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10-Helical conformations in oxetane β-amino acid hexamers

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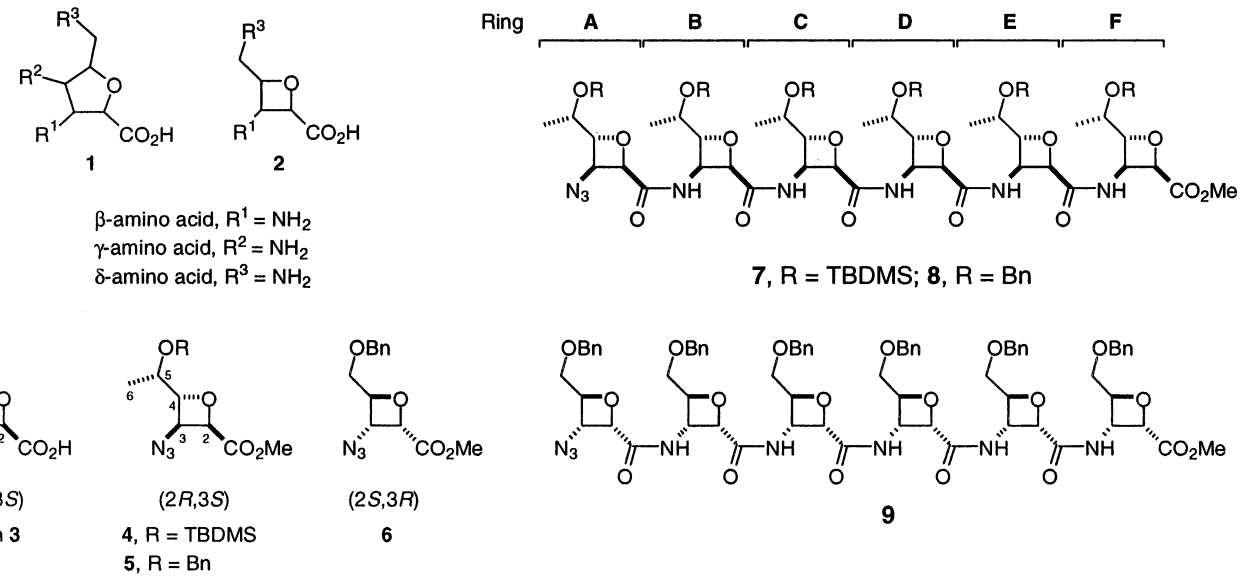
Abstract—The first structural investigations were undertaken on a β-hexapeptide **7** in which the peptide backbone is constrained by *cis*-substituted oxetane rings. Detailed NMR studies in $CDCl_3$ and C_6D_6 together with molecular mechanics conformational analysis identify a well-defined left-handed helical structure stabilised by 10-membered hydrogen-bonded rings. Comparison with two related hexapeptides **8** and **9** suggests a similar structural preference for these systems.

The identification of new polymeric backbones with specific folding propensities (foldamers[1]) has been proposed as the first stage in facilitating the design of entirely synthetic systems with a tertiary structure.[2] Peptides derived from carbohydrate amino acids ('carbopeptoids'[3]) bearing tetrahydrofuran motifs **1**[4] represent a novel approach to foldamer design. These densely functionalised scaffolds allow considerable structural diversity and both a repeating β-turn structure[5] and a left-handed 16-helix[6] have been generated in organic solvents from stereoisomers of the δ-amino acid template. To complement this research program, and in view of the absence of structural investigations on β-peptides constrained by 4-membered rings, the synthesis of carbopeptoids based on the smaller oxetane templates **2** was undertaken.

The naturally occurring β-amino acid antibiotic oxetin **3**[7] exemplifies the simplest oxetane 3-amino-2-carboxylic acid framework and the preceding paper[8] provided synthetic routes to the *C,N*-protected oxetin analogues **4**–**6**. This paper reports the results of NMR



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and molecular dynamics studies completed on a hexapeptide **7** synthesised from the oxetane azido ester **4** by established solution phase coupling procedures.[9] The repeating unit of **7** bears the same (2*R*,3*S*) absolute stereochemistry as oxetin, with the addition of a two carbon hydroxylated side-chain at C4. Comparisons are also made with the two related hexamers **8** and **9**, derivatives of the building blocks **5** and **6**, respectively.

