Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Varying nucleotide synthesis reaction conditions

In order to obtain maximum yields of the product, ratio of rMP to BA was varied in the reaction mixtures. BA concentration was fixed at 5mM and various concentrations of rMP were added (5, 10, 25 & 50 mM) to keep the ratios at 1:1, 1:2, 1:5 and 1:10, respectively. Much lower concentrations were used in comparison to previous related studies to keep the concentrations of the starting material more prebiotically realistic. The various reaction mixtures were subjected to heating under dry conditions at 90°C for 3 hours. Reaction mixtures were subsequently analyzed for the formation of nucleotide using anion exchange chromatography. Figure S1 shows the overlay of chromatograms obtained from the aforementionedfour reactions. Nucleotide formation was observed in all cases with increasing yields. Yields seem to level ofat1:5 ratio of BA to rMP as the addition of more rMP did not result in further increase in yields. Higher concentration of rMP may be required as a significant amount of thermal degradation of starting material (especially sugar) was evident from the browning of reaction mixtures. Variation in the relative intensities of the peaks between 8 and 10 minutes indicate that distribution of the resultant isomers could differ in the four samples.

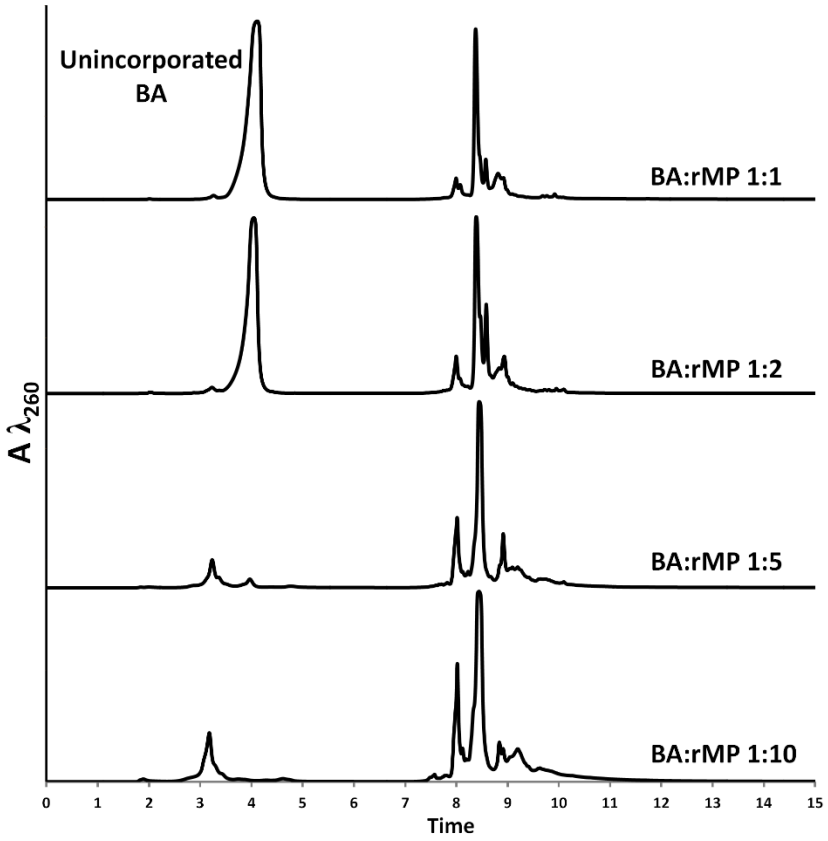


Figure S1 Varying ratio of BA to rMP for nucleotide synthesis. Unincorporated BA shows a peak in dead volume (before 5 min) and peaks obtained in the gradient indicate formation of isomers of the nucleotide in varying ratios depending on the starting reaction conditions.

