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TITLE: Synthesis and Characterization of γ-Peptide Foldamers

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ABSTRACT

Synthesis and Characterization of Constrained γ-Peptide Foldamers

Elucidating folding properties of unnatural amino acids has enabled the start of function-directed design. Two types of γ−amino acids are probed for their abilities to fold into helical secondary structure. Both γ-amino acids have backbone constraints that arise from a cyclohexane or cyclopentane respectively. The cyclohexyl monomer with and N-terminal Boc-protected gabapentin residue adopts a 14-helical structure in solution when extended beyond a trimer while an α/γ-oligomer of the cyclopentyl constrained γ-amino acid has not yet been show to adopt any helical folds from 2D-NMR experiments. Collaboration with the Timothy Zwier group at Purdue University has led to preliminary conclusions about the energetic contributions of amide stacking in γ-amino acids.

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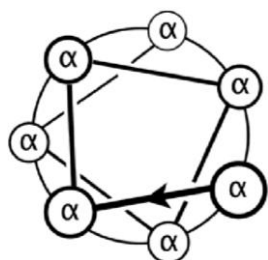Sam Gellman /Chemistry
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Abstract

Elucidating folding properties of unnatural amino acids has enabled the start of function-directed design. Two types of $\gamma-$amino acids are probed for their abilities to fold into helical secondary structure. Both γ-amino acids have backbone constraints that arise from a cyclohexane or cyclopentane respectively. The cyclohexyl monomer with and N-terminal Boc-protected gabapentin residue adopts a 14-helical structure in solution when extended beyond a trimer while an α/γ-oligomer of the cyclopentyl constrained γ-amino acid has not yet been show to adopt any helical folds from 2D-NMR experiments. Collaboration with the Timothy Zwier group at Purdue University has led to preliminary conclusions about the energetic contributions of amide stacking in γ-amino acids.

Introduction

The study of protein folding is an extraordinarily active area of research normally concerned with the folding of natural protein products derived from polymers of α-amino acids. Oligomers incorporating unnatural amino acids that adopt secondary structure have been dubbed foldamers.[1] Folding and activity of peptides is due in part to the side chains that protrude from the peptide backbone. Analogous secondary structures to α-peptides such as helices and sheet structures have been found in these unnatural oligomers.[2] These higher order structured foldamers can have interesting functional properties such as antibacterial activity.[3] Many of the small molecules designed for protein-protein interaction purposes have not been very successful, but foldamers show promise in this field.[4] The inability of proteases to degrade these unnatural peptides makes them attractive pharmaceutical candidates. In α-amino acids, the side chains are displayed

Figure 1. α-helical wheel diagram.

in a regular pattern shown in the helical wheel diagram in Figure 1. Upon incorporation of $\beta-$ and γ-

amino acids, the side chains are displayed in differing arrays. These different patterns allow for novel

designs when developing protein-protein antagonists. Expanding our knowledge of foldameric structure

will allow us to explore the role of foldamers in medicinal chemistry and other uses for foldamers such

as organocatalysis. As more is learned about how these oligomers fold, more can be done to engineer

their use for myriad purposes.
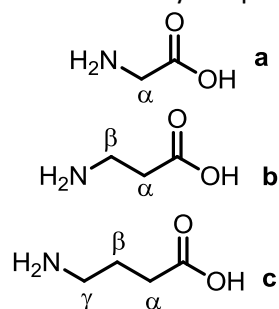
Figure 2. Comparison of α-(a), β-(b), and γ-(c) amino acid backbones.

Extensive research has been done on β-amino acids which have

an extra methylene unit in their backbone compared to an α-amino acid

(Figure 2b).[5] Having another carbon in the amino acid backbone increases

the amount of flexibility in the backbone making the entropic cost of

folding higher not regarding solvent and other folding parameters.

Peptides containing these β-residues have been shown to adopt helical

secondary structures, especially when the carbon backbone is constrained with a carbocyclic constraint,

and some of these α/β-peptides form quaternary structure.[6] Taking the β-peptide concept one step

further was the advent of incorporating γ-amino acids into the foldameric landscape. Hetero-oligomers

