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Synthesis and conformational analysis of novel water soluble macrocycles incorporating carbohydrates, including a β-cyclodextrin mimic

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Dedicated to Professor Amos B. Smith, III, on the occasion of his 60th birthday

Abstract—Rigid macrocyclic scaffolds based on carbohydrates have potential for the display of recognition groups with defined 3D structure and may have application in bioorganic and supramolecular chemistry. A series of water soluble macrocyclic structures containing two saccharide units was synthesised by ring closing metathesis of allyl and pentenyl glycosides derived from glucuronic acid. The 3D structure of the constrained systems was explored by NMR and computational methods. CD spectra were also recorded. On the basis of experimental observations we suggest that the carbohydrate presentation is constrained into a U-shape for the smaller ring size but can access an S-shape arrangement in the larger macrocycle. As an extension it is shown that the larger macrocycle displayed phenomena similar to β-cyclodextrin (β-CD). The binding of 8-anilino-1-naphthalenesulfonate (ANS) to β-CD is detectable by reversal of quenching of the ANS emission spectrum and a similar reversal of quenching was observed when this macrocycle was added to a solution of ANS; this was further supported by NMR. Furthermore molecular modelling suggests that the macrocyclic scaffolding has potential for the development of peptidomimetics.
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1. Introduction

Monosaccharides have been introduced and validated as biologically relevant scaffolds. They can mimic secondary structural features found in peptides[1] and have been useful as scaffolds for presentation of pharmacophore groups to receptors.[2] Advantages of using saccharides are that they display a high density of functional groups, are available as single enantiomers and contain multiple sites for attachment of recognition groups (multivalent or multifunctional scaffolds).[2b] Sugar amino acids have also been used as building blocks for synthesis of oligomeric or polymeric (carbopeptoid) structures[3] that have been of interest for studies of their secondary structural characteristics.[4] Once the principles governing the folding of such structures are understood the hydroxyl groups of such polymers could be further functionalised to create diverse structure and function. One of our interests has been the synthesis of systems containing two saccharide residues and the structural presentation of saccharides therein.[5] Herein we provide a full account of the synthesis and conformational analysis of novel macrocycles **2** containing two carbohydrate units. Macrocyclisation introduces conformational constraints to carbohydrate presentation. One macrocycle displays phenomena similar to cyclodextrin and the relationship of structure to the α-helical peptide backbone is discussed.

2. Results and discussion

2.1. Structure of glycocluster 1

Before proceeding to discuss the synthesis of macrocycles, it is useful to summarise what is known about the structure of glycocluster **1**, the synthesis and crystal structure of which has been described previously.[6] The amide nomenclature used is defined in Figure 1.

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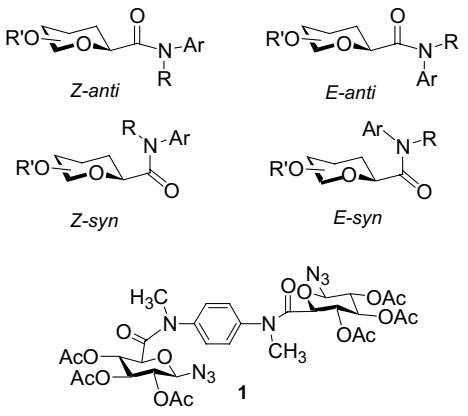


Figure 1. Amide nomenclature and glycocluster **1**.

In the solid state **1** adopted the folded structure **1a**, where each amide has different conformation: one is *E-anti* whereas the other is *E-syn* (Fig. 2).

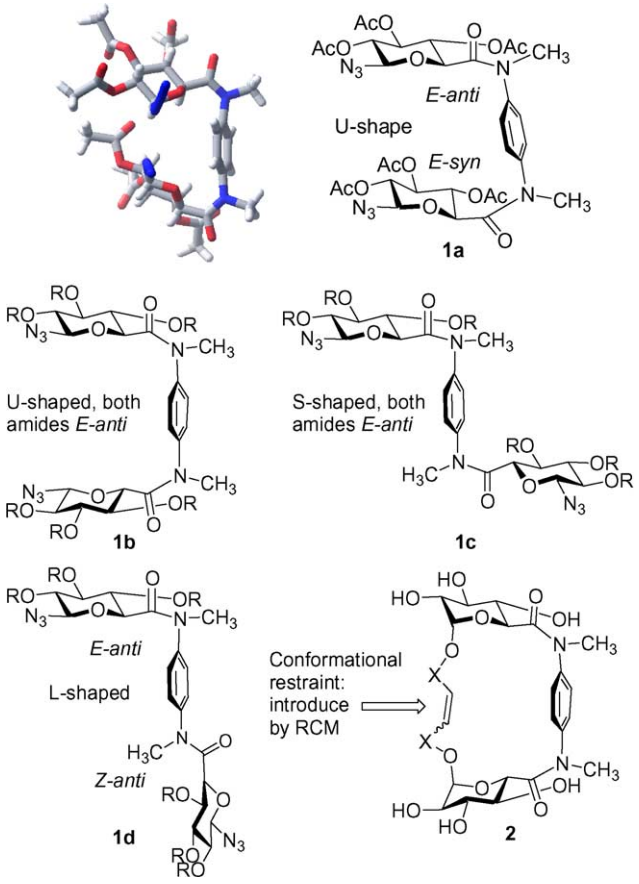
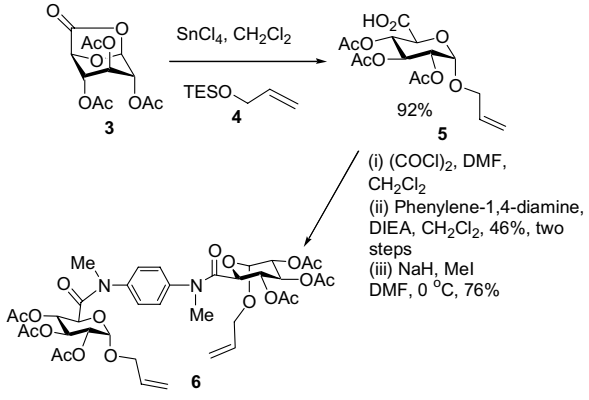


Figure 2. Crystal structure **1a** (top left) and structures **1** and **2**.

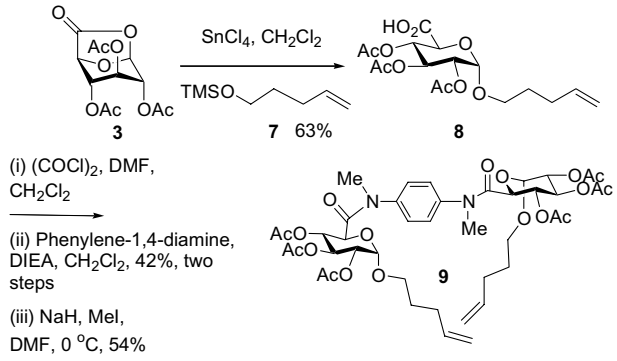
The observation of both *E-syn* and *E-anti* conformations in the crystal would indicate that two signal sets of equal intensity should be observed if the structure is stable in solution. However, this is not the case. The interconversion of *E*- and *Z*-configured amides is slow and the rotamers can be detected by ^{1}H NMR. There are multiple signal sets in the NMR spectrum of **1** and these are due to two slowly interconverting structural isomers; one, the major isomer, is C_2 symmetric, the minor isomer is not. The C_2 symmetric isomer can be explained if (i) both amides of **1** adopt the *E-anti*-conformation **1b** or **1c**; (ii) both amides adopt *E-syn*-conformation or (iii) *E-anti* to *E-syn* interconversion is dynamic but too rapid to be detected by NMR. Qualitative NOE data obtained for **1** in $CDCl_3$ indicates that the situation preferred is where the *E-anti* amides are adopted as strong NOE crosspeak is observed between H-5 of the sugar reside and aromatic protons but not between the methyl group and H-4 or H-5. An NOE enhancement between H-4 and aromatic proton would be expected if there was a significant population of *E-syn* in solution and it would be expected to give a stronger NOE than that between H-5 and the aromatic protons based on the distances measured in the solid state structure;[5] this NOE was not observed. The evidence suggests that the major structural isomer in $CDCl_3$ is the C_2-symmetric U-shaped **1b** and/or S-shaped **1c**. The NMR data for the minor structural isomer is explained by an isomer with the general features of the unsymmetric L-shaped **1d**, that is, it has one *E-anti* amide and one *Z*-configured amide. Monte-Carlo conformational searching techniques (Macromodel) found both S- and U-shape isomers **1b** and **1c** as low energy conformations. The fact that **1** adopts folded structures in the solid state and apparently also in solution, led us to design macrocyclic scaffolds **2** that could be prepared by metathesis. The idea was to constrain the carbohydrate presentation further and generate more rigid scaffolding. Some of the results described herein have been presented in a preliminary communication.[6]

2.2. Synthesis of metathesis substrates

Ring closing metathesis (RCM)[7] was expected for **6** and **9** because they have tertiary amides; the alkenes are preorganised for the ring forming reaction and this would lead to macrocycles.[6] The synthesis of these substrates was first carried out (Schemes 1 and 2). Glycosidation of the 6,1-anhydroglucopyranuronic acid (6,1-lactone) donor **3**[8] promoted by tin(IV) chloride gives α-glycosides with high stereoselectivity[9] and efficiency if an appropriate silyl ether is used as an acceptor.[10] The reaction of **3** with the TES[10] derivative **4** gave **5** in 92% yield.



