Supplementary Information

^{23}Na double-rotation NMR of sodium nucleotides leads to the discovery of a new dCMP hendecahydrate

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Experimental Description

NMR Experiments

Sodium deoxycytidine monophosphate (dCMP) was purchased from Aldrich. Sodium deoxyguanosine monophosphate (dGMP) and deoxyuridine monophosphate (dUMP) were purchased from Chem-Implex. Schematic representations of the structures are shown in Figure S1. All samples were recrystalized from D_2O by slow diffusion of acetone at room temperature (dCMP and dUMP) or at 52°C (dGMP). Freshly recrystallized samples were used for all NMR experiments; dry samples had severely degraded spectral quality. Similarly, if water was used for the crystallizations then the DOR lines were significantly broadened.

All ^{23}Na NMR experiments were performed on a Bruker AVANCE III 400 operating at 9.4 T ($\nu_0(^{23}Na) = 105.8$ MHz). The DOR experiments used a Bruker HP WB 73A DOR probe with a 4.3 mm inner rotor and a 14 mm outer rotor. The inner rotor spinning was monitored using an antenna which was attached to the stator and then connected to an oscilloscope. The outer rotor spin rate was varied from 700 to 900 Hz in order to identify the spinning sidebands and was set to 800 Hz or 850 Hz (dCMP) for the DOR experiments presented in this paper. The one dimensional experiments used a simple pulse and acquire method with outer rotor synchronization in order to remove the odd ordered sidebands.[1] The central transition selective excitation pulse length was set to 3.1 μs. 256 or 1024 transients were added with a recycle delay of 1 s for the 1D DOR experiments. 20-25 kHz SPINAL-64 ^1H decoupling was used.[2]

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[1] A. Samoson and E. Lippmaa, *J. Magn. Reson.*, 1989, **84**, 410.

[2] B. M. Fung, A. K. Khitrin and K. Ermolaev, *J. Magn. Reson.*, 2000, **142**, 97.

Rotor assisted population transfer (RAPT)[3] and double-frequency sweeps (DFS)[4] central transition signal enhancement schemes were tested under DOR conditions using dUMP. The pulse sequence diagrams are shown in Figure S2. These can then be used to reduce the length of 2D experiment times. For RAPT, 100 45 µs frequency selective Gaussian pulses were applied at offsets (i.e., ν_{off}) of ± 600 kHz from the transmitter frequency prior to the acquisition. In the case of DFS, a 160 µs convergent sweep running from 900 kHz to 175 kHz from the central transition was used. In both cases, signal enhancements well under the theoretical maxima of 2 and 3 for RAPT and DFS respectfully were obtained. Depending on the site, a signal enhancement of 1.6 to 1.7 was obtained when using RAPT, whereas an enhancement of 1.7 to 1.8 was obtained with DFS. The sidebands were uniformly enhanced, demonstrating that all crystallite orientations are affected equally (see Figure S3). As discussed by Kentgens,[5] the fact that in rotating solids the resonance frequency of the satellite transitions is time dependant affects the adiabaticity of the DFS sweeps and also reduces the control over the inversion. The fact that some crystallite orientations are inverted multiple times will cancel a part of the enhancement. Nonetheless, a signal enhancement of the order of 2 does reduce the time necessary for the acquisition of a 2D spectrum by a factor of 4, which is very significant.

[3] Z. Yao, H.-T. Kwak, D. Sakellariou, L. Emsley and P. J. Grandinetti, *Chem. Phys. Lett.*, 2000, **327**, 85.

[4] A. P. M. Kentgens and R. Verhagen, *Chem. Phys. Lett.*, 1999, **300**, 435.

[5] A. P. M. Kentgens, E. R. H. van Eck, T. G. Ajithkumar, T. Anupõld, J. Past, A. Reinhold and A. Samoson, *J. Magn. Reson.*, 2006, **178**, 212.

The 2D SD DOR NMR experiments used the 3 pulse experiment described by Dupree and coworkers[6] using rotor synchronization in both the direct and the indirect dimension,[7] and which is pictured in Figure S4. A DFS pulse was applied prior to the excitation pulse in all cases with the exception of dCMP. 64 t_1 increments of 100 μs were used. 20 kHz SPINAL-64 ^{1}H decoupling[2] was applied during both evolution periods. The States method was used to obtain purely absorptive 2D peaks. The four different ^{23}Na SD DOR NMR spectra collected for dUMP are shown in Figure S5.

