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VCD studies on cyclic peptides assembled from L-α-amino acids and a *trans*-2-aminocyclopentane- or *trans*-2-aminocyclohexane carboxylic acid

E. Vass,[a] U. Strijowski,[b] K. Wollschläger,[b] I. M. Mándity,[c] G. Szilvágyi,[a] M. Jewgiński,[d,e] K. Gaus,[b] S. Royo,[b] Z. Majer,[a] N. Sewald[b] and M. Hollósi[a]*

The increasing interest in peptidomimetics of biological relevance prompted us to synthesize a series of cyclic peptides comprising *trans*-2-aminocyclohexane carboxylic acid (Achc) or *trans*-2-aminocyclopentane carboxylic acid (Acpc). NMR experiments in combination with MD calculations were performed to investigate the three-dimensional structure of the cyclic peptides. These data were compared to the conformational information obtained by electronic circular dichroism (ECD) and vibrational circular dichroism (VCD) spectroscopy. Experimental VCD spectra were compared to theoretical VCD spectra computed quantum chemically at B3LYP/6-31G(d) density functional theory (DFT) level. The good agreement between the structural features derived from the VCD spectra and the NMR-based structures underlines the applicability of VCD in studying the conformation of small cyclic peptides.

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Keywords: vibrational circular dichroism; electronic circular dichroism; *trans*-2-aminocyclohexane carboxylic acid; *trans*-2-aminocyclopentane carboxylic acid; cyclic peptides; NMR

Introduction

There is an increasing interest in the synthesis and conformational analysis of peptides containing β-amino acids. A great number of β-amino acids occur naturally and display biological activity [1]. The presence of β-amino acids in peptide structures sometimes results in physiologically highly active compounds isolated from plants and marine organisms [2]. It was shown by several groups that linear peptides exclusively composed of β-amino acids form a number of stable secondary structures such as sheets and helices [3–7]. Kessler and coworkers designed carbohydrate derived β-amino acids that show the ability to constrain linear backbone conformations or form distinct β- or γ-turn structures [8]. Incorporation of a distinct β-amino acid in cyclic RGD peptides results in the stabilization of the overall secondary structure [9]. Peptides containing β-homo-amino acids often exhibit retarded metabolism and are sometimes stable against aminopeptidases [10]. Raines and coworkers incorporated β-amino acids into an enzyme to replace an existing β-turn and increase conformational stability [11].

β-Aminocycloalkane carboxylic acids represent a subgroup of β-amino acids. (1*R*,2*S*)-2-aminocyclopentane carboxylic acid (cispentacin) proved to be an antibiotic protecting against *Candida* infections [12]. Oligomers consisting of cyclic β-amino acid residues [e.g. (1*R*,2*R*)-2-aminocyclohexane carboxylic acid (*trans*-Achc) or (1*R*,2*R*)-2-amino cyclopentane carboxylic acid (*trans*-Acpc)] have been studied by Gellman and coworkers [13,14]. Cyclic β-amino acids have found a broad application in chemistry and peptide synthesis [15–18]. Similar to proline, they have a special role in peptides and proteins stabilizing folded structures [19,20].

Folded structures centered at β-amino acids cannot form C_{10} or C_7 intramolecular H-bondings (IHBs) frequently occurring in β- or γ-turns, respectively. However, IHBs of larger ring size (C_8, C_{11}, C_{12}, etc.) are possible. Qualitative comparison of the amide A region of the IR spectra in CH_2Cl_2 of the *N*-isobutyryl methylamide derivatives of *trans*-Achc and *trans*-Acpc indicated that the *trans*-Acpc derivative is more prone to C_8 IHB formation

* *Correspondence to: Prof. M. Hollósi, Institute of Chemistry, Eötvös Loránd University, 1117 Budapest, Pázmány Péter sétány 1/a, Hungary. E-mail: hollosi@chem.elte.hu*

a *Institute of Chemistry, Eötvös Loránd University, 1117 Budapest, Pázmány Péter sétány 1/a, Hungary*

b *Organic and Bioorganic Chemistry, Department of Chemistry, Bielefeld University, Universitätsstrasse 25, 33615 Bielefeld, Germany*

c *Institutes of Pharmaceutical Chemistry and Medical Chemistry, University of Szeged, Eötvös u. 6, 6720 Szeged, Hungary*

d *Institute of Chemistry, University of Opole, Oleska 48, Opole 45-052, Poland*

e *Department of Bioorganic Chemistry, Wrocław University of Technology, Wybrzeze Wyspianskiego 27, 50-370 Wrocław, Poland*

than the *trans*-Achc derivative [21]. The NH stretching contribution to the amide A band of the C_8 H-bonded derivatives of *trans*-Achc and *trans*-Acpc appears at 3357 and 3306 cm^{-1}, respectively, suggesting that the C═O and H–N in the *trans*-Achc model are not oriented as favorable for a strong hydrogen bond as in the *trans*-Acpc derivative.

The FT-IR spectra of the diasteromeric *cis*-(1*R*,2*S*)-2-aminocyclopentane carboxylic acid (*cis*-Acpc) oligomers have been studied in CH_2Cl_2 and DMSO [22]. DMSO is well known to destabilize weak IHBs. On the basis of its absence or decreased intensity in the FT-IR spectra in DMSO, the band at approximately 1645 cm^{-1} in CH_2Cl_2 was assigned to repeats of C_6 IHBs.

Vibrational circular dichroism (VCD) has become a well-known area of chiroptical spectroscopy over the past decades [23,24]. A wealth of vibrational transitions commonly occurs in the IR spectrum that are associated with a VCD band with a particular sign and intensity. IR and VCD spectra can be calculated directly using *ab initio* quantum chemical calculation programs such as Gaussian [25].

