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ARTICLE

Conformational preferences in the β-peptide oligomers of *cis*-2-amino-1-fluorocyclobutane-1-carboxylic acid

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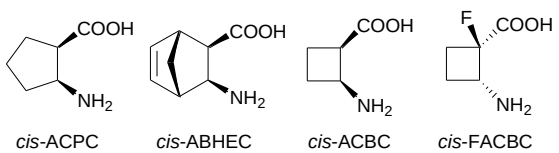
An efficient synthesis of *cis*-2-amino-1-fluorocyclobutane-1-carboxylic acid in single enantiomer form was established and protected homo-oligomers (2-, 4-, and 6-mers) of this cyclic *cis*-β-amino acid were prepared. Conformational analysis of these oligomers using IR, NMR and CD techniques in solution, supported by molecular modelling studies, suggested a strong conformational preference for a well-defined strand-like structure in which intra-residue hydrogen bonding is weak at best and is not consequential for adoption of the secondary structure. Single crystal x-ray analysis of the tetramer showed that the regular strand-like conformation is adopted in the solid state; only intermolecular hydrogen bonding networks are observed. The backbone topology and the 4-membered ring orientations are noticeably different from those of the tetramer of the corresponding non-fluorinated *cis*-β-amino acid.

Introduction

Foldamers are flexible oligomers, obtained from the condensation of a small number of organic building blocks, which adopt well-defined folded shapes thanks to favourable non-covalent interactions.[1] Among the diversity of the available building blocks, homologated amino acids have attracted much interest. Since the first examples of folded β-peptides reported by Gellman[2] and Seebach,[3] advances have been made in the control of secondary structure whereby β-peptides can now be conceived to fold into helices, sheet-forming strands, or turn-like features.[4] Theoretical analyses of β-peptides suggest that a number of periodic structures may be available,[5] and while a number of the predicted helical folding patterns have been demonstrated experimentally, more extended regular structures are less well studied.

In natural peptides, β-strand conformations are of importance since their assembly produces higher-level association of β sheets and has been implicated in formation of the protein aggregates and fibrils observed in diseases such as Alzheimer's disease. Foldamer-based mimetics of these materials are therefore of considerable interest. To date, however, the so-called C6-ribbon strand-like β-peptide secondary structure has only been described for homo-oligomers of three β-amino acids,[6] all of them cyclic (Fig. 1): *cis*-2-aminocyclopentane-1-carboxylic acid (*cis*-ACPC),[7] *exo*-3-aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid (*exo*-ABHEC),[8] and *cis*-2-aminocyclobutane-1-carboxylic acid (*cis*-ACBC).[9] It was proposed that these structures might be stabilised by a series of intra-residue 6-membered ring hydrogen bonds, even though the strength of such interactions is predicted to be low,[5] which suggests that their existence is not a prerequisite to the adoption of such a conformer.[10]

To further explore the propensity of oligomers of β-amino acids to adopt strand-like or related conformations, we decided to examine *cis*-2-amino-1-fluorocyclobutane-1-carboxylic acid (*cis*-FACBC).[11] (Fig. 1). This new building block adds to the growing inventory of *cis*-cyclic β-amino acids,[12] and the folding behaviour of its oligomers is not obvious: on the one hand, it has been shown that the electronic effects of a fluorine atom introduced at specific backbone positions in homologated amino acids may induce dramatic change in the preferred secondary structure of peptides which contain them;[13] on the other hand, oligomers of *cis*-oxetane β-amino acids are reported to fold into a 10-helix rather than a C6-ribbon.[14] In any case, it can be expected that an antiperiplanar dihedral angle F–C–C=O should be observed,[15] and in this study we evaluate the impact of this on any hydrogen-bonding proclivity and global secondary structure preferences in oligomers of *cis*-FACBC.



cis-ACPC *cis*-ABHEC *cis*-ACBC *cis*-FACBC

Fig. 1 Three β-amino acids whose oligomers fold into C6-ribbons, and the β-amino acid (*cis*-FACBC) considered in this work.

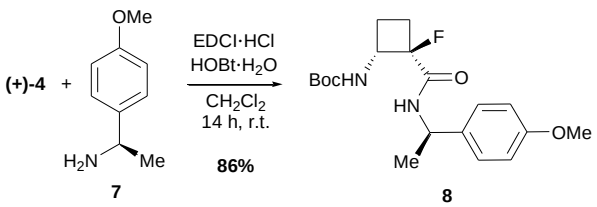
Results and discussion

Synthesis

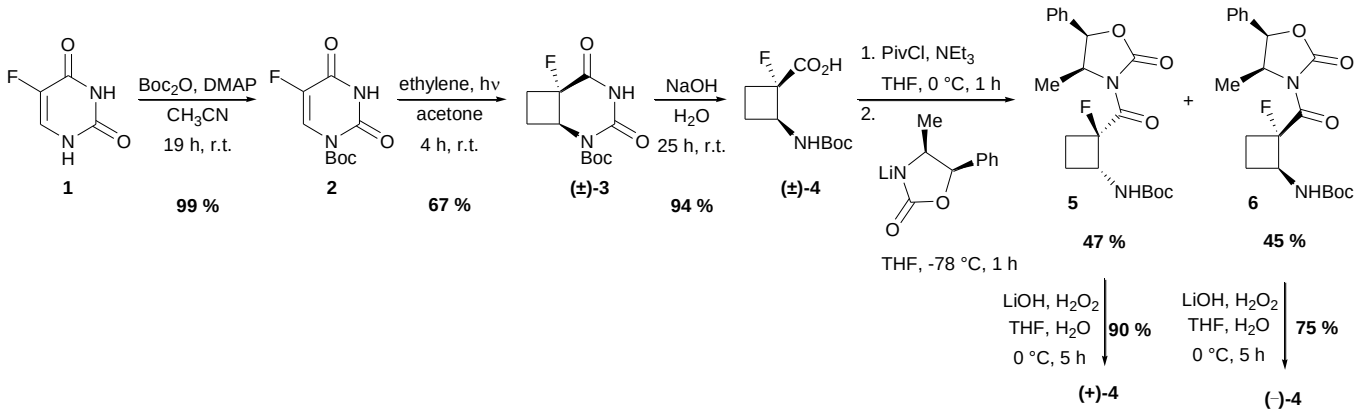
We first developed a robust and efficient synthesis of *cis*-FACBC in enantiomerically pure form (Scheme 1). It is known that the vicinal "donor-acceptor" functional group suite of a 2-aminocyclobutane carboxylate residue can, in certain circumstances, induce an irreversible ring opening reaction.[16] This process is likely to be enhanced by the presence of the electron-withdrawing fluorine atom on the α-carbon of *cis*-FACBC, so we took care to avoid working with the free amino acid, and adapted a synthetic approach recently described by our group for the preparation of the *cis*- and *trans*-ACBC.[17] 5-Fluorouracil **1** was selectively protected by a *tert*-butoxycarbonyl group at the N^1 position to give **2** in near quantitative yield. This compound was engaged in a [2+2] photocycloaddition reaction with ethylene, using a 400 W medium-pressure Hg-vapour lamp in a Pyrex® reaction vessel, to furnish the desired *cis*-cycloaddition adduct (+)-**3** in 67% yield after purification. Treatment with aqueous sodium hydroxide led smoothly to the *N*-protected racemic *cis*-FACBC (+)-**4** in a single step in 94 % yield.

Previously, we had found that chiral derivatization with oxazolidinones was a convenient way to achieve resolution of cyclic homologated amino acids;[17,18] gratifyingly, this also turned out to be the case with Boc-*cis*-FACBC (Scheme 1). Activation of (±)-**4** using pivaloyl chloride followed by the addition of the lithium salt of (4*S*,5*R*)-4-methyl-5-phenyloxazolidin-2-one provided the two diastereoisomers **5** and **6** which were easily separated by chromatography and isolated as pure materials in 47% and 45% yields. Treatment of each compound **5** and **6** with LiOOH in a mixture of water and THF allowed the smooth cleavage of the oxazolidinone, which could be recycled, and furnished enantiomerically pure Boc-*cis*-FACBC **(+)-4** and **(–)-4** in 90% and 75% yields, respectively. Overall, the pair of building blocks **(+)-4** and **(–)-4** were prepared on gram-scale in five steps from commercial 5-fluorouracil **1** in 47 % overall combined yield (26% and 21% respectively).

In order to determine the absolute configurations of **(+)-4** and **(–)-4**, we prepared crystalline amide **8** by condensation of the **(+)-4** with (*R*)-(*p*-methoxyphenyl)ethylamine **7** (Scheme 2). X-ray diffraction analysis of amide **8** revealed the (1*R*,2*R*) absolute configuration for **(+)-4** (Fig. 2). Interestingly, the dihedral angle F–C–C=O in the solid state is –169.8° and the deviation from the anticipated antiperiplanar arrangement actually displaces the carbonyl oxygen away from the amide hydrogen to a N–H···O=C distance of 2.97 Å, suggesting no importance for a 6-membered hydrogen bond for a *cis*-FACBC residue, at least in the solid state. The packing structure of **8** displayed some other interesting features (Fig. 2). An infinite one-dimensional network of hydrogen bonds is apparent, linking the amide NH of one molecule and the amide carbonyl of the next molecule in one direction (N–H···O=C distance = 2.16 Å), and linking the carbamate NH with the fluorine of the next molecule in the opposite direction (N–H···F distance = 2.20 Å). The hydrogen bond network forms a left-handed helix in the tetragonal lattice (space group $P4_3$).



