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Self-Assembled Peptide Tubelets with 7 Å Pores

Manuel Amorín, Luis Castedo, and Juan R. Granja*[a]

Abstract: Here we report the preparation and structural characteristics of self-assembling peptide tubelets composed of 32-membered rings formed of alternating α-amino acids and *cis*-3-aminocyclohexanecarboxylic acids. The tubelets possess a partial hydrophobic core environment, provided by the projection of the cyclohexane C2 methylene moiety into the lumen, and a Van der Waals pore diameter of about 7 Å.

Keywords: amino acids · dimerization · nanotubes · peptides · self-assembly

Introduction

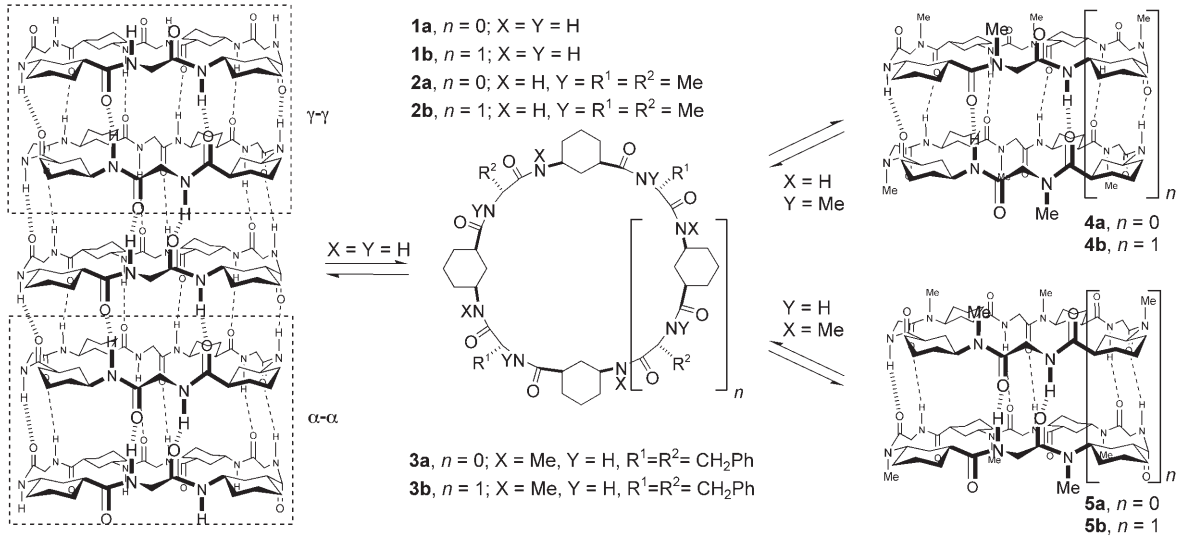
In recent years, considerable effort has been devoted to the synthesis of organic and inorganic nanotubes,[1] spurred by their potential utility in a wide variety of chemical, biological, and materials science applications.[1b,2,3] Proposed designs include hollow bundles of rod-like units, helically wound linear species, rolled-up sheets, helically juxtapositioned truncated wedges, and stacked rings. In particular, this last design in principle facilitates control of what in most applications would be one of the critical parameters of the nanotubes, their internal diameter.

One of the most promising implementations of the stacked ring approach is the self-assembling peptide nanotube (SPN).[4] The elementary components of SPNs are cyclic peptides, the chiralities of the amino acids of which allow the ring to adopt a quasiplanar conformation.[4–7] In this conformation, peptide backbone NH and C=O groups project perpendicularly from the plane of the ring on either side and are therefore able to form hydrogen bonds with those of neighboring rings, so constructing a nanotube. Specifically, self-assembling nanotubes have been designed based on cyclic peptides of alternating D,L-α-amino acids,[4,5] β-amino acids,[6] alternating α,β-amino acids,[7] δ-amino acids,[8] alternating α,ε-amino acids,[9] and oligoureas.[10] These design choices are not purely artistic, because the functional characteristics of the nanotubes made by this strategy depend on the nature of both their interior and exterior surfaces. The hydrophilic nature of the peptide nanotube interior has been an important feature in designing transmembrane ion channels and pores.[6b,11] The possibility of extending this class of nanotubes to include tubes with hydrophobic inner surfaces, a task that has not yet been widely performed because it requires peptide main-chain replacements that should not adversely affect the conformational requirements for the self-assembly process, has recently been shown in work on dimers that self-assemble from cyclic peptides composed of alternating D-α-amino acids and (1*R*,3*S*)-3-aminocyclohexanecarboxylic acids (D-α-Aas and L-γ-Achs, respectively), with the hydrophobic core environment being provided by the projection of one of the cyclohexane methylene moieties into the lumen.[12,13] In that work, each cyclic monomer consisted of three [L-γ-Ach-D-α-Aa] units (**1a**, Scheme 1) and the approximate Van der Waals internal diameter of the resulting dimeric "tubelets" was 4.3 Å. For analogous tubelets composed of 32-membered rings formed of four [L-γ-Ach-D-α-Aa] units (**1b**) or four [D-γ-Ach-L-α-Aa] units, a Van der Waals pore diameter of about 7 Å can be calculated. Here we report the preparation and structural characteristics of tubelets confirming these expectations.

Scheme 1 (left) shows how molecules of **1b** can stack to constitute a nanotube. As each constituent cyclic peptide has its γ-Ach NH and C=O groups on one face (the γ face) and its D-α-Aa NH and C=O groups on the other (the α face) and because the HN···C=O spacing of the α- and γ-amino acids is different, the orientations of the rings must alternate so that α faces bond to α faces and γ faces to γ

[a] M. Amorín, Prof. Dr. L. Castedo, Prof. Dr. J. R. Granja
Departamento de Química Orgánica e Unidade Asociada ó C.S.I.C.
Facultade de Química, Universidade de Santiago
15782 Santiago de Compostela (Spain)
Fax: (+34) 981-595-012
E-mail: qojuangg@usc.es

Supporting Information for this article (^{1}H and ^{13}C NMR spectra of L-Boc-^{Me}N-γ-Ach-OFm; ^{1}H and ^{13}C NMR, NOESY and/or ROESY, and FTIR spectra of peptides **2b**, **3b**, **6a**, **6b**) is available on the WWW under http://www.chemeurj.org/ or from the author.

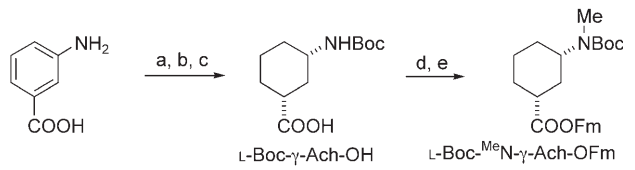


Scheme 1. Center: A generic cyclo[(D-α-Aa-L-γ-Ach)$_n$] peptide. Left: Structure of a nanotube self-assembled therefrom by Ach-to-Ach (γ–γ) and Aa-to-Aa (α–α) hydrogen bonding between antiparallel rings. Right: Dimers of derivatives with *N*-methylated D-α-Aa (top) or *N*-methylated L-γ-Ach (bottom) illustrating γ–γ and α–α bonding, respectively. For clarity, amino acid side chains have been omitted in the representations of the nanotube and dimers.

faces, in each case through the formation of a β-sheet-like set of hydrogen bonds between antiparallel rings. In this study, we investigated the stabilities of these two types of interring interaction (α–α and γ–γ) by preparing and characterizing tubelets formed of rings in which the stacking of more than two rings was blocked by *N*-methylation of either the α-amino acid (for formation of γ–γ-bound tubelets) or the γ-amino acid (for formation of α–α-bound tubelets).[14]