The ^{23}Na MAS NMR spectrum, shown in Figure S6, of dCMP was acquired using a Bruker triple resonance 4 mm MAS probe. A pulse and acquire method, using a 1.67 μs excitation pulse and an 8 kHz MAS spinrate, was used. A total of 128 transients were collected.

The ^{23}Na MQMAS NMR spectrum of dCMP, shown in Figure S7, was collected using the z-filtered method[8] and was sheared using the Bruker 'au' program "xfshear". 64 t_1 increments of 125 μs were collected and the States method was used to obtain purely absorptive 2D peaks.

Single Crystal X-ray Diffraction

The crystals of dCMP were mounted on thin glass fibers using paraffin oil and the sample was cooled to 200 K prior to data collection. Data were collected on a Bruker AXS SMART single crystal diffractometer equipped with a sealed Mo tube source

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[6] I. Hung, A. P. Howes, T. Anupõld, A. Samoson, D. Massiot, M. E. Smith, S. P. Brown and R. Dupree, *Chem. Phys. Lett.*, 2006, **432**, 152.

[7] A. Samoson and T. Anupõld, *Solid State Nucl. Magn. Reson.*, 2000, **15**, 217.

[8] J.-P. Amoureux, C. Fernandez and S. Steuernagel, *J. Magn. Reson. Ser. A*, 1996, **123**, 116.

(wavelength 0.71073 Å) APEX II CCD detector. Raw data collection and processing were performed with the APEX II software package from BRUKER AXS.[9] Diffraction data for the dCMP sample were collected with a sequence of 0.5° ω scans at 0, 90, 180, and 270° in φ. Initial unit cell parameters were determined from 60 data frames collected at the different sections of the Ewald sphere. Semi-empirical absorption corrections based on equivalent reflections were applied.[10] Systematic absences in the diffraction data-set and unit-cell parameters were consistent with the triclinic **P***1* (№1) space group. Solutions in a non-centrosymmetric space group yielded chemically reasonable and computationally stable results of refinement. The initial diffraction pattern suggested a twinned nature of a sample. Orientation matrices for non-merohedral twinning were found by analyzing positions of reflections in reciprocal space using the RLATT software package and confirmed by CELL-NOW software data analysis.[11] The structure was solved by direct methods, completed with difference Fourier synthesis, and refined with full-matrix least-squares procedures based on F^2. Twinning was accounted for by refining the final structural model versus split reflections/intensity data with a twining parameter refined to 13.7% for the smaller component. In the structure, the compound molecules are situated in general positions. All non-hydrogen atoms were refined anisotropically. Positions of all hydrogen atoms with the exception of solvent water molecules were obtained from the Fourier map analysis. Positions of the hydrogen atoms for the water solvent molecules were assigned with considerations for possible intermolecular interactions. After initial positioning, all hydrogen atoms were treated as

[9] APEX Software Suite v.2010; Bruker AXS: Madison, WI, **2005**.

[10] R. Blessing, *Acta Cryst.* 1995, **A51**, 33.