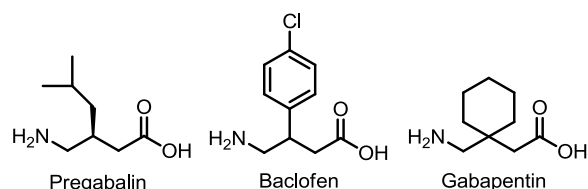were then developed containing various permutations of α-, β-, and γ–amino acids. Guo et al.[7]
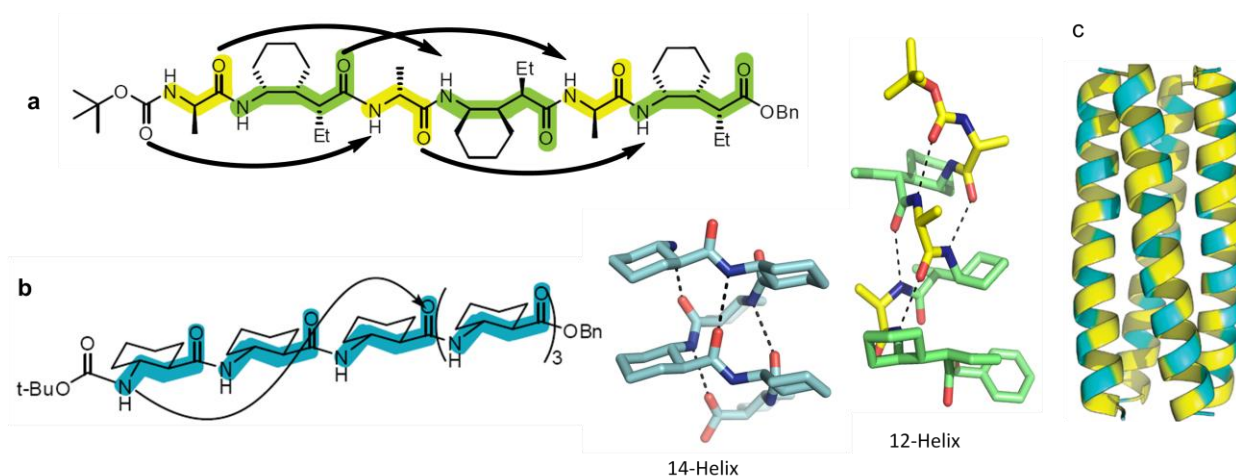
Figure 4. (a) α/γ 12-helix with H-bonds shown with arrows. (b) β-peptide 14-helix. (c) Four-helix bundle of α/β-peptides.

demonstrated that a 1:1 α/γ-peptide can adopt a 12-helix (Figure 4a).

If we can determine what structural constraints are necessary for oligomer folding, we can then tailor these oligomers to meet specific needs in areas like organocatalysis and protein-protein antagonist development. Although secondary structure has been found in unconstrained β− and γ-peptides, these structures are usually sheets rather than helices.[8] As it had been shown that folding propensity could be increased by adding cyclic structural constraints to β−residues, the same constraints would need to be applied to γ-residues that have even more freedom than β-residues.[9] Also, as α-amino acid chains become longer, they have been known to more strongly adopt secondary structure, and the same concept was applied to the creation of the γ-oligomers. If folding was not seen in shorter peptides, the length would be extended to enhance folding. Solvent also becomes of concern because hydrogen bond interactions intramolecularly will be stronger in a nonpolar solvent as opposed to a more polar protic solvent such as methanol. Although the foldameric properties of theses residues is of great interest, the γ-monomers themselves also show promise.

The lack of research in the realm of γ-amino acids has stemmed from a lack of stereochemically pure building blocks. In 2007, Chi, et al. developed a methodology that involves an asymmetric conjugate (1,4-) addition of aldehydes to nitroalkanes using a chiral pyrrolidine catalyst.[10] This methodology provided cyclically constrained γ-amino acid building block from which to create oligomers. Of note is the organocatalytic asymmetric nature of this reaction that sets three contiguous stereocenters with high selectivity which is fairly uncommon, and a metal catalyst is more often used to perform similar reactions.[11] An application of γ-amino acid monomers is their use as pharmaceuticals such as Pregabalin, gabapentin, and Baclofen, which are potent effectors of the nervous system (Figure 5). All of these drugs are derivatives of γ-aminobutyric


Figure 5. Derviatives of GABA.

acid (GABA, Figure 2c), a neurotransmitter.[12] Pregabalin is used as an anti-seizure drug and more recently to treat neuropathic pain.[13] These cyclopentyl and cyclohexyl γ-amino acid monomers studied in this thesis could lead to structural parameters for forming helical foldamers and to gaining more insight into designing foldamers with specific functions.

