The Organic Chemist's Guide to Peptide Stapling

Outline:

1. The Importance of Macrocyclic Peptides in Medicinal Chemistry
2. Synthetic Considerations for Stapling Methods
3. Amide Bond Formation
4. Metathesis
5. Cycloadditions and Click Chemistry
6. Cysteine Linkages
7. C-C σ-Linkages

Not covered: β-sheet mimetic staples, Glaser Coupling, and yne-yne metathesis.

A Few Useful Reviews:

For a comprehensive overview of peptide macrocyclization strategies:

Yudin, A. *Nature Chemistry*, **2011**, *509-524*.
https://doi.org/10.038/nchem.1062

Spring, D. *Chem. Soc. Rev.*, **2015**, 44, 91.
https://pubs.rsc.org/en/content/articlepdf/2015/cs/c4cs00246f

Lamers, C. *RSC Med. Chem.*, **2021**, 12, 1325.
https://doi.org/10.1039/D1MD00083G

For a comprehensive overview of macrocyclic peptides as therapeutics:

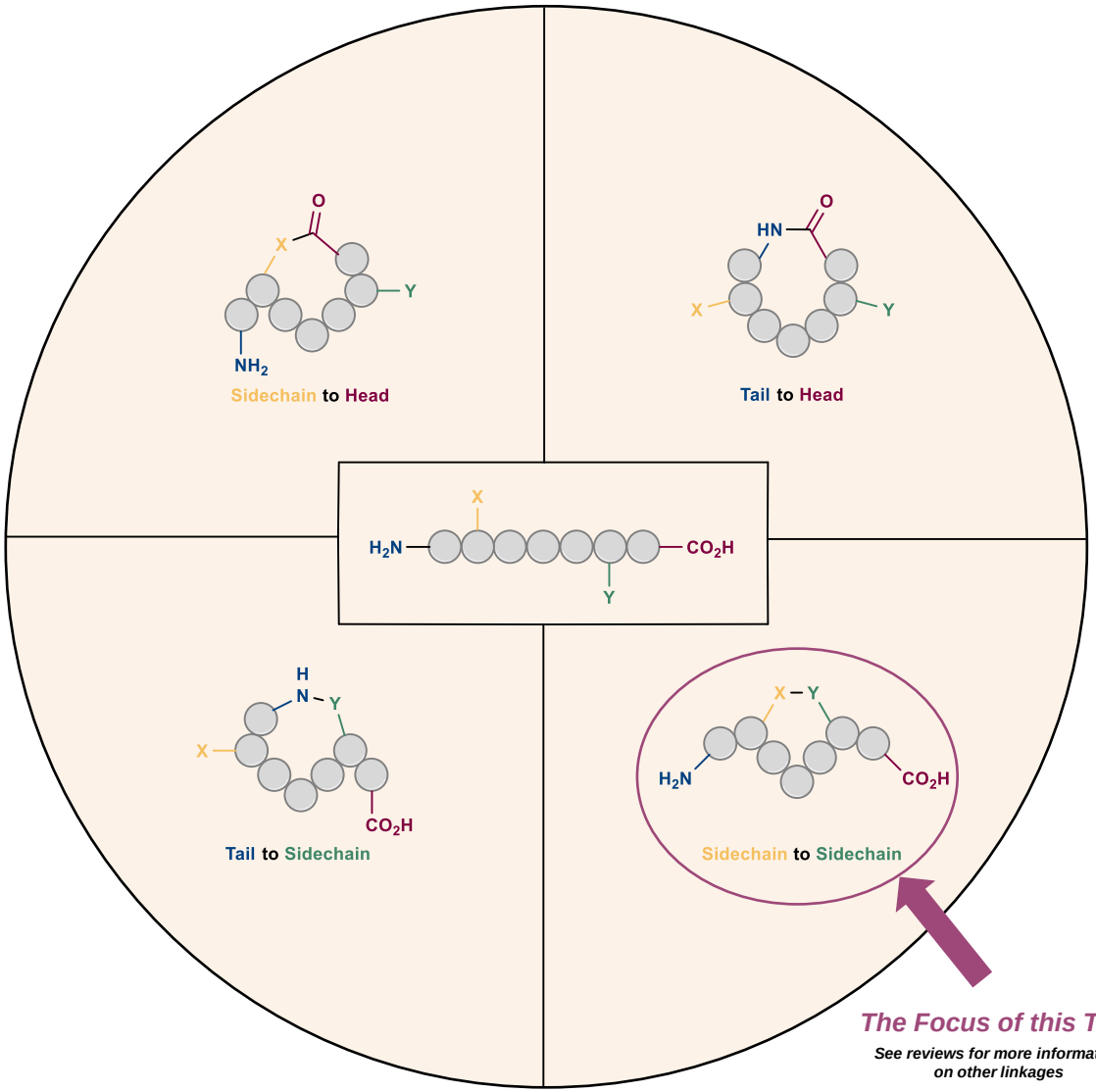
Suga, H. *J. Am. Chem. Soc.* **2019**, 141, 10, 4167–4181.
https://doi.org/10.1021/jacs.8b13178

Related Sarlah Group Topic Seminars:

Annie Hooper's (2020): *The Chemistry and Biology of Vancomycin*

David Ryffel's (2022): *Recent Syntheses of Complex Peptide Natural Products*

The Four Types of Peptide Macrocycles

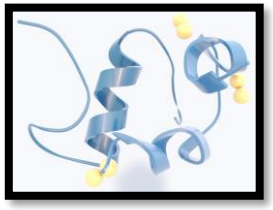


Peptides as Therapeutics

A Very Brief History of Peptides as Therapeutics:

1920-50s

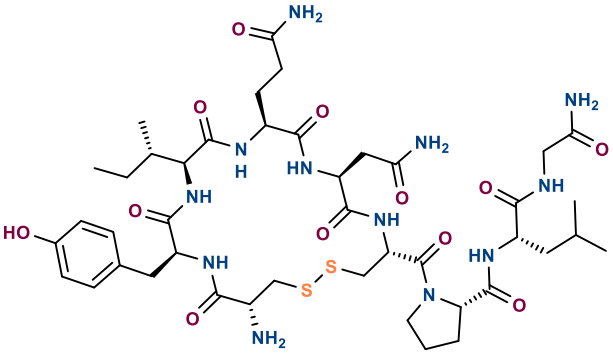
- *Endogenous peptides*
- *Technological limitations and fundamental understanding of peptide therapeutics limit their prevalence*



