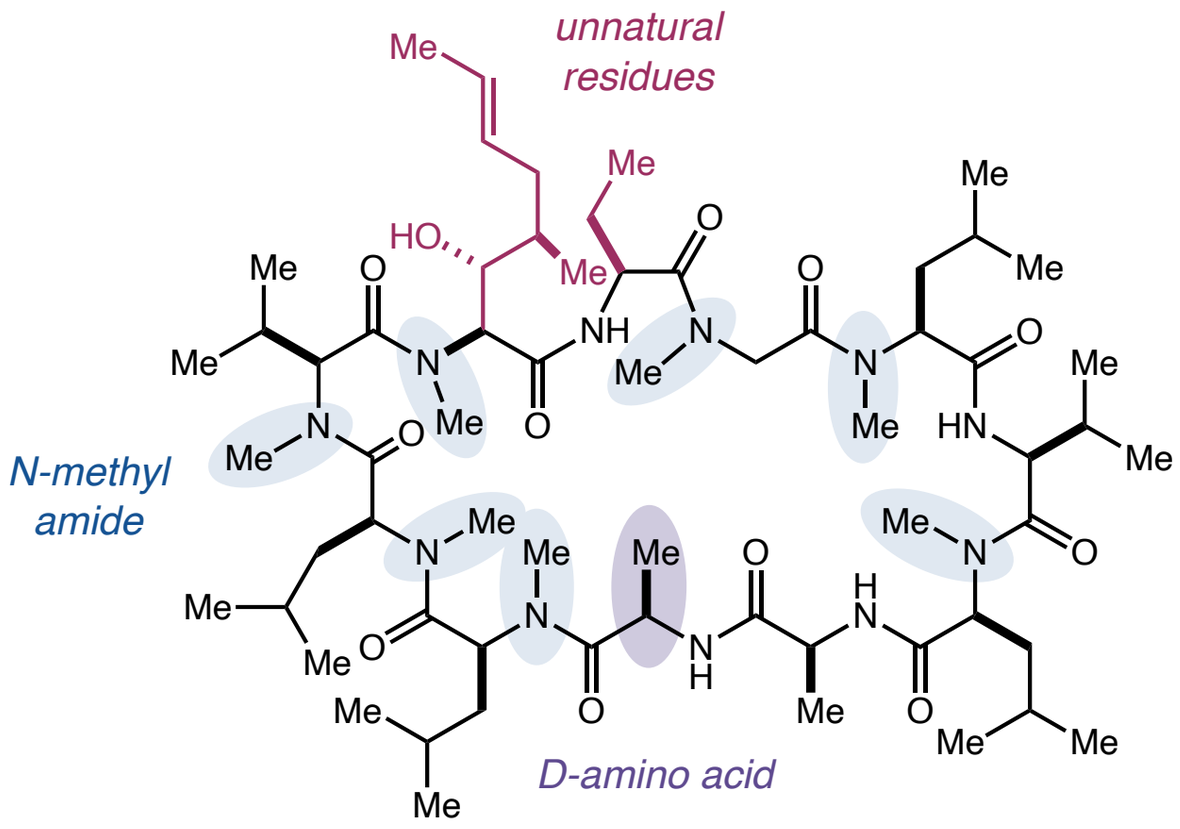
Crash Course in Macrocyclic Peptides

Structure, properties, synthesis, challenges



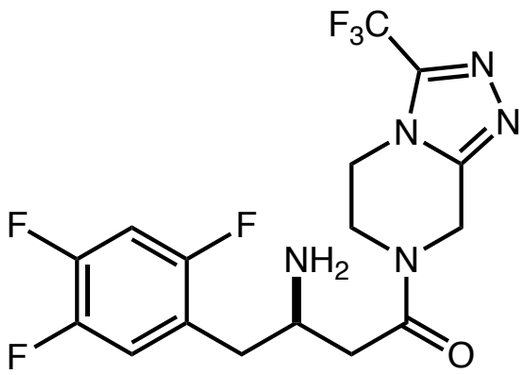
Vlad Băcăuanu

MacMillan Research Group

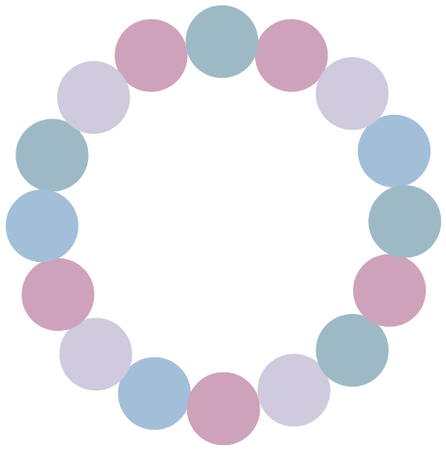
Group Meeting

January 22nd, 2020

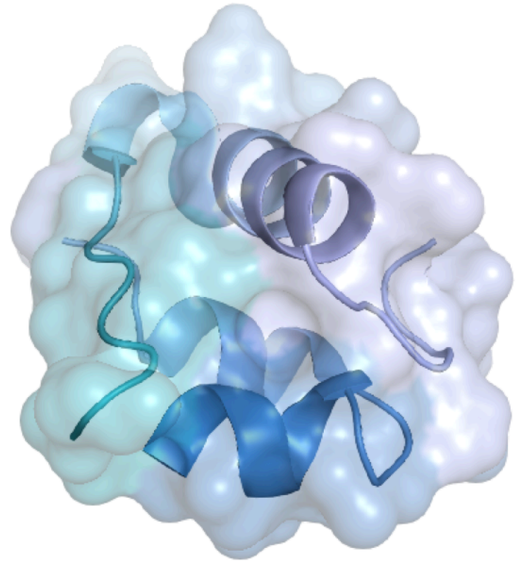
A Simplified Description of the Pharmaceutical Landscape



small molecule drugs



(cyclic) peptides, macrolides, etc.



biologics

molecular weight

500 Da

5000 Da

high bioavailability

low–moderate selectivity

chemical synthesis

rational design

best of both worlds (in an ideal scenario…)

poor bioavailability

exquisite selectivity

biological synthesis

high throughput screening

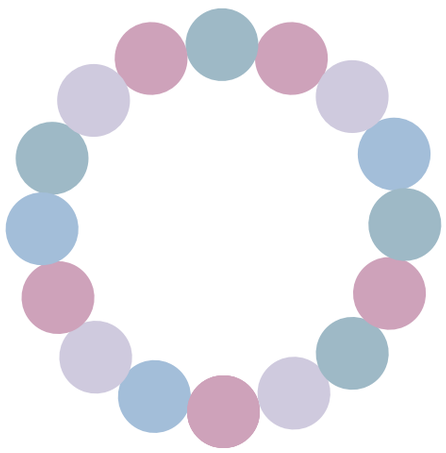
Craik, D. J.; Fairlie, D. P; Liras, S.; Price, D. *Chem. Biol. Drug. Des.* **2013**, *81*, 136

The Development of Macrocyclic Peptides

synthetic organic chemistry

combinatorial chemistry

medicinal chemistry

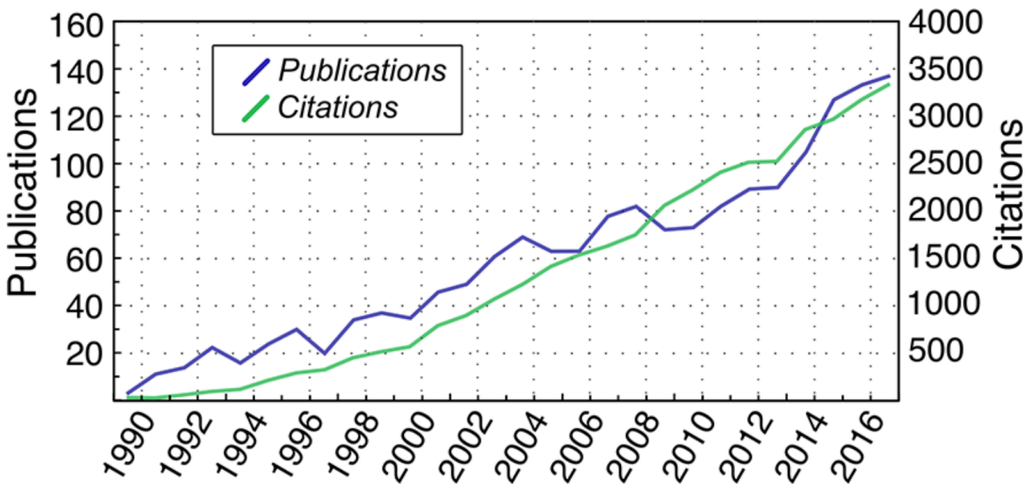


macrocyclic peptides

conformational analysis

computational chemistry

chemical biology



immense progress in the past 30 years

better high-throughput screening

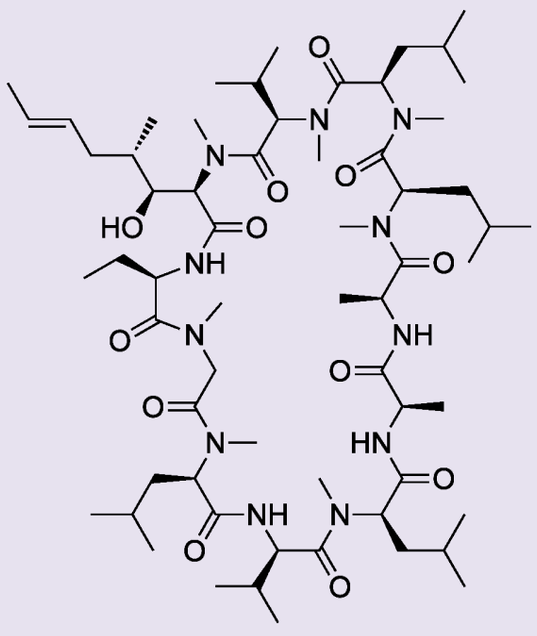
improved rational design

better understanding of pharmacokinetics

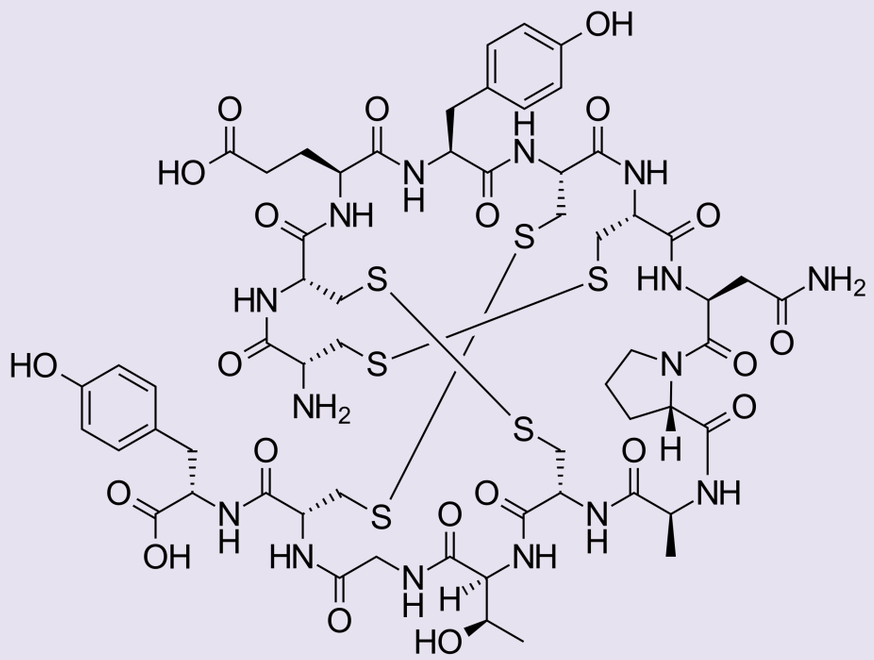
Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Macrocyclic Peptides in the Pharmaceutical Industry

examples of FDA-approved cyclic peptides

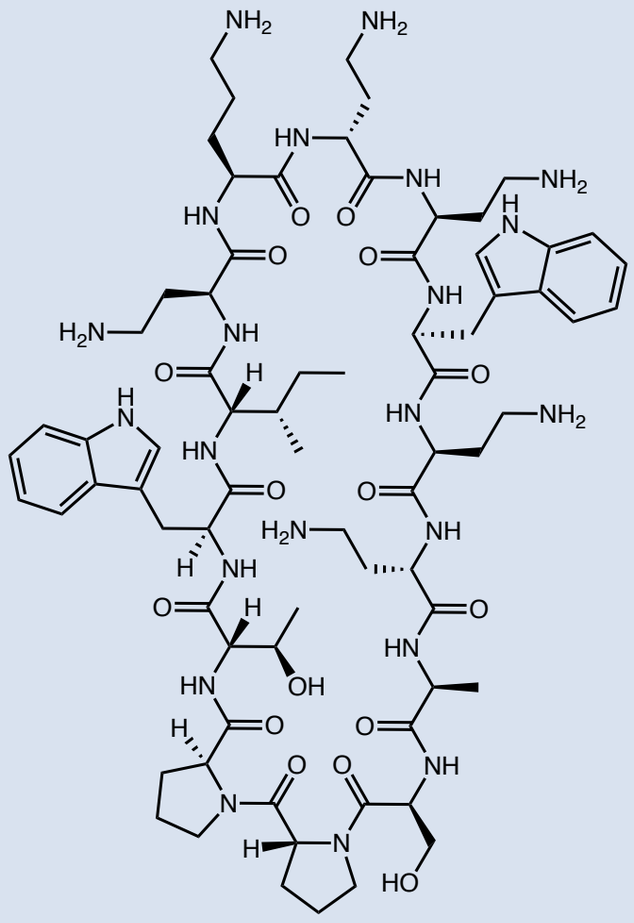


cyclosporin *(1983)*
immunosuppressant



linaclotide *(2012)*
irritable bowel syndrome
top 200 drug

example of peptides in trials



murepavadin *(phase III)*
antibacterial

- Over 40 cyclic peptides in clinical use; 7 in clinic trials
- In the past 10 years, nine cyclic peptides approved
- Traditionally inspired by or derived from natural products
- *De novo* synthesis becoming increasingly more common

Morisson, C. *Nat. Rev. Drug Discov.* **2018**, *17*, 531
Zorzi, A.; Deyle, K.; Heinis, C. *Curr. Opin. Chem. Biol.* **2017**, *38*, 24

Macrocyclic Peptides – Outline

Properties and structure

Why cyclic peptides?

Structural & conformational aspects

Macrocyclization

General considerations

Synthetic methods

cation-assisted, sulfur reagents,
ring contraction, click, RCM,
cross-coupling, C–H activation etc.

