Duplex of capped-cyclodextrins, synthesis and cross-linking behaviour with a biopolymer†

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Doubly connected cyclodextrin (CD) dimers, called duplexes, have the ability to cross-link hydrosoluble biopolymers grafted with a hydrophobic moiety, inducing a viscosity increase of the solution through formation of stable supramolecular assemblies. CD duplexes behave differently according to the nature of their inter-CD links. CD duplex with hydrophobic links has a better affinity for adamantane, but suffers from a lack of availability of its cavities, both phenomena being attributed to the hydrophobicity of its spacer arms. In the case of the CD duplex with hydrophilic links, all its cavities are available, but its affinity for adamantane is lower. We report here on the synthesis of a CD duplex displaying an original topology consisting of two capped and doubly connected concave rings. It possesses a hydrophobic arm on the CD primary face which induces an increased binding affinity for adamantane, and hydrophilic inter-CD links to keep all its cavities accessible. The cap was selected according to the binding efficiency of the monomeric capped-CD with adamantane determined by isothermal calorimetry (ITC) experiments. The synthesis of this challenging and unprecedented edifice consisting of two capped CDs doubly linked to each other was made possible thanks to our cyclodextrin debenzylation strategy, as well as the extensive use of metathesis reaction. ITC experiments with the duplex confirmed its better affinity for adamantane compared to the uncapped duplex. However, viscosity measurements revealed that capped and uncapped CD duplexes had similar cross-linking behaviour.

Introduction

Cyclodextrins (CDs) have been widely used in material sciences due to their ability to form inclusion complexes with hydrophobic guests, which allowed construction of smart materials displaying highly selective and specific molecular recognition properties.[1] However, use of native CDs limits their scope of interest, prompting synthetic chemists to alter their physical and chemical properties by modifying one, two, or all three types of hydroxyl groups of the macrocycle. While this homogeneous substitution strategy is often used to tune the solubility of CDs in aqueous or organic solutions, regioselective substitutions provide functionalized CDs, which could act as selective sensors, catalysts or cross-linking agents. Until recently, hetero-functionalization of CDs was particularly tedious,[2] but, for some time now, we have developed an original methodology relying on an efficient regioselective deprotection reaction of perbenzylated CDs giving access to CDs bearing two[3] or three[4] new functionalities on their primary rim. Furthermore, benzyl protection of the CDs allows the use of modern and efficient chemical reactions in apolar solvents and standard silica-gel flash chromatography. Multistep syntheses of CD-based structures with a high degree of complexity are now possible in practical overall yields.

Hence, using this new strategy, we were able to synthesize two doubly linked β-CD dimers **1** and **2**, called duplexes,[5] connected with two different kinds of spacer. (Fig. 1) These elaborate and original structures were obtained in good overall yields, 22 and 18% respectively, and on a large scale allowing the

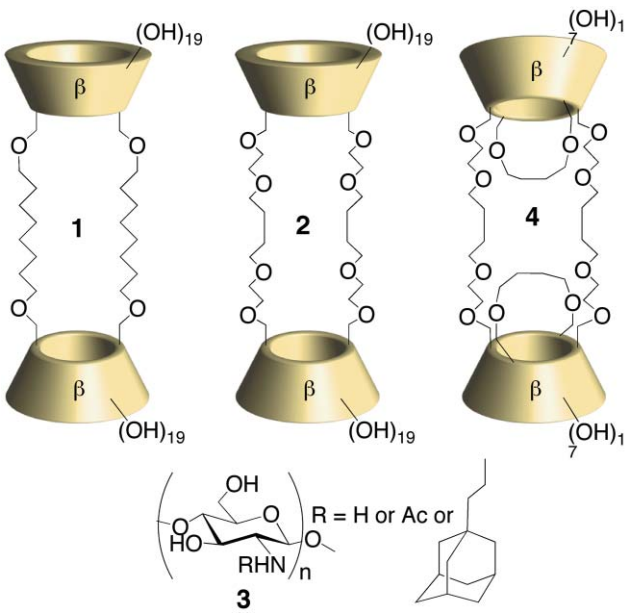

Fig. 1 Structures of β-cyclodextrin duplexes **1**, **2**, **4** and adamantane–grafted chitosan **3**.

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study of their physicochemical properties in combination with modified biopolymers.[6,7] Gratifyingly, we observed the formation of supramolecular assemblies *via* the physical cross-linking of a biopolymer functionalized with hydrophobic moieties, namely an adamantane-grafted chitosan (chit-AD) **3**, by the CD duplexes **1** and **2**.[6,7] A clear viscosity enhancement of the polymer solution in water was observed upon mixing the solutions of duplex **1** or **2** and biopolymer **3**. Such reversible system, also observed by others,[8] may have potential applications in various fields including drug delivery, coatings or personal care goods (Fig. 1).

