

# Preparation and guest binding of novel $\beta$ -cyclodextrin dimers linked with various sulfur-containing linker moieties†

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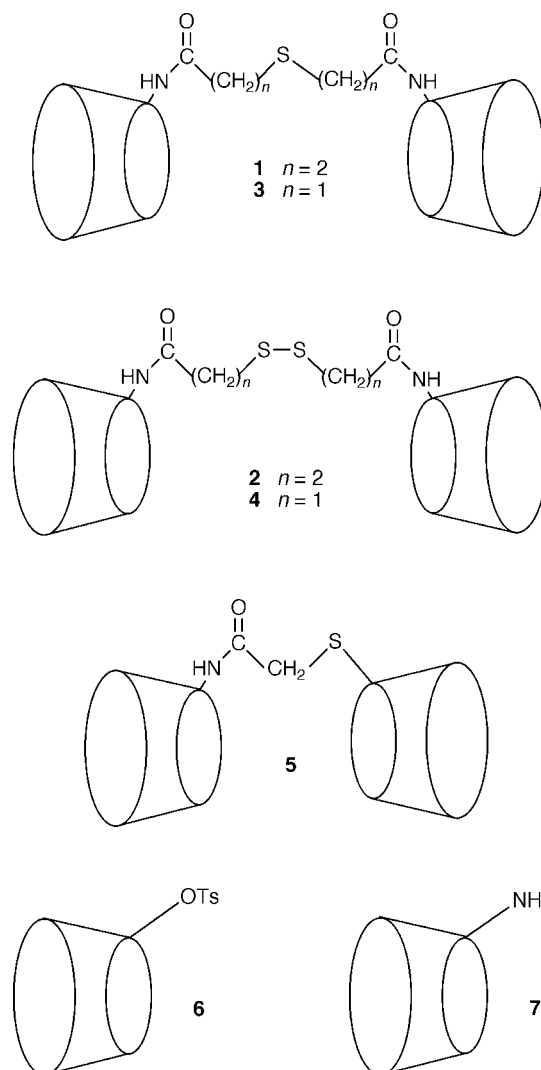
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Novel cyclodextrin dimers (**1**, **2**, **3** and **4**) whose cyclodextrins were linked with sulfur-containing linkers, namely a thiodipropanamide, a dithiodipropanamide, a thiodiethanamide, or a dithiodiethanamide linker, were synthesized by a reaction of 6-amino-6-deoxycycloheptaose **7** with the corresponding dicarboxylic acids. For their preparation, dicyclohexylcarbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and (benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate were examined as coupling reagents. <sup>1</sup>H NMR studies of the dimers suggested an intramolecular inclusion of the linker moiety to the cyclodextrin cavity, which affected the complexation of guest molecules. Dimer **4** was converted to another type of dimer **5** by reductive cleavage of the disulfide bond to generate thiol group-containing monomeric species followed by a substitution reaction with 6-*O*-tosyl derivative **6**.

## Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides composed of  $\alpha(1\rightarrow4)$ -linked glucose units. They can include a variety of guest molecules in their cavities.<sup>1</sup> However, the inclusion by native CDs is less efficient and also less specific, when we compare them with natural systems such as enzymes or antibodies. Chemical modification of CD would be essential in order to improve its inclusion ability. In 1984, Fujita *et al.*<sup>2</sup> reported that covalently linked CDs expressed fully collaborative guest binding. This was a turning point of the chemistry of CD dimers and there have been a number of studies<sup>3</sup> since then. Breslow demonstrated that the association constants of CD dimers reached the range of those of antigen-antibody complexes.<sup>4</sup> Now, by use of CD dimers, it might be possible to construct sophisticated molecular recognition systems such as artificial antibodies. Also, the chemistry of CD dimers should be extendable to further supramolecular systems such as CD tetramers.<sup>5</sup>

In our study of CD dimers, here we will describe the preparation of four cyclomaltoheptaose ( $\beta$ -CD) derivatives, **1–4**, in which the two CDs are linked at their C(6) atoms, with a thiodipropanamide, a dithiodipropanamide, a thiodiethanamide, and a dithiodiethanamide bridge, respectively. Also, we examined their inclusion properties using guest molecules such as Methyl Orange (MO), Tropaeolin OO (TR), sodium 6-(*p*-toluidino)naphthalene-2-sulfonate (TNS) and sodium 6-[4-(*tert*-butyl)anilino]naphthalene-2-sulfonate (BNS). They were chosen because their two aromatic moieties such as *tert*-butylanilino and naphthalene groups constitute a pair of separate binding sites. Reduction of the dimers **2** and **4** generates thiol compounds which permit further modification. As a particular example, here we will describe the conversion of **4** to another dimer **5** by reduction, followed by reaction with 6-*O*-tosyl- $\beta$ -CD **6**.



† COSY and HOHAHA spectra for compounds **1–3** are available as supplementary data from BLDSC (SUPPL. NO 57638, 9 pp.) or the RSC Library. See 'Instructions for Authors' available via the RSC web page (<http://www.rsc.org/authors>).

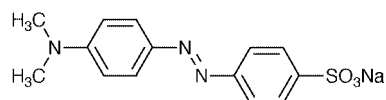
## Results and discussion

### Synthetic studies

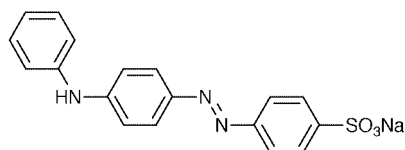
Each of the dimers described here was synthesized by a coupling reaction of the corresponding dicarboxylic acid and an amino derivative **7**. In order to prepare dimer **1**, thiodipropionic acid was treated with **7** by use of dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt). After 1 h TLC analysis of the reaction mixture indicated the presence of a spot with a lower  $R_F$ -value (0.03) than that of **7**, and after 24 h most of the amine **7** seemed to be consumed. By use of reversed-phase (RP) chromatography with an increasing MeCN gradient elution, a fraction containing the desired **1** was obtained, although HPLC analysis of the fraction showed the presence of minor impurities. Rechromatography with a gel permeation (GP) column gave pure compound **1** (30.3%). The dimer **2**‡ possessing a dithiodipropionamide moiety as a linker was similarly prepared by the DCC–HOBt method. By-products with higher  $R_F$ -values than that of **7** were observed on TLC and the reaction was terminated at the stage when some of the amine **7** remained unchanged. Chromatographic separation using a RP- and a GP column gave the desired **2** (46.7%).