[11] Sheldrick, G. M. *Cell_Now*, **2004**, Bruker-AXS, Inc., Madison, WI

idealized contributions. All scattering factors are contained in several versions of the SHELXTL program library, with the latest version used being v.6.12.[12] Crystallographic data and selected data collection parameters are reported in Tables S2.
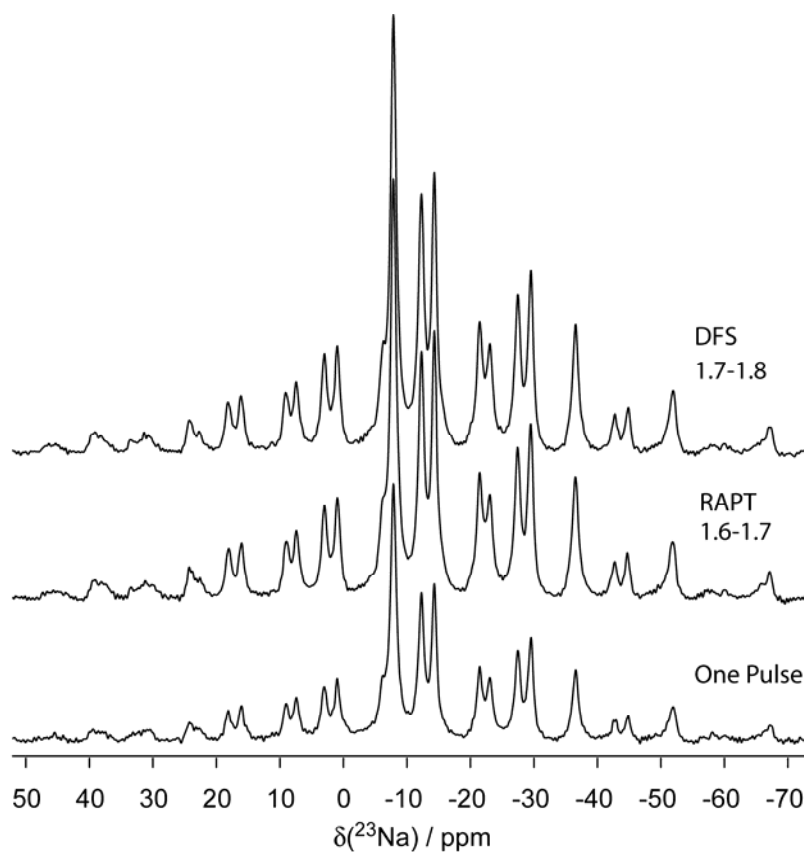
Figures
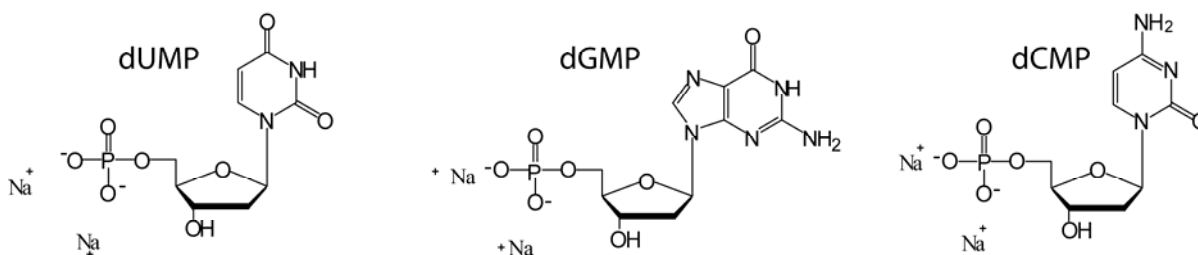
