Bioinspired Chemistry with Proline-rich Peptides

Ischia Advanced School of Organic Chemistry

Lacco Ameno, Ischia, September 27th 2016

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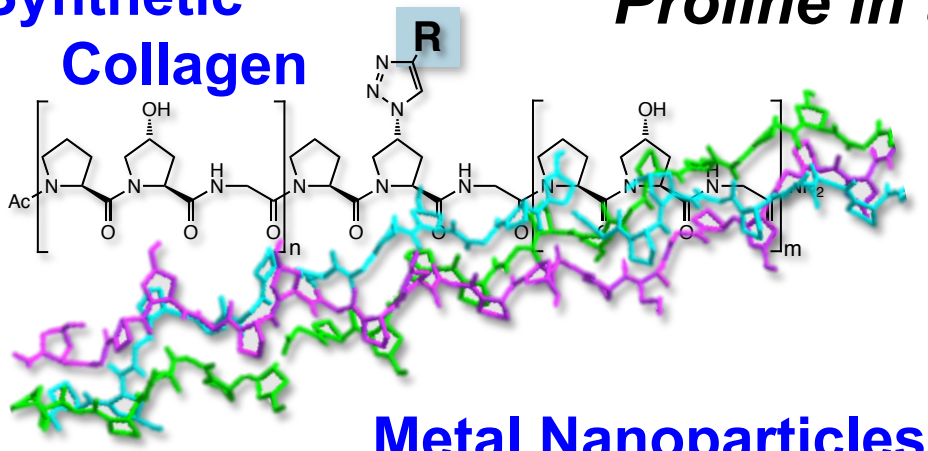
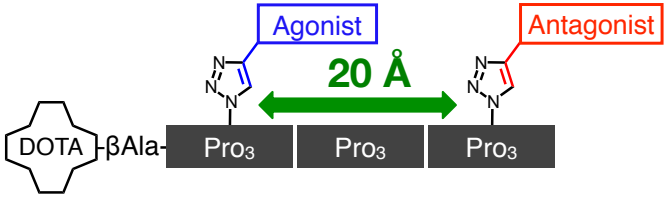
Laboratorium für Organische Chemie
ETH Zürich, Schweiz

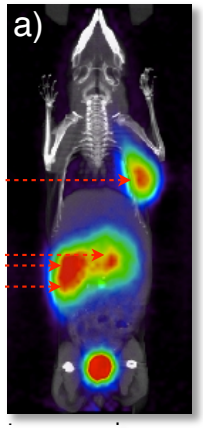
Proline in the Wennemers Lab

Synthetic Collagen

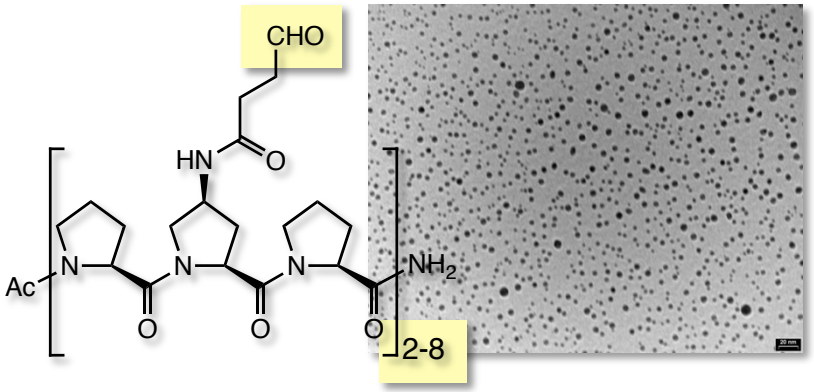


Tumor Targeting

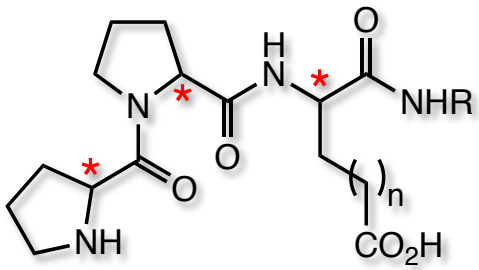




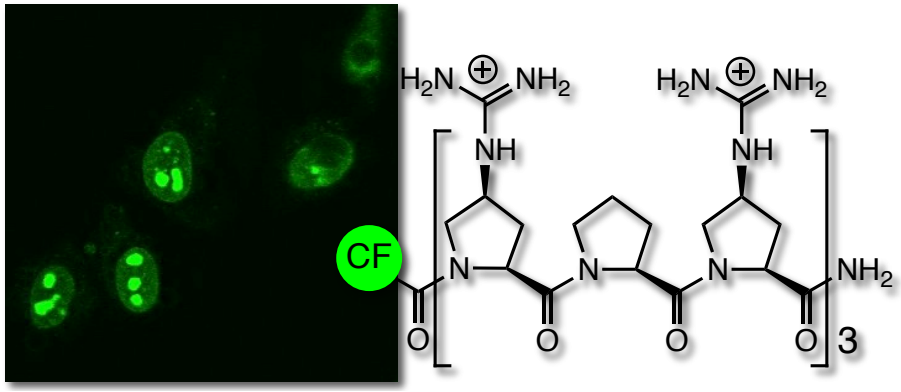
Metal Nanoparticles



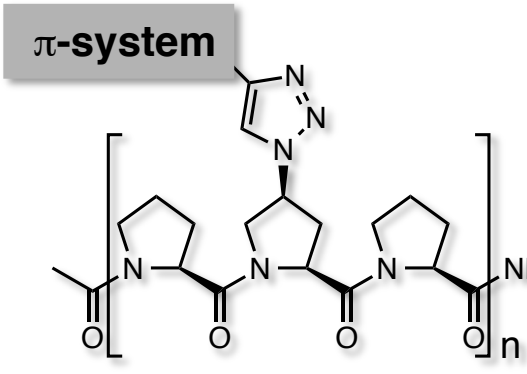
Asymmetric Catalysis

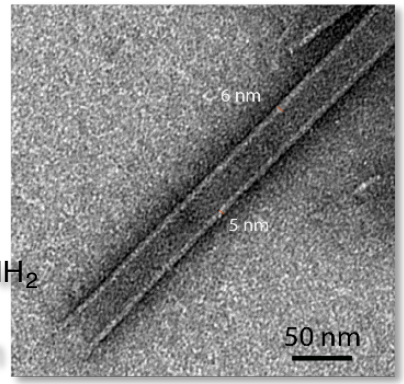


Cell-Penetration



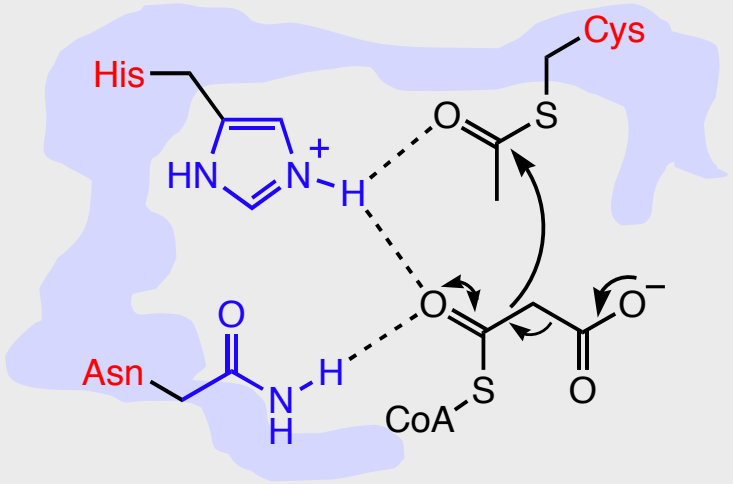
Supramolecular Assemblies



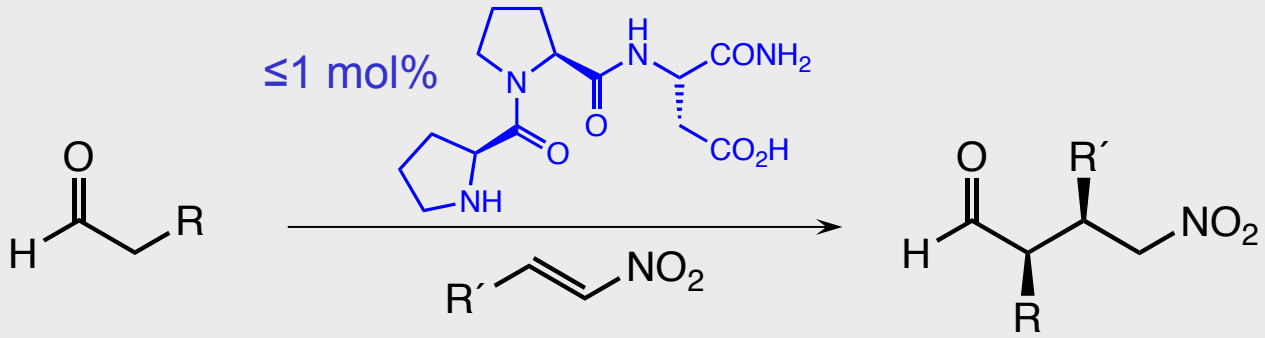


Bioinspired Catalysis

Catalysis Inspired by Polyketide Synthases

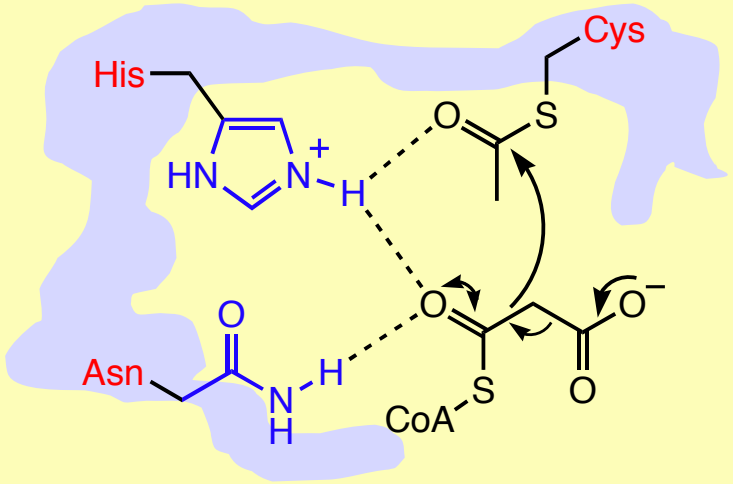


Catalytically Active Peptides

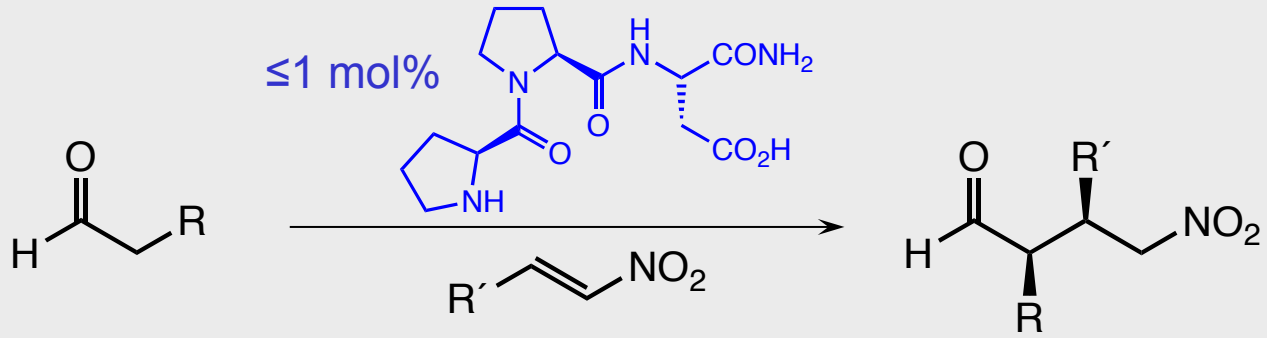


Bioinspired Catalysis

Catalysis Inspired by Polyketide Synthases

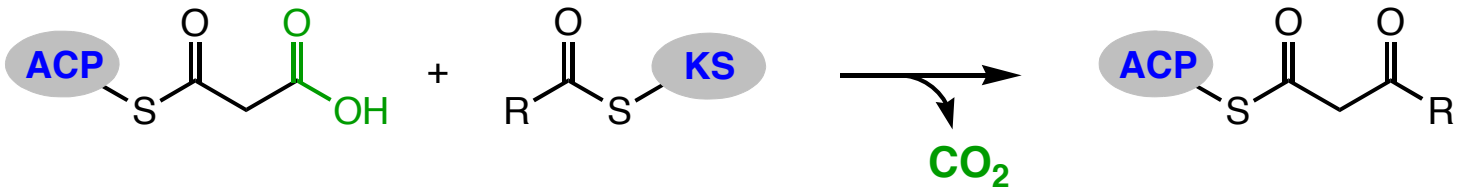


Catalytically Active Peptides

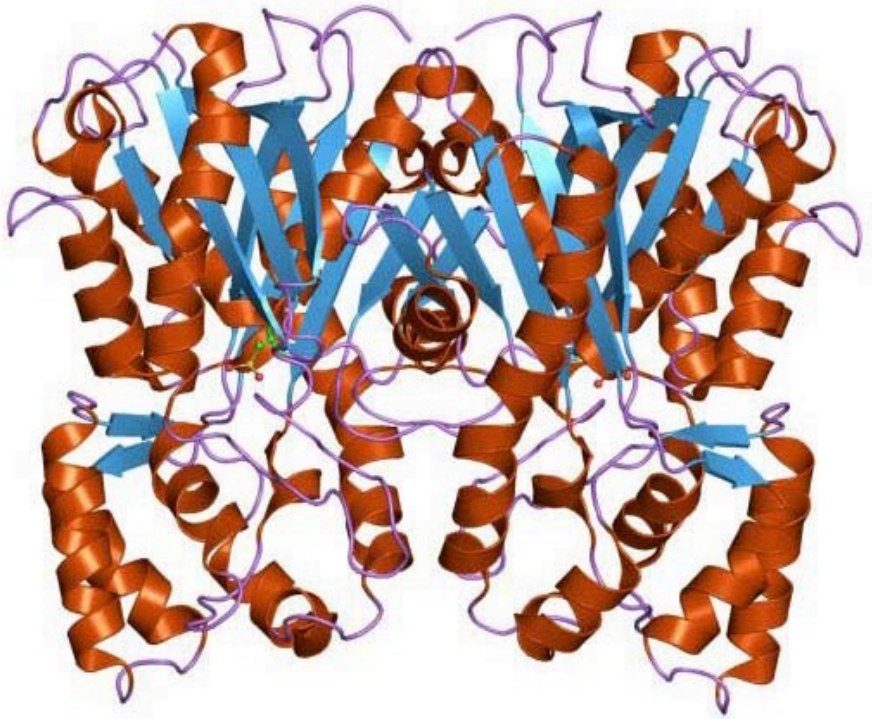


Catalysis Inspired by Polyketide Synthases

Polyketide and fatty acid biosynthesis



ACP = acyl carrier protein **KS** = ketosynthase



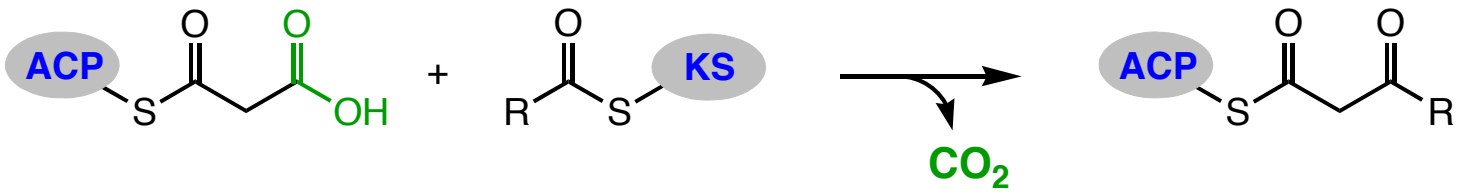
Type III Polyketide Synthase

40 – 45 kDa

S. Moore, J.P. Noel et al.,
J. Biol. Chem. **2004**, *43,* 45162.

Catalysis Inspired by Polyketide Synthases

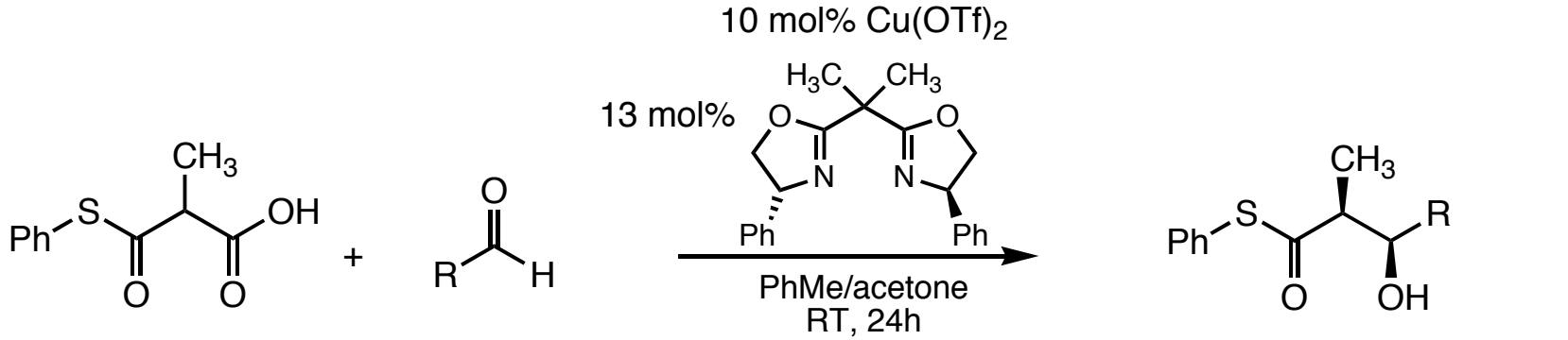
Polyketide and fatty acid biosynthesis



ACP = acyl carrier protein **KS** = ketosynthase

Synthetic, metal-catalyzed variants

Knoevenagel and Claisen Condensations: Kobuke (1978), Matile (2000), Thomas (2006)
Aldol reaction: Cozzi (2004)

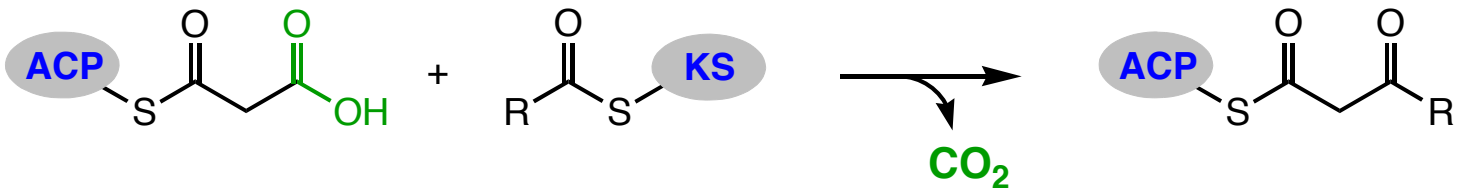


48-83% yield, up to 93% ee

M. D. Shair and coworkers:
J. Am. Chem. Soc. **2007**, *129*, 1032; *J. Am. Chem. Soc.* **2005**, *127*, 7284; *J. Am. Chem. Soc.* **2003**, *125*, 2852.

