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The Rational Design of Anion Host Compounds: An Exercise in Subtle Energetics**

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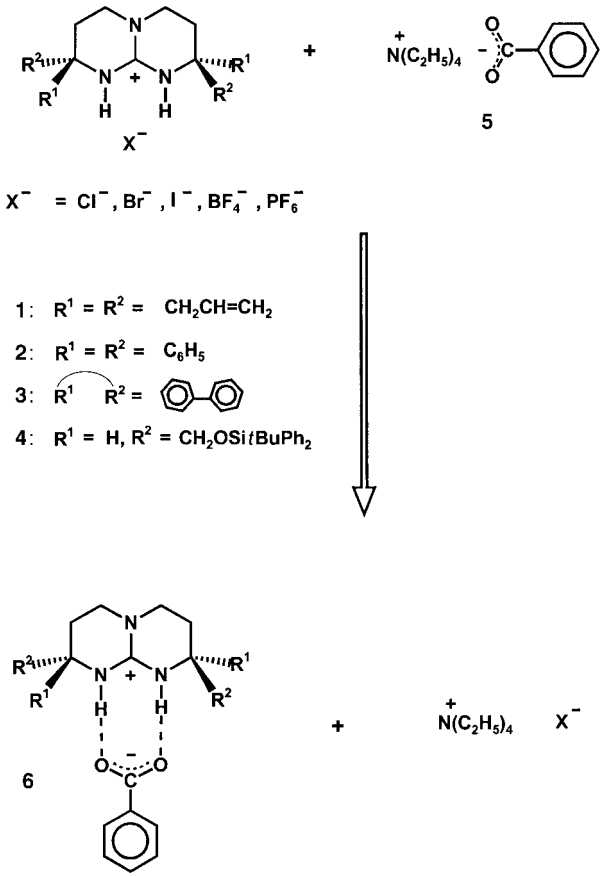
For more than 100 years the lock-and-key metaphor of Emil Fischer served as the fundamental concept to comprehend molecular recognition interactions.[1] The central idea rests on the assumption that the mutual geometric fit of a pair of supramolecular binding partners dominates their thermodynamic affinity.[2] Thus, generations of synthetic chemists have strived to optimize the direct interaction mode of hosts towards a given guest species by subtle modification of their covalent structures taking the Gibbs energy of complex formation ΔG° and the equilibrium constant for association (K_{ass}) as the ultimate judge. In many cases this approach did not live up to the expectations and met with only limited success. Here we describe an experimental attempt to evaluate the energetics of a simple host – guest system to develop more reliable guidelines for molecular recognition.

The failure of the lock-and-key model to rationalize general host – guest interactions can be traced to the premises of the concept which focuses exclusively on the enthalpic interactions of just two interaction partners, neglecting the entropic components as well as all solvent contributions. The latter point in particular gives rise to the phenomenon of enthalpy – entropy compensation[3, 4] that accompanies all weak interactions in solution. The enthalpic gain on binding of host and guest can thus be counteracted by an entropic influence. As a consequence, the free energy of association ΔG°_{ass}, which represents a combination of the state (enthalpic and entropic) functions ($\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$), may change only marginally and does not reflect the structural achievements. A remedy to connect the structural modification in a tailored host – guest relationship to the experimental energetics can be expected from isothermal titration calorimetry (ITC). This method allows the pertinent thermodynamic state functions to be obtained along with the stoichiometry of host – guest complex formation from a single experiment.[5] Since calorimetric measurements faithfully report on the cumulative heat response of the entire system it is mandatory to design a host – guest system simple enough to allow deconvolution of all the processes happening simultaneously in solution and ascribe the heat effect to just one association reaction. Studying a series of structurally related compounds should unfold the structure-affinity correlation, in addition, and the risk of misinterpretation is lowered by using trend analysis.

The association of bicyclic guanidinium cations (e.g. **1**) with carboxylates **5** (Scheme 1) may provide a suitable host – guest system. The interaction mode follows strict 1:1 stoichiometry



Scheme 1. Overview of the associations between **1** – **4**-X⁻ and **5**.

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and was characterized in solution and in the solid state in numerous examples.[6] The prime structural motif which is also frequently found in protein–substrate complexes[7] features an almost planar cation–anion arrangement, assisted by two parallel hydrogen bonds as shown by the X-ray crystal structures of the **2**-benzoate and **1**-trifluoroacetate complexes (Figure 1).

