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Backbone-modified amphiphilic cyclic di- and tetrasaccharides†

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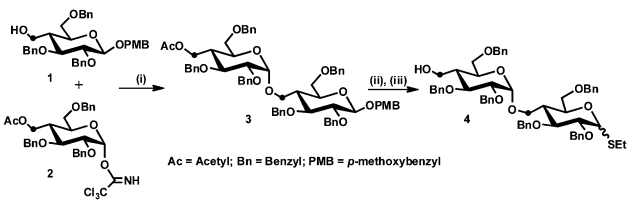
Synthesis of amphiphilic, cyclic di- and tetrasaccharides, which incorporate a methylene moiety at the inter-glycosidic bond, is reported. The amphiphilic properties of the new cyclic tetrasaccharide host were identified through assessing the solubilities of guests in aqueous and in organic solvents. The glycosidic bond stability of the cyclic tetrasaccharide under aqueous acidic condition was also verified.

Synthetic hosts, such as cyclodextrins (CDs),[1] calix[*n*]arenes,[2] cucurbiturils,[3,4] cavitands,[5,6] cyclophanes[7] and glycophanes,[8] and supramolecular assemblies exemplified by catenanes, rotaxanes, and molecular machines,[9–11] have greatly contributed to uncovering many facets of molecular recognition. Among synthetic hosts, the poor solubility of CDs in aqueous solutions and their practical insolubility in organic solvents limit their utilities, leading to their post-modification by functionalizing the hydroxyl groups. Synthetic macrocylic hosts soluble in both organic solvents and aqueous solutions would, in principle, expand their molecular recognition properties. With this perspective, we undertook synthesis of a new type of backbone-modified cyclic oligosaccharide. Backbone modification of the inter-glycosidic bond with a methylene moiety was anticipated to result in hydrophobicity of such cyclic oligosaccharides, differing from that of CDs, which, in turn, would modify their solubility properties. This report describes our achievement of this objective.

The synthesis of cyclic oligosaccharides, incorporating a methylene moiety at the inter-glycosidic bond, was initiated by identifying a disaccharide monomer suitable for cyclo-oligomerization. Synthesis of the disaccharide monomer is shown in Scheme 1. The protected derivative **1**[12] was subjected to a glycosylation with glycosyl donor **2** to afford the required disaccharide derivative **3**. The α-anomeric configuration of **3** was confirmed by the appearance of H-1′ of non-reducing *gluco*-pyranoside moiety at 4.80 ppm (J = 3.6 Hz). The following sequence of reactions was performed on **3** to secure the glycosyl donor **4**: (i) removal of PMB-group (CF_3CO_2H);[13] (ii) acetate protection of lactal (Ac_2O/pyridine); (iii) thioglycoside formation (EtSH, $BF_3{\cdot}Et_2O$ at 0 °C) and (iv) *O*-deacetylation (Scheme 1). The glycosidic bond expanded donor **4** was obtained as an anomeric mixture (α : β ∼ 1 : 1.7). Anomeric H_β-1 of the reducing end of **4** appeared at 5.49 ppm (J = 5.6 Hz) and the corresponding H_α-1 resonated at 4.50 ppm, as an apparent singlet, in the ^{1}H NMR spectrum.

The monomer **4**, equipped with an activated thioglycoside moiety at the reducing end and hydroxymethyl acceptor moiety at C-4 of the non-reducing end, was subjected subsequently to a cyclo-oligomerization reaction.[14] A thiophilic activator, either *N*-iodosuccinimide (NIS)/silver triflate (AgOTf) or trimethylsilyl trifluoromethane sulfonate (TMSOTf) in CH_2Cl_2, was used for the cyclo-condensation and the reaction was conducted in CH_2Cl_2 at 0 °C for 2 h and at room temperature for 4 h, then quenched with Et_3N and worked up. The reaction mixture was then purified (SiO_2) (pet. ether–EtOAc) to afford glycosidic bond expanded cyclic disaccharide **5** and cyclic tetrasaccharide **6** in 11 and 64%, respectively (Scheme 2). Concentrations of either 3 mM or 20 mM of **4** did not result in much difference in the ratio of product formation. Structures of **5** and **6** were established



Scheme 1 *Reagents and conditions*: (i) TMSOTf, Et_2O, MS (4 Å), rt, 30 min, 66%; (ii) (a) CF_3CO_2H, CH_2Cl_2–H_2O, 0 °C–rt, 2 h, 72%; (b) Ac_2O/pyridine, 0 °C, 12 h, 92%; (iii) (a) EtSH, BF_3OEt_2, CH_2Cl_2, 0 °C, 30 min; (b) NaOMe, MeOH, rt, 4 h, 85% (after two steps).

