CHEMISTRY
A EUROPEAN JOURNAL

ChemPubSoc
Europe

DOI: 10.1002/chem.201203319

Coordination-Driven Switching of a Preorganized and Cooperative Calix[4]pyrrole Receptor

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Abstract: The study of preorganization in receptors, particularly in cooperative receptors, and their reversible control by external stimuli is important for elucidating design strategies that can lead to increased sensitivity and external control of molecular recognition. In this work we present the design, synthesis, and operation of an asymmetric tetrathiafulvalene (TTF)–calix[4]pyrrole receptor appended with a pyridine moiety. ^{1}H NMR spectroscopy was employed to demonstrate that intramolecular complexation between the receptor and the pyridine moiety leads to a preorganized receptor. Absorption and ^{1}H NMR spectroscopy along with a computational investigation were used to demonstrate the ability of the receptor to complex the substrate 1,3,5-trinitrobenzene (TNB) and that the receptor can be reversibly modulated between negative and positive cooperativity by employing external stimuli in the form of Zn^{II}. Fitting procedures incorporating multiple datasets and fitting to multiple equilibria simultaneously have been employed to quantitatively determine the preorganization effects.

Keywords: allosterism · calix[4]pyrrole · molecular recognition · supramolecular chemistry · tetrathiafulvalene

Introduction

Allosteric controlled systems play a ubiquitous role in biological processes, in which events must be efficiently regulated in response to external stimuli. Well-known examples include the allosteric binding of oxygen to haemoglobin,[1] feed-back mechanisms of enzymes such as the inhibition of the biosynthesis of isoleucine in bacteria,[2] and positive stimulation of the lactose operon in the metabolism of *E. coli*.[2] A desire to mimic the key features of allosterism in artificial systems has long been a goal within the chemical community because it is appreciated that such efforts can lead to new advances in biomimetic design.[3] There are numerous examples[3b,4] of simple synthetic allosteric host–guest systems using ions or neutral guest as effectors. However, homotropic allosteric receptors[5] are considerably more difficult to achieve.[3b] In these systems the first binding event induces a nonlinear positive or negative response to subsequent guest binding.[5b,f] Some of the earliest systems were reported by Rebek,[6] Traylor,[7] and Tabushi.[8] In recent years Sessler,[5f,9] Setsune,[10] and Lee,[11] among others, have been successful in developing receptors that display a positive allosteric mechanism of binding.

Herein, we report the design, synthesis, and characterization of a tetrathiafulvalene (TTF)-substituted calix[4]pyrrole receptor **1** appended with a pyridine moiety and demonstrate how a coordination-driven switching mechanism can be utilized to control the function of the receptor between negative and positive cooperativity. The mechanistic scheme for the proposed coordination-driven switching of receptor **1** is illustrated in Scheme 1. Calix[4]pyrrole macrocycles are known to adopt four limiting conformations, namely the cone, partial cone, 1,2-alternate, and the 1,3-alternate conformation, in which the 1,3-alternate conformation is favored in solution. Theoretical studies[12] have provided insight into the conformational distribution of calix[4]pyrroles, for which the 1,3-alternate conformation is preferred, followed by the partial cone conformation, then the 1,2-alternate conformation and the least stabile conformation being the cone conformation. In solution, molecular receptor **1** exists predominantly in a preorganized[13] conformation (1,3-alternate) due to self-complexation between the host part (calix[4]pyrrole) and the guest part (pyridine) of the receptor. This leads to negative cooperativity of the receptor, whereby the first substrate (e.g., 1,3,5-trinitrobenzene, TNB) binds better (TNB⊂**1**) to the preorganized receptor than the second substrate (TNB_2⊂**1**), which has to compete with the pyridine guest for the second binding site. Addition of Zn^{II} results in a coordination-driven switching of the receptor from preorganized to random[13] conformation **1**·Zn^{II}. This event also switches the function of the receptor from negative to positive cooperativity. This can be observed as a weak binding of the first substrate and a strong binding of the second substrate.

