Deracemization and First CD Spectrum of a 3_{10}-Helical Peptide Made of Achiral α-Amino-Isobutyric Acid Residues in a Chiral Membrane Mimetic Environment

Francesca Ceccacci,[a] Giovanna Mancini[a,]*, Paola Rossi,[b] Paolo Scrimin,[c,]*Alessandro Sorrenti,[a] Paolo Tecilla[b,]*

[a] CNR, Istituto di Metodologie Chimiche, c/o University of Rome "Sapienza" Department of Chemistry, p.le A. Moro 5, I-00165 Roma, Italy

[b]University of Trieste, Department of Chemical and Pharmaceutical Sciences, via Giorgieri 1, I-34127 Trieste, Italy

[c]University of Padova, Department of Chemical Sciences, via Marzolo 1, I-35131 Padova, Italy

Supplementary Information

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Materials and general methods

All commercially available reagents were purchased from *Aldrich*, *Fluka* and *Strem Chemicals* and used without purification unless otherwise mentioned.

Solvents were purchased from *Aldrich*, *VWR*, *Fluka* and *Riedel*, and deuterated solvents from *Cambridge Isotope Laboratories* and *Aldrich*. Reactions were monitored by TLC on *Merck* silica

gel plates (0.25 mm). Chromatography was performed on *Merck* silica gel 60F-254 (230÷400 Mesh) and the solvents employed were of analytical grade.

NMR spectra were recorded on a Jeol 400 spectrometer (operating at 400 MHz for proton and at 100 MHz for carbon). Chemical shifts (δ) are reported in ppm using the solvent residual signal as an internal reference ($CDCl_3$: δH 7.26, δC 77.16; CD_3OD: δH 3.31, δC 49.00; DMSO-d_6 δH 2.50, δC 39.52) and the multiplicity of each signal is designated by the conventional abbreviations: *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet; *br*, broad; *dd*, doublet of doublets. Coupling constants (*J*) are quoted in Hz. Electrospray Ionization (ESI) measurements were performed on a *Perkin Elmer* APII at 5600 eV. Melting points (m.p.) were measured with a *Büchi* SHP-20 apparatus and are not corrected. IR spectra were recorded on a Shimadzu-FTIR 8400 S infrared spectrophotometer. Circular dichroism (CD) spectra were recorded on Jasco J-715 spectropolarimeter.

Sodium *N*-dodecanoylprolinate was prepared as previously described.[S1]

