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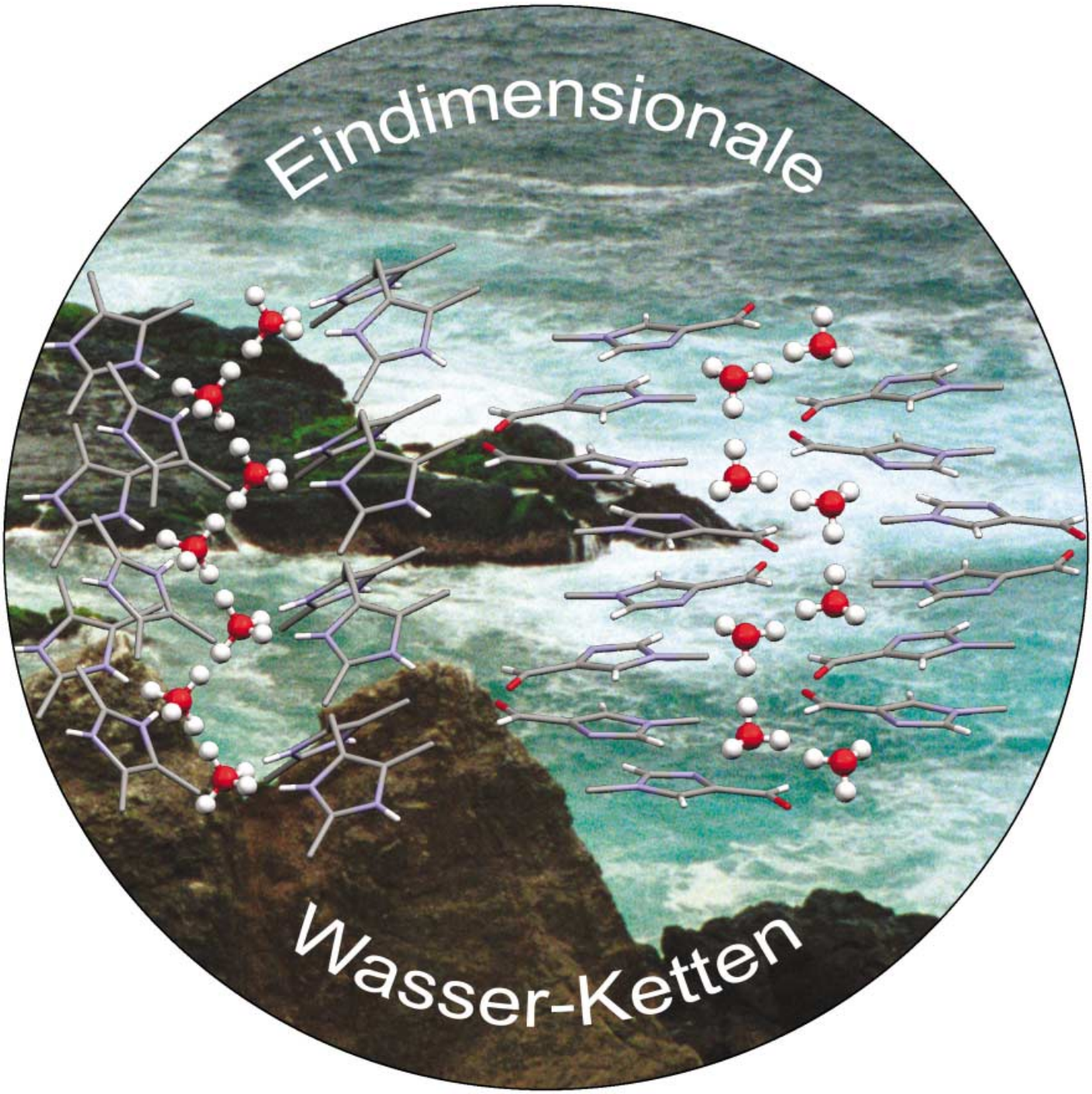

Eindimensionale Ketten aus Wassermolekülen („Protonendrähte“) sind an der Protonenpermeation durch Transmembranproteine wie Cytochrom-Oxidase beteiligt. R. Buchanan et al. berichten auf den folgenden Seiten über die Stabilisierung solcher Ketten durch biologisch relevante Imidazol-Verbindungen und über die Messung der Protonendynamik.

