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Electronic Supplementary Information

for

Fluorescent HTS assay for phosphohydrolases based on nucleoside 5'-fluorophosphates: application in screening for inhibitors of mRNA Decapping Scavenger and PDE-I

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Table S1. General guidelines for assay development.

	Optimal substrate concentration	30–60 μM
	F ⁻ conc. at maximal response	10–15 μM
۲.	Accepted buffers	MES, HEPES, Tris-HCl
sitic		рН 6.5–8
iple comp	Accepted additives	Metal ions: Ca^{2+} , Zn^{2+} , Mn^{2+} (up to 10 mM) ^[a]
amp		Mg ²⁺ , up to 2 mM
		Stabilising protein (e.g. BSA), up to 0.75 mg/mL
		Acetonitrile; 5 mM EDTA (quenching reagents)
	Factors to avoid	pH above 8, Mg ²⁺ above 2 mM, acetate ions, strong acids
	Sample volume optimal for F ⁻ quantification	10–30 μL
probe	Stable probe solutions	9:1 DMSO/Tris-HCl pH 7.6 (v/v)
AS-FL	Unstable probe	Pure DMSO
TBDN	solutions	DMSO:deionised water (9:1)
Reaction with	Required DMSO content after mixing the sample with probe solution	at least 80%
		1

 $^{\rm [a]}A$ previous study showed that the assay is also compatible with Fe^+/Fe^+.1

	No Compound Structure		DcpS	PDE-I
No			%inhibition	%inhibition
1	АТР	о- о- о- о- о о о NH2 - о о N о N о N о N о N о N о N о N о N о	3.62 ± 3.25	32.38 ± 4.45
2	ATPF	О ⁻ О ⁻ О ⁻ О ⁻ NH ₂ F ⁻ ^P ₋ O ⁻ ^P ₋ O ⁻ N N O ⁻ O ⁻ O ⁻ O ⁻ N O ⁻ O ⁻	-0.12 ± 3.49	67.75 ± 2.63
3	ATPαS D1	0 ⁻ 0 ⁻ ş ⁻ NH₂ -0 ⁻ 0 ⁻ 9 ⁻ 0 ⁻ 0 ⁻ N 0 ⁻ 0 ⁻	-1.58 ± 7.88	91.86 ± 4.48
4	Ap ₃ A	$\begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} $	9.72 ± 3.05	61.38 ± 5.56
5	Ap₄A		-8.86 ± 11.47	55.43±3.06
6	Ap₅A	$\begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} $	14.59 ± 8.57	65.36 ± 2.93
7	Ap₃U	$\begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ $	-3.56 ± 3.91	43.29 ± 3.78
8	Ap₄U		-6.44 ± 8.44	53.45±3.11
9	Up₃U		-9.55 ± 4.44	48.84 ± 3.56

Table S2. Compound library used for the fluorescence screening assay against DcpS and PDE-I enzymes with the determined screening scores. All compounds were in the form of sodium (Na^+) or ammonium (NH_4^+) salts.

10	ApCH₂pp	о- о- о- о- -о- р- о- м м -о- р- с- р- о- м о- р- о- р- с- р- о- м он он он	-6.66 ± 6.87	1.09 ± 6.42
11	AppCH₂pU	$\begin{array}{c} \begin{array}{c} 0H \\ 0H \\ 0 \end{array} \\ \begin{array}{c} 0H \\ 0H \\ 0 \end{array} \\ \begin{array}{c} 0H \\ 0H \\ 0H \end{array} \\ \begin{array}{c} 0H \\ 0H \\ 0H \end{array} \\ \end{array} \\ \begin{array}{c} 0H \\ 0H \\ 0H \\ 0H \\ 0H \end{array} \\ \begin{array}{c} 0H \\ 0H $	3.43 ± 10.37	47.68 ± 3.94
12	ApCH₂ppU	ОНОН О О О О О О О О О О О О О	11.88±3.01	33.81±4.32
13	ApCH₂ppA	$(\mathbf{N}_{\mathbf{N}}) = (\mathbf{N}_{\mathbf{N}}) = (\mathbf{N}_{\mathbf{N}}$	0.46 ± 11.82	37.69 ± 4.11
14	ApCH₂pF	0 ⁻ 0 ⁻ NH2 F- ^P ₀ -C- ^P ₂ O N N H2O N N N N N N N N	5.58 ± 4.84	100.34 ± 1.66
15	ApCH₂ppF	0 ⁻ 0 ⁻ 0 ⁻ N F ⁻ ^P ₂ O ⁻ ^P ₂ C ⁻ ^P ₂ O O H ₂ O O HOH	-8.03 ± 9.54	17.97±5.34
16	АрNНр		8.36 ± 7.40	36.95 ± 4.58
17	AMP BH₃F D1		-11.40 ± 5.31	-7.74 ± 9.91
18	AMPBH₃F D2		7.01 ± 5.31	-2.28±7.28
19	Ар _{внз} рF D1		-8.71 ± 4.44	5.02 ± 6.20

