Peptide Design I

Two Helical Conformations from a Single Foldamer Backbone: "Split Personality" in Short α/β-Peptides**

Ahlke Hayen, Margaret A. Schmitt, Felix N. Ngassa, Kathryn A. Thomasson, and Samuel H. Gellman**

Oligomers that adopt predictable conformations ("foldamers") are subjects of increasing interest from the perspectives of both fundamental research and applications.[1] The study of unnatural oligomers that display secondary structures analogous to those of proteins or nucleic acids provides new insight on the parameters that influence the "foldability" of a backbone, e.g., the relationships between conformational stability and number of residues or residue flexibility. As the rules that govern shape are elucidated for new backbones, this

[*] Dr. A. Hayen,[+] M. A. Schmitt,[+] Prof. Dr. S. H. Gellman
Department of Chemistry
University of Wisconsin
1101 University Avenue, Madison, WI 53706 (USA)
Fax: (+1) 608-265-4534
E-mail: gellman@chem.wisc.edu

F. N. Ngassa, Prof. Dr. K. A. Thomasson
Department of Chemistry
University of North Dakota
Grand Forks, ND 58202 (USA)

[+] These authors contributed equally.

[**] This research was supported by the NSF (CHE-0140621). A.H. was supported in part by a postdoctoral fellowship from the DAAD (German Academic Exchange Service), M.A.S. in part by an NIH Molecular Biophysics Training grant, and F.N.N. in part by a North Dakota EPSCoR Doctoral Dissertation Fellowship. NMR spectrometers were purchased with partial support from the NSF and the NIH.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200352125

information can be applied to create foldamers with interesting functions.[2] Most unnatural foldamers reported so far have homogeneous backbones, i.e., they are constructed from building blocks of a single class (e.g., α- or β-amino acids), as is also the case for biological foldamers.[1] Heterogeneous backbones, composed of two or more residue types, can also display well-defined folding behavior, although this strategy for foldamer design has received relatively little attention to date.[3] The exploration of heterogeneous backbones is important because mixing monomer classes leads to an exponential increase in the range of potential foldamers. Increasing the number of distinct shapes that can be predictably achieved should enhance opportunities to endow foldamers with desirable activities.

Here we show that combining α-amino acid and cyclic β-amino acid residues in a sequentially alternating pattern can lead to helix formation within relatively short heterooligomers.[4] Structural data suggest that these foldamers have a "split personality," simultaneously populating two different helical conformations. Both helices contain backbone C=O···H–N hydrogen bonds with N→C directionality, but they differ in the sequential spacing between C=O and H–N ($i,i+3$ in one case, $i,i+4$ in the other). This behavior parallels closely the split personality observed among peptides composed exclusively of α-amino acid residues, which frequently populate both α- and 3_{10}-helical conformations in solution.[5] Our α/β-peptides differ from α-peptides, however, in that folding is observed with fewer residues in the former than in the latter. The different monomer types in the heterogeneous α/β-peptide backbone offer complementary benefits to the new foldamers we have identified: the constrained β-residues provide conformational preorganization, while the α-residues allow facile introduction of specific side chains at specific positions.

Initial two-dimensional NMR studies[6] were conducted with diastereomeric hexamers **1a**,**b** in CD_3OH (Figure 1). Both contain (*S*,*S*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC)[7] residues in the β-amino acid positions. The two lysine residues were selected to promote solubility in polar media, and the central tyrosine residue was placed to promote ^{1}H NMR resonance dispersion (by means of the magnetic anisotropic effects of the aromatic side chain). Proteinogenic L-α-residues (*S* configuration) were used for **1a**, while D-α-residues were used for **1b**. The signals in the ^{1}H NMR spectrum of oligomer **1a** were moderately dispersed, and a number of NOEs were detected between residues that are not

