Selective Detection of ATP and ADP in Aqueous Solution by using a Fluorescent Zinc Receptor

Maria Strianese, Stefano Milione, Andrea Maranzana†, Alfonso Grassi, Claudio Pellecchia*

Dipartimento di Chimica e Biologia, Università di Salerno, via Ponte don Melillo. I-84084 Fisciano (SA), Italy

†Dipartimento di Chimica, Università di Torino, via Pietro Giuria 7. I-10125 Torino (TO), Italy.

Corresponding Author
* E-mail: smilione@unisa.it

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Experimental Section

General: All chemicals used for the synthetic work were obtained from Sigma-Aldrich or Strem Chemicals and were of reagent grade. They were used without further purification.

The ligand *pyr₂en*H₂ (*pyr₂en*H₂ = N,N'-ethylenebis(pyridoxylideneiminato) was prepared as previously reported.[1]

Elemental analyses were performed with a PERKIN-Elmer 240-C. Mass spectrometry analyses were carried out using a Micromass Quattro micro API triple quadrupole mass spectrometer equipped with an electrospray ion source (Waters, Milford, MA).

Room temperature NMR spectra were recorded on a Bruker AVANCE 400 NMR instrument (^{1}H, 400.13 MHz; ^{13}C, 100.62 MHz; ^{31}P 161.97 MHz). All spectra were recorded in 5 mm o.d. NMR tubes.

Chemical shifts (δ) are listed in parts per million and coupling constants *J* in hertz. ^{1}H NMR spectra are referenced to the residual solvent peak at δ = 4.79 for D_2O and δ = 3.34 for $(CD_3)_2SO$ (DMSO). ^{13}C NMR spectra are referenced externally to $SiMe_4$. ^{31}P NMR spectra are referenced externally to 85% H_3PO_4 at δ=0.00. Typically, 5 mg of the complex in 0.5 mL of D_2O or DMSO were used for each experiment.

Synthesis of pyr₂enZn: Pyr₂en (0.3 g, 0.84 mmol) was dissolved in methanol (10 mL) and NaOEt was added as solid (0.113 g, 1.7 mmol). The bright yellow reaction mixture was left under stirring for 1h. $Zn(CH_3COO)_2$ (0.184 g, 0.84 mmol) dissolved in methanol (5 mL) was slowly added and the mixture became pale yellow. The reaction mixture was left under stirring overnight. A yellow solid was collected by filtration and washed with methanol and diethyl ether, and dried under vacuum. Yield: 0.28 g, 80%. $C_{18}H_{20}N_4O_4Zn$: calcd. C 51.26, H 4.78, N 13.28; found C 51.3, H 4.6, N 13.3. MS (ESI methanol): *m/z* (%) 421.17 (100) [(pyr₂en)ZnH] ^{1}H NMR [400 MHz, DMSO-*d*₆]: *δ* =8.86 (s, 2H, C*H*$_{arom}$), 7.39 (s, 2 H, C*H*=N), 5.20 (t, 2H, *J* = 4.7 Hz, CH_2OH) 4.55 (d, 4H, *J* = 4.7 Hz, CH_2OH), 3.80 (s, 4H, CH_2CH_2), 2.36 (s, 6H, CH_3) ppm; ^{1}H NMR [400 MHz, D_2O] (pH~4.5): *δ* =8.19 (s, 2H, C*H*$_{arom}$), 6.77 (s, 2H, C*H*=N), 5.36 (d, 2H, *J* = 13.8 Hz, CH_2OH), 5.22 (d, 2H, *J* = 13.8 Hz, CH_2OH), 3.38 (s, 4H, CH_2CH_2), 2.68 (s, 6H, CH_3) ppm. ^{13}C NMR [100 MHz, D_2O] (pH~4.5): *δ* =15.2 (CH_3), 37.1 (CH_2CH_2), 70.4 (CH_2OH), 99.8 (*C*H=N), 119.6 (*C*H$_{arom}$), 137.8 (*C*$_{arom}$), 139.3 (*C*$_{arom}$), 145.7 (*C*$_{arom}$), 157.5 (*C*$_{arom}$).

Sample preparation for absorbance and fluorescence and NMR analysis. Aqueous saturated solutions of *pyr*$_2$*en*Zn were freshly prepared before each measurement and stored at 4 °C when not in use. Their concentrations were determined by flame atomic absorption spectrometry, on the basis of the metal content. Typically, $0.5 \div 3.0 \times 10^{-5}$ M solutions of complexes in ultra pure metal-free water were directly aspired into an air-acetylene flame without prior treatment. Concentrations were obtained by comparison with calibration curves. *pyr*$_2$*en*Zn aqueous solutions, analyzed for metal contents, were appropriately diluted and used for determining the extinction coefficient at 300 nm. *pyr*$_2$*en*Zn: $\varepsilon_{300} = 106.927\ mM^{-1}cm^{-1}$.

Absorbance and fluorescence measurements. Absorption spectra were recorded on a Cary-50 Spectrophotometer, using a 1 cm quartz cuvette (Hellma Benelux bv, Rijswijk, Netherlands) and a slit-width equivalent to a bandwidth of 5 nm. Fluorescence spectra were measured on a Cary Eclipse Spectrophotometer in a $10\times10 mm^2$ airtight quartz fluorescence cuvette (Hellma Benelux bv, Rijswijk, Netherlands) with an emission band-pass of 10 nm and an excitation band-pass of 5 nm. Both absorption and fluorescence measurements were performed in MilliQ water at room temperature.

Titration experiments. These experiments were performed as follows: the cuvette was filled with sample solutions in 20 mM MOPS. Then μL amounts of Na_2ADP (or Na_2ATP) solutions in 20 mM MOPS (to the end concentrations specified in the figure captions) were injected via a gas-tight syringe.

The relation between the observed fluorescence intensity, $F_{ADP/ATP}$, and K_d is given by

$$F_{ADP} = F_0 - \{(F_0 - F_\infty) \cdot [ADP]\}/\{[ADP] + K_d\}^2$$

where ADP(ATP) is the concentration of free ADP(ATP) in solution and F_0 and F_∞ denote the emission intensities of the ADP(ATP)-free and ADP(ATP)-bound *pyr*$_2$*en*Zn, respectively.

Computational Methods.

The potential energy surface (PES) was studied by Density Functional Theory (DFT), making use of the M06 functional,[3] which was recommended for organometallic and inorganometallic thermochemistry and noncovalent interactions.[4]

Solvent (water) effect was taken into account via the Self Consistent Reaction Field (SCRF) method, by using the IEF-PCM model.[5] Four explicit H_2O molecules were included in the

calculations of the ATP^{4-}, ADP^{4-} and $P_2O_7^{4-}$, and two H_2O molecules in the zinc complex of the Shiff base ligand (*pyr*$_2$*en*Zn). The number of water molecules was limited to 4, to avoid extremely demanding computations costs.

The 6-311G(2d) polarized basis set[6-8] was used in the geometry IEF-PCM optimizations. The nature of each critical point was determined by harmonic vibrational analysis. In order to assess a better estimate of the energy differences, single-point energy calculations were carried out by using the cc-pVTZ Dunning's correlation consistent basis sets.[9] The thermochemistry was calculated by combining 6-311G(2d) thermochemical corrections with single-point cc-pVTZ energies. The natural atomic orbital (NAO) charges were obtained by the natural population analysis.[10] All calculations were carried out by using Gaussian 09 program.[11] The MOLDEN program was exploited to display some optimized structures.[12]

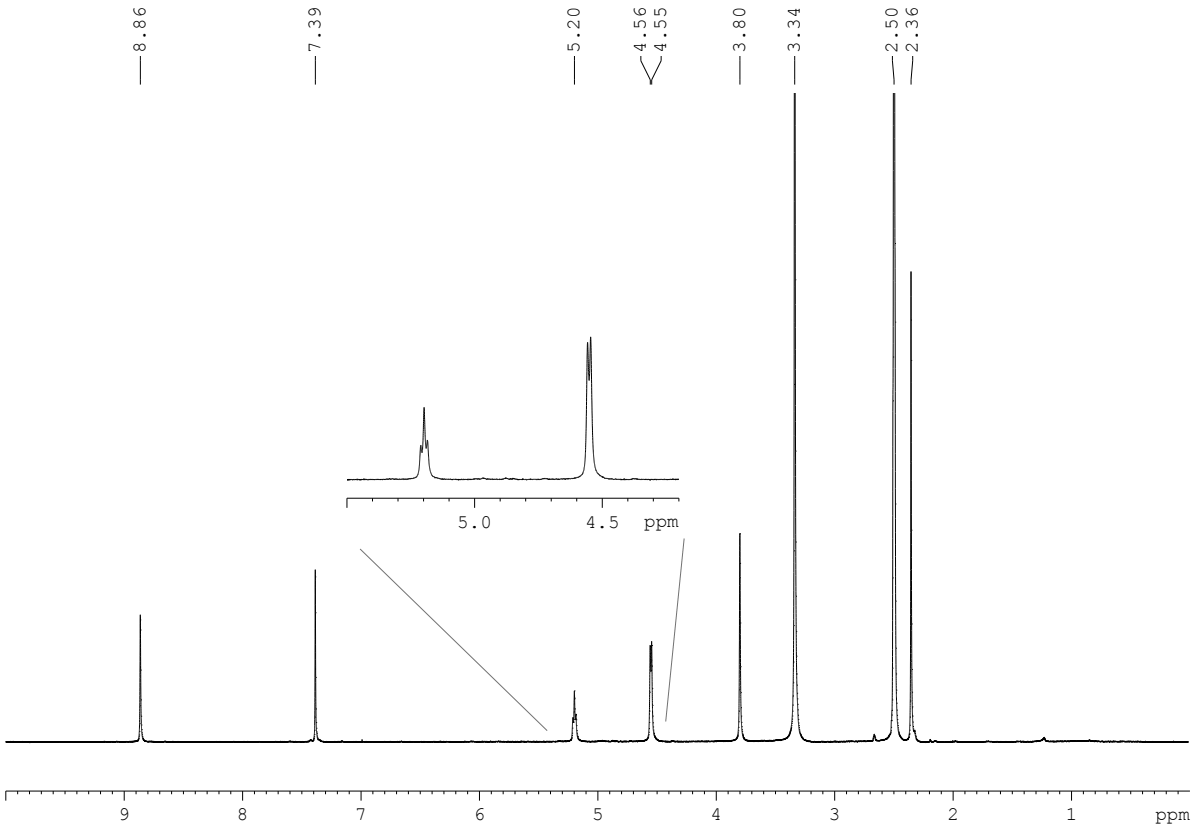

Figure S1. ^{1}H NMR spectrum of *pyr*$_2$*en*Zn in DMSO-d_6 (rt, 400.13 MHz)

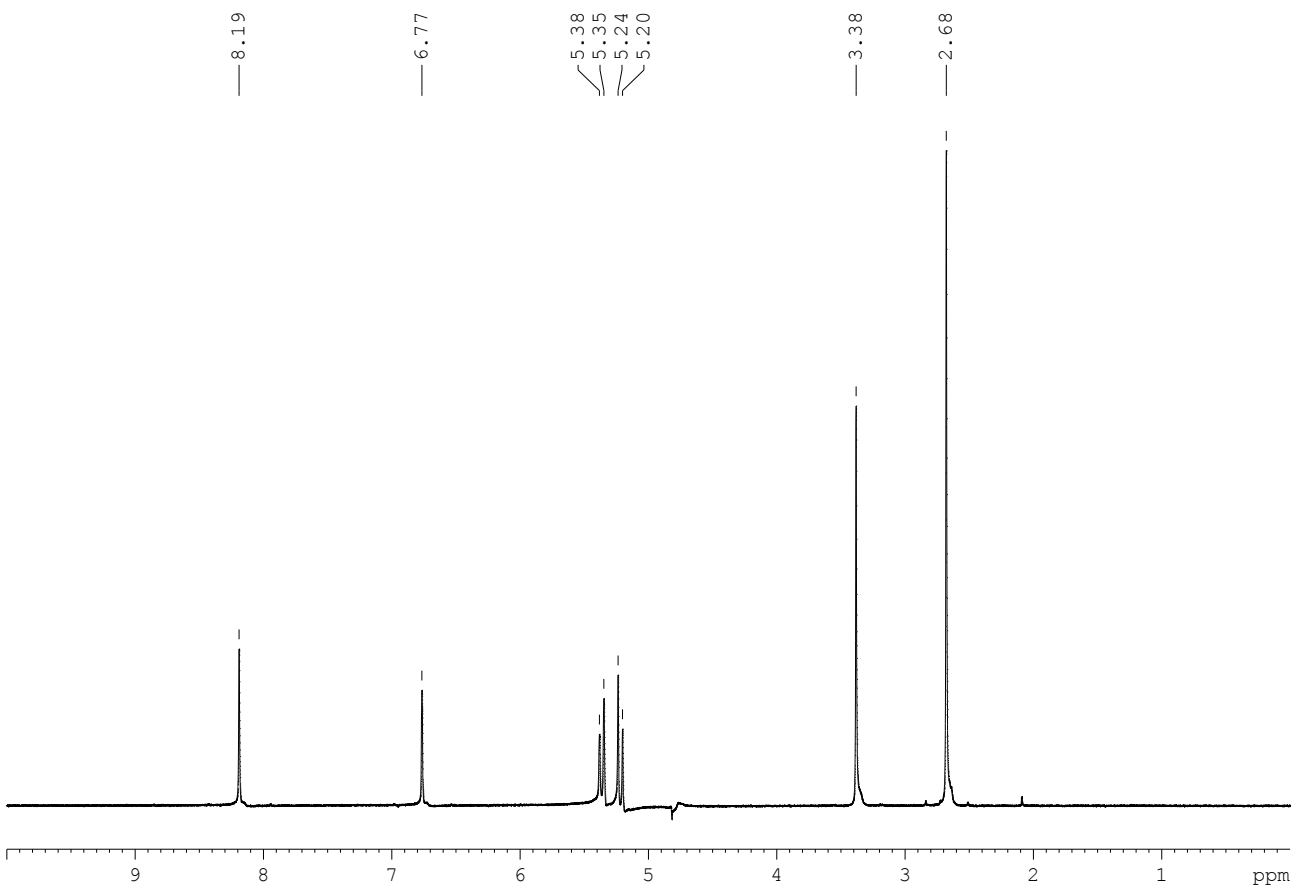

Figure S2. ^{1}H NMR spectrum of *pyr*$_2$*en*Zn in D_2O/HCl. A presaturation pulse sequence was used to suppress the water signal (pH~4.5, rt, 400.13 MHz)

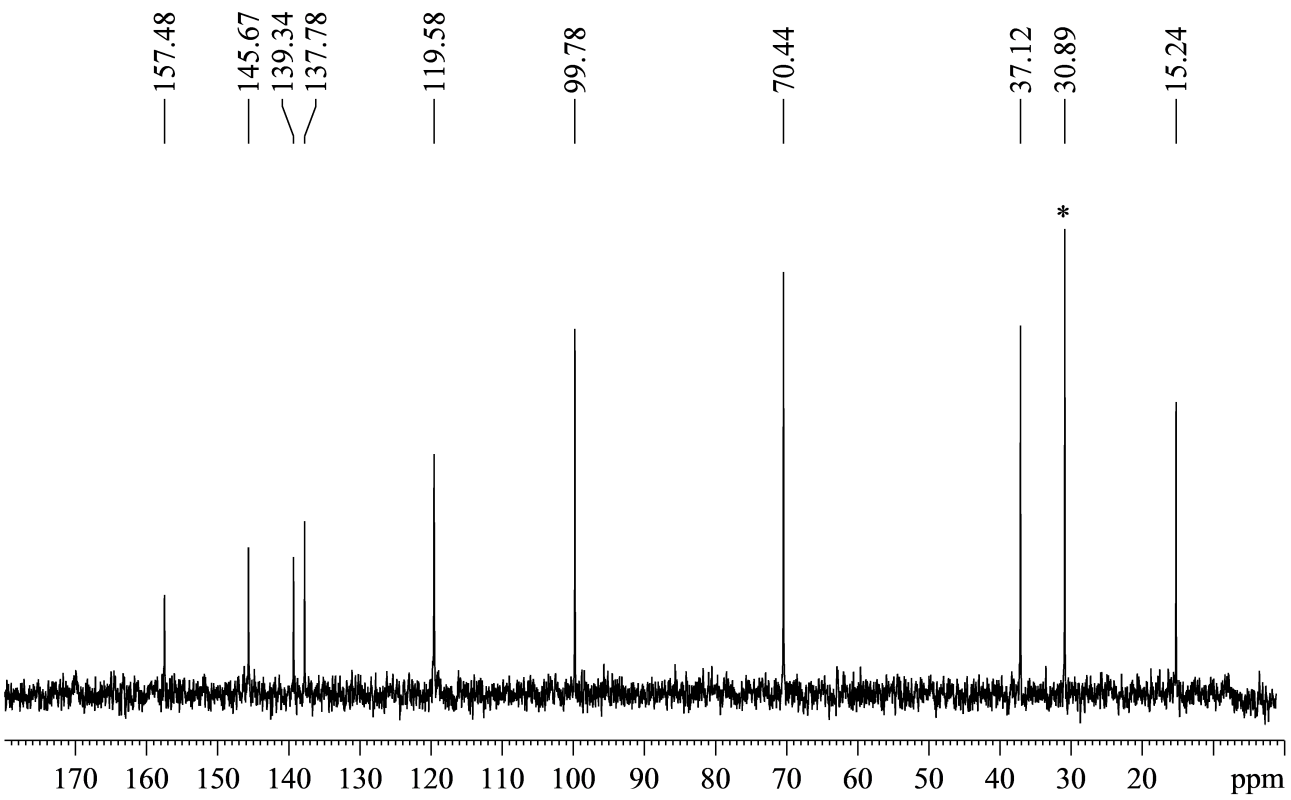

Figure S3. ^{13}C NMR spectrum of *pyr₂en*Zn in D_2O/HCl (pH~4.5, rt, 100.62 MHz). The signal marked with an asterisk is due to methanol.

