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Bis(phenylthienyl)ethene-tethered β-cyclodextrin dimers as photoswitchable hosts

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Two β-cyclodextrin dimers tethered by photoswitchable bis(phenylthienyl)ethene moieties were synthesized as potentially tunable receptor molecules. The cyclodextrin cavities of these dimers were linked *via* their secondary sides, with the photochromic bis(phenylthienyl)ethene unit either directly connected to the secondary rim (**7**) or *via* propyl spacers (**10**). By irradiation with light the dimers were reversibly switched between a relatively flexible (open) form and a rigid (closed) form. The photostationary states for both dimers consisted of 92% of the open and 8% of the closed form, enabling the nearly complete conversion between the two forms. The binding properties of the open and closed forms of dimers **7** and **10** were assessed by complexation studies with *meso*-tetrakis(4-sulfonatophenyl)-porphyrin (TSPP) using isothermal titration calorimetry. For the rigidly tethered dimer **7**, a factor 8 difference in binding affinity between the open and closed form of the dimer was found. This difference in binding affinity reflects the difference in enthalpy of binding for the two dimers, indicating that the β-cyclodextrin cavities of the closed dimer **7b** are spaced too far apart from each other by the rigid closed bis(phenylthienyl)ethene tether to cooperatively bind TSPP. The difference in binding affinity was sufficient to enable the phototriggered release of TSPP from dimer **7**. The thermodynamic parameters obtained for dimer **10** suggested that the closed tether substantially contributes to the binding of TSPP. The open and closed form of dimer **10** bound TSPP with similar association constants, although the enthalpy of binding for the complexation of TSPP by the closed form of dimer **10** was more favorable than that found for the open form of the dimer.

Introduction

Receptor molecules of which the binding affinity towards specific substrates can be altered by external stimuli have been subject of ongoing research for several years.[1–13] Work in this field was initiated in the late 1970s by Ueno[1] and Shinkai,[2] who synthesized cyclodextrins and crown-ethers capped with photoswitchable azobenzene moieties, respectively, to obtain tunable supramolecular receptors of which the binding affinity and selectivity could be altered by irradiation with light. In a later stage, Shinkai elegantly demonstrated the use of the photoswitchable crown-ethers for controlled transport of cations.[3] This paradigm of controlled release and uptake of guest molecules to and from solution by external stimuli, has inspired numerous groups to synthesize tunable receptors, and a variety of metal-[4] and photoswitchable[5] host molecules has been reported in the literature.

Among the host molecules considered for implementation in tunable receptors, cyclodextrins are of special interest. Cyclodextrins are able to complex hydrophobic substrates in aqueous solutions and this has led to their application in a wide variety of fields such as pharmaceuticals, artificial enzymes, and biomimetic materials.[6] In order to obtain tunable cyclodextrin host molecules, cyclodextrins have been functionalized with azobenzene moieties,[1,7] and β-cyclodextrin dimers have been tethered with metal chelating,[8,9] photoswitchable,[10] and photocleavable tethers.[11] Recently, we reported on tunable β-cyclodextrin dimers tethered by photoswitchable dithienylethene moieties.[12,13] Dithienylethenes are photoswitchable molecules that are able to undergo thermally irreversible, fatigue-resistant, photochromic cyclization reactions between two defined states: a relatively flexible open form and a rigid closed form (Fig. 1, top).[14,15] This subtle difference in flexibility between the two forms of dithienylethenes was used to achieve a surprisingly large difference in binding affinity (factor 35) between the open and closed states of the photoswitchable β-cyclodextrin dimer for binding a porphyrin guest molecule. In this dimer, the dithienylethene moiety was tethered directly to the secondary rim of the β-cyclodextrin cavities. Calorimetric experiments indicated that the decreased porphyrin affinity observed for the closed form of the dimer was due to diminished cooperativity between the two β-cyclodextrin cavities caused by the closed dithienylethene tether, which spaces the two β-cyclodextrin cavities apart from each other in a rigid fashion.

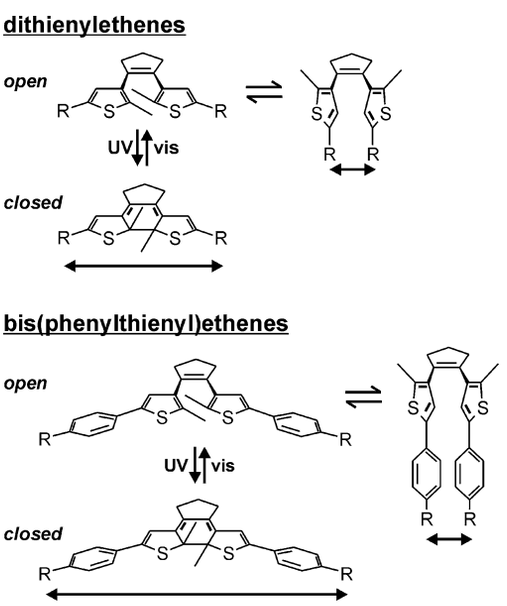

Fig. 1 Possible spacing of appended groups (R) for the different forms (open and closed) of dithienylethenes (top) and bis(phenylthienyl)-ethenes (bottom).

