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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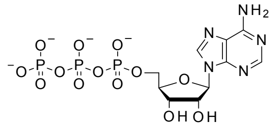
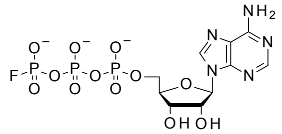
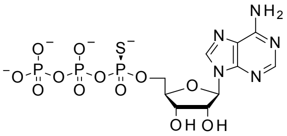
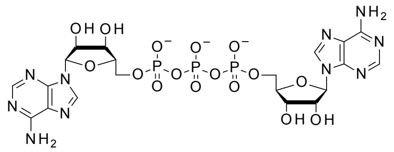
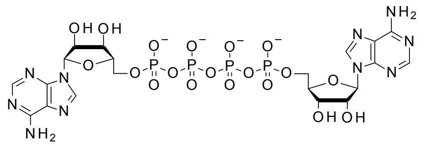
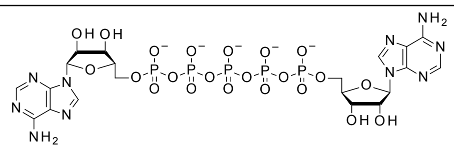
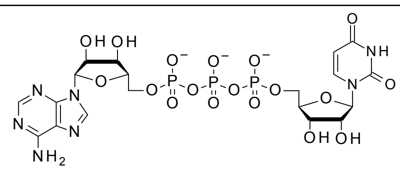
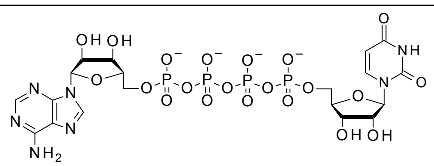
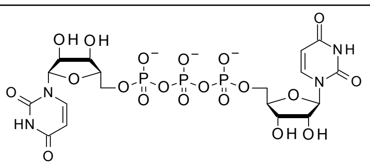
1. Supplementary tables

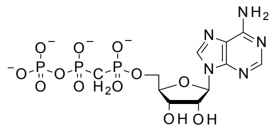
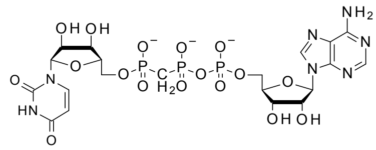
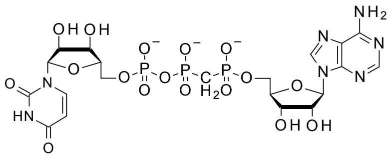
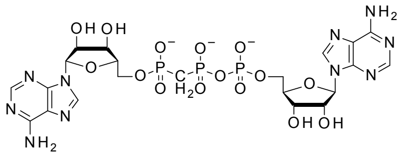
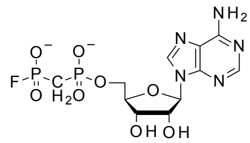
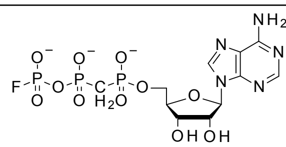
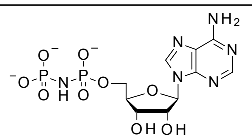
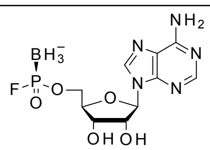
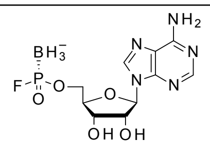
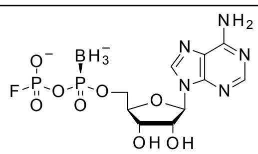
Table S1. General guidelines for assay development.

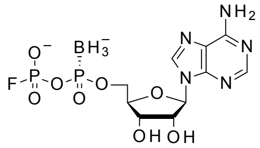
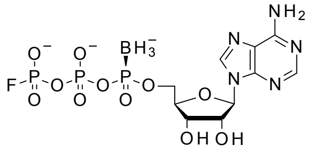
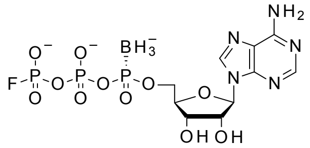
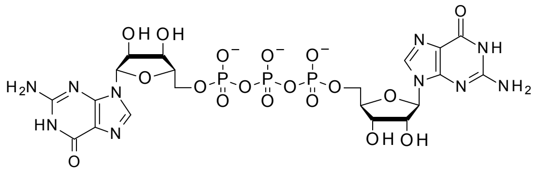
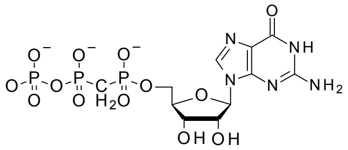
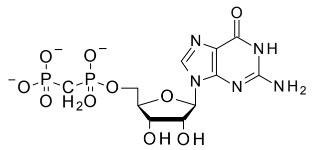
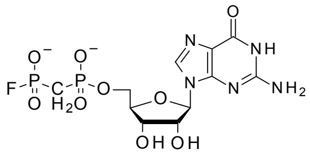
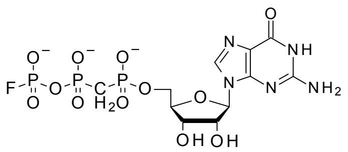
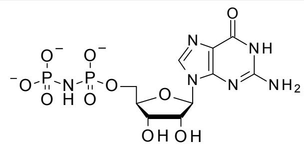
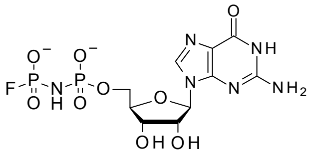
Sample composition	Optimal substrate concentration	30–60 µM
	F^- conc. at maximal response	10–15 µM
	Accepted buffers	MES, HEPES, Tris-HCl pH 6.5–8
	Accepted additives	Metal ions: Ca^{2+}, Zn^{2+}, Mn^{2+} (up to 10 mM)[a] Mg^{2+}, up to 2 mM Stabilising protein (e.g. BSA), up to 0.75 mg/mL Acetonitrile;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

