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β-Cyclodextrin–Glycerol Dimers: Synthesis and NMR Conformational Analysis

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The synthesis of new β-cyclodextrin dimers linked through their primary faces by different glycerol-like moieties by click chemistry is described. The unusual behaviour of these cyclodextrin–glycerol dimers in aqueous solution has been studied by NMR spectroscopy. We show that, depending on the length of the linking arm between the two cyclodextrins, the dimers could adopt very different conformations in water: symmetrical or pseudo[1]rotaxane-like structures through one D-glucopyranose unit tumbling in one β-cyclodextrin.

Introduction

Cyclodextrins (CDs) are versatile macrocyclic maltooligosaccharides composed of α-(1→4)-linked D-glucopyranose units in the $^4C_1$ chair conformation. The most common CDs have six, seven or eight D-glucopyranose units and are referred to as α-, β- and γ-CD, respectively. As a consequence of the structure, CDs are hydrophilic entities that feature a hydrophobic conical cavity. Due to their unique cup-like structures, CDs are known to form inclusion complexes in aqueous solution with a large variety of organic targets with a hydrophobic nature and a suitable size and geometry, and are involved in many supramolecular applications.[1,2] Because of such an ability to behave as hosts, CDs and their chemically modified derivatives find a wide range of applications that include the areas of drug delivery,[2–4] analytical chemistry,[5] artificial enzymes,[6] photochemical sensors,[7] food technology,[8] catalysis[9] and nanostructured functional materials.[10] However, the relatively low values for the binding constants of native CDs or simply modified CD monomers with model substrates appeared to be a limitation in some of their applications.

CD derivatives consisting of two CD units linked by tethers of different nature have received much attention because of their improved binding abilities and molecular selectivity compared with those of native CDs. In comparison with CD monomers, bridged bis(β-CD) derivatives clearly allow two hydrophobic cavities to be in close vicinity, which is an improvement; moreover, the presence of functional linkers between the two CDs can supply a well-organized pseudo-cavity that may afford supplementary binding properties.[11,12]

Since early work on the synthesis and cooperative binding of CD dimers by the groups of Breslow,[13] Tabushi,[14] Harada[15] and Fujita,[16] much effort has been devoted to making CD dimers with a variety of functional tethers.[11] Recently, click chemistry has proved its efficiency to access such dimeric structures in a straightforward manner.[17] Recent conformational analysis of bridged bis(β-CD) derivatives has highlighted that some CD dimers were able to adopt unusual conformations and behave as pseudo[1]-rotaxanes in water solution through tumbling of the pyranose units of CDs.[18] This inversion phenomenon results in limited accessibility to the CD cavities and is of major importance for understanding and controlling CD applications.

Herein, we report our contribution regarding the synthesis of β-CD dimers with glycerol-like structures as linking arms. Such compounds were obtained by click chemistry coupling reactions. To access CD dimers with different physical properties, (i) the length of the linking arm between the two CDs has been modified; (ii) an allyl group has been introduced on the secondary hydroxy group of the glycerol moiety, which should allow further modifications of the CD dimers by thiol–ene click reactions; and (iii) the behaviour of native and permethylated CDs in solution has been studied by NMR spectroscopy. As already observed in the literature for very few examples,[18] one of these CD dimers showed unusual conformations in aqueous solution.

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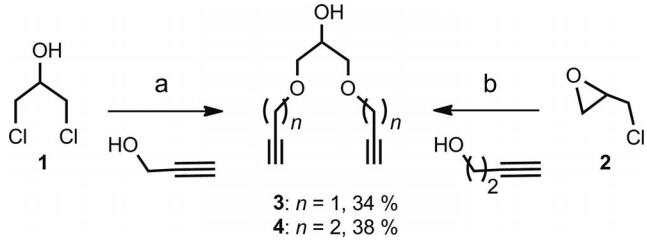
Results and Discussion

Synthesis

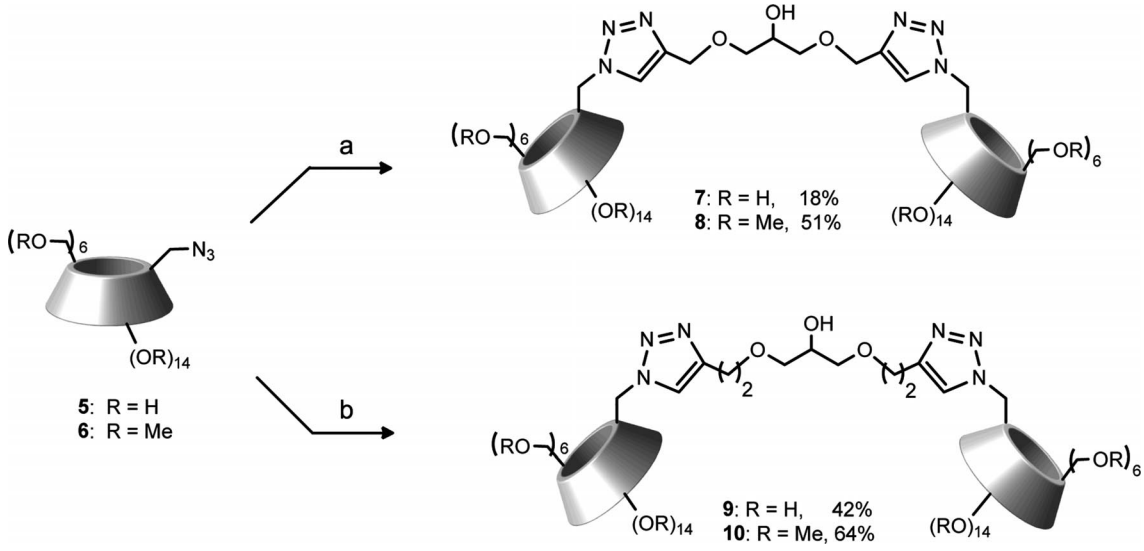
To access β-CD dimers in which CD moieties are linked by spacers of variable lengths, which could also allow further easy modifications, we turned our attention to glycerol-like moieties. Starting from 1,3-dichloro-2-propanol (**1**), we obtained the dipropargylated glycerol derivative **3** in 34% yield in one step by treatment with propargyl alcohol in sodium hydroxide at reflux.[19] Similarly, from epichlorhydrin (**2**) and butynol in the presence of aqueous sodium hydroxide and tetrabutylammonium bromide in petroleum ether, dialkyne **4** was obtained in moderate yield (Scheme 1).



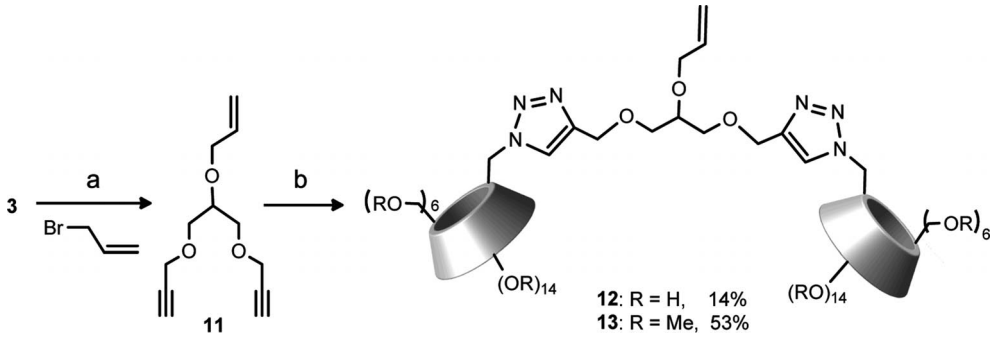
Scheme 1. Synthesis of glycerol-type linking arms **3** and **4**. Reagents and conditions: (a) NaOH (aq.), reflux, 3 h, 34%; (b) NaOH (aq.), Bu_4NBr, petroleum ether, room temp., 18 h, 38%.

