Supplementary Information for Thermodynamic Reaction Control of Nucleoside Phosphorolysis

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Author contributions (with definitions as recommended by Brand *et al.*[1])

Conceptualization, F.K., R.T.G., P.N., A.W. and M.G.; Data curation, F.K. and R.T.G.; Formal analysis, F.K. and R.T.G.; Funding acquisition, R.T.G., P.N. and A.W.; Investigation, F.K.; Methodology, F.K. and R.T.G.; Project administration, F.K., R.T.G. and A.W.; Resources, R.T.G., A.W., P.N., S.W. and N.K.; Software, - ; Supervision, R.T.G., P.N. A.W. and M.G.; Validation, - ; Visualization, F.K.; Writing—original draft, F.K.; Writing—review & editing, F.K., R.T.G., P.N., A.W. and M.G.

Calculation of K

The equilibrium constant K was calculated from the nucleobase/nucleobase ratio determined by spectral unmixing via

$$K = \frac{[B]^2}{([N]_0 - [B])([P]_0 - [B])}$$

where $[B]$ is the concentration of the free nucleobase, $[N]_0$ and $[P]_0$ are the initial concentrations of the nucleoside and phosphate, respectively.

Figure S1. Nucleosides in this work.

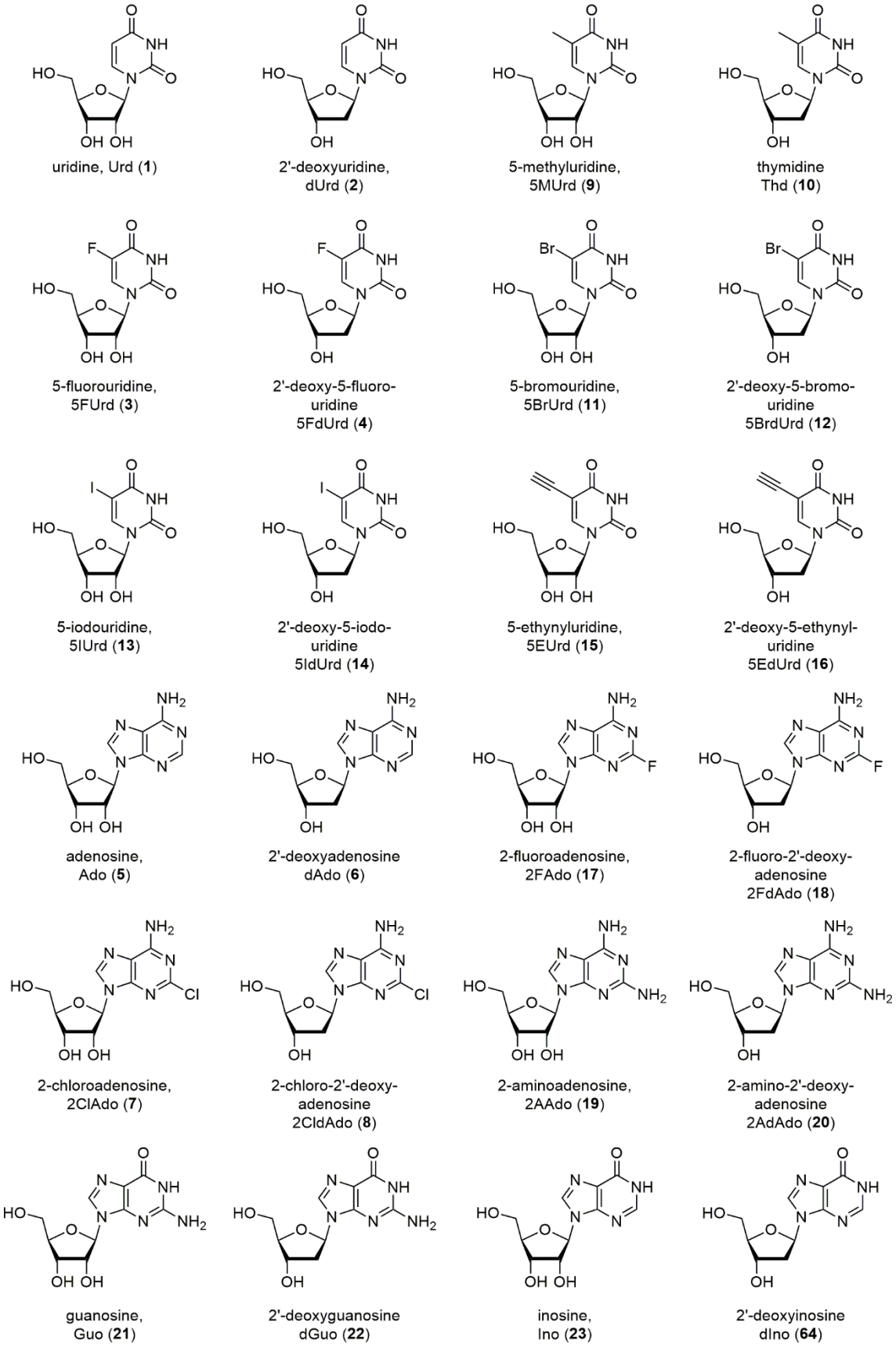

Table S1. Thermodynamic properties of nucleoside phosphorolysis determined at pH 9 and 25 °C in 50 mM glycine buffer (± SD).

Compound	$\Delta_R G'^*$ [kJ·mol^{-1}]	$\Delta_R H'^*$ [kJ·mol^{-1}]	$\Delta_R S'^*$ [J·mol^{-1}·K^{-1}]	K_{exp}(40 °C) [ ]	R^2
Urd (**1**)	4.65 ± 0.28	8.88 ± 0.31	14.20 ± 0.93	0.181 ± 0.002	0.976
dUrd (**2**)	4.08 ± 0.29	7.10 ± 0.32	10.13 ± 0.97	0.224 ± 0.003	0.958