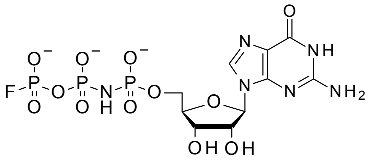
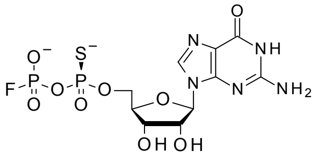
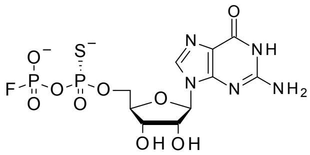
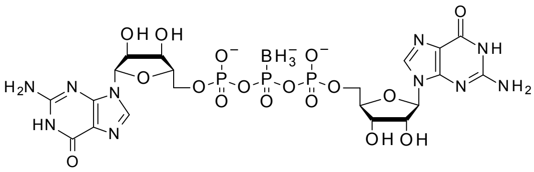
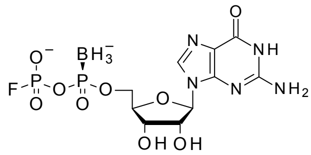
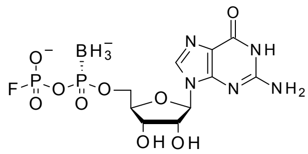
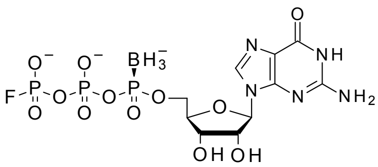
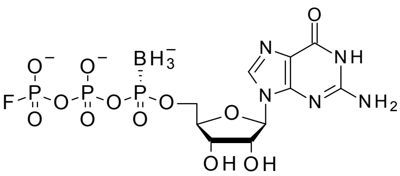
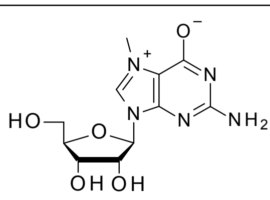
[a]A previous study showed that the assay is also compatible with Fe^{2+}/Fe^{3+}.[1]

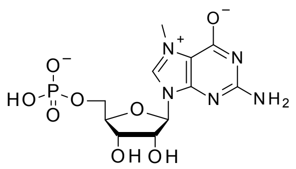
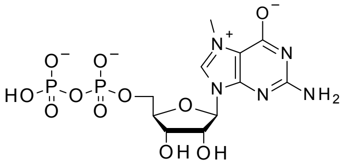
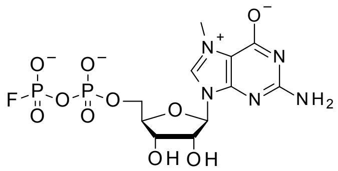
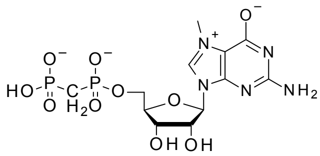
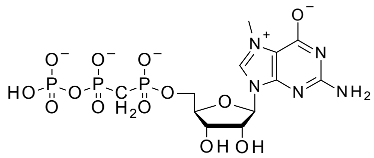
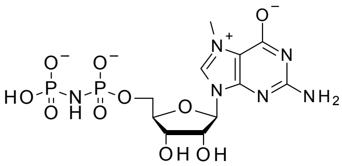
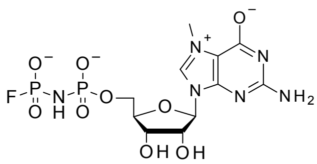
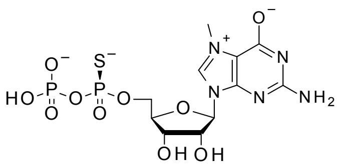
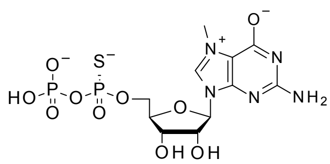
Table S2. Compound library used for the fluorescence screening assay against DcpS and PDE-I enzymes with the determined screening scores. All compounds were in the form of sodium (Na^+) or ammonium (NH_4^+) salts.

