Electronic Supplementary Material (ESI) for Faraday Discussions.

Proton transfer in guanine–cytosine base pair analogue studied by NMR spectroscopy and PIMD simulations

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Supporting Information

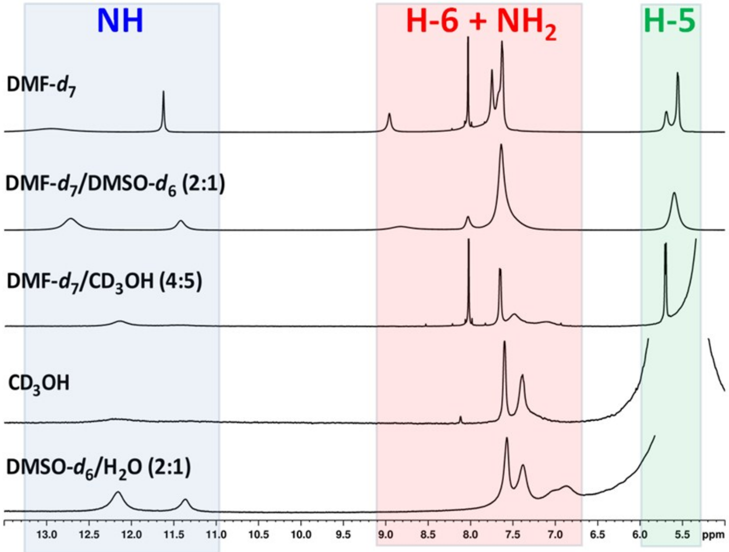

Figure S1. Part of ^{1}H NMR of isocytosine acquired in different solvents and their mixtures at -60 °C. In order to observe exchangeable NH and NH_2 signals, protonated (non-deuterated) protic solvents (CD_3OH, H_2O) were used. It was found that protic solvents or their mixtures are not suitable for the measurement due to their strong OH signal, which overlaps H-5 signals of isocytosine.

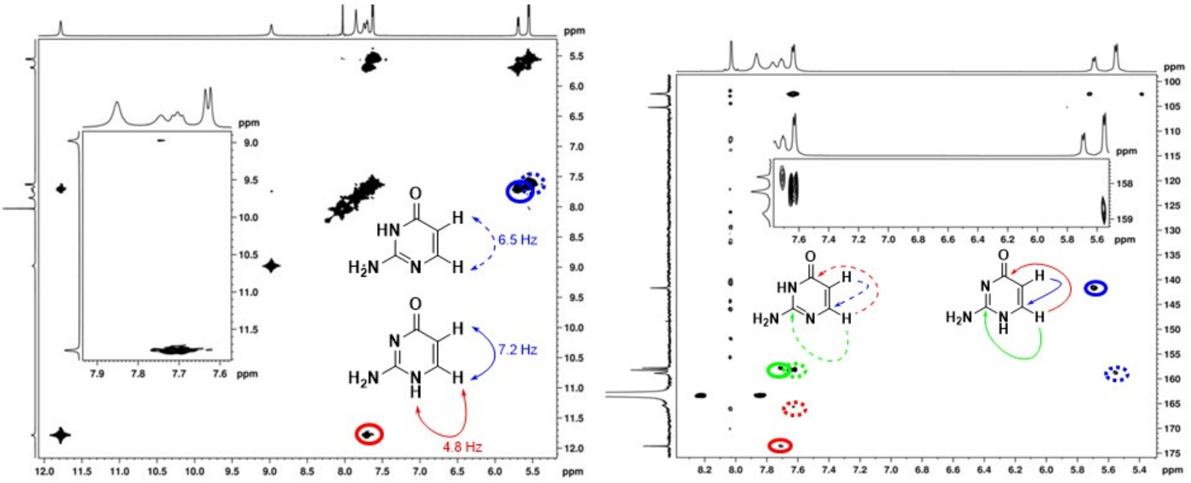

Figure S2. Part of H,H-COSY (left) and H,C-HMBC (right) spectrum of isocytosine measured in DMF-d_7 at -70 °C.

