Electronic Supplementary Information (ESI) for:

Fluorine NMR Reporter for Phosphate Anions

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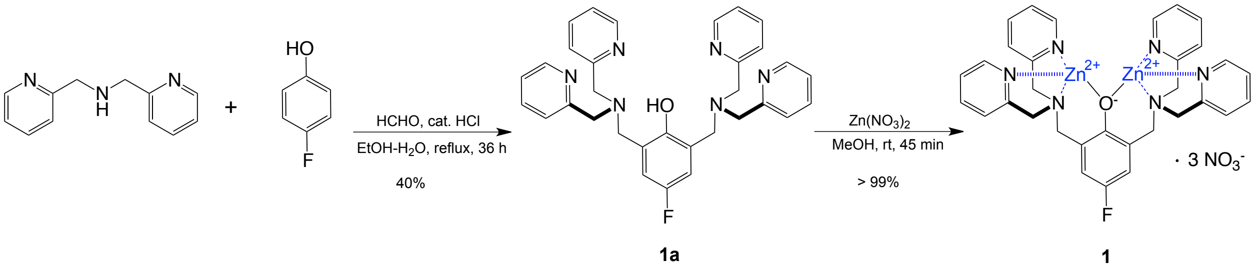
Synthesis



Scheme S1. Synthesis of **1**.

Formalin (37% in H_2O, 413 µL, 5.09 mmol) was added slowly to a solution of 4-fluorophenol (260 mg, 2.31 mmol), 2,2'-dipicolylamine (1.01 g, 5.07 mmol), and HCl (270 µL of 1M stock HCl solution) in EtOH:H_2O (2:1, 20 mL). The solution was refluxed for 36 h, cooled to room temperature and the ethanol removed under vacuum. The reaction mixture was washed with water/chloroform and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave an oily product that was purified by silica-gel column chromatography (98 : 1.9 : 0.1 $CHCl_3$: MeOH : $NH_3 \cdot H_2O$ mobile phase, v/v/v) to give the known compound **1a** [S1] (500 mg, 40% yield) as a pure yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 3.79 (s, 4H), 3.88 (s, 8H), 6.86 (d, *J* = 12.0 Hz, 2H), 7.14 (m, 4H), 7.47 (d, *J* = 12.0 Hz, 4H), 7.62 (m, 4H), 8.53 (m, 4H). ESI-MS: Found 535.2610, Calcd. $C_{32}H_{32}FN_6O$ 535.2616 $[M+H]^+$. A methanolic solution of compound **1a** (1.00 mmol) and zinc nitrate (2.10 mmol) was shaken for 45 min. The solvent was removed to afford reporter **1** in quantitative yield.[S1]

[S1] S. Torelli, C. Belle, I. Gautier-Luneau, S. Hamman, J. L. Pierre, *Inorg. Chim. Acta.*, **2002**, 333, 144-147.

X-ray Crystal Structure of 1$(H_2PO_4)(NO_3)_2 \cdot H_2O$

The complex crystallized as colorless block-like crystals from a water / KF / NaH_2PO_4 / $Zn(NO_3)_2$ solution. An arbitrary sphere of data were collected on a crystal having approximate dimensions of 0.207 × 0.164 × 0.120 mm, on a Bruker APEX-II diffractometer using a combination of ω- and φ-scans of 0.5°. Data were corrected for absorption and polarization effects and analyzed for space group determination. The structure was solved by Patterson methods and expanded routinely. The model was refined by full-matrix least-squares analysis of F^2 against all reflections. All non-hydrogen atoms were refined with anisotropic thermal displacement parameters. Unless otherwise noted, hydrogen atoms were included in calculated positions. Thermal parameters for the hydrogens were tied to the isotropic thermal parameter of the atom to which they are bonded (1.5 × for methyl, 1.2 × for all others).

There are four crystallographically independent complex molecules, eight nitrates and 10.5 waters of crystallization in the asymmetric unit of the primitive, acentric, monoclinic space group $P2_1$. Tests for higher symmetry revealed no symmetry other than the screw axis within the space group. Further analysis showed that the crystal was a racemic twin and was subsequently refined with a racemic twin law yielding a 0.471 twin component.