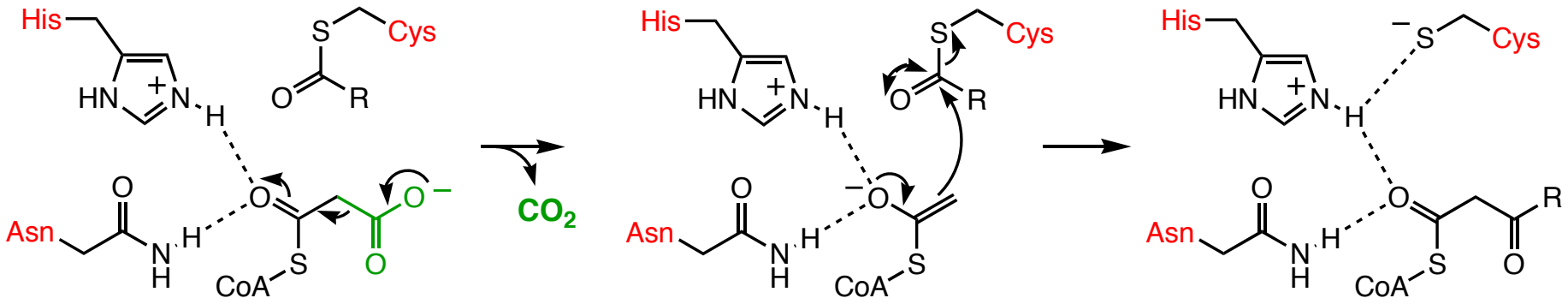
Active Site of Polyketide Synthases

polyketide and fatty acid biosynthesis



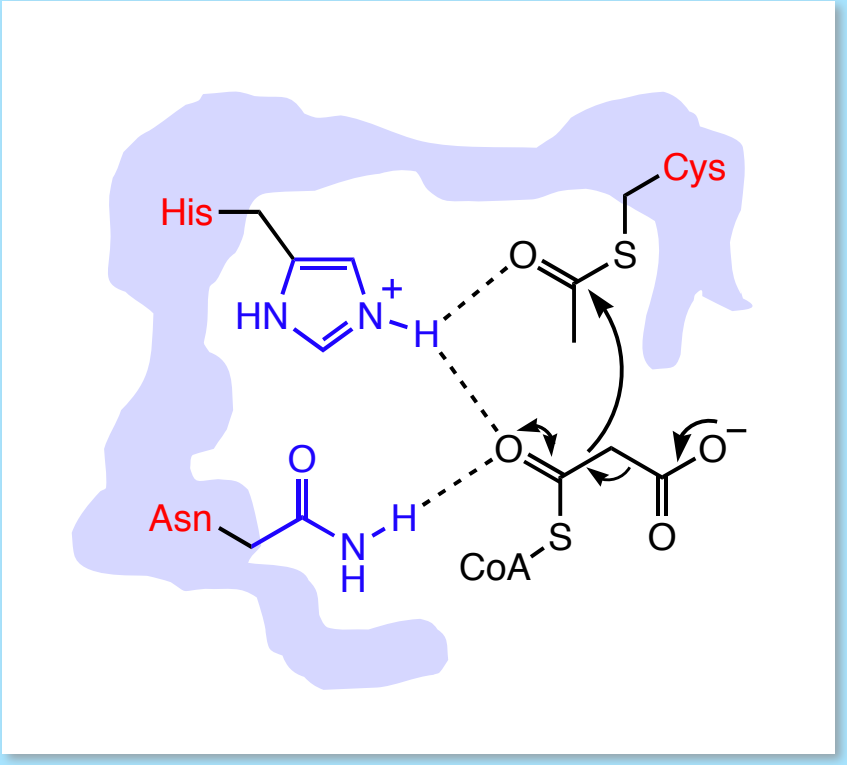
ACP = acyl carrier protein **KS** = ketosynthase

In the active site: catalytic triad Cys-His-Asn/His

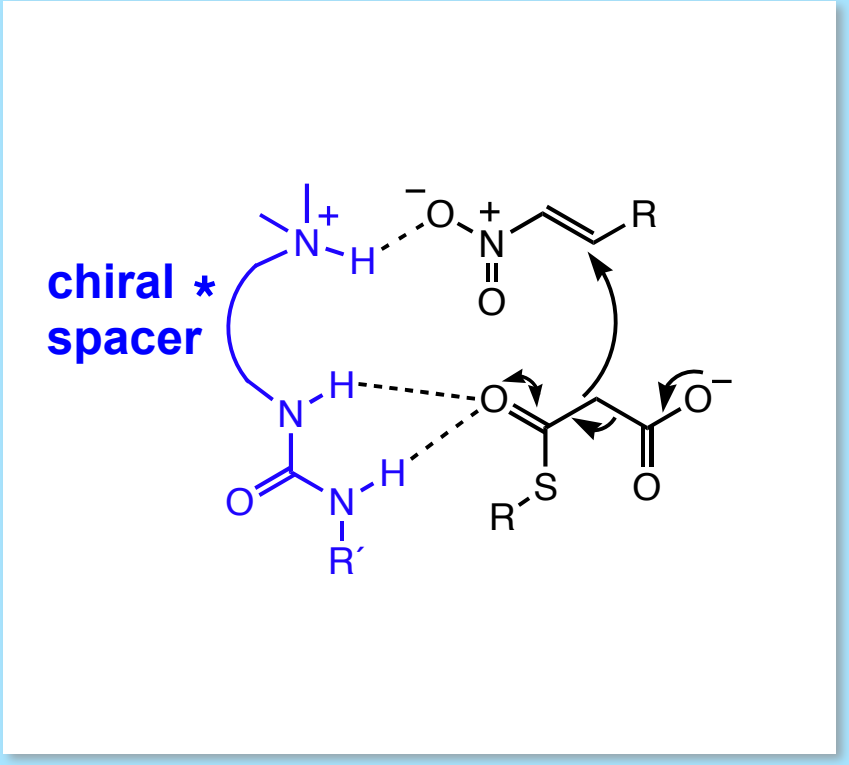


from: B.S. Moore, J.P. Noel et al., *J. Biol. Chem*. **2004**, *43,* 45162.

A Polyketide Synthase Inspired Organocatalyst?

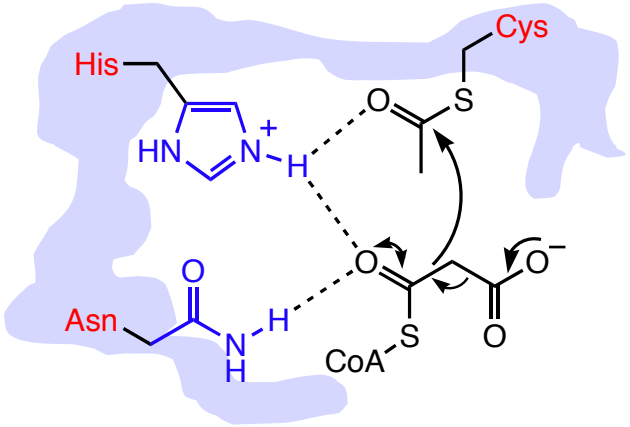


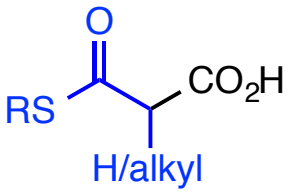
active site of polyketide synthases



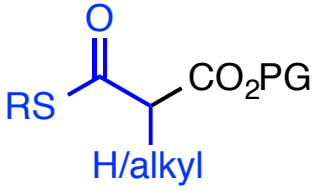
synthetic catalyst ?

Thioesterenolate Chemistry Inspired by Polyketide Synthases



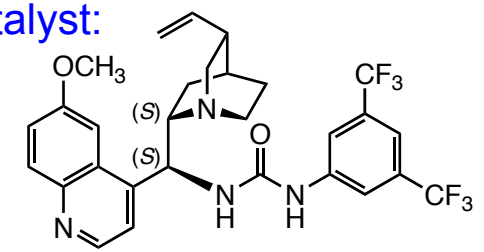


MAHT



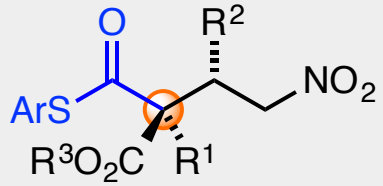
MTM

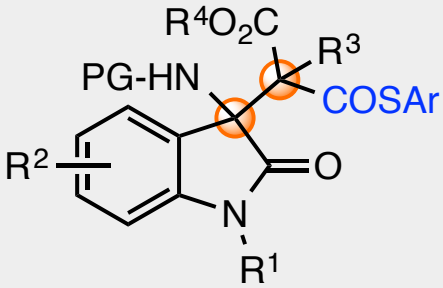
Catalyst:

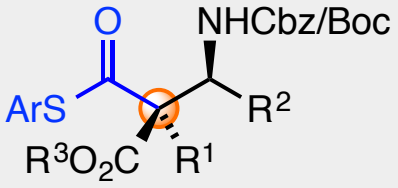


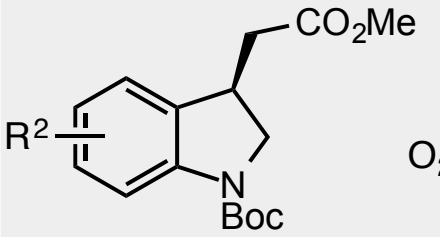
Connon, Dixon, Soos, Wu **2005**

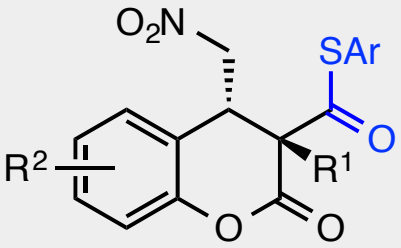
Examples:

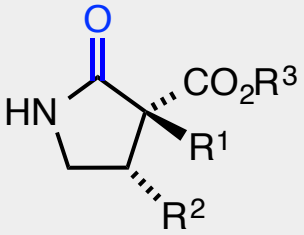




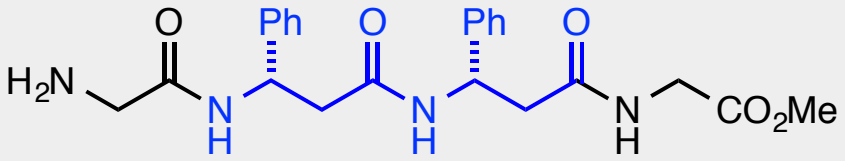








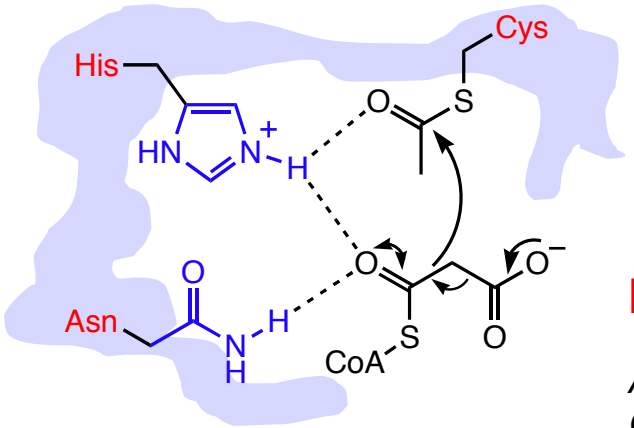
coupling reagent free peptide synthesis

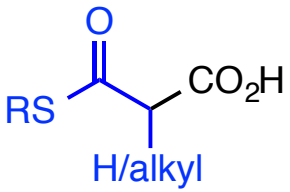


with Jana Lubkoll, Paolo Clerici, Yukihiro Arakawa, Andrej Kolarovic, Sven Fritz, Oliver Engl

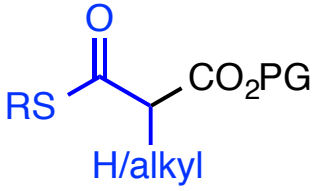
ACIE **2007**, 6841. *OBC* **2012**, 110. *JOC* **2014**, 3937. *ACIE* **2014**, 8779. *OL* **2014**, 4236. *OL* **2014**, 5454. *ACIE* **2015**, 8193.

Thioesterenolate Chemistry Inspired by Polyketide Synthases



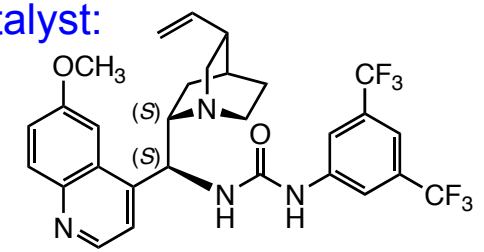


MAHT



MTM

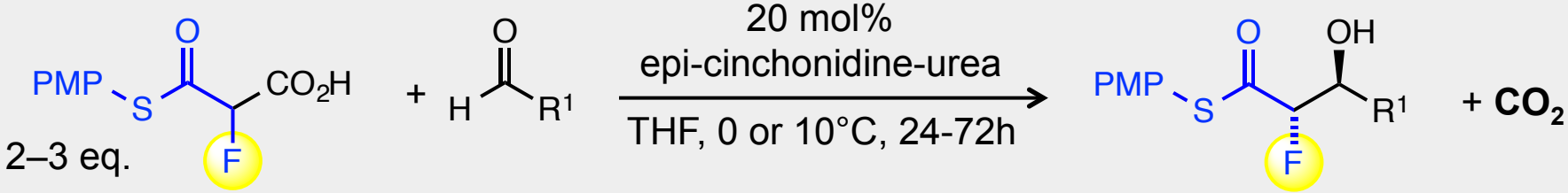
Catalyst:



Connon, Dixon, Soos, Wu **2005**

ACIE **2007**, 6841. *OBC* **2012**, 110. *JOC* **2014**, 3937. *ACIE* **2014**, 8779.
OL **2014**, 4236. *OL* **2014**, 5454. *ACIE* **2015**, 8193.

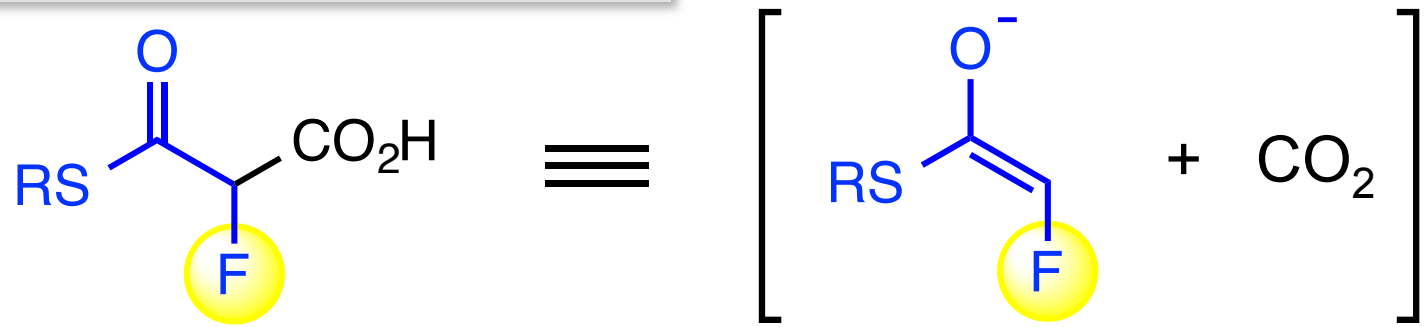
Enantioselective aldol reactions with fluoroacetate



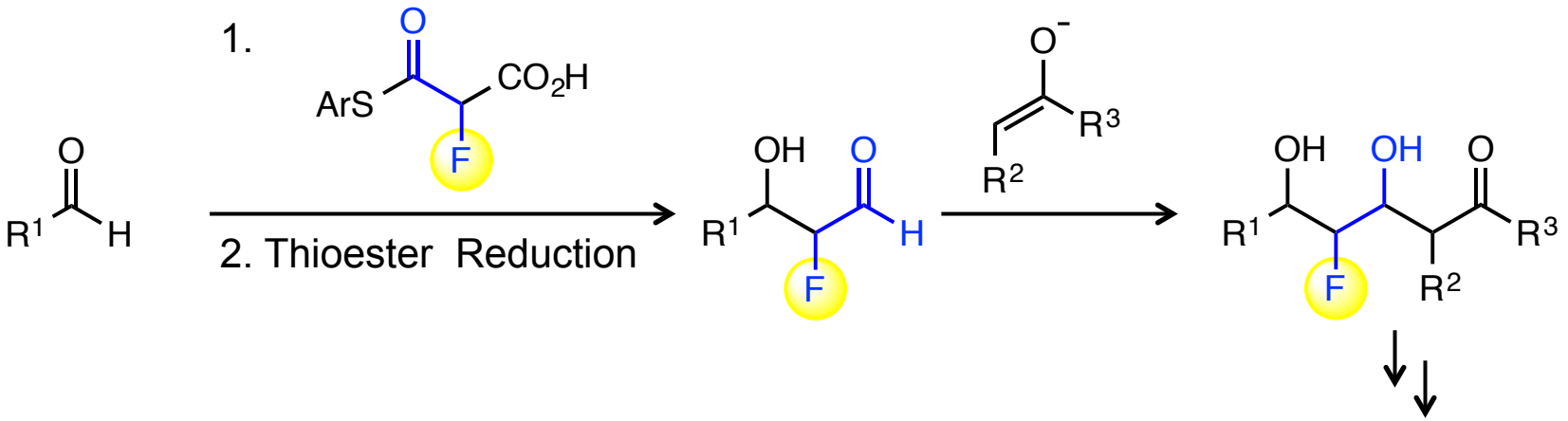
54–99% yield
d.r. 3:1–17:1, 65–99% ee

J. Saadi, H.W. *Nat. Chem.* **2016**, *8*, 276.

Synthon for Fluoroacetate



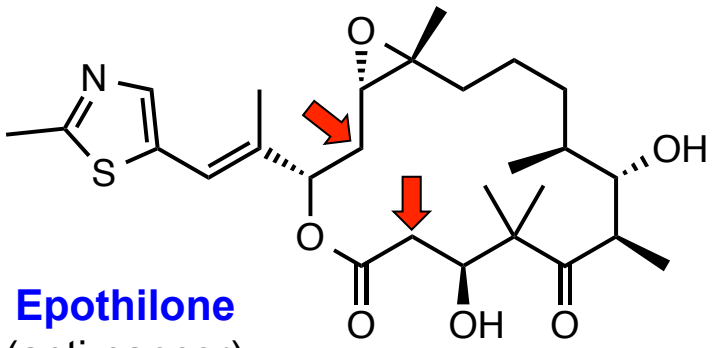
Synthon for Fluoroacetaldehyde



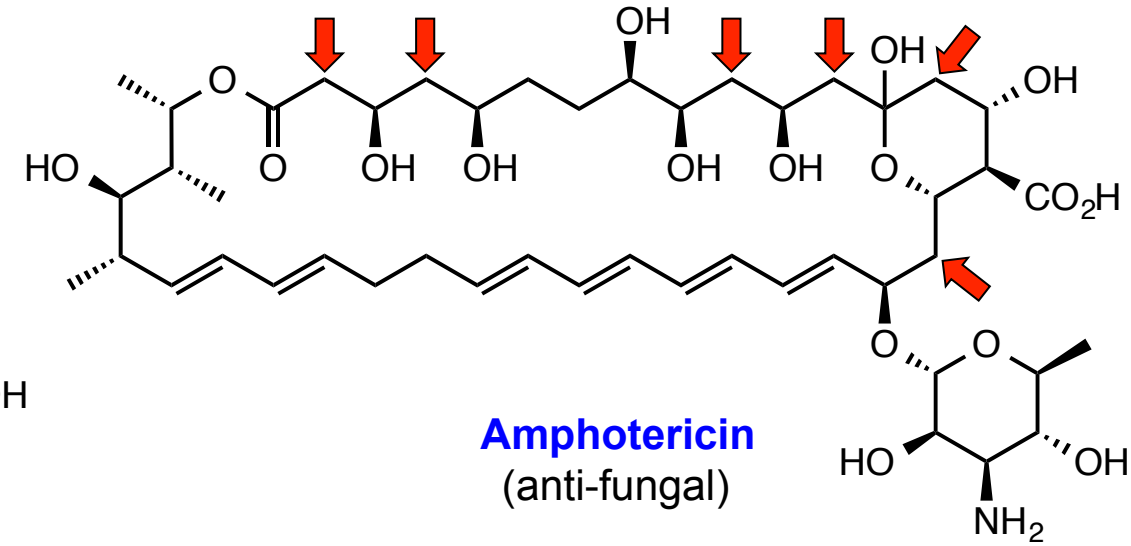
Fluorinated Polyketides

Acetate Derived Compounds

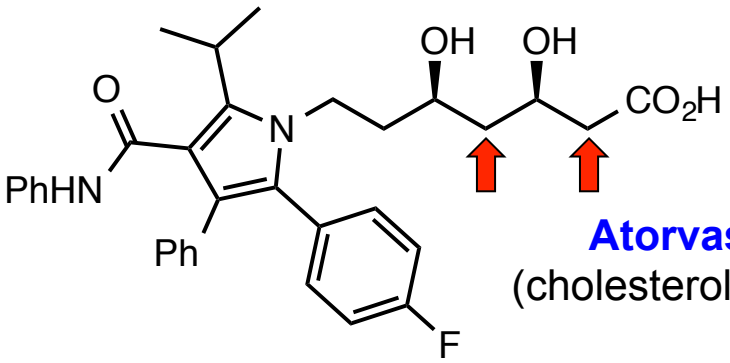
...many more examples



Epothilone
(anti-cancer)

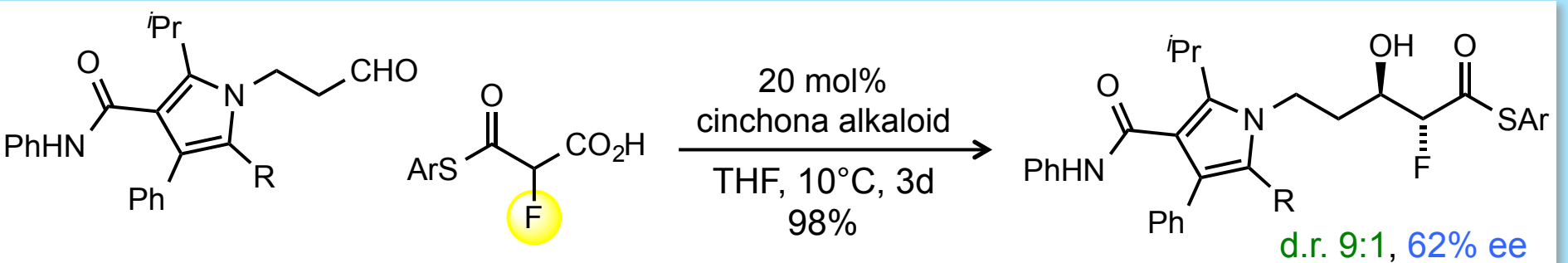


Amphotericin
(anti-fungal)



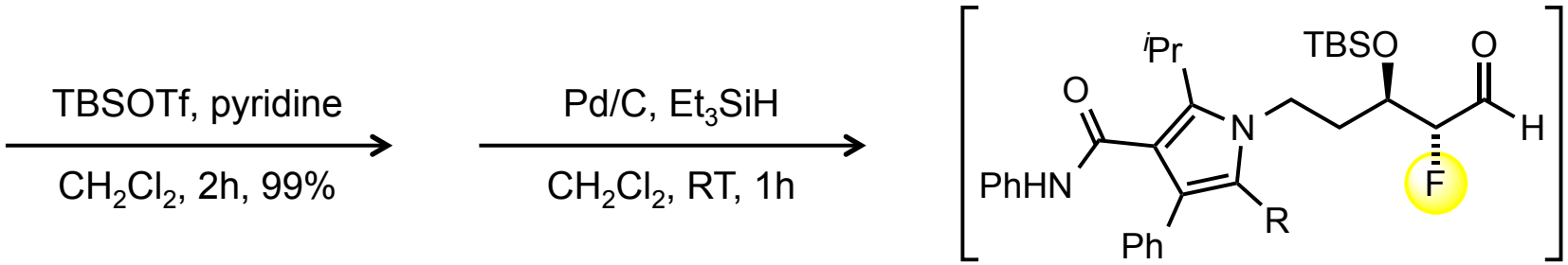
Atorvastatin
(cholesterol lowering)