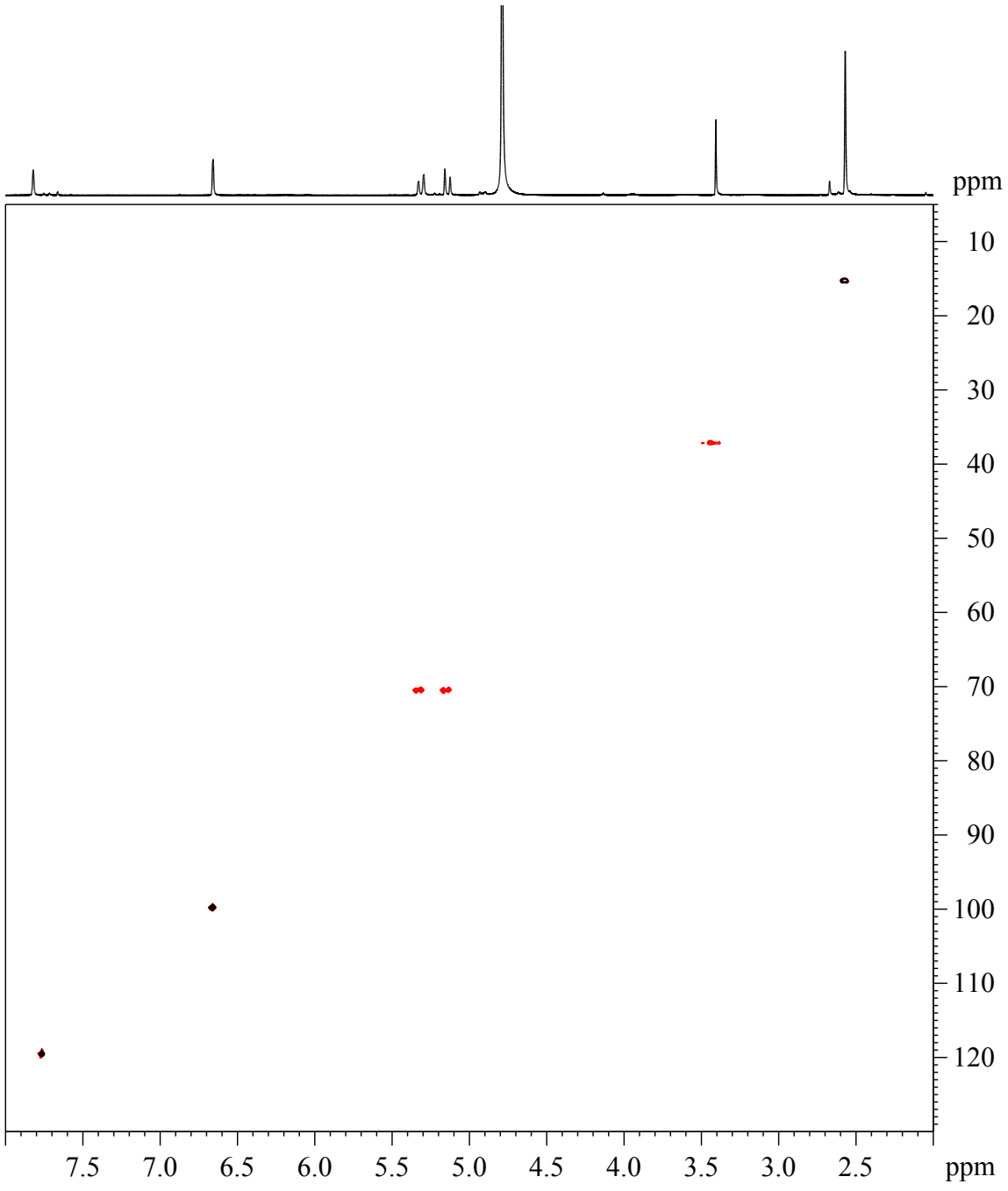

Figure S4. 2D ^{1}H-^{13}C HSQC of *pyr*$_2$*en*Zn in D_2O/HCl (pH~4.5, rt, 400MHz)

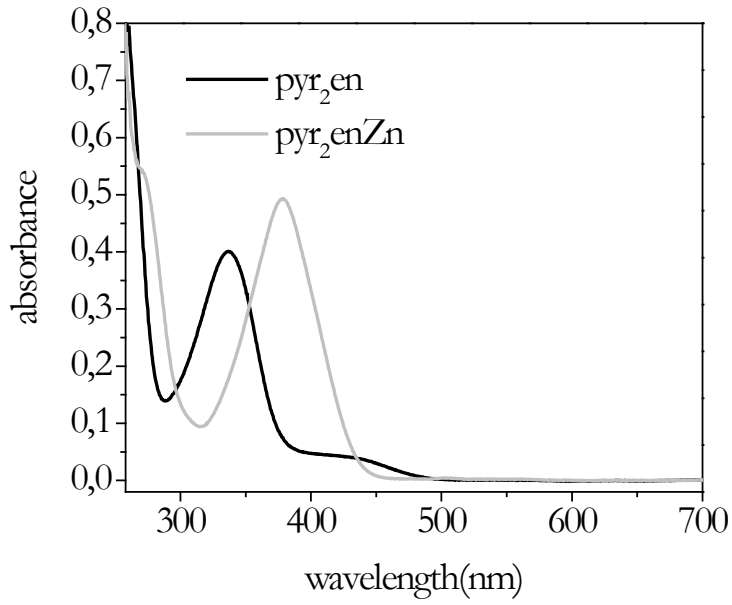

Figure S5. Electronic absorption spectra of pyr_2en and *pyr₂en*Zn (rt, DMSO). [pyr_2en] = 20µM [*pyr₂en*Zn] = 20µM**.**

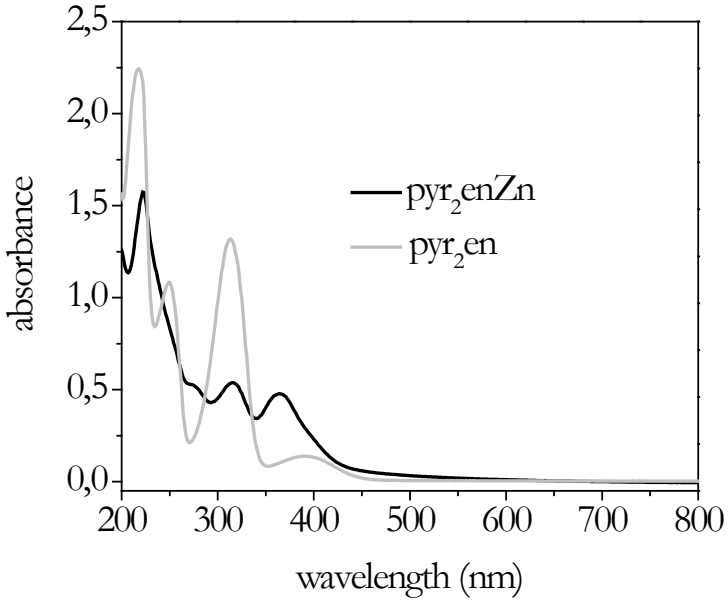

Figure S6. Electronic absorption spectra of pyr$_2$en and *pyr$_2$en*Zn (rt, MilliQ water). [pyr$_2$en] = 5µM [*pyr$_2$en*Zn] = 5µM**.**

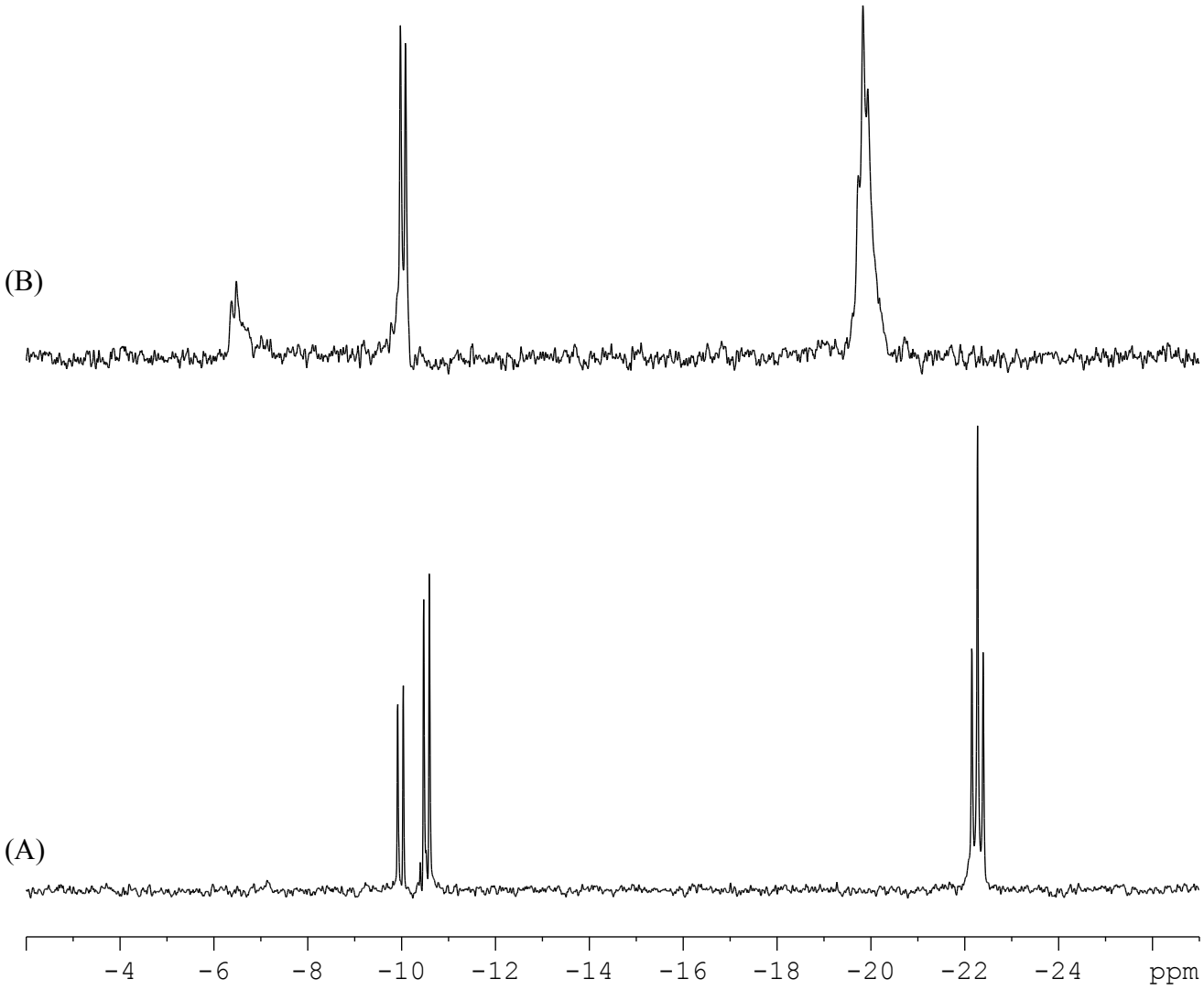

Figure S7. ^{31}P NMR spectra of Na_2CTP (A) and after addition of *pyr*$_2$*en*Zn (B) (rt, D_2O).

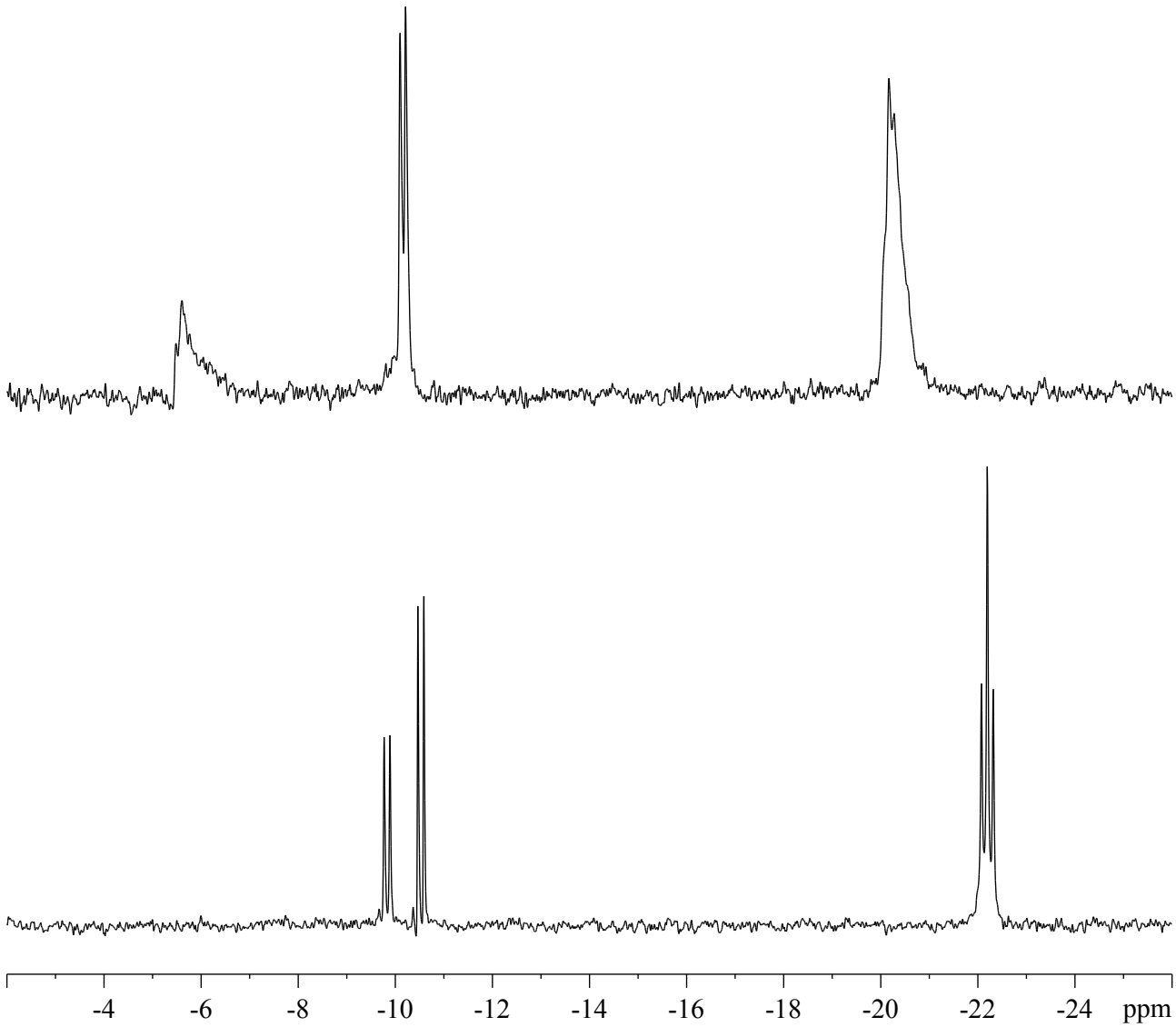

Figure S8. ^{31}P NMR spectra of Na_2UTP (A) and after addition of *pyr*$_2$*en*Zn (B) (rt, D_2O)