20	Ар _{внз} рF D2		1.19 ± 8.00	19.71 ± 5.30
21	Ар _{внз} ррF D1	0 ⁻ 0 ⁻ ВН ³ № № F ⁻ ^P ₀ O ⁻ ^P ₀ O ⁻ ^P ₂ O ⁻ ^N ₀ O ⁻ ^N ₀ № № OHOH	6.77±8.91	21.60 ± 5.08
22	Ар _{внз} ррF D2	О ⁻ О ⁻ ВН ³ F ⁻ P ⁻ O ⁻ P ⁻ O ⁻ P ⁻ O ⁻ N ⁻ N ⁻ N ⁻ F ⁻ O	-1.74 ± 6.06	19.73 ± 5.22
23	Gp₃G	$H_2N \xrightarrow{N}_{HN} \xrightarrow{N}_{N} \xrightarrow{N}_{N} \xrightarrow{N}_{O} \xrightarrow{-P_{-}O_{-}P_{-}O_{-}P_{-}O_{-}P_{-}O_{-}N_{-}N_{-}N_{-}N_{-}N_{-}N_{-}N_{-}N$	-4.71 ± 3.48	46.35 ± 3.56
24	GpCH₂pp	0 ⁻ 0 ⁻ 0 ⁻ 0 ⁻ № № № № № № № № № № № № № № № № № № №	-2.99 ± 5.91	2.18 ± 6.25
25	GpCH₂p		-9.06 ± 9.78	16.83 ± 5.42
26	GpCH₂pF	$ \begin{array}{c} $	7.94 ± 9.08	93.69 ± 1.13
27	GpCH ₂ ppF	0 ⁻ 0 ⁻ 0 ⁻ 0 ⁻ № № № F ⁻ ^P ₂ O ⁻ ^P ₂ C ⁻ ^P ₂ O № № № № № № № № № № № № № № № № № № №	-4.78 ± 4.82	17.97 ± 5.26
28	GpNHp		1.52 ± 3.51	-0.34 ± 6.50
29	GpNHpF	О- О- F- ^H -N- ^H -О- О H О O H O O H OH	-2.63 ± 3.67	3.56 ± 6.20

30	GpNHppF	О- О- О- F-P-O-P-N-P-O N NH NH2 NH2 NH2	9.05 ± 4.26	-0.63 ± 6.44
31	GpSpF D1	о- ş- F-P-O-P-O OO-NNH2 OHOH	11.94 ± 10.95	85.37 ± 1.79
32	GpSpF D2	о- §- F 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0- 0	7.84 ± 6.44	17.15 ± 5.38
33	Gpp _{внз} pG	$H_{2}N$ N N N N N N N N N	0.15 ± 3.55	59.71 ± 3.15
34	Gp _{BH3} pF D1	о- ВН3 F-B-O-P-O N NH F-N-N-NH2 OHOH	-1.98±8.74	10.45 ± 5.84
35	Gр _{внз} рF D2		-10.47 ± 5.76	20.45 ± 5.13
36	Gр _{внз} ррF D1	о- о- вн ₃ F- ^P -о- ^P -о- ^P -о- ^N -о- ^N NH ₂ o 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	2.35 ± 9.43	18.01 ± 5.29
37	Gр _{внз} ррF D2	$\begin{array}{c} O^{-} & O^{-} & BH_{3}^{-} \\ F^{-} B^{-} O^{-} B^{-} O^{-} B^{-} O^{-} \\ O^{-} O^{-} B^{-} O^{-} O^{-} O^{-} \\ O^{-} O^$	11.04 ± 4.56	18.97 ± 6.58
38	m ⁷ G		3.15 ± 2.96	-2.71 ± 6.65

