Supporting Information

Updating and Extending an UV/Vis Spectroscopy-Based Assay for Monitoring of Transformations Between Nucleosides and Nucleobases

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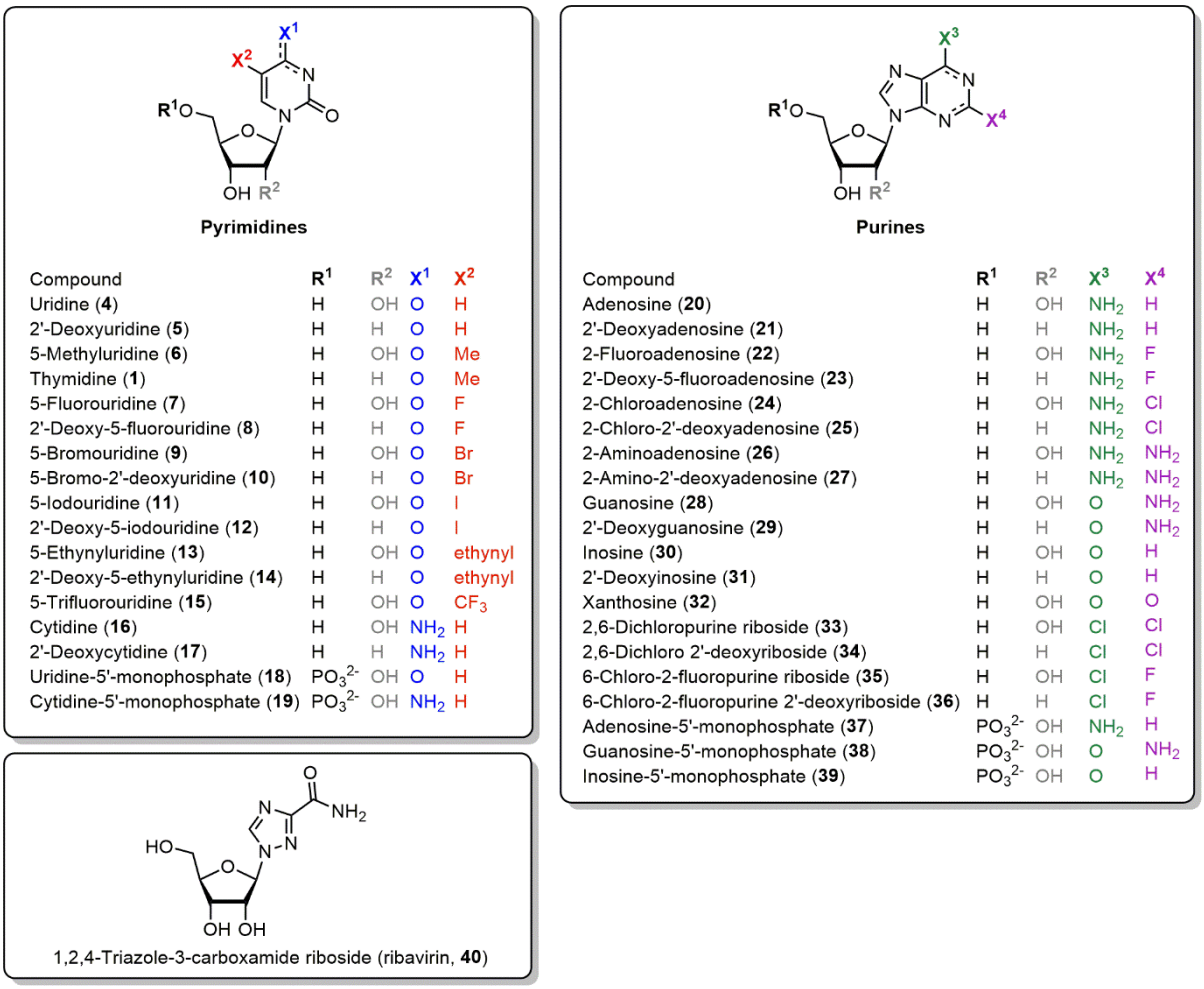
Author Contributions (with definitions as recommended by Brand *et al.*[1])