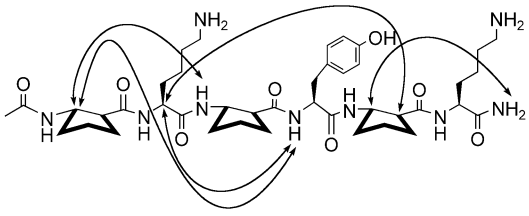


Figure 1. Graphical summary of unambiguously assigned NOEs involving sequentially nonadjacent residues for α/β-peptide **1a** in CD_3OH. α/β-Peptide **1b** is the diastereomer in which all α-residues have the opposite configuration (not shown); no nonsequential NOEs were detected for **1b**.

adjacent in sequence. Nonsequential NOEs of this type are strong evidence that the oligomer adopts a compact conformation, or perhaps multiple compact conformations, for at least part of the time. (Interconversion between folded and unfolded states, and between alternative folded states, is presumably rapid on the NMR timescale.) No nonsequential NOEs could be detected for **1b**, which suggests that this diastereomer has little propensity to fold under these conditions.

Five nonsequential NOEs could be assigned with certainty for **1a** (Figure 1); several other nonsequential NOEs were present but of ambiguous identity because of resonance overlap. The unambiguous nonsequential NOEs were consistent with formation of helices involving backbone hydrogen bonds from carbonyl groups to amide protons in the C-terminal direction. Each of the ambiguous nonsequential NOEs had a possible assignment that was consistent with this type of helix.[8] The same hydrogen-bond directionality is observed in the two most common helices formed by homogeneous L-α-residue backbones, the α-helix (13-membered-ring hydrogen bonds, C=O(i)···H–N($i+4$)) and the 3_{10}-helix (10-membered-ring hydrogen bonds, C=O(i)···H–N($i+3$)), and in the 12-helix[9] formed by homooligomers of (*S*,*S*)-ACPC (12-membered-ring hydrogen bonds, C=O(i)···H–N($i+3$)).

Structural conclusions based on the data for **1a** are tentative because relatively few nonsequential NOEs could be confidently assigned. Prior to the design of a second generation of α/β-peptides related to **1a**, intended to display greater NMR resonance dispersion, we used an ACPC/alanine dodecamer for computational modeling[10] of three helical conformations that contain backbone C=O···H–N hydrogen bonds with N→C directionality (Figure 2). These helices are named based on the characteristic hydrogen-bond ring size: 11-helix, 14/15-helix, and 18-helix (Figure 3). The model structures suggest that some nonsequential NOEs (or lack thereof) might allow distinctions to be drawn among the alternative helices (Table 1). For example, α-residue $H^{\alpha}(i)$/α-residue NH($i+2$) NOEs are expected for the 11-helix (3.8 Å average interproton distance predicted), but not for the 14/15- or 18-helix (>5 Å). In **1a**, one of the two possible NOEs of this class was observed. On the other hand, α-residue $H^{\alpha}(i)$/β-residue $H^{\alpha}(i+3)$ NOEs are expected for the 14/15-helix (3.0 Å) and the 18-helix (4.3 Å), but not for the 11-helix (>5 Å). In **1a**, the only possible NOE of this type was observed. (The other unambiguous NOEs observed for **1a** were two β-residue $H^{\beta}(i)$/β-residue NH($i+2$) NOEs and one β-residue $H^{\beta}(i)$/α-residue NH($i+3$) NOE, which the models indicate to be possible in all three helices). Model structures were constructed also for helices defined by hydrogen bonds with the opposite directionality, from carbonyl groups to amide protons in the N-terminal direction (the 9-, 12/13-, and 16-helices; not shown). This set of helical conformations could be ruled out because the NOEs predicted for these structures, mostly NH of residue i to H^{α} or H^{β} of residue $i+2$, $i+3$, or $i+4$, were not detected for **1a** or the oligomers discussed below.