I. Cyclohexane Constrained γ-Peptide Foldamers

A γ−amino acid monomer was synthesized that contained a cyclohexane moiety between the β− and γ-backbone carbons. The synthetic scheme for this monomer is shown in Figure 6a (characterizations for these and other γ-monomers can be found in the supporting information of Guo, et al.).[10] The asymmetric conjugate addition sets three stereocenters simultaneously with high selectivity. Ideally, a dipeptide of this residue would have been made and analyzed for its folding properties and possibly be used as a starting point for longer peptides; however, coupling conditions for dipeptide formation results in the formation of a γ-lactam from the N-Boc protected monomer. Avoiding γ-lactam formation meant incorporating of a Boc protected gabapentin residue at the N-terminus (Figure 6a, bottom). The hypothesis was that gabapentin has some backbone constraints with the 1,1-disubstituted cyclohexane on its β-carbon and should contribute to the folding of the oligomer overall.[14] **1** is the same γ-residue that was used in the α/γ-peptide shown in Figure 4a, and it was also used to make a β/γ-helix.[15] A trimer was made, composed of Boc-gabapentin/1/1-OBn (Figure 6b, **3**), and this molecule took on a 9-helical fold for both the gabapentin and γ−residue as corroborated by the crystal structures (Figure 6c).[16] As the peptide was extended, the gabapentin retained the 9-helical fold (C=O(i)- -H-N(i+2)), but our γ−monomer took on a 14-helix (i,i+3 C=O--H-N) H-bond (Figure 6b,c). This conformational change that arises from extending the peptide indicates a stronger intrinsic preference of **1** for the 14-helix over the 9-helix.
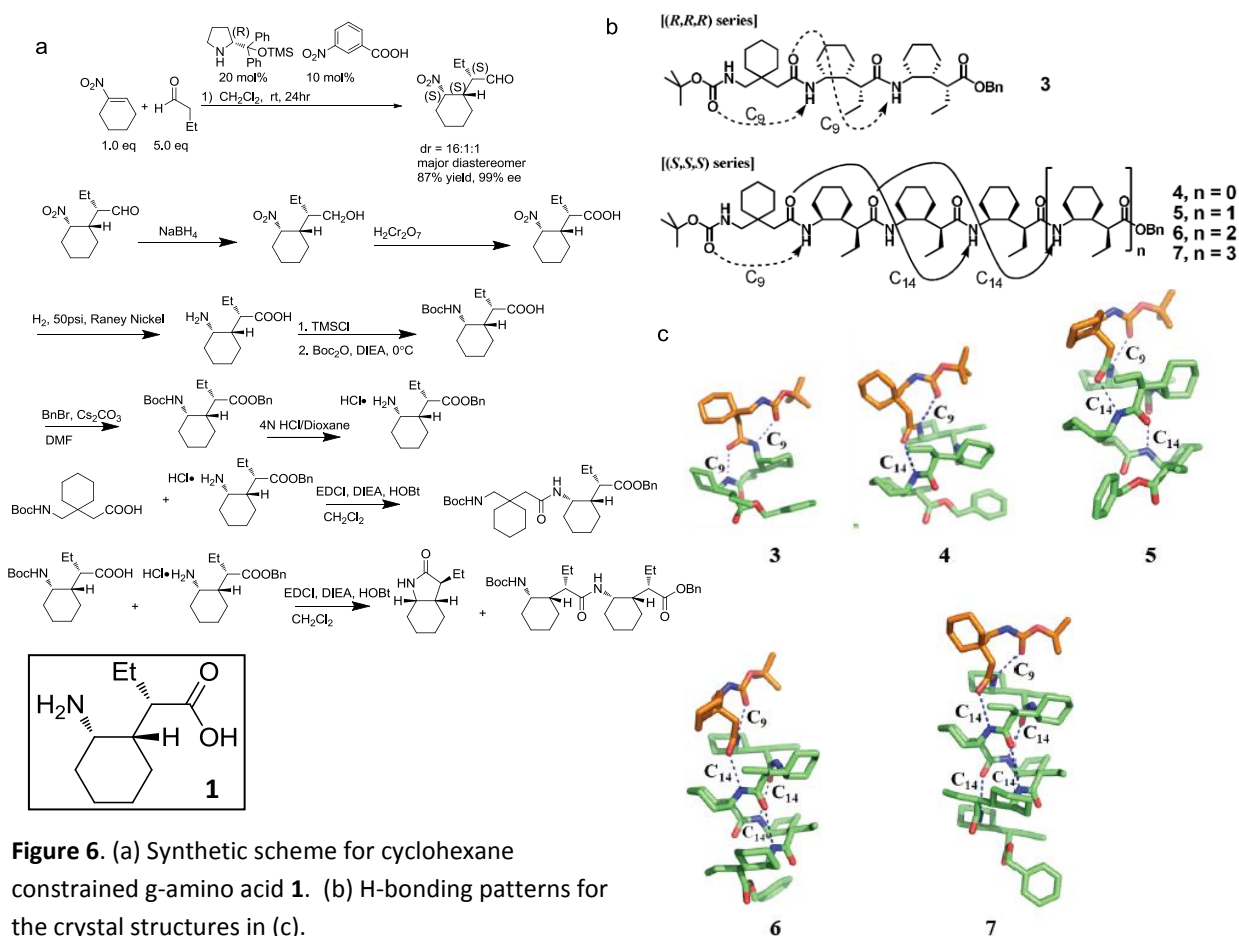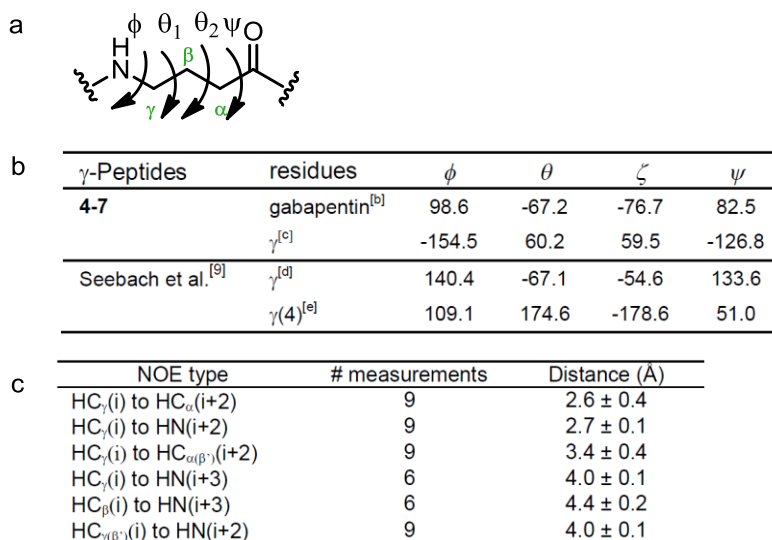
Figure 6. (a) Synthetic scheme for cyclohexane constrained g-amino acid **1**. (b) H-bonding patterns for the crystal structures in (c).

