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Supplementary Data

Pyrazolo[3,4-*d*]pyrimidine Ribonucleosides Related to 2-Aminoadenosine and Isoguanosine: Synthesis, Deamination and Tautomerism

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1. pK_a Measurements

The pK_a values of compounds **1a-d**, **3a-d**, **15** were determined spectrophotometrically according to Albert.[1] The UV spectra were recorded between 220-350 nm. The titration was performed between pH 2 and 12 at room temperature. The compounds were dissolved in phosphate buffer solution (7.8 g $NaH_2PO_4 \cdot H_2O$ in 500 ml water). An aliquot (50 ml) was placed in a beaker (100 ml) and the pH value was adjusted to different values. For pH values from 2.0 to 4.6 the solution was stepwise acidified with H_3PO_4 (first 10% and then 85%) and for pH from 4.6 to 12 with 3 M aq. NaOH. UV-spectra were recorded. The absorbance values were plotted against the pH values. The given wavelength corresponds to the maximal changes of the UV spectra. The first derivative of the absorption (dA/dpH) was calculated using the program Origin 6.0. For spectra see Figures S1-8.

Compound **1a**

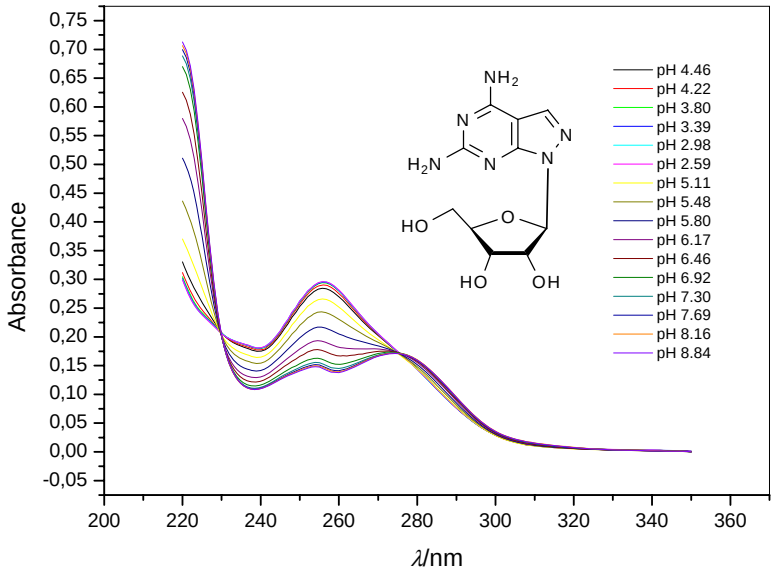

Figure S1 UV-spectral changes of compound **1a** in phosphate buffer solutions pH 2.59-8.84.

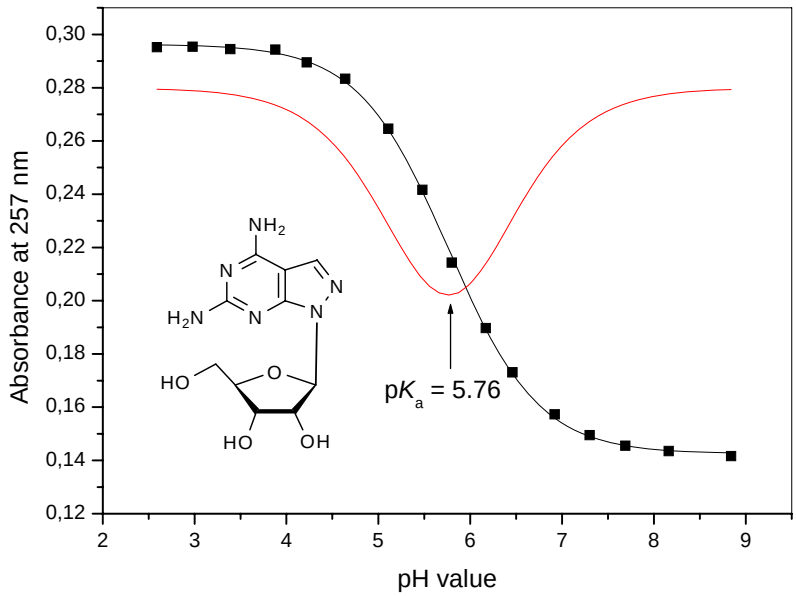

Figure S2 Titration profile and first derivative of absorbance (275nm) of compound **1a** against the pH value.

