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Folding Control in Cyclic Peptides through N-Methylation Pattern Selection: Formation of Antiparallel β-Sheet Dimers, Double Reverse Turns and Supramolecular Helices by 3α,γ Cyclic Peptides

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Abstract: Peptide foldamers constitute a growing class of nanomaterials with potential applications in a wide variety of chemical, medical and technological fields. Here we describe the preparation and structural characteristics of a new class of cyclic peptide foldamers (3α,γ-CPs) that, depending on their backbone N-methylation patterns and the medium, can either remain as flat rings that dimerize through arrays of hydrogen bonds of antiparallel β-sheet type, or can fold into twisted double reverse turns that, in the case of double γ-turns, associate in nonpolar solvents to form helical supramolecular structures. A 3α,γ-CP consists of a number of multiples of a repeat unit made up of four amino acid residues of alternating chirality: three corresponding to α-amino acids and one to a γ-amino acid (a *cis*-3-aminocycloalkanecarboxylic acid).

Keywords: beta sheets · foldamers · helical structures · peptides · self-assembly

Introduction

In pursuit of biomaterials with improved biocompatibility, rigidity, responsiveness, specificity and other properties that are desirable in medical devices and drug delivery systems, increasing attention is being paid to nanostructures.[1] These entities consist of hierarchies of substructures, the higher-ranking members of which are formed by the same kinds of self-assembly process as are involved in the formation of natural biopolymers that carry out sophisticated chemical operations such as catalysis or electron transport. To gain deeper insight into how to control the three-dimensional structures of such materials, chemists have in recent years devoted increasing research effort to synthetic foldamers, non-natural oligomers that exhibit the same kinds of secondary structural motifs as natural biomolecules (helices, sheets, loops etc.).[2–6]

A major goal of foldamer chemistry is to develop oligomers that spontaneously adopt higher-order structure—individually or by association—to form tertiary or even quaternary structures known as tyligomers.[2b,7] In this respect, although the most intensively studied foldamers have been helical, much attention has recently focused on sheet-forming foldamers, because of their relevance to pathological amyloid plaques[8] and their potential use for the manufacture of nanotapes or nanotubes.[9] Sheet-forming foldamers include certain types of cyclic peptides (CPs) designed to stack spontaneously through arrays of hydrogen bonds similar to those found in β-sheets, to form nanotubes [SPNs (self-assembling peptide nanotubes) such as **CP-A** ⇄ **SPN-A** (Figure 1)].[10]

Changes in the structure, number and distribution of the CPs allow for the modulation of the properties of the resulting SPNs and their subsequent development for chemical studies and technological applications.[10–13] For example, alternating N-methylation on a CP (**CP-B**) makes it form dimers $\mathbf{D_{CP\text{-}B}}$ (**CP-B** ⇄ $\mathbf{D_{CP\text{-}B}}$, Figure 1), the shortest possible SPNs, which have been successfully employed to estimate the relative stabilities of β-sheets' parallel and antiparallel arrangements.[14]

In recent years we have been exploring the possibilities of CPs composed of α-amino acids (α-Aas) alternating with a γ-amino acid, more specifically a *cis*-3-aminocycloalkanecarboxylic acid (γ-Aca).[15,16] In these α,γ-CPs (e.g., **CP-1–3** in Figure 1) the projection of one of the cycloalkane methylenes into the lumen (space within the tube) creates a hydrophobic region, and the relative rigidity of the cyclohexane

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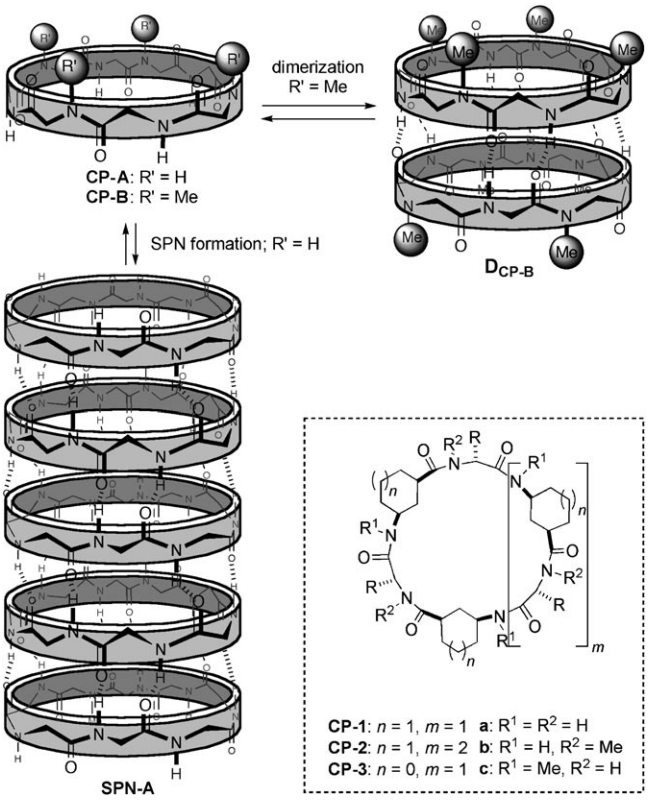

Figure 1. SPN and dimer formation by stacking of cyclic peptides, and the structures of α,γ-CPs.

ring ensures that the γ-amino acid segments of the peptide backbone have the all-*trans* conformation necessary for the peptide ring to be flat and stackable.[15,17] This conformational rigidity means that in CPs the cycloalkane ring of a γ-Aca can be regarded as a superatom, and (1*S*,3*R*)- and (1*R*,3*S*)-γ-Aca residues as equivalent to D- and L-α-amino acid residues, respectively (Figure 2).[18] Hereafter we avail ourselves of this equivalence by using D and L to describe the stereochemistry of γ-Aca residues.

In terms of the above equivalence, the α,γ-CPs with which we have been working in recent years can each be viewed as the result of taking a parent nanotube-forming CP consisting of alternating D- and L-α-Aas (D,L-α-CP) and replacing alternate α-Aa residues with γ-Aca residues of the same chirality (Figure 2), or L-γ-Aca residues for only half the L-α-Aa residues. The overall symmetry of the resulting α,γ-CP, when flat, is C_n, where n is the number of [L-γ-Aca-D-α-Aa] or [D-γ-Aca-L-α-Aa] units in the peptide ring, and thus also depends on the size of the ring.[15] However, the cavity properties of SPNs composed of CPs with γ-Aca residues, and hence their technological applications, may well depend not only on size and number or type of substituents on their inner surfaces but also on their symmetries. It is therefore of interest to be able to control the overall symmetry of CPs independently of their size. As part of an extensive program directed towards the design, synthesis and structural and functional evaluation of novel homo- and heterosupramolecular entities involving CPs,[15,16] but also with aim of checking the possibility described above, we have now synthesized a new class of CPs in which the α-amino acids (α-Aas) and *cis*-3-aminocyclohexanecarboxylic acid (γ-Ach) are combined in a three to one ratio ("3α,γ-CPs"), resulting from substitution of γ-Ach residues for only half the α-Aa residues of identical chirality to the formal D,L-α-CP parent. These CPs should retain the β-sheet-forming propensity and at the same time would be a simple model of novel SPNs with partial hydrophobic cavities of C_2 symmetry.

Surprisingly, the full structural characterization of the new CPs showed that their folding is dictated by their methylation patterns.[19,20] These novel 3α,γ-CPs can either retain their flat ring structures through hydrogen bonds in β-sheet-like arrays, or they can fold into double reverse turn motifs, which may associate as helical aggregates.[21]

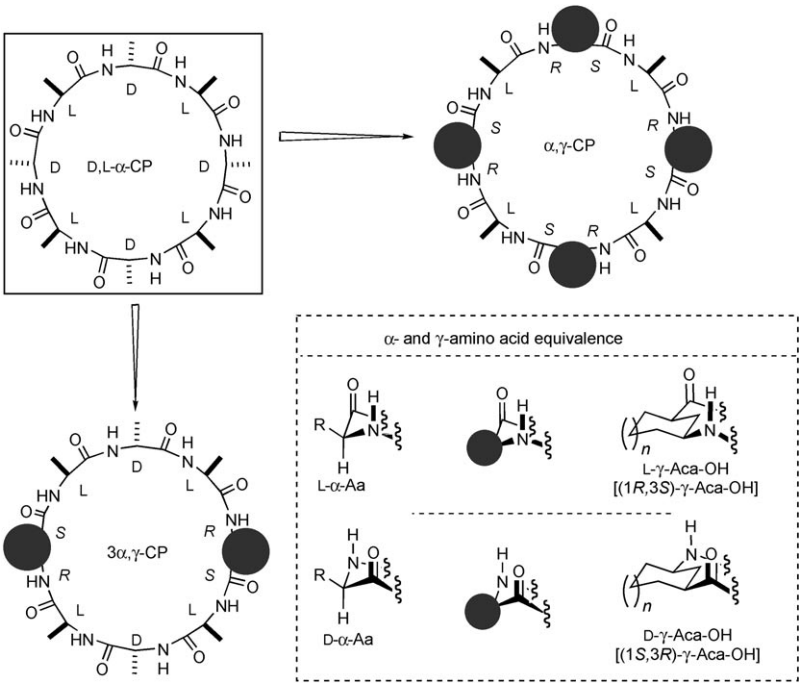

Figure 2. Equivalence between D-(L)-α-amino acids and (1*S*,3*R*)-[(1*R*,3*S*)]-γ-aminocycloalkanecarboxylic acids (α-Acas), and the generation of α,γ- or 3α,γ-CPs by substitution of D-γ-Acas for selected D-γ-Aas in a D,L-α-CP. Solid dishes indicate cycloalkane superatoms.

Results and Discussion

The replacement of α-Aa by γ-Aca residues has the result that in the flat conformation of the resulting CP (3α,γ-CPs)—as in α,γ-CPs, in one of which all the amide NH and C=O groups belong to α-Aas and on the other to γ-Acas—the two faces are distinct: at one face (α,α) all the amide NH and C=O groups belong to α-Aas, while at the other (α,γ) they belong to equal numbers of α-Aa and γ-Aca residues. All the residues with NH and C=O groups pointing to the same side have the same chirality. Because of the different spacings of the NH and C=O groups on the two faces, the association of CPs in SPNs through β-sheet-like hydrogen bonding requires successive CPs to lie antiparallel,[22] so that like faces face each other: α,γ-faces bond to α,γ-faces (AG-type bonding) and α,α-faces bond to α,α-faces (AA-type bonding) (Figure 3). To investigate these two types of association separately and to avoid the insolubility of multi-CP SPNs, we adopted the same strategy as in our work on α,γ-CP-based SPNs,[14,15] using CPs in which the backbone NH groups of either one CP face or the other were methylated so as to limit the β-sheet-like association of CPs to the β-strand dimer stage (Figure 4). Suspecting that methylation of α-Aas might be affecting dimerization efficiency by preventing the CP from adopting a sufficiently flat conformation, we also carried out experiments in which only half the NH groups of one face were methylated. It was in these latter experiments that the alternative folding properties of 3α,γ-CPs were manifested.

CPs with full amide N-methylation of one face: The C_2-symmetric CPs cyclo-[(L-γ-Ach-D-^{Me}*N*-Ala-L-Leu-D-^{Me}*N*-Ala)$_2$-] (**CP-4**) and cyclo-[(L-^{Me}*N*-γ-Ach-D-Phe-L-^{Me}*N*-Ala-D-Phe)$_2$-] (**CP-5**), in which γ-Ach is *cis*-3-aminocyclohexanecarboxylic acid, were prepared by standard procedures and characterized by NMR spectroscopy and mass spectrometry. We started our studies with peptide **CP-4** in which the L-Aas, both the γ-Ach and Ala, were methylated. Their NMR spectra in polar organic solvents showed the presence of several slowly interconverting conformers, presumably the results of *cis–trans* isomerization about peptide bonds. Under this assumption, the temperature dependence of the NMR data allowed the activation barrier for *cis–trans* isomerization in [D$_6$]DMSO be estimated as 16–17 kcal mol^{-1} at the coalescence temperature (358 K).