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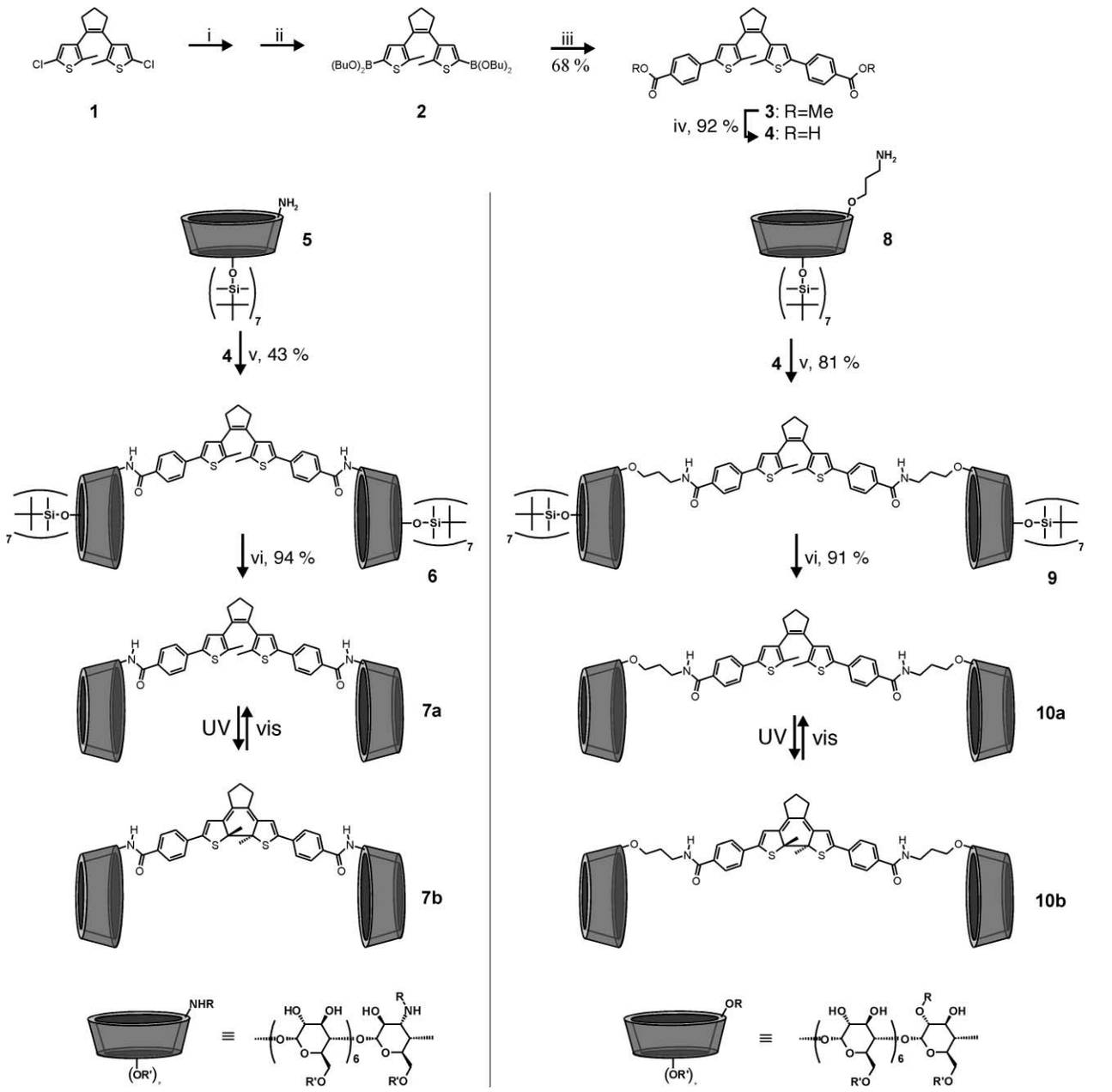

Scheme 1 Synthesis routes for dimers **7** and **10**. i, n-BuLi, THF, room temperature; ii, $B(OBu)_3$; iii, Me 4-bromobenzoate, $Pd(PPh_3)_4$, 2 M Na_2CO_3, ethylene glycol, THF, reflux; iv, 4 M NaOH, dioxane, reflux; v, HBTU, DIPEA, THF, room temperature; vi, TFA, room temperature.

Here, we describe the synthesis and photochromic properties of two β-cyclodextrin dimers tethered by a bis(phenylthienyl)-ethene (Fig. 1, bottom), in an attempt to obtain even larger differences in binding affinity. Compared to the previously used dithienylethenes, the bis(phenylthienyl)ethenes have an additional phenyl ring attached to the photoswitchable core. As a consequence, the difference in spacing of the photoswitch-appended moieties between the open and closed forms of the photoswitch is larger for the latter type of molecules (Fig. 1). Therefore, bis(phenylthienyl)ethene tethers potentially enable larger differences in binding affinity compared to dithienylethene tethers. An additional advantage of the bis(phenylthienyl)ethenes over the dithienylethenes is that they can be switched almost completely to the closed form: for bis(phenylthienyl)ethene switches the fraction of the closed form in the photostationary states often exceeds 90%,[16] which is substantially higher than that obtained for dithienylethenes.[17] Binding studies with *meso*-tetrakis-(4-sulfonatophenyl)porphyrin (TSPP) have been performed to assess the potential use of these dimers as tunable receptors.

Results and discussion

Synthesis and characterization of the β-cyclodextrin dimers

The synthesis of the β-cyclodextrin dimers is outlined in Scheme 1. The two synthesized bis(phenylthienyl)ethene-tethered β-cyclodextrin dimers (**7** and **10**) have a different connectivity between the cyclodextrins and the photochromic units. In dimer **7**, the bis(phenylthienyl)ethene moiety is attached directly at the secondary sides of the β-cyclodextrin cavities, giving a relatively rigid dimer, where most of the rotational freedom is present in the bis(phenylthienyl)ethene tether. Alternatively, the more flexible dimer **10** was synthesized in which the bis(phenylthienyl)ethene unit and the secondary sides of the β-cyclodextrin cavities are spaced by propyl spacers.

