Peptide stapling via C-H activation

2016/12/10 Hiroaki Matoba

Contents

1. Conventional methods of peptide stapling

2. Peptide stapling via C(sp^2)-H arylation

3. Peptide stapling via C(sp^3)-H arylation

α-helix in peptide

α-helical structures in peptides sometime have important role in protein-protein interaction.

To mimic these activities, truncated peptides, which have minimal structure of active sites, are synthesized.

However, truncated peptides somtime lose activities.

In small peptides, α-helix formation is unfavorable because entropic cost of forming α-helix is larger than its stabilization energy.

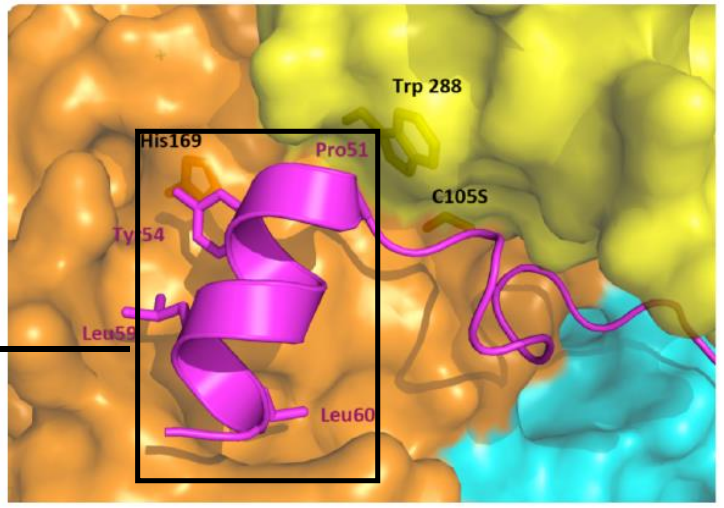
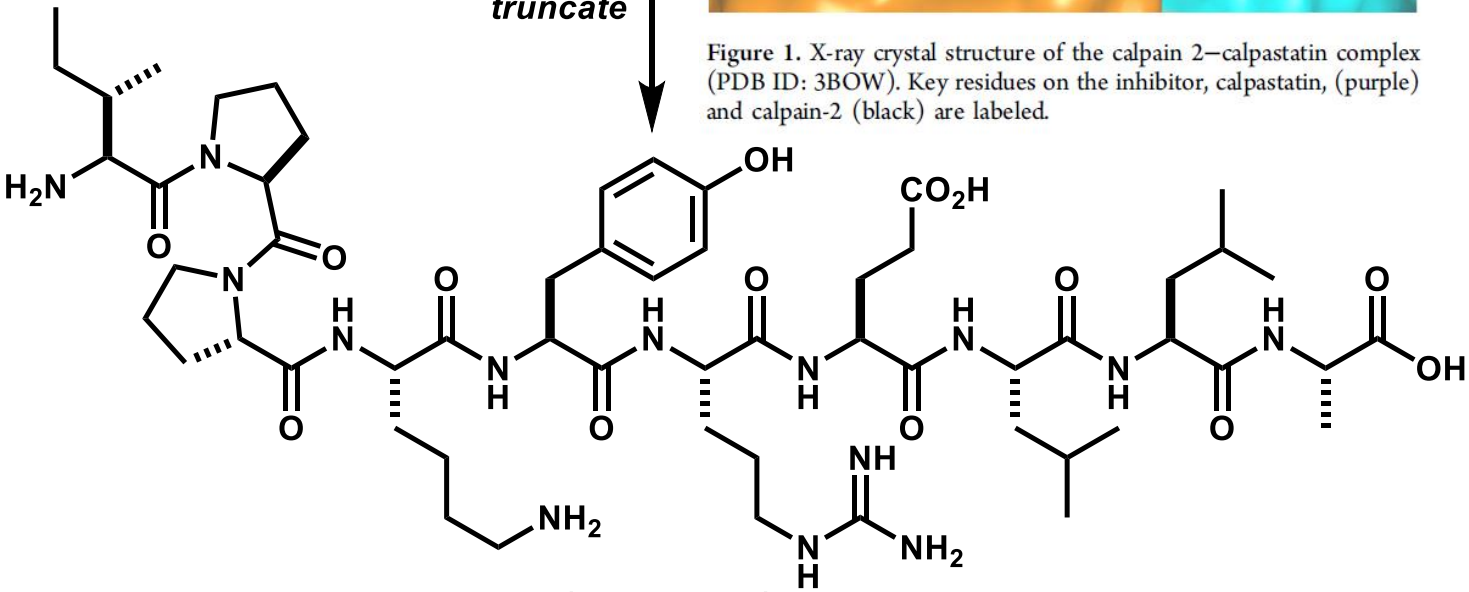


Figure 1. X-ray crystal structure of the calpain 2–calpastatin complex (PDB ID: 3BOW). Key residues on the inhibitor, calpastatin, (purple) and calpain-2 (black) are labeled.

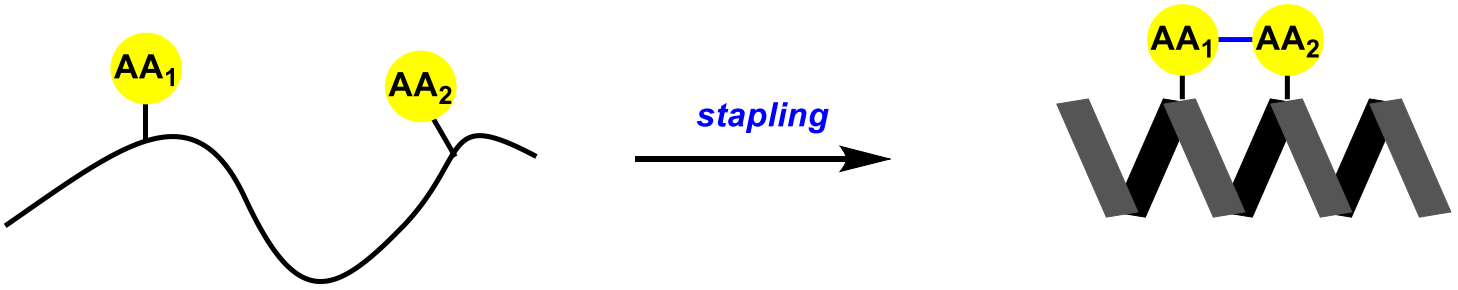
truncate



not active (K_i > 100 μM)

Greenbaum, D. C. et al. *J. Am. Chem. Soc.* **2012**, *134*, 17704.

Peptide stapling



Native peptide
flexible conformation

Stapled peptide
restricted conformation

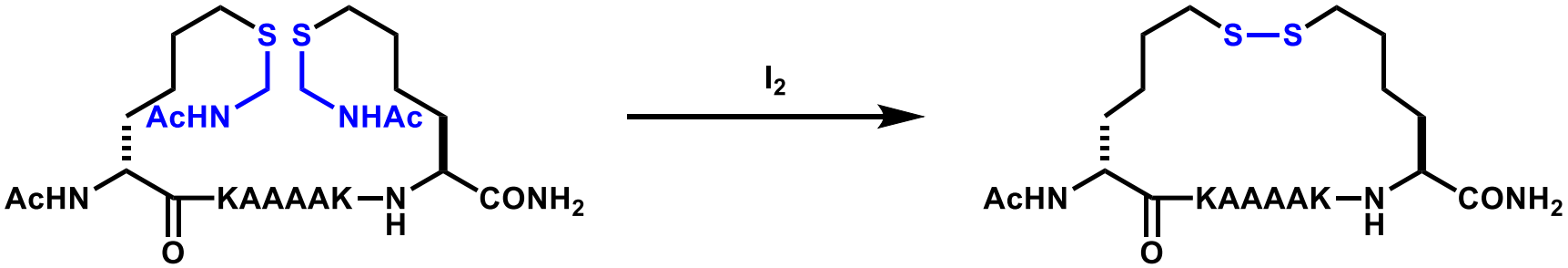
Peptide stapling:
Covalent bond formation between two-amino acids

Purpose of peptide stapling:
Stabilization of α-helical conformation

Effect of α-helix formation:
binding affinity
proteolytic stability
cellular uptake

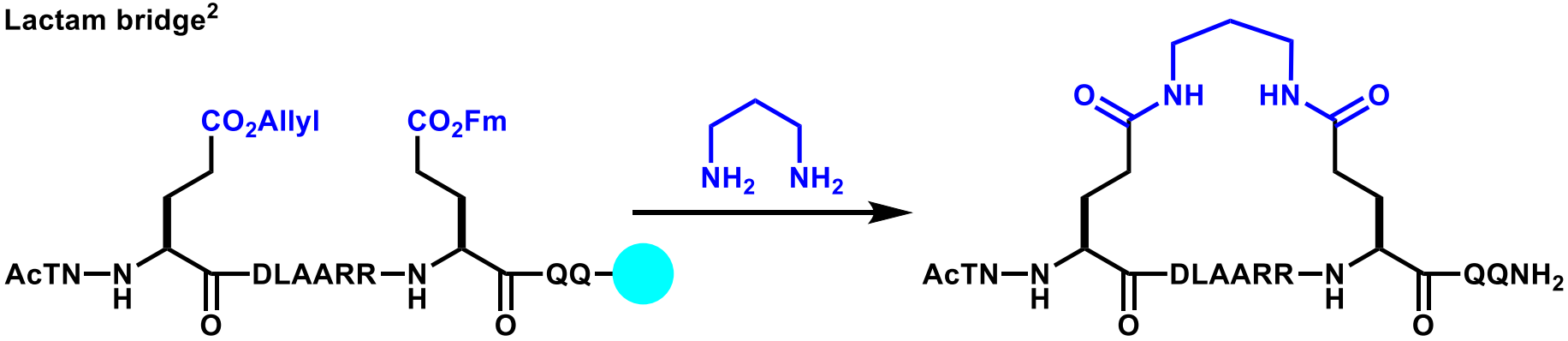
Methods of peptide stapling (1)

Disulfide bridge[1]



helicity **16%** **105%**

Lactam bridge[2]



helicity **n.d.**
(20%: OAllyl, OFm -> NHMe)

