Journal of Peptide Science
J. Peptide Sci. **5**: 425–441 (1999)

Review

Designing Secondary Structures: 5-Azidomethyl Tetrahydrofuran-2-carboxylates as Carbohydrate-derived Dipeptide Isosteres

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Received 2 July 1999
Accepted 4 July 1999

Abstract: The potential of carbohydrate-like tetrahydrofuran frameworks as building blocks with predictable conformational propensities useful in the design and synthesis of novel peptidomimetic materials which adopt well-defined secondary structures is discussed. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: secondary structure; design; carbohydrate; tetrahydrofuran; oligomers; peptidomimetic

INTRODUCTION

An examination of the relationship between form and function in biopolymers offers a tantalizing glimpse into the potential of their synthetic analogues; exquisite specificity of recognition and catalysis are enviable properties for any molecule to possess. These attributes emerge through folding and self-organization of flexible chains into specific and well-defined conformations – sometimes bringing covalently distant components into close spatial proximity. Domains within tertiary structures consist of particular secondary structural elements such as α-helices and β-sheets which are involved in the processes leading to the folding of proteins into functional conformations. Attempts to mimic the structure of natural polymers are varied [1], and unnatural oligomeric peptides which display secondary structure in relatively short chains, have been investigated intensively in recent years in the search for interesting catalytic or selective recognition properties. Polymers which have a tendency to adopt a specific compact conformation have been termed 'foldamers' [2]. *De novo* protein design is inherently an uphill task (as folding mechanisms are not completely understood) although the use of topological templates as built in folding appliances for the nucleation of secondary structures has been relatively successful [3]. There are also notable insights into the determinants of secondary (and tertiary) structure [4]. One approach to the problem of *de novo* protein design is the use of stereochemically constrained [5] non-proteinogenic amino acids

Abbreviations: Ac, acetyl; Boc, *tert*-butyloxycarbonyl; Bn, benzyl; CD, circular dichroism; COSY, correlation spectroscopy; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; DQF, double quantum filtered; DTT, dithiothreitol; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Fmoc, 9-fluorenylmethoxycarbonyl; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum correlation; HOBt, 1-hydroxybenzotriazole; HSQC, heteronuclear single quantum correlation; ^{i}Pr, *iso*-propyl; Ms, methanesulfonyl; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; ppm, parts per million; RMS, root mean square; ROESY, rotating-frame NOE spectroscopy; TOCSY, total correlation spectroscopy; T-ROESY, transverse rotating-frame NOE spectroscopy; PFT, protein: farnesyltransferase; TFA, trifluoroacetic acid; Tf, trifluoromethanesulfonyl; Ts, *p*-toluenesulfonyl.

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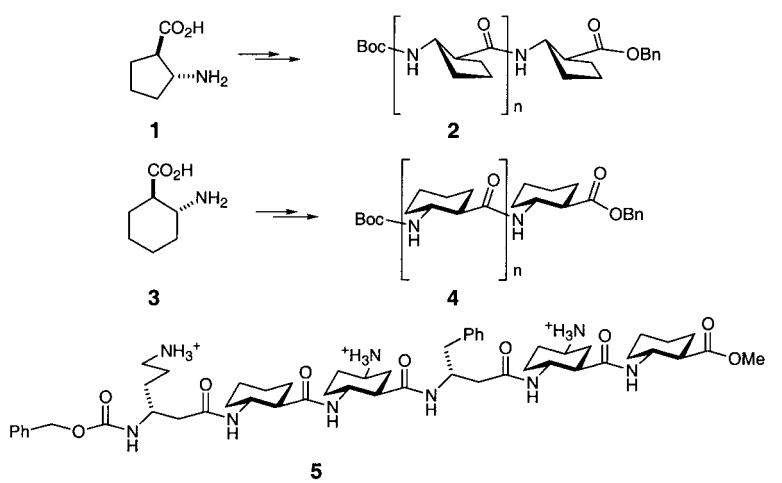
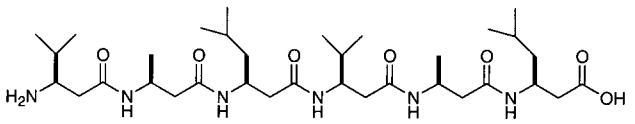
CCC 1075–2617/99/100425-17$17.50

[6]. However, the construction of novel macromolecules with a predetermined secondary structure in chains shorter than those required by the proteinogenic amino acids remains a challenging goal. The folding pattern of a protein cannot at present be predicted from its amino acid sequence, although individual residues have preferences for forming sheets, turns and helices. In cases such as these, protein folding is often driven by local stereochemical influences [7]. Hydrogen-bonding between sites in the polymeric backbone largely defines the regular secondary structure of polymers although other factors, such as intrinsic conformational preferences of the backbone, dispersion and polar interactions and solvation are clearly important. The first step in the creation of artificial protein-like structures (which might display specific tertiary structure) is the identification of novel scaffolds [8] that possess an inherent propensity to generate secondary structural elements.

ARTIFICIAL SECONDARY STRUCTURES

Gellman and co-workers [9] have synthesized peptides based upon rigidified cyclic β-amino acids. Oligomers **2** and **4** (based upon *trans*-2-aminocyclopentanecarboxylic acid **1** and *trans*-2-aminocyclohexanecarboxylic acid **3**, respectively) have been shown to exhibit stable 12- and 14-helical conformations, respectively, in organic solvents and the solid state [10]. Constraints imposed by the cyclohexane/cyclopentane ring on oligomeric derivatives confer high conformational stability. A related species derived from a combination of cyclic and acyclic β-amino acids **5** has been shown to exist with a high population of a specific helical conformation in aqueous solution [11]. Similarly the heterohexa-β-peptide **6** synthesized by Seebach and co-workers adopts a stable 14-helical conformation stabilized by hydrogen-bonds in methanol solution [12], and is also resistant to the action of proteases. Detailed 2D NMR spectroscopic analysis revealed that the hexapeptide **6** forms a secondary structure which is more stable then those adopted by α-peptides, based upon a *right*-handed helix stabilized by 14-membered ring C=O^{i}–NH^{i-3} hydrogen-bonds [13]. This is in marked contrast to natural α-amino acids which do not form stable secondary structures in chains less than 15–20 residues in length. Hanessian *et al.* [14] have also investigated the conformational preferences of γ-amino acids by the preparation of α-substituted derivatives derived by homologation of L-alanine and L-valine. Their findings were similar to those of Seebach, although they reported a helical tetrapeptide **7** which adopted a 14-helical structure, and others related to **8** which were found to adopt both helices and turns [15]. Utilizing a different approach, Gervay and co-workers [16] have synthesized the oligomer **9** based upon an amide-linked *N*-acetylneuraminic acid scaffold and studied its solution conformation to ascertain whether it adopted the same solution structure as its glycosyl-linked congener. Although the conformational preferences of these structures were not completely elucidated and varied with chain length, amide proton exchange rates were indicative of a stable secondary structure. Oligourea templates have been successfully employed by Nowick and co-workers in an elegant





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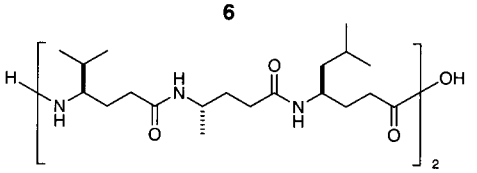

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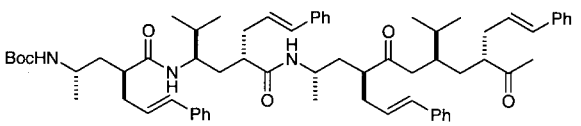

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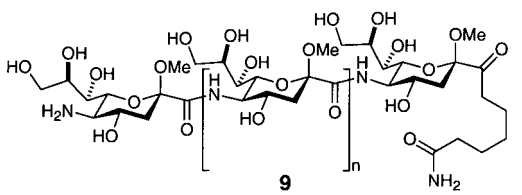
approach to the design of β-sheets [17]. The artificial sheet **10** was found to adopt approximately 50% sheet type structure in solution. Oligomers of *N*-substituted glycines **11** have been shown to adopt helical conformations in solution despite their lack of hydrogen-bond donors [18]. Peptides of α-aminoxy acids **12**, another peptide surrogate, have been shown to form novel turns and helices **13** in very short chains [19]. A detailed treatment of the development of compounds that mimic protein-like secondary structures is beyond the scope of this review; readers should consult references cited above for leading accounts.



