

Keywords: Molecular recognition / Thermodynamics / Lower-rim substitution / Calixarenes

The complex stability constants (K_D) and thermodynamic parameters (ΔH° and TΔS°) for the 1:1 complexation of two water-soluble calixarenes, p-sulfonatocalix[4]arene (SC4A) and 5,11,17,23-tetrasulfonato-25,26,27,28-tetrakis(n-butyl)calix[4]arene (SC4A-Bu), with organic ammonium cations and neutral spherical organic molecules, have been determined by means of isothermal titration calorimetry (ITC) in aqueous solutions at 298.15 K. The obtained results indicate that, upon complexation with these guests by SC4A-Bu, the enthalpy changes become less favorable, whereas the entropy changes become more favorable relative to SC4A complexation. These differences can be attributed to differential degrees of desolvation and removal of high-energy water as well as the change in conformation or conformational degrees of freedom upon complexation. The calorimetric investigations, accompanied by 1H NMR and UV/Vis spectroscopy and X-ray crystallography provide a thermodynamic explanation for the different complexation behavior of SC4A and SC4A-Bu towards charged and neutral organic guests. Binding ability and molecular selectivity are discussed from the viewpoint of the conformational geometry and electronic properties of hosts and guests.

Introduction

p-Sulfonatocalix[n]arenes (SCnAs) have become popular in the fields of molecular recognition/sensing,[1] crystal engineering,[2,3] catalysis,[4] enzyme mimics/enzyme assays,[5,6] and medicinal chemistry.[7–9] The diversity of the applications employing SCnAs is due to a number of favorable properties, including their water solubility, preorganized framework, fascinating binding ability, and especially biological compatibility.[10] As a class of versatile macrocyclic hosts, the SCnA family can efficiently include various guest molecules inside their three-dimensional, flexible, π-electron-rich cavities. Moreover, the upper-rim sulfonate groups provide additional anchoring points, which enable SCnA to display especially strong binding ability and high molecular selectivity towards a variety of organic ions.[11–14] Although the SCnA homologues have been extensively studied,[4,15–18] their binding behavior as a function of lower-rim modification has only been addressed to a small extent. For example, Arena and co-workers reported that lower-rim modification of SC4A with carboxylate groups provides specific preorganization of the host cavity, which affects its selectivity towards organic ammonium ions.[12,18,19] Da Silva et al. have built upon this concept by examining the selective affinities of lower-rim-modified SCnAs towards amino acids and bovine serum albumin.[17] Studies of SCnAs at different pH ranges have shown that SCnA macrocycles act as pH-dependent receptors owing to their protonatable phenolate groups.[11,20] The modification of phenolic hydroxy groups does, however, not only affect

![Figure 1. Structures of the calixarenes and guests employed in this paper.](image-url)

---

[a] Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P. R. China
E-mail: yuliu@nankai.edu.cn

[b] School of Engineering and Science, Jacobs University Bremen, Campus Ring 1, 28759 Bremen, Germany
E-mail: w.nau@jacobs-university.de

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200901361.
the pH-dependent binding behavior but also the π-electron densities of the cavities as well as the presumed calixarene conformation.\[12\]

In an attempt to clarify the influence of lower-rim modification on the binding ability and molecular selectivity of SCnAs, we have systemically investigated and compared the binding behavior and thermodynamics of SC4A and SC4A-Bu (Figure 1) upon complexation with organic ammonium cations as well as neutral spherical organic molecules. Specifically, we have compared the binding behavior of SC4A-Bu with the smallest and more commonly used, conformationally constrained analogue of the SCnA family, SC4A.

Results and Discussion

Structural Difference Between SC4A and SC4A-Bu

As can be seen from the $^1$H NMR spectra (Figure 2), the flexibility of the calixarene framework changes characteristically upon lower-rim modification with butyl groups. The bridging methylene protons of the parent SC4A show a single peak (Figure 2a), which broadens or splits into a doublet upon complexation with guest molecules\[21,22\] indicating that SC4A itself has a flexible conformation in aqueous solution but becomes a rigidified cone upon complexation. However, the $^1$H NMR spectrum of SC4A-Bu presents two peaks, assigned to the axial and equatorial bridge methylene protons (Figure 2b), where the $\Delta\delta_{ax-eq}$ value equals 1.0 ppm. This reveals that the framework of SC4A-Bu is much more rigid than that of the parent SC4A species, because steric hindrance from the lower-rim butyl substituents restrains a random flipping motion of the aromatic rings. Moreover, the signals of the aromatic protons of SC4A-Bu undergo a slight but distinct upfield shift compared to those of SC4A. This observation suggests that the ring-current effect of SC4A-Bu is not as strong as that of SC4A, corresponding to a lower π-electron density of the SC4A-Bu cavity. This can be rationalized in terms of the protonation constants of SC4A\[23,24\] which predict that at least one phenol group is deprotonated near neutral pH and, thus, is strongly electron-donating.