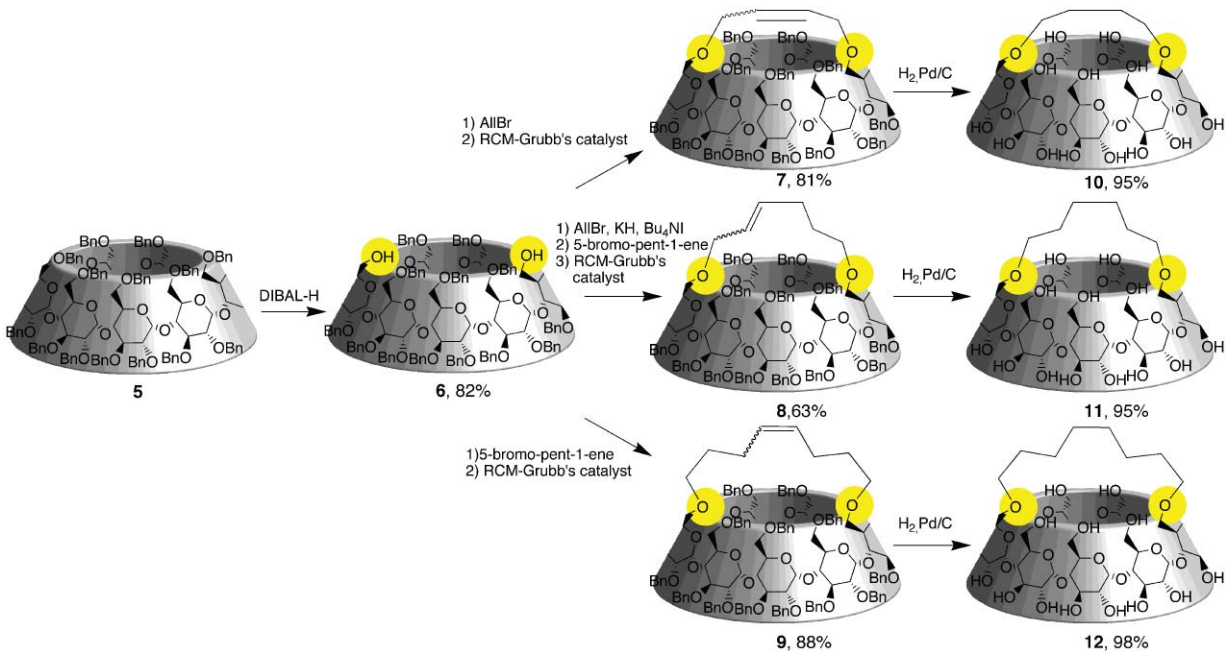
Investigation of the interaction between β-CD derivatives and chit-AD **3** through viscosity measurements[6] showed clear behaviour differences between duplexes **1** and **2**. When used as a cross-linking agent in the presence of a chit-AD **3** solution, duplex **1** led to higher viscosity values compared to duplex **2**. Calorimetric titration measurements indicated that binding of sodium adamantyl acetate (ADAC) by duplex **1** is 4.3 times stronger than for duplex **2** and 3 times stronger than for natural β-CD. We also observed that, in the case of duplex **1**, about one half of the CD cavities were apparently unavailable for complexation probably due to aggregation.[7] In the case of duplex **2**, however, in which the two CDs are connected with two hydrophilic arms, both cavities host adamantane. Based on these results,[6] we postulated that the higher efficiency of duplex **1** as a cross-linking agent could be partly explained by the greater binding strength of CD cavities of **1** toward adamantane compared to those of duplex **2** and this observation could be attributed to a strengthening of the hydrophobic interactions due to the presence of the two C_8 hydrophobic chains connecting the CD cavities in duplex **1**.

Taking the best of both worlds, we designed a new CD duplex **4** of unprecedented complexity (Fig. 1) which should exhibit both a high binding affinity toward hydrophobic guest molecules and a high availability of its cavities. Binding potency of the duplex can be enhanced by bridging the primary rim of the CD with a hydrophobic cap,[9,10] and use of hydrophilic arms doubly connecting the two cavities should avoid formation of aggregates as previously observed. Here, we report the synthesis of this new dimeric β-CD **4** and its use as a physical cross-linking agent of a guest biopolymer, adamantane-grafted chitosan **3**.

Selection of the capping unit to enhance the affinity of CD toward adamantane

In order to select the optimal capping chain length, we first prepared three β-CD derivatives **10**, **11** and **12** capped with either a C4-, C6- or C8- aliphatic chain and analyzed their complexation properties. The capped-CDs **10**, **11** and **12** were synthesized according to a previously described methodology,[11] based on the bis-alkylation of classical diol **6**[3] into unsaturated diethers followed by ring closing metathesis (RCM) using Grubbs catalyst affording the protected capped-CDs **7**, **8** and **9**. Final hydrogenolysis in the presence of Pd/C afforded the capped-CDs **10**, **11** and **12** in 63, 49 and 70% overall yield respectively from native β-CD. (Scheme 1)

The binding behaviour of the resulting CD derivatives was investigated by isothermal titration calorimetry (ITC) using sodium adamantyl acetate (ADAC) as a model guest. While titration of CDs **10** and **11** by ADAC gave exothermic peaks and a binding curve typical of a 1 : 1 complex formation, that of CD **12** led to a thermogram similar to that obtained for the dilution of ADAC. Changing the concentrations of CD **12** and ADAC or carrying out reverse titration could not demonstrate complex formation. Moreover, ROESY NMR spectroscopy provided no evidence for a spatial vicinity between protons of the C8 chain and those of the CD cavity. The absence of complexation between ADAC and CD **12** suggests that the CD cavity is unavailable, which can be tentatively explained by the formation of aggregates promoted by the C8 chain-CD complexation. In the case of CDs **10** and **11**, the binding curve was fitted using a 1 : 1 binding model, leading



Scheme 1 Synthesis of capped CDs **10**, **11** and **12**.