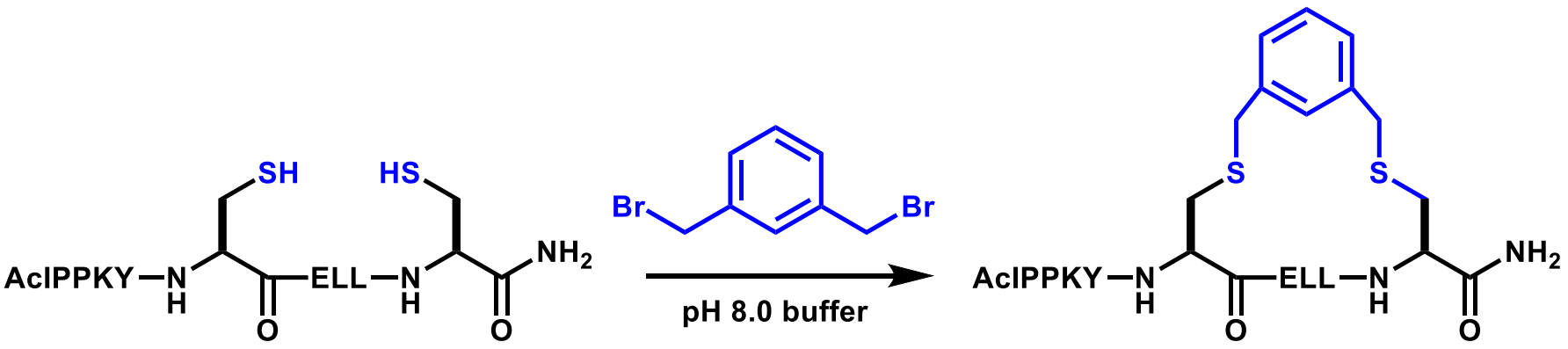
84%

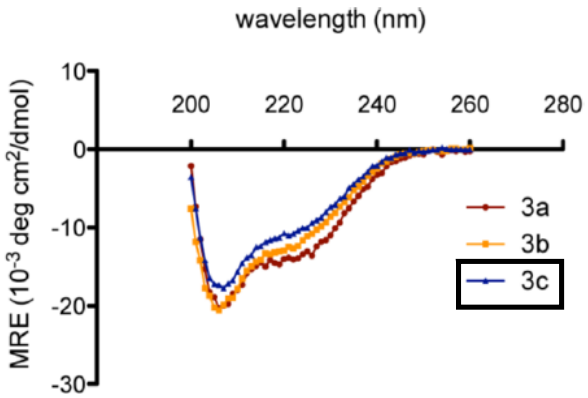
Disulfide and lactam bridges can stabilize α-helical structure, but these cross-links are pharmacologically labile. (e.g. reductive cleavage, hydrolysis)
-> More robust cross-links are required.

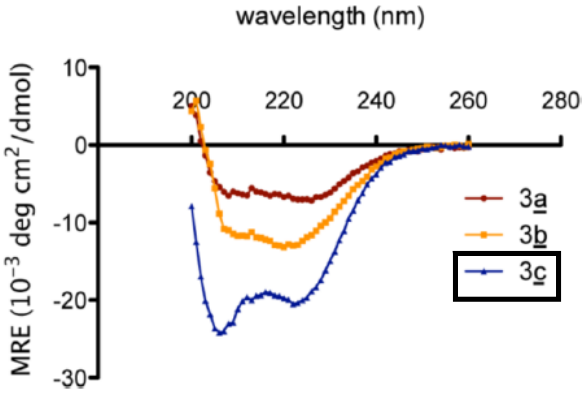
1. Schultz, P. G. et al. *J. Am. Chem. Soc.* **1991**, *113*, 9391.
2. Phelan, J. C. et al. *J. Am. Chem. Soc.* **1997**, *119*, 455.

Methods of peptide stapling (2)

Cysteine cross-linker







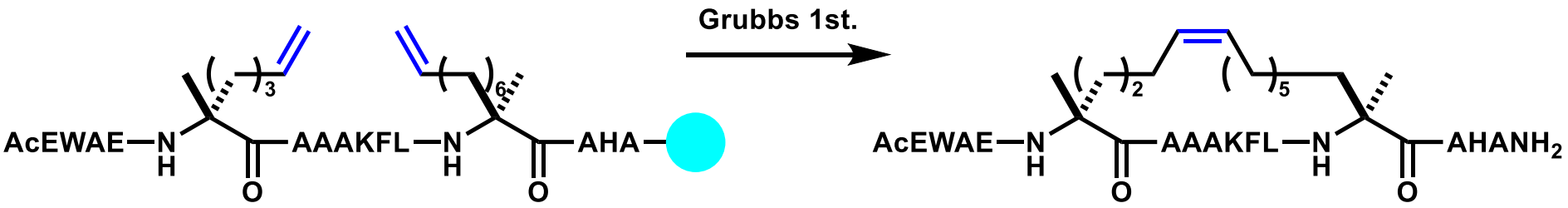
peptide	K_i against Calpain-1 (μM)
3c (native)	>100
3c (stapled)	10.2 ± 2.9

Alkyl bromides, benzyl bromides, and electron deficient aromatics are used as cross-linkers.

Greenbaum, D. C. et al. *J. Am. Chem. Soc.* **2012**, *134*, 17704.

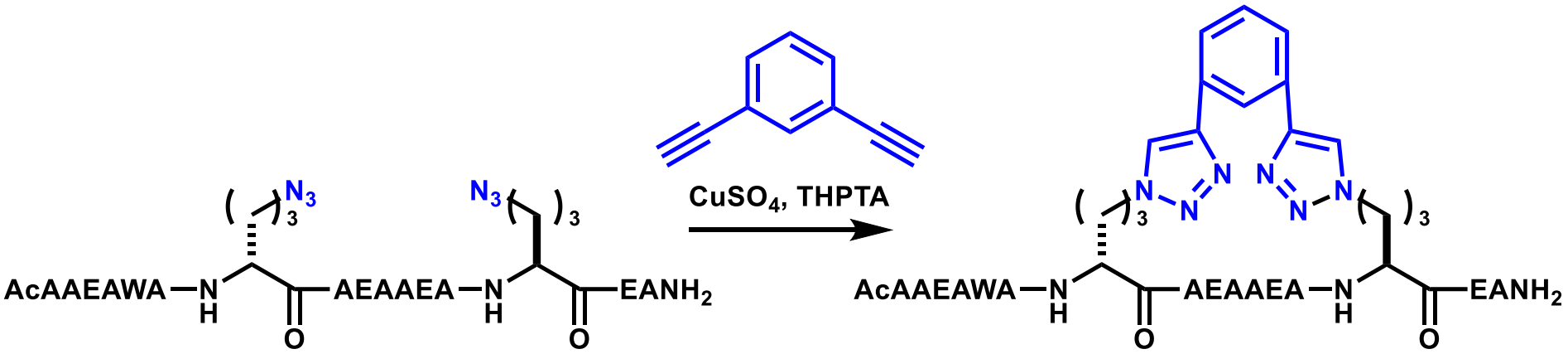
Methods of peptide stapling (3)

Ring-closing metathesis[1]



helicity	60%	84%
cleavage rate constant ($M^{-1}s^{-1}$) (trypsin)	0.05	0.058

Azide-alkyne cycloaddition[2]

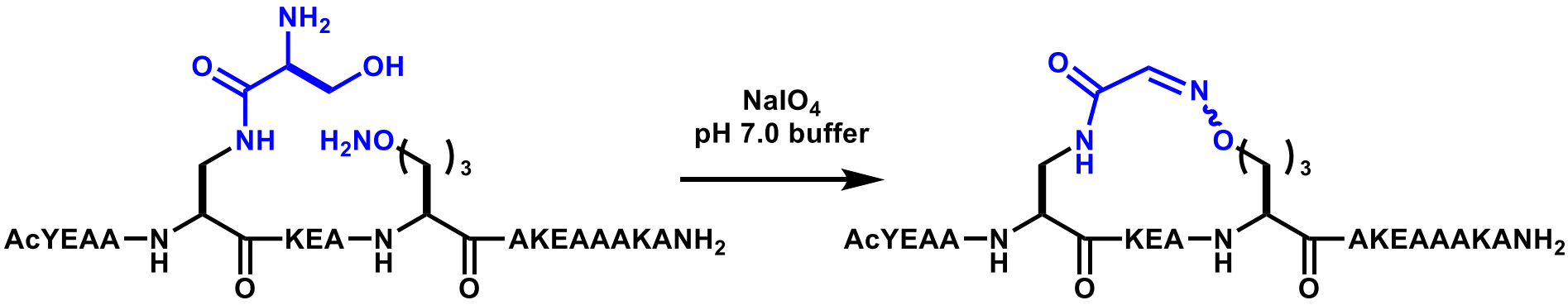


helicity	16%	51%

1. Verdine, G. L. et al. *J. Am. Chem. Soc.* **2000**, *122*, 5891.
2. Spring, D. R. et al. *Chem. Sci.* **2014**, *5*, 1804.

Methods of peptide stapling (4)

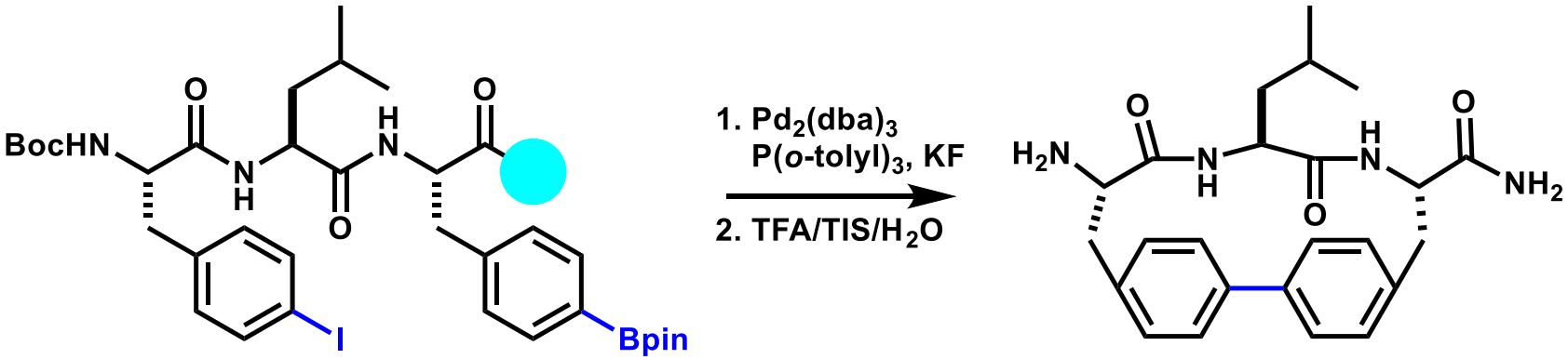
Oxime formation[1]



helicity n.d.