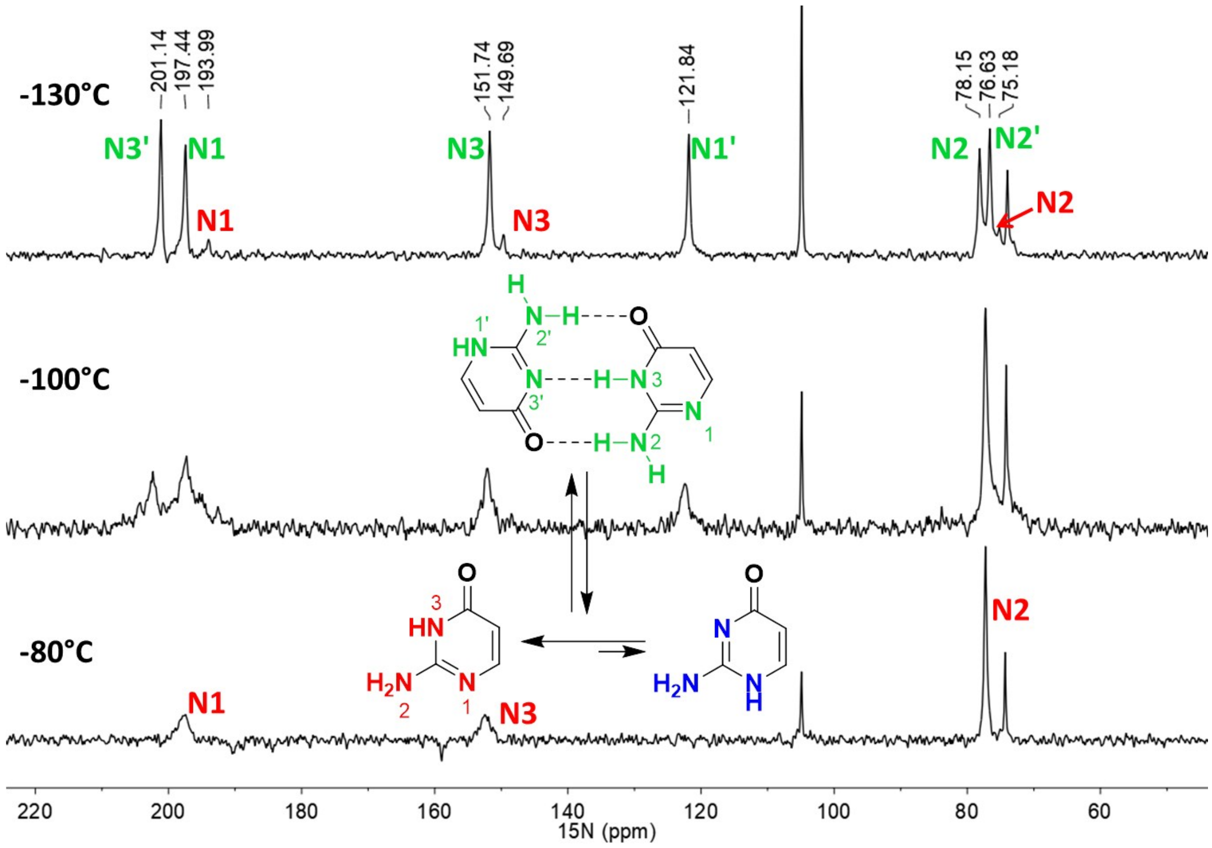

Figure S3. Variable temperature ^{15}N NMR spectra of ^{15}N-labelled isocytosine in DMF/DME mixture with signal assignment of the isocytosine dimer (blue) and monomer of 2,3-I form of isocytosine (red).