Table 1 Thermodynamic parameters for inclusion complex formation of sodium adamantyl acetate with natural β-CD, capped β-CD **10** and **11**, duplexes **1**, **2** and **4** derived from calorimetric titration experiments

CD derivative	CD derivative conc./mM	AD conc./mM	$K_a \times 10^{-4}/M^{-1}$	ΔH/kJ mol^{-1}	$T\Delta S$/kJ mol^{-1}	n (nAD:1CD derivative)
Natural β-CD[a]	0.56	6.59	7.96 (±0.05)	−26.2 (±0.04)	1.76 (±0.04)	0.90 (±0.01)
Capped β-CD **10**	0.105	0.945	19.5 (±1.02)	−25.2 (±0.23)	4.97 (±0.26)	0.77 (±0.005)
Capped β-CD **11**	0.4	4.5	23.6 (±0.72)	−29.8 (±0.08)	0.83 (±0.11)	0.87 (±0.001)
Duplex **1**[a]	0.6	4.4	26.42 (±0.50)	−28.2 (±0.40)	2.73 (±0.40)	0.80 (±0.01)
Duplex **2**[a]	0.35	7	6.38 (±0.50)	−20.11 (±0.20)	7.29 (±0.28)	2.03 (±0.01)
Duplex **4**	0.35	7	22.5 (±0.35)	−25.2 (±0.03)	5.32 (±0.50)	1.41 (±0.001)

[a] Data from reference 6

to association constants respectively 2.5 and 3 times higher than for natural β-CD (see Table 1). The best association constant is thus obtained with a C6 capped-CD. The entropy contribution is only slightly favourable, as in natural CD, indicating that a six-carbon tether does not perturb the CD ring flexibility in solution. The higher affinity is enthalpy driven with a gain of 2.6 kJ mol^{-1} compared to the native CD. The six-carbon chain triggers a favourable enthalpic contribution that could be rationalized either by a direct contact with the ADAC ligand or by a conformational effect on the CD ring. Interestingly, the enhanced binding of ADAC by CD **10** is due to a more favourable entropy contribution that goes up to 25% of the free energy for binding of **10** (6% for the natural CD). This suggests that the loss of flexibility of the CD upon binding has been reduced, and therefore that compound **10** is more rigid than native CD. The enthalpy term is slightly decreased, indicating that such "prearranged" conformation does not provide a perfect fit for the host.[12] Another likely hypothesis for the more favourable entropy contribution would be an increase of desolvation upon inclusion complexation due to the presence of the C4 chain as a cap of the CD. We have thus showed the possibility of enhancing the binding of ADAC by introducing a hydrophobic alkyl chain on the primary face of the CD. However, the length of the chain must be carefully chosen. Considering our objective in the present study, we decided to introduce a C4 chain on the CD cavities of duplex **2** based on the observation that both capped CD **10** and **11** form a complex with ADAC with a similar association constant. Moreover the synthesis of the C6-capped CD **11** is not as straightforward as for C4-capped CD **10**.

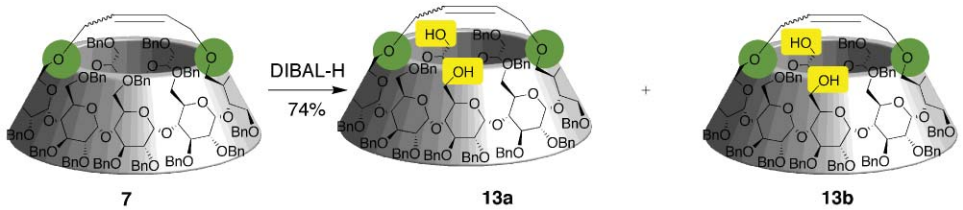
Synthesis of capped-CD duplex 4

Although a very large number of cyclodextrin dimers have been described,[13] only few CD-duplexes have been reported.[14] This is mainly due to their tedious and low-yielding synthetic access mainly because of the difficult preparation of the bifunctionalized CD precursors. Our deprotection methodology combined with the use of benzyl protecting groups overcomes this limitation and allowed us,[15] and others[16] to synthesize CD-duplexes in good yields. However, we here wish to go one step further, and synthesize a duplex of specifically functionalized CDs, namely of capped-CDs. Such goal can be met through a duplication of our deprotection strategy. Indeed, we have previously shown that a change of steric hindrance on the primary rim of the CD could induce selective deprotection reactions of the primary hydroxyl groups allowing the synthesis of CDs bearing three different orthogonal protecting groups on the primary rim.[2,4] For instance, capping of CD **7**, synthesized according to Scheme 1, induces a DIBAL-H-mediated regioselective deprotection affording a 4 : 1 mixture of diols **13a** and **13b** in 74% yield (Scheme 2). This reaction thus allows the synthesis of capped-duplex CD **4**.

