Organic & Biomolecular Chemistry

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Cite this: *Org. Biomol. Chem.*, 2012, **10**, 861

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PAPER

Designing hybrid foldamers: the effect on the peptide conformational bias of β- *versus* α- and γ-linear residues in alternation with (1*R*,2*S*)-2-aminocyclobutane-1-carboxylic acid†‡

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Received 14th September 2011, Accepted 11th October 2011
DOI: 10.1039/c1ob06575k

Several oligomers constructed with (1*R*,2*S*)-2-aminocyclobutane-1-carboxylic acid and glycine, β-alanine, and γ-amino butyric acid (GABA), respectively, joined in alternation have been synthesized and studied by means of NMR and CD experiments as well as with computational calculations. Results account for the spacer length effect on folding and show that conformational preference for these hybrid peptides can be tuned from β-sheet-like folding for those containing a C_2 or C_4 linear segment to a helical folding for those with a C_3 spacer between cyclobutane residues. The introduction of cyclic spacers between these residues does not modify the extended ribbon-type structure previously manifested in poly(*cis*-cyclobutane) β-oligomers.

Introduction

There is a growing interest in the design and use of bioinspired oligomers with well-defined conformational propensities, called foldamers. Their applications in the fields of protein-protein interactions, drug-discovery or self-assembling materials make these compounds relevant. Unnatural amino acids when incorporated in peptidomimetics offer manifold and broad possibilities to fold in several motifs mainly named helices, sheets and turns.[1] Among the most frequent oligomers used, α-, β, and γ-peptides currently differ in their folding preferences and, in addition, β-peptides usually need a shorter number of residues than α- and γ-oligomers to arrange in a helical folding.[2] Moreover, the use of carbocyclic amino acids confers rigidity to the corresponding oligomers thus determining their conformational bias in solution. Therefore, small rings[3] as well as bridged systems[4] have been included into conformationally constrained peptides with well-defined secondary structures in solution.

Combination of different types of cyclic or linear amino acids in heterogeneous backbones to afford hybrid peptides offers unique possibilities of folding.[5] For instance, some α,β-,[6] β,γ-,[7] and α,β,γ-peptides[8] adopt α-helix-like conformations which often are more stable than those of the analogous α-peptides probably because of the preorganized β or γ residues employed. Other α,β-peptides form β-sheets in aqueous solution.[9] In addition, some designed amphiphilic α,β-peptides self assemble giving highly stable pleated sheets.[10] Moreover, replacement of α-amino acids with β-residues have resulted in α,β-peptides with interesting biological activities as anti-HIV agents,[11] or as integrin ligands, which are involved in the control of several disorders.[12]

Some time ago, we reported on a 14-helix-like folding adopted by a tetrapeptide built with achiral β-alanine and (*R*,*S*)-2-aminocyclobutane-1-carboxylic acid joined in alternation.[13] This behaviour clearly differs from the analogous β-tetrapeptide made exclusively with the cyclobutane residues,[14] which displays a ribbon-type extended conformation as well as its homologous hexamer and octamer (Fig. 1).[15] We wanted to investigate the influence of the number of residues on the conformational propensity shown by that mixed β,β-tetramer as well as by the length of the achiral linear residue included.

For this reason, we have synthesized and studied tetra-, hexa-, and octamers constituted of 1 : 1 (*R*,*S*)-cyclobutane residues and glycine, β-alanine, and γ-aminobutyric acid (GABA), respectively, used as spacers between the cyclic building blocks. NMR, CD and computational calculation results are reported in this paper and show a similar conformational bias for these α,β- and β,γ-oligomers which folds giving β-sheet-like structures, whereas β,β-peptides prefer to arrange in a helical folding. The introduction of cyclic moieties between cyclobutane residues has also been investigated and it does not modify the extended secondary structure previously manifested by poly(*cis*-cyclobutane) β-oligomers.

Results and discussion

1. Synthesis of hybrid peptides

Convergent synthetic routes allowed us the easy and efficient preparation of the desired oligomers. The general strategy is

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† This article is part of an *Organic & Biomolecular Chemistry* web theme issue on Foldamer Chemistry.

‡ Electronic supplementary information (ESI) available: Full details on experimental procedures and product characterization, NMR studies, and theoretical calculations. See DOI: 10.1039/c1ob06575k

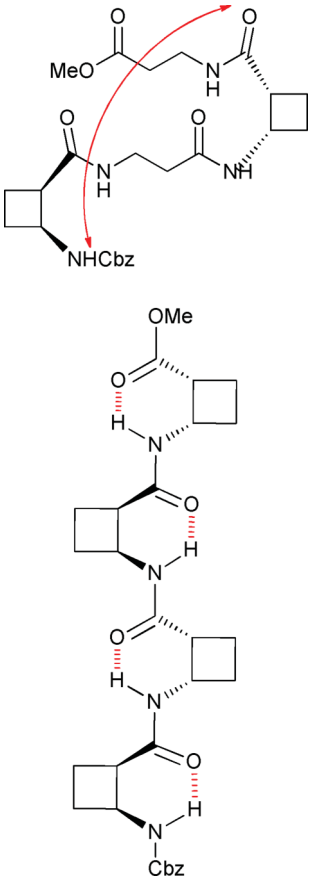
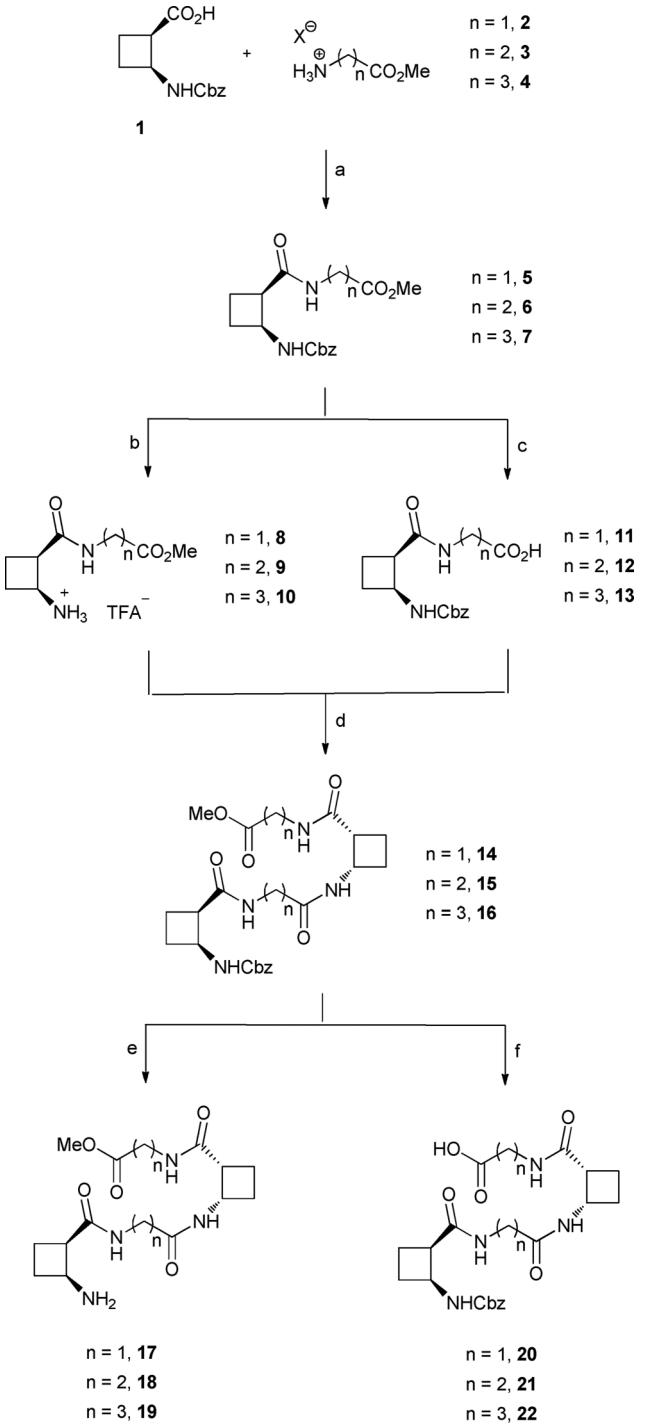