53%

Suzuki-Miyaura coupling[2]



1. Horne, W. S. et al. *Chem. Commun.*, **2011**, *47*, 10915.
2. Planas, M. et al. *Tetrahedron* **2011**, *67*, 2238.

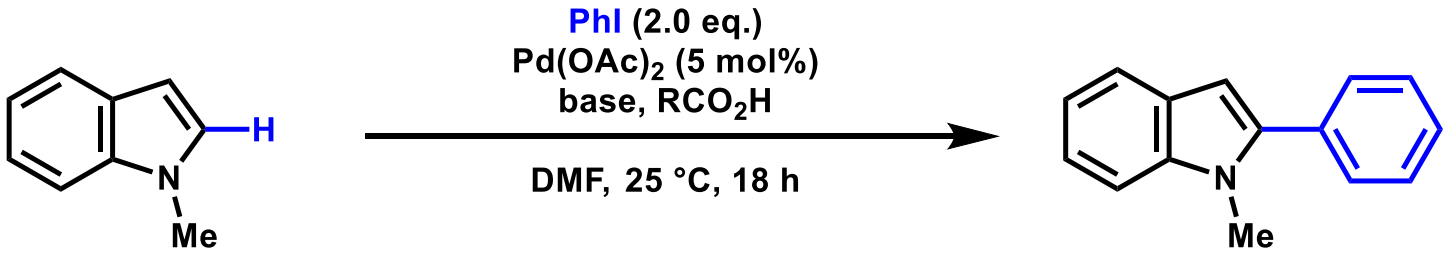
Contents

1. Conventional methods of peptide stapling

2. Peptide stapling via C(sp^2)-H arylation

3. Peptide stapling via C(sp^3)-H arylation

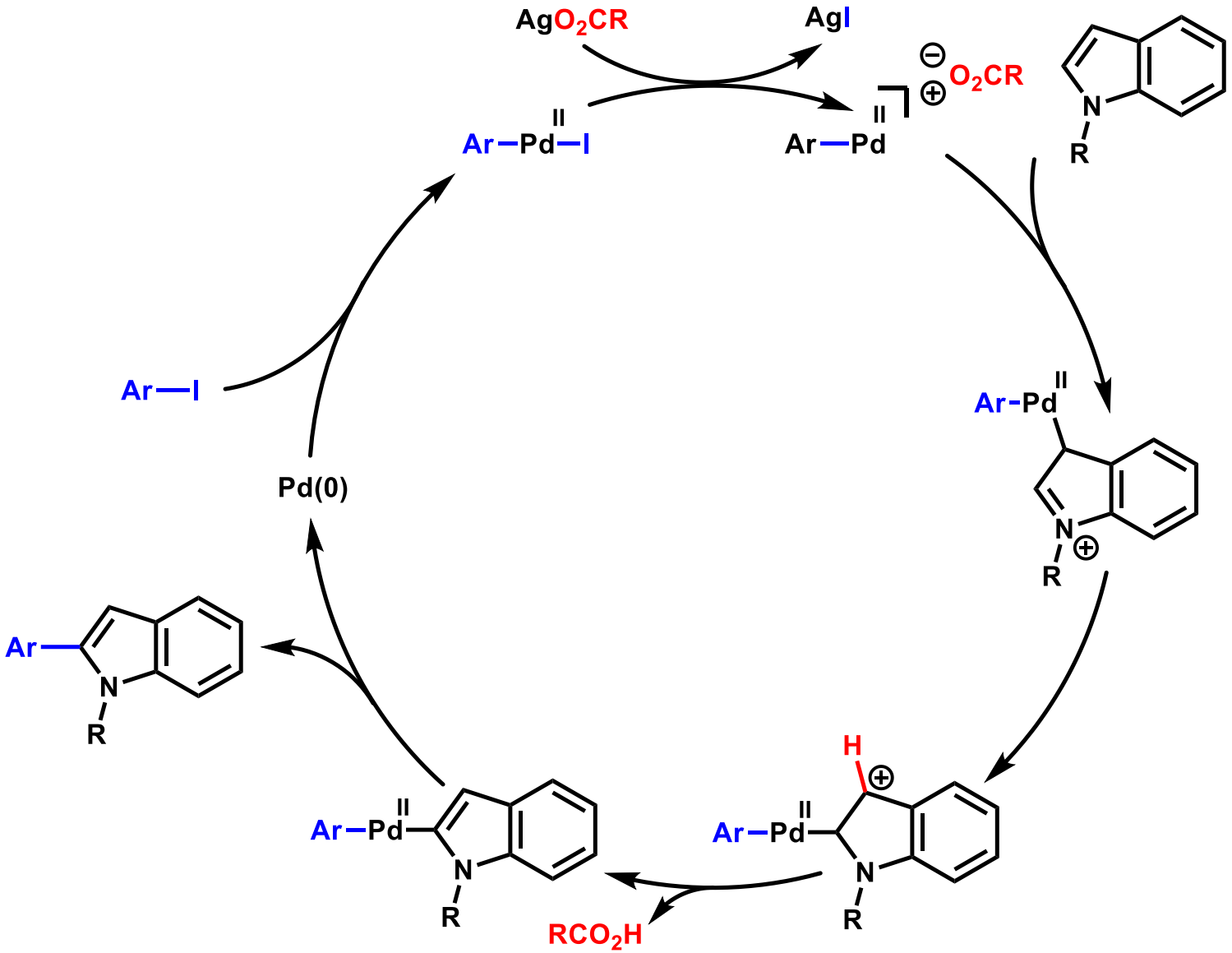
C-H arylation of indole



entry	base	RCO_2H	conv (%)
1	K_2CO_3	none	<5
2	AgOAc	none	53
3	Ag_2O	none	12
4	Ag_2O	AcOH	49
5	Ag_2O	*o*-MeO-C_6H_4-CO_2H	13
6	Ag_2O	*o*-Ph-C_6H_4-CO_2H	95
7	Ag_2O	*o*-NO_2-C_6H_4-CO_2H	>99

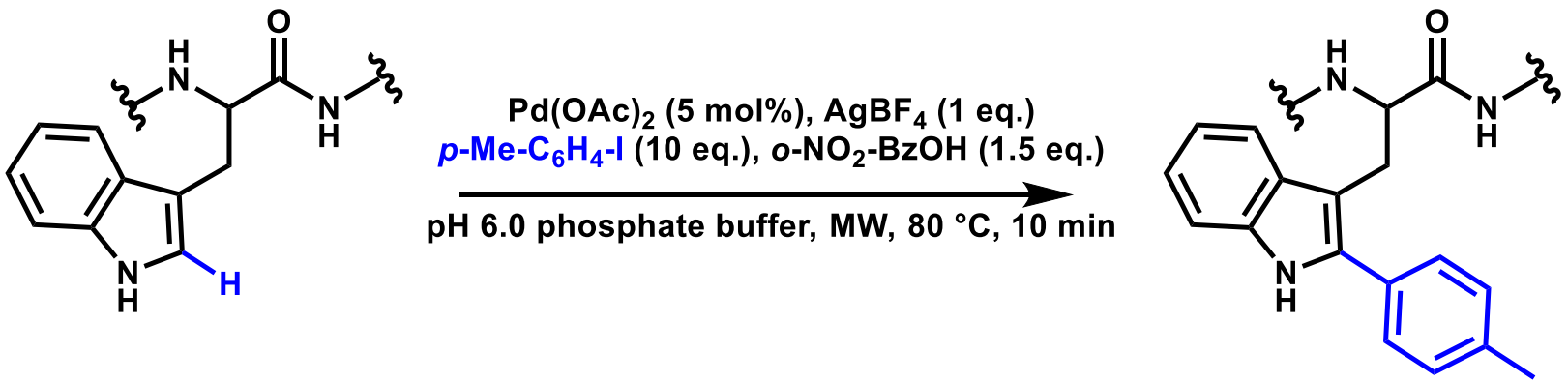
Lebrasseur, N. and Larrosa, I. *J. Am. Chem. Soc.* **2008**, *130*, 2926.

Proposed mechanism



Sames, D. et al *J. Am. Chem. Soc.* **2005**, *127*, 8050.
Lebrasseur, N. and Larrosa, I. *J. Am. Chem. Soc.* **2008**, *130*, 2926.