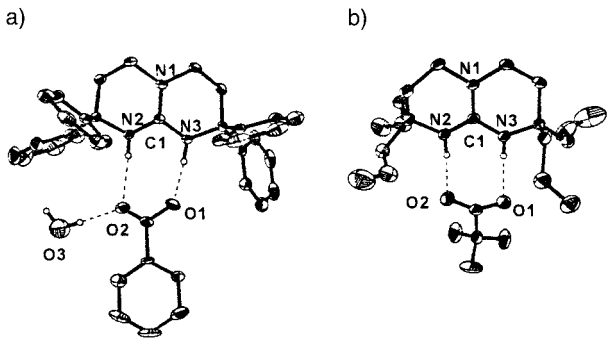

Figure 1. X-ray crystal structure of **2**-benzoate (a) and **1**-trifluoroacetate (b).[11] Selected bond lengths [Å] in (a): N2-O2 2.88, N3-O1 2.70, O2-O3 2.77; the orientation of the benzoate was refined using a split model: dihedral angle N1,N2,N3/O1,O2,C2 position 1 with 0.75 occupancy = 29.4°; position 2 with 0.25 occupancy = 59.0°.

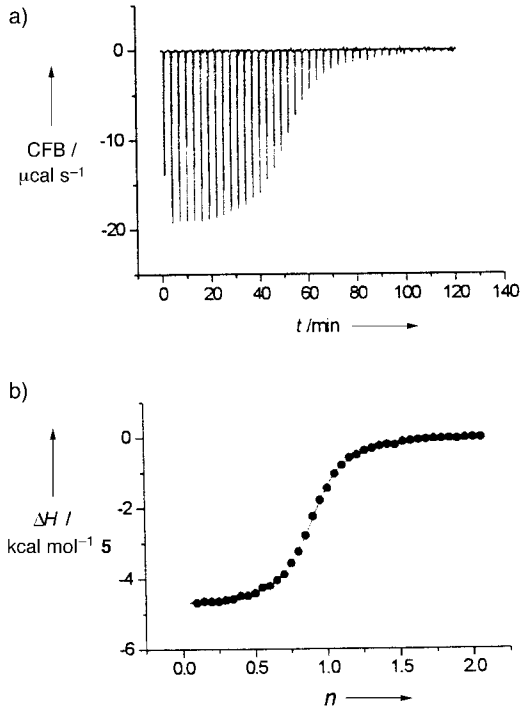

Figure 2. Typical ITC plot of the titration of **3**-PF_6^- (0.91 mM) with **5** (22.57 mM) in acetonitrile at 30 °C; CFB = cell feedback current.

Formation of the dedicated hydrogen-bonded ion-pair complex **6** is expected to depend on the competition with solvent. Thus, a non-hydrogen-bonding solvent was chosen that at the same time would minimize unspecific ion pairing by its high dielectric permittivity ε. Guided by these requirements and the excellent solubility of **5**, dry acetonitrile ($\varepsilon = 36$) appeared to be an optimal choice. A typical ITC plot from the titration of **3** with **5** in acetonitrile at 30 °C is depicted in Figure 2. The integration of the heat pulses obtained in each titration step in Figure 2a gives a titration curve (Figure 2b) rendering the molar enthalpy ΔH° as the step height and the free energy ΔG° from the slope in the inflection point. The stoichiometry n is derived as an independent parameter from the curve fit, and the molar entropy ΔS° may be calculated from the Gibbs–Helmholtz equation.

Competition of the desired guest species with the counter ions is inevitable when using abiotic hosts carrying a charge. Though straightforward deliberations can reduce this interference its influence has occasionally been noted[8] but has seldom been quantified. The calorimetric determination of host–guest binding in acetonitrile of the tetraallyl-substituted guanidinium compound **1** with **5** revealed a surprisingly strong dependence on the counter ion (Table 1). Switching the counter anion from chloride to hexafluorophosphate shifts the experimental host–guest affinity by more than a power of ten, with the larger, less hydrogen-bonding anions yielding the higher K_{ass} values. The anion influence is easily detectable even at submillimolar concentrations in high dielectric solvent acetonitrile. The clear-cut dependence on charge density points to an unspecific ion-pairing process which is also indicated by inspection of the entropy of host–guest binding. In all cases the association is accompanied by a positive entropy contribution testifying to the inadequacy of the lock-and-key principle[2] to explain the experimental outcome. While the combination of just two binding partners to form a tight complex must superficially result in a more negative ΔS° because of the loss of degrees of freedom for translation and rotation[3] the observation of the opposite result can be explained by the release of solvent molecules and counter ions engaged in the solvation of the binding sites. The contribution of the entropic component $T\Delta S^\circ$ to the free energy ΔG° is not a marginal factor but may even constitute the major part of ΔG° (e.g. in the case involving chloride; Table 1). Again a subtle enthalpy–entropy compensation ensures the more drastic change in the association enthalpy ΔH° is not expressed in the resulting Gibbs enthalpy ΔG°.