The top part of Scheme 1 details the synthesis of the photoswitchable tether, bis(carboxyphenylthienyl)ethene **4**, used for coupling of the β-cyclodextrin cavities. The synthesis of **4** was achieved by extension of the photoswitchable unit 2,2′-dichlorodithienylethene[18] **1** *via* a Suzuki coupling with methyl 4-bromobenzoate, analogous to a procedure recently reported by Feringa *et al.*[16] The resulting bis(methyl ester) **3** was purified

by column chromatography and subsequently hydrolyzed to give **4**. Direct coupling of tether **4** to the secondary rim of the β-cyclodextrin cavities was realized by an amide coupling of **4** with 3-amino-3-deoxy-heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin[19] **5** using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as a coupling agent analogous to a previously reported procedure.[13] The resulting protected dimer **6** was purified by gradient column chromatography. Deprotection of the primary hydroxyl groups, using trifluoroacetic acid, gave dimer **7** in the open form (**7a**). Similarly, dimer **10a** was synthesized from bis(carboxyphenylthienyl)ethene **4** and mono-(2-*O*-(3-aminopropyl))-2-deoxy-heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (**8**).[13]

Both dimers **7** and **10** were poorly water-soluble, which is probably inherent to their large hydrophobic tether. ^{1}H NMR spectroscopy indicated that the dimers strongly aggregated in aqueous solution. Fig. 2 (top) gives the ^{1}H NMR spectrum of dimer **7a** in D_2O, which is a typical example of the spectra obtained for the dimers **7** and **10** in aqueous solution. The spectrum is dominated by a set of broad peaks around 3–4 and 5 ppm, characteristic shifts for the β-cyclodextrin protons. The only indication of the presence of the bis(phenylthienyl)ethene tether is an extremely broad and barely visible hump in the aromatic region (~8 ppm). The spectra in aqueous solution sharpened only slightly at elevated temperatures. Spectra recorded with the dimers in DMSO-d_6 or mixtures of D_2O and MeOD (1 : 1, v/v) showed relatively sharp peaks for both the β-cyclodextrin protons and the bis(phenylthienyl)ethene tether with integral ratios in agreement with the molecular structure of the dimer (Fig. 2, center and bottom). Both ^{1}H NMR spectra of dimer **7** and **10** in DMSO-d_6 show three signals for the phenylthienyl moiety in accordance with the C_2 symmetry of the dimer, and the cyclopentene bridge protons occur at 2.1 and 2.8 ppm, characteristic shifts for these switching units.[16] Additionally, the spectrum of dimer **10** (Fig. 2, bottom) shows a multiplet around 1.8 ppm, which originates from the C2 methylene group of the propyl spacers. Only moderate sharpening of the spectra recorded for the D_2O solutions of the dimers is obtained when the dimer concentration is systematically decreased from 5 to 0.1 mM (spectra not shown), suggesting that intramolecular aggregation is responsible for the poor resolution observed in these ^{1}H NMR spectra.

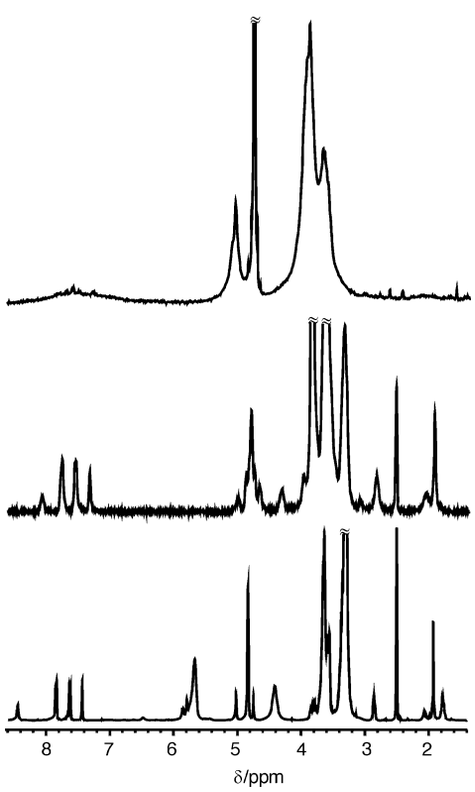

Fig. 2 ^{1}H NMR spectra of **7a** in D_2O (top) and in DMSO-d_6 (middle), and of **10a** in DMSO-d_6 (bottom).

Switching behavior of the dimers

The photochromic behavior of the dimers was studied by irradiation with a high-pressure mercury lamp with band-pass filters. The photochemical reactions were monitored by UV-vis spectroscopy. The absorption spectra of dimers **7** and **10** are shown in Fig. 3 (top and bottom, respectively). The open forms of both dimers showed strong absorption in the UV region with absorption maxima at 331 nm for dimer **7a** and at 298 and 334 nm for dimer **10a**, respectively (Fig. 3, solid lines). The colorless aqueous solutions turned purple upon irradiation at 313 nm and strong absorption bands appeared in the visible region of the absorption spectra with maxima at 564 nm for dimer **7b** and 553 nm for dimer **10b**.

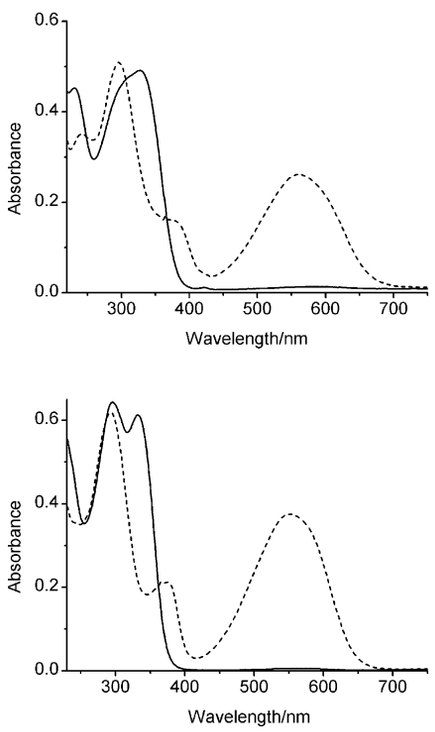

Fig. 3 Absorption spectra of 16 μM **7** (top) and 20 μM **10** (bottom) in water before (open form, ——) and after (PSS mixture, - - -) photoirradiation with 313 nm light.

Photostationary states (PSS) of both dimers were readily obtained, suggesting that aggregation, if present at these concentrations has little effect on the switching behavior of the dimers. UV-vis spectra recorded before reaching the PSS showed sharp isosbestic points, indicative of only two, interchanging species. The absorption spectra of the PSS mixtures of **7a/7b** and **10a/10b** are given by the dashed lines in Fig. 3. The PSS mixtures were stable at room temperature in the dark. Irradiation of the PSS mixtures with visible light (λ > 460 nm) led to the disappearance of the absorption bands in the visible region and to restoration of the absorption spectra of the open forms, demonstrating the reversibility of the photochemical ring-opening/ring-closure process. The compositions of the PSS mixtures were determined by modeling of the UV-vis spectra, as reported previously.[13] A minimum of ten UV-vis spectra, obtained during irradiation of the dimers, was fitted with a set of Gaussians representing the open form (directly obtained from the UV-vis spectra of the open forms, see solid lines Fig. 3) and a set of Gaussians representing the absorption spectra of the closed form (optimized in the fitting procedure).

