Secondary structure in oligomers of carbohydrate amino acids

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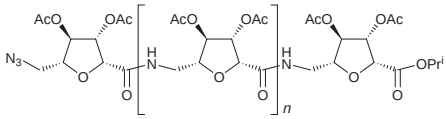
Short oligomeric chains of tetrahydrofuran amino acids exhibit a novel repeating β-turn type secondary structure in solution stabilised by hydrogen bonds and provide clear evidence that carbopeptoids will allow control of conformation in peptidomimetics.

Secondary structural elements such as α-helices and β-sheets are involved in the processes leading to the folding of proteins into functional conformations. The design and synthesis of novel materials which are predisposed to fold into these ordered structures has been an area of intense interest in recent[1] years as they may have interesting catalytic or selective recognition properties. Oligomers[2] based upon a range of templates have been shown to form helices in solution and the solid state. Our approach involves the use of carbohydrate-like frameworks bearing both an amino and a carboxylic acid functionality[3] which have been proposed as non-peptide peptidomimetics[4] by virtue of their rigidity and conformational influence on peptide backbones. Oligomers of pyranose sugar amino acids[5] ('carbopeptoids'[6]) have been synthesised by solution[7] and solid phase[8] methods, but there are few reports of their conformational preferences.[9] Here we describe oligomers of sugar amino acid derivatives based upon a β-D-*arabino*-furanose scaffold which adopt a novel repeating β-turn type structure stabilised by intramolecular hydrogen bonds in solution.

An efficient synthesis of the tetrameric **2** and hexameric **3** carbopeptoids utilising solution phase coupling procedures is reported in the previous paper.[10]



1 *n* = 0, dimer
2 *n* = 2, tetramer
3 *n* = 4, hexamer

Solution conformations in $CDCl_3$ were investigated by ^1H NMR spectroscopy. All resonances were unambiguously assigned by a combination of 2D NMR techniques. Proton spin systems within each residue were identified *via* DQF-COSY and T-ROESY[11] spectra, with the configuration within each sugar ring being confirmed by the observed NOE correlations (cross peaks in NOESY spectra were positive but rather weak, indicating the molecular correlation time, τ_c, to approach the $\omega_o\tau_c$ = *ca*. 1 condition). NOE data also allowed the sequential placement of each residue from the observation of $H2^i$ to HN^{i+1} interactions. To confirm that these were indeed sequential, rather than longer-range correlations brought about by folding of the molecule, semi-selective gradient-enhanced HMBC experiments[12] of the carbonyl region were used to establish unambiguous through-bond ^1H–^{13}C connectivities between adjacent residues *via* correlations with the carbonyl carbons (in particular, $H2^i$ to CO^i and CO^i to $H6^{i+1}$). Finally, the NOE data were further used to establish the solution conformation of the molecule in which tetramer **2** appears to adopt a novel repeating 'β-turn' type structure stabilised by (*i*, *i* − 2) inter-residue hydrogen bonds (Fig. 1). Each repeating tetrahydrofuran unit can be considered as a dipeptide isostere with each H-bond

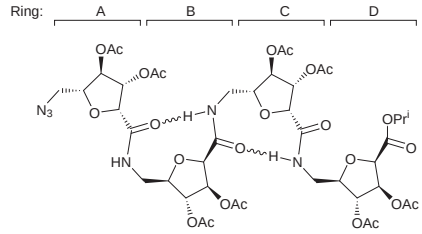


Fig. 1 Representation of the observed solution secondary structure of the tetramer **2** indicating ring labelling. Rings are identified by labelling each residue alphabetically from 'A' at the N-terminus.

completing a turn that is structurally reminiscent of a conventional peptide β-turn.[13]

Proton chemical shift dispersion of **2** is high despite the repeating unit, which is itself suggestive of a well defined solution structure. The ^1H NMR spectrum of the amide region for tetramer **2** and its hexameric homologue **3** is shown in Fig. 2. The chemical shifts of amide protons are sensitive to the presence of hydrogen bonding; a decrease in diamagnetic shielding due to the population of hydrogen bonded states should result in a high-frequency δ_{NH} shift:

For the tetramer **2**, such a shift is observed for two of the three amide protons (δ_H 8.19 and 8.03), subsequently identified as NH^D and NH^C, whose shifts are therefore indicative of involvement in hydrogen-bond formation. The remaining amide (NH^B) resonates at significantly lower frequency (δ_H 6.91), characteristic of an amide which experiences little or no hydrogen-bonding. This shift is similar to that observed for the dimeric unit **1** (δ_H 7.18) which is itself unable to form the inter-residue hydrogen-bond proposed herein for the higher homologues. An equivalent pattern is observed in the hexameric analogue **3** which exhibits four high-frequency amide protons and one again at lower frequency (Fig. 2). The chemical shifts of all three amide protons of the tetramer are, in contrast, similar in DMSO (Table 1), indicating similar solvent hydrogen-bonding interactions for all three. However, temperature coefficients of the amide protons of the tetramer **2** in DMSO indicate that NH^D and NH^C experience greater shielding from these solvent interactions than does NH^B (Table 1) and correlates with the higher chemical shifts of NH^D and NH^C observed in $CDCl_3$.

