https://doi.org/10.17952/37EPS.2024.P2281

Impact of backbone N-amination on the stability of parallel β-sheets

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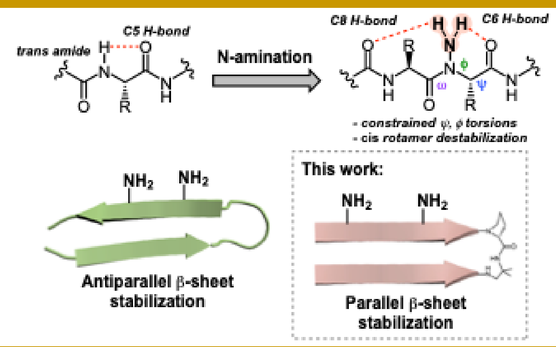
BACKGROUND and OBJECTIVES

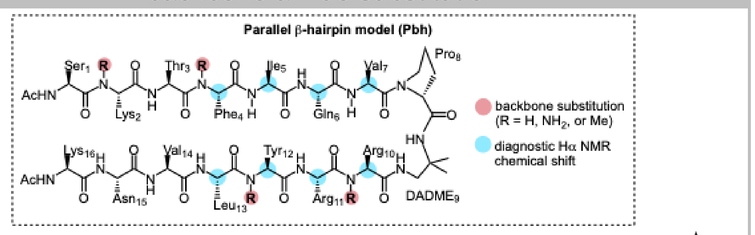
Homotypic interactions between parallel β-strands are characteristic of amyloids that underlie several neurodegenerative diseases.[1] Minimalist approaches to stabilize parallel sheet conformation can inform the design of β-strand mimics and selective ligands of pathological amyloids. Backbone amide substitution can be used to enforce specific peptide conformations without sacrificing side chain content. We previously demonstrated that backbone N-amination of linear peptides stabilizes antiparallel β-sheet folds through increased torsional strain, *cis* amide lone pair repulsion, and intraresidue hydrogen bonding.[2] Here, we investigate the impact of backbone amide N-amination on parallel β-sheet stability in a peptide parallel hairpin model, as well as in a miniprotein that exhibits a βαβ tertiary fold.

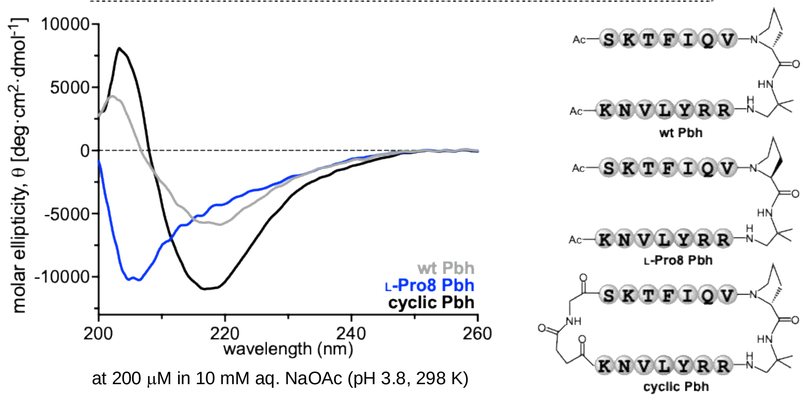
Study Objectives: (1) Determine the effect of α-hydrazino acid substitution on the folded population of a parallel β-hairpin model peptide[3]; **(2)** Incorporate α-hydrazino acids into the β-strand region of DS119 analogues and assess their impact on thermal stability[4]



RESULTS

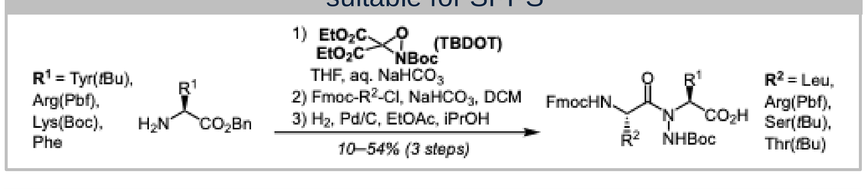
1) A parallel β-hairpin (Pbh) model for investigating the impact of backbone amide substitution



