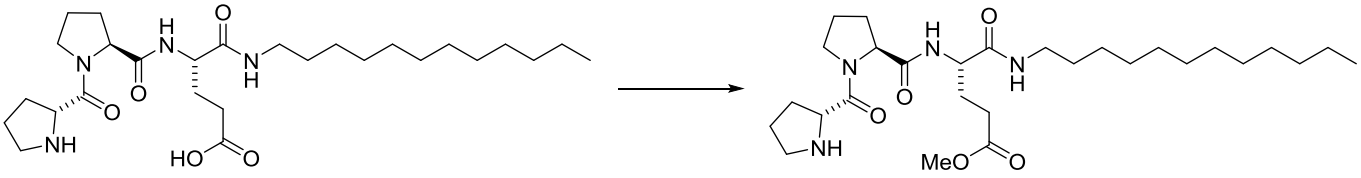
7.3.3 Synthesis of H-D-Pro-Pro-Glu(OCH$_3$)-NH$_2$ (2a)



Boc-D-Pro-Pro-OH (794 mg, 2.54 mmol, 1.0 eq) and EDC·HCl (584 mg, 3.05 mmol, 1.2 eq) were dissolved in EtOAc (12 mL) and DMF (1.2 mL). After adding *i*Pr$_2$NEt (508 µL, 3.05 mmol, 1.20 eq) the mixture was stirred at room temperature for 10 min. Then H-Glu(OMe)-NH$_2$ (500 mg, 2.54 mmol, 1.0 eq) was added and the resulting cloudy mixture was stirred at room temperature for 12 h. The mixture was diluted with EtOAc (20 mL) and washed with 0.1 M HCl (10 mL), H$_2$O (10 mL), 10 % NaHCO$_3$ (10 mL) and brine (2 × 10 mL). The aqueous layer was re-extracted with CH$_2$Cl$_2$ (30 mL). The combined organic layers were dried over Na$_2$SO$_4$. After removing all volatiles under reduced pressure, the crude product was filtered through a plug of silica gel eluting with 10 % MeOH in EtOAc. The solvents were removed under reduced pressure and the product dissolved in a mixture of TFA/CH$_2$Cl$_2$ 2:1 (5 mL). The mixture was stirred at room temperature for 2 hours. Then, the solvent was removed under reduced pressure and the peptide precipitated by the addition of Et$_2$O. The solvent was decanted and the resulting oil dried *in vacuo* to provide peptide H-D-Pro-Pro-Glu(OMe)-NH$_2$ as the TFA salt (570 mg, 45 %). The TFA was removed according to the general protocol 7.2.1 f).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.74 (d, *J* = 8.2 Hz, 1H), 6.82 (s, 1H), 6.18 (s, 1H), 4.53 – 4.38 (m, 2H), 3.96 – 3.83 (m, 2H), 3.68 (s, 3H), 3.51 (dt, *J* = 10.8, 7.8 Hz, 1H), 3.22 – 3.07 (m, 1H), 2.81 (dt, *J* = 10.7, 7.0 Hz, 1H), 2.53 – 2.36 (m, 2H), 2.32 – 1.93 (m, 7H), 1.90 – 1.61 (m, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ 174.8, 174.5, 173.8, 171.7, 61.6, 59.5, 52.4, 51.9, 47.2, 47.1, 30.6, 29.6, 29.3, 26.5, 25.8, 24.8. HRMS (ESI, [M+Na]$^+$) Calcd for C$_{16}$H$_{26}$N$_4$NaO$_5$: 377.1795. Found: 377.1807.

7.3.4 Synthesis of H-D-Pro-Pro-Glu(OCH_3)-NH-$C_{12}H_{25}$ (2b)



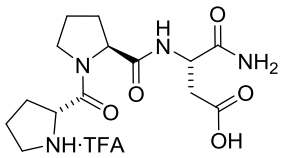
TFA·H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (150 mg, 240 mmol, 1.0 eq) was dissolved in MeOH (4 mL) and cooled to -5°C with an ice/NaCl bath. Thionyl chloride (68.0 µL, 960 mmol, 4.0 eq) was added carefully and the solution was stirred for 90 min at -5 to 15 °C. The solution was added to 10 % $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (5x 10 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Flash column chromatography on silica gel eluting with CH_2Cl_2:MeOH:NH_3(aq) 100:10:1 provided peptide H-D-Pro-Pro-Glu(OMe)-NH-$C_{12}H_{25}$ as a colorless solid (82 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 7.8 Hz, 1H), 6.88 (t, J = 5.6 Hz, 1H), 4.45 – 4.30 (m, 2H), 3.95 (dd, J = 8.6, 5.8 Hz, 1H), 3.90 – 3.80 (m, 1H), 3.66 (s, 3H), 3.50 (dt, J = 9.9, 7.7 Hz, 1H), 3.29 – 3.03 (m, 4H), 2.84 (dt, J = 10.8, 6.7 Hz, 1H), 2.53 – 2.32 (m, 1H), 2.23 – 2.02 (m, 5H), 2.04 – 1.91 (m, 2H), 1.89 – 1.63 (m, 3H), 1.58 – 1.39 (m, 2H), 1.35 – 1.15 (m, 18H), 0.85 (t, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 174.8, 171.5, 170.6, 61.5, 59.7, 53.2, 52.1, 47.6, 47.2, 39.8, 32.0, 30.5, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 29.2, 27.0, 26.5, 26.1, 24.9, 22.8, 14.2. HRMS (ESI, $[M+H]^+$) Calcd for $C_{28}H_{51}N_4O_5$: 523.3854. Found:523.3838.

7.3.5 Synthesis of Peptides 1c – g with Increasing Side Chain Length

Peptides H-D-Pro-Pro-Asp-NH_2 (**1c**), H-D-Pro-Pro-Aad-NH_2 (**1d**), H-D-Pro-Pro-Api-NH_2 (**1e**) and H-D-Pro-Pro-Asu-NH_2 (**1f**) were prepared on solid support as described previously.[92,130] Similarly, the analogue H-D-Pro-Pro-Ada-OH (**1g**) bearing a along alkyl spacer between the backbone of the peptide and the carboxylic acid was also obtained by solid phase peptide synthesis.

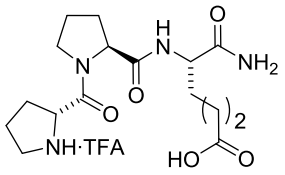
TFA·H-D-Pro-Pro-Asp-NH_2 (1c):



Peptide **1c** was prepared on Rink Amide AM resin (0.72 mmol/g) on a 215 µmol scale using general procedures 7.2.1 b – f. H-D-Pro-Pro-Asp-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.71 (dd, *J* = 5.3, 8.4 Hz, 1H), 4.64 (dd, *J* = 7.1, 8.7 Hz, 1H), 4.46 (dd, *J* = 3.7, 8.6 Hz, 1H), 3.73 (m, 1H), 3.60 (m, 1H), 3.42 (m, 2H), 2.97 (dd, *J* = 5.3, 16.9 Hz, 1H), 2.84 (dd, *J* = 8.4, 16.8 Hz, 1H), 2.55 (m, 1H), 2.31 (m, 1H), 2.11-1.97 (m, 6H); 13C NMR (100 MHz, D_2O) δ 175.2, 174.5, 174.3, 168.9, 61.4, 58.8, 50.4, 48.1, 47.1, 35.8, 29.9, 28.5, 24.7, 24.4; HRMS (ESI, $[M+H]^+$) Calcd for $C_{14}H_{23}N_4O_5$: 327.1668. Found, 327.1661.

TFA·H-D-Pro-Pro-Aad-NH_2 (1d):

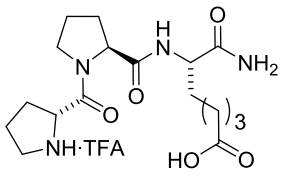


Peptide (**1d**) was prepared on Rink Amide AM resin (0.72 mmol/g) on a 215 µmol scale using general procedures 7.2.1 b – f. H-D-Pro-Pro-Aad-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.64 (dd, *J* = 7.0, 8.7 Hz, 1H), 4.48 (dd, *J* = 3.5, 8.9 Hz, 1H), 4.28 (dd, *J* = 5.4, 8.8 Hz, 1H), 3.76 (m, 1H), 3.61 (m, 1H), 3.43 (m, 2H), 2.56 (m, 1H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.33 (m, 1H), 2.12 – 1.93 (m, 6H), 1.91 – 1.62 (m, 4H); ^{13}C NMR (100

MHz, D_2O) δ 181.7, 179.6, 177.2, 171.4, 63.9, 62.4, 56.6, 50.8, 49.8, 36.5, 33.3, 32.7, 31.2, 27.4, 27.1, 23.9; MS (ESI, $[M+H]^+$) Calcd for $C_{16}H_{27}N_4O_5$: 355.4. Found 355.2.

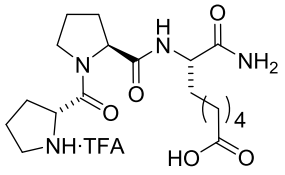
TFA·H-D-Pro-Pro-Api-NH_2 (1e):



Peptide (**1e**) was prepared on Rink Amide AM resin (0.72 mmol/g) on a 215 µmol scale using general procedures 7.2.1 b – f. H-D-Pro-Pro-Api-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.64 (m, 1H), 4.47 (dd, *J* = 3.5, 8.9 Hz, 1H), 4.27 (dd, *J* = 5.5, 9.0 Hz, 1H), 3.72 (m, 1H), 3.60 (m, 1H), 3.43 (m, 2H), 2.66 (m, 1H), 2.32 (m, 3H), 2.05 (m, 5H), 1.79 (m, 3H), 1.62 (m, 2H), 1.42 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 182.4, 179.7, 177.0 171.2, 63.7, 62.2, 56.5, 50.6, 49.6, 37.0, 33.4, 32.5, 31.0, 27.6, 27.2, 26.9, 26.9; MS (ESI, $[M+H]^+$) Calcd for $C_{17}H_{29}N_4O_5$: 369. 4. Found 369.2.

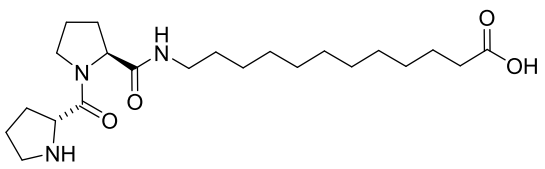
TFA·H-D-Pro-Pro-Asu-NH_2 (1f):



Peptide (**1f**) was prepared on Rink Amide AM resin (0.72 mmol/g) on a 215 µmol scale using general procedures 7.2.1 b - f. H-D-Pro-Pro-Asu-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.64 (dd, *J* = 7.3, 8.5 Hz, 1H), 4.48 (dd, *J* = 3.5, 9.0 Hz, 1H), 4.26 (dd, *J* = 5.6, 9.0 Hz, 1H), 3.73 (m, 1H), 3.61 (m, 1H), 3.43 (m, 2H), 2.55 (m, 1H), 2.37 – 2.26 (m, 3H), 2.04 (m, 5H), 1.78 (m, 3H), 1.59 (td, *J* = 7.3, 14.2 Hz, 2H), 1.30 (m, 4H); ^{13}C NMR (100 MHz, D2O) δ 183.0, 179.7, 176.8, 171.0, 63.5, 62.0, 56.4, 50.4, 49.4, 37.3, 33.4, 32.3, 30.8, 30.4, 27.5, 27.2, 27.0 26.7; MS (ESI, $[M+H]^+$) Calcd for $C_{18}H_{31}N_4O_5$: 382.5. Found 383.3.

H-D-Pro-Pro-Ada-OH (1g):



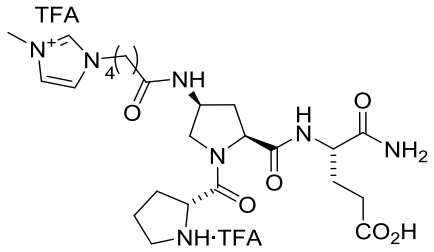
Peptide (**1g**) was prepared on Wang resin (0.80 mmol/g) on a 1.6 mmol scale using general procedures 7.2.1 a and c – f. H-D-Pro-Pro-Ada-OH was obtained as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (t, *J* = 5.8 Hz, 1H), 4.55 (dd, *J* = 7.9, 2.5 Hz, 1H), 4.22 (t, *J* = 8.3 Hz, 1H), 3.87 – 3.77 (m, 1H), 3.41 (dd, *J* = 9.4, 7.8 Hz, 1H), 3.34 – 3.19 (m, 2H), 3.11 – 3.01 (m, 2H), 2.37 – 2.14 (m, 4H), 2.13 – 1.92 (m, 5H), 1.75 (dq, *J* = 12.5, 8.0 Hz, 1H), 1.64 – 1.51 (m, 2H), 1.51 – 1.39 (m, 2H), 1.34 – 1.19 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.1, 170.4, 170.4, 61.2, 58.8, 46.7, 45.4, 39.8, 35.8, 29.6, 29.3, 29.0, 29.0, 28.9, 28.8, 28.8, 28.7, 26.7, 26.0, 25.4, 24.0. HRMS (ESI, $[M+H]^+$) Calcd for $C_{22}H_{40}N_3O_4$: 410.3013. Found: 410.3013.

7.3.6 Synthesis of Peptides 1h – j used in the ESI-MS Back Reaction Screening

Peptide H-D-Pro-Pro(4-NHCO-C_4H_8-MeIm)-Glu-NH_2 (**1h**) was prepared on solid support coupling (4*S*)-azidoproline as the second amino acid followed by Staudinger reduction and coupling of the charge tag. The peptides H-Pro-Pro-d-Glu-NH2 (1i) and H-Pro-Pro-d-Gln-OH (1j) were prepared by solid phase peptide synthesis as described previously.[130]

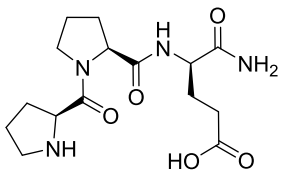
TFA_2·H-D-Pro-Pro(4-NHCO-C_4H_8-MeIm)-Glu-NH_2 (1h):



The peptide was constructed on Rink Amide AM resin (0.72 mmol/g) on a 180 µmol scale following general procedures 7.2.1 b – d using (4*S*)-azidoproline as the second amino acid. The azido-group was then reduced on solid support by treatment with 5 eq of Me_3P (1 M) and 65 eq of H_2O over night. To the resulting (4*S*)-aminoproline containing peptide was then coupled 1-(4-carboxybutyl)-3-methylimidazol-3-ium bromide following the general procedure 7.2.1 c. After cleavage from the solid support and isolation according to the general procedure e, the peptide was obtained as a colourless solid.

^{1}H NMR (400 MHz, DMSO) δ 12.17 (s, 1H), 9.52 (s, 1H), 9.12 (s, 1H), 8.59 (s, 1H), 8.34 (d, *J* = 7.9 Hz, 1H), 8.09 – 7.98 (m, 1H), 7.75 (s, 1H), 7.71 (s, 1H), 7.32 (s, 1H), 7.09 (s, 1H), 4.55 – 4.30 (m, 3H), 4.27 – 4.07 (m, 3H), 3.85 (s, 3H), 3.77 (dd, *J* = 10.5, 6.4 Hz, 1H), 3.40 – 3.31 (m, 1H), 3.31 – 3.21 (m, 1H), 3.21 – 3.07 (m, 1H), 2.48 – 2.17 (m, 4H), 2.17 – 2.02 (m, 2H), 2.02 – 1.63 (m, 8H), 1.57 – 1.33 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 174.0, 173.8, 173.0, 171.3, 166.6, 136.5, 123.7, 122.3, 58.2, 57.5, 52.1, 51.9, 48.6, 45. 8, 35.8, 34.5, 30.3, 28.9, 27.9, 27.3, 23.5, 21.6, 16.9; MS (ESI, $[M]^+$) Calcd for $C_{24}H_{38}N_7O_6$: 520.3. Found 520.4.

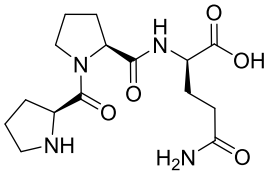
TFA·H-Pro-Pro-D-Glu-NH_2 (1i):



The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 200 µmol scale using general procedures 7.2.1 b – e. TFA·H- Pro-Pro- D-Glu-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.50 (dd, *J* = 6.4, 8.5 Hz, 1H), 4.35 (dd, *J* = 7.1, 7.7 Hz, 1H), 4.24 (dd, *J* = 4.7, 9.9 Hz, 1H), 3.59 (m, 1H), 3.45 (m, 1H), 3.27 (m, 2H), 2.50-2.30 (m, 3H), 2.21 (m, 1H), 2.09 (m, 1H), 2.02-1.75 (m, 7H); ^{13}C NMR (100 MHz, D_2O) δ 177.3, 176.3, 174.4, 168.4, 61.2, 59.5, 53.0, 48.1, 47.0, 30.4, 29.7, 28.7, 26.4, 25.1, 24.2; MS (ESI, $[M+H]^+$) Calcd for $C_{15}H_{25}N_4O_5$: 341.2. Found 341.2.

TFA·H-Pro-Pro-D-Gln-OH (1j):

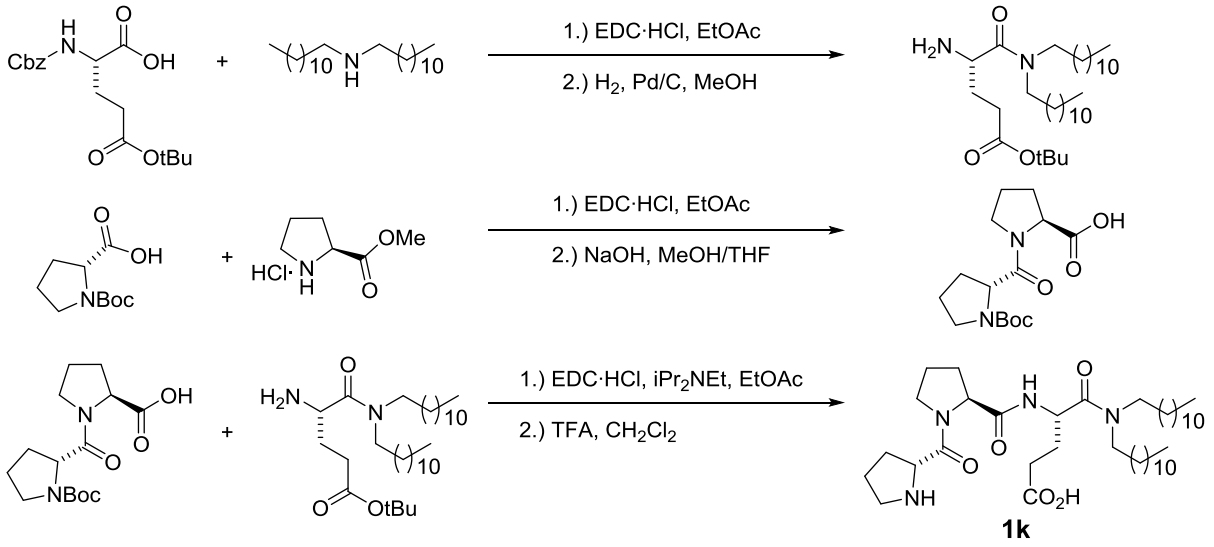


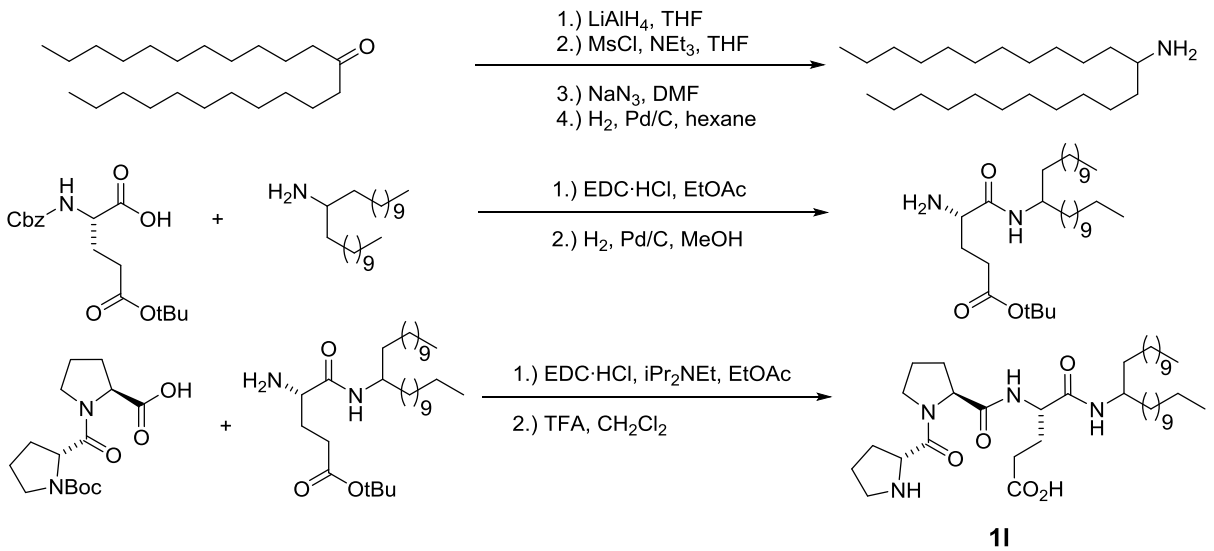
The peptide was prepared on Wang resin (0.80 mmol/g) on a 2.4 mmol scale using general procedures 7.2.1 a as well as c – e. TFA·H-D-Pro-Pro-Ada-OH was obtained as a white solid.

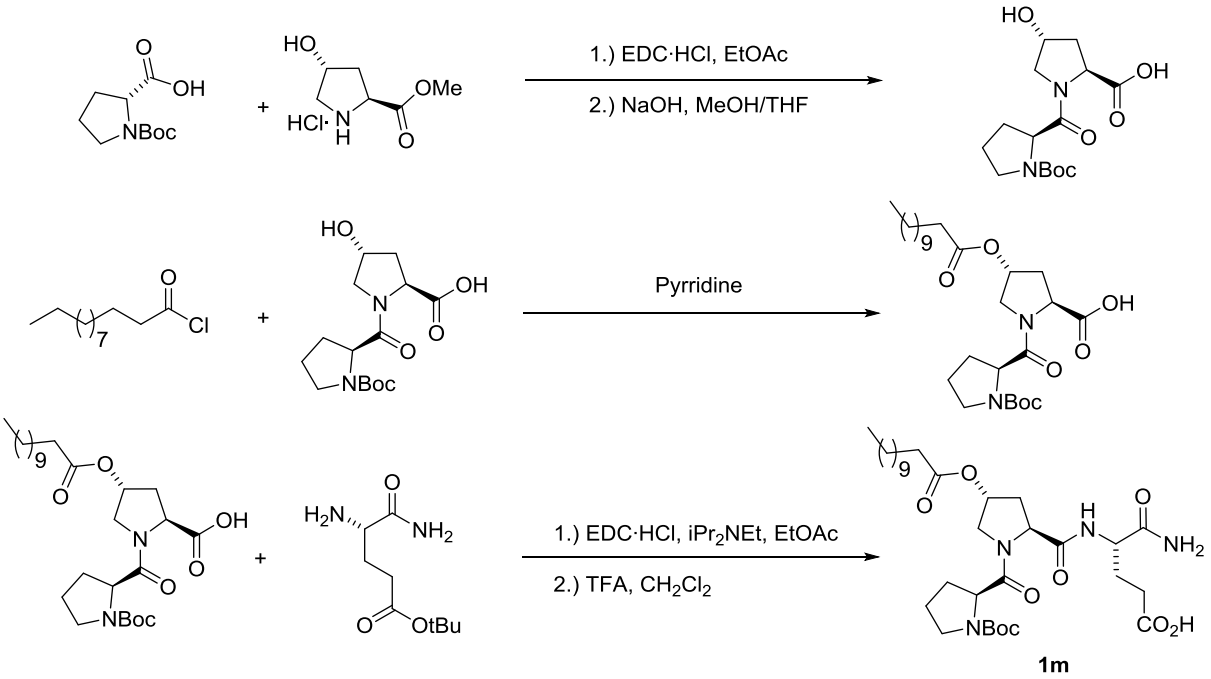
^{1}H NMR (400 MHz, D_2O): δ 4.71 – 4.58 (m, 1H), 4.58 – 4.49 (m, 1H), 4.43 (dd, *J* = 8.9, 4.6 Hz, 1H), 3.80 – 3.68 (m, 1H), 3.68 – 3.52 (m, 1H), 3.52 – 3.27 (m, 2H), 2.67 – 2.42 (m, 2H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.30 – 2.16 (m, 1H), 2.16 – 1.76 (m, 7H); ^{13}C NMR (100 MHz, D_2O): δ 178.3, 175.2, 174.0, 168.5, 61.0, 59.5, 52.6, 48.1, 47.0, 31.5, 29.9, 28.8, 27.0, 25.0, 24.3; HRMS (ESI): *m/z* calcd for $C_{15}H_{25}N_4O_5$: 341.1819 $[M+H^+]$; found: 341.1819.