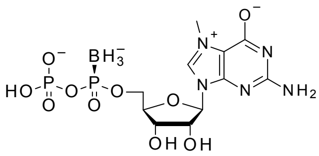
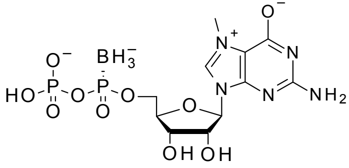
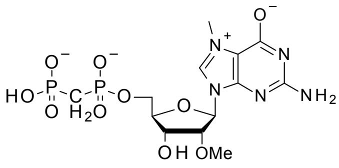
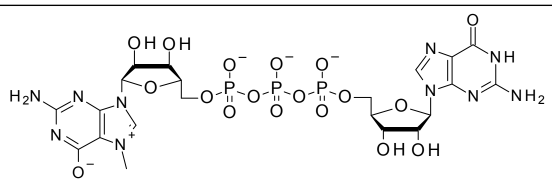
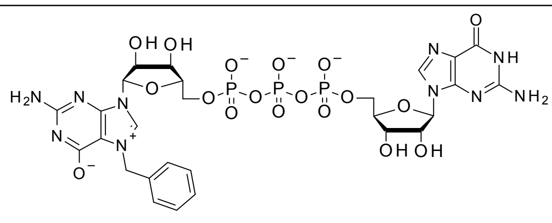
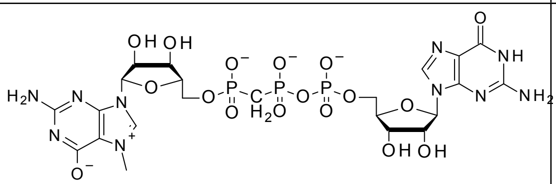
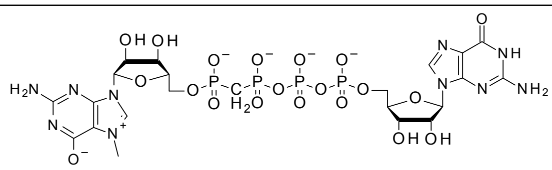
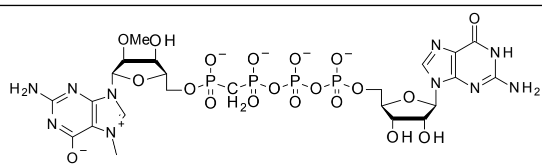
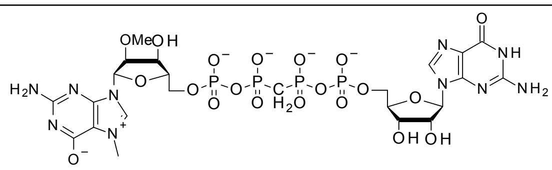
No	Compound	Structure	DcpS $\%_{inhibition}$	PDE-I $\%_{inhibition}$
1	ATP		3.62 ± 3.25	32.38 ± 4.45
2	ATPF		-0.12 ± 3.49	67.75 ± 2.63
3	ATPαS D1		-1.58 ± 7.88	91.86 ± 4.48
4	Ap$_3$A		9.72 ± 3.05	61.38 ± 5.56
5	Ap$_4$A		-8.86 ± 11.47	55.43 ± 3.06
6	Ap$_5$A		14.59 ± 8.57	65.36 ± 2.93
7	Ap$_3$U		-3.56 ± 3.91	43.29 ± 3.78
8	Ap$_4$U		-6.44 ± 8.44	53.45 ± 3.11
9	Up$_3$U		-9.55 ± 4.44	48.84 ± 3.56

