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New α,γ-cyclic peptides-nanotube molecular caps using α,α-dialkylated α-amino acids‡

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Abstract: We report here the preparation and structural characteristics of a novel self-assembling peptide composed of alternating α,α-dialkylated α-amino and *cis*-4-aminocyclopent-2-enecarboxylic acids. The use of α,α-dialkylated amino acids represents a novel method to prevent the formation of extensive β-sheet-like hydrogen-bonding networks that are characteristic of peptide nanotubes. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: self-assembly; peptide nanotubes; molecular caps; γ-amino acids

INTRODUCTION

In the pursuit of biomaterials with improved biocompatibility, responsiveness, specificity and other properties that are desirable in medical devices and drug delivery systems, increasing attention is being paid to nanostructures [1–7]. One of the most promising of this structures is the nanotube [8–11], which also has a wide variety of potential chemical and technological applications [12–23]. For this reason, considerable effort devoted to the synthesis of organic and inorganic nanotubes has been seen in recent years. One of the most promising nanotube designs is the self-assembling peptide nanotube (SPN), a stack of cyclic peptides (CPs) (Figure 1) [24–27]. The elementary components of SPNs are CPs in which the chiralities of the amino acids allow the ring to adopt a quasi-planar conformation [28–36]. In this conformation, peptide backbone N–H and C=O groups project in a perpendicular manner from the plane of the ring on either side and are therefore able to form hydrogen bonds with those of neighbouring rings, thus leading to the construction of a nanotube. This design not only offers considerable scope for facile elaboration to incorporate structural and functional properties of practical interest but also facilitates the pre-determination of the internal diameter of the nanotube, a critical parameter that is not easy to fix with other designs.

The possibility of extending this class of nanotubes to include tubes with hydrophobic inner surfaces, an approach that has not yet been widely studied, has recently been demonstrated through work on dimers that self-assemble from CPs composed of alternating *D*-α-amino and (1*R*,3*S*)-3-aminocycloalkanecarboxylic acids, such as (*cis*)-3-aminocyclohexanecarboxylic acid (γ-Ach) or (*cis*)-3-aminocyclopentanecarboxylic acid (γ-Acp) (**1**–**2**, Figure 1). The hydrophobic core environment is provided by the projection of one of the cycloalkane methylenes into the lumen [37–41]. The cycloalkane rings of these α,γ-CPs not only direct a hydrophobic, functionalizable methylene towards the interior of the CP ring (thus allowing manipulation of the behaviour of the cavity of α,γ-CP based SPNs) but also ensure the flatness and rigidity of the cycloalkane segments of the CP backbone. The success of this strategy is demonstrated by the large association constants of some of the α,γ-CPs that have been studied previously (which for convenience have substituents within the amide skeleton that limit their stacking to the dimer stage) [42–48]. *N*-methylated peptides have also been used as nanotube caps in synthetic ion channels, an application that leads to altered conductance and rectification properties [49]. The cap subunits can participate in backbone–backbone hydrogen bonding from only one face of the peptide ring structure, thus preventing nanotube growth. The properties of the mouth of the nanotube depend on the characteristics of the CP present at the ends of the assembly. We envisaged that the introduction of methyl substituents at axial α-positions of every second residue would provide an alternative means of sterically blocking one face of the peptide ring. Although previous studies have shown the preference of α,α-disubstituted residues for helical rather than β-sheet conformations [50], and also that α,α-disubstituted D,L-α-CPs failed to self-assemble into