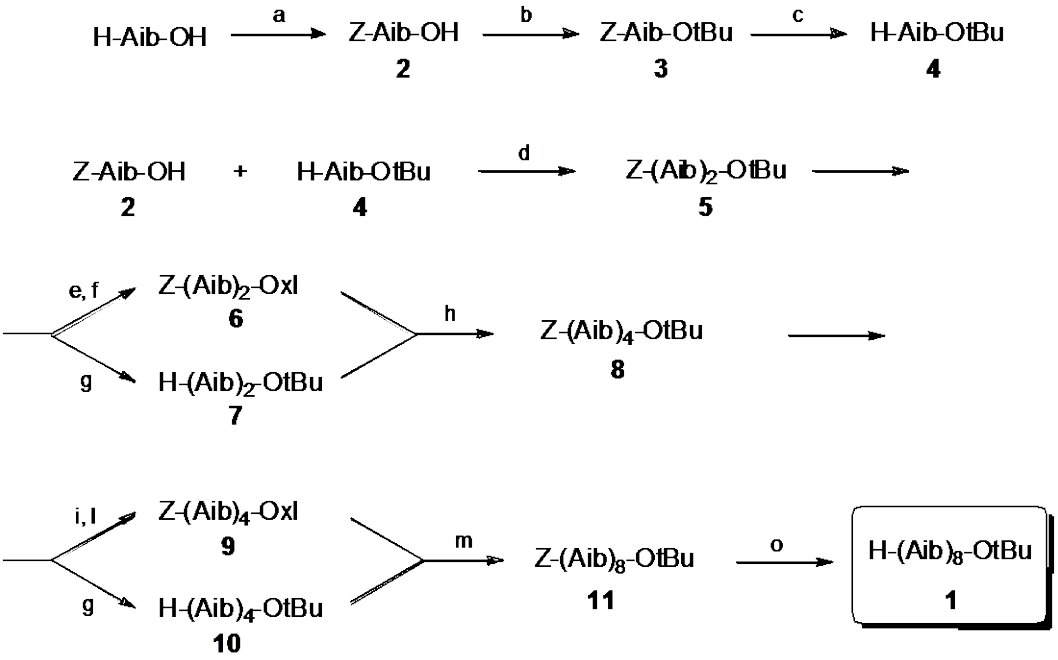
Circular Dichroism experiments

The CD spectra of the aqueous solutions of both enantiomers of surfactant **2** (25 mM) were recorded in the absence and in the presence of **H-(Aib)$_8$-OtBu** (0.5 mM) in quartz cuvettes (path length 0.02 cm); accumulations: 40; step size: 0.2 nm.

The samples containing **H-(Aib)$_8$-OtBu** were prepared by adding 2 mL of a 25 mM aqueous solution of surfactant to the proper amount of **H-(Aib)$_8$-OtBu** (prepared from 20 μL of a 50 mM ethanol **H-(Aib)$_8$-OtBu** stock solution, dried by a nitrogen flux) to obtain a concentration 0.5 mM of the peptide. The CD spectra of the **H-(Aib)$_8$-OtBu** helix were obtained by subtracting the spectrum of the surfactant aqueous solution in the absence of **H-(Aib)$_8$-OtBu** from that of **H-(Aib)$_8$-OtBu** in the solution of surfactant.

Synthesis

The H-(Aib)$_8$-OtBu peptide[S2] was synthesized by conventional solution chemistry using the procedure reported by Toniolo and coll.[S3] for the preparation of Z-(Aib)$_8$-OtBu followed by final removal of the Z-protecting group (Scheme 1). For an alternative approach see ref. S4.



Scheme 1. a) Z-OSu, CH_3CN/H_2O, r.t., 24 h, 54%; b) isobutene, H_2SO_4, CH_2Cl_2, r.t., 67 h, 85%; c) H_2, Pd/C, CH_2Cl_2, r.t., o.n., quant.; d) EDC, HOBt, NEt_3, CH_2Cl_2, 0 °C – r.t., 3 gg., 38%; e) TFA, CH_2Cl_2, r.t., 6 h, quant.; f) EDC, CH_2Cl_2, r.t., 24 h, 76%; g) H_2, Pd/C, CH_3OH, r.t., o.n., 97%; h) CH_3CN, reflux, 24 h, 82%; i) TFA, CH_2Cl_2, r.t., 4 h, quant.; l) EDC, CH_2Cl_2, 0 °C – r.t., 12 h, 75%; m) CH_3CN, reflux, 4 h, 86%; o) H_2, Pd/C, CH_3OH, r.t., o.n., 97%.

Z-Aib-OH (2)

To a solution of 15 g of H-Aib-OH (MW: 103.12; 0.14 mol) in 120 mL of water were first added 19.53 mL of NEt_3 (MW: 101.19; 0.14 mol) and then, drop by drop, a solution of 34.9 g of N-(benzyloxycarbonyloxy)succinimide (Z-OSu, MW: 249.22; 0.14 mol) in 80 mL of CH_3CN. The reaction mixture was stirred at r.t. for 24 h. monitoring the reaction by TLC (9/1, $CHCl_3/CH_3OH$). The reaction mixture was then concentrated to eliminate the acetonitrile, made basic by addition of 5% $NaHCO_3$ and extracted three times with Et_2O. The aqueous phase was acidified to pH 3 with 10% $KHSO_4$ and extracted with AcOEt (3 x 200 mL). The organic phase was washed with water (6 x 100 mL), dried over anhydrous Na_2SO_4 and the solvent removed. The solid obtained was recrystallized from Et_2O/hexane affording 17.97 g of **2** (MW: 237.25; 0.07 mol) as a white solid.

Yield 54 %. **m.p.** 84 - 85 °C. **R_f**: 0.6 (CH_3Cl/CH_3OH; 9/1). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ = 8.79 (br, 1H, COOH); 7.34 (m, 5H, Ph); 5.35 (s, 1H, NH); 5.1 (s, 2H, Ph*CH_2*O), 1.54 (s, 6H, $2CH_3$).

