Short Course:

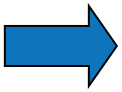
Solution Structure Determination In Organic Chemistry and Chemical Biology

Samuel H. Gellman
University of Wisconsin – Madison, USA

August 2012
University of Jyväskylä, Finland

V. Foldamers

FOLDAMERS -- Oligomers that adopt compact, predictable and well-defined conformations



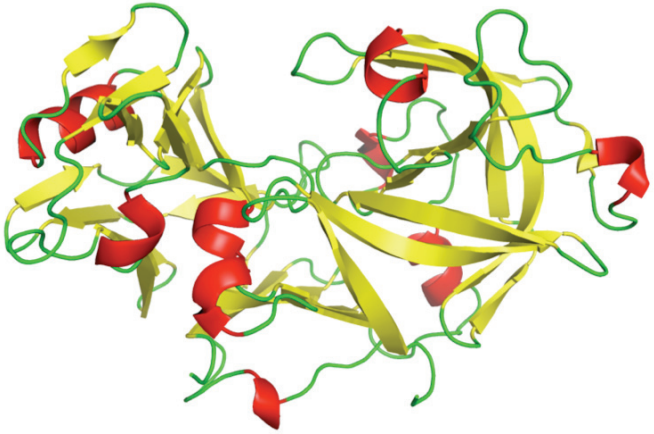
A “new” strategy for controlling molecular shape and function

Proteins: Workhorse Molecules in Biology

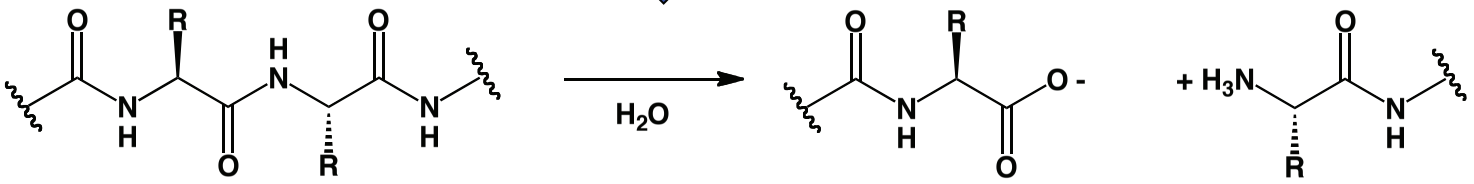
Proteins: Workhorse Molecules in Biology

Sequence (1D) → Shape (3D) → Function

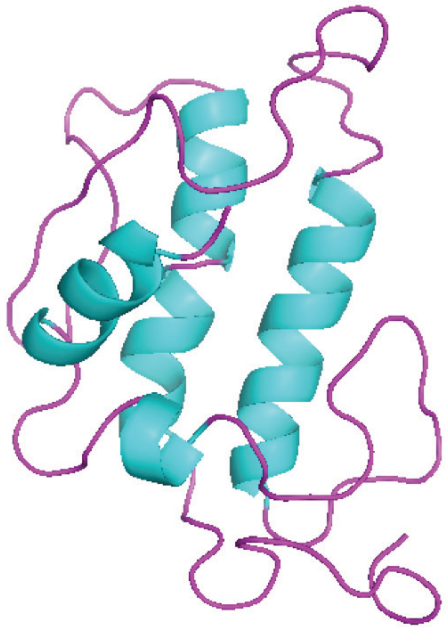
…Tyr – His – Ala – Leu – Phe – Leu – Gly – Lys…



Pepsin (PDB 6APR)



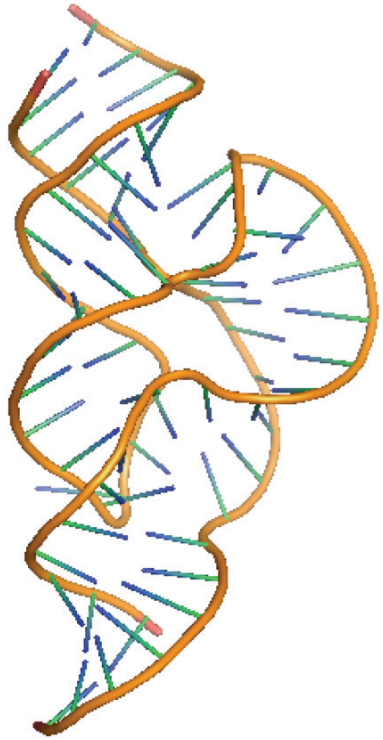
Proteins vs. RNA: Backbone Variation is Possible

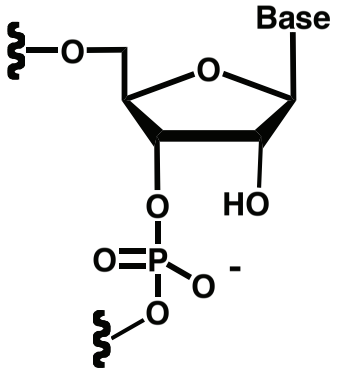




Porcine Pancreatic
Phospholipase A2

van den Berg et al. *Embo J* 14:4123 (1995)

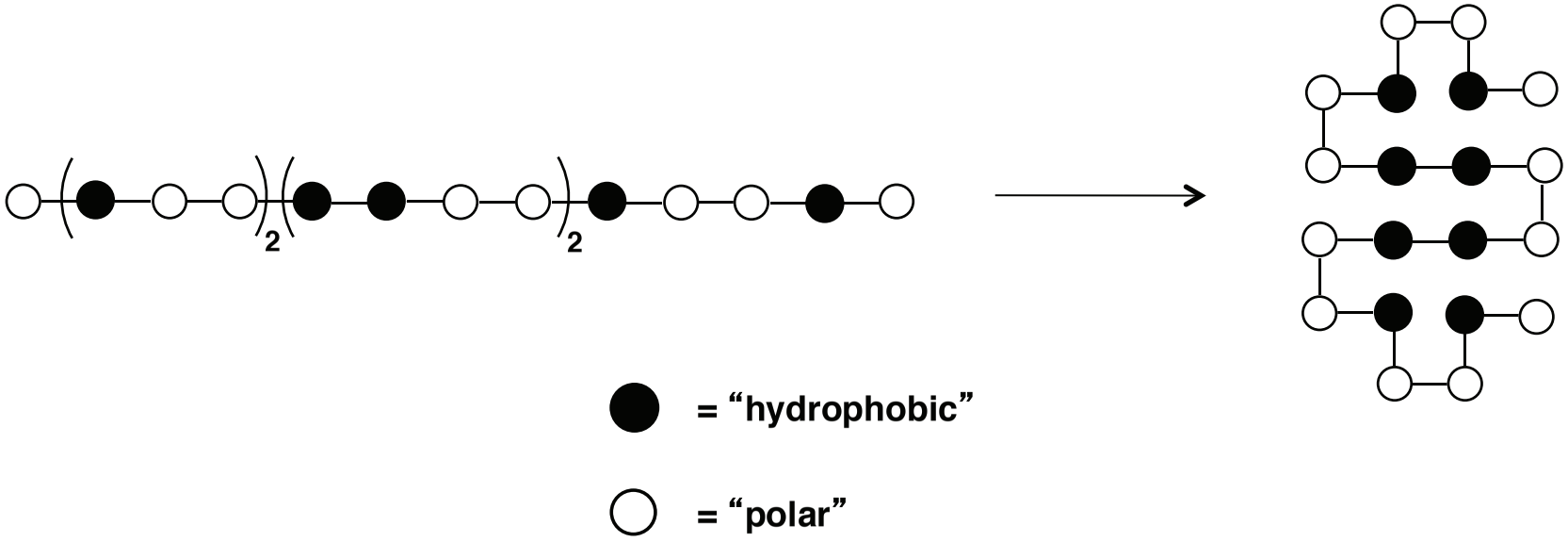




A Hammerhead Ribozyme

Martick, Scott *Cell* 126:309 (2006)

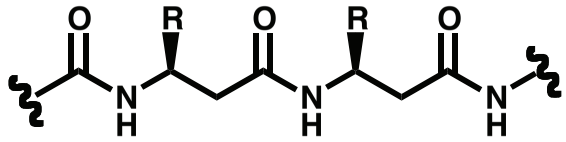
Biopolymer Folding Simulated with Simple Lattice models



Dill et al. *Protein Science* 4:561 (1995)

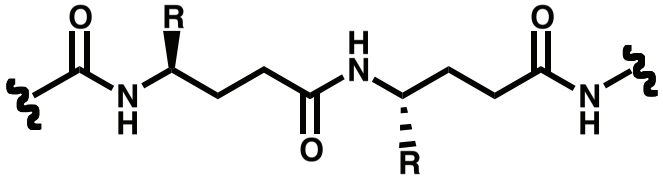
Foldamer Examples: Extended Peptidic Backbones

β-Peptides:



Seebach, Gellman, Gung, Diederichsen, DeGrado, Fleet, Fülöp, Winkler, Berkessel, Schepartz,...

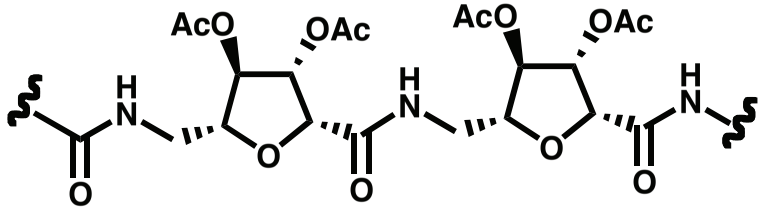
γ-Peptides:



Schreiber ("vinylogous peptides"),

Seebach, Hanessian, Gellman, Smith...

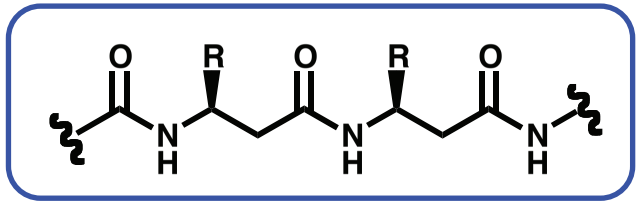
δ-Peptides:



Gervay, Fleet

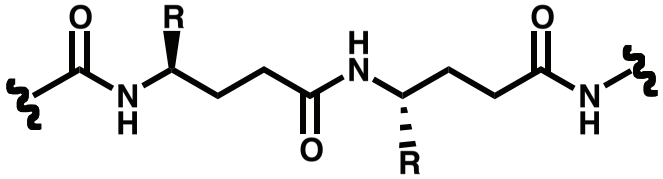
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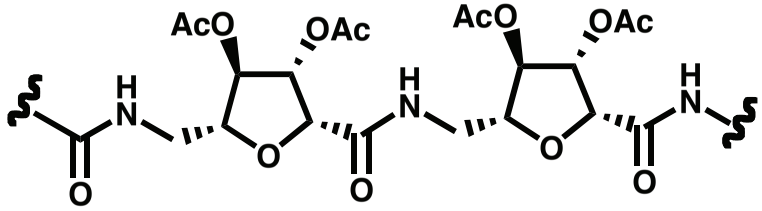
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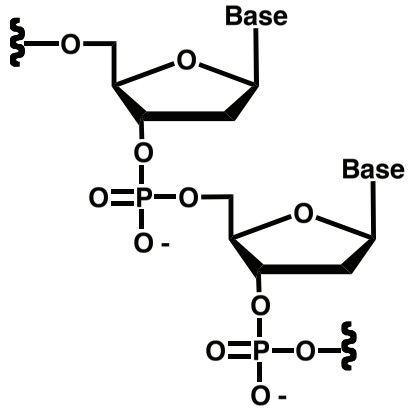
δ-Peptides:



Gervay, Fleet

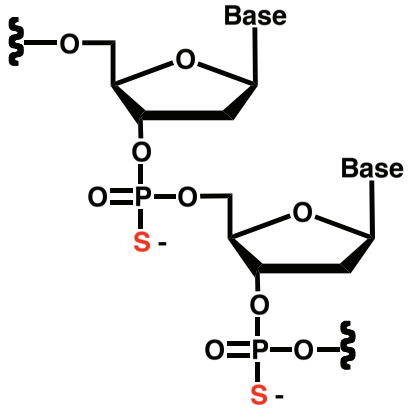
Foldamer Examples: Nucleotide Analogues

DNA:

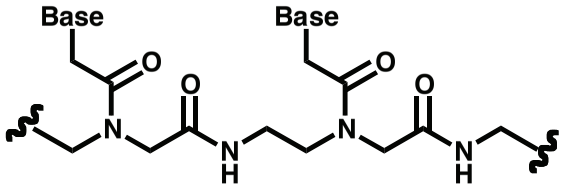


Phosphorothioates:

Milligan, Matteucci & Martin
J. Med. Chem. 36:1923 (1993)



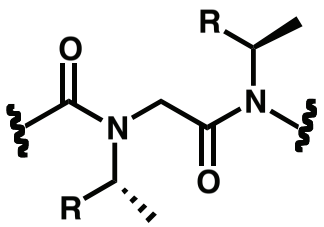
Peptide-Nucleic Acids (PNAs):



Nielsen, Acc. Chem. Res.
32:624 (1999)

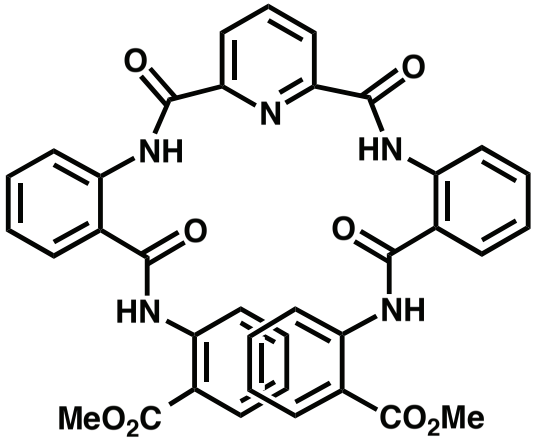
Foldamer Examples

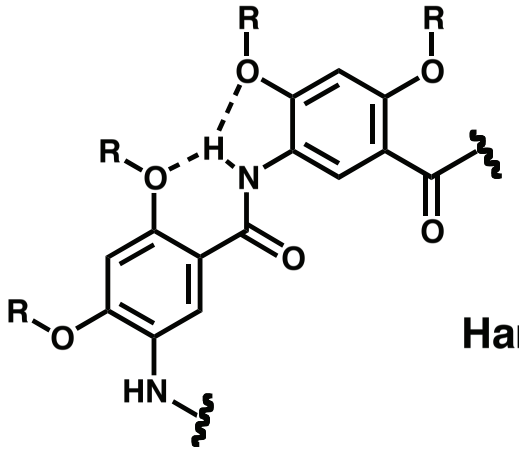
Helical Peptoids:



Dill, Zuckermann, Cohen, Barron, Kirshenbaum, Blackwell, Kodadek, Appella...

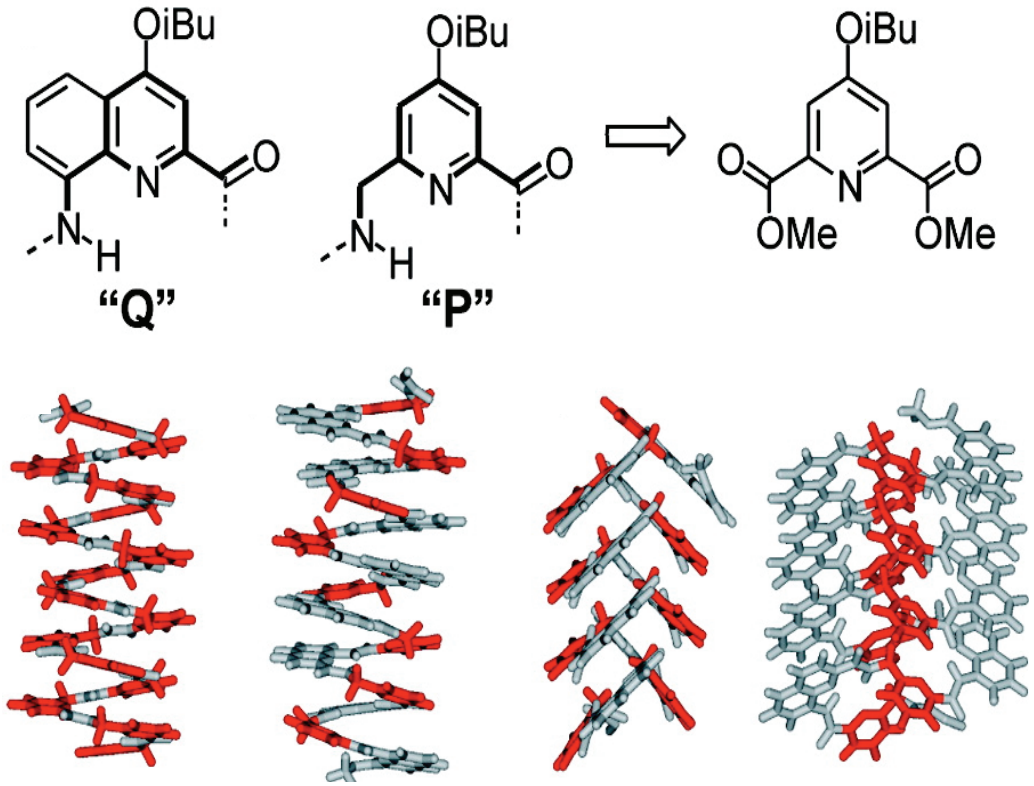
Aromatic Oligoamides





Hamilton, Gong, Huc,...

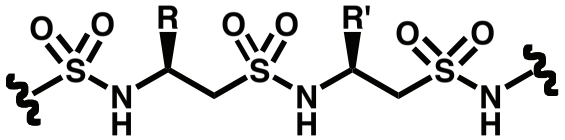
Aromatic Foldamer Design: Huc et al.



Huc et al. *J. Am. Chem. Soc.* **2007,** *129*, 11348.

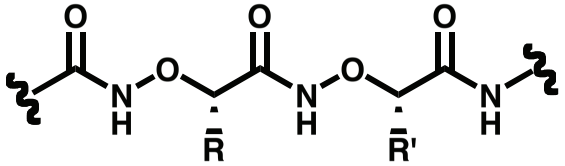
Foldamer Examples: Beyond Carboxamides

Sulfonamide Oligomers:



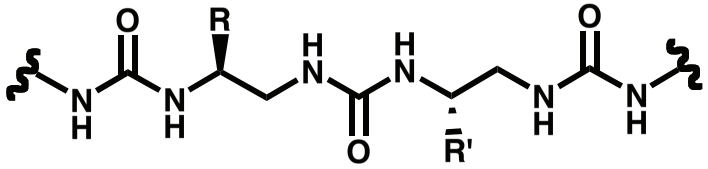
Gennari, Liskamp

α-Aminoxy Acid Oligomers:



Yang

Urea Oligomers:

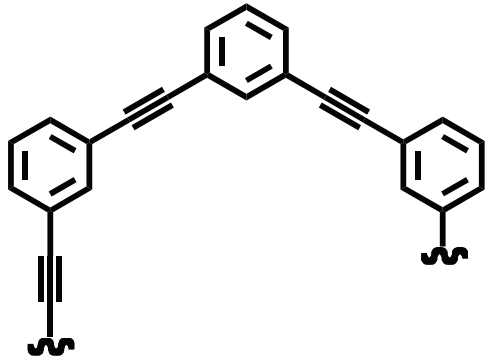


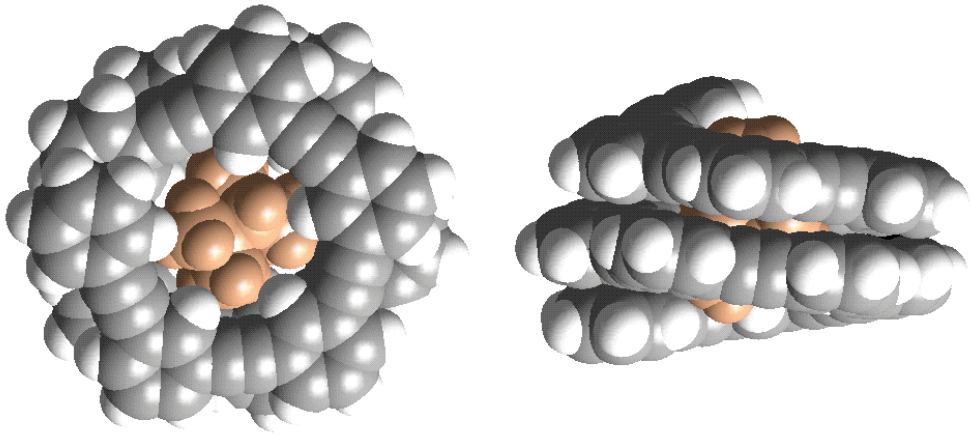
Guichard

Foldamer Examples: Purely Hydrocarbon Backbones

m-Phenylene-ethynylene Oligomers:

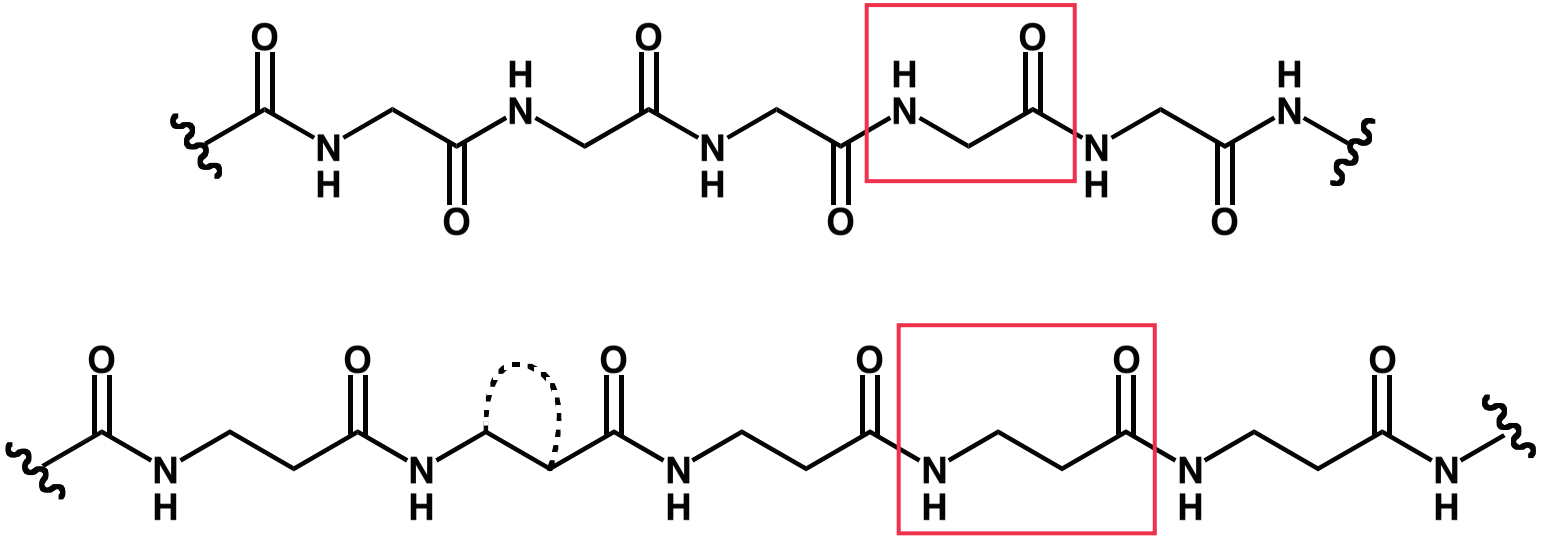
Moore





J. S. Moore et al., *Chem. Rev.* 101:3893 (2001)
"A Field Guide to Foldamers"

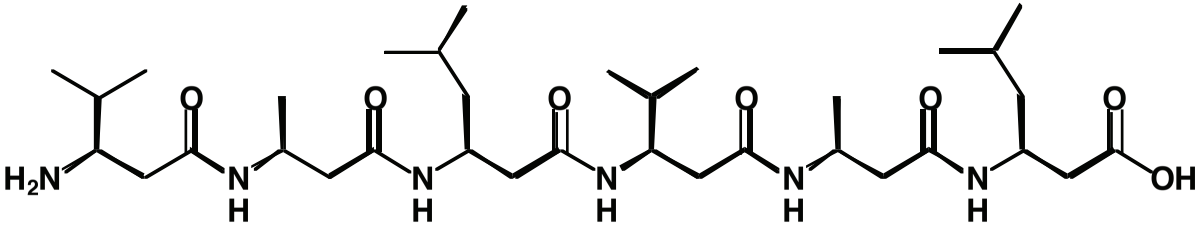
Extrapolation from α-Peptides: β-Peptides

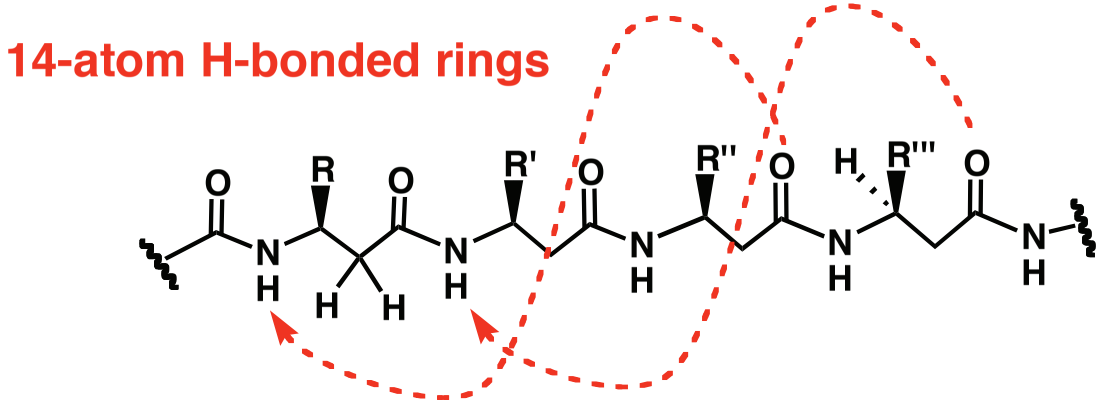


"β-Peptides"

Seebach et al.; Gung et al.; Fleet et al.; DeGrado et al.; Diederichsen et al.; Ichikawa et al.; Winkler, Klein et al.; Martinek, Fülöp et al.; Kessler et al.; Schepartz et al.; Berkessel et al.; Sharma, Kunwar et al., Chandrasekhar, Jagadeesh, Jagannadh et al.; Hofmann et al.; Wu et al.; Van Gunsteren et al.; Jorgensen et al.; Brunsveld et al., Ortuño et al.;…

β-Peptides with Acyclic Residues (β^3)

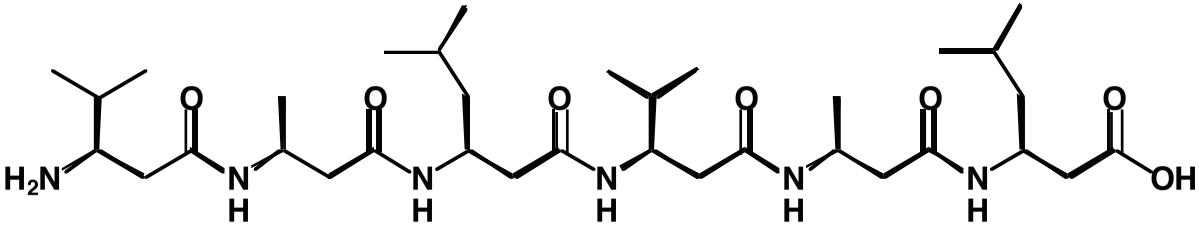


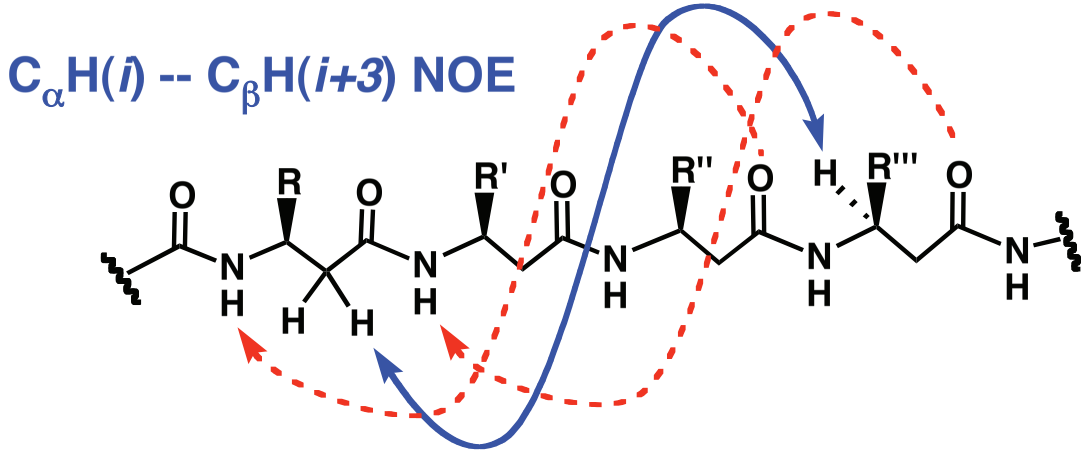


"14-helix" in organic solvents

D. Seebach et al. *Helv. Chim. Acta* **1996**, *79*, 913 and 2043.
D. Seebach and J. L. Matthews *Chem. Commun.* **1997**, 2015.