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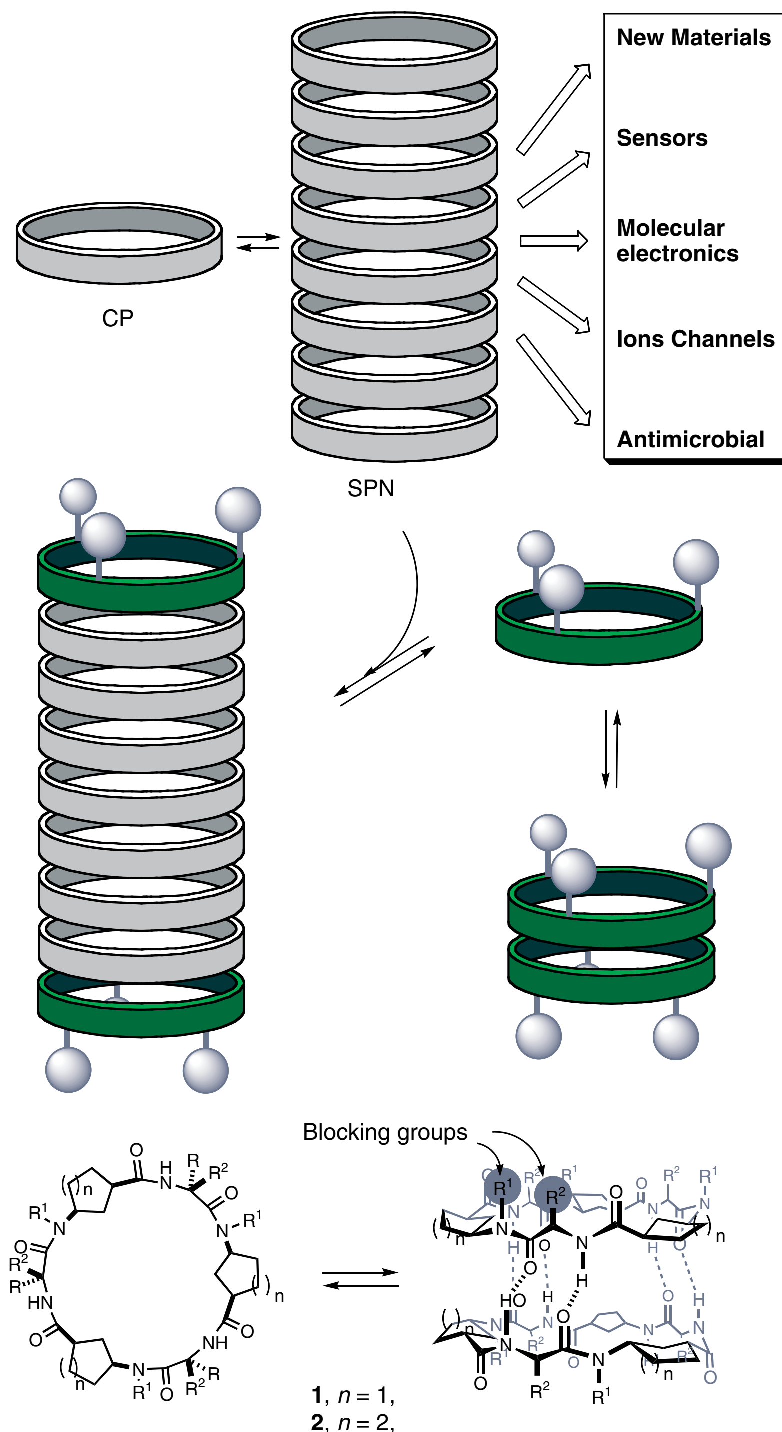

Figure 1 Strategy for nanotube formation through a self-assembly process of cyclic peptides. Nanotube caps are CPs that contain axially oriented substituents, such as *N*- or α-Alkyl groups, which block nanotube formation. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

dimers [45,46], we envisaged that the rigidity of the cycloalkane moiety of γ-Aa would ensure the flatness of the CP backbone. Thus, the effect of α,α-dialkylated α-amino acids was also examined. In addition, a new γ-Aca, the 4-aminocyclopent-2-enecarboxylic acid (γ-Ace), was also prepared and studied. The use of γ-Ace was chosen not only due to its simple preparation from Vince's lactam [51] but also because the angle (θ) defined by the C–N and C–C(O) bonds radiating from the cycloalkane ring is wider than the angles in γ-Acp or γ-Ach (Figure 2). This property makes γ-Ace more suitable for the construction of large α,γ-CPs [41].

The required *cis*-γ-Ace acid was prepared from Vince's lactam (Scheme 1) by acidic hydrolysis and

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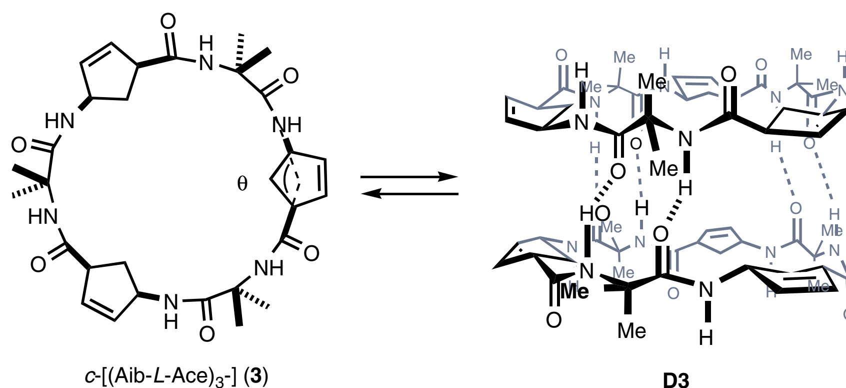
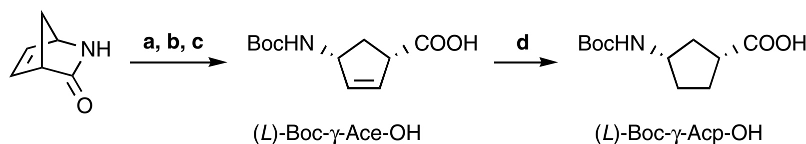


Figure 2 Structure of *cyclo*-[(Aib-L-Ace)$_3$-] (**3**) and its corresponding dimer (**D3**). This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.



