# Supporting Information for Detecting Biologically Relevant Phosphates with Locked Salicylaldehyde Probes in Water

Namita Kumari, Felix Zelder*

## Experimental Section

**Materials.** All solvents and reagents were purified and dried by usual methods. All of the starting materials were obtained from well-known commercial suppliers and used as received. A stock solution (1 mM) of compound (**1**) was made in water. A stock solution (1 mM) of compound (**2**) was made in DMSO. All solvents used for studies were of analytical grade and used without further purification. Each chemical used for synthesis or spectroscopic titrations was of the best available grade. TRIS buffer (10 mM, pH 7.4) was used for studies in aqueous medium. The sodium salts of ATP (Adenosine 5′-triphosphate disodium salt hydrate), ADP (Adenosine 5′-diphosphate sodium salt) and AMP (Adenosine 5′-triphosphate disodium salt) were used for the studies in aqueous medium, however tetrabutylammonium salts of ATP, ADP and AMP were used for studies performed in DMSO. All the other anions used in the UV-vis and fluorescence studies were sodium salts in aqueous medium and tetrabutylammonium salts in DMSO (except for PPi which was tributylammonium salt).

**Instrumentation.** $^{1}H$, $^{31}P$ and $^{13}C$ NMR spectra were recorded in ([D6]DMSO) or $D_2O$ with a Bruker AV DRX 400 spectrometer operating at 400.13, 161.98 and 100.61 MHz respectively. UV/Vis absorption spectra were obtained with a Varian Cary spectrophotometer. Fluorescence spectra were recorded with a Cary-Eclipse spectrofluorometer. The slit-widths for the fluorescence experiments were kept at 10 nm (excitation) and 10 nm (emission) and the excitation wavelength was set at 350 nm for all of the fluorescence experiments. High resolution electrospray mass spectra were recorded with Bruker maXis spectrometer. Binding constants were determined by nonlinear regression analysis of each binding isotherm using GraphPad Prism for Windows (GraphPad Software, San Diego, CA).

**Sensing Procedure**. The concentration of probes (**1** and **2**) used for UV-vis measurements were 20 μM in both water ([TRIS] = 10 mM, pH 7.4) and DMSO. The final concentrations for the fluorescence experiments were ([**1**] = 10 μM in water ([TRIS] = 10 mM, pH 7.4), and [**2**] = 2 μM in DMSO. The addition of the anions followed afterwards.

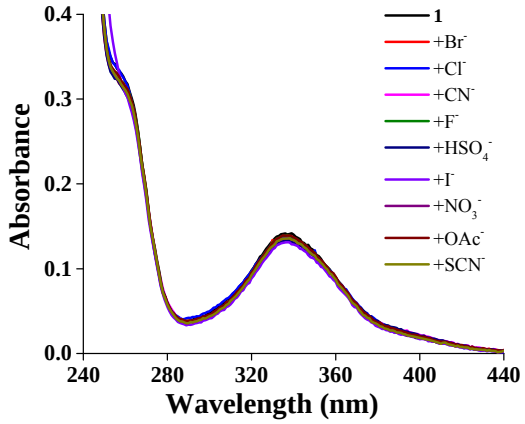


**Fig. S1** UV-vis absorption spectra of **1** (20 μM) in in water ([TRIS] = 10 mM, pH 7.4) with added anions (10 eq.).

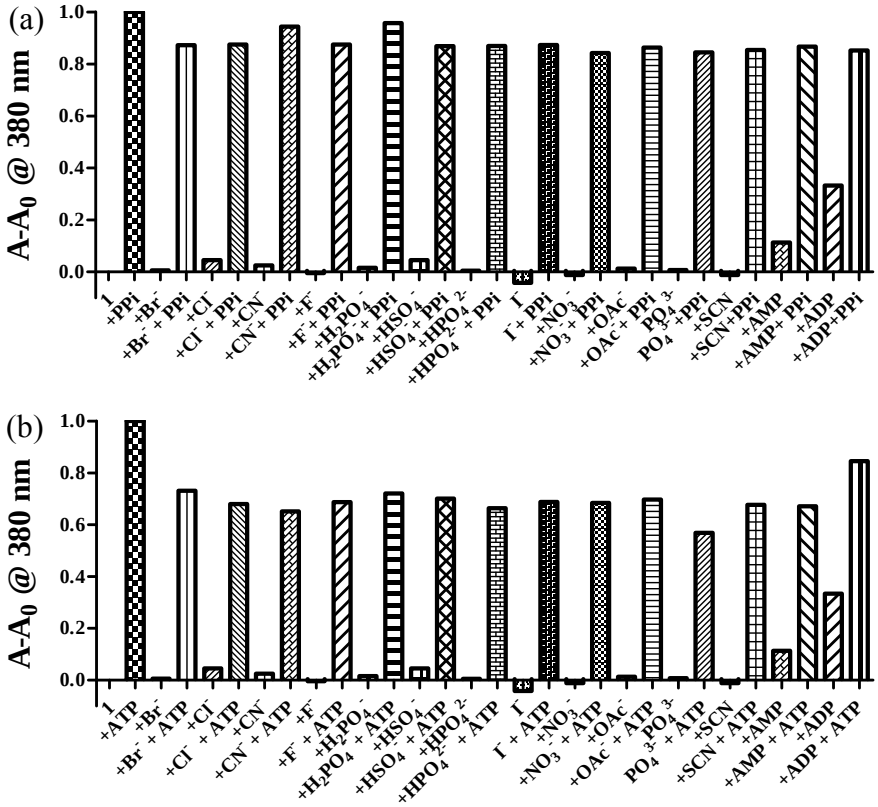


**Fig. S2** Normalized change in absorbance of **1** (20 μM) at 380 nm in water ([TRIS] = 10 mM, pH 7.4) upon addition of (a) PPi (10 eq.) and (b) ATP (10 eq.) in the presence of various anions (10 eq.).

