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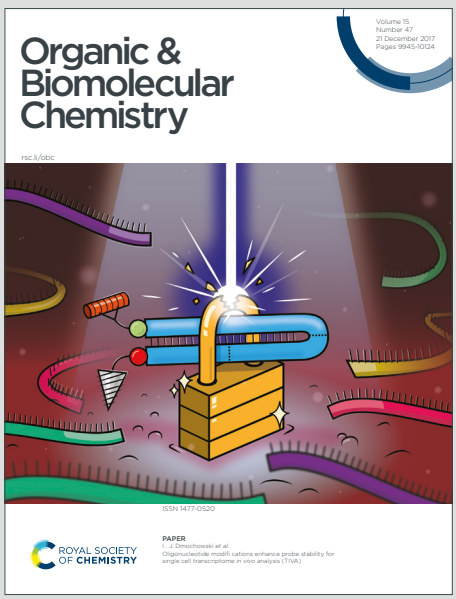

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Uncommon Strong Inhibition of α-Glucosidase by Multivalent Glycoclusters based on Cyclodextrins Scaffolds†

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The homeostasis disruption of D-glucose causes diabetes, a dramatic world wide chronic disease. The type 1 diabetes is a succesfully treatable form, where the blood D-glucose is regulated by insulin treatement. In contrast the type 2 diabetes, the non Insulin dependent one, is problematic. The control of the D-glucose blood level *via* intestinal α-D-glucosidase inactivation can be achieved by using competitive inhibitors as iminosugars (e.g. acarbose) or sulfonium sugar derivatives (e.g. salacinol). Recently, an unprecedented result showed that multivalent diamond nanoparticules grafted with unmodified sugars displayed α-glucosidase inhibition at low micromolar concentrations. We describe herein the synthesis of multivalent glycoclusters using cyclodextrines (CDs) as scaffolds and their assessment as inhibithors of α-D-glucosidase. The glycoclusters were efficiently obtained from per-azido α, β and γ-CDs derivatives and propargyl glycosides using click-chemistry under microwave irradiation. The methodology was successfully applied to various protected and non-protected propargylated monosaccharides, including both *O*- and *S*- glycosides, giving clear evidences of its versatility. The targeted 6-*per*-glycosylated CDs were isolated in moderate to excellent yields (30-90 %) by silica gel chromatography. The results showed inhibition of α-glucosidase from *Saccharomyces cerevisiae* with IC_{50} values in a 32-132 μM range, lower than that of acarbose (IC_{50} ~250μM), a well knowm competitive inhibitor used in clinical treatment of type 2 diabetes. Preliminary experiments suggest a mixed-type non-competitive inhibition mode of these new glycoclusters.

Keywords: cyclodextrin, click chemistry, microwaves, glycocluster, multivalent scaffold, glucosidase inhibitors

Introduction

Diabetes is a chronic disease that affected in 2014, 8.5% of adults aged 18 years and older according to the World Health Organization [1]. Type 2 diabetes comprises 90% of diabetic people around the world. It can be treated and its complications reduced by synthetic and natural product inhibitors of carbohydrate degrading enzymes, especially α-glucosidase involved in carbohydrate digestion process increasing the glucose level in blood [2-4].

It was demonstrated that α-O-glucosides and also α-O-mannosides, conjugated on nanodiamond particles (ND) as multivalent scaffolds are inhibitors of glycosidases at low micromolar concentrations [5]. Unfortunately, such type of scaffolds does not allow to evaluate the number of saccharides which are grafted so the efficiency of the inhibition. Multivalent interactions involving oligosaccharides and proteins are now well known as key factors in molecular recognition driving many biological events [6], where the protein-saccharide binding affinity is increased by the so-called cluster effect.

Cyclodextrins (CDs) are cyclic oligomers of α (1→4)–glucopyranose (Glcp) units able to form well-known host-guest complexes, driven by non-covalent interactions. [7,8]. Common CDs are classified according to their number of Glcp units as follows: α-CD (cyclohexaamylose, six Glcp units), β-CD (cycloheptaamylose, seven Glcp units) and γ-CD (cyclooctaamylose, eight Glcp units) [9]. A CD molecule is considered as a nanoparticle (outer diameter from 1.46 nm onwards) with a hydrophilic outer wall and a hydrophobic inner cavity, which can include non-polar compounds [8]. Among all CDs, β-CD is the most described in literature, due to its availability in large amounts and its low cost.

Moreover, CDs are appealing scaffolds to design multivalent glycoconjugates [10], as their primary hydroxyls can be regioselectively functionalized. Some saccharide-branched CDs synthesized chemically or chemo-enzymatically are monosubstituted derivatives, in which mono-, di- or oligosaccharides are grafted either directly or via a spacer arm [11]. Some CD derivatives have been previously described as suitable glycoclusters interacting with lectins [12,13]. Moreover, β-CD grafted with iminosugars were potent glycosidase inhibitors [14,15]. Recently, glucosyl and mannosyl-CD were reported as potential Trojan horses to improve effects of antibiotics in bacteria growth, opening new potential applications [16], a heptavalent β-CD tethered with n-heptyl α-D-mannoside as a nanomolar adhesin FimH antagonist [17]. β-CDs carrying GlcNAc, Gal, Lac and LacNAc residues exhibited enhanced inhibition of erythrocytes agglutination through binding to wheat germ agglutinin (WGA) and Erythrina corallodendron lectin (ECorL) [18]. Mannoside and rhamnoside β-CD derivatives have been tested as inhibitors of binding of ConA to yeast mannan ($0.1<IC_{50}<0.4$μM) [19].

Copper-catalyzed azide alkyne cycloaddition (CuAAC) has been extensively used for grafting sugar units onto oligosaccharides [20] but, to our knowledge, only few examples were described with CDs. Per-azido β-CD and alkyne functionalized mannoside have been coupled using a CuBr and 4,4'-bypyridine (bpy) catalyst in DMSO yielding a β-CD-$(Man)_7$ glycocluster [21]. β-CD substituted with the iminosugar 1-deoxynojirimycin (DNJ) has

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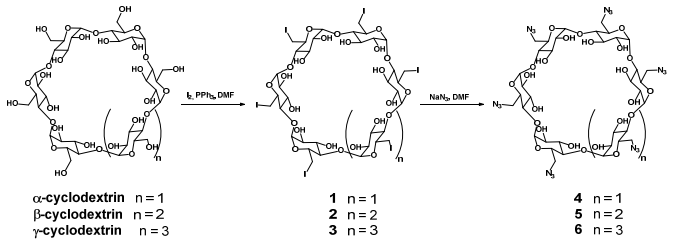
been prepared by CuAAC from a hepta-propargylated β-CD derivative and N-(ω-azidoalkyl)-DNJ [22].

