α-Cyclodextrins reversibly capped with disulfide bonds†

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Per-*O*-methyl-6^{I},6^{IV}-disulfanyl-6^{I},6^{IV}-dideoxy-α-cyclodextrin undergoes air oxidation at pH 9 to exclusively form a monomeric species having an intramolecular disulfide linkage capping the $C6^{I}$ and $C6^{IV}$ positions. In contrast, its non-methylated analogue, 6^{I},6^{IV}-disulfanyl-6^{I},6^{IV}-dideoxy-α-cyclodextrin, gives a mixture of monomeric and dimeric species under the same conditions. Molecular models show that in both cases of the monomeric species, the disulfide bonds effectively close the primary rims of the cyclodextrin cavities. The disulfide bonds of the capped cyclodextrins could be cleaved by reduction with dithiothreitol. Thus, the macrocyclic system can interchange the open-ended and cup-like forms by the application of external stimuli.

Introduction

Stimuli-responsive molecular devices have become a subject of intense research,[1,2] in particular because of their potential applications in industry and medicine. In principle, they can be realized by molecules and molecular assemblies that can perform a function through a reversible change of their conformation or constitution in response to chemical or physical changes in their environment. For example, various shuttles controlled by electrochemical,[3] photochemical[4] or chemical[5,6] stimuli have been described. Externally-controlled dimerizations of macrocyclic compounds into capsules by electrochemical inputs,[7,8] as well as chemical stimuli-induced releasing of guest molecules from cucurbiturils,[9] are examples of this concept that one day could be used in medicine for drug delivery. Dynamic combinatorial chemistry[10,11] has recently produced systems where chemical effectors (templates) are capable of switching, for example ring–chain equilibria, to produce strongly binding receptors[12–14] or induce morphological changes.[15,16]

We are particularly interested in external stimuli-controlled equilibria in host–guest systems through the manipulation of dynamic covalent bonds. In this context, the disulfide bond appears to be an interesting chemical switching element due to its capability of undergoing a reductive cleavage to give thiols, which can be followed by a backward oxygen-mediated oxidative formation of the initial disulfide state. Recently, we reported the synthesis of an α-cyclodextrin duplex, obtained by the oxidative dimerization of 6^{I},6^{IV}-disulfanyl-α-cyclodextrin.[17] Apart from the dimeric species (Scheme 1, path A), the monomeric intramolecular disulfide was also isolated (Scheme 1, path B) from the reaction mixture. The presence of an appreciable amount of the α-cyclodextrin capped with the disulfide bond over the primary rim was unexpected because of the presumed distortion of the macrocycle invoked by the formation of such a short intramolecular disulfide linkage. The analysis of a molecular model of the monomer revealed that the disulfide bond occupies the entrance at the narrower rim of the cavity. Provided that cleavage/reconstitution of the disulfide bridge can be achieved in a reversible manner, the system represents an interesting molecular analogue of a gate whose open and closed states can be controlled by means of external stimuli, in this case by the action of reductive or oxidative agents. The reversibility of that particular system was, however, hampered as the formation of the monomeric intramolecular disulfide (oxidative step) tended to be accompanied by the dimeric form. This dimer could be formed either directly from the disulfanyl precursor upon oxidation (Scheme 1, path A), or *via* a thiol–disulfide exchange (Scheme 1, path C) whose equilibrium was shifted in favor of the dimer within the 1 to 10 mM concentration range.[17] Consequently, we reasoned that a substitution of hydrogens on the remaining primary hydroxyl groups with more bulky methyl groups should suppress the dimerization reaction due to steric hindrance, shifting the equilibrium in favor of monomeric species (Scheme 1, path B).

In this paper, we describe the synthesis of the intramolecular disulfide of permethylated α-cyclodextrin **9** (Scheme 2). Next, we examine the propensity of **9** and its non-methylated analogue **10** (Scheme 3) to undergo cleavage by a reducing thiol (dithiothreitol) and their subsequent oxidative reformation. Further insight into the molecular structures of both intramolecular disulfides **9** and **10** is provided by an analysis of their energy-minimized structures calculated by *ab initio* methods.

Results and discussion

Synthesis

The initial discrimination of the 6^{A} and 6^{D} positions at the α-cyclodextrin macrocycle was achieved using the DIBAL-H-promoted cleavage[18] of two benzyl groups at the 6^{A} and 6^{D} positions of perbenzylated α-cyclodextrin **1** (Scheme 2). In the course of several repetitive experiments carried out according to a literature procedure,[18] we observed that reactions proceeding in Schlenk flasks at the scale of 0.5 mL of reaction mixture

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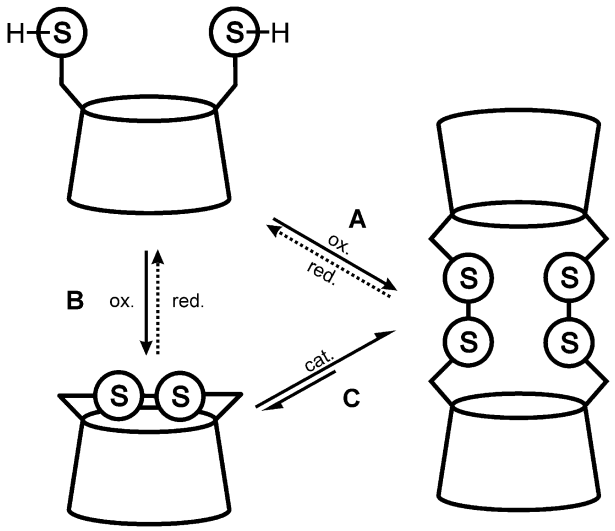