Library synthesis

Phage display

Split intein circular ligation

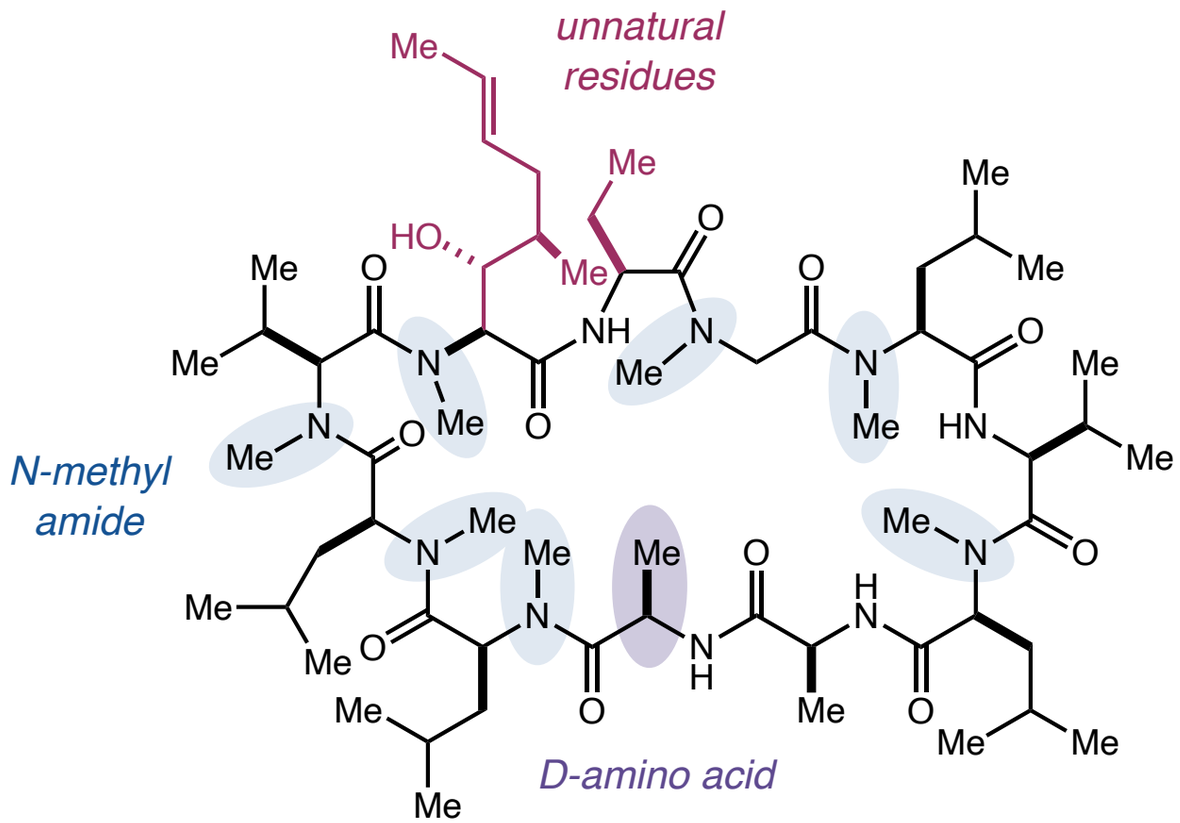
“Split and pool” approach

Challenges

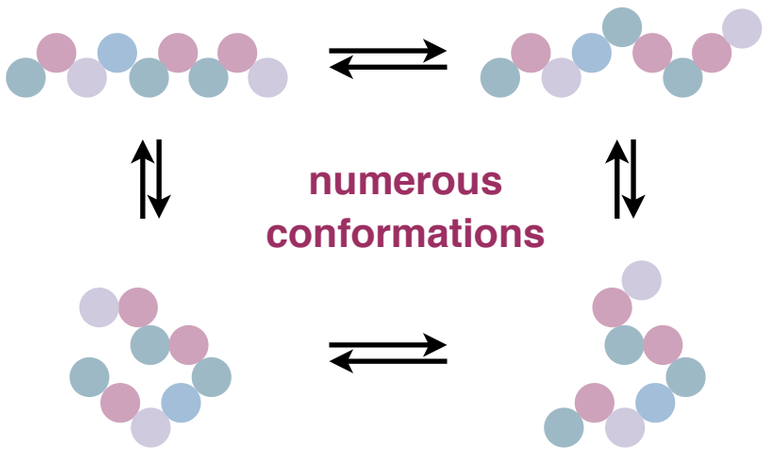
Metabolic stability

Cellular uptake & bioavailability

Roads to achieving lipophilicity



Why Macrocyclic Peptides?



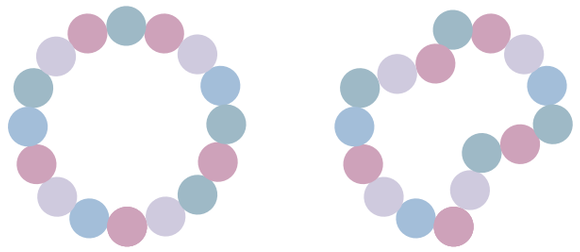
structural pre-organization

↓

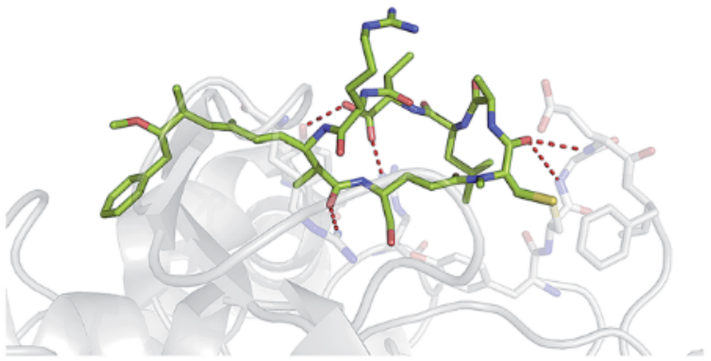
entropic cost built-in

↓

high potency, high selectivity



relatively rigid but still flexible



extended structure with both lipophilic and polar elements

→ intricate and extensive binding to target → *high potency* *high selectivity*

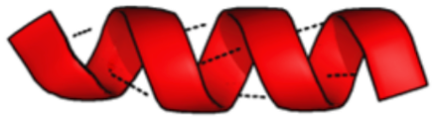
↓

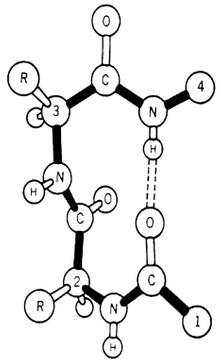
functionally a small "large molecule" rather than a large "small molecule"

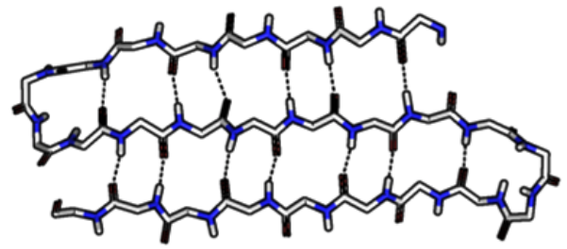
"functional sub-domains" (like a protein) → disrupt protein-protein interactions → *undruggable targets*

Driggers, E. M.; Hale, S. P.; Lee, J.; Terrett, N. K. *Nat. Rev. Drug Discov.* **2008**, *7*, 608

What Determines Structure in a Macrocyclic Peptide







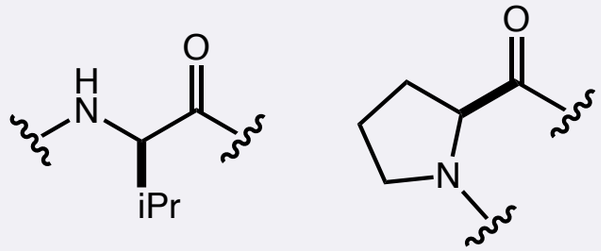
α-helix

β-turn

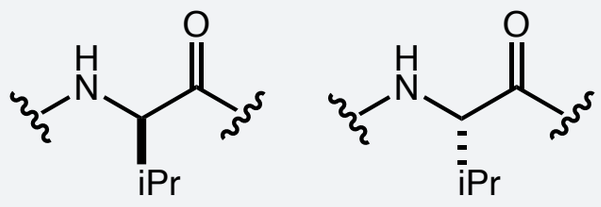
β-helix

basic conformation

secondary structure – can be "frozen" via macrocyclization



amino acid residue

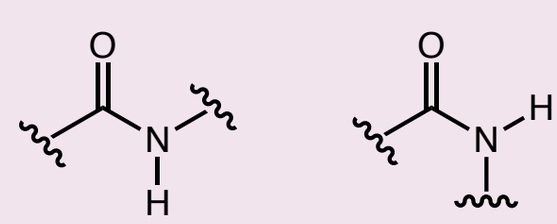


amino acid chirality

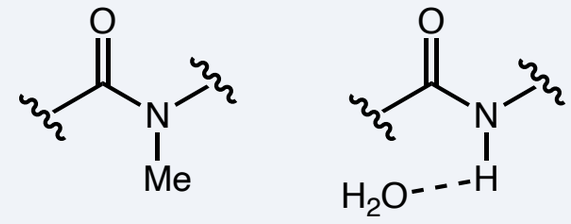
number of amino acids

sequence of amino acids

everything really…



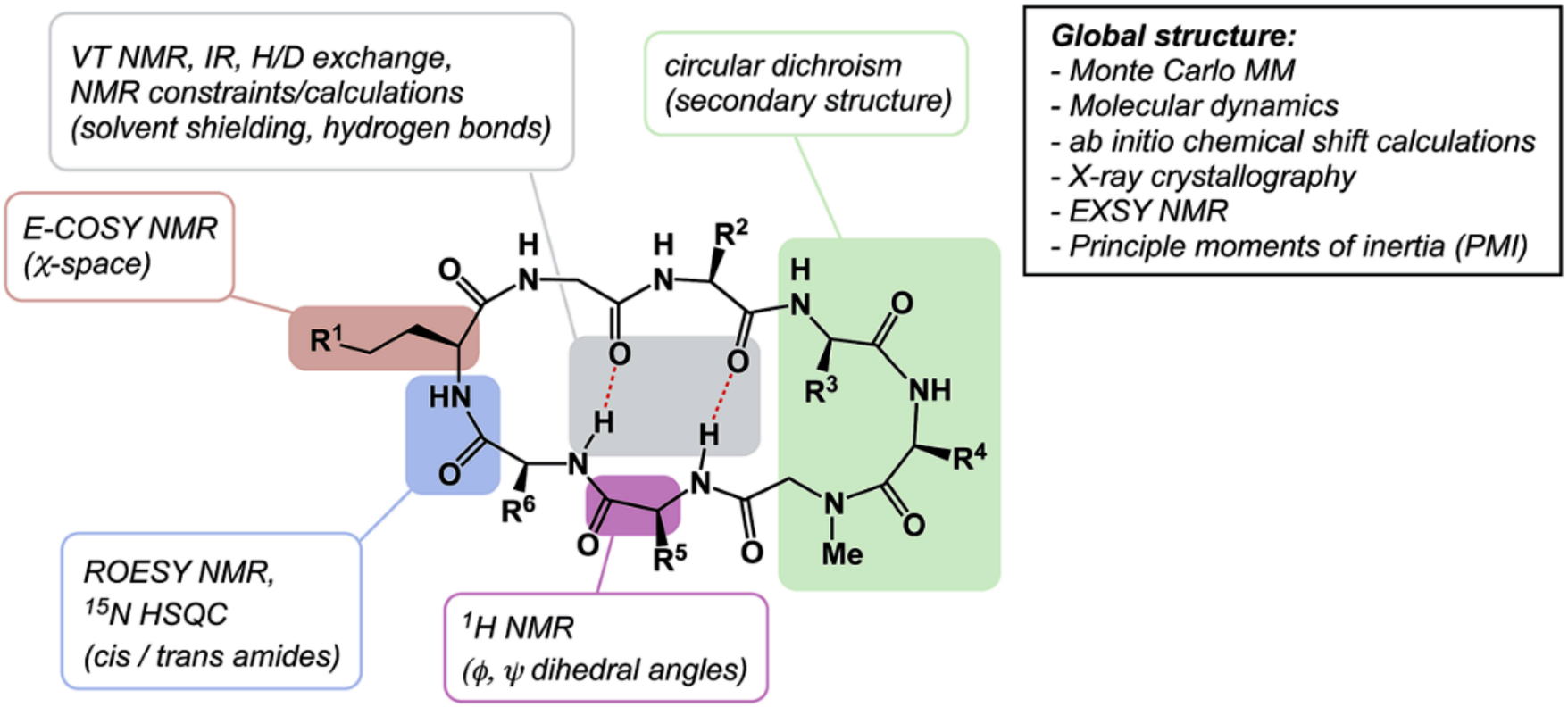
amide geometry



methylation of amide

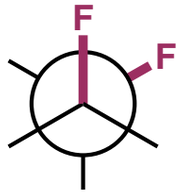
Yudin, A. K. *Chem. Sci.* **2015**, *6*, 30. Images pulled from Wikipedia.

List of Conformational Analysis Methods



Yudin, A. K. *Chem. Sci.* **2015**, *6*, 30

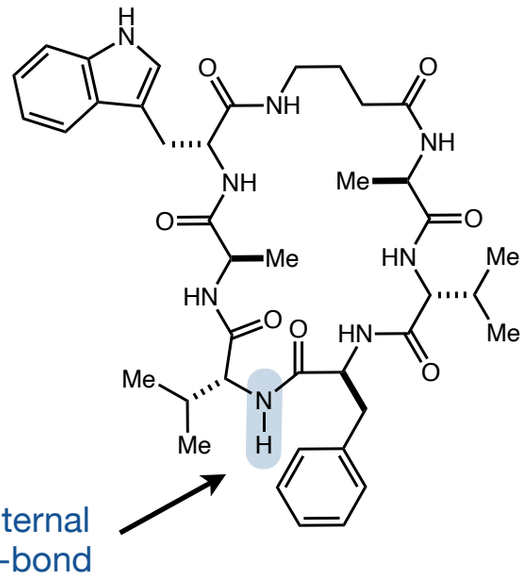
Influencing Geometry and Rigidity via Fluorine Incorporation



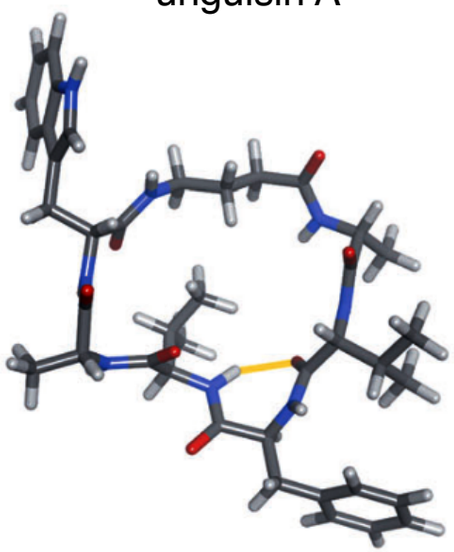
1,2-difluoroalkane

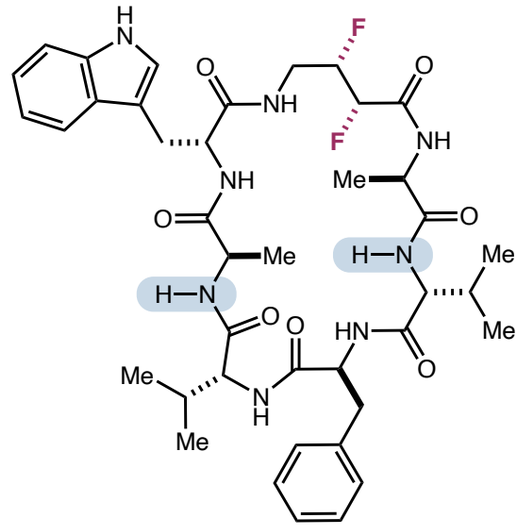
gauche conformation preferred

fluorinated backbone can strongly influence structure via conformational biasing



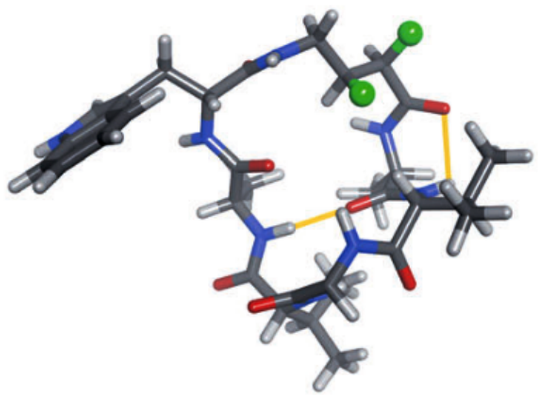
unguisin A

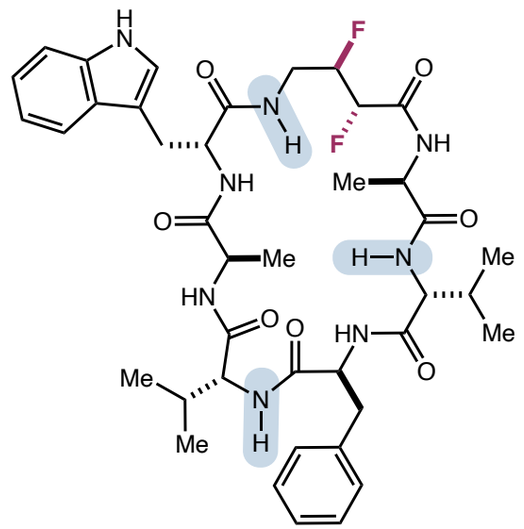




syn-difluoro

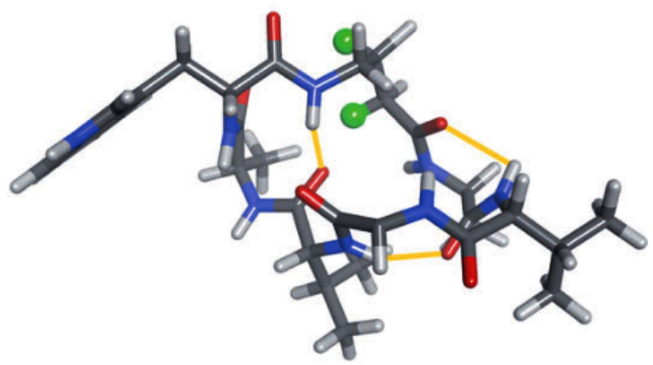
two internal H-bonds





anti-difluoro

three internal H-bonds



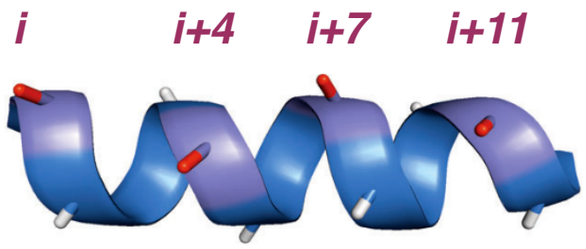
Hu, X.-G.; Thomas, D. S.; Griffith, R.; Hunter, L. *Angew. Chem. Int. Ed.* **2014**, *53*, 6176

Stabilizing α-Helix Structures via Peptide "Stapling"

α-helix

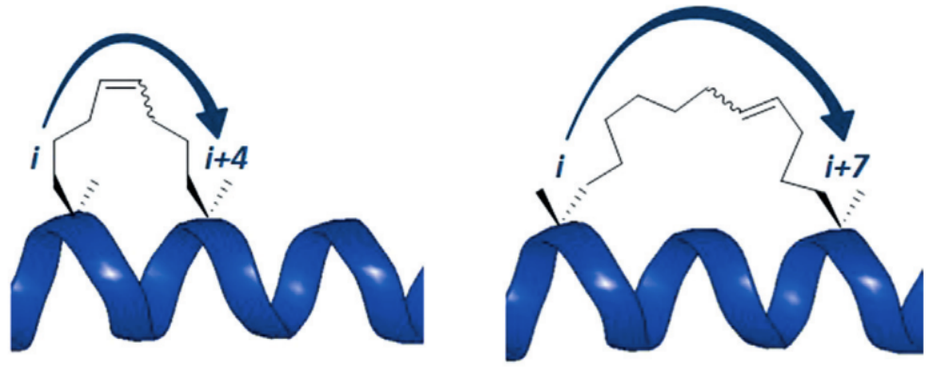
motif present at 2/3 of protein-protein interfaces

how to stabilize this structure in peptides?