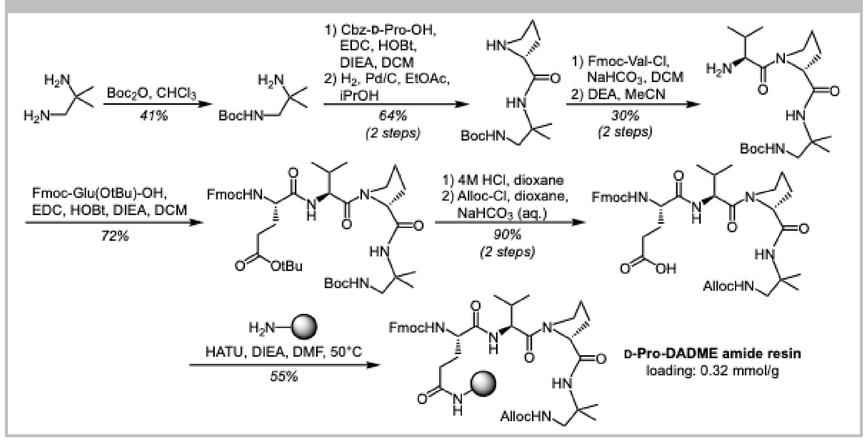


at 200 μM in 10 mM aq. NaOAc (pH 3.8, 298 K)

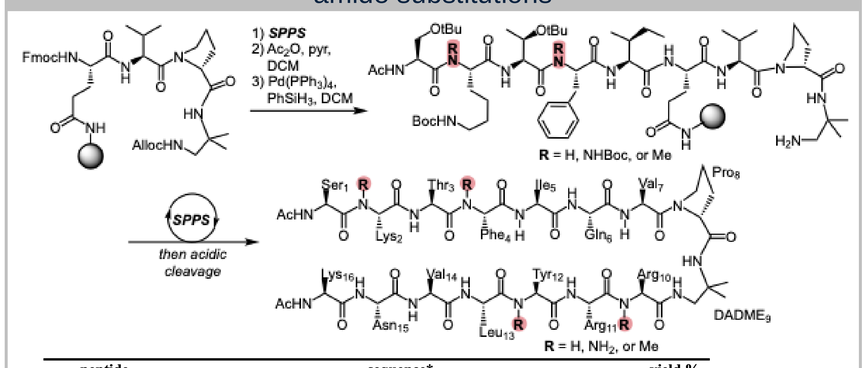
2) Synthesis of N-amino dipeptide building blocks suitable for SPPS



3) Synthesis of the resin-bound turn D-Pro-DADME motif



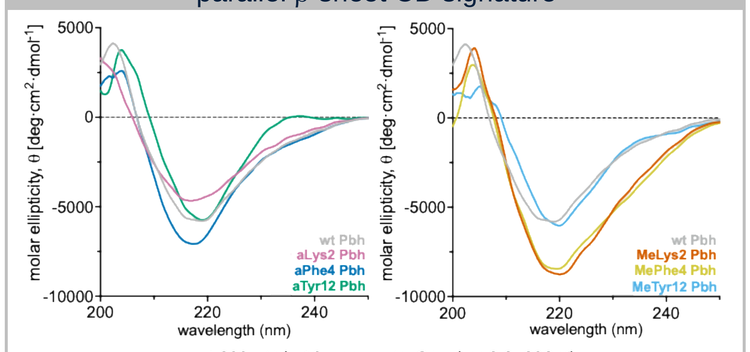
4) SPPS of parallel β-hairpin peptides featuring backbone amide substitutions



peptide	sequence*	yield %
wt Pbh	Ac-S K T F I Q V-^{D}P-dadme-R R Y L V N K-Ac	30
L-Pro8 Pbh	Ac-S K T F I Q V-^{L}**P**-dadme-R R Y L V N K-Ac	32
cyclic Pbh	**c[G**-S K T F I Q V-^{D}P-dadme-R R Y L V N K-**suc]**	10
aLys2 Pbh	Ac-**SaK** T F I Q V-^{D}P-dadme-R R Y L V N K-Ac	23
MeLys2 Pbh	Ac-**SMeK**T F I Q V-^{D}P-dadme-R R Y L V N K-Ac	31
aPhe4 Pbh	Ac-S K **TaF** I Q V-^{D}P-dadme-R R Y L V N K-Ac	15
MePhe4 Pbh	Ac-S K **TMeF**I Q V-^{D}P-dadme-R R Y L V N K-Ac	19
aTyr12 Pbh	Ac-S K T F I Q V-^{D}P-dadme-R R**aY L** V N K-Ac	12
MeTyr12 Pbh	Ac-S K T F I Q V-^{D}P-dadme-R R**MeYL** V N K-Ac	8
aArg10 Pbh	Ac-S K T F I Q V-^{D}P-dadme-**aRR** Y L V N K-Ac	11
MeArg10 Pbh	Ac-S K T F I Q V-^{D}P-dadme-**MeRR**Y L V N K-Ac	19