Scheme 1 A representation of reaction pathways allowing the interconversion of disulfanyl α-cyclodextrin, monomeric intramolecular disulfide and dimeric intermolecular disulfide.

gave—in comparison with 10 mL-scale experiments—more rapid cleavage of the first two benzyl groups in the 6^A and 6^D positions, while further cleavage was not accelerated. Consequently, the reaction mixture after quenching contained a lesser amount of the overreacted (triol) by-product. Careful observation revealed that small-volume reaction mixtures were prone to a considerable loss of solvent due to its condensation on the wall of the flask upon heating, increasing the concentration of the reactants. Subsequent experiments using more concentrated solutions of DIBAL-H confirmed this hypothesis. Moreover, lowering the temperature further increased selectivity. Thus, the reaction of an excess (22 equivalents) of a 3 M solution of DIBAL-H with perbenzylated α-cyclodextrin **1** at room temperature for 48 h furnished diol **2** virtually free from by-products in 94% yield (see the ^{1}H NMR spectrum of the crude product in Fig. S2 of the ESI†). The free hydroxyl groups were then protected with triisopropylsilyl (TIPS) groups. In contrast to *tert*-butyldimethylsilyl groups, which were also tested as protective moieties, TIPS groups turned out to be stable in the ensuing transformations, namely during the cleavage of the benzyl groups by hydrogenation. Thus, the reaction of diol **2** with triisopropylsilyl chloride using potassium hydride as a base in THF gave TIPS-protected derivative **3**. The use of potassium hydride as a base turned out to be essential; the usual reaction conditions for the introduction of TIPS groups, such as the use of imidazole as a base in DMF or even sodium hydride in THF, allowed only for partial substitution.

In the next step, the benzyl groups were removed by hydrogenation using palladium on charcoal as a catalyst in a mixture of DMF and ethanol.[17] The free hydroxyl groups of **4** were then methylated with methyl iodide using sodium hydride as a base to obtain permethylated derivative **5**. Subsequently, the TIPS groups were removed with tetrabutylammonium fluoride. Methylated diol **6** was then treated with tetrabromomethane and triphenylphosphine to obtain dibromide **7**, which was then converted to bis(acetylsulfanyl) derivative **8** by reaction with potassium thioacetate. In the next two-step sequence performed in one pot, the acetyl groups were first removed by alkaline hydrolysis under an inert atmosphere, followed by a dilution of the reaction mixture with water to adjust the concentration of the intermediate disulfanyl derivative to 1 mM. Then, the pH of the solution was lowered to 9 by the addition of solid carbon dioxide, and the mixture was allowed to react in an open flask under stirring for 48 h. The analysis of the reaction mixture by NMR and MS revealed that intramolecular disulfide **9** was formed as the *sole* product; no higher oligomers were detected. The product was isolated in 92% yield by reversed phase chromatography.

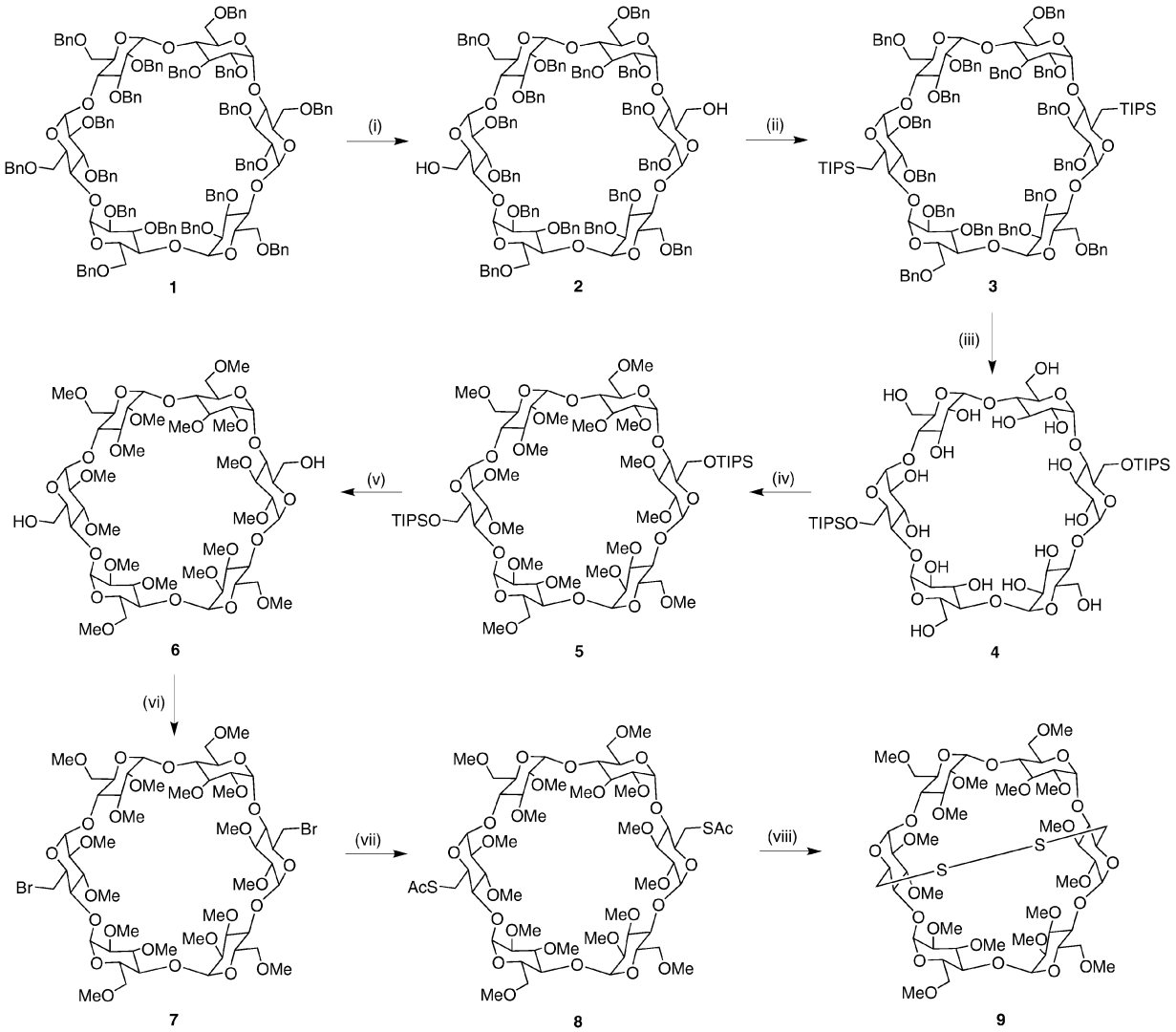
Reversible cleavage of disulfide bridges