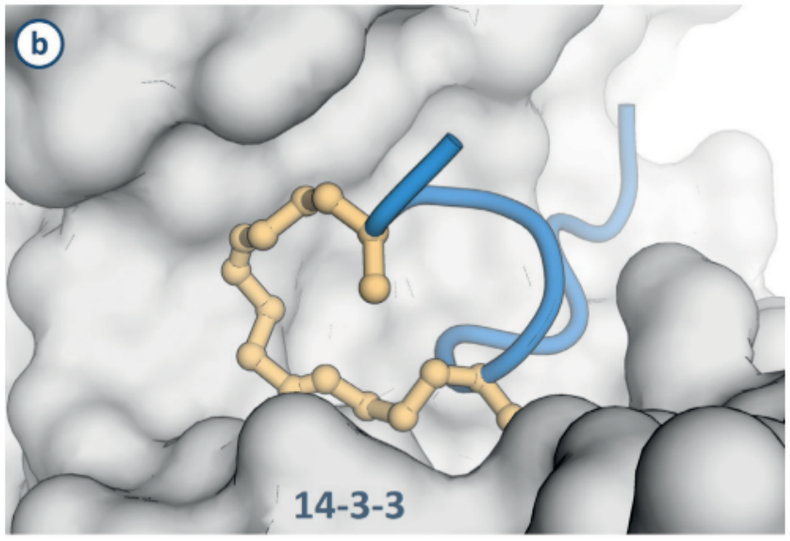
amino acids i, i+4, i+7 and i+11 are on same face

"stapled peptides"

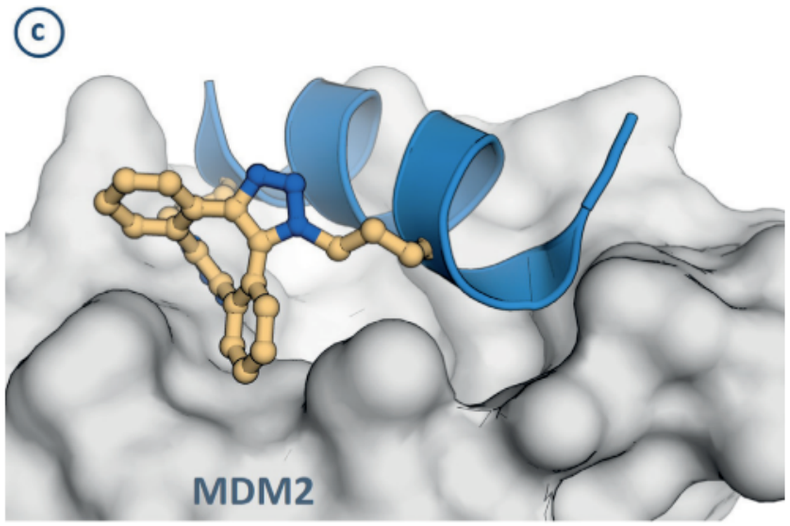


incorporate unnatural residues in synthesis → ring-closing metathesis, Click rxn, etc.

staples can provide beneficial binding to lipophilic surfaces



staple interacts with protein-protein surface



double Click staple involved in binding

Aileron
(biotech company)

focused on RCM-stapled peptides

candidate ALRN-692
Phase II clinical trials for lymphoma

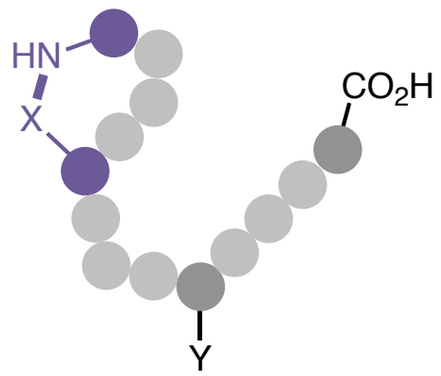
Valeur, E. *et al. Angew. Chem. Int. Ed.* **2017**, *56*, 10294

Lau, Y. H.; de Andrade, P.; Wu, Y.; Spring, D. R. *Chem. Soc. Rev.* **2015**, *44*, 91

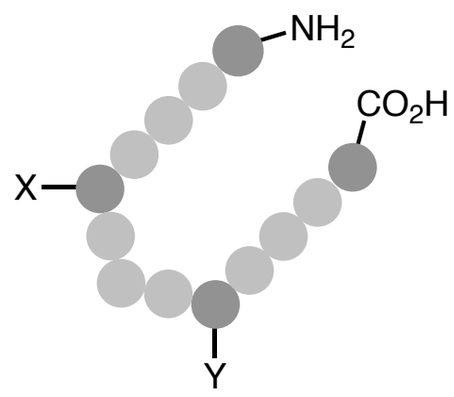
Four Possible Ways to Form Peptidic Macrocycle

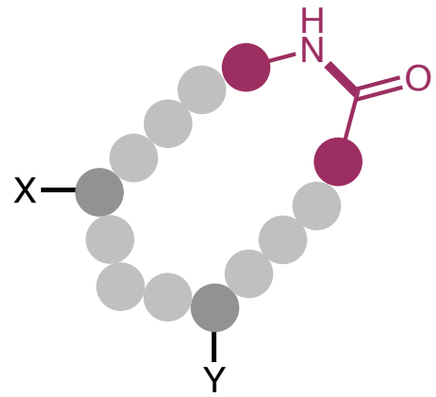
innate functional handles

N-terminus (tail)
C-terminus (head)
some natural residues

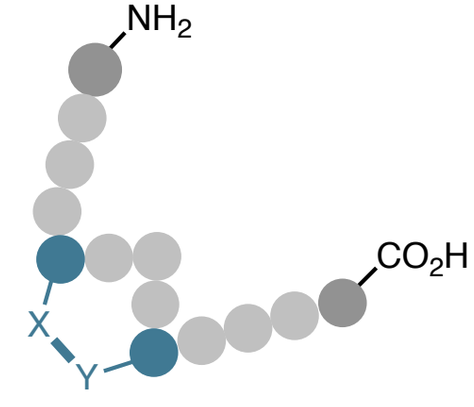


side-chain to tail



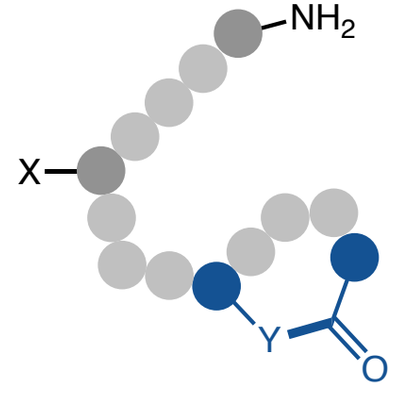


head to tail



side-chain to side-chain

unnatural amino acids are commonly incorporated for programmed side-chain reactivity



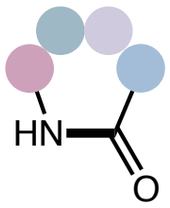
side-chain to head

White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509

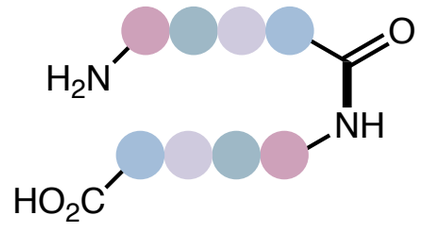
Successfully Inducing Macrocyclization

linear peptide

amide coupling



rate ~ k_{intra} [pept]



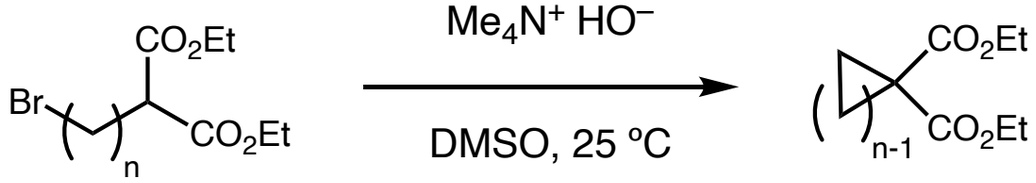
rate ~ k_{inter} [pept]^2

universally applied strategy – minimize peptide concentration

run at high dilution
(< 10 mM very common)

cyclization on solid support
(high pseudo-dilution)

biphasic reaction systems
(low [pept] in active phase)



high strain in TS for 8–12 membered rings

effects level off past ring sizes of 12+

cyclic peptides with 7+ amino acids are accessible

n	k_{intra} (s^{-1})	yield (%)	EM (M)
3	0.42	quantitative	1.5
4	6×10^{2}	quantitative	2.1×10^{3}
5	0.72	100	2.6
6	6.3×10^{-3}	99	2.3×10^{-2}
7	1.1×10^{-4}	13	3.9×10^{-4}
11	2.9×10^{-4}	29	1.0×10^{-6}
12	5.3×10^{-4}	46	1.9×10^{-3}
16	2.1×10^{-3}	73	7.5×10^{-3}
20	3.1×10^{-3}	77	1.1×10^{-2}

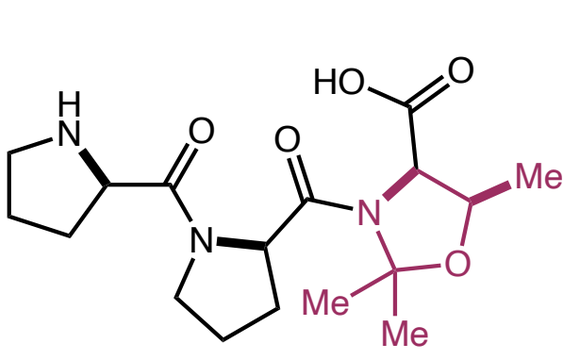
$k_{inter} = 0.28\ M^{-1}s^{-1}$

EM = k_{intra} / k_{inter}

Centelles-Marti, V.; Pandey, M. D.; Burguete, M. I.; Luis, S. V. *Chem. Rev.* **2015**, *115*, 8736

White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509

Conformational Control Strategies – Pseudoprolines



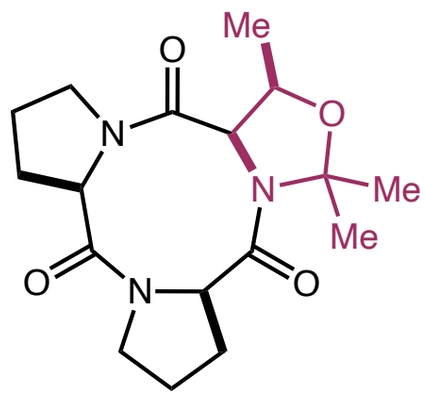
pseudoproline-containing peptide

PyBOP

DIPEA

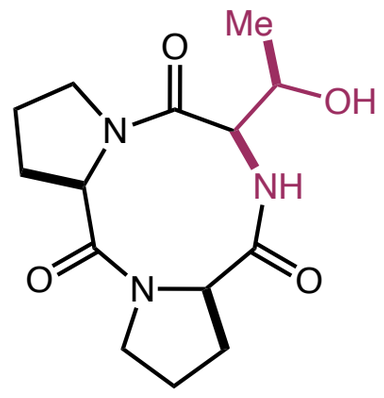
0.001 – 0.1 M

(86–80% yield)

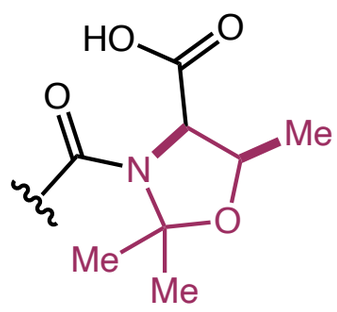


cyclic peptide

HCl

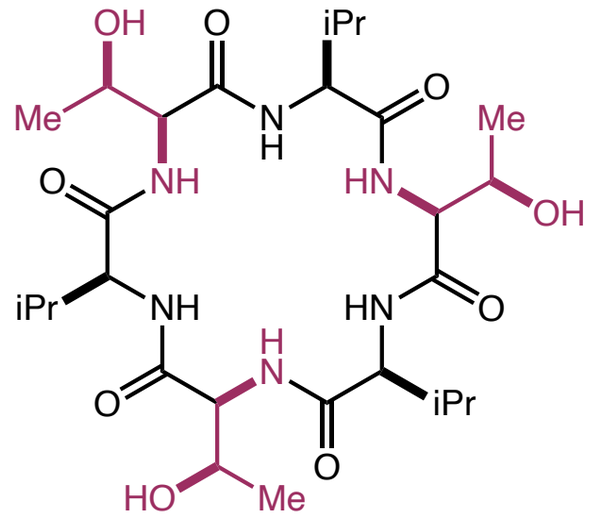


threonine residue unmasked



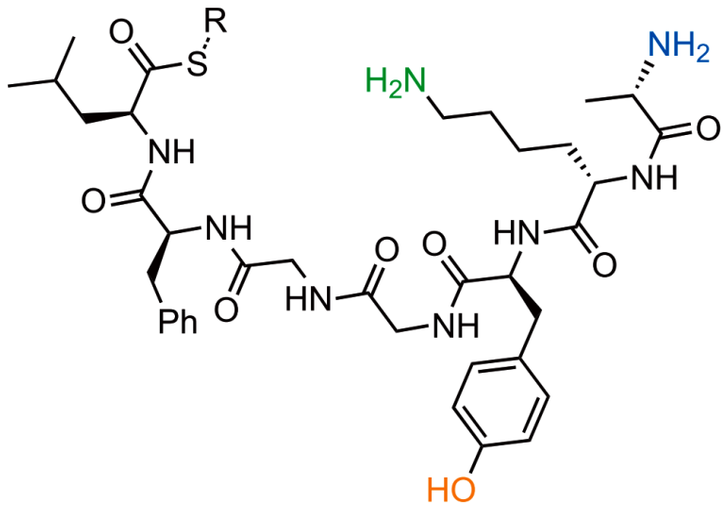
rigid proline motif
induces β-turn

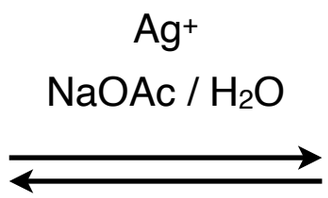
strategy applicable to many other
pseudoproline-containing chains



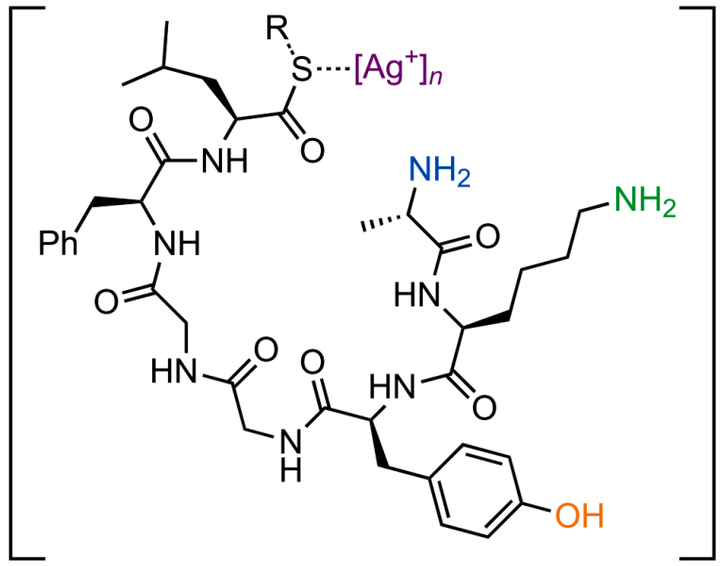
White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509