With these linkers in hand, the β-CD dimers could be obtained directly by using the Cu^I-catalysed azide–alkyne cycloaddition reaction (CuAAC). Indeed, this click chemistry reaction has been extensively used as an efficient tool to build mono-, di- and tritopic CDs from azido-CDs.[17] In our group, during the synthesis of CD–nucleobase derivatives, we also found that click reactions using mono-6-azido-β-CD were more efficient than the corresponding nucleophilic substitutions starting from mono-6-amino-β-CD.[20] The synthesis of mono-6-azido-β-CD **5** was performed in two steps from β-CD by following a standard literature procedure.[21] Permethylation of **5** with an excess of CH_3I in DMF was achieved as previously detailed to give mono-6-azido-2,3,6-per-*O*-methyl-β-CD **6**.[21] Bispropargylated glycerol arm **3** (1.2 equiv.) was then subjected to CuAAC with **5** or **6** (2 equiv.) and a mixture of $CuSO_4 \cdot 5H_2O$ (2 equiv.)/sodium ascorbate (NaAsc; 4 equiv.) in DMSO at 85 °C to give CD dimers **7** and **8** in 18 and 51% yield, respectively, after semi-preparative HPLC purification for **7** and classical silica gel column purification for **8** (Scheme 2). In a similar manner (Scheme 2), CuAAC reactions were performed starting from azido-CDs **5** or **6** and alkynyl glycerol arm **4** to give β-CD dimers **9** and **10** in 42 and 64% yield, respectively. These new CD dimers gave ^1H and ^{13}C NMR spectroscopy and mass spectrometry data consistent with their structures.

To introduce modifications of biological interest (penetrating or targeting agents) directly onto the linker moiety of the β-CD dimer, we synthesized linking arm **11** in 67% yield by direct alkylation of compound **3** using allyl bromide in the presence of sodium hydride in DMF (Scheme 3). Because thiol–ene reactions have emerged recently in the family of click reactions,[22] linker **11** was designed to allow orthogonal click reactions: first the CuAAC reaction, as described previously for the synthesis of dimers **7**–**10**, then a thiol–ene click reaction to introduce various modifications. Few examples of this click–click strategy have been reported in the literature,[23] and none on CD derivatives. CuAAC reactions between dialkyne **11** and native or permethylated monoazido-β-CD derivatives **5** and **6** were performed as described for the synthesis of other dimers in the presence of a mixture of $CuSO_4 \cdot 5H_2O$/sodium ascorbate in DMSO at 85 °C (Scheme 3). Dimers **12** and **13** were obtained in 14 and 53% yield, respectively, from **5** and **6**.



Scheme 2. Synthesis of β-CD dimers **7**–**10** with glycerol-type linkers. Reagents and conditions: (a) **3**, $CuSO_4 \cdot 5H_2O$, NaAsc (aq.), DMSO, 85 °C. (b) **4**, $CuSO_4 \cdot 5H_2O$, NaAsc (aq.), DMSO, 85 °C.



Scheme 3. Synthesis of β-CD dimers **12** and **13** with a functionalized glycerol linker. Reagents and conditions: (a) NaH, DMF, room temp., o/n, 67%; (b) **5** or **6**,$CuSO_4 \cdot 5H_2O$, NaAsc (aq.), DMSO, 85 °C.

Six new β-CD dimers were obtained: three with native CDs (**7**, **9**, **12**) and three that were permethylated (**8**, **10**, **13**). CDs **7** and **8** differed from **9** and **10** by the spacer length; CDs **12** and **13** possessed a functionalized glycerol linker. During characterization of these six new β-CD dimers by NMR spectroscopy, unexpected observations prompted us to undertake a detailed NMR spectroscopy study in solution.

Solution NMR Spectroscopy Conformational Analysis

^{1}H NMR spectra of the synthesized dimers **7**, **9** and **12** in [D_6]DMSO and **8**, **10** and **13** in $CDCl_3$ (see the Supporting Information) revealed only one singlet for the triazole protons H8-X and H8-Y (Figure 1), which was indicative of a symmetrical conformation.

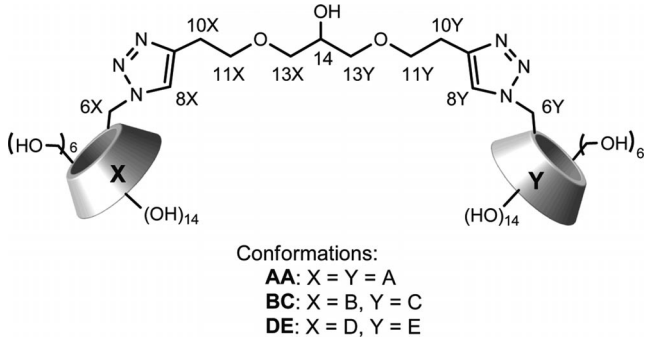


Figure 1. β-CD dimer **9**.

^{1}H NMR spectra of dimer **9** in D_2O or [D_6]DMSO are not similar, in contrast with NMR spectra of dimers **7**, **8**, **10**, **12** and **13**: whereas only one singlet was observed for H8 in [D_6]DMSO, the spectrum in D_2O revealed intriguing signals. Effectively, at 298 K in the dissociating solvent [D_6]-DMSO, both H8-X and H8-Y protons appear as a singlet at δ = 7.81 ppm (Figure 2, A), whereas the ^{1}H NMR spectrum in D_2O (Figure 2, B) is more complicated with two broad singlets at δ = 7.58 and 7.64 ppm and a large signal at δ = 7.90 ppm.

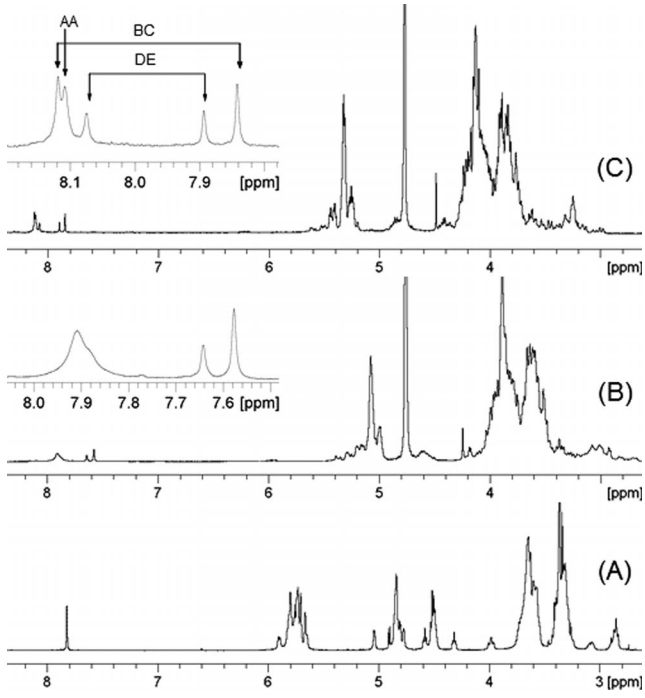


Figure 2. ^{1}H NMR spectra of β-CD dimer **9** (13 mM, 298 K) in [D_6]-DMSO (A) and D_2O at 298 (B) and 318 K (C). AA relates to the symmetrical conformation, BC and DE to the unsymmetrical forms.