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



Compound	R^1	R^2	X^1	X^2
Uridine (**4**)	H	OH	O	H
2'-Deoxyuridine (**5**)	H	H	O	H
5-Methyluridine (**6**)	H	OH	O	Me
Thymidine (**1**)	H	H	O	Me
5-Fluorouridine (**7**)	H	OH	O	F
2'-Deoxy-5-fluorouridine (**8**)	H	H	O	F
5-Bromouridine (**9**)	H	OH	O	Br
5-Bromo-2'-deoxyuridine (**10**)	H	H	O	Br
5-Iodouridine (**11**)	H	OH	O	I
2'-Deoxy-5-iodouridine (**12**)	H	H	O	I
5-Ethynyluridine (**13**)	H	OH	O	ethynyl
2'-Deoxy-5-ethynyluridine (**14**)	H	H	O	ethynyl
5-Trifluorouridine (**15**)	H	OH	O	CF_3
Cytidine (**16**)	H	OH	NH_2	H
2'-Deoxycytidine (**17**)	H	H	NH_2	H
Uridine-5'-monophosphate (**18**)	PO_3^{2-}	OH	O	H
Cytidine-5'-monophosphate (**19**)	PO_3^{2-}	OH	NH_2	H

Compound	R^1	R^2	X^3	X^4
Adenosine (**20**)	H	OH	NH_2	H
2'-Deoxyadenosine (**21**)	H	H	NH_2	H
2-Fluoroadenosine (**22**)	H	OH	NH_2	F
2'-Deoxy-5-fluoroadenosine (**23**)	H	H	NH_2	F
2-Chloroadenosine (**24**)	H	OH	NH_2	Cl
2-Chloro-2'-deoxyadenosine (**25**)	H	H	NH_2	Cl
2-Aminoadenosine (**26**)	H	OH	NH_2	NH_2
2-Amino-2'-deoxyadenosine (**27**)	H	H	NH_2	NH_2
Guanosine (**28**)	H	OH	O	NH_2
2'-Deoxyguanosine (**29**)	H	H	O	NH_2
Inosine (**30**)	H	OH	O	H
2'-Deoxyinosine (**31**)	H	H	O	H
Xanthosine (**32**)	H	OH	O	O
2,6-Dichloropurine riboside (**33**)	H	OH	Cl	Cl
2,6-Dichloro 2'-deoxyriboside (**34**)	H	H	Cl	Cl
6-Chloro-2-fluoropurine riboside (**35**)	H	OH	Cl	F
6-Chloro-2-fluoropurine 2'-deoxyriboside (**36**)	H	H	Cl	F
Adenosine-5'-monophosphate (**37**)	PO_3^{2-}	OH	NH_2	H
Guanosine-5'-monophosphate (**38**)	PO_3^{2-}	OH	O	NH_2
Inosine-5'-monophosphate (**39**)	PO_3^{2-}	OH	O	H

Figure S1. Nucleosides and nucleotides in this study.

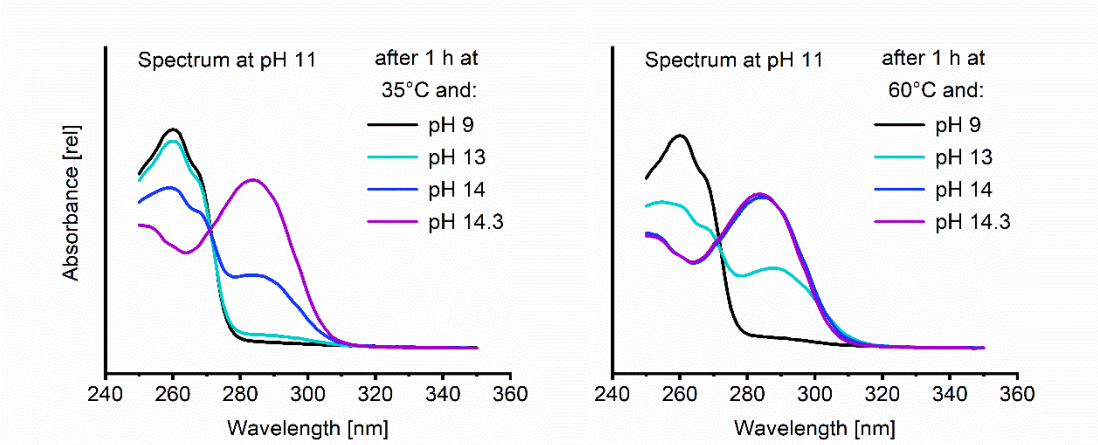


Figure S2. Degradation of fluorinated nucleoside **22**. 1 mM solutions of **22** were kept in either 50 mM glycine buffer (pH 9), 100 mM NaOH (pH 13), 1 M NaOH (pH 14) or 5 mM NaOH (pH 14.3) at 35 °C or 60 °C for 1 h. Samples of 40 µL were diluted in 460 µL 1 M H_2HPO_4 (pH 11) for measurement of UV/Vis spectra. A compound-to-compound conversion appears likely given the isosbestic point at 275 nm. Both base and temperature accelerated the apparent reaction. A similar behavior was observed for compound **23**.