The dynamic nature of the disulfide bond should allow reversible opening/closing of the capping over the narrower rim of the α-cyclodextrin macrocycle. In principle, the scission of the disulfide linkages could be achieved with a number of reducing reagents. However, reducing thiols that allow cleavage *via* thiol–disulfide exchange under mild conditions are most appealing, with respect to potential applications in living organisms. We examined the reversibility of the cleavage/closure steps by reduction of intramolecular disulfides **9** and **10**, respectively, with dithiothreitol (DTT), which was followed by air-promoted oxidative disulfide bond reformation (Scheme 3). Thus, disulfides **9** and **10** were each treated with two molar equivalents of DTT in deuterated phosphate buffer (pD 8.4) in flame-sealed NMR tubes under an argon atmosphere.[19] Monitoring of the reaction by ^{1}H NMR revealed that the reductive cleavage of disulfides **9** and **10** (Fig. 1a and Fig. 2a) to their corresponding disulfanyl derivatives **11** and **12** (Fig. 1b and Fig. 2b) proceeded cleanly within 4 h. Subsequently, the NMR tubes were opened to the air to allow the system to reoxidize. Complete oxidation of the sulfanyl groups to disulfide linkages proceeded within 48 h in both cases. However, the composition of the reaction mixture was different in each case, as evidenced by the ^{1}H NMR spectra. Whilst intermediate methylated disulfanyl derivative **11** was exclusively reoxidized to intramolecular disulfide **9** (*cf*. Fig. 1a and c), oxidation of non-methylated disulfanyl derivative **12** gave a mixture of both monomeric and dimeric species, **10** and **13** (*cf*. Fig. 2a and c). Thus, methylated intramolecular disulfide **9** is fully capable of reversible locking/unlocking the disulfide cap over the narrower rim of the cyclodextrin cavity.

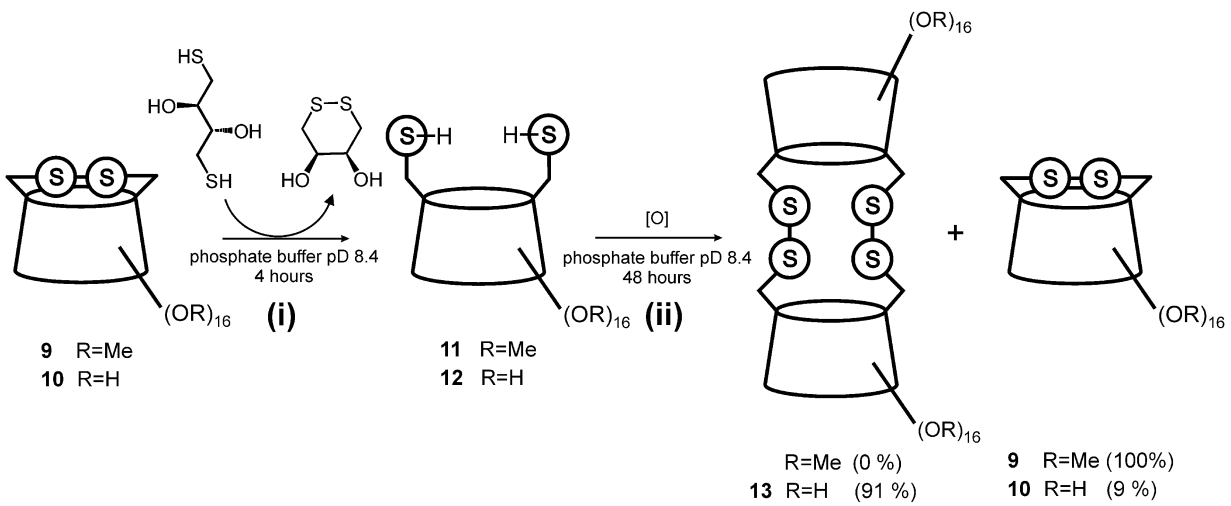
Molecular modelling

In contrast to our recently described α-cyclodextrin duplexes connected with two[17] or three[20] disulfide bonds, monomeric intramolecular disulfides **9** and **10** resisted to our efforts to obtain either of them in a crystalline state amenable to crystallographic analysis. Therefore, we used quantum chemical modelling to obtain an alternative insight into their molecular structure in the gas phase.