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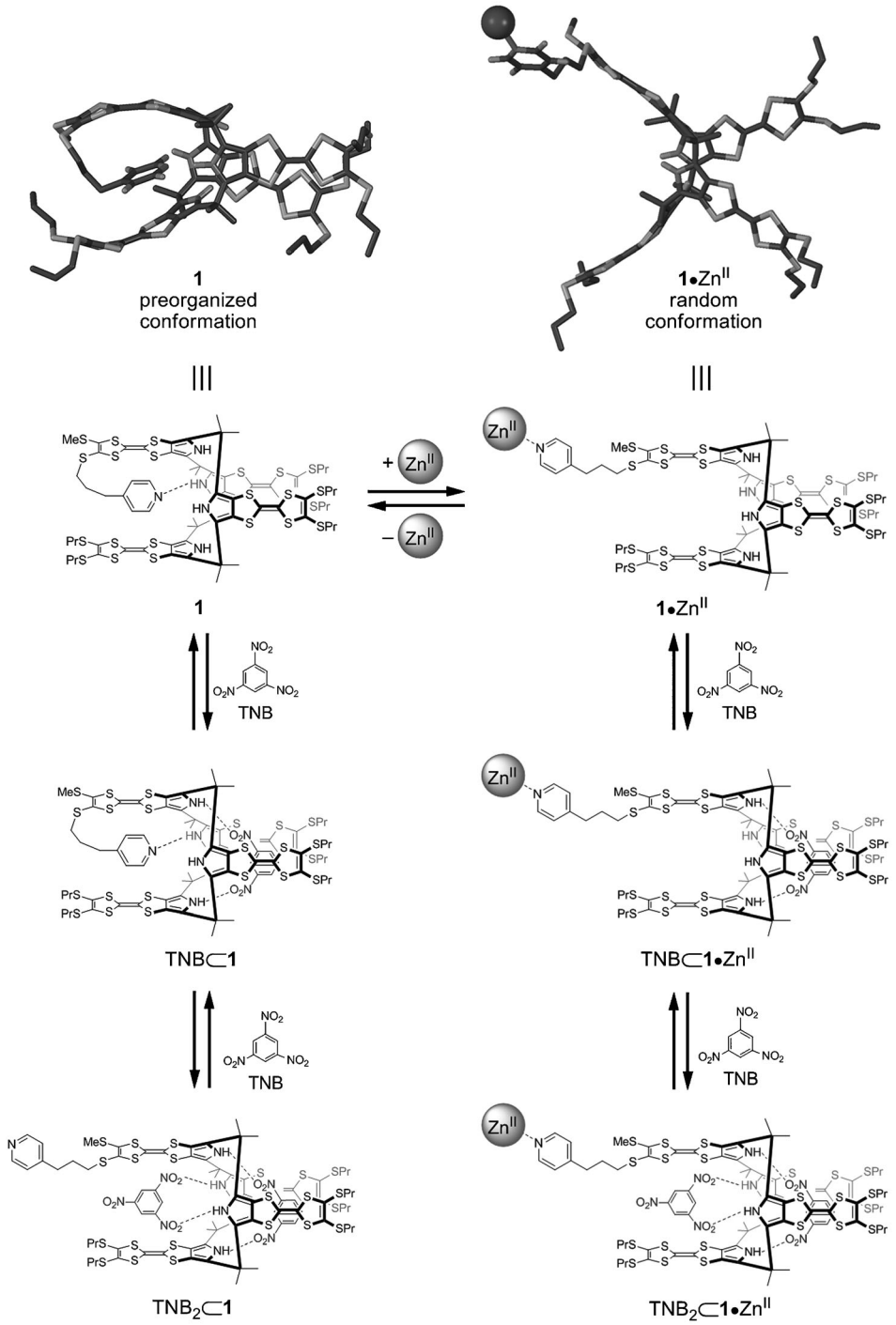
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201203319.



Scheme 1. Mechanistic scheme illustrating the Zn^{II} coordination-driven switching of receptor **1** and stepwise complexation of the analyte TNB with the receptor in its preorganized **1** and random **1**·Zn^{II} conformations, along with their energy-minimized structures.

Results and Discussion

To design a functional switching system, it is very important to know the relative energies of its conformations; therefore, we undertook a computational study on receptor **1** in its proposed preorganized (self-complexed) 1,3-alternate conformation and a conformationally rather mobile 1,3-alternate conformation, along with the intramolecular complexed 1,2-alternate and partial cone conformations to establish the most stable conformation (Figure 1). By employing the M05-2X/6-31G*[14] level of quantum chemical geometry optimization and single point energy calculations in CH_2Cl_2, we were able to show that the preorganized conformation of **1** is more stable than the random nonbinding conformation of **1** by 54.39 kJ mol^{-1} (Table 1). The stability of the proposed conformations follow the trend reported for calix[4]-pyrrole.[12a]

The receptor **1** was synthesized as outlined in Scheme 2. Treatment of 4-(2-cyanoethylthio)-5-methylthio-1,3-dithiolo-2-thione[15] (**2**) with CsOH·H_2O generated the corresponding thiolate, which was subsequently alkylated with 4-(3-bromopropyl)-pyridine[16] (**3**) affording the 4-methylthio-5-[3-(4-pyridyl)propylthio]-1,3-dithiolo-2-thione (**4**) in 66% yield. Triethyl phosphite mediated cross-coupling of **4** with *N*-tosyl-(1,3)-dithiolo[4,5-*c*]pyrrole-2-one[17] (**5**) in neat triethyl phosphite gave the tosyl-protected monopyrrolotetrathiafulvalene (MPTTF) derivative **6** in 52% yield. Removal of the tosyl protecting group was completed with an excess of NaOMe in a mixture of THF/MeOH to afford the MPTTF derivative **7** in 86% yield. Reaction of **7** and four equivalents of MPTTF derivative **8**[18] in a CH_2Cl_2/acetone mixture in the presence of tetrabutylammonium chloride (TBACl) and stoichiometric amounts of methanesulfonic acid (MSA) afforded a mixture of the desired asymmetric receptor **1** bearing one pyridine moiety in 16% yield, and the symmetric TTF–calix[4]pyrrole[5a,f,19] receptor **10** in 13% yield, after basic aqueous workup and column chromatographic purification.

The asymmetry of receptor **1** is readily seen in the ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K; Figure 1 and

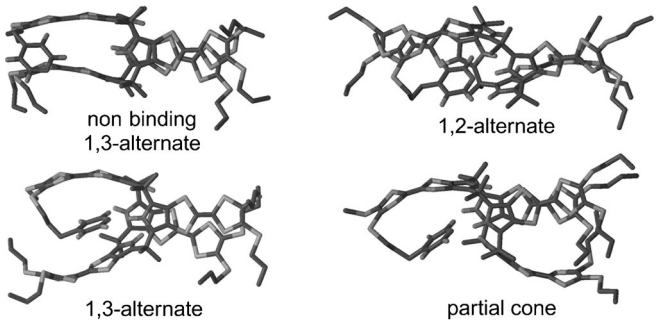
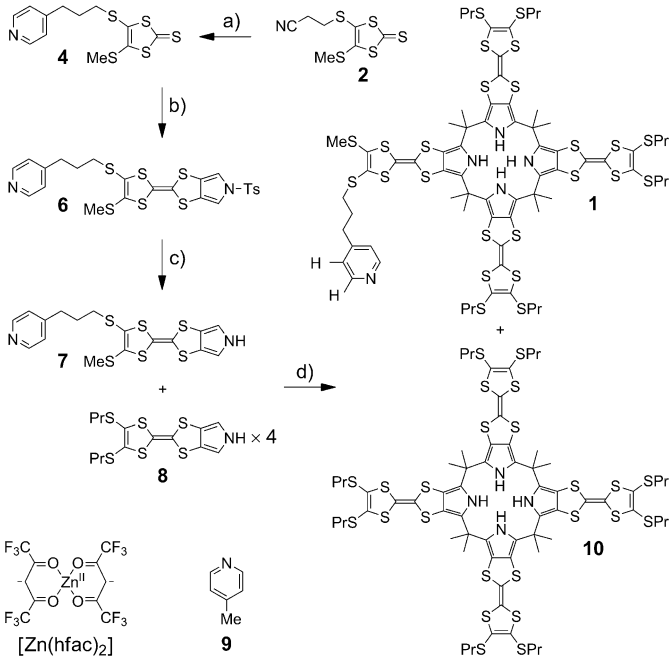