To clarify this phenomenon, ^{1}H NMR spectroscopy experiment was performed at 318 K (Figure 2, C). At this temperature, five separated signals are clearly observed (δ = 7.84, 7.89, 8.07, 8.11 and 8.12 ppm). Three species can be deduced from integral values: one symmetrical form, AA, with only one singlet at δ = 8.11 ppm and two unsymmetrical forms, a major one, BC, and a minor one, DE (Figure 2, C), with a distinct signal for both H8-X and H8-Y triazole protons (δ = 7.84 and 8.12 ppm for BC and δ = 7.89 and 8.07 ppm for DE). The H8 protons chemical shifts of unsymmetrical forms BC and DE are close, which suggest two neighbouring structures. Considering the very close downfield chemical shifts of H8 protons (δ = 8.07, 8.11 and 8.12 ppm) of the three forms, the assumption can be made

that all forms have one side with very similar chemical environments. Therefore, each unsymmetrical form BC and DE should have one AA-like side.

It has been reported that increasing the temperature has a detrimental effect on the CD-inclusion phenomenon;[24] the free form used to be favoured. The solution equilibrium of the AA/BC/DE forms in D_2O is modified upon increasing the temperature from 298 to 318 K, as observed in ^1H NMR spectra of dimer **9** at those temperatures. The ratios of symmetrical/unsymmetrical AA/BC/DE forms vary from 30:45:25 to 40:38:22 (Table 1). At 318 K, an increase in the integral value for the symmetrical free form is observed, as described before.

Table 1. Ratio of the three forms observed by ^1H NMR spectroscopy in D_2O, depending on the temperature, for dimer **9**.

T [K]	AA form[a]	BC form[a]	DE form[a]
298	30	45	25
318	40	38	22

[a] Percentage calculated from integrals of H8 signals in the ^1H NMR spectrum.

Although rarely evidenced, tumbling of a pyranose unit of CDs has been recently highlighted. Liu et al. described the synthesis of tetrakis(permethyl-β-CD)-modified zinc(II) porphyrin and showed that some CDs included the porphyrin by rotating 360° around the pivot D-glucopyranose unit in the methylated CD when dissolved in water.[18a] Harada et al. reported the formation of a pseudo[1]rotaxane dimer from an altro-α-CD dimer by using tumbling of an altropyranose unit.[18b,18c] They demonstrated that alkyl CD dimers with long and flexible chains formed the self-inclusion complex easily in aqueous solution. Monflier et al. also described an unusual inversion phenomenon of β-CD dimers in water.[18d] Through a detailed NMR conformational analysis, they clearly showed that one of the CD D-glucopyranose units underwent 360° rotation in water, so that the CD linker was included into one of the CD cavities. They concluded that tumbling strongly depended on the nature of the spacer.

Based on these data, we suppose that dimer **9** could behave as a pseudo-rotaxane through tumbling of one CD glucopyranose unit, as described previously. Regarding the two upfield H8 proton chemical shifts (δ = 7.84 and 7.89 ppm), it can be assumed that these signals attest to a chemical environment that is different from the AA-like side. Unsymmetrical forms BC and DE may have structures in which a part of the spacer is included in the cavity of the opposite CD. This might be possible only by a 360° rotation of one of the substituted D-glucopyranose units of the β-CD dimer. To confirm this hypothesis, we performed 2D-NOESY NMR spectroscopy experiments to observe long-range correlations between the triazole and/or spacer with the CD moiety (Figure 3). In the AA form, no long-range correlations have been observed. On the contrary, in the case of BC and DE, long-range dipolar couplings have been observed. For the BC form, proton H10-B correlates with proton H8-C of the linker and protons H3-C, H5-C and H6-C of the CD (see Figure 3 and the Supporting Information). This clearly demonstrates that BC folds as a hairpin because of the H10-B/H8-C correlation. We can also conclude that the triazole moiety linked to B-CD is included into the CD on the other side (C-CD) because H10-B correlates with internal protons of C-CD. For the minor DE form, the lack of resolution allows fewer correlations to be observed (see the Supporting Information). Nevertheless, interesting correlations can be distinguished that are different from those of the BC form. Indeed, a long-range correlation between H6-D of the CD and H13-D indicates different folding of the linker: the bend of the hairpin is shifted from the middle of the dimer in the case of the BC form to C11-D in the case of the DE form.

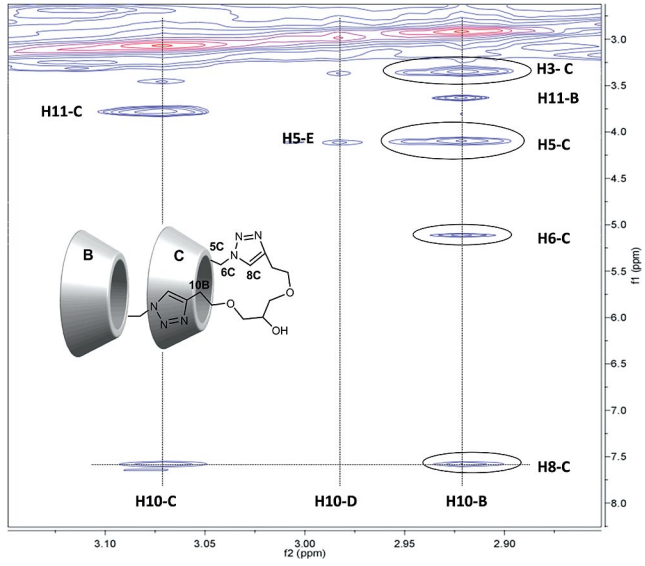


Figure 3. 2D-NOESY NMR spectrum enlargement (H10 region) at 298 K for dimer **9**, and the proposed structure of BC in water.

The BC and DE conformations are similar, but distinct. NMR spectroscopy results suggest strong interactions of the linker, either the triazole part for BC or the glycerol part for DE (see Figure 3 and the Supporting Information) with one of the two CD cavities. Tables summarising exhaustive NOE correlations for each unsymmetrical form are given in the Supporting Information. The inclusion of these hydrophobic moieties could explain the temperature stability of the three conformations in D_2O (Figure 2). Moreover, DE appears to be an intermediate form between AA and BC: the CD of the E side is partially blocked on the glycerol part of the linker thanks to the presence of a hydroxy group.