Scheme 2 Preparation of amide derivative **8**.



Scheme 1 Synthesis of both enantiomers of Boc-*cis*-FACBC: **(+)-4** and **(–)-4**.

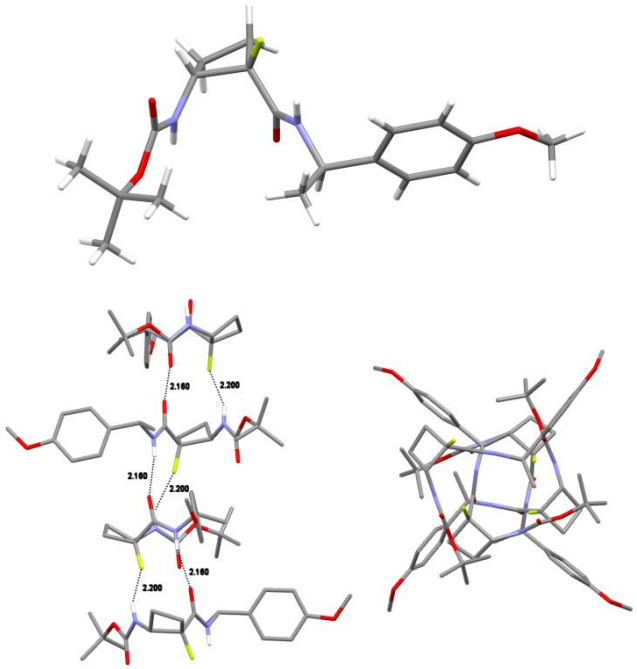
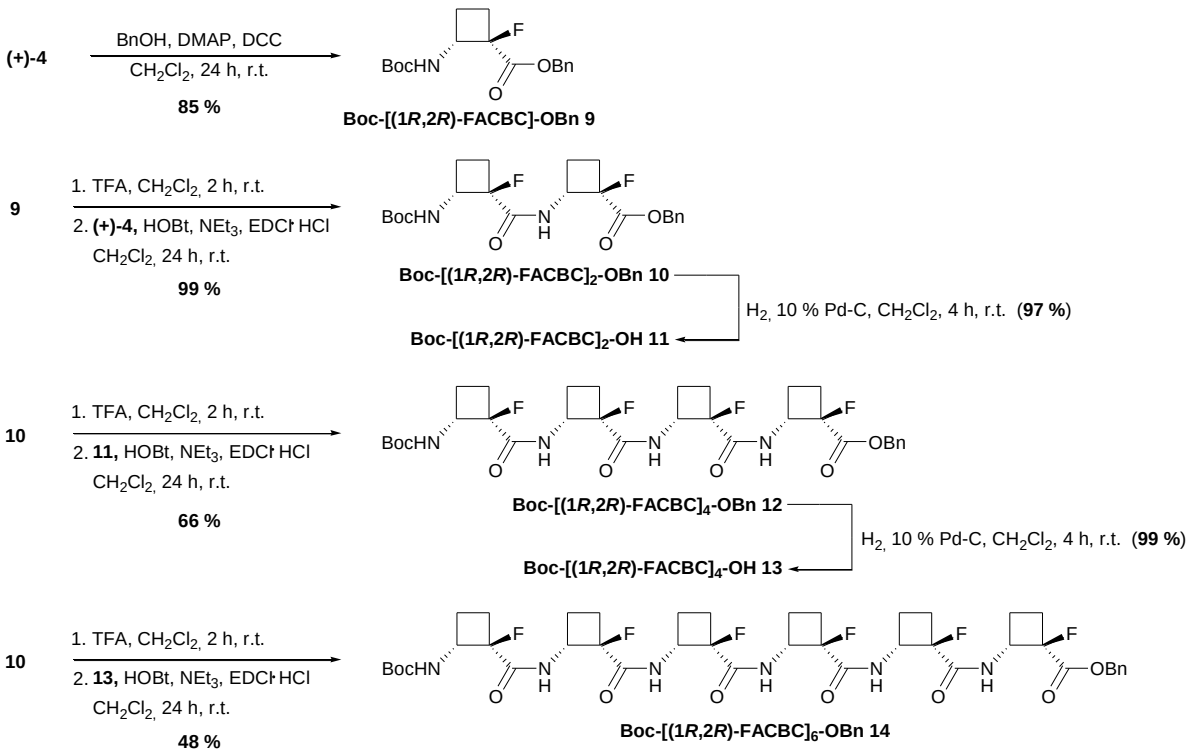


Fig. 2 X-Ray crystal diffraction analysis of amide **8**, showing the molecular structure (above) and the crystal packing features, highlighting the hydrogen bond network (below left) and an axial projection of the left-handed helical arrangement (bottom right). Some hydrogen atoms are removed for simplicity.

The preparation of homo-oligomers was carried out in solution phase, using Boc-(1*R*,2*R*)-*cis*-FACBC **(+)-4** as the building block and EDCI/HOBt as the coupling agents, as outlined in Scheme 3. The benzyl ester **9** was prepared initially by esterification of **(+)-4**. Treatment of **9** with trifluoroacetic acid followed directly by coupling with **(+)-4** allowed formation of the dimer Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OBn **10** in high yield. Hydrogenolysis (Pd-C, H_2) of dimer **10** provided the corresponding acid derivative **11**, which was coupled with the amine obtained by TFA treatment of dimer **10**. The resulting tetramer, Boc-[(1*R*,2*R*)-*cis*-FACBC]$_4$-OBn **12**, was treated to the same series of operations via the tetramer acid **13** to provide the hexamer Boc-[(1*R*,2*R*)-*cis*-FACBC]$_6$-OBn **14**.



Scheme 1 Synthesis of the oligomers Boc-[(1*R*,2*R*)-FACBC]$_n$-OBn: **10**, **12** and **14**.

Conformational Analysis

We began the solution state conformational analysis of oligomers **10**, **12** and **14**, using FTIR spectroscopy. The absorption spectra in the NH stretching frequency domain are shown for 10 mM solutions in chloroform (Fig. 3). The spectra were identical when recorded for 1 mM solutions, ruling out any intermolecular association effects. For each oligomer a single broad NH absorption band was observed in the range 3420-3430 cm^{-1}. Free amide absorption usually occurs above 3400 cm^{-1}, while a shift to lower frequencies (3350-3250 cm^{-1}) is observed when the NH is involved in a hydrogen bond. An initial conclusion would be that oligomers **10**, **12** and **14** show no significant hydrogen bonding features, in contrast to oligomers of *exo*-ABHEC[8] and *cis*-ACBC,[9] whose $CHCl_3$ solution state FTIR spectra display NH absorptions at 3300 cm^{-1} and 3310 cm^{-1} respectively, attributed in these cases to intra-residue 6-membered ring hydrogen-bonds.

However, it was important to rule out any electronic perturbation of the β-fluorine atom on the NH absorption frequency. Despite a wide coverage of the electronic effects of fluorine,[13,15,19] the literature seems bereft of solution state FTIR absorption studies for α-fluoro-β-peptides; in the occasional cases where IR data is provided, it is often reported as "neat" or for solid state samples, which cannot be equated with solution state behaviour.

Precious comparative data were forthcoming from an inspection of the work of Seebach. Recently,[20] data were reported for the three tripeptides **I, II** and **III** (Fig. 4) in $CHCl_3$ solution: they each showed a single NH absorption in the range 3425-3436 cm^{-1}. Previously,[21] the non-fluorinated tripeptides **IV** and **V** had been prepared and characterized with IR absorptions at 3445 and 3412 cm^{-1}, respectively. The tripeptides **I**-**V** were used in the course of Seebach's studies as intermediates for the preparation of larger peptides, and their conformational preferences were not examined as such. Nonetheless, the description of their IR absorption spectra allows us to deduce that, whatever hydrogen bonding is prevalent in this series (in fact, it is predicted to be very low), there is no apparent effect of the β-fluorine atom on the NH absorption frequency. We therefore conclude that the IR data obtained for **10**, **12** and **14** indicate that hydrogen bonding is negligible for these oligomers.

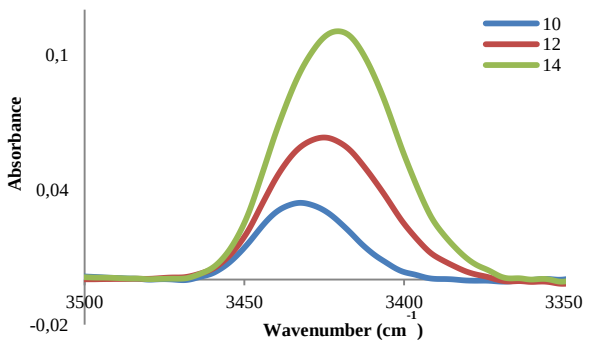
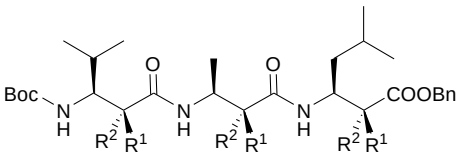


Fig. 3 FTIR absorption spectra in $CHCl_3$ (10 mM) of **10**, **12** and **14**.



I: R^1 = F, R^2 = H **IV**: R^1 = R^2 = H
II: R^1 = H, R^2 = F **V**: R^1 = Me, R^2 = H
III: R^1 = R^2 = F

Fig. 4 α-Fluorinated and non-fluorinated β-tripeptides from the literature.

