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ITC analysis of guest binding to a deep-cavity cavitand

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ITC analysis of guest binding to a deep-cavity cavitand

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Using the recommended modifications to isothermal titration calorimetry (ITC) analysis proposed by Turnbull and Daranas (*J. Am. Chem. Soc.* **2003**, *125* 14859–14866) and Tellinghuisen (*Anal. Biochem.* **2008**, *373*, 395–397), we determine the thermodynamic data for the binding of a series of halogenated guests to deep-cavity cavitand **1**. These modifications allowed the accurate determination of the thermodynamic data of systems with c values of between 0.2 and 5; values that are much lower than is often suggested as the lower limit of ITC. The compiled data allowed a more accurate glimpse of the strength of the unusual C—H···X—R hydrogen bonds observed between these hosts and halogenated guests.

Keywords: resorcinarene; deep-cavity cavitand; isothermal titration calorimetry

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Introduction

Deep-cavity cavitands (*1–6*) such as **1** are readily available hosts synthesised in three steps from the corresponding resorcinarene (*7*). The 12 aromatic rings that constitute the bulk of the host define an 8 Å wide and deep-binding pocket suitable for complexing guests as large as adamantanes or camphors. Guest molecules are capable of forming C—H···π interactions with the host, but the strongest non-covalent interactions that we have observed with these types of hosts involve the four benzal hydrogens that point into the binding pocket. These define a crown of relatively electron-deficient C—H groups that show good affinity for halogen atoms, particularly iodine atoms (*8*). Thus, a combination of X-ray crystallography (*8*), NMR and NMR titration studies, involving the protio host **1** (*5*) and the corresponding d_4-host (*9*) deuterated at the benzal positions, have shown that iodinated guests bind in an orientation-specific manner with the iodine atom inserted into the C—H crown to form four C—H···I—R interactions with the guest. In the case of iodine, the halogen is large enough to form four such hydrogen bonds simultaneously, but the smaller the halogen atom the fewer hydrogen bonds it can simultaneously form and the weaker the overall host–guest interaction.

In an effort to quantify these rather unusual C—H···I—R hydrogen bonds, we have previously used NMR titration experiments as well as NMR-based van't Hoff analysis of selected host–guest pairs (*5*, *9*). For example, these results have demonstrated that the $\Delta H°$ for complexing 1-iodoadamantane (in toluene-d_8) is $6.7\,\mathrm{kcal\,mol^{-1}}$ higher than adamantane; even though polar guests such as cyanoadamantane bind weakly, suggesting that molecular scale dipole–dipole interactions do not play a role in guest complexation. Unfortunately, the error in these measurements was relatively high (ca. 15%), a fact that precluded comparison between structurally similar guests. In an effort to improve on this situation, and hence provide more information about C—H···I—R hydrogen bonds, we have undertaken the isothermal titration calorimetry (ITC) studies presented here. ITC has had a major impact in biochemistry (*10–14*) because it directly measures the enthalpy ($\Delta H°$) and the association constant (K_a) of a binding event. This allows the calculation of the free energy change ($\Delta G°$) and entropy change ($\Delta S°$), and hence the determination of the complete thermodynamic data for binding in one single experiment. Hence, the errors arising from these measurements are typically much smaller than NMR spectroscopy where van't Hoff analyses are required.

Results and discussion

All the complexations discussed here involve host **1**-binding guests **2–10** (Figure 1) in dimethyl sulphoxide (DMSO) as the solvent. DMSO was chosen because it poorly solvates the cavity of this host, and as a result, competition from the solvent is low and binding constants are much larger than in a more competitive solvent such as chloroform. The poor solvating ability of DMSO does, however, mean that host **1** is not particularly soluble in DMSO. As we explain below, this fact can engender problems in ITC analysis where the heat of complexation is relatively small.