Herein, we introduce a robust and versatile approach using microwaves to synthesize a glycosyl-CD family by CuAAC from different propargyl glycosides and 6-deoxy-6-azido α-, β- or γ-CDs. Reaction conditions were carefully optimized to meet as much as possible the green chemistry criteria, especially to limit the amount of chemical reagents used. Two compounds revealed to be suitable inhibitors of α-glucosidase compared to iminosugars as acarbose. Finally, inhibition tests showed that α-glucosylated cyclodextrin acted not as a substrate but as a mixed-type non-competitive inhibitor showing the effect of multivalent presentation of the substrate on enzyme activity.

Results and discussion

The synthetic route starts by the halogenation of primary positions of unprotected cyclodextrins [23]. Thus, CDs were separately reacted with I_2-PPh_3 in DMF, leading to the corresponding per-6-deoxy-6-iodo-CD derivatives (Scheme 1). Characterization of the obtained products by ESI-MS revealed the presence of pseudo-molecular ions at m/z 1655.22, 1927.25 and 2198.80, corresponding to $[M+Na]^+$ of α-, β- and γ-CD derivatives, respectively. ^{13}C NMR spectra exhibited all the characteristic signals of CH_2-I at the C6 which are shifted downfield ($\Delta\delta$ = 50 ppm) with respect to the initial ones corresponding to CH_2OH. Moreover, a unique peak was observed for CH_2I as required by the symmetries of uniformly hexa-, hepta- and octa-substituted α-, β- and γ-CD, respectively. The main advantage of this Vilsmeier-type iodination method [24, 25] is that by-products are easily removed by treatment with sodium methanolate to cleave the formate esters previously formed. Our procedure for iodination was modified from previously reported ones [26-28].

First, the commercial CDs were thoroughly freeze-dried before use. Second, the addition of sodium methoxide 3 M in methanol to adjust the pH in the range 9-10 was performed at room temperature without further cooling, and the solution was kept at room temperature at least for 30 min to cleave the formate



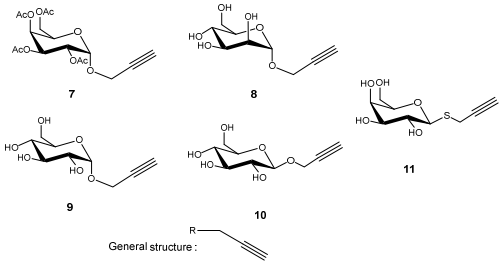
Scheme 1: Synthesis pathway of per-6-azido CDs from native CDs.

esters formed during the reaction. Finally, the expected iodo-CDs were isolated by overnight precipitation in dichloromethane. With these improved conditions, yields were 71, 88 and 79% of per-6–deoxy-6-iodo α-, β-, and γ-CDs, respectively.

The obtained per-6-halogenated derivatives were used for the synthesis of per-6-deoxy-6-azido α-, β-, γ-CDs intermediates via nucleophilic substitution with azide anion [27-31]. Highly pure α-, β-, and γ-CD azido-derivatives were obtained in 78, 90 and 87% yields, respectively. ^{13}C NMR chemical shifts of C6 within per-azido CDs at 51.60 ppm, 51.58 ppm and 51.79 ppm for per-6-deoxy-6-azido α-, β-, γ-CDs, respectively, were in good agreement with previous reported data [32].

On the other hand, the propargylated monosaccharides (Scheme 2) used in this study were synthesized according to procedures described elsewhere [33-38].

Click reaction in aqueous medium of $CuSO_4.5H_2O$/sodium ascorbate at room temperature failed to lead to expected clicked products. Further runs were attempted by varying the temperature (45-80 °C), the number of equivalents of the catalyst, and the reaction time (from several days to 2 weeks). Unfortunately, the targeted products were obtained in low yields and/or in the form of unresolving mixtures with by-products. To overcome such limitations, we noticed that microwaves were previously described as an efficient tool for click chemistry [39-41]. Herein, microwaves were combined with CuI - *N,N*-diisopropylethylamine (DIPEA) instead of common $CuSO_4.5H_2O$/sodium ascorbate couple. It is well documented that copper(I) iodide as the catalyst in the presence of DIPEA can significantly increase yields of CuAAC [38]. The click reactions were performed using both protected and unprotected propargyl glycosides, affording a library of glycosyl CDs (Scheme 3) in moderate to excellent yields (28-95%).



Scheme 2: Structures of propargyl glycopyranosides.

The results obtained for the various conjugation assays between the three CDs and the propargyl glycosides clearly showed that the reaction is quite versatile (Scheme 3), whatever the type of monosaccharide, the anomeric centre (α, β), the nature of the glycoside bond (O, S). Nevertheless, in some case no reaction occurred, such as for propargyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside **7** where the reaction was effective with α- and γ-CDN_3 (leading to **12** and **13**), but not for β-CDN_3 under the same conditions. Indeed, TLC, HRMS and, ^{13}C NMR only revealed the initial propargyl glycoside and some partially substituted intermediates. Such results can be due to the steric hindrance driven by acetylated groups avoiding any reactivity on the β-CD having the more rigid structure compared to α- and γ-CD. Although the reaction with protected glycosides

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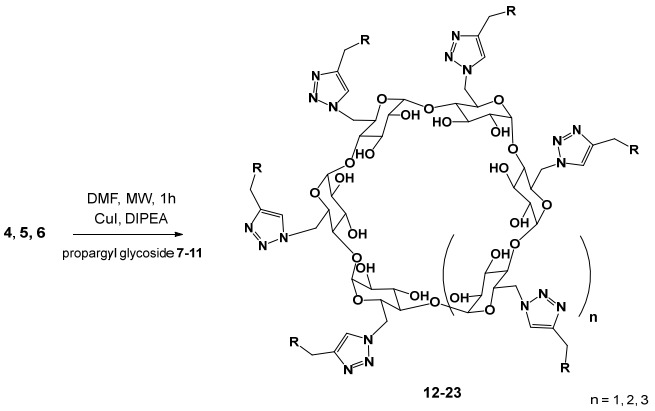
was efficient, the deprotection of **12** and **13** was difficult leading to mixture of partially deprotected glycosylated cyclodextrins.



Propargyl glycoside	Product (yield)		
	α-CD	β-CD	γ-CD
7	**12** (77%)	(<5%)	**13** (67%)
8	(<5%)	**14** (95%)	**15** (95%)
9	**16** (77%)	**17** (92%)	**18** (93%)
10	**19** (28%)	**20** (79%)	not synthesized
11	**21** (47%)	**22** (31%)	**23** (84%)

Scheme 3: Synthesis and yields of glycosyl-CDs by CuI /DIPEA based CuAAC reaction (with R=glycoside, see Scheme 2).

