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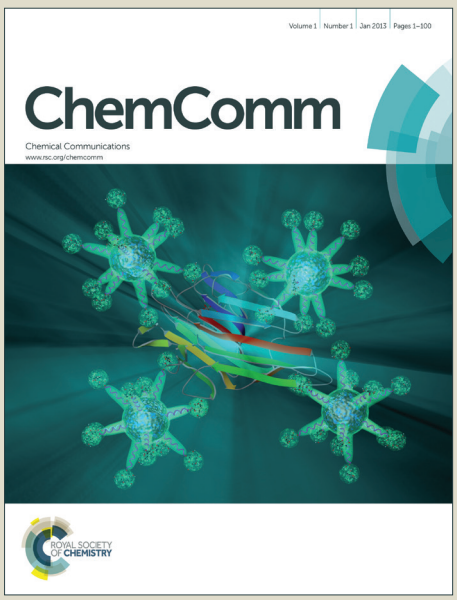

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ARTICLE TYPE

Backbone Modified Amphiphilic Cyclic Di- and Tetrasaccharides

Gour Chand Daskhan and Narayanaswamy Jayaraman*

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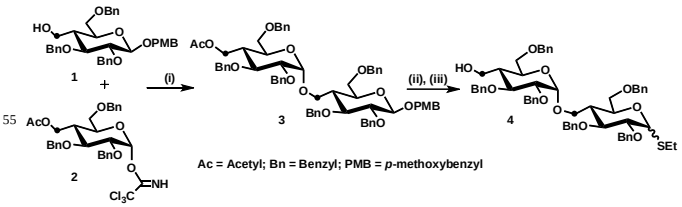
Synthesis of amphiphilic, cyclic di- and tetrasaccharides, incorporated with methylene moiety at the inter-glycosidic bond, is reported. The amphiphilic properties of new cyclic tetrasaccharide host were identified through assessing solubilities of guests in aqueous and in organic solvents. Glycosidic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

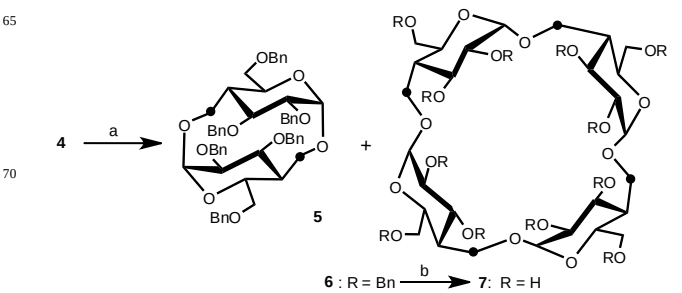
Synthetic hosts, such as, cyclodextrins (CDs),[1] calix[n]arenes,[2] cucurbiturils,[3,4] cavitands,[5,6] cyclophanes,[7] and glycophanes,[8] and supramolecular assemblies exemplified by catenanes, rotaxanes, and molecular machines,[9-11] have contributed greatly to uncover many facets of molecular recognitions.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

Synthesis of cyclic oligosaccharides, inserted with methylene moiety at 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] (iii) acetate protection of lactal (Ac_2O/pyridine); (iii) thioglycoside formation (EtSH, $BF_3.Et_2O$ 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 cyclo-oligomerization reaction.[14] Thiophilic activator either *N*-iodosuccinimide (NIS)/silver triflate (AgOTf) or trimethylsilyl



Scheme 1. Reagents and conditions: (i) TMSOTf, Et_2O, MS (4Å), 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).



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

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/hexane) to afford glycosidic bond expanded cyclic disaccharide **5** and cyclic tetrasaccharide **6** in 11 and 64 %, respectively (**Scheme 2**). Concentration of **4** either 3 mM or 20 mM led to the reaction without much difference in the ratio of product formation. Structures of **5** and **6** were established through ^{1}H and ^{13}C, COSY and HSQC NMR spectroscopic and mass spectrometric analyses. ESI-MS spectrum of **5** showed 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 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 (**Figures 1 and 2**). ESI-MS analysis showed molecular ion peak at 727.2617, as [M+Na]$^+$ adduct.

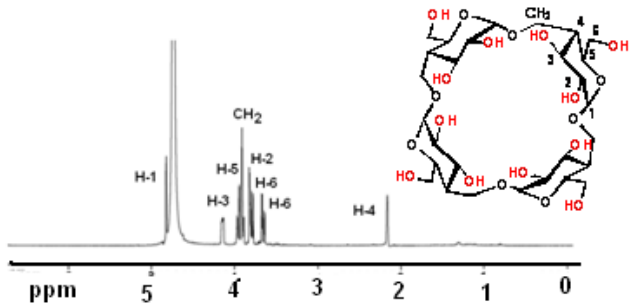


Figure 1. ^{1}H NMR spectrum of **7** (D_2O, 400 MHz).

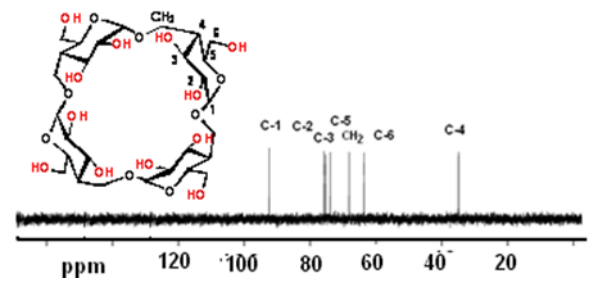


Figure 2. ^{13}C NMR spectrum of **7** (D_2O, 400 MHz).