Figure 1. M05-2X/6-31G* geometry optimized structures of the random nonbinding conformation and the three possible intramolecular hydrogen-bonding conformations.

Table 1. M05-2X/6-31G*[14] single point energy calculations of the three intramolecular hydrogen-bonding conformations and the non-hydrogen-bonding random conformation.

Conformation	Energy [a.u.]	Relative energy [a.u.]	Relative energy [kJ mol^{-1}]
nonbinding 1,3-alternate	−12583.780520	–	–
1,3-alternate	−12583.801042	-2.052×10^{-2}	−54.39
1,2-alternate	−12583.779758	-7.62×10^{-4}	2.020
Partial cone	−12583.792694	-1.217×10^{-2}	−32.27



Scheme 2. Synthesis of the receptors **1** and **10** along with the chemical structures of model compound **9** and $[Zn(hfac)_2]$. Reagents and conditions: a) 4-(3-bromopropyl)-pyridine (**3**; 1.1 equiv) and then $CsOH{\cdot}H_2O$ (1.05 equiv), DMF/MeOH, RT, 24 h, 66%; b) *N*-tosyl-(1,3)-dithiolo[4,5-*c*]pyrrole-2-one (**5**; 0.5 equiv), $P(OEt)_3$, 130 °C, 2 h, 52%; c) NaOMe (13 equiv), THF/MeOH, 35 °C, 0.5 h, 86%; d) MSA, TBACl, CH_2Cl_2/acetone, RT, 1 h, then excess Et_3N, 16%.

Figure S7 in the Supporting Information), which shows four singlets resonating at $\delta = 7.04$, 7.13, 7.24, and 9.50 ppm that can be assigned to the four chemically nonequivalent NH protons. The *meso*-methyl groups are found to split into three singlets in the ratio 1:1:2 at $\delta = 1.53$, 1.54, and 1.56 ppm, respectively, and multiplets are found to arise from the six thiopropyl groups in the aliphatic part of the spectrum.

Initial evidence for the interactions between the host part (calix[4]pyrrole) and the guest part (pyridine) came from comparing the ^{1}H NMR spectrum of receptor **1** with those of the model compound 4-methylpyridine (**9**) and receptor **10** (Figure 2). In the spectrum of receptor **1** the signals corresponding to the resonances from the aromatic protons of the guest part (pyridine) are found to be upfield shifted ($\Delta\delta = 0.14$–0.47 ppm), relative to those of model compound **9**, due to shielding of the guest between two TTF subunits.[5a,20] Furthermore, one of the four NH ($\delta = 9.50$ ppm) protons was found to be highly downfield shifted ($\Delta\delta = 2.36$ ppm), relative to that of the NH protons of receptor **10**. This pronounced shift is caused by hydrogen bonding taking place between the NH proton of the host part of receptor **1** and the nitrogen lone pair of the guest part of receptor **1**. Further support for the proposed complexation came from NOESY NMR studies; in the case of receptor **1** the presence of cross-peaks corresponding to close noncovalent contacts between the host and guest part were observed (Figure 2). To establish whether the preferred complexation

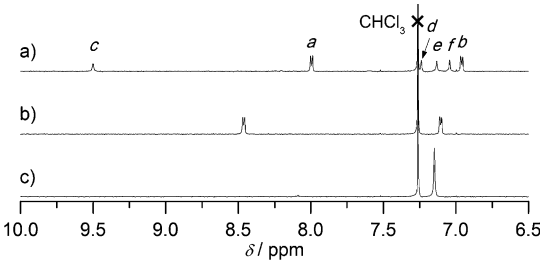


Figure 2. Partial ^{1}H NMR spectra (400 MHz, 298 K) recorded in $CDCl_3$ of a) receptor **1** (2.00 mM), b) **9** (2.00 mM), and c) receptor **10** (2.00 mM).

is intra- or inter-molecular in nature, a dilution experiment[21] was carried out and monitored by ^{1}H NMR spectroscopic analysis (Figure S17 in the Supporting Information). Upon dilution of a concentrated solution (30.0–0.10 mM) only small peak shifts ($\Delta\delta < 0.06$ ppm) were observed, indicating that intramolecular complexation (Scheme 1) is taking place. Collectively, these data provide support for a preorganization of receptor **1** that is affected by an intramolecular complexation between the guest part and one of the two host sites of the receptor. Importantly, the preorganization of the receptor leaves one of the two host sites vacant for substrate binding and, as a consequence of the positive homotropic allosteric nature of the receptor, an increased sensitivity for target substrate is to be expected.