α/β-Peptides **2** and **3** (Figure 4) were prepared in an effort to search for the $i,i+3$, $i,i+4$, and $i,i+5$ NOE patterns that

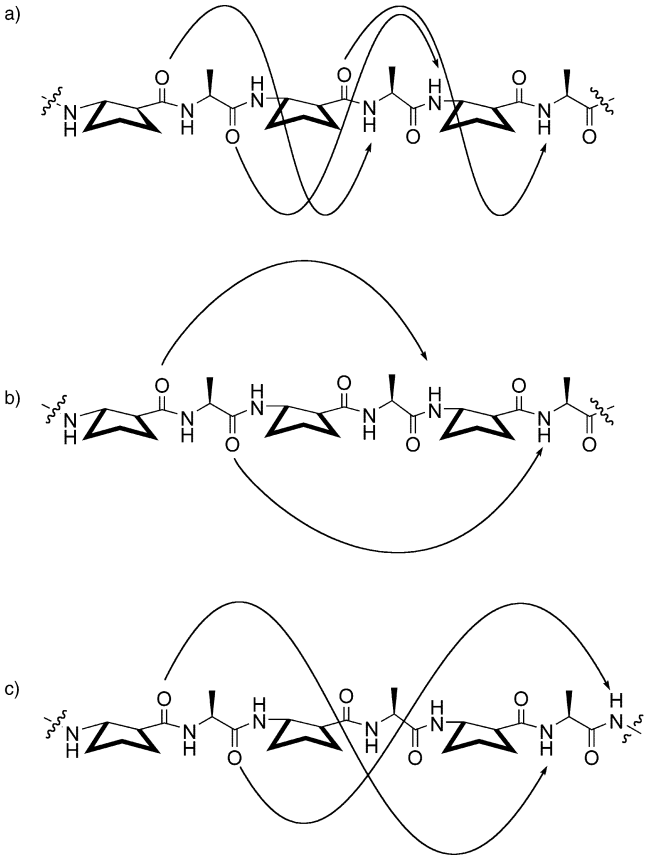


Figure 2. Hydrogen-bond patterns that define the helical secondary structures of the α/β-peptides considered here, with hydrogen bonds from carbonyl groups to amide protons in the C-terminal direction; a) 11-helix, b) 14/15-helix, c) 18-helix.

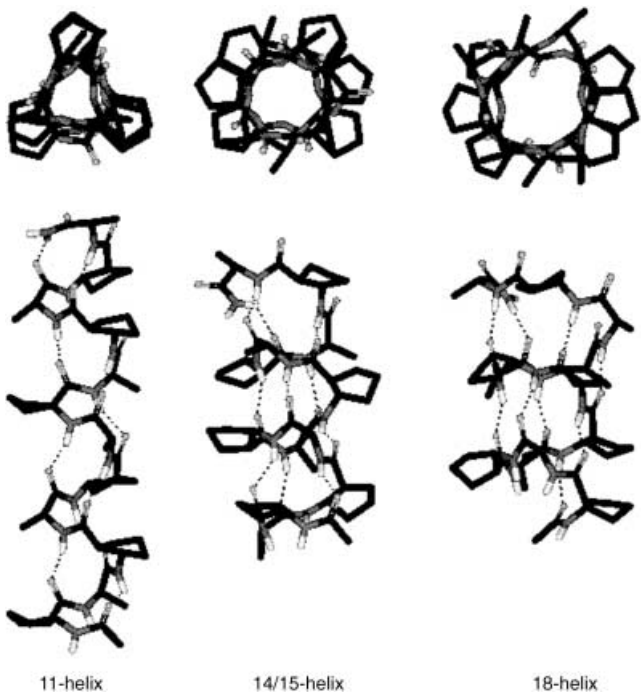


Figure 3. Model helices generated in SYBYL to examine possible conformations of α/β-peptides. Dotted gray lines indicate hydrogen bonds. The upper views are along the helical axis; the lower views are perpendicular to the helical axis.

would aid in identifying the helical conformation(s) adopted by this type of heterogeneous backbone. These new compounds contain eight residues, rather than the six in **1a**. They are stereochemical analogues of **1a**, but two of the β-residues are (3*R*,4*S*)-*trans*-3-aminopyrrolidine-4-carboxylic acid ((3*R*,4*S*)-APC) rather than (*S*,*S*)-ACPC. Four different L-α-residues were employed. Oligomers **2** and **3** differ only in the placement of the Lys and Glu residues.[11]