Insulin (diabetes) – 1922

1950-60s

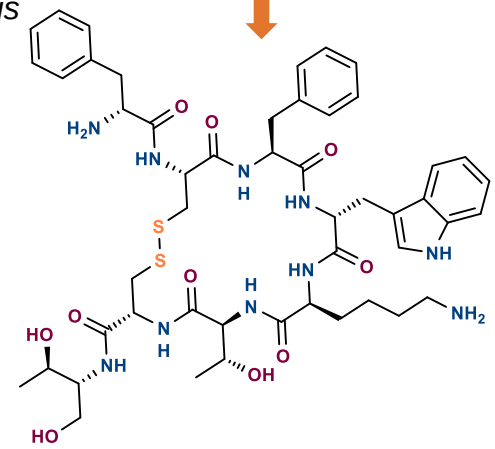
- *Sequencing technology advances*
- *Solid-phase peptide synthesis developed*
- *Isolation and purification techniques improve significantly (HPLC is developed)*



Oxytocin (painkiller) - 1962

1970-80s

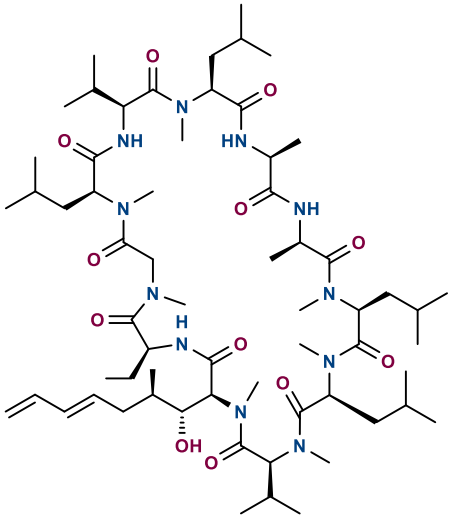
- *Synthetic analogues begin to boom*
- *Huge leaps in biotechnology (recombinant technology)*



Octreotide (anti-cancer) - 1988

1990-present

- *Begin to circumvent traditional limitations of peptide therapeutics*
- *Huge advances in computational capabilities*
- *Large synthetic and HTS advances*
- *First demonstration of "stapled peptides"*

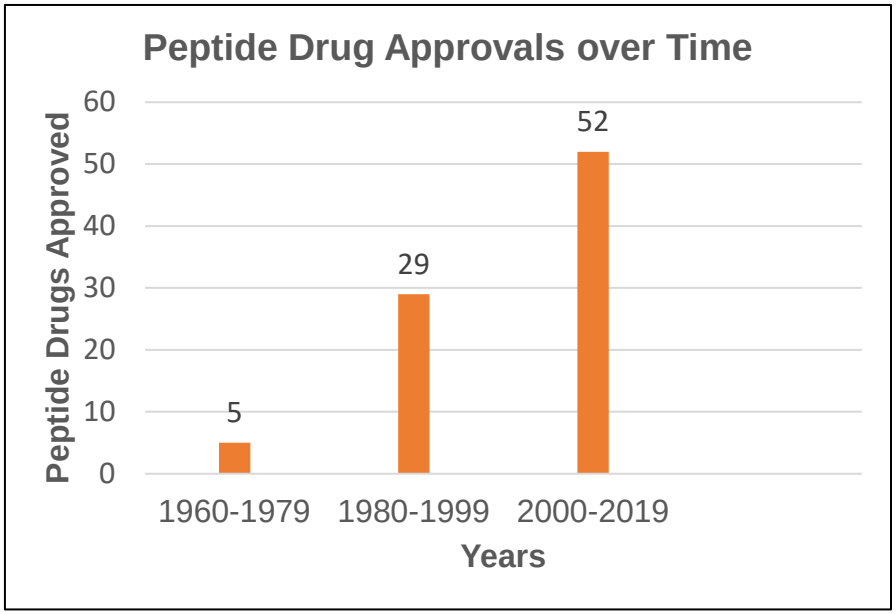


Voclosporin (lupus) - 2021

Dunn, M. *Bioorganic and Medicinal Chemistry*, **2018**, 26. http://dx.doi.org/10.1016/j.bmc.2017.06.052

A Closer Look at Peptide Therapeutic Development

Why did it take so long for peptide therapeutics to take off?



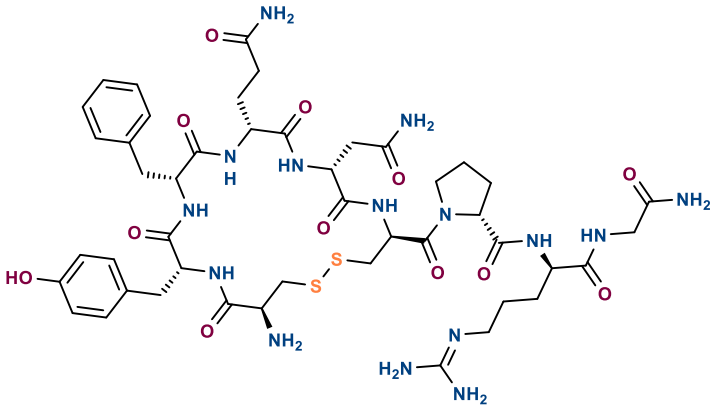
General Characteristics of Peptide Therapeutics:

- *Remarkable potency*
- *High selectivity*
- *Low toxicity*
- *Low oral bioavailability*
- *Low plasma stability*
- *Short circulation time*

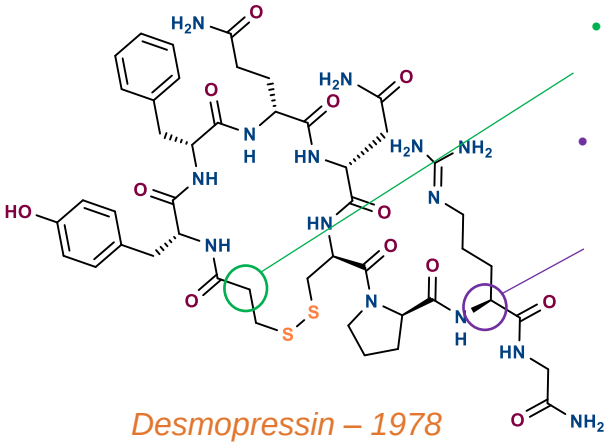
Associated limitations were previously quite difficult to address!