Z-Aib-OtBu (3)

A solution of 47 g of **2** (MW: 237.25; 0.20 mol) in 400 mL of CH_2Cl_2 containing 2 mL of concentrated H_2SO_4 was saturated with isobutene and kept in a pressure-vial at room temperature under stirring for 67 h. After leaving evaporate under the hood the excess of isobutene, the reaction mixture was washed with 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and the solvent removed. The residue was recrystallized from hexane affording 50.5 g of pure Z-Aib-OtBu (**3**) as a white solid (MW: 293.36; 0.17 mol). Yield = 85 %. **P.f.**: 60 - 61 °C. **IR** (KBr): 3371, 1714, 1520 cm^{-1}. **^{1}H-NMR** ($CDCl_3$; 400 MHz): δ = 7.32 (m, 5H, Ph); 5.46 (brs, 1H, NH); 5.08 (s, 2H, Ph*CH_2*O); 1.51 (s, 6H, $2CH_3$); 1.43 (s, 9H, tBu).

Z-$(Aib)_2$-OtBu (5)

To a solution of 13 g of **3** (MW: 293.36; 44 mmol) in 300 ml of anhydrous CH_2Cl_2, 1 g of 5% Pd/C was added. The flask was evacuated and flushed with hydrogen three times. The reaction mixture was stirred vigorously, under hydrogen atmosphere, overnight. It was filtered through a short pad of Celite® and the solvent removed to afford 7 g of H-Aib-OtBu (**4**) (MW: 159.23; 44 mmol) with quantitative yield. R_f: 0.2 ($CHCl_3/CH_3OH$; 95/5).

To a solution of 10.4 g of **2** (MW: 237.2; 44 mmol) in 10 mL of anhydrous CH_2Cl_2 cooled at O °C and kept under stirring were added 5.5 g of HOBt (MW: 135.12; 44 mmol) and 10.84 g of EDC (MW: 191.70; 52.8 mmol). After waiting ten minutes, 7 g of **4** (MW: 159.23; 44 mmol) and 6.12 mL di NEt_3 (MW: 101.19; 44 mmol) dissolved in 15 mL of anhydrous CH_2Cl_2 were added to the reaction mixture. The reaction mixture was stirred at r.t. for three days monitoring the reaction by TLC ($CHCl_3/CH_3OH$; 95/5). The solvent was then evaporated and the residue dissolved in AcOEt (500 mL) and washed with $KHSO_4$ 5% (3 x 200 mL), H_2O (2 x 100 mL) and $NaHCO_3$ 5% (3 x 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent removed. The residue was recrystallized from AcOEt/hexane affording 6.3 g of pure Z-$(Aib)_2$-OtBu (**5**) (MW: 378.46; 16.6 mmol) as a white solid. Yield = 38 %. **m.p.**: 134 - 135 °C. **R_f**: 0.9 ($CHCl_3/CH_3OH$, 95/5). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ = 7.36 (m, 5H, Ph); 6.93 (s, 1H, OCNH); 5.41 (s, 1H, OOCNH); 5.1 (s, 2H, Ph*CH_2*O); 1.47 (m, 21H, $4CH_3$ and tBu). **^{13}C-NMR** ($CDCl_3$,): 173.7, 173.0, 155.8, 136.2, 128.4, 128.0, 127.9, 81.4, 66.4, 56.7, 56.6, 27.7, 25.3, 25.2, 24.0.

Z-Aib$_2$-Oxl (6)

2.5 g of **5** (MW: 378.46; 6.6 mmol) were dissolved in 10 mL of a 1:1 CH_2Cl_2/TFA mixture and stirred at room temperature for 6 h monitoring the reaction by TLC ($CHCl_3$/CH_3OH; 9/1). The solvent was removed and the residue co-evaporated several times with Et_2O, until all the TFA traces have been removed, affording Z-(Aib)$_2$-H in quantitative yield (2.13 g, MW: 322.36; 6.6 mmol). The thus obtained acid was suspended in 15 mL of anhydrous CH_2Cl_2 and 1.39 g of EDC (MW: 191.70; 7.26 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed and the residue dissolved in 100 mL of AcOEt and washed with $KHSO_4$ 5% (2 x 50 mL), H_2O (1 x 50 mL), $NaHCO_3$ 5% (2 x 50 mL), H_2O (1 x 50 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent removed affording 1.6 g of pure Z-(Aib)$_2$-Oxl (**6**, MW: 304.34; 5.2 mmol) as a white solid. Yield = 76 %. **m.p.**: 121-122 °C. **R$_f$**: 0.9 ($CHCl_3$/CH_3OH; 9/1); 0.7 (toluene/EtOH; 7/1). **^{1}H-NMR** ($CDCl_3$; 400 MHz): δ=7.33 (m, 5H, Ph); 5.34 (br, 1H, NH); 5.07 (s, 2H, Ph*C*H_2O); 1.61 (s, 6H, 2CH_3); 1.41 (s, 6H, 2CH_3).