For these and other 1:1 complexes, the Wiseman isotherm (Equation (1)) (*15*) relates the stepwise change in heat normalised with respect to the moles of titrant added

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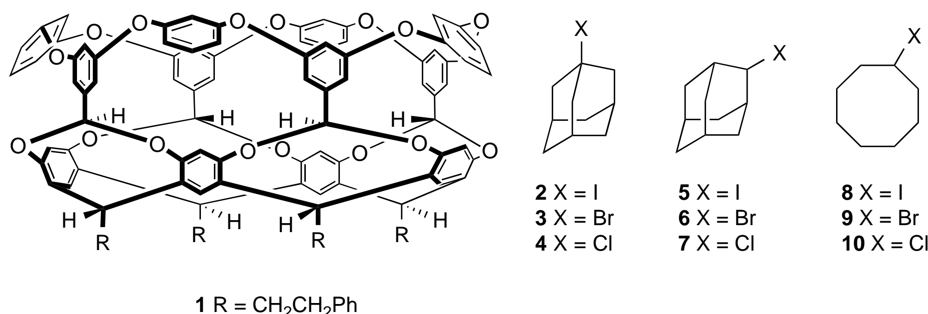

Figure 1. Structures of host **1** and guests **2**–**10**.

in each aliquot ($dq/d[X]_{0,m}$) to the absolute ratio of ligand to receptor concentration ($X_R = [X]_{0,m}/[M]_{0,m}$) at any point during the titration.

$$\frac{dq}{d[X]_{0,m}} = \Delta H^0 V_0 \left[\frac{1}{2} + \frac{1 - X_R - r}{2\sqrt{(1 + X_R + r)^2 - 4X_R}}\right], \quad (1)$$

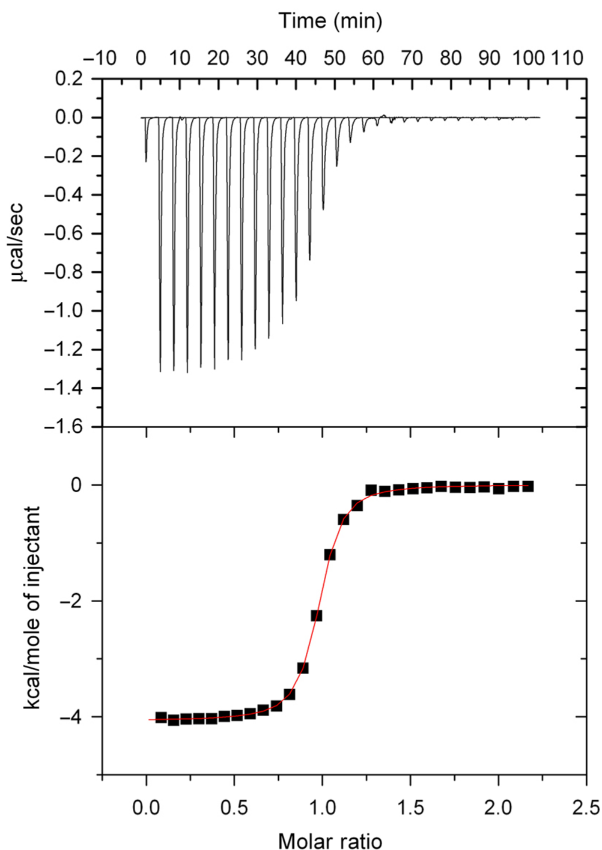

Figure 2. ITC isotherm for the titration of $CaCl_2$ into 0.1 mM EDTA ($K_a = 2.34 \times 10^6$, $c = 234$).