7.3.7 Synthesis of Amphiphilic Peptides 1k – p for Reactions in Aqueous Media

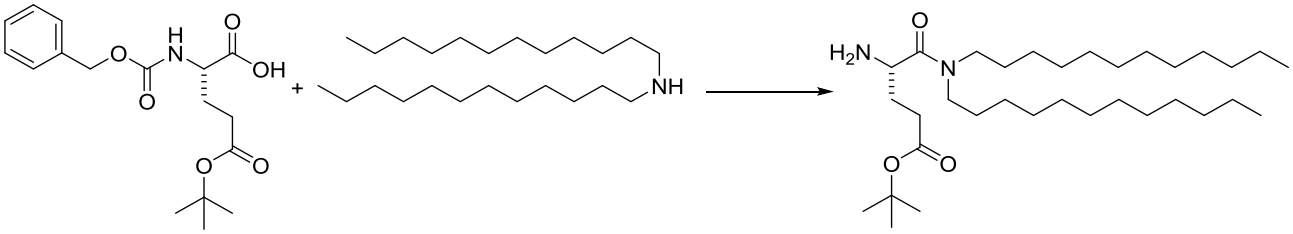
Peptides H-D-Pro-Pro-Glu-N($C_{12}H_{25}$)$_2$ (**1k**), H-D-Pro-Pro-Glu-NN-CH($C_{11}H_{23}$)$_2$ (**1l**) and H-D-Pro-Hyp(OCO$C_{11}H_{23}$)-Glu-NH_2 (**1m**) were prepared by solution phase peptide synthesis according to the strategy outlined below. The three peptides H-D-Pro-Pro-Glu-Ada-TEG (**1n**), H-D-Pro-Pro-Glu-Ada$_2$-TEG (**1o**) and H-D-Pro-Pro-Glu-Ada$_3$-TEG (**1p**) were prepared by solid phase peptide synthesis.







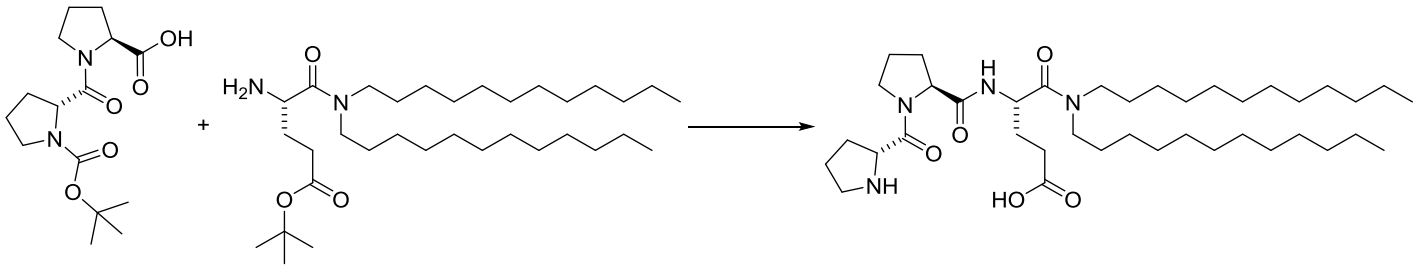
H-Glu(O*t*Bu)N($C_{12}H_{25}$)$_2$:



Z-Glu(OtBu)-OH (1.00 g, 2.96 mmol, 1.0 eq), didodecylamine (1.05 g, 2.96 mmol, 1.0 eq) and EDC·HCl (681 mg, 3.55 mmol, 1.2 eq) were suspended in EtOAc (15 mL) and stirred at room temperature over night. The reaction mixture was diluted with 80 mL EtOAc and washed with 0.1 M HCl (3 × 20 mL), 5 % Na_2CO_3 (2 × 20 mL) and brine (40 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting colourless solid was dissolved in 15 mL MeOH. Pd/C (10 % w/w, 150 mg) was added and the mixture was stirred under a hydrogen atmosphere at room temperature for 5 h. The reaction mixture was filtered over a pad of celite. The celite was washed with MeOH (3 × 5 mL). The solvent was removed under reduced pressure to give a colorless solid (1.23 g, 78 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 4.5 Hz, 1H), 3.59 (dd, *J* = 9.2, 4.1 Hz, 1H), 3.56 – 3.39 (m, 2H), 3.15 – 2.97 (m, 2H), 2.46 (ddd, *J* = 16.6, 8.5, 6.7 Hz, 1H), 2.39 – 2.25 (m, 1H), 1.92 – 1.80 (m, 1H), 1.67 – 1.45 (m, 5H), 1.43 (s, 9H), 1.37 – 1.14 (m, 36H), 0.86 (t, *J* = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.1, 172.8, 80.2, 50.2, 47.2, 46.1, 31.9, 31.3, 30.7, 29.6 - 29.5 (m), 29.4, 29.3, 29.3, 28.1, 28.1, 27.7, 27.0, 26.8, 22.7, 14.1; MS (ESI, $[M+H]^+$) Calcd for $C_{33}H_{67}N_2O_3$: 539.5. Found: 539.8.

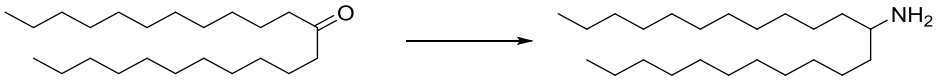
TFA·H-D-Pro-Pro-Glu-N($C_{12}H_{25}$)$_2$ (1k):



Boc-D-Pro-Pro-OH (380 mg, 1.22 mmol, 1.0 eq) and EDC·HCl (280 mg, 1.46 mmol, 1.2 eq) were suspended in 10 mL EtOAc and *i*Pr_2NEt (251 µL, 1.2 eq) followed by H-Glu(O*t*Bu)-N($C_{12}H_{25}$)$_2$ were added. The suspension was stirred at room temperature for 4h. The mixture was diluted with EtOAc (20 mL) and washed with 1 M HCl (10 mL), H_2O (10 mL), sat. $NaHCO_3$ (10 mL) and brine (2 × 10 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel eluting with a mixture of EtOAc and pentanes (2:1). The colourless oil was dissolved in 3 mL TFA/CH_2Cl_2 2:1 and stirred at room temperature for 60 min. Removal of all volatile components under reduced pressure provided the peptide as the TFA salt (579 mg, 70 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.4 Hz, 1H), 4.86 – 4.69 (m, 2H), 4.59 – 4.49 (m, 1H), 3.84 – 3.72 (m, 1H), 3.62 – 3.33 (m, 5H), 3.33 – 3.24 (m, 1H), 3.09 (dt, *J* = 13.0, 7.5 Hz, 1H), 2.64 – 2.33 (m, 3H), 2.29 – 1.78 (m, 9H), 1.47 – 1.53 (m, 2H), 1.54 – 1.42 (m, 2H), 1.39 – 1.12 (m, 36H), 0.87 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.9, 171.0, 170.0, 168.3, 60.9, 59.0, 49.5, 48.1, 47.1, 47.0, 46.7, 31.9, 29.7 – 29.4 (m), 29.3, 29.1, 29.0, 28.8, 28.6, 27.4, 26.9, 26.8, 26.5, 24.8, 24.4, 22.7, 14.1; HRMS (ESI): *m/z* calcd for $C_{39}H_{73}N_4O_5$: 677.5575 [*M*+H^+]; found: 677.5573.

12-Aminotricosane:

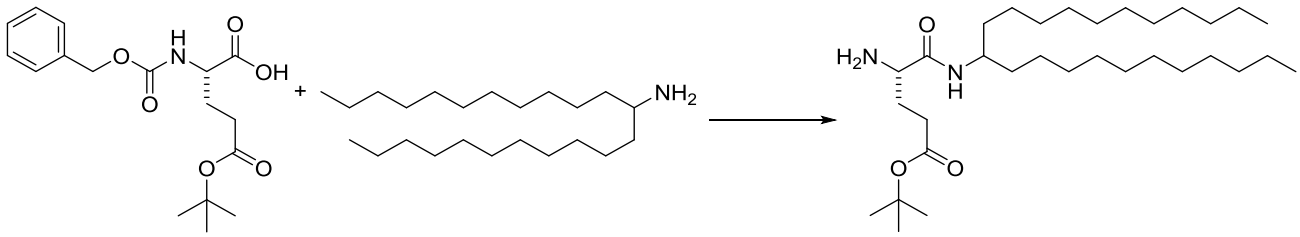


$LiAlH_4$ (682 mg, 18.0 mmol, 1.2 eq) was placed in 10 mL of THF. The mixture was cooled to 0°C and a suspension of 13-tricosanone (5.00 g, 14.8 mmol, 1.0 eq) in THF was added. The ice bath was removed and stirring was continued for 6 h. The reaction mixture was poured into 100 mL ice/water and extracted with CH_2Cl_2 (4x50 mL). The combined extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure to give 12-tricosanol as a colourless solid. The alcohol (3.00 g, 8.81 mmol, 1.0 eq) was dissolved in 60 mL THF, NEt_3 (1.35 mL, 9.69 mmol, 1.1 eq) was added followed by the dropwise addition of MsCl (1.96 mL, 25.3 mmol, 2.9 eq). The resulting suspension was stirred at room temperature for 90 min, filtered, washed with 15 mL of H_2O and 5 mL of brine and dried over Na_2SO_4. Removal of all volatiles under reduced pressure resulted in a colourless oil which was dissolved in 50 mL DMF. Sodium azide (2.36 g, 36.3 mmol, 4.8 eq) was slowly added in portions. The resulting suspension was heated to 85°C over night. The reaction mixture was allowed to cool to room temperature followed by the addition of 100 mL hexanes and 20 mL H_2O. The organic layer was washed with 20 mL sat. $NaHCO_3$ solution and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the resulting residue purified by column chromatography on silica gel eluting with pentanes. 12-azidotricosane was obtained as a colourless oil. The oil was dissolved in 20 mL hexane and Pd/C (10% w/w, 58 mg) was added. The resulting mixture was stirred under a H_2 atmosphere over night. Filtration through a plug of Celite and removal of the solvent under reduced pressure provided 12-aminotricosane as a colourless solid (2.23 g, 60 % overall yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.73 – 2.59 (m, 1H), 1.48 – 1.10 (m, 42H), 0.87 (t, J = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 51.2, 38.2, 31.9, 29.8, 29.7 – 29.6 (m), 29.3, 26.2, 22. 7, 14.1.

Spectroscopic data is in agreement with published data.[185]

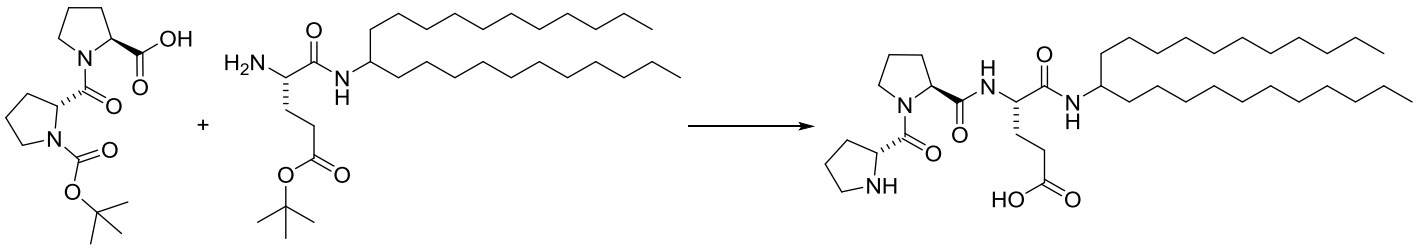
H-Glu(O*t*Bu)-NH-CH($C_{11}H_{23}$)$_2$:



Z-Glu(OtBu)-OH (100 mg, 295 µmol, 1.0 eq), 12-aminotricosane (100 mg, 295 µmol, 1.0 eq) and EDC·HCl (68.6 mg,354 µmol, 1.2 eq) were suspended in EtOAc (5 mL) and stirred at room temperature for 3 h. The reaction mixture was diluted with 15 mL of EtOAc and washed with 1 M HCl (2x10 mL), sat. $NaHCO_3$ (10 mL) and brine (10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting colourless solid was dissolved in 6 mL of a 5:1 mixture of MeOH and hexane. Pd/C (10% w/w, 5 mg) was added and the resulting mixture was stirred under a H_2 atmosphere for 3 h. Filtration through a plug of silica and removal of the solvents under reduced pressure provided 151 mg of a colourless solid (98 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (d, *J* = 9.3 Hz, 1H), 3.98 – 3.77 (m, 1H), 3.37 (dd, *J* = 7.5, 5.0 Hz, 1H), 2.41 – 2.31 (m, 2H), 2.12 – 2.02 (m, 1H), 1.87 – 1.75 (m, 1H), 1.59 – 1.04 (m, 40 H), 1.44 (s, 9H); 0.87 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.8, 172.9, 80.5, 54.8, 48.8, 35.2, 31. 9, 31.2, 30.5, 29.6, 29.6, 29.6 - 29.2 (m), 28.1, 26.4, 22. 7, 14.1; MS (ESI, $[M+H]^+$) Calcd for $C_{32}H_{65}N_2O_3$: 525.5. Found: 525.7.

TFA·H-D-Pro-Pro-Glu-NH-CH($C_{11}H_{23}$)$_2$ (1l):

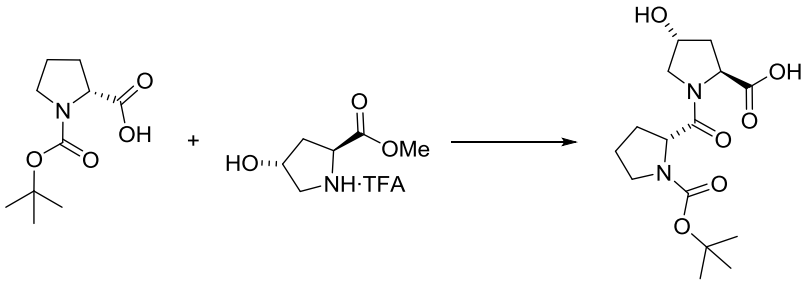


Boc-D-Pro-Pro-OH (59.5 mg, 191 µmol, 1.0 eq), H-Glu(O*t*Bu)-NH-CH($C_{11}H_{23}$)$_2$ (100 mg, 191 µmol, 1.0 eq) and EDC·HCl (43.9 mg, 229 µmol, 1.2 eq) were suspended in 3 mL EtOAc and *i*Pr_2NEt (39.2 µL, 229 µmol, 1.2 eq) was added. After stirring at room temperature over night the reaction mixture was diluted with 20 mL EtOAc and washed successively with 1M

HCl (3x10 mL), sat. $NaHCO_3$ (2x10 mL) and brine (10 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel eluting with EtOAc. The resulting colourless oil was dissolved in a mixture of TFA and CH_2Cl_2 2:1 and stirred at room temperature for 90 min. Removal of all volatile components under reduced pressure gave the peptide as the TFA salt (77.2 mg, 52 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (d, *J* = 7.5 Hz, 1H), 6.37 (d, *J* = 9.0 Hz, 1H), 4.78 (t, *J* = 7.8 Hz, 1H), 4.53 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.47 (td, *J* = 7.2, 2.2 Hz, 1H), 3.97 – 3.71 (m, 3H), 3.52 (td, *J* = 9.7, 6.9 Hz, 1H), 3.45 – 3.30 (m, 1H), 2.67 – 2.44 (m, 3H), 2.44 – 2.24 (m, 3H), 2.24 – 1.92 (m, 4H), 1.84 – 1.70 (m, 1H), 1.63 – 1.04 (m, 41H), 0.87 (t, *J* = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.5, 169.4, 169.1, 168.9, 62.0, 59.2, 53.1, 49.2, 47.5, 46.9, 42.8, 35.1, 34.8, 31.9, 31.9, 30.1, 29.9 – 29.2 (m), 28.8, 26.0, 25.6, 25.6, 24.9, 24.3, 23.9, 22.7, 14.1; HRMS (ESI): *m/z* calcd for $C_{38}H_{71}N_4O_5$: 663.5419 [M+H^+]; found: 663.5417.

Boc-D-Pro-Hyp-OH:

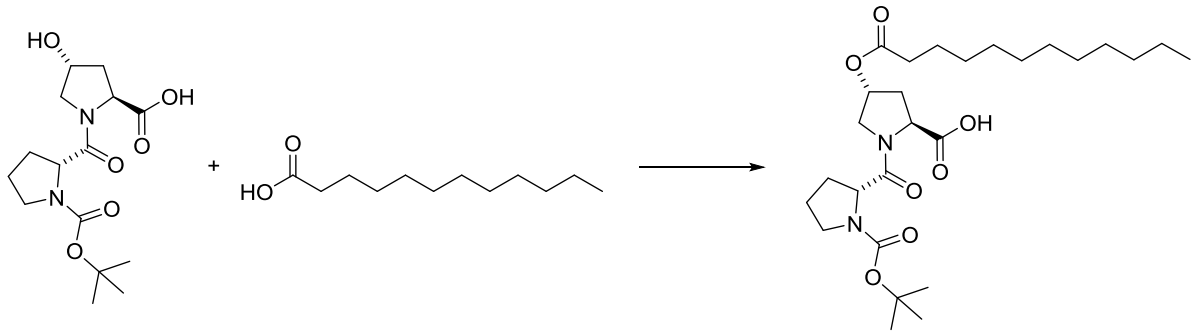


Boc-D-Pro-OH (1.77 g, 8.22 mmol, 1.0 eq), and EDC·HCl (1.89 g, 8.60 mmol, 1.05 eq) were suspended in EtOAc (10 mL). Then *i*Pr_2NEt (3.37 mL, 19.3 mmol, 2.3 eq) was added and the mixture was stirred for 10 min before H-Hyp-OMe·TFA (1.99 g, 8.22 mmol, 1.0 eq) was added. The mixture was stirred at room temperature for 6 h, deluted with 20 mL of EtOAc and washed with 1 M HCl (20 mL), water (20 mL), sat. $NaHCO_3$ (20 mL) and brine (20 mL). The combined aqueous layers were reextracted with EtOAc (10 × 50 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting colourless solid was purified by column chromatography on silica gel eluting with 10% MeOH in CH_2Cl_2 yielding 1.51 g of a colourless solid. The solid was suspended in a 1:1 mixture of THF and MeOH and 360 mg (9.0 mmol, 2.0 eq) of sodium hydroxide in a

minimal amount of water was added. The mixture was stirred for 1.5 h. The resulting solution was acidified with 10 mL of a 1M HCl solution and extracted with CH_2Cl_2 (5x50 mL). The combined organic layers were dryed over Na_2SO_4. Removal of the solvent under reduced pressure yielded 1.44 g of a colourless solid (54%).

^{1}H NMR (400 MHz, CD_3OD) δ 4.57 – 4.47 (m, 2H), 4.46 – 4.36 (m, 1H), 3.78 (dd, *J* = 10.9, 4.4 Hz, 1H), 3.68 – 3.47 (m, 2H), 3.47 – 3.35 (m, 1H), 2.43 – 2.17 (m, 2H), 2.17 – 2.03 (m, 2H), 2.03 – 1.70 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (101 MHz, CD_3OD) δ 175.1, 174.0, 155.9, 81.6, 71.0, 59.6, 55.8, 47.8, 38.5, 31.3, 28.7, 28. 5, 24.3; MS (ESI, $[M+Na]^+$) Calcd for $C_{15}H_{24}N_2NaO_6$: 351.2. Found: 351.3.

Boc-D-Pro-Hyp($COC_{11}H_{23}$)-OH:

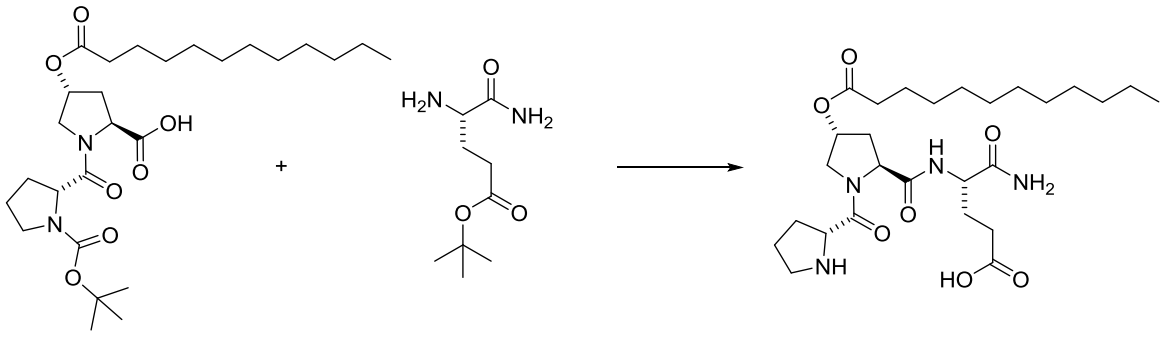


Lauric acid (1.22 g, 6.10 mmol, 2.0 eq) were added to thionyl chloride (3.12 mL, 42.6 mmol, 14 eq) and refluxed for 1.5 h. The reflux condenser was replaced by a distillation bridge and the excess thionyl chloride was removed under reduced pressure (100 mbar). To the resulting brown residue was added dropwise Boc-D-Pro-Hyp-OH (1.00 g, 3.05 mmol, 1.0 eq) in 1.5 mL pyridine at 0°C. The resulting mixture was diluted with 5 mL CH_2Cl_2 and stirred at room temperature for 40 h. The reaction mixture was added to a mixture of 50 mL 1M HCl and 10 g ice and extracted with CH_2Cl_2 (3x 50 mL). The combined organic layers were dried over $NaSO_4$ and evaporated to dryness. The resulting brown solid was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH/HOAc (100:10:1) providing 726 mg of a light brown solid (726 mg, 47%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 – 5.26 (m, 1H), 4.88 – 4.65 (m, 1H), 4.49 – 4.17 (m, 1H), 4.17 – 3.76 (m, 1H), 3.76 – 3.31 (m, 3H), 2.67 – 1.73 (m, 8H), 1.71 – 1.53 (m, 2H), 1.44 and 1.40 (2 x s, 9H), 1.35 – 1.19 (m, 16H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$)

δ 177.4, 174.5, 173.5, 153.7, 80.4, 72.3, 69.2, 58.9, 57.9, 53.6, 53.5, 46.9, 46.7, 35.3, 30.2, 29.7 – 29.5 (m), 28.4, 28.2, 28.2, 28.1, 24.8, 24.5, 23.6, 20.9, 14.1; (Mixture of two conformers in a ratio of approximately 2.5:1); MS (ESI, $[M+Na]^+$) Calcd for $C_{27}H_{46}N_2NaO_7$: 533.3. Found: 533.6.

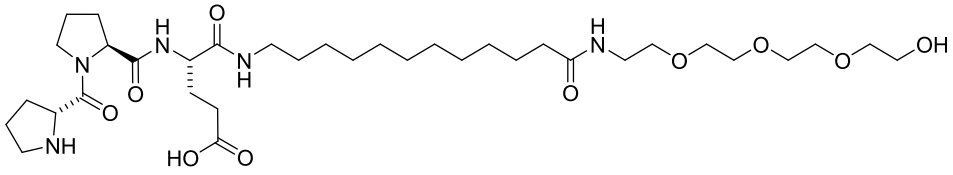
TFA·H-D-Pro-Hyp($COC_{11}H_{23}$)-Glu-NH_2 (1m):



Boc-D-Pro-Pro-OH (727 mg,1.42 mmol, 1.0 eq), HCl·H-Glu(O*t*Bu)-NH_2 (340 mg, 1.42 mmol, 1.0 eq) and EDC·HCl (326 mg, 1.70 mol, 1.2 eq) were suspended in 3 mL EtOAc and *i*Pr_2NEt (583 µL, 3.41 mmol, 1.2 eq) was added. The resulting suspension was stirred at room temperature over night. The reaction mixture was diluted with 20 mL EtOAc and washed with 1M HCl (2x15 mL), 10% $NaHCO_3$ (2x 15 mL) and dried over Na_2SO_4. The residue was purified by column chromatography on silica gel eluting with 5 % MeOH in EtOAc. The resulting colourless oil was The resulting colourless oil was dissolved in a mixture of TFA and CH_2Cl_2 2:1 and stirred at room temperature for 30 min. Removal of all volatile components under reduced pressure provided the peptide as the TFA salt (145 mg, 16 %).

^{1}H NMR (400 MHz, CD_3OD) δ 5.44 – 5.35 (m, 1H), 4.56 (t, *J* = 8.1 Hz, 1H), 4.51 (dd, *J* = 8.7, 7.1 Hz, 1H), 4.40 (dd, *J* = 9.4, 4.6 Hz, 1H), 3.94 (dd, *J* = 11.7, 4.4 Hz, 1H), 3.72 (dt, *J* = 11.7, 1.7 Hz, 1H), 3.43 (dt, *J* = 11.3, 7.0 Hz, 1H), 3.40 – 3.30 (m, 1H), 2.59 – 2.38 (m, 3H), 2.34 (t, J = 7.4 Hz, 2H), 2.27 (ddd, *J* = 13.6, 7.9, 5.1 Hz, 1H), 2.22 – 2.02 (m, 3H), 2.02 – 1.84 (m, 2H), 1.60 (p, *J* = 7.4 Hz, 2H), 1.41 – 1.21 (m, 18H), 0.92 – 0.86 (m, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 176.7, 175.9, 174.5, 173.4, 168. 8, 73.9, 60.5, 60.4, 53. 9, 53.6, 47.7, 36.0, 34.9, 33.1, 31.2, 30.7, 30.6, 30.5, 30.4, 30.2, 29.8, 28.6, 25.9, 25.2, 23.7, 14.4; HRMS (ESI): *m/z* calcd for $C_{27}H_{47}N_4O_5$: 539.3439 [*M*+H^+]; found: 539.3439.