β-Peptides with Acyclic Residues (β^3)





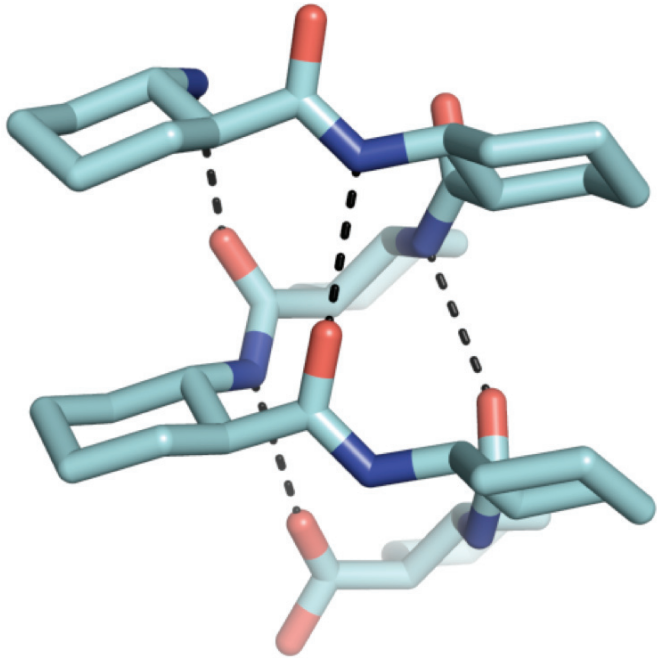
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D. Seebach and J. L. Matthews *Chem. Commun.* **1997**, 2015.

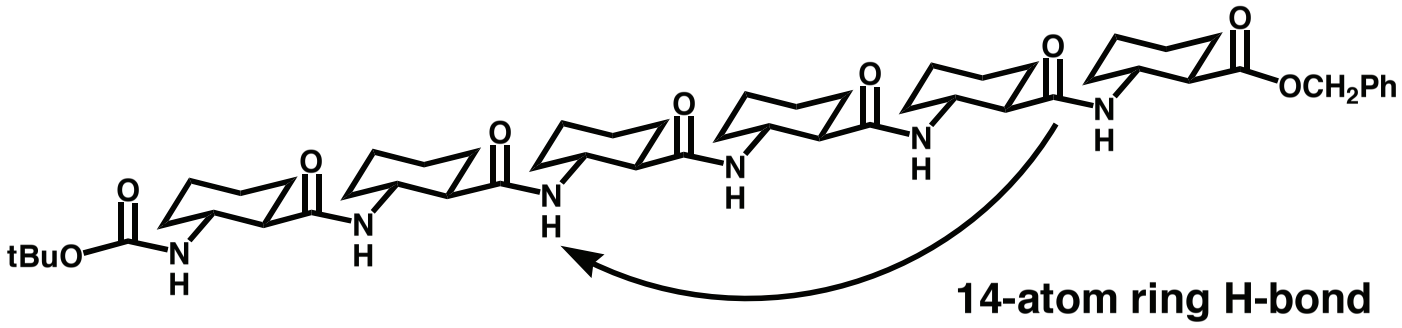
β-Peptide Secondary Structure: Stabilize Helix with Cyclic β-Amino Acid Residues?

14-Helix

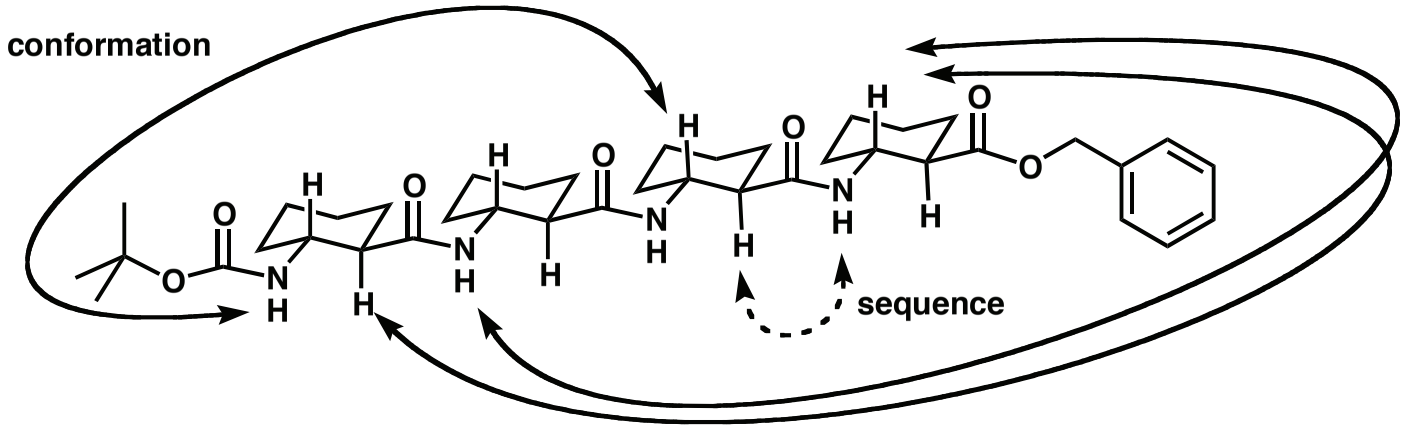
(Crystal structure)



Appella, Christianson et al.
JACS **1996**, *118*, 13071.



NOE Patterns for the β-Peptide 14-Helix

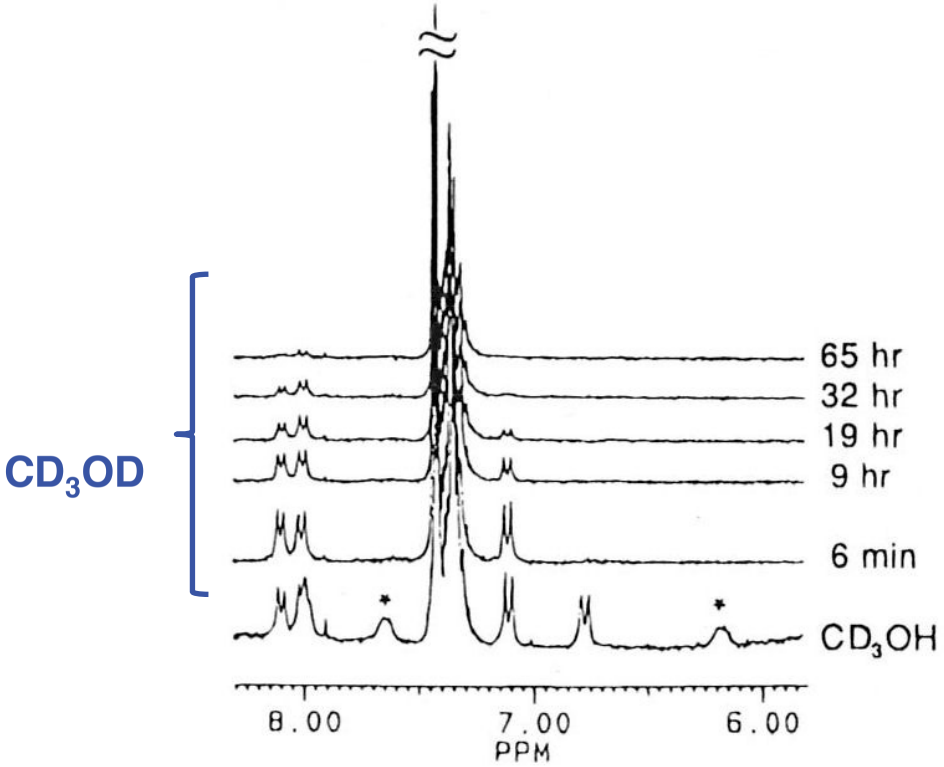


NOESY data (CD_3OH, 5°C, t_{mix} = 400 ms)

(Joe Barchi and Xiaolin Huang, NIH)

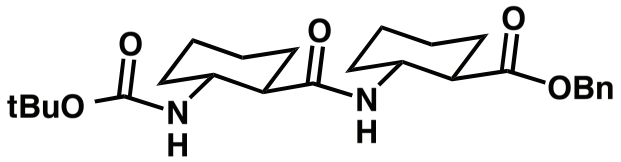
Barchi et al., *J. Am. Chem. Soc.* 122:2711 (2000)

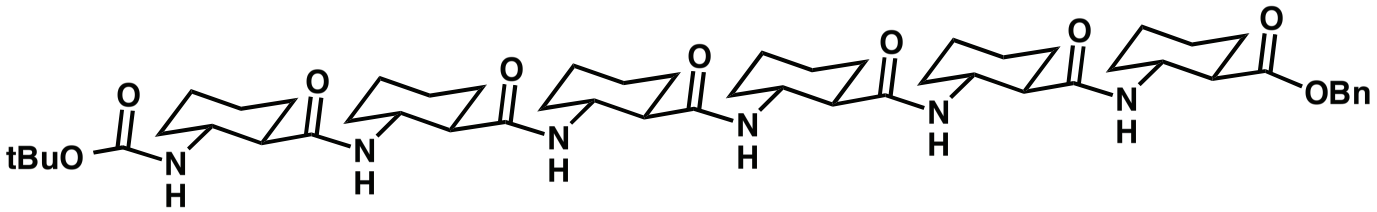
Qualitative Indication of 14-Helix Stability from Slow H/D Exchange (^{1}H NMR)



Appella, Christianson et al.
JACS **1996**, *118*, 13071.

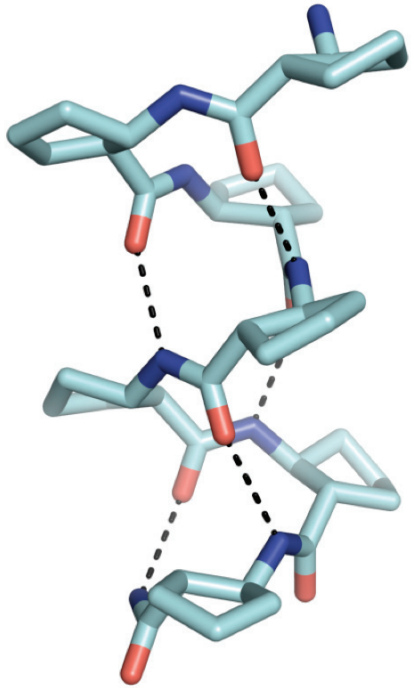
Non-folded control (*):



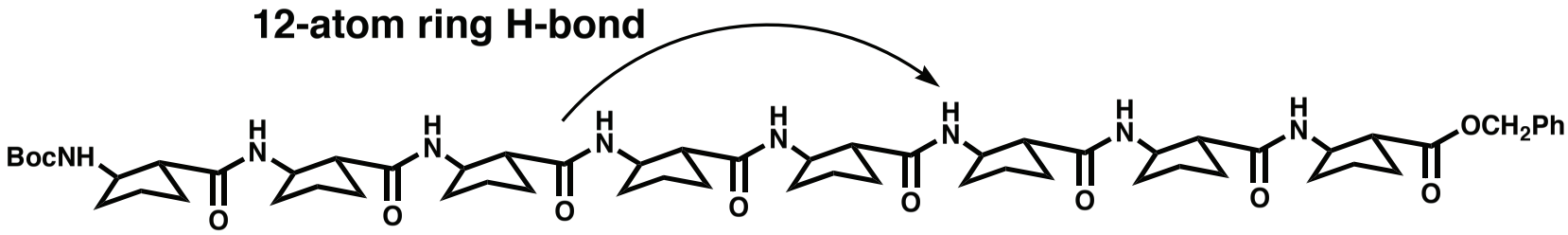


β-Peptide Secondary Structure: Control over Helix Shape via Choice of Cyclic Constraint

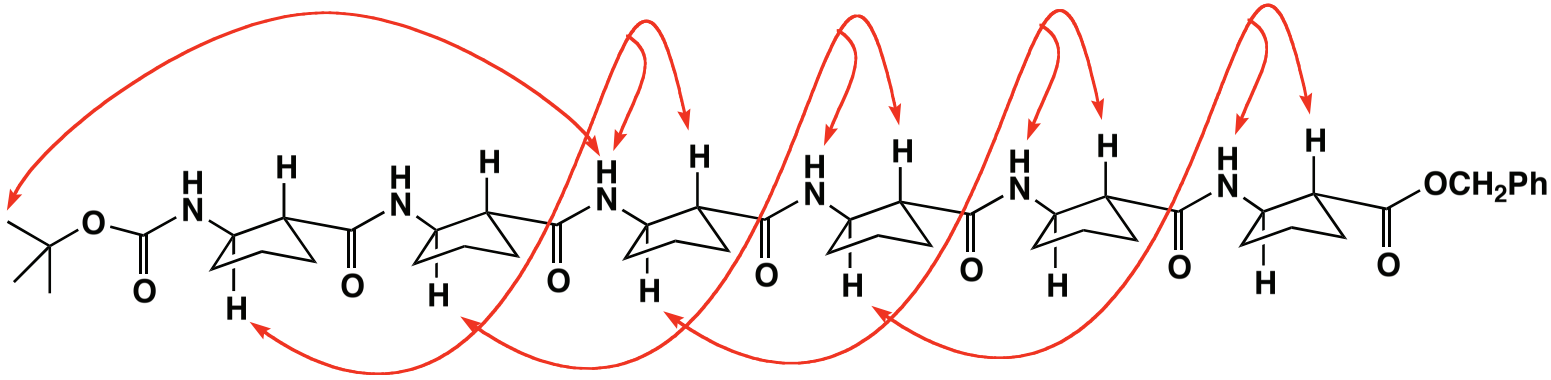
12-Helix



Appella,
Christianson et al.,
Nature **1997**, *387*, 381.



NOE Patterns for the β-Peptide 12-Helix



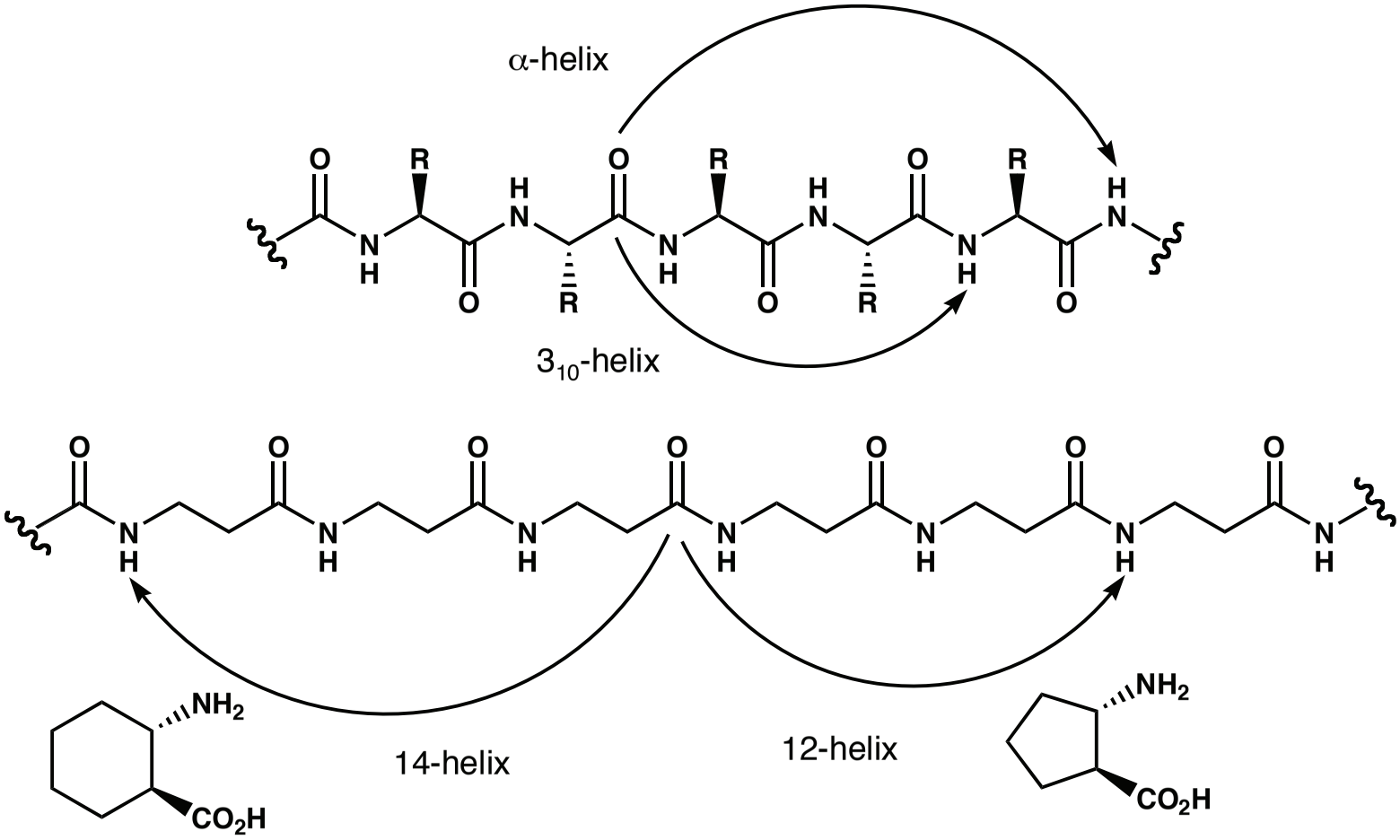
ROESY Data (pyridine-d_5 and CD_3OH)

(Joe Barchi and Xiaolin Huang, NIH)

Appella et al., *Nature* 387:381 (1997)

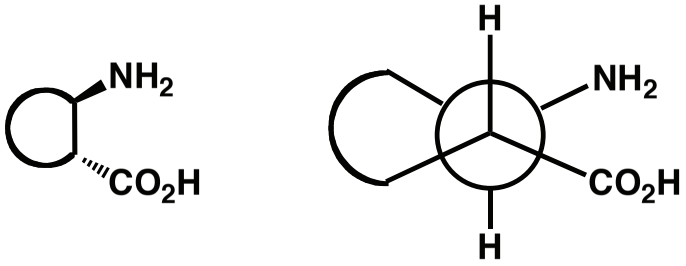
Barchi et al., *J. Am. Chem. Soc.* 122:2711 (2000)

α-Peptide vs. β-Peptide Helices



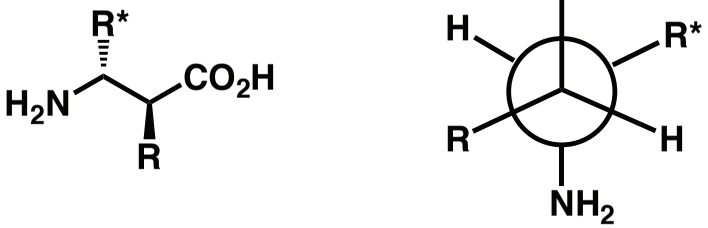
Sheet Secondary Structure in β-Peptides?

For helices…



C_α-C_β ~ gauche

For sheets…



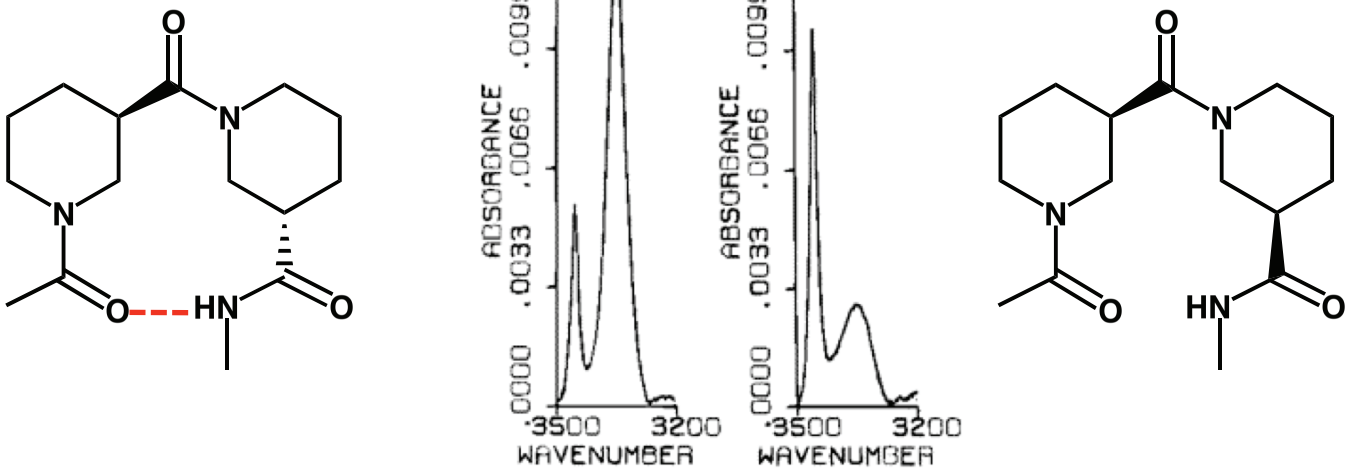
C_α-C_β ~ anti

Krauthäuser et al. *JACS* **1997**, *119*, 11719.
Chung et al. *JACS* **1998**, *120*, 10555.

See also:
Seebach et al. *Angew. Chem. Int. Ed.* **1999**, *38*, 1595.
Martinek et al. *Angew. Chem. Int. Ed.* **2002**, *41*, 1718.

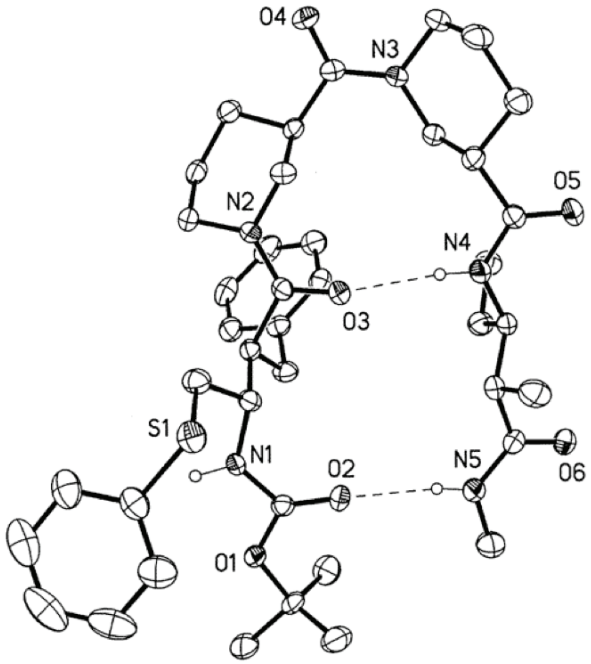
A β-Peptide Reverse Turn

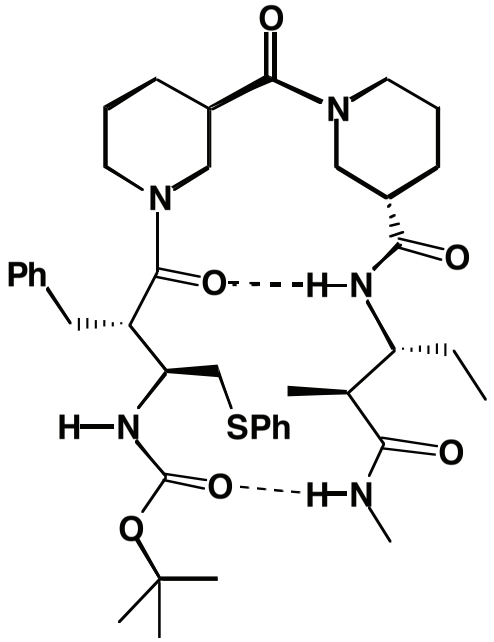
IR Data in CH_2Cl_2 (N-H Stretch Region)



L. Christianson, Y. Chung

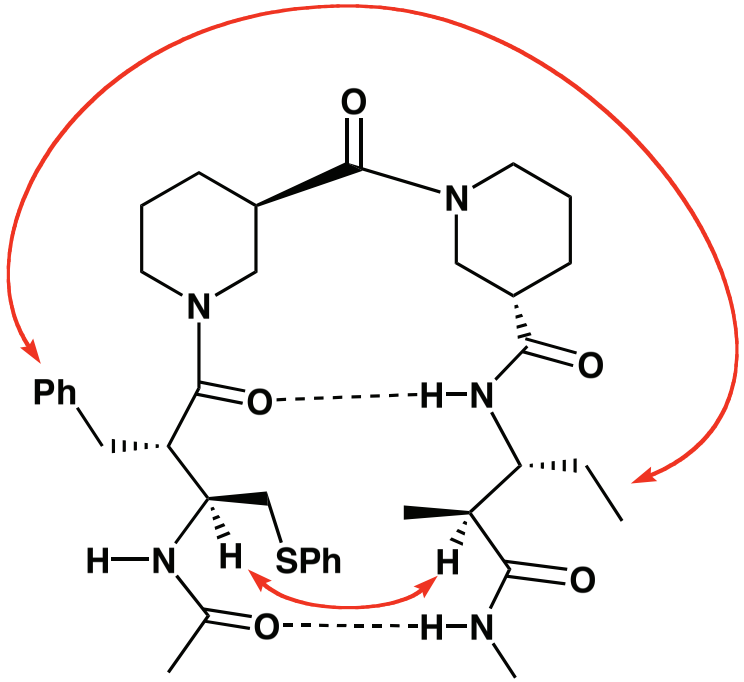
A Minimal β-Peptide Hairpin: One Residue in Each Strand and a Reverse Turn



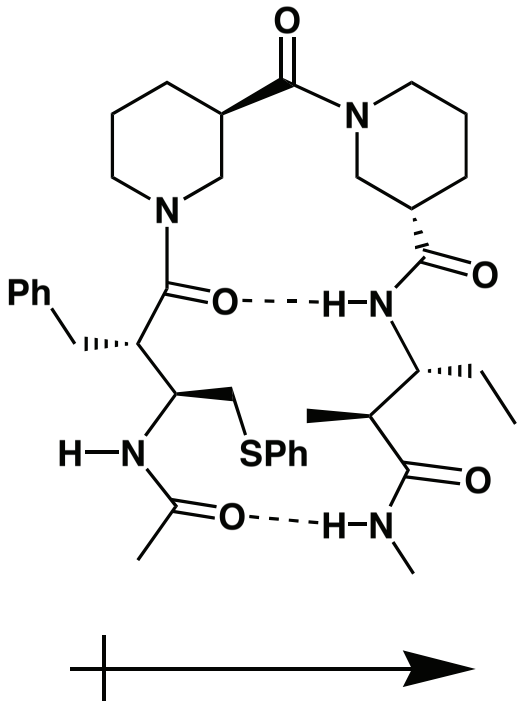


Chung, Christianson, Stanger, Powell, Gellman *J. Am. Chem. Soc.* **1998**, *120*, 10555.