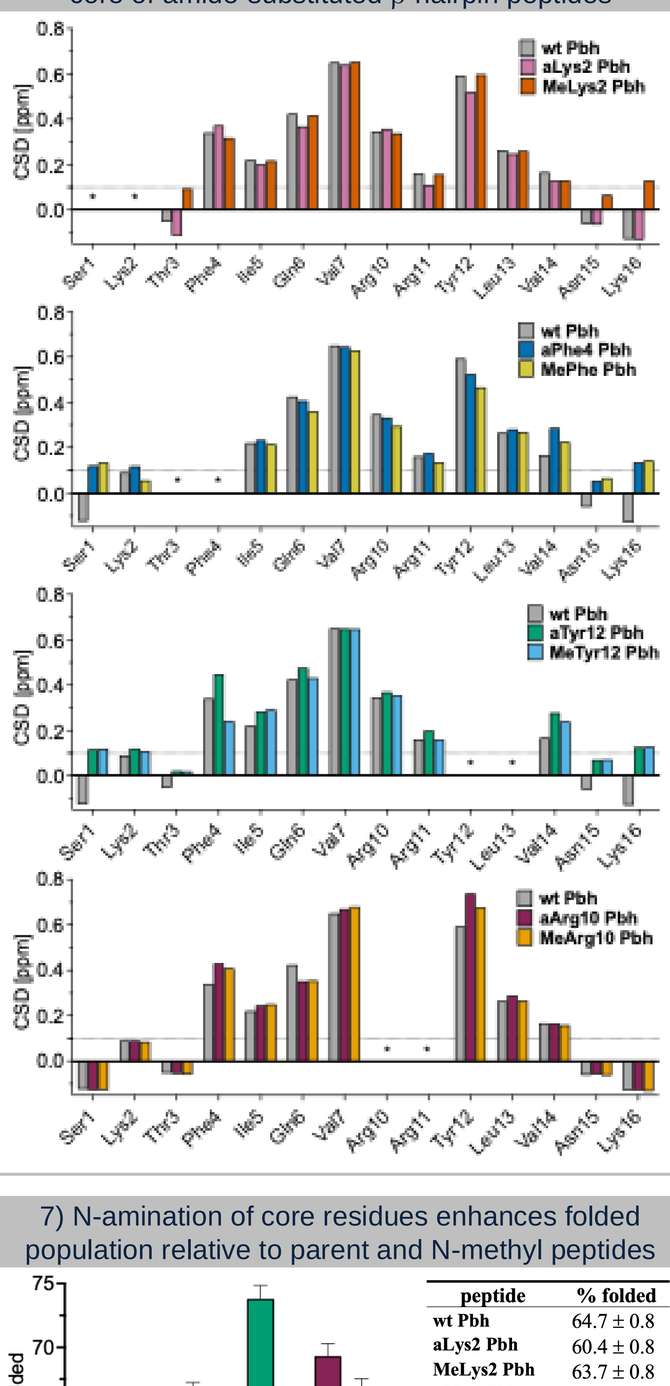
* aX = Nα-amino Xaa, MeX = Nα-methyl Xaa

5) Backbone N-amino and N-methyl peptides retain a parallel β-sheet CD signature

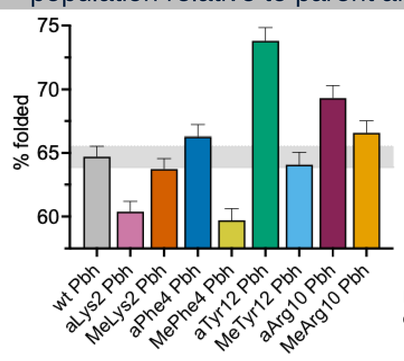


at 200 μM in 10 mM aq. NaOAc (pH 3.8, 298 K)

