Supporting Information for

Coupling of Functional Hydrogen Bonds in Pyridoxal-5’-phosphate-Enzyme Model Systems Observed by Solid State NMR

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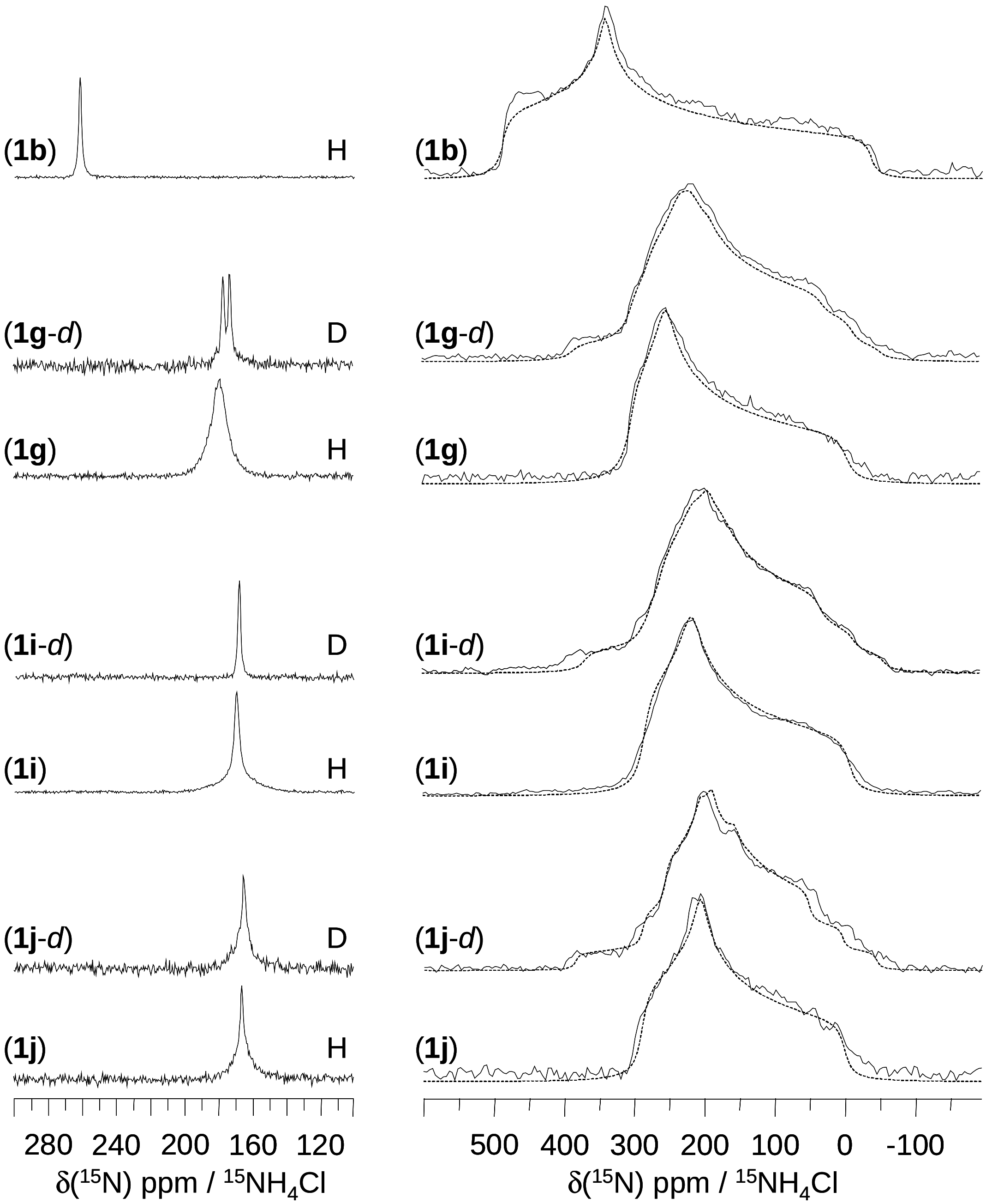

Figure S1. Superposed experimental and calculated 30.41 MHz ^{15}N{^{1}H} CP NMR static spectra solid powder samples of the aldenamine Schiff base and its 1 : 1 acid-base complexes of, **1b**, **1g**, **1i**, **1j** and deuterated **1g**-*d*, **1i**-*d*, **1j**-*d*, at room temperature. Additionally the MAS spectra of the mentioned samples are shown.

