# Improved syntheses of $bis(\beta$ -cyclodextrin) derivatives, new carriers for gadolinium complexes

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In the last decade a number of reports have been published on the synthesis and characterization of bridged cyclodextrin dimers (bis-CDs) connected with linkers of different lengths and structures. These dimers, having two hydrophobic cavities in close proximity, display much higher binding affinities and molecular selectivities than parent CDs, forming stable supramolecular adducts. We describe new synthetic protocols for the preparation of bis( $\beta$ -CDs) bearing 2–2', 3–3' and 6–6' bridges. Some of the critical steps were carried out either under high-intensity ultrasound (US) or microwave (MW) irradiation. Bis( $\beta$ -CDs) containing 6–6' ureido- and thioureido-bridges were prepared in high yields by a MW-promoted aza-Wittig reaction using polymer-bound triphenylphosphine, while those containing 2,2' and 3,3' bridges were prepared from mono-alkenyl  $\beta$ -CDs by the cross-metathesis reaction (homodimerization) in the presence of 2<sup>nd</sup>-generation Grubbs catalyst under sonochemical conditions. By these improved protocols CD dimers could be obtained in gram amounts to prepare stable adducts of bis-CDs with contrast agents (CAs) containing gadolinium(III) chelates. In the case of Gd(III) chleate "G-1" the inclusion complexes were found to be 2 to 3 orders of magnitude more stable than that formed by  $\beta$ -CD ( $K_{ass} = 4.3 \times 10^4$  M<sup>-1</sup> vs  $8.0 \times 10^2$  M<sup>-1</sup>). Relaxivity increased as well by factors of 3 and 4, *viz.* from 9.1 mM<sup>-1</sup> s<sup>-1</sup> ( $\beta$ -CD) to 27.7 and 35 mM<sup>-1</sup> s<sup>-1</sup>.

## Introduction

Cyclodextrins (CDs) play important roles in chemical, biological and pharmaceutical technology; among their numerous applications they are used for analytical separations,<sup>1</sup> in enzyme mimics,<sup>2</sup> as drug carriers3 and to deliver diagnostic agents.4,5 CDs are among the most promising and widely employed oligosaccharide hosts for drug complexation, as CD-encapsulated drugs usually have a better bioavailability, a longer half-life in the body, unhindered excretion and no extra toxicity.6 When they are used to host contrast agents (CAs), the resulting complexes have a much larger molecular mass; moreover the change from a quasispherical molecular shape (such as that of the classic [GdDOTA]-complex) to the more irregular shape of the CD:[GdDOTA]-complex, resulting in a slower rotation in water,<sup>7</sup> will enhance contrast. However, data published to date have shown but a poor binding to CDs of lanthanide(III) chelates with polyazamacrocyclic ligands. In fact even  $\gamma$ -CD, the largest commercially available member of the family, is too small to fully include these CAs; as the guest is only partially lodged in it, a weak complex results. To overcome the snag one might use substituted  $\gamma$ -CDs that associate more efficiently with the CA,8 or resort to CD dimers in association with CAs bearing at least two substituents (e.g. two phenyl or cyclohexyl

Since the pioneer works of Tabushi<sup>10</sup> and Harada<sup>11</sup> much effort has been devoted to making bis-CDs with a variety of functional tethers.<sup>12-14</sup> Although the synthesis and properties of bis-CDs that are linked through their primary face (positions 6) are well documented, few reports have appeared to date on dimers connected through their secondary face (positions 2 and 3).<sup>15-17</sup> The latter are actually harder to prepare because selective mono-substitution is not so easily achieved on the secondary as on the primary side and, moreover, their isolation has proved more troublesome.<sup>18</sup> While selective 6-monosubstitution can usually be achieved via monotosylation, monosubstitution on the secondary face is not straightforward, the resulting products generally requiring timeconsuming chromatographic purifications. Recently we described the preparation of 2,2' bis-CDs from 6-O-TBDMS-β-CD through the monotosyl and 2-monoazido derivatives, to end with an aza-Wittig reaction by treatment with CS<sub>2</sub> and triphenylphosphine (PPh<sub>3</sub>).<sup>17</sup> These dimers were linked by a thioureidic unit directly connected to the secondary rims. Although this synthetic procedure is very efficient, it suffers from the drawback of residual PPh<sub>3</sub> remaining trapped in the dimer cavities as a stable inclusion complex. The improved procedure presented here overcomes the hurdle by employing polymer-bound PPh<sub>3</sub> under MW irradiation, resulting in much shorter reaction times and higher yields (Scheme 1).

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Scheme 1 Synthesis of the 6-6' ureido- (1) and thioureido-tethered (2) bis-CDs.