Fig. S1 Schematic representation of the molecular structures of dUMP, dGMP and dCMP.
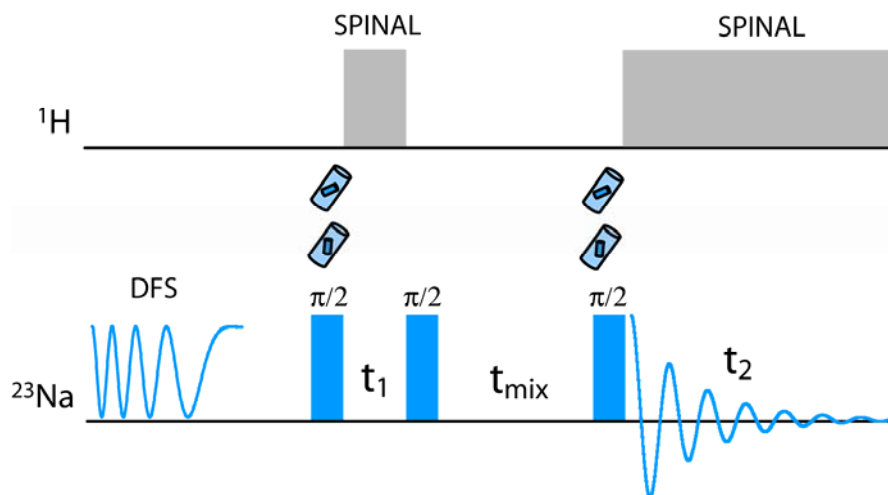
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[12] Sheldrick, G.M. *Acta Cryst.* **2008**, *A64*, 112.
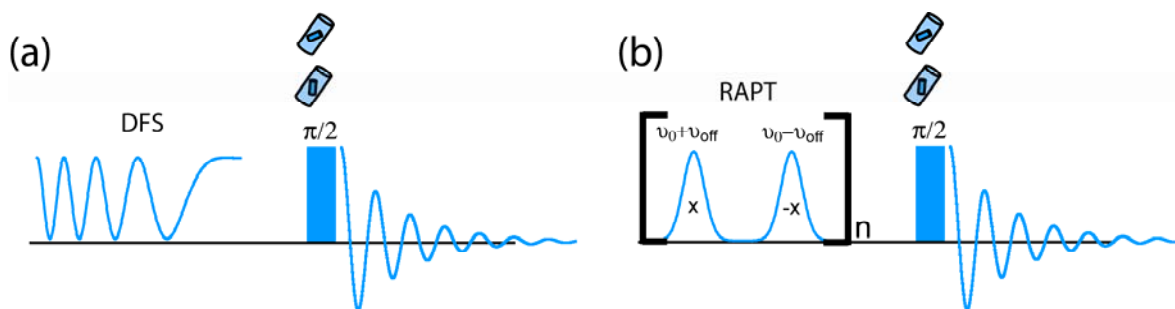
Fig. S2 Pictorial representation of the DFS (a) and RAPT (b) pulse sequences used for DOR NMR experiments with outer rotor synchronization.
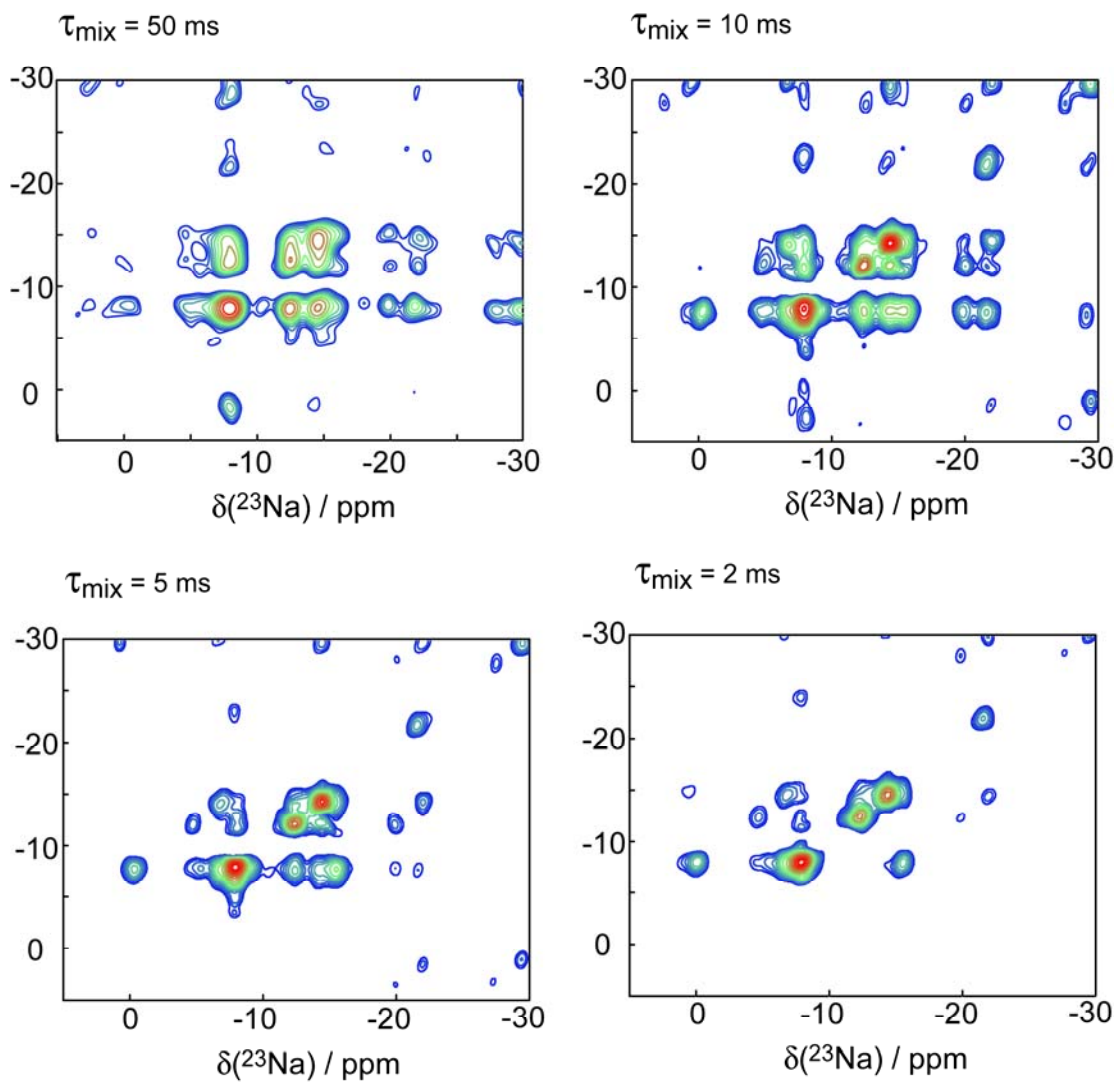