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One-Dimensional Water Chains

Structures and Solid-State Dynamics of One-Dimensional Water Chains Stabilized by Imidazole Channels**

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One-dimensional (1D) water chain structures constitute a potentially important form of water that is poorly understood.[1] Many fundamental biological processes[2–9] appear to depend on the unique properties of water chains. For example, water chains may assist in proton translocation through membranes by functioning as "proton wires".[10a] Such behavior is illustrated by the membrane protein gramicidin A (gA)[2] in which protons are envisioned[10b] to either hop along a single-file chain of water molecules according to the Grotthuss relay mechanism,[11] or migrate as $H_9O_4^+$[12] or $H_5O_2^+$[13] ionic water clusters.[14] In addition, water chains appear to be important to the proton pathways in the cytochrome b6f complex of plant chloroplast thylakoid membrane and mitochondrial ATPase,[3] and carbonic anhydrase II,[4] as well as in redox proteins such as cyctochrome oxidase,[5] bacteriorhodopsin,[6] and the photosynthetic reaction center of *Rhodobacter sphaeroides*.[7] Water chains are also found in membrane aquapores, which are important to the function of the nicotinic receptor M2δ_5[8] as well as the influenza A M2 virus.[9]

Curiously, little is known of the structural constraints required in stabilizing 1D water chains. Unlike bulk water or ice,[15] 1D water chains appear to be stabilized by strong H-bonding between neighboring water molecules along the chain as well as H-bonding between water molecules and donor–acceptor groups associated with channels.[2,8–9] Recently, we have discovered that certain imidazole compounds stabilize infinite 1D water chains relevant to the biological structures mentioned above. Herein we describe the crystal structures of the hydrated compounds and preliminary data from solid-state ^{2}H NMR studies and calorimetric analyses that establish the possibility of reorientation dynamics of the water-chain nanostructures.

The crystal structures of hydrates of 1-methylimidazole-4-carboxaldehyde (**1**)[16] and 4,4′-methylene-bis(2,5-dimethylimidazole) (**2**)[17] are shown in Figure 1, along with their respective packing representations in Figure 2, which also display the arrangement of imidazole N atoms in both compounds in relation to their infinite 1D water chains. The water channels in **1** are generated by weak π interactions between imidazole molecules in alternating layers along the crystallographic *c* axis. The closest intermolecular interactions between layers are 3.282 Å (C1′–C3), 3.297 Å (C1′–N1) and 3.323 Å (C3–N1′), and individual channels are linked together by weak interactions (C3···O1″ 3.273 Å) between neighboring imidazole molecules (see Supporting Information). The approximate dimension of the water channels in compound **1** is 6.62×3.44 Å^2.

The water molecules in **1** are trigonally distorted and form strong hydrogen bonds with symmetry related molecules in the chain as well as with imidazole nitrogen atoms directed towards the channel. Each water molecule has a full-occupancy H-atom (H1o) that is strongly hydrogen bonded to N2 (N2···O2 2.8424(16) Å). The other H-atoms (H2ao and H2bo) are positionally disordered and were treated as half-occupancy atoms during refinement. Both H2ao and H2bo are hydrogen bonded (see Supporting Information) to symmetry related waters, and the O2···O2′ ($-x+1,-y,-z+1$) and O2···O2″ ($-x+1$, y,$-z+3/2$) separations are 2.763(2) and 2.783(2) Å, respectively, thus indicating the presence of strong H-bonding between water molecules in the chain. Overall, the shape of the water chain resembles the "arm chair" motif proposed by Nagle and Morowitz[20] for 1D proton wires.

