

Synthesis of cyclic oligomers of a modified sugar amino acid utilising dynamic combinatorial chemistry

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Abstract - Dynamic combinatorial chemistry has been utilised for the rapid synthesis of a library cyclic oligomers based on a modified furanoid sugar amino acid repeat unit.

Keywords – dynamic combinatorial chemistry; sugar amino acid; macrocycle

Dynamic combinatorial libraries (DCLs) contain molecules that are generated from a set of building blocks, the connections between which are *reversible*. The connections may be covalent or noncovalent and suitably functionalised building blocks allow access to either DCLs of small molecules or cyclic oligomers. DCLs can change composition in the presence a guest by shifting the equilibrium to increase the concentration of the DCL member(s) that best recognises (through molecular recognition) the guest.¹ Dynamic combinatorial chemistry (DCC) proceeds not to generate high yielding pure compounds, as is most often the desired outcome of conventional synthesis, but rather to access diversity and exploit the effective amplification of the ‘best binder’ by the addition of a suitable guest *or* host in a screening protocol. There are now several examples of receptor amplification in systems derived from DCLs generated in the presence of a guest.² Drawing inspiration from the extensively explored cyclodextrins as ‘artificial’ receptors in host-guest chemistry, many studies pursuing the synthesis of unnatural cyclic carbohydrate oligomers have been reported.³ These macrocycles retain the cluster of

hydroxyl groups important for molecular recognition but incorporate unnatural covalent connections between building blocks. The unnatural connections impart different physical and chemical properties to the carbohydrate oligomers and in addition these modified connections can serve as a synthetic aid for cyclic oligomer synthesis.

Cyclic oligomers of sugar amino acids (SAAs) have been synthesised by others.^{4,5} The underlying premise for the work described was to retain the cyclic array of carbohydrate moieties, but incorporate both amine and carboxylic acid functional groups onto the sugar to allow iterative peptide coupling for oligomer synthesis. In the study reported here we have investigated DCC for the synthesis of unnatural carbohydrate cyclic oligomers. Specifically we describe an efficient, solution-phase one-pot synthesis, afforded by DCC, for the synthesis of a cyclic oligomer library derived from a modified furanoid SAA.

The DCC strategy used in this study was reversible acylhydrazone formation and exchange for the synthesis of cyclic oligomers based on a SAA repeat unit. Reversible acylhydrazone formation and exchange has been used previously for the synthesis of cyclic oligomers.⁶ The requirement is for building blocks to be functionalised with *both* a (protected) aldehyde and a hydrazide. The addition of acid to a dilute solution of building block catalyses deprotection of the aldehyde and subsequent hydrazone exchange resulting in a DCL of interchanging cyclic hydrazone oligomers, Scheme 1.

Insert Scheme 1

The furanoid SAA derivative **1** was functionalised with both hydrazide and aldehyde (protected as the dimethoxy acetal) moieties to give the modified SAA building block **2**. The seven step synthesis of **2** from D-ribose was adapted from published routes.⁷ The 2,3-*cis* diol of the D-ribose core was carried through the synthesis of **2** as its *O*-isopropylidene derivative.

Insert schematic of compounds 1 and 2

A DCL of interchanging cyclic carbohydrate oligomers was generated from **2** (5 mM) in aqueous dimethyl sulfoxide (H₂O/DMSO, 1 : 4, vol/vol), Scheme 2. Catalyses by both 5 μ L TFA and 25 μ L TFA were studied, with analysis of the DCL composition carried out by electrospray ionisation mass spectrometry (ESI MS), Figure 1.

Insert Scheme 2

The low concentration of **2** ensures that the reaction proceeds to direct the formation of cyclic oligomers. Linear oligomerisation would have led to products with molecular weights 18 mass units (= 1 molecule of water) higher than we observed by ESI MS. The results also identified a secondary role for TFA in generation of the DCL. In addition to deprotection of the aldehyde functionality and hydrazone exchange to form cyclic oligomers **A_n**, aqueous TFA also catalysed the irreversible loss of the 2,3-*O*-isopropylidene protecting group, thus unmasking the hydroxyl groups of the furanoid sugar and leading to alternative cyclic oligomers.