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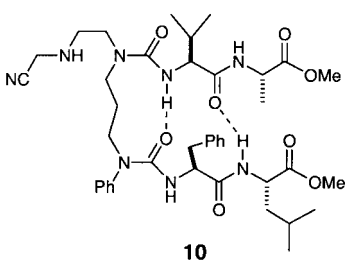

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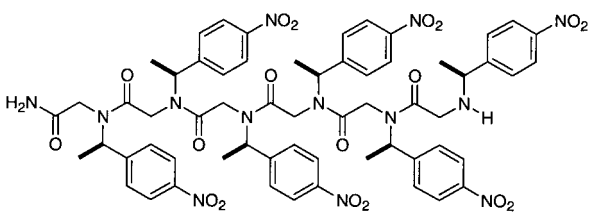
PEPTIDOMIMETIC DESIGN: DIPEPTIDE ISOSTERES

Peptides and proteins are attractive targets for mimicry [20] due to their involvement in a plethora of biological processes. However, there are obvious limitations of peptidic drugs, generally associated with mediocre absorption and poor metabolic stability amongst other factors. A successful approach for the imitation of secondary structures is the use of dipeptide analogues to induce a specific target conformation into a peptide [21]. In general, the requirements for a successful secondary structure mimic are the ability to induce the requisite conformation as exactly as possible whilst also permitting introduction of side chains and chemical modification. Most dipeptide analogues are based upon isosteric replacement of the amide bond with a suitable mimetic or bridging between two neighbouring amino acids in a peptide. Bridging leads to a compound with limited conformational flexibility in comparison with that of regular dipeptides composed of proteinogenic amino acids [22]. Whilst a combinatorial synthetic approach to peptide design may yield biologically active materials, a more fundamental consideration of the potential structural role of these mimics and their naturally occurring parents may provide important information. Before the generation and understanding of new tertiary structures is possible an increased knowledge of unnatural oligomers which are predisposed to adopt specific secondary structure is required.



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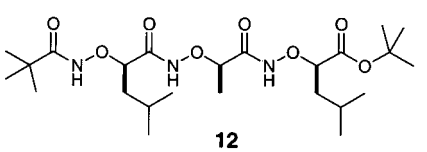

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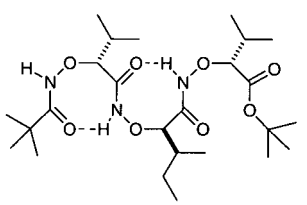

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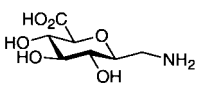
CARBOHYDRATE AMINO ACIDS

Carbohydrates bearing both an amino and a carboxylic acid functionality have been proposed as combinatorial building blocks [23,24] and peptidomimetics [25]; they are found as components of

both natural herbicides [26] and antibiotics [27], and in potent inhibitors of sugar-processing enzymes [28,29]. Oligomers of pyranose sugar amino acids ('carbopeptoids') [30] have been synthesized in solution [31,32] and on solid supports, and have been proposed as oligosaccharide mimics [33] and oligonucleotide backbone analogues [34]. Unnatural oligomers of this nature may have the ability to mimic conformations of natural biopolymers.

Kessler and co-workers [35,36] investigated the conformational influence of a range of pyranose carbohydrate amino acids **14**–**17** on peptide chains by utilizing them as a replacement for the natural Gly-Gly dipeptide in a linear Leu-enkephalin analogue **18**, in the preparation of cyclic somatostatin analogues **19**, **20** and in a model cyclic peptide **21**.



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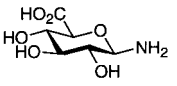

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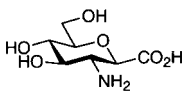

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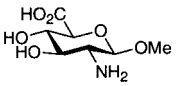

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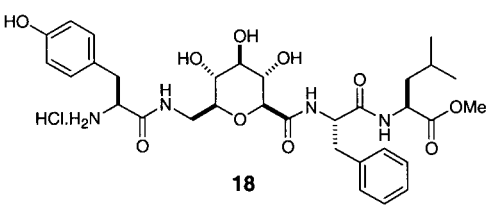
Conformational analysis of the resulting sugar-peptide hybrids by NMR and molecular dynamics simulations indicate that the pyranoside **16** functions as a γ-turn whilst **14** and **15** both induce a β-turn like conformation. The partially deoxygenated sugar amino acid **23** has been used as a replacement for the Xaa-Xaa dipeptide (where 'Xaa' can be any proteinogenic amino acid) in the natural K-Ras p21 substrate **22** of protein: farnesyltransferase (PFT) to afford the peptidomimetic **24** [37]. Inhibitors of PFT have potential therapeutic uses in the treatment of pancreatic and colonic cancers. The peptide **24** containing the 2,6-*cis* carbohydrate derivative was found to have an IC_{50} of 214 μM, significantly higher than the peptide containing the 2,6-*trans* isomer.

A similar approach based on the incorporation of 2,5-*cis* **25** and 2,5-*trans* **26** furanose sugar amino acids into Leu-enkephalin as a Gly-Gly replacement has also recently been reported [38]. The 2,5-*cis* sugar **25** introduced a folded turn-type structure into the linear peptide **27** (stabilized by aromatic–aromatic interactions) as shown by NMR, CD and molecular dynamics investigations. It was also shown to have similar analgesic activities to Leu-enkephalin methyl ester. In contrast, the peptide containing the 2,5-*trans* sugar **26** was inactive (presumably due to a lack of folded conformation). A 3-deoxy-β-D-glucose derivative has been used as a nonpeptide somatostatin mimetic [39]. A D-glucopyranose sugar amino acid has also been used in the synthesis of *C*-terminal modified Leu- and Met-enkephalins [40]. Introduction of the carbohydrate moiety into the Leu-enkephalin gave an analogue **28** that has significantly more potent analgesic activity than enkephalinamide.

TETRAHYDROFURAN AMINO ACIDS AS DIPEPTIDE ISOSTERES

Our approach to the synthesis of materials with well-defined secondary structure involves the use of carbohydrate-like tetrahydrofuran frameworks **29** bearing both an amino and a carboxylic acid functionality. This rigid scaffold (which is isosteric with a dipeptide) can be formally derived from a conventional dipeptide **30** by introducing an ether linkage as an amide surrogate and also bridging between the two amino acids.