At room temperature the ^{1}H NMR spectrum of **CP-4** in deuterochloroform showed a single set of signals that lacked amide proton signals because of coalescence, but spectra recorded at below 253 K showed two species that intercon-

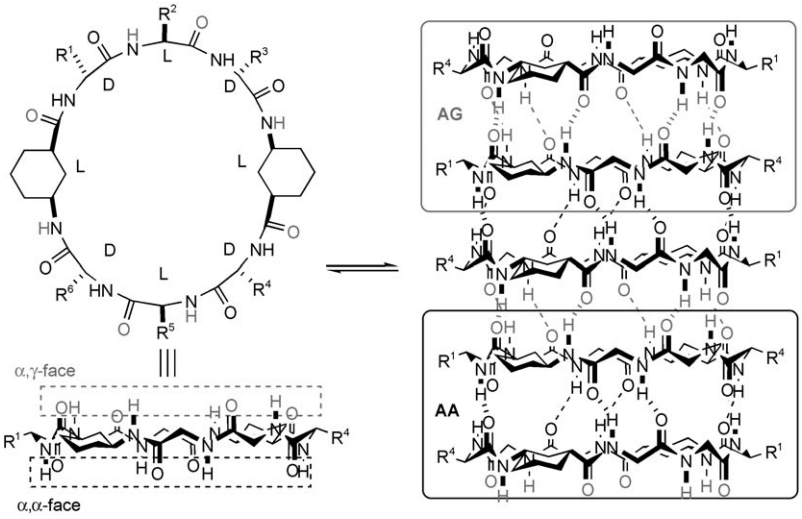

Figure 3. AA- and AG-type hydrogen bonding between cyclo-[(L-γ-Ach-D-Aa-L-Aa-D-Aa)$_2$-] units (for clarity, most amino acid side chains have been omitted in the representation of the nanotube).

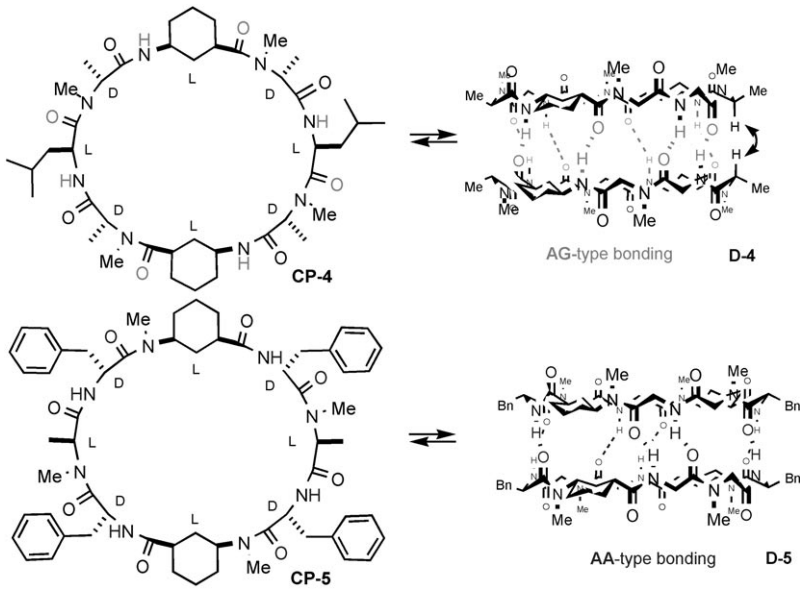

Figure 4. Cyclic peptides **CP-4** and **CP-5** and their corresponding dimers (for clarity, most amino acid side chains have been omitted from the representations of the dimers).

verted slowly on the NMR timescale. The assumption that one of the two species was the monomer **CP-4** and the other the AG-bonded dimer **D-4** is supported by the fact that their relative concentrations depended on total peptide concentration and by their interconversion rate, which was moderately slow on the NMR timescale, as befits a process involving the making or breaking of eight hydrogen bonds.[23] Also, the presence of dimers was confirmed by NOE cross-peaks between the α-carbon protons of the alanines (at 5.47 and 5.95 ppm), which are in close proximity to each other in the dimer but not in the monomer. ^{1}H NMR experiments in which total peptide concentration was varied afforded a value of at least of 10 M^{-1} for the association constant of the dimerization process at 243 K (see Table S1, Supporting Information).[24] Although the poor solubility of **CP-4** in chloroform at low temperature prevented reliable Van′t Hoff analysis, the strength of the dimerizing hydrogen bonds is reflected by the downfield shifts of the Leu and γ-Ach N−H signals from 7.02 and 7.66 ppm, respectively, in the monomer (**CP-4**) to 8.75 and 8.21 ppm, respectively, in the dimer (**D-4**). Their antiparallel β-sheet nature is attested to by the downfield shifts of the C_α–H signals (5.51, 5.15 and 5.95 ppm in the dimer, as against 5.22, 4.95 and 4.38 ppm in the monomer; see Table 1),[25] and also by the IR spectrum recorded in $CHCl_3$, which shows amide $I_\perp$, II_{II} and A bands at 1542, 1632 and 3310 cm^{-1}, respectively, close to those of previously reported peptide nanotubes (the band at 3413 cm^{-1} corresponds to the non-hydrogen-bonded amide proton).[14,15,22,26] Unfortunately no crystals suitable for X-ray diffractometry were obtained, which prevented crystallographic confirmation of dimerization.

Peptide **CP-5** behaved in the same way as **CP-4** in polar solvents such as deuterated DMSO, its ^{1}H NMR spectra showing the presence of several slowly interconverting conformers. Spectra run in nonpolar solvents such as $CDCl_3$ showed the presence of two slowly interconverting species, and their $^3J_{N,H}$ coupling constants of 7.1–8.2 Hz were consistent with the all-*trans* geometry required for dimerization. As in the case of **CP-4**, the conjecture that one of the two species was monomeric and the other dimeric was again supported by the dependence of their relative concentrations on total peptide concentration, which allowed the association constant in $CDCl_3$ at 303 to be estimated as 62 M^{-1} (Table S1, Supporting Information).[24] Van′t Hoff plots over the 263–303 K range afforded values of −47.9 kJ mol^{-1} for $\Delta H^{\ominus}_{298}$ and 123.8 J K^{-1} mol^{-1} for $\Delta S^{\ominus}_{298}$. These values are consistent with dimerization being essentially an enthalpy-driven[27] hydrogen-bonding process.[14] The β-sheet hydrogen-bonding network is supported by the $^3J_{N\alpha}$ values of the dimer, which are substantially greater than those of the monomer, as well as by the downfield shifts (Table 1) of the Phe N−H (8.31 and 8.80 ppm in the dimer as against 7.49 and 7.10 ppm, respectively, in the monomer) and α-carbon signals (5.26–5.89 ppm in the dimer as against 4.86–5.16 ppm in the monomer),[25] as well as by the amide bands at 1525, 1627 and 3310 cm^{-1} in the IR spectrum (Table 2).[15,22,26]

Table 1. Summary of NMR data for 3α,γ-peptides **CP-4** and **CP-5**.

Peptide[a]	Aa	H	δ_{dimer} ($^3J_{N\alpha}$ Hz)	Δ_{mon} ($^3J_{N\alpha}$ Hz)
CP-4	^{Me}N-Ala	H_α	5.51	5.22
(233 K)		N−Me	3.26–3.23	3.07–2.91
	Leu	N−H	8.75	7.02
		H_α	5.15	4.95
	^{Me}N-Ala	H_α	5.95	4.38
		N−Me	3.26–3.23	3.07–2.91
	Ach	N−H	8.21	7.66
		H_γ	3.70	3.83
CP-5	Phe	N−H	8.80 (7.5)	7.10
		H_α	5.48	4.86
	^{Me}N-Ala	H_α	5.89 (5.9)	5.16
		N−Me	3.06	2.61
	Phe	N−H	8.31 (8.2)	7.49 (7.1)
		H_α	5.26	4.86
	^{Me}N-Ach	H_γ	4.39	4.39
		N−Me	2.72	2.52

Table 2. Summary of IR data for 3α,γ-peptides **CP-4**–**10**.

Peptide **CP-1**[a]	Amide $I_\perp$[a]	Amide II_{II}	Amide A[b]
CP-4/D-4	1632 (1673)	1542	3310
			3413
CP-5/D-5	1627 (1669)	1525	3310
CP-6/D-6	1642 (1681)	1515	3296
			3403
CP-7	1652, 1687	1537	3324
CP-8/D-8	1616, 1651 (1683)	1543	3312
			3417
CP-9	1629 (1671)	1521	3326
			3432
CP-10	1655, 1684	1530	3327

[a] Values in parentheses are for putative amide $I_\perp$ bands arising from non-hydrogen-bonding N-methylated residues. [b] Upper values correspond to hydrogen-bonded residues, lower values non-hydrogen-bonded residues.

Conclusive confirmation of the AA-type dimerization of **CP-5** was provided by X-ray crystallography of the colourless prismatic crystals obtained by equilibration of a solution of the peptide in dichloromethane with hexane vapour. These crystals consisted of β-sheet dimers composed of essentially flat, slightly elliptical, antiparallel monomers linked by a β-sheet-like array of eight hydrogen bonds with N···O distances of 2.87–3.02 Å (Figure 5). The lumen of one of the monomers has approximate maximum and minimum van der Waals diameters of 8.83 Å (between Ala C_αs) and 4.34 Å (between Ach C_β protons), respectively, while those of the other are 9.16 and 3.81 Å. The cavity of the dimer has a van der Waals volume of approximately 167 $Å^3$. The crystals are body-centred, with the axes of the dimers approximately parallel to the *a* axis and each dimer making van der Waals contacts with its neighbours in this direction through its α,γ-face carbonyl and N-methyl groups. This body-centred structure creates channels along the *a* axis that are composed of two alternating types of segment with a period of about 13.8 Å: the dimer pore, and the space surrounded by the external surfaces of four coplanar dimers (Figure 5b).

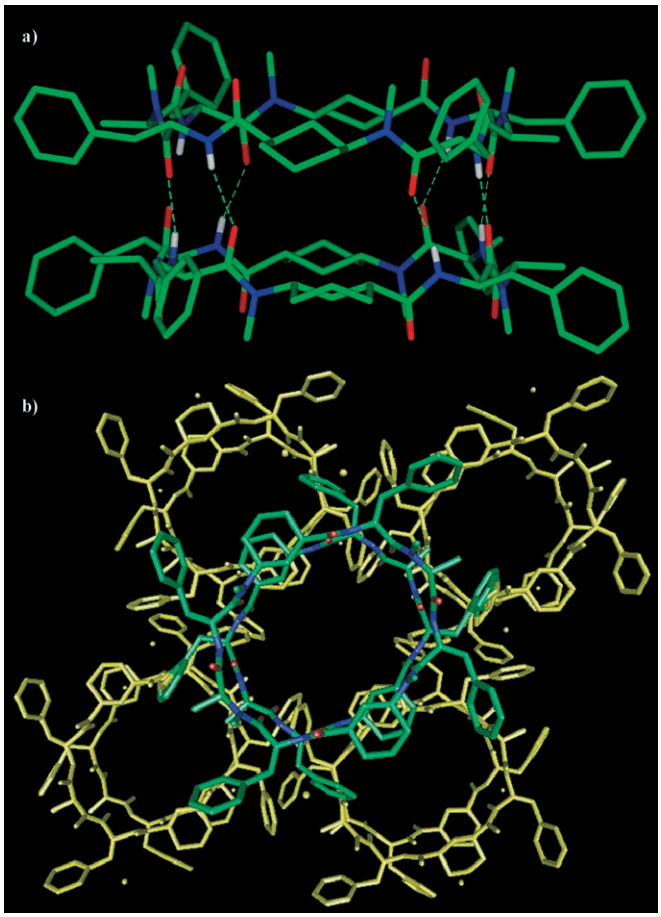

Figure 5. a) Side view, and b) view down the axis, of dimer **D-5**, with the dimers of the next layer in gold.

