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Bend ribbon-forming tetrahydrofuran amino acids †

Martin D. Smith,[a,b] Timothy D. W. Claridge,[a] Mark S. P. Sansom[c] and George W. J. Fleet*[a]

[a] *Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford, UK OX1 3QY. E-mail: george.fleet@chem.ox.ac.uk*

[b] *University Chemical Laboratory, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW*

[c] *Department of Biochemistry, University of Oxford, South Parks Road, Oxford, UK OX1 3QU*

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Short oligomeric chains of *C*-glycosyl β-D-*arabino*furanose configured tetrahydrofuran amino acids (where the C-2 and C-5 substituents of the tetrahydrofuran ring are *cis* to each other) exhibit a well-defined repeating turn secondary structure stabilised by (i, $i-2$) inter-residue hydrogen bonds. This is in contrast to the epimeric α-D-*arabino*furanose oligomer (where the C-2 and C-5 substituents of the tetrahydrofuran ring are *trans* to each other) in which there is no indication of any secondary structure in solution.

Introduction

The vibrancy and depth of carbohydrate research (accredited to the polyfunctional nature of the carbohydrate skeleton) attests to its importance within chemistry and biology. However, despite enormous advances, there are still difficulties in the routine synthesis of glycosidic bonds that render this polyfunctionality both an attribute and a burden.[1] One approach to simplify the routine incorporation of carbohydrates into libraries (for instance) is to superpose the functional groups of an amino acid onto the polyol backbone.[2,3] This concept was first explored in 1955[4] in the synthesis of pyranose carbohydrate mimetics and has since been expanded to encompass a wide range of applications[5] including combinatorial building blocks,[6] peptidomimetics[7–10] and inhibitors of glycogen phosphorylase. [11]An integral extension of this tenet involves the use of tetrahydrofuran (THF) frameworks[12–14] that can be formally derived from a conventional dipeptide by introducing an ether linkage as an amide surrogate and also bridging between the two amino acids. Several applications of THF amino acids have been demonstrated; a THF sugar amino acid was used as a β-turn inducing Gly-Gly substitute in the generation of a Leu-enkephalin analogue with equal pain reducing properties to those of the natural peptide.[15] THF amino acids have been incorporated into a functional cation channel with a biomimetic channel entrance and exit.[16] Oligomers of oxetane,[17] furanose[18] and pyranose[19–22] carbohydrate amino acids have been reported, and their utility in the generation of materials that possess well defined conformational properties ('foldamers'[23,24]) has also been described.[25,26]

This paper gives a full account of the synthesis and secondary structural investigation of oligomeric tetrahydrofuran amino acids related to **1** and **10** and illustrates the influence that backbone stereochemistry may exert on secondary structure. Certain aspects of this work have been published in a preliminary form.[27–29]

Results and discussion

1 Synthesis of oligomers based upon a *C*-glycosyl β-D-*arabino*-furanose template

The functionalised THF building blocks **1** and **2** can be synthesised from D-mannose in short, stereoselective sequences.[30] The strategy adopted for the synthesis of carbopeptoids utilises established peptide bond forming methodology. With this in mind, the methyl ester functionality in **2** was hydrolysed with aqueous sodium hydroxide in dioxane that gave the free acid **4** upon treatment with Amberlite IR-120 (H+). Reduction of the *N*-terminal azide in **1** was achieved by treatment with hydrogen in the presence of palladium black to afford the free amine **3** (characterised as its peracetate) that was used without further purification. With these components in hand, oligomerisations were performed using standard peptide coupling conditions. The two components were necessarily coupled in DMF as solvent for solubility reasons in the presence of EDCI, HOBt and DIEA. This enabled isolation of the dimeric unit **5** in 74% yield over three preparative steps without any protection being employed, Scheme 1.

Iteration of the coupling procedure gave ready access to the tetramer **8** and the hexamer **11**. The dimer **5** was treated with aqueous sodium hydroxide and purified by ion exchange chromatography to afford the free acid **6** in quantitative yield. Additionally, the *N*-terminal azide in **3** was reduced with hydrogen in the presence of palladium black to afford the amine **7**. Coupling of the dimeric building blocks **6** and **7** was performed using EDCI in DMF in the presence of HOBt, and the reaction mixture was treated with acetic anhydride in pyridine to facilitate isolation of the tetramer **8** [55% yield from the azide **5**] which was otherwise too polar to purify using conventional chromatographic techniques. The acetate groups in **8** can be removed with catalytic sodium methoxide in methanol to afford the deprotected carbopeptoid **9** in quantitative yield. Hydrogenation of the tetramer **8** in the presence of palladium gave the crude *N*-terminal amine **10** which was directly coupled to the dimeric acid **6** using EDCI in DMF in the presence of HOBt. Treatment of the reaction mixture with acetic anhydride in pyridine gave the hexamer **11** in 68% yield from the tetramer **8**.

2 Synthesis of oligomers based upon a *C*-glycosyl α-D-*arabino*-furanose template

In order to investigate the effects of backbone variation within the THF amino acid framework, it was decided to utilise the C-2 epimeric THFs **12** and **13** to prepare oligomers. This followed a scheme analogous to that utilised for the 2,5-*cis*-THF described above, Scheme 2. The building blocks **12** and **13** are readily synthesised from D-glucono-1,5-lactone.[30] Hydrogenation of the isopropyl azide **12** in isopropanol in the presence of palladium black allowed isolation of the polar amino

† This is one of a number of contributions from the current members of the Dyson Perrins Laboratory to mark the end of almost 90 years of organic chemistry research in that building, as all its current academic staff move across South Parks Road to a new purpose-built laboratory.

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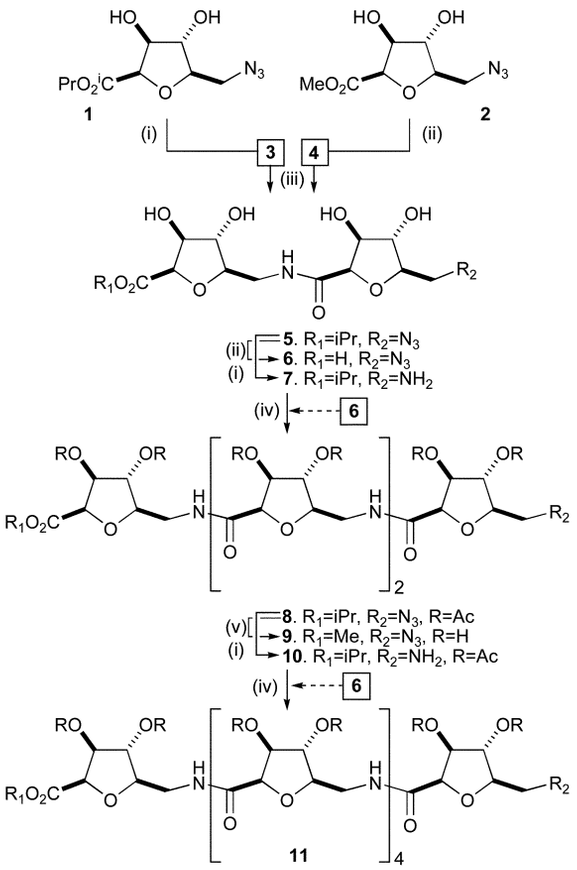
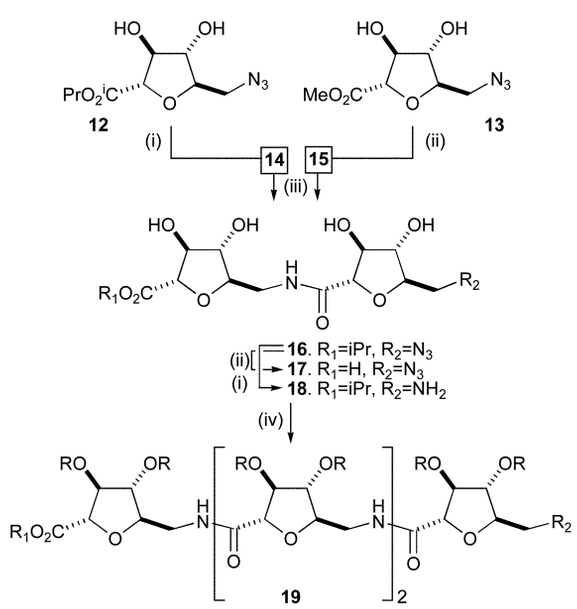


Scheme 1 *Reagents and conditions*: (i) H_2, Pd, IPA (ii) 0.5 M NaOH (aq.), dioxane; then Amberlite IR-120 (H+) (iii) EDCI, HOBt, DIEA, DMF (iv) 1 eq. of **6**, EDCI, HOBt, DIEA, DMF, then Ac_2O, py. (v) NaOMe, MeOH, then Amberlite IR-120 (H+).