Compound **2** has a different packing arrangement of imidazole molecules (Figure 2) that results in water channels oriented along the crystallographic *b* axis. The imidazole (N–H) and water hydrogen atoms where located and refined as half-occupancy atoms. Both water molecules associated with the asymmetric unit were found to be tetrahedrally disordered. The first water molecule containing O1 forms strong hydrogen bonds (see also Supporting Information) with imidazole N1 (O1···N1 2.828(2) Å) and N3 (O1···N3 2.854(2) Å) atoms, while the second water molecule, containing O2, is hydrogen bonded to N2′ ($x+1/2$, $y+1/2$, z) (O2···N2′ 2.827(2) Å) and N4″ (x, $-y+1$, $z-1/2$) (O2···N4″ 2.786(2) Å) associated with different imidazole molecules on opposite sides of the water channel. In addition, both water molecules are strongly hydrogen bonded to each other (O1···O2 2.778(2) Å) and form a "zig-zag" motif, which is the second probable proton wire structure proposed by Nagle and Morowitz.[20] The water channel formed by the stacking of **2** has an approximate rectangular shape ($\approx 4.80 \times 3.02$ Å^2) and a smaller pore size compared to **1** (Supporting Information). The apparent smaller pore diameter of **2** appears better suited for stabilizing the zig-zag arrangement of water molecules over the arm-chair motif.[21]

Curiously, there have been no comprehensive studies of water dynamics in systems containing infinite water-chain

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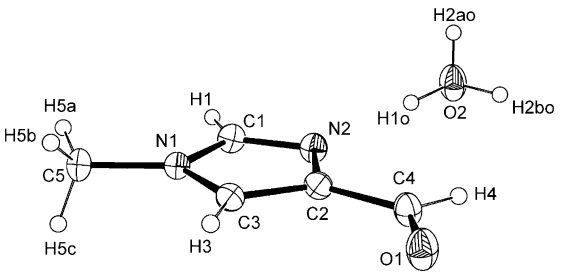
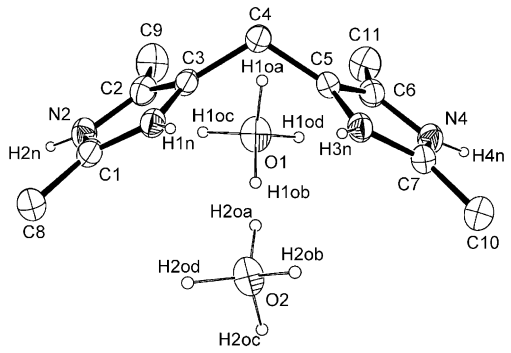


1



2

Figure 1. ORTEP[18] views of the molecular structures of compounds **1** ($C_5H_6N_2O{\cdot}H_2O$) and **2** ($C_{11}H_{16}N_4{\cdot}2\,H_2O$). Thermal ellipsoids are drawn at 40% probability level. The hydrates in both structures are disordered and form strong N–H···O and O–H···O hydrogen bonds. In compound **2**, the methyl and methylene H atoms are omitted for clarity.

structures. This fact may be due in part to the limited number of well-characterized continuous water chain structures involving small molecules.[22–25] To determine if **1** and **2** serve as models of biological proton wires, we have studied the dynamics of the water chains by using solid state ^2H and ^{17}O NMR spectroscopy and examined the calorimetric behavior of the compounds by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

In the context of proton transfer along an H-bonded chain of water molecules, dynamical disorder plays a central role. In particular, the proton positions between adjacent waters in the structures **1** and **2** are refined in the X-ray structure at half-occupancy and we probed the dynamics of this disorder by using ^2H and ^{17}O solid-state NMR. Water dynamics affect ^2H and ^{17}O spectra if O–H bonds reorient, thereby modulating the NMR spectrum in a manner completely analogous to chemical exchange. In both experiments, orientation dependent NMR frequencies arise from quadrupole coupling and the breadth of the powder spectrum defines the characteristic timescales for intermediate and fast exchange.[26] For ^2H ($S = 1$), this is the first-order quadrupole coupling with $\chi_{zz} \approx 170$ kHz. While for ^{17}O, in which the observed central transition of the $S = 5/2$ spin is effected only in second order, the spectrum breadth is ≈ 50 kHz at a field of 11.7 T for a typical hydrate water with $\chi_{zz} \approx 6$ MHz. For both experiments, we use an echo sequence to detect the broad powder spectra. Consequently, when intermediate exchange is present signals are substantially attenuated making their