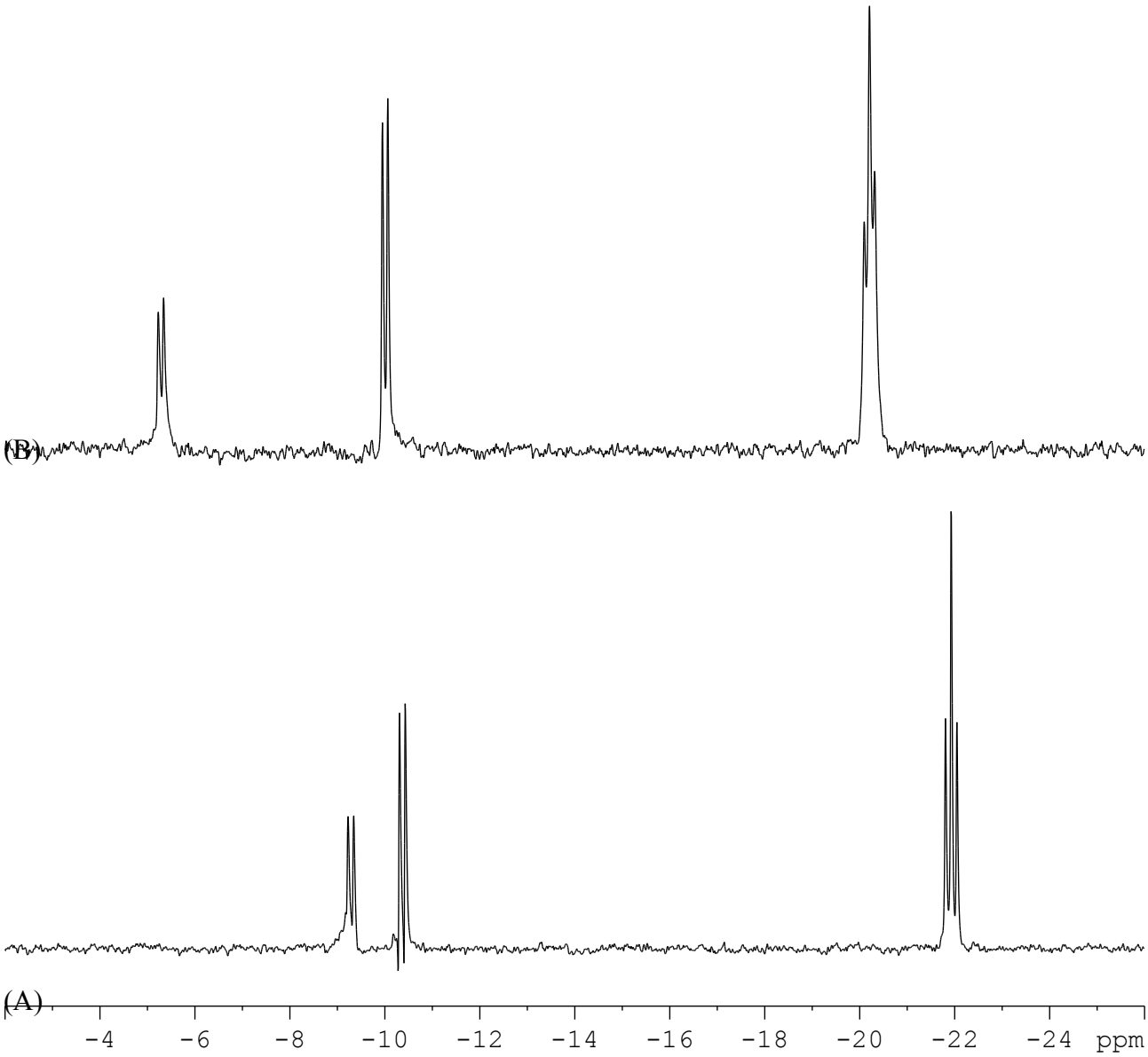

Figure S9. ^{31}P NMR spectra of Na_2GTP (A) and after addition of *pyr*$_2$*en*Zn (B) (rt, D_2O).

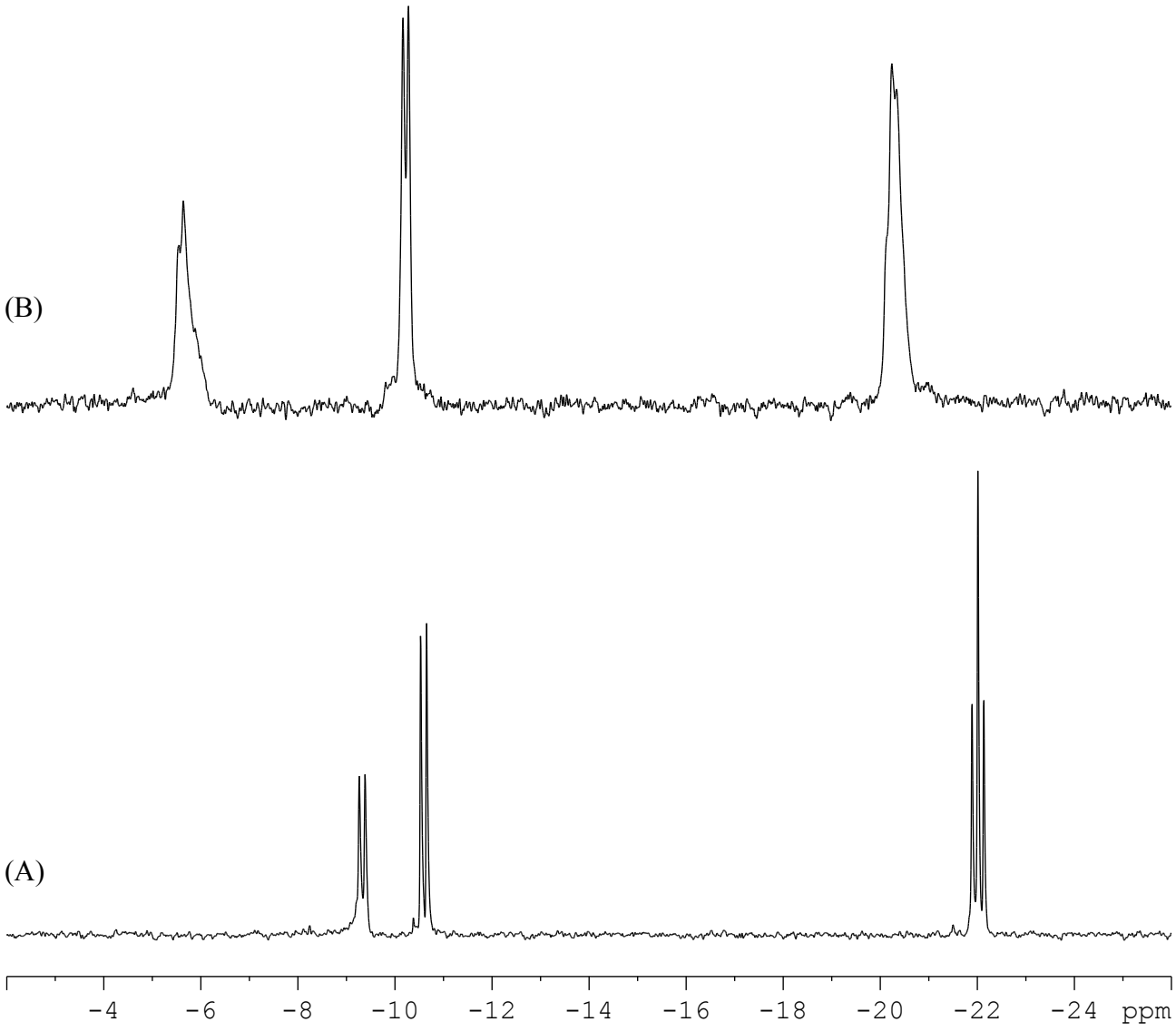

Figure S10. ^{31}P NMR spectra of Na_2TTP (A) and after addition of *pyr*$_2$*en*Zn (B) (rt, D_2O).

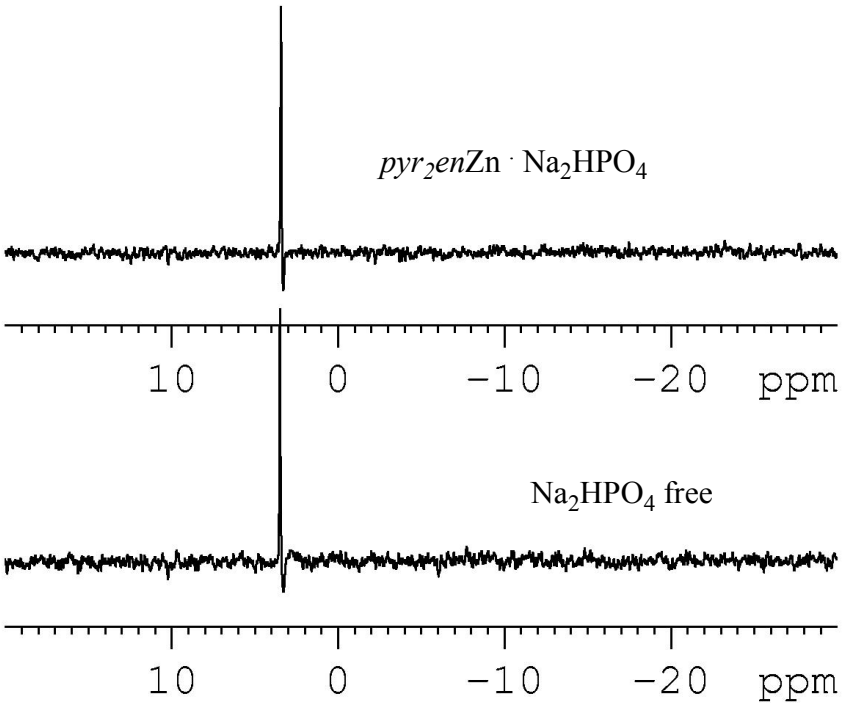

Figure S11. ^{31}P NMR spectra of Na_2HPO_4 free and after addition of *pyr*$_2$*en*Zn (rt, D_2O).

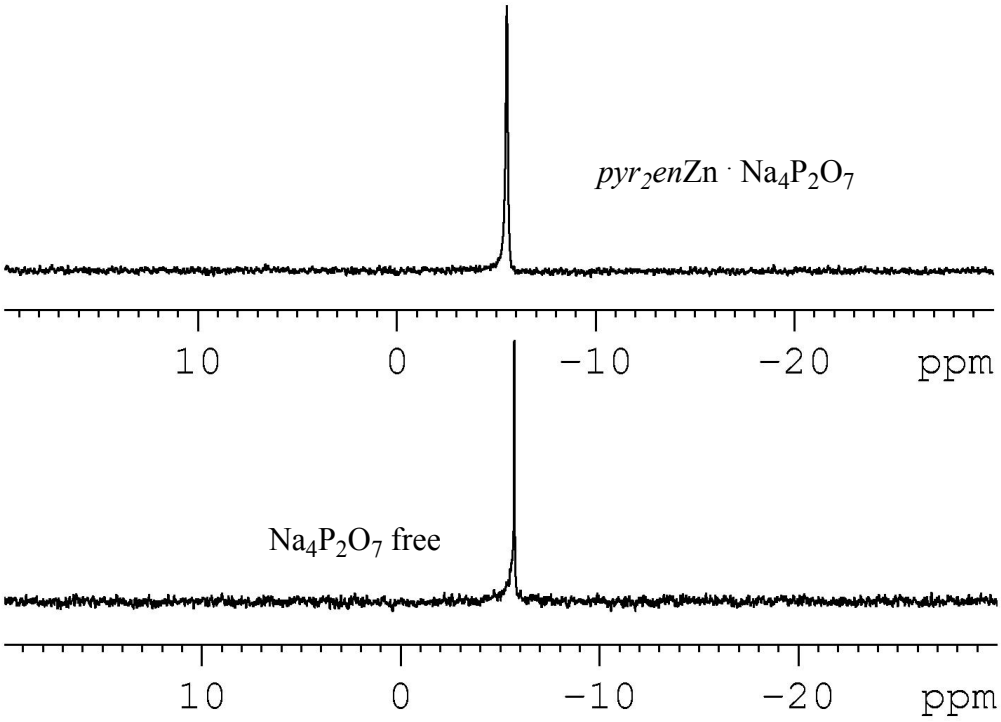

Figure S12. ^{31}P NMR spectra of $Na_4P_2O_7$ free and after addition of *pyr*$_2$*en*Zn (rt, D_2O).

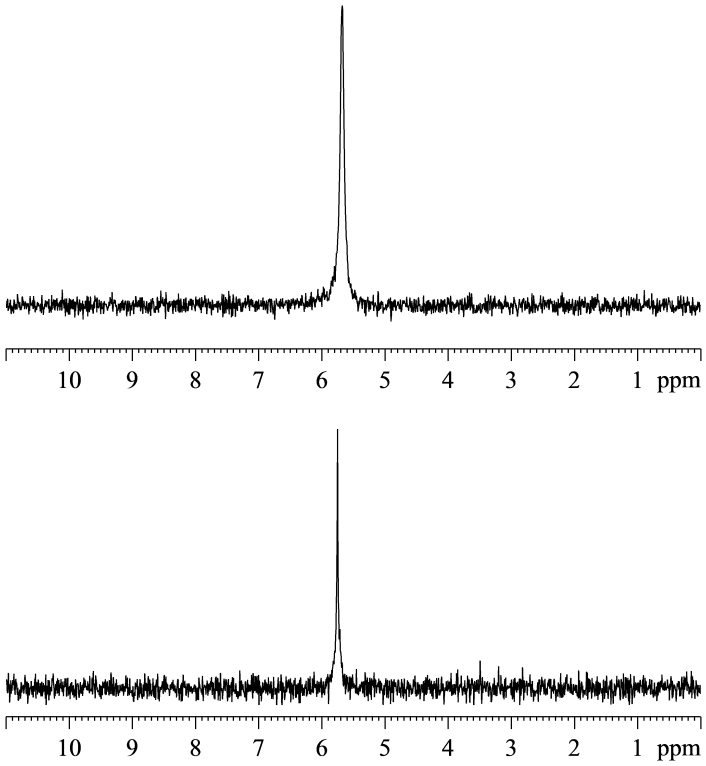

Figure S13. ^{31}P NMR spectra of Na_3PO_4 free and after addition of *pyr*$_2$*en*Zn (rt, D_2O).