Scheme 2 *Reagents and conditions:* (i) H_2, Pd, IPA (ii) 0.5 M NaOH (aq.), dioxane; then Amberlite IR-120 (H+) (iii) EDCI, HOBt, DIEA, DMF (iv) 1 eq. of **14**, EDCI, HOBt, DIEA, DMF, then Ac_2O, py.

ester **14** that was used without further purification. Hydrolysis of the ester functionality in **13** with aqueous sodium hydroxide in dioxane and purification by ion exchange chromatography gave access to the acid **15** that was coupled to the C-6 amino component under standard conditions with EDCI and HOBt in DMF in the presence of DIEA. This gave the unprotected dimer **16** (74% yield from the azide **14**) which is isolable by standard chromatographic techniques. Protection of the backbone hydroxy groups proved unnecessary during these coupling reactions. An iterative approach was adopted for the synthesis of the tetrameric carbopeptoid **19**; thus the dimer **16** was reduced by hydrogenation in isopropanol in the presence of palladium black to afford the *N*-terminal amine **18**. Treatment of the dimer **16** with aqueous sodium hydroxide in dioxane gave the acid **17** which could be coupled to the *N*-terminal dimeric amine **14** with EDCI and HOBt in DMF in the presence of DIEA. Treatment of the reaction mixture with acetic anhydride in pyridine facilitated isolation of the peracetylated tetramer **19** in 77% yield from the azide **16**.

3 Secondary-structure investigations: NMR studies on the 2,5-*cis* tetramer 8

The solution conformation of **8** in $CDCl_3$ was investigated by ^{1}H 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 Tr-ROESY spectra,[31] 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 approaches the $\omega_0\tau_c \approx 1$ condition). NOe data also allowed the sequential placement of each residue from the observation of H-2^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[32] 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, H-2^i to CO^i and CO^i to H-6^{i+1}). Finally, the NOE data were further used to establish the solution conformation of the molecule in which tetramer **8** appears to adopt a novel repeating turn-type ribbon structure stabilised by (i, $i-2$) inter-residue hydrogen-bonds (Fig. 1).

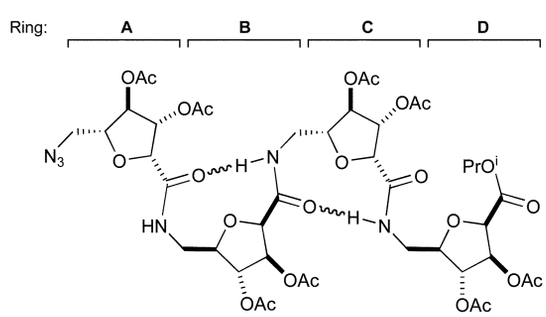


Fig. 1 Bend-ribbon type secondary structure of **8**.

This conformation is similar in some respects to that adopted by certain natural peptide antibiotics.[33] Each repeating tetrahydrofuran unit can be considered as a dipeptide isostere with each H-bond completing a turn that is structurally reminiscent of a conventional peptide β-turn. This turn structure is very similar to that described for an alanine-derived THF amino acid that exhibits a well defined 10-membered ring hydrogen bond in both solution and the solid phase.[34]

Proton chemical shift dispersion of **8** is high despite the repeating unit, which is itself suggestive of a well-defined solution structure. Dilution of the sample provided no changes in proton shifts indicating this dispersion is not a consequence of aggregation or intermolecular associations. The ^{1}H spectrum of the amide region for the tetramer **8** and its hexameric homologue **11** 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 **8**, such a

Table 1 Amide proton temperature coefficients and chemical shifts (at 298 K) for the tetramer **8**

	δ_H ($CDCl_3$)/ppm	$\Delta\delta$ ($CDCl_3$)/ppb K^{-1}	δ_H ([$^2H_6$]DMSO) /ppm	$\Delta\delta$ ([$^2H_6$]DMSO) /ppb K^{-1}
NH^D	8.19	−5.4	8.20	−4.3
NH^C	8.03	−4.4	8.29	−3.9
NH^B	6.91	−0.7	8.12	−5.3
H_2O				−5.1

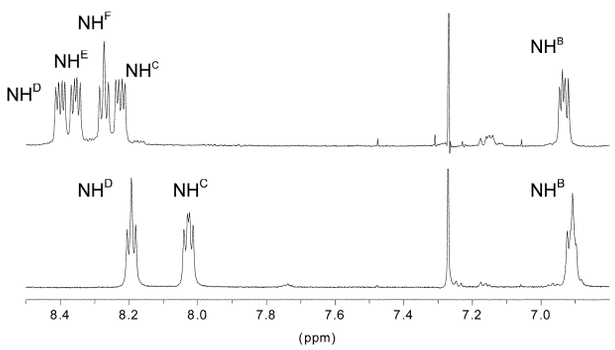


Fig. 2 Amide regions of the ^1H NMR (500 MHz) spectrum of tetramer **8** (lower plot) and hexamer **11** (upper plot).

shift is observed for two of the three amide protons (δ_H 8.19 and 8.03 ppm, subsequently identified as NH^D and NH^C respectively) 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 ppm), characteristic of an amide proton which experiences little or no hydrogen-bonding. This shift is similar to that observed for the dimeric unit (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 **11**, which exhibits four high-frequency amide protons and one again at lower frequency (Fig. 2). The amide proton temperature coefficients in $CDCl_3$ also demonstrate significant differences for residue B relative to C and D (Table 1). Coefficients larger than *ca.* 3 ppb K^{-1} are indicative of amide protons in equilibrium between hydrogen-bonded and non-hydrogen-bonded (solvent exposed) environments[35,36] and the data for **8** again indicate H-bond participation of NH^C and NH^D whereas, as indicated by the lower temperature coefficient, NH^B remains solvent exposed. 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. Likewise, temperature coefficients of the amide protons of the tetramer **8** in DMSO display significantly less variation than those recorded in $CDCl_3$, consistent with expectations that the turn stabilisation is disrupted in a strongly competitive hydrogen-bonding solvent such as DMSO. In competitive solvents, temperature coefficients are lower for those amide protons involved in intramolecular hydrogen bonds whereas larger coefficients are observed for solvent exposed protons. The coefficients for **8** indicate that NH^D and NH^C experience slightly greater shielding from solvent interactions than does NH^B, (Table 1) and suggest some intramolecular hydrogen-bonding remains for these protons in this solvent.

The pattern of deshielded *versus* shielded amide protons for **8**, **11** and the peracylated dimer 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. Similar shift patterns have been observed for a related 2,5-*cis* configured THF system where the backbone hydroxy groups were protected as a cyclohexylidene acetal rather than with acetyl groups.[25] A repeating structure for the tetramer **8** is further supported by the observed NOEs. With only one exception ($H\text{-}3^A$ to $H\text{-}6^C$*pro*-S), all NOEs that were observed *between* residues involved the amide protons and no

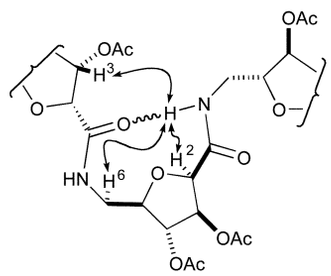


Fig. 3 Inter-residue NOE enhancements observed for each 'turn'.

inter-residue ring–ring interactions could be detected (Fig. 3). Significant inter-residue NOEs were NH^i to H-2 $^{i-1}$, NH^i to H-6^{i-1} (stereospecifically) and NH^i to H-3^{i-2} as observed from both NH^D and NH^C, and are suggestive of the proposed (i, $i-2$) inter-residue hydrogen-bonds.