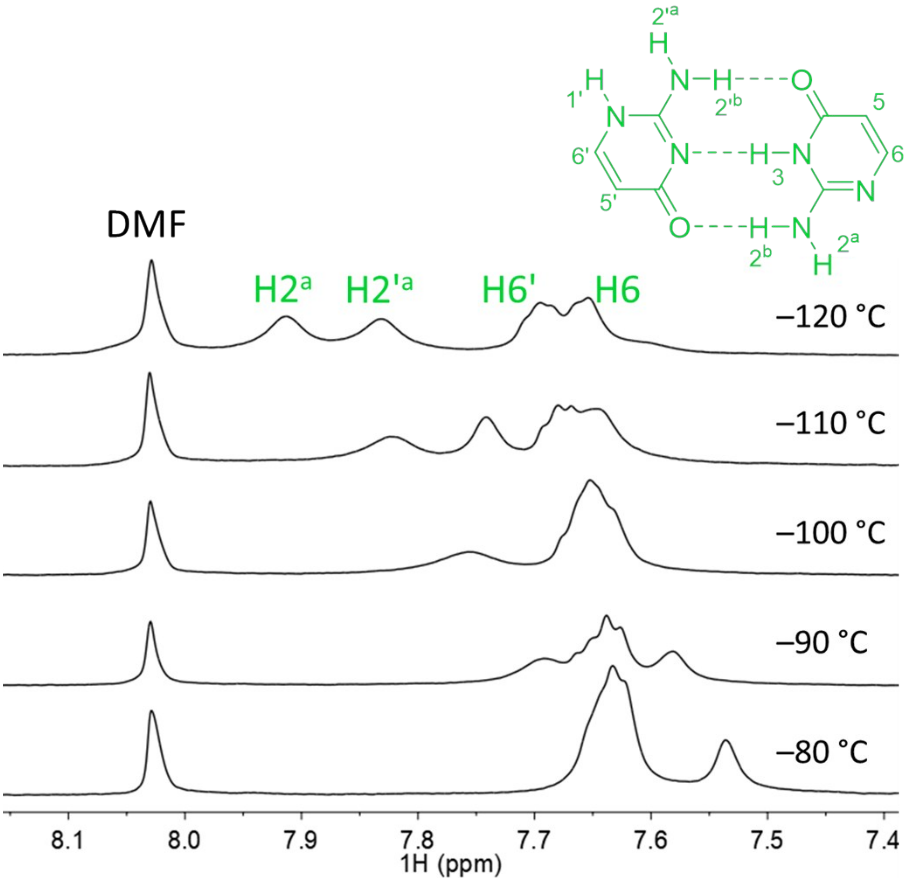

Figure S4. Part of ^{1}H NMR of isocytosine measured in DME/DMF-d_7 at variable temperatures.

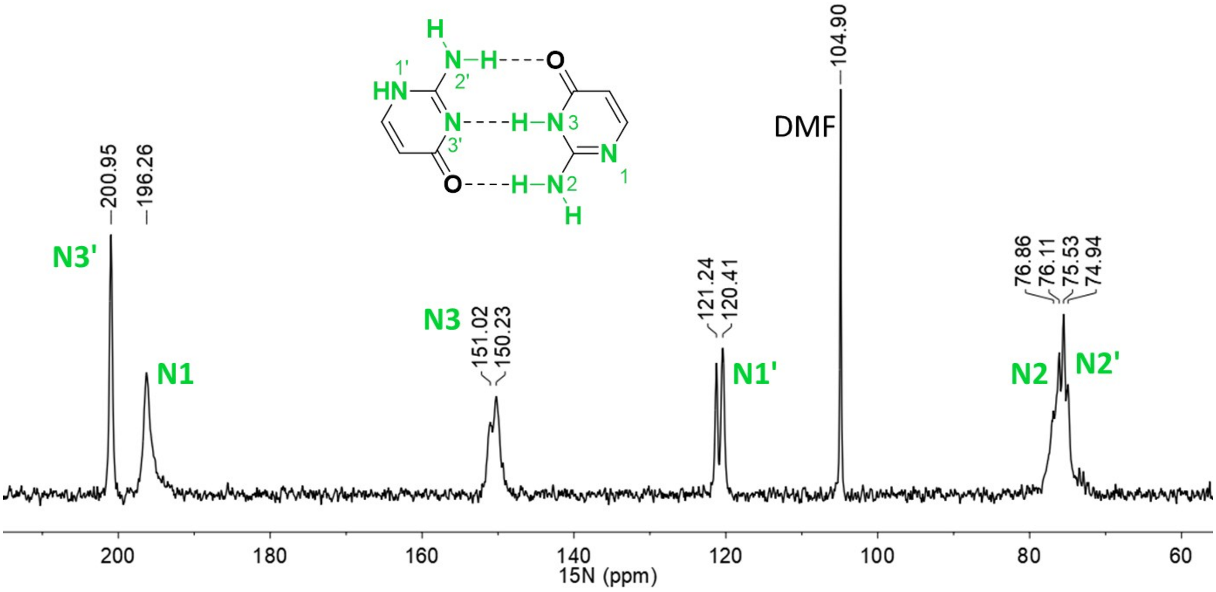

Figure S5. ^{15}N NMR spectra of ^{15}N-labelled and partially deuterated isocytosine in DMF/DME mixture measured at –120 °C with signal assignment.