Compound **3a**

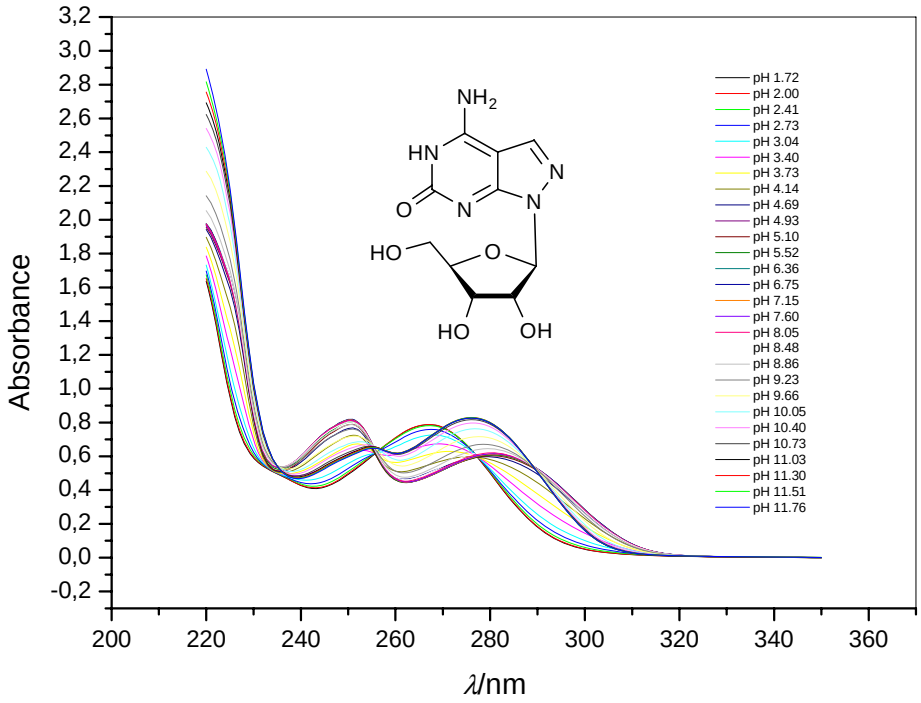

Figure S3 UV-spectral changes of compound **3a** in phosphate buffer solutions pH 1.72-11.76.

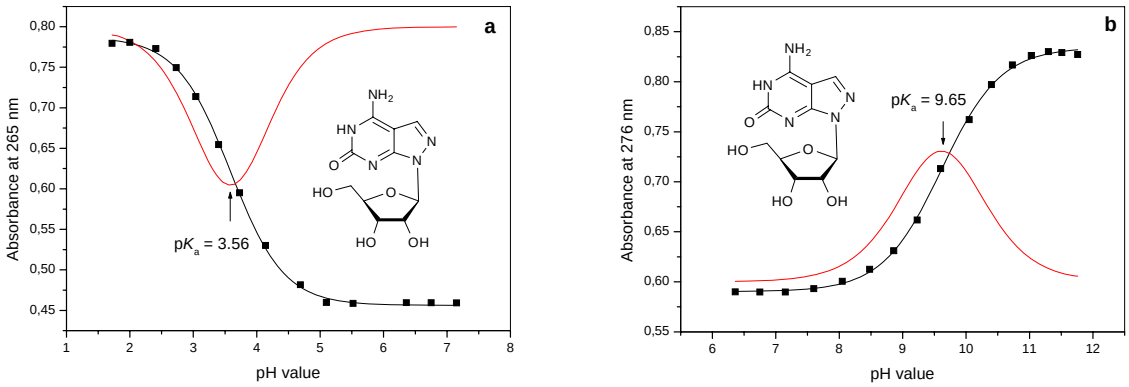

Figure S4 Titration profile and first derivative of absorbance (a: 265 nm; b: 276 nm) of compound **3a** against the pH value.

Compound **3c**

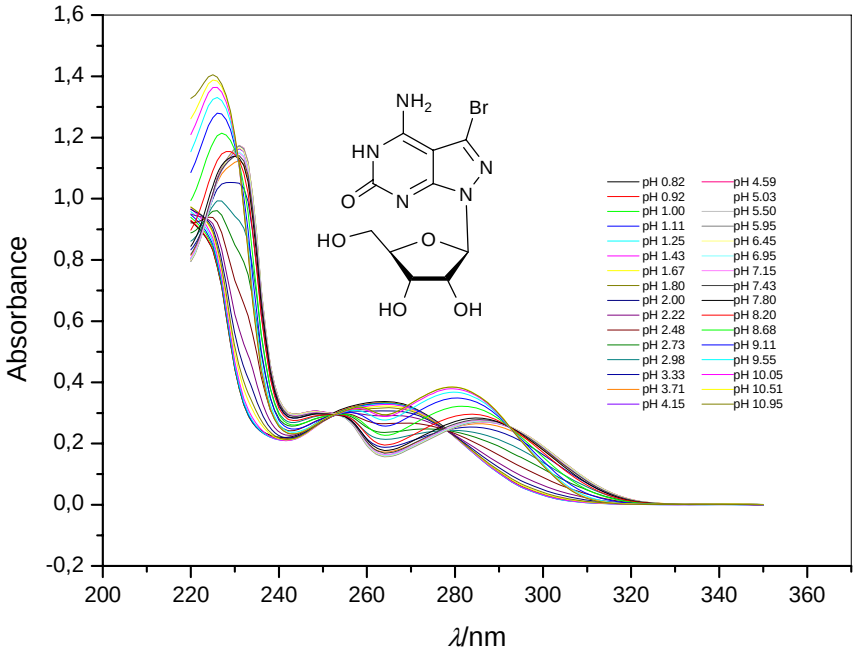

Figure S5 UV-spectral changes of compound **3c** in phosphate buffer solutions pH 0.82-10.95.