Determination of N...H/N...D - Distances by Dipolar ^{15}N Solid State NMR

The NMR theory of heteronuclear dipolar coupling[1,2,3] between ^{15}N nuclei and a deuteron has been described previously.[4,5] The N...L - Distances, with L = H, D, are calculated from the dipolar frequency of $\nu_d^{NL} = D^{NL}$ (1-3$cos^2\,\theta_d^{NL}$) is the dipolar coupling constant D^{NL} given by

$$\nu_d^{NL} = D^{NL}\,(1\text{-}3cos^2\,\theta_d^{NL}),\; D^{NL} = \frac{\gamma^N\gamma^L h\mu_0}{16\pi^3 r_c^3(\mathrm{NL})}. \tag{1}$$

γ^N and γ^L are the gyromagnetic ratios of the two nuclei, h Planck's constant, μ_0 the magnetic permeability. r_c(NL) represents the average reversal cubic internuclear distance N...L in expression in Å

$$D^{NH} = 12158\; r_c^{-3}(\mathrm{NH})\,[\mathrm{Hz}],\ \text{and}\ D^{ND} = 1868\; r_c^{-3}(\mathrm{ND})\,[\mathrm{Hz}]. \tag{2}$$

θ_d^{NL} is the angle between the magnetic field and the vector NL. This angle can more conveniently be described by the Euler angles in the coordinate system of the CSA tensor - ϕ^B and θ^B for the magnetic field and α_d^{NL} and β_d^{NL} for the vector NL, respectively. It can be shown that

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(1) Mehring M. In *NMR BasicPrinciples and Progress* **11**, *High Resolution NMR in Solids*, Springer: Berlin-Heidelberg-New York, 1978.

(2) Seth, N. K.; Alderman, D. W.; Grant, D. M. *Mol. Phys.* **1990**, *71*, 217-238.

(3) Zilm, K. W.; Grant, D. M. *J. Am. Chem. Soc.* **1981**, *103*, 2913-2922.

(4) Lorente, P.; Shenderovich, I. G.; Golubev, N. S.; Denisov, G. S.; Buntkowsky, G.; Limbach, H.-H. *Magn. Reson. Chem.* **2001**, *39*, 18-29.

(5) Hoelger, C.-G.; Limbach, H.-H. *J. Phys. Chem.* 1994, *98*, 11803-11810.

$$\cos\theta_{\mathrm{d}}^{\mathrm{NL}} = \sin\beta_{\mathrm{d}}^{\mathrm{NL}} \sin\theta^{\mathrm{B}} \cos\left(\phi^{\mathrm{B}} - \alpha_{\mathrm{d}}^{\mathrm{NL}}\right) + \cos\beta_{\mathrm{d}}^{\mathrm{NL}} \cos\theta^{\mathrm{B}} . \qquad (3)$$

The ^{15}N NMR spectrum of a polycrystalline powder is obtained from eqs. (1) - (3) by calculating ν^{N} for all possible orientations by variation of ϕ^{B} and θ^{B} using increments of 1-2°. For statistical reasons the signal intensity has to be weighted by a factor of $\sin\theta^{\mathrm{B}}$.

Experimentally, only relative chemical shifts $\delta_{\mathrm{ii}} = \delta_{\mathrm{ref}} - \delta_{\mathrm{ii}}$, ii = *XX, YY, ZZ* which are measured with respect to some standard reference, can be obtained. For the determination of the ^{15}N...D distances first the elements of the ^{15}N CSA tensor are determined by simulating the experimental solid state ^{15}N NMR spectrum of the protonated system, *i. e.* L = H, under proton decoupling. The values obtained are then corrected for H/D isotope effects on the elements of the ^{15}N CSA tensor. The new values have been used for a simulation of the proton decoupled ^{15}N NMR spectrum of the species deuterated in the hydrogen bond, by adapting the remaining parameters $\alpha_{\mathrm{d}}^{\mathrm{ND}}$, $\beta_{\mathrm{d}}^{\mathrm{ND}}$ and D^{ND}. The determination of the ^{15}N...H distances more difficult.[4] Hence, we could obtain the distances N...H by extrapolation from the observed strong dependence of the N...D distances on the isotropic chemical shifts.