Metal Ion-Assisted Cyclization of Peptides

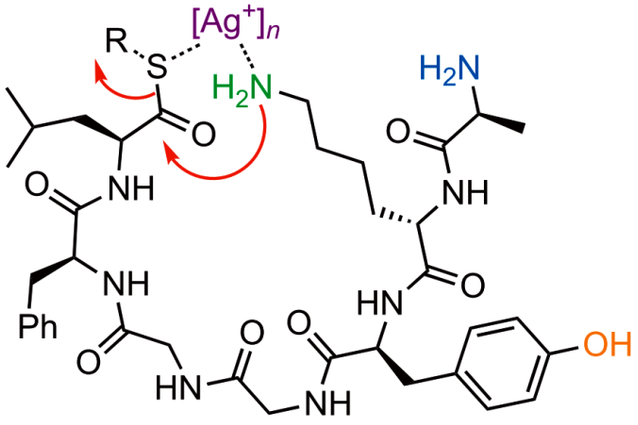




preferential *S*-coordination

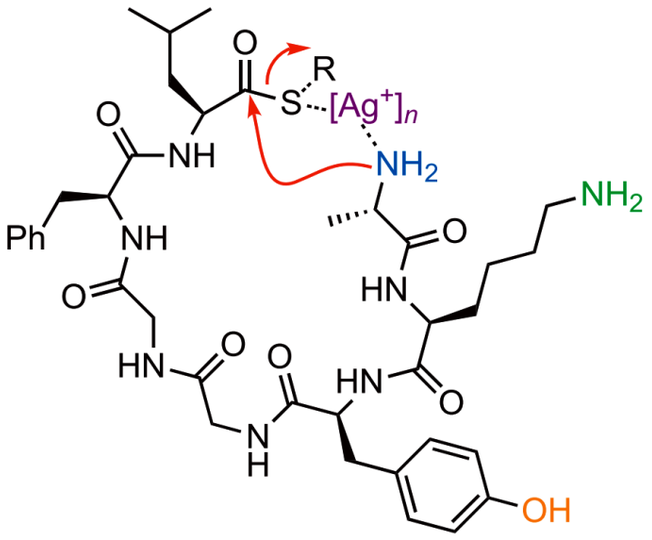


pH-dependent pre-organization mediated by Ag+



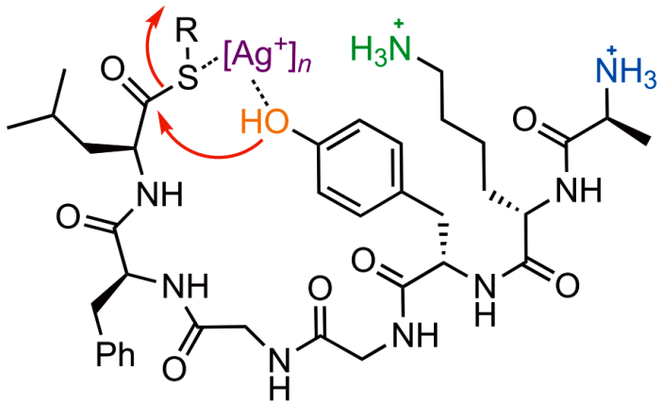
pH > 6

internal amine attack



pH = 5 – 5.7

terminal amine attack



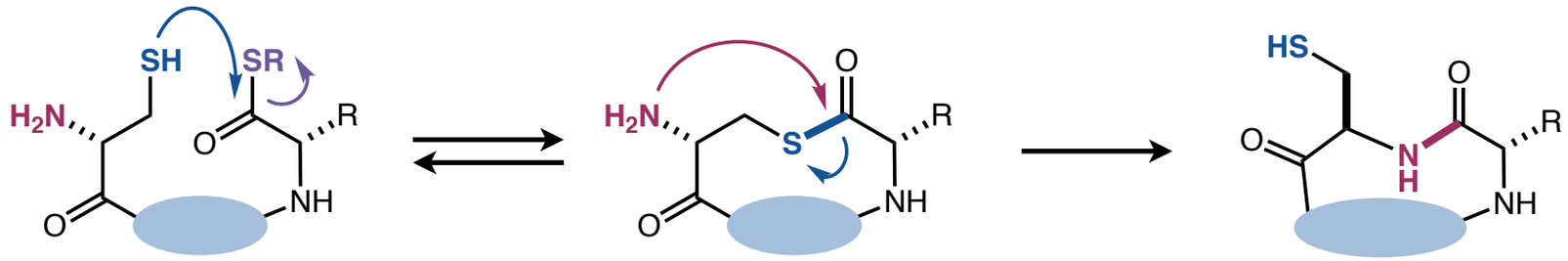
pH = 4

phenol attack

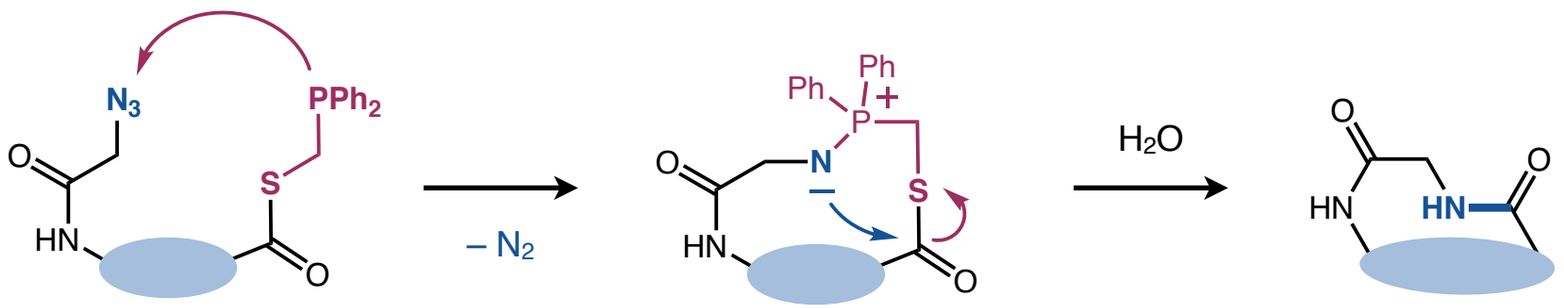
Zhang, L.; Tam, J. P. *J. Am. Chem. Soc.* **1999**, *121*, 3311

Sulfur Reagents and Ring Contractions

head-to-tail thioesterification strategy

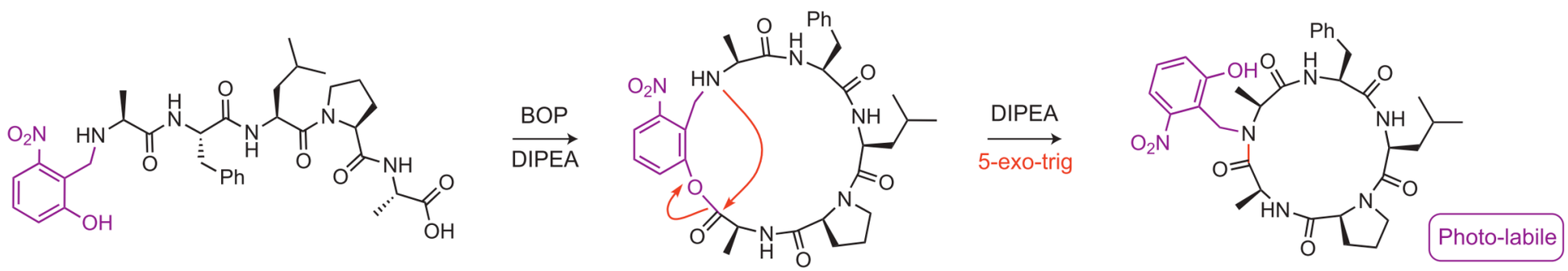


cysteine & thioester — *S*-to-*N* acyl migration — *amide product*



traceless Staudinger ligation

lactamization via ring contraction



White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509

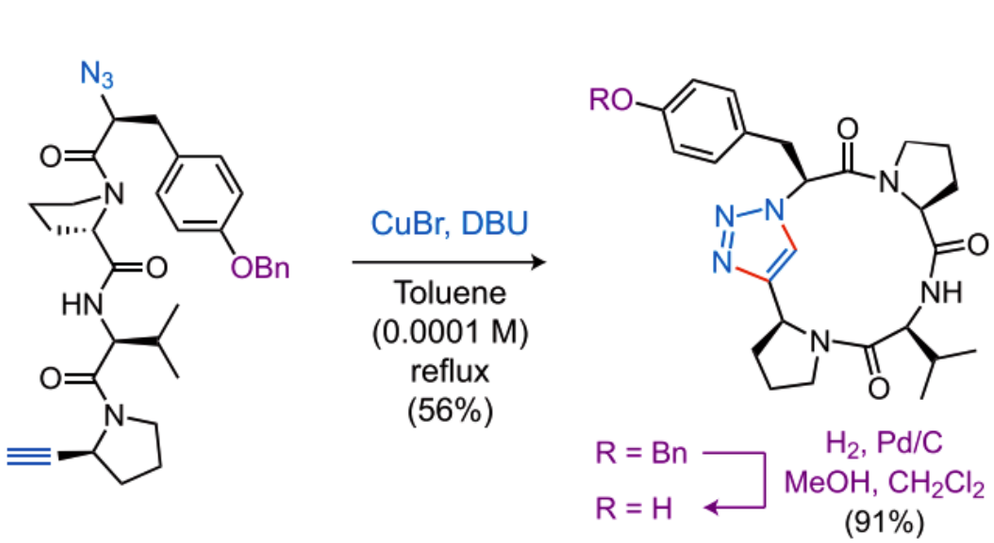
Click Reactions in Cyclic Peptide Synthesis

Click reactions

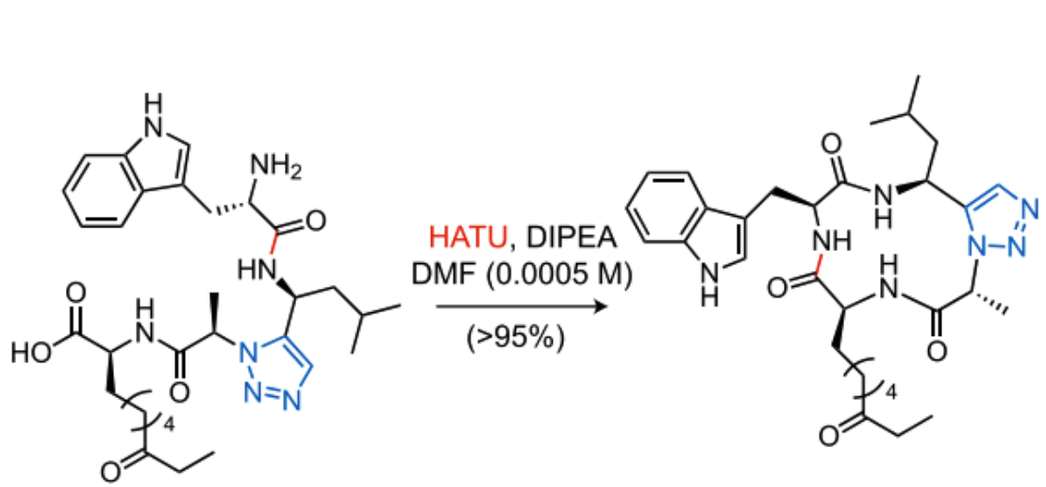
applicable to constrained rings

incorporate triazole heterocyclic motif

introduce geometrical constraints



synthesis of constrained ring



cis-amide bioisostere

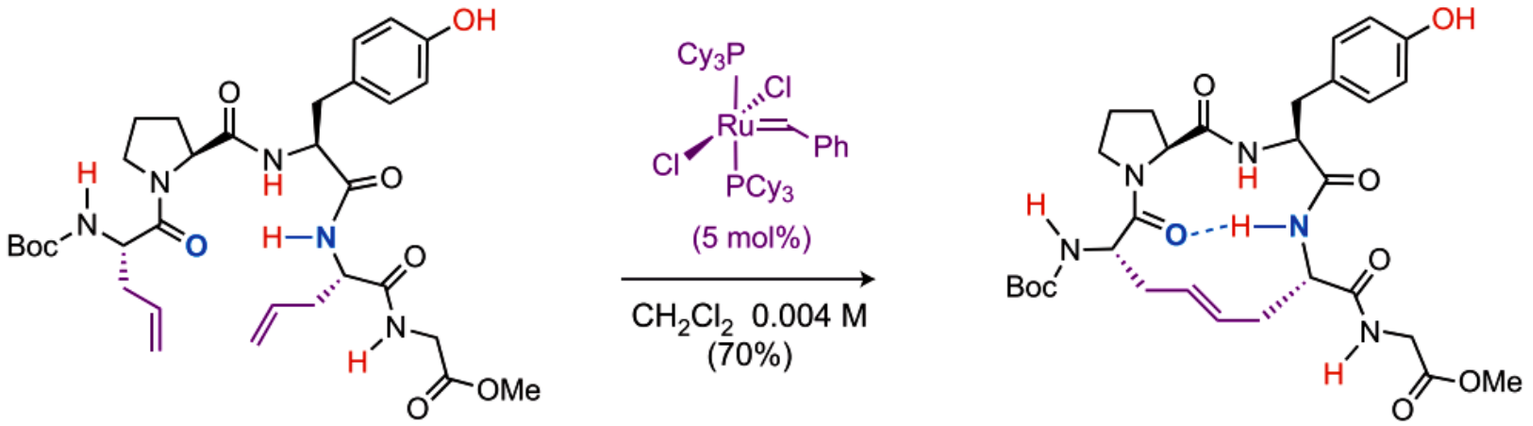
facile cyclization

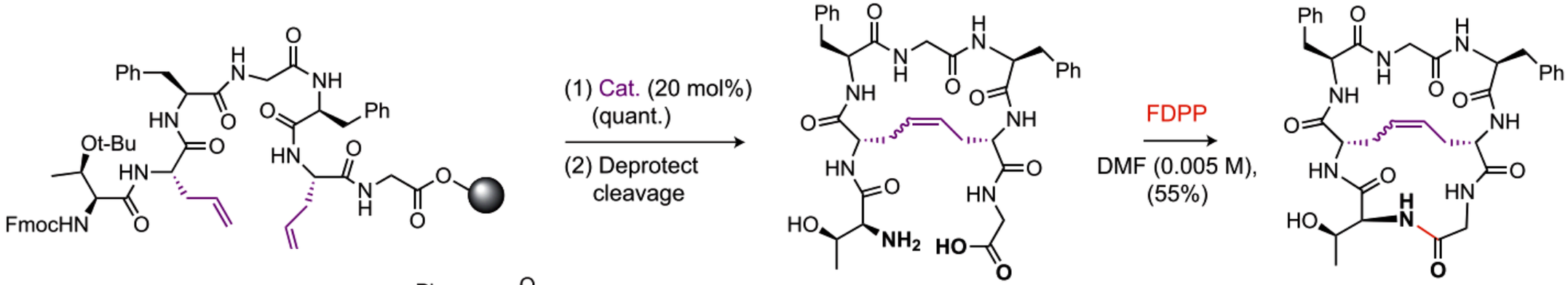
White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509