From the pattern that emerges with this γ–residue, we calculated average value for its torsion angles (torsion angle definitions in Figure 7a). The only other γ-residue that had been crystallized prior to ours had torsion angles that agreed with the average torsion angles found for our crystal structures Figure 7b. The expected mid-range NOEs from our crystal structures are shown in Figure 7c. 2D-NMR analysis by Seebach, et al.[17] of γ-peptides yielded NOEs that corresponded to the suspected hydrogen bonding patterns observed in our crystal structures. However, there were some observed NOEs from the Seebach et al. studies for a hexameric γ-peptide that were not seen in our crystal structure, giving evidence that there is some conformational flexibility when in solution (CDCl$_3$). One of these inconsistent NOEs displayed a strong C$_{\gamma(\beta')}$H(i)–NH(i+3) NOE, where C$_{\gamma(\beta')}$H indicates a proton on the first carbon (β') of a side chain attached to the backbone γ-carbon, but the crystallographic data indicate that this H—H distance is typically 5.3+0.2 Å in the 14-helix (six measurements). Another proton displayed a

medium-intensity $C_\gamma H(i)$–$C_\alpha H(i+3)$ NOE, but the crystallographic data indicate an H—H distance of 5.9+0.2 Å (six measurements). Interestingly, the gabapentin residue adopts torsion angles of opposite sign to (our $\gamma-$residue).

Collaborations with Professor Timothy Zwier of Purdue University have been ongoing since the research on α/β-peptides began. The Zwier group studies the folding effects of our short peptides in the gas phase. They have put great effort into elucidating the contributions of amide stacking interactions on folding in the single molecule state. The amide stacking interactions are predicted to be antiparallel and arise due to the juxtaposition of the amide groups based on the γ-peptide backbone structure. These interactions are much more difficult to detect than the standard C=O--HN H-bonds that are much more easily detected by NMR. Using double resonance spectroscopy (UV and IR spectral signatures) and computer analysis, they can assign specific folding

a

ϕ θ_1 θ_2 ψ O

b

γ-Peptides	residues	ϕ	θ	ζ	ψ
4-7	gabapentin[b]	98.6	-67.2	-76.7	82.5
	γ[c]	-154.5	60.2	59.5	-126.8
Seebach et al.[9]	γ[d]	140.4	-67.1	-54.6	133.6
	$\gamma(4)$[e]	109.1	174.6	-178.6	51.0

c

NOE type	# measurements	Distance (Å)
$HC_\gamma(i)$ to $HC_\alpha(i+2)$	9	2.6 ± 0.4
$HC_\gamma(i)$ to $HN(i+2)$	9	2.7 ± 0.1
$HC_\gamma(i)$ to $HC_{\alpha(\beta\cdot)}(i+2)$	9	3.4 ± 0.4
$HC_\gamma(i)$ to $HN(i+3)$	6	4.0 ± 0.1
$HC_\beta(i)$ to $HN(i+3)$	6	4.4 ± 0.2
$HC_{\gamma(\beta\cdot)}(i)$ to $HN(i+2)$	9	4.0 ± 0.1