Scheme 1 Synthesis of (1S,4R)-Boc-γ-Ace-OH and (1R,3S)-Boc-γ-Acp-OH from Vince's lactam; (a) HCl (10%), 100%; (b) $(Boc)_2O$, N,N-diisopropylethylamine, H_2O/dioxane 99%; (c) resolution: (+)-phenylethylamine, $CHCl_3$/hexane: $[\alpha]_D = -46.1$ ($c = 1.0$, MeOH) >97% ee; (d) H_2, Pd/C, EtOH, 99%.

protection to provide N-Boc-γ-Ace-OH). The resolution of Boc-γ-Ace-OH was achieved by crystallization from a chloroform solution containing 0.7 equiv of (+)-phenylethylamine to give, after a couple of crystallizations, (1R,4S)-Boc-γ-Ace-OH (**L-Boc-γ-Ace-OH**) with an enantiomeric purity higher than 97% [52]. Hydrogenation of L-Boc-γ-Ace-OH provided the Acp derivative in quantitative yield, representing a new method for this amino acid, with the main advantage that the synthesis of can be carried out using multi-gram quantities and in a more reproducible manner [39].

The CP was prepared by solution-phase methods as shown in Scheme 2, starting from the methyl ester of 2-aminoisobutyric acid (Aib-OMe). Coupling of Aib-OMe with L-Boc-γ-Ace-OH using HATU at 0°C proceeded in high yield to give dipeptide **4** (97%) [53,54]. Under standard coupling conditions, i.e. at room temperature and acid pre-activation [55], large amounts of epimer and/or double bond isomer products, which formed through a base-induced mechanism, were observed (Scheme 2, structures **A, B** and **C**). Treatment of **4** with lithium hydroxide in methanol provided the corresponding dipeptide **5** in good yield, but again a mixture of isomers was formed. The use of CsOH at 0°C and with short reaction times reduced the amounts of isomers formed. Under these conditions dipeptide **5** was obtained in 78% yield (90% taken into account recovered starting material). The resulting acid was coupled to dipeptide **6**, obtained by TFA treatment of **4**, using HATU in the presence of DIEA to give tetrapeptide **7** in 87% yield. TFA treatment of **7** followed by coupling with dipeptide **5** provided hexapeptide **9** in 79% yield. Double deprotection of **9** with CsOH followed by TFA, and cyclization of the resulting peptide, afforded CP **3** in 35% yield from **9**. Unfortunately, methyl ester deprotection again gave the corresponding acid together with a mixture of other isomers at the cyclopentenyl moieties. These isomers were easily removed by reverse-phase HPLC purification. The use of other protecting groups, such as the fluorenylmethyl ester, did not facilitate the peptide synthesis. Although we were able to reduce the level of peptide isomerization, the solubility problems associated with the resulting peptides (dipeptides and tetrapeptides) dramatically reduced the reaction yields.

The ^{1}H NMR spectra of the cyclopeptide **3**, in both polar and non-polar solvents, are well defined and are highly symmetrical, with a $J_{NH,H\gamma}$ coupling constant of 7.16 Hz (Figure 3). These observations indicate that the peptide exists in an all-*trans* conformation with a flat-ring-shaped backbone. In non-polar solvents the formation of dimer **D3** is reflected by the downfield shift on increasing the concentration of the N–H resonance of Aib from δ 6.1 to 6.6 ppm, while the N–H resonance of Ace remains unchanged. The corresponding association constant, determined at 293 K by dilution experiments, is 51 M^{-1} in $CDCl_3$ (see Supporting Information) [56]. Van't Hoff plots for the range 243–303 K afford values of −22.6 KJ mol^{-1} for ΔH°_{298} and −44.6 J K^{-1} mol^{-1} for ΔS°_{298} and these, like the fall in K_a with increasing solvent polarity, are consistent with dimerization being essentially an enthalpy-driven hydrogen-bonding process [57,58].

