ORIGINAL ARTICLE

Synthesis and structural study of highly constrained hybrid cyclobutane-proline γ , γ -peptides

Raquel Gutiérrez-Abad · Daniel Carbajo · Pau Nolis · Carles Acosta-Silva · Juan A. Cobos · Ona Illa · Miriam Royo · Rosa M. Ortuño

Received: 14 January 2011/Accepted: 2 April 2011/Published online: 4 May 2011 © Springer-Verlag 2011

Abstract Two diastereomeric series of hybrid γ , γ -peptides derived from conveniently protected derivatives of (1*R*,2*S*)- and (1*S*,2*R*)-3-amino-2,2-dimethylcyclobutane-1carboxylic acid and *cis*-4-amino-L-proline joined in alternation have efficiently been prepared through convergent synthesis. High-resolution NMR experiments show that these compounds present defined conformations in solution affording very compact structures as the result of intra and inter residue hydrogen-bonded ring formation. (*R*,*S*)cyclobutane containing peptides adopt more twisted conformations than (*S*,*R*) diastereomers. In addition, all these γ -peptides have high tendency to aggregation providing vesicles of nanometric size, which were stable when allowed to stand for several days, as verified by transmission electron microscopy.

Keywords Hybrid γ , γ -peptides · Cyclobutane · *cis*-4-amino-L-proline · Hydrogen bonds · Secondary structures · Self-assembly · Vesicles

Electronic supplementary material The online version of this article (doi:10.1007/s00726-011-0912-4) contains supplementary material, which is available to authorized users.

R. Gutiérrez-Abad \cdot C. Acosta-Silva \cdot J. A. Cobos \cdot O. Illa \cdot R. M. Ortuño (\boxtimes)

Departament de Química, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain e-mail: rosa.ortuno@uab.cat

D. Carbajo · M. Royo Combinatorial Chemistry Unit, Barcelona Science Park, University of Barcelona, Baldiri Reixac, 10, 08028 Barcelona, Spain

P. Nolis

Servei de RMN, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain

Abbreviations

11001010	
Boc	t-Butoxycarbonyl
^t Bu	<i>t</i> -Butyl
^t BuOH	<i>t</i> -Butanol
CD	Circular dichroism
Cbz	Benzyl carbamate
DIPEA	N,N-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
EDAC	1-Ethyl-3-(3-dimethylaminopropyl)
	carbodiimide
GABA	γ-Aminobutyric acid
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum correlation
Hz	Hertz
Me	Methyl
MeOH	Methanol
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect spectroscopy
ppm	Parts per million
PyBOP	Benzotriazol-1-yl-
	oxytripyrrolidinophosphonium
	hexafluorophosphate
ROESY	Rotational nuclear overhauser effect
	spectroscopy
TEM	Transmission electron microscopy
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TOCSY	Total correlation spectroscopy

Introduction

Foldamers with heterogeneous backbones (hybrid peptides) consisting, in most cases, of α,β -, α,γ -, or β,γ -amino acids

(DePol et al. 2004; Guo et al. 2009, 2010; Hecht and Huc 2007; Horne and Gellman 2008) have been prepared with the aim to enhance the ability of peptide oligomers to fold in defined manners. Also, combinations of different β , β -(Cheng et al. 2001; Izquierdo et al. 2004) and, in less extent, γ , γ -amino acids (Brenner and Seebach 2001; Seebach et al. 2002; Aguilera et al. 2008) have been used for these purposes. Moreover, replacement of linear residues by alicyclic ones has resulted, in some instances, in the formation of strong secondary structures and in changing their biological properties (Koglin et al. 2003; Lang et al. 2006).

We have previously synthesized β - and γ -cyclobutane amino acids and incorporated them into different oligomers. For β -peptides constituted by cyclobutane β -amino acids, we have observed secondary structures derived from the formation of six-membered hydrogen-bonded rings due to CO...HN intra-residual interactions in the case of all-ciscyclobutane derivatives, from a dimer up to an octamer (Torres et al. 2010). In contrast, for β -dipeptides containing two trans-cyclobutane amino acids or one cis and another trans, we have found the formation of eight-membered rings as the consequence of inter-residual CO⁽ⁱ⁾...HN⁽ⁱ⁺¹⁾ hydrogen bonds (Torres et al. 2009). In the case of hybrid β -peptides consisting of *cis*-cyclobutane amino acids and β -alanine joint in alternation, a 14-helix resulted as the preferred folding showing thus the ability of the cyclobutane ring to induce secondary structures (Izquierdo et al. 2004). Some of these peptides presented activity as metallocarboxypeptidase inhibitors (Fernández et al. 2009). In addition, some of these oligomers self-assemble hierarchically producing stable gels or fibres in common organic solvents (Rúa et al. 2007; Gorrea et al. 2011).

We have also prepared fully protected cyclobutane enantiomeric γ -amino acids **1** and **2** (Fig. 1), which have been used for the preparation of short γ -peptides consisting of all cyclobutane amino acids, with the same or different configuration, and in hybrid γ -peptides obtained by sequential alternation with GABA residues. Preliminary results show the tendency of these oligomers to adopt extended conformations or to form β -sheets (Aguilera et al. 2008).

4-Aminoprolines when included in oligomers, can behave both as an α - and as a γ -amino acid with an additional amino function. The key lies on which amino group is involved in the peptide bond. They are useful scaffolds for the construction of γ -oligomers that can be modified by introduction of alkyl chains or other functional groups by manipulation of the pyrrolidine nitrogen. The ability to fold into defined secondary structures of homopeptides containing some of these compounds has been investigated (Farrera-Sinfreu et al. 2004). To mention some applications, 4-aminoprolines and derivatives have been used for the synthesis of distamycin analogues with DNA-binding affinity (Woods et al. 2002), for the preparation of cell-penetrating peptides (Farrera-Sinfreu et al. 2005), and for the synthesis of helical dendronized polymers (Zhang and Schlüter 2007; Zhang et al. 2008; Rodríguez-Ropero et al. 2009).

We decided to combine *cis*-4-amino-L-proline derivative **3** (Fig. 1) (Torino et al. 2009) and enantiomeric amino acids **1** and **2** to afford diastereomeric hybrid γ , γ -peptides with the purpose to study the influence of both cyclic residues on the structural features of the resultant γ -peptides. In this article, we report the synthesis of several oligomers, dimers, tetramers and hexamers, for each diastereomeric series and their structural study mainly based in high resolution NMR techniques. The ability of some of these compounds to aggregate has been also investigated by TEM and results are reported herein.