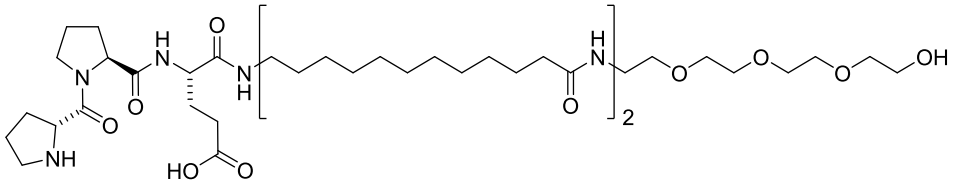
H-D-Pro-Pro-Glu-Ada-TEG (1n):



The peptide was prepared on PS-TEG-NH_2 resin (0.95 mmol/g) on a 250 µmol scale using general procedures 7.2.1 b – d. Cleavage of the peptide including the tetraethylene glycol moiety was performed with a mixture of TFA:triflic acid:TIS 8:1:1. Desalting according to general procedure f afforded the peptide as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (d, *J* = 6.0 Hz, 1H), 7.01 (t, *J* = 5.5 Hz, 1H), 6.90 (t, *J* = 5.1 Hz, 1H), 4.54 (t, *J* = 7.6 Hz, 1H), 4.41 (t, *J* = 6.1 Hz, 1H), 4.34 – 4.21 (m, 1H), 4.04 – 3.88 (m, 1H), 3.81 – 3.67 (m, 5H), 3.67 – 3.56 (m, 8H), 3.56 – 3.48 (m, 3H), 3.48 – 3.32 (m, 3H), 3.32 – 3.13 (m, 2H), 2.55 – 1.86 (m, 14H), 1.66 – 1.55 (m, 2H), 1.55 – 1.43 (m, 2H), 1.43 – 1.10 (m, 14H); 13C NMR (101 MHz, $CDCl_3$) δ 181.5, 173.7, 170.8, 170.1, 169.3, 72.7, 70.7, 70.5, 70.4, 70.1, 62.0, 61.5, 59.2, 54.7, 47.5, 45.2, 39.7, 39.2, 36.7, 32.6, 31.1, 29.7 - 29.3 (m), 27.9, 27.0, 25.9, 24.8, 24.6; MS (ESI, $[M+H]^+$) Calcd for $C_{35}H_{63}N_5NaO_{10:}$ 736.5. Found 736.7.

H-D-Pro-Pro-Glu-Ada$_2$-TEG (1o):

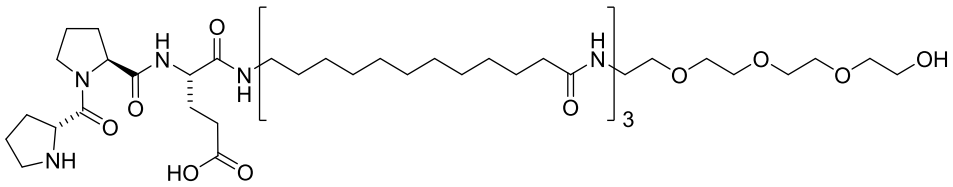


The peptide was prepared on PS-TEG-NH_2 resin (0.95 mmol/g) on a 250 µmol scale using general procedures 7.2.1 b – d. Cleavage of the peptide including the tetraethylene glycol moiety was performed with a mixture of TFA:triflic acid:TIS 8:1:1. Desalting according to general procedure f afforded the peptide as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 6.99 (s, 1H), 6.86 (s, 2H), 5.81 – 5.60 (m, 1H), 4.56 (t, *J* = 8.0 Hz, 1H), 4.43 (t, *J* = 6.1 Hz, 1H), 4.35 – 4.22 (m, 1H), 4.07 – 3.91 (m, 1H), 3.71 (dd, *J* = 5.7, 3.0 Hz, 5H), 3.68 – 3.57 (m, 9H), 3.56 – 3.48 (m, 3H), 3.47 – 3.35 (m, 3H),

3.29 – 3.13 (m, 5H), 2.57 – 1.83 (m, 16H), 1.70 – 1.55 (m, 4H), 1.55 – 1.40 (m, 4H), 1.40 – 1.13 (m, 28H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 181.4, 173.5, 173.1, 170.4, 169.9 169.4, 72.6, 70.6, 70.4, 70.3, 70.0, 69.9, 61.8, 61.5, 59.4, 54.7, 47.3, 44.9, 39.5, 39. 5, 39.1, 36.8, 36.6, 32.2, 29.6, 29.4 - 29.3 (m), 29.2, 27.7, 26.9, 26.8, 25.8, 25. 8, 25.5, 24. 9, 24.5; HRMS (ESI): *m/z* calcd for $C_{47}H_{87}N_6O_{11}$: 911.6427 [M+H^+]; found: 911.6429.

H-D-Pro-Pro-Glu-Ada$_3$-TEG (1p):



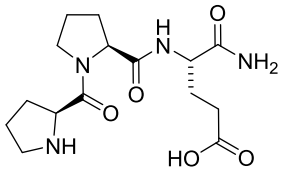
The peptide was prepared on PS-TEG-NH_2 resin (0.95 mmol/g) on a 250 µmol scale using general procedures 7.2.1 b – d. Cleavage of the peptide including the tetraethylene glycol moiety was performed with a mixture of TFA:triflic acid:TIS 8:1:1. Desalting according to general procedure f afforded the peptide as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.11 (s, 1H), 7.00 (s, 1H), 6.85 (s, 1H), 5.68 (d, J = 17.0 Hz, 2H), 4.55 (t, J = 8.2 Hz, 1H), 4.48 – 4.37 (m, 1H), 4.34 – 4.20 (m, 1H), 4.08 – 3.93 (m, 1H), 3.78 – 3.69 (m, 5H), 3.69 – 3.56 (m, 10H), 3.57 – 3.48 (m, 3H), 3.48 – 3.34 (m, 3H), 3.35 – 3.08 (m, 8H), 2.52 – 1.84 (m, 18H), 1.74 – 1.54 (m, 6H), 1.54 – 1.39 (m, 6H), 1.38 – 0.95 (m, 42H); ^{13}C NMR (101 MHz, 5% $CDCl_3$ in CD_3OD) δ 182.4, 176.1, 175.9, 172.8, 172.4, 169.6, 73.5, 71.4, 71.4, 71.1, 71.0, 70.4, 62.7, 62.0, 60. 9, 55.9, 45.8, 40.3, 40.2, 40.1, 37.1, 37.0, 33.8, 30.5, 30.7 – 29.9 (m), 27.9, 27.8, 27.8, 26.9, 26.8, 26.5, 25.2, 24.6; HRMS (ESI): *m/z* calcd for $C_{59}H_{109}N_7NaO_{12}$: 1130.8026 [M+Na^+]; found: 1130.8013.

7.3.8 Synthesis of Peptides Screened as Catalysts for Reactions of Aldehydes to α,β-Disubstituted Nitroolefins

All peptides screened in the conjugate addition reaction between aldehydes and α,β-disubstituted nitroolefins (chapter 5.2.2) were prepared by solid phase peptide synthesis.

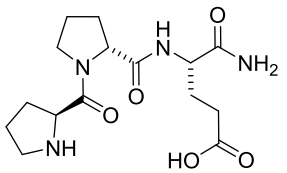
TFA·H-Pro-Pro-Glu-NH_2:



The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 200 µmol scale using general procedures 7.2.1 b – e. TFA·H-Pro-Pro-Glu-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.51 (dd, *J* = 6.3, 8.5 Hz, 1H), 4.36 (dd, *J* = 6.3, 8.2 Hz, 1H), 4.20 (dd, *J* = 5.5, 9.1 Hz, 1H), 3.57 (m, 1H), 3.45 (m, 1H), 3.29 (m, 2H), 2.42 (m, 3H), 2.21 (m, 1H), 2.07-1.72 (m, 8H); ^{13}C NMR (100 MHz, D_2O) δ 177.4, 176.2, 174.2, 168.4, 60.9, 59.5, 53.2, 48.1, 47.0, 30.2, 29.7, 28.7, 26.5, 25.0, 24.2; MS (ESI, $[M+H]^+$) Calcd for $C_{15}H_{25}N_4O_5$: 341.2. Found 341.2.

TFA·H-Pro-D-Pro-Glu-NH_2:

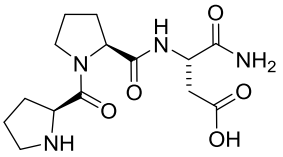


The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 200 µmol scale using general procedures 7.2.1 b – e. TFA·H-Pro- D-Pro-Glu-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.49 (dd, *J* = 7.1, 8.8 Hz, 1H), 4.34 (dd, *J* = 4.4, 8.8 Hz, 1H), 4.23 (dd, *J* = 4.8, 9.8 Hz, 1H), 3.60 (m, 1H), 3.47 (m, 1H), 3.29 (m, 2H), 2.47-2.37 (m, 3H),

2.19 (m, 1H), 2.07 (m, 1H), 2.01-1.78 (m, 7H); ^{13}C NMR (100 MHz, D_2O) δ 177.4, 176.3, 174.6, 168.4, 61.3, 59.6, 53.2, 48.0, 47.0, 30.5, 29.9, 28.5, 26.3, 24.7, 24.3; MS (ESI, $[M+H]^+$) Calcd for $C_{15}H_{25}N_4O_5$: 341.2. Found 341.2.

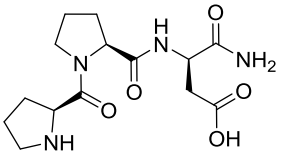
TFA·H-Pro-Pro-Asp-NH_2:



The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 200 µmol scale using general procedures 7.2.1 b – e. TFA·H-Pro-Pro-Asp-NH_2 was obtained as a white solid.

^{1}H NMR (500 MHz, D_2O): δ 4.63 (dd, *J*=8.3, 6.3 Hz, 1H), 4.53 (dd, *J*=7.3, 5.9Hz, 1H), 4.48 (dd, *J*=8.1, 6.1 Hz, 1H), 3.70 (m, 1H), 3.60 (m, 1H), 3.44 (m, 1H), 3.39(m, 1H), 2.68 (dd, *J* = 5.8, 3.9Hz, 1 H), 2.57 (m, 1 H), 2.35 (m, 1 H), 2.31 (m, 1H), 2.11–1.91 (m, 6H); ^{13}C NMR (125.8 MHz, D_2O): δ 177.0, 175.7, 173.4, 168.3, 60.6, 59.1, 51.1, 47.7, 46.5, 38.0, 29.2, 28.3, 24.5, 23.8; HRMS (ESI, $[M+H]^+$) Calcd for $C_{14}H_{23}N_4O_5$: 327.1668. Found: 327.1661.

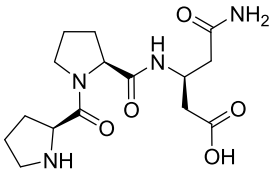
TFA·H-Pro-Pro-D-Asp-NH_2:



The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 200 µmol scale using general procedures 7.2.1 b – e. TFA·H-Pro-Pro-D-Asp-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O): δ 4.77 (m, 1 H), 4.64 (m, 1 H), 4.51 (dd, *J* = 5.9, 8.5 Hz, 1 H), 3.71 (m, 1 H), 3.62 (m, 1 H), 3.42 (m, 2 H), 2.89 (dd, *J*=5.1, 15.8 Hz, 1 H), 2.80 (dd, *J* = 7.6, 15.7 Hz, 1 H), 2.62 (m, 1 H), 2.32 (m, 1 H), 2.12 – 1.89(m, 6H); ^{13}C NMR (100 MHz, D_2O): δ 175.3, 174.6, 173.9, 168.8, 61.2, 59.8, 50.1, 48.4, 47.3, 37.1, 30.2, 29.1, 25.2, 24.6; HRMS (ESI, $[M+H]^+$) Calcd for $C_{14}H_{23}N_4O_5$: 327.1668. Found: 327.1668.

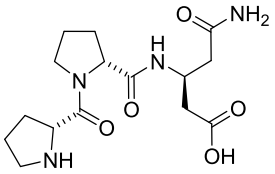
TFA·H-Pro-Pro-®-*homo*-Asp-NH_2:



The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 200 µmol scale using general procedures 7.2.1 b – e. TFA·H-Pro-Pro-β-*homo*-Asp-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.47 (dd, *J* = 6.6, 8.6 Hz, 1H), 4.41 (m, 1H), 4.25 (dd, *J* = 6.1, 8.4 Hz, 1H), 3.54 (m, 1H), 3.45 (m, 1H), 3.26 (m, 2H), 2.54 (dd, *J* = 2.3, 6.8 Hz, 2H), 2.45 (dd, *J* = 5.1, 14.6 Hz, 2H), 2.35 (dd, *J* = 9.2, 14.6 Hz, 1H), 2.15 (m, 1H), 2.00-1.80 (m, 5H), 1.75 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 175.8, 175.0, 173.2, 168.4, 61.2, 59.5, 48.0, 47.0, 44.5, 39.8, 38.7, 30.0, 28.7, 24.9, 24.3; MS (ESI, $[M+H]^+$): Calcd for $C_{15}H_{25}N_4O_5$: 341.2. Found: 341.3.

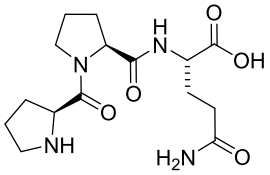
TFA·H-D-Pro-D-Pro-®-*homo*-Asp-NH_2:



The peptide was prepared on Rink Amide AM resin (0.72 mmol/g) on a 200 µmol scale using general procedures 7.2.1 b – e. TFA·H-D-Pro-D-Pro-β-*homo*-Asp-NH_2 was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.49 (dd, *J* = 6.3, 8.5 Hz, 1H), 4.42 (m, 1H), 4.26 (dd, *J* = 6.1, 8.4 Hz, 1H), 3.55 (m, 1H), 3.45 (m, 1H), 3.28 (m, 2H), 2.60 (dd, *J* = 4.8, 15.9 Hz, 1H), 2.45 (m, 4H), 2.15 (m, 1H), 2.00-1.80 (m, 5H), 1.75 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 175.8, 175.0, 173.3, 168.4, 61.2, 59.5, 48.0, 47.0, 44.5, 39.8, 38.6, 29.9, 28.7, 24.9, 24.3; MS (ESI, $[M+H]^+$): Calcd for $C_{15}H_{25}N_4O_5$: 341.2. Found: 341.2.

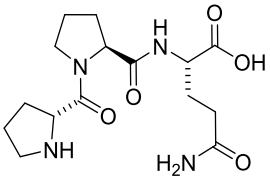
TFA·H-Pro-Pro-Gln-OH:



The peptide was prepared on Wang resin (0.80 mmol/g) on a 200 µmol scale using general procedures 7.2.1 a and c – e. TFA·H-Pro-Pro-Gln-OH was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O): δ 4.64 (dd, *J* = 8.4, 6.0 Hz, 1H), 4.51 (dd, *J* = 8.3, 5.9 Hz, 1H), 4.37 (dd, *J* = 9.2, 5.1 Hz, 1H), 3.71 (dt, *J* = 10.3, 6.6 Hz, 1H), 3.60 (dt, *J* = 10.1, 6.9 Hz, 1H), 3.49 – 3.35 (m, 2H), 2.64 – 2.50 (m, 1H), 2.42 (t, *J* = 7.5 Hz, 2H), 2.39 – 2.30 (m, 1H), 2.27 – 2.14 (m, 1H), 2.14 – 1.90 (m, 7H); ^{13}C NMR (100 MHz, D_2O): δ 178.4, 175.2, 174.2, 168.5, 60.9, 59.5, 52.6, 48.2, 47.1, 31.6, 29.7, 28. 8, 26.9, 25.0, 24.3; HRMS (ESI): *m/z* calcd for C15H24N4O5+H^{+}: 341.1819 [*M*+H^{+}]; found: 341.1817.

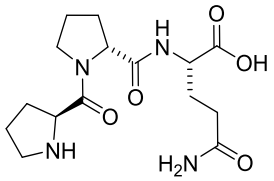
TFA·H-D-Pro-Pro-Gln-OH:



The peptide was prepared on Wang resin (0.80 mmol/g) on a 200 µmol scale using general procedures 7.2.1 a and c – e. TFA·H-Pro-Pro-Gln-OH was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O): δ 4.61 (dd, *J* = 8.7, 7.1 Hz, 1H), 4.47 (dd, *J* = 8.6, 3.5 Hz, 1H), 4.37 (dd, *J* = 9.4, 4.9 Hz, 1H), 3.75 – 3.66 (m, 1H), 3.63 – 3.53 (m, 1H), 3.49 – 3.31 (m, 2H), 2.60 – 2.47 (m, 1H), 2.40 (t, *J* = 7.5 Hz, 2H), 2.36 – 2.26 (m, 1H), 2.26 – 2.13 (m, 1H), 2.13 – 1.92 (m, 7H); ^{13}C NMR (100 MHz, D_2O): δ 178.3, 175.1, 174.4, 168.3, 61.0, 59.6, 52.5, 48.0, 47.0, 31.5, 29.8, 28.4, 26.8, 24.6, 24.3; HRMS (ESI): *m/z* calcd for $C_{15}H_{25}N_4O_5$: 341.1819 [M+H^{+}]; found: 341.1819.

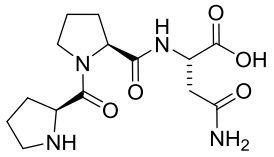
TFA·H-Pro-D-Pro-Gln-OH:



The peptide was prepared on Wang resin (0.80 mmol/g) on a 200 µmol scale using general procedures 7.2.1 a and c – e. TFA·H-Pro-D-Pro-Gln-OH was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O): δ 4.65 (dd, *J* = 7.4, 8.4 Hz, 1H), 4.57 – 4.45 (m, 1H), 4.41 (dd, *J* = 9.2, 5.0 Hz, 1H), 3.81 – 3.69 (m, 1H), 3.66 – 3.53 (m, 1H), 3.52 – 3.32 (m, 2H), 2.62 – 2.51 (m, 1H), 2.38 (t, *J* = 7.4 Hz, 2H), 2.44 – 2.28 (m, 1H), 2.28 – 2.15 (m, 1H), 2.14 – 1.96 (m, 7 H); ^{13}C NMR (100 MHz, D_2O): δ 178.4, 175.1, 174.3, 168.5, 61.3, 59.7, 52.6, 48.0, 47.1, 31.5, 30.1, 28.5, 26.7, 24.6, 24.3; HRMS (ESI): *m/z* calcd for $C_{15}H_{25}N_4O_5$: 341.1819 [M+H^+]; found: 341.1821.

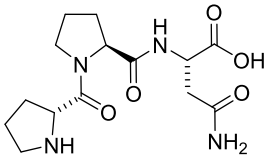
TFA·H-Pro-Pro-Asn-OH (1q):



The peptide was prepared on Wang resin (0.80 mmol/g) on a 2.4 mmol scale using general procedures 7.2.1 a and c – e. TFA·H-Pro-Pro-Asn-OH was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ = 4.58 (dd, *J* = 6.1, 6.2 Hz, 1H), 4.50 (dd, *J* = 6.1, 8.5 Hz, 1H), 4.37 (dd, *J* = 5.3, 8.5 Hz, 1H), 3.56 (m, 1H), 3.48 (m, 1H), 3.28 (m, 2H), 2.73 (d, *J* = 6.2, 2H), 2.44 (m, 1H), 2.20 (m, 1H), 2.02-1.78 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ = 175.0, 174.3, 173.8, 168.5, 60.9, 59.5, 49.8, 48.0, 47.0, 36.4, 29.6, 28.8, 24.9, 24.2; MS (ESI, $[M+H]^+$) Calcd for $C_{14}H_{23}N_4O_5$: 327.2. Found 327.2.

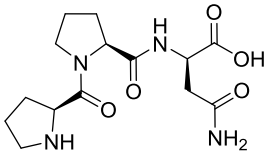
TFA·H-D-Pro-Pro-Asn-OH:



The peptide was prepared on Wang resin (0.80 mmol/g) on a 200 µmol scale using general procedures 7.2.1 a and c – e. TFA·H-D-Pro-Pro-Asn-OH was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.55 (dd, *J* = 6.4, 8.5 Hz, 1H), 4.51 (dd, *J* = 6.4, 8.6 Hz, 1H), 4.39 (dd, *J* = 5.4, 8.6 Hz, 1H), 3.46 (m, 1H), 3.40 (m, 1H), 3.33 (m, 2H), 2.70 (dd, *J* = 5.8, 15.4 Hz, 1H), 2.65 (dd, *J* = 6.4, 15.2 Hz, 1H), 2.35 (m, 1H), 2.18 (m, 1H), 2.12-1.70 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 175.0, 174.3, 173.7, 168.5, 60.9, 59.5, 49.8, 48.0, 47.0, 36.4, 29.6, 28.8, 24.9, 24.2; MS (ESI, $[M+H]^+$) Calcd for $C_{14}H_{23}N_4O_5$: 327.2. Found 327.2.

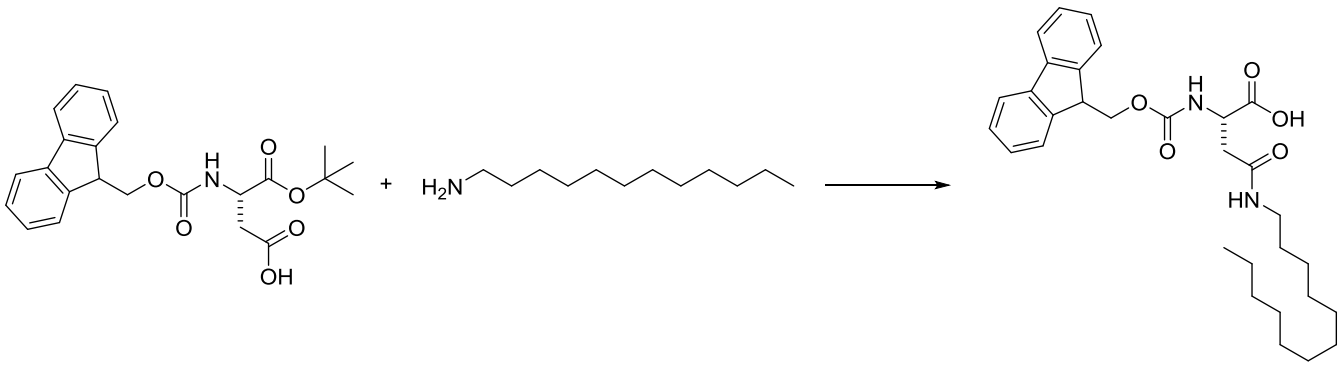
TFA·H-Pro-Pro-D-Asn-NH_2:



The peptide was prepared on Wang resin (0.80 mmol/g) on a 200 µmol scale using general procedures 7.2.1 a and c – e. TFA·H-Pro-Pro-D-Asn-OH was obtained as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.64 (dd, *J* = 5.1, 7.5 Hz, 1H), 4.51 (dd, *J* = 6.4, 8.5 Hz, 1H), 4.38 (dd, *J* = 5.9, 8.5 Hz, 1H), 3.58 (m, 1H), 3.48 (m, 1H), 3.28 (m, 2H), 2.76 (dd, *J* = 5.1, 15.7 Hz, 1H), 2.67 (dd, *J* = 7.6, 15.6 Hz, 1H), 2.44 (m, 1H), 2.18 (m, 1H), 2.06-1.72 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 175.0, 175.3, 173.6, 168.5, 60.9, 59.5, 49.8, 48.0, 47.0, 36.8, 29.9, 28.8, 24.9, 24.3; MS (ESI, $[M+H]^+$): Calcd for $C_{14}H_{23}N_4O_5$: 327.2. Found: 327.2.

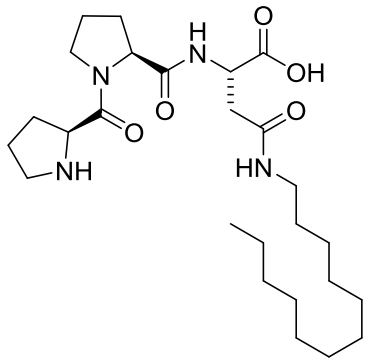
Fmoc-Asn($C_{12}H_{25}$)-OH:



Fmoc-Asp-O*t*Bu (500 mg, 1.22 mmol, 1.0 eq), dodecylamine (225 mg, 1.22 mmol, 1.0 eq) and EDC·HCl (280 mg, 1.46 mmol, 1.2 eq) were suspended in EtOAc (8 mL) and stirred at room temperature for 4 h. The reaction mixture was diluted with 60 mL of EtOAc and washed with 0.1 M HCl (2x40 mL), sat. $NaHCO_3$ (2x40 mL) water (40 mL) and brine (40 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel eluting with EtOAc. The resulting colourless solid was dissolved in 6 mL of a 2:1 mixture of trifluoroacetic acid and CH_2Cl_2 and stirred at room temperature for 1h. All volatile components were removed under reduced pressure. The colourless oil was coevaporated three times with toluene and three times with acetonitrile to provide a colourless solid (427 mg, 70%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 7.5 Hz, 2H), 7.53 (dd, *J* = 7.6, 4.3 Hz, 2H), 7.36 – 7.29 (m, 2H), 7.27 – 7.19 (m, 3H), 4.39 (t, *J* = 5.3 Hz, 1H), 4.36 – 4.20 (m, 2H), 4.15 (t, *J* = 7.2 Hz, 1H), 3.14 – 3.00 (m, 2H), 2.79 (dd, *J* = 15.5, 5.3 Hz, 1H), 2.66 (dd, *J* = 15.6, 5.3 Hz, 1H), 1.45 – 1.31 (m, 2H), 1.27 – 1.10 (m, 18H), 0.80 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 170.6, 156.4, 143.8, 143.6, 141.2, 127.7, 127.0, 125.1, 125.0, 119.9, 67.2, 50.7, 47.0, 39.7, 37.6, 31.8, 29.6 – 29.5 (m), 29.3 - 29.2 (m), 29.2, 26.9, 22.6, 14.0. MS (ESI, $[M+H]^+$) Calcd for $C_{31}H_{43}N_2O_5$: 523.3. Found: 523.6.

H-Pro-Pro-Asn($C_{12}H_{25}$)-OH (1r):



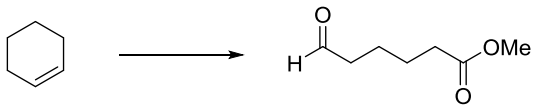
The peptide was prepared on Wang resin (0.80 mmol/g) on a 200 µmol scale using general procedures 7.2.1 a and c – f. H- Pro-Pro-Asn($C_{12}H_{25}$)-OH was obtained as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 8.3 Hz, 1H), 7.63 (s, 1H), 4.75 – 4.25 (m, 3H), 3.88 – 2.93 (m, 6H), 2.88 – 2.53 (m, 2H), 2.49 – 1.77 (m, 8H), 1.55 – 1.37 (m, 2H), 1.35 – 1.17 (m, 18H), 0.87 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.5, 171.6, 171.5, 171.1, 170.8, 169.0, 168.6, 61.9, 61.4, 60.5, 58.1, 58.1, 51.9, 47.5, 47.2, 46.4, 46.1, 39.7, 39.6, 39.0, 32.1, 31.9, 31.6, 29.8, 29. 7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 27. 7, 27.1, 25.0, 25.0, 24.8, 24.6, 24.5, 22.7, 22.3, 14.1 (Mixture of two conformers in a ratio of approximately 2:1); HRMS (ESI): *m/z* calcd for $C_{26}H_{47}N_4O_5$: 495.3541 [M+H^+]; found: 495.3550.