Fig. 1 14-Helical folding with a β,β-tetrapeptide consisting of two cyclobutane and two β-alanine residues joined in alternation in contrast with the extended ribbon-type conformation adopted by another β,β-tetrapeptide built with cyclobutane residues. The arrow and dashed lines, respectively, indicate the intramolecular hydrogen bonds responsible for these secondary structures.

illustrated in Scheme 1. Previously described *N*-Cbz-(1*R*,2*S*)-2-aminocyclobutane-1-carboxylic acid, **1**,[13] was reacted with *O*-Me-protected glycine, β-alanine, and GABA ammonium salts, **2–4**, respectively, in the presence of DIPEA and using PyBOP as coupling agent. In this way, hybrid dipeptides **5**,[16] **6**,[13] and **7**[16] were obtained in 80–90% yield under milder conditions and with better yield than those previously described. The new ammonium triflates **8–10** were quantitatively prepared by submitting **5–7** to hydrogenation of *N*-Cbz protection under 6–7 atmospheres pressure by using 10% $Pd(OH)_2$ as a catalyst and in the presence of TFA, at room temperature. Protonation of the amino function prevented the cyclobutane ring-opening which was previously observed in similar compounds.[17,18] Following an alternative route, mild saponification of the ester function in **5–7** afforded free acids **11–13** in nearly quantitative yields. Subsequent coupling of the amines with the corresponding carboxylic acids afforded hybrid tetrapeptides **14–16**, respectively, in 55–60% yield. These compounds were selectively deprotected to provide amines **17–19** and carboxylic acids **20–22**, respectively, which were used in the synthesis of hexamers **23–24** and octamers **25–27** (Chart 1) by iterative segment condensation following similar procedures than those described above for tetrapeptides **14–16**.



Scheme 1 *Reagents and conditions*: (a) DIPEA, PyBOP, CH_2Cl_2, rt, 1.5 h (80–90%); (b) H_2 (6–7 atm), 10% $Pd(OH)_2/C$, TFA, rt (quantitative); (c) 0.25 M NaOH, 1 : 1 THF-H_2O, 0 °C, 3 h (93–96%); (d) DIPEA, FDPP, 20 : 1 CH_2Cl_2-DMF, rt, overnight (55–60%); (e) H_2 (7–8 atm), 10% $Pd(OH)_2/C$, MeOH, rt (quantitative); (f) 0.25 M NaOH, 1 : 1 THF-H_2O, 0 °C to rt, (80–94%).

During our structural studies (*vide infra*), in order to establish the participation of the terminal carbonyl oxygen in the secondary structure observed for **16**, it seemed convenient to prepare compound **29** (Scheme 2). Therefore, carboxylic acid **1** was condensed with hexylamine giving an amide that, after hydrogenation of

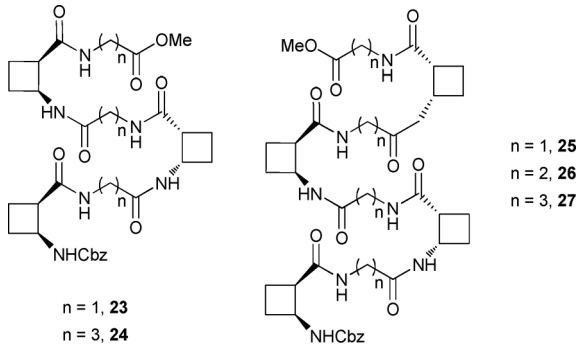
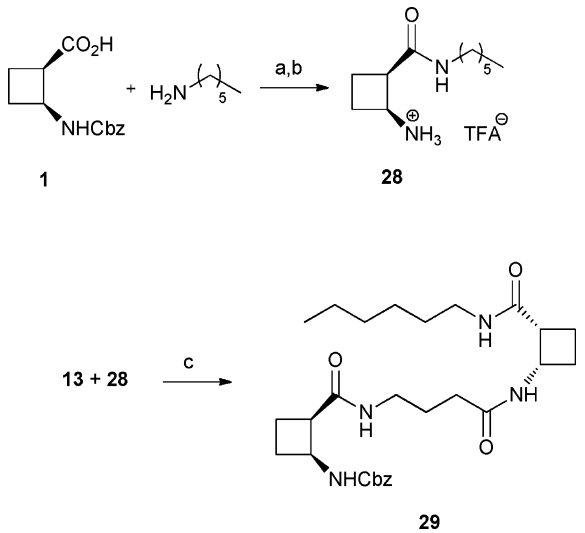
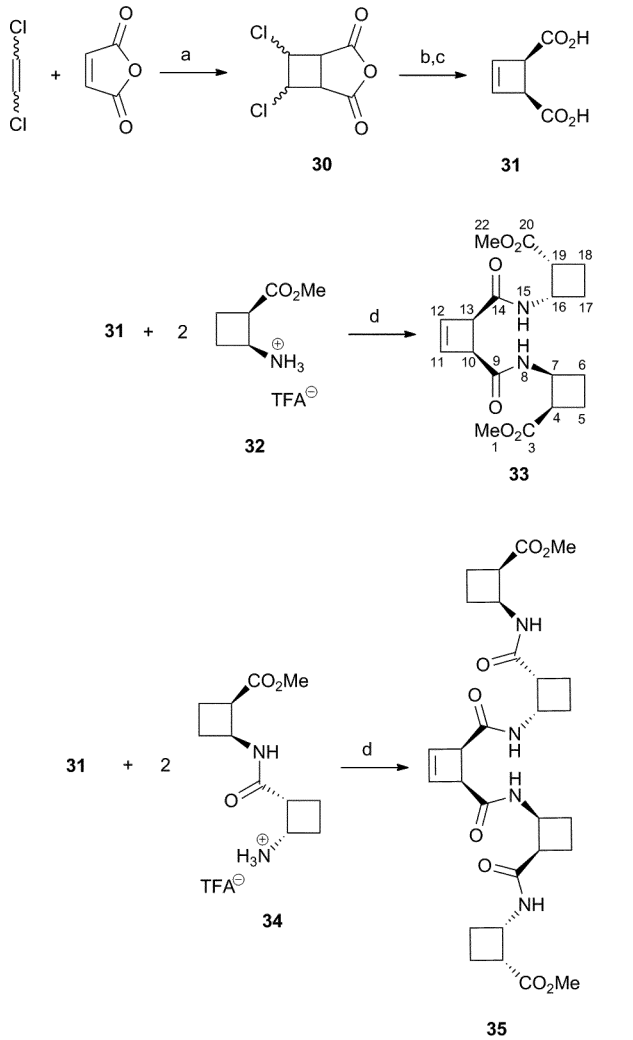