The different conformational preferences of SC4A and SC4A-Bu were also corroborated by X-ray crystallography. SC4A-Bu assumes a pinched-cone conformation of $C_{2v}$ symmetry in the solid state, having distances between the two pairs of sulfonate groups (S···S) of 5.15 Å and 12.95 Å, in contrast to the reported $C_{4v}$-symmetrical cone conformation of SC4A with equal S···S distances of about 10 Å (Figure 3).\[25\] Previous X-ray studies have indicated that in the absence of an organic guest molecule, SC4A can accommodate water molecules inside its cavity\[26\] whereas no water molecules can be accommodated in the pinched, conical cavity of SC4A-Bu, according to the obtained crystal structure. This suggests that, compared to the parent SC4A, the inner cavity of SC4A-Bu has a higher propensity to undergo π–π interactions. The observed differences in the single-crystal structures of SC4A and SC4A-Bu support the different binding abilities of the two hosts discussed in the thermodynamic section below.

Binding with Organic Ammonium Cations

Organic ammonium cations are the most commonly employed model guests for SCnAs. They show high complex stabilities on the order of 10$^5$ M$^{-1}$ due to strong ionic interactions with the sulfonate groups of the hosts, frequently accompanied by C–H–π interactions with the π-electron-rich cavity of the calixarene.\[27\] Herein, two quaternary ammonium cations, trimethyl(phenyl)ammonium chloride (TMA) and tetramethylammonium chloride (TEMA), were selected to compare the inclusion complexation of SC4A and SC4A-Bu. The complexation of SC4A and SC4A-Bu with TMA was measured at two pH values (pH = 2.0 and 6.0). According to the protonation constants of SC4A\[23,24\]...
at least one phenol group is deprotonated at pH = 6.0, whereas all the phenol groups are protonated at pH = 2.0. The thermodynamic results show that the complexation of SC4A-Bu with TMA gives not only comparable stability constants but also similar enthalpy and entropy changes at pH = 2.0 and 6.0 (Table 1), which is quite different from the case of SC4A. This demonstrates that the pH dependence of SC4A-Bu is much weaker than that of the SC4A analogue, because the lower-rim phenolic hydroxy groups are substituted and no longer ionizable. Additionally, its binding ability to organic ammonium ions (TMA and TEMA) decreases by up to three orders of magnitude relative to that of the parent SC4A analogue, resulting in only weak to medium stability constants of around 500–1000 M$^{-1}$. A close inspection of the calorimetric data revealed that a large difference in the enthalpy term is responsible for the decrease in the stability constants upon going from SC4A to SC4A-Bu, even though the complexation of SC4A-Bu is accompanied by favorable entropy changes. As mentioned above, the π-electron density of SC4A-Bu is on average lower than that of SC4A, resulting in weaker van der Waals, π–π, and C–H–π interactions. In addition, since the crystal structures indicate that water molecules are complexed by SC4A but not by SC4A-Bu, it is likely that less (or no) high-energy water is expelled during complexation by SC4A-Bu than by SC4A, which could also contribute to the observed enthalpy differences. The more favorable entropy change for SC4A-Bu is attributed to at least two factors. First, SC4A-Bu undergoes a smaller loss of conformational freedom upon going from the structured pinched cone to the conical host–guest complex than does SC4A on going from a flexible cone to the rigidified cone complex. Second, the cavity of SC4A-Bu becomes tighter than that of SC4A, as evidenced by the crystal structures which, in combination with the lower π-electron density, results in a shallower inclusion, also reflected in the lower affinity constants. In other words, the organic cations presumably interact more electrostatically with the sulfonate groups than through C–H–π interactions with the aryl rings, which may cause a larger degree of desolvation of the anionic groups, corresponding to an increased entropic driving force for complexation by SC4A-Bu relative to that by SC4A.