The key step in our synthesis of 2,2' and 3,3' bis-CDs is the metathesis reaction (homodimerization) with ruthenium catalysts, which we carried out under sonochemical conditions, reducing the reaction time (2 h *vs.* 8 h under conventional conditions) without significantly improving yields (Scheme 2). Olefin metathesis is now established as an important reaction of wide application in synthetic organic chemistry,<sup>19</sup> the most commonly used catalysts being first- and second generation Grubbs (Ru) reagents. The tethering of two  $\beta$ -CD molecules with chains of different lengths can afford a wide range of bis-CDs differing only in the spacing.

We are investigating all these water-soluble bridged bis( $\beta$ -CDs) as host molecules for CA-adducts containing gadolinium(III) chelates, and their fully substituted derivatives 6-*O*-TBDMS, 2,3-Me- $\beta$ -CD as chiral selectors for enantioselective gas chromatography (GC).

### **Results and discussion**

In the present work we improved the synthetic protocols for the preparation of 2-2', 3-3' and 6-6' bis( $\beta$ -CDs), testing the critical steps both under high-intensity ultrasound (US)<sup>20</sup> and under MW irradiation.<sup>21</sup> These techniques, that often complement each other, have emerged as effective promoters of organic reactions, cutting down reaction times to minutes or even seconds rather than hours or days. Against the vast literature on US- and MW-promoted reactions, only a couple of reports have so far concerned the modification of CDs under these non-conventional conditions.<sup>22,23</sup> In a previous paper we compared several CD functionalizations carried out both under conventional conditions and under US, showing that the sonochemical procedures were very advantageous in terms of yields and reaction times.<sup>22</sup>



Scheme 2 Synthesis of the 2–2' bridged bis( $\beta$ -CDs) 9 and 11. *Reagents and conditions: a*) TBDMSCl, imidazole, dry pyridine, stirring rt, 8 h; *b*) 5-bromopentane, LiH, dry THF–DMSO, reflux, 4 h; *c*) acetic anhydride, dry pyridine, MW, 50 °C, 1 h; *d*) Grubbs catalyst, Ar, dry CH<sub>2</sub>Cl<sub>2</sub>, US, 34 °C, 2 h; *e*) KOH, 2 M, MeOH, H<sub>2</sub>O, US, 40 °C, 30 min; *f*) AcCl 2% in MeOH, CH<sub>2</sub>Cl<sub>2</sub>, MW, reflux, 15 min.



Fig. 1 1,8-Bis-(β-CD-2'-yl)oct-4-ene (9).

For the sake of clarity bis-CD 9 is depicted in Fig. 1 both as bridged cyclic heptamer consisting of six equal  $\beta$ -D-glucose units and a monoalkylated one and according to the classic schematic representation.

The well-known procedure to prepare  $2-2^{'18}$  and  $6-6^{'13,14}$  ureidoor thioureido-bridged bis-CDs was greatly improved by us in terms of shorter reaction times (30–90 min *vs.* 10–20 h under conventional conditions), ease of purification and, better yields by using polymer-bound PPh<sub>3</sub> under MW irradiation. Conceivably this procedure is also suitable for the preparation of thioureabridged CD-glycoconjugates<sup>24</sup> and CD-glycodendrimers.<sup>25</sup> Our protocol also did away with the most serious drawback of the standard procedure, the formation of stable inclusion complexes of the dimers with PPh<sub>3</sub> and PPh<sub>3</sub> oxide, guests that proved difficult to remove even by competition with other aromatic or cyclohexyl compounds (under reflux or under US) or by HPLC purification (gradient CH<sub>3</sub>CN–H<sub>2</sub>O).

Olefin metathesis has been reported by Sinaÿ *et al.* as a useful reaction to prepare bis( $\alpha$ - or  $\beta$ -CD)s linked at 6–6' by one or two aliphatic bridges.<sup>26</sup> We were the first to carry it out sonochemically starting from 2- or 3-monoalkenyl  $\beta$ -CD. High-intensity ultrasound strongly accelerates olefin metathesis; this reaction of course requires suitable apparatus for work under modified atmosphere (argon).