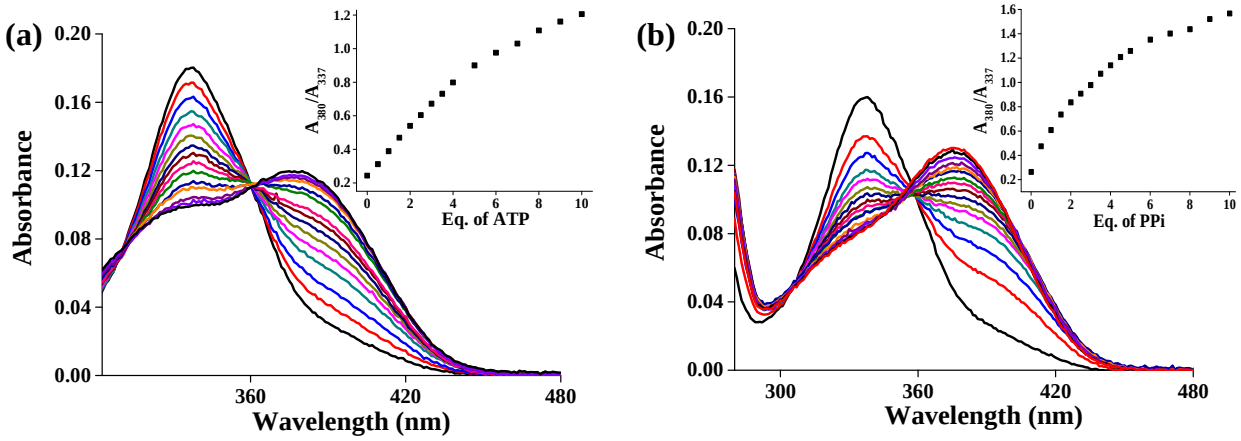


**Fig. S3** UV-Vis titration of **1** (20 μM) in water ([TRIS] = 10 mM, pH 7.4) with (a) ATP and (b) PPi.

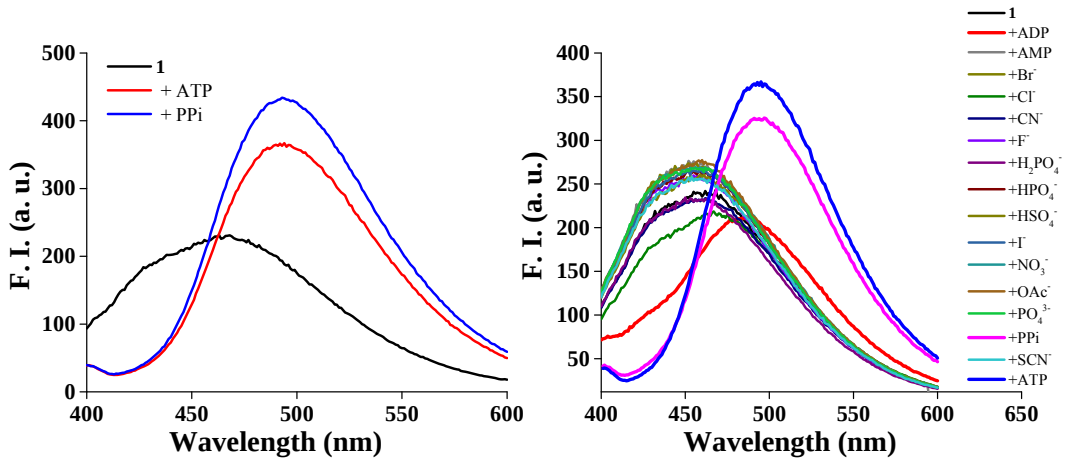


**Fig. S4** Emission spectra of **1** (10 μM) in in water ([TRIS] = 10 mM, pH 7.4) with added anions (10 eq.).

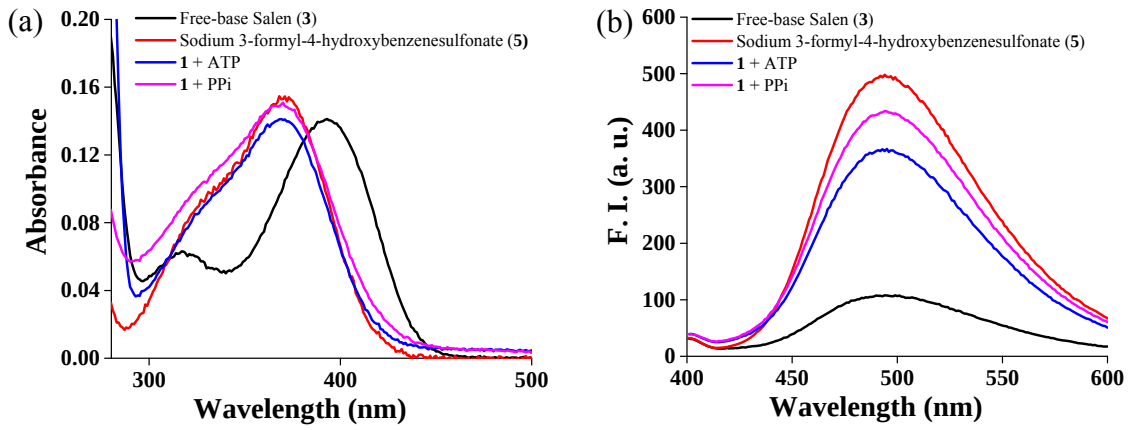


**Fig. S5** (a) UV-vis spectra of **1** (20 μM) in water ([TRIS] = 10 mM, pH 7.4) with added ATP and PPi compared with the absorption spectra of the corresponding free-base salen **3** and salicylaldehyde **5** (Sodium 3-formyl-4-hydroxybenzenesulfonate). (b) Emission spectra of **1** (10 μM) in water ([TRIS] = 10 mM, pH 7.4) upon addition of ATP and PPi (10 eq.), compared with free-base salen (**3**) (10 μM) and salicylaldehyde **5** (Sodium 3-formyl-4-hydroxybenzenesulfonate; 20 μM; $\lambda_{ex.}$ = 350 nm).

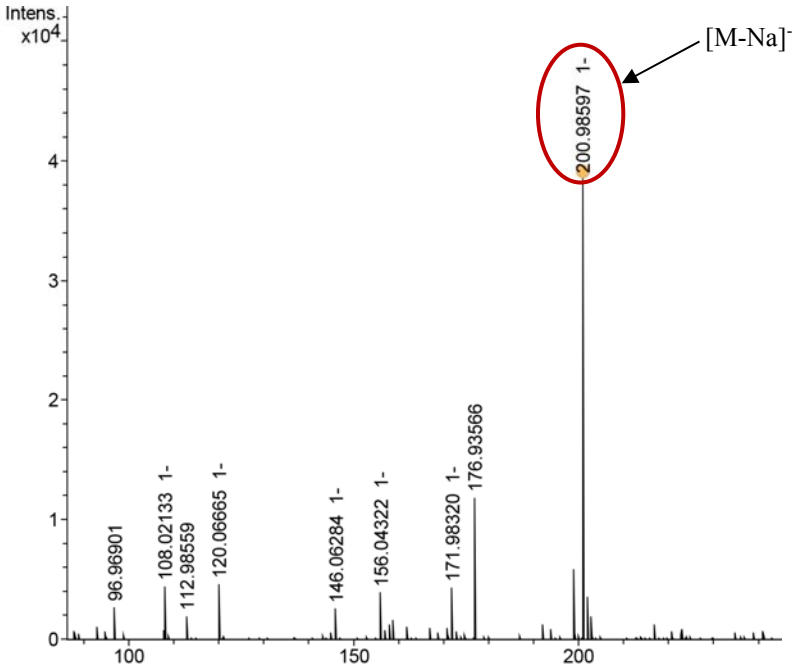


**Fig. S6** Mass spectra of **1** with added PPi in water (pH 7.4, [TRIS] =10 mM). Mass spectral peak 200.9860 corresponds to $[M\text{-}Na]^-$ ($m/z_{calcd.}$ = 200.9863); [M] corresponds to salicylaldehyde derivative **5**.