Table 1 Parameters for the sets of Gaussians used for the fitting of the absorption spectra of **7** and **10**[a]

7a			**7b**			**10a**			**10b**		
λ_{max}	FWHM	ε_{max}	λ_{max}	FWHM	ε_{max}	λ_{max}	FWHM	ε_{max}	λ_{max}	FWHM	ε_{max}
229.0	17.2	25.2	237.0	20.0	18.6	219.9	24.0	29.7	230.0	25.0	16.8
297.2	31.0	26.5	295.9	24.7	30.8	294.7	23.4	31.1	293.8	23.1	30.1
341.4	19.2	17.8	374.0	24.6	9.6	339.8	16.1	24.4	371.0	18.8	11.3
			561.2	53.0	17.1				549.5	50.3	20.8

[a] λ_{max} (nm), width (nm), ε_{max} (10^3 cm^{-1} M^{-1}).

Typical fits are given in Fig. 4, which shows the recorded and modeled absorption spectra of the open form and the PSS mixture of dimer **7** and the calculated absorption spectrum of the closed form **7b**. Equally good fits were obtained for dimer **10**. Table 1 lists the parameters for the sets of Gaussians used for the fitting procedure. For both dimers, the photostationary state was composed of 8% of the open and 92% of the closed form. These photostationary states are in accordance with results obtained for similar bis(phenylthienyl)ethene switches,[16] and indicate that the coupling with and close proximity of the cyclodextrin cavities do not interfere with the switching process.

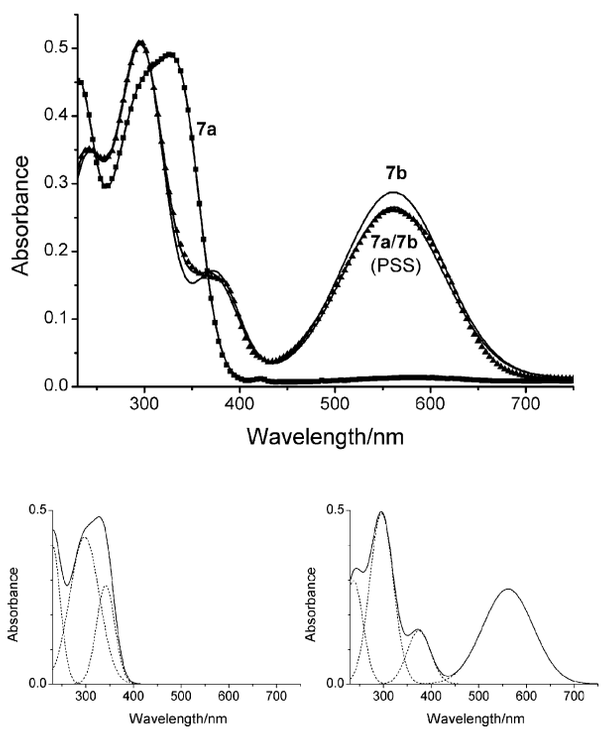

Fig. 4 Measured (markers) and modeled (lines) absorption curves of 16 μM **7** in water (top; **7a** (■), PSS mixture of **7a**/**7b** (▲), and **7b**). Set of Gaussians (- - -) that constitute the calculated absorption spectrum (—) of **7a** (bottom left) and **7b** (bottom right).

The nearly complete conversion between the open and closed forms of dimers **7** and **10** is ideal for their use as photoswitchable receptor molecules. In this respect, the bis(phenylthienyl)ethene-tethered β-cyclodextrin dimers reported here are superior to the dithienylethene β-cyclodextrin dimers reported previously, which showed a PSS composition of only 75% of the closed form.[13] Consequently, strong differences in binding affinity between the open and closed states of the bis(phenylthienyl)ethene-tethered β-cyclodextrin dimers might lead to a more pronounced release of guest molecules.

Complexation studies

Complexation studies were performed with *meso*-tetrakis(4-sulfonatophenyl)porphyrin (TSPP). TSPP is a well-studied guest molecule for complexation by β-cyclodextrin and β-cyclodextrin dimers.[20,21] The binding of TSPP by the open and closed forms of the dimers **7** and **10** was studied with isothermal titration microcalorimetry. Despite the aggregation behavior of dimers **7** and **10**, titrations were performed with aqueous solutions in order to enable comparison with the previously obtained calorimetric data for the shorter dithienylethene-tethered dimers. Titrations with dimer **7** were performed in water, whereas for dimer **10** mixtures of DMSO and water (1 : 9 v/v) were used to prevent precipitation of the dimer from solution.

Fig. 5 depicts two typical titration curves obtained for the titration of TSPP to **7a** (top) and a PSS mixture of **7** (bottom). The initial, less exothermic heat effects observed for the titration of TSPP to dimer **7a** (Fig. 5, top) were attributed to deaggregation of the dimer upon complexation of TSPP. Similar heat effects were seen for titrations with dimer **10** in the DMSO–water mixtures. The deaggregation only affects the heat effects for the first additions, and the remainders of the curves could be fitted well with a 1 : 1 interaction. The aggregation behavior of dimers **7a** and **10** was not further quantified.