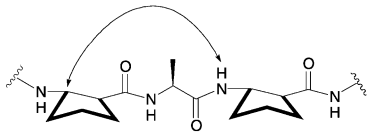
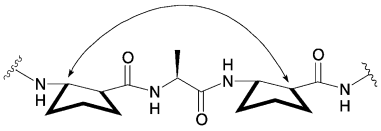
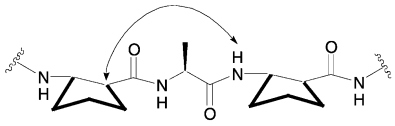
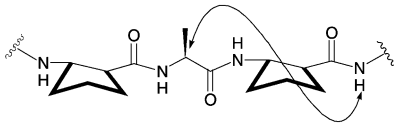
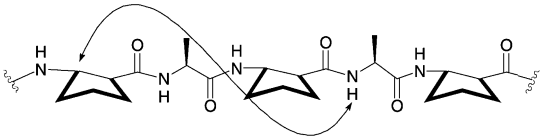
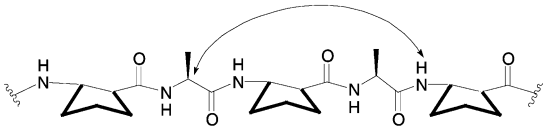
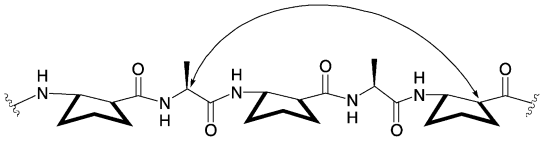
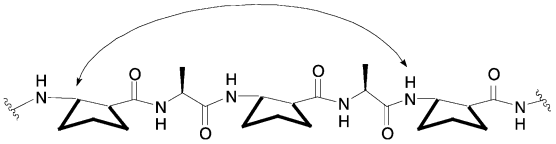
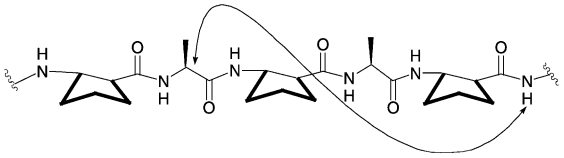
Oligomer **2** in CD_3OH displayed the best ^1H NMR resonance dispersion among the α/β-peptides we examined, although there was still some overlap. Eight of the 14 possible $i,i+2$ NOEs between backbone protons could be unambiguously identified (Table 2), including two of the four possible α-residue $H^{\alpha}(i)$/α-residue NH($i+2$) NOEs. As pointed out above, this type of NOE is suggested by our simple modeling to be consistent with only the 11-helix. Modeling suggests that three $i,i+3$ NOE patterns would be most likely among backbone protons in the three helical secondary structures considered: 1) β-residue $H^{\beta}(i)$/α-residue NH($i+3$), 2) α-residue $H^{\alpha}(i)$/β-residue NH($i+3$), and 3) α-residue $H^{\alpha}(i)$/β-residue $H^{\alpha}(i+3)$. Among these three patterns, the third is not expected for the 11-helix, but is consistent with the other two helices, as discussed above; two of the three possible NOEs of this type were observed for **2**. Our second predicted NOE pattern is consistent with the 11-helix (3.6 Å average interproton distance predicted) and the 14/15-helix

Table 1: Average interproton distances [Å] measured from model helices.[a]