Results and Discussion

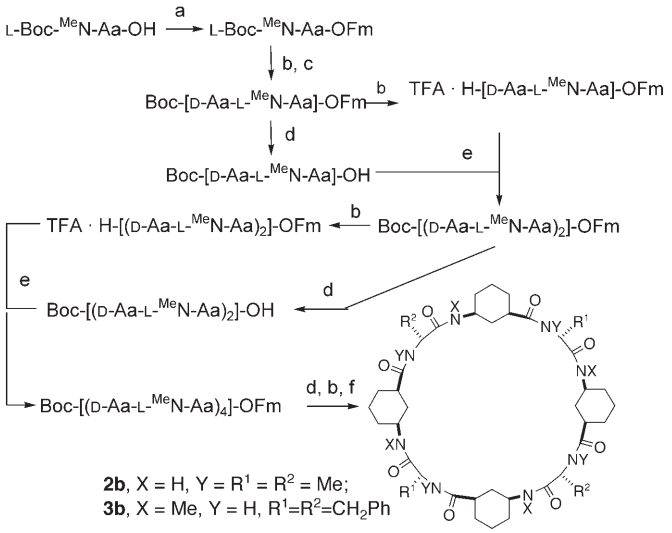
The *cis*-3-aminocyclohexanecarboxylic acids (Boc-γ-Ach and Boc-^{Me}N-γ-Ach; Boc = *tert*-butoxycarbonyl, ^{Me}N = *N*-methyl) needed for these studies were prepared by hydrogenation of the sodium salt of *m*-aminobenzoic acid in the presence of alkaline Raney nickel at 90–100 atm and 150 °C[15] followed by treatment with *tert*-butoxycarbonyl anhydride to provide the racemic acid Boc-γ-Ach-OH in 64% yield (Scheme 2). Resolution with successive recrystallizations from chloroform containing 1 equivalent of (+)-1-phenylethanamine gave (1*R*,3*S*)-*N*-Boc-3-aminocyclohexanecarboxylic acid (L-Boc-γ-Ach-OH) with an enantiomeric purity greater than 95%.[16] The *N*-methylated amino acid Boc-^{Me}N-γ-Ach-OH was then obtained by treatment of Boc-γ-Ach-OH with sodium hydride and methyl iodide in THF, and the fluorenyl-protected ester Boc-^{Me}N-γ-Ach-OFm was obtained by treatment of the resulting Boc-^{Me}N-γ-Ach-OH with 9-fluorenylmethanol (Scheme 2).



Scheme 2. Synthesis of the fluorenyl-protected ester of *cis*-*N*-Boc-*N*-methyl-3-aminocyclohexanecarboxylic acid (Boc-^{Me}N-γ-Ach-OFm): a) H_2 (95 atm), Raney Ni, NaOH, H_2O, 150 °C, 80%; b) $(Boc)_2O$, DIEA, H_2O/dioxane, 80%; c) resolution with (+)-1-phenylethylamine, $CHCl_3$/hexane; d) NaH, MeI, THF, 88%; e) FmOH, EDC, HOBt, DMAP, CH_2Cl_2, 89%. DIEA = *N*,*N*-diisopropylethylamine, THF = tetrahydrofuran, Fm = 9*H*-fluoren-9-ylmethyl, EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide, HOBt = 1-hydroxy-1*H*-benzotriazole, DMAP = 4-dimethylaminopyridine.

The cyclopeptides were prepared following the synthetic strategy shown in Scheme 3, by starting from the fluorenyl-protected ester of the Boc-protected *N*-methylamino acid, that is, D-Boc-^{Me}N-Ala-OFm for the cyclic-peptide precursors of γ–γ-bound tubelets and L-Boc-^{Me}N-γ-Ach-OFm for the cyclic-peptide precursors of α–α-bound tubelets. The corresponding dipeptides, tetrapeptides, and octapeptides were prepared by using standard solution-phase peptide-synthesis protocols, and treatment of the linear unprotected octapeptides with TBTU and DIEA at 6 mM concentrations in dichloromethane led to formation of the desired cyclic products in good yields (50–75%).

The *N*-methylated cyclopeptide cyclo[(D-^{Me}N-Ala-L-γ-Ach)$_4$] (**2b**) was characterized by NMR and FTIR spectroscopy and mass spectrometry. Its ^{1}H NMR spectrum shows it to have a flat, all-*trans* conformation in polar and nonpolar solvents. In nonpolar solvents, such as deuteriochloroform, the spectrum reflects two species that are moderately slow at exchanging on the NMR timescale, and the peaks representing these species are sharp enough at 273 K to show that both have $J_{NH-\gamma H}$ constants of 7.4 Hz, a value indicative of the flat all-*trans* backbone conformation. The concentration dependence of the ratio of these two species is consistent with one being monomeric **2b** and the other being the dimer **4b** (Scheme 1),[17] with the association constant in $CDCl_3$ at 273 K being 340 M^{-1}.[18] The β-sheet nature of the



Scheme 3. General synthetic strategy for cyclic peptide preparation: a) FmOH, EDC, HOBt, DMAP, CH_2Cl_2; b) 50% TFA in CH_2Cl_2; c) Boc-Aa-OH, HATU, DIEA, CH_2Cl_2; d) 20% piperidine in CH_2Cl_2; e) HBTU, DIEA, CH_2Cl_2; f) TBTU, DIEA, CH_2Cl_2. TFA = trifluoroacetic acid, HATU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, TBTU = 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate.

hydrogen bonding in the dimer is shown by the downfield shift of the Ala $C_\alpha H$ signal from $\delta = 5.31$ ppm in the NMR spectrum of the monomer to $\delta = 5.59$ ppm in that of the dimer (the δ shift of the γ-Ach $C_\gamma H$ signal from 3.59 to 3.82 ppm probably has the same significance). The β-sheet nature is also indicated by the FTIR spectrum in $CHCl_3$, which shows an amide I band at $\tilde{\nu} = 1627\ cm^{-1}$ and an amide II_{II} band at $\tilde{\nu} = 1540\ cm^{-1}$. Amide A FTIR bands near $\tilde{\nu} = 3315\ cm^{-1}$ suggest an intermonomer distance of 4.85–4.90 Å.[19]

The strength of the hydrogen bonds in **4b** is shown by the NMR signals for its NH groups lying considerably downfield of those of **2b**, at $\delta = 8.12$ ppm as against $\delta = 6.76$ ppm. However, the hydrogen bonding in **4b** is weaker than in the dimer (**4a**) of the hexapeptide cyclo[(D-^{Me}N-Ala-L-γ-Ach)$_3$]:[12] Van't Hoff plots for the range 243–283 K afford values of $-38.2\ kJ\,mol^{-1}$ for ΔH°_{298} and $-94.2\ kJ^{-1}\,mol^{-1}$ for ΔS°_{298}. These results show that although the dimerization process is enthalpy driven,[20] as in the case of the hexapeptide, the energy per hydrogen bond is only $1.27\ kJ\,mol^{-1}$ in **4b** as against $2.20\ kJ\,mol^{-1}$ for the hexapeptide dimer (**4a**), for which $\Delta H^\circ_{298} = -34.1\ kJ\,mol^{-1}$ and $\Delta S^\circ_{298} = -69.8\ kJ^{-1}\,mol^{-1}$.[12] The difference in energy per bond is due partly to the more negative ΔS°_{298} value of **4b**, which is attributable to the greater flexibility of its larger ring,[21] and partly to a disproportionately small increase in negative ΔH°_{298}, which is discussed below in relation to the crystallographic characterization of **4b**.[22]

To investigate the stability of α–α-bound tubelets, we prepared compound **3b**. In polar solvents, such as dimethyl sulfoxide (DMSO), its ^{1}H NMR spectrum reflects a slowly interconverting conformer equilibrium;[23] however, in nonpolar solvents the spectrum consists of a single set of signals and exhibits no concentration dependence ($J_{NH-\alpha H} = 8.6$ Hz), with the chemical shift of the Phe NH proton remaining constant ($\delta = 8.57$ ppm) at concentrations down to 1 mM. Addition of 35% or more of methanol, or heating to 333 K, does not alter this NH signal by more than about 0.1 ppm. Since the FTIR spectrum in $CHCl_3$ shows β-sheet-like hydrogen bonding (amide I, II_{II}, and A bands at $\tilde{\nu} = 1623$, 1525, and 3312 cm^{-1}, respectively),[19] compound **3b** must therefore be entirely dimerized as **5b**, and the α–α interaction must therefore be considerably stronger than the γ–γ interaction. This difference is probably due to the backbone conformation being better fitted for α–α than for γ–γ interactions and to the NH group of the α-amino acid being more polarized than that of the γ-amino acid.[22]