Scheme 1. Synthesis of metathesis substrate **6**.



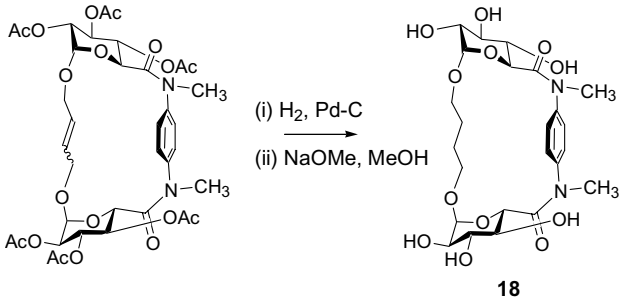
Scheme 2. Synthesis of metathesis substrate **9**.

The reason for using the TES ether in this case is because it is not as volatile as the TMS ether and therefore is easier to isolate and can be purified by distillation and can give better yields in these reactions. The acid **5** was then converted into its acid chloride and a subsequent coupling reaction with 1,4-phenylenediamine gave a secondary amide substrate, which was subsequently converted to **6** using sodium hydride and methyl iodide in DMF at 0 °C.

A similar sequence was used to prepare **9** from **3**. In this case the pentenyl glycoside **8** was prepared from the TMS ether **7**. The formation of the diamide **9** was accomplished as before by treating **8** and subsequent alkylation (24% for three steps) of the intermediate bis-amide gave **9** (Scheme 2).

2.3. Synthesis of macrocycles

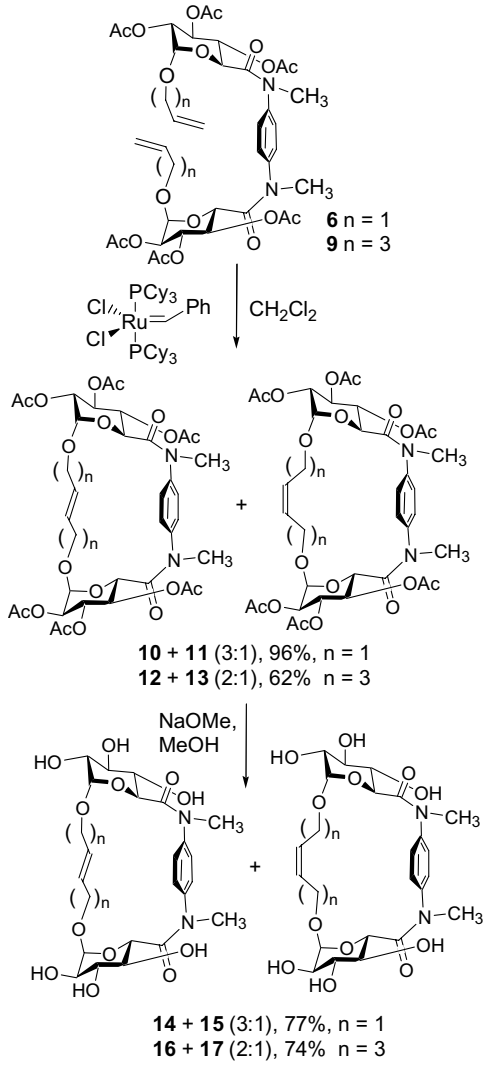
The reaction of the tertiary amide **6** in the presence of the Grubbs' catalyst gave a 3:1 mixture of the macrocycles **10** and **11** (Scheme 3). These compounds were converted to **14** and **15** by deacetylation. Catalytic hydrogenation of **14/15** followed by deprotection gave **18** (Scheme 4). Metathesis of **9** gave **12** and **13** and these were converted to **16** and **17** by removal of the protecting groups (Scheme 3). The alkene isomers could be separated by preparative reverse phase HPLC.



Scheme 4. Synthesis of **18**.

2.4. NMR and molecular modelling based structural analysis

Analysis of coupling constants in ^{1}H NMR spectra of **14**, **16** and **18** indicate the pyranoses have $^4C_1$ conformation and are not distorted. The spectrum recorded for **16** (Fig. 5, 5 °C, D_2O), as for **1**, showed two signal sets. There were signals, for example, at δ 4.17 (d, $J = 9.3$ Hz) and δ 5.08 (d, $J = 10$ Hz) for H-5 of **16a** and at δ 4.94 (br s) and δ 5.04 (d, $J = 4.2$ Hz) for H-1 of **16a** as well as signals at δ 4.40 (d, $J = 9.9$ Hz) for H-5 of **16b/c** and at δ 4.97 (d, $J = 3.9$ Hz) for H-1 of **16b/c**. The assignment assumes that interconversion of **16b** and **16c** is too rapid to be detected by ^{1}H NMR or that **16b** or **16c** is preferred. The **16a** to **16b/c** ratio can be determined by integration of signal in ^{1}H NMR spectra and found to be 15:85 at 25 °C indicating that **16b/c** is preferred. Evidence for interconversion of **16a** to **16b/c** can be obtained: there are crosspeaks of negative sign between the signals at δ 4.17, 4.40 and 5.08 in the phase sensitive 2D NOESY spectrum and these signals broaden and coalesce to a single signal at δ 4.37 at 65 °C. Similar phenomena were observed for other signals in the spectra. In contrast with **16** the ^{1}H NMR spectra of **14** (*E*-alkene), **15** (*Z*-alkene) and **18** showed only one signal set (see Section 4 for full details). This indicates that there is no access to an isomer related to **16a** where one amide has the *Z*-configuration.



Scheme 3. Synthesis of macrocycles.

However similar to **16** the evidence suggests a major structural isomer exists also for **14** and **18**, which is a C_2 symmetric macrocyle in each case. Monte-Carlo conformational searching protocols (SUMM method) available in Macromodel 8.5[11] were employed to search the conformational space accessible locating low energy structural isomers for **14**, **16** and **18**. These models were used to correlate observed crosspeaks in the 2D NOESY/ROESY spectra of the macrocyles (Figs. 3 and 4).

All calculations were carried out using the GB/SA solvation model[12] for water and the OPLS-AA force field[13] and only structural isomers within 20 kJ/mol of the lowest energy structure were retained. The lowest energy isomers retained for **14** were conformers where sugars had a U-shape. Isomers where the carbohydrates had an S-shape **14c** were calculated to be less stable than the lowest energy U-shaped isomer by 20 kJ/mol and the lowest energy L-shaped isomer was less stable than **14b** by ~70 kJ/mol. The SUMM method generated conformational isomers with S-shape **18c** within 20 kJ/mol of lowest energy U-shape **18b**. Similar calculations for **16** generated both **16b** and **16c** as well as isomers with the general features of **16a** (i.e., the isomers had one *E-anti* amide and one *Z-anti* amide). These calculations combined with the ^{1}H NMR discussed above and the NOESY/ROESY studies support the proposal that the amides in these macrocycles favour *E-anti* structure; significant NOE crosspeaks were observed between the glucuronic acid H-5 and aromatic protons in each case but not between the aromatic signals and H-4. The lack of crosspeaks observed between alkene protons and aromatic protons in ROESY of **14** suggests that **14b** is favoured. The lack of crosspeaks in ROESY of **18** between the two alkyl protons that appear at highest field (δ = 0.90–0.99 (m, 2H), 1.48–1.57 (m, 2H) in the ^{1}H NMR spectrum) and aromatic protons indicated that **18b** is favoured. If **14c** or **18c** was predominant we would have expected to see these NOEs as the alkene protons and alkyl protons would both have been within 3 Å according to the 3D models. The observation of an NOE crosspeak between alkene and aromatic protons for **16** is indicative that **16c** may be populated to a significant extent. For isomers **14** and **18** with *E-syn* and *Z*-configured amide arrangements are high energy structures and thus not likely to be easily accessible due to the constraints imposed by macrocyclisation and the short length of the hydrocarbon chain between the anomeric oxygen atoms. For **16** the longer chain facilitates access to *Z-anti* configured amide, and possibly also to the S-shaped isomer **16c** but not *E-syn* structures.

