Peptides derived from nucleoside β-amino acids form an unusual 8-helix†

Richard Threlfall,[a] Andrew Davies,[a] Nicola M. Howarth,[b] Julie Fisher*[c] and Richard Cosstick*[a]

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Peptides of varying length (dimers to octamers) were prepared from nucleoside β-amino acids and conformational studies, based on NOE observations, show that the β-peptides form an unusual 8-helix.

Unnatural oligomers that have a propensity to adopt well-defined compact conformations have been termed foldamers[1] and possess the potential to mimic structural motifs found in complex biopolymers such as proteins and RNA. A number of exciting applications can be envisaged for foldamers including peptidomimetics,[2] antimicrobial agents[3] and components in nanostructured materials.[4] Of all the unnatural oligomers under investigation, the folding preferences of β-peptides have been the most intensively studied. In particular, studies on β-amino acids derived from conformationally restrained systems such as cycloalkanes[5,6] and sugars,[7] show that they form peptides that adopt strongly helical structures. Gellman and co-workers have shown that β-peptides derived from *trans*-2-aminocyclopentanecarboxylic acid[5] (*trans*-ACPC, Fig. 1) adopt a 12-helix, whereas peptides prepared from *trans*-2-aminocyclohexanecarboxylic acid[6] (*trans*-ACHC), form a 14-helix.

Whilst inspiration for the construction of foldamers has largely been drawn from protein chemistry, nucleosides also possess a number of features that make them attractive monomers for this purpose. The most notable of which are well established methods for oligomer synthesis and predictive associative properties of the nucleobases. The association of peptide helices through base-pairing provides a potentially powerful method for controlling the assembly of complex tertiary structures. As proof of principle, Brückner *et al.* have demonstrated that helices containing β-homoalanine functionalised with nucleobases can associate through base pairing.[8] We were struck by the fact that nucleosides can be readily converted to β-amino acids which are closely related to Gellman's helix-forming *trans*-ACPC monomer and now report the synthesis of peptides derived from the thymidine β-amino acid **1** (Fig. 1) and their structural properties.

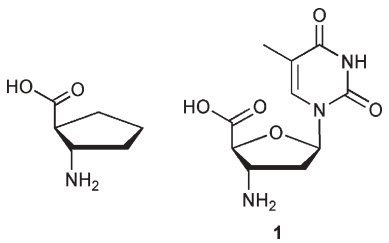

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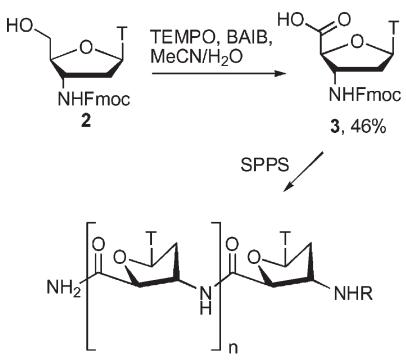
Fig. 1 *trans*-ACPC and thymidine β-amino acid (**1**).

The target monomer[9] **3** (Scheme 1), was synthesised from the previously reported[10] fluorenylmethoxycarbonyl (Fmoc)-protected amino alcohol **2** by oxidation with [bis(acetoxy)iodo]benzene (BAIB) and 2,2,6,6-tetramethypiperidinyloxy (TEMPO)[11] in aqueous acetonitrile. Although the oxidation proceeds in relatively low yield (46%) the method has the advantage that the Fmoc-protected amino acid precipitates out of the reaction mixture and is obtained pure after filtration and washing with diethyl ether.

The choice of resin for Fmoc solid-phase peptide synthesis (SPPS) was influenced by the sensitivity of the glycosidic bond to acid. With this in mind, the hyperacid-labile Sieber amide resin[12] was chosen, which, on cleavage, produces peptides with a primary amide at the carboxylate terminus. The peptide assembly used 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in *N*,*N*-dimethylacetamide as the coupling agent and followed standard Fmoc protocols.[13] Using this procedure, dimers, tetramers and octamers, both with and without the Fmoc group, were prepared (Scheme 1). Following cleavage from the support and washing with water, the oligomers gave clean NMR spectra and were also shown, by mass spectrometry, to be free of failure sequences.