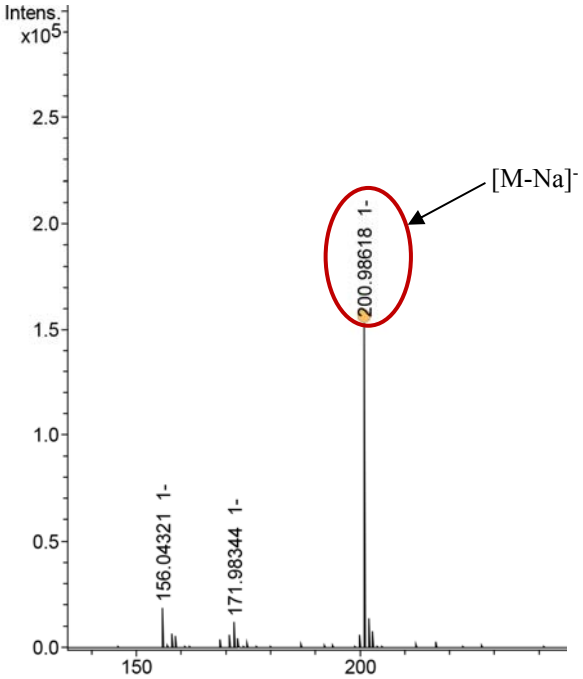


**Fig. S7** Mass spectra of **1** with added ATP in water (pH 7.4, [TRIS] =10 mM). Mass spectral peak 200.9862 corresponds to $[M\text{-}Na]^-$ ($m/z_{calcd.}$ = 200.9863); [M] corresponds to salicylaldehyde derivative **5**.

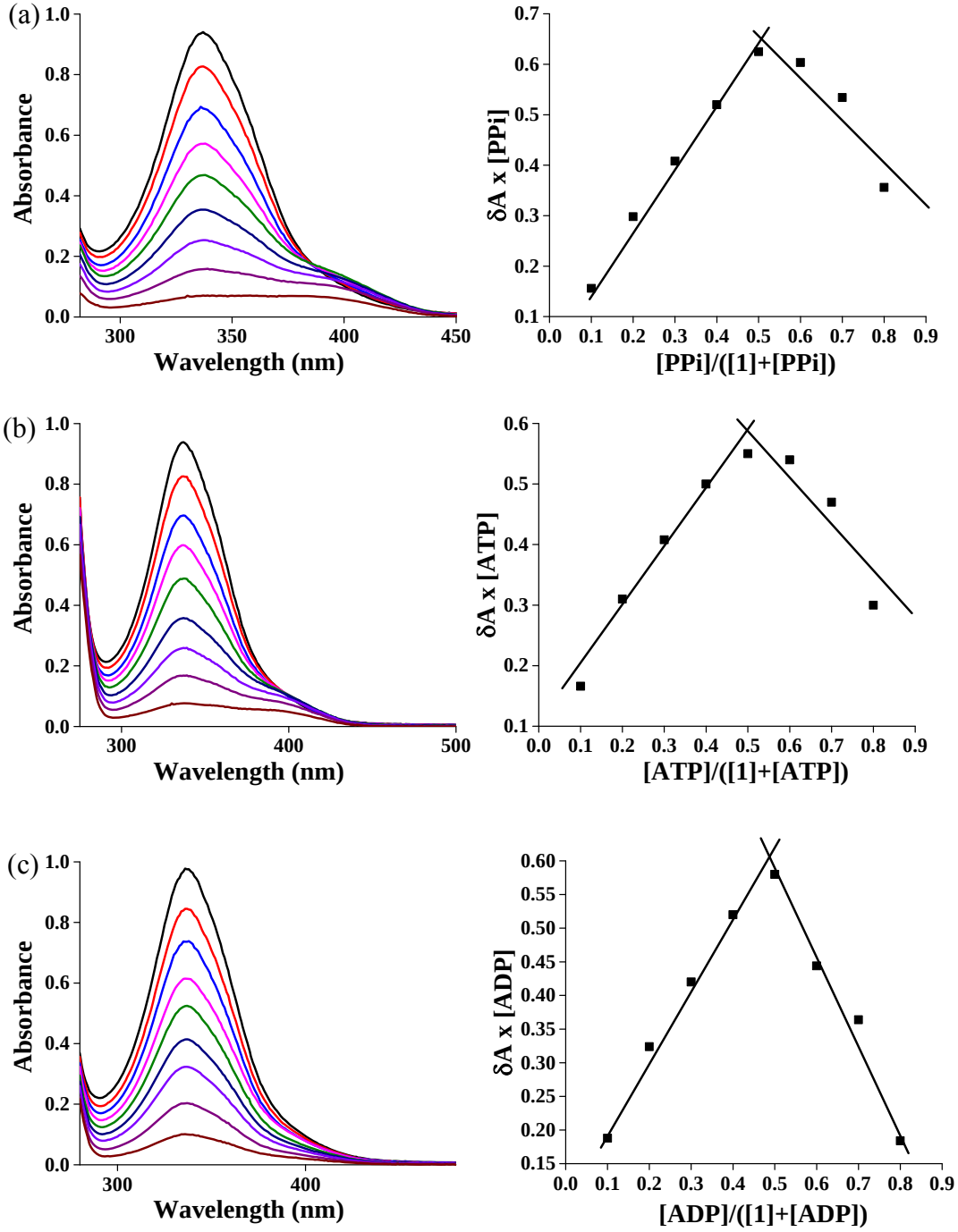


**Fig. S8** Job plot for stoichiometry determination for the reaction of **1** with (a) PPi, (b) ATP and (c) ADP in water ([TRIS] = 10 mM, pH 7.4). [(δA = change in absorbance at 380 nm); the total concentration [probe] + [phosphate oxo-anions] $= 1.0 \times 10^{-4}$ M].