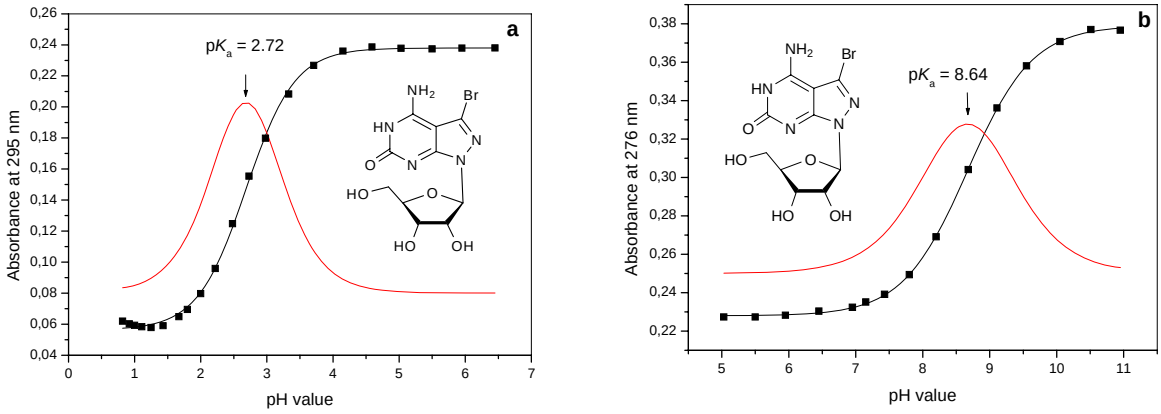

Figure S6 Titration profile and first derivative of absorbance (a: 295 nm; b: 276 nm) of compound **3c** against the pH value.

Compound **15**

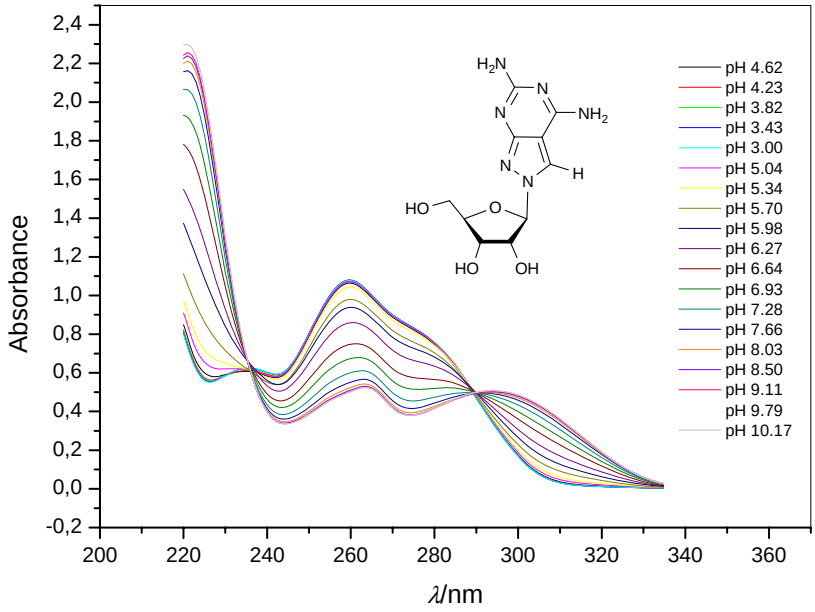

Figure S7 UV-spectral changes of compound **15** in phosphate buffer solution pH 3.00-10.17.

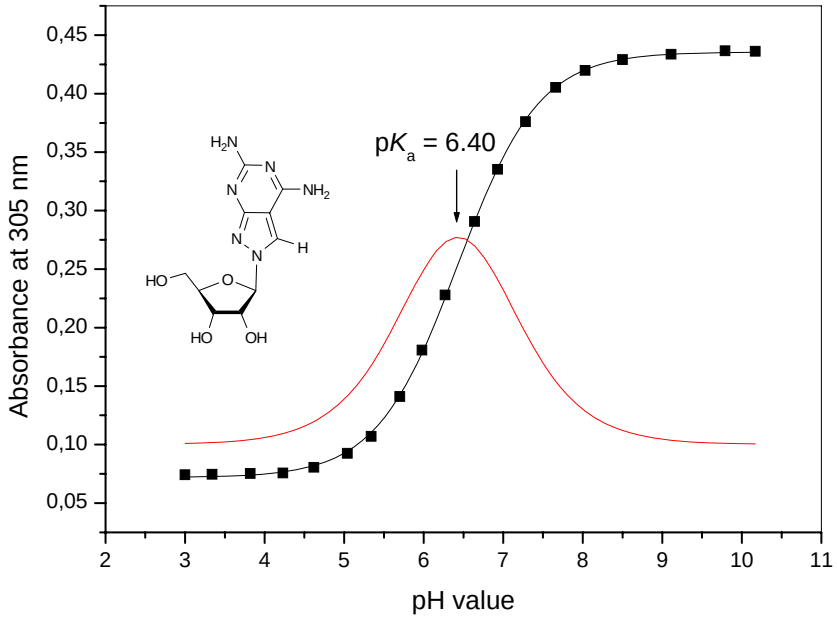

Figure S8 Titration profile and first derivative of absorbance (275nm) of compound **15** against the pH value.

2. Relative initial velocities of the deamination reaction catalyzed by adenosine deaminase

Adenosine deaminase (EC 3.5.4.4, Type V: from Bovine spleen, 0.16 unit per μl, Sigma) was diluted (0.004 unit per μl) with 0.06 M Sørensen buffer (pH 7.0). The substrate was dissolved in the same buffer. After the deaminase (0.004 units to 1 ml substrate solution) was added, the UV absorbance was recorded at a wavelength where the absorbance shows the maximal changes. The absorption at this wavelength was plotted against the time. The slop of the linear part of the curve was taken as slop *S*. At least two solutions with different concentrations were measured for each substrate to prove enzyme saturation. Only when the same *S* was observed for different concentration, the initial velocity of the deamination reaction can be the maximum enzyme velocity. The extinction coefficients of the substrate and the product at certain wavelengths were measured as ε_{sub} and ε_{pro}, the difference between them is defined as $\Delta\varepsilon = \varepsilon_{pro} - \varepsilon_{sub}$. The relative V_{max} of the sample was calculated according to the equation:

$V_{max(sample)} = V_{max(standard)} \times (S_{sample}\Delta\varepsilon_{standard}/S_{standard}\Delta\varepsilon_{sample})$.