Figure 7. (a) Definition of torsion angles for γ-amino acids. (b) Average torsion angles from the crystal structures in Figure 6 compared to Seebach et al.[17] [b] Average backbone torsion angles of gabapetin residues in **4-7**. [c] Average backbone torsion angles of γ-residues derived from **1** in **4-7**, excluding the C-terminal residue in each case (14 independent γ-residues from **4-7** were used to generate the torsion angle averages). [d] Average backbone torsion angles of the first three $\gamma-$residues in the tetra-γ-peptide crystal structure reported in ref. 16. [e] Torsion angles of the C-terminal residue in the tetra-γ-peptide crystal structure reported in ref. 17. (c) Average H—H distances (Å) in crystal structures of **4-7** corresponding to medium-range NOE patterns expected to be characteristic of γ-peptide 14-helix formation in solution.

conformations to specific IR signatures based on density functional theory calculations. These data provide information on the intrinsic folding behavior of the oligopeptide backbones in the absence of
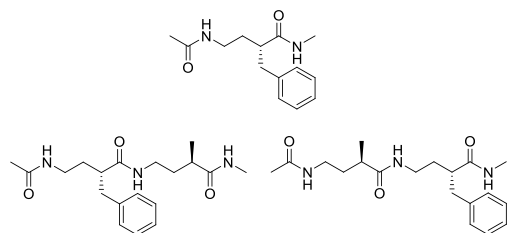
solvent effects. This information can be used in conjunction with the information obtained on the longer peptides in the solid and solution states.[18]

Molecules that were synthesized for these spectroscopic purposes are shown in Figure 8. These molecules are not necessarily expected to show the same helical propensities as the longer γ−peptides

Figure 8. γ−residues synthesized for spectroscopic analysis.

in the solution phase, but looking at these characteristics in the gas phase will provide more information as to the residue specific folding propensities at the most minimal level of structure, a single molecule. With the efforts of the Zwier group we will be able to determine the energy contributions of amide stacking interactions and their energetic competition with H-bonds. More information about amide-stacking interactions could lead to another parameter to consider when designing foldamers.

II. Cyclopentane Constrained γ-Amino Acid Foldamers

The creation of a heteropeptide with a repeating α/γ pattern is another way to probe the structural constraints that need to be in place for γ-residues to form helices. A precedent has been set with α/β-peptides and their propensity for helicity, so the next step is to make heterogeneous backbones incorporating γ-amino acids.[19] All of these strategies for foldamer design are ways to find molecules of value. According to Hofmann's calculations, a 12-helix is the lowest energy helical conformation for an α/γ-peptide octamer with no side chains or constraints in aqueous solution.[20] Our constraints impose specific torsion angles that should help promote some type of helical fold even if it is not a 12-helix. In peptides incorporating β-amino acids with a cyclopentyl constraint, unique helical structures have been observed, setting a precedent for using this constraint.[21] Hofmann's calculations can be taken as a starting point from which to design peptides with torsion angles that should lower the

energy required to adopt a helix. Our hypothesis was that our α/γ-peptide would fold into a 10/12-, 15/17- or 18/20-helix which was predicted from a crystal structure of a dipeptide (Boc-D-Alanine/**2**-OBn) whose torsion angles were compared to Hofmann's calculations.