7.4 Synthesis of Non-Commercially Available Substrates

7.4.1 Aldehydes

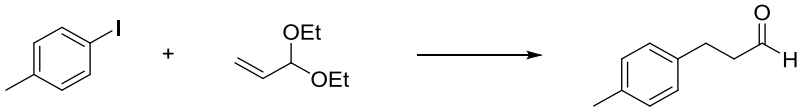
Methyl 6-oxohexanoate:



Cyclohexene (36.5 mmol, 3.70 mL, 1.0 eq) was dissolved in CH_2Cl_2 (100 mL) and MeOH (25 mL). Then $NaHCO_3$ (11.9 mmol, 1.00 g, 0.33 eq) was added and the resulting mixture was cooled to -78°C (acetone/dry ice). 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 40 mL of toluene and concentrated to a volume of 25 mL. 100 mL of CH_2Cl_2 was added and the resulting solution cooled to 0°C. Triethylamine (54.7 mmol, 7.26 mL, 1.5 eq) and acetic anhydride (110 mmol, 10.4 mL, 3.0 eq) were added and the solution stirred at 0°C for 20 min followed by 20 h at room temperature. The reaction mixture was washed with 1m HCl and 100 mL of 2m NaOH (100 mL each). The organic layer was dried over Na_2SO_4. The solvents were evaporated and the crude product was purified by distillation to isolate 3.66 g (70 %) of a colorless liquid.

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 (100 MHz, $CDCl_3$, 25°C): δ = 202.0, 173.7, 51.5, 43.5, 33.7, 24.4, 21.5.
Spectroscopic data is in agreement with published data.[186]

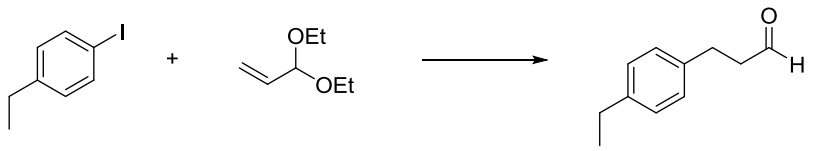
3-(4-Methylphenyl)propanal:



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 100 mL 2M 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 (3x100 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 colourless oil that was purified by column chromatography on silica gel (pentane/EtOAc 10:1) to provide 1.84 g (69 %) of a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) 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, $CDCl_3$) δ 201.6, 137.1, 135.7, 129.2, 128.1, 45.3, 27.6, 20. 9.

3-(4-Ethylphenyl)propanal:

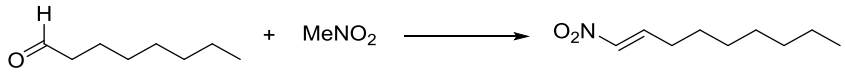


1-Iodo-4-methylbenzene (5.11 g, 22.0 mmol, 1.0 eq) was dissolved in 85 mL DMF. Acrolein diethyl acetal (9.44 mL, 61.8 mmol, 2.8 eq), tetrabutylammonium acetate (14.3 g, 47.4 mmol, 2.2 eq), potassium chloride (1.64 g, 22.1 mmol, 1.0 eq), potassium carbonate (4.27 g, 30.9 mmol, 1.4 eq) and palladium(II) 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 100 mL 2M 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 (3x100 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 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 4M HCl over night. 20 mL EtOAc were added and washed with water (3x10 mL). Column chromatography on silica gel (pentane/EtOAc 10:1) provided 2.57 g of a colourless liquid (72 %).

^{1}H NMR (400 MHz, $CDCl_3$) 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, $CDCl_3$) δ 201.6, 142.1, 137.4, 128.1, 128.0, 45.3, 28.3, 27.6, 15.5.

7.4.2 Nitroolefins

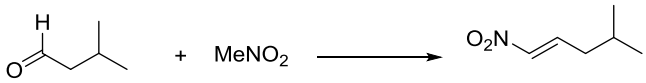
(*E*)-Nitronon-1-ene:



To a solution of octanal (2.44 mL, 20.0 mmol, 1.0 eq) and nitromethane (2.18 mL, 40.0 mmol, 2.0 eq) in toluene (10 mL) was added a functionalized mesoporous silica catalyst (500 mg, aminopropyl-MCM[187]). The resulting mixture was heated to 100°C for 6 h. The catalyst was removed by filtration and the resulting solution concentrated *in vacuo* on a rotary evaporator. Column chromatography on silica gel eluting with a mixture of pentane and EtOAc (10:1) provided 2.16 g of a yellow oil (76 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (dt, *J* = 13.4, 7.3 Hz, 1H), 6.97 (d, *J* = 13.4 Hz, 1H), 2.26 (q, *J* = 7.3, 2H), 1.50 (p, *J* = 7.2 Hz, 2H), 1.39 – 1.18 (m, 8H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 142. 8, 139.5, 31.6, 29.0, 28.9, 28.4, 27.7, 22.5, 14.0.
Spectroscopic data is in agreement with published data.[128]

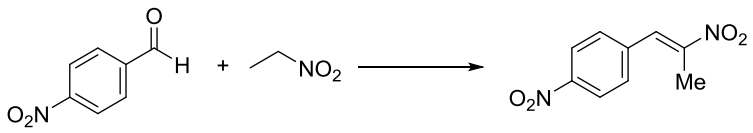
(*E*)-4-Methyl-nitropent-1-ene:



To a solution of *iso*-valeraldehyde (1.25 mL, 11.6 mmol, 1.0 eq) and nitroethane (1.27 mL, 23.2 mmol, 2.0 eq) in toluene (10 mL) was added a functionalized mesoporous silica catalyst (500 mg, aminopropyl-MCM[187]). The resulting mixture was heated to 100°C for 5 h. The catalyst was removed by filtration and the resulting solution concentrated *in vacuo* on a rotary evaporator. Column chromatography on silica gel eluting with a mixture of pentane and EtOAc (10:1) provided 902 mg of a yellow oil (60 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (td, *J* = 13.4, 9.0 Hz, 1H), 6.97 (td, *J* = 13.2 Hz, 1H), 2.21 – 2.13 (m, 2H), 1.77 - 1.89 (m, 1H), 0.96 (d, *J* = 6.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.5, 140.1, 37.2, 27.7, 22.2, 22.2.
Spectroscopic data is in agreement with published data.[128]

(*E*)-1-Nitro-4-(2-nitroprop-1-en-1-yl)benzene:

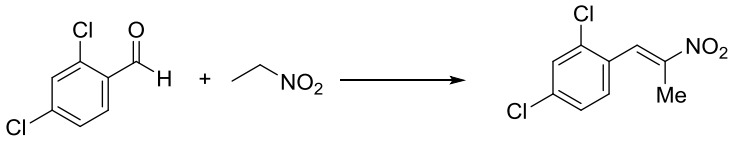


A suspension of 4-nitrobenzaldehyde (3.00 g, 19.9 mmol, 1.0 eq) and nitroethane (1.42 mL, 19.9 mmol, 1.0 eq) in Et_2O (20 mL) was cooled to 0°C. Then, a solution of KOH (1.12 g, 19.9 mmol,1.0 eq) in MeOH (20 mL) was added dropwise. The resulting mixture was stirred at room temperature for 8 h. Acetic acid (1.14 mL, 19.9 mmol, 1.0 eq) was added and the reaction mixture was poured into water (15 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was taken up in CH_2Cl_2 (20 mL) and cooled to 0°C. Trifluoroacetic anhydride (2.91 mL, 20.9 mmol, 1.05 eq) was added followed by the dropwise addition of triethylamine (5.81 mL, 41.8 mmol, 2.1 eq). The resulting mixture was stirred at 0°C for 1.5 h, diluted with CH_2Cl_2 (30 mL), washed with sat. NH_4Cl (2x20 mL) and 10% $NaHCO_3$ (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting brown solid recrystallised from MeOH/EtOAc to yield 2.66 g (64 %) of a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 – 8.27 (m, 2H), 8.08 (s, 1H), 7.64 – 7.55 (m, 2H), 2.45 (d, J = 1.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.2, 148.1, 138.8, 130.7, 130.5, 124.0, 14.0.

Spectroscopic data is in agreement with published data.[188]

(*E*)-2,4-Dichloro-1-(2-nitroprop-1-en-1-yl)benzene:

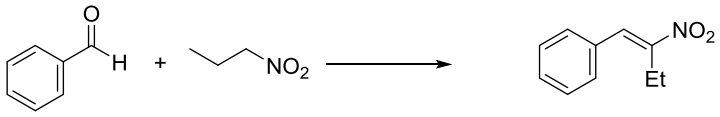


A solution of 2,4-dichlorobenzaldehyde (5.00 g, 28.6 mmol, 1.0 eq) and nitroethane (2.45 mL, 34.3 mmol, 1.2 eq) in Et_2O (30 mL) was cooled to 0°C. Then, a solution of KOH (1.61 g, 28.6 mmol, 1.0 eq) in MeOH (10 mL) was added dropwise. The resulting mixture was stirred at room temperature for 18 h. Acetic acid (1.64 mL, 28.6 mmol, 1.0 eq) was added and the

reaction mixture was poured into water (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting red oil was taken up in CH_2Cl_2 (30 mL) and cooled to 0°C. Trifluoroacetic anhydride (4.17 mL, 30.0 mmol, 1.05 eq) was added followed by the dropwise addition of triethylamine (8.35 mL, 60.1 mmol, 2.1 eq). The resulting mixture was stirred at 0°C for 1 h, diluted with CH_2Cl_2 (50 mL), washed with sat. NH_4Cl (2x20 mL) and 10% $NaHCO_3$ (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting yellow solid recrystallised from MeOH to yield 4.50 g (68 %) of a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.44 (d, J = 2.0 Hz, 1H), 7.27 (dd, J = 8.4, 2.0 Hz, 1H), 7.20 (d, J = 8.9 Hz, 1H), 2.26 (d, J = 1.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$,): δ 149.5, 136.3, 135.6, 130.9, 130.0, 129.8, 129.5, 127.4, 14.0; elemental analysis calcd (%) for $C_9H_7Cl_2NO_2$: C 46.58, H 3.04, N 6.04; found: C 46.55, H 3.00, N 5.97.

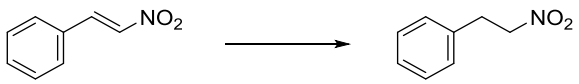
(*E*)-(2-Nitrobut-1-en-1-yl)benzene:



To a solution of benzaldehyde (49.5 mmol, 5.00 mL, 1.0 eq) and nitropropane (54.4 mmol, 4.86 mL, 1.1 eq) in toluene (10 mL) was added a functionalized mesoporous silica catalyst (1.00 g, aminopropyl-MCM[187]). The resulting mixture was heated to 100°C for 12 h. The catalyst was removed by filtration and the resulting solution concentrated *in vacuo* on a rotary evaporator. Distillation provided 6.61 g (75 %) of a yellow oil.

b.p. 148 °C (18 mbar); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 8.03 (s, 1H), 7.44 (s, 5H), 2.87 (q, J = 7.3 Hz, 2H), 1.28 (t, J = 7.3 Hz, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): δ = 153.5, 133.2, 132.5, 130.1, 129.7, 129.1, 20.9, 12.6; elemental analysis calcd (%) for $C_{10}H_{11}NO_2$: C 67.78, H 6.26, N 7.90; found: C 67.80, H 6.17, N 7.70.

(2-Nitroethyl)benzene:

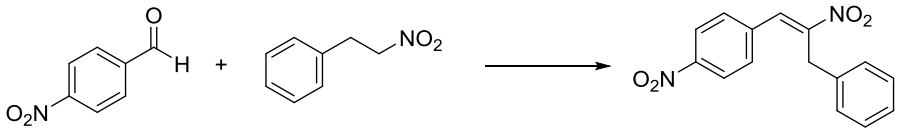


trans-β-Nitrostyrene (1.00 g, 6.70 mmol, 1.0 eq) dissolved in 13 mL dioxane was added dropwise to a suspension of $NaBH_4$ (507 mg, 13.4 mmol, 2.0 eq) in a mixture of dioxane (13 mL) and EtOH (4 mL) over the course of 60 min. While the reaction mixture was stirred at room temperature for a further 60 min a colourless precipitate formed. 150 mL of an ice/water mixture was added. 50% HOAc in water was added carefully until the bubbling ceased. The mixture was extracted with CH_2Cl_2 (3x80 mL) and the combined organic extracts were washed with water (3x80 mL) and brine (80 mL). Drying of the organic layer over Na_2SO_4 and removal of the solvents under reduced pressure resulted in a brown oil which was purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (20:1) to provide 924 mg of a colourless oil (91 %)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 – 7.24 (m, 3H), 7.23 – 7.18 (m, 2H), 4.60 (t, *J* = 7.2 Hz, 2H), 3.31 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 135.6, 128.9, 128.5, 127.4, 76.2, 33.4.

Spectroscopic data is in agreement with published data.[189]

(*E*)-1-Nitro-4-(2-nitro-3-phenylprop-1-en-1-yl)benzene:



A solution of 4-nitrobenzaldehyde (916 mg, 6.06 mmol, 1.0 eq) and (2-nitroethyl)benzene (998 mg, 6.06 mmol, 1.0 eq) in Et_2O (30 mL) was cooled to 0°C. Then, a solution of KOH (340 mg, 6.06 mmol, 1.0 eq) in MeOH (4 mL) was added dropwise. The resulting mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into water (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated

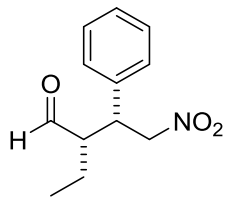
under reduced pressure. The resulting orange oil was taken up in CH_2Cl_2 (6 mL) and cooled to 0°C. Trifluoroacetic anhydride (0.798 mL, 6.06 mmol, 1.0 eq) was added followed by the dropwise addition of triethylamine (1.67 mL, 12.1 mmol, 2.0 eq). The resulting mixture was stirred at 0°C for 1 h, allowed to warm to room temperature and stirred for 2 h. The dark brown mixture was diluted with CH_2Cl_2 (50 mL), washed with sat. NH_4Cl (2x20 mL) and 10% $NaHCO_3$ (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure. The resulting yellow oil was purified by flash column chromatography on silica gel (P:EtOAc 10:1). Product containing fractions were united, evaporated and the resulting yellow solid recrystalised from MeOH to yield 1.33 g (38 %) of a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 – 8.25 (m, 3H), 7.59 (d, J = 8.5 Hz, 2H), 7.37 – 7.31 (m, 2H), 7.31 – 7.27 (m, 1H), 7.18 (d, J = 7.1 Hz, 2H), 4.23 (s, 2H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 152.4, 148.6, 138.5, 135.4, 132.6, 130.4, 129.3, 127.7, 127.5, 124.4, 33.0; elemental analysis calcd (%) for $C_{15}H_{12}N_2O_4$: C 63.38, H 4.25, N 9.85; found: C 63.10, H 4.22, N 9.76.

7.5 Analytical Data of γ-Nitroaldehydes

7.5.1 2,3-Disubstituted γ-Nitroaldehydes

(2*S*,3*R*)-2-Ethyl-4-nitro-3-phenylbutanal:



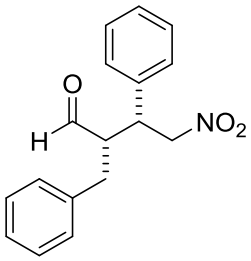
Prepared from butanal and *trans*-β-nitrostyrene according to general procedures 7.2.2 a) 7.2.2 b) or 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (10:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (d, J = 2.6 Hz, 1H), 7.32 (m, 3H), 7.18 (m, 2H), 4.72 (dd, J = 5.0 Hz, 12.7 Hz, 1H), 4.63 (dd, J = 9.7 Hz, 12.7 Hz, 1H), 3.79 (dt, J = 5.0 Hz, 9.8 Hz, 1H), 2.68 (dddd, J = 2.6 Hz, 5.0 Hz, 7.6 Hz, 10.1 Hz, 1H), 1.51 (m, 2H), 0.84 (t, J = 7.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.1, 136.8, 129.1, 128.1, 128.0, 78.5, 55.0, 42.7, 20.4, 10.7.

Spectroscopic data is in agreement with published data.[92]

The enantiomeric excess was determined by 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, (*syn*, major) = 47.9 min.

(2*S*,3*R*)-2-Benzyl-4-nitro-3-phenylbutanal:

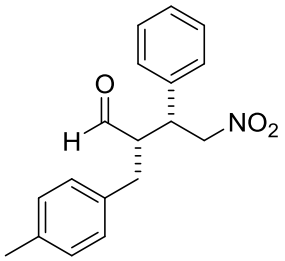


Prepared from 3-phenylpropanal and *trans*-β-nitrostyrene according to general procedures 7.2.2 b) or 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (10:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.72 (d, *J* = 2.3 Hz, 1H), 7.43 – 7.10 (m, 8H), 7.07 – 7.00 (m, 2H), 4.76 – 4.67 (m, 2H), 3.83 (td, *J* = 8.5, 6.3 Hz, 1H), 3.17 – 3.03 (m, 1H), 2.82 – 2.70 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.9, 137.1, 136.7, 129.2, 128.8, 128.7, 128.3, 128.0, 126.9, 78.0, 55.3, 43.4, 34.2.
Spectroscopic data is in agreement with published data.[92]

The enantiomeric excess was determined by HPLC using a Chiracel OD-H column (*n*-hexane/*i*-PrOH 80:20, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* major) = 27.5 min, (*syn*, minor) = 30.9 min.

(2*S*,3*R*)-2-(4-Methylbenzyl)-4-nitro-3-phenylbutanal (5a):

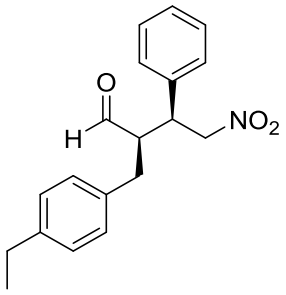


Prepared from 3-(4-methylphenyl)propanal and *trans*-β-nitrostyrene according to general procedure 7.2.2 b) in the presence of H-D-Pro-Pro-Glu-NH_2 at 5°C. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (10:1). The γ-nitroaldehyde was obtained as a colourless solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (d, *J* = 2.3 Hz, 1H), 7.48 – 7.29 (m, 3H), 7.29 – 7.18 (m, 2H), 7.08 (d, *J* = 7.7 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 2H), 5.04 – 4.59 (m, 1H), 3.82 (td, *J* = 8.6, 6.0 Hz, 1H), 2.87 – 2.65 (m, 2H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.1, 136.7, 136.5, 133.9, 129.4, 129.2, 128.6, 128.2, 128.0, 78.0, 55.3, 43.4, 33.8, 21.0; elemental analysis calcd (%) for $C_{18}H_{19}NO_3$: C 72.71, H 6.44, N 4.71; found: C 72.54, H 6.47, N 4.92.

The enantiomeric excess was determined by HPLC using a Chiracel OD-H column (*n*-hexane/*i*-PrOH 80:20, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* major) = 20.3 min, (*syn*, minor) = 23.5 min.

(2*R*,3*S*)-2-(4-Ethylbenzyl)-4-nitro-3-phenylbutanal (5b):

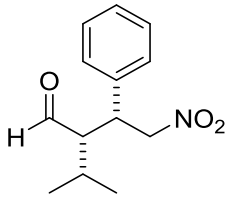


Prepared from 3-(4-ethylphenyl)propanal and *trans*-β-nitrostyrene according to general procedure 7.2.2 b) in the presence of H-Pro-D-Pro-D-Glu-NH_2 at 5°C. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (10:1). The γ-nitroaldehyde was obtained as a colourless solid.

^{1}H NMR (250 MHz, $CDCl_3$) δ 9.71 (d, *J* = 2.3 Hz, 1H), 7.45 – 7.31 (m, 3H), 7.25 – 7.18 (m, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.94 (d, *J* = 8.0 Hz, 2H), 4.76 – 4.68 (m, 2H), 3.82 (ddd, *J* = 9.2, 8.1, 6.4 Hz, 1H), 3.10 (dddd, *J* = 9.2, 8.0, 6.4, 2.3 Hz, 1H), 2.80 – 2.69 (m, 2H), 2.60 (q, *J* = 7.6 Hz, 2H), 1.20 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.2, 142.9, 136.7, 134.2, 129.2, 128.7, 128.2, 128.2, 128.0, 78.0, 55.3, 43.4, 33.8, 28.4, 15.5; elemental analysis calcd (%) for $C_{19}H_{21}NO_3$: C 73.29, H 6.80, N 4.50; found: C 73.15, H 6.81, N 4.50.

The enantiomeric excess was determined by HPLC using a Chiracel OD-H column (*n*-hexane/*i*-PrOH 80:20, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* minor) = 16.6 min, (*syn*, major) = 20.8 min.

(2*S*,3*R*)-2-Isopropyl-4-nitro-3-phenylbutanal:

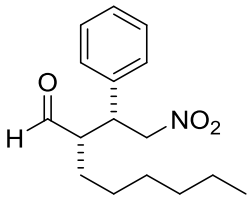


Prepared from *iso*-valeraldehyde and *trans*-β-nitrostyrene according to general procedure 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (10:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.92 (d, *J* = 2.6 Hz, 1H), 7.38 – 7.25 (m, 3H), 7.21 – 7.16 (m, 2H), 4.67 (dd, *J* = 12.5, 4.4 Hz, 1H), 4.57 (dd, *J* = 12.5, 9.9 Hz, 1H), 3.90 (td, *J* = 10.3, 4.4 Hz, 1H), 2.77 (ddd, *J* = 10.7, 4.2, 2.6 Hz, 1H), 1.72 (heptd, *J* = 7.0, 4.2 Hz, 1H), 1.09 (d, *J* = 7.2 Hz, 4H), 0.88 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.3, 137.1, 129.1, 128.1, 127.9, 79.0, 58.7, 41.9, 27. 9, 21.6, 17.0.
Spectroscopic data is in agreement with published data.[92]

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*-PrOH 95:5, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* minor) = 9.5 min, (*syn*, major) = 11.0 min.

(*S*)-2-((*R*)-2-Nitro-1-phenylethyl)octanal:

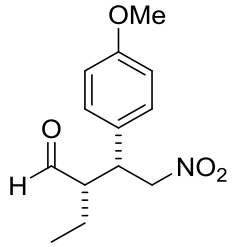


Prepared from octanal and *trans*-β-nitrostyrene according to general procedure 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.71 (d, *J* = 2.7 Hz, 1H), 7.40 – 7.28 (m, 3H), 7.22 – 7.13 (m, 2H), 4.71 (dd, *J* = 12.7, 5.5 Hz, 1H), 4.64 (dd, *J* = 12.7, 9.3 Hz, 1H), 3.77 (td, *J* = 9.3, 5.5 Hz, 1H), 2.76 – 2.63 (m, 1H), 1.53 – 1.05 (m, 10H), 0.86 – 0.78 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.3, 136.8, 129.1, 128.1, 128.0, 78.4, 53.9, 31.3, 29.0, 27.3, 26.3, 22.4, 13.9.
Spectroscopic data is in agreement with published data.[190]

The enantiomeric excess was determined by HPLC using a Chiracel OD-H column (*n*-hexane/*i*-PrOH 90:10, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* major) = 14.6 min, (*syn*, minor) = 18.9 min.

(2*S*,3*R*)-2-Ethyl-3-(4-methoxyphenyl)-4-nitrobutanal:

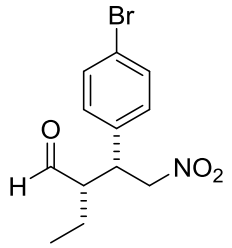


Prepared from butanal and *trans*-(4-methoxy)-β-nitrostyrene according to general procedure 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (10:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (d, *J* = 2.6 Hz, 1H), 7.11 – 7.05 (m, 2H), 6.88 – 6.83 (m, 2H), 4.68 (dd, *J* = 12.5, 5.0 Hz, 1H), 4.57 (dd, *J* = 12.5, 9.9 Hz, 1H), 3.77 (s, 3H), 3.76 – 3.67 (m, 1H), 2.62 (dddd, *J* = 10.2, 7.5, 4.7, 2.6 Hz, 1H), 1.57 – 1.41 (m, 2H), 0.81 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.3, 159.2, 129.0, 128.5, 114.4, 78.7, 55.2, 55.1, 42.0, 20.3, 10.6.
Spectroscopic data is in agreement with published data.[93]

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*-PrOH 99.5:0.5, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* minor) = 47.3 min, (*syn*, major) = 58.0 min.

(2*S*,3*R*)-2-Ethyl-3-(4-bromophenyl)-4-nitrobutanal:

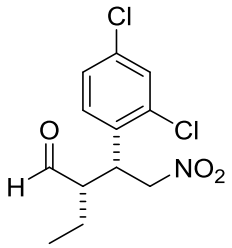


Prepared from butanal and *trans*-(4-bromo)-β-nitrostyrene according to general procedure 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (d, *J* = 2.3 Hz, 1H), 7.53 – 7.41 (m, 2H), 7.10 – 7.03 (m, 2H), 4.72 (dd, *J* = 12.8, 4.8 Hz, 1H), 4.59 (dd, *J* = 12.8, 9.9 Hz, 1H), 3.77 (td, *J* = 9.9, 4.8 Hz, 1H), 2.66 (dddd, *J* = 10.3, 8.2, 4.8, 2.3 Hz, 1H), 1.60 – 1.41 (m, 2H), 0.83 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.6, 135.9, 132.3, 129.7, 122.1, 78.2, 54.6, 42.1, 20.3, 10.5. Spectroscopic data is in agreement with published data.[128]

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*-PrOH 98.5:1.5, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* minor) = 28.5 min, (*syn*, major) = 40.1 min.

