N-Methylated Cyclic Peptides: Relationship between Structure and Property

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Supervisors: Prof. Wu, Yun-Dong

Dr. Zhang, Xin-Hao

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- **Improvement of pharmaceutical properties**
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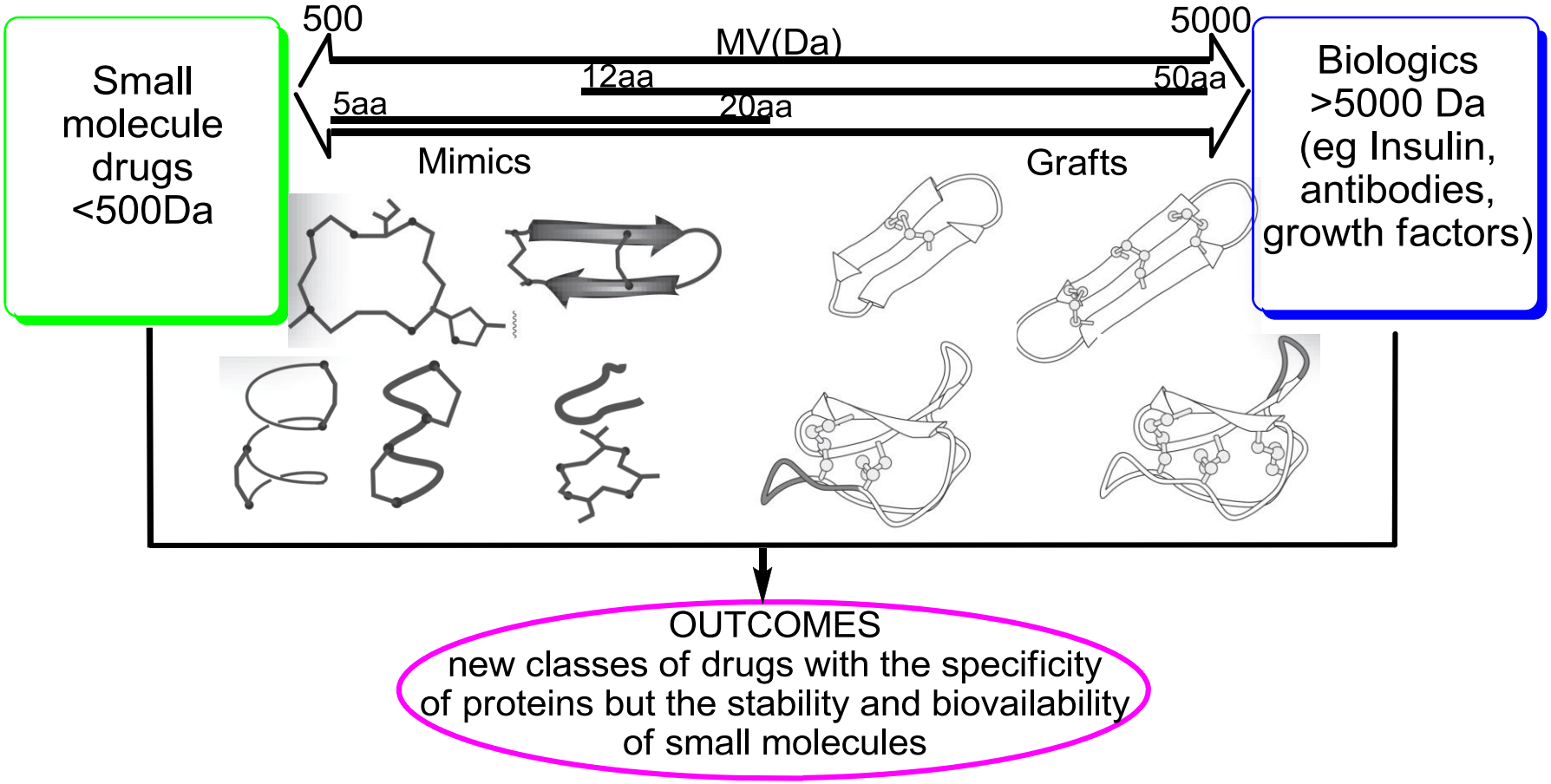
Changing Trends in Drug Design

Year	Technological era	Molecular classes and/or approaches	Regulatory environment
1960	Chemistry	Natural products, screening, rational design	Activity paramount
1980	Molecular biology	Biologics (insulin, growth factors, EPO)	↓
2000	Genome & proteomics	New target identification and validation	Safety paramount
2020	Peptide drugs?	Increased specificity	

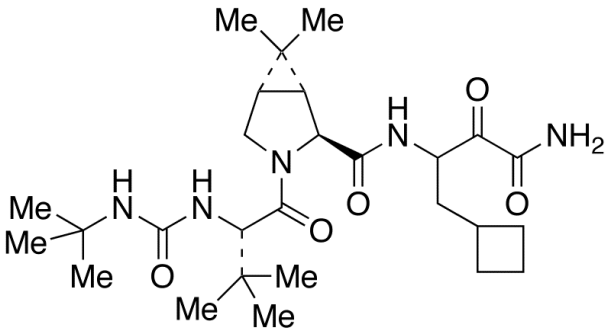
D. J. Craik, D. P. Fairlie, S. Liras, D. Price, *Chem Bio Drug Des.* **2013**, *81*, 136