NOE type	11-helix	14/15-helix	18-helix
β-residue $H^{\beta}(i)$/β-residue NH($i+2$)	3.3	3.9	4.6
β-residue $H^{\beta}(i)$/β-residue $H^{\alpha}(i+2)$	2.4	3.7	4.5
β-residue $H^{\alpha}(i)$/β-residue NH($i+2$)	4.3	4.1	3.8
α-residue $H^{\alpha}(i)$/α-residue NH($i+2$)	3.8	–	–
β-residue $H^{\beta}(i)$/α-residue NH($i+3$)	3.1	2.3	4.3
β-residue $H^{\beta}(i)$/α-residue $H^{\alpha}(i+3)$	–	4.6	–
α-residue $H^{\alpha}(i)$/β-residue NH($i+3$)	3.6	4.3	–
α-residue $H^{\alpha}(i)$/β-residue $H^{\alpha}(i+3)$	–	3.0	4.3
β-residue $H^{\beta}(i)$/β-residue NH($i+4$)	–	3.0	3.4
β-residue $H^{\alpha}(i)$/β-residue $H^{\alpha}(i+4)$	–	–	4.8
β-residue $H^{\beta}(i)$/β-residue $H^{\alpha}(i+4)$	–	–	2.4
α-residue $H^{\alpha}(i)$/α-residue NH(i+4)	–	4.0	3.1
β-residue $H^{\beta}(i)$/α-residue NH($i+5$)	–	–	3.7
α-residue $H^{\alpha}(i)$/β-residue NH($i+5$)	–	–	3.7

[a] Measurements less than 5 Å were used for predicting expected NOEs. "–" indicates that all distances were greater than 5 Å and no NOEs would be expected.

Table 2: Summary of NOEs involving nonsequentially adjacent residues for α/β-peptides **2** and **3** in CD_3OH.[a]

NOE type and residues	Oligomer **2**	Oligomer **3**	Structure
i_β $H^\beta \rightarrow i+2_\beta$ HN			
1→3	Yes*	?	
3→5	Yes	?	
5→7	Yes*	Yes	
7→9	Yes	Yes	
i_β $H^\beta \rightarrow i+2_\beta$ H^α			
1→3	Yes*	Yes*	
3→5	Yes	Yes	
5→7	Yes*	No	
i_β $H^\alpha \rightarrow i+2_\beta$ HN			
1→3	Yes	Yes*	
3→5	Yes	No	
5→7	Yes	No	
7→9	No	No	
i_α $H^\alpha \rightarrow i+2_\alpha$ HN			
0→2	Yes	No	
2→4	Yes	No	
4→6	No	No	
6→8	No	No	
i_β $H^\beta \rightarrow i+3_\alpha$ HN			
1→4	?	?	
3→6	Yes	Yes	
5→8	?	Yes	
i_α $H^\alpha \rightarrow i+3_\beta$ HN			
0→3	Yes	?	
2→5	Yes	?	
4→7	Yes	Yes	
6→9	Yes	Yes	
i_α $H^\alpha \rightarrow i+3_\beta$ H^α			
0→3	No	No	
2→5	Yes	No	
4→7	Yes	No	
i_β $H^\beta \rightarrow i+4_\beta$ HN			
1→5	No	?	
3→7	No	Yes	
5→9	No	?	
i_α $H^\alpha \rightarrow i+4_\alpha$ HN			
0→4	No	?	
2→6	No	?	
4→8	No	Yes	

[a] "Yes" indicates that an NOE was observed that had only one possible assignment. "Yes*" indicates that an NOE was observed that had two or more possible assignments, one of which was consistent with the indicated nonsequential NOE. Other assignments for this NOE were not consistent with other probable nonsequential NOEs. "No" indicates that no NOE was observed, but that the indicated NOE would have been observed if it had been present. "?" indicates that an NOE was observed, but that it could not be assigned because of overlap with either a sequential or intraresidue NOE, or that more than one probable nonsequential assignment for the NOE was possible.

