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Conformational analysis by HRMAS NMR spectroscopy of resin-bound homo-peptides from C^{α}-methyl-leucine

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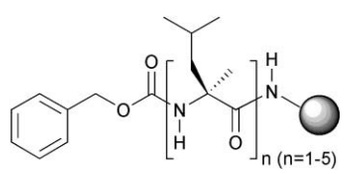
A series of [L-(αMe)Leu]$_n$ (n = 1–5) homo-peptides have been covalently linked to Tentagel and POEPOP resins and submitted to a conformational study using HRMAS NMR spectroscopy. Whereas the mono- and dipeptide are mainly fully-extended, stable 3_{10}-helical structures are formed beginning from the trimer.

High-resolution magic angle spinning (HRMAS) NMR spectroscopy is becoming an increasingly useful technique for the characterization of molecules linked to a solid support.[1] For example, it has been applied to the analysis of multi-step solid-phase organic reactions,[2–4] to the conformational studies of resin-bound peptides,[5–9] and to the investigation of synthetic ligand–receptor interactions.[10] In the field of solid-phase peptide chemistry we have recently characterized by HRMAS NMR the 3_{10}-helical conformation adopted by two Aib (α-aminoisobutyric acid) homo-peptides linked to the POEPOP resin.[5] The information obtained from that preliminary study allowed us to undertake the HRMAS NMR analysis of a complete series of resin-bound, L-(αMe)Leu homo-peptides, synthesized as potential catalysts in the Julia–Colonna asymmetric epoxidation reaction.[11–13] In such a reaction, high enantioselectivity was observed when short, resin-bound peptides based on Leu residues were present in the reaction mixture. As peptide helicity seems to play a major role in the induction of the enantiomeric discrimination, we reasoned that increasing the helix stability by C^{α}-methylation,[14] *i.e.* by replacing Leu with (αMe)Leu, would generate heterogenous catalysts with higher efficiency. Therefore, we covalently grafted to the Tentagel resin the homo-peptide series [L-(αMe)Leu]$_n$ (n = 1–5) and the homo-tetrapeptide only to the POEPOP resin.[15] With the aim of understanding the relationship between peptide conformation and catalytic efficiency of the conjugates, we investigated, by HRMAS NMR, the 3D-structure of the newly synthesised systems upon swelling them in deuterated dimethylformamide (DMF) and dimethysulfoxide (DMSO) (for experimental details, see ref. 5). We were also able to gain information on the influence exerted on peptide folding by main-chain length and type of swelling solvent and resin.

Direct build-up on a solid support of homo-peptides based on bulky C^{α}-tetrasubstituted α-amino acids, such as (αMe)Leu, is difficult. Therefore, starting from optically pure L-(αMe)Leu, produced on a large scale by a chemo-enzymatic procedure developed at DSM Research a few years ago,[16] we synthesized by solution methods the peptide series Z-[L-(αMe)Leu]$_n$–OH (n = 1–5) (Z: benzyloxycarbonyl).[17] Each compound was then coupled to the amino function of a Tentagel resin. Typically, a resin sample was added to a solution of peptide and HATU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium] hexafluorophosphate,[18] in DMF in the presence of 3% NMM (*N*-methylmorpholine) and was shaken for several days at room temperature. Coupling yields were monitored by means of a quantitative Kaiser test.[19] In all cases a peptide loading of at least 75% was obtained. A similar coupling procedure was used to link Z-[L-(αMe)]$_4$–OH to the amino–POEPOP resin.[7]



The peptide–resin conjugates, swollen in DMF-d_7 and/or DMSO-d_6, were analysed by 1D and 2D HRMAS NMR spectroscopy. To unambiguously assign all NH proton signals and get an insight into peptide secondary structure, we exploited the information obtained from the NOESY experiments. In the case of the Tentagel-bound peptides, DMSO turned out to be a poor swelling solvent. Therefore, a thorough conformational study on these conjugates was conducted in DMF. For each solid-supported peptide the resonances of the N-terminal (αMe)Leu residue were attributed by means of the NOE correlations between the (αMe)Leu NH proton and the aromatic and methylene Z protons. The remaining resonances were assigned from the N- to the C-terminal residue by analysing the cross-peaks of the ($i \longrightarrow i + 1$) type. A series of strong sequential NH($i \longrightarrow i + 1$) dipolar interactions was observed in the NOESY spectra of the tetra- (Fig. 1) and pentapeptides. The presence of these correlations is considered diagnostic of a helical conformation,[20] although it is not possible to assess whether a 3_{10}- or an α-helical structure would be present. To address this last issue, αN(i, i + 2) and αN(i, i + 4) NOE constraints are crucial because they are considered indicative of the presence of a 3_{10}- and an α-helical conformation, respectively.[20,21] Unfortunately, these interactions cannot be observed in peptides based exclusively on C^{α}-tetrasubstituted α-amino acids, as such residues lack any αCH proton. In the