- How do you modify a complex peptide to improve bioavailability and stability without solid phase synthesis or recombinant technology?
- How do you perform HTS without robust purification techniques?
- How do you reduce renal excretion and prevent proteolysis without the means to understand the pharmacokinetics?

Beginning to Address Some of the Problems through Synthetic Analogues:



Vasopressin – anti diuretic (1962)



Desmopressin – 1978

- *Deamination protects against exopeptidases*
- *L-Arg to D-Arg protects against trypsin-like cleavage of the Pro-Arg bond*

Adams, D.J., *Nat Rev Drug Discov.* **2021**, 20, 309–325. https://doi.org/10.1038/s41573-020-00135-8

Macrocycles and the α-helix

Macrocycles Afford a Number of Advantages in Peptide Therapeutics:

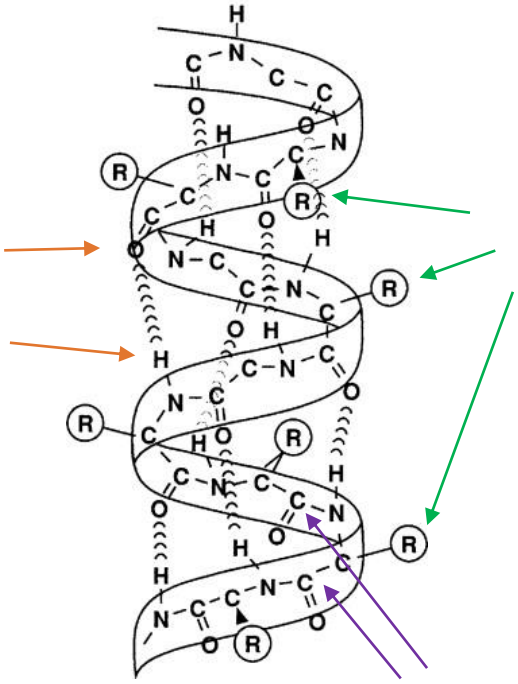
- *Macrocycles increase rigidity of the structure, reducing conformational flexibility*
- *The incorporation of macrocycles will also reduce susceptibility of proteolytic cleavage*
- *Allows a new frontier of targeting "undruggable" protein-protein interactions and surfaces*
- *Macrocycles generally increase membrane permeability, increasing drug viability*

- *Macrocyclic peptides offer many of the benefits of small molecules while also offering the broad target capabilities of monoclonal antibodies.*

Notice, the overlap in the benefits of macrocycles and α-helices

The α-helix:

Carbonyl O and Amidic H of every fourth residue engage in H-bonding, stabilizing the helix.



Polar functionality present on the AA residues interacting and forming the center of the helix will result in the often more polar side chains being found on the outside of the helix.

With the cleavable amides rigidly locked within the helix proteolytic cleavage becomes more difficult.

The Formation of α-helices:

While α-helix formation could be a topic seminar on its own, the important points to highlight:

- *Entropically, helices are not inherently favored and will require pre-cyclization conformations which can often be challenging to meet.*
- *Beyond these conformational considerations, α-helices can be destabilized in a polar medium which interacts with the polar functionality (hence in situ formation of α-helices generally occurring in nonpolar solvents).*

What's the fundamental overlap between these α-helices and macrocycles?

Macrocycles will introduce conformational rigidity which promotes α-helix formation in many peptides. This affords the benefits of both macrocycles and α-helices.

Passioura, T., *Biochemistry* **2020**, 59, 2, 139–145 https://doi.org/10.1021/acs.biochem.9b00802

Stapled Peptides and the α-helix

The Commonly Employed Definitions of Peptide Stapling:

"A strategy for constraining short peptides typically in an α-helical conformation." - Spring, D. (2015)

"Stapled peptides consist of peptide chains that bring an external brace that force the peptide structure into an α-helical one. The cross-link is obtained by the linkage of the side chains of opportune-modified amino acids posed at the right distance inside the peptide chain." – Quadrelli, P. (2019)

"The concept of "stapling" itself is not new and refers to the connection of two parts of a molecule using a cross-linker. The term tends to be used to indicate any cyclic peptides that are not N-to-C cyclic peptides." – Joo (2019)

Spring, D. *Chem. Soc. Rev.*, **2015**, 44, 91. https://pubs.rsc.org/en/content/articlepdf/2015/cs/c4cs00246f

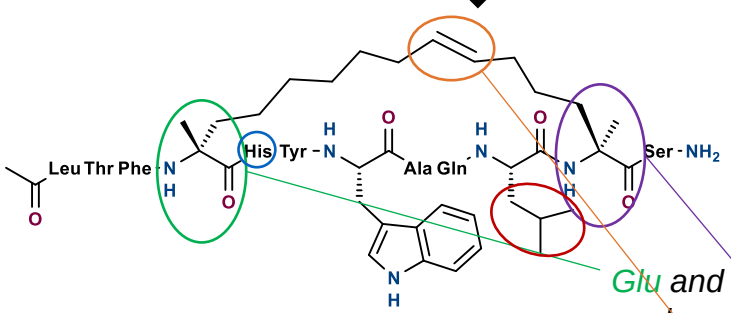
Quadrelli, P. *Molecules*, **2019**, 24, 3654. doi: 10.3390/molecules24203654

***An Example of the Power of Peptide Stapling*:**

MDM2 IC_{50} = **10 nM**

DMX IC_{50} = **110 nM**

Known pDI peptide with actvity against p53 inhibitory proteins MDM2 and MDMX (anti-cancer)



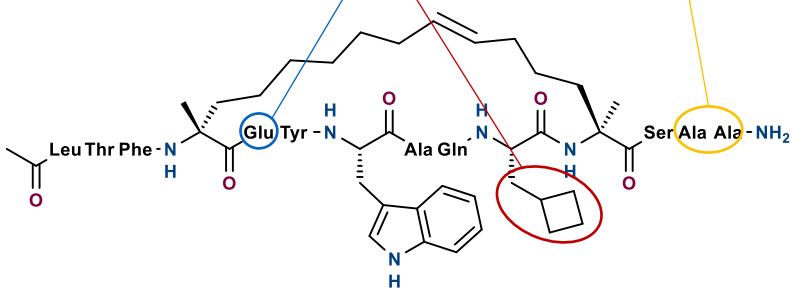
ATSP-3900

MDM2 K_i = **1.0 nM**

DMX K_i = **18.3 nM**

Glu and Thr residues were replace with α-methylated metathesis precursors to aid in cyclization before being stapled via metathesis to form ATSP-3900.