In the field of polypeptides and proteins, VCD spectroscopy can be applied for revealing the presence of protein periodic secondary structures such as α-helix and β-structure and evaluating their relative contribution [26]. VCD spectral characteristics of β-turns in linear and disulfide-cyclized tetrapeptides and longer natural and model sequences have been studied [24,27,28]. Lovas and coworkers studied the VCD spectra of disulfide bridged Ac-cyclo-(Cys-Xxx-Yyy-Cys)-NH_2 cyclic peptides, where Xxx and Yyy are amino acids, which were previously found to occur in different types of β-turns preferentially [28]. Diem and coworkers reported the VCD spectra of cyclo-(-Gly-Pro-Gly-D-Ala-Pro-) [29] and small disulfide-cyclized β-turn models [30]. They suggested that IR spectra with the amide I bands in the 1670–1690 cm^{-1} frequency region and the corresponding broad positive couplet-type VCD signal are associated with a type II β-turn whereas a positive–negative–positive shaped amide I VCD signal was proposed for type I β-turns [30]. Keiderling and coworkers [31] and Polavarapu and coworkers [32] reported that linear type II′ β-turns have a negative couplet-type amide I′ VCD signal in the 1670–1890 cm^{-1} region. The role of 2-aminocycloalkane carboxylic acids in inducing the adoption of unique H-bonded pseudo-turns in peptides has not yet been investigated in detail. Acpc and Achc occur in *cis*- and *trans*-form that increases the number of the possible H-bonded structures.

Strijowski and Sewald described the synthesis and structural studies on a series of cyclic peptides containing either *cis*- or *trans*-Acpc or *cis*- or *trans*-Achc moieties [33]. In this article, we report electronic circular dichroism (ECD), VCD and NMR spectroscopic investigations of cyclic peptides containing *trans*-Acpc and *trans*-Achc residues (Table 1). The main goal of this work was to enlarge the knowledge of the H-bond-forming and turn-stabilizing ability of the above cyclic β-amino acids.

Table 1. Cyclic peptides comprising *trans*-Acpc or *trans*-Achc moieties

Code	Sequence[a]
Hcp	cyclo-(-Ile-Asp-Ser-*trans*-Acpc-Leu-Asn-)
Hch	cyclo-(-Ile-Asp-Ser-*trans*-Achc-Leu-Asn-)
Pcp	cyclo-(-Asp-Ser-*trans*-Acpc-Leu-Asn-)
Pch	cyclo-(-Asp-Ser-*trans*-Achc-Leu-Asn-)

[a] *trans*-Acpc= *trans*-(1*S*,2*S*)-2-aminocyclopentane carboxylic acid; *trans*-Achc= *trans*-(1*S*,2*S*)-2-aminocyclohexane carboxylic acid.

Materials and Methods

Synthesis and Purification of the Peptides

Chemicals and solvents were obtained from Sigma-Aldrich (Hamburg, Germany) or Acros (Geel, Belgium). All amino acids and the Clt resin were purchased from Iris Biotech GmbH (Marktredwitz, Germany) or ACT (Cambridgeshire, UK).

Analytical RP-HPLC was carried out on a Thermo Separation Products system consisting of a UV-6000 diode array detector and a P-4000 pump equipped with a Phenomenex HPLC Guard Cartridge system (C12; 4 × 3.00 mm) and a Phenomenex Jupiter 4 μ Proteo 90 A column (C12; 250 × 4.60 mm). Preparative RP-HPLC was carried out on a Thermo Separation Products system consisting of a UV-1000 detector and a P-4000 pump equipped with a Vydac high-performance guard column (C18) and a Phenomenex Jupiter 10 μ Proteo 90 A column (C12; 250 × 21.20 mm).

MALDI-TOF mass spectra were recorded on a Voyager DE instrument (PerSeptive Biosystems, Weiterstadt, Germany), with 2,5-dihydroxybenzoic acid as the matrix.

The synthesis of *trans*-Acpc and *trans*-Achc was performed according to the procedure developed by Davies *et al*. [34]. The amino acids were Fmoc protected using 9-fluorenylmethyl succinimidyl carbonate according to Milton *et al*. [35] to give Fmoc-*trans*-Acpc-OH and Fmoc-*trans*-Achc-OH, respectively.

The synthesis of the peptides Pch and Hch (Table 1) containing *trans*-Achc was performed as published previously [33]. The linear precursors of all peptides were obtained by solid phase peptide synthesis on Clt resin preloaded with leucine according to the Fmoc chemistry using TBTU as coupling reagent. The linear peptides were cleaved from the resin using a solution of 1% TFA in dichloromethane and cyclized in solution under pseudo-high-dilution conditions using a dual syringe pump to avoid dimerization and HATU as coupling reagent [36]. After deprotection of the permanent protecting groups with 95% TFA, 2.5% triisopropyl silane and 2.5% water, the peptides were purified by RP-HPLC using TFA/acetonitrile/water elution gradients. The purified peptides were analyzed by MALDI-TOF MS (Supporting Information).