Initial coordinates of the cyclodextrin macrocycles in compounds **9** and **10** were obtained from the crystal structures of permethylated[21] and native[22] α-cyclodextrins, respectively. MCM molecular graphics[23] was used to arbitrarily rotate the terminal OH and OCH_3 residues of the molecules to adopt a



Scheme 2 The synthesis of intramolecular disulfide **9**. Reagents and conditions: (i) DIBAL-H (3 M), toluene, 94%; (ii) KH, triisopropylsilyl chloride, THF, 89%; (iii) Pd/C, H_2, DMF–EtOH, 82%; (iv) NaH, MeI, DMF, 90%; (v) tetrabutylammonium fluoride, THF, 89%; (vi) CBr_4, Ph_3P, DMF, 85%; (vii) CH_3COSNa, DMF, 90%; (viii) a. NaOH (1M), b. CO_2 to pH 9, air, 92%.



Scheme 3 Cleavage of the intramolecular disulfide bonds of **9** and **10** by DTT [step (i)]; subsequent re-oxidation of the reaction mixture with air oxygen [step (ii)].

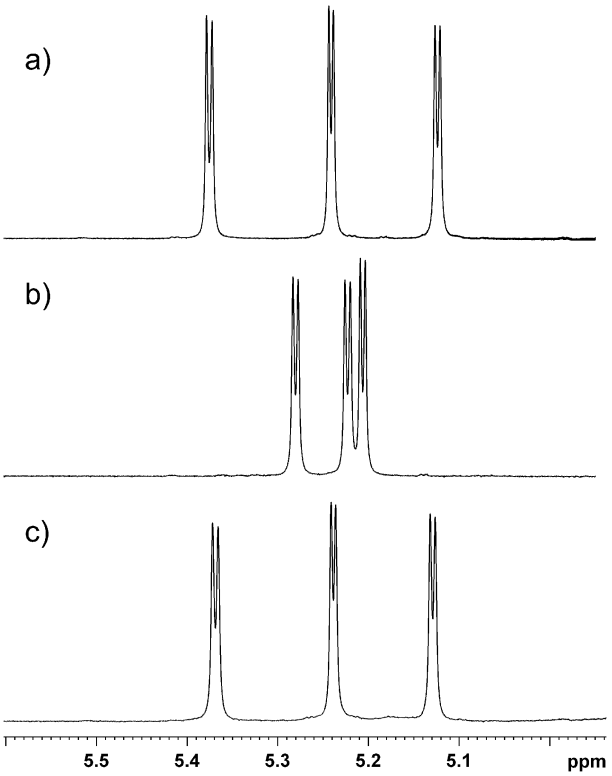


Fig. 1 Extracts of the ^{1}H NMR spectra (anomeric protons H-1) of a reaction mixture of (a) intramolecular disulfide **9** before reduction with DTT; (b) after reduction with DTT [step (i) in Scheme 3]—the spectrum is identical to that of thiol **11**; (c) after reoxidation with air–oxygen [step (ii) in Scheme 3]—the spectrum is identical to that of intramolecular disulfide **9**.

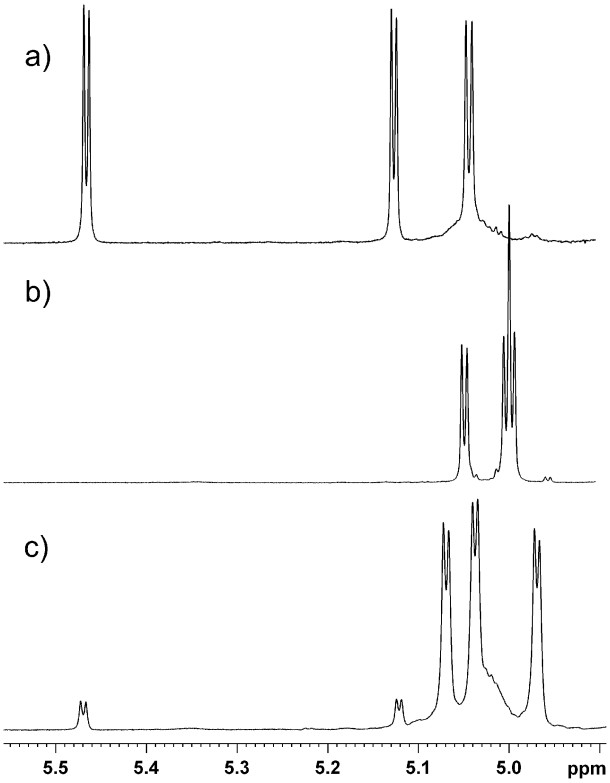


Fig. 2 Extracts of the ^{1}H NMR spectra (anomeric protons H-1) of a reaction mixture of (a) intramolecular disulfide **10** before reduction with DTT; (b) after reduction with DTT [step (i) in Scheme 3]—the spectrum is identical to that of thiol **12**; (c) after reoxidation with air–oxygen [step (ii) in Scheme 3]—the majority of the product corresponds to dimeric species **13**.