Conclusive proof of the dimerization of **2b** to **4b** and **3b** to **5b** was provided by X-ray diffractometry of single crystals obtained by crystallization from $CHCl_3/CCl_4$ (**2b**) or MeOH (**3b**). In both cases the colorless prismatic crystals were composed of units in which two antiparallel cyclooctapeptide rings were linked in β-sheet fashion by eight hydrogen bonds (Figure 1),[24] although in the case of **3b** the unit cell contained two nonequivalent dimers: one in which the range of hydrogen-bond N···O distances, 2.91–3.01 Å, was similar in breadth to that observed in **2b** (2.84–2.93 Å) and another in which the N···O distances were more irregular, ranging from 2.86–3.12 Å (Figure 1f). In the peptide rings of both dimers of **3b**, all four Phe C(O)–C_α–N angles are very similar (≈110°), thereby resulting in circular molecules (Figure 1e) and dimer lumina with approximate Van der Waals diameters of 6.6 Å ($H_{\beta\text{-Ach}}$–$H_{\beta\text{-Ach}}$), but in those of the dimer of **2b**, probably because of lattice packing forces, the Ala C(O)–C_α–N angle alternated between 107.6° and 112.9° in one monomer and between 110.3° and 111.6° in the other, thereby resulting in elliptical molecules (Figure 1a) and dimer lumina with approximate minimum and maximum diameters ($C_{\alpha\text{-Ala}}$–$C_{\alpha\text{-Ala}}$) of 8.06 and 12.05 Å for one cyclopeptide and 8.50 and 12.00 Å for the other. The cavities of the dimers **4b** and **5b** have Van der Waals volumes of approximately 232 and 286 $Å^3$,[25] respectively. In both cases they contain disordered solvent molecules, a fact that establishes the ability of **4b** to retain hydrophobic molecules ($CHCl_3$ and/or CCl_4) and the ability of **5b** to retain hydrophilic molecules (MeOH, H_2O);[26,27] in particular, the fact that the observed electron density for water molecules in the cavity of **5b** is the time average for molecules at several overlapping sites suggests that these molecules are loosely bound and can easily move around within the cavity. It may be noted that all the hydrogen-bonding amide groups in **4b** are slightly tilted towards the center of the cavity (Figure 1b); this may explain why the enthalpy of dimerization was only $38.2\ kJ\,mol^{-1}$, instead of about $46\ kJ\,mol^{-1}$ as would be suggested by the value for the corresponding D,L-cyclohexapeptide dimer.[12a] Finally, the body-centered structure of the crystals of **4b** and **5b**, together with the annular

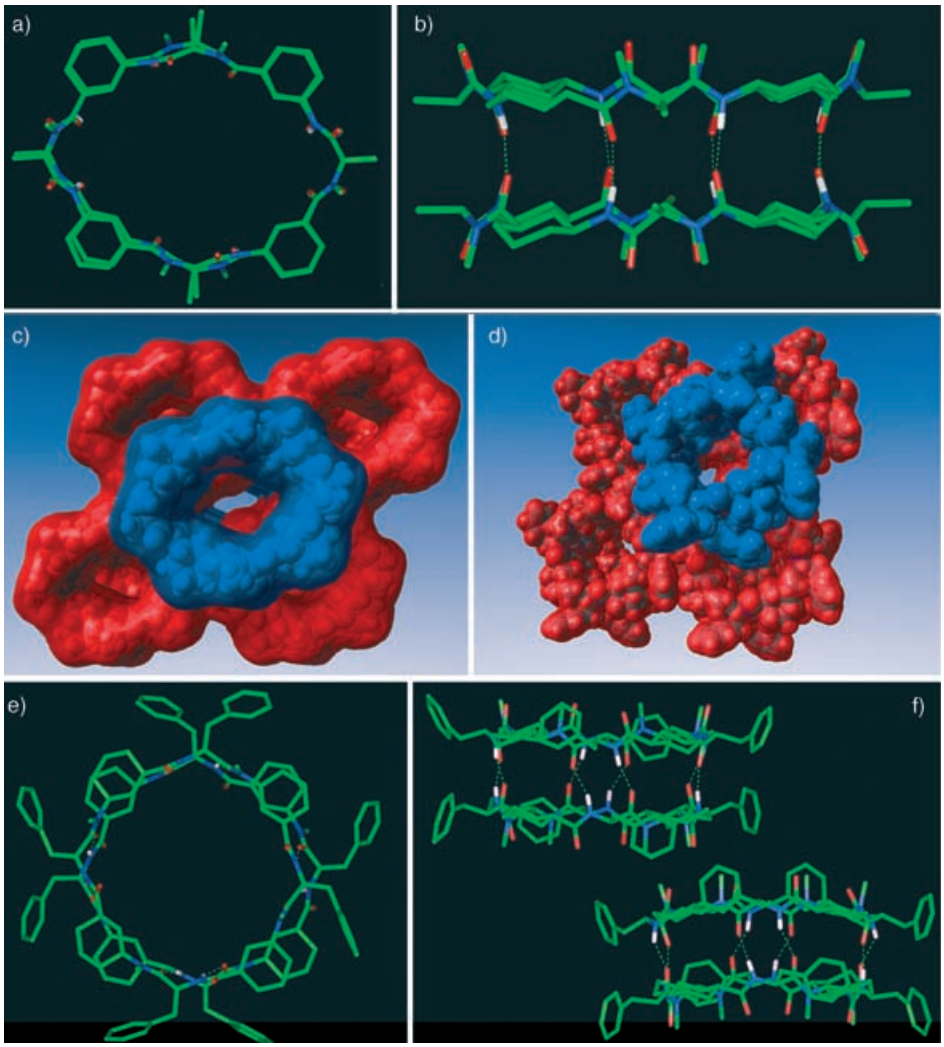


Figure 1. Crystal structures of dimers of a–c) cyclo[(D-^{Me}N-Ala-L-γ-Ach)$_4$] (**2b**) and d–f) cyclo[(D-Phe-L-^{Me}N-γ-Ach)$_4$] (**3b**), as viewed along (a and e) or perpendicular to (b and f) the dimer axis or along the crystallographic axis (c and d).

nature of the dimers themselves, creates channels along the crystallographic *b* (**2b**) and *c* (**3b**) axes that are composed of two alternating types of segment with a period of about 12.5 Å for **2b** and 10.5 Å for **3b**; this spacing is due to the dimer pore and the space surrounded by the external surfaces of four coplanar dimers (Figure 1c,d). In the case of **4b** these channels are straight, while those of **5b** are more sinuous.

To investigate whether self-assembly might be significantly affected by interactions between amino acid side chains on adjacent cyclopeptides, we prepared cyclo[(L-Ser-D-^{Me}N-γ-Ach-L-Phe-D-^{Me}N-γ-Ach)$_2$] (**6b**; Figure 2). This cyclopeptide has twofold symmetry instead of the fourfold symmetry of **2b** and **3b**, and can accordingly, in principle, form two kinds of α–α-bonded dimer with stabilities that might differ because of differences in between-monomer side-chain interactions: one in which Phe faces Phe and Ser faces Ser (**7b**), and the other in which Phe faces Ser (**7b***; Figure 2). We hypothesized that hydrogen bonding between mutually opposed Ser hydroxy groups would favor the formation of **7b**.[28]