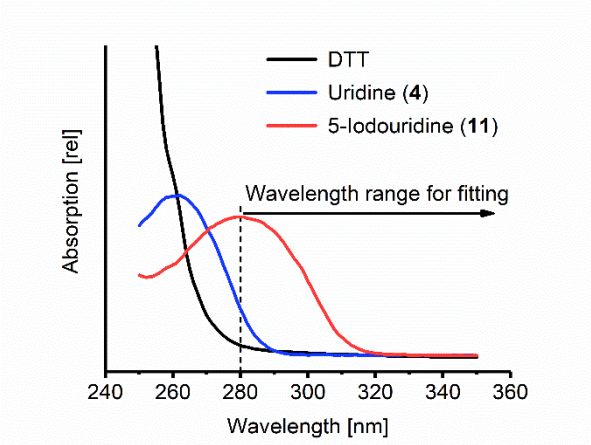


Figure S3. UV absorption spectra of nucleosides and dithiothreitol (DTT). With 5 mM DTT, the spectrum of DTT largely completely overlaps with that of 1 mM uridine (**4**, left), but the spectra of other nucleosides such as 5-iodouridine (**11**, right) can still be deconvoluted well from the reaction mixtures. A typical DTT spectrum (alkaline dilution factor of 9) and reference spectra for **4** (in 100 mM NaOH) and **11** (in 200 mM NaOH) are shown. All spectra in this figure are background corrected for multiwell plate absorption.

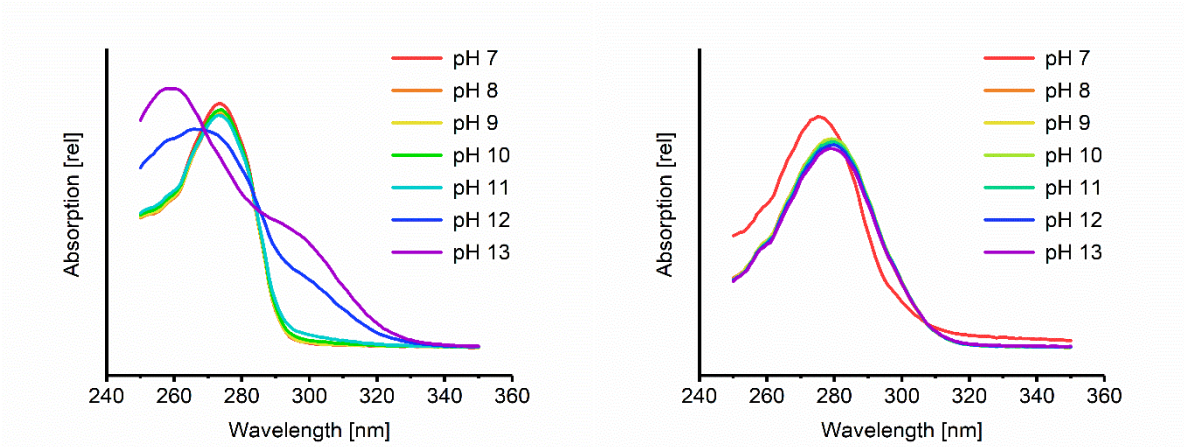


Figure S4. Dynamic UV absorption spectra of chlorinated purine scaffold **33**. Ribosyl nucleoside **33** (left) displays marked spectral "wobbling" above pH 11, compared to its free base (right). At pH 9 (yellow line), both compounds provide stable and reproducible spectra that allow robust unmixing.