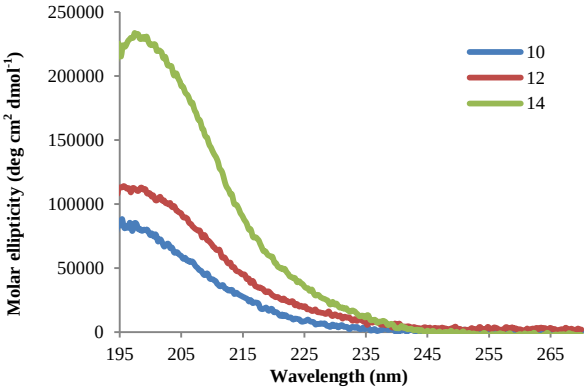


Fig. 5 Circular dichroism spectra in MeOH (0.2 mM) **10**, **12** and **14**.

The CD spectra of **10**, **12** and **14** were recorded as 0.2 mM solutions in methanol (Fig. 5). The three oligomers showed the same profile, with a single maximum band near 200 nm (**10**: 195.4 nm, **12**: 195.6 nm, **14**: 197.4 nm). These curves are similar to those described for the oligomers of *cis*-ACPC,[7] *exo*-ABHEC,[8] and *cis*-ACBC,[9] all of which were attributable to a strand-like conformation.

NMR spectroscopy was used to carry out NH/ND exchange experiments, following the evolution of the ^1H NMR spectra of 15 mM solutions of oligomers **10**, **12** and **14** in methanol-d_4 at 298 K. All amide protons disappeared very rapidly (<2 min) and the carbamate signals became imperceptible within < 90 min. This behaviour suggests that NH atoms are largely solvent accessible, indicating little or no hydrogen bonding within the molecular structures, in agreement with the conclusions from the FTIR experiments. This observation contrasts with the behaviour of *exo*-ABHEC oligomers in the same conditions,[8] where NH/ND exchange was a prolonged process (up to 24 h) attributed to intra-residue N-H···O=C hydrogen bonding in that case.

Further NMR studies were conducted on tetrapeptide **12** as a 15 mM solution in pyridine-d_5. This solvent allowed the best separation of ^1H signals, and standard 1D and 2D NMR experiments were used to assign unambiguously all proton, carbon and fluorine signals. The chemical shifts of **12** remained constant when the sample was diluted to 1 mM, indicating the absence of aggregation effects. On the other hand, the very large temperature coefficients for all NH signals (larger than –

13.8 ppb/K) clearly confirmed the absence of intramolecular hydrogen bonding. Furthermore, a combination of 2D ^{1}H–^{1}H NOESY and ^{1}H–^{19}F HOESY experiments facilitated the attribution process and revealed short intra- and inter-residue nOe connectivities (Fig. 6). The fully periodic pattern of NH_i–$C^{\beta}H_i$, $C^{\beta}H_i$–F_i and NH_i–F_{i-1} nOe interactions precludes both helical and random-coil conformations, and the molecule can thus be assigned a highly ordered strand-like secondary structure.

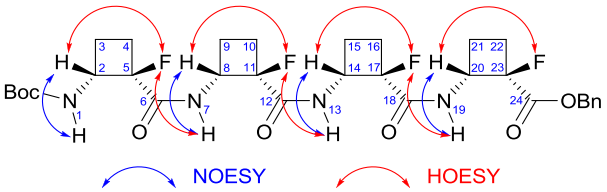


Fig. 6 Atom numbering and diagnostic 2D NOESY and HOESY interactions observed for **12**.

Collectively, these solution state experimental studies suggest that *cis*-FACBC oligomers adopt a preferred strand-like conformation, reminiscent of the C6-ribbon, but in which the influence of the intra-residue hydrogen bond is negligible.

In parallel with these solution state studies, the building block Boc-(1*S*,2*S*)-*cis*-FACBC **(–)-4** was used to construct the tetramer **12'** (enantiomer of **12**). Crystals which were suitable for X-ray diffraction analysis were grown successfully from an ethyl acetate-pentane solution. To the best of our knowledge, solid state data for strand-like β-peptide oligomers is almost unprecedented in the literature.[22] The molecular structure and the intermolecular hydrogen bonding network are shown in Fig 7. The molecule adopts a regular extended strand-like conformation with alternating orientations of the *trans* amide bonds in such a way that all intra-residue N-H···O=C distances are > 3 Å (see Table 1), with no intramolecular hydrogen bonding whatsoever. Instead, the packing is characterized by a parallel sheet-like alignment of molecules leading to an infinite network of intermolecular N-H···O=C hydrogen bonding interactions with an average distance of around 2.35 Å.

To complement this study and to facilitate comparisons between solid state and solution state conformations, a hybrid Monte Carlo Molecular Mechanics (MCMM) conformational search was carried out on tetramer **12'** using SPARTAN'06 software and the MMFF94 force field without restraints. The geometries of five low-energy conformers were optimized by DFT using GAUSSIAN 09 and the B3LYP/6-311G*(d,p) basis set in a chloroform medium. After refinement, the lowest energy conformer clearly showed the regular strand-like conformation (Fig. 8). The general shape of the molecule is similar to that in the solid state, although backbone torsion angles are not identical. In particular, C–N–C^{β}–C^{α} and C^{β}–C^{α}–C–N dihedral angles evolve so that intra-residue N-H···O=C distances are below 2.5 Å, within the recognised limit for hydrogen bonding distances.

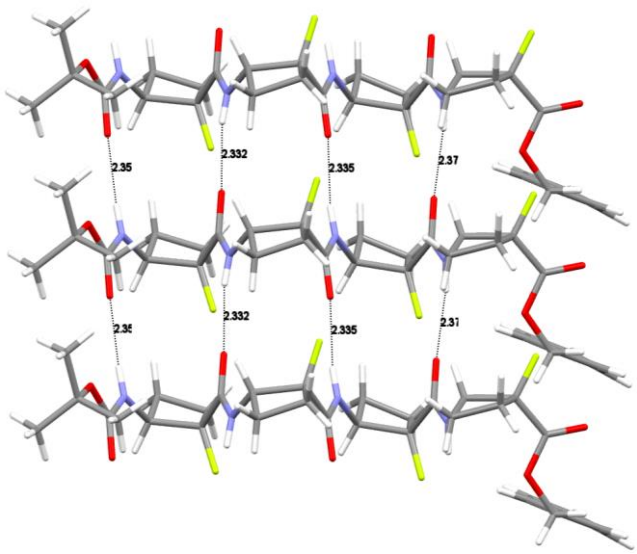


Fig 7 X-ray crystal diffraction analysis of tetramer **12'**.

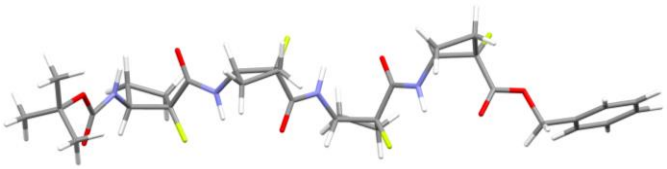


Fig. 8 Calculated lowest energy conformer of tetramer **12'**.

We therefore decided to extend the comparison to include a tetramer of *cis*-ACBC, Cbz-[(1*R*,2*S*)-*cis*-ACBC)]$_4$-OMe (**VI**) for which theoretical calculations have been disclosed.[9] Data are presented in Table 1. For **VI**, the calculated intra-residue N–H···O=C distances are in the range 1.92-2.11 Å with N–H···O angles in the range 131-138°. These values are significantly more propitious for 6-ribbon hydrogen bond formation than those calculated for the *cis*-FACBC tetramer **12'**, for which N–H···O=C distances are in the range 2.38-2.44 Å and N–H···O angles are in the range 113-115°.

	H···O distance (N-H···O bond angle)		
	N_1-H···O=C_6	N_7-H···O=C_{12}	N_{13}-H···O=C_{18}
12' crystal	3.126 Å (-)	3.149 Å (-)	3.069 Å (-)
12' calcd	2.444 Å (113°)	2.375 Å (115°)	2.382 Å (115°)
VI calcd	2.112 Å (131°)	1.918 Å (138°)	2.065 Å (131°)

Table 1 N-H···O=C distances in solid state **12'**, calculated **12'** and calculated **VI**. See Fig. 5 for atom numbering.

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	Dihedral angles X–C^{α}–C=O (deg)			Dihedral angles of C–N–C^{β}–C^{α} (deg)		
	X–C_5–C_6=O	X–C_{11}–C_{12}=O	X–C_{17}–C_{18}=O	C–N_1–C_2–C_5	C_6–N_7–C_8–C_{11}	C_{12}–N_{13}–C_{14}–C_{17}
12' crystal (X=F)	160.5	156.2	164.0	–85.1	–85.8	–82.8
12' calcd (X=F)	–175.1	–171.1	–173.6	–121.4	–127.9	–124.3
VI calcd (X=H)	173.0	–170.5	174.2	–165.2	179.0	–151.8

	Dihedral angles of N–C^{β}–C^{α}–C (deg)			Dihedral angles of C^{β}–C^{α}–C–N (deg)		
	N_1–C_2–C_5–C_6	N_7–C_8–C_{11}–C_{12}	N_{13}–C_{14}–C_{17}–C_{18}	C_2–C_5–C_6–N_7	C_8–C_{11}–C_{12}–N_{13}	C_{14}–C_{17}–C_{18}–N_{19}
12' crystal (X=F)	–12.6	–14.2	–13.8	105.0	98.8	105.9
12' calcd (X=F)	–24.0	–24.7	–23.8	133.9	139.5	135.8
VI calcd (X=H)	17.0	19.9	15.2	120.6	140.4	120.1

Table 2 Dihedral angles observed for solid state **12'**, calculated for **12'** and **IV.** See Fig. 5 for atom numbering.