We have thus preferred to click unprotected glycosides (**8-11**) because of their use in aqueous inhibition tests without any further modification. In most cases, yields were as high as yields with acetylated glycosides. Some low yields can be explained by a limited efficiency of purification steps of glycosylated CDs by flash chromatography. Silica gel column chromatography was performed using a acetone-methanol-water gradient to remove

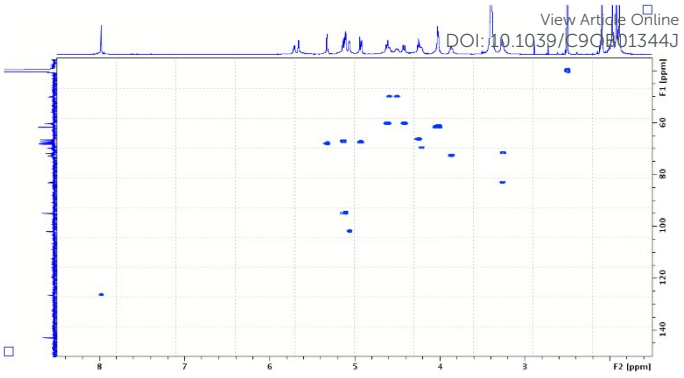


Figure 1: HSQC NMR experiment of **12** (DMSO d6, 25°C)

the excess of monosaccharides. Then the CDs derivatives were eluted in pure water. All the glycosyl-CDs were characterized by NMR and by ESI-HRMS to confirm their structure. As an example, HSQC NMR spectrum of compound **12** is shown in Figure 1.

The quaternary carbon atom of triazole was observed at 142 ppm as well as the CH-triazole at $^{1}H/^{13}C$ 8.02/125.1 ppm, the CH_2-triazole at 4.63/50.28 ppm, and the CH_2 of the linker at 4.67/60.69 ppm. In addition, the structure of this clicked product was confirmed by HRMS (ESI+): m/z 1743.0302, corresponding to the doubly sodiated ion (Figure 2).

Previous studies have shown that glycosides normally accepted as substrates by glycosyl hydrolases, can inhibit these enzymes when presented in multivalent structures on nanodiamonds [4]. The glycoCDs prepared in this study were therefore evaluated for α-glucosidase inhibition. As a model, we have chosen α-glucosidase from *Saccharomyces cerevisiae* because mammalian glucosidases are not commercially available [4]. Some of the glycoCDs showed strong inhibition of the enzyme, as the glucose derivatives of β-CD (**17**) and γ-CD (**18**), and the mannose derivative of γ-CD (**15**) with IC_{50} values about 2-7 fold lower than acarbose used as positive control inhibitor (Figure 3). IC_{50} values were 31.8 µM, 67.4 µM, 132.4 µM for compounds **15**, **17** and **18**, respectively, while values for acarbose were about 250 µM. As expected native β-CD and methyl-α-D-glucopyranoside showed no inhibition.

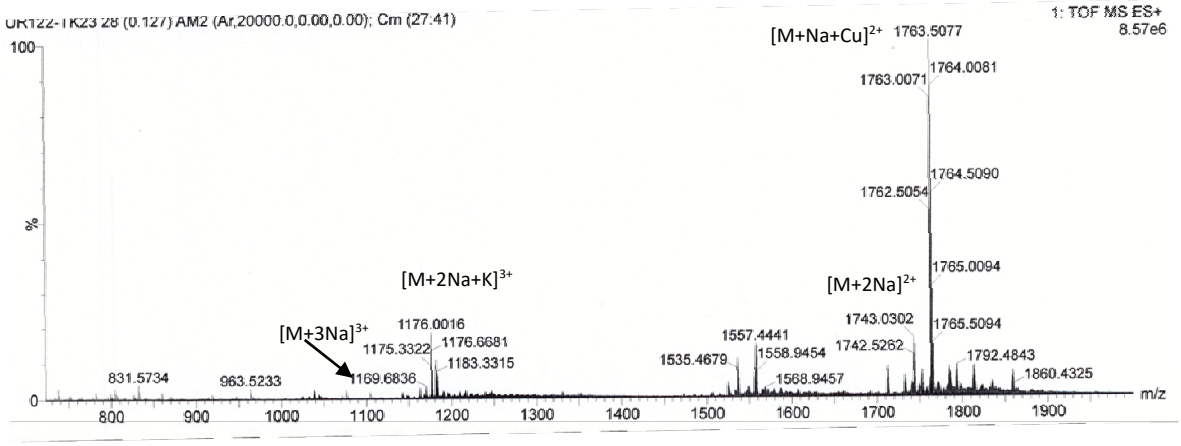


Figure 2: ESI+ HRMS of compound **12**

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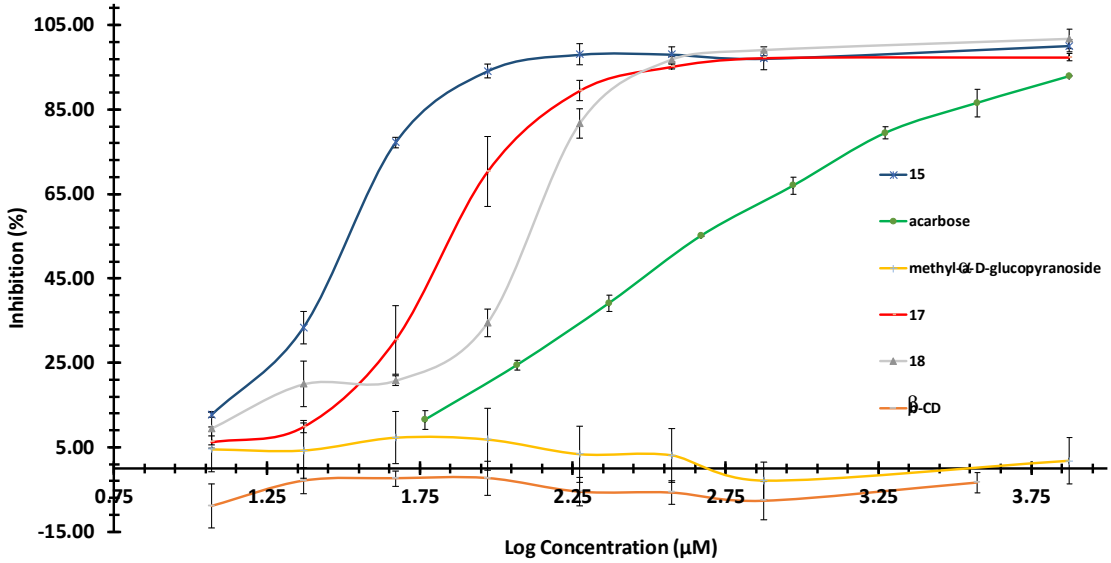

Figure 3: Percentage of inhibition of α-glucosidase by cyclodextrin derivatives (see experimental section for details). Acarbose is used as positive control, β-CD and methyl-α-D-glucopyranoside are negative controls (n=3).