Though the four Zn cation species are crystallographically independent they consist of the same chemical species. Each cation consists of two zinc centers, one adopting an octahedral coordination environment, the other a five-coordinate trigonal pyramidal geometry. The octahedrally coordinated Zn is coordinated by the bridging phenoxide oxygen, two pyridyl nitrogens a tertiary amine nitrogen, one oxygen of the bridging H_2PO_4 and a water molecule. The second Zn lacks a coordinating water; the phosphate oxygen and amine nitrogen occupy the apical positions of the trigonal bipyramid with the pyridyl nitrogens and phenoxide oxygen occupying equatorial positions. The primary difference between the cations is the orientation of the ligands. In all cases the amine and pyridyl nitrogens adopt a facial coordination at the Zn centers (in both the six- and five-coordinate centers). What differs is the arrangement of the coordinating atoms and hence orientation of the ligands. Although the four complex molecules are apparently different in coordination geometry, they are inversion isomers. However, the space group in which the compound crystallizes ($P2_1$) is acentric; thus the racemic mixture crystallizes without the presence of a crystallographic inversion center and has racemic twinning inherent within the lattice.

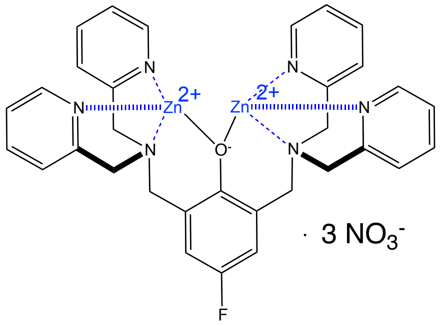
The data are of sufficiently high quality that hydrogen atoms on all but one full occupancy water and the half occupancy water could be located. The hydrogens on the dihydrogen phosphate groups were initially located from a difference Fourier map and were subsequently refined as a riding model. Water hydrogens were placed where located from a difference Fourier map and not refined further. The coordinated and free water, nitrates and dihydrogen phosphate groups all form an extended H-bonded network within the lattice. The H_2PO_4 moiety must be a dihydrogen phosphate for charge balance since there are two nitrates and one phenoxide present per pair of Zn centers. One nitrate exhibits positional disorder with about a 2/3 : 1/3 site occupancy for the two positions related by approximately 180°rotation.

The bond distances and angles within the molecules are as expected.

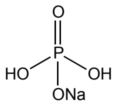
Table S1. Crystal data and structure refinement.

Empirical formula	$C_{32}H_{39.25}FN_8O_{14.62}PZn_2$	
Formula weight	950.67	
Temperature	120(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 20.2555(19) Å	α = 90°
	b = 19.4335(19) Å	β = 108.3970(10)°
	c = 20.627(2) Å	γ = 90°
Volume	7704.7(13) $Å^3$	
Z	8	
Density (calculated)	1.639 $g.cm^{-3}$	
Absorption coefficient (μ)	1.371 mm^{-1}	
F(000)	3906	
Crystal color, habit	colorless, block	
Crystal size	0.207 × 0.164 × 0.120 mm^3	
θ range for data collection	1.228 to 28.419°	
Index ranges	$-27 \le h \le 27$, $-26 \le k \le 25$, $-27 \le l \le 27$	
Reflections collected	211824	
Independent reflections	38661 [R_{int} = 0.0324]	
Completeness to θ = 25.242°	100.0 %	
Absorption correction	Numerical	
Max. and min. transmission	0.9197 and 0.8609	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	38661 / 1 / 2113	
Goodness-of-fit on F^2	1.015	
Final R indices [I>2σ(I)]	R_1 = 0.0295, wR_2 = 0.0660	
R indices (all data)	R_1 = 0.0361, wR_2 = 0.0687	
Absolute structure parameter	0.471(4)	
Extinction coefficient	n/a	
Largest diff. peak and hole	1.028 and -0.626 $e^{-}.Å^{-3}$	

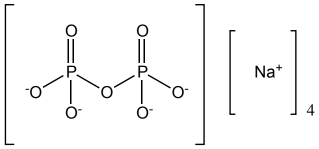
^{19}F NMR Titrations of Phosphate Anions with Reporter 1



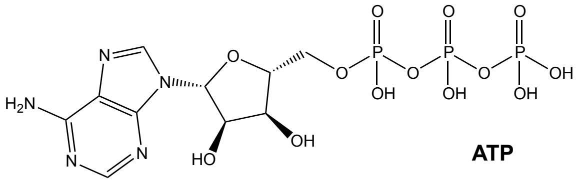
1



NaH_2PO_4 (NaPi)

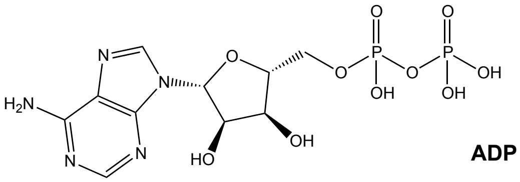


$Na_4O_7P_2$ (NaPPi)