Comparison of the dihedral angles in the three tetramer structures (**12'** solid, **12'** modelled, **VI** modelled) was also instructive (Table 2). The amide bonds were invariably *trans* in all structures, as expected. However some deviation from the anticipated antiperiplanar disposition of the F–C^{α}–C=O motifs in the **12'** structures were observed, with values in the range 156.2° to 164.0° in the crystal and –171.1° to –175.5° in the calculated structure. Indeed, the corresponding H$^{\alpha}$–C$^{\alpha}$–C=O dihedral angles for the *cis*-ACBC residues in **VI** (170.5° to 174.2°) are just as close to antiperiplanar.

Significant differences were observed between **12'** and **VI** in both the backbone torsion angles and the disposition of the cyclobutane rings, leading to quite different molecular topologies for these strand-like conformers The four-membered cycles all adopt regular out-of-plane puckering: 12.6-14.3° (**12'** crystal), 16.6-17.5° (**12'** calculated), 14.1-16.9° (**VI** calculated). However, the calculated **VI** conformer bears its N atoms in pseudo-axial positions and its C=O groups in pseudo-equatorial positions on any given residue. In contrast, in both the crystal and the calculated structures of **12'** the cyclobutane "butterfly" is inverted, placing the F and N atoms in pseudo-equatorial positions and the C=O groups in axial positions.

Concomitantly, the C–N–C^{β}–C^{α} and N–C^{β}–C^{α}–C dihedral angles diverge considerably between **12'** and **VI**. These differences in the backbone conformations are highlighted in Fig 9. The solid state structure of **12'** shows a highly regular zig-zag (or "step-up-step-down") arrangement of backbone atoms and the NH and C=O functions pointing away orthogonally. In the minimized conformer of **12'**, the zig-zag feature is still appreciable although some flattening has occurred. In the preferred conformer of **VI**, the structure is more extensively flattened, as regards both the backbone atoms and the amide group orientations.

Collectively, these results suggest that short oligomers of *cis*-FACBC prefer to adopt a regular strand-like conformation with alternating dispositions of the constituent β-amino acid residues and alternating amide bond orientations. This conformation resembles the so-called C6-ribbon secondary structure, with the important attribute that intra-residue 6-membered ring hydrogen bonds seem to have very little significance for the stabilisation of this structure. In theoretical studies, it was postulated that (at least for short oligomers) the strand-like conformer should be a stable β-peptide secondary structure resulting from a favourable combination of electrostatic, steric and dipole effects: the contribution of intra-residue hydrogen bonding was predicted to be weak, at best.[5,23] Our interpretation of the present results is in closer agreement with these predictions than previous investigations of oligomers of *cis*-cyclic β-amino acids, in which at least some stabilising effect, and perhaps even a directing effect, were imputed to a 6-membered ring hydrogen bond.

It is interesting to observe that despite the clear differences in the backbone torsion angles and the 3D topologies of **12'** and **VI**, they both prefer to adopt a strand-like secondary structure globally.

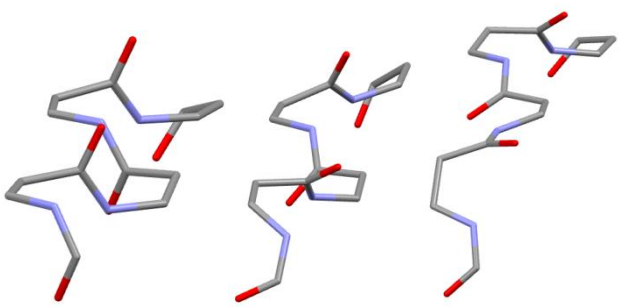

Fig. 9 Backbone conformations of **12'** in solid state (left), **12'** calculated minimum energy conformer (middle), **VI** calculated minimum energy conformer (right).

Conclusions

The β-amino acid *cis*-FACBC can be prepared in enantiomerically pure form using a reliable gram-scale procedure, and it joins the restrictive group of foldamer building blocks whose homo-oligomers preferentially adopt a strand-like β-peptide secondary structure. There appears to be little or no significance for an intra-residue hydrogen bond for the induction or stabilisation of this conformer, which suggests that the term "6-ribbon" for this might be somewhat misleading. Combinations of solution state spectroscopic studies, theoretical calculations and a valuable x-ray crystal structure of a *cis*-FACBC tetramer provided useful structural information, and elaborated on the features of strand-like secondary structures proposed previously. When compared with its closest analogue, *cis*-ACBC, the backbone shape and amide group orientations of *cis*-FACBC oligomers are quite different and the cyclobutane ring conformations are all inverted, giving a different topology to the peripheral parts of the strand. In a wider sense, the introduction of a fluorine atom did not dramatically change the category of the preferred secondary structure, thus allowing fluorine to serve as a helpful structural probe.

Experimental

General experimental

All reagents and solvents were of commercial grade and were used as received, except for those qualified as "dry" which were purified under argon as follows: dichloromethane was passed through an activated alumina column; THF was distilled from sodium/benzophenone. Flash column chromatography was performed with SDS silica gel (35–70 μm). Analytical thin-layer chromatography was performed on 0.25 mm commercial silica gel plates (EMD, 60F-254). Plates were visualized by UV fluorescence at 254 nm and then revealed using a phosphomolybdic acid solution (10% in EtOH) or a ninhydrin solution (0.3% in *n*-BuOH). Retention factors (R_f) are given for such analyses. Melting points (Mp) were obtained in open capillary tubes and are uncorrected. Optical rotations were measured in a 10 cm quartz cell using solutions of concentration (c) in units of g.100 mL^{-1}; values for $[\alpha]_D^T$ were deduced for the D-line of sodium at the indicated temperature *T*. Infrared spectroscopy (IR) analyses were recorded neat on a Vertex 70 FTIR spectrometer equipped with an ATR diamond accessory, or as solutions in $CHCl_3$ on a Perkin-Elmer Spectrum One instrument; maximum absorbances (υ) are given in cm^{-1}. Nuclear magnetic resonance (NMR) data were acquired on Bruker AC 250, AM 300, AM 360 or DRX 400 spectrometers using commercial software. Chemical shifts (δ) are reported in ppm with respect to tetramethylsilane (δ = 0 ppm) for ^1H and ^{13}C and $CFCl_3$ (δ = 0 ppm) for ^{19}F. Splitting patterns for ^1H NMR and ^{13}C NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), broad signal (br) or m (multiplet). High-resolution mass spectrometry (HRMS) data were recorded on a Bruker MicroTOFq instrument in positive or negative electrospray ionization mode (ESI+ or ESI–). Circular dichroism (CD) spectra were recorded at 20 °C for solutions in MeOH on a Jasco J-815 spectrometer using 1 mm cell length. X-ray diffraction data were collected by using a Kappa X8 APPEX II Bruker diffractometer with graphite-monochromated MoKα radiation (λ = 0.71073 Å); see ESI for details.

Synthetic procedures for the synthesis of the building-block

1-*tert*-butyl-5-fluoro-2,4-dioxone-3,4-dihydropyrimidine-1(2H)-carboxylate (2). To a solution of Boc_2O (1.36 g, 6 mmol) and 5-fluorouracil **1** (0.7825 g, 6 mmol) in acetonitrile (50 ml) was added the 4-dimethylaminopyridine (7.33 mg, 0.06 mmol). The reaction mixture was stirred under argon at room temperature for 19 h. The solvent was removed under reduced pressure to give N^1-Boc-5-fluorouracil **2** (1.37 g, 99%) as a white solid which was used in the following step without purification. R_f (EtOAc): 0.78; Mp: >250 °C; IR (ATR) υ 3250, 1760, 1635, 1468, 1389, 1150; ^1H NMR (250 MHz, $CDCl_3$) δ 8.4 (s, 1H), 8.0 (d, *J* = 6.5 Hz, 1H), 1.6 (s, 9H); HRMS (ESI+) *m/z*: theor. 253.0595 (calc. for $C_9H_{11}FN_2O_4Na$), meas. 253.0588.

(±)-*cis*-*tert*-butyl 6-fluoro-3,5-dioxo-2,4-diazabicyclo[4.2.0]-octane-2-carboxylate ((±)-3). A solution of N^1-Boc-5-fluorouracil **2** (1.28 g, 5.58 mmol) in acetone (200 ml) was placed in a cylindrical reactor. The reaction mixture was degassed with an argon stream for 30 min, and then saturated with ethylene for 30 min. The solution was then irradiated for 4 h with a 400 W medium-pressure Hg lamp fitted with a Pyrex filter and a water-cooling jacket while a slow stream of ethylene was bubbled through. The solution was then evaporated under reduced pressure. Flash column chromatography (20→35% EtOAc/PE) gave the adduct **(±)-3** (0.965 g, 67%) as a white solid. R_f (50% EtOAc/PE): 0.55; Mp: >250 °C; IR (ATR) υ 3206, 2987, 1720, 1689, 1434, 1409, 1369, 1316, 1147; ^1H NMR (360 MHz, $CDCl_3$) δ 8.89 (brs, 1H), 4.91-4.75 (m, 1H), 2.51-2.17 (m, 3H), 1.64-1.53 (m, 1H), 1.50 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –142.61; ^{13}C NMR (90 MHz, $CDCl_3$) δ 166.4 (d, *J* = 24.9 Hz, C), 150.1 (C), 147.9 (C), 87.0 (d, *J* = 233.5 Hz, C), 85.1 (C), 54.7 (d, *J* = 22.1 Hz, CH), 27.9 ($3CH_3$), 26.6 (d, *J* = 22.5 Hz, CH_2), 19.4 (d, *J* = 19.0 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 281.0908 (calc. for $C_{11}H_{15}FN_2O_4Na$), meas. 281.0901.