Catalysis with 5 μ L TFA produced cyclic oligomers **A_n** of **2**, with retention of the 2,3-*O*-isopropylidene protecting groups (Figure 1, entries a and b). With 25 μ L TFA cyclic oligomers **A_n** were initially formed (Figure 1, entry c), but in time, the full complement of mixed oligomers containing both the protected (**A**) and deprotected (**B**) 2,3-*cis* diol species **A_nB_n'** were observed (Figure 1, entry d). The presence of species **B** was clearly evident from the loss of 40 mass units per **B** repeat unit. The reaction with 25 μ L TFA proceeded from partially deprotected species to the fully deprotected 2,3-*cis* diol species (**B_n'**) over time (Figure 1, entry e). The dominant product in the DCL after 72 hours was **B₂**, indicating a greater thermodynamic stability for the cyclic dimer relative to other potential library members.

Insert Figure 1

We have demonstrated that hydrazone formation and exchange can be used efficiently to generate a library of cyclic oligomers with novel carbohydrate repeat units. From a

single building block, a DCL accessing >10 detectable cyclic oligomers was formed in a matter of hours. The DCC approach described here offers a rapid, one-step alternative to the multistep, iterative approach more commonly adopted for oligomer synthesis. It is anticipated from our experience with carbohydrate chemistry that the synthesis and purification of the cyclic oligomers described here would be significantly more demanding than that of **2** alone. This study is the first example of a DCL generated from a hybridisation of reversible covalent chemistry (to generate molecular diversity) and irreversible covalent chemistry (to unmask groups for molecular recognition). Importantly, the ability to selectively unmask the carbohydrate hydroxyl groups during the DCL generation step, simply by altering the amount of TFA catalyst employed, significantly enhances the potential scope for DCLs derived from carbohydrate building blocks. The simplified synthesis of protected carbohydrate building blocks and increased stability of such building blocks during long term storage are also both potential advantages. We expect this strategy will lead to a DCC approach towards discovery of potential macrocyclic host molecules based on the SAA scaffold, a scaffold that offers exceptional diversity itself.

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Notes and references -

§ To a 5 mM solution of **2** in H₂O/DMSO (1 : 4, vol/vol) was added TFA (5 μL or 25 μL). The reaction mixture was stirred at room temperature and then analysed by ESI MS at one, six, 24 and 72 hours. ESI MS spectra were recorded on a Micromass VG Platform 2, single quadrupole instrument fitted with a linear electrospray source. The source was heated to 90 °C and the sampling cone voltage was 30 eV. Samples were prepared by dilution of the DCL reaction with MeCN (1/10 dilution). Samples were introduced into the mass spectrometer source with an LC pump (Shimadzu LC-9A and mixing valve FCV-9AL.) at a rate of 1.0 mL/min in CH₃CN/H₂O 80%/20%.

Scanning was performed from 100 to 2000 over 4 s and multiple scans were summed to obtain the final spectrum which was processed using MassLynx V 3.4 software.

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7. The furanoid SAA derivative **1** was synthesised in five steps from D-ribose, adapted from Kessler *et al.*^{5b},⁸ This intermediate was coupled to 4-carboxybenzaldehyde dimethoxy acetal, followed by hydrazinolysis to give **2** in an identical manner to that described by us for commercially available amino acid methyl esters.⁶ For the complete synthesis of **2** from D-ribose please see electronic supplementary data.

