A shape-dependent hydrophobic effect for tetrazoles†

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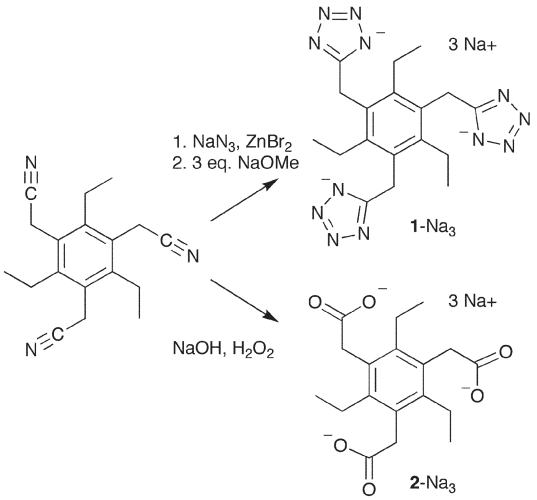
An adaptive tetrazole-derived host provides insight into tetrazolate-biomolecule interactions, and is the first member of a new family of receptors that function in pure water.

Tetrazoles have been used extensively in the creation of enzyme inhibitors; they are routinely pulled from the medicinal chemist's toolkit to serve as pharmacophore replacements of carboxylic acids, and are prized for their similar ionization and recognition properties at physiological pH.[1] In recent years, elegant studies on tetrazole recognition have underlined the subtle differences in hydrogen bonding between carboxylates and tetrazoles,[2,3] and tetrazoles have found use in such varied applications as stacked supramolecular oligomers,[4] functional polymers,[5] and drug-binding gels.[6] We report here a compact tetrazole-derived receptor that shows high selectivity in its binding of ammonium ions in aqueous solutions. Binding and structural studies reveal that the host's charged tetrazolate rings have a significant hydrophobicity only when their *faces* engage quaternary ammonium ions. Unlike closely related non-tetrazole analogs, the tetrazole host retains the ability to bind partners in pure buffered water.

Host **1** was designed to present three tetrazole substituents on the same face of its aromatic core,[7–9] and was synthesized by adaptation of a tetrazole-forming methodology that utilizes Zn^{II} to mediate the cycloaddition of nitriles with N_3^- (Scheme 1 — see the ESI† for synthetic procedures).[10] The crystal structure‡ of neutral host **1** reveals a water molecule at the focal point of the host's three tetrazole binding elements: one tetrazole NH donates a hydrogen bond to the water's lone pair, while the other two tetrazoles are oriented such that their lone pairs receive hydrogen bonds from the water's protons (Fig. 1). To study the cation-binding aptitude of **1** in water, the fully deprotonated host **1**-Na_3 is prepared by treatment with NaOMe. NMR titrations in buffered 60 : 40 CD_3OD–D_2O (pD* 8.65) show a steady trend of decreasing binding constants in the series starting with primary $MeNH_3^+$ (strongest) and proceeding one methyl group at a time to quaternary Me_4N^+ (weakest) (Table, entries 1–4). Despite the trianionic nature of the host under these conditions, Job plots for both the primary ammonium salt $MeNH_3Cl$ and the quaternary ammonium salt Me_4NI show a 1 : 1 binding stoichiometry (Fig. 2, inset—see the ESI† for pK_a determinations and additional Job plots). This suggests that all three of the host's convergent anionic



Scheme 1 Synthesis of hosts **1** and **2**.

groups cooperate to achieve effective binding of a single cation in this competitive medium. In analogy with a previous report of weak tetrazole–amidinium hydrogen bonding,[2] host **1**-Na_3 binds guanidinium cation below the limits of detection (K_{assoc} <10 M^{-1}) in all of the solvent systems studied.