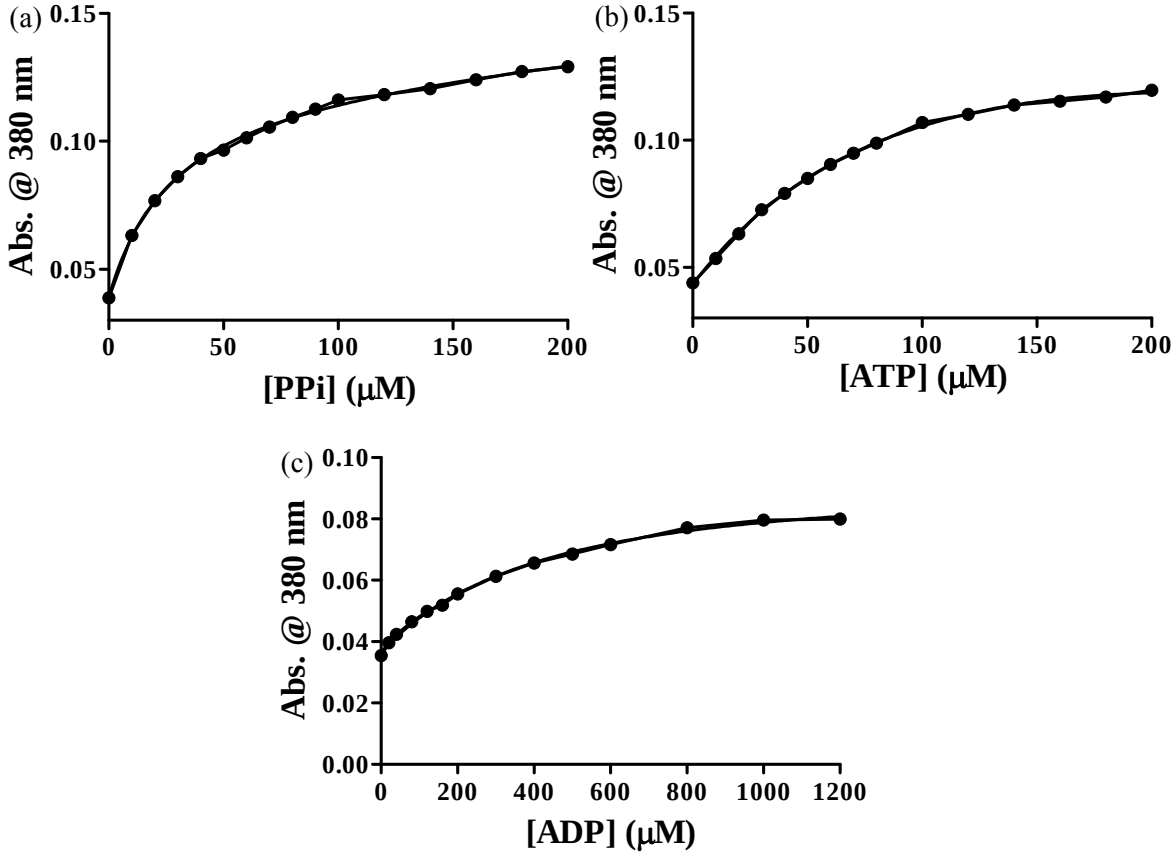


**Fig. S9** Equilibrium constant calculation for the displacement of $Zn^{II}$ from **1** with (a) PPi, (b) ATP and (c) ADP based on non-linear regression analysis for 1:1 stoichiometry using GraphPad Prism software for one site total binding.

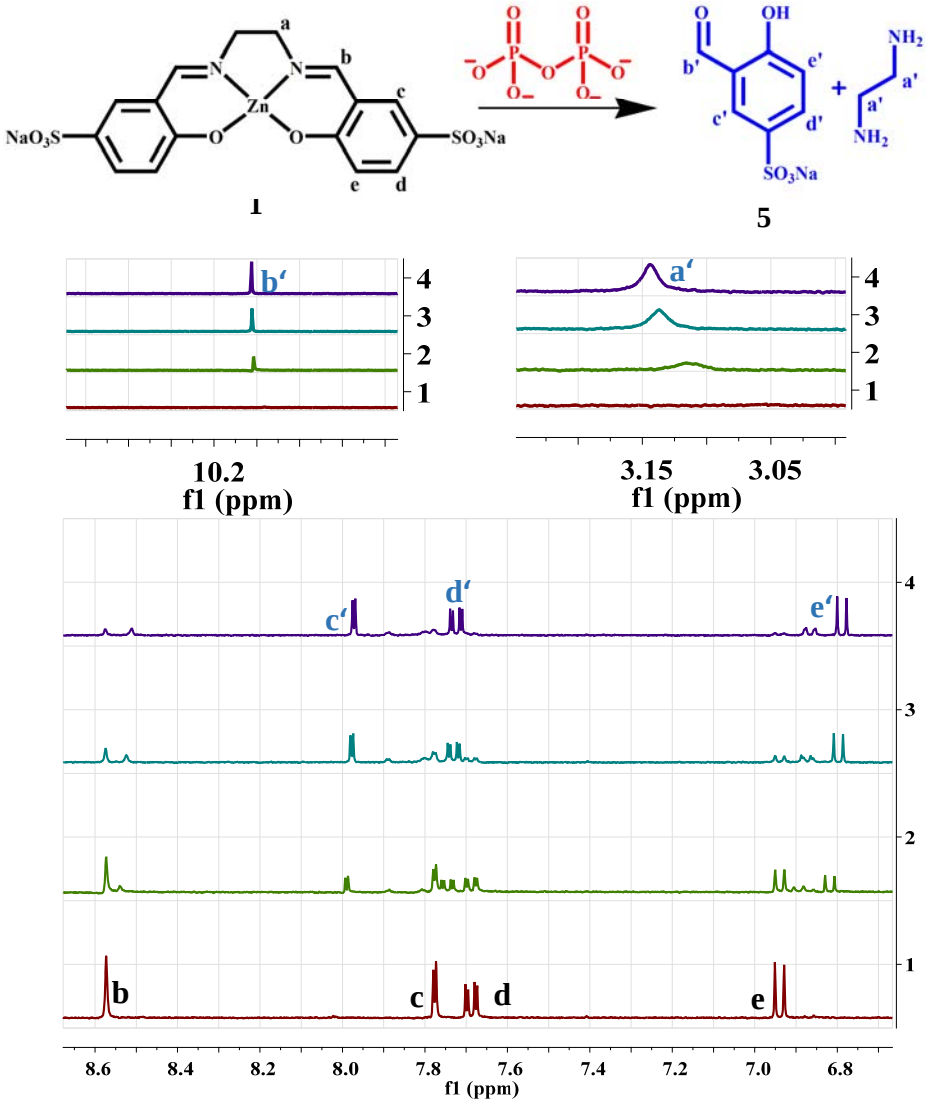


**Fig. S10** Reaction scheme for the disassembly of **1** with PPi to the salicylaldehyde derivative **5**. Lines 1-4: $^{1}$H NMR spectra of compound **1** (2 mM) in $D_2O$ ([TRIS] = 10 mM, pH 7.4; line 1) after the addition of PPi (1, 2 and 3 eq. in $D_2O$; lines 2-4). Characteristic peaks are indicated.