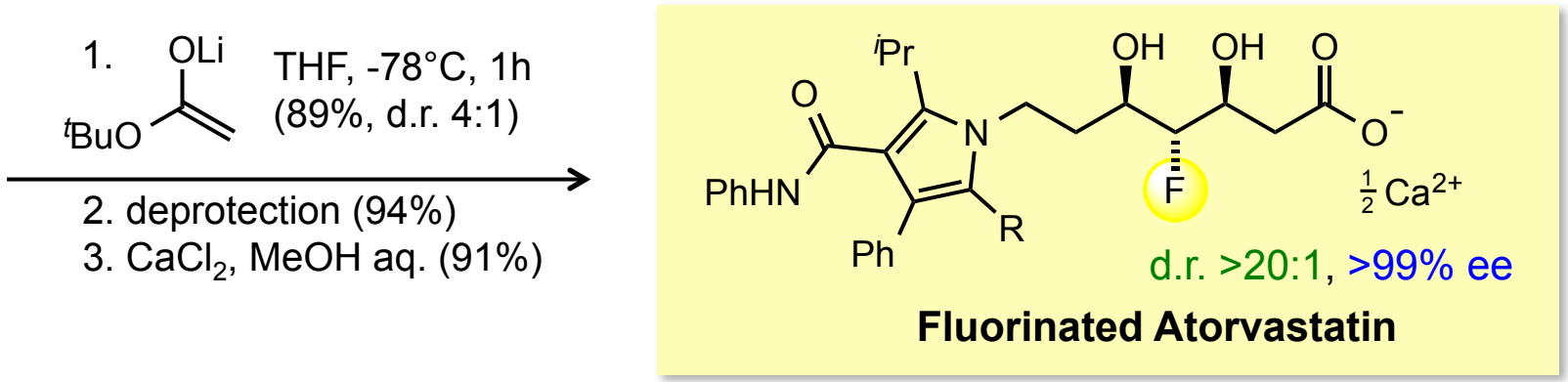
Synthesis of Fluorinated Atorvastatin



d.r. 9:1, 62% ee

Recrystallization: 50%, d.r. >20:1, >99% ee



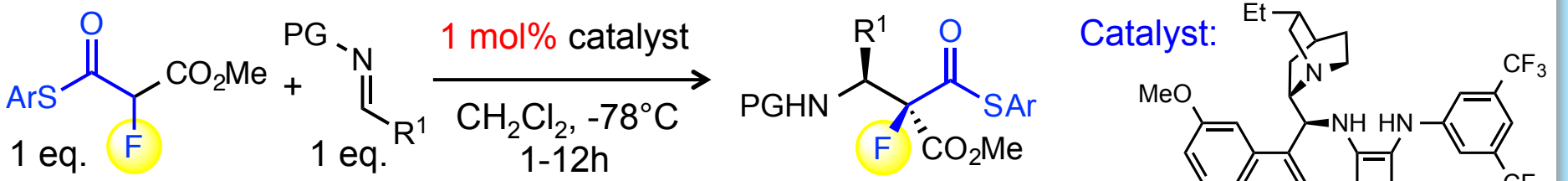


d.r. >20:1, >99% ee

Fluorinated Atorvastatin

J. Saadi, H.W. *Nat. Chem.* **2016**, *8*, 276.

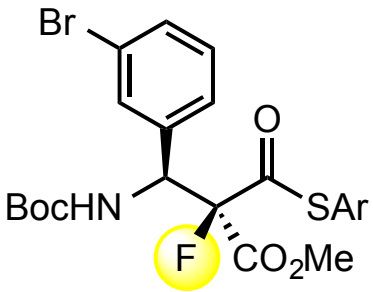
Stereoselective Synthesis of α-Fluorinated β-Amino Acids



80–99% yield

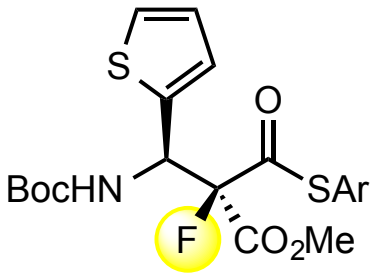
d.r. 9:1– >20:1, 87–99% ee

Rawal **2008**



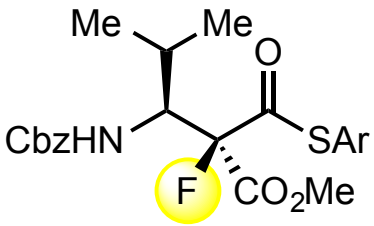
98% yield

d.r. >20:1, >99% ee



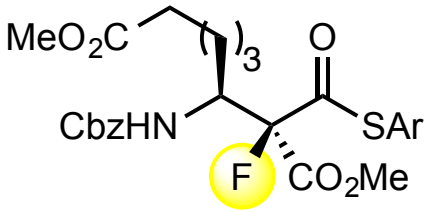
78% yield

d.r. >20:1, >99% ee



95% yield

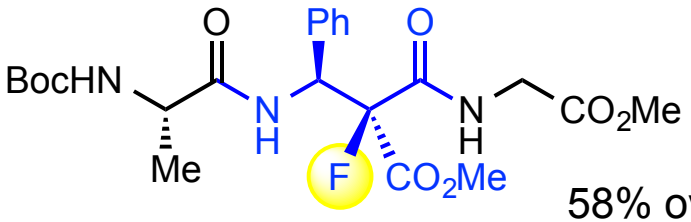
d.r. 9:1, 99% ee



71% yield

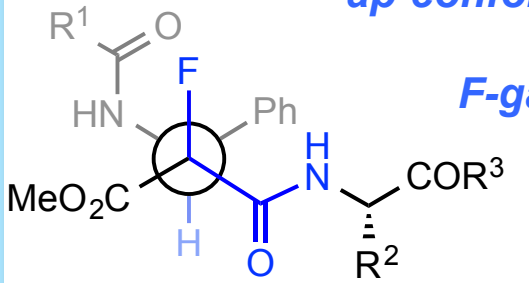
d.r. 4:1, 92% ee

coupling reagent free peptide synthesis



58% overall yield

conformational control



ap-conformation F–C–C=O
&
F-gauche effect

see also: Seebach, *HCA* **2011**

E. Cosimi, O. Engl, J. Saadi, H.W. *ACIE* **2016**, *asap*.

Inspiration from Polyketide Synthases

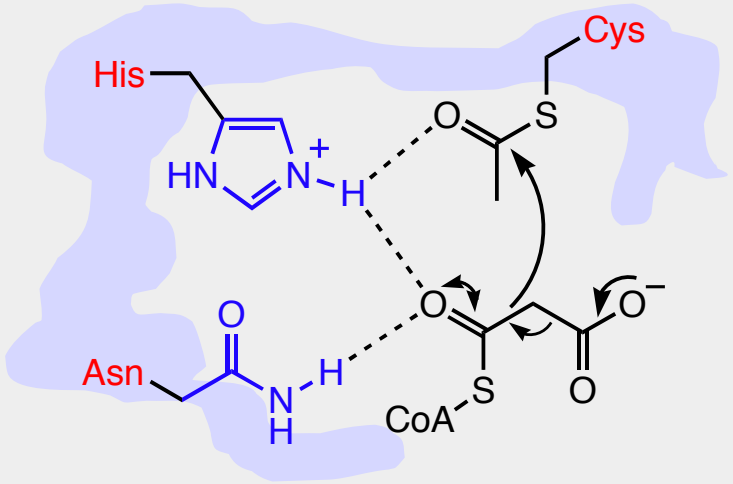
MAHT and MTM = Thioester Enolate Equivalents

- **Mild, organocatalytic asymmetric reactions**
- **Low catalyst loadings, clean reactions, scalable**

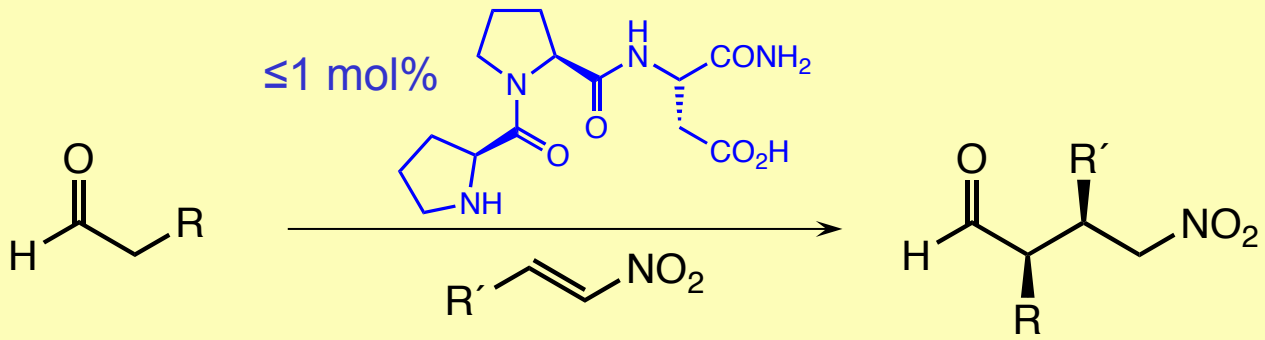
Natural Enzyme and Building Blocks → **Synthetic Methodology**

Bioinspired Catalysis

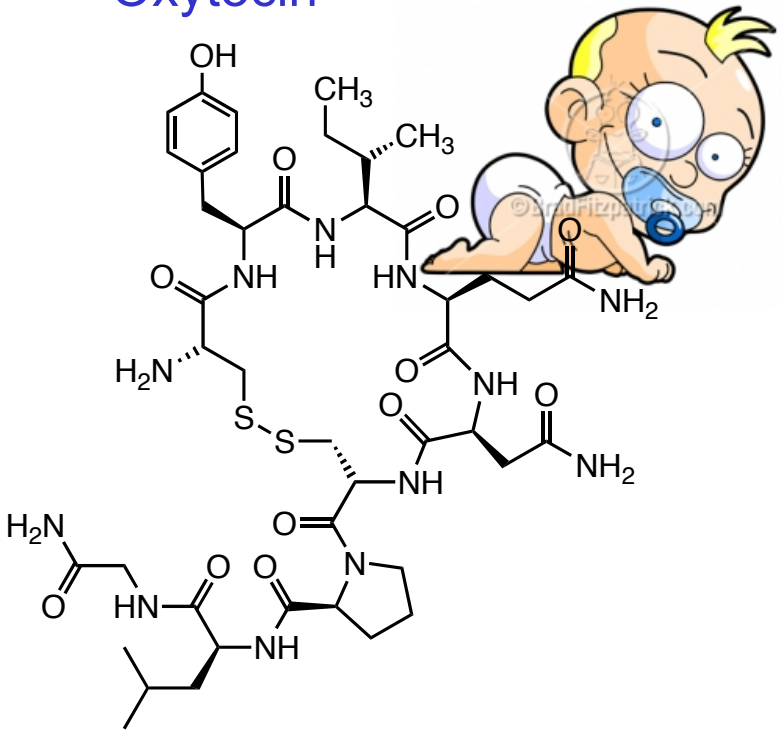
Catalysis Inspired by Polyketide Synthases



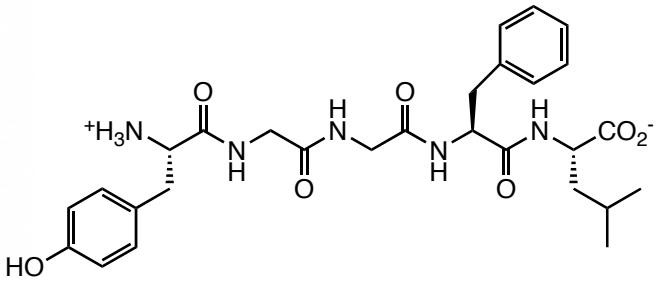
Catalytically Active Peptides



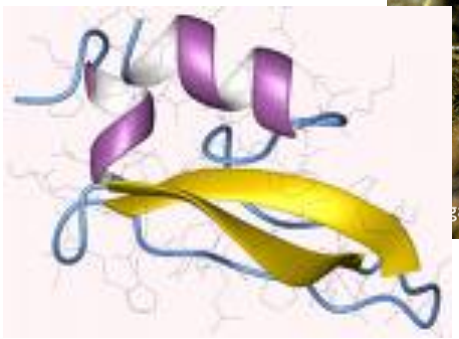
Oxytocin



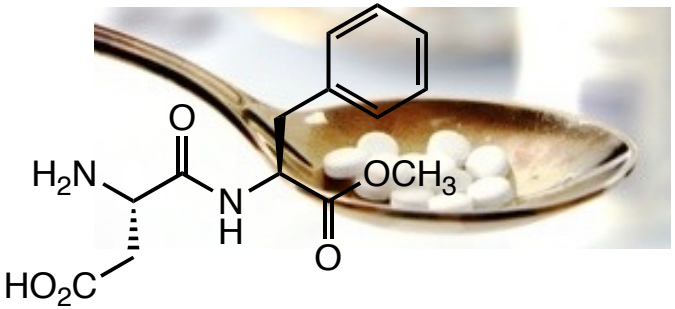
Enkephalines



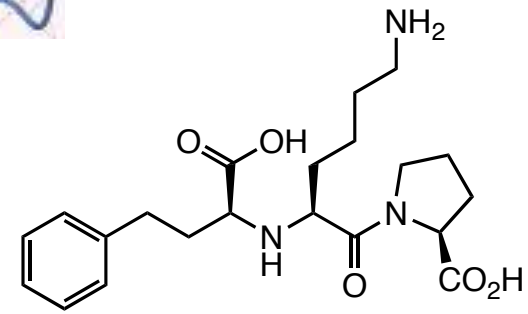
Dendrotoxin



Aspartam

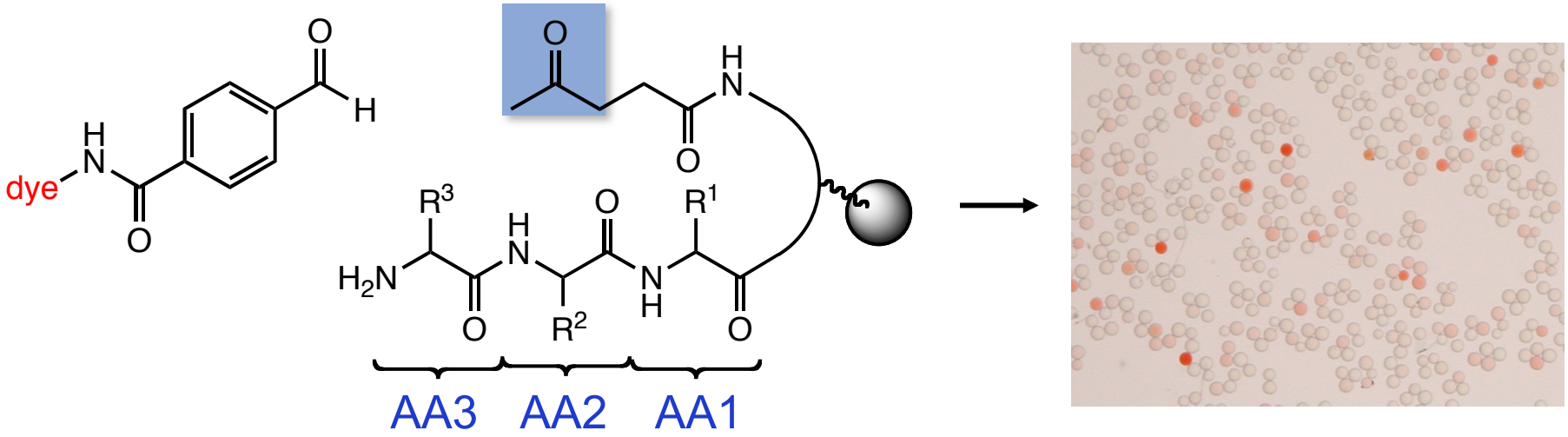


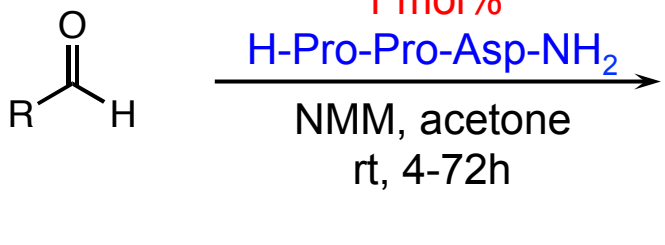
Lisinopril

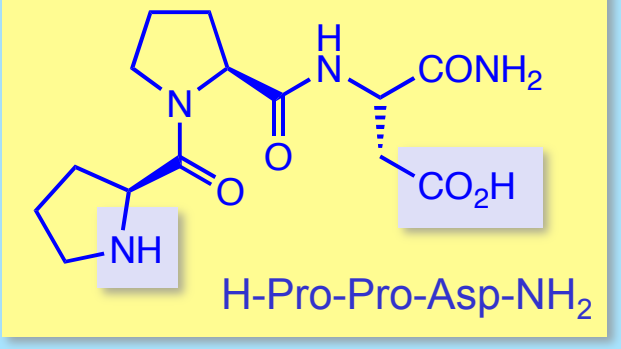


Numerous functions but not a single naturally occuring catalytically active peptide known

Screening Peptides for Catalysts of Aldol Reactions





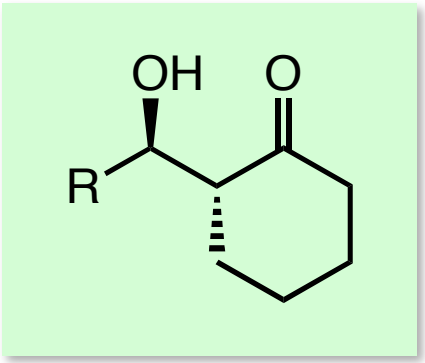


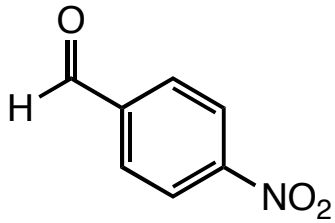
- Significantly higher activity compared to proline (30 mol% of Pro necessary)

Krattiger, McCarthy, Pfaltz, H.W., *Angew. Chem. Int. Ed.* **2003**, *42*, 1722.
Krattiger, Kovasy, Revell, Ivan, H.W, *OL* **2005**, *7*, 1101. Revell, H.W., *Tetrahedron* **2007**, *63*, 8420 & *ASC* **2008**, *350*, 1046.

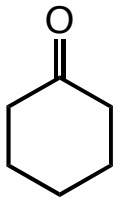
Opposite Enantioselectivities in Different Solvents

