Metal Species for Amide Hydrolysis

Literature Seminar, 20130511

Kiyomichi SHINODA (M1)

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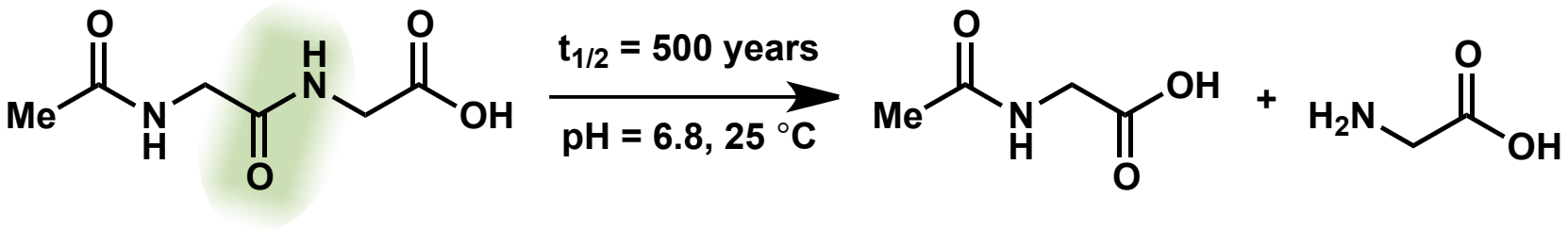
§1 Introduction

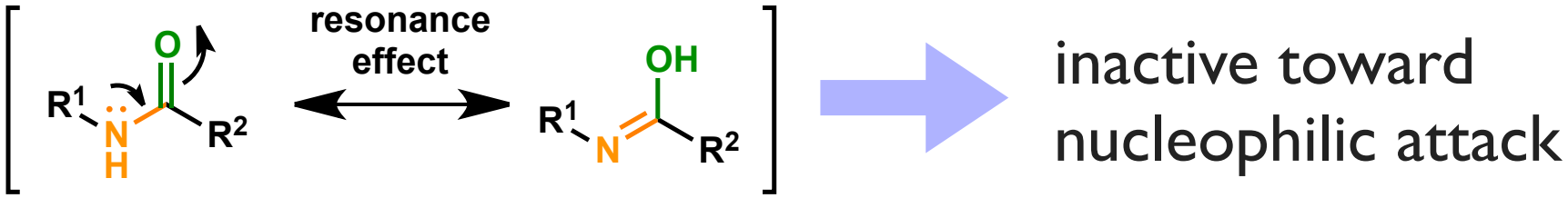
Characteristics of Amide Bond

- stability toward hydrolytic cleavage



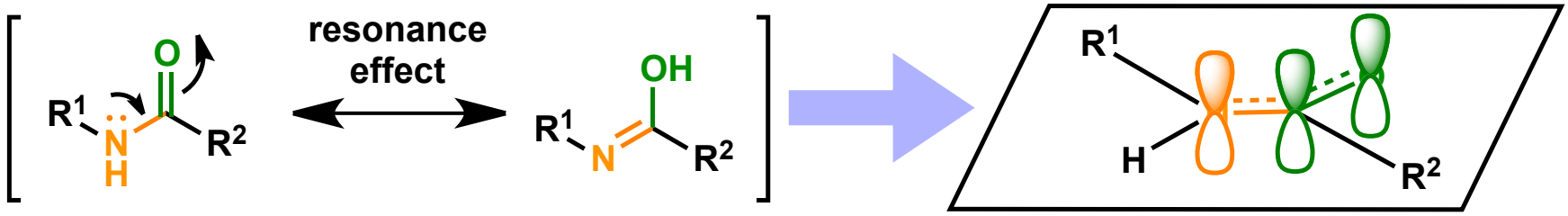
A. Radzicka, R. Wolfenden, *J. Am. Chem. Soc.* **1996,** *118,* 6105-6109.

- This stability derives from resonance effect.

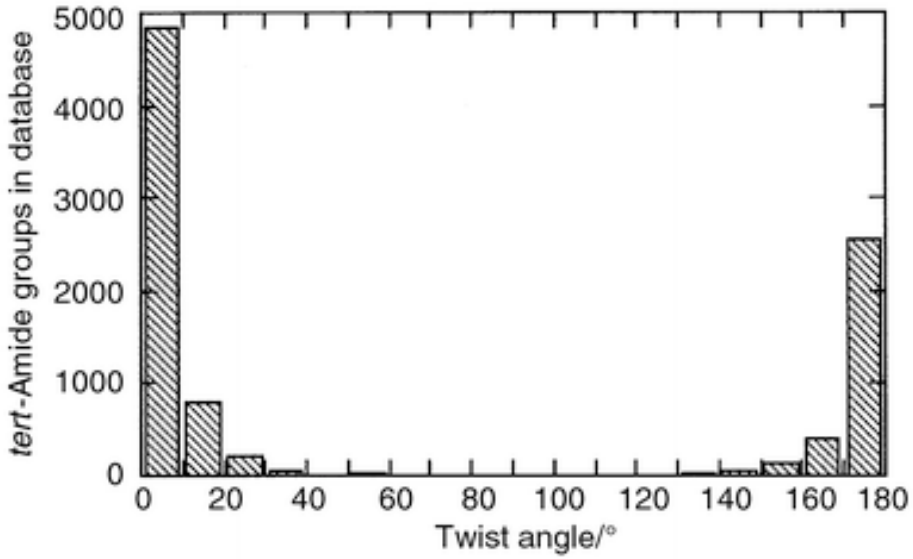


Characteristics of Amide Bond

- planar structure due to the resonance effect



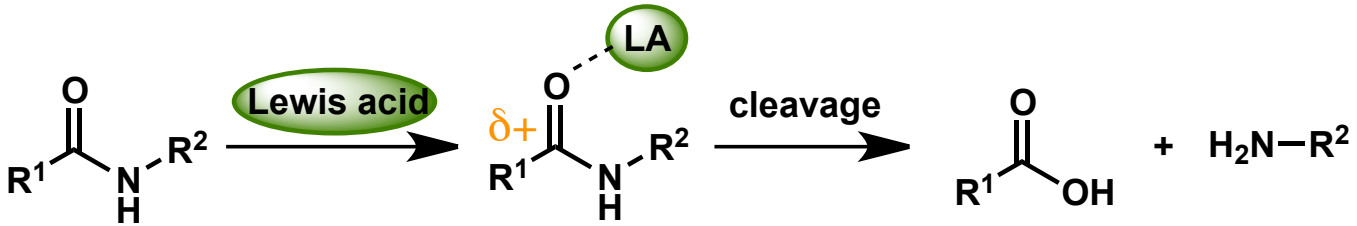
- twist angle distribution of tertiary amides



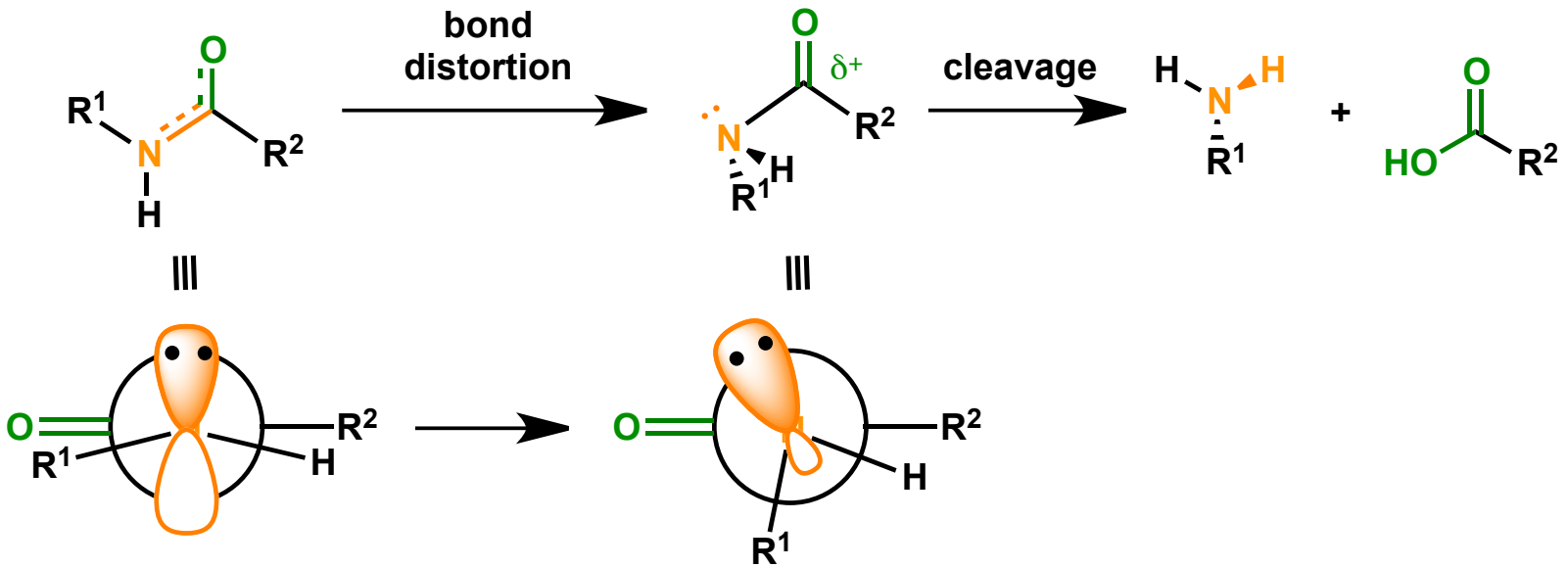
(A. J. Kirby *et al.*, *J. Chem. Soc., Perkin Trans. 2* **2001**, 522.)

Destabilizing Amide Bond

- activation by Lewis acid

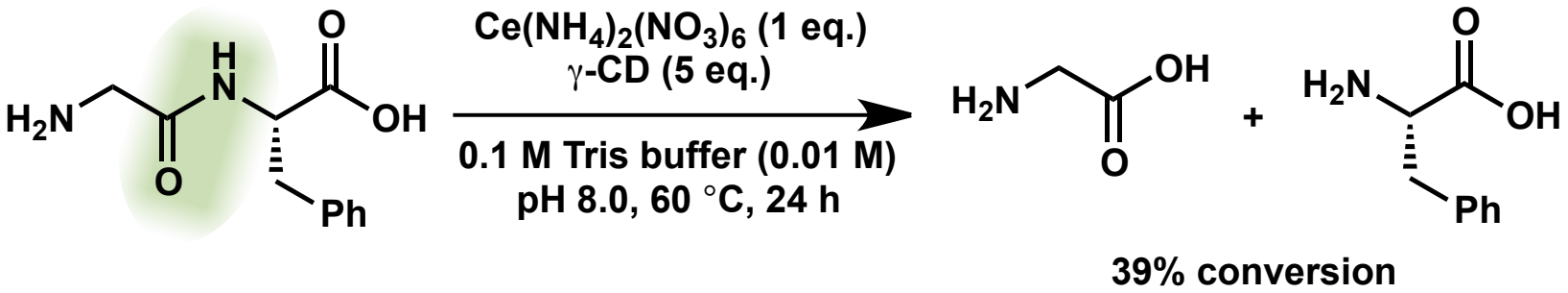


- activation by bond distortion



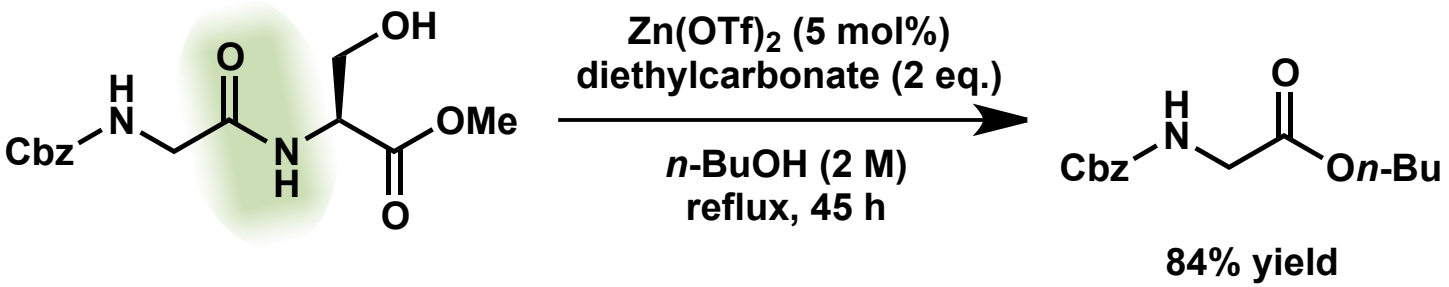
Precedents: Activation by Lewis Acid

- hydrolysis by cerium(IV)-cyclodextrin complex



M. Komiyama *et al.*, *J. Chem. Soc., Chem. Commun.* **1994**, 1758.

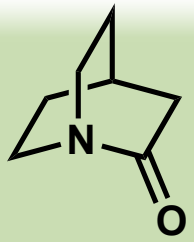
- Zinc-catalyzed solvolysis



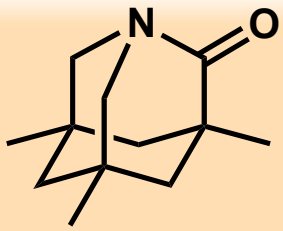
K. Mashima *et al.*, *Angew. Chem. Int. Ed.* **2012**, *51*, 5723.

Precedents: Activation by Bond Twisting

- molecules containing twisted amide



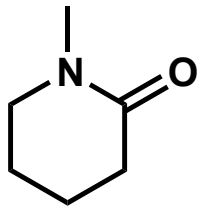
2-quinoclidone

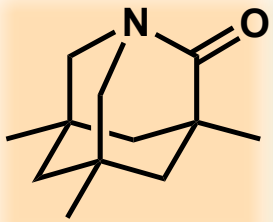


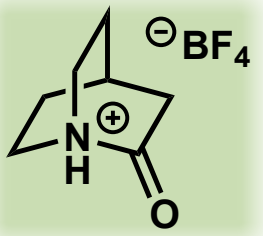
1-aza-2-adamantanone (Kirby's Amide)

total synthesis of
- the former: K. Tani & B. M. Stoltz, *Nature* **2006**, *441*, 731. (tetrafluoroborate salt)
- the latter: A. J. Kirby *et al.*, *J. Chem. Soc., Perkin Trans. 2*, **2001**, 522.

- characters





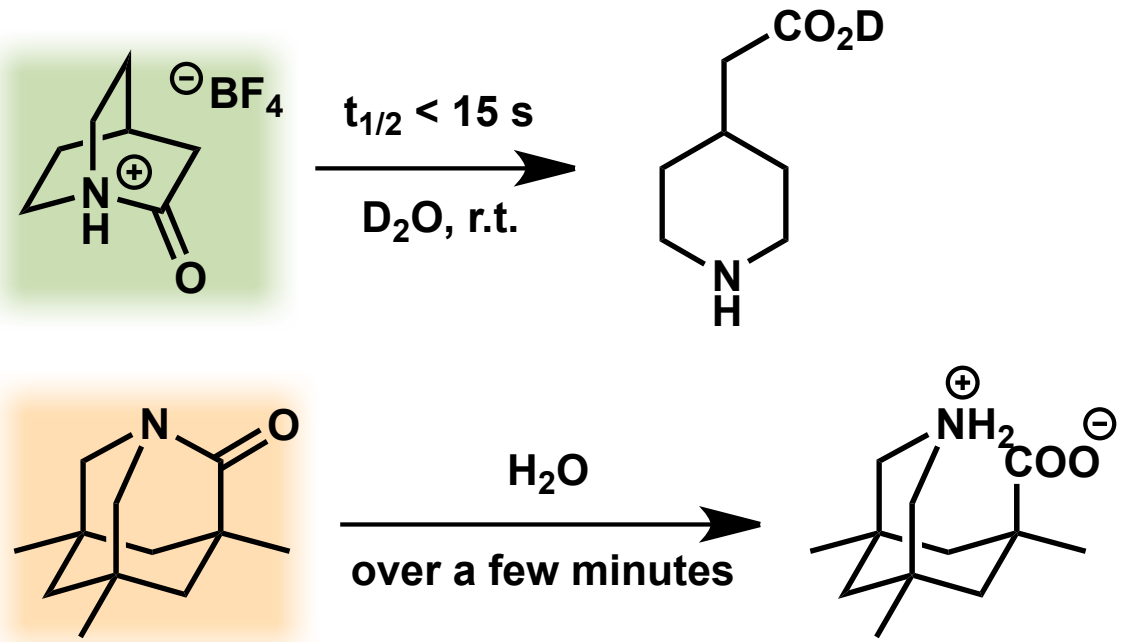


twist angle	**2.5°**	**90.5°**	**90.9°**
bond length C-N	**1.325 Å**	**1.475 Å**	**1.526 Å**

(Values are cited from the above 2 papers .)