6-*O*-TBDMS-β-CD<sup>27</sup> was reacted at room temperature in anhydrous THF with a stoichiometric amount of alkenyl bromide in the presence of lithium hydride, yielding the 2-monoalkenyl derivative as major product beside a small amount of the 3-monoalkenyl derivative. The use of US here was not appropriate, because it would lead to polyalkylation right away. These derivatives could be much more easily separated after CD derivatization

to acetates. The 6-O-TBDMS-2<sup>I-VI</sup>,3<sup>I-VII</sup>-O-acetyl-2<sup>I</sup>-O-alkenyl-β-CD was subjected to homodimerization in the presence of a commercially available second-generation Grubbs catalyst. In this manner two different  $bis(\beta$ -CD)s with a spacer arm of either 8 or 20 carbons were prepared in good yields. Residual ruthenium was removed from the crude reaction mixtures by oxidation with a small amount of Pb(OAc)<sub>4</sub> followed by filtration through a silica plug. The length of the alkenyl chain strongly influenced the reaction rate; in fact the short allyl chain of 6-O-TBDMS-2<sup>I-VI</sup>,3<sup>I-VII</sup>-O-acetyl-2<sup>I</sup>-O-propenyl-β-CD utterly failed to react. Successively the acetates could be rapidly hydrolyzed under US (30 min at 40 °C) and silyl groups cleaved off in a few minutes under acidic conditions and MW. Both deprotection reactions can be successfully carried out in any US device and domestic MW oven, respectively. Parallel syntheses following the same procedure as depicted in Scheme 2, afforded the dimer 9 isom [i.e., a 3-3'bis( $\beta$ -CD) linked by a C<sub>8</sub> bridge] starting from 5 and 9b [*i.e.*, a 2–2' bis( $\beta$ -CD) linked by a C<sub>20</sub> bridge] starting from **4b** (Fig. 2).

We studied the interactions of our bridged bis-CDs with some suitably functionalized CAs for diagnostic magnetic resonance imaging (MRI),<sup>28</sup> *e.g.* with the complex shown in Fig. 3. Ditopic guests can be expected to bind to bis-CDs more strongly than monotopic ones. The strength of a Gd(III) chelate as CA for MRI is related to its relaxivity ( $r_{1p}$ ), that can be much enhanced by attaching it to a macromolecular system such as a bis-CD. To minimize the toxicity of the resulting adduct, this should be non-covalent rather than covalent in nature, a condition that requires the Gd(III) chelate to contain suitable hydrophobic groups to behave as guests in the CD cavities.

Firstly, we investigated host–guest interactions of G-1 with 6–6' ureido- (1) and thioureido- (2) bis-CDs. Its relaxivity increased,





Fig. 2 3-3' Bis( $\beta$ -CD) with a C<sub>8</sub> bridge (9 isom) and 2-2' bis( $\beta$ -CD) with a C<sub>20</sub> bridge (9b).



Fig. 3 Gd(III) complex G-1.

respectively, by a factor of 3 and 4, *viz*. from 9.1 mM<sup>-1</sup> s<sup>-1</sup> to 27.7 and 35 mM<sup>-1</sup> s<sup>-1</sup>. The inclusion complexes formed by **1** and **2** with **G-1** were more stable ( $K_{ass} = 3.1 \times 10^3$  M<sup>-1</sup> and  $3.9 \times 10^3$  M<sup>-1</sup>, respectively) than that formed by  $\beta$ -CD ( $K_{ass} = 8.0 \times 10^2$  M<sup>-1</sup>). With dimer **9**  $K_{ass}$  was 4.3 × 10<sup>4</sup> M<sup>-1</sup>, still one order of magnitude higher, and its relaxivity was 27.3. These results show that these systems could be promising candidates for MRI applications.

## Conclusions

An easier access to bis-CDs could vastly broaden their field of application in research and for industrial purposes. Nonconventional techniques such as US and MW, widely used already in other synthetic fields, can play an important role in the synthesis of these compounds, cutting down reaction times and increasing yields. Stability constants and relaxivity values showed that adducts of Gd(III) chelates with our bis-CDs can be promising candidates for MRI applications.