(*R*,*S*)-enantiomer





+



+

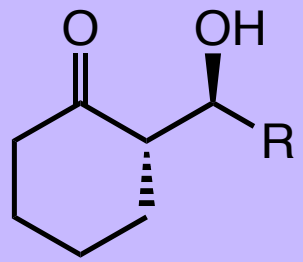
DMSO

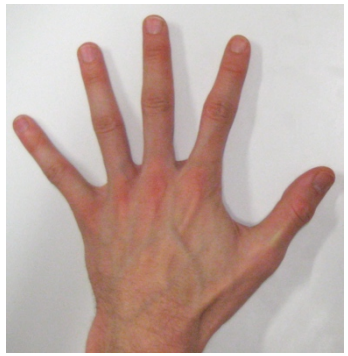
98% yield
dr: 1:3.5
55% ee

10% H_2O
DMSO

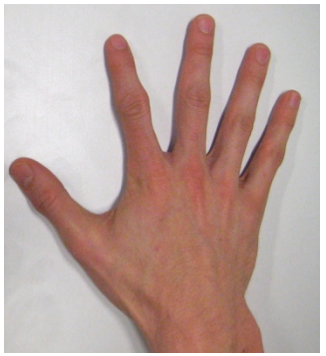
95% yield
dr: 1:2
69% ee

(*S*,*R*)-enantiomer



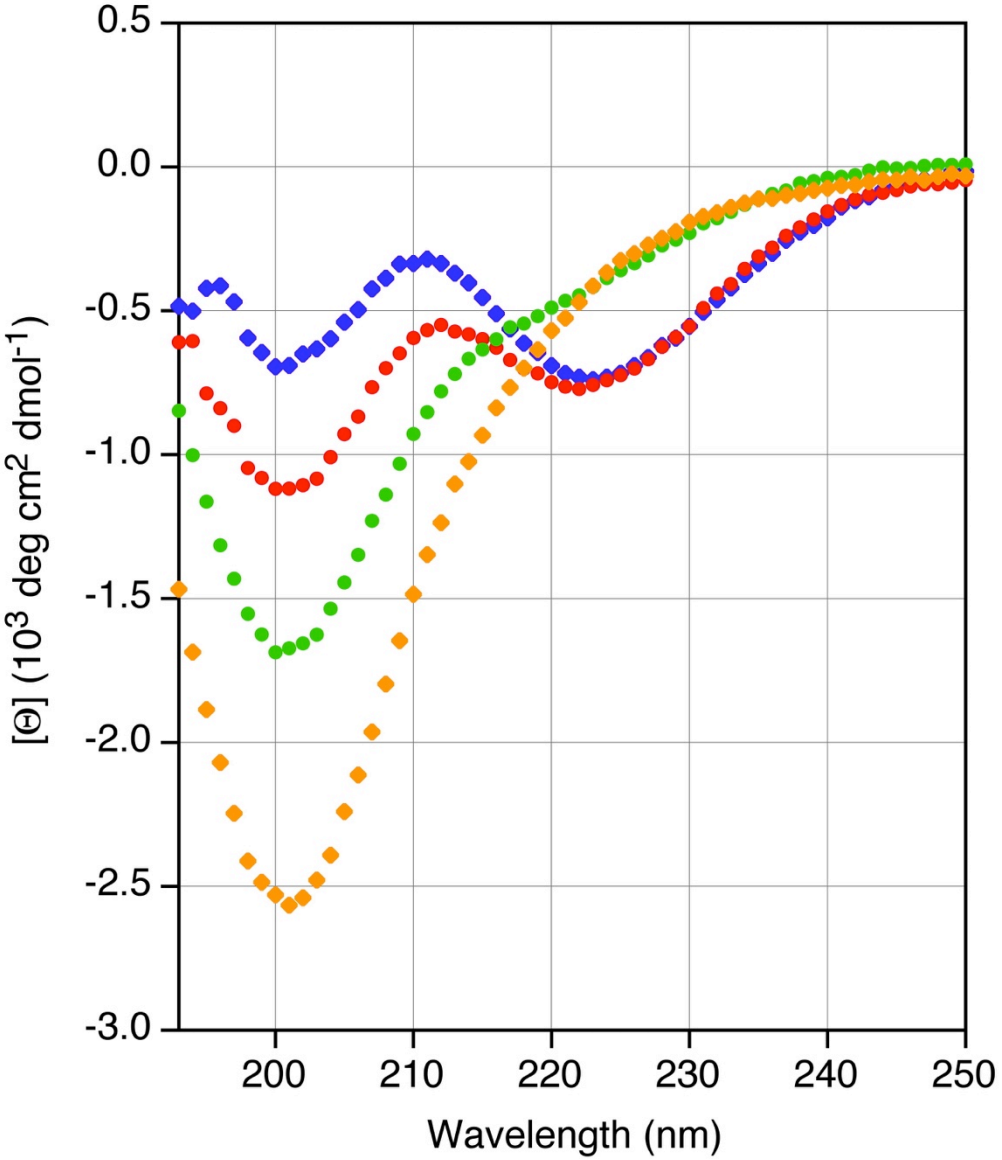


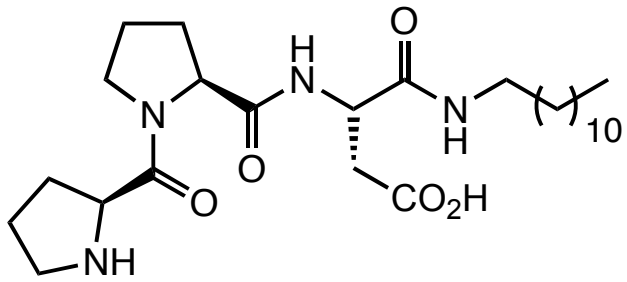
5 mol%
H-Pro-Pro-Asp-$NHC_{12}H_{25}$



M. Messerer, H.W., *Synlett* **2011**, 499.

CD-Spectra of H-Pro-Pro-Asp-NH_2 in Different Solvents





MeOH

10% H_2O in MeOH

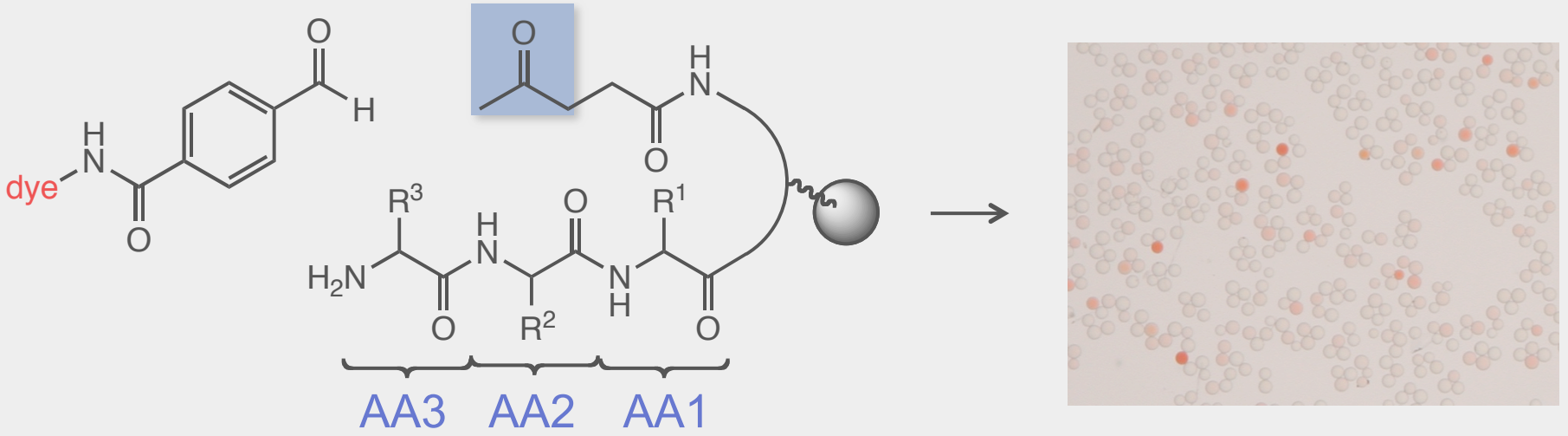
50% H_2O in MeOH

Water

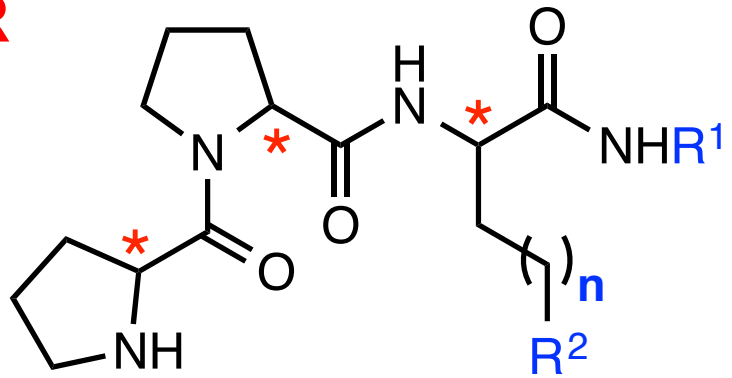
Different conformations are adopted in different solvents

M. Messerer, H.W., *Synlett* **2011**, 499.

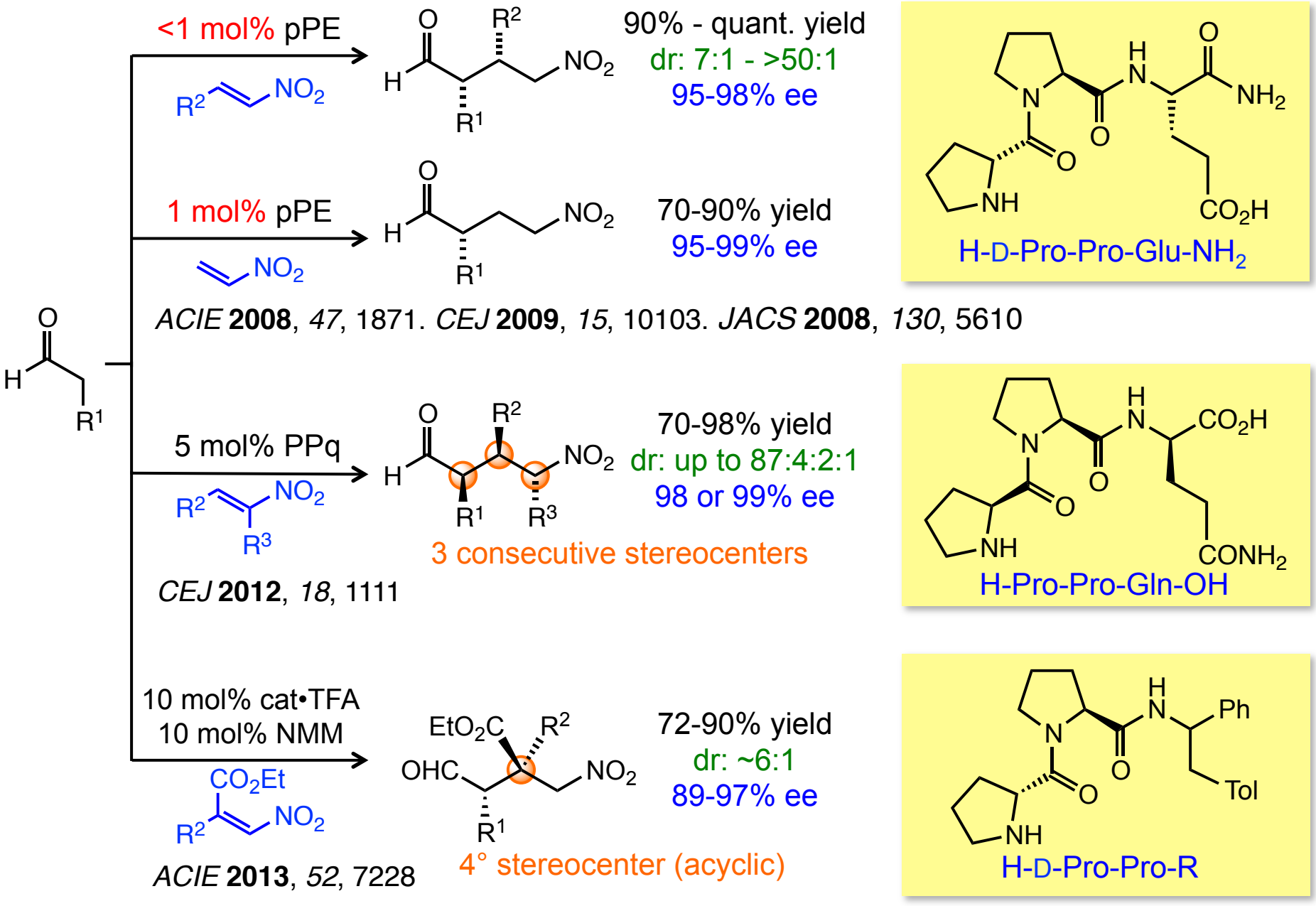
Combinatorial Chemistry – H-Pro-Pro-Xaa-R



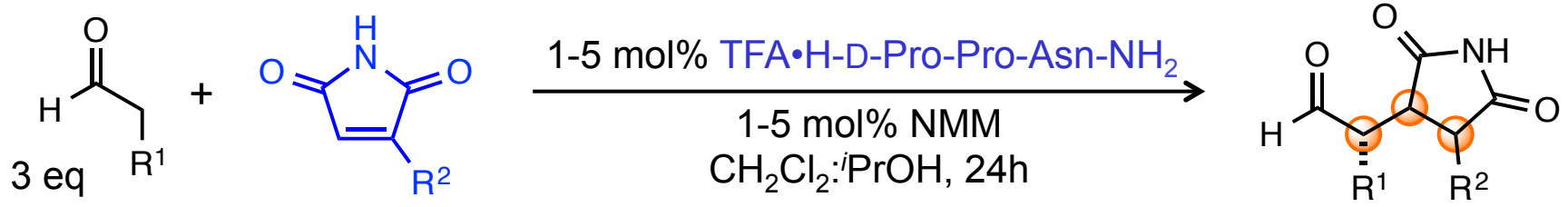
H-Pro-Pro-Xaa-R

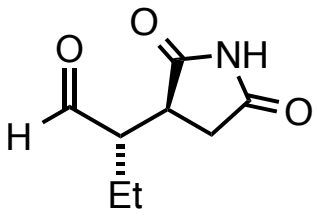


Tailored Peptidic Catalysts

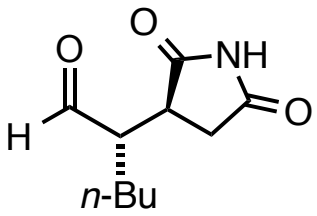


Maleimides as Electrophiles

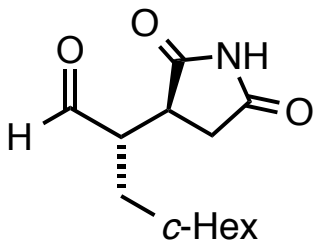




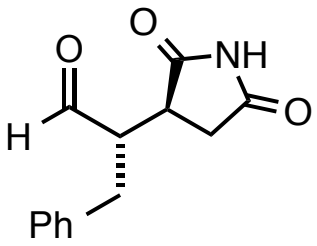
91%, d.r. 93:7
95% ee



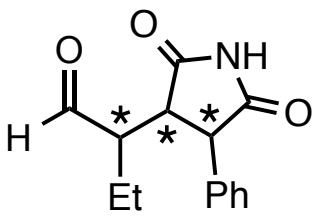
99%, d.r. 89:11
95% ee



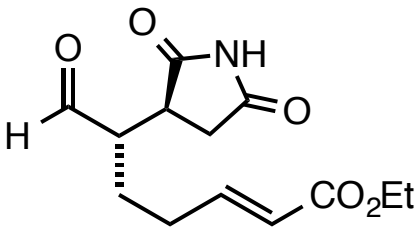
98%, d.r. 94:6
93% ee



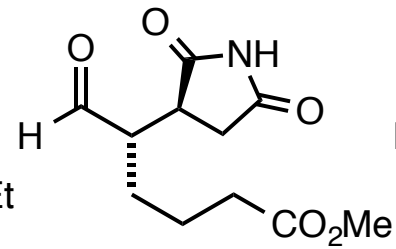
97%, d.r. 88:12
97% ee



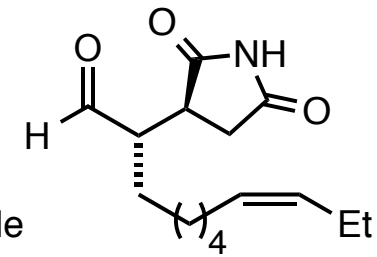
62%, d.r. 87:9:<1:4
98% ee



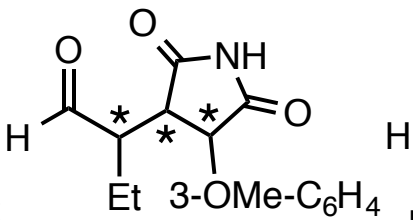
80%, d.r. 83:17
96% ee



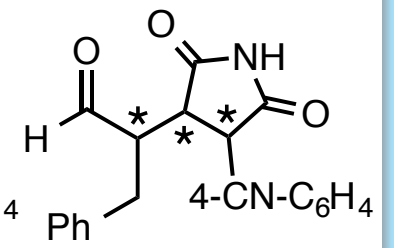
98%, d.r. 92:8
94% ee



86%, d.r. 93:7
97% ee



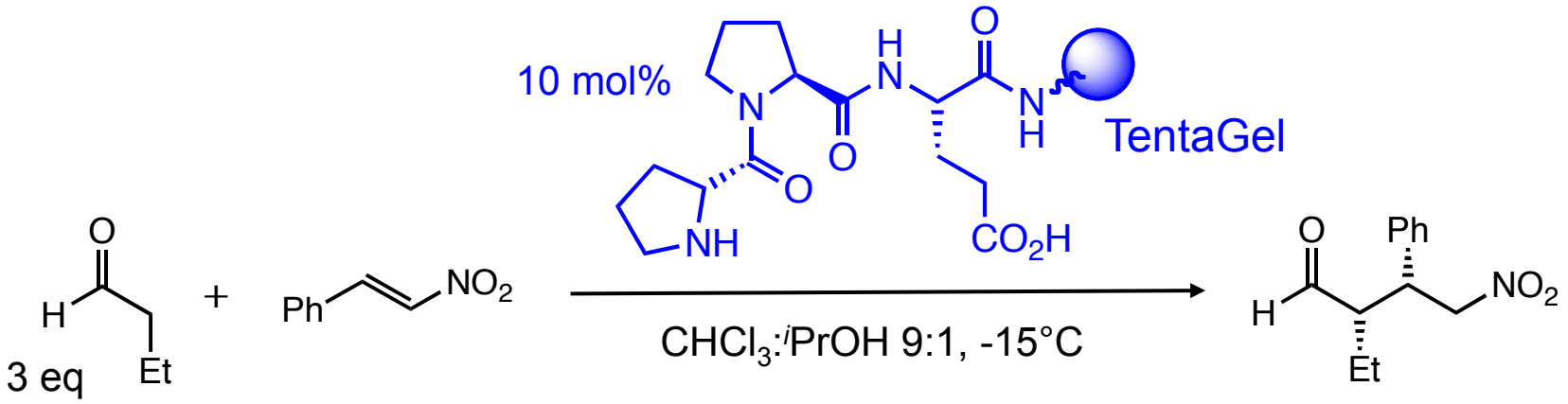
69%, d.r. 83:11:1:5
97% ee



quant., d.r. 83:8:4:5
95% ee

Claudio Grünenfelder

Solid-supported H-D-Pro-Pro-Glu-NH-TentaGel

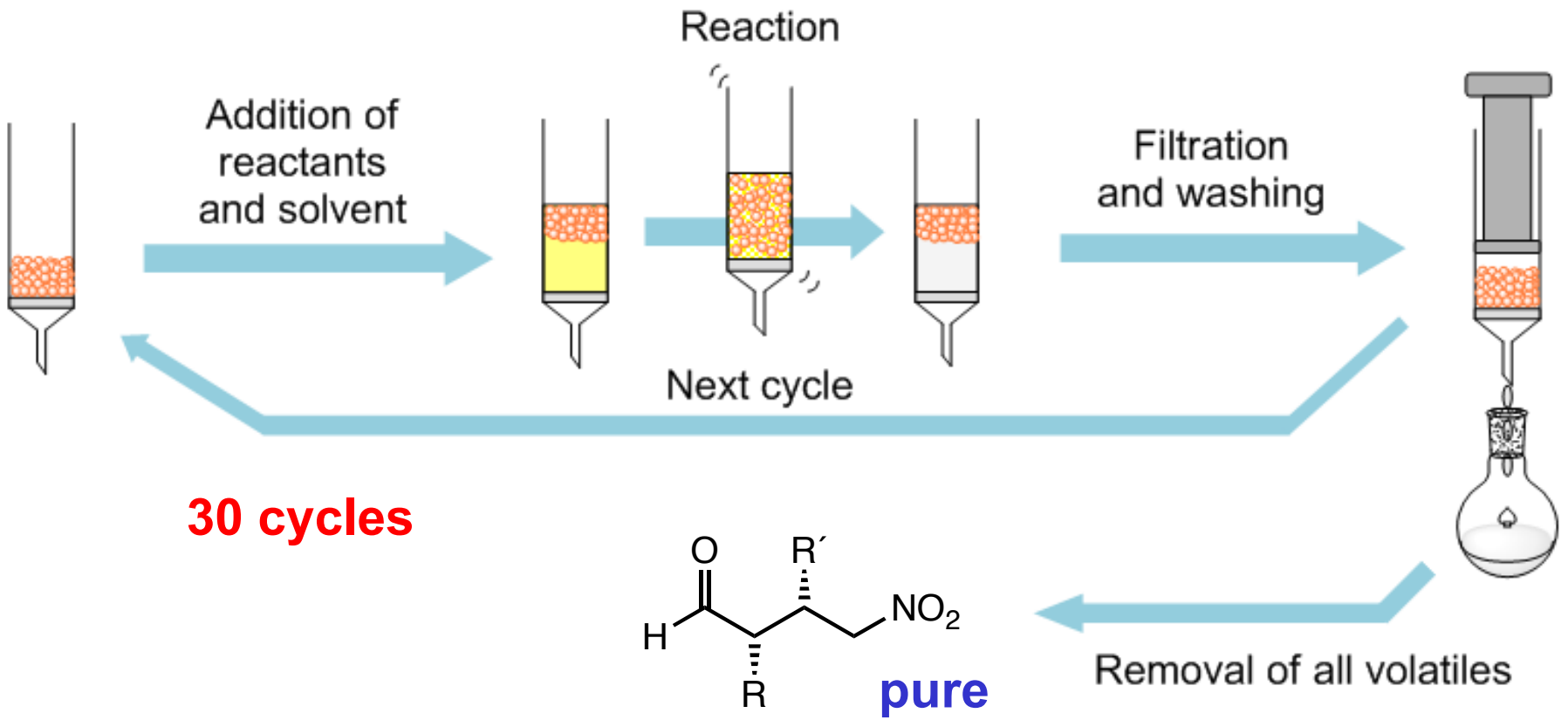


1st cycle	20h	>99% yield	*syn*:*anti* >99:1, 96% ee
2nd – 10th cycle	20-24h	96 – >99% yield	*syn*:*anti* >99:1, 96% ee
11th – 13th cycle	20-24h	quant. conv.	not determined
14th cycle	23h	>99% yield	*syn*:*anti* >99:1, 96% ee
15th – 25th cycle	20-24h	97 – >99% yield	*syn*:*anti* >99:1, 96% ee
26th cycle	24h	99% conv.	*syn*:*anti* >99:1, 96% ee
27th – **30th cycle**	24-36h	99 – >99% yield	*syn*:*anti* >99:1, 96% ee

Yukihiro Arakawa

Y. Arakawa, M. Wiesner, H.W. *Adv. Synth. Catal.* **2011**, *353*, 1201.

H-D-Pro-Pro-Glu-NH-TentaGel

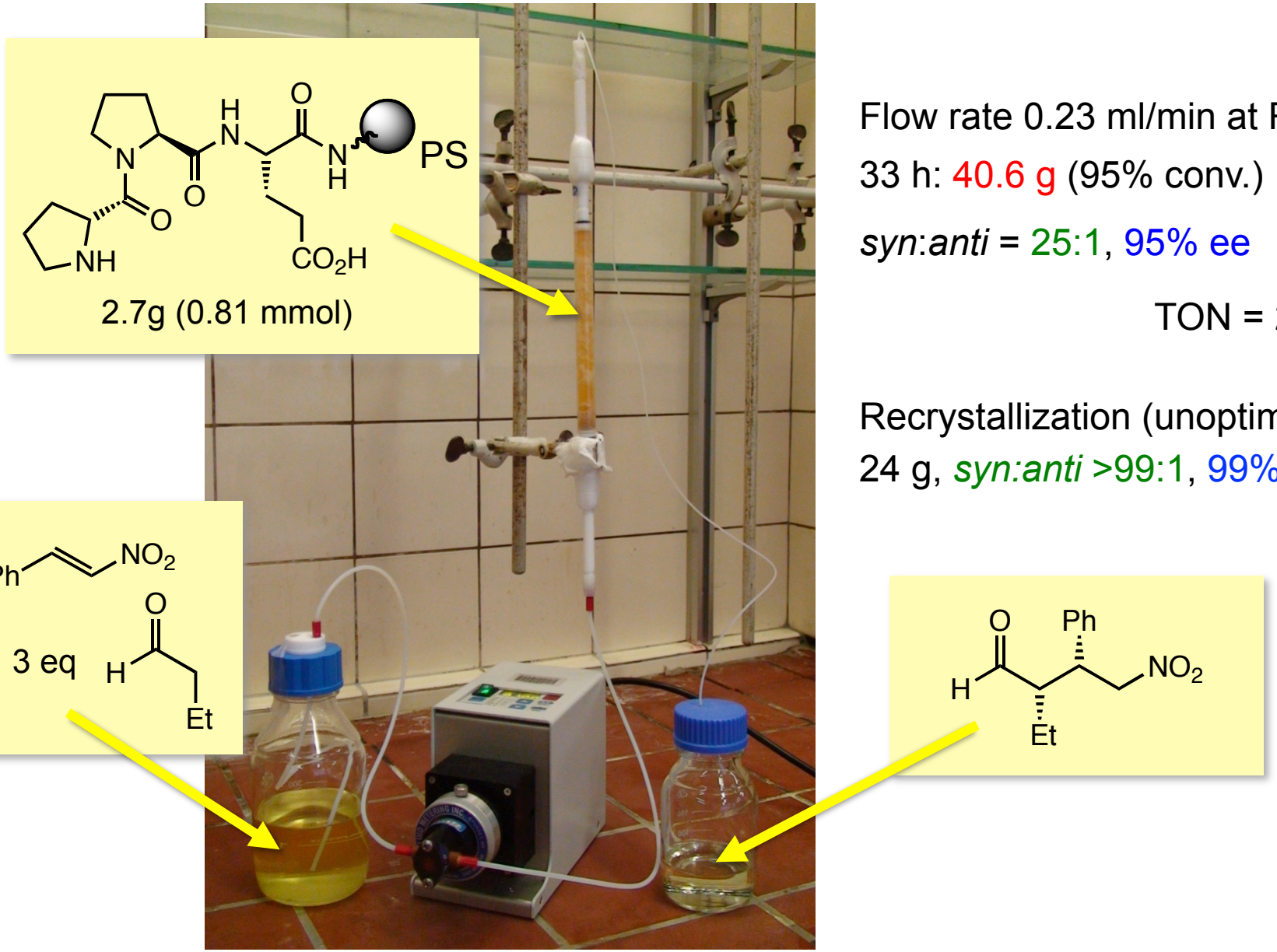


- Correct elemental analysis
- No chromatographic purification

Yukihiro Arakawa

Y. Arakawa, M. Wiesner, H.W. *Adv. Synth. Catal.* **2011**, *353*, 1201.

Enamine Catalysis in Flow



Flow rate 0.23 ml/min at RT

33 h: 40.6 g (95% conv.)

