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Reverse-CD mimics with flexible linkages offer adaptable cavity sizes for guest encapsulation†‡

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Atchutarao Pathigoolla and Kana M. Sureshan*

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Reverse-CD mimics with adaptable cavity sizes have been synthesized. The adaptability has been demonstrated through single crystal structure determination and guest binding studies.

Cyclodextrins (CDs) are toroidal shaped natural α-1,4-cyclo-oligoglucopyranosides with the ability to encapsulate hydrophobic guest molecules in their hydrophobic cavities.[1] CDs consisting of six (α-CD), seven (β-CD) and eight (γ-CD) glucose units are naturally abundant and are rigid with cavity diameters of approximately 5 Å, 7 Å and 8 Å, respectively. CDs and their derivatives[2–4] are widely used in molecular recognition,[5] molecular reactors,[6] catalysis,[7] drug delivery,[8] polymers,[9] *etc.* The main drawbacks of natural CDs and their derivatives are their fixed size of the cavity, fixed polarity of the cavity and rigid structure which limits the class of guests that can be encapsulated. Though many CD-analogs[10] with non-glycosidic linkages have been synthesized, in all these analogs, as in the natural CDs, the α-face of the pyranoses makes the inner wall of the cavity, which is responsible for the guest recognition (Fig. 1A). Fernandez *et al.* introduced the concept of reverse-CDs (*R*-CDs), wherein the β-face of the pyranoses forms the inner wall.[11] A few *R*-CD mimics *viz.* cyclotrehaloses (CTs, Fig. 1B) have been tactically synthesized by the head-to-head (6,6′) linkage of the C_2-symmetric disaccharide (trehalose) derivative. However, an ideal *R*-CD will have β-1,4-linkages (head-to-tail) between pyranoses with β-oriented 4-OH (*e.g.* D-galactopyranose) such that the endocyclic oxygens and the hydroxymethyl groups will orient inward the cavity (Fig. 1C). Though this will modify the polarity of the cavity, they are expected to be rigid, like CDs, due to the glycosidic linkages. We herein report flexible reverse-CD analogs with adaptable cavity sizes and provide evidence for their variable cavity sizes through crystallography and guest binding profiles.

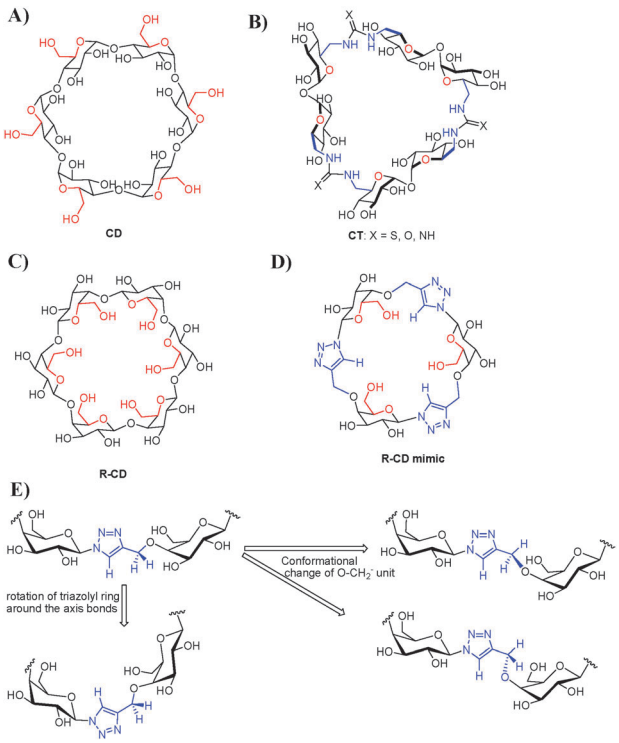


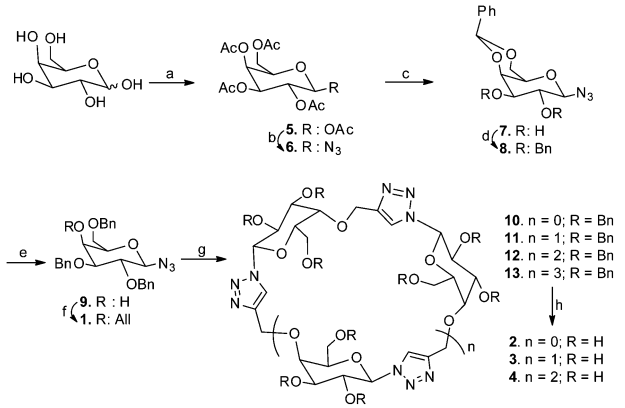
Fig. 1 Structural comparison of (A) CDs, (B) CTs, (C) *R*-CDs and (D) *R*-CD mimics. (E) Possible conformational freedoms for triazolylmethyl linkages.