## Experimental

#### General

**Materials and methods.** Reactions were monitored by TLC on Merck 60  $F_{254}$  (0.25 mm) plates, which were visualized by UV inspection and/or by heating after a spray with 5% H<sub>2</sub>SO<sub>4</sub> in ethanol. Merck silica gel was used for column chromatography (CC). IR spectra were recorded with a Shimadzu FT-IR 8001 spectrophotometer. Unless stated otherwise, NMR spectra were recorded on a Bruker 300 Avance (300 MHz and 75 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively) at 25 °C; chemical shifts are calibrated to the residual proton and carbon resonance of the solvent: CDCl<sub>3</sub> ( $\delta_{\rm H}$  = 7.26,  $\delta_{\rm C}$  = 77.0). For spectra recorded in D<sub>2</sub>O *t*butanol was added as external reference ( $\delta_{\rm H} = 1.29$ ). Chemical shifts ( $\delta$ ) are given in ppm, and coupling constants (J) in Hz. MALDI-TOF MS spectra were measured on a Bruker Reflex III spectrometer. Sonochemical apparatus with immersion horn developed in the author's laboratory<sup>28</sup> can control all critical parameters (power, frequency modified atmosphere and reaction temperature). For higher volumes (up to 120 mL) and power (up to 500 W) was employed a new type of thermostatted cup-horn, a thin cylinder machined from a titanium rod developed in collaboration with NTS (Italy) (Fig. 4). MW-promoted reactions were carried out in a professional oven, Microsynth-Milestone (Italy). HPLC separations were recorded with an Amersham AKTA purifier 10/100 equipped with Atlantis RP18 ( $4.6 \times 150$  and  $19 \times 100$  mm) columns (Waters). The longitudinal water proton relaxation rates were measured on a Stelar Spinmaster (Mede, Italy) spectrometer operating at 20 MHz, by means of the standard inversion-recovery technique (16 experiments, 2 scans). A typical 90° pulse width was 4  $\mu$ s and the reproducibility of the  $T_1$  data was  $\pm 0.5\%$ . The temperature was kept at 25±0.1 °C with a Stelar VTC-91 airflow heater equipped with a copper-constantan thermocouple. Commercially available reagents and solvents were used without further purification unless otherwise noted. β-CD was kindly provided by Wacker Chemie. Gd(III) complex G-1 (Fig. 3) was kindly provided by Bracco Imaging Spa (Italy).



Fig. 4 New high-power cup-horn reactor (cavitating tube).

#### Synthesis

*N*,*N*'-**Bis**[6-*O*-**TbDMS-mono(2-deoxy)**] **2–2**' thioureido-β-CD (2). The reaction was carried out in a professional MW oven with magnetic stirring and Milestone fibre-optic thermometer. In a 50 mL two-necked round-bottomed flask equipped with gas inlet and condenser, 6<sup>1</sup>-*O*-azido-β-CD (500 mg, 0.43 mmol), polymer-bound PPh<sub>3</sub> (loading: ~3.0 mmol g<sup>-1</sup>, 2% cross-linked with divinylbenzene, Aldrich) (1.146 g, 3.44 mmol) and dry DMF (18 mL) were added and stirred for a few minutes. After addition of a cold solution (0 °C) of CS<sub>2</sub> (150 µL, 2.15 mmol) in DMF (3 mL), the mixture was heated under MW for 30 min at 110 °C (130 W). The reacted mixture was filtered off and DMF removed by vacuum distillation. The residue was washed with ether (40 mL × 3) and dried under vacuum. 938 mg of **2** were obtained (white powder, 0.40 mmol, yield 93%). Spectroscopic data were in accordance with the literature.<sup>14</sup>

The same procedure was used for the preparation of 1, CS<sub>2</sub> being replaced with a stream of CO<sub>2</sub>. The mixture was heated under MW for 90 min at 110 °C (yield 84%).

**6-O-TBDMS-2<sup>1</sup>-O-pentenyl-β-CD (4).** In a 50 mL flame-dried round-bottomed flask, 6-*O*-TBDMS-β-CD (5 g, 2.6 mmol) and LiH (123 mg, 15.5 mmol) were dissolved in anhydrous THF (20 mL). The magnetically stirred mixture was left 2 h under reflux. After cooling to room temperature, a solution of 5-bromo-1-pentene (920 µL, 7.8 mmol) in THF (3 mL) was added dropwise. The reaction was stirred 4 h under reflux and monitored by TLC, eluent CHCl<sub>3</sub>–CH<sub>3</sub>OH 4 : 1. The reacted mixture was diluted with EtOAc, washed with 1 M H<sub>2</sub>SO<sub>4</sub> and brine, and finally dried (Na<sub>2</sub>SO<sub>4</sub>). The crude residue, purified by CC (CHCl<sub>3</sub>–CH<sub>3</sub>OH 19 : 1,9:1,4:1) yielded 2.61 g of monoalkylated product **4** (1.31 mmol, yield 51.1%), 0.87 g of monoalkylated product **5** (0.43 mmol, yield 17%), 0.97 g of dialkylated derivative (0.47 mmol, yield 18%) and traces of starting material.

**NB**. If carried out at this point, the separation of **4** and **5** would be quite laborious; it became much easier after acetylation.