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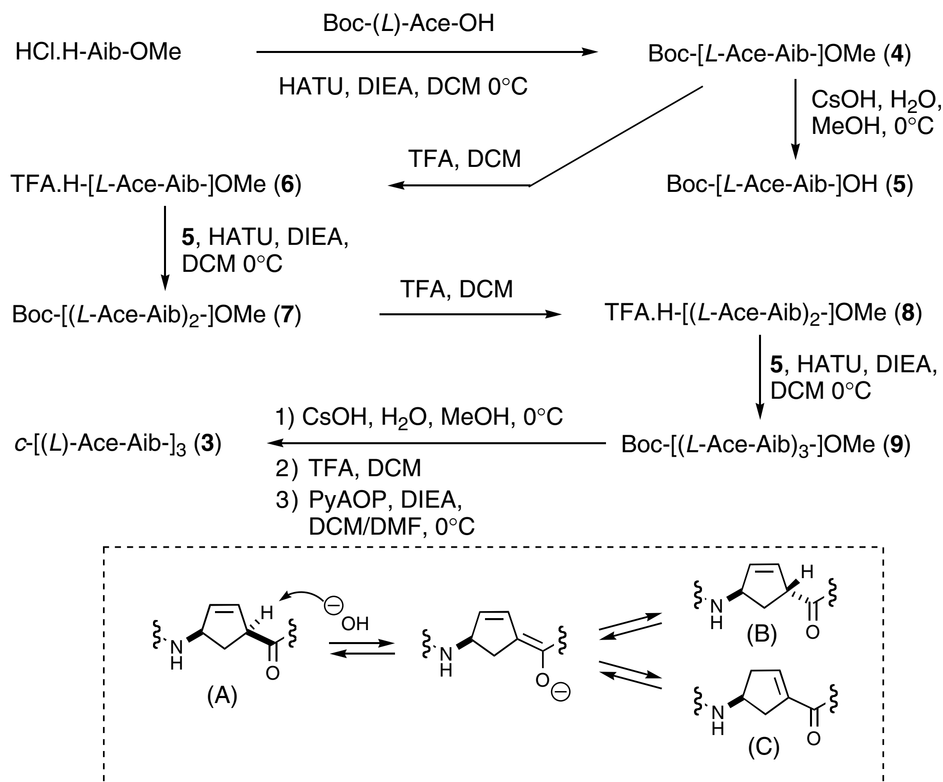

Scheme 2 Strategy for the synthesis of CP **3**, and proposed mechanism for Ace isomerization under basic conditions.

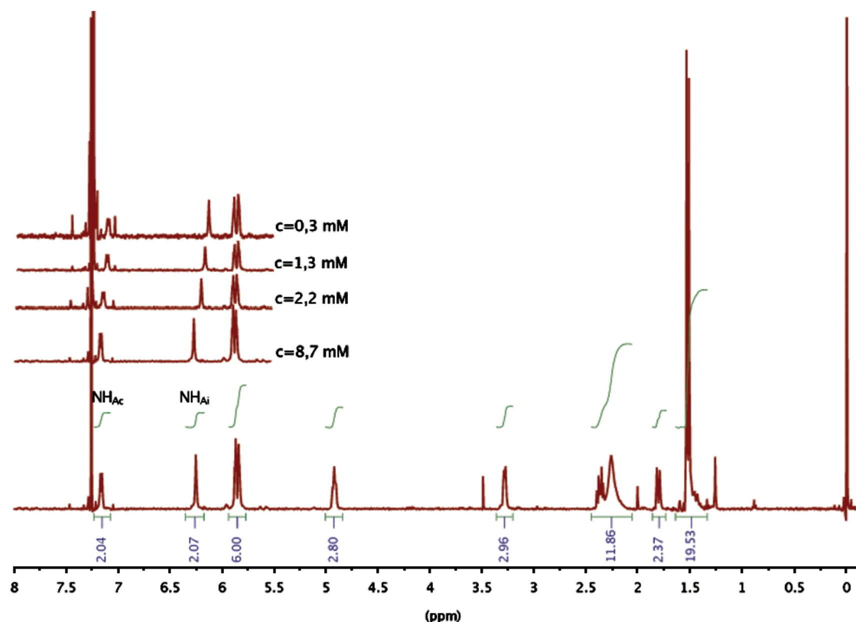

Figure 3 ^{1}H NMR spectrum of a 8.7 mM solution of peptide **3** in $CDCl_3$ with the 5.5–8.0 ppm region of the spectra of 0.3, 1.3, 2.2, and 8.7 mM solutions, showing the downfield shift of Aib N–H. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

These studies described above show that axially oriented amino acid side-chains can block one face of the peptide ring and the resulting hexapeptide **3** adopts the flat conformation and self-assembles to form dimeric structures. We decided to extend this strategy to peptides containing other γ-Aca. For this purpose γ-Acp containing CP **10** was easily prepared by hydrogenation of **3** (Scheme 3). Unfortunately, the resulting peptide did not present any of the characteristic features of dimer-forming CPs. The NMR