When we undertook a preparation of dimer **3** by use of DCC–HOBt, the coupling product was co-precipitated with dicyclohexylurea derived from DCC. The difficulty in extracting **3** with aq. MeOH and DMF from the precipitate resulted in a very low yield (14.9%). This could be due to the cyclohexyl moieties of the urea, whose shape and hydrophobicity facilitated an inclusion by the CD moieties of **3**. Next, we adopted 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC)<sup>7</sup> as a coupling reagent. As EDC is called ‘water-soluble carbodiimide,’ its tertiary amino group affords enough hydrophilicity to dissolve in water. By use of a chromatographic separation, we obtained the desired compound **3** in a good yield (84.3%).

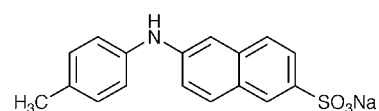
The reactions of amine **7** with dithiodipropionic acid by use of DCC and EDC gave a dimer **4** in 6.74 and 19.0% yield, respectively. Since the reactions using the carbodiimide reagents gave



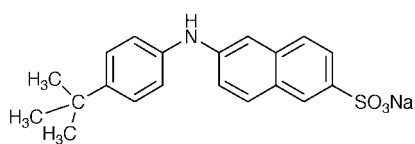
methyl orange (MO)



tropaeolin OO (TR)



sodium 6-(*p*-toluidino)naphthalene-2-sulfonate (TNS)



sodium 6-[4-(*tert*-butyl)anilino]naphthalene-2-sulfonate (BNS)

‡ Compound **2** was prepared previously by use of an activated ester of the corresponding dicarboxylic acid.<sup>6</sup>

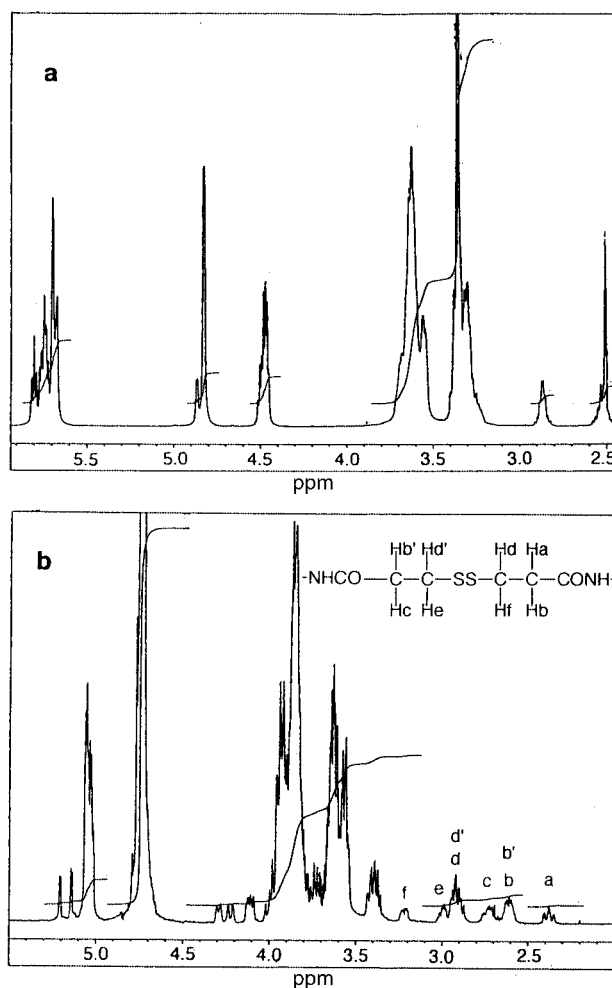


Fig. 1 400 MHz <sup>1</sup>H NMR spectra of **2** in DMSO-*d*<sub>6</sub> (a) and D<sub>2</sub>O (b).

unsatisfactory results, we attempted (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (PyBOP)<sup>8</sup> as a coupling reagent. This phosphonium-based activation reagent enables fast reactions to occur even between sterically hindered amino acid derivatives in peptide synthesis. According to TLC analysis after 6 h, the reaction using PyBOP–HOBt seemed to proceed much faster than that using the carbodiimide-based reagents. The reaction mixture was applied to GP chromatography followed by a RP chromatography to give the desired dimer **4** (48.4%).

### <sup>1</sup>H NMR studies

The dimer **2**, whose CDs are linked with a dithiodipropionamide moiety, was subjected to <sup>1</sup>H NMR analysis in DMSO-*d*<sub>6</sub> and in D<sub>2</sub>O solutions (Fig. 1a and b). In DMSO-*d*<sub>6</sub>, **2** exhibited a rather simple spectrum. Two kinds of anomeric H-1 signals [ $\delta$  4.83 (12H) and 4.87 (2H)] were observed, which resembled a typical pattern of a mono substituted CD derivative, suggesting that **2** was almost C<sub>2</sub>-symmetric.