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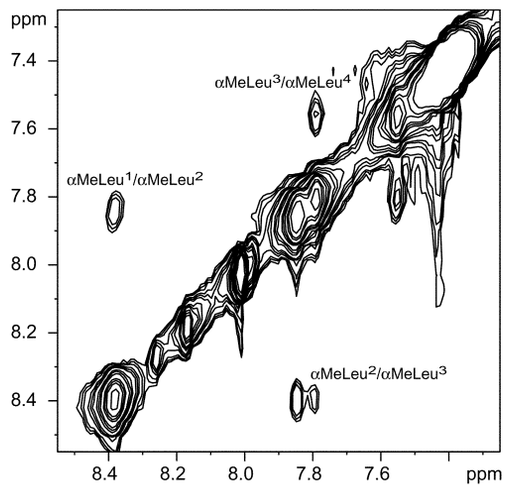

Fig. 1 Amide proton region of the HRMAS 2D NOESY (τ_m = 300 ms) spectrum for Z-[L-(αMe)Leu]$_4$-Tentagel swollen in DMF-d_7.

case of the tripeptide, only weak sequential NH($i \longrightarrow i + 1$) cross-peaks were observed, while for the shorter peptide–resin conjugates such signals were missing. Likely, this latter finding is due to a higher flexibility of the peptide moiety or to a non-helical spatial arrangement.

To more deeply investigate the 3D-structure of the resin-bound L-(αMe)Leu homo-peptides and, hopefully, to establish whether a 3_{10}- or an α-helical structure is present, a series of monodimensional spectra was recorded in DMF-d_7 at different temperatures (from 300 K to 340 K). The temperature coefficients for the amide protons of the five peptide-Tentagel conjugates are reported in Fig. 2. It is worth recalling here that the peptides examined are linked to the resin through an amide bond. Therefore, an additional amide NH group is available for possible contributions to the H-bonding scheme.

An inspection of Fig. 2 reveals two classes of NH protons: (i) the first class includes protons particularly sensitive to the increase in temperature; (ii) the second class involves all other amide protons, only marginally perturbed by heating. In the case of the monomer, the NH proton of (αMe)Leu is remarkably sensitive to the change of temperature. Therefore, this NH group is probably not involved in any H-bonding. The dipeptide has the possibility of folding into a β-turn by forming an intramolecular H-bond between the carbonyl of the Z-protecting group and the amide NH group on the resin. However, this proton displays a temperature coefficient typical of a free NH group, whereas, on the contrary, the amide protons of the two (αMe)Leu residues are significantly less perturbed by heating. It has been previously shown that *p*BrBz-[D-(αMe)Leu]$_3$–O*t*Bu, also characterized by three amide protons, adopts in the crystal state a fully-extended conformation stabilized by three intraresidue N–H$_i$ $\cdots$ O=C$_i$ H-bonds (C$_5$ or fully-extended conformation).[22] The same 3D-structural indication was extracted from a solution conformational analysis.[23] In agreement with these findings, the temperature coefficients of the C-terminal amidated dipeptide, linked to the Tentagel resin, point to the involvement of the NH protons of both (αMe)Leu residues in a C$_5$ structure.