Changing Trends in Drug Design

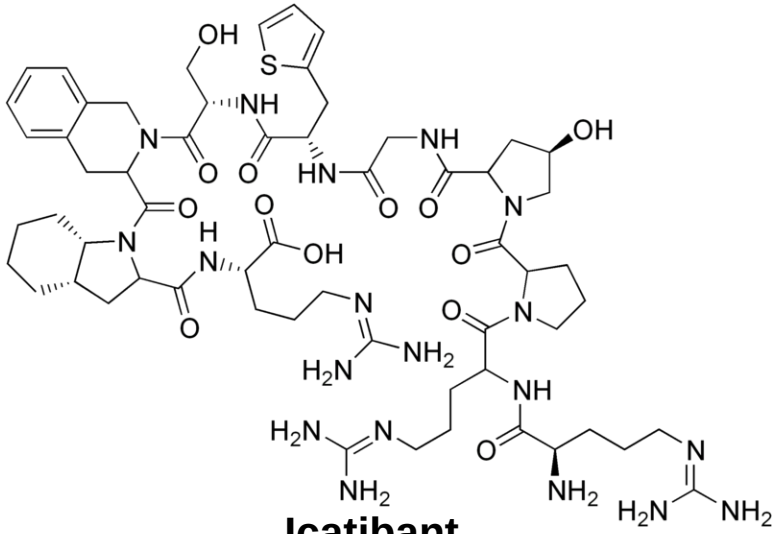


D. J. Craik, D. P. Fairlie, S. Liras, D. Price, *Chem Bio Drug Des.* **2013**, *81*, 136

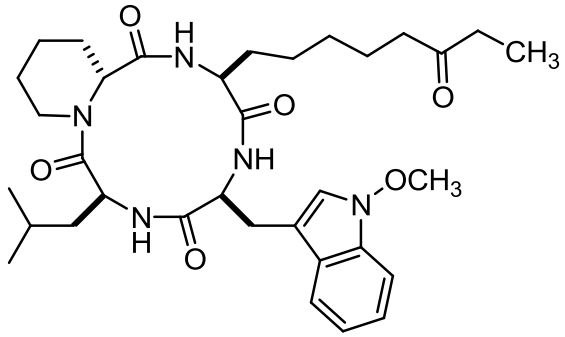
Peptide as Drugs



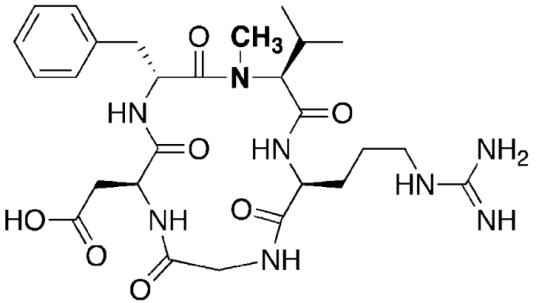
Boceprevir
(Victrelis,2011)
Protease inhibitor



Icatibant
(Firazyr,2011) Antagonist of bradykinin B2 receptors



Apicidin
Histone deacetylase inhibitor



Cilengitide
Integrin antagonist

Advantages and Disadvantages of Peptides as Drugs

Advantages	Disadvantages
High potency	Poor metabolic stability
High selectivity	Poor membrane permeability
Broad range of targets	Poor oral bioavailability
Potentially lower toxicity than small molecules	High production costs
Low accumulations in tissues	Rapid clearance
High chemical and biological diversity	Sometimes poor solubility
Discoverable at peptide/ nucleic acid levels	

D. J. Craik, D. P. Fairlie, S. Liras, D. Price, *Chem Bio Drug Des.* **2013**, *81*, 136

Cyclic Peptides

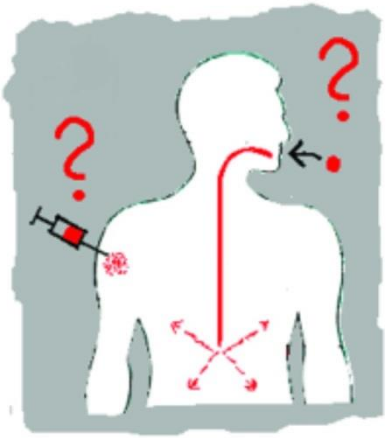
Poor oral bioavailability

Rapid clearance (Short half-life)

Poor metabolic stability

enzymatic degradation

(GI tract and the blood system)