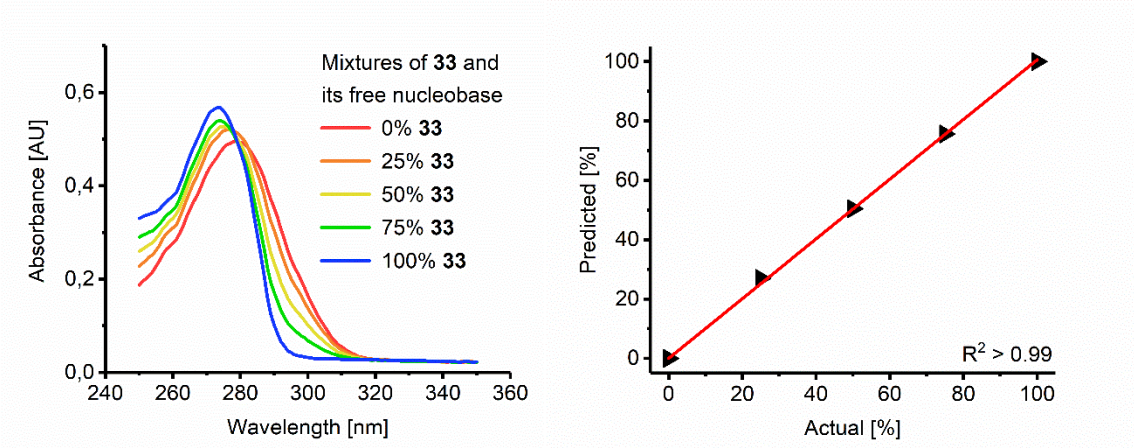


Figure S5. Spectral unmixing of mixtures of **33** and its free nucleobase. Mixtures of **33** with its base deliver stable and reproducible spectra at pH 9 with an isosbestic point of base cleavage at 278 nm (left; non-normalized raw data is shown), which allows for accurate unmixing under these conditions with reference spectra obtained at pH 9 (right).

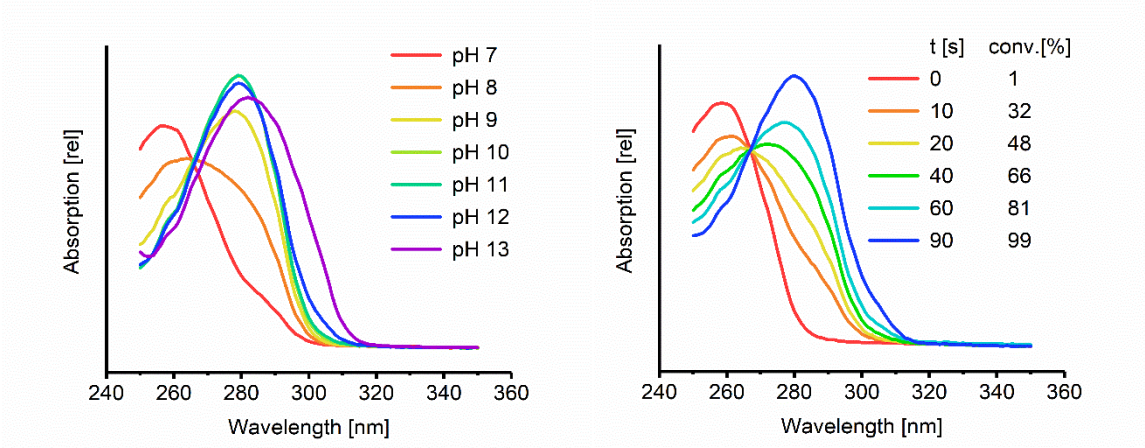


Figure S6. pH-dependent UV absorption spectra of 5-trifluoromethyluracil (the free base of **15**, left) and experimental spectra obtained from a reaction quenched in iPrOH and diluted in 100 mM glycine/NaOH buffer (pH 10) which allowed spectral unmixing-based reaction monitoring (right, spectra were normalized to the isosbestic point at 267 nm). The reaction was performed with 1 mM **15**, 50 mM phosphate and 10 µg·mL^{-1} pyrimidine nucleoside phosphorylase Y07 (BioNukleo GmbH, Berlin, Germany) in 100 mM glycine buffer at pH 9 and 80 °C. The reaction samples (50 µL) were quenched in 50 µL iPrOH and subsequently diluted with 400 µL 100 mM glycine/NaOH buffer (pH 10). Also see Figure S7.