Compound **4** is a white powder.  $R_f = 0.52$  (CHCl<sub>3</sub>–CH<sub>3</sub>OH 4 : 1). IR (KBr): v = 3420, 1471, 1254, 1040, 1086, 835 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.8$  (m, 1 H, 4'-H), 5.04–4.90 (m, 7 H, 1-H, overlapped 2 H, 5'-H), 4.14–3.89 (m, 13 H), 3.73–3.49 (m, 28 H, overlapped 2 H, 3'-H), 3.18 (d, J = 9.4 Hz, 1 H, 2-H), 2.11–2.09 (m, 2 H, 3'-H), 1.72–1.70 (m, 2 H, 2'-H), 0.88 (s, 63 H, *t*-Bu), 0.05 (s, 42 H, Si-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta = 137.8$  (C4'), 114.7 (C5'), 101.9 (C1), 81.6 (C4), 73.4 (C2), 73.2 (C3), 72.2 (C5), 61.5 (C6), 29.6 (C2'), 28.2 (C3'), 25.8 (C- $Me_3$ ), 18.2 (C- $Me_3$ ), -5.0, -5.2 (Si- $Me_2$ ) ppm. MALDI-TOF MS: m/z calcd. for [M + Na]<sup>+</sup> 2024.0; found 2025.1.

Compound **5** is a white powder.  $R_{\rm f} = 0.62$  (CHCl<sub>3</sub>–CH<sub>3</sub>OH 4 : 1). IR (KBr):  $\nu = 3420$ , 1471, 1254, 1040, 1086, 835 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.8$  (m, 1 H, 4'-H), 5.16–4.93 (m, 7 H, 1-H, overlapped 2 H, 5'-H), 3.94–3.90 (m, 14 H), 3.73–3.51 (m, 28 H, overlapped 2 H, 3'-H), 2.20–2.10 (m, 2 H, 3'-H), 1.86–1.88 (m, 2 H, 2'-H), 0.88 (s, 63 H, *t*-but), 0.05 (s, 42 H, Si-CH<sub>3</sub>) ppm. MALDI-TOF MS: m/z calcd. for [M + Na]<sup>+</sup> 2024.0; found 2026.2.

The same procedure was followed to synthesize other monoalkenyl analogues.

**6-O-TBDMS-2<sup>1</sup>-O-undecenyl-β-CD (4b).** Starting from 6-*O*-TBDMS-β-CD (1.5 g, 0.75 mmol), LiH (36 mg, 4.5 mmol) and 11-bromo-1-undecene (494 μL, 2.25 mmol). The following were obtained: 2-monoalkylated product (548 mg, 0.26 mmol, yield 34%) and dialkylated derivative (87 mg, 0.04 mmol, yield 5%), recovering 485 mg of the starting material. **4b** is a white powder.  $R_{\rm f} = 0.66$  (CHCl<sub>3</sub>-CH<sub>3</sub>OH 4 : 1). IR (KBr): v = 3420, 1472, 1253, 1041, 839 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.78$  (m, 1 H, 10'-H), 5.04–4.90 (m, 7 H, 1-H, overlapped 2 H, 11'-H), 4.14–3.89 (m, 13 H), 3.73–3.49 (m, 28 H, overlapped 2 H, 3'-H), 3.18 (d, J = 9.4 Hz, 1 H, 2-H), 2.05–2.03 (m, 2H, 9'-H), 1.59 (m, 2H, 2'-H), 1.37 (m, 2 H, 8'-H), 1.27 (s, 10 H, aliphatic chain), 0.88 (s, 63 H, *t*-Bu), 0.05 (s, 42 H, Si-CH<sub>3</sub>) ppm. MALDI-TOF MS: *m*/*z* calcd. for [M + Na]<sup>+</sup> 2108.12; found 2110.08.

6-O-TBDMS- $2^{I-VI}$ ,  $3^{I-VII}$ -O-acetyl- $2^{I}$ -O-pentenyl- $\beta$ -CD (6). In a 50 mL two-necked round-bottomed flask equipped with the Milestone fibre-optic thermometer probe, 6-O-TBDMS-2<sup>I</sup>-Opentenyl-\beta-CD (1.540 g, 0.77 mmol), acetic anhydride (4 mL, 42 mmol) and cat. DMAP (1 mmol) were dissolved in anhydrous pyridine (20 mL). The mixture was heated under MW at 50 °C for 1 h in a professional oven. The mixture was diluted with  $CH_2Cl_2$ , washed with 1 M  $H_2SO_4$  (×3) and brine, and finally dried (Na<sub>2</sub>SO<sub>4</sub>). The crude product, purified by CC (hexane–EtOAc 1 : 1, 2:3,3:7), yielded 1.773 g of 6 (0.69 mmol, yield 89%). 6 is a white powder.  $R_{\rm f}$  (hexane-EtOAc 1 : 1) = 0.25. IR (KBr): v = 1755, 1473,1373, 1250, 1044, 835 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.78$  (m, 1H, 4'-H), 5.42–5.33 (m, 6 H, 3-H), 5.20–5.19 (m, 7 H, 1-H, overlapped 1 H, 3-H), 4.98 (m, 2 H, 5'-H), 4.79-4.67 (m, 6 H, 2-H), 4.12-3.71 (m, 28 H, overlapped 2 H, 1'-H), 3.08 (d, J = 9.4 Hz, 1 H, 2-H), 2.07 (m, 39 H, Ac, overlapped 2 H, 3'-H), 1.72–1.70 (m, 2 H, 2'-H), 0.88 (s, 63 H, t-Bu), 0.05 (s, 42 H, Si-CH<sub>3</sub>) ppm. MALDI-TOF MS: m/z calcd. for  $[M + Na]^+$  2570.2; found 2570.0.