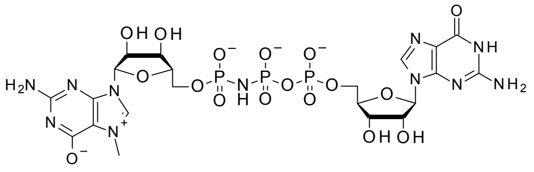
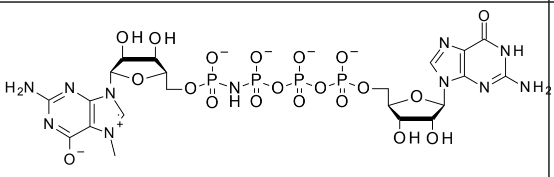
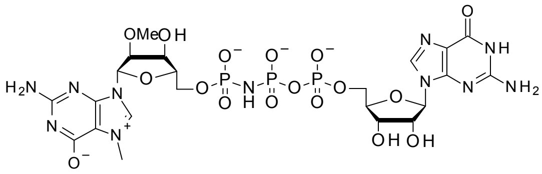
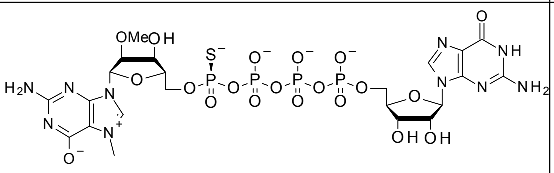
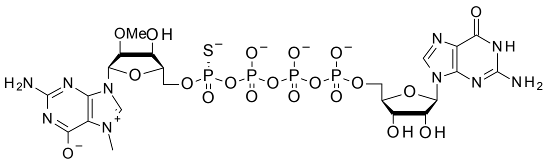
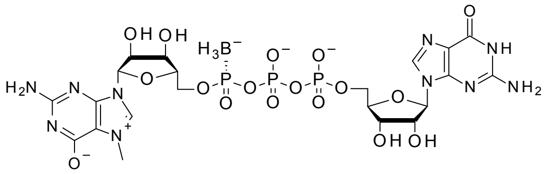
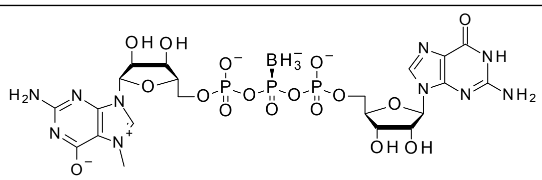
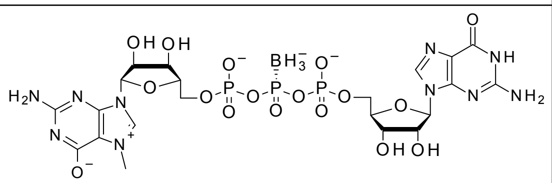
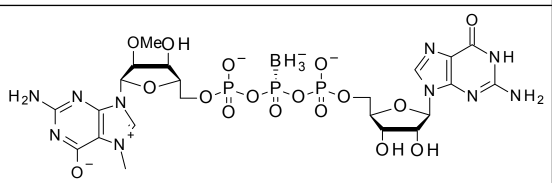
10	ApCH_2pp		-6.66 ± 6.87	1.09 ± 6.42
11	AppCH_2pU		3.43 ± 10.37	47.68 ± 3.94
12	ApCH_2ppU		11.88 ± 3.01	33.81 ± 4.32
13	ApCH_2ppA		0.46 ± 11.82	37.69 ± 4.11
14	ApCH_2pF		5.58 ± 4.84	100.34 ± 1.66
15	ApCH_2ppF		-8.03 ± 9.54	17.97 ± 5.34
16	ApNHp		8.36 ± 7.40	36.95 ± 4.58
17	AMP BH_3F D1		-11.40 ± 5.31	-7.74 ± 9.91
18	AMPBH_3F D2		7.01 ± 5.31	-2.28 ± 7.28
19	Ap$_{BH3}$pF D1		-8.71 ± 4.44	5.02 ± 6.20

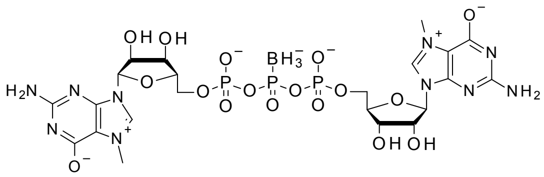
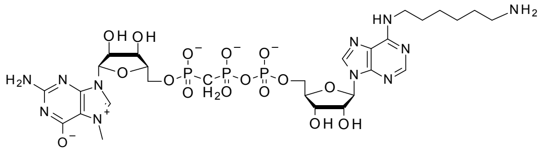
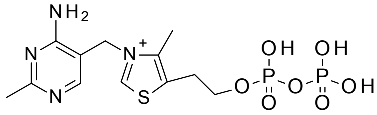
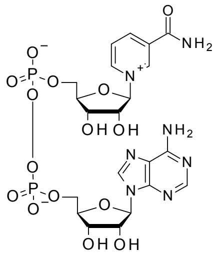
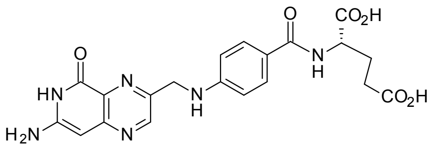
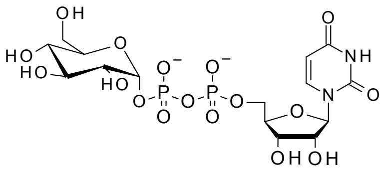
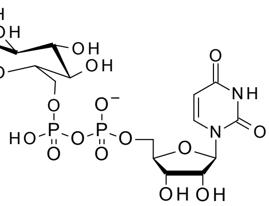
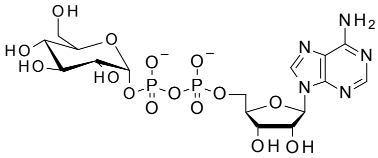
20	$\mathrm{Ap_{BH3}pF}$ D2		1.19 ± 8.00	19.71 ± 5.30
21	$\mathrm{Ap_{BH3}ppF}$ D1		6.77 ± 8.91	21.60 ± 5.08
22	$\mathrm{Ap_{BH3}ppF}$ D2		-1.74 ± 6.06	19.73 ± 5.22
23	$\mathrm{Gp_3G}$		-4.71 ± 3.48	46.35 ± 3.56
24	$\mathrm{GpCH_2pp}$		-2.99 ± 5.91	2.18 ± 6.25
25	$\mathrm{GpCH_2p}$		-9.06 ± 9.78	16.83 ± 5.42
26	$\mathrm{GpCH_2pF}$		7.94 ± 9.08	93.69 ± 1.13
27	$\mathrm{GpCH_2ppF}$		-4.78 ± 4.82	17.97 ± 5.26
28	GpNHp		1.52 ± 3.51	-0.34 ± 6.50
29	GpNHpF		-2.63 ± 3.67	3.56 ± 6.20