2.5. Circular dichroism studies on macrocyles and acyclic compounds

Circular dichroism (CD) is popular as an experimental tool for the study of conformational equilibria. We obtained CD spectra of the macrocycles and compared these spectra with acyclic **19** and **20**, prepared by deacetylation of intermediates described in Scheme 1. The NMR spectra for **19** shows similar pattern as **1** and **16** in that the major structural isomer is that which prefers both amides in *E-anti* orientation; there is also evidence for the presence of the minor *EZ*-isomer (~10%) related to **16a**. For **20** the *Z*-configured amides would be expected to predominate in solution and in this case there is only one signal set in the ^{1}H NMR spectrum. There

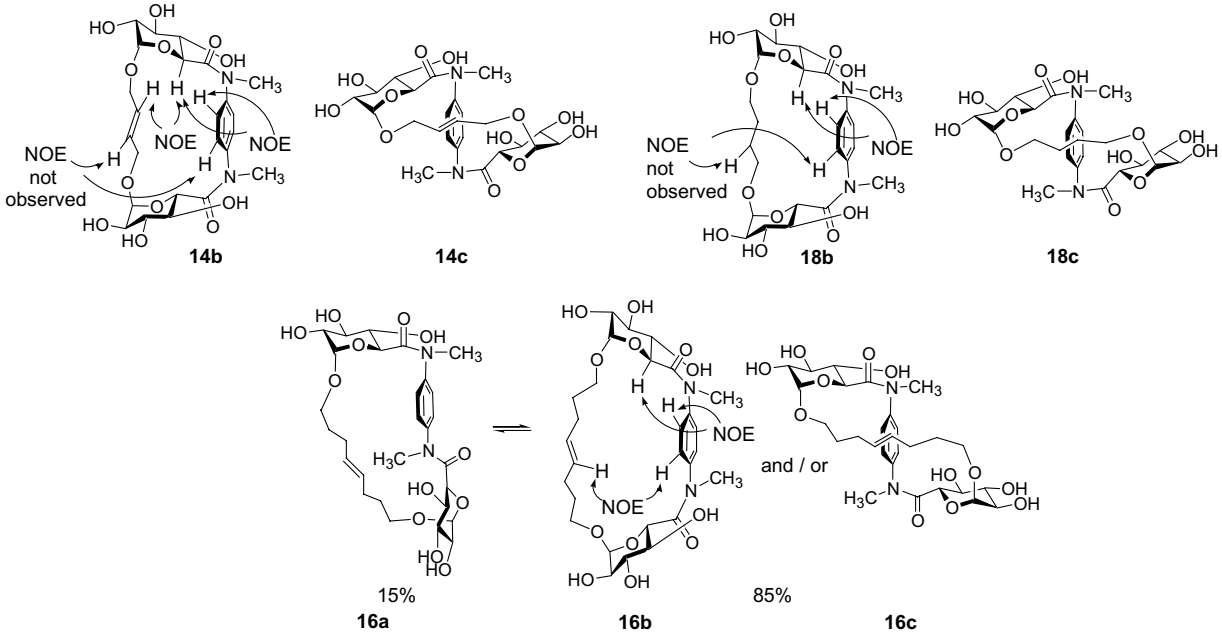


Figure 3. Structural isomers of carbohydrate containing macrocycles with possible L-shape **16a**, U-shape **14b**, **16b** and **18b** and S-shape **14c**, **16c** and **18c**. Selected NOE enhancements have been added. NOEs are not observed between alkene and aromatic protons for **14** indicating **14b** is favoured. NOEs are not observed between the high field alkyl and aromatic protons indicating **18b** is favoured. A significant NOE is observed between alkene and aromatic protons for **16** indicating that **16c** may be populated to a significant extent. See Figure 4 below for pictures of the 3D models.

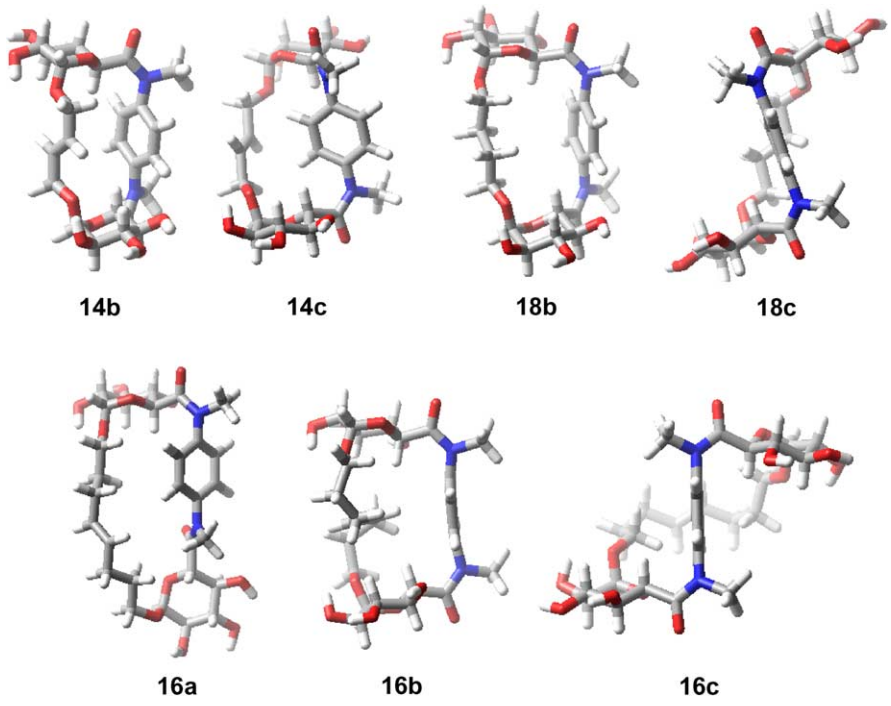


Figure 4. Structural models for macrocycles. These structures were generated by conformational searching techniques and correspond to local energy minima. Compound **14c** was calculated to be less stable than **14b** by 20 kJ/mol.

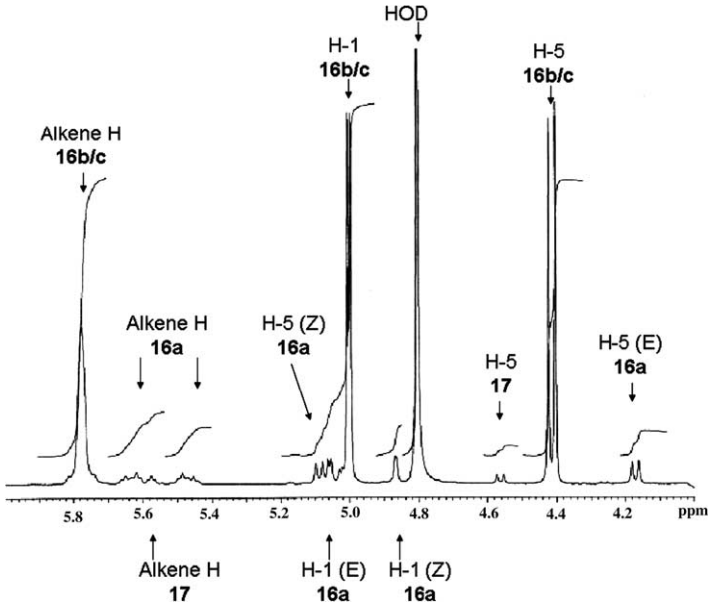


Figure 5. ^{1}H NMR spectrum for **16** containing a minor amount of isomer **17**.

would be a substantially different presentation of carbohydrates than for **19** and for the macrocycles.

The CD spectra for these compounds were recorded in water and are shown in Figure 6. Both **14** and **18** have similar spectra. They show positive maxima at 191.5 and 214.5 nm and 191.5 and 215.4 nm, respectively as well as negative maxima of significantly higher magnitude at 243.5 and 245 nm, respectively. In contrast the spectrum of **16** shows a significant positive maximum at 240 nm and two negative maxima at 189.5 and 209.5 nm. The acyclic compound **19** shows a positive maximum at 240 nm whereas 20 shows a negative maximum at 183.5 nm; no significant differential dichroic absorption was observed above 200 nm for either of these structures. Tentatively, we suggest that the difference observed arise from **14** and **18** adopting the U-shape whereas **16** adopts the S-shape preferentially. Acyclic **19** may adopt both U-shape and S-conformations and the differential dichroic absorption for each

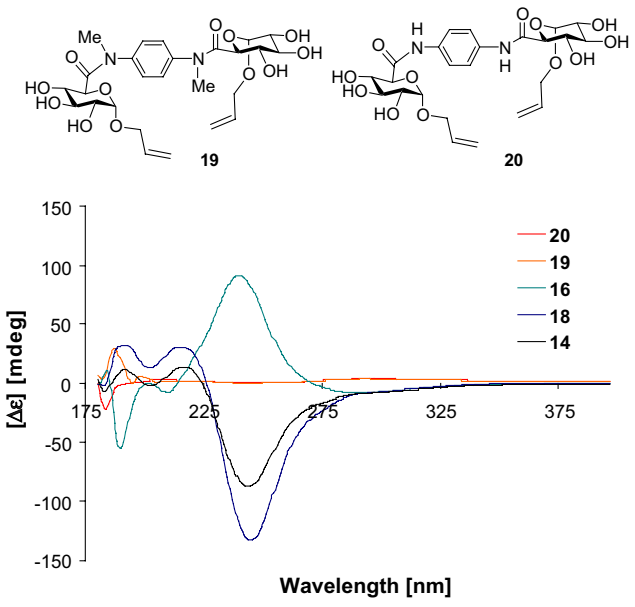


Figure 6. CD spectra for macrocycles and acyclic compounds.

conformational isomer may be cancelled out at regions above 190 nm. More studies would be required to prove that this is the case, however this seems to be a reasonable hypothesis.