NOE correlations have been used as constraints in Sybyl software to build, after molecular dynamic calculations, the illustrative 3D models outlined in Figure 4. In the case of the AA form, no constraints have been set. For the BC form, after iterative assays, four distance constraints are sufficient to obtain a structure that complies with every observed NOE. For the DE form, the lack of NOEs makes the 3D structure built with four constraints uncertain. Nev-

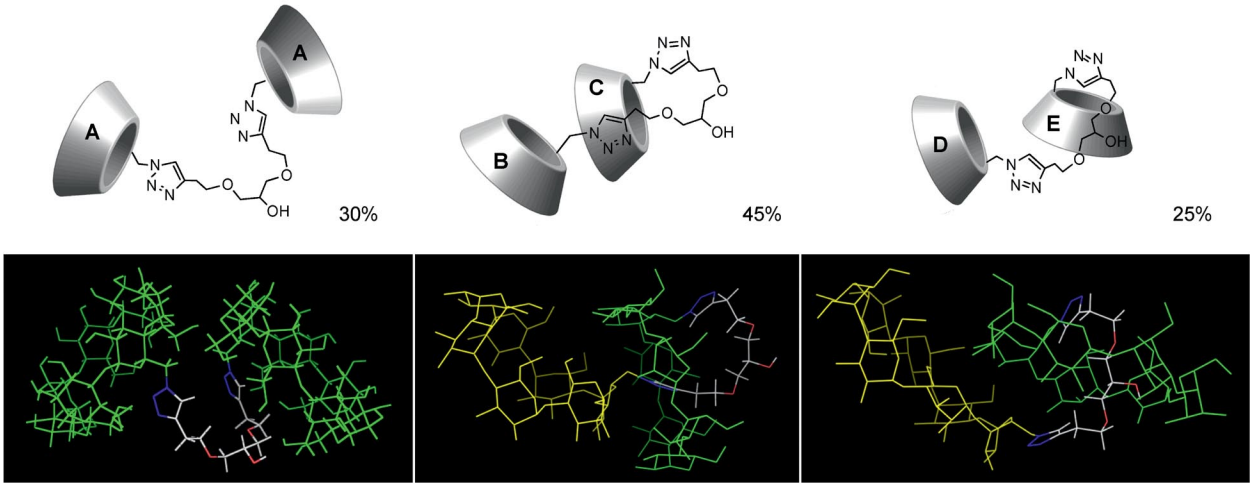
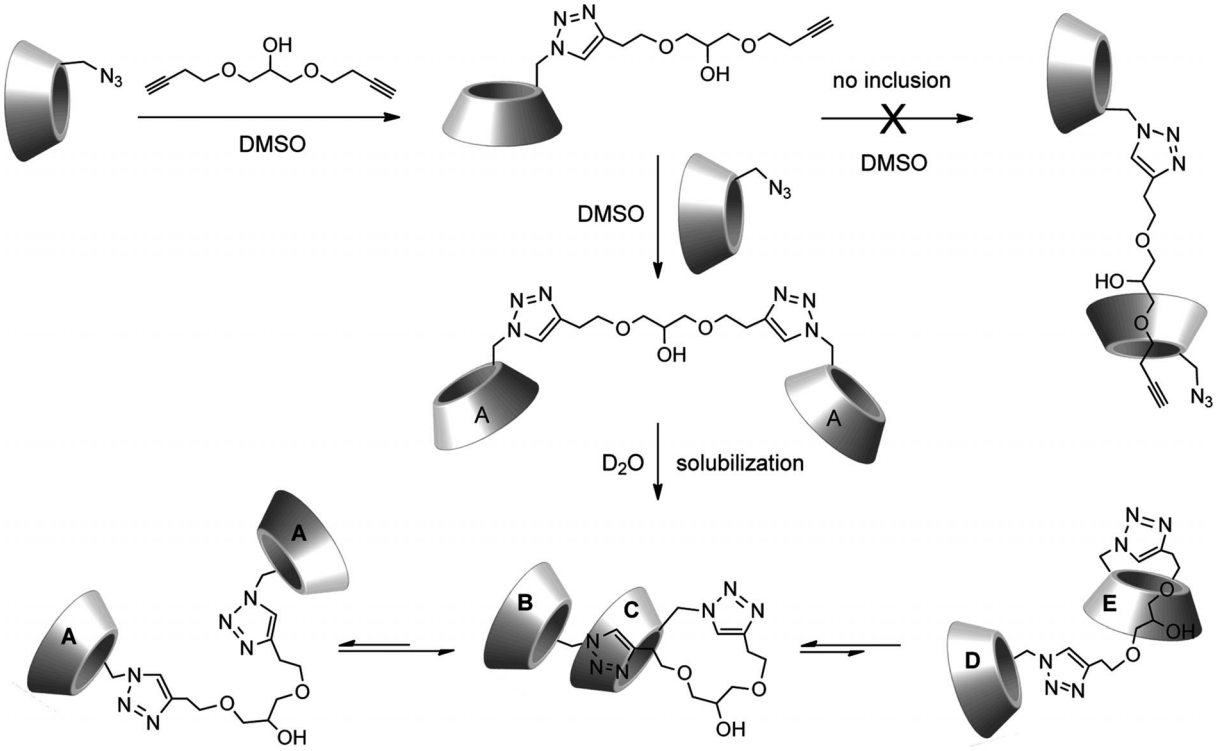


Figure 4. Top: Proposed structures of CD dimer **9** in water at 298 K from NMR spectroscopic data. Bottom: Corresponding models of the three conformations.

ertheless, in the DE conformation, the linking arm clearly appears to be less deeply included into the cavity of the CD than that of the BC form.

Conformational Equilibrium Study and the Inversion Process

From our NMR spectroscopic data, we demonstrated that, amongst six new CD dimers linked by glycerol-type arms of two different lengths, only one showed three different conformations in water: β-CD dimer **9**. The phenomenon is not observed when dimers are composed of permethylated β-CDs, probably due to a lack of space in the cavity. For dimers composed of native β-CDs, the only one able to give rise to tumbling of one glucopyranose unit is the one with the more flexible linking arm, as expected. Compared with the previously described CD dimers in which inversion processes underwent the formation of only two species in water solution (one symmetrical and one unsymmetrical),[18] for the first time, a third form can be described, probably due to the polar nature of the linker. The free hydroxy group of the glycerol moiety behaves as a temporary stopper during the pseudo-rotaxanation process.



Scheme 4. Proposed click chemistry in DMSO and inversion processes in D_2O.

As already mentioned in the literature by Monflier et al.,[18d] the inversion process occurs from the symmetrical conformation once the synthesis of the dimer has been achieved. Indeed, the synthesis was performed in DMSO, a known dissociating solvent, which does not allow any inclusion of the linking arm in the cavity of a monoazido-β-CD unit after the first click reaction occurred (Scheme 4).

Monoazido-β-CD **5** was added in two different ways, giving similar results: either 2 equiv. were directly added at the beginning of the reaction or **5** was added portionwise (equivalent by equivalent). A ^{1}H NMR spectrum of the obtained dimer **9** in [D_6]DMSO revealed a singlet for H8 triazole protons. After evaporation of [D_6]DMSO and dilution in D_2O, the ^{1}H NMR spectrum again showed signals in the triazole region as described before. Thus, the inversion process happens only once the dimer is diluted in water and not during the synthesis and gives rise to three dimers (Scheme 4).