4 R = H, n = 1
5 R = H, n = 3
6 R = Fmoc, n = 3
7 R = H, n = 7
8 R = Fmoc, n = 7

Scheme 1 T = thymin-1-yl.

[a]*Department of Chemistry, University of Liverpool, Crown Street, Liverpool, UK L69 7ZD. E-mail: rcosstic@liv.ac.uk; Fax: +44 (0) 151 794 3588; Tel: +44 (0) 151 794 3514*
[b]*Chemistry, School of Engineering & Physical Sciences, William H. Perkin Building, Heriot Watt University, Edinburgh, UK EH14 4AS*
[c]*University of Leeds, Leeds, UK LS2 9JT. E-mail: J.Fisher@chem.leeds.ac.uk; Fax: +44 (0) 113 343 6565; Tel: +44 (0) 113 343 6577*

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Table 1 Temperature-dependent chemical shifts of NH protons of **6** in d_6-DMSO solution

Residue number	25 °C	30 °C	35 °C	40 °C	45 °C	Average shift difference/ppb K^{-1}
1	8.845	8.818	8.790	8.764	8.729	5.80
2	8.910	8.882	8.857	8.830	8.783	6.35
3	8.980	8.951	8.926	8.902	8.851	6.45
4	8.117	8.098	8.084	8.069	8.054	3.15

Conformational features of the Fmoc-protected tetramer **6** and the fully deprotected octamer **7**, in d_6-DMSO and d_5-pyridine solution respectively, have been determined using NMR spectroscopy.

A complete ^{1}H resonance assignment has been achieved for **6** through the combined use of DQF-COSY, TOCSY and NOESY spectra (see ESI for numbering and NMR assignment details†). The amide NHs are well resolved and through their chemical shifts in the 2D spectra the remaining assignments could be made. The NH chemical shifts are worthy of note. The NH of residue 4 (the Fmoc-protected amino terminus) is almost identical to that observed for the Fmoc-protected monomer. This observation, together with the lowfield shift of the remaining secondary amide protons, is supportive of a hydrogen bonding network involving the three interresidue NHs.[14] Variable temperature experiments performed over the temperature range 25 to 45 °C resulted in temperature dependent chemical shift changes to highfield of approximately 6 ppb K^{-1} for the NHs of residues 1 to 3, but only 3 ppb K^{-1} for residue 4 (Table 1).

It is the convention to assume that small shift changes would arise for amide protons involved in intramolecular hydrogen bonds and large changes for solvent exposed NHs. However, for essentially linear systems, as here, if hydrogen bonding is intramolecular then a large temperature coefficient would be observed.[15] Thus these results are also consistent with hydrogen bonding within the tetramer. The possibility of a hydrogen bonding array is also suggested by the model, formed through consideration of a series of NOE connectivities identified in the NOESY spectrum, and built using Macro-Model.[16] More specifically, through-space connections are observed between NH(1)–H3′(2), 3H6–2H3′, NH(3)–H4′(4). These, together with a number of other interresidue NOEs and intraresidue connections (see Fig. 2 and ESI†), have been utilised to build a model for the tetramer (Fig. 3). All of the NOEs observed are consistent with this model. NMR studies of **4** had revealed the sugar puckers of the first and second rings to be 60% and 50% south, respectively. Consequently, in the modeling procedure sugar puckers were constrained to 50% south, with dihedral angles permitted to vary by $\pm 10°$.

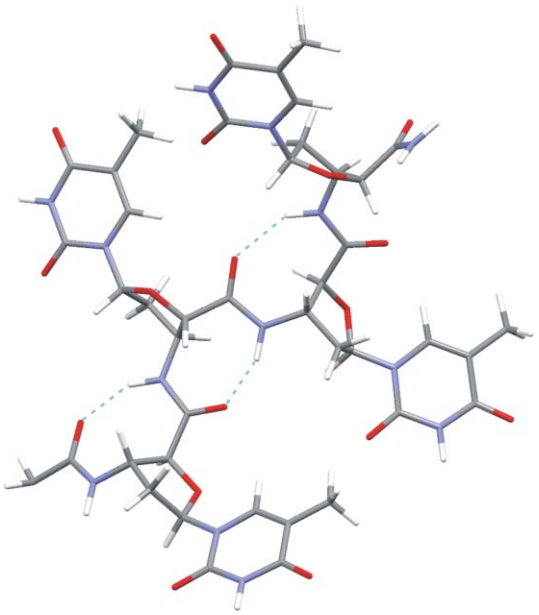

Fig. 3 Model of the structure formed by tetramer **6** produced using Macro-Model and NMR constraints.[18,19] The dashed lines are provided to indicate hydrogen bonded NHs and carbonyls. The Fmoc group has been deleted for ease of viewing.