where

$$\frac{1}{r} = c = K_a[M]_{0,m} = \frac{[M]_{0,m}}{K_d} \quad (2)$$

and V_0 is the effective volume of the calorimetry cell, and c (Equation (2)) is known as the Wiseman parameter. Quantitatively, the enthalpy change ($\Delta H°$) and association constant (K_a) are determined by iteration of Equation (1) to maximise the fit to the obtained ITC-binding isotherm (e.g. Figure 2). Qualitatively, $\Delta H°$ is derived from the height of the obtained isotherm, while the K_a-value is derived from its overall shape. Regarding the latter, it was Wiseman who first noted (*15*) that the shape of an ITC-binding isotherm is dictated by what is now called the Wiseman parameter c, the product of the titrate concentration ($[X]$) and the binding constant (K_a). It was initially understood that in order to obtain an isotherm corresponding to a smooth S-shaped curve and minimal errors in both $\Delta H°$ and K_a ($\pm$ 2–3%), the Wiseman parameter should lie in the range of 10–500. Figure 2 shows an example of $CaCl_2$ binding to 0.1 mM EDTA ($K_a = 2.34 \times 10^6$, c-value = 234). In most cases, changing the c-value to fit within this range is possible by changing the concentrations $[X]$ and $[M]$, and the $[X]/[M]$ ratio; however, such changes are bracketed by the overall affinity ($\Delta G°$) and the heat of association ($\Delta H°$) of the process under study. Thus, for relatively weak binding events in which one or more of the interacting partners is of limited solubility, identifying conditions that define a c-value of 10–500 may not be possible. For example, according to our previous NMR study (*5*), the K_a of **4** binding to **1** is 3600 M^{-1}. With a maximum host concentration in DMSO of approximately 0.1 mM,[1] an estimated c-value of 0.36 might suggest an issue in accurately measuring the binding data of this and other guests described here. However, as has recently been highlighted by Turnbull (*16*), the lower limit of $c = 10$ is in fact an artefact of restricting $[X]/[M] \leq 2$; a common strategy when binding is strong and only two equivalents of guest are required to saturate a host. Under these conditions if the binding interaction is quite weak, then the isotherm appears flat and featureless because only a small

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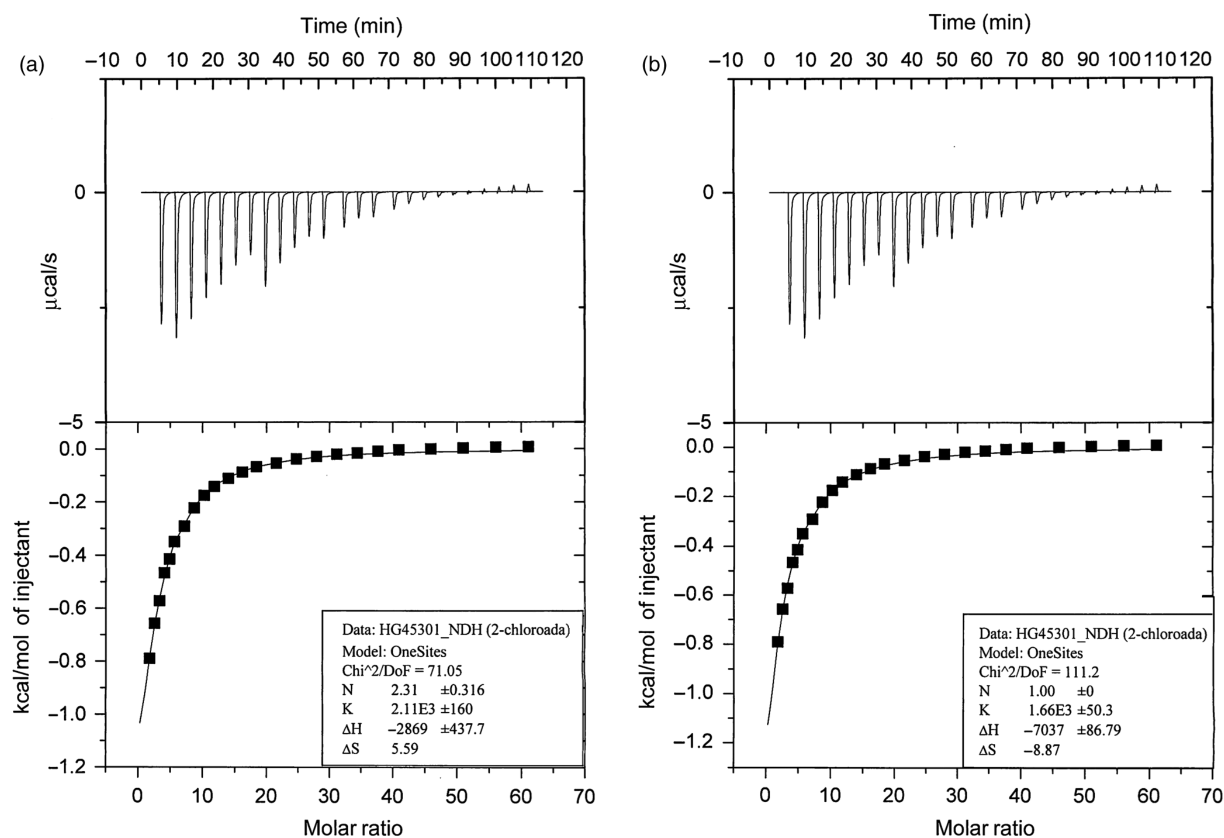

Figure 3. ITC titrations using guest (**7**) as titrant and host **1** in cell (DMSO, 298.15 K). In both (a) and (b), the concentration of host **1** and guest **7** was respectively 121.1 μM and 53.04 mM: (a) N-value treated as a variable (final value 2.31) and (b) N-value set to 1 (in accordance to 1:1 stoichiometry observed by NMR).