(2*S*,3*R*)-2-Ethyl-3-(2,4-dichlorophenyl)-4-nitrobutanal:

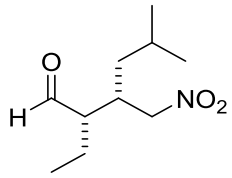


Prepared from butanal and *trans*-(2,4-dichloro)-β-nitrostyrene according to general procedure 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (10:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (d, *J* = 2.1 Hz, 1H), 7.43 (d, *J* = 2.2 Hz, 1H), 7.26 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 4.84 (dd, *J* = 13.0, 9.2 Hz, 1H), 4.68 (dd, *J* = 13.0, 4.4 Hz, 1H), 4.30 (td, *J* = 9.2, 4.4 Hz, 1H), 2.93 (dddd, *J* = 9.2, 7.4, 5.2, 2.1 Hz, 1H), 1.67 – 1.44 (m, 2H), 0.87 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.4, 135.1, 134.5, 133.2, 130.3, 127.8, 76.5, 53.7, 38.7, 20.4, 10.6.
Spectroscopic data is in agreement with published data.[128]

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*-PrOH 98.5:1.5, 25°C) at 1.0 mL/min, UV detection at 254 nm: t_R : (*syn,* minor) = 18.3 min, (*syn*, major) = 20.1 min.

(2*S*,3*S*)-2-Ethyl-5-methyl-3-(nitromethyl)hexanal:

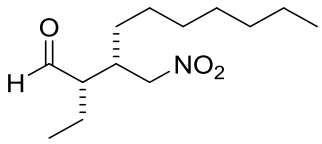


Prepared from butanal and (*E*)-4-methyl-nitropent-1-ene according to general procedure 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (40:1). The γ-nitroaldehyde was obtained as a colourless oil.

NMR (400 MHz, $CDCl_3$) δ 9.72 (d, *J* = 1.3 Hz, 1H), 4.47 (dd, *J* = 6.4, 12.5 Hz, 1H), 4.42 (dd, *J* = 6.6, 12.5 Hz, 1H), 2.73 (m, 1H), 2.43 (dtd, *J* = 1.3, 4.7, 6.0 Hz, 1H), 1.80 (m, 1H), 1.61 (m, 1H), 1.50 (dqd, *J* = 4.9, 7.4, 14.8 Hz, 1H), 1.24 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H), 0.92 (d, *J* = 4.9 Hz, 3H), 0.90 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.0, 77.1, 54.0, 38.3, 34.7, 25.2, 22.7, 22.0, 18.5, 12.2.
Spectroscopic data is in agreement with published data.[128]

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*-PrOH 90.25:0.75, 25°C) at 0.3 mL/min, UV detection at 210 nm: t_R : (*syn,* minor) = 35.5 min, (*syn*, major) = 40.1 min.

(2*S*,3*S*)-2-Ethyl-3-(nitromethyl)decanal:



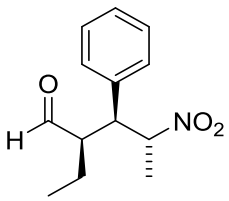
Prepared from butanal and (*E*)-nitronon-1-ene according to general procedure 7.2.2 c). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (40:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (d, *J* = 1.5 Hz, 1H), 4.46 (dd, *J* = 12.6, 6.8 Hz, 1H), 4.41 (dd, *J* = 12.4, 6.4 Hz, 1H), 2.63 (qd, *J* = 6.4, 4.8 Hz, 1H), 2.40 (ddt, *J* = 8.4, 4.9, 2.4 Hz, 1H), 1.78 (ddq, *J* = 14.5, 8.7, 7.4 Hz, 1H), 1.52 (dqd, *J* = 14.8, 7.5, 4.7 Hz, 1H), 1.45 – 1.16 (m, 12H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.90 – 0.81 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.1, 77.0, 53.9, 36.8, 31.7, 29.4, 29.1, 29.0, 26.7, 22.6, 18.6, 14.0, 12.1.
Spectroscopic data is in agreement with published data.[128]

The enantiomeric excess was determined by HPLC using a Chiracel AD-H column (*n*-hexane/*i*-PrOH 99.8:0.2, 25°C) at 0.6 mL/min, UV detection at 254 nm: t_R : (*syn,* major) = 56.3 min, (*syn*, minor) = 73.1 min.

7.5.2 2,3,4-Trisubstituted γ-Nitroaldehydes

(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-phenylpentanal:

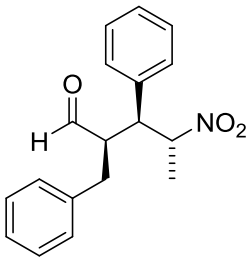


Prepared from butanal and *trans*-β-methyl-β-nitrostyrene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.83 (d, *J* = 1.8 Hz, 1 H), 7.35 – 7.24 (m, 3 H), 7.05 – 6.99 (m, 2 H), 5.06 - 4.97 (m, 1H), 3.39 (dd, *J* = 10.0, 5.3 Hz, 1H), 3.15 (ddt, *J* = 10.0, 3.4, 1.8 Hz, 1H), 1.39 (d, *J* = 6.6 Hz, 3H), 1.58 - 1.47 (m, 1H), 1.46 - 1.25 (m, 1H), 0.75 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.9, 134.9, 128.9, 128.7, 128.2, 83.5, 52.8, 48.3, 20.5, 17.4, 10.1; elemental analysis calcd (%) for $C_{13}H_{17}NO_3$: C 66.36, H 7.19, N 5.75; found: C 66.33, H 7.28, N 5.79;

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 99:1, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 25.0 min, (major) = 28.2 min.

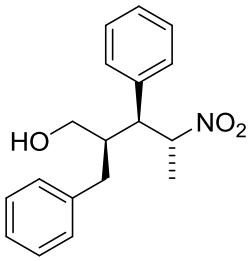
(2*R*,3*S*,4*R*)-2-Benzyl-4-nitro-3-phenylpentanal:



Prepared from 3-phenylpropanal and *trans*-β-methyl-β-nitrostyrene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.81 (d, *J* = 1.5 Hz, 1H), 7.38 - 7.29 (m, 3H), 7.24 - 7.14 (m, 3H), 7.11 - 7.07 (m, 2H), 6.96 (d, *J* = 6.8 Hz, 2H), 5.03 (dq, *J* = 6.7, 5.4 Hz, 1H), 3.54 (ddt, *J* = 9:9, 4.0, 1.5 Hz, 1H), 3.38 (dd, *J* = 9.9, 5.4 Hz, 1H), 2.73 (dd, *J* = 14.1, 4.0 Hz, 1H), 2.60 (dd, *J* = 14.1, 9.9 Hz, 1H), 1.38 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.4, 137.1, 134.8, 129.0, 128.9, 128.9, 128.7, 128.4, 126.8, 83.0, 53.4, 49.5, 35.2, 17.3.

(2*R*,3*S*,4*R*)-2-Benzyl-4-nitro-3-phenylpentan-1-ol:

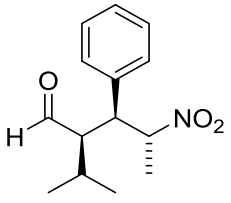


Prepared from (2*R*,3S,4R)-2-benzyl-4-nitro-3-phenylpentanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (2:1). The δ-nitroalcohol was obtained as a colourless solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 - 7.14 (m, 8H), 7.08 - 7.03 (m, 2H), 5.24 (dq, *J* = 7:8, 6.6 Hz, 1H), 3.75 (dd, *J* = 11.0, 3.7 Hz, 1H), 3.49 (dd, *J* = 7.8, 7.1 Hz, 1H), 3.35 (dd, *J* = 11.0, 6.6 Hz, 1H), 2.59 (dd, *J* = 13.6, 3.2 Hz, 1H), 2.43 - 2.35 (m, 1H), 2.26 (dd, *J* = 13.6, 11.0 Hz, 1H), 1.60 (d, *J* = 6.6 Hz, 3H); . ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.7, 136.2, 129.2, 128.8, 128.6, 128.5, 127.8, 126.3, 84.1, 61.4, 50.4, 42.9, 34.0, 18.2; elemental analysis calcd (%) for $C_{18}H_{21}NO_3$: C 72.22, H 7.07, N 4.68; found: C 72.21, H 7.14, N 4.53.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 95:5, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 40.2 min, (major) = 77.6 min.

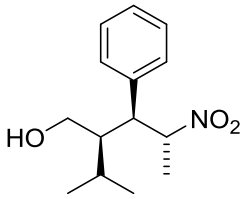
(2*R*,3*S*,4*R*)-2-Isopropyl-4-nitro-3-phenylpentanal:



Prepared from *iso*-valeraldehyde and *trans*-β-methyl-β-nitrostyrene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (d, *J* = 0.4 Hz, 1H), 7.33 - 7.27 (m, 3H), 7.03 - 6.97 (m, 2H), 4.96 - 4.89 (m, 1H), 3.38 - 3.35 (m, 2H), 1.67 - 1.58 (m, 1H), 1.28 (d, *J* = 6.6 Hz, 3H), 1.11 (d, *J* = 7.2 Hz, 3H), 0.68 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 205.4, 134.7, 129.0, 128.7, 128.1, 83.0, 56.6, 47.6, 28.7, 21.8, 17.1, 16.2.

(2*R*,3*S*,4*R*)-2-Isopropyl-4-nitro-3-phenylpentan-1-ol:

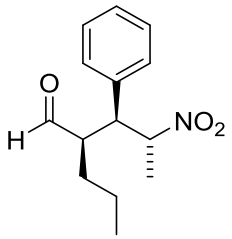


Prepared from (2*R*,3S,4R)-2-isopropyl-4-nitro-3-phenylpentanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (2:1). The δ-nitroalcohol was obtained as a colourless solid

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ 7.32 - 7.26 (m, 3H), 7.10 - 7.02 (m, 2H), 5.35 (dq, *J* = 6.7, 4.5 Hz, 1H), 4.11 (dd, *J* = 11.4, 3.7 Hz, 1H), 3.82 (dd, *J* = 11.4, 4.9 Hz, 1H), 3.22 (dd, *J* = 10.0, 4.5 Hz, 1H), 2.15 - 2.08 (m, 1H), 1.50 (dsept, *J* = 6.9, 2.5 Hz, 1H), 1.44 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.63 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.7, 129.0, 128.5, 127.6, 83.4, 60.4, 51.0, 45.8, 27.5, 22.6, 18.0, 16.7; elemental analysis calcd (%) for $C_{14}H_{21}NO_3$: C 66.91, H 8.42, N 5.75; found: C 66.95, H 8.25, N 5.39.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 97.5:2.5, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 41.5 min, (major) = 52.7 min.

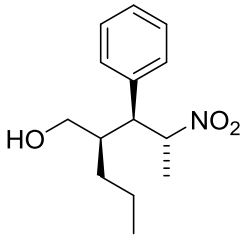
(2*R*,3*S*,4*R*)-4-Nitro-3-phenyl-2-propylpentanal:



Prepared from valeraldehyde and *trans*-β-methyl-β-nitrostyrene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture of cyclohexane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless solid.

colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.81 (d, *J* = 1.9 Hz, 1H), 7.36 - 7.24 (m, 3H), 7.11 - 6.99 (m, 2H), 5.01 (quint, *J* = 6.6 Hz, 1H), 3.39 (dd, *J* = 9.6, 5.8 Hz, 1H), 3.16 - 3.08 (m, 1H), 1.40 (d, *J* = 6.6 Hz, 3H), 1.38 - 1.05 (m, 4H), 0.75 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CDCl): δ 204.1, 135.0, 128.8, 128.7, 128.2, 83.6, 51.7, 49.0, 29.9, 19.4, 17.4, 14.1.

(2*R*,3*S*,4*R*)-4-Nitro-3-phenyl-2-propylpentan-1-ol:



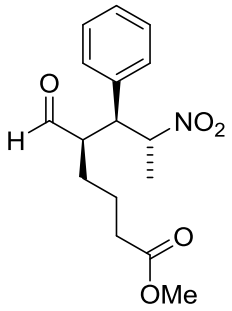
Prepared from (2*R*,3*S*,4*R*)-4-nitro-3-phenyl-2-propylpentanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (2:1). The δ-nitroalcohol was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 - 7.17 (m, 3H), 7.17 - 7.12 (m, 2H), 5.19 (dq, *J* = 9.2, 6.6 Hz, 1H), 3.79 (dd, *J* = 10.9, 3.7 Hz, 1H), 3.49 - 3.43 (m, 1H), 3.31 (dd, *J* = 10.9, 7.7 Hz, 1H), 2.08 - 1.99 (m, 1H), 1.58 (d, *J* = 6.6 Hz, 3H), 1.43 - 1.31 (m, 1H), 1.26 - 1.13 (m, 2H), 1.10 - 0.97 (m, 1H), 0.85 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.4, 129.1, 128.4,

127.6, 85.0, 62.5, 50.3, 40.6, 29.2, 20.9, 18.3, 14.3; elemental analysis calcd (%) for $C_{14}H_{21}NO_3$: C 66.91, H 8.42, N 5.75; found: C 66.64, H 8.22, N 5.66.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 97.5:2.5, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (major) = 61.5 min, (major) = 64.8 min.

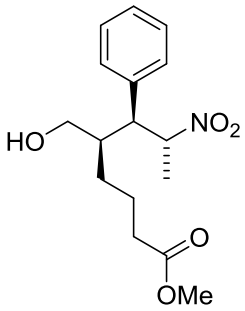
(5*R*,6*S*,7*R*)-Methyl 5-formyl-7-nitro-6-phenyloctanoate:



Prepared from methyl 6-oxohexanoate and *trans*-β-methyl-β-nitrostyrene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture of 0.5 % MeOH in CH_2Cl_2. The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.84 (d, *J* = 1.8 Hz, 1H), 7.36 – 7.23 (m, 3H), 7.06 – 6.99 (m, 2H), 5.01 (dq, *J* = 6.7, 5.8 Hz, 1H), 3.57 (s, 3H), 3.42 (dd, *J* = 9.6, 5.8 Hz, 1H), 3.19 – 3.11 (m, 1H), 2.17 – 2.10 (m, 2H), 1.61 – 1.37 (m, 4H), 1.42 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.5, 173.2, 134.8, 128.9, 128.9, 128.4, 83.7, 51.7, 51.7, 48.9, 33.7, 26.9, 21.5, 17.5.

5*R*,6*S*,7*R*)-Methyl 5-(hydroxymethyl)-7-nitro-6-phenyloctanoate:

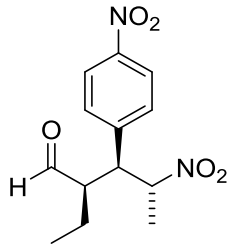


Prepared from (5*R*,6S,7R)-methyl 5-formyl-7-nitro-6-phenyloctanoate according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (2:1). The δ-nitroalcohol was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 – 7.05 (m, 5H), 5.28 – 5.14 (m, 1H), 3.87 (dd, *J* = 11.1, 3.7 Hz, 1H), 3.64 (s, 3H), 3.48 (dd, *J* = 11.1, 6.7 Hz, 1H), 3.40 (t, *J* = 7.4 Hz, 1H), 2.29 – 2.21 (m, 2H), 2.16 – 2.00 (m, 1H), 1.78 – 1.62 (m, 1H), 1.56 (d, *J* = 6.6 Hz, 3H), 1.56 – 1.46 (m, 1H), 1.34 – 1.19 (m, 1H), 1.19 – 1.02 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.1, 136.4, 129.2, 128.5, 127.8, 84.5, 62.1, 51.7, 50.5, 40.8, 33.9, 27.0, 22.8, 18.3; elemental analysis calcd (%) for $C_{16}H_{23}NO_5$: C 62.12, H 7.49, N 4.53; found: C 61.88, H 7.79, N 4.66;

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 92.5:7.5, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 45.6 min, (major) = 49.1 min.

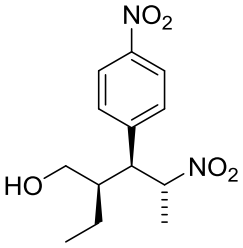
(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-(4-nitrophenyl)pentanal:



Prepared from butanal and (*E*)-1-nitro-4-(2-nitroprop-1-en-1-yl)benzene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (5:1 to 2:1). The γ-nitroaldehyde was obtained as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.86 (d, *J* = 1.4 Hz, 1H), 8.22 – 8.18 (m, 2H), 7.29 – 7.23 (m, 2H), 5.09 (dq, *J* = 6.7, 5.4 Hz, 1H), 3.56 (dd, *J* = 10.0, 5.4 Hz, 1H), 3.21 (dddd, *J* = 10.0, 8.5, 3.4, 1.4 Hz, 1H), 1.64 – 1.52 (m, 1H), 1.40 – 1.32 (m, 2H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.78 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.8, 142.6, 130.2, 130.1, 124.0, 83.2, 52.5, 48.0, 20.6, 17.6, 10.1.

(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-(4-nitrophenyl)pentan-1-ol:



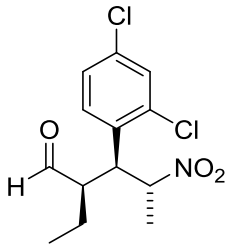
Prepared from (2*R*,3*S*,4*R*)-2-ethyl-4-nitro-3-(4-nitrophenyl)pentanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (2:1 to 1:1). The δ-nitroalcohol was obtained as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.7 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 2H), 5.23 (dq, *J* = 8.8, 6.6 Hz, 1H), 3.90 (dd, *J* = 10.8, 3.9 Hz, 1H), 3.67 (dd, *J* = 8.8, 6.0 Hz, 1H), 3.26 (dd, *J*

= 10.8, 8.1 Hz, 1H), 2.08 – 1.96 (m, 1H), 1.62 (d, *J* = 6.6 Hz, 3H), 1.38 – 1.22 (m, 1H), 1.07 – 0.94 (m, 1H), 0.91 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.5, 144.5, 130.3, 123.6, 84.6, 61.5, 49.9, 42.8, 20.2, 18.5, 12.4; elemental analysis calcd (%) for $C_{13}H_{18}N_2O_5$: C 55.31, H 6.43, N 9.92; found: C 55.19, H 6.66, N 9.70.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 92.5:7.5, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 50.9 min, (major) = 52.9 min.

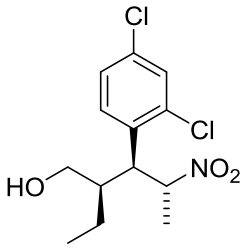
(2*R*,3*S*,4*R*)-3-(2,4-Dichlorophenyl)-2-ethyl-4-nitropentanal:



Prepared from butanal and (*E*)-2,4-dichloro-1-(2-nitroprop-1-en-1-yl)benzene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (20:1 to 5:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.88 (d, *J* = 1.5 Hz, 1H), 7.46 (d, *J* = 2.2 Hz, 1H), 7.24 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 5.15 – 5.04 (m, 1H), 4.15 (dd, *J* = 10.6, 4.8 Hz, 1H), 3.26 – 3.14 (m, 1H), 1.59 – 1.49 (m, 1H), 1.40 (d, *J* = 6.7 Hz, 3H), 1.38 – 1.30 (m, 1H), 0.76 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.9, 136.3, 134.7, 132.0, 130.0, 127.9, 83.3, 52.9, 42.2, 20.7, 16.7, 9.9.

(2*R*,3*S*,4*R*)-3-(2,4-Dichlorophenyl)-2-ethyl-4-nitropentan-1-ol:

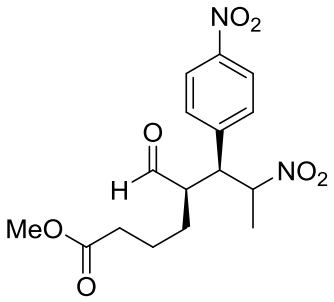


Prepared from (2*R*,3*S*,4*R*)-3-(2,4-dichlorophenyl)-2-ethyl-4-nitropentanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (5:1). The δ-nitroalcohol was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ7.43 (d, *J* = 2.2 Hz, 1H), 7.21 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 5.20 (p, *J* = 6.7 Hz, 1H), 4.06 – 3.94 (m, 2H), 3.70 (dd, *J* = 11.5, 5.0 Hz, 1H), 1.98 (ddt, *J* = 12.7, 8.6, 4.3 Hz, 1H), 1.52 (d, *J* = 6.7 Hz, 3H), 1.22 – 1.09 (m, 2H), 0.86 (t, *J* = 7.4 Hz, 4H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.5, 134.0, 133.9, 129.8, 127.6, 84.0, 61.6, 45.4, 43.4, 21.3, 17.7, 12.0; elemental analysis calcd (%) for $C_{13}H_{17}NCl_2O_3$: C 51.00, H 5.60, N 4.57; found: C 51.04, H 5.79, N 4.55.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 97.5:2.5, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 32.0 min, (major) = 38.3 min.

(5*R*,6*S*,7*R*)-Methyl 5-formyl-7-nitro-6-(4-nitrophenyl)octanoate:

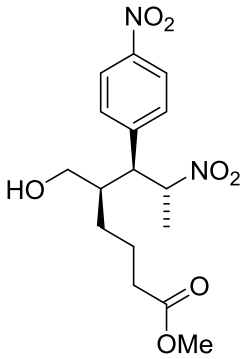


Prepared from methyl 6-oxohexanoate and (*E*)-1-nitro-4-(2-nitroprop-1-en-1-yl)benzene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel

eluting with a mixture pentane and Et_2O (4:1). The γ-nitroaldehyde was obtained as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$: δ 9.87 (d, *J* = 1.4 Hz, 1H), 8.22 – 8.17 (m, 2H), 7.28 – 7.23 (m, 2H), 5.12 – 5.04 (m, 1H), 3.69 – 3.64 (m, 1H), 3.58 (s, 3H), 3.25 – 3.17 (m, 1H), 2.20 – 2.13 (m, 2H), 1.59 – 1.32 (m, 4H), 1.44 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 202.4, 173.0, 147.9, 142.4, 130.1, 124.1, 83.3, 51.8, 51.4, 48.4, 33.5, 26.9, 21.3, 17.6.

(5*R*,6*S*,7*R*)-Methyl 5-(hydroxymethyl)-7-nitro-6-(4-nitrophenyl)octanoate:

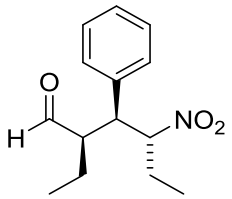


Prepared from (5*R*,6*S*,7*R*)-methyl 5-formyl-7-nitro-6-(4-nitrophenyl)octanoate according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (1:1). The δ-nitroalcohol was obtained as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 – 8.12 (m, 2H), 7.38 – 7.31 (m, 2H), 5.22 (dq, *J* = 8.2, 6.6 Hz, 1H), 3.91 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.64 (s, 1H), 3.61 (dd, *J* = 8.2, 6.6 Hz, 1H), 3.38 (dd, *J* = 11.0, 7.2 Hz, 1H), 2.29 – 2.22 (m, 2H), 2.17 – 2.07 (m, 1H), 1.75 – 1.62 (m, 1H), 1.59 (d, *J* = 6.6 Hz, 3H), 1.57 – 1.44 (m, 1H), 1.28 – 1.15 (m, 1H), 1.11 – 0.97 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.9, 147.6, 144.3, 130.3, 123.6, 84.2, 61.6, 51.8, 50.0, 40.8, 33.7, 27.0, 22.7, 18.4; elemental analysis calcd (%) for $C_{13}H_{17}NCl_2O_3$: C 55.73, H 6.05, N 7.65; found: C 56.00, H 6.32, N 7.30.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 90:10, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 43.9 min, (major) = 52.9 min.

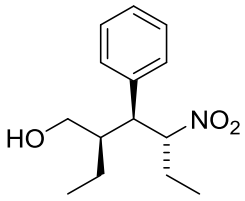
(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-phenylhexanal:



Prepared from butanal and (*E*)-(2-nitrobut-1-en-1-yl)benzene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture pentane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.81 (d, *J* = 1.8 Hz, 1H), 7.35 – 7.27 (m, 3H), 7.07 – 7.00 (m, 2H), 4.83 (ddd, *J* = 10.1, 5.9, 4.3 Hz, 1H), 3.49 (dd, *J* = 9.7, 5.9 Hz, 1H), 3.08 – 2.98 (m, 1H), 1.84 – 1.74 (m, 1H), 1.74 – 1.64 (m, 1H), 1.58 – 1.47 (m, 1H), 1.47 – 1.34 (m, 1H), 0.93 (t, *J* = 7.3 Hz, 3H), 0.76 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 203.8, 135.2, 129.0, 128.7, 128.2, 90.8, 53.0, 47.4, 25.1, 20.5, 10.6, 10.3.

(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-phenylhexan-1-ol:

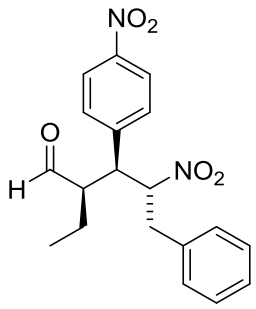


Prepared from (2*R*,3*S*,4*R*)-2-ethyl-4-nitro-3-phenylhexanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (2:1). The δ-nitroalcohol was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 – 7.25 (m, 3H), 7.20 – 7.14 (m, 2H), 5.04 (td, *J* = 8.8, 5.5 Hz, 1H), 3.83 (dd, *J* = 10.9, 3.8 Hz, 1H), 3.55 (dd, *J* = 9.3, 5.8 Hz, 1H), 3.35 (dd, *J* = 10.9, 7.7 Hz, 1H), 2.01 – 1.88 (m, 3H), 1.37 (dqd, *J* = 14.9, 7.5, 2.9 Hz, 1H), 1.13 – 1.05 (m, 1H), 1.02 (t, *J* = 7.3 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 136.5, 129.2, 128.3, 127.6, 91.8, 62.0, 49.2, 42.8, 25.4, 19.9, 12.4, 10.3; elemental analysis calcd (%) for $C_{14}H_{21}NO_2$: C 66.91, H 8.42, N 5.57; found: C 66.74, H 8.19, N 5.42.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 95:5, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 22.5 min, (major) = 26.3 min.

