Electronic Supplementary Information

for

A shape-dependent hydrophobic effect for tetrazoles in water

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1. Synthesis

Proton (^{1}H) NMR spectra were recorded on Brüker Avance-500 (500 MHz) or Brüker AC-300 (300 MHz) spectrometers, as indicated in each case. Carbon (^{13}C) NMR spectra were recorded on a Brüker Avance-500 spectrometer at 125 MHz. High-resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained on a Micromass Q-Tof Micro. Solvents and reagents were used as obtained from Acros Organics or Sigma-Aldrich without additional purification. Deuterated solvents were used as purchased from Cambridge Isotope Laboratories.

Tris(tetrazole) (1). 2,4,6-Triethyl-1,3,5-tris(cyanomethyl)benzene[1] (565 mg, 2.02 mmol), $ZnBr_2$ (1.364 g, 6.06 mmol), and NaN_3 (460 mg, 7.07 mmol) were combined in a pressure tube with H_2O (10 mL) and MeOH (10 mL). The tube was sealed and heated to 140 °C. After 24 h, the reaction mixture was cooled and transferred to a round-bottom flask. The volatiles were removed *in vacuo*, the aqueous mixture was acidified to pH 1 with 3 M HCl, and the resulting precipitate was collected by vacuum filtration. The solid was then dissolved in aqueous NaOH (1 M, 25 mL) and stirred for 30 min, resulting in a fine white precipitate of Zn salts that were removed by filtration. Reacidification with 3 M HCl produced a precipitate that was collected by filtration to give the product **1** (705 mg, 86%, 90–95% pure by ^{1}H NMR). Crystalline material of purity sufficient for binding studies was obtained by slow evaporation of a saturated methanolic solution of the product. M.p. 262 °C (dec.). ^{1}H-NMR (CD_3OD, 500 MHz): 0.95 (*t*, *J* = 7.5 Hz, 9H, $CH_2C\underline{H}_3$); 2.59 (*q*, *J* = 7.5 Hz, 6H, $C\underline{H}_2CH_3$); 4.41 (*s*, 6H, $C\underline{H}_2$-tetrazole). ^{13}C-NMR (CD_3OD, 125 MHz): 13.3; 23.2; 23.5; 130.2; 142.6; 156.2. HR-ESI-MS: 407.2208 ([*M*–H]$^-$, $C_{18}H_{23}N_{12}$; calc. 407.2169).

Tris(tetrazolate) (1-Na_3). Tris(tetrazole) **1** (10.0 mg, 0.0245 mmol) was dissolved in a solution of NaOMe in methanol (0.137 M, 0.54 mL, 0.074 mmol) with sonication, then concentrated *in vacuo* to yield the trisodium salt as a white powder. ^{1}H-NMR (D_2O, 500 MHz): 0.82 (*t*, *J* = 7.5 Hz, 9H, $CH_2C\underline{H}_3$); 2.59 (*q*, *J* = 7.5 Hz, 6H, $C\underline{H}_2CH_3$); 4.27 (*s*, 6H, $C\underline{H}_2$-tetrazole).

Tricarboxylic acid (2). 2,4,6-Triethyl-1,3,5-tris(cyanomethyl)benzene[1] (210 mg, 0.75 mmol) was dissolved in a mixture of aqueous NaOH (3 M, 20 mL) and aqueous H_2O_2 (30%, 8 mL), and heated to reflux with vigorous stirring. After 24 h, the mixture was cooled and acidified to pH 1 with 3 M HCl, and sufficient water was added to dissolve the precipitate that formed. The solution was extracted with EtOAc (3 × 25 mL), and the combined organics were washed with brine (25 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude material was purified by recrystallizion from EtOAc/hexanes (125 mg, 50%). M.p. 265 °C (dec.). ^{1}H-NMR (CD_3OD, 500 MHz): 1.10 (*t*, *J* = 8.0 Hz, 9H, $CH_2C\underline{H}_3$); 2.69 (*q*, *J* = 8.0 Hz, 6H, $C\underline{H}_2CH_3$); 3.75 (*s*, 6H, $C\underline{H}_2CO_2H$). ^{13}C-NMR (CD_3OD, 125 MHz): 13.1; 23.0; 34.4; 129.2; 141.6; 174.6. HR-ESI-MS: 335.1513 ([*M*–H]$^-$, $C_{18}H_{23}O_6$; calc. 335.1495).