NMR Studies and MD Simulations

The peptides were dissolved in DMSO-d_6 at a concentration of 4–10 mM, and NMR spectra were recorded at 300 K and processed using XWINNMR software (Bruker). NMR experiments were performed to obtain information on conformationally relevant parameters such as proton–proton distances using ROE, coupling constants and temperature gradients of the H^N chemical shift to identify possible IHBs. The details of the NMR experiments are given in Ref. 33; the full NMR data are compiled in the Supporting Information. The assignment of the diastereotopic β-protons and the population of different rotamers of torsion angle χ_1 as calculated by Pachler's equations [37] using $^3J_g = 2.60$ Hz and $^3J_a = 13.56$ Hz is given in the Supporting Information. The proton–proton distances were used as constraints for the generation of an initial structure for MD calculations. All structural calculations and MD simulations were carried out using the InsightII Software package (Accelrys, San Diego, USA) and the GROMACS3 software package. The simulations were performed

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using the force field with automatic evaluation and cluster analysis of the trajectories. Starting structures were generated by torsion angle variation (φ with 60° increment) under experimental constraints and resulted in 3600 conformers. The structural dynamics was simulated in a cubic solvent box with periodic boundary conditions (a = 3.5 Å, ≈300 DMSO molecules) for 10 ps with constraints and for 1.2 ns in a free MD simulation. Snapshots of the trajectory were clustered. All structures correspond to the investigated proton–proton distances within an error margin of about 10%.

For validation, the structure of Hch was recalculated using the workflow described by Guthöhrlein *et al*. [38]. The starting structure for MD calculations was obtained by distance geometry calculations. One thousand structures fulfilling the experimental distance restraints derived from the ROE spectrum were generated using the program Xplor-NIH [39]. The two central structures obtained by cluster analysis (41 and 31%) were used as starting structures in restrained MD simulations (2.5 ns, stepsize 2 fs, truncated octahedral solvent box) using the GROMOS software package [40], with the force field GROMOS 45a3d [41]. The two trajectories were clustered according to their torsion angles (every 10th structure, deviation ±60°) [38]. The central structures were applied as starting structures in unrestrained MD calculations (10 ns, stepsize 2 fs, truncated octahedral solvent box), resulting in two different structure proposals after a final torsion angle clustering.

ECD Spectroscopy

ECD spectra in TFE were recorded on a Jasco J-810 spectropolarimeter using 0.2 mm quartz cells and concentrations of 1.19, 1.10, 2.82 and 2.30 mM for Hcp, Hch, Pcp and Pch, respectively. The raw spectra were subsequently smoothed by the Savitzky–Golay algorithm [42].

VCD Spectroscopy

VCD spectra at a resolution of 4 cm^{-1} were recorded in DMSO-d_6 solution with a Bruker PMA 37 VCD/PM-IRRAS module connected to an Equinox 55 FT-IR spectrometer. The ZnSe photoelastic modulator of the instrument was set to 1600 cm^{-1} and an optical filter with a transmission range of 1960–1250 cm^{-1} was used in order to increase the sensitivity in the amide I–II spectral region. The instrument was calibrated for VCD intensity with a CdS multiple-wave plate. A CaF_2 cell of 0.2 mm pathlength and peptide concentrations of 10 mg ml^{-1} (15.3 mM for Hcp, 15.0 mM for Hch, 18.5 mM for Pcp and 18.0 mM Pch) were used. In order to improve the S/N ratio, the spectra were averaged for 7 h (corresponding to ∼24 500 accumulated interferograms). Baseline correction was achieved by subtracting the spectrum of the solvent obtained under the same conditions. IR spectra were calculated from the single-channel DC spectra of the sample and solvent, respectively.

The theoretical VCD spectra were computed with the Gaussian 03 quantum chemical software package [25] at B3LYP/6-31G(d) DFT level of theory, using an integral equation formalism-polarizable continuum model (IEF-PCM) solvent model for DMSO, based on geometries optimized at the same quantum chemical level. The VCD spectra were simulated from the calculated rotatory strengths and wave numbers (scaled by a factor of 0.97) with Lorentzian curves of 16 cm^{-1} half-width at half-height values.

In the case of Hcp and Hch, the structures obtained by the NMR analysis were directly used as starting geometries for the DFT optimizations, whereas in the case of Pcp and Pch the NMR-based geometries were slightly modified (supposing a C_{11} H-bonded $\psi\beta$-turn between the CO group of Leu and the NH group of Asp instead of a non-H-bonded $\psi\beta$-turn).

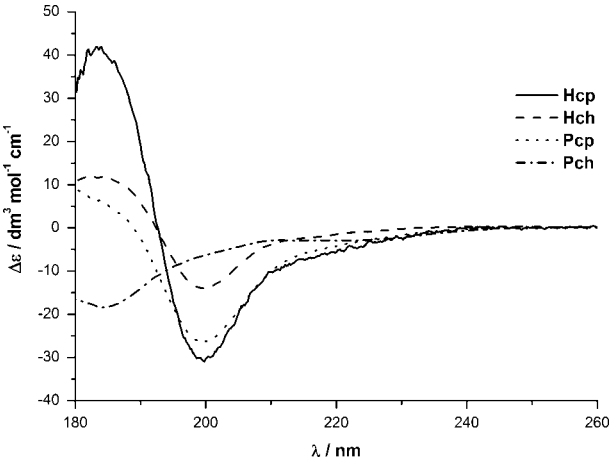

Figure 1. ECD spectra of Hcp, Hch, Pcp and Pch recorded in TFE.

Results and Discussion

ECD Spectroscopy

The ECD spectra in TFE of the peptides Hcp, Hch and Pcp show a negative band near 200 nm and a positive one below 190 nm (Figure 1). In the spectrum of the *trans*-Acpc hexapeptide Hcp, the positive band appears at approximately 185 nm, whereas in the case of the other peptides this band is much weaker and its position cannot be determined. The ECD spectra of the above three cyclic peptides are influenced more strongly by the *trans* geometry of the Acpc or Achc moieties than by the cyclic β-amino acid ring and the size of the macrocycle. The *trans*-Acpc or -Achc moieties appear to lock the local conformation of the -Ser-*trans*-(1*S*,2*S*)-Acpc-Leu- or -Ser-*trans*-(1*S*,2*S*)-Achc-Leu-segment. The ECD spectrum of the cyclic pentapeptide Pch comprising a *trans*-Achc moiety is significantly different. The main feature of the spectrum is a negative band at 184 nm accompanied by a shoulder at approximately 200 nm and a weak negative band at 222 nm.