As noted above, the spacing of the NH and C=O groups of **CP-4** and **CP-5** (and their methylation) means that their monomers must be antiparallel in their homodimers. By the same token, heterodimers of these two species must be formed as parallel monomers (Figure 6). To investigate the possible parallel dimer formation and its relative stability versus the antiparallel β-sheet-like dimer, we recorded the ^{1}H NMR spectrum of an equimolecular mixture of **CP-4** and **CP-5** in chloroform at 230 K. The spectrum showed the presence of both **D-4** and **D-5**, but no sign of heterodimer **D-4–5**. This result clearly confirms the preference of SPN-forming CPs for antiparallel dimerization.[22]

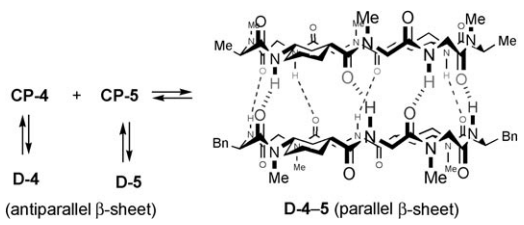

Figure 6. Theoretical heterodimer resulting from parallel β-sheet-like hydrogen bonding of cyclic peptides **CP-4** and **CP-5**.

Effect of incomplete methylation on dimerization: In our previous studies with eight-residue α,γ-CPs, we found that the CPs with N-methylated γ-Achs dimerized through the formation of eight hydrogen bonds between carbonyl and N–H components of α-Aas with association constants typically greater than $10^5\,M^{-1}$,[28] at least a thousand times greater than those of eight-residue α,γ-CPs with N-methylated α-Aas.[15] This has tentatively been attributed to steric interactions between the N-methyl and carbonyl groups of the latter, which might prevent the CP from adopting as flat a conformation as is required for strong dimerization in the β-strand form (effects of this kind have been observed in computational studies of β-conformations of model peptides).[29] The fact that in this work both **CP-4** and **CP-5** had association constants of the same order as eight-residue α,γ-CPs with N-methylated α-Aas was therefore tentatively attributed to their both having residues of this type. To investigate this, we prepared two 3α,γ-CPs that would both "theoretically" undergo AA-type dimerization, but which both had just two N-methylated residues on their α,γ-faces: cyclo-[(L-Ser(Bn)-D-^{Me}*N*-γ-Ach-L-Phe-D-Ala)$_2$-] (**CP-6**), in which the γ-Achs are methylated, and cyclo-[(L-γ-Ach-D-Phe-L-^{Me}*N*-Ala-D-Phe)$_2$-] (**CP-7**), in which the N-methylated Aas are the two α-Aas (Figure 7).[30] Compound **CP-6** was expected to undergo strong AA-type dimerization to β-band **D-6**, since it has no N-methylated α-AAs, while compound **CP-7** was expected to dimerize more weakly to **D-7**, because it does have such residues.

Our expectations for compound **CP-6** were totally fulfilled. That it adopts a flat, all-*trans* conformation in chloroform was corroborated by its ^{1}H NMR $^3J_{N\alpha}$ values (9.1, 7.6 and 6.5 Hz for Phe, Ser and Ala, respectively). The β-sheet-type hydrogen-bonded interaction to form **D-6** was confirmed by the positions of its Phe and Ser NH signals (8.66 and 8.58 ppm, respectively) and by its IR bands at 1642, 1515 and 3296 cm^{-1}.[15,22,26] The strength of this bonding was shown by the chemical shifts of all its NH groups being unchanged by addition of up to 30% of methanol or by dilution with chloroform to a concentration below $1\times10^{-3}\,M$, which suggests total dimerization and an association constant of at least $10^4\,M^{-1}$. Also, there appeared to be no additional association through the unmethylated α,γ-face Ala NH, the ^{1}H NMR signal and IR stretching band of which lay at 6.49 ppm and 3403 cm^{-1}, respectively.

Comparison of the results for **CP-5** and **CP-6** showed that when the γ-Ach was N-methylated, N-methylation of the α,γ-face α-Aas reduced the dimerization constant, as hypothesized. There was no sign that the γ-Ach N-methyl groups of **CP-6** hampered β-sheet formation. Quite different results were obtained with **CP-7** and no evidence of dimer **D-7** was found, suggesting that the γ-Ach N-methyl groups might not be irrelevant to CP conformation and dimerization.[31] The ^{1}H NMR spectrum of this compound in chloroform shows broad signals that are in some cases concentration-dependent (for example, the Phe C_αH signal shifts from 5.00 to 4.60 ppm upon raising of the concentration from 3.75 to 15.0 mM). Notably, one of the concentration-dependent

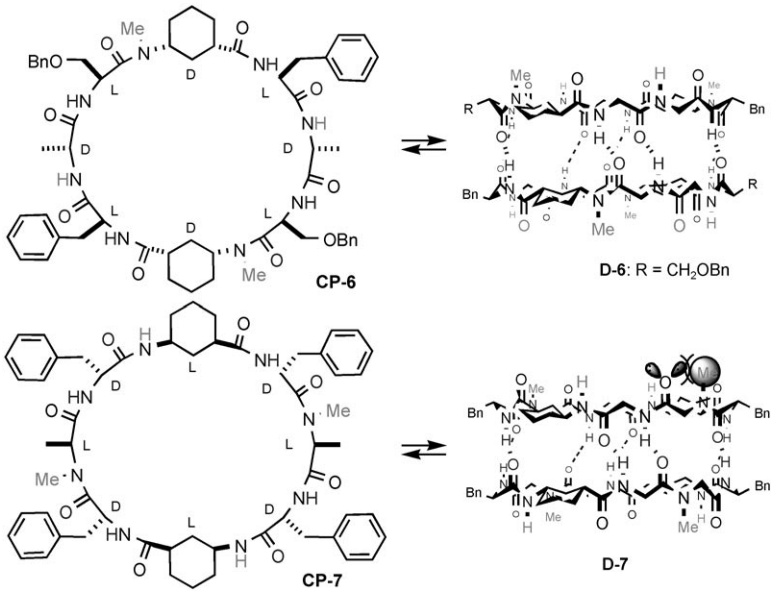

Figure 7. Compounds **CP-6** and **CP-7** and the corresponding theoretical AA-bonded dimers. Only peptide **CP-6** is able to form a stable dimer (**D-6**) in nonpolar solvents.

signals is that of Ach NH, which shows that the α,γ-face of **CP-7**, which would be expected to be solvent-exposed, is not fully protected against hydrogen bonding by its N-methylated alanines and suggests that the peptide does not adopt the flat conformation required for the β-sheet formation. **CP-7** also exhibited unexpected behaviour in polar solvents capable of acting as hydrogen bond acceptors. Though its ^{1}H NMR spectrum is well defined and concentration-independent, the signal of only two of its four Phe NH protons lies in the region expected for strong hydrogen bonding (at 8.81 ppm in deuteromethanol), and the position of the γ-Ach NH signal (7.68 ppm in deuteromethanol) shows that this proton is also involved in hydrogen bonding. Additionally, the chemical shifts of both amide NH protons do not significantly shift to higher magnetic field relative to the other NH protons with elevation of temperature.[32] All these data suggested that the species present appeared not to be the result of the expected almost flat CPs and raised the question of the identity of the CP structure. Unfortunately, two-dimensional NMR experiments failed to throw further light on the structure of **CP-7** in either polar or nonpolar solvents, and the failure of attempts to crystallize this CP precluded crystallographic characterization. We shall return below to the issue of the conformation and interactions of this CP.

The suspicion that the effects of N-methylation went beyond protection against multi-CP stacking or slight distortion of more or less flat CPs led us to extend our investigation to 3α,γ-CPs that were partially N-methylated on their α,α-faces. Specifically, we prepared cyclo-[(L-γ-Ach-D-Ala-L-Ser(Bn)-D-^{Me}*N*-Ala)$_2$-] (**CP-8**) and cyclo-[(L-γ-Ach-D-^{Me}*N*-Ala-L-Ser(Bn)-D-Ala)$_2$-] (**CP-9**) (Figure 8).

CP-9 behaved similarly to **CP-7**. Its ^{1}H NMR spectrum in chloroform was well defined and concentration-independent, but the position of its Ser NH signal—6.59 ppm—showed that it was not involved in hydrogen bonding as it would be in the AG-bonded dimer **D-9**. Also, the unmethylated α-Aa on its α,α-face, like the unmethylated γ-Ach of **CP-7** (7.97 ppm), appeared to be involved in a weak hydrogen bond, its NH signal lying at 7.3 ppm. An additional indication that **CP-9** had not dimerized was the location of its methylated Ala C_αH signal at surprisingly high field: 3.54 ppm.

In contrast, the well defined ^{1}H NMR spectrum of **CP-8** in chloroform showed that, like **CP-4**, and apparently unlike **CP-9**, it underwent AG-type dimerization: Ser and γ-Ach NH signals appeared at positions indicative of hydrogen bonding (8.70 and 7.91 ppm, respectively), the corresponding $^3J_{N\alpha}$ coupling constants (9.5 and 8.9 Hz, respectively) suggested that the hydrogen bonding was of the β-sheet type, and the position and $^3J_{N\alpha}$ value of the NH signal of the unmethylated Ala (6.55 ppm and 6.5 Hz) furthermore showed that this α,α-face proton was not involved in a hydrogen bond. The dimerization of **CP-8** to **D-8** was corroborated by its ROESY spectrum in $CDCl_3$,

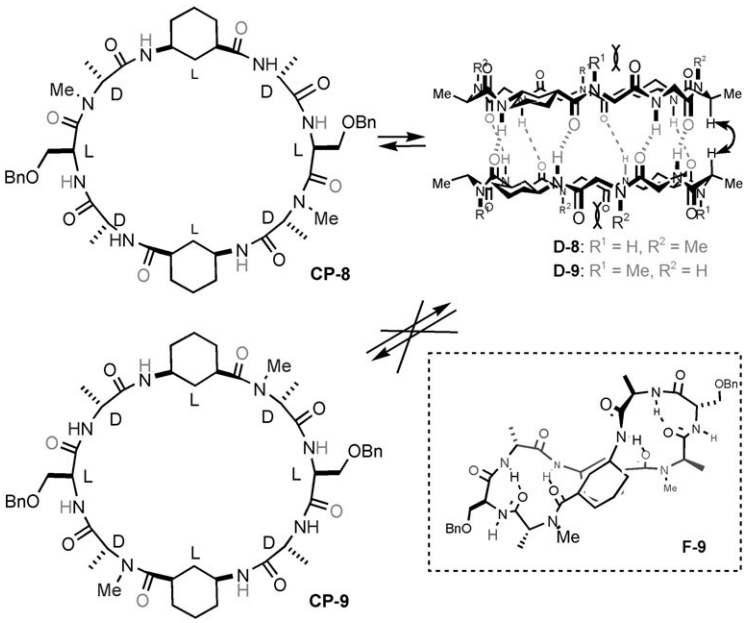

Figure 8. Compounds **CP-8** and **CP-9** and the corresponding theoretical AG-bonded dimers (**D-8** and **D-9**). Only peptide **CP-8** is able to form a stable dimer (**D-8**) in nonpolar solvents. Inset: Theoretical double reverse turn-type structure (**F-9**) proposed for **CP-9**.

which showed cross-peaks indicative of close spatial proximity between the C_α protons of the N-methylated and unmethylated Ala residues (Figure 8), while the presence of β-sheet-like hydrogen bonding was corroborated by the results of IR studies in chloroform, which showed amide I, II_{II} and A bands at 1543, 1616, 1651 and 3312 cm^{-1} (as well as a band at 3417 cm^{-1} attributed to the stretching mode of the unmethylated Ala NH).[15,22,26] The bonding in **D-8** was much stronger than in **D-4**: the chemical shifts of the Ser, γ-Ach and unmethylated Ala NH protons were unchanged by dilution down to 2×10^{-4} M, showing that the association constant for AG-type dimerization by **CP-8** is at least $10^4\,M^{-1}$. Methylation of the α-Aa nitrogen adjacent to the serine C=O thus appeared not to impede AG-type dimerization, while in the presence of this methyl group, additional methylation of the α-Aa nitrogen adjacent to the γ-Ach C=O reduced the strength of dimerization in the same way as N-methylation of the α,γ-face α-Aas appeared to weaken the AA-type dimerization of γ-Ach-methylated CPs such as **CP-6**.