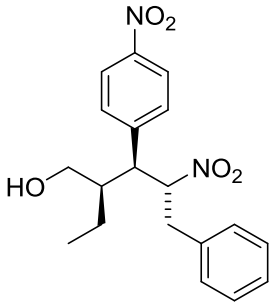
(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-(4-nitrophenyl)-5-phenylpentanal:



Prepared from butanal and (E)-1-nitro-4-(2-nitro-3-phenylprop-1-en-1-yl)benzene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture pentane and EtOAc (5:1). The γ-nitroaldehyde was obtained as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.87 (d, *J* = 1.2 Hz, 1H), 8.26 – 8.20 (m, 2H), 7.35 – 7.21 (m, 5H), 7.15 – 7.09 (m, 2H), 5.31 (dt, *J* = 9.7, 5.0 Hz, 1H), 3.69 (dd, *J* = 10.4, 5.0 Hz, 1H), 3.26 – 3.18 (m, 1H), 3.00 (dd, *J* = 14.6, 9.7 Hz, 1H), 2.93 (dd, *J* = 14.6, 5.0 Hz, 1H), 1.60 (ddd, *J* = 14.7, 7.5, 3.4 Hz, 1H), 1.41 – 1.29 (m, 1H), 0.76 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C): δ = 202.7, 148.1, 142.4, 135.0, 130.3, 129.1, 128.9, 127.7, 124.0, 89.1, 52.3, 46.7, 37.7, 20.7, 9.8.

(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-(4-nitrophenyl)-5-phenylpentan-1-ol:

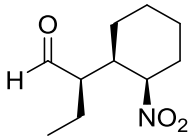


Prepared from (2R,3S,4R)-2-Ethyl-4-nitro-3-(4-nitrophenyl)-5-phenylpentanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (5:1). The δ-nitroalcohol was obtained as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ8.24 – 8.09 (m, 2H), 7.44 – 7.35 (m, 2H), 7.35 – 7.22 (m, 3H), 7.22 – 7.11 (m, 2H), 5.39 (ddd, *J* = 9.8, 8.2, 4.5 Hz, 1H), 3.97 (dd, *J* = 10.8, 3.8 Hz, 1H), 3.77 (dd, *J* = 8.2, 6.9 Hz, 1H), 3.35 (dd, *J* = 10.8, 7.3 Hz, 1H), 3.22 (dd, *J* = 14.5, 4.4 Hz, 1H), 3.14 (dd, *J* = 14.5, 9.9 Hz, 1H), 2.18 – 2.06 (m, 1H), 1.44 – 1.27 (m, 1H), 1.17 – 0.98 (m, 1H), 0.91 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.6, 144.2, 135.3, 130.4, 129.1, 128.9, 127.8, 123.6, 90.9, 61.4, 49.4, 42.9, 38.5, 20.6, 12.4; elemental analysis calcd (%) for $C_{19}H_{22}N_2O_5$: C 63.68, H 6.19, N 7.82; found: C 63.41, H 6.36, N 7.47;

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 90:10, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 36.4 min, (major) = 40.3 min.

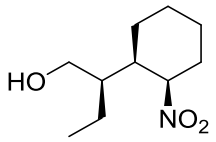
(*R*)-2-((1*R*,2*R*)-2-Nitrocyclohexyl)butanal:



Prepared from butanal and 1-nitrocyclohex-1-ene according to general procedure 7.2.2 d). Purified by column chromatography on silica gel eluting with a mixture pentane and EtOAc (20:1). The γ-nitroaldehyde was obtained as a colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.68 (d, *J* = 1.6 Hz, 1H), 4.87 (q, *J* = 3.4 Hz, 1H), 2.50 (dddd, *J* = 9.7, 8.0, 3.7, 1.6 Hz, 1H), 2.32 - 2.23 (m, 1H), 2.17 - 2.06 (m, 1H), 1.92 - 1.52 (m, 7H), 1.44 - 1.16 (m, 2H), 0.84 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.6, 83.5, 53.1, 37.3, 29.6, 25.0, 23.2, 20.1, 19.3, 9.9.

(*R*)-2-((1*R*,2*R*)-2-nitrocyclohexyl)butan-1-ol:



Prepared from (*R*)-2-((1*R*,2*R*)-2-nitrocyclohexyl)butanal according to the general procedure 7.2.2 e) for the reduction of γ-nitroaldehydes. Purified by column chromatography on silica gel eluting with a mixture of pentane and EtOAc (2:1). The δ-nitroalcohol was obtained as a colourless oil.

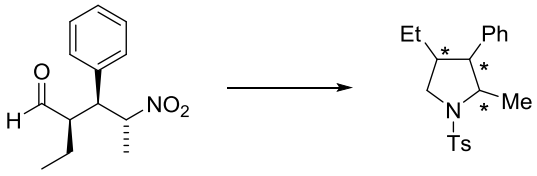
^{1}H NMR (400 MHz, $CDCl_3$,): δ 5.00 - 4.92 (m, 1H), 3.68 (dd, *J* = 11.2, 3.8 Hz, 1H), 3.64 (dd, *J* = 11.2, 4.3 Hz, 1H), 2.35 - 2.25 (m, 1H), 1.93 - 1.00 (m, 11H), 0.87 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 84.4, 61.6, 43.0, 40.3, 31.0, 25.4, 23.5, 20.8, 20.3, 11.4; elemental analysis calcd (%) for $C_{10}H_{19}NO_3$: C 59.68, H 9.51, N 6.96; found: C 59.58, H 9.41, N 6.81.

The enantiomeric excess was determined by HPLC using a Chiracel AS-H column (*n*-hexane/^{i}PrOH 94:6, 25°C) at 0.5 mL min^{-1}, UV detection at 210 nm: t_R (minor) = 18.7 min, (major) = 20.8 min.

7.6 Derivatization of γ-Nitroaldehydes

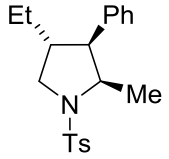
7.6.1 Derivatization of 1,3,4-Trisubstituted γ-Nitroaldehydes

Synthesis of Diastereoisomeric Pyrrolidines:



(2*R*,3*S*,4*R*)-2-Ethyl-4-nitro-3-phenylpentanal (145 mg, 616 μmol, 1.0 eq) was dissolved in 10 mL of MeOH and $Pd(OH)_2/C$ (15 – 20 % w/w, 29 mg) was added. The resulting mixture was hydrogenated under 4.5 bar of H_2 for 18 h. The reaction mixture was filtered through a pad of cellite and the volatiles were removed *in vacuo*. The residue was dissolved in 5 mL CH_2Cl_2 and cooled to 0°C. Tosyl chloride (129 mg, 678 μmol, 1.1 eq) and NEt_3 (256 μL, 1.85 mmol, 3.0 eq) were added. The reaction mixture was allowed to warm to room temperature and stirring was continued for 18 h. The solution was diluted with 20 mL CH_2Cl_2 and washed with water, 5 % $NaHCO_3$ and brine (10 mL each). The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated on a rotary evaporator. The resulting residue was purified by flash column chromatography on silica gel by eluting with pentane/EtOAc (10:1) to give the products (32 – 41 %).

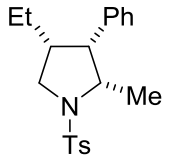
(2*R*,3*S*,4*R*)-4-ethyl-2-methyl-3-phenyl-1-tosylpyrrolidine:



^{1}H NMR (600 MHz, CD_3OD): δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.28 (t, *J* = 7.2 Hz, 2H), 7.25 – 7.17 (m, 2H), 7.08 (d, *J* = 7.2 Hz, 2H), 3.93 (dq, *J* = 7.9, 6.7 Hz, 1H), 3.75 (dd, *J* = 9.9, 7.2 Hz, 1H), 2.82 (t, *J* = 9.9 Hz, 1H), 2.71 – 2.60 (m, 1H), 2.50 – 2.40 (m, 1H), 2.47 (s, 3H), 1.40 (ddq, *J* = 15.1, 7.6, 3.6 Hz, 1H), 0.95 (ddq, *J* = 15.1, 7.6, 2.0 Hz, 1H), 0.89 (d, *J* = 6.7 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, CD_3OD): δ 145.1, 138.1,

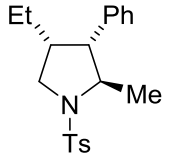
135.5, 130.8, 129.6, 129.3, 128.3, 127.8, 61.5, 54.9, 53.9, 41.3, 25.2, 21.4, 19.0, 11.9; MS (ESI, $[M+H]^+$) Calcd for $C_{25}H_{26}NO_2S$: 344.2. Found: 344.4.

(2*S*,3*R*,4*R*)-4-ethyl-2-methyl-3-phenyl-1-tosylpyrrolidine:



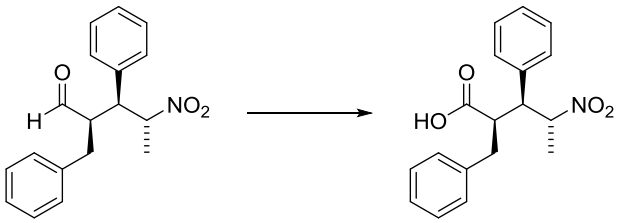
^{1}H NMR (600 MHz, C_6D_6): δ 7.84 (d, *J* = 8.3 Hz, 2H), 7.11 – 7.06 (m, 2H), 7.06 – 7.02 (m, 1H), 7.02 – 6.99 (m, 2H), 6.88 (d, *J* = 7.9 Hz, 2H), 3.72 (dd, *J* = 11.3, 8.8 Hz, 1H), 3.56 (p, *J* = 6.5 Hz, 1H), 3.31 (t, *J* = 11.3 Hz, 1H), 2.55 (t, *J* = 6.5 Hz, 1H), 1.45 – 1.39 (m, 1H), 1.95 (s, 3H), 1.37 (d, *J* = 6.5 Hz, 3H), 0.74 – 0.63 (m, 1H), 0.62 – 0.55 (m, 1H), 0.42 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, C_6D_6): δ 142.7, 137.6, 135.3, 130.2, 129.5, 128.2, 127.9, 126.7, 60.5, 54.3, 53.8, 43.3, 21.8, 20.9, 18.3, 12.0; MS (ESI, $[M+H]^+$) Calcd for $C_{25}H_{26}NO_2S$: 344.2. Found: 344.2.

(2*R*,3*R*,4*R*)-4-ethyl-2-methyl-3-phenyl-1-tosylpyrrolidine:



^{1}H NMR (600 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.2 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 1H), 7.11 (tt, *J* = 7.2, 1.1 Hz, 1H), 7.02 (t, *J* = 7.2 Hz, 1H), 6.51 (d, *J* = 7.2 Hz, 1H), 3.87 (qd, *J* = 6.3, 3.4 Hz, 1H), 3.67 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.02 (t, *J* = 9.6 Hz, 1H), 2.97 (dd, *J* = 7.1, 3.4 Hz, 1H), 2.50 (s, 2H), 2.45 – 2.36 (m, 1H), 1.48 (d, *J* = 6.3 Hz, 2H), 0.95 – 0.86 (m, 1H), 0.71 (t, *J* = 7.4 Hz, 2H), 0.57 – 0.47 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 143.3, 140.1, 135.2, 129.6, 128.0, 127.9, 127.4, 126.4, 61.9, 55.6, 52.3, 43.0, 23.5, 21.3, 21.5, 12.5; MS (ESI, $[M+H]^+$) Calcd for $C_{25}H_{26}NO_2S$: 344.2. Found: 344.3.

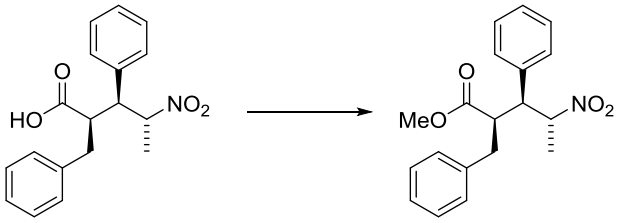
(2*R*,3*S*,4*R*)-2-benzyl-4-nitro-3-phenylpentanoic acid (6):



A solution of (2*R*,3*S*,4*R*)-2-benzyl-4-nitro-3-phenylpentanal (190 mg, 639 μmol, 1.0 eq) in 2 mL of acetone was cooled to 0°C. The solution was titrated carefully with Jones-Reagent (prepared as a standard reagent; 8 N) until the red coloration prevailed (560 μL). The reaction was quenched by the dropwise addition of 200 μL of ^{i}PrOH and the resulting suspension stirred at 0°C for 10 min. The reaction mixture was filtered. The filtrate was diluted with 5 mL of 1M HCl and extracted with Et_2O (5x10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography on silica gel by eluting with 5 % MeOH in CH_2Cl_2 to give 196 mg (98 %) of a colourless solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 – 7.30 (m, 3H), 7.26 – 7.10 (m, 5H), 7.01 – 6.95 (m, 2H), 4.99 (dq, *J* = 6.7, 4.9 Hz, 1H), 3.47 – 3.38 (m, 1H), 3.33 (dd, *J* = 10.6, 4.9 Hz, 1H), 2.70 (dd, *J* = 13.9, 4.0 Hz, 1H), 2.60 (dd, *J* = 13.9, 9.5 Hz, 1H), 1.44 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.4, 137.7, 135.0, 129.1, 129.1, 129.0, 128.6, 128.6, 127.0, 109.7, 84.3, 51.6, 48.9, 37.4, 17.5; elemental analysis calcd (%) for $C_{18}H_{19}NO_4$: C 69.00, H 6.11, N 4.47; found: C 69.38, H 6.15, N 4.24.

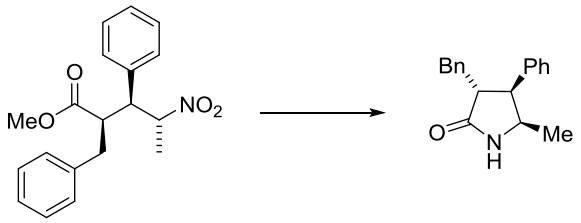
(2*R*,3*S*,4*R*)-Methyl 2-benzyl-4-nitro-3-phenylpentanoate:



(2*R*,3*S*,4*R*)-2-Benzyl-4-nitro-3-phenylpentanoic acid (40.0 mg, 129 µmol, 1.0 eq) was dissolved in a mixture of 250 µL of MeOH and 850 µL of toluene and trimethylsillyl diazomethane (as a 2m solution in hexane, 83.0 µL, 166 µmol, 1.3 eq) was added dropwise. The resulting yellow solution was stirred at room temperature for 20 min before being quenched with a solution of acetic acid in MeOH (0.5M, 100 µL). The colorless solution was evaporated to dryness. 44.7 mg (quant.) of a colorless oil resulted.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 – 7.30 (m, 3H), 7.25 – 7.12 (m, 5H), 6.98 – 6.91 (m, 2H), 4.92 (qd, *J* = 6.7, 4.7 Hz, 1H), 3.56 (s, 3H), 3.44 – 3.32 (m, 2H), 2.63 (dd, *J* = 13.6, 3.9 Hz, 1H), 2.56 (dd, *J* = 13.6, 9.3 Hz, 1H), 1.44 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 138.1, 135.5, 129.1, 129.1, 128.9, 128.5, 128.5, 126.8, 84.6, 52.0, 51.8, 49.4, 37.9, 17.4; HRMS (ESI): *m/z* calcd for $C_{19}H_{22}NO_4$: 328.1543 [*M*+H^+]; found: 328.1546.

(3*R*,4*S*,5*R*)-3-benzyl-5-methyl-4-phenylpyrrolidin-2-one (7):

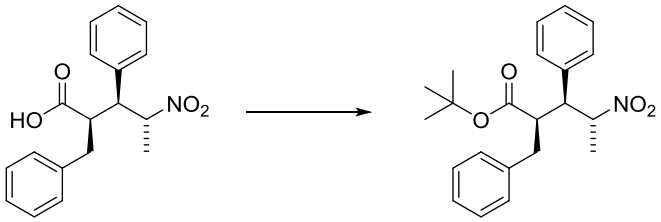


(2*R*,3*S*,4*R*)-Methyl 2-benzyl-4-nitro-3-phenylpentanoate (126 mg, 385 µmol, 1.0 eq) was dissolved in 13 mL of EtOAc and 2 mL of HOAc. Then freshly activated (washed with 2M HCl, H_2O, EtOH and Et_2O; dried under a flow of nitrogen) zinc powder (754 mg, 11.6 mmol, 30 eq) was added and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was filtered and the filtrate washed with 20 mL of sat. NH_4OH. The aqueous layer was extracted with EtOAc (3x15 mL). The combined organic extracts were dried over

Na_2SO_4 and the solvents were removed *in vacuo* on a rotary evaporator. The resulting crude material was purified by flash column chromatography on silica gel by eluting with 5 % MeOH in CH_2Cl_2 to give 83.0 mg (81 %) of a colorless, slowly crystallizing oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 – 7.06 (m, 10H), 6.21 (s, br, 1H), 3.73 (p, *J* = 6.8 Hz, 1H), 3.44 – 3.35 (m, 1H), 3.21 – 3.10 (m, 2H), 3.00 – 2.91 (m, 1H), 0.78 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0, 138.4, 138.1, 129.9, 128.7, 128.4, 128.4, 127.2, 126.5, 51.9, 48.3, 45.3, 34.0, 18.2; MS (ESI): *m/z* calcd for $C_{18}H_{19}NNaO$: 288.2 $[M+Na]^+$; found: 288.2.elemental analysis calcd (%) for $C_{18}H_{19}NO$: C 81.48, H 7.22, N 5.28; found: C 81.08, H 7.30, N 5.07.

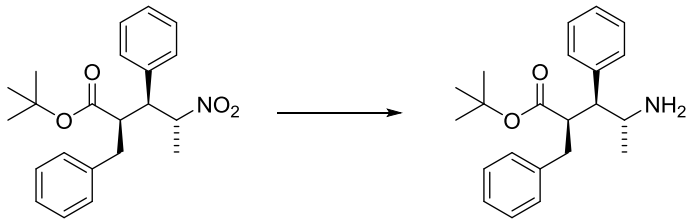
***tert*-Butyl (2*R*,3*S*,4*R*)-2-benzyl-4-nitro-3-phenylpentanoate:**



(2*R*,3*S*,4*R*)-2-Benzyl-4-nitro-3-phenylpentanoic acid (72.0 mg, 230 mmol, 1.0 eq) was dissolved in 1 mL of CH_2Cl_2. After the addition of conc. H_2SO_4 (10 mL, 188 mmol, 0.82 eq) 1 mL of *iso*-butene was condensed into the flask. The resulting mixture was stirred at room temperature for 24 h. Then the reaction mixture was poured into 10 mL of sat. $NaHCO_3$ and extracted with CH_2Cl_2 (3x 15 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The resulting residue was treated with pentane to yield 57.1 mg (67 %) of a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 – 7.29 (m, 3H), 7.25 – 7.13 (m, 5H), 7.05 – 6.99 (m, 2H), 4.98 (qd, *J* = 6.7, 5.0 Hz, 1H), 3.33 (dd, *J* = 10.4, 5.0 Hz, 1H), 3.26 (td, *J* = 10.4, 4.0 Hz, 1H), 2.60 (dd, *J* = 13.7, 4.0 Hz, 1H), 2.49 (dd, *J* = 13.7, 10.4 Hz, 1H), 1.45 (d, *J* = 6.7 Hz, 3H), 1.26 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7, 138.3, 135.7, 129.2, 129.2, 128.9, 128.3, 128.3, 126.6, 84.4, 81.6, 52.4, 49.6, 38.3, 27.9, 17.9; elemental analysis calcd (%) for $C_{22}H_{27}NO_4$: C 71.52, H 7.37, N 3.79; found: C 71.88, H 7.46, N 3.67.

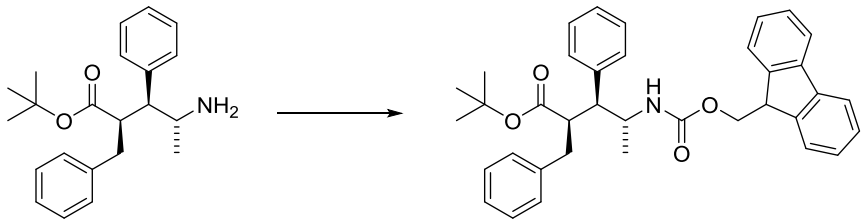
(2*R*,3*S*,4*R*)-*tert*-Butyl 4-amino-2-benzyl-3-phenylpentanoate:



tert-Butyl (2*R*,3*S*,4*R*)-2-benzyl-4-nitro-3-phenylpentanoate (57.0 mg, 154 mmol, 1.0 eq) was dissolved in 5 mL of EtOAc and 800 mL of HOAc. Then freshly activated (washed with 2M HCl, H_2O, EtOH and Et_2O; dried under a flow of nitrogen) zinc powder (303 mg, 4.63 mmol, 30 eq) was added and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was filtered and the filtrate washed with 20 mL of sat. NH_4OH. The aqueous layer was extracted with EtOAc (3x20 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents were removed *in vacuo* on a rotary evaporator. 51.7 mg (99 %) of a colorless solid resulted.

^{1}H NMR (400 MHz, $CDCl_3$: δ 7.40 – 7.33 (m, 2H), 7.33 – 7.27 (m, 3H), 7.20 (t, *J* = 7.3 Hz, 2H), 7.17 – 7.10 (m, 1H), 7.07 (d, *J* = 7.1 Hz, 2H), 3.24 – 3.17 (m, 1H), 3.15 (dd, *J* = 11.4, 3.5 Hz, 1H), 2.85 (dd, *J* = 11.3, 3.5 Hz, 1H), 2.62 (dd, *J* = 13.6, 11.6 Hz 1H), 2.49 (dd, *J* = 13.6, 3.5 Hz, 1H), 1.44 (s, br, 2H), 1.28 (s, 9H), 0.88 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 139.7, 138.2, 129.8, 129.0, 128.4, 128.2, 127.1, 126.2, 81.0, 55.8, 51.3, 48.3, 37.7, 28.0, 22.9; HRMS (ESI): *m/z* calcd for $C_{22}H_{30}NO_2$: 340.2271 [*M*+H^+]; found: 340.2267.

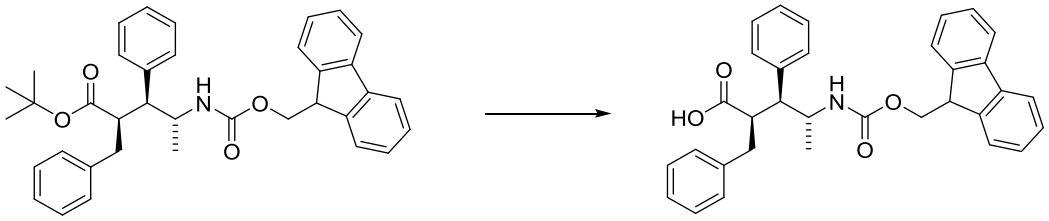
(2*R*,3*S*,4*R*)-*tert*-butyl 4-(9-fluorenylmethoxycarbonylamino)-2-benzyl-3-phenyl-pentanoate:



(2*R*,3*S*,4*R*)-*tert*-Butyl 4-amino-2-benzyl-3-phenylpentanoate (50.0 mg, 147 mmol, 1.0 eq) was suspended in 1 mL of 1:1 dioxane/water. To that mixture $NaHCO_3$ (37.1 mg, 256 mmol, 3.0 eq) and Fmoc-Cl (57.2 mg, 221 mmol, 1.5 eq) were added successively. The resulting mixture was stirred at room temperature for 20 min. Water (10 mL) was added and extracted with EtOAc (3x15 mL). The combined organic extracts were dried over Na_2SO_4 and the solvents removed *in vacuo* on a rotary evaporator. The resulting oil was purified by flash column chromatography on silica gel by eluting with pentane/EtOAc (10:1) to give 72.9 mg (88 %) of a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 – 7.75 (m, 2H), 7.62 – 7.55 (m, 2H), 7.47 – 7.38 (m, 4H), 7.33 (q, *J* = 7.4 Hz, 3H), 7.25 – 7.20 (m, 4H), 7.20 – 7.14 (m, 1H), 7.08 (d, *J* = 7.0 Hz, 2H), 4.54 – 4.40 (m, 1H), 4.38 – 4.28 (m, 1H), 4.24 (t, *J* = 6.8 Hz, 1H), 4.20 – 4.04 (m, 1H), 3.26 – 3.11 (m, 1H), 3.08 (dd, *J* = 11.4, 2.7 Hz, 1H), 2.63 (dd, *J* = 13.7, 3.6 Hz, 1H), 2.54 (dd, *J* = 13.7, 10.5 Hz, 1H), 1.28 (s, 9H), 1.06 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.4, 155.6, 144.2, 144.1, 141.5, 141.5, 139.1, 137.8, 129.6, 129.3, 129.0, 128.2, 127.8, 127.8, 127.5, 127.2, 126.3, 125.3, 125.1, 120.1, 120.1, 81.2, 66.5, 53.1, 49.5, 48.3, 47.5, 37.8, 27.9, 20.8; HRMS (ESI): *m/z* calcd for $C_{37}H_{40}NO_4$: 562.2952 [*M*+H^+]; found: 562.2953.