The same procedure was employed for the following acetylations.

Acetylation of 6-*O*-TBDMS-3<sup>1</sup>-*O*-pentenyl-β-CD (yield 90%). 6-*O*-TBDMS-2<sup>*i*-νπ</sup>,3<sup>*i*-ν1</sup>-*O*-acetyl-3<sup>*i*</sup>-*O*-pentenyl-β-CD (6 isom) is a white powder.  $R_{\rm f}$  (hexane–EtOAc 1 : 1) 0.51. IR (KBr):  $\nu = 1755$ , 1473, 1373, 1250, 1044, 835 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.8$  (m, 1 H, H-4'), 5.5–5.1 (m, 13 H), 4.98 (m, 2 H, 5'-H), 4.70–4.67 (m, 7 H, 2-H, overlapped 1 H, 3-H), 4.12–3.69 (m, 28 H, overlapped 2 H, 1'-H), 2.07 (m, 39 H, Ac, overlapped 2 H, 3'-H), 1.72–1.70 (m, 2 H, 2'-H), 0.88 (s, 63 H, *t*-Bu), 0.05 (s, 42 H, Si-CH<sub>3</sub>). MALDI-TOF MS: m/z calcd. for [M + Na]<sup>+</sup> 2570.2; found 2570.1.

Acetylation of 6-*O*-TBDMS-2<sup>1</sup>-*O*-undec-10-enyl-β-CD (yield 72%). 6-*O*-TBDMS-2<sup>1-V1</sup>,3<sup>1-V11</sup>-*O*-acetyl-2<sup>1</sup>-*O*-undec-10-enyl-β-CD (6b) is a white powder.  $R_{\rm f}$  (hexane–EtOAc 3 : 7) 0.61. IR (KBr):  $\nu = 1755$ , 1475, 1373, 1250, 1040, 838 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.78$  (m, 1 H, 10'-H), 5.39–5.33 (m, 6 H, 3-H), 5.18–5.10 (m, 7 H, 1-H, overlapped 1 H, 3-H), 4.98 (m, 2 H, 11'-H), 4.75–4.68 (m, 6 H, 2-H), 4.12–3.71 (m, 28 H, overlapped 2 H, 1'-H), 3.08 (d, J = 9.4 Hz, 1 H, 2-H), 2.07 (m, 39 H, Ac, overlapped 2 H, 9'-H), 1.59 (m, 2 H, 2'-H), 1.37 (m, 2 H, 8'-H), 1.27 (s, 10 H, aliphatic chain), 0.88 (s, 63 H, *t*-Bu), 0.05 (s, 42 H, Si-CH<sub>3</sub>). MALDI-TOF MS: m/z calcd. for [M + Na]<sup>+</sup> 2654.2; found 2650.9.

1,8-Bis-(6'-O-TBDMS-2'<sup>1-VI</sup>,3'<sup>1-VII</sup>-O-acetyl-β-CD-2'-yl)oct-4ene (7). US-promoted metathesis reactions were performed under argon atmosphere in a 1 mm thick Teflon® tube inserted in a Delrin<sup>®</sup> reactor thermostatted by two Peltier modules.<sup>29</sup> Compound 6 (520 mg, 0.20 mmol) and 2<sup>nd</sup>-generation Grubbs Ru catalyst (0.03 g, 0.04 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (35 mL) and sonicated (19.1 kHz, 50 W cm<sup>-2</sup>) for 2 h at 34 °C. After cooling down to rt, the reaction was stopped by adding Pb(OAc)<sub>4</sub> (0.05 mmol) and sonicating for additional 20 min at 25 °C. The mixture was dried under vacuum. Purification by CC, eluent (hexane-EtOAc 1 : 1, 2 : 3, 3 : 7) afforded 338 mg of dimer (0,07 mmol, yield 69%). 64 mg of the starting material were recovered. 7 is a white powder.  $R_{\rm f}$  (hexane–EtOAc 1 : 1) 0.04. IR (KBr): v = 1754, 1470, 1244, 1035, 832 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.42-5.33$  (m, 12 H, 3'-H, overlapped 2 H, 4,5-H), 5.17-5.11 (m, 14 H, 1'-H, overlapped 2 H, 3'-H), 4.79–4.67 (m, 12 H, 2'-H), 4.12–3.71 (m, 56 H, overlapped 4 H, 1,8-H), 3.08 (d, J = 9.4 Hz, 2 H, 2'-H,), 2.07 (m, 78 H, Ac, overlapped 4 H, 3,6-H), 1.72-1.70 (m, 4 H, 2,7-H), 0.88 (s, 126 H, *t*-Bu), 0.05 (s, 84 H, Si-CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  = 170.1 (Me<sub>3</sub>CO), 129.2 (C4,5), 97.3 (C1'), 77.8 (C4'), 72.1, 72.0, 71.8 (C2',3',5'), 61.5 (C6'), 29.6 (C2,7), 28.2 (C3,6), 26.2 (C-*Me*<sub>3</sub>), 21.2 (*Me*<sub>3</sub>CO), 18.6 (*C*-Me<sub>3</sub>), -4.5, -5.0 (Si-Me<sub>2</sub>). MALDI-TOF MS: *m*/*z* calcd. for [M + Na]<sup>+</sup> 5089.3; found 5092.1.