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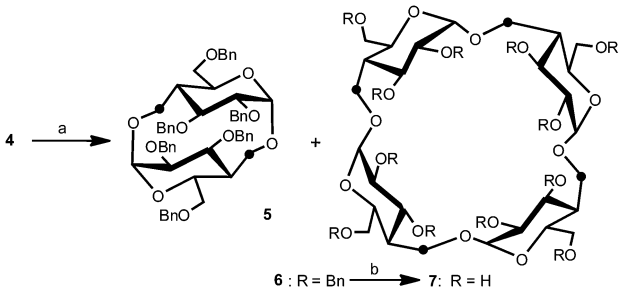


Scheme 2 *Reagents and conditions*: (a) NIS, AgOTf or TMSOTf (Cat.), CH_2Cl_2, MS (4 Å), 0 °C–rt, 6 h (**5**: 11%, **6**: 64%); (b) H_2, Pd/C (10%), MeOH/EtOH (1 : 1), 24 h, 91%.

through ^{1}H and ^{13}C, COSY and HSQC NMR spectroscopic and mass spectrometric analyses. The ESI-MS spectrum of **5** showed a molecular ion peak at 915.4088 ([M + Na]$^+$), along with a peak at 469.1786 as [M/2 + Na]$^+$ adduct. In ^{1}H NMR spectrum of **5**, the anomeric H-1 appeared at 4.92 ppm (J = 2.0 Hz), and in ^{13}C NMR spectrum, the anomeric carbon appeared at 90.7 ppm. MALDI-TOF mass spectral analysis of **6** showed the molecular ion peak at m/z = 1807.6888 ([M + Na]$^+$), along with peaks at m/z 1362.650, 915.533, corresponding to [M-(one monomer unit) + Na]$^+$ and [M/2 + Na]$^+$ ion peaks, respectively. In ^{1}H NMR spectrum of **6**, H-1 nucleus was observed at 5.04 ppm, as an apparent singlet, whereas in ^{13}C NMR spectrum, C-1 signal resonated at 91.2 ppm. Removal of benzyl protecting group in **6** (H_2, Pd/C (10%)) afforded a free hydroxyl group-containing cyclic tetrasaccharide **7**. In ^{1}H NMR spectrum of **7**, the H-1 nucleus was observed at 4.89 ppm (app. s) and in ^{13}C NMR spectrum, the C-1 nuclei appeared at 92.3 ppm (Fig. 1 and 2). ESI-MS analysis showed molecular ion peak at 727.2617, as [M + Na]$^+$ adduct.

Energy-minimized molecular modelling was performed in order to identify the size and shape of the backbone cyclic tetrasaccharide **7**, using Gaussian 03 software at B3LYP/6-311G* level.[15] In the optimized structure derived from modelling (Fig. 3), the pyranoside moieties were seen to adopt $^4C_1$ conformation. The distances between alternate glycosidic oxygen atoms in **7** were 8.42 and 6.80 Å. Interestingly, it was observed from the optimized structure that all the primary hydroxyl groups were placed on the top of upper rim and all the secondary hydroxyl groups placed on the lower rim, in contrast to CDs, where the reverse occurs.

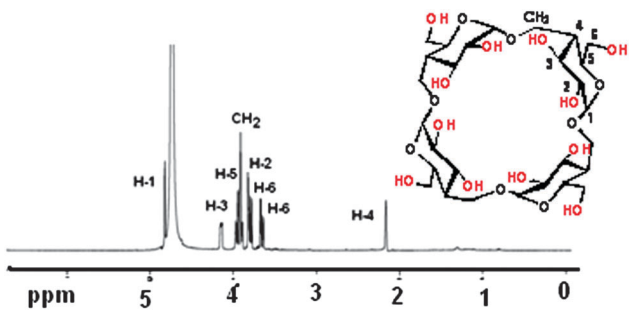


Fig. 1 ^{1}H NMR spectrum of **7** (D_2O, 400 MHz).