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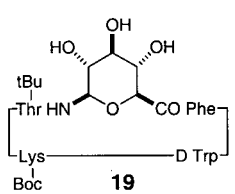

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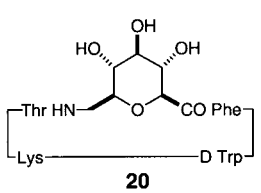

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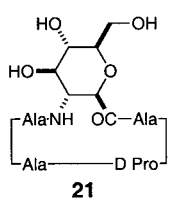

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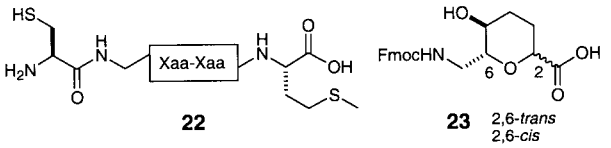
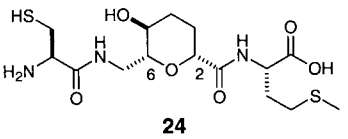
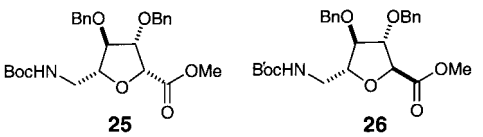
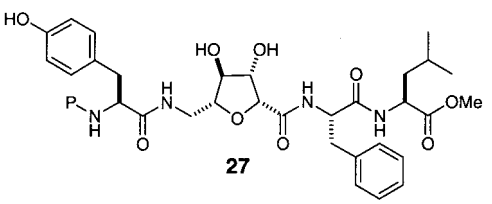
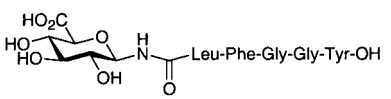
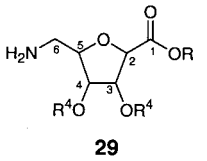


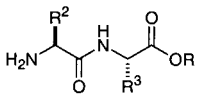








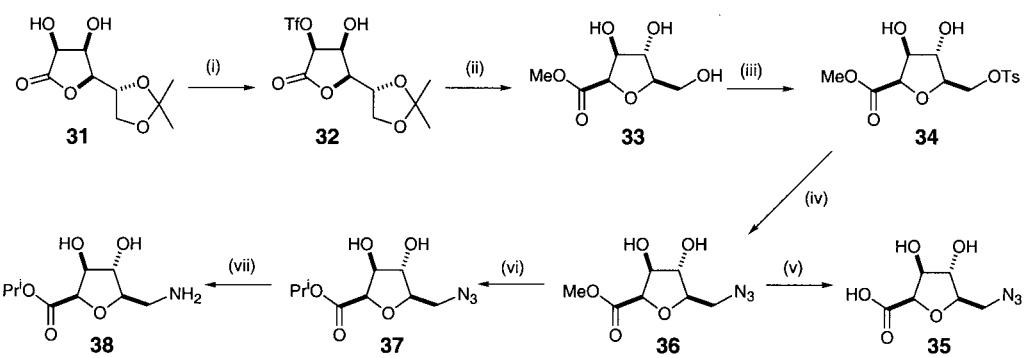




SYNTHESIS OF TETRAHYDROFURAN DERIVATIVES

The use of tetrahydrofuran amino acids as dipeptide isosteres depends crucially on the development of efficient syntheses of the monomer units. Ideally, easy access to all 16 stereoisomeric 5-azidomethyl tetrahydrofuran-2-carboxylates **29** would provide a family of materials for incorporation into peptidomimetic structures. Tetrahydrofuran rings are readily synthesized from carbohydrate derivatives, in particular from 2-*O*-triflates of carbohydrate lactones under either basic [41] or acidic [42] conditions. Such a procedure has recently been utilized for the synthesis of *C*-glycosides of glucofuranose [43], which have provided scaffolds for the generation of glucofuranose libraries.

Both the 2,5-*cis* and 2,5-*trans* monomers are accessible from readily available carbohydrate starting materials such as D-mannono-1,4-lactone and D-glucono-1,4-lactone. The 2,5-*cis* tetrahydrofuran carboxylate (THFC) derivative **33**, available via an efficient route utilizing this strategy was utilized as a framework which could be elaborated to an amino acid-like structure (Scheme 1). For the synthesis of the tetrahydrofuran **33**, the triflate **32** is required; in order to effect esterification at C-2, it was necessary to protect the primary hydroxyl group at C-6 in D-mannonolactone as its kinetic monoacetonide **31**. This is easily accessible in 74% yield from the diacetonide of D-mannose. Treatment of the diol **31** with trifluoromethanesulfonic anhydride in dichloromethane in the presence of pyridine caused highly regioselective esterification of the hydroxyl group at C-2 to give the stable triflate **32** which may be isolated in 85% yield. Treatment of the crude triflate **32** with hydrogen chloride in methanol gave the required ester **33** in 84% yield from **31**, providing multi-gram quantities of **33** in an overall yield of 62% from D-mannose. The key transformation of **32** to **33** by treatment with acidic methanol involves

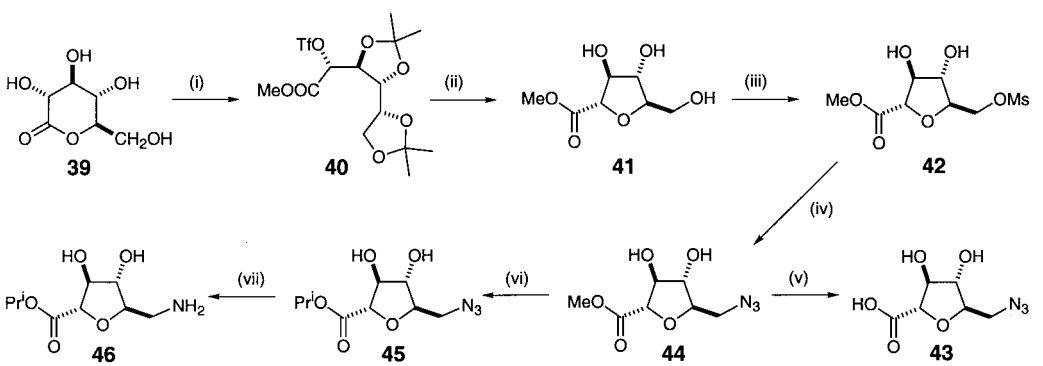


Scheme 1 Reagents and conditions: (i) Tf_2O, py, DCM; (ii) HCl in MeOH; (iii) TsCl, 3 Å sieve, py; (iv) NaN_3, DMF, 90°C; (v) 1 M NaOH (aq.), dioxane, then IR-120 (H^+); (vi) K_2CO_3, ^{i}PrOH; (vii) H_2, Pd, ^{i}PrOH.

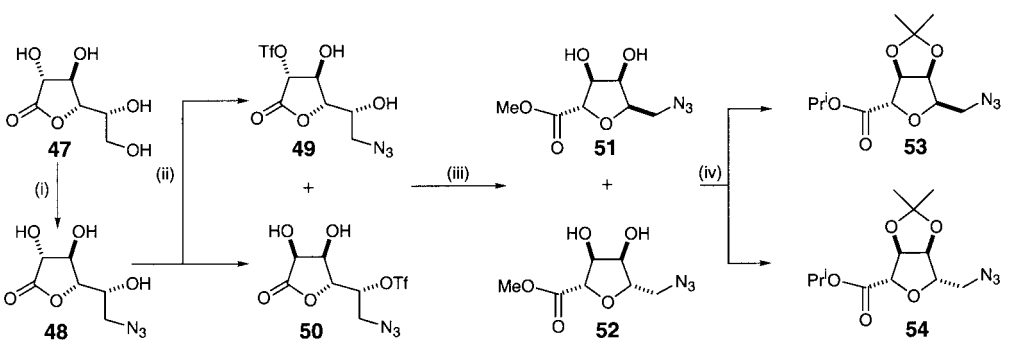
hydrolysis of the side chain acetonide and methanolysis of the lactone, followed by intramolecular S_N2-type closure of the resulting open chain hydroxy-triflate with inversion of configuration at C-2. Although it is possible that hydroxy-triflate intermediates such as this could undergo alternative closure to a tetrahydropyran, resulting from attack by the C-6 rather than the C-5 hydroxyl group, no *C*-glycopyranosides were isolated; ring closures to *C*-glycopyranoses by nucleophilic displacement at C-2 of a sugar are rare [44]. Introduction of nitrogen via tosylation to give **34** and displacement with azide gave the desired amino acid framework **36** in 78% yield over two steps. Attempts to reduce the azide in **36** led to closure across the THF ring to give a bicyclic lactam; a more hindered ester is required to enable isolation of a 6-amino species. Thus, transesterification of the methyl carboxylate **36** under basic conditions gave the more hindered isopropyl ester **37**.