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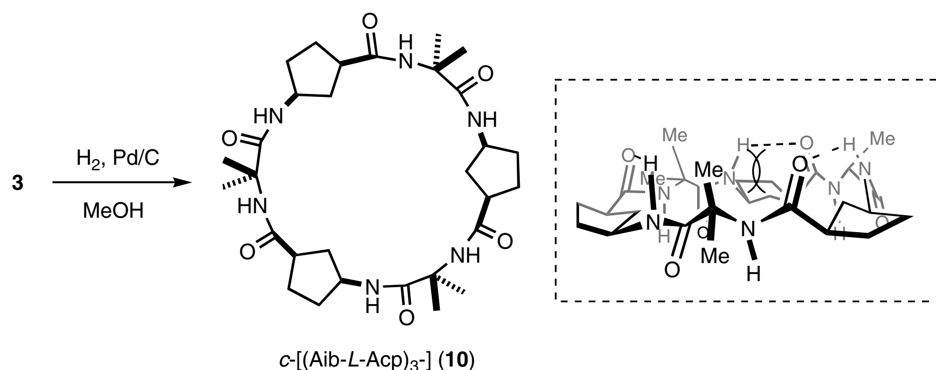

Scheme 3 Preparation of CP **10** by hydrogenation of **3** and proposed folded structure for **10**.

spectrum of **10** showed the N–H of the γ-Acp (7.62 ppm for CP **10** and 7.18 for CP **3**). The signal for the N–H proton that is projected towards the solvent-exposed ring face, which should be hydrogen-bonding protected by the axial methyl group of Aib, is shifted down field, while the N–H resonance of Aib, which should be participating in the β-sheet-like hydrogen bonding networks in the dimeric structure, is now up-field shifted (5.88 ppm). Furthermore, concentration-dependent shifts were not observed for any of the proton resonances. These features suggest that the peptide is not forming the dimeric structure because it is folded as a consequence of the steric interactions between the axially oriented α-methyl and carbonyl groups of the γ-Acp. Another reason for the absence of dimer formation might be the higher degree of flexibility of the cyclopentyl moiety of Acp compared to the cyclopentenyl ring, which might prevent the CP from adopting the flat conformation required for dimerization in the β-strand form.

Experimental Section

General methods. Commercially available amino acid, HATU and (7-azabenzotriazol-1-yloxy)tris(pyrrolidino) phosphonium hexafluorophosphate (PyAOP) were used as obtained from Novabiochem or GL Shanghai Biochem. All other reagents obtained from commercial suppliers were used without further purification unless otherwise stated. DCM and piperidine were dried and distilled over calcium hydride [59,60]. DIEA was dried and distilled over calcium hydride, and then re-distilled over ninhydrin [59,60]. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates. Compounds that were not UV-active were visualized by dipping the plates in a ninhydrin solution, and heating. Silica gel flash chromatography was performed using E. Merck silica gel (type 60SDS, 230–400 mesh). Solvent mixtures for chromatography are reported as v/v ratios. HPLC purification was carried out on Inertsil ODS-2 C18 columns with H_2O(0.1% TFA):ACN(0.1% TFA). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on 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 (δ 0.00). ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q) or quintuplet (p). 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). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Varian Mercury-300 MHz, Bruker WM-250 MHz or Bruker AMX-500 MHz spectrometers. Carbon resonances were assigned using distortionless enhancement by polarization transfer (DEPT) spectra obtained with phase angles of 135°. Chemical ionization (CI) mass spectra were carried out on a Finnigan TraceMS mass spectrometer. FAB mass spectra were recorded on a Micromass Autospec mass spectrometer. ESI-TOF mass spectra were recorded on a Bruker Microtof ESI-TOF. FTIR measurements were made on a JASCO FT/IR-400 spectrophotometer using 5–10 mM solutions in $CHCl_3$ placed in a NaCl IR cell.

^{1}H NMR assignments of cyclic peptides. The signals of the ^{1}H NMR spectra of the peptides in $CDCl_3$ were identified from the corresponding spectra acquired at the indicated concentrations and temperatures. Mixing times (~250 or 400 ms) were not optimized. Spectra were typically acquired using Bruker standard pulse sequences on 500 MHz spectrometers, and were referenced relative to residual proton resonances in $CDCl_3$ (at 7.26 ppm). ^{1}H NMR spectra were also obtained on a Bruker MX-500 spectrometer. Owing to conformational averaging on the NMR time scale, monomeric peptides with C_3 sequence symmetry generally had C_3-degenerate ^{1}H NMR spectra, and their dimers D_3-degenerate spectra.

cis-4-aminocyclopent-2-enecarboxylic acid hydrochloride (ClH.H-Ace-OMe). A solution of 2-azabicyclo[2.2.1]hept-5-en-3-one (25.0 g, 229.3 mmol)

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in 1.3 l of HCl (10%) was stirred for 24 h at room temperature and then concentrated *in vacuo*. Addition of acetone to the resulting yellow oil gave a white solid, *cis*-γ-Ace hydrochloride, that was filtered off and washed with acetone [29.08 g, 100%, $R_f = 0.46$ (CH_2Cl_2:MeOH (1 : 1))]. **^{1}H NMR** (D_2O, 250.13 MHz, δ) : 6.2-5.9 (dd, 2H), 4.37 (s, 1H), 3.71 (p, 1H), 2.71-2.58 (dt, 2H), 2.09-1.99 (dt, 2H) [51].