syn:*anti* = 25:1, 95% ee

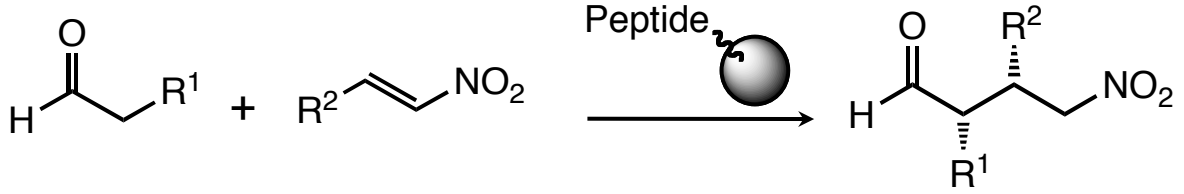
TON = 222

Recrystallization (unoptimized)

24 g, *syn:anti* >99:1, 99% ee

Y. Arakawa, H.W., *ChemSusChem* **2013**, *6*, 242.

Enamine Catalysis in Flow

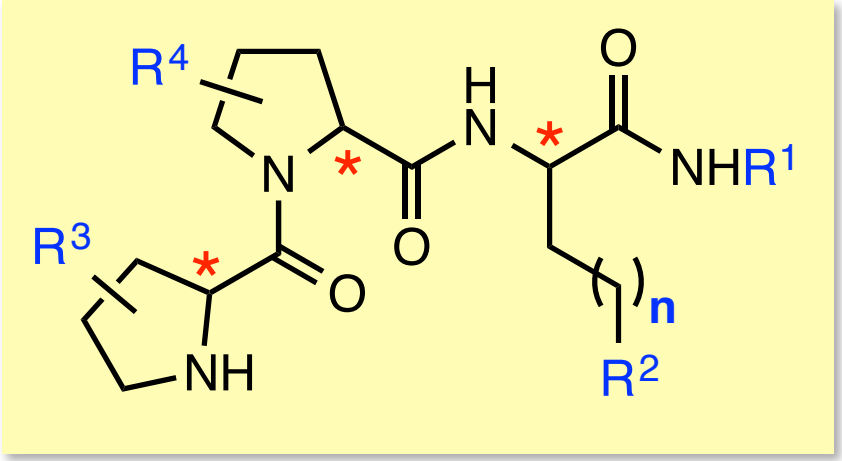


Run	R^1	R^2	Flow rate (ml/min)	Flow time (h)	Amount (g) [Conv (%)]	*syn*:*anti*	ee (%)	TON
1	Et	Ph	0.23	33	40.6 [95]	25:1	95	222
2 (0 °C)	Et	Ph	0.08	9	3.1 [95]	50:1	97	17
3	Et	Ph	0.23	2	1.4 [96]	23:1	95	7
4	Et	4-Br-C_6H_4	0.12	5	2.1 [quant.]	20:1	95	9
5	Me	Ph	0.12	6	2.7 [quant.]	13:1	93	16
6	Et	4-Cl-C_6H_4	0.12	4	1.5 [quant.]	25:1	95	7
7	Et	Ph	0.12	35	26 [96]	30:1	95	142

Total Turn Over Number: >600

Yukihiro Arakawa

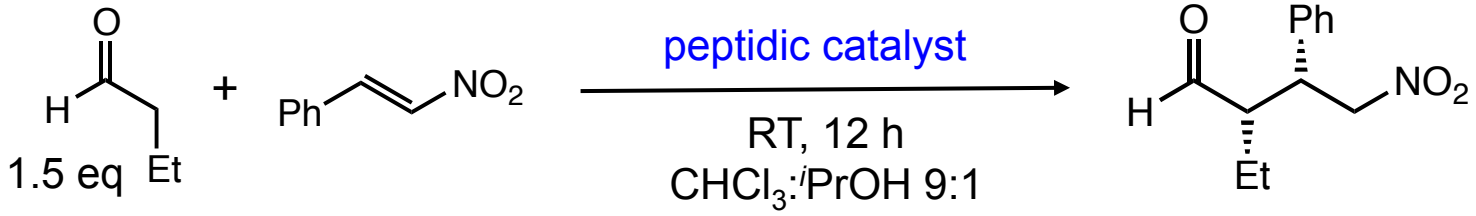
Peptidic Catalysts: H-Pro-Pro-Xaa-R



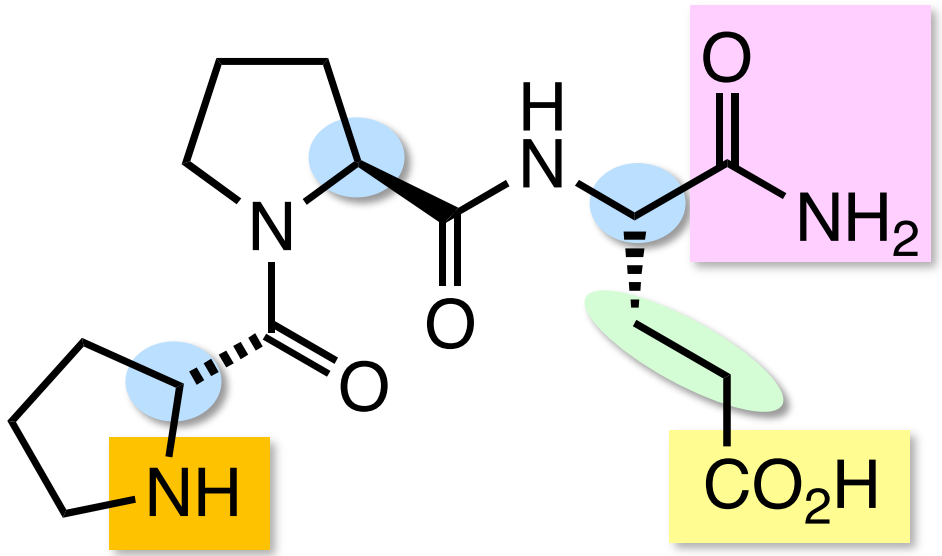
- High reactivity and robustness
- Modular structure – tunable and adaptable
- High Chemoselectivity and Stereoselectivity

Reaction Mechanism?

Structure–Activity Studies



96% yield
syn:anti 50:1
97% ee (syn)



2° amine:
Reactive center

Carboxylic acid:
Critical for reactivity and stereoselectivity

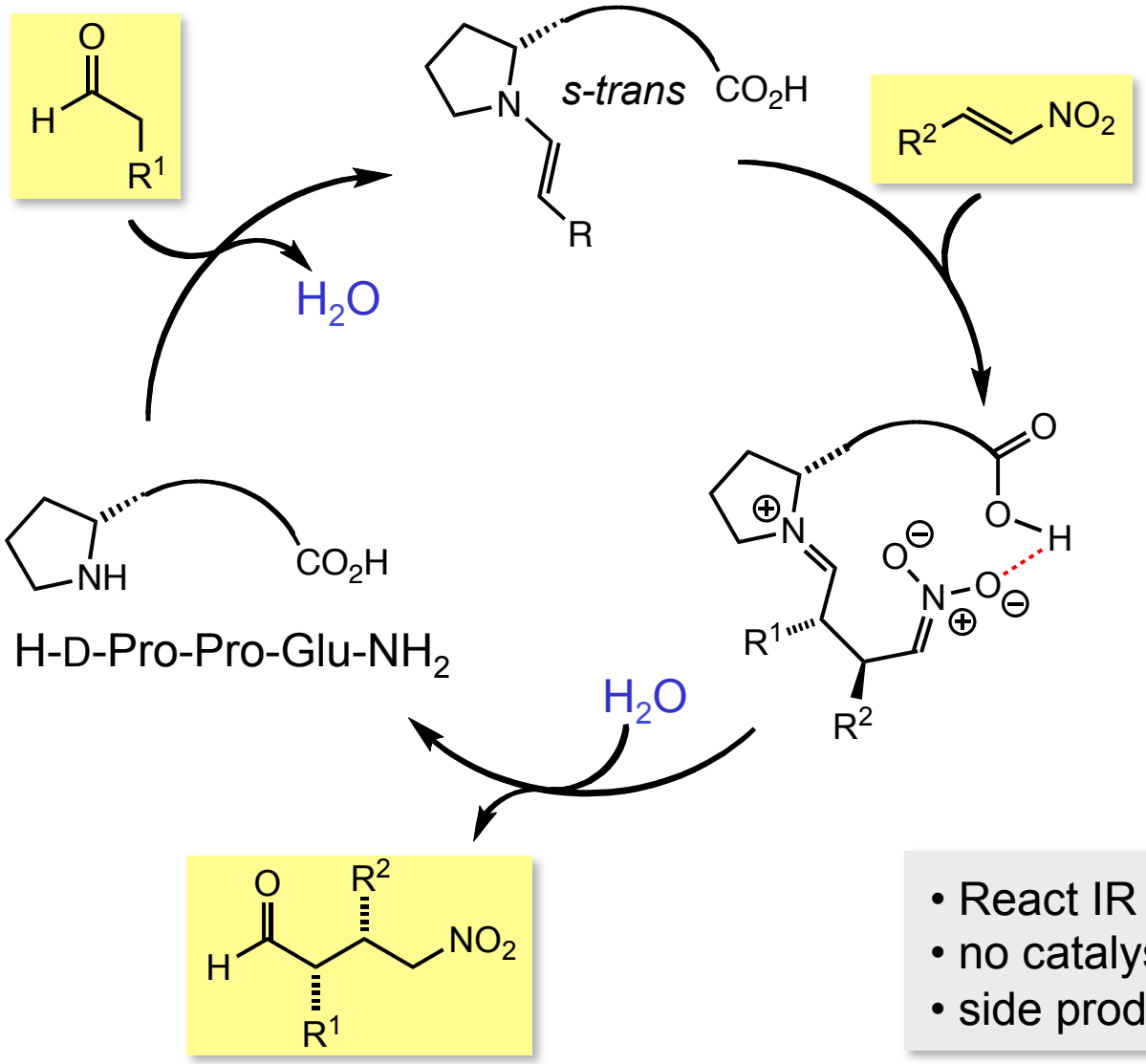
No additives necessary

Stereochemistry

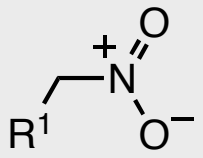
C-terminus

linker length

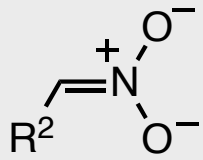
Proposed Mechanism



Interactions with R-CO_2H



Very weak!

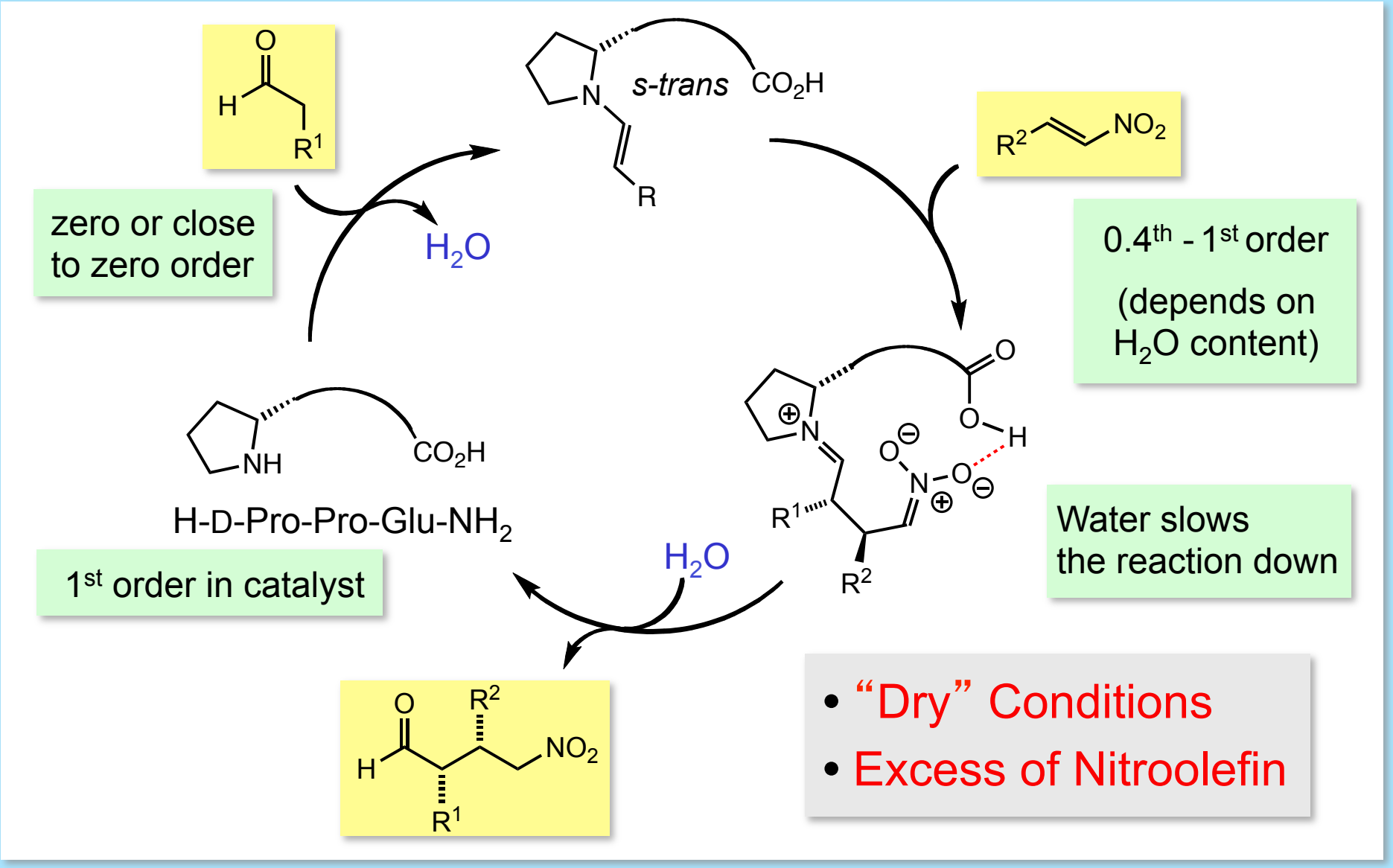


K_a ~4000 M^{-1}

Hamilton, *CEJ*, **2000**, *6*, 2449
Kelly, *JACS* **1994**, *116*, 7072

- React IR – in situ monitoring
- no catalyst deactivation
- side products ≤ 3%

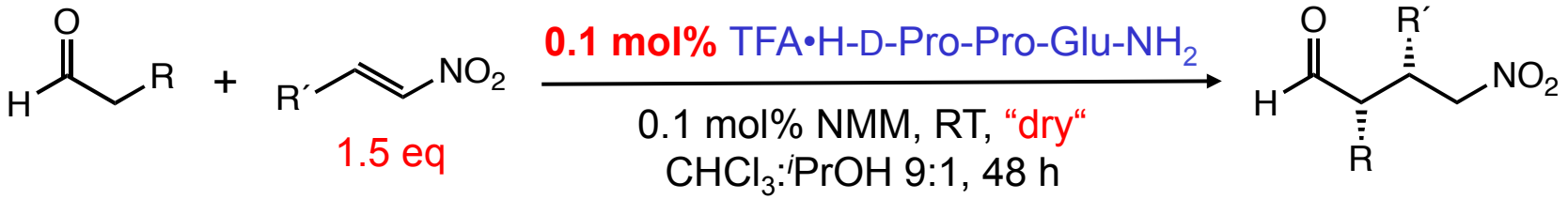
Kinetic Studies



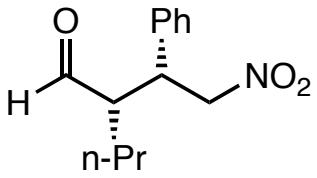
Markus Wiesner, Grégory Upert

M. Wiesner, G. Upert, G. Angelici, H.W., *JACS* **2010**, *132*, 6.

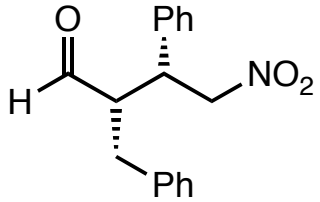
Improved Conditions: Catalyst Loading of 0.1 mol%!



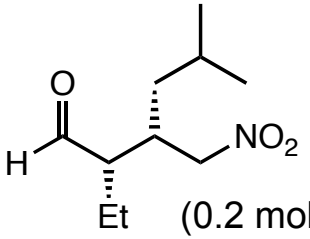
1g butanal (14 mmol) **catalyst: 5 mg (M = 340.4 g/mol)**



98% yield, syn:anti 95:5
96% ee (syn)

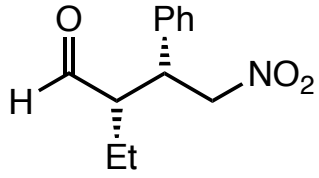


87% yield, syn:anti 94:6
98% ee (syn)

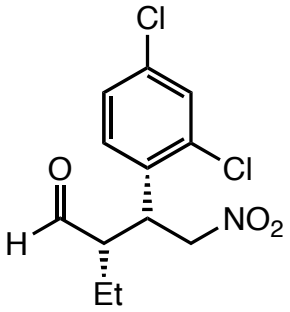


(0.2 mol% cat)

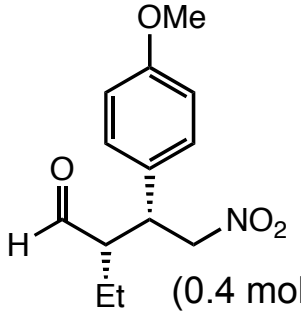
92% yield, syn:anti 91:9
99% ee (syn)



87% yield, syn:anti 94:6
97% ee (syn)

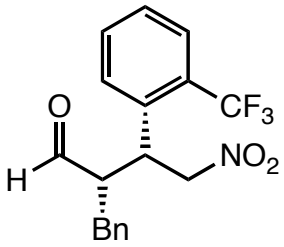


95% yield, syn:anti 95:5
96% ee (syn)

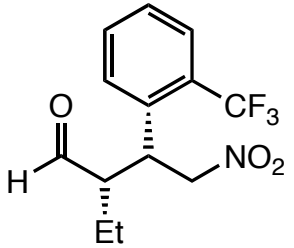


(0.4 mol% cat)

96%, syn:anti 93:7
95% ee (syn)



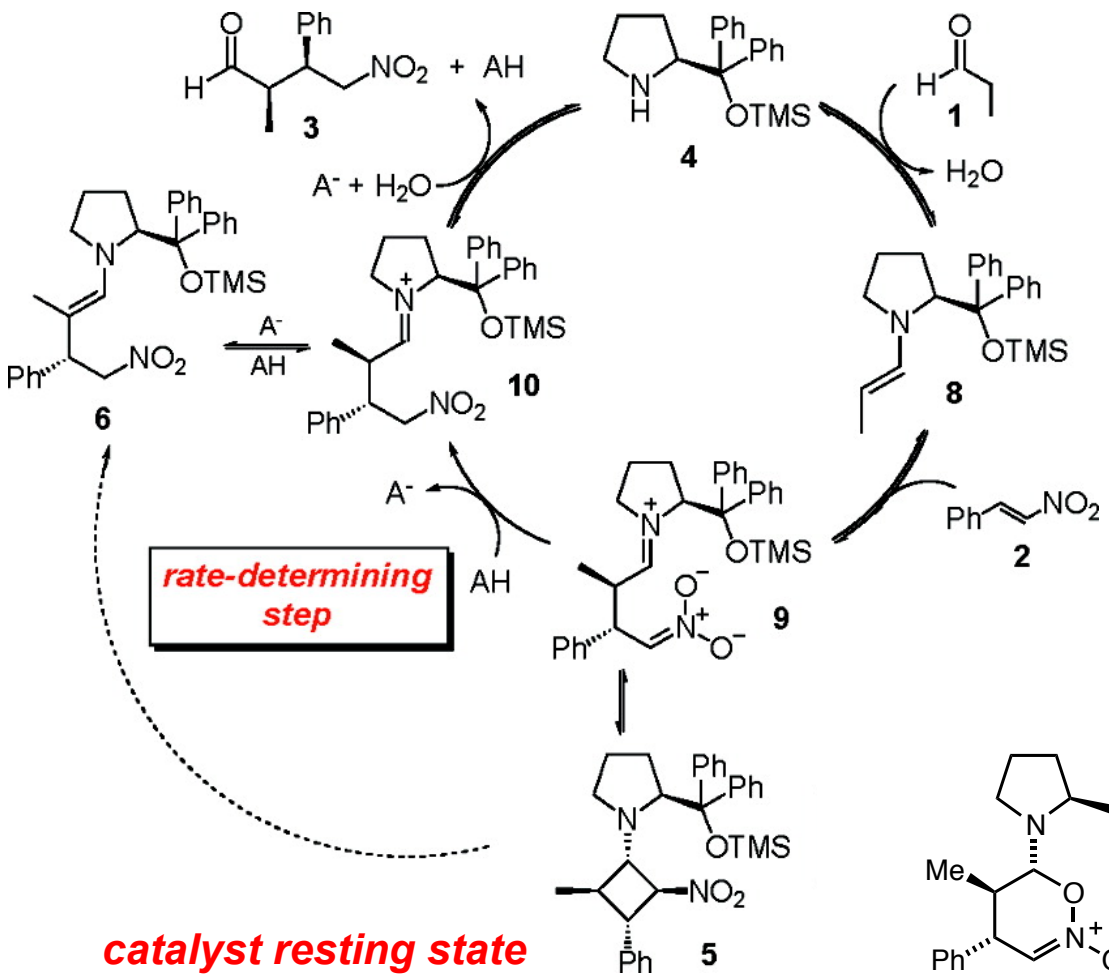
92% yield, syn:anti 98:2
99% ee (syn)