Twisted CPs: Two-dimensional NMR experiments carried out to throw further light on the structures adopted by **CP-7** and **CP-9** failed to achieve their objective, and in both cases the failures of attempts at crystallization also precluded crystallographic characterization. We therefore investigated the behaviour of cyclo-[(L-Ser(Bn)-D-γ-Ach-L-Phe-D-^{Me}*N*-Ala)$_2$-] (**CP-10**, Figure 9), in which two of the Phe residues of **CP-7** had been replaced with benzylated serines in the hope that it would behave in the same way as **CP-7** but also provide structural information through the chemical shifts of the protons on Ser C_α and C_β and through NOE cross-peaks.

In both polar and nonpolar solvents the ^{1}H NMR behaviour of **CP-10** was indeed similar to that of **CP-7** (Figure 10), with broad, poorly resolved, concentration-dependent signals in chloroform and sharp, concentration-independent signals in methanol, in which the Ser NH signal lay at 8.56 ppm and the γ-Ach NH signal at 7.49 ppm. In fact, as with compound **CP-7**, neither the NMR spectrum nor NOE experiments with **CP-10** afforded sufficient information to allow unequivocal inference of its structure from the data. Fortunately, however, we were able to crystallize compound **CP-10** from methanol and chloroform.

In crystals of **CP-10** obtained from a solution in methanol Figure 11a, the peptide adopts a structure akin to a pair of type II′ β-turns,[31c,33,34] with a hydrogen bond between each γ-Ach carbonyl and the nonadjacent Ser(Bn) NH, and with

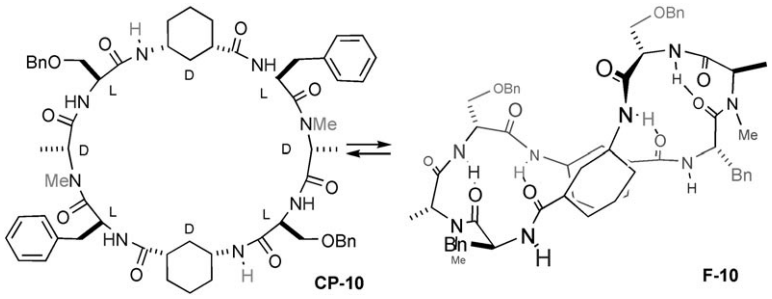

Figure 9. **CP-10** and its folded structure (**F-10**).

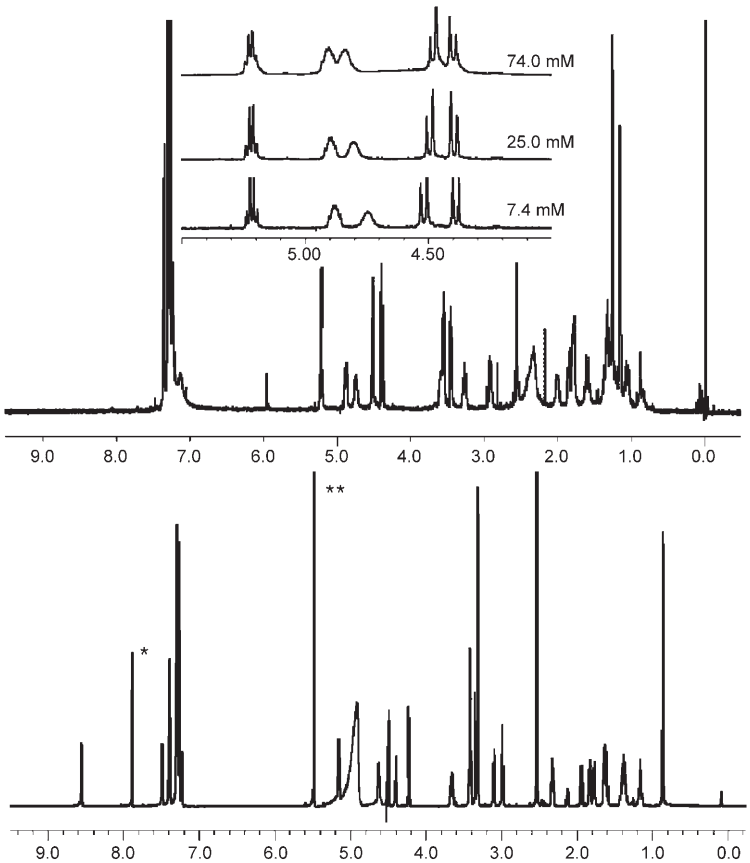

Figure 10. Top: ^{1}H NMR spectrum of a solution of peptide **CP-10** in $CDCl_3$ (7.4 mM) with the 4.0–5.5 ppm region of the spectra of 74.0, 25.0 and 7.4 mM solutions, showing the downfield shift of Phe H_α. Bottom: ^{1}H NMR spectrum of a solution of peptide **CP-10** in CD_3OH (10 mM). [* $CHCl_3$ (7.89 ppm), ** CH_2Cl_2 (5.48 ppm)].

Ramachandran plot coordinates (ϕ,ψ) of (60.4,−107.0) or (55.3,−107.4) for Phe (the two turns are not exactly identical) and (−110.0,47.9) or (−105.4,38.0) for ^{Me}N-Ala.[35] This double reverse turn structure avoids steric interactions between the N-methyl and carbonyl groups of ^{Me}N-Ala (the N−Me and C=O bonds making an angle of 52° with each other) and is stabilized not only by the β-turn hydrogen bonds but also by a pair of hydrogen bonds between the two γ-Achs. Layers of these double reverse turn structure are formed, in which methanol and water molecules form hydrogen bonds with carbonyl groups to stabilize the crystal structure (See Figure S2 in the Supporting Information).

When crystallized from chloroform (10% CS_2), **CP-10** adopts a structure like a couple of γ-turns (**F-10**), with a hydrogen bond between each Phe CO and the NH group of the Ser(Bn) on the other side of the adjacent ^{Me}N-Ala, which has (ϕ,ψ) coordinates of (93.7,−75.0) (Figure 11b).[33,36] As in the crystals obtained from methanol, there are also hydrogen bonds between the two γ-Ach residues. The receptor of the Phe NH is a Ser(Bn) belonging to another molecule of **CP-10**, giving rise, together with a water molecule, to the formation of a helical supramolecular structure with four CPs per turn (Figure 11c and d). A fast equilibrium between **F-10** and fragments of this helical structure in chloroform may explain the concentration dependence of NMR spectra of **CP-10** in this solvent (Figure 10); fitting of a LaPlanche model[37] of the relationship between total peptide concentration and the chemical shift of Phe $C_\alpha H$ afforded an value of 420 M^{-1} for the association constant at 298 K, and Van't Hoff plots for the 273–323 K range afforded values of −27.5 kJ mol^{-1} for $\Delta H^{\ominus}_{298}$ and −42.0 J K^{-1} mol^{-1} for $\Delta S^{\ominus}_{298}$ (Table S1 in the Supporting Information).

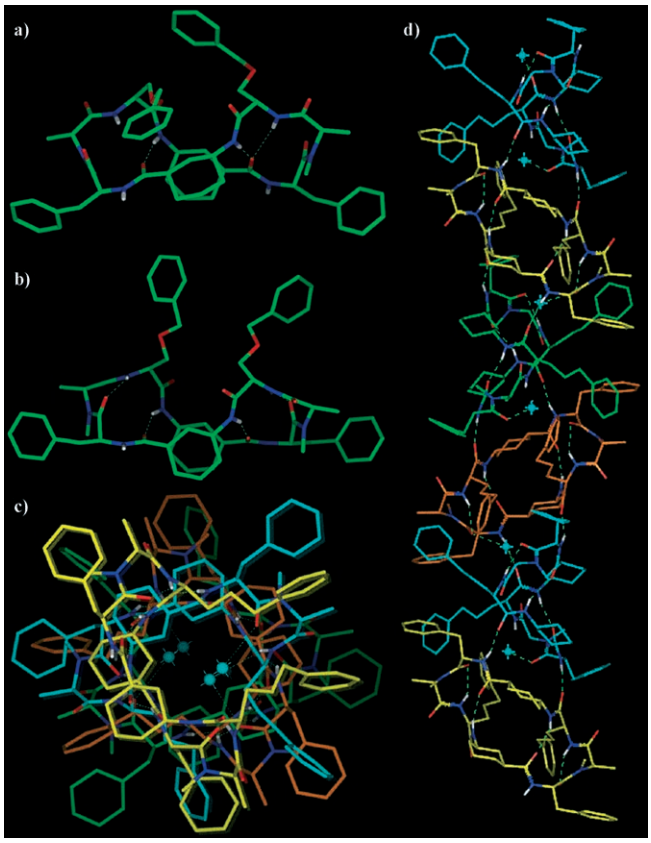

Figure 11. a) A monomer of **CP-10** as crystallized from methanol. b) A monomer of **CP-10** as crystallized from chloroform. c) Axial and d) lateral views of the helical supramolecular structure of **CP-10** as crystallized from chloroform, showing each monomer of the four-monomer pitch in a different colour, with the amide groups in blue (nitrogen), red (oxygen) and white (hydrogen).

The likelihood that **CP-7** may adopt a double reverse turn structure similar to that of **CP-10** in chloroform is supported by the similar concentration dependence of its ^{1}H NMR signals, and by its IR spectrum in chloroform having a band at 1687 cm^{-1}, a position typical of reverse turns and close to the band at 1684 cm^{-1} in the IR spectrum of **CP-10**. The possibility that **CP-9** may also adopt a double reverse turn conformation (**F-9**) is suggested by the positions (in chloroform solution) of its γ-Ach NH signal (7.97 ppm) and its α,γ-face exposed Ala NH (7.3 ppm), which suggest that both are involved in hydrogen bonding, while the Ser NH with its signal at 6.59 ppm is not, which is consistent with the double turn conformation adopted by **CP-10**. Interestingly, **CP-9** does not display concentration dependence of its ^{1}H NMR signals in nonpolar solvent, suggesting that the differences in the methylation patterns of those peptides (the methyl group in peptide **CP-9** is placed in the α-Aa after the γ-Ach, while in **CP-10** it is in the middle of the three α-Aas) might prevent the reverse turn structure self-assembling into a tyligomeric structure. Additionally all three peptides (**CP-7**, **9** and **10**) display dramatic conformational changes when dissolved in polar solvents such as methanol, denoted by ^{1}H NMR spectra with sharp and well defined signals, with some of the NH groups involved in strong hydrogen-bonding interactions (Phe NH at 8.81 ppm for peptide **CP-7**, Ser NH at 8.56 ppm and γ-Ach NH at 7.49 ppm for peptide **CP-10**, γ-Ach NH at 9.20 ppm for peptide **CP-9**) and in all three the γ-Ach NH (7.68 ppm for **7**, 7.49 ppm for **CP-10**, and 9.20 ppm for **CP-9**) are also involved in hydrogen-bonding interactions. All these data are consistent with the double β-turn conformation found for **CP-10**. These more closely tied structures in polar solvents must result from hydrophobic interaction between the two cyclohexyl moieties assisted by the formation of hydrogen bonds by the C=O and NH groups of the γ-Ach residues.