6) Hα NMR chemical shift deviations define the folded core of amide-substituted β-hairpin peptides



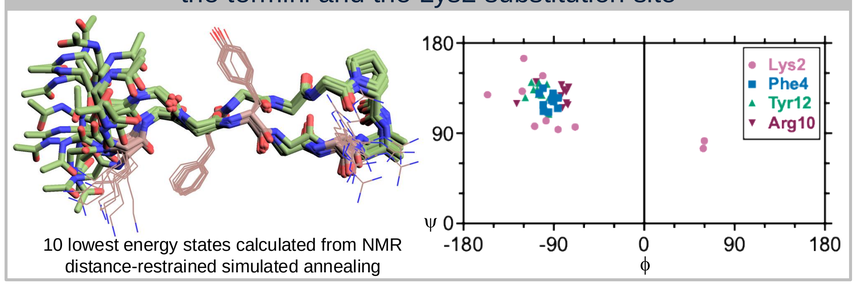
7) N-amination of core residues enhances folded population relative to parent and N-methyl peptides



peptide	% folded
wt Pbh	64.7 ± 0.8
aLys2 Pbh	60.4 ± 0.8
MeLys2 Pbh	63.7 ± 0.8
aPhe4 Pbh	66.3 ± 0.9
MePhe4 Pbh	59.7 ± 0.9
aTyr12 Pbh	73.8 ± 1.0
MeTyr12 Pbh	64.1 ± 1.0
aArg10 Pbh	69.3 ± 1.0
MeArg10 Pbh	66.6 ± 1.0

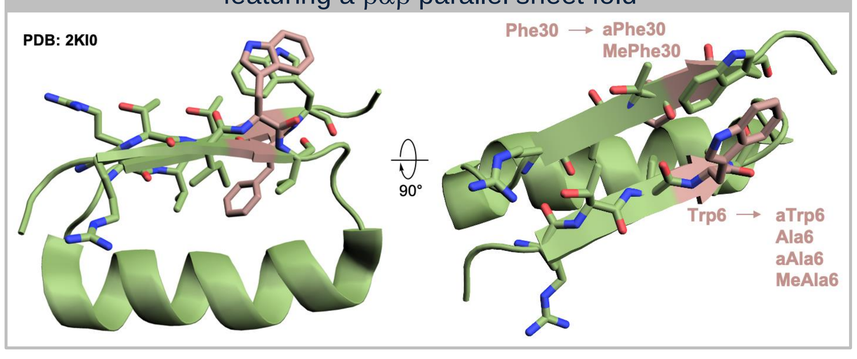
Derived from NMR CSD values of Hα of reporter residues in D_2O (800 MHz, 100 mM AcOD, pH 3.8, 280 K)

8) NMR-derived structures of wt Pbh reveal significant fraying at the termini and the Lys2 substitution site



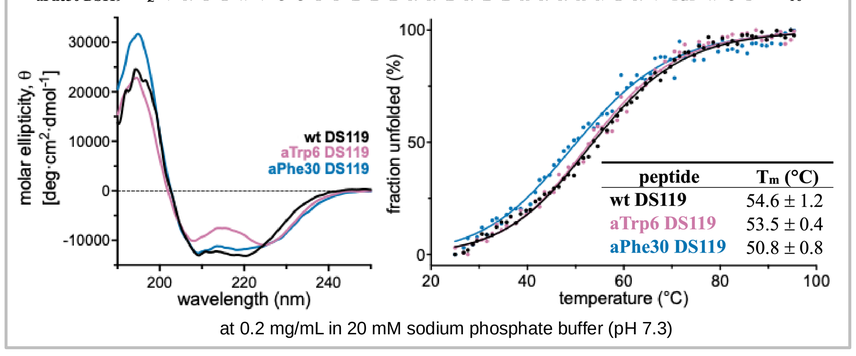
10 lowest energy states calculated from NMR distance-restrained simulated annealing

9) Backbone substituted analogues of DS119: a miniprotein featuring a βαβ parallel sheet fold