Minimal β-Peptide Hairpin: NOE Data (CD_2Cl_2)

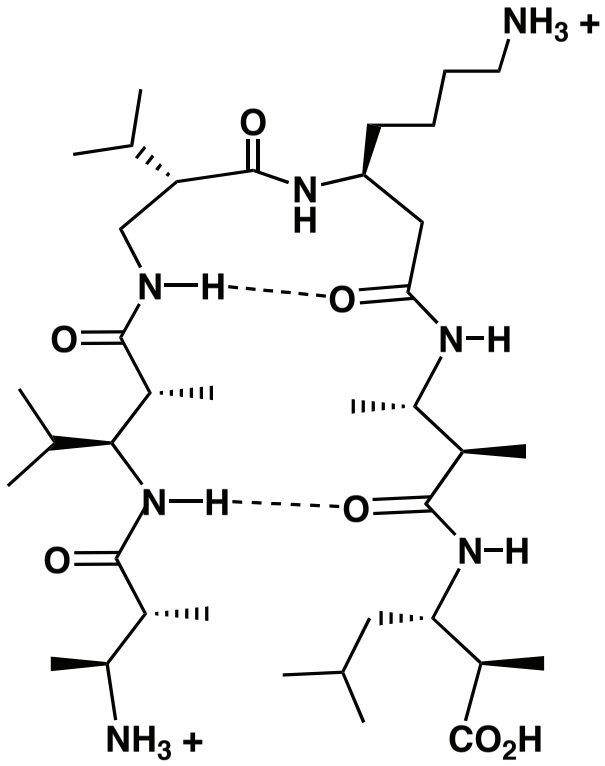


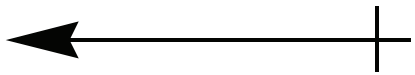
Alternative β-Peptide Reverse Turns



Strand residues: Krauthäuser et al., *J. Am. Chem. Soc.* 1997, *119*, 11719.

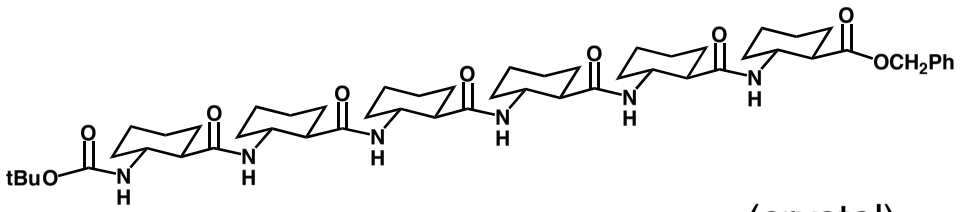
Reverse turn: Chung et al., *J. Am. Chem. Soc.* 1998, *120*, 10555.





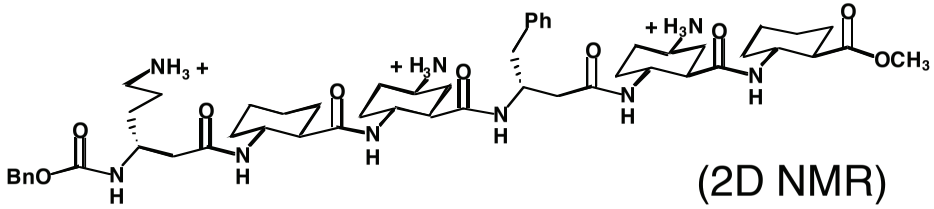
Seebach et al., *Angew. Chem. Int. Ed. Engl.* 1999, *38*, 1595.

14-Helix Formation by a β-Peptide Hexamer in Water (The Ultimate Challenge in Peptide Folding…)



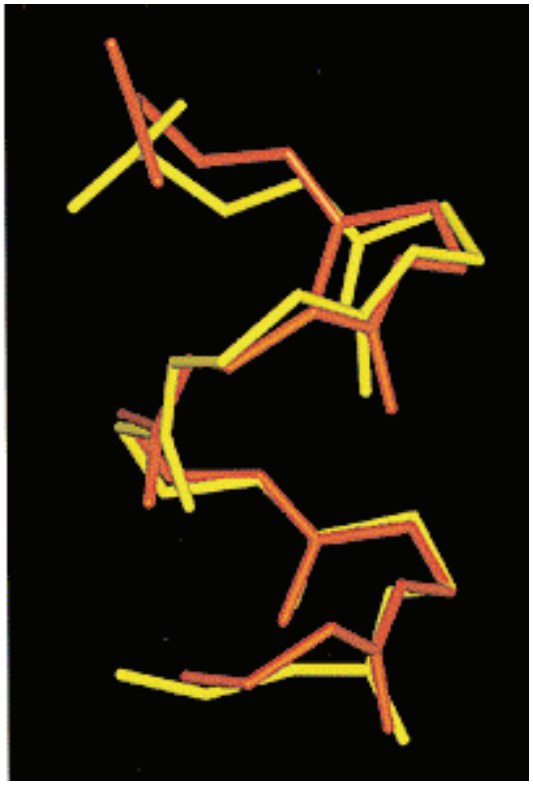
(crystal)

vs.



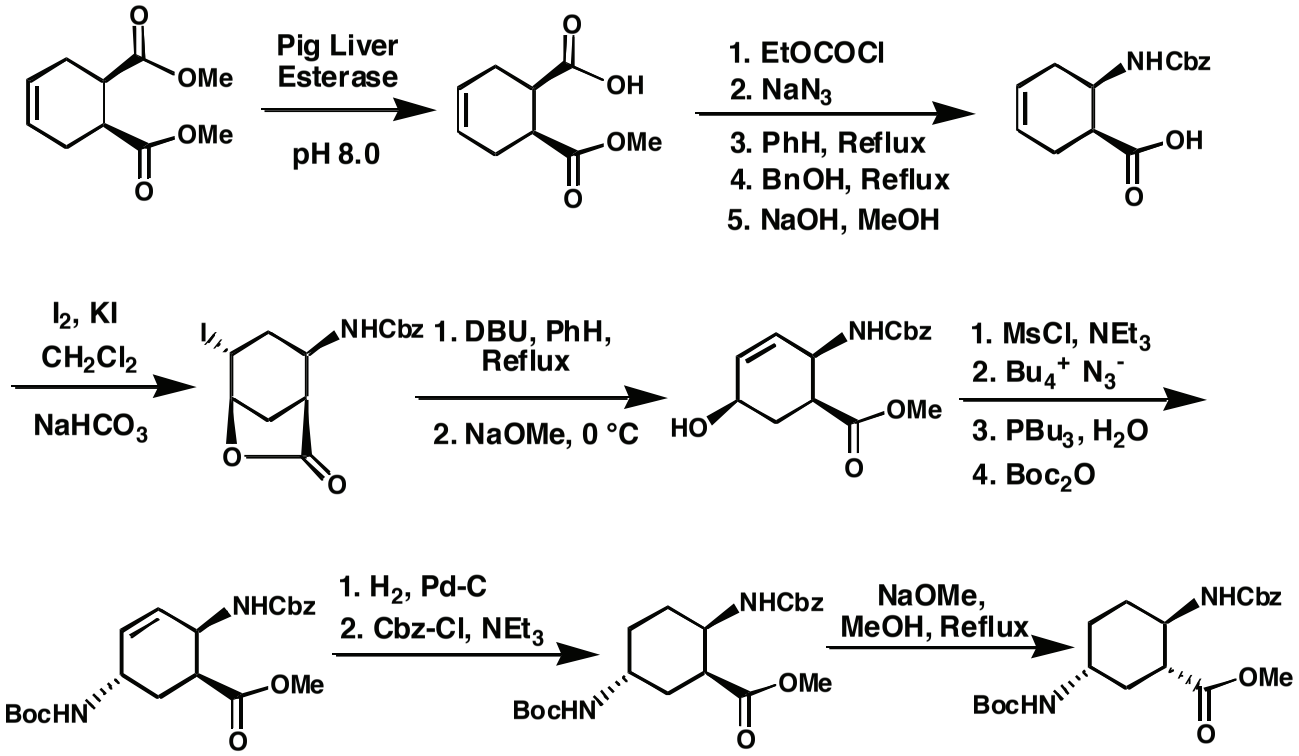
(2D NMR)

Backbone overlay: rmsd = 0.27 Å



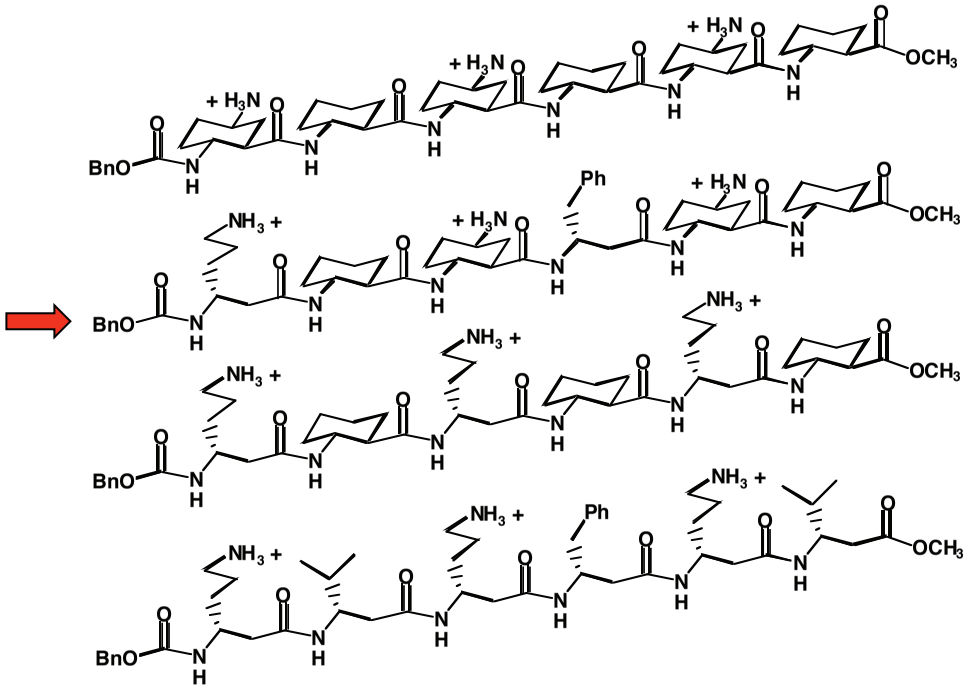
Appella, Barchi, Durell, Gellman *J. Am. Chem. Soc.* **1999**, *121*, 2309.

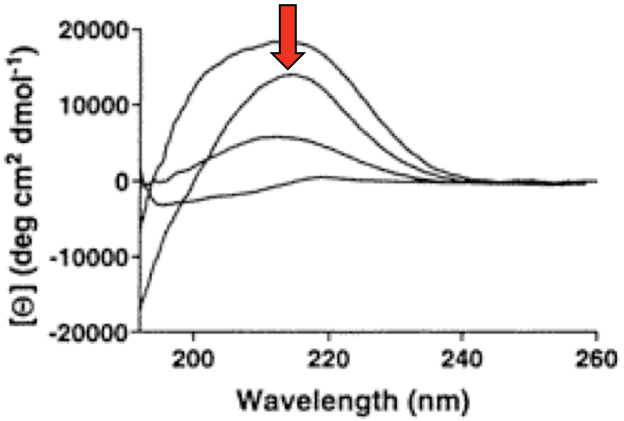
Synthesis Required…



Appella et al. *J. Org. Chem.* **2000**, *65*, 4766

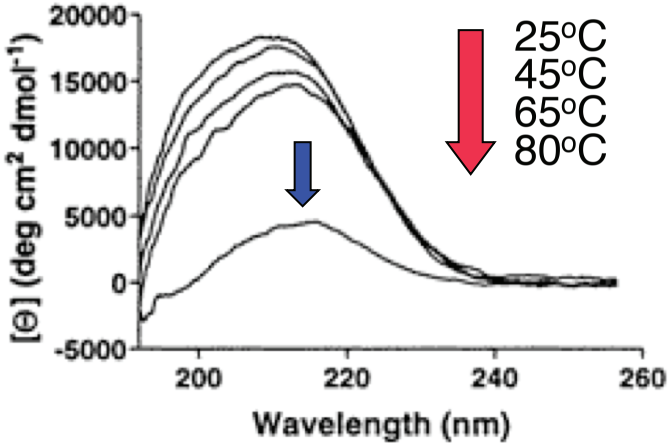
Cyclic vs. Acyclic Residues: Dramatic Conformational Effects in Water (Circular Dichroism)

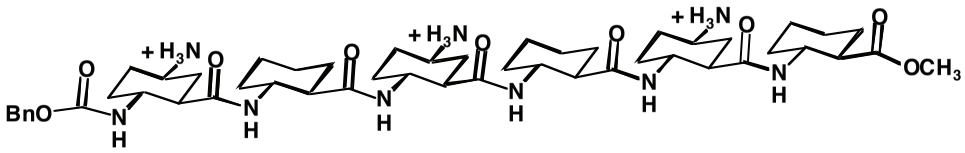


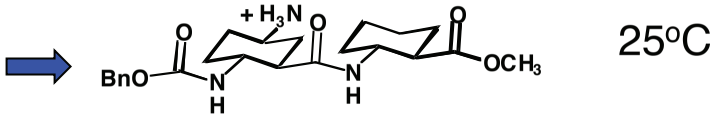


Appella, Barchi, Durell, Gellman *J. Am. Chem. Soc.* **1999**, *121*, 2309.

A Preorganized 14-Helix Shows High Thermal Stability

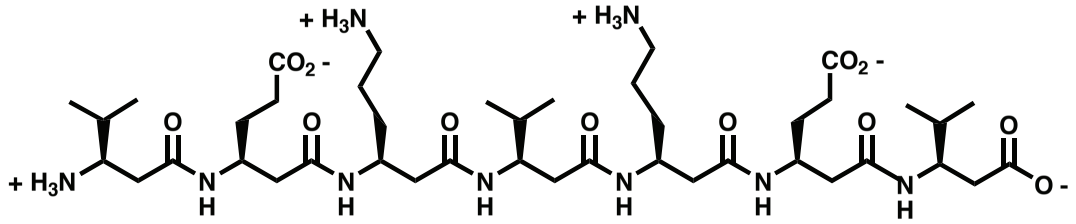


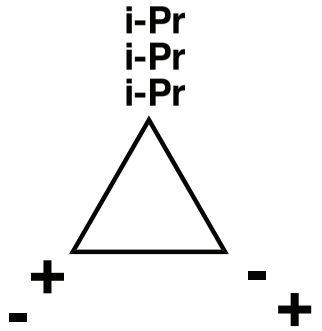




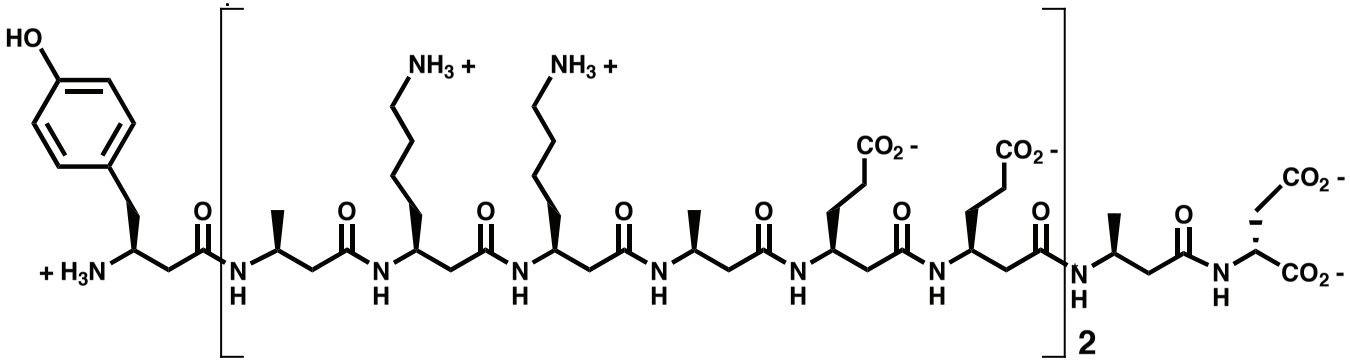
25°C

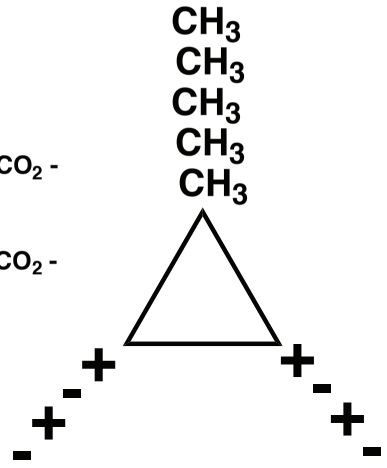
Ion Pairing Allows 14-Helix Formation in Water Without Cyclic Residues





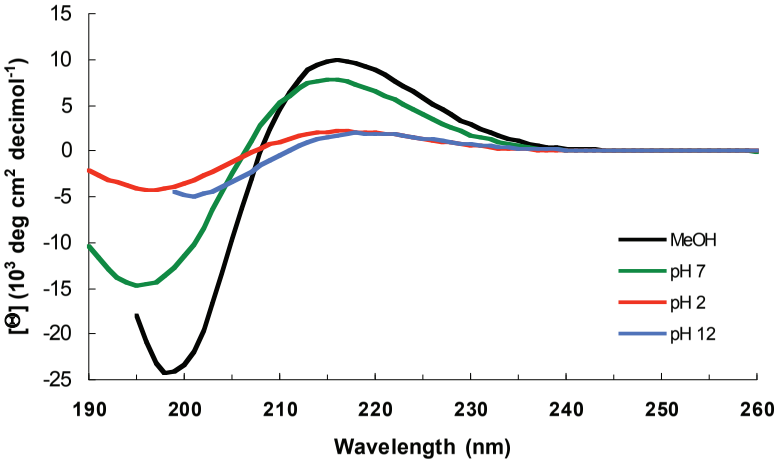
Seebach et al. *J. Chem. Soc., Chem. Commun.*, **2001**, 649.

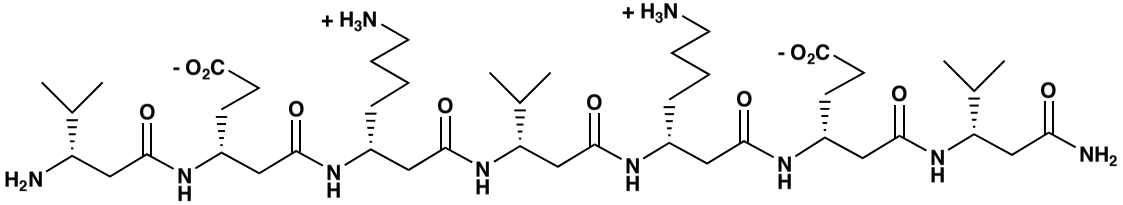




Cheng & DeGrado *J. Am. Chem. Soc.*, **2001**, *123*, 5162.

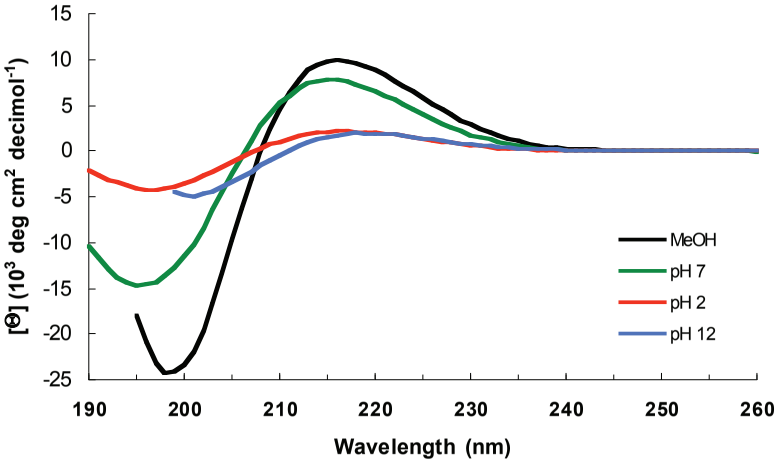
Circular Dichroism: Cyclic Residues Diminish Environmental Effects on Folding

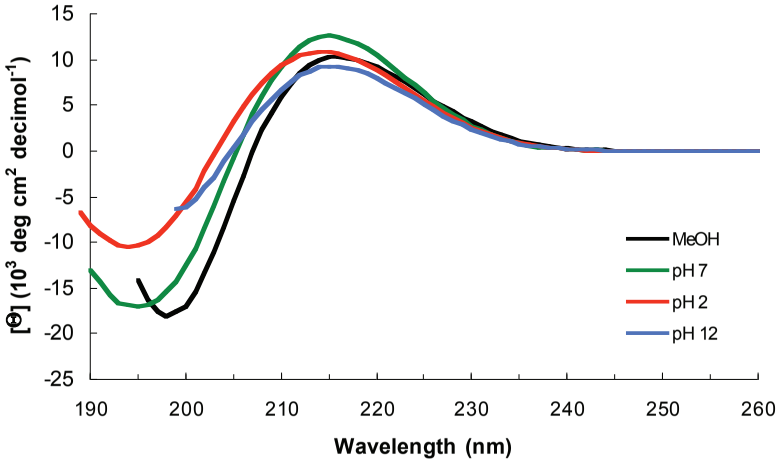


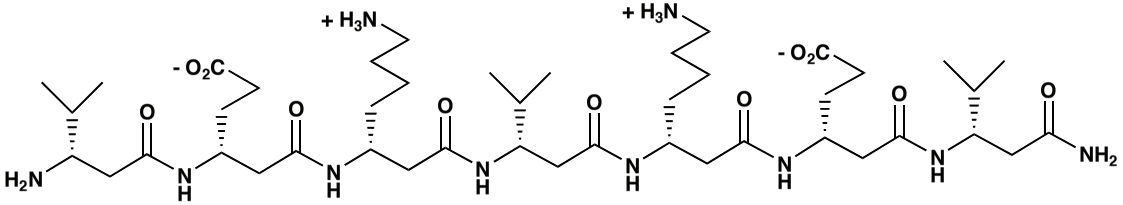


T. R. Raguse / Seebach sequence

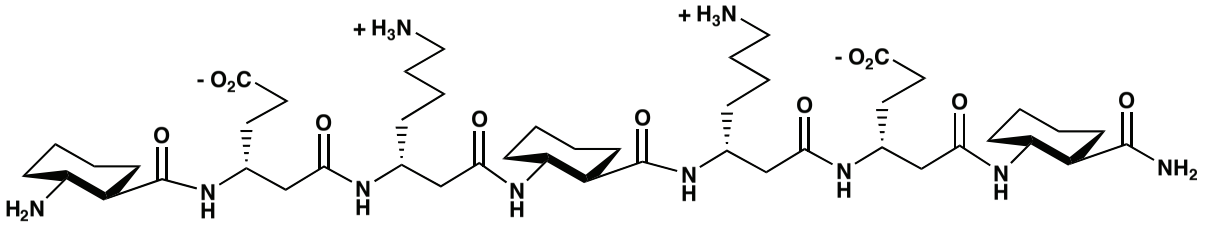
Circular Dichroism: Cyclic Residues Diminish Environmental Effects on Folding