(±)-*cis*-(2-*tert*-butyloxycarbonylamino)-1-fluorocyclobutane carboxylic acid ((±)-4). A solution of compound **(±)-3** (0.70 g, 2.7 mmol) in aqueous sodium hydroxide solution (3 M, 27 mL) was stirred at room temperature. After 25 h, a concentrated solution of HCl was added dropwise at 0 °C to pH = 1. The resulting aqueous phase was extracted with EtOAc and the combined organic phases were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure to give Boc-*cis*-FACBC **(±)-4** (0.59 g, 94%) as a white solid which was used in the following step without purification. R_f (50% EtOAc/PE): 0.78; Mp: 134-135 °C; IR (ATR, neat) υ 3311,

2979, 1729, 1666, 1405, 1105; ^{1}H NMR (360 MHz, $CDCl_3$) δ 12.41 (brs, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 4.52-4.31 (m, 1H), 2.55-2.35 (m, 1H), 2.32-2.15 (m, 1H), 2.12-1.76 (m, 2H), 1.47 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –144.57; ^{13}C NMR (90 MHz, $CDCl_3$) δ 173.4 (d, *J* = 24.9 Hz, C), 157.7 (C), 97.7 (d, *J* = 240.9 Hz, C), 82.8 (C), 55.2 (d, *J* = 25.3 Hz, CH), 28.2 ($3CH_3$), 26.1 (d, *J* = 21.2 Hz, CH_2), 20.4 (d, *J* = 15.6 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 256.0956 (calc. for $C_{10}H_{16}FNO_4Na$), meas. 256.0941.

***tert*-butyl-(1*R*,2*R*)-2-fluoro-2-((4*S*,5*R*)-4-methyl-2-oxo-5-phenyloxazolidine-3-carbonyl)cyclobutylcarbamate (5) and *tert*-butyl-(1*S*,2*S*)-2-fluoro-2-((4*S*,5*R*)-4-methyl-2-oxo-5-phenyloxazolidine-3-carbonyl)cyclobutylcarbamate (6).** To a cold (–78 °C) solution of racemic Boc-*cis*-FACBC **(±)-4** (1.25 g, 5.35 mmol) and triethylamine (0.89 mL, 6.42 mmol) in dry THF (54 mL) was added dropwise pivaloyl chloride (0.69 mL, 5.61 mmol). The mixture was stirred for 1 h at 0 °C to form the mixed anhydride, and then cooled to –78 °C. In a separate flask, a cold (ca. –40 °C) solution of (4*S*,5*R*)-4-methyl-5-phenyloxazolidin-2-one[24] (0.95 g, 5.35 mmol) in dry THF (26 mL) was treated with *n*-BuLi (1.6 M solution in hexane, 3.34 mL, 5.35 mmol) and stirred for 10 min. The resulting solution was cooled to –78 °C and added by rapid cannulation to the cooled (–78 °C) solution of the mixed anhydride. Residual metalated oxazolidinone was taken up by rinsing with dry THF (2 × 5 mL), and added to the cooled reaction mixture. After 1 h at –78 °C, the reaction mixture was quenched at this temperature using saturated $NaHCO_3$. After warming to room temperature, THF was removed under reduced pressure and the residual aqueous phase was extracted with CH_2Cl_2 (3×). The combined organic layers were washed successively with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Flash column chromatography (25→30% EtOAc/PE) gave diastereoisomers **5** (1.40 g, 47%) as a white solid and **6** (1.3 g, 45%) as colourless oil.

Compound **5**: R_f (30% EtOAc/PE): 0.65; Mp: 157-158 °C; $[\alpha]_D^{27}$ +64 (c 0.50, $CHCl_3$); IR (ATR) υ 3436, 2973, 1785, 1718, 1689, 1486, 1367, 1343, 1154; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.49-7.24 (m, 5H), 6.12 (d, *J* = 8.1 Hz, 1H), 5.74 (d, *J* = 6.5 Hz, 1H), 4.86- 4.48 (m, 2H), 2.60-2.39 (m, 1H), 2.37-2.12 (m, 2H), 1.68-1.53 (m, 1H), 1.46 (s, 9H), 1.02 (d, *J* = 6.6 Hz, 3H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –138.59; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1 (d, *J* = 28.8 Hz, C), 154.8 (C), 151.0 (C), 132.9 (C), 129.0 (CH), 128.9 (2CH), 125.7 (2CH), 96.7 (d, *J* = 241.9 Hz, C), 80.0 (CH), 79.8 (C), 56.7 (CH), 54.2 (d, *J* = 23.2 Hz, CH), 28.5 ($3CH_3$), 27.6 (d, *J* = 19.9 Hz, CH_2), 21.4 (d, *J* = 18.7 Hz, CH_2), 14.2 (CH_3); HRMS (ESI+) *m/z*: theor. 393.1820 (calc. for $C_{20}H_{26}FN_2O_5$), meas. 393.1803.

Compound **6**: R_f (30% EtOAc/PE): 0.52; $[\alpha]_D^{27}$ –13 (c 0.50, $CHCl_3$); IR (ATR) υ 3445, 2975, 1797, 1692, 1343, 1158; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.50-7.14 (m, 5H), 5.74 (d, *J* = 7.8 Hz, 1H), 5.68 (d, *J* = 7.4 Hz, 1H), 4.90-4.74 (m, 1H), 4.72-4.48 (m, 1H), 2.74-2.47 (m, 1H), 2.38-2.05 (m, 2H), 1.80-1.59 (m, 1H), 1.42 (s, 9H), 0.94 (d, *J* = 6.5 Hz, 3H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –139.10; ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5 (d, *J* = 28.8 Hz, C), 154.8 (C), 151.5 (C), 133.4 (C), 129.0 (CH), 128.7 (2CH), 125.9 (2CH), 97.8 (d, *J* = 240.1 Hz, C), 79.8 (C), 79.7 (CH), 55.5 (CH), 54.8 (d, *J* = 24.5 Hz, CH), 28.3 ($3CH_3$), 27.1 (d, *J* = 20.8 Hz, CH_2), 20.9 (d, *J* = 17.3 Hz, CH_2), 14.8 (CH_3); HRMS (ESI+) *m/z*: theor. 393.1820 (calc. for $C_{20}H_{26}FN_2O_5$), meas. 393.1810.

General procedure A for cleavage of the oxazolidin-2-one moiety. To an ice-cold solution of compound **5** or **6** (1 equiv.) in a 1:4 mixture of water and THF (30 ml/mmol) was added a 35% w/w solution of H_2O_2 (6 equiv.). The resulting mixture was stirred for 5 min at 0 °C, and then a solution of LiOH (2 equiv.) in water (4 ml/mmol) was added. The mixture was stirred for 6 h at 0 °C then a 1 M aqueous solution of Na_2SO_3 and a saturated aqueous solution of $NaHCO_3$ were added successively. THF was removed under reduced pressure and the aqueous residue was washed with CH_2Cl_2 (5×) to recover and recycle the chiral auxiliary. The aqueous phase was then acidified to pH 1 with concentrated HCl and extracted with CH_2Cl_2 (5×) The combined organic phases were dried over Na_2SO_4, filtrated and evaporated under reduced pressure to give single enantiomers of Boc-*cis*-FACBC, (+)-**4** or (⁻)-**4**, as white solids which were used in the following step without purification.

(+)-(1*R*,2*R*)-(2-tert-butyloxy-1-fluorocarbonylamino)cyclobutanecarboxylic acid ((+)-4). From a solution of compound **5** (3.27 g, 8.34 mmol), 35% w/w solution of H_2O_2 (4.86 g, 50 mmol, 4.3 mL), $LiOH.H_2O$ (701 mg, 16.68 mmol) in THF (200 mL) and H_2O (2 × 50 mL) following the general procedure A, **(+)-4** was obtained (1.75 g, 90%) as a white solid. R_f (30% EtOAc/PE): 0.78; Mp: 134-135 °C; $[\alpha]_D^{22}$ +25 (c 0.50, $CHCl_3$); IR (ATR) υ 3311, 2979, 1729, 1666, 1405, 1105; ^{1}H NMR (360 MHz, $CDCl_3$) δ 12.41 (brs, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 4.52-4.31 (m, 1H), 2.55-2.35 (m, 1H), 2.32-2.15 (m, 1H), 2.12-1.76 (m, 2H), 1.47 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –144.57; ^{13}C NMR (90 MHz, $CDCl_3$) δ 173.4 (d, *J* = 24.9 Hz, C), 157.7 (C), 97.7 (d, *J* = 240.9 Hz, C), 82.8 (C), 55.2 (d, *J* = 25.3 Hz, CH), 28.2 ($3CH_3$), 26.1 (d, *J* = 21.2 Hz, CH_2), 20.4 (d, *J* = 15.6 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 256.0956 (calc. for $C_{10}H_{16}FNO_4Na$), meas. 256.0947.