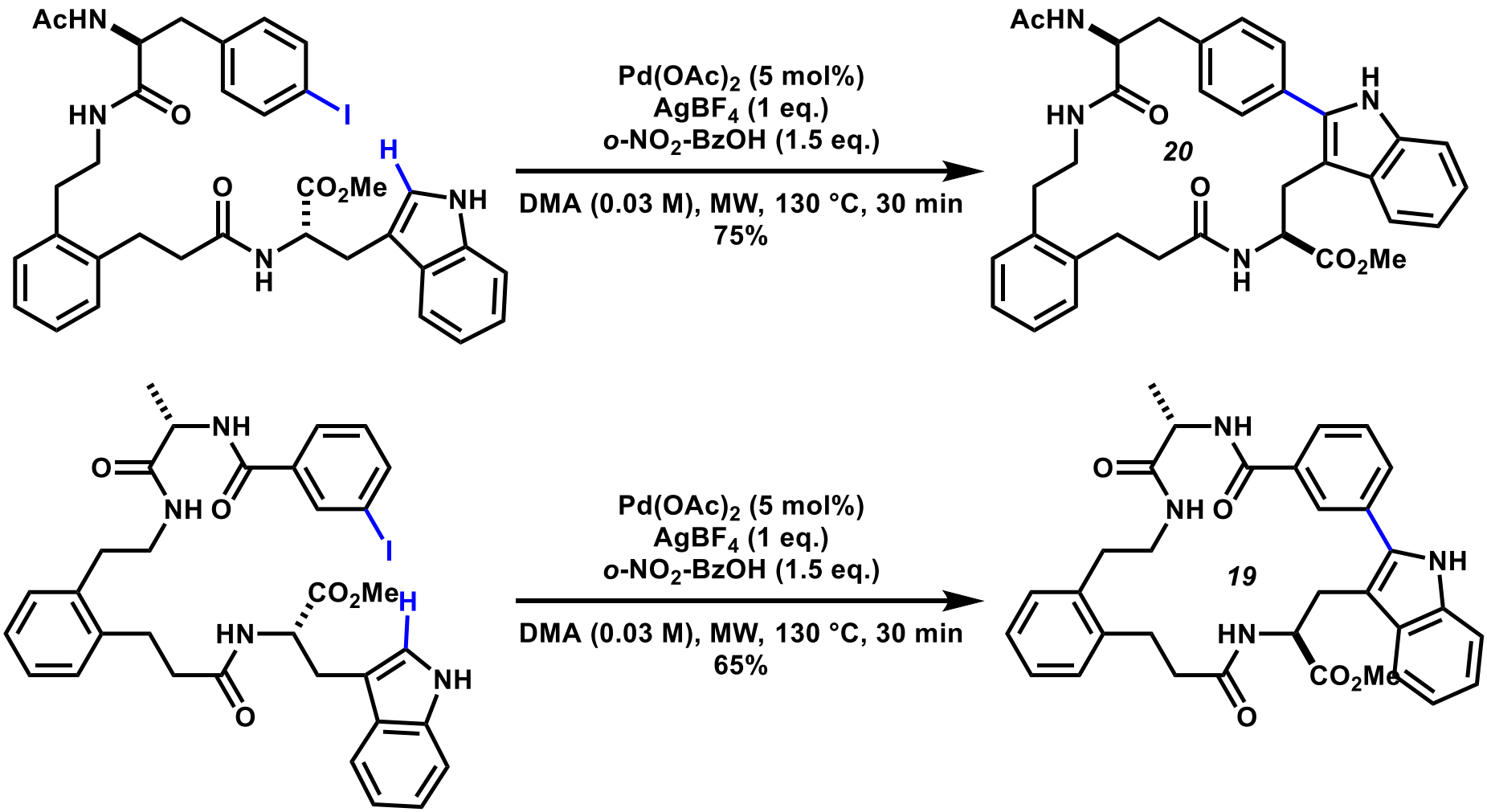
C(sp^2)-H arylation of Trp residue in peptides



entry	peptide	yield (%)	entry	peptide	yield (%)
1	Ac-Ala-Trp-Ala-OH	57	6	Ac-Lys-Gly-Trp-Ala-OH	95
2	Ac-Trp-Leu-Asp-Phe-OH	68	7	Ac-Ser-Gly-Trp-Ala-OH	62
3	Ac-Tyr-Pro-Trp-Phe-OH	75	8	Ac-Met-Gly-Trp-Ala-OH	-
4	Ac-Arg-Gly-Trp-Ala-OH	94	9	Ac-Gln-Phe-Ala-Trp-OH	88
5	Ac-His-Gly-Trp-Ala-OH	51			

Albericio, F. et al. *Chem. Eur. J.* **2010**, *16*, 1124.

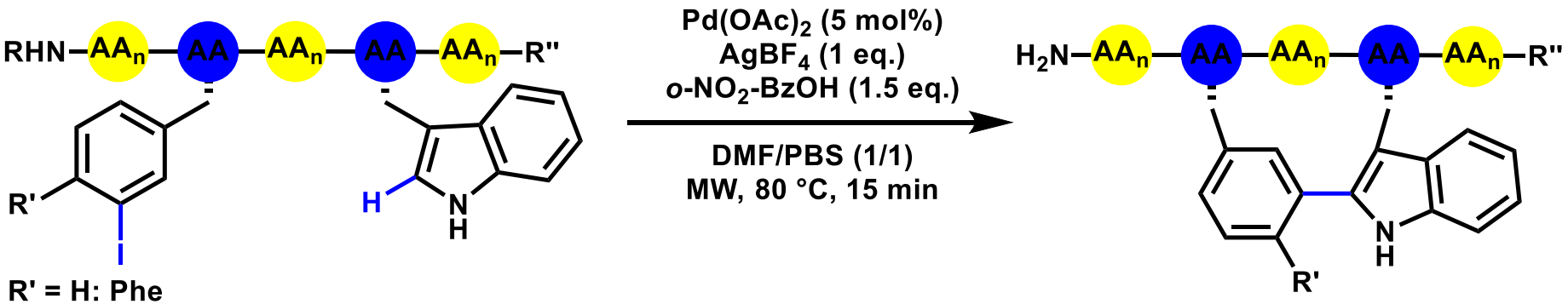
Macrocyclization via C(sp^2)-H arylation



***p*- or *m*-biaryl-bridged macrocyclizations were achieved**
15-25 membered biaryl macrocycles were synthesized (40-75% yield)

James, K. *Chem. Commun.*, **2012**, *48*, 11644.

Peptide stapling via C(sp^2)-H arylation

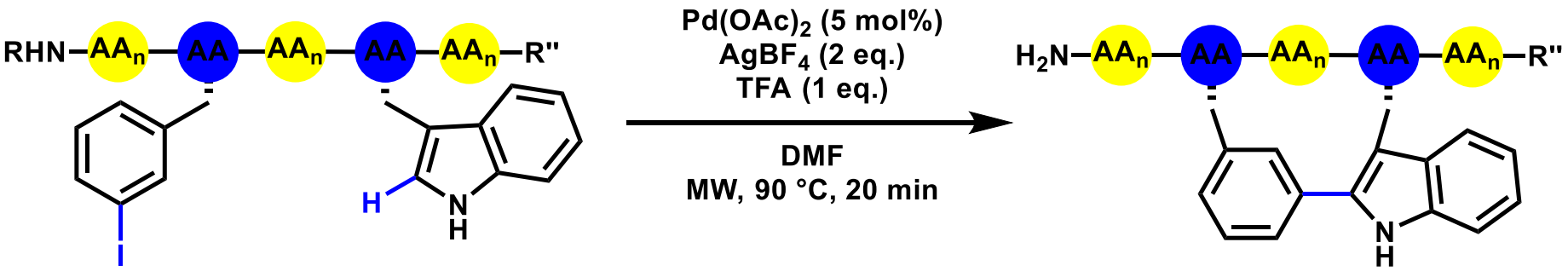


R' = H: Phe
R' = OAc: Tyr

entry	peptide	conv (%)
1	Ac-Ala-*m*-I-Phe-Ala-Trp-Ala-OH	38
2	Ac-Ala-*m*-I-Phe-Ala-Ala-Trp-Ala-OH	100
3	Ac-Ala-*m*-I-Phe-Ala-Ala-Ala-Trp-Ala-OH	100
4	Ac-Ala-*m*-I-Tyr-Ala-Trp-Ala-OH	100
5	Ac-Ala-*m*-I-Tyr-Ala-Ala-Trp-Ala-OH	100
6	Ac-Ala-*m*-I-Tyr-Ala-Ala-Ala-Trp-Ala-OH	100

Albericio, F. et al. *Nat. Commun.* **2015**, *6*, 7160.

Peptide stapling via C(sp^2)-H arylation

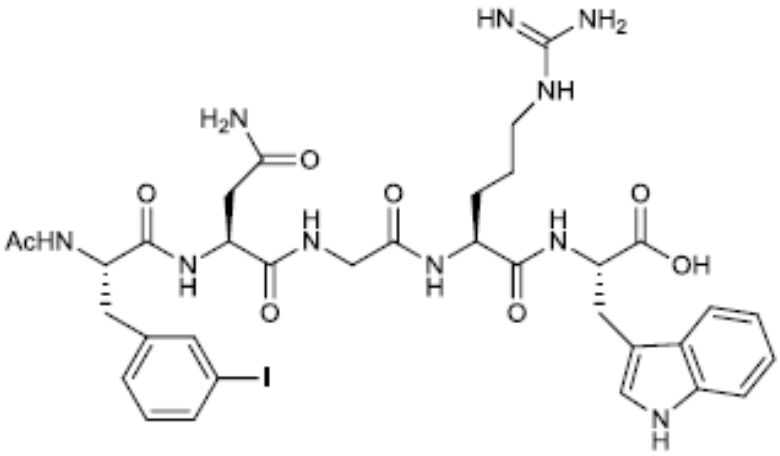


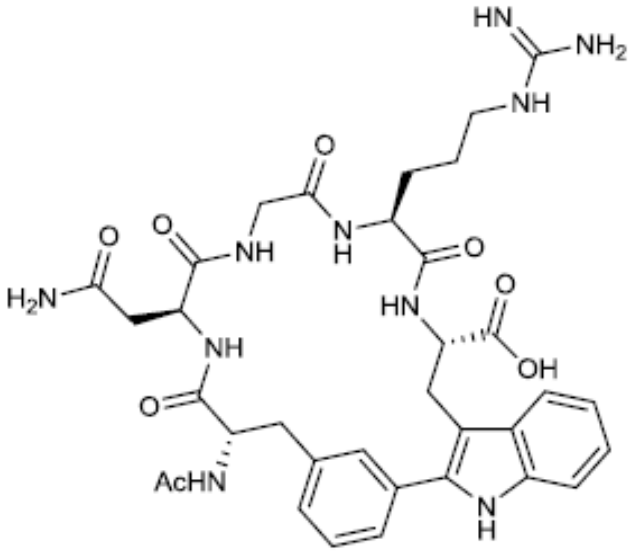
entry	peptide	conv (%)
1	Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-NH_2	77
2	Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-NH_2	70
3	H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH	39
4	Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH	71
5	Ac-Ala-*m*-I-Phe-Trp-Ala-OH	60*