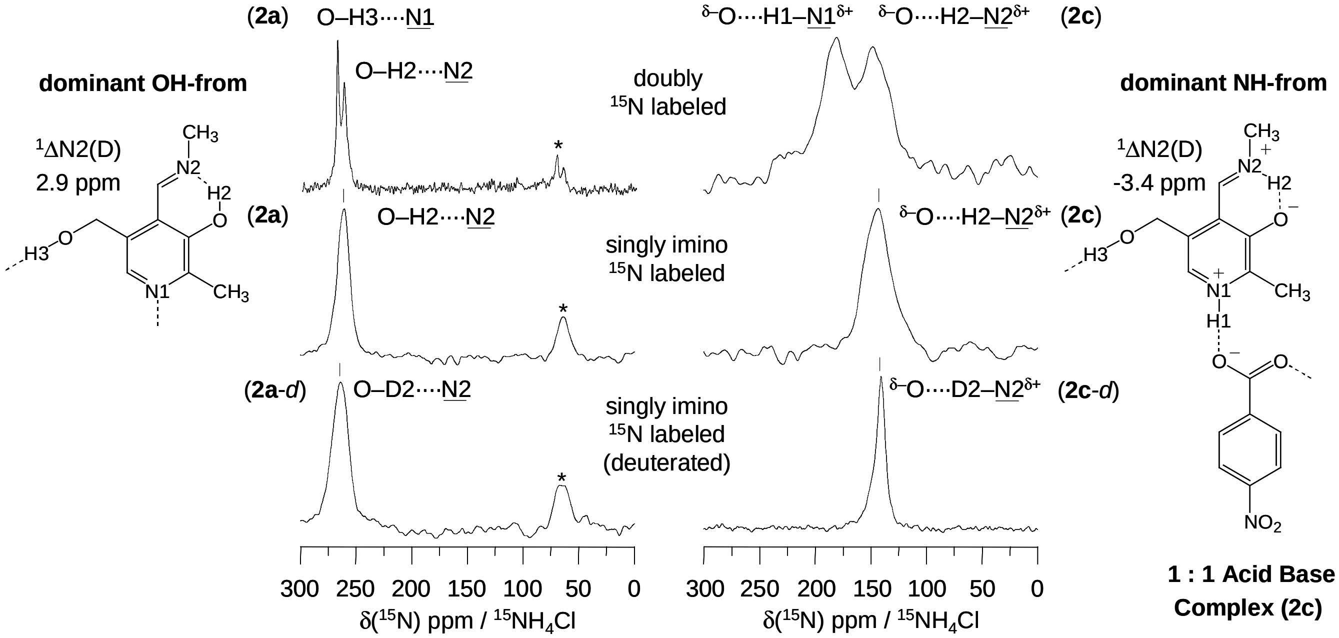

Figure S2. ^{15}N{^{1}H} CP MAS (30.41 MHz, 6 kHz) spectra of the aldimine Schiff base (**2a**), and its 1 : 1 acid-base complex **2a**-4-nitrobenzoic acid (**2c**). From the top of the figure are shown the ^{15}N labeled doubly, selectively imino, and the selectively imino of the deuterated compounds **2a**-*d* and **2c**-*d*. *: spinning sideband.