Conclusion

In this work we studied the behaviour of a new class of cyclic peptide—3α,γ-CPs—that were designed with overall C_2 symmetry with a view to the formation of SPNs featuring two functionalizable hydrophobic bands on opposite sides of their cavities. NMR, IR, MS, and X-ray diffraction data show that, depending on their backbone N-methylation patterns and the medium, monomers of eight-residue 3α,γ-CPs can remain as flat rings or fold into double reverse turns,

that flat monomers dimerize through sets of hydrogen bonds of antiparallel β-sheet type, forming a "β-band", and that monomers forming double γ-turns associate to form helical supramolecular structures.

N-Methylation just of their α,γ-face γ-Achs (as in **CP-6**), or of their α,α-face α-Aas with backbone nitrogens that are *not* adjacent to a γ-Ach C=O (as in **CP-8**), appears to ensure that eight-residue 3α,γ-CP monomers are flat and form tight dimeric β-bands. Additional N-methylation of the other residues on the same face (as in **CP-4** and **CP-5**) weakens dimerization, probably due to deviation from flatness caused by steric interaction between the N-methyl and C=O groups of the "newly" methylated α-Aas. N-Methylation of these other residues in the absence of the flatness-enforcing methyls (as in **CP-7**, **9** and **10**) appears to result in the monomers forming twisted, double reverse turn structures comprising a pair of β-turns (in methanol) or γ-turns (in chloroform). Monomers of **CP-10** with γ-turns associate in helical supramolecular structures. Given the structural and functional importance of reverse turns in molecular biology,[38] it is possible that the reverse-turn-forming propensity of these 3α,γ-CPs may find application in studies of biologically significant peptides.

Experimental Section

General: 2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), Boc-phenylalanine, Boc-serine and Boc-*N*-methylalanine were purchased from Novabiochem or Advanced ChemTech. All reagents and solvents were used as received unless otherwise noted. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Inova 750 MHz, Varian Mercury 300 MHz, Bruker AMX 500 MHz, or Bruker WM 250 MHz spectrometers. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane ($\delta = 0.00$ ppm). ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), or quartet (q). All first-order splitting patterns were assigned on the basis of the appearance of the multiplet. Splitting patterns that could not easily be interpreted are designated as multiplet (m) or broad (br). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian Mercury 300 MHz or Bruker WM 250 MHz spectrometers. Carbon resonances were assigned by use of distortionless enhancement by polarization transfer (DEPT) spectra obtained with phase angles of 135°. Mass spectra (MS) were obtained with Bruker Autoflex MALDI-TOF and Micromass Autospec mass spectrometers. Crystallographic data were collected in a Bruker–Nonius FR591-Kappa CCD2000 diffractometer or a Bruker SMART 1000 fitted with a sealed tube (Mo Kα) and a highly ordered graphite monochromator. FTIR measurements with 5–10 mM solutions in $CHCl_3$ placed in NaCl solution IR cells were made on a JASCO FT/IR-400 spectrophotometer. Column chromatography was performed on EM Science silica gel 60 (230–400 mesh). Solvent mixtures for chromatography are reported as *v*/*v* ratios. HPLC was carried out on Phenomenex Maxsil 10 silica columns with CH_2Cl_2/MeOH gradients between 100:0 and 90:10. CH_2Cl_2 and pyridine used as reaction solvents were distilled from CaH_2 over argon immediately prior to use.

Peptide synthesis: Unless otherwise noted, linear peptide synthesis was carried out by Boc solution-phase synthesis as previously described.[15] The resulting fully protected linear peptides were purified by flash column chromatography (CH_2Cl_2/MeOH).

cyclo-[(L-γ-Ach-D-^{Me}*N*-Ala-L-Leu-D-^{Me}*N*-Ala)$_2$-] (CP-4): A solution of Boc-[(L-Leu-D-^{Me}*N*-Ala-L-γ-Ach-D-^{Me}*N*-Ala)$_2$-]-OFm (124 mg, 0.10 mmol) in piperidine in CH_2Cl_2 (20%, 1 mL) was stirred at RT for 30 min. After removal of the solvent, the residue was dissolved in CH_2Cl_2 and washed with citric acid (10%), dried over Na_2SO_4, filtered, and concentrated. The resulting material was dissolved in TFA/CH_2Cl_2 (1:1) and stirred at RT for 10 min. After removal of the solvent, the residue was dried under high vacuum for 2 h and used without further purification. The linear peptide was dissolved in CH_2Cl_2 (165 mL, 0.6 mM) and treated with TBTU (35 mg, 0.11 mmol), followed by dropwise addition of DIEA (75 μL, 0.44 mmol). After 12 h, the solvent was removed under reduced pressure and the crude product was purified by HPLC (Phenomenex Maxsil 10, CH_2Cl_2/MeOH), affording **CP-4** as a white solid (27 mg, 33%). ^{1}H NMR ($CDCl_3$, 500 MHz, 233 K): $\delta = 8.75$ (brs, 1–0H; NH-Leu$_{dimer}$), 8.21 (brs, 1–0H; NH-γ-Ach$_{dimer}$), 7.66 (brs, 0–1H; NH-γ-Ach$_{mon}$), 7.02 (brs, 0–1H; NH-Leu$_{mon}$), 5.95 (m, 0–1H; Hα-^{Me}*N*-Ala$_{dimer}$), 5.51 (m, 0–1H; Hα-^{Me}*N*-Ala$_{dimer}$), 5.22 (m, 0–1H; Hα-^{Me}*N*-Ala$_{mon}$), 5.15 (m, 0–1H; Hα-Leu$_{dimer}$), 4.95 (m, 0–1H; Hα-^{Me}*N*-Leu$_{mo}$), 4.38 (m, 0–1H; Hα-Ala$_{mon}$), 3.83 (m, 0–1H; Hγ-Ach$_{mon}$), 3.70 (m, 0–1H; Hγ-Ach$_{dimer}$), 3.26 (s, 0–3H; *N*Me$_{dimer}$), 3.23 (s, 0–3H; *N*Me$_{dimer}$), 3.07 (s, 0–6H; *N*Me$_{mon}$), 2.91 ppm (s, 0–6H; *N*Me$_{mon}$); FTIR (293 K, $CHCl_3$): $\tilde{\nu} =$ 3413 and 3310 (amide A), 2955, 2937, 2860, 1673, 1632 (amide I), 1542 cm^{-1} (amide II$_{II}$); MALDI-TOF: *m*/*z* (%): 817.5 (40) $[M+H]^+$, 839.6 (49) $[M+Na]^+$, 855.6 (100) $[M+K]^+$; MALDI-TOF-HRMS: *m*/*z* (%): calcd for: 817.55461 $[M+H]^+$; found: 817.55054.

cyclo-[(L-^{Me}*N*-γ-Ach-D-Phe-L-^{Me}*N*-Ala-D-Phe)$_2$-] (CP-5): This compound was prepared in the same way as **CP-4**, from Boc-[(L-^{Me}*N*-γ-Ach-D-Phe-L-^{Me}*N*-Ala-D-Phe)$_2$-]-OFm (210 mg, 0.158 mmol). Yield after HPLC purification: 35 mg (21%). ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 8.80$ (d, $J =$ 7.5 Hz, 1–0H; NH-Phe$_{dimer}$), 8.31 (d, $J = 8.2$ Hz, 1–0H; NH-Phe$_{dimer}$), 7.49 (d, $J = 7.1$ Hz, 0–1H; NH-Phe$_{mon}$), 7.32–7.19 (m, 5H; Ar), 7.10 (0–1H; NH-Phe$_{mon}$), 5.89 (d, $J = 5.9$ Hz, 0–1H; Hα ^{Me}*N*-Ala$_{dimer}$), 5.48 (m, 0–1H; Hα Phe$_{dimer}$), 5.26 (m, 0–1H; Hα Phe$_{dimer}$), 5.16 (m, 0–1H; Hα ^{Me}*N*-Ala$_{mon}$), 4.86 (m, 0–2H; Hα Phe$_{mon}$), 4.39 (m, 0–1H; Hγ γ-Ach), 3.06 (s, 0–3H; ^{Me}N-Ala$_{dimer}$), 2.92 (m, 4H; CH_2β Phe), 2.72 (s, 0–3H; ^{Me}N-γ-Ach$_{dimer}$), 2.61 (s, 0–3H; ^{Me}N-Ala$_{mon}$), 2.52 ppm (s, 0–3H; ^{Me}N-γ-Ach$_{mon}$); FTIR (293 K, $CHCl_3$): $\tilde{\nu} = 3310$ (amide A), 2934, 2860, 1669, 1627 (amide I), 1525 cm^{-1} (amide II$_{II}$); MS-FAB$^+$: *m*/*z* (%): 1037.7 (100) $[M+H]^+$; HRMS: *m*/*z* (%): calcd for: 1037.59454 $[M+H]^+$; found: 1037.59087.

cyclo-[(L-Ser(Bn)-D-^{Me}*N*-γ-Ach-L-Phe-D-Ala)$_2$-] (CP-6): This compound was prepared in the same way as **CP-4**, from Boc-[(L-Ser(Bn)-D-^{Me}*N*-γ-Ach-L-Phe-D-Ala)$_2$-]-OFm (130 mg, 0.09 mmol). Yield after HPLC purification: 46 mg (35%). ^{1}H NMR ($CDCl_3$, 750 MHz): $\delta = 8.66$ (d, $J = 7.6$ Hz, 1H; NH-Phe), 8.58 (d, $J = 9.1$ Hz, 1H; NH-Ser), 7.23–7.11 (m, 10H; Ar), 6.49 (d, $J = 6.5$ Hz, 1H; NH-Ala), 5.36 (q, $J = 7.7$ Hz, 1H; Hα Ser), 4.68 (m, 1H; Hα Phe), 4.50 (m, 1H; Hγ γ-Ach), 4.43 (m, 1H; Hα Ala), 4.36 (d, $J = 12.3$ Hz, 1H; CH_2-Bn), 4.21 (d, $J = 12.3$ Hz, 1H; CH_2-Bn), 3.31 (m, 1H; CH_2-Ser), 3.26 (m, 1H; CH_2-Ser), 3.08 (m, 1H; CH_2-Phe), 2.95 (s, 3H; *N*Me), 2.82 (m, 1H; Hα-Ach), 2.76 (m, 1H; CH_2-Phe), 0.95 ppm (d, $J = 5.4$ Hz, 3H; Me-Ala); ^{13}C NMR ($CDCl_3$, 62.83 MHz): $\delta = 175.0$ (C=O), 171.5 (C=O), 170.3 (C=O), 168.9 (C=O), 137.7 (C), 136.6 (C), 129.4 (CH), 128.4 (CH), 128.3 (CH), 127.5 (CH), 126.8 (CH), 72.7 (CH_2), 71.1 (CH_2), 54.6 (CH), 51.6 (CH), 47.3 (CH), 47.3 (CH), 43.0 (CH), 41.1 (CH_2), 32.5 (CH_2), 29.9 (CH_3), 28.9 (CH_2), 27.5 (CH_2), 24.3 (CH_2), 21.3 ppm (CH_3); FTIR (293 K, $CHCl_3$): $\tilde{\nu} = 3403$ and 3296 (amide A), 2934, 2863, 1681, 1642 ppm (amide I), 1515 cm^{-1} (amide II$_{II}$); MALDI-TOF: *m*/*z* (%): 1069.6 (13) $[M+H]^+$, 1091.6 (100) $[M+Na]^+$, 1107.5 (46) $[M+K]^+$; MALDI-TOF-HRMS: *m*/*z* (%): calcd for: 1091.55768 $[M+Na]^+$; found: 1091.55636.