Cyclization

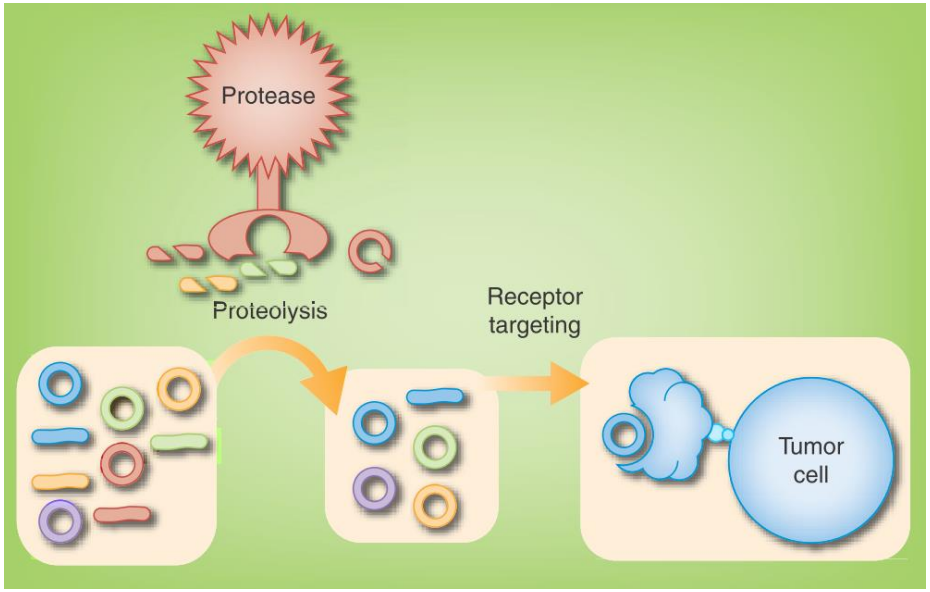
⇩

Constrained structures

⇩

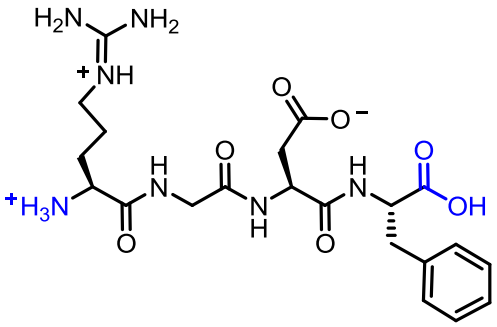
Higher proteolytic stability

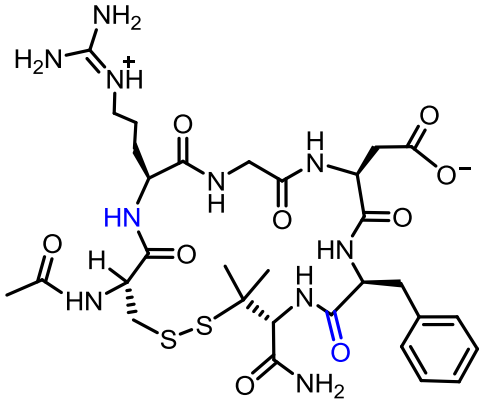
Higher selectivity



Á. Roxin, G. Zheng, *Future Med. Chem.* **2012**, *4*, 1601

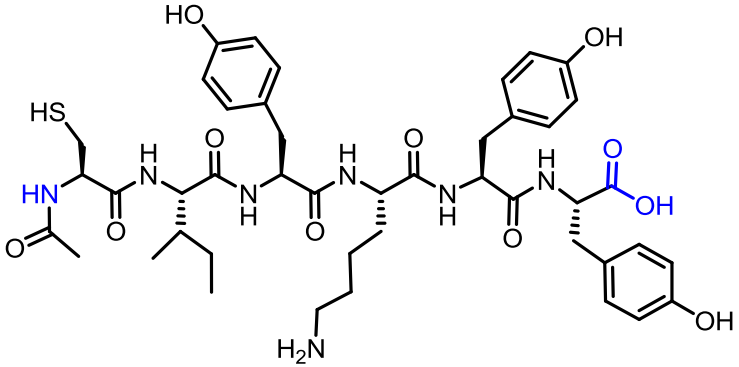
Cyclic Peptides

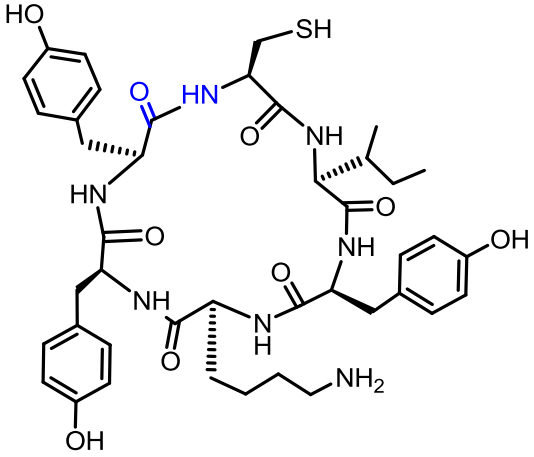




The cyclized RGD could stabilize the labile aspartic acid

Arg-Gly-Asp(RGD)





Inhibitory potency was improved

IC_{50}=400μM

IC_{50}=6.4μM

Kumar, G. Ye, Y. Wang, X. Lin, G. Sun, K. Parang, *J. Med. Chem.* **2006**, *49*, 3395

S. J. Bogdanowich-Knipp, S. Chakrabarti, T. J. Siahaan, T. D. Williams, R. K. Dillman, *J. Pep. Res.* **1999**, *53*, 530

N-Methylated Cyclic Peptides

Poor membrane permeability – intestinal permeability

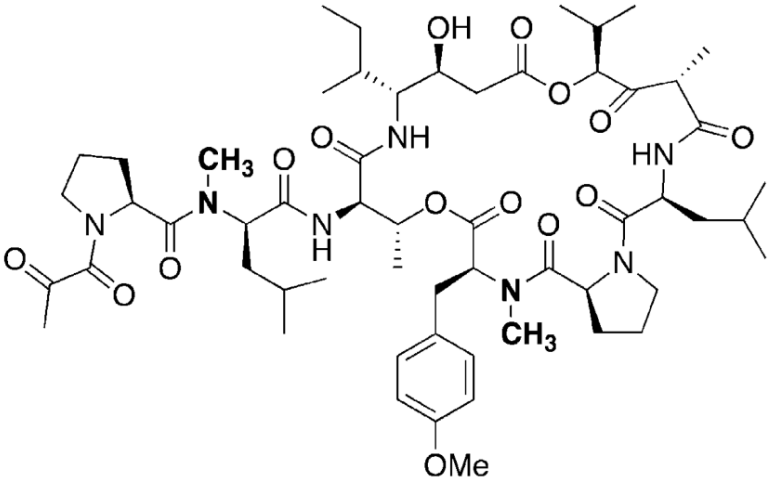
N-methylation

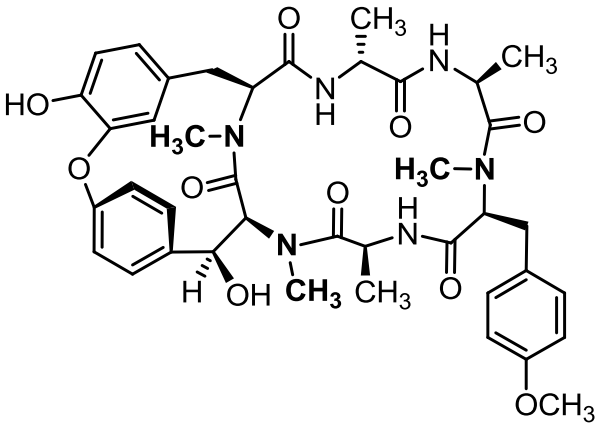
⇩

Improving membrane permeability

Achieving oral availability

Improving affinity and selectivity





Bouvardin
Rubiaceae

Aplidine
Aplidium albicans

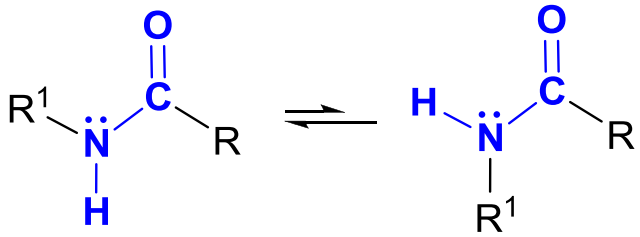
H. Kessler et al. *J. Med. Chem*. **1999**, *42*, 3033 M. D'Incalci et al. *Leukemia.* **2003**, *17*, 52

Conformational Impact

1. Steric hindrance

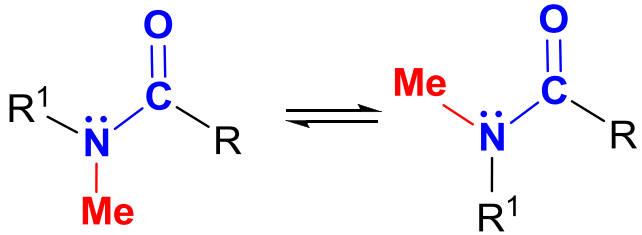
- Influences the cis–trans equilibrium of the amide bond
- Influences the adjacent residues

Horst Kessler



(a) (b)

(a) is dominant



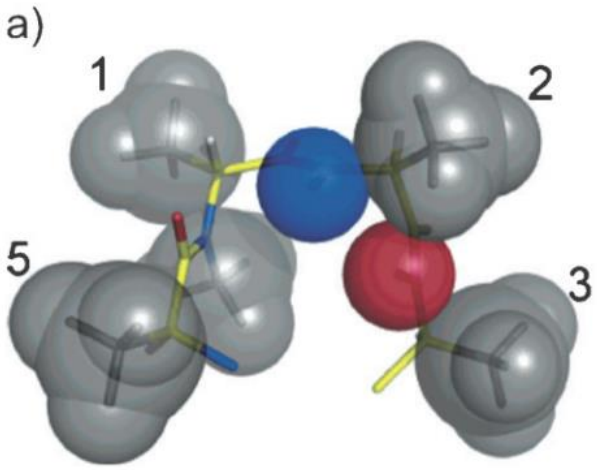
(a) (b)

