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A Solid Phase Approach to Oligomers of Carbohydrate Amino-Acids: Secondary Structure in a Trimeric Furanose Carbopeptoid

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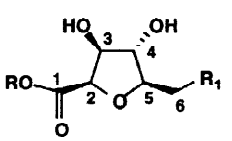
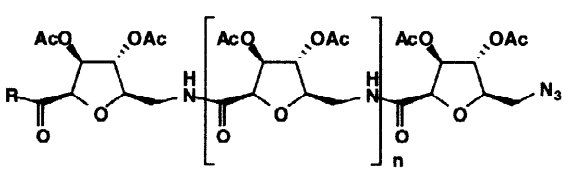
Abstract: The synthesis of oligomers of a *C*-arabinofuranosyl carbohydrate amino acid on a polystyrene support functionalised with a Rink linker is reported. Cleavage from the solid support gives ready access to dimeric and trimeric carbopeptoids bearing a *C*-terminal carboxamide. Investigations into the solution structure of these novel carbopeptoids utilising ^{1}H NMR indicate that they adopt well defined conformations based around a repeating β-turn like structure stabilised by (i, i-2) inter-residue hydrogen bonds.

The application of solid phase methodology to carbohydrate syntheses is an attractive approach for combinatorial library generation,[1] and has notably been exploited in the formation of novel glycopeptide libraries[2] and in the synthesis of oligosaccharides.[3] Carbohydrates bearing both an amino and a carboxylic acid functionality may be incorporated into combinatorial libraries by standard peptide coupling procedures[4] compatible with established solid phase methods.[5] Oligomers[6] of pyranose sugar amino acids (carbopeptoids')[7] have been synthesised in solution[8] and on solid support;[9] unnatural oligomers[10] of this nature may have the ability to mimic conformations of natural biopolymers. This approach has been extended to β-[11] and γ-[12] peptides and oligourea templates.[13]



1. $R_1 = N_3$, R = H
2. $R_1 = NH_2$, R=isopropyl



3. R = *O*-isopropyl, n = 0, 2, 4
4. R = NH_2, n = 0, 1

Figure 1. Structures of sugar amino acids and carbopeptoids showing numbering scheme.

The tetramer **3** (n=2) and hexamer **3** (n=4), derived from the *C*-glycofuranosyl sugar amino acid derivatives **1** and **2**,[14] have been shown to adopt a well defined solution state secondary structure.[15] For these carbopeptoids **3** the *C*-terminal ester appears to exert little influence on the solution conformation; the hydrogen bonding interaction between an amide proton of tetrahydrofuran (i) and the carbonyl of the penultimate sugar residue (i-2) stabilises the repeating β-turn type structure observed in solution. This paper describes a solid phase approach in which an amine linker is utilised to obtain a trimer **4** (n=1) which differs from **3** by substitution of the *C*-terminal ester for a carboxamide group. The primary amide at the *C*-terminus is shown to form an additional inter-residue hydrogen bond, so that the trimer **4** adopts the same well-defined solution state secondary structure as that observed in **3** (n=2,4) when one carbohydrate residue shorter.

The solid phase synthesis of the trimer **4** is shown in Scheme 1. The monomeric sugar amino acid derivative **1** was attached through the carboxyl function to a polystyrene support *via* a Rink[16] linker **5** (loading 0.56 mmol / g)[17] by treatment with diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) in dimethylformamide (DMF) to afford **6**. Treatment of the solid-supported azide **6** with diisopropylethylamine (DIPEA) and dithiothreitol (DTT)[18] in DMF at 50°C afforded the amine **7**. The progress of the coupling of **1** to the resin and the azide reduction was monitored by the bromophenol blue amine indicator test.[19]