To confirm this hypothesis for the inversion process, complementary NMR spectroscopy experiments were performed. Starting from ^{1}H NMR spectroscopic analysis in D_2O, gradual additions of [D_6]DMSO were performed (Figure 5) with increasing [D_6]DMSO/D_2O ratios. From an initial spectrum showing the three forms for dimer **9** (lane 0), we observed that, by adding small amounts of [D_6]-DMSO, initial upfield signals gradually disappeared. We observed that, once the [D_6]DMSO/D_2O ratio reached 0.68, the resulting ^{1}H NMR spectrum of the dimer showed one singlet at δ = 8.12 ppm for triazole H8 protons. Only one symmetrical conformation remains present in solution; thus demonstrating the reversibility of the inversion process.

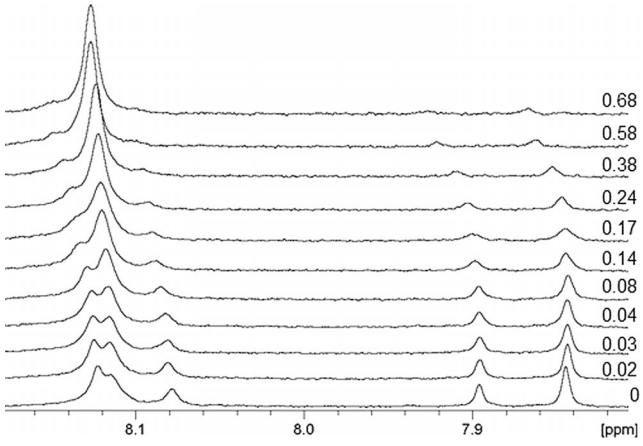


Figure 5. ^{1}H NMR spectra (magnification of the H8 region) of dimer **9** in D_2O (lane 0) with gradual addition of [D_6]-DMSO ([D_6]DMSO/D_2O ratio from 0.02 to 0.68).

Conclusions

Six new β-CD dimers linked by glycerol-type arms have been synthesized by click chemistry in a very straightforward manner. One linker was designed so that a thiol–ene click reaction could be performed directly on the obtained triazole-containing CD-dimers: this work is in progress. NMR spectroscopy experiments showed that, depending on the length of the linker between the two CDs, as well as the nature of the substituent borne by the β-CDs, these dimers could adopt very different conformations in water. Five compounds have only one linear and symmetrical conformation. Interestingly, the sixth could adopt either symmetrical or pseudo[1]rotaxane-like structures. In this case, native CDs are linked by the more flexible arm and three different conformations are present in water solution. Compared with the previously described CD dimers, in which tumbling afforded two species, for the first time, a third form could be observed. These results suggest that one of the D-glucopyranose units undergoes 360° rotation to induce more or less deep inclusion of the linker into the CD cavity. This behaviour in water might impact the physical properties of such dimers; our work in this direction will be reported soon.

Experimental Section

General: All reagents were used as purchased from commercial suppliers without further purification. Solvents (DMF, THF) were distilled under anhydrous conditions. TLC plates (Macherey–Nagel, ALUGRAM SIL G/UV_{254}, 0.2 mm silica gel 60 Å) were visualized under 254 nm UV light and/or by dipping the TLC plate into a solution of phosphomolybdic acid (3 g) in ethanol (100 mL) followed by heating with a heat gun. Purifications by column chromatography were performed using Macherey–Nagel silica gel 60 (15–40 μm). Semi-preparative HPLC purifications were performed by using a C18 reversed-phase column and a water/acetonitrile system as the eluent. NMR spectroscopy experiments were recorded with a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H nuclei and at 100 MHz for ^{13}C nuclei. The chemical shifts are expressed in ppm relative to tetramethylsilane (TMS; δ = 0 ppm) and the coupling constant, *J*, in Hz. NMR multiplicities are reported by using the following abbreviations: br = broad, s = singlet, d = doublet, t = triplet, q = quadruplet and m = multiplet. Microwave irradiations were performed with a CEM Discover apparatus. HRMS were obtained from the Mass Spectrometry Service, CRMPO, at the University of Rennes I, France, using a MICROMASS ZABSPEC-TOF spectrometer and a VARIAN MAT311 spectrometer.

Synthesis

1,3-Bis(prop-2-ynyloxy)propan-2-ol (3): 1,3-Dichloropropan-2-ol (0.37 mL, 3.88 mmol) was added dropwise to a mixture of propargyl alcohol (0.90 mL, 15.47 mmol, 4 equiv.) and sodium hydroxide (0.68 g, 17.10 mmol, 4.4 equiv.) in water (5 mL). The mixture was then heated at reflux for 3 h, cooled and neutralized with 2 M HCl. After extraction with dichloromethane (3 × 20 mL), the organic layers were dried and concentrated under vacuum. The residue was purified by silica-gel column chromatography (CH_2Cl_2) to give **3** as a clear yellow oil (0.22 g, 34%). Physical data are in accordance with the literature.[19] R_f = 0.18 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.17 (s, $H_{1'}$, 4 H), 3.87 (s, H_2, 1 H), 3.63–3.52 (m, H_1, H_3, 4 H), 2.44 (s, $H_{3'}$, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 79.4 ($C_{2'}$, 2 C), 74.9 ($C_{3'}$, 2 C), 70.9 (C_1, C_3, 2 C), 69.3 (C_2, 1 C), 58.6 ($C_{1'}$, 2 C) ppm.

1,3-Bis(but-3-ynyloxy)propan-2-ol (4): 3-Butynol (610 mg, 8.64 mmol, 4 equiv.) and tetrabutylammonium bromide (690 mg, 2.16 mmol) were added to a solution of sodium hydroxide (860 mg,

2.16 mmol) in water (1.5 mL). Petroleum ether (10 mL) was then added to the mixture, and finally 1-chloro-2,3-epoxypropane (200 mg, 21.6 mmol) was added dropwise. The mixture was stirred overnight at room temperature, diluted with water (20 mL) then extracted with dichloromethane (3× 20 mL). The combined organic layers were washed with saturated aq. NaCl (20 mL), water (20 mL), dried and the solvents evaporated to dryness. The residue was purified by silica-gel column chromatography [petroleum ether (PE)/EtOAc, 90:10 to 60:40] to give **4** as a colourless oil (0.16 g, 38%). R_f = 0.23 (80:20 PE/EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.97–3.92 (m, H_2, 1 H), 3.62–3.47 (m, H_1, H_3, $H_{1'}$, 8 H), 2.66 (br. s, OH, 1 H), 2.47–2.43 (m, $H_{2'}$, 4 H), 1.97 (t, J = 2.8 Hz, $H_{4'}$, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 81.2 ($C_{3'}$, 2 C), 71.9–69.3 (C_1, C_2, C_3, $C_{1'}$, $C_{4'}$, 7 C), 19.8 ($C_{2'}$, 2 C) ppm. ES-HRMS [M + Na]$^+$: calcd. for $C_{11}H_{16}O_3Na$: 219.0998; found 219.0997.