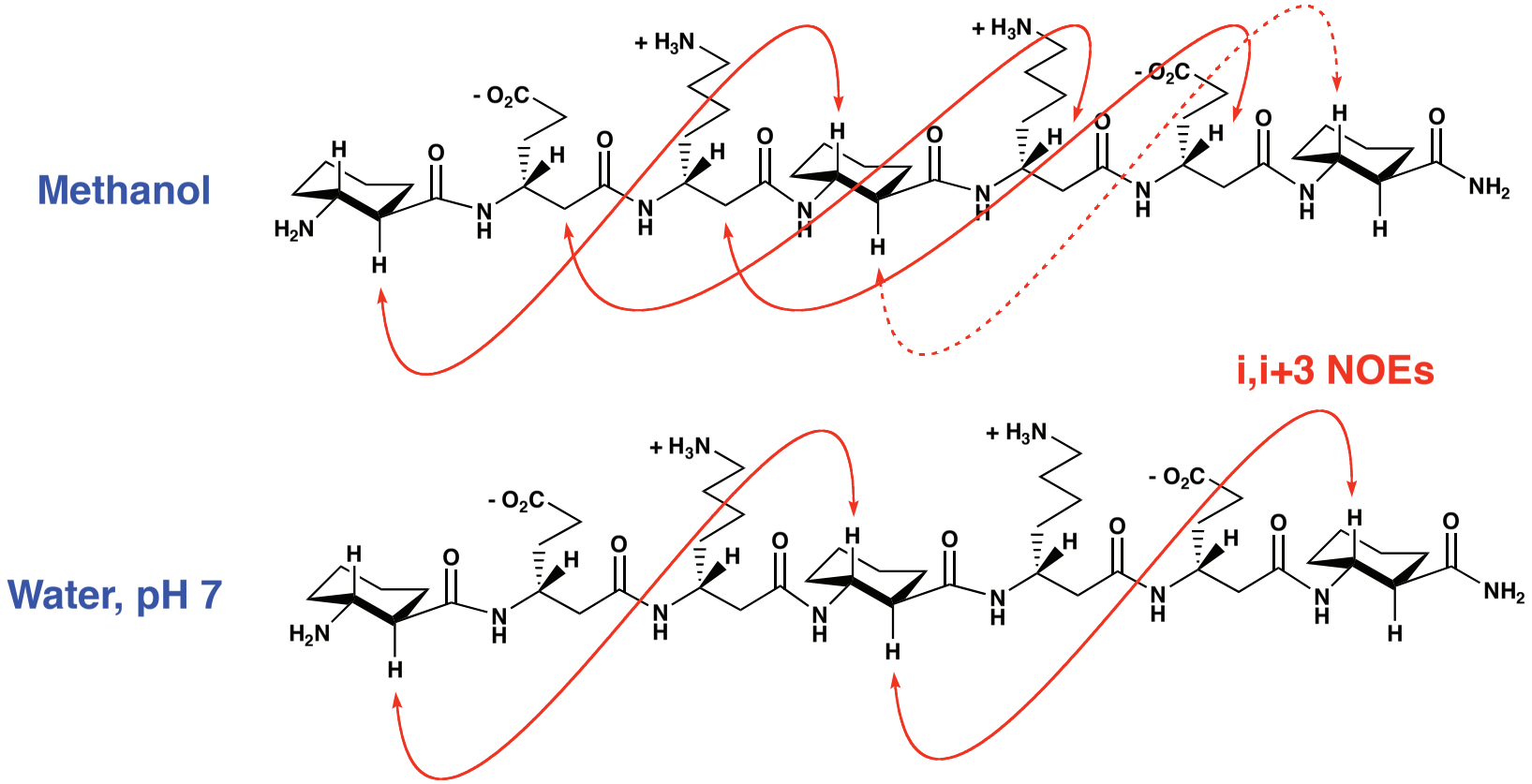




T. R. Raguse

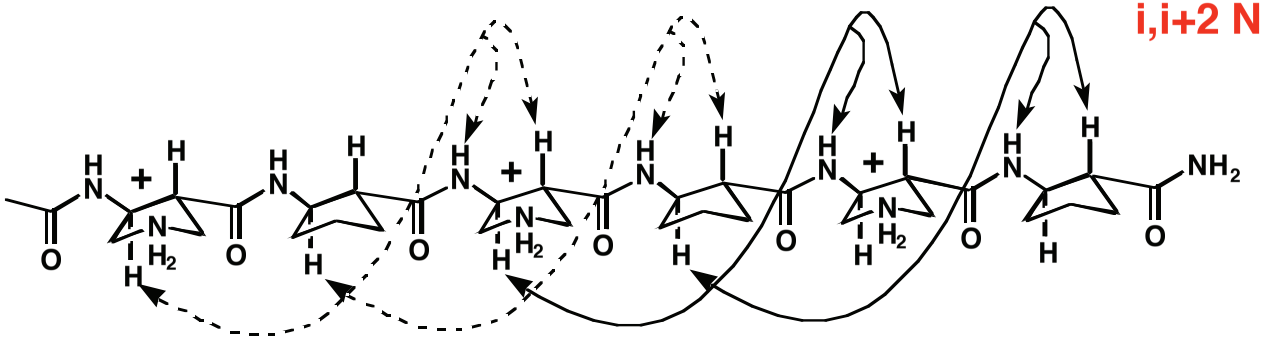


NOEs Demonstrate 14-Helix Formation in Solution

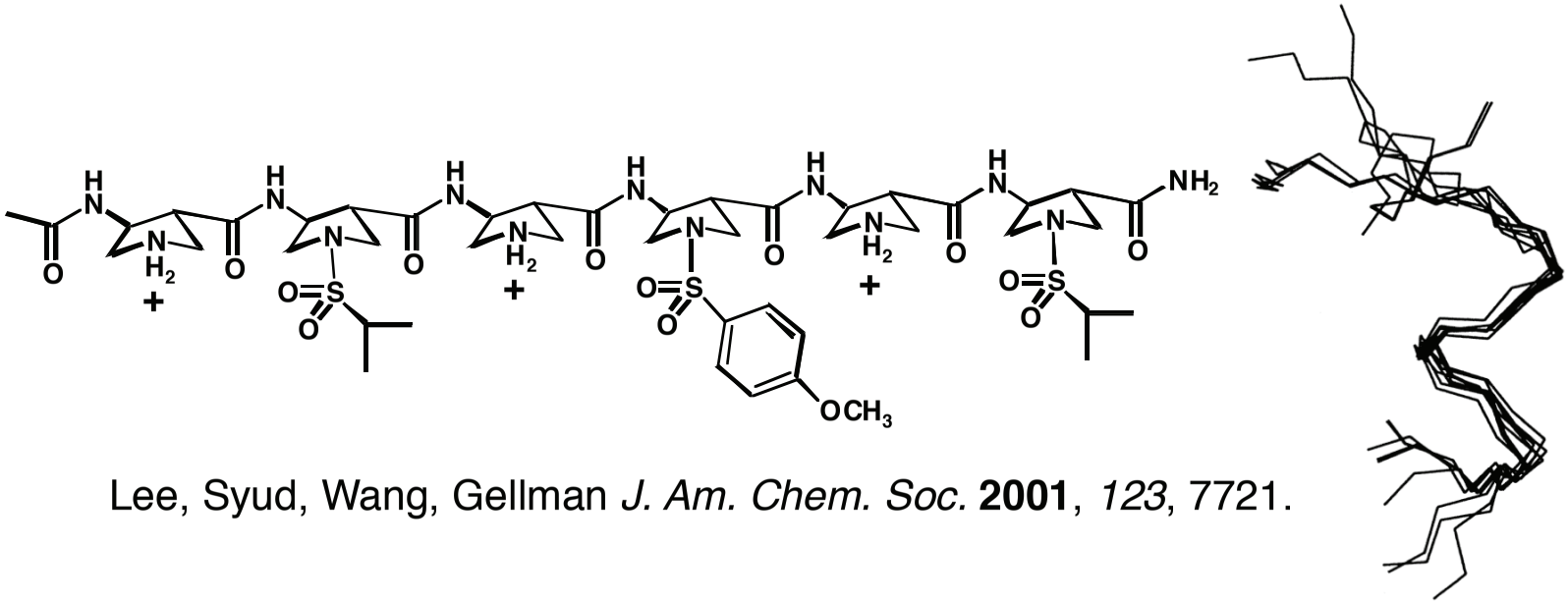


Raguse, Lai, Gellman *J. Am. Chem. Soc.* **2003**, *125*, 5592.

12-Helix Formation in Water by Hexamers (ROESY)

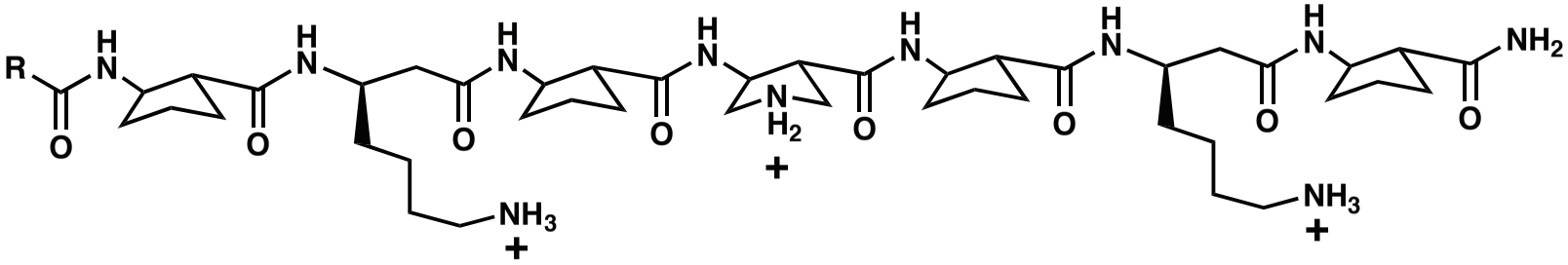


Wang, Espinosa, Gellman *J. Am. Chem. Soc.* **2000**, *122*, 4821.



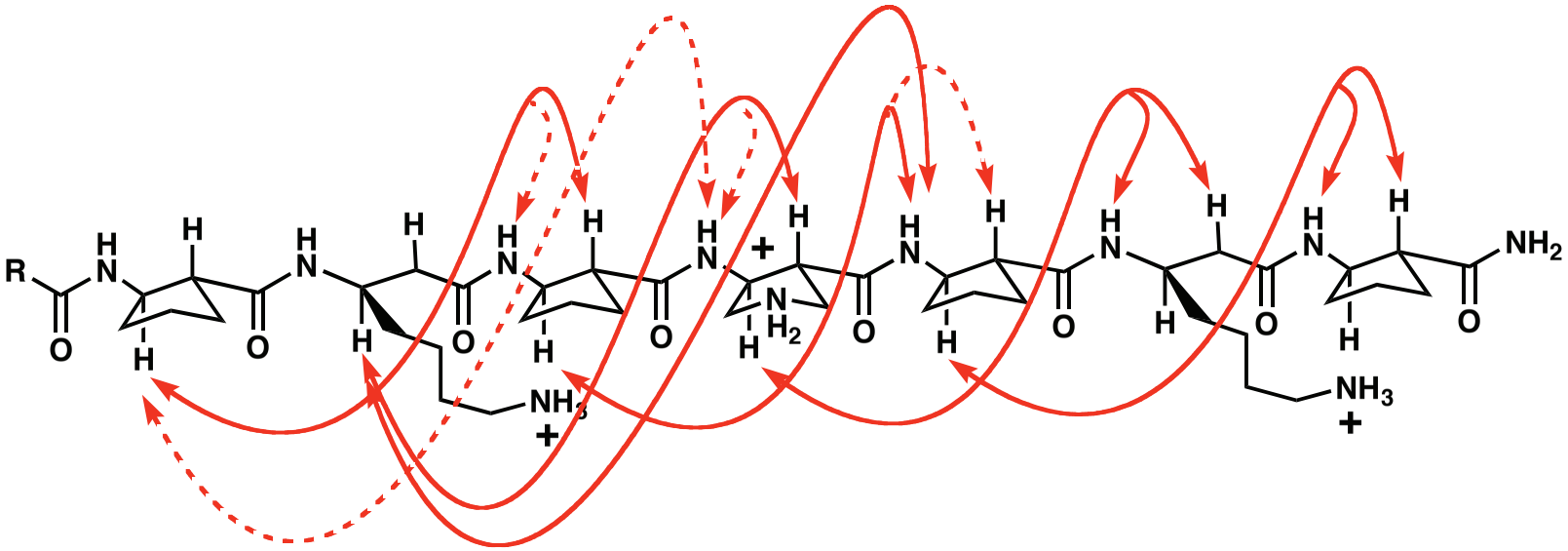
Lee, Syud, Wang, Gellman *J. Am. Chem. Soc.* **2001**, *123*, 7721.

Will the 12-Helix Tolerate Acyclic Residues?



Will the 12-Helix Tolerate Acyclic Residues?

Yes.



Numerous NOEs between sequentially non-adjacent residues (CD_3OH), all characteristic of the 12-helix

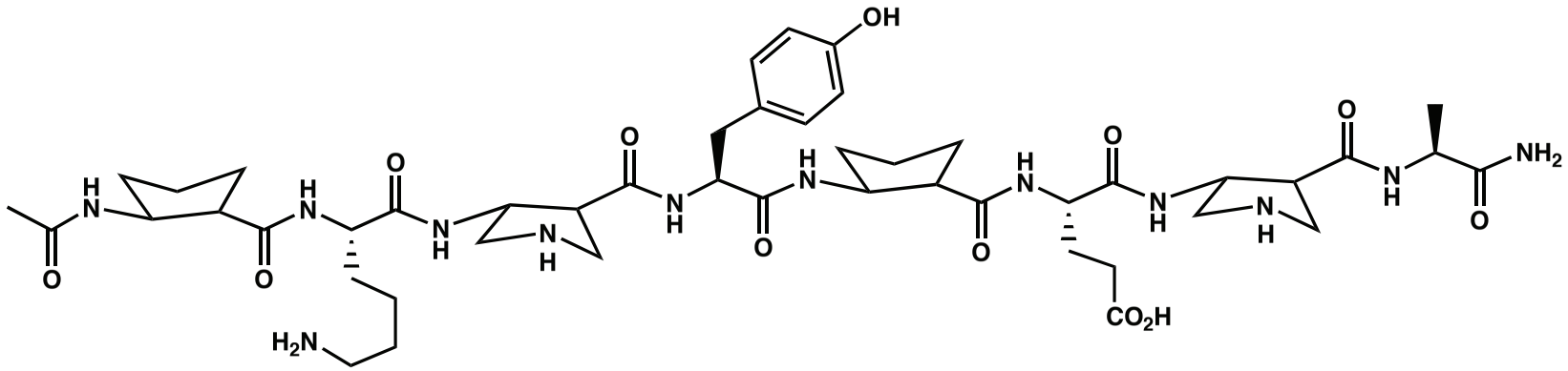
LePlae, Fisk, Porter, Weisblum, Gellman *J. Am. Chem. Soc.* **2002**, *124*, 6820

New Foldamers:
Homogeneous vs. Heterogeneous Backbones

Homogeneous Backbones: All residues in the same class
(e.g., conventional peptides, DNA, RNA, β-peptides, γ-peptides)

Heterogeneous Backbones: Residues of different classes
(e.g., …?)

α/β-Peptide Foldamers?

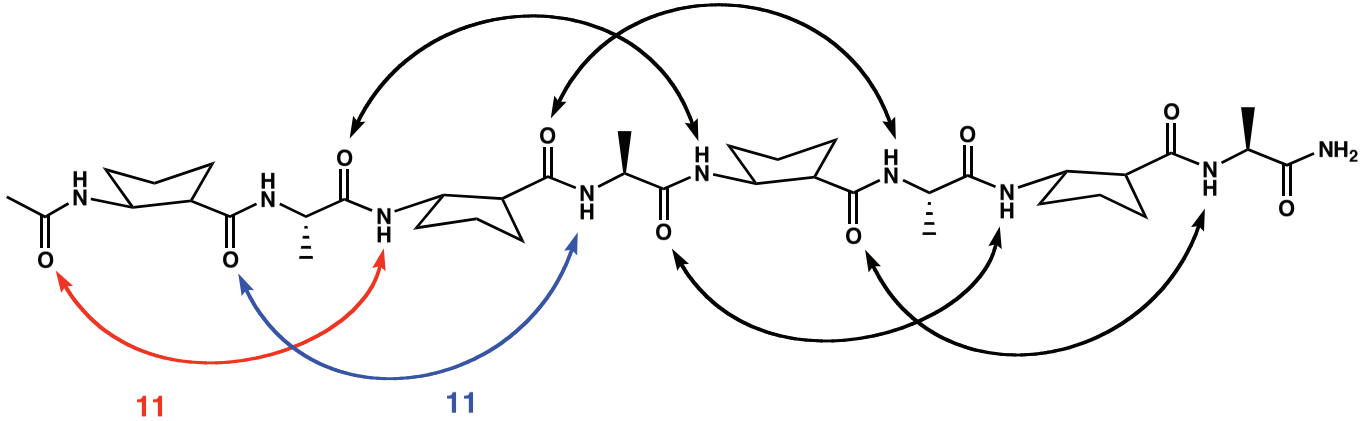


2D NMR Analysis (CD_3OH)

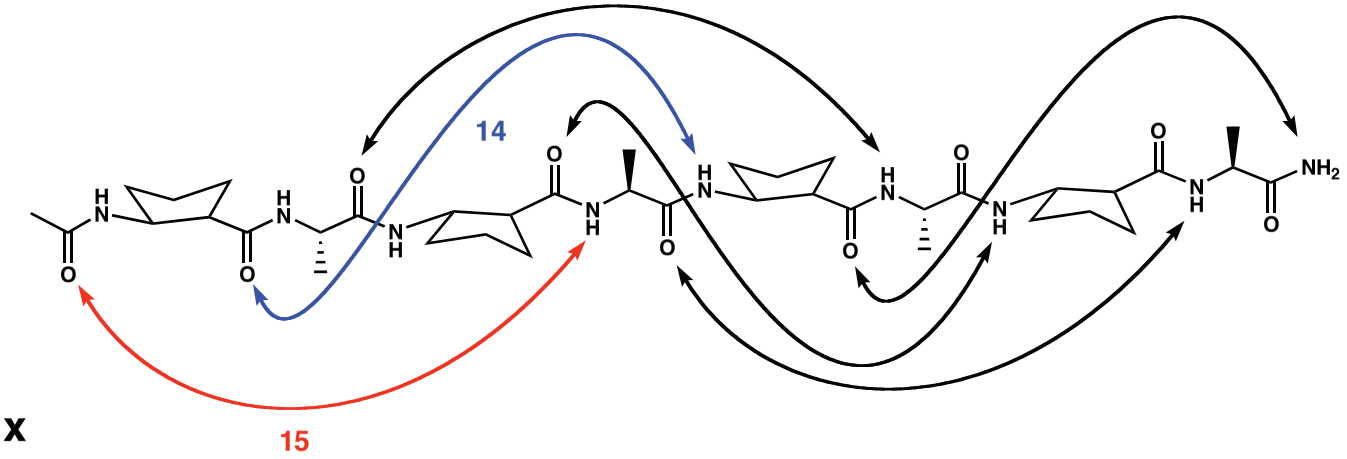
Numerous i --> i+2 and i --> i+3 NOEs

Hayen, Schmitt, Ngassa, Thomasson, Gellman
***Angew. Chem. Int. Ed.* 43:505 (2004)**

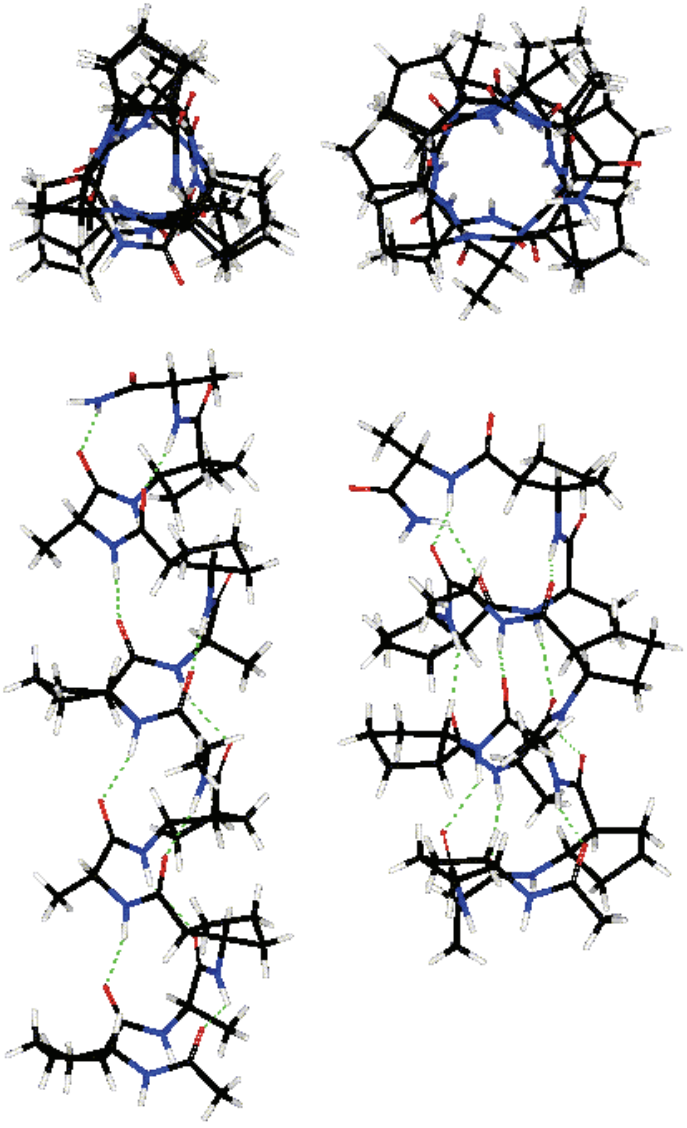
NOE Data Suggest Two Conformations Populated in Solution



11-Helix
[C=O(*i*) -- H-N(*i+3*)]



14/15-Helix
[C=O(*i*) -- H-N(*i+4*)]



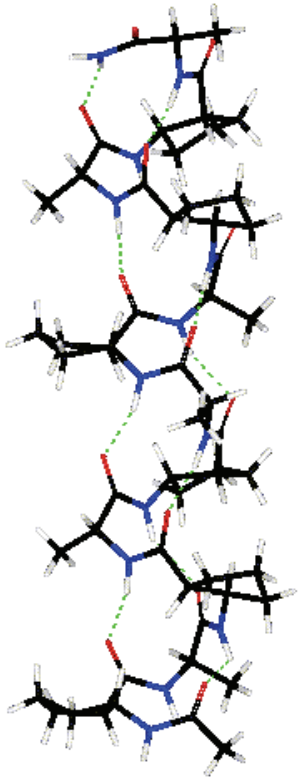
11-Helix 14/15-Helix

α/β-Peptide Helix Modeling

(Molecular Mechanics:
SYBYL / MMFF94s;
continuum MeOH model;
12 residues)

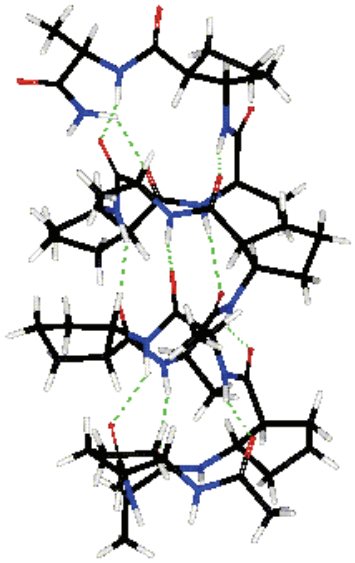
M. Schmitt

Neither α/β-Peptide Helix Accounts for All NOEs



11-Helix

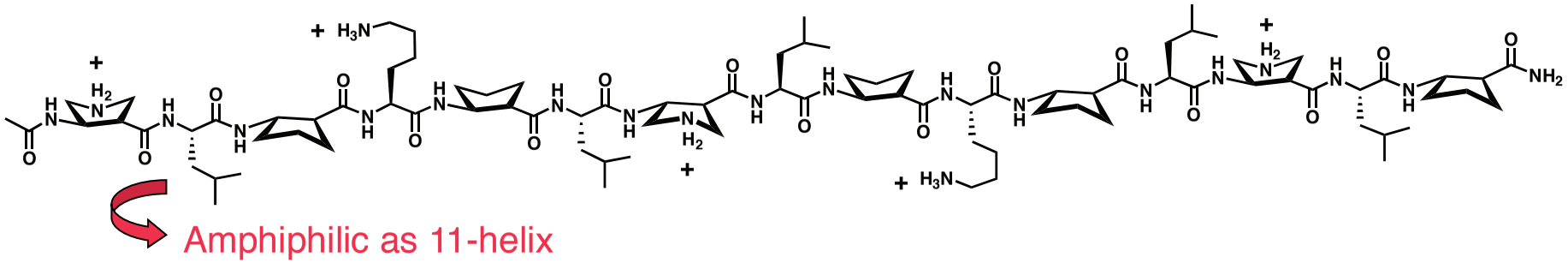
??



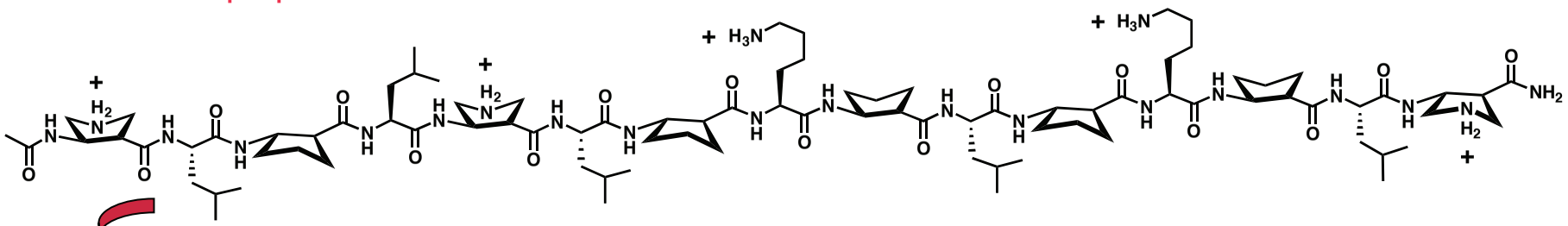
14/15-Helix

M. Schmitt

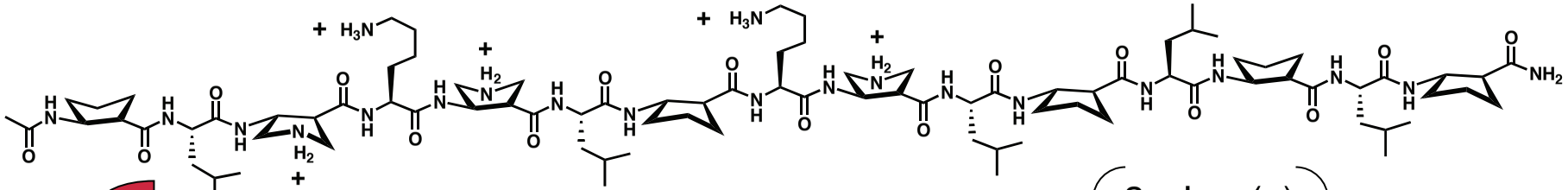
Amphiphilic α/β-Peptide 15-mers: Insight into Helix Preference from Physical Properties?