Chart 1 Structures of hexa- and octamers synthesized and studied in this work.



Scheme 2 *Reagents and conditions* (a) PyBOP, DIPEA, CH_2Cl_2, rt, 1.5 h (78%); (b) H_2, $Pd(OH)_2/C$, TFA (quantitative); (c) HATU, DIPEA, CH_2Cl_2-DMF, rt, overnight (40%).



Scheme 3 *Reagents and conditions*: (a) hν (quartz), CH_3CN, –35 °C, 4 h (quantitative); (b) Ac_2O, Zn, toluene, 85 °C, 24 h (50%); (c) 2 : 1 acetone-water, rt, overnight (quantitative); (d) DIPEA, FDPP, DMF, rt, overnight (20%).

benzyl carbamate, afforded compound **28**. This product was coupled with acid **13** in the presence of HATU to provide **29** in 40% yield.

With the aim to verify if the incorporation of a cyclic spacer could influence the formation of six-membered hydrogen-bonds inducing extended arrangements in cyclobutane β-homopeptides as shown in Fig. 1, related compounds **33** and **35** were synthesized (Scheme 3). Adduct **30** was prepared by photochemical cycloaddition of 1,2-dichloroethylene (isomeric mixture) to maleic anhydride. Zn reduction followed by hydrolysis afforded cyclobutene-1,2-dicarboxylic acid, **31**. Double condensation of this compound with 2 mol of amino acid **32**[13] in the presence of DIPEA and FDPP in DMF afforded **33** in 20% yield. The low yield is mainly due to the easy electrocyclic ring-opening of the cyclobutene moiety. Similarly, coupling of **31** with cyclobutane β-dipeptide **34**[17] (2 mol) provided **35**.

All new compounds were fully characterized by their physical constants and spectroscopic data (see the Experimental section and the ESI‡).

2. Structural studies

CD spectra were recorded for 0.5 mM methanol solutions of all hybrid peptides which exhibited negative peaks. Peaks at 223 nm were found for tetramer **15** and octamer **26** in the β-alanine series. This excellent concordance allowed us to assume a helical conformational preference for octamer **26** in view of previous results. For tetramer **15**, a 14-helical folding stabilized by hydrogen bonding interaction between NH(*1*) and CO(*3*) had been described, on the basis of NMR experiments and theoretical calculations.[13]

An accurate coincidence in the CD signatures was also observed for tetra-, hexa- and octamers in glycine and GABA series, respectively, showing that both linear residues induce a similar preferred conformation in solution (Fig. 2). According to the literature, the presence in the spectrum of negative peaks at 217–219 nm would suggest a β-sheet-like structure.[19] This motif is also in accordance with the NMR structures obtained (*vide infra*).

$CDCl_3$ is a solvent that has been successfully used in the NMR conformational analysis of related cyclobutane peptides.[13,14,15,17,20]

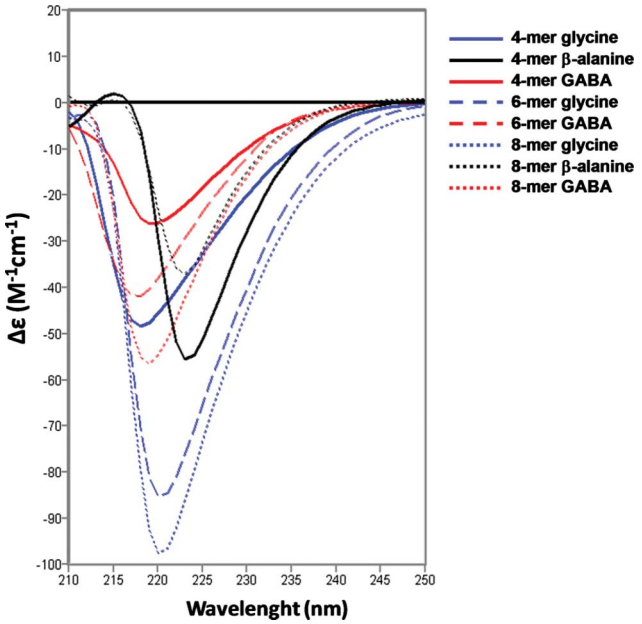


Fig. 2 CD spectra of 0.5 mM methanol solutions of hybrids of β-cyclobutane amino acids and glycine, β-alanine and GABA, respectively.

Nevertheless, hexamers **23** and **24** as well as octamers **25–27** (Chart 1) were insoluble in $CDCl_3$ and their ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 solutions only for characterization purposes (see ESI‡). Thus, a detailed structural study was carried out on α,β- and β,γ-tetrapeptides **14** and **16**, respectively. β,β-Tetrapeptide **15** had been previously studied.[13] Conclusions were extended to the corresponding hexa- and octapeptides in each series according to CD results.

NMR spectra of **14** and **16** allowed us to assign all protons and carbon atoms (see the ESI‡). Specifically, 2D ^1H-^1H ROESY experiments permitted intra- and inter-residue ROE contacts to be established. Some of these contacts and H–H coupling constants led us to secure a *trans* stereochemistry for all amide bonds in the major conformer for each peptide. In the N*H* region of the ^1H NMR spectrum, additional signals were observed, which could be attributed to minor conformers. Selective TOCSY experiments permitted the specific signals for each residue to be seen, while temperature coefficients, self-diffusion and MeOD exchange experiments gave an idea of the fixation and, therefore, of the involvement of each N*H* proton in hydrogen bonds. For **14**, results strongly supported the formation of an anti-parallel β-sheet-type folding, facing each other the two glycine residues (see ESI‡). Tetrapeptide **16** was less rigid because of the higher flexibility of the GABA linear segments. To verify if the terminal CO_{31} participated in the hydrogen bonding involved in a β-sheet formation (NH_{16}-OC_{31}) or, on the contrary, the terminal GABA segment was conformationally random we synthesized and studied pseudopeptide **29** (Scheme 2).

