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Conformational studies of oligomeric oxetane-based dipeptide isosteres derived from L-rhamnose or D-xylose

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Abstract: Conformational investigations have been undertaken on oligomers (dimers, tetramers, hexamers) of five closely related oxetane-based dipeptide isosteres. All the oligomers were subjected to a range of studies by NMR, FT-IR and CD spectroscopy. The oligomers derived from methyl 2,4-anhydro-5-azido-3-*O*-*tert*-butyldimethylsilyl-5-deoxy-L-rhamnonate 'monomer' all exhibited evidence of ordered conformations in chloroform and 2,2,2-trifluoroethanol (TFE) solution. 5-Acetamido and *N*-methylamide derivatives of the L-rhamnonate 'monomer', along with a 'dimer' lacking silyl protection at C-3, were synthesized to ascertain the role of intramolecular interactions. This led to the conclusion that, for the L-rhamnonate oligomers, steric interactions govern the conformational preference observed. The equivalent silyl-protected D-lyxonate oligomers gave ordered CD spectra in TFE solution, but NMR and FT-IR spectroscopy in chloroform solution suggested an irregular, non-hydrogen bonded system. The remaining silyl-protected 6-deoxy-L-altronate, 6-deoxy-D-gulonate and D-fuconate oligomers appear to be characterized by their lack of ordered conformation in TFE and chloroform solution.

Keywords: oxetane; dipeptide isostere; secondary structure; conformation

INTRODUCTION

It is well known that oligomers of many carbohydrate-derived peptidomimetics have the ability to display novel secondary structures. For example, the 'tetramer' of the unsaturated pyranoid system **1** is thought to display a turn-like conformation in chloroform solution [1], whilst the 'octamer' of the furanoid system **2** adopts a left-handed helical conformation [2,3] (see Figure 1). Oxetane-based amino acids are comparatively novel; consequently few conformational studies have been performed on oligomers of these systems. Where such studies have been carried out, the results have often been just as remarkable as for those seen in pyranoid- and furanoid-based oligomers. For example, the 'hexamer' of β-amino acid **3** has been shown to display a novel left-handed helical conformation in $CDCl_3$ and C_6D_6 solution stabilized by 10-membered hydrogen-bonded rings [4] (see Figure 1).

To date, however, there are no reports of conformational studies involving oligomers of oxetane-containing dipeptide isosteres derived from carbohydrates. The work outlined in this paper amends this by reporting a study of five closely related oxetane-based oligomeric series (shown in Figure 2). The oligomers were synthesized from their respective 'monomers' as outlined in the previous paper [5]. The dipeptide isostere 'monomers' were obtained from either L-rhamnose or D-xylose as reported earlier [6].

Only one of the oligomers produced, the D-fuconate dimer **10**, was a crystalline solid, however, crystals suitable for single crystal x-ray crystallography could not be obtained. All conformational investigations were therefore performed in solution using a combination of NMR, FT-IR and CD (circular dichroism) spectroscopy. In addition, due to the silyl protection, all studies took place in organic solvents. The results of these investigations are summarized by series/stereochemistry below.

The 6-deoxy-L-altronate (**4**–**6**), 6-deoxy-D-gulonate (**7**–**9**) and D-fuconate (**10**–**12**) series have been grouped together for discussion purposes, as the results obtained and hence the conclusions reached concerning their conformational preferences are essentially the same. The first assessment of their conformational preference was made following the comprehensive assignment of their ^{1}H NMR spectra. For these three oligomeric series, from the dimers through to the hexamers, δ_{NH} in $CDCl_3$ occurred in the range 6.6 to 7.2 ppm. Chemical shifts of this magnitude are normally associated with amide protons that are not involved in hydrogen bonding [7,8]. In addition, very little dispersion was visible within the ^{1}H NMR spectra. The ROESY spectra of both the tetramers and hexamers of the three series failed to reveal the presence of any long-range intramolecular nOe interactions. All the NMR evidence therefore pointed towards a lack of intramolecular hydrogen bonds and the absence

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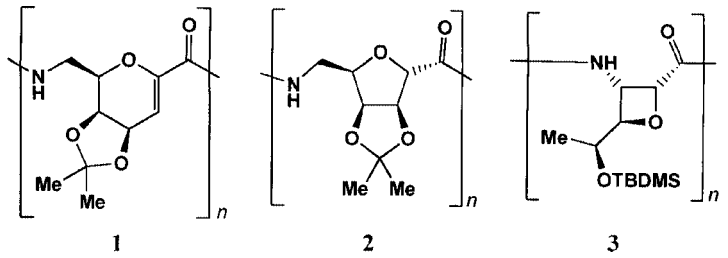


Figure 1 Oligomers of carbohydrate-derived peptidomimetics displaying secondary structure.