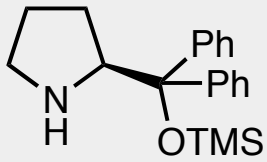


96% yield, syn:anti 97:3
97% ee (syn)

M. Wiesner, G. Upert, G. Angelici, H.W., *JACS* **2010**, *132*, 6.

Mechanism for Reactions with the Hayashi-Jorgensen Catalyst





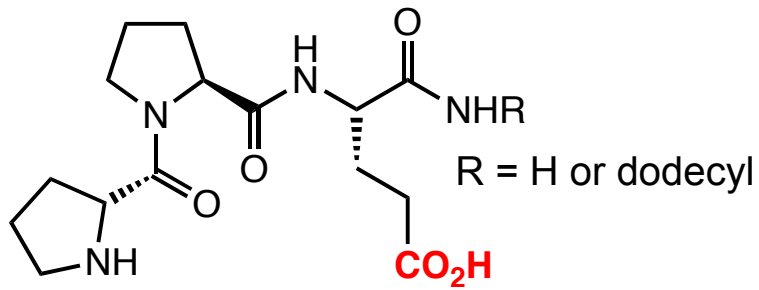
Hayashi-Jørgensen Catalyst

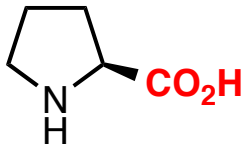
Differences to Peptide Catalyzed Reactions

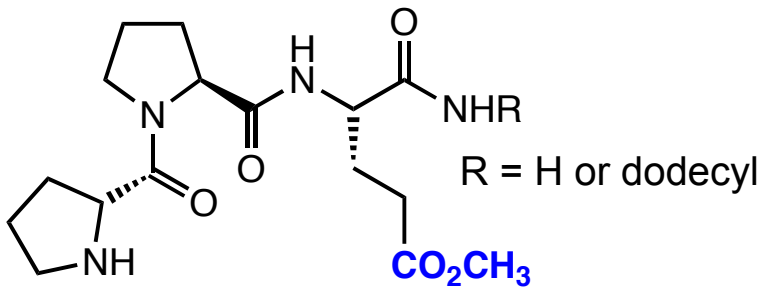
- role of water
- rate orders of nitroolefin and RCHO
- acidic additives

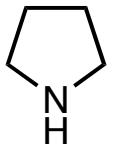
Hayashi, Seebach, *HCA* **2011**, *94*, 719. Pápai, Pihko, *ACIE* **2012**, *52*, 13144
Blackmond, *JACS* **2011**, *133*, 8822. *JACS* **2012**, *134*, 6741.

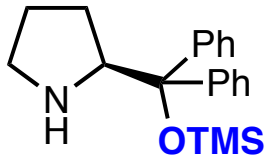
Intramolecular versus Intermolecular Carboxylic Acid







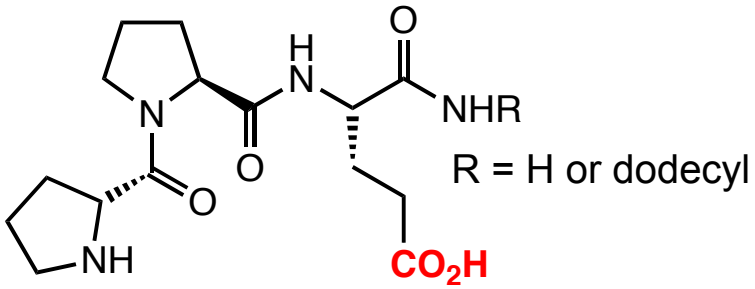


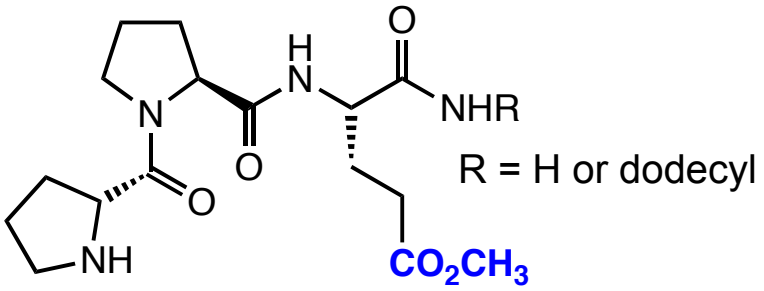


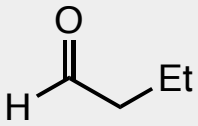
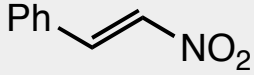
Does the presence or absence of an intramolecular proton donor control the reaction mechanism?

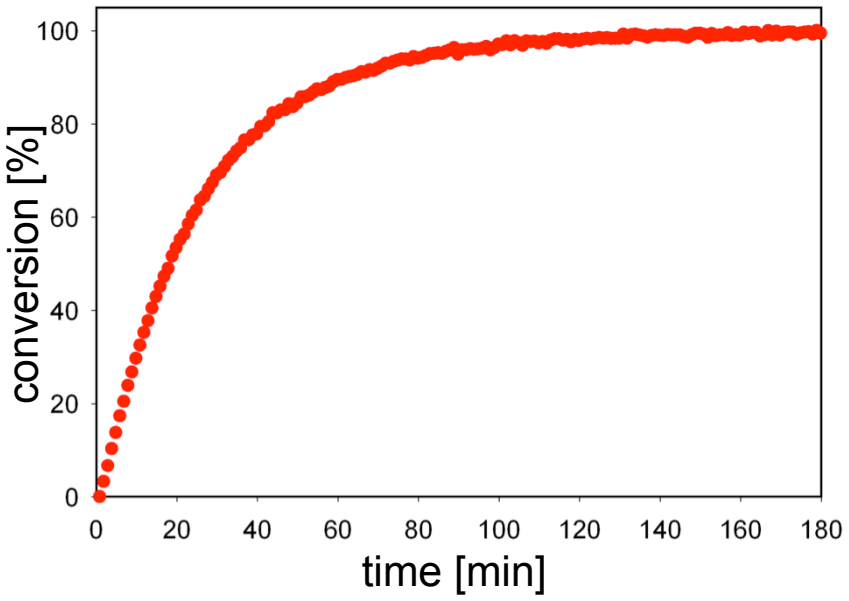
Jörg Duschmalé

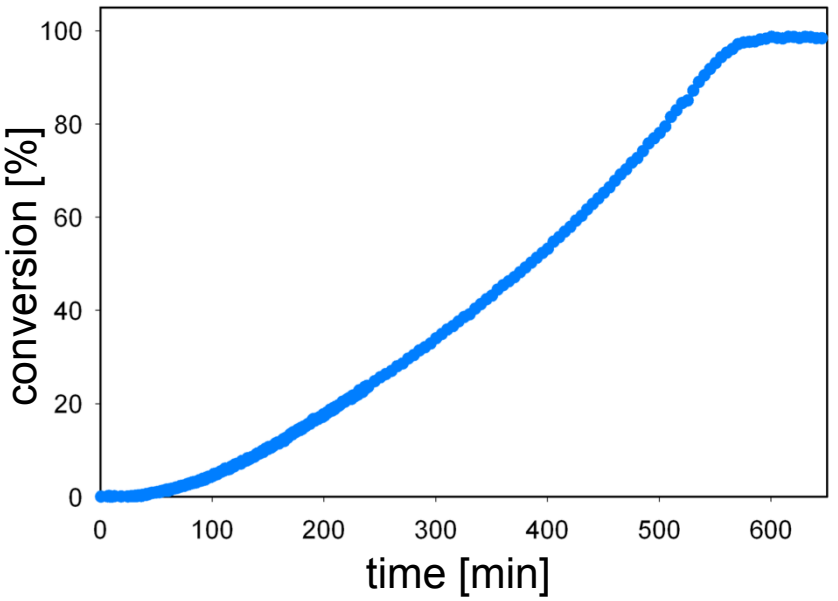
Kinetic Studies





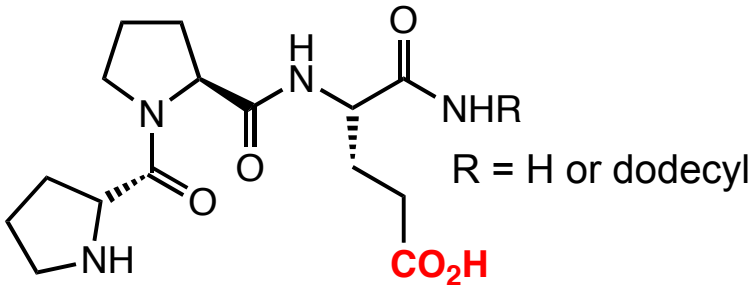
Reaction with 1.5 eq  and 1.0 eq 

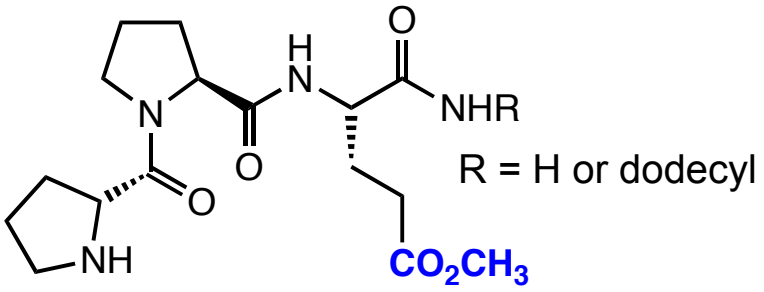


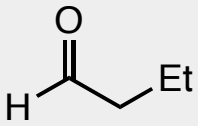
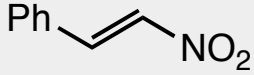


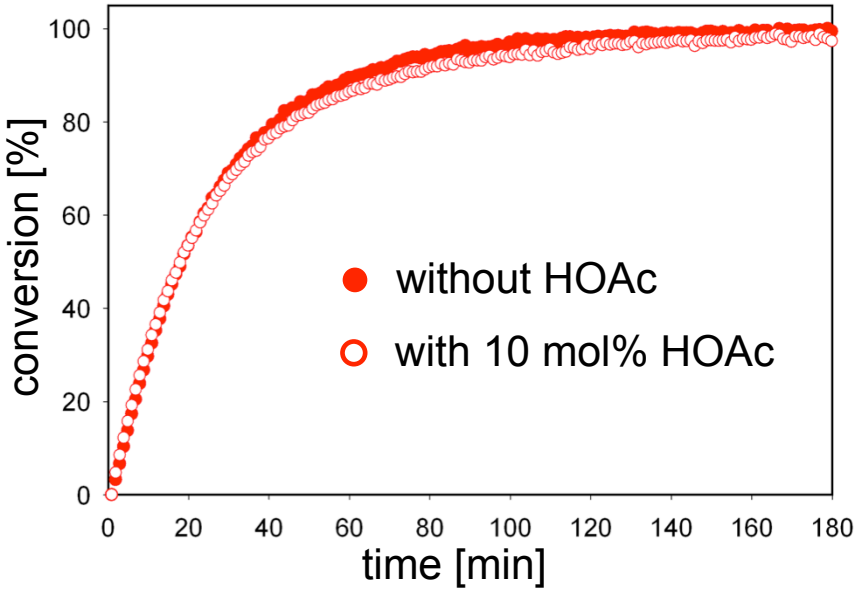
Jörg Duschmalé

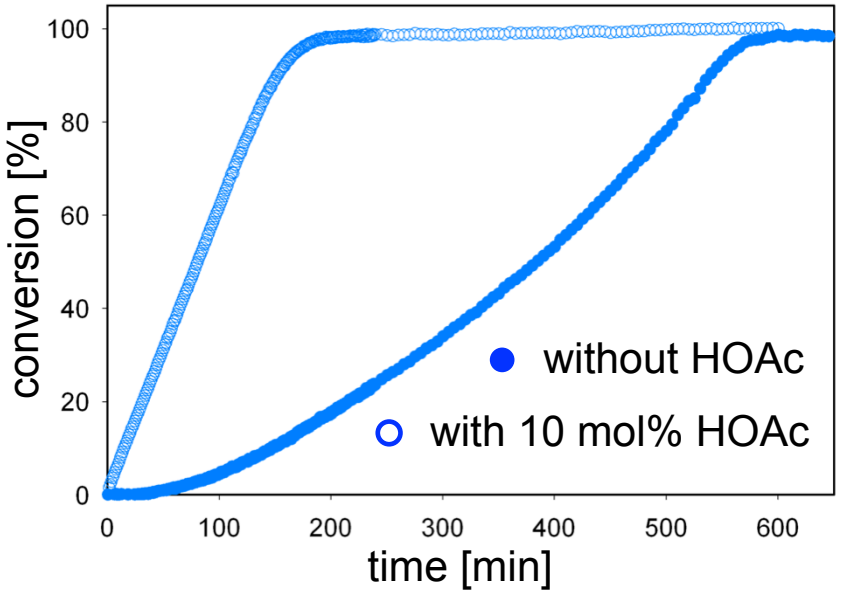
Kinetic Studies





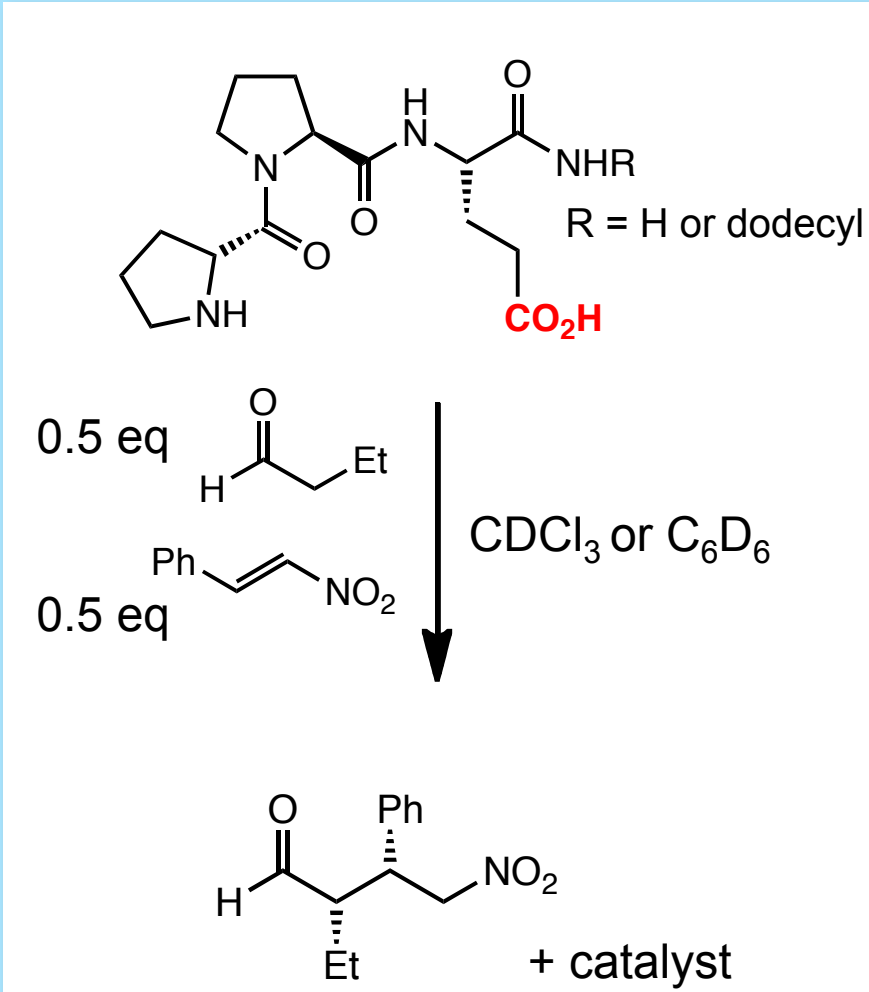
Reaction with 1.5 eq  and 1.0 eq 



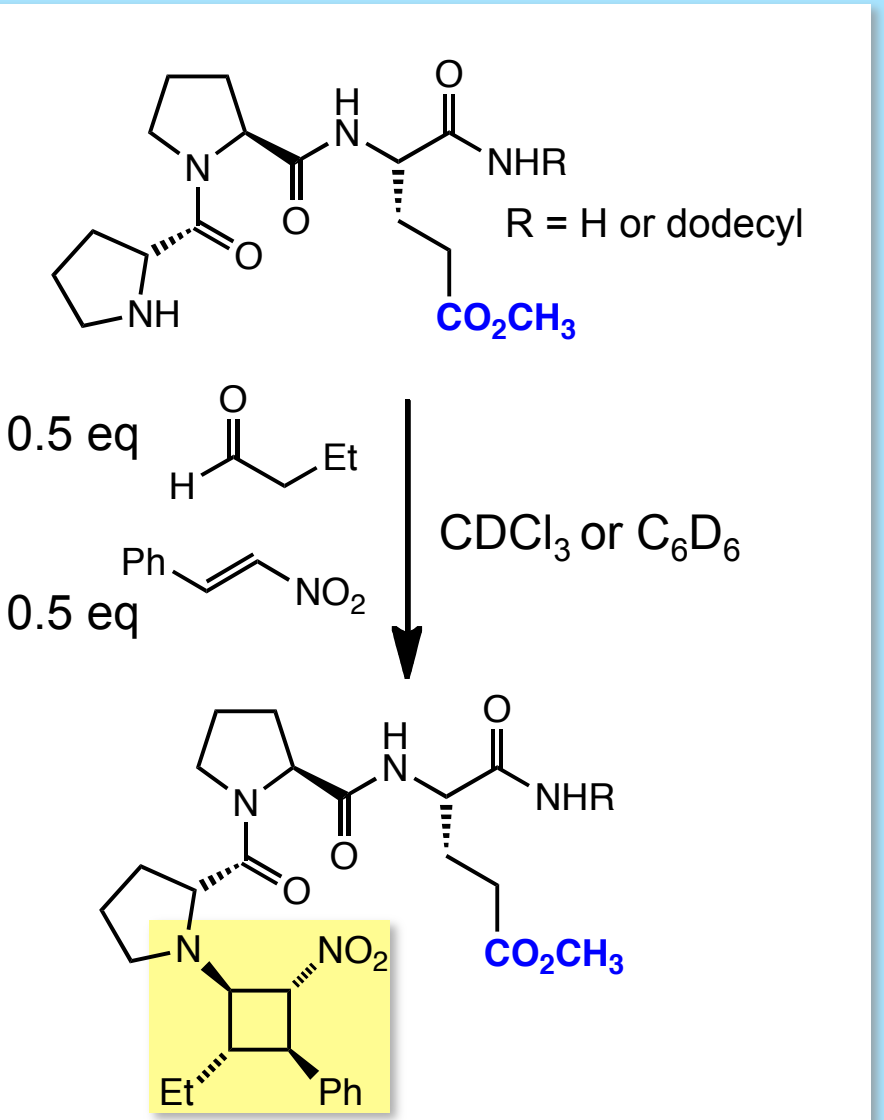


J. Duschmalé, J. Wiest, M. Wiesner, H.W. *Chem. Sci.* **2013,** *4*, 1312.

NMR-Spectroscopic Studies



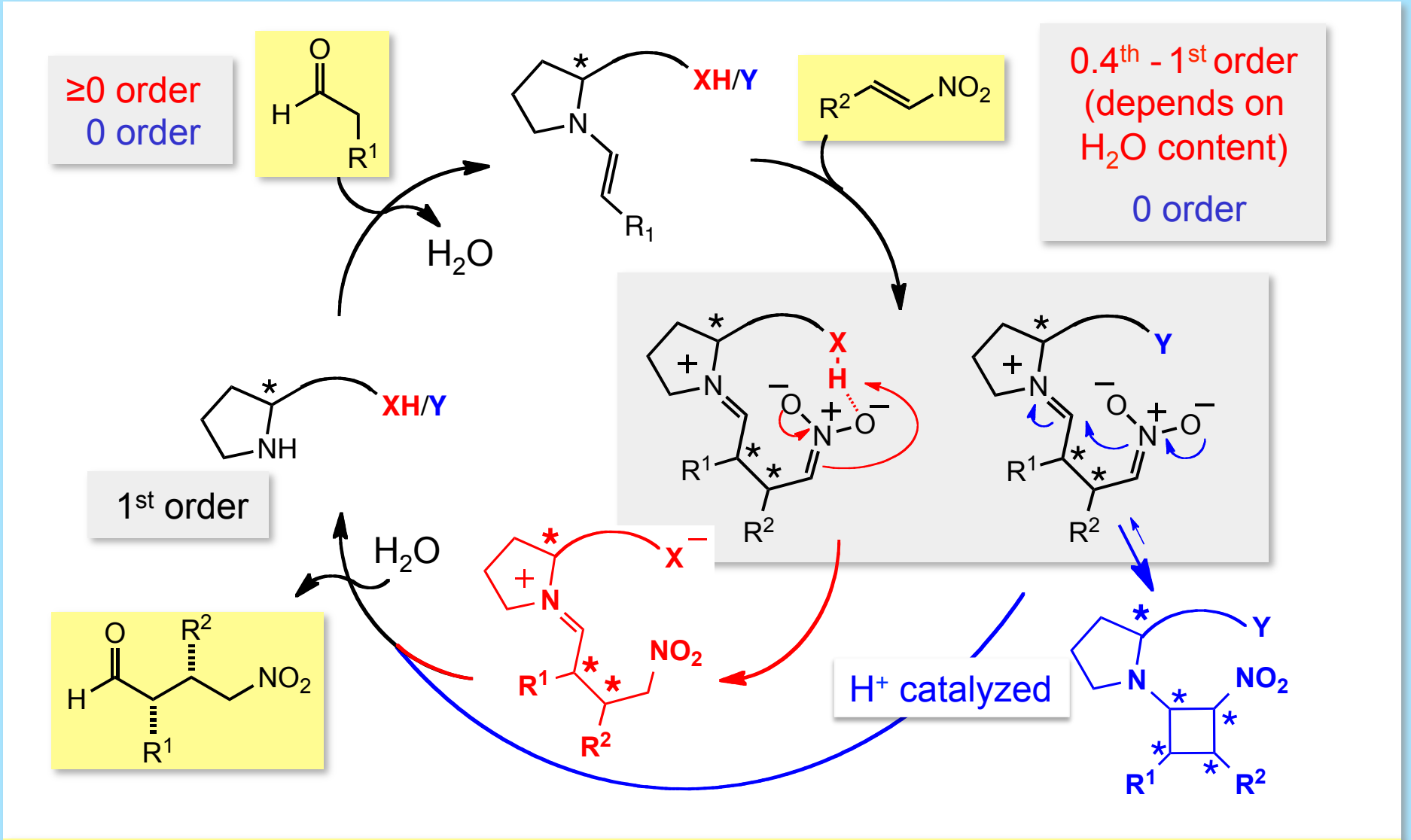
Full conversion to 1,4-addition product



1,4-addition product **not** formed

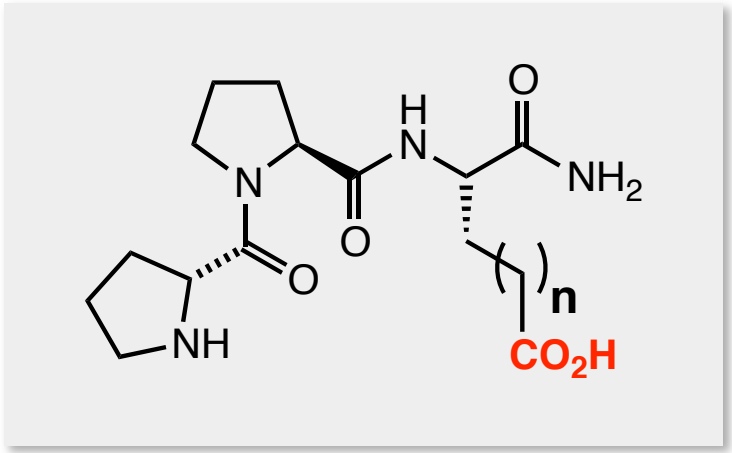
J. Duschmalé, J. Wiest, M. Wiesner, H.W. *Chem. Sci.* **2013,** *4*, 1312.