As expected, the ^{1}H NMR spectrum of **6b** in chloroform showed two sets of signals, both of which reflect β-sheet-like hydrogen bonding between all-*trans* monomers (Figure 2, bottom left). The ratio between these two species was 6:1 and did not depend on peptide concentration or temperature, thereby showing both forms to be completely dimerized.[29] Unexpectedly, however, 2D NOESY experiments showing NH_{Ser}–NH_{Phe} and $H\beta_{Ser}$–$H\beta_{Phe}$ cross-peaks (Figure 2, bottom right) implied that the major form of the dimer was **7b***. Hypothesizing that the predominance of **7b*** might be due to steric hindrance between the mutually opposed phenyl moieties of **7b** or to weak hydrogen bonds between phenyl (Phe) and hydroxy (Ser) groups in **7b***,[30] we examined the dimerization behavior of cyclopeptide **6a**, from which **6b** had been prepared. Although steric hindrance between phenyl groups must be similar for **7a*** and **7a**, and although there is no possibility of O−H···Ph hydrogen bonding in **7a***, it was again the "staggered" arrangement (**7a***) that predominated in a similar ratio. Examination of the chemical shifts of signals for the NH groups of **7b** and **7b*** suggests that, although the NH_{Phe} hydrogen bond is stronger in **7b** ($\delta(NH_{Phe}) = 8.65$ ppm) than in **7b*** ($\delta(NH_{Phe}) = 8.49$ ppm), the NH_{Ser} hydrogen bond is much weaker ($\delta(NH_{Ser}) = 8.14$ ppm in **7b**; $\delta(NH_{Ser}) = 8.96$ ppm in **7b***), so that the overall balance seems to favor the staggered form. The weakness of the NH_{Ser}–O_{Ser} bond is in keeping with Ser having a somewhat lower propensity to form β sheets than Phe;[31] in all the other dimerizing hydrogen bonds of **7b** and **7b*** (NH_{Ser}–O_{Phe}, NH_{Phe}–O_{Ser}, and NH_{Phe}–O_{Phe}) at least one Phe is involved. It may be pointed out that the above interpretation suggests that eclipsed α–α bonding of the kind modeled by **7b** results in the component cyclopeptides becoming buckled, with the Phe–Phe distances being shorter than the Ser–Ser distances.

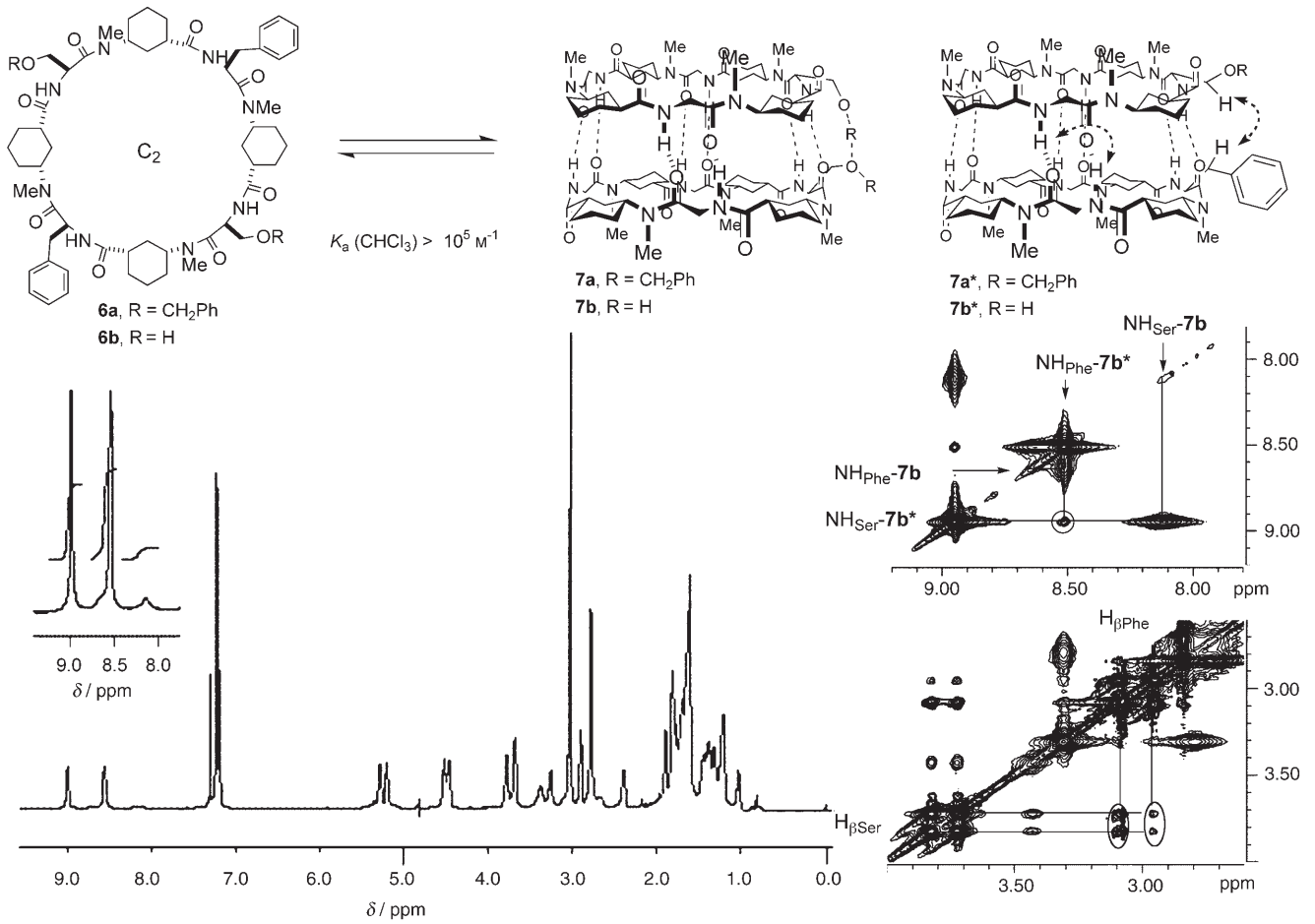


Figure 2. Top: Cyclooctapeptides **6a** and **6b** and the two dimers that can be formed from each: in one dimer the Phe of one monomer faces the Phe of the other, while in the second dimer Phe faces the other α-amino acid (Ser or benzylated Ser). For clarity, amino acid side chains have been omitted. Bottom left: ^{1}H NMR spectrum of **6b** showing the NH signals of both **7b** and **7b***. Bottom right: NOESY spectrum showing NH_{Ser}(**7b***)–NH_{Phe}(**7b***) and $H\beta_{Phe}$–$H\beta_{Ser}$ cross-peaks.

Conclusion

Like their hexapeptide analogues, cyclooctapeptides composed of four [L-γ-Ach-D-α-Aa] or four [D-γ-Ach-L-α-Aa] units can form dimers in which antiparallel rings are linked by a β-sheet-like set of eight hydrogen bonds. This suggests that the class of α,γ-SPNs extends at least to members composed of 32-membered rings with pore diameters of around 7 Å. The finding of both hydrophobic and hydrophilic solvent molecules in the cavities of the dimers synthesized in this work supports the possibility that α,γ-SPNs could be used for inclusion of both hydrophobic and hydrophilic molecules. As has been pointed out elsewhere, the fact that C2 of γ-Ach intrudes into the cavity (where it must contribute to hydrophobicity) means that α,γ-SPNs, unlike SPNs based on α- or β-amino acids, might be endowed with functionalized inner surfaces affording greater selectivity as ion channels, catalysts, receptors, or molecule containers.

Experimental Section

General: HBTU, TBTU, HATU, Boc-phenylalanine, serine, and *N*-methylalanine were purchased from Novabiochem or Advanced ChemTech. All reagents and solvents were used as received unless otherwise noted. ^{1}H NMR spectra were recorded on Varian Inova-750 MHz, Varian Mercury-300 MHz, Varian Inova-400 MHz, Bruker AMX-500 MHz, or Bruker WM-250 MHz spectrometers. Chemical shifts (δ) were reported in parts per million (ppm) 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 be easily interpreted are designated as multiplet (m) or broad (br). ^{13}C NMR spectra were recorded on Varian Mercury-300 MHz and Bruker WM-250 MHz spectrometers. Carbon resonances were assigned by using DEPT spectra obtained with phase angles of 135°. Mass spectra were obtained by using Bruker Autoflex MALDI-TOF and Micromass Autospec mass spectrometers. Crystallographic data were collected in a FR591-KappaCCD2000 Bruker-Nonius diffractometer. FTIR measurements were made on a JASCO FT/IR-400 spectrophotometer with solutions at a concentration of 5–10 mM in $CHCl_3$ and placed in an NaCl solution IR cell. Column chromatography was performed on EM Science silica gel 60 (230–400 mesh). Solvent mixtures for chromatography are re-

ported as v/v ratios. Reversed-phase HPLC was carried out on C_{18} columns with the use of water/acetonitrile/TFA gradients between 99:1:0.1 and 10:90:0.1, and HPLC was carried out on phenomenex maxsil-10 silica column with CH_2Cl_2/MeOH gradients between 100 and 90:10. THF was distilled from sodium/benzophenone under argon immediately prior to use. CH_2Cl_2 and pyridine to be used as reaction solvents were distilled from CaH_2 over argon immediately prior to use.