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percentage of the isotherm is explored. The conclusion from Turnbull's studies was that a large excess of guest was needed in order to saturate the host and give a full isotherm. Furthermore, Tellinghuisen (*17–19*) has also identified several protocols for improving accuracy of measuring ITC data, including in cases where a low affinity is measured. These include the use of less than 10 injections per titration, the use of variable injection volumes and the freezing of the stoichiometry parameter N when required. In combination, the Turnbull and Tellinghuisen modifications extend the Wiseman parameter range from $0.1 < c < 1000$; a range that theoretically covers the range of guests in this study.

For guests **2–10** binding to host **1** (Figure 1), we examined the fits obtained with and without the aforementioned modifications. The ones that proved most useful in our studies were as follows: first, we observed that for all guests, data were improved if the volume of guest solution added in each aliquot increased as the titration proceeded. In addition, for low affinity systems (low c-value), it was beneficial to follow the Turnbull modification and titrate in a large excess of guest to ensure that the host was fully saturated at the end of the experiment. Finally, it was noted for the weaker binding guests that it was necessary to set the stoichiometry parameter ($N = 1$) rather than allowing it to float free as another variable. Allowing the N-value to float free led to unrealistic N values (Figure 3). Fixing $N = 1$ is accepted practically if the stoichiometry of complexation is known, as is the case with these systems here (^{1}H NMR). In wishing to standardise the data collection protocol, we also investigated fixing or floating free the N-value of titrations involving strongly binding guests. In these instances, fixing $N = 1$ led to a poor fit (Figure 4(a)), while in calculations with N as a variable, the determined N values ranged from 0.90 to 0.96. Consequently, we determined the thermodynamics of the strongest binding guests (**1**, **2** and **5**) by carrying out the titrations at as low a host concentration as was practical. This lowered c-value for these titrations to <5 (the c-value range in this study was $0.2 \leq c \leq 5$) and allowed the N values for the titrations of the stronger guests also to be fixed (Figure 4(b)). The importance of running these experiments at low concentrations is emphasised in Figure 5, which shows plots of c-value vs. relative standard error in the determination of K_a (σ_{Ka}/K_a). As can be seen, triplicate determinations for the binding of guests **2**, **3** and **5** to host **1** revealed much improved data at lower vs. higher concentrations.