30	GpNHppF		9.05 ± 4.26	-0.63 ± 6.44
31	GpSpF D1		11.94 ± 10.95	85.37 ± 1.79
32	GpSpF D2		7.84 ± 6.44	17.15 ± 5.38
33	Gpp$_{BH3}$pG		0.15 ± 3.55	59.71 ± 3.15
34	Gp$_{BH3}$pF D1		-1.98 ± 8.74	10.45 ± 5.84
35	Gp$_{BH3}$pF D2		-10.47 ± 5.76	20.45 ± 5.13
36	Gp$_{BH3}$ppF D1		2.35 ± 9.43	18.01 ± 5.29
37	Gp$_{BH3}$ppF D2		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		70.47 ± 1.49	-4.15 ± 6.67
41	m^{7}GDPF		27.56 ± 10.20	15.66 ± 5.46
42	m^{7}GpCH$_2$p		20.16 ± 5.14	-4.40 ± 9.11
43	m^{7}GpCH$_2$pp		20.74 ± 3.22	-1.69 ± 6.55
44	m^{7}GpNHp		32.11 ± 6.04	-3.69 ± 6.67
45	m^{7}GpNHpF		3.33 ± 4.27	-6.97 ± 6.88
46	m^{7}GDPαS D1		53.91 ± 2.53	-1.95 ± 6.56
47	m^{7}GDPαS D2		35.52 ± 3.45	6.38 ± 6.02

48	m^7GDPαBH3 D1		43.15 ± 5.79	7.82 ± 6.43
49	m^7GDPαBH3 D2		67.69 ± 1.80	-1.64 ± 6.57
50	$m^{7,2'\text{-}O}$GpCH_2p		6.75 ± 3.18	0.61 ± 6.51
51	m^7Gp_3G		30.18 ± 11.84	34.48 ± 4.35
52	bn^7Gp_3G		8.90 ± 2.97	25.48 ± 4.87
53	m^7GpCH_2ppG		81.61 ± 5.52	26.71 ± 5.93
54	m^7GpCH_2pppG		70.63 ± 6.48	13.60 ± 5.58
55	$m^{7,2'\text{-}O}$GpCH_2pppG		-2.11 ± 6.07	20.54 ± 7.86
56	$m^{7,2'\text{-}O}$GppCH_2ppG		8.60 ± 6.44	14.61 ± 5.56

57	m^{7}GpNHppG		81.37 ± 1.42	12.21 ± 5.74
58	m^{7}GpNHpppG		71.83 ± 3.06	15.21 ± 5.64
59	m$^{7,2'-O}$GpNHppG		15.48 ± 3.44	11.56 ± 5.80
60	m$^{7,2'-O}$Gp$_{S}$pppG D1		17.91 ± 3.00	37.84 ± 5.22
61	m$^{7,2'-O}$Gp$_{S}$pppG D2		19.76 ± 6.15	36.66 ± 4.19
62	m^{7}Gp$_{BH3}$ppG D2		65.65 ± 9.75	38.84 ± 4.09
63	m^{7}Gpp$_{BH3}$pG D1		95.12 ± 1.07	39.12 ± 4.03
64	m^{7}Gpp$_{BH3}$pG D2		86.14 ± 2.34	43.79 ± 3.84
65	m$^{7,2'-O}$Gpp$_{BH3}$pG D2		69.29 ± 1.53	42.99 ± 3.81

66	m^{7}Gpp$_{BH3}$pm^{7}G		40.40 ± 2.47	13.74 ± 5.57
67	m^{7}GpCH$_2$ppA-N6-HDA		9.19 ± 3.25	-3.67 ± 6.69
68	Thiamine PP		-3.78 ± 4.56	-5.12 ± 6.75
69	NAD$^+$		-6.71 ± 3.47	9.01 ± 7.71
70	Folic acid		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