Z-(Aib)$_4$-OtBu (8)

To a solution of 2.5 g of **5** (MW: 378.46; 6.6 mmol) in 50 ml of CH_3OH, 0.25 g of 5% Pd/C was added. The flask was evacuated and flushed with hydrogen three times. The reaction mixture was stirred under hydrogen atmosphere, overnight. It was filtered through a short pad of Celite® and the solvent removed to afford 1.56 g of H-(Aib)$_2$-OtBu (**7**) (MW: 244.33; 6.4 mmol) with 97% yield. The deprotected dipeptide was dissolved in 10 mL of anhydrous acetonitrile and 1.65 g of oxazolone **6** (MW: 304.34; 5.2 mmol) were added. The reaction mixture was heated at reflux for 24 h monitoring the reaction by TLC ($CHCl_3$/CH_3OH; 98/2). The solvent was evaporated, the residue dissolved in 150 mL of AcOEt and washed with $KHSO_4$ 5% (4 x 70 mL) and H_2O (2 x 70 mL). The organic phase was dried over anhydrous Na_2SO_4, the solvent removed and the crude product was purified by column chromatography (silica, $CHCl_3$/MeOH from 100/0 to 98/2) affording 2.3 g of Z-(Aib)$_4$-OtBu (**8**) (MW: 548.67; 4.1 mmol) as a white solid. Yield = 82 %. **m.p.**: 172 -173°C. **R$_f$**: 0.4 ($CHCl_3$/CH_3OH; 98/2). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ = 7.34 (m, 5H, Ph); 7.20 (s, 1H, NH); 7.10 (s, 1H, NH); 6.31 (s,1H, NH); 5.20 (s, 1H, NH); 5.11 (s, 2H, Ph*C*H_2O); 1.46 (m, 33H, t-Bu, 8CH_3). **^{13}C-NMR**: δ 174.1, 174.0, 173.7, 172.7, 155.8, 136.2, 128.5, 128.3, 128.1, 79.8, 67.0, 57.1, 56.5, 56.0, 27.8, 25.3, 25.0, 24.9, 24.7. **ESI-MS** (CH_3OH): m/z 549.3 ($M+H^+$), 571.3 ($M+Na^+$), 587.2 ($M+K^+$).

Z-(Aib)$_4$-Oxl (9)

1.16 g of **8** (MW: 548.67; 2.1 mmol) were dissolved in 10 mL of a 1:1 CH_2Cl_2/TFA mixture and stirred at room temperature for 4 h monitoring the reaction by TLC ($CHCl_3$/CH_3OH; 9/1). The solvent was removed and the residue co-evaporated several times with Et_2O, until all the TFA traces have been removed, affording Z-(Aib)$_4$-H in quantitative yield (1.035 g, MW: 492.57; 2.1 mmol). The thus obtained acid was suspended in 20 mL of anhydrous CH_2Cl_2 and 0.443 g of EDC (MW: 191.70; 2.31 mmol) were added. The reaction mixture was stirred at room temperature for 12 h. The solvent was removed and the residue dissolved in 150 mL of AcOEt and washed with $KHSO_4$ 5% (2 x 80 mL), H_2O (1 x 80 mL), $NaHCO_3$ 5% (2 x 80 mL), H_2O (1 x 80 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent removed affording 0.747 g of pure Z-(Aib)$_4$-Oxl (**9**, MW: 474.55; 1.57 mmol) as a white solid. Yield = 75 %. **m.p.**: 152-153°C. **R_f**: 0.9 ($CHCl_3$/CH_3OH; 9/1). **^{1}H-NMR** ($CDCl_3$; 400 MHz): δ 7.36 (m, 5H, Ph); 7.31 (s, 1H, NH); 6.26 (s, 1H, NH); 5.24 (s, 1H, NH); 5.11 (s, 2H, Ph*CH$_2$*O); 1.45 (m, 24H, 8CH_3).