When hexyl group was introduced instead of the GABA residue, all protons became less unshielded, indicating a loss of secondary structure (rigidity). However, not all N*H* protons moved in the same proportion (Fig. 3). That is indicative of loss of ordered structure in only a part of the molecule. Concretely, NH_{27} proton was the most shifted one, so it has mostly lost its hydrogen bond, presumably formed with the chemically removed CO_{31}. Surprisingly, NH_{16} proton did not shift too much compared to NH_{27}. Assuming that a β-sheet type structure was present, we were expecting so because the interaction NH_{16}-OC_{31} is removed by introducing the hexyl group. Neither NH_{21} nor NH_{10} protons shifted too much (compared with NH_{27}), indicating they preserve most of the hydrogen bond character they had prior to the introduction of the hexyl moiety and, therefore, preserving the 6-membered rings. Nevertheless, these experimental results were explained by computational calculations (*vide infra*), which showed that the most stable conformation presents a stable intra-residue hydrogen bond between NH_{27}-OC_{31}.

To assess the feasibility of these β-sheet arrangements, data from NMR experiments were used in computational calculations to establish the structure of the predominant conformers for **14** and **16**. Thus, ROE values were used to define distances and *J* coupling values were used to extract dihedral angles applying a Karplus-type equation.[21] A MMFF conformational search was done with the NMR restrictions and including the effect of chloroform as solvent (see computational details in the Experimental section). The resultant structures selected were grouped into families and the most stable structure (lowest energy) in each family was optimized at the B3LYP[22]/6-31G(d) level of calculation. The energies of the computed structures were examined in terms of ΔG and, finally, the most stable structure was selected (see ESI for details‡).

In the case of α,β-peptides **14**, **23** and **25**, the ability of glycine to fold into β-sheets competed with the propensity of poly(*cis*-cyclobutane) β-peptides to form intra-residue six-membered hydrogen-bonded rings. Thus, from computational calculations for tetrapeptide **14**, four structures emerged within $\Delta G°$ values differing in less 2.5 kcal mol^{-1} (Fig. 4).

The most stable calculated conformer is consistent with the presence of hydrogen bonds between NH_{16}–OC_{27}, NH_{25}–OC_{18} and a bifurcated one between CO_{15} and NH_{10}/NH_{19}. A capped-sticks representation of this structure is provided in Fig. 5a. It fully agrees with the NMR data and represents an anti-parallel β-sheet-type folding, the two glycine residues facing each other and having the cyclobutane residue as a loop at the junction. However, β-sheet is not perfectly extended due to a tension induced by the formation of the NH_{18}–OC_{15} inter-residue hydrogen bond, which affords an extra fold to the peptide

For β,γ-tetrapeptide **16**, the most stable calculated conformer presents hydrogen bonds between NH_{10}–OC_{15}, NH_{27}–OC_{31} and a bifurcated one between CO_{26} and NH_{16}/NH_{21} (Fig. 5b). Thus, the β-sheet arrangement is disrupted by the formation of the intra-residue NH_{27}–OC_{31} bond. This interaction is in accordance with NMR observations when comparing **16** with **29** (Fig. 3). Nevertheless, the expected inter-residue NH_{16}-OC_{31} is found in the second most stable structure with relative $\Delta G°$ 1.9 kcal mol^{-1} higher (Fig. 6).

The intra-residue NH_{10}–OC_{15} bond in **14** and **16** and NH_{21}–OC_{26} bonding in **16** resemble those observed in the cyclobutane monomer and in the poly(*cis*-cyclobutane) β-peptides,[15] and, probably, the resultant NMR structure displays an average between this bonding and the inter–residue interactions with the linear segments.

To realize if this motif is perturbed by the intercalation of cyclic moieties, the prevalent conformations of pseudopeptides **33** and **35** (Scheme 3) were investigated.

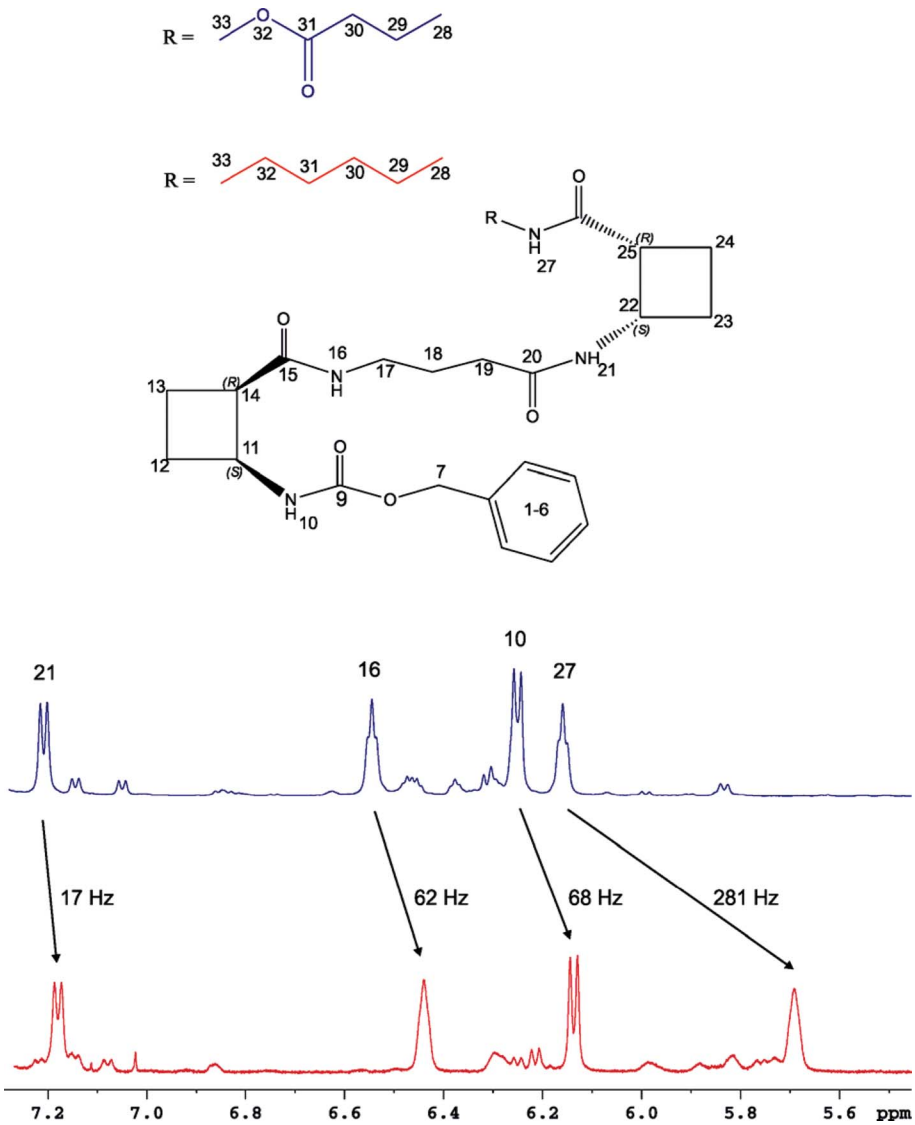


Fig. 3 Comparison of the N*H* region in the 600 MHz ^{1}H NMR spectra in $CDCl_3$ of compounds **16** (blue) and **29** (red).