An 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 (**Figure 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.

Whereas chemical modifications, such as, per-*O*-alkylation are exercised commonly 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, 18.5 mg mL^{-1}, respectively.[1] Realizing the 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

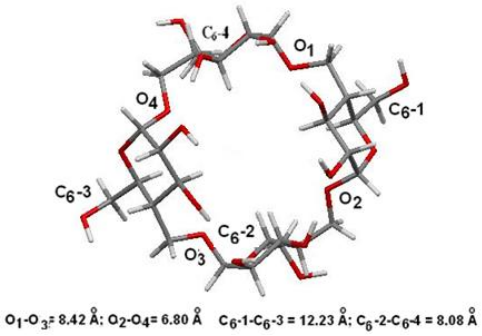


Figure 3. Energy minimized structure of **7,** derived from molecular modelling simulation.

extent of 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 concentration was admixed with pyrene, stirred at 45 ^{o}C for 24 h in dark and the solutions were 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 that in aqueous solutions of host were 1.5, 2.3, 7.7, and 14.7 µM, with at 0.2 mM; 0.5 mM; 0.7 mM and 0.9 mM of host **7**. Increased solubility of pyrene illustrated higher hydrophobicity of the host. Further, in order to assess the polarity of microenvironment, fluorescence spectra were recorded. Emission spectra (**Figure 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**.

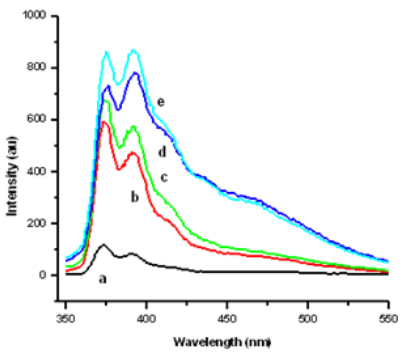


Figure 4. Emission spectra of pyrene: (a) in H_2O; (b) in the solution of **7**: (b) 0.2 mM; (c) 0.5 mM; (d) 0.7 mM and (e) 0.9 mM.

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 microenvironments,[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 of $[guest]_{with\ host}/1-[guest]_{without\ host}$ is proportional to [**7**] – critical aggregation concentration of **7**/n and the proportionality constant being K_a, a K_a of 1.77 x 10^5 M^{-1} was derived. Detailed description of the calculation is given in the Supplementary Information.

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, 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 (**Figure 5**) and broadening of the resonances was observed. The ROESY spectrum of the complex (**Figure 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 from 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.

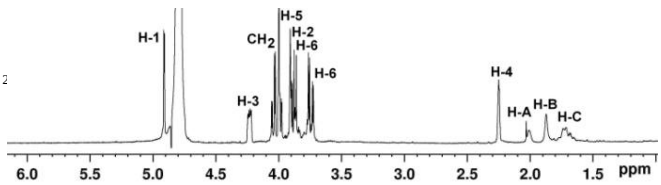


Figure 5. ^{1}H NMR (D_2O, 400 MHz) spectrum of the complex of **7** with adamantane-1-carboxylic acid (H-A – H-C).

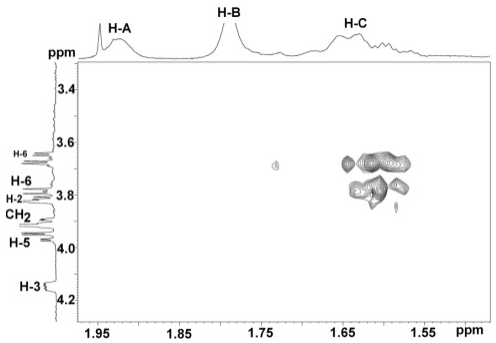


Figure 6. ROESY spectrum of the complex of **7** with adamantane-1-carboxylic acid (D_2O, 400 MHz).

Free hydroxyl group containing cyclodextrins are practically insoluble in organic solvents.[16] The amphiphilic nature of the cyclic tetrasaccharide **7** warranted further to assess the ability to solubilize hydrophilic guests in organic solvents. Thus, solubilization of **7** with hydrophilic guest, namely, L-tyrosine was 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. Appearance of signals of 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.

Further, an effort was undertaken to identify the stability of glycosidic bond in **7**, for which an acid-catalyzed hydrolysis was performed and the hydrolysis was 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 the same in the case of α-cyclodextrin 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 tetra-saccharides, inserted with methylene moiety at the inter-glycosidic bond, is achieved through a one-pot condensation of a disaccharide monomer, in good yields. Solubilities of free hydroxyl group containing amphiphilic cyclic tetrasaccharide in aqueous solution and in organic solvents provide a new platform for host-guest studies.

Notes and references

Indian Institute of Science, Bangalore 560012, India. Fax: 91-80-2360-0529; Tel: 91-80-2293-2578; E-mail: jayaraman@orgchem.iisc.ernet.in

† Electronic Supplementary Information (ESI) available: Experimental section, spectra and calculations. See DOI: 10.1039/b000000x/

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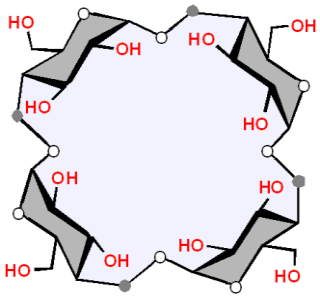

Backbone modified cyclic di- and tetrasaccharides, their guest solubilizations in aqueous and organic solutions, and glycosidic bond stabilities are reported.