The same procedure was followed to synthesize 1,20-bis-(6'-*O*-TBDMS-2'<sup>I-VI</sup>,3'<sup>I-VII</sup>-*O*-acetyl- $\beta$ -CD-2'-yl)eicos-10-ene (**7b**). Starting from 6-*O*-TBDMS-2<sup>I-VI</sup>,3<sup>I-VII</sup>-*O*-acetyl-2'-*O*-undecenyl- $\beta$ -CD (388 mg, 0.15 mmol). The reaction gave 184 mg of product (0.036 mmol, yield 48%). **7b** is a white powder.  $R_{\rm f} = 0.49$  (hexane-EtOAc 3 : 7). IR (KBr):  $\nu = 1754$ , 1472, 1245, 1036, 834 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.36-5.33$  (m, 12 H, 3'-H, overlapped 2H, 10,11-H), 5.17-5.12 (m, 14 H, 1'-H, overlapped 2 H, 3'-H), 4.75-4.68 (m, 12 H, 2'-H), 4.2–3.71 (m, 56 H, overlapped 4 H, 1,20-H), 3.8 (d, J = 9.4 Hz, 2 H, 2'-H), 2.07 (m, 78 H, Ac, overlapped 4 H, 9,12-H), 1.59 (m, 4 H, H-2,19), 1.37 (m, 4 H, 8, 13-H), 1.27 (s, 20 H, aliphatic chain), 0.88 (s, 126 H, *t*-Bu), 0.05 (s, 84 H, Si-CH<sub>3</sub>). MALDI-TOF MS: m/z calcd. for [M + Na]<sup>+</sup> 5257.5; found 5239.0.

1,8-Bis-(6'-O-TBDMS-β-CD-2'-yl)oct-4-ene (8). In a titanium cup-horn reactor (Fig. 1), 1,8-bis-(6'-O-TBDMS-2'<sup>I-VI</sup>,3'<sup>I-VII</sup>-Oacetyl-β-CD-2'-yl)-oct-4-ene (980 mg, 0.19 mmol), H<sub>2</sub>O (10 mL) 2 M KOH (2.5 mL) and CH<sub>3</sub>OH (60 mL) were added. The mixture was sonicated for 30 min at 40 °C (22 kHz, 100 W). The reaction was monitored by TLC, eluent hexane-EtOAc 1 : 1. The reacted mixture was diluted with EtOAc, washed with H<sub>2</sub>O and brine, and finally dried (Na<sub>2</sub>SO<sub>4</sub>). 732 mg of product were obtained (0.18 mmol, yield 96%). **8** is a white powder.  $R_{\rm f} = 0.61$ (CHCl<sub>3</sub>-CH<sub>3</sub>OH 4 : 1). IR (KBr): v = 3420, 1474, 1254, 1086, 1040, 835 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta = 5.5$  (m, 2 H, 4,5-H), 4.90 (brs, 14 H, 1'-H), 4.19-3.81 (m, 26 H), 3.73-3.60 (m, 56 H, overlapped 4 H, 1,8-H), 3.18 (m, 2 H, 2'-H), 2.11-2.09 (m, 4 H, 3,6-H), 1.72–1.70 (m, 4 H, 2,7-H), 0.88 (s, 126 H, t-Bu), 0.05 (s, 84 H, Si-CH<sub>3</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$  = 129.2 (C4,5), 97.3 (C1'), 77.8 (C4'), 72.1, 72.0, 71.8 (C2',3',5'), 67.0 (C1,8), 61.5 (C6'), 29.6 (C2,7), 28.2 (C3,6), 26.2 (C-Me<sub>3</sub>), 18.6 (C-Me<sub>3</sub>), -4.5, -5.0 (Si-Me<sub>2</sub>). MALDI-TOF MS: m/z calcd. for  $[M + Na]^+$  3997.0; found 3995.0.