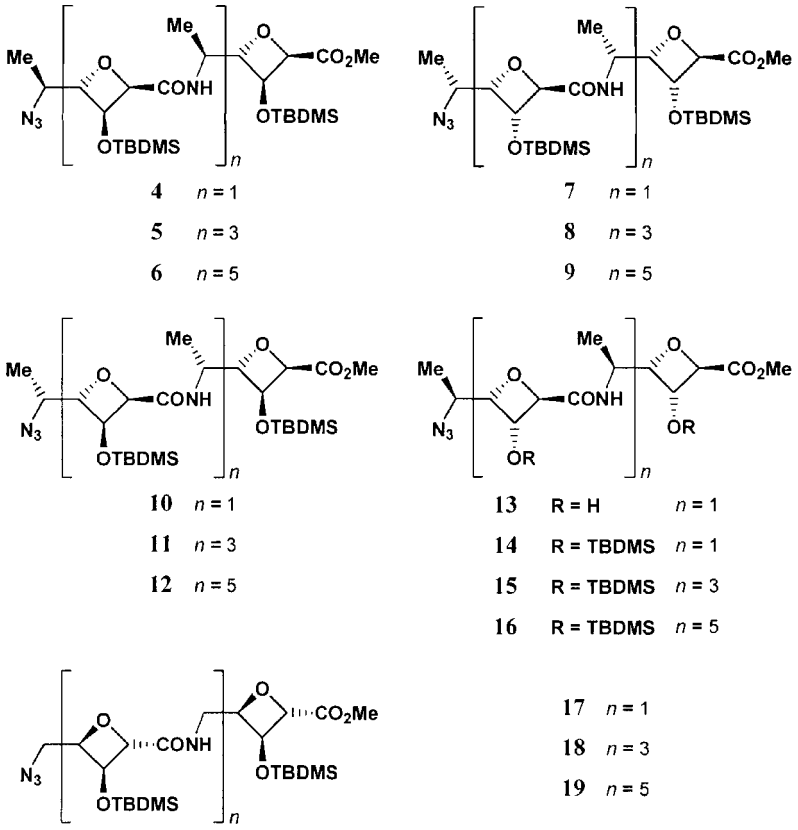


Figure 2 Oligomers of oxetane-based dipeptide isosteres studied.

of ordered secondary structures. Accordingly no more NMR experiments were performed.

Further verification of the NMR findings was sought *via* solution FT-IR experiments in $CHCl_3$. For the three oligomeric series, the amide proton stretch occurs as a sharp band at ν_{max} 3397–3402 cm^{-1} (e.g. Figure 3), with no change being observed between 2 mM and 0.2 mM. These features correspond well with that expected of a non-hydrogen bonded secondary amide (sharp band ca. 3500–3400 cm^{-1}) compared with that of a hydrogen bonded secondary species (broad band ca. 3330–3060 cm^{-1}) [9]. Furthermore, this interpretation is consistent with the spectra previously observed for hydrogen bonded and free species of a related oxetane series [4].

Lastly, CD studies were undertaken. CD is a technique frequently applied to proteins and peptides composed of α-amino acids. By using the basic principles of CD it has been possible to obtain conformational information on related carbopeptoids [10]; herein interpretation of the CD spectra will be achieved *via* a similar approach. The recording of CD spectra in $CHCl_3$ was not possible due to the requirement for transparency of the solvent within the wavelength range measured [11]. Preliminary studies by ourselves, however, have indicated that furanoid-based peptidomimetic oligomers behave in a similar manner in both $CDCl_3$ and CF_3CD_2OH (Claridge TDW, Perez-Victoria I, Edwards AA, unpublished results). It was therefore assumed that no significant effect from the change of solvent environment would occur for the closely related oxetane-based systems studied here. The CD spectra of the three oligomeric series (compounds **4**–**12**) were recorded in TFE. Unfortunately it was

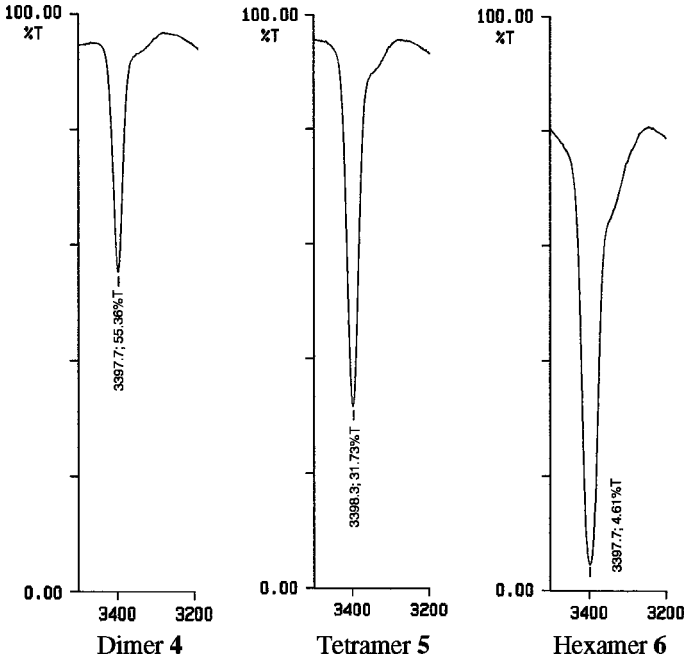


Figure 3 Solution FT-IR spectra of the 6-deoxy-L-altronate dimer **4**, tetramer **5** and hexamer **6**, respectively, at 2 mM concentration in $CHCl_3$.