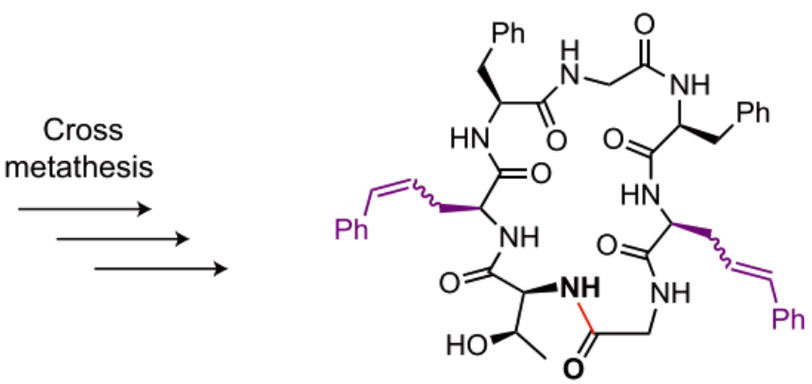
Ring-Closing Metathesis in Cyclic Peptide Synthesis

ring-closing metathesis for side-chain tethering

in this case, also stabilizes β-turn structure



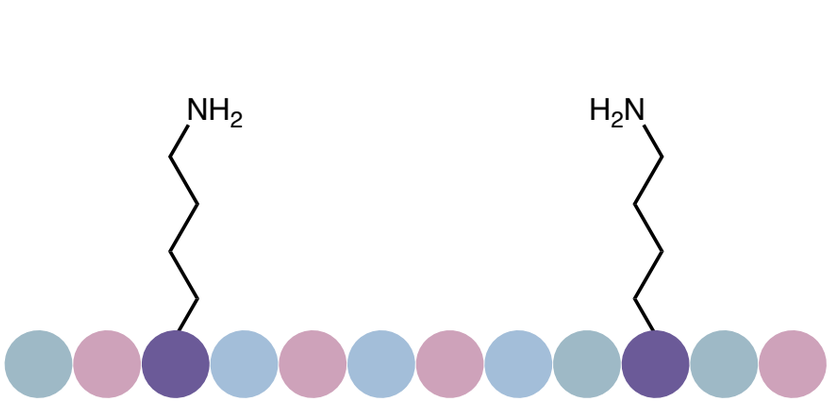




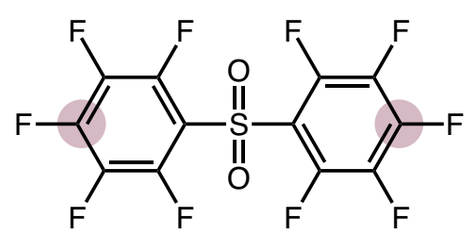
RCM as tool for pre-organizing peptide for cyclization

White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509

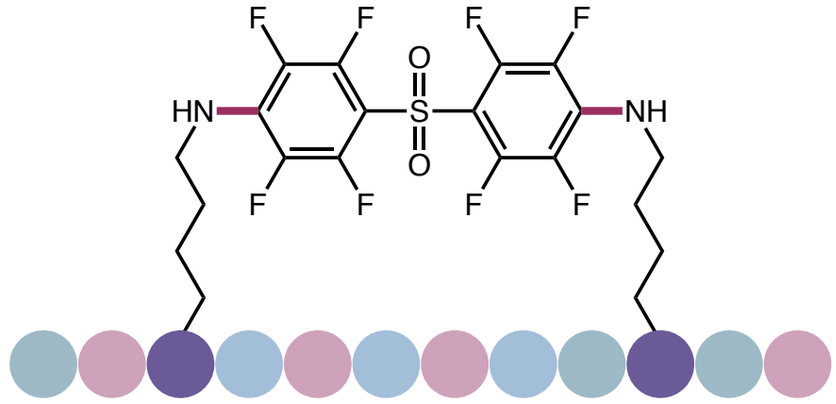
Peptide Stapling via Side-Chain S_NAr or Cross-Coupling



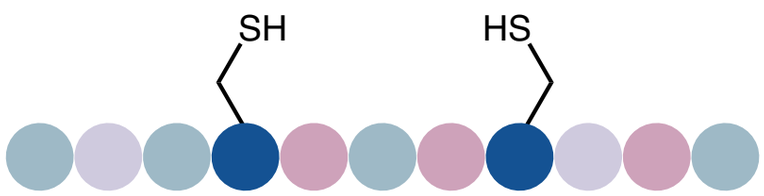
unprotected peptide (0.5 mM)



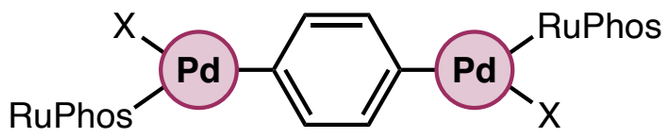
iPr_2NEt
DMF, rt, 4 h
70 – 95% yield



i, i+7 staple stabilizes α-helix

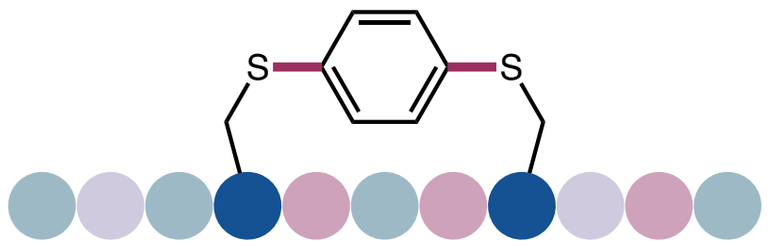


unprotected peptide (0.1 mM)



(0.2 mM)

Tris buffer (pH 7.5)
MeCN / H_2O, rt, 30 min
70 – 99% yield

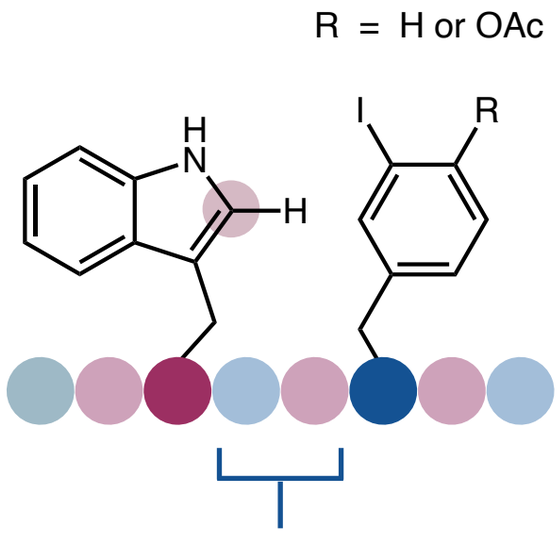


applicable to i, i+4 and i, i+7 staples

Rojas, A. J.; Zhang, C.; Vinogradova, E. V.; Buchwald, N. H.; Reilly, J.; Pentelute, B. L.; Buchwald, S. L. *Chem. Sci.* **2017**, *8*, 4257

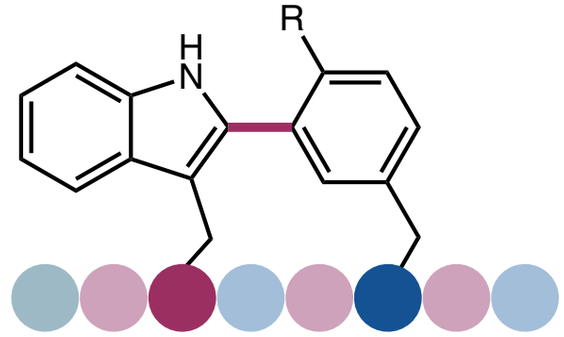
Lautrette, G.; Touti, F.; Lee, H. G.; Dai, P.; Pentelute, B. D. *J. Am. Chem. Soc.* **2016**, *138*, 8340

Peptide Stapling via Metal-Catalyzed C–H Activation

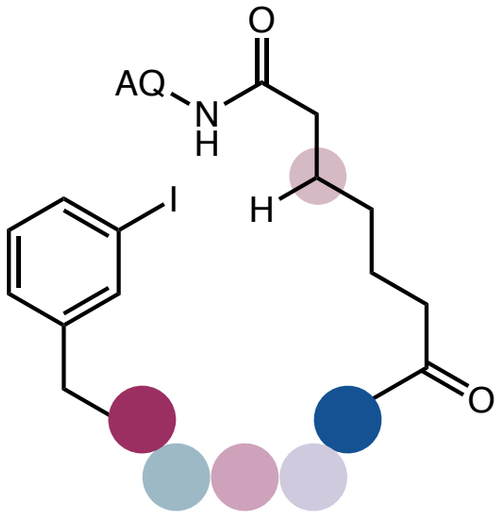


$Pd(OAc)_2$ (cat.)
$AgBF_4$, TFA

DMF, 80 °C, MW
15 min



can tolerate Asn, Arg, Asp, Ser

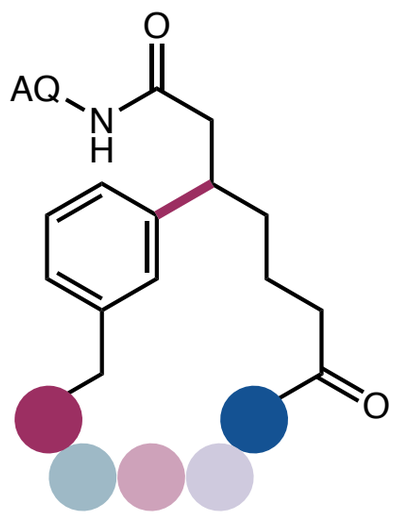


modified side-chains

$Pd(OAc)_2$ (cat.)
Ag_2CO_3, oPBA

tBuOH (5–25 mM)
100 °C, 12 h

ring sizes of
11–37 atoms accessible



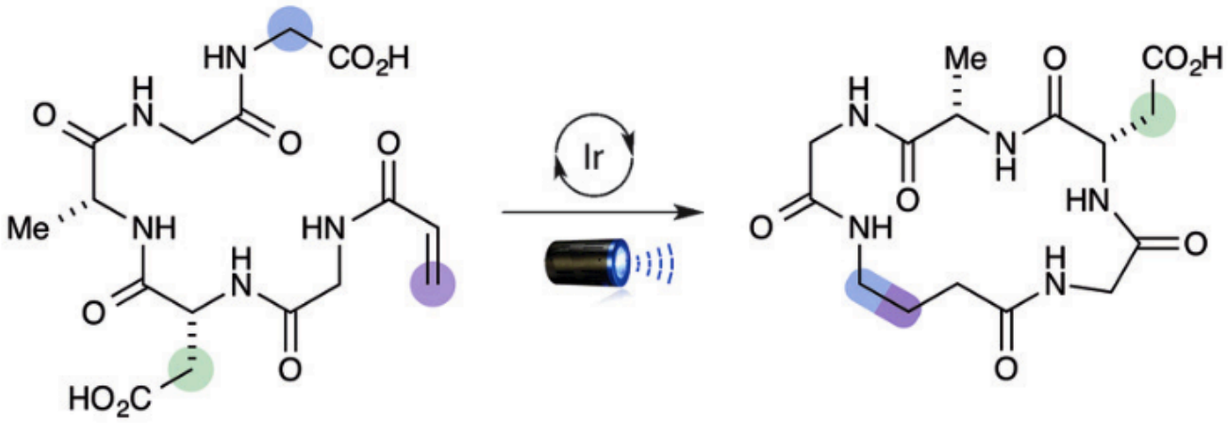
cyclophane products
anti-cancer activity (IC_{50} = 2 μM)

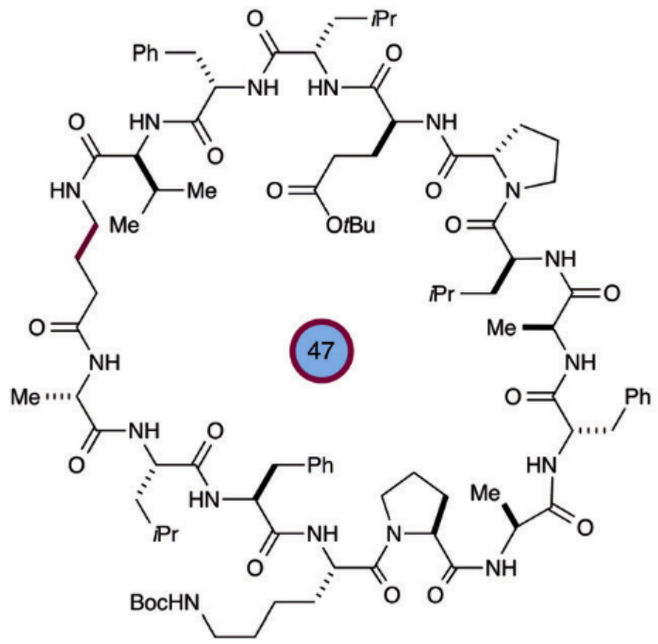
He, G.; Qi, X.; Shen, W.; Liu, P.; Chen, G. *et al. Nat. Chem.* **2018**, *10*, 540

Mendive-Tapia, L.; Preciado, S.; Garcia, J.; Ramon, R.; Kielland, N.; Albericio, F.; Lavilla, R. *Nat. Commun.* **2015**, *6*, 7160

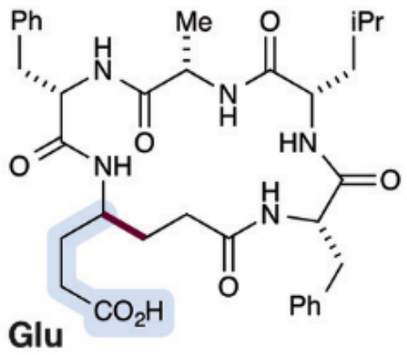
Photoredox-Catalyzed Peptide Cyclization

Photoredox decarboxylative macrocyclization: A selective route – harnessing native C-terminus carboxylate functionality

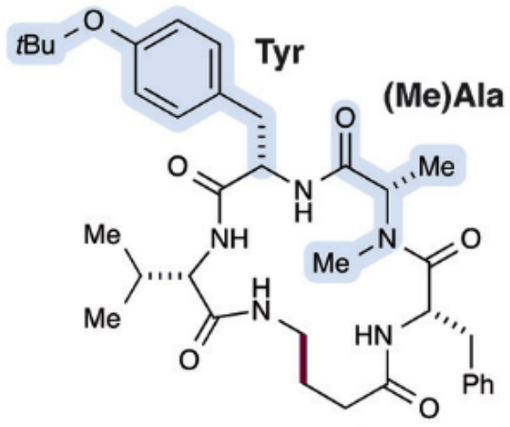




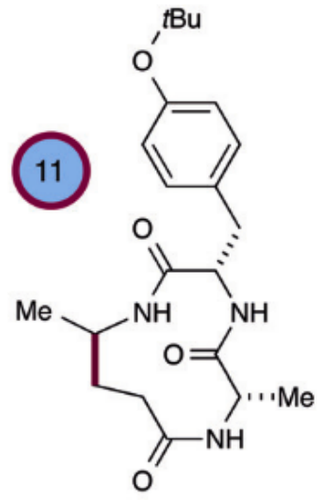
53% yield



50% yield, 2:1 dr



82% yield



36% yield, 4:1 dr

McCarver, S. J.; Qiao, J. X.; Carpenter, J.; Borzilleri, R. M.; Poss, M. A.; Eastgate, M. D.; Miller, M. M.; MacMillan, D. W. C. *Angew. Chem. Int. Ed.* **2017**, *56*, 728

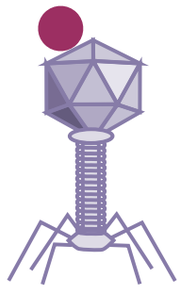
High-Throughput Screening of Cyclic Peptide Libraries

ribosomal synthesis

highest throughput methods
least variation (unnatural residues difficult)

phage display

10^9 peptides
express peptides, then cyclize

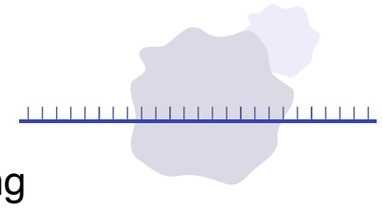


split intein circular ligation

10^9 peptides
spontaneous cyclization (splicing)

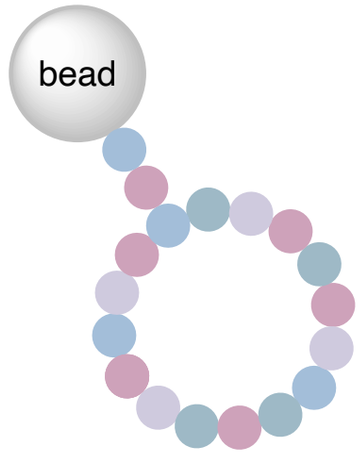
mRNA display

10^{14} peptides
in vitro method
combine with genetic reprogramming



chemical synthesis

quite laborious approaches
highest control over topology, residues, cyclization



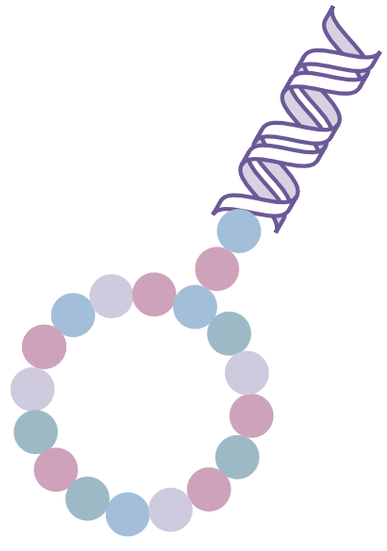
one-bead-one-compound

10^6 peptides
solid-support synthesis
“split and pool” diversification

DNA-encoded libraries

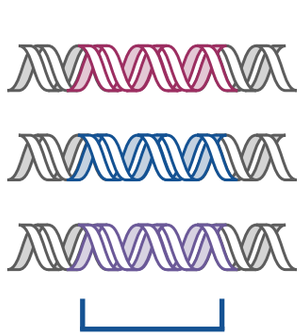
see David Liu’s work

JACS **2008**, *130*, 15611
Nat. Chem. **2018**, *10*, 704

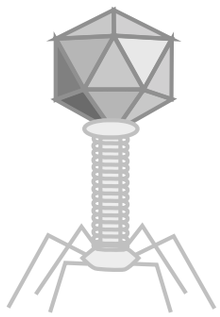


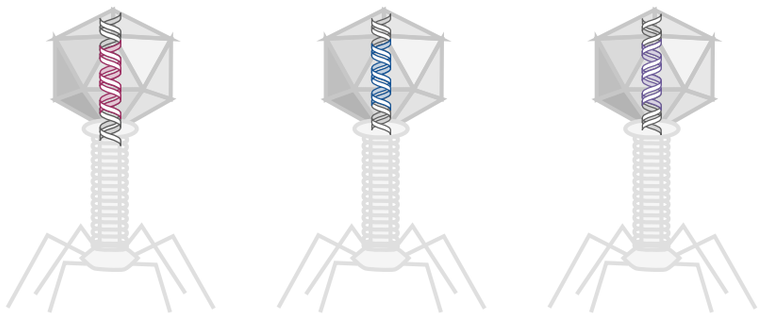
White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509
Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Phage Display for Library Synthesis in a Nutshell