C_2 symmetry. The symmetrization did not significantly change the geometry of the cyclodextrin core but allowed us to perform quantum computations efficiently on a higher approximation level. Using the Gaussian suite of programs,[24] the geometry was optimized by an energy minimization performed with the BPW91 GGA DFT functional[25] and the Pople type 6-31G* basis set. We suppose that such a relatively high level DFT approximation describes the most important trends in the molecular geometries of compounds **9** and **10** (Fig. 3 and Fig. 4), relevant also to their solutions. This is also indicated by the calculated NMR shifts (see Tables S3 and S4 in the ESI†), which correspond well with the measured values.

Analysis of the optimized geometry of intramolecular disulfide **10** (Fig. 4) reveals that the circular shape of the cyclodextrin macrocycle—usually observed in its native form—is preserved better than that of methylated analogue **9** (Fig. 3). This is not surprising, since it is known that the rigid shape of the native cyclodextrin is largely strengthened by a belt of hydrogen bonds linking the secondary hydroxyl groups OH-2(n) and OH-3($n-1$) of the adjacent glucose units.[26] Removing this extra support by methylation of the hydroxyl groups in disulfide **9** results in a somewhat larger distortion of the cyclodextrin macrocycle. Most of the deformations of both macrocycles that allow the relatively short disulfide bonds to form occur through distortions of dihedral angles φ and ψ, describing rotations about the glucosyl bonds;[26,27] in the case

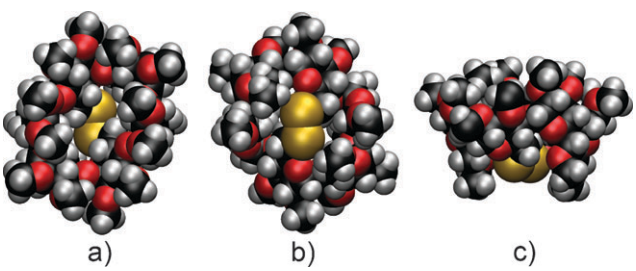


Fig. 3 The calculated (BPW91/6-31G**) equilibrium geometry of intramolecular disulfide **9**: (a) top-down view of the cavity; (b) view of the closed port; (c) side view.

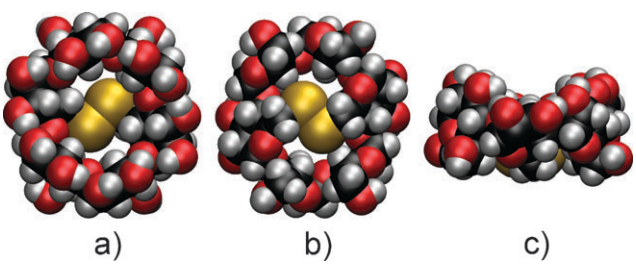


Fig. 4 The calculated (BPW91/6-31G**) equilibrium geometry of intramolecular disulfide **10**: (a) top-down view of the cavity; (b) view of the closed port; (c) side view.

of disulfide **10** particularly, dihedral angle $\psi(2)$, defined by the glucosyl bond between the sulfur-bearing and the neighbouring glucose residues G1 and G2, respectively (see the numbering of the glucose units in the ESI†), is the most distorted (*cf*. the computed value of 88° for disulfide **10** *vs*. the average value 131° found[28] for several crystal modifications of native α-cyclodextrins). This allows the sulfur-bearing glucose unit to tilt, with its primary carbon directed towards the center of the macrocycle (Fig. 4). The larger rigidity of macrocycle **10** inevitably results in a rather unfavorable value of the dihedral angle of the intramolecular disulfide bond (−151°). In contrast, the lack of the hydrogen bonding belt allows deformation of the methylated cyclodextrin macrocycle in disulfide **9** and hence a more energetically favorable dihedral angle of the disulfide bond (−104°). Thus, in common with the steric repulsion caused by the methyl groups, the absence of strain in the disulfide bridge in **9** is presumably responsible for the exclusive formation of the monomeric species.

Conclusions

We have developed the synthesis of a novel permethylated α-cyclodextrin, **9**, capped with an intramolecular disulfide linkage by means of the air-oxidation of the corresponding disulfanyl derivatives. Intramolecular disulfide **9** and its non-methylated analogue **10** could be quantitatively reduced to their corresponding disulfanyl derivatives **11** and **12**, respectively, by DTT, resulting in the opening of the narrower rim of the cavity. When exposed to the air again, both disulfanyl α-cyclodextrins **11** and **12** underwent oxidative coupling of the thiol moieties to the corresponding disulfide linkages. However, whereas the permethylated macrocycle exclusively yielded monomeric α-cyclodextrin **9** capped with the intramolecular disulfide bridge, the non-methylated analogue gave a mixture, in which dimeric form **13** predominated at millimolar concentrations. Among other known capped cyclodextrin-based systems,[29] permethylated cyclodextrin **9** is distinguished by its ability to reversibly switch between its open-ended and cup-like forms by the application of external (redox) stimuli.

As evidenced in our previous study,[17] the formation of non-methylated α-cyclodextrin intramolecular disulfide **10** is at least partly kinetically driven, and its quantitative formation can be expected at concentrations below 10^{-4} M. In contrast, methylated thiol **11** does not exhibit observable oligomerization, even at concentrations of 10^{-2} M. The unusually high effective molarity of intramolecular disulfide bond formation in compound **9** can be ascribed to the effect of steric hindrance caused by methyl groups and the loss of hydrogen bonding between the secondary hydroxyl groups.