The solution NMR spectra of the hexamer **7** were analysed in both $CDCl_3$ and C_6D_6 with benzene providing the more favourable resonance dispersion and reduced interference for analysis of NOE data (see below). In both solvents the 1D proton NMR spectra displayed high resonance dispersion, notably with the five amide protons clearly resolved from each other (Fig. 1). That the spectra remained largely invariant to concentration (up to and including a 40-fold dilution) indicates that this resonance dispersion is attributable to internal structure rather than molecular aggregation.

Proton assignments for individual residues were established from a combination of 2D (DQF-COSY, TOCSY, and HSQC) and selective 1D (DPFGSE-TOCSY[10]) NMR techniques. Sequential assignment of the six amino acid residues was initially made through the mapping of inter-residue NOEs, namely NH(i) to NH(i-1) and NH(i) to H2(i-1), observed in Tr-ROESY[11] experiments. The sequential nature of these NOEs was confirmed by the observation of through-bond ^1H, ^{13}C long-range correlations in a gradient-selected HMBC experiment.[12]

Full ^1H NMR assignment of the hexapeptide facilitated investigation of its solution structure. Titration of a strongly hydrogen-bonding solvent, here DMSO-d_6, into the chloroform solution is a convenient means of differentiating those amide protons that are solvent exposed over those involved in intramolecular H-bonds.[13] These data (Fig. 2) support a structure in which the C-terminal amide proton NH^F is the most exposed to the solvent.[14] Distinct amide environments could also be identified in the solution IR spectrum of **7** in $CHCl_3$ (Fig. 3). The strong absorption at 3300 cm^{-1} is characteristic of the N–H stretch of a strongly hydrogen-bonded amide functionality.[15]

Further structural analysis focused on the patterns of NOEs observed in ROESY and Tr-ROESY spectra.

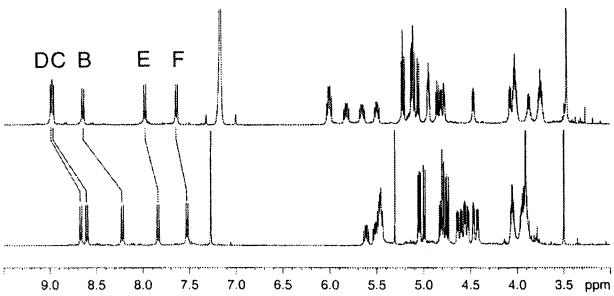

Figure 1. Partial NMR spectra of hexamer **7** in C_6D_6 (top plot) and $CDCl_3$ (bottom plot), including ring assignments for the amide NH protons.

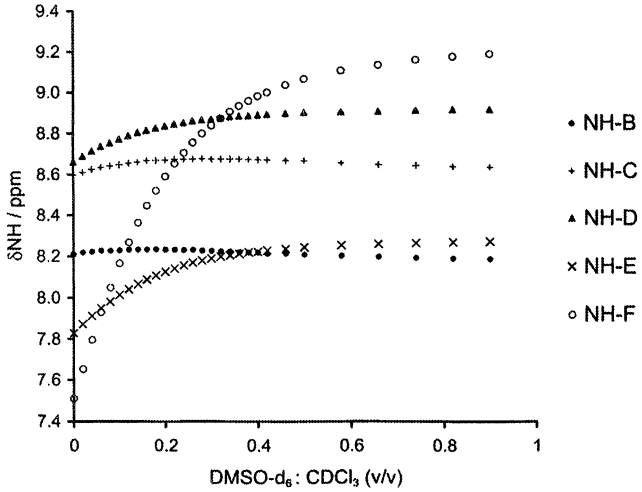

Figure 2. Solvent titration plot for the amide NH protons of hexapeptide **7** showing the variation of chemical shift with volume ratio of DMSO-d_6 to $CDCl_3$ at 298 K.

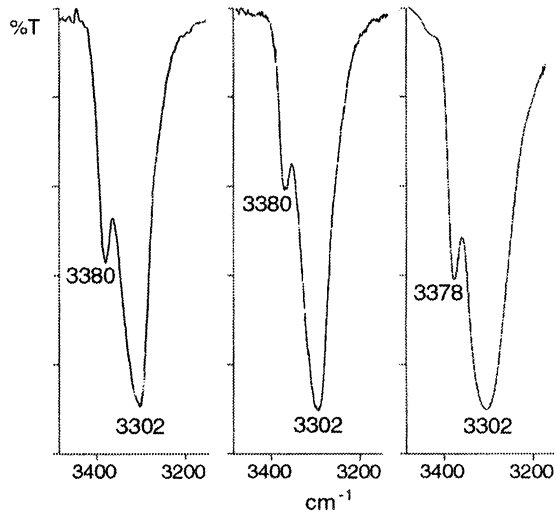

Figure 3. Solution IR spectrum of β-hexapeptides **7** (left plot), **8** (middle plot) and **9** (right plot) at 2 mM in $CHCl_3$ showing amide N-H stretching bands.