As for the signals of the linker moiety, two overlapping dt signals around  $\delta$  2.80–2.92 indicated that the two  $\beta$ -CH<sub>2</sub> groups are slightly nonequivalent. In the range  $\delta$  2.46–2.58 a similar pattern of  $\alpha$ -CH<sub>2</sub> signals was observed, which was obscured by a signal of CH<sub>3</sub> of DMSO. In contrast to the spectrum in DMSO-*d*<sub>6</sub>, compound **2** showed a peculiar spectrum in D<sub>2</sub>O. Concerning unique signals of glucose residues, three kinds of H-1 signals of glucose residues appeared, at  $\delta$  5.00–5.09, 5.14, and, 5.21, whose relative intensities were 12:1:1. In addition to the major peaks of oxygen-carrying methine and methylene groups in the range  $\delta$  3.5–4.0, several minor peaks also appeared around  $\delta$  3.3–3.5 and 4.0–4.3. The eight protons

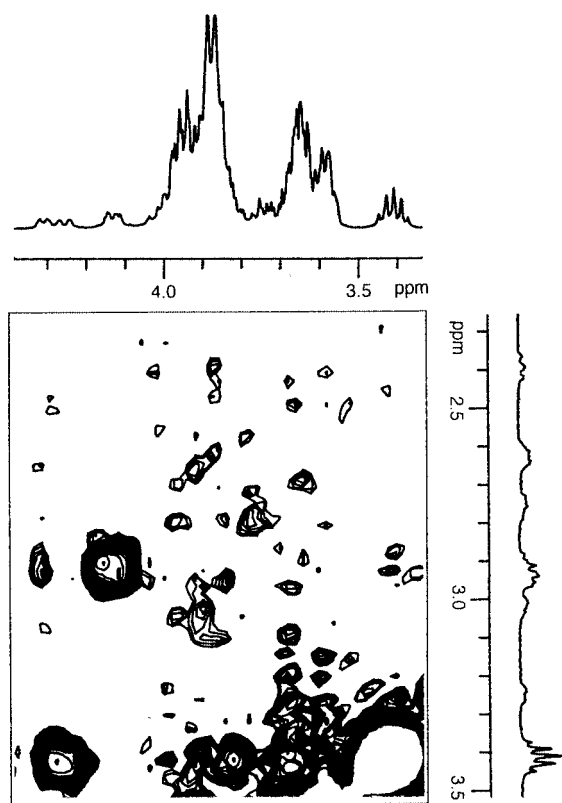


Fig. 2 ROESY spectrum of **2** in D<sub>2</sub>O. The mixing time is 300 ms.

of the dithiodipropylamide moiety are distinguishable as divergent peaks observed at  $\delta$  2.4–3.33. These data clearly indicated that the conformation of **2** is unsymmetrical in D<sub>2</sub>O. In a rotating-frame nuclear Overhauser and exchange spectroscopy (ROESY) experiment (Fig. 2), cross peaks were observed between the proton signals of the linker and the ones around  $\delta$  3.75–4.0 due to signals of H-3 and H-5 of glucoses located at the inner wall of a CD cavity. This reveals that an intramolecular inclusion of the linker moiety by a CD caused the unsymmetrical conformation. A complexation of alkyl chains by CDs is known to be an energetically favorable process.<sup>9</sup> A similar observation was reported in the case of a CD dimer connected through the secondary hydroxy-group sides by octanediamide moieties.<sup>10</sup> On varying the temperature from 20 to 70 °C, no significant spectral change was observed. On the other hand, the addition of DMSO-*d*<sub>6</sub> to the D<sub>2</sub>O solution ([DMSO-*d*<sub>6</sub>] = 25%) resulted in a spectrum similar to that obtained in the DMSO-*d*<sub>6</sub> solution. These suggested that a hydrophobic interaction between the linker and the CD cavity may be a driving force of the self-inclusion. The interaction is so strong in water that an intramolecular complex is stable even at high temperature, but in hydrophobic circumstances it does not work well enough to facilitate the self-inclusion.

Dimers **1**, **3** and **4** also gave simple spectra in DMSO-*d*<sub>6</sub>, which were similar to that of **2**. However, each of them demonstrated unique behavior in D<sub>2</sub>O (Fig. 3). In the spectrum of **1** linked with a thiodipropylamide moiety, major signals of H-1 protons ( $\delta$  5.0–5.1) were accompanied by minor ones at  $\delta$  5.15 and 5.20, and their relative intensities (1.0:0.87:31.1) suggested the presence of a self-included conformation in D<sub>2</sub>O. In the case of **3** with thiodiethanamide, almost the same signal patterns as those in DMSO-*d*<sub>6</sub> were observed. The spectrum of dimer **4** possessing a dithioethanamide moiety was unique. Three kinds of H-1 signals were observed at  $\delta$  5.0–5.1, 5.18 and 5.31 (relative intensities 21:2:1). The other region in its spectrum was also more complicated than those of other dimers. The NMR characteristics of these dimers with different linker moieties reflect

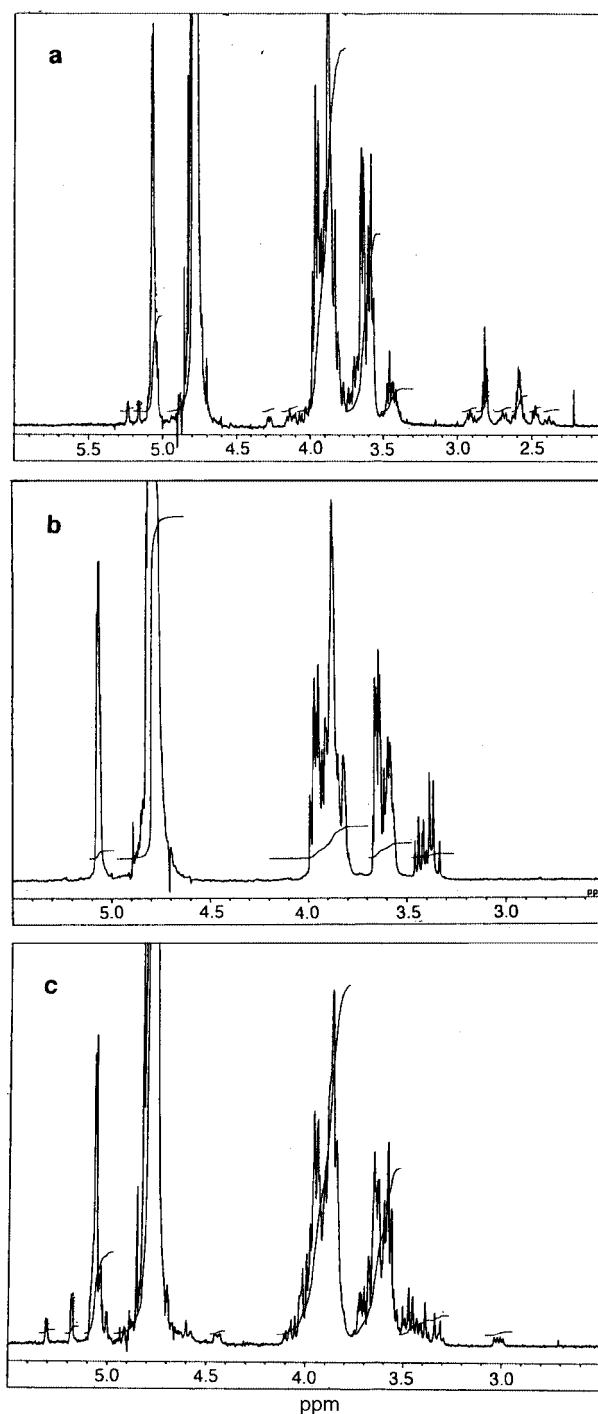
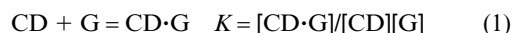