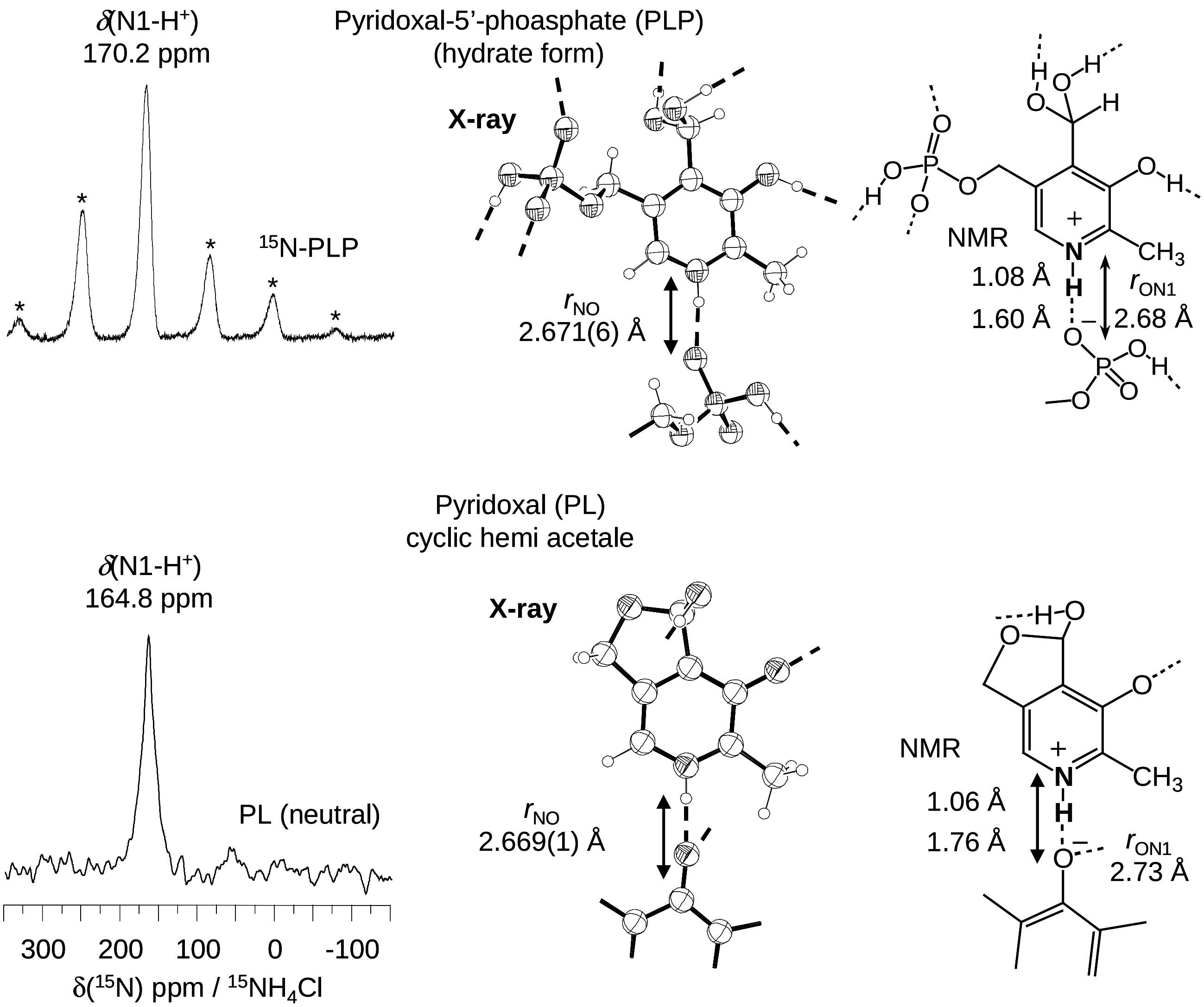

Figure S3. ^{15}N{^{1}H} CP MAS (30.41 MHz, 6 kHz) spectra of ^{15}N labeled pyridoxal-5'-phosphate (PLP), and its derivative pyridoxal (PL) in natural abundance. For the presented crystallographic structures and values see text. The spectra of ^{15}N labeled (PLP) is obtained at 60.8 MHz with a spinning speed of 5 kHz. *: spinning sidebands.