2-Aminoadenosine was taken as standard substrate with a relative V_{max} of 25 according to Hampton.[2] For details and calculations see Figures S9-12.

2-Aminoadenosine

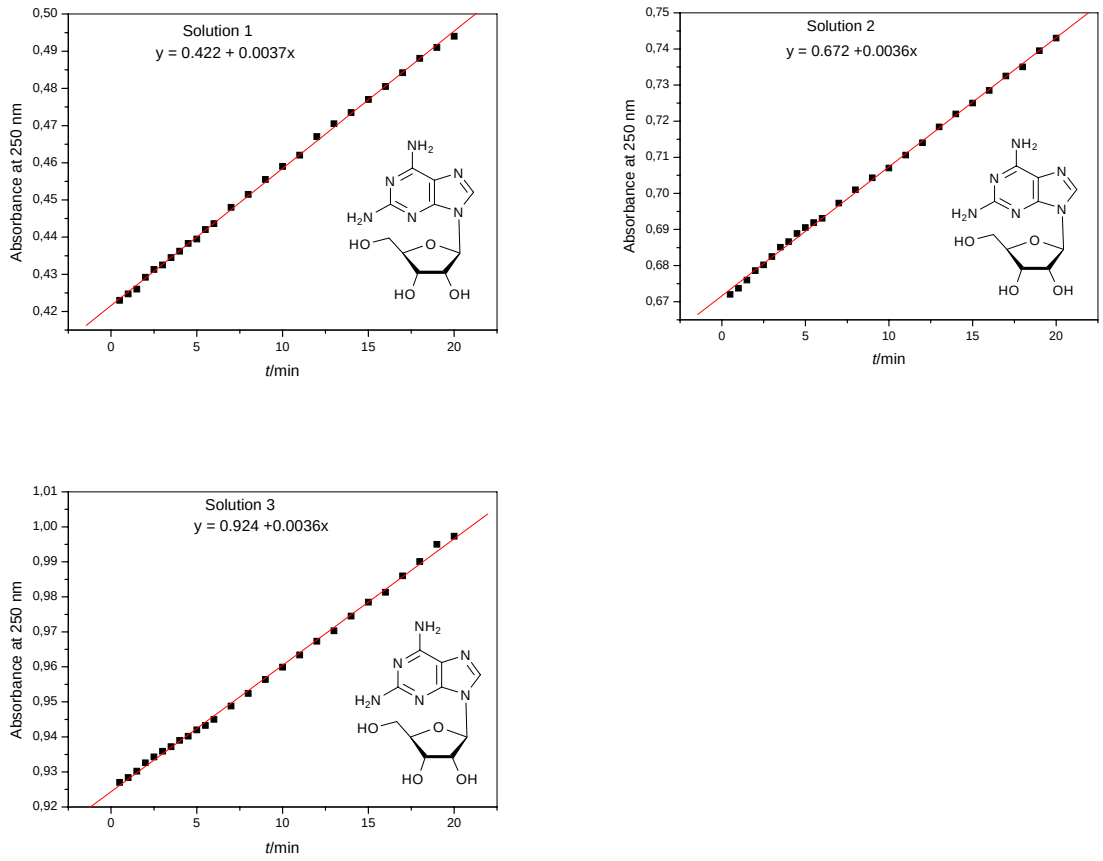

Figure S9 Deamination graph of 2-aminoadenosine measured at 250 nm as a function of time (three different concentrations). Slop = 0.0036, 2-aminoadenosine→guanosine: $\Delta\varepsilon_{250}$ = 11500-8800 = 2700.

Compound **1a**

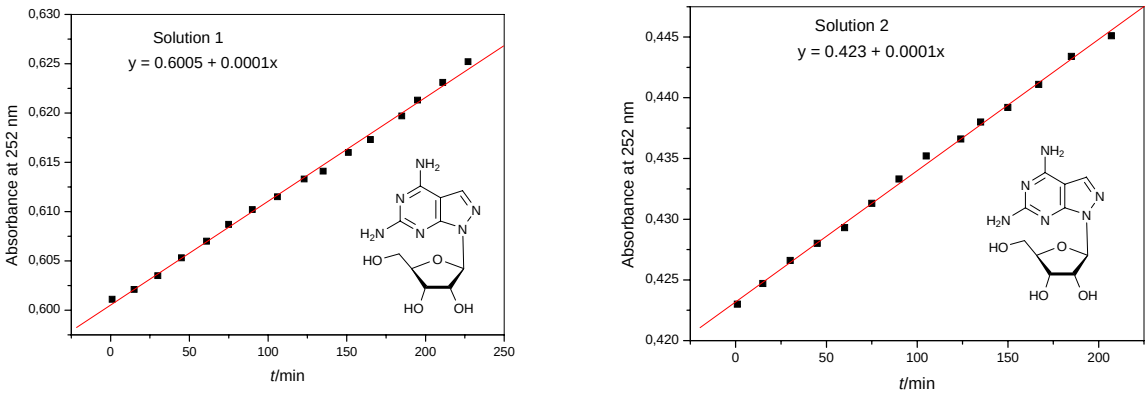

Figure S10 Deamination graph of compound **1a** measured at 252 nm as a function of time (two different concentrations). Slop = 0.0001, **1a**→ pyrazoloG: $\Delta\varepsilon_{252} = 13200\text{-}6200 = 7000$.