For **33**, 1D selective TOCSY experiments show the same pattern for both cyclobutane residues (Fig. 7). This fact and NOE contacts suggested the formation of two similar six-membered hydrogen-bonded rings as in the isolated monomers.

We then investigated the possible hydrogen bonding between N*H* protons and the extra carbonyl oxygen in the cyclobutene moiety, which would afford seven-membered rings. The estimated distance extracted from NOE contacts for H_8–H_{11} and H_{12}–H_{15} is 1.67 Å (see Scheme 3 for atom numeration), which fits nicely to the value 1.65 Å calculated by assuming the formation of two six-membered rings. On the contrary, calculations considering one six- and one seven-membered ring afforded a distance of 1.25 Å in clear discrepancy with the experimental value (see ESI for details‡).

For pseudotetrapeptide **35**, 1D selective TOCSY experiments showed two different pairs of NH-cyclobutane residues. In each pair, the two involved moieties were not completely distinguishable due to the presence of signal overlapping in most of the cyclobutane signals. This result jointly with the strong inter-residue NOE contacts observed between Hα(*i*) and NH(*i*+1)/(*i*−1) protons suggest the secondary structure depicted in Fig. 8, which is very similar to that of the β-tetrapeptide exclusively made with cyclobutane residues shown in Fig. 1.

Conclusions

We have synthesized and studied new hybrid α,β- β,β- and β,γ-peptides consisting of chiral cyclobutane cyclic residues and glycine, β-alanine, and GABA, respectively, joined alternately. The intercalation of C_2–C_4 linear residues with (*R*,*S*)-2-aminocyclobutane-1-carboxylic acid in hybrid peptides modifies the secondary structure observed in this cyclic monomer and in homo poly(*cis*-cyclobutane) β-peptides. This involves the formation of intra-residue six-membered hydrogen bonds resulting in an extended ribbon-type conformation. This strong bonding is only locally disrupted by the incorporation of cyclic spacers, like in pseudopeptides **33** and **35**. On the contrary, the hybrid foldamers with linear segments allow conformational-bias tuning to β-sheet-like or helix-type motifs depending on the length of the spacer

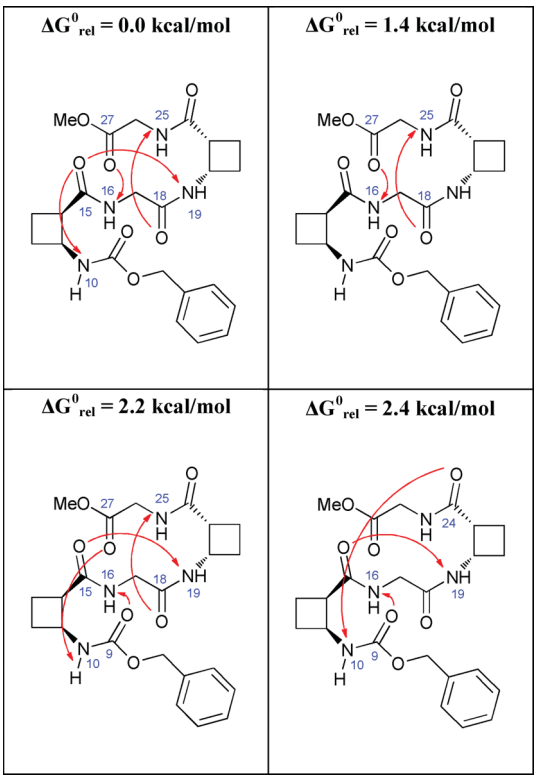


Fig. 4 Structures and relative ΔG° values for the most stable conformers of α,β-tetrapeptide **14**.

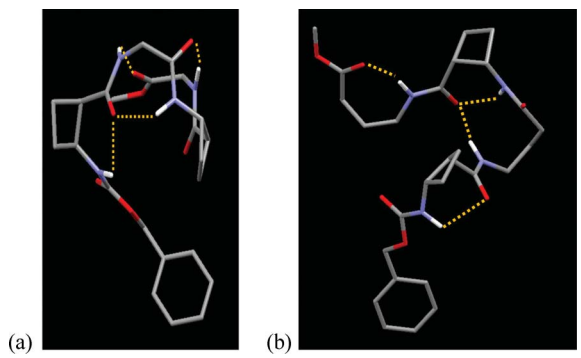


Fig. 5 Capped sticks representation of the calculated preferred conformation for (a) α,β-tetrapeptide **14** and (b) β,γ-tetrapeptide **16**. The hydrogens are omitted for clarity except for amide and carbamate protons.

between the cyclic residues. The new conformations result from the production of inter-residue N*H*–*O*C interactions, which often are in rapid equilibrium with the intra-residue ones affording the so-called bifurcated hydrogen bonds. So, the propensity to helical folding previously reported in the literature[6] for other hybrid α,β-peptides switches to the formation of β-sheet-like structures due to the local flatness imposed by the *cis*-cyclobutane residues. In fact, neighbouring side-chain juxtapositions in the 14-helix are similar to those encountered in a β-sheet.

CD and NMR spectroscopy suggested structures for β,γ-peptides similar to those of α,β-peptides. For GABA derivatives, NMR technique evidences the concurring presence of other related conformers, which can be explained by the higher flexibility of the C_4-fragment.

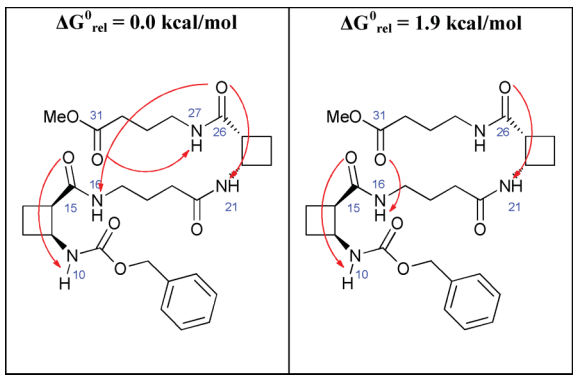


Fig. 6 Structures and relative ΔG° values for the most stable conformers of β,γ-tetrapeptide **16**.

