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Synthesis and Characterisation of Helical β-Peptide Architectures that Contain (*S*)-β^3-HDOPA(Crown Ether) Derivatives**

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Abstract: A new set of β-amino acids that carry various crown ether receptors on their side chains of the general formula (*S*)-β^3-HDOPA(crown ether) (HDOPA: homo-3,4-dihydroxyphenylalanine; (crown ether): [15]crown-5 ([15-C-5]), [18]crown-6 ([18-C-6]), [21]crown-7 ([21-C-7]), 1,2-Benzo-[24]crown-8 ([Benzo-24-C-8]) and (*R*)-Binol-[20]crown-6 ([(*R*)-Binol-20-C-6])) was prepared. Peptides that are based on these new crowned β-amino acids combined with (1*S*,2*S*)-ACHC (2-aminocyclohexanecarboxylic acid), which is known to be a potent 3_{14}-helix inducer, to the hexamer level, with two crowned residues at the *i* and *i*+3 positions of the main-chain, were synthesized in solution by stepwise coupling using Boc-N$^{\alpha}$-protection (Boc: *tert*-butoxycarbonyl) and the EDC/HOAt C-activation method. Their conformational analysis was performed by using FTIR absorption, NMR and CD spectroscopy techniques. Our results are in full agreement with a 3_{14}-helix conformation.

Keywords: amino acids · circular dichroism · crown ethers · IR spectroscopy · peptides

Introduction

β-Peptides have received considerable attention in recent years because of their remarkable ability to fold into well-defined and predictable secondary structures.[1,2] The most extensively studied β-peptide secondary structure is the 14-helix (also named 3_{14}- or 3_1-helix), which is defined by a 14-membered ring (*i*) N−H···O=C (*i*+2) hydrogen bond between backbone amide groups.[2,3] In particular, Seebach and co-workers have shown that β^3-peptides adopt the 14-helix conformation in organic solvents,[4] whereas Gellman et al. have discovered that the insertion of β-amino acids with a constrained six-membered ring, such as *trans*-2-aminocyclohexanecarboxylic acid (ACHC), into a peptide chain dramatically enhances the 14-helix stability compared to β^3-amino acids.[5] Moreover, the recently reported properties of β-peptides, such as resistance to proteolytic degradation,[6] somastatin antagonism,[7] antimicrobial activity,[8] membrane translocation[9] and disruption of protein–protein interactions,[10] should lead to important therapeutic applications for this class of "foldamers".[3]

We envisioned that β-peptides could also be used for the construction of molecular receptors and devices that are based on peptide frameworks.[11] We were particularly impressed by the pioneering work of Voyer et al.[11,12] who studied L-DOPA α-amino acid derivatives that bear a crown ether receptor on their side chain; these compounds could be easily assembled in well-defined amphiphilic α-helical nanostructures with multiple crown ethers that are aligned, one on top of the other, on the same side of the peptide backbone. Indeed, a 21-mer peptide was shown to act as an artificial ion channel that was capable of facilitating the transport of monovalent cations across bilayer membranes.[12c,e,f,h,l,m] In contrast to the 21-mer, the 14-mer analogue interacted as a powerful membrane-disrupting agent,[12i,n,o] and the 7-mer analogue only behaved as an ion carrier.[12c] The shorter versions of this series of compounds also selec-

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[**] HDOPA: homo-3,4-dihydroxyphenylalanine.

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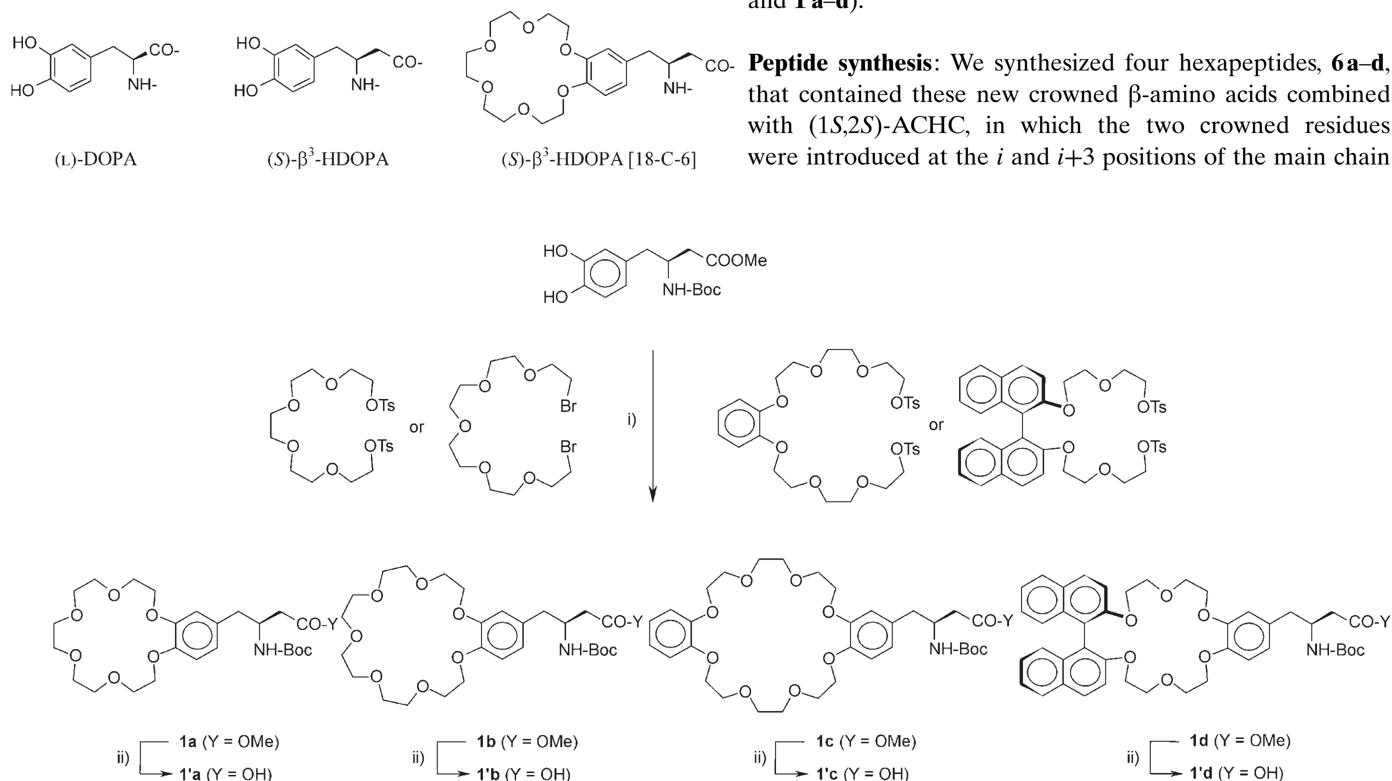
tively complexed ammonium[12b] and diammonium[12g] chains of various lengths. Moreover, depending on the peptide's secondary structure (helix or sheet), chiral recognition of either L- or D-amino acids could be induced as a result of the relative orientation of the crown ether receptors.[11a,c,d] We believe that extension of these properties to crowned 14-helical β-peptides could offer the same advantages that were found for the α-helical peptides,[12j] namely, ease of changing the size of the crown ether ring for modulation of the ion selectivity, ease of changing the amino acid components and/or peptide length by chemical synthesis, post-synthetic modifications and end-group engineering for tuning of the biological activity. Furthermore, because the 14-helix structurally differs from the α-helix in many respects, that is, it has a slightly wider radius and shorter rise for a given main-chain length than the α-helix, a net dipole that is opposite to that of the α-helix, and a 3-residue repeat with the side chains of the *i* and *i*+3 residues that are directly aligned atop one another along the same face of the peptide backbone instead of the 3.6-residue repeat for the α-helix, it is our view that the influence of these parameters could be worth examining in comparison with Voyer's results.