As the glycosidic linkages impart rigidity to the *R*-CDs, we planned to introduce flexible linkages between sugar units, which can impart conformational freedom to the macrocycle. Such conformationally flexible reverse-CD analogs can orchestrate diverse cavity sizes by adopting different conformations and consequently encapsulate guests of different sizes.[12] We planned to replace alternate β-(1,4)-galactopyranosyl units in *R*-CDs by flexible triazolylmethyl groups (Fig. 1D), which can adopt multiple conformations either through rotation of the triazolyl ring around the single bonds or through adoption of different conformation of

School of Chemistry, Indian Institute of Science Education and Research, Thiruvananthapuram, CET campus, Thiruvananthapuram-695016, Kerala, India. E-mail: kms@iisertvm.ac.in

† Dedicated to Dr Suresh Das on his 60th Birthday.

‡ Electronic supplementary information (ESI) available: Experimental procedures, characterization data for all new compounds, computational and binding studies of compounds **2–4** and crystallographic data for compound **3**. CCDC 960369. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc47734g



Scheme 1 Synthesis of *R*-CD analogs: (a) NaOAc, Ac_2O, 60 °C, 12 h, 90%; (b) Me_3SiN_3, $SnCl_4$, DCM, rt, Ar, 12 h, 95%; (c) (i) NaOMe, MeOH, rt, (ii) $PhCH(OMe)_2$, TsOH, MeCN, rt, Ar, 24 h, 83% (2 steps); (d) BnBr, NaH, DMF, 0 °C-rt, 3 h, 90%; (e) Et_3SiH, CF_3COOH, $(CF_3CO)_2O$, DCM, 0 °C-rt, 5 h, 78%; (f) propargyl bromide, NaH, DMF, −15 °C, 2 h, 87%; (g) CuI, DIPEA, THF, Ar, rt, 24 h, 90%; (h) Pd/C (w/w 10%), $HCOONH_4$, EtOAc : EtOH : H_2O (v/v/v 2 : 2 : 1), 50 °C, 20 h (>90%).

the sp^3 hybridized –O–CH_2– linkages (Fig. 1E). Also, the alternate aromatic triazolyl motifs in the host might render different guest selectivity for these reverse-CD analogs than the natural CDs.

We have synthesized 2,3,6-tri-*O*-benzyl-4-*O*-propargyl-β-D-galactosylazide (**1**) from D-galactose in seven steps in an overall yield of 43% as reported.[13] Among the various CuAAC[14] reaction conditions attempted, compound **1** underwent cyclooligomerization upon treatment with CuI and *N*,*N*-diisopropylethylamine in THF giving a mixture of cyclooligomers. Chromatographic separation of the oligomers afforded cyclic dimer **10** (15%), cyclic trimer **11** (28%), cyclic tetramer **12** (15%) and cyclic pentamer **13** (3%). Debenzylation of compounds **10–12** gave *R*-CD analogs **2–4** respectively (Scheme 1). Though the monomer undergoes topochemical oligomerization to linear oligomers in the solid state,[13] no linear oligomers were formed in solution. It is interesting to note the formation of the strained cyclic dimer, which has not been observed previously.[10j]

In order to get a clear insight into the conformation of these *R*-CD analogs, we have optimized the compounds **2**, **3** and **4** using DFT. We have used B3LYP and M05-2X functionalities with basis set 6-311+G(d,p). The relative stabilities of these molecules followed the same trend in both the methods. The dimer **2** adopted a rigid conformation with a slightly distorted chair conformation for the pyranose ring and showed no cavity space for guest encapsulation as anticipated (Fig. 2A). The trimer **3** showed a bowl shaped symmetrical cavity with an internal diameter of ~6 Å (Fig. 2B). But the tetramer **4** adopted a saddle-like conformation with a diameter of 5.5 Å (Fig. 2C). VT-NMR studies of trimer **3** and