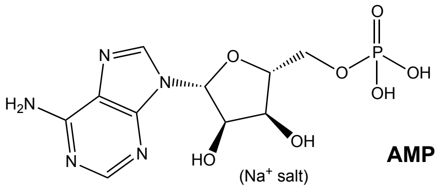
ATP

(Na$^+$ salt)



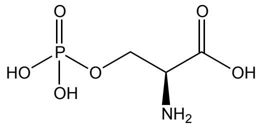
ADP

(Na$^+$ salt)



AMP

(Na$^+$ salt)



***o*-Phospho-L-Serine (P-Ser)**

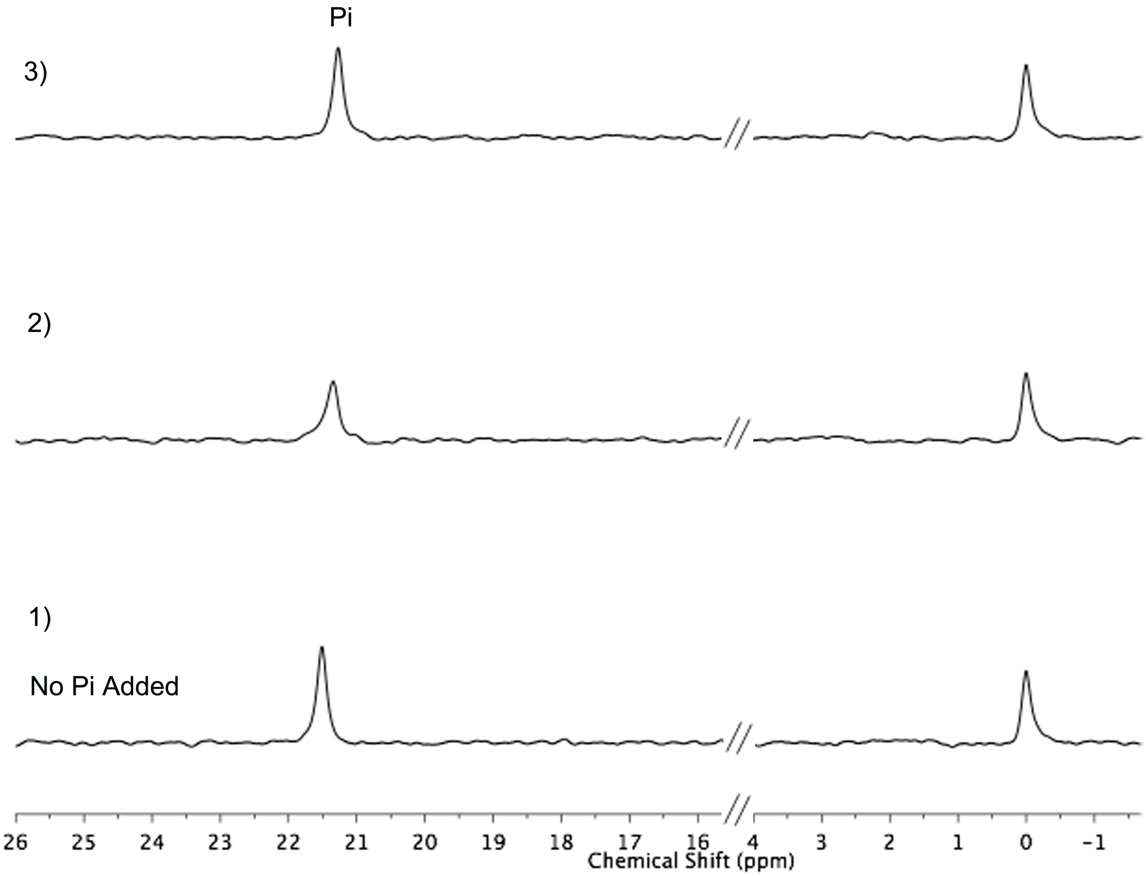


Figure S1. Partial ^{19}F NMR spectra (564 MHz) of reporter **1** (0.40 mM in HEPES buffer, pH 7.2, KBF_4 ref at 0 ppm) with (1) no anion added and in the presence of (2) 0.5 molar eq.; (3) 1.0 eq. NaPi.