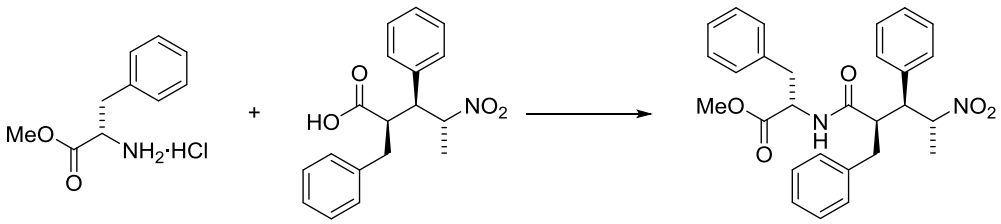
(2R,3S,4R)-4-(9-fluorenylmethoxycarbonylamino)-2-benzyl-3-phenylpentanoic acid (8):



The fully protected amino acid (68.0 mg, 121 mmol, 1.0 eq) was dissolved in a mixture of 2 mL CH_2Cl_2 and 1 mL of trifluoroacetic acid and stirred at room temperature for 15 min. All volatiles were removed by evaporation and the resulting residue treated with pentane. 59.3 mg (96 %) of a colorless solid resulted.

^{1}H NMR (400 MHz, CD_3CN): δ 8.02 – 7.90 (m, 2H), 7.80 – 7.68 (m, 2H), 7.59 – 7.38 (m, 7H), 7.38 – 7.21 (m, 5H), 7.13 (t, *J* = 17.3 Hz, 2H), 5.39 (d, *J* = 8.0 Hz, 1H), 4.59 (dd, *J* = 10.5, 6.6 Hz, 1H), 4.44 – 4.35 (m, 1H), 4.35 – 4.28 (m, 1H), 4.17 (s, 1H), 3.31 – 3.18 (m, 1H), 3.18 – 3.06 (m, 1H), 2.65 (d, *J* = 6.4 Hz, 2H), 1.14 (d, *J* = 6.3 Hz, 3H) ; ^{13}C NMR (100 MHz, CD_3OD): δ 178.2, 158.1, 145.4, 145.3, 142.6, 142.6, 140.5, 139.6, 130.9, 130.0, 129.4, 129.2, 128.7, 128.2, 128.1, 127.3, 126.4, 126.1, 120.9, 120.8, 67.4, 54.2, 50.7, 49.8, 48.6, 37.4, 20.2; MS (ESI): *m/z* calcd for $C_{33}H_{31}NNaO_4$: 528 [*M*+Na^+]; found: 528; MS (ESI): *m*/*z*: 528 [*M* + Na^+]; elemental analysis calcd (%) for $C_{33}H_{31}NO_4$: C 78.39, H 6.18, N 2.77; found: C 78.20, H 6.04, N 2.75.

Methyl ((2*R*,3*S*,4*R*)-2-benzyl-4-nitro-3-phenylpentanoyl)-L-phenylalaninate:



(2*R*,3*S*,4*R*)-2-Benzyl-4-nitro-3-phenylpentanoic acid (97.8 mg, 312 mmol, 1.0 eq) was dissolved in CH_2Cl_2 (1 mL). EDC·HCl (67.3 mg, 375 mmol, 1.2 eq) and *i*Pr_2NEt (118 mL, 687 mmol, 2.2 eq) were added and the resulting solution was stirred at room temperature for 5 min. Then, L-phenylalanine methyl ester hydrochloride (67.3 mg, 312 mmol, 1.0 eq) was

added and the resulting mixture stirred at room temperature for 18 h. CH_2Cl_2 (5 mL) was added and the resulting organic layer was washed with 5% $NaHCO_3$ (2 x 3 mL) and 1M HCl (3 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The resulting colorless oil was purified by flash column chromatography on silica gel by eluting with pentane/EtOAc (2:1) to give 31.3 mg (22 %) of a colorless solid. Crystals suitable for x-ray analysis were obtained by slow evaporation of a hexane solution.

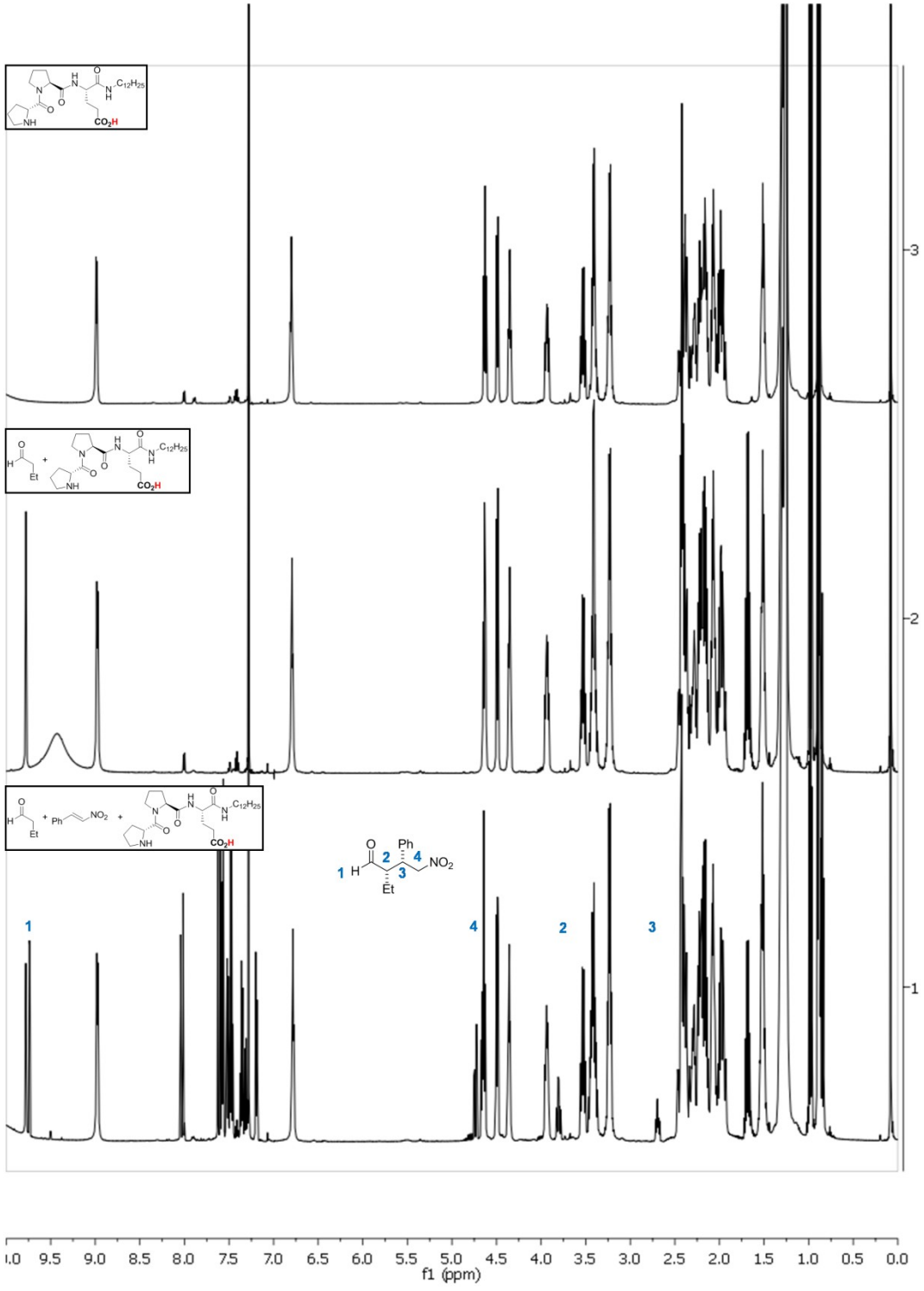
^{1}H NMR (250 MHz, $CDCl_3$): δ 7.38 – 7.28 (m, 3H), 7.25 – 7.10 (m, 8H), 7.05 – 6.99 (m, 2H), 6.99 – 6.92 (m, 2H), 6.08 (d, *J* = 9.0 Hz, 1H), 5.02 (ddd, *J* = 10.1, 9.0, 4.6 Hz, 1H), 3.79 (qd, *J* = 6.6, 2.1 Hz, 1H), 3.58 (s, 3H), 3.24 (dd, *J* = 14.1, 4.6 Hz, 1H), 3.14 (dd, *J* = 10.7, 3.1 Hz, 1H), 3.05 (dd, *J* = 11.5, 2.1 Hz, 1H), 2.77 (dd, *J* = 14.1, 10.1 Hz, 1H), 2.54 (dd, *J* = 13.2, 10.7 Hz, 1H), 2.32 (dd, *J* = 13.2, 3.1 Hz, 1H), 1.09 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9, 171.2, 139.0, 136.2, 134.7, 129.6, 129.1, 128.8, 128.4, 128.4, 128.4, 127.1, 126.4, 82.7, 52.9, 52.8, 52.4, 52.1, 38.8, 38.1, 17.3; HRMS (ESI): *m/z* calcd for $C_{28}H_{32}N_2O_5$: 475.227 [M+H^+]; found: 475.2224.

7.7 Identification of Reaction Intermediates

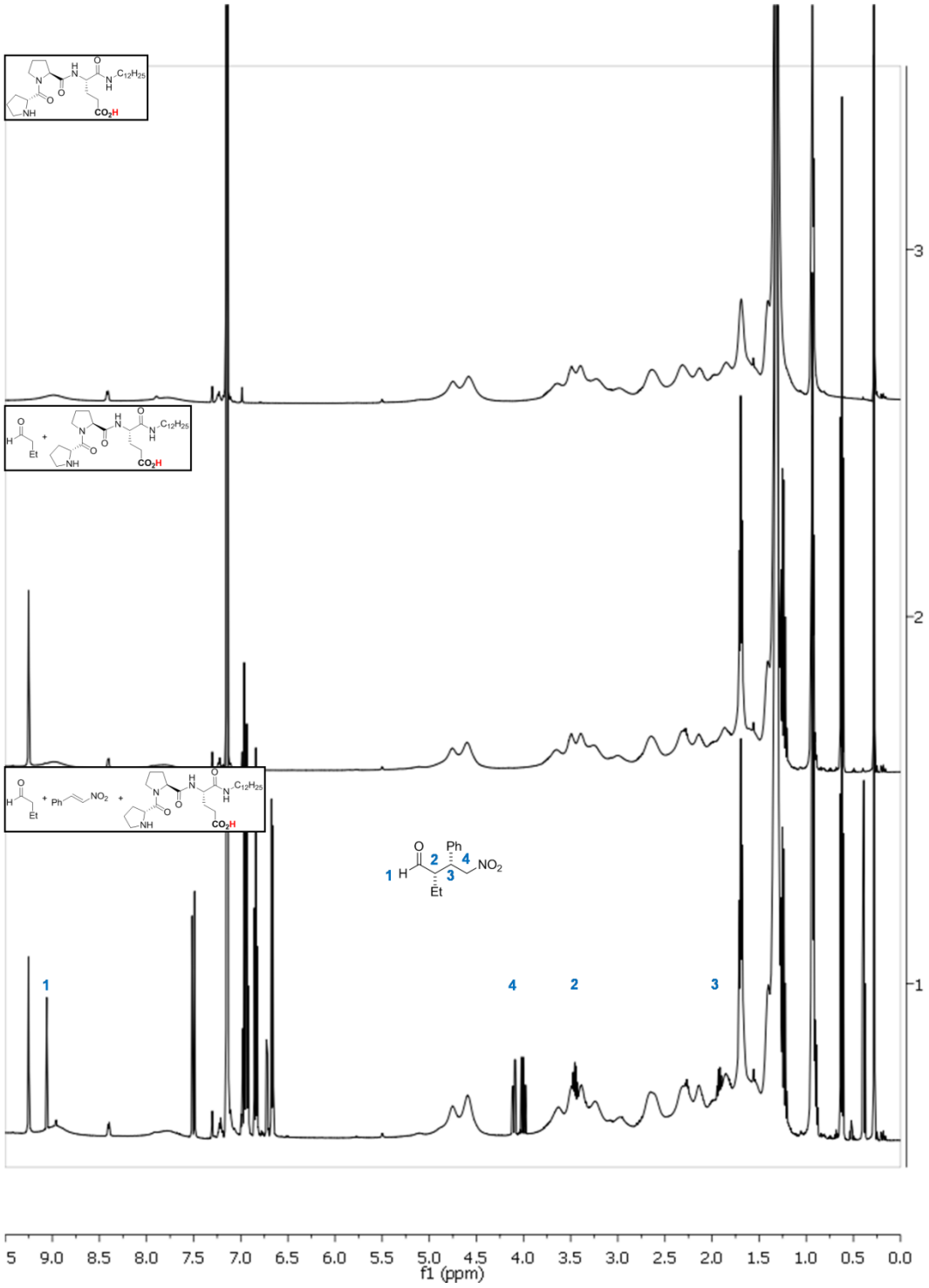
7.7.1 Comparison of H-D-Pro-Pro-Glu-$NHC_{12}H_{25}$ with H-D-Pro-Pro-Glu(OMe)-$NHC_{12}H_{25}$ Using 1H-NMR Spectroscopy

Experimental setup: To a solution of the peptide (20 μmol, 2.0 eq) in the respective solvent (0.5 mL) was added a solution of butanal (0.90 μL, 10 μmol, 1.0 eq) in the respective solvent (0.1 mL). After 5 min a ^{1}H NMR spectrum was recorded. Then *trans*-nitrostyrene (1.49 mg, 10 μmol, 1.0 eq) was added as a solution in the respective solvent and again a ^{1}H NMR spectrum was collected after 5 min.

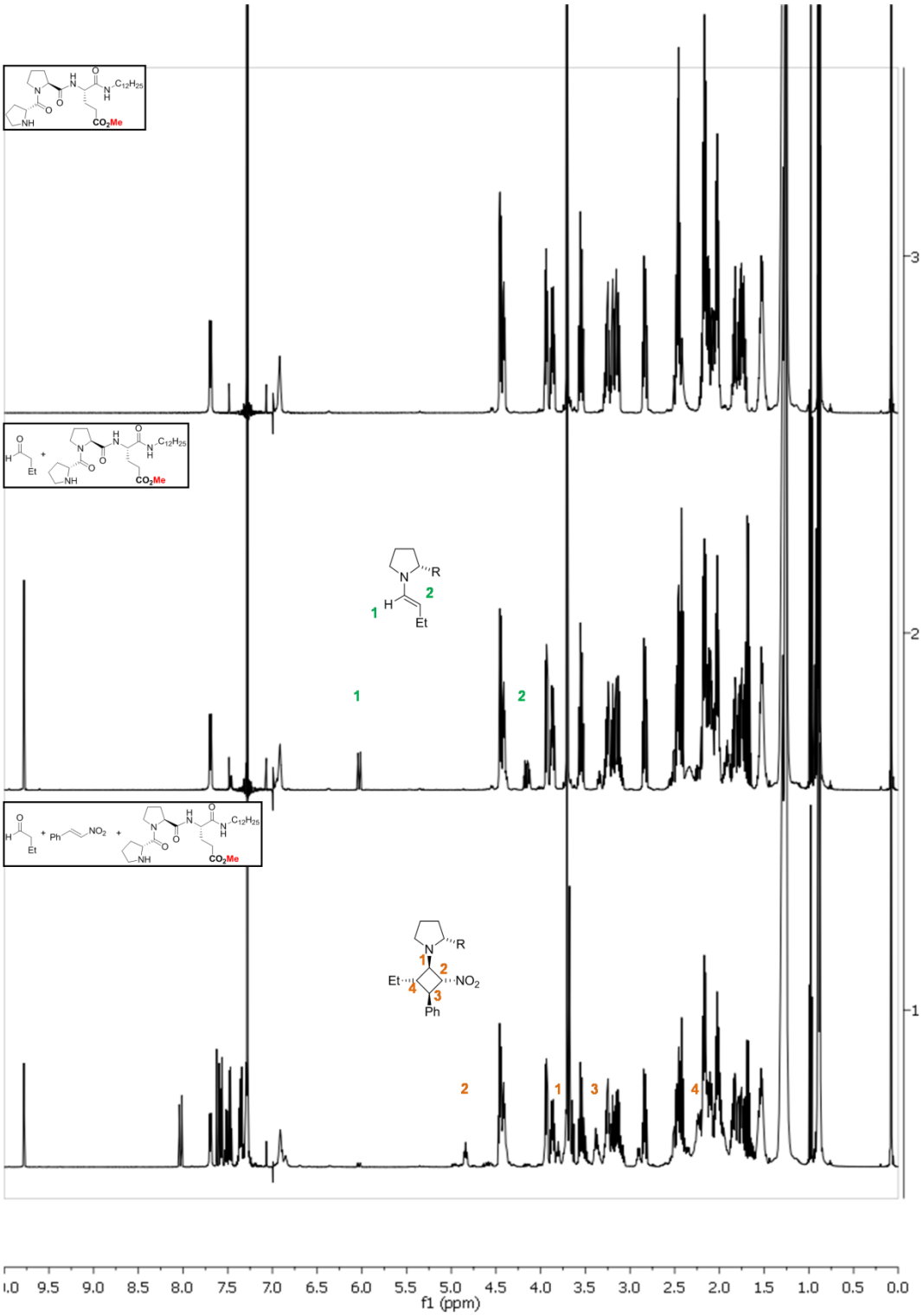
H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (1b) in $CDCl_3$



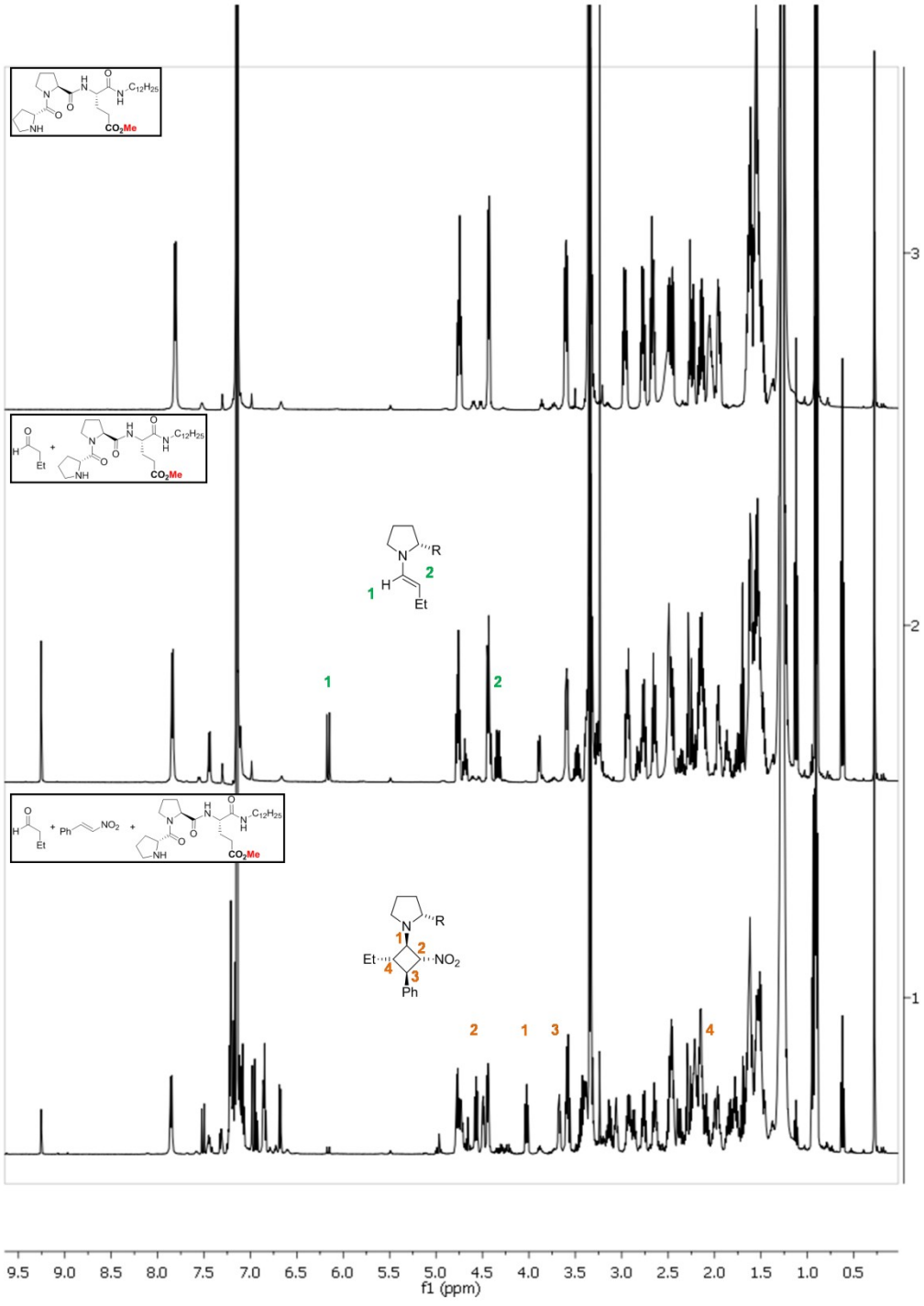
H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (1b) in C_6D_6



H-D-Pro-Pro-Glu(OMe)-NH-$C_{12}H_{25}$ (2b) in $CDCl_3$



H-D-Pro-Pro-Glu(OMe)-NH-$C_{12}H_{25}$ (2b) in C_6D_6

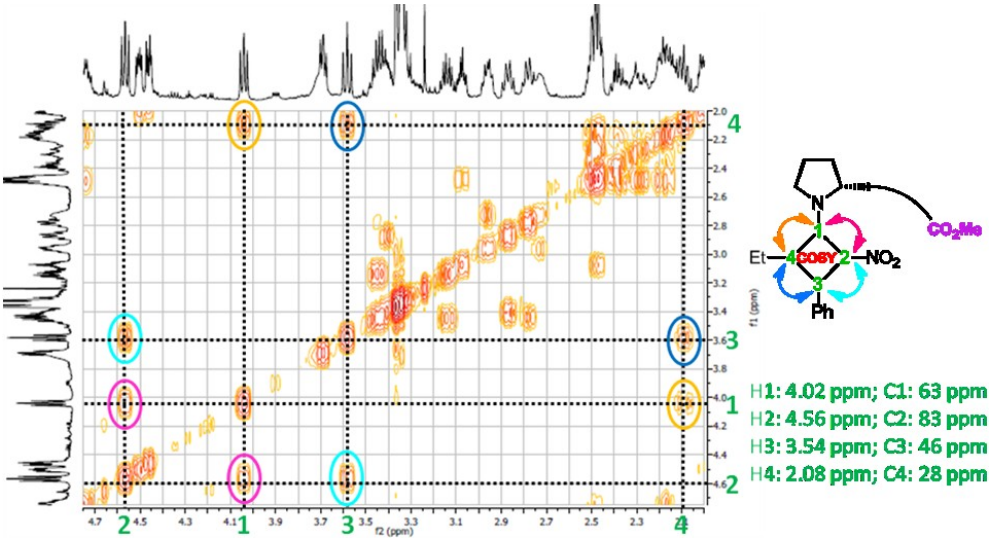


2D NMR spectroscopic analysis cyclobutane 2b-C:

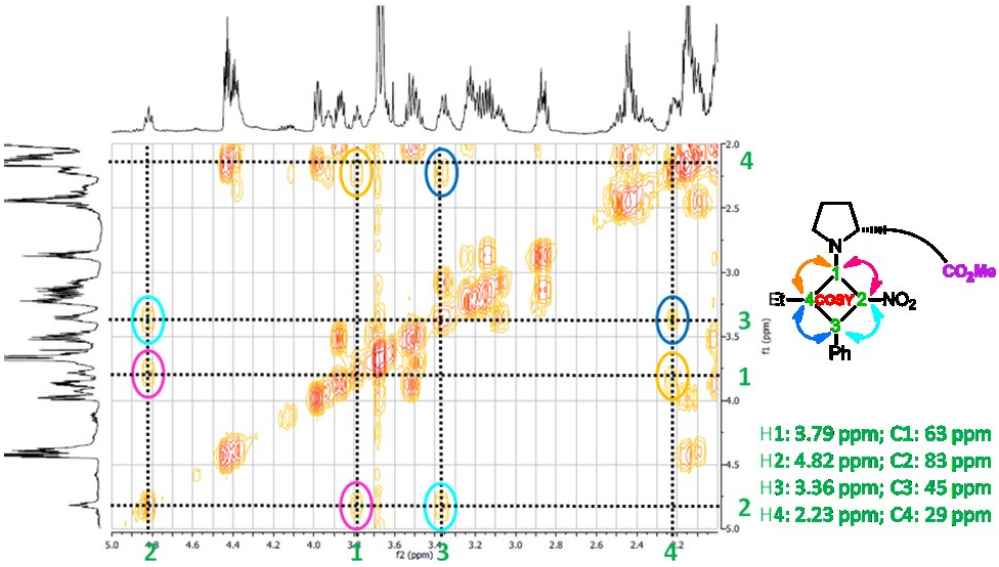
Conditions:

- 20 µmol of H-D-Pro-Pro-Glu(OMe)-$C_{12}H_{25}$ (2.0 eq)
- Freshly activated 4Å molecular sieves
- 0.6 mL of solvent
- 10 µmol of butanal (1.0 eq)
- 10 µmol of nitrostyrene (1.0 eq)
- Room temperature, 5 min