Surprisingly, the relative affinities of SC4A and SC4A-Bu towards the TMA and TEMA pair are inverted, revealing a different selectivity; whereas SC4A binds about two times stronger with TEMA, SC4A-Bu binds two times more tightly with TMA. The complexation of SC4A-Bu with TEMA is mainly entropy-driven with a minimal enthalpic contribution, which indicates that TEMA binds at the upper rim, surrounded by sulfonate groups. Therefore, the electrostatic attraction and desolvation may act as the dominant driving forces for complexation. The complexation of SC4A-Bu with TMA occurs, on the contrary, with a lower entropic contribution and is mainly enthalpy-driven, with the favorable enthalpy changes originating from π-stacking interactions between the aromatic rings of the calixarene and TMA. The enthalpy-driven binding confirms that the benzene portion of TMA becomes immersed in the cavity of SC4A-Bu. The enthalpy changes of TMA and TEMA with SC4A are not as distinguishable, because the cavity of SC4A can accommodate either methylammonium or aromatic groups equally well. Presumably, the higher propensity of SC4A-Bu to seek hydrophobic and π–π interactions, in combination with a decreased tendency to undergo C–H–π interactions (see above), as well as its preformed pinched-cone conformation, facilitate the cavity binding to the aromatic residue. This is independently reflected by the distinctly increased complexation enthalpy for TMA relative to that for TEMA. By comparison, SC4A has been reported to show no regioselectivity towards TMA at neutral pH, suggesting that π–π interactions (which favor inclusion of the aryl ring) and C–H–π interactions (which favor inclusion of the methyl groups) are just about balanced for this host.[11]

### Binding with Neutral Spherical Organic Molecules

Among neutral organic guest molecules, 2,3-diazabicyclo[2.2.1]hept-2-ene (DBH), 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO), and

---

**Table 1. Complex stability constants ($K_S$), enthalpy ($\Delta H^\circ$), and entropy ($\Delta S^\circ$) changes for the 1:1 complexation of SC4A and SC4A-Bu with charged and neutral organic guests at 298.15 K, pH = 6.0.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Host</th>
<th>Guest</th>
<th>$K_S$ [10$^3$ M$^{-1}$]</th>
<th>$\Delta H^\circ$ [kJ mol$^{-1}$]</th>
<th>$\Delta S^\circ$ [kJ mol$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SC4A</td>
<td>TMA$^{[a]}$</td>
<td>32 ± 0.10</td>
<td>-30.2 ± 0.32</td>
<td>-4.51 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>SC4A</td>
<td>TMA</td>
<td>180 ± 0.26</td>
<td>-28.9 ± 0.24</td>
<td>1.02 ± 0.17</td>
</tr>
<tr>
<td>3</td>
<td>SC4A</td>
<td>TEM$^{[b]}$</td>
<td>380 ± 0.34</td>
<td>-25.3 ± 0.11</td>
<td>6.51 ± 0.21</td>
</tr>
<tr>
<td>4</td>
<td>SC4A-Bu</td>
<td>TMA$^{[b]}$</td>
<td>1.2 ± 0.02</td>
<td>-15.5 ± 0.22</td>
<td>2.09 ± 0.17</td>
</tr>
<tr>
<td>5</td>
<td>SC4A-Bu</td>
<td>TMA</td>
<td>1.0 ± 0.01</td>
<td>-13.6 ± 0.13</td>
<td>3.49 ± 0.11</td>
</tr>
<tr>
<td>6</td>
<td>SC4A</td>
<td>TEM$^{[b]}$</td>
<td>0.53 ± 0.01</td>
<td>-1.88 ± 0.02</td>
<td>13.7 ± 0.26</td>
</tr>
<tr>
<td>7</td>
<td>SC4A</td>
<td>DBH</td>
<td>0.66 ± 0.01</td>
<td>-23.3 ± 0.44</td>
<td>-7.2 ± 0.48</td>
</tr>
<tr>
<td>8</td>
<td>SC4A</td>
<td>DBO</td>
<td>0.76 ± 0.02</td>
<td>-25.4 ± 0.56</td>
<td>-8.88 ± 0.51</td>
</tr>
<tr>
<td>9</td>
<td>SC4A</td>
<td>DBN</td>
<td>0.86 ± 0.00</td>
<td>-26.4 ± 0.36</td>
<td>-9.62 ± 0.34</td>
</tr>
<tr>
<td>10</td>
<td>SC4A-Bu</td>
<td>DBH</td>
<td>0.87 ± 0.00</td>
<td>-1.01 ± 0.12</td>
<td>15.8 ± 0.28</td>
</tr>
<tr>
<td>11</td>
<td>SC4A-Bu</td>
<td>DBO</td>
<td>1.0 ± 0.01</td>
<td>-1.19 ± 0.15</td>
<td>16.0 ± 0.13</td>
</tr>
<tr>
<td>12</td>
<td>SC4A-Bu</td>
<td>DBN</td>
<td>1.5 ± 0.01</td>
<td>-0.92 ± 0.11</td>
<td>17.2 ± 0.32</td>
</tr>
</tbody>
</table>