cyclo-[(L-γ-Ach-D-Phe-L-^{Me}*N*-Ala-D-Phe)$_2$-] (CP-7): This compound was prepared in the same way as **CP-4**, from Boc-[(L-γ-Ach-D-Phe-L-^{Me}*N*-Ala-D-Phe)$_2$-]-OFm (354 mg, 0.27 mmol). Yield after HPLC purification: 130 mg (48%). ^{1}H NMR (CD_3OH, 750 MHz): $\delta = 8.81$ (s, 1H; NH-Phe), 7.68 (d, $J = 8.0$ Hz, 1H; NH-Ach), 7.3–7.0 (m, 11H; NH-Phe and Ar), 4.90 (m, 1H; Hα-^{Me}*N*-Ala), 4.66 (m, 1H; Hα-Phe), 4.32 (m, 1H; Hα-Phe), 3.77 (m, 1H; Hγ-Ach), 3.2–2.90 (m, 4H; CH_2-Phe), 2.59 (m, 1H; Hα-Ach), 2.49 (s, 3H; *N*Me), 2.15 (d, $J = 11.9$ Hz, 1H; Ach), 2.03 (d, $J =$

11.7 Hz, 1H; Ach), 1.92 (d, J=13.1 Hz, 1H; Ach), 1.77 (d, J=10.0 Hz, 1H; Ach), 1.72 (d, J=12.2 Hz, 1H; Ach), 1.50 (d, J=13.1 Hz, 1H; Ach), 1.44 (d, J=12.1 Hz, 1H; Ach), 1.29 (d, J=10.1 Hz, 1H; Ach), 0.74 ppm (d, J=7.1 Hz, 3H; Me-Ala); ^{13}C NMR (CD_3OD, 75.4 MHz): δ=178.1 (C=O), 175.6 (C=O), 173.7 (C=O), 172.4 (C=O), 137.4 (C), 136.9 (C), 130.4 (CH), 129.9 (CH), 129.6 (CH), 129.5 (CH), 128.2 (CH), 128.0 (CH), 57.9 (CH), 53.7 (CH), 52.6 (CH), 48.9 (CH), 42.9 (CH), 40.9 (CH_2), 38.3 (CH_2), 35.5 (CH_2), 32.4 (CH_2), 31.9 (CH_2), 31.4 (CH_3), 25.8 (CH_2), 12.6 ppm (CH_3); FTIR (293 K, $CHCl_3$): $\tilde{\nu}$=3324 (amide A), 2939, 2860, 1687, 1652 (amide I), 1537 cm^{-1} (amide II$_{II}$); MS-FAB$^+$: m/z (%): 1009.5 (100) $[M+H]^+$, 1031.5 (11) $[M+Na]^+$; HRMS: m/z (%): calcd for: 1009.55514 $[M+H]^+$; found: 1009.55409.

cyclo-[(L-γ-Ach-D-Ala-L-Ser(Bn)-D-^{Me}*N*-Ala)$_2$-] (CP-8): This compound was prepared in the same way as **CP-4**, from Boc-[(L-Ser(Bn)-D-^{Me}*N*-Ala-L-γ-Ach-D-Ala)$_2$-]-OFm (140 mg, 0.116 mmol). Yield after HPLC purification: 74 mg (70%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ=8.70 (d, J=9.5 Hz, 1H; NH Ser), 7.91 (d, J=8.9 Hz, 1H; NH γ-Ach), 7.26–7.15 (m, 10H; Ar), 6.55 (d, J=6.5 Hz, 1H; NH-Ala), 5.54 (dd, J=7.0 Hz, 1H; Hα-Ser), 5.34 (q, J=7.1 Hz, 1H; Hα-^{Me}*N*-Ala), 5.06 (t, J=6.6 Hz, 1H; Hα-Ala), 4.41 and 4.28 (d, J=12.5 Hz, 2H; CH_2-Ser), 3.72 (m, 1H; Hγ-Ach), 3.39 (5H; CH_2-Ser and ^{Me}*N*-Ala), 2.09 (m, 1H; Hα-Ach), 1.30 (s, 3H; Me-Ala) and 1.28 ppm (s, 3H; Me-^{Me}N-Ala); ^{13}C NMR ($CDCl_3$, 62.83 MHz): δ= 173.9 (C=O), 172.3 (C=O), 171.5 (C=O), 169.9 (C=O), 137.5 (C), 128.4 (CH), 127.7 (CH), 127.1 (CH), 72.9 (CH_2), 71.0 (CH_2), 51.1 (CH), 48.9 (CH), 47.4 (CH), 46.4 (CH), 45.1 (CH), 34.4 (CH_2), 32.7 (CH_2), 31.4 (CH_3), 29.0 (CH_2), 24.1 (CH_2), 20.0 (CH_3), 17.2 ppm (CH_3); FTIR (293 K, $CHCl_3$): $\tilde{\nu}$=3417 and 3312 (amide A), 2935, 2862, 1683, 1651, 1616 (amide I), 1543 cm^{-1} (amide II$_{II}$); MS-FAB$^+$: m/z (%): 917.4 (100) $[M+H]^+$, 1833.4 (3) $[M+H]^+$; HRMS: m/z (%): calcd for: 917.51367 $[2M+H]^+$; found: 917.51484.

cyclo-[(L-γ-Ach-D-^{Me}*N*-Ala-L-Ser(Bn)-D-Ala)$_2$-] (CP-9): This compound was prepared in the same way as **CP-4**, from Boc-[(L-Ser(Bn)-D-Ala-L-γ-Ach-D-^{Me}*N*-Ala)$_2$-]-OFm (71.6 mg, 0.060 mmol). Yield after HPLC purification: 15 mg (28%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ=7.97 (d, J= 8.80 Hz, 1H; NH-Ach), 7.37–7.27 (m, 6H; NH-Ala and Ar), 6.59 (d, J= 8.3 Hz, 1H; NH-Ser), 4.61 (m, J=6.4 Hz, 2H; CH_2-Bn), 4.58–4.51 (m, 2H; Hα-Ala and Ser), 4.25 (m, 1H; Hγ-Ach), 3.96 (dd, J=6.1 and 10.1 Hz, 1H; CH_2-Ser), 3.78 (dd, J=5.1 and 10.1 Hz, 1H; CH_2-Ser), 3.54 (q, J=7.0 Hz, 1H; Hα-^{Me}*N*-Ala), 3.21 (s, 3H; *N*Me), 2.91 (m, 1H; Hα-Ach), 1.50 (d, J=7.0 Hz, 3H; Me-^{Me}*N*-Ala), 1.35 ppm (d, J=6.9 Hz, 3H; Me-Ala); ^{13}C NMR ($CDCl_3$, 62.83 MHz): δ= 179.2 (C=O), 173.2 (C=O), 170.5 (C=O), 169.2 (C=O), 137.7 (C), 128.5 (CH), 127.9 (CH), 127.8 (CH), 73.4 (CH_2), 66.9 (CH_2), 61.8 (CH_3), 52.7 (CH), 49.0 (CH), 42.9 (CH), 38.7 (CH), 31.7 (CH), 29.5 (CH_2), 29.2 (CH_2), 26.7 (CH_2), 17.6 (CH_3), 16.7 (CH_2), 13.6 ppm (CH_3); FTIR (293 K, $CHCl_3$): $\tilde{\nu}$=3432 and 3326 (amide A), 2935, 2855, 1671, 1629 (amide I), 1521 cm^{-1} (amide II$_{II}$); MALDI-TOF: m/z (%): 917 (9) $[M+H]^+$, 939 (100) $[M+Na]^+$; MALDI-TOF-HRMS: m/z (%): calcd for: 939.4952 $[M+Na]^+$; found: 939.4951.

cyclo-[(L-Ser(Bn)-D-γ-Ach-L-Phe-D-^{Me}*N*-Ala)$_2$-] (CP-10): This compound was prepared in the same way as **CP-4**, from Boc-[(L-Ser(Bn)-D-γ-Ach-L-Phe-D-^{Me}*N*-Ala)$_2$-]-OFm (71 mg, 0.05 mmol). Yield after HPLC purification: 20 mg (37%). ^{1}H NMR (CD_3OH, 750 MHz): δ=8.56 (s, 1H; NH-Ser), 7.49 (d, J=8.5 Hz, 1H; NH-Ach), 7.4–7.2 (m, 11H; NH Ar), 5.15 (q, J=7.0 Hz, 1H; Hα-^{Me}N-Ala), 4.63 (m, 1H; Hα-Ser), 4.50 (d, J= 12.7 Hz, 1H; CH_2-Bn), 4.40 (m, 1H; Hα-Phe), 4.22 (d, J=12.7 Hz, 1H; CH_2-Bn), 3.66 (m, 1H; Hγ-Ach), 3.42–3.35 (m, 4H; CH_2-Phe), 3.12 and 3.00 (m, 2H; CH_2-Ser), 2.53 (s, 3H; *N*Me), 2.33 (m, 1H; Hα-Ach), 1.95 (d, J=11.9 Hz, 1H; Ach), 1.83 (d, J=12.8 Hz, 1H; Ach), 1.78 (d, J= 12.5 Hz, 1H; Ach), 1.62 (d, 2H; Ach), 1.39 (d, 1H; Ach), 1.16 (d, J= 12.9 Hz, 1H; Ach), 0.86 ppm (d, J=7.0 Hz, 3H; Me-Ala); ^{13}C NMR (CD_3OH, 75.4 MHz): δ=178.0 (C=O), 175.7 (C=O), 172.9 (C=O), 171.3 (C=O), 138.9 (C), 137.2 (C), 130.4 (CH), 129.7 (CH), 129.5 (CH), 128.8 (CH), 128.7 (CH), 128.2 (CH), 73.8 (CH_2), 70.0 (CH_2), 56.4 (CH), 53.6 (CH), 52.5 (CH), 48.9 (CH) 43.1 (CH), 38.2 (CH_2), 35.2 (CH_2), 32.3 (CH_2), 31.0 (CH_3), 25.9 (CH_2), 12.6 ppm (CH_3); FTIR (293 K, $CHCl_3$): $\tilde{\nu}$=3327 (amide A), 2941, 2862, 1684, 1655 (amide I), 1530 cm^{-1} (amide II$_{II}$); MALDI-TOF: m/z (%): 1069.5 (5) $[M+H]^+$, 1091.5 (100) $[M+Na]^+$, 1107.5 (48) $[M+K]^+$; MALFI-TOF-HRMS: m/z (%): calcd for: 1091.5577 $[M+Na]^+$; found: 1091.5527.

^{1}H NMR assignments of cyclic peptides: The ^{1}H NMR spectra of peptides were identified with the aid of the corresponding double-quantum-filled 2D COSY (2QF-COSY) and/or NOESY and ROESY spectra, which were recorded at the indicated concentration and temperature. Mixing times (≈250 ms and 500 ms) were not optimized. Because of conformational averaging on the NMR timescale, CPs with C_2 sequence symmetry generally have ^{1}H NMR spectra that reflect the C_2 symmetry of the monomeric species and the D_2 symmetry of the dimeric species.

Measurement of association constants by variable-concentration ^{1}H NMR: The HPLC-purified peptides **CP-4**, **CP-5** and **CP-10** were dissolved in $CDCl_3$ and their association constants (K_a) were estimated as described in refs. [24,37].

Van't Hoff analysis of dimerization: The HPLC-purified cyclic peptides **CP-4**, **CP-5** and **CP-10** were each dissolved in $CDCl_3$ at concentrations ranging from 1 to 74 mM. ^{1}H NMR spectra were recorded at intervals of 5–10 K over the 223–323 K temperature range. K_a was estimated at each temperature as described in refs. [24,37]. Analysis of a plot of $1/T$ (K) vs. $\ln K_a$ then afforded the values $\Delta H^{\ominus}_{298}=-41.5$ kJ mol^{-1} and $\Delta S^{\ominus}_{298}=-152.5$ J K^{-1} mol^{-1} for **CP-4**, $\Delta H^{\ominus}_{298}=-47.8$ kJ mol^{-1} and $\Delta S^{\ominus}_{298}=-126.1$ J K^{-1} mol^{-1} for **CP-5**, and $\Delta H^{\ominus}_{298}=-27.5$ kJ mol^{-1} and $\Delta S^{\ominus}_{298}=-42.0$ J K^{-1} mol^{-1} for **CP-10**.

Preparation of single crystals for X-ray analysis: HPLC-purified peptide **CP-5** (3 mg) was dissolved in CH_2Cl_2 (1 mL) and equilibrated by vapour-phase diffusion against hexanes (5 mL), which resulted in spontaneous crystallization after 2–4 d.