$V_{max(sample)} = V_{max(standard)} \times (S_{sample}\Delta\varepsilon_{standard}/S_{standard}\Delta\varepsilon_{sample})$

$V_{max}(\mathbf{1a}) = 25 \times (0.0001\times2700)/(0.0036 \times7000) = 0.27$

Compound **1b**

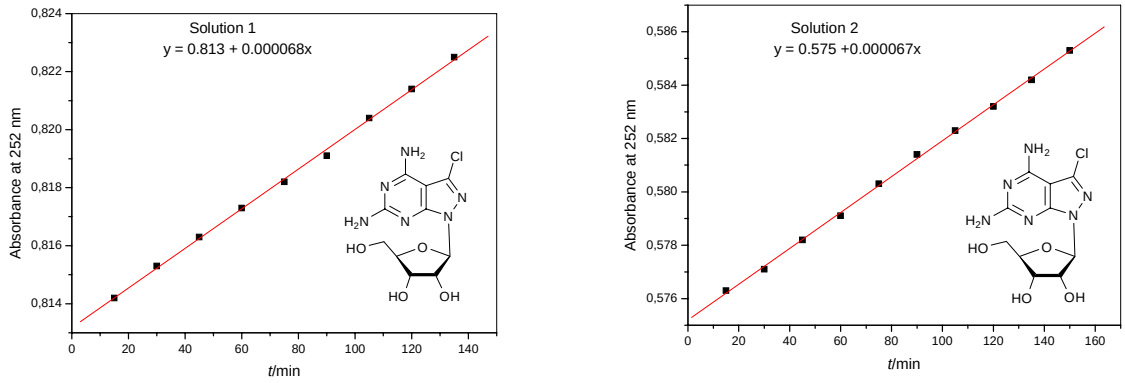

Figure S11 Deamination graph of compound **1b** measured at 252 nm as a function of time (two different concentrations). Slop = 0.000068, **1b**→ chloropyrazoloG: $\Delta\varepsilon_{252}$ = 12800-5800 = 7000.

$V_{max(sample)} = V_{max(standard)} \times (S_{sample}\Delta\varepsilon_{standard}/S_{standard}\Delta\varepsilon_{sample})$

V_{max}(**1b**) = 25 × (0.000068×2700)/(0.0036 ×7000) = 0.18

8-Aza-7-deazaadenosine (pyrazolo A)

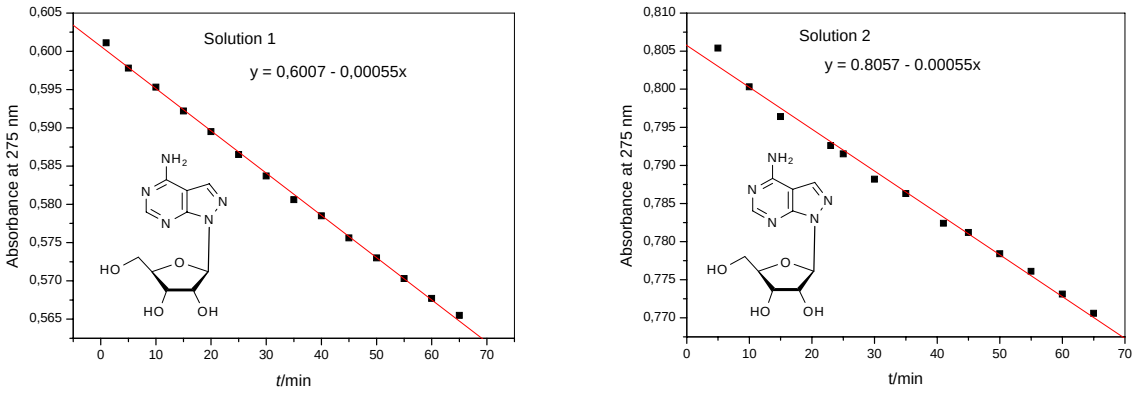

Figure S12 Deamination graph of 8-aza-7-deazaadenosine measured at 275 nm as a function of time (two different concentrations). Slop = -0.00055, 8-aza-7-deazaadenosine→chloropyrazolo G: $\Delta\varepsilon_{275}$ = 2300-10100 = -7800.

$V_{max(sample)} = V_{max(standard)} \times (S_{sample}\Delta\varepsilon_{standard}/S_{standard}\Delta\varepsilon_{sample})$

V_{max}(pyrazolo A) = 25 × ((-0.00055)×2700)/(0.0036 ×(-7800)) = 1.32

3. Determination of keto/enol populations of compounds 3a-d

Stock solutions of compounds **3a-d** were prepared (~4 mg of the nucleoside in 2 ml MeOH). Aliquots (100 µl) were introduced into a measuring flask (10 ml) and the solvent was allowed to evaporate. The solvent mixtures varying in the water/dioxane content (from 98% dioxane to 5% dioxane) were added. For details see Figures S13, 15, 18, 20. To obtain quantitative data, the ratio of the extinction coefficient at two wavelengths to maximize the difference between the keto and enol forms was chosen as a metric for the tautomeric equilibrium constant in dioxane/water mixture in varying proportions. The data were processed by taking the ratios of absorbance at the long wavelength (290 nm, corresponding to the keto form absorption) to the absorbance at the short wavelength (265 nm, corresponding to the enol form absorption), and plotting the logarithm of that value *vs* the $E_T(30)$ value of the various solvent mixtures (Figures S14, 16, 19, 21), the $E_T(30)$ values were interpolated from the data described by Jouanne *et al*.[3] The K_{TAUT} values were determined quantitatively by the method of Shugar *et al*.[4] and Voegel *et al*.[5] A plot of the logarithm of the tautomeric equilibrium constant for **3a-d** versus the polarity parameter $E_T(30)$ of a set of the dioxane-water mixture is shown in Figures S17, 22.