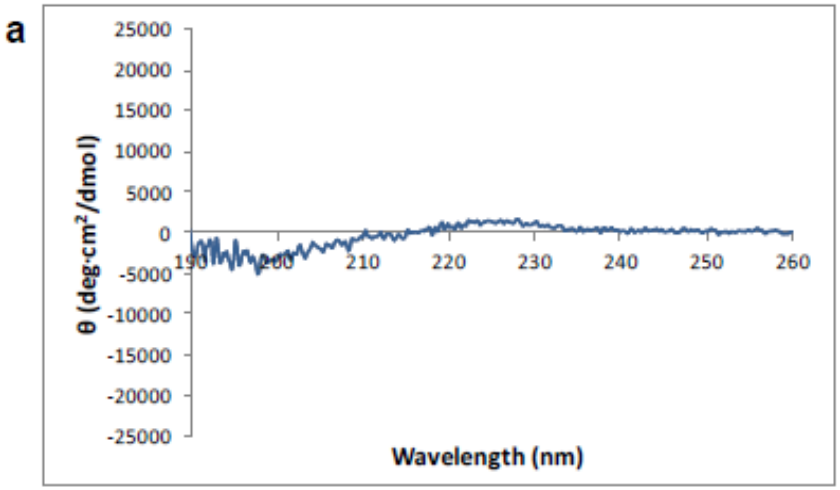
*: cyclodimer was obtained instead of monomeric macrocycle

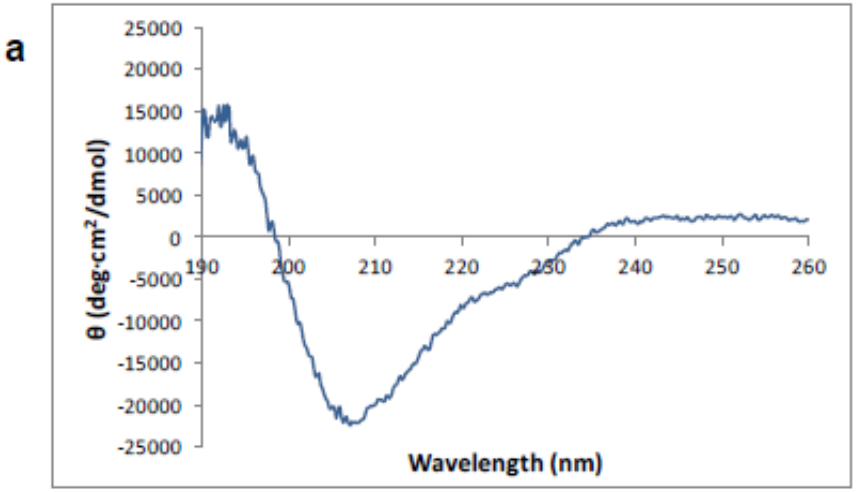
Albericio, F. et al. *Nat. Commun.* **2015**, *6*, 7160.

CD spectra of peptides





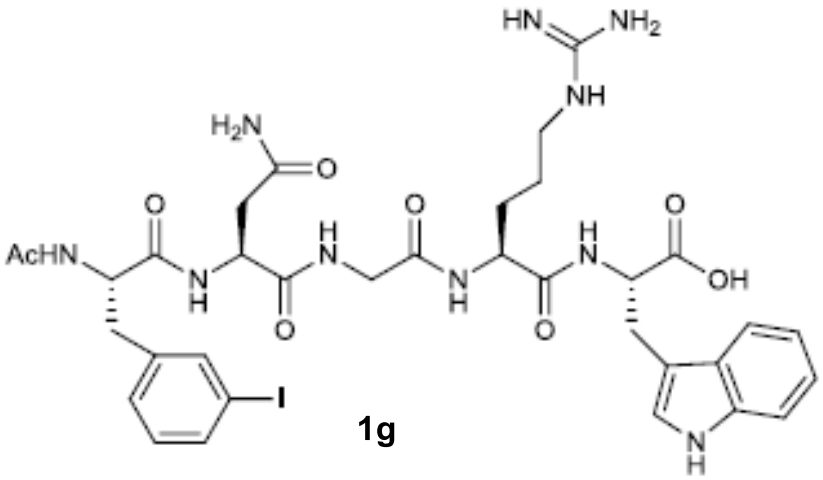




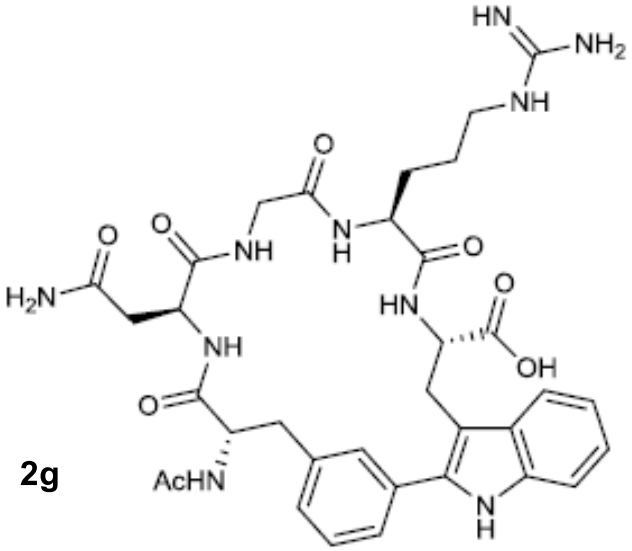
Stapled peptide shows a positive maximum at ~190 nm and minimum peak at 206 nm, which indicates structuring.

Albericio, F. et al. *Nat. Commun.* **2015**, *6*, 7160.

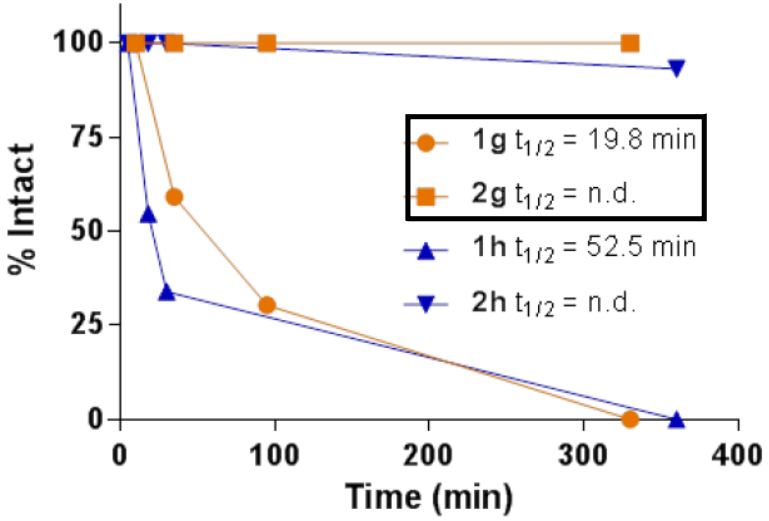
Proteolytic degradation assay



1g



2g



Conditions:
50 μM peptide, 50 μg/mL α-chymotrypsin in pH 7.8 buffer/DMSO (9/1)

Proteolytic degradation assay with α-chymotrypsin was conducted.
Stapled peptide 2g is stable under the condition at 6 h, while parent peptide 1 g is completely hydrolysed.

Albericio, F. et al. *Nat. Commun.* **2015**, *6*, 7160.

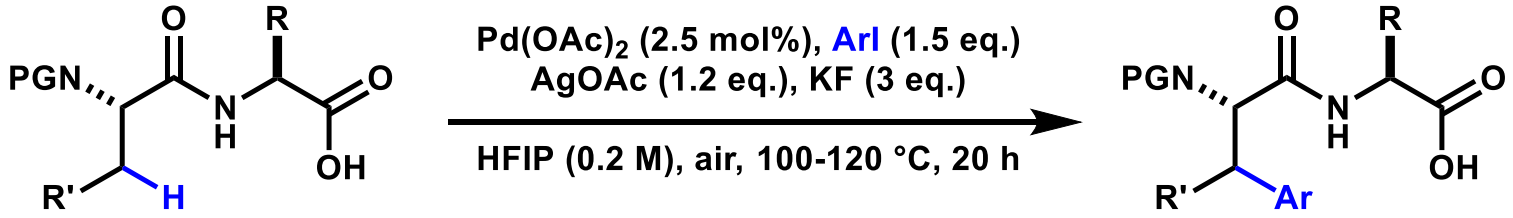
Contents

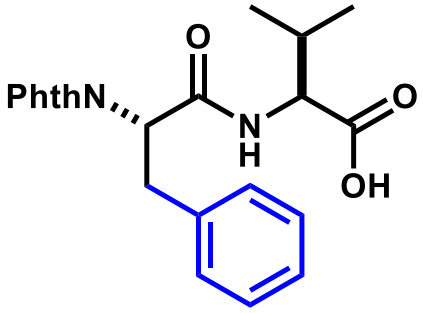
1. Conventional methods of peptide stapling

2. Peptide stapling via C(sp^2)-H arylation

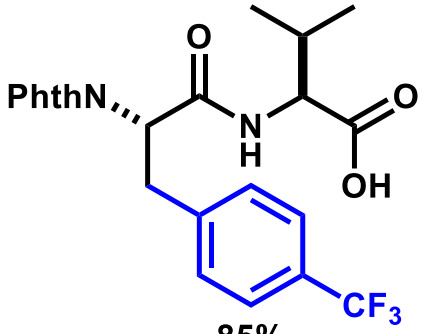
3. Peptide stapling via C(sp^3)-H arylation

