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An Octameric Carbopeptoid; Secondary Structure in Octameric and Tetrameric 5-Aminomethyl-Tetrahydrofuran-2-carboxylates

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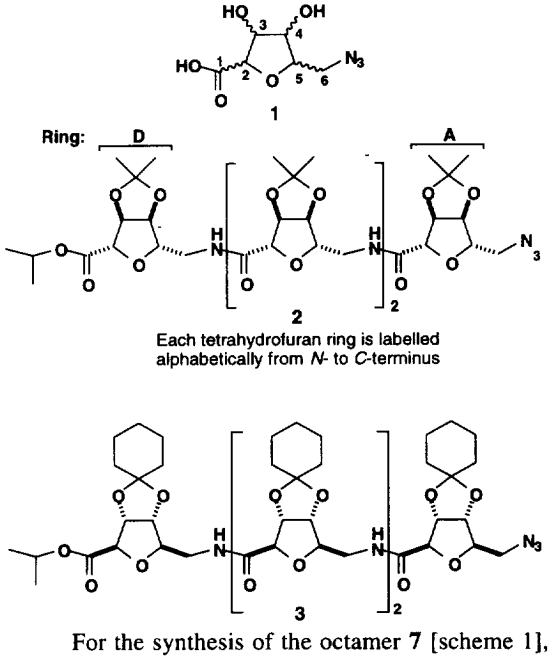
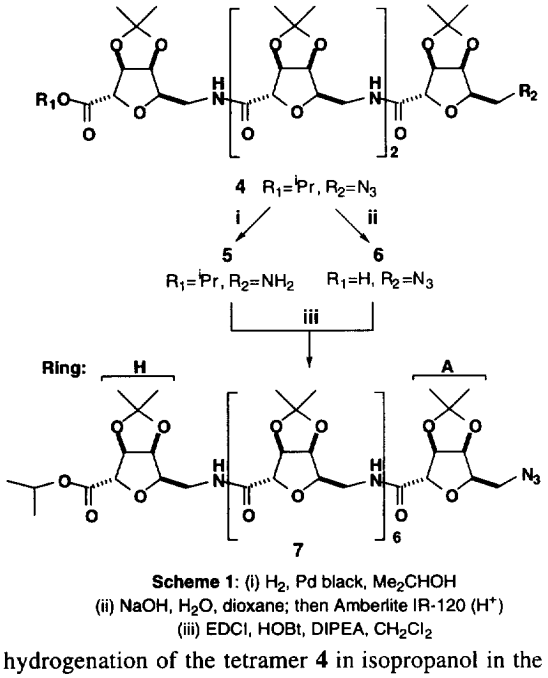
Abstract: The efficient synthesis of an octameric furanose carbopeptoid, readily purified by chromatography in ethyl acetate:hexane (2:1), is reported. Extensive NMR studies suggest that two tetrameric 5-aminomethyltetrahydrofuran-2-carboxylates are prone to adopt solution conformations reminiscent of a repeating β-turn, a third tetramer and the corresponding octamer may tend towards a left-handed α-helix.

The preceding paper describes the synthesis of stereoisomeric tetramers **2**, **3** and **4** derived from protected forms of three of the sixteen possible stereoisomeric azidoacids **1**;[1] each unit of **1** might be considered as a dipeptide isostere so that a tetramer is the equivalent of an octameric peptide. This paper describes the conversion of the 2,5-*trans*-disubstituted tetramer **4** to the octamer **7** [a hexadecameric peptide equivalent]; ^{1}H NMR studies on the three tetramers and the octamer indicate that all these oligomers may be predisposed towards the formation of secondary structures in solution and suggest that the complete family of tetrahydrofuran-azidoacids **1** may eventually provide a useful set of compounds for the induction of different secondary structures.





Scheme 1: (i) H_2, Pd black, Me_2CHOH
(ii) NaOH, H_2O, dioxane; then Amberlite IR-120 (H^+)
(iii) EDCI, HOBt, DIPEA, CH_2Cl_2

For the synthesis of the octamer **7** [scheme 1], hydrogenation of the tetramer **4** in isopropanol in the presence of palladium black gave the corresponding amine **5**, whereas hydrolysis by sodium hydroxide in aqueous dioxane followed by ion exchange purification gave the acid **6**. The tetrameric amine **5** and acid **6** were then efficiently coupled with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and