Tricarboxylate (2-Na_3). Tricarboxylic acid **2** (114 mg, 0.339 mmol) was dissolved in a solution of NaOMe in methanol (0.137 M, 7.45 mL, 1.02 mmol) with sonication, then concentrated *in vacuo* to yield the trisodium salt as a white powder. ^{1}H-NMR (D_2O, 500 MHz): 1.02 (*t*, *J* = 8.0 Hz, 9H, $CH_2C\underline{H}_3$); 2.51 (*q*, *J* = 8.0 Hz, 6H, $C\underline{H}_2CH_3$); 3.62 (*s*, 6H, $C\underline{H}_2CO_2H$).

2. NMR titrations and Job plots

Binding titrations carried out in "Water" were carried out in D_2O containing NaH_2PO_4/Na_2HPO_4 (total phosphate concentration 10 mM) whose pD value of 7.4 was determined by measurement on a pH meter (see the discussion on errors at the end of Section 3).

The mixed solvent for "Methanol/Water" titrations was prepared as follows: D_2O was buffered at pD 7.4 with a total NaH_2PO_4/Na_2HPO_4 concentration of 25 mM, and the resulting aqueous solution was mixed with CD_3OD to make up a solution nominally 60:40 (v/v) in CD_3OD:D_2O and having a total phosphate buffer concentration of ~ 10 mM (assuming only a small volume change upon mixing). The pD* of the mixed CD_3OD/D_2O/buffer system thus prepared was estimated by determination of pH* for an identically prepared CH_3OH/H_2O/buffer mixture. To achieve this measurement, a pH meter's electrode was first equilibrated in CH_3OH/H_2O for three days. The electrode was calibrated using buffers with pH* values precisely defined for 52.1% (w/w) methanol/water.[2] (The 60:40 (v/v) ratio used in the binding studies corresponds to 54.3% (w/w) methanol/water. By comparison with solvent-dependent pH* data reported in Reference 2 the error in pH* measurement arising from this small discrepancy can be estimated at ≤ 0.5.)

Host solutions (1–2 mM) were first prepared in the appropriate solvent, and a portion was used to make up Guest solutions (20–70 mM) in order to ensure that the Host concentrations were kept constant throughout the titrations. Titrations were carried out on a Brüker Avance-500 (500 MHz) NMR, and chemical shift data was fit to a 1:1 binding isotherm using Origin 7.0, Rockware, Inc.

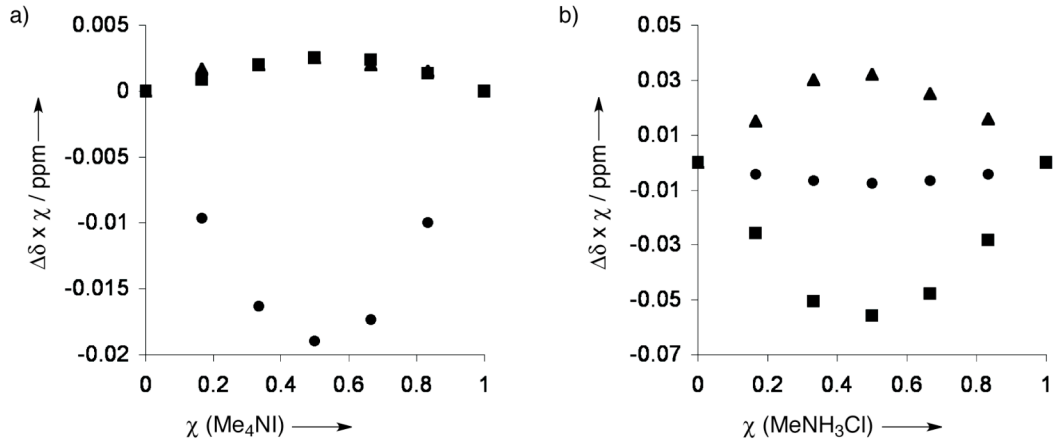

Fig. S1. Job plots at 298 K for host **1**-Na_3 binding a) Me_4NI and b) $MeNH_3Cl$ in 60:40 CD_3OD/D_2O (10 mM NaH_2PO_4 having pD* 8.65) show that signals arising from host $C\underline{H}_2CH_3$ (■), host $CH_2C\underline{H}_3$ (▲), and guest $C\underline{H}_3$ (●) protons all indicate a 1:1 binding stoichiometry. Total [Host] + [Guest] = 5 mM.