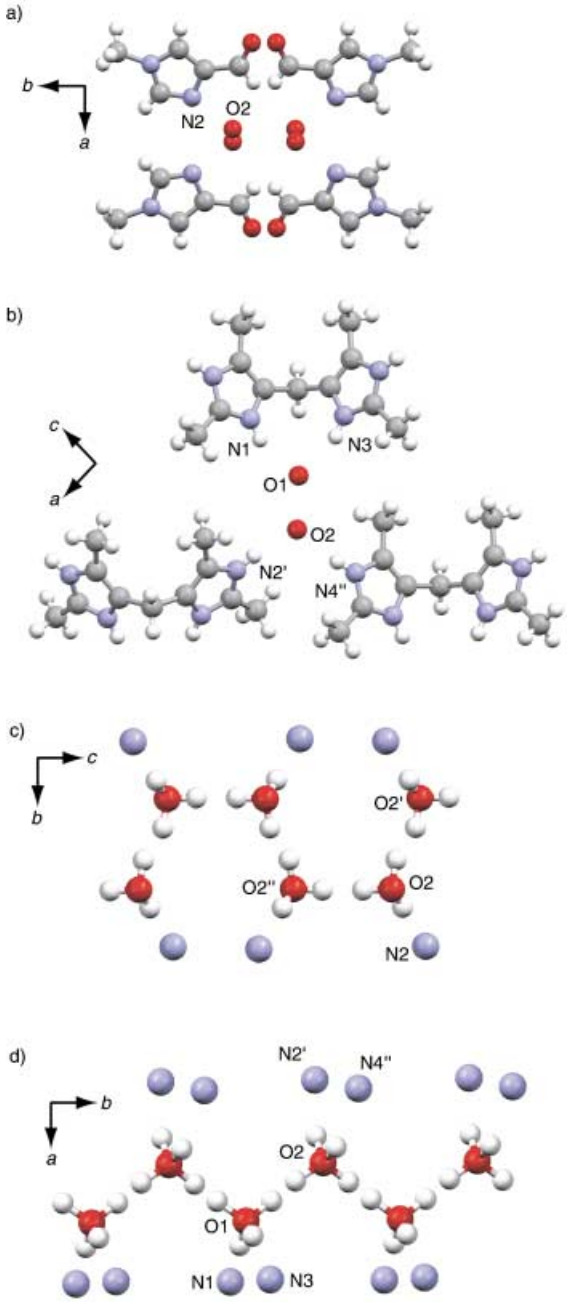


Figure 2. Packing representations[19] viewed down the water channel of compound **1** and **2** are shown in frames a) and b), respectively. In both representations water H atoms have been omitted for clarity. In addition, hydrogen-bonding representations illustrating the nanostructure of the water chains of **1** and **2** are shown in frames c) and d), respectively, with each channel oriented along the horizontal axis of the figure. Imidazole nitrogen atoms involved in hydrogen bonding are included in frames c) and d). Imidazole N–H atoms are omitted in frame d) for clarity. For more detailed packing representations of **1** and **2**, see the Supporting Information.

detection difficult. Reorientation of O–H bonds in both **1** and **2** occurs not only if waters reorient but also if a proton is transferred because, in contrast to the ice structure, the half-occupied O–H bonds for adjacent waters in **1** and **2** are not antiparallel. ^2H spectra are shown in Figure 3 and clearly