Our preliminary studies showed that even larger β- and γ-cyclodextrin analogues are capable of forming similar intramolecular disulfide bridges. Such systems could thus find numerous applications in supramolecular chemistry and material sciences. They can be used, for instance, as lockable end-pieces of molecular tubing with variable diameters of the input port. Suitable functionalization of the cyclodextrin macrocycle could bring about the formation of Langmuir–Blodgett films[30] or vesicles[31] with reversibly closable voids. Such systems are currently under study in our laboratory.

Experimental

General experimental methods

NMR spectra were measured on spectrometers Bruker AVANCE 500 (^{1}H at 500.1 MHz and ^{13}C at 125.8 MHz) and AVANCE 600 (^{1}H at 600.1 MHz and ^{13}C at 150.9 MHz) in $CDCl_3$, CD_3OD, CD_3SOCD_3 or D_2O at 300 K. Homonuclear 2D-NMR spectra (H,H-PFG-COSY, H,H-PFG-TOCSY and H,H-PFG-ROESY) and heteronuclear 2D-NMR spectra (H,C-PFG-HSQC a H,C-PFG-HMBC) were used for structural assignment of proton and carbon signals. Mass spectra (Finnigan, ESI ionization) were recorded in a full-scan mode with m/z = 50–2500 Da range of analyzed ions. High resolution ESI-MS were recorded using a Waters Q-TOF instrument. Optical rotations were recorded on AUTOPOL IV (Rudolph Research Analytical). Preparative reversed-phase chromatography (RP) was carried out on medium pressure columns containing C-18 modified silica (Phenomenex Luna, 15 μm). Thin-layer (TLC) and reversed-phase thin-layer chromatography (RPTLC) were performed using pre-coated silica gel 60F and RP-18 F plates (E. Merck), respectively, which were developed by spraying with an aqueous solution of phosphomolybdenic acid containing 5% H_2SO_4 and heating. All chemicals used were commercially available. Intramolecular disulfide **10** was prepared according to a known procedure.[17]

Syntheses

2^{I}, 2^{II}, 2^{III}, 2^{IV}, 2^{V}, 2^{VI}, 3^{I}, 3^{II}, 3^{III}, 3^{IV}, 3^{V}, 3^{VI}, 6^{II}, 6^{III}, 6^{V}, 6^{VI}-Hexadeca-*O*-benzyl-α-cyclodextrin (2). Diisobutylaluminium hydride (1.5 M solution in toluene, 46 mL, 69 mmol) was introduced into a calibrated Schlenk flask. The flask was placed into a water–ice cooling bath at ∼0 °C and a solution of DIBAL-H concentrated to half of the volume under reduced pressure. The concentrated solution of diisobutylaluminium hydride was added dropwise through a cannula to dried per-benzyl-α-cyclodextrin (**1**; 4 g, 1.54 mmol) with vigorous stirring and cooling to ∼0 °C under an argon atmosphere. It was then allowed to react at room temperature, the course of the reaction being regularly monitored by TLC (toluene : acetone 94 : 6). After about 48 h, only one spot corresponding to product **2** was visible on the TLC plate and the reaction was then quenched by pouring the mixture onto ice placed in a beaker. Toluene (100 mL) was added to the mixture and the excess hydride was decomposed by the slow addition of a 1 M aqueous solution of HCl (50 mL) with vigorous stirring. The aqueous phase was extracted with toluene (3 × 200 mL), and the combined organic layers washed with aqueous sodium carbonate and dried with sodium sulfate. Evaporation of the solvent gave a crude product that was subsequently purified by short-column chromatography (200 g silica gel, gradient elution from toluene to toluene : acetone 95 : 5). The product was isolated as an amorphous, colorless material (4.36 g, 94%). Analytical data were in accordance with the published literature.

2^{I}, 2^{II}, 2^{III}, 2^{IV}, 2^{V}, 2^{VI}, 3^{I}, 3^{II}, 3^{III}, 3^{IV}, 3^{V}, 3^{VI}, 6^{II}, 6^{III}, 6^{V}, 6^{VI}-Hexadeca-*O*-benzyl-6^{I},6^{IV}-di-*O*-triisopropylsilyl-α-cyclodextrin (3). Potassium hydride (30% w/w, 240 mg, 1.8 mmol) was placed in a Schlenk flask and washed three times with dry

THF under an argon atmosphere. The remaining THF was removed under reduced pressure. The solution of compound **2** (200 mg, 82.8 μmol) in dry THF (1 mL) was added dropwise to potassium hydride with stirring at room temperature under an argon atmosphere. The reaction mixture was allowed to react for 2 h and triisopropylsilyl chloride (71 μl, 0.33 mmol) was added over 1 h at room temperature. After 3 h, dry THF (10 mL) was added and the Schlenk flask placed in a water–ice cooling bath. The excess hydride was decomposed by the addition of ethanol (100 μl). Then, water was added (3 mL) and the crude product isolated by extraction into toluene (3 × 100 mL). The combined organic layers were washed with water (3 × 100 mL), brine (1 × 100 mL) and dried with sodium sulfate. After evaporation of the solvent, the product was purified by column chromatography (16 g silica, gradient elution from hexane to hexane : acetone 95 : 5). The pure product (205 mg, 89%) was isolated as a colorless amorphous material (found: C, 73.4; H, 7.4; calc. for $C_{166}H_{196}O_{30}Si_2$: C, 73.1; H, 7.2%); R_f 0.2, toluene : acetone 99 : 1; ^{1}H NMR: see Table S1, ESI†; ^{13}C-NMR: see Table S2, ESI†; *m*/*z* (FAB) 2750 $[M + Na]^+$. $C_{166}H_{196}O_{30}Si_2$ requires 2750.