***cis*-3-Aminocyclohexanecarboxylic acid (γ-Ach-OH)**:[15] A solution of 3-aminobenzoic acid (11.3 g, 82.5 mmol) and NaOH (3.3 g, 82.5 mmol) in H_2O (200 mL) in a pressure bottle was treated with Raney Ni (7.0 g). The resulting mixture was hydrogenated (90–100 atm) at 150 °C for 1 h in a Parr apparatus. The mixture was then filtered over celite, and the catalyst was washed with H_2O. Acidification of the resulting solution to pH 2 with 10% HCl and concentration under vacuum gave a residue that was desalted by Dowex AG50W-X4 chromatography (1 M pyridine) to provide γ-Ach-OH (9.73 g, 80%) as a white solid; $R_f = 0.48$ (MeOH); ^{1}H NMR (250 MHz, D_2O, 25 °C): $\delta = 3.04$ (m, 1H), 2.20–1.70 (m, 5H), 1.30–0.90 ppm (m, 4H).[32]

(1*R*,3*S*)-*N*-Boc-3-aminocyclohexanecarboxylic acid (L-Boc-γ-Ach-OH): Boc_2O (7.0 g, 32 mmol) and DIEA (14.7 mL, 84 mmol) were added to a solution of *cis*-3-aminocyclohexanecarboxylic acid (4.0 g, 28 mmol) in water (25 mL) and dioxane (25 mL). After being stirred at room temperature for 3 h, the solution was acidified to pH 2 and extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated, and the resulting oil was crystallized from CH_2Cl_2/hexane (2:1) to give racemic Boc-γ-Ach-OH (3.4 g initially and 2.1 g in a second crystallization, 80%); $R_f = 0.85$ (MeOH); m.p. 136–138 °C. The racemic Boc-γ-Ach-OH was redissolved by crystallization from $CHCl_3$/hexane (2:1) in the presence of (+)-1-phenylethanamine (1 equiv). The resulting white crystals were washed with $CHCl_3$/hexane (2:1), and the mixture was poured into a separation funnel, dissolved in CH_2Cl_2, and washed with 10% citric acid. This operation was repeated 2–3 times. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated, and the resulting oil was crystallized from CH_2Cl_2/hexane (2:1);[16] $[\alpha]_D^{20} = -50.5$ ($c = 1$ in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 5.56$ (m, 1H, NH), 4.47 (m, 1H), 3.44 (m, 1H), 1.42 ppm (s, 9H); ^{13}C NMR (62.83 MHz, CD_3OD, 25 °C): $\delta = 178.7$ (C=O), 157.6 (C=O), 79.8 (C), 50.2 (CH), 43.5 (CH), 36.5 (CH_2), 33.4 (CH_2), 29.4 (CH_2), 28.9 (CH_3), 25.4 ppm (CH_2); elemental analysis: calcd (%): C 58.99, H 8.68, N 5.73; found: C 58.53, H 9.03, N 5.75%; FAB$^+$-MS: *m*/*z* (%): 487 (17) [M_2H^+], 387 (18) [M_2H^+−Boc], 244 (66) [MH^+], 188 (94) [MH^+−CH_2=C(CH_3)$_2$], 144 (100) [MH^+−Boc].

(1*R*,3*S*)-*N*-Boc-*N*-methyl-3-aminocyclohexanecarboxylic acid (L-Boc-^{Me}N-γ-Ach-OH): A solution of L-Boc-γ-Ach-OH (1.38 g, 5.68 mmol) in dry THF (50 mL) was treated with NaH (680 mg, 60% in mineral oil, 17.1 mmol). The resulting mixture was stirred at 0 °C for 30 min, and then methyl iodide (1.06 mL, 17.1 mmol) was added. After the mixture had been stirred overnight at room temperature, an additional three equivalents of NaH (680 mg, 17.1 mmol) and methyl iodide (1.06 mL, 17.1 mmol) were added at 0 °C if starting material was detected by TLC, and the resulting mixture was stirred for 3 h at room temperature. After being quenched with water, the solution was concentrated to remove the THF and the resulting aqueous solution was washed with diethyl ether, acidified to pH 3 by addition of HCl (10%), and finally extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was crystallized from CH_2Cl_2/hexane to give the L-Boc-^{Me}N-γ-Ach-OH (1.29 g, 88%) as a white solid; $R_f = 0.58$ (10% MeOH in CH_2Cl_2); m.p. 141–143 °C; $[\alpha]_D^{20} = -51.7$ ($c = 1$ in MeOH); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 4.01$ and 3.77 (m, 1H), 2.69 (s, 3H), 2.42 (m, 1H), 1.42 ppm (s, 9H); ^{13}C NMR (62.83 MHz, $CDCl_3$, 25 °C): $\delta = 180.5$ (C=O), 155.6 (C=O), 79.6 (C), 54.0 and 52.6 (CH), 42.4 (CH), 31.9 (CH_2), 29.1 (CH_2), 28.4 (CH_3), 28.2(CH_3), 28.0 (CH_2), 24.4 ppm (CH_2); FAB$^+$-MS: *m*/*z* (%): 515 (6) [2MH^+], 258 (67) [MH^+], 158 (86) [MH^+−Boc$^+$]; HRMS: calcd: 258.17053 [MH^+]; found: 258.17042.

(1*R*,3*S*)-(9*H*-fluoren-9-yl)-methyl *N*-Boc-*N*-methyl-3-aminocyclohexanecarboxylate (L-Boc-^{Me}N-γ-Ach-OFm): A solution of L-Boc-γ-^{Me}N-Ach-OH (100 mg, 0.389 mmol) in dry CH_2Cl_2 (10 mL) was treated successively with EDC (112 mg, 0.584 mmol), HOBt (79 mg, 0.584 mmol), 9-fluorenylmethanol (92 mg, 0.467 mmol), and DMAP (70 mg, 0.584 mmol). After being stirred for 1 h at room temperature, the solution was washed with 10% citric acid and saturated $NaHCO_3$. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was purified by flash chromatography (50–80% CH_2Cl_2 in hexane) to give L-Boc-^{Me}N-γ-Ach-OFm (151 mg, 89%) as a yellow oil; $R_f = 0.95$ (10% MeOH in CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 7.70$ (d, J(H,H) = 7.2 Hz, 2H), 7.53 (d, J(H,H) = 7.2 Hz, 8H), 7.38–7.20 (m, 4H), 4.36 (d, J(H,H) = 6.5 Hz, 2H), 4.14 (t, J-(H,H) = 6.7 Hz, 1H), 3.99 and 3.74 (m, 1H), 2.67 (s, 3H), 2.43 (m, 1H), 1.42 ppm (s, 9H); ^{13}C NMR ($CDCl_3$, 62.83 MHz): $\delta = 174.9$ (C=O), 155.5 (C=O), 143.7 (C), 141.3 (C), 127.7–119.9 (CH), 79.3 (C), 66.6 (CH_2), 52.7 (CH), 46.9 (CH), 42.7 (CH), 32.2 (CH_2), 29.2 (CH_2), 28.4 (CH_3), 28.2 (CH_3), 28.1 (CH_2), 24.5 ppm (CH_2); FAB$^+$-MS: *m*/*z* (%): 436 (7) [MH^+], 336 (100) [MH−Boc$^+$]; HRMS: calcd: 436.24878 [MH^+]; found: 436.24997.