An examination of the amide chemical shifts in the tetramer **8** and the hexamer **11** reveal that the high frequency shift observed for hydrogen-bonded amide protons becomes larger as the chain length increases. This may be attributed to stronger hydrogen-bonds[37] as a consequence of 'cooperative', or 'resonance assisted' hydrogen-bonding.[38] Involvement of a hydrogen atom in a hydrogen-bond leads to a withdrawal of its electron density. If both the carbonyl and nitrogen substituents of an amide are involved in strong hydrogen-bonding, then they become both a better hydrogen-bond acceptor and a hydrogen-bond donor respectively. Longer oligomers in both 2,5-*cis* and 2,5-*trans* systems have continued this trend, with larger diamagnetic shifts being observed.[25,39]

4 Molecular dynamics simulations

Molecular dynamics simulations[40] utilising NOE derived distance constraints together with restraints for the two hydrogen-bonds described above were performed for the tetramer **8**, Fig. 4. Thus, a set of 22 distance restraints were generated from a total of 58 unambiguous NOEs, eliminating those that would provide no constraint over that imposed by the covalent structure. 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. 4a) 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. 4b. This structure is consistent with the lack of ring–ring NOEs and reflects the strong conformational preferences from each sugar ring stereochemistry. The NOE data are also suggestive of the presence of a minor conformer in which the *N* and *C* termini approach each other. Thus, weak but reproducible NOEs are observed for **8** between NH^D and $H2^A$ and $H3^A$ which cannot be accounted for in the repeating turn structure above. These weak, anomolous NOE patterns have been observed in various 2,5-*cis* THF tetramers (differing only in their hydroxyl protecting groups) but, interestingly, not in longer hexameric sequences. It can be speculated that these arise when only a single hydrogen-bond is formed within the tetramer (possibly between NH^D and the amide carbonyl of residue A) forcing the approach of the ends, whereas in the hexamer the larger number of hydrogen-bonds in the ribbon structure (four *versus* only two in the folded tetramer) more strongly favours the formation of the repeat-

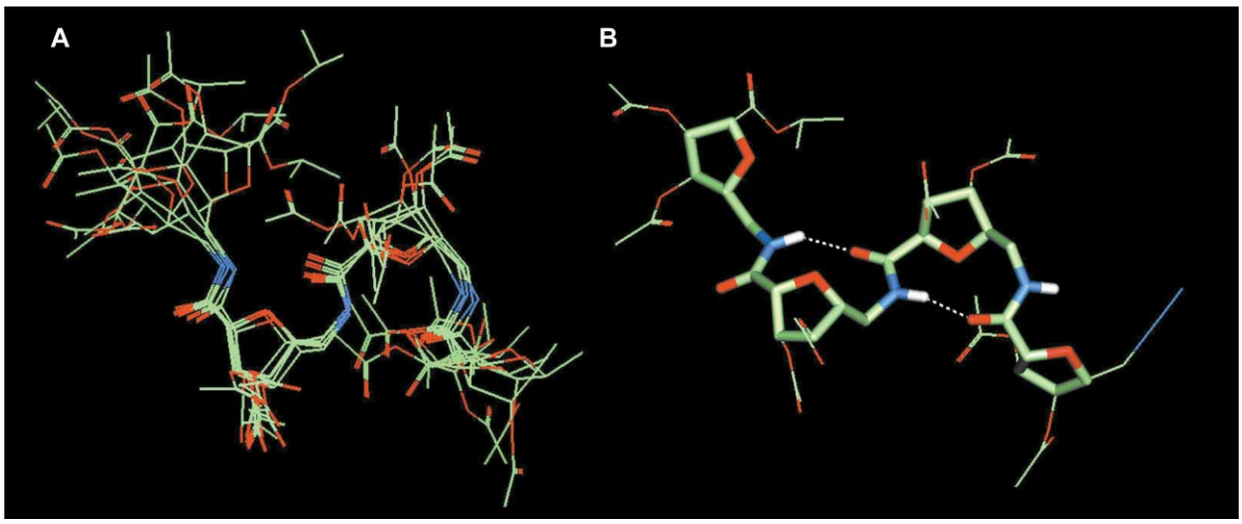


Fig. 4 Five lowest energy structures of the tetramer **8** generated by restrained molecular dynamics simulations performed using the program QUANTA with the CHARMM force-field. An initial model of the tetramer **8** was built using QUANTA, and was subjected to 50 cycles of steepest descent energy minimisation to regularise its local stereochemistry. Possible backbone conformations were explored using a RIS/filter conformational search, during which the 9 backbone torsion angles were varied between their t/g+/g− conformations with two distance filters used to reject those structures which did not satisfy the backbone H-bonding requirement. Fifty accepted structures were subjected to a short burst of MD in order to relax the conformations of the backbone acetate groups. The five lowest energy structures thus generated were examined in more detail, and one conformer with optimum backbone H-bonding selected as the starting point for a 400 ps MD simulation at 600 K, restrained to maintain the two backbone H-bonds. Structures were saved every 1 ps and the 5 lowest energy structures from the trajectory were energy minimised whilst maintaining the H-bond restraints. The lowest energy structure from these was subjected to further simulations in which 22 NOE derived distance restraints and 2 H-bond restraints were applied. A 1.2 ns MD simulation at 1000 K was performed, saving structures every 2 ps. All 500 structures from the final ns of the MD simulation were energy minimised. **A**. 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 **8**. The two H-bonds are indicated by broken lines.

ing bend. Solution infra-red studies in chloroform (at 2 mM concentration) were also undertaken, and these indicated the presence of two amide environments – non-hydrogen bonded amide at 3425 cm^{-1} and hydrogen bonded amide at 3297 cm^{-1}, Fig. 5A. This is completely consistent with the amide environments predicted through NMR studies and provides further evidence for the observed turn conformation.

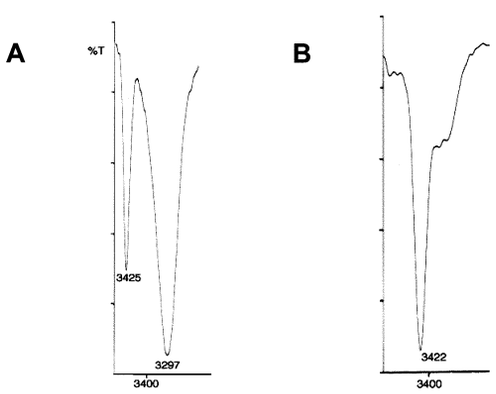


Fig. 5 Solution IR spectra (2 mM in $CHCl_3$, RT) **A** 2,5 *cis*-tetramer **8** **B**. 2,5-*trans* tetramer **19**.

5 Secondary-structure investigations: NMR studies on the 2,5-*trans* tetramer 19

In sharp contrast to the spectra of the 2,5-*cis* tetramer **8** the proton spectrum of the *trans* isomer **19** demonstrates very little shift distribution, with all protons clustered together according to their positions within the monomer units (Fig. 6). 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.[41] These amide proton shifts are similar to that observed for the *cis* dimer in $CDCl_3$

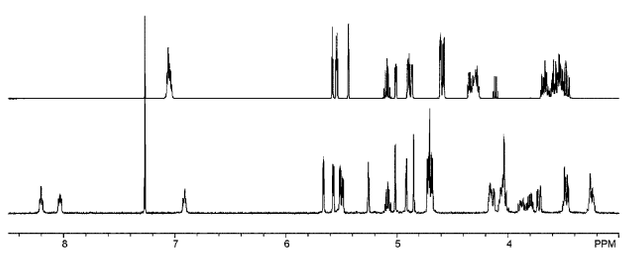


Fig. 6 1H NMR spectra ($CDCl_3$, 500 MHz) of 2,5-*cis* tetramer **8** (lower plot) and 2,5-*trans* tetramer **19** (upper plot).