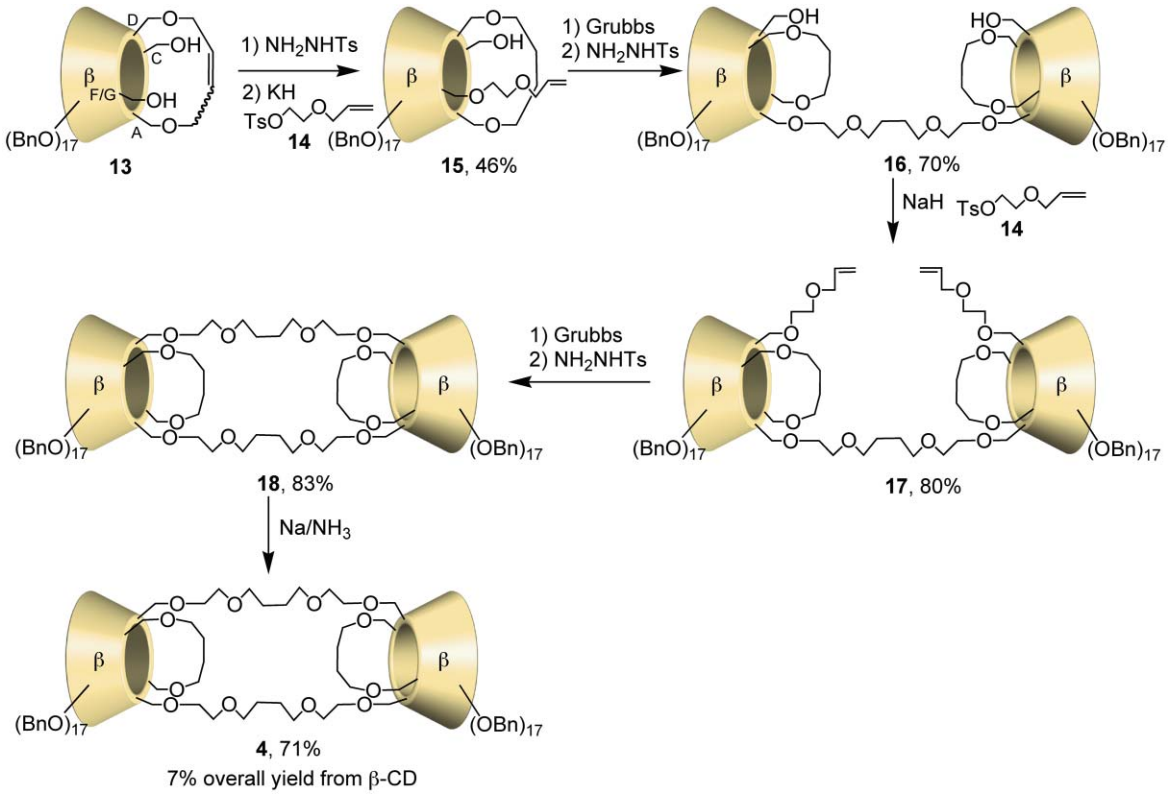
Saturation of the double bond in diols **13a** and **13b**, followed by mono-alkylation using tosylate **14** afforded alcohol **15** in 46% yield as a mixture of regioisomers, used as such in the following steps. Alkene **15** was dimerized upon action of Grubbs catalyst, through a cross-metathesis, followed by the reduction of the newly formed double bond to afford dimeric capped-CD **16** in 70% yield. Bis-alkylation of diol **16** furnished the bis-allyl derivative **17** in 80% yield which was submitted to a RCM-based macrocyclization followed by saturation of the resulting alkene to afford the protected capped-CD duplex **18** (83%). Final benzyl deprotection yielded the desired duplex **4** in 7% overall yield from native β-CD (Scheme 3).

Comparison of the complexation properties of duplex 4 toward free adamantane by isothermal titration calorimetry

Analysis of complex formation between duplex **4** and ADAC by ITC, performed under the same conditions as for duplex **2**, revealed an improved binding in the same range as for monomeric capped-CD **10** and similar to duplex **1**. Comparison of the complexation stoichiometry values obtained for the different duplex molecules with the theoretical value ($n = 2$) demonstrates that duplex **4** exhibits a better cavity availability for adamantane complexation than duplex **1**, albeit not as high as for **2** (Table 1).



Scheme 2 Regioselective deprotection of capped-CD **7**.



Scheme 3 Synthesis of capped-CD duplex **4**.

This series of experiments thus supports our assumption that duplex **4** displays a binding affinity for adamantane similar to duplex **1**, with almost all its cavities available.

Comparison of the cross-linking ability of CD duplexes 1, 2 and 4 toward adamantane-grafted chitosan

The ability of the β-CD dimeric species to form stable supramolecular assemblies with adamantane-grafted chitosan **3** was monitored by viscosity measurements. These were performed keeping the polymer concentration constant, while progressively increasing the concentration of CD derivatives **1**, **2** or **4**. Fig. 2 displays the dependence of the zero-shear viscosity of chit-AD solutions, at a concentration of 2.5 g L^{-1} or 3 g L^{-1}, on the [CD]/[AD] ratio. Viscosity of the solution clearly increases upon addition of the duplex **4** solution up to a maximum reached for a 1 : 1 [CD]/[AD] ratio. This maximum value is identical as for duplex **2**. Beyond this optimal [CD]/[AD] ratio, solution viscosities decrease. Viscosity enhancement can be attributed to the cross-linking of chit-AD chains through complexation by duplex **2** or **4**. However, when more duplex is added, the probability of effective interchain cross-linking decreases as a result of increasing duplex monocomplexation, which leads to a decrease of the viscosity solution. As reported previously,[6] the fact that this optimal [CD]/[AD] ratio is close to 1 for duplexes **2** and **4** and close to 2.5 for duplex **1** may be related to the percentage of CD cavities available for binding, which is ~ 100% for **2**, ~ 70% for **4** and, only ~ 40% for **1**. Surprisingly, the presence of the butyl caps on the primary face of duplex **4** does not significantly improve the viscosity value compared to duplex **2**. This result suggests that