Materials and methods

General

The chemicals and reagents (Sigma-Aldrich, Fluka, Madrid, Spain) were of analytical grade and were used without further purification. Anhydrous dichloromethane was freshly distilled when needed under a nitrogen atmosphere from calcium chloride. Anhydrous methanol was freshly distilled from calcium hydride. TLC on silica gelcoated aluminium plates was performed in the systems indicated for each product in the following description. The compounds were visualized by exposure to UV light at 254 nm, dipping in a basic potassium permanganate solution or in an acid vanillin solution. Flash chromatography purifications were carried out on silica gel (200-400 mesh). Melting points were recorded on a Reicher Klofler block and values are uncorrected. ¹H NMR and ¹³C NMR spectra were measured in a Bruker Avance 600 apparatus (¹H at 600.13 MHz, ¹³C at 150.9 MHz) in CDCl₃ solution at 298 or 273 K. The 2D-H,H-COSY, 2D-H,C-HSQC and

Fig. 1 Monomers used in this work for the synthesis of hybrid γ -peptides



1



isomer is marked C



2D-H,C-HMBC spectra were recorded and used for the structural assignment of proton and carbon signals. IR spectra were obtained from samples in neat form with an Attenuated Total Reflectance (ATR) accessory. High resolution mass spectra were recorded using a direct inlet system (ESI) in electrospray ionization mode.

Triprotected Proline (3)

Previously described product (Torino et al. 2009). Detailed NMR of conformers at 273 K in CDCl₃ (see Fig. 2 for numbering).

Cis-Conformer ¹H NMR (600 MHz; CDCl₃): 1.41 (s, 9H, H⁹), 1.98 (d, J = 14.9 Hz, 1H, H^{7 proR}), 2.48 (m, 1H, $H^{7 \text{ proS}}$), 3.58 (d, J = 11.4 Hz, 1H, $H^{5 \text{ proS}}$), 3.61 (m, 1H, $H^{5 \text{ proR}}$), 3.75 (s, 3H, H^{1}), 4.26 (dd, J = 9.8 Hz, J' = 2.5 Hz, 1H, H³), 4.42 (m, 1H, H⁶), 5.10 (m, 2H, $H^{11 \text{ CH2}}$), 5.86 (d, J = 9.2 Hz, 1H, H^{10}), 7.33–7.37 (m, 5H, H^{11 Ar}). ¹³C NMR (150 MHz; CDCl₃): 28.3 (C⁹), 35.8 or $36.8 (C^7), 49.7 (C^6), 52.6 (C^1), 53.1 (C^5), 57.7 (C^3), 66.8$ (C¹¹ CH2), 80.7 (C^{9q}), 128.2, 128.3 and 128.6 (C¹¹ Ar), 136.3 (C^{11q}), 153.5 (C⁸), 155.7 (C^{11 CO}), 174.7 (C²).

Trans-conformer ¹H NMR (600 MHz; CDCl₃): 1.46 (s, 9H, H⁹), 1.96 (d, J = 14.9 Hz, 1H, H^{7 proR}), 2.48 (m, 1H, $H^{7 \text{ proS}}$), 3.49 (d, J = 11.4 Hz, 1H, $H^{5 \text{ proS}}$), 3.61 (m, 1H, $H^{5 \text{ proR}}$), 3.77 (s, 3H, H^{1}), 4.34 (dd, J = 9.8 Hz, J' = 2.3 Hz, 1H, H³), 4.42 (m, 1H, H⁶), 5.10 (m, 2H, $H^{11 \text{ CH2}}$), 5.93 (d, J = 9.2 Hz, 1H, H^{10}), 7.33–7.37 (m, 5H, H¹¹ Ar). ¹³C NMR (150 MHz; CDCl₃): 28.3 (C⁹), 35.8 or 36.8 (C⁷), 50.7 (C⁶), 52.9 (C¹), 53.7 (C⁵), 57.6 (C³), 66.8 (C^{11 CH2}), 80.7 (C^{9q}), 128.2, 128.3 and 128.6 (C^{11 Ar}), 136.3 (C^{11q}), 154.2 (C⁸), 155.8 (C^{11 CO}), 174.8 (C²).

y-Dipeptide (9)

Acid (4) (292 mg, 0.58 mmol), DIPEA (0.30 mL, 1.57 mmol) and PyBOP (420 mg, 0.78 mmol) were dissolved in anhydrous dichloromethane (15 mL). The mixture was stirred for 5 min under nitrogen atmosphere, and then a solution of amine 8 (92 mg, 0.58 mmol) in anhydrous dichloromethane (10 mL) was added via cannula. After stirring at room temperature for 2 h the reaction crude was washed with a saturated aqueous sodium bicarbonate solution. The organic phase was dried over

magnesium sulfate and the solvents were removed under vacuum. The reaction crude was purified by column chromatography on neutral silica gel (ethyl acetate/hexane 1:1) to afford pure dipeptide (9). Yield 292 g (quantitative). White solid. mp 52–54°C (from Et₂O). $[\alpha]_D^{20}$ –13.8 (*c* 0.99, CH₂Cl₂) (see Fig. 6 for numbering). ¹H NMR (600 MHz; CDCl₃): 0.87 (s, 3H, H⁷), 1.27 (s, 3H, H⁸), 1.40 (s, 9H, H¹⁷), 2.12–2.21 (m, 2H, H^{4 proR}, H^{15 proS}), 2.25 (m, 1H, $H^{4 \text{ proS}}$), 2.40 (d, J = 13.7 Hz, 1H, $H^{15 \text{ proR}}$), 2.62 (dd, J = 10.0 Hz, J' = 8.0 Hz, 1H, H³), 3.47 (d, J = 11.6 Hz, 1H, H^{13 proS}), 3.55 (m, 1H, H^{13 proR}), 3.70 (s, 3H, H¹), 4.08 $(dd, J = 17.8 Hz, J' = 8.6 Hz, 1H, H^5), 4.30 (m, 1H, H^{14}),$ 4.43 (d, J = 8.7 Hz, 1H, H¹¹), 5.10 (m, 2H, H¹⁹ CH2), 6.85 (d, J = 6.2 Hz, 1H, H^{18}), 7.32–7.37 (m, 5H, $H^{19 \text{ Ar}}$), 7.72 (d, J = 8.0 Hz, 1H, H⁹). ¹³C NMR (150 MHz; CDCl₃): 17.0 (C⁷), 26.2 (C⁴), 28.3 (C¹⁷), 29.0 (C⁸), 31.5 (C¹⁵), 43.1 (C^3) , 46.3 (C^6) , 50.1 (C^5) , 50.7 (C^{14}) , 51.4 (C^1) , 55.1 (C^{13}) , 59.21 (C¹¹), 66.4 (C^{19 CH2}), 81.2 (C^{17q}), 128.0 and 128.4 (C^{19 Ar}), 136.7 (C^{19q}), 156.0 (C^{19 CO}), 156.2 (C¹⁶), 172.3 (C^{10}) , 172.8 (C^2) . IR (ATR, γ_{max} cm⁻¹) 3,312, 2,956, 2,876, 1,677, 1,521, 1,393. HRMS (ESI) calculated for $C_{26}H_{37}N_{3}O_{7}$ [M + Na]⁺ 526.2524; found: 526.2506.