6^I,6^{IV}-Di-*O*-triisopropylsilyl-α-cyclodextrin (4). Compound **3** (497 mg, 0.18 mmol) was dissolved in a de-gassed mixture of DMF–ethanol (1 : 1, 40 mL). Then, palladium on charcoal (10% w/w, 124 mg) was added and the reaction mixture placed in an autoclave equipped with a magnetic stirring bar. The autoclave was filled with hydrogen to a pressure of 40 bar. After stirring of the reaction mixture for 5 h at room temperature, the excess hydrogen was released and the catalyst removed from the reaction mixture by centrifugation. The solvents were evaporated under reduced pressure. The residue was dissolved in 40 mL of a mixture of ethylacetate : acetone : water : ethanol 15 : 3 : 3 : 4 and purified by short column chromatography through silica (15 g). The pure product was obtained as a colorless amorphous material (201 mg, 82% calc. for trihydrate) after evaporation of the solvents (found C, 48.8; H, 8.1; calc. for $C_{54}H_{100}O_{30}Si_2{\cdot}3H_2O$: C, 48.4; H, 8.0%); R_f 0.25, ethylacetate : acetone : water : ethanol 15 : 3 : 3 : 4; ^{1}H NMR: see Table S1, ESI†; ^{13}C NMR: see Table S2, ESI†; *m*/*z* (FAB) 1308 $[M + Na]^+$. $C_{54}H_{100}O_{30}Si_2$ requires 1308.

2^I, 2^{II}, 2^{III}, 2^{IV}, 2^V, 2^{VI}, 3^I, 3^{II}, 3^{III}, 3^{IV}, 3^V, 3^{VI}, 6^{II}, 6^{III}, 6^V, 6^{VI}-Hexadeca-*O*-methyl-6^I,6^{IV}-di-*O*-triisopropylsilyl-α-cyclodextrin (5). A solution of compound **4** (629 mg, 0.469 mmol, calculated for trihydrate) in dry DMF (26 mL) was added dropwise to sodium hydride (60% w/w, 931 mg, 23.2 mmol; washed with hexane) with stirring at room temperature under an argon atmosphere, followed by the addition of methyl iodide (1.95 mL, 31.3 mmol). After 12 h, the reaction was quenched by the addition of ethanol (0.5 mL). After dilution of the reaction mixture with water (20 mL), the product was isolated by extraction into hexane (3 × 100 mL). The combined organic layers were washed with water (3 × 100 mL) and brine (1 × 100 mL), and dried using sodium sulfate. Evaporation of the solvent gave the crude product, which was subsequently subjected to short column chromatography (15 g, gradient elution from chloroform to chloroform : methanol 98 : 2). The product (640 mg, 90%) was isolated as an amorphous colorless material (found: C, 55.3; H, 8.5; calc. for $C_{70}H_{132}O_{30}Si_2$: C, 55.7; H, 8.8%); R_f 0.3, chloroform :methanol 96 : 4; ^{1}H NMR: see Table S1, ESI†; ^{13}C NMR: see Table S2, ESI†; *m*/*z* (FAB) 1532 $[M + Na]^+$. $C_{70}H_{132}O_{30}Si_2$ requires 1532.

2^I, 2^{II}, 2^{III}, 2^{IV}, 2^V, 2^{VI}, 3^I, 3^{II}, 3^{III}, 3^{IV}, 3^V, 3^{VI}, 6^{II}, 6^{III}, 6^V, 6^{VI}-Hexadeca-*O*-methyl-α-cyclodextrin (6). Compound **5** (111 mg, 73.6 μmol) was dissolved in THF (5.2 mL) and then a solution of tetrabutylammonium fluoride (1.5 mL, 0.191 M, 0.28 mmol) was added with stirring. After 12 h, the solvent was taken up under reduced pressure, and the crude product dissolved in ethanol and charged onto a column of Dowex 50 in the NH_4^+ cycle. The eluate was collected, the solvents evaporated and the product purified by column chromatography (8 g over silica, gradient elution from chloroform to chloroform : methanol 94 : 6). The product (77 mg, 89%) was isolated as a colorless amorphous material (found: C, 51.9; H, 7.6; calc. for $C_{52}H_{92}O_{30}$: C, 52.2; H, 7.75%); R_f 0.45, chloroform : methanol 9 : 1; ^{1}H NMR: see Table S1, ESI†; ^{13}C NMR: see Table S2, ESI†; *m*/*z* (FAB) 1220 $[M + Na]^+$. $C_{52}H_{92}O_{30}$ requires 1220.