Typical Procedures for CuAAC Reactions Between Mono-6-azido-β-CD 5 or 6 and Alkyne Derivatives 3, 4 or 11: Mono-6-azido-β-CD (150 mg, 0.13 mmol, 2 equiv.), the alkyne derivative (100 mg, 0.08 mmol, 1.2 equiv.) and copper sulfate pentahydrate (30 mg, 0.13 mmol, 2 equiv.) were dissolved in DMSO (4 mL). A solution of sodium ascorbate (51 mg, 0.26 mmol, 4 equiv.) in water (1 mL) was added dropwise to this solution for 15 min, then the mixture was stirred at 85 °C for 24 h (for **8**, **9** and **13**), or at 85 °C for 80 min under microwave (MW) activation (for **7**, **10** and **12**). For the hydroxy CDs, acetone (20 mL) was added and the precipitate was filtered off. The crude was then purified by semi-preparative HPLC (gradient elution with H_2O/CH_3CN from 98:2 to 70:30 in 20 min for **7** and **12**, and from 15:95 to 50:50 in 20 min for **9**). For the per-*O*-methylated CDs, the crude mixture was concentrated then purified by silica-gel column chromatography (CH_2Cl_2/MeOH, 100:0 to 94:6). Yields are given after freeze-drying of the samples.

CD Dimer (7): From **3** (10 mg, 0.08 mmol) and **5** (150 mg, 0.13 mmol), dimer **7** was obtained as a white powder (30 mg, 18%). R_f = 0.42 (40:60 H_2O/CH_3CN, 1% NH_4OH). ^{1}H NMR ([D_6]-DMSO, 400 MHz): δ = 8.01 (s, H_8, 2 H), 5.88–5.65 (m, OH_{2CD}, OH_{3CD}, 28 H), 4.89–4.77 (m, H_{1CD}, $H_6{}^A{}_{CD}$, 16 H), 4.62–4.47 (m, $OH_6{}^{B-G}{}_{CD}$, H_{10}, 16 H), 4.29 (m, $H_{6'}{}^A{}_{CD}$, 2 H), 3.97 (m, $H_5{}^A{}_{CD}$, 2 H), 3.63–3.06 (m, H_{2CD}, H_{3CD}, H_{4CD}, $H_5{}^{B-G}{}_{CD}$, $H_6{}^{C-G}{}_{CD}$, H_{12}, H_{13}, 79 H), 3.08–2.84 (m, $H_6{}^B{}_{CD}$, 4 H) ppm. ^{13}C NMR ([D_6]DMSO, 100 MHz): δ = 143.9 (C_9, 2 C), 126.6 (C_8, 2 C), 101.9–101.4 (C_{1CD}, 14 C), 83.0–63.3 (C_{10}, C_{12}, C_{13}, C_{2CD}, C_{3CD}, C_{4CD}, C_{5CD}, 61 C), 60.3 ($C_6{}^{C-G}{}_{CD}$, 10 C), 59.1 ($C_6{}^B{}_{CD}$, 2 C), 51.1 ($C_6{}^A{}_{CD}$, 2 C) ppm. ES-HRMS [M + Na]$^+$: calcd. for $C_{93}H_{150}N_6O_{71}Na$: 2509.8264; found 2509.8209.

Per-*O*-methylated CD Dimer (8): From **3** (60 mg, 0.35 mmol) and **6** (1.0 g, 0.70 mmol), dimer **8** was obtained as a white powder (540 mg, 51%). R_f = 0.21 (CH_2Cl_2/MeOH 95/5). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64 (s, H_8, 2 H), 5.32–5.12 (m, H_{1CD}, 14 H), 4.93–4.79 (m, $H_6{}^A{}_{CD}$, 4 H), 4.68 (m, H_{10}, 4 H), 4.09 (m, $H_5{}^A{}_{CD}$, 2 H), 3.97–3.05 (m, H_{12}, H_{13}, H_{2CD}, H_{3CD}, H_{4CD}, $H_5{}^{B-G}{}_{CD}$, $H_6{}^{B-G}{}_{CD}$, OCH_{3CD}, 203 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 144.3 (C_9, 2C), 124.9 (C_8, 2C), 99.2–98.3 (m, C_{1CD}, 14C), 82.6–58.4 (C_{10}, C_{12}, C_{13}, C_{2CD}, C_{3CD}, C_{4CD}, C_{5CD}, $C_6{}^{B-G}{}_{CD}$, OCH_{3CD}, 113 C), 51.2 ($C_6{}^A{}_{CD}$, 2 C) ppm. ES-HRMS [M + Na]$^+$: calcd. for $C_{133}H_{230}N_6O_{71}Na$: 3070.4463; found 3070.4469.

CD Dimer (9): From **4** (30 mg, 0.13 mmol) and **5** (150 mg, 0.13 mmol), dimer **9** was obtained as a white powder (110 mg, 42%). R_f = 0.48 (40:60 H_2O/CH_3CN, 1% NH_4OH). ^{1}H NMR ([D_6]DMSO, 400 MHz): δ = 7.81 (s, H_8, 2 H), 5.90–5.64 (m, OH_{2CD}, OH_{3CD}, 28 H), 5.03–4.76 (m, H_{1CD}, $H_6{}^A{}_{CD}$, 16 H), 4.59–4.48 (m, $OH_6{}^{C-G}{}_{CD}$, $H_{6'}{}^A{}_{CD}$, 12 H), 4.31 (t, $OH_6{}^B{}_{CD}$, 2 H, J = 8 Hz), 3.97 (m, $H_5{}^A{}_{CD}$, 2 H), 3.63–3.06 (m, H_{2CD}, H_{3CD}, H_{4CD}, $H_5{}^{B-G}{}_{CD}$, $H_6{}^{C-G}{}_{CD}$, H_{11}, H_{13}, H_{14}, 83 H), 3.08–2.84 (m, H_{10}, $H_6{}^B{}_{CD}$, 8 H) ppm. ^{13}C NMR ([D_6]DMSO, 100 MHz): δ = 143.7 (C_9, 2 C), 123.7 (C_8, 2 C), 102.2 (C_{1CD}, 14 C), 82.0–71.9 (C_{2CD}, C_{3CD}, C_{4CD}, $C_5{}^{B-G}{}_{CD}$, 54 C), 70.0 ($C_5{}^A{}_{CD}$, 2 C), 69.7 (C_{11}, C_{13}, 4 C), 68.6 (C_{14}, 1 C), 59.9 ($C_6{}^{C-G}{}_{CD}$, 10 C), 58.9 ($C_6{}^B{}_{CD}$, 2 C), 50.3 ($C_6{}^A{}_{CD}$, 2 C), 25.8 (C_{10}, 2 C) ppm. ES-HRMS [M + Na]$^+$: calcd. for $C_{95}H_{154}N_6O_{71}Na$: 2537.8510; found 2537.8517.

Per-*O*-methylated CD Dimer (10): From **4** (20 mg, 0.11 mmol) and **6** (270 mg, 0.19 mmol), dimer **10** was obtained as a white powder (180 mg, 64%). R_f = 0.45 (CH_2Cl_2/MeOH: 94:6). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.47 (s, H_8, 2 H), 5.28–5.08 (m, H_{1CD}, 14 H), 4.87–4.67 (m, $H_6{}^A{}_{CD}$, 4 H), 4.04 ($H_5{}^A{}_{CD}$, 2 H), 3.93–2.93 (H_{10}, H_{11}, H_{13}, H_{14}, H_{2CD}, H_{3CD}, H_{4CD}, $H_5{}^{B-G}{}_{CD}$, $H_6{}^{B-G}{}_{CD}$, OCH_{3CD}, 211 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 144.3 (C_9, 2 C); 124.0 (C_8, 2 C); 99.2–98.8 (C_{1CD}, 14 C); 82.7–58.5 (C_{10}, C_{11}, C_{13}, C_{14}, C_{2CD}, C_{3CD}, C_{4CD}, C_{5CD}, $C_6{}^{B-G}{}_{CD}$, OCH_{3CD}, 115 C), 51.1 ($C_6{}^A{}_{CD}$, 2 C) ppm. ES-HRMS [M + Na]$^+$: calcd. for $C_{135}H_{234}N_6O_{71}Na$: 3098.4800; found 3098.4782.