C(sp^3)-H arylation of dipeptides

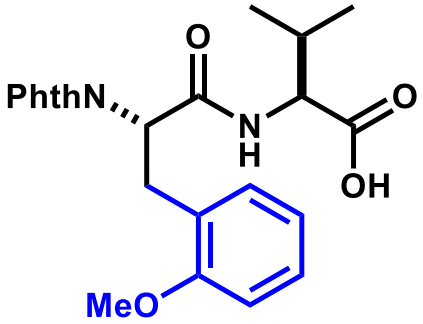




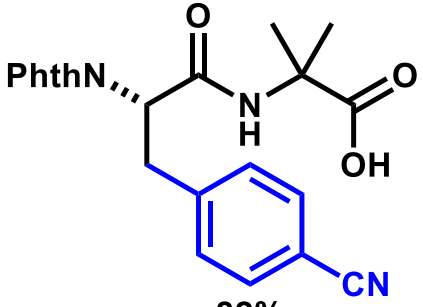
90%
mono:di = 10:1



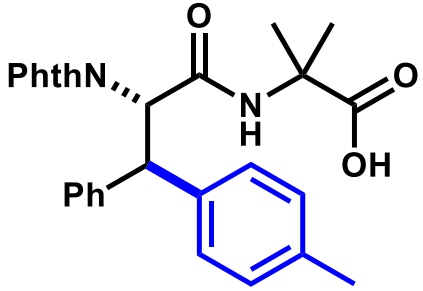
85%
mono:di = 7:1



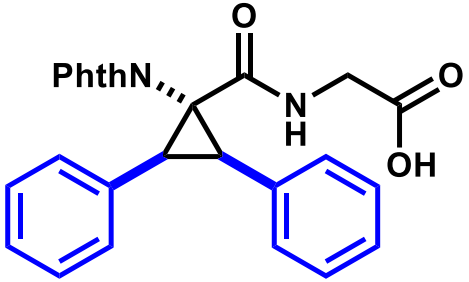
80%
mono:di = >20:1



82%
mono:di = 18:1



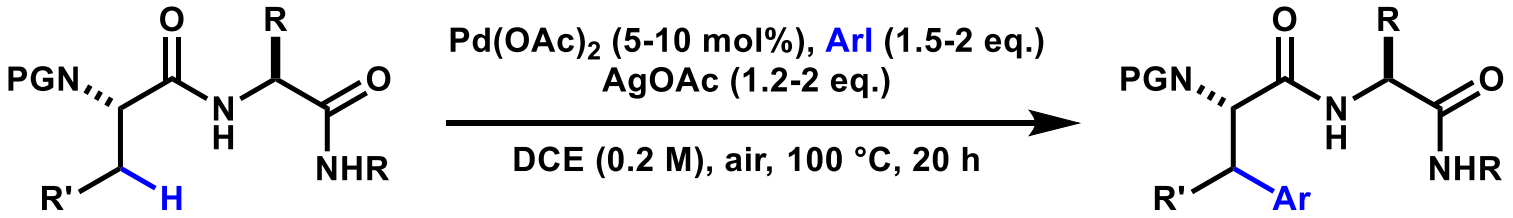
48%
dr = 9:1

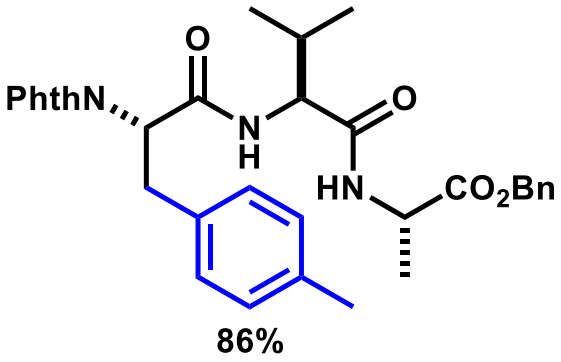


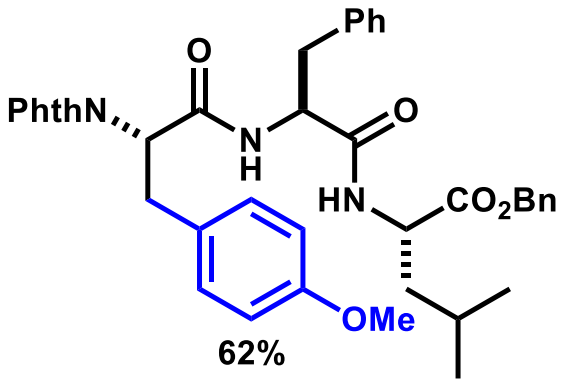
70%

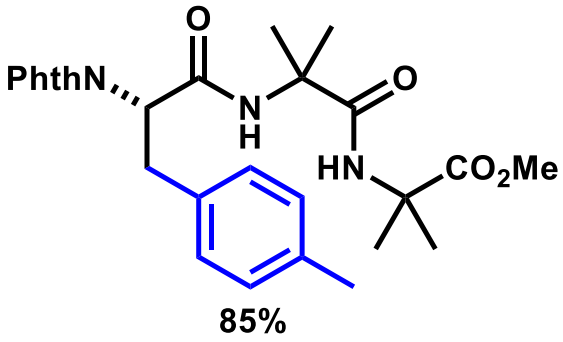
Yu, J.-Q. et al *J. Am. Chem. Soc.* **2014,** *136*, 16940.

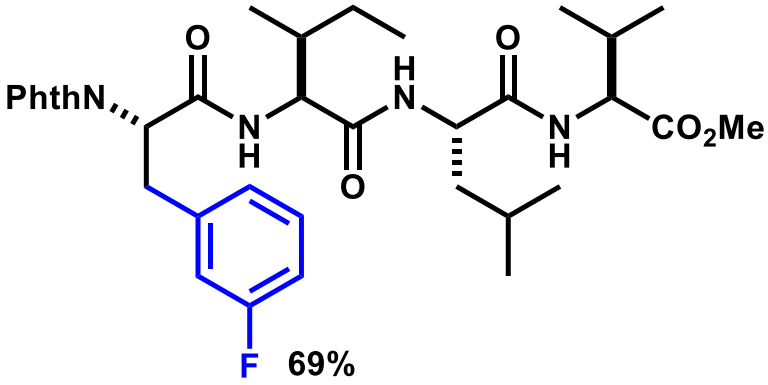
C(sp^3)-H arylation of tri- and tetrapeptides





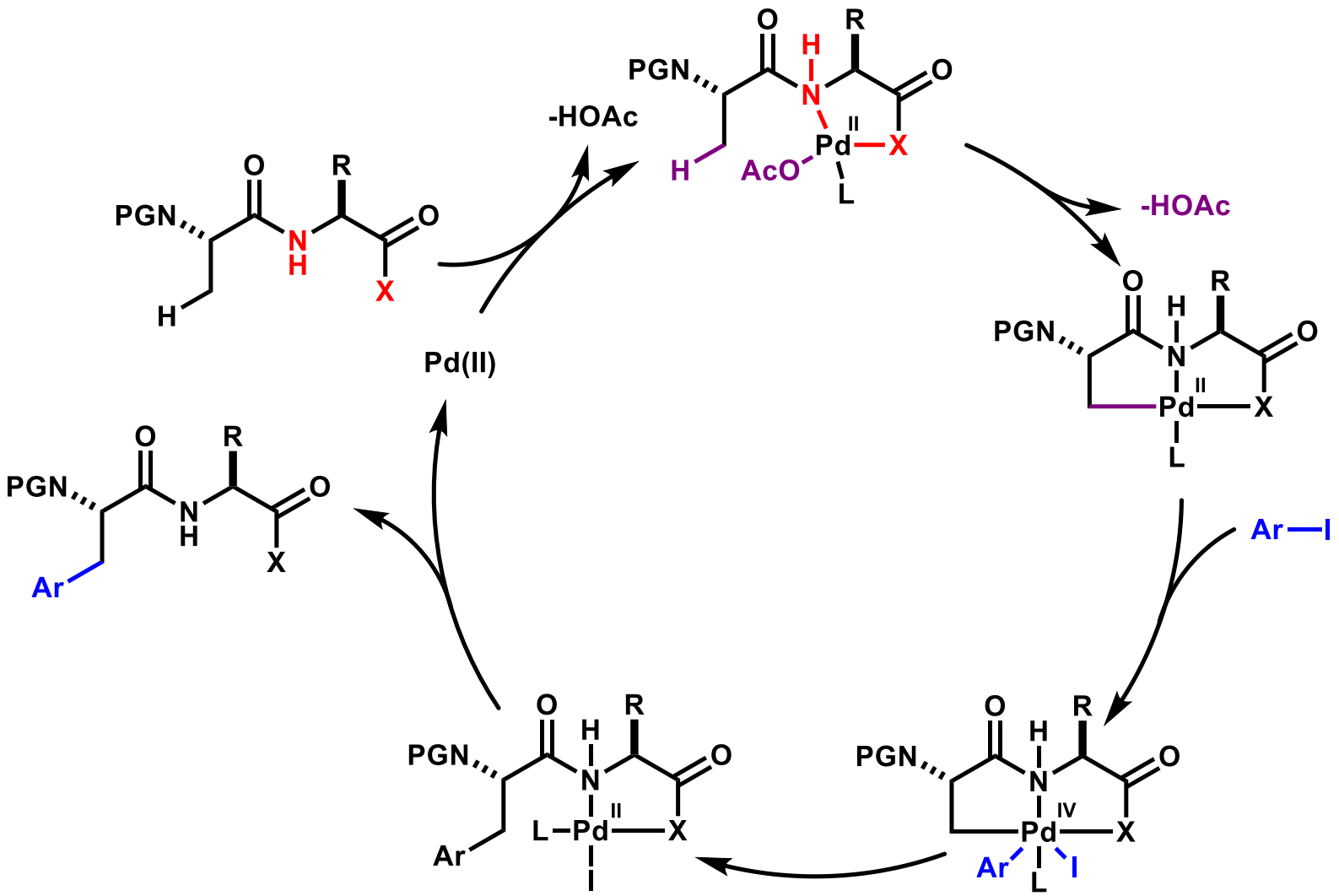






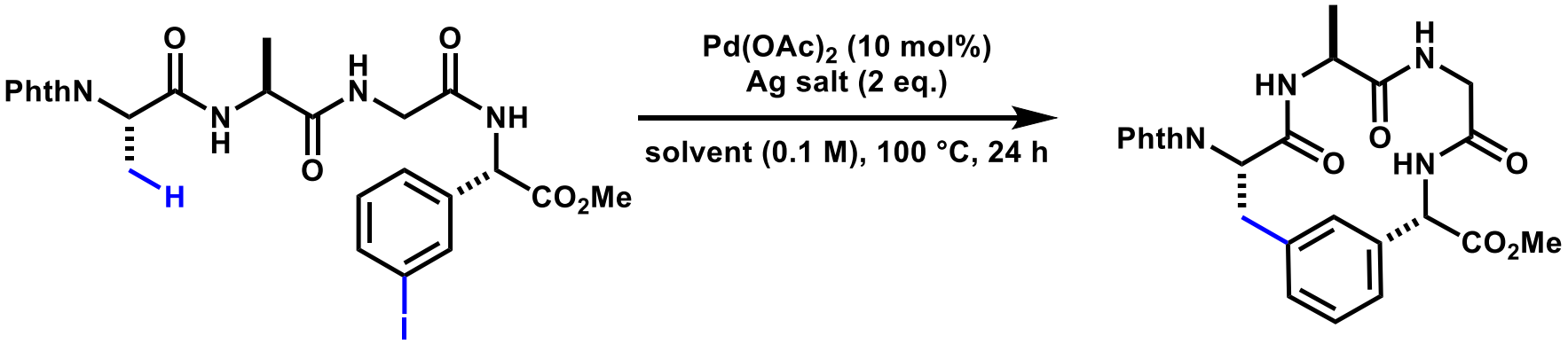
Yu, J.-Q. et al *J. Am. Chem. Soc.* **2014,** *136*, 16940.