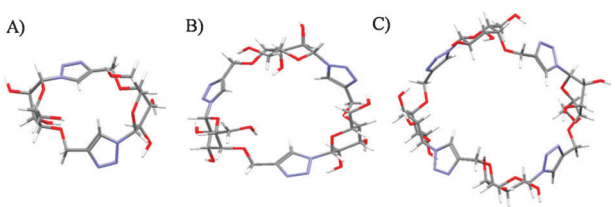


Fig. 2 Minimum energy (DFT-M05-2X) structures of (A) dimer **2**, (B) trimer **3** and (C) tetramer **4**.

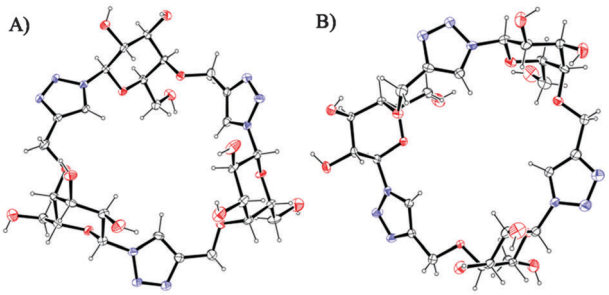


Fig. 3 ORTEP diagram of trimer **3** showing conformers A and B. Water molecules are omitted for clarity.

tetramer **4** suggested conformational flexibility of these macrocycles in solution too, as evident from the slight downfield shift of H-2 and the up-field shift of triazolyl protons upon increasing temperature.

The trimer **3** crystallized as colorless plate-like rectangular crystals from a mixture of ethanol and chloroform (7 : 3 v/v). Single crystal XRD analysis of these crystals revealed that the asymmetric unit has two conformers A and B along with nine water molecules (Fig. 3 and ESI‡). This is the first report on the crystal structure of a triazole-linked CD mimic. Interestingly, in support of our hypothesis of adaptability, the cavity sizes of these two conformers were found to be different. The inner diameter of conformer A is ~5 Å and that of conformer B is ~5.4 Å, whereas the natural α-CD has a diameter of 5.8 Å (Table 1). Thus we could show that the trimer **3** can adopt at least three stable conformations (two XRD structures and one DFT) with different cavity sizes due to its flexibility. This suggests that the higher analogs, due to their increased flexibility, might have many conformations of varying cavity sizes.

A comparison of the crystal structure of **3** with known crystal structures of α-CD revealed a clear difference in their hydration. While **3** crystallized with 4.5 water molecules per molecule of **3**, α-CD usually crystallizes with 6–7 molecules of water. Though **3** and α-CD have similar cavity sizes, the former encapsulates only a single water molecule in its cavity (crystal), but the latter entraps three molecules of water in its cavity (crystal). This suggests that the *R*-CD analogs have different cavity properties, and hence guest binding ability than the natural CDs. Such a comparative analysis of crystal structure of CD-analogs with natural CDs has not been reported earlier.

Inclusion properties of these *R*-CD analogs were investigated with different guest molecules using ^{1}H NMR spectroscopy and isothermal titration calorimetry (ITC). As expected, dimer **2**

Table 1 Comparison of cavity sizes of *R*-CD analogs with CDs

CD/*R*-CD analog	Diameter of the cavity[a] (Å)
Dimer **2**[b]	2.8
Trimer **3**[b]	6.0
Trimer **3** (conformer A)[c]	5.0
Trimer **3** (conformer B)[c]	5.4
Tetramer **4**[b]	5.4
α-Cyclodextrin[c]	5.8
β-Cyclodextrin[c]	7.4
γ-Cyclodextrin[c]	9.2

[a] The cavity diameter was measured using UCSF Chimera by considering the atoms distances from the centroid of the cyclic molecule.[15] [b] DFT. [c] Crystal structure.