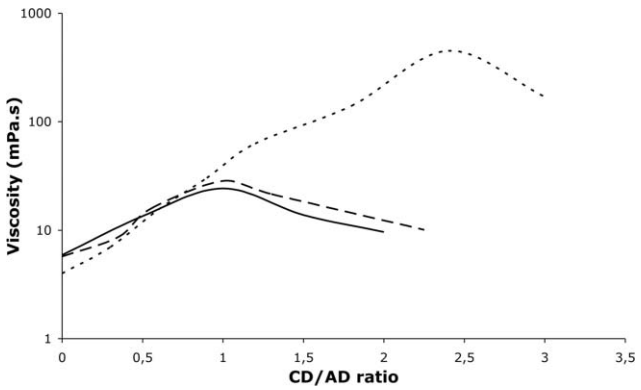


Fig. 2 Variation of the viscosity of solutions of AD-chitosan at a concentration of 2.5 or 3 g L^{-1} (0.3 M CH_3COOH/0. 03 M CH_3COONa, 25 °C) with the [CD]/[AD] ratio (*i.e.* with increasing concentration of CD dimeric species **1**, **2** and **4**). --- Duplex **2**/AD-chit 3 g L^{-1}, --- Duplex **4**/AD-chit 3 g L^{-1}, — Duplex **1**/AD-chit 2.5 g L^{-1}.

the gain in binding affinity for AD is not enough to improve the cross-linking efficiency of the CD duplex. Comparison with CD duplex **1** indicates that the cross-linking efficiency may be rather related to the aggregation of this CD duplex possibly allowing multivalent association with the polymer.[17]

Conclusion

We thus synthesized a CD duplex displaying an original topology consisting of two doubly connected and capped concave rings. The

hydrophobic arm on the primary face on the CD cavities induces an increased binding affinity for adamantane compared to its uncapped counterpart, while almost all its cavities are available for complexation thanks to the hydrophilic inter-CD linkers. This new CD duplex combines the advantages of both previously studied duplexes **1** and **2** to be an efficient cross-linking agent of an AD modified biopolymer. However, upon mixing **4** with modified biopolymer **3**, duplex **4** showed the same behaviour as its uncapped counterpart **2**. This result reveals that the peculiar behaviour of duplex **1** as cross-linking agent is not correlated with its affinity for adamantane.

Experimental section

Materials

Adamantane grafted chitosan with a degree of substitution equal to 0.05 was synthesized as previously reported.[18] The parent chitosan used has a weight-average molecular weight M_w of 195000; it is a commercial sample from Pronova (Norway) with a degree of *N*-acetylation equal to 0.12. The overlap concentration C^*, which characterizes the transition from the dilute concentration regime to the semi-dilute one, is around 0.9 g L^{-1} for this chitosan sample.[17] The β-CD dimeric species **1–2** were synthesized as described in detail elsewhere.[6,12] β-Cyclodextrin was kindly supplied by Cyclolab (Budapest, Hungary). All other chemical products and reagents were purchased from Fluka (Buchs, Switzerland).

Elemental analysis and optical rotation measurements

Elemental analyses were performed by Service de Microanalyse – CNRS ICSN, Av. de la Terrasse, 91198 Gif-sur-Yvette Cedex, France and Centre Régional de Mesures Physiques de l'Ouest (Rennes, France). Optical rotation was measured at 20 ± 2 °C with a Perkin Elmer Model 241 digital polarimeter, using a 10 cm, 1 mL cell.

NMR spectroscopy

^{1}H NMR spectra were recorded with a Bruker DRX 400 spectrometer for solutions in $CDCl_3$ or D_2O at ambient temperature. Assignments were done using COSY experiments. ^{13}C NMR spectra were recorded at 100.6 MHz with a Bruker DRX 400 spectrometer for solutions in $CDCl_3$ or D_2O adopting 77.00 ppm for the central line of $CDCl_3$. Assignments were derived from J-mod technique and HMQC experiments.

Mass spectrometry

Fast Atom Bombardment Mass Spectra (FAB-MS) were obtained with a JMS-700 spectrometer. MALDI mass spectra were recorded with a PerSeptive Biosystems Voyager Elite (Framingham MA - USA) time-of-flight mass spectrometer. This instrument was equipped with a nitrogen laser (337 nm), a delayed extraction and a reflector. PEG standards were used to calibrate the mass scale using the two points calibration software 3.07.1 from PerSeptive Biosystems. The matrix, 2,5-dihydroxybenzoic acid (2,5-DHB) was from Sigma (France) and used without further purification. ESI mass spectra were recorded with a Q-TOF1 (Micromass) time-of-flight mass spectrometer with a sample cone of 40 V.