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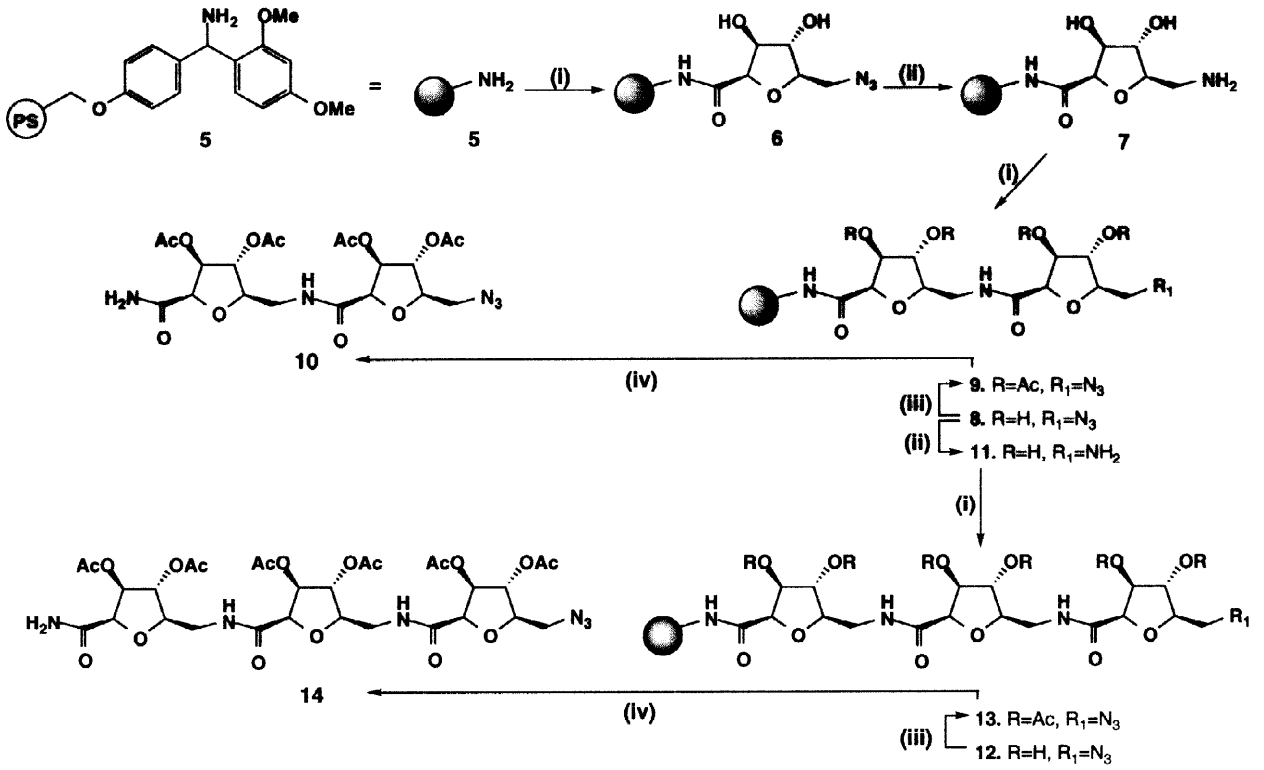

Reagents: (i) monomer **1**, DIC, HOBt, DMF. (ii) DTT, DIPEA, DMF, 50°C . (iii) Ac_2O, Pyridine . (iv) 50% TFA/CH_2Cl_2.

Scheme 1: Homo-Oligomerisations

The monomeric azidoacid **1** was coupled to the polymer-bound amine **7** by treatment with DIC and HOBt in DMF to afford the immobilised dimer **8**. The acetylated dimer **10** was isolated to prove that coupling had taken place and that the material could be removed from the support; the unprotected material is very polar. The polymer-bound dimer **8** was acylated by treatment with acetic anhydride in pyridine to afford **9**. Cleavage of the dimer **9** from the solid support with 50% v/v trifluoroacetic acid (TFA) / dichloromethane (CH_2Cl_2) and HPLC purification yielded pure dimer **10** in 30% yield over the 5 steps. The trimer **14** was prepared by an iterative sequence. Thus, reduction of the solid-supported dimer **8** with DTT and DIPEA in DMF at 50°C gave the amine **11**; subsequent coupling of **11** with acid **1** by treatment with DIC and HOBt in DMF gave the polymer-bound trimer **12**. Reaction of **12** with acetic anhydride and pyridine gave **13** which was released from the resin by treatment with 50% TFA/CH_2Cl_2; purification by HPLC gave the acetylated trimer **14**. The presence of a number of impurities, subsequently identified as dimer **10** and a reduced *N*-acetylated dimer was indicated by HPLC. Evidently, neither the azide reduction of **8** and nor the subsequent peptide coupling proceed to completion. The trimer **14** was thus obtained in a lower overall yield than the dimer **10** but in excellent purity. Further studies are in progress to monitor and improve the azide reduction and the peptide coupling; nonetheless, this work demonstrates the viability of the use of solid phase techniques for incorporation of tetrahydrofuran amino acids into oligomers.

^{1}H NMR studies of both the dimer **10** and the trimer **14**[20] in $CDCl_3$ show high proton chemical shift dispersion and significant variation in the chemical shifts of the amide protons. For the dimer **10**, amide NH shifts was 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, 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 in the dimer **10**. The ^{1}H NMR spectrum of trimer **14** 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 (Figure 2); 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 indicate intramolecular hydrogen bonding and two NH signals which indicate no intramolecular hydrogen bonding. Comparison between the spectra of the trimer **14** and the previously reported tetramer **3** (n=2) reveals a high correlation between proton chemical shifts indicating a very similar solution conformation. This is further supported by the similarities observed in the nOe data (2D NOESY and/or ROESY) obtained for the tetramer **14** and trimer **3**. 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 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.