(1S,4R)-4-Amino-N-t-butyloxycarbonylcyclopent -2-enecarboxylic acid (L-Boc-γ -Ace-OH). To a solution of *cis*-γ-Ace hydrochloride (37.0 g, 163.45 mmol) in water (750 ml) and dioxane (750 ml) were added Boc_2O (74.02 g, 339.55 mmol) and DIEA (118.6 ml, 679.1 mmol). After stirring at room temperature (rt) for 3 h, the solution was acidified to pH 3 by the addition of HCl (10%) and extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), filtered and concentrated, providing a yellow oil that was crystallized from 1 : 1 $CHCl_3$/hexanes, giving 41.9 g and 11.3 g of Boc-γ-Ace-OH in successive crystallizations. This racemic product was resolved by crystallization from 1 : 1 $CHCl_3$/hexane in the presence of (+)-α-phenylethylanamine (0.7–1 equiv.), and the resulting white crystals were washed with hexane, poured into a separation funnel and dissolved in CH_2Cl_2 and washed with HCl (5%) (this operation was repeated 2–3 times). The combined organic layers were dried (Na_2SO_4), filtered, concentrated and the resulting oil crystallized from $CHCl_3$/hexane (1 : 1) [99%, $R_f = 0.71$ (CH_2Cl_2: MeOH (1 : 1)), white crystals]. **^{1}H NMR** ($CDCl_3$, 250.13 MHz, δ) : 10.8 (s, 1H), 6.30 and 4.98 (s, 1H), 5.90 (s, 2H), 4.80-4.51 (m, 1H), 3.51 (s, 1H), 2.64-1.77 (m, 2H), 1.46 (s, 9H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz, δ) : 179.1 (CO_2H), 155.5 (CO), 135.1 (CH), 131.2 (CH), 79.8 (C), 56.1 (CH), 49.4 (CH), 34.4 (CH2), 28.6 (CH_3). **MS (CI)** [***m/z*** (%)]: 228 ($(M + H)^+$, 60), 171 (100), 127 (52), 110 (66). **[MH]$^+$ calculated** for $C_{11}H_{17}NO_4$ 228.1158, **found**: 228.1238. $[\alpha]_D = -46.1$ ($C = 1.0$, MeOH).

(1R,3S)-3-Amino-N-t-butyloxycarbonyl cyclopentane-carboxylic acid (L-Boc-γ -Acp-OH). A solution of L-Boc-γ-Ace-OH (2.58 mg, 11.36 mmol) in EtOH (40 ml) was treated with 10% Pd/C (120.9 mg, 1.36 mmol) and hydrogenated at balloon pressure for 12 h. The insolubles were separated by filtration through Celite, rinsed with EtOH and concentrated to provide L-Boc-γ-Acp-OH as a white solid [2.60 g, 99%, $R_f = 0.60$ (CH_2Cl_2:MeOH (9 : 1))] [39].

HCl.H-Aib-OMe. 2-Aminoisobutyric acid (1g, 9.70 mmol) was suspended in methanol (10 ml) and cooled with an ice bath. $SOCl_2$ (0.95 ml, 13.09 mmol) was added dropwise to the stirred solution. After the complete addition of thionyl chloride, the mixture was heated for 3 h at 50 °C. The solvent was evaporated *in vacuo* three times. Addition of $CHCl_3$ to the resulting yellow oil gave a white solid that was filtered off and washed with acetone. [1.40 g, 94%, $R_f = 0.17$ (CH_2Cl_2:MeOH (9 : 1))]. **^{1}H NMR** (D_2O, 250.13 MHz, δ) : 3.79 (s, 3H), 1.55 (s, 6H). **^{13}C NMR** (D_2O, 62.90 MHz, δ) : 173.4 (CO_2H), 57.2 (C), 54.2 (CH_3), 23.3 (CH_3). **MS (CI)** [***m/z*** (%)]: 117 ($(M + H)^+$, 63), 88 (15), 57.5 (100). **[MH]$^+$ calculated** for $C_5H_{11}NO_2$ 118.08608, **found**: 118.0866.