More interesting than the association constants themselves, a key indicator of binding geometry is found in the movement of the ^1H NMR signals for the host's methylene protons upon addition of different ammonium ions. Addition of $MeNH_3Cl$ causes an upfield shift in the host's CH_2CH_3 protons, while an opposing downfield shift is observed upon binding of Me_4NI (Fig. 2 and

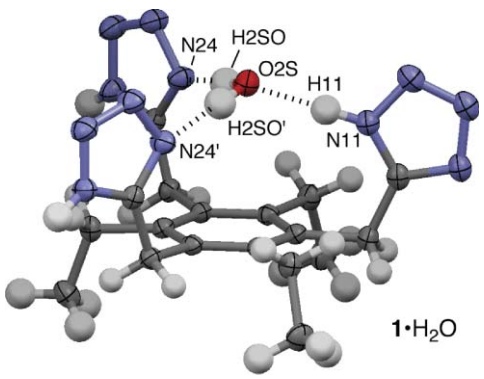


Fig. 1 X-Ray crystal structure of **1**·H_2O with thermal ellipsoids displayed at 50% probability. Selected hydrogen bonding distances and angles: N11–O2S 2.664(2) Å; N11–H11–O2S 179(3)°; N24–O2S 2.9374(14) Å; O2S–H2SO–N24 176.1(16)°. Primed atoms are disposed about a crystallographically imposed plane of symmetry. A solvent MeOH molecule on the exterior of the complex has been omitted for clarity.

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† Electronic supplementary information (ESI) available: Synthetic procedures and characterization, pK_a determinations, supplementary Job plot, NMR titration, and molecular modeling data. See DOI: 10.1039/b705256a

Table 1 Binding constants and binding-induced chemical shifts for hosts **1**-Na_3 and **2**-Na_3 determined by ^{1}H NMR titration at 298 K

Entry	Host	Guest	Solvent[a]	K_{assoc}[b]/M^{-1}	$\Delta\delta_{max}$[c]/ppm
1	**1**-Na_3	$MeNH_3Cl$	M/W	1030	−0.29
2	**1**-Na_3	Me_2NH_2Cl	M/W	180[d]	0[d]
3	**1**-Na_3	Me_3NHCl	M/W	50	0.06
4	**1**-Na_3	Me_4NI	M/W	50	0.17
5	**1**-Na_3	$MeNH_3Cl$	W	<10	0
6	**1**-Na_3	Me_4NI	W	65	0.07
7	**2**-Na_3	$MeNH_3Cl$	M/W	1900	−0.07
8	**2**-Na_3	$MeNH_3Cl$	W	<10	0
9	**2**-Na_3	Me_4NI	M/W	<10	0
10	**2**-Na_3	Me_4NI	W	<10	0

[a] M/W = 60 : 40 CD_3OD–D_2O, buffered with 10 mM Na_2HPO_4 at pD* 8.65. W = D_2O buffered with 10 mM Na_2HPO_4 at pD 7.4. [b] Values determined by fitting of ^{1}H NMR titration data to a 1 : 1 binding isotherm. All values are the average of 2–3 runs with [Host] = 1–2 mM, and include K_{assoc} values obtained from *all* host protons that undergo chemical shift during the titrations. Estimated uncertainty ±20%. Values reported as <10 are for titrations that produced negligible chemical shift of any host protons. [c] Maximum binding-induced chemical shift of the diagnostic host CH_2CH_3 protons. [d] The CH_2CH_3 protons did not shift during this titration. K_{assoc} was determined by observation of other host protons.

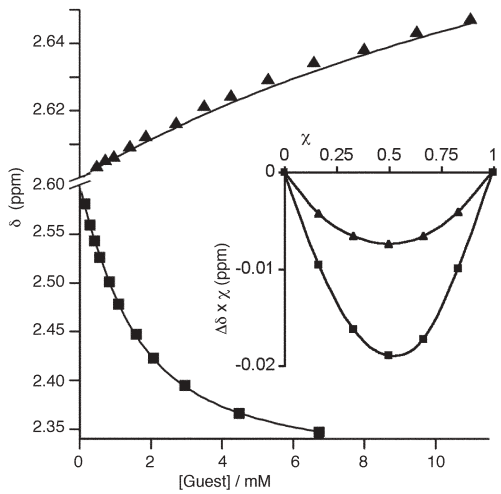

Fig. 2 ^{1}H NMR data showing divergent chemical shifts of the diagnostic host CH_2CH_3 signal upon titration with $MeNH_3Cl$ (■) and Me_4NI (▲) at 298 K. [Host] = 1 mM. Solvent = 60 : 40 CD_3OD–D_2O (10 mM Na_2HPO_4, pD* = 8.65). Fits of data to 1 : 1 binding isotherms are shown as solid lines. Inset: Job plots for $MeNH_3Cl$ (■) and Me_4NI (▲) demonstrate the formation of 1 : 1 complexes for both guests. [Host] + [Guest] = 5 mM. See the ESI† for representative titration curves in buffered water.