Structures Determined by NMR Spectroscopy and MD Calculations

For cyclic penta- or hexapeptides comprising a *cis*- or *trans*-Achc moiety, NMR experiments have been performed earlier in DMSO-d_6 solution [33]. In peptide Pch where *trans*-configured Achc is incorporated, no predominant secondary structural element could be located. In the peptide Hch, the structure of which has been previously reported [33], the ring is big enough and *trans*-Achc occupies position $i + 1$ of a β-turn-like structure (C_{11} conformer, $\psi\beta$-turn) with the sequence Ser-*trans*-Achc-Leu-Asn. This turn is stabilized by a strong hydrogen bond Ser CO ← Asn NH as proven by the positive value of $\Delta\delta/\Delta T$ (+0.6 ppb K^{-1}). However, frequency calculations at DFT level failed to confirm this structure proposal.

Thus, structure analysis was repeated with a modified workflow, where the structures are clustered according to the torsion

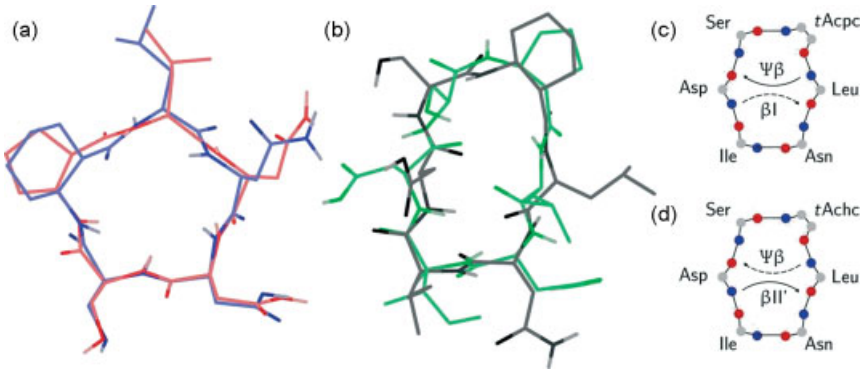

Figure 2. NMR structures, overlays of (a) Pcp (red) and Pch (blue), (b) Hcp (green) and Hch (black) and schematic representations of the secondary structure motifs found in (c) Hcp and (d) Hch.

clusters in the peptide backbone [38]. This method overcomes insufficiencies of conventional clustering according to the root mean square deviation of atomic coordinates in regard to the separation of different secondary structure motifs. Two structure proposals were obtained in this manner. The first one is similar to the previously calculated structure of Hch [33], but the second one differs from it, which allowed DFT-based geometry optimization and the calculation of VCD spectra. Further discussions of the hexapeptides are thus based on this second structure proposal.

An overlay of the hexapeptide structures is shown in Figure 2b. The structure proposals for the two hexapeptides Hcp and Hch show a β-turn with Asn in $i+1$ and Ile in $i+2$ position (Figure 2c and d). In Hcp, the β-turn is distorted but resembles type I, in Hch it is of type II′. The cyclic amino acid *trans*-Acpc in Hcp occupies the $i+2$ position of an H-bonded $\Psi\beta$-turn. A similar $\psi\beta$-turn is present in Hch, but it is distorted and thus, a C_{11} H-bonding cannot be observed.

The pentapeptides Pcp and Pch are flexible according to NMR and MD calculations and show no pronounced turn structure. However, the backbone conformation is similar in both peptides (Figure 2a).

VCD Spectroscopy

The FT-IR and VCD spectra of cyclic peptides Hcp, Hch, Pcp and Pch were measured in DMSO-d_6. The FT-IR spectra are dominated by an intense amide I band between 1668 and 1679 cm^{-1} accompanied by a definite short-wavenumber shoulder (Figure 3a). The amide II band near 1536 cm^{-1} also features a short-wavenumber shoulder. The band or shoulder at approximately 1717 cm^{-1} was tentatively assigned to the ν_{co} vibration of the side-chain COOH group of Asp. The common feature of the FT-IR spectra of cyclic peptides comprising a *trans*-Acpc moiety (Hcp and Pcp) is the somewhat better separation of the amide I band from the ν_{co}(COOH) band that is an indication of the less fixed local conformation of the Asp side chain that is not involved in strong IHB. The amide I band of the Asn side chain is not separated from the main amide I band of the backbone contribution.

The VCD spectra of the cyclic peptides in DMSO-d_6 are dominated by a negative amide I couplet between 1653 and 1682 cm^{-1} accompanied by a negative amide II band at approximately 1500 cm^{-1} and a weaker positive band near 1550 cm^{-1} (Figure 3b). In the spectrum of Hcp, the negative amide II band is much more intense than the negative wing of the amide I couplet. In the VCD spectrum of Pcp, the negative band of the couplet is more intense and two positive bands are present. In all VCD spectra, the negative amide II band is more intense than the positive one.

The cyclic models Hcp and Hch differ from Pcp and Pch in the size of the macrocycle. Hcp and Pcp contain *trans*-Acpc, whereas Hch and Pch contain *trans*-Achc. The common feature of the four cyclic peptides is the *trans*-configuration of the cyclic β-amino acids. The amide I and II regions of their VCD spectra appear to be determined by the *trans*-configuration of the Acpc and Achc moieties.