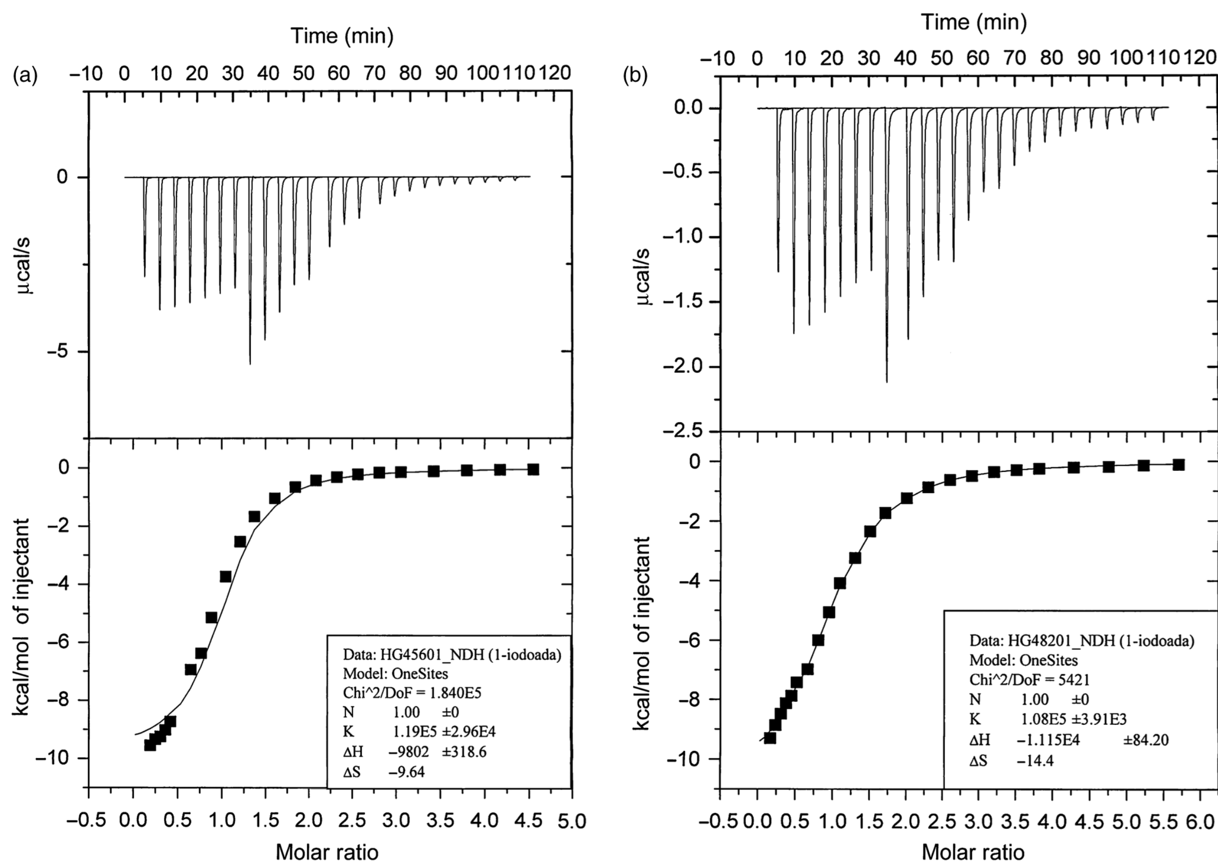

Figure 4. ITC titrations using guest (**2**) as titrant and host **1** in cell (DMSO, 298.15 K): (a) [**1**] = 127.7 μM, [**2**] = 4.17 mM (*c*-value = 10) and (b) [**1**] = 51.0 μM, [**2**] = 2.09 mM (*c*-value = 5).

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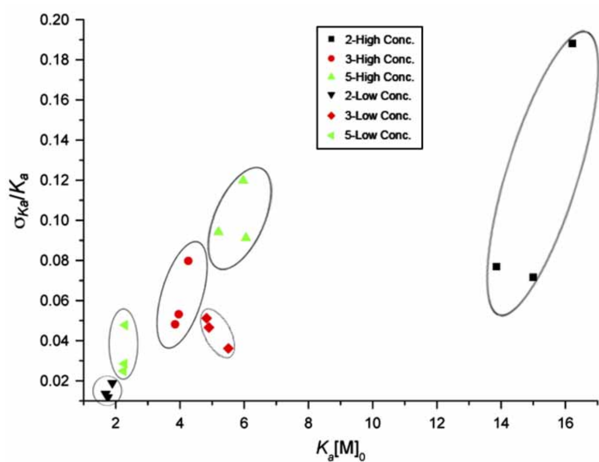

Figure 5. Plot of *c*-value vs. relative standard error in K_a (σ_{K_a}/K_a) for triplicate titrations involving guests **2**, **3** and **5**. High and low concentrations for each respective guest are as follows: guest **2**, [**1**] = 127.7 μM, [**2**] = 4.17 mM and [**1**] = 51.0 μM, [**2**] = 2.09 mM; guest **3**, [**1**] = 114.5 μM, [**3**] = 7.34 mM and [**1**] = 69.0 μM, [**3**] = 3.75 mM; guest **5**, [**1**] = 123.3 μM, [**5**] = 8.11 mM and [**1**] = 61.8 μM, [**5**] = 3.89 mM.