39	m ⁷ GMP		20.52 ± 3.40	-2.37±6.57
40	m ⁷ GDP	О ⁻ О ⁻ НО ⁻ Р-О ⁻ Р-О О О О О О О О О О О О О О О О О О О	70.47 ± 1.49	-4.15 ± 6.67
41	m ⁷ GDPF	О ⁻ О ⁻ F ⁻ ^P -O ⁻ ^P -O ⁻ Ö O ⁻ ^N -N ⁺ N ⁺ N ⁺ N ⁺ N ⁺ N ⁺ N ⁺ N ⁺	27.56 ± 10.20	15.66 ± 5.46
42	m ⁷ GpCH₂p		20.16 ± 5.14	-4.40±9.11
43	m ⁷ GpCH₂pp	О ⁻ О ⁻ О ⁻ О ⁻ HO ⁻ ^P ·O ⁻ O ⁻ O ⁻ N ⁺ N ⁺ O ⁻ O ⁻ O ⁻ N ⁺ N ⁺ N ⁺ HO ⁻ O ⁻ O ⁻ O ⁻ O ⁻ N ⁺ N ⁺ N ⁺ N ⁺ O ⁻ O ⁻ O ⁻ O ⁻ O ⁻ O ⁻ N ⁺	20.74 ± 3.22	-1.69 ± 6.55
44	m ⁷ GpNHp	$\begin{array}{c} O^{-} O^{-} \\ HO^{-} P^{-} N^{-} P^{-} O \\ O \\ H \\ O \\ O \\ H \\ O \\ O \\ H \\ O \\ O$	32.11±6.04	-3.69 ± 6.67
45	m ⁷ GpNHpF	О ⁻ О ⁻ F ⁻ ^P N ⁻ ^P O Ö H Ö O H OH	3.33 ± 4.27	-6.97 ± 6.88
46	m ⁷ GDPαS D1	0 ⁻ §- HO ⁻ P-O-P-O- O O O O O O O O O O O O O O O O O O	53.91 ± 2.53	-1.95 ± 6.56
47	m ⁷ GDPαS D2	0 ⁻ S ⁻ HO ⁻ ^P O ⁻ Ö ⁻ N N N N N N N N N N N N N N N N N N N	35.52 ± 3.45	6.38 ± 6.02