In Fig. 3 a zig-zag arrangement of the deoxyribose rings is apparent, leading to the thymine bases occupying opposite faces of the extended chain. In this conformation the NH of residue 1 comes close in space to the carbonyl attached 3′ to residue 2. Similarly the NH of residue 2 comes close in space to the carbonyl 3′ to residue 3 and finally the NH of residue 3 is close in space to the carbonyl of the Fmoc group. An atom count between these groups reveals an 8-helix. Whilst 12- and 14-helices are much more common, a number of examples of 8-helix have appeared in the literature.[14] Doerksen *et al.* found the 8-helix to be energetically most favourable for their conformationally-constrained oxanorbornene β-peptides. Whilst the current systems have more flexibility in the pentose ring compared to the oxanorbornene systems, the helical arrangement observed here may be due to steric factors imposed by the thymine nucleobase. In this respect, it has been suggested that *trans*-ACPC monomers with relatively small substituents on the 4 position (which is comparable to the anomeric position of **1**) perturb the 12-helix conformation.[17]

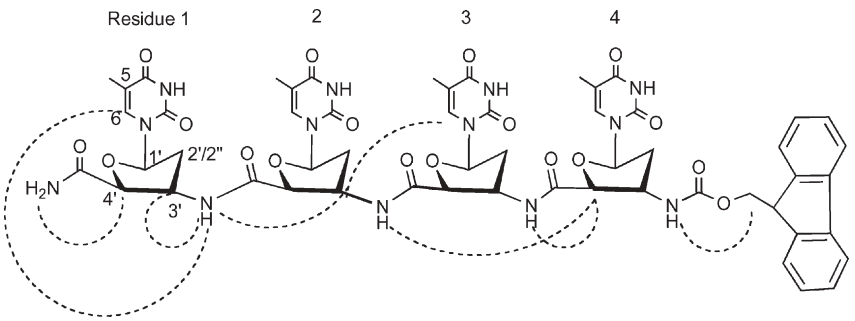

Fig. 2 Diagram of peptide **6** presenting some of the NOE connections used in determining the model for **6** shown in Fig. 3.

Similar studies have been performed for the deprotected octamer **7**, in d_5-pyridine solution, although resonance assignment is not yet complete.[18] Residues 1 and 8 have been unambiguously identified and despite the change in solvent, these look remarkably similar to the first and last residues of the tetramer **6**. A series of interresidue NH–H3′/H4′ NOE connections have been observed, together with a number of NH–NH, and NH–H6 (interresidue); the latter NOEs being more apparent at low temperature (10 °C). These NOE connectivities bear similarities to those observed for tetramer **6** and imply a similar helical arrangement for the octamer **7**, but more extensive resonance assignment is required for this to be confirmed. Variable temperature experiments over the range 20 to 45 °C have revealed chemical shift changes for the secondary amide protons of between 8.3 and 9.4 ppb K^{-1} to highfield. In contrast, the average chemical shift change for the thymine H6 protons was 3 ppb K^{-1}. These data are consistent with a hydrogen bonded system in which there is little or no base stacking (as suggested by Fig. 3). With the aid of the model derived for the tetramer **6** it is anticipated that full assignment and structure determination for the octamer **7** will be forthcoming. Similar features are observed for **7** in DMSO solution, however there is better resolution in the H6 chemical shifts for **7** in d_5-pyridine.

In conclusion, peptides derived from a thymidine β-amino acid have been prepared and their conformation studied by NMR spectroscopy. Interestingly, the tetramer **6** forms an 8-helical conformation, which differs considerably from the 12-helix derived from the related *trans*-ACPC. An important long term aim is to investigate association between helices derived from adenine and thymine and thus capable of nucleobase-pairing. These systems would offer a new structural motif which would have applications in the assembly of innovative macromolecular architectures.

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