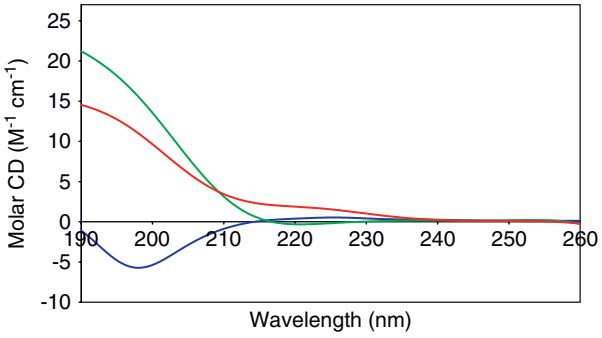


Figure 4 Inversion; L-altronate hexamer **6** (blue) versus the D-gulonate hexamer **9** (green) and the D-fuconate hexamer **12** (red).

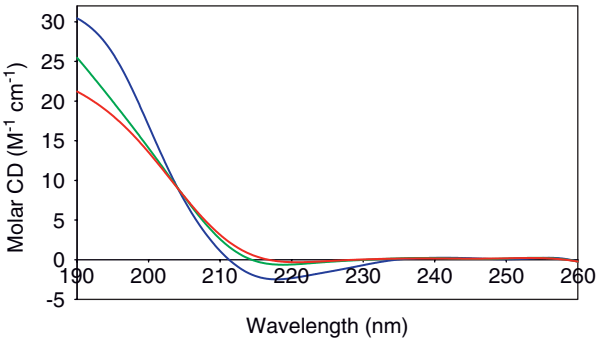


Figure 5 CD spectra of the D-gulonate oligomers: dimer **7** (blue), tetramer **8** (green), hexamer **9** (red).

not possible to record a comparable CD spectrum of the D-fuconate dimer **10** as it was not soluble in TFE. The three sets of spectra are largely similar in form (although the 6-deoxy-L-altronate spectra are inverted due to the change in stereochemistry at C-5, see Figure 4). Each has a maximum at ~190 nm and tails off to zero above 220 nm. For example, the spectra of the 6-deoxy-D-gulonate oligomers (**7**–**9**) are shown in Figure 5. For conventional proteins this type of spectrum is characteristic of a peptidic backbone possessing an irregular secondary structure [12]. It is likely that this is also the case for these three sets of oligomers, with allowance made for the differing nature of carbopeptoids vs peptides.

The L-rhamnonate oligomers (**13**–**16**) were also studied. These showed a number of differences when compared with the above three oligomeric series. The first anomaly was the chemical shift of the amide protons in the ^{1}H NMR spectra. Figure 6 shows the ^{1}H NMR spectra of the silyl-protected L-rhamnonate dimer **14** compared with the non-silylated dimer **13**.

For the silyl-protected L-rhamnonate oligomers (**14**–**16**), δ_{NH} occurs between 7.5 and 7.8 ppm, a value considerably greater than δ_{NH} for the previous three series (6.6–7.2 ppm). This is consistent with amide protons in a deshielded environment, which at first sight may be attributable to hydrogen-bonding. The relatively high NMR shift of the amide proton occurs even in the silyl-protected dimer **14**. Intriguingly, when the TBDMS protection is removed to give the de-silylated dimer **13**, δ_{NH} drops back to below 7 ppm. These combined observations strongly suggest that the amide protons in the silyl-protected L-rhamnonate oligomers are in a deshielded environment due to their conformational preferences rather than their atom connectivity.

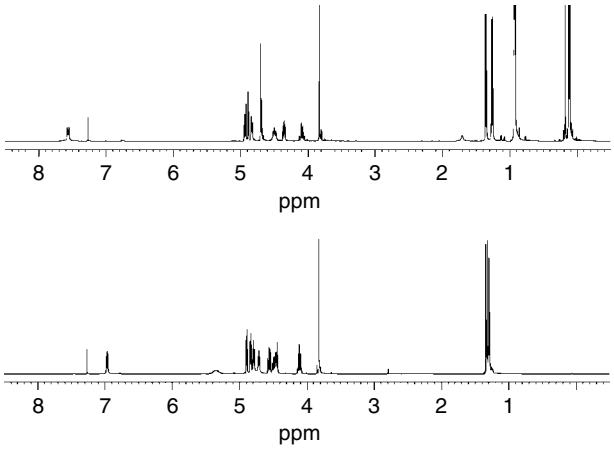


Figure 6 ^{1}H NMR spectra of the TBDMS protected L-rhamnonate dimer **14** (top) and the non-silyl protected dimer **13** in $CDCl_3$.