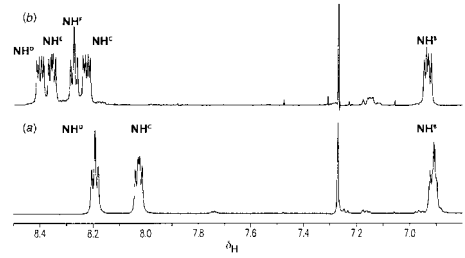


Fig. 2 Amide regions of the ^1H NMR (500 MHz) spectrum of (*a*) tetramer **2** and (*b*) hexamer **3**. Proton assignments are indicated. The spectra were recorded on a Bruker AMX-500 spectrometer at 298 K in $CDCl_3$ and referenced to residual solvent at δ 7.27.

Table 1 Amide proton temperature coefficients and chemical shifts for the tetramer **2**

	$\Delta\delta$([$^2H_6$]DMSO)/ ppb K^{-1}	δ_H([$^2H_6$]DMSO)/ ppm	δ_H($CDCl_3$)/ ppm
NH^D	4.3	8.20	8.19
NH^C	3.9	8.29	8.03
NH^B	5.3	8.12	6.91
H_2O	5.1	—	—

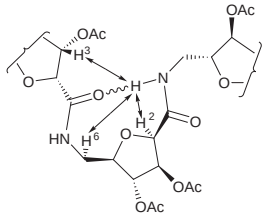


Fig. 3 Representation of the significant inter-residue NOE enhancements observed for each 'turn'. Relevant protons are numbered individually.

The pattern of deshielded *vs.* shielded amide protons for **1–3** is consistent with a repeating structural unit, rather than simply the formation of hydrogen bonds between amide protons and acetate groups on the same or adjacent residues, as is further supported by the NOE data. With only one exception ($H3^A$ to $H6^C$-*pro-S*), all NOEs that were observed *between* residues involved the amide protons and no inter-residue ring–ring interactions could be detected. Significant inter-residue NOEs were NH^i to $H2^{i-1}$, NH^i to $H6^{i-1}$ (stereospecifically) and NH^i to $H3^{i-2}$ (Fig. 3) as observed from both NH^D and NH^C, and are suggestive of the proposed (i, $i-2$) inter-residue hydrogen bonds.

Using these data, molecular dynamics simulations[14] utilising NOE derived distance constraints were performed for the tetramer (Fig. 4). This resulted in the generation of five low energy structures, all of which exhibit the anticipated geometry (backbone atom RMS deviation between the five structures is 0.6 Å). Superposition of these structures [Fig. 4(A)] shows the expected fraying at the C-terminus, which does not participate in hydrogen bonding. The conformer which most satisfies the distance restraints is shown in Fig. 4(B)). This structure is consistent with the lack of ring–ring NOEs and reflects the strong conformational preferences from each sugar ring stereochemistry.

In conclusion, we have shown that short oligomeric furanose sugar amino acid chains—even a tetramer—can adopt well-defined novel secondary structures stabilised by intramolecular hydrogen bonds; this is the first example of a 'carbopeptoid' of *any* length in which secondary structure has been experimentally demonstrated. The ease of synthesis of a wide range of structures such as the tetrahydrofuran **4** is likely to give flexibility and control in the design and applications of peptidomimetics with well-defined secondary structure, low molecular weights and thus good bioavailablity.

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Notes and References

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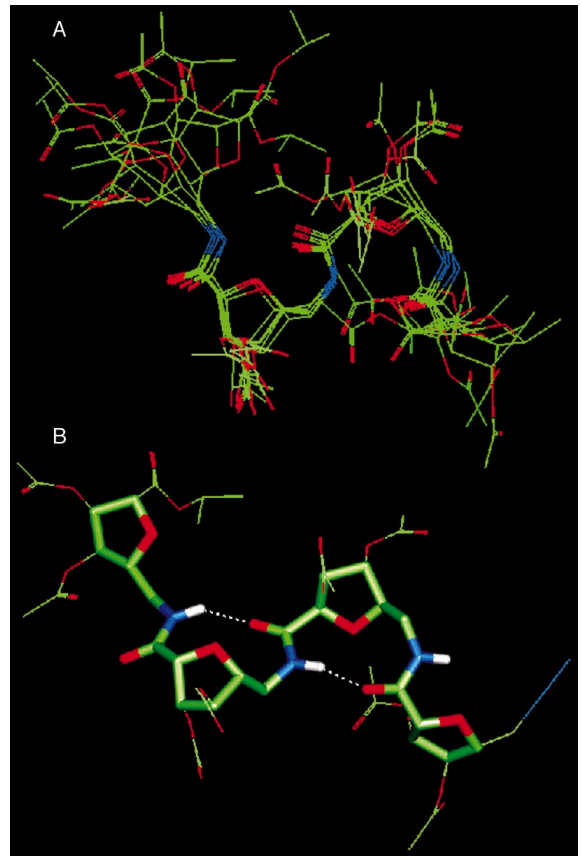

Fig. 4 (A) Five lowest energy structures of the tetramer **2** generated by restrained molecular dynamics simulations performed using the program QUANTA with the CHARMM forcefield. (B) The conformer in best agreement with the experimental restraints from the five structures of the tetramer **2** illustrated in (A). The two hydrogen bonds are indicated by broken lines.

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