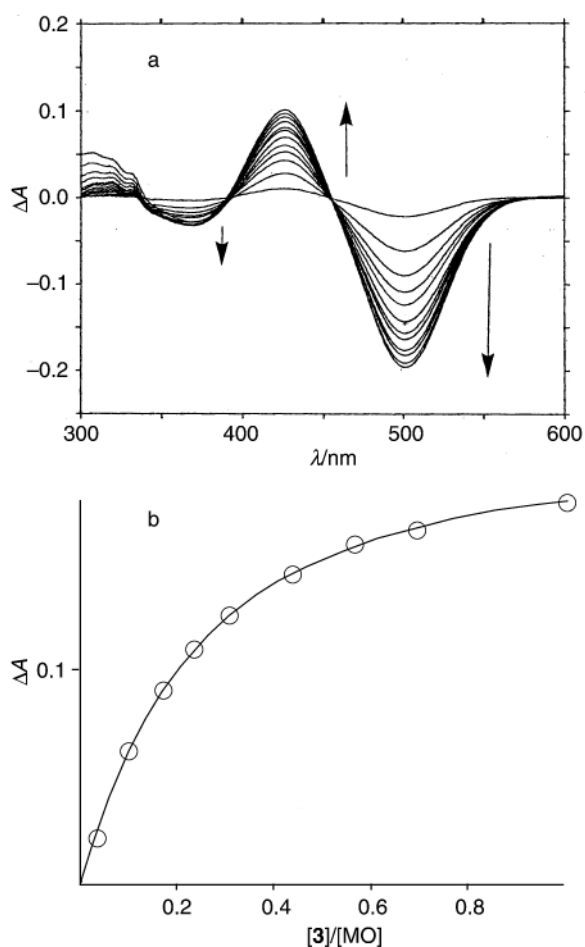
Fig. 3 400 MHz <sup>1</sup>H NMR spectra in D<sub>2</sub>O of **1** (a), **3** (b) and **4** (c).

the conformational properties of each dimer involving the self-inclusion of the linker moiety.

#### Guest binding

Inclusion of guest compounds by the dimers **1–4** was investigated. The guest molecules MO, TR, TNS, and BNS possess two aromatic moieties to be included by the two CDs in a dimer and have frequently been used in chemistry of CD dimers.<sup>2,4a,4b,11</sup> Inclusion ability of the dimers was examined in a buffered aq. solution at 25 °C, using spectral changes induced by complexation, namely, UV-visible spectral changes for MO and TR, and fluorescence spectral changes for BNS and TNS. The following equilibrium was assumed and the association constant *K* was defined according to equation (1) where CD is





**Fig. 4** Absorption spectral change of MO in 0.05 M sodium carbonate buffer (pH 10.6) with addition of the dimer **3** (25 °C; [MO] =  $3.05 \times 10^{-5}$  M, [3] =  $1.04$  to  $3.04 \times 10^{-4}$  M). (a) Difference spectra are given.  $\Delta A$  refers to the absorbance of the solution minus the absorbance of the component solution of MO and the dimer **3** at the corresponding concentration. (b) Plots of  $\Delta A$  vs. [3]/[MO] at 500 nm is shown.

the cyclodextrin dimer, G is the guest molecule, and CD·G is the inclusion complex.

On addition of the dimer **3** to MO ([3]/[MO] = 0.34–10), the absorption spectrum of MO showed a significant change, exhibiting isosbestic points at 392 and 456 nm (Fig. 4) which suggested the validity of the single equilibrium-assumption described above. Based on the absorbance change at 371, 425 and 500 nm over the range of [MO]:[3] from 1:3.3 to 1:24, nonlinear least-squares curve-fitting analysis<sup>12</sup> indicated the formation of a 1:1 complex with a  $K$ -value of 19,000 ( $M^{-1}$ ).§ In the case of BNS, the increase in the magnitude of BNS fluorescence and its blue shift with increasing [3] were observed (Fig. 5). These are characteristic of a transfer of a fluorophore from an aqueous environment to a hydrophobic CD cavity.<sup>13</sup> The association constant was determined by the curve-fitting analysis and the  $K$ -value was obtained as 740 000 ( $M^{-1}$ ). Similar experiments were performed for other combinations of the dimers and each of the guest molecules. The obtained association constants are listed in Table 1.