Therefore, we decided to apply the Arndt–Eistert homologation procedure of α-amino acids, which was previously improved by Seebach and co-workers,[13] to the synthesis of (*S*)-β^3-HDOPA (HDOPA: homo-3,4-dihydroxyphenylalanine), from which the first crown ether derivative of a β-amino acid, (*S*)-β^3-HDOPA-[18]crown-6 ((*S*)-β^3-HDOPA-[18-C-6]) was obtained.[14] In the present paper, we wish to report the preparation of a new set of (*S*)-β^3-HDOPA(crown ether) amino acid derivatives. We also describe the synthesis of peptides that are based on these new crowned β-amino acids combined with (1*S*,2*S*)-ACHC (which is a hydrophobic 3_{14}-helix inducer) to the hexamer level, with two crowned residues at the *i* and *i*+3 positions of the main chain (for alignment of the crown ether rings atop one another). Finally, we discuss the results of their conformational analysis by using FTIR absorption, NMR and CD spectroscopy.

Results and Discussion

Crown ether amino acid synthesis: The crown ether derivatives of (*S*)-β^3-HDOPA were prepared from the terminally protected derivative Boc-(*S*)-β^3-HDOPA-OMe (Boc, *tert*-butoxycarbonyl) according to the procedure of Voyer and co-workers[12j] at relatively high dilution (ca 0.1 M) with cesium carbonate (1.1 equiv) in DMF at 60 °C. Either pentaethylene glycol di-*p*-toluenesulfonate, hexaethyleneglycol dibromide,[12j] 1,2-bis(8-tosyloxy-3,6-dioxa-1-octyloxy)benzene,[15a] or (*R*)-2,2′-bis(5-tosyloxy-3-oxa-1-pentyloxy)-1,1′-binaphthyl[15b,16] were used as the alkylating agent (1.0 equiv) to afford **1a** (39 %), **1b** (22 %), **1c** (36 %) and **1d** (28 %), respectively, in reasonable yields after chromatography on silica gel (Scheme 1). Saponification of the ester function of **1a–d** was performed in methanol by using aqueous 1 M NaOH at room temperature for 24 h to give the corresponding N-protected amino acids: **1′a** (98 %), **1′b** (75 %), **1′c** (94 %) and **1′d** (40 %) (see Scheme 1 for structures of **1a–d** and **1′a–d**).

Peptide synthesis: We synthesized four hexapeptides, **6a–d**, that contained these new crowned β-amino acids combined with (1*S*,2*S*)-ACHC, in which the two crowned residues were introduced at the *i* and *i*+3 positions of the main chain



Scheme 1. Synthesis of the crown ether derivatives **1a–d** and **1′a–d** from Boc-(*S*)-β^3-HDOPA-OMe. i) Cs_2CO_3, DMF, 60 °C; ii) 1 M aq. NaOH/MeOH, RT.

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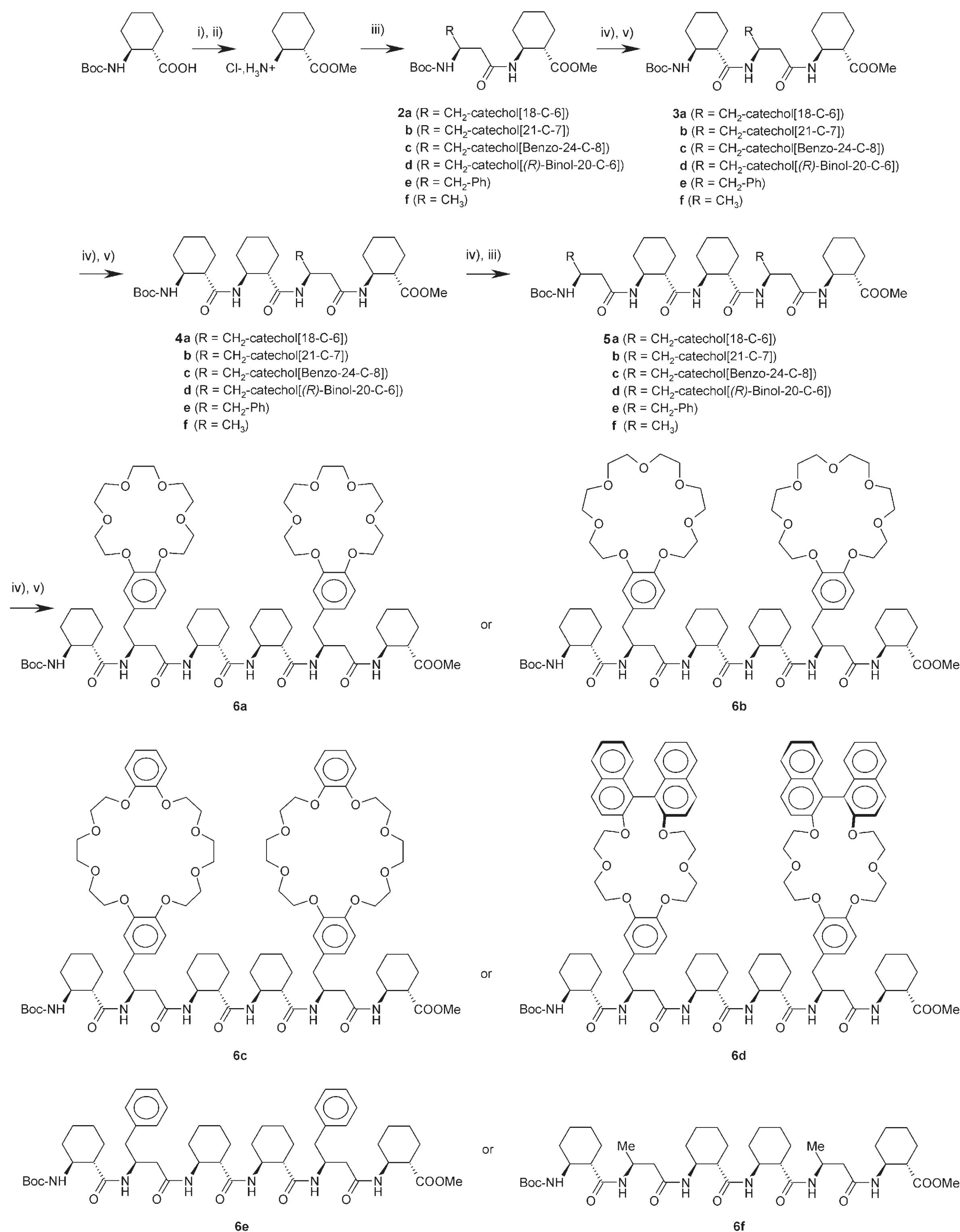
by a stepwise elongation strategy (Scheme 2). We also prepared the two model β-hexapeptides **6e** and **6f**, in which the absence of the crown ether carriers was expected to considerably simplify the NMR spectra.