The nucleotide synthesis reaction was also carried out at 60°C with 1:5 ratio of BA to rMP. HPLC analysis of the reaction mixture revealed formation of the BA-nucleotide even at low temperature (Figure S2). However, the yields of the product were smaller as is indicated by the peak intensity obtained for unincorporated BA. We also checked effect of presence of Mg^{2+}(in the form of $MgCl_2$) on the reaction.[2,3] Ratio of BA to rMP to Mg^{2+}in these reactions was kept at 1:2:2 and the mixture was heated at 90°C for 3 hours. As shown in figure S3, significant increase in nucleotide yields was observed when Mg^{2+} was present in the reaction mixture. Distribution of the gradient peaks did seem to change in the presence of magnesium ions indicating formation of the resultant isomers in varying ratios. This change of ratio of the resultant isomers was also observed in case of 6-aminouracil nucleoside synthesis under similar conditions as reported earlier.[8]

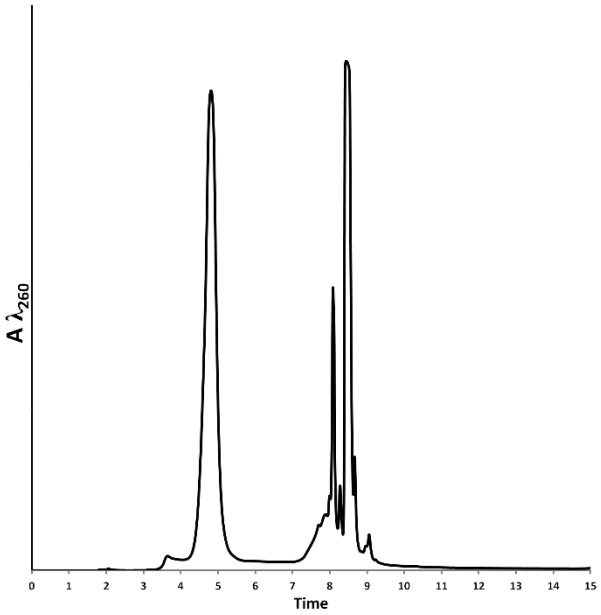


Figure S2 Formation of nucleotide from BA and rMP at low temperature (60°C).

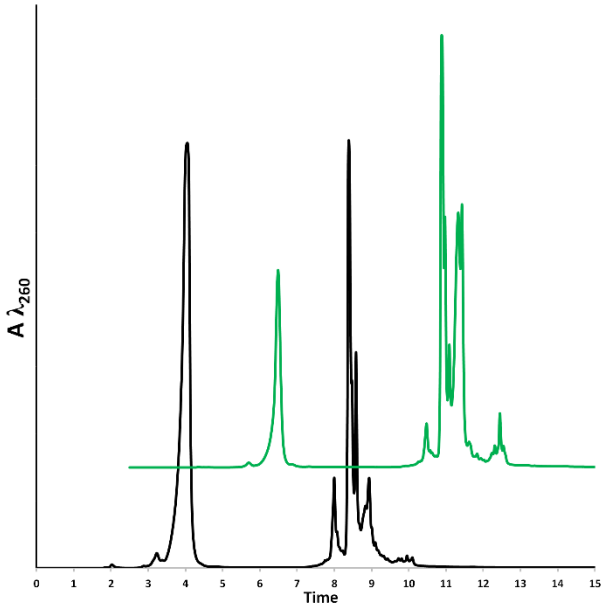


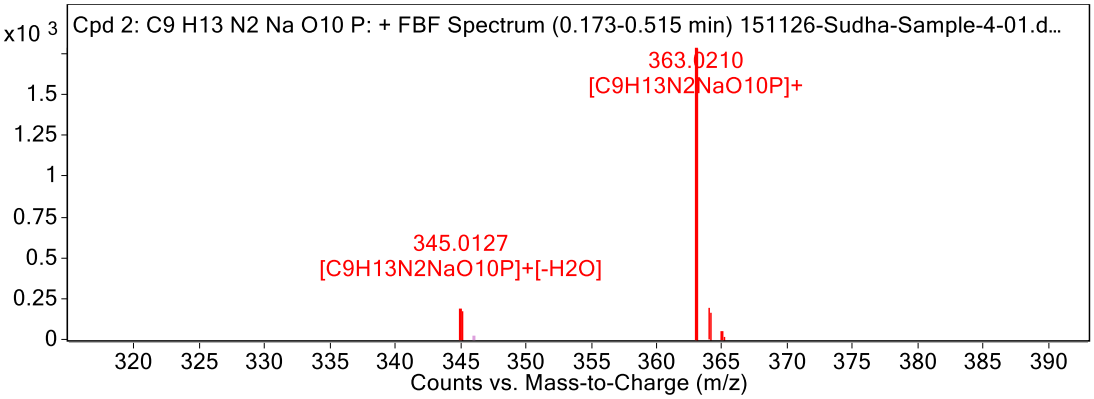
Figure S3 Nucleotide synthesis in presence of Mg^{2+}. Black chromatogram shows control synthesis (without Mg^{2+}) and green is the reaction with Mg^{2+}. Green trace is offset evenly from both axes for better comparison of peaks.