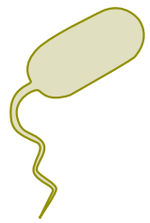


randomized DNA sequence
encodes peptide

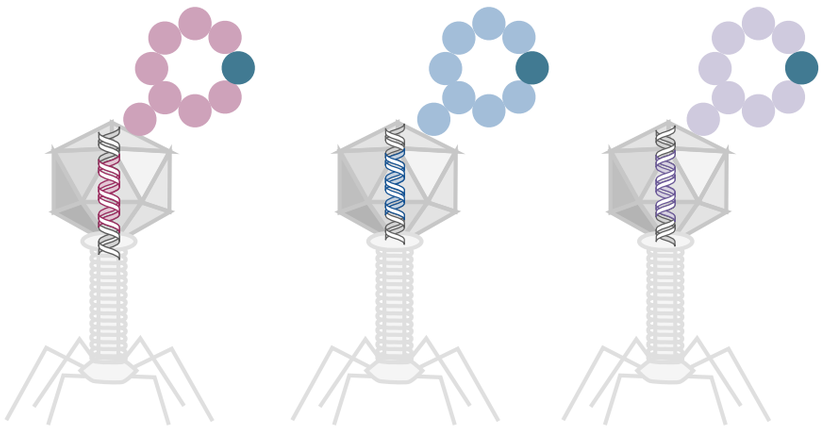




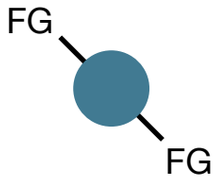
DNA sequence now in phage

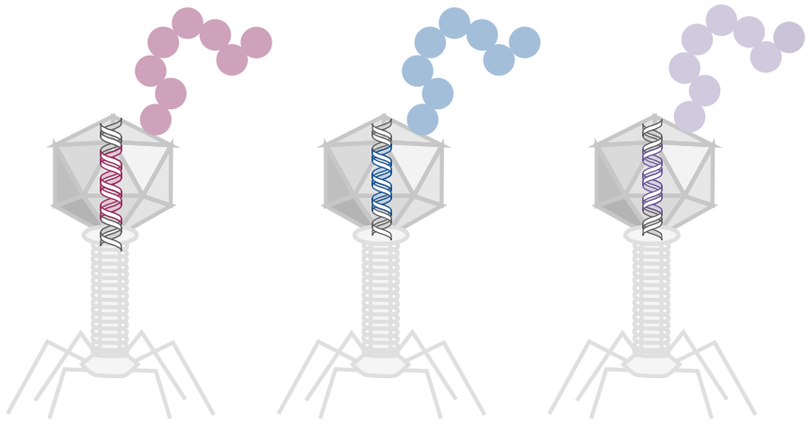


ribosomal synthesis
in E. Coli



macrocyclic peptides
attached to DNA that encoded them





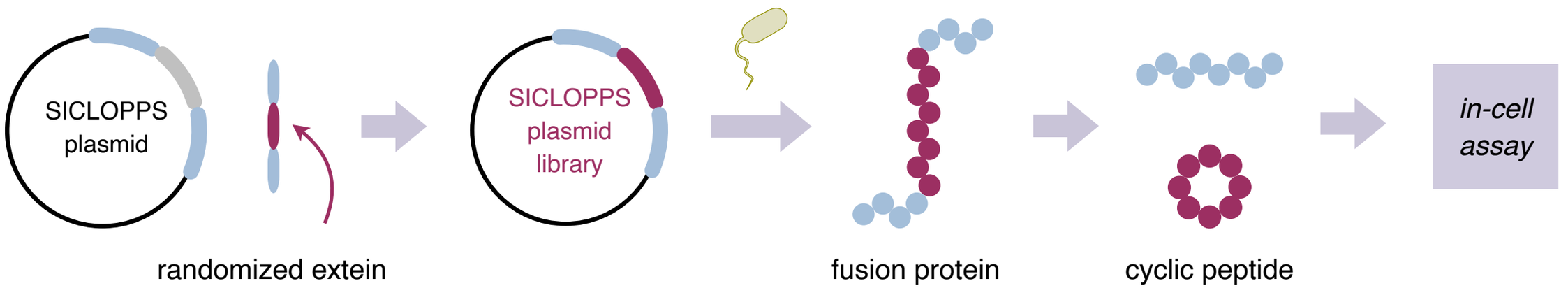
linear peptides displayed on phage surface

Heinis, C.; Winter, G. *Curr. Opin. Chem. Biol.* **2015**, *26*, 89

Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

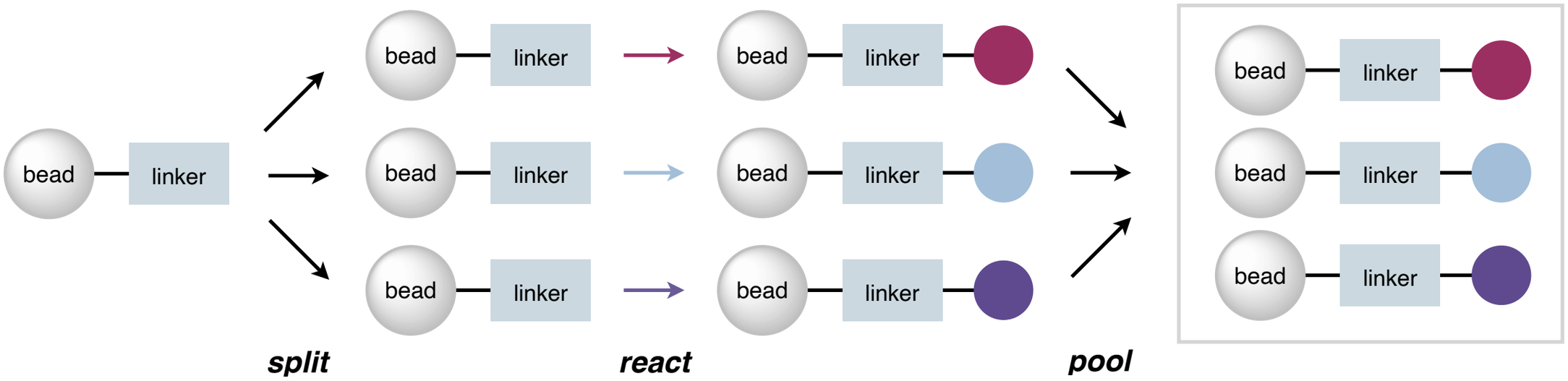
Cyclic Peptide Libraries via SICLOPPS

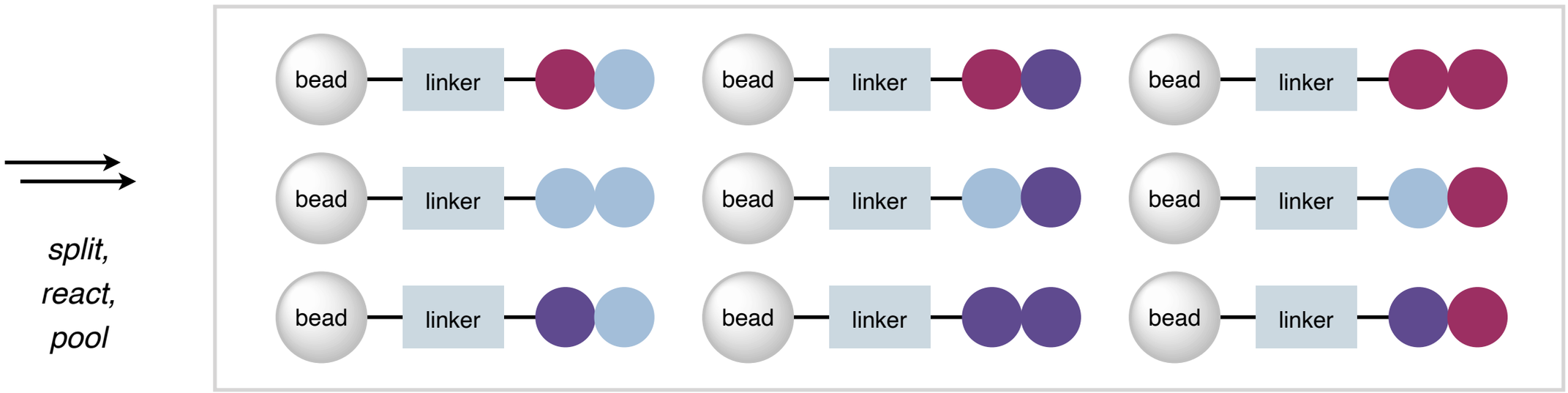
SICLOPPS = split-intein circular ligation of peptides and proteins



White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509
Tavassoli, A. *Curr. Opin. Chem. Biol.* **2017**, *38*, 30

Chemical Synthesis of Libraries via Split & Pool Approach





several → *iterations* **library of linear peptides** *cyclization* → **cyclic peptide library (up to 10^6 members)**

Kodadek, T.; McEnaney, P. J. *Chem. Commun.* **2016**, *52*, 6038

Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Challenges in Macrocyclic Peptide Development

Lipinski's Rule of 5

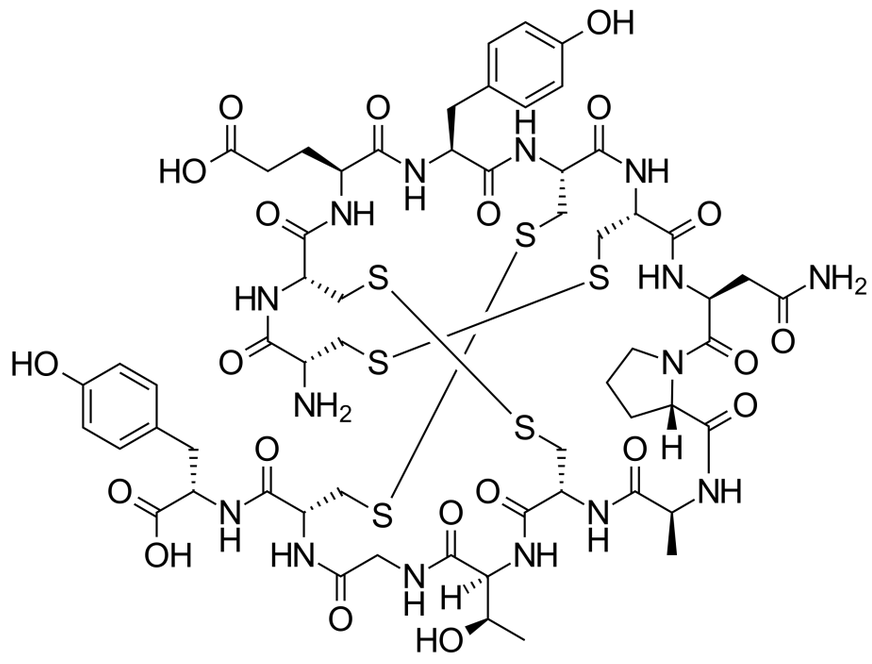
MW	<	500
cLogP	>	5
HBD	<	5
HBA	<	10

(somewhat) useful for small molecules

linaclotide *(2012)*
top 200 drug

1456	MW
–3.4	cLogP
21	HBD
22	HBA

inadequate for macrocyclic peptides



straightforward library synthesis

high-throughput screening for potency and selectivity

→ **very potent molecule**

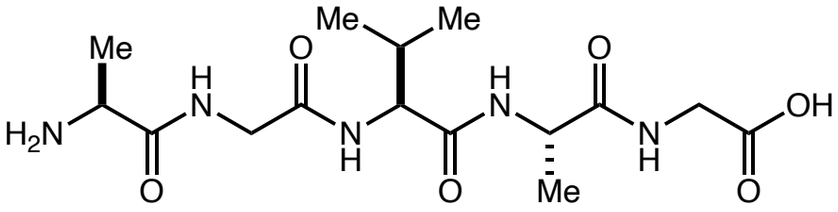
→ **bad drug candidate**

← suboptimal understanding of what determines good PK properties

← few well-established guidelines or rules-of-thumb

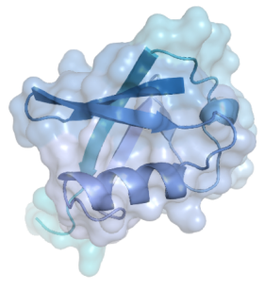
Craik, D. J.; Fairlie, D. P; Liras, S.; Price, D. *Chem. Biol. Drug. Des.* **2013**, *81*, 136

Combatting Metabolism – Not the Worst of Problems

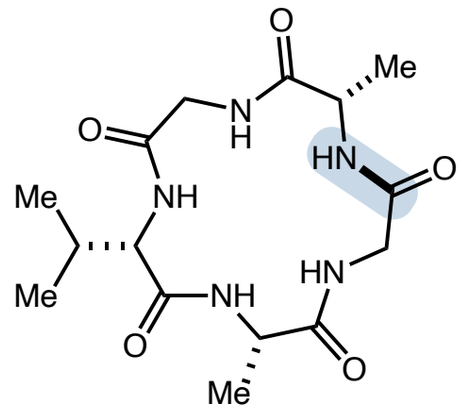


generic peptide
only proteogenic side-chains

$t_{1/2}$ < 5 minutes

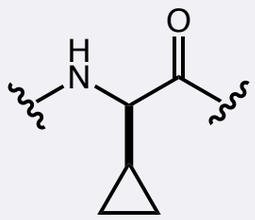


rapid degradation
by native proteases

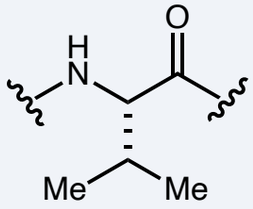


cyclized analogue – *uncommon motif*

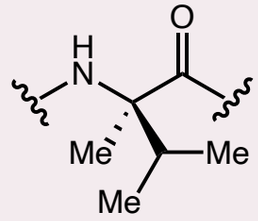
10x increase in $t_{1/2}$



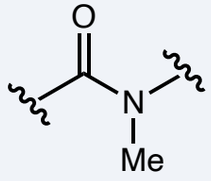
unnatural side-chains



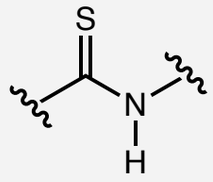
D-amino acids



α,α-disubstitution



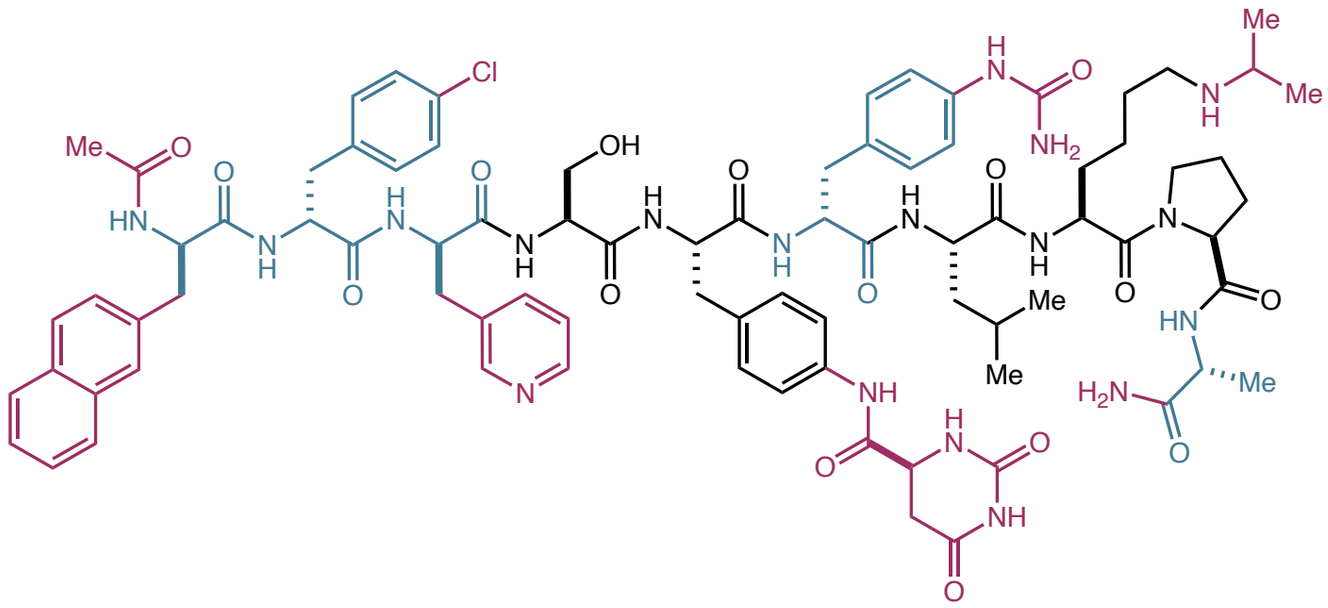
N-methyl amides



amide bioisosteres

Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Combatting Metabolism – Not the Worst of Problems



degarelix (prostate cancer drug)

numerous non-proteogenic side-chains

numerous D-amino acids

$t_{1/2}$ = 40 – 70 days

metabolism via proteolytic cleavage is generally avoidable by design

other pathways should not be discounted (e.g., oxidative degradation via P450)

Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Bigger Challenges: Cellular Uptake and Oral Bioavailability

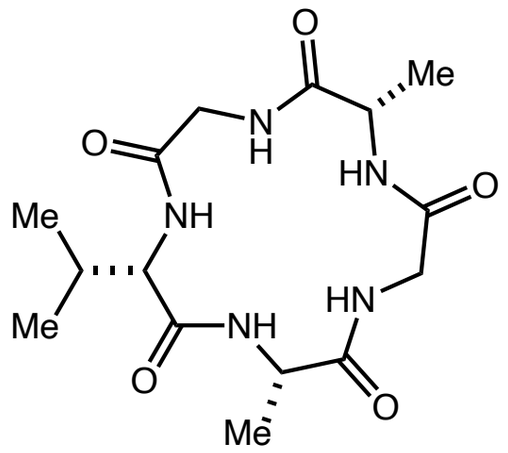
challenge: cellular uptake

cyclic peptides are inherently polar

difficult passive diffusion through cell membrane

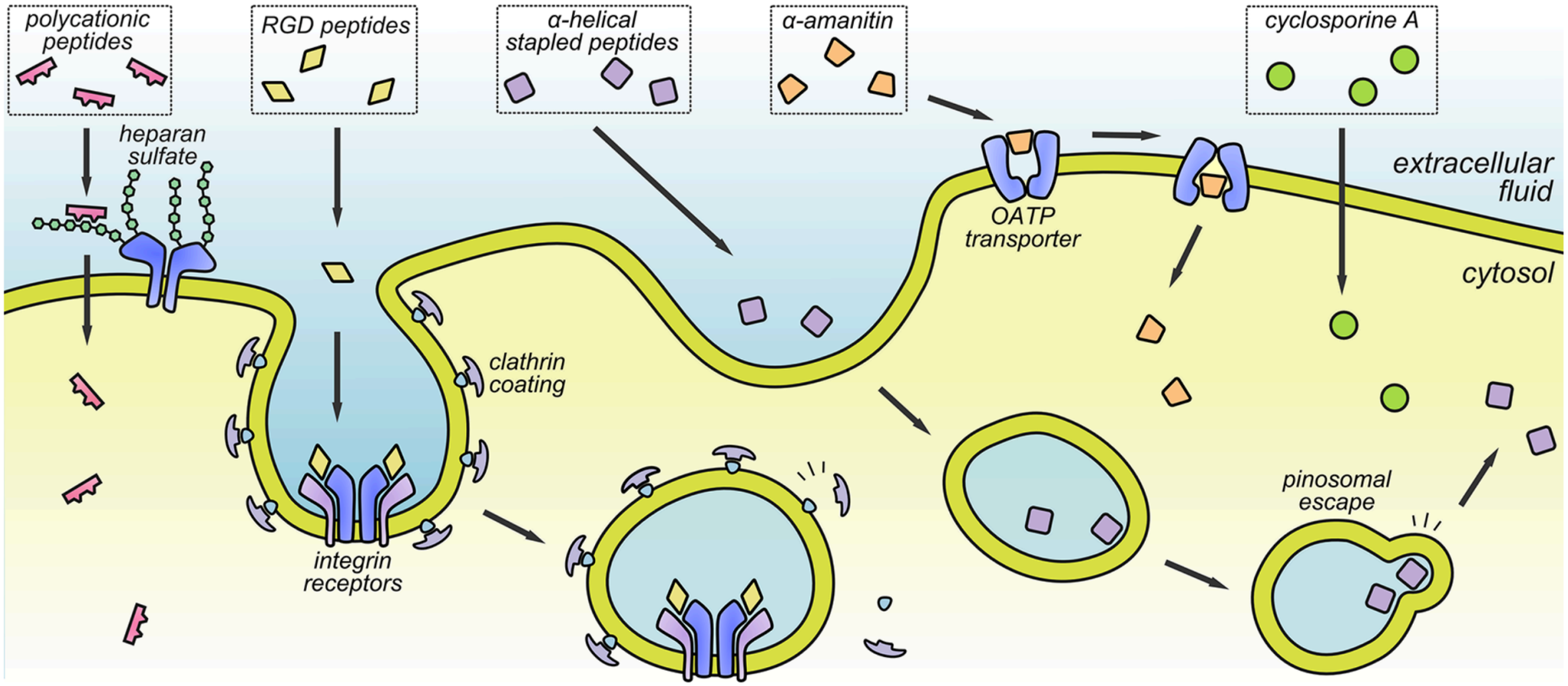
increase peptide lipophilicity

exploit other mechanisms



Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Overview of Canonical Cell Uptake Mechanisms



electrostatically facilitated passive diffusion *(poly-Arg peptides)*	receptor-mediated endocytosis *(RGD peptides)*	pinocytosis followed by pinosomal escape *(amphiphilic peptides)*	active transport	passive diffusion *(cyclosporin)*	combination of mechanisms

"Clearly, macrocyclic peptides can be efficiently uptaken by the cell, at least in principle. But at any rate, the general strategy for transforming a bioactive peptide into a cell-permeable compound remains elusive."

Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Bigger Challenges: Cellular Uptake and Oral Bioavailability

challenge: cellular uptake

cyclic peptides are inherently polar

difficult passive diffusion through cell membrane

increase peptide lipophilicity

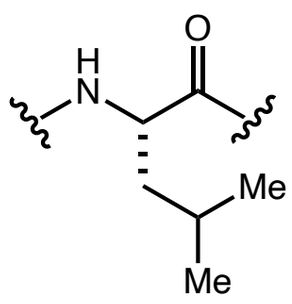
exploit other mechanisms

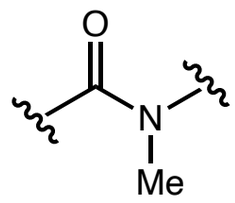
challenge: oral bioavailability

1) survive acidic stomach and proteases

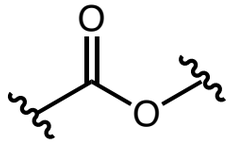
2) absorption via diffusion through intestine

recent efforts mostly dedicated to improving lipophilicity





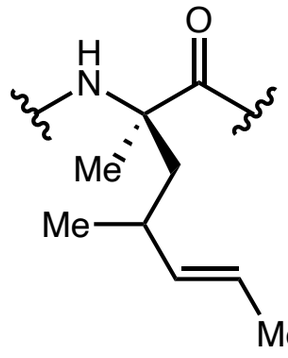
N-methyl-amide linkage



worse H-acceptors

lipophilic side chains

non-canonical amino acids



Naylor, M. R.; Bockus, A. T.; Blanco, M. J.; Lokey, R. S. *Curr. Opin. Chem. Biol.* **2017**, *38*, 141

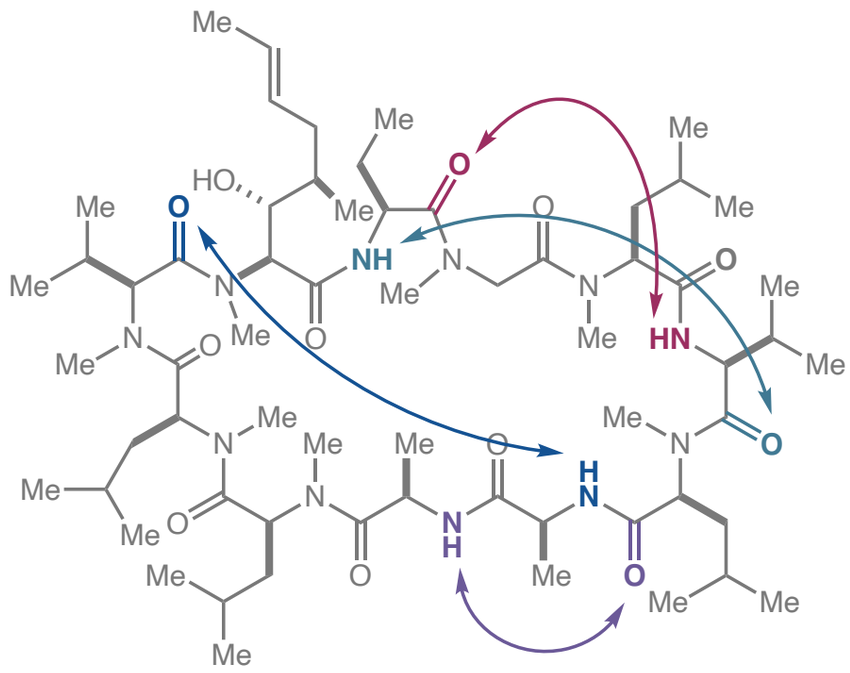
Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Cyclosporin – Remarkable Example of Cellular Uptake and Bioavailability

cyclosporin
(immunosuppressant)

seven *N*-Me amides

lipophilic side-chains



30% oral bioavailability
(0–5% standard for peptides)

readily diffuses through cell membranes
(10^{-7} vs 10^{-5} to 10^{-6} cm/s for small molecules)

in water – hydrophilic

6 conformations

intermolecular H-bonding of amides

considerable amount of exposed polar surface

in non-polar media – lipophilic

compact, folded into self

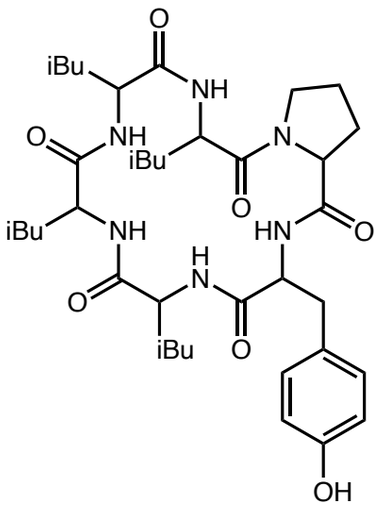
four internal hydrogen bonds

exposed polar surface minimized via intramolecular interactions

amphiphilic, flexible structure allows for distinct but optimal behavior in both aqueous and lipophilic media

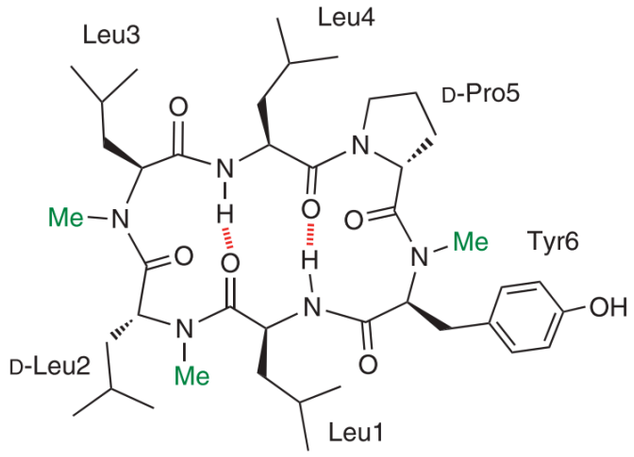
Tayar, N. E.; Mark, A. E.; Vallat, P.; Brunner, R. M.; Testa, B.; van Gunsteren, W. F. *J. Med. Chem.* **1993**, *36*, 3757
Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

Effect of Degree of N-Methylation on Lipophilicity

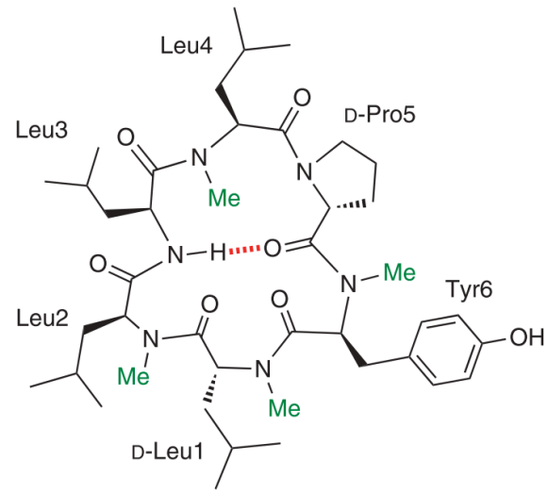


D- and L-amino acids

several degrees of N-methylation

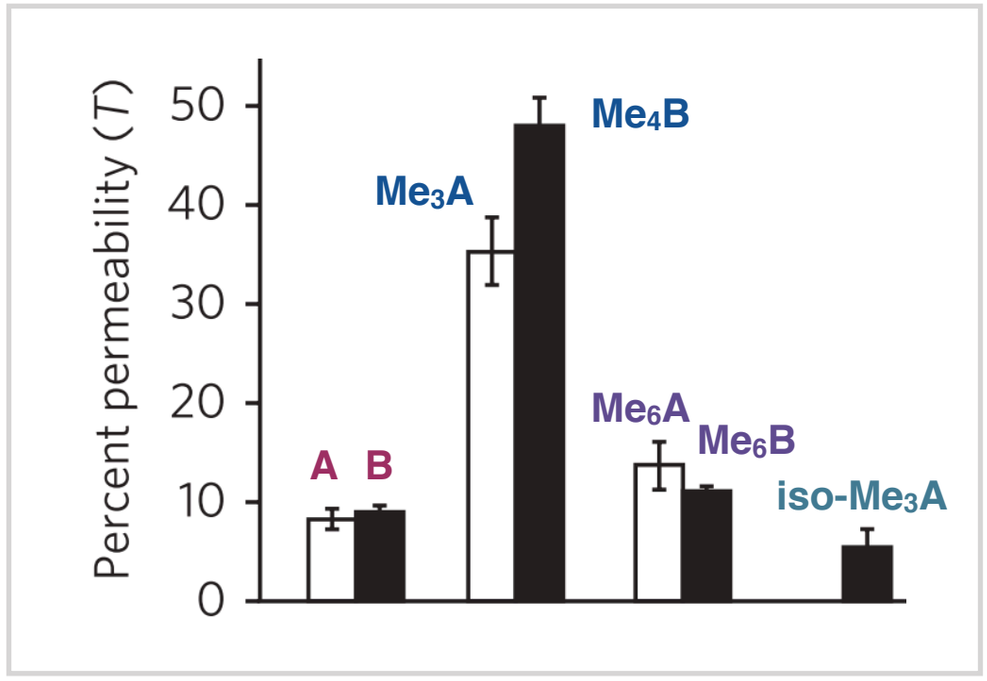


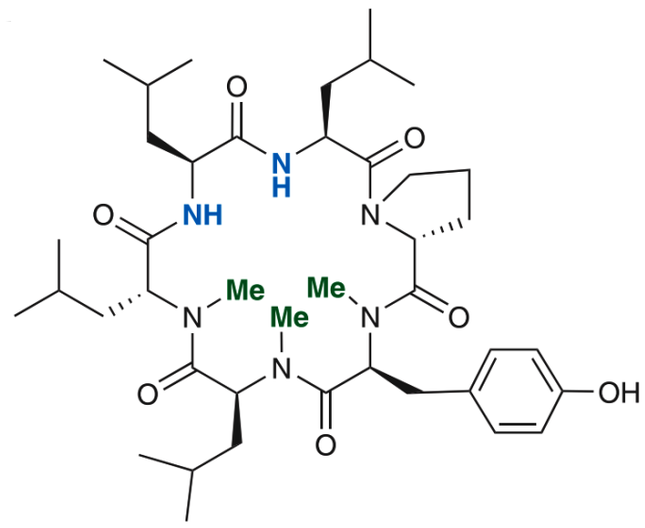
Me_3A



Me_4B

complex relation between membrane permeability and (degree + positions) of N-methylation