His residue was replaced by Glu, resulting in a fourfold increase in solubility. Replacement of Leu with cBu resulted in increased target binding and cell potency. Finally, increasing the length of the peptide N-termini with Ala-Ala residues, resulted in ATSP-7041, a more potent and more soluble anti-cancer peptide.



ATSP-7041

MDM2 K_i = **0.9 nM**

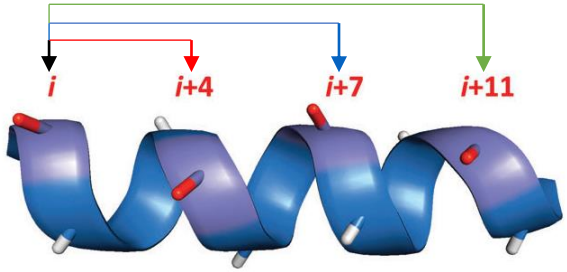
DMX K_i = **6.8 nM**

Peptide Stapling Methods – Amide Bond Formation

Considerations for Stapling Peptides:

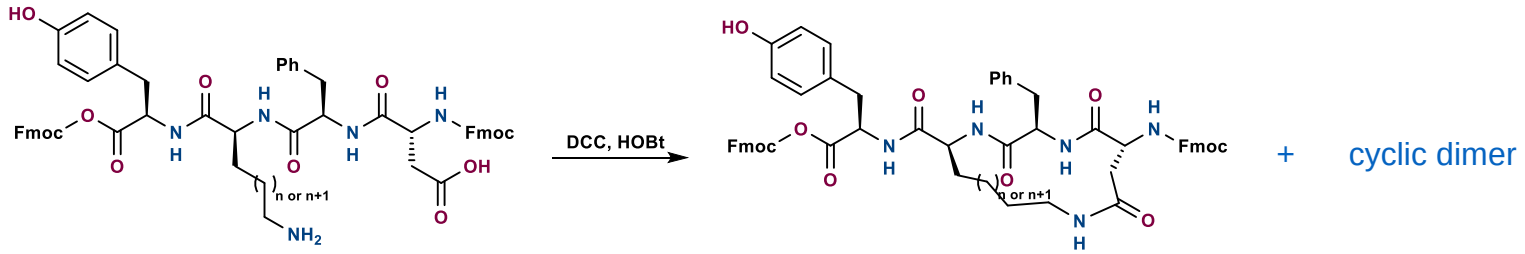
Stapled linkages should occur on residues which lie on the same face of the helix to promote or maintain the α-helical configuration. R groups generally compatible are depicted as ***i*** in the helix.

i,i + 4 = 0.5 nm i,i + 7 = 1.1 nm i,i + 11 = 1.6 nm



Spring, D. *Chem. Soc. Rev.*, **2015**, 44, 91. https://pubs.rsc.org/en/content/articlepdf/2015/cs/c4cs00246f

Schiller, 1985: *first example of sidechain macrolactamization (no helical stabilization, (i,i + 2)*

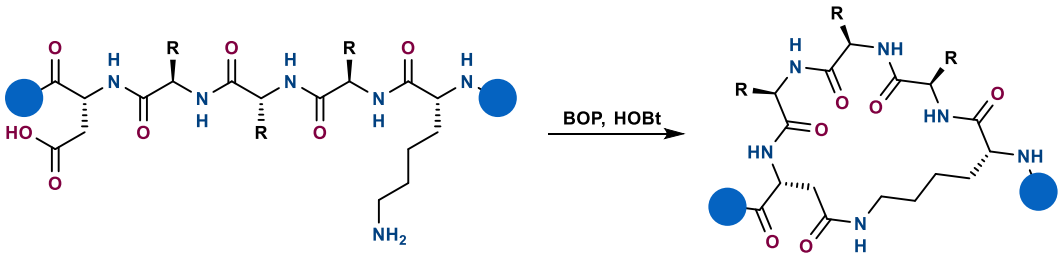


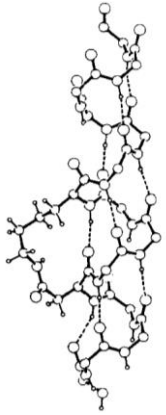
Ratios varied from 1.4:1 to 1:3

Demonstrated this cyclization with various combinations of one D and one L amino acid. Lys and Orn were utilized as amine sources while Glu and Asp were utilized as carboxylic acid sources.

Schiller, P., *J. Med. Chem.* **1985**, 28, 1766-1771. https://doi.org/10.1021/jm00150a005

Felix, 1988: *first example of sidechain stabilization of α-helix via macrolactamization (i,i + 4)*