Titration calorimetry

Isothermal titration microcalorimetry (ITC) was performed using a Microcal VP-ITC titration microcalorimeter (Northampton, USA). In individual titrations, injections of 10 μL of sodium adamantyl acetate were added from the computer-controlled 250-μL microsyringe at an interval of 5 min into the solutions of the β-CD dimeric species **4** (cell volume = 1.45 mL) containing the same solvent as sodium adamantyl acetate (phosphate buffer (pH 7)), while stirring at 297 rpm at 25 °C. The observed heat effects under identical injections of sodium adamantyl acetate into a cell containing only the solvent were identical to the heat signals at the end of titration, after the saturation was reached. The raw experimental data were presented as the amount of heat produced following each injection of sodium adamantyl acetate as a function of time. The amount of heat produced per injection was calculated by integration of the area under individual peaks by the instrument software, after taking into account heat of dilution. The experimental data were fitted to a theoretical titration curve using the instrument software (ORIGIN software (Microcal)), with ΔH^0 (the enthalpy change in kJ mol^{-1}), K_a (the association constant in M^{-1}), and n (complex stoichiometry) as adjustable parameters. In all cases, calculations were performed using the "one set of binding sites" model. The $T\Delta S^0$ values were calculated from the equation $\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT\ ln\ (K_a)$, where ΔG^0, ΔH^0, ΔS^0 are the changes in free energy, enthalpy, and entropy of binding, respectively, T is the absolute temperature, and $R = 8.314$ J K^{-1} mol^{-1}. Consequently, the standard errors for the $T\Delta S^\circ$ values depend on those for T, ΔG^0 and ΔH^0 (which are the adjustable parameters together with n). They were calculated as described in the literature.[19]

Viscometry

The steady shear flow properties of solutions of adamantane-chitosan in the absence and in the presence of CD-dimeric species **4** was measured using a low shear viscometer (LS30 from Contraves) or a cone-plate rheometer (ARES-RFS from TA Instruments), depending on the sample viscosity. In the latter case, the cone used has a diameter of 5 cm and an angle of 0.04 rad, and experiments were carried out with a film of silicone to avoid solvent evaporation. The solutions of adamantane grafted chitosan and CD-derivative **4** were prepared separately in 0.3 M CH_3COOH/0.03 M CH_3COONa, a good solvent of chitosan. The dissolution time of adamantane-chitosan was at least 1 day at room temperature. The CD-dimeric species were dissolved in the minimum amount of solvent (65 mg mL^{-1} for **4**) to avoid dilution of the polymer solution after their addition under stirring. After each addition, the samples were allowed to rest for at least 1 h before the measurement.

Synthesis

Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with

sulfuric acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).

Capped-CD duplex 4. The duplex **18** (152 mg, 26.5 mmol) was dissolved in a mixture of THF/NH_3 1 : 1 (15 mL) at −78 °C. Small pieces of Na (150 mg, excess) were added. The blue solution was refluxed for 1 h (−33 °C), carefully quenched by *i*PrOH (10 mL) and concentrated. A solution of the residue in water (15 mL) was neutralized with IR-120 H^+ resin, diluted with EtOAc (15 mL), vigorously stirred for 30 min and filtrated. The organic layer was separated, extracted with water (3 × 10 mL) and the combined aqueous layers were concentrated. The residue was purified by chromatography on Sephadex G25 column (water) to give duplex **4** (50 mg, 71%) as a white powder. ^{1}H NMR (400 MHz, D_2O): δ 1.59–1.73 (m, 16H, 16×$OCH_2CH_2CH_2CH_2O$), 3.40–4.25 (m, 152H, 84×CH, 34×OH, 16×OCH_2CH_2O, 16×$OCH_2CH_2CH_2CH_2O$), 5.05–5.30 (m, 14H, 14 × 1-H); ^{13}C NMR (100 MHz, D_2O): δ 25.8–26.7 (CH_2), 60.3–61.2 (CH_2), 69.8–71.1 (CH_2), 71.4–74.6 (CH), 79.4–82.4 (CH), 81.0–82.5 (CH), 101.6–102.8 (CH); HRMS (ESI): *m*/*z* (%): 1353.5059 (calc.); 1353.5061 (0 ppm) (obt.) ([M+2Na^+]).