All coupling steps were performed in solution by using the EDC/HOAt (EDC: *N*-ethyl,*N'*-(3-dimethylaminopropyl)carbodiimide; HOAt: 7-aza-1-hydroxy-1,2,3-benzotriazole) methodology.[17] To avoid epimerisation problems that



Scheme 2. Synthesis of the crowned β-hexapeptides **6a**–**6f**. i) TFA/CH_2Cl_2 1:1, 0°C to RT; ii) $SOCl_2$, MeOH, 0°C to RT; iii) **1'a**, **b**, **c** or **d** or Boc-(*S*)-β^3-HPhe-OH or Boc-(*S*)-β^3-HAla-OH, EDC, HOAt, NMM, CH_2Cl_2/THF; iv) TFA/CH_2Cl_2 1:3, 0°C to RT, or HCl/*p*-dioxane, 0°C to RT; v) Boc-(1*S*,2*S*)-ACHC-OH, EDC, HOAt, NMM, CH_2Cl_2/THF.

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have been encountered with triethylamine, we used the weaker base NMM (NMM: *N*-methyl morpholine).[4b] The cleavage of the Boc protecting group was performed with trifluoroacetic acid (TFA/CH_2Cl_2, 1:3) or HCl/*p*-dioxane. The peptides were usually obtained in 48–88% yield after purification either by column chromatography or by precipitation (the latter methodology for the less-soluble compounds). However, for the last coupling step, the yields of hexapeptides **6b** (15%), **6d** (29%) and **6e** (13%) were considerably lower, possibly for solubility reasons.

FTIR absorption analysis: The FTIR absorption spectra of the fully protected β-di- to β-hexapeptides **2a**–**6a**, **2b**–**6b**, **2c**–**6c**, **2d**–**6d** and **2e**–**6e** were recorded at room temperature in $CDCl_3$ by using diluted solutions to directly detect intramolecularly hydrogen-bonded and non-bonded NH groups by analysis of the conformationally sensitive N−H stretching (amide A) region (Figures 1–5).

The low-frequency band at about 3300 cm^{-1} indicates the formation of hydrogen-bonded NH groups, whereas the high-frequency band at about 3430 cm^{-1} arises from free, solvated NH groups.[18] We also observed an increasing amount of hydrogen-bonded NH groups when the length of the peptide chain was increased. Also, the low-frequency band of the β-hexapeptides **6a**–**6d** is of much higher intensity than the high-frequency band. However, we noticed the

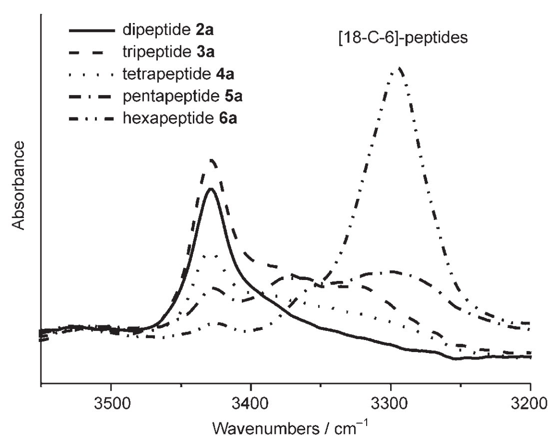

Figure 1. FTIR absorption spectra in $CDCl_3$ solution in the N−H stretching region for peptides **2a**–**6a**.

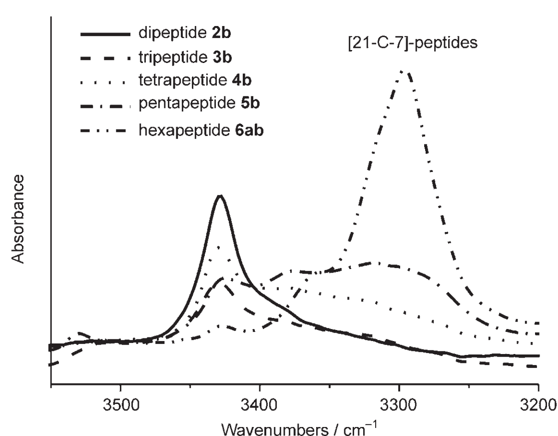

Figure 2. FTIR absorption spectra in $CDCl_3$ solution in the N−H stretching region for peptides **2b**–**6b**.

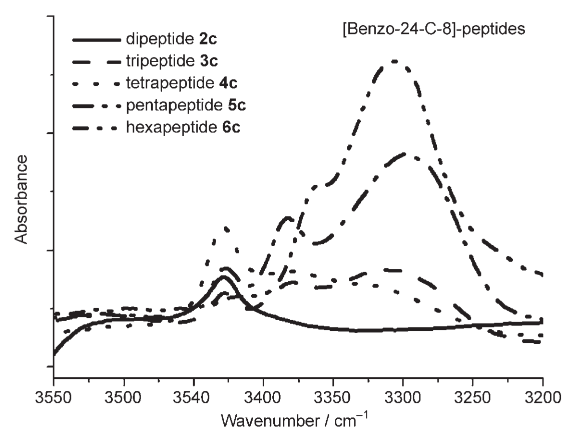

Figure 3. FTIR absorption spectra in $CDCl_3$ solution in the N−H stretching region for peptides **2c**–**6c**.