(7.18 ppm) which itself does not participate in internal hydrogen bonding. Overall, the low dispersion observed throughout the spectrum is generally suggestive of a lack of stable secondary structure. Solution infra-red studies (Fig. 5B) in chloroform indicated the presence of only non-hydrogen bonded amide environments (3422 cm^{-1}) which is consistent with observations from NMR studies. The 2,5-*trans* disposition of the groups across the tetrahydrofuran ring in **19** would not provide the optimum geometry for the formation of the hydrogen bonded turn-type structure observed for the *cis* isomer **8** but may not necessarily prevent the formation of alternative secondary structures; however, a larger oligomer than a tetramer is clearly necessary for this.

Conclusion

Tetrahydrofuran amino acids are potentially important building blocks for the formation of unnatural biopolymeric materials with interesting properties. It has been demonstrated that the inversion of a single stereocentre on the THF ring can have profound effects for solution conformation, emphasizing the different characteristics of each THF amino acid building block. This in-built conformational diversity bodes well for the generation of a monomer alphabet tailored for the formation of foldameric materials which may ultimately possess tertiary structure.

Experimental

1 General

1.1 Solvents. Tetrahydrofuran was distilled under an atmosphere of dry nitrogen from sodium benzophenone ketyl or purchased dry from the Aldrich chemical company in sure-seal™ bottles; dichloromethane was distilled from calcium hydride; pyridine was distilled from calcium hydride and stored over dried 4 Å molecular sieves; hexane refers to the fraction of petroleum ether which boils in the range 60–80 °C and was redistilled before use; water was distilled. *N*,*N*-Dimethylformamide was purchased dry from the Aldrich chemical company in sure-seal™ bottles. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification.

1.2 Reagents. Reactions performed under an atmosphere of nitrogen, argon or hydrogen gas were maintained by an inflated balloon. pH 7 Buffer was prepared by dissolving KH_2PO_4 (85 g) and NaOH (14.5 g) in distilled water (950 ml). All other reagents were used as supplied, without prior purification.

1.3 Chromatography. Thin layer chromatography (TLC) was performed on aluminium or plastic sheets coated with 60 F_{254} silica. Sheets were visualised using a spray of 0.2% w/v cerium (IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid or 0.5% ninhydrin in methanol (particularly for amines). Flash chromatography was performed on Sorbsil C60 40/60 silica. CMAW refers to the eluent system – chloroform–methanol–acetic acid–water (60 : 30 : 3:5).

1.4 Melting points. Melting points were recorded on a Kofler hot block and are uncorrected.

1.5 Nuclear magnetic resonance spectroscopy. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM 500 or AMX 500 (^{1}H: 500 MHz and ^{13}C: 125.3 MHz) or where stated on a Bruker AC 200 (^{1}H: 200 MHz and ^{13}C: 50.3 MHz) or Bruker DPX 400 (^{1}H: 400 MHz and ^{13}C: 100.6 MHz) spectrometer in the deuterated solvent stated. All chemical shifts (δ) are quoted in ppm and coupling constants (J) in Hz. Residual signals from the solvents were used as an internal reference and ^{13}C NMR spectra in D_2O were referenced to 1,4-dioxane (δ_C 67.4). ^{13}C multiplicities were assigned using a DEPT sequence.

Amide proton temperature coefficients were recorded at 500 MHz over a temperature range of 295–320 K in 5 K steps; all temperature dependencies exhibited linear correlations. Intramolecular rotating-frame NOEs were observed at 298 K and 500 MHz in transverse-ROESY (Tr-ROESY) spectra (so as to minimise interference from *J*-couplings) with mixing times of 200 and 500 ms. The 200 ms NOE data set was used to generate distant restraints through volume integration of cross peak intensities. Owing to cross peak overlap of the H6–H6′ NOEs, these geminal pairs proved unsuitable as reference distances for semi-quantitative analysis so instead the *cis*-H2–H3 intra-residue NOEs were employed with a reference distance of 2.35 Å. NOes were then classified to produce distance bonds of <3.0, 3.0–3.5, and 3.5–4.5 Å, as in Table 2. The stereospecific pro-*R* and pro-*S* assignments of the H6^B and H6^C geminal protons were derived from NOEs observed within each turn structure, notably those from H4 and H5 of the same ring and from the hydrogen-bonded amide proton of the following residue.

1.6 Infrared spectroscopy. Infrared spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform spectrophotometer using either thin films on NaCl plates (film) or KBr discs (KBr) as stated. Only the characteristic peaks are quoted.

1.7 Mass spectrometry. Low resolution mass spectra (*m*/*z*) were recorded on VG MassLab 20–250, Micromass BIOQ-II, Micromass Platform 1, Micromass TofSpec 2E, or Micromass Autospec 500 OAT spectrometers and high resolution mass spectra (HRMS *m*/*z*) on a Micromass Autospec 500 OAT spectrometer. Techniques used were electrospray (ES), matrix assisted laser desorption ionisation (MALDI), chemical ionisation (CI NH_3), or atmospheric pressure chemical ionisation (APCI) using partial purification by HPLC with methanol–acetonitrile–water (40 : 40 : 20) as eluent, as stated.

Table 2 NOE derived distance constraints for **8**[a]

Observed NOE		Distance/Å
NH^D to:	C-6 (pro-*R*)	3.5–4.5
	D-5	3.0–3.5
	C-2	3.5–4.5
	D-4	3.5–4.5
	B-3	3.5–4.5
NH^C to:	C-6′ (pro-*S*)	<3.0
	C-6 (pro-*R*)	3.0–3.5
	B-6 (pro-*R*)	3.0–3.5
	C-5	3.0–3.5
	B-2	3.5–4.5
	C-4	3.5–4.5
	A-3	3.5–4.5
NH^B to:	B-6′ (Pro-*S*)	<3.0
	A-6′	3.5–4.5
	A-6	3.5–4.5
	A-5	3.5–4.5
	A-2	3.5–4.5
	B-4	3.5–4.5
	C-6′ (pro-*S*)	<3.0
C-4 to:	C-6 (pro-*R*)	3.5–4.5
C-6′ (pro-*S*) to:	A-3	3.5–4.5
	C-5	<3.0

[a] Recorded at 500 MHz, 298 K.

1.8 Polarimetry. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g 100 ml^{-1}.

1.9 Elemental analysis. Elemental analyses were performed by the microanalysis service of the Dyson Perrins Laboratory, Oxford, or the Inorganic Chemistry Laboratory, Oxford.