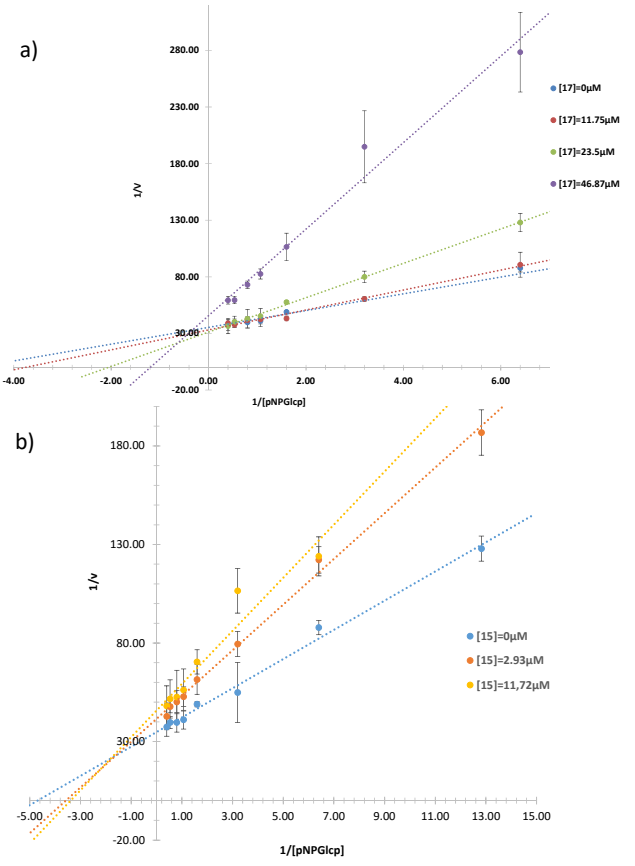

Figure 4 Lineweaver-Burk plots for different concentrations of a) **17** and b) **15** (n=3). V is in $\Delta Abs.min^{-1}$, [pNPGlcp] is the concentration of *p*-nitrophenyl α-D-glucopyranoside used as substrate in mM.

As previously described, multipresentation of α-D-mannopyranoside led to good α-glucosidases inhibitors [5,42]. In the α-D-glucopyranoside family, the β-CD derivative **17** is a better inhibitor than the γ-CD **18**. This could be explained by the higher flexibility of γ-CD compared to β-CD even if this scaffold can graft one additional monosaccharide.

In the case of α-D-glucopyranoside, variation of concentration of inhibitor **17** from 0 to 188 µM and **15** (0 to 11.72µM) was studied to evaluate their mode of inhibition. Kinetic study using the double reciprocal Lineweaver-Burk plot (Figure 4) revealed that **17** and **15** inhibited α-glucosidase thanks an unusual mixed-type non-competitive inhibition mode compared to acarbose well known as a competitive inhibitor.

Experimental

Materials and methods

Wacker Chemicals, Sigma- Aldrich, Fluka, Acros and TCI supplied reagents and solvents. DMF were distilled under argon atmosphere (on barium oxide) and stored with molecular sieves. Deuterated solvents ($CDCl_3$, D_2O, DMSO-d_6) were purchased from Eurisotop (France).

Microwave conditions

The apparatus is a Discover from CEM (France). It was used in dy namic mode with a pre-stirring of 2min. Pressure was controlled at 247 psi with power 200 W, temperature at 110°C for 1h.

Chromatography

Chromatographic separations were done in glass column filled with silica gel (GERUDAN®, MERCK). Eluent composition was specified for each compound in experimental part. Moreover,

flash chromatographic device (Reveleris iES Flash System) supplied by Grace was also used. The compound detection was achieved with an evaporative light scattering detector and a UV detector at two wavelengths (200 nm to 400 nm).

Mass Spectrometry

Stepwise control of the reactions has been readily achieved using electrospray ionisation mass spectrometry (ESI-MS) using a ZQ 4000 quadrupole mass spectrometer (Waters-Micromass, Manchester, U.K.), provided with a pneumatically assisted electrospray (Z-spray) ion source. The compounds were dissolved in MeOH, acetonitrile or water, alone or in mixture and were directly introduced into the ESI source at a flow rate of 20 µL/min through an integrated syringe pump. Source and desolvation temperatures were set to 80°C and 150°C, respectively. Nitrogen has been used as desolvation and nebulization gas with an output of 350 and 50 L/h, respectively. The capillary voltage was around 3.5 kV and the cone voltage was optimized from 20 to 150 V depending on analysed products. Spectra were recorded at 2s/scan rate. High resolution electrospray mass spectrometry (ESI-HRMS) analyses were performed on a SYNAPT G2-Si-Q-TOF hybrid quadrupole time-of-flight instrument (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source (Z-spray) and an additional sprayer for the reference compound (Lock Spray). Automated flow injections were performed using an ACQUITY UPLC H-Class system (Waters, Manchester, UK) coupled to the SYNAPT G2-Si-Q-TOF. The mobile phase composition was optimized according to the solvent used to dissolve the compounds and the flow rate was set to 0.4 mL/min. One microliter of each sample was injected and analyzed by ESI-HRMS in the positive ionization mode over the mass range 50-4000 at 0.4s/scan during an acquisition time set to 2 min.

The electrospray ionization conditions used a capillary voltage of 3 kV, a sampling cone voltage at 120 V and a source offset voltage at 40 V. Source and desolvation gas temperatures were 120 °C and 450 °C, respectively. Nitrogen (>99.5%) was employed as desolvation gas and cone gas with flows set to 600 L/h and 50 L/h, respectively. Mass calibration was performed using a solution containing a mixture of sodium and cesium iodide. A Leu-enkephalin solution was used for lock mass correction by using m/z 556.2771 in order to obtain accurate mass measurements. The time of flight (TOF) was operated in the resolution mode, with an average resolving power reaching 25,000 (FWHM). Data acquisition was performed with MassLynx software (V4.1, Waters).

Nuclear Magnetic Resonance (NMR)

Structure elucidation of compounds has been readily accomplished by recording standard ^{1}H, ^{13}C (or DEPTQ) spectra, and 2D experiments such as ^{1}H-^{1}H COSY, and ^{1}H-^{13}C HSQC. All NMR experiments were performed at 298 K on a Bruker Avance III 600 Mhz spectrometer or a Bruker Avance III HD 400 MHz spectrometer (Bruker, Wissembourg, France). Samples were dissolved in deuterated solvent (Methanol-d_4, $CDCl_3$, DMSO-d_6, D_2O, Pyridine-d_5). Chemical shift (δ) was expressed in ppm and ^{1}H and ^{13}C spectra were calibrated using the residual solvent signal. Spectra processing was performed thanks to the following softwares: MestRenova 6.0.2 (MestRelab) or Topspin 3.2 (Bruker).