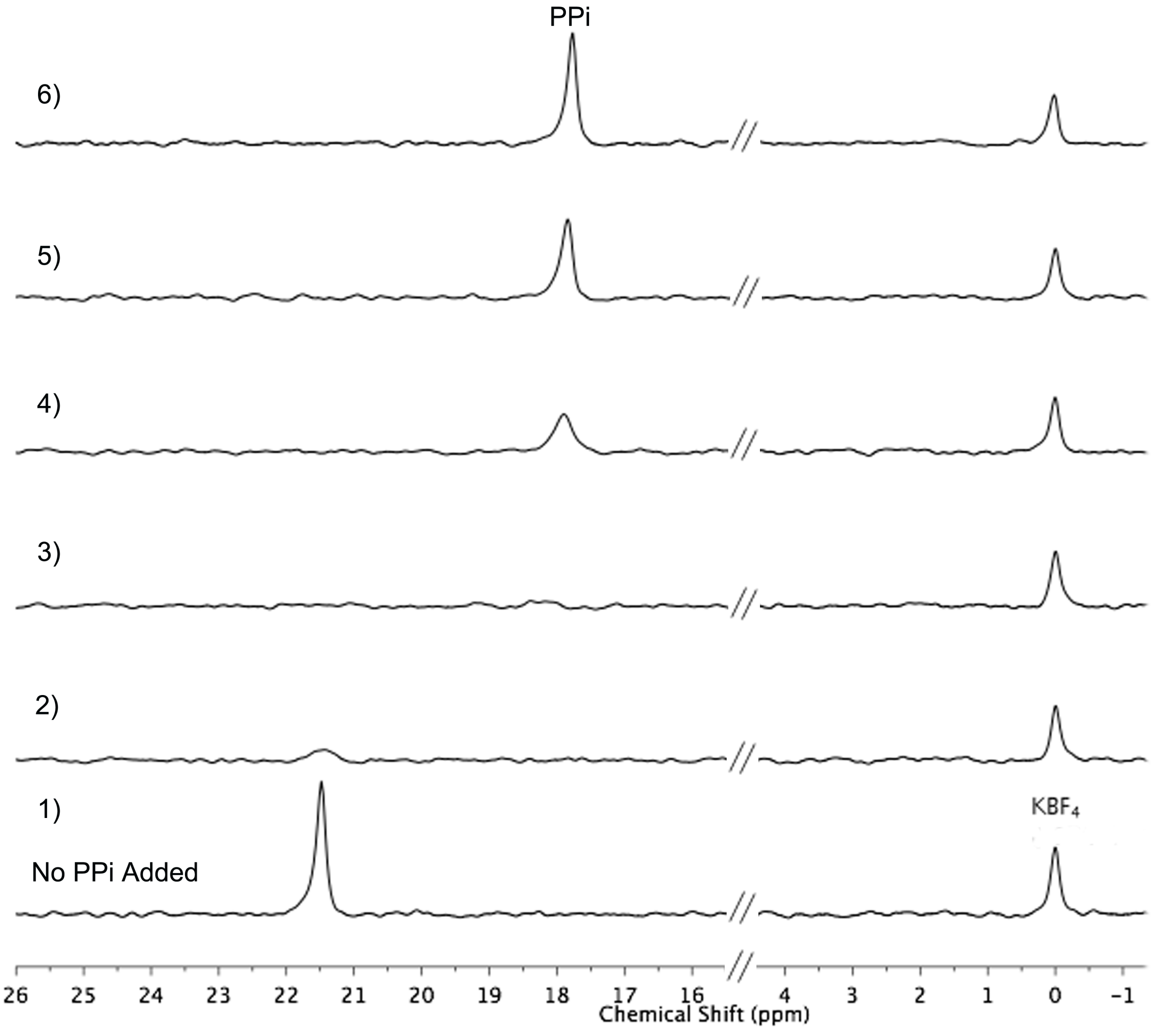


Figure S2. Partial ^{19}F NMR spectra (564 MHz) of reporter **1** (0.40 mM in HEPES buffer, pH 7.2) with (1) no anion added and in the presence of (2) 0.25 molar eq.; (3) 0.5 eq.; (4) 0.75 eq.; (5) 1.0 eq; (6) 1.5 eq. NaPPi.