Proposed mechanism



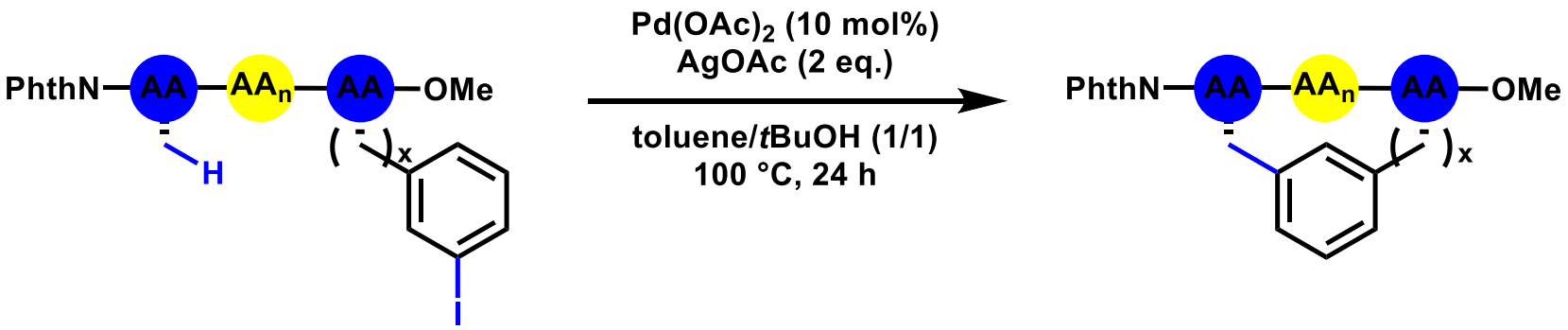
Chen, G. et al. *Org. Lett.* **2010**, *12*, 3414.
Yu, J.-Q. et al *J. Am. Chem. Soc.* **2014,** *136*, 16940.

Peptide stapling via C(sp^3)-H arylation

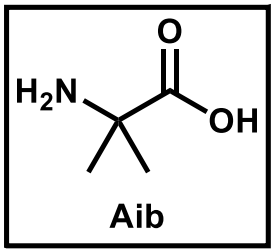


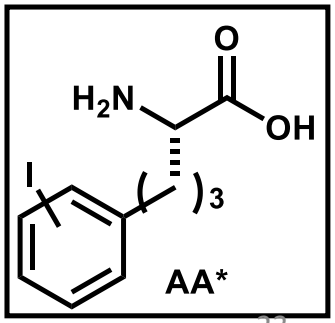
entry	Ag salt	solvent	conv (%)
1	AgOAc	DCE	63
2	$AgCO_3$	DCE	17
3	AgTFA	DCE	2
4	AgOAc	*t*BuOH	65
5	AgOAc	DCE/*t*BuOH (1/1)	69
6	AgOAc	toluene/*t*BuOH (1/1)	88

Peptide stapling via C(sp^3)-H arylation

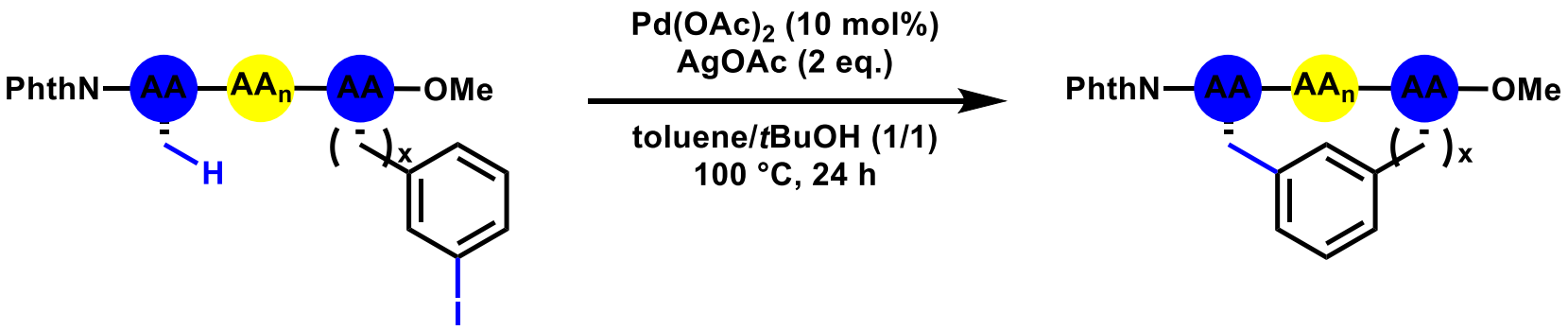


entry	x	peptide	conv (%)	yield (%)
1	1	Phth-Ala-Ala-Gly-*m*-I-Phe-OMe	88	29
2	1	Phth-Ala-Ser(O*t*Bu)-Gly-*m*-I-Phe-OMe	96	25
3	1	Phth-Ala-Lys(Boc)-Gly-*m*-I-Phe-OMe	77	53
4	1	Phth-Ala-Trp(Boc)-Gly-*m*-I-Phe-OMe	53	25
5	1	Phth-Ala-Met-Gly-*m*-I-Phe-OMe	26	17
6	1	Phth-Ala-Ala-Gly-*m*-I-D-Phe-OMe	88	23
7	1	Phth-Aib-Ser(O*t*Bu)-Gly-*m*-I-Phe-OMe	57	30
8	3	Phth-Ala-Ser(O*t*Bu)-Gly-*m*-I-AA*-OMe	57	23

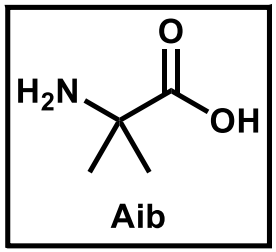


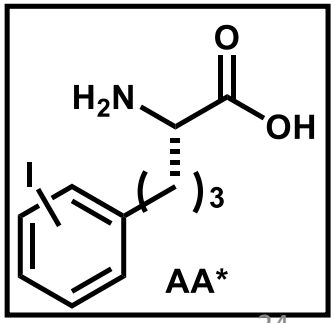


Peptide stapling via C(sp^3)-H arylation

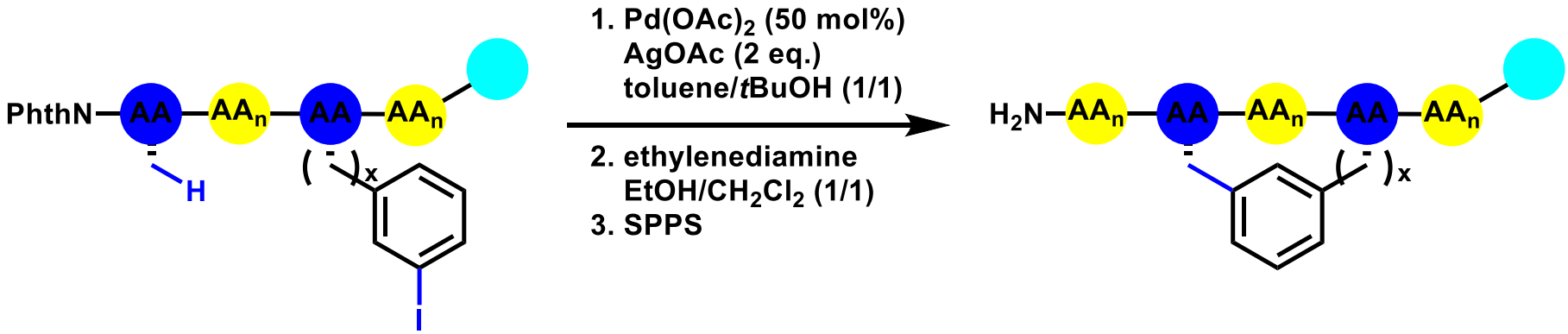


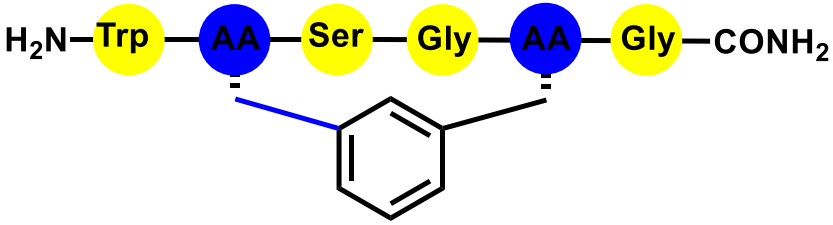
entry	x	peptide	conv (%)
1	1	Phth-Ala-Pro-Gly-*m*-I-Phe-OMe	no conv
2	1	Phth-Ala-Cys(Trt)-Gly-*m*-I-Phe-OMe	no conv
3	1	Phth-Ala-His(Trt)-Gly-*m*-I-Phe-OMe	complex
4	1	Phth-Phe-Ser(O*t*Bu)-Gly-*m*-I-Phe-OMe	no conv
5	1	Phth-Ala-Ala-Gly-*p*-I-D-Phe-OMe	complex
6	3	Phth-Ala-Ala-Ser(O*t*Bu)-*p*-I-AA*-OMe	complex
7	1	Phth-Ala-Gly-*m*-I-D-Phe-OMe	no conv