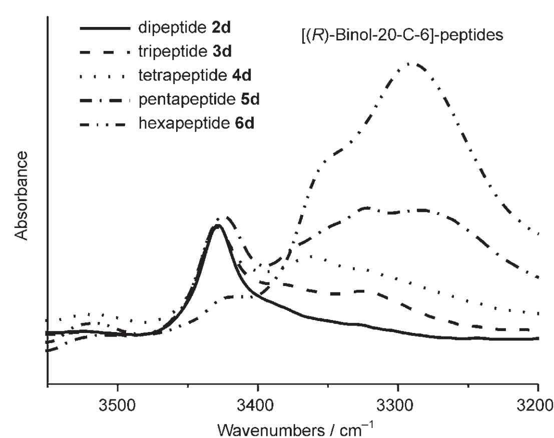

Figure 4. FTIR absorption spectra in $CDCl_3$ solution in the N−H stretching region for peptides **2d**–**6d**.

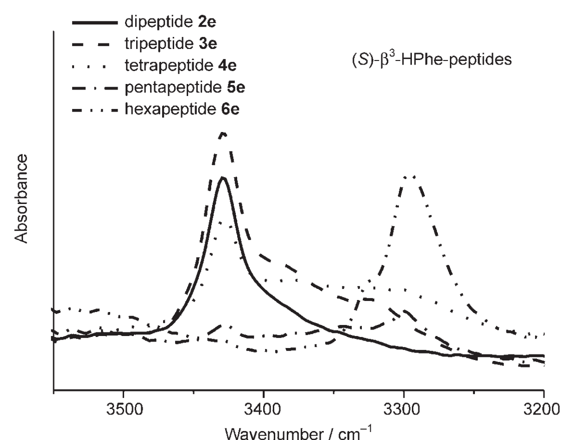

Figure 5. FTIR absorption spectra in $CDCl_3$ solution in the N−H stretching region for peptides **2e**–**6e**.

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presence of an additional band (shoulder) between 3325 and 3370 cm^{-1}, which suggests that at least two different kinds of hydrogen-bonded NH groups are present. Table 1 summarizes the FTIR absorption data in the N−H stretching region that was observed for the hexapeptides of each series.

Table 1. FTIR absorption data in the N−H stretching region for hexapeptides **6a**–**6e** in $CDCl_3$ solution.

β-Hexapeptides	Non-hydrogen-bonded N−H [cm^{-1}]	Shoulders [cm^{-1}]	Hydrogen-bonded N−H [cm^{-1}]
6a [18-C-6]	3427.2	3360.3	3295.8
6b [21-C-7]	3428.7	3359.7	3296.6
6c [Benzo-24-C-8]	3429.9	3326.8	3296.4
6d [(*R*)-Binol-20-C-6]	3426.6	3350.8	3292.7
6e β^3-HPhe	3429.3	3324.9	3296.4

In conclusion, the results of our FTIR absorption analysis clearly indicate the presence of two different classes of (non-bonded and hydrogen-bonded) NH groups for the highest oligomers. This information is consistent with well-organised β-peptide secondary structures.

Circular dichroism analysis: The peptides **2a**–**6a**, **2b**–**6b**, **2c**–**6c** and **2f**–**6f** were also studied by circular dichroism (CD), which is an extensively used technique for the 3D-structural investigation of peptides in solution.[4b,18d,19] As expected, we were not able to get any information on peptides **2d**–**6d** because their CD spectra were entirely dominated by contributions of the binaphthyl chromophore.[20] Experiments that were run at a 10^{-4} M concentration in 2,2,2-trifluoroethanol (TFE), which is a well-known solvent for stabilising peptide helical structures[21] gave satisfying results (Figure 6). Table 2 summarizes the characteristic parameters of the positive (λ_1) and negative (λ_2) Cotton effects that were observed for the longest members of each series. While no significant CD ellipticities were observed for the β-di- and β-tripeptides, a negative band near 215 nm and a positive band at about 192 nm were clearly seen in the spectra of the β-penta- and β-hexapeptides. These spectra show a typical pattern that is assigned to the (*M*)-3_{14} helix structure.[2] It should also be pointed out that the high intensity for the λ_2 value of the β-hexapeptide **6a** (−112.5) is probably related to a particularly good stability of the 3_{14}-helix.

^{1}H NMR spectroscopic analysis: NMR spectroscopy is one of the most widely used techniques for determination of the solution structure of β-peptides.[1a,22] The convention that is used throughout this study is the accepted labelling of $C_\alpha H$ and $C_\beta H$ for β-amino acids relative to standard α-amino acids, in which C_α bears the carbonyl group and C_β bears the nitrogen atom.[1a,2] The [(*R*)-Binol-20-C-6] series (**2d**–**6d**) could not be studied by ^{1}H NMR because of an overlapping of NH protons and aromatic CH protons from the binaphthyl nucleus. In contrast, the one-dimensional ^{1}H spectra of β-

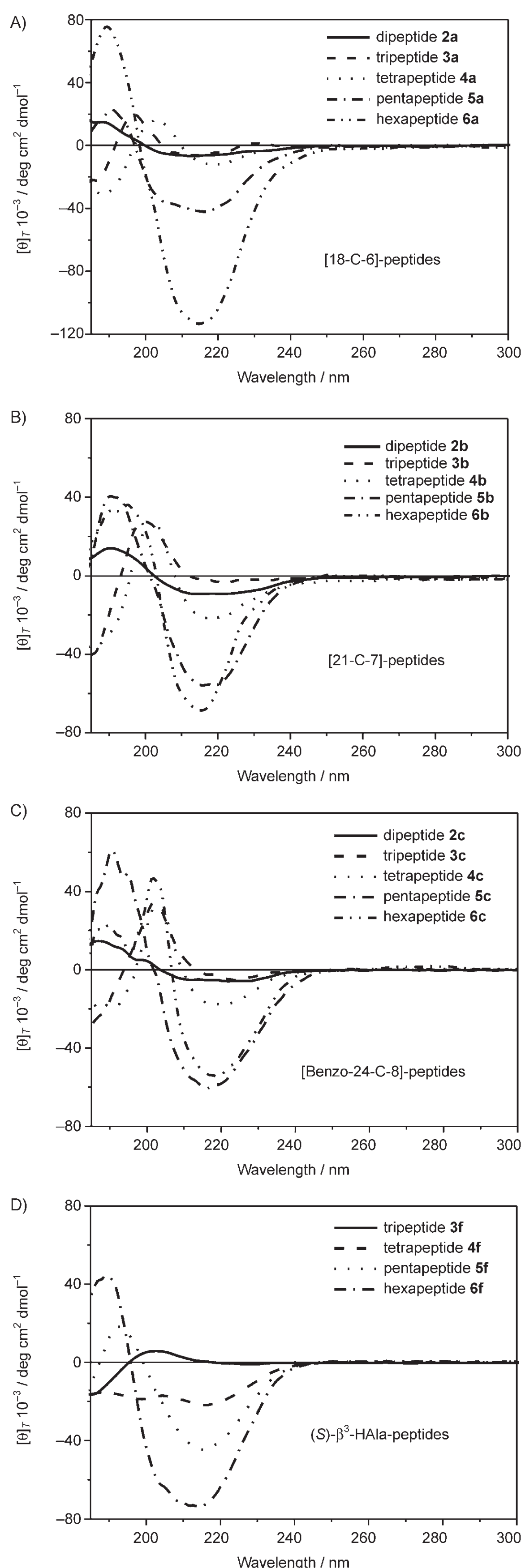

Figure 6. CD spectra for peptides **2a**–**6a** (A), **2b**–**6b** (B), **2c**–**6c** (C) and **2f**–**6f** (D) in TFE solution (peptide conc. 0.1 mM).