Transformation of the azido-ester scaffolds **36** and **37** into peptide building blocks was easily achieved: reduction of the C-6 azide in **37** gave the amine **38** and hydrolysis of the methyl ester in **36** with aqueous sodium hydroxide gave access to the acid **35**.

The 2,5-*trans*-THFC **41** is available via a similar strategy; this involved the acidic methanolysis of the known D-*gluco* triflate **40** [45] (derived from D-glucono-1,4-lactone **39** in two steps) to afford the D-*manno* configured tetrahydrofuran **41** (Scheme 2). The resultant triol **41** underwent a selective esterification with methanesulfonyl chloride in pyridine in the presence of DMAP at $-20°C$ to give the primary mesylate **42** in 72% yield which upon treatment with sodium azide in DMF at 65°C afforded the azido-ester **44** in 98% yield. Attempts to reduce the C-6 azide of **44** under standard hydrogenation conditions led to complex mixtures of products, probably arising from intermolecular condensations; as in the 2,5-*cis* series, a less reactive ester was required to enable isolation of the C-6 amine. Thus, transesterification of the methyl ester **44** to the more hindered isopropyl ester **45** was effected by heating at 70°C in isopropanol in the presence of potassium carbonate (84% yield). The azide **44** was converted into the building blocks **43** (by treatment with sodium hydroxide in aqueous



Scheme 2 Reagents and conditions: (i) Reference [45]; (ii) HCl in MeOH; (iii) MsCl, DMAP, py; (iv) NaN_3, DMF, 90°C; (v) 1 M NaOH (aq.), dioxane, then IR-120 (H^+); (vi) K_2CO_3, ^{i}PrOH; (vii) H_2, Pd, ^{i}PrOH.



Scheme 3 Reagents and conditions: (i) Reference [56]; (ii) Tf_2O, EtOAc, py; (iii) HCl in MeOH; (iv) HCl, ^{i}PrOH, then acetone.

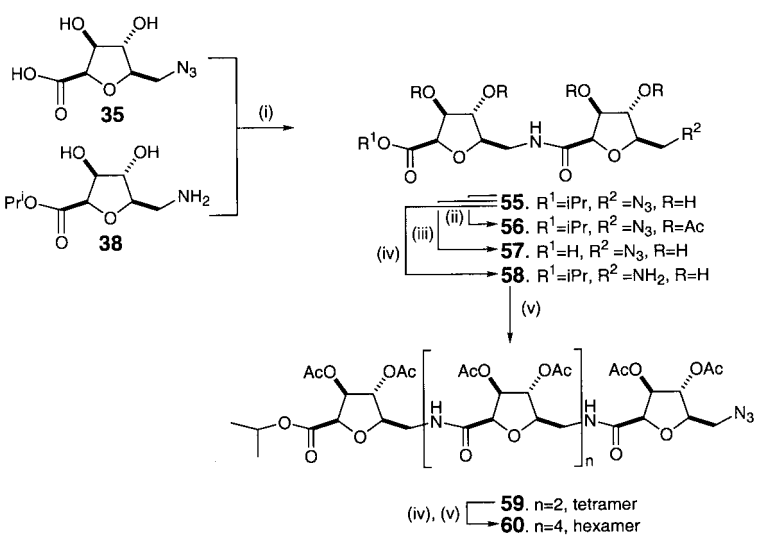
dioxane) and **46** (by hydrogenation of **45** in the presence of palladium black) required for the formation of oligomers.

The formation of THFCs with 3,4-*cis* stereochemistry was desirable as it would allow for facile protection regimes permitting easy isolation and purification. The strategy employed for the synthesis of this stereochemistry was based around introduction of nitrogen prior to closure of the THF ring. D-Galactono-1,4-lactone **47** was converted into the 6-azido derivative **48** in an overall yield of 61% in four preparative steps. Reaction of **48** with triflic anhydride in the presence of pyridine in ethyl acetate, followed by treatment with methanol, gave an inseparable mixture of two epimeric azidomethyl tetrahydrofuran carboxylates **51** and **52** in 46% yield in a ratio of 2.5:1 (Scheme 3). Formation of the D-*talo*-isomer **51** arises from the expected preferential triflation of **48** at the C-2 hydroxyl to give **49** followed by ring opening with methanol and subsequent THF ring formation by displacement of the C-2 triflate with inversion of configuration by the OH group at C-5. The formation of the L-*allo*-isomer **52** was unanticipated, as inversion of configuration at both C-2 and C-5 are required in its formation from **48**. A plausible pathway might involve initial epimerization at C-2 of **48** and triflate ester formation at C-5 to afford the D-*talo* triflate **50** which would then permit formation of the THF ring with the observed stereochemistry from closure by attack of the C-2 OH with inversion of configuration at C-5. Treatment of the mixture of **51** and **52** with HCl in isopropanol caused efficient transesterification to the isopropyl esters, still as an inseparable mixture; however, subsequent addition of acetone to the crude reaction mixture of yielded the separable isopropyl esters as D-*talo* **53** (65% yield) and L-*allo* **54** (23% yield).

A full account of the synthetic work is beyond the scope of this article, but the availability of many of the 16 required hexonolactones means that in principal the strategy described herein allows access to almost all the stereoisomeric THFCs. Additionally, other azido-THFCs are available [46]; thus, four stereoisomeric β-azido THFCs can be synthesized from diacetone glucose via a similar strategy. It is hoped that these and related THFCs will be useful as a family of amino acid building blocks with predictable conformational preferences.

OLIGOMERIC DERIVATIVES: CARBOPEPTOIDS

Initial studies evaluated the potential for secondary-structural predisposition of oligomeric tetrahydrofuran amino acids bearing 2,5-*cis* stereochemistry across the THF ring **36** (see previously for synthesis). Conventional solution phase coupling techniques were employed for oligomer formation, to afford the dimer **55** in 76% yield over three steps from **36** (Scheme 4) [47]. An iterative approach was adopted for the synthesis of higher oligomers; reduction of the azide in **55** gave an *N*-terminal amine **58** which was coupled to the acid **57** (derived from *C*-terminal ester hydrolysis of **55**) to give a tetramer which was isolated as its peracetate **59** in 55% yield. Extension of this methodology gave access to the hexamer **60** (68%).



Scheme 4 Reagents and conditions: (i) EDCl, HOBt, DMF, DIPEA, RT; (ii) Ac_2O, py; (iii) NaOH (aq.), dioxane, then Amberlite IR-120 (H^+); (iv) H_2, Pd, ^{i}PrOH; (v) EDCl, HOBt, DMF, DIPEA, RT, 1 eq. of **57**.

The solution conformation of the carbopeptoids in $CDCl_3$ was investigated by ^{1}H-NMR spectroscopy. Proton chemical shift dispersion of tetramer **59** is high despite the repeating unit, which is itself suggestive of a well-defined solution structure. 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 [48] spectra, with the configuration within each sugar ring being confirmed by the observed NOE correlations. 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 [49] of the carbonyl region were used to establish unambiguous through-bond ^{1}H–^{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}).