The calculated VCD spectra are in agreement with the measured ones concerning both the negative sign of the amide I couplet and the negative sign of the dominant amide II band (Figure 3b). The pattern of the weak bands, which can be assigned to the ν_{co} vibrations of the COOH group overlapping with the higher frequency amide I modes is slightly different in the spectra of the cyclic hexapeptides Hcp and Hch. Contrary to this, a well-defined negative ν_{co} band can be calculated at approximately 1751 cm^{-1} in the spectra of the cyclic pentapeptides Pcp and Pch. The carboxylic ν_{co} band, however, is not seen in the experimental VCD spectra (Figure 3b), which indicates a less definite orientation of the COOH group leading to cancellation of its VCD contribution.

Comparison of the Measured ECD and VCD Spectra

It is difficult to compare the ECD and VCD spectra because they were measured in TFE and DMSO-d_6, respectively. ECD spectra cannot be measured in DMSO because of its high absorbance below approximately 250 nm. The intensity of the negative band at approximately 200 nm is different in the spectra of Pcp, Hcp and Hch that, together with the different position and intensity of the positive band, may be a sign of different population of similar backbone conformers. The ECD spectrum of the cyclic pentapeptide Pch significantly differs from the ECD spectra of the other three peptides (Figure 1) that can be explained by a significantly different peptide backbone in TFE. Contrary to this, the VCD spectra in DMSO-d_6 of all four peptides are similar. It is probable that the VCD spectra are more influenced by the *trans*-configuration of the Acpc or Achc moieties, whereas the ECD spectra in TFE reflect a possible difference in the backbone conformation in the latter solvent.

Structures Derived from the Calculated VCD Spectra

In general, there is good agreement between the measured VCD spectra and those calculated for an NMR-based conformation

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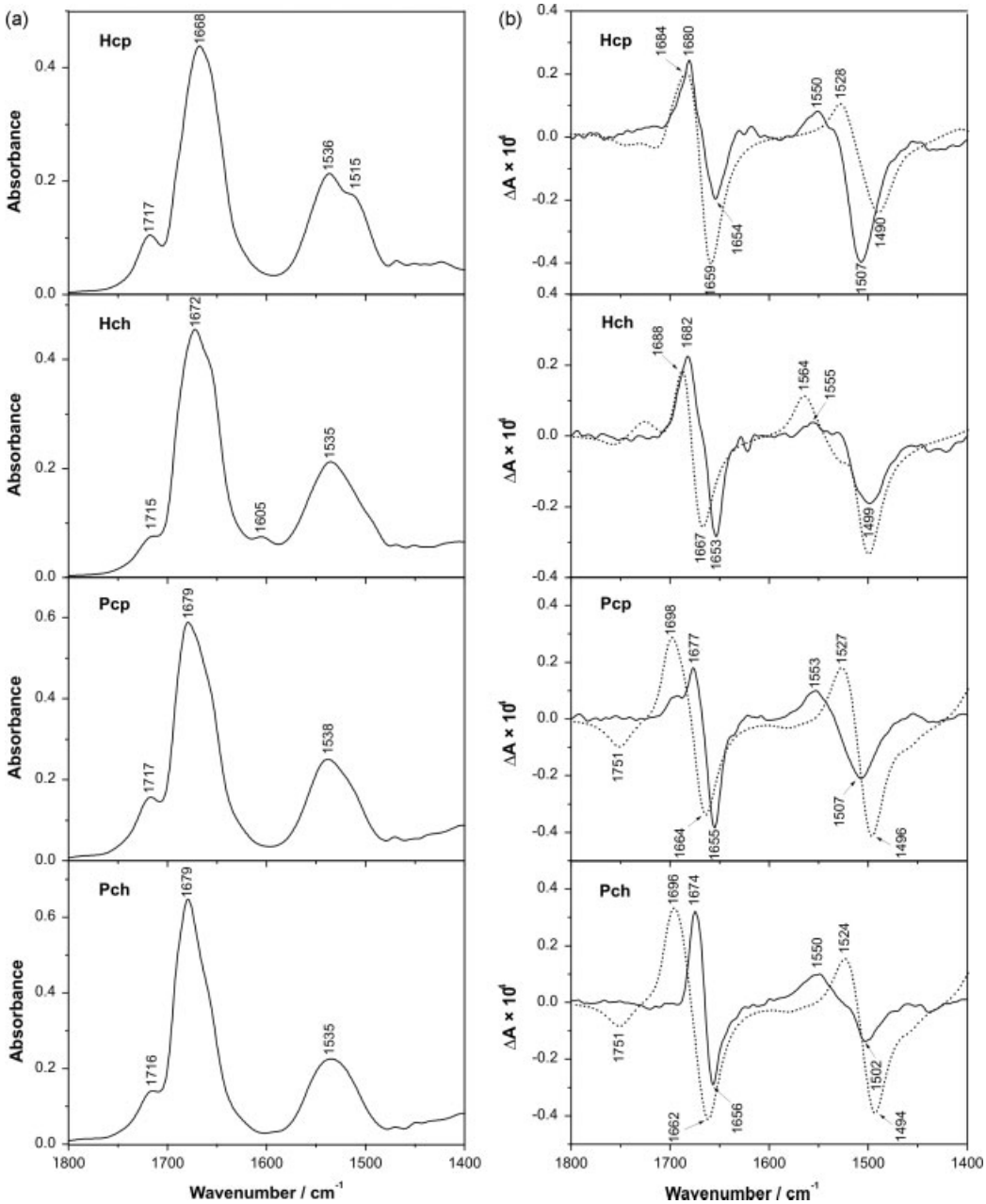

Figure 3. FT-IR (a) and VCD spectra (b) of Hcp, Hch, Pcp and Pch. Experimental spectra (obtained in DMSO-d_6) and calculated VCD spectra are shown with solid and dotted traces, respectively.

of Hcp (Figure 3). Figure 4 clearly demonstrates the close similarity between the structures obtained by NMR/MD and the conformations used for the calculation of the VCD spectra by DFT methods. Hcp features a distorted, non-H-bonded β-turn similar to type I (β I) encompassing residues Asn and Ile and a C_{11} H-bonded $\psi\beta$-turn encompassing Ser and *trans*-Acpc (Figure 4 and Supporting Information).