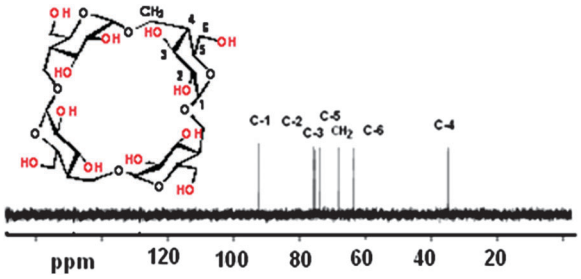
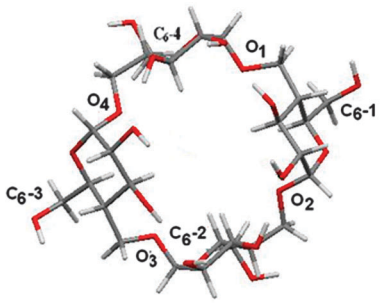


Fig. 2 ^{13}C NMR spectrum of **7** (D_2O, 100 MHz).



O_1-O_3= 8.42 Å; O_2-O_4= 6.80 Å C_6-1-C_6-3 = 12.23 Å; C_6-2-C_6-4 = 8.08 Å

Fig. 3 Energy minimized structure of **7**, derived from molecular modelling simulation.

Whereas chemical modifications, such as per-*O*-alkylation, are commonly performed in order to improve the solubility behavior of CDs[16] in water and in organic solvents, cyclic tetrasaccharide **7** is soluble in aqueous and in most organic solvents, as a result of its inherent amphiphilicity. Aqueous solubility of **7** is 74 ± 3 mg mL^{-1}, whereas that of α- and β-CDs are 145 and 18.5 mg mL^{-1}, respectively.[1] Given its amphiphilic nature, further studies were undertaken to assess the solubilization properties of **7**. Pyrene solubilization studies in aqueous solution were undertaken, in order to identify the extent of the microenvironment present in the host, for which UV-Vis absorption and fluorescence spectroscopies were used. In order to assess solubilization, an aqueous solution of **7** in varying concentrations was admixed with pyrene and stirred at 45 °C for 24 h in the dark. The solutions were then filtered and the extent of solubilization of pyrene in each aqueous solution of host was determined by UV-Vis spectroscopy (ε_{335} 50 730 mol^{-1} cm^{-1}).[17] Pyrene solubilization in water was found to be 0.7 μM, whereas in aqueous solutions of the host this was 1.5, 2.3, 7.7, and 14.7 μM with 0.2 mM; 0.5 mM; 0.7 mM and 0.9 mM of host **7**, respectively. Increased solubility of pyrene illustrated higher hydrophobicity of the host. Further, in order to assess the polarity of the microenvironment, fluorescence spectra were recorded. Emission spectra (Fig. 4) showed an enhancement of fluorescence intensity of pyrene upon increasing concentration of **7** in aqueous solutions. An excimer emission of pyrene was also apparent at higher concentrations (0.7 mM and 0.9 mM) of **7**.

The ratio of the intensities of third to first bands (I_3/I_1) in the fluorescence spectrum, which is a measure of the polarity of the

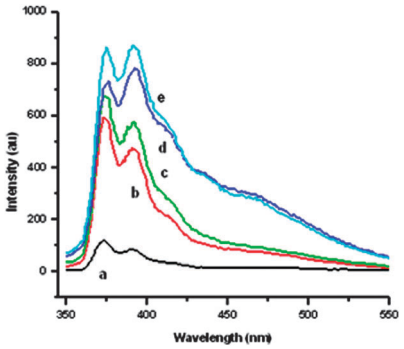


Fig. 4 Emission spectra of pyrene: (a) in H_2O; (b) in solutions of **7** of concentration (b) 0.2 mM; (c) 0.5 mM; (d) 0.7 mM and (e) 0.9 mM.

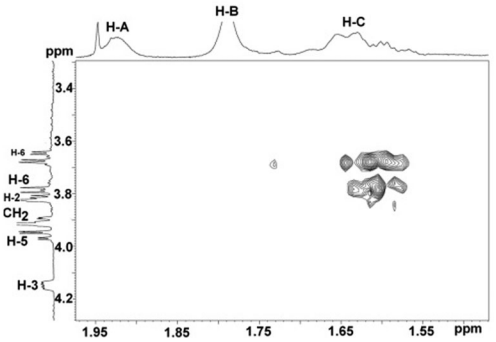


Fig. 6 ROESY spectrum of the complex of **7** with adamantane-1-carboxylic acid (D_2O, 400 MHz).