[a] All ITC titrations revealed a 1:1 binding stoichiometry from the curve fitting. [b] pH = 2.0.
2,3-diazabicyclo[2.2.3]non-2-ene (DBN), determined herein in H2O by isothermal titration calorimetry (ITC), are in good agreement with those previously obtained in D2O by 1H NMR spectroscopy.[28] A representative, calorimetric, titration curve is shown in Figure 4. As can be seen, each titration of DBO into the sample cell gave an apparent reaction heat caused by the formation of an inclusion complex between SC4A and DBO. The reaction heat decreased after each injection of DBO because less and less host become available to form the inclusion complex. The presently reported calorimetric details reveal that binding by SC4A is enthalpy-driven, over-ruling the unfavorable entropy changes. The favorable enthalpy changes for SC4A can be attributed to van der Waals and C–H–π interactions as well as the release of high-energy water molecules, triggered by the inclusion of the guests. Concurrently, the inclusion of guests leads to a complexation-induced loss in conformational freedom of SC4A (the signals of the bridging methylene protons change from a single peak to two peaks in the 1H NMR spectra,[28]), which likely accounts for the observed negative entropy changes.[14,28] The complexation of SC4A-Bu with the bicyclic azoalkane guests results in similar stability constants to those of SC4A, but the thermodynamic contributions are entirely different. The complexation of SC4A-Bu with the bicyclic azoalkanes is mainly entropy-driven, accompanied by small, favorable enthalpy changes. From these thermodynamic data, we can directly infer that SC4A-Bu adopts a different binding mode than does SC4A. In agreement with the arguments advanced above for the binding of organic ammonium ions, and based on the crystal structures, we project that the binding geometry is shallower for SC4A-Bu. Presumably, the bicyclic azoalkanes are not included inside the cavity of SC4A-Bu as deeply as they are in the cavity of SC4A, but instead bind near the upper rim in the proximity of the sulfonate groups. As a result, the partial desolvation of host (in particular the sulfonate groups) and guests acts as the dominant driving force of host–guest complexation.

NMR investigations provided supporting evidence for the distinctive mode of binding of the SC4A-Bu macrocycle. As shown in the 1H NMR spectra of DBO as guest in the absence and presence of SC4A-Bu at pD = 6.0 (Figure 5), only a very small upfield shift of the signals of the DBO protons was observed upon complexation with SC4A-Bu [δ(Hendo) = –0.04 ppm, δ(Hendo) = –0.08 ppm, and δ(Hendo) = –0.05 ppm]. This is typical for the formation of a very shallow complex, without full exposure to the ring current of the aryl groups. For comparison, the upfield shift upon the complexation of DBO by SC4A is very large [δ(Hendo) = –1.25 ppm, δ(Hendo) = –2.10 ppm, and δ(Hendo) = –1.46 ppm].[29] This is characteristic of deep immersion. 2D ROESY NMR spectra of the SC4A-Bu-DBO complex were also recorded at pD = 6.0 (Figure S2), and the results were compared with those of the SC4A-DBO complex.[28] There was hardly any correlation between the DBO protons and the aromatic protons of SC4A-Bu, whereas the correlations between the DBO protons and the aromatic protons of SC4A were strong.[28] The combined NMR and calorimetric data convincingly demonstrate that bicyclic azoalkane guests are included in the cavity of SC4A-Bu more superficially than they are in the SC4A cavity and may possibly even be near the sulfonate rim.[29]

In contrast to the observation made for the two investigated organic ammonium ions, the binding abilities of SC4A and SC4A-Bu towards the three azoalkanes are similar. However, the underlying driving forces responsible for this phenomenon are quite different. For SC4A, there is better space filling of the inner calixarene cavity with the larger guest, which results in improved van der Waals and C–H–π interactions due to closer proximity as well as a larger release of high-energy water molecules. Both of these effects contribute to much more favorable enthalpy changes with an entropic cost. For SC4A-Bu, on the other hand, there is more efficient desolvation of the sulfonate groups as the size of the guest increases, which contributes to much more favorable entropy changes with an enthalpy cost. It is obvious that the reason for the similar binding abilities of SC4A and SC4A-Bu towards the three azoalkanes is an enthalpy/entropy compensation effect in this system.