Boc-[L-γ-Ach-D-^{Me}N-Ala]-OFm: A solution of D-Boc-^{Me}N-Ala-OFm (160 mg, 0.570 mmol) in TFA/CH_2Cl_2 (1:1) was stirred at room temperature for 10 min. After removal of the solvent, the residue was dried under high vacuum for 2 h and then dissolved in dry CH_2Cl_2 (40 mM). L-Boc-γ-Ach-OH (138 mg, 0.570 mmol), HATU (229 mg, 0.601 mmol), and DIEA (400 mL, 2.280 mmol) were successively added. After being stirred for 30 min at room temperature, the solution was poured into a separation funnel and washed with 10% citric acid and saturated $NaHCO_3$. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was purified by flash chromatography (0–5% MeOH in CH_2Cl_2) to give the desired dipeptide (231 mg, 80%) as a white foam; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): $\delta = 7.72$ (d, J(H,H) = 7.3 Hz, 2H), 7.50 (t, J(H,H) = 9.2 Hz, 2H), 7.40–7.23 (m, 4H), 5.14 (q, J(H,H) = 7.2 Hz, 1H), 4.68 (d, J(H,H) = 7.3 Hz, 1H), 4.50 (m, 2H), 4.12 (t, J(H,H) = 5.7 Hz, 1H), 3.46 (m, 1H), 2.62 (s, 3H), 2.44 (m, H), 1.42 (s, 9H), 1.22 ppm (d, J(H,H) = 7.3 Hz, 3H); ^{13}C NMR (75.40 MHz, $CDCl_3$, 25 °C): $\delta = 174.6$ (C=O), 171.5 (C=O), 155.0 (C=O), 143.4 and 143.2 (C), 141.2 and 141.1 (C), 127.6–119.7 (CH), 78.8 (C), 66.4 and 65.9 (CH_2), 54.6 and 51.9 (CH), 48.7 (CH), 46.7 and 46.6 (CH), 39.5 and 39.2 (CH), 35.8 and 35.2 (CH_2); 32.8 and 32.6 (CH_2), 30.9 (CH_3), 28.6 and 28.2 (CH_3), 27.8 (CH_2), 24.2 (CH_2), 15.4 and 14.1 ppm (CH_3); FAB$^+$-MS: *m*/*z* (%): 507 (18) [MH^+]; 407 (100) [MH−Boc$^+$]; HRMS: calcd: 507.28590 [MH^+]; found: 507.28780.

Boc-[D-Phe-L-^{Me}N-γ-Ach]-OFm: This compound was prepared in the same way as dipeptide Boc-[L-γ-Ach-D-^{Me}N-Ala]-OFm by using L-Boc-^{Me}N-γ-Ach-OFm (145 mg, 0.334 mmol) and D-Boc-Phe-OH (88.5 mg, 0.334 mmol) to afford the desired dipeptide (164 mg, 84%); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 7.76$ (t, J(H,H) = 6.5 Hz, 2H), 7.57 (t, J(H,H) = 7.0 Hz, 2H), 7.41–7.16 (m, 9H), 5.43 (d, J(H,H) = 8.2 Hz, 1H), 4.79 (dd, J(H,H) = 7.8, 13.7 Hz, 1H), 4.44 (m, 2H), 4.18 (dd, J(H,H) = 6.8, 13.6 Hz, 1H), 3.25 (m, 1H), 2.96 (m, 2H), 2.70 and 2.44 (s, 3H), 1.42 ppm (s, 9H); ^{13}C NMR (62.83 MHz, $CDCl_3$, 25 °C): $\delta = 174.4$ (C=O), 171.1 (C=O), 154.8 (C=O), 143.4 and 143.4 (C), 141.1 (C), 136.5 and 136.2 (C), 129.3–119.7 (CH), 79.3 and 79.3 (C), 66.8 and 65.8 (CH_2), 54.9 (CH), 51.7 and 51.3 (CH), 46.8 and 46.7 (CH), 42.4 and 42.1 (CH), 40.4 and 40.1 (CH_2), 31.8 and 31.1 (CH_2), 29.5 and 29.4 (CH_2), 28.8 (CH_3), 28.1 and 27.2 (CH_3), 27.8 (CH_2), 24.0 ppm (CH_2); FAB$^+$-MS: *m*/*z* (%): 583 (100) [MH^+], 483 (70) [MH−Boc$^+$]; HRMS: calcd: 583.31273 [MH^+]; found: 583.31460.

Boc-[L-Phe-D-^{Me}N-γ-Ach]-OFm: This compound was prepared in the same way as dipeptide Boc-[L-γ-Ach-D-^{Me}N-Ala]-OFm by using D-Boc-^{Me}N-γ-Ach-OFm (1.335 g, 3.07 mmol) and L-Boc-Phe-OH (0.814 g, 3.07 mmol) to afford the desired dipeptide (1.61 g, 90%); FAB$^+$-MS: *m*/*z* (%): 583 (69) [MH^+], 483 (100) [MH−Boc$^+$].

Boc-[L-Ser(Bn)-D-^{Me}N-γ-Ach]-OFm (Bn = benzyl): This compound was prepared in the same way as dipeptide Boc-[D-γ-Ach-L-^{Me}N-Ala]-OFm by using D-Boc-^{Me}N-γ-Ach-OFm (1.055 g, 2.43 mmol) and L-Boc-Ser(Bn)-OH (0.717 g, 2.43 mmol) to afford the desired dipeptide (1.290 g, 90%); ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C): $\delta = 7.71$ (d, J(H,H) = 7.3 Hz, 2H), 7.54 (d, J(H,H) = 7.1 Hz, 2H), 7.39–7.18 (m, 9H), 5.51 (m, 1H), 4.85 (m, 1H), 4.48–4.37 (m, 4H), 4.23 (m, 1H), 3.80 (m, 1H), 3.57 (m,

2H), 2.86 and 2.78 (s, 3H), 2.47 (m, 1H), 1.42 ppm (s, 9H); ^{13}C NMR (62.83 MHz, $CDCl_3$, 25°C): δ = 174.5 and 174.1 (C=O), 170.2 and 170.0 (C=O), 155.0 and 154.9 (C=O), 143.5 (C), 141.1 (C), 137.6 and 137.3 (C), 128.1–119.8 (CH), 79.5 and 79.5 (C), 73.1 and 73.0 (CH_2), 71.1 and 70.0 (CH_2), 65.9 and 65.8 (CH_2), 54.7 (CH), 51.6 and 51.1 (CH), 50.1 and 49.8 (CH), 49.8 and 46.7 (CH), 42.2 and 42.1 (CH), 32.2 and 31.2 (CH_2), 29.5 and 28.5 (CH_2), 29.4 (CH_3), 28.2 and 27.6 (CH_3), 27.6 (CH_2), 24.1 ppm (CH_2); FAB$^+$-MS: *m/z* (%): 613 (48) [*M*H$^+$], 513 (100) [*M*H−Boc$^+$]; HRMS: calcd: 613.32776 [*M*H$^+$]; found: 613.32670.

Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_2$]-OFm: A solution of Boc-[L-γ-Ach-D-^{Me}N-Ala]-OFm (1.012 g, 2.0 mmol) in TFA/CH_2Cl_2 (1:1) was stirred at room temperature for 10 min. After removal of the solvent, the residue was dried under high vacuum for 2 h to give TFA·H-[L-γ-Ach-D-^{Me}N-Ala]-OFm, which was used without further purification.

A solution of dipeptide Boc-[L-γ-Ach-D-^{Me}N-Ala]-OFm (1.012 g, 2.0 mmol) in 20% piperidine in CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min, and then the solvent was removed. The residue was dissolved in CH_2Cl_2, and the solution was washed with 10% citric acid, dried over Na_2SO_4, filtered, and concentrated to give Boc-[L-γ-Ach-D-^{Me}N-Ala]-OH, which was used without further purification.