The VCD spectrum calculated for an optimized structure of Hch based on NMR data is in agreement with the experimental one (Figure 3). This structure features H-bonded type II′ β-turn and a $\psi\beta$-turn without IHB, where the *trans*-Achc is situated in the $i+2$ position (Figure 4 and Supporting Information).

The calculated conformation of Pcp is practically identical with that of Pch (Figures 2a and 4 and supporting information). The structures shown in Figure 4 differ only slightly with respect to amide bond orientations. The *trans*-Acpc and *trans*-Achc moieties of Pcp and Pch, respectively, are involved in $\psi\beta$-turn having a C_{11} H-bonding. Other backbone H-bonds are not formed. Side-chain IHB is possible but not likely because of the strong acceptor character of DMSO.

Conclusion

Two cyclic hexapeptides (Hcp and Hch) and two cyclic pentapeptides (Pcp and Pch) comprising *trans*-Acpc or *trans*-Achc, respectively, have been synthesized. NMR studies in DMSO-d_6 were performed to obtain information on conformationally relevant parameters such as proton–proton distances using ROE, coupling

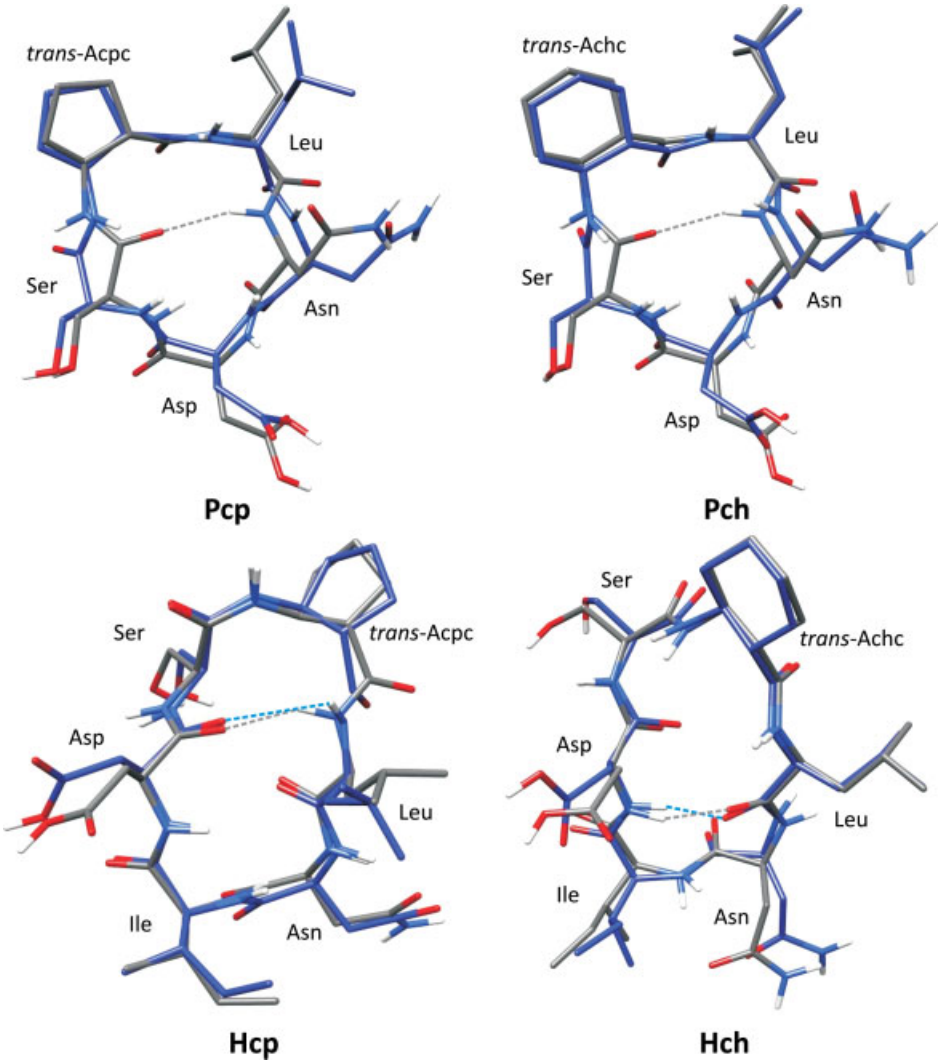

Figure 4. Overlay of the structure proposals of the cyclopeptides based on NMR spectroscopy in combination with MD calculations (blue) with the DFT-optimized structures used for the calculation of VCD spectra (gray).

constants and temperature gradients of the H^N chemical shift to identify possible IHBs. The proton–proton distances were used as constraints for the generation of an initial structure for MD calculations.

The ECD spectra of the peptides Hcp, Hch and Pcp in TFE show a characteristic negative band near 200 nm and a positive one below 190 nm (Figure 1). The ECD spectra of the three cyclic peptides are influenced more strongly by the *trans* geometry of the Acpc or Achc moieties. Contrary to this, the ECD spectrum in TFE of the constrained cyclic pentapeptide Pch is determined by the dominant conformation(s) of the peptide backbone.

The NMR and VCD spectra were measured in DMSO-d_6 at relatively high concentrations (4–10 and 18 mM, respectively). In this solvent, aggregation is not probable even at these high concentrations. This allows the comparison of the dominant NMR-based backbone conformations with those which were most suitable for calculating the VCD spectra. The calculated DFT-optimized VCD geometries of Hcp and Hch are very similar to those obtained by NMR/MD calculations (Figure 4). Both structures feature β-turns encompassing Asn and Ile and pseudo-β-turns ($\psi\beta$-turns) comprising *trans*-Acpc and *trans*-Achc, respectively.