Figure 4. Microcalorimetric titration of SC4A with DBO in aqueous solution (pH = 6.0) at 298.15 K. (a) Raw ITC data for 25 sequential injections (10 μL per injection) of a DBO solution (12.46 mM) into an SC4A solution (0.48 mM). (b) Apparent reaction heat obtained from the integration of the calorimetric traces.
Figure 5. $^1$H NMR spectra of DBO in the presence (a) and absence (b) of SC4A-Bu. DBO peak assignments H$_{exo}$ and H$_{endo}$ are taken from a previous publication.[28] Bands assigned to DSS-H refer to signals of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), which was added as an external reference.

The absorption (Figure 6) and emission (Figure 7) spectra of DBO upon complexation with SC4A and SC4A-Bu were also investigated at pH = 6.0. In particular, the n,$\pi^*$ absorption band in the near UV provides a sensitive probe for environmental effects, because the oscillator strength (extinction coefficient) of this band increases as the chromophore becomes immersed in more polarizable (electron-rich) environments.[30,31] Indeed, in the presence of SC4A, the absorbance of DBO increases by about 20%, suggesting its inclusion into a more polarizable microenvironment (i.e. near the aromatic rings, deep inside the cavity). Conversely, in the presence of SC4A-Bu, the absorbance of DBO increases by less than 5%, which indicates that the chromophore remains in an aqueous environment, as expected for complexation near the sulfonate rim. The larger fluorescence quenching of DBO by SC4A also confirms a deeper inclusion into the aromatic cavity, which facilitates quenching by exciplex formation.[32] These results are, therefore, nicely in line with the structural evidence obtained by NMR spectroscopy and the thermodynamic conclusions drawn from the ITC data.

Figure 6. UV/Vis spectra of DBO (2 mM) in the absence and presence of SC4A (5.5 mM) and SC4A-Bu (4.8 mM) at pH = 6.0.

Figure 7. Fluorescence spectra of DBO (2 mM) in the absence and presence of SC4A (5.5 mM) and SC4A-Bu (4.8 mM) at pH = 6.0 ($\lambda_{ex} = 365$ nm; $\lambda_{em} = 416$ nm).

Conclusions

The structural preferences and thermodynamic driving forces in the complexation of SC4A and SC4A-Bu with organic ammonium cations and neutral spherical organic molecules were systematically investigated. The alkylation of SC4A at its lower rim has large effects on the complex formation, the selectivity towards different guests, the structure of the isolated host and the corresponding host-guest complexes, and complexation thermodynamics. In general, SC4A-Bu favors the inclusion of flat aromatic residues over spherical ones, and it favors a shallower binding near the sulfonate groups. Compared to SC4A, the binding by
SC4A-Bu is accompanied by a relatively larger entropic driving force (due to desolvation and a smaller change in conformation) at the expense of a lower enthalpic driving force (caused by smaller contributions from C–H–π interactions). Overall, SC4A-Bu shows weaker binding by 2–3 orders of magnitude with organic ammonium ions but comparable binding with neutral organic guests. This new observation will allow us to rationally design a wide range of functional calixarene derivatives with enhanced binding abilities and molecular selectivities, as they are required, for example, for synthetic enzyme-mimetic systems.

**Experimental Section**

**Materials:** The host molecules, p-sulfonatocalix[4]arene (SC4A)[33] and 5,11,17,23-tetrasulfolanoo-25,26,27,28-tetrakis(n-butyl)calix[4]arene (SC4A-Bu),[40] were synthesized and purified according to the respective literature procedures. A crystal of SC4A-Bu was obtained by recrystallization from water/ethanol. The three guest molecules, DBH, DBO, and DBN, were obtained from a previous study.[34] The organic ammonium cations (TMA, TEMA) were commercially available from Acros Organics and used without further purification. Aqueous solutions of pH = 2.0 and 6.0 were prepared with distilled, deionized water, adjusted with 1 M hydrochloric acid (HCl) or 1 M sodium hydroxide (NaOH), and verified with a pH meter calibrated with two standard buffer solutions. D2O was used as the external reference.