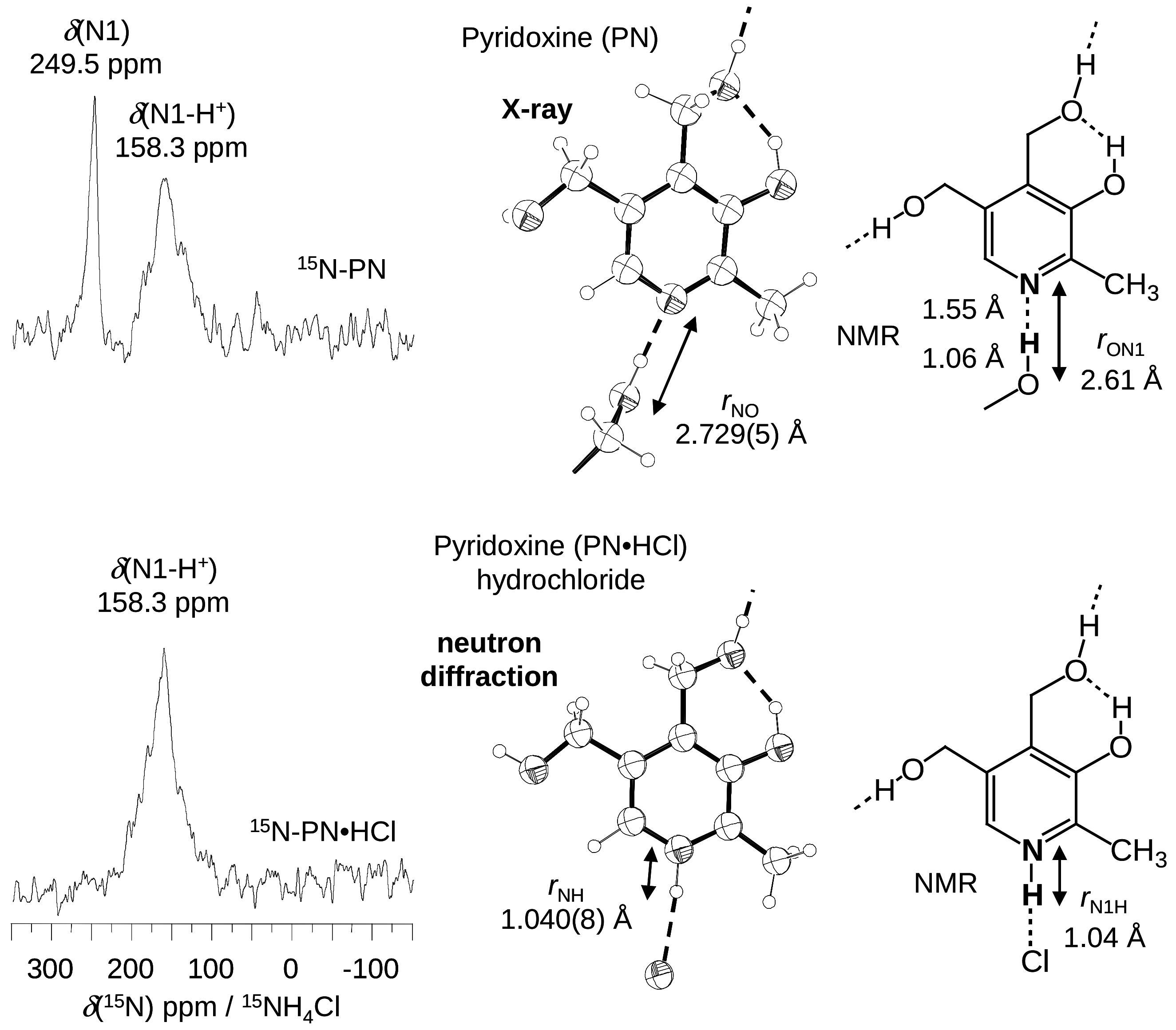

Figure S4. ^{15}N {^{1}H} CP MAS (30.41 MHz, 6 kHz) spectra of ^{15}N labeled pyridoxine (PN), pyridoxine hydrochloride (PN·HCl). For the presented crystallographic structures and values see text.