2.6. Binding studies with ANS

β-Cyclodextrin is water soluble, can bind molecules in its hydrophobic cavity and has found widespread application in supramolecular chemistry, including its use as a template for development of bilayer vesicles.[14] Synthetic variants are of interest because of difficulty in synthetic modification of cyclodextrins[15] and we considered if the macrocycles described herein, which are water soluble, would display any phenomena related to β-CD. The binding of 8-anilino-1-naphthalenesulfonate (ANS) to β-cyclodextrin (β-CD) is detectable by changes to the fluorescence of the dye. In water the fluorescence emission of ANS is quenched showing an emission maximum at 528 nm;[16] in the presence of excess β-CD there is a 4-fold increase in intensity in fluorescence emission and a blue shift to 517 nm in the emission maximum due to binding in the hydrophobic environment (Fig. 7).[17] The addition of excess of **16** caused a slight blue shift in the emission maximum (518 nm) and a 3-fold increase in emission intensity. In contrast with **16**, the macrocycles **14/15** had no effect on the ANS emission spectrum. This suggests that ANS associates with the hydrophobic environment of **16**.

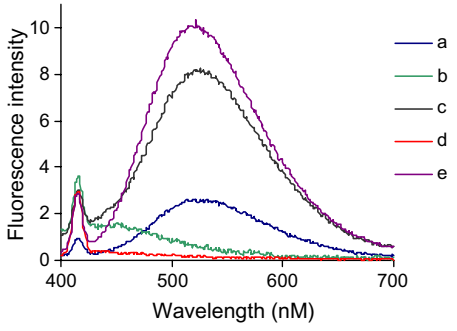


Figure 7. Fluorescence emission spectra in 0.1 M sodium phosphate buffer (λ_{ex} = 365 nm) of (a) ANS; (b) **16** (2:1); (c) ANS in presence of **16**; (d) β-CD; (e) ANS in presence of β-CD. Concentrations were ANS, 2.34×10^{-5} M; **16**, 3.2×10^{-4} M; β-CD, 2.8×10^{-4} M.

Furthermore the binding of **16** to ANS was detected by ^{1}H NMR (D_2O). The signals for protons of the macrocycle were shifted upfield and/or significantly broadened on the addition of ANS (see Fig. 8 for changes to signal to alkene proton of **16b/c**). In addition, the serial dilution of a mixture of **16** (0.053–0.0033 M) and ANS (0.065–0.004 M) led to a linear concentration dependant downfield shift of signals. The signal for alkene proton of **16b/c**, for example, shifted from δ 5.24 to δ 5.77. No change in the chemical shift of this signal (δ 5.78) was observed in the absence of ANS over the same concentration range (Fig. 9). These results suggest addition of the ANS may promote aggregation of **16** rather than including ANS into the cavity. The precise nature of the complex formed between **16** and ANS is unclear. There is no evidence to indicate that **16** self-associates, in absence of ANS, at the concentrations examined as there is no change to the chemical shift of the alkene proton on dilution of solutions of **16** alone.

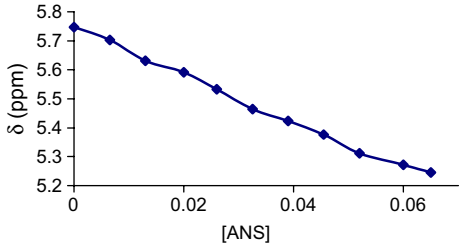


Figure 8. Chemical shift of the signal of alkene proton of **16** (0.053 M) in the presence of ANS.

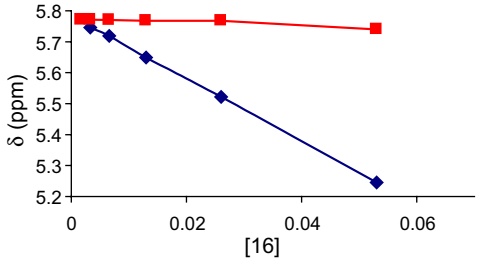


Figure 9. Variation of the chemical shift (δ) of the alkene proton of **16** upon dilution in absence (red) and presence (blue) of ANS.

2.7. Potential of macrocycles scaffolds for α-helical peptidomimetic development

We have examined, by molecular modelling, the structural relationship of **14** to the α-helix peptide backbone in order to determine if there is potential for peptidomimetic design.[18] The preliminary molecular modelling (Fig. 10) study indicated that it may be possible to use the O-2 and O-4 atoms of the saccharides and the nitro-

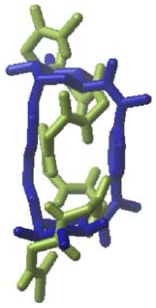


Figure 10. The superimposition of **14** (blue, hydrogen atoms not shown) and the backbone of an α-helical peptide (green, polar hydrogens only shown). The following atom pairs were superimposed to generate the overlapped structures: C_α of residue *i* and glucuronic acid O-2; C_α of residue *i* + 1 and glucuronic acid O-4; C_α of residue *i* + 2 and nitrogen; C_α of residue *i* + 5 and nitrogen; C_α of residue *i* + 6 and glucuronic acid O-4; C_α of residue *i* + 7 and glucuronic acid O-2.

gen atoms of the phenylenediamine unit to project side-chains of amino acids in orientations that may correspond to those projected from the C_α of residues *i*, *i* + 1, *i* + 2, *i* + 5, *i* + 6 and *i* + 7 of a helical peptide backbone.[19] By incorporating substitution on the aromatic ring or alkene of **14** it may also be possible to project amino acids to mimic side chains from C_α of residues *i* + 3 and *i* + 4.

3. Conclusion

A series of water soluble macrocyclic structures containing two saccharide units has been synthesised by ring closing metathesis. The 3D structure of these systems was explored using NMR, circular dichroism and computational methods. The results suggest that the carbohydrate presentation within the macrocycle may diverge depending on macrocycle size. Furthermore the larger macrocycle **16** derived from pentenyl glycosides showed switching phenomena similar to β-cyclodextrin. In addition the macrocycles can form a basis for efforts to develop novel peptidomimetics. Naturally occurring macrocyclic glycolipids[20] with antiviral and anti-cancer properties are known indicating macrocycles such as those described herein may be worth evaluating for their biological activity. This research is underway.

4. Experimental

4.1. General

Optical rotations were determined with a Perkin–Elmer 343 model polarimeter at the sodium D line at 23 °C. IR spectra were recorded with a Mattson Galaxy Series FTIR 3000 using either thin film between NaCl plates or KBr discs, as specified. Optical rotations were measured in a Perkin–Elmer 343 polarimeter. Low and high-resolution mass spectra were recorded on a Micromass LCT KC420 or Micromass Quattro. TLC was performed on aluminium sheets precoated with Silica Gel 60 (HF254, E. Merck) and spots visualised by UV and charring with 1:20 H_2SO_4–EtOH. Flash column chromatography was carried out with Silica Gel 60 (0.040–0.630 mm, E. Merck) and employed a stepwise solvent polarity gradient correlated with the TLC mobility. Chromatography solvents used were MeOH, dichloromethane (Riedel-de Haen), EtOAc (Fluka). Reaction solvents were dried and distilled before use when indicated.

4.2. NMR experiments

^1H and ^{13}C NMR spectra (75 or 125 MHz) were recorded with Varian 300 and 500 spectrometers. All ^{13}C spectra given are proton decoupled. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0) or HOD for D_2O (δ 4.79) for ^1H and (δ 77.16) for ^{13}C. ^{13}C signals were assigned with the aid of DEPT-135. ^1H signals were assigned with the aid of 2D-COSY. Coupling constants are reported in hertz. All spectra of macrocycles were recorded in D_2O at 303 K with a pulsed field gradient accessory. Standard gradient COSY spectra were recorded. The use of presaturation for solvent suppression was carried out for ROESY of **18**. NOESY (τ_{mix} = 600 ms) and ROESY spectra (τ_{mix} = 300 ms) were recorded as 2D-crossrelaxation experiments with solvent suppression by transmitter presaturation. All spectra were recorded in phase-sensitive mode, by using States-TPPI for F1-quadrature detection in the indirect dimension. Homonuclear coupling constants were determined from the corresponding ^1H spectra. Concentrations of macrocycles for NOESY and/or ROESY: **14**, 1.9 mg in 0.7 mL D_2O; **18**, 5 mg in 0.7 mL D_2O; **16**, 6 mg in 0.7 mL D_2O.

4.3. CD measurements

Circular-dichroism spectra of compounds **14**, **18**, **16a**, **19** and **20** were recorded a JASCO instrument in water in a cell of 1 cm path length at 25 °C. The sample solutions were prepared by dissolving the required amount of each compound in water to obtain the UV spectra of absorption intensity of 1.0 ± 0.05. This approximated to concentrations of $\sim 2.5 \times 10^{-4}$ M.