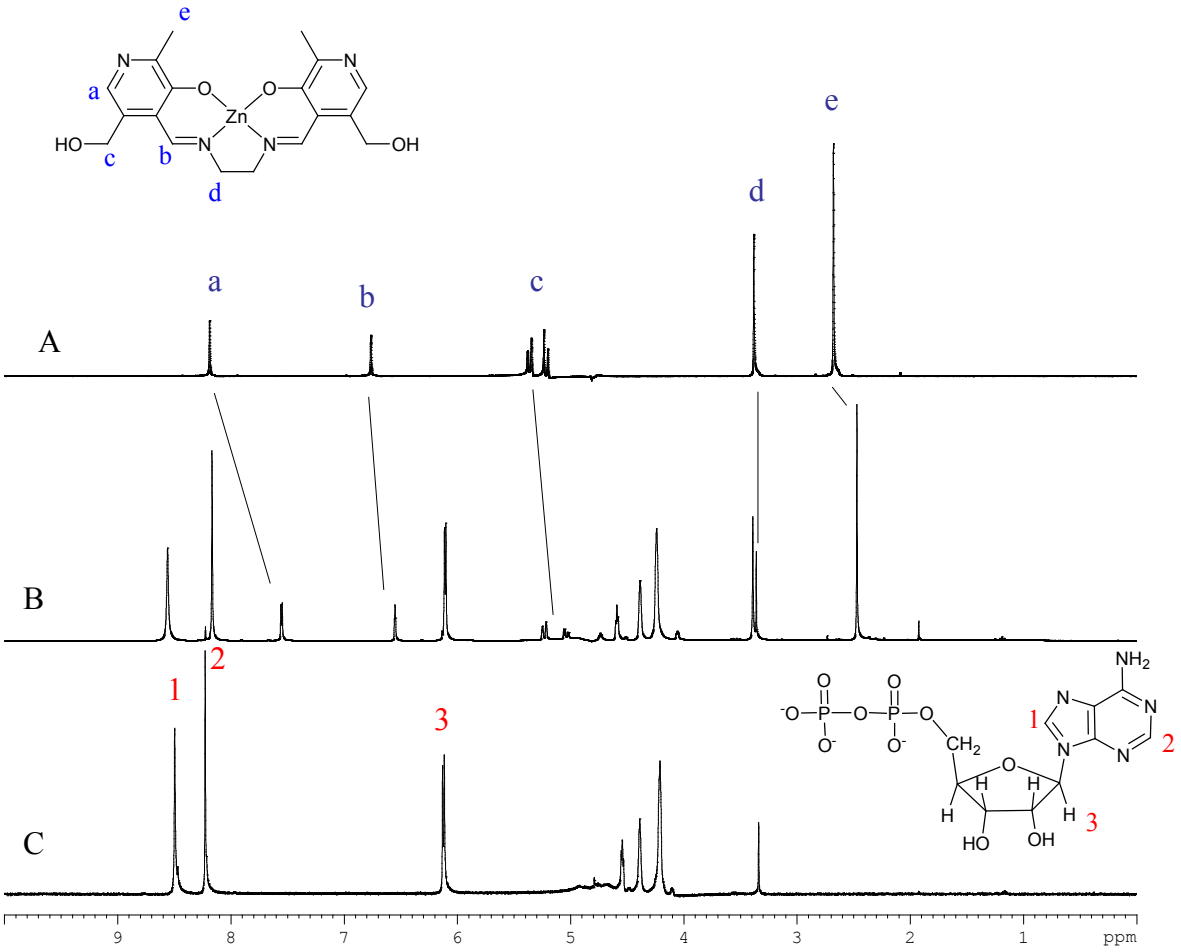

Figure S14. ^{1}H NMR spectrum of *pyr*$_2$*en*Zn (A), *pyr*$_2$*en*Zn /ADP (B) and ADPNa$_2$ (C) (D$_2$O, rt, 400MHz). A presaturation pulse sequence was used to suppress the water signal.

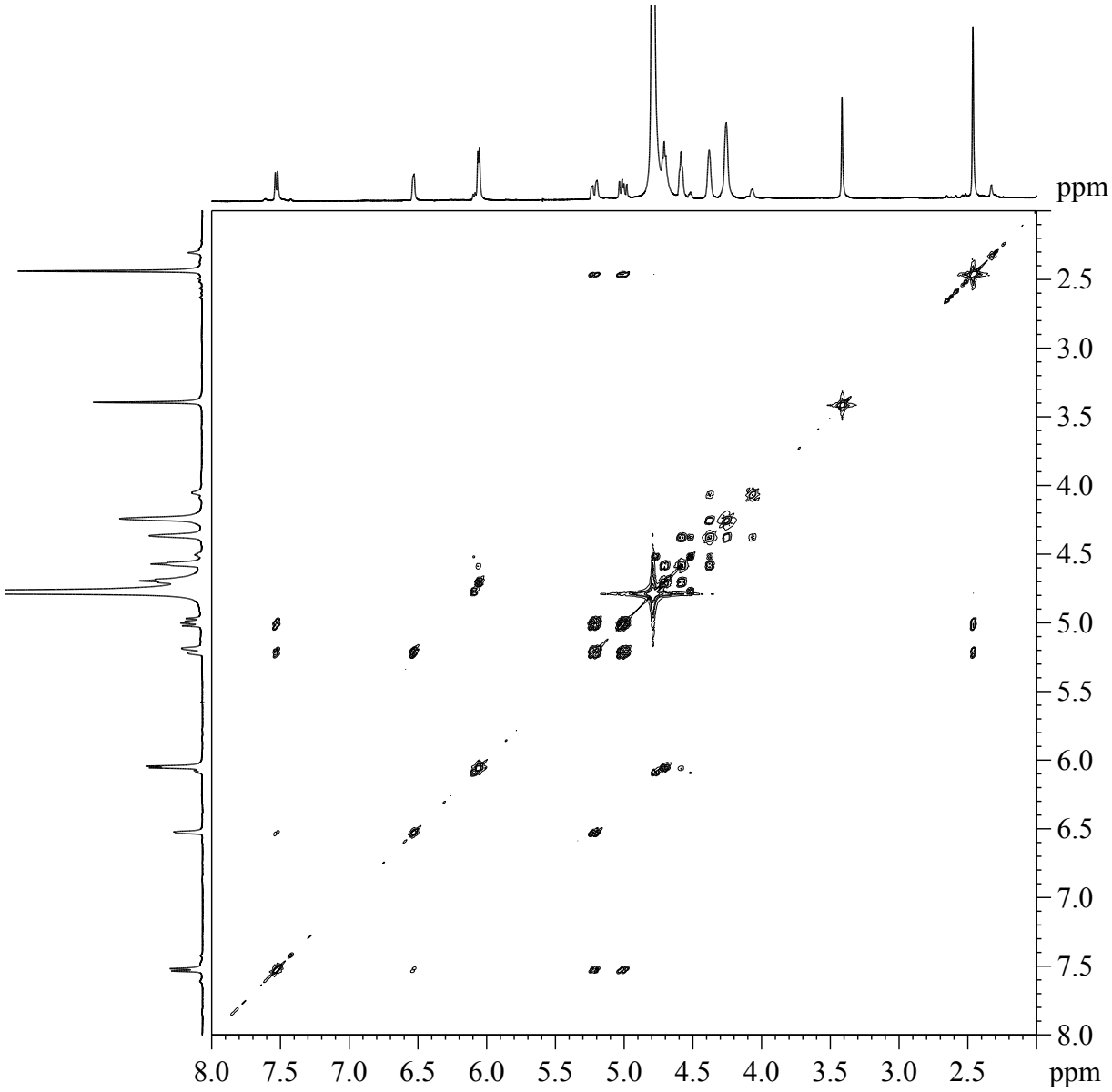

Figure S15. 2D ^{1}H-^{1}H COSY of *pyr*$_2$*en*Zn /ADP in D_2O (rt, 400MHz).

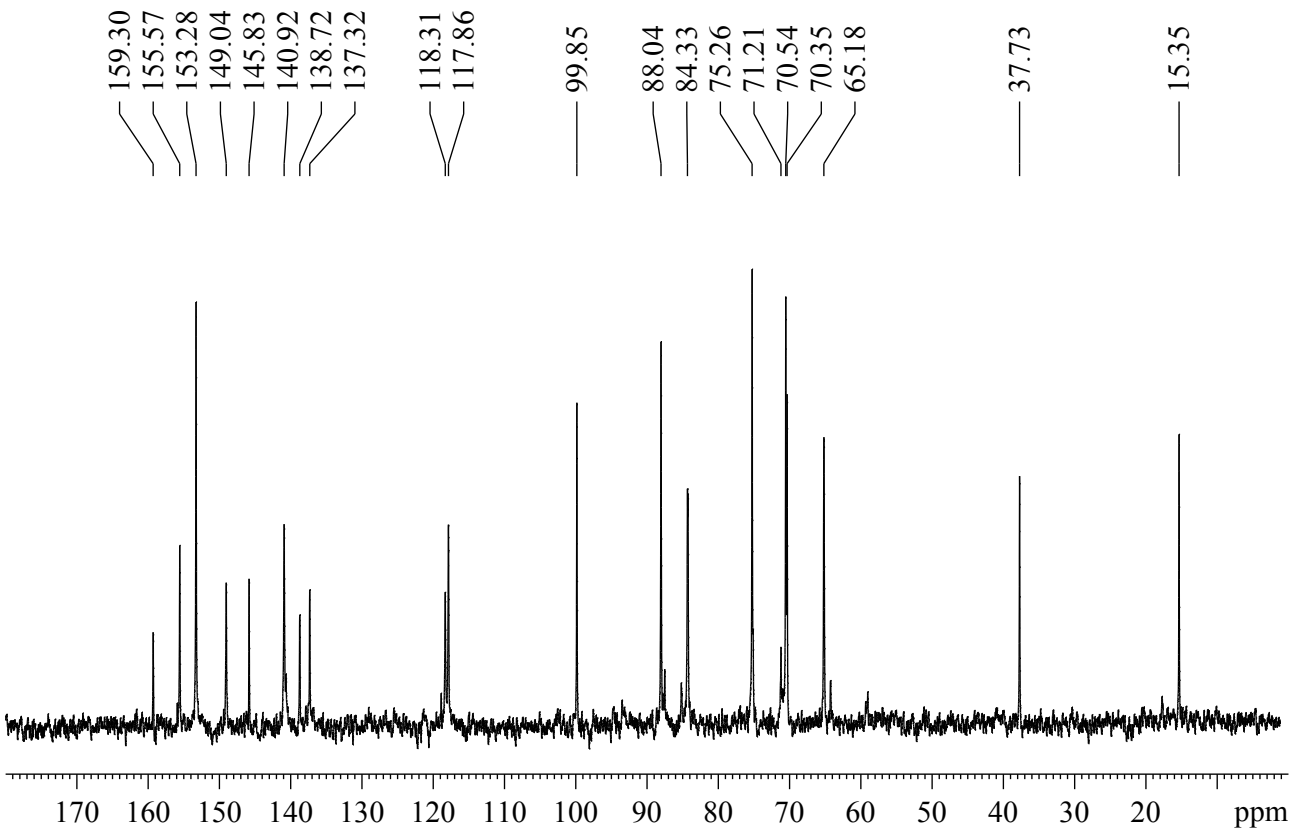

Figure S16. ^{13}C NMR spectrum of *pyr*$_2$*en*Zn /ADP in D_2O (rt, 100.62 MHz)

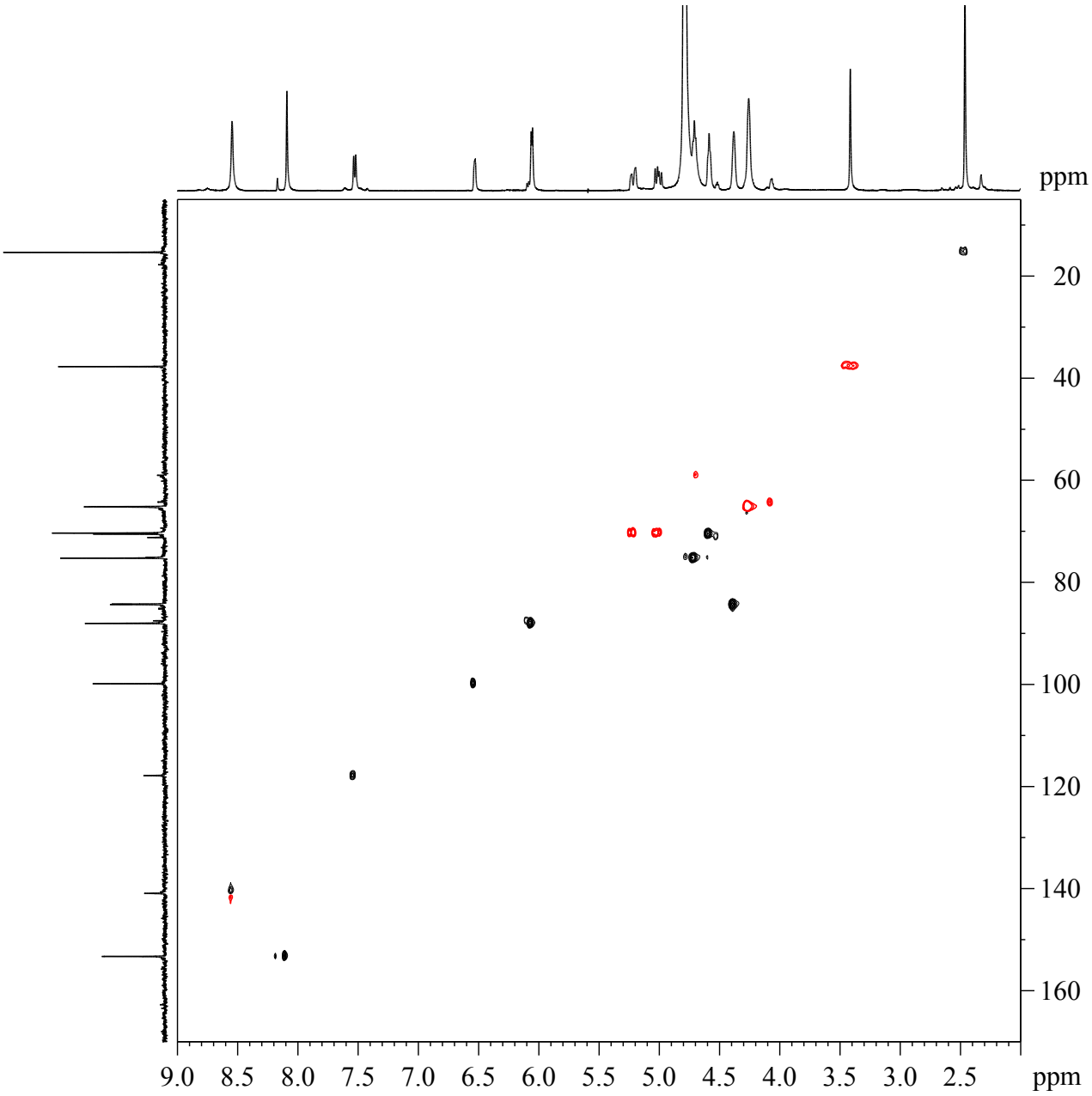

Figure S17. 2D ^{1}H-^{13}C HSQC of *pyr*$_2$*en*Zn /ADP in D_2O (rt, 400MHz)

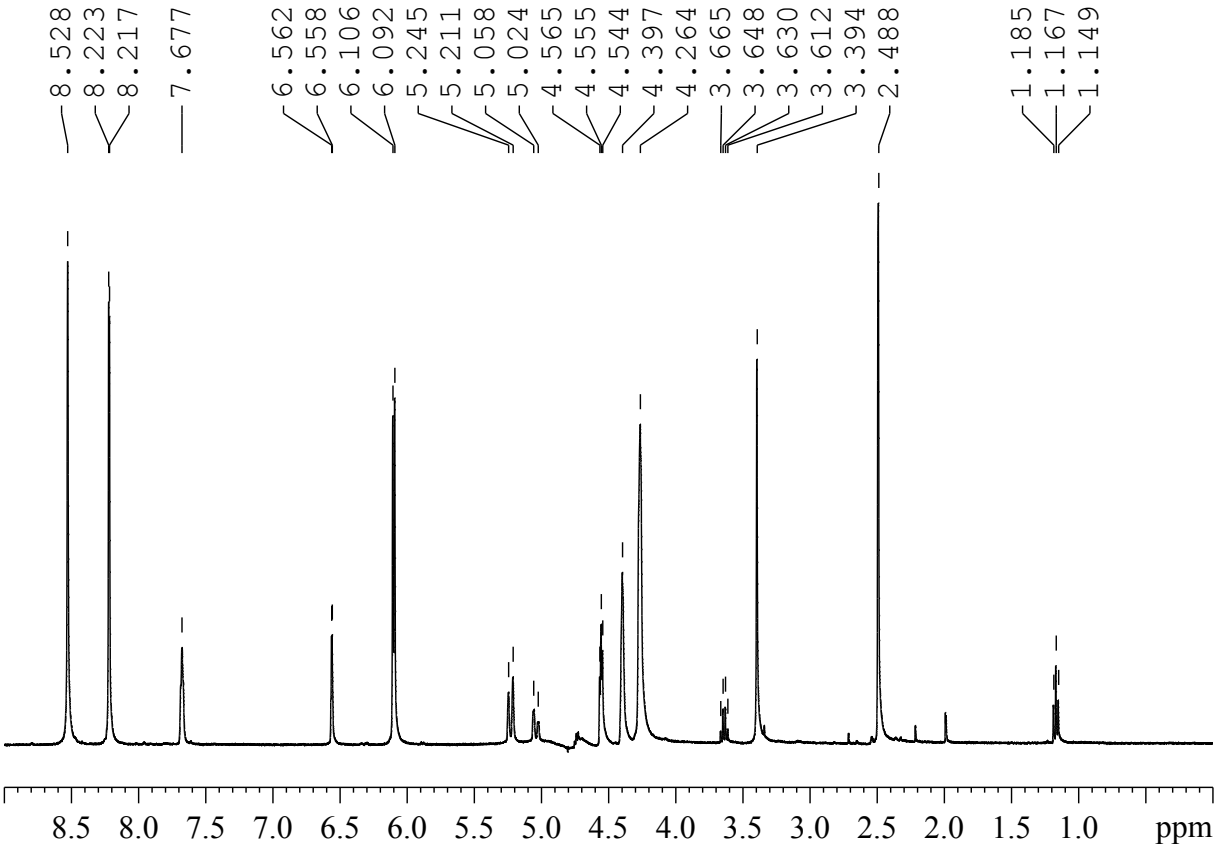

Figure S18. ^{1}H NMR spectrum of *pyr$_2$enZn* /ATP in D_2O. A presaturation pulse sequence was used to suppress the water signal.