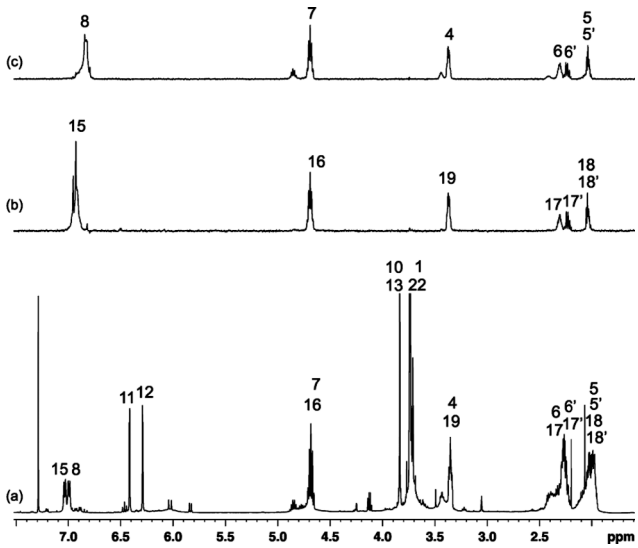


Fig. 7 1D selective TOCSY NMR experiments used for product characterization of pseudodipeptide **33**. TOCSY mixing time was set to 60 ms in all the experiments. Experiments were performed at 298 K in $CDCl_3$ (600 MHz). (a) ^{1}H-NMR for visual comparison purposes. (b) NH_{15} selective TOCSY. (c) NH_8 selective TOCSY.

The case of β-alanine seems to be special and the C_3-alternate fragment affords appropriate scenario for a genuine helical folding.

These results are relevant in the design of foldamers with well-defined and predictable conformations which currently are the object of active investigation in view of some possible applications in different fields.

Experimental

Computational details

Conformational search on peptides **14** and **16** was carried out with the restrictions deduced from NMR parameters. Thus, ROE values were used to define three groups of distances: 3, 4 and 5 Å, respectively, for strong, medium and weak signals. A margin of ±0.5 Å was allowed for all distance restrictions. *J* coupling values were used to extract dihedral angles applying Poulsen's equation.[21] A margin of ±20° was allowed for all of them. A mixed Monte-Carlo[23]/Low-Mode[24] Conformational Search was done using the MMFF (Merck Molecular Force Field)[25] force field implemented

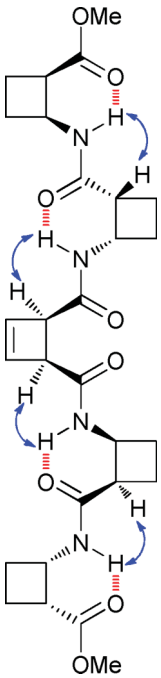


Fig. 8 NMR-suggested secondary structure for pseudotetrapeptide **35**. Red lines show hydrogen bonds and blue arrows indicate significant NOE contacts.

in the Macromodel 9.8 program.[26] The solvent effect was included using the GB/SA[27] method implemented in Macromodel with chloroform as solvent. For each of the starting geometries, if the number of structures computed within a 1 kcal mol^{-1} range was too big, a second conformational search was carried out limiting the margin of the angles to ±10°. If the number of structures obtained within 1 kcal mol^{-1} was too small, the conformers within 2 kcal mol^{-1} were also considered. After the conformational search was carried out, the selected structures within the defined energy range were grouped into different families if necessary, with the criteria of similar NH–CO interactions. The most stable structure (lowest energy) of each of these families was optimized at B3LYP[22]/6-31G(d) level of theory in gas phase with Gaussian09 package.[28] To ensure that the calculated structure was a minimum, a frequency calculation was also run. The energies of all the computed structures were compared in terms of ΔG to find the most stable conformer for each peptide (see ESI for results‡).

Synthetic procedures

The synthetic procedures used to prepare the different peptides are illustrated by the synthesis of tetramer **14**. Complete synthetic description for each product and full characterization of all new compounds is provided in ESI‡.

Dipeptide 5. DIPEA (3.6 mL, 21 mmol) and PyBOP (2.95 g, 5.7 mmol) were added to a solution of acid **1**[13] (1.28 g, 5.14 mmol) in anhydrous dichloromethane (20 mL). After five minutes stirring, methyl glycine hydrochloride, **2**, (0.71 g, 5.7 mmol) was added and the mixture was stirred at room temperature for 1.5 h. The solvent was removed *in vacuo* and the resulting solid purified by silica gel chromatography using hexane-ethyl acetate (1 : 5) as eluent to afford **5** (1.28 g, 78%) as a white solid. δ_H (250 MHz, $CDCl_3$): 1.94 (m, 1H), 2.08 (m, 1H), 2.19–2.41 (complex signal, 2H), 3.28 (m, 1H), 3.73 (s, 3H), 3.84–4.05 (complex signal, 2H), 4.51 (quint, J = 8.5 Hz, 1H), 5.07 (broad s, 2H), 5.78 (broad s, 1H), 6.00 (broad s, 1H), 7.27–7.38 (broad s, 5H). Spectroscopic data are consistent with those reported in the literature.[16]

Ammonium triflate 8. TFA (0.07 mL, 0.93 mmol) was added to a solution of dipeptide **5** (230 mg, 0.72 mmol) in EtOAc (20 mL). The mixture was hydrogenated over 10% $Pd(OH)_2/C$ (84 mg) at room temperature at 6–7 atm for 3 h. The catalyst was removed by filtration through Celite® and washed successively with ethyl acetate and methanol. The filtrate was evaporated *in vacuo* to provide **8** (216 mg, quantitative yield) as a yellow oil. This compound was used in next step without further purification. δ_H (250 MHz, MeOH-d_4) 2.15–2.48 (complex signal, 4H), 3.43 (m, 1H), 3.71 (s, 3H), 3.96 (broad s, 2H), 4.00 (m, 1H); δ_C (62.5 MHz, MeOH-d_4) 22.5, 27.5, 42.5, 48.2, 53.6, 173.0, 176.0.