**Measurements:** 1H and 2D ROESY NMR spectra were recorded with a Varian Mercury VX300 spectrometer by using DSS as an external reference. The host and guest were mixed in a 1:1 stoichiometry at 10 mm. UV/Vis spectra were recorded in a quartz cell (light path: 10 mm) with a Shimadzu UV-3600 spectrophotometer, equipped with a PTC-348WI temperature controller. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path: 10 mm) with a Varian Cary Eclipse instrument equipped with a single-cell Peltier accessory to keep the temperature at 25 °C. The X-ray intensity data for SC4A-Bu were collected with a Rigaku MM-007 rotating-anode diffractometer, equipped with a Saturn CCD Area Detector System, by using monochromated Mo-Κα radiation at T = 113(2) K. Data collection and reduction were performed with CRYSTAL CLEAR. The structures were solved by using direct methods and refined by employing full-matrix least squares on F2 (CRYSTALSTRUCTURE, SHEXLT-97). X-ray structural data for SC4A-Bu: C44H52Na3.67O30.17S4F0.3909, GOF on F2 = 0.1928, wR1 = 0.3841, R indices (all data): R1 = 0.2059, wR2 = 0.3909, GOF on F2 = 1.110. CDCDC-747027 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

**ITC:** A thermostatted and fully computer-operated isothermal calorimeter (VP-ITC) instrument, purchased from Microcal Inc. (Northampton, MA) was used for all microcalorimetric experiments. The VP-ITC instrument was calibrated chemically by the measurement of the complexation reaction of β-cycodextrin with cyclohexanol, and the obtained thermodynamic data were in good agreement (error < 2%) with the literature data.[50] All microcalorimetric titrations between hosts and guests were performed in aqueous solution (pH = 2.0 or 6.0) at atmospheric pressure and 298.15 K. Each solution was degassed and thermostatted by a Thermovac accessory before the titration experiment. Twenty-five successive injections were made for each titration experiment. A constant volume (10 µL/injection) of guest (or host) solution in a 0.250 mL syringe was injected into the reaction cell (1.4227 mL) charged with host (or guest) solution in the same aqueous solution. A control experiment was carried out in each run to determine the dilution heat by injecting a guest (or host) aqueous solution into a pure aqueous solution containing no host (or guest) molecules. The dilution heat determined in these control experiments was subtracted from the apparent reaction heat measured in the titration experiments to give the net reaction heat (Figure S1). The net reaction heat in each run was analyzed by using the “one set of binding sites” model (ORIGIN software, Microcal Inc.) to simultaneously compute the binding stoichiometry (N), complex stability constant (Kc), standard molar reaction enthalpy (ΔH°), and standard deviation from the titration curve. Generally, the first point of the titration curve was disregarded, as some liquid mixing near the tip of the injection needle is known to occur at the beginning of each ITC run. Knowledge of the complex stability constant (Kc) and molar reaction enthalpy (ΔH°) enabled the calculation of the standard free energy (ΔG°) and entropy changes (ΔS°) according to

$$
\Delta G° = -RT\ln K_c = \Delta H° - T\Delta S°,
$$

where R is the gas constant, and T is the absolute temperature. To check the accuracy of the observed thermodynamic parameters, two independent titration experiments were carried out to afford self-consistent thermodynamic parameters, and their average values with associated errors are listed in Table 1.

**Supporting Information** (see footnote on the first page of this article): Heat effects of dilution and complexation during the binding of DBO to SC4A, as determined by ITC; 2D ROESY NMR spectrum of the SC4A-Bu-DBO complex at pD = 6.0.

**Acknowledgments**

The Chinese team gratefully acknowledges support by the 973 Program (2006CB932900) and the National Natural Science Foundation of China (Nos. 20673061, 20721062, and 20703025). The German team thanks the Deutsche Forschungsgemeinschaft (DFG grant NA-686/5-1) and the Fonds der Chemischen Industrie for support. Both teams thank the Robert Bosch Foundation for collaborative project support (Wissenschaftsbrücke China).

---

[29] In addition, the $^1$H NMR spectra revealed no appreciable loss of conformational freedom of SC4A-Bu upon complexation with the bicyclic azoalkanes. The bridging methylene protons of conformational freedom of SC4A-Bu upon complexation with the bicyclic azoalkanes. The bridging methylene protons of SC4A-Bu showed two peaks in the absence and presence with the bicyclic azoalkanes. The bridging methylene protons of DBO, and more importantly, the $\Delta\delta_{ax-eq}$ values remained essentially the same (ca. 1 ppm).