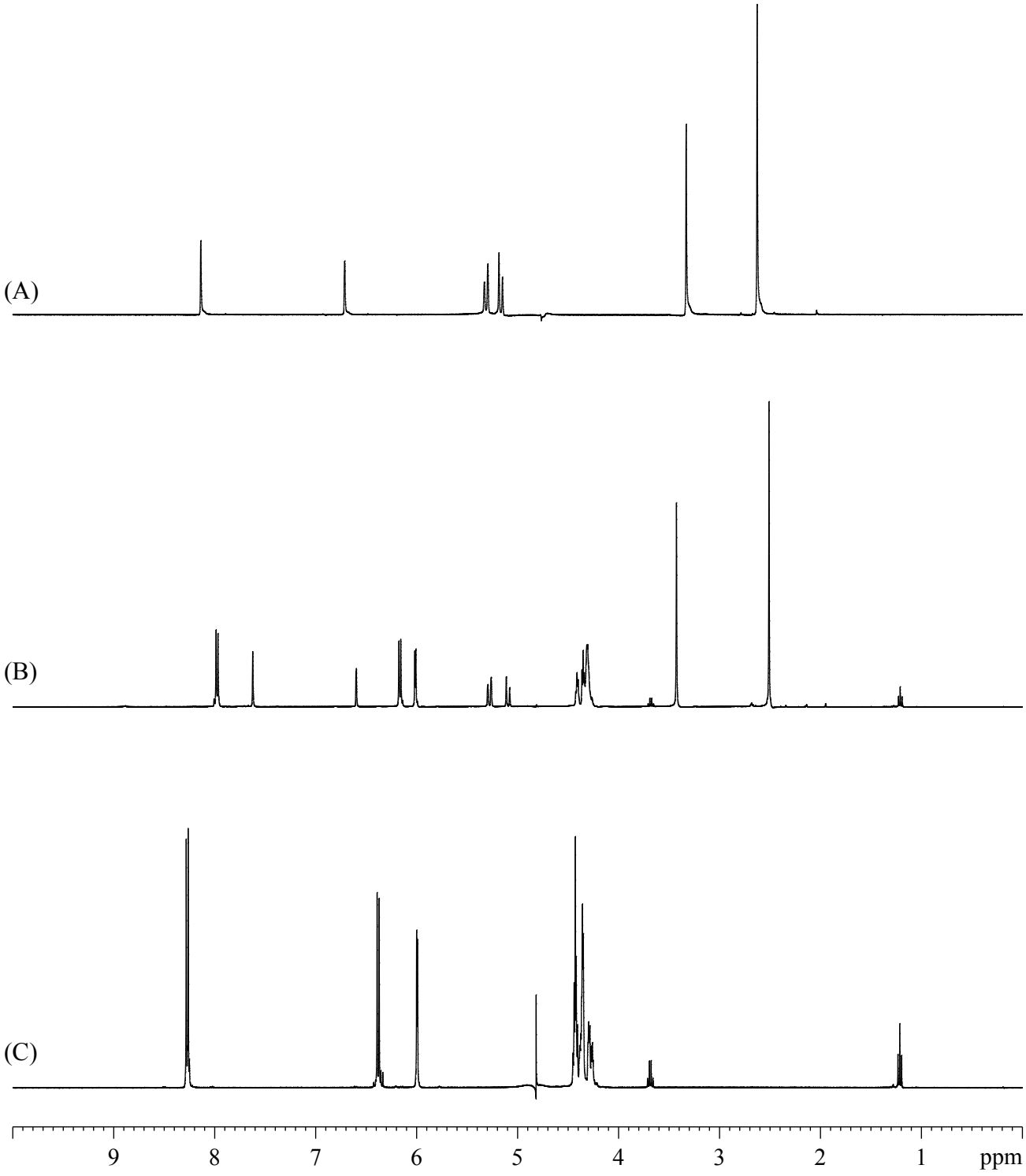

Figure S19. ^{1}H NMR spectrum of *pyr$_2$en*Zn (A), *pyr$_2$en*Zn /CTP (B) and Na_2CTP (C) (D_2O, rt, 400MHz). A presaturation pulse sequence was used to suppress the water signal.

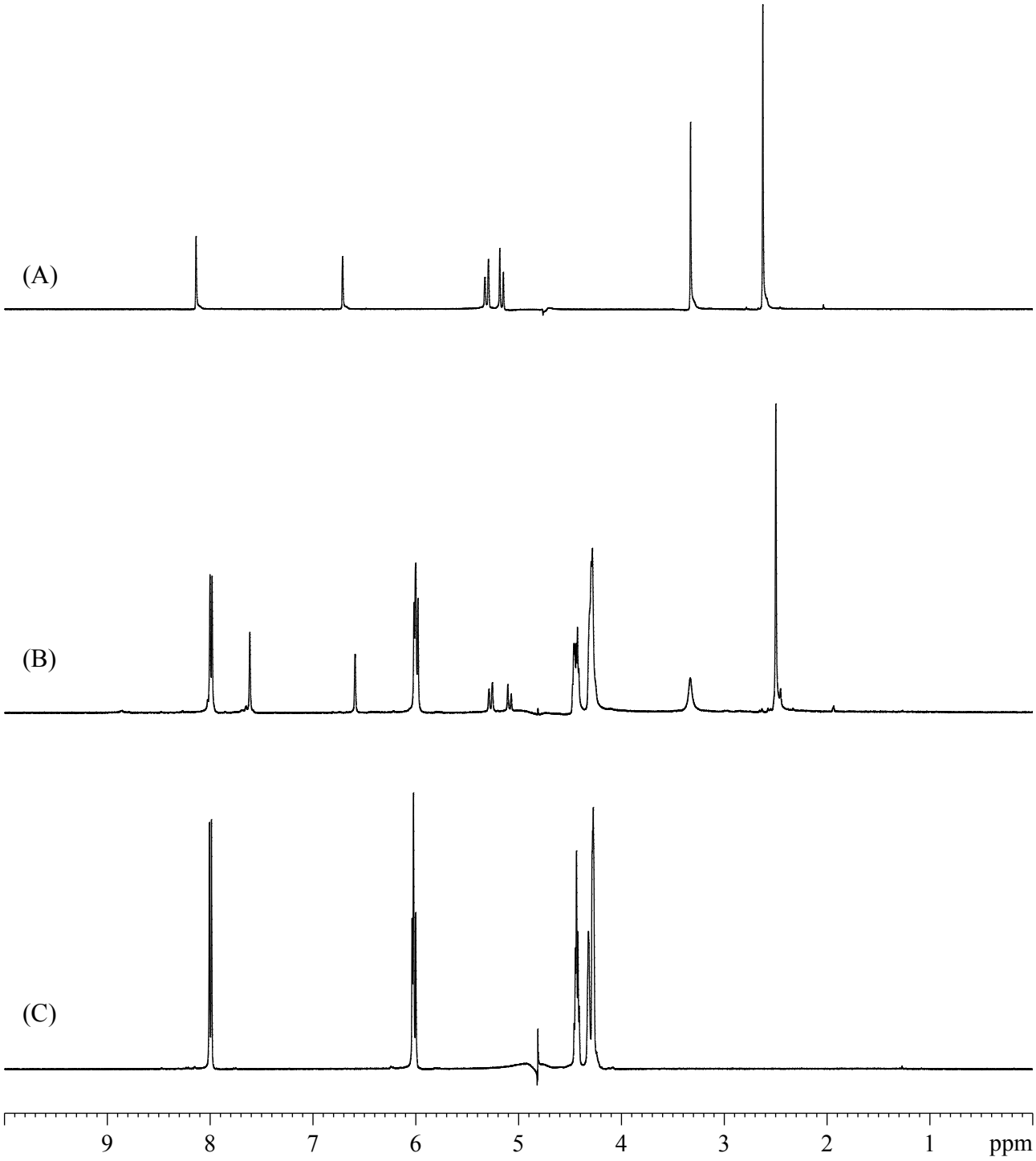

Figure S20. ^{1}H NMR spectrum of *pyr*$_2$*en*Zn (A), *pyr*$_2$*en*Zn /UTP (B) and Na_2UTP (C) (D_2O, rt, 400MHz). A presaturation pulse sequence was used to suppress the water signal.

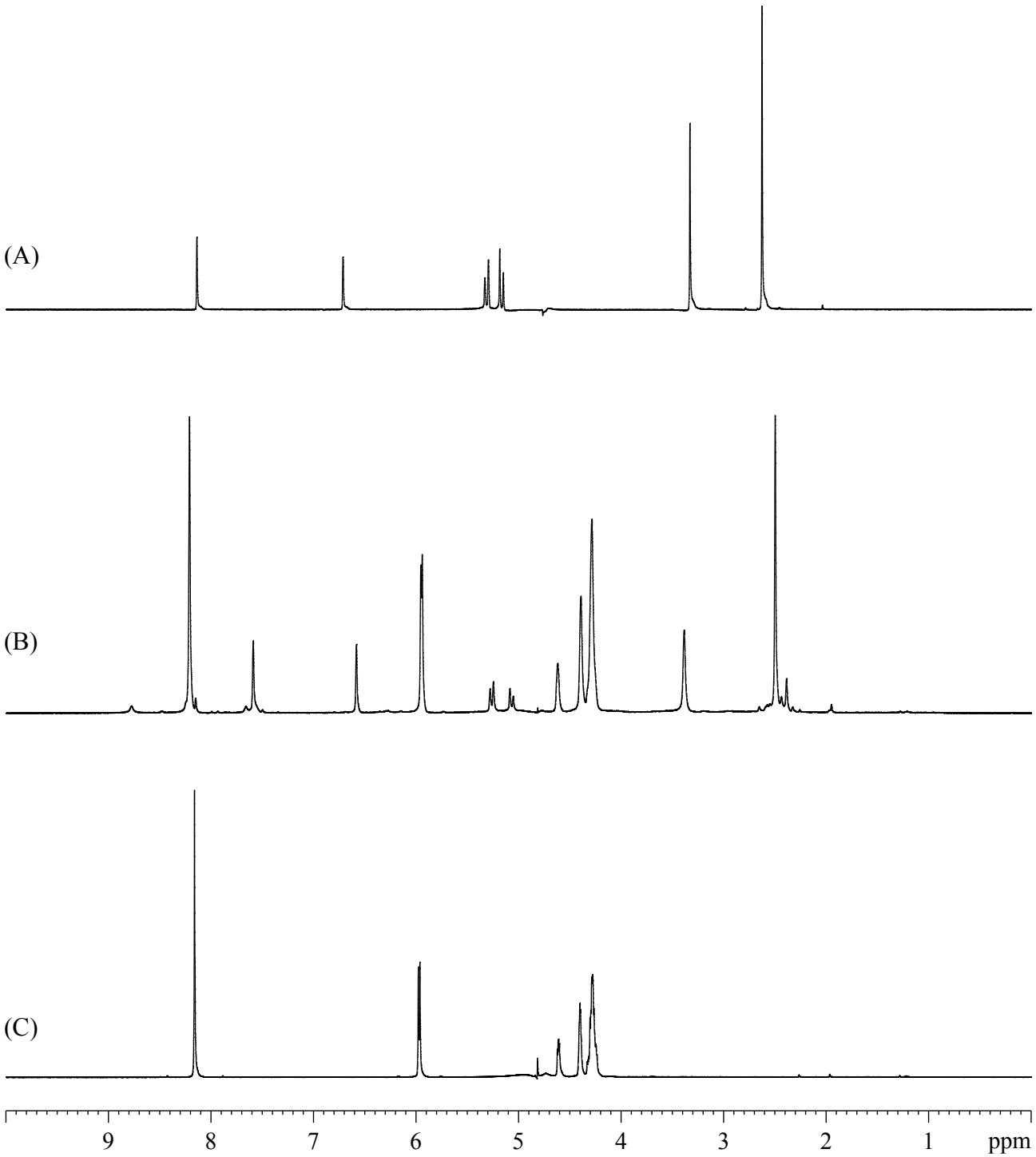

Figure S21. ^{1}H NMR spectrum of *pyr$_2$en*Zn (A), *pyr$_2$en*Zn /GTP (B) and Na_2GTP (C) (D_2O, rt, 400MHz). A presaturation pulse sequence was used to suppress the water signal.

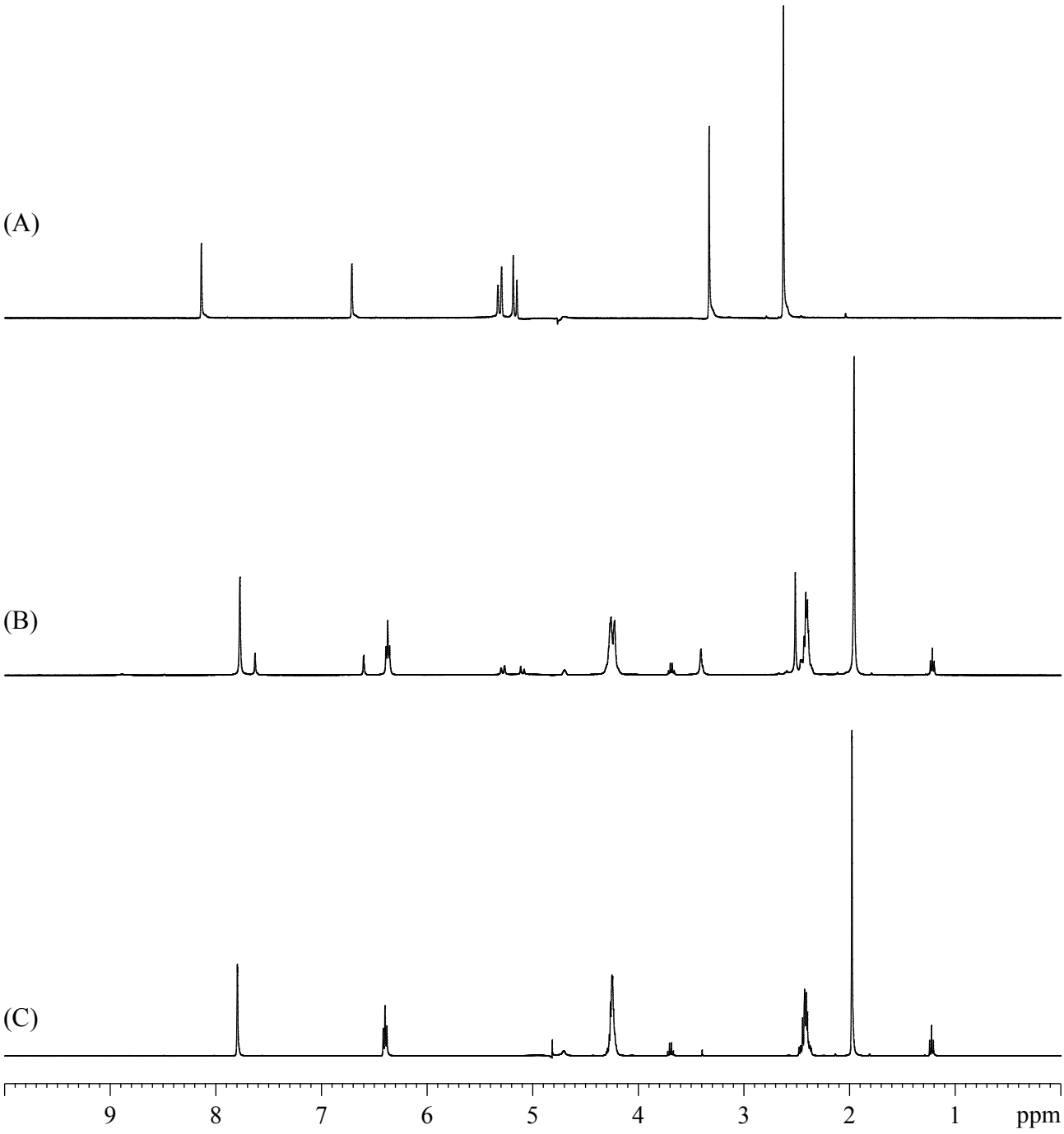

Figure S22. ^{1}H NMR spectrum of *pyr$_2$en*Zn (A), *pyr$_2$en*Zn /TTP (B) and Na_2TTP (C) (D_2O, rt, 400MHz). A presaturation pulse sequence was used to suppress the water signal.