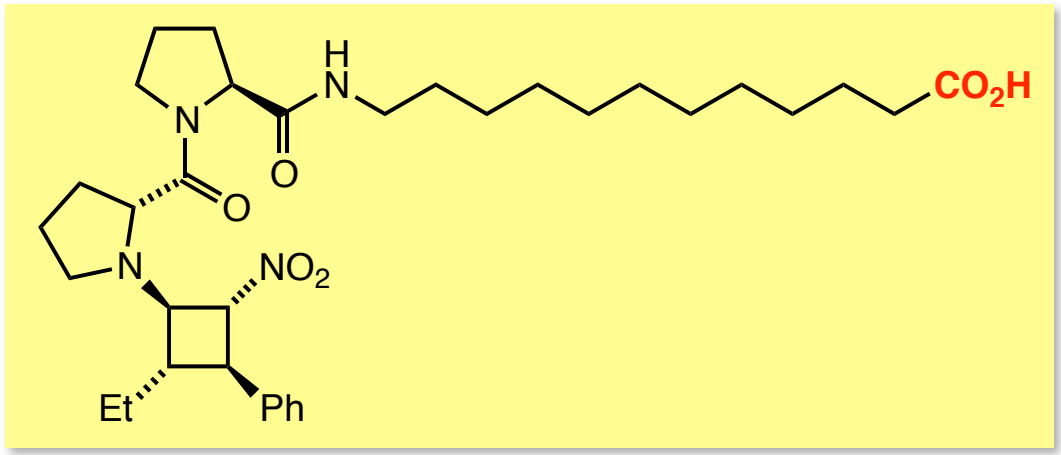
Proposed Reaction Mechanisms

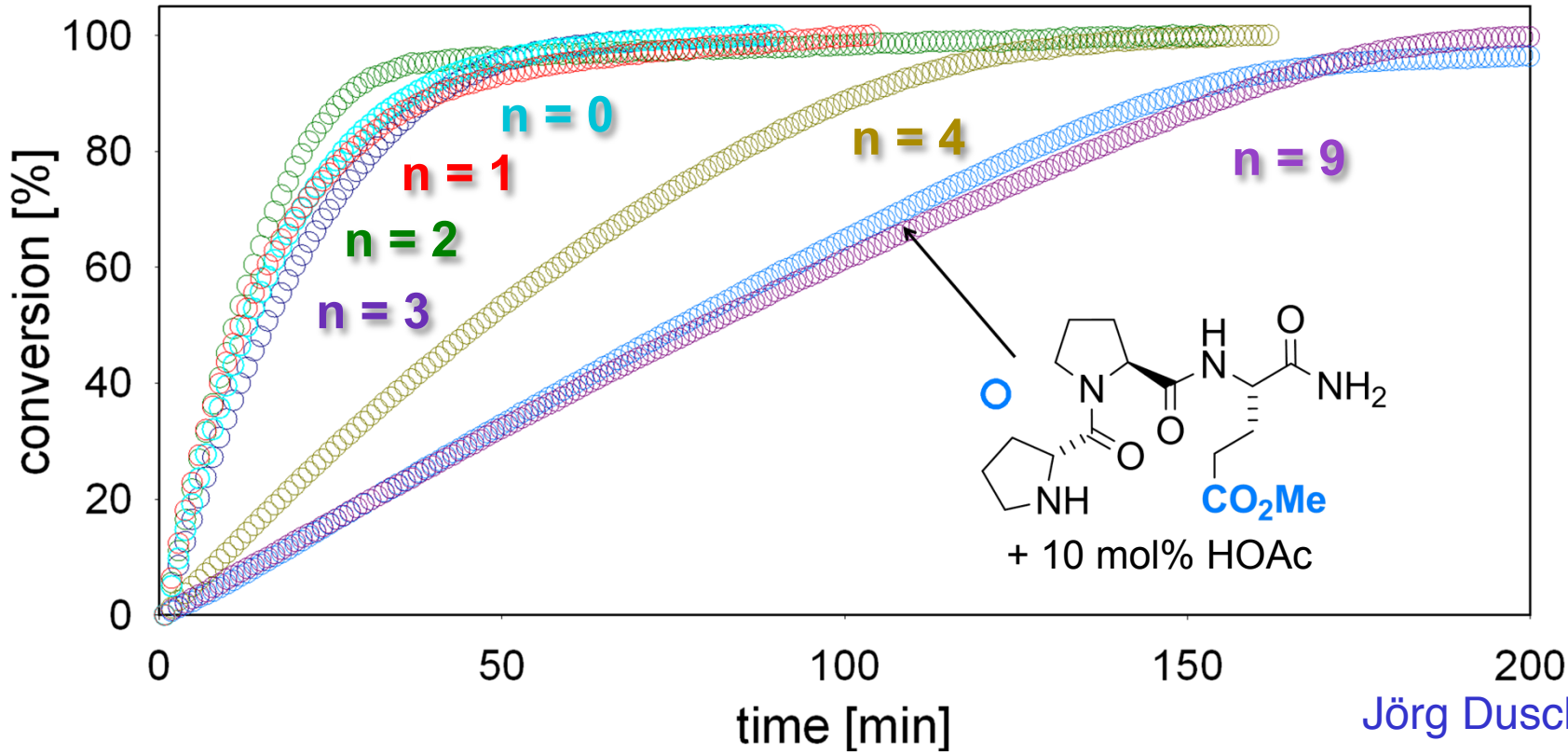


The rate determining step depends on the presence or absence of a suitably positioned intramolecular proton donor.

Probing the Reaction Pathways: Distant Proton Donor

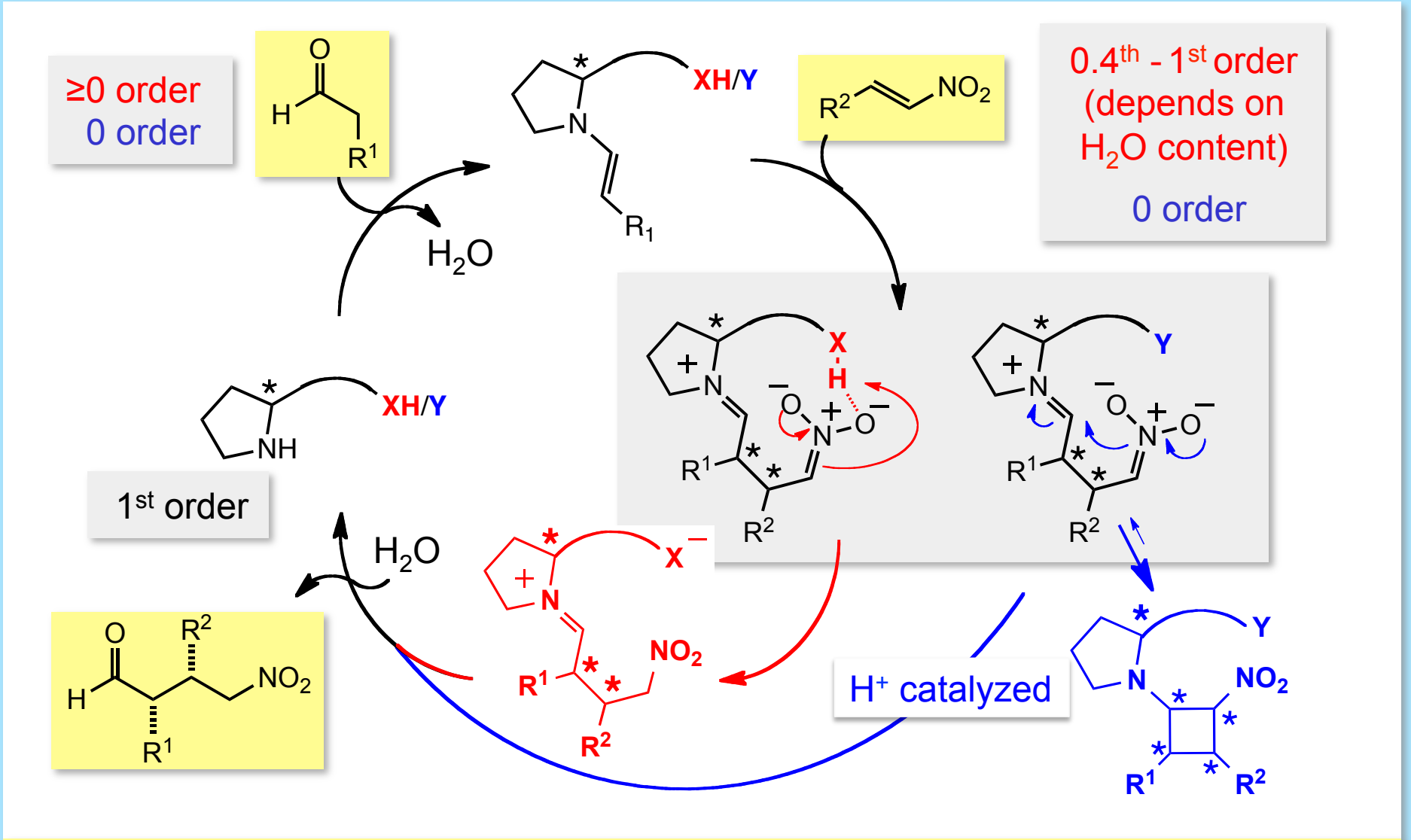






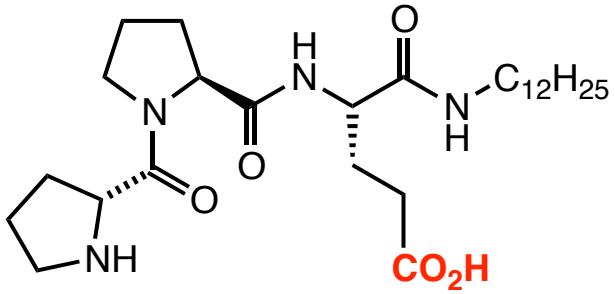
Jörg Duschmalé

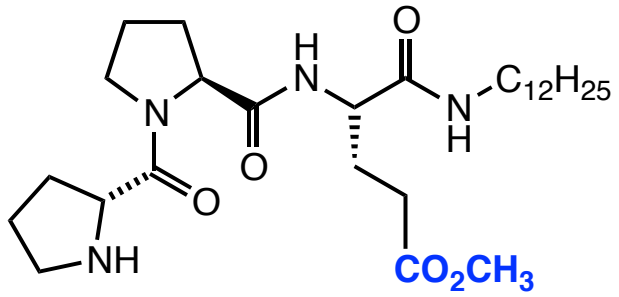
Proposed Reaction Mechanisms

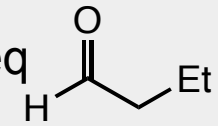
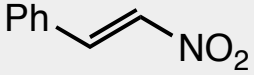


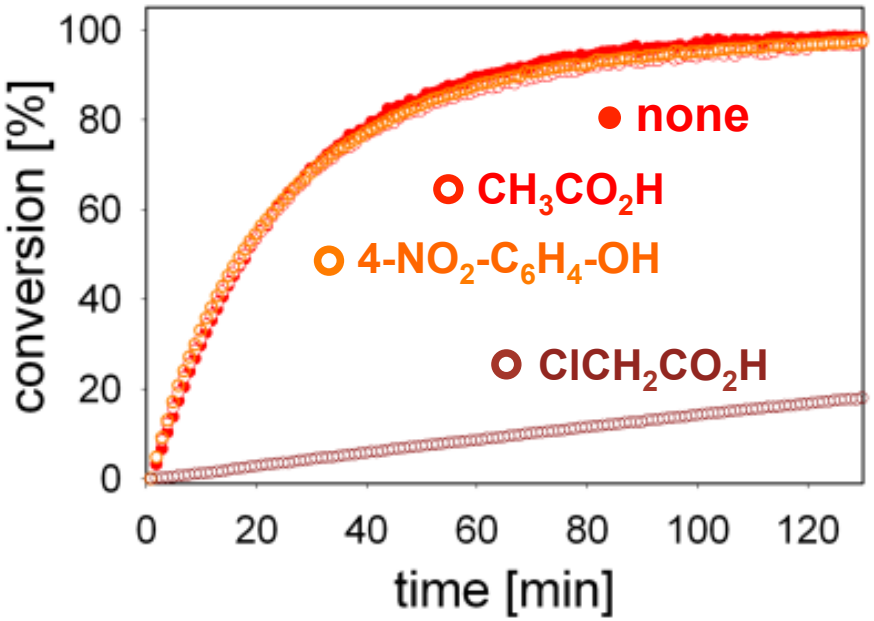
The rate determining step depends on the presence or absence of a suitably positioned intramolecular proton donor.

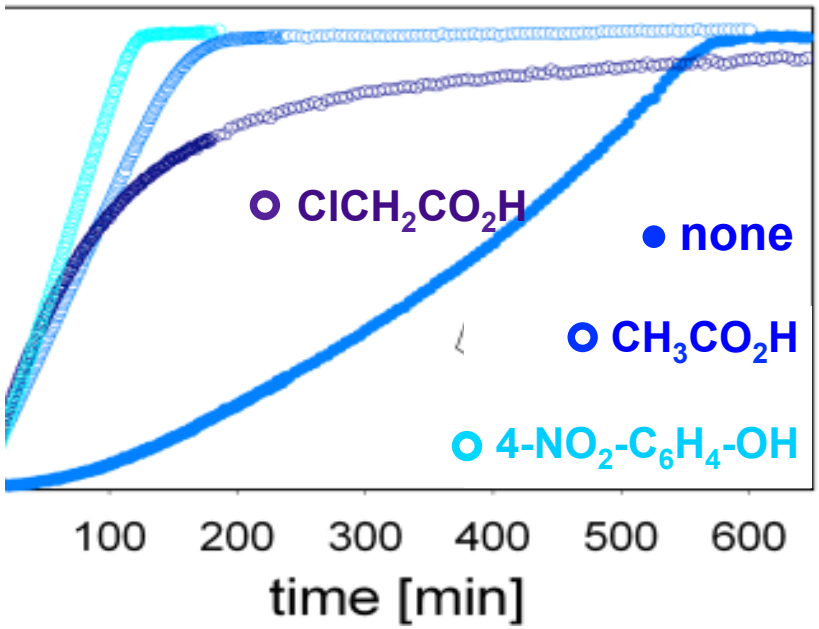
Probing the Reaction Pathways: Addition of Acid