Boc-(L-γ -Ace-Aib-)-OMe (4). A suspension of HCl.H-Aib-OMe (812 mg, 5.29 mmol) and L-Boc-γ-Ace-OH (600 mg, 2.64 mmol) in dry CH_2Cl_2 (30 ml) was cooled with an ice bath. The stirred solution was successively treated with HATU (1.10 g, 2.90 mmol) and DIEA (1.84 ml, 10.69 mmol). After 60 min stirring at 0 °C the solution was poured into a separation funnel and washed with HCl (5%) and $NaHCO_3$ (*dil. sat*). The organic layers were dried over Na_2SO_4, and concentrated under reduced pressure to give 858 mg of the target dipeptide (99%, white solid). **^{1}H NMR** (250.13 MHz, $CDCl_3$, δ) : 6.59 (s, 1H), 5.89-5.76 (m, 2H), 5.24 (s, 1H), 4.64 (t, 1H), 3.70 (s, 3H), 3.22 (d, 1H), 1.82 (t, 1H), 1.76 (t, 1H), 1.50 (d, 6H), 1.39 (s, 9H). **^{13}C NMR** (62.90 MHz, $CDCl_3$, δ) : 175.1 (C=O), 173.6 (C=O), 155.4 (C=O), 135.0 (CH), 131.7 (CH), 79.2 (C), 56.4 (C), 56.0 (CH), 52.7 (OCH_3), 51.4 (CH), 34.9 (CH_2), 28.5 (CH_3), 24.9 (CH_3). **MS-FAB$^+$** [***m/z*** (%)]: 327 (100) [MH^+], 227 (41), 154 (65). **HRMS [MH]$^+$ calculated** for $C_{16}H_{26}N_2O_5$: 327.1920, **found**: 327.1918.

Boc-(L-γ -Ace-Aib-)OH (5). A solution of Boc-[(*L*-γ-Ace-Aib)-]-OMe (**4**) (400 mg, 1.23 mmol) in MeOH (12 ml) was treated with CsOH (921 mg, 6.15 mmol) in water (4 ml) and stirred at rt for 3 h. After removal of the solvent, the residue was dissolved in water (25 ml) and this solution was acidified to pH 3 by the addition of 10% HCl, and finally extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give 290 mg of the target dipeptide (75%, white solid). **^{1}H NMR** (250.13 MHz, CD_3OD, δ) : 8.24 (s, 1H), 5.78 (s, 2H), 4.61 (t, 1H), 3.39 (dt, 1H), 2.48 (dt, 1H), 1.65 (dt, 1H), 1.44 (d, 15H). **MS (ESI.TOF)** [***m/z*** (%)]: 351 [$M + K^+$], 335 [$M + Na^+$], 313 [MH^+], 279. **HRMS [MH]$^+$ calculated** for $C_{15}H_{25}N_2O_5$: 327.17188, **found**: 313.1758.

Boc-((L-γ -Ace-Aib)$_2$-)OMe (7). A solution of Boc-[(*L*-γ-Ace-Aib)-]OMe (**4**) (420.0 mg, 1.29 mmol) in TFA/DCM (1 : 1, 13 ml) was stirred at rt for 15 min. After removal of the solvent, the residue was dried under high vacuum for 2 h and then dissolved in dry CH_2Cl_2:DMF (2 : 1, 15 ml) and **5** (403 mg, 1.29 mmol), HATU (539.5 mg, 1.42 mmol), DIEA (1.35 ml, 7.74 mmol) were successively added. After 60 min stirring at 0 °C the solution was poured into a separation funnel and washed with HCl (5%) and $NaHCO_3$ (*dil. sat*). The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give 586 mg of the target tetrapeptide (87%, white solid). **^{1}H NMR** (250.13 MHz, $CDCl_3$, δ) : 7.29 (d, 1H),

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6.80 (s, 1H), 6.40 (s, 1H), 5.96-5.85 (m, 4H), 5.39 (d, 1H), 4.99 (t, 1H), 4.70 (t, 1H), 3.73 (s, 3H), 3.28 (m, 2H), 2.44-2.24 (4 x t, 2H), 1.84-1.76 (m, 4H), 1.56-1.54 (d, 12H), 1.43 (s, 9H). **^{13}C NMR** ($CDCl_3$, 62.90 MHz): 175.0 (CO), 173.8 (CO), 173.7 (CO), 173.4 (CO), 155.4 (CO), 135.1 (CH), 134.8 (CH), 132.3 (CH), 132.1 (CH), 79.2 (C), 77.4 (C), 56.9 (C), 56.6 (CH), 56.0 (C), 54.4 (CH), 52.7 (CH), 51.9 (CH), 51.3 (CH), 35.1 (CH_2), 34.7 (CH_2), 28.6 (CH_3), 25.9 (CH_3). **MS-FAB$^+$** [***m/z*** (%)]: 521 (86) [MH^+], 394 (27), 325 (44), 231 (68). **HRMS [MH]$^+$ calculated** for $C_{26}H_{40}N_4O_7$: 521.297525, **found**: 521.298274.