3-[1,3-Bis(prop-2-ynyloxy)propan-2-yloxy]prop-1-ene (11): Sodium hydride (40 g, 1.78 mmol, 3 equiv.) was added to a solution of compound **3** (100 mg, 0.59 mmol.) in anhydrous DMF (5 mL) at 0 °C. After 10 min allyl bromide (220 mg, 1.78 mmol, 3 equiv.) was added dropwise then the mixture was stirred overnight at room temperature. A few drops of methanol were added then the mixture was concentrated and the residue purified by silica-gel column chromatography (CH_2Cl_2) to give **11** as a colourless oil (80 mg, 67%). R_f = 0.61 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.95–5.85 (m, CH-allyl, 1 H), 5.28–5.12 (m, CH_2-allyl, 2 H), 4.16 (s, $H_{1'}$, 4 H), 4.13–4.11 (m, O-CH_2-allyl, 2 H), 3.69–3.57 (m, H_1, H_2, H_3, 5 H), 2.42 (s, $H_{3'}$, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 135.0 (CH-allyl, 1 C), 117.1 (CH_2-allyl, 1 C), 79.7 ($C_{2'}$, 2 C), 76.8 (C_2, 1 C), 74.6 ($C_{3'}$, 2 C), 71.3 (O-CH_2-allyl, 1 C), 69.8 (C_1, C_3, 2 C), 58.7 ($C_{1'}$, 2 C) ppm. ES-HRMS [M + Na]$^+$: calcd. for $C_{12}H_{16}O_3Na$: 231.0999; found 231.0997.

CD Dimer (12): From **11** (17 mg, 0.083 mmol) and **5** (200 mg, 0.14 mmol), dimer **12** was obtained as a white powder (30 mg, 14%). R_f = 0.42 (40:60 H_2O/CH_3CN, 1% NH_4OH). ^{1}H NMR (D_2O, 400 MHz): δ = 8.03 (s, H_8, 2 H), 5.91–5.84 (m, CH-allyl, 1 H), 5.30–5.15 (m, CH_2-allyl, 2 H), 5.04–4.95 (m, H_{1CD}, $H_6{}^A{}_{CD}$, 16 H), 4.66–4.58 (m, $H_{6'}{}^A{}_{CD}$, H_{10}, 6 H), 4.14 (t, $H_5{}^A{}_{CD}$, 2 H, J = 9 Hz), 4.08 (d, O-CH_2-allyl, 2 H, J = 6 Hz), 3.95–3.46 (m, H_{2CD}, H_{3CD}, H_{4CD}, $H_5{}^{B-G}{}_{CD}$, $H_6{}^{C-G}{}_{CD}$, H_{12}, H_{13}, 79 H), 3.16–2.79 (m, $H_6{}^B{}_{CD}$, 4 H) ppm. ^{13}C NMR (D_2O, 100 MHz): δ = 134.1 (CH-allyl, C_9, 3 C), 126.4 (C_8, 2 C), 118.2 (CH_2-allyl, 1 C), 102.0–101.4 (C_{1CD}, 14 C), 83.0–70.7 (C_{13}, O-CH_2-allyl, C_{2CD}, C_{3CD}, C_{4CD}, C_{5CD}, 58 C), 69.3 (C_{12}, 2 C), 63.4 (C_{10}, 2 C), 60.1 ($C_6{}^{C-G}{}_{CD}$, 10 C), 59.0 ($C_6{}^B{}_{CD}$, 2 C), 51.1 ($C_6{}^A{}_{CD}$, 2 C) ppm. ES-HRMS [M + Na]$^+$: calcd. for $C_{96}H_{154}N_6O_{71}Na$: 2549.8500; found 2549.8522.

Per-*O*-methylated CD Dimer (13): From **11** (10 mg, 0.06 mmol) and **6** (150 mg, 0.10 mmol), dimer **13** was obtained as a white powder (80 mg, 53%). R_f = 0.32 (CH_2Cl_2/MeOH: 93:7). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.61 (s, H_8, 2 H), 5.90–5.80 (m, CH-allyl, 1 H), 5.31–5.27 (m, CH_2-allyl, 2 H), 5.14–5.09 (m, H_{1CD}, 14 H), 4.88–4.75 (m, $H_6{}^A{}_{CD}$, 4 H), 4.63 (s, H_{10}, 4 H), 4.20–4.06 (m, O-CH_2-allyl, $H_5{}^A{}_{CD}$, 4 H), 3.95–3.02 (m, H_{12}, H_{13}, H_{2CD}, H_{3CD}, H_{4CD}, $H_5{}^{B-G}{}_{CD}$, $H_6{}^{B-G}{}_{CD}$, OCH_{3CD}, 203 H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 144.6 (C_9, 2 C), 135.1 (CH-allyl, 1 C), 125.0 (C_8, 2 C), 117.0 (CH_2-allyl, 1 C), 99.3–98.4 (C_{1CD}, 14 C), 82.7–58.5 (C_{12}, C_{13}, O-CH_2-allyl, C_{2CD}, C_{3CD}, C_{4CD}, C_{5CD}, $C_6{}^{B-G}{}_{CD}$, OCH_{3CD}, 114 C), 51.3 ($C_6{}^A{}_{CD}$, 2 C). ES-HRMS [M + Na]$^+$: calcd. for $C_{136}H_{234}N_6O_{71}Na$: 3110.4764; found 3110.4782.

NMR Spectroscopy Measurements: NMR spectroscopy experiments were performed with a Bruker Avance 400 MHz spectrometer, except for NOESY analysis of compound **9**, which was performed on a Varian 500 MHz spectrometer. CD dimers were dissolved in D_2O or [D_6]DMSO. Because of the complexity of the spectra, the chemical shift assignment of compound **9** was determined starting from the assignment of compound **7**, which could be obtained more easily by classical 2D NMR spectroscopy experiments (COSY, HSQC, HMBC) and by selective TOCSY. Chemical shift assignments of **9** were completed with the experiments previously cited and by an additional 2D NOESY (with a mixing time of 300 ms) experiment. Only the protons of the linker and the CD pyranose unit connected to this linker could be assigned. To follow the chemical shift variations of dimer **9** protons upon addition of DMSO, increasing amounts of [D_6]DMSO were added directly to the NMR tube.

Molecular Modelling: Models of the three forms of CD dimer **9** were built in Avogadro software. Minimisations and molecular dynamics, with or without constraints, were run in Sybyl software. Force field: MMFF94s; dielectric constant: 80.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H and ^{13}C NMR spectra for glycerol derivatives **3**, **4** and **11** and β-CD dimers **7**–**10**, **12** and **13**, as well as tables showing NOE correlations and chemical shifts assignments for dimer **9**.