The flexible NMR-based backbone conformation of the cyclic pentapeptides Pcp and Pch is similar but lacks a definite H-bonded β- or $\psi\beta$-turn. Contrary to this, the structures that yield the best fit between the experimental and calculated VCD spectra feature C_{11} H-bonded $\psi\beta$-turns (Figure 4). The NMR- and VCD-based backbone conformations of Pcp and Pch are somewhat different, but the differences are mostly limited to the orientation of one peptide bond (between the Ser and Acpc or Achc, respectively; Figure 4). In summary, the same sign pattern of the amide I and II VCD bands in the experimental VCD spectra of the four models suggests that the VCD spectra in DMSO are determined by the *trans*-configuration of Acpc and Achc, respectively, and the local conformation of the $\psi\beta$-turns formed. The method used for calculating the VCD spectra is powerful enough to give rise to a good agreement between the experimental and calculated spectra.

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Supporting information

Supporting information may be found in the online version of this article.

References

1 Seebach D, Beck AK, Bierbaum DJ. The world of β- and γ-peptides comprised of homologated proteinogenic amino acids and other components. *Chem. Biodivers.* 2004; **1**: 1111–1238. DOI: 10.1002/chin.200533334.
2 Cardillo G, Tomasini C. Asymmetric synthesis of β-amino acids and α-substituted β-amino acids. *Chem. Soc. Rev.* 1996; **25**: 117–128.
3 De Grado WF, Schneider JP, Hamuro Y. The twists and turns of β-peptides. *J. Pept. Res.* 1999; **54**: 206–217.
4 Seebach D, Hook DF, Glättli A. Helices and other secondary structures of β- and γ-peptides. *Biopolymers* 2006; **84**: 23–37. DOI: 10.1002/bip.20391.
5 Seebach D, Schreiber JV, Abele S, Daura X, van Gunsteren WF. Structure and conformation of β-oligopeptide derivatives with simple proteinogenic side chains: circular dichroism and molecular dynamics investigations. *Helv. Chim. Acta* 2000; **83**: 34–57.
6 Möhle K, Günter R, Thormann M, Sewald N, Hofmann HJ. Basic conformers in β-peptides. *Biopolymers* 1999; **50**: 167–184.
7 Appella DH, Christianson LA, Karle IL, Powell DR, Gellman SH. β-Peptide foldamers: robust helix formation in a new family of β-amino acid oligomers. *J. Am. Chem. Soc.* 1996; **118**: 13071–13072. DOI: 10.1021/ja963290l.
8 Graf von Roedern E, Lohof E, Hessler G, Hoffman M, Kessler H. Synthesis and conformational analysis of linear and cyclic peptides containing sugar amino acids. *J. Am. Chem. Soc.* 1996; **118**: 10156–10167. DOI: 10.1021/ja961068a.
9 Schumann F, Müller A, Koksch M, Sewald N. Are β-amino acids γ-turn mimetics? Exploring a new design principle for bioactive cyclopeptides. *J. Am. Chem. Soc.* 2000; **122**: 12009–12010. DOI: 10.1021/ja0016001.
10 Frackenpohl J, Arvidsson PI, Schreiber JV, Seebach D. The outstanding biological stability of beta- and gamma-peptides toward proteolytic enzymes: an in vitro investigation with fifteen peptidases. *Chembiochem.* 2001; **2**: 445–455. DOI: 10.1002/1439–7633.
11 Arnold U, Hinderaker MP, Nilsson BL, Huck BR, Gellman SH, Raines RT. Protein prosthesis: a semisynthetic enzyme with a β-peptide reverse turn. *J. Am. Chem. Soc.* 2002; **124**: 8522–8523. DOI: 10.1021/ja026114n.
12 Iwamoto T, Tsujii E, Ezaki M, Fujie A, Hashimoto S, Okuhara M, Kohsaka M, Imanaka H, Kawabata K, Inamoto Y, Sakane K. FR109615. A new antifungal antibiotic from *Streptomyces setonii*. *J. Antibiot.* 1990; **43**: 1–7.
13 Gellman SH. Foldamers: a manifesto. *Acc. Chem. Res.* 1998; **31**: 173–180. DOI: 10.1021/ar960298r.
14 Cheng RP, Gellman SH, DeGrado WF. β-Peptides: from structure to function. *Chem. Rev.* 2001; **101**: 3219–3232. DOI: 10.1021/cr000045i.
15 Fülöp F. The chemistry of 2-aminocycloalkanecarboxylic acids. *Chem. Rev.* 2001; **101**: 2181–2204. DOI: 10.1021/cr000456z.
16 Martinek TA, Fülöp F. Side-chain control of β-peptide secondary structures: design principles. *Eur. J. Biochem.* 2001; **270**: 3657–3666.
17 Fülöp F, Martinek TA, Tóth GK. Application of alicyclic β-amino acids in peptide chemistry. *Chem. Soc. Rev.* 2009; **35**: 323–334.
18 Brückner AM, Schmitt HW, Diederichsen U. Nucleo-β-amino acids: synthesis and oligomerization to β-homoalanyl-PNA. *Helv. Chim. Acta* 2002; **85**: 3855–3866. DOI: 10.1002/1522-2675.
19 Sewald N, Jakubke HD. *Peptides: chemistry and biology*, 2nd edn, Weinheim: Wiley-VCH: 2009.