The preorganization of receptor **1** can be "turned off" by a coordination-driven switching event using zinc(II)-hexafluoro-acetylacetonate $[Zn(hfac)_2]$. This is evident from a

^{1}H NMR titration experiment (400 MHz, $CDCl_3$, 298 K; Figure S18–S20 in the Supporting Information) in which stepwise addition of $[Zn(hfac)_2]$[22] results in a downfield shift ($\Delta\delta = 0.48$ ppm) of the aromatic pyridine protons H_a and H_b ($\delta = 6.96$ and 7.99 ppm, respectively). After addition of one equivalent of $[Zn(hfac)_2]$, the protons were found to resonate close to the same position as the complex formed between the model compound **9** and one equivalent of $[Zn(hfac)_2]$ (Figure S18 in the Supporting Information). This corresponds to the guest part of receptor **1** being complexed with zinc (**1**·Zn^{II})[23] outside the host part of the receptor, showing that a coordination-induced intramolecular decomplexation has taken place.[24]

To examine whether the receptor can be switched between its preorganized **1** and random **1**·Zn^{II} conformations, a ^{1}H NMR experiment (400 MHz, $CDCl_3$, 298 K; Figure 3)

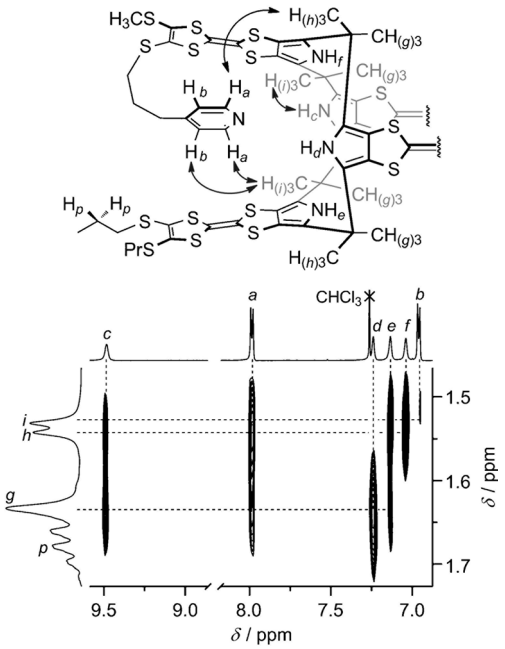


Figure 3. An expanded region of the 2D NOESY NMR spectrum of receptor **1**, showing the NOE between the aliphatic *meso*-Me protons and those of the aromatic pyridine and the four distinct NH protons. Some of the NOE signals have been omitted in the structure of receptor **1** for clarity.

was carried out that involved repeated addition of first $[Zn(hfac)_2]$ followed by aqueous extraction of zinc ions. Initially, the preorganized receptor **1** was converted into the random conformation (Figure 4b) by addition of one equivalent of $[Zn(hfac)_2]$. Extraction[25] of the zinc ions into an aqueous phase allows the system to revert to the preorganized conformation (Figure 4c). Repeated addition of $[Zn(hfac)_2]$ and aqueous extraction resulted in a continuation of the controlled switching of the receptor, as can be inferred from Figure 4.

The binding event between our substrate of choice, namely 1,3,5-trinitrobenzene (TNB), and the receptor in its two limiting conformations (Scheme 1) were studied by

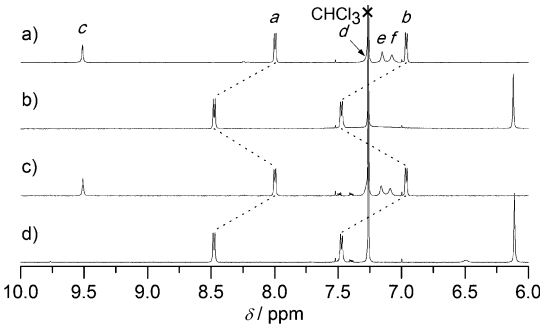


Figure 4. Partial ^{1}H NMR spectra (400 MHz, 298 K) recorded in $CDCl_3$ of a) **1** (1.0 mM), b) **1**+$[Zn(hfac)_2]$ (1.0 equiv), c) after washing the solution in b) with H_2O, and d) after addition of $[Zn(hfac)_2]$ (1.0 equiv) to the solution in c).

using ^{1}H NMR spectroscopy (400 MHz, $CDCl_3$, 298 K). Addition of up to one equivalent of TNB to a solution of receptor **1** caused a large downfield shift ($\Delta\delta = 0.23$–0.41 ppm; Figure 5a–d and Figure S35 in the Supporting Information)

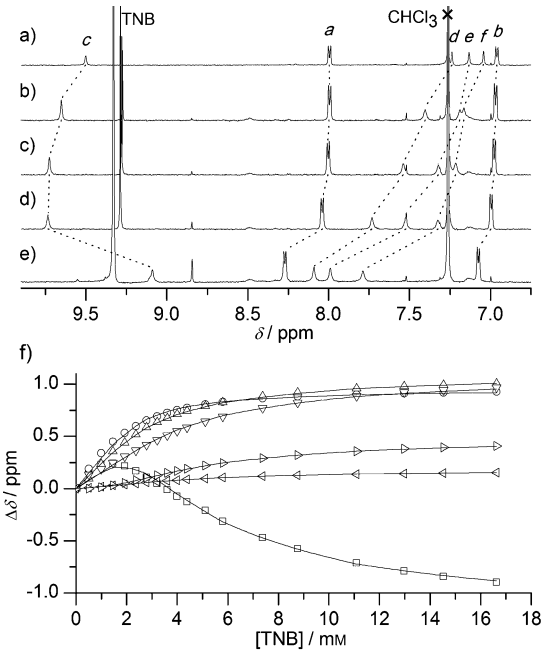


Figure 5. Partial ^{1}H NMR spectra (400 MHz, 298 K) recorded in $CDCl_3$ of **1** with addition of an increasing amount of TNB a) receptor **1** (2.0 mM), b) with TNB (0.25 equiv), c) with TNB (0.50 equiv), d) with TNB (1.0 equiv), and e) with TNB (3.7 equiv). f) Changes in chemical shift values of the aromatic protons H_a (▷), H_b (◁), H_c (□), H_d (○), H_e (△) and H_f (▽) together with the simultaneously fitted binding isotherm.

of the four NH protons as expected. However, when exceeding one equivalent of TNB, an upfield shift of the NH_c proton and a continued downfield shift of the remaining three NH protons was observed (Figure 5e). The upfield shift of the NH_c proton is rationalized as an increased competition between the pyridine "guest" and the substrate TNB for the second binding site (Scheme 1). In the second

experiment, addition of TNB to the receptor in the random conformation **1**·Zn^{II} was carried out under identical conditions (Figure S37 in the Supporting Information). The datasets for the receptor in its preorganized and random conformations, and the model receptor **10** titrated with TNB were fitted with multiple equilibria[26] (Figure 5 f and Figure S36, S38 and S39 in the Supporting Information) using the chemical shift changes of the NH protons and the aromatic pyridine protons H_a and H_b, using a downhill simplex method[27] with a least squares metric.