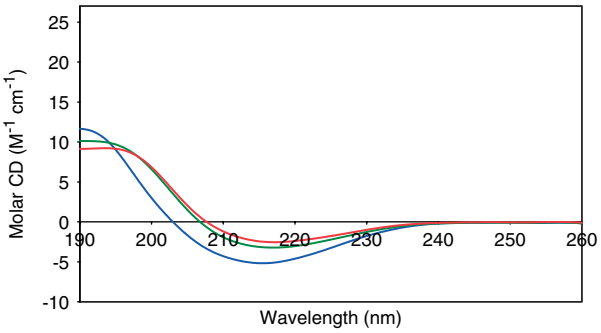


Figure 7 CD spectra of the silyl-protected L-rhamnonate oligomers in TFE: dimer **14** (blue), tetramer **15** (green), hexamer **16** (red).

Despite the high chemical shifts observed, very little dispersion was present within the ^{1}H NMR spectra. In addition, no long-range nOes were observed in either the tetramer **15** (in $CDCl_3$ and C_6D_6) or the hexamer **16** (in C_6D_6, lack of dispersion prevented assignment in $CDCl_3$). This data suggests that either there is no ordered secondary structure, or that the oligomers possess regular secondary structure that repeats identically with each dipeptide isostere residue and results in the residues being far apart in space, that is, not a helical or turn-like structure.

In an attempt to investigate the anomalous shift further and to elucidate conformational mobility, a variable temperature experiment was performed on the tetramer **15** in $CDCl_3$ solution. All the amide protons in this tetramer show relatively small values of $\Delta\delta_{NH}/\Delta T$ (in the range -1.3 to -3.0 ppbK^{-1}). These small numbers are typically interpreted as arising from amide protons that are fully exposed to the solvent, or that remain locked in a strong intramolecular hydrogen-bond over the temperature range studied [13–15]. Solution FT-IR experiments were then conducted to probe further for the presence of hydrogen bonding. All three L-rhamnonate oligomers give a sharp absorption at ~3393 cm^{-1}. This data, taken together with the NMR temperature coefficients, is only consistent with non-hydrogen bonded species for all the L-rhamnonate series.

Finally, the CD spectra of the silyl-protected L-rhamnonate oligomers (**14**–**16**) were recorded in TFE (see Figure 7). There are notable differences between these spectra and those of the previous oligomeric series (e.g. Figure 5). Although all have maxima at ~190 nm, the spectra for the silyl-protected L-rhamnonate oligomers gave a molar CD considerably less than zero above ~210 nm, possessing negative maxima at ~215 nm. Such features are indicative of ordered secondary structure in proteins and it is, by analogy, hypothesized that this is the case here. For conventional peptides, this type of CD spectrum is most closely associated with a β-sheet or β-strand structure [12]. Due to the differences in the amino acids, the same conclusion cannot be directly drawn for the silyl-protected L-rhamnonate oligomers, however, the amide chromophores must be in a similar environment, with comparable interactions, to those in an α-amino acid β-sheet or β-strand. It is also clear that the silyl-protected L-rhamnonate oligomers do not possess an irregular secondary structure in TFE solution. Interestingly, the most intense spectrum is displayed by dimer **14**, consistent with the ordered secondary structure being most prevalent in this molecule.

In order to ascertain if the observed secondary structure was the result of intra- rather than intermolecular interactions (e.g. molecular associations in solution), the CD spectrum of the dimer **14** was also recorded after 10-fold dilution to 20 µM in TFE. There was essentially no change in the spectrum on dilution, indicating that intermolecular interactions were not a significant force governing the secondary structure, or that the equilibrium constant for association is greater than 10^6 M^{-1}.

It was postulated that if an ordered conformation was occurring in dimer **14**, then perhaps it would also occur in simpler derivatives of the L-rhamnonate monomer **20** and if so, then a clearer evaluation of the conformation could be obtained. In view of this, 5-acetamido and *N*-methylamide derivatives (**21** and **22**, respectively) were prepared (see Figure 8). The 5-acetamido derivative **21** was obtained in 82% yield over two steps from the L-rhamnonate monomer **20** by reduction of the azido group (using hydrogen over palladium black) followed by reaction of the crude amine with acetic anhydride. Hydrolysis of the L-rhamnonate monomer **20** (using potassium carbonate in methanol) followed by TBTU mediated coupling of the resultant acid with methylamine led to the required *N*-methylamide derivative **22** in 51% yield (unoptimized).

The ^{1}H NMR spectra of these monomer derivatives revealed a relatively low chemical shift for both amide protons (6.3 and 6.7 ppm for **21** and **22**, respectively),

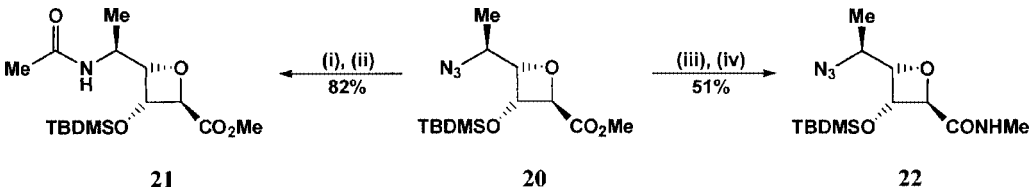


Figure 8 Reagents and conditions; (i) H_2, Pd black, MeOH, RT, 1 day; (ii) Ac_2O (2.0 eq.), Pyr., RT, 1.5 h; (iii) K_2CO_3 (1.3 eq.), MeOH:H_2O, 10 : 1, RT, 1 day; (iv) TBTU (1.2 eq.), TEA (2.5 eq.), $MeNH_3Cl$ (1.2 eq.), DMF, RT, 1 day.