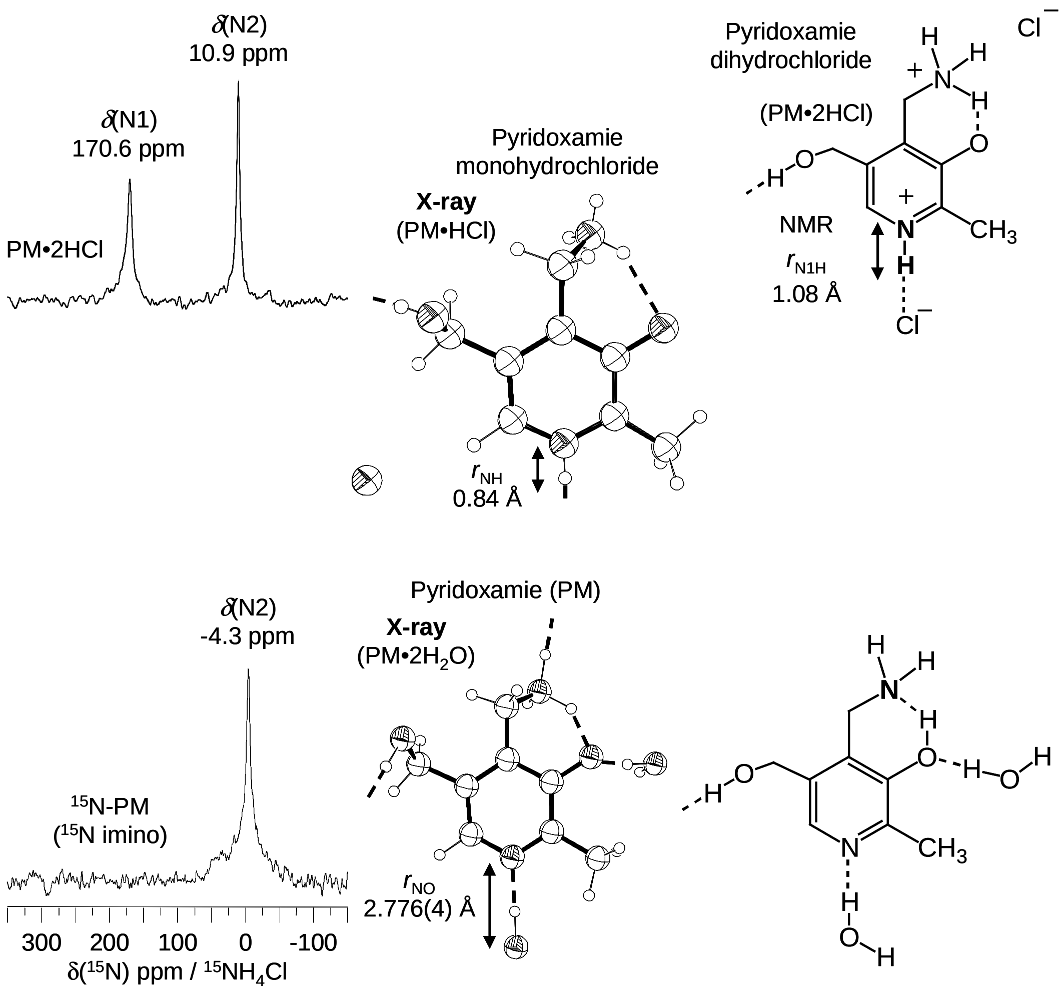

Figure S5. ^{15}N{^{1}H} CP MAS (30.41 MHz, 6 kHz) spectra of in imino position ^{15}N labeled pyridoxamine (PM) and pyridoxamine dihydrochloride (PN·2HCl) in natural abundance. For the presented crystallographic structures and values see text. We note that crystal structure is the pyridoxamine hydrochloride (PN·HCl).

Comment. The mono-hydrochloride of pyridoxamine (PM·HCl) was studied by *x*-ray crystallography, for which a value of $r_{\mathrm{HN}} = 0.84$ Å was reported, but the accuracy of the crystallographic finding is questionable. Also pyridoxamine dihydrate (PM·2 H_2O) was studied by *x*-ray diffraction, and a heavy atom distance of $r_{\mathrm{ON}} = 2.776(4)$ Å between the hydroxyl group of the adjacent water molecule and the pyridine ring was reported. For PM·2HCl we obtained by NMR a value of $r_{\mathrm{HN}} = 1.08$ Å for the pyridine-HCl hydrogen bond, indicating a zwitterionic structure. We observed for the amino nitrogen a ^{15}N chemical shift of 10.9 ppm, which we assign to the amino group in the protonated form, hydrogen bonded to chloride Cl^-. For neutral dihydrate of PM $2H_2O$, where the ammonium group is hydrogen bonded to water, a high field shift to -4.3 ppm was observed.