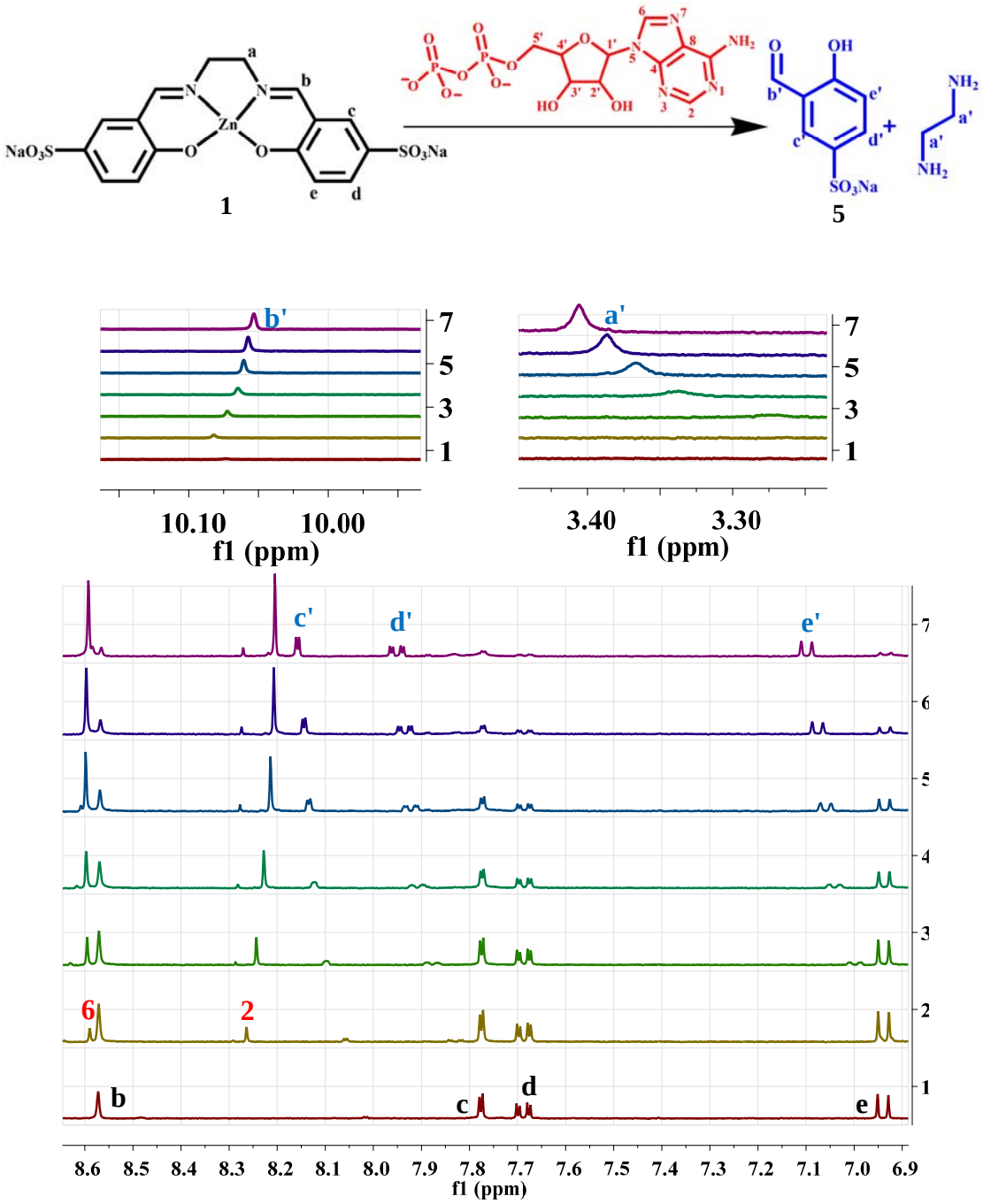


**Fig. S11** Reaction scheme for the disassembly of **1** with ADP to the salicylaldehyde derivative **5**. Lines 1-7: $^{1}$H NMR spectra of compound **1** (2 mM) in $D_2O$ ([TRIS] = 10 mM, pH 7.4; line 1) after the addition of ADP (1, 2, 3, 4, 5 and 6 eq. in $D_2O$; lines 2-7). Characteristic peaks are indicated.

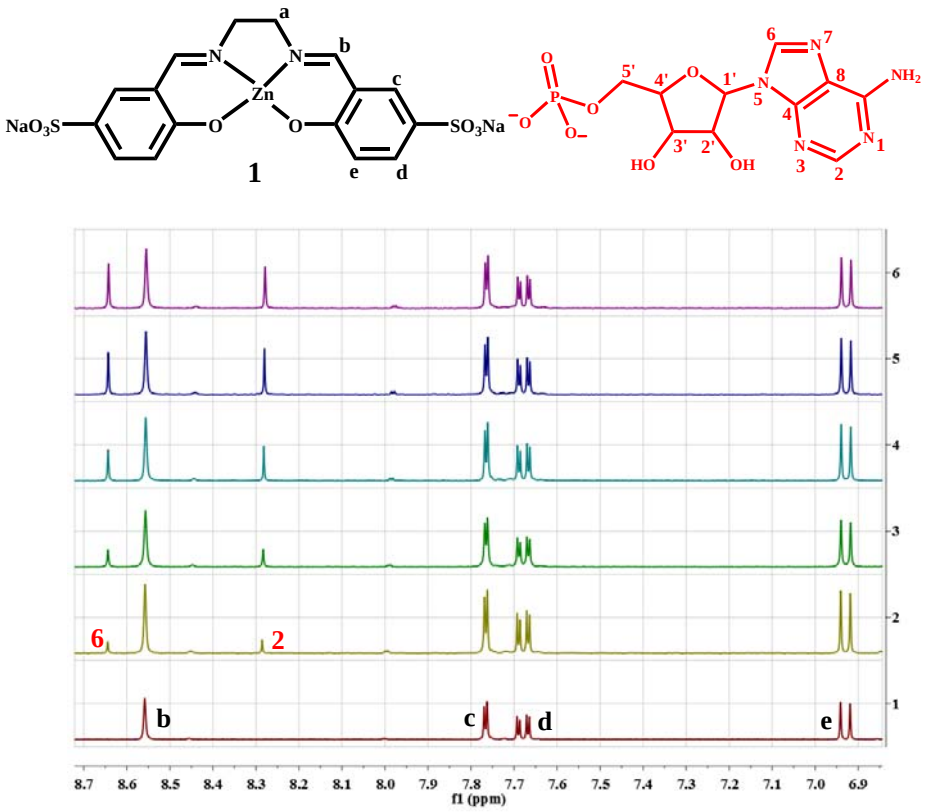


**Fig. S12** Molecular structures of **1** and AMP. Lines 1-6: $^{1}$H NMR spectra of compound **1** (8 mM in $D_2O$; line 1) after the addition of AMP (0.2, 0.4, 0.6, 0.8 and 1 eq. in $D_2O$; lines 2-6).

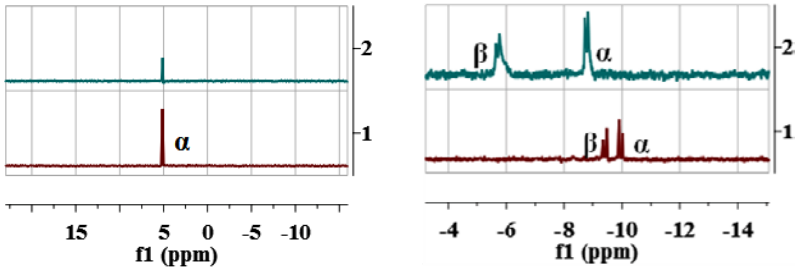


**Fig. S13** $^{31}$P NMR spectra of AMP (10 mM; left) and ADP (10 mM; right) in the presence (1 equiv.; line 2) and absence of compound **1** (line 1) in $D_2O$ ([TRIS] = 10 mM; pH 7.4).