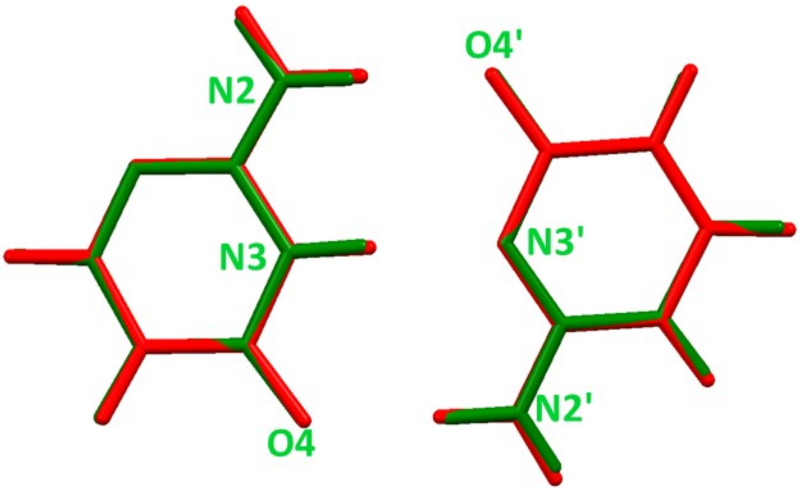

Figure S6. Overlay of X-ray structure of isocytosine (green) with geometry optimised structure from CASTEP (red). The distances between hydrogen bond donors and acceptors found by X-ray crystallography are: 2.857 Å (N2···O4'), 2.902 Å (N3···N3'), 2.903 Å (O4···N2').

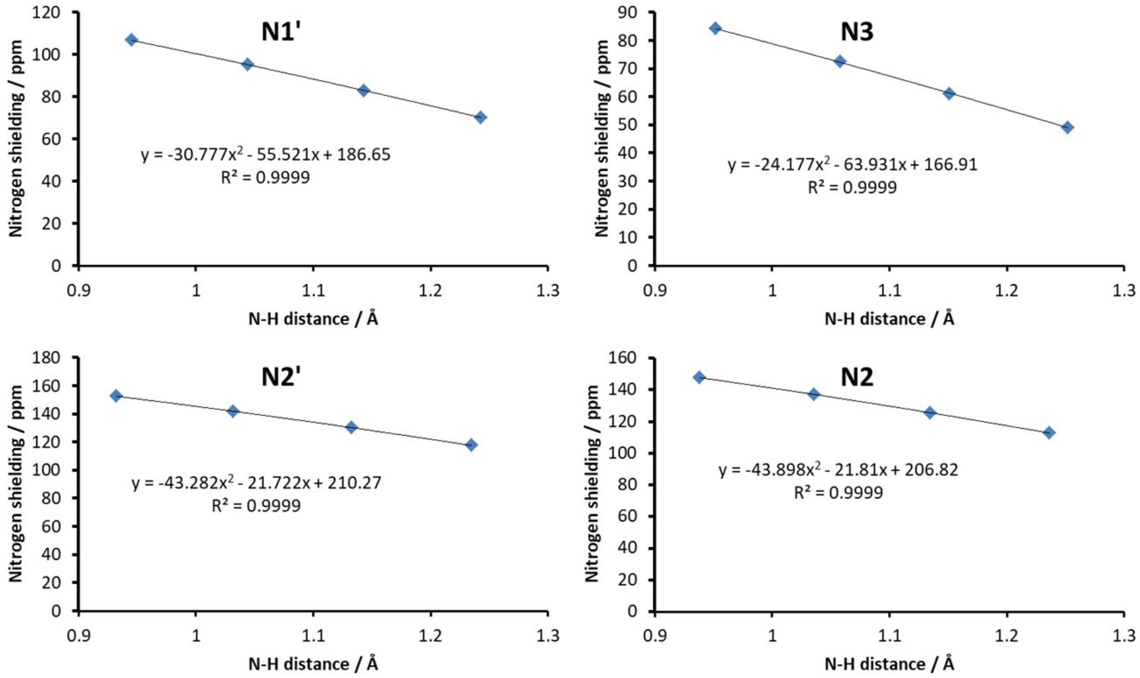

Figure S7. Selected dependences of nitrogen shieldings on N–H distances involving that nitrogen.

Table S1. ^{1}H, ^{13}C and ^{15}N chemical shifts (in ppm) of solid isocytosine and of isocytosine dimer in DME/DMF-d_7 solution.

		C2/C2'	C4/C4'	C5/C5'	C6/C6'	N1/N1'	N2/N2'	N3/N3'
Solution[a]	2,3-I	158.2	165.3	102.6	158.8	197.4	78.2	151.7
	1,2-I	157.9	173.6	105.3	141.6	121.8	76.6	201.1
Solid[b]	2,3-I	156.9	166.9	101.4	156.9	197.5	84.2	153.6
	1,2-I	156.9	173.0	105.4	137.6	124.1	80.8	203.5

		H3	H1'	H2/H2'	H2/H2'	H5/H5'	H6/H6'
Solution[a]	2,3-I	14.74	–	9.52	7.89	5.55	7.65
	1,2-I	–	11.93	9.15	7.82	5.70	7.69
Solid[b]	2,3-I	14.57	–	8–11	8–11	4.41	7.11
	1,2-I	–	11.62	8–11	8–11	5.44	7.11

[a]Solution: ^{13}C measured at –70 °C, ^{15}N at –130 °C, and ^{1}H at –120 °C; [b]Data from refs.[1-2]

Table S2. Average N–H distances (Å) obtained from PIMD simulations of isocytosine dimer.