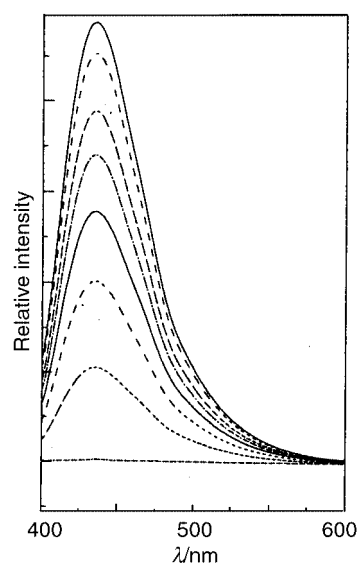
As shown in the  $K$ -values with BNS above, the greater

§ When **3** was added in excess to MO ([3]/[MO] > 10), the observed increase of difference absorption at 371 nm turned into a decrease and the observed isosbestic points disappeared. Based on the absorption change, neither a reasonable  $K$ -value nor a  $\Delta A_{\max}$ -value was attainable, assuming both 1:1 and 2:1 host-guest complex formation. Accordingly, all of the analyses described here were performed under conditions where curve-fitting analysis suggested the establishment of a 1:1 complex formation.

**Table 1** The association constants ( $M^{-1}$ ) between the dimers and MO, TR, TNS and BNS<sup>a</sup>

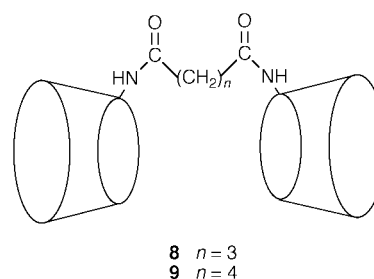
	MO	TR	TNS	BNS
<b>1</b>	6700 (2.4) <sup>b</sup>	42 000 (6.8)	6700 (3.7)	320 000 (9.1)
<b>2</b>	2200 (0.78)	30 000 (4.8)	2700 (1.5)	35 000 (1.0)
<b>3</b>	19 000 (6.8)	75 000 (12)	10 000 (5.6)	740 000 (21)
<b>4</b>	7300 (2.6)	27 000 (4.4)	4600 (2.6)	270 000 (7.7)
<b>8</b>	9200 (3.2)	69 000 (11)	8600 (4.8)	590 000 (17)
<b>9</b>	11 000 (3.9)	150 000 (24)	9300 (5.1)	710 000 (20)
<b>β-CD</b>	2800	6200	1800	35 000

<sup>a</sup> The experiment was performed at 25 °C, for MO in 0.05 M sodium carbonate buffer (pH 10.6) and for TR, TNS and BNS in 0.05 M potassium/sodium phosphate buffer (pH 6.86). <sup>b</sup> The values in parentheses are the ratio of the associated constant by the corresponding dimer to the one by β-CD.



**Fig. 5** Fluorescence spectra of BNS with addition of the dimer **3** (25 °C; [BNS] =  $4.06 \times 10^{-6}$  M, [3] =  $1.01$  to  $8.79 \times 10^{-6}$  M) in 0.05 M potassium/sodium phosphate buffer (pH 6.86). Excitation wavelength was 380 nm.

association constants of **3** compared with those of β-CD suggested a cooperative inclusion of a guest molecule by the two CD moieties. In contrast, the dimer **2** exhibited much smaller association constants. For example, the  $K$ -value with MO is 2200  $M^{-1}$ , which is 22% less than that of β-CD. The dithio-propanamide moiety caused an intramolecular complexation, as demonstrated by the NMR results, which disfavored an inclusion process of a guest species. The strong inclusion ability of **3** could be attributed to the non-existence of a self-inclusion complex and also to the proximity of the two CD moieties combined with the thiodiethanamide linker which is the shortest in this series. Comparison of the thioethanamide dimer





**3** and the dithioethanamide dimer **4** with the corresponding methylene-chain analogs, **8**<sup>14</sup> and **9**,<sup>15</sup> respectively, suggested a unique character of the sulfur-containing linkers that may cause self-inclusion, resulting in a weak guest inclusion. Reflecting the nature of the linker moieties, the dimers recognize slight differences in the structure of the guest compounds used here and discriminate between them, as shown in the quotient ( $K_{\text{TNS}}/K_{\text{BNS}}$ ) of **2** and **3**, *i.e.*, 13 and 74, respectively. Previous reports<sup>11b</sup> indicated that a linker plays an important role in a CD dimer to include a guest molecule cooperatively. The observed inclusion by dimers with a sulfur-containing moiety confirmed that linker moieties regulate a distance between the two CD moieties and also their relative orientation, which determines the inclusion characteristics for each guest molecule. We should seek a novel CD dimer with an optimum linker in order to construct a supramolecular recognition system for the target guest.

#### Preparation of a novel dimer **5** by a disulfide cleavage of **4**

Each of the dimers **2** and **4** with a disulfide bond generates CD monomers possessing a thiol group that can react with other compounds. As an example, the dithiodiethanamide dimer **4** was reduced by NaBH<sub>4</sub> and the product reacted with 6-*O*-tosyl ester **6** to produce a novel dimer **5** (26.8%). If we react the thiol with tosylmaltohexaose or tosylmaltooctaose, we can make "hetero" dimers<sup>16</sup> composed of different kinds of CDs. When the 2- or 3-*O*-tosyl ester is used, a head-to-tail dimer<sup>16b</sup> can be prepared. Moreover, the dimers **2** and **4** may be important materials in the construction of self-assembled monolayers on gold surfaces for a preparation of biosensors.<sup>6</sup>

In conclusion, we prepared CD dimers with sulfur-containing linkers through a coupling reaction between the amino group-containing CD and the corresponding dicarboxylic acid by use of a carbodiimide-based activation reagent or a phosphonium-based reagent. Each dimer demonstrated unique guest inclusion related to an intramolecular complexation, suggesting its conformation was affected by the linker moiety. Studies of these dimers to seek suitable guest compounds and also the preparation of novel dimers by use of the disulfide bond-containing dimers are in progress.