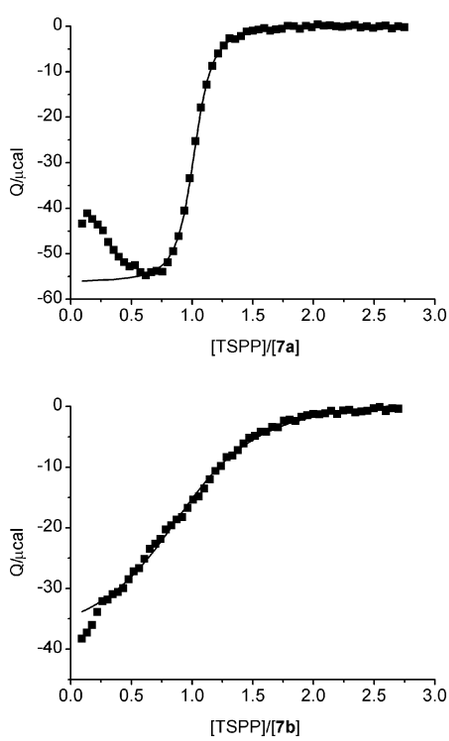

Fig. 5 Heat evolved per injection plotted against the [TSPP] : [**7**] ratio (markers) and fit (solid line) for the calorimetric titrations of TSPP to **7a** (top) and to the PSS mixture of **7** (bottom) in water at 298 K.

Interestingly, no pronounced heat effects due to deaggregation were observed for the titrations of TSPP to solutions of the PSS mixture of **7** (Fig. 5, below). The PSS of **7** mainly consists of **7b** (see above), and the titration curves obtained with the PSS mixtures of **7** showed no pronounced effects for the binding of TSPP by **7a**. The titration curves could be fitted well using a 1 : 1 model and a single host site **7b** (Fig. 5, below).

Table 2 Thermodynamic parameters of the complexation of TSPP by the open and closed forms of **7** and **10**, as determined by isothermal titration microcalorimetry at 298 K

Host	K (M^{-1})	$\Delta G°$ (kcal mol^{-1})	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})	Solvent
β-CD	$(3.1 \pm 0.4) \times 10^4$	-6.1 ± 0.1	-4.3 ± 0.2	1.8 ± 0.3	H_2O [13]
7a	$(2.8 \pm 1.3) \times 10^6$	-8.7 ± 0.3	-12.8 ± 0.6	-4.1 ± 0.9	H_2O
7b	$(3.4 \pm 2.0) \times 10^5$	-7.4 ± 0.5	-9.6 ± 0.3	2.1 ± 0.8	H_2O
10a	$(6.3 \pm 1.8) \times 10^5$	-7.9 ± 0.2	-10.7 ± 0.4	-2.8 ± 0.6	H_2O–DMSO 9 : 1
10b	$(6.6 \pm 2.4) \times 10^5$	-7.9 ± 0.2	-12.6 ± 0.4	-4.7 ± 0.6	H_2O–DMSO 9 : 1

Similar good fits were obtained when fitting titration curves obtained with the PSS mixture of dimer **10** taking into account only the closed dimer **10b**. Therefore, binding curves obtained with the PSS were considered to be the result of binding of TSPP by the closed form of the dimer, and both binding curves of the open and PSS mixtures of the dimers were fitted with a 1 : 1 binding model using one single association constant, K, and binding enthalpy, $\Delta H°$, as independent fitting parameters. The thermodynamic parameters obtained for the complexation of TSPP by the open and closed forms of dimers **7** and **10** are summarized in Table 2, together with those determined for the binding of TSPP by native β-cyclodextrin.

The thermodynamic parameters (K and $\Delta H°$) for the complexation of TSPP by dimer **7a** are indicative of strong 1 : 1 binding. The enthalpy of binding, -12.8 kcal mol^{-1}, is more than double that found for the complexation of TSPP by native β-cyclodextrin, and the association constant for complexation of TSPP with **7a**, 2.8×10^6 M^{-1}, is two orders of magnitude higher. It is noteworthy that the thermodynamic parameters obtained for the complexation of TSPP by dimer **7a** are within experimental error identical to the parameters determined for the corresponding binding of TSPP by the analogous dithienylethene dimer.[13] This similarity implies that both dimers bind TSPP in a similar fashion. Apparently the energetic cost for bringing the two β-cyclodextrin cavities together for complexation of TSPP is the same for both the short dithienylethene dimer and the longer bis(phenylthienyl)ethene dimer **7a**. Given the similarity of both dimers, *i.e.* connectivity and degrees of freedom present in the dimers, this seems sensible. The correspondence of these results verifies our assumption that the deaggregation observed in the titration curves only affects the initial part of the curve.

Dimer **7b** bound TSPP less effectively than dimer **7a**. The association constant obtained for the complexation of TSPP by dimer **7b**, 3.4×10^5 M^{-1}, was a factor 8 lower than that found for dimer **7a**. The strongly reduced enthalpy of binding, -9.6 kcal mol^{-1}, suggests that the weaker binding is likely due to less cooperative binding of TSPP by the two β-cyclodextrin cavities, which are spaced far apart from each other by the rigid bis(phenylthienyl)ethene tether. The less favorable enthalpy of binding is partly compensated by a relatively more favorable entropy of binding. This so-called enthalpy–entropy compensation[22] is a well-known phenomenon for complexation studies with cyclodextrins and cyclodextrin dimers,[23,24] and the more favorable entropy associated with less strong enthalpic binding is typically explained in terms of reduced fixation of the host–guest complex. It is noted that the enthalpy of binding found for the complexation of **7b** is considerably more favorable than that found with native β-cyclodextrin. Given the length and rigidity of the closed bis(phenylthienyl)ethene tether it is not likely that the second β-cyclodextrin cavity gives any contribution to the binding of TSPP by the first β-cyclodextrin cavity in the case of dimer **7b**.[25] A possible explanation for the relatively large enthalpy value found for **7b**, compared to native β-cyclodextrin, might be that the tether itself contributes to the binding of TSPP, *e.g.* by π–π interactions between tether and TSPP.