Mass analysis of products

Supplementary Data S4: Mass spectra of BA-nucleotide synthesis reaction mixture and details of the various chemical species observed.

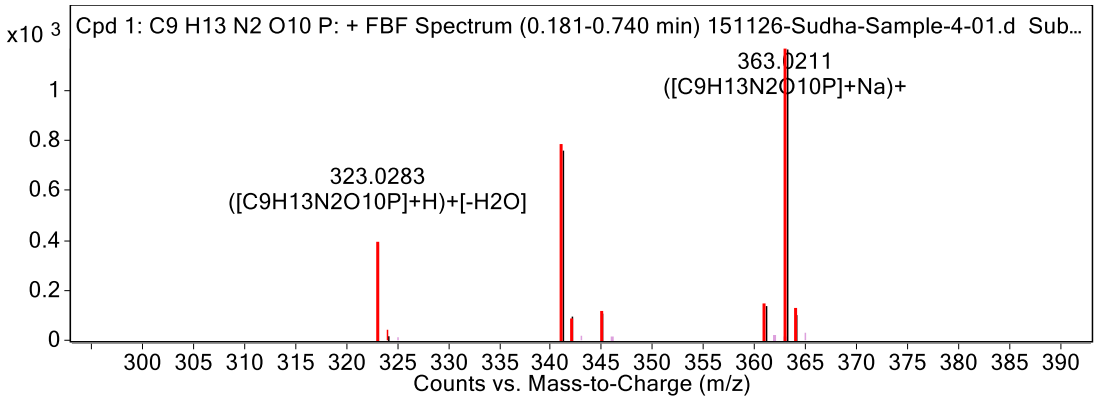
Mass Spectrum	Chemical species	Ref. Mass	Obs. Mass	Mass Error (ppm)
A	BA-nucleotide+Na	363.0206	363.0219	3.68
B	BA-nucleotide	340.0308	340.0311	1.07

A



Obs. m/z	Charge	Abundance	Formula	Ion/Isotope
345.0127	1	187.26	C9H13N2NaO10P	M+[-H2O]
363.021	1	1785.44	C9H13N2NaO10P	M+
364.0237	1	175.03	C9H13N2NaO10P	M+
365.0361	1	29.83	C9H13N2NaO10P	M+

