Notes on the Supporting Material

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

The reference spectra for all compounds can be obtained from the folder "Reference_Spectra" and the data used for figure creation is described below and can be obtained from the folder "Data_for_Figures".

The reference spectra for all substrates in this publication and their respective can be obtained from the folder "Reference_Spectra". The individual files are named either with a) the semi-trivial compound name according to IUPAC conventions, the compound number as listed in the main text of the publication and the medium in which it was obtained (e.g. "Uridine(4)_in_100mM_NaOH" also see the Supporting Information PDF file for the structures), or b) for nucleobases, the semi-trivial compound name according to IUPAC conventions and an indicator to which nucleoside(s) it belongs (e.g. "Uracil(base_of_4_and_5_and_18)_in_100mM_NaOH").
Importantly, several reference spectra can be applied to more than one species. Additional reference spectra for compounds **17** (use the one for **16**), **17** (**16**), **18** (**4**), **19** (**16**), **21** (**20**), **23** (**22**), **25** (**24**), **27** (**26**), **29** (**28**), **31** (**30**), **34** (**33**), **36** (**35**), **37** (**20**), **38** (**28**) and **39** (**30**) would be redundant and thus were not included. When fitting spectra of any of these compounds, we support using the above-mentioned alternative spectra that are, for all practical purposes, identical. Note, we did not include reference spectra for **40** or its free base, as this substrate does not display an isosbestic point of base cleavage and consequently does not allow normalization. For this substrate, we recommend reaction monitoring via the extinction coefficient at any given wavelength (< 260 nm) or performing careful manual calibration on the equipment to be used.

The data for figure 1 in the main text were generated from the reference spectra of **1** and **3** (accessible as "Thymidine(1)_in_100mM_NaOH" and "Thymine(base_of_1_and_6)_in_100mM_NaOH") and the data in panels **B**—**D** were created as linear combinations of these spectra for illustrative purposes.

The data for figure 2 in the main text are composed of the reference spectra of **1** and **3** (accessible as "Thymidine(1)_in_100mM_NaOH" and "Thymine(base_of_1_and_6)_in_100mM_NaOH") and several background spectra. For panel **B** we manually added a generic background spectrum which we

regularly employ for background correction of all reactions without atypical backgrounds (accessible in the file "FEK-2019-03-26-004_Spectrum", well A1). For panel **C** we manually shifted the spectrum of **1** by 0.1 AU by adding this value across the entire spectrum. For panel **D** we used the above mentioned generic backgrounds and background spectra obtained by 15-fold dilution of stocks of purified *Escherichia coli* thymidine phosphorylase in 100 mM NaOH to manually add these to the reference spectrum of **1** (background spectra available as raw data in file "FEK-2020-03-09-004", wells E1—E6, specifically E3 and E6). For panel **E** we employed previously mentioned background spectra and background spectra obtained from reaction mixtures containing DMF and manually added these to the reference spectrum of **1** (DMF background spectra available in the file "FEK-2019-12-03-001_Spectrum.tsv", wells C7—C12, specifically C8, C10 and C12).

The data for figure S2 in the Supporting Information are available as raw data in the file "FEK-2019-06-26-003" (wells F1—F8). Please note, this file also contains the data for the same experiment with the free base of **22** (2-fluoroadenine, wells G1—G8), for which no degradation was noticeable in the UV absorption spectra.

The data for figure S3 in the Supporting Information are composed of the reference spectra of nucleosides **4** and **11** (available as "Uridine(4)_in_100mM_NaOH" and "Iodouridine(11)_in_200mM_NaOH", respectively) and a background spectrum obtained by 9-fold dilution of a 5 mM solution od DTT (available as raw data in the file "FEK-2020-02-19-003", well F10).

The data for figure S4 in the Supporting Information are available as raw data in the file "FEK-2019-06-19-001" (wells A1—A7 and B1—B7 for the nucleoside **33** and its free base, respectively).

The data for figure S5 in the Supporting Information are available in the file "FEK-2019-11-28-009_Spectrum" (wells A1—A5) and described in the metadata file "FEK140". The data were fitted against the reference spectra provide herein ("Dichloropurine(base_of_33_and_34)_in_50mM_Tris_pH9" and "Dichloropurine-Riboside(33)_in_50mM_Tris_pH9" for the nucleoside **33** and its free base, respectively) and the results are available in the file "FEK140_results".

The data for figure S6 in the Supporting Information are provided as raw data in the file "FEK-2020-03-06-003" (wells B1—B7; the corresponding data for the nucleoside **15** is in wells A1—A7) and as processed data in the file "FEK-2020-03-09-001_Spectrum.tsv" as described by the metadata file

“FEK180”. Data were fitted against the reference spectra of **15** and its free base (“Trifluoromethyluridine(15)_in_100mM_glycine_pH10” and “Trifluoromethyluracil(base_of_15)_in_100mM_glycine_pH10”) and the results are given in the file “FEK180_results”.