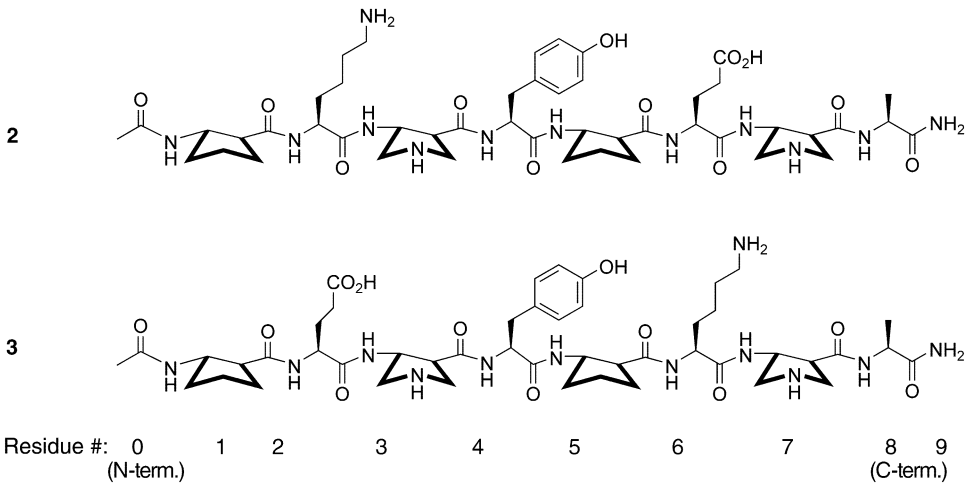


Figure 4. α/β-Peptides **2** and **3** shown with residue numbering used in Table 2.

(4.3 Å), but not with the 18-helix. All four of the possible NOEs of this type were observed for **2**.

Oligomer **3** displayed poorer ^{1}H NMR resonance dispersion than did isomer **2**, particularly in the N-terminal region, but it was nevertheless possible to identify ten unambiguous nonsequential NOEs (Table 2). These included two of the four possible α-residue $H^{\alpha}(i)$/β-residue NH($i+3$) NOEs (pattern #2 above), expected for the 11- and 14/15-helices but not the 18-helix. Also observed was a β-residue $H^{\beta}(i)$/β-residue NH($i+4$) NOE, which is consistent with the 14/15-helix (3.0 Å average interproton distance predicted), and the 18-helix (3.4 Å), but not with the 11-helix, and an α-residue $H^{\alpha}(i)$/α-residue NH($i+4$) NOE, which is consistent with the 14/15-helix (4.0 Å) and the 18-helix (3.1 Å), but not with the 11-helix.

The observed NOE data can be explained by proposing that α/β-peptides like **2** adopt both the 11-helix and the 14/15-helix, which interconvert rapidly and give rise to averaged NMR data.[12] Significant population of the 18-helix can be ruled out based on missing nonsequential NOEs. Specifically, modeling suggests that three types of nonsequential NOEs should be unique to the 18-helix among the secondary structures we considered: 1) β-residue $H^{\beta}(i)$/β-residue $H^{\alpha}(i+4)$ (2.4 Å average interproton distance predicted), 2) β-residue $H^{\beta}(i)$/α-residue NH($i+5$) (3.7 Å), and 3) α-residue $H^{\alpha}(i)$/β-residue NH($i+5$) (3.7 Å). None of these NOEs was observed for **2** or **3**, in which two of each type could have occurred, even though most of the NOEs could have been readily detected (no sequence overlap for 7 of the 12 diagnostic NOEs in **2** and **3**).

As a further probe of the folding preferences of α/β-peptides, we examined the analogue of **2** containing (*S*,*S*)-*trans*-2-aminocyclohexanecarboxylic acid (ACHC)[13] in place of ACPC, and the diastereomer containing (*S*,*S*)-ACHC and D-α-residues. Neither octamer displayed any nonsequential NOEs in methanol; thus, ACHC does not support α/β-peptide helix formation, in contrast to ACPC. This stark difference between these two types of β-amino acids, with five- and six-membered rings, may arise because homogeneous ACHC backbones favor a helix containing C=O···H–N hydrogen bonds with C→N directionality,[14] which is opposite to the hydrogen-bond directionality in the α-helix, the β-peptide 12-helix, and the α/β-peptide helices detected in this work.