Precedents: Activation by Bond Twisting

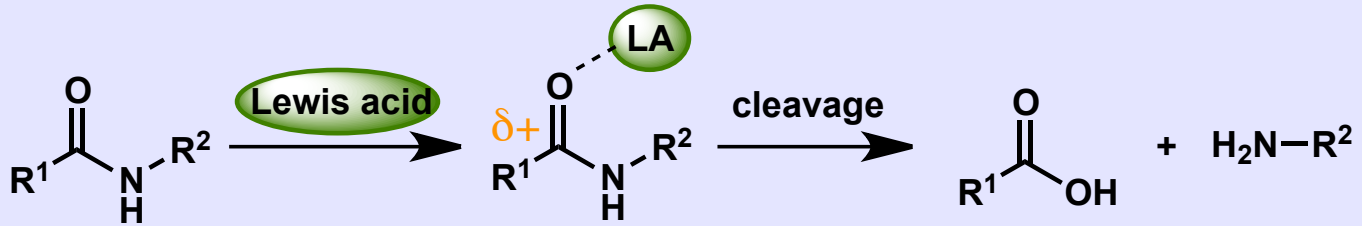
- hydrolysis of twisted amides



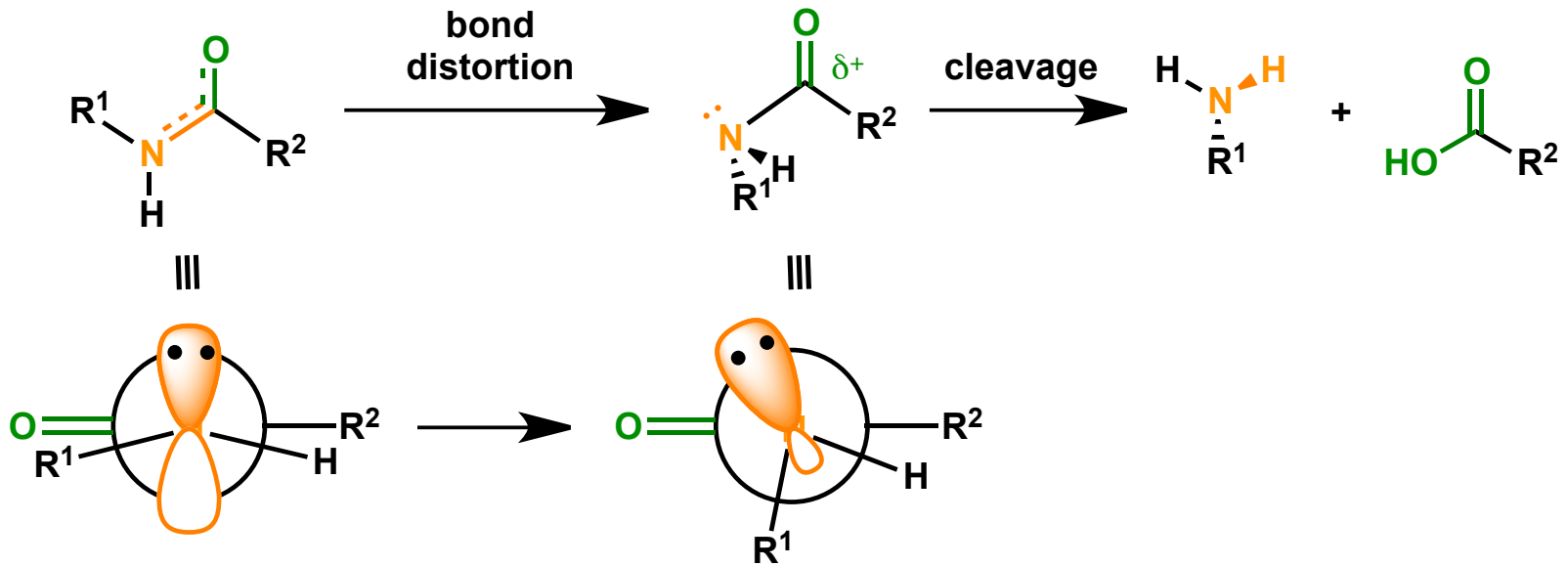
K. Tani & B. M. Stoltz, *Nature* **2006**, *441*, 731.
A. J. Kirby *et al.*, *J. Am. Chem. Soc.*, **1998**, *120*, 7101.

In This Seminar...

- activation by Lewis acid

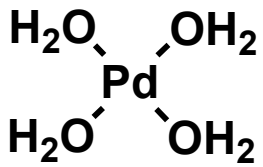


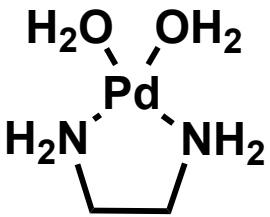
- activation by bond distortion

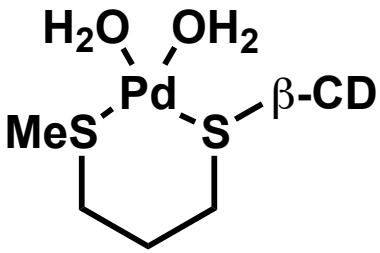


§2 Residue- or Sequence-Selective Hydrolysis of Amides

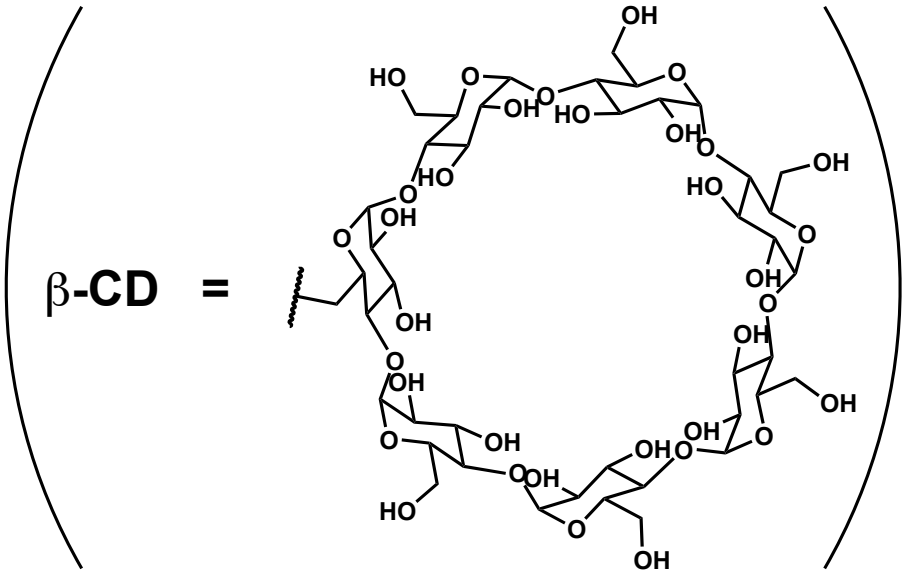
Palladium(II) Complexes







$[Pd(H_2O)_4]^{2+}$ $[Pd(en)(H_2O)_2]^{2+}$

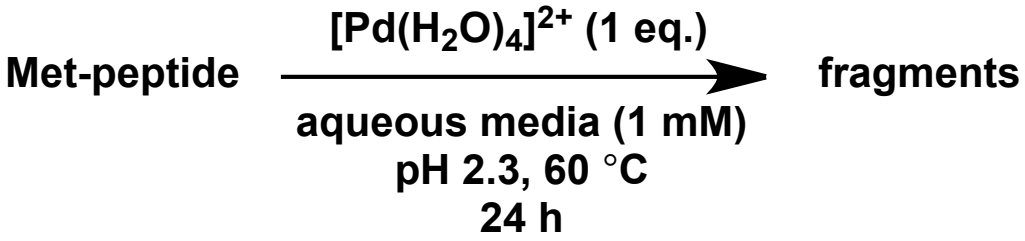


Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- substrate: Met-peptide

Ac-Ala-Lys-Tyr-Gly-Gly-Met-Ala-Ala-Arg-Ala-OH

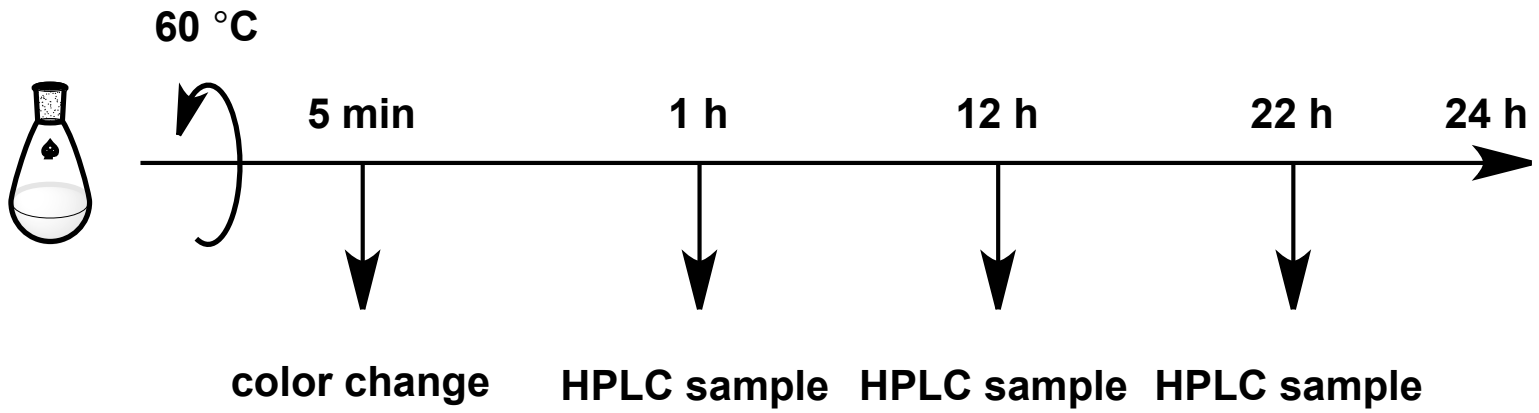
- hydrolysis condition



N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- experiment procedure

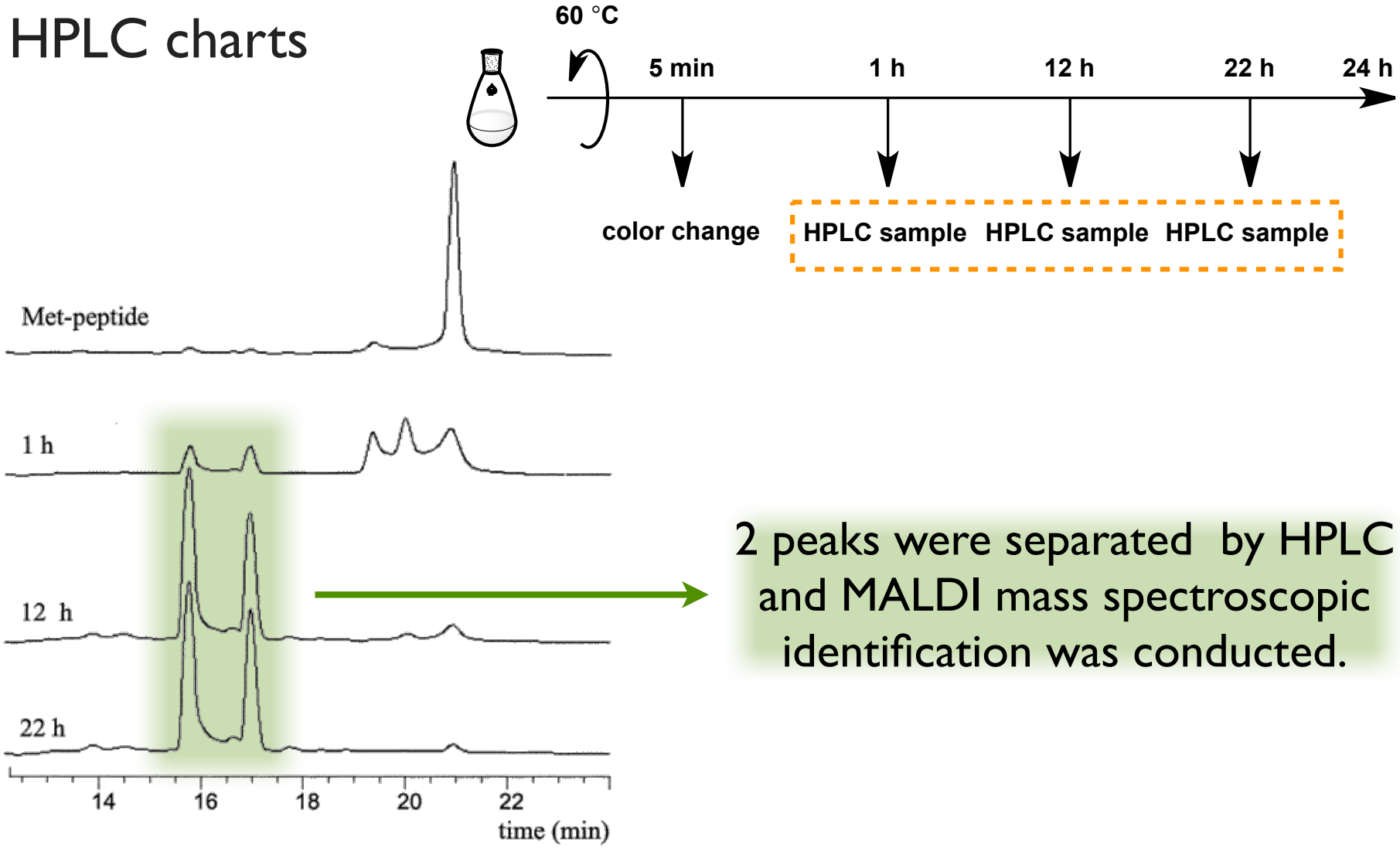


  - 5 min: $[Pd(H_2O)_4]^{2+}$ was bound to the peptide.
    (checked by HPLC and MALDI MS)
  - 24 h: two components were observed.

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- HPLC charts

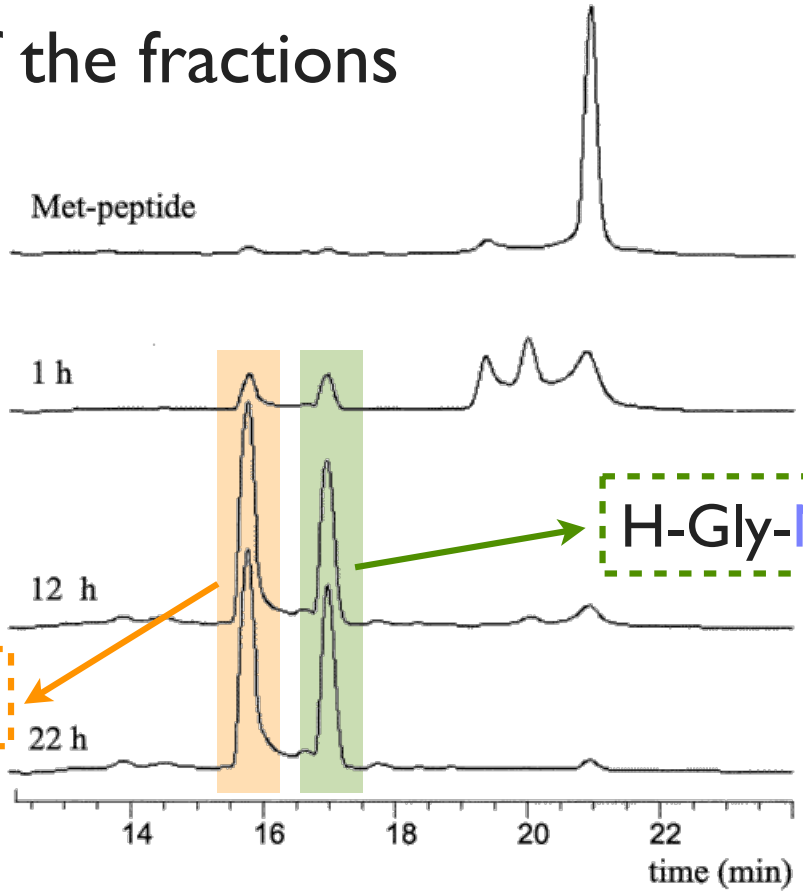


2 peaks were separated by HPLC and MALDI mass spectroscopic identification was conducted.