Table 1. Thermodynamic state functions, binding affinities K_{ass}, and experimental stoichiometries n of the reactions of **1**$^+$-X$^-$ with **5** in acetonitrile at 30 °C.[a]

X$^-$	Cl$^-$	Br$^-$	I$^-$	BF_4^-	PF_6^-
ΔH^o [kcal]	-2.93 ± 0.05	-4.13 ± 0.02	-4.52 ± 0.02	-4.58 ± 0.01	-5.21 ± 0.01
ΔG^o [kcal]	-6.35 ± 0.05	-7.03 ± 0.05	-7.55 ± 0.05	-7.78 ± 0.02	-7.73 ± 0.03
ΔS^o [e.u.]	$+11.3 \pm 0.3$	$+9.5 \pm 0.2$	$+10.0 \pm 0.2$	$+10.6 \pm 0.1$	$+8.3 \pm 0.1$
$T\Delta S^o$ [kcal]	+3.42	+2.89	+3.03	+3.20	+2.52
K_{ass} [M^{-1}]	38000	118000	280000	414000	380000
n	1.16	1.07	1.05	1.09	1.00

[a] From ITC titrations (the error limits refer to the fit of the data).

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The results listed in Table 1 suggest that there is a grading in the formation of ion-pairs between the guanidinium host and the series of counter anions. Chloride forms the most stable complex with the host, thus showing the lowest exothermicity ($-\Delta H^\circ$) in the exchange of chloride for benzoate, while the PF_6^- ion, at the other extreme, obviously enables the strongest enthalpic response because of its weak binding to the guanidinium group. There is no direct evidence that the limit of complete dissociation has been reached even with the least hydrogen-bonding ion PF_6^- nor that the second process to be considered in the trend analysis, the interaction of the counter anion, released on benzoate binding, with the tetraethylammonium cation, would perturb the result significantly. In fact tetraalkylammonium halides have been characterized as strong electrolytes in acetonitrile[9] and any ion pairing there would rather strengthen the differences within the series.

The impact of host design on binding affinity represents the ultimate goal of molecular-recognition studies and this was evaluated using an ensemble of custom-made guanidinium iodides **1**–**4**.[10] All of these compounds contain the same primary binding motif and are probed by complex formation with the same guest species **5**. However, the lining of the binding site was modified synthetically to introduce substituents with different steric properties, but without harming the preferential binding mode. The approach is analogous to the site-directed mutagenesis of biological receptors in which single amino acid residues in the substrate binding pocket may be deliberately exchanged to probe or alter the binding characteristics. Unlike the biological example, in the present artificial host–guest system the substituents available are not restricted to the natural amino acids.

The calorimetric results (Table 2) report a clear trend that assign a *decisive role* to solvation even in a typical organic solvent, such as acetonitrile. We observe a distinct variation of the binding free energy ΔG° with the substitution pattern of the host. The differences are manifest in the enthalpies as well as in the entropies of association. The less positive ΔS° values seen in **2** and **3** with the aromatic substituents indicate the release of fewer solvent molecules on benzoate binding than in **1** or **4**. In view of the considerable distance of the guest from the residues in the α-positions of the host (see Figure 1), a direct influence of the guest on the internal motility of the host is unlikely. Thus, it is most plausible to assume that the receptor site in **2** and **3**, lined by aromatic residues, is less solvated than the others and thus the enthalpic penalty paid to disrupt the solvation shell is smaller. This smaller unfavorable contribution results in an enhancement of the total exothermicity. The same arguments serve to explain the energetics of binding to the hosts **1** and **4**. The tetrasubstitution with "lean" residues directly adjacent to the binding site in **1** or by disubstitution with much more bulky silylether groups in **4** gives states of solvation that seem to be very much alike.