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Table 2. λ_1/λ_2 (nm) Values for the β-penta- and hexapeptides in TFE solution (the intensity $\times 10^{-3}$ of λ_2 is given in parentheses for each compound).

	β-Pentapeptides **5**	β-Hexapeptides **6**
[18-C-6] series (**a**)	191.3/216.3 (−41.6)	189.2/214.6 (−112.5)
[21-C-7] series (**b**)	191.3/218.2 (−55.0)	195.3/215.7 (−68.2)
[Benzo-24-C-8] series (**c**)	201.7/218.5 (−54.3)	194.6/216.4 (−60.4)
(*S*)-β^3-HAla series (**f**)	192.8/216.4 (−44.7)	189.3/213.3 (−73.4)

hexapeptides **6a–c** and **6e** in [D$_6$]DMSO solution revealed well-defined signals for the backbone, which suggests high-populations of a single well-organised conformation for each oligomer. At room temperature, the NH protons of each residue exhibit coupling constants J(NH,H$_\beta$) in the range of 7 to 10 Hz (Table 3). Those high values correspond to an *antiperiplanar* arrangement of the N−H$_{(i)}$ and C$_\beta$−H$_{\beta(i)}$ bonds for each residue, which are typically encountered with a 14-helix conformation.[1a]

Table 3. Coupling constant J(NH$_{(i)}$/H$_{\beta(i)}$) values [Hz] for the β-hexapeptides **6a–c**, and **e** in [D$_6$]DMSO solution.

Residue *i*	1	2	3	4	5	6
6a	9.1	9.0	7.1	7.7	8.7	7.9
6b	9.2	9.0	7.3	7.7	8.6	8.1
6c	–	9.2	8.7	10.4	8.7	8.7
6e	10.2	8.4	7.8	6.6	9.6	9.6

Because an NMR spectroscopic characterisation was only possible in [D$_6$]DMSO, as a result of the poor solubility of these compounds, we were not able to perform a [D$_6$]DMSO titration in CDCl$_3$ solution to evaluate the presence of intramolecular hydrogen bonds.[23] Alternatively, the extent of the intramolecular hydrogen bonding in the β-hexapeptides **6a–c** and **6e** was evaluated by temperature-dependent ^{1}H NMR spectroscopic measurements,[24] over a range of 30 K (Figure 7). The graphs reveal a generally higher variation of chemical shifts for the NH$_5$ and NH$_6$ protons than for the NH$_{1–4}$ protons. Some of the NH$_1$ and/or NH$_2$ chemical shifts could not be assigned because of their juxtaposition with aromatic CH signals. The temperature coefficients ($\Delta\delta\Delta T^{-1}$) (Table 4) range from −3.7 to −4.6 ppb K^{-1} for NH$_{1–4}$. Such relatively low values indicate poor solvent accessible and intramolecular hydrogen-bonded amide protons. However, the $\Delta\delta\Delta T^{-1}$ values fall between −5.3 to −6.7 ppb K^{-1} for NH$_5$ and NH$_6$, which suggests the onset of (DMSO) S=O···H−N (amide) hydrogen bonding interactions.[25] Therefore, these results provide evidence that the NH$_5$ and NH$_6$ protons of β-hexapeptides **6a–c** and **6e** are more solvent exposed than their NH$_{1–4}$ proton counterparts, as expected for 3$_{14}$-helical structures.

Residue-specific assignments of the β-hexapeptides **6a–c** and **6e** were based on a combination of COSY, DQFCOSY, TOCSY and ROESY experiments. We performed a "NOE walk" along the backbone, and assigned the NH, H$_\beta$ and H$_\alpha$ for each residue. Intraresidue assignments were confirmed

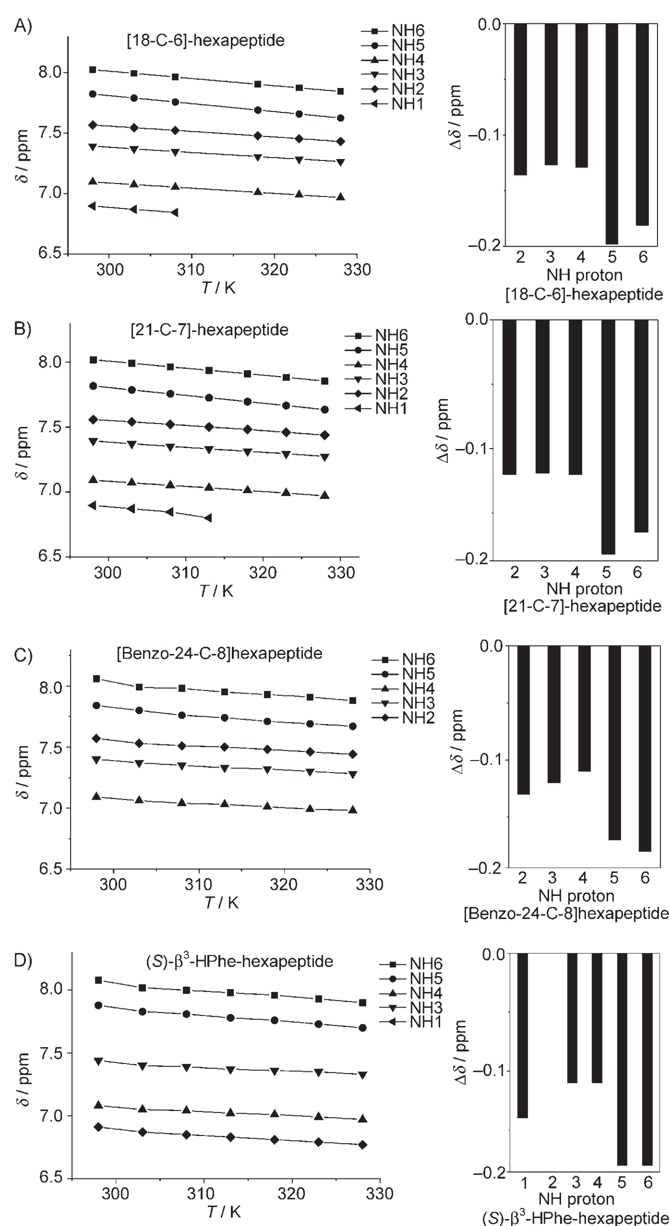

Figure 7. Plots and histograms showing the variations of the NH proton chemical shifts in the ^{1}H NMR spectra ([D$_6$]DMSO) of the β-hexapeptides **6a–c** and **6e** as a function of increasing temperature; A) β-hexapeptide **6a**; B) β-hexapeptide **6b**; C) β-hexapeptide **6c**; D) β-hexapeptide **6e**. The NH$_2$ proton of **6e** is overlapped by the aromatic CH protons.