5MUrd (**9**)	5.65 ± 0.24	10.28 ± 0.27	15.51 ± 0.79	0.125 ± 0.002	0.988
Thd (**10**)	5.02 ± 0.18	6.24 ± 0.20	4.12 ± 0.60	0.149 ± 0.001	0.979
5FUrd (**3**)	5.64 ± 0.36	9.29 ± 0.40	12.23 ± 1.20	0.122 ± 0.002	0.968
5FdUrd (**4**)	5.49 ± 0.32	6.03 ± 0.35	1.83 ± 1.07	0.123 ± 0.003	0.943
5BrUrd (**11**)	5.12 ± 0.47	10.96 ± 0.53	19.59 ± 1.59	0.157 ± 0.008	0.956
5BrdUrd (**12**)	4.63 ± 0.33	9.53 ± 0.37	16.44 ± 1.09	0.187 ± 0.003	0.972
5IUrd (**13**)	4.06 ± 0.35	8.55 ± 0.39	15.06 ± 1.37	0.233 ± 0.003	0.960
5IdUrd (**14**)	3.83 ± 0.40	8.60 ± 0.46	16.00 ± 1.38	0.280 ± 0.006*	0.958
5EUrd (**15**)	1.96 ± 0.95	15.89 ± 1.09	46.72 ± 3.20	0.607 ± 0.015	0.930
5EdUrd (**16**)	2.20 ± 0.64	12.33 ± 0.73	33.95 ± 2.15	0.345 ± 0.003	0.941
Ado (**5**)	11.33 ± 0.67	11.88 ± 0.76	1.83 ± 2.26	0.013 ± 0.001	0.932
dAdo (**6**)	12.06 ± 0.75	14.05 ± 0.85	6.67 ± 2.52	0.010 ± 0.003	0.933
2FAdo (**17**)	8.71 ± 0.43	14.28 ± 0.49	18.68 ± 1.45	0.039 ± 0	0.979
2FdAdo (**18**)	10.14 ± 1.57	18.69 ± 1.78	28.67 ± 5.27	0.022 ± 0	0.877
2ClAdo (**7**)	11.14 ± 1.28	23.19 ± 1.48	40.41 ± 4.30	0.011 ± 0.002	0.952
2CldAdo (**8**)	12.53 ± 1.84	24.95 ± 2.12	41.65 ± 6.16	0.009 ± 0	0.879
2AAdo (**19**)	12.63 ± 1.13	12.24 ± 1.27	-1.31 ± 3.80	0.008 ± 0.001	0.826
2AdAdo (**20**)	12.15 ± 1.05	10.19 ± 1.17	-6.57 ± 3.50	0.008 ± 0.001	0.810
Guo (**21**)	8.58 ± 0.52	9.15 ± 0.58	1.88 ± 1.75	0.037 ± 0.001	0.929
dGuo (**22**)	8.21 ± 0.69	8.56 ± 0.77	1.19 ± 2.32	0.043 ± 0.002	0.878
Ino (**23**)	6.00 ± 0.60	12.16 ± 0.68	20.67 ± 2.01	0.103 ± 0.001	0.948
dIno (**24**)	6.32 ± 0.60	13.67 ± 0.68	24.63 ± 2.02	0.114 ± 0.003	0.956