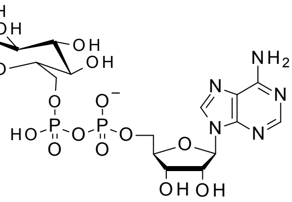
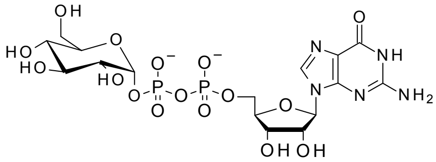
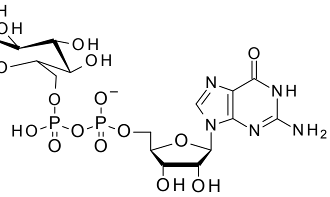
74	ADP-6-Glc		-2.19 ± 3.40	45.42 ± 3.66
75	GDP-1-Glc		6.22 ± 12.65	37.25 ± 4.11
76	GDP-6-Glc		-12.85 ± 7.65	35.21 ± 4.30

Table S3. Determined IC_{50} parameters for the selected DcpS and PDE-I inhibitors.

Compound	IC_{50} 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**	6.39 ± 0.92
66	15.2 ± 2.8		
m^7Gp_SppG D1	3.35 ± 0.67		
m^7Gp_SppG D2	2.39 ± 0.40		
RG3039	0.048 ± 0.010		

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

Tested compound		$\%_{inhibition}$	
Compound number	**Abbreviation**	**HPLC –based assay**	**Fluorescence assay**
39	**m^7GMP**	0.43 ± 12.93	20.52 ± 3.40
40	**m^7GDP**	45.55 ± 8.92	70.47 ± 1.49
49	**$m^7GDP\alpha BH_3$ D2**	37.29 ± 7.21	67.69 ± 1.80
53	**m^7GpCH_2ppG**	44.09 ± 3.96	81.61 ± 5.52
57	**$m^7GpNHppG$**	50.23 ± 0.45	81.37 ± 1.42
63	**$m^7Gpp_{BH3}pG$ D1**	79.17 ± 1.25	95.12 ± 1.07
64	**$m^7Gpp_{BH3}pG$ D2**	68.98 ± 2.29	86.14 ± 2.34
65	**$m^{7,2'\text{-}O}Gpp_{BH3}pG$ D2**	40.47 ± 5.32	69.29 ± 1.53
66	**$m^7Gpp_{BH3}pm^7G$**	25.48 ± 3.78	40.40 ± 2.47

2. Supplementary figures

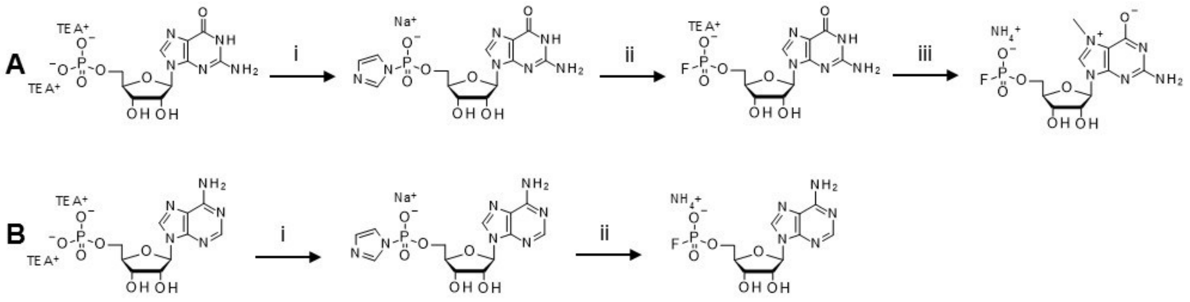


Figure S1. Synthesis of fluorophosphate nucleotide analogs from commercially available starting materials. **A**. Synthesis of 7-methyl guanosine 5’-fluoromonophosphate. **B.** Synthesis of adenosine 5’-fluoromonophosphate. i) 1. imidazole, 2’2-dithiodipyridine, Ph_3P, Et_3N, DMF; 2. $NaClO_4$, acetone; ii) TBAF, DMSO; iii) CH_3I, DMSO.

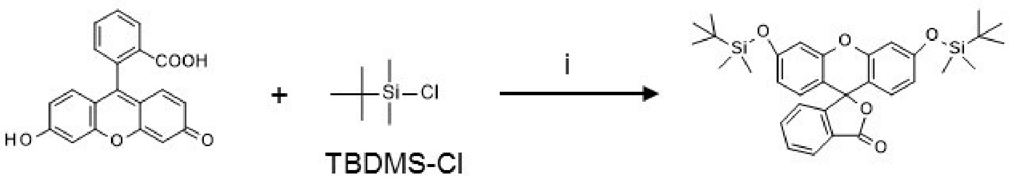


Figure S2. The synthesis of fluorogenic probe according to ref.[2] Conditions: i) imidazole, DMF.