Enzymatic tests

We herein examine the behaviour of some our CDs multivalent derivatives towards α-glucosidase inhibition from *Saccharomyces cerevisiae*. The inhibitory activity of our compounds and Acarbose was determined spectrophotometrically (UV-Visible, Tecan infinite M1000) on 96-well microplate reader by studying the reaction of hydrolysis of *p*-nitrophenyl-α-D-glucopyranoside. In the sample group (As), to a total of 60µL of reaction mixture contained 20µL of 100mM phosphate buffer pH 6.8, 20 µL of our compounds at different concentrations, 20 µL of 2.5 mM *p*-NPGlcp in phosphate buffer then 20 µL of 0.2 U/mL α-glucosidase in phosphate buffer were added and mixed well. In the control group (Ac), the composition of the reaction mixture was the same as in sample group except that phosphate buffer was used instead of our compounds, acarbose and methy-α-D-glucopyranoside. In the sample blank group (Asb) and in control blank group (Acb), the composition was the same as in sample and control except that phosphate buffer was used instead of α-glucosidase. After incubation at 37°C for 15 min, the reaction was stopped by adding 80 µL of 0.2 M sodium carbonate solution [43,44]. Then the absorbance was measured at 405 nm. Experiments were performed in triplicates.

The inhibitory activity was calculated using the following formula:

Inhibitory activity (%): (1-((As-Asb) /(Ac-Acb)))*100

Different concentrations of our products were prepared as follows:

- 30mM, 3mM, 1.5mM, 750µM, 375µM, 187.5µM, 93.7µM and 46.8µM for the compounds.
- 6mM, 3mM, 1.5mM, 750µM, 375µM, 187.5µM, 93.7µM and 46.8µM for our compounds and methy-α-D-glucopyranoside as negative controls.
- 30mM, 15mM, 7.5mM, 3.75mM, 1.87mM, 937µM, 468µM and 234µM for acarbose as standard reference.

To create the Lineweaver-Burk plot, a kinetical study in 30 min with measurements every 5 min is performed in same conditions to that used for the α-glucosidase inhibitory assay. The substrate was added at 8 concentrations: 10, 7.5, 5, 3.75, 2.5, 1.25, 0.625, and 0.312 mM. The mode of inhibition was defined as competitive, non-competitive or mixed-type non-competitive, according to the Michaelis-Menten constants and the maximum velocity on the Lineweaver-Burk plot. Experiments were performed in triplicates.

Synthetic procedures

Hexakis (6-deoxy-6-iodo)-cyclomaltohexaose (1)

Under argon, PPh_3 (3.9 g, 14.8 mmol) and I_2 (3.75 g, 14.8 mmol) were dissolved in anhydrous DMF (16 mL). The reaction mixture was stirred for 10 min then lyophilized α-cyclodextrin (0.8 g, 0.82 mmol) was added to the above mixture and stirred overnight at room temperature. After concentration under

vacuum to 5 mL, the pH was adjusted in the range 9-10 by the addition of 3 M sodium methoxide in methanol (30 mL) with continuous stirring at room temperature for 30 min. The mixture was poured into ice water, and the precipitate formed and filtrated. DCM was added to the solid and left overnight. The precipitate was separated and extensively washed with DCM until the filtrate becomes transparent, and finally with methanol. After drying at 60° for 24 h, a faint brown solid product was obtained (0.95 g, 71% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.53-5.68 (12H, m, OH), 4.92 (6H, s, H1), 3.80 (6H, bs, H2), 3.32 (6H, bs, H4), 3.61-3.67 (12H, 2s, H6), 3.34-3.51 (12H, m, H3, H5). ^{13}C NMR in agreement with reference 25. ESI-MS: m/z 1655.22 $[M+Na]^+$.

Heptakis (6-deoxy-6-iodo)-cyclomaltoheptaose (2)

The same procedure for the synthesis of **1** was used. DMF (16 mL), PPh_3 (4.2 g, 16 mmol) and I_2 (4.0 g, 16 mmol), lyophilized β-cyclodextrin (0.8 g, 0.71 mmol) gave 1.18 g (88% yield) of **2**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.29 (7H, m, H4), 3.33 (7H, m, H2), 3.44 (7H, m, H6a), 3.57 (7H, m , H5), 3.65 (7H, m, H3), 3.81(7H, bd, H6b), 4.99 (7H, bs, H1), 5.91-6.01 (14H, 3s, OH). ^{13}C NMR in agreement with reference 25. ESI-MS: m/z 1927.25 $[M+Na]^+$.

Octakis (6-deoxy-6-iodo)-cyclomaltooctaose (3)

The same procedure for the synthesis of **1** was used. DMF (16 mL), PPh_3 (4.4 g, 16.6 mmol) and I_2 (4.2 g, 16.6 mmol), lyophilized γ-cyclodextrin (0.9 g, 0.69 mmol) gave 1.19 g (79 % yield) of **3**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.98-5.74 (16H, 2 bs, OH), 5.02 (8H, s, H1), 3.80 (8H, bs , H2), 3.60 (16H, bs, H6) , 3.39 (16 H, bs, H3, H5), 3.27 (8H, bs, H4). ^{13}C NMR in agreement with references 27. ESI-MS: m/z 2198.80 $[M+Na]^+$.

Hexakis (6-azido-6-deoxy)-cyclomaltohexaose (4)

To a solution of compound **1** (0.95g, 0.58 mmol) in anhydrous DMF (34 mL), NaN_3 (0.567 g, 8.73 mmol) was added at room temperature, the reaction mixture was left stirring under argon atmosphere for 24 h. The suspension was evaporated from DMF to 3mL under reduced pressure, then about 100 mL from fresh deionized water was added to the above suspension, a faint yellowish precipitate was formed which was filtered and washed 5 times with deionized water then dried at 60° under vacuum, to afford compound **4** as a white solid (0.51 g, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.60-3.72 (12H, m, H6), 4.88 (6H, s, H1), 5.47-5.66 (12H, 3s, OH), 3.37-3.81 (18H, m, H2, H3, H5), 3.37 (6H, m, H4). ^{13}C NMR (101 MHz, DMSO-d_6): δ 102.22 (C1), 83.64 (C4), 72.96 (C3), 71.81 (C5), 70.65 (C2), 51.60 (C6). ESI-MS: m/z 1122 $[M+Na]^+$.

Heptakis (6-azido-6-deoxy)-cyclomaltoheptaose (5)

The same procedure for the synthesis of **4** was used. Compound **2** (0.33 g, 0.17 mmol), DMF (12 mL) and NaN_3 (0.169 g, 2.60 mmol) gave compound **5** as a white solid (0.205 g, 90% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.76-5.91 (14H, 3s, OH), 4.91 (7H, bs, H1), 3.34 (7H, m, H4), 3.74 (7H, m, H2), 3.60 (7H, m, H3), 3.38 (7H, m, H5), 3.60-3.78 (14H, m, H6). ^{13}C NMR (101 MHz, DMSO-d_6): δ 102.47 (C1), 83.62 (C4), 70.76 (C2), 73.04 (C3), 72.49 (C5), 51.73-51.77 (C6).
ESI-MS: m/z 1333 $[M+Na]^+$.