10) N-Aminated DS119 analogues retain wild-type fold and thermal stability in 1M Gdn·HCl

peptide	sequence	yield %
wt DS119	Q V R T I W V G G T P E E L K K L K E E A K K A N I R V T F W G D	9
aTrp6 DS119	Q V R T **IaW** V G G T P E E L K K L K E E A K K A N I R V T F W G D	6
aPhe30 DS119	Q V R T I W V G G T P E E L K K L K E E A K K A N I R V **TaF** W G D	10



peptide	T_m (°C)
wt DS119	54.6 ± 1.2
aTrp6 DS119	53.5 ± 0.4
aPhe30 DS119	50.8 ± 0.8

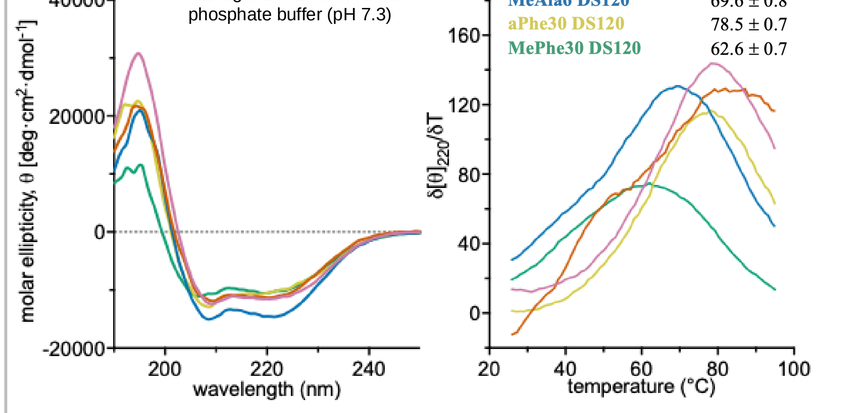
at 0.2 mg/mL in 20 mM sodium phosphate buffer (pH 7.3)

11) Backbone N-amination restores thermal stability in DS120

peptide	sequence	yield %
wt DS120	Q V R T I **A** V G G T P E E L K K L K E E A K K A N I R V T F W G D	7
aAla6 DS120	Q V R T **IaA** V G G T P E E L K K L K E E A K K A N I R V T F W G D	5
MeAla6 DS120	Q V R T **IMeA**V G G T P E E L K K L K E E A K K A N I R V T F W G D	14
aPhe30 DS120	Q V R T I **A** V G G T P E E L K K L K E E A K K A N I R V **TaF** W G D	12
MePhe30 DS120	Q V R T I **A** V G G T P E E L K K L K E E A K K A N I R V **TMeF**W G D	5

peptide	T_m (°C)
wt DS120	78.6 ± 0.6
aAla6 DS120	82.1 ± 0.1
MeAla6 DS120	69.6 ± 0.8
aPhe30 DS120	78.5 ± 0.7
MePhe30 DS120	62.6 ± 0.7

at 0.2 mg/mL in 20 mM sodium phosphate buffer (pH 7.3)



CONCLUSIONS

We incorporated α-hydrazino acids into models of folded secondary and tertiary structure. In the parallel β-sheet hairpin model, N-amination was well tolerated at residues in the structured core, but not at the frayed N-terminus. N-Amination of Arg10 and Tyr12 resulted in a significant increases in folded population relative to unsubstituted and N-methylated analogues. In the DS119 miniprotein, N-amination at Trp6 and Phe30 preserved the βαβ fold and had minimal effect on miniprotein stability. DS120, a DS119 analogue with a lower melting temperature, was stabilized upon N-amination of the Trp6 residue. As in the parallel β-hairpin model, α-hydrazino acids were better tolerated in the strand regions relative to N-methyl amino acids.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (CHE2109008) and the National Institute on Aging (NIH R01AG074570). SKS was supported by a Notre Dame CBBI fellowship through the NIH (T32GM145773). We thank the ND Nuclear Magnetic Resonance and Mass Spectrometry Core Facilities for access to instrumentation.

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[1] Taylor and Staniforth, *Front. Neurosci.* **2022**, *16*, e878869; [2] Angera et al., *Acc. Chem. Res.* **2024**, *57*, 1287; [3] Fisk et al., *J. Am. Chem. Soc.* **2006**, *128*, 7148; [4] Liang et al., *Angew. Chem.* **2009**, *48*, 3301.