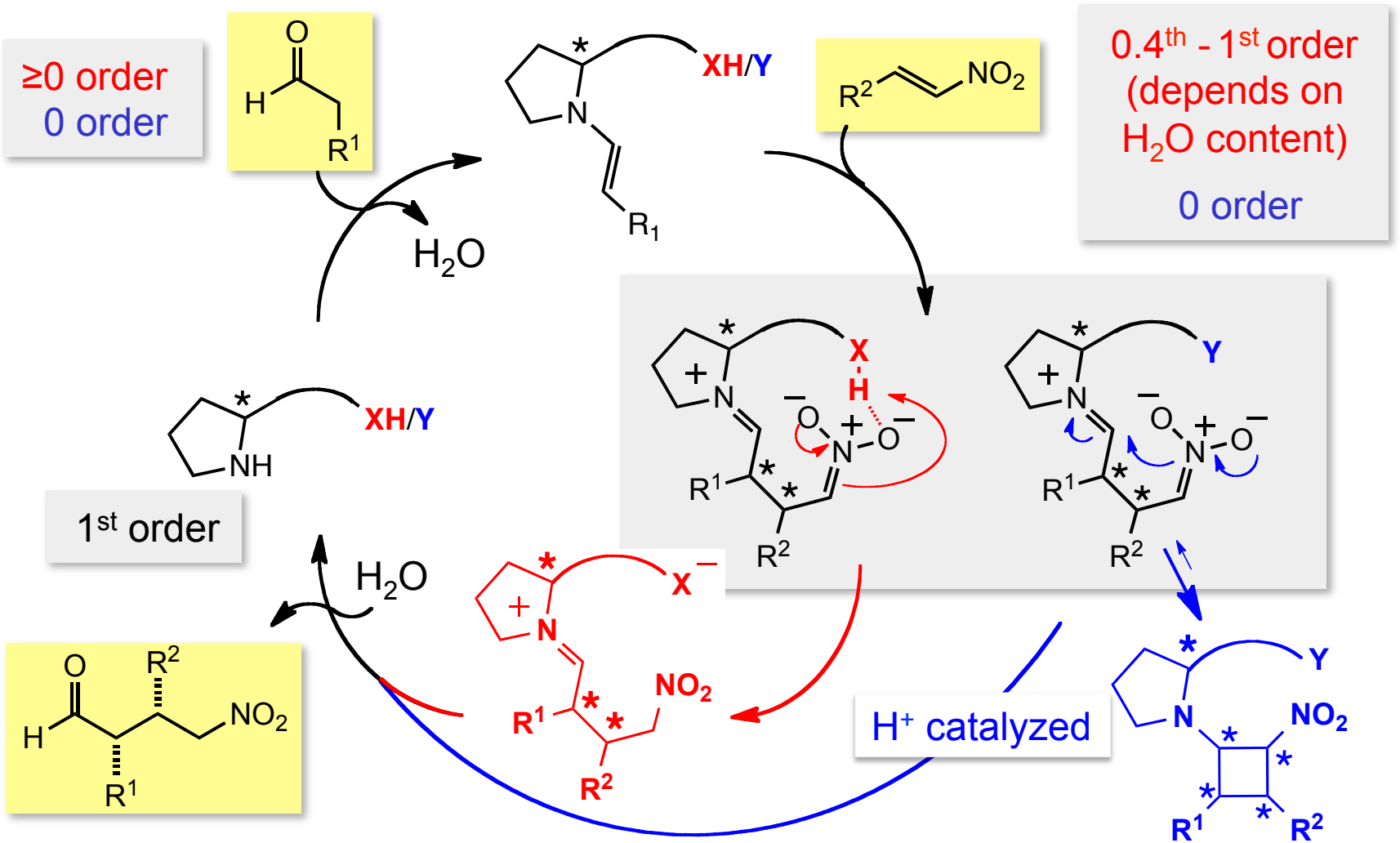


Reaction with 1.5 eq  and 1.0 eq  and **0.1 eq additive**





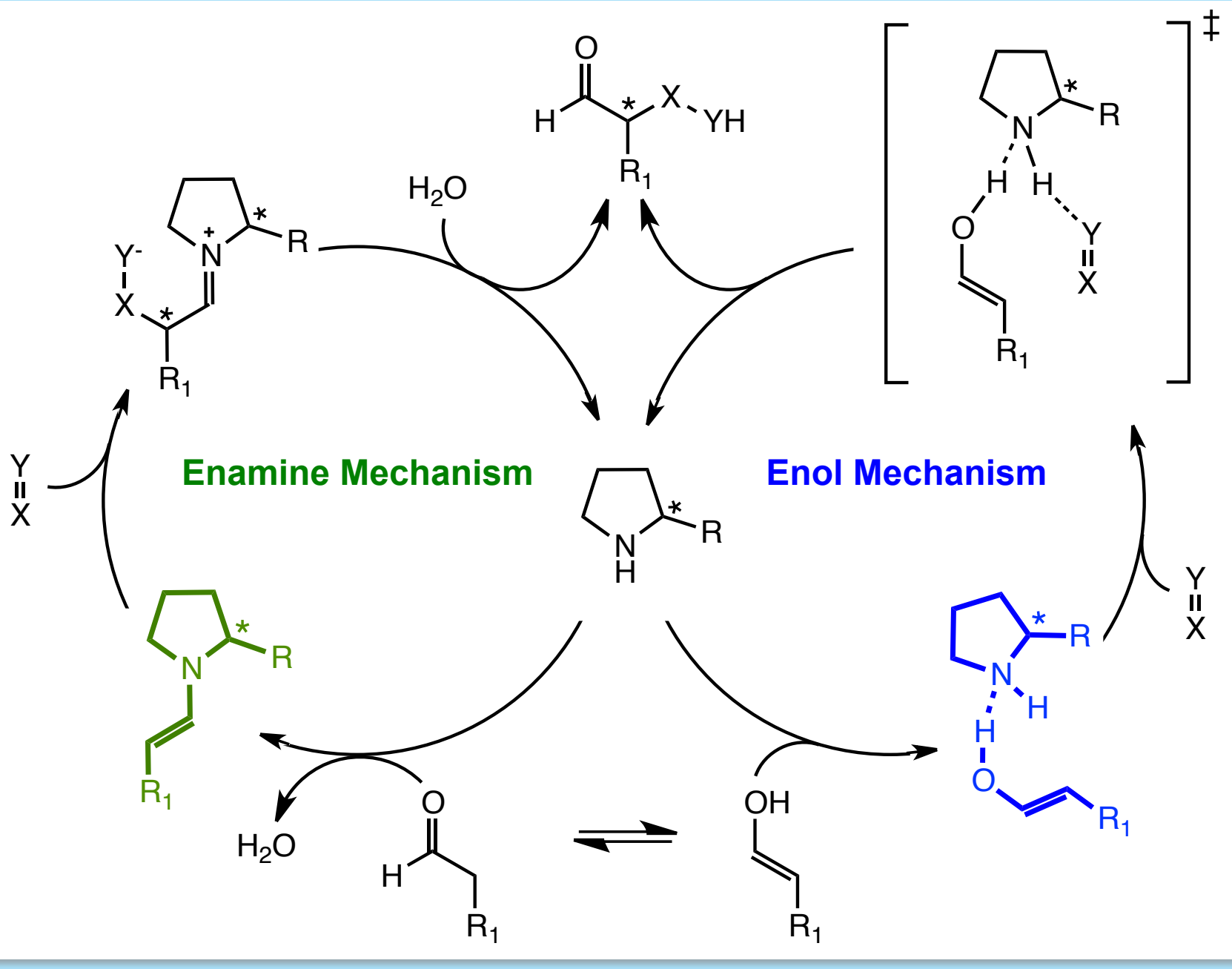
Proposed Reaction Mechanisms



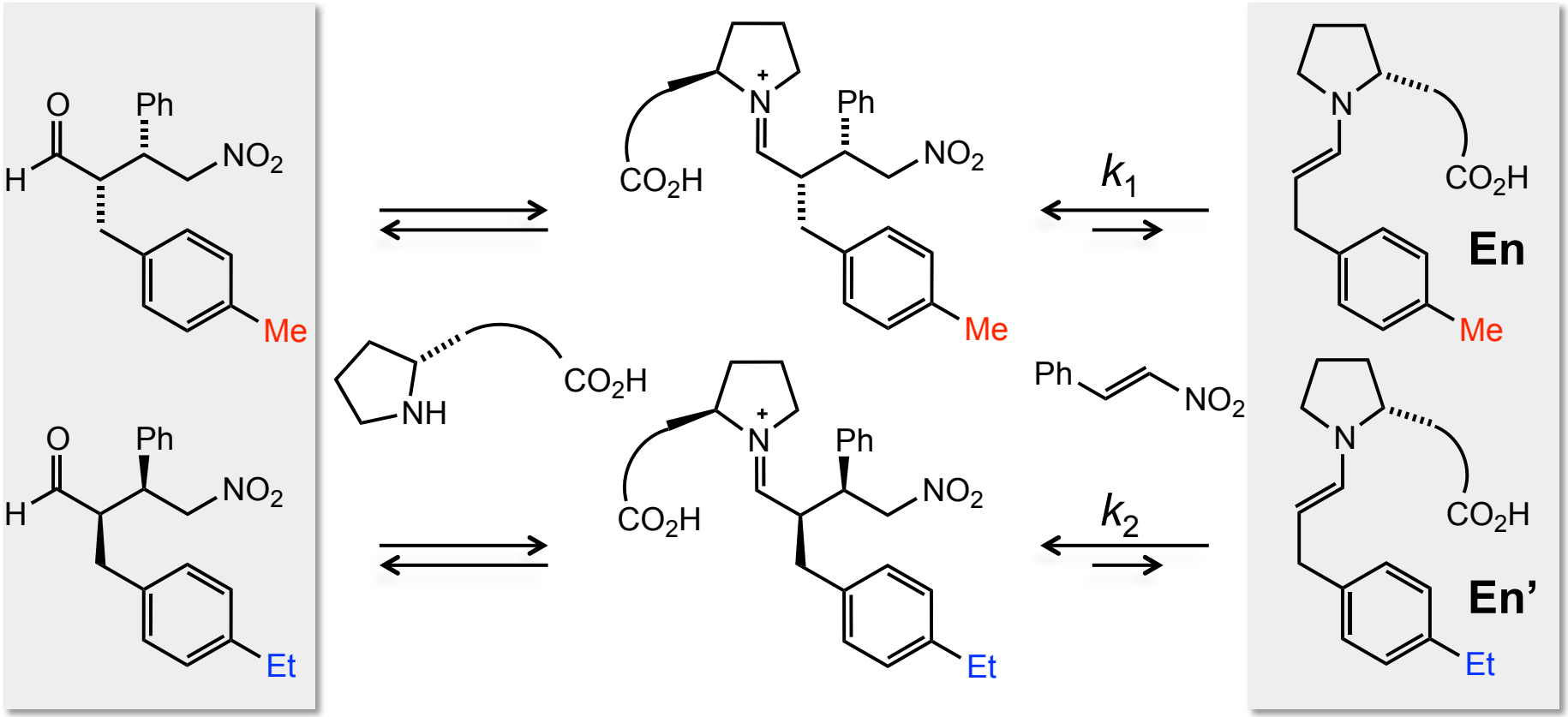
XH = CO_2H, **Y** = no proton donor

J. Duschmalé, J. Wiest, M. Wiesner, H.W. *Chem. Sci.* **2013,** *4*, 1312.

Enol Mechanism or Enamine Mechanism?



ESI-MS Back Reaction Monitoring with Quasienantiomers



En/En' of back reaction = **e.r.** of forward reaction

Enamine mechanism

C-C bond formation enantioselectivity determining step

$$\frac{k_1}{k_2} = \frac{\text{En}}{\text{En'}}$$

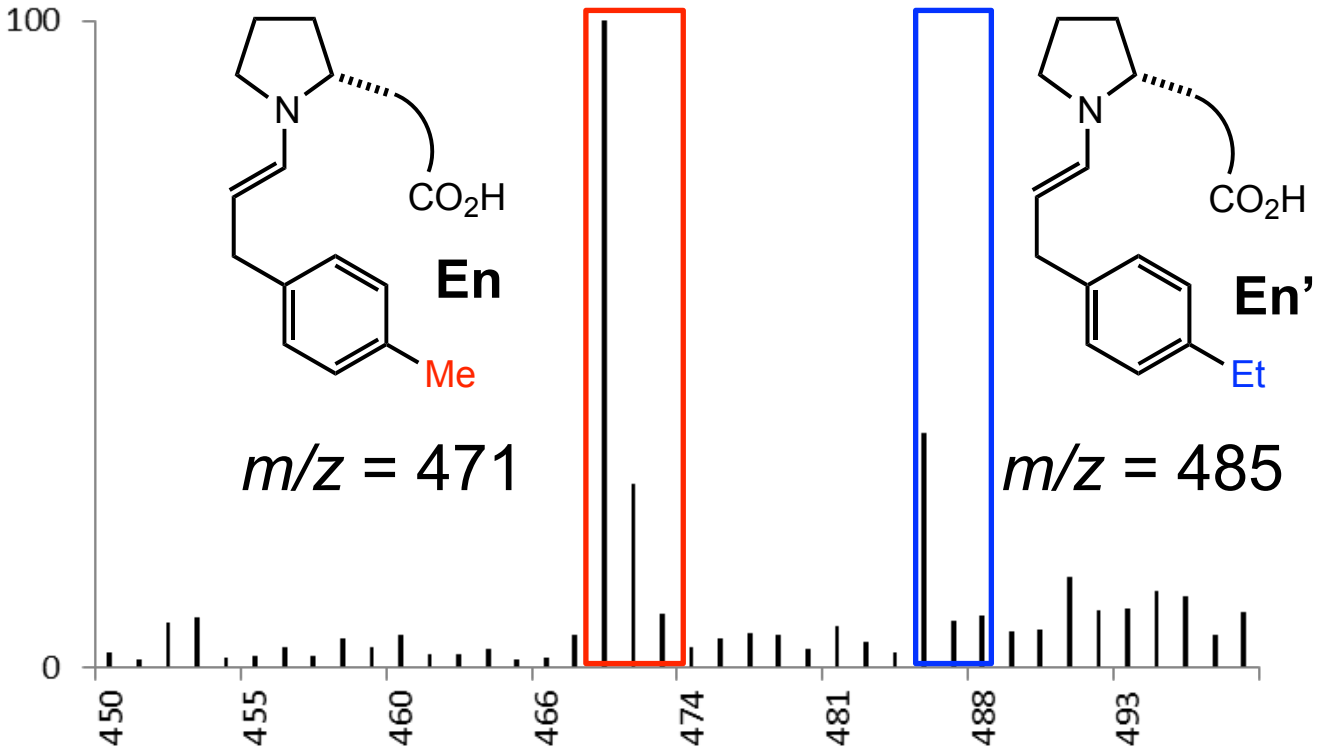
Jörg Duschmalé, with Prof. Andreas Pfaltz

Enamine *versus* Enol Mechanism

53% yield

dr = 5:1

e.r. 73:27



ESI-MS Back Reaction

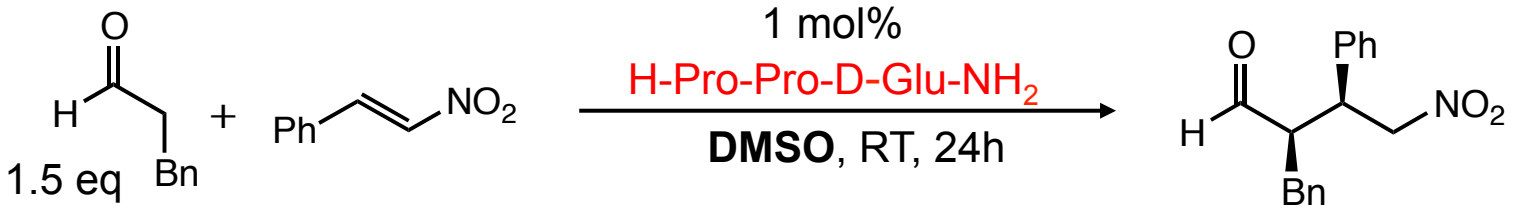
73:27

Enamin Mechanism

C-C bond formation enantioselectivity determining step

F. Bächle, J. Duschmalé, C. Ebner, A. Pfaltz, H.W. *Angew. Chem. Int. Ed.* **2013,** *52,* 12619.

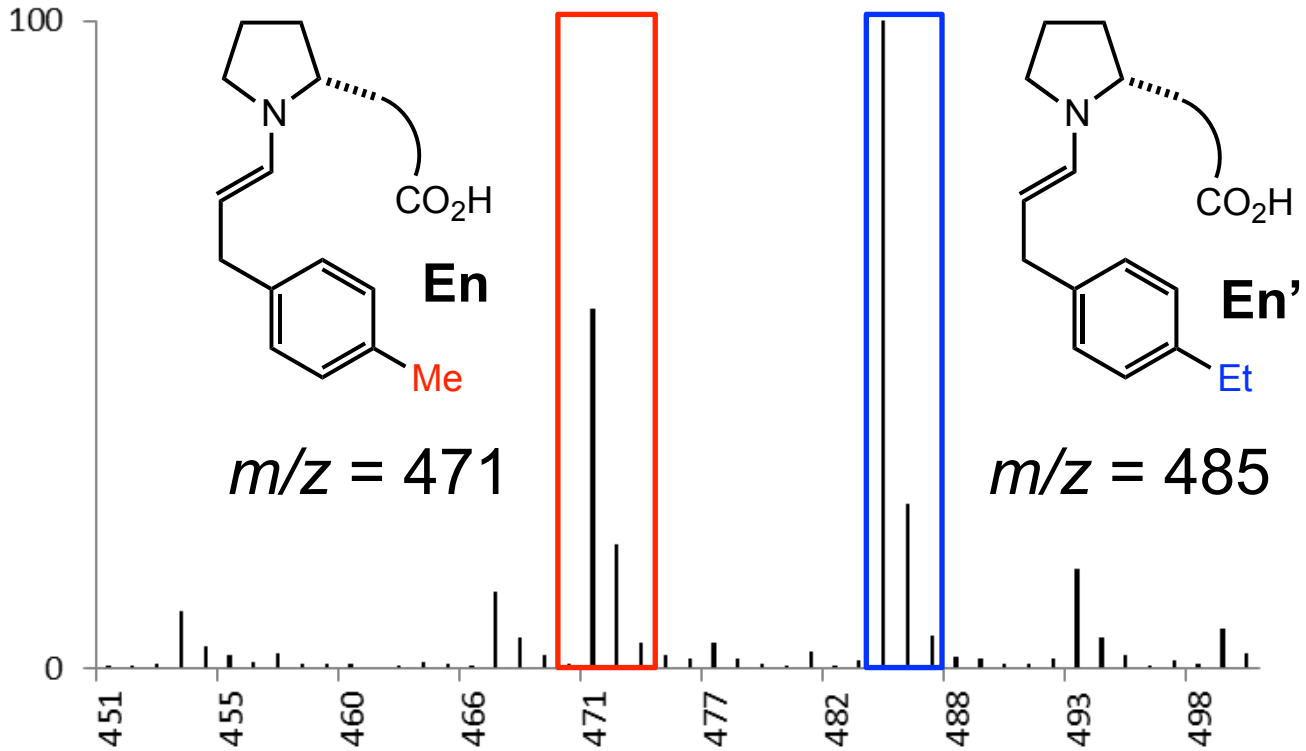
Enamine *versus* Enol Mechanism



48% yield

dr = 6:1

e.r. 35:65



ESI-MS Back Reaction

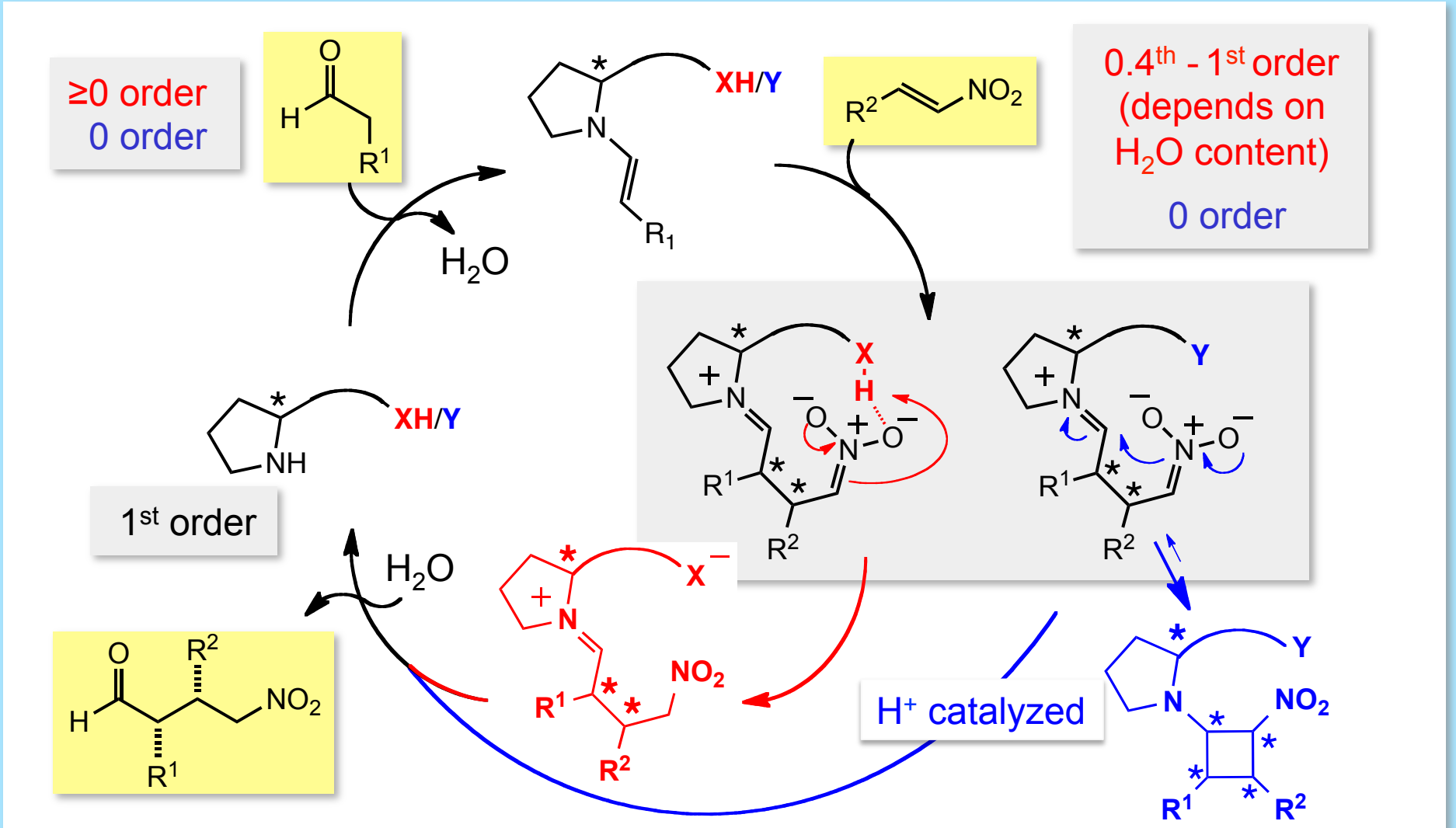
36:64

Enamin Mechanism

C-C bond formation enantioselectivity determining step

F. Bächle, J. Duschmalé, C. Ebner, A. Pfaltz, H.W. *Angew. Chem. Int. Ed.* **2013,** *52,* 12619.

Proposed Reaction Mechanism



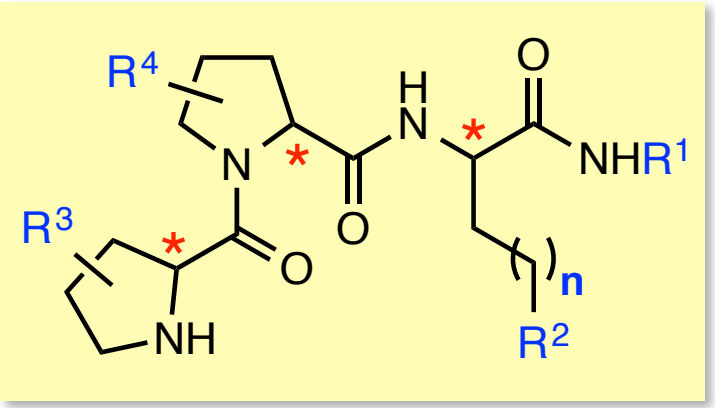
The rate determining steps depend on the presence or absence of an intramolecular proton source.

Jörg Duschmalé

Summary and Conclusions

H-Pro-Pro-Xaa-R (Xaa = acid amino acid)

- **Highly reactive, modular, robust**
  - mild reaction conditions
  - broad substrate scope
  - flow system
  - no additives necessary



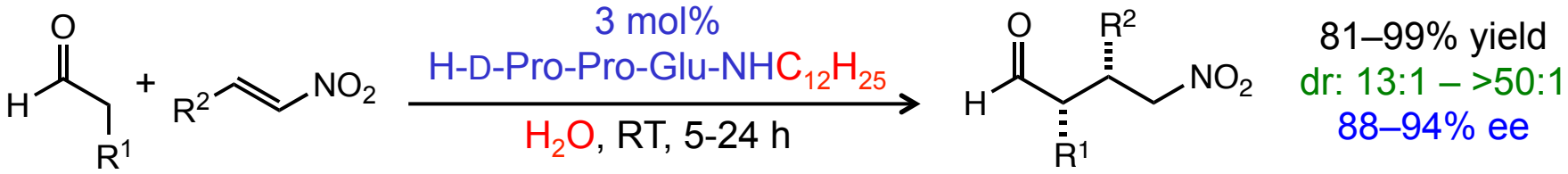
- **Chemo- and Stereoselectivity is tunable and adaptable**

- **Conformational flexibility is likely key to high effectiveness**

Peptidic Catalysts: Features of Enzymes and Synthetic Catalysts

Summary and Conclusions

Catalysis in Water

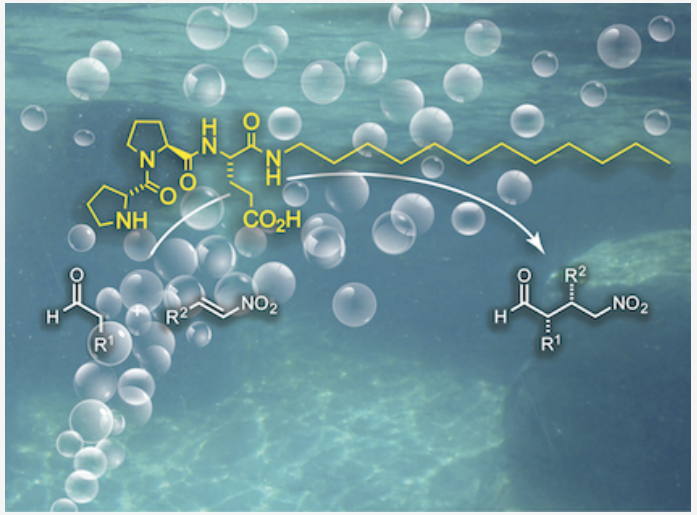


alkylated peptide in H_2O

foam

after addition of reactants

emulsion



J. Duschmalé, S. Kohrt, H.W., *Chem. Commun.*, **2014**, *50*, 8109.

Peptides might have played a role in the evolution of enzymes


Thank you!
Funding
Swiss National Science Foundation
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