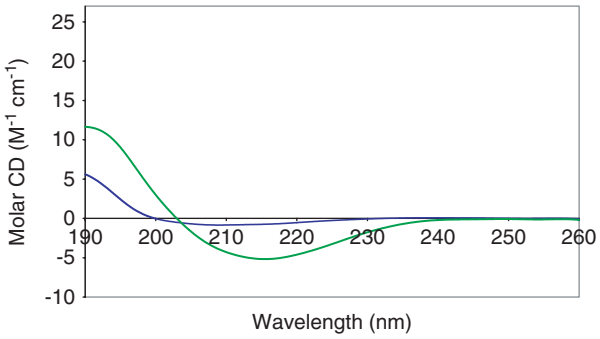


Figure 9 CD spectra of the non-silylated L-rhamnonate dimer **13** (blue) and the silyl-protected L-rhamnonate dimer **14** (green) in TFE.

suggesting that no hydrogen bonding exists and that the conformational features giving rise to the unusually high δ_{NH} values do not exist in these monomers. FT-IR experiments revealed sharp peaks at 3431 cm^{-1} (the 5-acetamido derivative **21**) and 3425 cm^{-1} (the *N*-methylamide derivative **22**) in 2 mM $CHCl_3$ solution, likewise consistent with the absence of hydrogen bonding. Both spectra were unchanged on 10-fold dilution. The NMR and FT-IR evidence confirm that the amides are not involved in hydrogen bonding.

As the ordered conformation evident in the silyl-protected L-rhamnonate dimer **14**, was not observed for the simpler silylated monomer derivatives, attention was focused on the dimer. In particular, the difference between the silylated and the non-silylated dimer (**14** and **13**) was investigated. It was apparent from the ^1H NMR spectrum that the non-silylated dimer **13** did not display any ordered conformation (see above). Further supporting evidence that this was the case came from the CD spectra. The TBDMS protected dimer **14** gives a CD spectrum corresponding to an ordered conformation in TFE, but the non-silylated dimer **13** produces a spectrum akin to a less ordered conformation (see Figure 9).

Detailed NMR studies were carried out on the two dimers to ascertain the differences between them. A variable temperature experiment was performed on both dimers in $CDCl_3$. The chemical shift of the amide proton of both dimers, whilst different in magnitude, varies little with temperature (−3.1 and −2.2 $ppbK^{-1}$ for **14** and **13**, respectively). These observations, combined with the solution IR studies (see above) and the absolute magnitude of δ_{NH} (7.6 ppm for **14** and 7.0 ppm for **13**), reaffirm the conclusion that neither of the dimers possess amide protons that participate in hydrogen-bonding.

The conformational properties of the two dimers were further probed *via* DPFGSE (double pulsed field gradient spin-echo) 1-D NOESY experiments in which the amide protons were targeted in order to investigate their surroundings, with the resultant nOe intensities classified as strong, medium or weak (Figure 10).

The nOe interactions between the amide proton and the oxetane ring adjacent to the azide terminus (dipeptide isostere A) are similar for both dimers. Differences do occur, however, between the amide proton and the oxetane ring adjacent to the methyl ester (dipeptide isostere B). Most apparent is the strong interaction present between the amide proton and the

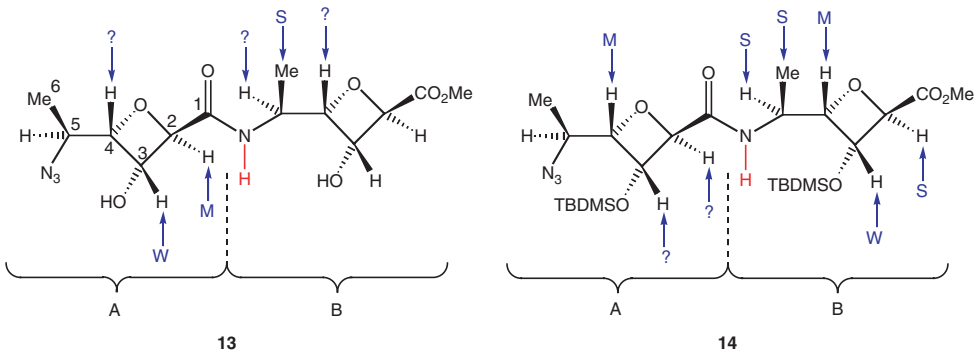
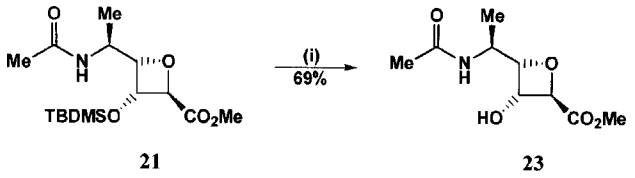


Figure 10 nOe interactions (shown in blue) upon irradiation of the amide proton shown in red in dimers **13** and **14**. S, strong interaction; M, medium; W, weak; ?, nOe interactions (signals overlapped).