**Fig. S3** Signal enhancements obtained in the ^{23}Na DOR NMR spectrum of dUMP when DFS or RAPT is used.

Fig. S4 Pulse sequence for the 2D spin diffusion DOR NMR experiments. A DFS pulse is applied prior to the acquisition and both the excitation and read pulses are synchronized with the outer rotor's rotation.
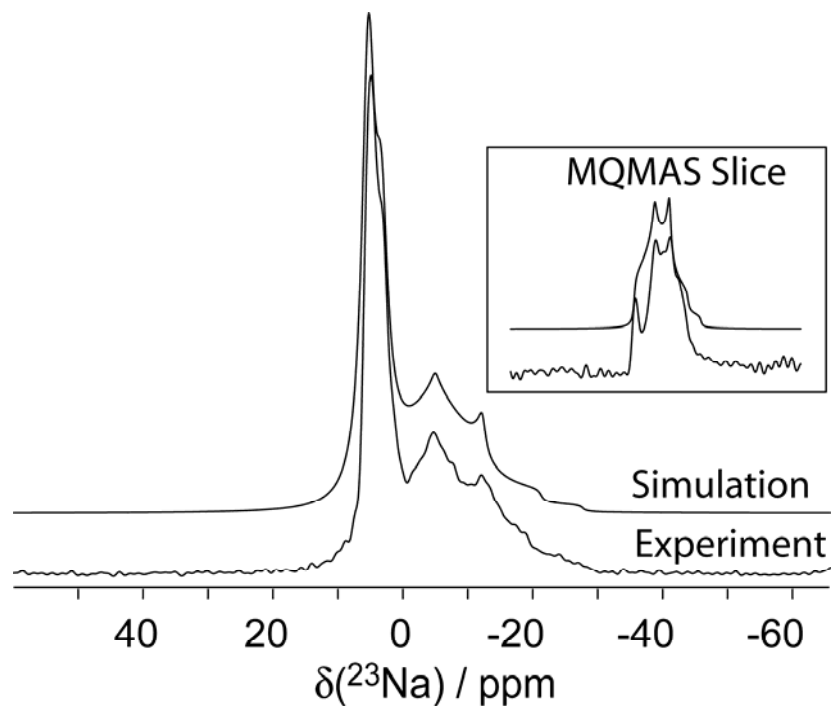
Fig. S5 ^{23}Na SD DOR NMR spectra of dUMP with various mixing times. The sideband intensities remain constant whereas the cross-peaks intensities grow.
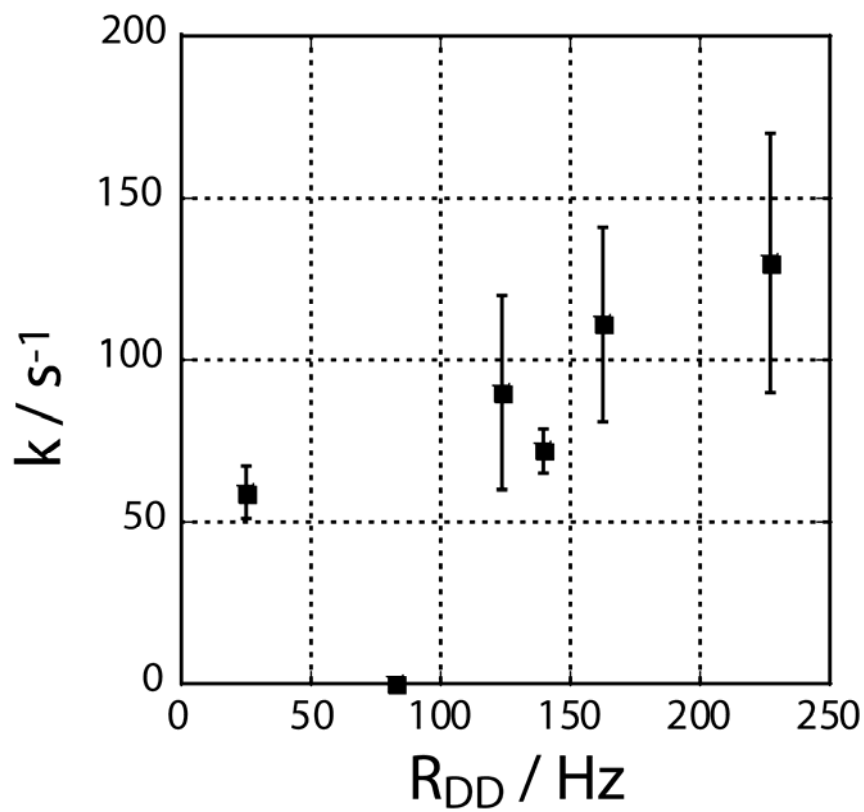
Fig. S6 ^{23}Na spin diffusion rate constants (k) as a function of the dipolar coupling constants in the case dUMP. The outlier on the far left is affected by proton mediated spin diffusion.