Amphiphilic as 11-helix



Amphiphilic as 14/15-helix



Not amphiphilic as either helix

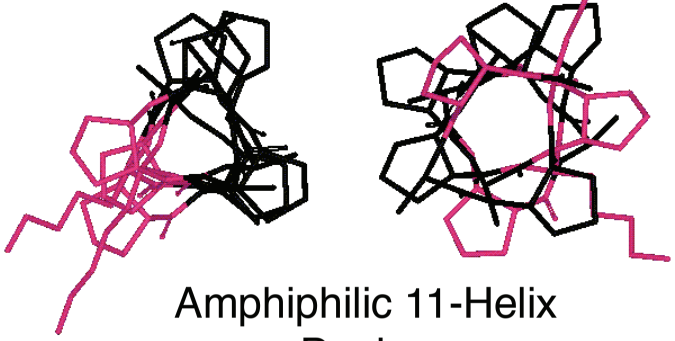
- 2 x Lys (+)
- 5 x Leu
- 3 x APC (+)
- 5 x ACPC

M. Schmitt

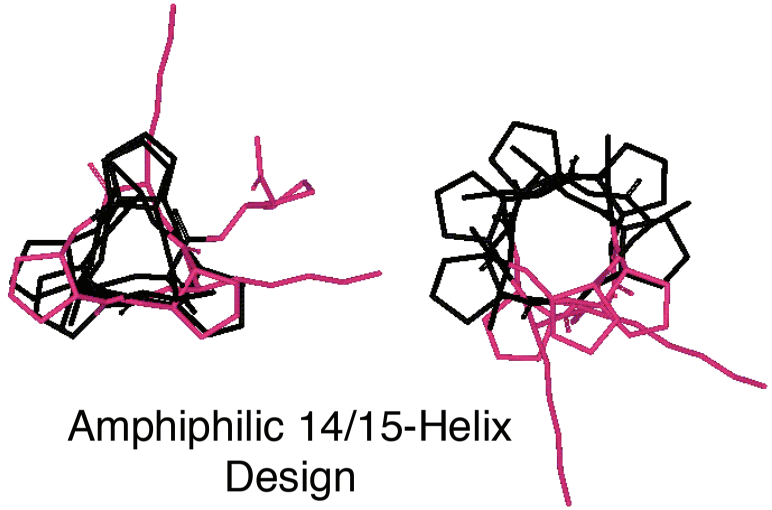
Amphiphilic α/β-Peptide 15-mer isomers: Axial Projections

11-helix

14/15-helix

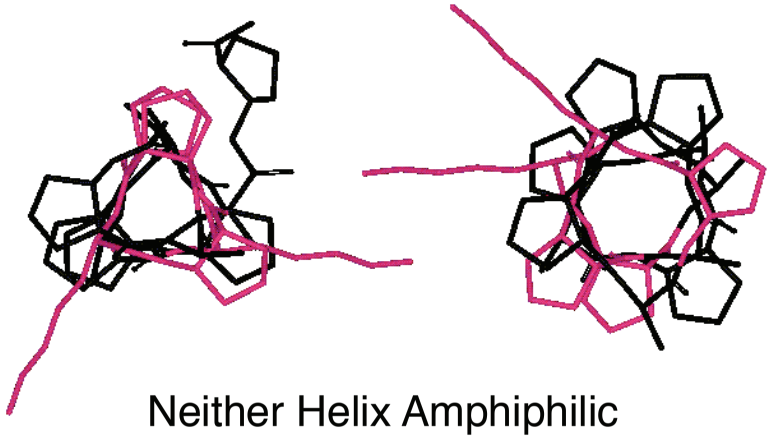


Amphiphilic 11-Helix Design



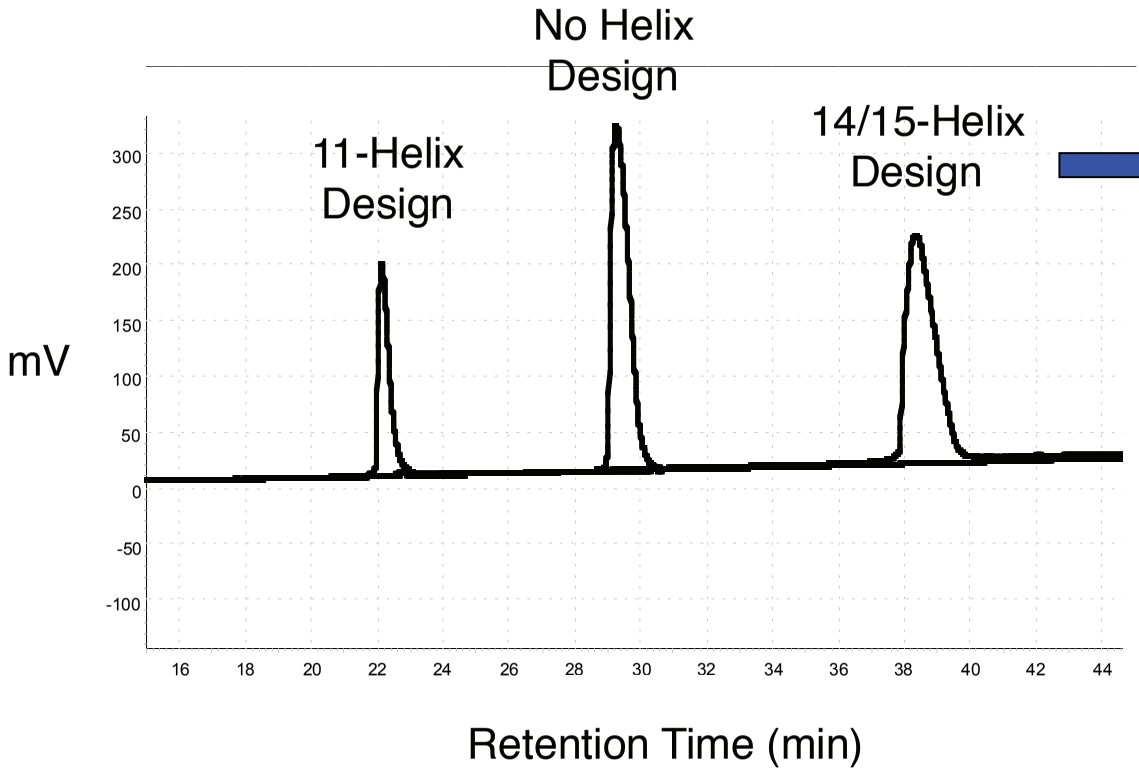
Amphiphilic 14/15-Helix Design

Purple = cationic
Black = hydrophobic



Neither Helix Amphiphilic Design

Amphiphilic α/β-Peptide 15-mer isomers: Reverse-Phase HPLC Retention

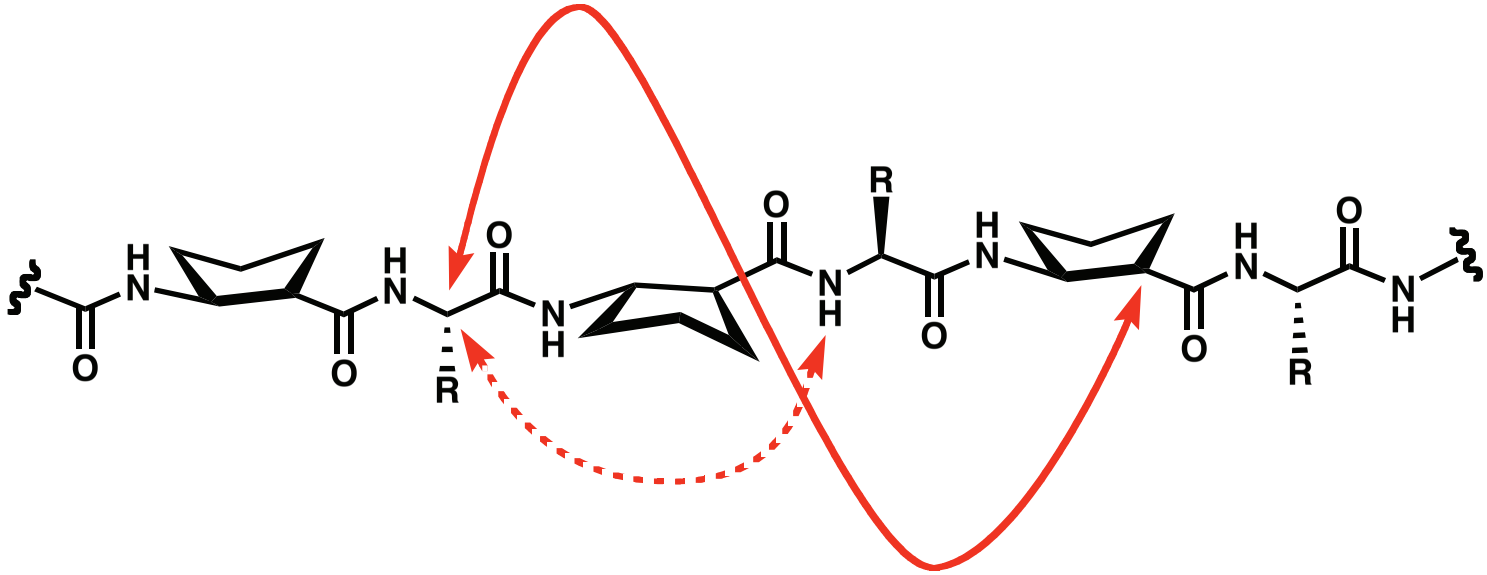


Strong HPLC retention suggests that 14/15-helix is Preferred?

Conditions: C8 column; linear gradient 20-->60% B over 40 min;
A = water, B = CH_3CN (both 0.1% CF_3CO_2H)

Schmitt, Weisblum, Gellman
***J. Am. Chem. Soc.* 126:6848 (2004)**

α/β-Peptide 15-mers: NOE Analysis

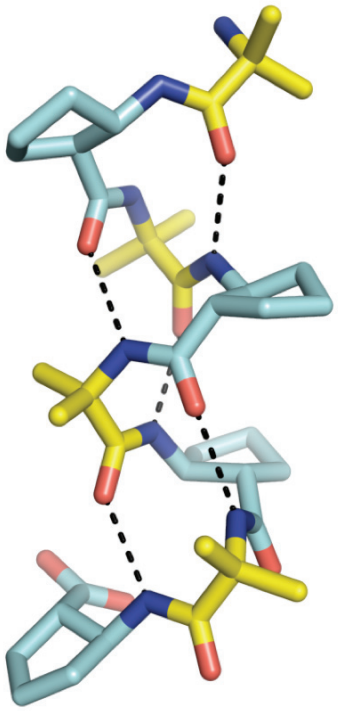


NOE pattern expected for 14/15-helix but not 11-helix [4 or 5 of 7 observed]

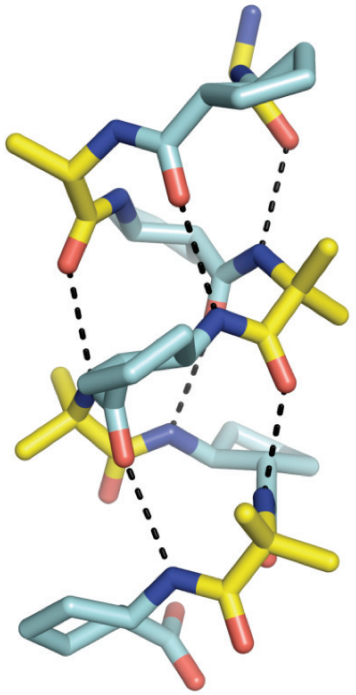
NOE pattern expected for 11-helix but not 14/15-helix [none observed]

Schmitt, Weisblum, Gellman
***J. Am. Chem. Soc.* 129:417 (2007)**

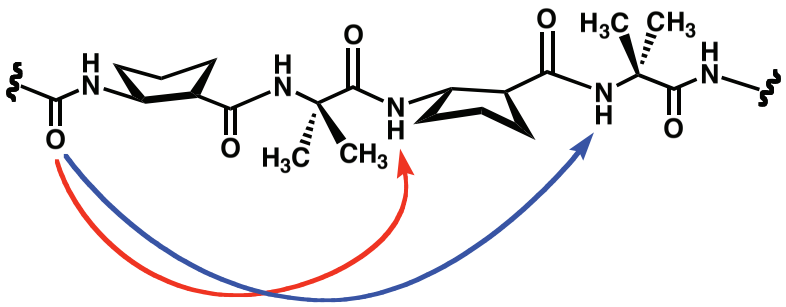
α/β-Peptide Crystal Structures Reveal Two Helical Conformations



11-Helix

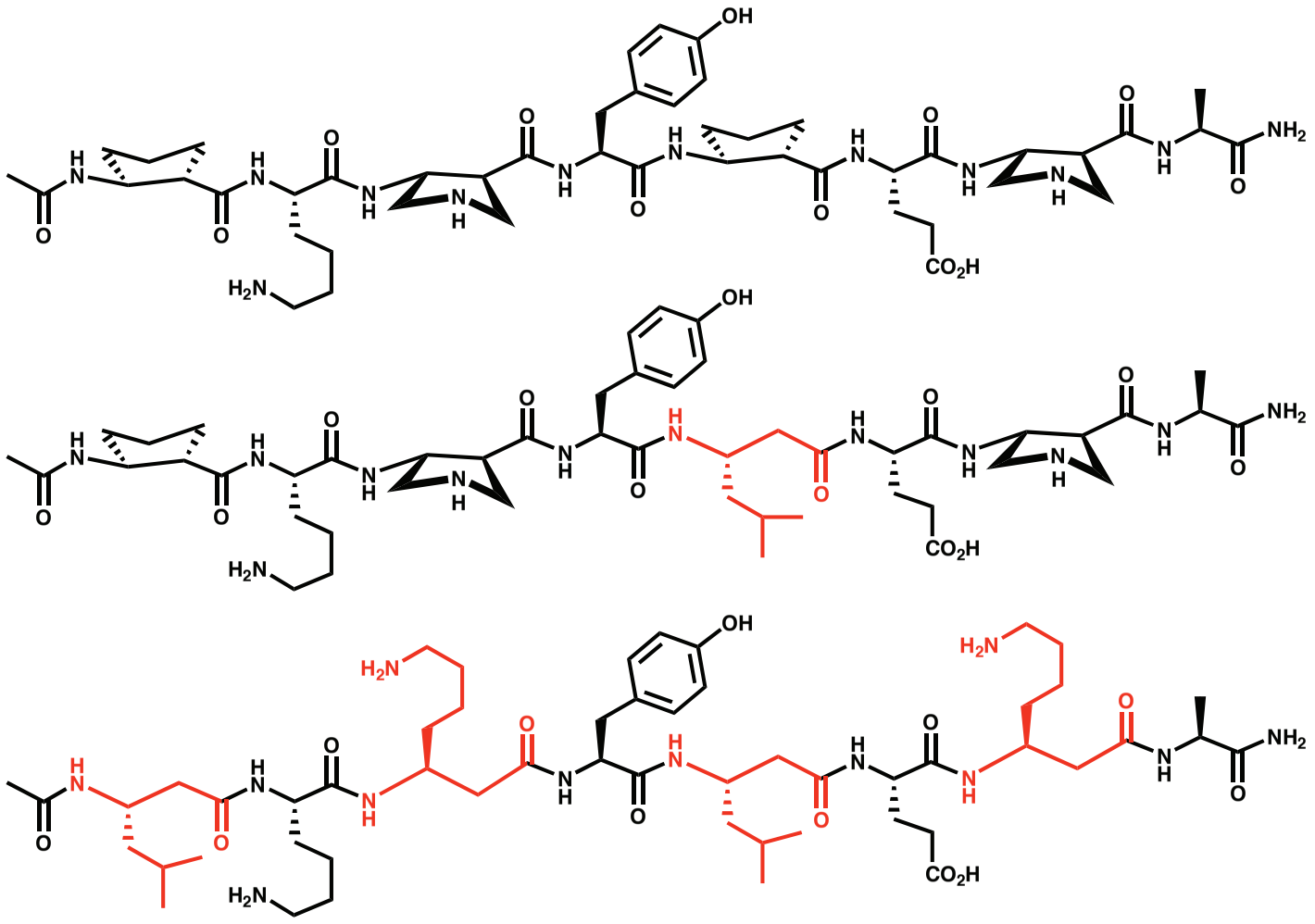


14/15-Helix



Choi, Guzei, Spence, Gellman
J. Am. Chem. Soc. **130:6544 (2008)**

Cyclically Constrained β-Residues Strongly Promote α/β-Peptide Helix Formation



Medium-range NOEs in CD_3OH
Many
Many
None

Schmitt, Choi, Guzei, Gellman *J. Am. Chem. Soc.* 127:13130 (2005)

Cyclically Constrained β-Residues Strongly Promote α/β-Peptide Helix Formation

Can we quantify this effect?

Cyclically Constrained β-Residues Strongly Promote α/β-Peptide Helix Formation

Can we quantify this effect?

1. **Population analysis is challenging for α-peptide secondary structures.**

2. **No examples of reliable population analysis for foldamer secondary structures to date.**

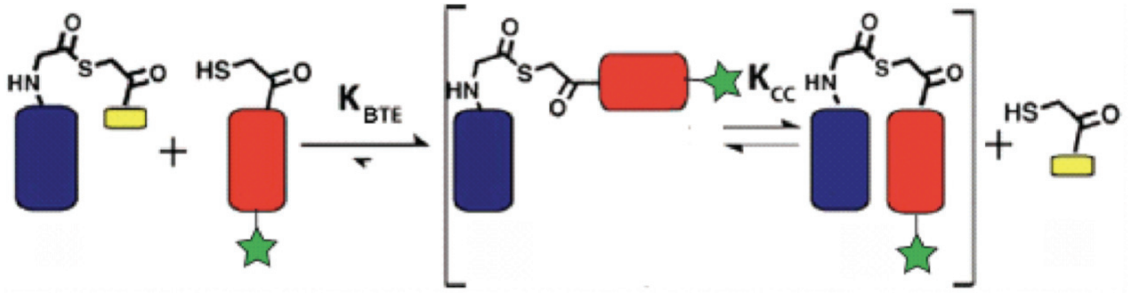
Cyclically Constrained β-Residues Strongly Promote α/β-Peptide Helix Formation

Can we quantify this effect?

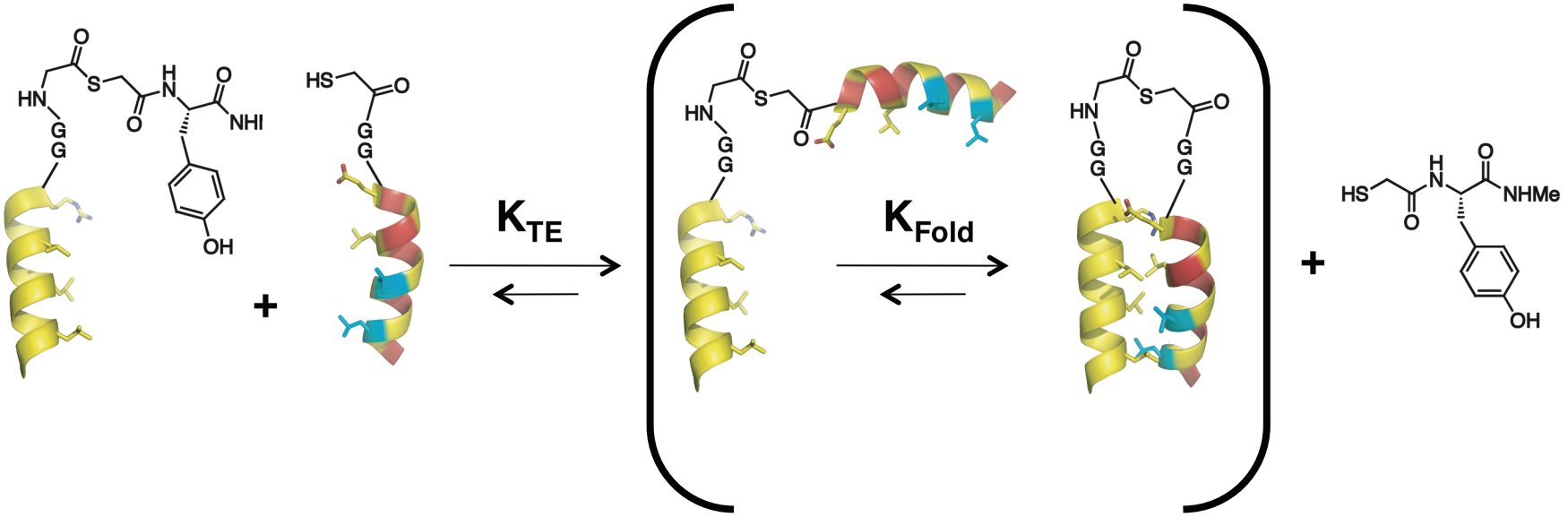
1. **Population analysis is challenging for α-peptide secondary structures.**

2. **No examples of reliable population analysis for foldamer secondary structures to date.**

3. **Use thioester exchange to address the role of cyclic residue constraints?**

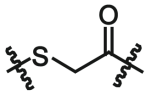


Thioester Exchange Design:
Packing of an α-Helix against an α/β-Peptide Helix



*bc**d**efg**a**bc**d**efg**a* *d**e**fg**a**bc**d**efg**a**bc*

Succ-EEL**RRR**L**EE**L**RRR**R**GGGGGGGEXEXLZEL**E**XE**L**RZY-NH$_2$**

G = 

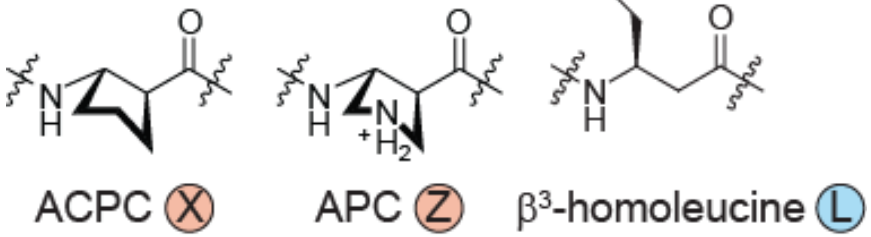
thioglycolic acid

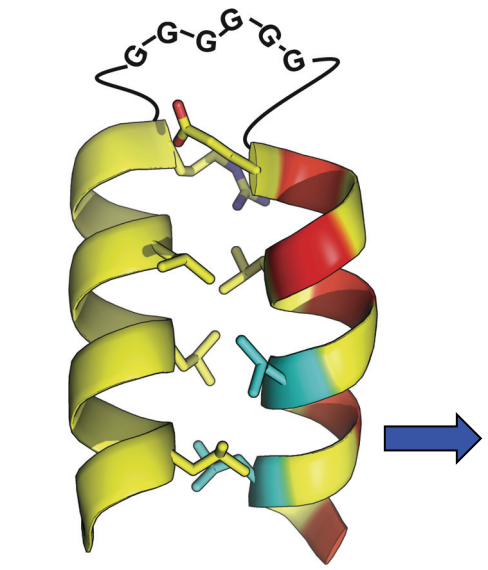
Price, Hadley, Steinkruger, Gellman *Angew. Chem. Int. Ed.* 49:368 (2010)

Thioester Exchange Design:
Packing of an α-Helix against an α/β-Peptide Helix

*bc**d**efg**a**bc**d**efg**a* *d**e**fg**a**bc**d**efg**a**bc*

Succ-EE**L**RRR**L**EE**L**RRR**R**GGGGGGGE**X**E**X****L**Z**E****L**E**X**E**L**R**Z**Y**-NH$_2$**



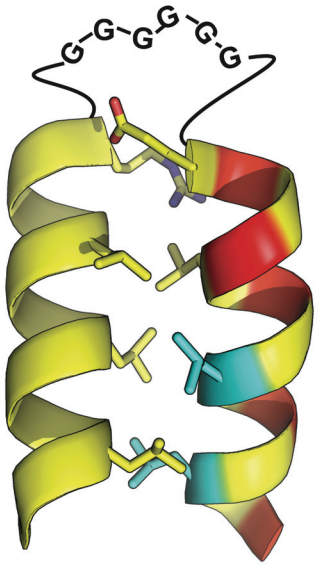


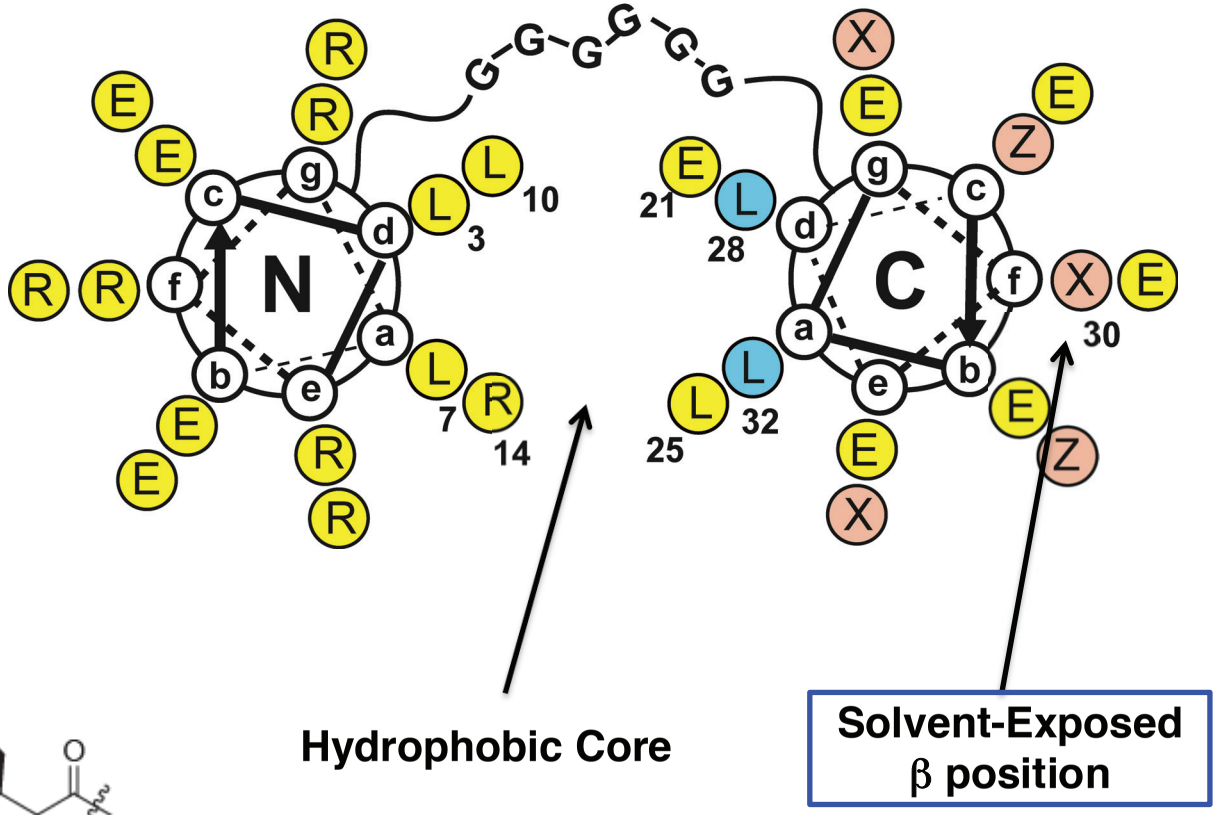
α-Helix

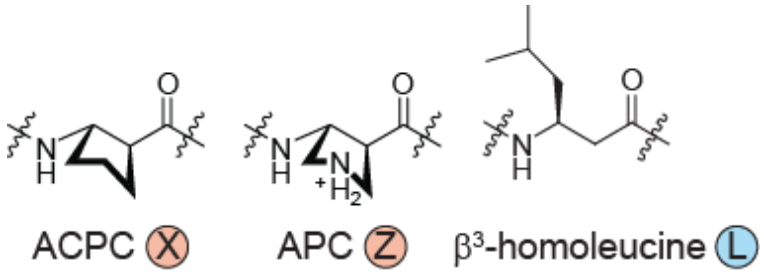
α/β-Peptide 14/15-Helix

Examine site on solvent-exposed side.