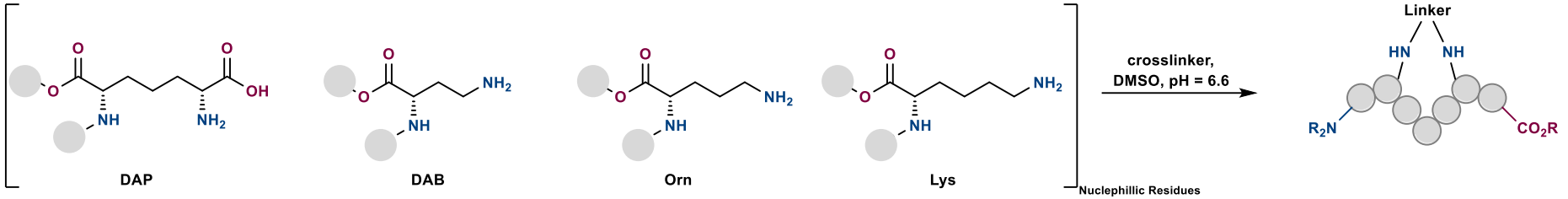




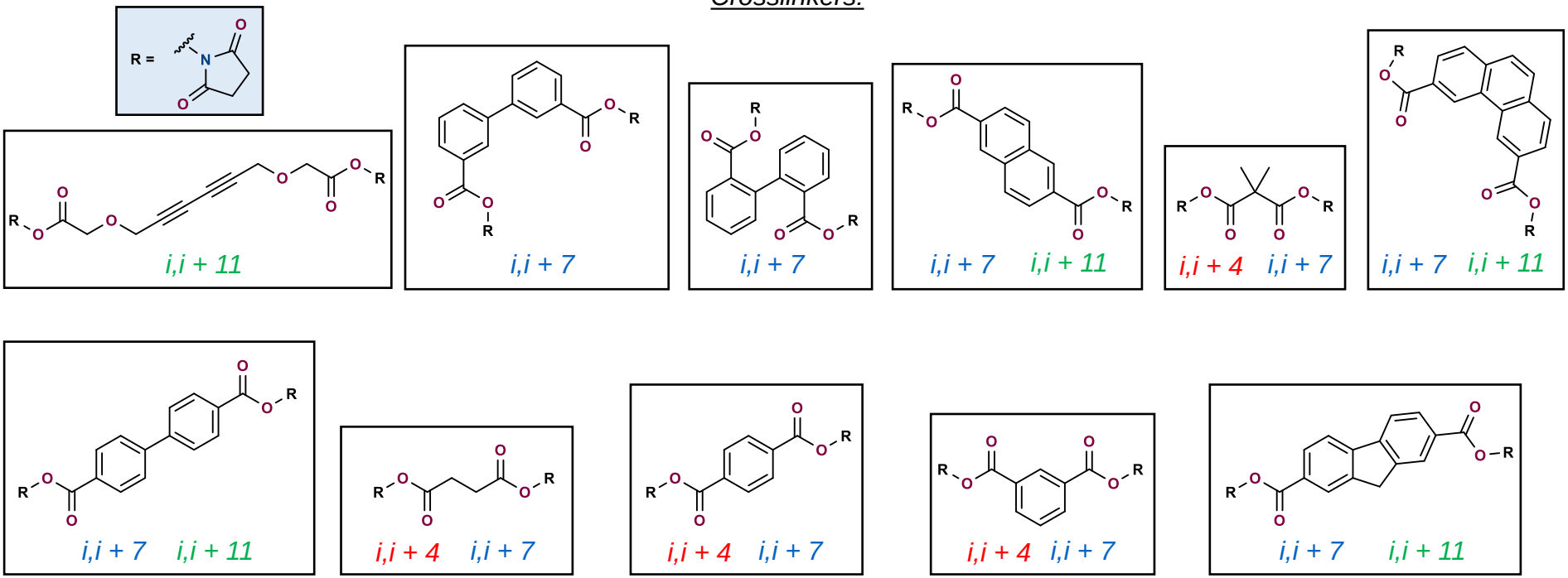
Felix, A. *Int. J. Peptide Protein Res.*, **1988**, 32, 441-454. doi: 10.1111/j.1399-3011.1988.tb01375.x.

Peptide Stapling Methods – Crosslinking Amides

Inouye, 2008: *Di-amide Stapling Various Linkers*



Crosslinkers:



Pros – Numerous amines and acids available and facile bond to form

Cons – Protecting group orthogonality is necessary and crosslinkers are needed to go beyond i, i+4

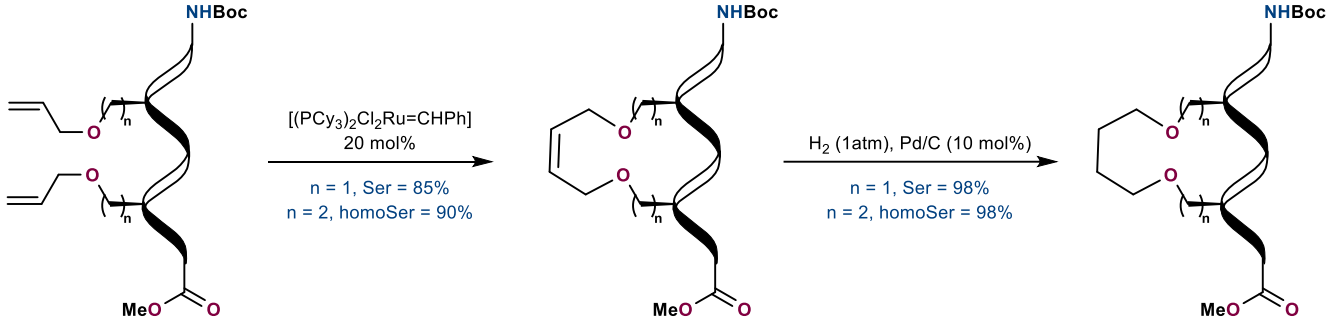
M. Inouye, *Chem. – Eur. J.*, **2008**, 14, 857–863. https://doi.org/10.1002/chem.200700843

Ene - Metathesis

Grubbs, 1998:

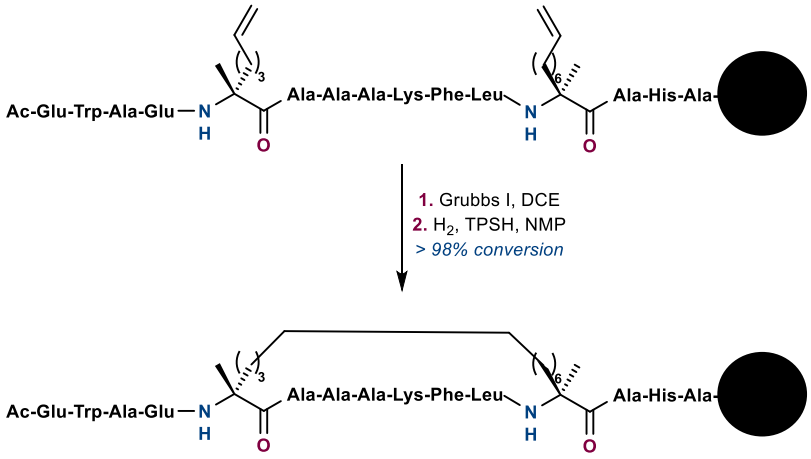
BocHN-Val-X-Leu-Aib-Val-X-Leu-OMe

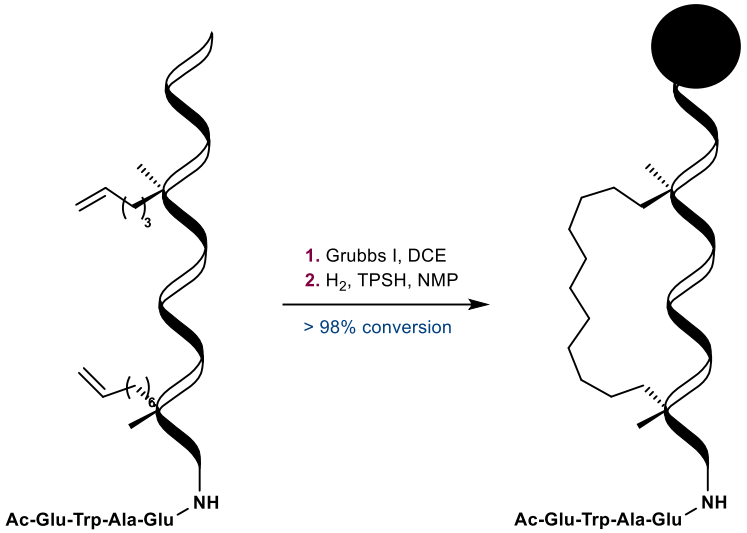
Starting from this heptapeptide, the allylated serine and homoserine residue were subjected to Grubbs metathesis to afford alky i, i+4 linked peptides in high yield.