γ -Tetrapeptide (12)

Acid (10) (90 mg, 0.19 mmol), DIPEA (0.12 mL, 0.60 mmol) and PyBOP (161 mg, 0.29 mmol) were dissolved in anhydrous dichloromethane (10 mL). The mixture was stirred for 5 min under nitrogen atmosphere, and then a solution of amine (11) (65 mg, 0.8 mmol) in anhydrous dichloromethane (5 mL) was added via cannula. After stirring at room temperature for 2 h, the reaction crude was washed with a saturated aqueous sodium bicarbonate solution. The organic phase was dried over magnesium sulfate and the solvents were removed under vacuum. The reaction crude was purified by column chromatography on neutral silica gel (ethyl acetate) to afford pure tetrapeptide (12). Yield 145 mg (96%). White solid. mp 79–81°C (from H₂O). $[\alpha]_{D}^{20}$ –310.8 (c 0.06, CH₂Cl₂) (see Fig. 7 for numbering). ¹H NMR (600 MHz; CDCl₃): 0.85 and 0.86 (s, 6H, H⁷ and H²⁴), 1.22 (s, 3H, H⁸), 1.26 (s, 3H, H²⁵), 1.45 (s, 18H, H¹⁷ and H³⁴), 2.10- $\begin{array}{l} 2.35 \ (m, \ 8H, \ H^4 \ ^{proS}, \ H^4 \ ^{proR}, \ H^{15 \ proS}, \ H^{15 \ proR}, \ H^{21 \ proS}, \\ H^{21 \ proR}, \ H^{32 \ proS}, \ H^{32 \ proR}), \ 2.48 \ (m, \ 1H, \ H^{20}), \ 2.60 \ (m, \ m, \ M^{21 \ proS}), \end{array}$ 1H, H³), 3.34 (m, 1H, H^{30 proS}), 3.44 (m, 1H, H^{13 proR}), 3.52 (m, 1H, H^{30 proR}), 3.68 (s, 3H, H¹), 4.00–4.08 (m, 2H, H⁵ and H²²), 4.30 (m, 1H, H³¹), 4.38–4.47 (m, 3H, H¹¹, H¹⁴ and H^{28}), 5.09 (m, 2H, $H^{36 \text{ CH2}}$), 6.93 (d, J = 6.1 Hz, 1H, H^{35}), 7.30–7.36 (m, 5H, H^{36} Ar), 7.56 (d, J = 7.9 Hz, 1H, H^{26}), 7.70 (d, J = 8.4 Hz, 1H, H^9), 7.81 (d, J = 6.4 Hz, 1H, H¹⁸). ¹³C NMR (150 MHz; CDCl₃): 16.5 (C²⁴), 17.0 (C^{7}) , 26.0 (C^{21}) , 26.2 (C^{4}) , 28.3 $(6C, C^{17} \text{ and } C^{34})$, 29.0 (C⁸), 29.3 (C²⁵), 31.1 (C¹⁵), 31.9 (C³²), 43.0 (C³), 44.3 (C²⁰), 45.8 (C²³), 46.2 (C⁶), 49.1 (C¹⁴), 50.0 and 50.1 (2C, C⁵, C²²), 50.7 (C³¹), 51.4 (C¹), 55.2 (C³⁰), 55.5 (C¹³), 59.2 (C¹¹), 59.3 (C²⁸), 66.4 (C³⁶ C^{H2}), 80.9 and 81.1 (2C, C^{17q}, C^{34q}), 127.9, 128.0 and 128.4 (C³⁶ A^r), 136.7 (C^{36q}), 156.1 and 156.3 (3C, C¹⁶, C³³, C³⁶ C^O), 171.2 (C¹⁹), 172.2 (C²⁷), 172.4 (C¹⁰), 172.7 (C²). IR (ATR, γ_{max} cm⁻¹) 3,355, 2,926, 2,854, 1,700, 1,671, 1,542, 1,459, 1,398. HRMS (ESI) calculated for C₄₃H₆₄N₆NaO₁₁ [M + Na]⁺ 863.4525; found: 863.4534.

γ -Hexapeptide (13)

Acid (12a) (140 mg, 0.17 mmol), DIPEA (0.12 mL, 0.60 mmol) and PyBOP (161 mg, 0.30 mmol) were dissolved in anhydrous dichloromethane (10 mL), the mixture was stirred for 5 min under nitrogen atmosphere, and then a solution of amine (11) (70 mg, 0.19 mmol) in anhydrous dichloromethane (5 mL) was added via cannula. After stirring at room temperature for 2 h the reaction crude was washed with a saturated aqueous sodium bicarbonate solution. The organic phase was dried over magnesium sulfate and the solvents were removed under vacuum. The reaction crude was purified by column chromatography on neutral silica gel (ethyl acetate to ethyl acetate/methanol 19:1) to afford pure hexapeptide (13). Yield 180 mg (81%). White solid. mp 128–131°C (from AcCN/H₂O). $[\alpha]_D^{20}$ 18.5 (c 0.1, CH₂Cl₂). (See Supplementary Material for numbering) ¹H NMR (600 MHz; CDCl₃): 0.76-0.81 (m, 9H, H^{7} , H^{24} , H^{24} and H^{41}), 1.12–1.17 (m, 9H, H^{8} , H^{25} and H^{42}), 1.35–1.38 (m, 27H, H¹⁷, H³⁴ and H⁵¹), 2.03–2.26 (m, 12H, H⁴, H¹⁵, H²¹, H³², H³⁸ and H⁴⁹), 2.41–2.44 (m, 2H, H²⁰ and H³⁷), 2.54 (m, 1H, H³), 3.26–3.44 (m, 6H, H¹³, H³⁰ and H⁴⁷), 3.61 (s, 3H, H¹), 3.96–3.97 (m, 3H, H⁵, H²² and H³⁹), 4.21 (m, 1H, H⁴⁸), 4.31 (m, 1H, H⁴⁵), 4.34 (m, 1H, H²⁸), 4.36–4.38 (m, 3H, H^{11} , H^{14} and H^{31}), 5.01 (m, 2H, H^{53} ^{CH2}), 6.93 (d, J = 6.8 Hz, 1H, H⁵²), 7.23–7.28 (m, 5H, H^{53} Ar), 7.56 (d, J = 8.1 Hz, 1H, H^{43}), 7.60 (d, J =8.9 Hz, 1H, H^{26}), 7.66 (d, J = 9.0 Hz, 1H, H^{9}), 7.94 (d, J = 7.1 Hz, 1H, H¹⁸), 8.03 (d, J = 7.2 Hz, 1H, H³⁵). IR $(ATR, \gamma_{max} \text{ cm}^{-1})$ 3,298, 2,957, 1,673, 1,520, 1,392, 1,367. HRMS (ESI) calculated for $C_{60}H_{91}N_9O_{15}$ [M + Na]⁺ 1,200.6527; found: 1,200.6521.