Capped-CD alcohol alkene 15. Bridged diol **13**[4] (772 mg, 304 mmol) was dissolved in 1,2-dimethoxyethane (40.5 mL) and treated by *p*-toluenesulfonhydrazide (4.38 g, 23.4 mmol). The reaction mixture was refluxed and a solution of sodium acetate in water (3.96 g in 27.5 mL H_2O) was added dropwise. After 6 h refluxing, the reaction mixture was concentrated, diluted in EtOAc (40 mL) and washed with H_2O (30 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL). Organic layers were combined, washed with brine (60 mL), dried over $MgSO_4$ and concentrated. The crude was purified by chromatography (cyclohexane/EtOAc, 2 : 1) to give the saturated diol (717 mg, 93%) as a white foam. To a solution of this diol (717 mg, 264 mmol) in THF (22 mL) were added nBu$_4$NI (9 mg, 26 mmol) and KH (40 mg, 290 mmol, w/w 30% in oil) at 0 °C under argon. The reaction mixture was stirred at 0 °C under argon for 20 min and tosyl-allyloxyethanol **14** (74 mg, 290 mmol) was added. The reaction mixture was stirred at r.t. under argon for 18 h, treated with MeOH (15 mL) and evaporated. The residue was diluted with CH_2Cl_2 (5 mL) and treated with a saturated solution of NH_4Cl (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). Organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography (cyclohexane/EtOAc, 3 : 1) to give **15** (336 mg, 46%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47–1.60 (m, 8H, 8×$OCH_2CH_2CH_2CH_2O$), 1.94 (br s, 1H, OH), 2.00 (br s, 1H, OH), 3.10–4.40 (m, 104H, 14 × 2-H, 14 × 3-H, 14 × 4-H, 14 × 5-H, 28 × 6-H, 16×CH_2O, 4×$OCH_2CH{=}CH_2$), 4.42–4.94 (m, 63H, 59×CHPh, 4 × 1-H), 4.97 (d, $^3J_{1,2}$ = 3.6 Hz, 1H, 1 × 1-H), 5.01–5.35 (m, 14H, 4 × 1-H, 6×CHPh, 4×$OCH_2CH{=}CH_2$), 5.43 (d, ^2J = 10.6 Hz, 1×CHPh), 5.44 (d, $^3J_{1,2}$ = 3.6 Hz, 2H, 2 × 1-H), 5.47 (d, $^3J_{1,2}$ = 4.0 Hz, 1H, 1 × 1-H), 5.51 (d, ^2J = 10.7 Hz, 1×CHPh), 5.52 (d, ^2J = 10.4 Hz, 1×CHPh), 5.76 (d, $^3J_{1,2}$ = 4.1 Hz, 1H, 1 × 1-H), 5.77 (d, $^3J_{1,2}$ = 4.2 Hz, 1H, 1 × 1-H), 5.80–5.97 (m, 2H, 2×$OCH_2CH{=}CH_2$), 7.15–7.37 (m, 85H, CH arom.); ^{13}C NMR (100 MHz, $CDCl_3$) : δ 26.8–26.9 (CH_2), 68.2–69.8 (CH_2), 70.3–71.8 (CH, CH_2), 71.9–76.4 (CH_2), 77.7–81.7 (CH), 97.7–100.3 (C-1), 116.9, 116.8 ($OCH_2CH{=}CH_2$), 126.7–128.3 (CH arom.), 134.9, 134.8 ($OCH_2CH{=}CH_2$), 137.9–139.5 (C arom. quat.); MS (FAB): *m*/*z* (%): 2826.7 (100) ([M+Na^+]); HRMS (ESI) *m*/*z* (%): 2826.26215 (calc.); 2826.2645 (1 ppm) (obt.) ([M+Na^+]).

Capped-CD dimer diol 16. A solution of compound **15** (336 mg, 120 mmol) in CH_2Cl_2 (1.5 mL), was degassed three times with argon. Grubbs catalyst (5 mg, 6 mmol) was added and the reaction mixture was refluxed under argon for 18 h. $Pb(OAc)_4$ (8 mg, 18 mmol) was added at r.t. The mixture was stirred for additional 3 h under argon at r.t. and concentrated. The residue was purified by chromatography (cyclohexane/EtOAc, 3 : 1, then 2 : 1, then 1 : 1) to give the unsaturated dimer (250 mg, 74%) as a white foam. This dimer (250 mg, 89 mmol) was dissolved in 1,2-dimethoxyethane (6.7 mL) and treated by *p*-toluenesulfonhydrazide (736 mg, 3.9 mmol). The reaction mixture was refluxed and a solution of sodium acetate in water (660 mg in 4.6 mL H_2O) was added dropwise. After 6 h refluxing, the reaction mixture was concentrated, diluted in EtOAc (20 mL) and washed with H_2O (15 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). Organic layers were combined, washed with brine (40 mL), dried over $MgSO_4$ and concentrated. The crude was purified by chromatography (cyclohexane/EtOAc, 2 : 1) to give the dimer **16** (237 mg, 95%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51–1.64 (m, 12H, 12×$OCH_2CH_2CH_2CH_2O$), 3.12–4.22 (m, 104H, 14 × 2-H, 14 × 3-H, 14 × 4-H, 14 × 5-H, 28 × 6-H, 20×OCH_2), 4.25–5.90 (m, 82H, 14 × 1-H, 68×CHPh), 7.10–7.34 (m, 170H, CH arom.); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.6 (CH_2), 25.7 (CH_2), 26.2 (CH_2), 26.8 (CH_2), 26.9 (CH_2), 61.7 (CH_2), 62.0 (CH_2), 68.0–68.8 (CH_2), 70.2–72.3 (CH, CH_2), 73.7–75.8 (CH_2), 75.9 (CH), 76.0–76.7 (CH_2), 77.8–81.7 (CH), 97.3–100.2 (C-1), 126.8–128.3 (CH arom.), 137.8–139.7 (C arom. quat.); HRMS (LSIMS): *m*/*z* (%): 5604.52224 (calc.); 5604.5237 (0 ppm) (obt.) ([M+Na^+]).