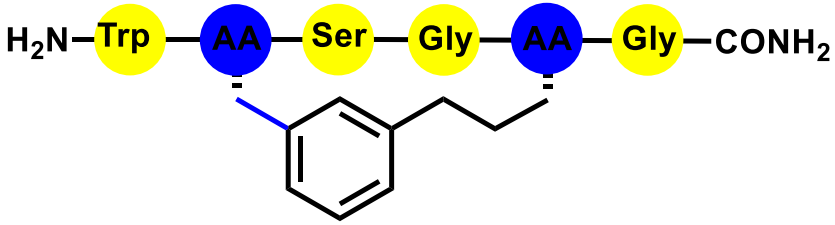


On resin peptide stapling

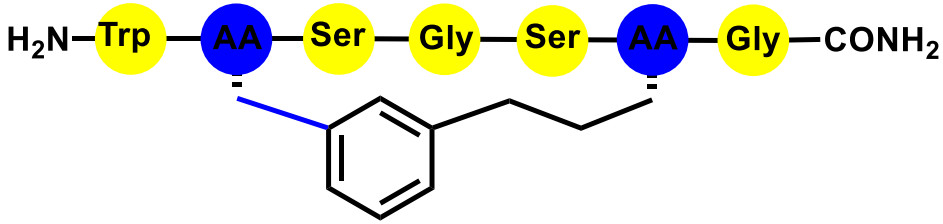




13%

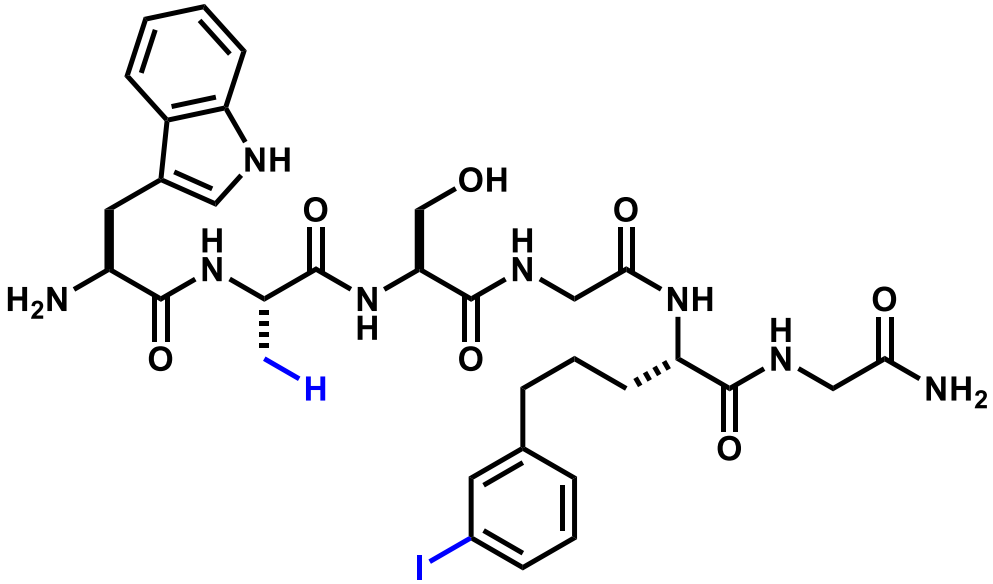


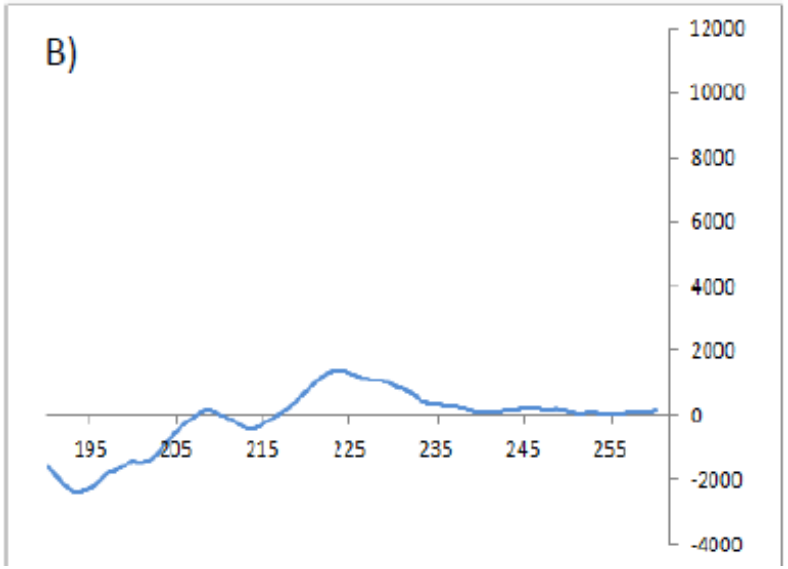
11%

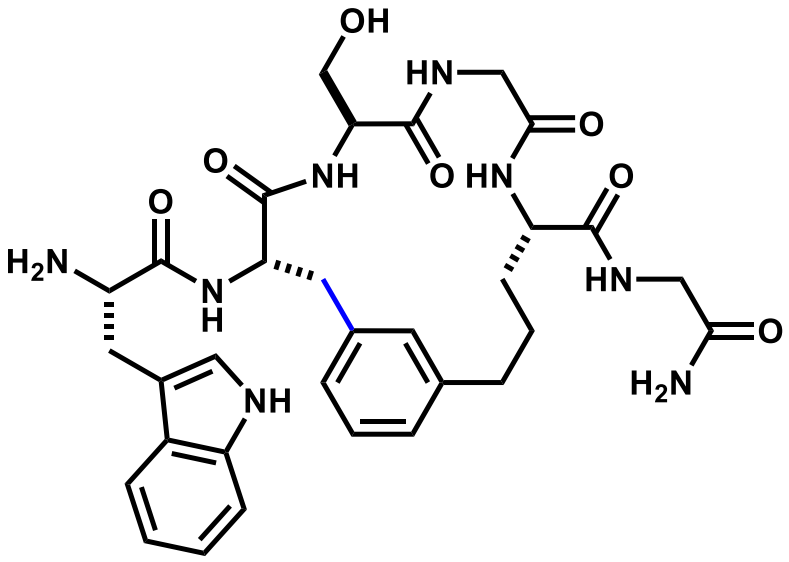


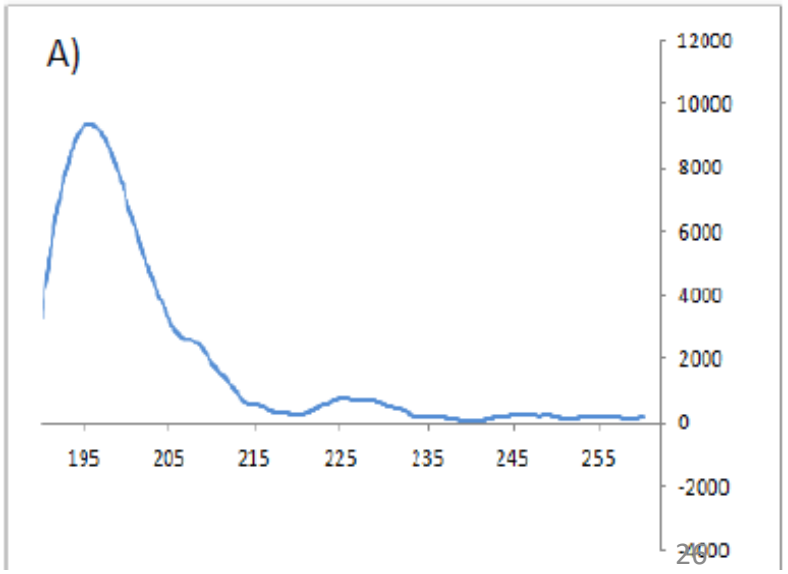
15%

CD spectra of peptides

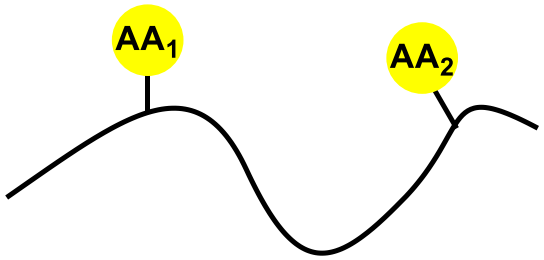






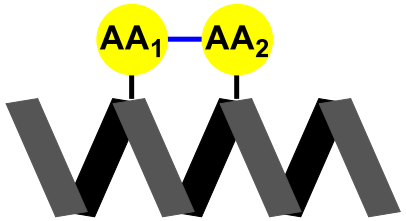


Summary

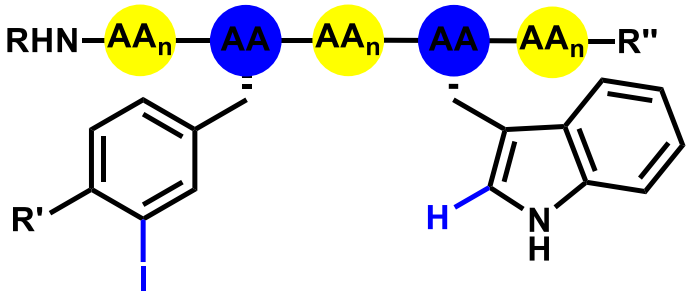


Native peptide
flexible conformation

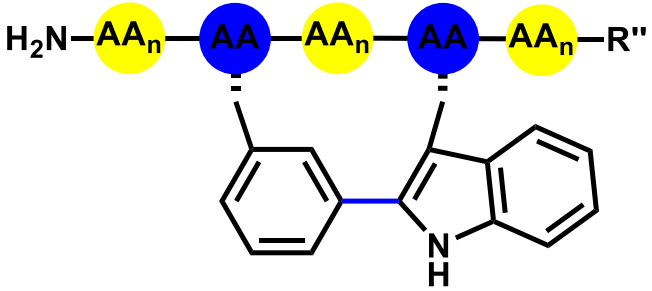
disulfide bridge
lactam bridge
RCM
CuAAC
etc...

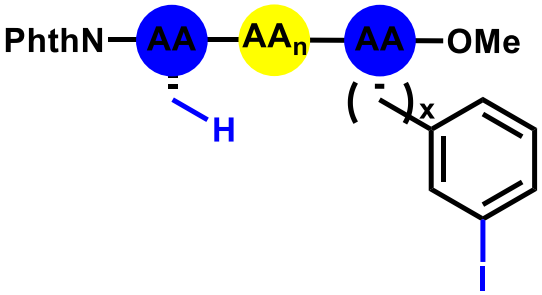


Stapled peptide
restricted conformation



C(sp²)-H arylation





C(sp³)-H arylation