γ -Dipeptide (14)

Acid (4) (210 mg, 0.57 mmol), DIPEA (0.34 mL, 1.71 mmol) and PyBOP (480 mg, 0.86 mmol) were dissolved in anhydrous dichloromethane (15 mL). The mixture was stirred for 5 min under nitrogen atmosphere, and then a solution of amine **ent-8** (90 mg, 0.57 mmol) in anhydrous dichloromethane (5 mL) was added via cannula. After

stirring at room temperature for 2 h the reaction crude was washed with a saturated aqueous sodium bicarbonate solution. The organic phase was dried over magnesium sulfate and the solvents were removed under vacuum. The reaction crude was purified by column chromatography on neutral silica gel (ethyl acetate/hexane 1:1) to afford pure dipeptide (14). Yield 276 mg (95%). White solid. mp 59- 62° C (from Et₂O). [α]_D²⁰ -109.4 (*c* 0.39, CH₂Cl₂) (see Fig. 6 for numbering). ¹H NMR (600 MHz; CDCl₃): 0.95 (s, 3H, H⁷), 1.28 (s, 3H, H⁸), 1.47 (s, 9H, H¹⁷), 2.11 (m, 1H, H^{4 proS}), 2.16 (m, 1H, H^{15 proS}), 2.32 (m, 1H, H^{4 proR}), 2.38 (m, 1H, $H^{15 \text{ proS}}$), 2.61 (dd, J = J' = 8.9 Hz, 1H, H^3), 3.45 (dd, J = J' = 10.8 Hz, 1H, H^{13} pro^S), 3.54 (dd, J = 10.8 Hz, J' = 4.8 Hz, 1H, H^{13 proR}), 3.69 (s, 3H, H¹), 4.06 (dd, J = 17.2 Hz, J' = 8.5 Hz, 1H, H⁵), 4.30 (m, 1H, H^{14}), 4.42 (d, J = 8.8 Hz, 1H, H^{11}), 5.06 (d, J = 12.3 Hz, 1H, $H^{19 \text{ CH2}}$), 5.13 (d, J = 12.3 Hz, 1H, $H^{19 \text{ CH2}}$), 6.68 (d, J = 6.4 Hz, 1H, H^{18}), 7.32–7.37 (m, 5H, $H^{19 \text{ Ar}}$), 7.67 (d, J = 7.6 Hz, 1H, H⁹). ¹³C NMR (150 MHz; CDCl₃): 17.2 (C⁸), 26.3 (C⁴), 28.3 (3C, C¹⁷), 28.8 (C⁷), 31.8 (C¹⁵), 43.1 (C³), 46.0 (C⁶), 50.0 (C⁵), 50.7 (C¹⁴), 51.5 (C¹), 55.1 $(C^{13}), 59.2 (C^{11}), 66.5 (C^{19 \text{ CH2}}), 81.2 (C^{17q}), 128.0, 128.2$ and 128.5 (C^{19 Ar}), 136.7 (C^{19q}), 156.0 (C^{19 CO}), 156.3 (C^{16}) , 172.1 (C^{2}) , 172.9 (C^{10}) . IR (ATR, $\gamma_{max} \text{ cm}^{-1}$) 3,305, 2,958, 1,679, 1,671, 1,541, 1,406. HRMS (ESI) calculated for $C_{26}H_{37}N_3O_7 [M + Na]^+$ 526.2524; found: 526.2504.

$\underline{\gamma}$ -Tetrapeptide (15)

Acid (14a) (160 mg, 0.33 mmol), DIPEA (0.2 mL, 1.00 mmol) and PyBOP (280 mg, 0.50 mmol) were dissolved in anhydrous dichloromethane (15 mL). The mixture was stirred for 5 min under nitrogen atmosphere, and then a solution of amine (14b) (120 mg, 0.33 mmol) in anhydrous dichloromethane (5 mL) was added via cannula. After stirring at room temperature for 2 h the reaction crude was washed with a saturated aqueous sodium bicarbonate solution. The organic phase was dried over magnesium sulfate and the solvents were removed under vacuum. The reaction crude was purified by column chromatography on neutral silica gel (ethyl acetate) to afford pure tetrapeptide (15) Yield 255 mg (92%). White solid. mp 129-131°C (from Et₂O). $[\alpha]_{D}^{20}$ –16.4 (c 0.57, CH₂⁻Cl₂) (see supplementary material for numbering). ¹H NMR (600 MHz; CDCl₃): 0.93 (s, 3H, H²⁵), 0.95 (s, 3H, H⁸), 1.27 (s, 3H, H⁷), 1.31 (s, 3H, H²⁴), 1.46 (s, 18H, H^{17} and H^{34}), 2.10–2.20 (m, 4H, $H^{4 \text{ proS}}$, $H^{15 \text{ proS}}$, $H^{21 \text{ proS}}$ and $H^{32 \text{ proS}}$), 2.24–2.27 (m, 1H, $H^{15 \text{ proR}}$), 2.32–2.45 (m, 4H, $H^{4 \text{ proR}}$, $H^{20 \text{ proR}}$, $H^{21 \text{ proR}}$ and $H^{32 \text{ proR}}$), 2.63 (dd, J = 10.1 Hz, J' = 7.8 Hz, 1H, H³), 3.38 (d, J = 11.6 Hz, 1H, $H^{13 \text{ proS}}$), 3.45 (d, J = 11.5 Hz, 1H, $H^{30 \text{ proS}}$), 3.50–3.55 (m, 2H, H^{13 proR} and H^{30 proR}), 3.70 (s, 3H, H¹), 4.02–4.07 (m, 2H, H⁵ and H²²), 4.29 (m, 1H, H³¹), 4.45–4.50 (m, 2H,

H¹¹ and H²⁸), 4.48 (m, 1H, H¹⁴), 5.04 (d, J = 12.3 Hz, 1H, H³⁶ CH2), 5.12 (d, J = 12.3 Hz, 1H, H³⁶ CH2), 6.80 (d, J = 7.1 Hz, 1H, H³⁵), 7.32–7.38 (m, 5H, H³⁶ Ar), 7.63–7.72 (m, 3H, H⁹, H¹⁸ and H²⁶). ¹³C NMR (150 MHz; CDCl₃): 17.0 (C²⁵), 17.3 (C⁸), 25.8 (C⁴), 26.3 (C²¹), 28.4 (6C, C¹⁷, C³⁴), 28.8 (C²⁴), 29.0 (C⁷), 31.9 (C¹⁵), 32.1 (C³²), 43.0 (C³), 44.7 (C²⁰), 45.6 and 45.9 (2C, C²⁵, C⁶), 49.0 (C¹), 49.9 (2C, C⁵, C²²), 50.7 (C³¹), 51.7 (C¹⁴), 55.2 (2C, C¹³, C³⁰), 59.2 (2C, C¹¹, C²⁸), 66.6 (C³⁶ CH²), 81.2 and 81.3 (2C, C^{17q}, C^{34q}), 128.1, 128.3 and 128.5 (C³⁶ Ar), 136.6 (C^{36q}), 156.1 and 156.2 (3C, C¹⁶, C³³, C³⁶ CO), 171.1 (C¹⁹), 172.1 (C²⁷), 172.5 (C¹⁰), 173.0 (C²). IR (ATR, γ_{max} cm⁻¹) 3,640, 3,571, 3,292, 2,956, 1,665, 1,524, 1,391, 1,368. HRMS (ESI) calculated for C₄₃H₆₅N₆O₁₁ [M + H]⁺ 841.4706; found: 841.4698.