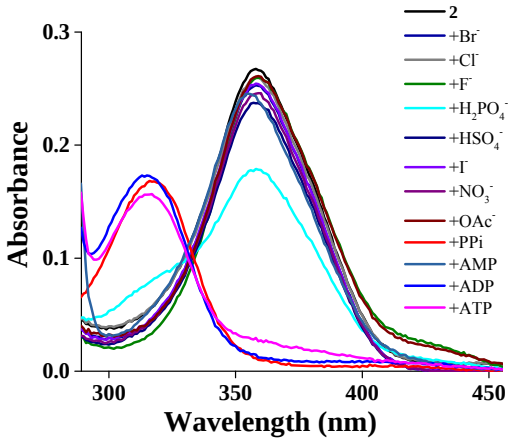


**Fig. S14** UV-vis absorption spectra of **2** (20 μM) in DMSO upon addition of various anions (10 eq., tetrabutylammonium salts except PPi which is a tributylammonium salt).

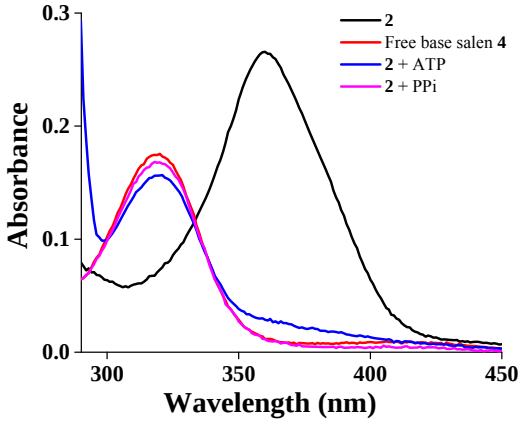


**Fig. S15** Comparison of the UV-vis spectra of **2** (20 μM) upon addition of ATP (10 eq.) and PPi (10 eq.) with free-base salen **4** (20 μM) in DMSO.

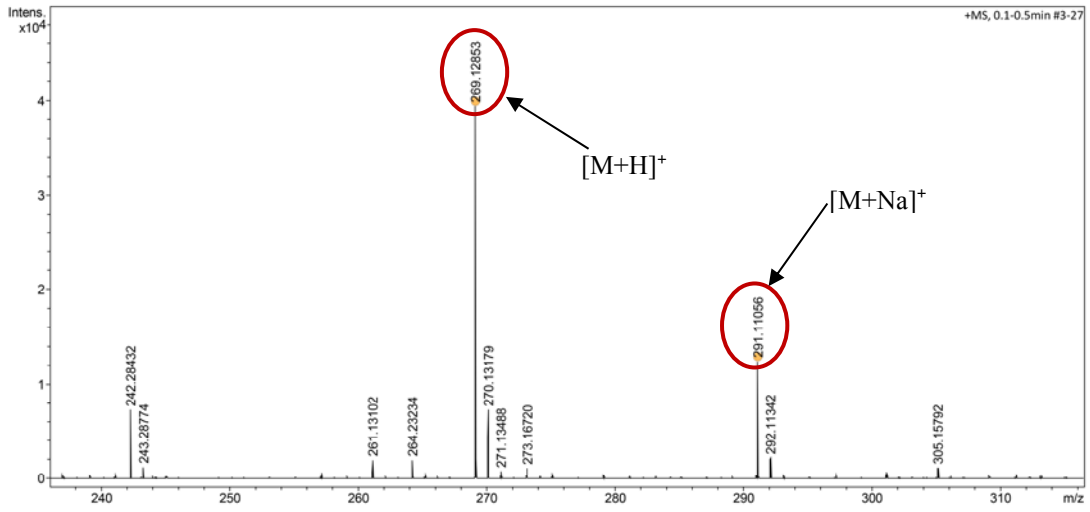


**Fig. S16** Mass spectra of **2** with added ATP in DMSO. Mass spectral peak 269.1285 corresponds to $[M+H]^+$ ($m/z_{calcd.}$ = 269.1285) and 291.1106 corresponds to $[M+Na]^+$ ($m/z_{calcd.}$ = 291.1104); [M] corresponds to free-base salen **4**.

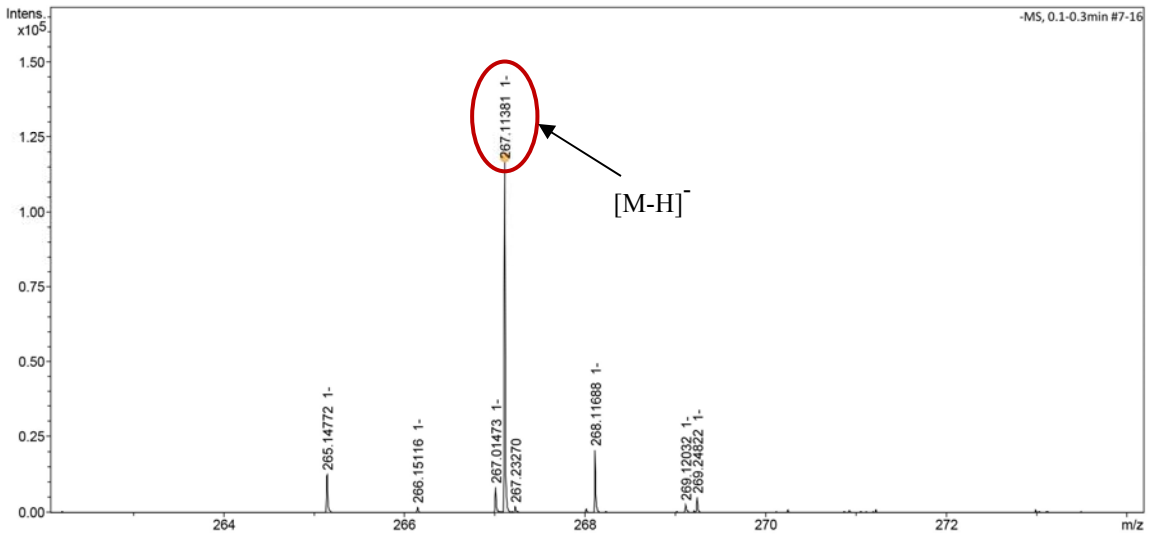


**Fig. S17** Mass spectra of **2** with added PPi (tributylammonium salt) in DMSO. Mass spectral peak 267.1138 corresponds to $[M-H]^-$ ($m/z_{calcd.}$ = 267.1139); [M] corresponds to free-base salen **4**.