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- composition of the fractions



H-Gly-Met-Ala-Ala-Arg-Ala-OH

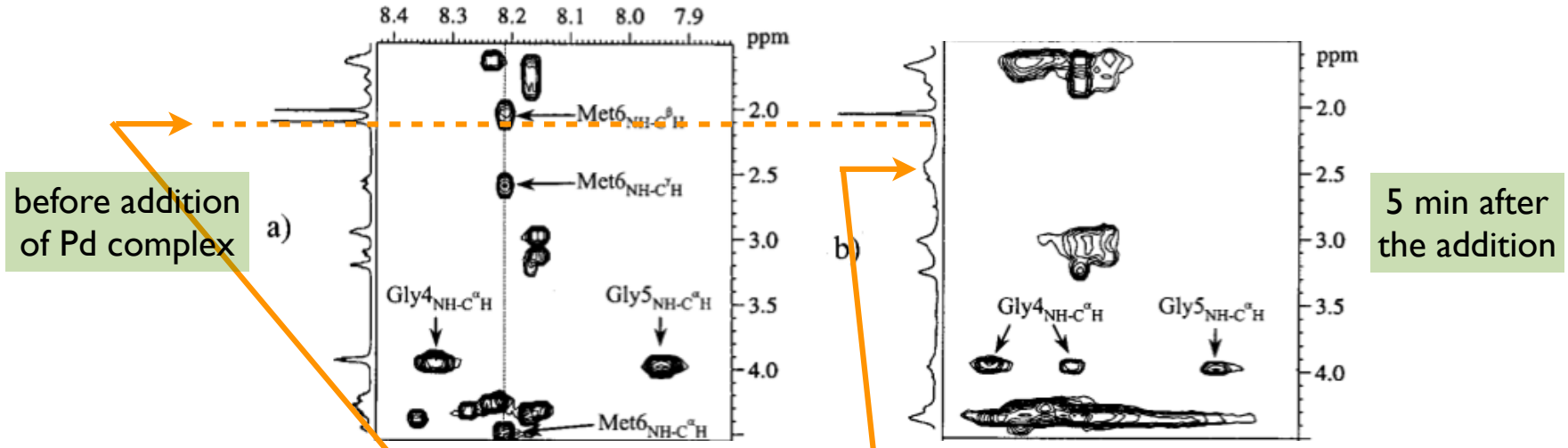
Ac-Ala-Lys-Tyr-Gly-OH

Ac-Ala-Lys-Tyr-Gly-Gly-Met-Ala-Ala-Arg-Ala-OH

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- TOCSY ^{1}H NMR spectrum



N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

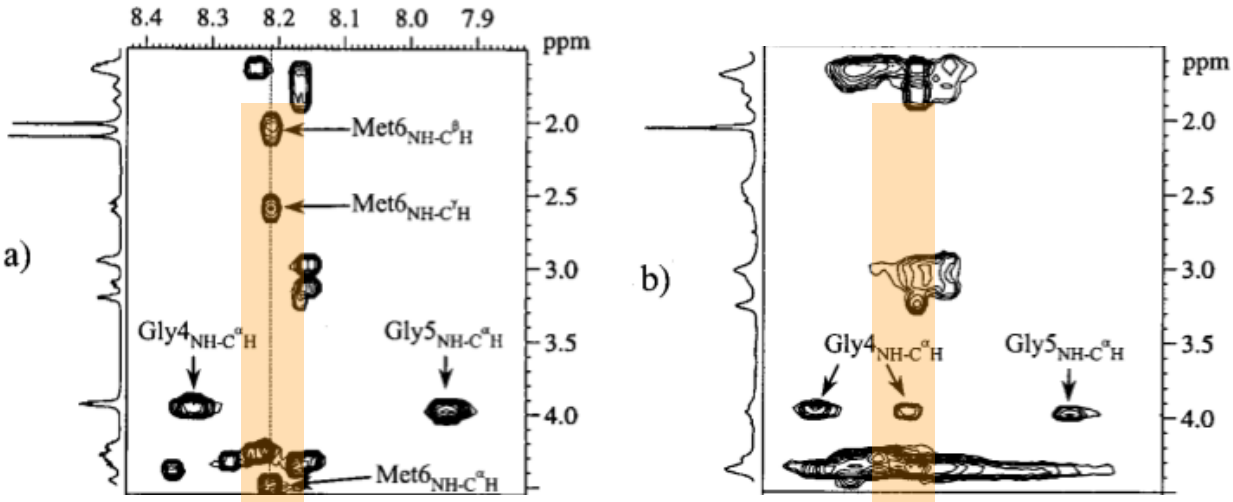
- SMe singlet: 2.12 ppm → 2.45 ppm

  = Pd(II) complex was bound to the Met6 side chain.

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- TOCSY ^{1}H NMR spectrum

before addition of Pd complex



5 min after the addition

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

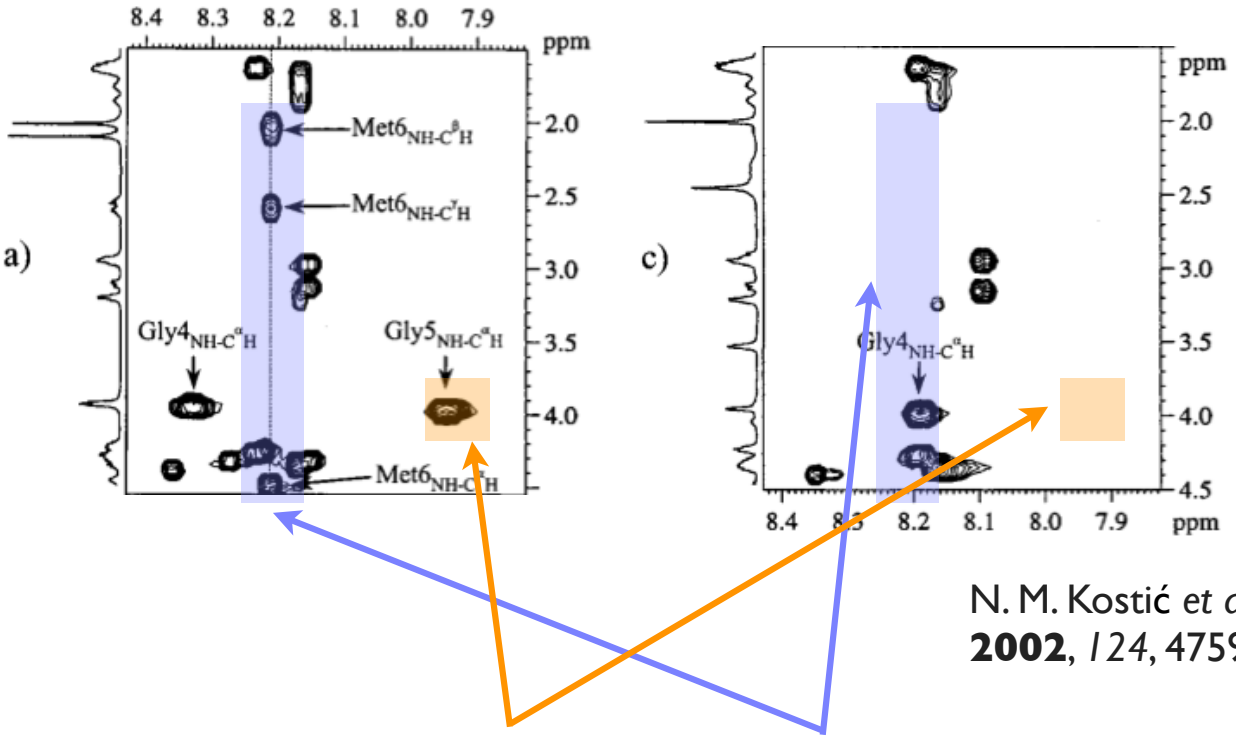
- Met6$_{NH\text{-}CH}$ signal disappeared: deprotonated

  = complete coordination of amide to the Pd(II) ion

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- TOCSY ^{1}H NMR spectrum

before addition of Pd complex



24 h after the addition (reaction finished)

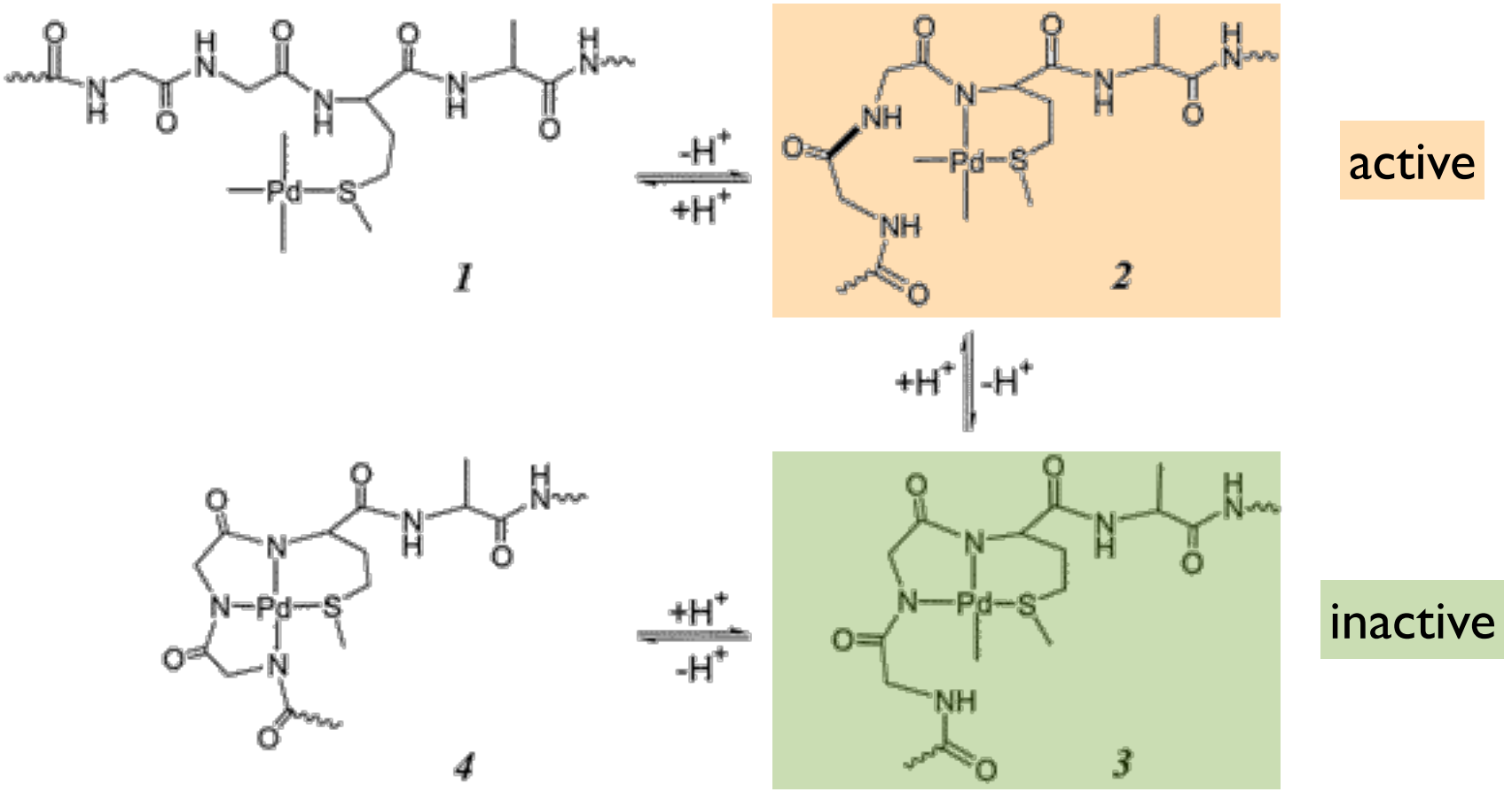
N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

- Signals derived from Gly5 and Met6 disappeared.

  = Amide cleavage occurred at Gly4-Gly5.

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- equilibrium among Pd(II) complexes

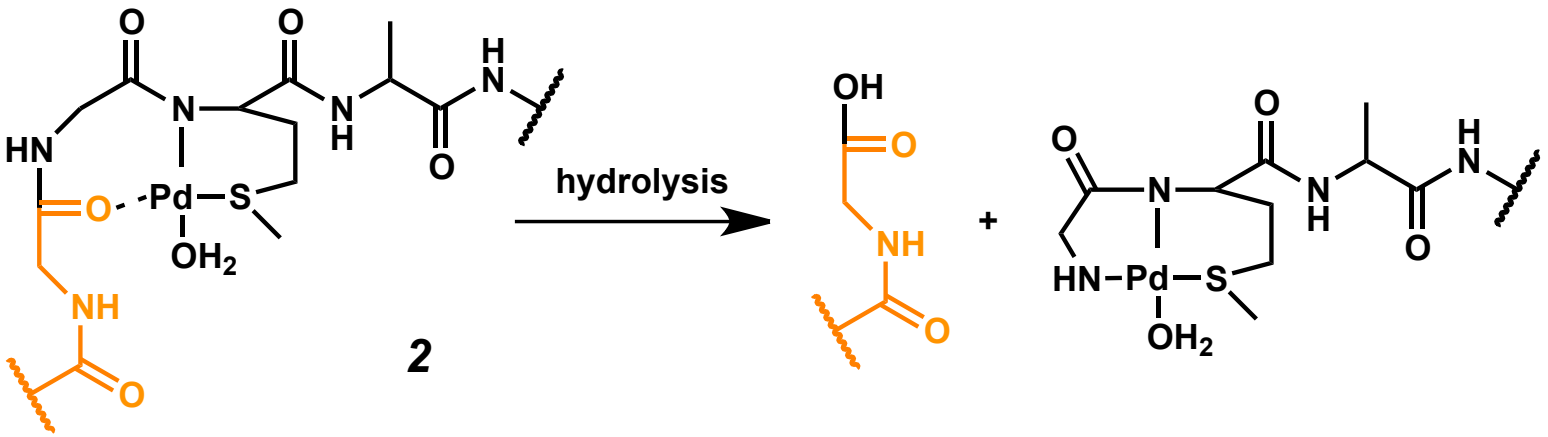


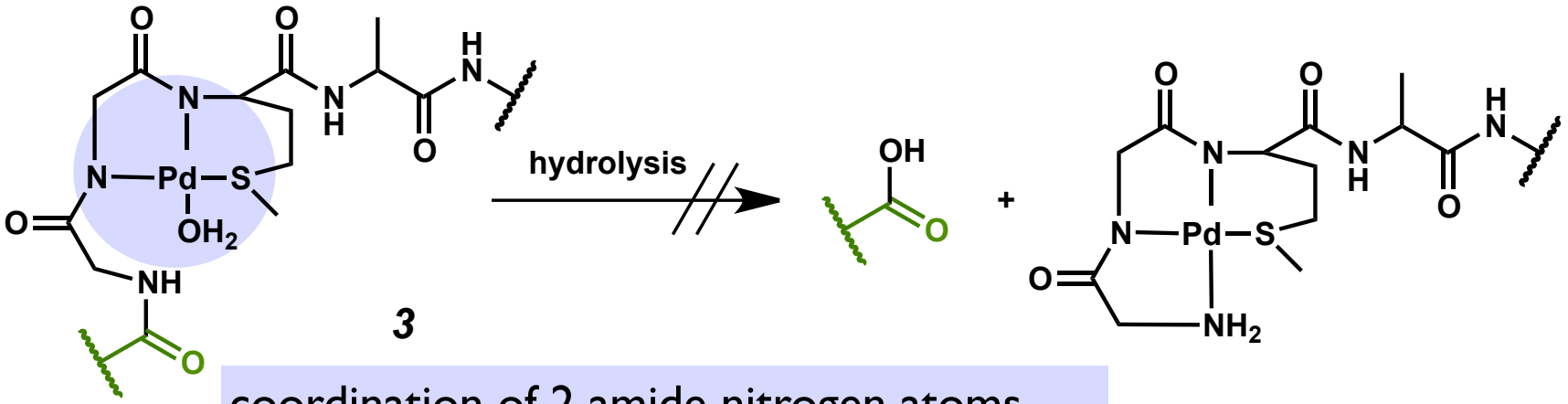
- **2** and ***3*** exists at pH 2.3, and ***3*** is major.