The fitting procedure for the Connors[26] 2:1 is based on that the actual host and guest concentrations ([H] and [G]) can be calculated from the starting concentrations ($[H]_0$ and $[G]_0$) via:

$$[H]_0 = (1 + K_a)[H] + (K_b + K_aK_d)[H][G] + K_bK_c[H][G]^2$$

$$[G]_0 = [G] + (K_b + K_aK_d)[H][G] + 2K_bK_c[H][G]^2$$

with the condition that $0 \le [H] \le [H]_0$ and $0 \le [G] \le [G]_0$. The chemical shift for a site (i) is obtained from:

$$\delta_i = \delta_{i,0} + K_a\frac{[H]}{[H]_0}\delta_{i,a} + K_b\frac{[H][G]}{[H]_0}\delta_{i,b} + 2K_bK_c\frac{[H][G]^2}{[H]_0}\delta_{i,c} + K_aK_d\frac{[H][G]}{[H]_0}\delta_{i,d}$$

for $i = a \ldots f$; and where $\delta_{i,0}$ are the chemical shift of the site at infinite dilution and $\delta_{i,a} \ldots \delta_{i,d}$ are the chemical shifts of the different complexes (Scheme 3). In the complexation event between the preorganized receptor **1** and TNB, multiple equillibria have been taken into consideration, in which the self-complexed preorganized receptor is in equilibrium with the noncomplexed conformation (Scheme 3), which also contributes to the complexation of TNB. Based on the fitted data, we were able to delineate the complex behavior of receptor **1** (Scheme 3). The fitted values of the macroscopic constants K_1 and K_2 (Table 2) show that receptor **1** in its preorganized conformation has a 291-fold increase in the first complexation (K_1) with TNB when compared with the random conformation. In the second complexation (K_2) with TNB, the competition between the pyridine "guest" and the TNB substrate results in a 38-fold decrease of the binding. These results demonstrate that a preorganization of the receptor—due to intramolecular complexation—leads to a large increase of the first substrate binding, and a decrease of the second substrate binding.

Table 2. Binding constants[a] [K_1 and K_2 (M^{-1})] corresponding to the interactions between the receptors **1**, **1**·Zn^{II}, and **10** and the analyte TNB as determined by ^1H NMR spectroscopy[b] at 298 K in $CDCl_3$ and Hill coefficient[c] (n) for the cooperative binding of TNB, determined by absorption spectroscopy[b] at 298 K in CH_2Cl_2.

Receptor	n	K_1	K_2	K_2/K_1
1	0.86	5.8×10^3	3.2×10^2	0.05
1·Zn^{II}	1.27	20	1.2×10^4	600
10	1.24	4.6×10^2	1.6×10^3	3.49

[a] Estimated errors are $<15\%$. [b] Receptors **1**, **1**·Zn^{II}, and **10** were titrated with a concentrated TNB solution containing the corresponding receptor at its initial concentration. [c] Estimated errors are $<10\%$.