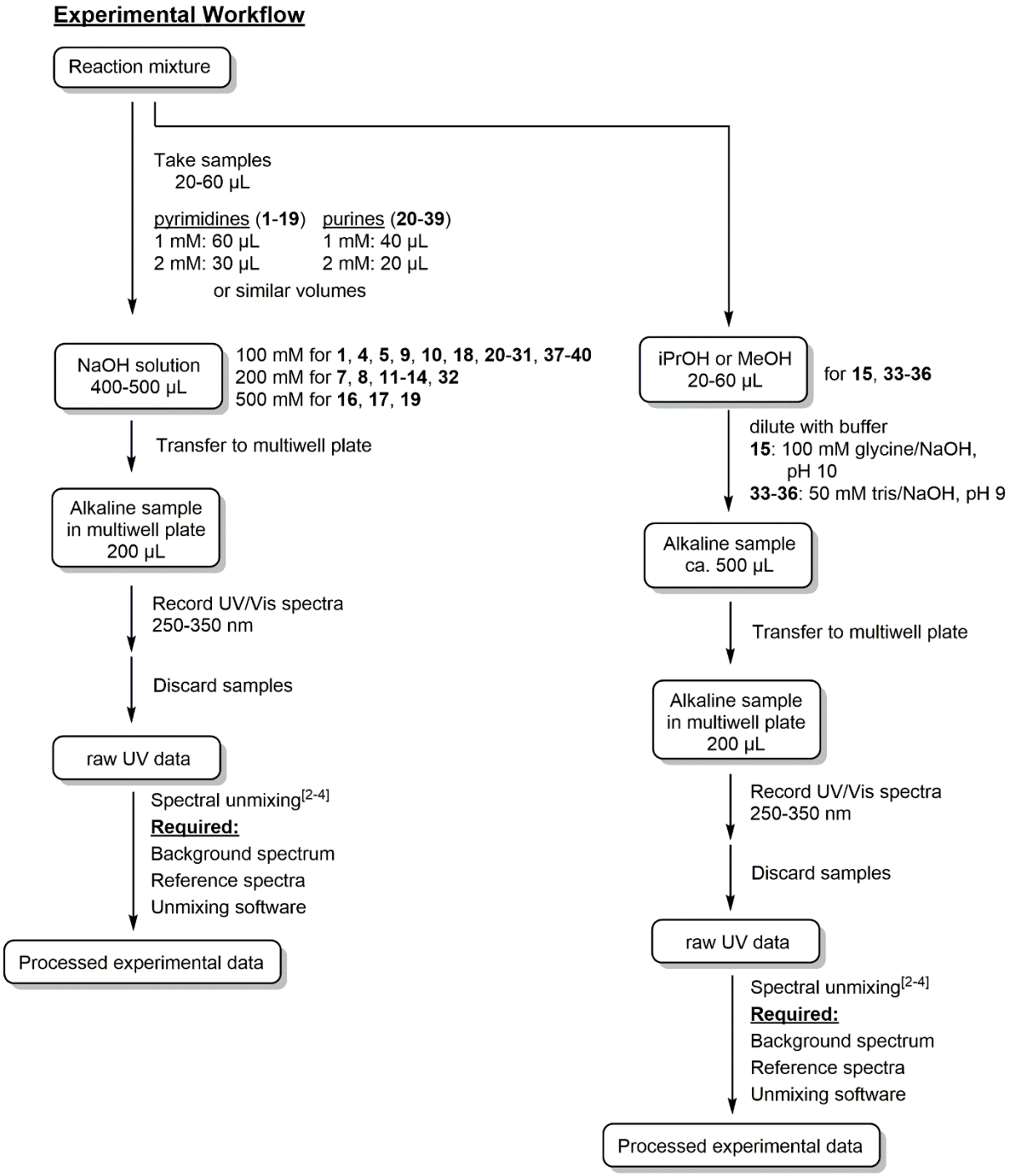


Figure S7. Flowchart for experimental sampling procedure. The process for sampling and sample processing is described. Please note that exact adherence to the suggested volumes is not required and, thus, purely optional. For the spectral unmixing procedure, please see the main text of the publication, the original report[2], as well as the associated external supplementary material[3] and the spectral unmixing Python code.[4] To facilitate the use of the method, reference spectra for substrates **1** and **4**—**40** can obtained from an external online repository.[3]

References

[1] A. Brand, L. Allen, M. Altman, M. Hlava, J. Scott, *Learn. Publ.* **2015**, *28*, 151–155.

[2] F. Kaspar, R. T. Giessmann, N. Krausch, P. Neubauer, A. Wagner, M. Gimpel, Methods Protoc. **2019**, *2*, 60.

[3] F. Kaspar, **2020**, DOI 10.5281/zenodo.3716126.

[4] R. T. Giessmann, N. Krausch, **2019**, DOI 10.5281/zenodo.3243376.