Figure 9. Synthetic scheme for γ-amino acid **2** and α/γ tetramer (Boc-D-Phe/**2**)$_2$
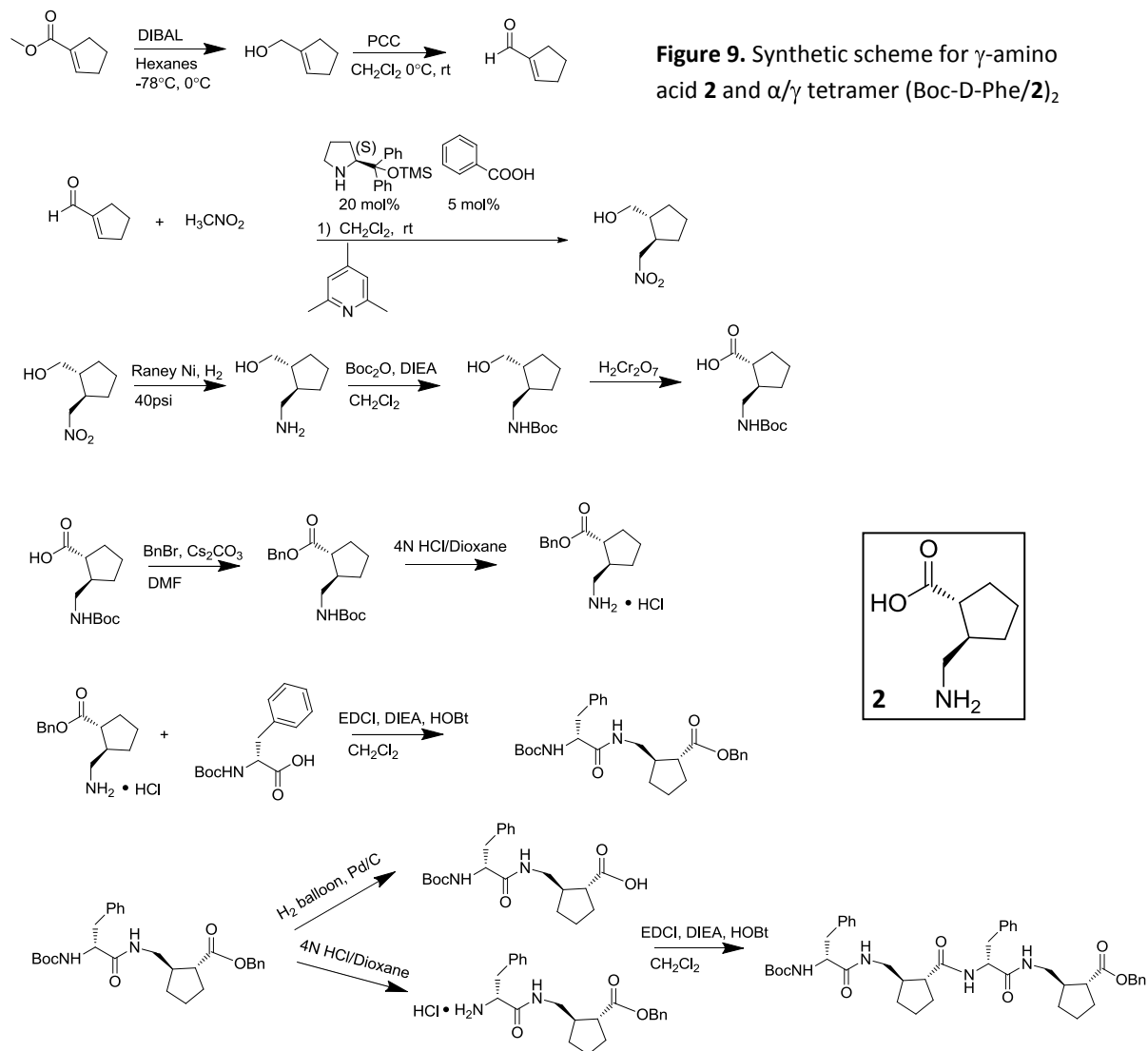
A different monomer containing a cyclopentane moiety between the α- and β-carbons (**2**) was synthesized. A different synthetic scheme needed to be employed to create this monomer, and there were two stereocenters set in the conjugate addition step as opposed to the three set during the aforementioned monomer synthesis (Figure 9). Also, **2** has no γ-carbon substituent which will contribute to the increased flexibility of this monomer compared to **1**. An α/γ tetramer, hexamer, and octamer of

D-alanine and **2** was made, but it was difficult to crystallize (Figure 10). In order to promote helical folds rather than unstructured local conformational effects, a longer α/γ-peptide a Boc-D-Phe/2 tetramer was made and coupled to the D-Ala/γ hexamer (Figure 10). The decamer formed from this coupling was purified, but it has not yet been possible to obtain a 2D-NMR or crystallographic structure of the molecule due to its low solubility in several deuterated solvents. [H]NMR can provide some preliminary evidence for folding if the amide NH protons are shifted downfield signifying interactions with other groups, but 2D-NMR and x-ray crystallography are more definitive ways of elucidating structure. A [H]NMR
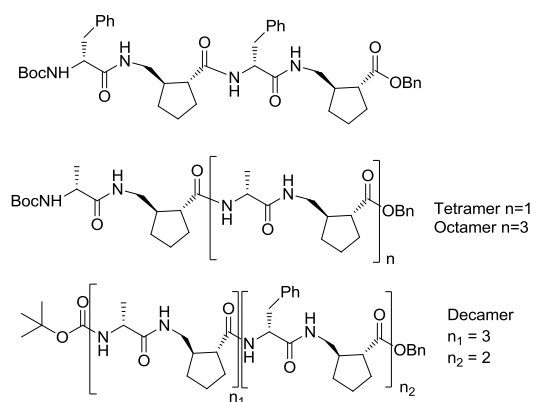
Figure 10. Structues for the Boc-D-Phe/**2**-OBn tetramer, Boc-D-Ala/**2**-OBn tetramer and octamer, and the Boc-(D-Ala-**2**)$_3$(D-Phe/**2**)$_2$-OBn decamer.