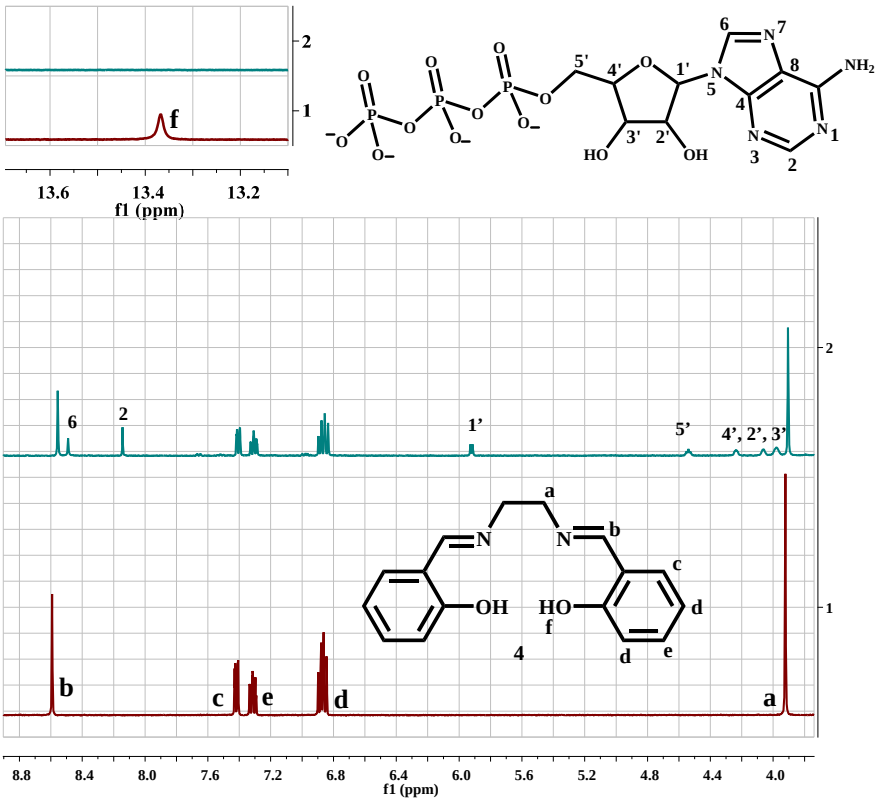


**Fig. S18** Comparison of $^{1}$H NMR spectra of free-base salen **4** (8 mM, in DMSO-$d_6$, line 1) upon addition of 1 eq. of ATP (in $D_2O$, line 2).

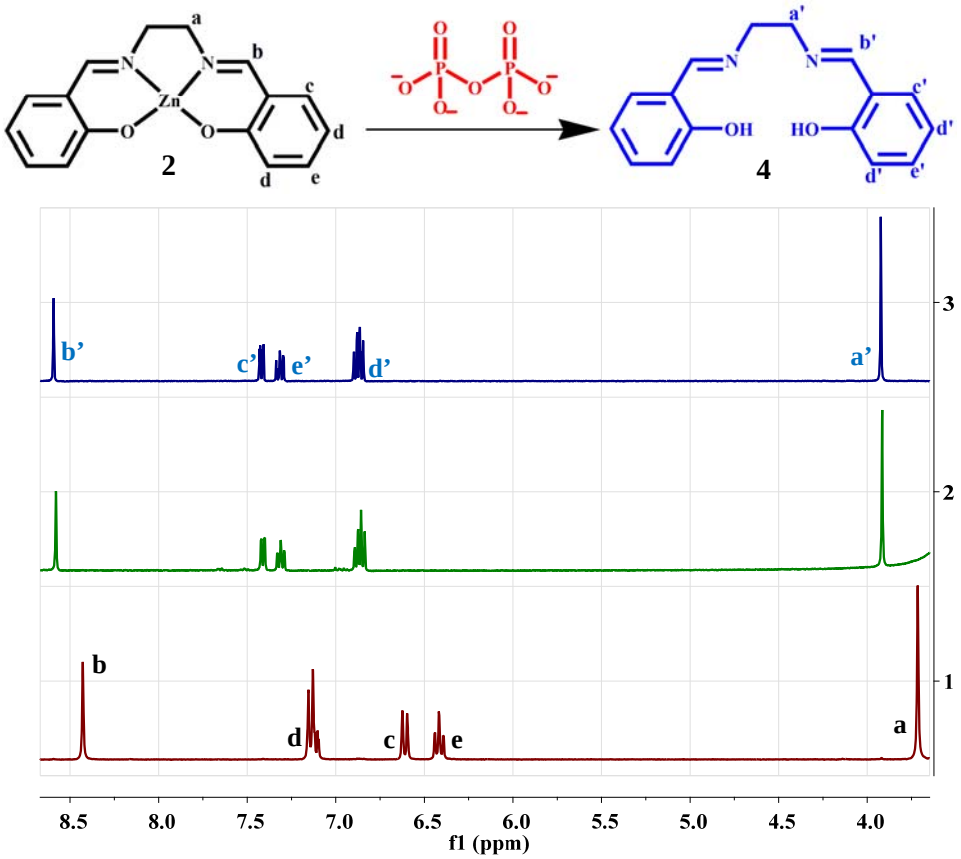


**Fig. S19** Displacement reaction scheme of **2** with PPi to the salen free-base salen **4**. Lines 1-3: $^{1}$H NMR spectra of compound **2** (10 mM in DMSO-$d_6$; line 1) after the addition of pyrophosphate (tributylammonium salt; 1 eq., line 2) and comparison with free base salen **4** (line 3). Characteristic peaks are indicated.