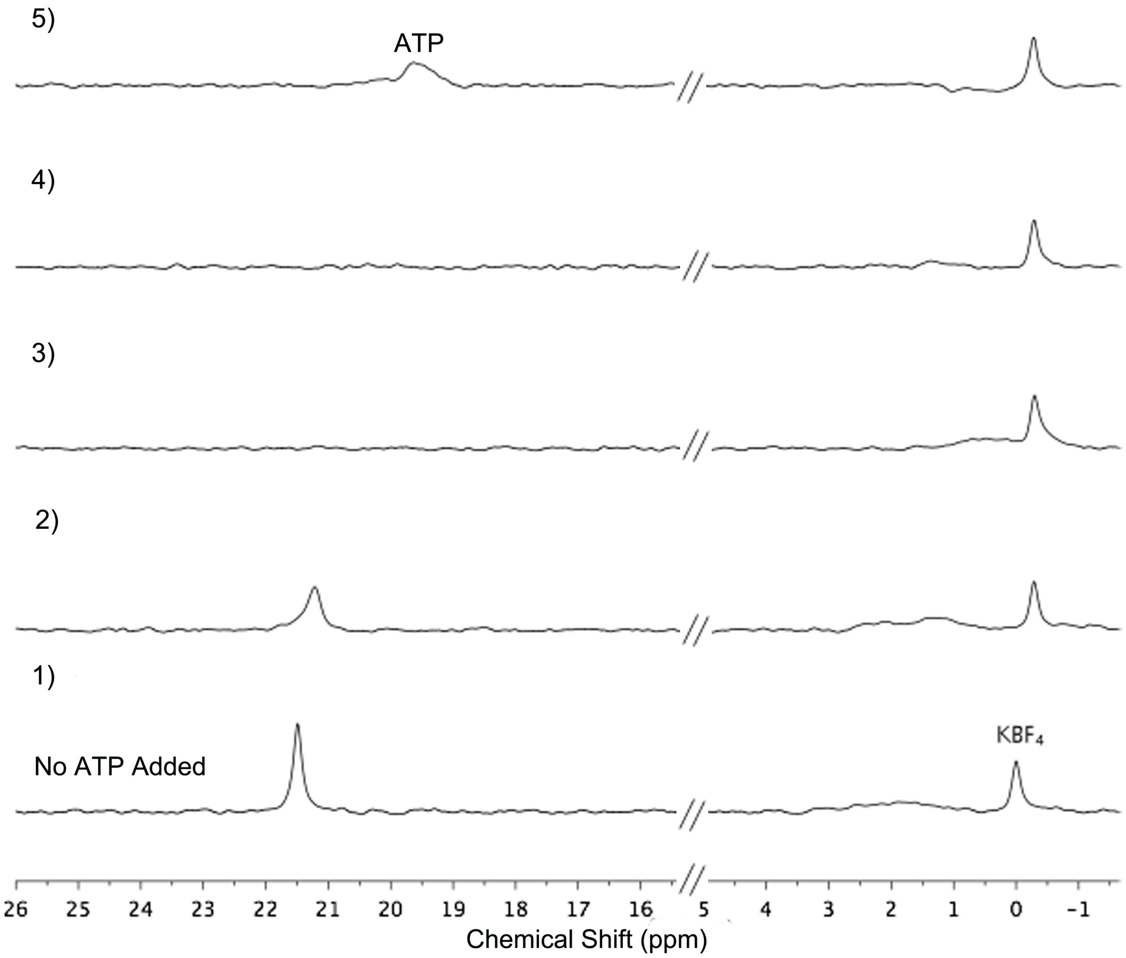


Figure S3. Partial ^{19}F NMR spectra (564 MHz) of reporter **1** (0.40 mM in HEPES buffer, pH 7.2) with (1) no anion added and in the presence of (2) 0.25 molar eq.; (3) 0.5 eq.; (4) 0.75 eq.; (5) 1.0 eq. ATP sodium salt.