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

Acidic Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- ***2*** is hydrolytically active, but ***3*** is hydrolytically inactive.





coordination of 2 amide nitrogen atoms
→ Lewis acidity of Pd(II) ion was quenched.

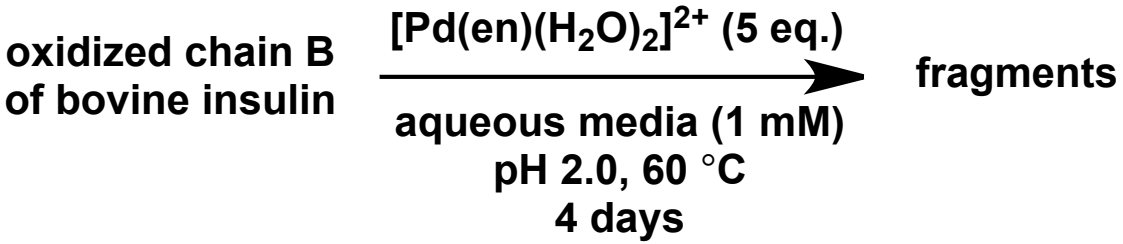
N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 4759.

Acidic Hydrolysis with $[Pd(en)(H_2O)_2]^{2+}$

- substrate: oxidized chain B of bovine insulin

  H-Phe-Val-Asn-Gln-His-Leu-Cys^{OX}-Gly-Ser-His-Leu-Val-
  -Glu-Ala-Leu-Tyr-Leu-Val-Cys^{OX}-Gly-Glu-Arg-Gly-Phe-
  -Phe-Tyr-Thr-Pro-Lys-Ala-OH

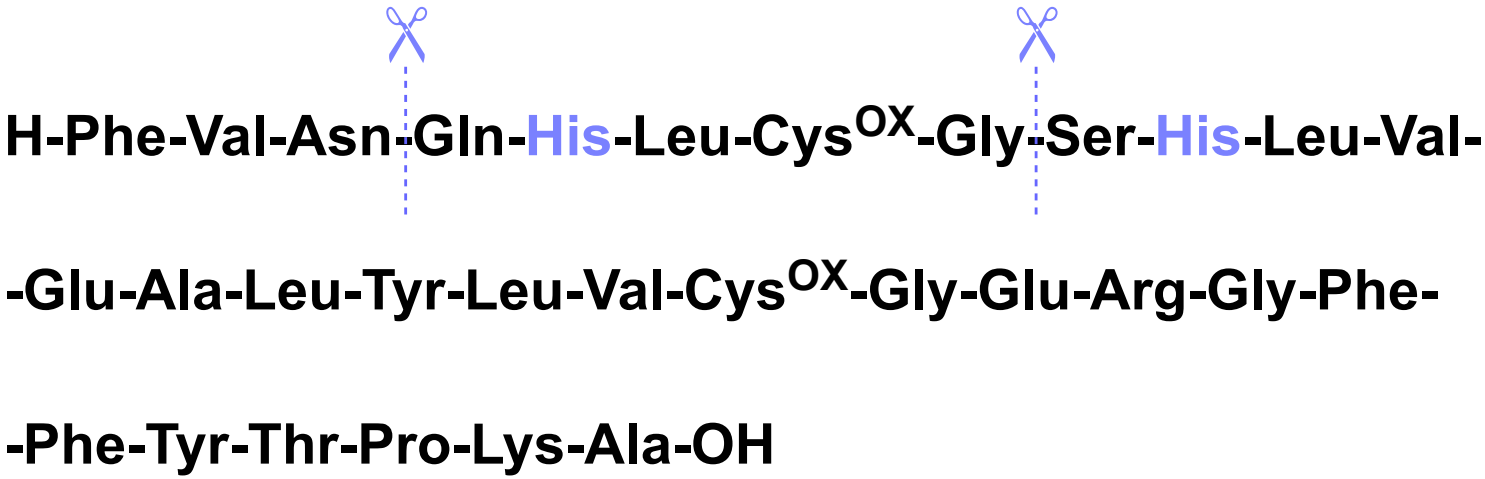
- hydrolysis condition



N. M. Kostić *et al.*, *Inorg. Chem.* **2002**, *41*, 7053.

Acidic Hydrolysis with $[Pd(en)(H_2O)_2]^{2+}$

- cleavage site



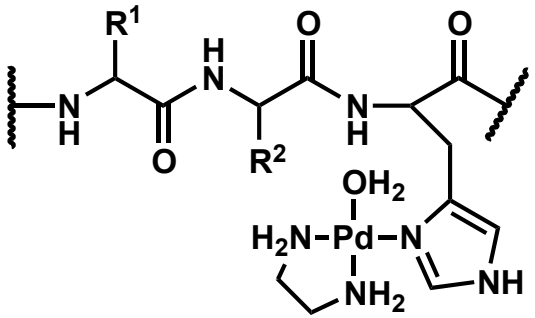
  - Pd(II) reagent promoted cleavage of the second bond upstream from the His5 and His10 anchor.

    (characterized by MALDI mass spectroscopic identification and amino acid analysis: N-terminal Edman degradation)

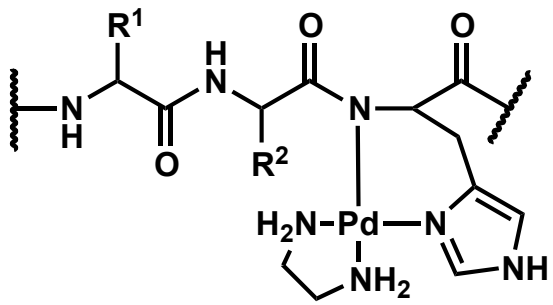
N. M. Kostić *et al.*, *Inorg. Chem.* **2002**, *41*, 7053.

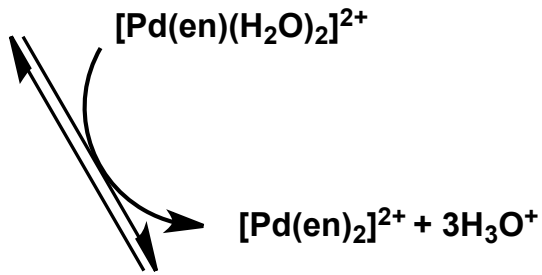
Acidic Hydrolysis with $[Pd(en)(H_2O)_2]^{2+}$

- equilibrium among Pd(II) complexes

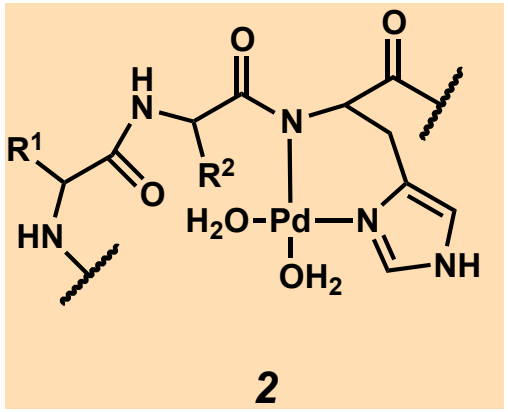


1



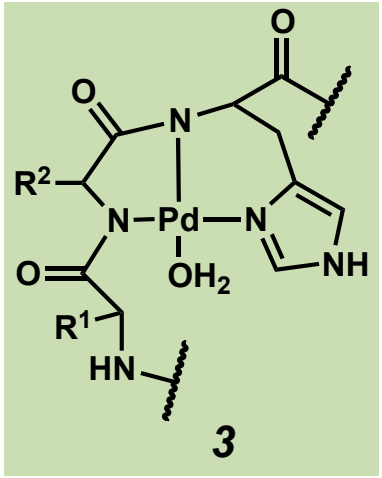


- ○ ***2*** and ***3*** exists at pH 2.0



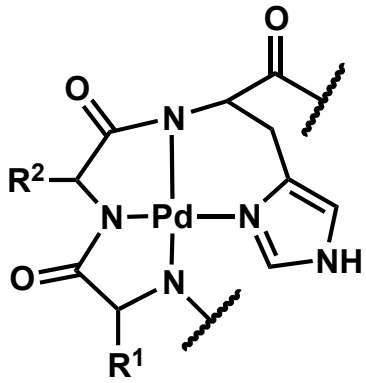
2

active



3

inactive



4

N. M. Kostić *et al.*, *Inorg. Chem.* **2002**, *41*, 7053.

Neutral Hydrolysis with $[Pd(H_2O)_4]^{2+}$

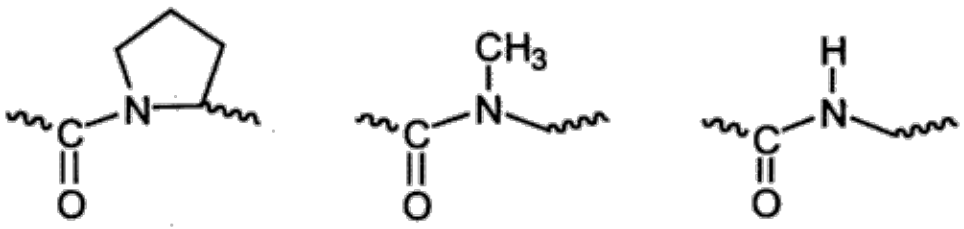
- substrates & cleavage site

GlyMet-peptide: Ac-Ala-Lys-Tyr-Gly-Gly-Met-Ala-Ala-Arg-Ala-OH

SarMet-peptide: Ac-Lys-Gly-Gly-Ala-Gly-Sar-Met-Ala-Ala-Arg-Gly-OH

ProMet-peptide: Ac-Lys-Gly-Gly-Ala-Gly-Pro-Met-Ala-Ala-Arg-Gly-OH

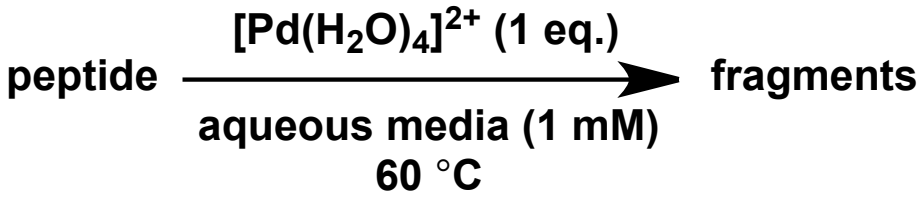
X-Pro X-Sar X-Gly



N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 781.

Neutral Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- dependence on pH of the 1st-order-rate const.
  - reaction:



pH	rate constant $k/10^{-4}\,min^{-1}$ ProMet-peptide	SarMet-peptide	GlyMet-peptide
0.9			95(2)
1.2			65(1)
1.5			48(2)
2.0	587(40)	146(8)	28(2)
2.5			17(1)
3.0	150(2)	93(3)	5(2)
4.0	67(3)	57(2)	0
5.0	47(2)	39(4)	0
6.0	43(2)	24(4)	0
7.0	41(3)	21(4)	0

Why?

ProMet > SarMet > GlyMet

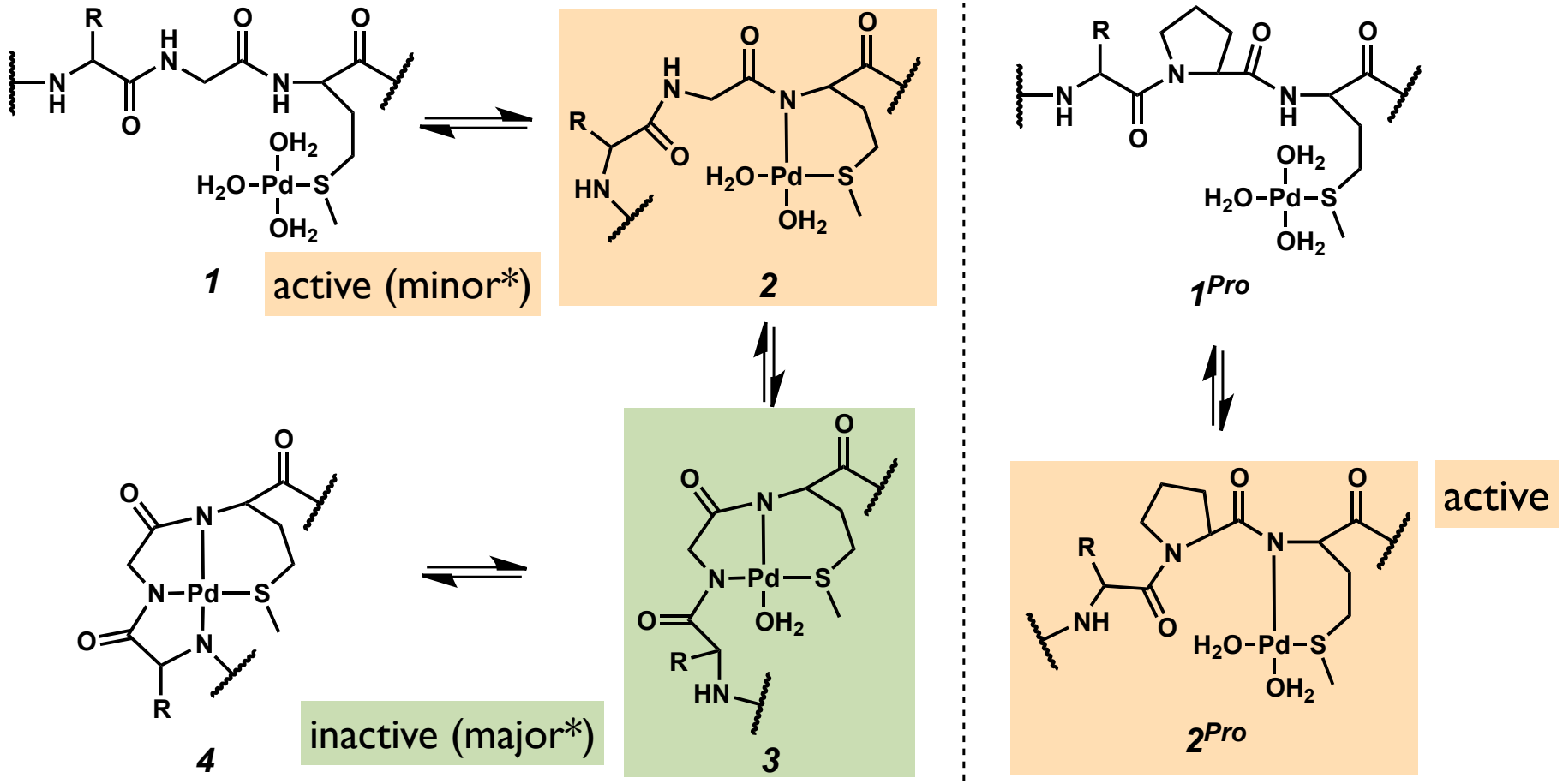
ProMet- and SarMet-peptide can be cleaved but GlyMet-peptide can't.

Why?

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 781.