The ^{1}H spectrum of the amide region for the tetramer **59** and its hexameric homologue **60** is shown in Figure 1. 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 **59**, such a displacement 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 **56** (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 **60** which exhibits four high-frequency amide protons and one again at lower frequency.

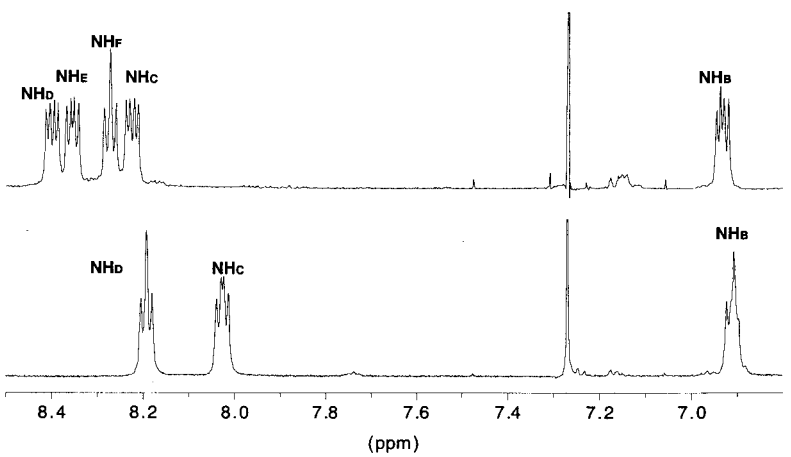


Figure 1 Amide regions of the ^{1}H-NMR ($CDCl_3$) of the tetramer **59** (lower plot) and the hexamer **60** (upper plot).

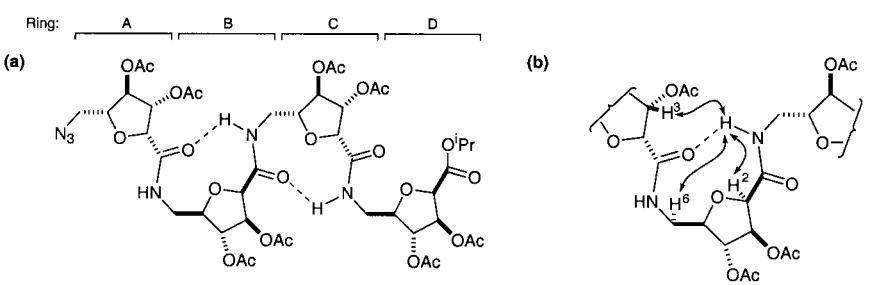


Figure 2 (a) Representation of the observed solution secondary structure of the tetramer **59** indicating ring labelling. Rings are identified by labelling each residue alphabetically from 'A' at the *N*-terminus. (b) Representation of the significant inter-residue NOE enhancements observed for each 'turn'. Relevant protons are numbered individually.

Temperature coefficients of the amide protons of the tetramer **59** in DMSO indicate that NH^D and NH^C experience greater shielding from these solvent interactions than does NH^B,and correlates with the higher chemical shifts of NH^D and NH^C observed in $CDCl_3$.

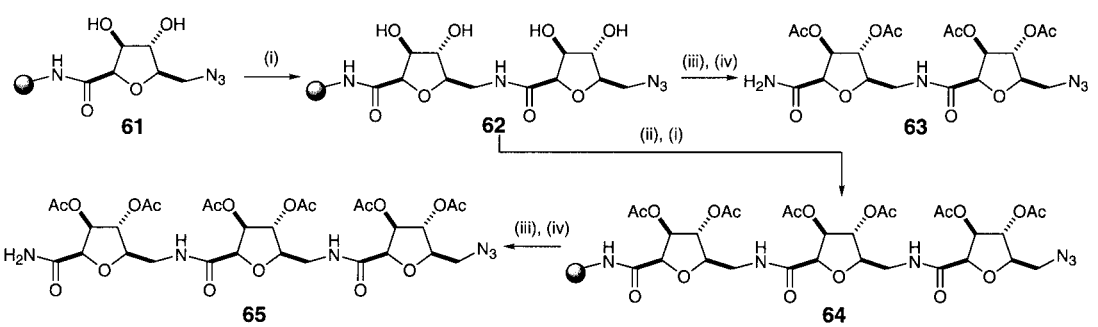
The pattern of deshielded *versus* shielded amide protons for **56**, **59** and **60** is consistent with a repeating structural unit, rather than simply the formation of hydrogen-bonds between amide protons and acetate groups on the same or adjacent residues. This is further supported by the NOE data which were also used to establish the solution conformation of the molecule in which tetramer **59** appears to adopt a novel repeating 'β-turn' type [50] structure stabilized by (i, $i-2$) inter-residue hydrogen-bonds (Figure 2a). 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 $H3^{i-2}$ (Figure 2b) as observed from both NH^D and NH^C, and are suggestive of the proposed (i, $i-2$) inter-residue hydrogen-bonds.

Molecular dynamics simulations [51] utilizing NOE-derived distance constraints were performed for the tetramer (Plate 1). This resulted in the generation of five low energy structures, all of which exhibit the anticipated β-turn type geometry (backbone atom RMS deviation between the five structures is 0.6 Å). Superposition of these structures (Plate 1A) shows the expected fraying at the *C*-terminus, which does not participate in hydrogen-bonding. The conformer that most satisfies the distance restraints is shown in Plate 1B. Comparison of the solution structure of **59** with a conventional peptidic β-turn reveals substantial similarities – both exhibit the same 10-membered ring hydrogen-bond and similar torsion angles. This is indicative of the effectiveness of the tetrahydrofuran scaffold as a dipeptide isostere and β-turn mimic.

CARBOPEPTOIDS: A SOLID PHASE APPROACH

The *C*-terminal ester appears to exert little influence on the solution conformation of the carbopeptoids **59** and **60**; the hydrogen-bonding interaction between an amide proton of tetrahydrofuran (i) and the carbonyl of the penultimate sugar residue ($i-2$) stabilizes the repeating β-turn type structure observed in solution. It was postulated that modification of the *C*-terminal ester to a group that could function as a hydrogen-bond donor would minimize the fraying at the *C*-terminus. Accordingly, a trimeric amide **64** was generated by a solid phase approach, based on a polystyrene resin functionalized with a Rink amide linker. Attachment of the amino acid derivative **35** to the solid support to give **61** and subsequent homo-oligomerizations enabled the synthesis of the dimer **63** and trimer **65** which bear a *C*-terminal carboxamide functionality [52].

The monomeric sugar amino acid derivative **35** was attached through the carboxyl function to a polystyrene support via a Rink [53] linker by treatment with DIC and 1-HOBt in DMF to afford **61** (Scheme 5). Treatment of the solid-supported azide **61** with diisopropylethylamine (DIPEA) and dithiothreitol (DTT) [54] in DMF at 50°C afforded an amine which was coupled to the acid **35** by treatment with DIC and HOBt in DMF to afford an immobilized dimer **62**. The polymer-bound dimer **62** was acylated by treatment with acetic anhydride in pyridine and cleaved from the solid support with 50% v/v TFA/DCM to give dimer **63** in 30% yield over the five steps. The trimer **65** was prepared by



Scheme 5 Reagents and conditions: (i) DTT, DIPEA, DMF, 50°C; (ii) DIC, DMF, HOBt, DIPEA, 1.5 eq. of **35**; (iii) Ac_2O, py; (iv) TFA/DCM.

an iterative sequence of reactions. Thus, reduction of the solid-supported dimer **62** with DTT and DIPEA in DMF at 50C gave an amine; subsequent coupling this amine with acid **35** by treatment with DIC and HOBt in DMF gave the polymer-bound trimer. Reaction with acetic anhydride and pyridine gave the acylated trimer **64** which was released from the resin by treatment with 50% TFA/CH_2Cl_2; purification by HPLC gave the acetylated trimer **65**.