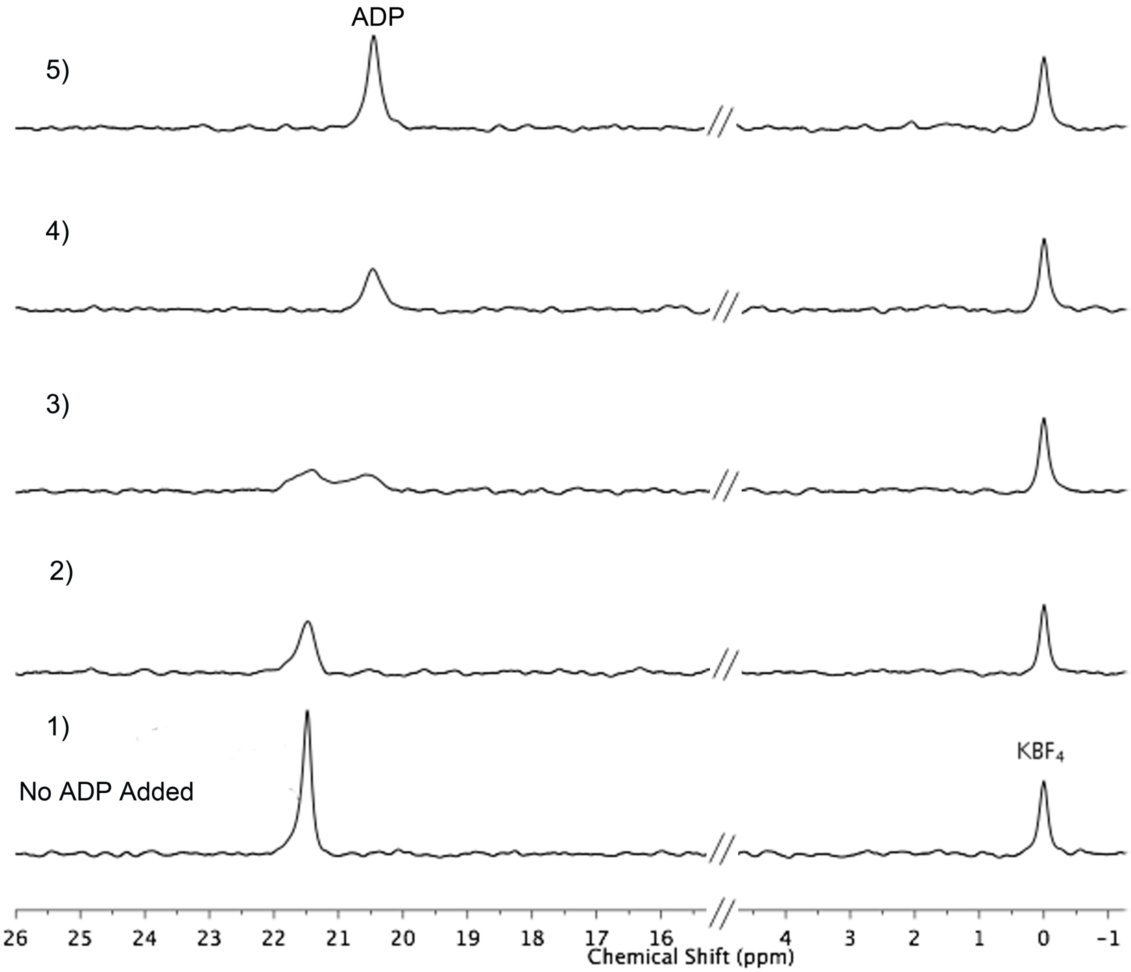


Figure S4. Partial ^{19}F NMR spectra (564 MHz) of reporter **1** (0.40 mM in HEPES buffer, pH 7.2) with (1) no anion added and in the presence of (2) 0.25 molar eq.; (3) 0.5 eq.; (4) 0.75 eq.; (5) 1.0 eq. ADP sodium salt.