4.4. Allyloxytriethylsilane 4

Chlorotriethylsilane (4 mL, 24.2 mmol) was added slowly to a solution of allyl alcohol (1.5 mL, 22 mmol) and NEt_3 (3.7 mL, 26.4 mmol) in dry dichloromethane (15 mL) under N_2 and cooled on an ice bath. The resulting suspension was allowed to stir on the ice bath for further 15 min and then reach rt and stir overnight. The salts formed were removed by filtration washing with dichloromethane (20 mL) and the filtrate was washed with satd $NaHCO_3$ solution and water. The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to give a colourless oil that was purified by distillation under reduced pressure (water pump, ~20 mbar, bp = 100–103 °C) to give the title compound **4** (2.85 g, 76%) as a colourless oil; ^1H NMR ($CDCl_3$, 300 MHz): δ = 0.62–0.66 [q, *J* = 3.5 Hz, 6H, $(CH_3CH_2)_3Si$], 0.94–0.98 [t, *J* = 3.5 Hz, 9H, $(CH_3CH_2)_3Si$], 4.16–4.18 (m, 2H, CH_2O), 5.06–5.31 (m, 2H, $=CH_2$), 5.87–5.99 (m, 1H, $=CH$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 4.5 [t,

($CH_3CH_2)_3Si$], 6.8 [q, ($CH_3CH_2)_3Si$], 63.9 (t, CH_2O), 114.2 (t, CH_2), 137.5 (d, CH); IR (film from dichloromethane): 2956, 2912, 2877, 2359, 1458, 1417 cm^{-1}.

4.5. Allyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosiduronic acid 5

Tin(IV) chloride (115 μL, 1 mmol) was added to a solution of 1,6-lactone **4** (300 mg, 1 mmol) and allyloxytrimethylsilane **5** (550 mg, 3.2 mmol) in dry dichloromethane (10 mL) under N_2 and at rt. The reaction mixture was allowed to stir at rt overnight and then aq satd $NaHCO_3$ (20 mL) was added and the mixture was stirred for 1 h. The slurry formed was filtered through Celite (washing with dichloromethane and aq $NaHCO_3$). The two phases of the filtrate were separated and the aqueous phase was acidified with 1 M HCl to pH ~2 and then extracted with EtOAc (5 × 15 mL). The combined extracts were dried ($MgSO_4$), filtered and evaporated to give **5** (330 mg, 92%) as a white foam; $[\alpha]_D$ = +141.9 (*c* 0.565, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ = 2.03, 2.04, 2.07 (each s, each 3H, $OCOCH_3$), 4.03–4.28 (m, 2H, $CHCH_2O$) 4.38–4.41 (d, *J* = 10 Hz, 1H, *H*-5), 4.89–4.94 (dd, *J* = 3.5 Hz, *J* = 7.1 Hz, 1H; *H*-2) 5.15–5.37 (m, 4H, *H*-4, *H*-1, CH_2=CH), 5.52–5.59 (t, *J* = 10 Hz, 1H, *H*-3), 5.81–5.94 (m, 1H, CH), 6.59 (br s, 1H, CO_2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.6, 20.7 (q, $COCH_3$), 67.8, (d, CH), 69.3 (t, CH_2O), 69.4, 69.5, 70.4, 95.0 (each d, CH), 118.5 (t, CH_2=CH), 132.8 (d, CH), 170.1, 170.2, 170.3 (each s, $COCH_3$), 171.3 (s, CO_2H); HRMS-ES: $[M+Na]^+$ Calcd 383.0954. Found 383.0971; IR (film from dichloromethane): 3533, 2662, 2360, 1754, 1429, 1371 cm^{-1}.

4.6. *N*,*N*-1,4-Di-(2,3,4-tri-*O*-acetyl-1-*O*-allyl-α-D-*N*-methyl-glucuronamide)benzene 6

To a solution of the acid **5** (65 mg, 0.18 mmol) in dry dichloromethane (2 mL) and under N_2 dry DMF (20 μL) was added. The solution was cooled on ice and oxalyl chloride (20 μL, 0.24 mmol) was added and an evolution of gas was observed. The reaction mixture was allowed to stir on the ice bath for 15 min and then it was removed and allowed to stir at rt for 1 h. A solution of 1,4-phenylenediamine (9 mg, 0.08 mmol) and dry DIEA (40 μL, 0.22 mmol) in dry dichloromethane (1.5 mL) was then cannulated into the reaction mixture. Evolution of gas was again observed and it was allowed to stir at rt overnight. The reaction mixture was diluted with further dichloromethane (5 mL) and washed with HCl 0.1 M (5 mL) and $NaHCO_3$ satd solution (5 mL). The basic aq phase was extracted further with dichloromethane (5 mL) and the combined extracts were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to give a mixture of products that was purified by column chromatography (dichloromethane/MeOH 100:2) and gave the intermediate bis-amide (25 mg, 35%) as a white solid; R_f = 0.5 (dichloromethane–MeOH 5%); $[\alpha]_D$ = +115.8 (*c* 0.91, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ = 2.03 (s, 6H, $OCOCH_3$), 2.09 (s, 12H, $OCOCH_3$), 4.07–4.28 (m, 4H, $CHCH_2O$) 4.33–4.36 (d, *J* = 10 Hz, 2H, *H*-5), 4.85–4.89 (dd, *J* = 3.5 Hz, *J* = 11 Hz, 2H, *H*-2), 5.14–5.21 (t, *J* = 10 Hz, 2H, *H*-4), 5.25–5.37 (m, 6H, *H*-1, CH_2=), 5.58–5.65 (t, *J* = 10 Hz, 2H, *H*-3), 5.81–5.94 (m, 2H, C=CH), 7.46 (s, 4H; ArH), 8.12 (s, 2H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 19.6, 19.7 (q, $COCH_3$), 67.5 (d), 68.2 (t, CH_2O), 68.4, 68.8, 69.7, 93.9 (each d), 117.6 (d, =CH_2), 120.0 (d, ArCH), 131.7 (s, ArC), 132.5 (d, CH), 164.1 (s, CONH), 168.8, 168.9, 169.3 (each s, $COCH_3$); HRMS: $[M+H]^+$ Calcd 793.2667. Found 793.2689; IR (film in dichloromethane): 3290, 2927, 2359, 1753, 1689, 1519 cm^{-1}. To a solution of bisamide intermediate (50 mg, 0.063 mmol) in dry DMF cooled on ice and under N_2, MeI (25 μL, 0.378 mmol) was added followed by NaH (60% in mineral oil, 15 mg, 0.378 mmol). The reaction mixture turned bright yellow and after 15 min was diluted with dichloromethane (10 mL) and washed with a NH_4OH solution (10 mL). The aq phase was extracted with dichloromethane (10 mL) and the combined organic layers dried ($MgSO_4$), filtered and the solvent removed under reduced pressure (co-evaporating with toluene to remove DMF) to give an oil. Column chromatography (eluant EtOAc–dichloromethane 4:1) gave **6** (38 mg, 73%) as a white solid; R_f = 0.45 (EtOAc–dichloromethane 4:1); $[\alpha]_D$ = +82.6 (*c* 1.18, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ = 2.04–2.01 (m, 18H, $OCOCH_3$), 3.31 (s, 6H, NCH_3), 3.46–3.76 (m, 4H, $CHCH_2O$), 4.42–4.45 (d, *J* = 9.6 Hz, 2H, *H*-5), 4.83–4.87 (dd, *J* = 3 Hz, *J* = 11 Hz, 2H, *H*-2), 4.99 (br s, 2H, *H*-1), 5.12–5.18 (m, 4H, =CH_2), 5.31–5.38 (t, *J* = 11.6 Hz, 2H, *H*-3), 5.44–5.60 (m, 4H, =CH, *H*-4), 7.34 (s, 4H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.6, 20.7, 2.10 (each q, $COCH_3$), 38.4 (q, NCH_3), 65.9 (d), 68.8 (t), 69.8, 70.2, 70.4, 95.3 (each d), 118.3 (t), 129.1, 132.5 (each d), 142.6 (s, ArC), 166.3 (s, $CONCH_3$), 168.7, 169.9, 170.3 (s, $COCH_3$); HRMS-ES: $[M+H]^+$ Calcd 821.2980. Found 821.3015; IR (film from dichloromethane): 3604, 3454, 2935, 2360, 2341, 1755, 1668, 1510 cm^{-1}.