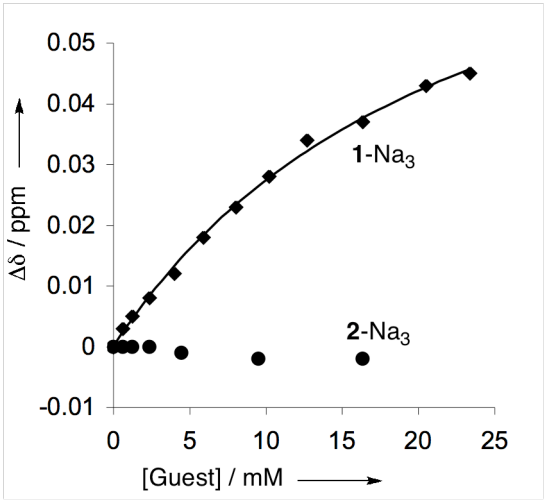

Fig. S2. ^{1}H NMR data for titration at 298 K of Me_4NI into D_2O (10 mM pD 7.4 NaH_2PO_4) solutions of **1**-Na_3 (◆, $K_{assoc.}$ = 65 M^{-1}) and **2**-Na_3 (●, no binding). [Host] = 1 mM.

3. pKa determinations and speciation curves

pKa titrations were performed using the titrimeter and calibration protocols recently described.[3] Solutions were prepared volumetrically using degassed deionized and distilled water and spectral grade methanol. In 60:40 M:W solutions a known volume of water (40%) was made to a total volume with methanol. This solution corresponds to 55.7 wt% methanol. Stock solutions of sodium nitrate and sodium hydroxide were prepared from the solids obtained commercially. Nitric acid titrant, diluted from concentrated HNO_3, was standardized versus sodium borate. Sodium hydroxide was standardized by direct titration with the secondary standard HNO_3 as titrant.

The titrations were performed using a Mettler DL21automatic titrimeter with data collection to a custom macro running under Microsoft Excel. The macro controlled the functions of the titrimeter via a serial port to the automatic titrimeter. The titrations were performed in a closed jacketed cell at a temperature of 25.0±0.2 °C under an atmosphere of nitrogen. The measurement circuit consider of: glass electrode | titration solution | glass frit| saturated KCl in water | AgCl/Ag. The electrode system was calibrated daily, according to the protocol outlined for the program GLEE[4] modified for the acid-into-base titration mode. Thus, electrolyte (0.1 M $NaNO_3$ in water or 0.1 M $NaNO_3$ in 60:40 M/W, 5.0 mL) and a known amount of sodium hydroxide was titrated with HNO_3, and the resultant strong acid–strong base curve of potential versus volume was converted via the standard concentrations to a curve of potential versus pcH. The required pK_w value for 0.1 M $NaNO_3$ in water was taken from Martell[5] (13.78). The pK_w value in 0.1 M $NaNO_3$ in 60:40 M/W (13.70) was determined by the procedure of Jameson and Wilson[6] using the pH meter calibrated using buffers prepared in 52.1 wt% methanol.[2] The curve of potential versus pcH was linear between 2.4–11.4 in both solvent systems, and gave a slope of >99% of the Nernstian value and an intercept value for the electrode. Triplicate values of the slope and intercept were determined daily for use in calculation of the formation constants determined that day.

Solutions for titration were prepared from stock solutions of the using microliter syringes and calibrated 10.0 mL volumetric flasks. Solutions in methanol/water utilized aqueous stock solutions of the components, plus additional water as required, made to the final volume with methanol. All solutions included a sufficient volume of standard NaOH to fully deprotonate the species present. A 4.0 mL sample was titrated within 2 h of solution preparation. Concentrations and aliquot volumes were chosen to produce 20–50 significant data points from each titration to give 60–150 points per system including triplicates. Significant precipitation was noted at approximately 1.4 equivalents of added acid for the tetrazole titrations in water and about 2.2 equivalents of acid added in M:W. Data more acidic than these points was discarded.

The titrimeter macro exported a formatted file for direct input to HYPERQUAD.[7] The refined parameters differed by less than the computed standard deviations in all cases of direct duplicates prepared from a common stock solution. HYPERQUAD produces a goodness-of-fit statistic (chi-squared) that indicates if the residuals (calculated-experimental) are normally distributed relative to a determined measurement precision. All systems gave a chi-squared value better than that expected for the 95% confidence level. The pKa for the third protonation of $\mathbf{1}^{3-}$ could not be determined due to precipitation, but the value must be below that of the second protonation (4.9) and therefore the concentration of triply protonated form in the solutions used for binding studies is negligible.

Discussion of binding measurements as related to pH, pD, and pK_a errors

Two significant sources of pH/pD errors can be identified: 1) The solvent isotope effect at the glass electrode when using pure water and 2) the effect of methanol content on proton and buffer activities when using methanol/water mixtures.