B

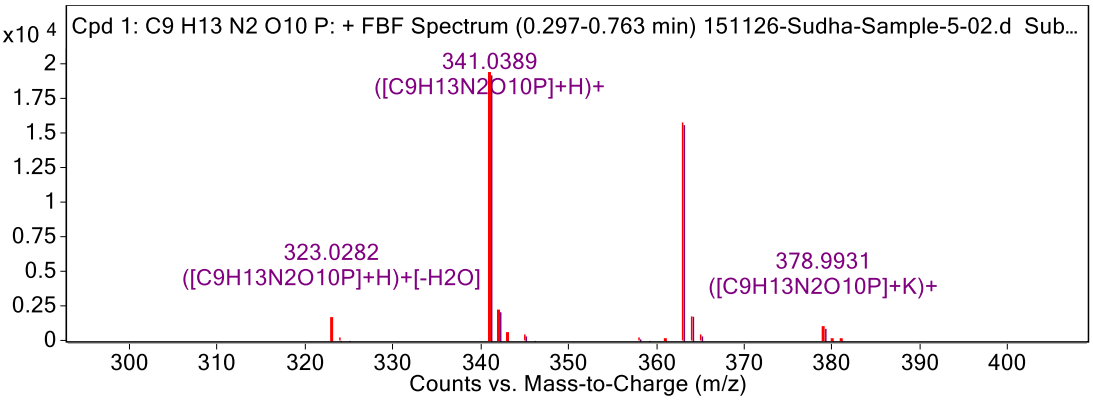


Obs. m/z	Charge	Abundance	Formula	Ion/Isotope
323.0283	1	394.33	C9H13N2O10P	(M+H)+[-H2O]
324.0287	1	23.24	C9H13N2O10P	(M+H)+[-H2O]
341.0379	1	766.77	C9H13N2O10P	(M+H)+
342.0396	1	102.75	C9H13N2O10P	(M+H)+
345.0127	1	115.66	C9H13N2O10P	(M+Na)+[-H2O]
360.9788	1	145.35	C9H13N2O10P	(M+K)+[-H2O]
363.0211	1	1169.23	C9H13N2O10P	(M+Na)+
364.0234	1	111.34	C9H13N2O10P	(M+Na)+

Supplementary Data S5: Mass spectra of BA-nucleotide polymerization reaction mixture and various species observed.

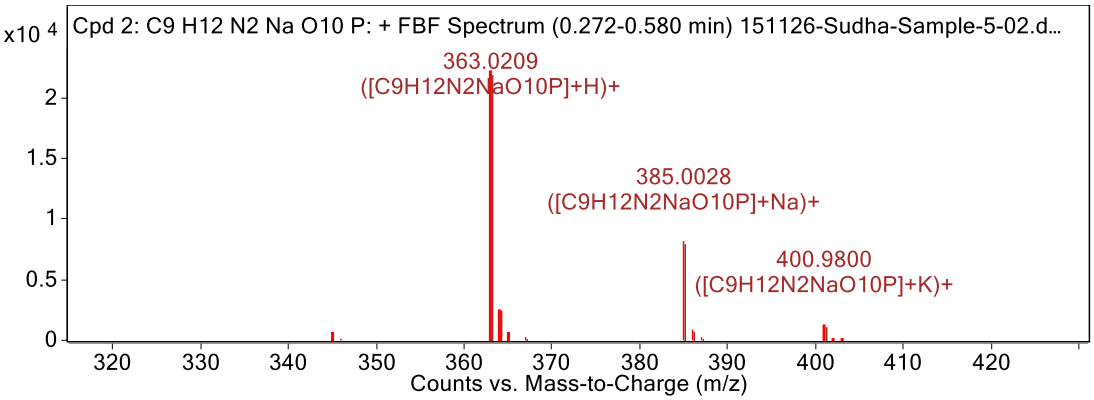
Mass Spectrum	Chemical Species	Ref. Mass	Obs. Mass	Mass Error (ppm)
C	BA-nucleotide	340.0308	340.0316	2.36
D	BA-nucleotide+Na	362.0127	362.0137	2.72
E	BA-nucleotide-rMP dimer	552.0394	552.0405	2.11
F	BA-nucleotide dimer	662.051	662.0518	1.29

C



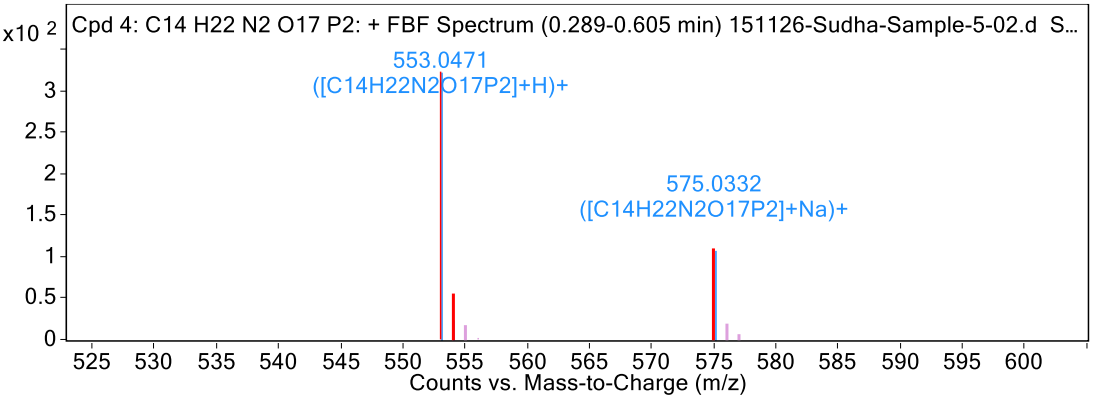
Obs. m/z	Charge	Abundance	Formula	Ion/Isotope
323.0282	1	1624.98	C9H13N2O10P	(M+H)+[-H2O]
341.0389	1	19189.47	C9H13N2O10P	(M+H)+
342.0417	1	2154.43	C9H13N2O10P	(M+H)+
343.0428	1	629.9	C9H13N2O10P	(M+H)+
345.0109	1	394.93	C9H13N2O10P	(M+Na)+[-H2O]
358.0684	1	222.11	C9H13N2O10P	(M+NH4)+
360.9789	1	104.12	C9H13N2O10P	(M+K)+[-H2O]
363.021	1	15621.86	C9H13N2O10P	(M+Na)+
364.0239	1	1814.92	C9H13N2O10P	(M+Na)+
378.9931	1	997.56	C9H13N2O10P	(M+K)+