γ -Hexapeptide (16)

Acid (15a) (90 mg, 0.10 mmol), DIPEA (0.06 mL, 0.30 mmol) and PyBOP (80 mg, 0.15 mmol) were dissolved in anhydrous dichloromethane (10 mL). The mixture was stirred for 5 min under nitrogen atmosphere, and then a solution of amine (14b) (60 mg, 0.10 mmol) in anhydrous dichloromethane (5 mL) was added via cannula. After stirring at room temperature for 2 h the reaction crude was washed with a saturated aqueous sodium bicarbonate solution. The organic phase was dried over magnesium sulfate and the solvents were removed under vacuum. The reaction crude was purified by column chromatography on neutral silica gel (ethyl acetate to ethyl acetate/methanol 19:1) to afford pure hexapeptide (16). Yield 100 mg (84%). White solid. mp 144-146°C (from AcCN/H₂O). $[\alpha]_{D}^{20}$ -131 (c 0.13, CH₂Cl₂) (see supplementary material for numbering). ¹H NMR (600 MHz; CDCl₃): 0.93–0.97 (m, 9H, H⁸, H²⁵ and H⁴²), 1.21–1.31 (m, 9H, H⁷, H²⁴ and H⁴¹), 1.44–1.46 (m, 27H, H¹⁷, H³⁴ and H^{51}), 2.10–2.44 (m, 14H, H⁴, H¹⁵, H²¹, H³², H³⁸, H⁴⁹, H²⁰ and H³⁷), 2.64 (m, 1H, H³), 3.37-3.53 (m, 6H, H¹³, H³⁰ and H^{47}), 3.69 (s, 3H, H^{1}), 4.02–4.07 (m, 3H, H^{5} , H^{22} and H^{39}), 4.29 (m, 1H, H⁴⁸), 4.37-4.43 (m, 3H, H¹¹, H²⁸ and H⁴⁵), 4.47–4.51 (m, 2H, H^{14} and H^{31}), 5.04 (d, J = 12.3 Hz, 1H, $H^{53 \text{ CH2}}$), 5.12 (d, J = 12.3 Hz, 1H, $H^{53 \text{ CH2}}$), 6.81 (d, J = 7.2 Hz, 1H, H⁵²), 7.33–7.37 (m, 5H, H^{53 Ar}), 7.62 (d, J = 7.9 Hz, 1H, H²⁶), 7.67–7.75 (m, 4 H, H⁹, H¹⁸, H³⁵ and H^{43}). IR (ATR, γ_{max} cm⁻¹) 3,292, 2,957, 1,666, 1,534, 1,392, 1,368. HRMS (ESI) calculated for C₆₀H₉₁N₉O₁₅ $[M + Na]^+$ 1,200.6527; found: 1,200.6509.

Results and discussion

Scheme 1 shows the synthesis of both enantiomers 1 and 2 from (–)-verbenone as a common chiral precursor.

This compound is commercially available and the double bond was oxidatively cleaved to afford quantitatively (-)-cis-pinononic acid without epimerization (Moglioni et al. 2000). Activation of the carboxyl group by formation of a mixed anhydride through reaction with ethyl chloroformate and subsequent reaction with sodium azide afforded an acyl azide which was submitted to Curtius rearrangement by heating in toluene in the presence of benzyl alcohol to afford compound 5. Lieben degradation of the methyl ketone and esterification of the resulting carboxylic acid produced orthogonally protected amino acid 2. Alternatively, (-)-cis-pinononic acid was protected as a tert-butyl ester and the ketone was submitted to Lieben degradation to give carboxylic acid 6, which was transformed into benzyl carbamate 7 following the same transformations as in the case of 2. Removal of the tertbutyl ester in 7 and subsequent methylation provided enantiomer 1 (Aguilera et al. 2008).

Benzyl carbamate in this compound was reductively cleaved by reaction with ammonium formate in the presence of 10% Pd/C in refluxing methanol affording free amine 8 (Scheme 2). This compound was reacted with partially protected 4-amino proline 4 (Fisher et al. 2006) under usual coupling conditions by using PyBOP and DIPEA in anhydrous dichloromethane to provide hybrid γ -dipeptide 9 in quantitative yield.

Tetrapeptide 12 was prepared through a convergent synthesis by coupling acid 10, obtained from 9 by mild saponification with 1 M LiOH, and the amine resulting from removal of the benzyl carbamate in a second molecule of 9. In this way, 12 was obtained in 96% yield. Finally, deprotection of the carboxyl group in 12 followed by coupling with amine 11 afforded hexamer 13 in 81% yield.

Similarly, the diastereometric series of hybrid γ -peptides **14–16** (Fig. 3) was synthesized.

Next, we decided to investigate the conformational bias of these products in solution. Full details are provided in

the supplementary material. We started studying fully protected 4-aminoproline **3** itself because no detailed data were provided in the literature on this compound. 600 MHz ¹H-NMR spectrum of **3** acquired at 273 K clearly showed split resonances in most of the protons. It is widely known that dynamic rotation of the conjugated NC bond in Boc group is very slow within the NMR time scale giving rise to *cis/trans* conformers. The key point for the unambiguous assignment of both conformers was the NOE contacts observed in *tert*-Bu group. While *trans* isomer correlates *tert*-Bu with H₅ protons, *cis* isomers does it with Me₁ and H₃ proton. Both conformers are almost equally populated (Fig. 2).

1D selective TOCSY experiments irradiating at H¹⁰ protons allowed us to obtain *cis/trans* proline protons in separate subspectra thus facilitating the *cis/trans* chemical shift assignment (see the supplementary material). ¹³C-NMR spectrum acquired at 273 K also exhibited split resonances for most of the signals due to *cis/trans* isomers. It is noticeable that CO^8 signal difference between *cis* and trans isomer was about 113 Hz, while the difference found between *cis/trans* isomers in CO² in the methyl ester and CO^{11} in the Cbz group was much lower (16 and 9 Hz, respectively), therefore confirming that conformational rotational barrier is due to NC bond in the Boc group. 2D-NOESY spectrum acquired at 273 K was the key point to assign unequivocally cis and trans isomers. The expanded region showing tert-Bu cross peaks indicated that the slightly major component has cross peaks with H^3 and Me^1 protons and, therefore, can be attributed to *cis* conformer. On the other hand, minor component correlates with H⁵ protons, consequently assigned to *trans* conformer.