Neutral Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- difference between GlyMet- (left) and ProMet- (right) peptide in equilibrium among Pd(II) complexes

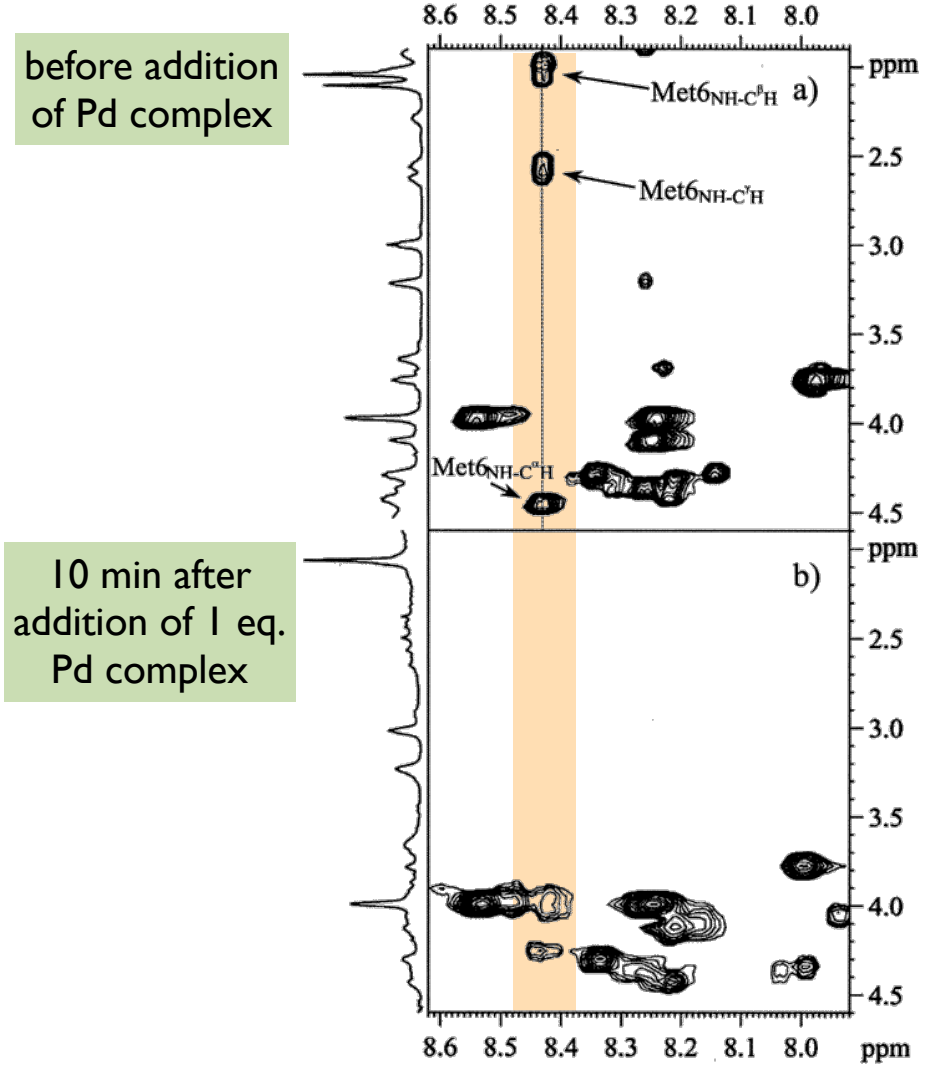


(* at pH 2.3)

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 781.

Neutral Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- TOCSY ^{1}H NMR spectrum (ProMet-peptide)



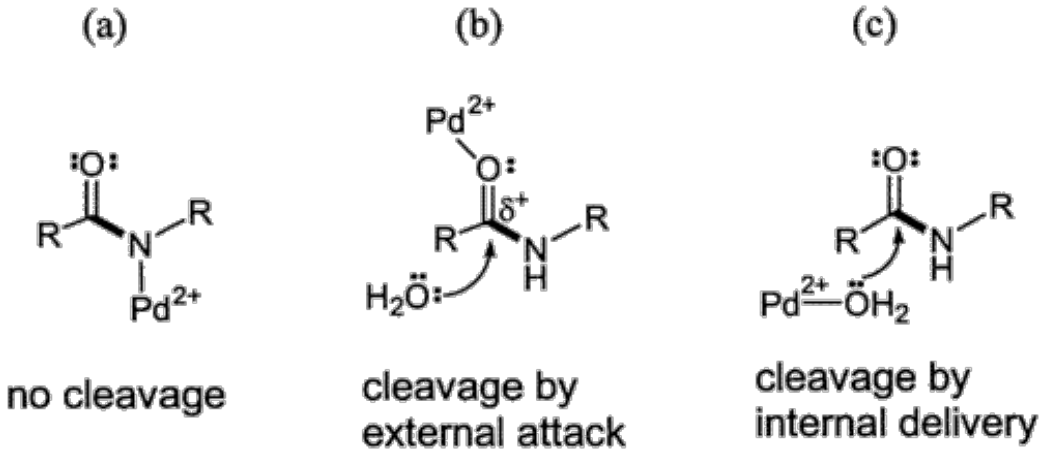
- signals derived from $Met7_{NH}$ disappeared.
  ↓
  $Met7_{NH}$ group coordinates to Pd(II) ion

- NH signals of other residues persisted.
  ↓
  Other amide groups were not bound either.

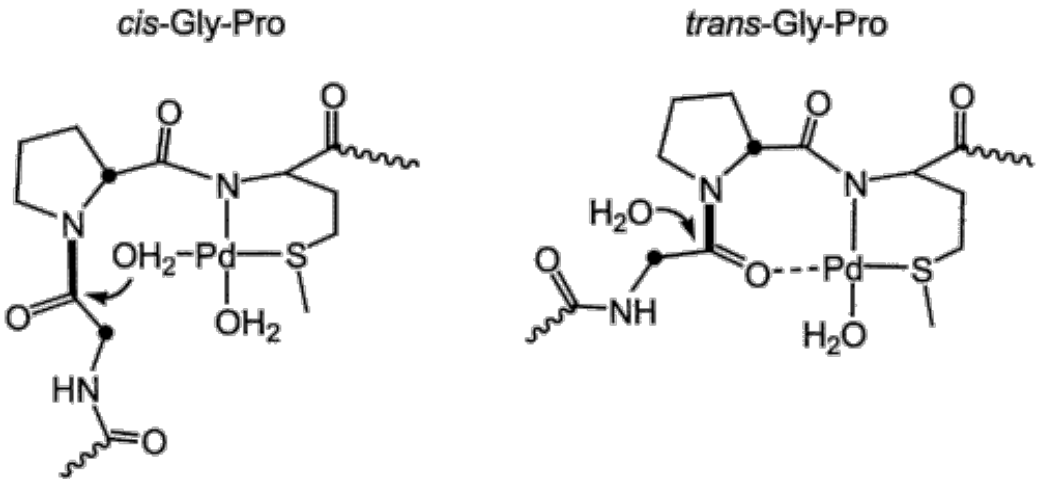
N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 781.

Neutral Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- external attack vs internal delivery



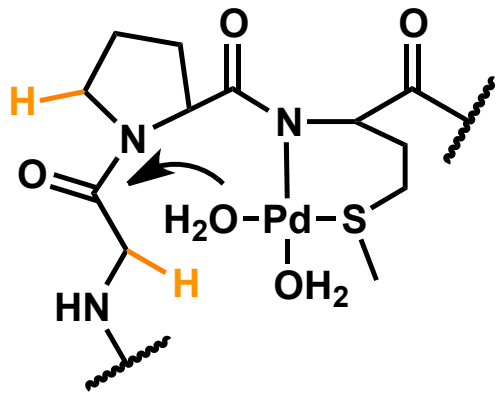
- in this case...



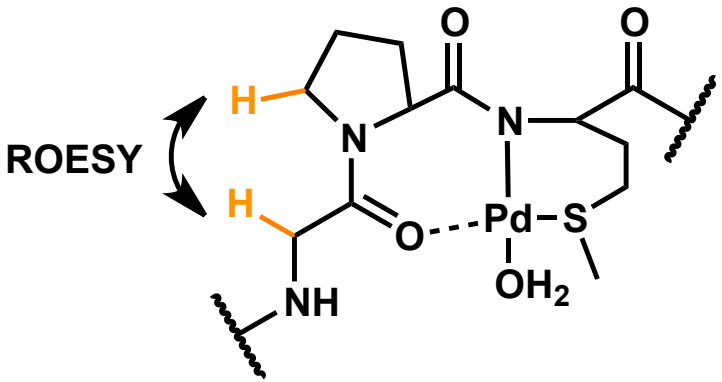
N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 781.

Neutral Hydrolysis with $[Pd(H_2O)_4]^{2+}$

- external attack vs internal delivery



cis-Gly-Pro



trans-Gly-Pro

  - ROESY 1H NMR spectrum showed the cross peak between the Gly5$_{\alpha\text{-CH}}$ and Pro6$_{\delta\text{-CH}}$

    → external attack (*trans*-Gly-Pro)

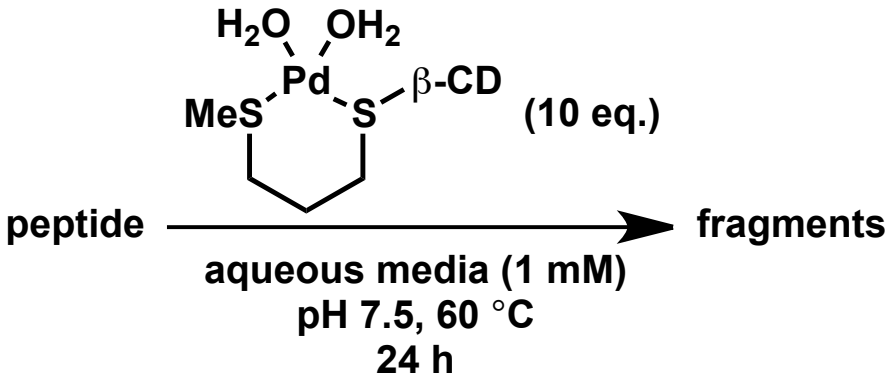
N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 781.

Neutral Hydrolysis with Pd(II)-β-CD complex

- substrate: Met-peptide

Ac-Lys-Gly-Gly-Phe-Ser-Pro-Phe-Phe-Ala-Ala-Arg-Ala-OH

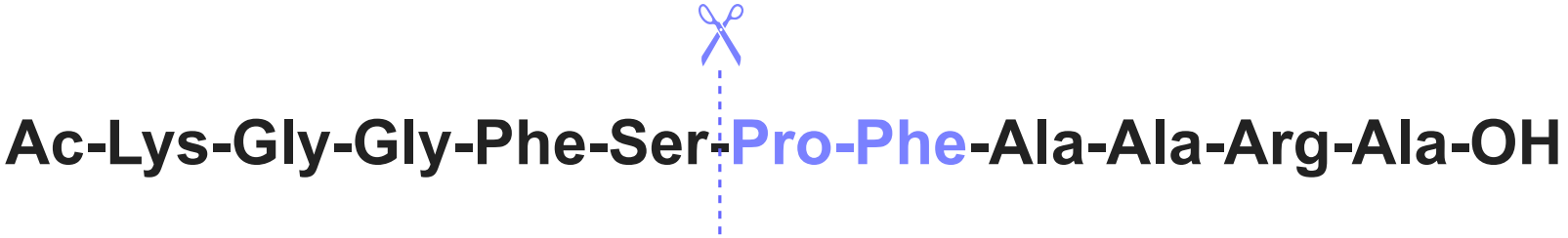
- hydrolysis condition



N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2004**, *126*, 696.

Neutral Hydrolysis with Pd(II)-β-CD complex

- cleavage site



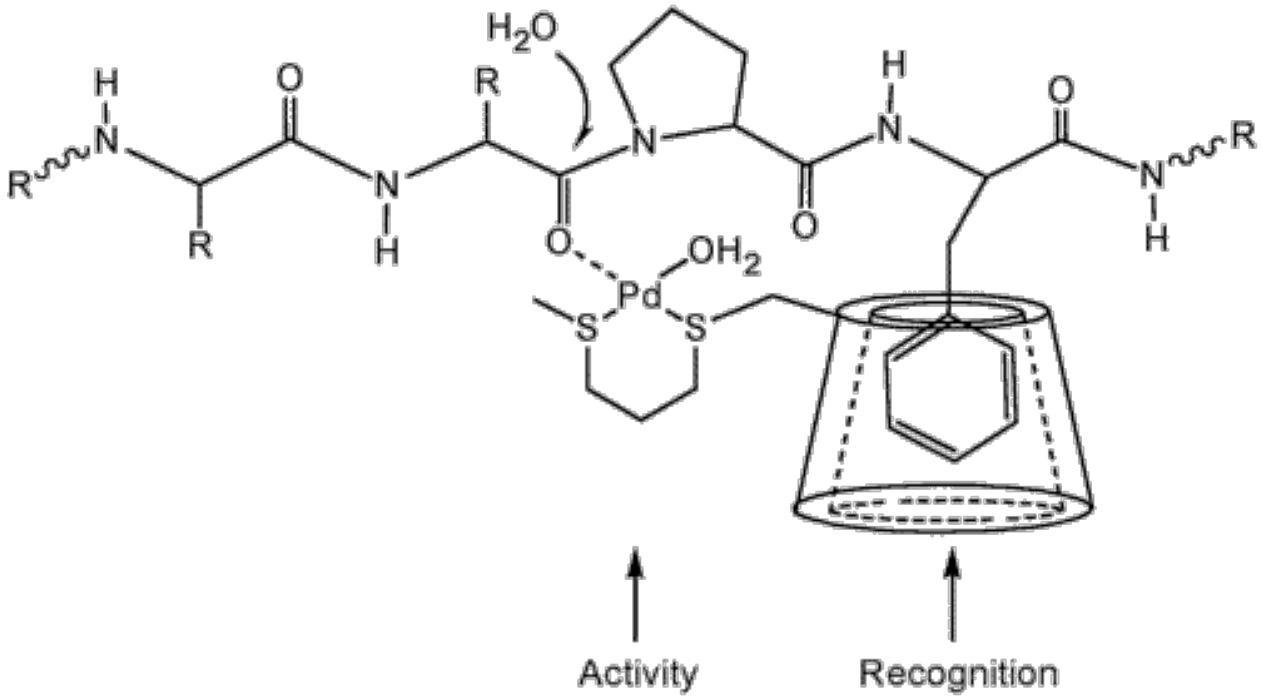
  - Pd(II)-β-CD complex promoted cleavage of the first one upstream from the Pro6-Phe7 sequence.

    (characterized by MALDI mass spectroscopic identification)

N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2004**, *126*, 696.

Neutral Hydrolysis with Pd(II)-β-CD complex

- possible interaction toward sequence selectivity

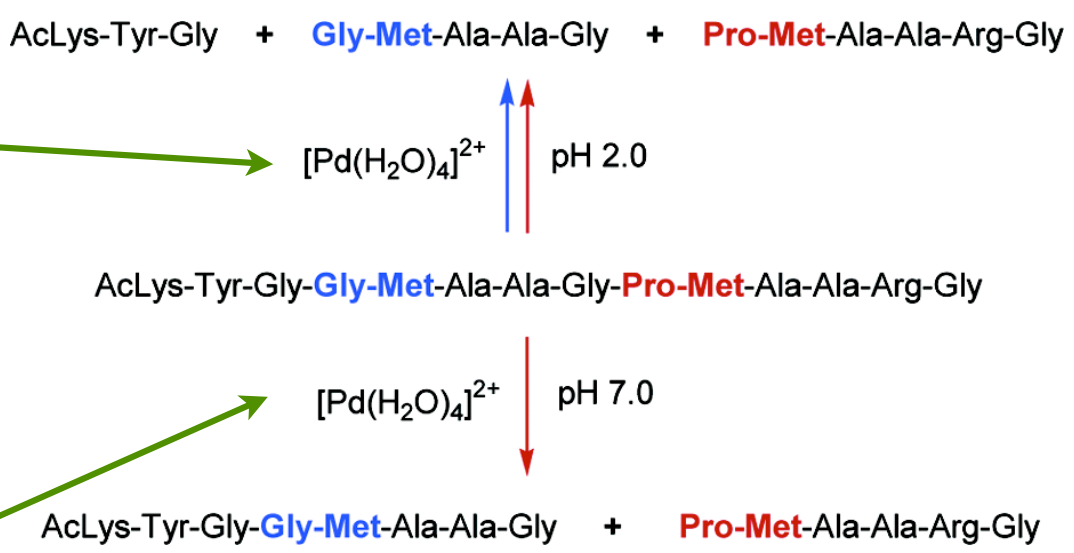


  - CD cavities bind hydrophobic substrates in aqueous media.