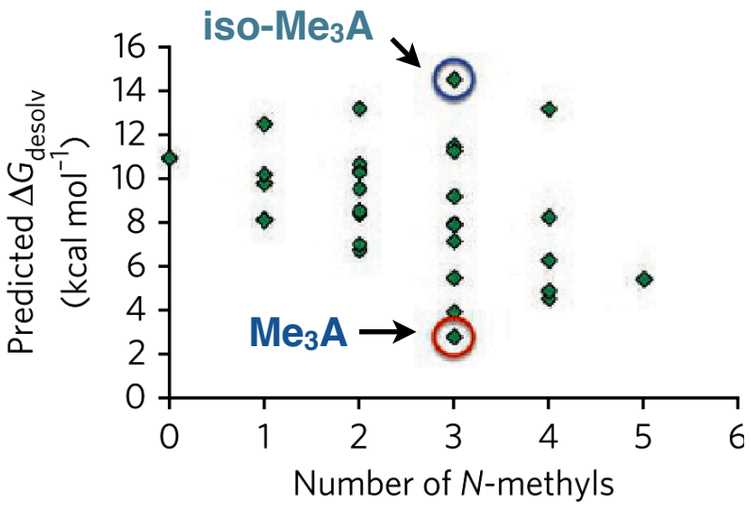
$iso\text{-}Me_3A$

Lokey, R. S. *et al. Nat. Chem. Bio.* **2011**, *7*, 4167

Effect of Degree of N-Methylation on Lipophilicity

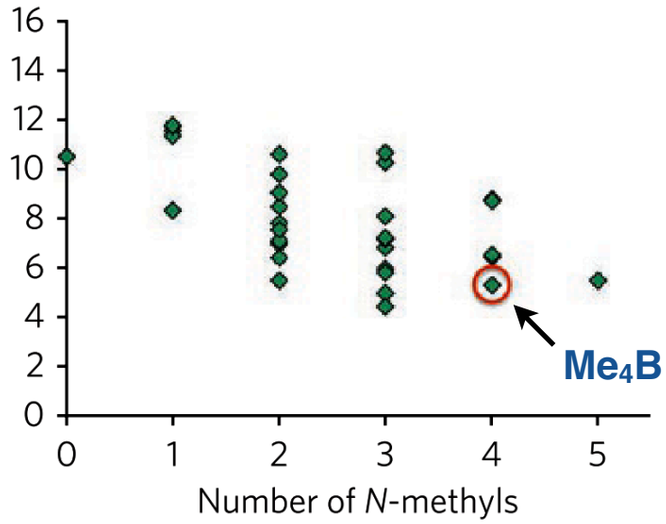
analysis for scaffold **A**

best and worst with 3 x N-Me



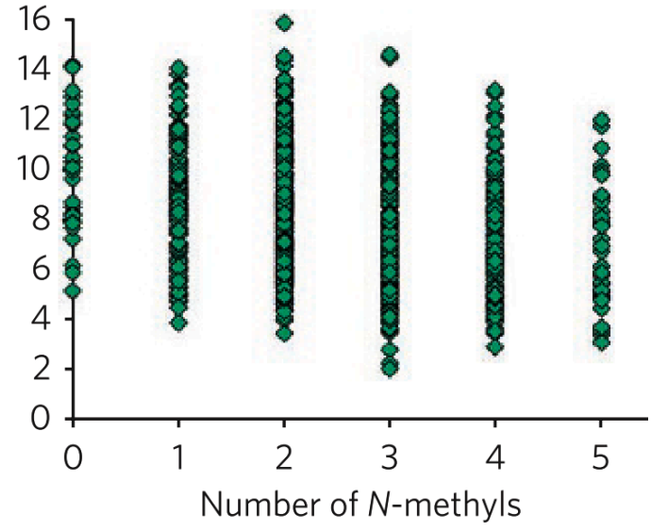
analysis for scaffold **B**

relatively linear correlation

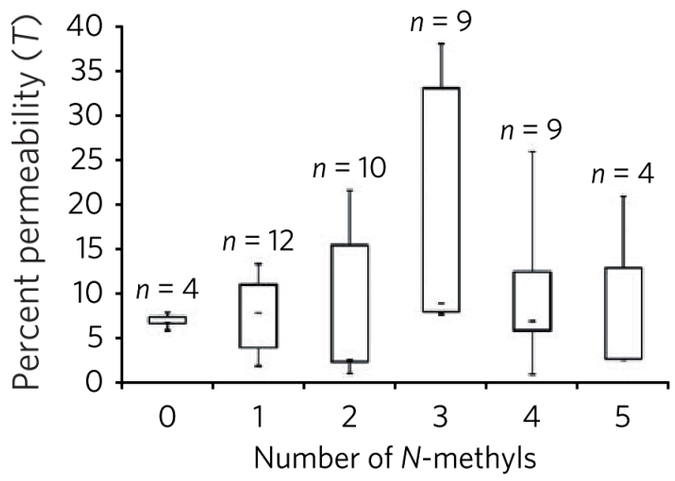


analysis for all scaffolds

relatively no correlation



experimental measurement for synthesized isomers



N-Me helps, but the degree of permeability varies

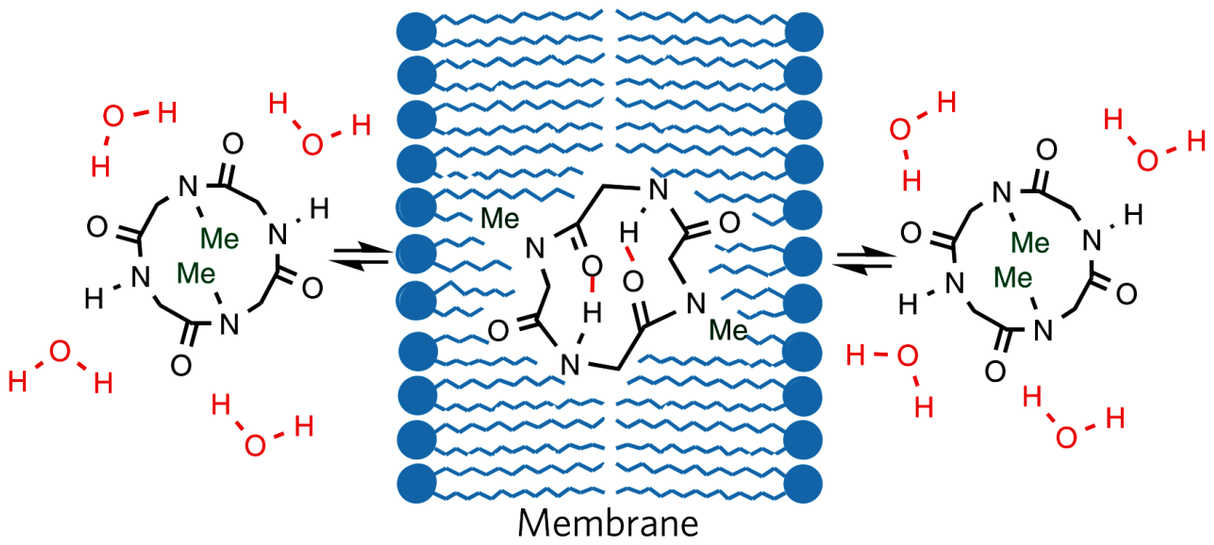
rat pharmacokinetics – comparable to cyclosporin!

	Intravenous administration		
	CL (ml min^{-1} kg^{-1})	V_{dss} (l kg^{-1})	$t_{1/2}$ (h)
Me$_3$A	4.5	1.1	2.8
cyclosporin	3.5	1.2	6.0

	Oral administration		
	AUC (ng h ml^{-1})	C_{max} (ng ml^{-1})	% *F*
Me$_3$A	10.5	852	28
cyclosporin	13.8	1440	29

Lokey, R. S. *et al. Nat. Chem. Bio.* **2011**, *7*, 4167

Effect of Degree of N-Methylation on Lipophilicity



ability to form intramolecular H-bonds can improve permeability by reducing exposed polar surface while in membrane

N–methylation inherently modulates permeability by eliminating N–H bonds

N–methylation modulates macrocyclic structure, leading to intramolecular H bonding

number of *N*-Me, position of *N*-Me and chirality of amino acids are critical

a priori predictions still difficult…

Lokey, R. S. *et al*. *Nat. Chem. Bio.* **2011**, *7*, 4167

A Disconnect in Macrocyclic Peptide Development

straightforward library synthesis

high-throughput screening for potency and selectivity

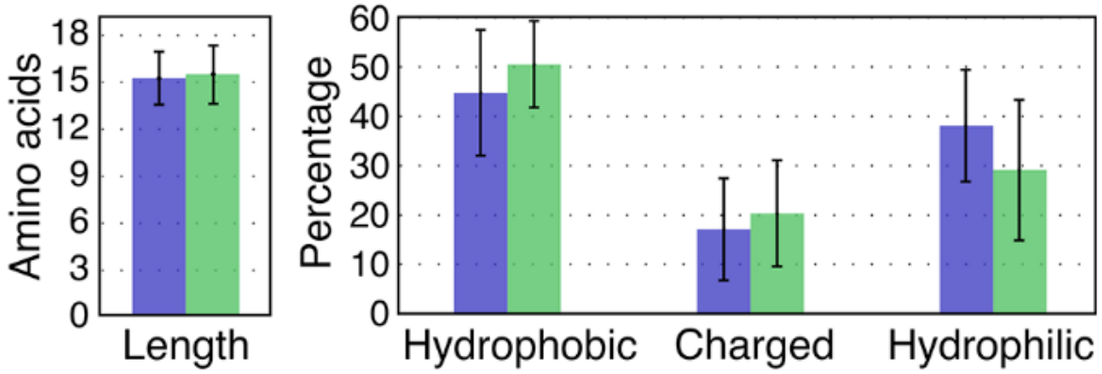
→ **very potent molecule**

→ **bad drug candidate**

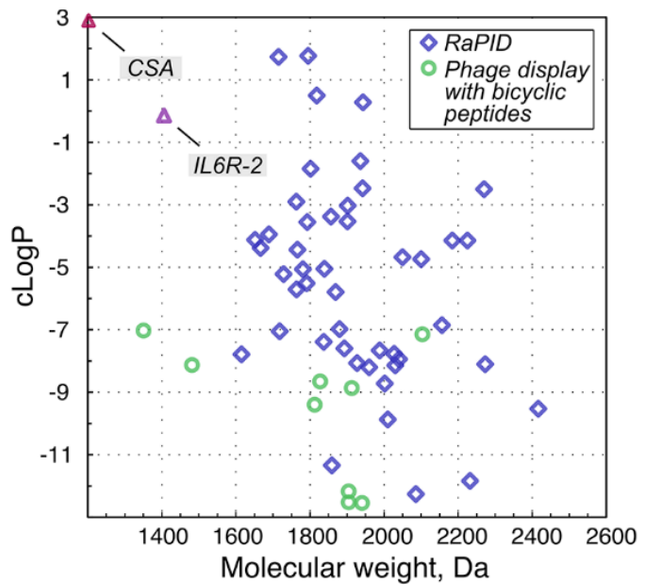
suboptimal understanding of what determines good PK properties

few well-established guidelines or rules-of-thumb

analysis – 16 targets – three most potent ligands for each (discovered via **RaPID mRNA** or **phage** display screen)



interpretation: we know the correct "recipe" for potency

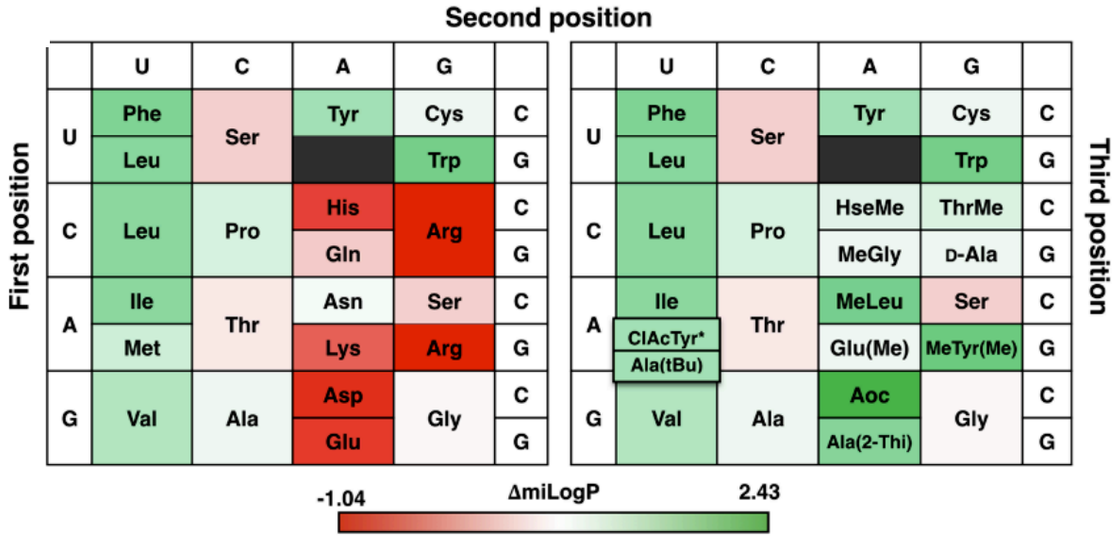


but pharmacokinetics are an afterthought…

Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc.* **2019**, *141*, 4167

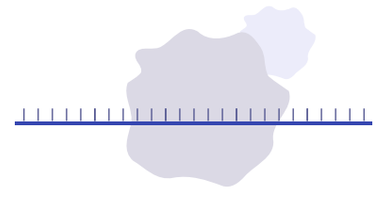
Craik, D. J.; Fairlie, D. P; Liras, S.; Price, D. *Chem. Biol. Drug. Des.* **2013**, *81*, 136

High-Throughput Screening with Genetically Reprogrammed Library



replace charged & polar amino acids

translation with 23 relatively non-polar amino acids



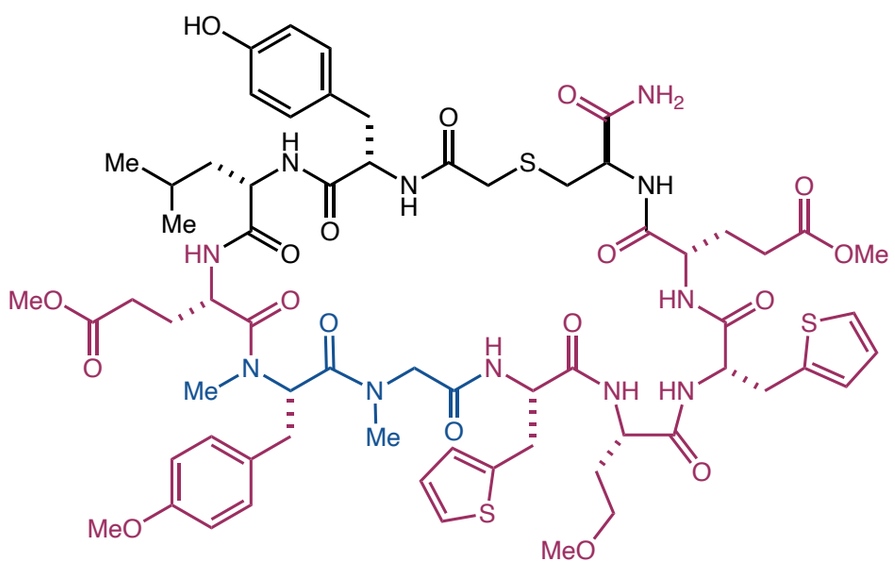
RaPID
(mRNA display)

“hydrophobic”
cyclic peptide library
(10^{12} members)

interleukin-6
receptor ligand

K_D = 350 nM

cLogP = 0.2



Passioura, T.; Liu, W.; Dunkelmann, D.; Higuchi, T.; Suga, H. *J. Am. Chem. Soc.* **2018**, *140*, 11551

Outlook

genetically reprogrammed libraries?

multi-layered screening?

leveraging other diffusion mechanisms?

better understanding of cellular uptake?

Macrocyclic Peptides – Questions?

Properties and structure

Why cyclic peptides?

Structural & conformational aspects

Macrocyclization

General considerations

Synthetic methods

cation-assisted, sulfur reagents,
ring contraction, click, RCM,
cross-coupling, C–H activation etc.

Library synthesis

Phage display

Split intein circular ligation

“Split and pool” approach

Challenges

Metabolic stability

Cellular uptake & bioavailability

Roads to achieving lipophilicity