microenvironment,[18] was found to increase with increasing concentrations of **7**, reflecting the hydrophobicity of **7** in aq. solutions. Further, a molar ratio of 4 : 1 of **7** to pyrene was identified from the integration of the ^{1}H NMR spectrum in $CDCl_3$, which reflected the creation of a hydrophobic microenvironment in aqueous solution of **7** and thus its solubilization property. Association constant (K_a) of the host–guest interaction was derived by utilizing the aggregation number (n), as is used with micelle host–guest interaction.[19] By utilizing the equation $[\text{guest}]_{\text{with host}}/1 - [\text{guest}]_{\text{without host}} \propto [7] -$ critical aggregation concentration of $7/n$ with the proportionality constant being K_a, a K_a of 1.77×10^5 M^{-1} was derived. A detailed description of the calculation is given in the ESI.†

Solubilization of adamantane-1-carboxylic acid (AdCA) in aqueous solution of **7** was also evaluated. For this purpose, an aq. solution of guest and host (5 : 1 host-to-guest molar equiv.) was stirred for 12 h at 30 °C, filtered, and the filtrate concentrated *in vacuo*. The ^{1}H NMR spectrum of the resulting solution in D_2O showed new peaks at 2.01, 1.87 and 1.73–1.65 ppm, corresponding to AdCA protons (Fig. 5), and broadening of the resonances was observed. The ROESY spectrum of the complex (Fig. 6) showed cross-peaks between H-C of AdCA and $H_{a,b}$-6 of **7**, from which the complexation of AdCA appeared to occur on the primary hydroxyl group side of the host. Complexation of AdCA with **7** causes up-field shift of H-B and H-C by 0.04 and 0.02 ppm. Analysis of ^{1}H NMR spectrum and the proton integration values revealed a molar ratio of 2 : 1 of **7**-to-AdCA.

Free hydroxyl group-containing cyclodextrins are practically insoluble in organic solvents.[16] The amphiphilic nature of the cyclic tetrasaccharide **7** warranted further assessment of its ability to solubilize hydrophilic guests in organic solvents. Solubilization of **7** with a hydrophilic guest, namely L-tyrosine, was thus assessed through ^{1}H NMR analysis in $CDCl_3$. A mixture of **7** and L-tyrosine (5 : 1 molar equiv.) was stirred in $CDCl_3$ for 24 h at room temperature, filtered and the ^{1}H NMR spectrum was recorded. The appearance of signals of the aromatic moiety of the guest at 7.52 and 6.98 ppm as doublets and methylene protons at 2.12 ppm as a multiplet in the spectrum reflected the solubilization of L-tyrosine in $CDCl_3$ solution. Integration of ^{1}H NMR values revealed a 1 : 1 molar ratio of **7**-to-L-tyrosine in the complex.

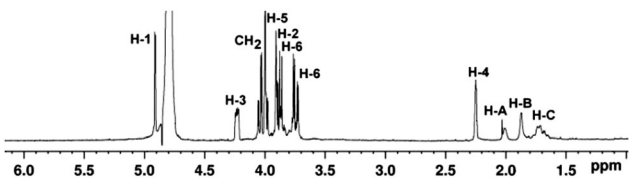


Fig. 5 ^{1}H NMR (D_2O, 400 MHz) spectrum of the complex of **7** with adamantane-1-carboxylic acid (H-A–H-C).

Further, an effort was undertaken to identify the stability of the glycosidic bond in **7**, for which an acid-catalyzed hydrolysis was performed, followed by ^{1}H NMR spectroscopy. The hydrolysis of **7** and α-cyclodextrin was performed using DCl in D_2O (2 N) at 60 °C and ^{1}H NMR spectra were recorded periodically. The analysis showed complete hydrolysis of **7** within 30 min, whereas in the case of α-cyclodextrin this required 6 h. The deoxygenation at C-4 led to faster hydrolysis, following a general trend that glycosides undergo faster hydrolysis when the hydroxyl group is substituted by a carbon substituent.[12,20,21]

In conclusion, synthesis of new cyclic di- and tetrasaccharides, incorporating a methylene moiety at the interglycosidic bond, has been achieved in good yields through a one-pot condensation of a disaccharide monomer. Solubility of free hydroxyl group-containing amphiphilic cyclic tetrasaccharide in aqueous solution and in organic solvents provides a new platform for host–guest studies.

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