Table 1 The Non-oxetane Backbone Coupling Constants of L-Rhamnonate Dimers **13** and **14** in $CDCl_3$, and 5-acetamido Monomer Derivatives **21** and **23** in $CDCl_3$ and CD_3CN, respectively

Coupling constant	TBDMS-protected dimer **14**	Non-silylated dimer **13**	TBDMS-protected 5-acetamido derivative **21**	Non-silylated 5-acetamido derivative **23**
$J_{B4,5}$ (Hz)	3.2	7.8	3.2	9.0
$J_{B5,NH}$ (Hz)	9.2	8.4	8.8	n.d.



21 23

Figure 11 Reagents and conditions; (i) NH_4F (5.0 eq.), MeOH, RT, 1 day.

proton α to the methyl ester (B-2) in the TBDMS protected dimer **14**; this interaction is absent in the non-silylated dimer **13**. This suggests that the TBDMS group is causing a conformational change in dipeptide isostere B (adjacent to the methyl ester), resulting in the α-proton becoming closer in proximity to the amide proton. In light of this information, the non-oxetane backbone coupling constants (i.e. $J_{B4,5}$ and $J_{B5,NH}$) were examined. In addition, they were compared with the equivalent coupling constants in the 5-acetamido derivative **21**. A summary of these coupling constants is shown in Table 1.

As can be seen, there is little change in the value of $J_{B5,NH}$ between the different compounds. The value of $J_{B4,5}$ does, however, show a marked change between those compounds with TBDMS protection and those without. This suggests that the TBDMS group(s) displace the conformation adopted by the unprotected dimer, resulting in rotation about the B4-5 bond. Further evidence for this was sought by preparing a de-silylated 5-acetamido derivative. Accordingly, the TBDMS protected 5-acetamido derivative **21** was treated with ammonium fluoride in methanol to give the de-silylated derivative **23** in 69% yield (see Figure 11).

Unfortunately, it was impossible to establish $J_{B4,5}$ for **23** in $CDCl_3$ due to a lack of dispersion in the spectrum. In CD_3CN, however, $J_{B4,5} = 9.0$ Hz. This suggests that the same conformational change takes place between the TBDMS and non-silylated 5-acetamido derivatives (**21** and **23**) as takes place between the TBDMS and non-silylated dimers (**14** and **13**), assuming the molecule behaves in a similar way in both $CDCl_3$ and CD_3CN. This interpretation is further reinforced by the comparative values of δ_{NH} in $CDCl_3$ for the two 5-acetamido derivatives; 6.30 ppm for **21** versus 5.60 ppm for **23**.

The CD studies on all the L-rhamnonate monomer derivatives were also carried out (see Figure 12). Both the silylated *N*-methylamide derivative **22** and the de-silylated 5-acetamido derivative **23** possess unordered conformations in TFE. The TBDMS protected 5-acetamido derivative **21**, however, produces a spectrum approximately halfway between that produced by the TBDMS protected dimer **14** and an irregular spectrum. This again underlines the importance of the ring adjacent to the methyl ester in conjunction with TBDMS protection in inducing conformational restriction in the protected dimer **14**.

In conclusion, the TBDMS protected L-rhamnonate dimer **14**, tetramer **15** and hexamer **16** do possess ordered conformations in chloroform solution, whereas de-silylated dimer **13** and the monomer derivatives **21, 22** and **23** do not. Where it occurs, the ordered conformation exhibited is a result of steric interactions, with no hydrogen bonding taking place. Detailed analysis of the TBDMS protected L-rhamnonate dimer **14** has revealed that a restricted conformation (across the B4-5 bond) is enforced by the TBDMS protection on the oxetane-ring adjacent to the methyl ester. This, in conjunction with the TBDMS protected oxetane-ring adjacent to the azide terminus, has in turn resulted in the amide proton being deshielded (relative to an unordered peptide) giving a higher δ_{NH} value. The

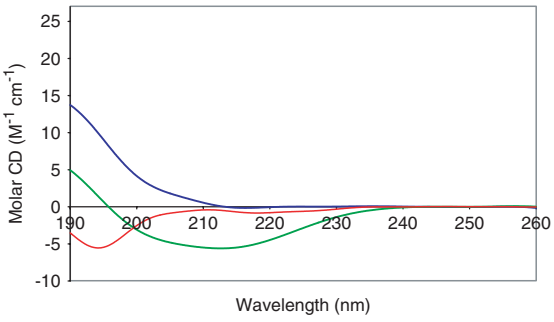


Figure 12 CD spectra of the *N*-methylamide derivative **22** (blue) the TBDMS protected 5-acetamido derivative **21** (green) and the de-silylated 5-acetamido derivative **23** (red) in TFE.