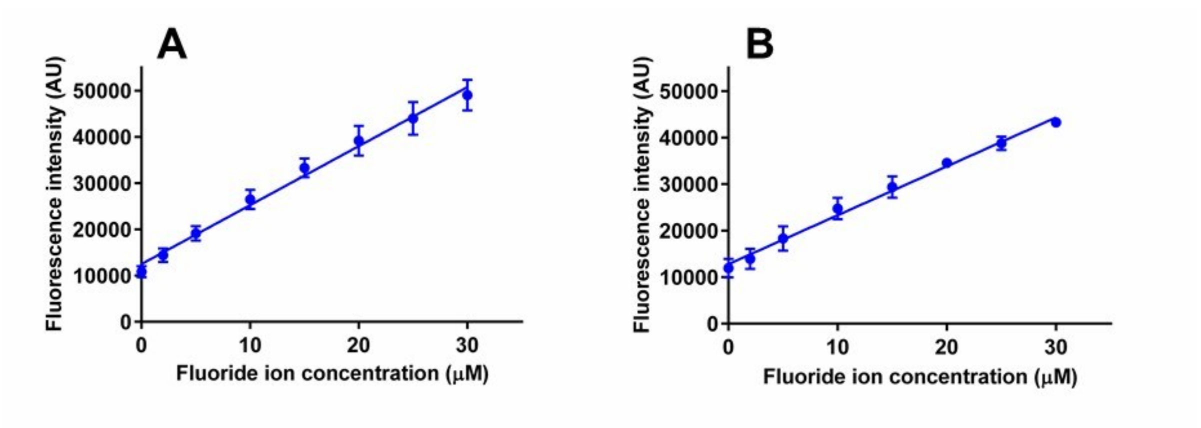


Figure S3. The calibration curves from hDcpS (A) and PDE-I (B) assays under optimized conditions.

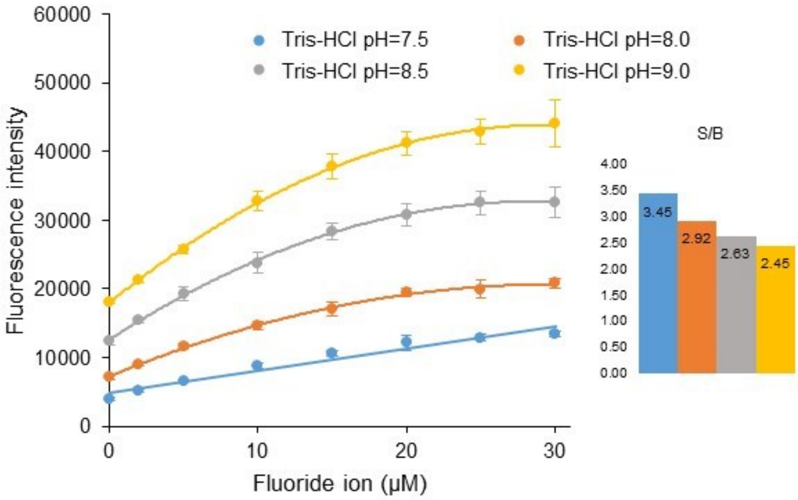


Figure S4. Influence of pH on calibration curve (left) and max. signal (30 μM) to background (0 μM) ratio (right). Each curve was repeated in triplicates.

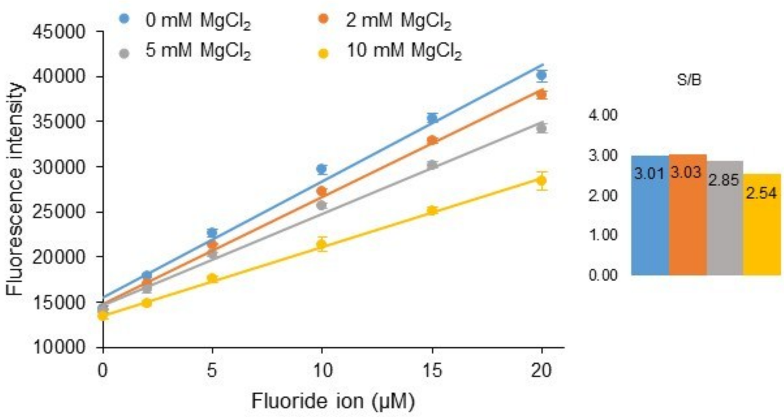


Figure S5. Influence of various concentrations of magnesium ions (in Tris-HCl pH=7.5) on the calibration curve (left) and max. signal (30 μM) to background (0 μM) ratio (right). Each curve was repeated in triplicates.