4.7. 4-Pentenoxytrimethylsilane 7

Chlorotrimethysilane (5.6 mL, 44.1 mmol) was added slowly to a solution of 4-penten-1-ol (4 mL, 38.73 mmol) and NEt_3 (8 mL, 57.4 mmol) in dry dichloromethane (20 mL) under N_2 and cooled on an ice bath. The resulting suspension was stirred on an ice bath for further 15 min and then allowed to reach rt and stirred overnight. The salts formed were removed by filtration, washing with dichloromethane (20 mL) and the filtrate was washed with satd $NaHCO_3$ and water. The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure (without heating) to give a dark oil that was purified by distillation under reduced pressure (water pump, ≃20 mbar, bp = 49–51 °C) to give **7** (4.08 g, 67%) as a colourless oil; ^1H NMR ($CDCl_3$, 300 MHz): δ = 0.01 [s, 9H, $(CH_3)_3Si$], 1.47–1.56 (m, 2H, CH_2CH_2CH=), 1.65–2.00 (m, 2H, $CH_2CH_2CH_2$), 3.45–3.50 (t, *J* = 6.5 Hz, 2H, CH_2CH_2OTMS), 4.81–4.95 (m, 2H, =CH_2), 5.64–5.77 (m, 1H, =CH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 0.0 [q, $(CH_3)_3Si$], 30.5, 32.3 (t), 62.5 (t, CH_2O), 115.0 (t), 138.9 (d).

4.8. 2,3,4-Tri-*O*-acetyl-1-*O*-4-pentenyl-α-D-glucuronic acid 8

Tin(IV) chloride (200 μL, 1.9 mmol) was added to a solution of **3** (600 mg, 2 mmol) and 4-pentenoxytrimethylsilane **7** (1.024 g, 6.5 mmol) in dry dichloromethane (20 mL) under N_2 and at rt. The reaction mixture was stirred at rt overnight. Then satd $NaHCO_3$ (20 mL) was added and the mixture stirred for 1 h. The slurry formed was filtered through Celite, washing with dichloromethane and satd $NaHCO_3$. The two phases of the filtrate were separated and the aqueous phase acidified with HCl (1 N) to pH 2 and then extracted with EtOAc (5 × 15 mL). The combined extracts were dried ($MgSO_4$), filtered and evaporated and dried at high vacuum to give **8** (486 mg, 63%) as an off white solid; $[\alpha]_D$ = +68.7 (*c* 0.146, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.65–1.77 (m, 2H, CH_2CH_2CH=), 2.03, 2.04, 2.07 (each s, each 3H, $OCOCH_3$), 2.09–2.16 (m, 2H, $CH_2CH_2CH_2$), 3.43–3.80 (m, 2H, CH_2CH_2O) 4.34–4.37 (d, *J* = 10 Hz, 1H, *H*-5), 4.88–4.90 (dd, *J* = 3.5, 10 Hz, 1H, *H*-2), 4.98–5.28 (m, 4H, *H*-4 and *H*-1 and CH_2=), 5.50–5.66 (t, *J* = 9.7 Hz, 1H, *H*-3), 5.74–5.87 (m, 1H, CH=), 6.22 (br s, 1H, CO_2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.6, 20.7, 20.7 (each t, $COCH_3$), 28.4, 30.0 (t), 68.4 (d), 69.6 (t, CH_2O), 70.6, 95.9 (d), 115.3 (t), 137.7 (d), 170.2, 170.3, 170.4 (each s, $COCH_3$); HRMS-ES: $[M-H]^-$ Calcd 387.1291. Found 387.1281; IR (film from dichloromethane): 3465, 2941, 1750, 1371, 1234, 1050 cm^{-1}.

4.9. *N*,*N*-1,4-Di-(2,3,4-*O*-acetyl-1-*O*-4-pentenyl-α-D-*N*-methyl-glucuronamide)benzene 9

To a solution of the acid **8** (489 mg, 1.27 mmol) in dry dichloromethane (15 mL) and under N_2 dry DMF (25 μL) was added. The solution was cooled on an ice bath and when oxalyl chloride (142 μL, 1.63 mmol) was added evolution of gas was observed. The reaction mixture was allowed to stir on the ice bath for 15 min and then it was removed and allowed to stir at rt for 1 h. A solution of 1,4-phenylenediamine (60 mg, 0.58 mmol) and dry DIEA (290 μL, 1.64 mmol) in dry dichloromethane (6 mL) was then cannulated into the reaction mixture. Evolution of gas was again observed and it was allowed to stir at rt overnight. The reaction mixture was diluted with further dichloromethane (15 mL) and washed with HCl 0.1 N (20 mL) and satd $NaHCO_3$ (20 mL). The basic aqueous phase was extracted further with dichloromethane (3 × 10 mL) and the combined extracts were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to give a mixture of products that was purified by column chromatography (dichloromethane/MeOH 100:2). The required bisamide intermediate (160 mg, 33%) was obtained as a white solid: R_f = 0.65 (dichloromethane/MeOH 5%); $[\alpha]_D$ = +98.0 (*c* 3.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.68–1.78 (m, 4H, CH_2CH_2CH=), 2.04, 2.09, 2.10 (each s, each 6H, $OCOCH_3$), 2.14–2.19 (m, 4H, $CH_2CH_2CH_2$), 3.44–3.80 (m, 4H, CH_2CH_2O) 4.30–4.33 (d, *J* = 10 Hz, 2H, *H*-5), 4.82–4.87 (dd, *J* = 3.5 Hz, *J* = 10 Hz, 2 H, *H*-2), 4.98–5.23 (m, 8H, *H*-4 and *H*-1 and CH_2=), 5.57–5.72 (t, *J* = 10 Hz, 2H, *H*-3), 5.75–5.86 (m, 2H, CH=), 7.45 (s, 4H, Ar*H*), 8.07 (s, 2H, N*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.7, 20.7, 20.8 (each q, $COCH_3$), 28.4, 30.0 (t), 68.5 (d), 68.6 (t, CH_2O), 69.3, 69.9, 70.6, 95.9 (each d), 115.4 (t), 121.0 (d, ArCH), 133.5 (s, ArC), 137.5 (d), 165.2 (s, CONH), 169.8, 169.9, 170.4 (s, $COCH_3$); HRMS-ES: $[M+H]^+$ Calcd 849.3293. Found 849.3325; IR (film from dichloromethane): 3313, 3078, 2941, 2360, 1755, 1691, 1520, 1370 cm^{-1}. To a solution of this bis-amide (300 mg, 0.366 mmol) in dry DMF cooled on an ice bath and under N_2, MeI (0.14 mL, 2.14 mmol) followed by NaH (60% in mineral oil, 90 mg, 0.378 mmol) was added. The reaction mixture turned bright yellow and the completion was monitored by TLC. After 20 min, the reaction mixture was diluted with dichloromethane (20 mL) and washed with aq NH_4OH (15 mL). The aqueous phase was extracted further with dichloromethane (15 mL) and the combined organic layers dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure (coevaporating with toluene to remove DMF) to give an oil that after chromatography (eluent ethyl acetate/dichloromethane 2:1) gave **9** (172 mg, 54%) as a white solid. R_f = 0.8 (ethyl acetate/dichloromethane 4:1); $[\alpha]_D$ = +67.5 (*c* 0.41 $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.33–1.42 (m, 4H, CH_2CH_2CH), 1.98–2.06 (m, 22H, $CH_2CH_2CH_2$ and $OCOCH_3$), 3.15–3.32 (m, 10H, CH_2CH_2O and NCH_3), 4.38–4.41 (d, *J* = 9.6 Hz, 2H; *H*-5), 4.81–4.84 (dd, *J* = 3 Hz, *J* = 10.2 Hz, 2H, *H*-2), 4.96–4.99 (m, 6H; *H*-1 and =CH_2), 5.29–5.33 (t, *J* = 9.6 Hz, 2H, *H*-3), 5.49–5.55 (t, *J* = 9.6 Hz, 2H, *H*-4), 5.65–5.74 (m, 2H, =CH–), 7.31 (s, 4H, Ar*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.6, 20.8, 20.9 (each q, $COCH_3$), 28.4, 29.7 (t), 38.4 (q, NCH_3), 65.8 (d), 68.1 (t, CH_2O), 69.8, 70.4, 70.5, 96.3 (each d), 115.6 (t), 129.0 (d, ArCH), 137.2 (d), 145.3 (s, ArC), 166.4 (s, CONH), 168.7, 170.0, 170.6 (each s, $COCH_3$); HRMS-ES: $[M+H]^+$ Calcd 877.3606. Found 877.3621; IR (film from dichloromethane): 3489, 2828, 1755, 1669, 1247, 1225 cm^{-1}.

4.10. Metathesis of 6

A degassed solution of **6** (36 mg, 0.043 mmol) in dry dichloromethane (20 mL, 2 mM) and under argon was treated with metathesis catalyst (9 mg, 25%) for 40 h at rt. The solvent was evaporated to give a black residue that was purified by column chromatography (eluant dichloromethane–EtOAc 1:4) and gave a mixture (ratio 1:3) of **10** and **11** (33 mg, 96%); HRMS-ES: $[M+H]^+$ Calcd 793.2667. Found 793.2700. The mixture of isomers was partially separated by HPLC (eluant H_2O–MeCN, 35:65, flow rate 10 mL/min, C4-reverse phase preparative column).