The open and closed dimers of **10** did not show any difference in binding affinity for TSPP. Both states bound TSPP with an association constant of 6×10^5 M^{-1}. These somewhat lower association constants, compared to those found with dimer **7a**, are probably caused by the presence of DMSO in solution. Although the association constants are similar, there are some striking differences between the binding enthalpies and entropies found for the complexation of the open and closed forms of dimer **10**. Dimer **10a** is the most flexible dimer of the dimers discussed in this paper and consequently it is expected to effectively bind TSPP with both β-cyclodextrin cavities. The thermodynamic parameters found for the complexation of TSPP by **10a**, a strongly negative enthalpy value accompanied by a negative entropy value, support this idea. Interestingly, the enthalpy of binding found for the closed dimer **10b** was 1.9 kcal mol^{-1} more favorable compared to the open dimer **10a**. This is remarkable because CPK modeling suggests that the rigidity imposed on the dimer by the closed bis(phenylthienyl)ethene tether, and the therewith associated spacing of the β-cyclodextrin cavities, can not be completely overcome by the flexible propyl spacers between the β-cyclodextrin cavities and the tether. Therefore, **10b** is probably not able to bind TSPP using both β-cyclodextrin cavities to a full extent. Nevertheless, a more favorable binding enthalpy is found for complexation of TSPP by the closed dimer **10b**, compared to **10a**, which is able to use both its β-cyclodextrin cavities for the complexation of TSPP.[26] These results indicate that additional interactions between the closed bis(phenylthienyl)ethene tether and TSPP substantially contribute to the binding of TSPP by the dimers. These findings might also explain the moderate difference in binding affinity found for the open and closed form of dimer **7**. The more favorable binding enthalpy found for **10b** is counteracted by a less favorable, *i.e.* more negative entropy term, to give a binding energy that is similar to that found for the complexation of TSPP by **10a**, rendering dimer **10** unsuitable for phototriggered release of TSPP.

Phototriggered release

To test whether the binding difference between the open and closed state of dimer **7** would be sufficient to allow phototriggered release of TSPP, the absorption of the dimer–TSPP complex was followed during irradiation at 313 nm. UV-vis spectroscopy allows the real-time determination of the ratio of uncomplexed and complexed TSPP upon irradiation of dimer–TSPP complexes. It is known that the absorption maximum of TSPP shifts to the red and the absorbance decreases upon complexation by cyclodextrin.[20b] Fig. 6 shows part of the absorption spectra of the complexes of TSPP and dimers **7** upon irradiation at 313 nm. The absorption maximum of TSPP in aqueous solution at 413 nm showed a red shift to 424 nm upon addition of dimer **7a**, indicative of complex formation between TSPP and the dimer (most top right absorbance band). The shoulder around 413 nm indicated the presence of excess TSPP. Irradiation of the solution at 313 nm led to a decrease of absorption of complexed TSPP (424 nm), and simultaneously an increase of the absorbance at lower wavelengths. Comparison of the separate absorption curves suggests that the increase of absorbance at lower wavelengths is due to an increase in absorbance of uncomplexed TSPP and an additional, less pronounced increase in absorbance around

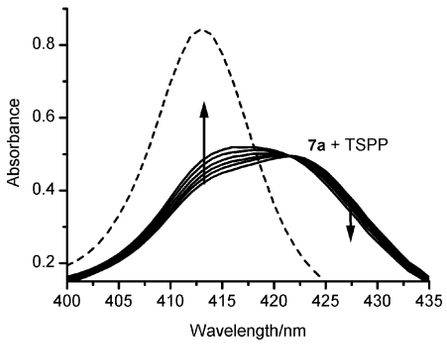

Fig. 6 Absorption spectra (0 to 10 min) of a 2 μM complex of TSPP with **7a** in water upon irradiation at λ = 313 nm. Also shown is the spectrum of 2 μM TSPP in water (- - -).

420 nm. The latter can be explained by assuming that the absorbance for the complex of TSPP with the closed dimer **7b** is different from that with open dimer **7a** and that its absorbance maximum lies at lower wavelength. The appearance of two new TSPP-species upon irradiation of the complex of TSPP and dimer **7a** corroborates the data obtained by calorimetry. At the concentrations used for the experiment, **7b** should still be able to give considerable complexation of TSPP, and consequently only a moderate amount of TSPP is released from the dimer upon closure of the bis(phenylthienyl)ethene tether.[27]

Conclusions

The implementation of a bis(phenylthienyl)ethene tether in β-cyclodextrin dimers gives photoswitchable receptor molecules that can be reversibly switched between a flexible open and a more rigid closed form. Compared to β-cyclodextrin dimers tethered by the shorter dithienylethene tethers, these dimers have a number of advantages and disadvantages. The advantages of the bis(phenylthienyl)ethene tethers are the near complete conversion between the two forms of the β-cyclodextrin dimer that can be achieved and the more pronounced spacing of the β-cyclodextrin cavities of the dimers obtained upon ring-closure of the bis(phenylthienyl)ethene tether. Disadvantages are the low water solubility of the β-cyclodextrin dimers, inherent to the large hydrophobicity of the tethers, and the substantial participation of the closed bis(phenylthienyl)ethene tether in the binding of guest molecules as observed for TSPP. The latter leads to a partial compensation for the possible diminished cooperativity of the β-cyclodextrin cavities achieved by the rigid and distant spacing by the closed tether. Consequently, less pronounced binding differences between the open and closed forms of the dimers were observed compared to the corresponding dithienylethene dimers, and only moderate release of complexed TSPP upon irradiation of the complexes could be achieved. This does not imply that dimers **7** and **10** are only moderately tunable receptor molecules. They are for TSPP, but strong differences in binding between the two forms of the bis(phenylthienyl)ethene dimers might be achieved with small ditopic guest molecules that have weaker interactions with the closed form of the tether, *i.e.* guest moieties tethered by relatively hydrophilic non-aromatic linkers.

Taken together, the results given in this paper illustrate that full addressability of both forms of the tunable receptor and the minimization of possible cooperativity between two host sites for one of the forms of the receptor are not the only criteria in the design of tunable ditopic receptors. The relative contributions of the tethers to the binding process should also be considered, as these can severely diminish potentially large differences in binding. On the other hand, the tether contributions to binding might be dependent on the form or state of the tether, and this could possibly be used to achieve tunable binding.