The data reported here demonstrate a strong propensity for helical secondary structure among short oligomers with heterogeneous backbones of alternating proteinogenic α- and cyclic β-amino acid residues. This folding is noteworthy since peptides of similar length composed exclusively of proteinogenic α-amino acids do not adopt helical conformations in methanol.[15] The apparent rapid interconversion between two alternative helical conformations is particularly interesting because of parallels with behavior that is well-documented among peptides containing exclusively α-amino acid residues.[5] We are currently trying to identify residue and sequence design strategies that will favor the 11-helix relative to the 14/15-helix and vice versa; some success has been achieved with analogous efforts to disentangle the α- and 3_{10}-helices among α-peptides.[5] At a general level, our findings suggest that heterogeneous oligomers containing two or more types of amino acid residues will be fruitful sources of new foldamers.

Received: June 12, 2003 [Z52125]

Keywords: α-amino acids · β-amino acids · foldamers · helical structures · NMR spectroscopy

[1] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173; b) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893.

[2] Examples: a) M. Werder, H. Hauser, S. Abele, D. Seebach, *Helv. Chim. Acta* **1999**, *82*, 1774; b) Y. Hamuro, J. P. Schneider, W. F. DeGrado, *J. Am. Chem. Soc.* **1999**, *121*, 12200; c) E. A. Porter, X. Wang, H.-S. Lee, B. Weisblum, S. H. Gellman, *Nature* **2000**, *404*, 565; d) D. Liu, W. F. DeGrado, *J. Am. Chem. Soc.* **2001**, *123*, 7553; e) E. A. Porter, B. Weisblum, S. H. Gellman, *J. Am. Chem. Soc.* **2002**, *124*, 7324; f) K. Gademann, T. Kimmerlin, D. Hoyer, D. Seebach, *J. Med. Chem.* **2001**, *44*, 2461; g) U. Arnold, M. P. Hinderaker, B. L. Nilsson, B. R. Huck, S. H. Gellman, R. T. Raines, *J. Am. Chem. Soc.* **2002**, *124*, 8522; h) T. L. Raguse, E. A.

Porter, B. Weisblum, S. H. Gellman, *J. Am. Chem. Soc.* **2002**, *124*, 12774.

[3] Selected examples: a) M. Hagihara, N. J. Anthony, T. J. Stout, J. Clardy, S. L. Schreiber, *J. Am. Chem. Soc.* **1992**, *114*, 6568; b) R. S. Lokey, B. L. Iverson, *Nature* **1995**, *375*, 303; c) Y. Hamuro, S. J. Geib, A. D. Hamilton, *J. Am. Chem. Soc.* **1996**, *118*, 7529; d) B. R. Huck, J. D. Fisk, S. H. Gellman, *Org. Lett.* **2000**, *2*, 2607; e) B. Gong, H. Q. Zeng, J. Zhu, L. H. Yua, Y. H. Han, S. Z. Cheng, M. Furukawa, R. D. Parra, A. Y. Kovalevsky, J. L. Mills, E. Skrzypczak-Jankun, S. Martinovic, R. D. Smith, C. Zheng, T. Szyperski, X. C. Zeng, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11583.

[4] α/β-Peptides designed to mimic neuropeptide Y have recently been shown to bind selectively to the Y_1 receptor:N. Koglin, C. Zorn, R. Beumer, C. Cabrele, C. Bubert, N. Sewald, O. Reiser, A. G. Beck-Sickinger, *Angew. Chem.* **2003**, *115*, 212; *Angew. Chem. Int. Ed.* **2003**, *42*, 202.

[5] A. K. Bolin, G. L. Millhauser, *Acc. Chem. Res.* **1999**, *32*, 1027, and references therein.

[6] COSY: W. P Aue, E. Bartholdi, R. R. Ernst, *J. Chem. Phys.* **1976**, *64*, 2229; TOCSY: A. Bax, D. G. Davis, *J. Magn. Reson.* **1985**, *65*, 355; ROESY: A. A. Bothner-By, R. L. Stephens, J. Lee, C. D. Warren, R. W. Jeanloz, *J. Am. Chem. Soc.* **1984**, *106*, 811.