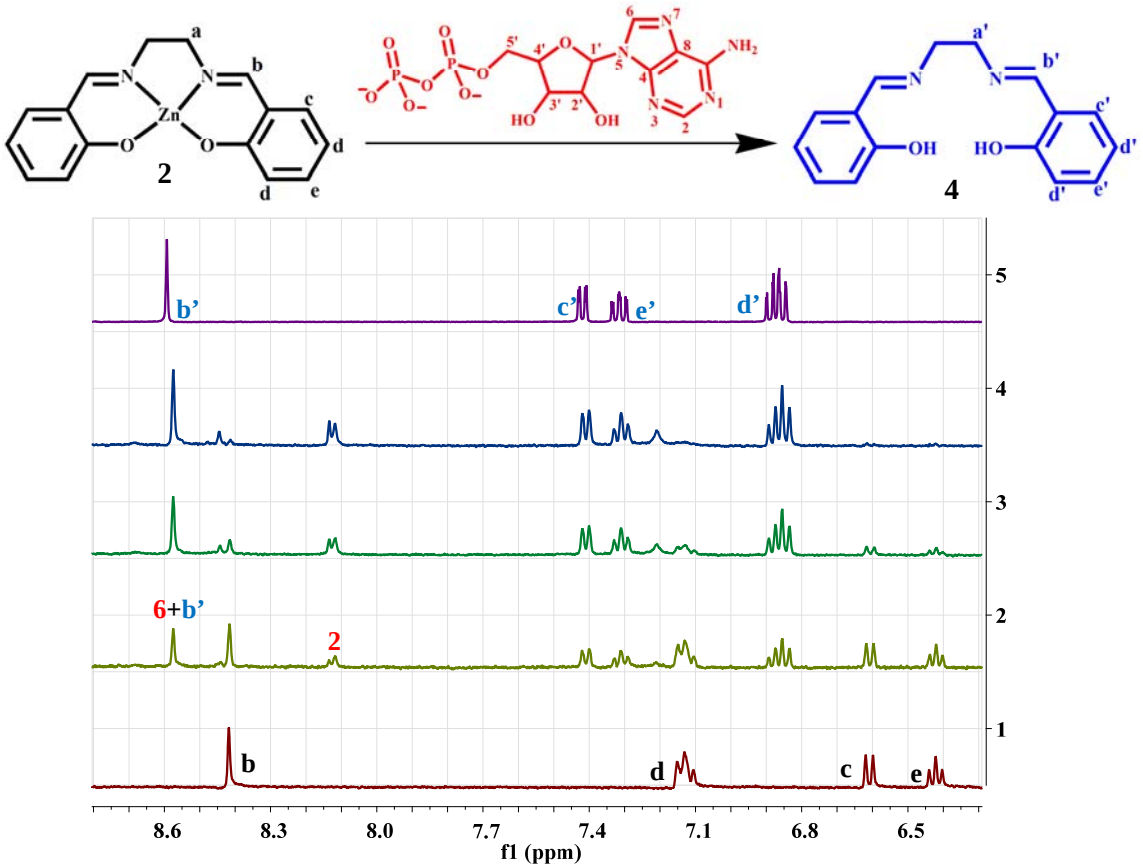


**Fig. S20** Displacement reaction scheme of **2** with ADP to free-base salen **4**. Lines 1-5: $^{1}$H NMR spectra of compound **2** (2 mM in DMSO-$d_6$; line 1) after the addition of tetrabutylammonium adenosine diphosphate (1, 2 and 2.5 eq., lines 2-4) comparison with free-base salen **4** (line 5).

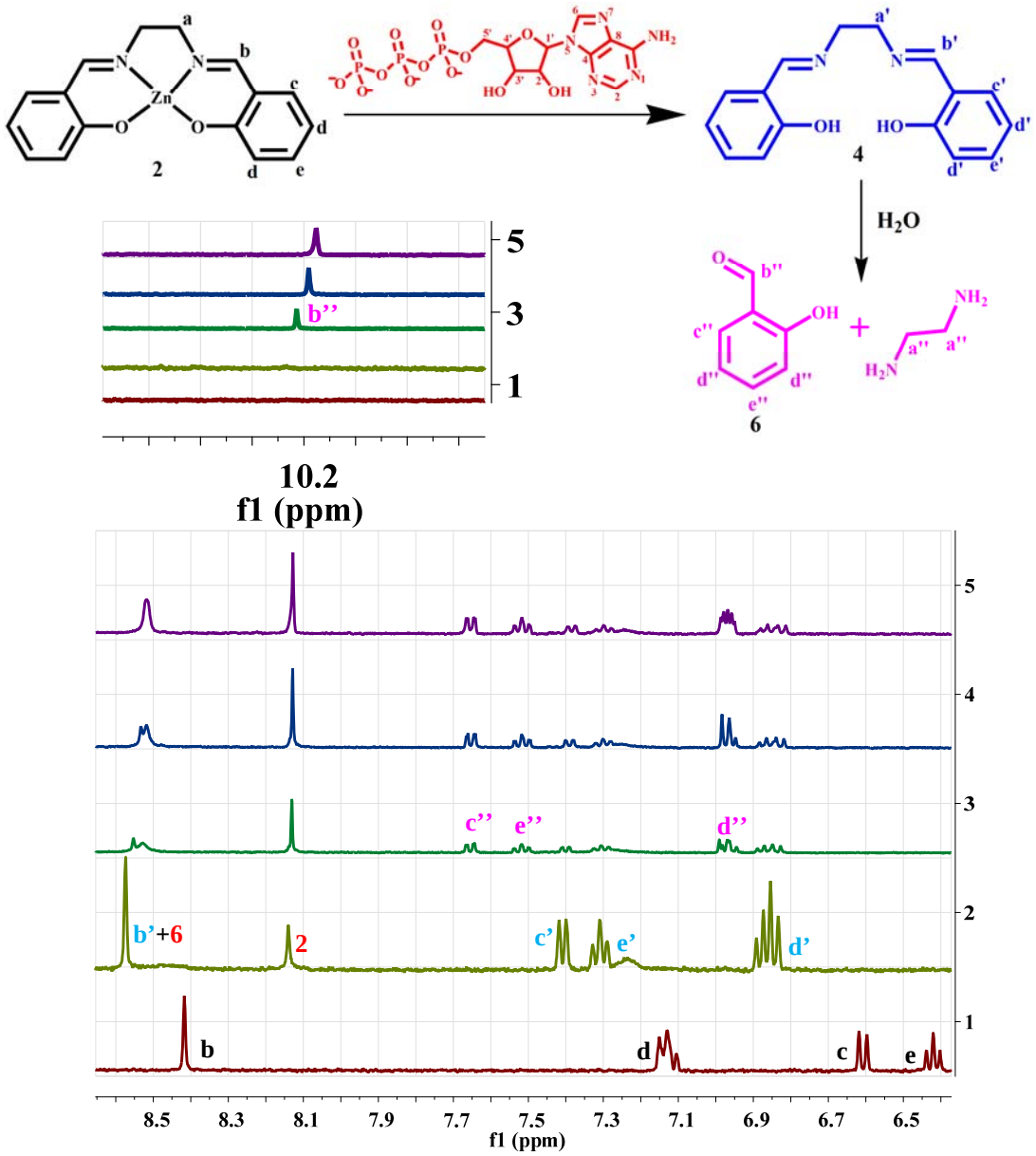


**Fig. S21** Reaction scheme for the demetallation of **2** with ATP to the free-base salen ligand **4** followed by disassembly to form salicylaldehyde **6** after addition of $D_2O$. Lines 1-5: $^1H$ NMR spectra of compound **2** (2 mM in DMSO-$d_6$; line 1) after the addition of tetrabutylammonium adenosine triphosphate (1 eq., line 2) and upon addition of $D_2O$ (4%, 8% and 12 %; lines 3-5).

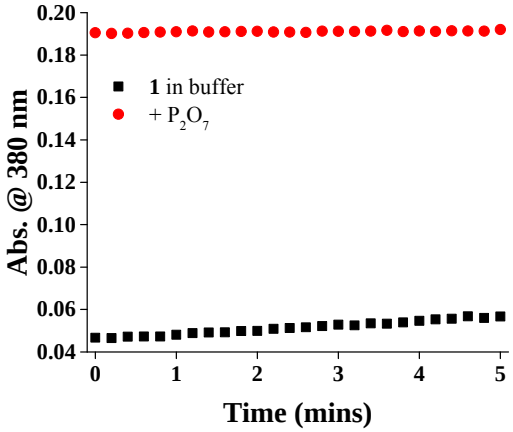


**Fig. S22** Comparison of change in absorbance @ 380 nm of **1** (20 μM) in water ([TRIS] = 10 mM, pH 7.4) upon addition of PPi (10 eq.) with time.