Experimental section

Materials and methods

All chemicals were used as received, unless stated otherwise. Solvents were purified according to standard laboratory methods. Thin-layer chromatography was performed on aluminium sheets precoated with silica gel 60 F254 (Merck). The cyclodextrin spots were visualized by dipping the sheets in 5% sulfuric acid in ethanol and subsequent heating. Chromatographic separations were performed on silica gel 60 (Merck, 0.040–0.063 mm, 230–240 mesh). 2,2′-(Dichlorodithienylethene)-cyclopentene (**1**),[18] 3-amino-3-deoxy-heptakis(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (**5**),[19] and mono-(2-*O*-(3-aminopropyl))-heptakis-(6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (**8**)[13] were prepared according to literature procedures.

FAB mass spectra were recorded with a Finnigan MAT90 spectrometer with *m*-nitrobenzyl alcohol as a matrix. MALDI-TOF mass spectra were recorded using a PerSpective Biosystems Voyager-DE-RP MALDI-TOF mass spectrometer. NMR spectra were recorded at 25 °C using a Varian Inova 300 spectrometer. ^{1}H NMR chemical shifts (300 MHz) are given relative to residual $CHCl_3$ (7.25 ppm) or DMSO-d_6 (2.50 ppm). ^{13}C NMR chemical shifts (75 MHz) are given relative to $CDCl_3$ (77.0 ppm) or to DMSO-d_6 (39.5 ppm).

All synthesized compounds containing the dithienylethene moiety are light-sensitive and were therefore exclusively handled in the dark using brown-stained glassware.

1,2-Bis[5′-(4″-methoxycarbonylphenyl)-2′-methylthien-3′-yl]-cyclopentene 3

2,2′-(Dichlorodithienylethene)-cyclopentene **1** (0.8 g, 2.4 mmol) was converted to 1,2-bis(5′-dibutoxyboryl-2′-methylthien-3′-yl)cyclopentene **2** by reaction with n-BuLi (2 mL, 5.1 mmol), and subsequently B(n-OBu)$_3$ (2 mL, 7.3 mmol) in freshly distilled anhydrous THF (10 mL) as previously reported by Feringa *et al.*[16] The boronic ester **2** was not isolated because it tends to hydrolyze during work-up. In the meantime methyl 4-bromobenzoate (1.56 g, 7.26 mmol) was dissolved in freshly distilled anhydrous THF (15 mL), and $Pd(PPh_3)_4$ (0.3 g, 0.23 mmol) was added to the stirred solution. The suspension was stirred at room temperature for 15 min, after which 2 M Na_2CO_3 (15 mL) and 10 drops of triethylene glycol were added. The solution of the boronic acid **2** (see above) was slowly added to this suspension. After the addition was complete the suspension was heated to reflux for 2 h, and allowed to cool to room temperature. Diethyl ether (40 mL) and water (40 mL) were added, and the organic layer was isolated and dried over Na_2SO_4. After evaporation of the solvent the product was purified by column chromatography (eluent hexane–CH_2Cl_2 1 : 9) to give **3** as a white solid in 68% overall yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01 (d, J = 6.6 Hz, 4H), 7.55 (d, J = 6.6 Hz, 4H), 7.17 (s, 2H), 3.90 (s, 6H), 2.87 (t, J = 7.5 Hz, 4H), 2.18–2.07 (m, 2H), 2.05 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.8, 138.6, 138.5, 137.0, 136.3, 134.7, 130.2, 128.2, 125.4, 124.8, 52.0, 38.5, 23.0, 14.5. FAB-MS: *m*/*z* calcd for [M+H] 528.1, found 528.1.

1,2-Bis[5′-(4″-carboxyphenyl)-2′-methylthien-3′-yl]cyclopentene 4

Compound **3** (0.4 g, 0.76 mmol) was dissolved in dioxane (10 mL) and 4 M NaOH (10 mL) was added to the solution. The stirred suspension was heated to reflux for 10 h, and allowed to cool to room temperature. The aqueous layer was isolated and carefully acidified by dropwise addition of 12 M HCl. The resulting precipitate was isolated by filtration and extensively washed with water. The residue was dried over $CaCl_2$ in a vacuum oven at 60 °C to give **4** as a white solid in 92% yield. ^{1}H NMR (300 MHz, THF-d_8): δ = 7.95 (d, J = 8.4 Hz, 4H), 7.52 (d, J = 8.4 Hz, 4H), 7.20 (s, 2H), 2.89 (t,

J = 7.5 Hz, 4H), 2.19–2.11 (m, 2H), 2.01 (s, 6H). ^{13}C NMR (75 MHz, THF-d_8): δ = 166.9, 139.5, 138.9, 137.8, 136.4, 135.4, 130.8, 129.9, 126.1, 125.6, 125.1, 39.0, 23.5, 14.3. FAB-MS: *m*/*z* calcd for [*M*] 500.1, found 500.0.

TBDMS-protected bis(phenylthienyl)ethene-tethered β-cyclodextrin dimer 6

To a cooled solution of **4** (98 mg, 0.2 mmol) in dry DMF (50 mL) were added HBTU (223 mg, 0.6 mmol) and DIPEA (0.17 mL, 0.98 mmol). The solution was stirred for 30 min and then allowed to warm to room temperature. **5** (950 mg, 0.5 mmol) was added and the solution was stirred for 3 days at room temperature. The solvent was removed *in vacuo*, and chloroform was added. The solution was washed twice with 1 M HCl and brine. After removal of the solvent, the crude product was purified by gradient column chromatography (ethyl acetate–ethanol–water 100 : 2 : 1 to 100 : 8 : 4) to give **6** (open form) as a white powder in 43% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.23 (d, J = 7.6 Hz, 2H), 7.86 (d, J = 8.4 Hz, 4H, H-Ar), 7.45 (d, J = 8.4 Hz, 4H), 7.05 (s, 2H), 4.94–4.86 (m, 12H), 4.69 (d, J = 7.3 Hz, 2H), 4.13–3.38 (m, 84H), 2.84 (t, J = 7.5 Hz, 4H), 2.14 (m, 2H), 2.00 (s, 6H), 1.1–0.7 (m, 126H), 0.05–0.00 (m, 84H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.3, 138.6, 137.8, 136.1, 135.1, 134.2, 131.5, 127.9, 126.3, 124.7, 105.9, 104.8, 101.9–100.2, 82.7–79.4, 73.4–71.6, 62.6–60.3, 51.9, 38.0, 26.0–25.8, 23.4, 15.4, −4.8 – −5.1. MALDI-TOF: *m*/*z* calcd for $[M+Na]^+$ 4351.1, found 4353.5.