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[1] a) J. Szejtli, *Chem. Rev.* **1998**, *98*, 1743–1754; b) J. Szejtli, T. Osa, *Comprehensive Supramolecular Chemistry*, Pergamon Press, Oxford, UK, **1996**, vol. 3; c) for a special issue on cyclodextrins, see: *Chem. Rev.* **1998**, *98*.
[2] J. Defaye, J. M. Garcia Fernandez, C. Ortiz Mellet, *Ann. Pharm. Fr.* **2007**, *65*, 33–49.
[3] B. Perly, S. Moutard, F. Djedaini-Pilard, *Pharm. Chem. J.* **2005**, *4*, 4–9.
[4] J. Li, H. Xiao, Y. P. Zhong, *Int. J. Pharm.* **2004**, *278*, 329–342.
[5] R. Bhushan, R. Kumar, *J. Chromatogr. A* **2009**, *1216*, 3413–3417.
[6] R. Breslow, S. D. Dong, *Chem. Rev.* **1998**, *98*, 1997–2012.
[7] G. G. Surpateanu, M. Becuwe, N. C. Lungu, P. I. Dron, S. Foumentin, D. Landy, *J. Photochem. Photobiol. A: Chem.* **2007**, *185*, 312–320.
[8] G. Astray, C. Gonzalez-Barreiro, J. C. Mejuto, R. Rial-Otero, J. Simal-Gandara, *Food Hydrocolloids* **2009**, *23*, 1631–1640.
[9] a) P. Blach, D. Landy, S. Fourmentin, G. Surpateanu, H. Bricout, A. Ponchel, F. Hapiot, E. Monflier, *Adv. Synth. Catal.* **2005**, *347*, 1301–1307; b) J. Bjerre, C. Rousseau, L. Marinescu, M. Bols, *Appl. Microbiol. Biotechnol.* **2008**, *81*, 1–11.
[10] a) A. Harada, *Acc. Chem. Res.* **2001**, *34*, 456–464; b) Y. Takashima, Y. Yang, M. Otsubo, H. Yamaguchi, A. Harada, *Beilstein J. Org. Chem.* **2012**, *8*, 1594–1600.
[11] Y. Liu, Y. Chen, *Acc. Chem. Res.* **2006**, *39*, 681–691.
[12] S. Aime, E. Gianolio, F. Arena, A. Barge, K. Martina, G. Heropoulos, G. Cravatto, *Org. Biomol. Chem.* **2009**, *7*, 370–379.
[13] R. Breslow, N. Greenspoon, T. Guo, R. Zarzycki, *J. Am. Chem. Soc.* **1989**, *111*, 8296–8297.
[14] I. Tabushi, Y. Kuroda, K. Shimokawa, *J. Am. Chem. Soc.* **1979**, *101*, 1614–1615.
[15] A. Harada, M. Furue, S. Nozakura, *Polym. J.* **1980**, *12*, 29–33.
[16] K. Fujita, S. Ejima, T. Imoto, *J. Chem. Soc., Chem. Commun.* **1984**, 1277–1278.
[17] a) J. M. Casas-Solvas, M. C. Martos-Maldonado, A. Vargas-Berenguel, *Tetrahedron* **2008**, *64*, 10919–10923; b) M. Mourer, F. Hapiot, E. Monflier, S. Menuel, *Tetrahedron* **2008**, *64*, 7159–7163; c) M. Mourer, F. Hapiot, S. Tilloy, E. Monflier, S. Menuel, *Eur. J. Org. Chem.* **2008**, 5723–5730; d) G. Cravatto, F. Mendicuti, K. Martina, S. Tagliapietra, B. Robaldo, A. Barge, *Synlett* **2008**, 2642–2646; e) M. Munteanu, S. Choi, H. Ritter, *J. Inclusion Phenom. Macrocyclic Chem.* **2008**, *62*, 197–202; f) D. N. Tran, C. Blaszkiewicz, S. Menuel, A. Roucoux, K. Philippot, F. Hapiot, E. Monflier, *Carbohydr. Res.* **2011**, *346*, 210–218; g) M. C. Martos-Maldonado, I. Quesada-Soriano, J. M. Casas-Solvas, L. Garcia-Fuentes, A. Vargas-Berenguel, *Eur. J. Org. Chem.* **2012**, 2560–2571; h) P.-A. Faugeras, B. Boëns, P.-H. Elchinger, F. Brouillette, D. Montplaisir, R. Zerrouki, R. Lucas, *Eur. J. Org. Chem.* **2012**, 4087–4105.
[18] a) Y. Liu, C.-F. Ke, H.-Y. Zhang, J. Cui, F. Ding, *J. Am. Chem. Soc.* **2008**, *130*, 600–605; b) K. Yamauchi, A. Miyawaki, Y. Takashima, H. Yamaguchi, A. Harada, *Org. Lett.* **2010**, *12*, 1284–1286; c) K. Yamauchi, A. Miyawaki, Y. Takashima, H. Yamaguchi, A. Harada, *J. Org. Chem.* **2010**, *75*, 1040–1046; d) S. Menuel, N. Azaroual, D. Landy, N. Six, F. Hapiot, E. Monflier, *Chem. Eur. J.* **2011**, *17*, 3949–3955; e) Y. Takashima, Y. Fukui, M. Otsubo, N. Hamada, H. Yamaguchi, H. Yamamoto, A. Harada, *Polym. J.* **2012**, *44*, 278–285.
[19] M. G. Veliev, N. M. Agaev, M. I. Shatirova, A. Z. Chalabieva, G. D. Geidarova, *Russ. J. Appl. Chem.* **2010**, *83*, 1957–1961.
[20] a) F. Hamon, B. Violeau, F. Turpin, M. Bellot, L. Bouteiller, F. Djedaini-Pilard, C. Len, *Synlett* **2009**, *17*, 2875–2879; b) V. Legros, F. Hamon, B. Violeau, F. Turpin, F. Djedaini-Pilard, J. Désiré, C. Len, *Synthesis* **2011**, *2*, 235–242.
[21] a) C. Bertolla, S. Rolin, B. Evrard, L. Pochet, B. Masereel, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1855–1858; b) L. Jicsinszky, R. Ivanyi, *Carbohydr. Polym.* **2001**, *45*, 139–145.
[22] a) A. Dondoni, *Angew. Chem.* **2008**, *120*, 9133; *Angew. Chem. Int. Ed.* **2008**, *47*, 8995–8997; b) C. E. Hoyle, A. B. Lowe, C. N. Bowman, *Chem. Soc. Rev.* **2010**, *39*, 1355–1387.
[23] a) G. Franc, A. K. Kakkar, *Chem. Soc. Rev.* **2010**, *39*, 1536–1544; b) A. S. Goldmann, A. Walther, L. Nebhani, R. Joso, D. Ernst, K. Loos, C. Barner-Kowollik, L. Barner, A. H. E. Müller, *Macromolecules* **2009**, *42*, 3707–3714.
[24] Y. Chen, T. Xu, X. Shen, H. Gao, *J. Photochem. Photobiol. A: Chem.* **2005**, *173*, 42–50.

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