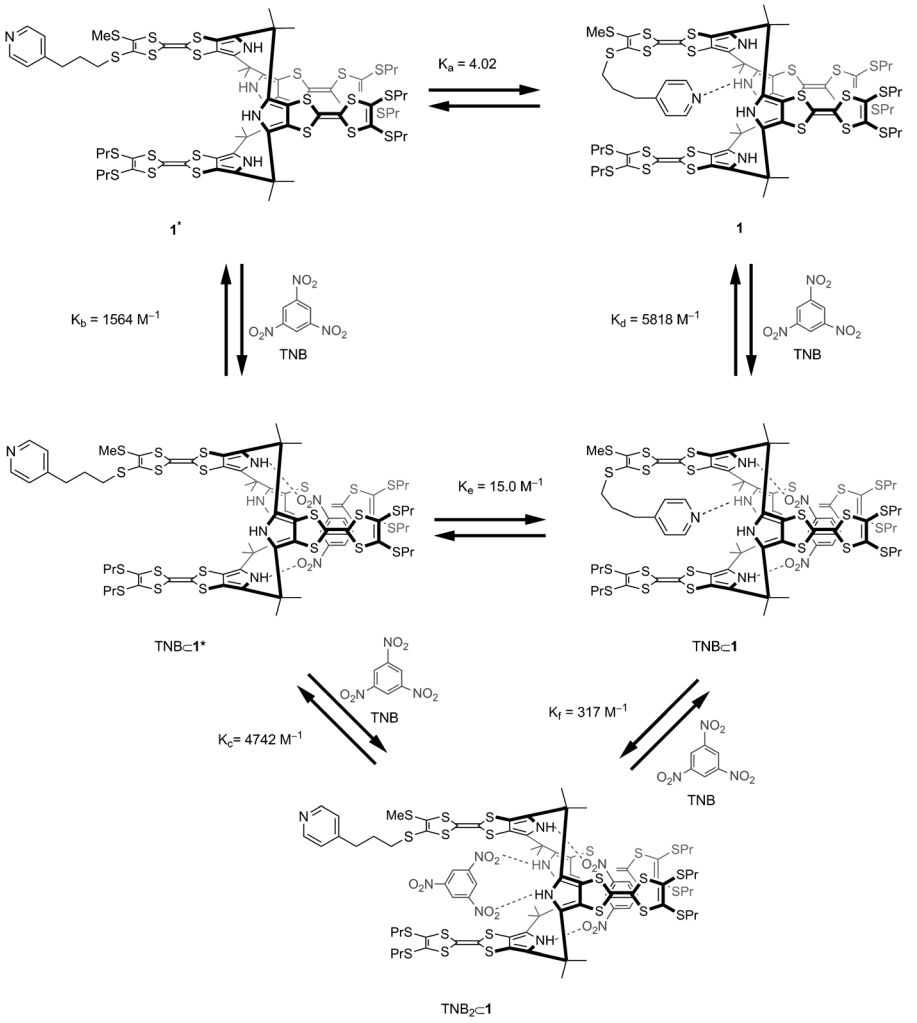
The complexation between receptor **1**—in its two limiting conformations—and the substrate TNB were also investigated in CH_2Cl_2 by using absorption spectroscopy. Addition of ten equivalents of TNB to a solution of **1** (Figure 6 b) resulted in an immediate color change from yellow to green, due to the presence of charge transfer (CT) interactions between the electron-donating TTF subunits and the electron-deficient substrate. The intensity of the CT band centered at 680 nm is approximately half that formed between **10** and ten equivalents of TNB (Figure 6 f). However, addition of one equivalent of [$Zn(hfac)_2$] to the solution of receptor **1** containing ten equivalents of TNB resulted in a coordination-driven intramolecular decomplexation event, which was observed as an increase of the CT band (Figure 6 d) to almost the same intensity as that of the model receptor **10** containing ten equivalents of TNB. This is in agreement with **1** being preorganized, having only one free binding site for TNB, whereas **1**·Zn^{II} and **10** has two binding sites. Fitting the titration data to the Hill equation[28] gave the Hill coefficients (Table 1, and Figure S45 and Table S1 in the Supporting Information) with a good correlation coefficient ($R^2 > 0.999$). From Table 1 it can be seen that a coordination-driven switching of the receptor between its two limiting conformations results in switching between positive and negative cooperativity.

Conclusion

We have demonstrated the operation of a molecular receptor **1** that—in the presence or absence of Zn^{II}—adjusts its ability to complex guest molecules. In the absence of Zn^{II} a self-complexation between the pyridine guest part and the calix[4]pyrrole host part occurs, resulting in a higher level of preorganization of the receptor. In this conformation the receptor shows negative cooperativity with initial high binding towards TNB guest molecules. Addition of Zn^{II} results in a coordination-induced switching of the receptor into its random conformation, in which it shows positive cooperativity towards TNB.

Experimental Section

General methods: All chemicals were purchased from Sigma–Aldrich and used as received unless indicated otherwise. Compounds **2**,[15] **3**,[16] **5**,[17] and **8**[18] were synthesized according to literature procedures. All reactions were carried out under an atmosphere of anhydrous nitrogen. THF and MeOH were distilled from sodium-benzophenone and Mg, respectively, immediately prior to use. CH_2Cl_2 was distilled immediately prior to use and, if necessary, stored over molecular sieves (4 Å). $(EtO)_3P$ was distilled prior to use and stored over molecular sieves (4 Å). Acetone was stored over Drierite for at least three days. Analytical thin-layer chromatography (TLC) was performed by using aluminum sheets precoated with silica gel 60F (Merck 5554), which were inspected by UV-light (254 nm) prior to development with I_2 vapor. Column chromatogra-



Scheme 3. Mechanistic scheme illustrating the equilibria present in the complexation of receptor 1 and TNB. K_e is calculated based on K_a, K_b and K_d. K_f is calculated based on K_c and K_e.

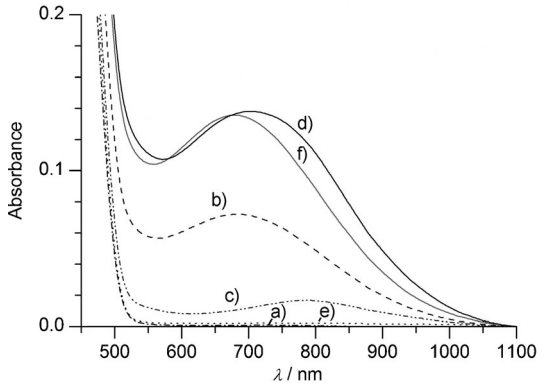


Figure 6. Absorption spectra recorded in CH_2Cl_2 at 298 K of a) receptor **1** (0.20 mM; –•–•), b) **1**+TNB (10 equiv; – – –), c) receptor **1**·Zn^{II} (0.20 mM; –••••–), d) **1**·Zn^{II}+TNB (10 equiv; black ——), e) receptor **10** (0.20 mM; •••••), and f) **10**+TNB (10 equiv; dark grey ——).

phy was performed by using silica gel 60F (Merck 9385, 0.040–0.063 mm). Melting points (mp) were determined with a Büchi melting point apparatus and are uncorrected. 1D and 2D NMR spectra were recorded with a Bruker AVANCE III 400 MHz spectrometer; ^{1}H NMR spectra were recorded at 400 MHz (298 K) and ^{13}C NMR was recorded at 100 MHz (298 K). Low temperature ^{1}H NMR spectra were recorded with a Varian Unity 500 MHz spectrometer. The NMR samples were dissolved in deuterated solvents purchased from Cambridge Isotope Labs or Sigma–Aldrich, and TMS or the residual solvent were used as internal standard. Solvent signals were assigned according to Nudelman et al.,[29] IR spectra were recorded with a Perkin-Elmer 580 spectrophotometer. Isothermal titration calorimetry was performed with a VP-ITC from MicroCal. Matrix-assisted laser-desorption (MALDI)/ionization mass spectrometry was performed with a Bruker Autoflex III Smartbeam (MALDI-TOF) utilizing a 2,5-dihydroxybenzoic acid (DHB) matrix. Electrospray ionization mass spectra (HR-ESI) were recorded with a Thermo Finnigan MAT SSQ710 single stage quadropole. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Georgia, USA.