Table 4. Temperature coefficients ($\Delta\delta\Delta T^{-1}$) [ppb K^{-1}] determined in [D$_6$]DMSO solution between 298 and 328 K for the β-hexapeptides **6a–c** and **e**.

	NH$_1$	NH$_2$	NH$_3$	NH$_4$	NH$_5$	NH$_6$
6a	–	−4.6	−4.3	−4.3	−6.7	−6.0
6b	–	−4.0	−4.0	−4.0	−6.0	−5.3
6c	–	−4.3	−4.0	−3.7	−5.7	−6.0
6e	−4.6	–	−3.7	−3.7	−6.0	−6.0

from 2D COSY and TOCSY data. The crown ether free hexapeptide **6e** was chosen as a model system because no

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signal overlap was observed in the H_β region of its ^{1}H NMR spectrum. A list of chemical shifts for **6e** is given in Table 5.

Table 5. ^{1}H NMR chemical shifts (δ/ppm) of the β-hexapeptide **6e** in [D$_6$]DMSO solution at room temperature.

Residues	H_α	H_β	NH
ACHC^1	2.36	3.42	6.91
β^3-HPhe^2	2.32	4.18	7.64
ACHC^3	2.35	3.77	7.42
ACHC^4	2.12	3.87	7.08
β^3-HPhe^5	2.17 and 2.20	4.61	7.88
ACHC^6	2.44	3.96	8.07

The NH/H_α, NH/H_β and H_α/H_β regions of the ROESY spectra for **6e** are shown in Figure 8. In the NH/H_α region, correlations $NH_{(i)}$–$H_{\alpha(i)}$ and $NH_{(i)}$–$H_{\alpha(i-1)}$ were observed between all possible pairs, such as ACHC^{1}NH–ACHC$^1H_\alpha$, ACHC$^1H_\alpha$–Phe^{2}NH, Phe^{2}NH–Phe$^2H_\alpha$, Phe$^2H_\alpha$–ACHC^{3}NH, ACHC^{3}NH–ACHC$^3H_\alpha$, ACHC$^3H_\alpha$–ACHC^{4}NH, ACHC^{4}NH–ACHC$^4H_\alpha$, ACHC$^4H_\alpha$–Phe^{5}NH, Phe^{5}NH–Phe$^5H_\alpha$, Phe$^5H_\alpha$–ACHC^{6}NH and ACHC^{6}NH–ACHC$^6H_\alpha$ (Figure 8A).

In the NH/H_β region, we noted correlations $NH_{(i)}$–$H_{\beta(i+2)}$ between the residue pairs ACHC^{1}NH–ACHC$^3H_\beta$, ACHC^{3}NH–Phe$^5H_\beta$ and ACHC^{4}NH–ACHC$^6H_\beta$. Correlations $NH_{(i)}$–$H_{\beta(i+3)}$ were also obtained between the residue pairs ACHC^{3}NH–ACHC$^6H_\beta$ (Figure 8B). Finally, in the H_α/H_β region, $H_{\alpha(i)}$–$H_{\beta(i+3)}$ and $H_{\alpha(i)}$–$H_{\beta(i-1)}$ cross-peaks, respectively, are present between the residue pairs ACHC$^1H_\alpha$–ACHC$^4H_\beta$, Phe$^2H_\alpha$–Phe$^5H_\beta$, ACHC$^3H_\alpha$–ACHC$^6H_\beta$ and ACHC$^6H_\alpha$–ACHC$^5H_\beta$ (Figure 8C).

Integration of the signals in the ROESY spectrum allowed us to determine intramolecular distances. Calibration of the spectrum was elaborated from a reference length that was calculated with the WebLab Viewer Pro 3.7 program. For this study, this length was 3.09 Å, which corresponds to the distance between the H_α and H_β protons of the ACHC^4 residue. From that calibration, distance (d) values were obtained. These data are directly correlated with the observed NOE strengths and classified into four categories, $d<2.5$ Å (strong interactions), $2.5<d<3.0$ Å (medium to strong interactions), $3.0<d<3.5$ Å (weak to medium interactions) and $d>3.5$ Å (weak interactions) (Figure 9).

2D COSY and TOCSY experiments were also performed on the crown ether β-hexapeptides **6a–c** to assign the chemical shifts for the H_α, H_β and NH protons of all residues of these hexapeptides (Table 6).

Then, each region of the ROESY spectra of **6a–c** was analysed to evaluate the observed NOEs for the backbone atoms of these β-hexapeptides. In the NH/H_α region, correlations $NH_{(i)}$–$H_{\alpha(i)}$ and $NH_{(i)}$–$H_{\alpha(i-1)}$ were seen between all possible pairs for **6a–c**. As for the NH/H_β region, we observed correlations $NH_{(i)}$–$H_{\beta(i+2)}$ between the residue pairs ACHC^{1}NH–ACHC$^3H_\beta$ for **6a** and **6b** and between ACHC^{4}NH–ACHC$^6H_\beta$ for **6c**. Correlations $NH_{(i)}$–$H_{\beta(i+3)}$ were obtained between the residue pairs ACHC^{3}NH–