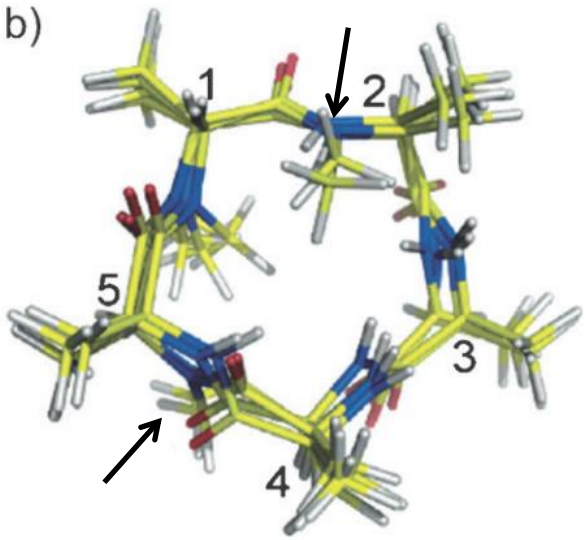
(a) and (b) have very similar energy

The energy barrier for rotation increases.

J. Chatterjee, F. Rechenmacher, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 254

Conformational Impact





cyclo(-NMe-d-Ala^1-Ala^2-Ala^3-Ala^4-Ala^5-)
blue : allowed sites for a N-methyl group
red : disallowed sites for a N-methyl group

Analogues of cyclo(-NMe-d-Ala-L-Ala$_4$-),
flip in the Ala^4–Ala^5 peptide bond (arrow)

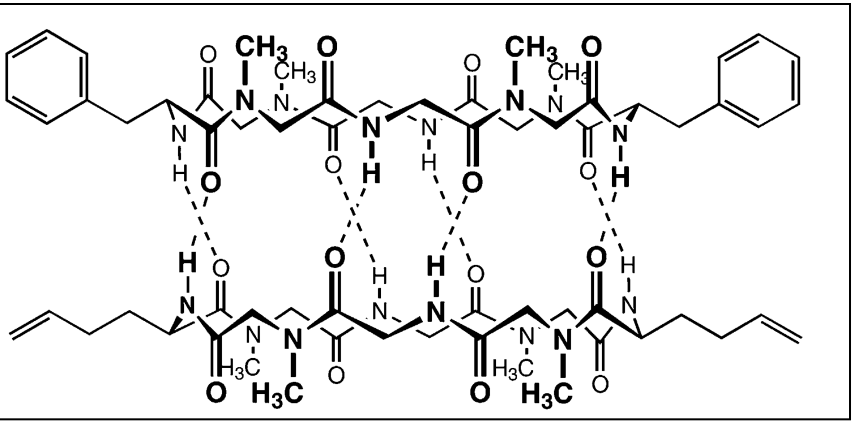
Steric interactions cause conformational interchange

J. Chatterjee, F. Rechenmacher, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 254
J. Chatterjee, D. Mierke, H. Kessler, *J. Am. Chem. Soc*. **2006**,*128*,15164

Conformational Impact

2. Hydrogen bonds

Removal of the hydrogen-bonding capability



Disrupts the stabilizing secondary structural elements

N-methylation prevents the association of further cyclic peptides on either face of the dimer by blocking the hydrogen bond formation

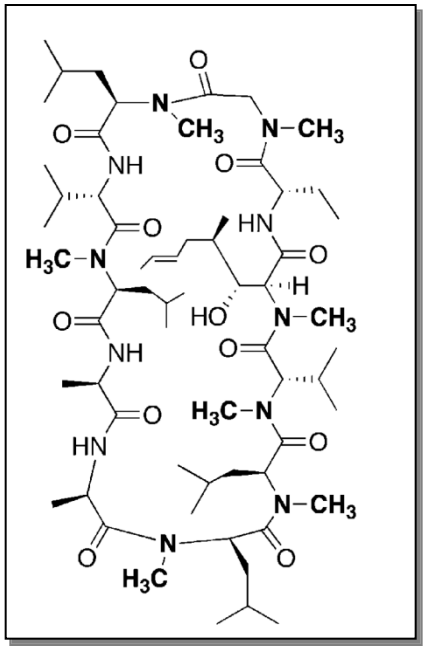
J. Chatterjee, F. Rechenmacher, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 254
T. D. Clark, M. R. Ghadiri, *J. Am. Chem. Soc.* **1995**, *117*, 12364

Improvement of Pharmaceutical Properties

- Improving permeability

Cyclosporine A (环孢霉素)

Immunosuppressive drug



key structural features：

a. Cyclic backbone

Protects against proteolytic degradation

Buries polar groups in the interior of the molecule

b. Seven N-methyl groups

Reduce the number of amide hydrogen bond donors

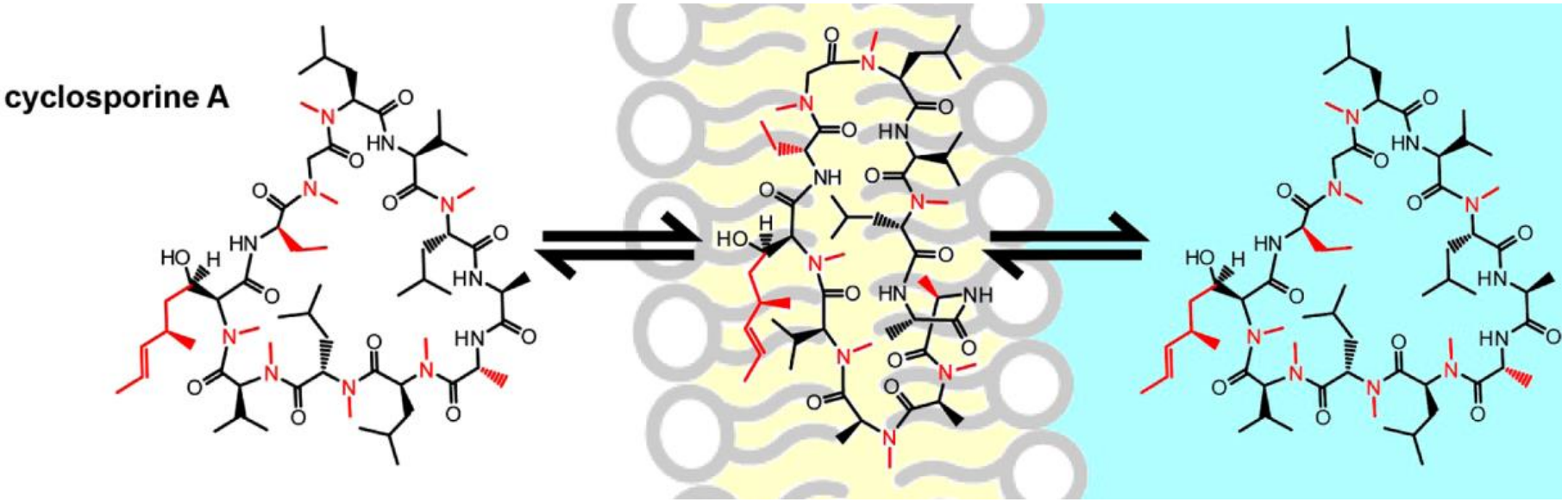
c. Four intramolecular hydrogen bonds

Tie up the remaining four amide NH protons to reduce their hydrogen bonding potential for solvation by water