R. H. Grubbs, *Angew. Chem., Int. Ed.,* **1998**, 37, 3281–3284. https://doi.org/10.1002/(SICI)1521-3773(19981217)37:23<3281::AID-ANIE3281>3.0.CO;2-V

Schafmeister, 2000:





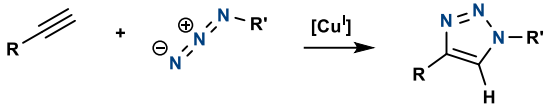
Did the metathesis and hydrogenation sequence on resin (Yields varied). Were able to demonstrate i, i+4 and i, i+7 linkages.

The stapled peptide here demonstrated increased helicity and higher resistance to proteolysis relative to the native peptide!

Schafmeister, C. *J. Am. Chem. Soc.,* **2000**, 122, 24, 5891–5892. https://doi.org/10.1021/ja000563a

Cycloadditions and Click Chemistry

Sharpless 2001: *Click Chemistry*



The fundamental "click" reaction depicted which will be highlighted in this topic is the Sharpless advances of the Huisigen 1,3-dipolar cycloaddition between a substituted azide and alkyne (CuAAC). For more information on this Nobel Prize winning reaction and its implications, see attached.

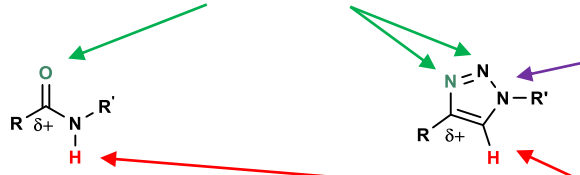
K. B. Sharpless, *Angew. Chem., Int. Ed.*, **2001**, 40, 2004–2021. https://doi.org/10.1002/1521-3773(20010601)40:11<2004::AID-ANIE2004>3.0.CO;2-5

The Structural Relevance of this Triazole: An impressive *trans* Amide Bond Isostere

The triazole maintains the planarity of the amide, though more rigidly due to its aromaticity

The N-2 and N-3 lone pairs are weak H-bond acceptors, comparable to the amide bond

Due to the aromaticity, the triazole is additionally able to engage in π-stacking (intra or inter)



C-4 proton is polarized enough to serve as an H-bond donor like an amide

- *Amide Bond Dipole~ 3.5 Debye*
- *Triazole Moiety Dipole ~ 4.5 Debye*

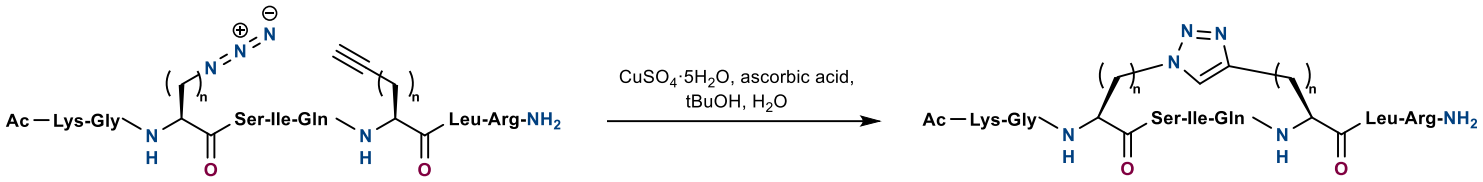
The triazole surrogate is slightly larger than that of an amide subunit tethering two amino acids (~5Å vs ~4Å)

Beyond its geometric and electronic similarities allowing it to function as an amide isostere, biologically the triazole moiety is highly resistance to enzymatic cleavage, acid or base hydrolysis, and oxidative or reductive conditions. While this makes it an appealing scaffold for stability and geometric considerations, this high chemical stability has left numerous questions as to the biological fate of the triazole and studies are still ongoing.

Mindt, T. *Molecules* **2020**, 25, 3576. https://doi.org/10.3390/molecules25163576

Click Staples

Scrima, 2010: *i, i+4 staples*

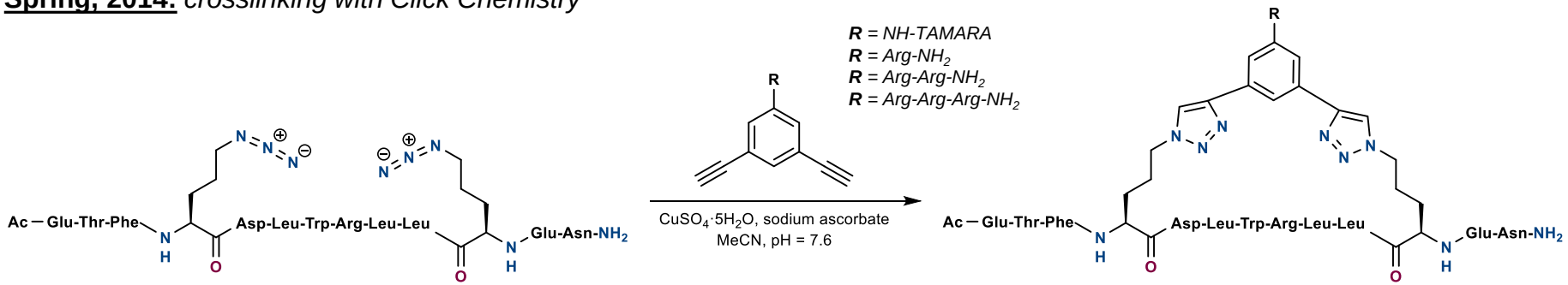


n = 1,2,3, or 4

n = 2,4 demonstrated exceptional helical stabilization

Scrima, M. *Eur. J. Org. Chem.*, **2010**, 446–457. https://doi.org/10.1002/ejoc.200901157