Interestingly, while inverting H^{10} proton in 1D selective NOE experiment, independently of *cis* or *trans* isomer, NOE effects were observed at *pro-S* H⁵ (H^{5S}) and *pro-R* H⁷ (H^{7R}) protons, suggesting that NH¹⁰ proton in both conformers is pointing to the carbonyl oxygen of the methyl

Scheme 1 Reagents, conditions, yields: (*a*) RuCl₃, NaIO₄, CH₂Cl₂/CH₃CN/H₂O, rt, quantitative; (*b*) ClCO₂Et; (*c*) NaN₃ (90% for **4**, 98% for **6**, two steps); (*d*) BnOH, toluene, reflux (92%); (*e*) NaOBr, dioxane/H₂O (80% for **2**, 93% for **5**); (*f*) CH₃I, Cs₂CO₃, DMF (95%); (*g*) 'BuOH, DMAP, EDAC, Et₃N, anhydrous THF, 0°C (60%); (*h*) TFA, Et₃SiH, CH₂Cl₂ (94%)





ester group probably due to the presence of a hydrogen bond. Also, conformational exchange signals were detected in that experiment between *cis* and *trans* isomers (see the supplementary material). Furthermore, two small signals resonating ~0.5 ppm downfield with respect to the major signals were observed and were assigned to minor conformations without such hydrogen bonding. According to all NMR experiments performed, the conformational equilibrium of modified proline **3** can be depicted as shown in Fig. 4.

Finally, variable ¹H-NMR temperature experiments led to visualize the coalescence temperature between major *cis/trans* Boc isomers (see the supplementary material). As conformer populations are almost equal, Eyring's equation was used to approximately determine a rotational barrier of 18.1 kcal/mol for the *cis/trans* equilibrium.

The next step was to investigate if the presence of the cyclobutane residues in the hybrid oligomers has any influence on this equilibrium and if preferred conformations change. Therefore, the two diastereomeric dimers 9 and 14 were studied. Differently to 4-aminoproline 3, the respective ¹H-NMR spectra of both 9 and 14 clearly showed a single major conformation. Strongly deshielded position of H⁹ suggested a hydrogen bond between NH⁹ and CO^{16} building a seven-membered ring stacked to the five-membered proline ring fixing, therefore, Boc rotamer to trans position (see Fig. 6 for atom numeration). NOE experiments confirmed such hypothesis as described below. 1D selective TOCSY experiment irradiating at the NH protons allows the separation of proline and cyclobutane subspectra, which affords a clear visualization of the correct proton assignment of the molecule.

Fig. 3 Structures of hybrid *γ*-peptides **14, 15, 16**



Considering dimer 14, *J* coupling values together with 1D selective NOE experiments (Fig. 5) over N*H* protons give an excellent visualization of the conformational structure of the molecule. Selective inversion of H⁹ gives a strong NOE effect with H¹¹. This fact, together with the highly deshielded position found for that proton, clearly indicates a strong hydrogen bond formation with Boc carbonyl group. Furthermore, coupling constant ${}^{3}J_{H9H5} =$ 7.8 Hz (dihedral angle ~ 150°) and NOE effects observed with H⁵, H^{4S} and Me⁸ indicate the spatial disposition of the cyclobutane ring.

Similarly to that observed in 4-aminoproline 3, NH^{18} proton in Cbz group has an unusual deshielded position suggesting the formation of a hydrogen bond with CO^{10} . A similar hydrogen bond was previously reported for a γ -dipeptide consisting of two residues of 4-aminoproline protected in a different manner than compound 3 (Farrera-Sinfreu et al. 2004). In our case, this is corroborated by NOE effects observed to H^{15R} and H^{13S} protons. However, there are slight differences in NOE intensities compared with 3. In the dimer 14, comparatively, more NOE signal is observed in H^{15R} with respect to H^{13S} proton indicating a shift toward this proton. This can be explained due to the conformational restriction of CO^{10} that belongs to the new seven-membered ring, which consequently shifts a bit the hydrogen bond CO^{10} -N H^{18} . The slight change is also noticeable in ${}^{3}J_{\rm H14H18}$ coupling value, which changed from 8.8 Hz (dihedral angle $\sim 160^{\circ}$) in triprotected proline to 6.5 Hz in the dimer (dihedral angle $\sim 140^{\circ}$).

Furthermore, exchange peaks are observed in selective NOE experiment indicating the presence of minor conformations during the NMR experiment time scale (500 ms). Those conformers can be seen in the ¹H-NMR spectrum background but close to noise level, making their study difficult. However, the broadness and the chemical shift position of the exchange peaks observed in NOE experiments give an idea of the nature of the different conformers. For instance, NH^9 exchanges with a broad peak (\sim 40 Hz at half height) that resonates at 6.2, 1.5 ppm far away from its initial 7.7 ppm position. The large displacement and the broadness of the line suggest that finding H^9 in this position is due to the loss of the hydrogen bond. On the other hand, NH^{18} behavior is more difficult to explain because several exchange lines are observed. Two signals (6.4 and 6.5 ppm), which are very close to the initial peak position (6.7 ppm) are observed. Those signals are equally broad at half height of the inverted peak $(\sim 20 \text{ Hz})$. Both slight chemical shift displacement and very similar signal broadness suggest that peaks come from a conformational exchange without hydrogen bond breaking, thus from another part of the molecule. This is probably due to Cbz rotation from a trans to a cis position and the above mentioned H⁹ hydrogen bond loss. Also, although small, a broad peak appears at 5.9 ppm (\sim 40 Hz at half height), which is attributed to hydrogen bond breaking. Low intensity is explained because once the bond is broken water can have access to that proton and exchange with it, which is in fact seen in the spectrum.

Fig. 4 Expanded region of

values for each conformer

¹H-NMR spectrum at 273 K with the detailed integration







A pictorial conformational structure of the major conformer is presented in Fig. 6a.

A similar study was carried out on dipeptide **9** leading to the same conclusions. The conformation for **9** deduced from NOEs and J coupling values is depicted in Fig. 6b.

In order to qualitatively compare hydrogen bonding strength of NH^9 and NH^{18} in these dipeptides, 50 µL of deuterated methanol were added into the NMR tube. The tube was then hand-shaken and left to equilibrate for 10 min. The spectrum clearly shows that, while approximately half of the signal of NH^9 prevails, NH^{18} has completely disappeared indicating a total deuterium exchange, therefore being experimentally demonstrated that NH^9 hydrogen bond is less accessible than NH^{18} one, thus suggesting a stronger hydrogen bond. Variable ¹H-NMR temperature experiments corroborate such hypothesis (see the supplementary material).