Z-(Aib)$_8$-OtBu (11)

To a solution of 1.16 g of **8** (MW: 548.67; 2.1 mmol) in 30 ml of CH_3OH, 0.12 g of 5% Pd/C was added. The flask was evacuated and flushed with hydrogen three times. The reaction mixture was stirred under hydrogen atmosphere, overnight. It was filtered through a short pad of Celite® and the solvent removed to afford 0.807 g of H-(Aib)$_4$-OtBu (**10**) (MW: 414.54; 1.94 mmol) with 92 % yield. The deprotected tetrapeptide was dissolved in 5 mL of anhydrous acetonitrile and 0.747 g of oxazolone **9** (MW: 474.55; 1.5 mmol) dissolved in 15 mL of CH_3CN were added. The reaction mixture was heated at reflux for 24 h monitoring the reaction by TLC ($CHCl_3$/CH_3OH; 9/1). The solvent was concentrated to about 10 mL and a white precipitate was filtered off. The precipitate was dissolved in 150 mL of $CHCl_3$ and washed with $KHSO_4$ 5% (2 x 90 mL), H_2O (1 x 90 mL), $NaHCO_3$ 5% (2 x 90 mL) and H_2O (1 x 90 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent removed to afford 1.2 g of Z-(Aib)$_8$-OtBu (**11**) (MW: 889.09; 1.35 mmol) as a white solid. Yield = 86 %. **m.p.**: 247 - 248°C. **R_f**: 0.5 (CH_3Cl/CH_3OH; 9/1). **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ 7.62 (s, 1H, NH); 7.57 (s, 1H, NH); 7.55 (s, 1H, NH); 7.33 (m, 7H, 2NH, 5H Ph); 6.38 (s, 1H, NH); 5.43 (s,1H, NH); 5.12 (s, 2H, Ph*CH$_2$*O); 1.47 (m, 57H: 9H, tBu, 48H, 16CH_3). **^{13}C-NMR** ($CDCl_3$): δ 175.6; 175.3; 175.3; 174.4; 174.3; 174.2; 174.2; 174.2; 156.1; 136.1; 128.6; 128.5; 128.1; 79.6; 67.3; 57.2; 56.7; 56.6; 56.5; 56.4; 56; 27.8; 25.4; 25.3; 24.9; 24.8; 24.8; 24.7; 24.7; 24.6; 24.6. **ESI-MS** (CH_3OH): m/z 889.6 ($M+H^+$), 906.7 ($M+NH_4^+$), 911.8 ($M+Na^+$), 927.7 ($M+K^+$), 464.5 ($M+K^+$)/2.

H-(Aib)$_8$-OtBu (1)

To a solution of 0.60 g of **11** (MW: 889.09; 0.65 mmol) in 15 ml of CH_3OH, 0.06 g of 5% Pd/C was added. The flask was evacuated and flushed with hydrogen three times. The reaction mixture was stirred under hydrogen atmosphere, overnight. It was filtered through a short pad of Celite® and the solvent removed to afford 0.47 g of H-(Aib)$_8$-O*t*Bu (**1**) (MW: 754.98; 0.62 mmol) as a white solid. Yield = 97 %. **m.p.**: 231 - 232°C. **R_f**: 0.1 (CH_2Cl_2/CH_3OH; 9/1). **^{1}H-NMR** (CD_3OD, 300 MHz): δ 8.01 (s, 1H, NH); 7.97 (s, 1H, NH); 7.91(s, 1H, NH); 7.89 (s, 1H, NH); 7.82 (s, 1H, NH); 7.72 (s, 1H, NH); 7.67 (s, 1H, NH); 1.50 (s, 6H, $2CH_3$); 1.49 (s, 6H, $2CH_3$); 1.48 (s, 6H, $2CH_3$); 1.47 (s, 6H, $2CH_3$); 1.46 (s, 6H, $2CH_3$); 1.45(s, 12H, $4CH_3$); 1.43 (s, 9H, tBu); 1.42 (s, 6H, $2CH_3$).
^{13}C-NMR (CD_3OD): δ 177.6; 177.5; 177.4; 176.9; 176.8; 176.4; 176.3; 175.5; 81.1; 58.0; 57.9; 57.8; 57.7; 57.6; 57.5; 56.9; 28.2; 26.4; 25.9; 25.6; 25.4; 25.3; 25.2; 25.1; 24.9.
ESI-MS (CH_3OH): m/z 755.4 ($M+H^+$), 777.4 ($M+Na^+$), 793.4 ($M+K^+$).
IR ($CHCl_3$): (cm^{-1})= 3323, 2990, 2936, 1716, 1663,1533, 1456, 1385, 1364, 1151.