^{1}H-NMR studies of both the dimer **63** and the trimer **65** in $CDCl_3$ show high proton chemical shift dispersion and significant variation in the chemical shifts of the amide protons. For the dimer **63**, amide NH shifts were observed at δ_H 7.55 and δ_H 5.48 as single broad singlets corresponding to each of the two terminal carboxamide NH protons, and the secondary amide NH was a broad triplet at δ_H 6.93. The carboxamide shift at δ_H 7.55 is indicative of involvement in hydrogen-bonding. The ^{1}H-NMR spectrum of trimer **65** indicates the presence of two hydrogen-bonded amide protons at δ_H 8.04 and δ_H 8.14 corresponding to a single carboxamide NH and the secondary amide NH^C one residue along, respectively. In contrast, the amide NH^B shift is observed at δ_H 6.95 and the remaining carboxamide NH' at δ_H 5.73. Thus, there are two NH signals which are indicative of intramolecular hydrogen-bonding and two NH signals are not. Comparison between the spectra of the trimer **65** and the previously reported tetramer **59** ($n = 2$) (Figure 3) reveals a high correlation between proton chemical shifts. This is further supported by the similarities observed in the NOE data obtained for the tetramer **59** and trimer **65** (Figure 4b). Notably, the amide NH^C proton displayed a strong NOE to only *one* of the $H6^B$ protons together with a weaker NOE to $H2^A$. Similarly, the 8.04 ppm carboxamide proton demonstrated a

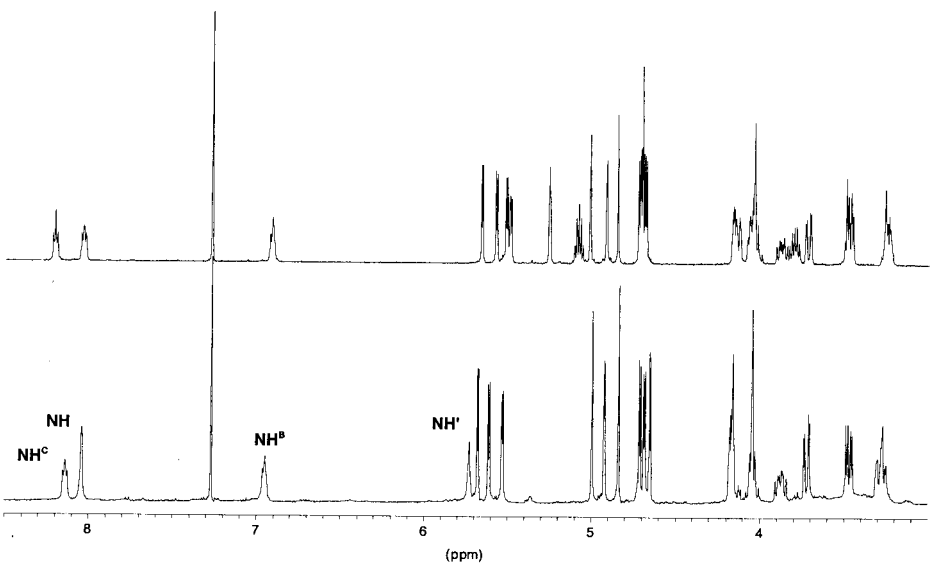


Figure 3 ^{1}H-NMR ($CDCl_3$) of *cis* tetramer **59** (upper plot) and the trimer **65** (lower plot).

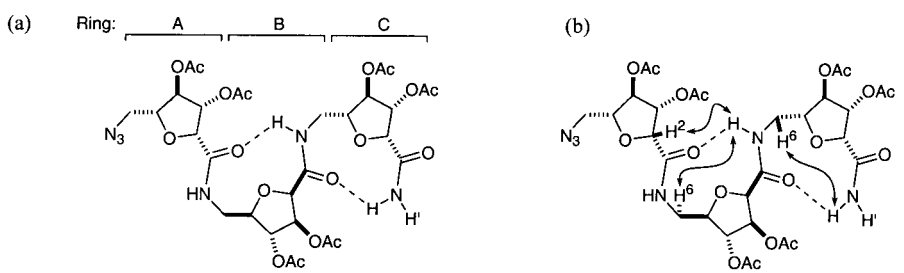
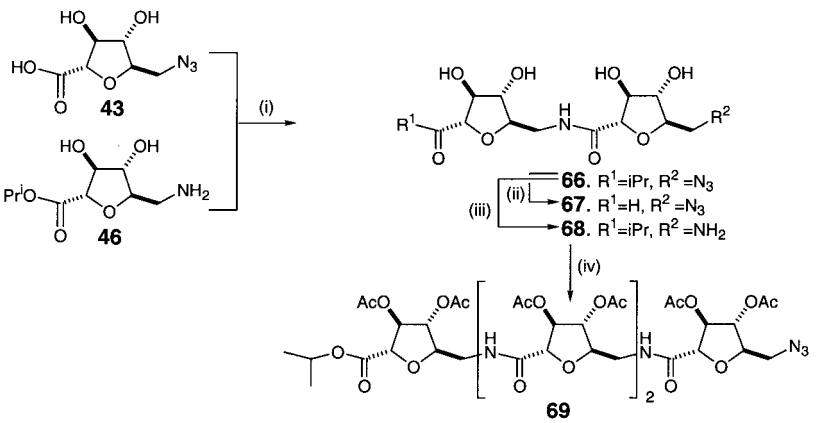


Figure 4 (a) Representation of the observed solution secondary structure of the trimer **65** indicating ring labelling. (b) Representation of the significant inter-residue NOE enhancements observed for each 'turn'. Relevant protons are numbered individually.

strong NOE to $H6^C$, again stereospecifically, whereas that at 5.73 ppm gave an NOE only to its geminal partner. In contrast to the behaviour of the high-frequency amide protons, NH^B displayed only rather weak NOEs of similar intensity to both $H6^A$ protons, consistent with a lack of conformational restriction about residue A. It would appear that the trimer **65** adopts the same type of conformation as that exhibited by **59** with participation of one of the carboxamide NH protons in a hydrogen-bond directly analogous to that of the amide NH^D proton of tetramer **59** (Figure 4a).

2,5-*TRANS* CARBOPEPTOIDS

It was decided to next turn our attentions to the synthesis of the C-2 epimer in which the C-2 and C-5 substituents of the tetrahydrofuran ring are *trans* to each other. It was anticipated that oligomers of this material would generate a different secondary structure to that exhibited by the 2,5-*cis* congener. The C-2 epimer **41**, in which the C-2 and C-5 substituents of the tetrahydrofuran ring are *trans* to each other, is efficiently synthesized from D-glucono-1,5-lactone (as described herein), allowing access to multi-gram quantities of **44**. Homo-oligomerization proceeded under standard conditions with EDCI and HOBt in DMF in the presence of DIPEA. This gave the unprotected dimer **66** (74% over three steps) which is isolable by standard chromatographic techniques (Scheme 6) [55]. An iterative approach was adopted for the synthesis of the tetrameric carbopeptoid **69**; thus the dimer **66** was reduced by hydrogenation in isopropanol in the presence of palladium black to



Scheme 6 Reagents and conditions: (i) EDCl, HOBt, DMF, DIPEA, RT; (ii) NaOH (aq.), dioxane, then Amberlite IR-120 (H^+); (iii) H_2, Pd, ^{i}PrOH; (iv) EDCl, HOBt, DMF, DIPEA, RT, 1 eq. of **67**, then Ac_2O, py.