1) A correction value of + 0.4 has been suggested to allow for the isotope effect of D^+ at a glass electrode,[8] but due to offsetting pK_a changes in D_2O it has been suggested that it is better not to use the correction factor.[9] We did not apply this correction to our reported pD values, but had we chosen to do so the actual pD of the measurements in pure water would be 7.8 — a value that is further from the pK_a of the first protonation of $\mathbf{1}^{3-}$ (see Fig. S3) and therefore less likely to result in a proton transfer that would interfere with the binding titrations.

2) Practical considerations and limited availability of mixed-solvent buffer data demanded the use of methanol/water compositions varying from 52.1 to 55.7 wt% at various stages of the pH* and pK_a determinations described above, and the cumulative errors expected from these are at most ± 1 pH*/pK_a unit. Given the spread of the pK_a values determined for methylammonium ion (10.19) and for the first protonation of $\mathbf{1}^{3-}$ (6.6) it is unlikely that proton transfer is a significant source of the observed chemical shifts upon titration. Speciation curves (Fig. S3) show < 2% contribution from the protonated $(\mathbf{1}\bullet H)^{2-}$ under the conditions of the binding studies, and even accounting for the errors in pK_a determination this value is not likely to rise above 10%.

Table S1. Log β_{XH} (proton binding equilibrium constants) and pK_a values determined for Host and Guest water and in the mixed methanol/water solvent system employed for binding studies.

Species	stoichiometry	log β_{XH} (W)	pK_a (W)	log β_{XH} (M/W[a])	pK_a (M/W[a])
$MeNH_2$	11	11.15	11.15 ± 0.05	10.19	10.19 ± 0.05
Host $\mathbf{1}^{3-}$	11	5.8	5.8 ± 0.1	6.6	6.6 ± 0.1
	12	10.7	4.9 ± 0.2	11.5	4.9 ± 0.2
	13	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]

[a]Mixed methanol/water solvent system prepared as described in the text. [b] n.d. = not determined due to precipitation of the neutral $(\mathbf{1}\bullet H_3)$ form of the host.

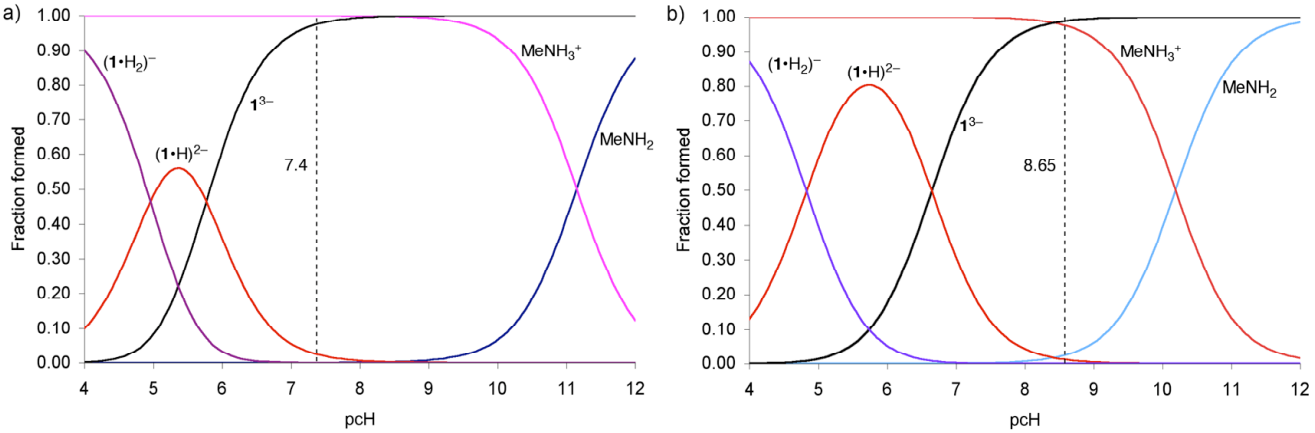

Fig. S3. Speciation curves generated using the pK_a values for host and guest determined in a) water and b) 60:40 (v/v) methanol:water. The pD values in which binding studies were carried out are indicated with dotted lines.