With the aforementioned modifications to the ITC titrations, we examined the binding of guests **2**–**10** to host **1** (Table 1). As the table reveals, experimental errors for individual determinations of the association constants, free energy changes, enthalpy changes and entropy changes for binding were no more than ± 3%, with the spread of each set of triplicate experiments ± 5%. With the exceptions discussed below, overall, the binding behaviour is in accord with the results from our previous NMR studies in DMSO-d_6 (*5*). That said, the binding of both **2** and **3** to host **1** was too strong to be obtained directly by NMR, and competition experiments with adamantane were required to calculate the reported binding constants (± 15% error). Furthermore, van't Hoff analyses could only be used to determine the thermodynamic parameters of the weaker binding guests, e.g. **4** and **6** (and was not carried out with **8**) (*20*). The errors in these determinations were also ± 15%. Where the ITC data differed from the NMR studies was for halogenated cyclooctyl guests **8** and **9**. According to ^{1}H NMR, in DMSO-d_6, the association constant for these guests was respectively 5958 and 1326 M^{-1} (*9*). Using ITC, the binding of **8** was measurably weaker (2330 M^{-1}), and we could not fit the data for the binding of **9** (nor **10**). For these two guests, we also

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Table 1. Binding constants and thermodynamic data obtained from NMR (*5*, *9*) and ITC.

Guest	Technique	K_a [a,b] (M^{-1})	ΔH° [a,c] (kcal mol^{-1})	ΔG° [d] (kcal mol^{-1})	$-T\Delta S^{\circ}$ [e] (kcal mol^{-1})
2	ITC	9.96×10^4	−11.50	−6.79	4.68
2	NMR [f]	1.40×10^5	– [g]	−7.01	– [g]
3	ITC	2.57×10^4	−9.70	−5.99	3.70
3	NMR [f]	3.30×10^4	– [g]	6.16	– [g]
4	ITC	5.72×10^3	−8.19	−5.11	3.09
4	NMR [f]	3.60×10^3	−9.30	−4.85	4.40
5	ITC	3.65×10^4	−10.5	−6.20	4.26
6	ITC	9.28×10^3	−8.87	−5.39	3.48
6	NMR [f]	9.80×10^3	−8.60	−5.50	3.10
7	ITC	1.66×10^3	−6.93	−4.38	2.55
8	ITC	2.32×10^3	−7.71	−4.57	3.14
8	NMR [f]	5.95×10^3	– [h]	−5.15	– [h]

[a] Reported values of K_a and ΔH° from ITC titrations are an average of three titrations in which the fitting error was ±5%.
[b] Experimental error for individual K_a determinations is ±3%.
[c] Experimental error for individual ΔH° determinations is ±1%.
[d] Calculated error for ΔG° is ±0.2%.
[e] Calculated error for $-T\Delta S^{\circ}$ is ±2%.
[f] Data are derived in DMSO-d_6, see references (*5*) and (*9*).
[g] Binding was too strong to determine thermodynamic parameters.
[h] ΔH° and $-T\Delta S^{\circ}$ are not determined (see reference (*9*)).

examined both the 10-injection procedure outlined by Tellinghuisen and increasing the host concentration, but neither of these resulted in any improvement in the data. It is probably the case that the amount of heat released from these complexations is too weak to be determined by ITC. In short, the calorimetry approach allowed a far more accurate determination of the thermodynamic data for the complexation of **2**–**8**.