Acid 11. To an ice-cooled solution of dipeptide **5** (120 mg, 0.37 mmol) in a 1 : 2 mixture of THF– water (21 mL), a 0.25 M NaOH (3.5 mL) was added. The mixture was stirred at 0 °C for 3 h. The mixture was washed with CH_2Cl_2 (1 × 20 mL) before being acidified to pH 2 with 2 M HCl. The aqueous layer was extracted with EtOAc (3 × 20 mL) and the organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under reduced pressure to afford the corresponding carboxylic acid **11** (110 mg, 96%) as a white solid. This compound was used directly in next step without further purification. Crystals, mp 124–128 °C (from EtOAc). $[\alpha]_D$ −44 (c 0.40, EtOAc). IR (ATR): ν 3316, 2951, 1695, 1650, 1537 cm^{-1}. δ_H (250 MHz, $CDCl_3$) 1.90 (m, 1H), 2.10 (m, 1H), 2.15–2.35 (complex signal, 2H), 3.32 (m, 1H), 3.61–4.30 (complex signal, 2H), 4.49 (quint, J = 8.5 Hz, 1H), 5.02 (d, J = 12.2 Hz, 1H), 5.08 (d, J = 12.2 Hz, 1H), 5.89 (d, J = 8.5 Hz, 1H), 6.35 (broad s, 1H), 7.27–7.41 (broad s, 5H); δ_C (62.5 MHz, $CDCl_3$) 18.2, 29.1, 41.4, 46.3, 46.7, 66.9, 128.2, 128.3, 128.6, 136.6, 157.9, 171.4, 172.4; m/z (ESI): Found, 329.1115 [M + Na]$^+$. Calcd. for $C_{15}H_{18}N_2O_5Na$: 329.1108.

α,β-Tetrapeptide 14. DIPEA (0.4 mL, 2.3 mmol) and FDPP (0.21 g, 0.55 mmol) were added to a solution of acid **11** (150 mg, 0.49 mmol) in a 20 : 1 mixture of anhydrous CH_2Cl_2–DMF (21 mL). After five minutes stirring dipeptide ammonium salt **8** (147 mg, 0.49 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and DMF lyophilized. The crude was dissolved in EtOAc (20 mL) and the resulting solution was washed once with saturated aqueous $NaHCO_3$ (15 mL) and H_2O (15 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and the solvent removed under vacuum. The resulting residue was then purified by Et_2O washes, stirring and disaggregating the solid to provide **14** (120 mg, 52%) as a white solid. Crystals, mp 175–179 °C (from Et_2O). $[\alpha]_D$ −124 (c 0.29, CH_2Cl_2). ν_{max} (ATR)/cm^{-1} 3307, 3067, 2950, 1703, 1650, 1536; δ_H (600 MHz, $CDCl_3$) 1.83–1.93 (complex signal, 2H), 2.03 (m, 1H), 2.09 (m, 1H), 2.12–2.20 (complex signal, 3H), 2.31 (m, 1H), 3.19 (m, 1H), 3.28 (m, 1H), 3.67 (dd, J = 17 Hz, J = 4.8 Hz, 1H), 3.74 (s, 3H), 3.76 (dd, J = 18 Hz, J = 5.0 Hz, 1H), 4.01 (dd, J = 17 Hz, J = 6.0 Hz, 1H), 4.20 (dd, J = 18 Hz, J = 6.2 Hz, 1H), 4.49 (quint, J = 8.5 Hz, 1H), 4.67 (quint, J = 8.5 Hz, 1H), 5.01 (d, J = 12 Hz, 1H), 5.06 (d, J = 12 Hz, 1H), 6.28–6.35 (complex signal, 2H), 6.50 (broad s, 1H),

7.15 (d, J = 8.5 Hz, 1H), 7.28–7.39 (complex signal, 5H); δ_C (150 MHz, $CDCl_3$) 18.1, 18.4, 28.9, 29.6, 41.4, 43.1, 44.9, 45.6, 46.0, 46.7, 52.7, 66.7, 128.1, 128.2, 128.6, 136.5, 156.0, 169.3, 171.4, 173.2, 173.5; m/z (ESI): Found, 497.2007 $[M + Na]^+$. Calcd. for $C_{23}H_{30}N_4O_7Na$: 497.2007.

Acknowledgements

The authors thank financial support from Spanish Ministerio de Ciencia e Innovación (grant CTQ2010-15408/BQU) and Generalitat de Catalunya (grant 2009SGR-733). They are also grateful to European Union for COST Action CM0803. Time allocated in the Servei de Ressonància Magnètica Nuclear (UAB) is gratefully acknowledged.

References

1 For a definition of secondary and tertiary structures in peptides, see, for instance: S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173. For recent reports on helix bundle quaternary structures of β- and α-peptides, respectively, see: D. S. Daniels, E. J. Petersson, J. X. Qiu and A. Schepartz, *J. Am. Chem. Soc.*, 2007, **129**, 1532; W. S. Horne, J. L. Price, J. L. Keck and S. H. Gellman, *J. Am. Chem. Soc.*, 2007, **129**, 4178; S. H. Gellman, Abstracts of Papers, 237th ACS National Meeting, Salt Lake City, UT, United States, 2009.

2 See, for example: D. Seebach, A. K. Beck and D. J. Bierbaum, *Chem. Biodiversity*, 2004, **1**, 1111; M. A. Gellman and S. H. Gellman, in *Enantioselective Synthesis of* β*-Amino Acids, Second Edition*, ed. E. Juaristi and V. A. Soloshonok, John Wiley and Sons, New Jersey, 2005, pp 585–725; D. Seebach, D. F. Hook and A. Glattli, *Biopolymers*, 2006, **84**, 23; P. Le Grel and G. Guichard, in *Foldamers: Structure, Properties and Applications*, ed. S. Hecht and I. Huc, Wiley-VCH, 2007, pp 35–74; D. Seebach and J. Gardiner, *Acc. Chem. Res.*, 2008, **41**, 1366; W. S. Horne and S. H. Gellman, *Acc. Chem. Res.*, 2008, **41**, 1399; I. M. Mándity, E. Wéber, T. Martinek, G. Olajos, G. K. Tóth, E. Vass and F. Fülöp, *Angew. Chem., Int. Ed.*, 2009, **48**, 2171.