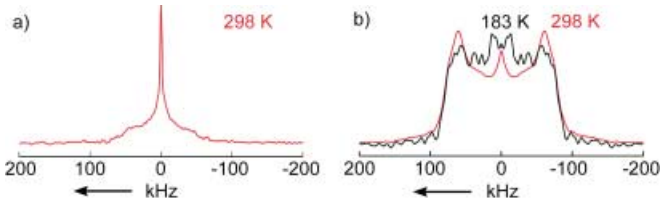


Figure 3. ^{2}H NMR powder spectra of a) compound **1** and b) compound **2**.

display different water dynamics in **1** and **2.** The room-temperature spectrum of **2** is dominated by features for stationary deuterons, supported on a lower time-scale by the ^{17}O spectrum of **2** (see Supporting Information). In contrast, the spectrum of **1** has a different shape with substantially reduced breadth and intensity indicative of intermediate exchange dynamics. Similar spectra were observed for ice where they could be uniquely attributed to molecular rotation.[26]

Interestingly, on lowering the temperature of **2** to 183 K, the corresponding spectrum (obtained with long recycle delay to avoid spectral distortion from saturation) shows the appearance of a narrow central feature indicative of a dynamic water. This suggests that one of the two water molecules in **2** is dynamically disordered and not seen in the ^{2}H spectrum at 298 K owing to intermediate exchange. Upon lowering the temperature, water motion slows and approaches slow exchange resulting in increased signal intensity. Over the range of temperature studied, the amine deuteron signals are saturated and not observed because of long T_1 values (>60 s). Similar proton dynamics are observed for other small molecule proton wire models, which include discontinuous water chain structures[27] and hydrated tripeptide crystals.[28] In the latter system, for example, Pometun et al.[28] have demonstrated the presence of dynamic deuterium exchange consistent with the Grotthuss mechanism.[10,11]

Crystalline samples of **1** and **2** also were examined by TGA and DSC under open conditions (see Supporting Information). TGA analyses show weight losses of 14.2 and 15.2 % for **1** and **2**, respectively. These results are consistent with the loss of one (**1**) and two (**2**) water molecules per imidazole molecule, which is in good agreement with the X-ray crystal structures. DSC for **1** shows a single sharp endotherm centered at 37 °C with a molar enthalpy of 16 kJ, which corresponds to 16 kJ per water molecule and 5 kJ per H-bond. The DSC measurements for compound **2** show the dehydration endotherm has two peaks indicating that the water molecules are in different environments, which is consistent with the X-ray crystal structure and differences in the ^{2}H exchange dynamics observed for the two water molecules. The total molar enthalpy was 44 kJ per water molecule and corresponds to 11 kJ per H-bond. Results similar to **2** have been reported previously for hydrated tripeptides[28] and theophylline monohydrate[29] and are in good agreement with the strength of a hydrogen bond.[30]

In conclusion, the X-ray crystal structures, solid state ^{2}H reorientation dynamics and calorimetry measurements suggest that compound **1** and **2** are potential models of biologically relevant proton wires. Future studies on these and related 1D water chain structures will include proton conductivity measurements, more detailed examination of deuterium exchange and hydrate reorientation dynamics in crystalline and powdered samples, as well as molecular modeling.

Experimental Section

Compound **1** was prepared by using a procedure reported previously and a ^{1}H NMR spectrum of **1** in $CDCl_3$ was in good agreement with previously reported results.[16] The off-white powder was crystallized by dissolving the compound (40 mg, 0.31 mmol) in 9:1 MeOH:H_2O (0.5 mL) layered with diethyl ether and cooled to 270 K. Colorless block crystals were isolated from the crystallization solution prior to the X-ray diffraction analysis.

Compound **2** was prepared by using a procedure reported elsewhere[17] and isolated in approximately 60 % yield. The white powder was crystallized by dissolving **2** (0.2 g, 0.83 mmol) in 9:1 MeOH:H_2O (1 mL) followed by slow evaporation of the crystallization solution. Colorless block crystals were isolated from the crystallization solution prior to the X-ray diffraction analysis. Characterization: ESIMS: 205.28 $[M+H]^+$; ^{1}H NMR (500 MHz, CD_3OD, 25 °C): $\delta = 3.65$ (s, 2H, -CH_2-), 2.15 (s, 3H, -CH_3), 2.05 ppm (s, 3H, -CH_3).