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1-hydroxy-benzotriazole (HOBt) in the presence of diisopropyl-ethylamine (DIPEA) in dichloromethane to give the octamer **7**, $[\alpha]_D^{25}$ +41.6 (*c*, 0.45 in $CHCl_3$), in 75% overall yield, readily purified by flash chromatography (ethyl acetate : hexane 2:1). The MALDI mass spectrum [Figure 1] of the octamer **7** was particularly informative, as it contains 75 carbon atoms so that the isotope distribution is significant; ^{13}C isotope constitutes 1.1% of carbon and for a compound containing over 100 carbon atoms the base peak of the mass spectrum is accordingly shifted one Dalton higher. A full analysis of all the contributing isotope effects was calculated and the expected distribution of the mass peaks for the octamer **7** ($M+H^+$ and $M+Na^+$) is shown in relation to the experimental observation [Figure 1].

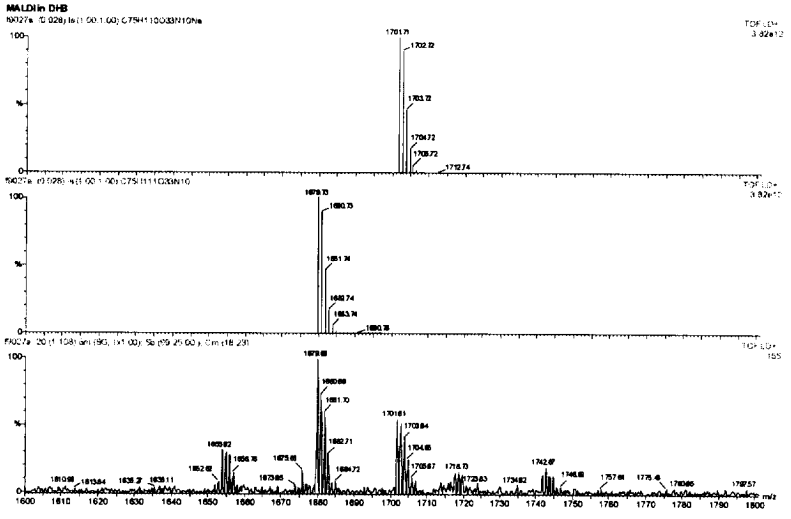


Figure 1. MALDI Mass spectrum for the octamer **7** (bottom) and calculated isotope distribution for the octamer, $M+H^+$ signal (middle) and $M+Na^+$ signal (top).

The solution conformations of the tetramers and the octamer were investigated by ^1H NMR spectroscopy.[2] Intra-residue assignments were derived from MLEV-17 TOCSY and gradient-selected HSQC[3] spectra, whilst ROESY and T-ROESY[4] spectra were used to establish sequential inter-residue connectivities[5] and to probe solution conformations.

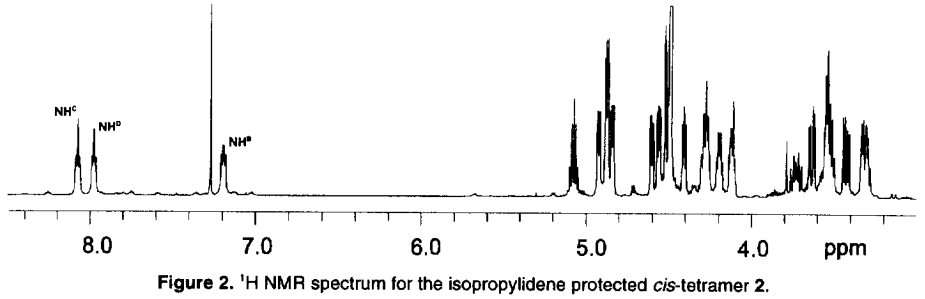
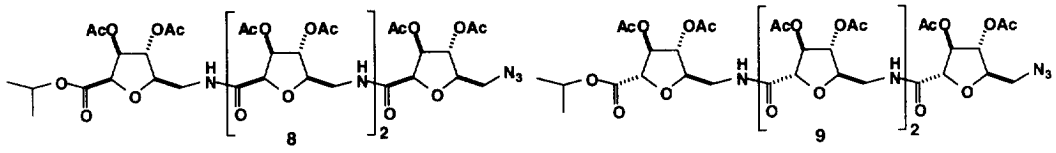


Figure 2. ^1H NMR spectrum for the isopropylidene protected *cis*-tetramer **2**.