## Experimental

<sup>1</sup>H NMR (200 or 500 MHz) spectra were recorded on a Varian Gemini 200 or a JEOL  $\alpha$  500 spectrometer. *J*-Values are given in Hz. Mass measurements were carried out with a Hitachi M-2000 (LSIMS) or a Shimadzu-Kratos Concept 32IH (FAB). TLC was run on precoated silica-gel plates (Art 5554, Merck) with the solvent system 1-PrOH–AcOEt–H<sub>2</sub>O–28% aq. NH<sub>3</sub> [3:3:2:1 (v/v/v/v)]. Spot detection was carried out by UV light and/or staining with 0.1% naphthalene-1,3-diol in EtOH–H<sub>2</sub>O–H<sub>2</sub>SO<sub>4</sub> [200:157:43 (V/V/V)]. A prepacked ODS column [LiChroprep RP-18, size B (25 × 310 mm), or size C (37 × 410 mm), Merck] was used for low-pressure RP column chromatography. GP chromatography was performed using Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). RP HPLC was carried out with a J'sphere ODS-M80 (4  $\mu$ m; 4.6 × 150 mm or 2.0 × 150 mm, YMC Inc., Kyoto, Japan). GP HPLC was performed using a YMC-Pack Diol-60 (5  $\mu$ m; 8.0 × 500 mm, YMC Inc., Kyoto, Japan). DMF was dried over molecular sieves 4 Å before use.

#### Thiodipropanamide dimer **1**

3,3'-Thiodipropanoic acid (26.8 mg,  $1.51 \times 10^{-4}$  mol) and amine **7** (527 mg,  $4.51 \times 10^{-4}$  mol) were dissolved in dry DMF (3 cm<sup>3</sup>). The reaction mixture was neutralized with triethylamine, followed by the addition of HOBt (20.8 mg,  $1.54 \times 10^{-4}$  mol) and DCC (125 mg,  $6.06 \times 10^{-4}$  mol) under ice cooling. After stirring of the reaction mixture at rt for 24 h,

the solvent was evaporated off *in vacuo* and to the residue was added 20% aq. MeOH (20 cm<sup>3</sup>). After removal of precipitated dicyclohexylurea by filtration, the filtrate was concentrated *in vacuo*. The residue was dissolved in water and applied to a low-pressure RP chromatography. After elution with 5% aq. MeCN (400 cm<sup>3</sup>), gradient elution from H<sub>2</sub>O (500 cm<sup>3</sup>) to 50% aq. CH<sub>3</sub>CN (500 cm<sup>3</sup>) gave the crude title product **1**, which was subjected to GP chromatography to give the dimer **1** (110 mg, 30.3%); *R*<sub>f</sub> 0.03; *t*<sub>R</sub> [column, J'sphere ODS-M80; gradient, 0–40% aq. MeCN (40 min); flow rate 1.0 cm<sup>3</sup> min<sup>-1</sup>] 22.5 min, [column Diol-60; H<sub>2</sub>O; flow rate 1.0 cm<sup>3</sup> min<sup>-1</sup>] 14.2 min;  $\delta_{\text{H}}$  (500 MHz; DMSO-*d*<sub>6</sub>) 2.33–2.45 (4 H, dt × 2, SCH<sub>2</sub>CH<sub>2</sub>CO), 2.60–2.70 (4 H, t, SCH<sub>2</sub>CH<sub>2</sub>CO), 4.39–4.60 (12 H, 6-OH), 4.83 [12 H, d, *J* 2.8, C(1)H of glucose], 4.87 [1 H, d, *J* 3.0, C(1)H of amidoglucose], 5.56–5.95 (28 H, 2- and 3-OH), 7.73 (2 H, br s, NH); *m/z* (LSIMS) 2409.0 (M<sup>+</sup>) (+FAB); 2411.3 (–FAB) 2408.3 [(M – H)<sup>–</sup>], 1220.4 [(SCH<sub>2</sub>CH<sub>2</sub>CONH- $\beta$ -CD)<sup>–</sup>] and 1186.4 [(CH<sub>2</sub>CH<sub>2</sub>CONH- $\beta$ -CD)<sup>–</sup>] (+FAB, peak match) 2409.7 [(M + H)<sup>+</sup>]. C<sub>90</sub>H<sub>149</sub>N<sub>2</sub>O<sub>70</sub>S requires *m/z*, 2409.8] (–FAB, peak match) 2407.7 [(M – H)<sup>–</sup>]. C<sub>90</sub>H<sub>147</sub>N<sub>2</sub>O<sub>70</sub>S requires *m/z*, 2407.8].

#### Dithiodipropanamide dimer **2**

To a solution of 3,3'-dithiodipropanoic acid (30.8 mg,  $1.47 \times 10^{-4}$  mol) and amine **7** (529 mg,  $4.52 \times 10^{-4}$  mol) in dry DMF (3 cm<sup>3</sup>) were added triethylamine, HOBt (19.6 mg,  $1.45 \times 10^{-4}$  mol) and DCC (113 mg,  $5.48 \times 10^{-4}$  mol) under ice cooling. After reaction for 24 h, the reaction mixture was worked up according to the previous procedure. Low-pressure RP chromatography and elution with 5% aq. MeCN (400 cm<sup>3</sup>), followed by a gradient elution from H<sub>2</sub>O (500 cm<sup>3</sup>) to 50% aq. MeCN (500 cm<sup>3</sup>), gave the crude product **2**, which was purified by GP chromatography to give the dimer **2** (168 mg, 46.7%); *R*<sub>f</sub> 0.03; *t*<sub>R</sub> [column J'sphere ODS-M80; gradient, 5–30% aq. MeCN (25 min); flow rate 1.0 cm<sup>3</sup> min<sup>-1</sup>] 16.0 min, [column Diol-60; H<sub>2</sub>O; flow rate, 1.0 cm<sup>3</sup> min<sup>-1</sup>] 14.2 min;  $\delta_{\text{H}}$  (500 MHz; DMSO-*d*<sub>6</sub>) 2.45–2.59 (4 H, dt × 2, SCH<sub>2</sub>CH<sub>2</sub>CO), 2.81–2.92 (4 H, dt × 2, SCH<sub>2</sub>CH<sub>2</sub>CO), 4.40–4.55 (12 H, 6-OH), 4.83 [12 H, d, *J* 2.2, C(1)H of glucose], 4.87 [2 H, d, *J* 2.7, C(1)H of amidoglucose], 5.60–5.85 (28 H, 2- and 3-OH), 7.79 (2 H, br s, NH); *m/z* (LSIMS) 2441.4 [(M + H)<sup>+</sup>] (+FAB) 2442.9 and 1222.4 [(HSCH<sub>2</sub>CH<sub>2</sub>CONH- $\beta$ -CD + H)<sup>+</sup>] (–FAB) 2441.1 [(M – H)<sup>–</sup>], 1220.4 [(SCH<sub>2</sub>CH<sub>2</sub>CONH- $\beta$ -CD + H)<sup>–</sup>] and 1186.5 [(CH<sub>2</sub>CH<sub>2</sub>CONH- $\beta$ -CD + H)<sup>–</sup>] (+FAB, peak match) 2441.7 [(M + H)<sup>+</sup>]. C<sub>90</sub>H<sub>149</sub>N<sub>2</sub>O<sub>70</sub>S<sub>2</sub> requires *m/z* 2441.7] (–FAB, peak match) 2439.7 [(M – H)<sup>–</sup>]. C<sub>90</sub>H<sub>147</sub>N<sub>2</sub>O<sub>70</sub>S<sub>2</sub> requires *m/z* 2439.7].