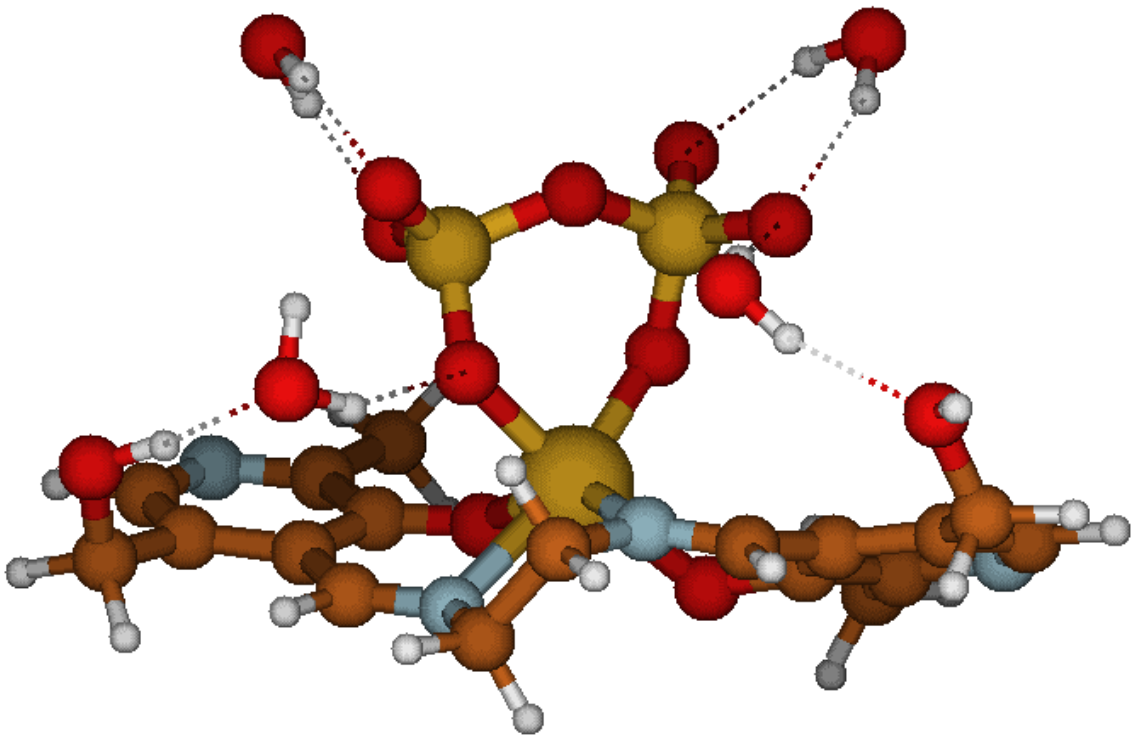

Figure S23. View of the optimized structure of the *pyr*$_2$*en*Zn /$P_2O_7^{4-}$ adduct

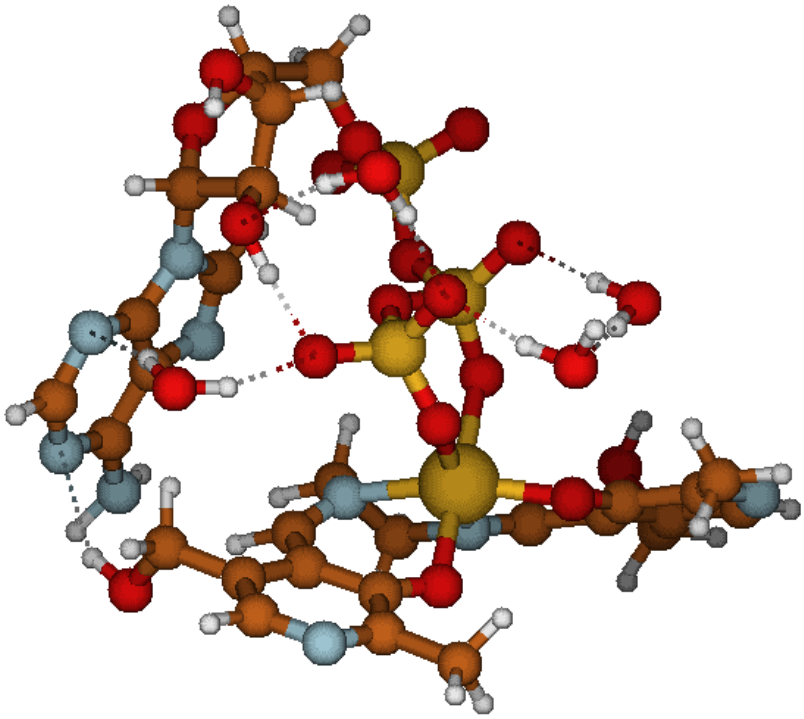

Figure S24. View of the optimized structure of the *pyr*$_2$*en*Zn /ATP adduct

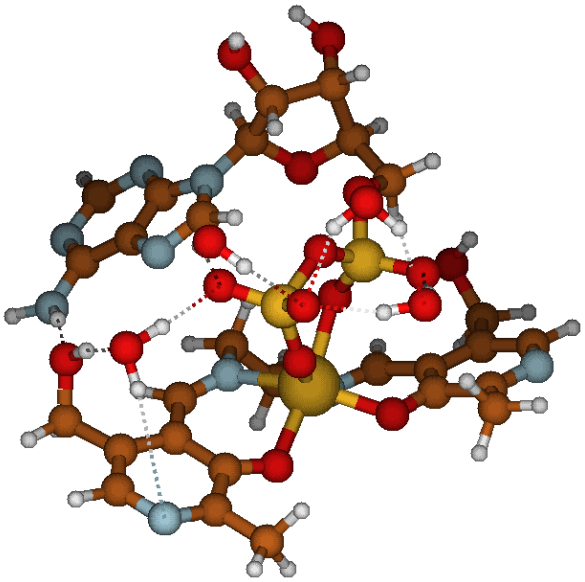

Figure S25. View of the optimized structure of the *pyr*$_2$*en*Zn /ADP adduct

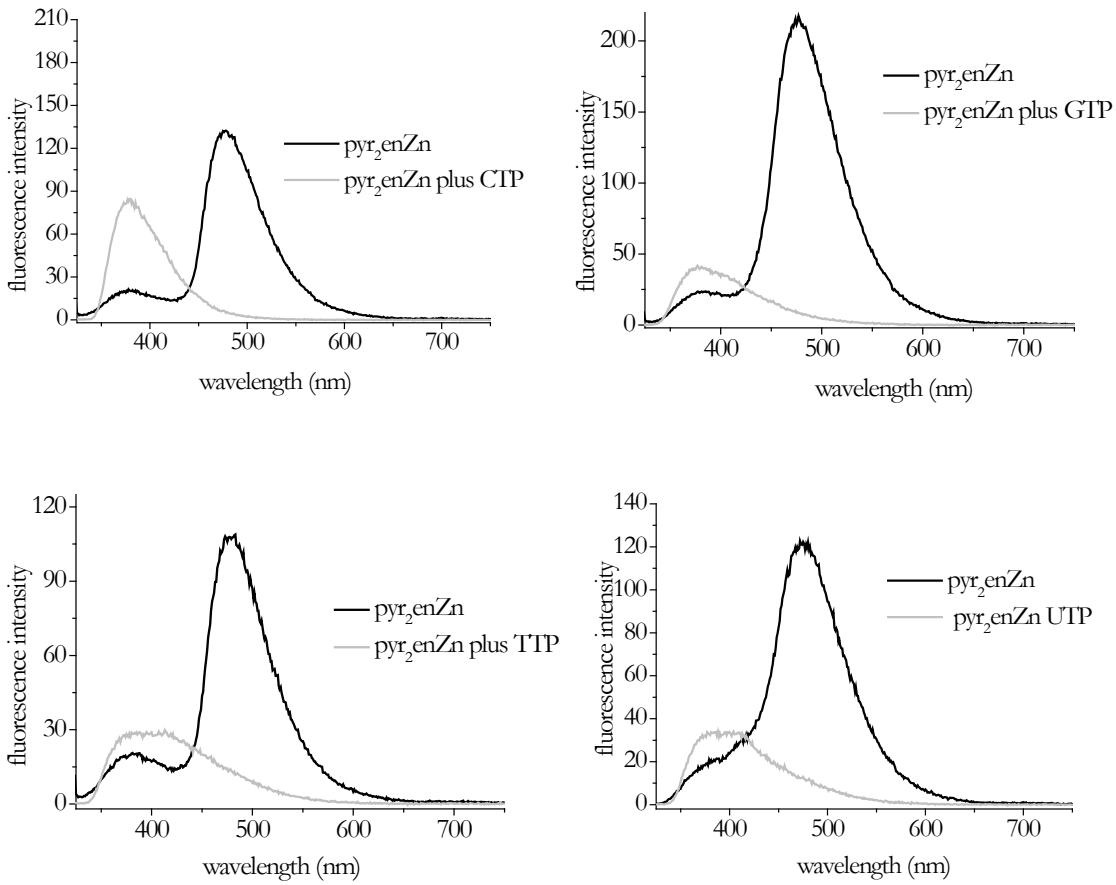

Figure S26. Emission spectra of *pyr₂en*Zn (λ_{exc} 315 nm) after addition of Na_2CTP, of Na_2GTP, Na_2TTP, Na_2UTP (rt, MilliQ water). [*pyr₂en*Zn] = 20 μM**.**

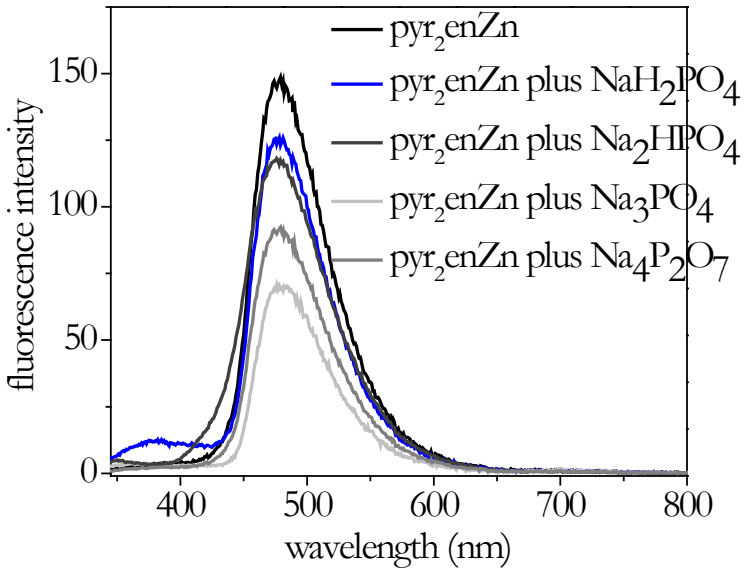

Figure S27. Emission spectra of *pyr₂en*Zn (λ_{exc} 315 nm) after addition of an excess of NaH_2PO_4 or of Na_2HPO_4 or of $Na_4P_2O_7$ or of Na_3PO_4 (rt, MilliQ water). [*pyren*Zn] = 15µM**.**

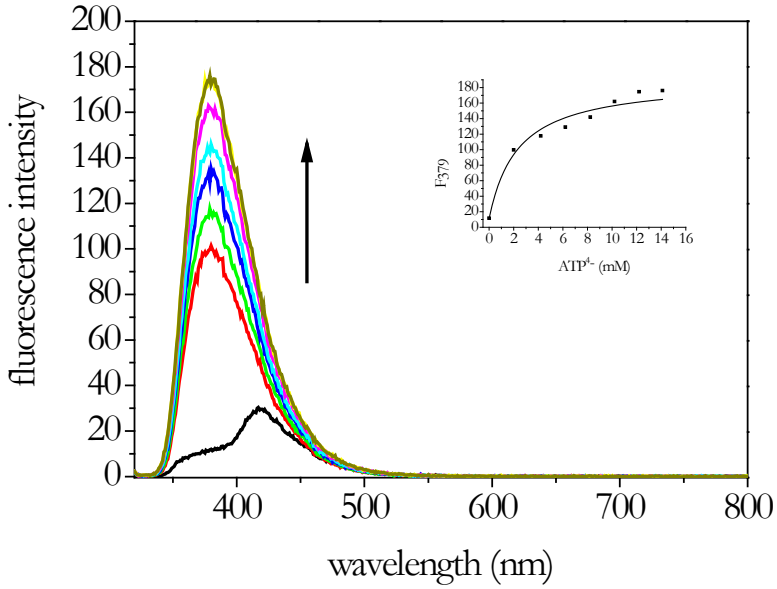

Figure S28. Emission spectra of *pyr*$_2$*en*Zn (λ_{exc} 315 nm) after additions of 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 eq. of Na_2ATP (rt, MOPS). [*pyr*$_2$*en*Zn] = 4.3 mM**.**

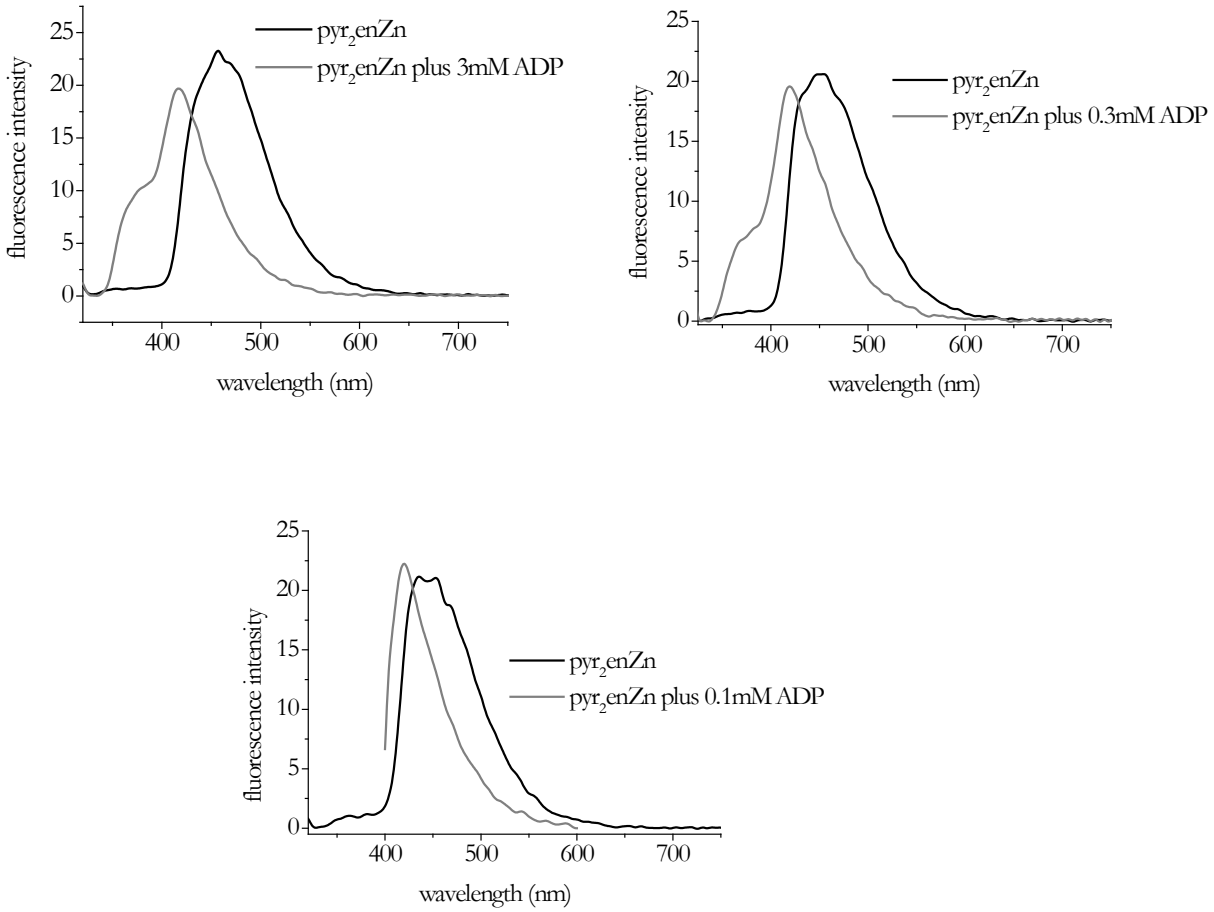

Figure S29. Sensitivity experiments for detection of Na_2ADP. *Pyr₂en*Zn concentration: 20 μM in 200 mM MOPS buffer.