Analytical data for **10**: R_f = 0.35 (dichloromethane–EtOAc 1:4); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.03, 2.04, 2.10 (each s, each 3H, $OCOCH_3$), 3.30 (s, 6H, NCH_3), 3.82–3.92 (m, 4H, $CHCH_2O$), 4.77 (d, *J* = 9.6 Hz, 2H, *H*-5), 5.00–5.04 (dd, *J* = 3.3 Hz, *J* = 10 Hz, 2H, *H*-2), 5.13 (d, *J* = 3.6 Hz, 2H, *H*-1), 5.44–5.51 (t, *J* = 10 Hz, 2H, *H*-3), 5.57 (s, 2H, =CH), 5.61–5.68 (t, *J* = 9.6 Hz, 2H, *H*-4), 7.28 (s, 4H, Ar*H*);

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.6, 20.7, 2.08 (each q, CO*C*H$_3$), 39.5 (q, N*C*H$_3$), 64.7 (d), 65.2 (t), 70.1, 70.3, 70.8, 94.8 (each d), 123.9 (d, Ar*C*H), 128.0 (d, *C*H=), 142.2 (s, Ar*C*), 166.5 (s, *C*ONCH$_3$), 168.4, 170.0, 170.5 (each s, *C*OCH$_3$).

Analytical data for **11**: R_f = 0.35 (dichloromethane–EtOAc 1:4); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.02, 2.03, 2.07 (each s, each 3H, OCOC*H*$_3$), 3.32 (s, 6H, NC*H*$_3$), 3.49–3.55 (m, 2H, CHC*H*$_2$O), 4.05–4.09 (m, 2H, CHC*H*$_2$O), 4.77 (d, J = 9.6 Hz, 2H, *H*-5), 4.88–4.93 (dd, J = 3.3, 12.9 Hz, 2H, *H*-2), 5.03 (d, J = 3.6 Hz, 2H, *H*-1), 5.38–5.48 (t, J = 9.6 Hz, 2H, *H*-3), 5.48–5.51 (m, 2H, =CH), 5.52–5.56 (t, J = 9.3 Hz, 2H, *H*-4), 7.37 (s, 4H, Ar*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.6, 20.7 (each q, CO*C*H$_3$), 39.3 (q, N*C*H$_3$), 64.5 (d, CH), 65.9 (t, *C*H$_2$O), 69.7, 70.6, 97.5 (each d), 127.9 (d, *C*H=), 128.3 (d, Ar*C*H), 142.1 (s, Ar*C*), 166.4 (s, *C*ONCH$_3$), 168.7, 169.9, 170.3 (each s, *C*OCH$_3$).

4.11. Macrocycles 14 and 15

Sodium methoxide from a 1 M solution freshly prepared (0.1 mL) was added to a mixture of **10** and **11** (48 mg, 0.06 mmol) in methanol (7 mL) that was precooled on ice. The reaction mixture was allowed to stir at 0 °C for 30 min. The solvent was evaporated under reduced pressure to give a yellow solid that was dissolved in water and acidified to pH 6 by addition of Amberlite (H^+). The resin was removed by filtration and the filtrate was freeze-dried to give a white solid corresponding to a mixture of title compounds (25 mg, 77%); HRMS-ES: $[M+H]^+$ Calcd 541.2034. Found 541.2056. The mixture was partially separated by HPLC (eluant water/acetonitrile 85:15, flow rate 3 mL/min, C4-reverse phase preparative column).

Analytical data for **14**: $[\alpha]_D$ = −77 (*c* 0.2, methanol); ^{1}H NMR (D_2O, 300 MHz): δ = 3.18 (s, 6H, NC*H*$_3$), 3.56–3.60 (dd, J = 3, 9.6 Hz, 2H, *H*-2), 3.70–3.84 (m, 8H, *H*-3, *H*-4, CHC*H*$_2$O), 4.43 (d, J = 9.3 Hz, 2H, *H*-5), 4.86 (d, J = 3.3 Hz, 2H, *H*-1), 5.50 (s, 2H, =C*H*), 7.40 (s, 4H, Ar*H*); ^{13}C NMR (D_2O, 125 MHz): δ = 42.1 (q, NCH$_3$), 66.8 (t), 70.5, 74.0, 74.8, 75.3, 99.5, 125.6, 131.5 (each d), 144.7 (s, Ar*C*), 172.8 (s, *C*O); IR (KBr): 3454, 3340, 2929, 2361, 1640, 1510, 1451, 1400 cm^{-1}.

Analytical data for **15**: ^{1}H NMR (D_2O, 300 MHz): δ = 3.22–3.29 (m, 8H, NC*H*$_3$ and CHC*H*$_2$O), 3.46–3.51 (dd, J = 3.6, 9.9 Hz, 2H, *H*-2), 3.56–3.62 (t, J = 9.6 Hz, 2H, *H*-3), 3.73–3.86 (t, J = 9 Hz, 2H, *H*-4), 4.10–4.17 (m, 2H, CHC*H*$_2$O), 4.47 (d, J = 9.9 Hz, 2H, *H*-5), 4.80 (d, J = 3.3 Hz, 2H, *H*-1), 5.45 (m, 2H, =C*H*), 7.53 (s, 4H, Ar*H*).

4.12. Macrocycle 18

Pd–C (10%, 7 mg) was added to a solution of **14/15** (47 mg, 0.059 mmol) in EtOAc (2 mL) and the mixture stirred under H_2 atmosphere at rt for 18 h. The reaction mixture was then filtered through Celite and the filtrate evaporated and purified by chromatography (dichloromethane/methanol 98:2) to give acetylated intermediate (34 mg, 72%) as a white solid; R_f = 0.35 (dichloromethane/methanol 5%); $[\alpha]_D$ = −177.6 (*c* 0.85, $CHCl_3$); ^{1}H NMR (D_2O, 300 MHz): δ = 0.90–0.99 (m, 2H, C*H*$_2$), 1.48–1.57 (m, 2H, C*H*$_2$), 2.03 (s, 3H, OCOC*H*$_3$), 2.11 (s, 6H, OCOC*H*$_3$), 3.32 (m overlapping with s, 10H, NC*H*$_3$ and C*H*$_2$O), 4.78 (d, J = 9.6 Hz, 2H, *H*-5), 4.95–4.99 (dd, J = 3.3, 9.6 Hz, 2H, *H*-2), 5.03 (d, J = 3.6 Hz, 2H, *H*-1), 5.38–5.44 (t, J = 9.6 Hz, 2H, *H*-3), 5.61–5.67 (t, J = 9.3 Hz, 2H, *H*-4), 7.35 (s, 4H, Ar*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.6, 20.8, 20.9 (each q, CO*C*H$_3$), 26.3 (t, *C*H$_2$), 39.5 (q, N*C*H$_3$), 64.5, (d, CH), 66.4 (t, *C*$_2$O), 70.2, 70.4, 70.9, 95.4 (each d), 128.3 (d, Ar*C*H), 142.3 (s, Ar*C*), 166.7 (s, *C*ONCH$_3$), 168.4, 169.9, 170.6 (each s, *C*OCH$_3$); LRMS-ES: $[M+H]^+$ 795.3; IR (CH_2Cl_2): 3625, 3515, 3418, 2933, 1755, 1664, 1506, 1243, 1041 cm^{-1}; HRMS-ES: $[M+H]^+$ Calcd 795.2824. Found 795.2852. Sodium methoxide from a 1 M solution freshly prepared (0.1 mL) was added to a solution of this intermediate (25 mg, 0.031 mmol) in methanol (2 mL) that had been pre-cooled on ice. The reaction mixture was stirred at 0 °C for 30 min. The solvent was evaporated under reduced pressure to give a yellow solid that was dissolved in water and acidified to pH 6 by addition of Amberlite (H^+). The resin was removed by filtration and the filtrate was freeze-dried to give **18** as a white solid (12 mg, 70%). The mixture was partially separated by HPLC (eluant water/acetonitrile 85:15, flow rate 10 mL/min, C4-reverse phase preparative column). $[\alpha]_D$ = −16 (*c* 0.25, MeOH); ^{1}H NMR (D_2O, 300 MHz): δ = 0.92–0.97 (m, 2H, C*H*$_2$), 1.41–1.59 (m, 2H, C*H*$_2$), 3.33–3.39 (m overlapping with s, 10H, NC*H*$_3$ and C*H*$_2$O), 3.62–3.66 (dd, J = 3 Hz, J = 9.6 Hz, 2H, *H*-2), 3.72–3.78 (t, J = 9.6 Hz, 2H, *H*-3), 3.87–3.93 (t, J = 9 Hz, 2H, *H*-4), 4.62 (d, J = 9.6 Hz, 2H, *H*-5), 4.89 (d, J = 3 Hz, 2H, *H*-1), 7.39 (s, 4H, Ar*H*); LRMS-ES: $[M+H]^+$ 543.2; HRMS-ES: $[M+Na]^+$ Calcd 565.2009. Found 565.2006.