Octakis (6-azido-6-deoxy)-cyclomaltooctaose (6)

The same procedure for the synthesis of **4** was used. Compound **3** (1.2 g, 0.55 mmol), DMF (43 mL) and NaN_3 (0.536 g, 8.25 mmol) gave compound **6** as a white solid (0.72 g, 87% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.86-5.92 (16H, 3s, OH), 4.94 (8H, m , H1), 3.35 (8H, m, H4), 3.74 (8H, m, H2), 3.57 (8H, m, H3), 3.40 (8H, m, H5),3.58-3.72 (16H, m, H6). ^{13}C NMR in agreement with reference 27. ESI-MS: m/z 1520 $[M+Na]^+$.

General procedure for the CuAAC

In a microwave reactor (10mL) under argon, to a solution of per-azido α-, β- or γ-CD (**4**, **5**, or **6**, respectively) (0.0445 mmol) and the corresponding propargyl glycoside (18, 21 or 24 equivalents, respectively) in 2 mL of degassed dry DMF, CuI (5.1 mg, 0.0267 mmol) was added, followed by DIPEA (34 µL, 0.2 mmol). The mixture was irradiated at 200 W, and 110°C for 1h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography.

Hexakis [6-deoxy-6-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxyethyl)triazol-1-yl]-cyclomaltohexaose (12)

The excess of propargyl glycoside was eluted in 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was then washed with deionized water then the filtrate was lyophilized to give a white solid product (0.118 g, 77% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.86-2.11 (72H, m, CH_3CO), 3.88 (6H, m, H'3), 3.87 (6H, m, H'5), 3.26 (6H, m, H'4), 4.02 (12H, s, H6), 4.23 (6H, m, H4), 4.25 (6H, m, H'2), 4.26 (6H, m, H'2), 4.63 (12H, m, H'6) , 4.67 (12H, m, CH_2-linker), 4.93 (6H, s, H3), 5.06 (6H, s, H'1), 5.11 (6H, m, H1), 5.11 (6H, m, H2), 5.32 (6H, s, H5), 5.66-5.72 (12H, OH), 8.08 (6H, s, CH-triazole). ^{13}C NMR (151 MHz, DMSO-d_6) δ 20.82-20.85 ($\underline{C}H_3CO$), 169.05-169.54 (C=O), 70.15 (C'2), 72.87 (C'5), 83.15 (C'4), 61.71 (C6), 66.67 (C4), 73.48 (C'3), 50.28 (C'6), 60.69 (CH_2-linker), 67.38 (C2), 67.87 (C3), 68.03 (C5), 94.89 (C1), 101.74 (C'1), 125.08 (CH-triazole), 142.08 (qC triazole). HRMS (ESI+): $[M + 2Na]^{2+}$ 1743.0302 (calculated $[M + 2Na]^{2+}$ 1743.0332).

Octakis [6-deoxy-6-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyloxyethyl)triazol-1-yl]-cyclomaltooctaose (13)

The excess of propargyl glycoside was eluted in 1:1 Acetone/H_2O. The silica gel was then washed with deionized water then the filtrate was lyophilized to give a white solid product (0.102 g, 67% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (8H, s, CH-triazole), 1.88-2.09 (96H, 4s, CH_3CO), 4.40 (8H, m, H6a), 4.95 (8H, m, H6b), 4.04 (16H, m, CH_2-linker), 4.25 (8H, m, H3), 4.93 (8H, m, H5), 5.14 (8H, m, H4), 5.33 (8H, m, H2), 4.02 (8H, m, H'3), 3.70 (8H, m, H'5), 3.40 (8H, s, H'2), 3.72 (8H, m, H'4), 5.17 (8H, m, H1), 5.15 (8H, m, H'1), 4.39 (16H, m, H'6), 5.99-6.01 (16H, OH). ^{13}C NMR (101 MHz, DMSO-d_6) δ 20.93-21.06 ($\underline{C}H_3CO$), 61.70 (CH_2-linker), 60.21-60.51 (C6), 66.38 (C3), 67.18 (C5), 66.88 (C4), 67.48 (C2), 69.72 (C'3), 72.31 (C'5), 72.46 (C'2), 81.72 (C'4), 95.11 (C1), 102.08 (C'1), 126.53 (CH-triazole), 49.79 (C'6), 170.09-170.61 (C=O), 143.10 (qC triazole). HRMS (ESI+): $[M + 3Na]^{3+}$ 1552.1418 (calculated $[M + 3Na]^{3+}$ 1552.1384).

Heptakis [6-deoxy-6-(α-D-mannopyranosyloxyethyl)triazol-1-yl]-cyclomaltoheptaose (14)

The excess of propargyl glycoside was eluted in 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was washed with

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deionized water then the filtrate was lyophilized to give a white solid product (0.103 g, 95% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.17-4.36 (14H, m, H'6), 4.36-4.59 (14H, m, CH_2-linker), 8.00 (7H, s, CH-triazole), 3.66-3.75 (14H, m, H6), 3.53 (7H, m, H4), 4.19 (7H, m, H2), 3.93 (7H, m, H3), 3.32 (7H, m, H'2), 3.46 (7H, m, H'3), 3.57 (7H, m, H5), 3.66 (7H, m, H'5), 3.32 (7H, m, H'4), 4.71 (7H, m, H1), 5.15 (7H, s, H'1). ^{13}C NMR (101 MHz, D_2O) δ 72.94 (C'3), 82.61 (C'4), 71.62 (C5), 69.93 (C'5), 72.33 (C'2), 72.35 (C3), 70.05 (C2), 66.63 (C4), 60.83-60.87 (C6), 50.45-50.49 (C'6), 99.34 (C1), 59.51-59.53 (CH_2-linker), 101.77 (C'1), 126.87 (CH-triazole), 143.73 (qC triazole). HRMS (ESI+): [M+ 2Na]$^{2+}$ 1441.4779 (calculated [M+2Na]$^{2+}$ 1441.4755).