Table).§ Molecular modeling[11] reveals the source of these opposing chemical shifts. The complex $\mathbf{1}^{3-}\cdot MeNH_3^+$ assumes a geometry similar to that observed in the crystal structure of $\mathbf{1}\cdot H_2O$; the edge of each tetrazolate ring forms hydrogen bonds with the primary ammonium ion, and the protons of the host's adjacent methylene group are thus forced into the (shielding) face of the aromatic tetrazolate (Fig. 3(a)). Modeling suggests that in complex $\mathbf{1}^{3-}\cdot Me_4N^+$ the tetrazolate binding elements can adopt a variety of stable edge-on and face-on geometries (see the ESI†), but the NMR data is most consistent with a structure in which the tetrazolate arms have rotated to present their aromatic faces to the guest. In this geometry, the downfield shift of the host's adjacent

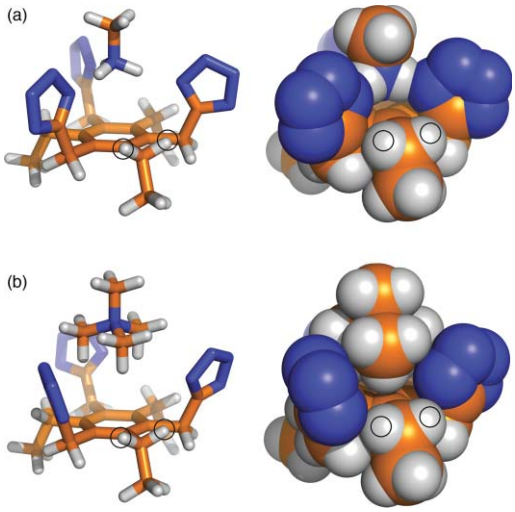

Fig. 3 HF energy-minimized stick diagram and space-filling models obtained for (a) $\mathbf{1}^{3-}\cdot MeNH_3^+$ and (b) $\mathbf{1}^{3-}\cdot Me_4N^+$ using the 6-31+G* basis set. The host's methylene protons indicated with circles are those whose ^{1}H NMR signals shift upfield (for $MeNH_3^+$) or downfield (for Me_4N^+) upon binding.

methylene protons is explained by their location at the deshielding edge of the host's tetrazolate (Fig. 3(b)).

Why does the host choose to direct the tetrazolate faces toward the guest, and what is the nature of the interaction between an anionic tetrazolate face and a quaternary ammonium ion? Titrations in the more competitive pure D_2O show that primary $MeNH_3^+$ is not bound to **1**-Na_3 at all, while Me_4N^+ forms a complex slightly stronger than in CD_3OD–D_2O mixtures (Table 1, entries 5 and 6). This result suggests that the tetrazolate rings can participate in the hydrophobic effect (that is, experience favorable dispersive interactions with the guest in place of weaker alternative interactions with water) when they present their faces to the more hydrophobic quaternary ammonium guest. Further evidence for the significance of the hydrophobic nature of the tetrazolate faces comes from the study of tricarboxylate host **2**-Na_3, an analog with similar overall charge, geometry, and hydrogen bonding capability, but lacking the faces of the tetrazolate host. As expected, the higher charge density of **2**-Na_3 relative to tetrazole **1**-Na_3 provides stronger electrostatic binding—in the less polar CD_3OD–D_2O mixtures, K_{assoc} for $\mathbf{2}^{3-}\cdot MeNH_3^+$ is almost double that of $\mathbf{1}^{3-}\cdot MeNH_3^+$ (Table, entries 1 and 7). However, in pure D_2O, host **2**-Na_3 *does not bind* the quaternary ammonium guest Me_4N^+ (Table, entry 10) despite its electrostatic superiority over host **1**-Na_3. Only the tetrazole host in its face-on orientation provides a complementary hydrophobic surface for interaction with Me_4N^+.