Isopropyl 6-N-acetamido-3,4-di-*O*-acetoxy-2,5-anhydro-6-deoxy-D-gluconate. A solution of isopropyl 2,5-anhydro-6-azido-6-deoxy-D-gluconate (100 mg, 0.41 mmol) in propan-2-ol (2 ml) was vigorously stirred under an atmosphere of hydrogen in the presence of palladium black (15 mg). After 2 h, TLC (ethyl acetate) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.5). The reaction was degassed, purged with nitrogen and filtered through Celite (eluent: propan-2-ol). The solution was concentrated *in vacuo* and dissolved in pyridine (1 ml) under an atmosphere of nitrogen. Acetic anhydride (1 ml) was added and the mixture was stirred at room temperature. After 12 h, TLC (ethyl acetate) indicated the presence of a major product (R_f 0.3) and no starting material (R_f 0.0). The mixture was concentrated *in vacuo* and purified by flash column chromatography (ethyl acetate–hexane, 3 : 1 increasing polarity to ethyl acetate) to afford isopropyl 6-*N*-acetamido-3,4-di-*O*-acetoxy-2,5-anhydro-6-deoxy-D-gluconate as an amorphous solid (127 mg, 90% over two steps). $[\alpha]_D^{24}$ −0.82 (*c*, 1.1 in $CHCl_3$). ν_{max} (film): 1752 (s, C=O), 1659 (C=O, amide I), 1546 (amide II). δ_H ($CDCl_3$, 500 MHz): 1.23 (3H, d, *J* 6.3, $(CH_3)_2CH$–), 1.27 (3H, d, *J* 6.3 $(CH_3)_2CH$–), 2.00, 2.05, 2.08 (3 × 3H, 3 × s, 2 × $OCOCH_3$, 1 × $NHCOCH_3$), 3.47 (1H, apparent (a)-dt, *J* 6.1, $J_{6,6'}$ 14.1, H-6), 3.68 (1H, a-dt, *J* 5.2, $J_{6',6}$ 14.1, H-6′), 4.10 (1H, a-dt, *J* 4.5, *J* 6.4, H-5), 4.70 (1H, d, $J_{2,3}$ 5.6, H-2), 5.02 (1H, dd, $J_{4,3}$ 2.6, $J_{4,5}$ 3.7, H-4), 5.08 (1H, septet, *J* 6.3, $(CH_3)_2CH$–), 5.56 (1H, dd, $J_{3,4}$ 2.5, $J_{3,2}$ 5.6, H-3), 6.57 (1H, br s, NH). δ_C ($CDCl_3$, 50 MHz): 20.6, 20.6, 21.6, 21.7, 23.1 (5 × q, 2 × $OCOCH_3$, 1 × $NHCOCH_3$ $(CH_3)_2CH$–), 40.4

(1 × t, C-6), 69.5 (1 × d, $(CH_3)_2CH$), 76.4, 77.4, 78.4, 82.4 (4 × d, C-2, C-3, C-4, C-5), 167.7, 168.9, 169.5, 170.4 (4 × s, 4 × C=O, C-1, 2 × $OCOCH_3$, 1 × $NHCOCH_3$). *m/z* (APCI+): 346 ($M + H^+$, 100%). HRMS (CI+): Found 346.150285; $C_{15}H_{24}NO_8$ ($M + H^+$) requires 346.150192.

Isopropyl 6-amino-2,5-anhydro-6-deoxy-6-N-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate ***5***. Sodium hydroxide solution (0.5 M aq., 1.02 ml, 0.51 mmol) was added to a stirred solution of methyl 6-azido-6-deoxy-2,5-anhydro-D-gluconate **2** (110 mg, 0.51 mmol) in 1,4-dioxane (2 ml) at room temperature. After 10 min, TLC (ethyl acetate) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.8). The mixture was concentrated *in vacuo* and purified by ion exchange chromatography (Amberlite IR-120 (H^+) to afford 2,5-anhydro-6-azido-6-deoxy-D-gluconic acid **4** as a hygroscopic glassy solid.

A solution of isopropyl 2,5-anhydro-6-azido-6-deoxy-D-gluconate **1** (120 mg, 0.49 mmol) in propan-2-ol (3 ml) was vigorously stirred under an atmosphere of hydrogen in the presence of palladium black (15 mg). After 1 h, TLC (ethyl acetate) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.8). The reaction was degassed, purged with nitrogen and filtered through Celite (eluent: propan-2-ol). The solution was concentrated *in vacuo* to afford isopropyl-6-amino-2,5-anhydro-6-deoxy-D-gluconate **3** as a colourless oil which was used without further purification.

N-Ethyldiisopropylamine (85 µl, 0.49 mmol), hydroxybenzotriazole (73 mg, 0.54 mmol) and EDCI (94 mg, 0.54 mmol) were added to a stirred solution of crude 2,5-anhydro-6-azido-6-deoxy-D-gluconic acid **4** (105 mg, 0.51 mmol) in DMF (0.6 ml) at 0 °C under an atmosphere of nitrogen. The mixture was left stirring for 10 min when a solution of crude isopropyl 6-amino-2,5-anhydro-6-deoxy-D-gluconate **3** (107 mg, 0.49 mmol) in DMF (0.6 ml) was added. After 18 h, TLC (ethyl acetate–methanol, 9 : 1) indicated the presence of a major product (R_f 0.3). The reaction mixture was concentrated *in vacuo* to give a residue which was purified by flash column chromatography (ethyl acetate, increasing polarity to ethyl acetate–methanol, 9 : 1) to afford isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate **5** as a white solid (155 mg, 76% from the azide 153). Mp 145–146 °C (ethyl acetate). $[a]_D^{25}$ +82.2 (*c*, 0.5 in methanol). ν_{max} (KBr): 3392 (br, OH), 2110 (N_3), 1744 (C=O, ester), 1662 (C=O, amide I), 1540 (C=O, amide II). δ_H (CD_3OD, 500 MHz, major rotameric component): 1.30 (6H, a-t, *J* 6.3, $(CH_3)_2CH$–), 3.53 (1H, dd, *J* 6.1, *J* 12.6), 3.58 (1H, dd, *J* 6.4, *J* 14.0), 3.63 (1H, dd, *J* 13.4, *J* 4.4), 3.69 (1H, dd, *J* 6.7, *J* 12.6), 3.93–4.01 (4H, m), 4.25 (1H, dd, *J* 3.9, *J* 1.5), 4.28 (1H, dd, *J* 5.0, *J* 2.4), 4.55 (1H, d, *J* 3.9), 4.59 (1H, d, *J* 5.0), 5.10 (1H, septet, *J* 6.3, $(CH_3)_2CH$–). δ_C (50 MHz, CD_3OD): 22.2 (1 × q, 2 × $(CH_3)_2CH$–), 47.7, 53.8 (2 × t, C-6^A, C-6^B), 70.2, 79.2, 79.5, 79.9, 81.9, 83.9, 85.7, 86.8 (8 × d, C-2^A, C-3^A, C-4^A, C-5^A, C-2^B, C-3^B, C-4^B, C-5^B, $(CH_3)_2CH$–)), 171.5, 171.8 (2 × s, 2 × C=O, C-1^A, C-1^B). *m/z* (APCI+): 405 ($M + H^+$, 100%). Found: C 44.60, H 6.02, N 13.64%; $C_{15}H_{24}N_4O_9$ requires: C 44.55, H 5.98, N 13.86%.