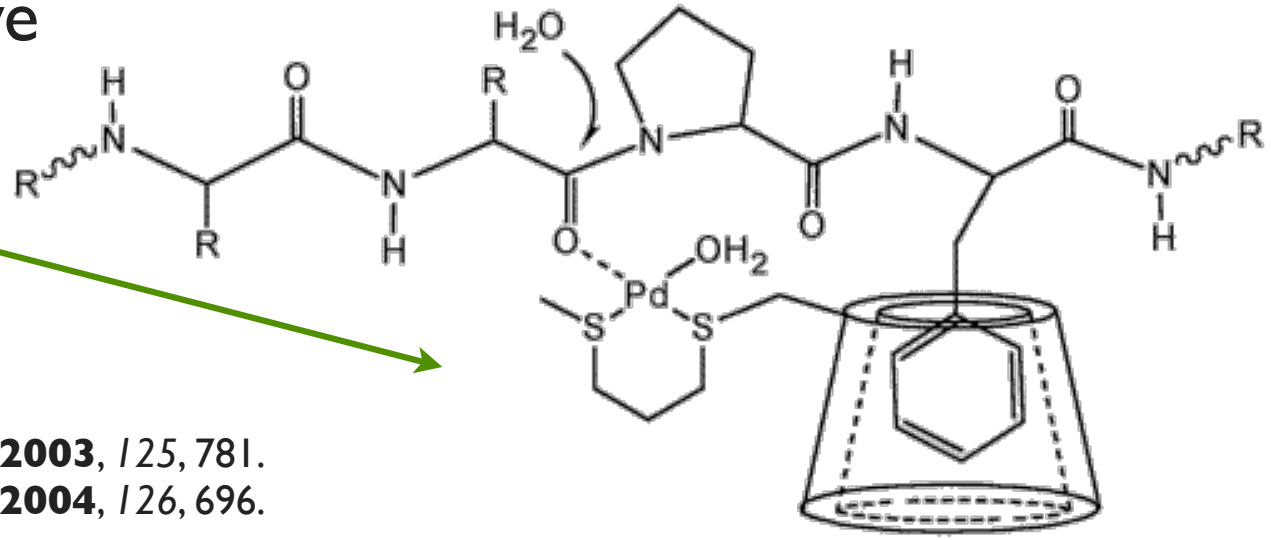
N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2004**, *126*, 696.

Summary of This Section

- residue-selective hydrolysis



- sequence-selective hydrolysis

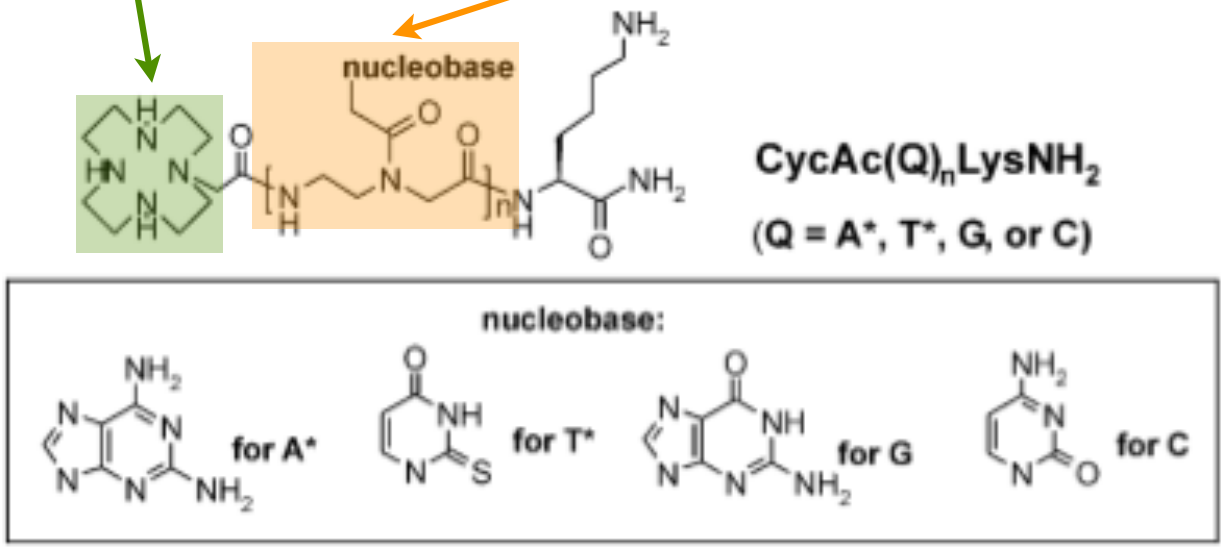


N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2003**, *125*, 781.
N. M. Kostić *et al.*, *J. Am. Chem. Soc.* **2004**, *126*, 696.

§3 Protein-Selective Hydrolysis of Amides

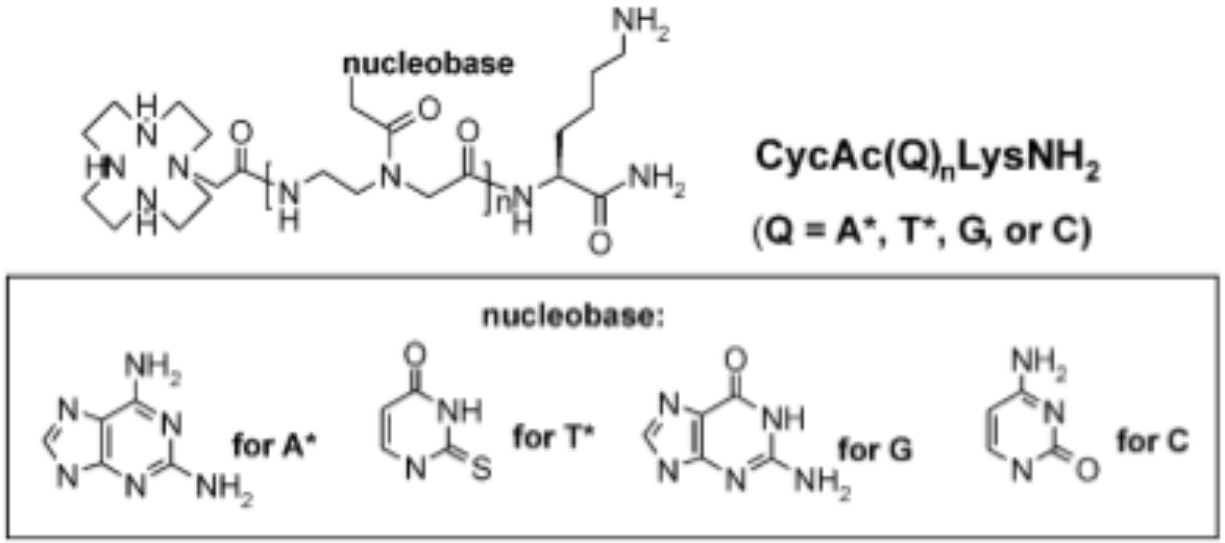
Mb-Selective Artificial Protease

- catalyst design
  - catalyst site
    : Cyc-metal complex
  - binding site
    : peptide nucleic acid (PNA)



J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

Mb-Selective Artificial Protease



- Based on this design, the library of Cyc-containing PNA oligomers was constructed.
  - n=7, 8: no activities* toward Mb (Cu(II)-complex)
  - n=9: active* toward Mb (Cu(II)-complex)

(* checked by SDS-PAGE)

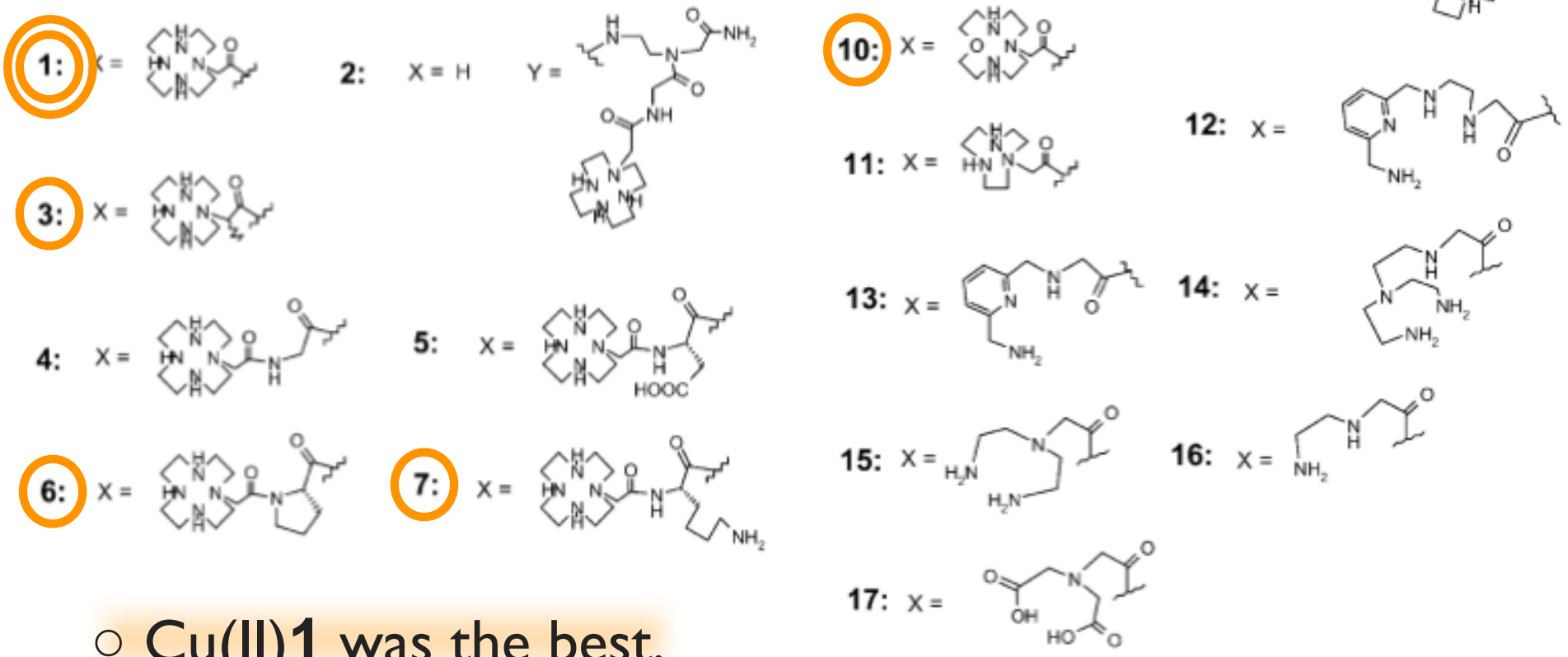
J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

Mb-Selective Artificial Protease

- their own combinatorial library

Ligand of Catayst: **X**-A*-A*-T*-T*-C-G-A*-A*-C-**Y**

Y = L-Lys-NH_2 for **1**, **3-17**

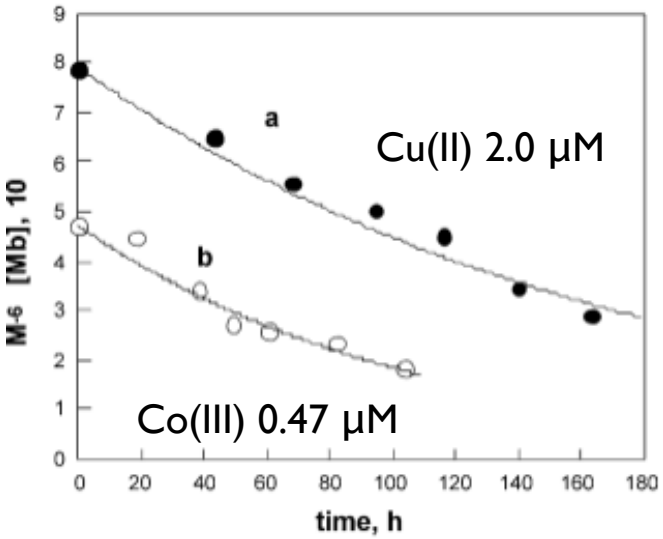


- Cu(II)**1** was the best.

J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

Mb-Selective Artificial Protease

- changing metal center from Cu(II) to Co(III)
  - Metal transfer to metal-abstracting materials in living body should be slower for Co(III) complexes because of the exchange-inertness of Co(III)



decrease of [Mb]
(pH 7.5, 37 ℃)

- Furthermore, Co(III)**1** shows high reactivity than Cu(II)**1**.

$k_0(Cu) = 5.3 \times 10^{-3}\ h^{-1}$

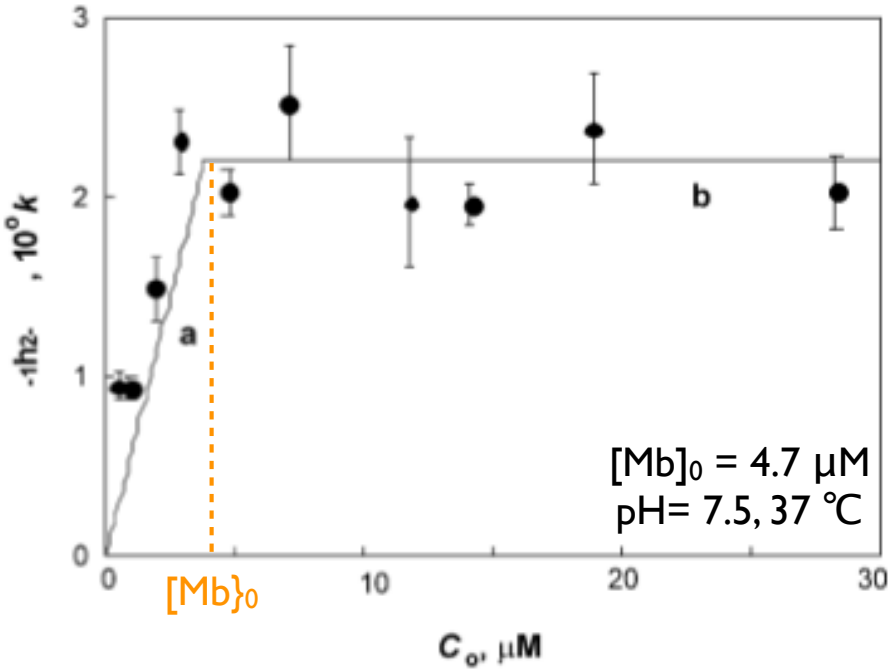
$k_0(Co) = 9.4 \times 10^{-3}\ h^{-1}$

(pseudo-1st-order kinetics)

J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

Mb-Selective Artificial Protease

- affinity of Co(III)**1** complex to Mb



- Two straight lines intersect at $C_0 = [Mb]_0 = 4.7$ μM.

↓

- $K_m < 4.7$ μM < 5.0 μM
  (cf. K_m of fumarase = 5.0 μM)

→ Affinity of Co(III)**1** complex to Mb was high enough.

→ k_{cat} (not k_{cat}/K_m) should be considered!!

J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

Mb-Selective Artificial Protease

- k_{cat} comparison between Co(III) complexes

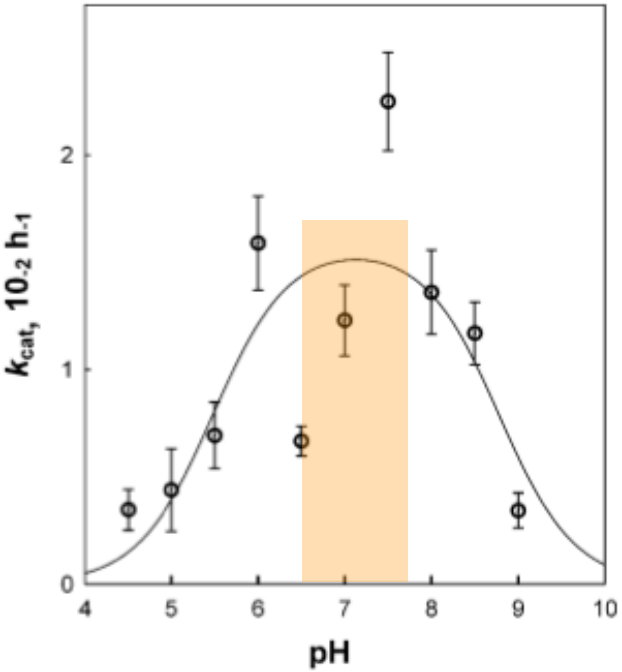
Table 1. Values of k_{cat} measured at optimum pH and 37 °C for the Co(III) complexes of **1**, **3**, **6**, **7**, **9**, and **10**

Catalyst	k_{cat} (10^{-3} h^{-1})[a]	Optimum pH
Co(III)**1**	22	7.5
Co(III)**3**[b]	2.8	7.5
Co(III)**6**	2.4	7.5
Co(III)**7**	2.4	7.5
Co(III)**9**	4.4	7.5
Co(III)**10**	8.9	8.0

→ Co(III)**1** was the best!