Spectroscopic data for methyl 3,6-anhydro-7-amino-2,7-dideoxy-4,5-*O*-isopropylidene-D-*allo*-heptulonate **1**: ¹H NMR (200 MHz, CDCl₃): δ = 4.57 (m, 2 H, H4, H5), 4.26 (m, 1 H, H3), 3.95 (m, 1 H, H6), 3.71 (s, 3 H, OMe), 2.92 (dd, 1 H, *J*_{7a,6} = 4.2 Hz, *J*_{7a,7b} = 19.7 Hz, H7a), 2.82 (dd, 1 H, *J*_{7b,6} = 5.3 Hz, *J*_{7a,7b} = 19.8 Hz, H7b), 2.69 (dd, 1 H, *J*_{2a,3} = 6.0 Hz, *J*_{2a,2b} = 15.8 Hz, H2a), 2.66 (dd, 1 H, *J*_{2a,2b} = 15.8 Hz, *J*_{2b,3} = 7.2 Hz, H2b), 1.56 (s, 3H, CH₃ isoprop), 1.36 (s, 3H, CH₃ isoprop); ¹³C NMR (80 MHz, CDCl₃): δ = 171.0 (C1), 114.9 (Cq isoprop), 84.5, 83.1, 82.7 (C4, C5, C6), 80.6 (C3), 52.0 (CH₃ OMe), 44.2 (C7), 38.4 (C2), 27.6 (CH₃ isoprop), 25.7 (CH₃ isoprop); R_f = 0.0 (silica gel, EtOAc/hexane, 1 : 1, vol/vol); HRMS (ESI) 246.1335, calculated for C₁₁H₁₉NO₅.H⁺: 246.1336.

Spectroscopic data for hydrazinocarbonyl 3,6-anhydro-2,7-dideoxy-7-[(4-

dimethoxymethyl-benzoyl)amino]-4,5-*O*-isopropylidene-*D*-*allo*-heptulonate **2**: ¹H NMR (200 MHz, CDCl₃): δ = 7.81 (bs, 1 H, CONHNH₂, NH), 7.62 (dd, 4 H, aromatic), 7.25 (bs, 1 H, CONH), 5.42 (s, 1 H, CH(OMe)₂), 4.57 (dd, 2 H, H₄, H₅), 4.18 (m, 4 H, H₃, H₆, NH₂), 3.70 (m, 2H, H_{7a}, H_{7b}), 3.32 (s, 6 H, CH₃ dimethoxy), 2.62 (dd, 1 H, *J*_{2,3} = 7.4 Hz, H_{2a}), 2.56 (dd, 1 H, *J*_{2,2'} = 15.9 Hz, *J*_{2,3} = 5.9 Hz, H_{2b}), 1.52 (s, 3 H, CH₃ isoprop), 1.31 (s, 3 H, CH₃ isoprop); ¹³C NMR (80 MHz, CDCl₃): δ = 170.8 (C₈), 168.1 (C₁), 128.9, 128.8, 126.0, 125.9 (C aromatic), 115.0 (C_q isoprop), 102.6 (C_q, aromatic), 84.3, 83.5, 82.6, 81.8 (C₃, C₄, C₅, C₆), 54.4 (CH₃ OMe), 42.1 (C₇), 38.2(C₂), 27.6 (CH₃ isoprop), 25.7 (CH₃ isoprop); R_f = 0.25 (silica gel, EtOAc/hexane, 1 : 1, vol/vol); HRMS (ESI) 446.1900, calculated for C₂₀H₂₉N₃O₇.Na⁺: 446.1898.