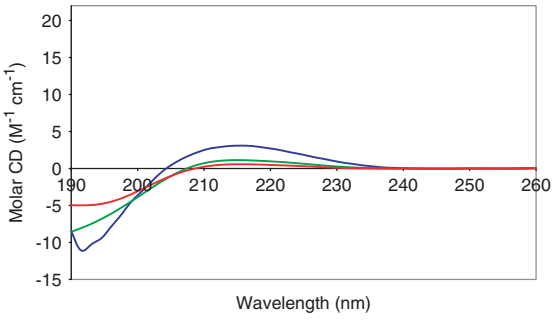


Figure 13 CD spectra of the D-lyxonate oligomers in TFE: dimer **17** (blue), tetramer **18** (green), hexamer **19** (red).

precise nature of the restricted conformation awaits to be established *via* molecular modelling experiments.

Finally, the D-lyxonate series (**17**–**19**) was studied. The NMR and FT-IR evidence for this series is broadly similar to that found for the 6-deoxy-L-altronate (**4**–**6**), 6-deoxy-D-gulonate (**7**–**9**) and D-fuconate (**10**–**12**) oligomers. Once again, a low δ_{NH} in $CDCl_3$ (~7.1 ppm) was observed with little dispersion in the ^1H NMR spectrum. The FT-IR spectra in $CHCl_3$ solution gave a sharp absorption at around υ_{max} 3410 cm^{-1}. Both these factors suggest an irregular, non-hydrogen bonded system in chloroform solution. Interestingly, however, the CD spectra of these oligomers in TFE (see Figure 13) was similar to those displayed by the L-rhamnonate oligomers (see Figure 7), albeit less pronounced. This suggests that, in TFE solution, the D-lyxonate oligomers (**17**–**19**) do weakly exhibit the same kind of ordered structure as is found in the silyl protected L-rhamnonate series (**14**–**16**).

In summary, detailed conformational studies of all five oligomeric series in chloroform and TFE solution have established that the 6-deoxy-L-altronate (**4**–**6**), 6-deoxy-D-gulonate (**7**–**9**) and D-fuconate (**10**–**12**) oligomers show no regular secondary structure in chloroform solution or TFE. The non-silylated L-rhamnonate dimer **13** and the monomer derivatives **21, 22** and **23** also do not possess regular secondary structure in chloroform solution. The TBDMS protected L-rhamnonate dimer **14**, tetramer **15** and hexamer **16** do, however, possess ordered conformations in chloroform and TFE solution. The conformation exhibited appears to be a result of steric interactions enforced by the bulky TBDMS protection, with no hydrogen-bonding taking place. Finally, the D-lyxonate oligomers (**17**–**19**) show no preference for regular secondary structure in chloroform solution by NMR and IR spectroscopy, however, CD spectroscopy suggests that they weakly exhibit an analogous ordered conformation to the L-rhamnonate oligomers in TFE solution.

EXPERIMENTAL

Exhaustive NMR and IR spectroscopic data were collected for all the compounds described [16], confirming their formulation as pure single isomers of the structures indicated. DMF was purchased dry from the Aldrich chemical company in Sure-Seal™ bottles capped with Oxford-Caps™. 'Hexane' refers to the fraction of petroleum ether that boils in the range 60°–80 °C. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Reactions performed under an atmosphere of nitrogen or hydrogen gas were maintained by an inflated balloon. All other reagents were used as supplied, without prior purification. Thin layer chromatography (TLC) was performed on aluminium backed sheets coated with 60 F_{254} silica. Sheets were developed using a spray of 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2 M sulphuric acid, or 0.5% ninhydrin in methanol (for amines). Column chromatography was performed using C60 40/60 silica. Melting points were recorded on a Kofler hot block. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g/100 ml. Low resolution mass spectra (m/z) were recorded either on a Micromass Platform I mass spectrometer using atmospheric pressure chemical ionization (APCI), or on a Micromass BioQ II-ZS LCT mass spectrometer using electrospray ionization (ESI), or on a micromass Autospec 500 OAT spectrometer using chemical ionization (CI). High resolution mass spectra (HRMS) were recorded on a micromass Autospec 500 OAT spectrometer using chemical ionization, or on a Waters 2790-Micromass LCT mass spectrometer using electrospray ionization. NMR spectra were recorded on a Bruker DPX 200 (^1H: 200 MHz and ^{13}C: 50.3 MHz) spectrometer, or a Bruker DPX 400 (^1H: 400 MHz and ^{13}C: 100.6 MHz) spectrometer, or a Bruker AMX500 or DRX500 (^1H: 500 MHz and ^{13}C: 125.7 MHz) spectrometer in the deuterated solvent stated. Solution infrared spectra were recorded at room temperature on a Perkin-Elmer 1750 IR Fourier Transform spectrophotometer in $CHCl_3$ (dried over 4 Å molecular sieves) at a concentration of 2 mM or 0.2 mM as stated, in cells equipped with CaF_2 windows with a path length of 1.0 cm. The spectra were recorded over 64 scans at a resolution of 1 cm^{-1}, with the final spectra being obtained by the subtraction of a background spectrum obtained using the same parameters for the neat solvent. Circular dichroism (CD) spectra were recorded on a Jasco J600 circular dichroism spectrometer fitted with a bespoke thermostated cell holder. The sample cell was a quartz Suprasil cylindrical cell with a path length of 1.0 cm. Sample spectra were measured at 293 K in 2,2,2-trifluoroethanol (TFE) at an amide concentration of 200 μM (except where stated otherwise). Amide concentration refers to the concentration of amide linkages in solution, for example a 40 μM solution of a hexamer (five amide linkages per molecule) has an amide bond concentration of 200 μM. The following acquisition parameters were used: scan speed = 10 nm/min; time constant = 4 s; spectral band width = 1 nm; data interval = 0.1 nm; scan range = 260–185 nm. A baseline spectrum was recorded in the same cell at proximal time and subtracted from the sample spectrum. The resultant spectra were normalized for path length and mean amide concentration to give molar circular dichroism spectra. The spectra were subsequently Fourier transform noise reduced and compared with the original spectrum to ensure that smoothing gave a representative spectrum.