*50 °C (data for 40 °C for this substrate were excluded from analysis)

Table S2. Enzymes applied in this study.

Enzyme	Type	Abbreviation	EC number	Concentration of the stock solution stored at 4 °C [mg·mL^{-1}]
E-PyNP-0001	Py-NPase	Py-NPase Y01	EC 2.4.2.2	3.63
E-PyNP-0002	Py-NPase	Py-NPase Y02	EC 2.4.2.2	1.61
Bacillus subtilis pyrimidine phosphorylase	Py-NPase	*B. subtilis* Py-NPase	EC 2.4.2.2	1.00
E-UP-0001	Py-NPase	*E. coli* UP	EC 2.4.2.3	1.30
E-TP-0001	Py-NPase	*E. coli* TP	EC 2.4.2.4	3.57
E-PNP-01	Pu-NPase	Pu-NPase N01	EC 2.4.2.1	1.30
E-PNP-02	Pu-NPase	Pu-NPase N02	EC 2.4.2.1	6.63
E-PNP-04	Pu-NPase	*E. coli* Pu-NPase	EC 2.4.2.1	0.37

Table S3. Sampling times for equilibrium constant determination at different temperatures for reactions performed as described in the Experimental section.

Substrate	sampling times [min] for reactions at 40 °C	50 °C	60 °C	70 °C
Urd (**1**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
dUrd (**2**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
5MUrd (**9**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
Thd (**10**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
5FUrd (**3**)	8, 10, 12	2, 3, 4	1, 1.5, 2	0.5, 1, 1.5
5FdUrd (**4**)	8, 10, 12	2, 3, 4	1, 1.5, 2	0.5, 1, 1.5
5BrUrd (**11**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
5BrdUrd (**12**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
5IUrd (**13**)	100, 120, 180	80, 70, 60,	30, 40, 50	20, 25, 30
5IdUrd (**14**)	180, 210, 240	80, 100, 120	40, 50, 60	20, 25, 30
5EUrd (**15**)	60, 70, 80	30, 35, 40	18, 20, 22	10, 12, 14
5EdUrd (**16**)	60, 70, 80	30, 35, 40	18, 20, 22	10, 12, 14
Ado (**5**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
dAdo (**6**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
2FAdo (**17**)	12, 13, 14	5, 5.5, 6	2, 2.5, 3	1, 1.5, 2
2FdAdo (**18**)	12, 13, 14	5, 5.5, 6	2, 2.5, 3	1, 1.5, 2
2ClAdo (**7**)	16, 17, 18	7, 7.5, 8	3, 3.5, 4	1.5, 2, 2.5
2CldAdo (**8**)	16, 17, 18	7, 7.5, 8	3, 3.5, 4	1.5, 2, 2.5
2AAdo (**19**)	12, 13, 14	5, 5.5, 6	2, 2.5, 3	1, 1.5, 2
2AdAdo (**20**)	12, 13, 14	5, 5.5, 6	2, 2.5, 3	1, 1.5, 2
Guo (**21**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
dGuo (**22**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
Ino (**23**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15
dIno (**24**)	20, 30, 40	20, 30, 40	10, 15, 20	5, 10, 15

References

[1] A. Brandt, L. Allen, M. Altman, M. Hlava, J. Scott, *Learned Publishing* **2015**, *28*, 151—155.