48	m ⁷ GDPαBH3 D1	$\begin{array}{c} O^{-} & BH_{3}^{-} \\ HO^{-} & O^{-} & O^{-} \\ O & O$	43.15 ± 5.79	7.82 ± 6.43
49	m ⁷ GDPαBH3 D2		67.69 ± 1.80	-1.64±6.57
50	m ^{7,2′-0} GpCH₂p	$\begin{array}{c} 0^{-} & 0^{-} \\ HO^{-} \overset{P}{_{u}} C^{-} \overset{P}{_{u}} O \\ O & H_{2}O \end{array} \xrightarrow{\begin{array}{c} 0 \\ 0 \\ 0 \end{array}} \overset{N}{_{u}} \overset{N}{_{u}} \overset{N}{_{u}} \overset{N}{_{u}} N_{H_{2}} \\ \end{array}$	6.75 ± 3.18	0.61 ± 6.51
51	m²Gp₃G	$H_2N \xrightarrow{N}_{O^-} N \xrightarrow{N}_{O^-} O^- O^- O^- O^- O^- O^- O^- O^- O^- O^-$	30.18 ± 11.84	34.48±4.35
52	bn ⁷ Gp₃G	$H_2N \xrightarrow{N}_{O^-} N \xrightarrow{N}_{O^-} O^- O^- O^- O^- N \xrightarrow{N}_{N+N+2} NH$	8.90 ± 2.97	25.48±4.87
53	m ⁷ GpCH₂ppG	$H_2N \xrightarrow{N}_{O^-} N \xrightarrow{N_1}_{O^-} O^- O^- O^- O^- O^- O^- O^- O^- O^- O^-$	81.61 ± 5.52	26.71±5.93
54	m ⁷ GpCH₂pppG	$H_{2}N \xrightarrow{N} \stackrel{N}{\underset{O^{-}}{\overset{N}}} \stackrel{O^{+} O^{+}}{\underset{O^{-}}{\overset{O^{-} O^{-} O^{-} O^{-}}{\underset{O^{+} O^{-} O^{-} O^{-} O^{-}}{\overset{O^{-} O^{-} O^{-} O^{-}}{\underset{O^{+} O^{-} O^{-} O^{-} O^{-} O^{-}} \stackrel{N}{\underset{O^{+} O^{+} O^{-} O^{-} O^{-}}{\overset{O^{+} O^{+} O^{-} O^{-} O^{-} O^{-} O^{-} O^{-}} \stackrel{N}{\underset{O^{+} O^{+} O^{+} O^{-} O^{-} O^{-} O^{-}}{\overset{O^{+} O^{+} O^{-} O^{-$	70.63 ± 6.48	13.60 ± 5.58
55	m ^{7,2'-} ^o GpCH ₂ pppG	$H_{2}N \xrightarrow{N} N^{+} \underbrace{N_{1}}_{O^{-}} N$	-2.11±6.07	20.54 ± 7.86
56	m ^{7,2′-} ⁰ GppCH₂ppG	$H_{2}N \xrightarrow{N}_{N} \xrightarrow{N}_{0^{-}}^{N} \xrightarrow{O}_{0^{-}}^{P} \xrightarrow{O}_{0^{-}}^{P} \xrightarrow{O}_{0^{-}}^{P} \xrightarrow{O}_{0^{-}}^{P} \xrightarrow{O}_{0^{-}}^{P} \xrightarrow{O}_{0^{-}}^{P} \xrightarrow{O}_{0^{-}}^{N} \xrightarrow{O}_{0^{+}}^{N} \xrightarrow{N}_{N} \xrightarrow{N}_{N} \xrightarrow{N}_{N} \xrightarrow{N}_{N}$	8.60 ± 6.44	14.61 ± 5.56