#### Thiodiethanamide dimer **3**

To a solution of thiodiethanoic acid (12.8 mg,  $8.52 \times 10^{-5}$  mol) in dry DMF (5 cm<sup>3</sup>) were added EDC·HCl (49.2 mg,  $2.57 \times 10^{-4}$  mol), HOBt (27.7 mg,  $2.05 \times 10^{-4}$  mol), and amine **7** (301 mg,  $2.57 \times 10^{-4}$  mol) under ice cooling, followed by neutralization with triethylamine. The reaction mixture was stirred for 2 h under ice cooling and for an additional 15 h at rt. After the addition of water, the reaction mixture was evaporated *in vacuo* and the residue was dissolved in 15% aq. MeOH (20 cm<sup>3</sup>) and submitted to low-pressure RP chromatography. After elution with 15% aq. MeOH (300 cm<sup>3</sup>), gradient elution from 15% aq. MeCN (1 dm<sup>3</sup>) to 25% aq. MeCN (1 dm<sup>3</sup>) gave the dimer **3** (169 mg, 84.3%); *R*<sub>f</sub> 0.03; *t*<sub>R</sub> [column J'sphere ODS-M80; gradient, 0–40% aq. MeCN (40 min); flow rate 0.2 cm<sup>3</sup> min<sup>-1</sup>] 22.2 min (Found: C, 38.55; H, 6.4; N, 1.1; S, 1.32. C<sub>88</sub>H<sub>144</sub>N<sub>2</sub>O<sub>70</sub>S·20H<sub>2</sub>O requires C, 38.55; H, 6.75; N, 1.0; S, 1.15%);  $\delta_{\text{H}}$  (500 MHz; DMSO-*d*<sub>6</sub>) 4.40–4.60 (12 H, 6-OH), 4.83 [12 H, C(1)H of glucose], 4.87 [2 H, C(1)H of amidoglucose], 5.55–5.95 (28 H, 2- and 3-OH), 7.85 (2 H, br s, NH); *m/z* (+FAB) 2382.1 [(M + H)<sup>+</sup>] and 2363.6 [(M – H<sub>2</sub>O + H)<sup>+</sup>] (–FAB) 2380.2 [(M – H)<sup>–</sup>], 1206.3 [(SCH<sub>2</sub>CONH- $\beta$ -CD)<sup>–</sup>]

and 1174.7  $[(\text{CH}_2\text{CONH-}\beta\text{-CD})^-]$  (+FAB, peak match) 2381.8  $[(\text{M} + \text{H})^+]$ .  $\text{C}_{88}\text{H}_{145}\text{N}_2\text{O}_{70}\text{S}$  requires  $m/z$  2381.7] (–FAB, peak match) 2379.7  $[(\text{M} - \text{H})^-]$ .  $\text{C}_{88}\text{H}_{143}\text{N}_2\text{O}_{70}\text{S}$  requires  $m/z$  2379.7].

#### Dithiodiethanamide dimer 4

To a solution of dithiodiethanoic acid (32.4 mg,  $1.78 \times 10^{-4}$  mol) in dry DMF (1.5  $\text{cm}^3$ ) were added PyBop (185 mg,  $3.56 \times 10^{-4}$  mol), amine **7** (500 mg,  $4.28 \times 10^{-4}$  mol), HOBt (24.0 mg,  $1.78 \times 10^{-4}$  mol), and diisopropylamine (182 mg,  $1.41 \times 10^{-3}$  mol) under ice cooling. The reaction mixture was stirred for 2 h under ice cooling and for an additional 24 h at rt. After the addition of water, the reaction mixture was concentrated *in vacuo* and the residue was dissolved in  $\text{H}_2\text{O}$  (90  $\text{cm}^3$ ) and submitted to low-pressure RP chromatography. After elution with both  $\text{H}_2\text{O}$  (100  $\text{cm}^3$ ) and 13% aq. MeOH (1  $\text{dm}^3$ ), gradient elution with from 13% aq. MeCN (500  $\text{cm}^3$ ) to 25% aq. MeCN (500  $\text{cm}^3$ ) gave the dimer **4** (208 mg, 48.4%);  $R_f$  0.03;  $t_R$  [column J'sphere ODS-M80; gradient, 0–40% aq. MeCN (40 min); flow rate 0.2  $\text{cm}^3 \text{min}^{-1}$ ] 23.1 min (Found: C, 37.82; H, 5.8; N, 1.08; S, 2.28.  $\text{C}_{88}\text{H}_{144}\text{N}_2\text{O}_{70}\text{S}_2 \cdot 20\text{H}_2\text{O}$  requires C, 38.1; H, 6.68; N, 1.01; S, 2.31%);  $\delta_H$  (500 MHz; DMSO- $d_6$ ) 4.40–4.55 (12 H, 6-OH), 4.83 [12 H, C(1)H of glucose], 4.87 [2 H, C(1)H of amidoglucose], 5.65–5.85 (28 H, 2- and 3-OH), 7.90 (2 H, br s, NH);  $m/z$  (+FAB) 2415.4, 2396.2  $[(\text{M} - \text{H}_2\text{O} + \text{H})^+]$  and 1208.5  $[(\text{M} + 2\text{H})^{2+}]$  (–FAB) 2412.1  $[(\text{M} - \text{H})^-]$  and 1206.4  $[(\text{SCH}_2\text{CONH-}\beta\text{-CD})^-]$ ; (+FAB, peak match) 2413.7  $[(\text{M} + \text{H})^+]$ .  $\text{C}_{88}\text{H}_{145}\text{N}_2\text{O}_{70}\text{S}_2$  requires  $m/z$  2413.7] (–FAB, peak match) 2411.6  $[(\text{M} - \text{H})^-]$ .  $\text{C}_{88}\text{H}_{143}\text{N}_2\text{O}_{70}\text{S}$  requires  $m/z$  2411.7].