Compound **3a**

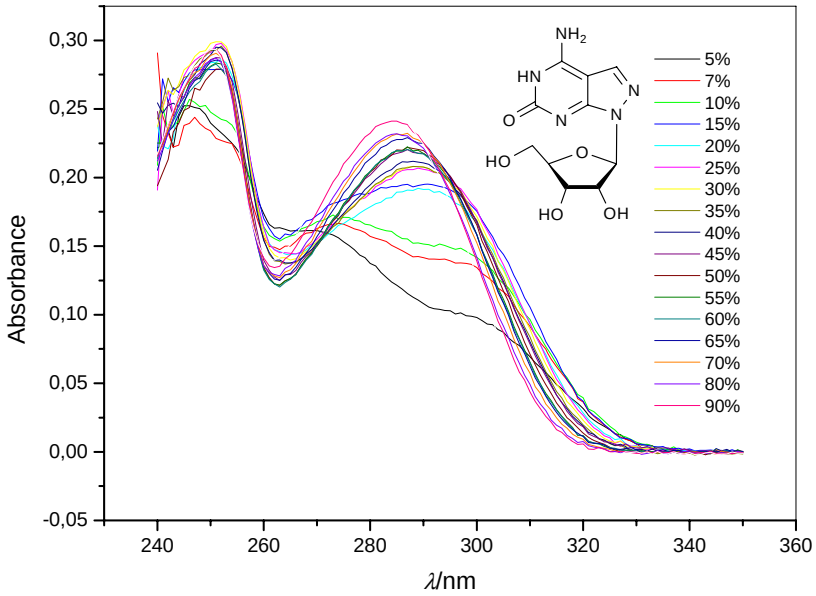

Figure S13 UV profile for compound **3a** measured in water-dioxane mixtures. The water content is indicated in the figure.

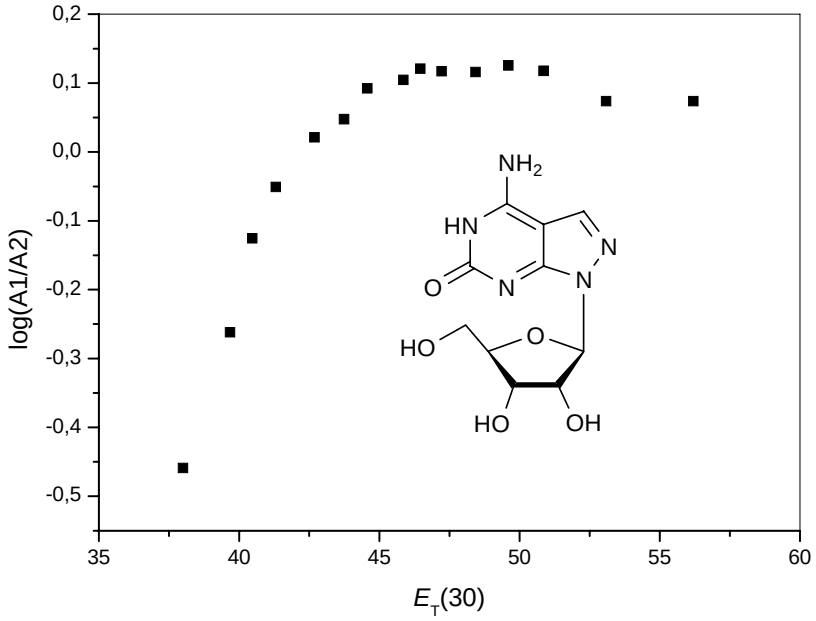

Figure S14 Graph of log (A1/A2) vs E_T(30) of the solvents (A1 = absorbance at 290 nm, A2 = absorbance at 265 nm).

Compound **3b**

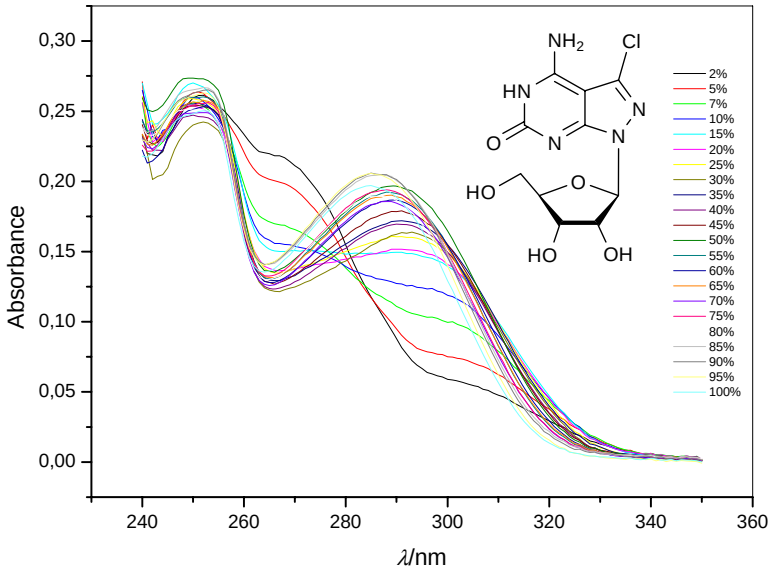

Figure S15 UV profile for compound **3b** measured in water-dioxane mixtures. The water content is indicated in the figure.