Bis(phenylthienyl)ethene-tethered β-cyclodextrin dimer 7

TBDMS-protected dimer **6** (245 mg, 0.09 mmol) was dissolved in trifluoroacetic acid (25 mL). The solution was stirred at room temperature for 10 min. The solvent was removed *in vacuo*. Methanol was added and evaporated *in vacuo* for azeotropic removal of any residual trifluoroacetic acid. The residue was dissolved in water and washed three times with diethyl ether. After freeze-drying dimer **7a** was obtained as a white solid in 94% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.12 (br., 2H), 7.82 (d, J = 8.4 Hz, 4H), 7.60 (d, J = 8.4 Hz, 4H), 7.39 (s, 2H), 4.89–4.76 (m, 12H), 4.67 (d, J = 6.6 Hz), 4.35 (br., 2H), 3.97 (br., 2H), 3.78–3.29 (m, 82H), 2.85 (br., 4H), 2.10 (m, 2H), 2.00 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.4, 139.8, 139.0, 138.5, 137.3, 136.4, 133.3, 129.6, 126.7, 126.0, 105.8, 103.9–102.8, 82.9–80.4, 74.9–73.0, 61.8–61.3, 53.3, 38.1, 23.2, 14.7. MALDI-TOF: *m*/*z* calcd for $[M+Na]^+$ 2753.9, found 2755.4.

TBDMS-protected bis(phenylthienyl)ethene-tethered β-cyclodextrin dimer 9

The same procedure as described for dimer **6** was used starting from **4** (28 mg, 0.06 mmol), HBTU (64 mg, 0.16 mmol) and DIPEA (48 μl, 0.28 mmol), and followed by addition of **8** (275 mg, 0.14 mmol). The crude product was purified by gradient column chromatography (ethyl acetate–ethanol–water 100 : 2 : 1 to 100 : 8 : 4) to give **9** (open form) as a white powder in 81% yield.^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.05 (br., 2H), 7.81 (d, J = 8.4 Hz, 4H), 7.58 (d, J = 6.6 Hz, 4H, H-Ar), 7.06 (s, 2H), 5.04–4.99 (m, 14H), 3.98–3.44 (m, 84H), 3.18 (d, J = 9.9 Hz, 2H), 2.80 (t, J = 7.5 Hz, 4H), 2.09–1.98 (m, 8H), 1.86 (m, 4H), 0.9–0.7 (m, 126H), 0.05–0.00 (m, 84H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.2, 143.6, 138.7, 136.9, 136.1, 135.3, 132.6, 127.9, 125.5, 125.1, 103.2–102.2, 82.0–80.1, 74.0–72.7, 71.8, 61.9–60.2, 37.8, 32.1, 26.1–24.5, 22.8, 14.8, −4.8 – −5.2. MALDI-TOF: *m*/*z* calcd for $[M+Na]^+$ 4467.1, found 4468.4.

Bis(phenylthienyl)ethene-tethered β-cyclodextrin dimer 10

Analogous to the procedure outlined for the deprotection of dimer **6**, dimer **9** (201 mg, 0.05 mmol) was deprotected using trifluoroacetic acid (25 mL) to give dimer **10** after freeze-drying as a white solid in 91% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.43 (br., 2H), 7.83 (d, J = 8.4 Hz, 4H), 7.64 (d, J = 8.4 Hz, 4H), 7.42 (s, 2H), 5.84–5.66 (m, 14H), 4.82–3.07 (m, 84H), 2.85 (t, J = 7.5 Hz, 4H), 2.06 (m, 2H), 1.92 (s, 6H), 1.77 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.1, 138.5, 137.4, 136.5, 135.2, 134.7, 133.3, 128.5, 125.9, 124.9, 102.6–101.9, 100.8, 82.7, 82.3–81.7, 81.3, 73.8–71.8, 70.3, 60.7–60.1, 36.5, 30.0, 23.9, 14.6. MALDI-TOF: *m*/*z* calcd for $[M+Na]^+$ 2871.8, found 2872.4.

UV-vis spectroscopy

UV-vis spectra were recorded on a Hewlett Packard HP 8452 UV-vis spectrophotometer. Irradiation experiments were performed *in situ* by irradiation of the samples in a 1 cm quartz cuvette in the UV-vis setup, using a 200 W mercury lamp with a 313 nm band-pass or a 460 nm high pass filter.

Preparation of the PSS mixtures

Solutions of the open form of the dimer in Millipore water (1 to 10 mM) in a quartz cuvette were irradiated with a high intensity mercury lamp for 10 to 15 min. UV-vis spectra of diluted samples were used to follow the photochromic reaction. Once the PSS was reached, samples were freeze dried to give the PSS mixture as a purple solid.

Calorimetry

Calorimetric titrations were performed at 25 °C using a Microcal VP-ITC titration microcalorimeter. Sample solutions were prepared in pure water (Millipore Q2) for dimer **7**, and in mixtures of DMSO and water (1 : 9, v:v) for dimer **10**. Titrations were performed by adding aliquots of a TSPP solution to the host solution. The titrant typically contained 0.1 to 1 mM of guest, while the cell solutions contained 10 to 100 μM of host. All calorimetric titrations were corrected for dilution heats by subtraction of the calorimetric dilution experiments from the calorimetric titration experiments. The titrations were analyzed with a least-squares curve fitting procedure. The thermodynamic data reported in Table 2 are based on three independent titrations.

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26 The complexation studies performed with the corresponding dithienylethene-tethered dimer with propyl spacers and TSPP also gave similar association constants for the open and closed forms of the dimer, but showed no pronounced differences in binding enthalpy (see ref. 13).

27 In this respect dimer **7** is inferior to the corresponding dithienylethene-tethered β-cyclodextrin dimer, which displayed a more substantial release of TSPP upon irradiation at 313 nm (see ref. 13).