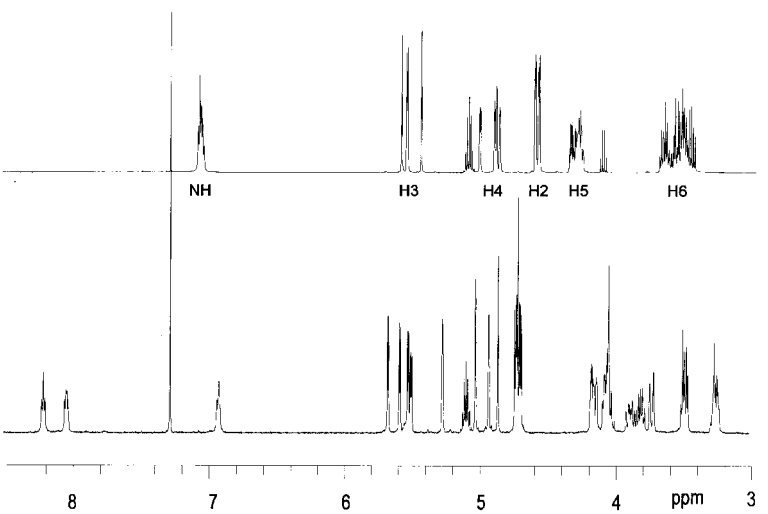


Figure 5 ^{1}H-NMR ($CDCl_3$) spectra of *trans* tetramer **69** (upper plot) and *cis* tetramer **59** (lower plot).

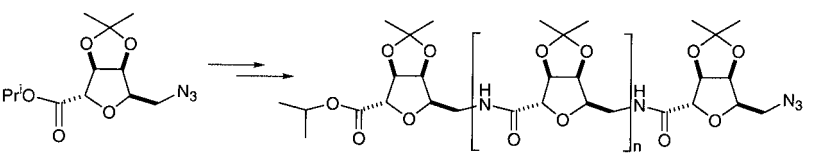
afford the *N*-terminal amine **68**. Treatment of the dimer **66** with aqueous sodium hydroxide in dioxane gave the acid **67** that could be coupled to the *N*-terminal dimeric amine **68** under the standard coupling conditions previously employed. Treatment of the reaction mixture with acetic anhydride in pyridine facilitated isolation of the peracetylated tetramer **69** in 77% yield from the azide **66**.

Partial proton spectra of the *cis* **59** and *trans* **69** tetramers are depicted in Figure 5 for comparison. In sharp contrast to the proton spectrum of the *cis* tetramer **59**, the proton spectrum of the *trans* isomer **69** demonstrates very little shift distribution, with all protons clustered together according to their positions within the monomer units. Outlying resonances from these clusters can be attributed to chemical differences at the termini, in particular the presence of the *C*-terminal ester functionality. The distinct lack of dispersion and similar chemical shifts for the amide protons indicates an absence of hydrogen-bonding interactions. These amide proton shifts are similar to that observed for the *cis* dimer **56** in $CDCl_3$ (7.18 ppm) which itself does not participate in intramolecular hydrogen-bonding. Overall, the low dispersion observed throughout the spectrum is generally suggestive of a random coil type conformation. The 2,5-*trans* disposition of the groups across the tetrahydrofuran ring in **69** would not provide the optimum geometry for the formation of the hydrogen-bonded turn-type structure observed for the *cis* isomer **59** but may not necessarily prevent the formation of alternative secondary structures; however, a larger oligomer than a tetramer is clearly necessary for this. Thus, the inversion of a single stereocentre from **36** to **44** confers significant changes upon the solution state conformation of oligomers such that in **69** no secondary structure is apparent. Synthesis of longer oligomers was troublesome due to the acetate protecting groups on the backbone; additionally the ester chromophore complicated CD studies.

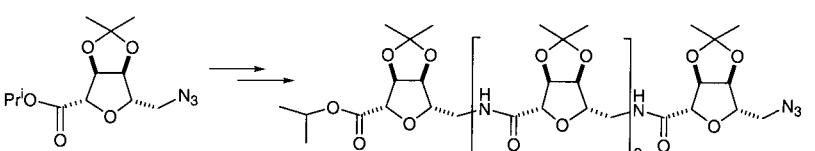
Oligomers derived from monomers in which there is a *cis*-diol unit would allow protection as an isopropylidene derivative, which should confer rigidity and ease of purification upon the resulting oligomers, as well as making CD studies more reliable. The requisite amino acid monomers, 2,5-*trans* **53** and 2,5-*cis* **54** were synthesized from D-galactono-1,4-lactone [56] as described herein and elaborated to tetrameric **70**, **72** (Scheme 8) and octameric oligomers **71** (Scheme 7), respectively [57], under the same conditions utilized for previous oligomerizations.

The solution conformations of the tetramers and the octamer were investigated by ^{1}H-NMR spectroscopy [58]. Intra-residue assignments were derived from TOCSY and gradient-selected HSQC [59] spectra, whilst ROESY and T-ROESY spectra were used to establish sequential inter-residue connectivities and to explore solution conformations.

The partial proton spectrum (Figure 6) of the isopropylidene-protected *cis*-tetramer **72** in $CDCl_3$ displays high chemical shift dispersion for both the amide and sugar-ring protons. The distribution of the amide protons is particularly noticeable, 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 **59**. Comparison of the NOE patterns revealed further