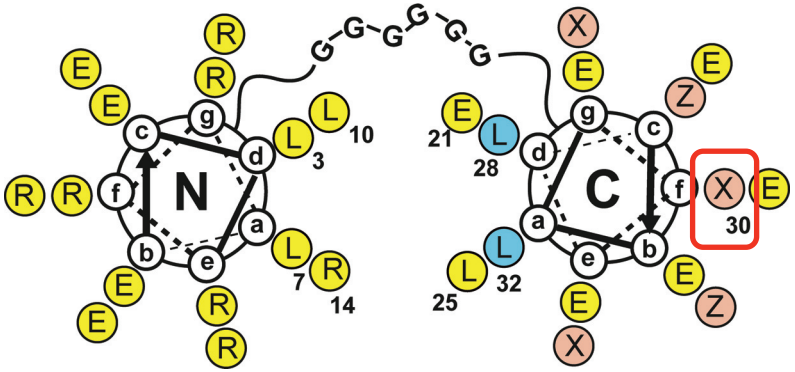
Thioester Exchange Design:
Packing of an α-Helix against an α/β-Peptide Helix





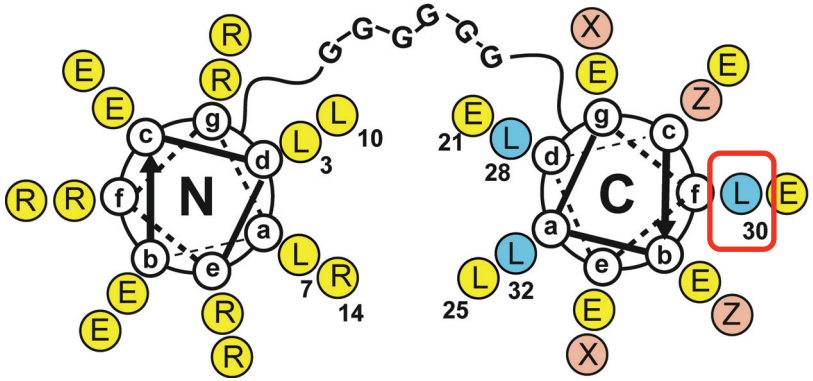


Quantitative Evaluation of Residue Preorganization to α/β-Peptide Helix Stability (1 Site)

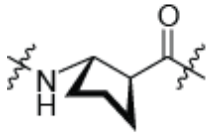


ΔG_f = -1.1 kcal/mol

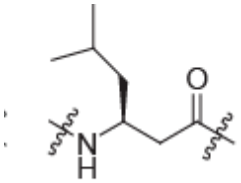
vs.



$\Delta\Delta G_f$ = +0.3 kcal/mol

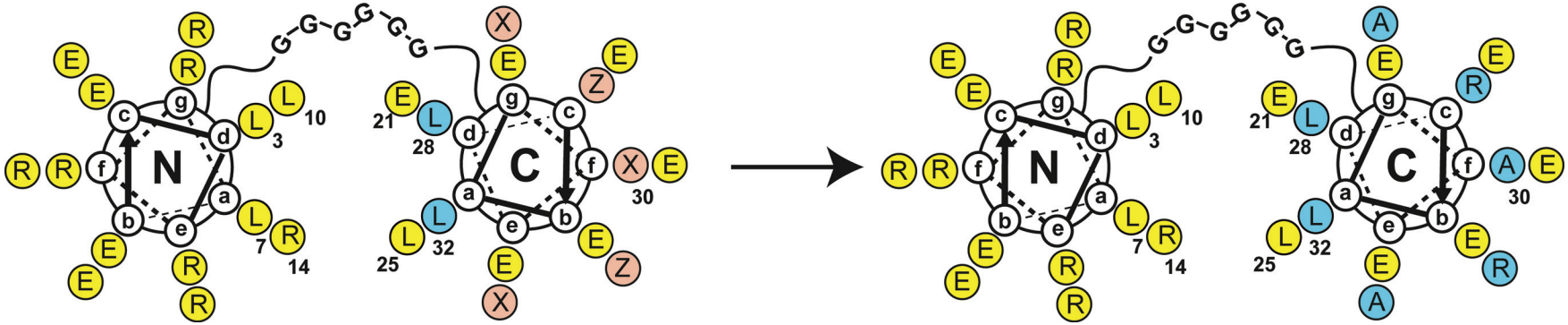


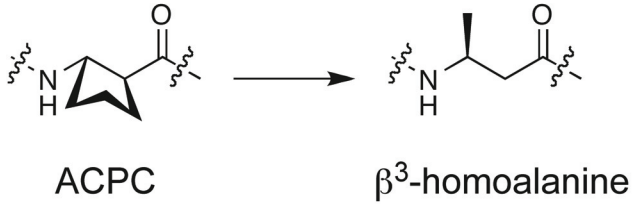
ACPC (X)

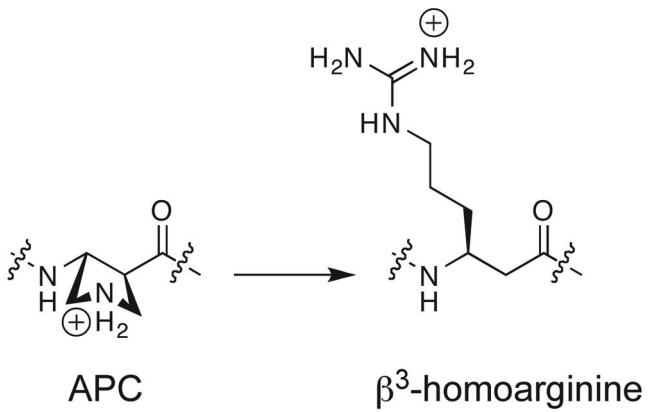


β³-homoleucine (L)

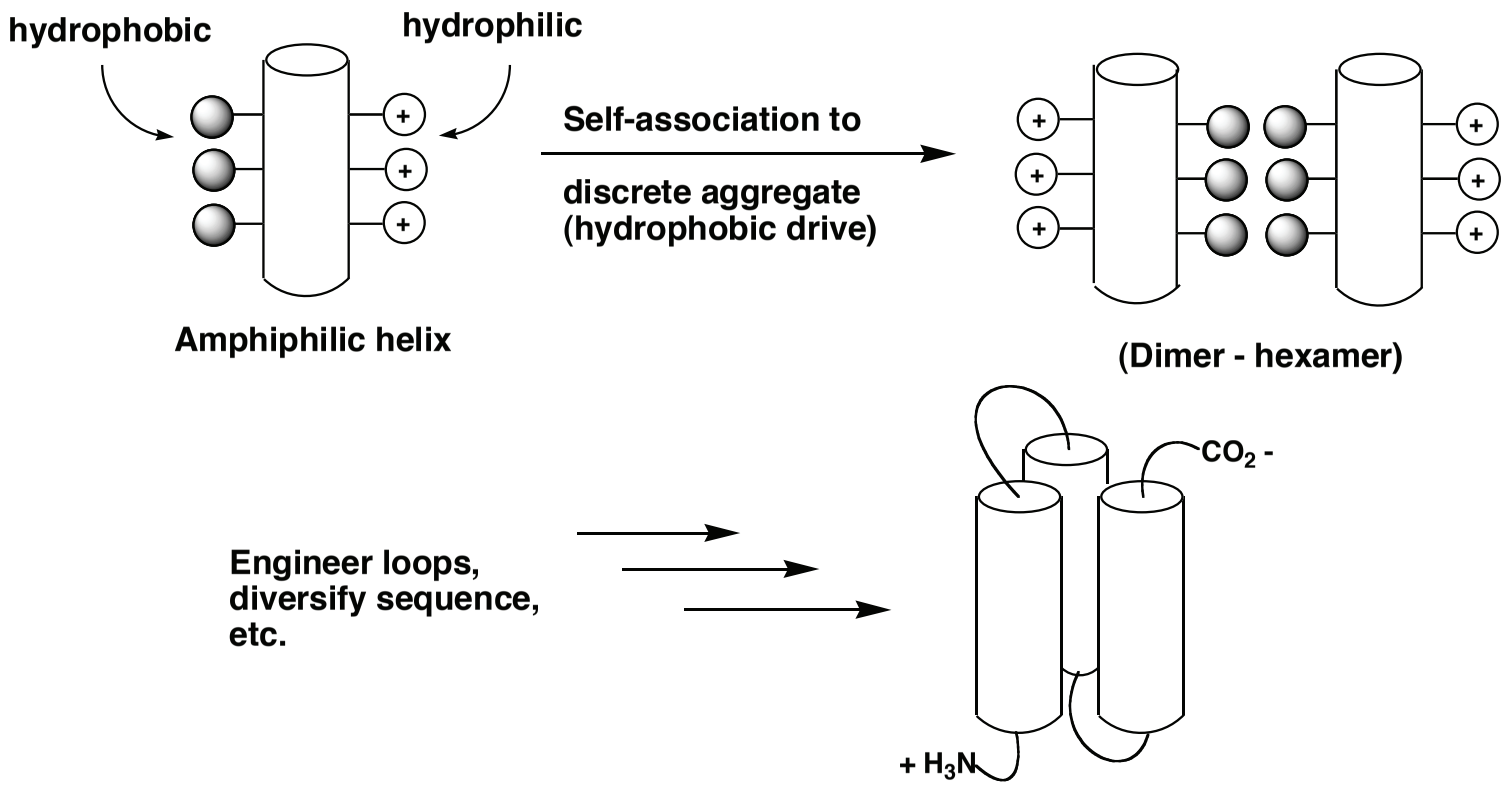
Quantitative Evaluation of Residue Preorganization to α/β-Peptide Helix Stability (5 Sites)







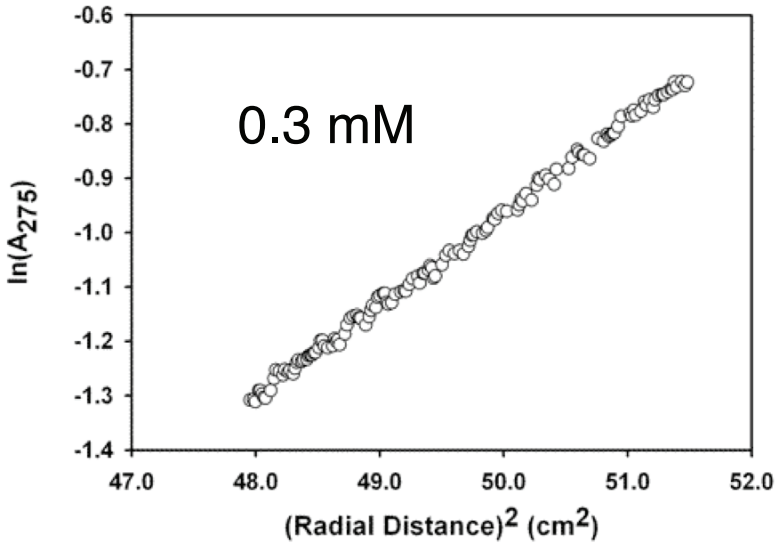
Toward Helix Bundle Foldamer Tertiary Structure?

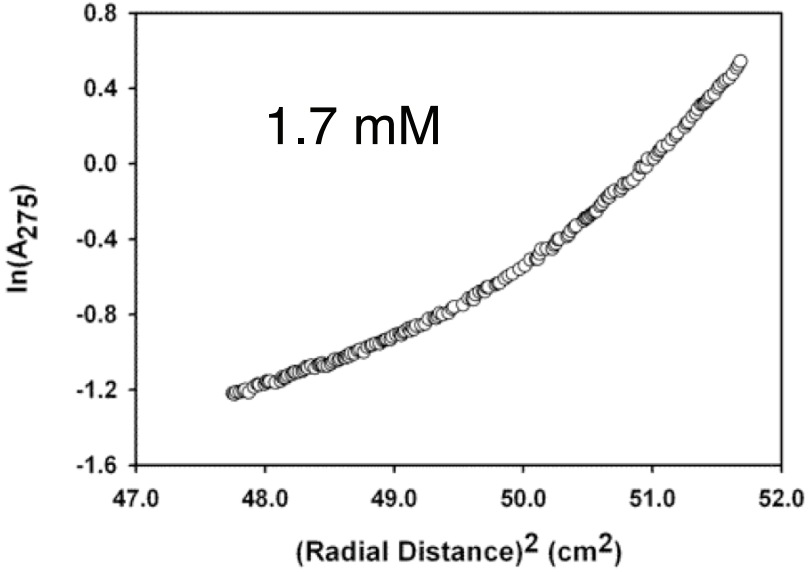


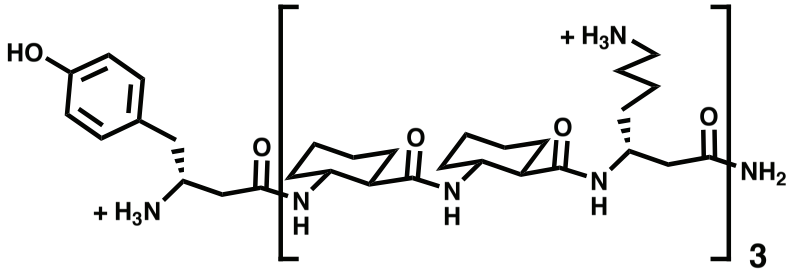
Protein precedent: DeGrado et al., *Science* 270:935 (1995)

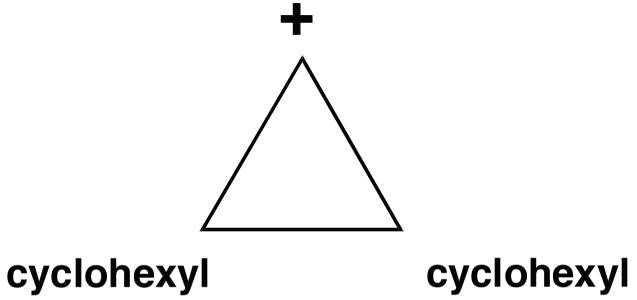
Amphiphilic 14-Helix: Analytical Ultracentrifugation

(Sedimentation Equilibrium; 60 krpm, 10 mM Tris, pH 8.0)

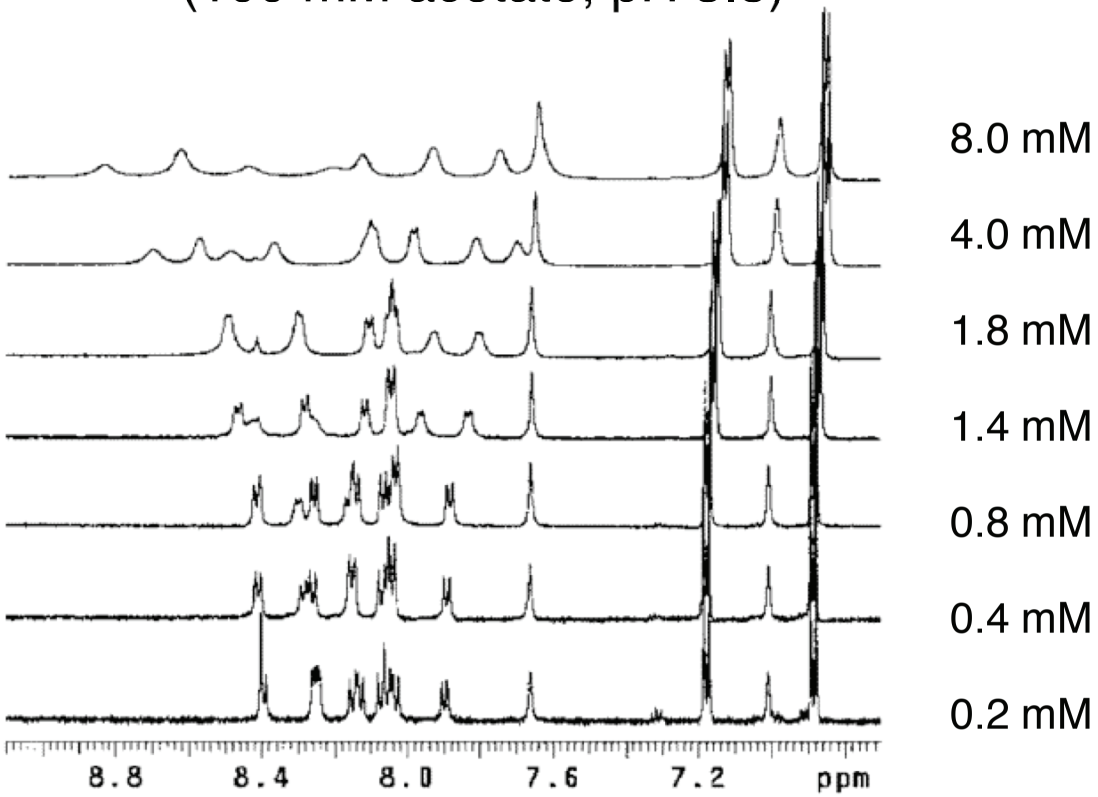


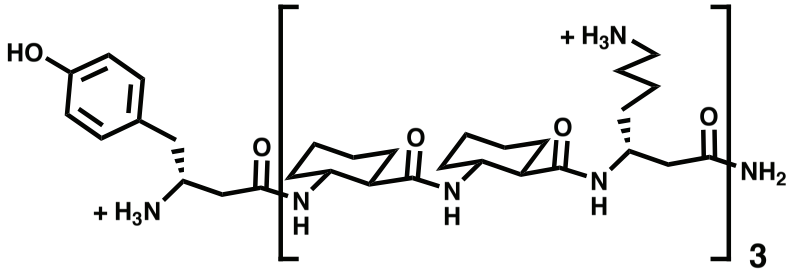


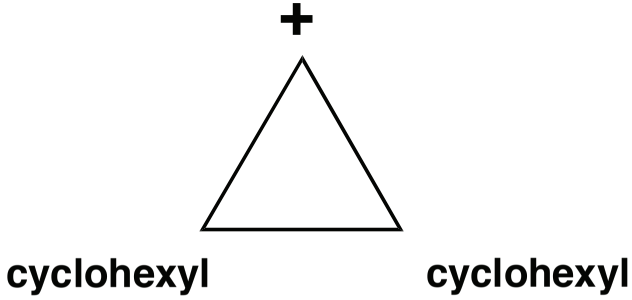




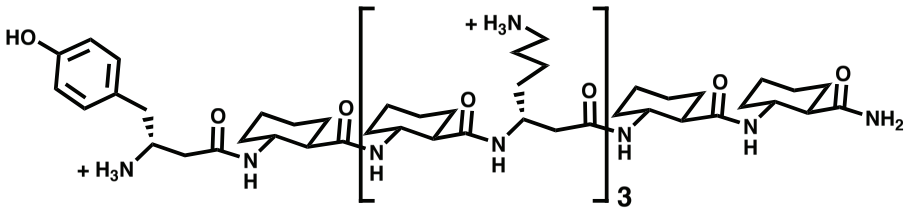
Detection of β-Peptide Self-Association by NMR
(100 mM acetate, pH 3.8)

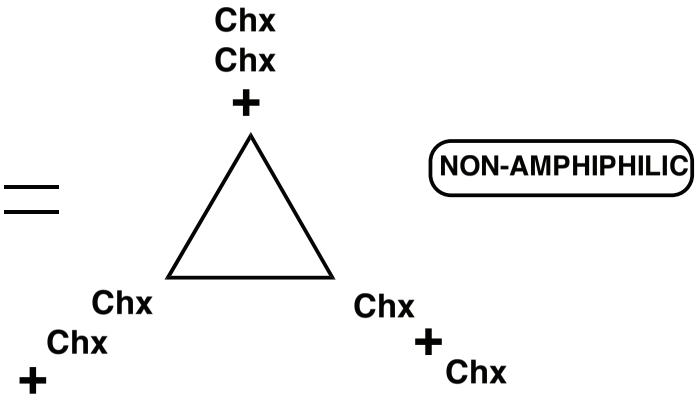




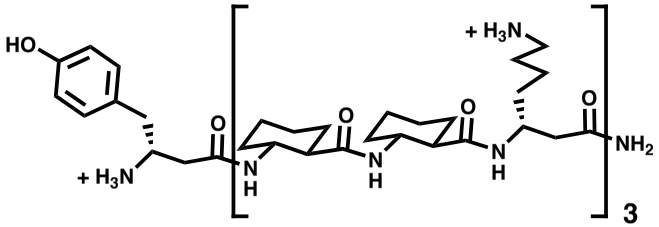


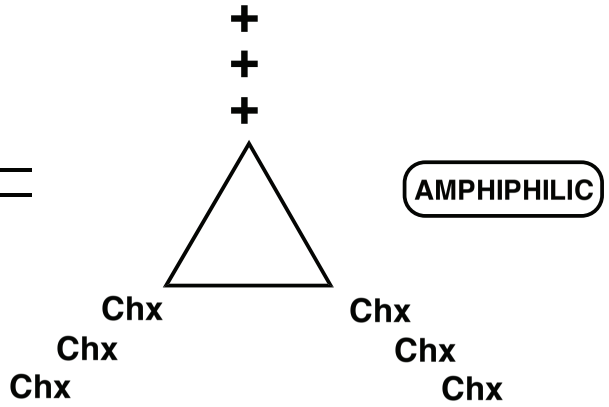
Non-Aggregating Control Compound



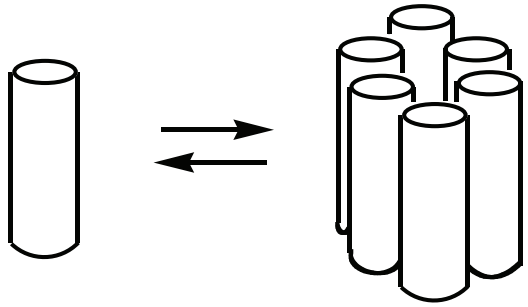


VS.





Monomer-Hexamer Equilibrium?



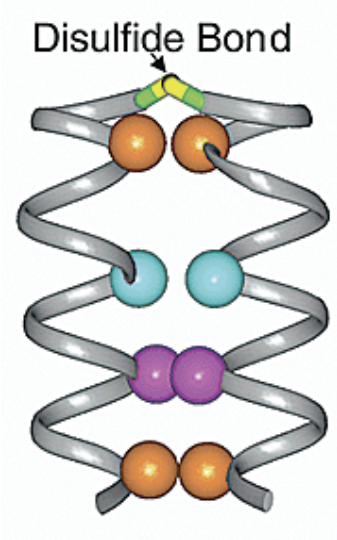
Sedimentation equilibrium results:

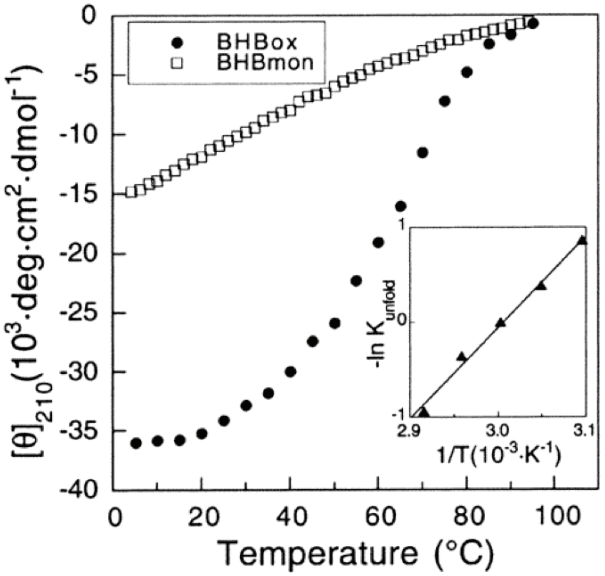
0.3 mM **0.6 mM**	**Monomer**
0.8 mM **1.3 mM** **1.8 mM**	**Monomer + Hexamer**

(10 mM Tris, pH 8, 60,000 rpm)

Raguse, Lai, LePlae, Gellman *Org. Lett.* **2001**, *3*, 3963.

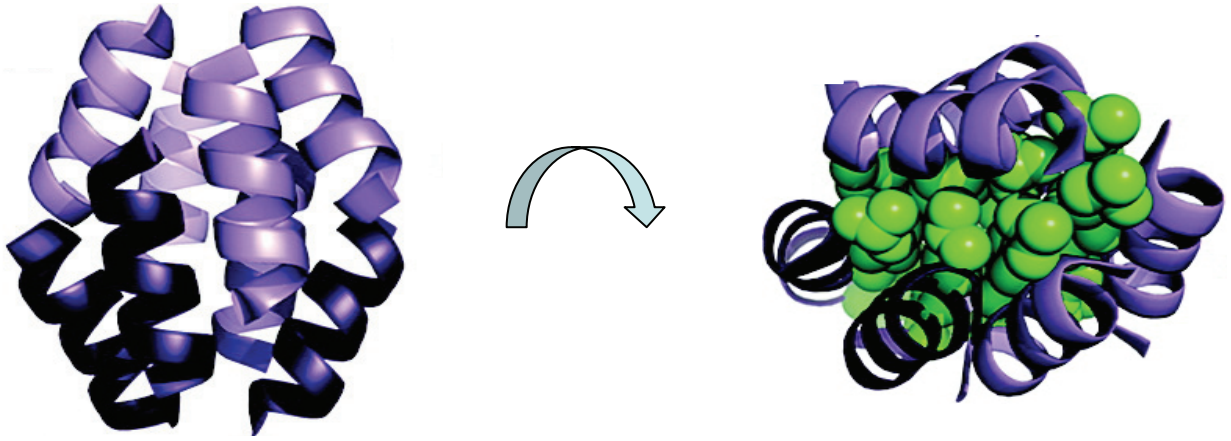
Unimolecular β-Peptide Folding?





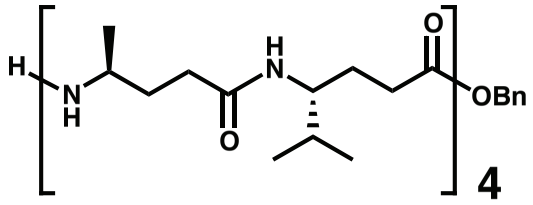
Cheng, DeGrado *J. Am. Chem. Soc.* **2002**, *124*, 11564

Crystal Structure of a β-Peptide Helix Bundle



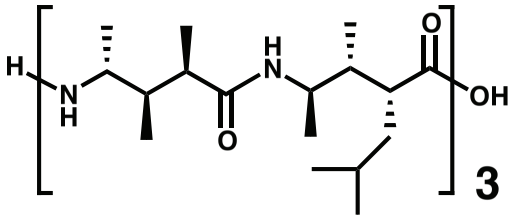
Schepartz et al. *J. Am. Chem. Soc.* **2007,** *129*, 1532.