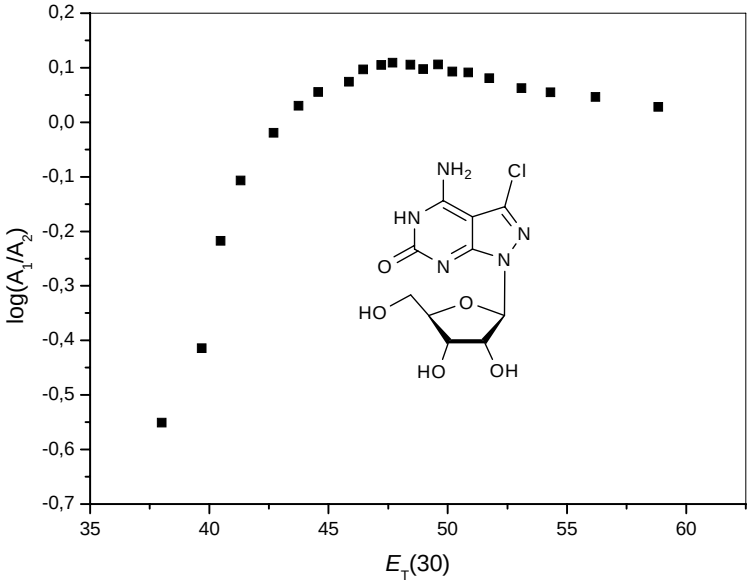

Figure S16 Graph of log (A1/A2) vs $E_T(30)$ of the solvents (A1 = absorbance at 290 nm, A2 = absorbance at 265 nm).

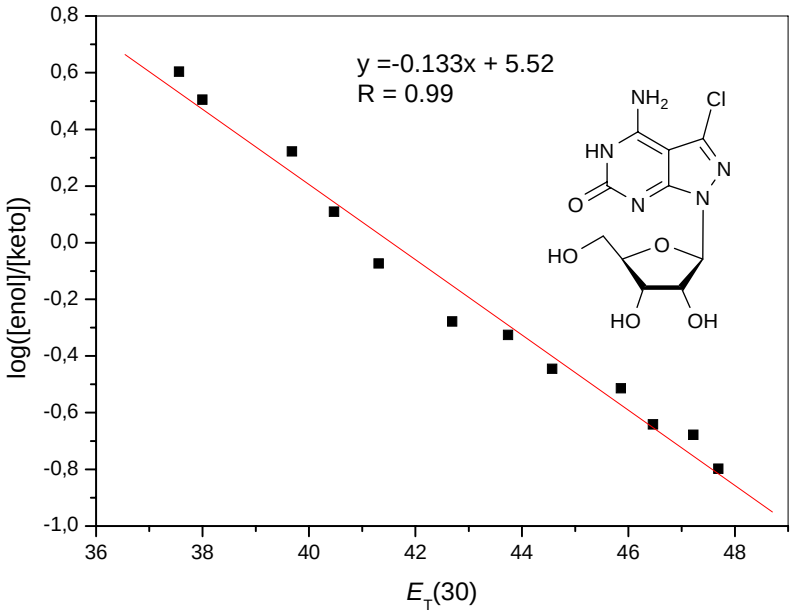

Figure S17 Plot of log([enol]/[keto]) versus $E_T(30)$ for compound **3b** in mixtures of dioxane ($E_T(30) = 36.0$) and water ($E_T(30) = 63.1$).

Compound **3c**

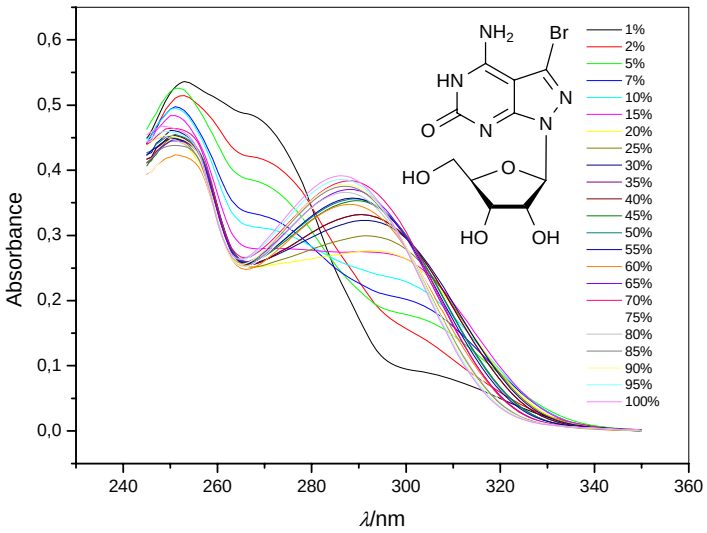

Figure 18 UV profile for compound **3c** measured in water-dioxane mixtures. The water content is indicated in the figure.

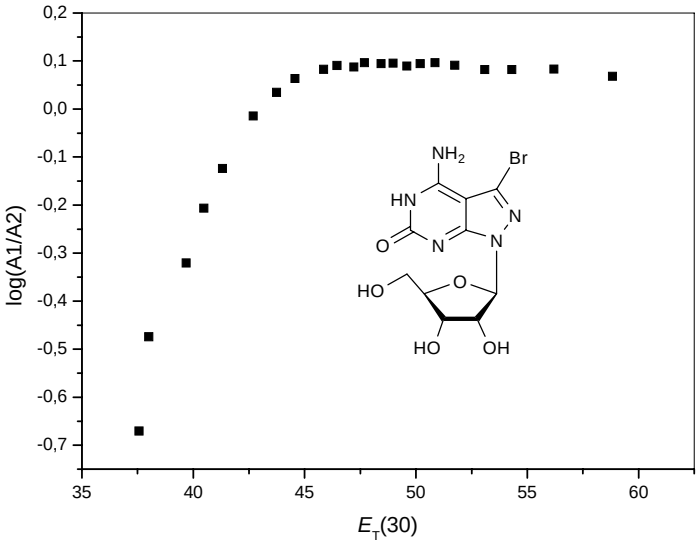

Figure S19 Graph of log (A1/A2) vs $E_T(30)$ of the solvents (A1 = absorbance at 290 nm, A2 = absorbance at 265 nm).