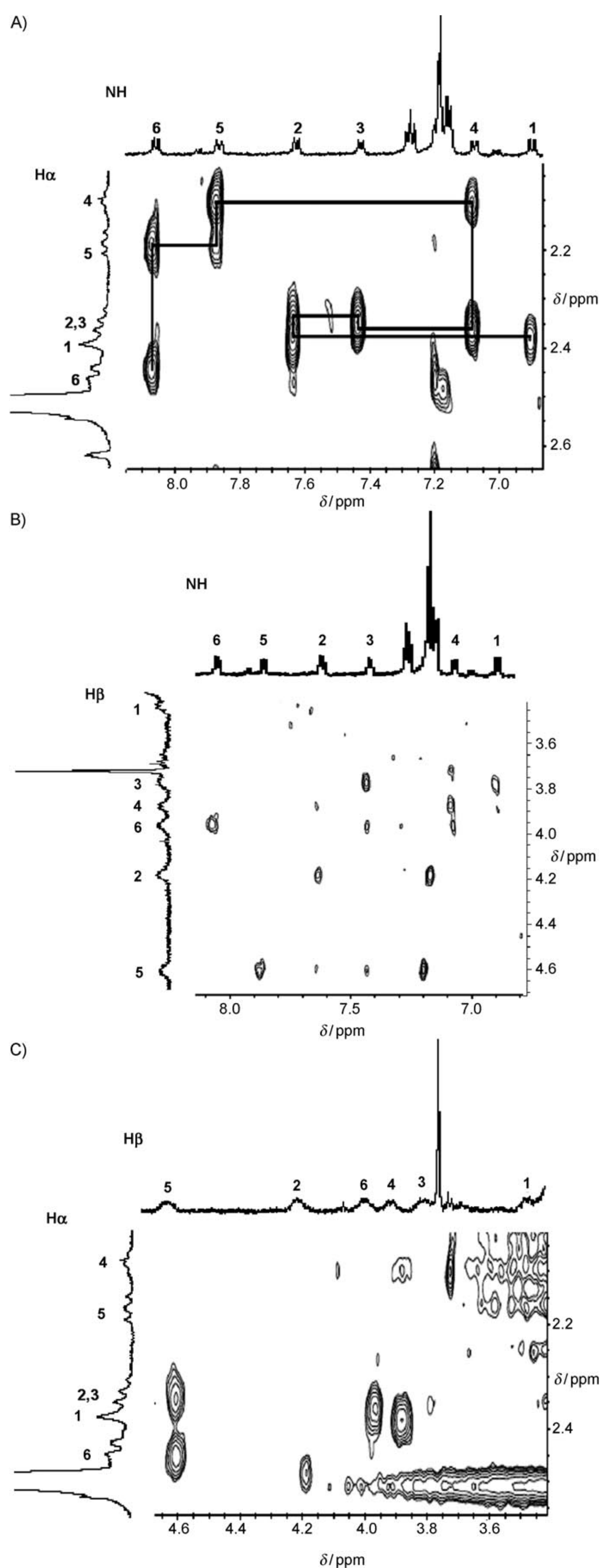

Figure 8. ROESY spectrum of the β-hexapeptide **6e** in the A) NH/H_α region, B) NH/H_β region and C) H_α/H_β region ([D$_6$]DMSO solution).

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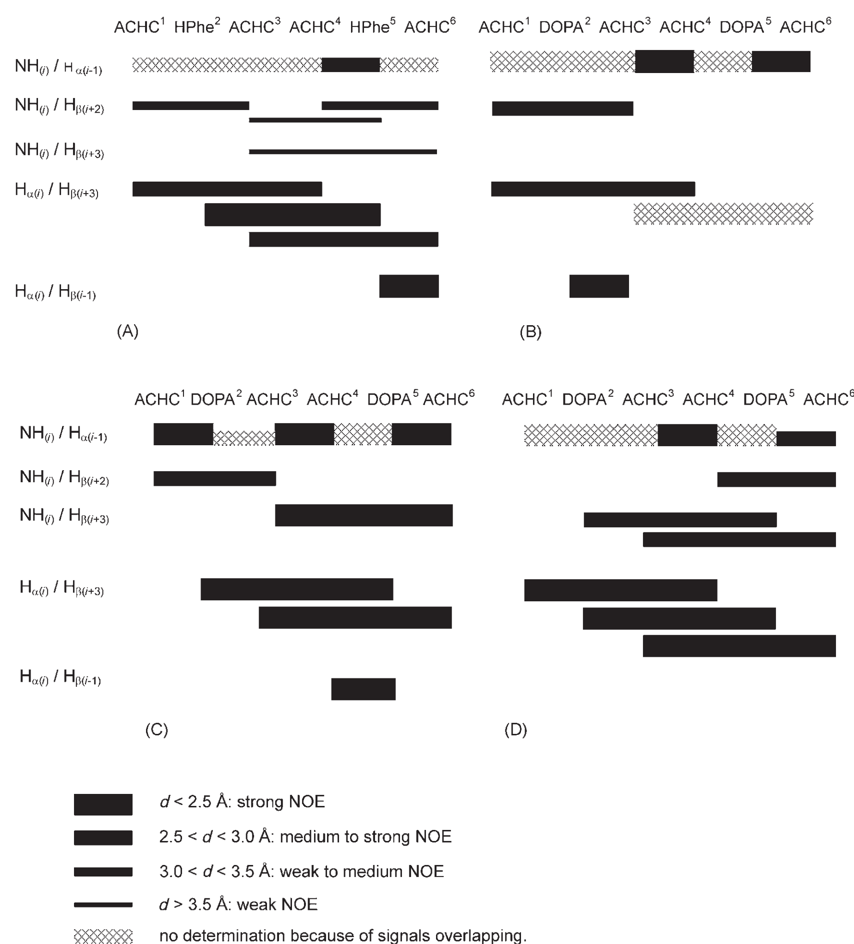

Figure 9. Weak, medium and strong NOEs observed in the ROESY spectra for **6e** (A), **6a** (B), **6b** (C) and **6c** (D) in [D$_6$]DMSO solution.

ACHC^{6}H$_\beta$ for **6b** and β-DOPA^{2}NH–β-DOPA^{5}H$_\beta$ and ACHC^{3}NH–ACHC^{6}H$_\beta$ for **6a**. The correlation NH$_{(i)}$–H$_{\beta(i-1)}$ was observed between ACHC^{3}NH–β-DOPA^{2}H$_\beta$, ACHC^{4}NH–β-DOPA^{3}H$_\beta$ and β-DOPA^{5}NH–ACHC^{4}H$_\beta$ for **6b** and β-DOPA^{2}NH–ACHC^{1}H$_\beta$, ACHC^{3}NH–β-DOPA^{2}H$_\beta$, ACHC^{4}NH–β-DOPA^{3}H$_\beta$ and ACHC^{6}NH–DOPA^{5}H$_\beta$ for **6a**. Finally, in the H$_\alpha$/H$_\beta$ region, H$_{\alpha(i)}$–H$_{\beta(i+3)}$ and H$_{\alpha(i)}$–H$_{\beta(i-1)}$ cross-peaks appeared between the residue pairs ACHC^{1}H$_\alpha$–ACHC^{4}H$_\beta$, β-DOPA^{2}H$_\alpha$–β-DOPA^{5}H$_\beta$ and ACHC^{3}H$_\alpha$–ACHC^{6}H$_\beta$ for **6c**, β-DOPA^{2}H$_\alpha$–β-DOPA^{5}H$_\beta$ and ACHC^{3}H$_\alpha$–ACHC^{6}H$_\beta$ for **6b**, and ACHC^{1}H$_\alpha$–ACHC^{4}H$_\beta$ and ACHC^{3}H$_\alpha$–ACHC^{6}H$_\beta$ for **6a**.