The previously prepared dipeptides Boc-[L-γ-Ach-D-^{Me}N-Ala]-OH and TFA·H-[L-γ-Ach-D-^{Me}N-Ala]-OFm were dissolved in dry CH_2Cl_2 (25 mL), and HBTU (758 mg, 2.00 mmol), and DIEA (1.354 mL, 0.80 mmol) were successively added. After being stirred 30 min at room temperature, the solution was washed with 10% citric acid and saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting material was purified by flash chromatography (0–5% MeOH in CH_2Cl_2) to give Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_2$]-OFm (1.156 g, 81%) as a white foam; FAB$^+$-MS: *m/z* (%): 717 (42) [*M*H$^+$], 617 (100) [*M*H−Boc$^+$]; HRMS: calcd: 717.42273 [*M*H$^+$]; found: 717.42222.

Boc-[(D-Phe-L-^{Me}N-γ-Ach)$_2$]-OFm: This compound was prepared in the same way as tetrapeptide Boc-[L-γ-Ach-D-^{Me}N-Ala]$_2$-OFm from dipeptide Boc-[D-Phe-L-^{Me}N-γ-Ach]-OFm (280 mg, 0.481 mmol) to afford Boc-[(D-Phe-L-^{Me}N-γ-Ach)$_2$]-OFm (380 mg, 91%); FAB$^+$-MS: *m/z* (%): 869 (10) [*M*H$^+$], 769 (100) [*M*H−Boc$^+$]; HRMS: calcd: 869.48532 [*M*H$^+$]; found: 869.48466.

Boc-[L-Phe-D-^{Me}N-γ-Ach-L-Ser(Bn)-D-^{Me}N-γ-Ach]-OFm: This compound was prepared in the same way as tetrapeptide Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_2$]-OFm from dipeptides Boc-[L-Phe-D-^{Me}N-γ-Ach]-OFm (387 mg, 0.663 mmol) and Boc-[L-Ser(Bn)-D-^{Me}N-γ-Ach]-OFm (392 mg, 0.663 mmol) to afford Boc-[L-Phe-D-^{Me}N-γ-Ach-L-Ser(Bn)-D-^{Me}N-γ-Ach]-OFm (477 mg, 80%); FAB$^+$-MS: *m/z* (%): 899 (14) [*M*H$^+$], 799 (100) [*M*H−Boc$^+$]; HRMS: calcd: 899.49589 [*M*H$^+$]; found: 899.49656.

Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_4$]-OFm: This compound was prepared in the same way as tetrapeptide Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_2$]-OFm from tetrapeptide Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_2$]-OFm (303 mg, 0.492 mmol) to afford Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_4$]-OFm (512 mg, 91%); FAB$^+$-MS: *m/z* (%): 1138 (80) [*M*H$^+$], 1038 (100) [*M*H−Boc$^+$]; HRMS: calcd: 1137.70002 [*M*H$^+$]; found: 1137.69933.

Boc-[(D-Phe-L-^{Me}N-γ-Ach)$_4$]-OFm: This compound was prepared in the same way as Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_4$]-OFm by using tetrapeptide Boc-[(D-Phe-L-^{Me}N-γ-Ach)$_2$]-OFm (174 mg, 0.20 mmol) to afford Boc-[(D-Phe-L-^{Me}N-γ-Ach)$_4$]-OFm (201 mg, 73%); MALDI-TOF MS: *m/z* calcd for $C_{87}H_{108}N_8O_{11}Na$ [*M*Na$^+$]: 1463.8; found: 1462.4.

Boc-[(L-Phe-D-^{Me}N-γ-Ach-L-Ser(Bn)-D-^{Me}N-γ-Ach)$_2$]-OFm: This compound was prepared in the same way as Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_4$]-OFm from tetrapeptide Boc-[L-Phe-D-^{Me}N-γ-Ach-L-Ser(Bn)-D-^{Me}N-γ-Ach]-OFm (477 mg, 0.531 mmol) to afford Boc-[(L-Phe-D-^{Me}N-γ-Ach-L-Ser(Bn)-D-^{Me}N-γ-Ach)$_2$]-OFm (541 mg, 68%); FAB$^+$-MS: *m/z* (%): 1502 (10) [*M*H$^+$]; 1402 (100) [*M*H−Boc$^+$]; HRMS: calcd: 1402.79364 [*M*H−Boc$^+$]; found: 1402.79118.

cyclo[(L-γ-Ach-D-^{Me}N-Ala)$_4$] (2b): A solution of Boc-[(L-γ-Ach-D-^{Me}N-Ala)$_4$]-OFm (170 mg, 0.15 mmol) in 20% piperidine in CH_2Cl_2 (2 mL) was stirred at room temperature for 30 min. After removal of the solvent, the residue was dissolved in CH_2Cl_2, and the mixture was washed with 10% citric acid, dried over Na_2SO_4, filtered, and concentrated. The resulting material was dissolved in TFA/CH_2Cl_2 (1:1) and stirred at room temperature 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 (25 mL) and treated with TBTU (53 mg, 0.165 mmol). This was followed by dropwise addition of DIEA (105 mL, 0.6 mmol). After 12 h, the solvent was removed under reduced pressure, and the crude residue was purified by reversed-phase HPLC to afford **2b** (125 mg, 50%) as a white solid; ^{1}H NMR (400 MHz, DMSO, 25°C): δ = 7.54 (d, *J*(H,H) = 8.2 Hz, 1H), 4.86 (d, *J*(H,H) = 7.2 Hz, 1H), 3.63 (m, 1H), 2.90 (s, 3H), 2.69 (m, 1H), 1.17 ppm (d, *J*(H,H) = 7.2 Hz, 3H); ^{13}C NMR (100.53 MHz, DMSO, 25°C): δ = 183.9 (C=O), 179.6 (C=O), 60.9 (CH), 56.4 (CH), 49.5–48.5 (CH), 44.0 (CH_2), 41.8 (CH_2), 40.0 (CH_3), 37.5 (CH_2), 33.4 (CH_2), 24.2 ppm (CH_3); FTIR ($CHCl_3$): $\tilde{\nu}$ = 3315 (amide A), 2934, 2859, 1672, 1627 (amide I), 1540 cm^{-1} (amide II$_{II}$); FAB$^+$-MS: *m/z*: 841 [*M*H$^+$]; HRMS: calcd: 841.55514 [*M*H$^+$]; found: 841.55643.

cyclo[(D-Phe-L-^{Me}N-γ-Ach)$_4$] (3b): This compound was prepared in the same way as **2b** from Boc-[(D-Phe-L-^{Me}N-γ-Ach)$_4$]-OFm (201 mg, 0.140 mmol) to give, after HPLC purification, **3b** (112 mg, 70%); ^{1}H NMR (250 MHz, $CDCl_3$, 25°C): δ = 8.57 (d, *J*(H,H) = 8.6 Hz, 1H), 7.17 (m, 5H), 5.30 (dd, *J*(H,H) = 8.0 and 13.6 Hz, 1H), 4.45 (m, 1H), 3.0–2.7 (m, 3H), 2.64 ppm (s, 3H); ^{13}C NMR (75.40 MHz, $CDCl_3$, 25°C): δ = 175.1 (C=O), 171.3 (C=O), 136.5 (C), 129.6 (CH), 128.2 (CH), 126.8 (CH), 51.4 (CH), 49.9 (CH), 43.3 (CH), 40.4 (CH_2), 30.8 (CH_2), 30.1 (CH_2), 29.5 (CH_3), 28.5 (CH_2), 24.7 ppm (CH_2); FTIR ($CHCl_3$): $\tilde{\nu}$ = 3312 (amide A), 2935, 2864, 1660, 1623 (amide I), 1525 cm^{-1} (amide II$_{II}$); FAB$^+$-MS: *m/z*: 1145 [*M*H$^+$]; HRMS: calcd: 1145.68034 [*M*H$^+$]; found: 1145.68176.