Compound **3d**

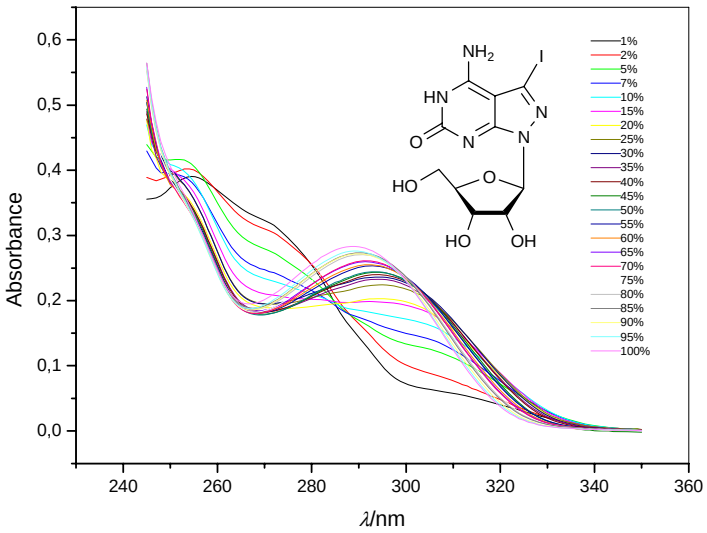

Figure S20 UV profile for compound **3d** measured in water-dioxane mixtures. The water content is indicated in the figure.

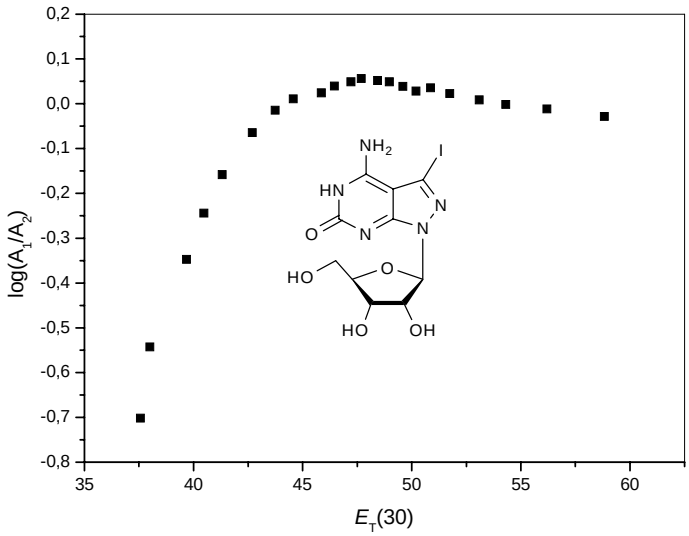

Figure S21 Graph of log (A1/A2) of compound **3d** vs $E_T(30)$ of the solvents (A1 = absorbance at 305 nm, A2 = absorbance at 270 nm).

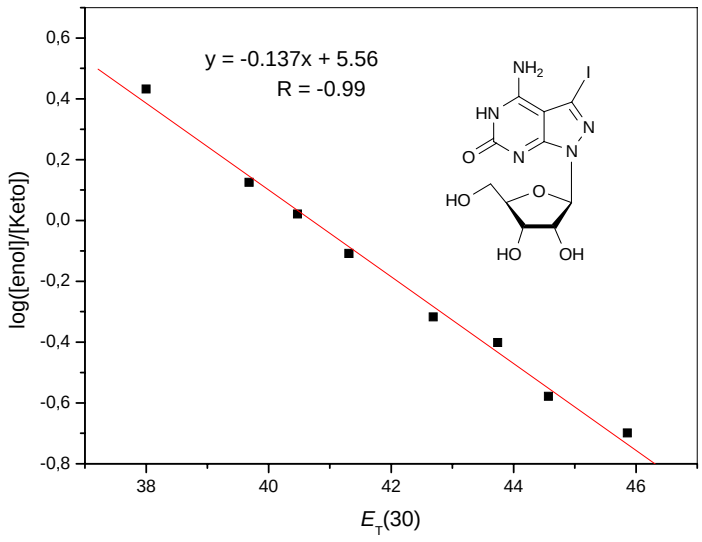

Figure S22 Plot of log([enol]/[keto]) versus $E_T(30)$ for compound **3d** in mixtures of dioxane ($E_T(30) = 36.0$) and water ($E_T(30) = 63.1$).

References

1 A. Albert and E. P. Serjeant, in *The Determination of Ionization Constants*, Chapman and Hall, Ltd., London, 1971, pp. 44-64.

2 A. Hampton, in *Chemistry of Nucleosides and Nucleotides*, ed. L. B. Townsend, Plenum, New York and London, 1991, vol. 2, ch. 5, pp. 405.

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4 J. Sepiol, Z. Kazimierczuk and D. Shugar, *Z. Naturforsch. C*, 1976, **31**, 361-370.

5 J. J. Voegel, U. Von Krosigk and S. A. Benner, *J. Org. Chem.*, 1993, **58**, 7542-7547.