HPLC-purified peptide **CP-10** (3 mg) was dissolved in a mixture of $CHCl_3$ and CS_2 (10:1, 1 mL) and equilibrated by vapour-phase diffusion against hexanes (5 mL), which resulted in spontaneous crystallization after 3–4 d.

HPLC-purified peptide **CP-10** (5 mg) was dissolved in MeOH (0.5 mL), resulting in spontaneous crystallization after 2 d.

X-ray crystallographic analysis: Data were collected either on a Bruker Nonius FR591 KappaCCD2000 fitted with an FR591 rotating anode ($Cu_{K\alpha}$) and Osmic multilayer confocal optics (**CP-5** and **CP-10** from $CHCl_3$) or on a Bruker SMART 1000 fitted with a sealed tube ($Mo_{K\alpha}$) and a highly ordered graphite monochromator at low temperature (**CP-5** at 100 K, **CP-10** ($CHCl_3$) at 100 K and **CP-10** (MeOH) at 120 K). All calculations were performed on an IBM-compatible PC with use of the programs COLLECT,[40] HKL Denzo and Scalepack,[41] ORTEP3,[42] PARST,[43] PLATON (SQUEEZE),[44] SORTAV,[45] SADABS and SHELX-97,[46] SIR2002,[47] SMART[48] and WinGx.[49]

CCDC 612329 (**CP-5**), 612330 [**CP-10**·$CHCl_3$] and 612331 [**CP-10**·MeOH] contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[1] a) R. Langer, D. A. Tirrell, *Nature* **2004**, *428*, 487–492; b) D. Cui, H. Gao, *Biotechnol. Prog.* **2003**, *19*, 683–692; c) T. M. Allen, P. R. Cullis, *Science* **2004**, *303*, 1818–1822; d) R. Langer, *Science* **2001**, *293*, 58–59; e) J. P. Vacanti, R. Langer, *Lancet* **1999**, *354*, SI32–34; f) S. Föster, M. Konrad, *J. Mater. Chem.* **2003**, *13*, 2671–2688; g) S. Föster, T. Plantenberg, *Angew. Chem.* **2002**, *114*, 712–739; *Angew. Chem. Int. Ed.* **2002**, *41*, 688–714.

[2] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180; b) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893–4011; c) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219–3232; d) A. R. Sanford, B. Gong, *Curr. Org. Chem.* **2003**, *7*, 1649–1659.

[3] For examples of reversible folding properties of foldamers induced by changes in temperature, solvent, pH or light, see: a) A. Khan, C. Kaisher, S. Hecht, *Angew. Chem.* **2006**, *118*, 1912–1915; *Angew. Chem. Int. Ed.* **2006**, *45*, 1878–1881; b) A. Khan, C. Kaisher, S. Hecht, *Angew. Chem.* **2003**, *115*, 6203–6206; *Angew. Chem. Int. Ed.* **2003**, *42*, 6021–6024; c) C. Dolain, V. Maurizot, I. Huc, *Angew. Chem.* **2003**, *115*, 2844–2846; *Angew. Chem. Int. Ed.* **2003**, *42*, 2738–2740; ; d) E. Kolomiets, V. Berl, I. Odriozola, A.-M. Stadler, N. Kyritsatas, J. M. Lehn, *Chem. Commun.* **2003**, 2868–2869; e) M. Barboiu, J. M. Lehn, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5201–6206; f) R. B. Prince, T. Okada, J. S. Moore, *Angew. Chem.* **1999**, *111*, 245–249; *Angew. Chem. Int. Ed.* **1999**, *38*, 233–236; .

[4] For some examples of foldamer-based reactivity, see: a) R. A. Smaldone, J. Moore, *J. Am. Chem. Soc.* **2007**, *129*, 5444–5450; b) T. J. Peelen, C. Yonggui, S. H. Gellman, *J. Am. Chem. Soc.* **2005**, *127*, 11598–11599; c) C. Dolain, C. Zhan, J.-M. Daniels, I. Huc, *J. Am. Chem. Soc.* **2005**, *127*, 2400–2401; d) J. M. Heemstra, J. Moore, *J. Am. Chem. Soc.* **2004**, *126*, 1648–1649.

[5] a) T. Beke, I. G. Csizmadia, A. Perczel, *J. Am. Chem. Soc.* **2006**, *128*, 5158–5167; b) D. Seebach, A. K. Beck, D. J. Bierbaum, *Chemistry & Biodiversity.* **2004**, *1*, 1111–1239; Biodiversity. **2004**, 1, 1111–1239.

[6] For some examples of γ-peptide foldamers, see: a) S. Hanessian, X. Lou, R. Schaum, *Tetrahedron Lett.* **1999**, *40*, 4925–4929; b) M. G. Woll, J. R. Lai, I. A. Guzei, S. J. C. Taylor, M. E. B. Smith, S. H. Gellman, *J. Am. Chem. Soc.* **2001**, *123*, 11077–11078; c) D. Seebach, A. Beck, M. Brenner, C. Gaul, A. Heckel, *Chimia* **2001**, *6*, 831–838; d) D. Seebach, L. Schaeffer, M. Brenner, D. Hoyer, *Angew. Chem.* **2003**, *99*, 800–802; *Angew. Chem. Int. Ed.* **2003**, *42*, 776–778; e) J. Farrera-Sinfreu, L. Zaccaro, D. Vidal, X. Salvatella, E. Giralt, M. Pons, F. Albericio, M. Royo, *J. Am. Chem. Soc.* **2004**, *126*, 6048–6057; f) G. V. M. Sharma, V. B. Jadhav, V. S. Ramakrishna, P. Jayaprakash, K. Narsimulu, V. Subash, A. C. Kunwar, *J. Am. Chem. Soc.* **2006**, *128*, 14657–14668.

[7] a) J. L. Price, W. S. Horne, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 6376–6377; b) D. S. Daniels, E. J. Petersson, J. X. Qiu, A. Schepartz, *J. Am. Chem. Soc.* **2007**, *129*, 1532–1533; c) N. Delsuc, J.-M. Léger, S. Massip, I. Huc, *Angew. Chem.* **2007**, *99*, 218–221; *Angew. Chem. Int. Ed.* **2007**, *46*, 214–217; d) B.-C. Lee, R. N. Zuckermann, K. A. Dill, *J. Am. Chem. Soc.* **2005**, *127*, 10999–11009; e) R. P. Cheng, W. F. J. DeGrado *J. Am. Chem. Soc.* **2002**, *124*, 11564–11565.

[8] M. Stefani, C. M. Dobson, *J. Mol. Med.* **2003**, *81*, 678–699, and references therein.

[9] a) J. M. Smeenk, M. B. J. Otten, J. Thies, D. A. Tirrell, H. G. Stunnenberg, J. C. M. van Hest, *Angew. Chem.* **2005**, *117*, 2004–2007; *Angew. Chem. Int. Ed.* **2005**, *44*, 1968–1971; b) M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, *366*, 324–327; c) C. Whitehouse, J. Fang, A. Aggeli, M. Bell, R. Brydson, C. W. G. Fishwick, J. R. Henderson, C. M. Knobler, R. W. Owens, N. H. Thomson, D. A. Smith, N. Boden, *Angew. Chem.* **2005**, *117*, 2001–2004; *Angew. Chem. Int. Ed.* **2005**, *44*, 1965–1968.

[10] a) N. Khazanovich, J. R. Granja, D. E. McRee, R. A. Milligan, M. R. Ghadiri *J. Am. Chem. Soc.* **1994**, *116*, 6011–6012; b) J. D. Hartgerink, J. R. Granja, R. A. Milligan, M. R. Ghadiri, *J. Am. Chem. Soc.* **1996**, *118*, 43–50; c) K. Rosenthal-Aiman, G. Svensson, A. Undén, *J. Am. Chem. Soc.* **2004**, *125*, 3372–3373.

[11] a) R. J. Brea, J. R. Granja, *Self-Assembly of Cyclic Peptides in Hydrogen-Bonded Nanotubes*, In Dekker Encyclopedia of Nanoscience and Nanotechnology, Marcel Dekker Inc., **2004**, pp. 3439–3457; b) G. R. Patzke, F. Krumeich, R. Nesper, *Angew. Chem.* **2002**, *114*, 2554–2571; *Angew. Chem. Int. Ed.* **2002**, *41*, 2446–2461; c) D. T. Bong, T. D. Clark, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 1016–1041; *Angew. Chem. Int. Ed.* **2001**, *40*, 988–1011; d) P. M. Ajayan, O. Z. Zhou, *Top. Appl. Phys.* **2001**, *80*, 391–425.

[12] For an example of biotechnological applications of nanotubes, see: C. R. Martin, P. Kohli, *Nature Reviews Drug Discovery* **2003**, *2*, 29–37.

[13] a) For transmembrane transport of molecules or ions by peptide nanotubes, see: M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature* **1994**, *369*, 301–304; J. R. Granja, M. R. Ghadiri, *J. Am. Chem. Soc.* **1994**, *116*, 10785–10786; J. Sanchez-Quesada, H. S. Kim, M. R. Ghadiri, *Angew. Chem.* **2001**, *99*, 2571–2574; *Angew. Chem. Int. Ed.* **2001**, *40*, 2503–2506; J. Sanchez-Quesada, H. S. Kim, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 2571–2574; *Angew. Chem. Int. Ed.* **2001**, *40*, 2503–2506; b) for biological application of peptide nanotubes, see: S. Fernández-López, H.-S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. Wilcoxen, M. R. Ghadiri, *Nature* **2001**, *412*, 452–455; V. Dartois, J. Sanchez-Quesada, E. Cabezas, E. Chi, C. Dubbelde, C. Dunn, C. Gritzen, D. Weinberger, J. R. Granja, M. R. Ghadiri, T. R. Parr Jr. , *Antimicrob. Agnt. Chemother.* **2005**, *49*, 3302–3310; W. S. Horne, C. M. Wiethoff, C. Cui, K. M. Wilcoxen, M. Amorín, M. R. Ghadiri, G. R. Nemerow, *Bioorg. Med. Chem.* **2005**, *13*, 5145–5153; c) for photoswitchable materials based on self-assembled peptides, see: C. Steinem, A. Janshoff, M. S. Vollmer, M. R. Ghadiri, *Langmuir* **1999**, *15*, 3956–3964; M. S. Vollmer, T. D. Clark, C. Steinem, M. R. Ghadiri, *Angew. Chem.* **1999**, *111*, 1703–1706; *Angew. Chem. Int. Ed.* **1999**, *38*, 1598–1601; d) for size-selective ion sensing based on SAM-supported peptide nanotubes, see: K. Motesharei, M. R. Ghadiri, *J. Am. Chem. Soc.* **1997**, *119*, 11306–11312; e) for hybrids of peptide nanotubes with polymers, see: J. Couet, J. D. J. S. Samuel, A. Kopyshev, S. Santer, M. Biesalski, *Angew. Chem.* **2005**, *117*, 3361–3365; *Angew. Chem. Int. Ed.* **2005**, *44*, 3297–3301; f) for potential applications in molecular electronics, see: N. Ashkenasy, W. S. Horne, M. R. Ghadiri, *Small* **2006**, *2*, 99–102.

[14] a) M. R. Ghadiri, K. Kobayashi, J. R. Granja, R. K. Chadha, D. E. McRee, *Angew. Chem.* **1995**, *107*, 76–78; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 93–95; b) D. T. Bong, M. R. Ghadiri, *Angew. Chem.* **2001**, *113*, 2221–2224; *Angew. Chem. Int. Ed.* **2001**, *40*, 2163–2166; c) M. Saviano, A. Lombardi, C. Pedone, B. Di Blasio, X. C. Sun, G. P. Lorenzi, *J. Incl. Phenom.* **1994**, *18*, 27–36; d) X. C. Sun, G. P. Lorenzi, *Helv. Chim. Acta* **1994**, *77*, 1520–1526; e) W. S. Horne, N. Ashkenasy, M. R. Ghadiri, *Chem. Eur. J.* **2005**, *11*, 1137–1144.