Boc-((L-γ-Ace-Aib)$_3$-)OMe (9). Prepared in the same way as tetrapeptide Boc-[(L-γ-Ace-Aib)$_2$-]OMe using Boc-[(*LL*-γ-Ace-Aib)$_2$-]OMe (192 mg, 0.370 mmol) and Boc-[(L-γ-Ace-Aib)-]OH (121 mg, 0.388 mmol) to afford 208 mg of the desired hexapeptide (79%). **^{1}H NMR** (300 MHz, $CDCl_3$, 25 °C, δ) : 7.38 (d, 1H), 7.31 (d, 1H), 6.90 (s, 1H), 6.77 (s, 1H), 6.43 (s, 1H), 5.90-5.70 (m, 6H), 5.37 (s, 1H), 4.90 (t, 2H), 4.64 (t, 1H), 3.65 (s, 3H), 3.24 (t, 3H), 2.30-2.10 (m, 3H), 2.10-1.56 (m, 8H), 1.47 (d, 18H), 1.36 (s, 9H), 1.15 (H_2O). **MS (ESI-TOF)** [m/z (%)]: 738 ($M + Na^+$), 715 (M^+), 615, 327. **HRMS [MH]$^+$ calculated** for $C_{36}H_{55}N_6O_9$: 715.3988, **found**: 715.4025.

cyclo-((L-γ-Ace-Aib)$_3$-) (3). A solution of Boc-[(L-γ-Ace-Aib)$_3$-]-OMe (**9**) (65 mg, 0.091 mmol) in MeOH (4 ml) was treated with CsOH (136 mg, 0.91 mmol) in water (1.5 ml) and stirred at rt for 3 h. After removal of the solvent, the residue was dissolved in water (10 ml) and this solution was acidified to pH 3 by the addition of 10% HCl, and finally extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was dissolved in 4 ml of TFA/DCM (1 : 1) and stirred at rt for 15 min. After removal of the solvent, the residue was dried under high vacuum for 3 h and used without further purification. The linear peptide was dissolved in DCM : DMF (2 : 1, 90 ml) and treated with PyAOP (78 mg, 0.14 mmol), followed (dropwise) by DIEA (95 μL, 0.55 mmol). After 12 h, the solvent was removed under reduced pressure and the crude product was purified by HPLC, affording 9 mg of **3** as a white solid {17%, $R_t = 18$ min [Inertsil ODS 2 semi-preparative column, 5% ACN (0.1% TFA) 5 min, 5%-50% ACN (0.1% TFA) in H_2O (0.1% TFA), 25 min]}. **^{1}H NMR** ($CDCl_3$, 500.13 MHz, δ) : 7.16 (d, 2H), 6.25 (s, 2H), 5.86 (d, 6H), 4.92 (t, 3H), 3.27 (d, 3H), 2.38-2.36 (dt, 3H), 1.79 (d, 3H), 1.52 (d, 18H). **MS (ESI-TOF)** [m/z (%)]: 605 ($M + Na^+$), 583 (M^+), 507. **HRMS (ESI-TOF) calculated** for $C_{30}H_{43}N_6O_6$: 583.3199, **found**: 583.3239.

cyclo-((L-γ-Acp-Aib)$_3$-) (10). A solution of *cyclo*-[(L-γ-Ace-Aib)$_3$-] (4 mg, 7 μmol) in MeOH (2 ml) was treated with 10% Pd/C (1.5 mg, 3.5 μmol) and the mixture was stirred at rt under hydrogen overnight. The resulting mixture was filtered through a Celite pad, the residue was washed with methanol, and the combined filtrates and washings were concentrated under reduced pressure, affording 4 mg of *c*-[(L-γ-Acp-Aib)$_3$-] as a white solid (99%). **^{1}H NMR** ($CDCl_3$, 500.13 MHz, δ) : 7.59 (d, 3H), 5.88 (s, 3H), 4.33 (m, 3H), 2.67 (m, 3H), 2.12-1.67 (m, 18H), 1.62 (s, H_2O), 1.52 (d, 18H).

Measurement of association constants by variable-concentration ^{1}H NMR. The HPLC-purified peptide **3** was dissolved in $CDCl_3$ and their association constants K_a estimated as described in Ref. 56.

Van't Hoff analysis of dimerization. The HPLC-purified CP **3** was dissolved in $CDCl_3$ at concentrations ranging from 0.1 to 9.0 mM. ^{1}H NMR spectra were recorded at intervals of 10 K in the temperature range 243–303 K. K_a was estimated at each temperature as described in Ref. 56. Analysis of a plot of $1/T$ (K) *vs* ln K_a then afforded the values $\Delta H^\circ_{298} = -22.6$ kJ mol^{-1} and $\Delta S^\circ_{298} = -44.6$ J K^{-1} mol^{-1}.

In conclusion, we have shown definitively that the CPs that consist of α,α-dialkylated α-amino acids alternated with *cis*-3-aminocyclopent-2-enecarboxylic acid (γ-Ace) form dimers in which anti-parallel peptide rings are linked by a β-sheet-like set of six hydrogen bonds. Axially oriented methyl groups have been shown to block peptide aggregation and limit self-assembly to dimer formation. These CPs represent a novel class of nanotube caps that may find utility in the design of ligand-gated channels and stochastic sensing.

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Supplementary Material

Supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/1075-2617/suppmat/

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