Synthesis of 4-methylthio-5-(3-(4-pyridyl)propylthio)-1,3-dithiolo-2-thione (4): 4-(2-Cyanoethylthio)-5-methylthio-1,3-dithiolo-2-thione[15] (**2**, 8.87 g, 33.2 mmol) and 4-(3-bromopropyl)pyridine[16] (**3**, 7.36 g, 36.8 mmol) were dissolved in anhydrous DMF (300 mL) and degassed with N_2 for 15 min, before a solution of $CsOH{\cdot}H_2O$ (5.89 g, 35.1 mmol) in anhydrous MeOH (5 mL) was added dropwise over 1 h. The reaction was stirred at RT overnight before being concentrated in vacuo to a red oil, which was redissolved in CH_2Cl_2 (400 mL) and washed with brine (3×200 mL) and H_2O (2×200 mL) before being dried ($MgSO_4$). The solution was evaporated onto silica and purified by column chromatography (800 mL SiO_2, 8 cm Ø; CH_2Cl_2/MeOH 49:1 v/v). The orange band (R_f=0.3) was collected and concentrated to give compound **4** (7.26 g, 66%) as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ=2.01 (quintet, J=7.0 Hz, 2H), 2.50 (s, 3H), 2.78 (t, J=7.0 Hz, 2H), 2.86 (t, J=7.0 Hz, 2H), 7.13 (d, J=6.0 Hz, 2H), 8.52 ppm (d, J=6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 298 K, TMS): δ=19.2, 29.9, 33.5, 35.9, 123.8, 131.4, 140.3, 149.5, 149.9, 210.7 ppm; HRMS (ESI): m/z: calcd for $C_{12}H_{14}NS_5^+$ 331.9724 [M^+]; found 331.9739; IR (KBr): $\tilde{\nu}$=3065 (w), 3023 (w), 2917 (m), 1673 (w), 1600 (s), 1414 (s), 1067 cm^{-1} (s); elemental analysis calcd (%) for $C_{12}H_{13}NS_5+1/3\times CH_2$: C 44.06, H 4.10, N 4.17, S 47.68; found: C 44.07, H 4.05, N 4.30, S 47.31.

Synthesis of monopyrrolotetrathiafulvalene 6: *N*-Tosyl-(1,3)-dithiolo[4,5-*c*]pyrrole-2-one[17] (**5**, 3.15 g, 10.1 mmol) was dissolved in triethyl phosphite (50 mL) and heated to 130 °C, whereafter 4-methylthio-5-(3-(4-pyridyl)propylthio)-1,3-dithiolo-2-thione (**4**) was added in portions after 0 min (4.98 g, 14.9 mmol), 7 min (1.15 g, 3.45 mmol) and 14 min (0.96 g, 2.88 mmol). The reaction mixture was stirred for 2 h before being cooled to RT and concentrated in vacuo to a dark-yellow oil, which was redissolved in CH_2Cl_2 (200 mL) and washed with brine (200 mL) and H_2O (3×200 mL) before being dried ($MgSO_4$). After evaporation of the solvent, the dark-yellow oil was purified by column chromatography

(500 mL SiO_2, 6 cm Ø; CH_2Cl_2/MeOH 49:1 v/v). The yellow band (R_f = 0.4) was collected and concentrated to give **6** (3.14 g, 52%) as a yellow solid (m.p. 100–105 °C). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, TMS) δ = 1.96 (quintet, J = 7.0 Hz, 2H), 2.41 (s, 3H), 2.42 (s, 3H), 2.76 (t, J = 7.0 Hz, 2H), 2.79 (t, J = 7.0 Hz, 2H), 6.94 (s, 2H), 7.11 (dd, J = 5.9, 1.6 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 8.50 ppm (dd, J = 5.9, 1.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 298 K, TMS) δ = 19.3, 21.8, 30.0, 33.6, 35.4, 111.4, 114.6, 117.5, 124.1 (two signals), 127.1, 127.2, 127.2, 130.3, 130.8, 135.5, 145.7, 150.0 ppm, two signals are overlapping or missing; HRMS (ESI): m/z: calcd for $C_{24}H_{23}N_2O_2S_7^+$ 594.9799 [M^+]; found 594.9806; IR (KBr): $\tilde{\nu}$ = 3154 (w), 3132 (w), 3064 (w), 2985 (m), 2916 (m), 1599 (s), 1493 cm^{-1} (m); elemental analysis calcd (%) for $C_{24}H_{23}N_2O_2S_7$: C 48.45, H 3.73, N 4.71, S 37.73; found: C 48.32, H 3.77, N 4.67, S 37.54.