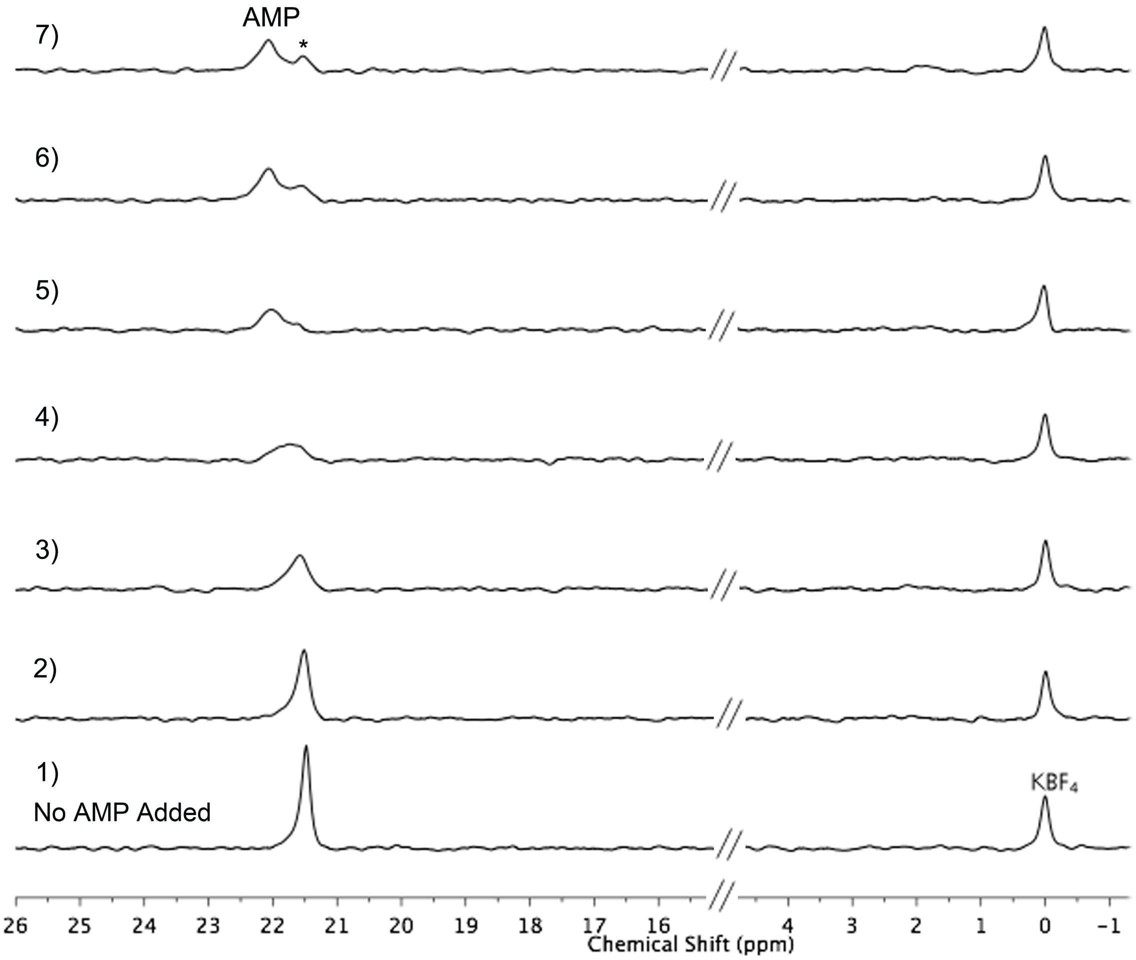


Figure S5. Partial ^{19}F NMR spectra (564 MHz) of reporter **1** (0.40 mM in HEPES buffer, pH 7.2) with (1) no anion added and in the presence of (2) 0.25 molar eq.; (3) 0.5 eq.; (4) 0.75 eq.; (5) 1.0 eq; (6) 1.5 eq.; (7) 2.0 eq. AMP sodium salt. * denotes free reporter.