2^I, 2^{II}, 2^{III}, 2^{IV}, 2^V, 2^{VI}, 3^I, 3^{II}, 3^{III}, 3^{IV}, 3^V, 3^{VI}, 6^{II}, 6^{III}, 6^V, 6^{VI}-Hexadeca-*O*-methyl-6^I,6^{IV}-dibromo-6^I,6^{IV}-dideoxy-α-cyclodextrin (7). Triphenylphosphane (100.6 mg, 0.40 mmol) was dissolved in dry DMF (1.4 mL) in a Schlenk flask under an argon atmosphere, and tetrabromomethane (127.4 mg, 0.40 mmol) and compound **6** (115 mg, 96 μmol) subsequently added. The reaction mixture was heated to 50 °C for 12 h and then quenched by the addition of methanol (100 μl). The mixture was allowed to cool to room temperature and the solvents evaporated under a reduced pressure. The residue was dissolved in chloroform (300 mL), the organic phase washed with water (4 × 200 mL) and brine (200 mL), and dried using sodium sulfate. The crude product obtained after evaporation of the solvent was purified by column chromatography (150 g of silica, isocratic elution with hexane : acetone 3 : 2). The product (107 mg, 85%) was obtained as a colorless amorphous material (found: C, 46.9; H, 6.7; calc. for $C_{52}H_{90}Br_2O_{28}$: C, 47.2; H, 6.9%); R_f 0.65, hexane : acetone 3 : 2; ^{1}H NMR: see Table S1, ESI†; ^{13}C NMR: see Table S2, ESI†; *m*/*z* (ESI) 1343 $[M + Na]^+$. $C_{52}H_{90}Br_2O_{28}$ requires 1343.

2^I, 2^{II}, 2^{III}, 2^{IV}, 2^V, 2^{VI}, 3^I, 3^{II}, 3^{III}, 3^{IV}, 3^V, 3^{VI}, 6^{II}, 6^{III}, 6^V, 6^{VI}-Hexadeca-*O*-methyl-6^I,6^{IV}-bis(acetylsulfanyl)-6^I,6^{IV}-dideoxy-α-cyclodextrin (8). Compound **7** (68 mg, 51.4 μmol) was dissolved in a Schlenk flask in dry DMF (1 mL) equipped with a magnetic stirring bar under an argon atmosphere. The mixture was deoxygenated by the application of a vacuum–argon cycle, and a solution of potassium thioacetate (23.4 mg, 0.205 mmol) in dry de-gassed DMF (0.5 mL) was added at room temperature. After 12 h, the DMF was evaporated with stirring under a reduced pressure at 40 °C. The crude product was purified by column chromatography (50 g of silica, isocratic elution with hexane : acetone 7 : 3). The product (61 mg, 90%) was obtained as a colorless amorphous material (found C, 51.4; H, 7.5; calc. for $C_{56}H_{96}O_{30}S_2$: C, 51.2; H, 7.4%); R_f 0.4, hexane : acetone 6 : 4; ^{1}H NMR: see Table S1, ESI†; ^{13}C NMR: see Table S2, ESI†; *m*/*z* (HRMS-ESI) 1335.532 $[M + Na]^+$. $C_{56}H_{96}O_{30}S_2$ requires 1335.53.

Intramolecular disulfide (9). Compound **8** (39 mg, 30 μmol) was dissolved in water (4 mL) in a Schlenk flask. The mixture was deoxygenated by means of a triple vacuum–argon cycle. Then, a de-gassed 1 M aqueous solution of sodium hydroxide (294 μl) was added, and the mixture allowed to react for 5 h at room temperature under an argon atmosphere with stirring. Subsequently, the flask was opened and additional water (28 mL) was added. The pH of the solution was adjusted to 9.0 by the addition of solid carbon dioxide. Next, the mixture was allowed to react under vigorous stirring in an open flask, the course of the reaction being followed by RPTLC. After 48 h, the solution was neutralized with 1 M HCl. The solution was then charged onto a C-18 reversed-phase column (gradient elution from methanol : water 1 : 1 to 9 : 1). The volume of the combined fractions was partly reduced under a reduced pressure and the remaining solution lyophilized. The product (34 mg, 92%) was obtained as a white amorphous material (found: C, 50.5; H, 7.3; calc. for $C_{52}H_{90}O_{28}S_2$: C, 50.9; H, 7.4%); R_f 0.25, RPTLC, methanol : water 8 : 2; $[\alpha]_D^{20} = +112$ (*c* 0.068, $CHCl_3$); ^{1}H NMR: see Table S1, ESI†; ^{13}C NMR: see Table S2, ESI†; *m*/*z* (HRMS-ESI) 1249.495 $[M + Na]^+$. $C_{52}H_{90}O_{28}S_2$ requires 1249.50.

Equilibration of intramolecular disulfide 9 with dithiothreitol

Intramolecular disulfide **9** (2.00 mg, 1.63 μmol) was dissolved in deuterated 5 mM phosphate buffer (0.5 mL, pD = 8.4) and the solution de-gassed by a triple vacuum–argon cycle. Next, a de-gassed 0.24 M solution of dithiothreitol (0.135 mL, 3.3 μmol) was added in the same buffer. The mixture was transferred into an NMR tube under argon and the tube flame-sealed. The progress of the reaction was monitored by ^{1}H NMR measurements.

Equilibration of intramolecular disulfide 10 with dithiothreitol

Intramolecular disulfide **10** (2.33 mg, 2.17 μmol, calculated for tetrahydrate) was dissolved in deuterated 5 mM phosphate buffer (0.5 mL, pD = 8.4) and the solution de-gassed by a triple vacuum–argon cycle. Next, a de-gassed 0.24 M solution of dithiothreitol (0.180 mL, 4.3 μmol) in the same buffer was added. The mixture was transferred into an NMR tube under argon and the tube flame-sealed. The progress of the reaction was monitored by ^{1}H NMR measurements.

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