What is the contribution of the C—H···X—R hydrogen bonds to the free energy of association of these complexes? Unfortunately, the ideal comparison of adamantane with the three halo-adamantanes (**2**, **3** and **4**) was not possible by NMR because guests **2** and **3** were bound too strongly in DMSO. Furthermore, it was not possible to study adamantane complexation with ITC because binding did not yield a sufficiently large amount of heat upon binding. Hence, only an indirect comparison – between the previously determined NMR data (DMSO-d_6, 25°C, 1 mM) for binding adamantane and the ITC date obtained here (DMSO-d_6, 25°C, 128 μM) – is possible. It should be stated that this must be treated with caution as the two techniques do of course collect the same data. The binding of adamantane was previously determined to release 4.0 kcal mol^{-1} of free energy in DMSO-d_6 ($\Delta H^{\circ} = -8.7$ kcal mol^{-1} and $-T\Delta S^{\circ} = 4.7$ kcal mol^{-1}) (*9*). Comparing these values to those obtained for **2** (Table 1), it is apparent that relative to adamantane an additional 2.79 kcal mol^{-1} of free energy is released in the binding of **2**, and that this is mostly enthalpic in nature (the difference in the entropic changes for the two guests is negligible). Previously, we had shown that the entropic penalty for binding adamantane in toluene-d_8 was significantly smaller than substituted adamantanes, but that this was not the case in DMSO-d_6 (*9*). The rational for this observation was that adamantane and halo-adamantanes are solvated similarly in toluene, but that there is an intrinsic difference between their respective solvations by DMSO. As a result, the fact that bound, pseudo-spherical adamantane can tumble freely in the cavity whereas guests such as **2** can only spin around their C_3-axis coincident with the C_4-axis of the host (*8*) was not apparent in DMSO. Hence, it is not surprising that the ITC-derived entropic cost for the binding of **2** is similar to the NMR-derived entropic cost of binding adamantane. As we have noted before, the large enthalpic difference in the binding of the two guests cannot be accounted for by dipole differences between the guests because many highly polar guests bind weakly to **1** (*5*). Instead, the difference arises through the four C—H···X—R hydrogen bonds that **1** can simultaneously form with **2**; each hydrogen bond liberates 0.70 kcal mol^{-1} of enthalpy. Furthermore, the data in Table 1 also reveal that the presence of an iodine atom gives a 1.63–1.80 kcal mol^{-1} enthalpic boost to complexation compared to a bromine atom, while a bromine atom leads to a 1.51–1.86 kcal mol^{-1} enthalpy boost compared to a chlorine. These differences must primarily be driven by the fact that an iodine, bromine and chlorine atom can only respectively form four, three and two C—H···X—R hydrogen bonds simultaneously.

The free energy differences between the complexation of 1-adamantane and 2-adamantane derivatives reflect the fact that the latter cannot form C—H···X—R hydrogen bonds without the adamantane cage impacting on the walls of the cavity. This leads to free energy differences for the guests **2**/**5**, **3**/**6** and **4**/**7** of between 0.59 and 0.73 kcal mol^{-1}. The importance of the goodness of fit

between the host and guest is also emphasised by the weak binding of smaller and more flexible **8** relative to **2** and **5**. Finally, it should be noted that all of these complexations are entropically penalised, and that there is reproducible enthalpy–entropy compensation observed for all the guests. For example, 1-adamantanes **2**, **3** and **4** all bind more exothermically than their corresponding 2-adamantane isomers **5**, **6** and **7**, but the entropy cost to the complexation of the former set is also larger. Similarly, when comparing data within each set (**2**, **3** and **4**, or **5**, **6** and **7**), it is apparent that the greater the number of simultaneous C—H···X—R hydrogen bonds that a guest can form to host **1**, the stronger the enthalpy bonus to complexation but the higher the accompanying entropy penalty.

In conclusion, by using the recommended modifications to ITC analysis proposed by Turnbull and Tellinghuisen, we have been able to accurately determine the binding of a series of halogenated guests to deep-cavity cavitand **1**. These modifications allowed the accurate determination of thermodynamic data for systems with *c* values of between 0.2 and 5; values that are much lower than is often suggested as the lower limit of ITC. In turn, these data have allowed a more accurate glimpse of the strength of unusual C—H···X—R hydrogen bonds.

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Experimental

General

With the exception noted below, all reagents and guests were purchased from Aldrich Chemical Company (St Louis, MO, USA) and used without further purification. Melting points are determined using a hot-stage apparatus and are uncorrected. Routine ^{1}H NMR experiments were performed at 400 MHz (Varian Unity INOVA instrument).

2-Iodoadamantane (5)

2-Bromoadamantane (500 mg) was dissolved in 30 ml pyridine and to this stirring solution was added 20 equiv. sodium iodide. The solution was refluxed for 7 days. After this time, the reaction was cooled to room temperature, and the solvent was removed under reduced pressure. The crude product was then dried under high vacuum (16 h). The crude mixture was then taken up in a chloroform–water mixture. The chloroform solution was separated, and the aqueous layer was washed twice with further volumes of chloroform. The organic solutions were combined, dried with anhydrous Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure. The crude solid obtained was purified by column chromatography with hexanes as the mobile phase. The pure product was dried under vacuum to give a white solid in 20% yield, mp = 45°C. The ^{1}H NMR of the product was identical to that quoted by Olah et al. (*21*). ^{1}H NMR (chloroform-*d*, 400 MHz) δ (ppm) 4.97 (s, 1H), 2.36–2.39 (m, 2H), 2.16 (br s, 2H), 1.69–1.95 (m, 10H).