(⁻)-(1*S*,2*S*)-(2-tert-Butyloxy-1-fluorocarbonylamino)cyclobutanecarboxylic acid ((-)-4). From a solution of compound **6** (2.65 g, 6.75 mmol), 35% w/w solution of H_2O_2 (3.93 g, 50 mmol, 3.5 mL), $LiOH{\cdot}H_2O$ (567 mg, 13.5 mmol) in THF (150 mL) and H_2O (2 × 40 mL) following the general procedure A, **(⁻)-4** was obtained (1.18 g, 75%) as a white solid. R_f (30% EtOAc/PE): 0.78; Mp: 134-135 °C; $[\alpha]_D^{22}$ –25 (c 0.50, $CHCl_3$); IR (ATR) υ 3311, 2979, 1729, 1666, 1405, 1105; ^{1}H NMR (360 MHz, $CDCl_3$) δ 12.41 (brs, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 4.52-4.31 (m, 1H), 2.55-2.35 (m, 1H), 2.32-2.15 (m, 1H), 2.12-1.76 (m, 2H), 1.47 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –144.57; ^{13}C NMR (90 MHz, $CDCl_3$) δ 173.4 (d, *J* = 24.9 Hz, C), 157.7 (C), 97.7 (d, *J* = 240.9 Hz, C), 82.8 (C), 55.2 (d, *J* =

25.3 Hz, CH), 28.2 ($3CH_3$), 26.1 (d, J = 21.2 Hz, CH_2), 20.4 (d, J = 15.6 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 256.0956 (calc. for $C_{10}H_{16}FNO_4Na$), meas. 256.0953.

(+)-*tert*-butyl-(1*R*,2*R*)-2-fluoro-2-((*R*)-1-(4-methoxyphenyl)-ethylcarbamoyl)cyclobutylcarbamate (8). To an ice-cold solution of **(+)-4** (50.8 mg, 0.22 mmol) in dry CH_2Cl_2 (5 mL) were added 1-hydroxybenzotriazole monohydrate (HOBt·H_2O) (41.2 mg, 0.31 mmol) and (*R*)-α-(*p*-methoxyphenyl)ethylamine **7** (35 µL, 0.24 mmol). The mixture was stirred for 10 min at 0 °C then 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI·HCl) (62.7 mg, 0.33 mmol) was added. The resulting mixture was then stirred for 14 h at room temperature then washed successively with 1 M aqueous solution of $KHSO_4$ and a saturated aqueous solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. Flash column chromatography (30% EtOAc/PE) gave amide **8** (69 mg, 86%) as a white solid. R_f (30% EtOAc/PE): 0.33; Mp: 151-153 °C; $[\alpha]_D^{24}$ +121 (c 0.50, $CHCl_3$); IR (ATR) υ 3389, 2930, 1692, 1658, 1510, 1160; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.20 (m, 2H), 6.98-6.61 (m, 2H), 6.57 (s, 1H), 5.28 (d, J = 8.0 Hz, 1H), 5.22-5.18 (m, 1H), 4.66-4.41 (m, 1H), 3.79 (s, 3H), 2.40-2.15 (m, 2H), 2.12-1.90 (m, 1H), 1.90-1.73 (m, 1H), 1.49 (d, J = 6.9 Hz, 3H), 1.43 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –142.26; ^{13}C NMR (90 MHz, $CDCl_3$) δ 168.6 (d, J = 22.9 Hz, C), 159.1 (C), 154.8 (C), 134.7 (C), 127.4 (2CH), 114.2 (2CH), 99.2 (d, J = 237.4 Hz, C), 79.7 (C), 55.3 (CH_3), 53.8 (d, J = 24.1 Hz, CH), 48.1 (CH), 28.4 ($3CH_3$), 26.6 (d, J = 20.9 Hz, CH_2), 22.0 (CH_3), 21.7 (d, J = 17.5 Hz, CH_2).

Boc-[(1*R*,2*R*)-FACBC]-OBn (9). To a solution of **(+)-4** (1.17 g, 5.0 mmol) and benzyl alcohol (1.6 mL, 15.0 mmol) in dry CH_2Cl_2 (100 mL) at 0 °C were added 4-dimethylaminopyridine (65 mg, 0.5 mmol) and *N,N'*-dicyclohexylcarbodiimide (1.24 g, 6.0 mmol). The reaction mixture was stirred for 1 h at 0 °C then 20 h at room temperature. After filtration, the solvent was removed under reduced pressure and EtOAc was added. The organic layer was washed successively with brine, 1 M HCl, brine, 5% $NaHCO_3$, and brine. The organic layer was then dried over Na_2SO_4 and evaporated under reduced pressure. Flash column chromatography (15% EtOAc/PE) gave Boc-(1*R*,2*R*)-*cis*-FACBC-OBn **9** (1.37 g, 85%) as a colorless oil. R_f (15% EtOAc/PE): 0.3; $[\alpha]_D^{25}$ +65 (c 0.50, $CHCl_3$); IR (ATR) υ 2976, 1707, 1500, 1455, 1164, 1129; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.27 (m, 5H), 5.36 (AB quartet, J = 12.2 Hz, 1H), 5.32 (brs, 1H), 5.27 (AB quartet, J = 12.2 Hz, 1H), 4.73-4.43 (m, 1H), 2.45-2.17 (m, 2H), 2.13-1.97 (m, 1H), 1.86-1.71 (m, 1H), 1.43 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –143.62; ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4 (brd, J = 25.7 Hz, C), 154.2 (C), 135.0 (C), 128.6 (2CH), 128.4 (CH), 128.2 (2CH), 97.0 (d, J = 238.8 Hz, C), 79.8 (br, C), 67.1 (CH_2), 53.8 (d, J = 22.0 Hz, CH), 28.2 ($3CH_3$), 26.6 (d, J = 21.5 Hz, CH_2), 21.2 (brd, J = 16.2 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 324.1606 (calc. for $C_{17}H_{23}FNO_4$), meas. 324.1595.

Synthetic Procedures for the preparation of homooligomers

General procedure B for *tert*-butoxycarbonyl group removal followed by peptide coupling. To a solution of Boc-[(1*R*,2*R*)-*cis*-FACBC]$_n$-OBn (1.0 equiv.) in dry CH_2Cl_2 (20 mL/mmol) at 0 °C was added TFA (30 equiv.). After 2 h at room temperature, the volatiles were evaporated under reduced pressure to leave the appropriate TFA salt. This material was added directly to a solution of Boc-[(1*R*,2*R*)-*cis*-FACBC]$_m$-OH (1 equiv.) in DMF (15 mL/mmol) under an argon atmosphere. 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) (1.2 equiv.) and triethylamine (3.0 equiv.) were added and the mixture was stirred for at least 5 min or until all reagents had dissolved, then 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI·HCl) (1.5 equiv.) was added. The solution was stirred at room temperature for 3 days. The solution was concentrated under reduced pressure then the residue was taken up in EtOAc (30 mL/mmol). The organic solution was washed successively with 1 M aqueous $KHSO_4$ solution (2×), saturated $NaHCO_3$ solution (2×) and brine. The organic solution was then dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was then purified by flash column chromatography to give the corresponding peptide Boc-[(1*R*,2*R*)-*cis*-FACBC]$_{n+m}$-OBn.

General procedure C for hydrogenolysis of benzyl esters. To a solution of Boc-[(1*R*,2*R*)-*cis*-FACBC]$_n$-OBn (1.0 equiv.) in dry CH_2Cl_2 (40 mL/mmol) was added carefully the stated quantity of 10% Pd-C. The mixture was stirred under a H_2 atmosphere (rubber balloon) until the reaction was complete (TLC monitoring). The mixture was filtered through a Celite pad and washed with EtOAc. The filtrate was concentrated under reduced pressure to give the corresponding Boc-[(1*R*,2*R*)-*cis*-FACBC]$_n$-OH which was engaged in the next peptide coupling step without purification.

Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OBn (10). From Boc-[(1*R*,2*R*)-*cis*-FACBC]-OBn **9** (1.21 g, 3.75 mmol) and TFA (9.0 mL, 12.8 g, 112.5 mmol) in CH_2Cl_2 (75 mL) and HOBt·H_2O (608 mg, 4.5 mmol), triethylamine (1.6 mL, 1.14 g, 11.25 mmol), EDCI·HCl (1.08 g, 5.63 mmol) and Boc-[(1*R*,2*R*)-FACBC]-OH **5** (874 mg, 3.75 mmol) in DMF (60 mL) according to the general procedure B, followed by flash column chromatography (0.5→5% MeOH/CH_2Cl_2), Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OBn **10** (1.63 g, 99%) was obtained as a colourless oil. R_f (1% MeOH/CH_2Cl_2): 0.24; $[\alpha]_D^{24}$ +79 (c 0.50, $CHCl_3$); IR ($CHCl_3$) υ 3432, 3019, 2965, 1712, 1679, 1524, 1501; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43-7.28 (m, 5H), 6.94-6.79 (m, 1H), 5.30-5.10 (m, 1H), 5.29 (A of AB quartet, J = 12.1 Hz, 1H), 5.24 (B of AB quartet, J = 12.1 Hz, 1H), 5.01-4.83 (m, 1H), 4.62-4.36 (m, 1H), 2.47-2.35 (m, 1H), 2.34-2.11 (m, 4H), 2.00-1.72 (m, 3H), 1.40 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –143.18, –143.27; ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9 (brd, J = 27.5 Hz, C), 168.8 (brd, J = 26.9 Hz, C), 154.5 (C), 134.7 (C), 128.6 (2CH), 128.5 (CH), 128.3 (2CH), 98.7 (d, J = 237.9 Hz, C), 96.2 (d, J = 239.3 Hz, C), 79.5 (C), 67.3 (CH_2), 53.5 (brd, J =

25.2 Hz, CH), 51.4 (brd, *J* = 25.5 Hz, CH), 28.1 ($3CH_3$), 27.0 (d, *J* = 21.7 Hz, CH_2), 26.5 (d, *J* = 20.8 Hz, CH_2), 21.3 (d, *J* = 17.4 Hz, CH_2), 20.4 (d, *J* = 16.1 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 439.2039 (calc. for $C_{22}H_{29}F_2N_2O_5$), meas. 439.2038.

Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OH (11). From Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OBn **10** (556 mg, 1.27 mmol) and Pd-C (300 mg) in CH_2Cl_2 (60 mL) according to the general procedure C, Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OH **11** was obtained (400 mg, 97%) as a white solid. R_f (30% EtOAc/PE): 0.12; Mp: 148-150 °C; $[\alpha]_D^{25}$ +87 (c 0.50, $CHCl_3$); IR ($CHCl_3$) υ 3432, 3357, 3022, 2982, 1711, 1676, 1528, 1502; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.54 (brd, *J* = 4.4 Hz, 1H), 6.42 (brs, 1H), 5.24 (d, *J* = 8.6 Hz, 1H), 5.07-4.90 (m, 1H), 4.48-4.33 (m, 1H), 2.51-2.39 (m, 2H), 2.26-2.09 (m, 4H), 2.02-1.74 (m, 2H), 1.40 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –141.71, –142.75; ^{13}C NMR (90 MHz, $CDCl_3$) δ 171.7 (d, *J* = 26.9 Hz, C), 168.9 (d, *J* = 23.4 Hz, C), 155.6 (C), 98.9 (d, *J* = 237.8 Hz, C), 96.6 (d, *J* = 240.9 Hz, C), 81.0 (C), 54.1 (brd, *J* = 25.0 Hz, CH), 51.6 (brd, *J* = 25.4 Hz, CH), 28.3 ($3CH_3$), 27.6 (d, *J* = 22.0 Hz, CH_2), 26.4 (d, *J* = 20.6 Hz, CH_2), 20.6 (d, *J* = 19.7 Hz, CH_2), 20.4 (d, *J* = 18.48 Hz, CH_2); HRMS (ESI–) *m/z*: theor. 347.1424 (calc. for $C_{15}H_{21}F_2N_2O_5$), meas. 347.1422.

Boc-[(1*R*,2*R*)-*cis*-FACBC]$_4$-OBn (12). From Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OBn **10** (478 mg, 1.09 mmol) and TFA (2.5 mL, 3.73 g, 32.7 mmol) in CH_2Cl_2 (50 mL) and HOBt·H_2O (177 mg, 1.31 mmol), triethylamine (0.46 mL, 330 mg, 3.27 mmol), EDCI·HCl (313 mg, 1.64 mmol) and Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OH **11** (379 mg, 1.09 mmol) in DMF (20 mL) according to the general procedure B, followed by flash column chromatography (30→40% EtOAc/PE), Boc-[(1*R*,2*R*)-*cis*-FACBC]$_4$-OBn **12** was obtained (477 mg, 66%) as a white solid. R_f (50% EtOAc/PE): 0.52; Mp: 88-90 °C; $[\alpha]_D^{24}$ +114 (c 0.50, $CHCl_3$); IR ($CHCl_3$) υ 3425, 3020, 2962, 1712, 1681, 1531, 1508, 1217; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.27 (m, 5H), 7.08-6.98 (m, 2H), 6.93-6.87 (m, 1H), 5.23 (AB quartet, *J* = 12.1 Hz, 1H), 5.19 (AB quartet, *J* = 12.1 Hz, 1H), 5.23-5.20 (m, 1H), 4.93-4.71 (m, 3H), 4.57-4.35 (m, 1H), 2.39-1.69 (m, 16H), 1.35 (s, 9H); ^{19}F NMR (376 MHz, $CDCl_3$) δ –143.06, –143.10, –143.14, –143.31; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1 (d, *J* = 26.3 Hz, 2C), 168.6 (brd, *J* = 23.2 Hz, C), 168.4 (d, *J* = 23.3 Hz, C), 154.6 (C), 134.7 (C), 128.7 (3CH), 128.4 (2CH), 98.7 (d, *J* = 238.0 Hz, C), 98.0 (d, *J* = 238.9 Hz, C), 97.9 (d, *J* = 238.7 Hz, C), 96.1 (d, *J* = 239.5 Hz, C), 79.6 (C), 67.5 (CH_2), 53.6 (brd, *J* = 23.1 Hz, 2CH), 51.2 (d, *J* = 25.5 Hz, CH), 51.1 (d, *J* = 24.7 Hz, CH), 28.2 ($3CH_3$), 27.1 (d, *J* = 21.2 Hz, $3CH_2$), 26.7 (d, *J* = 25.6 Hz, CH_2), 21.6 (brd, *J* = 17.4 Hz, CH_2), 20.9 (brd, *J* = 15.5 Hz, CH_2), 20.8 (d, *J* = 16.3 Hz, CH_2), 20.6 (d, *J* = 16.3 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 669.2906 (calc. for $C_{32}H_{41}F_4N_4O_7$), meas. 669.2888.

Boc-[(1*S*,2*S*)-*cis*-FACBC]$_4$-OBn (12'). The enantiomer of **12** was prepared from the building block **(-)-4**, following the above-described sequences with comparable yields.

Boc-[(1*R*,2*R*)-*cis*-FACBC]$_4$-OH (13). From Boc-[(1*R*,2*R*)-*cis*-FACBC]$_4$-OBn **12** (260 mg, 0.38 mmol) and Pd-C (200 mg) in CH_2Cl_2 (25 mL) according to the general procedure C, Boc-[(1*R*,2*R*)-*cis*-FACBC]$_4$-OH **13** was obtained (220 mg, 99%) as a white solid. Mp: 110-112 °C; $[\alpha]_D^{23}$ +95 (c 0.50, $CHCl_3$); IR ($CHCl_3$) υ 3425, 3346, 3022, 2929, 1712, 1677, 1528, 1506; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (brd, *J* = 5.7 Hz, 1H), 7.17 (brs, 1H), 7.08 (brs, 1H), 6.04 (brs, 1H), 5.28 (d, *J* = 9.5 Hz, 1H), 5.03-4.72 (m, 3H), 4.63-4.41 (m, 1H), 2.53-2.39 (m, 2H), 2.39-2.12 (m, 6H), 2.12-1.88 (m, 6H), 1.88-1.70 (m, 2H), 1.39 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –141.81, –142.77, –142.93, –143.43; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3 (brd, *J* = 25.4 Hz, C), 169,5-169.0 (overlapped signals, 2C), 168.6 (d, *J* = 22.9 Hz, C), 155.0 (C), 98.7 (d, *J* = 238.0 Hz, C), 98.1 (d, *J* = 238.7 Hz, C), 98.0 (d, *J* = 238.6 Hz, C), 96.3 (d, *J* = 239.5 Hz, C), 80.2 (C), 53.8-53.4 (br, CH), 51.5 (d, *J* = 25.0 Hz, CH), 51.3 (d, *J* = 25.4 Hz, CH), 51.3 (d, *J* = 24.6 Hz, CH), 28.2 ($3CH_3$), 27.2 (d, *J* = 21.4 Hz, CH_2), 27.2 (d, *J* = 20.9 Hz, CH_2), 27.1 (d, *J* = 21.0 Hz, CH_2), 26.7 (d, *J* = 20.5 Hz, CH_2), 21.4 (brd, *J* = 16.6 Hz, CH_2), 20.8 (brd, *J* = 16.3 Hz, CH_2), 20.4 (d, *J* = 16.3 Hz, CH_2), 20.3 (d, *J* = 16.0 Hz, CH_2); HRMS (ESI–) *m/z*: theor. 577.2291 (calc. for $C_{25}H_{33}F_4N_4O_7$), meas. 577.2298.