1,8-Bis-(\beta-CD-2'-yl)oct-4-ene (9). In a 100 mL two-necked round-bottomed flask equipped with a condenser and the Milestone fibre-optic probe, 1,8-bis-(6'-O-TBDMS-β-CD-2'-yl)-oct-4ene (640 mg, 0.16 mmol), AcCl (10 mL, 2% in CH<sub>3</sub>OH) and CH<sub>2</sub>Cl<sub>2</sub> (40 mL) were added. The mixture was irradiated with MW under reflux for 15 min. The reacted mixture was concentrated, ether (50 mL) was added; the precipitate was filtered, washed with ether (40 mL) and dried under vacuum. 377 mg of product were obtained (0.15 mmol, yield 98%). 9 is a white powder (Found: C, 46.6; H, 6.3; Calcd for  $C_{92}H_{152}O_{70}$ : C, 46.4; H, 6.4%).  $R_f = 0.23$  $(CH_3CN-H_2O \ 4 \ : \ 1.6)$ . IR (KBr): v = 3420, 1472, 1245, 1036,836 cm<sup>-1</sup>. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta = 5.5$  (m, 2 H, 4,5-H), 5.03 (brs, 14 H, 1'-H), 4.19-3.81 (m, 26 H), 3.73-3.60 (m, 56 H, overlapped 4 H, 1,8-H), 3.49 (m, 2 H, 2'-H), 2.11-2.09 (m, 4 H, 3,6-H), 1.70–1.66 (m, 4 H, 2,7-H). <sup>13</sup>C-NMR (150 MHz, D<sub>2</sub>O):  $\delta = 130.6 (C4,5), 102.1 (C1'), 81.5 (C4'), 73.2, 72.6, 71.4 (C2',3',5'),$ 60.3 (C6'), 29.6 (C2,7), 28.2 (C3,6). MALDI-TOF MS: *m*/*z* calcd. for  $[M + Na]^+$  2399.8; found 2399.1.

The same procedure was followed to obtain the following final products.

**1,8-Bis-(6'-O-TBDMS-β-CD-3'-yl)oct-4-ene** (9 isom) (yield 97%). 9 isom is white powder (Found: C, 46.5; H, 6.3; Calcd for C<sub>92</sub>H<sub>152</sub>O<sub>70</sub>: C, 46.4; H, 6.4%).  $R_f = 0.23$  (CH<sub>3</sub>CN-H<sub>2</sub>O 4 : 1.6). IR (KBr):  $\nu = 3420$ , 1472, 1245, 1036, 835 cm<sup>-1</sup>. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta = 5.5$  (m, 2 H, 4,5-H), 4.97 (brs, 14 H, 1'-H), 3.78–3.49 (m, 42 H, overlapped 4 H, 1,8-H), 1.99 (m, 4 H, 3,6-H), 1.64 (m, 4 H, 2,7-H). MALDI-TOF MS: *m*/*z* calcd. for [M + Na]<sup>+</sup> 2399.8; found 2399.0.

**1,20-Bis-(\beta-CD-2'-yl)eicos-10-ene (9b) (yield 92%). 9b** is a white powder (Found: C, 49.1; H, 6.9; Calcd for C<sub>104</sub>H<sub>176</sub>O<sub>70</sub>: C, 49.1; H, 7.0%).  $R_{\rm f} = 0.46$  (CH<sub>3</sub>CN-H<sub>2</sub>O 4 : 1.6). IR (KBr):  $\nu = 3420, 1472, 1245, 1120, 1030, 834 \,{\rm cm}^{-1}$ . <sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta = 5.5$  (m, 2 H, 10,11-H), 5.07 (s, 14 H, H-1'), 3.86 (m, 26 H), 3.70–3.55 (m, 56 H, overlapped 4 H, 1,20-H), 2.09 (m, 4 H, 9,12-H), 1.59 (m, 4 H, 2,19-H), 1.37 (m, 4 H, 8,13-H), 1.31–1.15 (m, 20 H, aliphatic chain). MALDI-TOF MS: m/z calcd. for [M + Na]<sup>+</sup> 2568.0; found 2568.0.

#### **Relaxometric measurements**

The affinity constants  $K_{ass}$  for the binding of Gd(III) complexes to CD-dimers, and the relaxivities of the resulting adducts were determined by the PRE method.<sup>30</sup>

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