Isopropyl 3,4-di-O-acetoxy-6-amino-2,5-anhydro-6-deoxy-6-N-(3,4-di-O-acetoxy-2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate. Acetic anhydride (1.5 ml) was added to a stirred solution of isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate (15 mg, 0.04 mmol) in pyridine (1.5 ml) at room temperature under an atmosphere of nitrogen. After 18 h, TLC (ethyl acetate–hexane, 3 : 1) indicated the presence of a single compound (R_f 0.5) and no starting material (R_f 0.0). The mixture was concentrated *in vacuo* to give a residue which was purified by flash column chromatography (ethyl acetate–hexane, 2 : 1) to afford isopropyl 3,4-di-*O*-acetoxy-6-amino-2,5-anhydro-6-deoxy-6-*N*-(3,4-di-*O*-acetoxy-2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate as an amorphous solid (21 mg, 99%). $[a]_D^{24}$ +43.0 (*c*, 1.4 in $CHCl_3$). ν_{max} (film): 2104 (N_3), 1750 (C=O), 1680 (amide I), 1534 (amide II). δ_H ($CDCl_3$, 500 MHz): 1.23 (3H, d, *J* 6.3, $(CH_3)_2CH$–), 1.27 (3H, d, *J* 6.3, $(CH_3)_2CH$–), 2.07, 2.07, 2.10, 2.11 (4 × 3H, 4 × s, 4 × CH_3CO–), 3.43 (1H, ddd, *J* 4.9, *J* 7.3, $J_{6',6}$ 12.2, H-6′^{B}), 3.56 (1H, dd, $J_{6',5}$ 5.7, $J_{6,6'}$ 13.2, H-$6^{A'}$), 3.67 (1H, dd, $J_{6,5}$ 3.4, $J_{6,6'}$ 13.1, H-6^A), 3.87 (1H, m, H-6^B), 4.06–4.11 (2H, m, H-5^A, H-5^B), 4.66 (1H, d, $J_{2,3}$ 4.0, H-2^A), 4.72 (1H, d, $J_{2,3}$ 5.5, H-2^B), 4.95 (1H, a-d, *J* 3.0, H-4^A), 5.05 (1H, a-t, *J* 2.8, H-4^B), 5.08 (1H, septet, *J* 6.3, $(CH_3)_2CH$–), 5.50 (1H, dd, *J* 0.5, $J_{3,2}$ 4.0, H-3^A), 5.57 (1H, dd, *J* 2.4, $J_{2,3}$ 5.5, H-3^B), 7.18 (1H, a-dd, *J* 5.4, *J* 6.6, NH^B). δ_C ($CDCl_3$, 125 MHz): 20.6, 20.6, 20.7, 20.7 21.6, 21.7 (6 × q, 4 × CH_3CO–, 2 × $(CH_3)_2CH$–), 40.3, 51.5 (2 × t, C-6^A, C-6^B), 69.3, 76.4, 76.5, 77.5, 78.1, 78.5, 80.7, 82.2, 84.4 (9 × d, C-2^A, C-3^A, C-4^A, C-5^A, C-2^B, C-3^B, C-4^B, C-5^B, $(CH_3)_2CH$–)), 166.5, 167.3 (2 × s, 2 × C=O, C-1^A, C-1^B), 169.0, 169.3, 169.6, 169.8 (4 × C=O, CH_3CO–). *m/z* (APCI+): 573 ($M + H^+$, 100%), 531 (60%). HRMS (CI+): found 573.204168; $C_{23}H_{33}N_4O$ ($M + H^+$) requires 573.204413.

Peracetylated D-gluco tetramer ***8***. Sodium hydroxide solution (0.5 M aq., 174 µl, 0.17 mmol) was added to a stirred solution of isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate **5** (70 mg, 0.17 mmol) in 1,4-dioxane (2 ml) at room temperature. After 10 min, TLC (ethyl acetate–methanol, 9 : 1) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.3). The mixture was concentrated *in vacuo* and purified by ion exchange chromatography (Amberlite IR-120 (H^+)) to afford 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconic acid **6** as a hygroscopic glassy solid.

A solution of isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate **5** (70 mg, 0.17 mmol) in propan-2-ol (5 ml) was vigorously stirred under an atmosphere of hydrogen in the presence of palladium black (10 mg). After 2 h, TLC (ethyl acetate–methanol, 9 : 1) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.3). The reaction was degassed, purged with nitrogen and filtered through Celite (eluent: propan-2-ol). The solution was concentrated *in vacuo* to afford isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(6-amino-2,5-anhydro-6-deoxy-D-gluconyl)-D-gluconate **7** as a colourless oil which was used without further purification.

N-Ethyldiisopropylamine (30 µl, 0.17 mmol), hydroxybenzotriazole (26 mg, 0.19 mmol) and EDCI (37 mg, 0.19 mmol) were added to a stirred solution of 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconic acid **6** (65 mg, 17 mmol) in DMF (0.5 ml) at 0 °C under an atmosphere of nitrogen. The mixture was stirred for 10 min when isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-amino-6-deoxy-D-gluconyl)-D-gluconate **7** (63 mg, 17 mmol) in DMF (0.5 ml) was added dropwise. The mixture was stirred for 18 h at room temperature and concentrated *in vacuo*. The residue was dissolved in pyridine (3 ml) and acetic anhydride (3 ml) was added to the stirred solution under an atmosphere of nitrogen. After 18 h, TLC (ethyl acetate) indicated the presence of a major product (R_f 0.3). The reaction was concentrated *in vacuo* and purified by flash column chromatography (ethyl acetate–hexane, 3 : 1, increasing polarity to ethyl acetate) to afford the *peracetylated D-gluco tetramer* **8** (102 mg, 55%) as a white amorphous solid. $[a]_D^{24}$ +56.0 (*c*, 0.7 in $CHCl_3$). ν_{max} (film): 2104 (N_3), 1750 (C=O), 1672 (amide I), 1548 (amide II). δ_H ($CDCl_3$, 500 MHz, residual $CHCl_3$ at 7.270 ppm) and δ_C ($CDCl_3$, 125 MHz, $CDCl_3$ at 77.00 ppm), see Table 3. *m/z* (APCI+): 1059 ($M + H^+$, 10%). Found: C 48.63 H 5.48 N 8.16%; $C_{43}H_{58}N_6O_{25}$ requires: C 48.77, H 5.52, N 7.94%.

Unprotected D-gluco tetramer ***9***. Sodium methoxide (2 mg, cat.) was added to a stirred solution of the *peracetylated D-gluco tetramer* **8** (38 mg, 0.36 mmol) in methanol (3 ml) under an atmosphere of nitrogen. After 4 days, TLC (ethyl acetate–methanol, 9 : 1) indicated the presence of a major product (R_f 0.0) and no starting material (R_f 0.3). Amberlite IR-120 (H^+)

Table 3

	Ring:	A	B	C	D	iPr
	Me					1.254, 21.71
	Me					1.211, 21.63
	CH					5.070, 69.00
C^1	δ_C	167.81	168.11	167.68	167.15	
C^2	δ_H	4.669	4.692	4.708	4.687	
	δ_C	81.02	81.74	81.38	78.93	
C^3	δ_H	5.646	5.559	5.495	5.475	
	δ_C	76.08	75.57	76.01	76.66	
C^4	δ_H	4.904	4.837	5.003	5.250	
	δ_C	78.40	78.06	77.68	77.57	
C^5	δ_H	4.153	4.027	4.123	4.055	
	δ_C	85.00	85.18	85.00	83.19	
C^6	δ_H	3.708, 3.461	4.027, 3.224	3.861, 3.228	3.781, 3.481	
	δ_C	51.43	41.28	41.28	40.48	
NH	δ_H	–	6.910	8.025	8.191	
	δ_H	2.028	2.033	2.061	2.083	
C^3-OAc	δ_C	≈20.5	≈20.5	≈20.5	≈20.5	
	δ_C C=O	169.00	168.92	168.84	169.17	
	δ_H	2.122	2.122	2.094	2.061	
C^4-OAc	δ_C	≈20.5	≈20.5	≈20.5	≈20.5	
	δ_C C=O	169.86	169.73	169.39	169.14	

(approx. 50 mg dry) was added and the mixture was stirred at room temperature for 1 h before being filtered and concentrated *in vacuo* to afford *the unprotected D-gluco tetramer* **9** as a colourless oil (25 mg, quant.). $[\alpha]_D^{24}$ +111.5 (*c*, 1.3 in MeOH). ν_{max} (film): 3342 (OH), 2106 (N_3), 1741 (C=O, ester), 1646 (C=O, amide I), 1552 (amide II). δ_H (CD_3OD, 500 MHz): 3.40 (1H, dd, *J* 3.7, *J* 10.9, H-6), 3.43 (1H, dd, *J* 4.2, *J* 11.2, H-6), 3.47 (1H, dd, *J* 7.0, *J* 13.8, H-6), 3.49 (1H, dd, *J* 5.9, *J* 12.6, H-6), 3.63–3.67 (2H, m, 2 × H-6), 3.77 (3H, s, $COOCH_3$), 3.83 (1H, dd, *J* 8.3, *J* 13.8, H-6), 3.96–4.03 (7H, m, 4 × H-4, 3 × H-5), 4.07 (1H, ddd, *J* 1.2, *J* 4.1, *J* 8.2, H-5), 4.24–4.28 (4H, m, H-3^A, H-3^B, H-3^C, H-3^D), 4.43 (1H, dd, *J* 4.3, *J* 11.2, H-6), 4.52 (1H, d, *J* 3.7, H-2), 4.54 (1H, d, *J* 3.8, H-2), 4.56 (1H, d, *J* 3.9, H-2), 4.64 (1H, d, *J* 4.6, H-2). δ_C (D_2O, 125 MHz): 42.3, 42.5, 42.6 (3 × t, C-6^A, C-6^B, C-6^C), 52.6 (1 × q, $COOCH_3$), 53.7 (1 × t, C-6^D), 79.1, 79.2, 79.5, 79.6, 79.9, 80.0, 80.0, 82.1, 83.8, 84.0, 86.0, 86.8, 87.3, 87.7 (14 × d, C-2^A, C-3^A, C-4^A, C-5^A, C-2^B, C-3^B, C-4^B, C-5^B, C-2^C, C-3^C, C-4^C, C-5^C, C-2^D, C-3^D, C-4^D, C-5^D), 172.2, 172.2, 172.3 (3 × s, 3 × C=O, C-1^A, C-1^B, C-1^C, C-1^D). *m*/*z* (APCI+): 695 (M + H^+, 10%). *m*/*z* (FAB+): 695 (M + H^+, 100%).