The repeating sequence of ^1H–^1H close-contacts identified along the molecule (Fig. 4) hinted strongly towards a stable, repeating secondary structure, as consistent with the high proton dispersion displayed in the 1D proton NMR spectra. A highly populated folded conformation stabilised by intramolecular NH–CO hydrogen bonds (Fig. 5) is also compatible with the solvent titration data. A more detailed analysis of the NOE data provided distance restraints suitable for molecular dynamics calculations. Thus, NOE intensities were semi-quantitatively analysed and classified into distance bounds of <0.27, <0.35 and <0.4 nm.

The conformation space of the molecules was investigated using MacroModel 5.5,[16] and the MM2* force field,[17] using Monte Carlo methods[18] and a genetic algorithm approach.[19] The lowest energy structures were not consistent with the NOE distance restraints. These were added for the two closest of the three

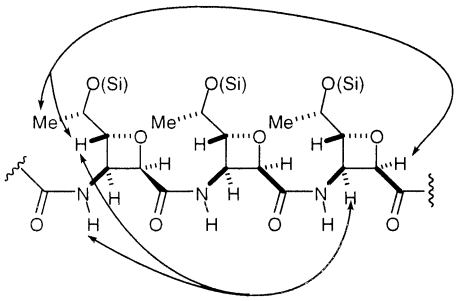

Figure 4. Typical (i) to (i+2) intra-residue close contacts from analysis of ROESY and Tr-ROESY spectra.

groups (<0.27 and <0.35 nm) and with these constraints the lowest energy structures were helical. These helices are present in the unconstrained conformation searches, but not as the lowest energy structures. This may be because the MM2* force field was overestimating the effect of attractive non-bonded intramolecular interactions. A molecular dynamics simulation, constrained by the NOE data as described above and starting from the lowest energy structure was carried out at 300 K for 1 ns. One hundred structures were sampled from this simulation, and all were reminimised. The ten lowest energy minima are overlayed, aligned to give the best fits to the carbon and nitrogen atoms of each amide, and are shown in Fig. 6. These show that the silicon protecting group can move around, but the helical structure of the molecule only shows small movements.

The NOE constrained modelling presents a left-handed helical structure stabilised by four 10-membered intramolecular hydrogen bonds between neighbouring residues (Fig. 6). In such a conformation only the amide proton NH^F is unable to participate in a hydrogen bond (Fig. 5) and would be solvent exposed, as implied by the titration data above. The hydrogen bonds are observed to point toward the C-terminus (as defined by the N–H vector), in contrast to those of the α- and 3_{10} helices of α-peptides. Although single 10-membered turns have been found in β-peptides,[20,21] a 10-helix has not been described before and in particular complements those secondary structures conferred by other cyclically constrained residues. By comparison *trans*-substituted cyclopentane and cyclohexane β-amino acids prefer 12- and 14- helical structures, respectively,[22] and a ribbon-type arrangement of 8-membered hydrogen-bonded rings has been reported in short peptides derived from a cyclopropane β-amino acid.[23]

Preliminary NMR studies in $CDCl_3$ and C_6D_6[24] of the hexapeptides **8** and **9** indicate comparable propensities to adopt the 10-helical structure proposed for hexamer **7**. The pattern of dispersion of amide proton resonances for the three hexapeptides **7**–**9** is very similar (Fig. 7). In all three cases the lowest frequency amide proton belonged to the same residue, the C-terminal residue F, and occurred at a similar frequency to the amide proton of the corresponding dipeptides. This is in accord with the postulated pattern of hydrogen bonds since such interactions will cause deshielding of the amide protons involved.[25] The repeating unit of hexapeptide **9** has the opposite absolute configuration

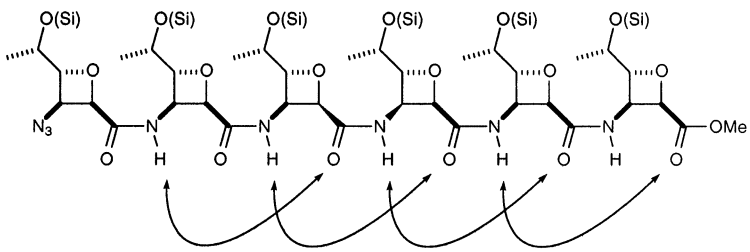

Figure 5. 10-Membered H-bonds consistent with NMR and modelling investigations.