D



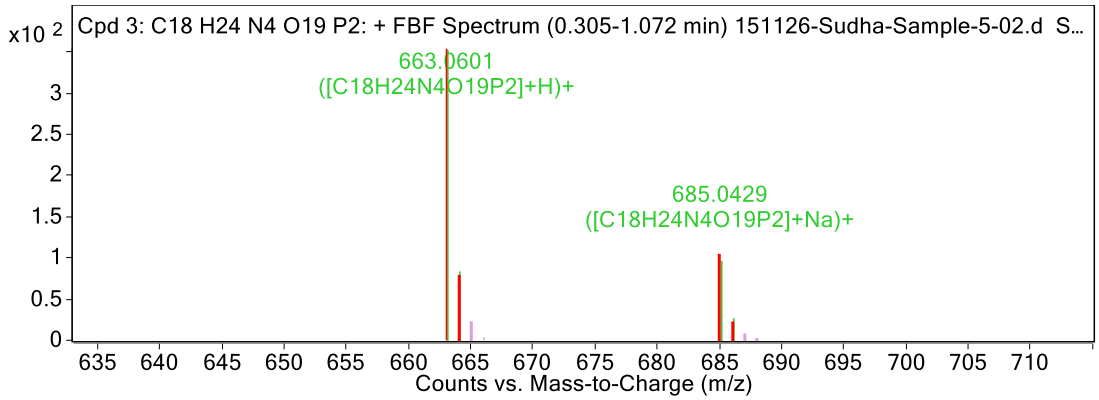
Obs. m/z	Charge	Abundance	Formula	Ion/Isotope
345.0107	1	556.22	C9H12N2NaO10P	(M+H)+[-H2O]
363.0209	1	21946.35	C9H12N2NaO10P	(M+H)+
364.0239	1	2573.73	C9H12N2NaO10P	(M+H)+
365.025	1	704.55	C9H12N2NaO10P	(M+H)+
366.9922	1	215.73	C9H12N2NaO10P	(M+Na)+[-H2O]
385.0028	1	8091.43	C9H12N2NaO10P	(M+Na)+
386.0059	1	909.6	C9H12N2NaO10P	(M+Na)+
387.0066	1	248.64	C9H12N2NaO10P	(M+Na)+
400.98	1	1269.69	C9H12N2NaO10P	(M+K)+
402.9817	1	157.9	C9H12N2NaO10P	(M+K)+

E



Obs. m/z	Charge	Abundance	Formula	Ion/Isotope
553.0471	1	323.65	C14H22N2O17P2	(M+H)+
554.0447	1	24.61	C14H22N2O17P2	(M+H)+
575.0332	1	108.77	C14H22N2O17P2	(M+Na)+

F



Obs. m/z	Charge	Abundance	Formula	Ion/Isotope
663.0601	1	353.87	C18H24N4O19P2	(M+H)+
664.0617	1	85.13	C18H24N4O19P2	(M+H)+
685.0429	1	97.61	C18H24N4O19P2	(M+Na)+
686.0276	1	28.99	C18H24N4O19P2	(M+Na)+