Scheme 7



Scheme 8

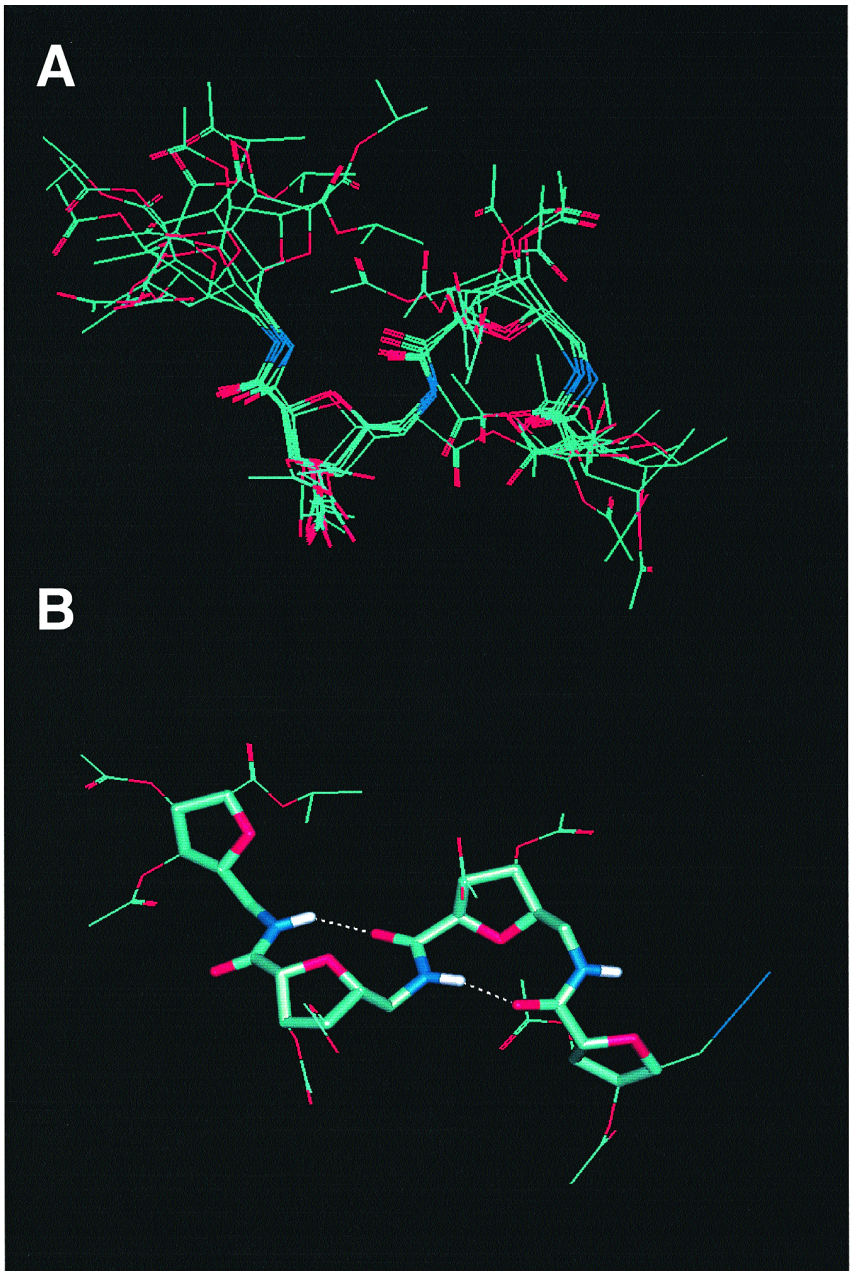


Plate 1 (A) Five lowest energy structures of the tetramer ***59*** generated by restrainedmoleculardynamicssimulationsperformedusingtheprogram QUANTA with the CHARMM forcefield. The five lowest energy conformers, superimposed on their backbone atoms, are shown. (B) The conformer in best agreement with the experimental restraints from the five structures of the tetramer ***59*** illustrated in (A). The two hydrogen-bonds are indicated by broken lines.

similarities indicative of i, $i-2$ inter-residue hydrogen-bonds. 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 **72** resembles that previously reported for **59**, adopting a similar repeating 'β-turn' type conformation. In such a conformer, the isopropylidene 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.

In the case of the *trans*-tetramer **70**, neighbouring residues were also identified via a semi-selective, gradient-enhanced HMBC experiment 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. The partial ^{1}H-NMR (Figure 7) of **70** also shows high dispersion in $CDCl_3$, although the resonance distribution of the amide region is quite different to that of **72** 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 **69** which displays no such dispersion and appears to have no conformational preference. The long-range inter-residue NOE patterns observed for the *trans*-isomer **70** are also quite different to those observed for the *cis*-isomer **72**; these data suggest **70** favours a solution conformation different to that of **72**. This conformational preference appears to be stronger in the octamer **71** in which NH^B and

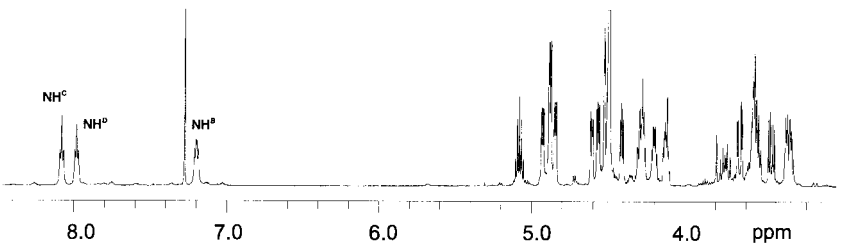


Figure 6 ^{1}H-NMR ($CDCl_3$) spectrum of the tetramer **72**.

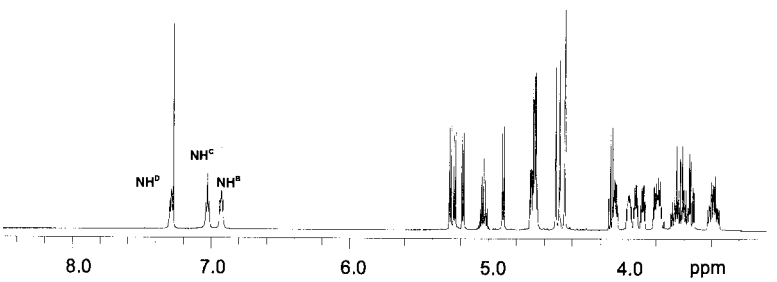


Figure 7 ^{1}H-NMR ($CDCl_3$) spectrum of the tetramer **70**.

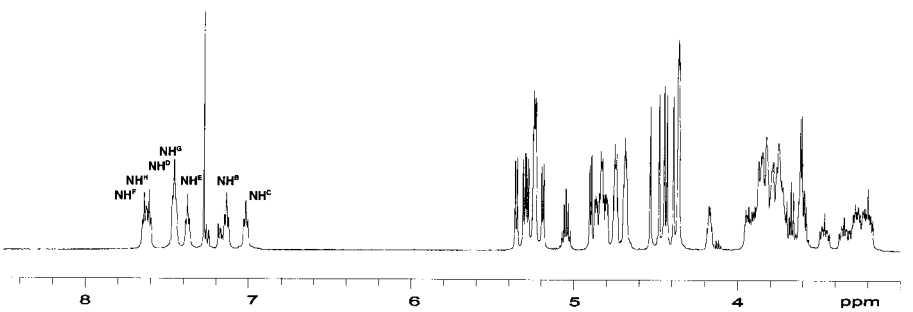


Figure 8 ^{1}NMR ($CDCl_3$) spectrum of the octamer **71**.

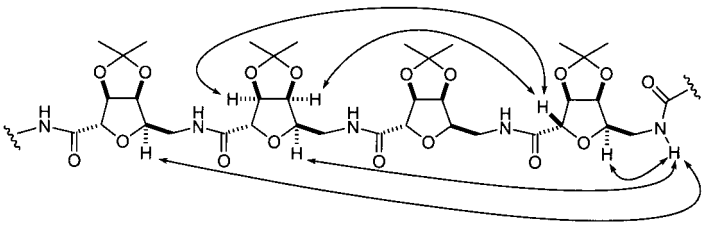


Figure 9 Representation of the typical long-range NOEs observed for the octamer **71**.

NH^C resonate at low frequency ($\delta < 7.2$ ppm), again corresponding to protons that are solvent exposed, having little or no participation in hydrogen-bonding (Figure 8). 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, suggestive of a repeating structural motif in the molecule. Extensive long-range NOE analysis of the octamer **71** 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 **72** and **59** 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 (Figure 9). At least one set of these NOE patterns were 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 suggests the presence of a helical structure stabilized by inter-residue NH^i–CO^{i-3} hydrogen-bonds. 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. In a helical structure for the octamer **71**, 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 **70**, 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 stabilizing H-bond, the conformation is likely to be averaging between the 'folded' and open forms, resulting in a smaller time-averaged amide proton shift.

CONCLUSIONS AND OUTLOOK

We have shown that it is possible to generate molecules with well-defined secondary structures based upon carbohydrate-like tetrahydrofuran frameworks in chains as short as a trimer. Manipulation of the tetrahydrofuran amino acid backbone stereochemistries also enables manipulation of the solution conformation. Thus, by inversion of one stereocentre per monomer unit from **59** to **69** the predisposition of folding is changed from a repeating β-turn type structure (in **59**) to one which has no conformational preferences (**69**). In contrast, inversion of one stereocentre, from **69** to **71** (with a protecting group change) leads from a molecule with a random solution conformation (**59**) to one which exhibits a well-defined left handed-helical conformation stabilized by hydrogen-bonds (**71**). The ease of synthesis of dipeptide isosteres of this nature augurs well for future rational design of molecules that adopt conformations related to those seen in natural biopolymers, ultimately leading to synthetic protein-like structures with tertiary structure.

Acknowledgements

We acknowledge the efforts of co-workers in the studies from our group summarized herein; their contributions are documented in the references cited. Financial support from GlaxoWellcome, EPSRC and the EU is gratefully acknowledged.

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