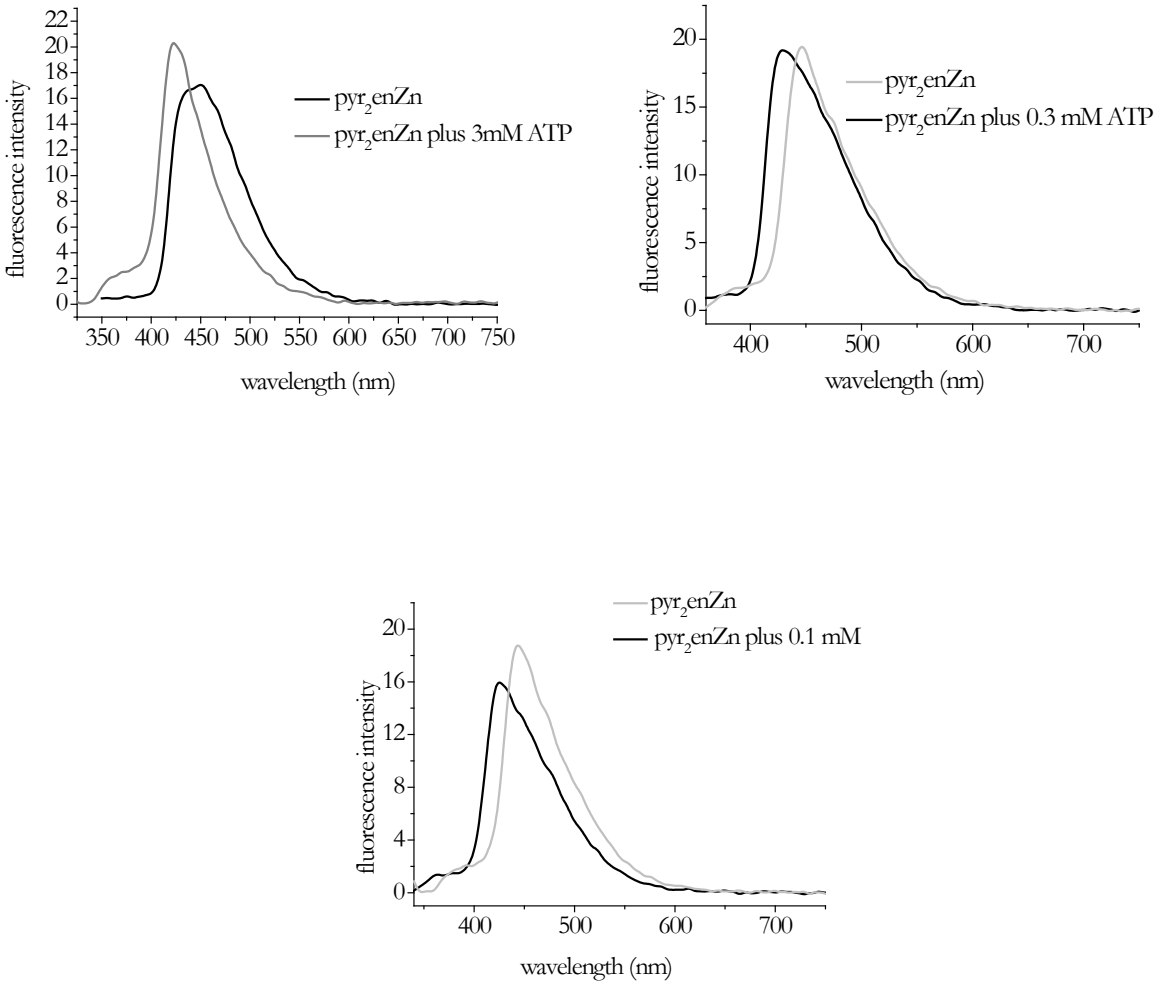

Figure S30. Sensitivity experiments for detection of Na_2ATP. *Pyr₂en*Zn concentration: 20 μM in 200 mM MOPS buffer.

Table S1. Resulting ^{31}P NMR Spectroscopic Resonances of nucleoside (AMP, ADP, ATP, CTP, UTP, TTP and GTP) and *pyr*$_2$*en*Zn /nucleoside adducts in D_2O solvent

	Signal	Multiplicity	Free Nucleoside ppm (*Hz*)	*pyr*$_2$*en*Zn /nucleoside ppm (*Hz*)
AMP	α	singlet	4.71	4.71
ADP	β	doublet	-9.54 (*20.8*)	-5.94 (*19.9*)
	α	doublet	-10.33 (*20.8*)	-9.34 (*20.4*)
ATP	γ	doublet	-9.84 (*18.6*)	-8.34 (*17.6*)
	α	doublet	-10.39 (*19.5*)	-10.20 (*18.3*)
	β	triplet	-22.07 (*19.2*)	-20.94 (*17.9*)
CTP	γ	doublet	-9.94 (*19.3*)	-6.43 (*16.6*)
	α	doublet	-10.53 (*19.7*)	-10.03 (*17.7*)
	β	triplet	-22.27 (*19.4*)	-19.83 (*16.4*)
UTP	γ	doublet	-9.82 (*19.3*)	-5.60 (-)
	α	doublet	-10.52 (*19.9*)	-10.15 (*18.4*)
	β	triplet	-22.19 (*19.6*)	-20.17 (-)
TTP	γ	doublet	-9.32 (*19.5*)	-5.64
	α	doublet	-10.22 (*18.3*)	-10.22 (*18.3*)
	β	triplet	-20.01 (*19.6*)	-20.24 (-)
GTP	γ	doublet	-9.28 (*19.3*)	-5.28 (*18.3*)
	α	doublet	-10.37 (*19.6*)	-10.01 (*18.3*)
	β	triplet	-21.93 (*19.5*)	-20.21 (*17.8*)

Cartesian coordinates

M06/6-311G(2d) IEF-PCM(solvent=water)

pyr$_2$*en*Zn

Atom	X	Y	X	(Angstrom)
6	-0.33152	0.19733	0.25235	
6	-0.40428	-0.36937	1.55014	
6	0.60630	-0.11590	2.43910	
7	1.67846	0.64550	2.15449	
6	1.77210	1.18900	0.96491	
6	0.78256	1.01985	-0.06730	
6	-1.55001	-1.23028	1.99358	
1	0.56660	-0.54032	3.44232	
6	2.96190	2.03084	0.65712	
8	0.99249	1.62877	-1.17785	
1	-1.44285	-1.43515	3.06790	
1	-2.50772	-0.71208	1.87051	
8	-1.66965	-2.43628	1.26079	
1	-0.81241	-2.87610	1.29642	
1	2.66895	3.04873	0.37864	
1	3.62303	2.07446	1.52427	
1	3.52038	1.63591	-0.19848	
6	-1.38711	-0.09495	-0.68814	
7	-1.48176	0.40872	-1.85751	
1	-2.14209	-0.82110	-0.36499	
6	-2.55953	0.03989	-2.75088	
6	-1.97720	-0.20662	-4.13629	
1	-3.11148	-0.83888	-2.39488	
1	-3.26358	0.88060	-2.80354	
1	-2.77284	-0.30818	-4.88474	
7	-1.06288	0.87126	-4.45287	
1	-1.40871	-1.14589	-4.12029	
6	-1.01655	1.37705	-5.62462	
6	-0.14423	2.45317	-6.02625	
1	-1.67118	0.96020	-6.39842	
6	-0.17253	2.88035	-7.37852	
6	0.65356	3.90110	-7.76628	
7	1.49277	4.54111	-6.93137	
6	1.53508	4.17727	-5.67272	
6	0.73130	3.11573	-5.12303	
6	-1.06455	2.24899	-8.40596	
1	0.65188	4.23734	-8.80312	
6	2.46721	4.89012	-4.75526	
8	0.87490	2.86590	-3.87312	
1	-0.97124	2.80918	-9.34682	
8	-0.80505	0.87237	-8.60992	
1	-2.11859	2.31241	-8.11200	
1	0.13479	0.78701	-8.80694	
1	3.19448	4.20223	-4.31052	
1	3.00255	5.67036	-5.29888	
1	1.93084	5.34278	-3.91468	
30	-0.04666	1.51167	-2.81146	

Energy	-2996.660985	(Hartree)
E+zpe	-2996.296031	(Hartree)
Enthalpy	-2996.268844	(Hartree)
Free Energy	-2996.352772	(Hartree)

pyr$_2$*en*Zn **-2H$_2$O**

Atom	X	Y	X	(Angstrom)
6	-2.72668	1.01843	-0.23763	
6	-3.31964	-0.25885	-0.03989	
6	-4.72249	-0.33313	0.14475	
6	-5.45589	0.82395	0.10800	
7	-4.92263	2.03972	-0.10248	
6	-3.62490	2.14214	-0.27118	
6	-2.55828	-1.49007	-0.05341	
7	-1.28588	-1.55253	-0.08488	
30	0.01545	0.02572	0.12534	
8	-1.47356	1.25328	-0.40751	
6	-5.43988	-1.62945	0.38078	
8	-5.37432	-2.51984	-0.71888	
6	-3.04488	3.49390	-0.50766	
6	-0.59270	-2.82059	-0.13739	
6	0.61050	-2.76005	0.79503	
7	1.31168	-1.51559	0.56807	
6	2.57888	-1.47988	0.43786	
6	3.34842	-0.27275	0.22419	
6	4.74544	-0.38486	0.01807	
6	5.48299	0.75836	-0.14879	
7	4.96107	1.99659	-0.10926	
6	3.67013	2.13362	0.08551	
6	2.76805	1.02594	0.25247	
6	5.44948	-1.70819	-0.03587	
8	4.98878	-2.55051	-1.07603	
6	3.09910	3.50876	0.12943	
8	1.52339	1.29596	0.42969	
1	6.55768	0.69045	-0.31922	
1	6.53043	-1.52998	-0.12440	
1	5.30551	-2.27449	0.89156	
1	5.05762	-2.05019	-1.89715	
1	2.59992	3.70322	1.08497	
1	3.88919	4.24747	-0.01561	
1	2.33324	3.64826	-0.64111	
1	3.14069	-2.42056	0.48591	
1	1.25741	-3.63675	0.65971	
1	0.25344	-2.76229	1.83379	
1	-1.24575	-3.66598	0.11591	
1	-0.23536	-2.96699	-1.16600	
1	-3.13875	-2.42120	-0.06711	
1	-6.53568	0.78687	0.25387	
1	-6.48451	-1.41318	0.64516	
1	-5.00998	-2.17291	1.22947	
1	-5.67987	-2.03570	-1.49466	
1	-2.50326	3.53640	-1.45875	
1	-3.83825	4.24322	-0.51728	
1	-2.31497	3.75766	0.26519	
8	0.46305	0.04870	-2.05172	
1	1.27712	0.52654	-2.24864	
1	-0.24555	0.65936	-2.29482	
8	-0.44345	0.40376	2.25548	
1	-1.24243	0.93207	2.36626	
1	0.28184	1.01532	2.43876	

Energy	-3149.519848	(Hartree)
E+zpe	-3149.103824	(Hartree)
Enthalpy	-3149.071176	(Hartree)
Free Energy	-3149.165429	(Hartree)

ADP^{3-}-4H$_2$O

Atom	X	Y	X	(Angstrom)
6	0.46332	-1.85882	0.70018	
6	1.72648	-1.70875	-0.13429	
8	1.41938	-2.09596	-1.44796	
6	0.12424	-2.71292	-1.49175	
6	-0.25258	-2.97920	-0.04959	
6	-0.83260	-1.82253	-2.24650	
8	-0.85027	-0.54548	-1.65988	
15	-2.24063	0.12988	-1.14753	
8	-1.52540	1.08183	-0.10910	
15	-2.18677	2.01131	1.15733	
8	-3.53069	2.46867	0.64546	
7	2.30156	-0.38411	-0.10513	
6	1.67786	0.84599	-0.08433	
7	2.50930	1.85043	-0.07697	
6	3.75507	1.25934	-0.10176	
6	3.64869	-0.12168	-0.12272	
7	4.65660	-0.99356	-0.14345	
6	5.82946	-0.37585	-0.13301	
7	6.08698	0.93290	-0.10719	
6	5.05279	1.78248	-0.09342	
7	5.29630	3.11256	-0.11547	
8	0.29973	-4.21223	0.35627	
8	0.79918	-2.20527	2.01017	
8	-3.07828	-0.92883	-0.48730	
8	-2.87211	0.85604	-2.28520	
8	-1.13582	3.08819	1.27801	
8	-2.26024	1.06612	2.33241	
1	-0.49532	-1.73178	-3.28686	
1	-1.82997	-2.28023	-2.25280	
1	0.21851	-3.66671	-2.02837	
1	2.50372	-2.36871	0.27737	
1	0.59423	0.93573	-0.07262	

1	6.22051	3.39809	0.17858
1	4.54622	3.71824	0.18784
1	6.70763	-1.02195	-0.14471
1	-1.33863	-2.96199	0.10796
1	-0.14500	-0.94609	0.67112
1	0.06566	-1.85080	2.57825
1	0.54095	-4.06983	1.28669
8	-5.48828	0.05894	-1.62887
1	-4.75578	0.52391	-2.07020
1	-4.95249	-0.50464	-1.04934
8	-2.25965	4.42124	-0.88672
1	-2.98581	3.86951	-0.53925
1	-1.59714	4.14916	-0.22188
8	-3.10795	-2.58256	1.63165
1	-4.00971	-2.53540	1.96210
1	-3.07381	-1.91299	0.90704
8	-1.33196	-1.24538	3.25732
1	-1.54605	-0.34302	2.90700
1	-1.98961	-1.79686	2.78557

Energy	-2403.045218	(Hartree)
E+zpe	-2402.672162	(Hartree)
Enthalpy	-2402.638266	(Hartree)
Free Energy	-2402.736383	(Hartree)