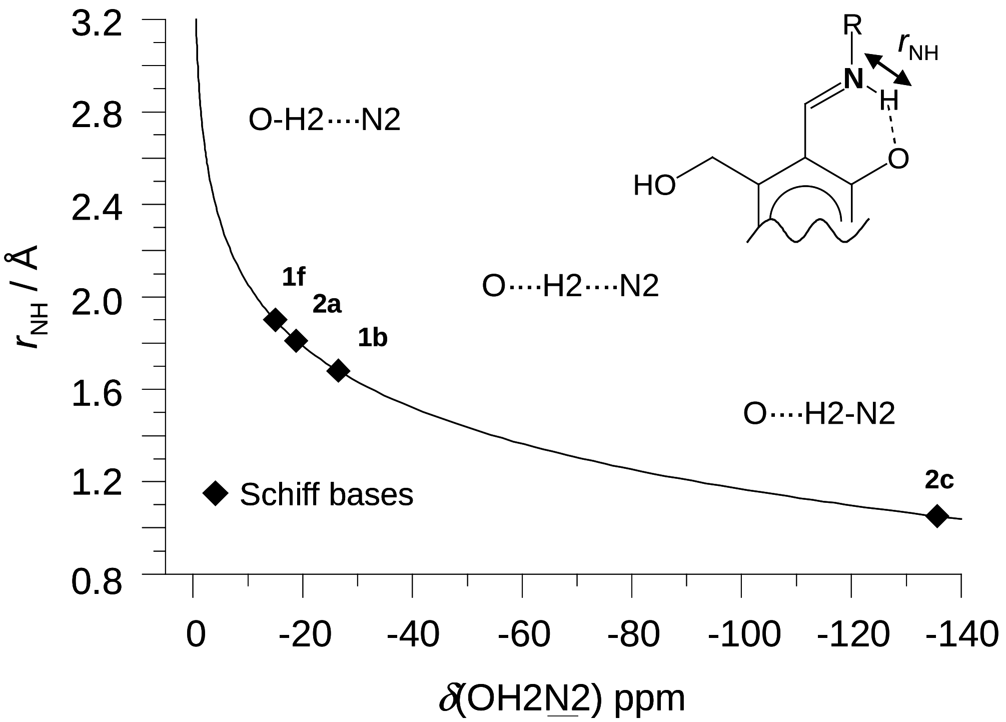

Figure S6. Plot of the estimated intramolecular r_{NH} distances against the intrinsic ^{15}N chemical shift of the imino nitrogen in the Schiff bases **1b** and **2a** as well as their acid-base adducts **1f** and **2c**. For the calculation of the solid line see the main text.

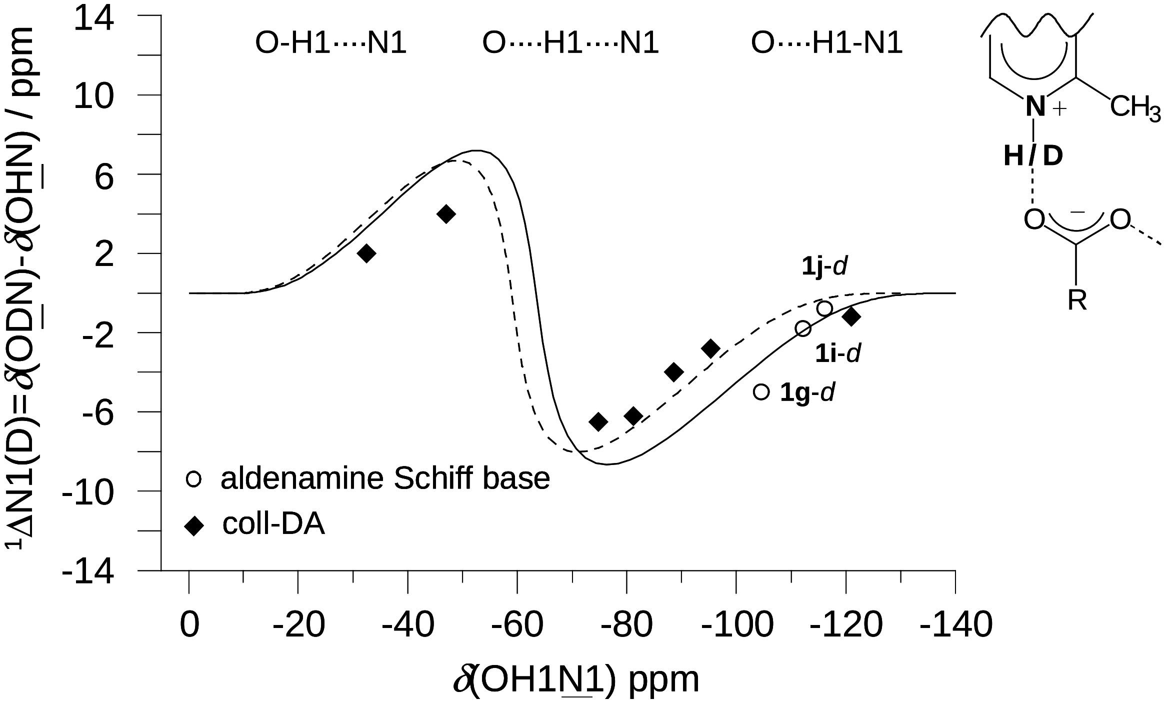

Figure S7. Secondary isotopic effect $^1\Delta$N1(D) *vs.* intrinsic ^{15}N chemical shift of the intermolecular OH1N1 hydrogen bond. The open circles represented the aldimine Schiff base-adducts **1g**-*d*, **1i**-*d*, and **1j**-*d*, the solid curve correspond to this data. The filled diamonds represented the collidine-DA complexes which were obtained from literature, the dash curve correspond to this data. For further details see the main text.