Octakis [6-deoxy-6-(α-D-mannopyranosyloxyethyl)triazol-1-yl]-cyclomaltooctaose (15)

The excess of propargyl glycoside was eluted in 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was washed with deionized water then the filtrate was lyophilized to give a white solid product (0.123 g, 95% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.20-4.32 (16H, m, H'6), 4.42-4.64 (16H, m, CH_2-linker), 8.05 (8H, s, CH-triazole), 3.78-3.87 (16H, m, H6), 3.66 (8H, m, H4), 4.27 (8H, m, H2), 3.98 (8H, m, H3), 3.60 (8H, m, H'2), 3.68 (16H, m, H'3, H5), 3.66 (8H, m, H'5), 3.43 (8H, m, H'4), 4.83 (8H, m, H1), 5.16 (8H, bs, H'1). ^{13}C NMR (101 MHz, D_2O) δ 71.89 (C'3, C5), 82.26 (C'4), 70.46 (C'5), 72.86 (C'2), 72.15 (C3), 69.89 (C2), 66.60(C4), 60.82-60.84(C6), 50.28-50.32 (C'6), 99.26 (C1), 59.38-59.39 (CH_2-linker), 101.62 (C'1), 126.60 (CH-triazole), 143.49 (qC triazole). HRMS for the purified product showed at 1643.5400 [M+2Na]$^{2+}$(calculated [M+2Na]$^{2+}$ 1643.5431).

Hexakis [6-deoxy-6-(α-D-glucopyranosyloxyethyl)triazol-1-yl]-cyclomaltohexaose (16)

The excess of propargyl glycoside was eluted in 1:1 Acetone/Methanol. The silica gel was washed with deionized water then the filtrate was lyophilized to give a pale yellow solid product (0.083 g, 77% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.30-4.63 (12H, m, H'6), 4.47-4.64 (12H, m, CH_2-linker), 3.71 (12H, m, H6), 3.56-3.68 (12H, m, H2-H5), 3.35 (6H, m, H'2), 4.29 (6H, m, H'5), 4.02 (6H, m, H'3), 3.46-3.47 (12H, m, H3, H4), 3.38 (6H, m, H'4), 4.85 (6H, s, H1), 5.15 (6H, s, H'1), 8.09 (6H, s, CH-triazol). ^{13}C NMR (101 MHz, D_2O) δ 50.89-50.90 (C'6), 60.38-60.42 (CH_2-linker), 60.92 (C6), 69.95 (C2,C5), 69.94 (C'2), 70.58 (C'5), 73.10 (C'3), 71.64-73.51 (C3, C4), 82.85 (C'4), 98.25 (C1), 101.80 (C'1), 127.62 (CH-triazole), 154.74 (qC triazole). HRMS (ESI+): [M+ 2Na]$^{2+}$ 1238.9033 (calculated [M+2H]$^{2+}$ 1238.9064).

Heptakis [6-deoxy-6-(α-D-glucopyranosyloxyethyl)triazol-1-yl]-cyclomaltoheptaose (17)

The excess of propargyl glycoside was eluted in 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was washed with deionized water then the filtrate was lyophilized to give a white solid product (0.100 g, 92% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.22-4.47 (14H, m, H'6), 3.70 (14H, m, CH_2-linker), 4.41-4.62 (14H, m, H6) , 4.21 (7H, m, H'5), 3.95 (7H, m, H3), 3.42 (7H, m, H'3), 3.64 (7H, m, H'2), 3.34-3.53 (21H, m, H2, H4, H5), 3.36 (7H, m, H'4), 5.03 (7H, s, H1), 5.10 (7H, s, H'1). ^{13}C NMR (101 MHz, D_2O) δ 50.28-50.30 (C'6), 60.38 (CH_2-linker), 59.84-59.85 (C6), 69.89 (C'5), 72.32 (C3), 72.93 (C'3), 69.34 (C'2), 69.36-69.37 (C2, C4, C5), 82.28 (C'4), 97.12 (C1), 101.59 (C'1), 143.56 (qC triazole). HRMS (ESI+): [M+Na+H]$^{2+}$ 1430.4838; [M+2H]$^{2+}$ 1419.4931; [M+ 2Na]$^{2+}$ 1441.4745 (calculated [M+2H]$^{2+}$ 1419.4932).

Octakis [6-deoxy-6-(α-D-glucopyranosyloxyethyl)triazol-1-yl]-cyclomaltooctaose (18)

The excess of propargyl glycoside was eluted in 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was washed with deionized water then the filtrate was lyophilized to give a white solid product (0.102 g, 93% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.19-4.33 (16H,m, H'6), 4.40-4.60 (16H, m, CH_2-linker), 8.02 (8H, s, CH-triazole), 3.72 (16H, m, H6), 3.58 (16H, m, H3, H4), 4.27 (8H, m, H2), 3.58 (16H, m, H3), 3.36 (8H, m, H'2), 3.56 (16H, m, H2, H5), 4.21 (8H, m, H'5), 3.38 (8H, m, H'4), 4.85 (8H, m, H1), 5.12 (8H, bs, H'1), 3.93 (8H, m, H'3). ^{13}C NMR (101 MHz, D_2O) δ 72.22 (C'3), 82.04 (C'4), 69.80 (C'5), 69.45 (C'2), 71.90 (C3, C4), 69.46 (C2, C5), 60.46 (C6), 50.24-50.25 (C'6), 97.81 (C1), 59.92-59.94 (CH_2-linker), 101.55 (C'1), 126.93 (CH-triazole), 143.65 (qC triazole). HRMS (ESI+): 1643.5422 [M+2Na] $^{2+}$ (calculated [M+2Na]$^{2+}$ 1643.5431).

Hexakis [6-deoxy-6-(β-D-glucopyranosyloxyethyl)triazol-1-yl]-cyclomaltohexaose (19)

The excess of propargyl glycoside was eluted in Acetone. The silica gel was washed with deionized water then the filtrate was lyophilized to give a white solid product (0.030 g, 28% yield). ^{1}H NMR (400 MHz, D_2O/MeOD d4) δ 4.70 (12H, m, H'6), 3.74-3.92 (12H, m, CH_2-linker) 4.74-4.86 (12H, m, H6), 3.37 (6H, m, H'2), 4.33 (6H, m, H'5), 3.24 (6H, m, H'3), 3.46-4.04 (24H, m, H2, H3, H4, H5), 3.42 (6H, m, H'4), 4.50 (6H, m, H1), 5.22 (6H, m, H'1), 8.14 (6H, s, CH-triazole). ^{13}C NMR (101 MHz, D_2O/MeOD d4) δ 50.46 (C'6), 60.93-60.96 (CH_2-linker), 61.76-61.86 (C6), 69.80 (C'2), 70.26 (C'5), 73.21 (C'3), 71.45-76.07 (C2, C3, C4, C5), 82.55 (C'4), 101.82 (C1), 101.64 (C'1), 127.07 (CH-triazole), 151.03 (qC triazole). HRMS (ESI+): [M+Na+H]$^{2+}$ 1227.4132; [M+2H]$^{2+}$ 1216.9239; [M+ 2Na]$^{2+}$ 1238.9056 (calculated [M+2H]$^{2+}$ 1216.9240).