Interestingly, the addition of one more residue results in a conformational bias towards a helical structure. Indeed, Z-[L-(αMe)Leu]$_3$-Tentagel appears to fold into an incipient helix, stabilized by two intramolecular H-bonds that involve the NH protons (low temperature coefficients, Fig. 2) of (αMe)Leu^3 and the resin. Again, this result is in agreement with the crystal structures of the homo-tripeptide *p*BrBz-[D-(αMe)Leu]$_3$–OH and the homo-tetrapeptide *p*BrBz-[D-(αMe)Leu]$_4$–O*t*Bu.[24] Both peptides fold into an incipient, regular 3_{10}-helix stabilized by two β-turn conformations. It is worth noting that in the crystal structure of *p*BrBz-[D-(αMe)Leu]$_3$–OH the C-terminal OH

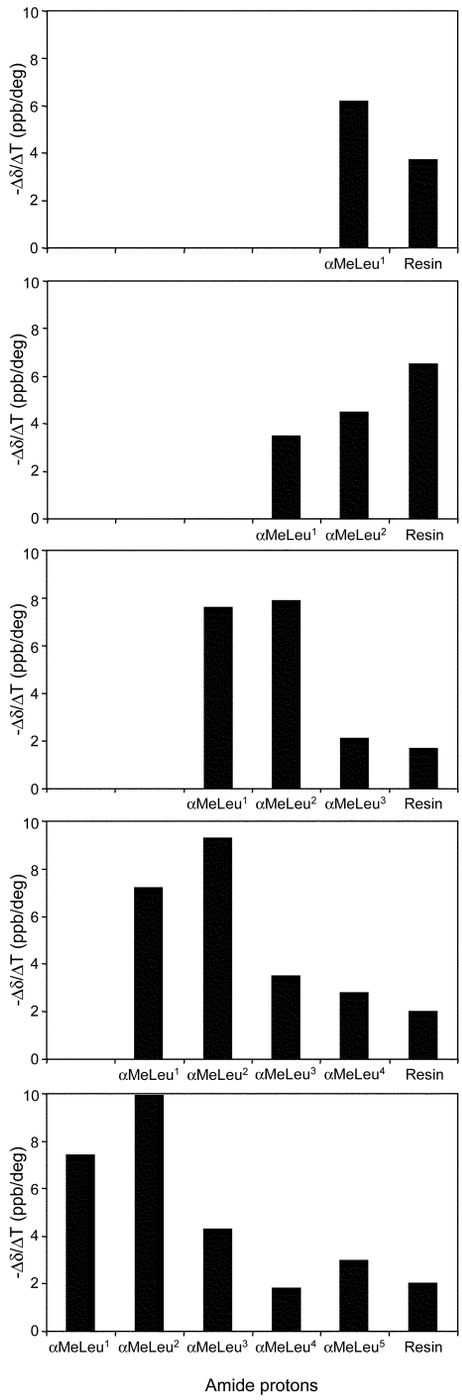

Fig. 2 Temperature coefficients of the amide NH protons of the Z-[L-(αMe)Leu]$_n$-Tentagel (n = 1–5, top to bottom) swollen in DMF-d_7, measured in the range 300–340 K. The residues are ordered from the N- to the C-terminus.

group acts as a H-bond donor, thus allowing this tripeptide to behave as a tetrapeptide ester. Our HRMAS NMR findings strongly support the conclusion that the Tentagel-supported L-(αMe)Leu trimer also folds, in DMF, into a 3_{10}-helix rather than into an α-helix. As a matter of fact, the latter structural motif would have required the first *three* backbone NH protons not being involved in the intramolecular H-bonding pattern, while the first *two* only are expected to be solvent-exposed in a 3_{10}-helix.[14]

Also for the resin-bound, L-(αMe)Leu tetramer and pentamer the temperature coefficients indicate the onset of a 3_{10}-helical structure (Fig. 2).

In order to assess the influence of the resin type on peptide conformation we performed a detailed 1D and 2D HRMAS

NMR analysis of Z-[L-(αMe)Leu]$_4$–POEPOP. With this resin spectra of good quality could be collected in DMF-d_7 as well as in DMSO-d_6 (Fig. 3). Interestingly, the temperature coefficients in the two solvents (Fig. 4) display a remarkably similar behaviour, which, in turn, matches perfectly that observed for the same oligomer covalently bound to the Tentagel resin (Fig. 2). In addition, a comparable temperature effect has already been observed for *p*BrBz-(Aib)$_4$-POEPOP and has been ascribed to the onset of a stable 3_{10}-helical conformation.[5]