ITC titrations

A MicroCal VP-ITC calorimeter (cell volume = 1.4711 ml) was used for all titrations. All experiments were run at 25°C. The curve-fitting model used was the single set of identical site model, and the obtained curve was analysed using Origin 7.0. All of the ITC titration experiments applied the 26-injection procedure in DMSO with variable injection volumes ($V_1 = 1\,\mu l$; V_2–$V_8 = 2.5\,\mu l$; V_9–$V_{12} = 5\,\mu l$; V_{13}–$V_{15} = 7\,\mu l$; V_{16}–$V_{22} = 10\,\mu l$; V_{23}–$V_{26} = 15\,\mu l$).

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Note

1. The host is soluble up to approximately 1 mM (the concentration used for binding constant determination by NMR). However, at this concentration, the ITC data obtained was poor. We suspect that over the time frame of the ITC experiment (which is longer than that of the NMR experiment) some amount of the host precipitated out of solution causing poor fitting of the data.

References

(1) Xi, H.; Gibb, C.L.D.; Stevens, E.D.; Gibb, B.C. *Chem. Commun.* **1998**, 1743–1744.
(2) Xi, H.; Gibb, C.L.D.; Gibb, B.C. *J. Org. Chem.* **1999**, *64*, 9286–9288.
(3) Green, J.O.; Baird, J.-H.; Gibb, B.C. *Org. Lett.* **2000**, *2*, 3845–3848.
(4) Gibb, C.L.D.; Xi, H.; Politzer, P.A.; Concha, M.; Gibb, B.C. *Tetrahedron* **2002**, *58*, 673–681.
(5) Laughrey, Z.R.; Gibb, C.L.D.; Senechal, T.; Gibb, B.C. *Chem. Eur. J.* **2003**, *9*, 130–139.
(6) Gibb, C.L.D.; Li, X.; Gibb, B.C. *Proc. Natl Acad. Sci. USA* **2002**, *99*, 4857–4862.
(7) Timmerman, P.; Verboom, W.; Reinhoudt, D.N. *Tetrahedron* **1996**, *52*, 2663–2704.
(8) Gibb, C.L.D.; Stevens, E.D.; Gibb, B.C. *J. Am. Chem. Soc.* **2001**, *123*, 5849–5850.
(9) Laughrey, Z.R.; Upton, T.; Gibb, B.C. *Chem. Commun.* **2006**, 970–972.
(10) Salim, N.N.; Feig, A.L. *Methods* **2009**, *47*, 198–205.
(11) Pulido, N.O.; Chavelas, E.A.; Torner, F.J.; Garcia-Hernandez, E. *Adv. Protein Phys. Chem.* **2008**, 115–138.
(12) Roselin, L.S.; Lin, M.-S.; Lin, P.-H.; Chang, Y.; Chen, W.-Y. *Biotechnol. J.* **2010**, *5*, 85–98.
(13) Freyer, M.W.; Lewis, E.A. *Methods Cell Biol.* **2008**, *84*, 79–113.
(14) Bianconi, M.L. *Biophys. Chem.* **2007**, *126*, 59–64.

(15) Wiseman, T.; Williston, S.; Brandts, J.F.; Lin, L.-N. *Anal. Biochem.* **1989**, *179*, 131–139.
(16) Turnbull, W.B.; Daranas, A.H. *J. Am. Chem. Soc.* **2003**, *125*, 14859–14866.
(17) Tellinghuisen, J. *Anal. Biochem.* **2008**, *373*, 395–397.
(18) Tellinghuisen, J. *J. Phys. Chem.* **2007**, *111*, 11531–11537.
(19) Tellinghuisen, J. *Anal. Biochem.* **2003**, *321*, 79–88.
(20) Laughrey, Z.R.; Gibb, C.L.D.; Senechal, T.; Gibb, B.C. *Chem. Eur. J.* **2003**, *9*, 130–139.
(21) Olah, G.A.; Welch, J.T.; Vankar, Y.D.; Nojima, M.; Kerekes, I.; Olah, J.A. *J. Org. Chem.* **1979**, *44*, 3872–3881.

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