*Peracetylated D-gluco hexamer **11***. Sodium hydroxide solution (0.5 M aq., 45 µl, 0.018 mmol) was added to a stirred solution of isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconate **5** (9 mg, 0.02 mmol) in 1,4-dioxane (2 ml) at room temperature. After 10 min, TLC (ethyl acetate–methanol, 9 : 1) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.3). The mixture was concentrated *in vacuo* and purified by ion exchange chromatography (Amberlite IR-120 (H^+)) to afford 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconic acid **6** as a hygroscopic glassy solid

A solution of the *peracetylated D-gluco tetramer* **8** (20 mg, 0.018 mmol) in dioxane (2 ml) was stirred vigorously under an atmosphere of hydrogen gas in the presence of palladium black (5 mg). After 4 h, TLC (ethyl acetate) indicated the presence of a major product (R_f 0.0) and no starting material (R_f 0.3). The mixture was filtered through Celite (eluent: propan-2-ol) and concentrated *in vacuo* to afford the *6-amino D-gluco tetramer* **10** which was used without further purification.

N-Ethyldiisopropylamine (4 µl, 0.018 mmol), hydroxybenzotriazole (3 mg, 0.02 mmol) and EDCI (4 mg, 0.02 mmol) were added to a stirred solution of 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-gluconyl)-D-gluconic acid **6** (8 mg, 0.018 mmol) in DMF (0.1 ml) at 0 °C under an atmosphere of nitrogen. The mixture was stirred for 10 min when the *6-amino D-gluco tetramer* **10** (19 mg, 0.018 mmol) in DMF (0.1 ml) was added dropwise. The mixture was stirred for 18 h at room temperature and concentrated *in vacuo*. The residue was dissolved in pyridine (1 ml), and acetic anhydride (1 ml) was added to the stirred solution under an atmosphere of nitrogen. After 18 h, TLC (ethyl acetate–methanol, 19 : 1) indicated the presence of a major product (R_f 0.3). The reaction was concentrated *in vacuo* and purified by flash column chromatography (ethyl acetate, increasing polarity to ethyl acetate–methanol, 19 : 1) to afford the *peracetylated D-gluco hexamer* **11** as a white amorphous solid (19 mg, 68% from the azide **5**). ν_{max} (film): 2104 (N_3), 1750 (C=O, ester), 1665 (C=O, amide). $[\alpha]_D^{24}$ −100.8 (*c*, 0.1 in $CDCl_3$). δ_H (CD_2Cl_2 at 5.32 ppm, 298 K, 500 MHz), see Table 4. δ_C ($CDCl_3$, 125 MHz): 20.4, 20.5, 20.6, 20.7, 20.8, 20.9, 21.7, (12 × CH_3CO–, $(CH_3)_2CH$), 40.3, 41.3, 41.4 (C-6^B, C-6^C, C-6^D, C-6^E, C-6^F), 51.4 (C-6^A), 68.9 ($(CH_3)_2CH$), 75.0, 75.3, 75.9, 76.0, 77.6, 77.9, 78.2, 78.5, 79.0, 81.1, 81.5, 81.8, 82.0, 82.1, 83.5, 85.0, 85.1, 85.1, 85.1, 85.5 (C-5^A, C-5^B, C-5^C, C-5^D, C-5^E, C-5^F, C-4^A, C-4^B, C-4^C, C-4^D, C-4^E, C-4^F, C-3^A, C-3^B, C-3^C, C-3^D, C-3^E, C-3^F, C-2^A, C-2^B, C-2^C, C-2^D, C-2^E, C-2^F), 167.2, 167.8, 167.9, 168.6, 168.7, 168.7, 168.9, 168.9, 169.1, 169.2, 169.3, 169.4, 169.7, 169.8 (12 × CH_3CO–, C-1^A, C-1^B, C-1^C, C-1^D, C-1^E, C-1^F). *m*/*z* (FAB+): 1567 (M + Na^+, 100%), 1545 (M + H^+, 40%). *m*/*z* (ES+): 792 ([M + 2K]$^{++}$, 100%). *m*/*z* (MALDI-TOF+): 1583 (M + K^+, 20%), 1657 (M + Na^+, 30%), 1545 (M + H^+, 90%), 1519 (100%).

Table 4

	Ring:	A	B	C	D	E	F	iPr
	Me							1.24
	Me							1.20
	CH							5.045
C^2	δ_H	4.67	4.72	4.70	4.68	4.66	4.67	
C^3	δ_H	5.66	5.67	5.63	5.54	5.47	5.41	
C^4	δ_H	4.93	4.86	4.93	4.93	4.97	5.24	
C^5	δ_H	4.18	4.09	4.27	4.20	4.14	4.00	
C^6	δ_H	3.70	4.09	4.00	3.99	3.86	3.69	
		3.48	3.23	3.09	3.04	3.12	3.36	
NH	δ_H	—	6.96	8.20	8.44	8.41	8.27	

*Isopropyl 6-amino-2,5-anhydro-6-deoxy-6-N-(2,5-anhydro-6-azido-6-deoxy-D-mannonyl)-D-mannonate **16***. Sodium hydroxide solution (1 M aq., 2.26 ml, 2.26 mmol) was added to a stirred solution of methyl 6-azido-6-deoxy-2,5-anhydro-D-mannonate **13** (490 mg, 2.26 mmol) in 1,4-dioxane (5 ml) at room temperature. After 10 min, TLC (ethyl acetate–hexane, 2 : 1) indicated the presence of a single product (R_f 0.0) and no

starting material (R_f 0.3). The mixture was concentrated *in vacuo* and purified by ion exchange chromatography (Amberlite IR-120 (H^+)) to afford 2,5-anhydro-6-azido-6-deoxy-D-mannonic acid **15** as a hygroscopic glassy solid.

A solution of isopropyl 2,5-anhydro-6-azido-6-deoxy-D-mannonate **12** (550 mg, 2.25 mmol) in propan-2-ol (10 ml) was vigorously stirred under an atmosphere of hydrogen gas in the presence of palladium black (40 mg). After 3 h, TLC (ethyl acetate–hexane, 2 : 1) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.5). The reaction was degassed, purged with nitrogen and filtered through Celite (eluent: propan-2-ol). The solution was concentrated *in vacuo* to afford isopropyl 2,5-anhydro-6-amino-6-deoxy-D-mannonate **14** which was used without further purification.