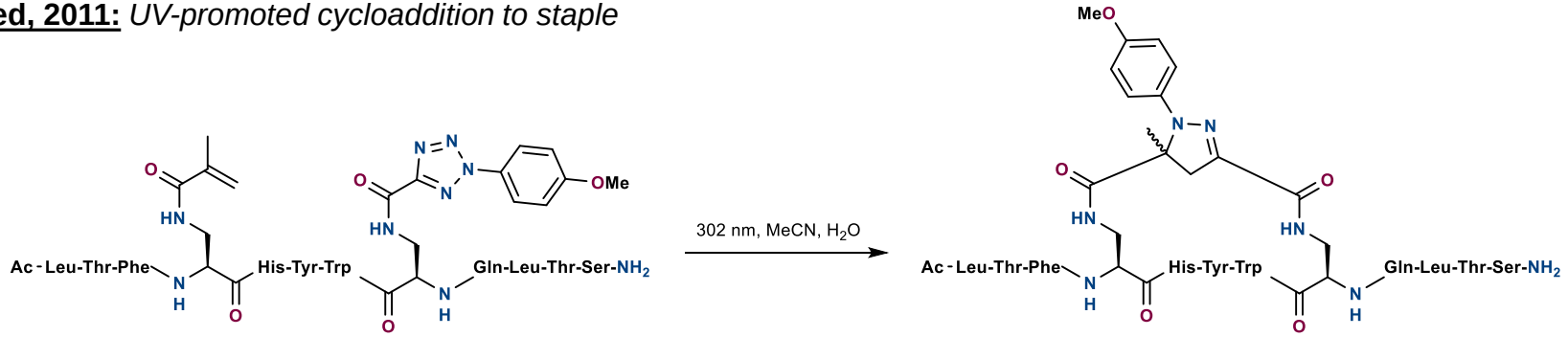
Spring, 2014: *crosslinking with Click Chemistry*



Were able to develop a more soluble anti-cancer peptide by adding cationic groups to the linker, aiding in helical formation, and importantly, without actually changing the sequence of the remainder of the peptide.

Spring, D. *Chem. Sci.*, **2014**, 5, 1804. https://doi.org/10.1039/C4SC00045E

Madded, 2011: *UV-promoted cycloaddition to staple*



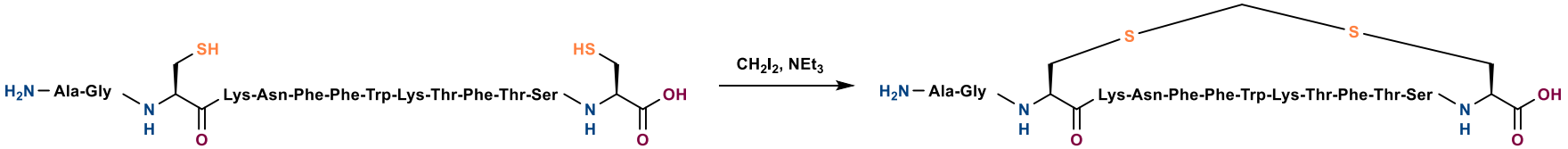
Madden, M. *Bioorg. Med. Chem. Lett.*, **2011**, 21, 1472–1475. doi: 10.1016/j.bmcl.2011.01.004

Cysteine Linkages

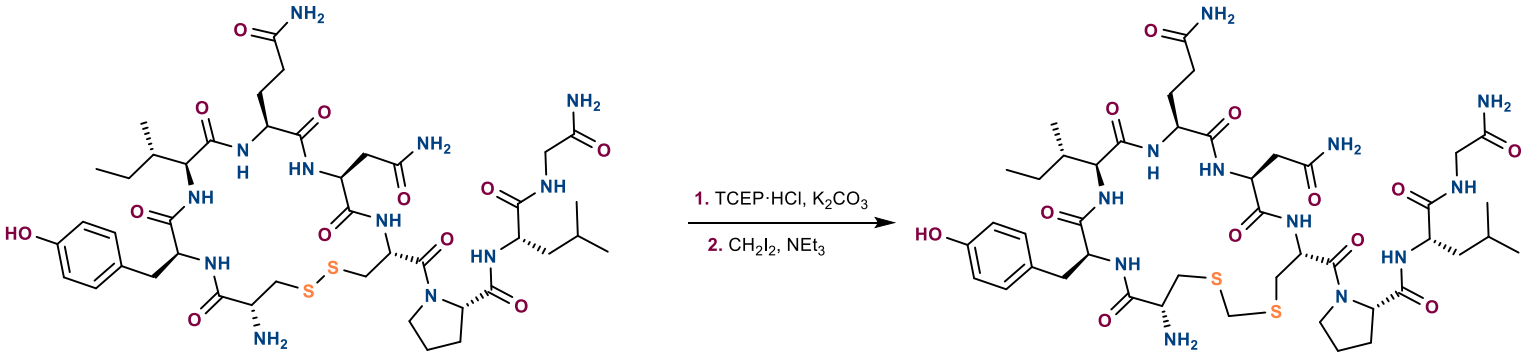
Thiol Crosslinks:

While nature utilizes disulfide bridges quite effectively, it is not a linkage we commonly employ. Disulfide linkages are not stable under reducing conditions and are susceptible to other nucleophiles, undergoing either substitution or thiol exchange. Instead, thioethers or thioacetals are often preferred.

Cramer, 2016: *Thioacetal Formation*



Performed reductive cleavage of disulfide linkage on Somatostatin and then i, i+11 stapling via thioacetal formation.



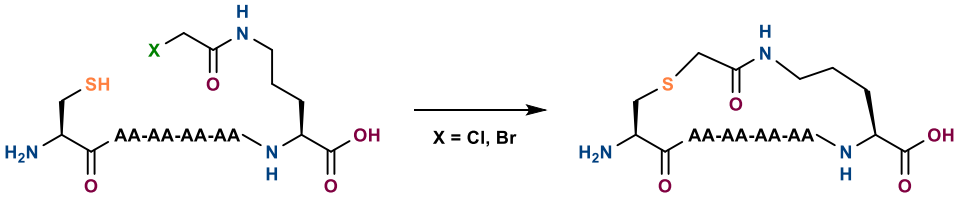
The same sequence was performed on oxytocin, proving an analogue that eliminates reductive lability and increases the serum pH and temperature stability of an important peptide hormone.