Fig. S7 ^{23}Na MAS NMR spectrum of sodium dCMP. The spectrum was fit using a single site for the broad pentacoordinate sites. A slice from the ^{23}Na MQMAS NMR spectrum is shown, demonstrating the agreement with the fit.
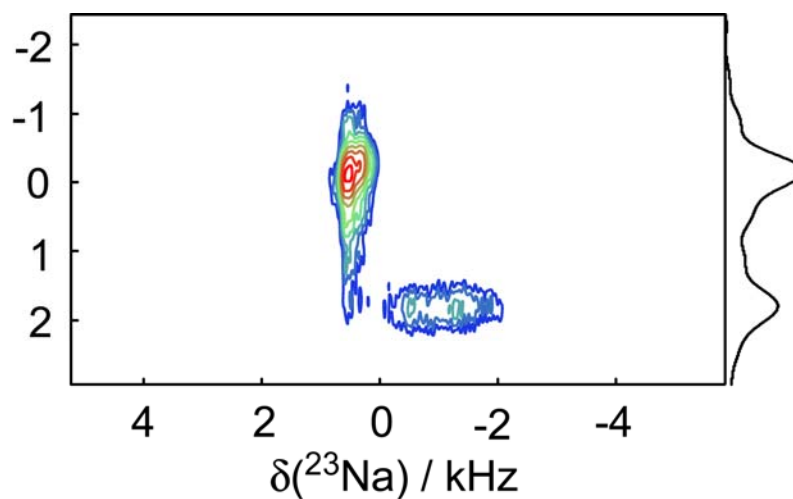
Fig. S8 ^{23}Na MQMAS NMR spectrum of sodium dCMP. The individual hexacoordinate and pentacoordinate sites are not resolved in this spectrum.

Table S1. Revised ^{23}Na NMR parameters

Compound	Site	C_Q / MHz (± 0.05)	η_Q (± 0.02)	δ_{iso} / ppm (± 0.2)
dUMP	Na1	2.42	0.70	3.0
	Na2	1.75	0.95	1.1
	Na3	2.45	0.65	0.6
	Na4	3.12	0.35	1.2
dGMP	Na1	1.82	0.90	4.9
	Na2	2.45	0.80	3.7
dCMP	Na1/Na3	2.55	0.55	7.5
	Na2/Na4	< 1.5	-	δ_{peak} = 3.3 & 5.2

Table S2. Crystallographic data and selected data collection parameters.

Compound	Sodium dCMP hendecahydrate
Empirical formula	$C_{18}H_{68}N_6Na_4O_{36}P_2$
Formula weight / g/mol	1098.68
Crystal size / mm	0.29 x 0.27 x 0.17
Crystal system	Triclinic
Space group	**P1** (№1)
Z	1
a / Å	7.3224(9)
b / Å	10.0224(12)
c / Å	16.631(2)
α / °	102.112(5)
β / °	92.853(6)
γ / °	91.309(6)
Volume / Å^3	1191.2(2)
Calculated density / Mg/m^3	1.532
Absorption coefficient / mm^{-1}	0.237
F(000)	580
Θ range for data collection / °	2.08 to 28.64
Limiting indices	$h = \pm9$, $k = \pm13$, $l = 0$ to $+22$
Reflections collected / unique	7378 / 5528
R(int)	0.0000
Completeness to $\Theta = 28.32$ / %	90.3
Max. and min. transmission	0.9608 and 0.9345
Data / restraints / parameters	5528 / 3 / 596
Goodness-of-fit on F^2	1.061
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0568$, $wR_2 = 0.1482$
R indices (all data)	$R_1 = 0.0632$, $wR_2 = 0.1544$
Largest diff. peak/hole / e·Å^{-3}	1.436 / -0.519