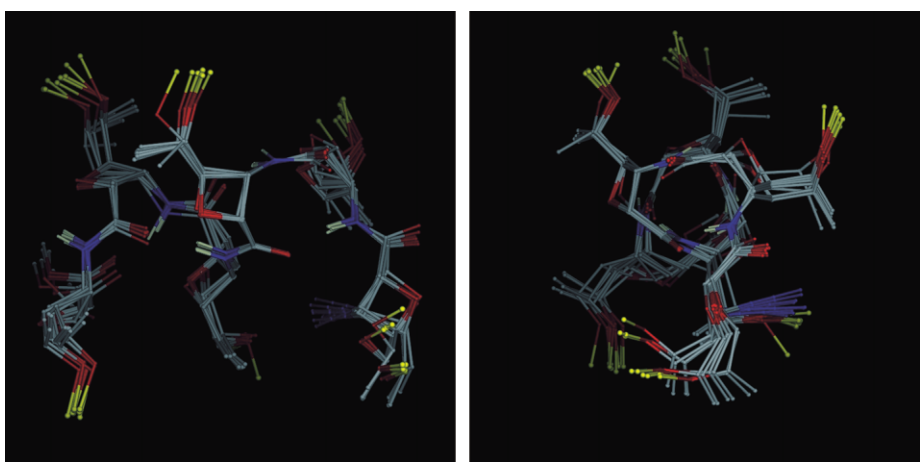

Figure 6. Overlays of ten low-energy minimum energy conformations of **7** viewed from the side and down the axis of the helix from the N-terminus (Only the silicon atoms of each TBDMS group are shown).

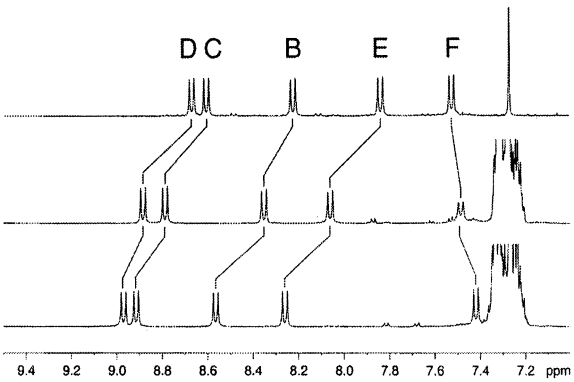

Figure 7. Partial NMR spectra ($CDCl_3$) for hexamers **7** (top plot), **8** (middle plot) and **9** (bottom plot) with ring assignments for amide NH protons.

at all stereogenic centres to that of **7** and **8**, and should therefore adopt the mirror image helical conformation, a right-handed 10-helix.

The solution IR spectra of hexamers **8** and **9** in $CHCl_3$ (Fig. 3) also show two distinct N–H stretches, a weak absorption at approximately 3380 cm^{-1} and a much stronger, broader absorption at 3302 cm^{-1}. Similar spectra recorded for the corresponding dipeptides show a single absorption at 3387 cm^{-1} which is unchanged upon 10-fold dilution. Since free amide groups are expected to give an NH absorption[26] above 3430 cm^{-1} and the 10-membered hydrogen-bonded rings postulated for the hexamers **7**–**9** cannot be formed in an isolated dimer molecule, we assign the amide bands observed as follows:

(i) the 3302 cm^{-1} band is attributed to amides engaged in strong hydrogen bonds within 10-membered rings.

(ii) the 3380 cm^{-1} band is attributed to amides engaged in much weaker, if any, intramolecular interactions and possibly within 6-membered rings. Such interactions have been deduced from crystallographic data for a dipeptide containing a *cis*-cyclobutane β-amino acid.[27]

In summary short β-peptides based on *cis*-oxetane β-amino acids are predisposed to forming a novel 10-helical conformation in organic solvents. Such oxetane-templated peptides are the most recent addition to an expanding array of foldamer families and may enhance our understanding of the principles behind the design of functional folded architectures in larger synthetic structures.[28]

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