Characterization data for the same compound can also be found in ref. S4. We note a discrepancy in the m.p. In ref S4 the reported m.p. is 212-214 °C.

IR spectra of H-(Aib)$_8$-OtBu

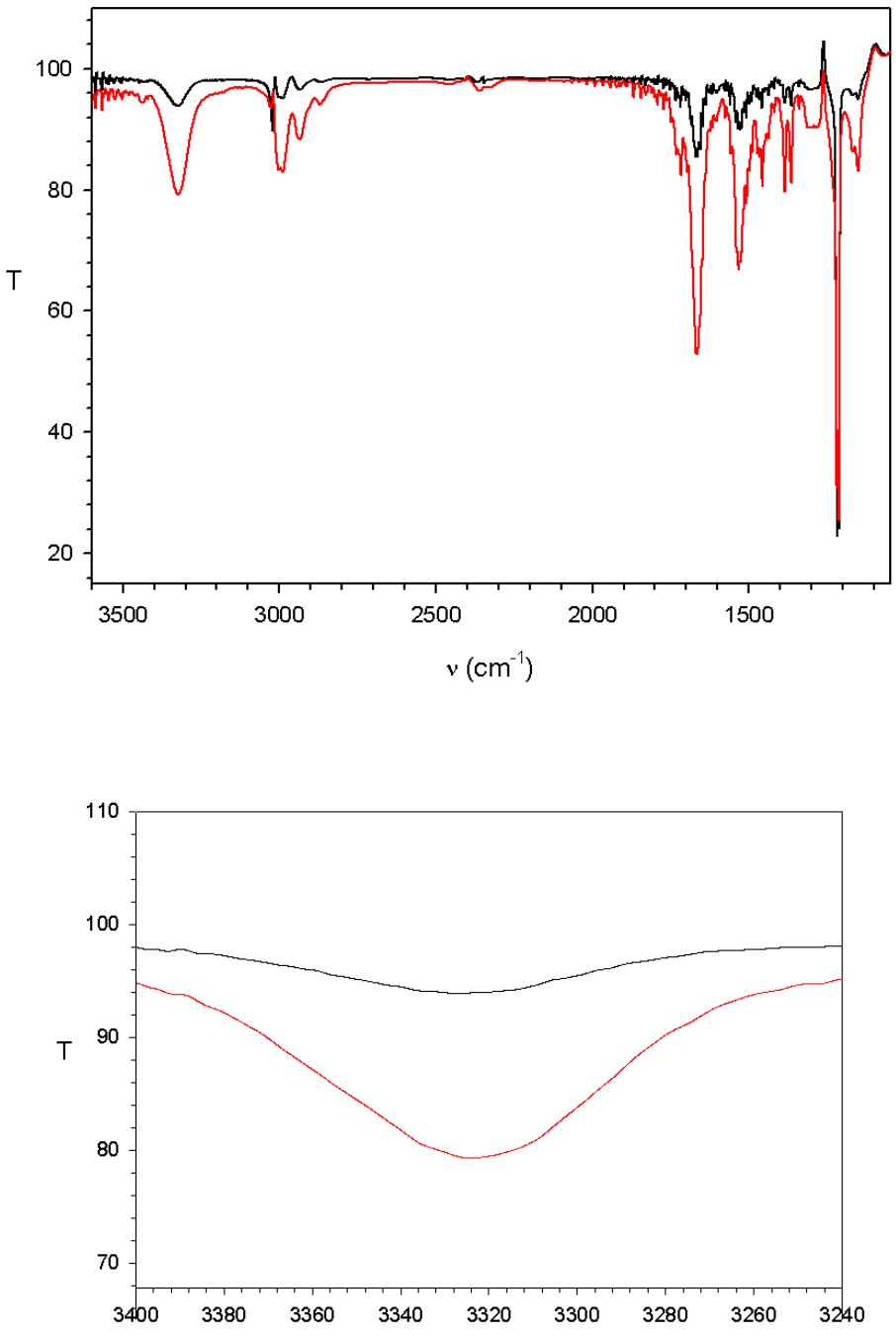


Figure S1. Top: IR spectra in $CHCl_3$ of H-(Aib)$_8$-OtBu (**1**) at 4 mM (black trace) and 18 mM (red trace) concentrations. Bottom: enlargement of the NH resonance region showing independence of the resonance frequency from the concentration of peptide.

CD Spectra

The choice of the experimental conditions to perform the CD experiments has been a very delicate issue because we had to use a concentration of surfactant higher than cmc (1 mM), to ensure the formation of the micelles, and a low peptide/surfactant ratio to solubilize completely the otherwise water insoluble peptide **1**. This was further complicated by the fact that the carboxylic group of the surfactant absorbs in the same spectral region of the peptide bond and also by its strong contribution to the CD signal which has to subtracted to the overall CD signal measured in the case of the peptide/surfactant co-micellar solution. After several attempts the better conditions were found using 0.5 mM **1**, 25 mM **2** and a 0.02 cm pathlength quartz cuvette. In this condition the contribution of **2** to the CD signal is about 25 times higher than that of the helical peptide (Fig S2) but the difference spectra show clearly and reproducibly the peptide band with a reasonable signal to noise ratio (Figure 1). The use of cuvettes with longer