[15] a) M. Amorín, L. Castedo, J. R. Granja, *J. Am. Chem. Soc.* **2003**, *125*, 2844–2845; b) for eight-residue α,γ-CPs, see: M. Amorín, L. Castedo, J. R. Granja, *Chem. Eur. J.* **2005**, *11*, 6539–6547; c) for four-residue α,γ-CPs, see: M. Amorín, R. J. Brea, L. Castedo, J. R. Granja, *Org. Lett.* **2005**, *7*, 4681–4684; d) for larger-size α,γ-CPs, see: R. J. Brea, L. Castedo, J. R. Granja, *Chem. Com.* **2007**, 3267–3269.

[16] For α,γ-CP heterodimer formation and applications in electron- and energy-transfer processes, see: R. J. Brea, M. Amorín, L. Castedo, J. R. Granja, *Angew. Chem.* **2005**, *117*, 5856–5859; *Angew. Chem. Int. Ed.* **2005**, *44*, 5710–5713; R. J. Brea, L. Castedo, J. R. Granja, M. Á. Herranz, L. Sanchez, N. Martín, W. Seitz, D. M. Guldi, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 5291–5294.

[17] The flexibility of its 30-membered ring has been blamed for the non-association of an N-methylated cyclic decapeptide composed of alternating D- and L-α-amino acids: T. D. Clark, J. M. Buriak, K. Kobayashi, M. P. Isler, D. E. McRee, M. R. Ghadiri, *J. Am. Chem. Soc.* **1998**, *120*, 8949–8962.

[18] For peptides made of alternating α-amino acid and 3-aminobenzoic acid derivatives: as ion channels, a) H. Ishida, Z. Qi, M. Sokabe, K. Donowaki, Y. Inoue, *J. Org. Chem.* **2001**, *66*, 2978–2989; as ion pair receptors, b) S. Kubik, *J. Am. Chem. Soc.* **1999**, *121*, 5846–5855.

[19] a) Alkylation of the amide nitrogen reduces the energy difference between the *cis* and *trans* forms: D. E. Stewart, A. Sarkar, J. E. Wampler, *J. Mol. Biol.* **1990**, *214*, 253–260; b) *N*-modified amino acids would influence polypeptides containing them by adopting an extended conformation and ensuring the adoption of a similar conformation by their *N*-terminal neighbours: P. Manavalan, F. A. Momany, *Biopolymers* **1980**, *19*, 1943–1973; but also promoting the

formation of β-turns: c) Y. Takeuchi, G. R. Marshall *J. Am. Chem. Soc.* **1998**, *120*, 5363–5372.

[20] For a recent study of structural behaviour of N-methylated cyclic peptide, see: J. Chatterjee, D. Mierke, H. Kessler, *J. Am. Chem. Soc.* **2006**, *128*, 15164–15172, and references therein.

[21] For a recent study on β-turns structures by insertion of β, γ or δ-amino acids, see: R. Rai, P. G. Vasudev, K. Ananda, S. Raghothama, N. Shamala, I. L. Karle, P. Balaram, *Chem. Eur. J.* **2007**, *13*, 5917–5926.

[22] Even in the case of CPs formed of alternate L- and D-α-Aas, hydrogen bonding between antiparallel CPs is thermodynamically more favoured than hydrogen bonding between parallel CPs: K. Kobayashi, J. R. Granja, M. R. Ghadiri, *Angew. Chem.* **1995**, *107*, 79–81; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 95–98.

[23] J. K. M. Sanders, B. K. Hunter, *Modern NMR Spectroscopy*, Oxford University Press, Oxford, **1987**, p. 210.

[24] The dimerization constant (K_a) was estimated by fitting the equation: $[P]_t = I_{obs}/K_a[2I_{sat}(1-I_{obs}/I_{sat})^2]$ to data for the integral ratio I_{obs} (the ratio of the integral of the Leu (**4**) NH or Phe (**6**) $C_\alpha H$ signal of the dimer to the sum of those of dimer and monomer) at various total peptide concentrations $[P]_t$ in the range $(1.0–30.0)\times10^{-3}$ M, where I_{sat}, the integral ratio at saturation, was also optimized in the fitting process.

[25] A. Pardi, M. Billeter, K. Wutrich, *J. Mol. Biol.* **1984**, *180*, 741–751.

[26] a) P. I. Haris, D. Chapman, *Biopolymers (Peptide Sci.)* **1995**, *37*, 251–263; b) S. Krimm, J. Bandekar, *Adv. Protein Chem.* **1986**, *38*, 181–364; c) J. Bandekar, *Biochim. Biophys. Acta* **1992**, *1120*, 123–143; d) J. Kubelka, T. A. Keiderling, *J. Am. Chem. Soc.* **2001**, *123*, 12048–12058.

[27] a) M. S. Searle, M. S. Westwell, D. H. Williams, *J. Chem. Soc. Perkin Trans. 2* **1995**, 141–151; b) J. D. Dunitz, *Chem. Biol.* **1995**, *2*, 709–712.

[28] For a recent study on dimerization constants of α,γ-CPs by fluorescence measurements, see: R. J. Brea, M. E. Vázquez, M. Mosquera, L. Castedo, J. R. Granja, *J. Am. Chem. Soc.* **2007**, *129*, 1653–1657.

[29] a) D. Möhle, H.-J. Hofman, *J. Pept. Res.* **1998**, *51*, 19–28; b) B. Vitoux, A. Aubry, M. T. Cung, M. Marraud, *Int. J. Pept. Protein Res.* **1986**, *27*, 617.

[30] Because of the unavailability of D-Boc-Ser(Bn)-OH, we prepared compound **CP-7** as the enantiomer of the CP strictly corresponding to peptides **CP-6** and **CP-10**, using D-^{Me}N-γ-Ach instead of L-^{Me}N-γ-Ach.

[31] a) Alkylation of the amide nitrogen reduces the energy difference between the *cis* and *trans* forms to approximately 2.1 kJ mol^{-1} as against around 10.9 kJ mol^{-1} for conventional amino acids: D. E. Stewart, A. Sarkar, J. E. Wampler, *J. Mol. Biol.* **1990**, *214*, 253–260; b) N-modified amino acids would influence the polypeptides containing them by adopting an extended conformation and ensuring the adoption of a similar conformation by their *N*-terminal neighbours: P. Manavalan, F. A. Momany, *Biopolymers* **1980**, *19*, 1943–1973; but also by c) promoting the formation of β-turns: W. Braun, J. Kallen, V. Mikol, M. D. Walkinshaw, K. Wüthrich, *FASEB* **1995**, *9*, 63–72; N. Fusetani, S. Matsunaga, *Chem. Rev.* **1993**, *93*, 1793–1806; Y. Takeuchi, G. R. Marshall, *J. Am. Chem. Soc.* **1998**, *120*, 5363–5372.

[32] Chemical shifts of NH protons involved in strong hydrogen-bonding interactions stay unchanged with temperature change, while the chemical shifts of solvent-exposed NH protons (no hydrogen bonding involved) change with the temperature. T. Cierpici, J. Otlewski, *J. Biomol. NMR* **2001**, *21*, 249–261.

[33] a) J. A. Smith, L. G. Pease, *CRC Crit. Rev. Biochem.* **1980**, *8*, 315–399; b) G. D. Rose, W. B. Young, L. M. Gierasch, *Nature* **1983**, *304*, 654–657; c) G. D. Rose, L. M. Gierasch, J. A. Smith, in *Turns in Peptides and Proteins, Vol. 37* (Eds.: C. B. Anfinsen, J. T. Edsall, F. M. Richards), Academic Press, New York, **1985**, pp. 1–109; d) J. S. Richardson, D. C. Richardson, in *Prediction of Protein Structure and Principles of Protein Conformation* (Ed.: G. D. Fasman), Plenum, New York, **1989**, pp. 1–98; e) C. K. Smith, L. Regan, *Acc. Chem. Res.* **1997**, *30*, 153–166; f) E. Lacroix, T. Kortemme, M. L. de la Paz, L. Serrano, *Curr. Opin. Struct. Biol.* **1999**, *9*, 487–493.

[34] It has been shown that D-α-amino acids induce type II′ β-turns in solution, see: a) V. Bobde, C. Sadasivan, S. Durani, *Int. J. Peptide Protein Res.* **1994**, *44*, 209–218; b) V. Bobde, S. Durani, V. Pattabhi, C. Sadasivan, *Int. J. Peptide Protein Res.* **1995**, *45*, 332–336; c) Y. Yan, B. W. Erickson, A. Tropsha, *J. Am. Chem. Soc.* **1995**, *117*, 7592–7599.

[35] β Hairpins design by insertion of β-, γ- and δ-amino acid residues at the position *i*+2, see: R. S. Roy, H. N. Gopi, S. Raghothama, I. L. Karle, P. Balaram, *Chem. Eur. J.* **2006**, *12*, 3295–3302; R. Rai, P. G. Vaused, K. Anada, S. Raghothama, N. Shamala, I. L. Karle, P. Balaram, *Chem. Eur. J.* **2007**, *13*, 5917–5926.

[36] a) B. L. Sibanda, J. M. Thornton, *Nature*, **1985**, *116*, 170–174; b) C. M. Wilmot, J. M. Thornton *J. Mol. Biol.* **1988**, *203*, 221–223; c) E. G. Hutchinson, J. M. Thornton, *Prot. Sci.* **1994**, *3*, 2207–2216; d) C. Mattos, G. A. Petsko, M. Karplus, *J. Mol. Biol.* **1994**, *238*, 733–747; e) R. L. Dunbrack, M. Karplus, *Nat. Struct. Mol. Biol.* **1994**, *1*, 334–340.

[37] A. M. Andrianov, Y. A. Sokolov, *J. Biomol. Struct. Dyn.* **2003**, *20*, 603–614.

[38] The chemical shift (δ_{obs}) of the $C_\alpha H$ group was measured at concentrations *c* ranging from 7.4×10^{-3} to 7.5×10^{-2} M, and the association constant (K_a), together with the shifts in the ($\delta_{monomer}$) and dimer (δ_{dimer}), were estimated by using a nonlinear regression program to fit the (δ, *c*) data with the equation $\delta_{obs}=\delta_{dimer}+(\delta_{monomer}-\delta_{dimer})[(1+8K_ac)^{1/2}-1]/(4K_ac)$, L. A. LaPlanche, H. B. Thompson, M. T. Rogers, *J. Phys. Chem.* **1965**, *69*, 1482.

[39] a) R. L. Stanfield, T. M. Fieser, R. A. Lerner, I. A. Wilson, *Science* **1990**, *248*, 712–719; b) G. R. Marshall, *Curr. Opin. Struct. Biol.* **1992**, *2*, 904–919.

[40] Nonius BV, Delft (The Netherlands) **1997–2000**.

[41] Z. Otwinowski, W. Minor, *Methods Enzymol.* **1997**, *276*, 307–326.

[42] L. J. Farrugia *J. Appl. Crystallogr.* **1997**, 30, 565.

[43] M. J. Nardelli, *Appl. Crystallogr.* **1995**, *28*, 659.

[44] A. L. Spek, PLATON, University of Utrecht (The Netherlands) (**2001**); P. van der Sluis, A. L. Spek, *Acta Crystallogr. Sect. A* **1990**, *46*, 194–201.

[45] R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *A51*, 33–38.

[46] G. M. Sheldrick, Institute für Anorganische Chemie, Universität Göttingen, Göttingen (Germany).

[47] M. C. Burla, M. Camalli, B. Carrozzini, G. L. Cascarano, C. Giacovazzo, G. Polidori, R. Spagna, *J. Appl. Crystallogr.* **2003**, *36*, 1103.

[48] Bruker AXS, Madison (USA).

[49] L. J. Farrugia, *J. Appl. Crystallogr.* **1999**, *32*, 837–838.

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