H. Tscherter, etc. *Eur. J. Appl. Microbiol.* **1976**, *3*, 125
H. Kessler, etc. *J. Am. Chem. Soc.* **1985**, *107*, 1083

Improvement of Pharmaceutical Properties

Improving permeability by shape shifting



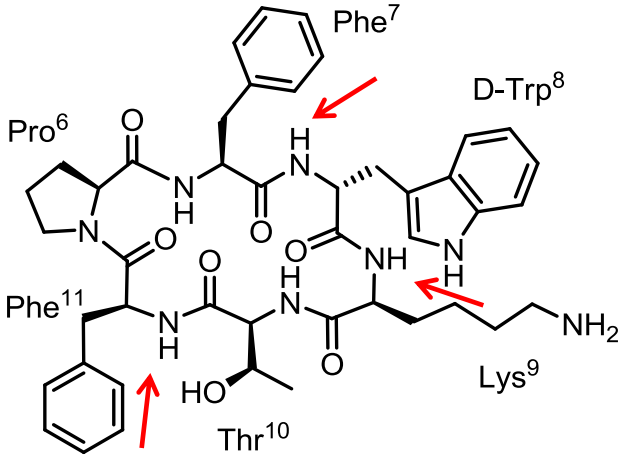
In aqueous solvent
conformation similar to the co-crystal structure

In organic solvent
forming internal backbone hydrogen bonds

J. E. Bock, J. Gavenonis, J. A. Kritzer, *ACS Chem. Bio.* **2012**, *8*, 488.

Improvement of Pharmaceutical Properties

- Improving bioavailability



Veber-Hirschmann peptide
(not orally available)

Analogs showing binding affinity

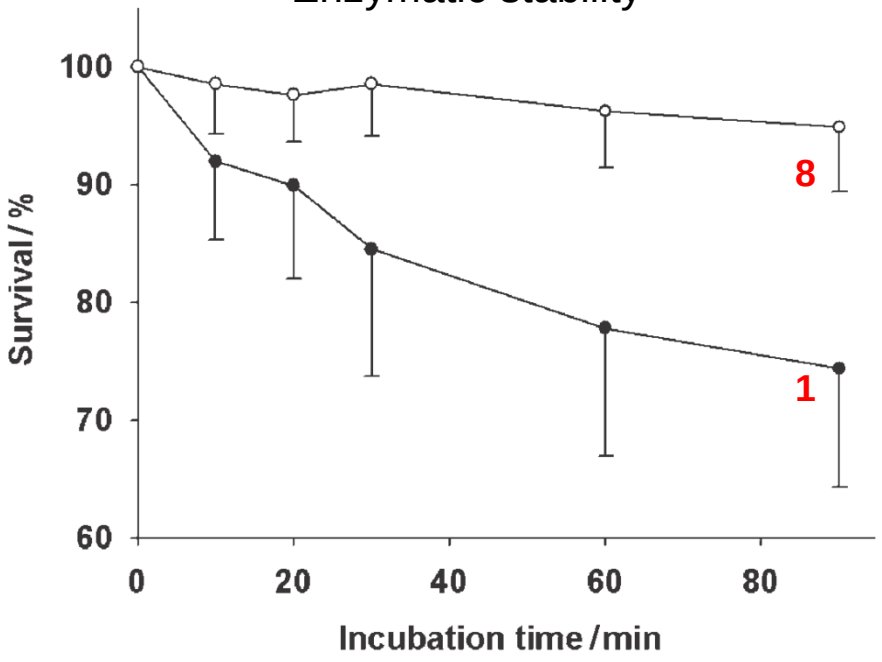
Peptide	N-methylated amino acid	hsst 2 (pK_d)	hsst 5 (pK_d)
Octreotide	–	9.18	7.71
1	–	8.01	7.82
2	Lys^9	8.60	8.19
3	Phe^{11}	7.93	8.28
4	D-Trp^8	7.61	7.87
5	Lys^9, Phe^{11}	7.96	7.39
6	D-Trp^8, Lys^9	7.60	7.19
7	D-Trp^8, Phe^{11}	7.16	7.47
8	D-Trp^8, Lys^9, Phe^{11}	7.21	7.22

N-me Lys^9 enhances the bioactivity
N-me Trp^8, Phe^{11} reduces the bioactivity

E. Biron, J. Chatterjee, O. Ovadia, D. Langenegger, J. Brueggen, D. Hoyer, H. A. Schmid, R. Jelinek, C. Gilon, A. Hoffman, H. Kessler, *Angew. Chem. Int. Ed.* **2008**, *47*, 2595.

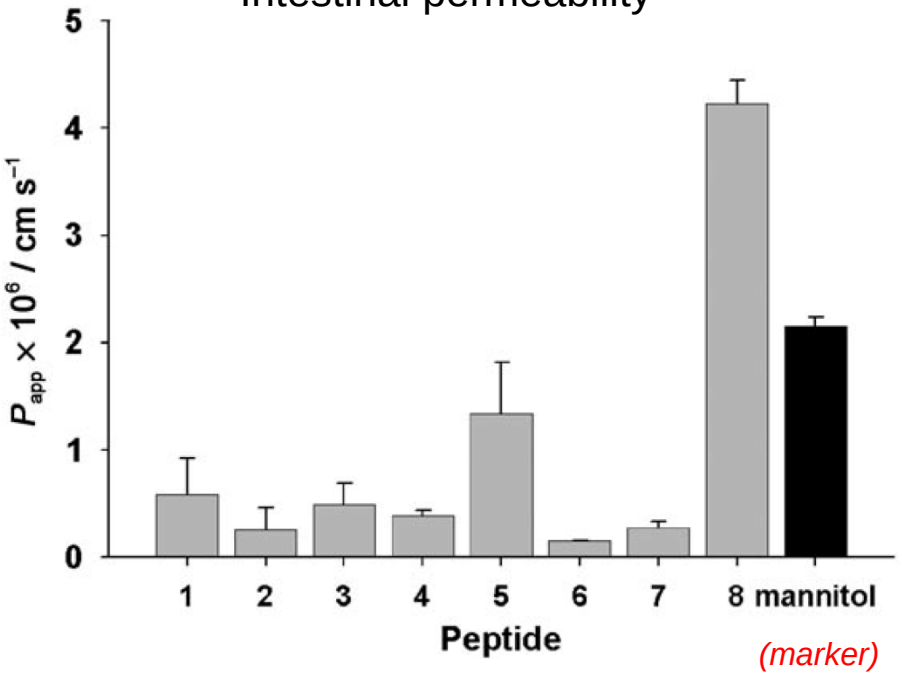
Improvement of Pharmaceutical Properties