Integration of the signals in the ROESY spectra allowed us to determine intramolecular distances between protons that are bound to the backbone carbon atoms and evaluate the strength of their observed NOEs. Calibration of the spectra was based on the well-known interaction between the two geminal protons H$_\alpha$ of β-DOPA^2 for **6a** and **6b**, and between the H$_\alpha$–H$_\beta$ protons of ACHC^4 for **6c**. The resulting NOE cross-peaks are presented in Figure 9. In conclusion, the overall patterns of NOEs observed with **6a–c** and **6e** are in full agreement with a 3_{14}-helix conformation.

Conclusion

We have prepared a new series of β-amino acids **1a–d** that carry various crown ether hosts on their side chains. Subsequently, four β-hexapeptides **6a–d** that contain two crowned β-amino residues at the *i* and *i*+3 positions of the main chain, combined with (1*S*,2*S*)-ACHC, and two β-hexapeptides **6e** and **6f**, which were used as models, were synthesised by a stepwise elongation strategy. FTIR absorption spectra in CDCl$_3$ solution in the N–H stretching (amide A) region for the series of β-di- to β-hexapeptides show a low-frequency band at about 3300 cm^{-1} which indicates the occurrence of hydrogen-bonded N–H groups. The CD spectra of the highest oligomers of all the series of peptides are characterized by an intense negative Cotton effect that is centred near 215 nm, followed by a positive Cotton effect at about

Table 6. ^{1}H NMR chemical shifts (δ/ppm) of the H$_\alpha$, H$_\beta$ and NH protons for the β-hexapeptides **6a–c** in [D$_6$]DMSO solution at room temperature.

δ [ppm]	**6a**			**6b**			**6c**		
	H$_\alpha$	H$_\beta$	NH	H$_\alpha$	H$_\beta$	NH	H$_\alpha$	H$_\beta$	NH
ACHC^1	2.38	3.40	6.89	2.37	3.41	6.89	2.38	3.42	6.89
DOPA^2	1.97, 2.28	4.18	7.56	1.97, 2.28	4.19	7.55	2.31	4.19	7.56
ACHC^3	2.35	3.76	7.39	2.33	3.75	7.39	2.35	3.77	7.39
ACHC^4	2.12	3.88	7.08	2.13	3.88	7.08	2.12	3.88	7.09
DOPA^5	2.13, 2.40	4.53	7.81	2.12, 2.39	4.54	7.81	2.17, 2.42	4.54	7.84
ACHC^6	2.41	3.95	8.01	2.40	3.94	8.01	2.41	3.95	8.05

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200 nm. ^{1}H NMR spectroscopy results from temperature-dependent DMSO titrations provide evidence that the NH_5 and NH_6 protons of β-hexapeptides are more solvent-exposed than their NH_{1-4} proton counterparts. At room temperature, the typically recorded high $J_{(NH,H\beta)}$ values are in favour of an *antiperiplanar* arrangement for the NH and $H_{\beta(i)}$ protons of each residue. Intraresidue assignments were confirmed by 2D COSY and TOCSY experiments. A "NOE walk" along the backbone allowed us to assign the NH, H_α and $H_{\beta(i)}$ protons for each residue of the β-hexapeptides. Altogether, our data sets are in full agreement with a 14-helix structure for all of the crowned β-hexapeptides; the positioning of the (*S*)-β^3-HDOPA(crown ether) residues at the *i* and $i+3$ positions of the main-chain should allow for a parallel orientation of their side-chain receptors with the opportunity for a cooperative cation binding. This structural study represents one of the initial steps towards functional uses of this new class of amphiphilic, either 14-helical or 3_{10}-helical,[26] crowned peptides as potential ion channels and/or cytolytic agents, as well as potential peptide-based catalysts[27] for enantioselectively catalysed reactions,[28] which will now be explored by our groups.

Experimental Section

General methods: Melting points were determined by using either a Mettler FP61 apparatus with a temperature rise of 3 °C min^{-1} or a Büchi melting point B545 apparatus with a temperature rise of 20 °C min^{-1}, and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 300, 400 or 600 MHz, and 77 MHz, respectively, the solvents $CDCl_3$ ($\delta=7.27$ for ^{1}H and 77.00 ppm for ^{13}C NMR) and [D$_6$]DMSO ($\delta=2.49$ for ^{1}H and 40.45 ppm for ^{13}C NMR) were used as internal standards. For conformational analysis by ^{1}H NMR spectroscopy, the spectra were recorded on a 600 MHz Bruker Advance DMX-600 spectrometer. The spin systems of the amino acid residues were identified by using standard DQF-COSY and TOCSY experiments. In the latter case, the spin-lock pulse sequence was 70 ms long. The mixing time of the ROESY experiment that was used for interproton distance determination was 150 ms. Interproton distances were obtained by integration of the ROESY spectrum with the Sparky software package. Distances were calibrated on the peak between the two H_α/H_β protons of ACHC or the two β^3-HDOPA H_β protons, which were set to a distance of 3.09 and 1.78 Å, respectively. The CD spectra were calculated on a Jasco J-710 spectropolarimeter by using quartz cells of 0.1 to 1.0 mm pathlength (Hellma). The values are expressed in terms of $[\theta]_T$, the total molar ellipticity (deg × cm^2 × dmol^{-1}). FTIR absorption spectra were recorded with a Perkin–Elmer 1720X spectrophotometer that was nitrogen-flushed and equipped with a sample shuttle device at a nominal resolution of 2 cm^{-1} for an average of 20 scans. Solvent (baseline) spectra were recorded under the same conditions. Cells with pathlengths of 1 and 10 mm (CaF_2) were used. The optical rotations were measured with an accuracy of 0.3 % in a 1 dm thermostatted cell. Analytical TLC and preparative column chromatography were performed on Kieselgel F254 and 60 (0.040–0.063 mm) (Merck), respectively, with the following eluant systems: EtOAc/CH_2Cl_2 98:2 (A), MeOH/CH_2Cl_2 2:98 (B), MeOH/CH_2Cl_2 3:97 (C), MeOH/CH_2Cl_2 5:95 (D), MeOH/CH_2Cl_2 1:9 (E), MeOH/CH_2Cl_2 2:8 (F), MeOH/CH_2Cl_2 1:1 (G), CH_2Cl_2/1-BuOH/EtOAc/MeOH/AcOH/H_2O 8:4:3:2.5:1:0.5 (H), solution H/MeOH 8:2 (I), solution H/MeOH 1:1 (J). UV light ($\lambda=254$ nm) allowed visualisation of the spots after TLC runs for all compounds, even at low concentration. H-(1*S*,2*S*)-ACHC-OMe·HCl was prepared from the corresponding N$^\alpha$-protected β-amino acid Boc-(1*S*,2*S*)-ACHC-OH by reaction with $SOCl_2$ and MeOH, according to the Ressler's procedure.[29] The synthesis and characterisation of all compounds (**1a**–**6a**, **1b**–**6b**, **1c**–**6c**, **1d**–**6d**, **1e**–**6e** and **1f**–**6f**) are reported in the Supporting Information.

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