Structures of Barbituric acid tautomers

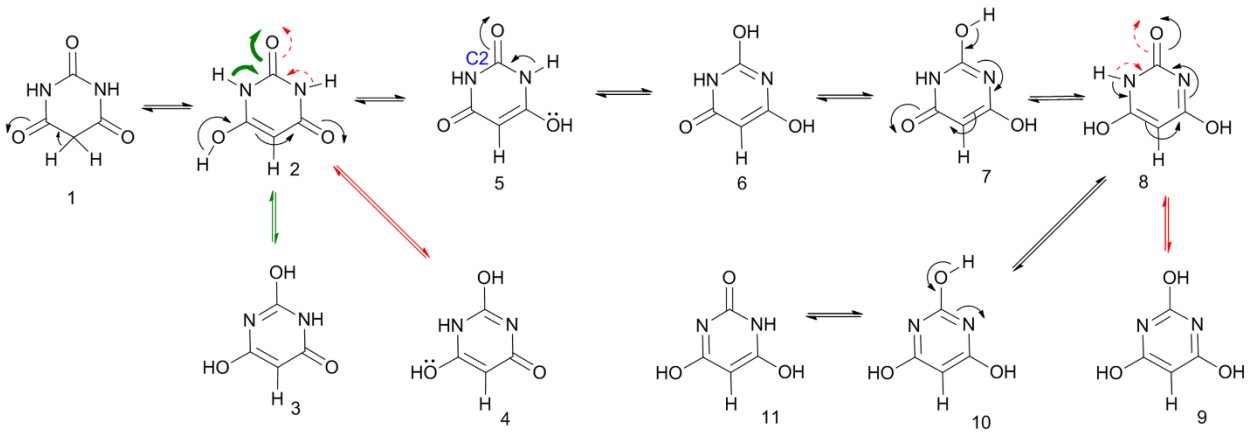


Figure S6 Barbituric acid can undergo extensive tautomerism. Above figure shows some of the tautomers that can be exhibited by BA.

Mechanism of formation of nucleotides between BA and rMP

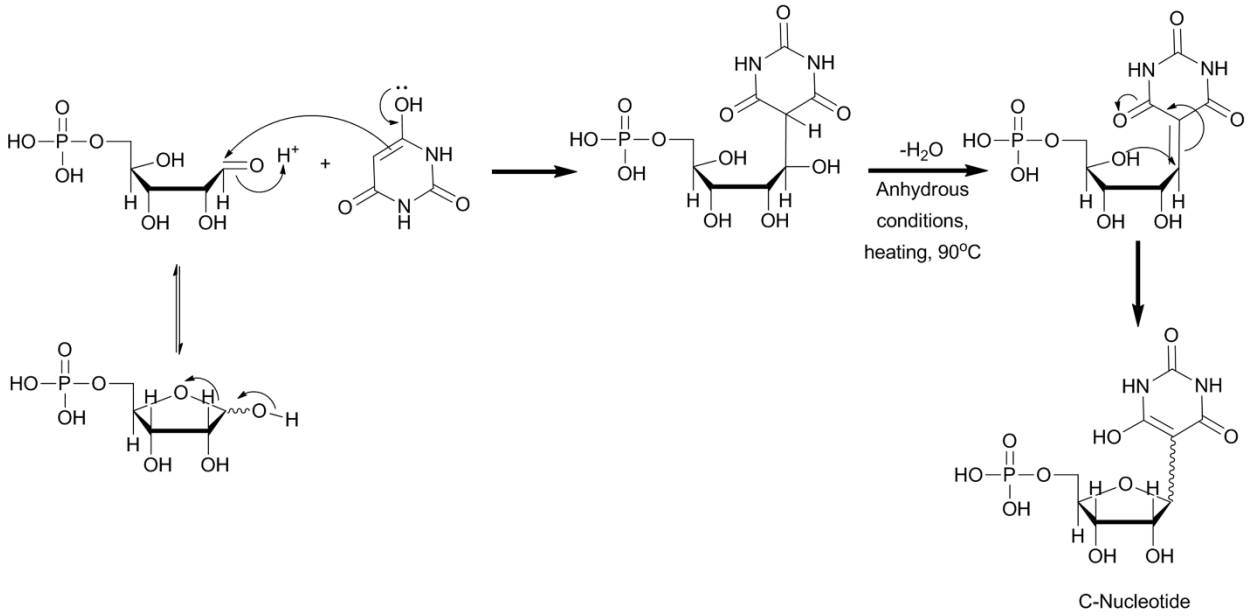


Figure S7a Possible mechanism for formation of C-nucleoside between BA and rMP (adapted and modified from formation of nucleoside with TAP[11] and 6-aminouracil[10]).