pathlengths lead to difference spectra showing similar profiles but with an unacceptable signal to noise ratio (see Fig S3). On the other hand, attempts to lower the peptide/surfactant ratio lead to the precipitation of the peptide from the solution.

Because the lower intensity (4% less intense) of the CD spectra obtained in the presence of **1** (dotted lines in Figure S2) with respect to the spectra of the pure surfactants (solid lines in Figure S2) could be ascribed to the dilution of the surfactants due to an increase of volume induced by the presence of **1**, we simulated such a dilution by multiplying the spectrum of surfactant L-**2** by 0.96. We then generated a difference spectrum by subtracting of the spectrum of a 25 mM aqueous solution of L-**2** from the trace obtained by multiplying the same spectrum for 0.96. The result obtained is shown in Figure S4. The spectrum differs substantially from that of Figure 1 clearly demonstrating that the 3_{10} helix signature observed is not due to artefacts generated by the subtraction of the contribution of the chiral surfactant from the total CD spectrum.

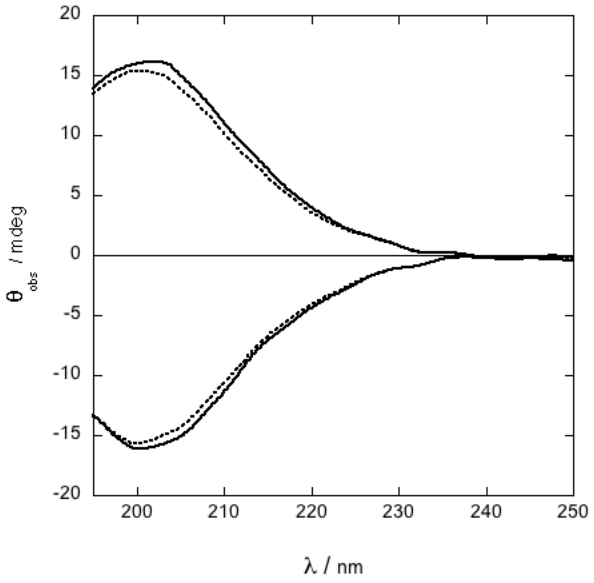


Figure S2. CD spectra of 0.5 mM **1** in an aqueous solution of 25 mM **2**. The positive traces are relative to L-**2** (solid line) and to L-**2** in the presence of **1** (dotted line). The negative traces are relative to D-**2** (solid line) and to D-**2** in the presence of **1** (dotted line). The data are expressed in terms of $[\theta]_{obs}$. Path length=0.02 cm.