Enzymatic stability



Multiple N-methylation enhance the resistance to enzyme

Intestinal permeability



Multiple N-methylation improved the intestinal permeability

1 cyclo(-PFwKTF)-
8 cyclo(-PF(NMe)w(NMe)KT(NMe)F)-

E. Biron, J. Chatterjee, O. Ovadia, D. Langenegger, J. Brueggen, D. Hoyer, H. A. Schmid, R. Jelinek, C. Gilon, A. Hoffman, H. Kessler, *Angew. Chem. Int. Ed* **2008**, *47*, 2595.

Improvement of Pharmaceutical Properties

	elimination half-life, min	volume of distribution at steady state Vss, L/Kg
1	(15.5±2)	(0.3±0.1)
8	(74±6)	(3.7±1.3)

Elimination half-life (5-fold) enhanced

Volume of distribution (10-fold) enhanced

N-methylation

Conformational impact

from **1** to **8**

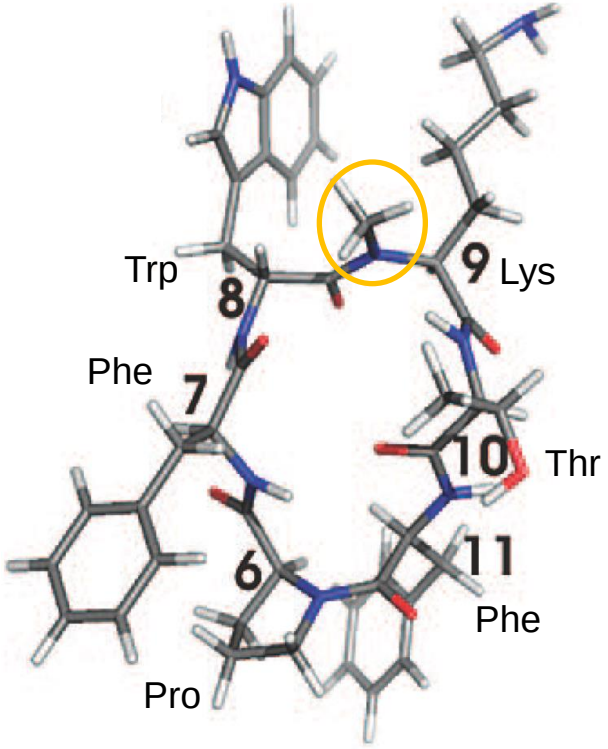
Enzymatic stability increased

Intestinal permeability increased

E. Biron, J. Chatterjee, O. Ovadia, D. Langenegger, J. Brueggen, D. Hoyer, H. A. Schmid, R. Jelinek, C. Gilon, A. Hoffman, H. Kessler, *Angew. Chem. Int. Ed* **2008**, *47*, 2595.

Improvement of Pharmaceutical Properties

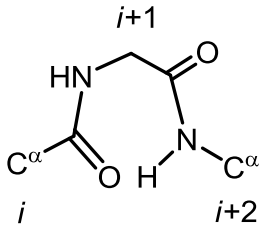
Conformational analysis



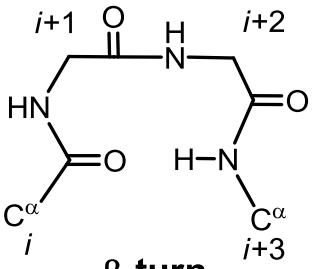
2 "bend"
bioactive conformation

But low permeability !