N-Ethyldiisopropylamine (400 µl, 2.24 mmol), hydroxybenzotriazole (340 mg, 2.51 mmol) and EDCI (455 mg, 2.51 mmol) were added to a stirred solution of 2,5-anhydro-6-azido-6-deoxy-D-mannonic acid **15** (275 mg, 2.24 mmol) in DMF (1 ml) at 0 °C under an atmosphere of nitrogen. The mixture was left stirring for 10 min when a solution of crude isopropyl 2,5-anhydro-6-amino-6-deoxy-D-mannonate **14** (490 mg, 2.25 mmol) in DMF (1 ml) was added. After 18 h, TLC (ethyl acetate : methanol, 9 : 1) indicated the presence of a major product (R_f 0.3). The reaction mixture was concentrated *in vacuo* to give a residue which was purified by flash column chromatography (ethyl acetate, increasing polarity to ethyl acetate–methanol, 9 : 1) to afford isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-mannonyl)-D-mannonate **16** as a white solid (670 mg, 74%). Mp 142–143 °C (ethyl acetate). $[\alpha]_D^{23}$ +46.6 (*c*, 0.5 in methanol). ν_{max} (film): 3379 (br, OH), 2106 (N_3), 1728 (C=O, ester), 1654 (CONH, amide I), 1545 (amide II). δ_H (CD_3OD, 500 MHz): 1.28, (6H, d, *J* 6.2, $(CH_3)_2CH$–), 3.46 (1H, dd, *J* 6.0, 13.9), 3.48–3.53 (2H, m), 3.55 (1H, dd, *J* 4.8, *J* 13.9), 3.91 (1H, a-t, *J* 3.5), 3.98 (1H, a-t, *J* 3.8), 4.12 (1H, m), 4.16 (1H, m), 4.27–4.29 (2H, d), 4.31 (1H, d, *J* 3.5), 4.39 (1H, d, *J* 2.7). 5.31 (1H, septet *J* 6.2, $CH_3)_2CH$–). δ_C (CD_3OD, 50 MHz): 20.5 (2 × q, $(CH_3)_2CH$–), 40.6, 51.7 (2 × t, C-6^A, C-6^B), 68.8 (1 × d, $(CH_3)_2CH$–), 77.6, 78.4, 80.3, 80.6, 82.8, 84.0, 84.4 (8 × d, C-2^A, C-3^A, C-4^A, C-5^A, C-2^B, C-3^B, C-4^B, C-5^B), 171.0, 172.4 (2 × s, 2 × C=O, C-1^A, C-1^B). *m/z* (APCI+): 405 (M+H$^+$, 100%), 363.1 (60%). Found: C 44.16, H 5.83, N 13.75%; $C_{15}H_{24}N_4O_9$ requires: C 44.55, H 5.98, N 13.86%.

*Peracetylated D-manno tetramer **19**.* Sodium hydroxide solution (0.5 M aq., 247 µl, 0.25 mmol) was added to a stirred solution of isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-mannonyl)-D-mannonate **16** (100 mg, 0.25 mmol) in 1,4-dioxane (2 ml) at room temperature. After 10 min, TLC (ethyl acetate–methanol, 9 : 1) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.3). The mixture was concentrated *in vacuo* and purified by ion exchange chromatography (Amberlite IR-120 (H^+)) to afford 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-mannonyl)-D-mannonic acid **17** as a hygroscopic glassy solid.

A solution of isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-mannonyl)-D-mannonate **16** (100 mg, 0.25 mmol) in propan-2-ol (5 ml) was vigorously stirred under an atmophere of hydrogen in the presence of palladium black (10 mg). After 2 h, TLC (ethyl acetate–methanol, 9 : 1) indicated the presence of a single product (R_f 0.0) and no starting material (R_f 0.3). The reaction was degassed, purged with nitrogen and filtered through Celite (eluent: propan-2-ol). The solution was concentrated *in vacuo* to afford isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(6-amino-2,5-anhydro-6-deoxy-D-mannonyl)-D-mannonate **18** as a colourless oil which was used without further purification *N*-Ethyldiisopropylamine (43 µl, 0.25 mmol), hydroxybenzotriazole (37 mg, 0.27 mmol) and EDCI (53 mg, 0.27 mmol) were added to a stirred solution of 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-azido-6-deoxy-D-mannonyl)-D-mannonic acid **17** (90 mg, 0.25 mmol) in DMF (0.5 ml) at 0 °C under an atmosphere of nitrogen. The mixture was stirred for 10 min when isopropyl 6-amino-2,5-anhydro-6-deoxy-6-*N*-(2,5-anhydro-6-amino-6-deoxy-D-mannonyl)-D-mannonate **18** (94 mg, 0.25 mmol) in DMF (0.5 ml) was added dropwise. The mixture was stirred for 18 h at room temperature and concentrated *in vacuo*. The residue was dissolved in pyridine (3 ml) and acetic anhydride (3 ml) was added to the stirred solution under an atmosphere of nitrogen. After 18 h, TLC (ethyl acetate) indicated the presence of a major product (R_f 0.3). The reaction was concentrated *in vacuo* and purified by flash column chromatography (ethyl acetate–hexane, 3 : 1, increasing polarity to ethyl acetate) to afford the *peracetylated D-manno tetramer* **19** (222 mg, 85%) as a white amorphous solid. Mp 74–77 °C (ethyl acetate). $[\alpha]_D^{23}$ +58.6 (*c*, 0.51 in $CHCl_3$). ν_{max} (film): 2103 (N_3), 17244 (C=O, ester), 1678 (amide I), 1530 (amide II). δ_H ($CDCl_3$, 400 MHz): 1.33 (6H, d, *J* 6.3, $(CH_3)_2CH$–), 2.07 (3H, s, OAc), 2.08 (6H, s, 2 × OAc), 2.10 (3H, s, OAc), 2.18 (3H, s, OAc), 2.19 (3H, s, OAc), 2.19 (6H, 2 × OAc), 3.52 (1H, dd, *J* 6.4, *J* 13.1, H-6), 3.54–3.75 (7H, m, 7 × H-6), 4.14–4.40 (4H, m, 4 × H-5), 4.62 (1H, d, *J* 4.7, H-2), 4.63 (1H, d, *J* 5.2, H-2), 4.64 (1H, d, *J* 3.1, H-2), 4.65 (1H, d, *J* 3.7, H-2), 4.91 (1H, m, H-4), 4.94 (1H, br s, H-4), 4.95 (1H, dd, *J* 1.7, *J* 2.1, H-4), 5.05 (1H, dd, *J* 1.8, *J* 3.4, H-4), 5.13 (1H, septet, *J* 6.3, $(CH_3)_2CH$–), 5.48 (1H, a-t, *J* 1.5, H-3), 5.58–5.59 (2H, m, 2 × H-3), 5.62 (1H, a-t, *J* 1.9, H-3), 7.07–7.11 (3H, m, NH^B, NH^C, NH^D). δ_C ($CDCl_3$, 100 MHz): 20.6, 20.6, 20.7, 20.7, 20.8, 20.8, 21.6 (7 × q, 8 × OAc, $(CH_3)_2CH$–), 40.5, 40.7, 51.7 (3 × t, C-6^A, C-6^B, C-6^C, C-6^D), 69.7 (1 × d, $(CH_3)_2CH$–), 78.0, 78.1, 78.2, 79.5, 79.6, 81.1, 82.5, 82.5, 82.7, 83.0, 83.1, 83.3, 83.8 (13 × d, C-2^A, C-3^A, C-4^A, C-5^A, C-2^B, C-3^B, C-4^B, C-5^B, C-2^C, C-3^C, C-4^C, C-5^C, C-2^D, C-3^D, C-4^D, C-5^D), 168.2, 168.7, 168.8, 169.5, 169.6, 169.6, 169.7, 169.7, 169.9, 169.9, 170.2 (11 × s, $CO_2CH(CH_3)_2$, 3 × CONH, 8 × OAc). *m/z* (APCI+): 1059 (M + H$^+$, 100%), 1081 (M + Na$^+$, 15%), 1097 (M + K$^+$, 10%). Found: C 48.38 H 5.46 N 8.11%; $C_{43}H_{58}N_6O_{25}$ requires: C 48.77, H 5.52, N 7.94%.

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