spectrum has been obtained in CDCl$_3$ and CD$_3$OH, but the broad peaks indicate aggregation of the peptide which is borne out by the white precipitates that form after a few hours in an NMR tube (Figure 11 in Supplementary Figures). A mass for this compound has been obtained M+Na of 1366.1 Da and the compounds expected exact mass is 1340.78 Da. Although the decamer was insoluble, 2D-NMR and [H]NMR data has been obtained for the tetramer, hexamer, and octamer (Figure 12 in Supplementary Figures).

The NOEs for the hexamer and octamer are not consistent with any known structure and certainly not consistent with any of the expected helical patterns. These peptides have been difficult to crystallize so no x-ray data is available on them, and this also provides support to the notion that they are unstructured because unstructured molecules will have more difficulty packing into an ordered lattice. The hypothesized 15/17- and 18/20-helical patterns may require longer peptides due to the large H-bond rings that need to be formed for those helical patterns. Also, incorporating other α-amino acids into the backbone could help with ordering the structure. Inability to crystallize, difficulty in obtaining

longer peptides, and decreasing solubility with increasing length has hindered further progress with these peptides.

The unfolded nature of these peptides indicates that this residue might not be constrained in the correct way to promote helical structures. This provides us with important information as to the propensities of **2**. Constructing a homopeptide of **2** may lead to more definitive characterization of its ability or inability to adopt a helical conformation.

Conclusion

With the data obtained in these studies it is evident that there are certain constraints that need to be applied to obtain helical folds. The γ-amino acid **1** does show helical propensity in the crystalline state in a way that is mostly consistent with other Seebach's 2D-NMR data.[15] Regardless of the necessary gabapentin residue at the N-terminus, **1** still adopted a 14-helical structure after extending beyond the tripeptide. **2** did not show any folding propensities after 2D-NMR analysis but this could be a consequence of the peptide being too short to form any of the helices predicted by Hofmann et al.[20]

Expanding the synthetic methodology of these monomers to include more diverse side chains is in progress. Accessing more building blocks with which to create these monomers will allow for even more in depth elucidation of the specific folding parameters. One area that has not been studied with these $\gamma-$monomers is their application as GABA derivatives and their effects in mammals. The precedent for compound with similar structures has already been set, but these monomers are not simply 3-substituted GABA analogues. Also being able to control the stereochemistry of these molecules allows for even more possibilities in the medicinal chemistry and foldamer realms. As more is discovered about the folding parameters for these molecules, the simpler it will become to create unnatural peptides with specific functional properties.