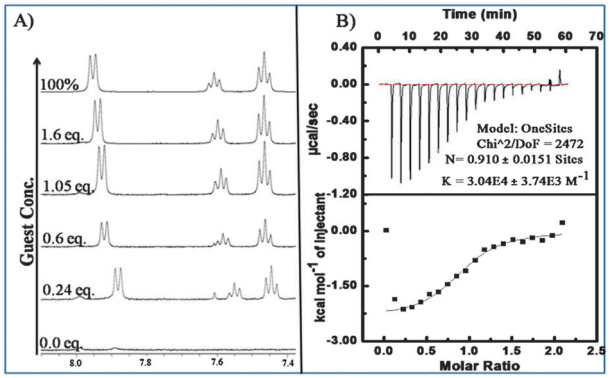


Fig. 4 ^{1}H NMR titration (A) and ITC (B) of benzoic acid with tetramer **4**.

could not encapsulate even small organic molecules like ethanol or propargyl alcohol. As the conformational analysis of **3** suggested variable cavity sizes, ranging from cavity size smaller than that of α-CD to a size intermediate between the cavity sizes of α-CD and β-CD, it is expected to bind guests of varying sizes. ^{1}H NMR titration experiments suggested the binding of trimer **3** with small organic molecules such as 1-propanol, 1-butanol, propargyl alcohol and but-3-yn-1-ol (ESI‡), as evident from the down-field shift (0.02–0.01 ppm) of the guest protons. The ITC experiments also confirmed the guest binding. It is noteworthy that the alkynes showed better binding than the corresponding alkanes. For instance, propargyl alcohol and but-3-yn-1-ol bound better than 1-propanol and 1-butanol respectively. The plausible CH$\cdots\pi$ hydrogen bonding between the acidic triazolyl protons and the alkyne might be responsible for the slightly improved binding of alkynes over alkanes. Interestingly, trimer **3** also showed guest binding with bigger guests, such as aromatic molecules. For instance, it binds with benzoic acid with an association constant (K_a) of 3550 M^{-1} (ESI‡) which is intermediate between the K_as of benzoic acid binding with α-CD (1000 M^{-1}) and β-CD (64 500 M^{-1}).[5] These results suggest that **3** could attain a cavity size better than that of α-CD for binding relatively large guests. Thus the binding of **3** with small and large organic guests provides proof of concept for the adaptability of these *R*-CD analogs.

The ^{1}H NMR titration of tetramer **4** with aromatic compounds such as phenol and benzoic acid showed shifts in the guest protons (0.01–0.1 ppm, Fig. 4A and ESI‡), suggesting that **4** is a host for these compounds. In every case, the ratio of the tetramer **4** to the guest was almost 1 : 1 for complete saturation suggestive of 1 : 1 complexation, which was further confirmed by ITC experiments. Though the energy minimized conformation of **4** showed a smaller cavity size than that of **3** and α-CD, interestingly the ITC experiment suggested a better binding of **4** with benzoic acid with an association constant of ~30 000 M^{-1}, which is of the order of the K_a of β-CD:benzoic acid binding (Fig. 4B). This also supports the guest-dependent adaptability of these *R*-CD analogs (Table 2).

Table 2 Binding constants of *R*-CD analogs and CDs[5] with various guests in M^{-1}

Guest	α-CD	β-CD	**3**	**4**
Benzoic acid	1000	64 500	3550	30 000
But-3-yn-1-ol	NR	NR	7	ND
1-Butanol	80	15	3	ND
Propargyl alcohol	NR	NR	5	ND

NR: not reported; ND: not determined.

In conclusion, for the first time we have synthesized *R*-CD analogs with flexible triazolylmethyl linkages through CuAAC click reaction of 4-*O*-propargyl-β-galactosyl azide. To the best of our knowledge, this is the first report on β-(1,4)-galactopyranosyl linked *R*-CD analogs. This is also the first report on the head-to-tail linked *R*-CD analog synthesized from a monosaccharide derivative. DFT calculations and single crystal X-ray crystallography provided proof-of-concept for (i) the polarity difference between *R*-CD and normal CD and (ii) cavity-size adaptability of *R*-CD analogs. Inclusion studies of these *R*-CD analogs also confirmed that they bind guests of different sizes supportive of the adaptable nature of their cavity size. Flexible *R*-CDs offer a new class of CD analogs with a different cavity property and adaptable cavity sizes.

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