cyclo[(L-Phe-D-^{Me}N-γ-Ach-L-Ser(Bn)-D-^{Me}N-γ-Ach)$_2$] (6a): This compound was prepared in the same way as **2b** from Boc-[(L-Phe-D-^{Me}N-γ-Ach-L-Ser(Bn)-D-^{Me}N-γ-Ach)$_2$]-OFm (188 mg, 0.125 mmol) to give, after HPLC purification, **6a** (112 mg, 75%); ^{1}H NMR (750 MHz, $CDCl_3$, 25°C): δ = 8.67 (d, *J*(H,H) = 8.0 Hz, 1H), 8.59 (d, *J*(H,H) = 9.2 Hz, 1H), 7.22–7.16 (m, 10H), 5.39 (dd, *J*(H,H) = 6.9 and 12.0 Hz, 1H), 5.25 (dd, *J*(H,H) = 9.1 and 15.6 Hz, 1H), 4.50 (m, *J*(H,H) = 15.3 Hz, 2H), 4.39 (s, 2H), 3.64 (m, 1H), 3.54 (m, 1H), 3.18 (m, 1H), 3.08 (m, 1H), 2.98 (s, 3H), 2.88 (m, 1H), 2.80 (s, 3H), 2.46 ppm (m, 1H); ^{13}C NMR (62.83 MHz, $CDCl_3$, 25°C): δ = 175.9 (C=O), 174.5 (C=O), 171.5 (C=O), 169.9 (C=O), 137.6 (C), 137.2 (C), 127–126 (CH), 73.2 (CH_2), 71.6 (CH_2), 51.9 (CH), 51.0 (CH), 49.8 (CH), 48.5 (CH), 44.2 (CH), 42.4 (CH), 38.9 (CH_2), 31.8 (CH_2), 31.0 (CH_2), 30.3 (CH_2), 29.9 (CH_3), 29.6 (CH_3), 28.7 (CH_2), 28.4 (CH_2), 24.6 ppm (CH_2); FTIR ($CHCl_3$): $\tilde{\nu}$ = 3311 (amide A), 2934, 2861, 1660, 1625 (amide I), 1523 cm^{-1} (amide II$_{II}$); FAB$^+$-MS: *m/z*: 1206 [*M*H$^+$]; HRMS: calcd: 1205.70147 [*M*H$^+$]; found: 1205.70536.

cyclo[(L-Phe-D-^{Me}N-γ-Ach-L-Ser-D-^{Me}N-γ-Ach)$_2$] (6b): A solution of peptide **6a** (49 mg, 0.041 mmol) in ethanol (1.5 mL) was treated with 10% Pd/C (10–30 mg) and stirred at room temperature under a hydrogen atmosphere overnight. The resulting mixture was filtered through a celite pad and washed with ethanol to afford, after concentration under reduced pressure, **6b** (41 mg, 100%); ^{1}H NMR (750 MHz, $CDCl_3$, 25°C): δ = 8.96 (br s, 1H), 8.49 (d, *J*(H,H) = 9.8 Hz, 1H), 7.17 (m, 5H), 5.27 (br s, 1H), 5.19 (br s, 1H), 4.52 and 4.47 (m, 2H), 3.79 and 3.70 (m, 2H), 3.28 (m, 1H), 3.05 (m, 4H), 3.94 (m, 1H), 2.80 (s, 3H), 2.42 ppm (m, 1H); ^{13}C NMR (75.40 MHz, $CDCl_3$, 25°C): δ = 177.9 (C=O), 174.2 (C=O), 171.8 and 171.6 (C=O), 168.6 and 168.6 (C=O), 136.8 and 136.8 (C), 129.8–126.7 (CH), 67.0 (CH_2), 53.3 (CH), 52.2 (CH), 51.2 (CH), 50.0 (CH), 44.4 (CH), 42.5 (CH), 39.3 (CH_2), 32.2 and 32.1 (CH_2), 30.8 (CH_2), 29.9 and 29.8 (CH_3), 29.1 and 28.7 (CH_2), 24.7 ppm (CH_2); FTIR ($CHCl_3$): $\tilde{\nu}$ = 3317 (amide A), 2935, 2863, 1660, 1620 (amide I), 1524 cm^{-1} (amide II$_{II}$); FAB$^+$-MS: *m/z*: 1026 [*M*H$^+$]; HRMS: calcd: 1025.60757 [*M*H$^+$]; found: 1025.60908.

^{1}H NMR Assignments of cyclic peptides: ^{1}H NMR spectra ($CDCl_3$) of peptides were assigned from the corresponding double-quantum-filled 2D COSY (2QF-COSY) and/or NOESY and ROESY spectra acquired at the concentration and temperature indicated (see Supporting Information). Mixing times (≈250 ms or 400 ms) were not optimized. Due to conformation averaging on the NMR timescale, peptides with C_n sequence symmetry (n = 2 or 4) generally display C_n symmetrical ^{1}H NMR

spectra for monomeric species and D_n symmetrical spectra for dimeric species.

Measurement of solution association constants by variable-concentration ^{1}H NMR spectroscopy: The HPLC-purified peptide was dissolved in $CDCl_3$ and the association constant, K_a, was estimated with a nonlinear regression fitting program by using the concentration-dependent shifts of the NH amide group between 28.6×10^{-3}–1.7×10^{-3}. The set of observed shifts (δ_{obs}) for each titration experiment was adjusted to the equation $[P]_t = I_{obs}/K_a[2I_{sat}(1-I_{obs}/I_{sat})^2]$,[18] where P corresponds to the set of concentrations and K_a is the calculated value.

Van't Hoff analysis of dimerization: The HPLC-purified cyclic peptide **2b** was dissolved in $CDCl_3$ at concentrations of 28.6, 14.3, 7.1, 7.7, 3.6, and 1.78 mM. ^{1}H NMR (Van't Hoff) spectra of the resulting samples were acquired at intervals of 10 K in the temperature range 233–313 K. Single-point determinations of the K_a value were estimated at each temperature by using nonlinear regression to fit the equation shown above[15] to δ(NH)C data obtained from ^{1}H NMR titrations at total monomer concentrations C. A plot was then made of $1/T$ (in K) versus $\ln K_a$, from which the following thermodynamic parameters were established for the dimerization of **2b**: $\Delta H^\circ_{298} = -38.2$ kJ mol^{-1} and $\Delta S^\circ_{298} = -94.2$ J K^{-1} mol^{-1}.

Preparation of peptide single crystals for X-ray analysis: In a typical experiment, HPLC-purified peptide **2b** (3 mg) was dissolved in a mixture of $CHCl_3$ and CCl_4 (1:1; 1 mL) and equilibrated by vapor-phase diffusion against hexane (5 mL), a process that resulted in spontaneous crystallization after 1 day.

X-ray crystallographic analysis: Data were collected at low temperatures (**2b** at 120 K and **3b** at 100 K) on a Bruker diffractometer equipped with a rotating FR591 KappaCCD 2000 anode ($Cu_{K\alpha}$) and Osmic multilager confocal optics. All calculations were performed on an IBM-compatible PC by using the programs COLLECT,[33] HKL Denzo and Scalepack,[34] SORTAV,[35] SHELX-97,[36] WinGx,[37] SIR2002,[38] ORTEP3,[39] PLATON (SQUEEZE),[27] and PARST.[40]

CCDC-265524 (**2b**) and CCDC-265525 (**3b**) 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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[24] Crystallization of **2b** from chloroform by vapor-phase equilibration with hexane afforded crystals in which **2b** had a bowl-shaped structure and was not dimerized, a result in keeping with **2b** being more flexible than the corresponding cyclohexapeptide.
[25] Van der Waals volumes were estimated for the spherical volume for which the radius was the distance measured from the dimer centroid to the closer hydrogen atom attached to C2.
[26] Recent studies of the cyclohexapeptide **3a** ($n=0$, Scheme 1) have shown that its core is, in fact, amphipathic.[12b]
[27] As the disorder caused high final *R* factors in the structure refinement (0.18 for **2b** and 0.12 for **3b**), the contribution of the solvent molecules to the diffractometric data was eliminated by using the SQUEEZE function of the PLATON program (see: PLATON, A. L. Spek, University of Utrecht, The Netherlands, **2001**; P. van der Sluis, A. L. Spek, *Acta Crystallogr. Sect. A* **1990**, *46*, 194–201). This reduced the final *R* values to 0.11 for **2b** and 0.07 for **3b**.
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