γ-Peptide Helices: Pioneering Studies of Helix Formation in Organic Solvents

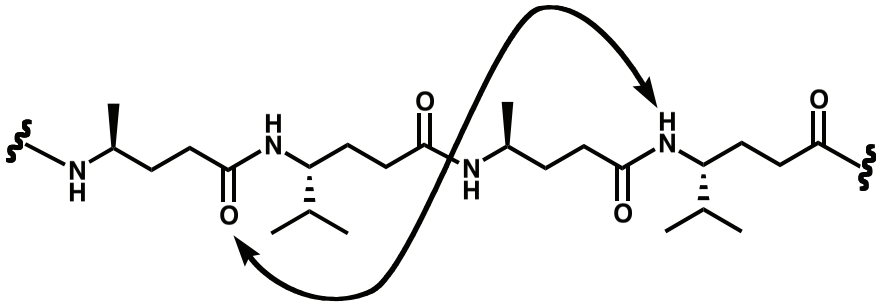


Hanessian et al.
J. Am. Chem. Soc. **1998, *120*, 8569.**

Seebach et al.
Helv. Chim. Acta **1998, *81*, 983.**

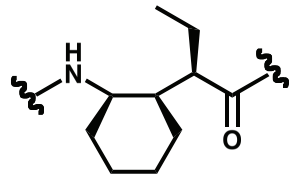


Seebach et al.
Chem. Commun. **2001, 207.**



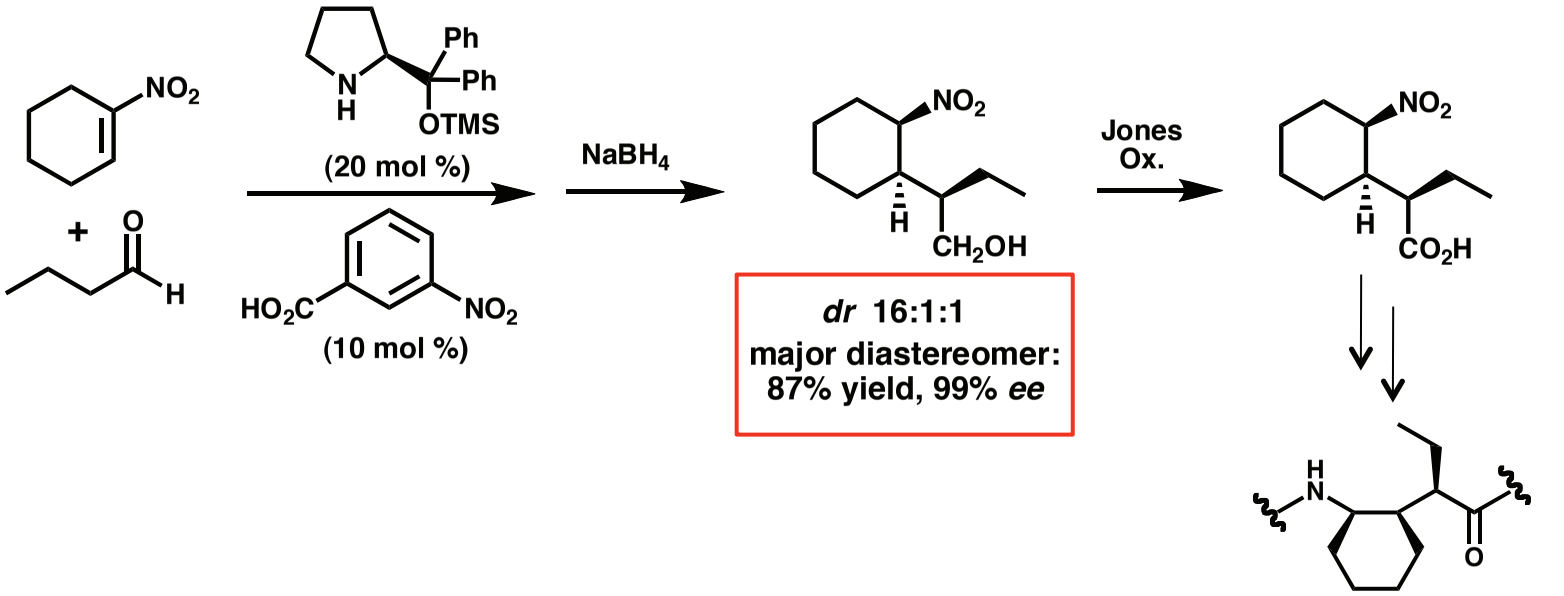
14-Helix

Improved Helix Stability with Constrained γ-Amino Acids?



γ-Residue with cyclic constraint

Improved Helix Stability with Constrained γ-Amino Acids?



Synthesis Precedents:

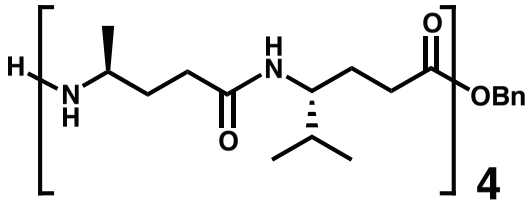
(mostly nitrostyrenes)

List, Pojarliev, Martin *Org. Lett.* 3:2423 (2001)
Betancort, Barbas *Org. Lett.* 3:3737 (2001)
Santanu et al. *Chem. Rev.* 107:5471 (2007)

γ-Residue with cyclic constraint

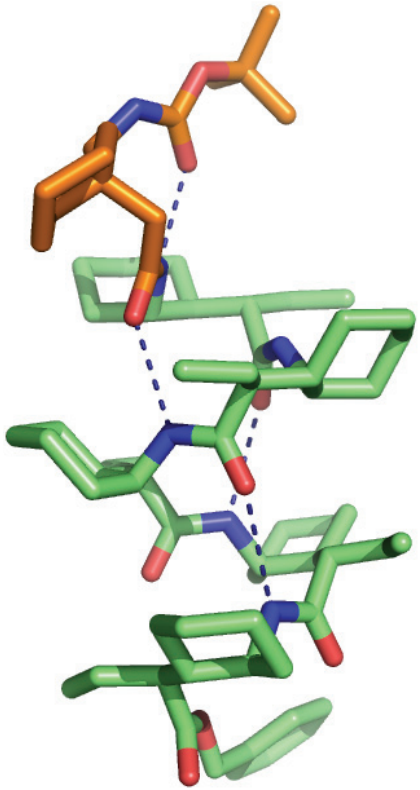
Guo, Chi, Almeida, Guzei, Parker, Gellman
J. Am. Chem. Soc. 131:16018 (2009).

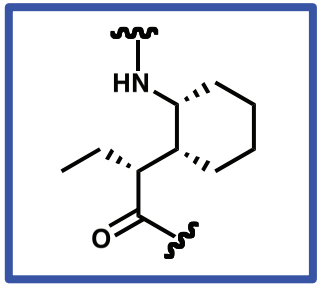
Homo-oligomers of the Constrained γ-Amino Acid Retain 14-Helix Propensity



Hanessian et al.
J. Am. Chem. Soc. **1998, *120*, 8569.**

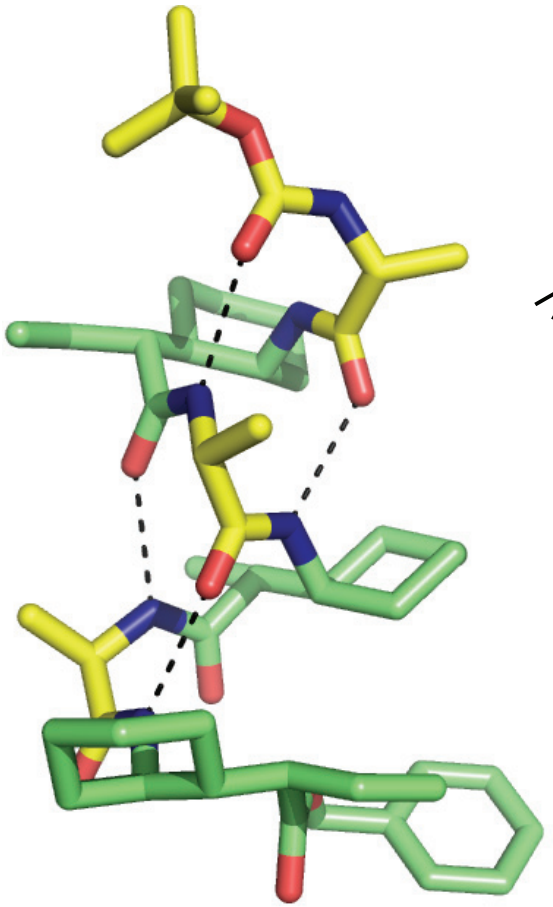
Seebach et al.
Helv. Chim. Acta **1998, *81*, 983.**





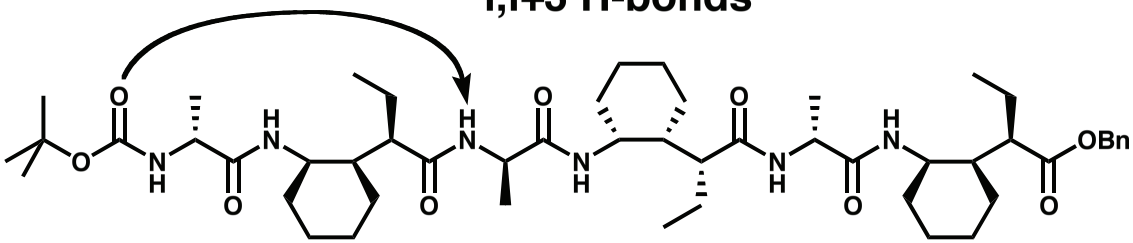
Guo, Zhang, Reidenbach,
Giuliano, Guzei, Spencer, Gellman
Angew. Chem. Int. Ed. 50:5843 (2011)

New Foldamers: α/γ-Peptide Helix

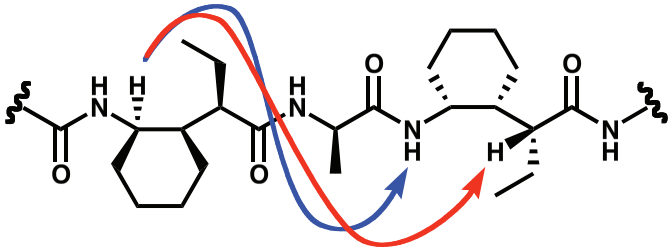


"12-Helix"
(Crystal Structure)

i,i+3 H-bonds



Structure-defining NOEs ($CDCl_3$):

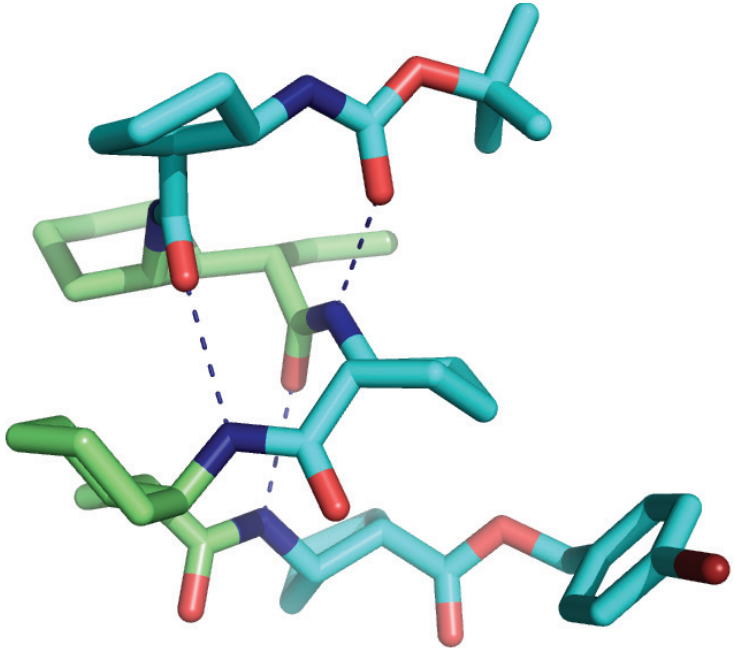


Guo, Chi, Almeida, Guzei, Parker, Gellman
J. Am. Chem. Soc. 131:16018 (2009).

New Foldamers: β/γ-Peptide Helix

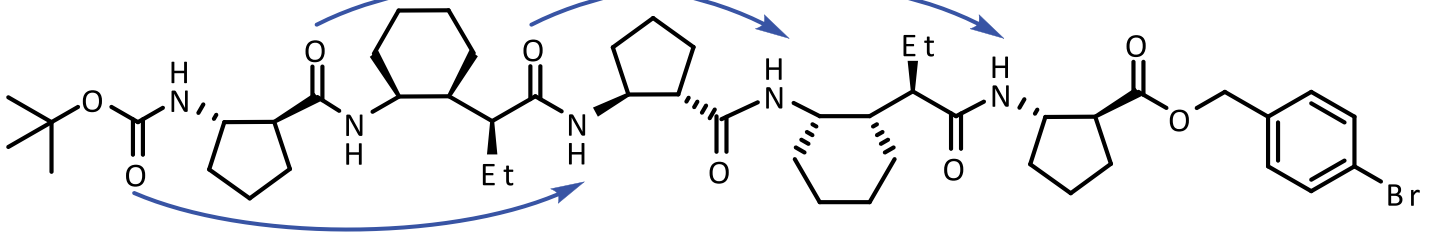
Structure-defining NOEs in $CDCl_3$ (not shown)

Guo, Almeida, Zhang, Reidenbach, Choi, Guzei, Gellman
J. Am. Chem. Soc. 132:7868 (2010)

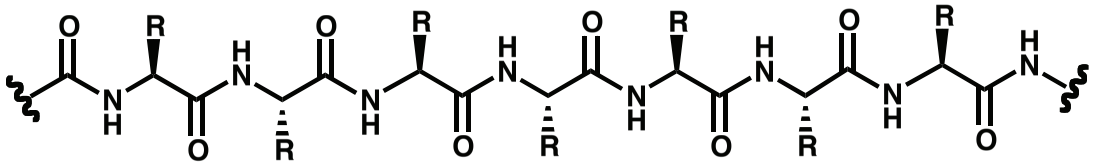


"13-Helix" (Crystal Structure)

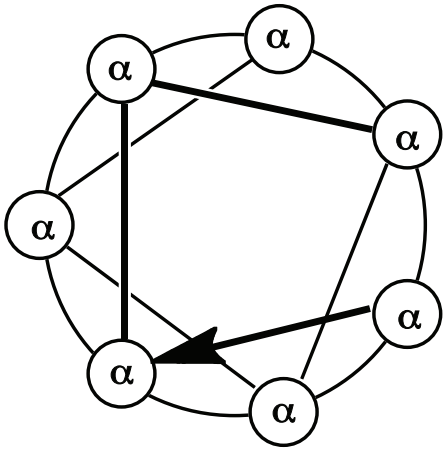
i,i+3 H-bonds



α-Helix Mimicry from α/β/γ-Peptides?



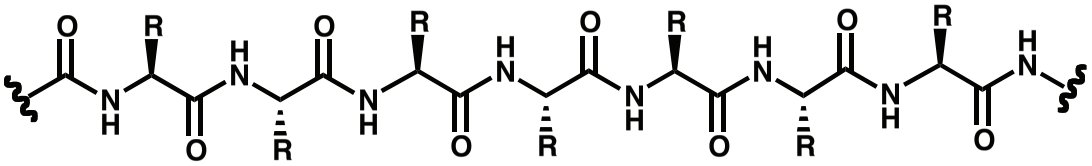
αααααααα



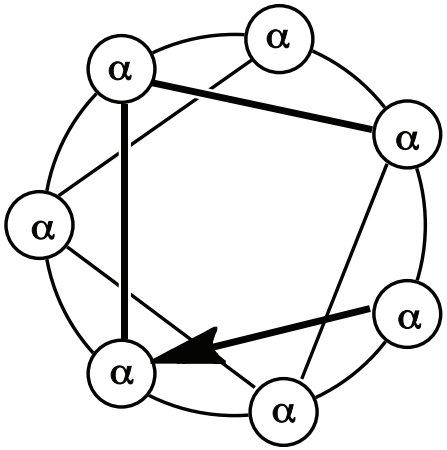
Seven α residues =
Two turns of α-helix

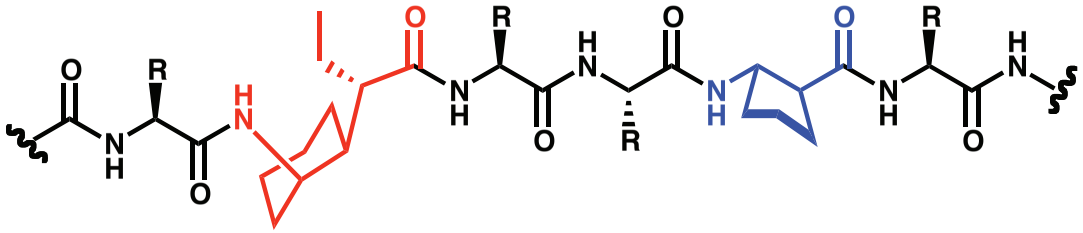
"α-helix wheel"

α-Helix Mimicry from α/β/γ-Peptides?



αααααααα

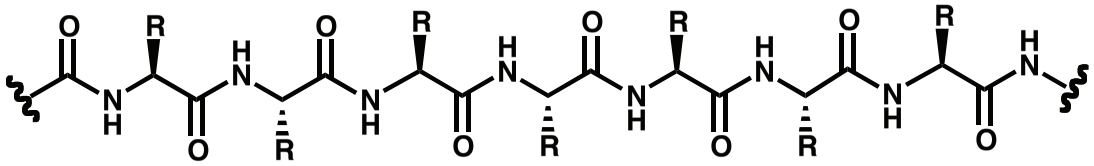




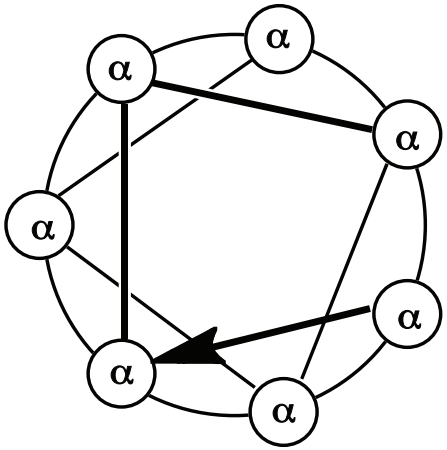
αγααβα

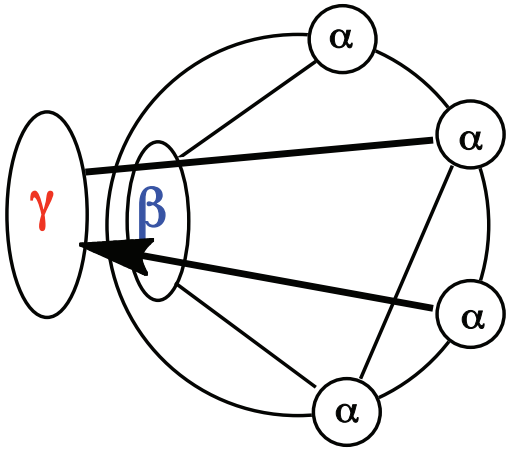
Same number of backbone atoms as seven α residues

α-Helix Mimicry from α/β/γ-Peptides?

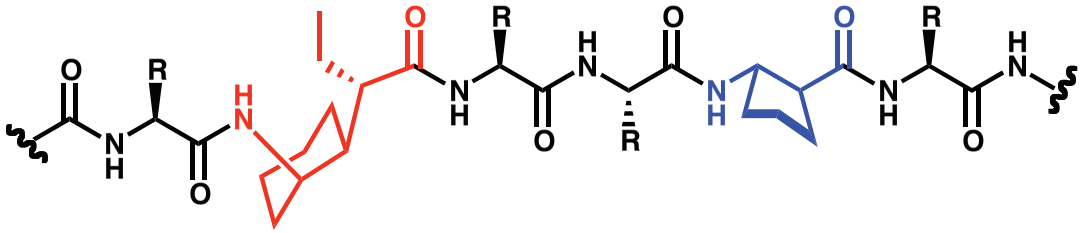


αααααααα





α/β/γ-Peptide Helix wheel?

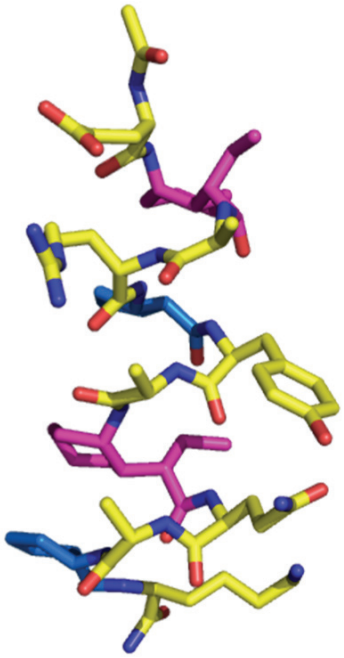


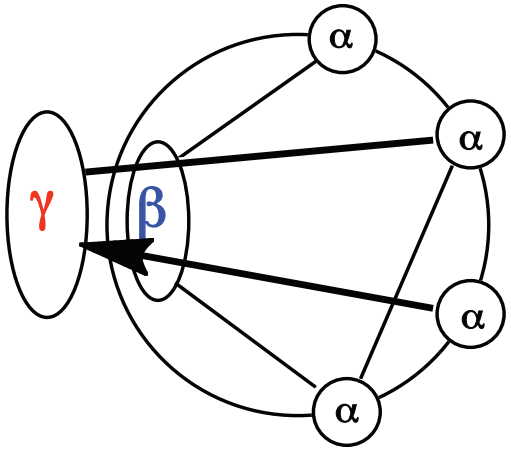
αγααβα

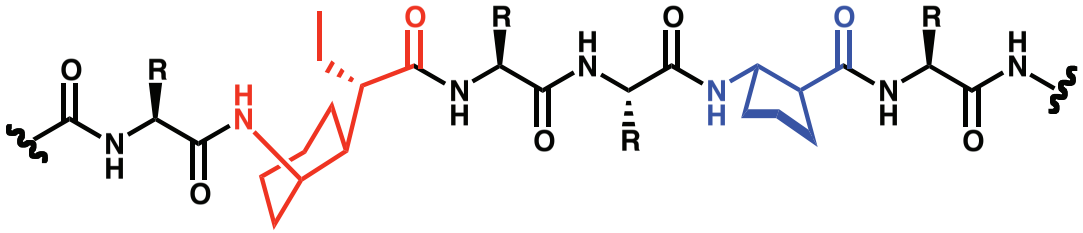
Same number of backbone atoms as seven α residues

α-Helix Mimicry from α/β/γ-Peptides?

α/β/γ-Peptide helical conformation in H_2O
(NOE-restrained dynamics; 12-mer)







αγααβα

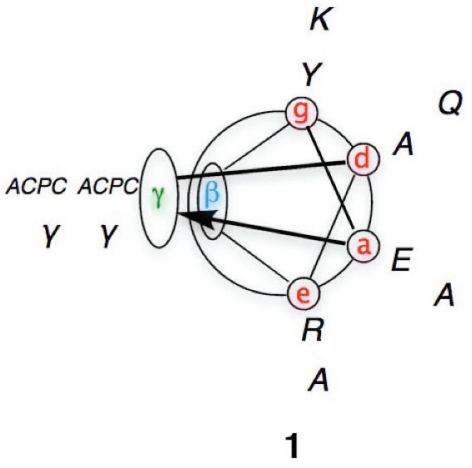
Sawada, Gellman *J. Am. Chem. Soc.* 133:7336 (2011)

CD Data Suggest High α/β/γ-Peptide Helix Stability

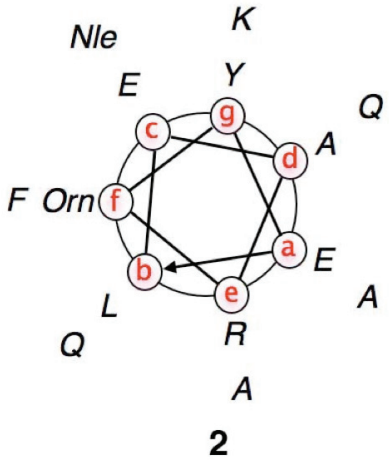
(H_2O vs. 1:1 H_2O:CH_3OH)

α/β/γ-peptide (12-mer) 1 : *Ac -E-γ-A-R-β(ACPC)-Y-A-γ-Q-A-β(ACPC)-K- NH_2*

α-peptide (14-mer) 2 : *Ac -E-L-E-A-R-Orn-Y-A-Q-Nle-Q-A-F-K- NH_2*



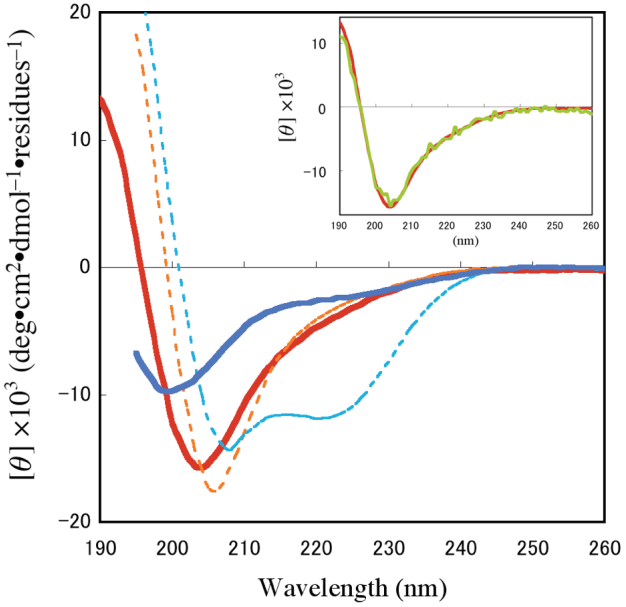
1



2

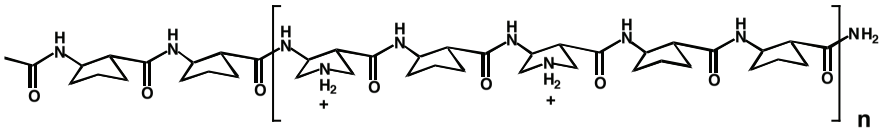
α/β/γ

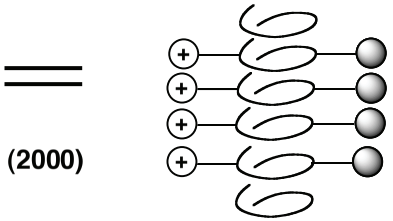
α



Structure → Function

Host-Defense Peptide Mimicry





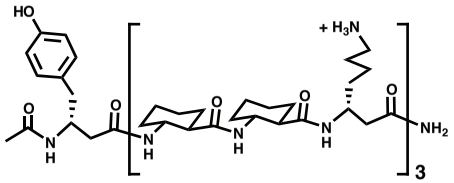
Porter et al. *Nature* 404:565 (2000)

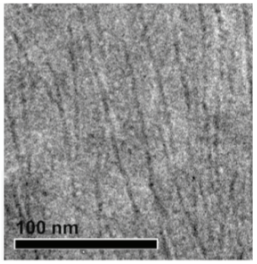


Antibacterial random copolymers

Mowery et al. *JACS* 129:15474 (2007)

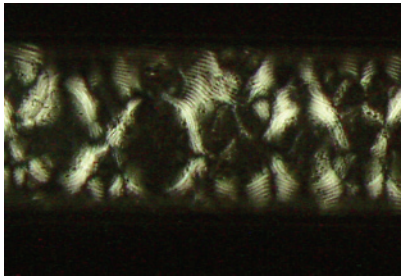
Nanofiber Assembly





(Cryo-TEM; Hartgerink, Rice)

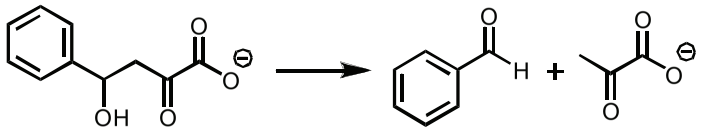




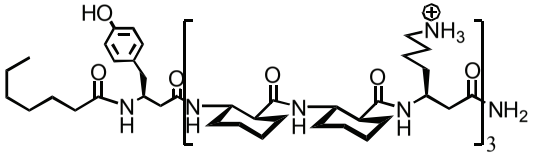
Pomerantz, Abbott, Gellman *JACS* 128:8730 (2006)

Lyotropic liquid crystalline phases

Catalysis



Aldolase-like activity



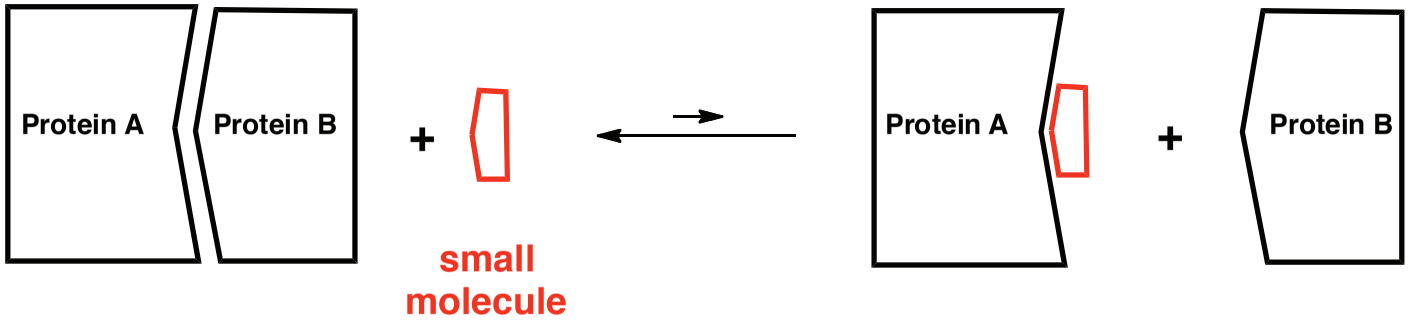
Müller, Windsor, Pomerantz, Gellman, Hilvert
Angew Chem Int Ed 48:922 (2009)

Folding and assembly required

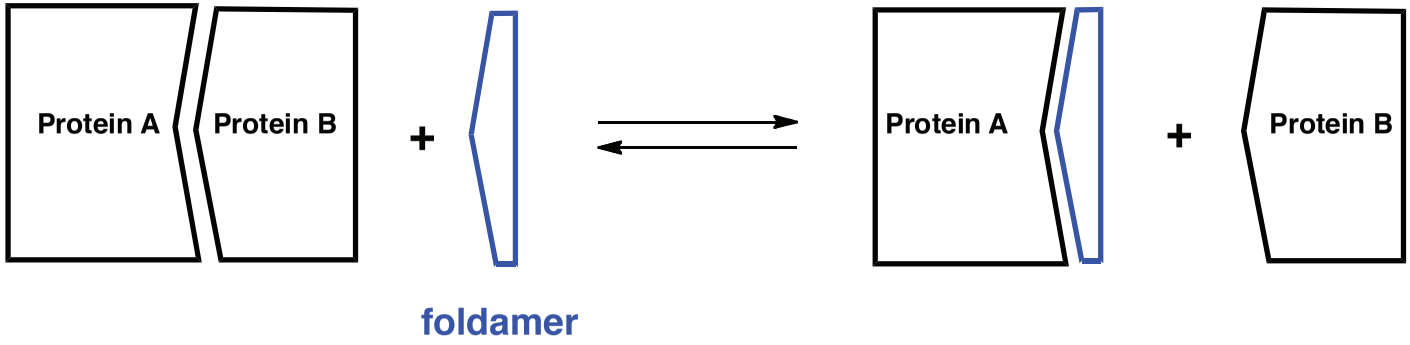
STRUCTURE → FUNCTION

General Platform for Mimicry of Protein-Recognition Surfaces?