3 For some leading references, see: D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 13071; D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 6206; D. H. Appella, L. A. Christianson, D. A. Klein, M. R. Richards, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 7574; J. J. Barchi Jr., X. Huang, D. H. Appella, L. A. Christianson, S. L. Durrell and S. H. Gellman, *J. Am. Chem. Soc.*, 2000, **122**, 2711; T. D. W. Claridge, J. M. Goodman, A. Moreno, D. Angus, S. F. Barker, C. Taillefumier, M. P. Watterson and G. W. Fleet, *Tetrahedron Lett.*, 2001, **42**, 4251; T. A. Martinek, G. Tóth, E. Vass, M. Hollósi and F. Fülöp, *Angew. Chem., Int. Ed.*, 2002, **41**, 1718; R. J. Doerksen, B. Chen, J. Yuan, J. D. Winkler and M. L. Klein, *Chem. Commun.*, 2003, 2534; M. A. Schmitt, S. H. Choi, I. A. Guzei and S. H. Gellman, *J. Am. Chem. Soc.*, 2006, **128**, 4538; F. Fülöp, T. A. Martinek and G. K. Tóth, *Chem. Soc. Rev.*, 2006, **35**, 323; A. Hetényi, I. M. Mándity, T. A. Martinek, G. K. Tóth and F. Fülöp, *J. Am. Chem. Soc.*, 2005, **127**, 547; T. A. Martinek, I. M. Mándity, L. Fülöp, G. K. Tóth, E. Vass, M. Hollósi, E. Forró and F. Fülöp, *J. Am. Chem. Soc.*, 2006, **128**, 13539; T. A. Martinek, A. Hetényi, L. Fülöp, I. M. Mándity, G. K. Tóth, I. Dékány and F. Fülöp, *Angew. Chem., Int. Ed.*, 2006, **45**, 2396; C. Fernandes, S. Faure, E. Pereira, V. Théry, V. Declerck, R. Guillot and D. J. Aitken, *Org. Lett.*, 2010, **12**, 3606; R. Gutiérrez-Abad, D. Carbajo, P. Nolis, C. Acosta-Silva, J. A. Cobos, O. Illa, M. Royo and R. M. Ortuño, *Amino Acids*, 2011, **41**, 673; E. Gorrea, E. Torres, P. Nolis, E. Da Silva, D. B. Amabilino, V. Branchadell and R. M. Ortuño, *Chem.–Eur. J.*, 2011, **17**, 4588.

4 K. Möhle, R. Günther, M. Thormann, N. Sewald and H.-J. Hofmann, *Biopolymers*, 1999, **50**, 167; R. J. Doerksen, B. Chen, J. Yuan, J. D. Winkler and M. L. Klein, *Chem. Commun.*, 2008, 2534; M. U. Kiran, A. Sudhakar, J. Klages, G. Kummerlöwe, B. Luy and B. Jagadeesh, *J. Am. Chem. Soc.*, 2009, **131**, 15590.

5 For a review, see: W. S. Horne and S. H. Gellman, *Acc. Chem. Res.*, 2008, **41**, 1399.

6 D. Seebach, B. Jaun, R. Sebesta, R. I. Mathad, O. Flögel, M. Limbach, H. Sellner and S. Cottens, *Helv. Chim. Acta*, 2006, **89**, 1801; S. De Pol, C. Zorn, C. D. Klein, O. Zerbe and O. Reiser, *Angew. Chem., Int. Ed.*, 2004, **43**, 511; W. S. Horne, L. M. Johnson, T. Ketas, J. Thomas, P. J. Klasse, M. Lu, J. P. Moore and S. H. Gellman, *J. Am. Chem. Soc.*, 2009, **106**, 14751.

7 L. Guo, A. M. Almeida, W. Zhang, A. G. Reidenbach, S. H. Choi, I. A. Guzei and S. H. Gellman, *J. Am. Chem. Soc.*, 2010, **132**, 7868.

8 T. Sawada and S. H. Gellman, *J. Am. Chem. Soc.*, 2011, **133**, 7336.

9 G. A. Lengyel, R. C. Frank and W. S. Gellman, *J. Am. Chem. Soc.*, 2011, **133**, 4246.

10 S. Segman, M. Lee, V. Vaiser, S. H. Gellman and H. Rapaport, *Angew. Chem. Int. Ed.*, 2010, **49**, 716.

11 W. S. Horne, S. H. Gellman and L. M. Johnson, PCT Int. Appl. (2010) WO 2010045342 A1 20100422.

12 S. Urman, K. Gaus, Y. Yang, U. Strijowski, S. De Pol, O. Reiser and N. Sewald, *Angew. Chem., Int. Ed.*, 2007, **46**, 3976.

13 S. Izquierdo, M. J. Kogan, T. Parella, A. G. Moglioni, V. Branchadell, E. Giralt and R. M. Ortuño, *J. Org. Chem.*, 2004, **69**, 5093.

14 F. Rúa, S. Boussert, T. Parella, I. Díez-Pérez, V. Branchadell, E. Giralt and R. M. Ortuño, *Org. Lett.*, 2007, **9**, 3643.

15 E. Torres, E. Gorrea, K. K. Burusco, E. Da Silva, P. Nolis, F. Rúa, S. Boussert, I. Díez-Pérez, S. Dannenberg, S. Izquierdo, E. Giralt, C. Jaime, V. Branchadell and R. M. Ortuño, *Org. Biomol. Chem.*, 2010, **8**, 564.

16 D. Fernández, E. Torres, F. X. Avilés, R. M. Ortuño and J. Vendrell, *Bioorg. Med. Chem.*, 2009, **17**, 3824.

17 S. Izquierdo, F. Rúa, A. Sbai, T. Parella, Á. Álvarez-Larena, V. Branchadell and R. M. Ortuño, *J. Org. Chem.*, 2005, **70**, 7963.

18 D. J. Aitken, C. Gauzy and E. Pereira, *Tetrahedron Lett.*, 2004, **45**, 2359.

19 M. López de la Paz, E. Lacroix, M. Ramírez-Alvarado and L. Serrano, *J. Mol. Biol.*, 2001, **312**, 229; A. M. Smith, R. J. Williams, C. Tang, P. Coppo, R. F. Collins, M. L. Turner, A. Saiani and R. V. Ulijn, *Adv. Mater.*, 2008, **20**, 37; A. M. Fernández-Escamilla, S. Ventura, L. Serrano and M. A. Jiménez, *Protein Sci.*, 2006, **15**, 2278; L. Chen, K. Morris, A. Laybourn, D. Elias, M. R. Hicks, A. Rodger, L. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 5232.

20 E. Torres, E. Gorrea, E. Da Silva, P. Nolis, V. Branchadell and R. M. Ortuño, *Org. Lett.*, 2009, **11**, 2301.

21 S. Ludvigsen, K. V. Andersen and F. M. Poulsen, *J. Mol. Biol.*, 1991, **217**, 731.

22 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648; C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785.

23 G. Chang, W. C. Guida and W. C. Still, *J. Am. Chem. Soc.*, 1989, **111**, 4379; G. Chang, W. C. Guida and W. C. Still, *J. Am. Chem. Soc*, 1990, **111**, 1419.

24 I. Kolossváry and W. C. Guida, *J. Am. Chem. Soc.*, 1996, **118**, 5011; I. Kolossváry and W. C. Guida, *J. Comput. Chem.*, 1999, **20**, 1671.

25 T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 490.

26 F. Mohamadi, N. G. H. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, D. Qiu, P. S. Shenkin, F. P. Hollinger and W. C. Still, *J. Phys. Chem. A*, 1997, **101**, 3005; *MacroModel 9.8* (2010) Schrodinger, LLC, New York, NY.

27 D. Qiu, P. S. Shenkin, F. P. Hollinger and W. C. Still, *J. Phys. Chem. A*, 1997, **101**, 3005.

28 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, Jr. J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox,(2009) *Gaussian 09*, Revision B.01, Gaussian, Inc., Wallingford CT.