57	m ⁷ GpNHppG	$H_{2}N \xrightarrow{N}_{O^{-}} N^{+}_{O^{-}} O^{-} O^{-}_{O^{-}} O^{-}_{O^{-}} O^{-}_{O^{-}} N^{+}_{N} O^{-}_{O^{-}} O^{-}_$	81.37 ± 1.42	12.21 ± 5.74
58	m ⁷ GpNHpppG	$H_{2}N \xrightarrow{N}_{O^{-}} N^{+}_{O^{-}} O^{-} O^{-}_{O^{-}} O^$	$\textbf{71.83} \pm \textbf{3.06}$	15.21 ± 5.64
59	m ^{7,2'-0} GpNHppG	$H_{2}N \xrightarrow{N}_{N} \xrightarrow{N}_{O}^{+} \xrightarrow{N}_{O}^{+} \xrightarrow{O}_{O}^{+} \xrightarrow{O}_{O}^{-} \xrightarrow{O}_{O}^{-} \xrightarrow{N}_{O}^{-} \xrightarrow{N}_{O}^{-} \xrightarrow{N}_{O}^{-} \xrightarrow{N}_{O}^{-} \xrightarrow{N}_{O}^{+} $	15.48±3.44	11.56 ± 5.80
60	m ^{7,2'-0} Gp _s pppG D1	$H_2N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}{\overset{N}}}} N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}{\overset{N}}} N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}{\overset{N}}}} N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}{\overset{N}}} N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}}} N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}{\overset{N}}} N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}}} N \xrightarrow{N}_{N} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{\underset{O^-}{\overset{N}} \stackrel{N}{\underset{O^-}{O^$	17.91 ± 3.00	37.84 ± 5.22
61	m ^{7,2′-0} Gp₅pppG D2	$H_{2N} \xrightarrow{N}_{O^{-}} N^{+}_{O^{-}} \xrightarrow{O^{-}}_{O^{-}} O^{-}_{O^{-}} \xrightarrow{O^{-}}_{O^{-}} O^{-}_{O^{-}} \xrightarrow{N}_{O^{+}} NH_{2}} O^{-}_{O^{-}} \xrightarrow{N}_{O^{-}} O^{-}_{O^{-}} \xrightarrow{N}_{O^{-}} O^{-}_{O^{-}} \xrightarrow{N}_{O^{-}} O^{-}_{O^{-}} \xrightarrow{N}_{O^{+}} O^{-}_{O^{+}} O^{-}_{O^{+}} \xrightarrow{N}_{O^{+}} O^{-}_{O^{+}} \xrightarrow{N}_{O^{$	19.76 ± 6.15	36.66 ± 4.19
62	m ⁷ Gp _{внз} ррG D2	$\begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ $	65.65 ± 9.75	38.84 ± 4.09
63	m ⁷ Gpp _{внз} pG D1	$H_{2}N \xrightarrow{N}_{O^{-}} N \xrightarrow{N}_{O^{-}} O^{+} O^{-} \xrightarrow{B}_{O^{-}} O^{-} \xrightarrow{N}_{O^{-}} O^{+} O^$	95.12 ± 1.07	39.12 ± 4.03
64	m ⁷ Gpp _{внз} pG D2	$H_{2}N \xrightarrow{N}_{O^{-}} N \xrightarrow{N}_{O^{-}} O^{-} \xrightarrow{B}H_{3}^{-} O^{-} \xrightarrow{N}_{O^{-}} NH_{N}H_{2}$	86.14±2.34	43.79±3.84
65	m ^{7,2'-0} Gpp _{BH3} pG D2	$H_{2}N \xrightarrow{N}_{O} N \xrightarrow{N}_{O} N \xrightarrow{V}_{O} O \xrightarrow{P}_{O} O \xrightarrow{P}_{O} O \xrightarrow{P}_{O} O \xrightarrow{P}_{O} O \xrightarrow{V}_{O} O \xrightarrow{N}_{O} O $	69.29 ± 1.53	42.99 ± 3.81

66	m ⁷ Gpp _{внз} pm ⁷ G	$H_{2}N \xrightarrow{N}_{O} N \xrightarrow{N}_{O} N \xrightarrow{V}_{O} N $	40.40 ± 2.47	13.74 ± 5.57
67	m ⁷ GpCH₂ppA- N6-HDA	$H_{2N} \xrightarrow{N}_{N \to N} \xrightarrow{N}_{O^{-}} \xrightarrow{O^{-}}_{O^{-}} \xrightarrow{O^{-}}_{O^{-}} \xrightarrow{N}_{O^{-}} \xrightarrow$	9.19 ± 3.25	$\textbf{-3.67}\pm6.69$
68	Thiamine PP	NH2 N OH OH N S O-P-OH O OH O OH	-3.78 ± 4.56	-5.12 ± 6.75
69	NAD⁺	$O^{-} \qquad NH_{2}$	-6.71 ± 3.47	9.01 ± 7.71
70	Folic acid	$H_{2}N$	1.33 ± 8.51	74.39 ± 6.09
71	UDP-1-Glc		-1.07 ± 9.57	19.36 ± 5.29
72	UDP-6-Glc		1.61 ± 5.90	44.60 ± 3.71
73	ADP-1-Glc		10.44 ± 3.95	46.25 ± 5.76

74	ADP-6-Glc	$\begin{array}{c} H \\ O \\ O \\ H \\ O \\ H \\ O \\ O \\ O \\ O \\$	-2.19 ± 3.40	45.42 ± 3.66
75	GDP-1-Glc	$H_{HO}^{O} \xrightarrow{O}_{HO}^{O} \xrightarrow{O}_{O}^{-} \xrightarrow{O}_{O}^{-} \xrightarrow{N}_{O} \xrightarrow{N}_{NH}_{NH_{2}}$	6.22 ± 12.65	37.25±4.11
76	GDP-6-Glc	$\begin{array}{c} 0H \\ 0H \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0$	-12.85 ± 7.65	35.21±4.30

Table S3.	Determined IC ₅₀	parameters f	for the selected	DcpS and PDE-	I inhibitors.
	200000000000000000000000000000000000000	parameters.			