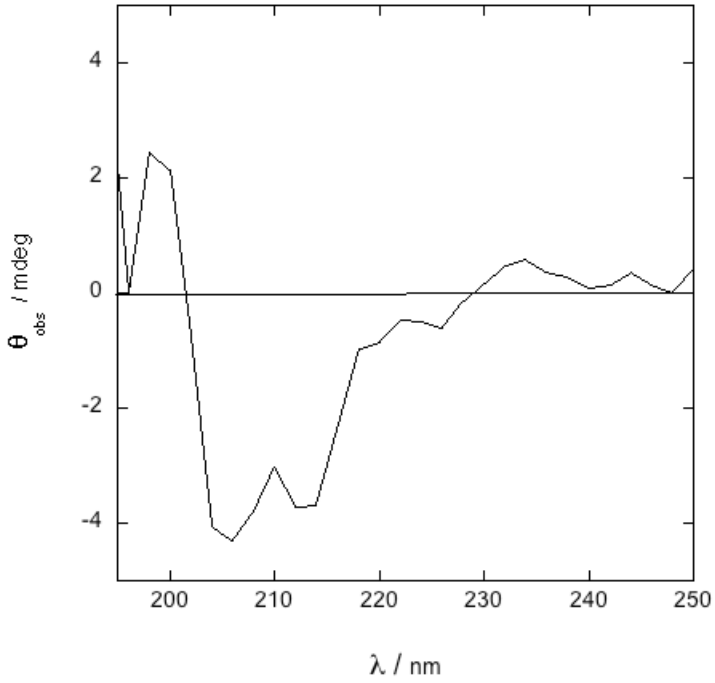


Figure S3. CD spectrum obtained by subtracting the CD spectrum of 25 mM aqueous L-**2** from the spectrum of 25 mM aqueous L-**2** in the presence of 0.5 mM **1.** Path length=0.1 cm. (Photomultiplier out of range between 195 and 210).

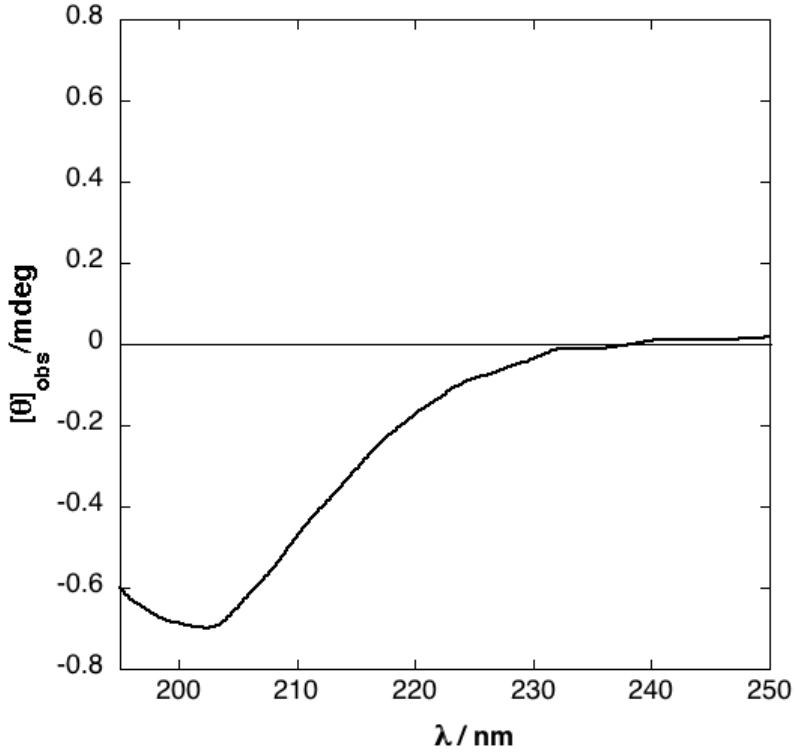


Figure S4. Difference CD spectrum obtained by subtracting the CD spectrum of a 25 mM aqueous solution of L-**2** from the trace obtained by multiplying the same spectrum by 0.96. The resulting spectrum is different from that obtained by subtracting the spectrum of a 25 mM aqueous solution of L-**2** from that of **1** in the same solution of surfactant L-**2** since it does not show the pattern of a 3_{10} helix.

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