Phe[11], Trp[8] destabilize the γ turns

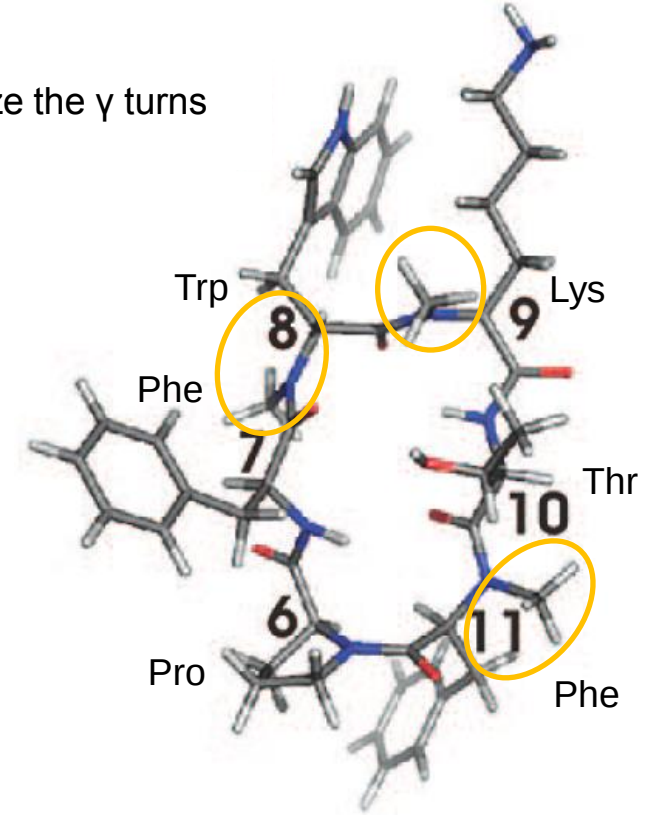


γ-turn



β-turn

Lys[9] stabilizes the β turn

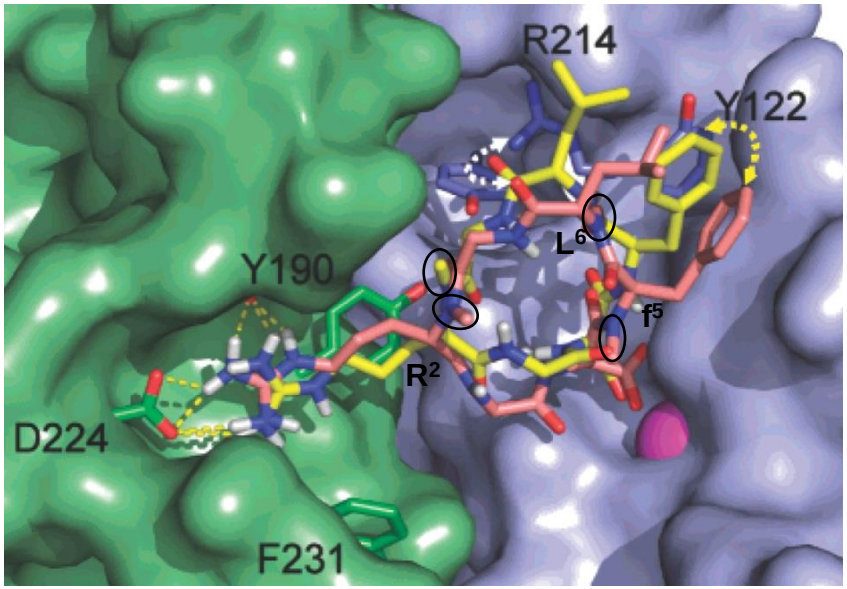


8 "flat"

Phe[11] -- Maintaining the permeability

Improvement of Pharmaceutical Properties

- Enhancing activity and receptor selectivity



Docked **R4** (yellow) and **R8** (pink) in the αIIbβ3 integrin

R1: cyclo(-GRGDfL-)

(IC_{50}(nM)=195, αVβ3/αIIbβ3=0.5)

R4: cyclo(-G**R**GDfL-) high activity

(IC_{50}(nM)=12, αVβ3/αIIbβ3=64)

R8: cyclo(-G**R**GD**fL**-) high selectivity

(IC_{50}(nM)=30, αVβ3/αIIbβ3=406)

		R4	R8
H-bond	R^2…αIIb-Asp224	Y	Y
π-π interaction	f^5…β3-Tyr122	Y	N
H-bond	L^6…β3-Arg214	Y	N

J. Chatterjee, O. Ovadia, G. Zahn, L. Marinelli, A. Hoffman, C. Gilon, H.J. Kessler, *Med. Chem*. **2007**, *50*, 5878

Synthesis of N-Methylated Peptides

- **Biosynthesis--** Non-ribosomal peptide synthetases (NRPSs)

  Not screening at the nucleic acid level

- **Ribosomal Synthesis--** Purified recombinant factors from *E. coli*

  Low yields

- **Chemical Synthesis**

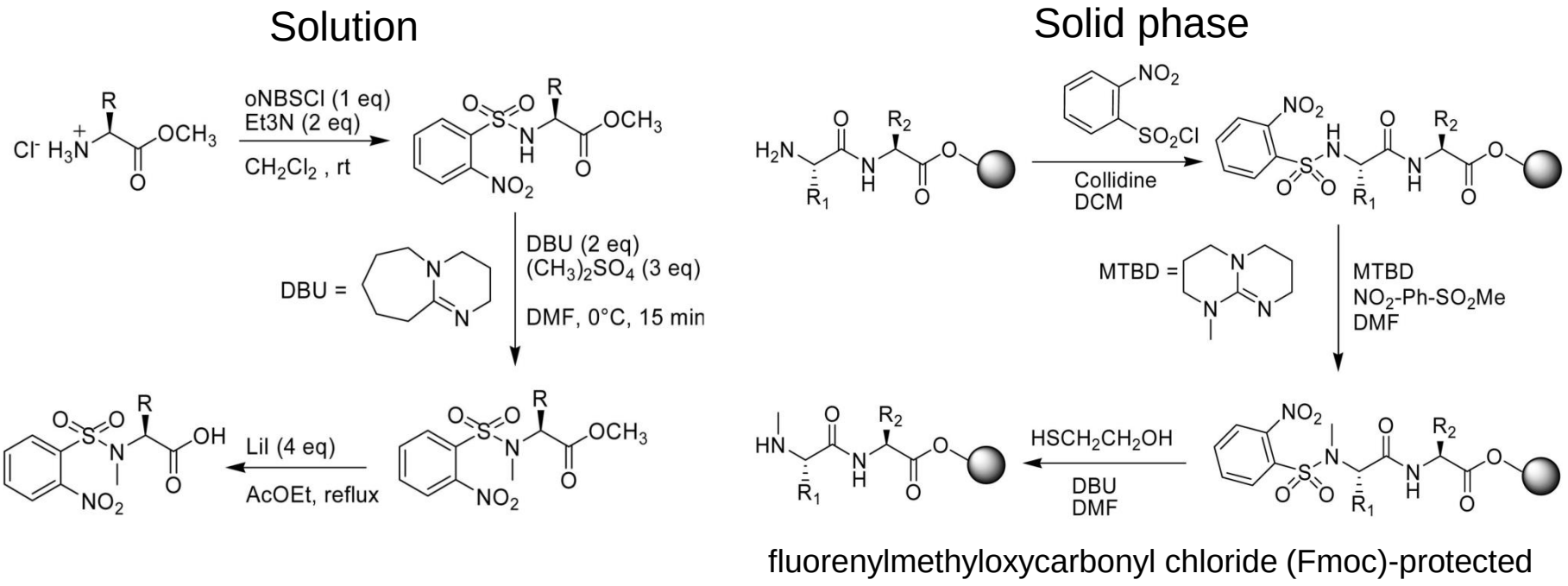
  Difficult to synthesis N-methylated amino acid and coupling

J. Chatterjee, F. Rechenmacher, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 254
R. Finking, M. A. Marahiel, *Annu. Rev. Microbiol.* **2004**, *58*, 453
R. M. Freidinger, J. S. Hinkle, D. S. Perlow, *J. Org. Chem.* **1983**, *48*, 77
A. O. Subtelny, M. C. Hartman, J. W. Szostak, *J. Am. Chem. Soc.* **2008**, *130*, 6131