Compound	IC₅₀ DcpS [µM]	Compound	IC ₅₀ PDE-I [μM]
·			
39	97 ± 21	3	4.58 ± 0.76
40	5.2 ± 1.2	14	1.21 ± 0.26
49	6.3±1.6	16	35 ± 8
53	4.0 ± 1.1	25	85 ± 23
57	3.22 ± 0.92	26	1.82 ± 0.24
63	1.02 ± 0.23	31	7.1 ± 1.8
64	1.44 ± 0.26	33	10.9 ± 3.5
65	4.36 ± 0.89	70	$\textbf{6.39} \pm \textbf{0.92}$
66	15.2 ± 2.8		
m ⁷ Gp _s ppG D1	3.35 ± 0.67		
m ⁷ Gp _s ppG D2	2.39 ± 0.40		
RG3039	0.048 ± 0.010		

Tested compound		%inhibition	
Compound number	Abbreviation	HPLC –based assay	Fluorescence assay
39	m ⁷ GMP	0.43 ± 12.93	20.52 ± 3.40
40	m ⁷ GDP	45.55 ± 8.92	70.47 ± 1.49
49	m ⁷ GDPαBH ₃ D2	37.29 ± 7.21	67.69 ± 1.80
53	m ⁷ GpCH₂ppG	44.09 ± 3.96	81.61 ± 5.52
57	m ⁷ GpNHppG	50.23 ± 0.45	81.37 ± 1.42
63	m ⁷ Gpp _{внз} pG D1	79.17 ± 1.25	95.12 ± 1.07
64	m ⁷ Gpp _{внз} pG D2	68.98 ± 2.29	86.14±2.34
65	m ^{7,2'-0} Gpp _{вн3} pG D2	40.47 ± 5.32	69.29 ± 1.53
66	m ⁷ Gpp _{вн3} pm ⁷ G	25.48 ± 3.78	40.40 ± 2.47

 Table S4. Comparison of HPLC and fluorescence DcpS screening results for selected compounds.

2. Supplementary figures



Figure S1. Synthesis of fluorophosphate nucleotide analogs from commercially available starting materials. **A**. Synthesis of 7-methyl guanosine 5'-fluoromonophosphate. **B**. Synthesis of adenosine 5'-fluoromonophosphate. i) 1. imidazole, 2'2-dithiodipyridine, Ph₃P, Et₃N, DMF; 2. NaClO₄, acetone; ii) TBAF, DMSO; iii) CH₃I, DMSO.



Figure S2. The synthesis of fluorogenic probe according to ref.² Conditions: i) imidazole, DMF.



Figure S3. The calibration curves from hDcpS (A) and PDE-I (B) assays under optimized conditions.



Figure S4. Influence of pH on calibration curve (left) and max. signal (30 µM) to background (0 µM) ratio (right). Each curve was repeated in triplicates.



Figure S5. Influence of various concentrations of magnesium ions (in Tris-HCl pH=7.5) on the calibration curve (left) and max. signal (30 μ M) to background (0 μ M) ratio (right). Each curve was repeated in triplicates.



Figure S6. Influence of manganese ions (in Tris-HCl pH=7.5) on the calibration curve (left) and max. signal (30 μ M) to background (0 μ M) ratio (right). Each curve was repeated in triplicates.



Figure S7. A. m⁷GMPF cleavage to m⁷GMP and fluoride catalyzed by hDcpS enzyme. **B.** Example HPLC profiles from the enzymatic reaction. **C.** Michaelis-Menten plot showing the kinetics of m⁷GMPF cleavage by hDcpS enzyme at 20 °C.

Supplementary references

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2. Yang, X.-F.; Ye, S.-J.; Bai, Q.; Wang, X.-Q., A fluorescein-based fluorogenic probe for fluoride ion based on the fluoride-induced cleavage of tert-butyldimethylsilyl ether. *Journal of Fluorescence* **2007**, *17* (1), 81-87.