ATP^{4-}-4H$_2$O

Atom	X	Y	X	(Angstrom)
6	-3.93315	2.57569	0.55126	
6	-3.03360	1.50041	0.49561	
6	-3.57932	0.23450	0.36567	
7	-4.87759	-0.06888	0.31175	
6	-5.62560	1.02407	0.39959	
7	-5.24829	2.29747	0.50816	
7	-2.50166	-0.61511	0.29927	
6	-1.38099	0.18123	0.38999	
7	-1.65921	1.44575	0.51640	
6	-2.55382	-2.03095	0.06078	
6	-1.97090	-2.44622	-1.27806	
6	-1.64497	-3.90878	-1.01163	
6	-1.21276	-3.88482	0.44535	
8	-1.81241	-2.71629	1.02940	
8	-2.93894	-2.25994	-2.27718	
6	0.27390	-3.83696	0.65794	
8	0.82237	-2.77275	-0.08639	
15	2.00630	-1.89285	0.59785	
8	1.65899	-1.63657	2.02035	
8	-2.81912	-4.67452	-1.13203	
7	-3.52849	3.84763	0.66804	
8	1.75242	-0.60750	-0.32458	
15	2.79817	0.65958	-0.52478	
8	3.54427	0.40430	-1.78918	
8	3.32535	-2.52918	0.29124	
8	3.56477	0.83158	0.74143	
8	1.66662	1.74797	-0.71959	
8	3.47966	-2.27582	-2.50828	
8	1.62362	1.86953	2.49489	
8	4.25181	-2.22990	2.94739	
1	0.48009	-3.70379	1.72595	
1	0.71556	-4.79131	0.33849	
1	-1.61171	-4.77556	0.94922	
1	-3.62097	-2.30115	0.08707	
1	-0.37989	-0.24069	0.35233	
1	-4.23925	4.55091	0.52156	
1	-2.56207	4.11552	0.42662	
1	-6.70390	0.86093	0.37240	
1	-0.84820	-4.29024	-1.66632	
1	-1.05279	-1.87827	-1.47223	
1	-2.49052	-2.14285	-3.12160	
1	-3.31266	-4.27655	-1.86535	
1	3.32781	-1.92704	2.96240	
1	4.29978	-2.45522	2.00416	
1	2.30780	1.34730	2.04046	
1	1.51139	2.60172	1.85086	
1	3.46860	-1.30549	-2.39589	
1	3.49802	-2.54791	-1.57406	
15	1.83290	3.44124	-0.76852	
8	1.44547	3.87733	0.64086	
8	3.25875	3.71235	-1.13651	
8	0.79350	3.81785	-1.80049	
8	-0.97467	4.94058	-0.05541	
1	-0.58828	4.59007	-0.88918	
1	-0.22532	4.66067	0.51309	

Energy	-2970.323534	(Hartree)
E+zpe	-2969.933863	(Hartree)
Enthalpy	-2969.896723	(Hartree)
Free Energy	-2969.999757	(Hartree)

P$_2$O$_7^{4-}$-4H$_2$O

Atom	X	Y	X	(Angstrom)
8	0.11449	0.12113	0.03143	
15	0.06471	0.09204	1.56091	
8	1.45496	0.05674	2.18360	
8	-0.63256	1.47669	2.08224	
15	-0.77363	2.98934	1.42258	
8	0.54205	3.35262	0.76747	
8	-0.85177	-0.99479	2.09702	
8	-1.93680	2.91773	0.44586	
8	-1.08760	3.83127	2.65159	
8	2.80750	0.03641	-0.13034	
8	3.18140	2.78808	0.33607	
8	-1.28734	-2.20173	-0.31482	
8	-3.75522	3.22822	2.48505	
1	-0.77461	-1.39943	-0.53778	
1	-1.30370	-2.01763	0.64753	
1	-2.89798	3.53897	2.84650	
1	-3.39150	3.01122	1.60141	
1	2.58301	-0.01798	0.83075	
1	1.86617	0.09938	-0.41188	
1	2.25338	3.01455	0.55391	
1	3.11260	1.84594	0.09606	

Energy	-1515.714006	(Hartree)
E+zpe	-1515.580628	(Hartree)
Enthalpy	-1515.562325	(Hartree)
Free Energy	-1515.627197	(Hartree)

*pyr$_2$en*Zn **-ADP-4H$_2$O**

Atom	X	Y	X	(Angstrom)
6	-5.75368	-1.87214	-0.37809	
6	-4.97508	-0.82046	-0.79799	
6	-3.62432	-1.06293	-1.12249	
6	-3.13137	-2.40205	-1.08709	
6	-4.06637	-3.40028	-0.62842	
7	-5.30646	-3.13849	-0.28393	
8	-1.96966	-2.77081	-1.45238	
30	-0.31938	-1.63094	-0.95242	
7	-1.51561	-0.04792	-1.63101	
6	-2.78722	0.00453	-1.62342	
6	-3.57453	-4.80237	-0.52039	
6	-5.58363	0.55017	-0.84942	
8	-4.96067	1.48303	0.00480	
8	1.18575	-0.36925	-0.14368	
15	1.80359	-0.55268	1.20616	
8	0.71038	-0.40764	2.35282	
15	-0.79604	-1.12804	2.29914	
8	-0.83627	-1.94440	1.02729	
8	2.69166	-1.72239	1.44925	
8	2.61276	0.81213	1.55320	
6	3.65424	1.15149	0.66107	
6	3.66152	2.63552	0.42218	
8	2.50129	3.00904	-0.33109	
6	1.70437	3.89633	0.40854	
6	2.14595	3.76592	1.85509	
6	3.63500	3.48200	1.68196	
7	0.31090	3.63188	0.17963	
6	-0.48366	2.61744	0.67990	
7	-1.69760	2.63625	0.21455	
6	-1.71310	3.69858	-0.66022	
6	-0.48260	4.33020	-0.70002	
7	-0.15805	5.39060	-1.44045	
6	-1.18550	5.79182	-2.17797	
7	-2.41168	5.27546	-2.26350	
6	-2.70581	4.20383	-1.51095	
7	-3.91082	3.62264	-1.62745	
8	4.31141	4.69019	1.43771	
8	1.88513	4.97172	2.52520	
8	-1.72891	0.05824	2.35574	
8	-0.81247	-1.99125	3.54718	

8	0.90656	-3.20915	-1.14789
6	2.14627	-3.08619	-1.42286
6	2.70471	-2.11993	-2.30495
6	4.10655	-2.11728	-2.50412
6	4.87011	-3.05808	-1.86156
7	4.36694	-3.99118	-1.03043
6	3.07370	-4.00351	-0.80946
6	1.89871	-1.11389	-2.95618
7	0.62872	-1.01943	-2.87558
6	-0.01575	0.12607	-3.47631
6	-0.74517	0.90830	-2.38595
6	2.52662	-4.98907	0.16256
6	4.80462	-1.10425	-3.35991
8	4.81055	0.19756	-2.78849
1	3.51922	0.64502	-0.30441
1	4.62351	0.84126	1.07646
1	4.55491	2.88911	-0.16507
1	1.86540	4.93310	0.07692
1	-0.10883	1.87432	1.37898
1	-4.63056	4.15434	-2.09598
1	-4.21000	2.94454	-0.92252
1	-1.00387	6.66327	-2.80785
1	4.06254	2.94862	2.54336
1	1.65504	2.91096	2.33198
1	1.80787	4.78762	3.46760
1	3.86278	5.35300	1.98487
1	2.44900	-0.35927	-3.53837
1	0.69380	0.77851	-4.00924
1	-0.75992	-0.22704	-4.20388
1	-1.37056	1.69911	-2.82765
1	-0.00656	1.37345	-1.72054
1	-3.28898	0.88355	-2.05691
1	1.72573	-5.59412	-0.27500
1	3.32190	-5.64919	0.51636
1	2.08627	-4.46863	1.02232
1	-2.69558	-4.86112	0.13148
1	-4.35913	-5.45048	-0.12465
1	-3.24842	-5.18950	-1.49231
1	5.95038	-3.07640	-2.01390
1	5.83294	-1.44422	-3.54737
1	4.33085	-0.99533	-4.34090
1	-6.79234	-1.70008	-0.09308
1	-6.65641	0.46301	-0.61803
1	-5.52391	0.96724	-1.86503
1	5.23365	0.11602	-1.92562
1	-4.72717	1.01764	0.84003
8	-2.22561	-0.17688	5.12626
1	-1.84038	-1.02900	4.85690
1	-2.16952	0.26318	4.26134
8	-4.39344	-0.08096	2.18670
1	-3.40446	-0.08248	2.24372
1	-4.60386	-0.91320	1.74552
8	1.29815	-1.36270	5.35719
1	0.47555	-1.26629	4.84790
1	1.69868	-2.09602	4.86151
8	1.59389	-3.41346	3.38624
1	2.02456	-2.86270	2.69985
1	0.67801	-3.06436	3.39181

Energy	-5399.768295	(Hartree)
E+zpe	-5399.025865	(Hartree)
Enthalpy	-5398.965681	(Hartree)
Free Energy	-5399.117338	(Hartree)

*pyr₂en*Zn **-ATP-4H₂O**

6	3.17881	2.35616	-3.00823
6	3.18480	1.05455	-2.50372
6	4.03774	0.80386	-1.43649
7	4.82865	1.69975	-0.83633
6	4.73320	2.89463	-1.40190
7	3.97922	3.26917	-2.43904
7	2.51573	-0.08496	-2.88322
6	2.96088	-1.00080	-2.06546
7	3.89131	-0.52956	-1.16925
6	4.58634	-1.29273	-0.14861
8	4.85680	-2.57340	-0.63552
6	4.56060	-3.55856	0.37176
6	4.33097	-2.78158	1.65142
6	3.77699	-1.47203	1.12962
6	3.40543	-4.41919	-0.07003
8	2.21346	-3.66623	-0.09516
15	1.12688	-4.01511	-1.26360
8	0.38553	-5.24666	-0.92144
8	3.95891	-0.41656	2.02833
8	5.57518	-2.56438	2.28388
7	2.36171	2.73744	-4.01550
8	0.21313	-2.68097	-1.11526
15	-0.55008	-2.05581	0.16880
8	0.60020	-1.05950	0.67917
15	0.41042	0.02869	1.91623
8	0.37987	-0.77187	3.20072
8	-0.90323	0.73489	1.66715
30	-2.08619	0.85962	0.01784
8	-1.67584	-1.24455	-0.38270
7	-0.66098	1.68860	-1.28204
6	-0.74327	1.05845	-2.57506
6	-2.20874	1.15165	-3.00245
7	-3.06495	0.74018	-1.91122
6	-4.05926	-0.01996	-2.14828
6	-4.97501	-0.53734	-1.15462
6	-4.79756	-0.33810	0.24667
6	-5.80464	-0.92119	1.10401
7	-6.83626	-1.60203	0.66307
6	-6.97640	-1.77663	-0.66430
6	-6.10032	-1.27776	-1.59392
8	-3.85394	0.31101	0.80329
6	-5.64403	-0.74119	2.57421
6	-6.38174	-1.54613	-3.04247
8	-5.40564	-2.36207	-3.66873
6	-0.08563	2.81635	-1.13803
6	-0.27423	3.66569	0.02302
6	-1.51296	3.64717	0.72906
6	-1.65675	4.64851	1.75766
7	-0.71372	5.50141	2.09032
6	0.45380	5.46973	1.42176
6	0.71109	4.60257	0.38938
8	-2.48153	2.85516	0.49721
6	2.04692	4.62399	-0.27951
8	1.94553	5.13345	-1.59712
6	-2.94611	4.68760	2.50514
8	1.65488	0.88228	1.76103
8	-0.83623	-3.11441	1.16773
8	1.79919	-3.89778	-2.58392
8	2.71429	-1.67309	4.31117
8	-1.95162	-0.39481	4.51881
8	-2.83620	-2.61580	2.99910
8	3.67196	2.78914	1.84197
1	2.74632	5.22532	0.32181
1	3.63178	-4.81752	-1.06744
1	3.30553	-5.26974	0.61991
1	5.44477	-4.19932	0.48248
1	5.50915	-0.73524	0.07230
1	2.67165	-2.04923	-2.09194
1	2.62032	3.58348	-4.50617
1	2.00054	2.00121	-4.60749
1	5.33917	3.68416	-0.95675
1	3.62573	-3.28478	2.32688
1	2.72382	-1.60930	0.86498
1	3.12020	0.12003	1.99615
1	5.49566	-1.72348	2.75512
1	-4.24904	-0.34154	-3.18181
1	-2.39212	0.57247	-3.91994
1	-2.42130	2.20724	-3.22851
1	-0.09690	1.54144	-3.32245
1	-0.44641	0.00757	-2.48170
1	0.53255	3.22615	-1.95234
1	-5.62652	0.31911	2.84996
1	-6.46417	-1.23271	3.10245
1	-4.68945	-1.16529	2.90509
1	-3.13316	3.74307	3.02895
1	-2.93594	5.49998	3.23480
1	-3.79668	4.82477	1.82824
1	-7.84726	-2.34714	-0.98946
1	-7.37858	-2.00215	-3.12971
1	-6.41167	-0.61898	-3.62648
1	1.21774	6.18063	1.73958
1	2.45931	3.60494	-0.28584

1	-5.25984	-3.11850	-3.08919
1	4.17195	2.33538	1.14916
1	2.83684	2.28670	1.85993
1	1.84334	-1.40589	3.94539
1	3.31757	-1.22047	3.70516
1	-1.09077	-0.44183	4.03456
1	-1.77125	-0.84637	5.34824
1	-2.11351	-2.77526	2.36122
1	-2.54924	-1.82831	3.49519
1	2.72939	4.79715	-2.06559

Energy	-5967.057164	(Hartree)
E+zpe	-5966.300773	(Hartree)
Enthalpy	-5966.236575	(Hartree)
Free Energy	-5966.395259	(Hartree)

*pyr₂en*Zn **-P_2O_7-4H_2O**

Atom	X	Y	X	(Angstrom)
6	2.50108	-1.22400	1.48920	
6	3.16475	-1.67906	0.31171	
6	4.57046	-1.54845	0.23481	
6	5.24692	-1.03120	1.31259	
7	4.65214	-0.63045	2.44987	
6	3.34413	-0.71669	2.53969	
6	2.43289	-2.20364	-0.81836	
7	1.16667	-2.33712	-0.88223	
30	-0.08855	-0.77368	0.23038	
8	1.07636	0.48687	-0.89869	
15	1.38850	1.97063	-0.64738	
8	2.23468	2.21525	0.57640	
6	5.36251	-1.87725	-0.99980	
8	5.40071	-0.82057	-1.93088	
6	2.68229	-0.23429	3.78522	
8	1.24287	-1.25008	1.68432	
6	0.58259	-2.72427	-2.14538	
6	-0.39334	-1.63708	-2.59688	
7	-1.22922	-1.32284	-1.46839	
6	-2.48288	-1.51937	-1.52107	
6	-3.33988	-1.45336	-0.36103	
6	-4.72884	-1.27673	-0.50664	
6	-5.51936	-1.28510	0.61601	
7	-5.03821	-1.47645	1.85710	
6	-3.74884	-1.67861	2.01754	
6	-2.79441	-1.69341	0.93653	
6	-5.35885	-1.08546	-1.84704	
8	-4.86766	0.10540	-2.44739	
8	-1.57357	-1.94623	1.18337	
6	-3.22517	-1.88144	3.39796	
8	-0.87324	0.84022	1.16213	
15	-1.17927	2.26905	0.72413	
8	-1.02761	3.26307	1.84885	
8	-2.49444	2.39266	-0.03131	
8	-0.06729	2.67826	-0.42374	
8	1.92867	2.61401	-1.91544	
8	-3.33305	4.66764	1.31528	
8	2.93759	0.16264	-2.79708	
8	-2.32961	1.56706	-2.65006	
8	3.60425	4.33207	-0.53919	
1	3.03356	-2.46104	-1.70626	
1	1.34169	-2.90503	-2.92469	
1	0.01770	-3.65636	-2.00435	
1	-0.97486	-1.97952	-3.46640	
1	0.17001	-0.73976	-2.87609	
1	-2.94486	-1.79220	-2.48353	
1	2.09735	-1.02513	4.26817	
1	3.43045	0.13429	4.49038	
1	1.97133	0.57072	3.56538	
1	-2.45885	-1.13657	3.64142	
1	-4.03623	-1.81128	4.12588	
1	-2.73575	-2.85642	3.50346	
1	6.33230	-0.92759	1.26916	
1	6.40234	-2.06397	-0.70551	
1	5.01004	-2.81178	-1.46354	
1	-6.59513	-1.12882	0.52777	
1	-6.44953	-1.03115	-1.72824	
1	-5.15214	-1.94957	-2.49726	
1	4.49050	-0.52431	-2.14658	
1	-5.25346	0.16850	-3.32828	
1	2.86664	1.13859	-2.75741	
1	2.25436	-0.01309	-2.10878	
1	3.11459	3.94286	-1.28836	
1	3.29187	3.68545	0.12382	
1	-3.07352	0.95097	-2.61535	
1	-2.29255	1.89524	-1.72313	
1	-2.48897	4.39492	1.72721	
1	-3.35575	3.94563	0.66104	

Energy	-4512.443544	(Hartree)
E+zpe	-4511.942970	(Hartree)
Enthalpy	-4511.897827	(Hartree)
Free Energy	-4512.020597	(Hartree)

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