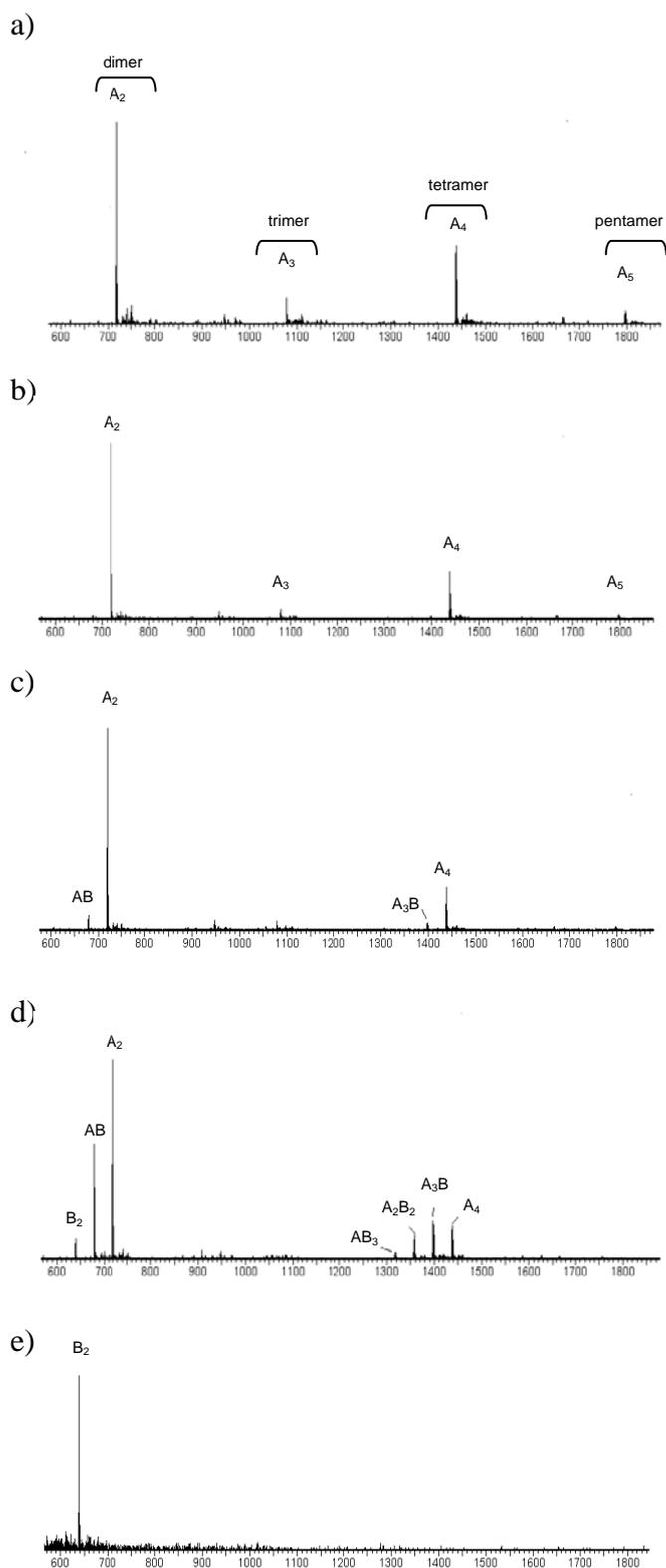
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Footnotes -

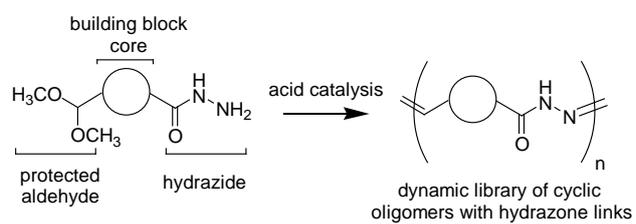
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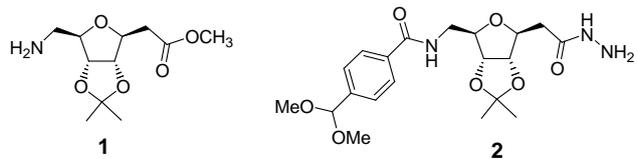
Figure 1 ESI MS spectra demonstrating a DCL of cyclic oligomers generated from hydrazone exchange of **2**. a) Catalysis with 5 μ L TFA after 6 h, b) 72 h. c) Catalysis with 25 μ L TFA after 1 h, d) 6 h, e) 72 h. **A** designates the 2,3-*O*-isopropylidene protected repeat unit while **B** designates the 2,3-*cis* diol repeat unit.



Scheme 1 Reversible hydrazone exchange for the synthesis of a DCL of cyclic hydrazone oligomers.⁶



Schematic of compounds 1 and 2



Scheme 2 Generation of a DCL of cyclic carbohydrate oligomers utilising hydrazone exchange. **A** designates the 2,3-*O*-isopropylidene protected repeat unit while **B** designates the 2,3-*cis* diol repeat unit. Reagents and conditions: TFA, H₂O/DMSO (1 : 4, vol/vol), rt§.