Crystallographic Studies: Single crystals of **1** and **2** were mounted on a 0.05 mm CryoLoop with Paratone oil for collection of X-ray data using omega scans on a Bruker SMART APEX CCD diffractometer. The following programs were used during data collection and refinement: SMART (V 5.625 to acquire frame data),[31a] SAINT (V 6.22 to determine final unit cell parameters and integrate frame data)[31b] and structures were solved by direct methods with SHELXS-90[31c] and refined by least square methods on F^2 with SHELXL-97[31d] incorporated into the SHELXTL (V 6.12)[31e] suite of programs.

Crystal structure analysis of $C_5H_6N_2O{\cdot}H_2O$ (**1**): $0.32\times0.25\times0.14$ mm, monoclinic, space group $C2/c$, $a = 20.149(4)$ Å, $b = 8.5299(17)$ Å, $c = 7.3447(15)$ Å, $\beta = 96.41(3)°$, $V = 1254.5(4)$ Å^3, $\rho_{calcd} = 1.357$ Mg m^{-3}, $Z = 8$, $\mu = 0.106$ mm^{-1}, $2\theta_{max} = 4.08$ to 56.52, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $T = 100$ K, F(000) 544, total reflections = 5325, independent reflections = 1466 ($R_{int} = 0.023$), observed data = 1350 ($I > 2\sigma(I)$), adsorption correction: SADABS (V 2.02),[31f] transmission factors max: 0.99, min: 0.90. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms of the water molecule are disordered. One full occupancy hydrogen atom (H1o) and two half-occupancy hydrogen atoms (H2ao and H2bo) were located by difference maps and refined isotropically in a trigonal arrangement about O2. Imidazole and carbonyl hydrogen atoms also were located by difference maps and refined isotropically. Methyl hydrogen atoms were placed in their geometrically generated positions and refined as a riding model. The final anisotropic full matrix least-squares refinement on F^2 for 102 variables converged at $R1 = 0.043$ and $wR2 = 0.107$ with a GOF of 1.04, 0.33 e Å^{-3}, residual based on $I > 2\sigma(I)$.

Crystal structure analysis of $C_{11}H_{16}N_4{\cdot}2H_2O$ (**2**): $0.36\times0.28\times0.25$ mm, monoclinic, space group Cc, $a = 17.9264(19)$ Å, $b = 4.5526(5)$ Å, $c = 17.8364(19)$ Å, $\beta = 105.623(2)°$, $V = 1401.9(3)$ Å^3, $\rho_{calcd} = 1.139$ Mg m^{-3}, $Z = 4$, $\mu = 0.081$ mm^{-1}, $2\theta_{max} = 4.72$ to 56.38, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $T = 296$ K, $F(000)$ 520, total reflections = 5680, independent reflections = 2963 ($R_{int} = 0.0224$), observed data = 2597 ($I > 2\sigma(I)$), adsorption correction: SADABS,[31f] transmission factors max: 0.98, min: 0.97. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms on the water molecules and the imidazole NH atoms are disordered and were located by difference electron density maps. Both sets of hydrogen atoms were refined isotropically as half-occupancy atoms and constrained by using the DFIX command in SHELXTL. Methyl and methylene

hydrogen atoms were placed in their geometrically generated positions and refined as a riding model. The final anisotropic full matrix least-squares refinement on F^2 for 184 variables converged at $R1 = 0.045$ and $wR2 = 0.096$ with a GOF of 1.02, 0.17 e Å^{-3}, residual based on $I > 2\sigma(I)$.

CCDC-212688 (**1**) and -212689 (**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Supporting Information available: Views showing important intermolecular contacts in **1** and illustrations of the water channels in **1** and **2**, ^{17}O NMR spectrum of **2**, TGA and DSC data for compound **1** and **2**, and H-bonding distances and angles for both compounds.

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