Methyl 5-acetamidamido-2,4-anhydro-3-*O*-*tert*-butyldimethylsilyl-5-deoxy-L-rhamnonate 21

A solution of methyl 2,4-anhydro-5-azido-3-*O*-*tert*-butyldimethylsilyl-5-deoxy-L-rhamnonate **20** (0.025 g, 0.079 mmol) in MeOH (0.5 ml) was stirred under H_2 in the presence of Pd black (0.006 g). After 1 day, TLC (EtOAc) indicated the presence of one product (R_F 0.18). The reaction mixture was degassed, flushed with N_2, and then filtered through Celite. The solvent was removed, and the residue was dissolved in pyridine (0.3 ml). The solution was stirred under N_2 at room temperature and Ac_2O (0.015 ml, 2 eq.) was added dropwise. After 1.5 h, TLC (EtOAc) indicated the formation of a major product (R_F 0.43). The solvent was removed, and the residue was purified by column chromatography (EtOAc : hexane, 1 : 1) to give *methyl 5-acetamidamido-2,4-anhydro-3*-O-tert-*butyldimethylsilyl-5-deoxy-L-rhamnonate* **21**

(0.021 g, 80% yield) as a clear oil; $[\alpha]_{365}^{25} + 18.4$ (*c* 0.80 in $CHCl_3$); MS (ESI^+) *m/z*: 331.93 (MH^+, 100%), 353.95 (MNa^+, 80%), 685.18 ($2M.Na^+$, 62%). HRMS: MNa^+ found 354.1715, $C_{15}H_{29}NO_5SiNa^+$ requires 354.1713.

2,4-anhydro-5-azido-3-*O*-*tert*-butyldimethylsilyl-5-deoxy-*N*-methyl-L-rhamnonamide 22

$MeNH_3Cl$ (0.006 g, 1.2 eq.) was added to a solution of 2,4-anhydro-5-azido-3-*O*-*tert*-butyldimethylsilyl-5-deoxy-L-rhamnonic acid [4] (0.024 g, 0.080 mmol), TEA (0.028 ml, 2.5 eq.), and TBTU (0.030 g, 1.2 eq.) in DMF (0.5 ml). The solution was stirred at room temperature under N_2 for 19 h, after which TLC (EtOAc : hexane, 1 : 1) revealed the formation of a major product (R_F 0.47). The solvent was removed, and the residue was purified by column chromatography (EtOAc : hexane, 1 : 2) to give *2,4-anhydro-5-azido-3*-O-tert-*butyldimethylsilyl-5-deoxy*-N-*methyl*-L-*rhamnonamide* **22** (0.013 g, 52% yield) as a clear oil; $[\alpha]_D^{27}$ +75.4 (*c* 0.32 in $CHCl_3$); MS ($APCI^+$) *m/z*: 315.5 (MH^+, 100%). HRMS: MH^+ found 315.1852, $C_{13}H_{27}N_4O_3Si$ requires 315.1852.

Methyl 5-acetamidamido-2,4-anhydro-5-deoxy-L-rhamnonate 23

NH_4F (0.008 g, 5 eq.) was added to a stirred solution of methyl 5-acetamidamido-2,4-anhydro-3-*O*-*tert*-butyldimethylsilyl-5-deoxy-L-rhamnonate **21** (0.014 g, 0.042 mmol) in MeOH (0.5 ml) at room temperature under N_2. After 1 day, TLC (EtOAc) revealed the formation of a major product (R_F 0.19). The solvent was removed, and the residue was purified by column chromatography (EtOAc : MeOH, 19 : 1) to give *methyl 5-acetamidamido-2,4-anhydro-5-deoxy-L-rhamnonate* **23** as a white crystalline solid; m.p. 101°–103 °C and 124°–126 °C (2 phases); $[\alpha]_D^{25}$ +13.1 (c, 0.32 in $CHCl_3$); MS (ESI^+) *m/z*: 239.71 (MNa^+, 100%), 456.96 ($2M.Na^+$, 54%). HRMS: MNa^+ found 240.0860, $C_9H_{15}NO_5Na^+$ requires 240.0848. Found C, 49.67; H, 6.65; N, 6.20; $C_9H_{15}NO_5$ requires C, 49.76; H, 6.96; N, 6.45%.

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