Boc-[(1*R*,2*R*)-*cis*-FACBC]$_6$-OBn (14). From Boc-[(1*R*,2*R*)-*cis*-FACBC]$_2$-OBn **10** (85 mg, 0.19 mmol) and TFA (445 µL, 663 mg, 5.82 mmol) in CH_2Cl_2 (10 mL) and HOBt·H2O (31 mg, 0.23 mmol), triethylamine (106 µL, 77 mg, 0.76 mmol), EDCI·HCl (55 mg, 0.29 mmol) and Boc-[(1*R*,2*R*)-*cis*-FACBC]$_4$-OH **13** (110 mg, 0.19 mmol) in DMF (5 mL) according to the general procedure B, followed by flash column chromatography (40→60% EtOAc/pentane), Boc-[(1*R*,2*R*)-*cis*-FACBC]$_6$-OBn **14** was obtained (82 mg, 48%) as a white solid. R_f (50% EtOAc/PE): 0.40; Mp: 221-223 °C; $[\alpha]_D^{24}$ +124 (c 0.50, $CHCl_3$); IR ($CHCl_3$) υ 3421, 3020, 2962, 1712, 1681, 1533, 1506, 1219; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.36-7.30 (m, 5H), 7.07-6.97 (m, 4H), 6.92-6.85 (m, 1H), 5.30-5.16 (m, 3H), 4.94-4.74 (m, 5H), 4.58-4.37 (m, 1H), 2.43-1.70 (m, 24H), 1.38 (s, 9H); ^{19}F NMR (235 MHz, $CDCl_3$) δ –143.08, –143.11, –143.16, –143.18, –143.19, –143.34; ^{13}C NMR (90 MHz, $CDCl_3$) δ 169.2 (d, *J* = 26.3 Hz, 2C), 168.7 (d, *J* = 23.3 Hz, C), 168.64 (d, *J* = 23.3 Hz, C), 168.57 (brd, *J* = 23.2 Hz, C), 168.5 (d, *J* = 23.3 Hz, C), 154.6 (C), 134.7 (C), 128.9 (3CH), 128.6 (2CH), 98.7 (d, *J* = 238.5 Hz, C), 98.1 (d, *J* = 238.8 Hz, C), 97.93 (d, *J* = 238.7 Hz, C), 97.89 (d, *J* = 238.7 Hz, C), 97.85 (d, *J* = 238.9 Hz, C), 96.2 (d, *J* = 239.8 Hz, C), 79.8 (C), 67.7 (CH_2), 53.6 (brd, *J* = 23.2 Hz, 2CH), 51.24 (d, *J* = 25.5 Hz, CH), 51.20 (d, *J* = 24.8 Hz, CH), 51.16 (d, *J* = 24.6 Hz, CH), 51.1 (d, *J* = 24.7 Hz, CH), 28.3 ($3CH_3$), 27.33 (d, *J* = 21.0 Hz, $2CH_2$), 27.27 (d, *J* = 21.7 Hz, $3CH_2$), 26.8 (brd, *J* = 20.8 Hz, CH_2), 21.7 (brd, *J* = 17.9 Hz, CH_2), 21.1 (d, *J* = 16.0 Hz, $2CH_2$), 21.0 (d, *J* = 16.5 Hz, $2CH_2$), 20.8 (d, *J* = 16.4 Hz, CH_2); HRMS (ESI+) *m/z*: theor. 921.3592 (calc. for $C_{42}H_{52}F_6N_6O_9Na$), meas. 921.3600.

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Notes and references

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†Electronic Supplementary Information (ESI). Copies of ^{1}H and ^{13}C NMR spectra for all new compounds, spectra of NH/ND exchange for compounds **10**, **12** and **14**, temperature coefficients and 2D NMR correlations for compound **12**, x-ray acquisition data for compounds **8** and **12'** (PDF file). Structural coordinates for the minimum energy conformer of **12'** (PDB file). Crystallographic diffraction data for **8** and **12'** (CIF file). X-ray crystallographic data have been deposited with the Cambridge Crystallographic Data Centre with deposition numbers CCDC 1028349 (**8**) and CCDC 1028350 (**12'**).

1 S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173; D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.*, 2001, **101**, 3893; *Foldamers: Structure, Properties, and Applications*, S. Hecht, I. Huc, Eds., Wiley-VCH: Weinheim, 2007; G. Guichard, I. Huc *Chem. Commun*., 2011, **47**, 5933; A. Roy, P. Prabhakaran, P. K. Baruah, G. J. Sanjayan, *Chem. Commun*., 2011, **47**, 11593.

2 D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 13071.

3 D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 913.

4 F. Fülöp, T. A. Martinek, G. Tóth, *Chem. Soc. Rev.*, 2006, **35**, 323; D. Seebach, J. Gardiner, *Acc. Chem. Res.*, 2008, **41**, 1366; W. S. Horne, S. H. Gellman, *Acc. Chem. Res.*, 2008, **41**, 1399; P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaram, *Chem. Rev*., 2011, **111**, 657; T. A. Martinek, F. Fülöp, *Chem. Soc. Rev.*, 2012, **41**, 687.

5 K. Möhle, R. Günther, M. Thormann, N. Sewald, H.-J. Hofmann, *Biopolymers*, 1999, **50**, 167; R. Günther, H.-J. Hofmann, K. Kuczera, *J. Phys. Chem. B*, 2001, **105**, 5559; Y.-D. Wu, W. Han, D.-P. Wang, Y. Cao, Y.-L. Zhao, *Acc. Chem. Res.*, 2008, **41**, 1418.

6 It has been tentatively suggested that a tetramer of *cis*-2-aminocyclohexane-1-carboxylic acid (*cis*-ACHC) may adopt a similar conformation: S. H. Choi, M. Ivancic, I. A. Guzei, S. H. Gellman, *Eur. J. Org. Chem*. 2013, 3464.

7 T. A. Martinek, G. K. Tóth, E. Vass, M. Hollósi, F. Fülöp, *Angew. Chem., Int. Ed.*, 2002, **41**, 1718.

8 S. Chandrasekhar, B. N. Babu, A. Prabhakar, A. Sudhakar, M. S. Reddy, M. U. Kiran, B. Jagadeesh, *Chem. Commun.*, 2006, 1548.

9 F. Rúa, S. Boussert, T. Parella, I. Díez-Pérez, V. Branchadell, E. Giralt, R. M. Ortuño, *Org. Lett.*, 2007, **9**, 3643; 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, R. M. Ortuño, *Org. Biomol. Chem.*, 2010, **8**, 564.

10 This contrasts with the behaviour of substituted aromatic oligoamides, in which intra-residue 6-membered ring hydrogen bonds play a decisive role: Y. Hamuro, S. J. Geib, A. D. Hamilton, *Angew. Chem. Int. Ed.*, 1994, **33**, 446; Y. Hamuro, S. J. Geib, A. D. Hamilton, *J. Am. Chem. Soc.*, 1996, **118**, 7529. D.-W. Zhang, X. Zhao, J.-L. Hou, Z.-T. Li, *Chem. Rev.*, 2012, **112**, 5271; G. M. Burslem, A. J. Wilson, *Synlett*, 2014, **25**, 3245.

11 Previously, *cis*-FACBC was described only once and in racemic form, without experimental details: C. Gauzy, B. Saby, E. Pereira, S. Faure, D. J. Aitken, *Synlett*, 2006, 1394.

12 L. Kiss, F. Fülöp, *Chem. Rev*. 2014, **114**, 1116.

13 R. I. Mathad, C. Gessier, D. Seebach, B. Jaun, *Helv. Chim. Acta*, 2005, **88**, 266; B. Jaun, D. Seebach, R. I. Mathad, *Helv. Chim. Acta*, 2011, **94**, 355; L. Hunter, S. Butler and S. B. Ludbrook, *Org. Biomol. Chem*., 2012, **10**, 8911; V. Peddie, R. J. Butcher, W. T. Robinson, M. C. J. Wilce, D. A. K. Traore, A. D. Abell, *Chem. Eur. J.*, 2012, **18**, 6655; X.-G. Hu, D. S. Thomas, R. Griffith, L. Hunter, *Angew. Chem. Int. Ed.*, 2014, **53**, 6176.

14 T. D. W. Claridge, J. M. Goodman, A. Moreno, D. Angus, S. F. Barker, C. Taillefumier, M. P. Watterson, G. W. Fleet, *Tetrahedron Lett.*, 2001, **42**, 4251.

15 J. W. Banks, A. S. Batsanov, J. A. K. Howard, D. O'Hagan, H. S. Rzepa, S. Martin-Santamaria, *J. Chem. Soc. Perkin Trans. 2*, 1999, 2409; C. R. S. Briggs, D. O'Hagan, J. A. K. Howard, D. S. Yufit, *J. Fluorine Chem.*, 2003, **119**, 9; D. O'Hagan, *Chem. Soc. Rev.*, 2008, **37**, 308.

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

17 V. Declerck, D. J. Aitken, *Amino Acids*, 2011, **41**, 587.

18 V. Declerck, D. J. Aitken, *J. Org. Chem*, 2011, **76**, 708; H. Awada, S. Robin, R. Guillot, O. Yazbeck, D. Naoufal, N. Jaber, A. Hachem, D. J. Aitken, *Eur. J. Org. Chem.* 2014, 7148.

19 T. L. March, M. R. Johnston, P. J. Duggan, J Gardiner, *Chem. Biodiversity*, 2012, **9**, 2410; L. E. Zimmer, C. Sparr, R. Gilmour, *Angew. Chem., Int. Ed.*, 2011, **50**, 11860.

20 T. Yoshinari, F. Gessier, C. Noti, A. K. Beck, D Seebach, *Helv. Chim. Acta*, 2011, **94**, 1908.

21 D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. **L.** Matthews, J. **V.** Schreiber, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta*, 1998, **81**, 932.

22 Previously, only a dipeptide derivative of *cis*-ACBC was described in the solid state: S. Izquierdo, F. Rúa, A. Sbai, T. Parella, Á. Álvarez-Larena, V. Branchadell, R. M. Ortuño, *J. Org. Chem.*, 2005, **70**, 7963.

23 Y.-D. Wu, D.-P. Wang, *J. Am. Chem. Soc*., 1999, **121**, 9352; Y.-D. Wu, J.-Q. Lin, Y.-L. Zhao, *Helv. Chim. Acta*, 2002, **85**, 3144.

24 G. Bégis, D. E. Cladingboel, L. Jerome, W. B. Motherwell, T. D. Sheppard, *Eur. J. Org. Chem.*, 2009, 1532.