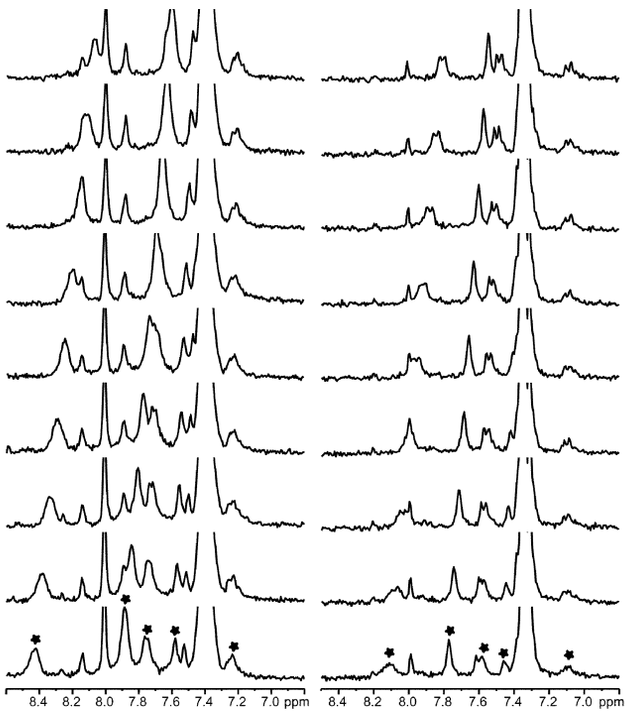

Fig. 3 Amide proton region of the HRMAS NMR spectra of Z-[L-(αMe)Leu]$_4$-POEPOP swollen in DMF-d_7 (left) and DMSO-d_6 (right) in the range 300–340 K. Stars indicate the NH protons sensitive to the increase of temperature.

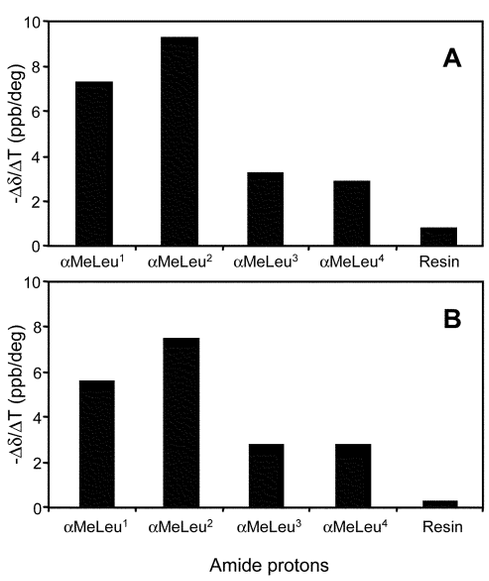

Fig. 4 Temperature coefficients of the amide NH protons of Z-[L-(αMe)Leu]$_4$-POEPOP swollen in DMF-d_7 (A) and DMSO-d_6 (B), measured in the range 300–340 K. The residues are ordered from the N- to the C-terminus.

In summary, we have successfully linked a series of L-(αMe)Leu homo-peptides, from monomer to pentamer, to Tentagel and POEPOP resins. Our conformational studies, performed by HRMAS NMR spectroscopy in two deuterated solvents, indicate that in the shortest sequences (monomer and dimer) a significant population of fully-extended conformers is present. However, starting from the trimer stable 3_{10}-helical structures are formed. These results are in remarkably good agreement with the conformations previously observed in the crystal state for three (αMe)Leu homo-oligomers. We conclude that the L-(αMe)Leu residue does promote formation of β-turn and 3_{10}-helical conformations also when grafted to a solid support, provided that at least four peptide NH protons are present in the sequence. This assumption is corroborated by the observation that no major conformational variations take place when the swelling solvent and the resin are changed. In view of the high 3D-structural stability of peptides heavily based on C^{α}-tetrasubstituted α-amino acids, even when bound to a resin, we hope to be able to shed light on the role of peptide folding in the catalytic mechanism of the Julia–Colonna asymmetric epoxidation. Studies in this direction are currently under way in our laboratory. Finally, we would like to stress that HRMAS NMR is probably the most powerful tool currently available to investigate details of the preferred conformation of resin-bound peptides.

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