4. Molecular modeling of host-guest complexes

A series of calculations was carried out using Spartan '04 in order to better understand the conformational preferences of the host in the free and bound state.

a) Free Host $\mathbf{1}^{3-}$. The free host was assumed to be in a conformation in which all tetrazolate binding elements are on the same face of the central benzene scaffold, in analogy with related compounds.[10–12] Dihedral driving calculations (PM3) show that the barrier to rotation of a single tetrazolate binding

element in the absence of guest is ~ 2.5 kJ/mol, with the slightly preferred minimum energy conformation ($\theta = 60°$) being intermediate between the idealized "face-on" ($\theta = 90°$) and "edge-on" ($\theta = 0°$) geometries (Fig. S4). Both the face-on and edge-on binding conformations are in fact local maxima in the absence of guests, but the extreme smoothness of this energy profile means that the tetrazolate binding elements of the free host are best described as rotating rapidly without any strong conformational preference.

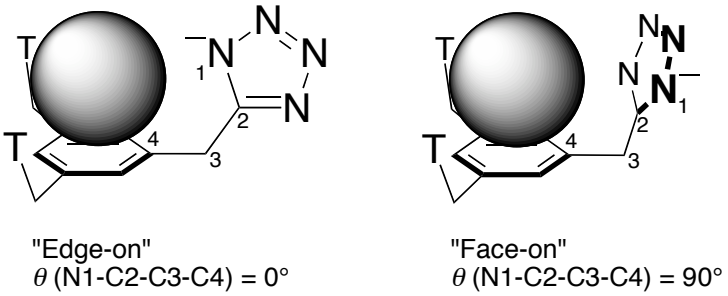

Fig. S4. Definition of "edge-on" and "face-on" binding geometries and the accompanying values for the dihedral angle θ. Ethyl substituents have been omitted and some tetrazoles have been indicated with a "T" for clarity.

b) Methylammonium complex $\mathbf{1}^{3-}$•$MeNH_3^+$. This complex exclusively adopts the edge-on geometry depicted in Fig. 3a of the paper. No other structures are found to be local minima at any level of theory.

c) Tetramethylammonium complex $\mathbf{1}^{3-}$•Me_4N^+. Two local minima were located using semi-empirical (PM3) energy minimizations. One most closely resembles an "edge-on" approach of tetrazolates to the quaternary ammonium ion guest (Fig. S5a), while the other is a clear representation of a "face-on" binding geometry (Fig. S5c). Calculations at a higher level of theory (HF/6-31+G*) reveal a single minimum energy structure (Fig. S5b) with an intermediate tetrazolate geometry ($\theta = 48°$). *None of these calculations are likely to be accurate predictions of the actual binding mode because they don't involve explicit water molecules and they therefore can't incorporate the influence of the hydrophobic effect and peripheral water molecules.* But such host-guest models are very good predictors of steric clash and size matching. In this context, the results suggest that the host and guest are free to adopt a variety of relative orientations and that the host's conformation is not driven toward face-on binding simply by the large size of the tetramethylammonium guest.

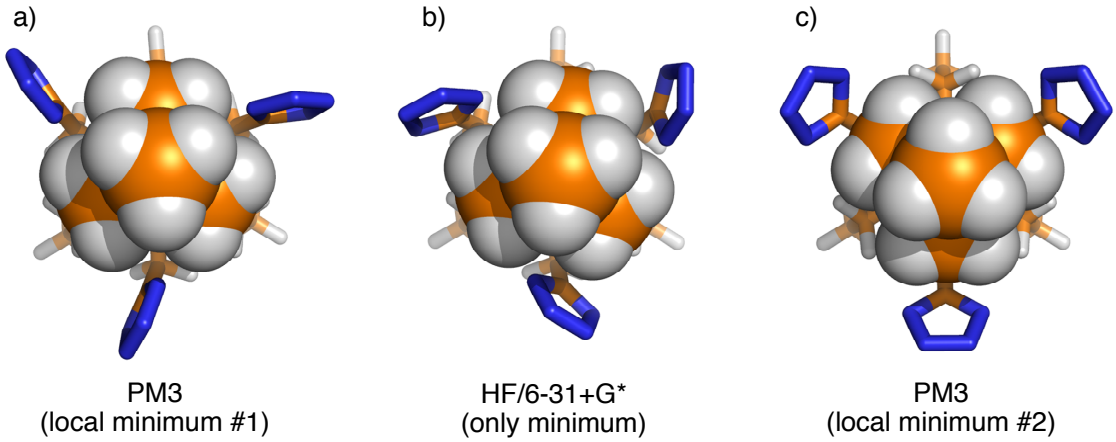

Fig. S5. Calculated structures for $\mathbf{1}^{3-}$•Me_4N^+ showing the variety of stable interaction geometries between the tetrazolate binding elements and the guest.

References

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