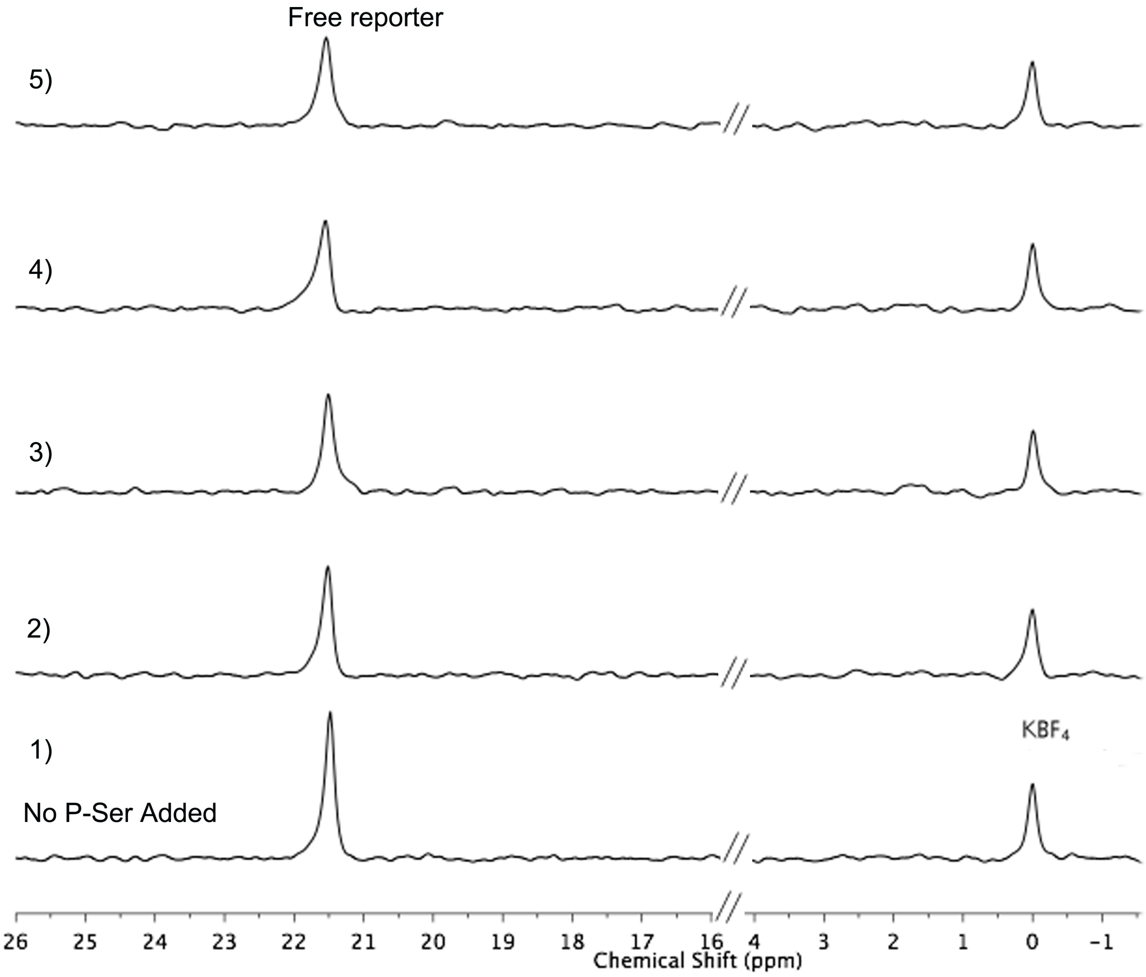


Figure S6. Partial ^{19}F NMR spectra (564 MHz) of reporter **1** (0.40 mM in HEPES buffer, pH 7.2) with (1) no anion added and in the presence of (2) 0.25 molar eq.; (3) 0.5 eq.; (4) 0.75 eq.; (5) 1.0 eq. P-Ser

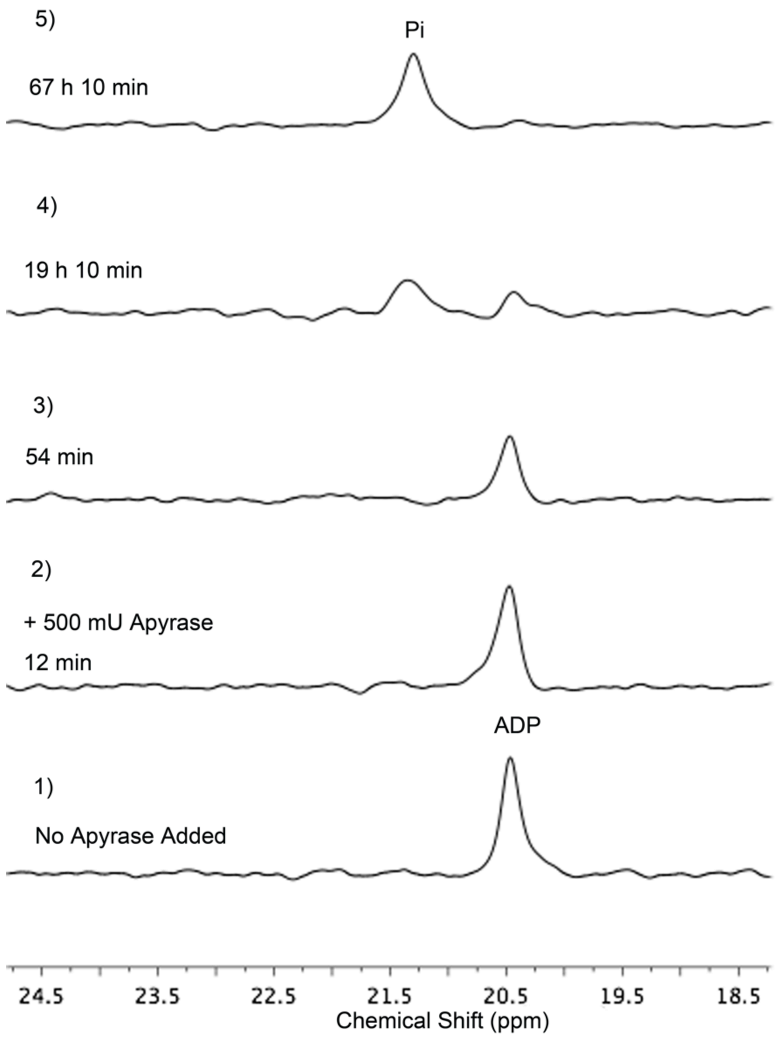


Fig S7. Partial ^{19}F NMR spectra (564 MHz) of a mixture of reporter **1** and ADP (both species 0.40 mM in HEPES buffer, pH 7.2) before addition of apyrase (*bottom spectrum*) and at increasing time points thereafter.