Heptakis [6-deoxy-6-(β-D-glucopyranosyloxyethyl)triazol-1-yl]-cyclomaltoheptaose (20)

The excess of propargyl glycoside was eluted in 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was washed with deionized water then the filtrate was lyophilized to give a pale yellow solid product (0.048 g, 79% yield). ^{1}H NMR (400 MHz, D_2O/MeOD d4) δ 4.08–4.49 (14H, m, H'6), 3.74-3.92 (14H, m, CH_2-linker), 3.52-3.68 (14H, m, H6), 3.69-4.32 (21H, m, H'2, H'5, H'3), 3.54- 4.37 (28H, m, H2, H3, H4, H5), 3.04 (7H, m, H'4), 4.88 (7H, m, H1), 5.05 (7H, m, H'1), 7.61 (7H, s, CH-triazole). ^{13}C NMR (101 MHz, D_2O/MeOD d4) δ 49.42 (C'6), 61.16 (CH_2-linker), 62.71 (C6), 72.10-74.95 (C'2, C'5, C'3), 70.94-77.05 (C2, C3, C4, C5), 81.07 (C'4), 102.07 (C1), 102.22 (C'1), 124.04 (CH-triazole), 143.59 (qC triazole). HRMS (ESI+): [M+ 2Na]$^{2+}$ 1441.4738 (calculated [M+2Na]$^{2+}$ 1440.9842).

Hexakis [6-deoxy-6-(1-thio-β-D-galactopyranosyloxyethyl)triazol-1-yl]-cyclomaltohexaose (21)

The crude coupled product was purified by crystallization using methanol as the best solvent for dissolving the impurities and the excess of propargyl thioglycoside. The product was filtrated and dried under vacuum to give a reddish brown powder (0.064 g, 47% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.07 (12H, t, CH_2-S-

linker), 4.30-4.61(12H, m, H'6), 3.54-3.69 (12H, m, H6), 3.60 (6H, m, H'2), 3.97 (6H, m, H5), 4.08 (6H, m, H'3), 3.44 (6H, m, H'4), 3.64 (6H, m, H'5), 3.60-3.63 (18H, m, H2, H3, H4), 3.44 (6H, m, H'3), 4.38 (6H, m, H1), 5.19 (6H, m, H'1), 8.06 (6H, m, CH-triazole). ^{13}C NMR (101 MHz, D_2O) δ 23.60 (CH_2-S- linker), 50.50-50.52 (C'6), 60.98-61.11 (C6), 69.50 (C'2), 71.30 (C'5), 73.95-79.02 (C2, C3, C4), 82.47 (C'4), 68.75 (C5), 72.66 (C'3), 85.23 (C1), 101.47 (C'1), 126.45 (CH-triazole), 144.98 (qC triazole). HRMS (ESI+): $[M+Na+H]^{2+}$ 1275.8466; $[M+2H]^{2+}$ 1264.8559; $[M+2Na]^{2+}$ 1286.8374 (calculated $[M+2H]^{2+}$ 1264.8555).

Heptakis [6-deoxy-6-(1-thio-β-D-galactopyranosyloxyethyl)triazol-1-yl]-cyclomaltoheptaose (22)

The excess of propargyl glycoside was eluted using a gradient from 1:2 to 1:4 EtOAc/MeOH and finally 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was washed with deionized water then the filtrate was lyophilized to give a white solid product (0.042 g, 31% yield). ^{1}H NMR (400 MHz, D_2O) δ 3.78-3.97 (14H, m, CH_2-S-linker), 4.25-4.47 (14H, m, H'6), 3.70 (14H, m, H6), 3.61 (7H, m, H'2), 3.67 (7H, m, H'5), 3.61-3.65 (21H, m, H2, H3, H4), 3.46 (7H, m, H'4), 4.40 (7H, m, H1), 5.18 (7H, s, H'1), 7.99-8.08 (7H, 2s, CH-triazole), 3.98 (7H, m, H5), 4.04 (7H, m, H'3). ^{13}C NMR (101 MHz, D_2O) δ 23.17-23.32 (CH_2-S-linker), 50.40-50.44 (C'6), 61.07 (C6), 69.42 (C'2), 71.64 (C'5), 73.95-79.07 (C2, C3, C4), 82.48 (C'4), 85.12 (C1), 101.67 (C'1), 126.29-126.35 (CH-triazole), 144.72 (qC triazole), 68.76 (C5), 72.48 (C'3). HRMS (ESI+): $[M+2Na]^{2+}$ 1497.3910 (calculated $[M+2Na]^{2+}$ 1497.3955).

Octakis [6-deoxy-6-(1-thio-β-D-galactopyranosyloxyethyl)triazol-1-yl]-cyclomaltooctaose (23)

The excess of propargyl glycoside was eluted using a gradient from 1:2 to 1:4 EtOAc/MeOH and finally 9:1:0.2 Acetone/Methanol/H_2O. The silica gel was washed with deionized water then the filtrate was lyophilized to give a white solid product (0.113 g, 84% yield). ^{1}H NMR (600 MHz, D_2O) δ 3.75-3.84 (16H, m, CH_2-linker), 4.13-4.23 (16H, m, H'6), 3.67 (16H, s, H6), 3.86 (8H, s, H5), 4.04 (8H, m, H'3), 3.98 (8H, m, H'5), 3.59 (8H, m, H'2), 3.53-3.59 (24H, m, H2, H3, H4), 3.37 (8H, m, H'4), 4.33 (8H, m, H1), 5.08 (8H, s, H'1), 7.91 (8H, s, CH-triazole). ^{13}C NMR (151 MHz, D_2O) δ 23.39-23.40 (CH_2-S- linker), 50.49 (C'6), 61.19 (C6), 68.80 (C5), 69.47 (C'3), 71.90 (C'5), 71.88 (C'2), 69.48-78.98 (C2, C3, C4), 82.08 (C'4), 85.13 (C1), 101.68 (C'1), 126.25 (CH-triazole), 144.76 (qC triazole). HRMS (ESI+): $[M + 2Na]^{2+}$ 1708.4569 (calculated $[M + 2Na]^{2+}$ 1708.4535).

Conclusions

An efficient route to synthesize multivalent glycosylated compounds with microwave-assistance was introduced. We demonstrated that the synthesis of azido α- and γ-CDs as new scaffolds proceeds as easily as for β-CD. In spite of the differences observed in their reactivity, this work showed that the three CDs can be used as scaffolds for the synthesis of multivalent glycoclusters. Furthermore, the synthesized CDs multivalent derivatives showed significant IC_{50} (32-132 μM) towards α-glucosidase from *Saccharomyces cerevisiae*. The permannosylated β-CD and perglucosylated β-CD were identified as the best α-glycosidase inhibitors are the interacting *via* a mixed non-competitive, inhibition mode.

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Conflicts of interest

There are no conflicts to declare.

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