The partial proton spectrum [Figure 2] of the isopropylidene-protected *cis*-tetramer **2** in $CDCl_3$ displays high chemical shift dispersion for the amide and sugar-ring protons. Particularly noticeable is the distribution of the amide protons, in which NH^C (δ 8.08 ppm) and NH^D (δ 7.98 ppm) appear to participate in intramolecular hydrogen bonding whilst NH^B (δ 7.20 ppm) does not. This pattern bears a striking similarity to that observed for **8**. Comparison of the nOe patterns revealed further similarities indicative of i, i-2 inter-residue hydrogen bonds.[6] Whilst unambiguous assignment was not always possible, significant inter-residue nOes for NH^i to $H2^{i-1}$ and NH^i to $H6^{i-1}$ (stereospecifically) were observed for both NH^C and NH^D. It thus appears that the gross secondary structure of **2** resembles that previously reported for **8**, adopting a similar

repeating "β-turn" type conformation. In such a conformer, the secondary hydroxyl protecting groups sit away from the hydrogen-bond of each turn and on the outer edges of the molecule, so modest chemical changes at these positions appear to have little effect on the secondary structure. This is further confirmed by studies on the tetramer **3** which is related to **2** as the enantiomer (but with a cyclohexylidene rather than an isopropylidene protecting group); the ^{1}H NMR of **3** is very similar to that of **2**, indicating that a different ketal protecting group has very little effect on the solution conformation.



In the case of the *trans*-tetramer **4**, neighbouring residues were also identified *via* a semi-selective, gradient-enhanced HMBC experiment[7] of the carbonyl region. The observation of simultaneous $H2^i$ and $H6^{i+1}$ correlations to CO^i in this confirmed the presence of sequential $H2^i$ to NH^{i+1} nOes in the ROESY spectra and provided sequential assignments for all rings.

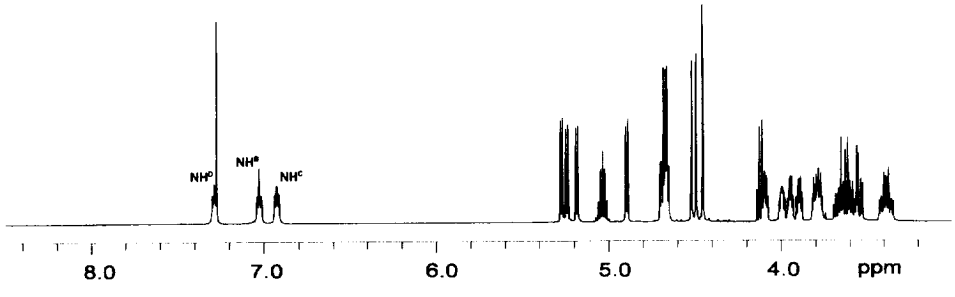


Figure 3. ^{1}H NMR spectrum for the *trans*-tetramer **4**

The ^{1}H NMR [Figure 3] of **4** also shows high dispersion in $CDCl_3$, although the resonance distribution of the amide region is quite different to that of **2** described above. Here, only the amide proton of a single residue (ring D) displays a moderate shift to higher frequency perhaps indicative of a weak H-bonding interaction, whilst the other two appear to be essentially solvent exposed. These data should be contrasted with the *trans*-tetrahydrofuran isomer **9**[8] which displays no such dispersion and appears to have no conformational preference. The long-range inter-residue nOe patterns observed for the *trans*-isomer **4** are also quite different to those observed for the *cis*-isomer **2**; these data suggest **4** favours a solution conformation(s) that it is quite different to that of **2**. This conformational preference appears to be stronger in the octamer **7** in which NH^B and NH^C resonate at low frequency (δ<7.2 ppm), again corresponding to protons that are solvent exposed, having little or no participation in hydrogen bonding [Figure 4]. Five NH resonances fall above 7.3 ppm which suggest these experience H-bonding interactions; all the amide protons display an alternating sequence of high-frequency/low-frequency shifts from the *C*- toward the *N*- terminus which is perhaps suggestive of a repeating structural motif in the molecule. Extensive long-range nOe analysis of the octamer **7** revealed a number of NH^i-$H5^{i-1}$and NH^i- $H5^{i-2}$ nOes and a series of NH^i-$H3^{i-3}$nOes. A collection of long-range nOes could also be identified between sugar ring protons, in contrast to the *cis*-isomers **2** and **3** where nOes between sugar protons were very rarely observed. In particular, sequences of $H2^i$-$H4^{i-2}$ and $H2^i$-$H3^{i-2}$ nOes were observed along the length of the molecule, although again a few of these could not be unambiguously assigned due to resonance overlap. At least one set of these nOe patterns was observed for each residue 'B' to 'H', whereas none were observed involving residue 'A'. The repeating pattern of nOes observed provides evidence for a repeating structure along the molecule. Taking into account the spread of amide protons and the probable involvement of those of residues 'D' to 'H' in H-bonding interactions, simple modelling suggests the presence of a helical structure stabilised by inter-residue NH^i-CO^{i-3} hydrogen bonds. Two possible helices can then be