- pH dependence of k_{cat} for cleavage of Mb by Co(III)**1** complex

  → most active at physiological pH!



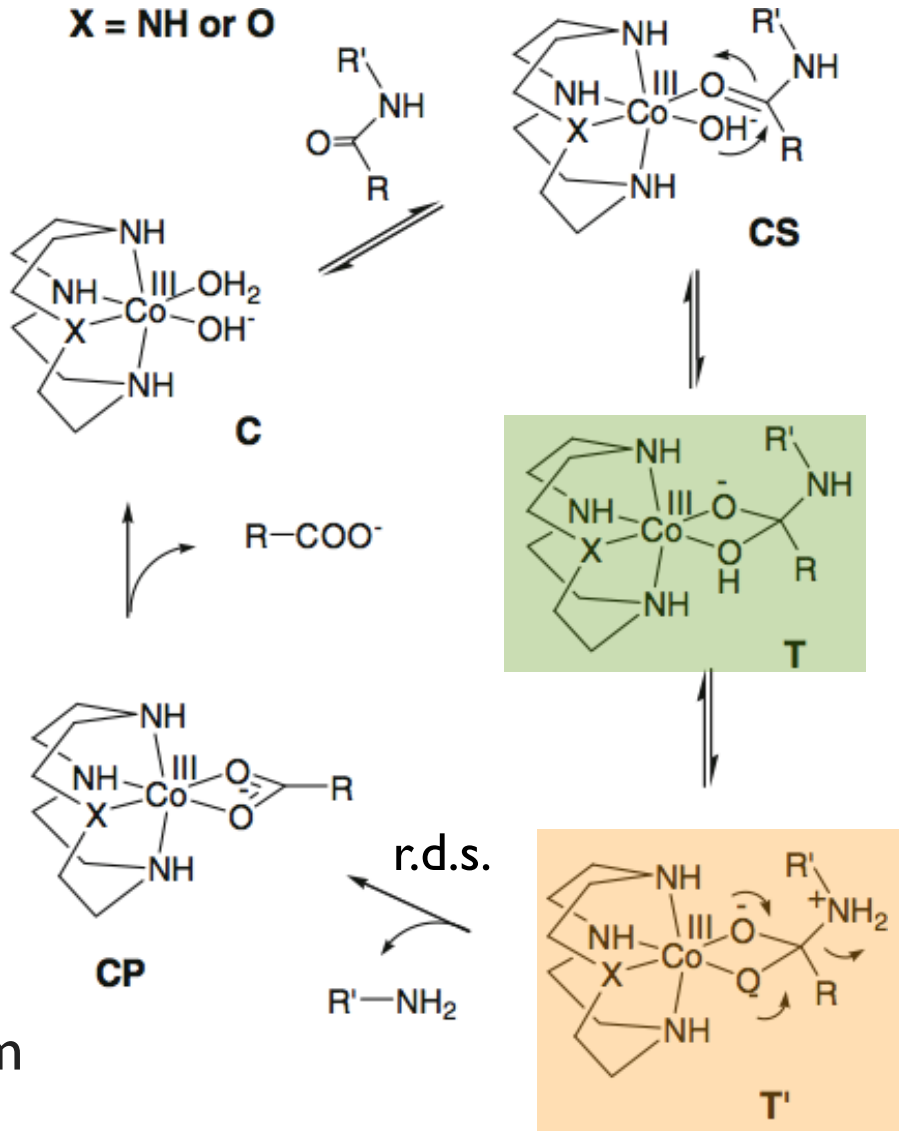
J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

Mb-Selective Artificial Protease

- possible cleavage mechanism
  - This mechanism consistents with the pH dependence of Co(III)**1** complex.

    ||

  - The following two would reduce the reactivity of Co(III)**1** complex.
    - ✓ protonation of the hydroxo (**T**) or oxo (**T'**) ligand at low pH
    - ✓ deprotonation of the ammonium ion (**T'**) at high pH



J. Suh *et al.*, *J. Biol. Inorg. Chem.* **2009**, *14*, 151.

Mb-Selective Artificial Protease

- cleavage site characterization

Table 2. Molecular weights of protein fragments disclosed by MALDI-TOF MS and cleavage sites proposed to account for the protein fragments

Catalyst	*m*/*z* of fragments	Cleavage site proposed on the basis of *m*/*z* values[a]
Co(III)**1**	7074, 9891, 8045, 8909	Leu_{89}-Ala_{90}, Leu_{72}-Gly_{73}
Co(III)**3**	7066, 9897	Leu_{89}-Ala_{90}
Co(III)**6**	7066, 9898	Leu_{89}-Ala_{90}
Co(III)**7**	7068, 9888	Leu_{89}-Ala_{90}
Co(III)**9**	7068, 9884	Leu_{89}-Ala_{90}
Co(III)**10**	7075, 9896	Leu_{89}-Ala_{90}

- *N*-terminal sequencing (Edman degradation) was conducted to product solution of Co(III)**3**.

→ Leu_{89}-Ala_{90} was the cleavage site.

J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

Mb-Selective Artificial Protease

- selectivity (control experiment)

albumin, γ-globulin, elongation factor P,
gelatin A, gelatin B

  - Above 5 proteins were incubated with Cu(II)**1** or Co(III)**1**, but protein cleavage was not observed.

Target selectivity can be expected!

J. Suh *et al.*, *Bioorg. Med. Chem.* **2003**, *11*, 2901.

PDF-Selective Artificial Protease

- PDF: peptide deformylase
  - involved in deformylation of the formyl-methionyl derivative of proteins formed in the prokaryotic translational systems.

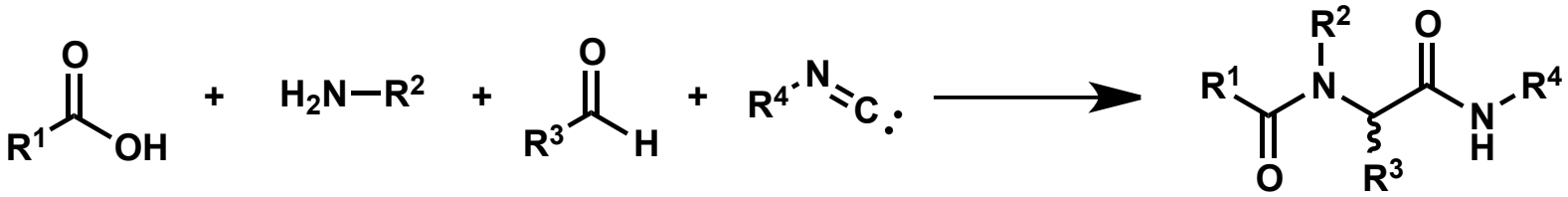
  - Its inhibitors are searched as candidates for new antibiotic drugs.

way toward "catalytic drugs"

J. Suh *et al.*, *J. Am. Chem. Soc.* **2005**, *127*, 2396.

PDF-Selective Artificial Protease

- construction of a library of catalyst candidates
  - synthesized by the Ugi reaction (product was racemic mixture)

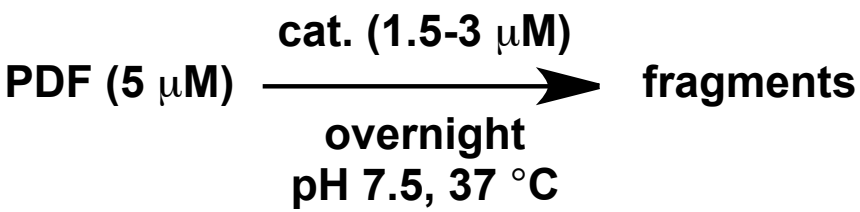


- analysis of the result (PDF-cleaving activity)
  - examined by MALDI-TOF mass spectrum

J. Suh *et al.*, *J. Am. Chem. Soc.* **2005**, *127*, 2396.

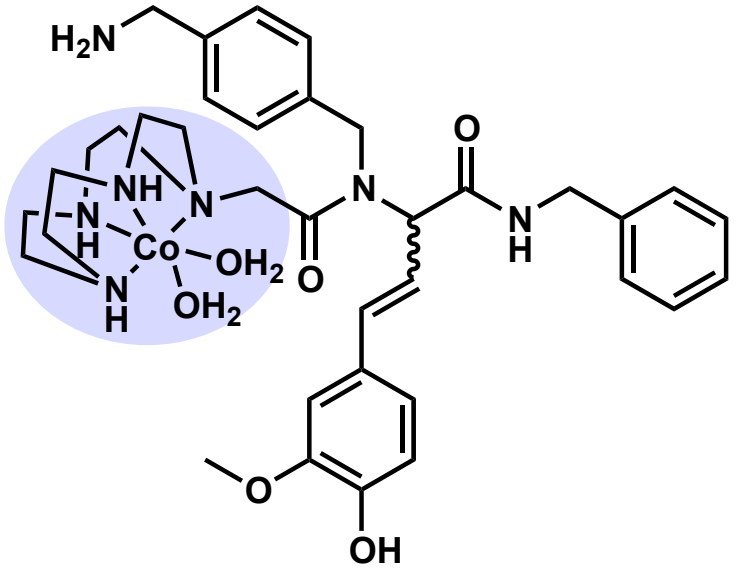
PDF-Selective Artificial Protease

- property of screening



  - examined by MALDI-TOF mass spectrum

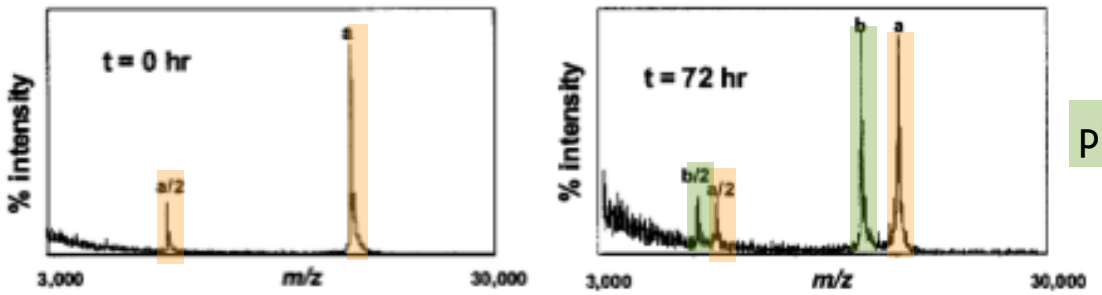
- the result of screening
  - most active complex:



J. Suh *et al.*, *J. Am. Chem. Soc.* **2005**, *127*, 2396.

PDF-Selective Artificial Protease

- cleavage site



pH 7.5, 37 ℃

  - ○ orange: PDF / green: fragment
  - ○ *C*-terminal sequencing by carboxy peptidase A

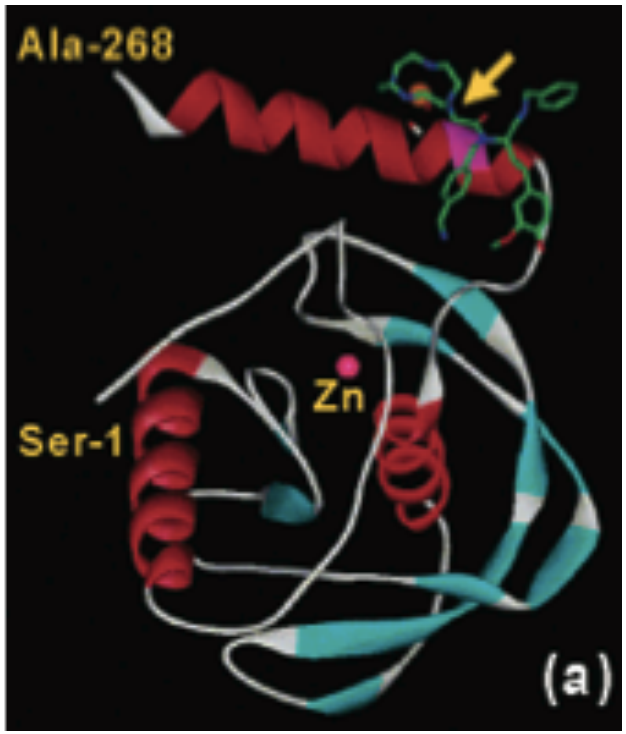
    → Glu152-Arg153 was the cleavage site.

- optimum pH = 7.5 / lower limit of k_{cat} = 0.05 h^{-1}

J. Suh *et al.*, *J. Am. Chem. Soc.* **2005**, *127*, 2396.

PDF-Selective Artificial Protease

- docking study result

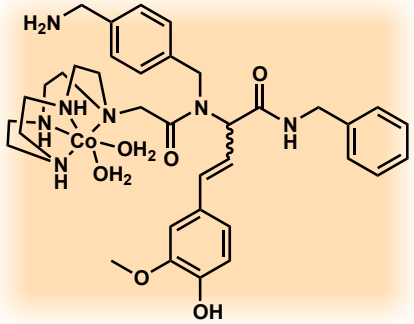


- *S*-isomer of the catalyst with PDF was more stable than with the *R*-isomer.
- Central acyclic chain of the catalyst interacted with the C-terminal α-helix, while the 3 aromatic tails made contact with the helical and the loop structures residing above the active site.

J. Suh *et al.*, *J. Am. Chem. Soc.* **2005**, *127*, 2396.

PDF-Selective Artificial Protease

- selectivity (control experiment)
  - 15 other proteins were examined, but none of them were cleaved by the Co(III) complex.



Application to Drug is possible!

list:

1. human cyclin-dependent kinase 2
2. human kinase insert domain receptor
3. human farnesyl transferase
4. hepatitis C virus protease
5. YacM
6. N-acetylglucosamine 1-phosphate uridyltransferase
7. UDP-N-acetylmuramoyl-L-alanine: D-glutamate ligase
8. non-structural protein 5B of hepatitis C virus
9. human protein-tyrosine phosphatase 1B
10. human retinoic X receptor α
11. human peroxisome proliferator activated receptor α
12. human peroxisome proliferator activated receptor γ
13. human liver X receptor α
14. human liver X receptor β
15. human Caspase-8

J. Suh *et al.*, *J. Am. Chem. Soc.* **2005**, *127*, 2396.

AmPs-Selective Artificial Protease

- AmPs: amyloidgenic peptides or proteins
  - lacking active sites

    → Conventional approaches can't be applied.
  - related to diseases

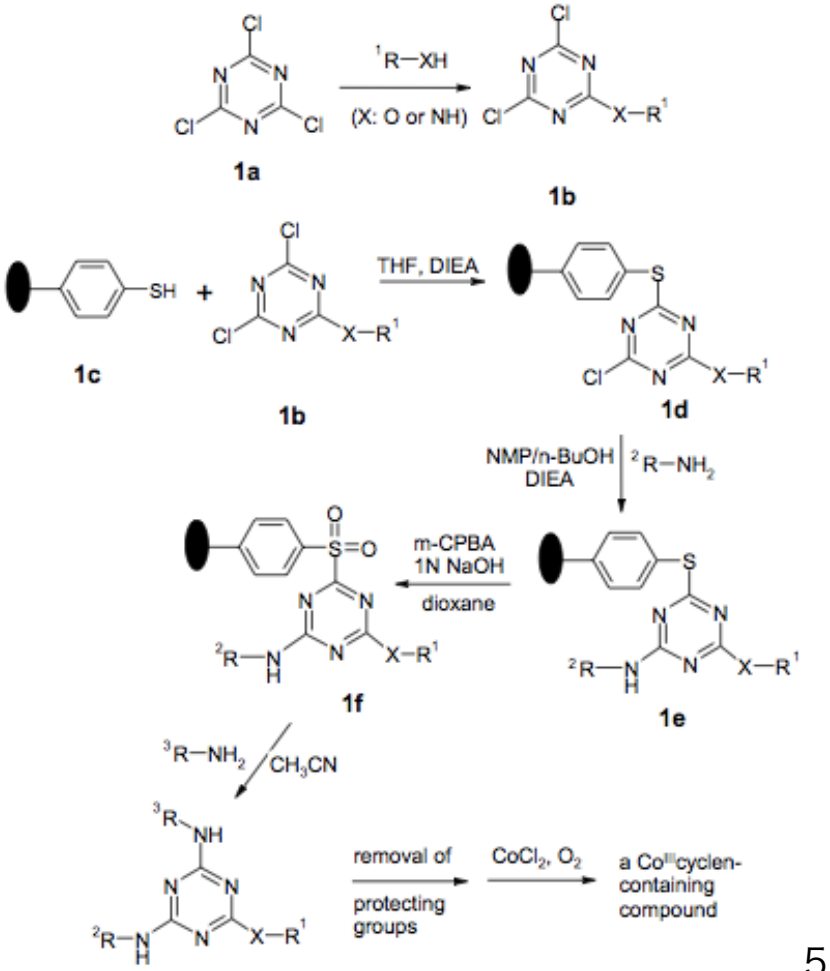
→ Peptide-cleaving catalysts is suitable for their drug!