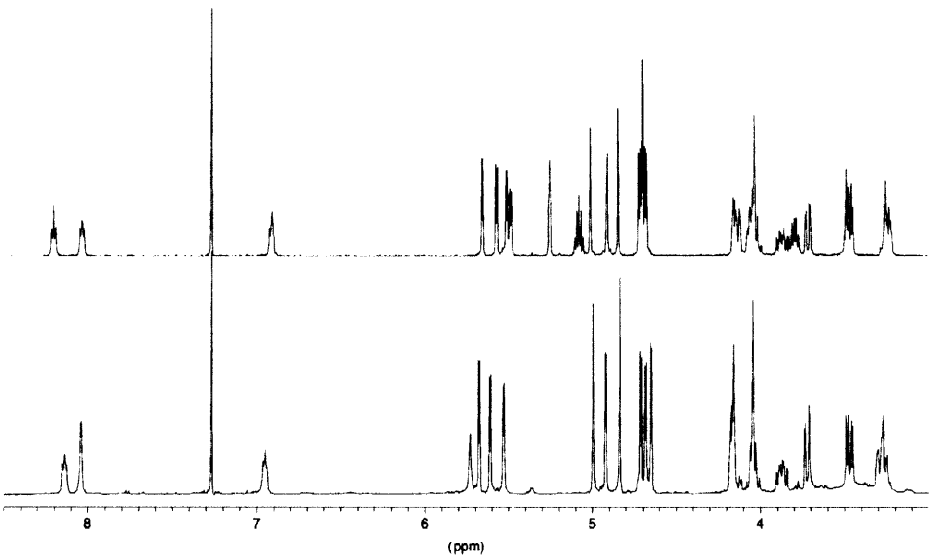

Figure 2. ^{1}H NMR (500MHz) spectra of tetramer **3**(upper plot) and trimer **14** (lower plot) in $CDCl_3$

On the basis of ^{1}H NMR studies and molecular dynamics, tetramer **3** (n=2) has been shown to exhibit a novel repeating β-turn type structure stabilised by (i, i-2) inter-residue hydrogen bonds. It would appear that the trimer **14** adopts the same type of conformation by participation of one of the carboxamide NH protons in a hydrogen bond which is directly analogous to that of the amide NH^D proton of tetramer **3** (Figure 3).

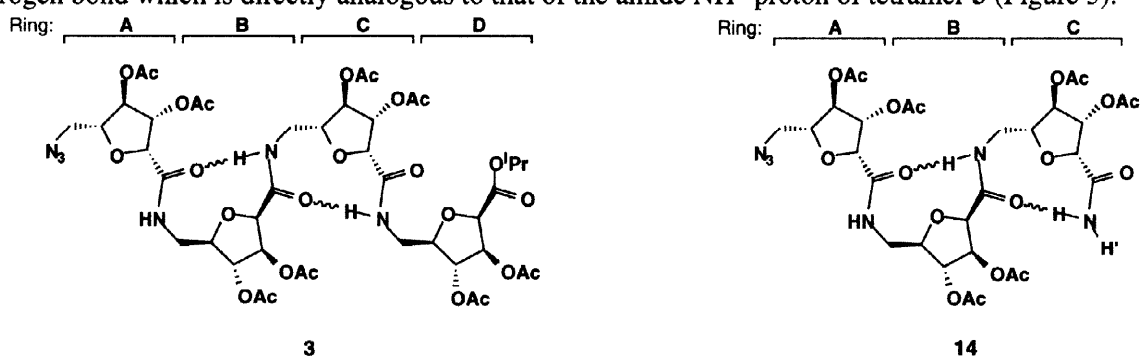

Figure 3: Representation of the observed solution secondary structure of the tetramer **3** and trimer **14** indicating ring labelling. Rings are identified by labelling each residue alphabetically from 'A' at the *N*-terminus

The involvement of one of the carboxamide NH protons (δ_H 7.55) of the dimer **10** in a hydrogen bond indicates the general propensity of these structures to adopt the well-defined conformation observed in higher oligomers in chloroform. As a result of the observed similarities between **14** (n=1) and **3** (n=2) we propose that carbopeptoids **4** bearing a *C*-terminal carboxamide adopt the same repeating β-turn type structure as oligomers **3**. This relationship is expected to be general between oligomers **3** and **4** where **3** bears one more carbohydrate residue than **4**.

In summary, this paper reports the first use of a solid phase for the incorporation of tetrahydrofuran amino acids into amide products; additionally further evidence is present that short tetrahydrofuran aminoacid sequences induce secondary structures. The flexibility of the synthesis of stereoisomers of **1** indicate that these materials may form a family of related aminoacids which ultimately may allow the design of secondary structures and will change tetrahydrofuran amino acids from sequencamers to foldamers.[6,21]

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[20] Selected data for trimer **3**. (500MHz, $CDCl_3$, 298K)

	A	B	C	NH_2
H^2	4.65	4.71	4.68	
H^3	5.68	5.61	5.53	
H^4	4.92	4.84	5.00	
H^5	4.16	4.05	4.17	
H^6	3.72, 3.47	4.04, 3.26	3.88, 3.29	
NH	-	6.95	8.14	8.04, 5.73

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