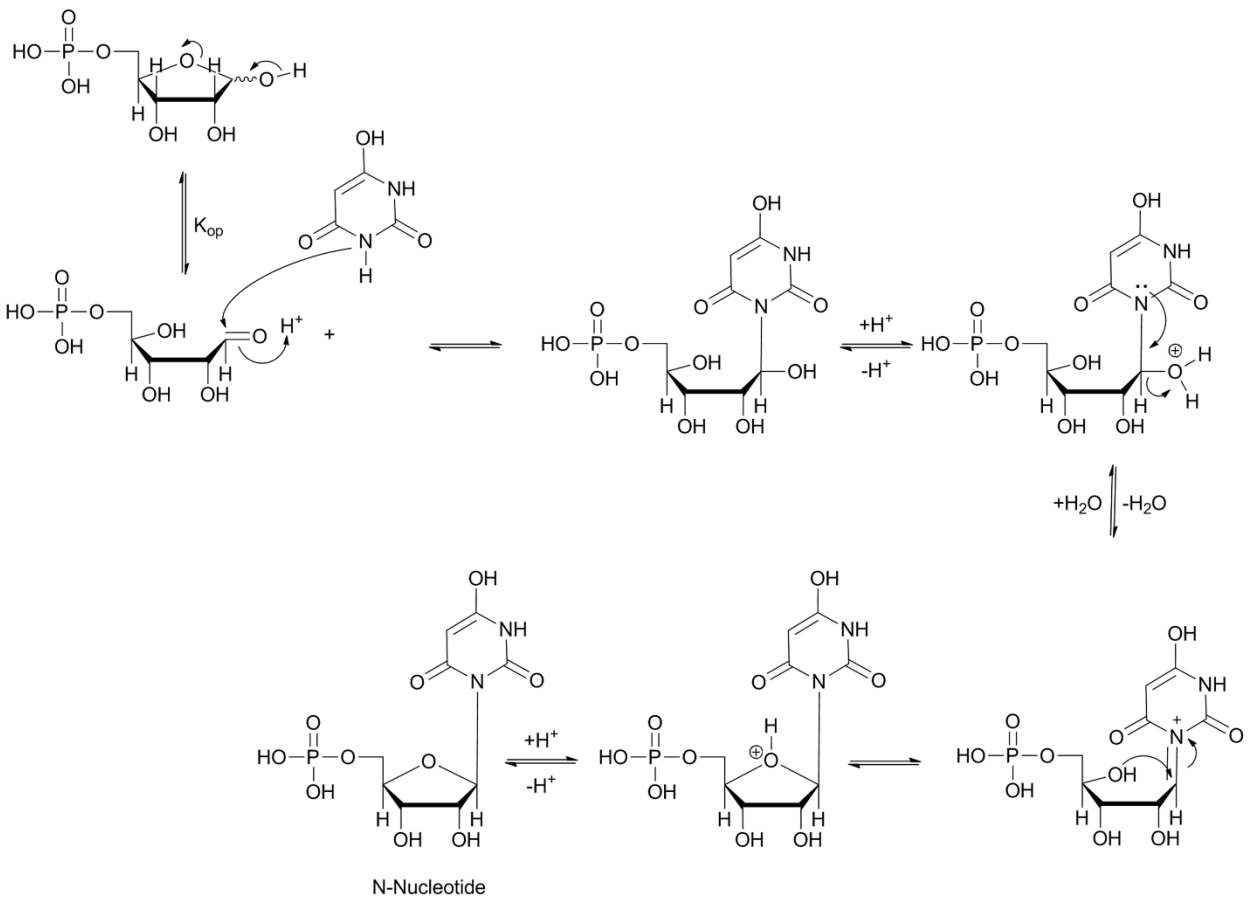


Figure S7b Possible mechanism for formation of N-nucleoside between BA and rMP (adapted and modified from formation of nucleoside with 2-Pyrimidinone[9] and Urazole[8]).