To ascertain if this conformation was preserved in tetramers, γ -tetrapeptides **12** and **15** were investigated. The respective ¹H-NMR spectra clearly showed a single major conformation although several minor conformers are visible in the spectrum and as exchange peaks in 2D ROESY spectra. All ¹H and ¹³C resonances have been assigned with the help of standard 2D NMR experiments which are reported in the supplementary material (TOCSY, ROESY, HSQC and HMBC). The result of these studies was the confirmation for the same preferred conformation found for γ -dipeptides **9** and **14**. These conformers are depicted in

Fig. 6 Conformation of a dipeptide 14 and b dipeptide 9 deduced from NOEs and J coupling values. NOE effects are indicated with *arrows* and hydrogen bonds indicated with *lines*



Fig. 7 in which the inter residue strongest ROE contacts are indicated by bold arrows.

 γ -Hexapeptides **13** and **16** were also examined by ¹H NMR. A similar preferential conformation as that for dipeptides and tetrapeptides was concluded. Nevertheless, other minor conformers were also observed.

Compared analysis of the amide N*H* region in the ¹H NMR spectra of these γ -tetra- and γ -hexapeptides showed that **12** and **13** in one diastereomeric series, and **15** and **16** in the other one presented different peak splitting. Thus, while all N*H* signals presented differentiated chemical shifts for **12** and **13**, overlapped N*H* signals were observed for **15** and **16**. On the basis of these results, we could suggest different molecular arrangements for the two γ -peptide series in good agreement with the observed signatures in the CD spectra (see below).

Figure 8 shows the CD spectra of all the synthesized γ -peptides as 0.01 M solutions in MeOH. This concentration is low enough to avoid self-aggregation. We can observe a different pattern and also a different sign of the maximum absorption peaks in each series thus reflecting the opposite chirality of the cyclobutane residues in 9, 12 and 13 with respect to diastereomers 14–16. Well-defined Cotton effects were observed for tetra- and hexapeptides 12 and 13, respectively.

Theoretical calculations were done to understand the differences observed for both diastereomeric series (see the supplementary material for details). Molecular Dynamics were performed on the significant structures obtained from the conformational search. It is remarkable that the inter residue hydrogen bond is always present during the dynamics while the proline intra residue one is not. This fact accounts for the higher strength of the former as deduced from ¹H NMR experiments. The geometries of the resultant structures were optimized at B3LYP/6-31G(d) level of theory in chloroform solution for tetrapeptides **12** and **15** (see the supplementary material) and in gas phase for hexapeptides **13** and **16** (Fig. 9). Structural trends are similar for tetra- and hexapeptides in each series.

Comparing the calculated structures for 13 and 16 some features are remarkable. The first one is that, in 13, each cyclobutane NH proton is involved, in average, in a bifurcated hydrogen bond, that means an inter residual NH⁽ⁱ⁾...OC⁽ⁱ⁻¹⁾ hydrogen bond with the carbamate of the sequentially preceding proline moiety and a second one with the amide carbonyl of the same residue, $NH^{(i)}$... $OC^{(i)}$. This last interaction is not observed in the terminal cyclobutane residue. In contrast, for hexapeptide 16, only inter residual hydrogen bonding is predicted and the molecule is more twisted than 13. The presence of the intra and inter residue hydrogen bonds in 13 originates a differentiated chemical environment for each NH proton, which in turn explains the splitted pattern in the NH region of the ¹H NMR spectra. In contrast, the preferred conformation for 16 presents a similar environment for all NH protons, thus resulting in an overlapping of the corresponding NH signals.

Once the conformational bias in solution was verified, we explored the ability of some of these compounds to selfassemble. Thus, 2 mM solutions of the hybrid γ -peptides in MeOH were prepared and incubated for 24 h. TEM micrographs showed the formation of nicely defined sphere vesicles of nanometric size, as shown in Fig. 10 for compounds **9**, **12** and **14**. Incubation of samples for a week did

Fig. 7 Main conformation for a tetrapeptide 12 and b tetrapeptide 15 deduced from ROE connections and ${}^{3}J_{NHCH}$ coupling values. Hydrogen bonds are indicated with *lines*. ROE contacts are indicated with *arrows*. The *bold ones* correspond to the strongest contacts in each peptide



Fig. 8 CD spectra of **a** γ-peptides **9**, **12** and **13**, and **b 14–16** as 0.01 mM solutions in MeOH



Fig. 9 Preferred conformations for hexapeptides **13 (a)** and **16** (**b**) as obtained at B3LYP/ 6-31G(d) level of theory in gas phase. Distances are in Å. All hydrogen atoms except *NH* have been omitted for clarity



Fig. 10 TEM images of the vesicles formed by a dipeptide 9, b tetrapeptide 12, c hexapeptide 13, d dipeptide 14, e tetrapeptide 15, f hexapeptide 16 from 2 mM solutions in MeOH (1 day incubation

for **a**, **b**, **d**, **e** and 4 days incubation for **c**, **f** placed onto a carbon-film coated copper grid

not alter the shape of these assemblies. Hexamers **13** and **16** required longer incubation times to form defined aggregates.

Conclusions

Two diastereomeric series of hybrid γ , γ -peptides have been synthesized from enantiomeric cyclobutane amino acids 1 or 2 and 4-aminoproline 3 joined in alternation. The presence of the cyclobutane moiety in these compounds leads to very defined conformations in solution affording compact structures as the result of two kinds of hydrogenbonded ring formation. The intra residue interaction between the Cbz–NH and the amide CO, equivalent to that already observed in the monomer, was retained. Furthermore, a new and strong hydrogen bond was formed by inter residue interaction between the Boc CO of a proline moiety and the NH of the cyclobutane residue giving a second seven-membered ring. Moreover, in the (S,R)-cyclobutane containing series (peptides 12 and 13), this NH proton is also involved in an intra residue hydrogen bond with the amide CO producing, in average, a third seven-membered ring. As a result, (S,R)-cyclobutane peptides are more rigid than the (R,S) diastereomers 15 and 16. The latter peptides adopt more twisted conformations according to ¹H NMR spectroscopy and theoretical calculations. In addition, all these y-peptides show tendency to aggregation providing vesicles of nanometric size, which were stable when allowed to stand for several days. Possible applications of these and other related hybrid peptides are under active investigation.

Acknowledgments Authors thank financial support from Spanish Ministerio de Ciencia e Innovación (grants CTQ2007-61704/BQU, CTQ2008-00177/BQU, and CTQ2010-15408/BQU) and Generalitat de Catalunya (grant 2009SGR-733). Time allocated in the Servei de Ressonància Magnètica Nuclear and Servei de Microscòpia Electrónica (UAB) is gratefully acknowledged. R G-A thanks Ministry of Education for a predoctoral fellowship.