Synthesis of monopyrrolotetrathiafulvalene 7: Monopyrrolotetrathiafulvalene **6** (1.35 g, 1.78 mmol) was dissolved in a mixture of anhydrous THF (150 mL) and anhydrous MeOH (50 mL) and degassed with N_2 for 20 min. Sodium methoxide (25 wt% in MeOH, 1.22 g, 22.7 mmol) was added in one portion, and the reaction mixture was heated to 35 °C. After 1 h, the reaction was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (2×100 mL). The combined organic phases were washed with H_2O (2×100 mL) and dried ($MgSO_4$). After evaporation of the solvent, the dark-yellow oil was purified by column chromatography (500 mL SiO_2, 6 cm Ø; CH_2Cl_2/MeOH 49:1 v/v). The yellow band (R_f = 0.3) was collected and concentrated to give **7** (678 mg, 86%) as a yellow solid (m.p. 157–158 °C). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ = 1.98 (quintet, J = 7.2 Hz, 2H), 2.43 (s, 3H), 2.77 (t, J = 7.2 Hz, 2H), 2.81 (t, J = 7.2 Hz, 2H), 6.61 (d, J = 2.8 Hz, 2H), 7.13 (dd, J = 4.5, 1.5 Hz, 2H), 8.33 (br. s, 1H), 8.50 ppm (d, J = 4.5, 1.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 298 K, TMS): δ = 19.2, 29.9, 33.5, 35.3, 109.8, 119.9, 119.9, 121.3, 124.0, 130.7, 149.9, 150.1 ppm, three signals are overlapping or missing; HRMS (ESI): m/z: calcd for $C_{17}H_{17}N_2S_6^{\cdot+}$ 440.9710 [M^+]; found 440.9710; IR (KBr): $\tilde{\nu}$ = 3401 (s), 3122 (s), 3058 (m), 2919 (s), 2857 (s), 1604 (s), 1417 cm^{-1} (s); elemental analysis calcd (%) for $C_{17}H_{17}N_2S_6$: C 46.33, H 3.66, N 6.36, S 43.65; found: C 46.54, H 3.67, N 6.36, S 43.46.

Synthesis of tetrathiafulvalene–calix[4]pyrrole 1: A solution of monopyrrolotetrathiafulvalene **7** (345 mg, 0.783 mmol), monopyrrolotetrathiafulvalene **8**[18] (915 mg, 2.34 mmol), and tetrabutylammonium chloride (0.227 g, 0.817 mmol) in anhydrous CH_2Cl_2 (300 mL) and anhydrous acetone (150 mL) was degassed with argon for 20 min, before methanesulfonic acid (1.4 mL, 2.1 g, 30 mmol) was added to the yellow solution. The reaction mixture was stirred at RT for 45 min, whereupon triethylamine (2.0 mL, 1.5 g, 14 mmol) was added slowly. H_2O (100 mL) was added and the solution was extracted with CH_2Cl_2 (3×100 mL). The combined organic phases were washed with H_2O (5×100 mL) before being dried ($MgSO_4$). After evaporation of the solvent, the dark-yellow oil was purified by column chromatography (600 mL SiO2, 6 cm Ø; CH_2Cl_2 until the first band eluted, then CH_2Cl_2/MeOH 33:1 v/v). The first band containing the symmetric tetrathiafulvalene–calix[4]pyrrole[5a,f,19] **10**, was isolated. The yellow band (R_f = 0.3) was collected and concentrated to give **1** (220 mg, 16%) as an orange solid (m.p. 124–126 °C). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ = 0.99–1.05 (m, 18H), 1.53 (s, 6H), 1.54 (s, 6H), 1.56 (s, 12H), 1.63–1.70 (m, 12H), 2.01 (quintet, J = 7.0 Hz, 2H), 2.49 (s, 3H), 2.73 (t, J = 7.0 Hz, 2H), 2.74–2.83 (m, 14H), 6.96 (dd, J = 5.9, 1.3 Hz, 2H), 7.04 (s, 1H), 7.13 (s, 1H), 7.24 (s, 1H), 7.99 (dd, J = 5.9, 1.3 Hz, 2H), 9.50 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 298 K, TMS): δ = 13.2, 19.3, 23.2, 27.8, 28.0, 28.1, 28.4, 29.7, 32.7, 36.0, 36.1, 36.2 (two signals), 38.1, 38.2, 109.2, 111.9, 112.2, 114.6, 115.1, 115.3, 115.7, 116.5, 123.8, 127.1, 127.2 (two signals), 127.4, 128.0, 128.3, 129.1, 130.6, 130.9, 148.4, 150.0 ppm, signals are overlapping and/or missing; HRMS (ESI): m/z: for $C_{71}H_{83}N_5S_{24}^{\cdot+}$ 1772.9940 [M^+]; found 1772.980; IR (KBr): $\tilde{\nu}$ = 3401 (s), 3122 (s), 3058 (m), 2919 (s), 2857 (s), 1604 (s), 1417 cm^{-1} (s); elemental analysis calcd (%) for $C_{71}H_{83}N_5S_{24}+C_5H_{10}$: C 49.44, H 5.08, N 3.79, S 41.68; found: C 49.14, H 5.03, N 3.75, S 41.22.

Acknowledgements

This work was supported by the Danish Natural Science Research Council (FNU, projects no. 272-08-0047), the Center for Fundamental Living Technology (FLinT), the University of Southern Denmark (SDU), and the Villum Foundation.

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[13] The random conformation exists in an equilibrium of the four limiting conformations: 1,3-alternate, partial cone, 1,2-alternate, cone, and all conformations in between. The preorganized conformation consists of all the same conformations. However, the equilibrium is shifted in favor of the 1,3-alternate conformation, due to the intramolecular complexation between the guest (pyridine)and the host (calix[4]pyrrole) parts of the receptor.
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[20] For the intermolecular complex **10**⊂**9**$_2$ small shifts were observed in the ^{1}H NMR spectrum (Figure S23 in the Supporting Information).

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[22] Association constants for Zinc hexafluoroacetylacetonate [Zn(hfac)$_2$] and 4-methylpyridine **9** are $K_1 = 4.27 \times 10^4\,\text{M}^{-1}$ and $K_2 = 2.95 \times 10^3\,\text{M}^{-1}$ based on ITC titrations (see the Supporting Information).

[23] 1.1 equivalents of [Zn(hfac)$_2$] were generally added to rule out the formation of a **1**$_2$·Zn^{II} complex.

[24] The labile NH protons were not observed under these slightly basic conditions.

[25] The ^{1}H NMR sample was extracted with water, followed by separation of the organic phase and drying over $MgSO_4$ before a subsequent spectrum was recorded.

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Received: September 17, 2012
Published online: January 7, 2013