Acknowledgements

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Supporting Figures
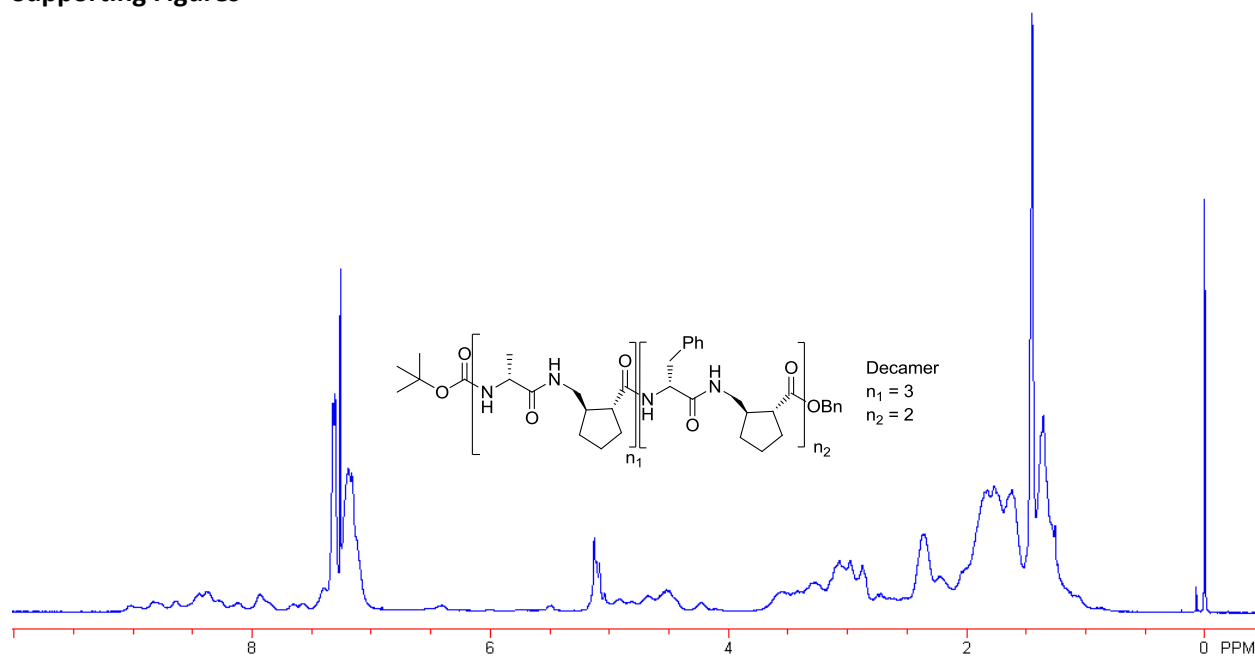
Figure 11. ^{H}NMR of decamer.
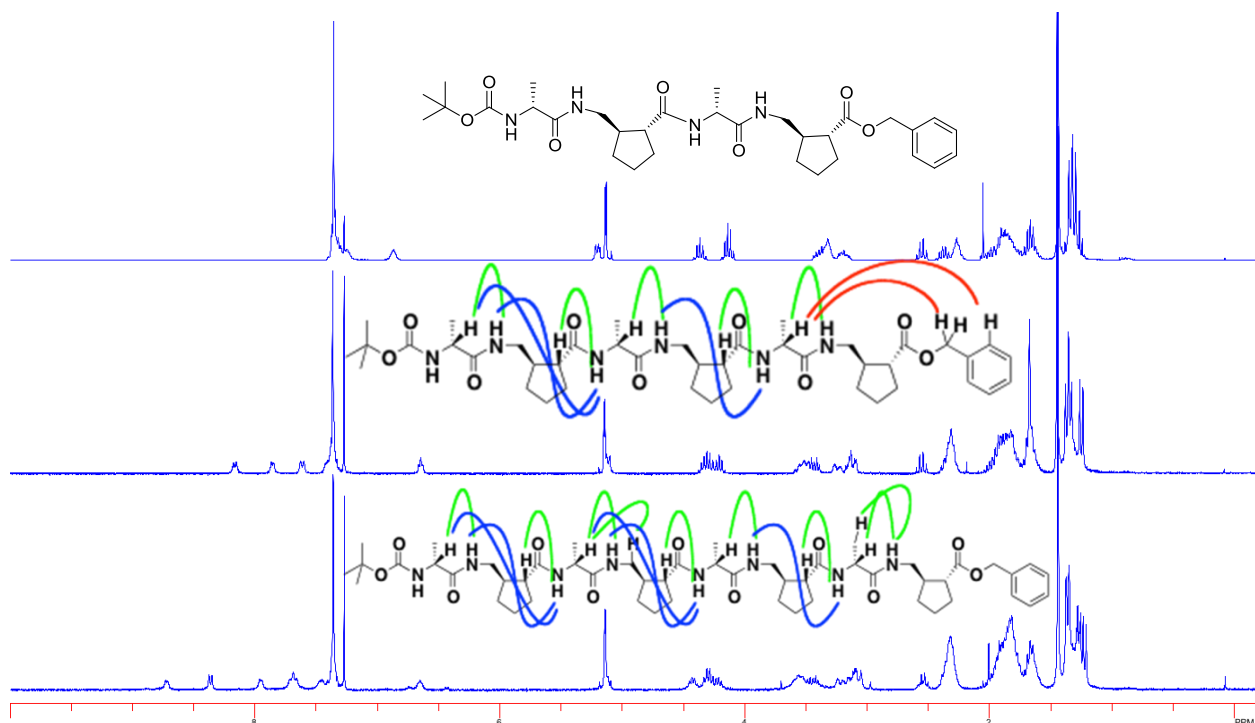
Figure 12. ^{H}NMR of the tetramer, hexamer, and octamer, of the D-Ala/γ peptide. The colored lines indicate NOEs obtained from 2D-NMR experiments. Green indicates sequential, blue indicates mid-range, and red indicates nonsequential NOEs respectively.