It is well known to medicinal chemists that tetrazolate is generally more hydrophobic than carboxylate, but tetrazolate-for-carboxylate substitutions are only sometimes beneficial for binding.[1] Does the shape-dependent hydrophobic character of **1** help explain the interactions of tetrazolate ligands with biomolecules? Losartan, the first marketed AT_1 receptor antagonist for the treatment of hypertension, is an example of a tetrazolate-containing drug that displays enhanced potency (11-fold improved IC_{50}[1,12]) relative to its carboxylate-containing analog.[13] An early study of losartan SAR/mutation data showed clear differences between carboxylate- and tetrazolate-receptor interactions that the

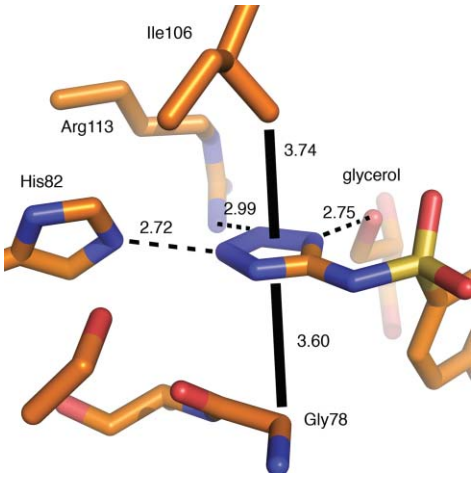

Fig. 4 A portion of Type II dehydroquinase-inhibitor complex (PDB code 2C4W[18]) showing edge-on hydrogen bonds (dashed lines) and face-on hydrophobic contacts (solid lines) between the tetrazolate of the inhibitor and neighboring fragments. Selected distances (Å) between heavy atoms are indicated.

authors could only attribute to a structurally undefined "non-conventional salt bridge" between losartan's tetrazolate and the AT_1 receptor's Lys199.[14] More than ten years later, the nature of the interaction between the tetrazolate of losartan (and those of newer tetrazolate-containing AT_1 antagonists) and the protein is still debated,[15,16] but our current results are most consistent with a recently published model[15] in which the γ, δ, ε methylenes of Lys199 make a hydrophobic contact with losartan's tetrazolate face.[17] Further evidence for the relevance of geometry-dependent hydrophobicity for tetrazolates comes from one of the few crystal structures of a bound anionic tetrazolate ligand in the Protein Data Bank. The structure of a tetrazolate ligand bound to Type II DHQase from *H. pylori* (PDB code 2C4W)[18] reveals a significant hydrophobic contact on each face of the inhibitor's tetrazolate ring and an equator of hydrogen bonding contacts to polar residues positioned around the tetrazolate's edges (Fig. 4).

In terms of binding capabilities in physiologically relevant solutions, our tetrazole-derived host occupies a middle ground between related hosts built using neutral heterocycles[19–26] (which rely on uncharged hydrogen bonds too weak to bind cationic guests in pure water without extensive hydrophobic decoration[27]) and carboxylate host **2**-Na_3 (which can employ electrostatic attraction for binding in less polar solvents, but is too well solvated to bind ammonium ion guests in pure water). We are currently seeking hosts with higher affinity for quaternary ammonium ions in order to take advantage of the attractive features of tetrazole-derived hosts (low molecular weight, ease of synthesis, and good pharmacological properties) not found in classical cyclophane-derived quaternary ammonium ion receptors.[28–30]

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Notes and references

‡ Crystals of **1**·H_2O·MeOH grown by slow evaporation of a wet methanol solution of **1**: $C_{19}H_{30}N_{12}O_2$, M_r = 458.55 g mol^{-1}; colorless prism, 0.65 × 0.41 × 0.40 mm; orthorhombic, $Cmc2_1$ (no. 36); a = 11.9746(7), b = 18.4624(11), c = 10.3004(6) Å, V = 2277.2(2) Å^3; D_c = 1.337 Mg m^{-3}; μ = 0.094 mm^{-1}; Mo-Kα radiation, λ = 0.71073 Å; T = 193 K; $2\theta_{max}$ = 52.76°; 8287 reflections measured (2437 independent); structure solution and refinement using *SHELXS-97* and *SHELXL-97* (G. M. Sheldrick, University of Göttingen, Göttingen (Germany), 1997); 183 parameters, hydrogen atoms generated in idealized positions; R_1 = 0.0262 (for 2386 reflns with $I > 2\sigma(I)$), wR_2 = 0.0709 (for all 2437 independent reflections). CCDC 637204. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b705256a.

§ As expected, the host's other protons all move downfield upon binding any cationic guest and the guests' methyl protons move upfield upon binding the anionic host.

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