Relevant excerpt in C_6D_6

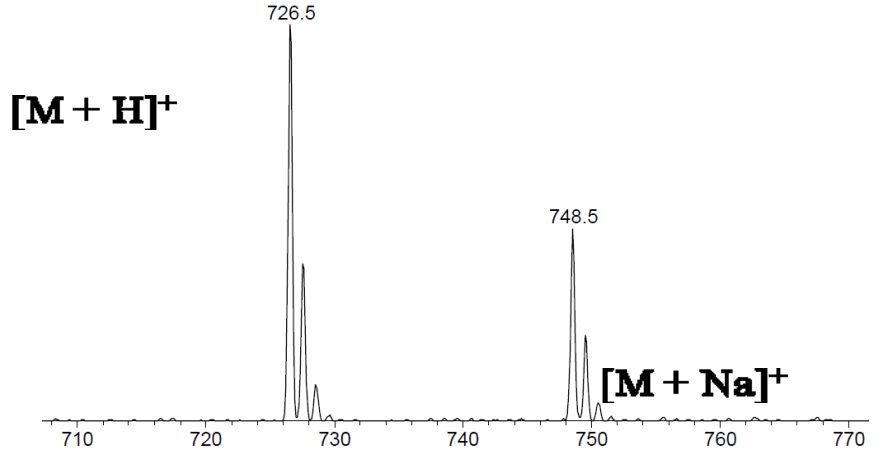


Relevant excerpt in $CDCl_3$



Electron spray mass spectrometric analysis of NMR sample

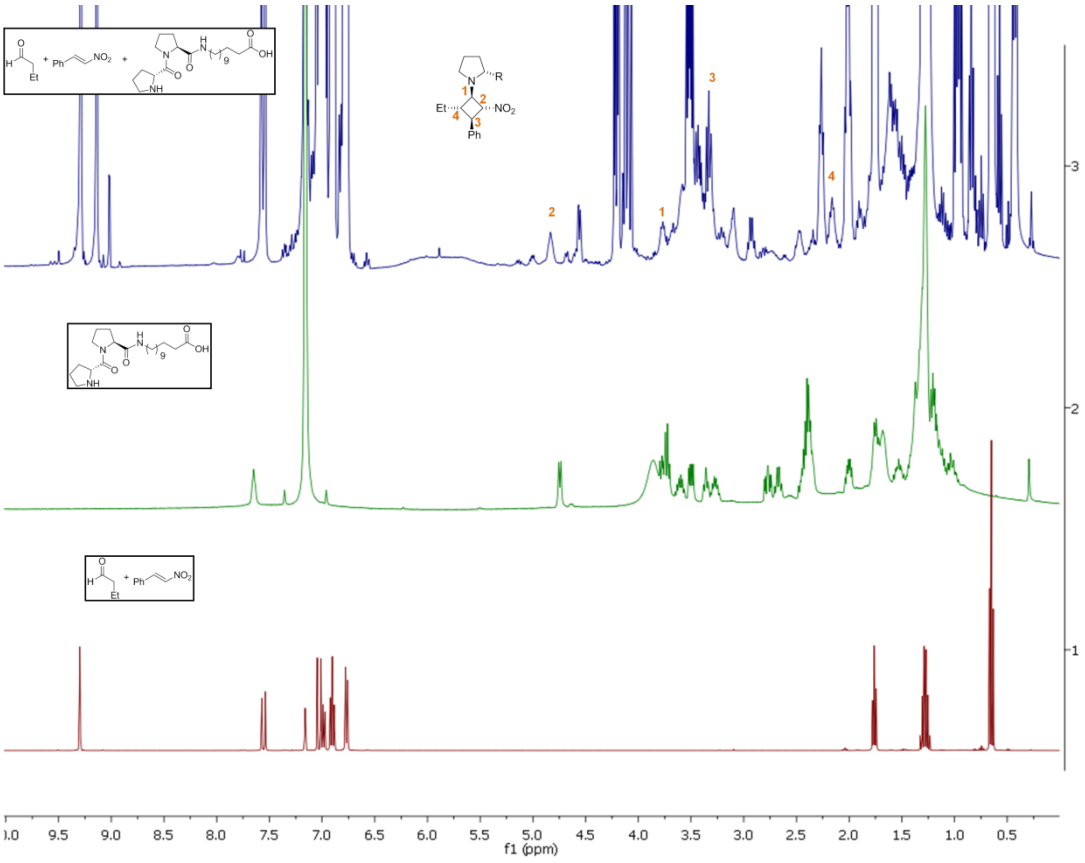
A small aliquot of the NMR sample in C_6D_6 was diluted with MeOH and injected into an ESI-MS spectrometer. In the resulting spectra the mass of a protonated cyclobutane intermediate as well as the corresponding sodium adduct are observed.

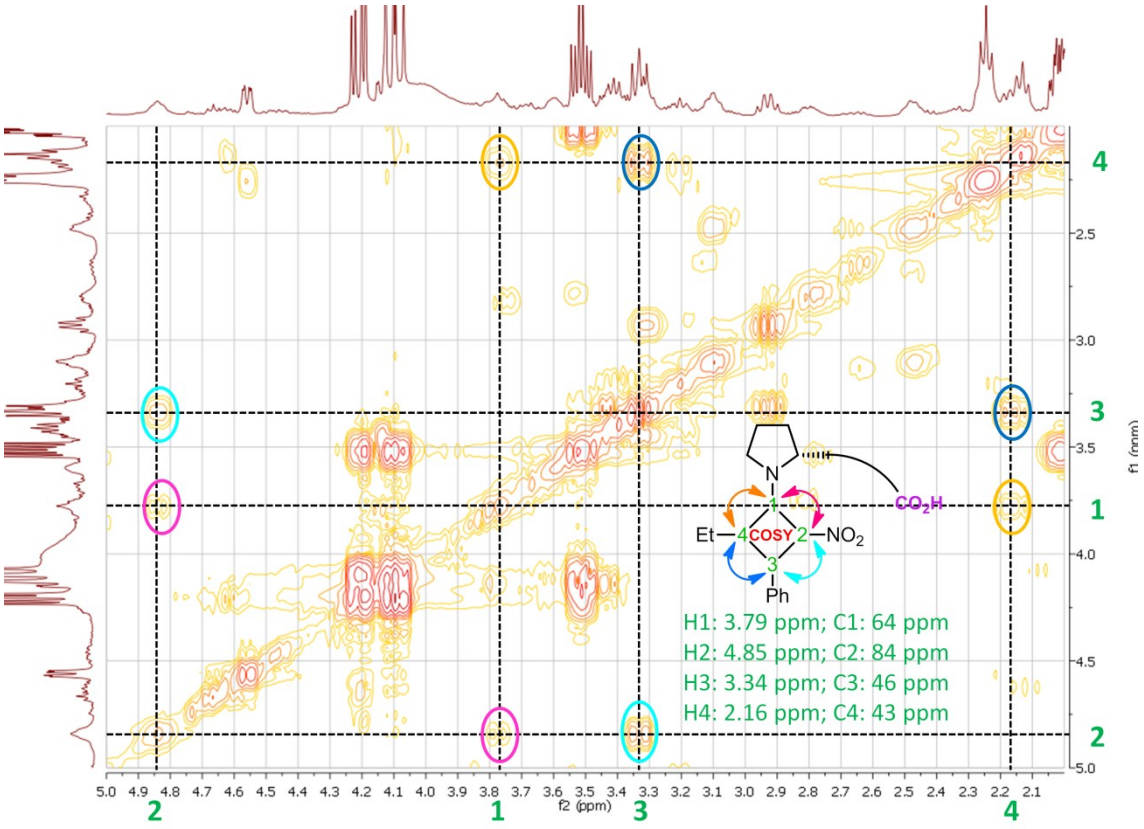


7.7.2 ^{1}H-NMR Spectroscopic Investigation on the Conjugate Addition Reaction in the Presence of H-D-Pro-Pro-Ada-OH (1g)

Conditions:

- 20 µmol of peptide H-D-Pro-Pro-Ada-OH (10 mol%)
- 0.6 mL of C_6D_6
- 200 µmol of butanal (1.0 eq)
- 200 µmol of nitrostyrene (1.0 eq)
- Room temperature, 60 min



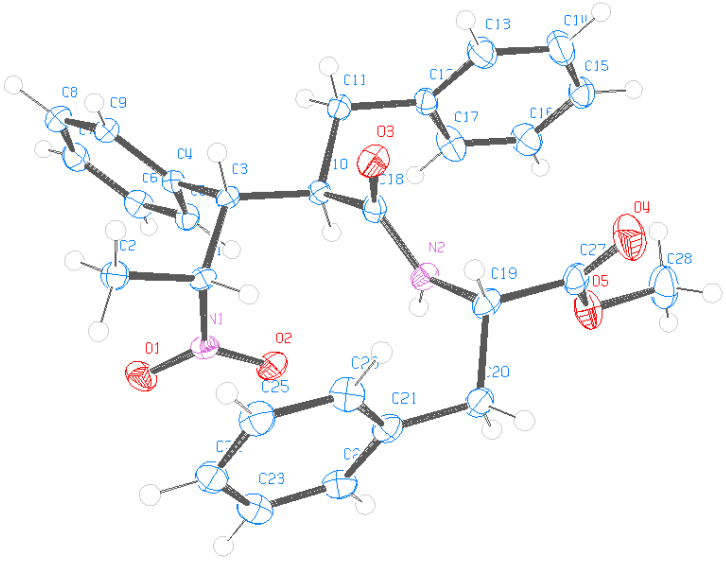


7.8 X-Ray Crystallography

Methyl ((2*R*,3*S*,4*R*)-2-benzyl-4-nitro-3-phenylpentanoyl)-L-phenylalaninate:

Crystal data for methyl ((2R,3S,4R)-2-benzyl-4-nitro-3-phenylpentanoyl)-L-phenylalaninate: formula $C_{28}H_{30}N_2O_5$, M = 474.56, F(000) = 252, colourless plate, size 0.020 · 0.130 · 0.290 mm^3, triclinic, space group P 1, Z = 1, a = 5.9193(4) Å, b = 9.9781(8) Å, c = 11.2851(9) Å, α = 73.794(5)°, β = 88.447(5)°, γ = 74.073(5)°, V = 614.60(8) $Å^3$, $D_{calc.}$ = 1.282 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} = 30.507°. Minimal/maximal transmission 0.99/1.00, μ = 0.088 mm^{-1}. The Apex2 suite has been used for datacollection and integration. From a total of 12551 reflections, 3729 were independent (merging r = 0.036). From these, 2886 were considered as observed (I>2.0σ(I)) and were used to refine 316 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 = 0.0352 (observed data), wR = 0.0539 (all data), GOF = 1.1422. Minimal/maximal residual electron density = -0.20/0.23 e $Å^{-3}$. Chebychev polynomial 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 838662. 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].



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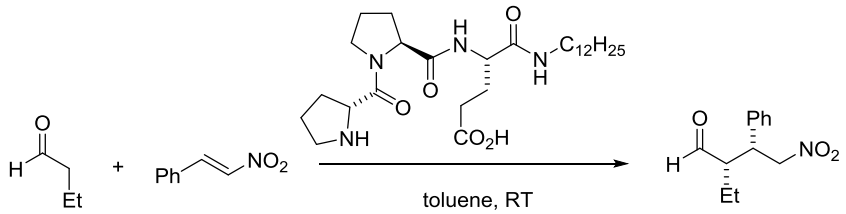
9 Appendix

9.1 Determination of Reaction Orders

9.1.1 Reaction of Butanal with Nitrostyrene in the Presence of H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (1b) in Toluene



Variation of catalyst concentration:

Experiments were carried out at constant initial concentrations of nitrostyrene (0.44 M) and butanal (0.44 M) varying the initial concentrations of H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (2.2 m M, 4.4 m M, 6.6 m M, 8.8 m M, 11.0 m M, 12.1 m M). To obtain initial rates, the first derivative of the product concentration vs. time curve was calculated at t = 15 min.

The product concentration vs. time curves, as well as the resulting log-log plot are shown in Figure 9-1: *first order dependence on the catalyst concentration is observed.*

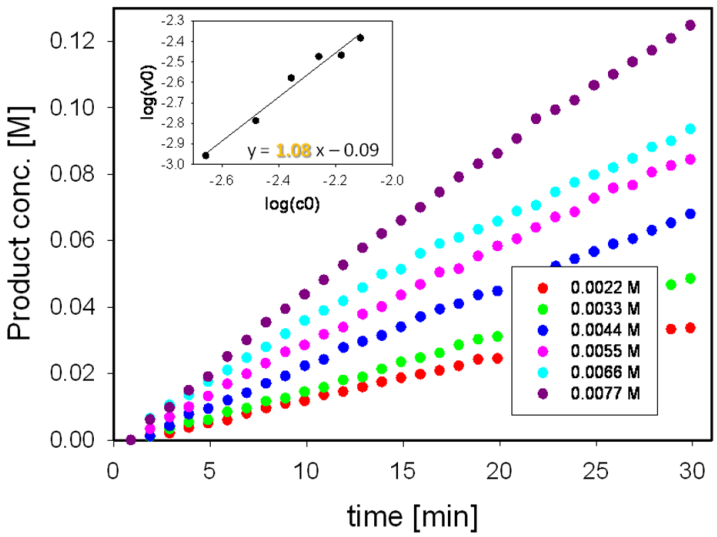


Figure 9-1 Product formation curves at a constant concentration of butanal and nitrostyrene at varying catalyst concentrations. Inlet: plot of log(initial rate) vs. log [catalyst].

Variation of nitrostyrene concentration:

Experiments were carried out at constant initial concentrations of H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (4.4 m M) and butanal (0.44 M) varying the initial concentrations of nitrostyrene (0.22 M, 0.44 M, 0.66 M, 0.88 M, 1.10 M, 1.21 M). To obtain initial rates, the first derivative of the product concentration vs. time curve was calculated at t = 15 min.

The product concentration vs. time curves, as well as the resulting log-log plot are shown in Figure 9-2: *a reaction order of 0.5 is observed*.

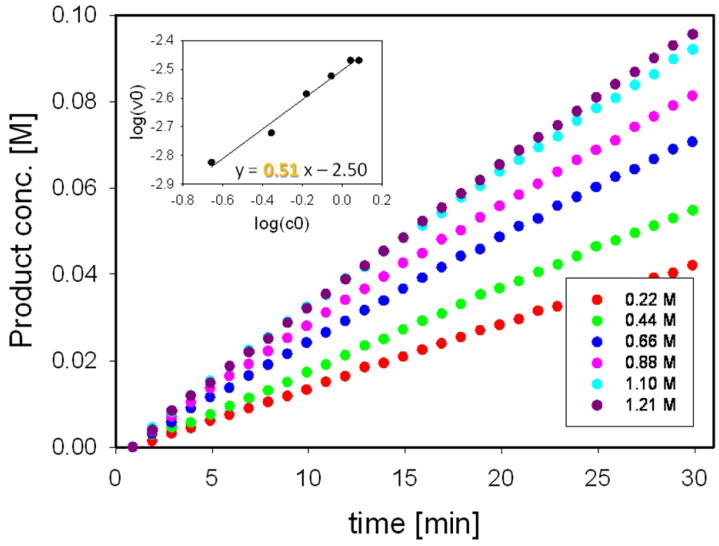


Figure 9-2 Product formation curves at a constant concentration of the peptidic catalyst and butanal at varying concentrations of nitrostyrene. Inlet: plot of log(initial rate) vs. log [nitrostyrene].

Variation of butanal concentration:

Experiments were carried out at constant initial concentrations of H-D-Pro-Pro-Glu-NH-$C_{12}H_{25}$ (4.4 mM) and nitrostyrene (0.44 M) varying the initial concentrations of butanal (0.22 M, 0.33 M 0.44 M, 0.66 M, 0.88 M, 1.10 M, 1.21 M). To obtain initial rates, the first derivative of the product concentration vs. time curve was calculated at t = 15 min.

The product concentration vs. time curves, as well as the resulting log-log plot are shown in Figure 9-3: *a zero order dependence on the aldehyde concentration is observed*.

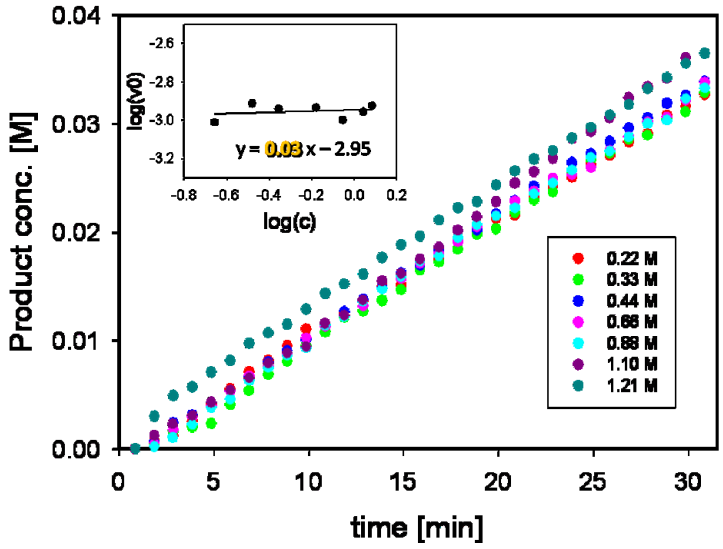
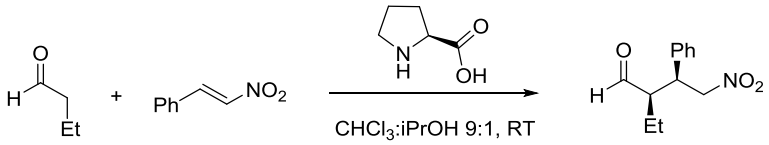


Figure 9-3 Product formation curves at a constant concentration of the peptidic catalyst and nitrostyrene at varying concentrations of butanal. Inlet: plot of log(initial rate) vs. log [butanal].

9.1.2 Reaction of Butanal with Nitrostyrene in the Presence of Proline



Variation of proline concentration:

Experiments were carried out at constant initial concentrations of nitrostyrene (0.44 M) and butanal (0.44 M) varying the initial concentrations of proline (13 mM, 22 mM, 35 mM, 44 mM, 66 mM). To obtain initial rates, the first derivative of the product concentration vs. time curve was calculated at t = 15 min.

The product concentration vs. time curves, as well as the resulting log-log plot are shown Figure 9-4: *0.9th order dependence on the catalyst concentration is observed*.

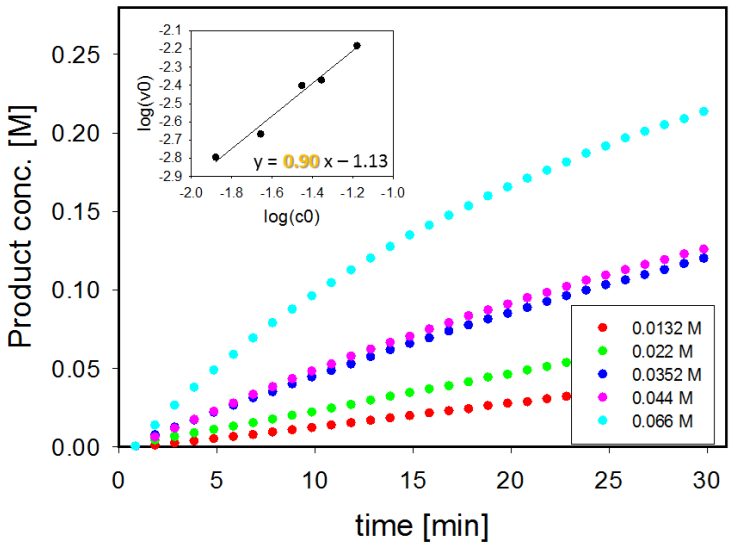


Figure 9-4 Product formation curves at a constant concentration of butanal and nitrostyrene at varying concentrations of proline. Inlet: plot of log(initial rate) vs. log [proline].

Variation of nitrostyrene concentration:

Experiments were carried out at constant initial concentrations of proline (44 mM) and butanal (0.44 M) varying the initial concentrations of nitrostyrene (0.22 M, 0.44 M, 0.88 M, 1.21 M). To obtain initial rates, the first derivative of the product concentration vs. time curve was calculated at t = 15 min.

The product concentration vs. time curves, as well as the resulting log-log plot are shown in Figure 9-5: *a reaction order of 0.7 is observed.*

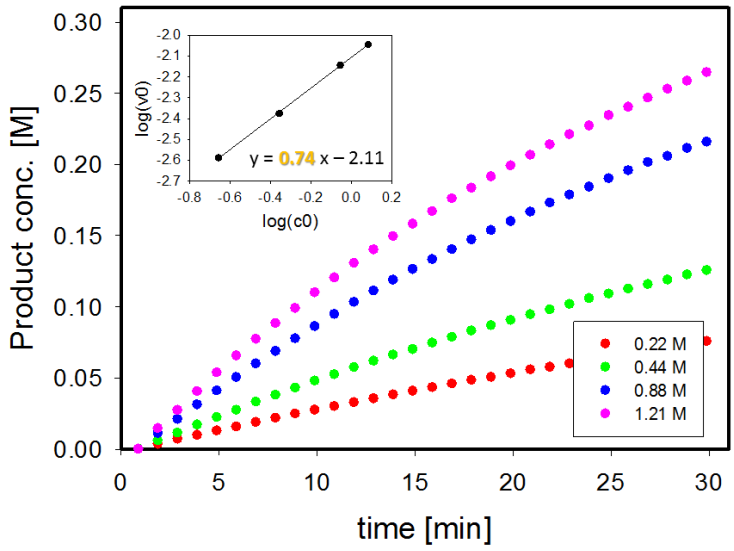


Figure 9-5 Product formation curves at a constant concentration of proline and butanal at varying concentrations of nitrostyrene. Inlet: plot of log(initial rate) vs. log [nitrostyrene].

Variation of butanal concentration:

Experiments were carried out at constant initial concentrations of proline (44 mM) and nitrostyrene (0.44 M) varying the initial concentrations of butanal (0.22 M, 0.44 M, 0.66 M, 0.88 M, 1.21 M). To obtain initial rates, the first derivative of the product concentration vs. time curve was calculated at t = 15 min.

The product concentration vs. time curves, as well as the resulting log-log plot are shown in Figure 9-6: *a reaction order of 0.45 is observed that becomes a near zero order dependence at higher aldehyde concentrations.*

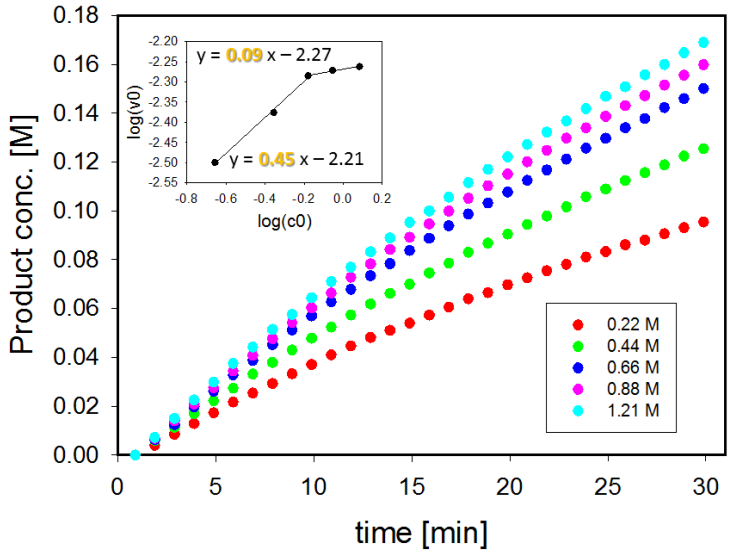
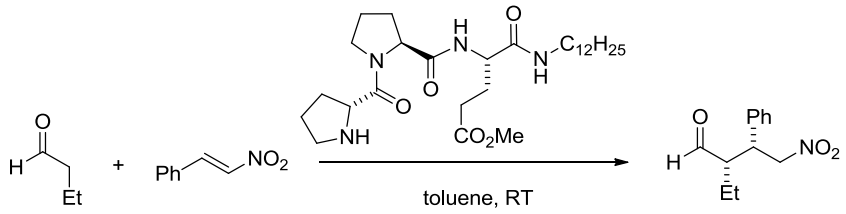


Figure 9-6 Product formation curves at a constant concentration of proline and nitrostyrene at varying concentrations of butanal. Inlet: plot of log(initial rate) vs. log [butanal].

9.1.3 Reaction of Butanal with Nitrostyrene in the Presence of H-D-Pro-Pro-Glu(OMe)-NH-$C_{12}H_{25}$ (2b) in Toluene



Two reactions using different initial concentrations of butanal (0.66 M and 0.88 M) and nitrostyrene (0.44 M and 0.66 M) at a constant catalyst concentration (22 mM) show identical reaction progress confirming a zero order dependence on both substrates.

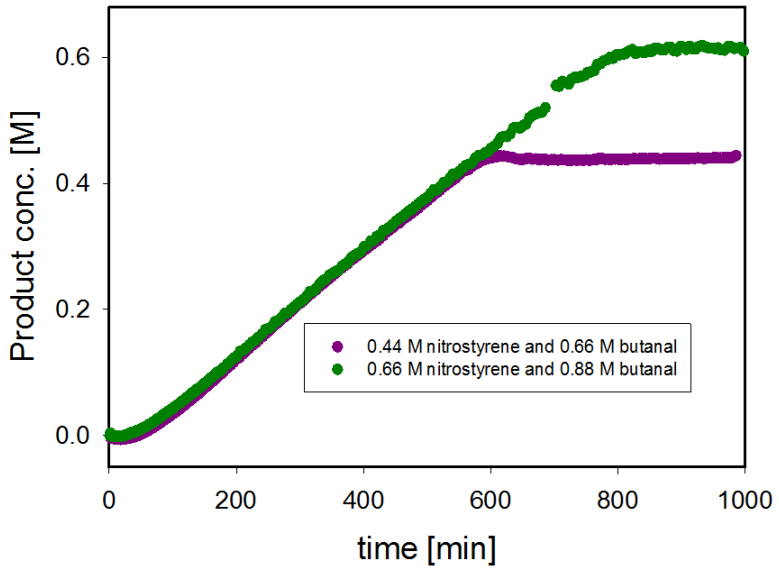


Figure 9-7 Product formation curves in the presence of methyl ester peptide **2b** using different initial concentrations of butanal and nitrostyrene.

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Zivilstand	ledig

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