- target AmPs of this research
  - amyloid β protein (Aβ): Alzheimer's Disease
  - human islet amyloid polypeptide (h-IAPP): type 2 diabetes

J. Suh *et al.*, *Chem. Soc. Rev.* **2008**, *38*, 1949.

AmPs-Selective Artificial Protease

- construction of a library of catalyst candidates
  - synthesized according to the route shown below

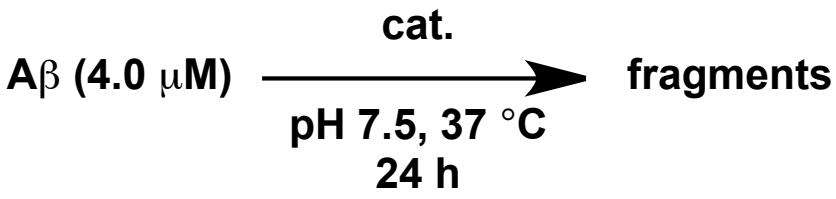


  - Aromatic moieties were employed as auxiliary binding components in the combinatorial library.

J. Suh *et al.*, *Angew. Chem.* **2007**, *119*, 7194.

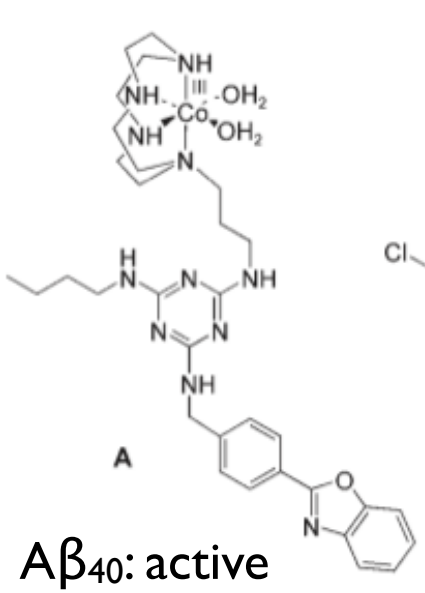
AmPs-Selective Artificial Protease

- property of screening

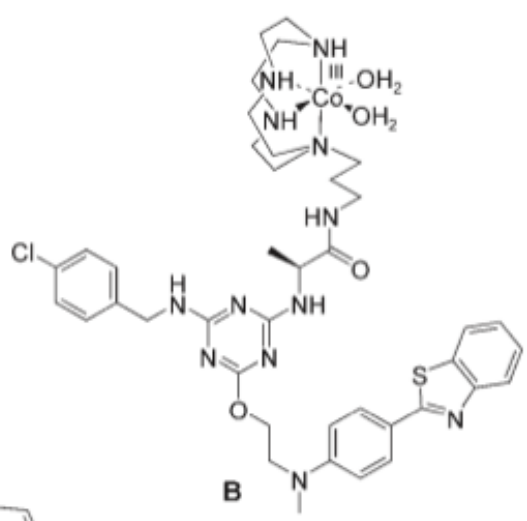


○ examined by MALDI-TOF mass spectrum

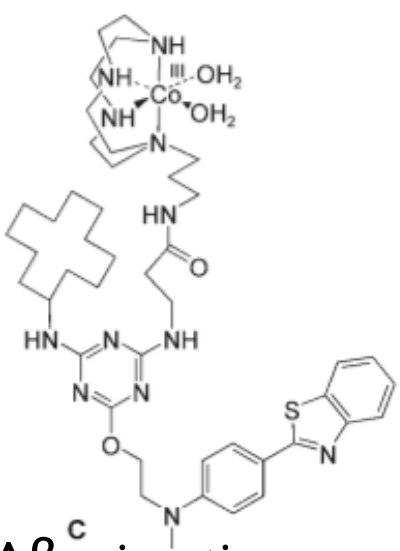
- 4 complexes were selected.



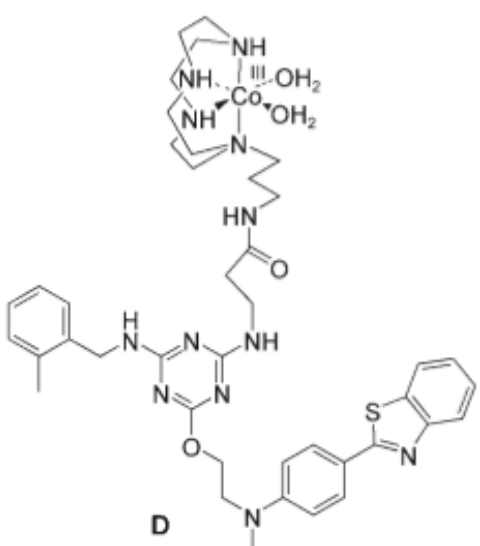
$A\beta_{40}$: active
$A\beta_{42}$: active



$A\beta_{40}$: active
$A\beta_{42}$: active



$A\beta_{40}$: inactive
$A\beta_{42}$: active

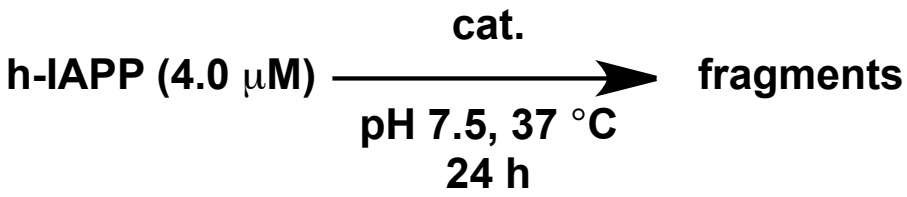


$A\beta_{40}$: inactive
$A\beta_{42}$: active

J. Suh *et al.*, *Angew. Chem.* **2007**, *119*, 7194.

AmPs-Selective Artificial Protease

- property of screening



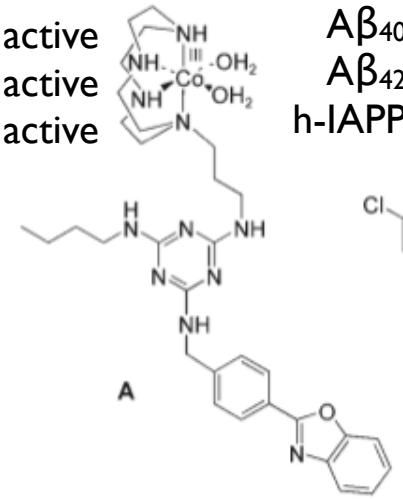
- examined by MALDI-TOF mass spectrum

J. Suh *et al.*, *J. Biol. Inorg. Chem.* **2008**, *13*, 693.

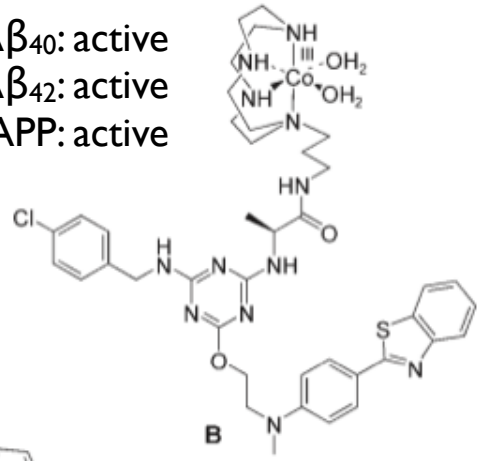
AmPs-Selective Artificial Protease

- 8 complexes were selected.

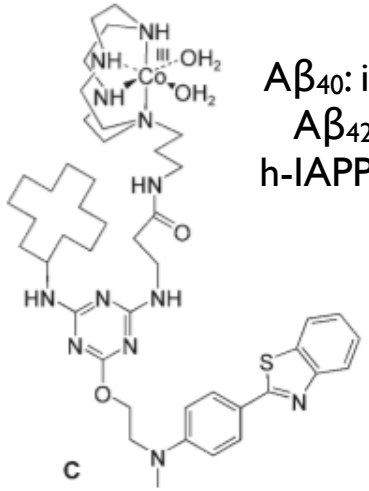
Aβ40: active
Aβ42: active
h-IAPP: active



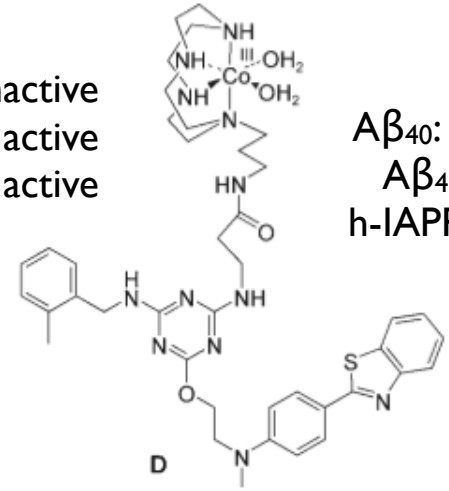
Aβ40: active
Aβ42: active
h-IAPP: active

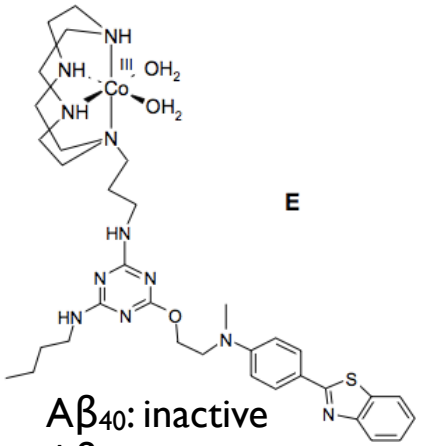


Aβ40: inactive
Aβ42: active
h-IAPP: active



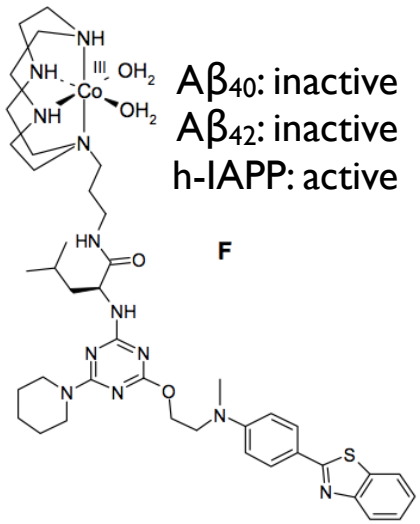
Aβ40: inactive
Aβ42: active
h-IAPP: active



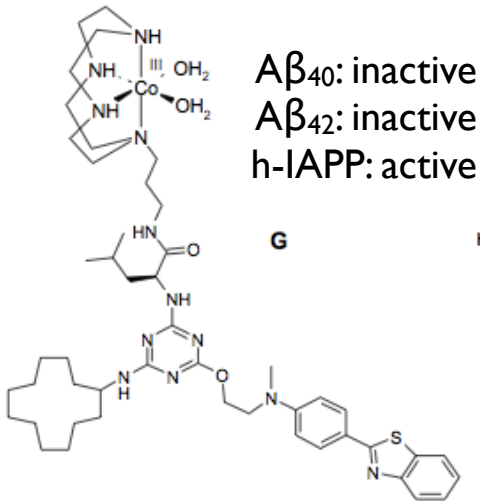


Aβ40: inactive
Aβ42: inactive
h-IAPP: active

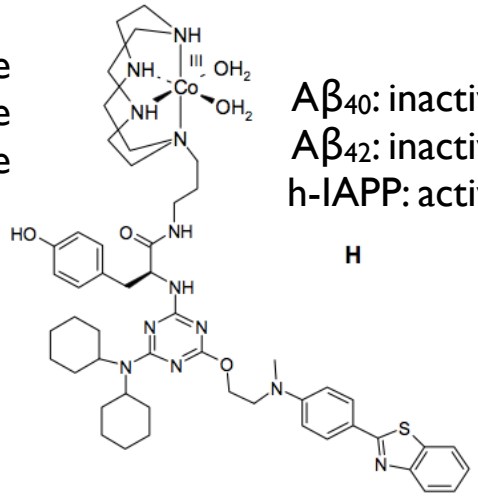
Aβ40: inactive
Aβ42: inactive
h-IAPP: active



Aβ40: inactive
Aβ42: inactive
h-IAPP: active



Aβ40: inactive
Aβ42: inactive
h-IAPP: active



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AmPs-Selective Artificial Protease

- selectivity (control experiment)

horse heart myoglobin, bovine serum γ-globulin,
bovine serum albumin, human serum albumin,
chicken egg white lysozyme, chicken egg ovalbumin,
bovine pancreas insulin

  - Above 7 proteins were incubated with from **A** to **H**, but protein cleavage was not observed.

Application to Drug is possible!
(Drug for all AmPs may be possible...?)

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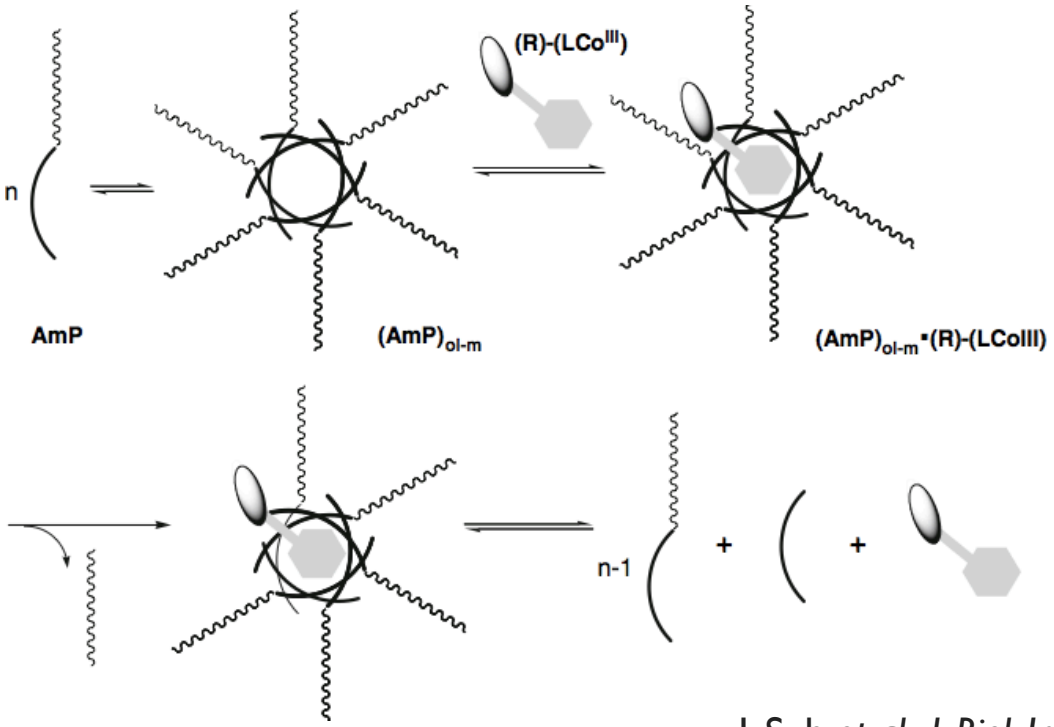
Summary of This Section

- Mb- or PDF-selective artificial protease
- AmPs-selective artificial protease
  - Conventional methods can't get these fruits.



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