20 Maleševi' M, Majer Z, Vass E, Huber T, Strijowski U, Hollósi M, Sewald N. Spectroscopic detection of Pseudo-turns in homodetic cyclic penta- and hexapeptides comprising β-homoproline. *Int. J. Pept. Res. Ther.* 2006; **12**: 165–177. DOI: 10.1007/s10989-006-9013-8.
21 Christianson LA, Lucero MJ, Appella DH, Klein DA, Gellman SH. Improved treatment of cyclic β-amino acids and successful prediction of β-peptide secondary structure using a modified force field: AMBER*C. *J. Comput. Chem.* 2000; **21**: 763–773. DOI: 10.1002/(SICI)1096-987X.
22 Martinek TA, Tóth GK, Vass E, Hollósi M, Fülöp F. cis-2-Aminocyclopentanecarboxylic acid oligomers adopt a sheetlike structure: switch from helix to nonpolar strand. *Angew. Chem. Int. Ed.* 2002; **41**: 1718–1721. DOI: 10.1002/1521-3773.
23 Nafie LA, Freedman TB. Vibrational circular dichroism: an incisive tool for stereochemical applications. *Enantiomer* 1998; **3**: 283–297.
24 Bouo P, Kim J, Kapitán J, Hammer RP, Huang R, Wu L, Keiderling TA. Vibrational circular dichroism and IR spectral analysis as a test of theoretical conformational modeling for a cyclic hexapeptide. *Chirality* 2008; **20**: 1104–1119.
25 Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA. *Gaussian 03, Revision D.01*. Wallingford, CT: Gaussian, Inc.: 2004.
26 Keiderling TA. Protein and peptide secondary structure and conformational determination with vibrational circular dichroism. *Curr. Opin. Chem. Biol.* 2002; **6**: 682–688. DOI: 10.1016/S1367-5931(02)00369-1.
27 Vass E, Hollósi M, Besson F, Buchet R. Vibrational spectroscopic detection of beta- and gamma-turns in synthetic and natural peptides and proteins. *Chem. Rev.* 2003; **103**: 1917–1954. DOI: 10.1021/cr000100n.
28 Borics A, Murphy RF, Lovas S. Optical spectroscopic elucidation of β-turns in disulfide bridged cyclic tetrapeptides. *Biopolymers* 2006; **85**: 1–11.
29 Wyssbrod HR, Diem M. IR (vibrational) CD of peptide β-turns: A theoretical and experimental study of cyclo-(-Gly-Pro-Gly-D-Ala-Pro-). *Biopolymers* 1992; **31**: 1237–1242.
30 Xie P, Zhou Q, Diem M. IR circular dichroism of turns in small peptides. *Faraday Discuss.* 1994; **99**: 233–243. DOI: 10.1039/FD9949900233.
31 Hilario J, Kubelka J, Keiderling TA. Optical spectroscopic investigations of model β-sheet hairpins in aqueous solution. *J. Am. Chem. Soc.* 2003; **125**: 7562–7574. DOI: 10.1021/ja030039e.
32 Zhao C, Polavarapu PL, Das C, Balaram P. Vibrational circular dichroism of α-hairpin peptide. *J. Am. Chem. Soc.* 2000; **122**: 8228–8231. DOI: 10.1021/ja000451j.
33 Strijowski U, Sewald N. Structural properties of cyclic peptides containing *cis*- or *trans*-2-aminocyclohexane carboxylic acid; *Org. Biomol. Chem.* 2004; **2**: 1105–1109. DOI: 10.1039/b312432k. Corrigendum: www.rsc.org/suppdata/OB/b3/b312432k/addition.htm.
34 Davies SG, Ichihara O, Lenoir I, Walters IAS. Asymmetric synthesis of (−)-(1*R*,2*S*)-cispentacin and related *cis*- and *trans*-2-amino cyclopentane- and cyclohexane-1-carboxylic acids. *J. Chem. Soc. Perkin Trans. 1*. 1994; 1411–1415.
35 Milton RC, Becker E, Milton SCF, Baxter JEJ. Improved purities for Fmoc-amino acids from Fmoc-ONSu. *Int. J. Pept. Prot. Res.* 1987; **30**: 431–432. DOI: 10.1111/j.1399–3011.1987.tb03351.x.
36 Malešević M, Strijowski U, Bächle D, Sewald N. An improved method for the solution cyclization of peptides under pseudo-high dilution conditions. *J. Biotechnol.* 2004; **112**: 73–77. DOI: 10.1016/j.jbiotec.2004.03.015.
37 Pachler KGR. The dependence of vicinal proton-proton coupling constants on dihedral angle and substituents. *J. Chem. Soc. Perkin Trans. 2*. 1972; 1936–1940. DOI: 10.1039/P29720001936.
38 Guthöhrlein EW, Malešević M, Majer Z, Sewald N. Secondary structure inducing potential of β-amino acids: torsion angle

clustering facilitates comparison and analysis of the conformation during MD trajectories. *Biopolymers* 2007; **88**: 829–839. DOI: 10.1002/bip.20859.

39 Schwieters CD, Kuszewski JJ, Tjandra N, Clore GM. The Xplor-NIH NMR molecular structure determination package. *J. Magn. Res.* 2003; **160**: 65–73.

40 Schuler LD, Daura X, van Gunsteren WF. An improved GROMOS96 force field for aliphatic hydrocarbons in the condensed phase. *J. Comput. Chem.* 2001; **22**: 1205–1218.

41 Geerke DP, Oostenbrink C, van der Vegt NFA, van Gunsteren WF. An effective force field for molecular dynamics simulations of dimethyl sulfoxide and dimethyl sulfoxide-water mixtures. *J. Phys. Chem. B.* 2004; **108**: 1436–1445.

42 Savitzky A, Golay MJE. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* 1964; **36**: 1627–1639.