References

- Aguilera J, Moglioni AG, Moltrasio GY, Ortuño RM (2008) Stereodivergent and efficient synthesis of the first bis(cyclobutane) gamma-dipeptides. Tetrahedron Asymmetry 19:302–308
- Brenner M, Seebach D (2001) Design, synthesis, NMR-solution and X-ray crystal structure of N-acyl- γ -dipeptide amides that form a $\beta \Pi'$ -type turn. Helv Chim Acta 84:2155–2166
- Cheng RP, Gellman SH, DeGrado WF (2001) β -Peptides: from structure to function. Chem Rev 101:3219–3232
- DePol S, Zorn C, Klein CD, Zerbe O, Reiser O (2004) Surprisingly stable helical conformations in α/β -peptides by Incorporation of *cis* β -aminocyclopropane carboxylic acids. Angew Chem Int Ed 43:511–514
- Farrera-Sinfreu J, Zaccaro L, Vidal D, Salvatella X, Giralt E, Pons M, Albericio F, Royo M (2004) A new class of foldamers based on cis-γ-amino-L-proline. J Am Chem Soc 126:6048–6057
- Farrera-Sinfreu J, Giralt E, Castel S, Albericio F, Royo M (2005) Cell-penetrating cis-γ-amino-L-proline-derived peptides. J Am Chem Soc 127:9459–9468

- Fernández D, Torres E, Avilés FX, Ortuño RM, Vendrell J (2009) Cyclobutane-containing peptides: evaluation as novel metallocarboxypeptidase inhibitors and modelling of their mode of action. Bioorg Med Chem 17:3824–3828
- Fisher A, Mann A, Verma V, Thomas N, Mishra RK, Johnson RL (2006) Design and synthesis of photoaffinityl-labeling ligands of the L-Prolyl-L-leucylglycinamide binding site involved in the allosteric modulation of the dopamine receptor. J Med Chem 49:307–317
- Gorrea E, Torres E, Nolis P, DaSilva E, Amabilino DB, Branchadell V, Ortuño RM (2011) Self-assembly of chiral *trans*-cyclobutane containing β -dipeptides into ordered aggregates. Chem Eur J 17:4588–4597
- Guo L, Chi Y, Almeida AM, Guzei IA, Parker BK, Gellman SH (2009) Stereospecific synthesis of conformationally constrained γ -amino acids: new foldamer building blocks that support helical secondary structure. J Am Chem Soc 131:16018–16020
- Guo L, Almeida AM, Zhang W, Reidenbach AG, Choi SH, Guzei IA, Gellman SH (2010) Helix formation in preorganized β/γ -peptide foldamers: hydrogen-bond analogy to the α -helix without α -amino acid residues. J Am Chem Soc 132:7868–7869
- Hecht S, Huc I (2007) Foldamers: structure, properties and applications. Wiley-VCH, Weinheim
- Horne WS, Gellman SH (2008) Foldamers with heterogeneous backbones. Acc Chem Res 41:1399–1408
- Izquierdo S, Kogan MJ, Parella T, Moglioni AG, Branchadell V, Giralt E, Ortuño RM (2004) 14-Helical folding in a cyclobutane containing β -tetrapeptide. J Org Chem 69:5093–5099
- Koglin N, Zorn C, Beumer R, Cabrele C, Bubert C, Sewald N, Reiser O, Beck-Sickinger AG (2003) Analogues of neuropeptide y containing β -aminocyclopropane carboxylic acids are the shortest linear peptides that are selective for the Y1 receptor. Angew Chem Int Ed 42:202–205
- Lang M, Bufe B, DePol S, Reiser O, Meyerhof W, Beck-Sickinger AG (2006) Structural properties of orexins for activation of their receptors. J Pept Sci 12:258–266
- Moglioni AG, García-Expósito E, Aguado GP, Parella T, Moltrasio GY, Branchadell V, Ortuño RM (2000) Divergent routes to chiral cyclobutyl synthons from (-)-α-pinene and their use in the

stereoselective synthesis of cyclobutane dehydro amino acids. J Org Chem 65:3934–3940

- Rodríguez-Ropero F, Canales M, Zanuy D, Zhang A, Schlüter D, Alemán C (2009) Helical dendronized polymers with chiral second-generation dendrons: atomistic view and driving forces for structure formation. J Phys Chem B 113:14868–14876
- Rúa F, Boussert S, Parella T, Diez-Pérez I, Branchadell V, Giralt E, Ortuño RM (2007) Self-assembly of a cyclobutane β -tetrapeptide to form nano-sized structures. Org Lett 9:3643–3645
- Seebach D, Brenner M, Rueping M, Jaun B (2002) γ2-, γ3-, and γ2, 3, 4-Amino acids, coupling to γ-hexapeptides: CD spectra, NMR solution and X-ray crystal structures of γ-peptides. Chem Eur J 8:573–584
- Torino D, Mollica A, Pinnen F, Feliciani F, Spisani S, Lucente G (2009) Novel chemotactic For-Met-Leu-Phe-OMe (fMLF-OMe) analogues based on Met residue replacement by 4-amino-proline scaffold: synthesis and bioactivity. Bioorg Med Chem 17:251–259
- Torres E, Gorrea E, DaSilva E, Nolis P, Branchadell V, Ortuño RM (2009) Prevalence of eight-membered hydrogen-bonded rings in some bis(cyclobutane) β -dipeptides with *trans* stereochemistry. Org Lett 11:2301–2304
- Torres E, Gorrea E, Burusco KK, DaSilva E, Nolis P, Rúa F, Boussert S, Díez-Pérez I, Dannenberg S, Izquierdo S, Giralt E, Jaime C, Branchadell V, Ortuño RM (2010) Folding and self-assembling with β-oligomers based on (1R, 2S)-2-aminocyclobutane-1-carboxylic acid. Org Biomol Chem 8:564–575
- Woods CR, Ishii T, Boger DL (2002) Synthesis and DNA binding properties of iminodiacetic-acid-linked polyamides: characterization of cooperative extended 2:1 side-by-side parallel binding. J Am Chem Soc 124:10676–10682
- Zhang A, Schlüter AD (2007) Multigram solution-phase synthesis of three diastereomeric tripeptidic second-generation dendrons based on (2S, 4S)-, (2S, 4R)-, and (2R, 4S)-4-aminoprolines. Chem Asian J 2:1540–1548
- Zhang A, Rodríguez-Ropero F, Zanuy D, Alemán C, Meijer EW, Schlüter AD (2008) A rigid, chiral, dendronized polymer with a thermally table, right-handed helical conformation. Chem Eur J 14:6924–6934