#### Dimer 5 linked with 2-thioethanamido moiety

The dithiodiethanamide dimer **4** (30.5 mg,  $1.26 \times 10^{-5}$  mol) was treated with  $\text{NaBH}_4$  (15.0 mg,  $3.95 \times 10^{-4}$  mol) in  $\text{H}_2\text{O}$  (0.4  $\text{cm}^3$ ) at rt for 1 h. The reaction mixture was acidified with dil. HCl followed by neutralization with  $\text{NaHCO}_3$ , to which were added  $\text{CsCO}_3$  (3.1 mg,  $9.39 \times 10^{-6}$  mol) and a solution of tosyl ester **6** (32.7 mg,  $2.54 \times 10^{-5}$  mol) in pyridine (0.2  $\text{cm}^3$ ). The mixture was stirred for 22 h under argon atmosphere. After neutralization with dil. HCl, the solution was concentrated *in vacuo* and the residue, as a mixture in  $\text{H}_2\text{O}$  (1  $\text{cm}^3$ ), was applied to GP chromatography. The obtained crude dimer **5** was dissolved in  $\text{H}_2\text{O}$  (5  $\text{cm}^3$ ) and re-chromatographed on an RP column. After elution with both  $\text{H}_2\text{O}$  (100  $\text{cm}^3$ ) and 10% aq. MeOH (200  $\text{cm}^3$ ), gradient elution from 10% aq. MeOH (300  $\text{cm}^3$ ) to 25% aq. MeOH (300  $\text{cm}^3$ ) gave the dimer **5** (15.7 mg, 26.8%);  $R_f$  0.03;  $t_R$  [column J'sphere ODS-M80; gradient, 0–40% aq. MeCN (40 min); flow rate 1.0  $\text{cm}^3 \text{min}^{-1}$ ] 15.1 min;  $\delta_H$  (200 MHz; DMSO- $d_6$ ), 4.30–4.70, 4.83, 5.50–5.95 and 7.67;  $m/z$  (+FAB) 2325.0  $[(\text{M} + \text{H})^+]$  and 2306.0  $[(\text{M} - \text{H}_2\text{O} + \text{H})^+]$  (–FAB) 2323.2  $[(\text{M} - \text{H})^-]$ , 1206.5  $[(\text{SCH}_2\text{CONH-}\beta\text{-CD})^-]$ , 1174.4  $[(\text{CH}_2\text{CONH-}\beta\text{-CD})^-]$  and 1149.4  $[(\text{M} - (\text{CH}_2\text{CONH-}\beta\text{-CD})^-)]$  (+FAB, peak match) 2324.7  $[(\text{M} + \text{H})^+]$ .  $\text{C}_{86}\text{H}_{142}\text{NO}_{69}\text{S}$  requires  $m/z$  2324.7] (–FAB) 2322.7  $[(\text{M} - \text{H})^-]$ .  $\text{C}_{86}\text{H}_{140}\text{NO}_{69}\text{S}$  requires  $m/z$  2322.7].

#### Guest-binding analyses

Analyses were repeated several times. Typical examples in the case of dimer **3** are given below.

**MO.** To a solution of MO ( $3.05 \times 10^{-5}$  M; 2.5  $\text{cm}^3$ ) in 0.05 M sodium carbonate buffer (pH 10.6) were added aliquots of an aqueous solution of dimer **3** ( $5.21 \times 10^{-3}$  M) containing MO ( $3.05 \times 10^{-5}$  M) at 25 °C. Thus, while the concentration of MO was kept constant, that of dimer **3** was changed from 1.04 to  $3.04 \times 10^{-4}$  M. After each addition, the absorption spectrum was recorded and the absorbance changes at 371, 425 and 500 nm were subjected to curve-fitting analysis.<sup>13</sup>

**TR.** The concentration of TR was  $3.08 \times 10^{-5}$  M in 0.05 M potassium/sodium phosphate buffer (pH 6.86) and the concentration of dimer **3** ranged from 0.922 to  $1.35 \times 10^{-4}$  M. The absorption changes at 417 and 488 nm were used for the analysis.

**TNS.** To a solution of TNS ( $1.00 \times 10^{-5}$  M; 2.5  $\text{cm}^3$ ) in 0.05 M potassium/sodium phosphate buffer (pH 6.86) was added an aqueous solution of dimer **3** ( $5.19 \times 10^{-3}$  M) containing TNS ( $1.00 \times 10^{-5}$  M) at 25 °C. The concentration of dimer **3** ranged from 0.207 to  $6.69 \times 10^{-4}$  M. Excitation wavelength was 366 nm and the emission changes at 417, 440 and 470 nm were used for the analysis.

**BNS.** The concentration of BNS was  $4.06 \times 10^{-6}$  M in 0.05 M potassium/sodium phosphate buffer (pH 6.86) and the concentration of dimer **3** ranged from 1.01 to  $8.79 \times 10^{-6}$  M. Excitation wavelength was 380 nm and the emission changes at 435, 464 and 500 nm were used.

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