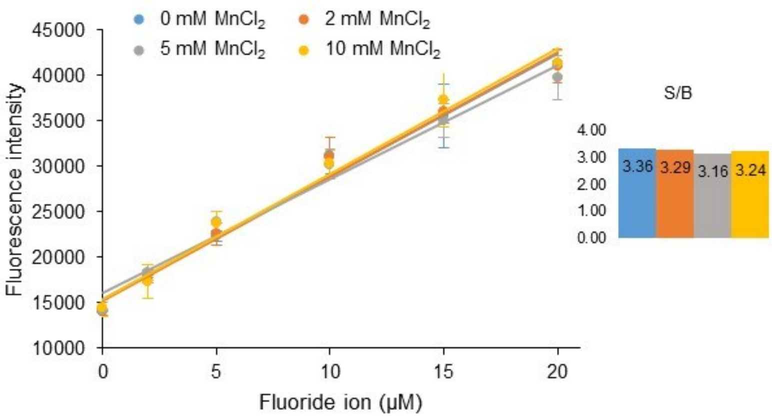


Figure S6. Influence of manganese ions (in Tris-HCl pH=7.5) on the calibration curve (left) and max. signal (30 μM) to background (0 μM) ratio (right). Each curve was repeated in triplicates.

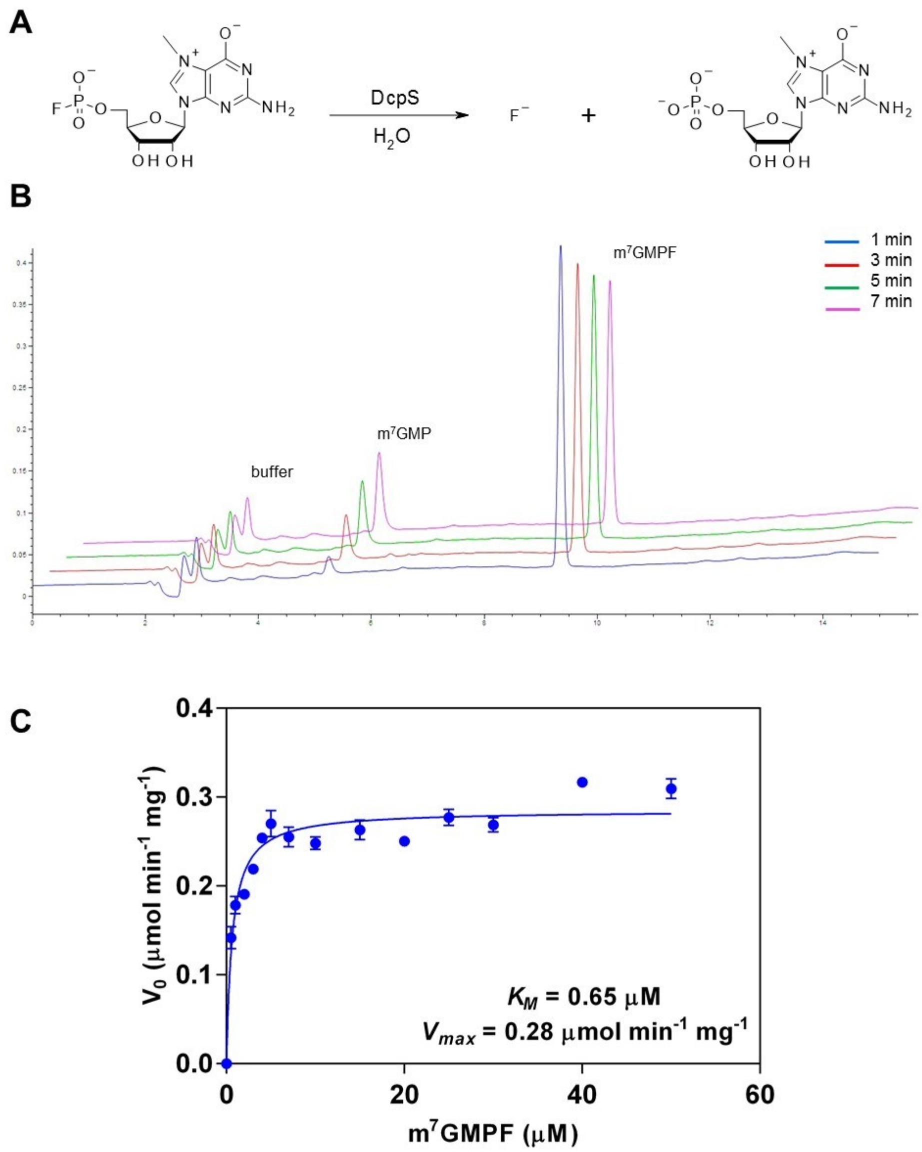


Figure S7. A. m^{7}GMPF cleavage to m^{7}GMP and fluoride catalyzed by hDcpS enzyme. **B.** Example HPLC profiles from the enzymatic reaction. **C.** Michaelis-Menten plot showing the kinetics of m^{7}GMPF cleavage by hDcpS enzyme at 20 ºC.

Supplementary references

1. Rydzik, A. M.; Leung, I. K. H.; Kochan, G. T.; Thalhammer, A.; Oppermann, U.; Claridge, T. D. W.; Schofield, C. J., Development and Application of a Fluoride-Detection-Based Fluorescence Assay for gamma-Butyrobetaine Hydroxylase. *Chembiochem* **2012,** *13* (11), 1559-1563.
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.