Synthesis of N-Methylated Peptides

- **Chemical Synthesis**

Step1: Synthesis of N-methylated amino acids



fluorenylmethyloxycarbonyl chloride (Fmoc)-protected

S. T. Cheung, N. L. Benoiton, *Can. J. Chem.* **1977,** 55906

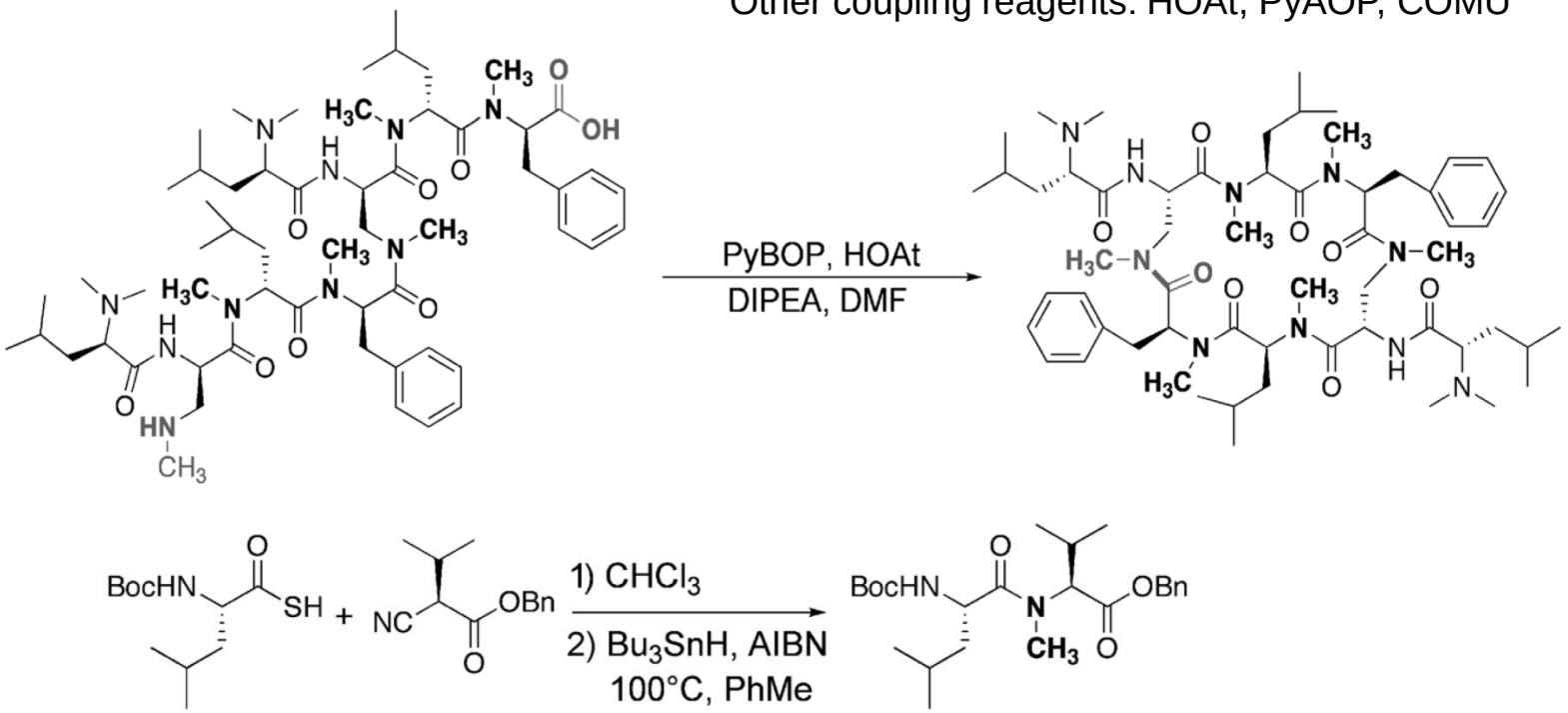
S. C. Miller, T. S. Scanlan, *J. Am. Chem. Soc.* **1997,** *119,* 2301

Synthesis of N-Methylated Peptides

Step 2: Coupling to cyclic peptides

Side reactions: Epimerization, formation of diketopiperazine, loss of peptide segments from resin

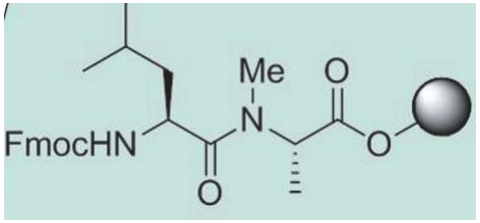
Other coupling reagents: HOAt, PyAOP, COMU



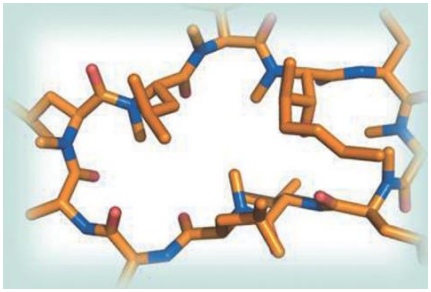
E. Marcucci, J. Tulla-Puche, F. Albericio, Org. Lett. **2012***, 14, 612*

V. R. Pattabiraman, J. W. Bode, *Nature***. 2011**, *480*, 471

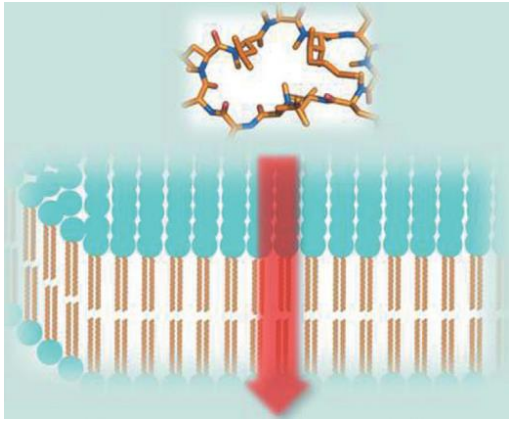
Summary



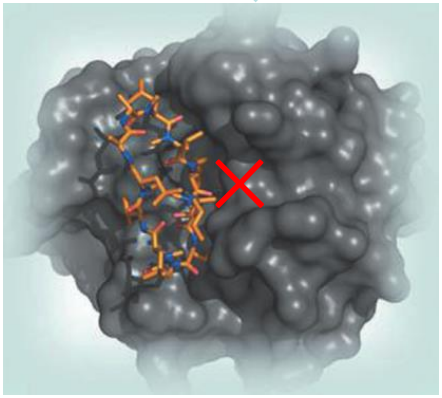
N-methylation



Conformation adjustment



Membrane permeability



Resistance of degradation
Oral availability

Perspectives

- Cheaper methods for their synthesis and purification need to be developed

- Computational methods need to be developed

Acknowledgements

- Prof. Wu, Yun-Dong; Dr. Zhang, Xinhao
- All the members in our group
- All the members in SCBB

Thank you for your attention !

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