Cramer, N. *Chem. Sci.*, **2016**, 7, 7007. https://doi.org/10.1039/C6SC02285E

Thioether Formation

Representative Examples of Thioether Formation:

α-chlorocarbamate Substitution



F. M. Brunel, *Chem. Commun.*, **2005**, 2552. https://doi.org/10.1039/B419015G

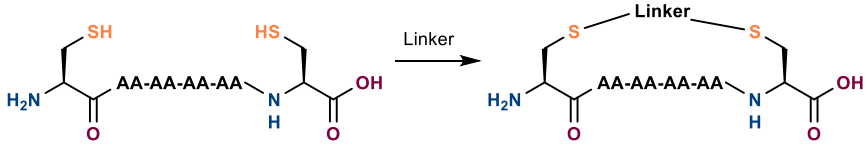
Radical Addition into an Alkyne

Y. Tian, *Chem. Rec.* , **2017**, 17, 874. doi: 10.1002/tcr.201600137.

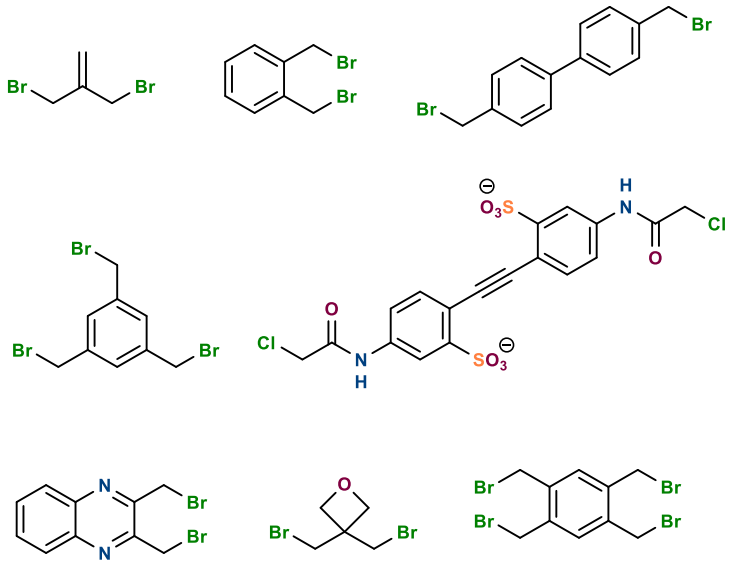
Radical Addition into an Alkene

Y. Tian, *Chem. Sci.*, **2016**, 7, 3325. https://doi.org/10.1039/C6SC00106H

Crosslinking with Electrophillic Linkers



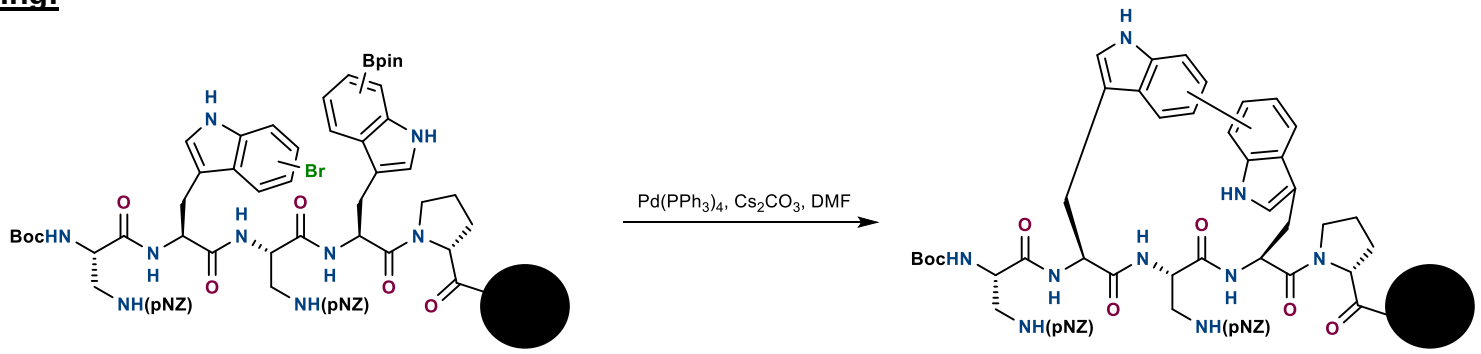
A Few Representative Linkers:



W. Liu, *Angew. Chem. Int. Ed.*, **2017**, 56, 4458. https://doi.org/10.1002/anie.201702381

C-C σ-bond Formation

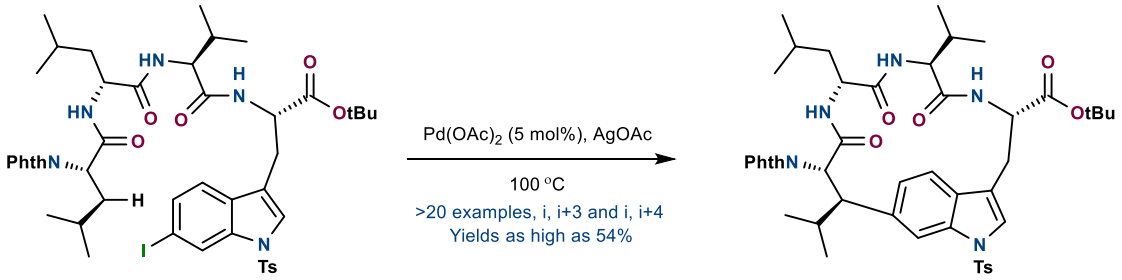
Cross Coupling:



Though not widely adopted, cross coupling has been demonstrated to perform sidechain-sidechain macrocyclizations. However, fundamentally it is restrictive due to necessary protecting groups and sensitivity to lewis basic functionality abundant on peptides.

M. Teixidó, *Biopolymers*, **2018**, 109, e23112. https://doi.org/10.1002/bip.23112 **For other macrocyclization examples, see:** S. Ballet, *Catalysts,* **2017**, 7, 74. https://doi.org/10.3390/catal7030074

C-H Activation:



While not commonly employed, C-H functionalization has demonstrated the capacity to staple peptides at various linker lengths. Many of the same restrictions apply when using Pd, in addition to stereochemistry of the β- functionalized position being controlled largely by the substrate in very complex systems.

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