4.13. Macrocycle 16

A degassed solution of **9** (172 mg, 0.19 mmol) in dry dichloromethane (65 mL, 3 mM) and under argon was treated with metathesis catalyst (34 mg, 20%) for 40 h. The solvent was evaporated to give a black solid that was purified by column chromatography (eluant dichloromethane/ethyl acetate 1:4) to give corresponding to a 2:1 mixture of the **12** and **13** (99 mg, 62%). R_f = 0.5 (dichloromethane/ethyl acetae 1:4); ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.61–1.81 (m, 4H, CH$_2$C*H*$_2$CH), 1.98–2.19 (m, 22H, CH$_2$C*H*$_2$CH$_2$ and OCOC*H*$_3$), 3.29–3.66 (br m, 10H, CH$_2$C*H*$_2$O– and NC*H*$_3$), 4.56–4.66 (m, 2H, *H*-5), 4.84–4.92 (m, 2H, *H*-2), 5.08–5.52 (m, 8H, *H*-1 and *H*-3 and *H*-4 and =C*H*$_2$), 7.23 (br s, 4H, Ar*H*); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.7, 20.8 (each q, CO*C*H$_3$), 28.5, 29.8 (t), 38.5 (q, N*C*H$_3$), 65.5 (d), 69.1 (t, *C*H$_2$O), 69.9, 70.6, 70.9, 96.1 (each d), 127.6, 127.9 (d, Ar*C*H), 129.9, 130.0 (d), 142.5, 145.2 (s, Ar*C*), 166.6, 166.9 (s, *C*ONH), 168.6, 170.2, 170.4 (each s, *C*OCH$_3$); LRMS-ES$^+$: 849.3 $[M+H]^+$. Sodium methoxide from a 1 M solution freshly prepared (0.1 mL) was added to a

solution of the mixture (80 mg, 0.094 mmol) in methanol (11 mL) cooled on an ice bath. The reaction mixture was allowed to stir at 0 °C for 35 min when no starting material could be observed by TLC. The solvent was evaporated under reduced pressure to give a yellow solid that was dissolved in water and acidified to pH 6 by addition of Amberlite-H^+ resin. The resin was removed by filtration and the filtrate was freeze-dried to give a white solid corresponding to a 2:1 mixture of **16** and **17** (40 mg, 71%).

Analytical data for **16** (isolated by crystallisation from ethanol); $[\alpha]_D = +110$ (*c* 0.08, methanol); ^{1}H NMR (**16b/c**, D_2O, 300 MHz, 25 °C): $\delta = 1.64$–1.89 (m, 4H, $CH_2CH_2CH_2$), 2.04–2.25 (m, 4H, $CH_2CH_2CH{=}$), 3.42 (s, 6H, NCH_3), 3.56–3.64 (m, 6H, *H*-2 and *H*-4 and OCH_2), 3.80–3.89 (m, 4H, *H*-3 and OCH_2), 4.40 (d, $J = 9.9$ Hz, 2H, *H*-5), 4.97 (d, $J = 3.9$ Hz, 2H, *H*-1), 5.77 (s, 2H, CH=), 7.42 (br s, 4H, Ar*H*); ^{13}C NMR (**16b/c**, D_2O, 125 MHz, 5 °C): $\delta = 30.6$, 31.4 (t), 40.3 (q, NCH_3), 70.2 (d), 71.5 (t, C_2O), 73.2, 74.6, 74.7 (each d), 101.5 (d), 130.3, 132.1 (d, Ar*C*H), 132.6 (d), 144.5 (s, Ar*C*), 172.9 (s, *C*O); ^{13}C NMR (major conformation **16b/c**, D_2O, 125 MHz, 25 °C): $\delta = 30.9$, 31.7 (t), 40.8 (q, NCH_3), 70.8, 72.2 (d), 73.7 (t, CH_2O), 74.9, 75.1, 102.0 (each d), 130.9, 132.0 (d, Ar*C*H), 133.0 (d), 144.8 (s, Ar*C*), 173.2 (s, *C*O); selected data for **16a**: ^{1}H NMR (D_2O, 300 MHz, 25 °C): $\delta = 3.39$ (s, NCH_3), 3.41 (s, NCH_3), 4.17 (d, $J = 9.3$ Hz, *H*-5), 4.94 (s, *H*-1), 5.04 (d, $J = 4.2$ Hz, *H*-1), 5.08 (d, $J = 10$ Hz, *H*-5), 5.60–5.56 (overlapping with signals of **16b/c**, *H*C=), 5.50–5.42 (m, *H*C=), 7.50, 7.47 (br s, *H*Ar); ^{13}C NMR, D_2O, 125 MHz, 5 °C) $\delta = 30.9$, 30.2 (t), 39.9, 41.3 (q, NCH_3), 71.0, 73.4, 73.6, 74.9, 75.8 (each d), 101.2, 101.8 (d, –*C*-1), 130.6 (d), 143.2, 145.1 (s, Ar*C*), 172.8, 173.4 (s, *C*O); HRMS-ES: $[M+H]^+$ Calcd 597.2660. Found 597.2672; IR (KBr): 3436, 3366, 2829, 1645, 1510, 1439 cm^{-1}; HRMS-ES: $[M+H]^+$ Calcd 597.2660. Found 597.2672.

The ratio between **16a** and **16b/c** was 85:15 at 25 °C.

Selected analytical data for **17**: ^{1}H NMR (D_2O, 300 MHz): $\delta = 3.41$ (s, NCH_3), 4.55 (d, $J = 9.9$ Hz, *H*-5), 5.00 (d, $J = 3.9$ Hz, *H*-1), 5.55–5.57 (t, $J = 4.8$ Hz, C*H*=), 7.52–7.54 (m, Ar*H*); ^{13}C NMR (D_2O, 125 MHz): $\delta = 25.8$, 31.3 (t), 70.5, 71.2, (d), 74.8 (t, CH_2O), 75.4, 101.7 (d), 132.6 (d), 144.7 (s, Ar*C*), 173.0 (s, *C*O).

4.14. Variable temperature experiments for 16

A variable temperature study was carried out to ascertain that both signal sets observed for **16** coalesce. ^{1}H NMR spectra were recorded at variable temperatures. The following was observed. The signals at $\delta = 7.50$ (s, *H*Ar, major isomer) and $\delta = 7.35$ (s, *H*Ar, minor isomer) at 5 °C coalesced into a single peak at δ=7.43 at 65 °C. The signals at $\delta = 5.78$ (s, =CH, major isomer and 5.41–5.65 (m, =C*H*, minor isomer) at 5 °C coalesced into a single peak at $\delta = 5.71$ at 65 °C. The signals at $\delta = 4.40$ (d, *H*-5, major isomer) and $\delta = 5.08$, 4.17 (d and br s, respectively, *H*-5, minor isomer) at 25 °C coalesce to a single peak at $\delta = 4.37$ (under D_2O signal) at 65 °C. The signals at $\delta = 3.42$ (s, NCH_3, major isomer) and $\delta = 3.39$, 3.41 (each s, NCH_3, minor isomer) at 25 °C coalesce to a single peak at $\delta = 3.41$ at 65 °C.

4.15. Fluorescence binding studies

Solutions were prepared using volumetric diluting methods. The 0.1 M sodium phosphate buffer was prepared by dissolving 640 mg of $NaH_2PO_4{\cdot}H_2O$ in 50 mL of distilled water and adjusting the pH to 7.4 with saturated NaOH solution. The different solutions were prepared by dissolving the corresponding solid products in the above buffer, sonicating if necessary. Fluorescence emission spectra were recorded on a Varian Cary-Eclipse fluorescence spectrophotometer with excitation at a fixed wavelength of 365 nm.

4.16. NMR binding studies and dilution experiments

A mixture of macrocycles **16/17** (22 mg) was dissolved in D_2O and solvent removed by freeze-drying. The residue was then dissolved in 0.7 mL of 0.1 M sodium phosphate buffer (prepared by dissolving 64 mg of $NaH_2PO_4{\cdot}H_2O$ in 5 mL of D_2O and adjusting the pH to 7.4 with saturated NaOH in D_2O solution) to give a solution of **16/17** = 0.053 M. To this solution, 1.5 mg portions of solid $Mg_{0.5}ANS{\cdot}H_2O$ were serially added and ^{1}H NMR spectra were recorded after each addition. The chemical shifts of the signals corresponding to the macrocycle **16** moved upfield and broadened when increasing ANS. The variation in the chemical shift of the signal of the alkene proton of **16** with increasing concentration of ANS is shown in Figure 7.

Dilution experiments of the mixture of **16/17** and ANS were also carried out. A solution containing **16/17** (0.35 mL of a 0.053 M solution of **16/17** and 0.065 M ANS) was added to 0.35 mL of sodium phosphate buffer (0.1 M) thus halving the concentration. This process was repeated a further four times and the ^{1}H NMR spectra recorded after each dilution. The chemical shifts of the peaks belonging to **16** reverted, in a linear concentration dependant manner to the original values recorded in the absence of ANS.

The ^{1}H NMR spectrum of **16** was recorded also over a range of concentrations in absence of any ANS. A solution containing **16/17** (0.35 mL of 0.026 M) was added to 0.35 mL of 0.1 M sodium phosphate buffer (prepared as described above) thus halving the concentration. This process was repeated consecutively four times thus giving a range of concentrations from 0.0017 M to 0.026 M. The chemical shift of alkene proton did not change on dilution with buffer.

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