considered, the right- and left-handed versions. Again, from simple models, only a left handed conformation would be consistent with the repeating nOe patterns observed. In such a structure all H5 protons point into the centre of the helix and produce close NH^{i}-$H5^{i-2}$ proximities. Likewise, close proximities between NH^{i}-$H3^{i-3}$ result. The handedness of the helix is confirmed by the observation of the $H2^{i}$-$H3^{i-2}$/$H4^{i-2}$ nOes along the molecule. In the left-handed form, the H2 proton sits below and between the H3 and H4 protons of the next sugar above it in the turn of the helix, consistent with these observations. However, in the right-handed form the H2 protons point instead towards the *C*-terminus of the helix, and would be expected to give rise to $H2^{i}$-$H3^{i+2}$/$H4^{i+2}$ nOes, unlike those observed. Moreover, the observed NH^{i}-$H3^{i-3}$nOes would not be anticipated for this form, whereas NH^{i}-$H2^{i-3}$ nOes may well be, but again these are not seen.

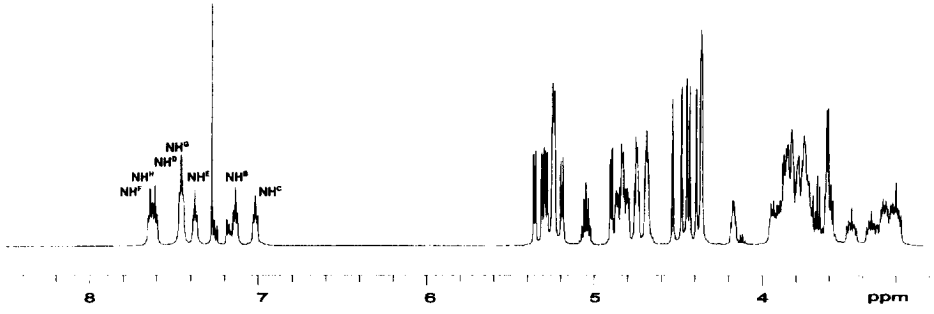


Figure 4. ^{1}H NMR spectrum for the *trans*-octamer **7**

In a helical structure for the octamer **7**, the amide protons of residues 'B' and 'C' would be unable to participate in the i, i-3 hydrogen bonds; this is also consistent with the observed amide proton shifts. The other amide proton shifts suggest a general increase in the hydrogen-bonding towards the *C*-terminus. Residue 'A' would not be restrained by H-bond interactions prior to its carbonyl group, and its relative freedom is consistent with the lack of sugar proton nOes. In the tetrameric equivalent **4**, only a single H-bond could be formed if a similar pseudo-helical structure existed, and indeed only a single amide proton is shifted to a slightly higher frequency. With only one stabilising H-bond, the conformation is likely to be averaging between the 'folded' and open forms, resulting in a smaller time-averaged amide proton shift. Modelling studies and circular dichroism investigations of the octamer **7** are in progress and will hopefully confirm the helical secondary solution structure of this class of carbopeptoid.

The studies reported in this and the two preceding papers indicate that relatively large oligomers of 5-aminomethyltetrahydrofuran-2-carboxylates are easy to synthesise and purify, that a range of different stereoisomers of the monomers are available and that short homooligomers appear to possess defined secondary structure. Such furanose aminoacids may give rise to a general class of foldamers.[9,10]

References.

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[2] All spectra were recorded at 298K on a Bruker AMX500 spectrometer equipped with a inverse broadband z-gradient probe. TOCSY spectra were collected with an MLEV-17 spin-lock and a mixing time of 70 ms. ROESY spectra were collected with either a 200 ms CW spin-lock or a phase-alternating spin-lock to suppress TOCSY contributions (T-ROESY).

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[5] Each sugar residue of a carbopeptoid is labelled alphabetically from the *N*- to the *C*- terminus. Protons on each sugar ring are numbered according to IUPAC recommendations on carbohydrate nomenclature.

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[10] Support from the EPSRC and GlaxoWellcome is gratefully acknowledged.