[7] P. L. LePlae, N. Umezawa, H.-S. Lee, S. H. Gellman, *J. Org. Chem.* **2001**, *66*, 5629.

[8] Ambiguous NOEs were assigned by using the program DYANA (P. Guntert, C. Mumenthaler, K. Wuthrich, *J. Mol. Biol.* **1997**, *273*, 283) in an iterative process. Each ambiguous NOE had one assignment that was consistent with all other nonsequential NOEs.

[9] a) D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi, S. H. Gellman, *Nature* **1997**, *387*, 381; b) D. H. Appella, L. A. Christianson, D. A. Klein, M. R. Richards, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 7574.

[10] Alternative helices were generated for an ACPC-Ala dodecamer using SYBYL (Tripos, St. Louis, MO) by imposing distance constraints for hydrogen bonds between appropriate C=O/H–N pairs. These structures were minimized using a continuum dielectric constant of 32.6, to simulate methanol, with the MMFF94s force field (T. A. Halgren, *J. Comput. Chem.* **1999**, *20*, 720.). Each of the resulting helical structures was taken to represent a possible local minimum in conformational energy for the dodecamer.

[11] It seemed possible that this design might bias each oligomer into either an 11- or 14/15-helical conformation based upon charge–charge interactions between side chains, allowing us to use rational principles to design α/β-oligomers with specific conformational preferences. A potential salt bridge between APC3 and Glu6 in **2** may preferentially stabilize an 11-helical conformation (helical wheel diagrams are included in the Supporting Information). In contrast, an interaction between APC3 and Lys6 in **3** may destabilize the 11-helical conformation, possibly favoring the 14/15-helical conformation. Complete evaluation of this hypothesis was not possible because of the severe resonance overlap observed for **2** and **3** in aqueous solution. In methanol, we do not expect the Glu side chain to be significantly deprotonated, minimizing the chance that salt-bridge formation would influence conformational preferences. Oligomers **2** and **3** show slightly different NOE patterns, suggesting that one helical conformation may be slightly more populated in one oligomer than in the other. Further investigations are under way to explore these possibilities.

[12] A helical conformation containing three-center backbone hydrogen bonds, simultaneous 11-membered ring and 14/15-membered ring hydrogen-bonding patterns, could also explain the nonsequential NOEs observed for the α/β-peptides described here. NOE-restrained analysis with the program DYANA (P. Guntert, C. Mumenthaler, K. Wuthrich, *J. Mol. Biol.* **1997**, *273*, 283) suggests that a helix containing three-center hydrogen bonds could explain the NMR data. The DYANA-generated structures resemble this "hybrid" helix in shape; however, hydrogen bonds are not observed in these structures. As pointed out in the text, a rapidly interconverting mixture of 11- and 14/15-helices can also explain the NOE data. We do not favor the helix with three-center hydrogen bonds because there is little precedent for analogous hydrogen bonds in α-peptide helices and none for β-peptide helices. Three-center hydrogen bonds in which an amide proton interacts simultaneously with two acceptor groups, such as oxygen atoms, are rare in proteins: E. N. Baker, R. E. Hubbard, *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97. For crystallographic detection of a single three-center hydrogen bond at the transition point between α- and 3_{10}-helical domains in a synthetic α-peptide, see: V. Pavone, E. Benedetti, B. Di Blasio, C. Pedone, A. Santini, A. Bavoso, C. Toniolo, M. Crisma, L. Sartore, *J. Biomol. Struct. Dyn.* **1990**, *7*, 1321. It has been shown in one system that a three-center C=O···H···O=C interaction is higher in energy than an alternative two-center C=O···H hydrogen bond: J. Yang, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 9090.

[13] a) A. Berkessel, K. Glaubitz, J. Lex, *Eur. J. Org. Chem.* **2002**, *17*, 2948; b) M. Schinnerl, J. K. Murray, J. M. Langenhan, S. H. Gellman, *Eur. J. Org. Chem.* **2003**, *4*, 721.

[14] a) D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 13071; b) D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1999**, *121*, 6206.

[15] D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.