Dialkylated capped-CD dimer 17. At 0 °C and under argon, nBu$_4$NI (1.6 mg, 4.2 mmol) and NaH (60% dispersion in oil, 10 mg, 250 mmol) were added to a solution of dimer **16** (237 mg, 42 mmol) in THF (1.6 mL). The reaction mixture was stirred at 0 °C under argon for 20 min, then tosyl-allyloxyethanol (65 mg, 250 mmol) was added. The mixture was stirred at r.t. under argon for 18 h, then NaH (60% dispersion in oil, 10 mg, 250 mmol) and tosyl-allyloxyethanol (65 mg, 250 mmol) were added. After 8 h, the reaction mixture was treated with MeOH (5 mL), evaporated and finally diluted with CH_2Cl_2 (15 mL). The organic layer was washed with a saturated solution of NH_4Cl (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). Organic layers were combined, washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. The crude mixture was purified by chromatography (cyclohexane/EtOAc, 6 : 1, then 4 : 1, then 2 : 1) to give dimer **17** (191 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50–1.62 (m, 12H, 12×$OCH_2CH_2CH_2CH_2O$), 3.15–4.29 (m, 116H, 12×$OCH_2CH_2CH_2CH_2O$, 16×OCH_2CH_2O, 4×$OCH_2CH{=}CH_2$, 14 × 2-H, 14 × 3-H, 14 × 4-H, 14 × 5-H, 28 × 6-H), 4.30–5.45 (m, 82H, 66×CHPh, 12 × 1-H, 4×$OCH_2CH{=}CH_2$), 5.56 (d, 2H, ^2J = 10.6 Hz, 2×CHPh), 5.77–5.82 (m, 2H, 2 × 1-H), 5.90 (m, 2H, 2×$OCH_2CH{=}CH_2$), 7.07–7.32 (m, 170H, CH arom.); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.5 (CH_2), 26.2 (CH_2), 26.8 (CH_2), 26.9 (CH_2), 68.6–70.9 (CH_2), 71.0–71.8 (CH), 71.9–73.5 (CH_2), 74.6–74.9 (CH_2), 75.4–75.6 (CH), 76.0–76.5 (CH_2), 77.8–81.5 (CH), 97.9–99.3 (C-1), 116.8, 116.85 ($OCH_2CH{=}CH_2$), 126.1–128.3 (CH arom.), 134.8, 134.9 ($OCH_2CH{=}CH_2$),

137.9–139.5 (C arom.quat.); HRMS (ESI): *m/z* (%): 5771.63392 (calc.); 5771.6343 (0 ppm) (obt.) ([M+Na$^+$]); elemental analysis calcd. for $C_{348}H_{386}O_{74}$: C, 72.66; H, 6.76; found C 72.37 H 6.72.

Protected capped-CD duplex 18. A solution of dimer **17** (190 mg, 33 mmol) in CH_2Cl_2 (33 mL, 0.001 M) was degassed three times with argon. Grubbs catalyst (2.7 mg, 3.2 mmol) was added and the reaction mixture was refluxed under argon for 18 h. Additional Grubbs catalyst (2.7 mg, 3.2 mmol) was added and the reaction mixture was refluxed under argon for 18 h. $Pb(OAc)_4$ (5.3 mg, 12 mmol) was added at r.t. The mixture was stirred for additional 3 h under argon at r.t. and concentrated. The residue was purified by chromatography (cyclohexane/EtOAc, 2 : 1) to give the unsaturated duplex (175 mg, 93%) as a white foam. This duplex (170 mg, 29 mmol) was dissolved in 1,2-dimethoxyethane (4.8 mL) and treated by *p*-toluenesulfonhydrazide (530 mg, 2.8 mmol). The reaction mixture was refluxed and a solution of sodium acetate in water (475 mg in 3.3 mL H_2O) was added dropwise. After 6 h refluxing, the reaction mixture was concentrated, diluted with EtOAc (20 mL) and washed with H_2O (15 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). Organic layers were combined, washed with brine (40 mL), dried over $MgSO_4$ and concentrated. The crude was purified by chromatography (cyclohexane/EtOAc, 3 : 1, then 2 : 1) to give duplex **18** (152 mg, 89%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51–1.70 (m, 16H, 16×OCH$_2$CH_2CH_2CH$_2$O),.15–4.30 (m, 116H, 16×OCH_2CH$_2$CH$_2$CH_2O, 16×OCH_2CH_2O, 14 × 2-H, 14 × 3-H, 14 × 4-H, 14 × 5-H, 28 × 6-H), 4.31–5.61 (m, 78H, 68×CHPh, 10 × 1-H), 5.62–5.85 (m, 4H, 4 × 1-H), 7.09–7.34 (m, 170H, CH arom.).; ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.2 (CH_2), 26.5 (CH_2), 26.6 (CH_2), 26.8 (CH_2), 26.9 (CH_2), 71.0–71.8 (CH), 68.6–71.0 (CH_2), 71.2–71.9 (CH), 72.0–73.7 (CH_2), 75.6–76.5 (CH_2), 77.5–81.6 (CH), 97.8–99.3 (C-1), 126.1–128.2 (CH arom.), 137.9–139.6 (C arom. quat.); HRMS (ESI): *m/z* (%): 5759.57656 (calc.); 5759.6149 (7 ppm) (obt.) ([M+K$^+$]); elemental analysis calcd. for $C_{346}H_{382}O_{74}$: C, 72.59; H, 6.73; found C 72.79 H 6.83.

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