Table 2. Energetics of host–guest binding of the compounds **1**–**4**-I^- with benzoate in acetonitrile at 30 °C.[a]

	1	**2**	**3**	**4**
ΔH^o [kcal]	-4.52 ± 0.02	-5.18 ± 0.02	-6.03 ± 0.03	-4.40 ± 0.02
ΔG^o [kcal]	-7.55 ± 0.05	-6.99 ± 0.04	-8.00 ± 0.04	-7.35 ± 0.04
ΔS^o [e.u.]	$+10.0 \pm 0.2$	$+6.0 \pm 0.2$	$+6.5 \pm 0.2$	$+9.8 \pm 0.3$
$T\Delta S^o$ [kcal]	+3.03	+1.81	+1.97	+2.95
K_{ass} [M^{-1}]	280000	111000	592000	203000
n	1.05	1.01	1.06	0.90

[a] From ITC titrations.

The solvation effects may be supplemented to some degree by a field effect that lowers the dielectric constant in the vicinity of the charged guanidinium function and thereby enhances the coulombic attraction for the anionic guest. For instance, the fluorenyl compound **3** shows solvation changes almost identical to those of the tetraphenyl analogue **2** (cf. ΔS° values), but exhibits a significantly enhanced enthalpic contribution. The result relates to the presumed higher rigidity of **3** which opposes the reorientation of the solvent dipoles to cope with the change of the electrostatic field on complex formation.

Comparison of **1** and **2** also provides a good example for the benefit of dissecting ΔG° into the enthalpy and entropy components. Considering affinity only (K_{ass}) the allyl compound **1** would be a better host than **2**. Most molecular recognition studies, however, would rather select for better structural definition of the noncovalent complex since this constitutes the basis of goals from selectivity to self-assembly. With respect to precise structuring of the host–guest complex, **2** holds an edge over **1** because of the stronger enthalpic interaction and thus provides a strong argument to take enthalpy instead of the almost exclusively used free energy as a ranking criterion.

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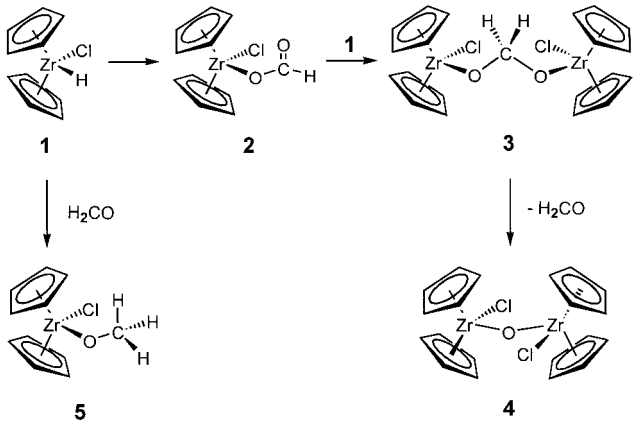
[11] X-ray crystal-structure determination: The data for 2,2,8,8-tetraallyl-1,3,4,6,7,8-hexahydro-2*H*-pyrimidino[1,2-a]pyrimidine trifluoroacetic acid salt (**1**-CF_3COO^-) and 2,2,8,8-tetraphenyl-1,3,4,6,7,8-hexahydro-2H-pyrimidino[1,2-a]pyrimidine benzoic acid salt (**2**-$C_6H_5COO^-$) were collected on a Nonius DIP 2020 diffractometer at 143(2) K with graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). The structures were solved by direct methods and refined against F^2 by full matrix least-squares with SHELXS-97 (SHELXS-97, G. Sheldrick, Universität Göttingen, **1997**). **1**-CF_3COO^- ($C_{21}H_{30}F_3N_3O_2$): $M_r = 413.48$, monoclinic, space group $C2/c$, $a = 26.1220(3)$, $b = 11.5845(2)$, $c = 24.8791(3)$ Å, $\beta = 117.8239(6)^\circ$, $V = 6658.25(16)$ Å^3, $Z = 12$, $\rho_{calcd} = 1.237$ g cm^{-3}, $F(000) = 2640$, $\mu(Mo_{K\alpha}) = 0.097$ mm^{-1}. A total of 144 785 reflections were measured in the range $7 \le 2\theta \le 53^\circ$, of which 6785 were unique ($R_{int} = 0.038$). Final R indices: $R_1 = 0.1144$ ($I > 2\sigma(I)$), w$R_2 = 0.3096$ (all data); max./min. residual electron density 1.97/ – 0.73 e Å^{-1}. **2**-$C_6H_5COO^-$ ($C_{38}H_{37}N_3O_3$): $M_r = 583.71$, monoclinic, space group $P2_1/c$, $a = 10.9899(3)$, $b = 16.4570(4)$, $c = 16.9609(4)$ Å, $\beta = 97.0971(13)$, $V = 3044.06(13)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.274$ g cm^{-3}, $F(000) = 1240$, $\mu(Mo_{K\alpha}) = 0.081$ mm^{-1}. A total of 91 531 reflections were measured in the range $3.4 \le 2\theta \le 55.2^\circ$, of which 6989 were unique ($R_{int} = 0.081$). Final R indices: $R_1 = 0.061$ ($I > 2\sigma(I)$), w$R_2 = 0.1369$ (all data); max./min. residual electron density 0.25/ – 0.40 e Å^{-1}. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-163362 (**1**-CF_3COO^-) and -163363 (**2**-$C_6H_5COO^-$). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