D in positions	Method	N3–H3	N2'–H2'b	N2–H2	N2'–H2'a
–	B3LYP/H_2O	1.066	1.050	1.050	1.033
NH in H bond	B3LYP/H_2O	1.057	1.043	1.043	1.033
–	B3LYP/vacuum	1.061	1.070	1.047	1.031
NH in H bond	B3LYP/vacuum	1.052	1.059	1.040	1.031
All NH	B3LYP/H_2O	1.056	1.042	1.043	1.027
All NH	B3LYP/vacuum	1.052	1.059	1.040	1.025
–	PBE/vacuum	1.090	1.108	1.062	1.039
CASTEP-dimer-D	PBE/vacuum	1.082	1.095	1.056	1.035

Modelling of isocytosine dimer

Geometry optimisation of the isolated dimer revealed significant dependence of the intermolecular hydrogen bond geometries on the computational method. Hybrid B3LYP functional with polarisable continuum model of solvation provides an optimised dimer geometry close to the MP2-optimised structure. On the other hand, the PBE functional (belonging to the general-gradient-approximation, GGA, family of functionals) leads to significantly shorter N2'···O4 hydrogen bond distance and an overall distortion of the dimer geometry. Empirical dispersion correction changes the geometry negligibly and the effect of the DMF solvent is almost identical to that of water. The geometrical parameter most affected by the choice of optimisation protocol is the H2'^{b}···O4 distance, where the influence of both the functional and solvation is large (and with the same sign); the difference B3LYP(H_2O)–PBE(vac) is 0.2 Å. The largest effect on a covalent bond is observed for the N2'–H2'^{b} distance, where the B3LYP(H_2O)–PBE(vac) difference is –0.04 Å (Table S3).

Table S3. Interatomic distances (Å) in intermolecular hydrogen bonds of geometry optimised isocytosine dimer. Distances obtained by other methods are shown in the SI.

Method	PBE	B3LYP	B3LYP	B3LYP	MP2
Solvation	–	–	DMF	H_2O	DMF
N3–H3	1.053	1.036	1.040	1.040	1.040
H3···N3'	1.805	1.870	1.879	1.879	1.854
N2–H2^{b}	1.037	1.024	1.029	1.029	1.024
H2^{b}···O4'	1.854	1.907	1.839	1.838	1.873
N2'–H2'^{b}	1.067	1.043	1.029	1.029	1.025
H2'^{b}···O4	1.637	1.721	1.839	1.841	1.869

Table S4. Interatomic distances (Å) in intermolecular hydrogen bonds of geometry optimised isocytosine dimer.

Method	PBE			B3LYP			MP2
Solvation	–	DMF	H_2O	–	DMF	H_2O	DMF
N3–H3	1.053	1.058	1.058	1.036	1.040	1.040	1.040
H3···N3'	1.805	1.813	1.813	1.870	1.879	1.879	1.854
N2–H2	1.037	1.043	1.043	1.024	1.029	1.029	1.024
H2···O4'	1.854	1.786	1.784	1.907	1.839	1.838	1.873
N2'–H2'	1.067	1.045	1.045	1.043	1.029	1.029	1.025
H2'···O4	1.637	1.766	1.768	1.721	1.839	1.841	1.869

Table S5. Interatomic distances (Å) in intermolecular hydrogen bonds of geometry optimised isocytosine dimer. Empirical correction for dispersion according to Grimme was applied.

Functional	PBE			B3LYP		
Solvation	–	DMF	H_2O	–	DMF	H_2O
N3–H3	1.054	1.059	1.059	1.038	1.042	1.042
H3···N3'	1.792	1.798	1.799	1.841	1.846	1.846
N2–H2	1.038	1.043	1.043	1.025	1.030	1.030
H2···O4'	1.840	1.774	1.772	1.876	1.813	1.812
N2'–H2'	1.068	1.046	1.046	1.045	1.031	1.030
H2'···O4	1.627	1.751	1.754	1.697	1.806	1.808

References

1 M. Dračínský, P. Jansa, K. Ahonen and M. Buděšínský, *Eur. J. Org. Chem.*, 2011, 1544-1551.

2 M. Dračínský and P. Hodgkinson, *Rsc Adv*, 2015, **5**, 12300-12310.