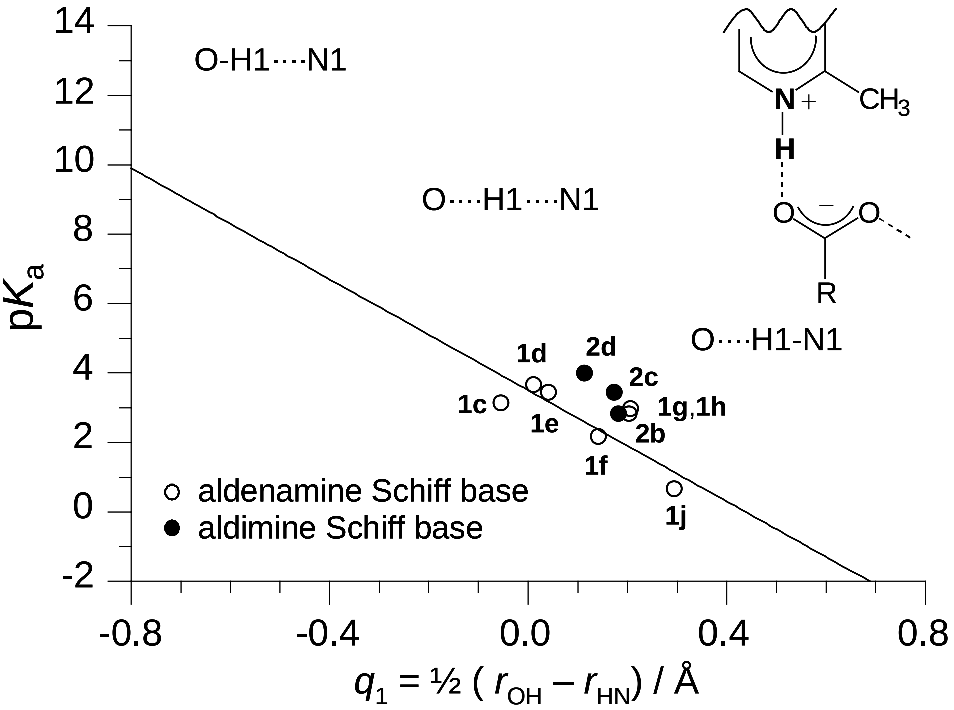

Figure S8. pK_a values *vs*. proton transfer coordinate q_1^H correlation of the acid components in the organic solids of the aldenamine Schiff base-adducts **1b** to **1j** (open circles) and aldimine Schiff base-adducts **2b** to **2e** (filled circles). The pK_a values is a function of q_1^H with the relation pK_a = A – B q_1^H by using the parameters A = 3.5 and B = 8.

Table S1. NMR crystallographic values. [a,b,c,d]

	pK_a	δ(OH1N1) [a] / ppm	r_{NH} [b] / Å	r_{OH} [b] / Å	q_1^{H} [c] / Å	q_2^{H} [d] / Å
1c	3.14	-60.2	1.32	1.21	-0.05	2.53
1d	3.66	-71.6	1.25	1.27	0.01	2.52
1e	3.44	-77.0	1.22	1.30	0.04	2.52
1f	2.17	-95.1	1.14	1.43	0.14	2.57
1g	2.82	-104.6	1.10	1.51	0.20	2.62
1h	2.97	-104.9	1.10	1.52	0.21	2.62
1i	-7	-112.2	1.08	1.60	0.26	2.68
1j	0.66	-116.2	1.06	1.65	0.29	2.72
2b	3.99	-90.1	1.16	1.39	0.11	2.55
2c	3.44	-100.2	1.12	1.47	0.17	2.59
2d	2.82	-101.4	1.12	1.48	0.18	2.60
2e	-6.65	-118.7	1.06	1.69	0.32	2.75

[a] Values of the pyridine nitrogen are with respect to solid **1a**, resonating at 282.5 ppm. [b] NMR crystallographic values are extrapolated from the experimental isotropic ^{15}N chemical shifts. [b] $q_1^H = ½ (r_{ON} - r_{NH})$. [c] $q_2 = r_{HN} + r_{OH}$.