(Goal = inhibitors of deleterious protein-protein interactions)

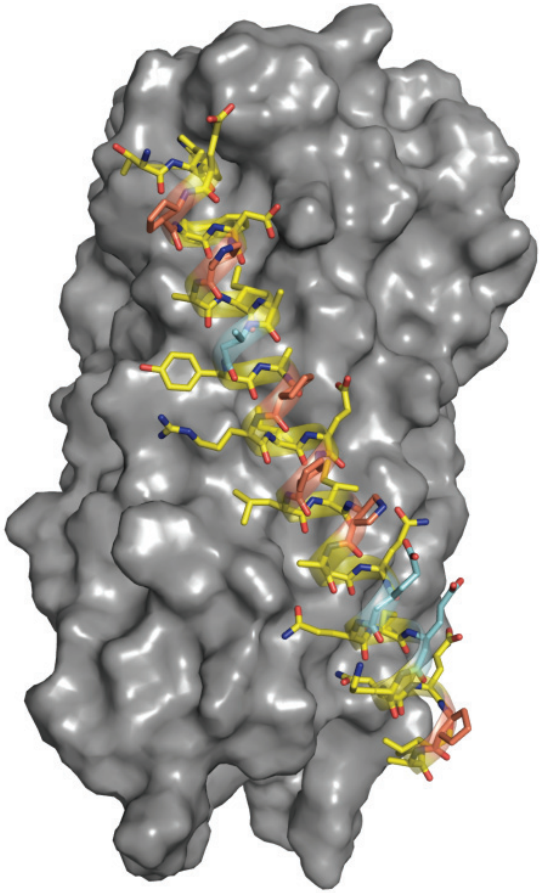


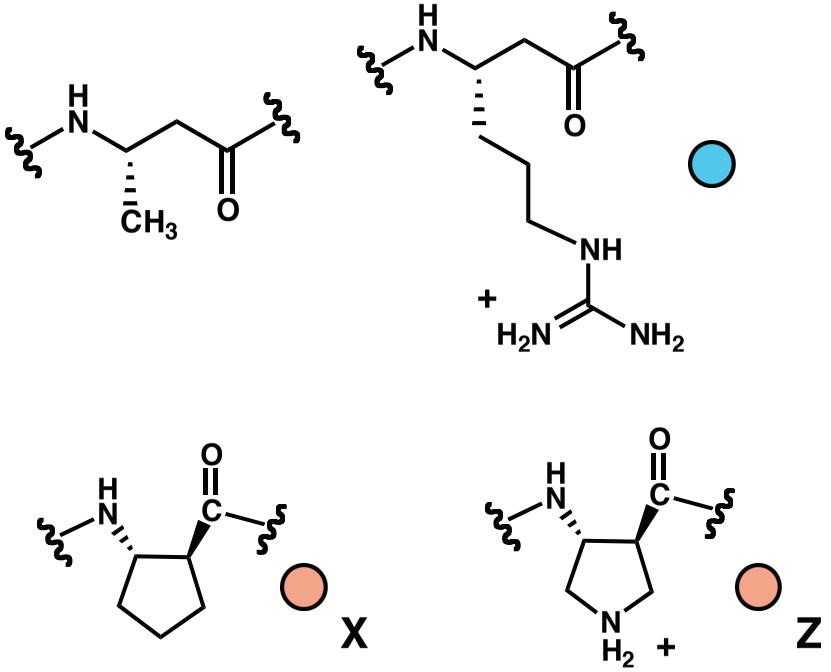
vs.



An α/β-Peptide that Mimics an α-helix from HIV Protein gp41 and Inhibits Viral Entry

Ac-TTWEXWDZAIAEYAXRIEXLIZAAQEQQEKNEXALZEL-NH_2





Horne, Johnson, Ketas, Klasse, Lu, Moore, Gellman *PNAS* 106:14751 (2009)

Conclusions:

Conformational mimicry of proteins with foldamers offers many opportunities for solution structure determination.

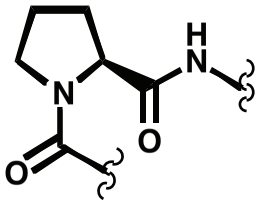
Establishing “folding rules” for new backbones enables function-based engineering.

New Shapes?

Polyproline Helices: No Hydrogen Bonds!

Polyproline I

3.3 residues/turn
1.9 Å rise/residue

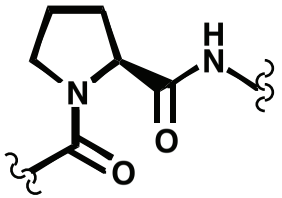


“cis” amide

(Helix-forming peptoids, Armand et al. *Proc. Natl. Acad. Sci.* **1998**, *95*, 4309)

Polyproline II

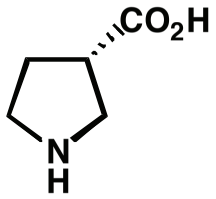
3.0 residues/turn
3.1 Å rise/residue



“trans” amide

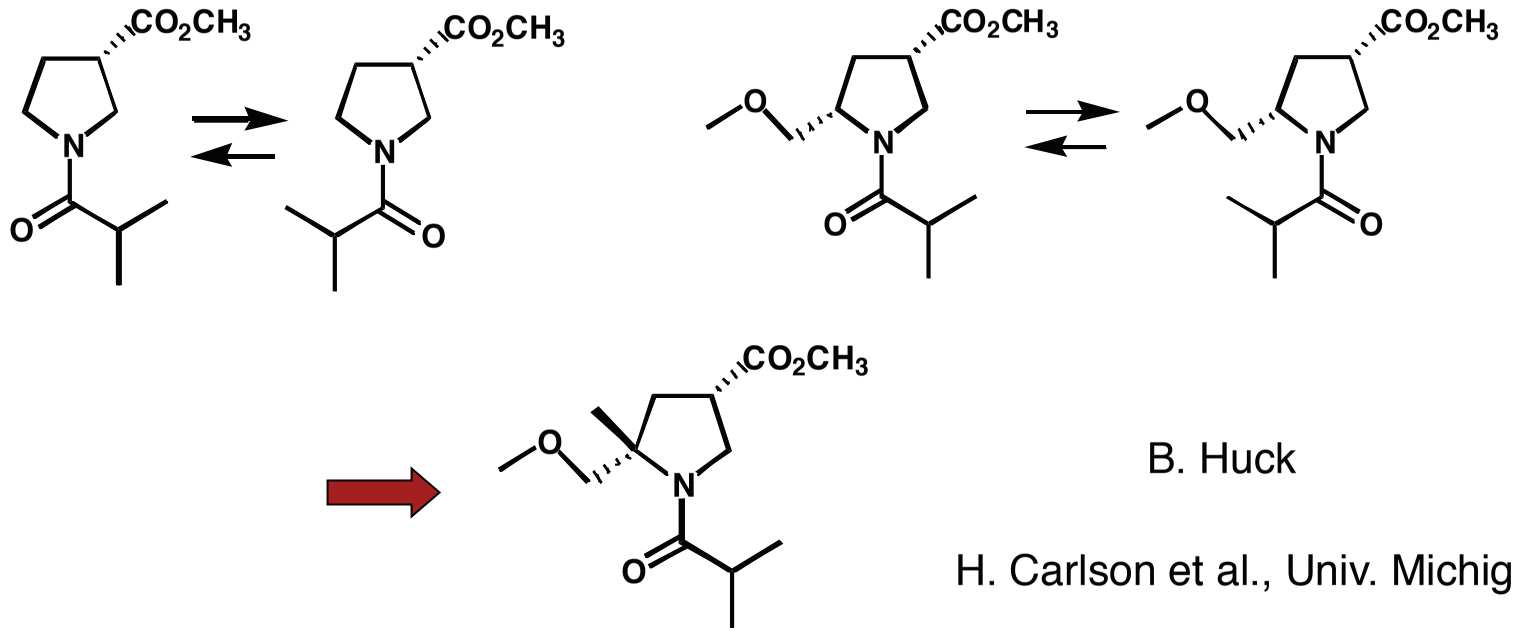
(collagen)

A β-Peptide Analogue of a Polyproline Helix?



pyrrolidine-3-carboxylic acid

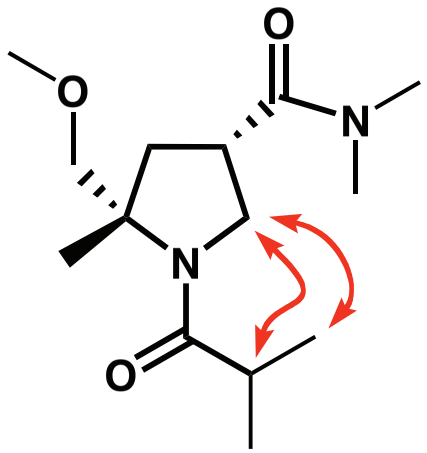
The rotamer problem:



B. Huck

H. Carlson et al., Univ. Michigan

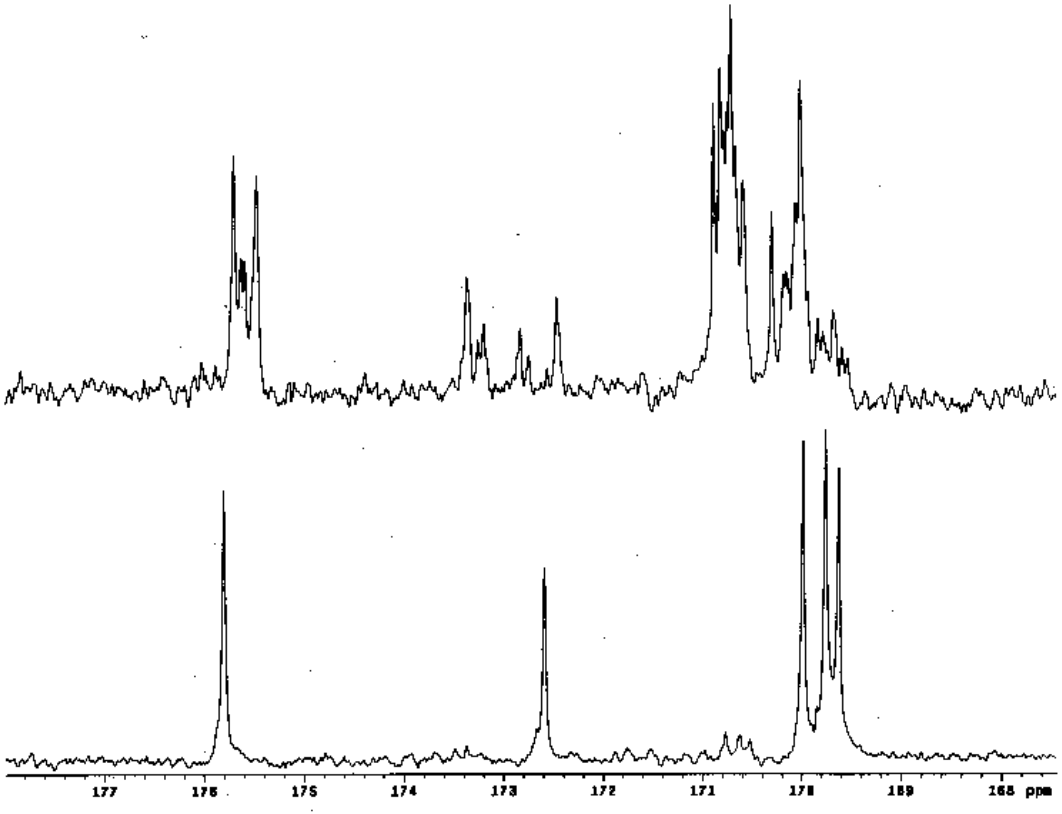
NOE Analysis of Monomer Consistent with Expected Rotamer Preference

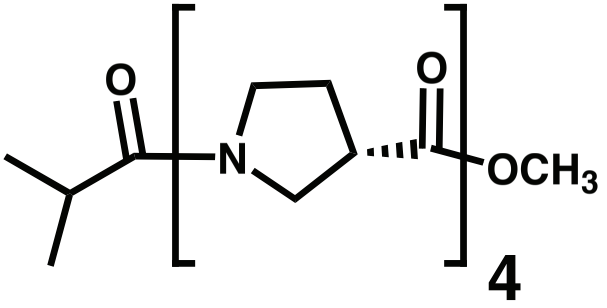


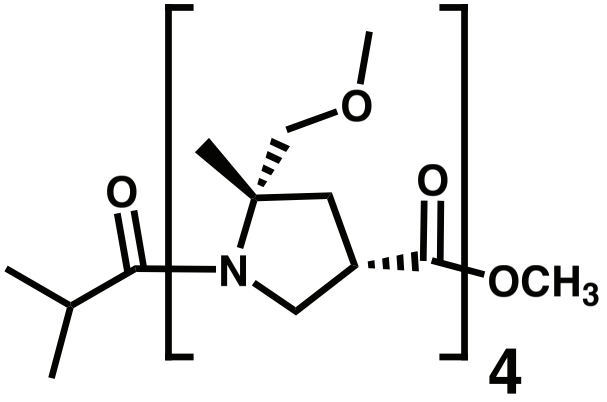
($CDCl_3$, rm temp)

B. Huck

Counting Amide Rotamer States by ^{13}C NMR (Carbonyl Region)

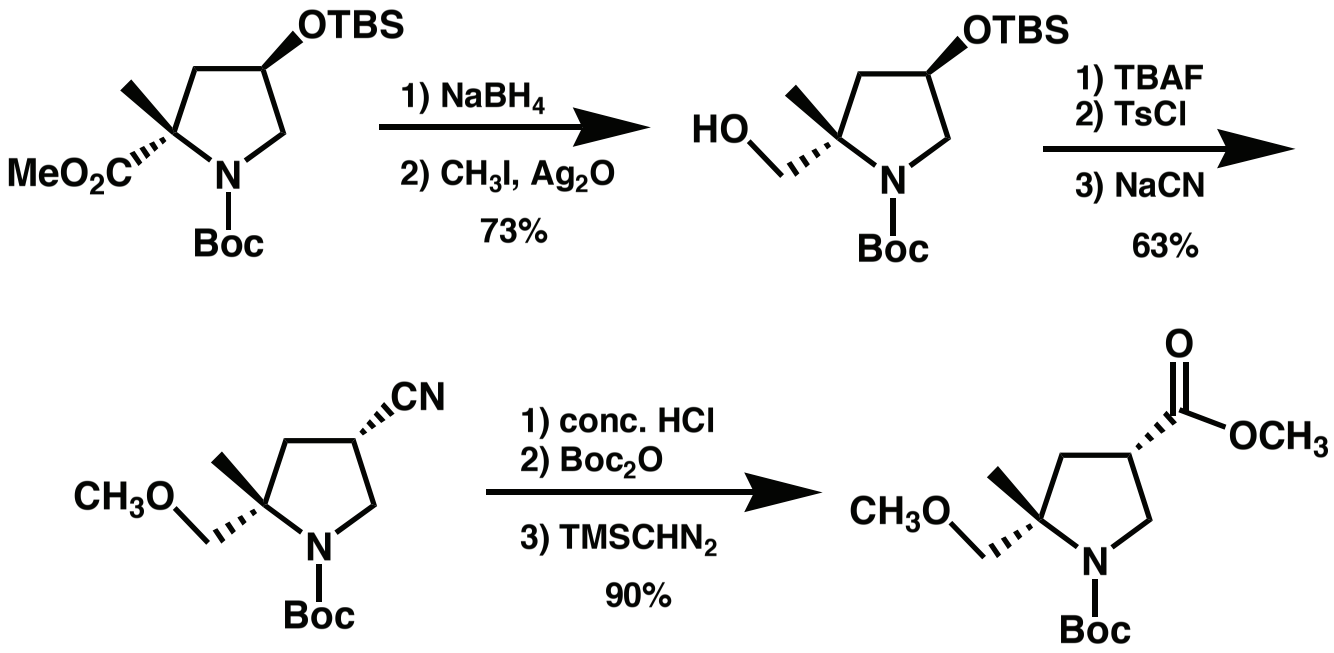






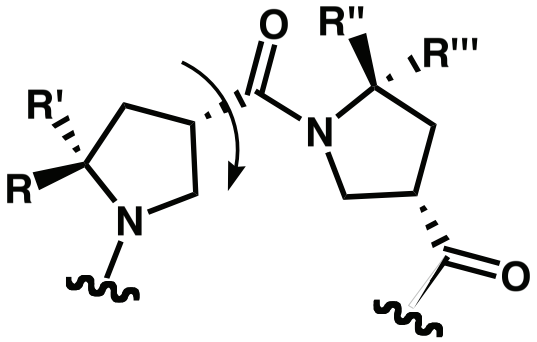
B. Huck

Synthesis: 5,5’-Disubstituted Monomers

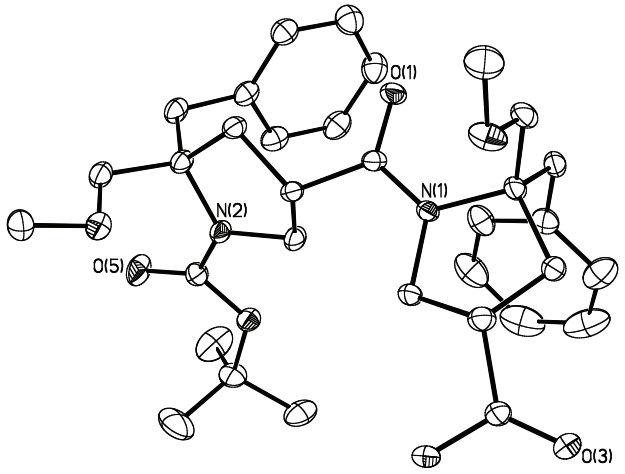


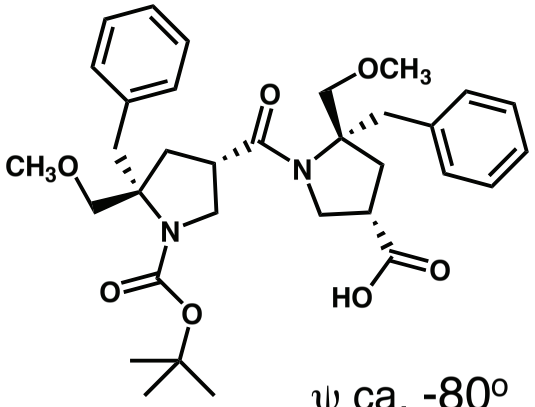
Starting material: Ohtake et al., *Bull. Chem. Soc. Japan* 1999, *72*, 2737.

Limited Torsional Options



Simulations suggest two minima, -80° and -160°



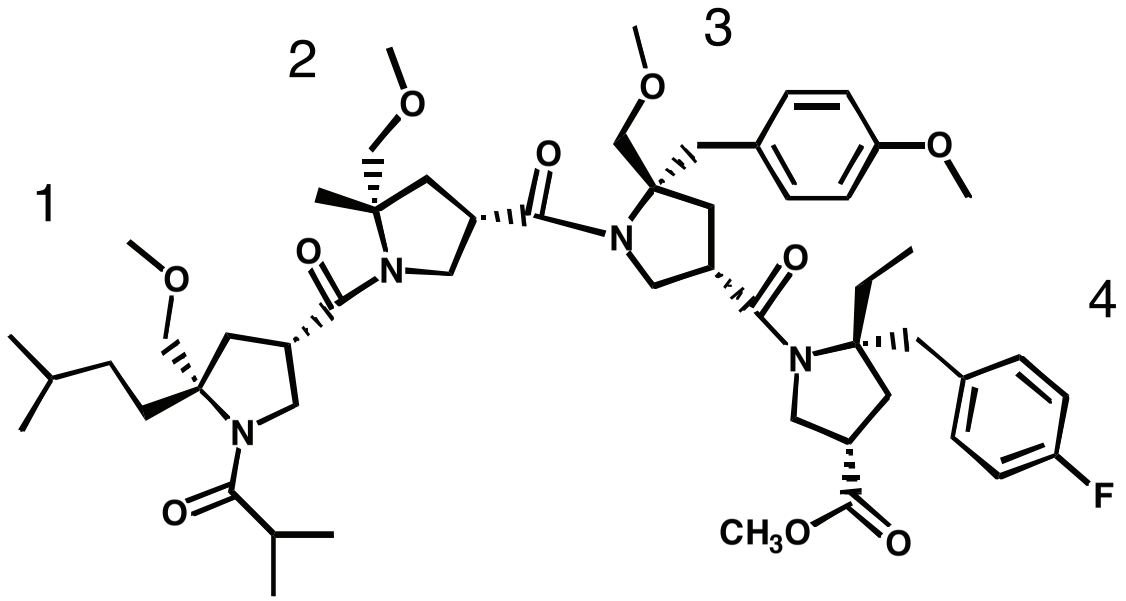


ψ ca. -80°

N. Fisk, B. Huck

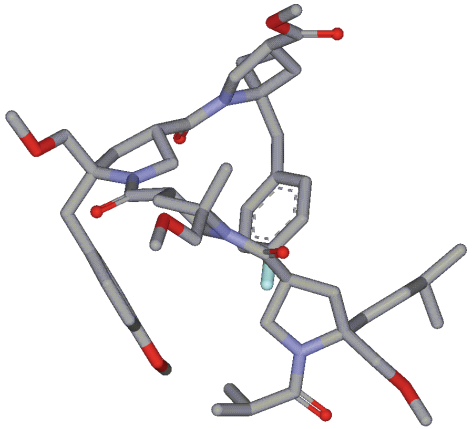
NOESY Analysis of a Heterotetramer ($CDCl_3$): NOEs Between Non-Adjacent Residues

Residue 1 ⟷ Residue 3 Residue 2 ⟷ Residue 4

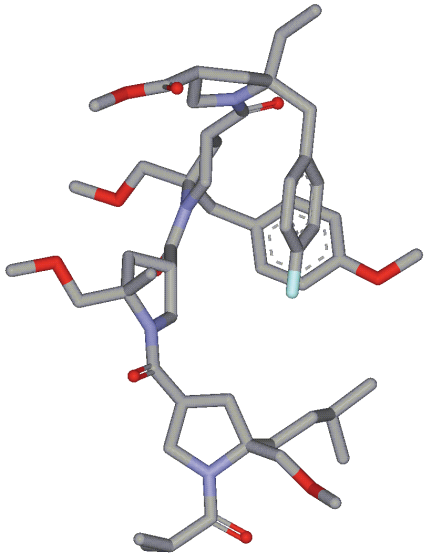


N. Fisk, B. Huck

Tetramer Structures: Simulation vs. NMR

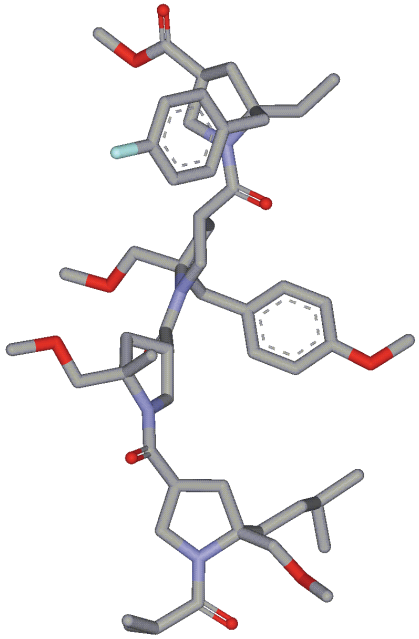


ψ: -80°, -80°, -80°



ψ: -160°, -160°, -80°

(Major, by NMR)



ψ: -160°, -160°, -160°