able advance.[3] By presenting the results in a „2D spectrum"—the chemical shift is displayed in one dimension, the diffusion in the other—the resolution and assignment of even complex mixtures are greatly facilitated. The surprising similarity of this concept as well as the manner of presentation led to the description of DOSY NMR spectroscopy as „NMR chromatography". Although this technique is also extraordinarily well-suited for applications in organometallic and inorganic chemistry in solution, it has attracted little attention.[4] Herein we demonstrate the usefulness of DOSY NMR spectroscopy by presenting a new application.

In the course of the present investigation, DOSY NMR spectroscopy has been successfully applied for the first time to determine the molecularity of an intermediate in the reaction of [$Cp_2Zr(Cl)H$] (**1**) with CO_2 (Scheme 1). Recently, we



Scheme 1. The investigated reaction of [$Cp_2Zr(Cl)H$] (**1**) with CO_2.

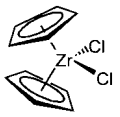
Characterization of Reactive Intermediates by Diffusion-Ordered NMR Spectroscopy: A Snapshot of the Reaction of $^{13}CO_2$ with [$Cp_2Zr(Cl)H$]**

Nils E. Schlörer, Eurico J. Cabrita and Stefan Berger*

Dedicated to Professor Anton Rieker on the occasion of his 70th birthday

In cases in which the structural characterization of reagents generated in situ or of reactive intermediates is not feasible with conventional methods, DOSY (diffusion ordered spectroscopy) NMR spectroscopy provides a valuable method to obtain additional information.[1] Until now, pulsed field gradient (PFG) spin-echo NMR experiments were employed mainly in organic and pharmaceutical chemistry for the study of aggregation and binding processes.[1d, 2] More recently, however, with the introduction of DOSY NMR spectroscopy the traditional diffusion experiment was subject to a remarksucceeded in elucidating the long-standing postulated mechanism of this reaction by low-temperature NMR spectroscopy studies.[5, 6] We were able to observe in situ both the formiato complex **2**, which is formed by insertion of CO_2 into the Zr–H bond of the hydride, and the binuclear diolato complex **3**, which results from reaction with another equivalent of **1**. Although complex **2** was synthesized in an earlier study,[7] the existence of the extremely unstable complex **3** was previously only postulated. However, all our attempts to structurally characterize this species by methods other than ^{1}H and ^{13}C NMR spectroscopy or to isolate it for a determination of the structure by X-ray structure analysis, failed. Since it was not possible to prove convincingly by spectroscopy that the observed intermediate is a binuclear zirconium compound, we tried to extract additional structural information by means of DOSY NMR spectroscopy.

Prior to studies aimed to characterize intermediate **3**, we examined the suitability of DOSY NMR spectroscopy for such a system by examining the behavior of two model compounds. With the purpose of determining whether the differences between the mono- and binuclear species lead to a resolution in the diffusion dimension in DOSY spectra, we chose a mixture consisting of the binuclear complex **4**, which has a molecular mass that is almost identical to that of the proposed intermediate, and the mononuclear complex **6**. The ^{13}C



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